

VU Learning HUB

BIO732 Quiz File

Chapter-wise Solved MCQs

vulearninghub.xyz

Lesson 1: Introduction to Gene manipulation and genetic engineering

Q1. What is the primary definition of gene manipulation provided in the handout?

- A) The natural evolution of novel traits in wild bacterial populations
- B) The alteration of genes of an organism by manually adding new DNA to introduce novel traits
- C) The chemical breakdown of cellular RNA during protein synthesis
- D) The random mutation of bacterial cell walls using organic solvents

Q2. Which of the following is NOT listed in Lesson 1 as a basic component covered in this course?

- A) Isolation of target genes and PCR amplification
- B) Gel electrophoresis and transformation of target organisms
- C) Cutting and joining of target DNA molecules or vectors
- D) Crystallization of cellular lipid bilayers

Q3. According to Lesson 1, what types of organisms have been generated with novel traits using gene manipulation?

- A) Microorganisms, plants, and animals
- B) Gram-negative bacteria exclusively
- C) Agricultural plants only
- D) Synthetic viral particles only

Q4. What is the ultimate application goal of genetic engineering mentioned in Lesson 1?

- A) To shorten the overall length of host genomic DNA
- B) To obtain beneficial end products or cure different types of diseases
- C) To eliminate the necessity of restriction enzymes in molecular biology
- D) To stop cell division in recipient organisms

Answer Key

1. B — Gene manipulation refers to altering an organism's genes by manually adding new DNA to introduce novel traits
2. D — The course covers gene isolation, gel electrophoresis, PCR, vector cutting/joining, and transformation, but not lipid crystallization
3. A — Novel traits have been successfully introduced into a variety of microorganisms, plants, and animals
4. B — Applications focus on obtaining beneficial end products or curing various diseases
-
-

Lesson 2: What is genetic engineering?

Q1. What is the primary goal of genetic engineering as defined in Lesson 2?

- A) To double the chromosomal copy number in recipient host cells
- B) To manually add one or more new traits that are not already found in that organism
- C) To synthesize purine bases directly from inorganic minerals
- D) To permanently inhibit the action of host DNA ligase

Q2. Which alternative term for genetic engineering is specifically noted in Lesson 2?

- A) Recombinant DNA technology

- B) Thermal denaturation
- C) Splicing inhibition
- D) Isopycnic centrifugation

Q3. According to the Western legal definition cited in Lesson 2, how are new combinations of heritable material formed?

- A) By natural meiotic crossing over inside intact gametes
- B) By inserting nucleic acid molecules produced outside the cell into a vector system
- C) By spontaneous degradation of viral capsids in host cytoplasm
- D) By heat-shock treatment without foreign nucleic acids

Q4. How is genetic engineering applied in basic research?

- A) To develop commercial crop insecticides
- B) To study mechanisms of gene replication and expression in prokaryotes, eukaryotes, and their viruses
- C) To manufacture industrial alcohol from yeast
- D) To test patient antibody levels in blood banks

Answer Key

1. B — Genetic engineering aims to manually add new DNA to confer traits not naturally present in the organism
2. A — Equivalent terms listed include gene manipulation, gene cloning, recombinant DNA technology, and genetic modification
3. B — The legal definition specifies inserting nucleic acid molecules produced outside the cell into a vector system
4. B — Basic research utilizes genetic engineering to study gene replication and expression mechanisms
-
-

Lesson 3: Genes and genome

Q1. How is a genome defined in Lesson 3?

- A) A sequence of three bases specifying an amino acid
- B) An organism's complete set of DNA, including all of its genes
- C) A single polypeptide chain synthesized during translation
- D) An extrachromosomal circular plasmid molecule

Q2. Which pyrimidine base is common to both DNA and RNA?

- A) Thymine
- B) Uracil
- C) Cytosine
- D) Guanine

Q3. What is a gene defined as in Lesson 3?

- A) A sequence of three amino acids forming a protein chain
- B) The complete set of lipid molecules in a cell
- C) The nucleic acid sequence necessary for the synthesis of a functional gene product
- D) A artificial polylinker construct containing restriction sites

Q4. What are the only known functions of DNA stated in Lesson 3?

- A) Enzymatic cleavage and active membrane transport
- B) Storage and transmission of biological information
- C) Ribosome assembly and amino acid catabolism
- D) Cellular respiration and ATP generation

Answer Key

1. B — A genome is an organism's complete set of DNA containing all genetic instructions
2. C — Cytosine is found in both DNA and RNA, whereas thymine is unique to DNA and uracil to RNA
3. C — A gene is a nucleic acid sequence required to synthesize a functional polypeptide or RNA product
4. B — Storage and transmission of biological information represent the only known functions of DNA

Lesson 4: The flow of genetic information**Q1. What concept describes the flow of genetic information from DNA to RNA to protein?**

- A) In vitro recombination
- B) Central dogma of molecular biology
- C) Genetic transformation
- D) Insertional inactivation

Q2. What defines the relationship between the base sequence in mRNA and the amino acid sequence in a protein?

- A) Restriction endonuclease cleavage specificity
- B) The genetic code (a sequence of triplet bases)
- C) Isoschizomer recognition sites
- D) Capillary buffer transfer rate

Q3. Which molecule serves as an adaptor during protein synthesis by reading mRNA codons?

- A) rRNA
- B) tRNA
- C) DNA polymerase
- D) DNA ligase

Q4. Where does protein synthesis occur within the cell according to Lesson 4?

- A) On ribosomes, which are assemblies of rRNAs and over 50 kinds of proteins
- B) Exclusively inside the nuclear envelope
- C) On restriction enzyme complexes
- D) Within pure plasmid vector backbones

Answer Key

1. B — The central dogma of molecular biology governs the directional flow of genetic information
2. B — The genetic code relies on triplet base codons specifying particular amino acids
3. B — tRNA molecules act as adaptors reading mRNA codons sequentially during translation
4. A — Translation occurs on ribosomes composed of rRNAs and more than 50 distinct proteins

Lesson 5: Molecular cloning

Q1. What is the fundamental strategy of molecular cloning outlined in Lesson 5?

- A) To double the total chromosomal length of the host organism
- B) To move a desired gene from a large, complex genome to a small, simple one
- C) To hydrolyze proteins using digestive enzymes
- D) To convert eukaryotic mRNA directly into infectious virus coats

Q2. Which three key tools are highlighted as essential for in vitro recombination?

- A) Restriction enzymes, DNA ligase, and synthetic DNA
- B) Phenol, chloroform, and ethanol
- C) Ethidium bromide, agarose, and ultraviolet light
- D) Reverse transcriptase, RNase, and SDS

Q3. How are source DNA fragments joined to a cloning vector when both are cut with the same restriction enzyme?

- A) By high-temperature baking at 80°C
- B) By annealing complementary single-stranded "sticky ends" mediated by DNA ligase
- C) By high-speed centrifugation at 15,000 g
- D) By alkaline hydrolysis of purine bases

Q4. What is formed when source DNA fragments are joined to vector molecules to yield a mixture of different clones?

- A) A central dogma
- B) A DNA library or gene library
- C) A homoduplex complex
- D) A polyacrylamide gel matrix

Answer Key

1. B — Molecular cloning involves moving a desired gene from a complex genome into a small, simple vector system
2. A — In vitro recombination relies on restriction enzymes, DNA ligase, and synthetic DNA
3. B — Matching restriction enzymes create complementary sticky ends that anneal and are covalently linked by DNA ligase
4. B — A mixture of recipient host cells carrying various foreign DNA inserts forms a gene/DNA library

Lesson 6: Basic techniques: Introduction

Q1. What three major technical developments in the early 1970s provided the initial impetus for gene manipulation in vitro?

- A) DNA sequencing, automated synthesis, and X-ray crystallography
- B) Genetic transformation of Escherichia coli, cutting and joining DNA molecules, and monitoring cutting/joining reactions
- C) Mass spectrometry, Western blotting, and protoplast fusion

D) Polymerase chain reaction, cDNA synthesis, and microinjection

Q2. Why do unintegrated exogenous DNA fragments fail to be maintained during host cell multiplication?

- A) They trigger immediate apoptosis in the host cell
- B) They lack an origin of replication (replicon) and are diluted out
- C) They are rapidly converted into RNA molecules
- D) They precipitate spontaneously due to cytoplasmic pH

Q3. According to Lesson 6, how many origins of replication are usually present per genome in bacteria and viruses?

- A) One per genome
- B) Two per chromosome
- C) Hundreds along the DNA strand
- D) None, replication is random

Q4. In early transformation attempts, what was a primary cause of failure when detection depended on gene expression?

- A) Lack of suitable fluorescent dyes
- B) Lack of accurate transcription or translation in recipient cells
- C) Excessive synthesis of viral capsids
- D) Immediate degradation of host cell ribosomes

Answer Key

1. B — The initial impetus came from E. coli transformation, cutting and joining DNA, and monitoring those reactions
2. B — Exogenous DNA fragments lack an origin of replication and are diluted out during host cell division
3. A — Bacteria and viruses usually contain only one origin of replication per genome
4. B — Detection relying on gene expression fails if recipient host cells lack accurate transcription or translation

Lesson 8: Isolation of genomic DNA from E. coli

Q1. In traditional chemical methods of DNA extraction, which combination of organic solvents was generally relied upon despite being toxic and multi-step?

- A) Methanol–acetone
- B) Phenol–chloroform
- C) Ethanol–ether
- D) Benzene–xylene

Q2. During genomic DNA isolation from bacterial cells, at what centrifugal force and time is the cell pellet initially separated?

- A) 5000 g for 10 min
- B) 10000 g for 5 min
- C) 15000 g for 2 min
- D) 20000 g for 1 min

Q3. What is the specific function of sodium dodecyl sulphate (SDS) in the bacterial cell lysis solution?

- A) To digest cellular RNA into individual nucleotides
- B) To disrupt membranes and denature proteins
- C) To precipitate genomic DNA out of solution
- D) To synthesize phosphodiester bonds

Q4. According to Lesson 8, how are DNA threads made visible during the final isolation steps?

- A) By boiling the solution at 100°C
- B) By dissolving the DNA pellet in 70% ethanol
- C) By adding ethidium bromide under UV light
- D) By treating the sample with proteinase K

Answer Key

1. B — Traditional extraction methods generally relied on phenol–chloroform, which is toxic and time-consuming
2. C — Bacterial cells cultivated overnight are pelleted by centrifugation at 15000 g for 2 min
3. B — Lysis solution contains the anionic detergent SDS to disrupt cell membranes and denature proteins
4. B — DNA threads can be made visible by dissolving the DNA pellet in 70% ethanol

Lesson 9: Agarose gel electrophoresis

Q1. What size range of DNA fragments is agarose gel electrophoresis convenient for separating?

- A) 1 bp to 50 bp
- B) A few hundred base pairs to about 20 kb
- C) 50 kb to 500 kb
- D) 1 Mb to 10 Mb

Q2. What technique was historically used to monitor cutting and joining reactions before gel electrophoresis superseded it?

- A) Velocity sedimentation in sucrose gradients
- B) Isopycnic centrifugation in CsCl
- C) Thin-layer chromatography
- D) Paper chromatography

Q3. Through what process do DNA molecules larger than ~20 kb move through an agarose gel matrix?

- A) Free solution diffusion
- B) Active transport
- C) Reptation
- D) Capillary action

Q4. Which technique allows the separation of DNA molecules as large as 10 Mb in agarose gels by periodically altering the electric field direction?

- A) SDS-PAGE

- B) Pulsed-field gel electrophoresis (PFGE)
- C) Affinity chromatography
- D) Isoelectric focusing

Answer Key

1. B — Agarose gel electrophoresis is suitable for separating DNA fragments from a few hundred base pairs to about 20 kb
2. A — Velocity sedimentation in sucrose gradients monitored early reactions before gel electrophoresis superseded it
3. C — Once the globular volume exceeds the gel pore size (around 20 kb), DNA moves through the gel by reptation
4. B — Pulsed-field gel electrophoresis (PFGE) separates DNA up to 10 Mb by periodically changing the electric field direction

Lesson 10: Nucleic acid blotting

Q1. What solid support matrix is generally used to immobilize sample nucleic acids during blotting?

- A) Polyacrylamide gel slabs
- B) Sucrose gradient tubes
- C) Nylon or nitrocellulose membranes
- D) Agarose gel blocks

Q2. What parameters determine the stringency of nucleic acid hybridization on a membrane?

- A) Gel voltage and electrophoresis run time
- B) Hybridization temperature and salt concentration in the buffer
- C) Centrifugation speed and rotor angle
- D) Concentration of SDS and lysozyme

Q3. What is defined as a fragment of DNA or RNA used to detect complementary target DNA sequences?

- A) Replicon
- B) Vector
- C) Probe
- D) Isoschizomer

Q4. Which option correctly identifies the three main types of nucleic acid hybridization blotting covered in Lesson 10?

- A) Southern (Protein), Northern (DNA), Western (RNA)
- B) Southern (DNA), Northern (RNA), Western (Protein)
- C) Southern (RNA), Northern (Protein), Western (DNA)
- D) Southern (DNA), Northern (Protein), Western (RNA)

Answer Key

1. C — Blotting immobilizes sample nucleic acids onto solid supports, generally nylon or nitrocellulose membranes

2. B — Hybridization stringency is determined by the hybridization temperature and salt concentration in the buffer
3. C — A probe is a fragment of DNA or RNA used to detect sequences of target DNA
4. B — Nucleic acid hybridization includes Southern blotting (DNA), Northern blotting (RNA), and Western blotting (Protein)

Lesson 11: Autoradiography

Q1. What medium is used in autoradiography to visualize radioactively labeled molecular fragments?

- A) Nitrocellulose filter paper
- B) X-ray film
- C) Agarose gel matrix
- D) Polyacrylamide slab

Q2. What type of information does autoradiography provide when analyzing biological specimens?

- A) Only structural and morphological data
- B) Both qualitative and quantitative information
- C) Exclusively nucleotide sequence order
- D) Only pH and temperature kinetic curves

Q3. Which of the following can autoradiography be used to analyze after gel electrophoresis?

- A) The precise amino acid sequence of proteins
- B) The length and number of radioactively labeled DNA fragments
- C) The melting temperature of unlabelled primers
- D) The concentration of ethidium bromide dye

Q4. Which specimens can be imaged using autoradiography according to Topic 11?

- A) Only synthetic oligonucleotide primers
- B) Only purified plasmid DNA in solution
- C) Large, small, and microscopic specimens containing a radiolabelled compound
- D) Unlabelled bacterial cell walls exclusively

Answer Key

1. B — Autoradiography uses X-ray film to visualize radioactively labeled molecular fragments
2. B — Autoradiography provides both qualitative and quantitative information such as images and amounts present
3. B — Autoradiography is used to analyze the length and number of DNA fragments separated by gel electrophoresis
4. C — It can image sectioned whole organisms, organs, tissues, cellular structures, and nucleic acids containing radiolabelled compounds

Lesson 12: Southern blotting

Q1. Who developed the original blotting method in 1975 to detect DNA fragments complementary to a given sequence?

- A) Alwine
- B) Southern
- C) Maxam and Gilbert
- D) Benton and Davis

Q2. Why is gel pretreatment with 0.25 mol/l HCl and alkali performed prior to Southern transfer for large DNA fragments (>10 kb)?

- A) To covalently bind the DNA directly to the filter paper wick
- B) To shorten fragments by alkaline hydrolysis at depurinated sites and denature them into single strands
- C) To synthesize complementary RNA probes directly on the gel matrix
- D) To prevent ethidium bromide from intercalating into the double helix

Q3. What main advantage do supported nylon membranes have over traditional nitrocellulose membranes in Southern blotting?

- A) They do not require any buffer or capillary action for transfer
- B) They have greater binding capacity for nucleic acids and high tensile strength
- C) They eliminate the need for probe hybridization washes
- D) They automatically emit fluorescence without radioactive labeling

Q4. What recommended fixation method is used for fixing transferred nucleic acids onto nitrocellulose membranes?

- A) Washing in 70% ethanol at room temperature
- B) Oven baking at 80°C
- C) Soaking in ice-cold calcium chloride for 30 minutes
- D) Autoclaving at 121°C

Answer Key

1. B — Southern developed the original DNA blotting technique in 1975
2. B — Acid depurination followed by alkali hydrolyzes large DNA fragments to shorten them and ensure single-stranded denaturation for efficient transfer
3. B — Supported nylon membranes offer higher tensile strength and greater binding capacity than fragile nitrocellulose
4. B — Oven baking at 80°C is the recommended method for fixing nucleic acids onto nitrocellulose membranes

Lesson 13: Northern blotting

Q1. What is the primary function of Northern blotting as described in Topic 13?

- A) To determine the amino acid sequence of primary proteins
- B) To detect specific RNA molecules among an RNA mixture and measure gene expression
- C) To cleave double-stranded genomic DNA at palindromic restriction sites
- D) To amplify target DNA sequences exponentially using thermal cycling

Q2. Why was Southern's original technique initially thought to be inapplicable to RNA transfers?

- A) RNA molecules are too large to pass through agarose pores
- B) RNA was found not to bind to nitrocellulose
- C) RNA lacks purine bases needed for capillary action
- D) RNA degrades completely at temperatures above 4°C

Q3. What chemically reactive paper was devised by Alwine and coworkers (1979) to covalently bind transferred RNA?

- A) Diazobenzylloxymethyl (DBM) paper
- B) Polyacrylamide-coated Whatman cellulose
- C) Ethidium bromide-impregnated nylon
- D) Sodium dodecyl sulphate membrane

Q4. How is DBM paper prepared according to Lesson 13?

- A) By treating Whatman 540 paper through diazotization of aminobenzylloxymethyl paper
- B) By baking nitrocellulose filters at 100°C in an alkali bath
- C) By washing nylon membranes with proteinase K and SDS
- D) By soaking filter paper in ice-cold 50 mmol/l calcium chloride

Answer Key

1. B — Northern blotting detects specific RNA molecules in a mixture to measure gene expression
2. B — It was initially thought inapplicable because RNA was found not to bind to nitrocellulose membranes
3. A — Alwine and coworkers used diazobenzylloxymethyl (DBM) paper to covalently bind transferred RNA
4. A — DBM paper is prepared by diazotization of aminobenzylloxymethyl paper derived from Whatman 540 paper

Lesson 14: Western blotting**Q1. What type of gel is typically used for electrophoresing protein bands prior to Western blotting?**

- A) Agarose gel
- B) Polyacrylamide gel
- C) Silica gel
- D) Sucrose gradient gel

Q2. What probes are most commonly used in Western blotting to detect specific antigen targets?

- A) Single-stranded oligodeoxynucleotides
- B) Antibodies
- C) Diazobenzylloxymethyl papers
- D) Radiolabelled tRNA molecules

Q3. Which reagents are mentioned in Lesson 14 as being used to specifically identify glycoproteins on Western blots?

- A) Lectins

- B) Restriction endonucleases
- C) Alkaline phosphatases
- D) Isoschizomers

Q4. What is a primary advantage of using a "sandwich" reaction with a labelled second molecule in Western blotting?

- A) It eliminates the need for polyacrylamide gel electrophoresis
- B) A single preparation of labelled second molecule can serve as a general detector for different probes
- C) It prevents antibodies from binding to nitrocellulose membranes
- D) It converts target proteins directly into complementary DNA

Answer Key

1. B — Western blotting involves transferring protein bands electrophoresed on a polyacrylamide gel onto a membrane
2. B — Antibodies are most commonly employed as probes to detect specific target antigens on Western blots
3. A — Lectins are specifically used to identify glycoproteins bound to the membrane
4. B — The sandwich method allows one preparation of labelled second molecule to detect many different primary antibody probes

Lesson 15: Transformation of E. coli

Q1. What chemical treatment was discovered to render E. coli cells competent for taking up bacteriophage λ and plasmid DNA?

- A) Phenol-chloroform
- B) Calcium chloride (CaCl_2)
- C) Sodium dodecyl sulphate (SDS)
- D) Isopropanol

Q2. What cell concentration and buffer condition are used when preparing log-phase E. coli competent cells in the standard procedure?

- A) 10^{10} cells/ml in ice-cold 50 mmol/l CaCl_2
- B) 10^5 cells/ml in boiling 100 mmol/l HCl
- C) 10^{12} cells/ml in 70% ethanol
- D) 10^2 cells/ml in pure isopropanol

Q3. What exact temperature and duration are specified for the heat-shock step during E. coli transformation?

- A) 37°C for 10 minutes
- B) 42°C for 2 minutes
- C) 65°C for 30 seconds
- D) 100°C for 5 minutes

Q4. Why are transformed cells incubated in nutrient medium following the heat-shock step?

- A) To denature the incoming plasmid vector into single strands
- B) To allow phenotypic properties like antibiotic resistance to be expressed before selection
- C) To digest excess host chromosomal DNA with restriction enzymes

D) To induce depurination of single-stranded overhangs

Answer Key

1. B — Treatment with calcium chloride (CaCl₂) makes E. coli competent to take up DNA
2. A — Log-phase cells are prepared at approximately 10¹⁰ cells/ml in ice-cold 50 mmol/l CaCl₂
3. B — The heat-shock step is carried out at 42°C for exactly 2 minutes
4. B — Incubation in nutrient medium allows expression of plasmid-encoded phenotypic markers such as antibiotic resistance

Lesson 16: Transformation with other organisms

1. Which Gram-positive bacterium is highlighted in Lesson 16 as a particularly attractive alternative cloning host because it is naturally competent for transformation? A) *Escherichia coli* B) *Bacillus subtilis* C) *Staphylococcus aureus* D) *Streptomyces coelicolor*
2. What term is used to describe the delivery of nucleic acids into host cells using cationic lipid liposomes? A) Transduction B) Electroporation C) Lipofection D) Microinjection
3. Which physical approach mentioned in Lesson 16 can be used to transform intact plant and animal cells? A) Heat-shock treatment at 42°C B) Microprojectiles shot from a gun C) Calcium phosphate co-precipitation D) Alkaline lysis treatment
4. What cellular preparation is specifically required to enable artificial transformation in yeast cells? A) Removal of the nuclear envelope B) Preparation of protoplasts C) High-voltage electroporation only D) Sonication with intense ultrasound

Answer Key

- * **1. B** — *Bacillus subtilis* is naturally competent for transformation, making it a prominent alternative prokaryotic host.
- * **2. C** — Lipofection refers to using cationic lipid liposomes as a transformation or transfection system.
- * **3. B** — Microprojectiles shot from a gun provide a physical delivery method for plant and animal transformation.
- * **4. B** — Yeast transformation specifically requires the preparation of wall-less protoplasts.

Lesson 17: Polymerase chain reaction (PCR)

1. What length are the two oligonucleotide primers used in PCR to flank the DNA target sequence?
 - A) 5 to 10 nucleotides
 - B) 17 to 30 nucleotides
 - C) 50 to 100 nucleotides
 - D) 150 to 200 nucleotides
2. During PCR, which cycle first produces two double-stranded molecules that comprise precisely the target region in double-stranded form?
 - A) First cycle

- B) Second cycle
- C) Third cycle
- D) Twenty-second cycle

3. What fold amplification of the specific target DNA fragment is expected after 22 cycles of PCR?

- A) About (10^2) fold
- B) About (10^4) fold
- C) About (10^6) fold
- D) About (10^9) fold

4. In forensic science, what procedure specifically exploits the high sensitivity of PCR?

- A) Prenatal tissue screening
- B) DNA profiling
- C) Southern blot hybridization
- D) Western blot analysis

Answer Key

* **1. B** — The two oligonucleotide primers that flank the target DNA sequence in PCR are 17 to 30 nucleotides in length.

* **2. C** — The third cycle produces two double-stranded molecules that comprise precisely the target region in double-stranded form.

* **3. C** — Repeated cycles of PCR result in an expected amplification of about (10^6) fold after 22 cycles.

* **4. B** — In forensic science, the sensitivity of PCR-based procedures is exploited in DNA profiling.

Lesson 18: Cutting and joining DNA molecules

1. To what approximate length is duplex DNA reduced when subjected to intense sonication with ultrasound?

- A) About 50 nucleotide pairs
- B) About 300 nucleotide pairs
- C) About 800 nucleotide pairs
- D) About 4000 nucleotide pairs

2. What mean size of DNA fragments is produced by controlled mechanical shearing using high-speed stirring in a blender at 1500 rev/min for 30 minutes?

- A) About 300 bp
- B) About 2 kb
- C) About 8 kb
- D) About 20 kb

3. Why was the restriction endonuclease of *Escherichia coli* K12, purified in 1968, perverse in its properties for precise gene manipulation?

- A) It degraded DNA exclusively into single nucleotides
- B) It bound to a recognition sequence but cleaved DNA at a random site several kilobases away
- C) It required single-stranded RNA templates instead of duplex DNA

D) It only cleaved DNA at the exact ends of linear chromosomes

4. What major breakthrough occurred in 1970 with the discovery of a restriction enzyme in *Haemophilus influenzae*?

- A) The discovery of an enzyme that recognizes a specific target sequence and cleaves within it to give discrete fragments
- B) The development of automated DNA sequencing
- C) The discovery of T4 DNA ligase
- D) The introduction of PCR thermal cycling

Answer Key

***1. B** — Intense sonication with ultrasound reduces duplex DNA length to about 300 nucleotide pairs.

***2. C** — High-molecular-weight DNA stirred at 1500 rev/min for 30 minutes is sheared to a mean size of about 8 kb.

***3. B** — The *E. coli* K12 enzyme binds to a defined recognition sequence but cleaves at a random site several kilobases away.

***4. A** — The *H. influenzae* enzyme recognizes a specific target sequence and breaks the polynucleotide chain within that sequence to yield discrete fragments.

Lesson 19: Host-controlled restriction/ modification

1. What effect does host-controlled restriction have when foreign bacteriophage DNA enters a restrictive bacterial host?

- A) It increases the phage burst size
- B) It reduces the efficiency of plating (EOP) and number of plaques formed
- C) It permanently integrates the phage genome into host ribosomes
- D) It prevents the phage from adsorbing to the cell wall

2. What occurs when phage lambda that survived and reproduced in *E. coli* strain K is replated directly on *E. coli* strain K?

- A) It is restricted with an efficiency of (10^{-4})
- B) It is no longer restricted because it received host modification during propagation in *E. coli* K
- C) It fails to inject its DNA into the host cell
- D) It mutates into a completely different viral strain

3. In experiments with ^{32}P -labelled restricted phages, what happens to the donor phage DNA shortly after injection into a restrictive host?

- A) The DNA is converted into single-stranded RNA
- B) The DNA is degraded soon after injection by restriction endonucleases
- C) The DNA is immediately integrated into the host chromosome
- D) The DNA remains completely intact in the cytoplasm for weeks

4. How does a restrictive host bacterium protect its own genomic DNA from cleavage by its restriction endonucleases?

- A) By removing all purine bases from its chromosome
- B) By methylating certain bases at its recognition sequences

- C) By wrapping its DNA in a protective lipid bilayer
- D) By completely eliminating all restriction sites from its genome

Answer Key

- * **1. B** — Restriction endonucleases cleave incoming foreign phage DNA, reducing the efficiency of plating.
- * **2. B** — The phages receive non-heritable modification from *E. coli* K, allowing them to re-infect *E. coli* K without restriction.
- * **3. B** — ^{32}P -labelled phage DNA is degraded soon after injection by restriction endonucleases.
- * **4. B** — The host modifies its own DNA by methylating certain bases at the enzyme's recognition sequences.

Lesson 20: Types of restriction and modification

1. What subunit structure characterizes the active Type I restriction-modification enzyme complex from *E. coli* K12?

- A) Four restriction subunits and four recognition subunits
- B) Two restriction subunits, two modification subunits, and one recognition subunit
- C) One restriction subunit and two lipid binding subunits
- D) Three modification subunits and three ATP synthase subunits

2. Why are Type I restriction-modification systems considered of little value for gene manipulation?

- A) They cannot cleave double-stranded DNA under any conditions
- B) They require RNA primers and destroy vector backbones
- C) They cut DNA at a distance from asymmetric recognition sites and may modify target DNA before cleavage
- D) They only function at temperatures above 90°C

3. What major advantage do Type II restriction endonucleases possess over Type I and Type III systems in genetic engineering?

- A) They require ATP and S-adenosylmethionine for restriction cleavage
- B) Restriction and modification are mediated by separate enzymes, and they cut within defined, symmetrical recognition sites
- C) They contain fused modification and cleavage subunits in a single polypeptide
- D) They cleave DNA randomly at distances exceeding 10 kb

4. What characteristic of Type II restriction-modification systems limits their usefulness in gene manipulation?

- A) They lack recognition subunits completely
- B) Restriction cleavage occurs at a distance from the recognition site
- C) They require high concentrations of phenol to function
- D) They degrade host DNA preferentially over foreign DNA

Answer Key

- * **1. B** — Type I active enzymes consist of two restriction subunits, two modification subunits, and one recognition subunit.

* **2. C** — Type I enzymes cut at a distance from asymmetric recognition sites and can modify target DNA before cleavage.

* **3. B** — Type II systems use separate restriction and modification enzymes, require no cofactors like ATP or S-adenosylmethionine, and cut within defined symmetrical sites.

* **4. B** — In Type II systems, restriction occurs at a distance from the recognition site, limiting their usefulness in cloning.

Lesson 21: Nomenclature

1. Who introduced the simplified uniform system of nomenclature for restriction and modification systems in 1973?

- A) Meselson and Yuan
- B) Smith and Nathans
- C) Alwine and coworkers
- D) Grunstein and Hogness

2. According to the nomenclature system in Topic 21, how is the species name of a host organism represented in the three-letter abbreviation?

- A) First two letters of the genus name and first letter of the specific epithet in bold
- B) First letter of the genus name and first two letters of the specific epithet in italics
- C) First three letters of the genus name in uppercase
- D) First letter of the family name and first two letters of the strain name

3. What do the Roman numerals at the end of a restriction enzyme's name (such as *HindI*, *HindII*, *HindIII*) represent?

- A) The number of base pairs in its recognition sequence
- B) The optimum temperature required for enzyme activity
- C) The specific R-M system when a host strain has several different R-M systems
- D) The number of subunits making up the active restriction complex

4. Which host organism and enzyme order does the restriction endonuclease **HaeIII** originate from according to Table 1?

- A) **Haemophilus aegyptius**, 3rd enzyme
- B) **Serratia marcescens**, 1st enzyme
- C) **Haemophilus influenzae**, strain d, 2nd enzyme
- D) **Bacillus amyloliquefaciens**, strain H, 1st enzyme

Answer Key

* **1. B** — Smith and Nathans introduced the uniform nomenclature system for restriction enzymes in 1973.

* **2. B** — The three-letter abbreviation consists of the first letter of the genus and first two letters of the specific epithet written in italics.

* **3. C** — Roman numerals designate the distinct R-M system when a host strain possesses multiple systems.

* **4. A** — Table 1 identifies **HaeIII** as the 3rd enzyme isolated from **Haemophilus aegyptius**.

Lesson 22: Target sites

1. What term is used for sequences of four to eight nucleotides that have a two-fold axis of rotational symmetry recognized by most Type II restriction enzymes?
 - A) Replicons
 - B) Palindromes
 - C) Promoters
 - D) Polylinkers
2. What type of termini are generated when DNA is digested with the restriction enzyme *SmaI* (recognition site CCC/GGG)?
 - A) Protruding 5' cohesive ends
 - B) Protruding 3' cohesive ends
 - C) Blunt or flush ends
 - D) Single-stranded homopolymer tails
3. What are restriction enzymes called if they share the exact same sequence specificity and cut site?
 - A) Neoschizomers
 - B) Isoschizomers
 - C) Isotype endonucleases
 - D) Heteroduplexes
4. How do neoschizomers differ from isoschizomers according to Topic 22?
 - A) Neoschizomers recognize the same sequence but cleave at different points
 - B) Neoschizomers cut randomly several kilobases away from their recognition site
 - C) Neoschizomers require ATP and S-adenosylmethionine cofactors
 - D) Neoschizomers only act on single-stranded RNA templates

Answer Key

* **1. B** — Recognition sequences possessing a two-fold axis of rotational symmetry are referred to as palindromes.

* **2. C** — *SmaI* cleaves symmetrically at CCC/GGG to produce blunt or flush ends.

* **3. B** — Restriction enzymes possessing identical sequence specificity and cut sites are defined as isoschizomers.

* **4. A** — Neoschizomers (such as *SmaI* and *XmaI*) recognize the same target sequence but cleave at different positions.

Lesson 23: Number and Size of restriction fragments

1. Statistically, assuming a random distribution of bases and a 50% G+C content, how frequently is a six-base recognition site expected to occur?
 - A) Every 256 bp
 - B) Every 4096 bp
 - C) Every 2000 bp
 - D) Every 65,536 bp

2. How frequently does an eight-base recognition site occur under the assumption of random base distribution and 50% G+C content?

- A) Every 256 bp
- B) Every 4096 bp
- C) Every 15,000 bp
- D) Every 65,536 bp

3. Why do actual average restriction fragment sizes produced by six-base cutting endonucleases differ significantly from the statistical expectation of 4096 bp?

- A) Bases are not randomly distributed and genomes vary in A+T or G+C content
- B) Restriction enzymes degrade bases randomly during incubation
- C) DNA ligase rapidly re-seals nicks during digestion
- D) Agarose gel electrophoresis alters the physical length of DNA strands

4. According to Table 2 in Topic 23, what is the average fragment size generated by *SpeI* (target ACTAGT) in *E. coli* DNA?

- A) 2,000 bp
- B) 6,000 bp
- C) 10,000 bp
- D) 60,000 bp

Answer Key

* **1. B** — Statistically, a six-base site occurs once every $(4^6) = 4096$ bp in a random genome with 50% G+C.

* **2. D** — An eight-base recognition site occurs once every $(4^8) = 65,536$ bp under random distribution assumptions.

* **3. A** — Non-random base distribution and genome G+C variations cause actual fragment sizes to diverge from theoretical values.

* **4. D** — Table 2 specifies that *SpeI* generates an average fragment size of 60,000 bp in *E. coli* DNA.

Lesson 24: Summary of restriction endonucleases

1. What is the biological function of restriction-modification systems in bacteria?

- A) To initiate translation on host cell ribosomes
- B) To continuously monitor and degrade incoming foreign DNA
- C) To repair damaged mitochondrial membranes
- D) To synthesize RNA primers for DNA replication

2. Why are Type I restriction-modification systems considered to be of little value for gene manipulation?

- A) They require heavy metal ions that precipitate DNA
- B) They lack cleavage specificity because they cut DNA at a distance from their recognition sites
- C) They can only cleave single-stranded cDNA molecules
- D) They destroy vector backbones irreversibly via alkaline hydrolysis

3. Which type of restriction-modification system is described as the most useful for genetic engineering because it recognizes a defined sequence and cleaves within it?

- A) Type I
- B) Type II
- C) Type III
- D) Type IIs

4. How do Type IIs restriction endonucleases differ from standard Type II systems, limiting their utility in cloning?

- A) Type IIs systems require RNA templates rather than DNA
- B) Type IIs cleavage occurs at a distance from the recognition site
- C) Type IIs systems irreversibly modify the host genome
- D) Type IIs systems lack recognition subunits completely

Answer Key

- * **1. B** — Restriction-modification systems allow bacteria to monitor and degrade incoming foreign DNA.
- * **2. B** — Type I systems cut at a distance from recognition sites and lack the specificity needed for gene manipulation.
- * **3. B** — Type II systems are the most useful because they recognize specific target sequences and cut within them.
- * **4. B** — In Type IIs systems, restriction occurs at a distance from the recognition site, limiting their cloning usefulness.

Lesson 25: DNA modifying enzymes

1. How do exonucleases differ from endonucleases in their mode of nucleic acid degradation?

- A) Exonucleases break phosphodiester bonds from the termini, whereas endonucleases cut within the DNA strand
- B) Exonucleases only digest RNA, whereas endonucleases only digest DNA
- C) Exonucleases require ATP, whereas endonucleases require NAD⁺
- D) Exonucleases remove methyl groups, whereas endonucleases add phosphate groups

2. In what direction do all polymerase enzymes synthesize nucleic acid strands?

- A) 3' → 5' direction
- B) 5' → 3' direction
- C) Bi-directionally without a primer
- D) Randomly from either terminus

3. What chemical group must be exposed at the growing 3' end of a primer for a DNA polymerase to catalyze phosphodiester bond formation?

- A) A free 5'-phosphate group
- B) A free 3'-OH group
- C) A 2'-deoxyribose ring closure
- D) A methylated cytosine base

4. What is the enzyme source and primary function of T4 DNA ligase as described in Topic 25?

- A) Purified from *Taq* bacteria; amplifies target DNA during thermal cycling
- B) Purified from *E. coli* infected with phage T4; seals discontinuities in sugar-phosphate chains
- C) Purified from calf thymus; adds homopolymeric tails to 3' ends
- D) Purified from *H. influenzae*; removes 5' phosphate groups from linearized vectors

Answer Key

- * **1. A** — Exonucleases degrade DNA starting from the termini, while endonucleases cleave within the strand.
- * **2. B** — Nucleic acid synthesis catalyzed by polymerases proceeds strictly in the (5' → 3') direction.
- * **3. B** — Polymerases require a free 3'-OH group on the primer to form phosphodiester bonds.
- * **4. B** — T4 DNA ligase is purified from T4 phage-infected *E. coli* and seals sugar-phosphate backbone gaps.
-

Lesson 26: Method of joining DNA fragments

1. How many main methods are used for joining DNA in vitro according to Topic 26?

- A) Two
- B) Three
- C) Five
- D) Ten

2. What chemical bond does DNA ligase catalyze to join DNA strands together?

- A) Hydrogen bond
- B) Glycosidic bond
- C) Phosphodiester bond
- D) Peptide bond

3. Which specific DNA ligase source can join blunt-ended DNA fragments under appropriate conditions?

- A) Uninfected *E. coli* DNA ligase
- B) T4 DNA ligase from phage T4-infected *E. coli*
- C) Calf thymus DNA polymerase
- D) Reverse transcriptase

4. Which enzyme is utilized in the third method of joining DNA fragments to synthesize homopolymeric 3' single-stranded tails?

- A) Alkaline phosphatase
- B) Terminal deoxynucleotidyltransferase
- C) Poly A polymerase
- D) Polynucleotide kinase

Answer Key

- * **1. B** — Three main methods are used for joining DNA in vitro.
- * **2. C** — DNA ligase facilitates joining by catalyzing the formation of phosphodiester bonds.
- * **3. B** — T4 DNA ligase purified from phage T4-infected *E. coli* can join blunt-ended DNA molecules.

* **4. B** — Terminal deoxynucleotidyltransferase synthesizes homopolymeric 3' single-stranded extensions at fragment ends.

Lesson 27: DNA ligase to create covalent recombinant DNA

1. What cofactor is specifically required by *E. coli* DNA ligase versus T4 DNA ligase?

- A) ATP for *E. coli*, NAD⁺ for T4
- B) NAD⁺ for *E. coli*, ATP for T4
- C) GTP for *E. coli*, CTP for T4
- D) S-adenosylmethionine for both

2. What functional groups must be exposed at a single-stranded nick for DNA ligase to form a covalent bond?

- A) 5' OH and 3' phosphate
- B) 5' phosphate and 3' OH
- C) 2' OH and 5' OH
- D) 3' phosphate and 3' OH

3. What is the optimum temperature for the enzymatic activity of ligating nicked DNA?

- A) 4°C
- B) 15°C
- C) 37°C
- D) 65°C

4. What experimental temperature range is used for ligating cohesive termini as a compromise between enzyme action and base-pair association stability?

- A) 0°C–2°C
- B) 4°C–15°C
- C) 25°C–30°C
- D) 42°C–50°C

Answer Key

* **1. B** — *E. coli* DNA ligase requires NAD⁺, whereas T4 DNA ligase requires ATP as a cofactor.

* **2. B** — A nick must expose a 5' phosphate and a 3' OH group for DNA ligase to seal it.

* **3. C** — The optimum temperature for ligation of nicked DNA is 37°C.

* **4. B** — The compromise temperature range for ligating cohesive termini is 4–15°C.

Lesson 28: Alkaline phosphatase

1. What is the primary enzymatic function of alkaline phosphatase in genetic engineering?

- A) Adding 5' phosphate groups to double-stranded DNA
- B) Removing 5' terminal phosphate groups from nucleic acids
- C) Methylating adenine residues in palindromes

D) Synthesizing cDNA from mRNA templates

2. Why is linearized plasmid vector DNA treated with alkaline phosphatase prior to ligation?

- A) To increase the rate of *EcoRI* cleavage
- B) To prevent vector recircularization and plasmid dimer formation
- C) To induce thermal denaturation of sticky ends
- D) To destroy host chromosomal DNA

3. In dilute DNA solutions during a ligation reaction, which process is relatively favored?

- A) Intermolecular reactions
- B) Circularization of linear fragments
- C) Homopolymer tailing
- D) Exonuclease cleavage

4. When foreign DNA is ligated into an alkaline phosphatase-treated vector, how many nicks remain unligated at each join before host transformation?

- A) None
- B) One nick at each join
- C) Two nicks at each join
- D) Four nicks at each join

Answer Key

* **1. B** — Alkaline phosphatase removes 5' terminal phosphate groups from DNA molecules.

* **2. B** — Removing 5' phosphates prevents self-recircularization and dimer formation of linearized vectors.

* **3. B** — Dilute solutions favor circularization of linear fragments due to reduced frequency of intermolecular collisions.

* **4. B** — One nick at each join remains unligated because only the foreign insert provides a 5' terminal phosphate.

Lesson 29: Blunt end ligation via linker molecules

1. Under what special condition will *E. coli* DNA ligase catalyze blunt end ligation?

- A) At 65°C incubation
- B) Under conditions of macromolecular crowding
- C) In the presence of 100% ethanol
- D) After treatment with S1 nuclease

2. Who synthesized self-complementary decameric oligonucleotides (linkers) containing restriction sites in 1977?

- A) Southern and Alwine
- B) Scheller and co-workers
- C) Maxam and Gilbert
- D) Birnboim and Doly

3. What length of self-complementary oligonucleotides was synthesized by Scheller and co-workers as linker molecules?

- A) Tetrameric (4 bp)
- B) Hexameric (6 bp)
- C) Decameric (10 bp)
- D) 100 bp

4. What major advantage is gained by inserting foreign DNA into a vector using linker molecules?

- A) It eliminates the need for T4 DNA ligase
- B) It creates restriction target sites at each end of the foreign DNA, enabling easy excision and recovery
- C) It prevents transformation of recipient bacteria
- D) It converts double-stranded DNA into single-stranded RNA

Answer Key

- * **1. B** — *E. coli* DNA ligase only catalyzes blunt end ligation under special conditions of macromolecular crowding.
- * **2. B** — Scheller and co-workers synthesized self-complementary decameric linkers in 1977.
- * **3. C** — Scheller and co-workers synthesized decameric (10-mer) oligonucleotides for use as linkers.
- * **4. B** — Linkers create target sites at each flank of foreign DNA, allowing it to be excised and recovered after cloning.

Lesson 30: Adaptors

1. What structural feature distinguishes an adaptor from a linker molecule?

- A) An adaptor has a preformed cohesive end, whereas a linker has blunt ends
- B) An adaptor is single-stranded RNA, whereas a linker is double-stranded DNA
- C) An adaptor cannot be phosphorylated by kinases
- D) An adaptor is synthesized by reverse transcriptase

2. What potential problem occurs when using linkers if the foreign DNA contains internal restriction sites for the restriction enzyme used?

- A) The vector will fail to replicate
- B) The foreign DNA will be cleaved into two or more subfragments during linker digestion
- C) The host cell will degrade the ligase enzyme
- D) The linkers will convert into circular plasmids

3. In the *BamHI* adaptor example, which end of the adaptor molecule is unphosphorylated when added to foreign DNA?

- A) The blunt end
- B) The *BamHI* cohesive end
- C) Both ends are phosphorylated
- D) Neither end has 3' OH groups

4. Which restriction sites are designed into the example *BamHI* adaptor to allow the foreign DNA to be recovered intact if internal *BamHI* sites exist?

- A) *EcoRI* and *HindIII*
- B) *SmaI* and *HpaII*

- C) *PstI* and *NotI*
- D) *Sall* and *XhoI*

Answer Key

- * **1. A** — The only difference between an adaptor and a linker is that an adaptor has cohesive ends, whereas a linker has blunt ends.
- * **2. B** — Cleaving linkers with restriction enzymes can cut foreign DNA at internal sites, fragmenting the insert.
- * **3. B** — The *BamHI* cohesive end of the adaptor is initially unphosphorylated to prevent adaptor dimerization.
- * **4. B** — The example *BamHI* adaptor incorporates *SmaI* and *HpaII* sites to permit intact recovery of foreign DNA.
-

Lesson 31: Homopolymer tailing

1. Which enzyme, purified from calf thymus, is used to synthesize homopolymeric extensions at the 3'-OH termini of DNA molecules?

- A) T4 DNA ligase
- B) Reverse transcriptase
- C) Terminal deoxynucleotidyltransferase
- D) Alkaline phosphatase

2. In homopolymer tailing, what occurs when oligo(dA) sequences are added to one DNA population and oligo(dT) blocks to another?

- A) The molecules undergo irreversible alkaline hydrolysis
- B) The two types of molecules anneal to form mixed dimeric circles
- C) The DNA is cleaved into blunt-ended subfragments
- D) The 5' terminal phosphate groups are completely removed

3. Which DNA substrate is particularly suitable for terminal deoxynucleotidyltransferase?

- A) Blunted 5'-P ends lacking overhangs
- B) DNA with exposed 3'-OH groups, such as from PstI restriction
- C) Internal RNA-DNA hybrid loops
- D) Dephosphorylated 5' overhangs

4. What requirement must be provided for terminal transferase to repeatedly add nucleotides to 3'-OH termini?

- A) All four deoxynucleotide triphosphates simultaneously
- B) A single deoxynucleotide triphosphate
- C) ATP and S-adenosylmethionine cofactors
- D) NAD⁺ and a hexameric RNA primer

Answer Key

- * **1. C** — Terminal deoxynucleotidyltransferase, purified from calf thymus, repeatedly adds nucleotides to exposed 3'-OH termini.
- * **2. B** — Annealing complementary oligo(dA) and oligo(dT) homopolymer extensions forms mixed dimeric circles.

* **3. B** — DNA containing exposed 3'-OH groups (such as after PstI digestion or lambda exonuclease treatment) is a very good substrate.

* **4. B** — When presented with a single deoxynucleotide triphosphate, terminal transferase repeatedly adds that nucleotide to 3'-OH ends.

Lesson 32: Cloning of cDNA by Homopolymer tailing

1. What natural feature of most eukaryotic mRNAs allows an oligo(dT) primer to hybridize for first-strand cDNA synthesis?

- A) A 5' 7-methylguanosine cap
- B) A polyadenylated tail [poly(A)] of up to 200 adenylate residues at the 3'-terminus
- C) Intron-exon junction palindromes
- D) A terminal hairpin loop structure

2. How is the RNA template strand destroyed from the immediate RNA-DNA hybrid without damaging the single-stranded cDNA?

- A) Phenol-chloroform extraction
- B) Alkaline hydrolysis
- C) Treatment with S1 nuclease at 65°C
- D) Digestion with EcoRI

3. In homopolymer tailing for cDNA cloning into pBR322, which complementary homopolymers are paired between insert and vector?

- A) Oligo(dA)-tailed cDNA and oligo(dT)-tailed vector
- B) Oligo(dC)-tailed cDNA and oligo(dG)-tailed vector cut with PstI
- C) Oligo(dU)-tailed cDNA and oligo(dA)-tailed vector
- D) Oligo(dG)-tailed cDNA and oligo(dC)-tailed vector cut with BamHI

4. What major advantage is provided by annealing oligo(dC)-tailed cDNA to PstI-cleaved, oligo(dG)-tailed vector DNA?

- A) It prevents translation of the cloned cDNA in host bacteria
- B) It reconstructs PstI target sites in the recombinant, allowing easy excision of the insert
- C) It eliminates the need for host cell repair enzymes
- D) It causes immediate integration into the host bacterial chromosome

Answer Key

* **1. B** — Most eukaryotic mRNA naturally contains a polyadenylated tail of up to 200 adenylates at its 3'-terminus.

* **2. B** — Alkaline hydrolysis destroys the RNA strand while leaving the alkali-resistant cDNA intact.

* **3. B** — Oligo(dC)-tailed cDNA is annealed with pBR322 vector cut open with PstI and tailed with oligo(dG).

* **4. B** — Homopolymers added at the PstI site reconstruct PstI target sites, providing a simple means to excise inserted sequences.

Lesson 33: Plasmid biology

1. What is the typical size range of naturally occurring bacterial plasmids stated in Topic 33?

- A) 100 to 1,000 bp
- B) 5,000 to 400,000 bp
- C) 1 Mb to 10 Mb
- D) 10 Mb to 50 Mb

2. In traditional transformation of E. coli, what temperature shift constitutes the heat shock after incubation at 0°C in CaCl₂?

- A) Rapidly raising the temperature to 20°C–25°C
- B) Rapidly raising the temperature to 37°C to 43°C
- C) Heating the mixture to 65°C for 10 minutes
- D) Boiling the suspension at 100°C for 2 minutes

3. Which technique uses a high-voltage pulse to transiently render the bacterial membrane permeable to large plasmid molecules?

- A) Lipofection
- B) Microinjection
- C) Electroporation
- D) Particle bombardment

4. How do plasmids replicate within host bacterial cells according to Topic 33?

- A) They replicate strictly as integrated prophages during cell lysis
- B) They replicate separately from the host chromosome
- C) They require host chromosome degradation before replication can begin
- D) They only replicate during meiotic recombination

Answer Key

* **1. B** — Naturally occurring bacterial plasmids range in size from 5,000 to 400,000 bp.

* **2. B** — After incubation on ice in CaCl₂, cells are heat-shocked by rapidly raising the temperature to 37–43°C.

* **3. C** — Electroporation uses a high-voltage pulse to transiently make bacterial membranes permeable to large molecules.

* **4. B** — Plasmids are circular DNA molecules that replicate separately from the host chromosome.

Lesson 34: Interconversion of plasmid DNA

1. What term describes double-stranded circular plasmid DNA in which both strands are intact closed circles?

- A) Open circular (OC) DNA
- B) Covalently closed circular (CCC) DNA
- C) Linear Form III DNA
- D) Denatured single-stranded DNA

2. How is open circular (OC) plasmid DNA generated from covalently closed circular (CCC) DNA?

- A) By complete cleavage of both strands with a restriction enzyme
- B) By a single-stranded nick in only one of the two strands
- C) By heating to 100°C in the presence of SDS
- D) By methylation of all adenine residues

3. Which structural feature protects the ends of linear plasmids in *Borrelia* species against nuclease degradation?

- A) Covalent attachment of terminal lipid bilayers
- B) Repeated sequences ending in a terminal DNA hairpin loop
- C) Integration into host ribosomal RNA
- D) Circularization via homopolymer dG tails

4. How are the ends of linear plasmids protected in *Streptomyces* species according to Topic 34?

- A) By terminal DNA hairpin loops
- B) By covalent attachment of a protein
- C) By methylation of cytosine bases
- D) By binding to host cell histones

Answer Key

* **1. B** — Plasmid molecules in which both strands are intact circles are described as covalently closed circular (CCC) DNA.

* **2. B** — Open circular (OC) DNA occurs when only one strand is intact due to a single-strand nick.

* **3. B** — Linear plasmid ends in *Borrelia* are protected by repeated sequences ending in a terminal DNA hairpin loop.

* **4. B** — Linear plasmid ends in *Streptomyces* are protected by covalent attachment of a protein.

Lesson 35: Supercoiling of DNA

1. Which intercalating agent is used to visualize DNA bands in gels and causes supercoiled plasmid DNA to unwind?

- A) Isopropanol
- B) Ethidium bromide
- C) Polyethylene glycol
- D) Sodium dodecyl sulphate

2. What occurs to a supercoiled plasmid when an excess amount of ethidium bromide is added beyond the point of complete unwinding?

- A) It degrades permanently into mononucleotides
- B) It rewinds in the opposite direction
- C) It forms a linear double-stranded chromosome
- D) It precipitates out of solution as an insoluble protein complex

3. Why do supercoiled CCC DNA and open circular (OC) DNA separate from each other during agarose gel electrophoresis?

- A) Because they have different G+C content percentages
- B) Because of differences in their structural configurations
- C) Because OC DNA carries a net positive electrical charge
- D) Because supercoiled DNA lacks phosphodiester bonds

4. In plasmid isolation, how does ethidium bromide binding assist in separating CCC plasmid DNA from linear chromosomal DNA?

- A) Ethidium bromide binds only to single-stranded RNA
- B) Topological constraints limit ethidium bromide binding in CCC DNA, making it denser than linear DNA at saturation
- C) Ethidium bromide covalently cleaves linear chromosomal DNA at restriction sites
- D) Ethidium bromide causes supercoiled DNA to float to the top of an aqueous gradient without centrifugation

Answer Key

* **1. B** — Ethidium bromide is an intercalating agent that binds DNA, allowing visual detection and unwinding supercoiled plasmids.

* **2. B** — Adding excess ethidium bromide causes the plasmid to unwind completely and then rewind in the opposite direction.

* **3. B** — Supercoiled and OC DNA separate during agarose gel electrophoresis due to differences in their structural configurations.

* **4. B** — Topological constraints restrict ethidium bromide binding in CCC plasmids, giving them a higher density than linear DNA at saturating dye levels.

Lesson 36: Phenotypic traits exhibited by plasmids

1. What size range do plasmids generally fall into according to Topic 36?

- A) 10 to 50 bp
- B) 1×10^3 to 1×10^4 daltons
- C) Less than 1×10^4 daltons to greater than 200×10^4 daltons
- D) 500×10^4 to 1000×10^4 daltons

2. Which of the following phenotypic traits is NOT specifically listed in Topic 36 as being conferred by plasmids?

- A) Heavy metal resistance
- B) Degradation of aromatic compounds
- C) Haemolysin production
- D) Active cell wall synthesis during mitosis

3. What term is used to describe plasmids to which phenotypic traits have not yet been ascribed?

- A) Stringent plasmids
- B) Cryptic plasmids
- C) Promiscuous plasmids
- D) Expression vectors

4. Which plasmid-conferred phenotypic trait is noted in Topic 36 as being especially useful for selecting transformants in gene cloning experiments?

- A) Haemolysin production
- B) Degradation of aromatic compounds
- C) Antibiotic resistance
- D) Heavy metal resistance

Answer Key

* **1. C** — Plasmids vary in size from less than 1×10^3 daltons to greater than 200×10^3 daltons.

* **2. D** — Phenotypic traits listed include antibiotic resistance/production, heavy metal resistance, degradation of aromatic compounds, and haemolysin production.

* **3. B** — Plasmids to which no phenotypic traits have been ascribed are called cryptic plasmids.

* **4. C** — Antibiotic resistance traits are particularly used in gene cloning experiments to select transformants.

Lesson 37: Properties of conjugative and non-conjugative plasmids

1. What specific set of genes determines whether a plasmid is classified as conjugative or non-conjugative?

- A) *par* genes
- B) *tra* genes
- C) *vir* genes
- D) *rec* genes

2. What term refers to plasmids that are maintained as a limited number of copies per cell?

- A) Relaxed plasmids
- B) Stringent plasmids
- C) Cryptic plasmids
- D) Shuttle plasmids

3. In general, what are the typical molecular weight and copy number properties of non-conjugative plasmids?

- A) High molecular weight and 1 to 3 copies per chromosome
- B) High molecular weight and multiple copies per chromosome
- C) Low molecular weight and multiple copies per chromosome
- D) Low molecular weight and strictly 1 copy per cell

4. Which conjugative plasmid is highlighted in Topic 37 as a notable exception because it has a molecular weight of 25×10^3 daltons and is maintained as a relaxed plasmid?

- A) pBR322
- B) pSC101
- C) R6K
- D) ColE1

Answer Key

- * **1. B** — Classification as conjugative depends on carrying transfer genes called **tra** genes that mediate conjugation.
- * **2. B** — Plasmids maintained as a limited number of copies per cell are categorized as stringent plasmids.
- * **3. C** — Non-conjugative plasmids are generally of low molecular weight and present as multiple copies per chromosome.
- * **4. C** — The conjugative plasmid R6K is an exception, having a molecular weight of 25×10^6 daltons while being maintained as a relaxed plasmid.
-
-

Lesson 38: Host range of plasmids

1. Which specific region of a plasmid determines its host range?

- A) The **tra** region
- B) The **ori** (origin of replication) region
- C) The polylinker region
- D) The **par** locus

2. Plasmids whose **ori** region is derived from ColE1 exhibit a restricted host range limited primarily to which group of bacteria?

- A) Enteric bacteria such as **E. coli** and **Salmonella**
- B) Gram-positive spore formers like **Bacillus**
- C) Extreme thermophilic Archaea
- D) Cyanobacteria and plant chloroplasts

3. Which non-conjugative plasmid mentioned in Topic 38 has a broad host range and can be transformed and stably maintained in both Gram-negative and Gram-positive bacteria?

- A) pUC19
- B) RSF1010
- C) pBR322
- D) pSC101

4. What key feature allows broad-host-range plasmids to replicate in a wider variety of host species compared to narrow-host-range plasmids?

- A) They lack restriction sites completely
- B) They encode most, if not all, of the proteins required for their own replication
- C) They contain linear terminal hairpin loops
- D) They contain **tra** genes derived from bacteriophage lambda

Answer Key

- * **1. B** — The host range of a plasmid is determined by its **ori** region.
- * **2. A** — ColE1-derived plasmids have a restricted host range and replicate only in enteric bacteria like **E. coli** and **Salmonella**.
- * **3. B** — RSF1010 is a non-conjugative, broad-host-range plasmid that can be transformed into a wide range of Gram-negative and Gram-positive bacteria.
- * **4. B** — Plasmids with a broad host range encode most, if not all, of the proteins required for their replication.

Lesson 39: Partitioning and segregative stability of plasmids

1. What term is used to describe the loss of plasmids from host cells due to defective partitioning during cell division?

- A) Structural instability
- B) Segregative instability
- C) Incompatibility
- D) Insertional inactivation

2. Which plasmid's **par** (partitioning) region can be cloned into pBR322 to increase its stability under nutrient limitation or stress conditions?

- A) ColE1
- B) pSC101
- C) pUC19
- D) RSF1010

3. What is the effect of **topA** mutations on **par**-defective mutants of pSC101 in **Escherichia coli**?

- A) They decrease negative supercoiling and cause rapid plasmid loss
- B) They increase negative DNA supercoiling and stabilize the **par**-defective mutants
- C) They convert closed circular plasmids into open circular molecules
- D) They induce immediate host cell lysis

4. What is the effect of DNA gyrase inhibitors or mutations in DNA gyrase on **par**-defective pSC101 derivative plasmids?

- A) They increase the rate of loss of the plasmid
- B) They increase plasmid copy number
- C) They restore wild-type partitioning functionality
- D) They seal single-stranded nicks in the plasmid backbone

Answer Key

* **1. B** — The loss of plasmids due to defective partitioning is called segregative instability.

* **2. B** — Cloning the **par** region from pSC101 into pBR322 stabilizes the plasmid.

* **3. B** — Partition-defective mutants of pSC101 are stabilized in **E. coli** by **topA** mutations, which increase negative DNA supercoiling.

* **4. A** — DNA gyrase inhibitors and mutations in DNA gyrase increase the rate of loss of **par**-defective pSC101 derivatives.

Lesson 40: Incompatibility of plasmids

1. How is plasmid incompatibility defined in Topic 40?

- A) The inability of a plasmid to undergo restriction endonuclease digestion
- B) The inability of two different plasmids to coexist in the same cell in the absence of selection pressure

- C) The failure of a plasmid to express antibiotic resistance markers
- D) The degradation of foreign DNA inserts by host cell exonucleases

2. What prerequisite conditions must be verified before describing two plasmids as incompatible?

- A) Both plasmids must be linearized and carry *par* loci
- B) Entry of the second plasmid has occurred and DNA restriction is not involved
- C) Both plasmids must contain identical polylinker restriction sites
- D) The cell host must be a Gram-positive bacterium

3. How many incompatibility groups have been defined for plasmids in *Escherichia coli* and *Staphylococcus aureus*, respectively?

- A) Over 30 in *E. coli* and 13 in *S. aureus*
- B) 10 in *E. coli* and 50 in *S. aureus*
- C) 100 in *E. coli* and 5 in *S. aureus*
- D) 3 in *E. coli* and 3 in *S. aureus*

4. What is the fundamental biological reason why two plasmids belong to the same incompatibility group?

- A) They share identical antibiotic resistance genes
- B) They possess the same mechanism of replication control
- C) They carry different *tra* gene operons
- D) They both contain terminal protein caps

Answer Key

* **1. B** — Plasmid incompatibility is the inability of two different plasmids to coexist in the same cell in the absence of selection pressure.

* **2. B** — Incompatibility can only be declared when entry of the second plasmid has taken place and restriction is not involved.

* **3. A** — Topic 40 states that over 30 incompatibility groups have been defined in *E. coli* and 13 in *S. aureus*.

* **4. B** — Plasmids will be incompatible if they have the same mechanism of replication control.

Lesson 41: Purification of plasmid DNA

1. What step in plasmid DNA isolation is highlighted in Topic 41 as the trickiest, where incomplete lysis or total dissolution severely reduces recovery?

- A) Ethanol precipitation
- B) Lysis of host cells
- C) Gel filtration
- D) Isopycnic centrifugation

2. In CsCl-ethidium bromide density gradient centrifugation, why does covalently closed circular (CCC) plasmid DNA have a higher density than linear chromosomal DNA at saturating dye concentrations?

- A) CCC plasmid DNA binds more ethidium bromide because it lacks supercoiling
- B) Topological constraints limit ethidium bromide binding in CCC DNA, so its density decreases less than that of linear DNA

- C) Linear chromosomal DNA is dephosphorylated by lysozyme, making it denser
- D) CCC plasmid DNA co-precipitates with SDS and sodium acetate

3. What specific pH range is utilized in the Birnboim and Doly (1979) alkaline lysis method to selectively denature linear chromosomal DNA while leaving CCC plasmid DNA native?

- A) pH 7.0–7.5
- B) pH 9.5–10.0
- C) pH 12.0–12.5
- D) pH 14.0–14.5

4. What occurs when the alkaline lysate is neutralized with acidic sodium acetate in the Birnboim and Doly method?

- A) Plasmid DNA aggregates into an insoluble network and precipitates
- B) Chromosomal DNA renatures and aggregates to form an insoluble network, co-precipitating with protein-SDS complexes and high-MW RNA
- C) Plasmid DNA is degraded into mononucleotides by lysozyme
- D) Linear chromosomal DNA remains in solution while CCC plasmid DNA precipitates

Answer Key

* **1. B** — Topic 41 notes that host cell lysis is the trickiest stage, as incomplete lysis or total cell dissolution severely reduces recovery.

* **2. B** — Topological constraints limit ethidium bromide intercalation in CCC plasmid DNA, resulting in a higher density than linear DNA at saturation.

* **3. C** — The Birnboim and Doly method operates within pH 12.0–12.5, where linear DNA denatures while CCC plasmid DNA remains native.

* **4. B** — Acidic sodium acetate neutralization causes denatured chromosomal DNA, protein-SDS complexes, and high-MW RNA to co-precipitate, leaving CCC plasmid DNA in solution.

Lesson 42: Desirable properties of plasmids

1. Why is small size considered a desirable property of plasmid cloning vectors according to Topic 42?

- A) It prevents the plasmid from undergoing supercoiling
- B) It makes the DNA easy to isolate and manipulate
- C) It prevents restriction enzymes from cleaving the vector
- D) It forces host cells into lytic cycle multiplication

2. What advantage does circular DNA structure provide to plasmids during extraction?

- A) It makes the DNA more stable during chemical isolation
- B) It allows the plasmid to integrate permanently into host ribosomes
- C) It prevents the plasmid from replicating in host cytoplasm
- D) It eliminates the need for an origin of replication

3. How does an independent origin of replication benefit a plasmid vector?

- A) Plasmid replication proceeds independently from direct chromosomal control
- B) It causes immediate cell lysis after transformation

- C) It inactivates host restriction endonucleases automatically
- D) It converts double-stranded DNA into single-stranded RNA

4. What is the primary advantage of a multiple copy number in plasmid vectors?

- A) It makes amplification of DNA possible by providing several or numerous copies per cell
- B) It prevents the use of antibiotic selection markers
- C) It restricts host range exclusively to Gram-positive bacteria
- D) It increases susceptibility to alkaline hydrolysis

Answer Key

* **1. B** — Small size is desirable because it makes plasmid DNA easy to isolate and manipulate without shearing.

* **2. A** — Circular DNA structure provides greater stability during chemical isolation procedures.

* **3. A** — An independent origin of replication allows plasmid replication to proceed free from direct chromosomal control.

* **4. A** — A high/multiple copy number enables DNA amplification because many vector copies are maintained in each host cell.

Lesson 43: Natural plasmids as cloning vehicles

1. What bacteriocin production and immunity are specified by the naturally occurring plasmid ColE1?

- A) Ampicillin and kanamycin
- B) Colicin E1
- C) Chloramphenicol
- D) Tetracycline

2. Which antibiotic resistance transposon is carried by RSF 2124, a derivative of ColE1?

- A) Tetracycline resistance
- B) Ampicillin resistance
- C) Chloramphenicol resistance
- D) Streptomycin resistance

3. What host organism is now known to be the natural source of plasmid pSC101?

- A) *Escherichia coli* strain K12
- B) *Salmonella panama*
- C) *Staphylococcus aureus*
- D) *Bacillus subtilis*

4. In early cloning experiments using pSC101, which restriction enzyme was used to digest plasmid and foreign DNA before selecting transformants on tetracycline?

- A) *BamHI*
- B) *PstI*
- C) *EcoRI*
- D) *HindIII*

Answer Key

- * **1. B** — ColE1 specifies the production of the bacteriocin colicin E1 and carries a gene for colicin E1 immunity.
- * **2. B** — RSF 2124 is a derivative of ColE1 that carries a transposon specifying ampicillin resistance.
- * **3. B** — The origin of pSC101 was determined to be a natural plasmid isolated from *Salmonella panama*.
- * **4. C** — Plasmid pSC101 and foreign DNA were digested with *EcoRI*, ligated, and used to transform hosts to tetracycline resistance.

Lesson 44: Use of PSC101 for cloning

1. Which *Staphylococcus aureus* plasmid was used by Chang and Cohen (1974) to construct interspecies molecular chimeras with pSC101?

- A) pBR322
- B) pI258
- C) pUC19
- D) pJB8

2. Into how many fragments did *EcoRI* cleave the *S. aureus* plasmid pI258 according to agarose gel electrophoresis?

- A) Two
- B) Four
- C) Eight
- D) Twelve

3. What type of *E. coli* strain was used as the host recipient for the interspecies chimeric DNA constructed by Chang and Cohen?

- A) A wild-type restrictive strain
- B) A restrictionless strain of *E. coli*
- C) A temperature-sensitive *topA* mutant
- D) A lysogenic P2 prophage strain

4. How were transformed *E. coli* cells carrying the pSC101–pI258 chimera initially selected and then screened?

- A) Selected for ampicillin resistance and screened for tetracycline resistance
- B) Selected for chloramphenicol resistance and screened for kanamycin resistance
- C) Selected for colicin immunity and screened for ampicillin sensitivity
- D) Selected for galactose utilization on blue/white X-gal plates

Answer Key

- * **1. B** — Chang and Cohen used the *Staphylococcus aureus* plasmid pI258 to construct interspecies chimeras with pSC101.
- * **2. B** — Agarose gel electrophoresis demonstrated that pI258 was cleaved into four easily identifiable fragments by *EcoRI*.
- * **3. B** — The chimeric DNA was transformed into a restrictionless strain of *E. coli* to prevent host degradation of foreign DNA.

* **4. A** — Transformants were selected for ampicillin resistance (from pI258) and screened for tetracycline resistance (from pSC101).

Lesson 45: PBR322, a purpose built cloning vehicle

1. From which three parent genetic elements was the in vitro constructed plasmid pBR322 derived?

- A) λ (Ap^R) from RSF2124, λ (Tc^R) from pSC101, and replication element of pMB1
- B) ColE1, lambda phage *cos* site, and M13 intergenic region
- C) pUC19 polylinker, pI258, and SV40 promoter
- D) λ (Kan^R) from pTiC58, λ (Cm^R) from pBR325, and YAC centromere

2. How many unique restriction enzyme cleavage sites exist within the tetracycline resistance (λ (Tc^R)) gene coding region and its promoter on pBR322?

- A) 2 in coding region, 6 in promoter
- B) 11 in coding region, 2 in promoter (*ClaI* and *HindIII*)
- C) 6 in coding region, 11 in promoter
- D) 40 total within the promoter alone

3. What phenomenon occurs when foreign DNA is inserted into one of the 19 unique restriction sites located within the λ (Ap^R) or λ (Tc^R) genes/promoters of pBR322?

- A) Homopolymer tailing
- B) Insertional inactivation
- C) Segregative instability
- D) Runaway replication

4. What is the approximate maximum length of DNA segments that can be successfully cloned using standard plasmid vectors like pBR322 before transformation efficiency declines significantly?

- A) 500 bp
- B) 2,000 bp
- C) 15,000 bp
- D) 100,000 bp

Answer Key

* **1. A** — pBR322 combines the ampicillin resistance gene of RSF2124, tetracycline resistance gene of pSC101, and replication element of pMB1.

* **2. B** — On pBR322, 11 unique restriction sites lie within the λ (Tc^R) gene coding region and 2 (*ClaI* and *HindIII*) within its promoter.

* **3. B** — Inserting foreign DNA into these unique antibiotic resistance gene sites causes insertional inactivation of that resistance marker.

* **4. C** — Transformation becomes increasingly difficult with plasmids when cloning DNA segments longer than about 15,000 bp.

Lesson 46: Cloning with PBR 322

1. What is the main function of the origin of replication (*ori*) in pBR322?

- A) It encodes resistance to ampicillin and tetracycline
- B) It is a sequence where replication is initiated by cellular enzymes to propagate and maintain the plasmid
- C) It cleaves foreign DNA at palindromic restriction sites
- D) It packages plasmid DNA into bacteriophage heads

2. At what level of copies per cell is pBR322 typically maintained in host *E. coli* cells?

- A) 1 to 3 copies
- B) 10 to 20 copies
- C) 100 to 200 copies
- D) 1000 to 2000 copies

3. Why does pBR322 contain two genes conferring resistance to different antibiotics?

- A) To increase the overall physical length of the vector
- B) To allow the identification of cells containing the intact plasmid or a recombinant version of the plasmid
- C) To enable the vector to replicate in eukaryotic hosts without an origin of replication
- D) To prevent restriction enzymes from cleaving the plasmid backbone

4. From what general approach was pBR322 developed as a cloning vector?

- A) By isolating intact linear plasmids from *Borrelia* species
- B) By modifying naturally occurring plasmids to develop suitable vectors for cloning
- C) By synthesizing a completely artificial chromosome from purine bases
- D) By fusing a bacteriophage coat protein with ribosomal RNA

Answer Key

* **1. B** — The *ori* sequence is required to initiate replication via cellular enzymes and maintain the plasmid in host cells.

* **2. B** — pBR322 is maintained at a level of 10 to 20 copies per cell in host bacteria.

* **3. B** — Carrying two antibiotic resistance genes allows identification of cells containing intact or recombinant plasmids.

* **4. B** — pBR322 was developed by modifying naturally occurring plasmids to optimize cloning features.

Lesson 47: Improved vectors derived from PBR322**1. Which additional antibiotic resistance marker and unique restriction site were incorporated into the pBR322 derivative pBR325?**

- A) Kanamycin resistance with a unique *BamHI* site
- B) Chloramphenicol resistance (Cm^R) with a unique *EcoRI* site
- C) Streptomycin resistance with a unique *HindIII* site
- D) Ampicillin resistance with a unique *PstI* site

2. What is a multiple cloning site (MCS) or polylinker as described in Topic 47?

- A) A short DNA sequence carrying sites for many different restriction endonucleases
- B) A single-stranded homopolymer tail of up to 200 adenylates

- C) A protein complex that seals phosphodiester nicks in DNA
- D) A temperature-sensitive origin of replication

3. What advantage is provided by making restriction sites contiguous within a multiple cloning site (MCS)?

- A) It prevents host restriction endonucleases from recognizing foreign DNA
- B) Any two sites within the MCS can be cleaved simultaneously without excising vector sequences
- C) It causes the plasmid to replicate via a lytic cycle
- D) It eliminates the need for selectable antibiotic markers

4. Which pBR322 derivative is mentioned in Topic 47 alongside pUC vectors as having been extensively used as a cloning vehicle?

- A) pSC101
- B) pAT153
- C) pI258
- D) pJB8

Answer Key

* **1. B** — pBR325 encodes chloramphenicol resistance and carries a unique *EcoRI* site in the Cm^R gene.

* **2. A** — An MCS is a short DNA sequence carrying sites for many different restriction endonucleases.

* **3. B** — Contiguous sites allow any two restriction sites within the MCS to be cut simultaneously without losing vector sequences.

* **4. B** — pAT153 is another derivative of pBR322 that has been extensively used as a cloning vehicle.

Lesson 48: Runaway plasmid vectors

1. Why is cloning certain genes on high copy number vectors sometimes impossible or lethal to the cell?

- A) High copy plasmids cannot undergo transformation
- B) Excess gene product synthesized from high copy vectors is lethal to the cell
- C) High copy plasmids are digested by host T4 DNA ligase
- D) High copy vectors convert host cells into obligate autotrophs

2. How do runaway plasmid vectors behave when host cells are maintained at 30°C?

- A) They undergo immediate cell lysis within 10 minutes
- B) They are present in a moderate number of copies per cell
- C) They lose all replication control and copy numbers increase without limit
- D) They degrade host chromosomal DNA completely

3. What happens to replication control and copy number in runaway plasmid vectors when the temperature is raised above 35°C?

- A) Replication stops completely and plasmids are degraded
- B) All control of plasmid replication is lost and copy numbers increase continuously
- C) Plasmids integrate permanently into the bacterial chromosome as prophages
- D) Plasmids form linear hairpins protected by terminal proteins

4. At the final stage of runaway replication after 2–3 hours at higher temperatures, what percentage of total cell DNA can plasmid DNA account for?

- A) 1%
- B) 10%
- C) 50%
- D) 99%

Answer Key

* **1. B** — Overproduction of excess gene product from high copy vectors can be lethal to the host cell.

* **2. B** — At 30°C, runaway plasmid vectors are maintained at a moderate copy number per cell.

* **3. B** — Above 35°C, control of plasmid replication is lost and plasmid copy number increases continuously.

* **4. C** — Plasmid DNA can account for up to 50% of the total cellular DNA during the final stage of runaway replication.

Lesson 49: Bacteriophage λ as cloning vector

1. What proportion of the 48,502 bp bacteriophage λ genome is non-essential and can be replaced with foreign DNA?

- A) One-tenth
- B) One-third
- C) One-half
- D) Three-quarters

2. What is the genome size constraint for DNA to be packaged into infectious bacteriophage λ particles?

- A) 1,000 to 5,000 bp
- B) 10,000 to 20,000 bp
- C) 40,000 to 53,000 bp
- D) 100,000 to 300,000 bp

3. What maximum size of foreign DNA fragments can be cloned using bacteriophage λ vectors?

- A) Up to 2,000 bp
- B) Up to 15,000 bp
- C) Up to 23,000 bp
- D) Up to 500,000 bp

4. What is the process called when recombinant λ DNA is packaged into viable phage particles using crude bacterial cell extracts containing head and tail assembly proteins?

- A) Transfection
- B) Electroporation
- C) In vitro packaging
- D) Homopolymer tailing

Answer Key

* **1. B** — Approximately one-third of the phage λ genome is non-essential and can be substituted with foreign DNA.

* **2. C** — DNA is packaged into infectious phage heads only if its total length falls between 40,000 and 53,000 bp.

* **3. C** — Bacteriophage λ vectors permit the cloning of foreign DNA fragments up to 23,000 bp in size.

* **4. C** — Assembling viable phage particles from recombinant DNA and cell extracts in vitro is termed in vitro packaging.

Lesson 50: Replication of phage λ DNA in lytic and lysogenic cycles

1. What defines lysogeny in bacteriophage λ compared to the lytic cycle?

- A) The immediate lysis and destruction of the host cell within minutes
- B) The integration of λ DNA into the host bacterium genome
- C) The degradation of host ribosomes by the cI gene product
- D) The synthesis of homopolymeric single-stranded tails

2. Which gene product directs the switch from early to middle stage transcription by acting as an anti-terminator?

- A) cI product
- B) cro product
- C) N gene product
- D) Q gene product

3. What function does the cI gene product perform in a lysogenic cell?

- A) It anti-terminates late transcripts across the cos site
- B) It represses early transcription from P_L and P_R , providing immunity to superinfecting λ
- C) It cleaves host DNA into 8 kb fragments
- D) It converts double-stranded DNA into single-stranded cDNA

4. Which gene product is responsible for the middle-to-late transcription switch by specifically anti-terminating the short P_R transcript into late genes across the cohered cos region?

- A) N product
- B) Q product
- C) cro product
- D) red product

Answer Key

* **1. B** — Lysogeny is characterized by the integration of phage λ DNA into the host bacterial genome.

* **2. C** — The N gene product acts as an anti-terminator to direct the transition from early to middle gene transcription.

* **3. B** — The cI repressor suppresses transcription from P_L and P_R , granting immunity against superinfecting phage.

* **4. B** — The *Q* gene product operates by anti-termination to extend transcription into late packaging genes across the *cos* region.

Lesson 51: Modified phages

1. What percentage of non-essential DNA deletion in the lambda genome yields the shortest lambda DNA molecules capable of producing plaques of nearly normal size?

- A) 10%
- B) 25%
- C) 50%
- D) 75%

2. How do replacement vectors (such as Charon 4A) differ from insertional vectors (such as Charon 16)?

- A) Replacement vectors contain a stuffer fragment that is replaced by foreign DNA, whereas insertional vectors contain a single insertion site
- B) Replacement vectors lack a *lacZ* reporter gene
- C) Replacement vectors can only carry single-stranded RNA
- D) Replacement vectors do not require *in vitro* packaging

3. What is the genome size of the modified lambda phage derivative Charon 4A compared to wild-type lambda (48.5 kbp)?

- A) 30.0 kbp
- B) 41.7 kbp
- C) 45.4 kbp
- D) 53.0 kbp

4. Which reporter gene present in Charon 4A and Charon 16 permits the detection of phages containing inserted clones?

- A) *ampR*
- B) *tetR*
- C) *lacZ*
- D) *cl*

Answer Key

* **1. B** — The shortest λ DNA molecules that produce plaques of nearly normal size are 25% deleted.

* **2. A** — Replacement vectors contain a pair of sites defining a stuffer fragment that is replaced by foreign DNA, while insertional vectors have a single target site.

* **3. C** — Wild-type lambda genome is 48.5 kbp, while Charon 4A is 45.4 kbp and Charon 16 is 41.7 kbp.

* **4. C** — Both Charon vectors carry the *lacZ* gene encoding β -galactosidase to allow detection of recombinant phages.

Lesson 52: Steps in cloning with ■

1. Why is selection of recombinants less of a problem with lambda replacement vectors like Charon 4A compared to plasmids?

- A) Lambda replacement vectors do not infect *E. coli* host cells
- B) Unrecombined lambda fragments lacking new DNA are too small to be incorporated into phage particles
- C) Plasmids are always destroyed by host restriction systems
- D) Recombinant lambda vectors convert host cells into obligate autotrophs

2. In Charon replacement vectors carrying the β -galactosidase reporter gene, how are recombinant plaques (containing inserted foreign genes) detected?

- A) Recombinants form blue/colored plaques
- B) Recombinants form colorless (Lac-) plaques among a background of colored plaques
- C) Recombinants cause complete destruction of the agar medium
- D) Recombinants produce glowing fluorescent colonies under UV light

3. Which *E. coli* host strain genotype is used to detect β -galactosidase expression from Charon vectors as colored (Lac+) plaques?

- A) Lactose-positive (Lac+)
- B) Lactose-negative (Lac-)
- C) Ampicillin-resistant (AmpR)
- D) Restriction-positive (R+)

4. What is the final step listed in Lesson 52 for cloning with lambda replacement vectors?

- A) In vitro packaging of naked vector DNA
- B) Digestion of total genomic host DNA with *EcoRI*
- C) Checking recombinant phage for the presence of the desired foreign DNA sequence
- D) Heat-shock transformation of competent bacterial cells

Answer Key

* **1. B** — Lambda fragments that have not received foreign DNA are too small to be incorporated into viable phage particles.

* **2. B** — Inserting a foreign gene into the β -galactosidase gene inactivates it, yielding colorless (Lac-) plaques.

* **3. B** — The vectors replicate in a lactose-negative (Lac-) strain of *E. coli* so that active β -galactosidase yields Lac+ colored plaques.

* **4. C** — Recombinant phages are checked for the target sequence using hybridization, sequencing, or genetic observation.

Lesson 53: Packaging phage- $\square$ DNA in vitro

1. What plaque yield per microgram of vector DNA is typically achieved when introducing restricted and ligated recombinant DNA via transfection of competent bacteria?

- A) $10^1 - 10^2$
- B) $10^3 - 10^4$
- C) $10^6 - 10^8$
- D) $10^9 - 10^{10}$

2. What is the expected plaque yield per microgram of vector DNA when using *in vitro* packaging following a ligation reaction?

- A) About (10^2) plaques/ μ g
- B) About (10^4) plaques/ μ g
- C) About (10^6) plaques/ μ g
- D) About (10^9) plaques/ μ g

3. How does *in vitro* packaged recombinant DNA enter the host bacterial cell?

- A) Via calcium chloride heat-shock transformation
- B) Via high-voltage electroporation
- C) By normal processes of phage infection (adsorption followed by DNA injection)
- D) By protoplast fusion with polyethylene glycol

4. Why does vector DNA that has been restricted and ligated with foreign DNA show a reduced plaque yield during standard transfection compared to unmanipulated phage DNA?

- A) Random association of fragments produces many inviable combinations
- B) DNA ligase permanently degrades the *cos* sites
- C) Competent cells reject all foreign DNA fragments
- D) Heat-shock treatment denatures double-stranded vector DNA

Answer Key

* **1. B** — *In vitro* restricted and ligated recombinant vector DNA yields about $(10^3 - 10^4)$ plaques/ μ g via transfection.

* **2. C** — Packaging recombinant DNA into phage coats *in vitro* yields about (10^6) plaques/ μ g of vector DNA.

* **3. C** — Enclosing recombinant DNA in a phage coat allows entry via normal phage adsorption and DNA injection.

* **4. A** — The random association of fragments during ligation creates a variety of fragment combinations, many of which are inviable.

Lesson 54: Vectors for DNA sequencing: bacteriophage M13

1. What type of genome structure and morphology characterizes bacteriophage M13?

- A) Double-stranded circular DNA in an icosahedral capsid
- B) Single-stranded DNA in a filamentous bacteriophage
- C) Double-stranded linear DNA with terminal protein caps
- D) Single-stranded RNA in a spherical envelope

2. How are mature M13 virions released from host bacterial cells without causing cell lysis?

- A) By a budding process
- B) By osmotic explosion
- C) By enzymatic digestion with lysozyme
- D) By holin-endolysin pore formation

3. How much foreign DNA can be cloned into the M13 intergenic region without affecting phage viability?

- A) Up to 200 bp
- B) Up to about 5 kbp
- C) Up to 23 kbp
- D) Up to 50 kbp

4. What artificial substrate is cleaved by β -galactosidase in M13mp18-infected cells to yield a blue color on indicator plates?

- A) IPTG
- B) Xgal
- C) Ethidium bromide
- D) Ampicillin

Answer Key

- * **1. B** — M13 is a filamentous bacteriophage containing single-stranded DNA.
- * **2. A** — Mature M13 particles are released continuously from host cells without lysing by a budding process.
- * **3. B** — Variable lengths of foreign DNA, up to about 5 kbp, can be cloned into the intergenic region without affecting viability.
- * **4. B** — β -galactosidase cleaves Xgal to produce a blue color, allowing easy identification of infected cells.

Lesson 55: Cosmid vectors

1. How are cosmid vectors constructed?

- A) By fusing bacterial ribosomes with viral capsids
- B) By ligating the lambda *cos* region to plasmid DNA
- C) By inserting yeast centromeres into M13 single-stranded DNA
- D) By digesting bacterial chromosomes with *EcoRI*

2. What maximum size of foreign DNA inserts can be accepted by cosmid cloning systems?

- A) Up to 5 kbp
- B) Up to 15 kbp
- C) As large as 50 kbp
- D) Up to 300 kbp

3. What key efficiency advantage do cosmids offer over standard plasmid vectors when cloning large DNA fragments?

- A) They eliminate the necessity of having to transform *E. coli*, which is inefficient with larger plasmids
- B) They allow direct translation of eukaryotic introns in bacteria
- C) They do not require DNA ligase for joining
- D) They replicate without needing any host cell enzymes

4. Why is storing recombinant DNA in cosmid phage particles advantageous compared to plasmid storage?

- A) Phage particles are more stable than plasmids, allowing long-term storage
- B) Phage particles undergo continuous runaway replication at 30°C
- C) Phage particles prevent host cells from undergoing cell division

D) Phage particles automatically correct base pair mismatches

Answer Key

- * **1. B** — Cosmids are constructed from plasmids by ligating the lambda *cos* region to plasmid DNA.
- * **2. C** — Cosmids can accept large fragments of DNA, with inserts as large as 50 kbp.
- * **3. A** — Using cosmids avoids the necessity of having to transform *E. coli*, which is especially inefficient with larger plasmids.
- * **4. A** — Phage particles are more stable than plasmids, so recombinant DNA can be kept for long periods of time.

Lesson 56: Modified scheme for cloning in cosmid vectors

1. Why are cosmids particularly attractive vectors for constructing libraries of eukaryotic genome fragments?

- A) Because of their capacity to carry large fragments of DNA generated by partial restriction digests
- B) Because they integrate permanently into host ribosomal RNA
- C) Because they require no host bacterial cell enzymes for replication
- D) Because they rely exclusively on single-stranded RNA templates

2. What major potential problem is associated with using partial restriction digests in cosmid cloning?

- A) Foreign DNA is completely degraded by T4 DNA ligase
- B) Two or more non-contiguous genome fragments may join together, giving an incorrect picture of chromosomal organization
- C) The cosmid vector loses its antibiotic resistance markers permanently
- D) Host bacterial cells undergo immediate lytic explosion

3. In the cosmid cloning procedure devised by Ish-Horowicz and Burke (1981) using vector pJB8, how is vector self-ligation prevented?

- A) By heating the vector to 100°C in SDS
- B) By purifying left-hand and right-hand vector ends that are incapable of self-ligation but accept dephosphorylated foreign DNA
- C) By adding ethidium bromide to saturate all palindromic target sites
- D) By digesting the foreign DNA with pancreatic RNase A

4. What key structural feature of cosmid c2XB (constructed by Bates and Swift, 1983) prevents vector self-ligation?

- A) A single-stranded poly(A) tail at the 5' terminus
- B) A *BamHI* insertion site and two *cos* sites separated by a blunt-end restriction site
- C) A temperature-sensitive origin of replication from phage lambda
- D) An integrated viral coat protein from tobacco mosaic virus

Answer Key

- * **1. A** — Cosmids can accommodate large DNA fragments from partial digests, making them ideal for eukaryotic genomic libraries.
- * **2. B** — Non-contiguous DNA fragments can ligate together into a single insert, misrepresenting true chromosomal organization.

* **3. B** — Purified left-hand and right-hand pJB8 vector ends cannot self-ligate but successfully accept dephosphorylated foreign DNA.

* **4. B** — Cosmid c2XB has two *cos* sites separated by a blunt-end site, creating blunt ends that ligate inefficiently to prevent self-ligation.

Lesson 57: Phasmid vectors

1. How are phasmid vectors defined in Topic 57?

- A) Combinations of bacterial ribosome sequences and plant viral capsids
- B) Combinations of plasmid and lambda phage sequences, carrying a lambda attachment (λ att) site
- C) Artificial chromosomes containing yeast centromeres and telomeres
- D) Double-stranded RNA molecules that replicate exclusively in chloroplasts

2. How can phasmid vectors be propagated in appropriate *E. coli* strains?

- A) Strictly as integrated prophages inside the host cell membrane
- B) Either as a plasmid or as a phage, because they contain functional origins of replication for both
- C) Only as single-stranded RNA viruses
- D) Only under anaerobic conditions at 65°C

3. What practical advantage of storing recombinant DNA as phage particles compared to bacterial colonies is highlighted in Topic 57?

- A) Phage particles do not require any nucleic acids
- B) Phage particles are easy to store, have an effectively infinite shelf life, and plaque screening gives cleaner results
- C) Phage particles eliminate the need for restriction enzymes
- D) Phage particles replicate without using host amino acids

4. Which highly developed phasmid vector incorporates components from lambda, M13, T3, and T7 phages?

- A) pBR322
- B) pUC19
- C) λ ZAP
- D) pJB8

Answer Key

* **1. B** — Phasmids combine plasmid and lambda phage sequences, including a lambda attachment (λ att) site.

* **2. B** — Phasmids carry functional origins of replication for both plasmids and phage lambda, allowing propagation as either form.

* **3. B** — Phage particles offer an effectively infinite shelf life and cleaner hybridization signals during plaque screening.

* **4. C** — λ ZAP is a novel, highly developed phasmid containing elements from lambda, M13, T3, and T7 phages.

Lesson 58: Bacterial artificial chromosomes (BACs)

1. What insert size range are Bacterial Artificial Chromosomes (BACs) designed to clone?

- A) 100 to 500 bp
- B) 1,000 to 5,000 bp
- C) 100,000 to 300,000 bp
- D) 10,000,000 to 50,000,000 bp

2. Which antibiotic resistance selectable marker is specifically mentioned in Topic 58 as being included on BAC vectors?

- A) Ampicillin resistance (Ap^R)
- B) Tetracycline resistance (Tc^R)
- C) Chloramphenicol resistance (Cm^R)
- D) Kanamycin resistance (Km^R)

3. At what copy number per cell are BAC vectors maintained by their origin of replication (ori)?

- A) One or two copies per cell
- B) 10 to 20 copies per cell
- C) 100 to 200 copies per cell
- D) Over 1,000 copies per cell

4. How are large recombinant BAC molecules introduced into host bacteria?

- A) Calcium phosphate precipitation on ice
- B) Electroporation into host bacteria with mutations that compromise cell wall structure
- C) Microinjection using glass capillary needles
- D) Conjugation using *Agrobacterium tumefaciens*

Answer Key

1. C — BACs are specialized plasmid vectors engineered to clone very long DNA segments ranging from 100,000 to 300,000 bp.

2. C — BAC vectors generally carry selectable markers such as chloramphenicol resistance (Cm^R).

3. A — BACs possess a stable replication origin maintaining the plasmid at a low copy number of one or two per cell.

4. B — Large circular BAC constructs are introduced into cell-wall-compromised mutant bacteria via electroporation.

Lesson 59: Yeast artificial chromosomes (YACs)

1. What is the genome size of the commonly used yeast *Saccharomyces cerevisiae* as stated in Topic 59?

- A) $1.5 \times 10^3 \text{ bp}$
- B) $4.5 \times 10^5 \text{ bp}$

- C) $(14 \times 10^6 \text{ bp})$
- D) $(3 \times 10^9 \text{ bp})$

2. How does the genome size of *S. cerevisiae* compare to that of the *E. coli* chromosome?

- A) It is exactly equal to the *E. coli* chromosome
- B) It is less than four times the size of the *E. coli* chromosome
- C) It is one hundred times smaller than the *E. coli* chromosome
- D) It is ten times larger than the human genome

3. What term is used to describe plasmids that incorporate multiple replication origins allowing them to be propagated in cells of two or more different species?

- A) Cryptic plasmids
- B) Cosmid vectors
- C) Shuttle vectors
- D) Expression vectors

4. Why is *Saccharomyces cerevisiae* described as a particularly convenient eukaryotic host for genetic engineering?

- A) Its entire genome sequence is known and it is easy to maintain and grow on a large scale
- B) It lacks cell membranes and cell walls entirely
- C) It does not require any carbon or nitrogen source for growth
- D) It converts all foreign DNA directly into infectious retroviruses

Answer Key

1. C — The genome of *Saccharomyces cerevisiae* contains $(14 \times 10^6 \text{ bp})$.

2. B — *S. cerevisiae* has a simple eukaryotic genome that is less than four times the size of the *E. coli* chromosome.

3. C — Plasmids capable of replicating and being maintained in two or more different host species are defined as shuttle vectors.

4. A — Yeast is convenient because its full sequence is determined, genetics are well developed, and large-scale growth is straightforward.

Lesson 60: Shuttle and expression vectors

1. What is the primary defining feature of a shuttle vector?

- A) It can only replicate in cell-free in vitro translation systems
- B) It can replicate and be stably maintained in two or more unrelated host organisms
- C) It cleaves foreign DNA at non-palindromic sites
- D) It automatically degrades host chromosomal DNA upon entry

2. Why must a bacterial plasmid vector be modified with a yeast origin of replication when being adapted as a yeast shuttle vector?

- A) Bacterial origins of replication do not function in eukaryotic cells
- B) Yeast cells degrade all bacterial DNA ligases
- C) Bacterial origins cause immediate lysis of yeast cells
- D) Yeast ribosomes cannot bind to plasmids lacking chloramphenicol resistance

3. What is the main design purpose of an expression vector?

- A) To increase the length of foreign DNA inserts up to 1 Mb
- B) To allow the experimenter to control and drive the expression of cloned genes in a host cell
- C) To remove all promoters and ribosome binding sites from the construct
- D) To prevent transcription of the cloned gene under any conditions

4. Which of the following is listed in Topic 60 as a synthetic hybrid promoter used in *E. coli* expression vectors?

- A) *lac*
- B) *trp*
- C) *tac*
- D) lambda \(\text{P}_{\text{L}}\)

Answer Key

* **1. B** — Shuttle vectors carry origins and markers allowing replication and maintenance in two or more unrelated species.

* **2. A** — Bacterial origins of replication are inactive in eukaryotes, so a functional yeast origin must be added.

* **3. B** — Expression vectors contain strong, correctly positioned regulatory sequences to control and drive transgene expression.

* **4. C** — The *tac* and *trc* promoters are synthetic hybrids constructed from the *trp* and *lac* operon promoters.

Lesson 61: Comparison of different cloning vectors-Summary**1. What main conclusion is drawn regarding different cloning vectors in Topic 61?**

- A) All vectors carry identical sizes of foreign DNA into recipient cells
- B) Different vectors can carry variable sizes of foreign DNA into host organisms
- C) Plasmids carry larger foreign DNA inserts than YACs and BACs
- D) Cloning vectors cannot be used in prokaryotic host systems

2. Which group of vectors is specifically listed in Topic 61 as being compared?

- A) Plasmids, cosmids, phasmids, BACs, and YACs
- B) Retroviruses, adenoviruses, liposomes, and transposons
- C) Lambda gt10, Lambda gt11, Charon 4A, and M13mp18
- D) pBR322, pUC19, pSC101, and pI258

3. What factor primarily dictates the choice of a specific vector type when constructing a gene library?

- A) The G+C content of the host bacterial ribosome
- B) The size capacity for foreign DNA that the vector can accommodate
- C) The temperature required for thermal denaturation in PCR
- D) The concentration of ethidium bromide in the agar gel

4. Which vector type discussed in the preceding topics offers the highest DNA insert capacity among plasmids, cosmids, BACs, and YACs?

- A) Plasmids

- B) Cosmids
- C) BACs
- D) YACs

Answer Key

- * **1. B** — Topic 61 states that different vectors can carry variable sizes of foreign DNA into host organisms.
- * **2. A** — Topic 61 specifically lists plasmids, cosmids, phasmids, BACs, and YACs for detailed comparison.
- * **3. B** — Vector types vary in their capacity to carry foreign DNA fragments into host organisms.
- * **4. D** — YACs provide the largest insert capacity (up to 1 Mb or more) compared to plasmids, cosmids, and BACs.

Lesson 62: CLONING STRATEGIES: Introduction

1. Which of the following is NOT listed in Topic 62 as one of the four essential parts of a cell-based cloning procedure?

- A) A method for generating the DNA fragment for cloning
- B) A reaction that inserts the fragment into the chosen cloning vector
- C) Direct crystallization of target proteins prior to transformation
- D) A method for selecting recipient cells that have acquired the recombinant

2. What is a library defined as in Topic 62?

- A) A collection of synthetic primers designed for real-time quantitative PCR
- B) A collection of clones representative of the entire starting population of source DNA
- C) A purified preparation of T4 DNA ligase and restriction buffer
- D) An array of X-ray films showing autoradiographic banding patterns

3. What is the first major cell-based strategy described in Topic 62 for isolating sequences from complex sources?

- A) To divide the source DNA into manageable fragments and clone everything
- B) To amplify the target sequence directly using degenerate PCR primers
- C) To subject intact whole cells to high-voltage electroporation
- D) To sequence the entire genome using automated slab-gel sequencers

4. What is the second major strategy for isolating target sequences directly from source DNA mentioned in Topic 62?

- A) To selectively amplify the target sequence directly using PCR, and then clone this individual fragment
- B) To perform 2-D gel electrophoresis on total cellular proteins
- C) To treat genomic DNA with alkaline phosphatase to prevent all ligation
- D) To synthesize single-stranded homopolymer tails using terminal transferase

Answer Key

- * **1. C** — The four parts include generating fragments, vector insertion, host introduction/replication, and selecting recombinants, but not protein crystallization.
- * **2. B** — A library is a collection of clones that is representative of the entire starting population of source DNA.

* **3. A** — The cell-based cloning strategy divides source DNA into manageable fragments and clones the entire population into a library.

* **4. A** — The second strategy is to selectively amplify the target sequence directly from source DNA via PCR before cloning.

Lesson 63: Genomic DNA libraries

1. Assuming average EcoRI fragments of 4 kb, how many independent recombinants must be screened to isolate a single-copy gene from the human haploid genome (2.8×10^6 kb)?

- A) About 1,000
- B) About 15,000
- C) Over 7×10^6
- D) Exactly 10^6

2. Why might digesting total genomic DNA with EcoRI fail to yield a complete single-copy gene as a single fragment?

- A) EcoRI cannot digest eukaryotic genomic DNA
- B) The gene may be cut internally one or more times by EcoRI if it is large
- C) EcoRI only cuts single-stranded cDNA templates
- D) EcoRI destroys all vector replication origins

3. What fragment size is typically cloned in lambda replacement vectors to overcome internal cleavage and short fragment limitations?

- A) Approximately 500 bp
- B) Approximately 4 kb
- C) Approximately 20 kb
- D) Over 300 kb

4. What advantage is gained by cloning randomly fragmented large DNA segments when creating a genomic library?

- A) It guarantees that all clones express functional proteins in *E. coli*
- B) There is no systematic exclusion of any sequence from the library
- C) It eliminates the need to screen recombinants with nucleic acid probes
- D) It prevents host restriction systems from recognizing foreign DNA

Answer Key

* **1. C** — Screening a 2.8×10^6 kb genome cut into 4 kb fragments requires over 7×10^6 independent recombinants for a complete library.

* **2. B** — Large genes are often cleaved internally one or more times by EcoRI, splitting the gene into subfragments.

* **3. C** — Lambda replacement vectors typically accept random DNA fragments of approximately 20 kb in size.

* **4. B** — Random fragmentation ensures that no sequences are systematically excluded from the genomic library.

Lesson 64: ■ EMBL vectors for genomic library construction

1. Which restriction enzyme is used to partially digest high-molecular-weight genomic DNA when constructing a library in phage- λ vector EMBL3A?

- A) EcoRI
- B) Sau3AI
- C) HindIII
- D) NotI

2. Why are the partially digested genomic DNA fragments treated with phosphatase during EMBL3A library construction?

- A) To remove their 5' phosphate groups and prevent genomic DNA fragments from ligating together
- B) To degrade host cell RNA before packaging
- C) To attach oligo(dC) homopolymer tails to the 3'-OH ends
- D) To induce mutations in the red and gam genes

3. How is non-recombinant vector re-formation prevented in the EMBL3A system?

- A) The tiny BamHI/EcoRI polylinker fragments are discarded during isopropanol precipitation or gel electrophoresis
- B) The vector arms are denatured with heat shock at 100°C
- C) The host cells are treated with ampicillin prior to transfection
- D) S1 nuclease is added to digest all sticky ends

4. What selection mechanism ensures the recovery of recombinant phages lacking red and gam genes on a P2 lysogen of E. coli?

- A) Blue/white X-gal screening
- B) Spi⁻ selection
- C) Kanamycin resistance selection
- D) HAT medium selection

Answer Key

* **1. B** — High-molecular-weight genomic DNA is partially digested with Sau3AI for EMBL3A library construction.

* **2. A** — Phosphatase treatment removes 5' phosphate groups, preventing genomic DNA fragments from ligating to each other.

* **3. A** — Small polylinker fragments are discarded, preventing non-recombinant vector arms from re-ligating together.

* **4. B** — Spi⁻ selection on a P2 lysogen host ensures recovery of recombinant phages lacking the red and gam genes.

Lesson 65: Genomic libraries in high capacity vectors

1. What major advantage do high-capacity vectors (cosmids, BACs, PACs, YACs) offer over lambda replacement vectors for genomic library construction?

- A) They allow direct translation of eukaryotic introns in bacterial hosts
- B) Their average insert size is much larger, so fewer recombinants need to be screened
- C) They do not require DNA ligase during in vitro recombination
- D) They eliminate the need for selectable markers in recipient cells

2. How does a larger insert size in high-capacity vectors benefit the study of large genes and chromosome walking?

- A) Large genes are more likely to be contained within a single clone and fewer steps are needed for chromosome walking
- B) It converts double-stranded DNA into single-stranded RNA automatically
- C) It increases the frequency of meiotic recombination in host bacteria
- D) It prevents DNA polymerase from detaching during thermal cycling

3. Which specialized electrophoresis techniques are devised to separate large DNA fragments over 30 kb for high-capacity vector library construction?

- A) Standard SDS-PAGE and agarose gel filtration
- B) Pulsed field gel electrophoresis (PFGE) and field-inversion gel electrophoresis (FIGE)
- C) Paper chromatography and thin-layer chromatography
- D) Isoelectric focusing and capillary zone electrophoresis

4. How can high-molecular-weight donor DNA fragments be prepared without mechanical shearing?

- A) By digesting genomic DNA with restriction enzymes that cut very rarely
- B) By treating DNA with boiling sodium hydroxide for 30 minutes
- C) By adding ethidium bromide to saturate all palindromic sites
- D) By exposing DNA to intense ultrasound sonication

Answer Key

* **1. B** — High-capacity vectors take much larger inserts, significantly reducing the total number of recombinants needed to cover a genome.

* **2. A** — Larger inserts increase the chance that a complete gene fits on one clone and reduce the number of steps required in chromosome walks.

* **3. B** — PFGE and FIGE are specialized electrophoresis methods designed to separate DNA fragments exceeding 30 kb in length.

* **4. A** — High-molecular-weight donor fragments can be produced using rare-cutting restriction endonucleases.

Lesson 66: PCR as an alternative to genomic DNA cloning

1. What is a major advantage of using PCR directly on genomic DNA as discussed in Lesson 66?

- A) It completely eliminates the need for specific primers
- B) It isolates target genes directly without producing genomic libraries
- C) It guarantees 100% error-free proofreading of long templates
- D) It replaces reverse transcriptase during cDNA synthesis

2. What is stated as a serious limitation of standard PCR conditions when isolating genes from genomic DNA?

- A) Standard PCR requires RNA templates exclusively
- B) Standard PCR is suitable only for amplifying short products, typically 1–2 kb and up to ~5 kb
- C) Standard PCR cannot operate at temperatures above 37°C
- D) Standard PCR requires vector ligation prior to amplification

3. Which two deficiencies of enzymes such as *Taq* polymerase contribute to their poor performance on long templates?

- A) Lack of 5' to 3' synthesis activity and inability to bind dNTPs
- B) Poor processivity and lack of proofreading activity
- C) Excessive exonuclease activity and thermal instability
- D) Inability to denature double-stranded DNA and requirement for RNA primers

4. Besides enzyme limitations, what additional factor during PCR causes damage to bases and generates nicks in long DNA templates?

- A) Extreme reaction conditions
- B) Low concentration of dNTPs
- C) Overuse of DNA ligase
- D) Excessively low denaturation temperatures

Answer Key

* **1. B** — Direct PCR on genomic DNA obviates the need for constructing genomic libraries.

* **2. B** — Standard PCR conditions typically amplify short products of 1–2 kb up to a maximum of about 5 kb.

* **3. B** — Enzymes like *Taq* polymerase suffer from poor processivity and lack of proofreading activity on long templates.

* **4. A** — Extreme reaction conditions during PCR cause base damage and nicks that lead to premature termination.

Lesson 67: Properties of cDNA

1. How is complementary DNA (cDNA) prepared?

- A) By digesting genomic DNA with restriction endonucleases
- B) By reverse-transcribing cellular RNA
- C) By mechanical shearing of nuclear chromatin
- D) By chemical synthesis of purine bases

2. What key structural feature distinguishes cloned eukaryotic cDNAs from their corresponding genomic DNA?

- A) cDNAs contain extra promoter regions
- B) cDNAs lack introns and other non-coding sequences
- C) cDNAs contain poly(A) tails attached to every exon
- D) cDNAs are double-stranded RNA molecules

3. In mammals, what proportion of the human dystrophin gene sequence is represented by its 79 introns?

- A) Less than 10%

- B) Exactly 50%
- C) Over 99%
- D) 100%

4. What is the approximate size of the cDNA corresponding to the nearly 2.5 Mb human dystrophin gene?

- A) Just over 11 kb
- B) 100 kb
- C) 500 kb
- D) 2.5 Mb

Answer Key

- * **1. B** — cDNA is synthesized by reverse-transcribing cellular RNA using reverse transcriptase.
- * **2. B** — Cloned cDNAs lack introns and other non-coding sequences present in genomic DNA.
- * **3. C** — The 79 introns of the human dystrophin gene represent over 99% of its total sequence.
- * **4. A** — Despite the gene being nearly 2.5 Mb long, the corresponding dystrophin cDNA is just over 11 kb.

Lesson 68: cDNA libraries

1. What advantage did phage λ vectors offer over plasmid vectors for constructing early cDNA libraries?

- A) They could be stored indefinitely and prepared to much higher titres
- B) They required no host cells for propagation
- C) They eliminated the need for EcoRI restriction sites
- D) They allowed translation without ribosomes

2. Which two phage λ insertion vectors served as standard vectors for cDNA cloning until about 1990?

- A) EMBL3A and EMBL4
- B) λ gt10 and λ gt11
- C) Charon 4A and Charon 16
- D) M13mp18 and pUC19

3. In λ gt10, at which unique restriction site is foreign cDNA inserted, interrupting the phage *cl* gene to allow selection by plaque morphology?

- A) *BamHI*
- B) *HindIII*
- C) *EcoRI*
- D) *NotI*

4. How are recombinant clones containing cDNA inserts detected in λ gt11 expression libraries?

- A) By measuring fluorescence of ethidium bromide
- B) As β -galactosidase fusion proteins detected by immunological screening
- C) By antibiotic resistance to chloramphenicol
- D) By blue plaque formation on X-gal plates

Answer Key

- * **1. A** — Phage λ libraries replaced early plasmid libraries because they can be stored indefinitely and reach higher titres.
- * **2. B** — λ gt10 and λ gt11 were the standard insertion vectors for cDNA cloning until around 1990.
- * **3. C** — Foreign cDNA is inserted into a unique *EcoRI* site in λ gt10, interrupting the *cl* gene.
- * **4. B** — cDNA inserted into λ gt11 is expressed as a β -galactosidase fusion protein detected immunologically.
-
-

Lesson 69: Preparation of cDNA for library construction

1. What is the first major step in the synthesis of double-stranded cDNA from an mRNA template?

- A) Second strand DNA synthesis using DNA polymerase I
- B) First strand DNA synthesis carried out with reverse transcriptase
- C) Tailing with terminal deoxynucleotidyltransferase
- D) Digestion of mRNA with restriction endonucleases

2. What enzyme is typically used to carry out second strand cDNA synthesis after removal of the RNA template?

- A) Reverse transcriptase
- B) A DNA-dependent DNA polymerase, such as *E. coli* DNA polymerase I
- C) T4 DNA ligase
- D) Alkaline phosphatase

3. What universal requirement must be provided for all DNA polymerases (whether using RNA or DNA as template) to initiate strand synthesis?

- A) An S-adenosylmethionine cofactor
- B) A primer
- C) High concentrations of ethidium bromide
- D) A 5' terminal cap structure

4. What is the second step in preparing double-stranded cDNA prior to second strand synthesis?

- A) Phosphorylation of 3' termini
- B) Removal of the RNA template
- C) Ligation of linkers to single-stranded RNA
- D) Heat-shock treatment at 100°C

Answer Key

- * **1. B** — The initial step of cDNA preparation is first strand DNA synthesis on the mRNA template using reverse transcriptase.
- * **2. B** — Second strand synthesis uses a DNA-dependent DNA polymerase such as *E. coli* DNA polymerase I.

* **3. B** — All DNA polymerases require a primer base-paired to the template to initiate nucleic acid synthesis.

* **4. B** — The second step in cDNA preparation involves removing the RNA template from the RNA-DNA hybrid.

Lesson 70: Improved methods for cDNA cloning

1. Why is it advantageous for cDNA to be inserted in a specific, correct orientation in expression libraries?

- A) To allow proper transcription and translation of the cloned sequence
- B) To force the cDNA to form circular plasmids spontaneously
- C) To prevent DNA polymerase I from detaching from the template
- D) To eliminate the need for reverse transcriptase

2. What serious disadvantage is associated with the early hairpin self-priming method of second strand cDNA synthesis?

- A) It converts double-stranded DNA into single-stranded RNA
- B) Cleavage with S1 nuclease results in the loss of sequence at the 5' end of the clone
- C) It requires heating the cDNA to 100°C in alkali
- D) It prevents terminal transferase from binding

3. In the improved second-strand priming method devised by Land and co-workers (1981), what enzyme is used to tail the first-strand cDNA with cytidine residues?

- A) Reverse transcriptase
- B) Terminal transferase
- C) *E. coli* DNA ligase
- D) Alkaline phosphatase

4. What synthetic primer is annealed to the artificial oligo-dC tail during Land and co-workers' method to allow synthesis of the second strand?

- A) Oligo-dT primer
- B) Oligo-dG primer
- C) Oligo-dA primer
- D) Random hexamer primer

Answer Key

* **1. A** — Inserting cDNA in the correct orientation is essential for expression libraries to produce functional protein.

* **2. B** — Cleaving the hairpin loop with S1 nuclease causes loss of 5' sequence information from the clone.

* **3. B** — Terminal transferase adds a string of cytidine residues (oligo-dC) to the 3' end of the first-strand cDNA.

* **4. B** — A synthetic oligo-dG primer anneals to the oligo-dC tail to prime second strand DNA synthesis.

Lesson 71: PCR as an alternative for cDNA cloning

1. What is the main requirement for template generation in RT-PCR compared to standard PCR?

- A) The template is generated in the same reaction tube in a prior reverse-transcription reaction
- B) The template must be purified by cesium chloride gradient centrifugation
- C) The template must be cloned into an M13 vector before amplification
- D) The template requires digestion with EcoRI and ligation with T4 ligase

2. Which primer is used to carry out the initial reverse-transcription step in RT-PCR to generate the first cDNA strand?

- A) A specific 5' primer
- B) A specific 3' primer
- C) A random degenerate hexamer only
- D) An unphosphorylated BamHI adaptor

3. When is PCR amplification initiated during RT-PCR?

- A) Before adding reverse transcriptase
- B) Following the addition of a 5' primer to the reaction mix
- C) After baking the sample on nitrocellulose at 80°C
- D) After S1 nuclease digestion of the template

4. What makes RT-PCR an attractive approach for obtaining a specific cDNA sequence for cloning?

- A) Its ability to eliminate all 3' untranslated regions
- B) The speed with which RT-PCR can be carried out
- C) Its complete reliance on genomic DNA libraries
- D) Its ability to perform high-stringency hybridization without primers

Answer Key

* **1. A** — The template for PCR amplification in RT-PCR is generated in the same reaction tube in a prior reverse-transcription reaction.

* **2. B** — Reverse transcription is carried out using a specific 3' primer that generates the first cDNA strand.

* **3. B** — PCR amplification is initiated following the addition of a 5' primer to the reaction mix.

* **4. B** — RT-PCR is attractive for obtaining specific cDNA sequences due to the speed with which it can be carried out.

Lesson 72: SCREENING STRATEGIES: Selection for the presence of vector**1. Which types of libraries can be screened using genetic methods or sequence-dependent screening?**

- A) Genomic libraries only
- B) cDNA libraries only
- C) Any type of library, genomic or cDNA
- D) Synthetic oligonucleotide libraries exclusively

2. What type of libraries specifically allow product screening using antibodies or ligands?

- A) Genomic DNA libraries in pBR322
- B) Expression libraries
- C) Jumping libraries
- D) Phage M13 sequencing libraries

3. What property serves as the selected marker for genetic selection in phage vectors?

- A) Chloramphenicol resistance
- B) Plaque formation
- C) Ampicillin resistance
- D) Histidine auxotrophy

4. What is described as a prerequisite stage in obtaining the recombinant population during library screening?

- A) Genetic selection for the presence of the vector
- B) Digestion of total RNA with proteinase K
- C) Electron microscopy of recombinant capsids
- D) Direct sequencing of the entire host chromosome

Answer Key

* **1. C** — Genetic methods and sequence-dependent screening apply to any type of library, whether genomic or cDNA.

* **2. B** — Product screening using antibodies or ligands applies specifically to expression libraries.

* **3. B** — In the case of phage vectors, plaque formation itself is the selected property.

* **4. A** — Genetic selection for the presence of the vector is a prerequisite stage in obtaining the recombinant population.

Lesson 73: Screening by hybridization

1. Who developed the in situ colony hybridization screening procedure using radioactive RNA probes in 1975?

- A) Benton and Davis
- B) Grunstein and Hogness
- C) Ish-Horowicz and Burke
- D) Alwine and coworkers

2. Why are transformed colonies treated with alkali after replica plating onto nitrocellulose filters?

- A) To induce protein synthesis from the lac promoter
- B) To lyse the bacterial colonies and denature the DNA they contain
- C) To remove radioactive RNA probes
- D) To synthesize oligo-dC homopolymer tails

3. What treatment is applied to the nitrocellulose filter to remove cellular protein before baking?

- A) Lysozyme
- B) Proteinase K
- C) S1 nuclease

D) Restriction endonuclease EcoRI

4. At what temperature is the nitrocellulose filter baked to fix the denatured DNA firmly prior to hybridization?

- A) 37°C
- B) 42°C
- C) 80°C
- D) 100°C

Answer Key

* **1. B** — Grunstein and Hogness (1975) developed the in situ screening procedure for transformed colonies.

* **2. B** — Alkali treatment lyses the bacterial colonies and denatures their contained DNA.

* **3. B** — The filter is treated with proteinase K to remove protein and leave denatured DNA bound to the filter.

* **4. C** — The DNA is fixed firmly to the nitrocellulose filter by baking at 80°C.

Lesson 74: Benton and Davis' plaque lift procedure

1. In Benton and Davis' plaque lift procedure (1977), how is the nitrocellulose filter applied to obtain DNA prints?

- A) By immersing the filter in boiling SDS solution
- B) By applying the filter directly to the upper surface of agar plates to make contact with plaques
- C) By electroporating phage particles into the filter matrix
- D) By centrifuging the agar plate at 15000 g

2. Besides intact phage particles, what else present in plaques binds to the nitrocellulose filter during a plaque lift?

- A) Unpackaged recombinant DNA
- B) Cell wall peptidoglycan fragments
- C) Active reverse transcriptase enzymes
- D) Ethidium bromide dye complexes

3. What major advantage does the plaque lift procedure offer regarding the number of prints obtainable from a single phage plate?

- A) It eliminates the need for any nitrocellulose membranes
- B) Several identical DNA prints can easily be made from a single phage plate
- C) It automatically sequences all recombinant inserts in parallel
- D) It converts single-stranded RNA into double-stranded DNA

4. How does making duplicate prints from a single plate benefit library screening?

- A) It increases screening reliability and allows screening a single set of recombinants with two or more probes
- B) It prevents host restriction endonucleases from degrading the vector
- C) It guarantees 100% expression of fusion proteins
- D) It reduces the required hybridization temperature to 0°C

Answer Key

* **1. B** — The nitrocellulose filter is applied directly to the upper surface of agar plates to contact plaques.

* **2. A** — Plaques contain phage particles as well as a considerable amount of unpackaged recombinant DNA, both of which bind to the filter.

* **3. B** — A major advantage is that several identical DNA prints can easily be made from a single phage plate.

* **4. A** — Duplicate prints permit duplicate screening for increased reliability and enable testing with multiple probes.

Lesson 75: Probe design

1. What hybridization stringency conditions are required to identify genomic clones that are identical to a specific cDNA probe?

- A) Very low stringency
- B) Very high stringency
- C) Neutral pH with no salt
- D) High concentration of ethidium bromide

2. Under what stringency conditions can a probe from one species be used to isolate a homologous clone from another species?

- A) Less stringent conditions
- B) Maximum possible stringency
- C) Boiling conditions at 100°C
- D) In the presence of SDS detergent only

3. What region of a gene can be targeted at lower stringency to cross-hybridize with several different clones and identify members of a gene family?

- A) A non-conserved intron sequence
- B) A conserved functional domain
- C) An unphosphorylated 5' linker terminus
- D) A poly(A) tail sequence

4. Which developmental gene family in vertebrates was identified using cross-species hybridization based on Drosophila developmental genes?

- A) Dystrophin genes
- B) Hox genes
- C) Immunoglobulin genes
- D) Beta-galactosidase genes

Answer Key

* **1. B** — Very stringent hybridization conditions are used when identifying sequences identical to the probe.

* **2. A** — Less stringent conditions allow cross-species hybridization to identify homologous clones.

* **3. B** — Probes corresponding to a conserved functional domain can identify related members of a gene family at lower stringency.

* **4. B** — Vertebrate Hox genes were identified using cross-species hybridization based on sequences conserved in Drosophila genes (*fushi tarazu* and *Antennapedia*).

Lesson 76: Chromosome walking

1. What feature of genomic libraries constructed from random DNA fragments enables chromosome walking?

- A) The fragments are all equal in length and lack non-coding sequences
- B) The resulting DNA fragments overlap with one another
- C) The fragments contain preformed synthetic linkers at both ends
- D) The fragments integrate into the bacterial chromosome via homologous recombination

2. What term refers to a technique used to isolate a gene of unknown function based solely on its known genetic location?

- A) Expression cloning
- B) Positional cloning
- C) Sub-cloning by homopolymer tailing
- D) Reverse transcriptase PCR

3. What starting material is required to initiate a chromosome walk toward a target gene?

- A) A purified synthetic oligo(dT) primer
- B) A genomic clone known to lie very close to the suspected location of the target gene
- C) An antibody that specifically recognizes the target gene's protein product
- D) A single-stranded RNA probe derived from a poly(A) tail

4. How are successive overlapping clones identified during a chromosome walk?

- A) By exposing the library to high doses of X-ray radiation
- B) By using an isolated overlapping clone as a probe in subsequent rounds of hybridization screening
- C) By measuring the rate of migration in SDS-PAGE gel electrophoresis
- D) By digesting the library with EcoRI to remove non-overlapping fragments

Answer Key

* **1. B** — Genomic libraries made from random DNA fragments contain overlapping sequences that allow contig assembly.

* **2. B** — Positional cloning is the isolation of a gene whose function is unknown but whose genetic location is known.

* **3. B** — A chromosome walk requires a starting genomic clone that is mapped very close to the target gene location.

* **4. B** — Isolated overlapping clones are labelled and used as probes to screen the library for adjacent overlapping clones.

Lesson 77: Chromosome jumping

1. Which restriction endonuclease cutting at very rare target sites is specifically mentioned in Topic 77 for generating very large genomic fragments in chromosome jumping?

- A) EcoRI
- B) NotI
- C) BamHI
- D) Sau3AI

2. What key step in constructing a jumping library brings together DNA sequences that were originally located a considerable distance apart?

- A) Alkaline hydrolysis of single-stranded cDNA
- B) Circularization of very large genomic fragments followed by sub-cloning of the region covering the closure
- C) Addition of oligo(dC) homopolymer extensions using terminal transferase
- D) Digestion of vector arms with S1 nuclease

3. What is the primary purpose of constructing a jumping library as described in Topic 77?

- A) To convert double-stranded eukaryotic DNA into single-stranded viral RNA
- B) To perform long-distance chromosome walks across large genomic distances
- C) To eliminate all intron sequences from genomic DNA fragments
- D) To select for antibiotic resistance markers in competent E. coli cells

4. How does chromosome jumping fundamentally differ from standard chromosome walking?

- A) Chromosome jumping relies on protein antibodies instead of nucleic acid probes
- B) Chromosome jumping circumvents stepping through short overlaps by circularizing large fragments to span long distances
- C) Chromosome jumping can only be performed on prokaryotic organisms lacking cell walls
- D) Chromosome jumping does not require restriction endonucleases at any stage

Answer Key

* **1. B** — Chromosome jumping utilizes rare-cutting endonucleases such as NotI to generate very large genomic fragments.

* **2. B** — Circularizing large fragments and sub-cloning the closure region brings distant sequences together.

* **3. B** — A jumping library is constructed specifically to enable long-distance chromosome walks.

* **4. B** — Chromosome jumping spans large distances by sub-cloning circularized fragment closure junctions rather than stepping through adjacent overlaps.

Lesson 78: Screening by PCR

1. What essential information is required to identify or screen a specific clone from a library using PCR?

- A) The exact tertiary 3D structure of the target protein
- B) Sufficient sequence information about the target to design suitable primers
- C) The exact melting temperature of the host cell wall
- D) The complete sequence of the vector's lacZ reporter gene

2. What type of primer consists of a mixture of similar sequences containing variations at one or more positions?

- A) Universal oligo(dT) primer
- B) Degenerate primer
- C) Phosphorylated BamHI adaptor
- D) Self-complementary decameric linker

3. In what common situation is the use of degenerate primers necessary during PCR screening?

- A) When target primer sequences must be deduced from amino acid sequences
- B) When plasmid vectors carry chloramphenicol resistance markers
- C) When double-stranded DNA templates are denatured at 42°C
- D) When transforming competent cells via electroporation

4. How are gene-specific primers positioned when performing PCR to isolate a specific clone?

- A) They bind to the poly(A) tail and 5' cap simultaneously
- B) They flank a unique sequence in the target DNA
- C) They align parallel to the host cell's 16S rRNA sequence
- D) They bind exclusively to the origin of replication (ori)

Answer Key

* **1. B** — Screening a clone by PCR requires sufficient sequence information to synthesize appropriate primers.

* **2. B** — Degenerate primers are mixtures of primers with similar sequences containing variations at specific positions.

* **3. A** — Degenerate primers are commonly required when primer sequences are deduced from amino acid sequences due to code degeneracy.

* **4. B** — Specific clones are isolated using gene-specific primers that flank a unique target sequence.

Lesson 79: Expression cloning

1. Why are expression libraries particularly useful when the DNA sequence of a target clone is completely unknown?

- A) They eliminate the need for host cell ribosomes during translation
- B) They allow screening based on structural or functional properties of the expressed polypeptide
- C) They automatically correct base-pair mutations introduced by PCR
- D) They convert single-stranded RNA directly into double-stranded plasmids

2. Why are all expression libraries derived from higher eukaryotes constructed as cDNA libraries rather than genomic libraries?

- A) Eukaryotic genomic DNA cannot be cut by restriction enzymes
- B) Eukaryotic cDNAs lack introns and are of a reasonable size for expression
- C) Eukaryotic genomic DNA lacks promoter sequences
- D) Eukaryotic cDNAs naturally contain ampicillin resistance markers

3. Why are bacterial and many yeast expression libraries usually constructed from genomic DNA rather than cDNA?

- A) Bacterial and yeast genomes contain few or no introns

- B) Bacterial genomic DNA cannot be transcribed by reverse transcriptase
- C) Bacteria lack origins of replication
- D) Yeast genomes consist entirely of single-stranded RNA

4. How can efficient expression libraries be generated so that all genes are represented at approximately the same frequency?

- A) By heating total cellular RNA to 100°C in alkaline buffer
- B) By cloning randomly sheared genomic DNA or partially digested DNA
- C) By treating vectors with terminal deoxynucleotidyltransferase
- D) By selecting transformants exclusively on HAT medium

Answer Key

* **1. B** — Expression libraries enable screening by exploiting structural or functional properties of the expressed protein product.

* **2. B** — Eukaryotic expression libraries use cDNA because cDNAs lack introns and are of reasonable size for expression.

* **3. A** — Bacterial and yeast expression libraries can use genomic DNA because their genes contain few or no introns.

* **4. B** — Cloning randomly sheared or partially digested DNA ensures all genes are represented at equal frequency.

Lesson 80: Immunological screening

1. What molecular probes are used in immunological screening to recognize specific target clones in an expression library?

- A) Single-stranded cDNA probes
- B) Antibodies that recognize antigenic determinants
- C) Degenerate oligonucleotide primers
- D) Radiolabelled T-DNA border sequences

2. What major advantage does immunological screening offer regarding the target protein product?

- A) The protein must be a functional enzyme with 100% catalytic activity
- B) The protein does not need to be biologically functional
- C) The protein must be secreted into the extracellular growth medium
- D) The protein must be less than 50 amino acids in length

3. What specific molecular target on the protein surface is generally recognized by antibodies during immunological screening?

- A) An origin of replication
- B) An epitope
- C) A polylinker
- D) A 5' terminal phosphate

4. Why can epitopes still be recognized by antibodies even when the expressed polypeptide chain is incomplete or part of a fusion protein?

- A) Epitopes fold independently of the rest of the protein
- B) Epitopes consist entirely of poly(A) adenylate residues
- C) Epitopes are resistant to heat denaturation at 100°C
- D) Epitopes are synthesized directly by DNA polymerase I

Answer Key

- * **1. B** — Immunological screening employs antibodies that specifically recognize antigenic determinants on synthesized polypeptides.
- * **2. B** — Immunological screening can be applied to any protein for which an antibody exists, without requiring the protein to be functional.
- * **3. B** — Antibodies recognize an epitope, which is a short sequence of amino acids folding into a specific 3D conformation.
- * **4. A** — Epitopes fold independently of the rest of the polypeptide chain, allowing detection even in incomplete or fusion proteins.
-

Lesson 81: Immunochemical screening of λ gt11

1. Who introduced immunological screening with phage- λ cDNA libraries in 1983 using the expression vector λ gt11?

- A) Benton and Davis
- B) Young and Davis
- C) Grunstein and Hogness
- D) Ish-Horowicz and Burke

2. Which enzyme is fused to foreign cDNA sequences when expressed in the vector λ gt11 under the control of the lac promoter?

- A) Alkaline phosphatase
- B) Reverse transcriptase
- C) β -galactosidase
- D) DNA polymerase I

3. Why is the *E. coli* host strain Y1090 used when plating λ gt11 libraries for expression screening?

- A) It lacks all restriction endonucleases and cell wall peptidoglycan
- B) It overproduces the lac repressor and is deficient in the lon protease
- C) It carries an endogenous plasmid encoding chloramphenicol resistance
- D) It converts single-stranded RNA into double-stranded plasmids spontaneously

4. Which chemical inducer is presented to infected host cells to trigger expression of the cloned cDNA fusion protein in λ gt11 plaque screening?

- A) Ethidium bromide
- B) Isopropyl- β -D-thiogalactoside (IPTG)
- C) X-gal
- D) Cyanogen bromide

Answer Key

- * **1. B** — Young and Davis (1983) introduced immunological screening of phage- λ cDNA libraries using λ gt11.
- * **2. C** — Vector λ gt11 generates fusion proteins with β -galactosidase driven by the lac promoter.
- * **3. B** — Strain Y1090 overproduces lac repressor to prevent premature expression and lacks lon protease to stabilize fusion proteins.
- * **4. B** — Presentation of IPTG to infected cells induces transcription of the cloned cDNA fusion protein.
-
-

Lesson 82: South-Western and North-Western blotting

1. What type of target clones is South-Western screening specifically designed to identify and isolate?

- A) Clones expressing cell-wall synthesis enzymes
- B) Clones expressing sequence-specific DNA-binding proteins
- C) Clones carrying ribosomal RNA genes
- D) Clones encoding viral coat proteins exclusively

2. What probe is incubated with the nitrocellulose membrane print during South-Western screening instead of an antibody?

- A) A radiolabelled double-stranded DNA oligonucleotide probe containing the target recognition sequence
- B) A fluorophore-tagged secondary antibody
- C) A single-stranded poly(A) RNA probe
- D) An unphosphorylated BamHI adaptor

3. Why is the technique named "South-Western" screening?

- A) Because it was developed in the southwestern region of the United States
- B) Because it combines the principles of Southern (DNA detection) and Western (protein detection) blotting
- C) Because it uses South-type restriction enzymes and West-type polyacrylamide gels
- D) Because it isolates proteins that migrate toward the southwestern pole of a gel

4. South-Western screening has been particularly successful in isolating cDNA clones corresponding to which class of proteins in mammals?

- A) Ribosomal structural proteins
- B) Mammalian transcription factors
- C) Mitochondrial ATP synthases
- D) Cell membrane ion channels

Answer Key

- * **1. B** — South-Western screening isolates clones expressing sequence-specific DNA-binding proteins.
- * **2. A** — The membrane print is incubated with a radiolabelled double-stranded DNA oligonucleotide containing the target recognition sequence.
- * **3. B** — It is termed South-Western screening because it combines the principles of Southern and Western blotting.

* **4. B** — The method has been especially successful in isolating cDNA clones for mammalian transcription factors.

Lesson 83: Screening by functional complementation

1. How is functional complementation defined in Lesson 83?

- A) The cleavage of target DNA using restriction endonucleases to yield blunt ends
- B) The process by which a particular DNA sequence compensates for a missing function in a mutant cell to restore the wild-type phenotype
- C) The degradation of foreign mRNA templates by host cell proteases
- D) The binding of radiolabelled antibodies to inactive enzyme subunits

2. Who provided an early example in 1977 of cloning eukaryotic genes based on their ability to complement auxotrophic mutations in *E. coli*?

- A) Young and Davis
- B) Ratzkin and Carbon
- C) Ish-Horowicz and Burke
- D) Maxam and Gilbert

3. Which plasmid and method were used by Ratzkin and Carbon to clone mechanically sheared yeast DNA fragments?

- A) pBR322 via EcoRI restriction digestion
- B) ColE1 via a homopolymer-tailing procedure
- C) Charon 4A via in vitro packaging
- D) M13mp18 via polylinker insertion

4. Which specific auxotrophic mutation in *E. coli* was complemented by yeast DNA on minimal medium in Ratzkin and Carbon's experiment?

- A) *leu2* mutant unable to synthesize leucine
- B) *hisB* mutant unable to synthesize histidine
- C) *lacZ* mutant unable to utilize lactose
- D) *ampR* mutant sensitive to ampicillin

Answer Key

* **1. B** — Functional complementation occurs when a DNA sequence compensates for a missing mutant function and restores the wild-type phenotype.

* **2. B** — Ratzkin and Carbon (1977) demonstrated cloning yeast genes by complementing auxotrophic mutations in *E. coli*.

* **3. B** — Mechanically sheared yeast DNA fragments were inserted into plasmid ColE1 using a homopolymer-tailing procedure.

* **4. B** — Transformation of *E. coli hisB* mutants selected for complementation of histidine auxotrophy on minimal medium.

Lesson 84: Requirement for expression in *E. coli*

1. According to Lesson 84, what three major cellular processes are necessary for the synthesis of a functional foreign protein in *E. coli*?

- A) Reverse transcription, PCR amplification, and depurination
- B) Transcription of the gene, efficient translation of mRNA, and post-translational processing/compartimentalization
- C) Homopolymer tailing, ligation with T4 ligase, and electroporation
- D) Alkaline lysis, ethanol precipitation, and isopycnic centrifugation

2. What essential element recognized by host RNA polymerase is required for transcription of a cloned insert in *E. coli*?

- A) An oligo(dT) primer
- B) A promoter
- C) A 25-bp direct repeat border sequence
- D) A polyadenylated tail

3. What structural feature is ideally required at the 3' end of a cloned gene to properly end transcription in *E. coli*?

- A) A terminal protein cap
- B) A transcription terminator
- C) An EcoRI restriction site
- D) A 5' phosphate overhang

4. What element must be present on the mRNA transcript to ensure efficient translation initiation by *E. coli* ribosomes?

- A) A T-DNA border sequence
- B) A ribosome binding site
- C) An origin of replication
- D) A 3'-OH homopolymer tail

Answer Key

* **1. B** — Synthesis of functional protein depends on gene transcription, mRNA translation, and post-translational processing/compartimentalization.

* **2. B** — Transcription of the cloned insert requires a promoter recognized by host RNA polymerase.

* **3. B** — Ideally, a transcription terminator is located at the 3' end of the gene to end transcription.

* **4. B** — Efficient translation requires a ribosome binding site on the mRNA transcript.

Lesson 85: Secretion of proteins

1. What structural compartments compose the cell envelope of Gram-negative bacteria such as *E. coli*?

- A) A single lipid monolayer without a cell wall
- B) An inner cytoplasmic membrane separated from an outer membrane by a cell wall and periplasmic space
- C) A thick peptidoglycan wall with no inner membrane

D) A chitin cell wall surrounding a nuclear envelope

2. What universal pathway mediates protein export through the inner membrane to the periplasm or outer membrane in *E. coli*?

- A) General export pathway (GEP)
- B) Lytic cycle cascade
- C) Homopolymer tailing pathway
- D) Reverse transcription pathway

3. Which group of genes encodes the components of the general export pathway (GEP) in *E. coli*?

- A) *tra* genes
- B) *sec* genes
- C) *vir* genes
- D) *par* genes

4. What feature at the N-terminus of nascent proteins directs them into the GEP, and what enzyme cleaves it during transport?

- A) A C-terminal poly(A) tail cleaved by RNase H
- B) A signal sequence at the N-terminus cleaved by signal peptidase
- C) A 5' cap structure cleaved by alkaline phosphatase
- D) An epitope tag cleaved by lon protease

Answer Key

* **1. B** — Gram-negative bacteria possess an inner cytoplasmic membrane, cell wall, periplasmic space, and outer membrane.

* **2. A** — Protein export through the inner membrane to the periplasm or outer membrane occurs via the general export pathway (GEP).

* **3. B** — The general export pathway (GEP) involves the *sec* gene products.

* **4. B** — Proteins entering the GEP carry an N-terminal signal sequence that is cleaved by signal peptidase during transport.

Lesson 86: Protein trafficking

1. What length range do N-terminal signal sequences typically span according to Topic 86?

- A) 5 to 10 amino acids
- B) 15 to 30 amino acids
- C) 50 to 100 amino acids
- D) 100 to 200 amino acids

2. How many hydrophobic amino acids are included in an N-terminal signal sequence, preceded by a short hydrophilic stretch?

- A) 3
- B) 7
- C) 11
- D) 20

3. What occurs to the signal sequence of proteins destined for the periplasm or outer membrane following export?

- A) It remains permanently attached as a membrane anchor
- B) It is cleaved following export
- C) It is phosphorylated by kinase enzymes
- D) It is converted into a poly(A) tail

4. How many general mechanisms of protein export manage the process of inserting proteins into membranes and passing hydrophilic proteins through hydrophobic membrane barriers?

- A) Two
- B) Five
- C) Seven
- D) Twelve

Answer Key

* **1. B** — N-terminal signal sequences range from 15 to 30 amino acids in length.

* **2. C** — Signal sequences include 11 hydrophobic amino acids preceded by a short stretch of hydrophilic residues.

* **3. B** — Unlike integral membrane proteins, proteins for the periplasm or outer membrane have their signal sequences cleaved following export.

* **4. C** — There are seven general mechanisms of protein export that traffic different sets of exported proteins.

Lesson 87: Stability of foreign proteins in E. coli

1. In the case of somatostatin, how was degradation prevented when expressed in *E. coli*?

- A) By incubating host cells at 65°C
- B) By producing a fused protein consisting of somatostatin and β -galactosidase
- C) By deleting the origin of replication
- D) By adding ethidium bromide to the culture

2. Which chemical reagent is used to cleave somatostatin from its β -galactosidase fusion partner?

- A) Isopropanol
- B) Cyanogen bromide
- C) Sodium dodecyl sulphate
- D) Phenol-chloroform

3. An alternative strategy to prevent degradation of foreign proteins uses *E. coli* mutants deficient in which specific intracellular protease?

- A) Signal peptidase
- B) Lon protease
- C) T4 DNA ligase
- D) Reverse transcriptase

4. What enzymatic activity and property characterize the *E. coli lon* protease according to Topic 87?

- A) A DNA-dependent RNA polymerase
- B) A DNA binding protein with ATP-dependent proteolytic activity
- C) An exonuclease requiring S-adenosylmethionine
- D) A cell-wall degrading lysozyme

Answer Key

- * **1. B** — Degradation of somatostatin was prevented by producing a fusion protein with β -galactosidase.
- * **2. B** — Somatostatin is cleaved from the fusion protein using cyanogen bromide.
- * **3. B** — Mutants lacking the lon protease have a reduced complement of intracellular proteases to protect foreign proteins.
- * **4. B** — The lon protease is a DNA binding protein with ATP-dependent proteolytic activity.

Lesson 88: Constructing the optimal promoter

1. What consensus sequence is defined for the -10 region (Pribnow box) of *E. coli* promoters?

- A) 5'-TTGACA
- B) 5'-TATAAT
- C) 5'-GGAGG
- D) 5'-ACTAGT

2. What consensus sequence is defined for the -35 region of *E. coli* promoters?

- A) 5'-TTGACA
- B) 5'-TATAAT
- C) 5'-CCCGGG
- D) 5'-AAGCTT

3. What percentage of total cell protein can be represented by the cloned gene product when expressed from a strong promoter?

- A) 1% to 5%
- B) 5% to 10%
- C) 20% to 40%
- D) 80% to 90%

4. How can expression driven by the *trp* promoter be induced in host cells according to Topic 88?

- A) By adding IPTG
- B) By tryptophan starvation
- C) By raising the temperature to 42°C
- D) By adding cyanogen bromide

Answer Key

- * **1. B** — The consensus sequence for the -10 region (Pribnow box) is 5'-TATAAT.
- * **2. A** — The consensus sequence for the -35 region is 5'-TTGACA.

* **3. C** — Expression from a strong promoter can result in 20–40% of total cell protein being the cloned gene product.

* **4. B** — Expression from the trp promoter can be induced specifically by tryptophan starvation.

Lesson 89: Optimizing translation initiation

1. Which sequence element is described as central to translational initiation in *E. coli*?

- A) Pribnow box (-10 region)
- B) Shine-Dalgarno (S-D) sequence or ribosome binding site
- C) Polyadenylated tail
- D) 25-bp direct repeat border

2. With which cellular component does the Shine-Dalgarno sequence base-pair during translation initiation?

- A) 23S rRNA
- B) 16S rRNA
- C) T4 DNA ligase
- D) 5S rRNA

3. What exact sequence exhibits perfect complementarity to the 16S rRNA according to Topic 89?

- A) 5'-TTGACA-3'
- B) 5'-GGAGG-3'
- C) 5'-TATAAT-3'
- D) 5'-AAGCTT-3'

4. How much can a single base change within the Shine-Dalgarno consensus sequence reduce translation efficiency?

- A) Twofold
- B) Fivefold
- C) Tenfold
- D) One hundredfold

Answer Key

* **1. B** — The Shine-Dalgarno (S-D) sequence or ribosome binding site is central to factors affecting translation initiation.

* **2. B** — The degree of complementarity of the Shine-Dalgarno sequence with 16S rRNA affects the rate of translation.

* **3. B** — Perfect complementarity with 16S rRNA occurs with the sequence 5'-GGAGG-3'.

* **4. C** — A single base change within the Shine-Dalgarno region can reduce translation tenfold.

Lesson 90: Stability of mRNA and codon choice

1. Provided translational machinery elements are present in excess, what determines the rate of synthesis of a particular protein?

- A) Plasmid size in base pairs
- B) The steady state level of mRNA in the cell
- C) Concentration of calcium chloride in the medium
- D) Number of restriction sites in the polylinker

2. The steady state level of mRNA in a cell reflects a balance between which two processes?

- A) Denaturation and renaturation
- B) Synthesis and degradation
- C) Methylation and restriction
- D) Ligation and transformation

3. Besides maximizing the rate of synthesis with strong promoters, how can a similar increase in steady state mRNA levels be achieved?

- A) By reducing the degradation of messenger RNA
- B) By deleting ribosome binding sites
- C) By increasing cell wall thickness
- D) By digesting mRNA with S1 nuclease

4. A strong positive correlation has been found between codon choice and which cellular factor?

- A) tRNA abundance
- B) Plasmid copy number
- C) Exonuclease activity
- D) DNA polymerase processivity

Answer Key

* **1. B** — The rate of protein synthesis depends on the steady state level of mRNA in the cell when translation machinery is in excess.

* **2. B** — The steady state level of mRNA reflects the balance between synthesis and degradation.

* **3. A** — A similar effect to strong promoters can be achieved by reducing mRNA degradation.

* **4. A** — A strong positive correlation has been found between tRNA abundance and codon choice.

Lesson 91: The effect of plasmid copy number

1. What is described in Lesson 91 as a major rate-limiting step in protein synthesis?

- A) The binding of ribosomes to mRNA molecules
- B) The transcription of ribosomal RNA
- C) The degradation of dNTPs by endonucleases
- D) The phosphorylation of 5' ends by kinase

2. Why can increasing the number of corresponding mRNA transcripts increase the expression of a cloned gene?

- A) Because cell wall thickness increases proportionally
- B) Because the number of ribosomes in a cell far exceeds any one class of messenger
- C) Because tRNA molecules are degraded during transcription

D) Because DNA polymerase I requires multiple mRNA templates

3. What two factors affect the rate of transcription according to Lesson 91?

- A) Promoter strength and gene copy number
- B) Ribosome size and cell wall composition
- C) Temperature and centrifugation speed
- D) Intron length and poly(A) tailing

4. Which high copy number plasmid is specifically mentioned in Lesson 91 as an example for increasing gene dosage?

- A) pSC101
- B) pBR322
- C) ColE1
- D) RSF1010

Answer Key

* **1. A** — Binding of ribosomes to mRNA molecules is described as a major rate-limiting step in protein synthesis.

* **2. B** — The number of ribosomes in a cell far exceeds any single class of messenger, so increasing transcript numbers boosts expression.

* **3. A** — Promoter strength and gene copy number are the two factors that dictate the rate of transcription.

* **4. B** — Plasmid pBR322 is cited as an example of a high copy number plasmid used to increase gene dosage.

Lesson 92: Plasmid stability

1. What effect can increased expression of recombinant genes have on host bacteria according to Lesson 92?

- A) Reductions in cell growth rates and morphological changes such as filamentation and increased fragility
- B) Increased resistance to all heavy metals
- C) Immediate conversion into Gram-positive endospores
- D) Elimination of all host restriction endonucleases

2. Why can a mutant cell that has lost its recombinant plasmid quickly predominate in a culture?

- A) It has a faster growth rate than cells harbouring the recombinant plasmid
- B) It secretes colicin E1 to kill surrounding plasmid-bearing cells
- C) It replicates its chromosome ten times faster
- D) It converts surrounding media into solid agar

3. What term is used to describe the loss of plasmids due to defective partitioning during cell division?

- A) Structural instability
- B) Segregative instability
- C) Incompatibility

D) Insertional inactivation

4. What function do naturally occurring plasmids possess to ensure accurate segregation at each cell division?

- A) *par* (partitioning) function
- B) *tra* transfer genes
- C) *lacZ* reporter gene
- D) *cos* packaging site

Answer Key

* **1. A** — High recombinant gene expression leads to reduced cell growth rates and morphological changes like filamentation and fragility.

* **2. A** — Plasmid-free mutants grow faster and can rapidly take over the culture.

* **3. B** — The loss of plasmids resulting from defective partitioning is termed segregative instability.

* **4. A** — Naturally occurring plasmids contain a *par* (partitioning) function that ensures accurate segregation during division.

Lesson 93: Structural instability

1. By which three mechanisms may structural instability of plasmids arise according to Lesson 93?

- A) Deletion, insertion, or rearrangements of DNA
- B) Methylation, depurination, or alkaline hydrolysis
- C) Phosphorylation, ligation, or electroporation
- D) Transcription, translation, or reverse transcription

2. In which type of plasmids were some of the earliest reports of DNA deletions observed?

- A) Chimaeric plasmids which can replicate in both *E. coli* and *B. subtilis*
- B) Wild-type single-stranded M13 phages
- C) High-capacity YAC vectors carrying human centromeres
- D) Linear plasmids protected by terminal proteins in *Streptomyces*

3. Spontaneous deletions have been observed in which of the following genetic elements according to Lesson 93?

- A) Plasmid, virus, and chromosomal DNAs
- B) Single-stranded ribosomal RNA exclusively
- C) Synthetic polyplexes and liposomes
- D) tRNA adaptors only

4. What is a common feature involved in spontaneous deletions across various DNAs?

- A) Homologous recombination between short direct repeats
- B) Cleavage by restriction enzyme *NotI*
- C) Tailing with terminal deoxynucleotidyltransferase
- D) Binding of ethidium bromide to open circular DNA

Answer Key

- * **1. A** — Structural instability arises through deletion, insertion, or rearrangement of plasmid DNA.
- * **2. A** — Early deletion reports occurred in chimeric plasmids capable of dual replication in *E. coli* and *B. subtilis*.
- * **3. A** — Spontaneous deletions occur across a wide range of plasmid, viral, and chromosomal DNAs.
- * **4. A** — Deletions commonly involve homologous recombination between short direct repeats.
-
-

Lesson 94: Host cell physiology can affect the level of expression

1. Besides promoter strength and gene dosage, what less tangible factor can control gene expression according to Lesson 94?

- A) The physiology of the host cell
- B) The concentration of agarose in gel electrophoresis
- C) The length of the polyadenylated tail on cDNA
- D) The number of restriction sites in the vector backbone

2. Which parameters are specifically listed in Lesson 94 as important host cell physiological factors?

- A) Choice and supply of nutrients, temperature, and dissolved oxygen
- B) Centrifugation speed and rotor angle
- C) Exposure time to ultraviolet light
- D) Type of X-ray film used in autoradiography

3. What does Lesson 94 state regarding systematic studies on growth conditions and foreign protein synthesis in *E. coli*?

- A) So far there has been no systematic study of the effect of different growth conditions on the synthesis of foreign proteins in *E. coli*
- B) Thousands of exhaustive studies have defined the exact growth parameters for every protein
- C) Dissolved oxygen has been proven to have zero effect on gene expression
- D) Growth conditions only affect Gram-positive hosts, not *E. coli*

4. Which environmental parameter is explicitly named in Lesson 94 alongside temperature?

- A) Dissolved oxygen
- B) Nitrogen gas pressure
- C) Sodium chloride salinity
- D) Magnetic field strength

Answer Key

- * **1. A** — Host cell physiology is a key, less tangible factor controlling gene expression.
- * **2. A** — Choice and supply of nutrients, temperature, and dissolved oxygen are key physiological parameters.
- * **3. A** — Lesson 94 notes that no systematic study of different growth conditions on foreign protein synthesis in *E. coli* has been conducted.
- * **4. A** — Dissolved oxygen is explicitly cited alongside temperature as an environmental parameter affecting expression.
-

Lesson 95: DNA sequencing: benefits and applications

1. Why is DNA sequencing considered a fundamental requirement for modern gene manipulation?

- A) It allows computer searching for restriction target sites to provide precise restriction maps and planning for DNA manipulation
- B) It eliminates the need for host cell culture and selection markers
- C) It automatically synthesizes foreign proteins without transcription
- D) It converts double-stranded DNA into single-stranded RNA

2. When did techniques for large-scale DNA sequencing first become available according to Lesson 95?

- A) Late 1970s
- B) Early 1950s
- C) Late 1990s
- D) 2010

3. On what principle does the Maxam and Gilbert DNA sequencing technique rely?

- A) Base-specific chemistry
- B) Dideoxynucleotide chain termination
- C) High-voltage electroporation
- D) Homopolymer tailing

4. Which sequencing approach gained widespread popularity following the Maxam and Gilbert technique?

- A) Chain-termination techniques
- B) Alkaline lysis procedures
- C) Protein microarrays
- D) Two-dimensional gel electrophoresis

Answer Key

* **1. A** — Sequence data is essential for planning DNA manipulations, such as computer-generating restriction maps.

* **2. A** — Large-scale DNA sequencing techniques became available in the late 1970s.

* **3. A** — The Maxam and Gilbert sequencing method relies on base-specific chemical cleavage.

* **4. A** — Chain-termination techniques gained popularity after Maxam and Gilbert sequencing.

Lesson 96: Maxam-Gilbert method

1. Which chemical reagent is used in the Maxam-Gilbert method to cleave the DNA backbone at sugar residues lacking a base?

- A) Dimethylsulphate
- B) Piperidine
- C) Sodium dodecyl sulphate
- D) Cyanogen bromide

2. How is the DNA strand to be sequenced radioactively labelled at the start of the Maxam-Gilbert procedure?

- A) At both 3' ends with an $\alpha\text{-}^{35}\text{S}$ group
- B) At one end with a $\gamma\text{-}^{32}\text{P}$ -phosphate group
- C) Internally at cytosine bases with $\gamma\text{-}^{33}\text{P}$
- D) At the poly(A) tail with tritium

3. Which base modification is specifically brought about by dimethylsulphate in the Maxam-Gilbert sequencing method?

- A) Methylation of guanine
- B) Depurination of adenine
- C) Deamination of cytosine
- D) Phosphorylation of thymine

4. Why is the Maxam-Gilbert method currently less favored for large-scale sequencing compared to enzymatic methods?

- A) It requires single-stranded RNA templates exclusively
- B) It relies on base-specific chemical cleavage rather than the enzymatic dideoxy procedure
- C) It cannot resolve DNA fragments larger than 10 base pairs
- D) It requires thermal cycling prior to chemical modification

Answer Key

* **1. B** — Piperidine cleaves the sugar-phosphate backbone specifically at sugar residues lacking a base.

* **2. B** — The DNA strand to be sequenced must be radioactively labelled at one end with a $\gamma\text{-}^{32}\text{P}$ -phosphate group.

* **3. A** — Dimethylsulphate is a base-specific reagent that methylates guanine residues in DNA.

* **4. B** — Maxam-Gilbert relies on base-specific chemical cleavage and is less favored for large-scale work than the enzymatic dideoxy method.

Lesson 97: Chain Termination or dideoxy procedure

1. Why does the incorporation of a 2', 3'-dideoxynucleotide cause chain termination during DNA synthesis?

- A) Its 3' end lacks a hydroxyl group, preventing further nucleotide additions
- B) It cleaves the phosphodiester backbone via alkaline hydrolysis
- C) It inhibits the 5' $\rightarrow$ 3' polymerase activity of DNA polymerase
- D) It binds irreversibly to the oligonucleotide primer

2. Why is the Klenow fragment used in the classic dideoxy procedure instead of intact *E. coli* DNA polymerase I?

- A) It lacks 3' $\rightarrow$ 5' polymerase activity
- B) It lacks the 5' $\rightarrow$ 3' exonuclease activity associated with the intact enzyme
- C) It functions efficiently at 95°C without heat denaturation
- D) It synthesizes complementary DNA directly from RNA templates

3. What is required to initiate DNA synthesis in the chain-termination sequencing method?

- A) A 25-bp direct repeat border sequence
- B) A chemically synthesized oligonucleotide primer annealed close to the target sequence
- C) A polyadenylated tail at the 3' terminus
- D) A dG homopolymer tail added by terminal transferase

4. How are the incubation mixtures set up in the classic Sanger dideoxy sequencing procedure?

- A) As a single reaction mix containing equal concentrations of all four dideoxynucleotides
- B) As four separate incubation mixes, each containing a low concentration of one of the four dideoxynucleoside triphosphates
- C) As two reaction tubes containing high concentrations of radiolabelled piperidine
- D) As eight reaction tubes containing varying concentrations of T4 DNA ligase

Answer Key

- * **1. A** — Once incorporated, a dideoxynucleotide lacks a 3'-OH group, making it no longer a substrate for chain elongation.
- * **2. B** — The Klenow fragment is used because it lacks the 5' $\rightarrow$ 3' exonuclease activity of intact DNA polymerase I.
- * **3. B** — Initiating synthesis requires an oligonucleotide primer annealed complementary to the template strand near the sequence being analyzed.
- * **4. B** — Four separate incubation mixes are prepared, each containing the four dNTPs and a low concentration of one specific ddNTP analogue.

Lesson 98: Modifications of chain termination sequencing

1. How can the sharpness of autoradiographic images in dideoxy sequencing be improved over traditional ^{32}P labelling?

- A) By replacing ^{32}P with lower-energy radiolabels such as ^{33}P or ^{35}S
- B) By baking the sequencing gel at 120°C in alkali
- C) By adding saturating concentrations of ethidium bromide to the gel matrix
- D) By substituting reverse transcriptase for DNA polymerase

2. Which phage DNA polymerase ('Sequenase') is widely used in place of the Klenow fragment because of its high processivity?

- A) Phage T4 DNA polymerase
- B) Phage T7 DNA polymerase
- C) Phage lambda DNA polymerase
- D) Phage M13 DNA polymerase

3. What major advantage does T7 DNA polymerase ('Sequenase') offer regarding band intensities on sequencing gels?

- A) It produces bands only at guanine residues
- B) Its incorporation of dideoxynucleotides is less affected by local nucleotide sequence, yielding more even band intensities
- C) It eliminates the need for gel electrophoresis during fragment separation
- D) It prevents single-stranded DNA from denaturing during electrophoresis

4. Which thermostable DNA polymerase from a thermophilic bacterium is cited in Lesson 98 as a replacement for Klenow polymerase?

- A) *Escherichia coli* DNA polymerase III
- B) *Thermus aquaticus* (*Taq*) DNA polymerase
- C) *Bacillus subtilis* DNA polymerase I
- D) Calf thymus terminal transferase

Answer Key

***1. A** — Lower-energy radiolabels like P^{33} or S^{35} (e.g., $\alpha\text{-}^{35}\text{S}$ -dNTP) significantly sharpen autoradiographic band images.

***2. B** — Natural or modified forms of phage T7 DNA polymerase ('Sequenase') are highly processive replacements for the Klenow fragment.

***3. B** — T7 DNA polymerase incorporates dideoxynucleotides more uniformly regardless of local sequence, producing ladders with even band intensities.

***4. B** — *Taq* DNA polymerase from *Thermus aquaticus* is a heat-stable enzyme used in modified chain-termination sequencing.

Lesson 99: Automated DNA sequencing

1. How does four-dye automated sequencing enable all four chain-terminating reactions to be run together in a single gel lane?

- A) By using four spectrally different fluorescent dyes attached to the chain-terminating nucleotides or primers
- B) By labelling each reaction with different radioactive isotopes of carbon
- C) By running the gel at four different electrical voltage gradients simultaneously
- D) By digesting the fragments with four different restriction endonucleases

2. What double function is accomplished when a fluorescently tagged dideoxynucleotide is incorporated by DNA polymerase?

- A) It terminates chain synthesis and attaches the fluorophore tag to the 3' end of the molecule
- B) It cleaves the DNA backbone and methylates adjacent purines
- C) It amplifies the target sequence and removes the primer
- D) It converts double-stranded DNA into single-stranded cDNA

3. Why is sophisticated software required when analyzing four-dye automated sequencing data from a single lane?

- A) To remove background radioactive noise from the detector
- B) To compensate for different fluorophores affecting the electrophoretic mobility of DNA fragments to different extents
- C) To synthesize missing purine bases during the gel run
- D) To translate DNA codons directly into amino acid sequences

4. What alternative strategy is described for automated sequencing if only a single fluorophore dye is used?

- A) Running the four different chain-terminating reactions in separate gel lanes
- B) Treating the DNA fragments with piperidine prior to detection

- C) Substituting radioactive ^{32}P for fluorescence detection
- D) Exposing the gel directly to X-ray film

Answer Key

- **1. A** — Four spectrally distinct fluorophores allow all four chain-terminating reactions to be distinguished in a single electrophoresis lane.
- **2. A** — Incorporation of a dye-labelled ddNTP accomplishes two steps at once: terminating synthesis and attaching a fluorophore tag.
- **3. B** — Software corrects for mobility shifts caused by the different fluorophores to ensure bands are read in the exact order.
- **4. A** — When using a single fluorophore, the four chain-terminating reactions are electrophoresed in four separate lanes.

Lesson 100: Sequencing accuracy

1. In the sequence accuracy study by Wilson et al. (1992) analyzing mouse DNA, what was the average error frequency for raw sequence reads beyond 400 bp?

- A) 0.1%
- B) 1.0%
- C) 3.2%
- D) 10.5%

2. According to Wilson et al. (1992), what proportion of errors in raw sequence data was attributed to ambiguities in sequence reading?

- A) At least one-third
- B) Exactly one-tenth
- C) More than nine-tenths
- D) None

3. In raw sequences longer than 400 bp, where did most errors occur and in what specific form were they often present?

- A) At the 5' end as deleted adenine bases
- B) Late in the sequence, often as extra bases in a run of two or more of the same nucleotide
- C) Uniformly across all positions as inverted palindromes
- D) Exclusively in the vector polylinker region as single-stranded nicks

4. How was the final estimated error frequency reduced to less than 0.1% in the complete sequence analysis?

- A) By analyzing the complete sequence with an average 5.9-fold redundancy and mostly on both strands
- B) By re-sequencing all fragments using Maxam-Gilbert chemical cleavage
- C) By using high-voltage electroporation to eliminate secondary structures
- D) By discarding all sequence reads longer than 200 bp

Answer Key

- **1. C** — Raw sequences read beyond 400 bp contained an average error rate of 3.2% (compared to 2.8% for reads under 400 bp).

* **2. A** — At least one-third of the observed errors were caused by ambiguities in sequence reading.

* **3. B** — Most errors occurred late in reads beyond 400 bp and manifested as extra bases in homopolymeric runs of identical nucleotides.

* **4. A** — High coverage (5.9-fold average redundancy) and double-stranded analysis reduced the final overall error rate to under 0.1%.

Lesson 101: DNA sequencing databases

1. Which three major international database centers collect and maintain accumulated DNA sequence data worldwide?

- A) NCBI (USA), DDBJ (Japan), and EBI (UK)
- B) GenBank, Swiss-Prot, and EMBL
- C) BLAST, FASTA, and PubMed
- D) TIGR, PIR, and PDB

2. How frequently do the three main database groups exchange new and updated sequence entries among themselves?

- A) Hourly
- B) On a daily basis
- C) Weekly
- D) Monthly

3. How many specialized bacterial genome databases are explicitly noted in Topic 101?

- A) Three
- B) Five
- C) Seven
- D) Twelve

4. Of the specialized bacterial genome databases mentioned in Topic 101, how many are dedicated to *E. coli* and *B. subtilis*, respectively?

- A) Four for *E. coli* and two for *B. subtilis*
- B) Two for *E. coli* and four for *B. subtilis*
- C) Three for *E. coli* and three for *B. subtilis*
- D) One for *E. coli* and six for *B. subtilis*

Answer Key

* **1. A** — DNA sequence data are maintained in the NCBI (USA), DDBJ (Japan), and EBI (UK) databases.

* **2. B** — All new and updated database entries are exchanged between the three groups on a daily basis.

* **3. C** — Seven specialized databases exist for bacterial genomes.

* **4. A** — Topic 101 specifies that of the bacterial databases, four are for *E. coli* and two are for *B. subtilis*.

Lesson 102: SITE DIRECTED MUTAGENESIS

1. How do gene-manipulation mutagenesis methods fundamentally differ from traditional mutagenesis methods?

- A) Mutations are induced across the entire host genome randomly
- B) Mutations are restricted to the gene of interest or a small portion of it
- C) They require radiation exposure rather than chemical mutagens
- D) They function exclusively on single-stranded viral RNA

2. Which strategy is recommended in protein engineering when the goal is to improve an operating parameter but the specific required amino acid changes are unknown?

- A) Single-primer extension
- B) Random mutagenesis strategy
- C) Cassette mutagenesis using decameric linkers
- D) Restriction digestion with EcoRI

3. What term is used for proteins generated by combining functional domains derived from different proteins?

- A) Cryptic proteins
- B) Chimeric proteins
- C) Fusion lipoplexes
- D) Auxiliary proteases

4. Which three distinct methods of site-directed mutagenesis are listed in Topic 102?

- A) Maxam-Gilbert, Sanger dideoxy, and automated four-dye sequencing
- B) Cassette mutagenesis, primer extension, and procedures based on the PCR
- C) Alkaline lysis, CsCl density gradient, and gel filtration
- D) Southern blotting, Northern blotting, and Western blotting

Answer Key

* **1. B** — Gene manipulation methods differ by restricting mutations specifically to the gene of interest or a small part of it.

* **2. B** — A random mutagenesis strategy provides a route when the required amino acid changes are unknown.

* **3. B** — Genetic engineering allows the generation of chimeric proteins where each domain comes from a different protein.

* **4. B** — Topic 102 explicitly names cassette mutagenesis, primer extension, and PCR-based procedures as the three methods.

Lesson 103: Cassette mutagenesis**1. How is cassette mutagenesis carried out on a target gene?**

- A) By exposing single-stranded RNA to chemical mutagens
- B) By using a synthetic DNA fragment containing the desired mutant sequence to replace the corresponding wild-type sequence
- C) By performing thermal cycling with degenerate primers
- D) By tailing the 3' end with terminal deoxynucleotidyltransferase

2. Which enzyme was originally used as a model to generate improved variants using cassette mutagenesis?

- A) Subtilisin
- B) Beta-galactosidase
- C) Taq polymerase
- D) Lysozyme

3. What efficiency of mutagenesis is achieved with cassette mutagenesis?

- A) Around 10%
- B) Close to 50%
- C) Close to 100%
- D) Variable between 1% and 5%

4. What is a major requirement and limitation of cassette mutagenesis mentioned in Topic 103?

- A) Requirement for single-stranded cDNA and limitation of high reaction temperatures
- B) Requirement for unique restriction sites flanking the region of interest and limitation on the number of synthetic oligonucleotides
- C) Requirement for ATP and S-adenosylmethionine cofactors and limitation to Gram-negative hosts
- D) Requirement for radioactive labelling with tritium and limitation to small plasmids

Answer Key

* **1. B** — Cassette mutagenesis replaces a section of the wild-type gene with a synthetic DNA fragment carrying the mutant sequence.

* **2. A** — The method was originally used to generate improved variants of the enzyme subtilisin.

* **3. C** — Cassette mutagenesis is a simple method with an efficiency close to 100%.

* **4. B** — Disadvantages include needing unique flanking restriction sites and limitations on the number of oligonucleotides that can be synthesized.

Lesson 104: Primer extension: the single primer method

1. What length range is specified in Topic 104 for the chemically synthesized oligonucleotide used in single-primer extension mutagenesis?

- A) 3 to 5 bp
- B) 7 to 20 bp
- C) 50 to 100 bp
- D) 200 to 500 bp

2. Which vector system is highlighted as making it easy to obtain the single-stranded template DNA required for single-primer extension?

- A) pBR322
- B) M13-based vectors
- C) Charon 4A
- D) pUC19

3. What type of DNA molecule is initially formed when the synthetic oligonucleotide primes DNA synthesis on the single-stranded template?

- A) A homoduplex molecule
- B) A heteroduplex molecule
- C) A triple-stranded RNA loop
- D) A linear protein-bound dimer

4. How can mutant clones be detected and selected from wild-type clones after host transformation if the mutation frequency is low?

- A) By blue/white screening on X-gal plates
- B) By nucleic acid hybridization screening using a ^{32}P -labelled oligonucleotide probe under suitable stringency
- C) By measuring resistance to chloramphenicol
- D) By subjecting the lysate to CsCl density gradient centrifugation

Answer Key

* **1. B** — The method uses a chemically synthesized oligonucleotide of 7 to 20 bp bearing a base mismatch.

* **2. B** — Single-stranded DNA is required, and cloning the gene in M13-based vectors makes obtaining it easy.

* **3. B** — The synthetic oligonucleotide primes synthesis and is incorporated into a resulting heteroduplex molecule.

* **4. B** — Mutants are picked out by hybridization with a ^{32}P -labelled oligonucleotide probe under stringency conditions where only mutants give a signal.

Lesson 105: PCR method for site directed mutagenesis

1. How many primers and PCR reactions are required by the method of Higuchi et al. (1988) to introduce a mutation anywhere along a DNA fragment?

- A) Two primers and one PCR
- B) Three primers and two PCRs
- C) Four primers and three PCRs
- D) Six primers and four PCRs

2. In the method of Higuchi et al. (1988), why is one of the two hybrid molecules effectively lost from the reaction mixture?

- A) It is degraded by T4 DNA ligase
- B) It has recessed 5' ends and is not a substrate for DNA polymerase
- C) It contains a poly(A) tail that precipitates in ethanol
- D) It binds irreversibly to the reaction tube wall

3. What term is used in the simpler PCR mutagenesis method by Sarkar and Sommer (1990) for the product of the first PCR when used in the second PCR?

- A) Linker
- B) Adaptor
- C) Megaprimer
- D) Stuffer

4. What efficiency is achieved for obtaining the desired mutation using PCR-based mutagenic protocols?

- A) 25%
- B) 50%
- C) 75%
- D) 100%

Answer Key

- * **1. C** — The method of Higuchi et al. (1988) requires four primers and three PCRs.
- * **2. B** — One hybrid has recessed 5' ends and, not being a substrate for polymerase, is effectively lost.
- * **3. C** — Sarkar and Sommer (1990) described a method where the product of the first PCR acts as a megaprimer for the second PCR.
- * **4. D** — PCR-based mutagenic protocols offer the advantage of obtaining the desired mutation with 100% efficiency.

Lesson 106: Basic PCR reaction**1. Who developed the Polymerase Chain Reaction (PCR) in 1983?**

- A) Kary Mullis
- B) Frederick Sanger
- C) Maxam and Gilbert
- D) Alec Jeffreys

2. What process does PCR rely on to achieve DNA melting and enzymatic replication?

- A) Isopycnic centrifugation
- B) Thermal cycling
- C) High-voltage electroporation
- D) Alkaline hydrolysis

3. What short DNA fragments serve as key components to enable selective amplification by providing sequences complementary to the target region?

- A) Promoters
- B) Primers
- C) Linkers
- D) Adaptors

4. How is the target DNA amplified as PCR progresses through cycles?

- A) Linearly
- B) Exponentially
- C) Logarithmically in a 1:1 ratio
- D) Parabolically at a fixed rate of 10 copies per cycle

Answer Key

- * **1. A** — PCR was developed by Kary Mullis in 1983.
- * **2. B** — The method relies on thermal cycling, consisting of repeated cycles of heating and cooling for DNA melting and enzymatic replication.

* **3. B** — Primers are short DNA fragments complementary to the target region that enable selective amplification.

* **4. B** — The DNA generated serves as a template for subsequent cycles, setting in motion an exponential amplification chain reaction.

Lesson 107: PCR principles and procedure

1. To which ends of the target DNA strands are the two PCR primers complementary?

- A) The 5' ends of each strand
- B) The 3' ends of each strand
- C) Internal palindromic sites only
- D) The poly(A) tail of both strands

2. Why is *Taq* polymerase specifically used in the basic PCR reaction mixture?

- A) Because it contains a 3'-5' proofreading exonuclease
- B) Because it is a heat-resistant enzyme that can withstand high-temperature denaturation
- C) Because it attaches to single-stranded DNA without needing primers
- D) Because it functions at 0°C on ice

3. What components serve as the building blocks from which DNA polymerase synthesizes a new DNA strand?

- A) Deoxynucleoside triphosphates (dNTPs)
- B) Dideoxynucleoside triphosphates (ddNTPs)
- C) Ribonucleoside monophosphates (rNMPs)
- D) Short RNA oligonucleotides

4. Which cations are listed in Topic 107 as essential bivalent and monovalent cation components of the PCR buffer?

- A) Sodium (bivalent) and Calcium (monovalent)
- B) Magnesium or manganese (bivalent) and potassium (monovalent)
- C) Zinc (bivalent) and Lithium (monovalent)
- D) Iron (bivalent) and Ammonium (monovalent)

Answer Key

* **1. B** — The two primers are complementary to the 3' ends of each strand of the DNA target.

* **2. B** — *Taq* polymerase is heat-resistant and can withstand the high temperatures of the denaturation step.

* **3. A** — Deoxynucleoside triphosphates (dNTPs) are the building blocks used by DNA polymerase to synthesize new DNA.

* **4. B** — The reaction requires bivalent cations (magnesium or manganese ions) and a monovalent cation (potassium ions).

Lesson 108: DNA polymerases

1. Which DNA polymerase was used in the original description of the PCR method, requiring fresh enzyme to be added during each cycle?

- A) *Taq* DNA polymerase
- B) Klenow DNA polymerase
- C) T7 DNA polymerase
- D) Reverse transcriptase

2. From which thermophilic organism was *Taq* DNA polymerase isolated?

- A) *Thermus aquaticus*
- B) *Escherichia coli*
- C) *Bacillus subtilis*
- D) *Thermotoga maritima*

3. How did the heat resistance of *Taq* DNA polymerase impact the practical execution of PCR?

- A) It required manual addition of enzyme after every denaturation step
- B) It allowed PCR to be easily automated using a heating block with a thermal cycling program
- C) It restricted amplification to temperatures below 37°C
- D) It completely eliminated the need for buffer solutions and dNTPs

4. Which enzymatic activity is absent in *Taq* DNA polymerase, contributing to potential misincorporation errors during amplification?

- A) 5' to 3' polymerase activity
- B) 3' to 5' proofreading exonuclease activity
- C) 5' to 3' ligase activity
- D) Terminal transferase activity

Answer Key

* **1. B** — In the original description of PCR, Klenow DNA polymerase was used and fresh enzyme had to be added each cycle due to heat denaturation.

* **2. A** — *Taq* DNA polymerase was introduced from the thermophilic bacterium *Thermus aquaticus*.

* **3. B** — Employing a heat-resistant enzyme enabled simple automation of PCR using thermal cycling heating blocks.

* **4. B** — *Taq* DNA polymerase lacks 3' to 5' proofreading exonuclease activity, which can lead to misincorporation errors.

Lesson 109: Primers

1. What length range is recommended for effective PCR primers according to Topic 109?

- A) 5 to 10 bp
- B) 17 to 30 bp
- C) 50 to 100 bp
- D) 150 to 200 bp

2. What ideal GC content is specified for PCR primer design?

- A) About 20%

- B) About 50%
- C) About 80%
- D) Exactly 100%

3. What design strategy is recommended when designing a primer with a low GC content to prevent a low melting temperature?

- A) Choosing a shorter primer
- B) Choosing a longer primer
- C) Adding extra dideoxynucleotides
- D) Using single-stranded RNA templates

4. Which of the following features should be avoided when selecting effective PCR primers?

- A) A GC content of 50%
- B) Complementarity between the two primers
- C) Primer lengths between 17 and 30 bp
- D) Equal melting temperatures between primers

Answer Key

- * **1. B** — Topic 109 states that primers should be 17 to 30 bp in length.
- * **2. B** — A GC content of about 50% is ideal for effective PCR primers.
- * **3. B** — With low GC content, it is desirable to choose a longer primer to avoid a low melting temperature.
- * **4. B** — There should be no complementarity between the two primers to prevent primer-dimer formation.

Lesson 110: Degenerate primers

1. What is a degenerate primer as defined in Topic 110?

- A) A primer containing radioactive isotopes at the 5' terminus
- B) A mixture of primers of similar sequence with variations at one or more positions
- C) A single-stranded RNA primer that binds to poly(A) tails
- D) A primer methylated at all palindromic restriction target sites

2. What common situation requires the use of degenerate primers when designing PCR experiments?

- A) When target sequences must be deduced from amino acid sequences
- B) When using high concentrations of ethidium bromide
- C) When amplifying circular plasmid vectors with EcoRI
- D) When digesting template DNA with S1 nuclease

3. Besides deducing primers from amino acid sequences, what is another application of degenerate primers mentioned in Topic 110?

- A) Removing 5' phosphate groups from linearized vectors
- B) Searching for novel members of a known family of genes
- C) Inhibiting host cell RNA polymerase activity
- D) Inducing lytic cycles in bacteriophage lambda

4. What selection strategy is recommended when designing degenerate primers based on amino acid sequences?

- A) Selecting amino acids with high codon degeneracy
- B) Selecting amino acids with low codon degeneracy
- C) Using only synthetic decameric linkers
- D) Selecting long runs of a single nucleotide

Answer Key

* **1. B** — A degenerate primer is a mixture of primers, all of similar sequence but with variations at one or more positions.

* **2. A** — Degenerate primers are commonly required when primer sequences must be deduced from amino acid sequences.

* **3. B** — Degenerate primers may also be employed to search for novel members of a known family of genes.

* **4. B** — Selecting amino acids with low codon degeneracy is desirable when considering genetic code degeneracy for primer design.

Lesson 111: Types of PCR

1. Which variant of PCR simultaneously amplifies several DNA sequences, usually exonic sequences?

- A) Real time PCR
- B) Multiplex PCR
- C) Inverse PCR
- D) Nested PCR

2. How does Nested PCR increase the specificity of the amplified product?

- A) By utilizing reverse transcriptase prior to thermal cycling
- B) By using a second set of primers that hybridize within the amplified fragment from the first PCR
- C) By adding ethidium bromide to quantify product accumulation in real time
- D) By circularizing fragments with T4 DNA ligase

3. Which PCR variant allows an approximation of the relative amount of nucleic acids present in a sample?

- A) Inverse PCR
- B) Semiquantitative PCR
- C) Multiplex PCR
- D) RAPD-PCR

4. Which technique performs absolute or relative quantification of nucleic acid copies obtained by PCR?

- A) RT-PCR
- B) Real time PCR
- C) Multiplex PCR
- D) Inverse PCR

Answer Key

- * **1. B** — Multiplex PCR simultaneously amplifies several DNA sequences, usually exonic sequences.
- * **2. B** — Nested PCR uses a second set of primers that hybridize within the fragment amplified in the first PCR to increase specificity.
- * **3. B** — Semiquantitative PCR allows an approximation of the relative amount of nucleic acids in a sample.
- * **4. B** — Real time PCR performs absolute or relative quantification of nucleic acid copies obtained by PCR.
-
-

Lesson 112: Competitor RT-PCR

1. What primer is often used to initiate first-strand cDNA synthesis from mRNA during RT-PCR?

- A) Degenerate hexamer
- B) Oligo(dT) primer
- C) Unphosphorylated BamHI adaptor
- D) Decameric linker

2. In competitor RT-PCR, how is the amount of target mRNA in the original sample estimated?

- A) By measuring the rate of migration in SDS-PAGE gel electrophoresis
- B) By adding a known amount of competitor RNA and comparing target and competitor product band intensities on a gel
- C) By measuring the absorbance of the solution at 260 nm
- D) By digesting the reaction mixture with S1 nuclease

3. What condition indicates that the amount of target sequence in the original sample equals the amount of competitor RNA added?

- A) When the target band is twice as intense as the competitor band
- B) When the target and competitor bands are of equal intensity
- C) When the competitor band completely disappears
- D) When no amplification product is formed in the first cycle

4. Why might the first few cycles of RT-PCR be biased?

- A) In favor of copying the single-stranded cDNA product until enough second-strand copies are generated
- B) In favor of degrading the dNTP building blocks
- C) In favor of methylating adenine bases in the target
- D) In favor of circularizing the template with DNA ligase

Answer Key

- * **1. B** — First-strand cDNA synthesis from mRNA in RT-PCR is commonly initiated using an oligo(dT) primer.
- * **2. B** — Competitor RT-PCR estimates target mRNA amounts by adding known amounts of competitor RNA and comparing band intensities on a gel.
- * **3. B** — Equal intensity between target and competitor bands on a gel indicates equal initial amounts of target and competitor RNA.
- * **4. A** — The early cycles of RT-PCR may be biased toward copying the single-stranded cDNA until sufficient second-strand copies accumulate.

Lesson 113: Real time quantitative PCR (qPCR)

1. What is the principal drawback of using ethidium bromide to quantify PCR products as they accumulate?

- A) Ethidium bromide inhibits Taq polymerase activity completely
- B) Both specific and non-specific products generate a fluorescence signal
- C) Ethidium bromide binds exclusively to single-stranded RNA
- D) Ethidium bromide requires radioactive ^{32}P labelling

2. How are oligonucleotide probes structured in probe-based real-time qPCR methods?

- A) With an oligo-dC tail at the 5' end and an oligo-dG tail at the 3' end
- B) With a reporter fluorescent dye attached at the 5' end and a quencher dye at the 3' end
- C) With a terminal protein cap at both ends
- D) With a radiolabelled ^{32}P group at the 3' end

3. Which enzymatic activity of Taq polymerase cleaves the probe during primer extension to separate the reporter dye from the quencher?

- A) 3' to 5' proofreading exonuclease activity
- B) 5' nuclease activity
- C) Terminal transferase activity
- D) Reverse transcriptase activity

4. What causes the increase in reporter-dye signal intensity during each cycle of probe-based qPCR?

- A) The binding of ethidium bromide to single-stranded primers
- B) The separation of the reporter dye from the quencher dye upon enzymatic cleavage of the probe
- C) The incorporation of ^{35}S -dNTPs into the template
- D) The phosphorylation of 5' ends by polynucleotide kinase

Answer Key

* **1. B** — Ethidium bromide fluoresces upon binding all double-stranded DNA, so both specific and non-specific products generate a signal.

* **2. B** — Probes in qPCR carry a reporter fluorescent dye at the 5' end and a quencher dye at the 3' end.

* **3. B** — The 5' nuclease activity of Taq polymerase cleaves the annealed probe during primer extension.

* **4. B** — Cleavage of the probe physically separates the reporter dye from the quencher dye, resulting in increased fluorescence signal.

Lesson 114: Nested PCR

1. Why does Nested PCR involve a double process of amplification using two sets of primers?

- A) To convert double-stranded DNA into single-stranded RNA
- B) To increase sensitivity when small amounts of target are present
- C) To attach homopolymeric tails to 3'-OH termini

D) To induce site-directed mutagenesis at restriction target sites

2. Where do the primers used in the second PCR of Nested PCR anneal?

- A) To the vector origin of replication
- B) To an internal sequence within the product amplified in the first PCR
- C) To the poly(A) tail of the host mRNA
- D) To the 5' cap of the primary transcript

3. What is stated as a primary disadvantage of Nested PCR?

- A) Complete lack of Taq polymerase processivity
- B) High probability of contamination during transfer from the first amplified product to the second reaction tube
- C) Requirement for high-voltage electroporation
- D) Inability to amplify fragments longer than 100 bp

4. How can contamination in Nested PCR be controlled according to Topic 114?

- A) By adding ethidium bromide to the master mix
- B) By using primers designed to anneal at different temperatures
- C) By treating the sample with alkaline phosphatase
- D) By performing the reaction at 0°C on ice

Answer Key

* **1. B** — Nested PCR uses two sequential rounds of amplification to detect very small amounts of target DNA with increased sensitivity.

* **2. B** — Primers in the second round of Nested PCR anneal to an internal sequence within the first PCR product.

* **3. B** — The main disadvantage of Nested PCR is the risk of contamination when transferring the first PCR product to the second reaction tube.

* **4. B** — Contamination risk in Nested PCR can be controlled by using primers engineered to anneal at different temperatures.

Lesson 115: Inverse PCR

1. What problem does Inverse PCR (IPCR) solve in molecular biology?

- A) Amplifying a target sequence that lies outside a known stretch of DNA sequence
- B) Removing 5' terminal phosphate groups from linearized plasmid vectors
- C) Synthesizing cDNA from ribosomal RNA without reverse transcriptase
- D) Preventing non-specific binding of ethidium bromide

2. What initial structural modification is performed on the restriction fragment in Inverse PCR before amplification?

- A) Tailing with terminal transferase
- B) Circularizing the fragment and cutting inside the known sequence to invert it
- C) Dephosphorylating with alkaline phosphatase
- D) Methylating with EcoRI methylase

3. How must primers be oriented in the original known sequence for Inverse PCR?

- A) Facing toward each other
- B) Facing away from each other (with respect to direction of product synthesis)
- C) Aligned in parallel 5' to 3' orientation on the same strand
- D) Bound exclusively to 3' homopolymer tails

4. What additional technique can Inverse PCR be combined with using sets of primers?

- A) Maxam-Gilbert chemical cleavage
- B) Nested PCR
- C) CsCl-ethidium bromide density gradient centrifugation
- D) Western blotting

Answer Key

- * **1. A** — Inverse PCR allows the amplification of unknown DNA sequences that lie outside (flanking) a known region.
- * **2. B** — IPCR circularizes a restriction fragment containing the known and flanking regions, then cuts within the known region to invert it.
- * **3. B** — Primers in IPCR must face away from each other in the original known sequence so that circularization places them in the proper orientation for amplification.
- * **4. B** — Inverse PCR can be combined with sets of primers designed for nested PCR.
-

Lesson 116: Multiplex PCR

1. What defines Multiplex PCR as an adaptation of the basic PCR method?

- A) It uses a single degenerate primer to amplify non-specific targets
- B) It allows simultaneous amplification of many sequences in a single tube using multiple sets of specific primers
- C) It relies on reverse transcriptase to copy RNA before thermal cycling
- D) It uses radioactive isotopes to quantify product accumulation in real time

2. Which of the following is NOT listed in Topic 116 as an application of Multiplex PCR?

- A) Detection of different pathogens in a single sample
- B) Identification of exonic and intronic sequences in specific genes
- C) Determination of gene dosage
- D) Sequencing of whole eukaryotic genomes without primers

3. What critical characteristic must primers possess when designing a Multiplex PCR assay?

- A) They must adhere to specific DNA sequences at similar temperatures
- B) They must contain oligo(dC) single-stranded overhangs
- C) They must bind exclusively to 3' poly(A) tails
- D) They must be labelled with ^{32}P at both 5' ends

4. Why might standardizing a Multiplex PCR procedure require several experimental trials?

- A) Because Taq polymerase is completely destroyed during the first cycle
- B) Because multiple primer sets must be balanced to ensure specific binding and prevent cross-reactivity at shared annealing temperatures
- C) Because it requires restriction digestion with EcoRI after every cycle
- D) Because it relies on RNA templates that denature at 0°C

Answer Key

- * **1. B** — Multiplex PCR simultaneously amplifies multiple target sequences in a single reaction tube using specific primer sets.
- * **2. D** — Multiplex PCR is used for pathogen detection, gene dosage, and identifying exonic/intronic sequences, not whole-genome sequencing.
- * **3. A** — Primers in Multiplex PCR must be characterized by adherence to specific target sequences at similar temperatures.
- * **4. B** — Designing sets of specific primers that function effectively under similar annealing temperatures requires careful standardization trials.

Lesson 117: RAPD

1. What does the acronym RAPD stand for in molecular biology?

- A) Rapid Amplification of Protein Domains
- B) Random Amplification of Polymorphic DNA
- C) Restriction-Assisted Primer Degradation
- D) Reverse Alignment of Polynucleotide Duplexes

2. What alternative name is also used to refer to RAPD-PCR according to Topic 117?

- A) Real-time quantitative PCR (qPCR)
- B) Arbitrarily primed PCR (AP-PCR)
- C) Inverse PCR (IPCR)
- D) Nested PCR

3. How is the binding stringency managed during the early versus later cycles of a RAPD-PCR protocol?

- A) Stringency is kept at 100°C throughout all 40 cycles
- B) Single primers bind at low stringency initially, and stringency can be increased after a few cycles to amplify the best mismatches
- C) High stringency is used in early cycles, followed by alkaline hydrolysis
- D) Primers are digested with S1 nuclease after the second cycle

4. What is cited in Topic 117 as a major limitation or challenge associated with RAPD-PCR?

- A) Inability to amplify double-stranded DNA
- B) Poor reproducibility due to mismatched binding of primers at low stringency
- C) Extreme cost of fluorescent reporter dyes
- D) Requirement for high-voltage electroporation equipment

Answer Key

- * **1. B** — RAPD stands for Random Amplification of Polymorphic DNA.
- * **2. B** — RAPD-PCR is also known as arbitrarily primed PCR (AP-PCR).
- * **3. B** — Low-stringency annealing initially allows primers to bind multiple sites, and increasing stringency later selects the best mismatches for full amplification.
- * **4. B** — Mismatched binding at low stringency makes it difficult to achieve consistent levels of primer binding, leading to reproducibility problems.

Lesson 118: RFLP

1. What does Restriction Fragment Length Polymorphism (RFLP) exploit to detect genetic differences?

- A) Variations in homologous DNA sequences that alter the locations of restriction enzyme target sites
- B) Differences in the length of 3' poly(A) tails on mRNA
- C) Variations in the temperature required for Taq polymerase activation
- D) The rate of RNA degradation by proteinase K

2. How are DNA fragments separated in RFLP analysis after digestion with restriction endonucleases?

- A) By CsCl density gradient centrifugation
- B) According to their lengths by gel electrophoresis
- C) By paper chromatography
- D) By affinity column chromatography

3. What status does RFLP analysis hold in historical context according to Topic 118?

- A) It was the first DNA profiling technique inexpensive enough to see widespread application
- B) It was developed after next-generation sequencing
- C) It cannot be performed on eukaryotic DNA
- D) It relies exclusively on fluorescently tagged ddNTPs

4. Which of the following is listed as a primary application of RFLP analysis before it was largely superseded by sequencing?

- A) Genome mapping, localization of genes for genetic disorders, disease risk determination, and paternity testing
- B) Direct translation of mRNA in cell-free systems
- C) Synthesis of synthetic decameric linkers
- D) Removal of 5' terminal phosphate groups

Answer Key

* **1. A** — RFLP refers to differences between homologous DNA samples caused by variations in restriction site locations.

* **2. B** — After restriction enzyme digestion, RFLP fragments are separated according to length using gel electrophoresis.

* **3. A** — RFLP analysis was the first DNA profiling technique inexpensive enough to achieve widespread practical application.

* **4. A** — RFLP was an important tool in genome mapping, gene localization for genetic disorders, assessing disease risk, and paternity testing.

Lesson 119: AFLP-PCR

1. What primary genetic variations give rise to Amplified Fragment Length Polymorphisms (AFLPs)?

- A) Variations in single-stranded RNA poly(A) tails
- B) Differences in restriction fragment lengths caused by SNPs or INDELs
- C) Changes in the concentration of bivalent magnesium ions
- D) Incomplete methylation by EcoRI methylase

2. What initial step is performed on genomic DNA in the AFLP procedure before PCR amplification?

- A) Digestion with restriction enzymes followed by ligation of adaptors to the sticky ends
- B) Alkaline denaturation at pH 12.5
- C) Tailing with terminal transferase using dGTP
- D) Microinjection into xenopus oocyte nuclei

3. How is the selective subset of restriction fragments targeted for amplification during AFLP-PCR?

- A) By using primers complementary to the adaptor sequence, the restriction site sequence, and a few nucleotides inside the restriction site fragments
- B) By adding ethidium bromide to inhibit non-specific fragments
- C) By digesting non-target fragments with S1 nuclease
- D) By selecting fragments that contain 25-bp direct repeats exclusively

4. On what gel medium are amplified AFLP fragments separated and visualized?

- A) Low-melting agarose tube gels
- B) Denaturing polyacrylamide gels, visualized via autoradiography or fluorescence
- C) Sucrose gradient sucrose beds
- D) Paper chromatography sheets

Answer Key

* **1. B** — AFLPs represent differences in restriction fragment lengths caused by single nucleotide polymorphisms (SNPs) or insertions/deletions (INDELs).

* **2. A** — Genomic DNA is first digested with restriction enzymes and then ligated to adaptors at the sticky ends.

* **3. A** — Selective amplification uses primers extending a few nucleotides beyond the adaptor and restriction site sequence into the fragment.

* **4. B** — Amplified AFLP fragments are separated and visualized on denaturing polyacrylamide gels using autoradiography or fluorescence.

Lesson 120: Applications of PCR

1. Over what timeframe have molecular techniques like PCR undergone rapid development and widespread implementation?

- A) The past 5 years
- B) During the past 30 years
- C) Over the past 150 years
- D) Since the 19th century

2. Which role of PCR is specifically highlighted regarding public health impact in Topic 120?

- A) Providing clues for research into the genetics and biochemistry of antimicrobial resistance
- B) Eliminating all bacterial infections without drugs
- C) Synthesizing artificial blood cells in vitro
- D) Crystallizing viral capsids for X-ray diffraction

3. How have PCR and its variants contributed to the study of human, animal, and plant biology?

- A) By enabling identification/characterization of organisms and understanding the physiopathology of diverse diseases
- B) By eliminating the need for nucleic acid extraction
- C) By replacing all light and electron microscopy
- D) By converting all RNA viruses into DNA autotrophs

4. Which three major applied fields are explicitly named in Topic 120 as benefiting from PCR applications?

- A) Human medicine, forensic sciences, and agricultural science and environment
- B) Metallurgy, geology, and astronomy
- C) Petroleum engineering, textiles, and architecture
- D) Organic synthesis, glass manufacturing, and cosmetics

Answer Key

* **1. B** — Topic 120 notes that molecular techniques have been under rapid development during the past 30 years.

* **2. A** — PCR has provided key insights into public health topics such as the genetics and biochemistry of antimicrobial resistance.

* **3. A** — PCR has contributed to identifying and characterizing organisms and elucidating disease physiopathology across humans, animals, and plants.

* **4. A** — Topic 120 explicitly lists human medicine, forensic sciences, and agricultural science and environment as key application fields.

Lesson 121: PCR-Medicine**1. How has molecular technology in clinical microbiology directly benefitted patient care according to Topic 121?**

- A) By allowing the chemical synthesis of synthetic red blood cells
- B) By enabling the identification of microorganisms that are difficult to culture or dangerous to laboratory personnel
- C) By eliminating the need for antibiotic treatment in bacterial infections
- D) By completely replacing traditional blood transfusions with recombinant protein solutions

2. What role does PCR play in blood banks as described in Topic 121?

- A) It synthesizes hemoglobin in vitro
- B) It is used in the selection and quality assurance of blood for patients
- C) It destroys white blood cells to prevent host-versus-graft disease
- D) It converts type O blood into universal donor plasma

3. Which virus is specifically mentioned in Topic 121 as being identified and characterized through rapid molecular diagnosis to allow early treatment?

- A) Rabies virus
- B) Influenza virus
- C) Hepatitis C virus
- D) Dengue virus

4. What is the major overall benefit generated by PCR in medicine as outlined in Topic 121?

- A) It generates advancements across all fields of medicine and transforms clinical microbiology
- B) It prevents the occurrence of all genetic mutations in human offspring
- C) It replaces all surgical interventions with gene therapy
- D) It allows the direct sequencing of whole organ tissues without DNA extraction

Answer Key

* **1. B** — Molecular technology allows identification of microorganisms that are difficult to culture or dangerous for laboratory personnel.

* **2. B** — PCR is utilized for the selection and quality assurance of blood used in blood banks.

* **3. B** — Molecular diagnosis of influenza allows early treatment and control of this high-impact infection.

* **4. A** — Molecular techniques, especially PCR, have transformed clinical microbiology and driven advances in medical science.

Lesson 122: PCR-Forensic sciences

1. What limitation of classic morphology in forensic pathology does molecular biology help overcome?

- A) Morphology cannot determine the physical weight of organs
- B) Morphology cannot detect systemic changes involved in the pathophysiological process of death
- C) Morphology is unable to examine bone fractures
- D) Morphology requires DNA sequencing prior to tissue sectioning

2. Why has practical application of RNA analysis historically been challenging in post-mortem research?

- A) RNA is resistant to all enzymes
- B) RNA undergoes rapid decomposition after death
- C) RNA cannot be amplified by PCR under any conditions
- D) RNA is present only in living plant cells

3. What term is used for the application of relative quantification of RNA transcripts (via qPCR and RT-PCR) to research deaths in molecular pathology?

- A) Molecular autopsy
- B) Karyotype analysis
- C) Positional cloning
- D) Western blotting

4. In addition to investigating deaths, how are molecular methods used in forensic science regarding individuals?

- A) To predict exact future lifespan
- B) To establish filiations of a person (paternity testing) or analyze minimal tissue debris

- C) To synthesize artificial fingerprint patterns
- D) To alter chromosomal numbers in skin samples

Answer Key

- * **1. B** — Molecular biology helps research systemic changes in death that cannot be detected by classic morphology.
- * **2. B** — RNA analysis was limited in post-mortem research due to the rapid decomposition of RNA after death.
- * **3. A** — Applying relative quantification of RNA transcripts to research deaths is termed molecular autopsy.
- * **4. B** — Molecular methods establish filiations (paternity testing) and obtain evidence from minimal samples of saliva, semen, or tissue debris.

Lesson 123: PCR-Agricultural sciences and environment

1. What is highlighted in Topic 123 as one of the most important contributions of molecular techniques like PCR in agricultural sciences?

- A) Synthesizing artificial soil minerals
- B) Identification and characterization of multiple infectious agents impacting human and animal health
- C) Eliminating the need for photosynthesis in crops
- D) Increasing the physical size of domestic livestock ten-fold

2. How has knowing the genome of domestic animals and major infectious agents aided animal health research?

- A) It eliminates all viral infections automatically
- B) It facilitates the study of mutations associated with disease
- C) It allows animals to synthesize human growth hormone naturally
- D) It prevents any environmental pathogens from entering farms

3. Which techniques are specifically mentioned in Topic 123 as facilitating pathogen detection in plants, animals, and the environment?

- A) Maxam-Gilbert sequencing and paper chromatography
- B) Conventional PCR or qPCR
- C) Southern blotting and sucrose gradient centrifugation
- D) Microinjection and electroporation

4. What additional outcome is achieved by applying molecular techniques in agricultural and environmental research besides pathogen detection?

- A) Understanding pathogen epidemiology and developing new diagnostic tests, treatments, or vaccines
- B) Converting all dicot plants into monocots
- C) Completely replacing animal feeds with nitrogen gas
- D) Removing all cell walls from plant tissues permanently

Answer Key

- * **1. B** — A major contribution is identifying and characterizing infectious agents that affect human and animal health.

* **2. B** — Genomic knowledge of domestic animals and pathogens facilitates studying disease-associated mutations.

* **3. B** — Conventional PCR or qPCR facilitates detection of pathogens in plants, animals, and the environment.

* **4. A** — Molecular tools aid in understanding epidemiology and developing diagnostic tests, treatments, and vaccines.

Lesson 124: PCR-Molecular Paleontology

1. What is the definition of molecular paleontology according to Topic 124?

- A) The study of living bacterial cell walls under electron microscopy
- B) The recovery and analysis of DNA and protein from ancient human, animal, and plant remains
- C) The chemical synthesis of new mineral fossils
- D) The carbon-dating of inorganic rock formations

2. What major benefit does molecular paleontology provide when studying evolutionary relationships?

- A) It allows evolutionary questions to be pursued on a genetic level rather than relying on phenotypic variation alone
- B) It allows extinct organisms to be cloned and revived immediately
- C) It proves that ancient organisms lacked nucleic acids
- D) It eliminates the need for fossil discovery

3. What metric can be quantified by applying molecular analytical techniques to DNA in fossils?

- A) The exact atmospheric temperature at the time of fossilization
- B) The level of relatedness between any two organisms for which DNA has been recovered
- C) The amount of sunlight absorbed by ancient leaves
- D) The exact weight of the living organism millions of years ago

4. Which set of biotechnological techniques is explicitly listed in Topic 124 as enabling new insights into divergence and evolutionary history?

- A) Alkaline lysis, SDS-PAGE, and Western blotting
- B) DNA isolation, amplification, and sequencing
- C) Homopolymer tailing, ligation, and transformation
- D) Restriction digestion, sonication, and colony lift

Answer Key

* **1. B** — Molecular paleontology is the recovery and analysis of DNA and protein from ancient human, animal, and plant remains.

* **2. A** — It allows scientists to investigate evolutionary questions on a genetic level rather than relying solely on phenotypic variation.

* **3. B** — DNA from fossils enables quantification of the level of relatedness between organisms.

* **4. B** — Insights into evolutionary history are gained using DNA isolation, amplification, and sequencing.

Lesson 125: Mapping and Sequencing Genome

1. What is the primary function of a genome map as described in Topic 125?

- A) To measure the total weight of chromosomal proteins
- B) To pinpoint the location of specific features on the chromosomes of an organism
- C) To destroy non-coding regions of DNA
- D) To synthesize artificial tRNA molecules

2. What term is used to collectively refer to the features located on a genome map?

- A) Promoters
- B) Markers
- C) Adaptors
- D) Vectors

3. Which technique is cited in Topic 125 as the ONLY method that allows the position of markers in the genome to be observed directly?

- A) Fluorescence in situ hybridization (FISH)
- B) Restriction fragment length polymorphism (RFLP)
- C) Polymerase chain reaction (PCR)
- D) Agarose gel electrophoresis

4. How do indirect methods infer the order and spacing of markers on a genome map?

- A) By measuring the rate of protein translation on ribosomes
- B) By isolating a portion of the genome (e.g., by cloning), identifying markers present, and analyzing overlapping samples
- C) By subjecting whole cells to high-voltage electroporation
- D) By digesting total cellular RNA with RNase H

Answer Key

* **1. B** — Genome maps define the relative positions of specific features (markers) on chromosomes.

* **2. B** — Features located on a genome map (including genes and noncoding sequences) are called markers.

* **3. A** — Fluorescence in situ hybridization (FISH) is the only method allowing direct observation of marker positions in the genome.

* **4. B** — Indirect mapping isolates genomic portions (cloning), identifies linked markers, and infers order from overlapping samples.

Lesson 126: Markers for genome mapping

1. What term is used for short, unique fragments of known DNA sequence that can be amplified and detected in a DNA sample using PCR?

- A) Sequence tagged sites (STSs)
- B) Expressed sequence tags (ESTs)
- C) Restriction fragment length polymorphisms (RFLPs)
- D) Single nucleotide polymorphisms (SNPs)

2. When a sequence tagged site (STS) originates specifically from a coding sequence, such as by sequencing a clone from a cDNA library, what is it called?

- A) An expressed sequence tag (EST)
- B) A microsatellite
- C) A restriction fragment length polymorphism (RFLP)
- D) A contig

3. What structural feature defines a microsatellite marker?

- A) A tandem repetition of a simple, short motif flanked by a unique sequence
- B) A 200–300 bp double-stranded plasmid vector
- C) A single-stranded RNA hairpin loop
- D) A 25-bp direct repeat border sequence

4. Which type of marker is mapped using genetic linkage mapping when an anonymous cloned fragment contains a restriction site whose location differs between alleles?

- A) Restriction fragment length polymorphism (RFLP)
- B) Expressed sequence tag (EST)
- C) YAC centromere
- D) T-DNA border sequence

Answer Key

- ***1. A** — Sequence tagged sites (STSs) are short, unique fragments of known DNA sequence amplified and detected via PCR.
- ***2. A** — An STS originating from a coding sequence is designated an expressed sequence tag (EST).
- ***3. A** — Microsatellites consist of tandem repetitions of a simple, short motif flanked by unique sequences.
- ***4. A** — RFLPs involve restriction sites whose locations differ from one allele to the next and can be mapped by genetic linkage mapping.

Lesson 127: Genetic linkage mapping

1. What do genetic linkage maps illustrate regarding genes or genetic markers on a chromosome?

- A) Their exact mass in kilodaltons
- B) The order of genes or genetic markers and the relative distances between them
- C) Their rate of transcription by RNA polymerase
- D) Their tertiary 3D protein conformation

2. What biological process during sexual reproduction breaks parental chromosomes at random, recombines fragments, and segregates shuffled chromosomes into gametes?

- A) Mitosis
- B) Meiosis
- C) Lipofection
- D) Electroporation

3. How do two markers that lie very close together on a chromosome typically behave during meiosis?

- A) They recombine 100% of the time
- B) They seldom recombine and usually cosegregate together

- C) They are degraded by host restriction endonucleases
- D) They segregate independently 100% of the time

4. What maximum cosegregation frequency is expected by chance for distant markers lying at opposite ends of a chromosome?

- A) 1%
- B) 10%
- C) 50%
- D) 100%

Answer Key

- * **1. B** — Genetic linkage maps illustrate the order of genes or markers on a chromosome and their relative distances.
- * **2. B** — Meiosis breaks, recombines, and segregates parental chromosomes into gametes during sexual reproduction.
- * **3. B** — Closely linked markers seldom recombine and usually cosegregate to the same gametes and offspring.
- * **4. C** — Distant markers recombine often and cosegregate no more than expected by chance, which is 50% of the time.

Lesson 128: Physical mapping

1. What term describes a contiguous series of cloned DNA fragments containing overlapping portions of the genome in physical mapping?

- A) Contig
- B) Intron
- C) Haptens
- D) Transposon

2. Which vectors are noted as carrying large inserts in excess of 100 kb for physical mapping of large genomes?

- A) pBR322 and pUC19
- B) P1 artificial chromosomes (PACs) and bacterial artificial chromosomes (BACs)
- C) Single-stranded M13 bacteriophages
- D) Charon 16 lambda replacement vectors

3. How are clones screened to identify all members containing a specific STS marker in STS content mapping?

- A) Either by PCR using appropriate primers or by probing arrayed membrane-bound clone DNA with labelled DNA
- B) By measuring resistance to ampicillin and tetracycline on X-gal plates
- C) By subjecting whole cells to sonication and high-voltage electroporation
- D) By sequencing single-stranded RNA templates with reverse transcriptase

4. What is measured during restriction fingerprinting to help construct a physical map?

- A) The exact amino acid sequence of translated proteins

- B) The sizes of restriction fragments resulting from digesting clone library members with restriction enzymes
- C) The level of ethidium bromide intercalation in supercoiled plasmid DNA
- D) The rate of bacterial cell division in liquid culture

Answer Key

- * **1. A** — A contig is a contiguous series of cloned DNA fragments with overlapping portions of the genome.
- * **2. B** — PACs and BACs can carry inserts in excess of 100 kb for physical mapping of large genomes.
- * **3. A** — STS content mapping identifies clones using PCR with specific primers or hybridization probing on arrayed membranes.
- * **4. B** — Restriction fingerprinting measures the sizes of fragments generated by restriction enzyme digestion of clone library members.

Lesson 129: Physical versus linkage maps

1. How were original genetic-linkage maps constructed before modern molecular techniques?

- A) By measuring base-pair distances using slab-gel electrophoresis
- B) By tracing the inheritance of multiple traits through several generations
- C) By automated four-dye fluorescent sequencing
- D) By performing restriction fingerprinting on BAC libraries

2. What biological process during meiosis enables genetic-linkage mapping by allowing chromosomes to exchange equivalent pieces of themselves?

- A) Crossing over
- B) Lipofection
- C) Homopolymer tailing
- D) Insertional inactivation

3. What distance measurement unit is uniquely provided by physical maps compared to genetic-linkage maps?

- A) Recombination frequency percentage
- B) Physical, DNA-base-pair distances
- C) Centimorgans per chromosome arm
- D) Number of meiotic breaks per cell division

4. What defines a sequence-tagged site (STS) used as a landmark in physical mapping?

- A) A unique DNA sequence a few hundred base pairs long found in only one place in the genome
- B) A single-stranded RNA primer that binds to poly(A) tails
- C) A 25-bp direct repeat flanking the T-DNA of Ti plasmids
- D) A 10-mer blunt-ended oligonucleotide carrying an EcoRI site

Answer Key

- * **1. B** — Original genetic-linkage maps were constructed by tracing trait inheritance across generations.
- * **2. A** — Crossing over during meiosis involves chromosome pairing and exchange of equivalent pieces.
- * **3. B** — Physical maps give physical, DNA-base-pair distances from one landmark to another.

* **4. A** — An STS is a unique DNA sequence a few hundred base pairs long present in only one place in the genome.

Lesson 130: Use of RFLPs in physical maps

1. Who were the first researchers to recognize in 1980 that DNA probes targeting RFLPs can be used for mapping?

- A) Botstein et al.
- B) Maxam and Gilbert
- C) Ish-Horowicz and Burke
- D) Chang and Cohen

2. What requirement must be met by RFLP loci and probes to be useful for constructing an RFLP map?

- A) They must be highly informative and very polymorphic
- B) They must be located exclusively on single-stranded cDNA
- C) They must contain EcoRI restriction sites every 10 base pairs
- D) They must be present in 100% of bacterial plasmids

3. Why is an RFLP where one allele exists in 99.9% of the population and another in 0.1% considered of little utility for mapping?

- A) Because it is unstable during gel electrophoresis
- B) Because it is seldom informative due to the extreme rarity of one allele
- C) Because it cannot be digested by restriction endonucleases
- D) Because it causes immediate host cell lysis

4. As a general rule, how many alleles with equivalent frequencies should RFLPs have to be effectively used in map construction?

- A) Exactly ten alleles
- B) Two, or perhaps three, alleles with equivalent frequencies
- C) Over 100 rare alleles
- D) Only one invariant allele

Answer Key

* **1. A** — Botstein et al. (1980) first recognized that DNA probes targeting RFLPs could be used for mapping.

* **2. A** — To generate an RFLP map, probes and loci must be highly informative and very polymorphic.

* **3. B** — A polymorphism with an allele frequency of 99.9% vs 0.1% is seldom informative.

* **4. B** — RFLPs used for map construction generally should have two, or perhaps three, alleles with equivalent frequencies.

Lesson 131: STS in physical maps

1. What is the typical length range of a sequence tagged site (STS) region in DNA?

- A) 10 to 50 bases

- B) 200 to 300 bases
- C) 1,000 to 2,000 bases
- D) 10,000 to 15,000 bases

2. Which vector is highlighted in Topic 131 for creating a chromosome-specific library to develop STS markers?

- A) Plasmid pBR322
- B) Phage M13
- C) Cosmid pJB8
- D) YAC vector

3. In developing STS markers, what length of sequence is generated from random M13 clones to identify unique regions?

- A) 10 to 20 bases
- B) 50 to 80 bases
- C) 200 to 400 bases
- D) 1,000 to 2,000 bases

4. What result on an electrophoretic gel indicates a functional STS marker after PCR amplification?

- A) A continuous smear of DNA fragments across all lanes
- B) A single band corresponding to the size of the target region
- C) Exactly four distinct bands representing four dideoxynucleotides
- D) No visible bands until stained with silver nitrate

Answer Key

* **1. B** — An STS is a short region of DNA about 200–300 bases long whose exact sequence is unique in the genome.

* **2. B** — A recommended method to develop STS markers is to create a chromosome-specific library in phage M13.

* **3. C** — Random M13 clones are selected and 200–400 bases are sequenced to identify unique regions for primer selection.

* **4. B** — A functional STS marker amplifies a single target region of the genome and produces a single band on a gel.

Lesson 132: SNPs as physical markers

1. In highly outbred populations like humans, what minimum frequency must the least abundant allele possess to be formally classified as a SNP?

- A) 0.01% or more
- B) 0.1% or more
- C) 1% or more
- D) 10% or more

2. What term is used for single base variants originating specifically in cDNAs?

- A) indels

- B) cSNPs
- C) STSs
- D) ESTs

3. What alternative name ("snip-SNPs") is given to the special subset of SNPs that alters sensitivity to cleavage by a restriction endonuclease?

- A) Expressed sequence tags (ESTs)
- B) Restriction fragment length polymorphisms (RFLPs)
- C) Randomly amplified polymorphic DNAs (RAPDs)
- D) Sequence-tagged sites (STSs)

4. Which of the following sequence markers is cited in Topic 132 as probably being the most important for physical mapping of genomes?

- A) Single nucleotide polymorphisms (SNPs)
- B) Polyadenylated tails
- C) Decameric linkers
- D) T-DNA border sequences

Answer Key

* **1. C** — In outbred populations like humans, polymorphisms are considered SNPs only if the least abundant allele has a frequency of 1% or more.

* **2. B** — Single base variants in cDNAs are referred to as cSNPs.

* **3. B** — SNPs that alter a restriction endonuclease cleavage site are known as restriction fragment length polymorphisms (RFLPs) or "snip-SNPs".

* **4. A** — SNPs are probably the most important sequence markers for physical mapping of genomes.

Lesson 133: Polymorphic DNA detection in the absence of sequence information

1. How long are the single arbitrary primers used to amplify genomic DNA for RAPD detection?

- A) 3 or 4 nucleotides
- B) 9 or 10 nucleotides
- C) 25 to 30 nucleotides
- D) 100 to 200 nucleotides

2. What GC composition constraint is specified for the single arbitrary primers used in RAPD analysis?

- A) 10 to 20% G + C
- B) 30 to 40% G + C
- C) Between 50 and 80% G + C
- D) Exactly 100% G + C

3. What structural feature must be absent from the arbitrary primers used in RAPD amplification?

- A) Purine bases
- B) Phosphodiester bonds

- C) Palindromes
- D) 3'-OH groups

4. Why are RAPDs widely used by plant molecular biologists to construct maps?

- A) Because they require complete genome sequence data prior to primer synthesis
- B) Because they provide very large numbers of markers and are very easy to detect by agarose gel electrophoresis
- C) Because they convert single-stranded RNA into double-stranded plasmids
- D) Because they rely exclusively on Southern blotting with radioactive probes

Answer Key

- * **1. B** — Arbitrary primers used in RAPD analysis are 9 or 10 nucleotides in length.
- * **2. C** — The primers are chosen within the constraint of being between 50 and 80% G + C composition.
- * **3. C** — Arbitrary primers for RAPD must contain no palindromes.
- * **4. B** — Plant molecular biologists widely use RAPDs because they yield large numbers of markers that are easily detected on agarose gels.

Lesson 134: AFLPs detection in the absence of sequence information

1. What technique is used to detect restriction fragments in AFLP, making it distinct from Southern blotting in RFLP?

- A) Isopycnic centrifugation
- B) PCR amplification
- C) Autoradiography of X-ray films only
- D) Paper chromatography

2. Why should AFLP not be used as an acronym according to Topic 134?

- A) Because it is a registered trademark of a commercial biotech kit
- B) Because the technique detects presence or absence of restriction fragments and not length differences
- C) Because it requires radioactive isotopes instead of fluorescence
- D) Because it functions only on eukaryotic RNA templates

3. Why has AFLP been widely adopted by plant geneticists while not proving useful in mapping animal genomes?

- A) Plant genomes lack restriction sites completely
- B) AFLP depends on high rates of substitutional variation in DNA, which causes RFLPs to be much more common in plants than animals
- C) Animal genomes cannot be digested by restriction endonucleases
- D) Plant cell walls prevent PCR amplification

4. What key operational advantage makes the AFLP approach particularly powerful for target genomes?

- A) It requires no previous sequence characterization of the target genome
- B) It eliminates the need for DNA ligase or adaptors
- C) It allows direct translation of proteins without mRNA

D) It converts double-stranded DNA into single-stranded RNA

Answer Key

* **1. B** — Unlike RFLP which uses Southern blotting, AFLP detects restriction fragments using PCR amplification.

* **2. B** — AFLP should not be used as an anagram because the method detects presence or absence of fragments, not length differences.

* **3. B** — AFLP relies on high rates of substitutional variation in DNA, making RFLPs much more common in plant genomes than in animal genomes.

* **4. A** — AFLP is particularly powerful because it requires no prior sequence characterization of the target genome.

Lesson 135: Fluorescence in situ hybridization (FISH)

1. In conventional FISH, why is a high proportion of cells required to be in metaphase?

- A) Because metaphase is the only stage where RNA polymerase is active
- B) Because chromosomes are condensed as distinct bodies at metaphase
- C) Because cell walls dissolve completely during metaphase
- D) Because metaphase cells lack nuclear membranes permanently

2. Which mitogen is used to culture lymphocytes or fibroblasts, and which agent blocks the cell cycle at metaphase for FISH?

- A) Phytohaemagglutinin (mitogen) and colcemid (blocking agent)
- B) Ampicillin (mitogen) and cyanogen bromide (blocking agent)
- C) Isopropyl- β -D-thiogalactoside (mitogen) and lysozyme (blocking agent)
- D) Sodium dodecyl sulphate (mitogen) and piperidine (blocking agent)

3. What is the effect of treating chromosomes with bromodeoxyuridine and ethidium bromide prior to FISH?

- A) It digests non-target DNA into 100 bp fragments
- B) It improves the morphology of the chromosomes for FISH
- C) It seals phosphodiester nicks in the chromatids
- D) It removes all hapten tags from the probe

4. How does multicolor FISH improve map accuracy over single-probe experiments?

- A) By allowing simultaneous hybridization and detection of two or more probes to determine their order and spacing
- B) By eliminating the need for denaturing chromosome DNA
- C) By converting fluorescent signals into radioactive decay counts
- D) By increasing the cell cycle speed past metaphase

Answer Key

* **1. B** — Metaphase is required because it is the cell cycle stage where chromosomes are condensed as distinct bodies.

* **2. A** — Cells are cultured with the mitogen phytohaemagglutinin and treated with colcemid to block the cell cycle at metaphase.

* **3. B** — Treatment with bromodeoxyuridine and ethidium bromide improves chromosome morphology for FISH.

* **4. A** — Multicolor FISH uses combinations of haptens and fluorophores to detect multiple probes simultaneously, determining their order and spacing.

Lesson 136: Radiation hybrid (RH) mapping

1. What agent is used in Radiation Hybrid (RH) mapping to break human chromosomes into fragments?

- A) X-rays
- B) Restriction endonucleases
- C) Sonication ultrasound
- D) Ultraviolet light

2. In RH mapping, in which host cells are the human chromosomal fragments recovered?

- A) Escherichia coli cells
- B) Rodent cells
- C) Yeast spheroplasts
- D) Plant protoplasts

3. What resolution range can be achieved with radiation hybrid mapping compared to 1–3 Mb in genetic mapping?

- A) 10–20 bp
- B) 100–500 kb
- C) 5–10 Mb
- D) 50–100 Mb

4. Which of the following is an advantage of RH mapping over conventional genetic (meiotic) mapping?

- A) It requires double-stranded RNA templates
- B) It can use monomorphic markers such as STSs
- C) It relies exclusively on polymorphic microsatellites
- D) It exhibits strong gender-specific recombination hotspots

Answer Key

* **1. A** — RH mapping uses a high dose of X-rays to break human chromosomes into fragments.

* **2. B** — Human chromosomal fragments are recovered in rodent cells using somatic cell hybrids.

* **3. B** — Radiation hybrid mapping achieves a higher resolution of 100–500 kb compared to 1–3 Mb in genetic mapping.

* **4. B** — Unlike meiotic mapping, RH mapping does not require polymorphic markers and can use monomorphic markers like STSs.

Lesson 137: Mapping mapping

1. Why does the presence of a host genome in rodent–human somatic cell hybrids restrict RH mapping?

- A) It precludes the use of generic markers such as AFLPs and RAPDs
- B) It destroys all Taq polymerase enzymes
- C) It causes immediate cell lysis under UV light
- D) It prevents PCR amplification of all STSs

2. How are genomic DNA fragments segregated in HAPPY mapping after random breakage by irradiation or shearing?

- A) By high-voltage capillary electrophoresis
- B) By diluting fragments to give aliquots containing one haploid genome equivalent
- C) By CsCl density gradient centrifugation with ethidium bromide
- D) By hybridization on nitrocellulose membrane overlays

3. What technique is used to detect markers in aliquots during HAPPY mapping?

- A) Southern blotting
- B) PCR
- C) Maxam-Gilbert sequencing
- D) Western blotting

4. Which plant genome is explicitly mentioned in Topic 137 as having been mapped using HAPPY mapping?

- A) Arabidopsis
- B) Oryza sativa
- C) Zea mays
- D) Nicotiana tabacum

Answer Key

* **1. A** — The host genome in RH hybrids precludes the use of generic markers like AFLPs and RAPDs.

* **2. B** — Fragments are segregated by diluting them to give aliquots containing one haploid genome equivalent.

* **3. B** — Markers in HAPPY mapping aliquots are detected using PCR.

* **4. A** — HAPPY mapping has been used to map the human genome, animal genomes, and the Arabidopsis genome.

Lesson 138: Map integration

1. Why is it essential to integrate different mapping methods according to Topic 138?

- A) Because each mapping method has its own strengths and weaknesses
- B) Because single mapping methods destroy DNA templates during analysis
- C) Because map integration converts physical maps directly into amino acid sequences
- D) Because restriction enzymes cannot function without integrated maps

2. What factors can greatly influence the methodologies employed in constructing a genome map?

- A) The concentration of IPTG and X-gal in the agar medium

- B) The size and complexity of the genome being analyzed
- C) The pH of the sodium acetate neutralization buffer
- D) The type of X-ray film used during autoradiography

3. In constructing the human cytogenetic map, what feature of the BAC clones provides a reference to radiation hybrid and linkage maps?

- A) Poly(A) tails
- B) STSs
- C) Decameric linkers
- D) T-DNA border sequences

4. What vectors carrying large inserts were used to construct the human cytogenetic map mentioned in Topic 138?

- A) BAC clones
- B) M13 single-stranded phages
- C) Charon 4A replacement vectors
- D) pUC19 plasmids

Answer Key

* **1. A** — Map integration is essential because each mapping method possesses its own strengths and weaknesses.

* **2. B** — Genome size and complexity greatly influence the specific methodologies chosen for map construction.

* **3. B** — BAC clones used for the cytogenetic map contain STSs that reference radiation hybrid and linkage maps.

* **4. A** — BAC clones carrying large inserts were used to construct the human cytogenetic map.

Lesson 139: Sequencing genome

1. What was the duration and completion year of the Human Genome Project?

- A) 5 years, completed in 1995
- B) 13 years, completed in 2003
- C) 20 years, completed in 2010
- D) 10 years, completed in 1990

2. Which sequencing technology, developed in 1975, was used to accomplish the Human Genome Project?

- A) Maxam-Gilbert chemical sequencing
- B) Sanger sequencing (chain-termination method)
- C) Ion torrent semiconductor sequencing
- D) Nanopore sequencing

3. What cost estimate is associated with the Human Genome Project in Topic 139?

- A) \$100 million
- B) \$1 billion
- C) \$3 billion

D) \$10 billion

4. What has driven the development of second-generation sequencing methods (next-generation sequencing, NGS)?

- A) Demand for cheaper and faster sequencing methods
- B) The elimination of PCR in all clinical laboratories
- C) The complete ban on using fluorescent reporter dyes
- D) The requirement to sequence RNA without converting it to cDNA

Answer Key

* **1. B** — The Human Genome Project was a 13-year-long endeavor completed in 2003.

* **2. B** — Sanger sequencing (the chain-termination method developed in 1975) was used to accomplish the Human Genome Project.

* **3. C** — The Human Genome Project was a \$3 billion endeavor.

* **4. A** — Greatly increased demand for cheaper and faster sequencing methods drove the development of NGS.

Lesson 140: High throughput sequencing

1. What read length is typically obtained from a single traditional Sanger sequencing reaction before improvements?

- A) 50 to 100 bases
- B) 200 to 300 bases
- C) 500 to 600 bases
- D) 2,000 to 3,000 bases

2. How many runs per 24-hour period can be achieved by switching from slab gels to capillary systems in automated sequencers?

- A) Two runs
- B) Five runs
- C) Nine runs
- D) Twenty runs

3. What read length can be achieved when improvements to reaction biochemistry and gel matrix chemistry are applied to automated sequencing?

- A) Up to 100 bases
- B) Up to 800 bases
- C) Up to 5,000 bases
- D) Up to 25,000 bases

4. What sequencing capacity per 24 hours is claimed by Amersham Biosciences for their MegaBACE 4000 instrument?

- A) 100,000 bases
- B) 500,000 bases
- C) 2.8 million bases
- D) 50 million bases

Answer Key

- * **1. C** — A single standard Sanger sequencing reaction allows 500–600 bases to be read.
- * **2. C** — Capillary systems reduce electrophoresis run time, allowing nine runs per 24-hour period.
- * **3. B** — Read length can be extended from 500–600 bases to 800 bases with improved biochemistry and gel chemistry.
- * **4. C** — Amersham Biosciences claims 2.8 million bases in 24 hours for the MegaBACE 4000 instrument.
-

Lesson 141: Shotgun sequencing

1. What method is used to sequence the randomly broken small DNA segments in shotgun sequencing?

- A) Maxam-Gilbert chemical cleavage
- B) Chain termination method
- C) Mass spectrometry
- D) Pyrosequencing without primers

2. How are the individual sequencing reads assembled into a continuous sequence in shotgun sequencing?

- A) By using restriction endonucleases to rejoin sticky ends
- B) By computer programs using the overlapping ends of different reads
- C) By ligating all fragments with T4 DNA ligase before analysis
- D) By hybridization to a macroarray nylon membrane

3. Why are multiple rounds of fragmentation and sequencing performed in shotgun sequencing?

- A) To convert double-stranded DNA into single-stranded cDNA
- B) To obtain multiple overlapping reads for the target DNA
- C) To remove all intron sequences from the genome
- D) To destroy vector polylinkers

4. For what length of DNA strands is shotgun sequencing specifically used?

- A) Sequencing long DNA strands
- B) Sequencing single dinucleotides only
- C) Short synthetic 10-bp linkers
- D) Purified 20-bp PCR primers exclusively

Answer Key

- * **1. B** — In shotgun sequencing, small random segments are sequenced using the chain termination method to obtain reads.
- * **2. B** — Computer programs assemble overlapping ends of different reads into a continuous sequence.
- * **3. B** — Multiple rounds of fragmentation and sequencing are performed to obtain overlapping reads for assembly.
- * **4. A** — Shotgun sequencing is used specifically for sequencing long DNA strands.
-
-

Lesson 142: Clone-by-clone sequencing

1. What two problems arise when applying a whole-genome shotgun approach to large genomes according to Topic 142?

- A) High cost of fluorescent dyes and loss of Taq polymerase activity
- B) Presence of dispersed repeated sequences and gaps between contigs needing closure
- C) Inability to cut DNA with restriction enzymes and lack of DNA ligase
- D) Rapid degradation of RNA and loss of host cell viability

2. What target size was assumed to represent the practical limit of the shotgun approach before map-based strategies were developed?

- A) Single base pairs
- B) Targets the size of cosmids
- C) Whole human chromosomes
- D) YAC inserts over 1 Mb

3. Which two organisms are explicitly mentioned as having their complete genome sequences generated using the "clone-by-clone" or "map-based" approach?

- A) **Escherichia coli** and **Bacillus subtilis**
- B) **Saccharomyces cerevisiae** and **Caenorhabditis elegans**
- C) **Drosophila melanogaster** and **Arabidopsis thaliana**
- D) **Homo sapiens** and **Mus musculus**

4. What is the accurate read length range of Sanger sequencing stated in Topic 142?

- A) 10 to 50 bases
- B) 100 to 200 bases
- C) 500 to 800 bases
- D) 2,000 to 5,000 bases

Answer Key

* **1. B** — Whole-genome shotgun assembly is confounded by dispersed repeats and gaps between contigs.

* **2. B** — Targets the size of cosmids were assumed to be the limit of the shotgun approach.

* **3. B** — The clone-by-clone approach was used successfully for **Saccharomyces cerevisiae** and **Caenorhabditis elegans**.

* **4. C** — Sanger sequencing provides an accurate read length of 500–800 bases.

Lesson 143: Comparative Genomics: Orthologs and paralogs

1. How are orthologs defined in Topic 143?

- A) Homologous genes within the same organism encoding proteins with identical 3D structures
- B) Homologous genes in different organisms that encode proteins with the same function and evolved by direct vertical descent
- C) Non-homologous genes that carry identical promoter sequences
- D) Synthetic oligonucleotide primers used for PCR mutagenesis

2. How do paralogs arise according to the definitions in Topic 143?

- A) By direct vertical descent without any gene duplication
- B) By gene duplication followed by mutation accumulation
- C) By horizontal gene transfer mediated exclusively by retroviruses
- D) By alkaline hydrolysis of mitochondrial RNA

3. What mechanism drives the evolution of orthologs as described in Topic 143?

- A) Gene duplication followed by exon shuffling
- B) Gradual accumulation of mutations
- C) Immediate chromosome jumping
- D) T-DNA integration into chloroplast genomes

4. What feature distinguishes paralogs from orthologs regarding their function and occurrence?

- A) Paralogs are homologous genes within an organism encoding proteins with related but non-identical functions
- B) Paralogs are found only in organelles and lack protein-coding capacity
- C) Paralogs never accumulate mutations after gene duplication
- D) Paralogs are non-coding EST markers mapped by FISH

Answer Key

- * **1. B** — Orthologs are homologous genes in different organisms with the same function that evolved by direct vertical descent.
- * **2. B** — Paralogs arise by gene duplication followed by the accumulation of mutations.
- * **3. B** — Orthologs evolve simply by the gradual accumulation of mutations over time.
- * **4. A** — Paralogs exist within an organism and encode proteins with related but non-identical functions.

Lesson 144: Comparative genomics of bacteria

1. What is the approximate gene density observed across all sequenced bacterial species analyzed in Topic 144?

- A) 1 gene per 100 kilobases of DNA
- B) About 1 gene per kilobase of DNA
- C) 10 genes per base pair
- D) 1 gene per 50 Mb

2. Which bacterium is cited in Topic 144 as having the largest archaeal genome (5.8 Mb) because it thrives in a broad range of environments?

- A) *Nanoarchaeum equitans*
- B) *Methanosarcina acetivorans*
- C) *Bradyrhizobium japonicum*
- D) *Streptomyces coelicolor*

3. What genome size variation range was revealed among the 173 bacterial genomes listed by NCBI in mid-2004?

- A) From 0.01 Mb to 0.05 Mb (5-fold difference)

- B) From 0.49 Mb (*Nanoarchaeum equitans*) to 9.1 Mb (*Bradyrhizobium japonicum* and *Streptomyces* species), an 18-fold difference
- C) From 10 Mb to 100 Mb (10-fold difference)
- D) Exactly 4.6 Mb across all species with zero variation

4. Where are the smallest eubacterial genomes typically found according to Topic 144?

- A) In soil bacteria capable of degrading aromatic compounds
- B) In free-living autotrophic cyanobacteria
- C) In organisms normally associated with animals or humans (e.g., mycoplasmas, rickettsias, chlamydiae)
- D) In thermophilic archaea living in hydrothermal vents

Answer Key

- * **1. B** — Gene density across bacterial species is remarkably consistent at about 1 gene per kilobase of DNA.
- * **2. B** — *Methanosarcina acetivorans* thrives in diverse environments and has the largest archaeal genome at 5.8 Mb.
- * **3. B** — Bacterial genome sizes ranged from 0.49 Mb to 9.1 Mb, representing a greater than 18-fold difference.
- * **4. C** — The smallest eubacterial genomes occur in host-associated organisms such as mycoplasmas, rickettsias, and chlamydiae.

Lesson 145: Comparative genomics of organelles

1. What primary metabolic function is performed by the mitochondrion?

- A) Photosynthetic carbon fixation via C4 pathways
- B) Generation of ATP via oxidative phosphorylation
- C) Synthesis of peptidoglycan cell walls
- D) Production of colicin bacteriocins

2. Why has functional gene transfer from mitochondria to the nucleus stopped in animals?

- A) Animals lack nuclear DNA polymerases
- B) Transfer is blocked by changes in the mitochondrial genetic code
- C) Animal mitochondria contain no nucleic acids
- D) Animal nuclear membranes are impermeable to all proteins

3. In which groups of organisms does functional gene transfer from mitochondria to the nucleus continue to occur?

- A) Animals and mammals
- B) Plants and protists
- C) Eubacteria and Archaea
- D) Filamentous phages and cosmids

4. How many genes encoding proteins critical for oxidative phosphorylation, and how many encoding ribosomal proteins, are expected in mitochondrial DNA?

- A) 2 genes for phosphorylation, 50 for ribosomes

- B) At least 21 genes for oxidative phosphorylation and 14 for ribosomal proteins
- C) 100 genes for phosphorylation, zero for ribosomes
- D) Exactly 1 gene for each function

Answer Key

- * **1. B** — The principal function of mitochondria is ATP generation through oxidative phosphorylation.
- * **2. B** — In animals, further gene transfer is blocked by changes in the mitochondrial genetic code.
- * **3. B** — Gene transfer to the nucleus continues in plants and protists because there is no genetic code barrier.
- * **4. B** — At least 21 genes encode oxidative phosphorylation proteins and 14 encode ribosomal proteins needed for mtRNA translation.

Lesson 146: Comparative genomics of Eukaryotes

1. What is the smallest eukaryotic genome that has been sequenced, with a genome size of 2.9 Mb?

- A) *Schizosaccharomyces pombe*
- B) *Encephalitozoon cuniculi*
- C) *Neurospora crassa*
- D) *Drosophila melanogaster*

2. How is genome compaction achieved in *Encephalitozoon cuniculi*?

- A) By removing all ribosomal RNA genes
- B) By reduction in the length of intergenic spacers and shortness of most putative proteins
- C) By integrating into host mitochondrial DNA
- D) By circularizing chromosomes into plasmid forms

3. Approximately how many Open Reading Frames (ORFs) does the genome of *Encephalitozoon cuniculi* contain?

- A) 200 ORFs
- B) 500 ORFs
- C) 2000 ORFs
- D) 10,000 ORFs

4. How does the number of ORFs in the multicellular fungus *Neurospora crassa* (~10,000 ORFs) compare to that of the fruit fly *Drosophila melanogaster*?

- A) It has twice as many ORFs
- B) It has about 25% fewer ORFs
- C) It has the exact same number of ORFs
- D) It has ten times fewer ORFs

Answer Key

- * **1. B** — *Encephalitozoon cuniculi* has a genome size of only 2.9 Mb, making it the smallest eukaryotic genome sequenced.
- * **2. B** — Compaction in *E. cuniculi* is achieved by shorter intergenic spacers and shorter putative proteins.

* **3. C** — *E. cuniculi* contains approximately 2000 ORFs, which is 7–8 times the number in a minimal bacterial genome.

* **4. B** — *Neurospora crassa* has approximately 10,000 ORFs, which is about 25% fewer than *Drosophila melanogaster*.

Lesson 147: DNA microarrays

1. What is the primary purpose of using DNA microarrays?

- A) To determine the 3D tertiary structure of folded proteins
- B) To study the expression of many genes at once
- C) To synthesize artificial amino acids in vitro
- D) To cleave double-stranded DNA at palindromic sites

2. What alternative strategy to robotic deposition of PCR products is used to synthesize DNA directly on a solid chip surface?

- A) Alkaline hydrolysis
- B) Photolithography
- C) Isopycnic centrifugation
- D) Homopolymer tailing

3. Which two major types of DNA arrays are used in gene expression analysis according to Topic 147?

- A) Southern blots and Northern blots
- B) Spotted DNA arrays and printed oligonucleotide chips
- C) Cosmid vectors and phasmid vectors
- D) Density gradient gels and tube gels

4. What length of DNA segments from known genes is typically deposited onto microarrays after PCR amplification?

- A) 1 to 5 nucleotides long
- B) A few dozen to hundreds of nucleotides long
- C) 10,000 to 50,000 nucleotides long
- D) Over 1 million nucleotides long

Answer Key

* **1. B** — Microarrays are designed to study the expression of thousands of genes simultaneously.

* **2. B** — Oligonucleotide sequences can be synthesized directly on the solid surface using photolithography.

* **3. B** — The two main types of DNA arrays used for expression analysis are spotted DNA arrays and printed oligonucleotide chips.

* **4. B** — Microarrays utilize DNA segments from known genes ranging from a few dozen to hundreds of nucleotides in length.

Lesson 148: Spotted DNA arrays

1. How are spotted DNA arrays manufactured?

- A) By exposing glass slides to high-energy X-rays
- B) By printing or transferring individual DNA clones or PCR products onto a treated solid support
- C) By converting RNA directly into protein crystals
- D) By fusing bacterial protoplasts with plant cell walls

2. What term is used to describe the early high-density cDNA arrays created on nitrocellulose or nylon membranes in precise grid patterns?

- A) Microarrays
- B) Macroarrays
- C) Polyplexes
- D) Contigs

3. In macroarray expression analysis, what does hybridizing complete libraries with complex probes generate for a particular RNA source?

- A) A "fingerprint" specific to that RNA source
- B) A single restriction fragment length polymorphism
- C) A double-stranded plasmid vector
- D) An inactivated lacZ reporter gene

4. Which two mammalian tissues are cited in Topic 148 as early examples of differential gene expression studies using macroarrays?

- A) Mouse liver and human kidney
- B) Mouse thymus and human muscle
- C) Rat brain and bovine heart
- D) Human skin and canine spleen

Answer Key

* **1. B** — Spotted DNA arrays are made by transferring/spotting individual DNA clones or PCR products onto a treated microscope slide.

* **2. B** — The early high-density cDNA arrays transferred to membrane grids are described as macroarrays.

* **3. A** — Complex probes hybridized with complete libraries produce a specific expression "fingerprint" for the RNA source.

* **4. B** — Early macroarray expression studies investigated differential gene expression in mouse thymus and human muscle.

Lesson 149: Oligonucleotide chips

1. What length range and strand structure characterize the targets on high-density oligonucleotide chips?

- A) Double-stranded targets of 1,000 to 5,000 bp
- B) Single-stranded targets ranging from 25 to 70 nt
- C) Circular double-stranded plasmids of 2.8 kb
- D) Triple-stranded RNA loops of 10 nt

2. What material is used to construct probes for hybridization onto oligonucleotide chips during expression profiling?

- A) Dual-labelled double-stranded genomic DNA
- B) Cleaved, biotinylated cRNA transcribed in vitro from cDNA
- C) Unlabelled ribosomal RNA
- D) Radiolabelled T-DNA border sequences

3. How is comparative expression analysis performed using oligonucleotide chips?

- A) By dual hybridization of two different fluorescent samples on the same chip
- B) By hybridizing alternative cRNA samples to identical chips and comparing signal intensities
- C) By measuring the rate of migration in SDS-PAGE gel electrophoresis
- D) By digesting the chip with EcoRI after hybridization

4. By how much is maximum array density increased when oligonucleotides are printed directly onto a glass surface rather than transferred from microtiter dishes?

- A) Twofold
- B) Almost tenfold
- C) Fiftyfold
- D) One hundredfold

Answer Key

* **1. B** — Oligonucleotide chips contain single-stranded target sequences ranging from 25 to 70 nucleotides in length.

* **2. B** — Probes for oligo chip hybridization consist of cleaved, biotinylated cRNA transcribed in vitro from cDNA.

* **3. B** — Comparative analysis on oligo chips relies on hybridizing separate cRNA samples to identical chips and comparing signal intensities.

* **4. B** — Direct printing of oligos onto the glass surface increases the maximum array density almost tenfold.

Lesson 150: Microbial gene expression analysis

1. How many total genes of the *S. cerevisiae genome were represented as PCR-amplified open reading frames on early spotted arrays?**

- A) 1,000 genes
- B) 2,500 genes
- C) Most of the 6200 genes
- D) Over 20,000 genes

2. What percentage of interrogated yeast genes showed highly significant expression changes when cells were shifted from fermentation to aerobic metabolism or heat shock?

- A) Less than 0.1%
- B) About 5%
- C) Exactly 50%
- D) 95%

3. In the study by Spellman and colleagues on the yeast cell cycle, how many cell cycle-regulated genes were identified, and how many were inducible by cyclins?

- A) 100 genes identified, 10 inducible
- B) 800 genes identified, about 400 inducible by cyclins
- C) 2,000 genes identified, 1,500 inducible
- D) 5,000 genes identified, 2,500 inducible

4. Which genomic property affecting gene expression in yeast was identified using arrays alongside sporulation and drug exposure studies?

- A) Cell wall thickness
- B) Cell ploidy (number of chromosome sets)
- C) Length of poly(A) tail
- D) Plasmid copy number

Answer Key

* **1. C** — Spotted arrays for yeast included PCR-amplified ORFs representing most of the 6200 genes in the *S. cerevisiae* genome.

* **2. B** — Approximately 5% of interrogated genes exhibited significant expression changes during metabolic shifts and heat shock.

* **3. B** — Spellman et al. identified 800 cell cycle-regulated genes, about 400 of which were inducible by cyclins.

* **4. B** — Arrays helped identify genes whose expression levels depend on the ploidy (chromosome set count) of the cell.

Lesson 151: Profiling in human diseases

1. In an early study using cDNA arrays to investigate cancer, human chromosome 6 was tested for its ability to suppress the tumorigenic phenotype of which melanoma cell line?

- A) HeLa
- B) UACC-903
- C) MCF-7
- D) PC-3

2. Which type of array technology was utilized to discover novel tumor-suppressor genes in the melanoma suppression study?

- A) Printed oligonucleotide chips
- B) Spotted cDNA arrays
- C) Mass spectrometry arrays
- D) Protein microarrays

3. Besides cancer, spotted cDNA arrays have been used to investigate global gene expression profiles in which pair of inflammatory conditions?

- A) Rheumatoid arthritis and inflammatory bowel disease
- B) Diabetes mellitus and hypertension
- C) Cystic fibrosis and sickle cell anemia
- D) Parkinson's disease and Alzheimer's disease

4. Which metabolic disorder is explicitly listed in Topic 151 alongside asthma as being investigated using spotted cDNA arrays?

- A) Phenylketonuria
- B) Insulin resistance
- C) Hypercholesterolemia
- D) Gout

Answer Key

* **1. B** — Chromosome 6 was tested for suppressing the tumorigenic phenotype of the melanoma cell line UACC-903.

* **2. B** — Spotted cDNA arrays were used in one of the earliest studies to identify novel tumor-suppressor genes.

* **3. A** — Spotted cDNA arrays were used to study global gene expression in rheumatoid arthritis and inflammatory bowel disease.

* **4. B** — Insulin resistance and asthma are explicitly mentioned as being investigated using spotted cDNA arrays.

Lesson 152: Phage display

1. Which vector is most commonly used to create random peptide display libraries in phage display technology?

- A) Bacteriophage lambda
- B) Filamentous phage M13
- C) Cosmid pJB8
- D) Plasmid pBR322

2. What is the fundamental concept underlying phage display technology?

- A) Expressing exogenous polypeptides on the surface of phage particles to select those that bind a desired target
- B) Integrating phage genomes permanently into the bacterial chromosome via homologous recombination
- C) Cleaving single-stranded viral RNA with restriction endonucleases
- D) Synthesizing cDNA from ribosomal RNA templates using reverse transcriptase

3. How can the affinity and selectivity of a known peptide that binds a target be increased when constructing phage display libraries?

- A) By exposing the phage to high-dose X-ray radiation
- B) By creating libraries with limited mutagenesis of the peptide
- C) By treating the library with alkaline phosphatase
- D) By digesting the target with S1 nuclease

4. What process is used to enrich and isolate high-affinity ligands with greater target specificity from phage display libraries?

- A) Electroporation
- B) Biopanning
- C) Isopycnic centrifugation

D) Western blotting

Answer Key

* **1. B** — The filamentous phage M13 is the most commonly used vector to create random peptide display libraries.

* **2. A** — Phage display allows expression of exogenous polypeptides on the surface of phage particles to select target-binding peptides.

* **3. B** — Limited mutagenesis of a known binding peptide allows screening for variants with higher affinity and selectivity.

* **4. B** — Initial screening by basic biopanning and extended methods are used to isolate high-affinity ligands.

Lesson 153: Screening phage display libraries

1. How are target molecules typically immobilized during the first step of biopanning?

- A) Covalent binding to gold nanoparticles
- B) Passive adsorption to modified 96-well polystyrene microtiter plates
- C) Microinjection into nuclear membranes
- D) Precipitation with cold ethanol and sodium acetate

2. Why are washes in the first round of biopanning made less stringent compared to subsequent rounds?

- A) To achieve a higher yield of the fittest phage clones over the background
- B) To destroy all non-specific bacterial cell walls
- C) To induce lytic cleavage of the M13 coat protein
- D) To prevent DNA polymerase from detaching during thermal cycling

3. Which of the following conditions can be used for non-specific elution of target-bound filamentous phage due to their high stability?

- A) Extremes of pH, denaturants, ionic strength, limited proteolysis, or sonication
- B) Heating the solution to 120°C in concentrated sulfuric acid
- C) Digestion with EcoRI and HindIII restriction enzymes
- D) Treatment with ethidium bromide under UV light

4. How many times is the biopanning process typically repeated before analyzing phage clones by DNA sequencing?

- A) Only once
- B) Three to six times
- C) Exactly 20 times
- D) 50 to 100 times

Answer Key

* **1. B** — Target molecules are immobilized by passive adsorption to modified 96-well polystyrene microtiter plates.

* **2. A** — Less stringent washes in the first round ensure a higher yield of the fittest phage clones over background.

* **3. A** — Filamentous phages withstand extremes of pH, denaturants, ionic strength, limited proteolysis, or sonication for non-specific elution.

* **4. B** — The biopanning cycle is repeated three to six times before analyzing positive phage clones by DNA sequencing.

Lesson 154: Applications of phage display

1. Why are filamentous phages particularly useful for identifying substrates of various proteases?

- A) They contain double-stranded RNA genomes
- B) They are resistant to a broad range of proteases
- C) They replicate exclusively in chloroplasts
- D) They lack coat proteins entirely

2. Which antibody derivative produced via phage display is useful in proteomics, specific drug delivery, and analyzing intracellular processes?

- A) Full-length IgM pentamers
- B) Phage display of antibody fragments
- C) Polyclonal antiserum from rabbits
- D) Monoclonal antibodies produced by hybridomas

3. What key feature makes phage display an advantageous method for mapping antigen epitopes?

- A) It is a cheap and rapid method
- B) It eliminates the need for DNA sequencing
- C) It relies exclusively on radioactive ^{32}P labelling
- D) It converts target proteins into double-stranded cDNA

4. In enzymology, how has phage display been applied regarding enzyme regulation?

- A) To determine substrate specificity and develop modulators of active and allosteric sites
- B) To eliminate all allosteric regulation in metabolic pathways
- C) To synthesize artificial tRNA molecules
- D) To digest host cell DNA during transformation

Answer Key

* **1. B** — Filamentous phages are resistant to a broad range of proteases, making them ideal for identifying protease substrates.

* **2. B** — Display of antibody fragments generates target-specific antibodies for proteomics, drug delivery, and intracellular analysis.

* **3. A** — Phage display is described as a cheap and rapid method to map antigen epitopes for interaction studies.

* **4. A** — Phage display is used in enzymology to determine substrate specificity and develop active and allosteric site modulators.

Lesson 155: Knock out and ins

1. How is a gene knockout defined in Topic 155?

- A) A genetic technique in which one of an organism's genes is made inoperative
- B) A technique that duplicates a target gene ten times in the host genome
- C) The insertion of a viral promoter upstream of an active gene
- D) The chemical degradation of all cellular mRNA molecules

2. What defines a gene knock-in procedure as distinguished from a knockout?

- A) The complete deletion of a chromosome arm
- B) The insertion of a protein-coding cDNA sequence at a particular locus in an organism's chromosome
- C) The random point mutation of a single base pair using alkylating agents
- D) The transformation of competent cells using calcium chloride

3. What is a common practical application of knock-in technology in scientific research?

- A) Creation of disease models and studying promoter regulatory machinery
- B) Purification of large quantities of plasmid DNA
- C) Direct sequencing of single-stranded RNA
- D) Production of synthetic restriction endonucleotides

4. Which high-capacity vectors are specifically mentioned in Topic 155 for transferring large DNA fragments in knock-in technology?

- A) BACs and YACs
- B) pBR322 and pUC19
- C) M13mp18 and Charon 4A
- D) pJB8 and c2XB

Answer Key

- * **1. A** — A gene knockout is a genetic technique in which one of an organism's genes is made inoperative (knocked out).
- * **2. B** — A knock-in involves inserting a protein-coding cDNA sequence at a specific locus in an organism's chromosome.
- * **3. A** — Knock-in technology is commonly used to create disease models and study the function of regulatory promoters.
- * **4. A** — BACs and YACs are used in knock-in procedures so that large DNA fragments can be transferred.
-

Lesson 156: siRNA technology

1. What type of gene silencing is typically induced by small interfering RNA (siRNA) according to Topic 156?

- A) Long-term permanent genomic deletion
- B) Short-term silencing of protein coding genes
- C) Permanent methylation of ribosomal RNA
- D) Indefinite integration of viral cDNA

2. What is the structural nature of siRNA as described in Topic 156?

- A) A single-stranded DNA hairpin loop

- B) A synthetic RNA duplex designed to specifically target a particular mRNA for degradation
- C) A double-stranded DNA plasmid vector
- D) A circular triple-stranded RNA molecule

3. What outcome makes siRNAs a promising tool for novel therapeutic procedures?

- A) Successful knock-downs of disease-related genes
- B) Complete elimination of all cellular ribosomes
- C) Reversion of eukaryotic cells to prokaryotic phenotypes
- D) Synthesis of artificial amino acids in vitro

4. What molecular feature allows siRNAs to target specific genes according to Topic 156?

- A) High specificity and efficiency of the underlying molecular mechanism of gene silencing
- B) Non-specific binding to total genomic DNA
- C) Indiscriminate cleavage of all cellular tRNA molecules
- D) Binding exclusively to 3' poly(A) tails of ribosomal RNA

Answer Key

* **1. B** — Small interfering RNA (siRNA) is the most commonly used RNAi tool for inducing short-term silencing of protein coding genes.

* **2. B** — siRNA is defined as a synthetic RNA duplex designed to specifically target a particular mRNA for degradation.

* **3. A** — Successful knock-downs of disease-related genes demonstrate that siRNAs open the door for novel therapeutic procedures.

* **4. A** — The underlying molecular mechanism of gene silencing enables siRNAs to target any gene with high specificity and efficiency.

Lesson 157: Applications of siRNA

1. Why were long dsRNAs (>30 nt) NOT feasible for gene silencing applications in mammals?

- A) They cannot be synthesized in vitro
- B) They induce a sequence-nonspecific interferon response resulting in global inhibition of mRNA translation
- C) They degrade immediately upon contact with cell culture media
- D) They bind permanently to the host cell wall

2. What discovery enabled efficient gene silencing of endogenous genes in mammalian cell lines without inducing global translation inhibition?

- A) The use of chemically synthesized siRNAs transfected into mammalian cells
- B) The microinjection of intact viral capsids
- C) The application of high-voltage electroporation to genomic DNA
- D) The digestion of cellular RNA with restriction endonucleases

3. Over what timeframe can the transient reduction in mRNA levels be detected following siRNA transfection?

- A) Immediately within 5 seconds
- B) In less than a day, lasting for several days depending on turnover rates

- C) Only after 6 to 8 weeks of continuous culture
- D) Exactly 30 days after transfection

4. In which organisms were long dsRNAs initially used for gene silencing before mammalian applications were developed?

- A) Mammals and humans
- B) Plants, Drosophila, and other species
- C) E. coli and B. subtilis exclusively
- D) Bacteriophages M13 and Lambda

Answer Key

* **1. B** — Long dsRNAs (>30 nt) induce a sequence-nonspecific interferon response in mammals, causing global inhibition of mRNA translation.

* **2. A** — Transfection of chemically synthesized siRNAs into mammalian cells allows efficient silencing of endogenous genes.

* **3. B** — Transient reduction in mRNA levels is detectable in less than a day and persists for several days.

* **4. B** — Long dsRNAs were initially applied in plants, Drosophila, and other species prior to short siRNA mammalian methods.

Lesson 158: Proteomics

1. How is the proteome defined in Topic 158?

- A) The entire complement of DNA sequences in a nucleus
- B) The entire complement of proteins synthesized by a given cell or organism
- C) The total mRNA transcript population in a ribosome
- D) The collection of non-coding intergenic spacers in a chromosome

2. Besides sequence and abundance, which additional parameters are relevant in proteomics analysis?

- A) Three-dimensional structure, modification, localization, biochemical/physiological function, and molecular interactions
- B) Melting temperature of PCR primers and GC content
- C) Number of restriction sites in the plasmid vector backbone
- D) Rate of bacterial cell wall peptidoglycan synthesis

3. Why is protein expression analysis described as more challenging than mRNA profiling?

- A) Because proteins cannot be amplified like nucleic acids
- B) Because proteins lack amino acid residues
- C) Because proteins are present only in dead host tissue
- D) Because proteins cannot be stained on gels

4. What is proteomics defined as in Topic 158?

- A) The study of the expression of genetic information at the level of the proteome
- B) The automated sequencing of whole eukaryotic genomes
- C) The chemical synthesis of synthetic restriction endonucleases
- D) The alignment of cDNA clones using Southern blotting

Answer Key

- * **1. B** — The proteome is defined as the entire complement of proteins synthesized by a given cell or organism.
- * **2. A** — Relevant proteomic features include 3D structure, modifications, localization, function, and interactions.
- * **3. A** — Protein expression analysis is more challenging because proteins, unlike nucleic acids, cannot be amplified.
- * **4. A** — Proteomics is the study of the expression of genetic information at the level of the proteome.
-
-

Lesson 159: Two dimensional (2-D) electrophoresis

1. On what property are proteins separated during the first dimension of 2-D gel electrophoresis (isoelectric focusing)?

- A) Molecular weight / size
- B) Charge (migrating to their isoelectric point in a pH gradient)
- C) Hydrophobicity of N-terminal signal sequences
- D) Degree of glycosylation

2. What role does the detergent sodium dodecylsulfate (SDS) play before the second-dimension separation?

- A) It cleaves proteins at specific methionine residues
- B) It binds non-specifically to all proteins and confers a uniform negative charge for size fractionation
- C) It amplifies protein copy numbers by ten-fold
- D) It induces fluorescent light emission under UV

3. How many proteins from Escherichia coli were resolved on a single gel in the first application of 2-D gel electrophoresis?

- A) 100 proteins
- B) 1000 proteins
- C) 5000 proteins
- D) 10,000 proteins

4. What modern gel format has largely replaced cumbersome tube gels in 2-D gel electrophoresis to provide easier handling and better reproducibility?

- A) Agarose tube gels
- B) Strip gels
- C) Nitrocellulose macroarrays
- D) Sucrose gradient beds

Answer Key

- * **1. B** — First-dimension isoelectric focusing separates proteins according to charge as they migrate to their isoelectric point in a pH gradient.
- * **2. B** — SDS binds non-specifically to proteins, conferring a uniform negative charge so they can be separated by size in the second dimension.
- * **3. B** — In the original application, 1000 E. coli proteins were resolved on a single 2-D gel.

* **4. B** — Strip gels have largely replaced tube gels because they are easier to handle and yield more reproducible separations.

Lesson 160: Mass spectrometry

1. What are the three component parts of a mass spectrometer as outlined in Topic 160?

- A) An ionizer, a mass analyzer, and an ion detector
- B) A laser diode, a capillary gel, and a CCD camera
- C) A restriction enzyme, a ligase, and a polymerase
- D) A heating block, a cooler, and a thermal sensor

2. According to what ratio does a mass analyzer separate gas phase ions?

- A) Charge/length ratio
- B) Mass/charge ratio (m/z)
- C) Volume/density ratio
- D) Absorbance/fluorescence ratio

3. What technique allows the ionization of large molecules such as proteins without significant degradation in modern mass spectrometry?

- A) Hard electron bombardment
- B) Soft ionization
- C) Alkaline hydrolysis
- D) High-voltage electroporation

4. How are the accurate mass measurements obtained from mass spectrometry used in protein identification?

- A) To synthesize artificial tRNA molecules
- B) To search protein databases to identify particular proteins
- C) To induce site-directed mutagenesis in plasmid vectors
- D) To calculate the exact GC percentage of genomic DNA

Answer Key

* **1. A** — A mass spectrometer comprises three main component parts: an ionizer, a mass analyzer, and an ion detector.

* **2. B** — The mass analyzer separates gas phase ions according to their mass/charge ratio (m/z).

* **3. B** — Soft ionization allows large molecules like proteins and peptides to be ionized without significant degradation.

* **4. B** — Mass data of whole proteins and peptide fragments are used to search protein databases to identify specific proteins.

Lesson 161: Protein microarrays

1. What is described in Topic 161 as a key advantage of closed systems (like DNA or protein arrays) compared to open systems (like 2D-electrophoresis)?

- A) They allow post-translational modifications to be ignored

- B) They do not require downstream characterization of features by sequencing or mass spectrometry because immobilized sequences are already known
- C) They enable unlimited PCR amplification of sample proteins
- D) They eliminate the need for glass slides or solid supports

2. Which major limitation complicates the manufacture and use of protein microarrays compared to DNA microarrays?

- A) DNA is composed of 22 different purines, whereas proteins contain only 4 amino acids
- B) While PCR allows rapid amplification of any nucleic acid sequence, no equivalent amplification procedure exists for proteins
- C) Proteins bind irreversibly to glass slides without hybridization
- D) DNA sequences require high-voltage electroporation to adhere to arrays

3. How many amino acids specified by the genetic code compose proteins, contributing to their diverse chemical properties?

- A) 4
- B) 12
- C) 22
- D) 64

4. Why can single complex probes be used in highly parallel formats on DNA arrays but not as easily on protein arrays?

- A) All DNA sequences share the same four nucleotides and behave similarly chemically based on complementary base pairing
- B) DNA arrays use radioactive isotopes that attract all nucleic acids equally
- C) Protein arrays lack solid surfaces
- D) DNA molecules possess no net electric charge

Answer Key

* **1. B** — Closed systems do not require downstream feature characterization because the immobilized sequences are already known.

* **2. B** — Unlike nucleic acids which can be amplified by PCR, there is no amplification procedure for proteins.

* **3. C** — Proteins are composed of 22 amino acids specified by the genetic code plus post-translational modifications.

* **4. A** — DNA sequences share the same four nucleotides and behave similarly chemically via complementary base pairing.

Lesson 162: Cloning in gram negative other than E. coli

1. Why is *E. coli* sometimes impracticable as a recipient host for cloning foreign genes?

- A) It lacks auxiliary biochemical pathways for functions such as degrading aromatic compounds, antibiotic synthesis, or sporulation
- B) It cannot take up plasmid vectors under any experimental conditions
- C) Its cell wall is completely resistant to lysozyme and detergent lysis
- D) It lacks RNA polymerase and ribosomes for transcription and translation

2. Which of the following is NOT listed in Topic 162 as one of the three basic prerequisites for cloning genes in a new host?

- A) A method for introducing the DNA into the potential recipient
- B) A mechanism to ensure the introduced DNA is maintained in the host
- C) Expression of the uptake and maintained cloned genes
- D) Complete elimination of all host cell ribosomes prior to transformation

3. When *Pseudomonas putida* is transformed with plasmid RSF1010 isolated from a host other than *P. putida*, what is the observed outcome?

- A) Transformants are recovered at 10^7 transformants/ μg DNA
- B) No transformants are obtained due to host restriction
- C) The plasmid integrates permanently into the 16S rRNA gene
- D) The plasmid is converted into single-stranded RNA

4. What efficiency of transformation is achieved when plasmid RSF1010 is prepared from another *P. putida* strain and used to transform *P. putida*?

- A) 10^2 transformants/ μg DNA
- B) 10^5 transformants/ μg DNA
- C) 10^{12} transformants/ μg DNA
- D) Zero transformants

Answer Key

* **1. A** — *E. coli* lacks auxiliary biochemical pathways essential for phenotypic expression of traits like aromatic compound degradation or sporulation.

* **2. D** — The three prerequisites are a method of introduction, maintenance in the host, and gene expression.

* **3. B** — Host restriction prevents recovery of any transformants unless the plasmid is prepared from another *P. putida* strain.

* **4. B** — Transformation of *P. putida* with RSF1010 prepared from another *P. putida* strain yields 10^5 transformants/ μg DNA.

Lesson 163: Cloning in gram +ve bacteria: *B. subtilis*

1. Which plasmid construct was created by inserting the TcR gene of pT127 into *Staphylococcus aureus* plasmid pC194 for use in *Bacillus subtilis*?

- A) pBR322
- B) pHV11
- C) pTA1060
- D) pUC19

2. What problem occurs when exogenous DNA is inserted into *S. aureus* derived plasmids in *B. subtilis*?

- A) Segregative stability is greatly reduced
- B) The host cell wall dissolves immediately
- C) The plasmid converts into a linear retrovirus
- D) Tetracycline resistance is lost permanently

3. Which cryptic *Bacillus* plasmid was developed as a vector to improve host-vector stability in *B. subtilis*?

- A) pUB110
- B) pTA1060
- C) pC194
- D) pBR325

4. Why are hybrid plasmids constructed as fusions between *E. coli* plasmids (e.g. pBR322) and *B. subtilis* plasmids (e.g. pC194 or pUB110) useful?

- A) They allow *E. coli* to be used as an efficient intermediate host before transforming *B. subtilis* competent cells with multimeric plasmids
- B) They eliminate the need for selectable markers in both hosts
- C) They degrade host DNA upon entry into *B. subtilis*
- D) They prevent *B. subtilis* from growing on solid media

Answer Key

***1. B** — Plasmid pHV11 is an improved vector consisting of pC194 carrying the TcR gene of pT127.

***2. A** — Insertion of exogenous DNA into these plasmids in *B. subtilis* greatly reduces their segregative stability.

***3. B** — Cryptic *Bacillus* plasmid pTA1060 was developed to improve host-vector system stability.

***4. A** — Chimeric shuttle plasmids allow *E. coli* to serve as an intermediate host to generate multimeric DNA for *B. subtilis* transformation.

Lesson 164: Cloning in Streptomyces

1. What defines the G+C content of *Streptomyces* DNA, and how does it affect restriction target sites?

- A) It is 20–25% G+C, making GC-rich sites very rare
- B) It is 70–75% G+C, making AT-rich recognition sites rare
- C) It is exactly 50% G+C, resulting in random restriction site distribution
- D) It contains no G or C bases, preventing restriction cleavage

2. Which restriction endonucleases with high GC content in their recognition sequences are most commonly used to construct gene libraries in *Streptomyces*?

- A) EcoRI, HindIII, and DraI
- B) BamHI, BglII, and BclI
- C) PaeI, PmeI, and SmaI
- D) NotI, SalI, and XhoI

3. Why are *Streptomyces* genes usually difficult to express in *E. coli*?

- A) Most *Streptomyces* promoters do not function in *E. coli*, and translation may be inefficient unless initial amino acid codons are changed
- B) *E. coli* secretes enzymes that digest all G+C-rich DNA
- C) *Streptomyces* genes lack initiation codons entirely
- D) *E. coli* ribosomes translate only single-stranded RNA

4. What structural feature of *Streptomyces* promoters can cause them to be lost during gene cloning?

- A) Promoters may lie several hundred base pairs upstream of the start of the gene
- B) Promoters are located within the poly(A) tail
- C) Promoters consist exclusively of thymine repeats
- D) Promoters are covalently attached to cell wall lipids

Answer Key

- * **1. B** — *Streptomyces* DNA has a G+C content of 70–75%, which makes AT-rich recognition sites rare.
- * **2. B** — Enzymes with high GC content in their recognition sequence, such as BamHI, BglII, and BclI, are commonly used.
- * **3. A** — *Streptomyces* promoters function poorly in *E. coli*, and translation is inefficient without codon modification to lower G+C content.
- * **4. A** — Promoters in *Streptomyces* may be located several hundred base pairs upstream of the gene start site.

Lesson 165: Cloning in Archea

1. Which method is most commonly used to introduce DNA into most Archaea species, yielding low efficiencies of $\sim 10^2$ transformants/ μg ?

- A) Electroporation
- B) Particle bombardment
- C) Pronuclear microinjection
- D) Transfection with DEAE-dextran

2. In which group of Archaea have much higher transformation efficiencies (e.g. 10^7 transformants/g) been observed using polyethylene glycol- or liposome-mediated transformation?

- A) Extreme halophiles
- B) Hyperthermophiles
- C) Methanogens
- D) Acidophiles

3. What are the three basic requirements for gene cloning in any organism listed in Topic 165?

- A) A means of introducing DNA into recipients, suitable plasmid vectors, and selectable markers
- B) High-voltage electroporation, radioactive probes, and sucrose gradient centrifugation
- C) Reverse transcriptase, S1 nuclease, and poly(A) tails
- D) Phenol extraction, ethanol precipitation, and agarose gels

4. What environmental conditions characterize many organisms within the domain Archaea compared to eubacteria?

- A) Extreme environments such as very high temperatures, high pH, high salt, or strictly anaerobic conditions
- B) Obligate dependence on mammalian blood serum
- C) Growth exclusively on minimal agar at 0°C
- D) Inability to synthesize nucleic acids naturally

Answer Key

- * **1. A** — Electroporation is the main method for introducing DNA into most Archaea, yielding low efficiencies around 10^2 transformants/ μg .
- * **2. C** — High efficiencies ($\sim 10^7$ transformants/g) have been achieved in some methanogens using PEG- or liposome-mediated transformation.
- * **3. A** — Gene cloning in any organism requires a DNA delivery method, suitable plasmid vectors, and selectable markers.
- * **4. A** — Many Archaea thrive in extreme environments like high temperature, high pH, high salt, or anaerobic methanogenic niches.
-

Lesson 166: Cloning in Saccharomyces and other Fungi

1. What was the genome size of *Saccharomyces cerevisiae*, the sequencing of which was completed in 1996?

- A) 4.6 Mb
- B) 12 Mb
- C) 48.5 Mb
- D) 100 Mb

2. Which shift in biological research approach occurred following the complete sequencing of the *S. cerevisiae* genome?

- A) A switch from a holistic approach to a reductive approach
- B) A switch from a reductive approach to a holistic approach
- C) A complete abandonment of gene manipulation techniques
- D) A focus exclusively on non-coding tRNA genes

3. Which methylotrophic yeast is highlighted for its ability to produce very high yields of recombinant proteins?

- A) *Schizosaccharomyces pombe*
- B) *Pichia pastoris*
- C) *Neurospora crassa*
- D) *Candida albicans*

4. Which of the following is listed as a major advantage offered by fungi for overproducing proteins of commercial value?

- A) Presence of bacterial lipopolysaccharides
- B) Ability to glycosylate proteins and absence of pyrogenic toxins
- C) Inability to form disulfide bonds
- D) Requirement for high-voltage electroporation

Answer Key

- * **1. B** — The entire 12 Mb genome of *S. cerevisiae* was completely sequenced in 1996.
- * **2. B** — Research shifted from analyzing individual genes (reductive approach) to studying the whole genome (holistic approach).
- * **3. B** — *Pichia pastoris* is a methylotrophic yeast capable of yielding very high levels of recombinant proteins.

* **4. B** — Fungi provide advantages such as protein glycosylation capability and the absence of pyrogenic toxins.

Lesson 167: Transformation of fungi with exogenous DNA

1. What type of cells are used in the enzymatic cell wall removal method developed for transforming *S. cerevisiae*?

- A) Callus cells
- B) Spheroplasts
- C) Protoplasts with intact chitin walls
- D) Endospores

2. Which chemical agent is used to fuse spheroplasts with foreign DNA in the presence of CaCl_2 ?

- A) Ethylene glycol
- B) Diethylaminoethyl dextran
- C) Cyanogen bromide
- D) Isopropyl- β -D-thiogalactoside

3. In what medium percentage of agar are spheroplasts allowed to regenerate new cell walls after transformation?

- A) 0.5% agar
- B) 1% agar
- C) 3% agar
- D) 10% agar

4. What advantage does electroporation offer over the spheroplast technique for transforming fungi?

- A) It eliminates the need for any selection markers
- B) It provides a simpler and more convenient alternative allowing selection on the surface of solid media
- C) It permanently integrates viral genomes into chloroplasts
- D) It operates without electrical current

Answer Key

* **1. B** — Spheroplasts are wall-less cells produced enzymatically for fungal transformation.

* **2. A** — Spheroplasts are fused with foreign DNA using ethylene glycol in the presence of CaCl_2 .

* **3. C** — Spheroplasts generate new cell walls in a stabilizing medium containing 3% agar.

* **4. B** — Electroporation is simpler and convenient because transformed cells can be selected directly on solid media surfaces.

Lesson 168: Vectors for use in *S. cerevisiae*

1. Which four types of plasmid vectors have been developed for use in *S. cerevisiae*?

- A) pBR322, pUC19, pSC101, and pC194
- B) YEps, YRps, YCps, and YACs
- C) Ti-plasmids, Ri-plasmids, pGV3850, and pJB8
- D) M13mp18, Charon 4A, Charon 16, and λ gt11

2. Why is the ability of all yeast plasmid vectors to replicate in *E. coli* an important feature?

- A) It allows yeast cells to synthesize bacterial peptidoglycan
- B) It enables the vector DNA to be amplified in *E. coli* before transforming yeast recipients
- C) It prevents restriction endonucleases from cutting the vector
- D) It converts eukaryotic mRNA directly into viral cDNA

3. Which four selectable markers are listed as the most widely used in yeast vectors?

- A) AmpR, TcR, CmR, and NeoR
- B) His3, Leu2, Trp1, and Ura3
- C) LacZ, Cat, NptII, and Nos
- D) Octopine, Nopaline, Luc, and GFP

4. How do the selectable markers used in yeast vectors typically interact with *E. coli* host mutations?

- A) They induce lytic cell death in *E. coli*
- B) They complement the corresponding mutations in *E. coli*
- C) They degrade *E. coli* chromosomal DNA completely
- D) They inhibit *E. coli* RNA polymerase activity

Answer Key

- * **1. B** — The four types of vectors developed for yeast are YEps, YRps, YCps, and YACs.
- * **2. B** — Replication in *E. coli* allows easy amplification of vector DNA prior to transforming yeast.
- * **3. B** — The four most widely used yeast markers are His3, Leu2, Trp1, and Ura3.
- * **4. B** — Yeast selectable markers often complement corresponding auxotrophic mutations in *E. coli*.

Lesson 169: Properties of different yeast vectors

1. Which vector type or linearized vector form is specifically used for conventional genetic analysis by means of recombination in yeast?

- A) Circular YEpl vectors only
- B) Ylp vectors or linearized YRp vectors
- C) Intact YAC vectors exclusively
- D) Phage M13 intergenic vectors

2. Which group of vectors can be used to perform complementation studies in yeast?

- A) YEpl, YRp, YCp, or YAC vectors
- B) ColE1 and pBR322 plasmids only
- C) Single-stranded M13 phages exclusively
- D) Disarmed Ti-plasmids

3. Why are experimentally induced changes made to cloned yeast genes in *E. coli* returned to yeast cells?

- A) To destroy all host ribosomes
- B) To determine their effects on the function and expression of yeast genes
- C) To prevent plasmid DNA from supercoiling
- D) To convert *E. coli* into a eukaryotic fungus

4. What operational advantage is gained by initially cloning yeast DNA sequences in *E. coli*?

- A) Sequences can be obtained easily in large amounts and modified in vivo or in vitro
- B) Yeast DNA becomes completely immune to restriction enzymes
- C) It eliminates the need for promoters in expression
- D) It causes single-stranded RNA to form triple helices

Answer Key

- * **1. B** — Recombination analysis in yeast uses YIp vectors or linearized YRp vectors.
- * **2. A** — Complementation in yeast can be carried out using YEp, YRp, YCp, or YAC vectors.
- * **3. B** — Returning modified genes to yeast allows researchers to observe the effects on gene function and expression.
- * **4. A** — Cloning in *E. coli* permits easy bulk preparation and precise genetic or enzymatic modification.

Lesson 170: Promoter system for yeast

1. Which consensus sequence motif located 60–120 nucleotides before the initiation site in yeast promoters is functionally equivalent to the Pribnow box of *E. coli*?

- A) The Shine-Dalgarno sequence
- B) The TATA box
- C) The poly(A) signal
- D) The 25-bp direct repeat border

2. What structural feature is characteristic of Upstream Activating Sequences (UASs) in yeast promoters?

- A) Presence of one or more regions of dyad symmetry
- B) High G+C content exceeding 95%
- C) Terminal protein caps attached to 5' ends
- D) Presence of EcoRI restriction sites every 10 bp

3. What effect does the deletion of Upstream Activating Sequences (UASs) have on gene transcription in yeast?

- A) It increases the transcription rate ten-fold
- B) It abolishes transcription
- C) It converts mRNA into double-stranded DNA
- D) It shifts the transcription start site by 500 bp

4. Which two transcription-initiation consensus sequences account for more than half of all known yeast initiation sites?

- A) 5'-TTGACA-3' and 5'-TATAAT-3'

- B) TC(G/A)A and PuPuPyPuPu
- C) 5'-GGAGG-3' and 5'-AAGCTT-3'
- D) CCCGGG and GGCC

Answer Key

- * **1. B** — The TATA box in yeast promoters is functionally equivalent to the *E. coli* Pribnow box.
- * **2. A** — An important structural feature of UASs is the presence of one or more regions of dyad symmetry.
- * **3. B** — Binding of positive-control proteins to UASs increases transcription, and their deletion abolishes transcription.
- * **4. B** — Sequences TC(G/A)A and PuPuPyPuPu account for more than 50% of known yeast initiation sites.

Lesson 171: Multipurpose vectors for use in yeast

1. What organelle receives proteins destined for export from the cell after they are synthesized in yeast?

- A) Nucleolus
- B) Endoplasmic reticulum
- C) Peroxisome
- D) Vacuole

2. Which proteins naturally synthesized and secreted by yeast end up directly in the growth medium?

- A) Invertase and acid phosphatase
- B) Mating pheromone α factor and killer toxin
- C) Ribosomal RNA and tRNA
- D) Cytochrome c and DNA polymerase

3. Where do natural yeast proteins like invertase and acid phosphatase remain after crossing the plasma membrane?

- A) In the growth medium
- B) Within the periplasmic space or associated with the cell wall
- C) In the nucleus
- D) Inside the vacuole

4. How long is the hydrophobic amino-terminal signal extension that directs polypeptides to the endoplasmic reticulum in yeast?

- A) About 5 amino acids
- B) About 20 amino acids
- C) About 100 amino acids
- D) About 500 amino acids

Answer Key

- * **1. B** — Proteins destined for export are synthesized on and translocated into the endoplasmic reticulum.
- * **2. B** — Of naturally secreted yeast proteins, only a few like α factor mating pheromone and killer toxin end up in the growth medium.

* **3. B** — Invertase and acid phosphatase cross the plasma membrane but remain within the periplasmic space or associated with the cell wall.

* **4. B** — Signal extensions composed of about 20 amino acids precede mature yeast secreted polypeptides to direct translocation.

Lesson 172: Cloning for large DNA fragments

1. What factor determines the segregative stability of early YACs?

- A) Their G+C ratio
- B) Their size
- C) The presence of ampicillin resistance genes
- D) The culture temperature

2. At what YAC size was centromere function found to be impaired?

- A) Less than 20 kb
- B) Greater than 350 kb
- C) Exactly 1 Mb
- D) Less than 2 kb

3. Why were large DNA molecules difficult to keep intact during early YAC library construction?

- A) They are difficult to manipulate in the liquid phase prior to transformation
- B) They are degraded by reverse transcriptase
- C) They bind permanently to glass slides
- D) They dissolve in 70% ethanol

4. How was the average insert size of YAC libraries increased from 50–100 kb to 350 kb?

- A) By removing small DNA fragments by PFGE fractionation prior to cloning
- B) By treating the library with cyanogen bromide
- C) By adding ethidium bromide to the growth medium
- D) By increasing the incubation temperature to 42°C

Answer Key

* **1. B** — The segregative stability of YACs is determined by their size.

* **2. A** — Centromere function was impaired if the YAC size was less than 20 kb.

* **3. A** — Large DNA molecules are difficult to manipulate in the liquid phase prior to transformation.

* **4. A** — Removing small DNA fragments using PFGE fractionation before cloning raised the average insert size to 350 kb.

Lesson 173: Deficiencies and advantages of YACs

1. What percentage of clones in existing YAC libraries is estimated to represent chimeric DNA sequences?

- A) 1–5%

- B) 10–60%
- C) 75–90%
- D) Exactly 100%

2. How can chimeric YAC clones arise during library construction?

- A) By co-ligation of DNA inserts in vitro or by recombination between two DNA molecules in the same cell
- B) By digestion with S1 nuclease
- C) By exposure to ultraviolet light in agarose gels
- D) By treatment with sodium hypochlorite

3. Which technique can be used to detect chimeric YACs by identifying hybridization to two or more metaphase chromosomes?

- A) In situ hybridization
- B) Western blotting
- C) Polyacrylamide gel electrophoresis
- D) Column chromatography

4. What structural defect besides chimerism commonly affects YAC clones?

- A) They tend to delete internal regions from their inserts
- B) They convert into circular RNA molecules
- C) They synthesize bacterial cell walls
- D) They lose all origins of replication

Answer Key

- * **1. B** — An estimated 10–60% of clones in existing YAC libraries represent chimeric DNA sequences.
- * **2. A** — Chimeras arise via co-ligation of inserts in vitro or recombination between DNA molecules in the same yeast cell.
- * **3. A** — In situ hybridization to metaphase chromosomes detects chimeras if signals appear on multiple chromosomes or disparate regions.
- * **4. A** — Many YAC clones are unstable and tend to delete internal regions from their inserts.

Lesson 174: GENE TRANSFER TO PLANT CELLS-Introduction

1. What major limitation restricts traditional plant breeding and selection?

- A) Breeders are restricted to the existing gene pool in each species or sexually compatible group
- B) Plant cells cannot perform cell division
- C) Plants lack DNA and RNA
- D) Seeds cannot germinate in soil

2. In what year was stable foreign DNA transmission through the germline first demonstrated in transgenic tobacco plants using *Agrobacterium tumefaciens*?

- A) 1953
- B) 1970
- C) 1981
- D) 2003

3. Which soil bacterium was used in 1981 to generate the first confirmed transgenic tobacco plants?

- A) *Escherichia coli*
- B) *Agrobacterium tumefaciens*
- C) *Bacillus subtilis*
- D) *Thermus aquaticus*

4. How are plant viruses utilized in plant genetic engineering?

- A) As versatile episomal vectors allowing high-level transient gene expression
- B) To permanently integrate transgenes into chloroplast genomes
- C) To remove cell walls from leaf mesophyll cells
- D) As restriction enzymes to cut genomic DNA

Answer Key

* **1. A** — Plant breeding is limited because breeders are restricted to the existing gene pool of sexually compatible groups.

* **2. C** — Stable transformation and germline transmission were first demonstrated in 1981 in transgenic tobacco.

* **3. B** — The soil bacterium *Agrobacterium tumefaciens* was used to generate the first transgenic tobacco plants.

* **4. A** — Plant viruses serve as versatile episomal vectors that allow high-level transient gene expression.

Lesson 175: Callus culture

1. What term describes the undifferentiated mass of cells that develops when an explant is cultured on a suitable nutrient medium?

- A) Callus
- B) Plasmid
- C) Protoplast
- D) Vector

2. Why are explants treated with disinfectants such as sodium hypochlorite before tissue culture?

- A) Because nutrient media support the growth of microorganisms as well as plant cells
- B) To dissolve the nuclear membrane
- C) To induce immediate shoot formation
- D) To synthesize opines

3. Why are callus cultures usually maintained in the dark?

- A) Because light can induce differentiation of callus cells
- B) Because dark conditions prevent DNA replication
- C) Because light destroys phytohormones instantly
- D) Because chloroplasts require darkness to function

4. What morphological outcome is favored when culturing callus with a low auxin:cytokinin ratio?

- A) Root formation
- B) Shoot formation
- C) Complete cell lysis
- D) Seed dormancy

Answer Key

- * **1. A** — An undifferentiated mass of cells grown from an explant is known as a callus.
- * **2. A** — Explants are washed in disinfectant because nutrient media also support microorganism growth.
- * **3. A** — Callus cultures are usually kept in the dark because light can induce cell differentiation.
- * **4. B** — A low auxin:cytokinin ratio leads to shoot formation, whereas a high ratio favors root formation.

Lesson 176: Cell-suspension culture

1. What type of callus tissue breaks up when placed in a liquid medium and agitated to form a cell-suspension culture?

- A) Hard callus
- B) Friable callus
- C) Compact callus
- D) Sclerotized callus

2. What term describes the accumulation of mutations resulting from genetic instability during long-term cell-suspension culture?

- A) Somaclonal variation
- B) Position effect
- C) Functional complementation
- D) Transgene silencing

3. What effect can somaclonal variation accumulated during long-term culture have on regenerated plants?

- A) It increases root growth ten-fold
- B) It can adversely affect their vitality and fertility
- C) It prevents cell division in liquid medium permanently
- D) It converts dicot plants into monocots

4. Which cellular components are present in liquid cell-suspension cultures following agitation of friable callus?

- A) Isolated cells, small clusters of cells, and larger aggregates
- B) Enucleated protoplasts exclusively
- C) Single-stranded cDNA molecules only
- D) Chloroplasts stripped of cell membranes

Answer Key

- * **1. B** — Friable callus is soft and easily breakable, disintegrating into a cell suspension when agitated in liquid medium.
- * **2. A** — Accumulation of mutations during long-term tissue culture is known as somaclonal variation.
- * **3. B** — Somaclonal variation resulting from genetic instability can adversely affect the vitality and fertility of regenerated plants.

* **4. A** — Cell suspensions consist of a heterogeneous mixture of isolated cells, small cell clusters, and larger aggregates.

Lesson 177: Protoplasts

1. What defines protoplasts in plant tissue culture?

- A) Undifferentiated callus cells grown in dark conditions
- B) Cells from which the cellulose cell walls have been removed
- C) Embryonic stem cells isolated from animal blastocysts
- D) Bacterial cells containing disarmed Ti plasmids

2. Which enzyme is specifically required during protoplast preparation to digest away the cell wall?

- A) Pectinase
- B) Cellulase
- C) Signal peptidase
- D) Reverse transcriptase

3. How are protoplasts separated from intact cells and cell debris following enzymatic treatment?

- A) By paper chromatography
- B) By flotation on a cushion of sucrose
- C) By high-voltage electroporation
- D) By precipitation with cold isopropanol

4. How long after being plated onto nutrient medium do protoplasts typically synthesize new cell walls before initiating division?

- A) Within 5 to 10 days
- B) In 24 to 48 hours
- C) After 30 to 40 days
- D) Immediately within 5 minutes

Answer Key

* **1. B** — Protoplasts are plant cells that have had their cellulose cell walls removed.

* **2. B** — Cellulase is used to digest away the cellulose cell wall, while pectinase breaks up cell aggregates.

* **3. B** — Protoplasts are separated from intact cells and debris by flotation on a cushion of sucrose after centrifugation.

* **4. A** — Protoplasts synthesize new cell walls within 5–10 days of being plated on nutrient medium.

Lesson 178: Regeneration of fertile plants

1. What term describes the formation of roots and shoots on callus tissue in plant tissue culture?

- A) Organogenesis
- B) Somatic embryogenesis
- C) Bactofection
- D) Transduction

2. Increasing the level of which phytohormone in the culture medium specifically induces shoot formation from callus tissue?

- A) Auxin
- B) Cytokinin
- C) Absciscic acid
- D) Ethylene

3. What effect does increasing the level of auxin in the growth medium have on callus tissue?

- A) It promotes root formation
- B) It causes immediate cell lysis
- C) It inhibits DNA replication
- D) It induces total darkness tolerance

4. How do plantlets ultimately arise during organogenesis from callus tissue?

- A) Through adventitious roots developing on shoot buds, or shoot buds developing from rootlet bases
- B) Through the direct fusion of two unfertilized gametes
- C) By transforming cell walls with Agrobacterium vir genes
- D) By precipitating proteins with sodium dodecyl sulphate

Answer Key

* **1. A** — Organogenesis is the process of forming roots and shoots on callus tissue.

* **2. B** — Increasing cytokinin levels available to the callus induces shoot formation, whereas auxin promotes root formation.

* **3. A** — Higher auxin concentrations in the culture medium promote root formation.

* **4. A** — Plantlets develop via adventitious roots forming on shoot buds or shoot buds forming at the base of rootlets.

Lesson 179: Major strategies of gene transfer to plants

1. Which gene transfer method is the most widely used for transforming dicotyledonous plants?

- A) Agrobacterium-mediated transformation
- B) Calcium phosphate co-precipitation
- C) Lipofection with DEAE-dextran
- D) Microinjection into nuclear membranes

2. Which physical method is particularly popular for transforming monocotyledonous plants such as cereals?

- A) Particle bombardment
- B) Electroporation of intact seeds
- C) Sonication with ultrasound

D) Alkaline lysis

3. With which type of plant cells are chemical transfection methods compatible?

- A) Protoplasts exclusively
- B) Hard woody explants
- C) Intact cereal pollen grains
- D) Dry seed coats

4. Why are plant viruses used as episomal vectors rather than for stable genomic transformation?

- A) No known plant viruses integrate their genetic material into the plant genome during their natural infection cycle
- B) Plant viruses contain no nucleic acids
- C) Plant viruses degrade host plant DNA completely
- D) Plant viruses operate only in animal host cells

Answer Key

* **1. A** — Agrobacterium-mediated transformation is the most widely used method, especially for dicotyledonous plants.

* **2. A** — Particle bombardment is the leading physical method for transforming monocot plants like cereals.

* **3. A** — Chemical transfection methods are compatible only with plant protoplasts.

* **4. A** — Plant viruses do not integrate into the host genome naturally, so they serve as episomal vectors for transient expression.

Lesson 180: Agrobacterium- mediated transformation

1. What plant disease is naturally caused by gene transfer from the soil bacterium

***Agrobacterium tumefaciens*?**

- A) Crown gall disease
- B) Hairy-root disease
- C) Bacterial blight
- D) Damping off

2. What unusual amino acid derivatives synthesized by crown gall tumor tissues serve as a hallmark of *Agrobacterium* transformation?

- A) Opines (such as octopine and nopaline)
- B) Beta-galactosides
- C) Chloramphenicol esters
- D) Polyamines

3. What key property distinguishes crown gall callus tissue from normal plant callus in tissue culture?

- A) It can grow indefinitely in vitro even in the absence of exogenous phytohormones
- B) It requires light exposure 24 hours a day
- C) It cannot synthesize proteins
- D) It contains no nuclear DNA

4. What size range characterizes the tumor-inducing plasmids (Ti-plasmids) harbored by virulent strains of *A. tumefaciens*?

- A) 140 to 235 kbp
- B) 2 to 5 kbp
- C) 500 to 800 kbp
- D) Over 10,000 kbp

Answer Key

- * **1. A** — *Agrobacterium tumefaciens* naturally causes crown gall disease, a plant tumor.
- * **2. A** — Transformed crown gall tissues synthesize opines (e.g., octopine and nopaline), which are unusual amino acid derivatives.
- * **3. A** — Crown gall tissue exhibits autonomous, unlimited growth in culture without needing added phytohormones.
- * **4. A** — Ti-plasmids harbored by virulent *A. tumefaciens* strains range from 140 to 235 kbp in size.

Lesson 181: T-DNA transfer

1. What is the approximate size of the T-DNA segment that integrates into the plant nuclear genome from the Ti-plasmid?

- A) 5 kbp
- B) 23 kbp
- C) 100 kbp
- D) 235 kbp

2. Where in the plant nuclear genome does T-DNA integrate during *Agrobacterium*-mediated transformation?

- A) Exclusively at the 18S ribosomal RNA gene locus
- B) At an apparently random site
- C) Always inside the chloroplast genome
- D) Strictly within telomeric repeat regions

3. What structural elements flank the T-DNA in the Ti-plasmid?

- A) 10-bp blunt-ended restriction sites
- B) 25-bp imperfect direct repeats known as border sequences
- C) Single-stranded 5' overhangs containing BamHI sites
- D) 100-bp inverted terminal repeats containing promoter sequences

4. What function do the genes carried within the T-DNA confer upon transformed plant tissue?

- A) Unregulated growth and the ability to synthesize opines
- B) Sensitivity to carbenicillin and resistance to cold temperatures
- C) Inability to synthesize cell wall cellulose
- D) Direct translation of viral coat proteins

Answer Key

- * **1. B** — The T-DNA is a specific segment of the Ti-plasmid about 23 kbp in size that integrates into plant nuclear DNA.
- * **2. B** — T-DNA integrates into the plant nuclear DNA at an apparently random site.

* **3. B** — In the Ti-plasmid, the T-DNA is flanked by 25-bp imperfect direct repeats known as border sequences.

* **4. A** — T-DNA carries genes conferring unregulated growth and opine synthesis upon transformed plant tissue.

Lesson 182: Function of T-DNA genes

1. How were genetic maps of T-DNA initially obtained by researchers?

- A) By sequencing with automated four-dye capillary sequencers
- B) By studying spontaneous and transposon-induced mutants that affect tumour morphology
- C) By performing Southern blotting on purified chloroplast DNA
- D) By digesting whole plasmids with EcoRI and HindIII

2. T-DNA mutants displaying a "shooty" phenotype were found to affect genes encoding enzymes for the biosynthesis of which phytohormone?

- A) Cytokinin
- B) Auxin
- C) Absciscic acid
- D) Ethylene

3. T-DNA mutants displaying a "rooty" phenotype were found to be involved in the production of which phytohormone?

- A) Auxin
- B) Cytokinin
- C) Gibberellin
- D) Ethylene

4. Why do wild-type T-DNA transformed tumour cells grow in culture without added auxins and cytokinins?

- A) Because T-DNA oncogenes encode enzymes involved in phytohormone synthesis, resulting in high internal hormone levels
- B) Because plant cell walls synthesize hormones when exposed to light
- C) Because Agrobacterium secretes hormones continuously into the agar
- D) Because opines are converted directly into auxins by host ribosomes

Answer Key

* **1. B** — Genetic maps of T-DNA were obtained by analyzing spontaneous and transposon-induced mutants affecting tumour morphology.

* **2. B** — Functional analysis confirmed that T-DNA genes with "shooty" mutant phenotypes encode enzymes for auxin biosynthesis.

* **3. B** — Genes associated with "rooty" mutant phenotypes are involved in cytokinin production.

* **4. A** — T-DNA oncogenes encode enzymes for phytohormone synthesis, producing high internal levels of auxins and cytokinins.

Lesson 183: Disarmed Ti vectors

1. Why are wild-type Ti plasmids unsuitable as general gene vectors for plant genetic engineering?

- A) They lack restriction enzyme sites
- B) The T-DNA contains oncogenes that cause disorganized, tumorous growth
- C) They cannot enter host plant cells
- D) They contain no selectable markers or origins of replication

2. How is a Ti plasmid vector "disarmed" to render it nononcogenic for plant regeneration?

- A) By deleting all of its oncogenes from the T-DNA
- B) By treating the plasmid with hydrogen peroxide
- C) By adding ethidium bromide to the culture medium
- D) By mutating the origin of replication

3. In the disarmed plasmid construct pGV3850, what sequence replaced almost all of the T-DNA between the border regions?

- A) pBR322 sequences
- B) Phage lambda filler DNA
- C) Chloroplast tRNA genes
- D) M13mp18 polylinker

4. Which genes have been widely used as screenable markers to identify plant cells transformed by disarmed Ti vectors?

- A) *ampR* and *tcR*
- B) *ocs* and *nos*
- C) *lacZ* and *cat*
- D) *his3* and *leu2*

Answer Key

* **1. B** — Wild-type Ti plasmids cause disorganized tumorous growth due to oncogenes in the T-DNA, making plant regeneration difficult.

* **2. A** — Disarming a Ti vector is achieved by deleting its oncogenes to make it nononcogenic.

* **3. A** — In pGV3850, pBR322 sequences substituted for almost all of the T-DNA, leaving the border regions and *nos* gene.

* **4. B** — Opine production genes like *ocs* and *nos* have been widely used as screenable markers for transformed plant cells.

Lesson 184: Two plasmid strategy for transformation

1. In the two-plasmid (binary) transformation strategy, what is the role of the altered Ti plasmid from which T-DNA was removed?

- A) It supplies the *vir* gene products needed to direct T-DNA transfer
- B) It encodes kanamycin resistance for plant selection
- C) It provides the foreign gene flanked by 25-bp repeats
- D) It induces crown gall tumor formation at wound sites

2. What structural feature flanks the foreign gene and selectable marker in the shuttle vector of a binary system?

- A) 25-bp T-DNA repeat border sequences
- B) 100-bp inverted terminal repeats
- C) EcoRI restriction sites
- D) Polyadenylated tails

3. Which selectable marker is commonly included alongside the foreign gene in the shuttle vector to identify transformed plant cells?

- A) Resistance to kanamycin
- B) Sensitivity to carbenicillin
- C) Loss of beta-galactosidase activity
- D) Auxotrophic histidine complementation

4. Why are crown galls NOT formed when plants are transformed using the two-plasmid strategy?

- A) Because T-DNA genes for auxin, cytokinin, and opine biosynthetic enzymes are absent from both plasmids
- B) Because Agrobacterium is killed by high temperature before infection
- C) Because plant cell walls become immune to bacterial infection
- D) Because kanamycin destroys all bacterial vir proteins

Answer Key

* **1. A** — The altered Ti plasmid provides *vir* gene products that direct the transfer of the foreign gene from the second plasmid.

* **2. A** — In the shuttle vector, the foreign gene and selectable marker are flanked by the 25-bp T-DNA repeats.

* **3. A** — Transformed plant cells are selected using a marker such as resistance to the antibiotic kanamycin.

* **4. A** — Crown galls do not form because genes for auxin, cytokinin, and opine biosynthesis are deleted from both plasmids.

Lesson 185: Leaf-disk transformation by *A. tumefaciens*

1. What antibiotic is added to the culture medium after leaf-disk inoculation to selectively kill *Agrobacterium* cells?

- A) Kanamycin
- B) Carbenicillin
- C) Chloramphenicol
- D) Tetracycline

2. Which chimeric gene was included in the foreign DNA during leaf-disk transformation to confer resistance to kanamycin as a selectable marker?

- A) *neo* gene
- B) *lacZ* gene
- C) *cat* gene

D) *nos* gene

3. How long are leaf disks typically cultured with *A. tumefaciens* before being transferred to selection medium containing antibiotics?

- A) 2 days
- B) 10 days
- C) 4 weeks
- D) 7 weeks

4. Approximately how long after the initial inoculation step are rooted plantlets ready to be transplanted into soil in the leaf-disk protocol?

- A) 1 to 2 days
- B) 4 to 7 weeks
- C) 6 to 8 months
- D) 1 year

Answer Key

* **1. B** — Carbenicillin is added to the culture medium to kill *Agrobacterium* after the initial co-cultivation step.

* **2. A** — The foreign DNA contained a chimeric *neo* gene conferring resistance to kanamycin to select for transformed cells.

* **3. A** — Disks are cultured for 2 days before transfer to medium containing selection and antibacterial agents.

* **4. B** — Rooted plantlets are transplanted to soil approximately 4 to 7 weeks after the initial inoculation step.

Lesson 186: Agrobacterium and monocots

1. Why was Agrobacterium infection of monocotyledonous plants traditionally considered inefficient?

- A) Monocot tissues produce excessive amounts of carbenicillin
- B) Wounded monocot tissues do not produce phenolic compounds such as acetosyringone at sufficient levels to induce vir gene expression
- C) Monocot cell walls lack cellulose completely
- D) Monocots degrade Ti plasmids with restriction enzyme EcoRI

2. Pretreatment of Agrobacterium suspension with wound exudate from which source enabled tumor induction in yam?

- A) Tobacco leaves
- B) Potato tubers
- C) Onion epidermis
- D) Rice seeds

3. Which cereal was the first monocot species successfully transformed using Agrobacterium?

- A) Wheat
- B) Maize
- C) Rice
- D) Barley

4. Which two monocot species were shown to form tumors in laboratory tests during early studies on Agrobacterium infection?

- A) Asparagus and yam
- B) Wheat and oat
- C) Tomato and tobacco
- D) Arabidopsis and mustard

Answer Key

* **1. B** — Infection was inefficient because wounded monocot tissues fail to produce sufficient levels of phenolics like acetosyringone to activate vir genes.

* **2. B** — Pretreatment of Agrobacterium with potato tuber wound exudate facilitated tumor induction in yam.

* **3. C** — Rice was the first cereal species to be transformed using Agrobacterium.

* **4. A** — Laboratory experiments demonstrated tumor induction in the monocot species asparagus and yam.

Lesson 187: A. rhizogenes and Ri plasmids

1. What plant condition is naturally induced by Agrobacterium rhizogenes infection?

- A) Crown gall disease
- B) Hairy-root disease
- C) Damping off
- D) Bacterial blight

2. Why are root-inducing (Ri) plasmids considered functionally equivalent to disarmed Ti plasmids?

- A) They lack border sequences completely
- B) Opine-producing roots induced by Ri plasmids can be regenerated into whole plants without deleterious effects, and Ri T-DNA is transmitted sexually
- C) They do not transfer any DNA into the host genome
- D) They contain chloramphenicol resistance genes instead of oncogenes

3. What advantage do hairy root cultures offer over cell-suspension cultures for producing secondary metabolites?

- A) They grow without carbon or nitrogen sources
- B) They can produce certain valuable secondary metabolites at higher levels and are much more genetically stable
- C) They do not require aseptic culture media
- D) They convert eukaryotic DNA into single-stranded viral RNA

4. How can whole plants be recovered from opine-producing root tissue induced by Ri plasmids?

- A) By exposing roots to high-voltage electricity
- B) By manipulation of phytohormones in the culture medium
- C) By treating roots with cyanogen bromide
- D) By bombarding roots with gold microprojectiles

Answer Key

- * **1. B** — Agrobacterium rhizogenes naturally induces hairy-root disease in susceptible plants.
- * **2. B** — Ri plasmids act like disarmed Ti plasmids because transformed root tissues can regenerate into fertile, non-deleterious plants that transmit Ri T-DNA sexually.
- * **3. B** — Hairy root cultures offer greater genetic stability and higher yield potential for valuable secondary metabolites than cell suspensions.
- * **4. B** — Transformed root tissues are regenerated into whole fertile plants by manipulating phytohormone levels in culture.

Lesson 188: Protoplasts transformation

1. Which chemical compound is most widely used to promote direct gene transfer across the plant protoplast membrane?

- A) Diethylaminoethyl dextran (DEAE-dextran)
- B) Polyethylene glycol (PEG)
- C) Isopropyl- β -D-thiogalactoside (IPTG)
- D) Sodium dodecyl sulphate (SDS)

2. Besides chemical transfection, which physical method is commonly used to induce DNA uptake into plant protoplasts?

- A) Electroporation
- B) Particle bombardment with shotgun pellets
- C) Sonication with high-frequency X-rays
- D) Microinjection with glass micropipettes into chloroplasts

3. Why is a selectable marker gene required alongside the transgene during protoplast transformation?

- A) To digest cell-wall cellulose after transformation
- B) To identify and isolate stable transformants from non-transformed cells
- C) To activate host RNA polymerase II
- D) To prevent the plasmid from circularizing

4. What vector format is used to deliver both the selectable marker and the target transgene into protoplasts?

- A) Phage M13 single-stranded DNA
- B) Plasmid vectors carrying both the marker and transgene
- C) Intact retroviral capsids
- D) Linearized YAC arms without centromeres

Answer Key

- * **1. B** — Polyethylene glycol (PEG) is the most widely used chemical to promote DNA uptake across protoplast membranes.
- * **2. A** — Electroporation is a favoured physical technique for inducing direct DNA uptake into protoplasts.
- * **3. B** — Selectable markers are required to distinguish and select stable transformants from untransfected protoplasts.

* **4. B** — Plasmid vectors carrying both the selectable marker and the transgene of interest are used for co-delivery.

Lesson 189: Particle bombardment

1. What size range and velocity characterize the metal particles used in particle bombardment of plant cells?

- A) 1–4 μm diameter at a velocity of ~ 250 m/s
- B) 10–20 μm diameter at a velocity of ~ 1000 m/s
- C) 50–100 μm diameter at a velocity of ~ 10 m/s
- D) 0.1–0.5 μm diameter at a velocity of ~ 50 m/s

2. In the initial test system for particle bombardment (1987), which tissue and viral nucleic acid were used?

- A) Tobacco leaf disks coated in CaMV DNA
- B) Intact onion epidermis bombarded with tungsten particles coated in tobacco mosaic virus (TMV) RNA
- C) Rice calli coated in Agrobacterium T-DNA
- D) Carrot protoplasts coated in brome mosaic virus RNA

3. Which reporter gene driven by the CaMV 35S promoter was used to demonstrate DNA delivery via particle bombardment?

- A) *neo* gene
- B) *cat* (chloramphenicol acetyltransferase) gene
- C) *lacZ* gene
- D) *luc* (luciferase) gene

4. What percentage of onion cells containing tungsten particles showed evidence of TMV replication 3 days after bombardment?

- A) Approximately 10%
- B) Approximately 40%
- C) Exactly 75%
- D) 100%

Answer Key

* **1. A** — Small metal particles of 1–4 μm are accelerated to ~ 250 m/s to penetrate plant cell walls.

* **2. B** — Intact onion epidermal tissue was bombarded with tungsten particles coated in TMV RNA.

* **3. B** — The *cat* reporter gene driven by the CaMV 35S promoter demonstrated successful plasmid DNA delivery.

* **4. B** — Approximately 40% of particle-containing onion cells exhibited evidence of viral replication after 3 days.

Lesson 190: Chloroplast transformation

1. How much higher can transgene expression levels be in chloroplast transformation compared to nuclear transformation?

- A) Up to 2 times higher
- B) Up to 10 times higher
- C) Up to 50 times higher
- D) Up to 500 times higher

2. Why does chloroplast transformation provide a natural biological containment mechanism for transgenic plants?

- A) Chloroplast DNA dissolves upon seed harvest
- B) The transgene cannot be transmitted through pollen
- C) Chloroplasts inhibit seed germination in wild plants
- D) Transgenic chloroplasts undergo autolysis in darkness

3. Which expression phenomenon commonly affecting nuclear transgenes is avoided in chloroplast integrated transgenes?

- A) Gene silencing and position effects
- B) Polyadenylation
- C) Translation initiation
- D) Transcription termination

4. Why can high expression levels be achieved when targeting chloroplasts for genetic transformation?

- A) Photosynthetic cells contain thousands of chloroplasts per cell
- B) Chloroplasts lack RNA polymerases and rely on cytoplasm translation
- C) Chloroplast DNA contains no restriction sites
- D) Chloroplast transgenes bypass the genetic code

Answer Key

* **1. C** — Transgene expression levels in chloroplasts can reach up to 50 times higher than nuclear transformation levels.

* **2. B** — Transgenes in chloroplast DNA are not transmitted through pollen, providing natural gene containment.

* **3. A** — Transgenes integrated into chloroplast DNA do not undergo gene silencing or suffer from position effects.

* **4. A** — The presence of thousands of chloroplast copies in photosynthetic cells enables very high transgene expression levels.

Lesson 191: In planta transformation

1. Which model organism led the way in developing in planta transformation techniques to minimize or eliminate tissue culture?

- A) *Escherichia coli*
- B) *Arabidopsis thaliana*
- C) *Nicotiana tabacum*
- D) *Oryza sativa*

2. What key characteristic of *Arabidopsis thaliana* helps compensate for the very low efficiency of in planta transformation methods?

- A) Its inability to undergo sexual reproduction
- B) Its ability to produce over 10 000 seeds per plant
- C) Its complete lack of nuclear DNA
- D) Its resistance to all chemical antibiotics

3. Into which cells or stage must DNA be incorporated during in planta transformation to contribute to the germ line?

- A) Lignified cell walls of xylem vessels
- B) Cells contributing to the germ line, germ cells around fertilization, or the very early plant embryo
- C) Fully differentiated mesophyll protoplasts exclusively
- D) Root cap cells in mature taproots

4. What major requirement of traditional plant gene transfer is minimized or eliminated altogether by in planta transformation?

- A) The use of restriction endonucleases
- B) An obligatory tissue-culture step for regenerating whole fertile plants
- C) Polymerase chain reaction amplification of vector DNA
- D) Agarose gel electrophoresis of plasmid inserts

Answer Key

* **1. B** — *Arabidopsis thaliana* led the way in developing in planta transformation methods that minimize tissue culture.

* **2. B** — *Arabidopsis* produces over 10 000 seeds per plant, helping overcome the low efficiency of in planta methods.

* **3. B** — DNA must be incorporated into cells contributing to the germ line, germ cells, or early plant embryos.

* **4. B** — In planta transformation eliminates or minimizes the need for an obligatory tissue-culture regeneration step.

Lesson 192: Plant viruses as vectors

1. How can naked viral DNA or RNA recombinant vectors be introduced directly into intact plant cells?

- A) High-voltage electroporation of seeds
- B) Methods such as leaf rubbing
- C) Microinjection into vacuolar membranes
- D) Isopycnic centrifugation in CsCl

2. Why are plants NOT stably transformed when infected with plant viruses?

- A) Viruses dissolve the host plant genome completely
- B) All known plant viruses replicate episomally and do not integrate into or pass through the germ line
- C) Viral proteins inhibit plant cell division permanently
- D) Plant cells digest viral RNA using restriction enzyme EcoRI

3. What advantage does episomal replication of plant viruses provide regarding transgene expression?

- A) Transgenes are not subject to position effects that often influence integrated transgenes
- B) Transgenes integrate permanently into the host chloroplast genome
- C) Transgenes undergo immediate de novo methylation
- D) Transgenes require co-transformation with disarmed Ti plasmids

4. What feature of viral infections allows transgene expression to occur throughout the entire plant?

- A) Infections are strictly localized to root tip caps
- B) Viral infections are often systemic
- C) Viruses infect only guard cells around stomata
- D) Viral vectors transform pollen grains exclusively

Answer Key

- * **1. B** — Naked viral DNA or RNA can be directly introduced into plants by simple methods such as leaf rubbing.
- * **2. B** — Plant viruses replicate episomally and do not integrate into or pass through the germ line, preventing stable lines.
- * **3. A** — Episomal replication ensures that transgenes are not influenced by genomic position effects.
- * **4. B** — Systemic viral infection allows the virus to spread and express transgenes throughout all cells of the plant.

Lesson 193: Genetic modification of animals**1. Why are mammalian cells the most widely used hosts for delivering human genes to produce therapeutic proteins?**

- A) They allow authentic post-translational modifications not carried out by bacteria, yeast, or plants
- B) They divide ten times faster than *E. coli* in simple salt media
- C) They do not contain cell membranes or organelle structures
- D) They lack nuclear DNA, preventing native protein contamination

2. Which of the following product classes is explicitly listed in Topic 193 as being synthesized on a commercial scale in cultured mammalian cells?

- A) Synthetic restriction enzymes and ligases
- B) Antibodies, hormones, growth factors, cytokines, and vaccines
- C) Beta-galactosidase reporter enzymes exclusively
- D) Callus-inducing auxins and cytokinins

3. What type of experiments can be conducted by introducing DNA or RNA into hundreds of cultured cell types?

- A) Studying gene function and regulation, producing recombinant proteins, and manipulating the endogenous genome
- B) Converting eukaryotic chromosomes into circular bacterial plasmids
- C) Generating artificial plant cell walls from cellulose
- D) Preventing RNA polymerase II from binding promoters

4. On what broad categories of vectors has research into animal cell transformation focused?

- A) Viral, bacterial, and synthetic delivery vectors
- B) Cosmid vectors and phasmid vectors exclusively
- C) M13 filamentous phages and lambda Charon vectors
- D) Ti-plasmids and Ri-plasmids

Answer Key

* **1. A** — Mammalian cells allow production of recombinant human proteins with authentic post-translational modifications.

* **2. B** — Mammalian cell cultures synthesize valuable commercial products including antibodies, hormones, growth factors, cytokines, and vaccines.

* **3. A** — Delivering nucleic acids into cultured cells enables studying gene function, producing proteins, and manipulating genomes.

* **4. A** — Animal cell transformation vector systems are based on viral, bacterial, and synthetic delivery vectors.

Lesson 194: Strategies to transform animal cells**1. What term is used for gene delivery using a bacterial vector that invades animal cells and carries a plasmid?**

- A) Transduction
- B) Bactofection
- C) Lipofection
- D) Electroporation

2. How is viral vector-mediated delivery of genetic material to animal cells designated?

- A) Transfection
- B) Transduction
- C) Transformation
- D) Conjugation

3. What term distinguishes non-biological delivery mechanisms from those involving infection with a bacterium or virus?

- A) Transduction
- B) Transfection
- C) Lysogeny
- D) Infection

4. How do positively charged chemical transfection complexes interact with target animal cells to promote DNA uptake?

- A) By cutting the cell membrane with S1 nuclease
- B) By interacting with the negatively charged cell membrane to promote uptake by endocytosis
- C) By bombarding the nucleus with high-velocity tungsten particles
- D) By dissolving nuclear membranes with sodium dodecyl sulphate

Answer Key

- * **1. B** — Gene delivery using bacterial vectors carrying plasmid transgenes into animal cells is termed bactofection.
- * **2. B** — Delivery of transgenes into animal cells using viral vectors is known as transduction.
- * **3. B** — Non-biological delivery methods that do not use viral or bacterial infection are termed transfection.
- * **4. B** — Synthetic complexes with an overall positive charge interact with negatively charged cell membranes to trigger endocytosis.
-
-

Lesson 195: Chemical transfection techniques-Calcium phosphate method

1. What selective medium was used to identify rare HPRT-positive human cells transfected with functional HPRT gene fragments?

- A) Minimal glucose agar
- B) HAT medium
- C) Ampicillin-Xgal agar
- D) Kanamycin-carbenicillin broth

2. What physical form must the DNA/calcium phosphate complex take at the time of transfection to achieve successful DNA transfer?

- A) A baked dry powder
- B) A fine co-precipitate formed freshly at the time of transfection
- C) A boiled liquid solution stored at 100°C
- D) A frozen crystalline gel block

3. By what cellular mechanism are the small granules of DNA-associated calcium phosphate thought to be taken up by recipient cells?

- A) Active transport through sodium-potassium pumps
- B) Endocytosis
- C) Direct mechanical injection
- D) Osmotic lysis

4. What enzyme deficiency was present in the human cell line used in early total uncloned genomic DNA calcium phosphate transfection experiments?

- A) Adenosine deaminase (ADA)
- B) Hypoxanthine-guanine phosphoribosyltransferase (HPRT)
- C) Thymidine kinase (TK)
- D) Beta-galactosidase (LacZ)

Answer Key

- * **1. B** — Rare HPRT-positive cells taking up functional gene fragments were identified using selection on HAT medium.
- * **2. B** — Successful transfection depends on a fine DNA/calcium phosphate co-precipitate formed freshly at transfection time.
- * **3. B** — Small granules of calcium phosphate associated with DNA settle on cells and are internalized by endocytosis.

* **4. B** — Total uncloned genomic DNA was initially used to transfect human cells deficient for the HPRT enzyme.

Lesson 196: Transfection with polyplexes

1. What was the earliest polycationic compound used to form soluble complexes (polyplexes) for transfection?

- A) DEAE-dextran
- B) Polyethylene glycol
- C) Ethidium bromide
- D) Sodium dodecyl sulphate

2. What major limitation caused DEAE-dextran to fall out of favor for gene transfer studies?

- A) It causes immediate cell wall synthesis in animal cells
- B) It cannot be used to generate stably transformed cell lines
- C) It degrades plasmid DNA into single-stranded mononucleotides
- D) It functions only at temperatures above 65°C

3. How do polycationic compounds form soluble polyplex complexes with DNA?

- A) Through covalent phosphodiester bonding
- B) Through spontaneous electrostatic interactions with DNA
- C) Through alkaline hydrolysis at depurinated sites
- D) Through enzymatic ligation by T4 DNA ligase

4. Which post-mitotic cell types can be stably transformed using the newer generation of polycationic compounds?

- A) Erythrocytes and platelets
- B) Neurons and myocytes
- C) Spheroplasts and calli
- D) Lymphocytes and megakaryocytes

Answer Key

* **1. A** — DEAE-dextran was the earliest soluble polycationic carbohydrate used to form polyplex complexes for nucleic acid transfer.

* **2. B** — DEAE-dextran fell out of favor because it is efficient only for transient transfection and cannot generate stably transformed cell lines.

* **3. B** — Polycationic compounds form polyplexes through spontaneous electrostatic interactions with DNA.

* **4. B** — Newer polycationic compounds allow stable transformation of primary cells and post-mitotic cells such as neurons and myocytes.

Lesson 197: Transfection with liposomes and lipoplexes

1. In the first example of liposome-mediated gene delivery, what agent was used to induce fusion between bacterial protoplasts and mammalian cell monolayers?

- A) Polyethylene glycol
- B) Calcium phosphate
- C) DEAE-dextran
- D) Sodium hypochlorite

2. What key advantage is provided by bacterial protoplast or yeast spheroplast fusion when delivering DNA into mammalian cells?

- A) It converts double-stranded DNA into retroviral RNA
- B) It is gentle and allows the transfer of large DNA fragments without shearing
- C) It eliminates the need for incubation at 37°C
- D) It prevents endocytosis in recipient cells

3. What term is used for the artificial phospholipid vesicles used to package DNA for cellular uptake?

- A) Polyplexes
- B) Liposomes
- C) Contigs
- D) Replicons

4. What breakthrough structure is spontaneously formed when cationic/neutral lipid mixtures are combined with DNA to promote endocytosis?

- A) Lipoplexes
- B) Spheroplasts
- C) Cosmid inserts
- D) Decameric linkers

Answer Key

* **1. A** — Bacterial protoplasts prepared with lysozyme were fused with mammalian cell monolayers using polyethylene glycol.

* **2. B** — Protoplast/spheroplast fusion is gentle, permitting the transfer of large DNA fragments (such as YACs) without shearing.

* **3. B** — Artificial phospholipid vesicles used for packaging DNA are called liposomes.

* **4. A** — Cationic/neutral lipid mixtures spontaneously form stable complexes with DNA called lipoplexes, which undergo endocytosis.

Lesson 198: Physical transfection techniques-Electroporation and ultrasound

1. What structural modification in the cell membrane is induced during electroporation by an electric pulse?

- A) Complete dissolution of the lipid bilayer
- B) Generation of transient, nanometer-sized pores
- C) Covalent cross-linking of membrane proteins
- D) Synthesis of a cellulose outer cell wall

2. Under what cell condition does the most efficient electroporation frequently occur according to Topic 198?

- A) When there is up to 50% cell death
- B) When cells are incubated at 0°C in pure glycerol
- C) When cell membranes are completely uncharged
- D) When cells are in stationary phase after 4 weeks

3. What physical phenomenon causes membrane cavity formation during ultrasound transfection?

- A) Endocytosis
- B) Cavitation
- C) Depurination
- D) Isopycnic banding

4. Which equipment requirement contributes to high input costs for electroporation?

- A) High-power X-ray diffractometers
- B) Specialized capacitor discharge machines capable of controlling pulse length and amplitude
- C) Automated ultracentrifuges with CsCl rotors
- D) UV transilluminators with photographic filters

Answer Key

* **1. B** — Electroporation exposes cells to a brief electric pulse that generates transient, nanometer-sized pores in the membrane.

* **2. A** — Electroporation efficiency is often highest under conditions that result in up to 50% cell death.

* **3. B** — Ultrasound waves produce bubble formation and collapse in liquids and cell membranes, a process known as cavitation.

* **4. B** — Electroporation requires a specialized capacitor discharge machine to precisely control pulse intensity and duration.

Lesson 199: Microinjection: Direct transfer of DNA

1. What distinguishes microinjection from chemical transfection methods?

- A) It delivers DNA directly into target cells without requiring a synthetic carrier
- B) It relies on viral capsids to penetrate nuclear membranes
- C) It can process millions of cells simultaneously in a single tube
- D) It works exclusively on bacterial endospores

2. What is stated as a primary operational limitation of microinjection in any single experiment?

- A) It can only be applied to a few cells in any one experiment
- B) It degrades foreign DNA prior to nuclear entry
- C) It requires high concentrations of calcium phosphate
- D) It can only transfer single-stranded RNA molecules under 20 nt

3. For which type of cultured cells is microinjection particularly useful?

- A) Cells that are recalcitrant to other transfection methods
- B) Cells that divide every 10 minutes

- C) Protoplasts lacking cell membranes
- D) Anucleated mammalian red blood cells

4. In in vivo applications, which tissues or routes are explicitly listed for direct needle injection of DNA?

- A) Skin, muscle, internal organs, or blood
- B) Chloroplast stroma and plant vacuolar sap
- C) Hair shafts and nail beds exclusively
- D) Bacterial peptidoglycan cell walls

Answer Key

- * **1. A** — Microinjection directly transfers DNA into the cell without using a synthetic carrier.
- * **2. A** — While guaranteed to yield successful hits, microinjection can only be applied to a few cells per experiment.
- * **3. A** — Microinjection is applied to cultured cells that are recalcitrant to other transfection procedures.
- * **4. A** — In vivo needle injection can deliver DNA directly into skin, muscle, internal organs, or the blood.

Lesson 200: Selectable markers for animal cells-Endogenous markers

1. What prerequisite condition is required when using endogenous selectable markers in animal cell transformation?

- A) The recipient cells must be mutant cell lines lacking the endogenous enzyme function
- B) The host cells must be grown in 10% sodium dodecyl sulphate
- C) The vector must carry a viral coat protein gene
- D) The cells must be pre-treated with lysozyme and PEG

2. Which viral gene was transfected into thymidine kinase-deficient (TK-) mouse cells to restore a wild-type phenotype?

- A) Herpes simplex virus (HSV) Tk gene
- B) Tobacco mosaic virus coat protein gene
- C) SV40 T-antigen gene
- D) Bacteriophage lambda cl gene

3. What mechanism of nucleotide synthesis is inhibited by the drug aminopterin in HAT selection medium?

- A) The salvage pathway
- B) The de novo pathway
- C) The general export pathway
- D) The pentose phosphate pathway

4. Which three components compose the HAT selective medium used to select Hprt+ and Tk+ transformants?

- A) Histidine, Ampicillin, and Tetracycline
- B) Hypoxanthine, Aminopterin, and Thymidine
- C) Hepes, Acridine, and Trypsin
- D) Hydrolyzed casein, Avidin, and Thiamine

Answer Key

- * **1. A** — Endogenous selectable markers are already present in the genome, so mutant cell lines are required.
- * **2. A** — TK- deficient mouse cells were stably transformed to a wild-type phenotype using the HSV Tk gene.
- * **3. B** — Aminopterin blocks the de novo synthesis of nucleotides by inhibiting key enzymes in that pathway.
- * **4. B** — HAT selective medium contains Hypoxanthine, Aminopterin, and Thymidine.

Lesson 201: Bacterial vectors for the transfection of animal cell

1. What was often used as a nonspecific carrier to bulk up donor DNA in early calcium phosphate transfection experiments?

- A) Cleaved salmon sperm DNA
- B) Synthetic polyadenylated RNA
- C) DEAE-dextran
- D) Bovine serum albumin

2. Which early expression vectors incorporated transcriptional control sequences from SV40 and Rous sarcoma virus into pBR322?

- A) pSV and pRSV plasmids
- B) pUC19 and pBR325
- C) pJB8 and c2XB
- D) YEps and YACs

3. Which selectable marker gene was included in the pSV2 series of vectors alongside pSV2-gpt and pSV2-dhfr?

- A) pSV2-neo
- B) pSV2-cat
- C) pSV2-tet
- D) pSV2-amp

4. What feature allows certain plasmid vectors for animal gene transfer to function as shuttle vectors maintained as episomal replicons?

- A) Origins of replication functional in animal cells
- B) Poly(A) tails attached to the plasmid backbone
- C) Unphosphorylated BamHI adaptors
- D) Retroviral capsids surrounding the plasmid DNA

Answer Key

- * **1. A** — Early calcium phosphate experiments bulked up donor DNA using a nonspecific carrier such as cleaved salmon sperm DNA.
- * **2. A** — The pSV and pRSV plasmids contain transcriptional control sequences from SV40 and Rous sarcoma virus.
- * **3. A** — The pSV2 series of vectors included pSV2-neo, pSV2-gpt, and pSV2-dhfr as selectable markers.

* **4. A** — Shuttle vectors carry origins of replication functional in animal cells to allow episomal maintenance.

Lesson 202: Viruses as vector for gene transfer

1. What natural capability of virus particles is exploited to deliver recombinant DNA into animal cells?

- A) Natural ability to adsorb to cell surfaces and gain entry
- B) Ability to digest cellulose cell walls
- C) Synthetic binding to calcium phosphate crystals
- D) Formation of lipoplex vesicles in vitro

2. Besides cultured cells, to what recipients can viral vectors deliver nucleic acids for high-level gene expression and replication?

- A) Living animals
- B) Dry plant seeds
- C) Isolated cell wall matrices
- D) Synthetic liposomes

3. How many classes of viral vectors have been used in clinical trials for human gene therapy according to Topic 202?

- A) At least eight
- B) Exactly two
- C) Over one hundred
- D) Zero

4. In addition to gene therapy in humans and expression in cultured cells, what additional application of viral vectors is highlighted in Topic 202?

- A) Recombinant vaccines
- B) Production of synthetic restriction enzymes
- C) Cleavage of double-stranded RNA
- D) Isopycnic density gradient centrifugation

Answer Key

* **1. A** — Viral delivery exploits the natural ability of virus particles to adsorb to the surface of cells and gain entry.

* **2. A** — Viral vectors are used for gene transfer both in cultured cells and in living animals.

* **3. A** — At least eight classes of viral vectors have been used in clinical trials for human gene therapy.

* **4. A** — Topic 202 notes that viral vectors have also been developed as recombinant vaccines.

Lesson 203: Methods for the production of transgenic mice

1. What application of genetically modified mammals is highlighted alongside studying gene function and regulation in Topic 203?

- A) Functioning as bioreactors producing valuable recombinant proteins (e.g. in their milk)
- B) Degrading synthetic plastics in soil
- C) Synthesizing artificial blood in cell-free systems
- D) Producing commercial antibiotics via fermentation

2. What essential sequence of handling steps is shared by methods for mouse germline transformation?

- A) Removal of fertilized eggs/embryos, brief in vitro culture, and return to foster mothers
- B) Electroporation of adult bone marrow directly in vivo
- C) Chemical precipitation of sperm with calcium phosphate
- D) Direct injection of naked plasmid into adult ovaries

3. While traditional germline transformation methods were developed for nuclear transgenesis, which organellar genome has recently been targeted for transgenic production?

- A) Mitochondria
- B) Chloroplast
- C) Peroxisome
- D) Endoplasmic reticulum

4. What impact did developing germline transformation in mice have on animal biotechnology according to Topic 203?

- A) It paved the way for the transformation of other mammals
- B) It eliminated the need for embryo culture in all species
- C) It made all transgenic animals sterile
- D) It completely replaced cell culture methods in research

Answer Key

* **1. A** — Transgenic mammals serve as bioreactors to produce valuable recombinant proteins in fluids like milk.

* **2. A** — All germline transformation methods require removing fertilized eggs/embryos, brief in vitro culture, and transfer to foster mothers.

* **3. A** — Methods have recently been developed for producing mitochondrial transgenics.

* **4. A** — Introducing DNA into the germline of mice paved the way for transforming other mammals.

Lesson 204: Pronuclear microinjection

1. In the first strategy used to generate transgenic mice, what viral DNA was injected into the blastocoele cavities of preimplantation embryos?

- A) Simian virus 40 (SV40) DNA
- B) Tobacco mosaic virus (TMV) RNA
- C) Rous sarcoma virus (RSV) DNA
- D) Bacteriophage T4 DNA

2. Why is the male pronucleus usually chosen over the female pronucleus for direct microinjection just after fertilization?

- A) Because the male pronucleus (sperm nucleus) is larger than the female pronucleus

- B) Because the female pronucleus lacks a nuclear membrane
- C) Because the male pronucleus contains no histones
- D) Because the female pronucleus degrades foreign DNA immediately

3. What volume of DNA solution is typically transferred into the target pronucleus during microinjection?

- A) About 2 pL
- B) About 2 μ L
- C) About 2 mL
- D) About 200 nL

4. What outcome can occur if injected exogenous DNA remains extrachromosomal for one or more cell divisions before integrating?

- A) The resulting animal may be mosaic for transgene insertion
- B) The embryo undergoes immediate cell death
- C) The transgene converts into double-stranded RNA
- D) The animal develops an extra pair of chromosomes

Answer Key

* **1. A** — SV40 DNA was injected into blastocoele cavities in the first strategy used to generate transgenic mice.

* **2. A** — The larger male pronucleus (sperm nucleus) is usually chosen as the target for injection.

* **3. A** — Approximately 2 pL of DNA solution is transferred into the nucleus through a fine needle.

* **4. A** — Delayed integration can result in animals that are mosaic for transgene insertion.

Lesson 205: Recombinant retroviruses-germline transformation

1. What is an advantage of retroviral germline transformation over pronuclear microinjection?

- A) Only a single copy of the retroviral provirus is integrated, leaving surrounding genomic DNA generally intact
- B) Retroviruses can carry inserts up to 500 kb in size
- C) Retroviruses are completely immune to de novo methylation
- D) Retroviral vectors require no foster mothers for development

2. What structural form of the retrovirus integrates stably into the host animal genome?

- A) Retroviral provirus
- B) Single-stranded RNA capsid
- C) Circular plasmid dimer
- D) Polycationic polyplex

3. Which disadvantage of retroviral vectors can lead to the loss of transgene expression (transgene silencing)?

- A) Susceptibility of the virus to de novo methylation
- B) Absence of viral promoters
- C) Shearing of genomic DNA into 100 bp fragments
- D) High toxicity to pseudopregnant foster mothers

4. Which limitation restricts the size of the transgene that can be delivered using recombinant retroviruses?

- A) The limited amount of foreign DNA that can be carried by the virus
- B) The absence of reverse transcriptase in early embryos
- C) The complete lack of viral envelope proteins
- D) The requirement for high-voltage electroporation

Answer Key

- * **1. A** — Retroviral vectors integrate a single provirus copy and keep surrounding genomic DNA intact.
- * **2. A** — A single copy of the retroviral provirus integrates into the genome.
- * **3. A** — Susceptibility to de novo methylation can lead to transgene silencing.
- * **4. A** — Recombinant retroviruses are limited in the amount of foreign DNA they can carry.

Lesson 206: Transfection of ES cells**1. From which structure of the mouse blastocyst are embryonic stem (ES) cells derived?**

- A) The outer trophectoderm
- B) The inner cell mass
- C) The zona pellucida
- D) The blastocoele fluid

2. In what year was the ability of ES cells to contribute to the germline first demonstrated?

- A) 1971
- B) 1981
- C) 1984
- D) 1996

3. How are transformed ES cells identified after DNA introduction by transfection or transduction?

- A) By observing fluorescent signals under UV light directly in the petri dish
- B) By selecting transformed cells using standard selection markers
- C) By destroying all non-transformed cells with high-voltage electricity
- D) By performing Southern blotting on whole blastocysts

4. Why must potential transgenic mice generated by pronuclear microinjection be individually tested by Southern blot or PCR?

- A) Because microinjection destroys all cell markers
- B) Because there is no convenient way to select for eggs or embryos that have taken up foreign DNA
- C) Because ES cells cannot survive transfection in vitro
- D) Because microinjection only transfers single-stranded RNA

Answer Key

- * **1. B** — ES cells are derived from the inner cell mass of the mouse blastocyst.
- * **2. C** — The ability of ES cells to contribute to the germline was first demonstrated in 1984.
- * **3. B** — Since ES cells can be serially cultured, transformed cells can be selected using standard markers.

* **4. B** — Potential transgenic mice must be tested individually because there is no convenient way to select eggs or embryos that incorporated foreign DNA.

Lesson 207: Gene transfer to *Xenopus*

1. Which researchers first showed in 1971 that *Xenopus* oocytes synthesized large amounts of globin after microinjection with rabbit globin mRNA?

- A) Gurdon et al.
- B) Melton et al.
- C) Young and Davies
- D) Benton and Davis

2. What is the common name and species of the frog used in the *Xenopus* expression system?

- A) *Xenopus tropicalis* (Western clawed frog)
- B) *Xenopus laevis* (African clawed frog)
- C) *Rana temporaria* (Common European frog)
- D) *Lithobates catesbeianus* (American bullfrog)

3. What is the large nucleus of a fully grown *Xenopus* oocyte called, and in which hemisphere is it located?

- A) Nucleolus, located in the unpigmented vegetal hemisphere
- B) Germinal vesicle, located in the darkly pigmented hemisphere
- C) Polar body, located in the equatorial zone
- D) Blastomere, located in the transparent cytoplasm

4. Up to what embryonic stage can the store of eukaryotic RNA polymerases in the *Xenopus* oocyte nucleus furnish the needs of the developing embryo?

- A) Up to the 100-cell stage
- B) Up to the 1,000-cell stage
- C) At least until the 60,000-cell stage
- D) Indefinitely into adulthood

Answer Key

* **1. A** — Gurdon et al. (1971) first demonstrated globin synthesis in *Xenopus* oocytes microinjected with rabbit globin mRNA.

* **2. B** — *Xenopus laevis* is the African clawed frog.

* **3. B** — The large nucleus is called the germinal vesicle and is located in the darkly pigmented hemisphere.

* **4. C** — The oocyte nucleus contains a store of all three eukaryotic RNA polymerases sufficient until at least the 60,000-cell stage.

Lesson 208: Rabbit reticulocytes

1. What type of cells are reticulocytes?

- A) Mature white blood cells
- B) Immature red blood cells
- C) Differentiated platelets
- D) Embryonic stem cells

2. What unique cellular characteristic makes reticulocytes particularly useful for studying protein translation in vitro?

- A) They lack ribosomes and tRNA
- B) They contain all translation machinery but lack a nucleus
- C) They possess double-stranded RNA genomes
- D) They divide rapidly every 5 minutes in culture

3. What system do scientists establish using extracted reticulocyte mRNA and enzymes to study translation under controlled conditions?

- A) A cell-free, in vitro translation system
- B) A whole-organ culture system
- C) A retroviral transduction pathway
- D) A high-voltage electroporation cell array

4. From which animal species are reticulocytes commonly collected to study protein translation in vitro according to Topic 208?

- A) Mice
- B) Rabbits
- C) Horses
- D) Chickens

Answer Key

* **1. B** — Reticulocytes are immature red blood cells.

* **2. B** — Reticulocytes contain all the translation machinery but lack a nucleus, providing greater control for translation studies.

* **3. A** — Scientists extract mRNA and translation enzymes to study protein translation in a cell-free, in vitro system.

* **4. B** — Reticulocytes are collected from animals such as rabbits to study translation in vitro.

Lesson 209: Frog oocyte system**1. Which human medical condition's basic science (initiation and progression) is directly studied using *Xenopus* research models?**

- A) Diabetes mellitus
- B) Cancer
- C) Sickle cell anemia
- D) Cystic fibrosis

2. What specific class of proteins can be rapidly and easily assayed for functional activity using expression in *Xenopus* oocytes?

- A) Nuclear histones
- B) Channel and transporter proteins
- C) Ribosomal structural proteins
- D) Collagen and elastin fibers

3. Which three modes of *Xenopus* research are listed in Topic 209 for studying human disease genes?

- A) Calli, protoplasts, and explants
- B) Embryos, cell-free extracts, and oocytes
- C) Reticulocytes, lymphocytes, and myocytes
- D) Spheroplasts, endospores, and capsids

4. What main insight into human disease has expression in *Xenopus* oocytes provided regarding cellular physiology?

- A) Understanding channel and transporter protein activity
- B) Measuring cell wall peptidoglycan synthesis
- C) Tracking mitochondrial DNA replication rates
- D) Analyzing antibody heavy-chain gene rearrangement

Answer Key

* **1. B** — *Xenopus* research modes are used to study the basic science underlying the initiation and progression of cancer.

* **2. B** — A major strength of the *Xenopus* oocyte system is the ability to rapidly assay channel and transporter proteins.

* **3. B** — The three modes of *Xenopus* research mentioned are embryos, cell-free extracts, and oocytes.

* **4. A** — Assaying channel and transporter protein activity in oocytes has led to important insights into human disease.

Lesson 210: The different ways that recombinant DNA technology has been exploited

1. What term is used for the medical field that applies gene transfer technology to humans to prevent, treat, or cure diseases?

- A) Gene medicine
- B) Pharmacogenomics
- C) Positional cloning
- D) Molecular autopsy

2. Which of the following is explicitly listed in Topic 210 as a concept included under gene medicine?

- A) DNA vaccines, gene transfer to kill cancer cells, and repairing genetic defects
- B) Chemical synthesis of artificial red blood cells
- C) Conversion of human cells into plant protoplasts
- D) Removal of all nuclear membranes from somatic cells

3. Why are animal genomes deliberately mutated using gene-transfer techniques as described in Topic 210?

- A) To produce synthetic plastic-degrading enzymes
- B) To recreate mutations seen in human inherited diseases and develop disease models to test novel drugs
- C) To prevent animals from undergoing meiosis
- D) To eliminate the need for cellular ribosomes

4. How does the application of trait modification in humans compare to that in animals and plants according to Topic 210?

- A) It is practiced universally without any restrictions
- B) It is applied to humans in a more limited sense to prevent or cure diseases
- C) It is strictly forbidden for all cell types including somatic cells
- D) It is used exclusively to change phenotypic hair and eye color

Answer Key

* **1. A** — Applying gene transfer to humans to prevent or cure disease is known as gene medicine.

* **2. A** — Gene medicine includes DNA vaccines, targeted killing of cancer cells, and repairing genetic defects.

* **3. B** — Animal genomes are mutated to recreate human disease mutations and build models for studying diseases and testing drugs.

* **4. B** — Trait modification is applied to humans in a more limited sense specifically aimed at preventing or curing diseases.

Lesson 211: Commercial production of recombinant therapeutic proteins**1. Which two human therapeutic proteins were the first recombinant versions produced commercially in bacteria?**

- A) Insulin and hemoglobin
- B) Human growth hormone and insulin
- C) Erythropoietin and interferon
- D) Tissue plasminogen activator and factor VIII

2. What serious risk was associated with human growth hormone obtained from cadaver pituitary glands prior to recombinant DNA technology?

- A) Hepatitis B transmission
- B) Contracting Creutzfeld–Jacob syndrome
- C) Severe allergic anaphylaxis
- D) Autoimmune destruction of the pancreas

3. Blood components, anticoagulants, and growth factors isolated from human blood carry the risk of which infectious disease if not produced recombinantly?

- A) Tuberculosis
- B) HIV infection
- C) Malaria
- D) Creutzfeld–Jacob syndrome

4. In addition to growth hormone and insulin, which group of proteins is specifically mentioned as being identified and produced in bacteria as cloning methods became more sophisticated?

- A) Cytokines, interferons, and interleukins
- B) Monoclonal IgA immunoglobulins
- C) Ribosomal structural proteins
- D) Collagen and elastin fibers

Answer Key

* **1. B** — Human growth hormone and insulin were the first recombinant versions of human therapeutic proteins produced in bacteria.

* **2. B** — Obtaining human growth hormone from cadaver pituitaries caused some recipients to contract Creutzfeld–Jacob syndrome.

* **3. B** — Recombinant production eliminates the risk of HIV infection associated with products isolated from human blood.

* **4. A** — Sophisticated gene cloning enabled the identification and bacterial production of cytokines, interferons, and interleukins.

Lesson 212: Biopharmaceuticals approved in different countries

1. How do second-generation protein biopharmaceuticals differ from first-generation protein drugs?

- A) First-generation drugs were synthetic small molecules, whereas second-generation drugs are monoclonal antibodies
- B) First-generation drugs were exact copies of human molecules, whereas second-generation drugs are engineered for improved properties
- C) Second-generation drugs are extracted exclusively from plant cell walls
- D) Second-generation drugs require higher doses due to lower stability

2. Which structural modifications are used in second-generation protein engineering to improve efficacy or longevity?

- A) Replacing certain amino acids or removing particular protein domains
- B) Adding bacterial peptidoglycan tails to the C-terminus
- C) Converting proteins into double-stranded DNA plasmid vectors
- D) Methylating all internal lysine residues with EcoRI methylase

3. What combination is frequently used in novel antibody-based drugs to achieve targeted therapeutic effects?

- A) Combining viral capsid proteins with bacterial endonucleases
- B) Combining antibody binding specificity with the activity of other proteins like cytokines and lymphokines
- C) Combining plant chlorophyll with mammalian cell receptors
- D) Combining tRNA anti-codons with synthetic lipid bilayers

4. Which regions are explicitly mentioned in Topic 212 as having approved biopharmaceuticals such as vaccines, hormones, and blood factors?

- A) Asia and South America

- B) The United States and Europe
- C) Australia and Africa
- D) Canada and Japan

Answer Key

- * **1. B** — First-generation protein drugs were exact copies of human molecules, while second-generation drugs are modified via protein engineering.
- * **2. A** — Second-generation proteins are engineered by replacing specific amino acids or removing protein domains to enhance efficacy or longevity.
- * **3. B** — Novel therapeutics combine antibody binding specificity with active proteins like cytokines/lymphokines to target specific cells.
- * **4. B** — Approved biopharmaceuticals in the United States and Europe include blood factors, anticoagulants, hormones, interferons, vaccines, and antibodies.

Lesson 213: Transgenic animals and plants to produce recombinant proteins

1. In which fluid of transgenic mice was continuous production of recombinant growth hormone first demonstrated?

- A) Saliva
- B) Serum
- C) Urine
- D) Cerebrospinal fluid

2. Which promoter was joined to transgenes to direct the secretion of recombinant proteins like tPA into mouse milk?

- A) CaMV 35S promoter
- B) Mammary-specific promoter (such as from the casein gene)
- C) Lac promoter
- D) SV40 early promoter

3. What were the first two recombinant proteins reported to be produced in mouse milk using mammary-specific promoters?

- A) Insulin and growth hormone
- B) Sheep β -lactoglobulin and human tissue plasminogen activator (tPA)
- C) Human serum albumin and factor IX
- D) Interferon-alpha and rabies glycoprotein

4. Why are larger animals such as sheep and goats investigated as bioreactors instead of mice for producing proteins in milk?

- A) Mice milk contains proteases that degrade all recombinant proteins
- B) Mice produce a very small volume of milk
- C) Larger animals do not require mammary-specific promoters
- D) Casein promoters do not function in rodents

Answer Key

* **1. B** — Growth hormone production in the serum of transgenic mice provided the first evidence of continuous recombinant protein production in body fluids.

* **2. B** — Transgenes were joined to a mammary-specific promoter, such as the casein gene promoter, to direct protein expression into milk.

* **3. B** — Sheep β -lactoglobulin and human tissue plasminogen activator (tPA) were the first proteins produced in mouse milk.

* **4. B** — Mouse milk yields high protein concentrations but in very small volumes, making sheep and goats preferable bioreactors.

Lesson 214: Therapeutic recombinant proteins expressed in genetically modified plants

1. What advantage do genetically modified plants offer as subunit vaccine production systems regarding administration?

- A) Vaccines can be administered via inhalation without any plant tissue
- B) Orally administered vaccines can be ingested directly by eating the plant, limiting the need for processing and purification
- C) Vaccines do not induce mucosal immune responses
- D) Plant tissue eliminates the need for host immune cells

2. The expression of a surface antigen from which bacterium in tobacco represented the earliest demonstration of plant-derived subunit vaccines?

- A) *Streptococcus mutans*
- B) *Escherichia coli*
- C) *Bacillus thuringiensis*
- D) *Xanthomonas oryzae*

3. Which disease-causing bacterium or virus antigen is correctly matched with its plant expression host in Topic 214?

- A) Rabies glycoprotein in tomato
- B) Hepatitis B virus antigen in potato
- C) Cholera antigen in lettuce
- D) Influenza virus antigen in tobacco

4. In addition to viral and bacterial antigens, which type of human health-related antigens have been produced in transgenic plants according to Topic 214?

- A) Autoantigens associated with diabetes
- B) Synthetic hemoglobin chains
- C) Prion proteins causing CJD
- D) Oncogenic Ras mutants

Answer Key

* **1. B** — Edible plant vaccines allow oral administration by eating the plant, reducing processing and purification requirements.

* **2. A** — Surface antigen of *Streptococcus mutans* (the cause of tooth decay) expressed in tobacco was the earliest demonstration.

* **3. A** — Topic 214 specifies rabies glycoprotein in tomato, hepatitis B antigen in lettuce, and cholera antigen in potato.

* **4. A** — Autoantigens associated with diabetes have been produced in transgenic plants alongside animal virus antigens.

Lesson 215: Metabolic engineering-production of small molecules in bacteria

1. When large-scale production of penicillin began in the 1940s, how were yields initially measured?

- A) Kilograms per liter
- B) Micrograms per liter
- C) Grams per milliliter
- D) Milligrams per gallon

2. Because nothing was known about the penicillin biosynthetic pathway in the 1940s, what approach was used for strain improvement?

- A) Site-directed cassette mutagenesis
- B) Random mutation and screening
- C) Automated four-dye DNA sequencing
- D) Direct transformation with Ti plasmids

3. What level of penicillin yield per liter has been achieved today through iterative cycles of selection and improvement?

- A) Micrograms per liter
- B) Tens of grams per liter
- C) Hundreds of kilograms per liter
- D) Exactly 1 milligram per liter

4. What scientific knowledge was required before researchers could understand how antibiotic yields might be rationally improved?

- A) Elucidation of the details of gene regulation and metabolic pathway regulation
- B) Complete sequencing of the human nuclear genome
- C) Synthesis of decameric linkers with BamHI sites
- D) Development of 2D gel electrophoresis

Answer Key

* **1. B** — Large-scale penicillin production in the 1940s initially yielded only micrograms per liter of culture.

* **2. B** — Strain improvement was carried out via random mutation and screening without prior knowledge of the biosynthetic pathway.

* **3. B** — Strain selection systematically increased penicillin yields from micrograms per liter up to tens of grams per liter today.

* **4. A** — Rational improvement of antibiotic yields became possible only after understanding gene regulation and metabolic pathways.

Lesson 216: Metabolic engineering-in plant to produce diverse chemical structures

1. How are plant secondary metabolites defined in Topic 216?

- A) Primary cellular components essential for basic mitosis and cell division
- B) Products of biochemical pathways not involved in synthesizing essential cellular components, providing functions like attracting pollinators or pest resistance
- C) Single-stranded RNA molecules involved in protein translation
- D) Cell wall polysaccharides responsible for mechanical support

2. Which set of secondary metabolites is explicitly listed in Topic 216 as well-known examples having potent pharmaceutical properties in humans?

- A) Penicillin, streptomycin, tetracycline, and ampicillin
- B) Caffeine, nicotine, morphine, and cocaine
- C) Aspirin, ibuprofen, paracetamol, and naproxen
- D) Taxol, vincristine, vinblastine, and artemisinin

3. Why is transferring secondary metabolic pathways from plants into microbes often impossible for large-scale production?

- A) Microbes lack ribosomes for translating plant mRNA
- B) The secondary metabolic pathways of plants are extremely extensive and complex
- C) Plant genes are destroyed by bacterial restriction endonucleases
- D) Microbial cell walls prevent endocytosis of plant enzymes

4. How can large-scale plant cell cultures be utilized according to Topic 216?

- A) In the same manner as microbial cultures for the production of important phytochemicals
- B) Exclusively for converting diploid cells into haploid spores
- C) To synthesize bacterial peptidoglycan cell wall layers
- D) To eliminate the need for carbon dioxide in photosynthesis

Answer Key

* **1. B** — Secondary metabolites are complex molecules from pathways not involved in essential cellular synthesis, serving functions like attracting pollinators or resisting pests.

* **2. B** — Caffeine, nicotine, morphine, and cocaine are explicitly listed as well-known plant secondary metabolites with potent pharmaceutical properties.

* **3. B** — Plant secondary metabolic pathways are so extensive and complex that transferring all necessary components into microbes is usually impossible.

* **4. A** — Advances in plant transformation allow large-scale plant cell cultures to be used like microbial cultures for phytochemical production.

Lesson 217: Herbicide resistance in commercial transgenic plants

1. Which two plant-unique processes are explicitly mentioned in Topic 217 as target processes affected by herbicides?

- A) Photosynthesis or amino acid biosynthesis
- B) Cell wall pectin degradation and spore formation
- C) DNA replication and RNA splicing
- D) Cellular respiration and glycolysis

2. Why is developing herbicides that selectively kill weeds without harming crops very difficult?

- A) Weeds do not contain cellulose cell walls
- B) Both crops and weeds share the same fundamental plant processes affected by herbicides
- C) Herbicides are inactivated by soil bacteria before reaching weeds
- D) Crop plants absorb herbicides faster than weed species

3. What is the first strategy outlined in Topic 217 for engineering herbicide resistance in crop plants?

- A) Rendering the cellular target molecule insensitive or overproducing it
- B) Eliminating the target molecule from the plant genome completely
- C) Increasing the thickness of the leaf cuticle
- D) Inhibiting root growth to reduce chemical absorption

4. What is the second strategy described in Topic 217 to confer herbicide resistance?

- A) Introducing a pathway into the plant that degrades or detoxifies the herbicide
- B) Applying high-voltage electricity to destroy herbicide molecules
- C) Culturing plant cells exclusively in total darkness
- D) Deleting all amino acid biosynthetic pathways from the crop

Answer Key

* **1. A** — Herbicides generally target processes unique to plants, such as photosynthesis or amino acid biosynthesis.

* **2. B** — Developing selective herbicides is difficult because crops and weeds share the same biochemical processes.

* **3. A** — The first strategy renders the intracellular target molecule insensitive to the herbicide or overproduces it.

* **4. A** — The second strategy introduces a metabolic pathway that degrades or detoxifies the broad-spectrum herbicide within the plant.

Lesson 218: Production of virus resistance crops

1. What annual financial loss does Tobacco Mosaic Virus (TMV) cause in the tomato industry according to Topic 218?

- A) Over \ \$5 million
- B) Over \ \$50 million
- C) Over \ \$500 million
- D) Over \ \$5 billion

2. What phenomenon describes the protection conferred against a second viral strain when a plant is infected with a related first strain?

- A) Cross-protection
- B) Position effect
- C) Somaclonal variation
- D) Functional complementation

3. In which tissues must the TMV coat protein be expressed to confer systemic virus resistance in transgenic plants?

- A) Root tip caps and root hairs only
- B) Epidermis and vascular tissue
- C) Chloroplast stroma exclusively
- D) Seed coat aleurone layer

4. Which plant was the first virus-resistant transgenic crop containing multiple viral coat protein genes to reach commercial production?

- A) Transgenic tomato
- B) Transgenic squash
- C) Transgenic tobacco
- D) Transgenic potato

Answer Key

* **1. B** — TMV infection causes losses of over \$50 million per year in the tomato industry alone.

* **2. A** — Cross-protection is the phenomenon where infection with one virus strain protects a plant against superinfection by a related strain.

* **3. B** — To protect against TMV, coat protein expression is required in the epidermis and vascular tissue for systemic protection.

* **4. B** — Transgenic squash expressing coat proteins against cucumber mosaic, watermelon mosaic 2, and zucchini yellow mosaic viruses was the first commercial virus-resistant crop.

Lesson 219: Resistance against fungal pathogens

1. Expression of which heterologous protein was shown to protect tobacco and oilseed rape from post-emergent damping off caused by *Rhizoctonia solani*?

- A) Bean chitinase
- B) Maize PEPC
- C) Bacterial citrate synthase
- D) Viral coat protein

2. What collective term is used for plant proteins like chitinases and glucanases that are induced by microbial infection?

- A) Pathogenesis-related proteins (PR proteins)
- B) Opine-catabolizing proteins (OC proteins)
- C) Heat-shock elements (HS proteins)
- D) Upstream activating factors (UAF proteins)

3. Which three crops are explicitly named in Topic 219 as being engineered to express both chitinase and glucanase simultaneously for synergistic fungal resistance?

- A) Wheat, rice, and corn
- B) Tobacco, carrot, and tomato
- C) Squash, potato, and lettuce
- D) Arabidopsis, soybean, and cotton

4. What name is given to the anti-fungal peptides synthesized by plants that confer pathogen resistance when overexpressed?

- A) Defensins
- B) Colicins
- C) Opines
- D) Histones

Answer Key

* **1. A** — Expressing bean chitinase protects tobacco and oilseed rape against damping off caused by *Rhizoctonia solani*.

* **2. A** — Proteins such as chitinases and glucanases induced upon microbial infection are called pathogenesis-related (PR) proteins.

* **3. B** — Tobacco, carrot, and tomato have been engineered to express both chitinase and glucanase simultaneously for synergistic protection.

* **4. A** — Plants synthesize anti-fungal peptides called defensins, which confer resistance when overexpressed.

Lesson 220: Resistance to bacterial diseases

1. How much annual yield loss is caused by bacterial blight of rice in Asia alone?

- A) Over \$25 million
- B) Over \$250 million
- C) Over \$2.5 billion
- D) Over \$25 billion

2. From which wild rice species was the resistance gene complex against bacterial blight originally identified?

- A) *Oryza sativa*
- B) *Oryza longistaminata*
- C) *Oryza glumaepatula*
- D) *Oryza rufipogon*

3. What type of enzyme is encoded by the *Xa21* resistance gene isolated from the wild rice species?

- A) Receptor tyrosine kinase
- B) DNA-dependent RNA polymerase
- C) Chitinase
- D) Phosphoenolpyruvate carboxylase

4. Which bacterial pathogen is responsible for causing bacterial blight of rice?

- A) *Rhizoctonia solani*
- B) *Xanthomonas oryzae pv. oryzae*
- C) *Agrobacterium tumefaciens*
- D) *Bacillus thuringiensis*

Answer Key

* **1. B** — Bacterial blight causes losses exceeding \$250 million per year in Asia alone.

* **2. B** — The resistance gene complex was discovered in the related wild rice species *Oryza longistaminata*.

* **3. A** — The *Xa21* gene encodes a receptor tyrosine kinase that confers resistance to multiple isolates of the pathogen.

* **4. B** — Bacterial blight of rice is caused by *Xanthomonas oryzae pv. oryzae*.

Lesson 221: Bacillus thuringiensis-source of insect resistance genes**1. What estimated monetary loss in crop value occurs globally in rice production due to insect pests each year according to Topic 221?**

- A) Nearly \$1.5 billion
- B) Nearly \$10 billion
- C) Nearly \$50 billion
- D) Nearly \$100 billion

2. From which type of organism do all commercially produced insect-resistant transgenic crops derive their toxin proteins?

- A) Gram-negative soil bacterium *Agrobacterium tumefaciens*
- B) Gram-positive bacterium *Bacillus thuringiensis*
- C) Filamentous fungus *Rhizoctonia solani*
- D) Wild rice species *Oryza longistaminata*

3. What molecular weight is characteristic of the protoxin crystal proteins produced during sporulation by *Bacillus thuringiensis*?

- A) ~20 kDa
- B) ~50 kDa
- C) ~130 kDa
- D) ~350 kDa

4. By what physiological mechanism do active Bt toxins cause midgut epithelial cell death in susceptible insects?

- A) By inhibiting DNA-dependent RNA polymerase II
- B) By forming pores in the plasma membrane that cause cell death through osmotic lysis
- C) By preventing the synthesis of ribosomal RNA
- D) By blocking the de novo pathway of nucleotide synthesis

Answer Key

* **1. C** — Insect pests cause global losses in rice production estimated at nearly \$50 billion annually.

* **2. B** — All commercially produced insect-resistant transgenic crops express toxin proteins from *Bacillus thuringiensis*.

* **3. C** — *B. thuringiensis* produces crystals during sporulation comprising protoxins of approximately 130 kDa.

* **4. B** — Active Bt toxins bind midgut cell receptors and form plasma membrane pores, leading to osmotic lysis and cell death.

Lesson 222: Drought resistant transgenic crops

1. By what maximum percentage could global food yields potentially be increased through the development of drought-resistant transgenic crops?

- A) Up to 10%
- B) Up to 30%
- C) Up to 50%
- D) Up to 75%

2. What collective term is used for small, soluble molecules like betaines, sugars, amino acids, and polyamines synthesized by plants under drought or salinity stress?

- A) Pathogenesis-related proteins
- B) Opines
- C) Compatible solutes
- D) Defensins

3. How do compatible solutes protect plants during periods of prolonged dehydration?

- A) By degrading cell wall cellulose to release bound water
- B) By increasing the osmotic potential within the plant, preventing water loss and maintaining ion balance
- C) By inhibiting transpiration permanently through stomatal destruction
- D) By blocking the action of plant RNA polymerases

4. What natural accumulation level range of glycine betaine (in $\mu\text{mol/g}$ fresh weight) is observed in plants that naturally accumulate this solute under salt stress?

- A) 0.1 to 0.5 $\mu\text{mol/g}$
- B) 1 to 3 $\mu\text{mol/g}$
- C) 5 to 40 $\mu\text{mol/g}$
- D) 100 to 500 $\mu\text{mol/g}$

Answer Key

* **1. B** — Developing transgenic crops with built-in drought resistance could increase global food yields by up to 30%.

* **2. C** — Small, highly soluble molecules like betaines, sugars, amino acids, and polyamines are collectively termed compatible solutes.

* **3. B** — Compatible solutes increase internal osmotic potential to prevent water loss and maintain physiological ion balance.

* **4. C** — Natural salt-stress accumulator plants exhibit glycine betaine levels ranging from 5 to 40 $\mu\text{mol/g}$ fresh weight.

Lesson 223: Transgenic plants to cope poor soil quality

1. What percentage of the world's potential arable land consists of marginal soils characterized by pH extremes, nutrient limitations, or toxic metal ions?

- A) About 25%
- B) About 40%
- C) About 65%
- D) About 90%

2. What toxic metal ion present at high levels in acidic soils directly inhibits plant root development and sequesters phosphorus?

- A) Calcium
- B) Aluminum
- C) Magnesium
- D) Sodium

3. What common physiological adaptation is shared by naturally tolerant plants adapted to marginal soils?

- A) Increased exudation of organic acids (such as citrate, malate, and oxalate) from roots
- B) Conversion of dicotyledonous vascular tissues into C4 Kranz anatomy
- C) Complete degradation of cell wall pectin in taproots
- D) Accumulation of high concentrations of sodium hypochlorite

4. Transgenic plants engineered for improved soil tolerance have been transformed with genes encoding which enzyme to boost organic acid production?

- A) Phosphoenolpyruvate carboxylase
- B) Citrate synthase
- C) Chitinase
- D) Reverse transcriptase

Answer Key

* **1. C** — Marginal soils make up approximately 65% of the world's potential arable land.

* **2. B** — Aluminum in acidic soils is toxic, directly inhibiting root development and sequestering phosphorus.

* **3. A** — Tolerant plants exude higher levels of organic acids like citrate, malate, and oxalate from their roots.

* **4. B** — Several plant species have been transformed with bacterial or plant citrate synthase genes to increase organic acid exudation.

Lesson 224: Plant biotechnology to increase food yields

1. Which biological process is targeted for genetic intervention to increase intrinsic crop yield potential because it determines the rate of carbon fixation?

- A) Nitrogen fixation
- B) Photosynthesis
- C) Glycolysis
- D) Transpiration

2. Which enzyme catalyzes the key step in C4 photosynthesis by converting CO₂ into C4 organic acids in mesophyll cells?

- A) Phosphoenolpyruvate carboxylase (PEPC)
- B) Ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO)
- C) Citrate synthase
- D) Pyruvate kinase

3. From which crop was the PEPC-encoding gene isolated and transferred into C3 crops like potato and rice?

- A) Wheat
- B) Maize
- C) Tobacco
- D) Barley

4. What range of yield increase was observed in preliminary field trials in Korea for transgenic rice plants expressing pyruvate orthophosphate dikinase (PPDK)?

- A) 5–10%
- B) 10–30%
- C) 30–35%
- D) 50–70%

Answer Key

* **1. B** — Photosynthesis determines the rate of organic carbon fixation and is the primary target for intrinsic yield enhancement.

* **2. A** — Phosphoenolpyruvate carboxylase (PEPC) converts CO₂ into C4 organic acids in mesophyll cells during C4 photosynthesis.

* **3. B** — The maize gene encoding PEPC was transferred into C3 crop species to enhance carbon fixation.

* **4. C** — Field trials in Korea demonstrated yield increases of 30–35% for PPDK transgenic rice plants.

Lesson 225: Transgenic animals as model of human disease

1. Prior to transgenic animal technology, how were animal models of inherited diseases primarily obtained?

- A) By microinjecting viral capsids into bone marrow
- B) As spontaneously occurring mutants, from mutagenesis screens, or by selective breeding
- C) By growing animal cells in plant tissue culture media
- D) By treating adult animals with high-dose X-ray radiation

2. What type of disease were the earliest transgenic animal models designed to mimic by introducing exogenously derived oncogenes into the germline of mice?

- A) Severe combined immunodeficiency
- B) Particular forms of cancer
- C) Type 1 diabetes
- D) Cystic fibrosis

3. Cancer models created by adding a dominantly acting allele to a normal genome exemplify which class of genetic diseases?

- A) Loss-of-function diseases
- B) Gain-of-function diseases
- C) Auxotrophic mutant diseases
- D) Recessive chromosomal deletions

4. How are dominant gain-of-function disease alleles typically introduced into recipient mouse genomes to generate transgenic disease models?

- A) By electroporation of unfertilized sperm
- B) By microinjection of the allele into eggs
- C) By leaf-disk co-cultivation
- D) By treating blastocysts with DEAE-dextran

Answer Key

* **1. B** — Inherited disease models were originally identified as spontaneous mutants, from mutagenesis screens, or via selective breeding.

* **2. B** — The earliest transgenic disease models were mice predisposed to cancer through germline introduction of oncogenes.

* **3. B** — Dominantly acting alleles that cause disease when added to a genome exemplify gain-of-function diseases.

* **4. B** — Gain-of-function alleles are typically modeled by adding the allele to the genome via egg microinjection.

Lesson 226: Gene medicine to prevent, treat, or cure disease

1. Which human tissue is explicitly noted as being amenable to culture for the ex vivo gene transfer approach?

- A) Cardiac muscle
- B) Bone marrow
- C) Retinal epithelium
- D) Renal parenchyma

2. What term describes gene transfer performed directly in the patient, as opposed to transferring DNA into cultured cells that are then reintroduced?

- A) Ex vivo
- B) In vivo
- C) In vitro
- D) In silico

3. In addition to inherited disorders and cancers, what other category of diseases can be treated using gene therapy?

- A) Prion encephalopathies
- B) Infectious diseases
- C) Nutritional deficiencies
- D) Autoimmune dermatoses

4. Which combination of applications is explicitly listed under the scope of gene medicine?

- A) DNA vaccines, targeted killing of disease cells, and repairing genetic defects
- B) Whole-organ xenotransplantation, chemical mutagenesis, and dietary restriction
- C) Protein crystallization, peptide synthesis, and radioactive decay tracing
- D) Selective plant breeding, callus culture, and opine catabolism

Answer Key

* **1. B** — The ex vivo approach can be applied only to tissues such as bone marrow where cells are amenable to culture.

* **2. B** — Transferring DNA directly into the patient (directly or using viral vectors) is called in vivo transfer.

* **3. B** — Gene therapy can be used to treat inherited disorders, cancers, and infectious diseases.

* **4. A** — Gene medicine includes DNA vaccines, targeted killing of disease cells, and repairing genetic defects.

Lesson 227: DNA vaccines

1. In what year was the principle of DNA vaccination first demonstrated in animals?

- A) 1981
- B) 1990
- C) 1993
- D) 2003

2. What vector structure generally forms the backbone of a DNA vaccine?

- A) A single-stranded RNA viral vector
- B) A bacterial plasmid carrying an antigen-encoding gene under a strong host promoter
- C) A linear YAC carrying centromeric repeats
- D) An intact bacteriophage lambda virion

3. How does a DNA vaccine stimulate the host immune system?

- A) The injected DNA molecule itself acts as a direct surface antigen
- B) The expressed protein encoded by the introduced DNA stimulates the immune system
- C) The plasmid backbone destroys host antibodies directly
- D) The DNA binds irreversibly to serum albumin to trigger immunity

4. Which of the following delivery methods is explicitly listed for administering DNA vaccines?

- A) Injection, liposomes, or particle bombardment
- B) Leaf rubbing, root dipping, or seed soaking
- C) Electrophoresis, CsCl centrifugation, or sonication
- D) Microinjection into germinal vesicles exclusively

Answer Key

* **1. C** — DNA vaccination was first demonstrated in 1993.

* **2. B** — DNA vaccines generally comprise a bacterial plasmid carrying an antigen gene under a strong host-recognized promoter.

* **3. B** — Introduction of DNA yields an expressed protein, which then stimulates the immune system.

* **4. A** — DNA vaccines may be administered by injection, using liposomes, or by particle bombardment.

Lesson 228: Gene augmentation therapy

1. Which marker gene was used to genetically mark tumor-infiltrating lymphocytes in the first human gene marking experiment?

- A) Neomycin resistance gene
- B) Ampicillin resistance gene
- C) Tetracycline resistance gene
- D) Chloramphenicol resistance gene

2. Who was the four-year-old female patient in the first clinical trial involving a therapeutic gene transfer procedure?

- A) Ashanthi DeSilva
- B) Louise Brown
- C) Karen Ann Quinlan
- D) Alexis St. Martin

3. Severe combined immune deficiency (SCID) treated in the first therapeutic gene trial resulted from a deficiency in which enzyme?

- A) Hypoxanthine-guanine phosphoribosyltransferase (HPRT)
- B) Adenosine deaminase (ADA)
- C) Thymidine kinase (TK)
- D) Citrate synthase

4. What functional type of cells were isolated, marked, and re-injected in the first human genetic engineering experiment?

- A) Tumor-infiltrating lymphocytes
- B) Bone marrow erythroblasts
- C) Epidermal keratinocytes
- D) Pancreatic beta cells

Answer Key

* **1. A** — Tumor-infiltrating lymphocytes were genetically marked with a neomycin resistance gene.

* **2. A** — Ashanthi DeSilva was the four-year-old patient in the first clinical trial using a therapeutic gene transfer procedure.

* **3. B** — The trial treated SCID resulting from the absence of the enzyme adenosine deaminase (ADA).

* **4. A** — Tumor-infiltrating lymphocytes were isolated, marked with a neomycin resistance gene, and returned to the patient.

Lesson 229: Gene therapy strategies for cancer

1. Which gene was introduced into tumor-infiltrating leukocytes alongside the neomycin resistance gene to enhance tumor killing?

- A) Tumor necrosis factor (TNF)
- B) Interleukin-2 (IL-2)
- C) Erythropoietin (EPO)
- D) Insulin-like growth factor (IGF)

2. At what dosage level is TNF reported to be highly toxic to humans?

- A) As low as 10 µg/kg body weight
- B) 10 mg/kg body weight
- C) 1 g/kg body weight
- D) 100 mg/kg body weight

3. What is the goal of transforming tumor cells directly in cancer gene therapy?

- A) To make them more susceptible to the immune system through expression of cytokines or a foreign antigen
- B) To convert them into benign fibroblasts
- C) To stimulate rapid division of normal host cells
- D) To prevent all protein synthesis in cytoplasm

4. Why are fibroblasts transformed and co-injected with tumor cells in assisted-killing strategies?

- A) Fibroblasts are easier to grow in culture and co-injection provokes an immune response against the tumor
- B) Fibroblasts secrete carbenicillin into the tumor mass
- C) Fibroblasts integrate into the host germline
- D) Fibroblasts digest the tumor extracellular matrix

Answer Key

* **1. A** — Leukocytes were transformed with a gene for tumor necrosis factor (TNF) in addition to the neomycin-resistance gene.

* **2. A** — TNF is highly toxic to humans at levels as low as 10 µg/kg body weight.

* **3. A** — Transforming tumor cells directly aims to make them more susceptible to the immune system via cytokine or foreign antigen expression.

* **4. A** — Fibroblasts are easy to culture and, when co-injected with tumor cells, provoke an immune response against the tumor.

Lesson 230: Ethical issues in manipulation

1. In the medical applications of genetic research, what is a major ethical dilemma regarding molecular diagnosis of genetically based disease?

- A) Whether or not a fetus should be aborted if a disease-causing trait is detected
- B) Whether genetic tests should be performed in dark rooms
- C) Whether DNA can be extracted from hair follicles
- D) Whether PCR machines consume too much electricity

2. Prior to public concerns over GM foods in the late 1990s, what was traditionally the major source of difficulty in transgenesis debates?

- A) Animal welfare
- B) Plant tissue culture
- C) Bacterial resistance
- D) Viral transduction

3. What are the two main areas of public concern regarding genetically modified plants?

- A) Effect of GMO-derived foods on health and effect on the environment
- B) Cost of laboratory equipment and duration of gel electrophoresis
- C) Soil color and water evaporation rates
- D) Seed size and leaf shape

4. What specific mechanism is cited as a major environmental concern that could potentially cause ecological issues with GM plant technology?

- A) Cross-pollination with wild or non-transgenic populations
- B) Excessive absorption of atmospheric nitrogen
- C) Overproduction of oxygen during photosynthesis
- D) Bacterial transformation of soil minerals

Answer Key

* **1. A** — Major ethical dilemmas center around whether a fetus should be aborted if a disease-causing trait is detected.

* **2. A** — Traditionally, animal welfare was the major source of difficulty between pressure groups, scientists, and regulators.

* **3. A** — Public concerns regarding GM plants focus on health effects of GMO-derived foods and environmental effects.

* **4. A** — Cross-pollination of GM plants with wild or non-transgenic populations is a major environmental concern.

End of Quiz

BIO732 — 916 solved MCQs
Prepared by VU Learning HUB
vulearninghub.xyz