VU Learning HUB

BIO203 Quiz File

Chapter-wise Solved MCQs

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Lesson 1: Polymerase Chain Reaction (PCR)

Q1. Which component gives Polymerase Chain Reaction (PCR) its name? A) Thermocycler B) DNA polymerase C) RNA primer D) Microfuge tube

Q2. What serves as the template for replication as PCR progresses? A) Only the original genomic DNA B) Synthetic RNA probes C) The DNA generated during the reaction itself D) Restriction endonucleases

Q3. Which organism is the source of the heat-stable Taq polymerase used in PCR? A) Pyrococcus furiosus B) Thermus aquaticus C) Thermococcus litoralis D) Escherichia coli

Q4. What type of amplification does the PCR chain reaction set in motion? A) Linear amplification B) Exponential amplification C) Decreasing amplification D) Random amplification

Answer Key

B — PCR derives its name from one of its key components, DNA polymerase

C — As PCR progresses, the DNA generated during the reaction is itself used as a template for replication

B — Taq polymerase is a heat-stable DNA polymerase derived from the bacterium Thermus aquaticus

B — PCR sets in motion a chain reaction in which the DNA template is exponentially amplified

Lesson 2: Steps of PCR

Q1. What temperature range and time duration are typically used during the initialization step of PCR? A) 40°C–65°C for 20–40 seconds B) 70°C–80°C for 5–15 minutes C) 93°C–96°C for 1–10 minutes D) 4°C–15°C for an indefinite time

Q2. What occurs during the denaturation step of a PCR cycle? A) Primers anneal to the single-stranded DNA B) DNA polymerase adds dNTPs to synthesize new strands C) The DNA template melts, yielding single strands of DNA D) The reaction is cooled for short-term storage

Q3. What is the optimal activity temperature for Taq polymerase during the extension step? A) 40°C–65°C B) 70°C–80°C (commonly 72°C) C) 93°C–98°C D) 4°C–15°C

Q4. Why is a final elongation step performed at 70°C–74°C for 5–15 minutes after the last PCR cycle? A) To lower the reaction temperature for storage B) To melt non-specific primer dimers C) To ensure that any remaining single-stranded DNA is fully extended D) To denature the DNA polymerase enzyme

Answer Key

C — The initialization step consists of heating the reaction to 93°C–96°C for 1–10 minutes

C — Denaturation causes melting of the DNA template, yielding single strands of DNA

B — Taq polymerase has its optimum activity temperature at 70°C–80°C, with 72°C commonly used

C — Final elongation ensures that any remaining single-stranded DNA is fully extended

Lesson 3: Ingredients of PCR

Q1. Which mechanism allows the thermal cycler to raise and lower temperatures in a pre-programmed manner? A) Peltier effect B) Joule heating C) Seebeck induction D) Electrophoretic drag

Q2. Which of the following is NOT listed as a major ingredient of PCR? A) DNA template B) Primers C) MgCl2 D) Sodium bisulfite

Q3. What molecule can act as the template in PCR when reverse transcriptase is used? A) Double-stranded RNA only B) Single-stranded cDNA only C) RNA D) Protein

Q4. Which of the following enzymes is listed as a DNA polymerase used in PCR? A) EcoRI B) Pfu polymerase C) DNA ligase D) Alkaline phosphatase

Answer Key

A — The thermocycler works on the principle of the Peltier effect to raise and lower block temperatures

D — The major ingredients include microfuge tube, thermal cycler, DNA template, primers, buffer, and MgCl2

C — The template can be DNA or RNA in the case of reverse transcriptase

B — Pfu polymerase (along with Taq and Vent) is explicitly listed as a DNA polymerase for PCR

Lesson 4: Primers for PCR

Q1. What is the typical length of single-stranded DNA primers used in PCR? A) 5–10 bp B) 18–30 bp C) 100–700 bp D) 1000–2000 bp

Q2. What determines the size of the resulting PCR product? A) The concentration of MgCl2 B) The distance between the primer binding sites C) The brand of thermal cycler used D) The initialization temperature duration

Q3. What is the formula given for calculating the melting temperature (Tm) of primers? A) Tm=2(G+C)+4(A+T) B) Tm=4(G+C)+2(A+T) C) Tm=4(A+G)+2(C+T) D) Tm=2(G+C)×4(A+T)

Q4. What G/C content percentage range is recommended for PCR primers? A) 5%–10% B) 20%–80% C) 85%–95% D) Exactly 50%

Answer Key

B — Primers are single-stranded 18–30 bp long DNA fragments

B — The distance between primer binding sites determines the size of the PCR product

B — The formula provided for calculating melting temperature is Tm=4(G+C)+2(A+T)

B — Primers should have a G/C content of 20%–80% and avoid runs longer than GGGG

Lesson 5: DNA Polymerases

Q1. What is the extension rate of standard Taq polymerase? A) 33 nt/sec B) 60 nt/sec C) 75 nt/sec D) >80 nt/sec

Q2. Which organism is the source of Pfu DNA polymerase? A) Thermus aquaticus B) Thermococcus litoralis C) Pyrococcus furiosus D) Thermus thermophilus

Q3. Which DNA polymerase exhibits Reverse Transcriptase (RT) activity and an extension rate of >33 nt/sec? A) Vent B) Tth C) Amplitaq (Stoffel fragment) D) Taq pol

Q4. Which polymerase is isolated from Thermococcus litoralis and has an extension rate of >80 nt/sec? A) Vent B) Pfu C) Taq pol D) Tth

Answer Key

C — Taq pol has an extension rate of 75 nt/sec

C — Pfu polymerase is sourced from Pyrococcus furiosus

B — Tth polymerase has RT activity, an extension rate of >33 nt/sec, and originates from T. thermophilus

A — Vent polymerase originates from Thermococcus litoralis and has an extension rate >80 nt/sec

Lesson 6: Standard PCR Reaction

Q1. What is the standard concentration of each primer in a standard PCR reaction mix? A) 0.2 mM B) 0.25 mM C) 1.5 mM D) 50 mM

Q2. What is the recommended concentration of MgCl2 in a standard PCR reaction mixture? A) 0.2 mM B) 1.5 mM C) 10 mM D) 50 mM

Q3. How many units of polymerase enzyme are included in a standard 50 µl PCR reaction volume? A) 0.25 units B) 1.5 units C) 2.5 units D) 10 units

Q4. A standard PCR amplification program typically consists of how many cycles? A) 1–5 cycles B) 5–10 cycles C) 20–50 cycles D) 100–200 cycles

Answer Key

B — Standard reaction conditions specify 0.25 mM of each primer

B — Standard reaction mixture contains 1.5 mM MgCl2

C — A standard 50 µl reaction includes 2.5 units of polymerase

C — The amplification program consists of a series of 20–50 PCR cycles

Lesson 7: Interpretation of Results

Q1. What is the primary expectation regarding the size of a successful PCR product? A) It should always be larger than 10 kb B) It should be of the expected size C) It should vary in size across every lane D) It should match the size of the primer

Q2. What causes misprimes during PCR? A) Excessive extension time at 72°C B) Non-specific hybridization of primers C) Using Pfu instead of Taq polymerase D) Heating the reaction above 100°C

Q3. How can mispriming affect PCR interpretation? A) It generates non-specific bands of unexpected sizes B) It destroys the DNA template completely C) It increases the fidelity of the enzyme D) It prevents denaturation from occurring

Q4. What confirms that targeted amplification occurred during gel analysis? A) The product matches the expected size B) The reaction tube changes colour C) The Peltier block cools to 4°C D) The dNTPs are completely depleted

Answer Key

B — When interpreting PCR results, the PCR product should be of the expected size

B — Misprimes occur due to non-specific hybridization of primers to non-target regions

A — Non-specific primer hybridization leads to misprimes and unwanted non-specific amplification products

A — Confirming that the PCR product is of the expected size verifies correct targeted amplification

Lesson 8: Types of PCR

Q1. Which variant of PCR uses two pairs of primers to enhance specificity? A) Multiplex PCR B) Nested PCR C) Asymmetric PCR D) Colony PCR

Q2. Which PCR technique allows for simultaneous amplification of multiple targets using more than one primer pair? A) Multiplex PCR B) Reverse-transcriptase PCR C) In situ PCR D) Long-range PCR

Q3. Which technique is used when starting with an RNA template? A) RAPD PCR B) Touchdown PCR C) Reverse-transcriptase PCR D) Suicide PCR

Q4. Which PCR variant is also referred to as AP-PCR? A) AFLP PCR B) RAPD PCR C) Real-time PCR D) Assembly PCR

Answer Key

B — Nested PCR uses two pairs of primers to amplify a fragment

A — Multiplex PCR enables simultaneous amplification of many targets in one reaction

C — Reverse-transcriptase PCR uses RNA as the initial template

B — RAPD PCR is also known as AP-PCR (Arbitrarily Primed PCR)

Lesson 9: Nested PCR

Q1. How do the primers used in the second round of Nested PCR relate to the first PCR product? A) They are identical in sequence to the first primer pair B) They lie / are nested within the fragment amplified by the first pair C) They bind outside the flanking region of the first pair D) They contain random 10-mer sequences

Q2. What is the main advantage of using Nested PCR? A) It amplifies RNA directly without reverse transcription B) It produces single-stranded DNA exclusively C) It dramatically increases specificity by reducing non-specific amplification D) It eliminates the need for thermal cycling

Q3. How many total primers (individual oligonucleotides) are used in a complete Nested PCR strategy? A) 2 (1 pair) B) 4 (2 pairs) C) 8 (4 pairs) D) 1 (single primer)

Q4. How does the probability of non-specific amplification in Nested PCR compare to standard PCR? A) It is significantly higher B) It is identical C) It has a very low probability of non-specific amplification D) It cannot be determined

Answer Key

B — The second pair of primers (nested primers) lie or are nested within the first amplified fragment

C — Nested PCR increases specificity by reducing background due to non-specific amplification

B — Nested PCR uses two pairs (4 individual primers) instead of one pair

C — The key advantage of Nested PCR is a very low probability of non-specific amplification

Lesson 10: Multiplex PCR

Q1. What is produced in a Multiplex PCR reaction? A) A single uniform product length only B) Amplicons of varying sizes specific to different DNA sequences C) Only single-stranded RNA

fragments D) Circularized plasmid vectors

Q2. What parameter must be carefully optimized across all primer pairs in a Multiplex PCR? A) Annealing temperature B) Extension time to >10 minutes C) Denaturation temperature above 100°C D) Concentration of sodium bisulfite

Q3. What is a major practical benefit of Multiplex PCR? A) It uses fewer reagents and less time than running multiple separate reactions B) It eliminates the need for DNA polymerase C) It bypasses gel electrophoresis completely D) It prevents any primer-dimer formation automatically

Q4. In Multiplex PCR, how many primer pairs are present in the single reaction mixture? A) Strictly one pair B) More than one pair of unique primers C) No primers (uses random hexamers only) D) Only 1 forward primer with multiple reverse primers

Answer Key

B — Multiplex PCR produces amplicons of varying sizes specific to different target DNA sequences

A — Primer design must be optimized so all primer pairs work at the same annealing temperature in the single reaction

A — Multiplexing yields additional information from a single test run, saving time and reagents

B — Multiplex PCR uses multiple, unique primer sets within a single PCR reaction

Lesson 11: Reverse Transcriptase PCR

Q1. What process serves as the foundation for Reverse Transcriptase PCR (RT-PCR)? A) Translating mRNA into functional proteins B) Reverse transcribing RNA into DNA C) Methylating unmethylated cytosines D) Cleaving DNA at palindrome recognition sites

Q2. Which primer type is typically used during the first strand cDNA reaction of RT-PCR at 37°C? A) Random 10-mer primers B) Oligo dT primers C) Degenerate primers D) Hexacutter linkers

Q3. Which enzyme is used in the second strand reaction to digest the cDNA:RNA hybrid? A) Taq polymerase B) RNase H C) DNA ligase D) Alkaline phosphatase

Q4. What is a primary application of Reverse Transcriptase PCR? A) Measuring DNA fingerprint band migration rates B) Gene expression profiling and identifying RNA transcript sequences C) Self-ligating circular restriction fragments D) Screening bacterial colonies with toothpicks

Answer Key

B — Reverse Transcriptase PCR is based on reverse transcription, which transcribes RNA into DNA

B — The first step involves synthesis of cDNA using oligo dT primers at 37°C for one hour

B — The second strand reaction utilizes RNase H for digestion of the cDNA:RNA hybrid

B — RT-PCR is widely used in gene expression profiling and identifying sequence of RNA transcripts

Lesson 12: Real Time PCR

Q1. How does Real Time PCR detect and measure amplicons during the reaction? A) By measuring radioisotope emission on X-ray film B) By using fluoroprobes that bind to target regions of amplicons to produce fluorescence C) By weighing the PCR microfuge tube after

every cycle D) By digesting double-stranded products with EcoRI

Q2. Which instrument module in a Real Time PCR cycler detects the generated fluorescence signal? A) Inbuilt filter fluorometer B) Mass spectrometer C) Scintillation counter D) Spectrophotometer cuvette

Q3. Which fluorescent dye is explicitly listed in the source for QRT-PCR methods? A) Ethidium bromide B) SYBR Green C) Methylene blue D) Bromophenol blue

Q4. Which fluorophore-containing DNA probe is explicitly mentioned for measuring amplified product in real time? A) Alu probe B) TaqMan C) Oligo dT probe D) Pfu probe

Answer Key

B — Real Time PCR relies on fluoroprobes binding to specific amplicon targets to emit fluorescence during PCR

A — Fluorescence measured in real time is detected in a PCR cycler equipped with an inbuilt filter fluorometer

B — QRT-PCR methods utilize fluorescent dyes such as SYBR Green to measure amplified product

B — Fluorophore-containing DNA probes like TaqMan are used to quantify product in real time

Lesson 13: Hot Start PCR

Q1. What is the primary purpose of Hot Start PCR? A) To speed up the extension rate to >100 nt/sec B) To reduce non-specific amplification during the initial setup stages C) To amplify RNA directly without cDNA synthesis D) To digest genomic DNA using restriction endonucleases

Q2. At what temperature can Hot Start PCR be manually initiated before adding the polymerase? A) 4°C–15°C B) 37°C C) Up to the melting temperature (e.g. 95°C) D) 60°C–65°C

Q3. How do specialized enzyme systems keep DNA polymerase inactive prior to thermal activation? A) By binding an antibody or covalently bound inhibitors that dissociate only after high heating B) By permanently denaturing the active site of the enzyme C) By removing MgCl2 ions from the reaction buffer D) By freezing the reaction mix at -80°C

Q4. Which group of DNA polymerases shows very small activity at room temperature in Hot Start setups? A) Klenow fragment polymerases B) Thermophilic DNA polymerases (Eubacterial type I, Pfu) C) T4 RNA polymerases D) Reverse transcriptases

Answer Key

B — Hot Start PCR is a technique that reduces non-specific amplification during initial setup stages

C — The technique can be performed manually by heating reaction components up to melting temperature (e.g., 95°C)

A — Inactivation occurs via antibody binding or covalently bound inhibitors that dissociate after high-temperature activation

B — Thermophilic DNA polymerases like Eubacterial type I DNA pol and Pfu exhibit very small activity at room temperature

Lesson 14: Asymmetric PCR

Q1. What is the main characteristic that defines Asymmetric PCR? A) It uses four different primer pairs in one tube B) It amplifies one strand of the original DNA more than the other C) It

requires a constant temperature of 37°C throughout D) It relies entirely on RNA templates

Q2. How is Asymmetric PCR set up to achieve differential strand amplification? A) By adding equal amounts of four different DNA polymerases B) By conducting PCR with a great excess of one primer for the chosen strand C) By omitting dNTPs from the initial master mix D) By using degenerate primers only

Q3. Why are extra cycles of PCR required in Asymmetric PCR? A) Because Taq polymerase degrades rapidly at 95°C B) Due to slow amplification later in the reaction after the limiting primer is used up C) Because single-stranded DNA cannot be detected by gel electrophoresis D) To allow self-ligation of circularized fragments

Q4. In which applications is Asymmetric PCR particularly ideal? A) Colony screening and plasmid digestion B) Sequencing and hybridization probing where having only one complementary strand is needed C) Whole genome amplification from single cells D) Paternity testing using VNTRs

Answer Key

B — Asymmetric PCR is used to amplify one strand of original DNA more than the other

B — The reaction is carried out as usual but with a great excess of one primer for the chosen strand

B — Extra PCR cycles are required due to slow amplification after the limiting primer has been consumed

B — Having only one of the two complementary strands is ideal for sequencing and hybridization probing

Lesson 15: Long PCR

Q1. Long PCR is defined as amplifying DNA fragments of what size? A) Under 500 base pairs B) Over 5 kilobases (frequently over 10 kb) C) Exactly 18 to 30 base pairs D) 100 to 700 base pairs

Q2. What enzyme formulation is often utilized in Long PCR to ensure efficiency and accuracy? A) Pure restriction endonucleases B) Special mixtures of proficient polymerases along with accurate polymerases like Pfu C) Single-stranded RNA polymerases D) Alkaline phosphatases combined with T4 ligase

Q3. According to the source, Long PCR is useful only if it meets which key condition? A) If it uses oligo dT primers B) If it is accurate C) If it is performed inside a living cell D) If it runs for under 5 cycles

Q4. What is a key practical application of Long PCR? A) To clone large genes not possible with conventional PCR B) To convert unmethylated cytosine to uracil C) To identify single-base mismatches in 10-mer primers D) To measure fluorescence in real time using fluoroprobes

Answer Key

B — Long PCR refers to amplification longer than standard PCR, meaning over 5 kb and frequently over 10 kb

B — Mixtures of proficient polymerases alongside accurate polymerases such as Pfu are often mixed together

B — Long PCR is useful only if it is accurate

A — Long PCR is applied to clone large genes that are not possible to amplify with conventional PCR

Lesson 16: Allele Specific PCR

Q1. What type of genetic variation is Allele Specific PCR primarily used to identify? A) Variable Number Tandem Repeats (VNTRs) B) Single Nucleotide Polymorphisms (SNPs) C) Chromosomal translocations D) Large gene duplications over 10 kb

Q2. Where are the SNP-specific target regions positioned on the primers in Allele Specific PCR? A) At the 5' end of the forward primer only B) Encompassing the SNP at their 3' ends C) In the middle of a 100 bp linker sequence D) Within an arbitrary 10-mer overhang

Q3. How does a template-primer mismatch affect PCR amplification under stringent conditions? A) It increases the extension rate to >80 nt/sec B) It makes amplification much less efficient C) It causes immediate self-ligation of the template D) It converts the DNA polymerase into a reverse transcriptase

Q4. What outcome confirms the presence of the targeted single-nucleotide variant in Allele Specific PCR? A) Successful amplification with the SNP-specific primer B) Complete failure of Taq polymerase to extend C) Degradation of all single-stranded template DNA D) Formation of primer-dimers on an agarose gel

Answer Key

B — Allele-Specific PCR is a diagnostic/cloning technique used to identify single-nucleotide polymorphisms (SNPs)

B — This method uses primers whose 3' ends encompass the SNP

B — Amplification under stringent conditions is much less efficient when a mismatch exists between template and primer

A — Successful amplification with an SNP-specific primer signals the presence of that specific SNP

Lesson 17: Colony PCR

Q1. What is the main purpose of Colony PCR? A) To amplify unknown flanking sequences of transposable elements B) To screen bacterial or yeast clones for correct ligation or plasmid products C) To detect methylation patterns at CpG islands D) To measure real-time fluorescence using TaqMan probes

Q2. What tool is explicitly mentioned for picking a bacterial colony in Colony PCR methodology? A) Platinum wire loop B) Autoclaved toothpick C) Glass capillary tube D) Micropipette tip with filter

Q3. In Colony PCR, for how long and at what temperature is the colony-water mixture heated in a boiling bath? A) 37°C for 30 minutes B) 90–100°C for 2 minutes C) 72°C for 15 minutes D) 4–15°C for 1 hour

Q4. How much supernatant is taken as template for a standard 25 µl Colony PCR reaction? A) 1–2 µl B) 10–15 µl C) 20 µl D) 25 µl

Answer Key

B — Colony PCR is used for screening bacterial or yeast clones for correct ligation or plasmid products

B — The procedure begins by picking a bacterial colony with an autoclaved toothpick

B — The mix is heated in a boiling water bath (90–100°C) for 2 minutes

A — Taking 1–2 µl of the supernatant serves as template in a 25 µl standard PCR reaction

Lesson 18: In Situ PCR

Q1. Where does the amplification reaction take place in In Situ PCR (ISH)? A) Inside a microfuge tube containing 1.5 mM MgCl2 B) Directly inside the cell on a slide C) On a nylon membrane following capillary transfer D) In a polyacrylamide gel matrix

Q2. What sample state can be used to perform In Situ PCR amplification? A) Purified plasmid DNA in TE buffer B) Fixed tissue or cells C) Boiling cell lysates centrifuged at high speed D) Synthesized decameric linkers

Q3. In Situ PCR combines PCR methodology with which molecular biology principle? A) Bisulfite conversion of cytosine B) Hybridization of nucleic acids C) Blunt-end ligation by T4 ligase D) Restriction fragment self-ligation

Q4. According to the source, In Situ PCR detection is limited to which type of cellular target? A) Double-stranded genomic DNA only B) Non-genomic material such as RNA C) Covalently bound protein inhibitors D) Free dNTPs in the cytoplasm

Answer Key

B — In Situ PCR (ISH) is a polymerase chain reaction that actually takes place inside the cell on a slide

B — In situ PCR amplification can be performed on fixed tissue or cells

B — In Situ PCR applies the methodology of nucleic acid hybridization

B — In Situ PCR detection is limited to non-genomic material such as RNA

Lesson 19: Inverse PCR

Q1. How are standard PCR primers oriented in Inverse PCR compared to their usual orientation? A) In the same parallel direction B) In the reverse direction of the usual orientation C) Perpendicular to the double helix D) Attached to 3' poly-A tails

Q2. What serves as the template for reverse primers in Inverse PCR? A) A single-stranded RNA transcript B) A restriction fragment that has been self-ligated C) A linear genomic DNA fragment with blunt ends D) A 10-mer random oligonucleotide

Q3. What is the primary function of Inverse PCR? A) To amplify cDNA from mRNA templates B) To clone sequences flanking a known sequence C) To quantify copy number of target genes D) To measure fluorescence using SYBR Green

Q4. Inverse PCR is especially useful for identifying flanking sequences of what genetic entities? A) Single nucleotide polymorphisms (SNPs) B) Transposable elements and genomic inserts C) Oligo dT tails D) Unmethylated CpG islands

Answer Key

B — Inverse PCR uses standard PCR primers oriented in the reverse direction of the usual orientation

B — The template for reverse primers is a restriction fragment that has been self-ligated

B — Inverse PCR functions to clone sequences flanking a known sequence

B — It is especially useful in identifying sequences flanking transposable elements and genomic inserts

Lesson 20: AFLP PCR

Q1. Which restriction enzymes are specified in the AFLP PCR methodology as a tetracutter and a hexacutter? A) EcoRI (tetracutter) and HindIII (hexacutter) B) MseI (tetracutter) and EcoRI (hexacutter) C) BamHI (tetracutter) and SmaI (hexacutter) D) HaeIII (tetracutter) and Pfu (hexacutter)

Q2. What is ligated to all restriction fragments prior to pre-selective PCR in AFLP? A) Fluorophores B) Small DNA oligonucleotide linkers (adaptors) C) TaqMan probes D) Poly-A tails

Q3. How are amplicons visualized following PCR in AFLP? A) By measuring fluorescence in an inbuilt filter fluorometer B) Electrophoretic separation on a gel matrix followed by band pattern visualization C) By radioactive X-ray film exposure of living colonies D) By centrifuging microfuge tubes at high speed

Q4. What makes AFLP PCR particularly valuable in genetic studies? A) It is a highly sensitive method for detecting DNA polymorphisms and genotyping across a large number of loci B) It eliminates the need for restriction digestion of genomic DNA C) It works exclusively on single-stranded RNA templates D) It relies entirely on 9–10 nt mini-primers

Answer Key

B — Genomic DNA is digested with a tetracutter (MseI) and a hexacutter (EcoRI)

B — Small DNA oligonucleotide linkers (adaptors) are ligated to all restriction fragments

B — Amplicons undergo electrophoretic separation on a gel matrix followed by band pattern visualization

A — AFLP is a highly sensitive method for detecting DNA polymorphisms and genotyping individuals for a large number of loci

Lesson 21: Assembly PCR

Q1. What is the primary function of Assembly PCR? A) To digest genomic DNA into small fragments B) To assemble two or more pieces of DNA into one piece C) To convert unmethylated cytosine to uracil D) To amplify RNA without reverse transcriptase

Q2. What serves as the starting pool of material in Assembly PCR? A) Circular plasmids with sticky ends B) Single-stranded cDNA:RNA hybrids C) A pool of long oligonucleotides with short overlapping segments D) Random 10-mer primers only

Q3. How many PCR stages are involved in the methodology of Assembly PCR? A) An initial PCR with overlapping primers followed by a second PCR using those products as templates B) Three rounds of nested PCR using four primer sets C) A single isothermal incubation at 37°C D) A reverse transcriptase step followed by real-time fluorimetry

Q4. What is generated at the end of the second PCR stage in Assembly PCR? A) Degraded single nucleotides B) The final full-length DNA product C) Unlinked adaptor linkers D) Methylated CpG islands

Answer Key

B — Assembly PCR is used to assemble two or more pieces of DNA into one piece

C — Assembly PCR performs PCR on a pool of long oligonucleotides with short overlapping segments

A — It involves an initial PCR with overlapping primers and a second PCR using the products as a template

B — The second PCR generates the final full-length DNA product

Lesson 22: Suicide PCR

Q1. In which fields of study is Suicide PCR typically used? A) Recombinant plasmid expression in E. coli B) Paleogenetics or studies where avoiding false positives is top priority C) Real-time quantitative RNA expression profiling D) Digesting sticky-ended DNA with EcoRI

Q2. What strict rule regarding primer combinations does Suicide PCR prescribe? A) Any primer combination must be used only once in a PCR B) Primer combinations must be reused in every positive control C) Primers must contain 20%–80% G/C content with poly-A tails D) Primers must be pre-heated to 95°C for 1 hour

Q3. In Suicide PCR, primer combinations should NEVER have been used in which type of reaction? A) Negative control PCR B) Any positive control PCR reaction C) Reverse transcriptase PCR D) Agarose gel electrophoresis

Q4. What is the primary purpose of ensuring primers target genomic regions never amplified before in the lab? A) To increase Taq polymerase extension speed B) To ensure no contaminating DNA from previous PCR reactions is present to cause false positives C) To allow annealing at 37°C D) To generate single-stranded RNA probes

Answer Key

B — Suicide PCR is typically used in paleogenetics or studies where avoiding false positives is the highest priority

A — The method prescribes the use of any primer combination only once in a PCR

B — The primer combination should never have been used in any positive control PCR reaction

B — Targeting previously unamplified regions ensures no contaminating DNA from past PCRs is present to generate false positives

Lesson 23: Methylation Specific PCR

Q1. What is Methylation-Specific PCR (MSP) used to identify in genomic DNA? A) Single nucleotide polymorphisms in exons B) Patterns of DNA methylation at CpG islands C) Length variations in microsatellites D) Cleavage sites of type II restriction enzymes

Q2. Which chemical reagent is used to treat target DNA in Methylation-Specific PCR? A) Ethidium bromide B) Sodium bisulfite C) Alkaline phosphatase D) T4 DNA ligase

Q3. What chemical conversion occurs when target DNA is treated with sodium bisulfite? A) Adenine is converted to guanine B) Unmethylated cytosine bases are converted to uracil C) Methylated cytosines are converted to thymine D) Uracil is converted to cytosine

Q4. How many PCR amplifications are carried out on bisulfite-treated DNA in MSP? A) One amplification using a single primer set B) Two amplifications: one primer set for DNA with cytosines and another for DNA with uracil C) Four rounds of nested PCR D) None, because bisulfite treatment eliminates the need for PCR

Answer Key

B — MSP is used to identify patterns of DNA methylation at CpG islands in genomic DNA

B — Target DNA is first treated with sodium bisulfite

B — Sodium bisulfite converts unmethylated cytosine bases to uracil

B — Two amplifications are carried out: one set anneals to DNA with cytosines (methylated) and the other to DNA with uracil (unmethylated)

Lesson 24: InterSequence Specific PCR

Q1. What is InterSequence Specific PCR (ISSR) used for? A) DNA fingerprinting using primers selected from segments repeated throughout a genome B) Amplifying full-length mRNA transcripts C) Cleaving palindromic recognition sequences D) Measuring real-time fluorescence with TaqMan probes

Q2. What is the specific name given to InterSequence Specific PCR when primers are derived from a commonly repeated genomic segment? A) AFLP-PCR B) Alu-PCR C) RAPD-PCR D) RT-PCR

Q3. What do the primers in Alu-PCR amplify? A) Sequences adjacent to or between repeated Alu segments B) Blunt-ended linkers only C) 16S rRNA genes only D) Single-stranded cDNA tails

Q4. What type of output pattern does InterSequence Specific PCR generate for identification? A) A single uniform band size for all species B) A unique fingerprint of amplified product lengths C) Total degradation of genomic DNA D) Fluorescent emission standard curves

Answer Key

A — ISSR is a method for DNA fingerprinting using primers selected from segments repeated throughout a genome

B — The use of primers from a commonly repeated segment is called Alu-PCR

A — Alu-PCR helps to amplify sequences adjacent to or between these repeated segments

B — ISSR produces a unique fingerprint of amplified product lengths

Lesson 25: Ligation Mediated PCR

Q1. What molecules are first ligated to target DNA fragments in Ligation-Mediated PCR? A) Single-stranded RNA probes B) Small DNA oligonucleotide 'linkers' (or adaptors) C) Fluorophores D) Thermostable DNA polymerases

Q2. Where do the PCR primers anneal during Ligation-Mediated PCR? A) Directly to internal exon-intron boundaries B) To the ligated linker sequences to amplify target fragments C) To poly-A tails exclusively D) To random 10-mer sites

Q3. Which of the following is explicitly listed as an application of Ligation-Mediated PCR? A) DNA footprinting B) Colony screening with toothpicks C) Bisulfite conversion of cytosine D) Real-time quantitation with SYBR Green

Q4. Besides DNA footprinting, which other applications are explicitly mentioned for Ligation-Mediated PCR? A) DNA sequencing and genome walking B) Paleogenetic ancient DNA isolation C) Methylation profiling of CpG islands D) Multiplex amplicon sizing

Answer Key

B — Ligation-mediated PCR uses small DNA oligonucleotide 'linkers' (or adaptors) ligated to target DNA

B — Primers that anneal to the linker sequences are used to amplify target fragments

A — DNA footprinting is explicitly listed as an application of ligation-mediated PCR

A — Applications of ligation-mediated PCR include DNA sequencing, genome walking, and DNA footprinting

Lesson 26: Whole Genome Amplification PCR

Q1. What is Whole Genome Amplification (WGA)? A) A procedure that amplifies only one specific gene locus B) A group of procedures that allow amplification to occur at many locations in a genome C) A technique for digesting whole genomes into 4 bp fragments D) A method to convert double-stranded DNA into single-stranded RNA

Q2. What type of primers can be designed for Whole Genome Amplification to initiate replication across many target locations? A) Oligo dT primers B) 'Degenerate' primers C) Fluorophore-labeled TaqMan probes D) Decameric linker primers

Q3. What capability makes Whole Genome Amplification PCR unique? A) Whole genomes can be amplified by WGA B) It requires prior sequence knowledge of every locus C) It eliminates the need for DNA polymerase D) It only amplifies 18–30 bp fragments

Q4. How do degenerate primers function in Whole Genome Amplification? A) They bind to a single unique locus in the genome B) They are able to initiate replication from a large number of target locations C) They inhibit Taq polymerase at room temperature D) They cleave DNA at palindromic recognition sites

Answer Key

B — WGA is a group of procedures allowing amplification at many locations in a genome

B — Primers in WGA can be designed to be 'degenerate'

A — Whole genomes can be amplified using WGA procedures

B — Degenerate primers are able to initiate replication from a large number of target locations

Lesson 27: Mini-Primer PCR

Q1. What special enzyme is utilized in Mini-Primer PCR? A) Taq polymerase B) S-Tbr thermostable polymerase C) Pfu polymerase D) Reverse transcriptase

Q2. How short can primers be in Mini-Primer PCR while still being extended by the enzyme? A) 1 to 3 nucleotides B) 9 or 10 nucleotides C) 18 to 30 nucleotides D) 50 to 100 nucleotides

Q3. What advantage does Mini-Primer PCR offer regarding target binding sites? A) It requires long 100 bp binding regions B) It permits PCR targeting to smaller primer binding regions C) It prevents primer binding completely D) It binds only to poly-A tails

Q4. Which conserved gene is explicitly mentioned as a target amplified by Mini-Primer PCR? A) Human beta-globin gene B) 16S (or eukaryotic 18S) rRNA gene C) EcoRI restriction enzyme gene D) TaqMan probe gene

Answer Key

B — Mini-Primer PCR uses a thermostable polymerase called S-Tbr

B — S-Tbr polymerase can extend from short primers as short as 9 or 10 nucleotides

B — This method permits PCR targeting to smaller primer binding regions

B — It is used to amplify conserved DNA sequences, such as the 16S (or eukaryotic 18S) rRNA gene

Lesson 28: Advantages and Limitations of PCR

Q1. Which of the following is listed as an advantage of PCR in the source? A) It requires large, high-gram quantities of starting DNA B) It amplifies from low quantities of DNA and works on damaged DNA C) It eliminates all risk of contamination D) It requires expensive radioisotopes for basic operation

Q2. Which characteristic describes PCR as simple, rapid, and relatively inexpensive? A) It is a major advantage of PCR B) It is a key limitation of PCR C) It applies only to Western blotting D) It is true only when using radioactive probes

Q3. Which of the following is explicitly listed as a limitation of PCR? A) Contamination risk and primer complexities B) Inability to work on low quantities of DNA C) High cost compared to all other methods D) Requirement for living cell cultures during cycling

Q4. What primer-related limitation is noted for PCR? A) Primers cannot be synthesized artificially B) Primer complexities and primer-binding site complexities C) Primers must be longer than 1,000 bp D) Primers must be made of RNA

Answer Key

B — Advantages include amplifying from low quantities and working on damaged DNA

A — Being simple, rapid, and relatively inexpensive is listed among the advantages of PCR

A — Limitations of PCR include contamination risk and primer complexities

B — Primer complexities and primer-binding site complexities are key limitations

Lesson 29: Applications of PCR

Q1. Which group of infectious diseases is explicitly listed as detectable by PCR applications? A) AIDS, TB, CMV, and H1N1 B) Scurvy and rickets C) Type 1 and Type 2 diabetes D) Hypertension and atherosclerosis

Q2. In clinical microbiology, PCR is used to detect antibiotic resistance genes in which organisms? A) Viruses B) Bacteria C) Prions D) Synthetic polymers

Q3. Which forensic application of PCR is highlighted in the source? A) Karyotyping B) DNA fingerprinting C) Western blot immunodetection D) Bisulfite sequencing

Q4. Which fungal genus is explicitly listed under clinical microbiology applications of PCR? A) Aspergillus spp (and Candida spp) B) Escherichia spp C) Thermus spp D) Pyrococcus spp

Answer Key

A — Applications include detection of infectious diseases such as AIDS, TB, CMV, and H1N1

B — In clinical microbiology, PCR identifies bacterial antibiotic resistance genes

B — Forensic applications include DNA fingerprinting

A — Clinical microbiology fungus applications include Aspergillus spp and Candida spp

Lesson 30: Polymerase Chain Reaction (PCR) - Applications Overview

Q1. PCR has widespread applications across which fields of life sciences? A) Genetic engineering, medical, forensic, agriculture, and environment B) Theoretical physics and astronomy C) Organic synthesis of heavy metals D) Classical thermodynamics

Q2. In gene cloning, how is PCR utilized according to Lesson 30? A) For gene cloning and screening of genomic libraries B) For removing 5' phosphate groups from vectors C) For digesting circular plasmids with tetracutters D) For measuring Peltier block electrical current

Q3. How does PCR impact molecular studies in agriculture and the environment? A) By facilitating research in the detection of pathogens in plants, animals, and environment B) By eliminating the need for seed planting C) By measuring total soil mass D) By synthesizing inorganic fertilizers directly

Q4. What role does PCR play in medical diagnostics as introduced in general applications? A) Identifying pathogens and diagnosing genetic disorders B) Replacing surgical procedures completely C) Manufacturing synthetic erythrocytes D) Preventing all bacterial mutations

Answer Key

A — PCR has widespread applications in genetic engineering, medical, forensic, agriculture, and environment

A — PCR has been used in gene cloning and screening of genomic libraries

A — PCR facilitates research in detecting pathogens in plants, animals, and environment

A — PCR plays a major role in detecting pathogens and diagnosing genetic disorders

Lesson 31: PCR - Medicine

Q1. In which primary medical fields has PCR made a major impact according to Lesson 31? A) Physical therapy and orthopedics B) Clinical microbiology and diagnosis C) General dermatology and cosmetic surgery D) Herbal medicine and nutrition

Q2. What medical application does PCR enable prior to birth? A) Synthetic organ manufacturing B) Prenatal genetic diagnosis C) Surgical bone grafting D) Blood pressure regulation

Q3. Which of the following can be detected using PCR in clinical diagnosis? A) Pathogens, latent viruses, and inherited mutation carriers B) Essential nutrient levels in erythrocytes C) Skeletal muscle density D) Bone mineral density

Q4. How do molecular tools in PCR contribute to genetic medicine? A) By tracking DNA abnormalities and diagnosing genetic disorders B) By eliminating the need for all therapeutic drugs C) By producing synthetic red blood cells D) By preventing cell division in healthy tissue

Answer Key

B — PCR has a major impact on medicine, especially in clinical microbiology or diagnosis

B — Molecular tools allow clinicians to perform prenatal genetic diagnosis

A — PCR is utilized for the diagnosis of latent viruses, detection of pathogens, and screening of inherited mutation carriers

A — Molecular PCR tools enable tracking of DNA abnormalities and prenatal diagnosis of genetic disorders

Lesson 32: PCR - Forensic Sciences

Q1. How is forensic science defined in Lesson 32? A) The study of ancient fossilized plant remains B) The application of scientific procedures to solve criminal and legal matters C) The classification of industrial enzymes D) The chemical synthesis of synthetic nucleotides

Q2. Which biological sample types are explicitly mentioned as sources of minimal evidence for PCR in forensic science? A) Saliva, semen, or other tissues B) Plant bark and xylem sap only C) Synthesized lipid membranes D) Purified mineral dust

Q3. What purpose do molecular methods serve in forensic investigations regarding personal identity? A) Establishing the filiations of a person and identifying individuals B) Measuring bone growth rates C) Synthesizing artificial hormones D) Calculating metabolic rates

Q4. What name is given to the forensic technique that identifies individuals by unique characteristics of their DNA? A) Western blot immunodetection B) DNA profiling or DNA fingerprinting C) Sodium bisulfite conversion D) Isothermal nick translation

Answer Key

B — Forensic science is defined as the application of scientific procedures to solve criminal and legal matters

A — Molecular evidence can be obtained from minimal samples of saliva, semen, or other tissues

A — Molecular methods establish the filiations of a person and identify suspects from crime scene evidence

B — DNA profiling or DNA fingerprinting is used to identify individuals by unique characteristics of their DNA

Lesson 33: PCR - Agricultural Sciences and Environment

Q1. How has PCR facilitated research in agricultural sciences and the environment? A) By replacing soil nutrients with synthetic minerals B) By detecting pathogens in plants, animals, and the environment C) By eliminating the need for light in photosynthesis D) By preventing insect metamorphosis

Q2. Which environmental application of PCR involves studying disease distribution and transmission in populations? A) Molecular epidemiology B) Polymerase degradation C) Blunt-end ligation D) Restriction mapping

Q3. In plant pathology, what is a primary role of PCR? A) Measuring root depth directly without sampling B) Identifying and detecting plant pathogens rapidly C) Synthesizing chlorophyll artificially D) Increasing atmospheric nitrogen fixation

Q4. Which field connects PCR technology with ecological research as mentioned in the source? A) Quantum chemistry B) Molecular ecology C) Inorganic metallurgy D) Structural geology

Answer Key

B — PCR facilitates research in detecting pathogens in plants, animals, and the environment

A — Molecular epidemiology uses PCR to track pathogen spread and distribution in populations

B — PCR enables rapid detection and identification of plant pathogens

B — Molecular ecology uses PCR-based molecular tools to study environmental and ecological interactions

Lesson 34: PCR - Molecular Paleontology

Q1. How is molecular paleontology defined in Lesson 34? A) The chemical synthesis of new artificial proteins B) The recovery and analysis of DNA and protein from ancient human, animal, and plant remains C) The study of modern bacterial plasmid expression D) The measurement of current soil pH values

Q2. Which biological macromolecules are explicitly stated as recovered and analyzed in molecular paleontology? A) Lipids and carbohydrates only B) DNA and protein C) Free dNTPs and buffer salts D) Synthetic oligonucleotide linkers

Q3. What property of PCR makes it indispensable for molecular paleontology? A) Its ability to amplify DNA from low quantities and damaged ancient samples B) Its requirement for live cellular cultures C) Its ability to prevent RNA degradation completely D) Its reliance on radioactive isotopes

Q4. From which ancient sources can genetic material be extracted for molecular paleontology analysis? A) Ancient human, animal, and plant remains B) Fresh viral isolates only C) Synthetic plastic polymers D) Recombinant expression vectors

Answer Key

B — Molecular paleontology is the recovery and analysis of DNA and protein from ancient human, animal, and plant remains

B — Ancient DNA and proteins are the two target macromolecules recovered and analyzed

A — PCR can amplify trace amounts of DNA from ancient or damaged specimens

A — Remains of ancient humans, animals, and plants serve as sources of target genetic material

Lesson 35: Types of Restriction and Modification (R-M) System

Q1. How many Restriction-Modification (R-M) systems are explicitly listed in Lesson 35? A) One B) Two C) At least four (Type I, Type II, Type III, Type IIs) D) Exactly ten

Q2. Which bacterial strain was the source from which Type I R-M systems were first characterized? A) Thermus aquaticus B) Escherichia coli K12 C) Pyrococcus furiosus D) Haemophilus influenzae

Q3. What subunit composition makes up an active Type I restriction enzyme? A) One restriction subunit and five recognition subunits B) Two restriction subunits, two modification subunits, and one recognition subunit C) Four restriction subunits and no modification subunits D) Three ligase subunits and one polymerase subunit

Q4. Why are Type I restriction-modification systems of little value for recombinant gene manipulation? A) They cannot bind to DNA at all B) They cleave DNA at random locations distant from their recognition sites C) They require incubation at 100°C D) They destroy the vector antibiotic resistance genes

Answer Key

C — At least four R-M systems are known: Type I, Type II, Type III, and Type IIs

B — Type I systems were the first to be characterized from E. coli K12

B — An active Type I enzyme consists of 2 restriction, 2 modification, and 1 recognition subunit

B — Type I systems are of little value for gene manipulation because cleavage occurs at random sites away from recognition sequences

Lesson 36: Nomenclature of Restriction Enzymes

Q1. Who proposed the standard system for restriction enzyme nomenclature in 1973? A) Edwin Southern B) Smith and Nathans C) Kary Mullis D) Watson and Crick

Q2. In restriction enzyme nomenclature, how is the species name of the host organism represented? A) By the full genus name in capital letters B) By the first letter of the genus and first two letters of the specific epithet C) By a random three-digit Roman numeral D) By the author's initials

Q3. What is the derivation of the enzyme designation "Eco" according to the nomenclature rules? A) Enterobacter cloacae B) Escherichia coli C) Echinocandins origin D) Euglena gracilis

Q4. What is the host strain and enzyme order source for the restriction enzyme BamHI? A) Bacillus amyloliquefaciens, strain H, 1st enzyme B) Haemophilus influenzae, strain d, 3rd enzyme C) Serratia marcescens, 1st enzyme D) Escherichia coli, strain RY13, 1st enzyme

Answer Key

B — A suitable nomenclature system was proposed by Smith and Nathans in 1973

B — The species name is identified by the first letter of the genus and the first two letters of the specific epithet

B — Escherichia coli yields the abbreviation "Eco"

A — BamHI originates from Bacillus amyloliquefaciens, strain H, 1st enzyme

Lesson 37: Target Sites of Restriction Endonucleases

Q1. Type II restriction endonucleases recognize target sequences of what typical length? A) 1 to 2 nucleotides B) 4 to 8 nucleotides C) 18 to 30 nucleotides D) 100 to 500 nucleotides

Q2. What structural feature characterizes the target sites of Type II restriction endonucleases? A) Poly-A tail repeats B) Twofold axis of rotational symmetry (palindromes) C) Random non-repetitive single strands D) Triple-stranded RNA loops

Q3. Which 6 bp palindromic sequence is recognized and cleaved by the restriction enzyme EcoRI? A) 5'-GGATCC-3' B) 5'-GAATTC-3' C) 5'-AAGCTT-3' D) 5'-CCCGGG-3'

Q4. What type of DNA termini are produced when EcoRI makes staggered single-stranded cuts in its target site? A) Blunt non-overlapping ends B) Cohesive (sticky) single-stranded ends C) Circularized RNA loops D) Methylated cap structures

Answer Key

B — Type II endonucleases recognize target sequences of 4 to 8 nucleotides in length

B — Target sites exhibit a twofold axis of rotational symmetry, known as palindromes

B — EcoRI specifically recognizes the palindromic target site 5'-GAATTC-3'

B — Staggered cleavage by EcoRI produces cohesive or sticky single-stranded overhangs

Lesson 38: Number and Size of Restriction Fragments

Q1. What factor primarily determines the number and size of fragments generated by a restriction enzyme? A) The concentration of agarose in the gel B) The frequency of occurrence of the target site in the target DNA C) The extension rate of Taq polymerase D) The voltage applied during electrophoresis

Q2. On average, how frequently does a 4-base recognition sequence occur in a random DNA sequence? A) Every 16 bp B) Every 256 bp (4■) C) Every 4,096 bp (4■) D) Every 65,536 bp (4■)

Q3. How frequently is a 6-base restriction site expected to occur by chance in genomic DNA? A) Every 256 bp B) Every 4,096 bp (4■) C) Every 10,000 bp D) Every 65,536 bp

Q4. How often does an 8-base recognition site occur statistically in a DNA sequence? A) Every 256 bp B) Every 1,024 bp C) Every 4,096 bp D) Every 65,536 bp (4■)

Answer Key

B — Fragment number and size depend on the frequency of occurrence of the target site in the DNA

B — A 4-base recognition site occurs on average every 4■=256 bp

B — A 6-base recognition site occurs on average every 4■=4,096 bp

D — An 8-base recognition site occurs on average every 4■=65,536 bp

Lesson 39: Summary of Restriction Endonucleases

Q1. Which class of restriction enzymes is heavily responsible for the revolution in recombinant gene manipulation? A) Type I restriction enzymes B) Type II restriction enzymes C) Type III restriction enzymes D) Single-stranded exonucleases

Q2. What essential role do Type II restriction endonucleases play in molecular cloning? A) Sealing phosphodiester nicks in DNA backbones B) Forming recombinant DNA molecules by cutting DNA at predictable target sites C) Adding poly-A tails to mRNA transcripts D) Converting cytosine to uracil

Q3. Why are Type II enzymes preferred over Type I and Type III enzymes in genetic engineering? A) Because they require ATP hydrolysis to cut randomly B) Because they cleave DNA reliably within or near specific recognition sequences without random cleavage C) Because they only digest single-stranded RNA D) Because they operate exclusively at 0°C

Q4. What outcome is made possible by digesting vector and insert DNA with the same Type II restriction enzyme? A) Complete destruction of target DNA B) Generation of compatible ends that allow joining into recombinant DNA C) Irreversible denaturation of DNA strands D) Inactivation of antibiotic resistance markers

Answer Key

B — Type II restriction enzymes are heavily responsible for the current expansion in gene manipulation

B — Type II enzymes are essential for forming recombinant DNA molecules

B — Type II enzymes cut predictably at specific target sites rather than randomly

B — Digesting vector and insert with the same Type II enzyme produces complementary ends for recombinant DNA formation

Lesson 40: DNA Modifying Enzymes

Q1. Which four major classes of DNA modifying enzymes are outlined in Lesson 40? A) Helicases, Topoisomerases, Gyrases, and Primases B) Nucleases, DNA Polymerases, Reverse transcriptases, and DNA ligases C) Proteases, Lipases, Amylases, and Nucleases D) Kinases, Phosphatases, Transferases, and Dehydrogenases

Q2. What are the two broad functional classes of nucleases based on their site of cleavage? A) Exonucleases and Endonucleases B) Polymerases and Ligases C) Tetracutters and Hexacutters D) Acidic and Basic nucleases

Q3. What chemical bond is synthesized by DNA ligase between adjacent DNA fragments? A) Hydrogen bond between bases B) Phosphodiester bond between 3'-OH and 5'-phosphate ends C) Peptide bond between amino acids D) Glycosidic bond between sugars

Q4. What activity defines a reverse transcriptase enzyme? A) Degrading single-stranded DNA exonucleolitically B) RNA-directed DNA polymerase capable of making DNA complementary to RNA C) Adding methyl groups to unmethylated cytosines D) Cleaving palindromic sites in double-stranded DNA

Answer Key

B — The four classes listed are Nucleases, DNA Polymerase, Reverse transcriptases, and DNA ligases

A — Nucleases are broadly divided into Exonucleases and Endonucleases

B — DNA ligase creates a phosphodiester bond between the 3' end of one DNA segment and the 5' end of another

B — Reverse transcriptase is an RNA-directed DNA polymerase that synthesizes cDNA from RNA

Lesson 41: Methods of Joining DNA Fragments

Q1. What are the three main methods used for joining DNA fragments in vitro? A) Polymerization, reverse transcription, and bisulfite conversion B) Joining cohesive ends by DNA ligase, blunt ends by T4 DNA ligase, and synthesizing homopolymeric 3' tails C) Denaturation, annealing, and extension D) Nick translation, random priming, and end-labeling

Q2. Which enzyme seals single-stranded nicks between adjacent nucleotides in a duplex DNA chain? A) Restriction endonuclease B) DNA ligase C) Alkaline phosphatase D) Terminal transferase

Q3. Which enzyme synthesizes homopolymeric 3' single-stranded tails at the ends of DNA fragments? A) T4 DNA ligase B) Reverse transcriptase C) Terminal deoxynucleotidyltransferase D) Taq DNA polymerase

Q4. Which organisms encode the DNA ligase enzyme commonly used to seal nicks in duplex DNA? A) Thermus aquaticus and Pyrococcus furiosus B) Escherichia coli and phage T4 C) Bacillus amyloliquefaciens and Haemophilus influenzae D) Saccharomyces cerevisiae and Aspergillus spp.

Answer Key

B — The three main in vitro DNA joining methods are joining cohesive ends by DNA ligase, joining blunt ends by T4 DNA ligase, and synthesizing homopolymeric 3' single-stranded tails using terminal transferase

B — DNA ligase seals single-stranded nicks between adjacent nucleotides in a duplex DNA chain

C — Terminal deoxynucleotidyltransferase synthesizes homopolymeric 3' single-stranded tails at fragment ends

B — Both E. coli and phage T4 encode the DNA ligase enzyme used for sealing duplex DNA nicks

Lesson 42: DNA Ligase to Create Covalent Recombinant DNA

Q1. What structural defect does DNA ligase repair after complementary cohesive ends of DNA associate? A) Double-stranded chromosomal breaks B) Nicks produced after the association of cohesive ends C) Mismatched SNP base pairs D) Depurinated sugar-phosphate sites

Q2. What is created when DNA ligase covalently seals nicks between a linearized plasmid vector and a foreign DNA fragment? A) A single-stranded RNA probe B) Covalent recombinant DNA C) A linear homopolymer tail D) A digested restriction linker

Q3. Following transformation of bacterial cells with recombinant plasmid DNA, how are transformants selected in the presented workflow? A) By heat-shock survival at 95°C B) By tetracycline (Tc) selection C) By autoradiography of living colonies D) By blue-white colony picking with toothpicks

Q4. What initial process brings the vector and foreign DNA sticky ends together before DNA ligase acts? A) Thermal denaturation at 95°C B) Association/annealing of complementary cohesive ends C) Bisulfite conversion of cytosine D) Alkaline phosphatase dephosphorylation

Answer Key

B — DNA ligase repairs the single-stranded nicks produced after the association of cohesive ends of DNA strands

B — Sealing nicks between vector and foreign DNA strands yields a functional covalent recombinant DNA plasmid

B — Bacterial transformation selection is carried out using antibiotic selection such as tetracycline (Tc) selection

B — Complementary cohesive ends associate by base-pairing (annealing) prior to covalent sealing by DNA ligase

Lesson 43: Alkaline Phosphatase

Q1. What is the primary function of the enzyme alkaline phosphatase? A) Synthesizing cDNA from mRNA templates B) Removing phosphate groups from many types of molecules, including DNA C) Ligating blunt-ended DNA fragments D) Cleaving palindromic DNA target sites

Q2. Why is linearized plasmid vector DNA treated with alkaline phosphatase prior to ligation? A) To accelerate T4 DNA ligase activity B) To remove 5'-terminal phosphate groups and prevent recircularization and dimer formation C) To introduce cohesive sticky ends onto blunt vectors D) To convert unmethylated cytosine to uracil

Q3. How many 5'-terminal phosphate groups are removed from a linearized plasmid vector during alkaline phosphatase treatment? A) One phosphate group B) Two inorganic phosphate (2 Pi) groups C) Four phosphate groups D) All internal backbone phosphate groups

Q4. How does a host bacterial cell handle a recombinant plasmid that contains nicks after transformation? A) The cell degrades the plasmid immediately B) The host repairs one nick at

each join C) The cell converts the plasmid into single-stranded RNA D) The plasmid recircularizes without host intervention

Answer Key

B — Alkaline phosphatase is an enzyme responsible for removing phosphate groups from molecules including DNA

B — Removing 5'-terminal phosphate groups from linearized vector DNA prevents self-recircularization and plasmid dimer formation

B — Treatment releases two inorganic phosphate groups (2 Pi) from the 5' terminals of the linearized plasmid vector

B — Following transformation, the host bacterium repairs one nick at each join in the recombinant plasmid

Lesson 44: Blunt End Ligation via Linker Molecules

Q1. Which enzyme is used to join blunt-ended DNA molecules to linker molecules? A) Taq DNA polymerase B) T4 DNA ligase C) E. coli DNA polymerase I D) Alkaline phosphatase

Q2. What are decameric linker molecules? A) Single-stranded 10-mer primers for RAPD PCR B) Chemically synthesized short double-stranded DNA oligonucleotides containing a restriction site C) Poly-A RNA adaptors D) Fluorophore-labeled probes

Q3. How are cohesive sticky ends generated after linkers are ligated to blunt-ended foreign DNA? A) By heating to 95°C for 10 minutes B) By digesting the ligated linkers with the corresponding restriction enzyme (e.g., EcoRI) C) By treating with alkaline phosphatase D) By adding terminal deoxynucleotidyltransferase

Q4. In the decameric linker example provided in the source (CCGAATTCGG), which restriction enzyme site is embedded? A) BamHI B) EcoRI C) HindIII D) SmaI

Answer Key

B — T4 DNA ligase is used to join blunt-ended DNA molecules directly to linker molecules

B — Linkers are synthetic double-stranded oligonucleotides (e.g., decamers) that carry specific restriction enzyme recognition sites

B — Cleaving the ligated linkers with restriction endonucleases such as EcoRI exposes cohesive single-stranded sticky ends

B — The sequence CCGAATTCGG contains the internal palindromic recognition site for EcoRI (GAATTC)

Lesson 45: Adaptors

Q1. What is the main structural difference between a linker molecule and an adaptor molecule? A) Linkers are single-stranded, whereas adaptors are triple-stranded B) Linkers have blunt ends, whereas adaptors have one preformed cohesive end C) Linkers contain RNA, whereas adaptors contain DNA only D) Linkers are radioactive, whereas adaptors are fluorescent

Q2. What advantage do chemically synthesized adaptors offer when inserting blunt-ended foreign DNA into a vector? A) They possess a preformed cohesive end, eliminating the need to digest foreign DNA with restriction enzymes after ligation B) They permanently prevent vector ligation C) They require no DNA ligase enzyme for attachment D) They convert foreign DNA into cDNA automatically

Q3. Which enzyme is used to phosphorylate the 5'-OH ends of adaptor-modified DNA before vector ligation? A) Alkaline phosphatase B) Polynucleotide kinase C) RNase H D) Taq polymerase

Q4. In the adaptor ligation workflow shown in the source, which restriction adaptor molecule is specifically illustrated? A) EcoRI adaptor B) BamHI adaptor C) HindIII adaptor D) TaqI adaptor

Answer Key

B — The main difference is that linkers have blunt ends while adaptors have one preformed cohesive end

A — Adaptors carry a preformed cohesive end so foreign DNA can be inserted into a vector without subsequent restriction digestion

B — Polynucleotide kinase and ATP are used to add 5'-phosphate groups to the adaptor ends prior to vector ligation

B — The source explicitly illustrates the use of a BamHI adaptor molecule

Lesson 46: Southern Blotting

Q1. What is the primary purpose of the Southern blotting technique? A) To detect a specific protein in a complex mixture using antibodies B) To detect the presence of a particular piece of DNA in a sample using a molecular probe C) To measure real-time mRNA expression levels D) To sequence whole genomes directly without electrophoresis

Q2. Who invented Southern blotting and in what year was it published? A) Karry Mullis in 1983 B) Edwin Southern in 1975 C) Smith and Nathans in 1973 D) Alec Jeffreys in 1985

Q3. Which specific biological macromolecule is targeted and detected in Southern blotting? A) RNA B) DNA C) Protein D) Carbohydrate

Q4. How were subsequent blotting techniques (such as Northern and Western blotting) named? A) Based on the cardinal directional location of the gel apparatus B) In reference to Edwin Southern's name C) After the universities where they were invented D) According to the geographic origin of the research teams

Answer Key

B — The Southern blot is used to detect the presence of a particular piece of DNA in a sample by a molecular probe

B — Southern blotting is named after its inventor, British biologist Edwin Southern (1975)

B — Southern blotting specifically targets and detects DNA sequences

B — Other blotting methods using RNA (Northern) or protein (Western) were named in reference to Edwin Southern's name

Lesson 47: Southern Blotting Procedure

Q1. What is the first step in preparing DNA for Southern blotting after extraction from cells? A) Hybridizing with radioactive probes in wash buffer B) Cleaving the DNA into fragments using restriction enzymes (e.g., BamHI, EcoRI) C) Transferring intact chromosomes to paper towels D) Exposing gel directly to X-ray film

Q2. By what method are restriction-cleaved DNA fragments separated on the basis of size? A) Centrifugation at high speed B) Gel electrophoresis C) Column chromatography D) Bisulfite

precipitation

Q3. What must be done to double-stranded DNA fragments in the gel before transferring them to a nitrocellulose membrane? A) Ligation with decameric linkers B) In situ denaturation to produce single-stranded DNA C) Phosphorylation with polynucleotide kinase D) Digestion with RNase H

Q4. How is excess unbound labeled probe removed from the membrane following hybridization? A) By boiling the membrane in water at 100°C B) By washing the blot with wash buffers containing NaCl and detergent C) By exposing the membrane to UV light D) By centrifuging the membrane in a microfuge tube

Answer Key

B — Extracted DNA is cleaved into many fragments by restriction endonucleases such as BamHI or EcoRI

B — Resulting DNA fragments are separated on the basis of size by gel electrophoresis

B — DNA fragments are denatured into single strands before or during transfer to a nitrocellulose membrane

B — The blot is incubated with wash buffers containing NaCl and detergent to wash away excess unbound probe

Lesson 48: Southern Blotting Probes

Q1. What is the typical length of single-stranded DNA probes used in Southern blotting? A) 2 to 6 nucleotides B) 20 to 30 nucleotides C) 100 to 500 nucleotides D) >1,000 nucleotides

Q2. By what mechanism does a labeled probe bind to target DNA immobilized on a membrane? A) Covalent peptide bond formation B) Hybridization between single-stranded probe and complementary target sequence C) Blunt-end ligation by T4 ligase D) Non-specific hydrophobic adsorption

Q3. Which radioisotope is explicitly specified for labeling radioactive Southern blot probes? A) Carbon-14 B) Phosphorus-32 C) Tritium D) Iodine-125

Q4. What non-radioactive molecule is explicitly listed as a probe label for Southern blotting? A) Ethidium bromide B) Biotin C) SYBR Green D) Luminol

Answer Key

B — DNA probes for Southern blotting are typically 20–30 nucleotides long and complementary to the target region

B — Probe binding occurs via hybridization between the single-stranded labeled probe and a complementary sequence on target DNA

B — Phosphorus-32 is explicitly listed as the radioactive label for probes

B — Biotin is explicitly listed as a non-radioactive probe labeling option

Lesson 49: Transfer Methods in Southern Blotting

Q1. Which of the following is NOT listed as a transfer method for moving DNA to a membrane in Southern blotting? A) Upward capillary transfer B) Downward capillary transfer C) Electrophoretic transfer D) Osmotic vapor distillation transfer

Q2. How does buffer move through the gel during upward capillary transfer? A) It is forced downward by centrifugal gravity B) Buffer drawn from a reservoir passes through the gel into a stack of paper towels C) It flows by high-voltage electrical current only D) It evaporates into a vacuum chamber

Q3. What role does the moving stream of buffer play in upward capillary transfer? A) It denatures the agarose matrix completely B) It elutes DNA from the gel and deposits it onto the membrane C) It synthesizes new complementary DNA strands D) It activates radioactive 32P probes

Q4. Which membrane material options are explicitly listed for receiving transferred DNA in Southern blotting? A) Cellulose acetate or glass fiber B) Nylon or nitrocellulose membrane C) Polyacrylamide or starch matrix D) Polyethylene or Teflon film

Answer Key

D — Listed transfer methods include upward capillary, downward capillary, simultaneous transfer to two membranes, electrophoretic, and vacuum transfer

B — Buffer drawn from a reservoir passes upward through the gel and membrane into a stack of paper towels

B — DNA is eluted from the gel by the moving stream of buffer and deposited onto the membrane

B — Membrane materials explicitly listed include nylon or nitrocellulose

Lesson 50: Mutations Identification by Southern Blotting

Q1. How does a mutation affecting a restriction site alter the outcome of a Southern blot? A) It prevents probe hybridization entirely across all chromosomes B) It causes the pattern, size, and number of restriction bands to differ from a normal gene C) It converts DNA into single-stranded RNA D) It inactivates the radioactive label on the probe

Q2. What happens when a single nucleotide substitution alters a restriction site sequence? A) The restriction endonuclease fails to recognize and cleave at that site B) The restriction enzyme cleaves the site twice as fast C) The DNA strand is destroyed by exonucleases D) The Taq polymerase skips the site during extension

Q3. What are the sequential steps for identifying mutations using Southern blotting? A) PCR amplification → bisulfite conversion → colony picking → sequencing B) Digestion of genomic DNA → size-separation → in situ denaturation → transfer to solid support → hybridization to labeled probe → detection C) Western blotting → antibody probing → ECL detection → autoradiography D) Ligation of adaptors → kinase phosphorylation → transformation → selection

Q4. How does the band pattern of a mutant gene compare to a wild-type gene after restriction digestion and Southern blotting? A) They are always completely identical in size and number B) Estimation of the size and number of bands generated will be different C) The mutant gene yields no visible bands under any condition D) The wild-type gene produces double-stranded RNA bands

Answer Key

B — The presence of a mutation affecting a restriction site causes the pattern of bands to differ from those seen in a normal gene

A — A nucleotide change may alter the site sequence so that the restriction endonuclease fails to recognize and cleave it

B — Mutation identification follows digestion, size-separation, denaturation, transfer, hybridization with a labeled probe, and detection

B — Estimation of the size and number of bands generated after digestion will be different for a mutated site compared to normal DNA

Lesson 51: Northern Blotting

Q1. What is the primary purpose of the Northern blotting technique? A) Detection of a specific protein sequence in serum B) Detection of a specific RNA sequence in RNA samples C) Amplification of genomic DNA fragments D) Cleavage of single-stranded DNA probes

Q2. Which type of gel is used during Northern gel electrophoresis to separate RNA according to size? A) Non-denaturing polyacrylamide gel B) Denaturing agarose gel C) Starch gel matrix D) Silica gel column

Q3. What process immobilizes RNA sequences onto a solid support prior to hybridization? A) Fixation of the RNA B) Digestion with EcoRI C) Phosphorylation with kinase D) Centrifugation at high speed

Q4. How are specifically targeted RNA sequences identified following probe hybridization in Northern blotting? A) By measuring tube weight B) Removal of non-specifically bound probes followed by detection, capture, and image analysis C) By boiling the membrane in 100°C water D) By adding T4 DNA ligase

Answer Key

B — Northern blotting is a method used for the detection of a specific RNA sequence in RNA samples

B — In Northern blotting, RNA is separated according to size through a denaturing agarose gel

A — The procedure includes transferring RNA to a solid support followed by fixation of the RNA

B — Detection requires removal of nonspecifically bound probe molecules followed by capture and image analysis

Lesson 52: Northern Blot Applications

Q1. Which of the following is listed as a primary application of Northern blotting? A) Study of gene expression in eukaryotic cells B) Sequencing total chromosomal DNA C) Generating blunt-ended adapter linkers D) Measuring bacterial cell wall density

Q2. What feature of transcribed RNA can be measured using Northern blotting? A) The exact tertiary folding angles B) The amount and size of RNAs transcribed from eukaryotic genes C) The amino acid sequence of translated proteins D) The degree of DNA methylation at CpG islands

Q3. Why are housekeeping genes used as controls in Northern blot assays? A) To digest non-target RNA transcripts B) To equalize the amounts of RNA loaded into lanes and normalize samples C) To amplify single-stranded DNA primers D) To prevent probe hybridization

Q4. What type of gene is selected as a housekeeping gene for sample normalization? A) An inducible viral promoter gene B) An endogenous, constitutively-expressed gene C) A synthetic

antibiotic resistance gene D) A restriction endonuclease gene

Answer Key

A — Applications of Northern blotting include the study of gene expression in eukaryotic cells

B — Northern blotting is used to measure the amount and size of RNAs transcribed from eukaryotic genes

B — Housekeeping genes are used to equalize the amounts of RNA loaded into gel lanes and normalize samples

B — A housekeeping gene is defined as an endogenous, constitutively-expressed gene

Lesson 53: Western Blotting

Q1. What is Western blotting designed to detect? A) A specific DNA sequence in genomic extracts B) The presence of a specific protein in a complex protein mixture C) The rate of RNA transcription in real time D) Single nucleotide polymorphisms in CpG islands

Q2. Besides detecting target proteins, what physical property of a protein can be determined using Western blotting? A) The melting temperature (Tm) B) The molecular weight of the protein C) The exact nucleotide sequence of its gene D) The number of intron-exon boundaries

Q3. Western blotting is commonly used as an analytical tool for detecting proteins from which pathogen class? A) Prions B) Viral proteins C) Synthetic vectors D) Plasmids

Q4. What capability of Western blotting allows characterization of antibody preparations? A) Determining the specificity of the immune response to viral antigens and antibody preparations B) Converting monoclonal antibodies into DNA probes C) Cleaving heavy chains with restriction enzymes D) Methylating unmethylated cytosines

Answer Key

B — Western blotting is a technique used to detect the presence of a specific protein in a complex protein mixture

B — Western blotting is used to determine the molecular weight of a protein

B — Western blots have become one of the most common analytical tools for the detection of viral proteins

A — It is used for characterization of monoclonal and polyclonal antibody preparations and determining immune response specificity

Lesson 54: Western Blotting Procedure

Q1. What type of gel matrix is used for separating protein samples in Western blotting? A) Agarose gel B) Formaldehyde-agarose gel C) Polyacrylamide gel D) Silica gel

Q2. What is the correct order of initial steps in the Western blotting procedure? A) Blocking → Sample preparation → Antibody probing → Gel Electrophoresis B) Sample preparation → Gel Electrophoresis → Blotting (transfer) → Blocking → Antibody probing → Detection C) Blotting → Detection → Blocking → Sample preparation D) Hybridization → Sample preparation → Electrophoresis → Transfer

Q3. Why is a blocking step performed during the Western blotting procedure? A) To denature double-stranded DNA templates B) To prevent non-specific binding of antibodies to the membrane surface C) To digest excess RNA transcripts D) To ligate protein fragments together

Q4. What molecular probes are utilized to detect target proteins on the membrane in Western blotting? A) Radioactive DNA oligomers B) Specific antibodies (primary/secondary) C) Single-stranded cDNA probes D) Decameric linker molecules

Answer Key

C — Western blotting separates proteins using polyacrylamide gel electrophoresis

B — The procedure follows Sample preparation, Gel Electrophoresis, Blotting (transfer), Blocking, Antibody probing, and Detection

B — Blocking is required prior to antibody probing to prevent non-specific binding to the transfer membrane

B — Antibodies are used as specific probing agents in Western blotting

Lesson 55: Applications of Western Blotting

Q1. What alternative name is frequently given to the Western blotting technique? A) Southern hybridization B) Protein blotting or immunoblotting C) Northern transcript profiling D) Polymerase chain reaction

Q2. In which biological discipline is Western blotting explicitly described as a widely used analytical technique? A) Theoretical astronomy B) Immunogenetics, molecular biology, and biochemistry C) Structural geology D) Inorganic metallurgy

Q3. What clinical role does Western blotting serve according to Lesson 55? A) Analysis of various kinds of diseases in medical diagnostics B) Measuring bone density directly C) Synthesizing artificial hormone lipids D) Calculating metabolic oxygen consumption

Q4. What matrix is used to resolve and identify unknown proteins in Western blotting? A) Agarose gel B) Polyacrylamide gel C) Nylon membrane stack D) Paper towel reservoir

Answer Key

B — Western blotting is sometimes called protein blotting or immunoblotting

B — It is widely used in molecular biology, immunogenetics, and biochemistry disciplines

A — Western blotting is used in medical diagnostics for the analysis of various kinds of diseases

B — Western blotting is a way to identify unknown proteins on a polyacrylamide gel

Lesson 56: Difference Between Different Blotting Techniques

Q1. Which transfer method is specifically used for Western blotting compared to Southern and Northern blotting? A) Upward capillary transfer B) Electric transfer C) Downward capillary transfer D) Vacuum distillation transfer

Q2. Which combination correctly pairs the blotting technique with its target molecule? A) Northern = DNA; Southern = Protein; Western = RNA B) Northern = RNA; Southern = DNA; Western = Protein C) Northern = Protein; Southern = RNA; Western = DNA D) Northern = DNA; Southern = RNA; Western = Protein

Q3. What gel matrix is used in Northern blotting to separate RNA molecules? A) Polyacrylamide gel B) Agarose/formaldehyde gel C) Plain agarose gel D) Starch gel

Q4. What probe type is used for Western blotting detection, as listed in the comparison table? A) DNA probes labeled radioactively B) cDNA probes labeled non-radioactively C) Primary

antibodies D) Oligo dT primers

Answer Key

B — Northern and Southern use capillary transfer, whereas Western blotting uses electric transfer

B — Northern detects RNA, Southern detects DNA, and Western detects protein

B — Northern blotting uses an Agarose/formaldehyde gel

C — Western blotting utilizes primary antibodies as probes

Lesson 57: Single Nucleotide Polymorphism

Q1. How is a Single Nucleotide Polymorphism (SNP) defined in Lesson 57? A) A large deletion of over 1,000 base pairs B) A single base change in a DNA sequence occurring in >1% of a large population C) A tandem repeat of 2–6 base pair sequences D) An artificial insertion of a decameric linker

Q2. What minimum population threshold frequency must a single base change meet to be classified as a SNP? A) 0.01% B) More than 1 percent (>1%) C) Exactly 50 percent D) 100 percent

Q3. Single Nucleotide Polymorphisms represent variation in what type of biological molecule? A) Amino acid chains B) DNA sequence C) Ribosomal RNA subunits D) Phospholipid bilayers

Q4. Compared to large structural mutations, what is the scale of genetic alteration in a SNP? A) Entire chromosomal translocation B) Single base change C) Insertion of 10 kb transposable element D) Triplication of whole genome

Answer Key

B — A SNP is defined as a single base change in a DNA sequence occurring in a significant proportion (>1%) of a population

B — The single base change must occur in more than 1 percent of a large population

B — SNPs represent variations occurring directly in DNA sequences

B — A SNP involves a change of a single base in the DNA sequence

Lesson 58: Single Nucleotide Polymorphism-2

Q1. What is an "SNP map" as described in Lesson 58? A) A physical map of bacterial colony growth on agar B) A single map of the human genome containing all possible SNPs discovered by sequencing genomes C) A diagram showing gel restriction band migration rates D) A schematic of Peltier block thermal cycles

Q2. What clinical application makes individual SNP profiles particularly valuable? A) Identifying response to drug therapy B) Measuring body temperature during thermal cycling C) Determining blood glucose levels D) Synthesizing artificial blood vessels

Q3. How are people grouped when constructing population genetic frameworks in Lesson 58? A) By blood pressure readings B) Based on their SNP profile C) By gel electrophoresis voltage D) By height and weight

Q4. What relationship can emerge from analyzing SNP profiles across patient cohorts? A) Correlations between certain SNP profiles and specific responses to treatment B) Total

destruction of genomic DNA C) Automatic resistance to all viral infections D) Conversion of heterozygous alleles to homozygous alleles

Answer Key

B — SNP maps are generated by comparing base sequences across many people to map all possible SNPs in the genome

A — SNP profiles are important for identifying individual response to drug therapy

B — People can be grouped based on their distinct SNP profile patterns

A — Correlations might emerge between certain SNP profiles and specific responses to treatment

Lesson 59: How to Detect SNPs

Q1. Which of the following is listed as a hybridization technique for detecting KNOWN polymorphisms? A) Direct Sequencing B) Microarrays and Real-time PCR C) Electrophoretic mobility assays D) Bisulfite conversion

Q2. Which method is listed for detecting UNKNOWN polymorphisms? A) Direct Sequencing (e.g. Sanger dideoxysequencing) B) Oligo dT tailing C) Colony picking with toothpicks D) Alkaline phosphatase dephosphorylation

Q3. What limitation of Sanger dideoxysequencing is noted when detecting unknown polymorphisms? A) It misses polymorphisms and mutations when the DNA is heterozygous B) It cannot sequence double-stranded DNA C) It works only on RNA samples D) It requires incubation at 100°C

Q4. Why does direct sequencing have limited utility for analyzing solid tumors or pooled samples? A) Due to high toxicity of reagents B) Due to low sensitivity C) Because tumors do not contain DNA D) Because sequencing takes over ten years

Answer Key

B — Known polymorphism detection techniques include hybridization techniques such as microarrays and real-time PCR

A — Unknown polymorphisms can be detected using direct sequencing, microarrays, cleavage/ligation, and mobility assays

A — Sanger dideoxysequencing misses polymorphisms and mutations when the DNA is heterozygous

B — Direct sequencing has limited utility for solid tumors or pooled samples due to low sensitivity

Lesson 60: SNP Screening

Q1. What are the two main SNP screening strategies outlined in Lesson 60? A) High temperature vs. low temperature screening B) "Many SNPs in a few individuals" and "A few SNPs in many individuals" C) Single-stranded vs. double-stranded screening D) Bacterial screening vs. viral screening

Q2. In complex genetic studies, what type of population study is used to detect genes contributing to disease susceptibility? A) Case-control association analysis B) Western blot immunodetection C) Upward capillary transfer D) Random amplified primer ligation

Q3. Which field of clinical medicine benefits directly from SNP screening as listed under "Significance of SNPs"? A) Disease diagnosis and finding predisposition to diseases B) Bone

fracture casting C) Surgical suture design D) Physical rehabilitation therapy

Q4. How does SNP screening contribute to pharmacology? A) Assisting in drug discovery & development and understanding drug responses B) Replacing all chemical drugs with protein vaccines C) Preventing all adverse drug reactions automatically D) Synthesizing custom antibiotics in vitro

Answer Key

B — The two screening strategies are "Many SNPs in a few individuals" and "A few SNPs in many individuals"

A — Detecting genes contributing to multifactorial disease susceptibility utilizes case-control association analysis

A — Significance of SNPs includes disease diagnosis and finding predisposition to diseases

A — SNPs play a key role in drug discovery & development as well as evaluating individual drug responses

Lesson 61: SNP-Haplotype

Q1. How is a haplotype defined in Lesson 61? A) A single base mutation occurring in over 50% of a population B) A set of closely linked genetic markers present on one chromosome that tend to be inherited together C) A double-stranded RNA fragment synthesized during transcription D) A restriction fragment digested by EcoRI

Q2. What feature of haplotypes makes them difficult to separate during inheritance? A) High rate of enzymatic degradation by nucleases B) They are not easily separable by recombination C) They are located on separate non-homologous chromosomes D) They bind permanently to poly-A tails

Q3. What approach combines information from adjacent SNPs into composite multilocus haplotypes? A) The "Haplotype-centric" approach B) The "Colony-centric" approach C) Asymmetric PCR strategy D) Nested primer design

Q4. Which of the following is listed as a major SNP database resource in Lesson 61? A) dbSNP (or TSC, JSNP, GeneSNPs) B) GenBank-Protein-Only C) PCR-Master-DB D) EcoRI-Base

Answer Key

B — A haplotype is defined as a set of closely linked genetic markers present on one chromosome that tend to be inherited together

B — Markers on a haplotype tend to be inherited together because they are not easily separable by recombination

A — The haplotype-centric approach combines information from adjacent SNPs into composite multilocus haplotypes

A — dbSNP (along with TSC, JSNP, and GeneSNPs) is explicitly listed as a key SNP database resource

Lesson 62: DNA Fingerprinting

Q1. What percentage of human DNA sequences are identical across all individuals? A) 50% B) 75% C) 99.9% D) 100%

Q2. What highly variable repetitive DNA sequences are primarily analyzed in DNA profiling? A) Single nucleotide overhangs B) Variable Number Tandem Repeats (VNTRs) C) Palindromic EcoRI recognition sites D) Polynucleotide kinase adaptors

Q3. What length range defines the repeat units of microsatellites (SSRs or STRs)? A) 1 base pair only B) 2 to 6 base pairs C) 10 to 60 base pairs D) 100 to 500 base pairs

Q4. What length range characterizes minisatellite repeat sequences (referred to as VNTRs)? A) 2 to 6 base pairs B) 10 to 60 base pairs C) 100 to 200 base pairs D) 1,000 to 5,000 base pairs

Answer Key

C — Human DNA sequences are 99.9% identical in every person

B — DNA profiling relies on highly variable repetitive sequences called Variable Number Tandem Repeats (VNTRs)

B — Microsatellites (STRs/SSRs) consist of repeating sequences of 2 to 6 base pairs of DNA

B — Minisatellites consist of a short series of bases 10 to 60 base pairs long

Lesson 63: DNA Profiling Process

Q1. What is the most desirable non-invasive sample collection method for obtaining reference DNA? A) Muscle biopsy B) Buccal swab C) Bone marrow aspiration D) Hair follicle excision

Q2. Which restriction enzyme is explicitly cited as an example used to cut DNA at specific sites during profiling? A) BamHI B) EcoRI C) HindIII D) SmaI

Q3. What material is used as the solid support matrix onto which DNA pieces are transferred overnight? A) Polyacrylamide gel sheet B) Nylon sheet C) Starch block D) Filter paper disk

Q4. How many probes simultaneously are typically used to construct a final DNA fingerprint barcode pattern? A) Exactly 1 probe B) 2 to 3 probes C) Several probes (5–10 or more) D) Over 1,000 probes

Answer Key

B — The most desirable method for collecting a reference sample is a buccal swab because it reduces contamination risk

B — EcoRI is explicitly cited as an enzyme that cuts DNA when the 5'..GAATTC..3' sequence occurs

B — DNA fragments are transferred to a nylon sheet by soaking them overnight

C — The final DNA fingerprint pattern is built using several probes (5–10 or more) simultaneously

Lesson 64: Types of DNA Fingerprinting Methods

Q1. Which of the following is listed as a major type of DNA fingerprinting method in Lesson 64? A) RFLP (Restriction Fragment Length Polymorphism) B) Western Blotting Immunodetection C) Bisulfite cytosine conversion D) Nick translation

Q2. How does electrophoresis separate ions and DNA fragments during gel analysis? A) Based on mobility of ions in an electric field according to charge, size, and shape B) By centrifuging fragments through a sucrose gradient C) By boiling fragments in alkaline detergent solution D) By filtering fragments through paper towels

Q3. Towards which electrode do negatively charged DNA ions migrate during electrophoresis? A) Negative electrode (cathode) B) Positive electrode (anode) C) Ground wire terminal D) Peltier cooling block

Q4. In RFLP analysis, what determines the length of detected fragments on a membrane? A) Length of strands with repeating base pair patterns digested by restriction enzymes B) Extension rate of thermostable polymerases C) Number of dNTPs in the reaction buffer D) Amount of SDS added to the polyacrylamide gel

Answer Key

A — RFLP (along with electrophoresis, PCR, RAPD, and AFLP) is explicitly listed as a DNA fingerprinting method

A — Electrophoresis separates ions based on their mobility in an electric field depending on total charge, size, and shape

B — Negatively charged ions migrate toward the positive electrode during gel electrophoresis

A — RFLP analyzes the length of DNA strands containing repeating base pair patterns cut by restriction digest

Lesson 65: Types of DNA Fingerprinting Methods-2

Q1. What length range characterizes the arbitrary primers used in Random Amplified Polymorphic DNA (RAPD)? A) 2 to 4 nucleotides B) 8 to 12 nucleotides C) 18 to 30 nucleotides D) 50 to 100 nucleotides

Q2. Which advantage is explicitly attributed to RAPD in Lesson 65? A) It requires no DNA probes or prior sequence information B) It identifies co-dominant alleles effortlessly C) It is completely immune to changes in PCR cycling conditions D) It uses radioactive 32P isotopes exclusively

Q3. Why are nearly all RAPD markers classified as dominant markers? A) Because they cannot distinguish whether an amplified segment is heterozygous (1 copy) or homozygous (2 copies) B) Because they bind only to dominant phenotypes C) Because they require 2 pairs of nested primers D) Because they convert unmethylated cytosines to uracil

Q4. Why is the RAPD technique notoriously laboratory-dependent? A) Because it relies on enzymatic PCR reactions sensitive to template quality, component concentrations, and cycling conditions B) Because it requires radioactive decay counters C) Because it uses single-stranded RNA templates D) Because it requires high-gram quantities of genomic DNA

Answer Key

B — RAPD creates arbitrary, short primers (8–12 nucleotides long) for PCR

A — Advantages of RAPD include requiring no DNA probes or prior sequence information

A — RAPD markers are dominant because it is impossible to distinguish between heterozygous (1 copy) and homozygous (2 copies) states

A — RAPD is sensitive to template quality, component concentrations, and PCR conditions, making results lab-dependent

Lesson 66: Applications of Fingerprinting

Q1. Which inherited medical conditions are explicitly listed as diagnosable using DNA fingerprinting? A) Cystic fibrosis, hemophilia, Huntington's disease, sickle cell anemia, and thalassemia B) Type 1 diabetes and essential hypertension C) Scurvy, rickets, and pellagra D) Asthma and seasonal allergies

Q2. Since what year has biological evidence from DNA fingerprinting been used in legal courts to solve criminal cases? A) 1953 B) 1975 C) 1987 D) 2010

Q3. What degree of accuracy do DNA fingerprints bring to legal paternity and child custody determinations? A) Low accuracy B) Moderate accuracy requiring secondary tests C) Unprecedented, nearly perfect accuracy D) 50% statistical probability

Q4. Why is DNA fingerprinting ideal for personal identification across all body tissues? A) All body tissues and organs contain the same DNA type B) Tissue samples change DNA sequence daily C) Only liver tissue contains usable genomic DNA D) It requires kilograms of tissue samples

Answer Key

A — Listed inherited disorders include cystic fibrosis, hemophilia, Huntington's disease, sickle cell anemia, and thalassemia

C — Innumerable legal cases have been solved using DNA fingerprint evidence since the year 1987

C — In paternity disputes, DNA fingerprints bring an unprecedented, nearly perfect accuracy to court determinations

A — DNA is ideal for personal identification because all body tissues and organs contain the same DNA type

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