VU Medical Zone

Admin: Amaan Khan

METHODS IN MOLECULAR BIOLOGY

BIO 203 MIDTERM MERGE PPT

LECTURE 1 TO 34

Essentials of Genetics

POLYMERASE CHAIN REACTION

LESSON NO 1

Polymerase Chain Reaction

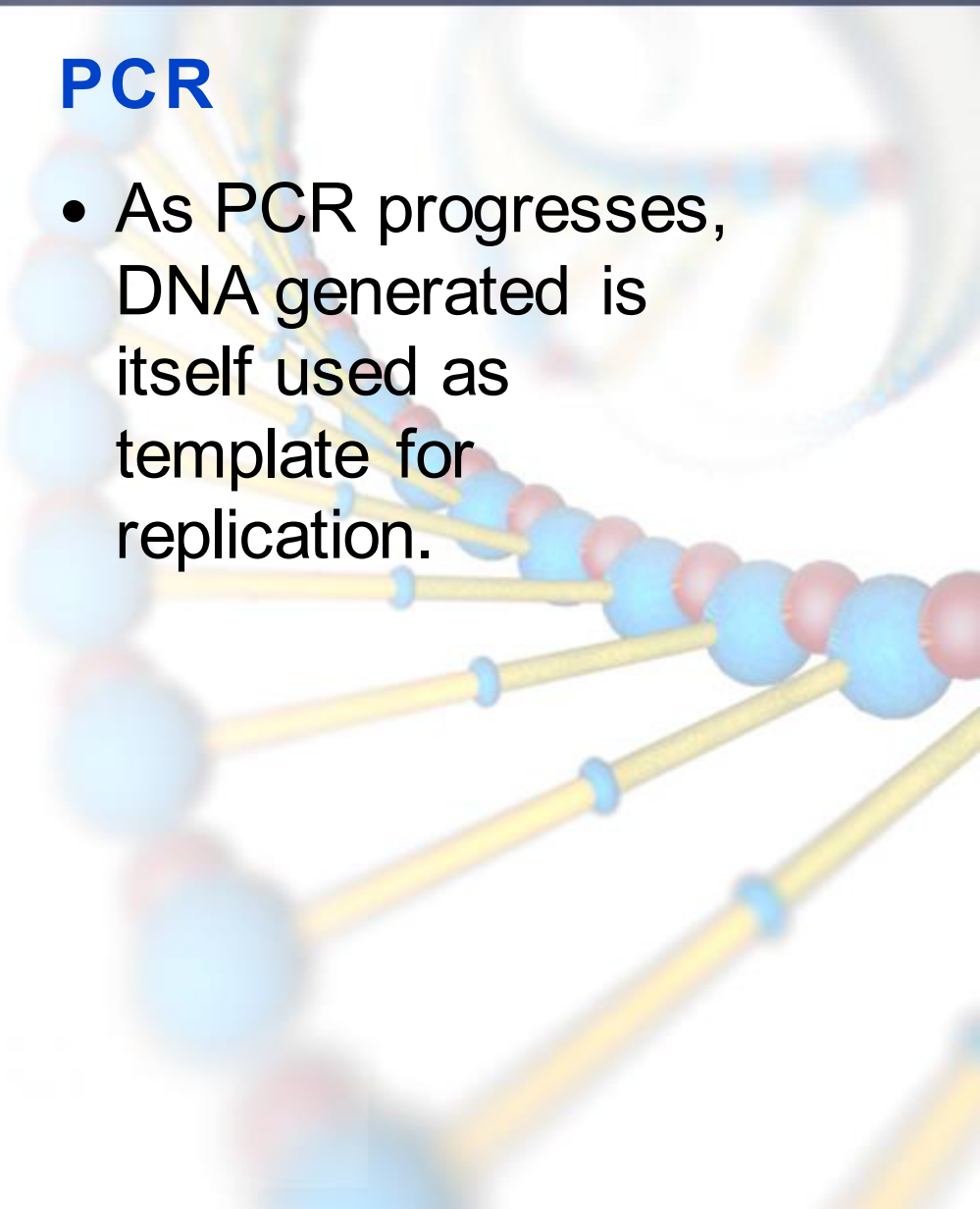
PCR

- A technique widely used in Molecular Biology and Biotechnology.
- Its name is from one of its key component - DNA polymerase.

Polymerase Chain Reaction

PCR

- As PCR progresses, DNA generated is itself used as template for replication.



Polymerase Chain Reaction

PCR- A CHAIN REACTION

- This sets in motion of a chain reaction in which the DNA template is exponentially amplified.

Polymerase Chain Reaction

HEAT STABLE DNA POLYMERASE

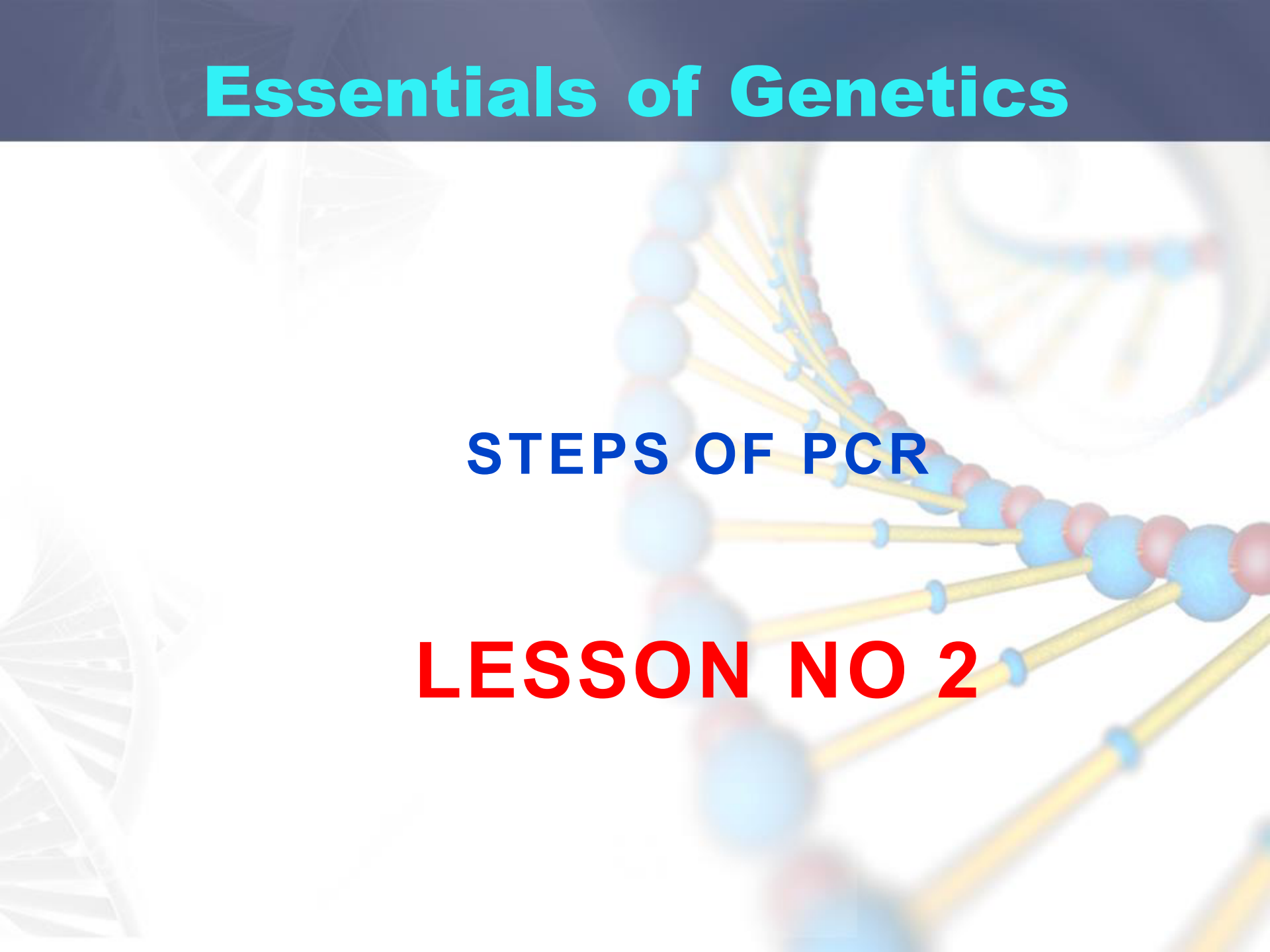
During PCR, a heat-stable DNA polymerase, such as Taq polymerase - an enzyme derived from the bacterium Thermus aquaticus.

Polymerase Chain Reaction

CONCLUSION

- PCR can be performed to amplify DNA.
- It can be extensively modified to perform a wide array of genetic manipulations.

Essentials of Genetics



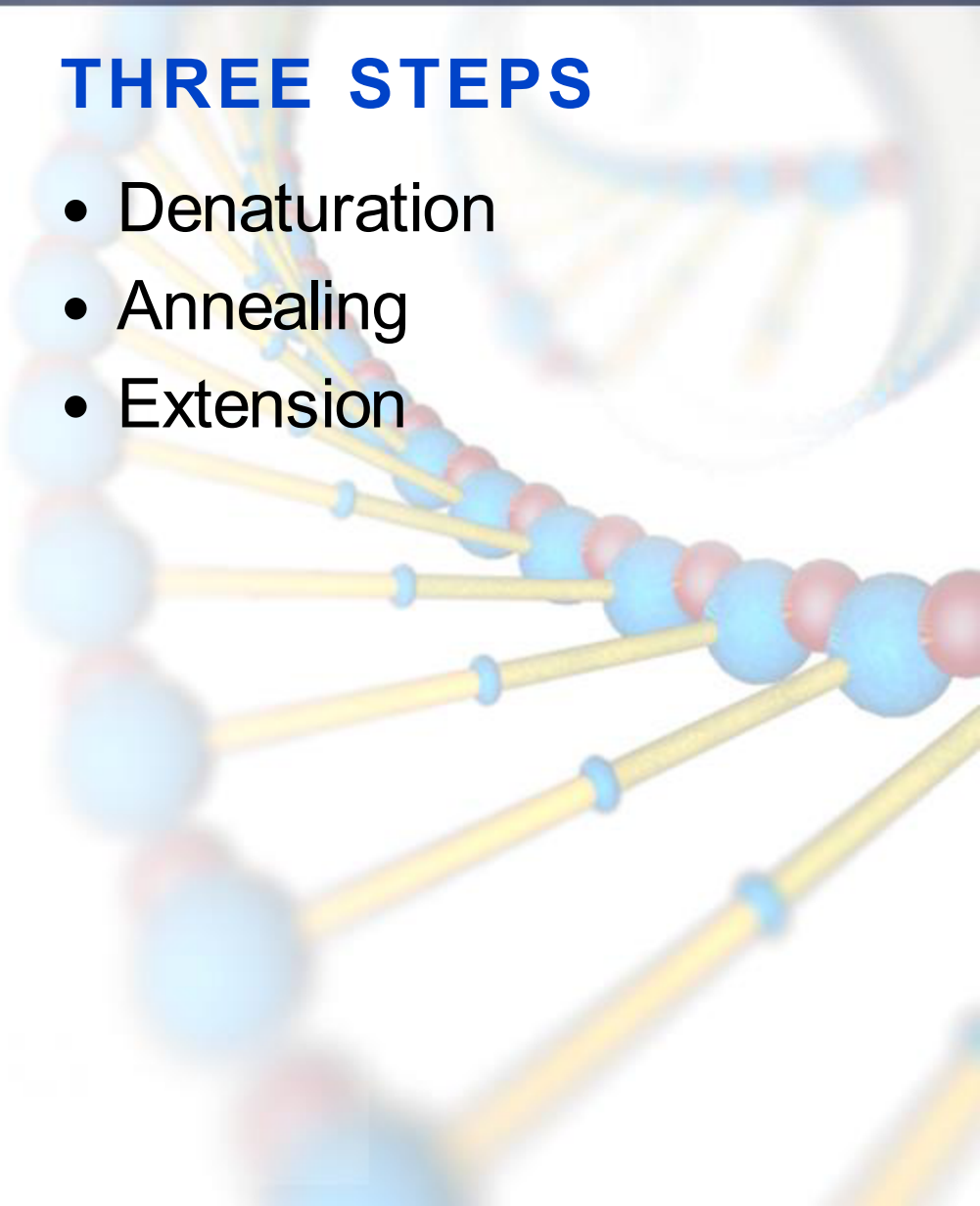
STEPS OF PCR

LESSON NO 2

Steps of PCR

THREE STEPS

- Denaturation
- Annealing
- Extension



Steps of PCR

INITIALIZATION STEP

- This step consists of heating the reaction to a temperature of 93°C - 96°C, which is held for 1-10 minutes.

Steps of PCR

DENATURATION

- First regular cycling event and consists of heating the reaction to 93°C - 98°C for 20-45 seconds.
- It causes melting of DNA template yielding single strands of DNA.

Steps of PCR

ANNEALING

- The reaction temperature is lowered to 40-65°C for 20-40 seconds.
- Annealing of the primers to the single-stranded DNA template.

Steps of PCR

EXTENSION

- The temperature at this step depends on the type of DNA polymerase used.
- Taq polymerase has its optimum activity temperature at 70-80°C.
- Commonly 72°C is used.

Steps of PCR

EXTENSION

- At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTP's.

Steps of PCR

FINAL ELONGATION

- Single step is performed at a temperature of 70-74°C for 5-15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

Steps of PCR

FINAL HOLD TEMPERATURE

- This step at 4-15°C for an indefinite time may be employed for short-term storage of the reaction.

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INGREDIENTS OF PCR

LESSON NO 3

Ingredients of PCR

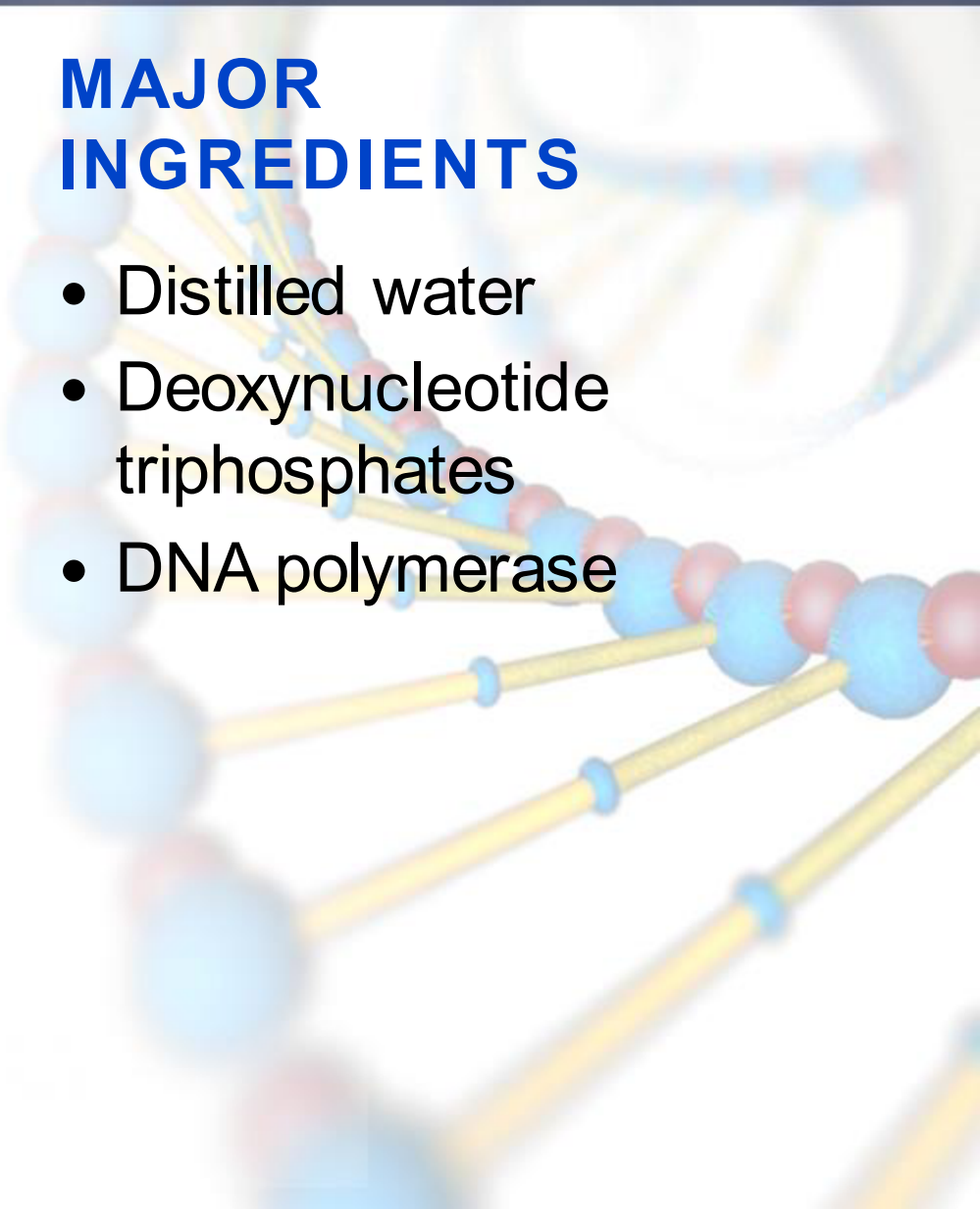
MAJOR INGREDIENTS

- Microfuge tube
- Thermal cycler
- DNA template
- Primers
- Buffer
- MgCl2

Ingredients of PCR

MAJOR INGREDIENTS

- Distilled water
- Deoxynucleotide triphosphates
- DNA polymerase



Ingredients of PCR

MICROFUGE TUBES

- These are small cylindrical plastic tubes with conical bottoms.





Ingredients of PCR

THERMOCYCLER

- The thermocycler works on the principle of Peltier effect, which raises and lowers the temperature of the block in a pre-programmed manner.



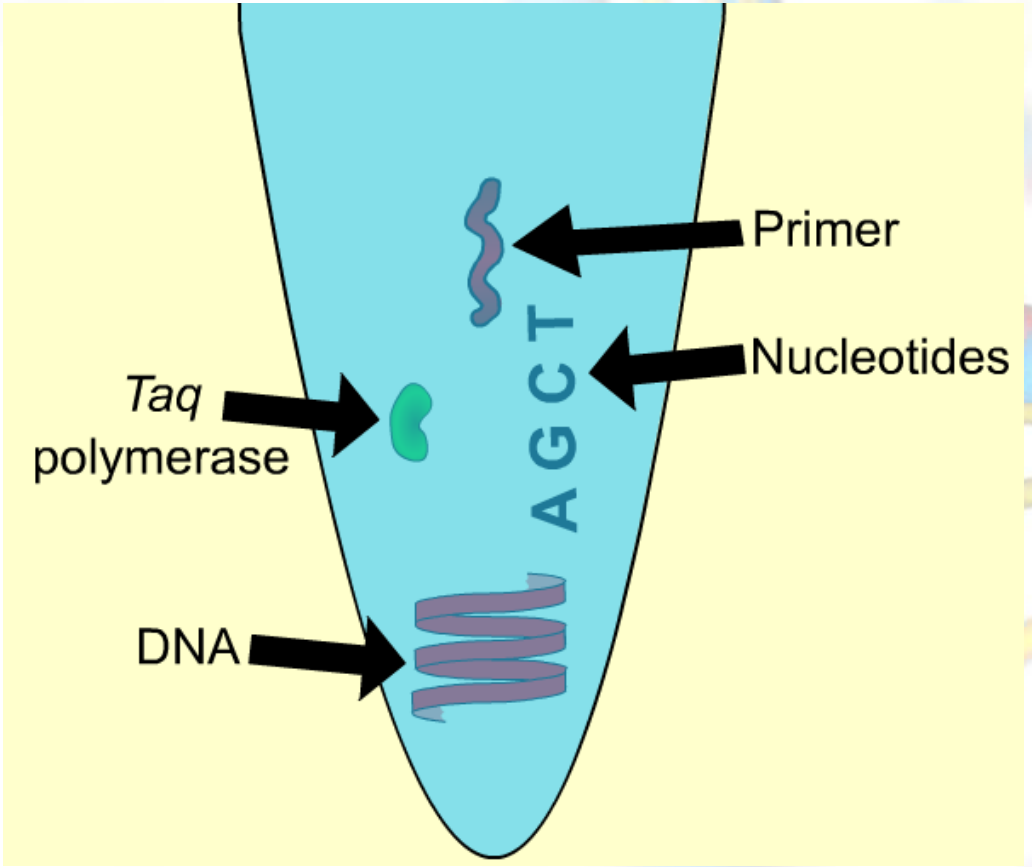
Ingredients of PCR

DNA POLYMERASES

- Polymerases
  - Taq polymerase
  - Pfu polymerase
  - Vent polymerase

Ingredients of PCR

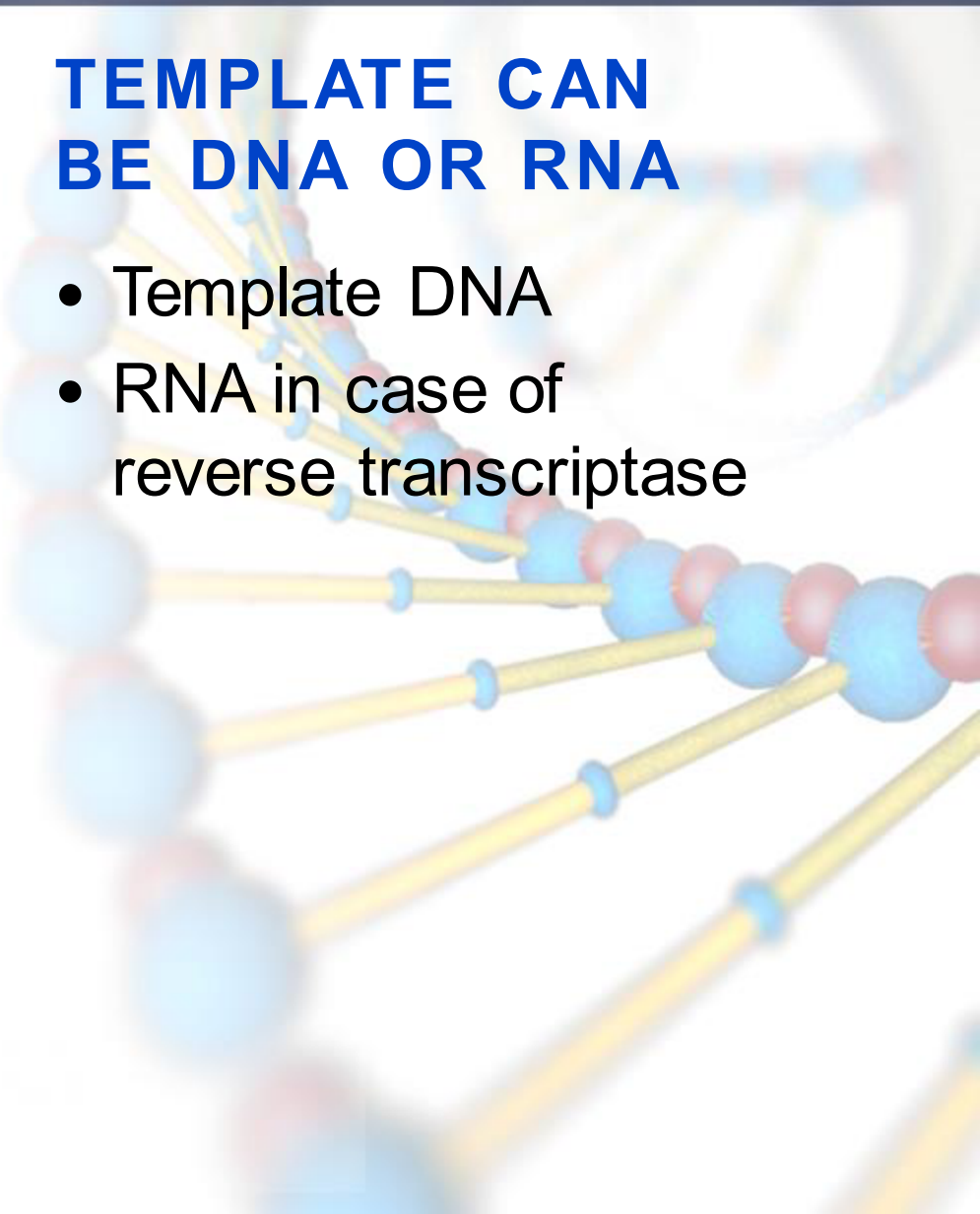
INGREDIENTS IN PCR TUBE



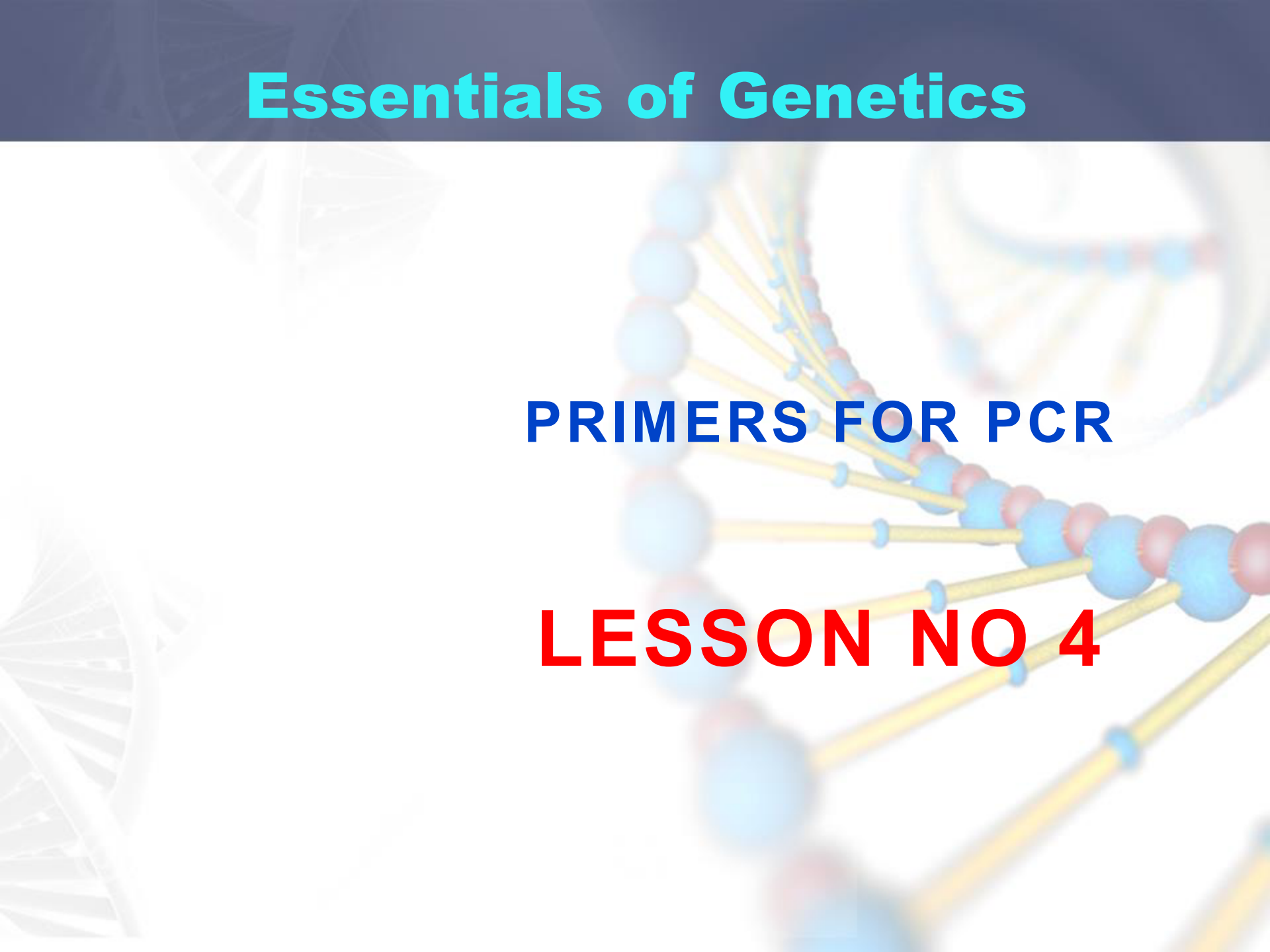
Ingredients of PCR

TEMPLATE CAN BE DNA OR RNA

- Template DNA
- RNA in case of reverse transcriptase



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PRIMERS FOR PCR

LESSON NO 4

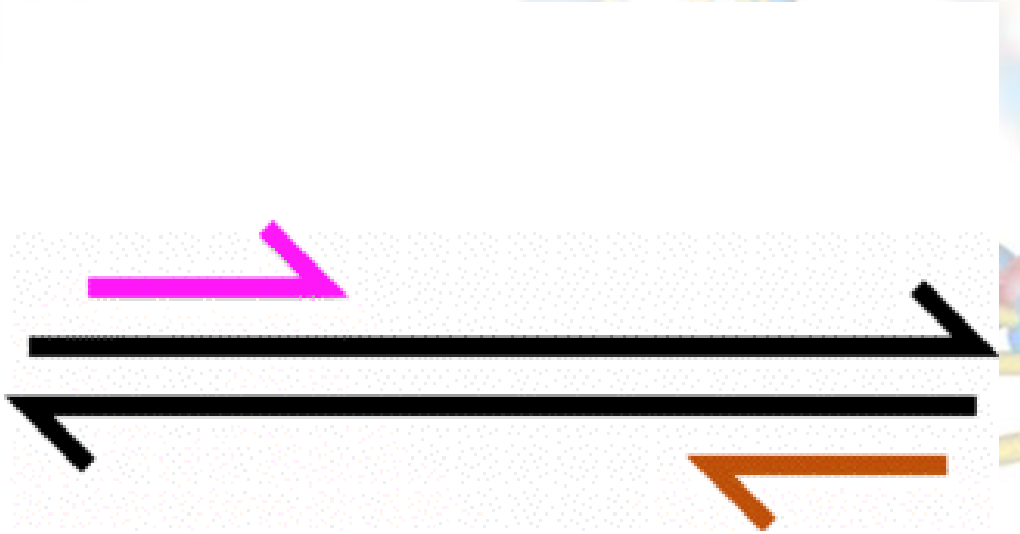
PRIMERS FOR PCR

PRIMERS

- Primers are single-stranded 18–30 bp long DNA fragments
- Complementary to sequences flanking the region to be amplified.

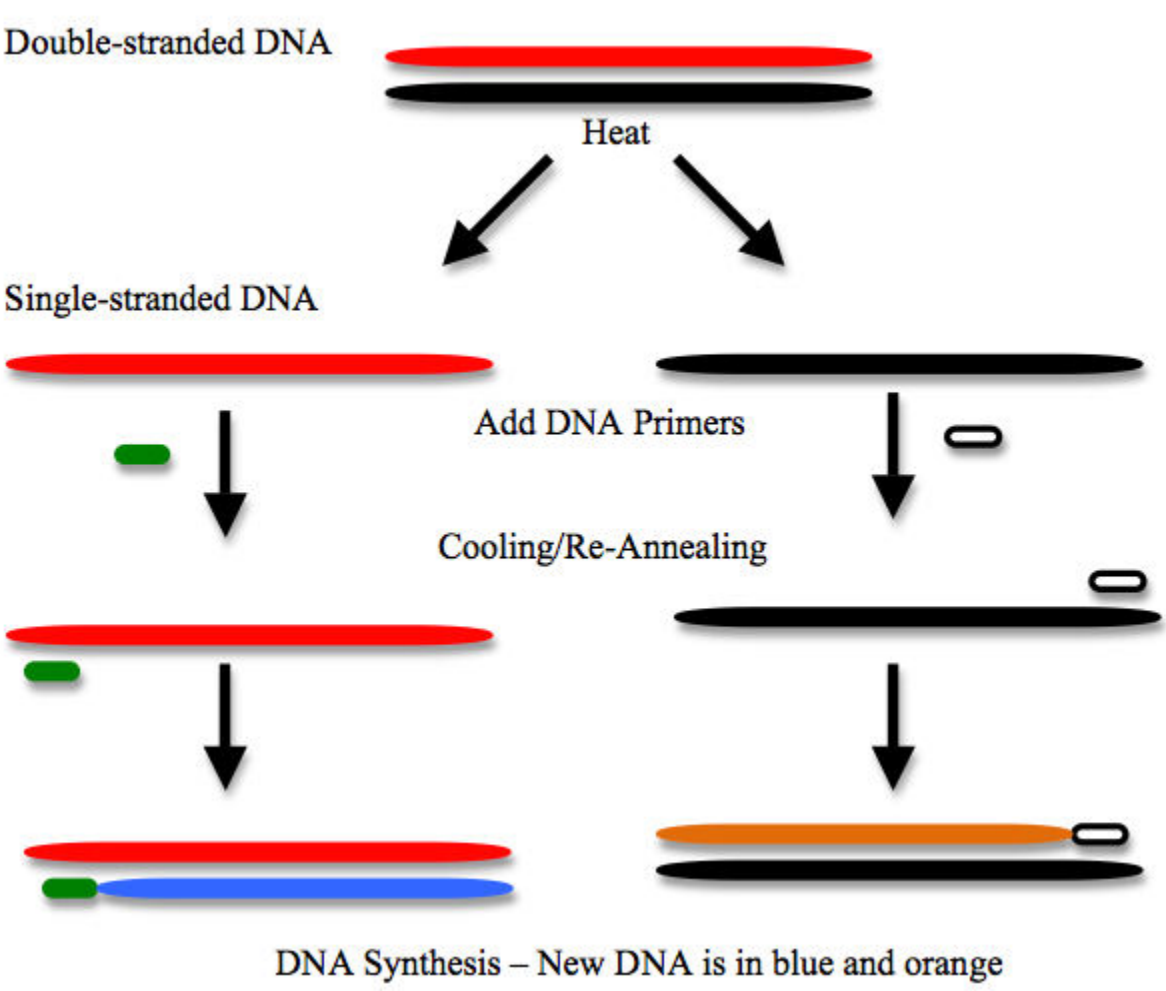
PRIMERS FOR PCR

PRIMERS



PRIMERS FOR PCR

PRIMERS



DNA Synthesis – New DNA is in blue and orange

PRIMERS FOR PCR

PRIMERS CAN BE SPECIFIC OR RANDOM

- Primers determine the specificity of the PCR reaction.
- Distance between the primers binding sites will determine the size of PCR product.

PRIMERS FOR PCR

FEATURES OF PRIMERS

- Types of primers- random or specific
- Primer length
- Annealing temperature
- Specificity
- Nucleotide composition

PRIMERS FOR PCR

PRIMERS

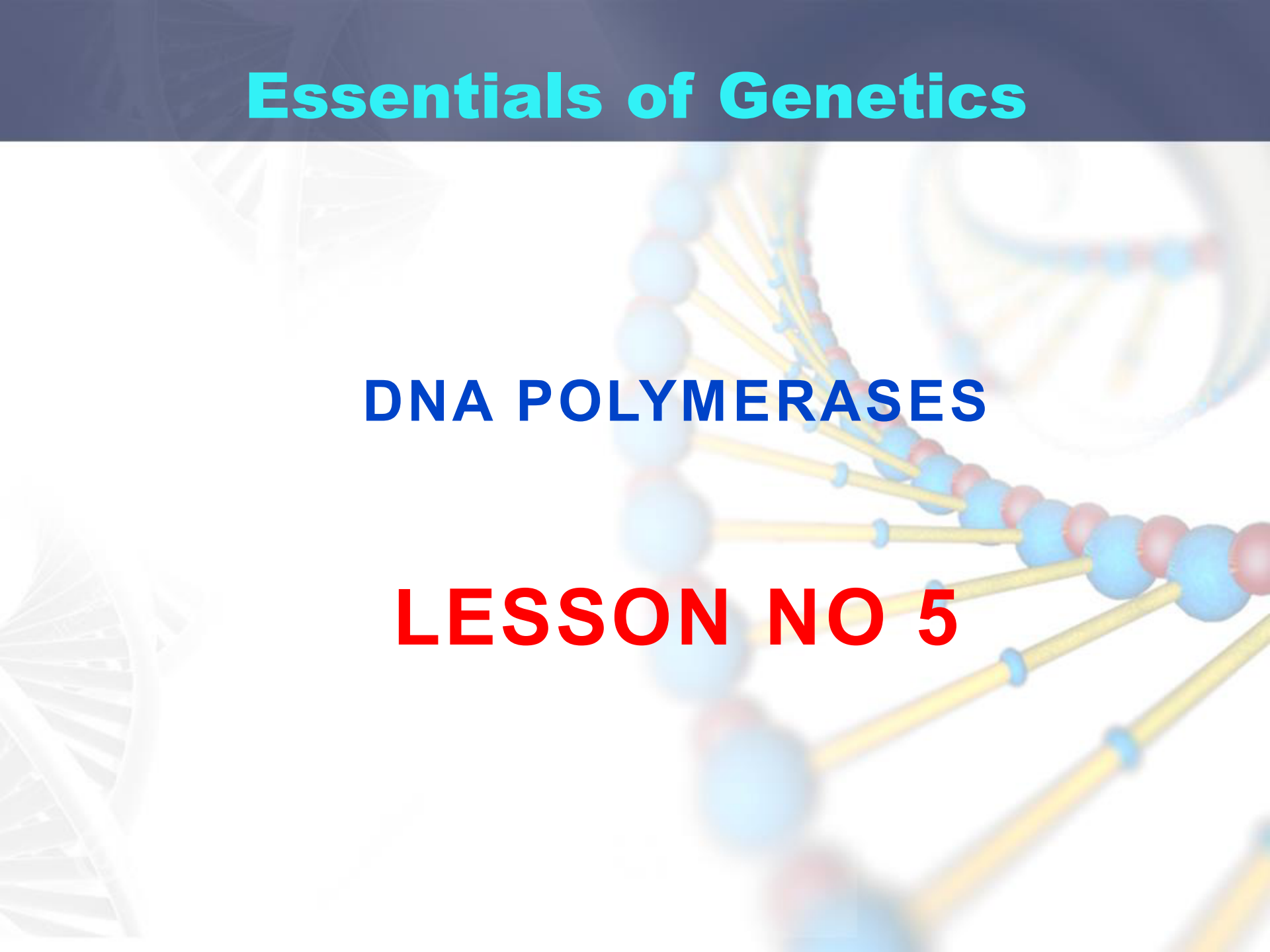
- Avoid inter-strand homologies
- Avoid intra-strand homologies
- T_m of forward primer = T_m of reverse primer
- G/C content of 20–80%; avoid longer than GGGG
- Product size (100–700 bp)
- Target specificity

PRIMERS FOR PCR

FORMULA FOR CALCULATING MELTING TEMPERATURE OF PRIMERS

- Tm = 4(G+C) + 2(A+T)

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DNA POLYMERASES

LESSON NO 5

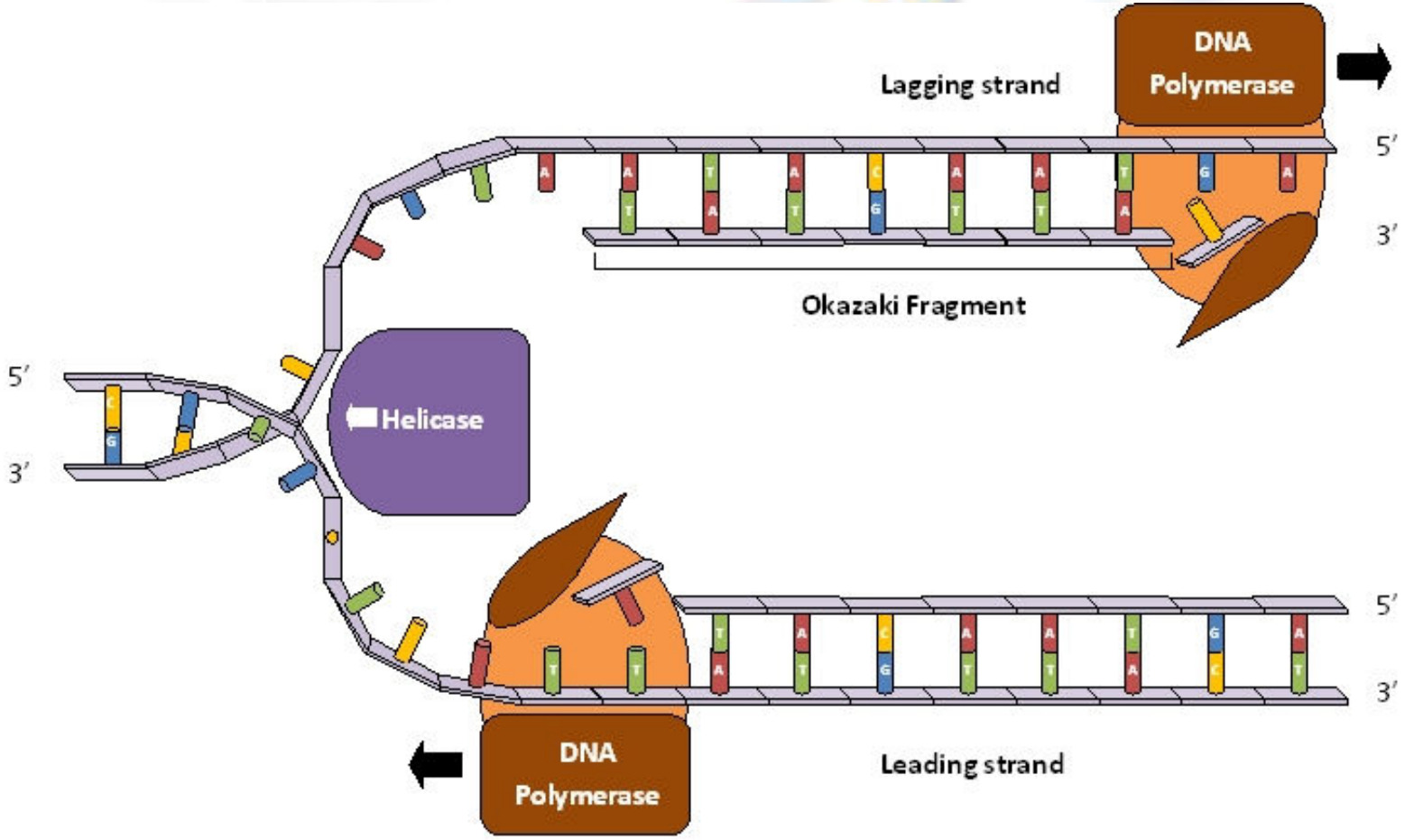
DNA Polymerases

POLYMERASES

Polymerase	Extension Rate (nt/sec)	Source
Taq pol	75	*T. aquaticus*
Amplitaq (Stoffel fragment)	>50	*T. aquaticus*
Vent	>80	*Thermococcus litoralis*
Pfu	60	*Pyrococcus furiosus*
Tth (RT activity)	>33	*T. thermophilus*

DNA Polymerases

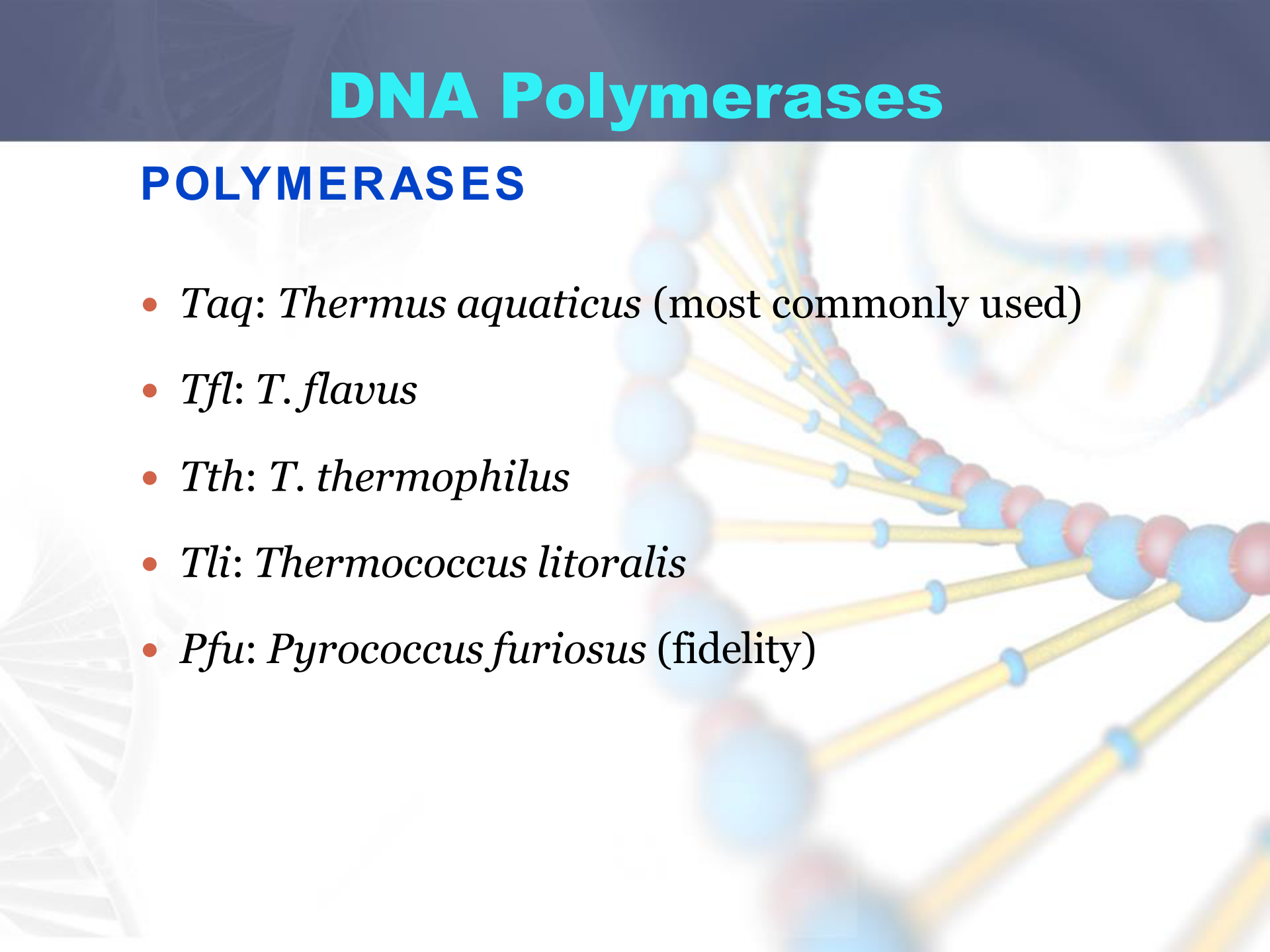
POLYMERASES



DNA Polymerases

POLYMERASES

- *Taq*: *Thermus aquaticus* (most commonly used)
- *Tfl*: *T. flavus*
- *Tth*: *T. thermophilus*
- *Tli*: *Thermococcus litoralis*
- *Pfu*: *Pyrococcus furiosus* (fidelity)



DNA Polymerases

DNA POLYMERASES

- Polymerases
  - Taq polymerase
  - Pfu polymerase
  - Vent polymerase

Essentials of Genetics

STANDARD PCR REACTION

LESSON NO 6

Standard PCR Reaction

STANDARD REACTION

0.25 mM each primer

0.2 mM each dATP, dCTP, dGTP, dTTP

50 mM KCl

10 mM Tris

1.5 mM $MgCl_2$

2.5 units polymerase

10^2 - 10^5 copies of template

50 ul reaction volume

Standard PCR Reaction

PCR TEMPERATURES

- Denaturation temperature
  - Reduces double stranded molecules to single stranded
  - 90–96°C, 20-45 seconds
- Annealing temperature
  - Controls specificity of hybridization
  - 40–68°C, 20-30 seconds
- Extension temperature
  - Optimized for individual polymerases
  - 70–75°C, 30-45 seconds

Standard PCR Reaction

TEMPERATURES

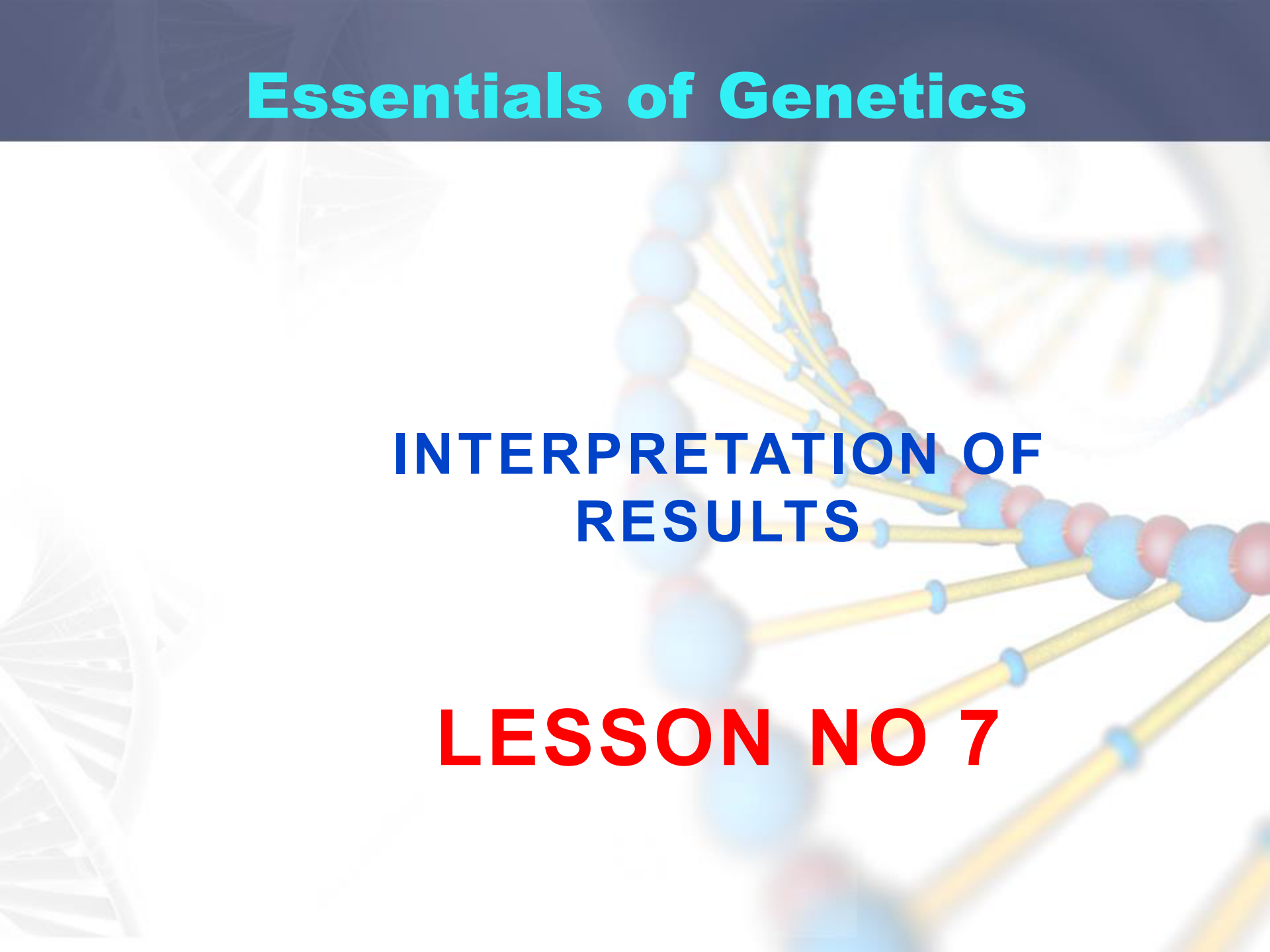
Temp	For	Comments
94-60-72	Perfect, long primers	Higher temp can be used; maximum annealling temp
94-55-72	Good or perfectly matched primers between 19-24 nt	Standard conditions
94-50-72	Adequate primers	Allows 1-3 mismatches/20 nt
94-48-68	Poorly matched primers	Allows 4-5 mismatches/20 nt
94-45-65	Unknown match, likely poor	Primers of questionable quality

Standard PCR Reaction

TEMPERATURES

- Amplification takes place as the reaction mix is subject to an amplification program and temperatures.
- The amplification program consists of a series of 20–50 PCR cycles.

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INTERPRETATION OF RESULTS

LESSON NO 7

Interpretation of Results

INTERPRETATION

- The PCR product should be of the expected size.
- Misprimes may occur due to non-specific hybridization of primers.

Interpretation of Results

PRIMERS DIMERS MAY REDUCE AMPLIFICATION

- Primer dimers may occur due to hybridization of primers to each other.

Interpretation of Results

BLANK REACTION

- Controls for contamination

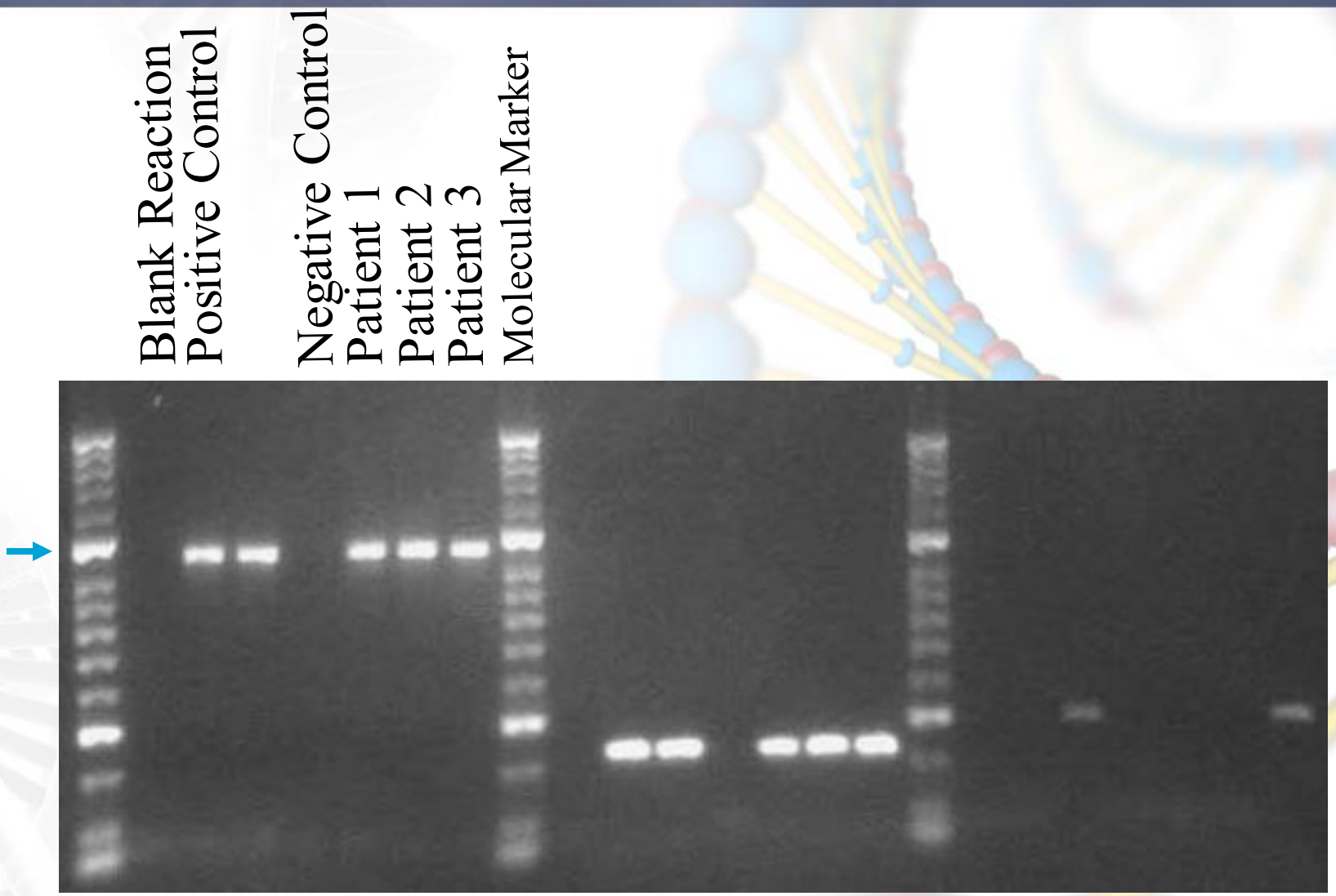
  contains all reagents except DNA template.

Interpretation of Results

NEGATIVE CONTROL

- Controls for specificity of the amplification reaction contains all reagents and a DNA template lacking the target sequence.

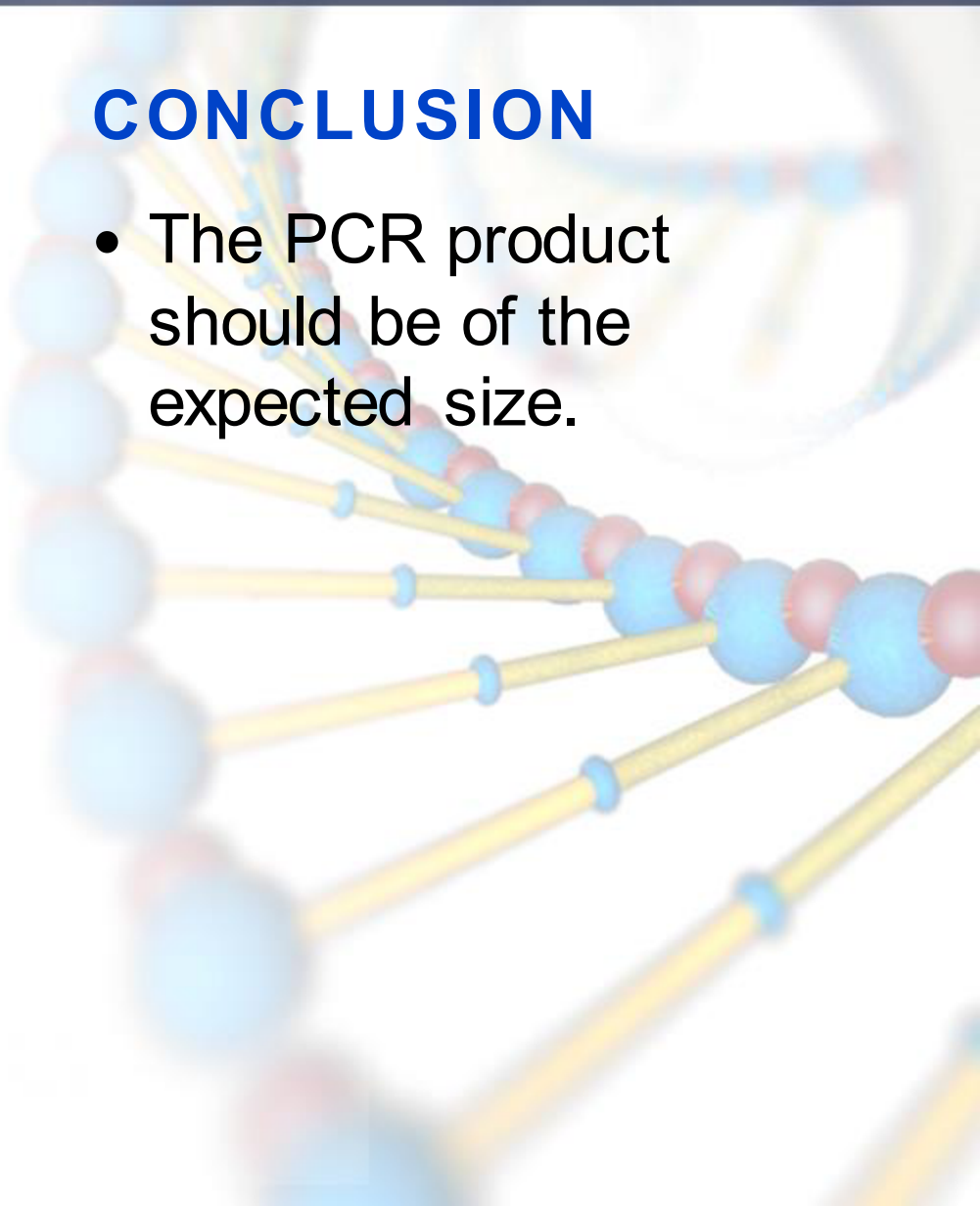
Interpretation of Results



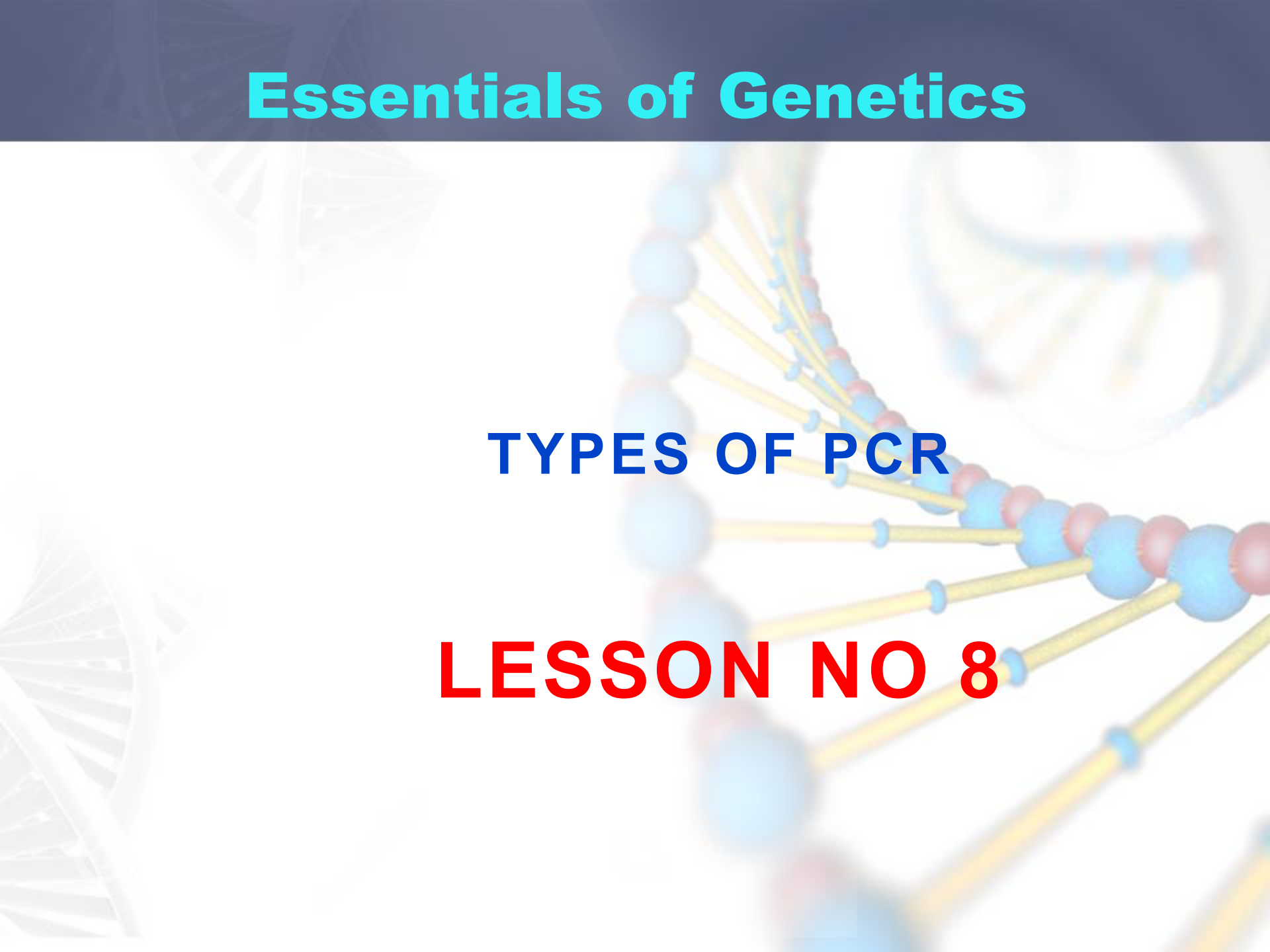
Interpretation of Results

CONCLUSION

- The PCR product should be of the expected size.



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TYPES OF PCR

LESSON NO 8

Types of PCR

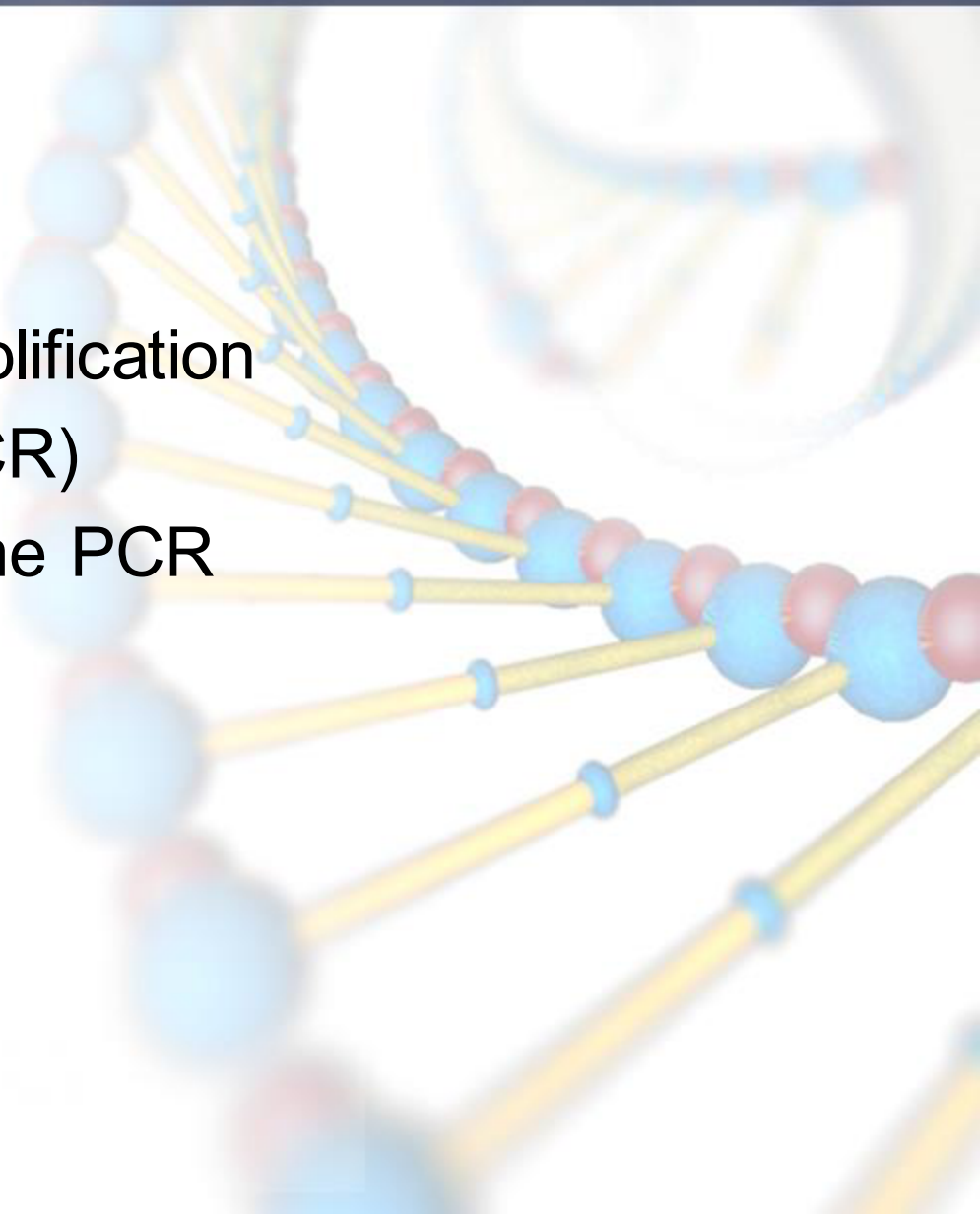
TYPES

- Nested PCR
- Multiplex PCR
- Touchdown PCR
- Sequence-specific PCR
- Reverse-transcriptase PCR

Types of PCR

TYPES

- Long-range PCR
- Whole-genome amplification
- RAPD PCR (AP-PCR)
- Quantitative real-time PCR

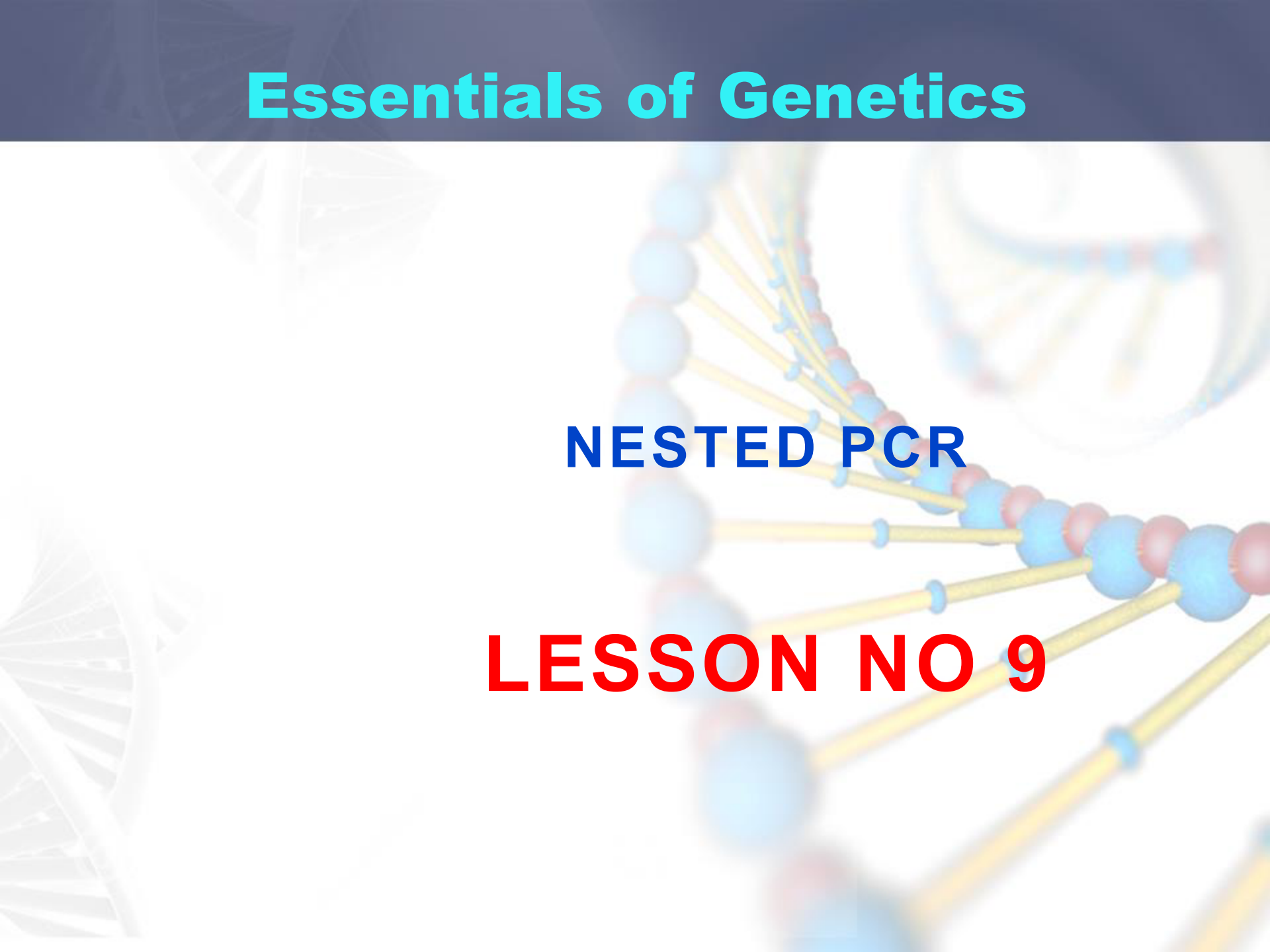


Types of PCR

TYPES

- Long-range PCR
- Whole-genome amplification
- RAPD PCR (AP-PCR)
- Quantitative real-time PCR

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NESTED PCR

LESSON NO 9

Nested PCR

NESTED PCR

- Two pairs (instead of one pair) of PCR primers are used to amplify a fragment.
- First pair amplify a fragment similar to a standard PCR.

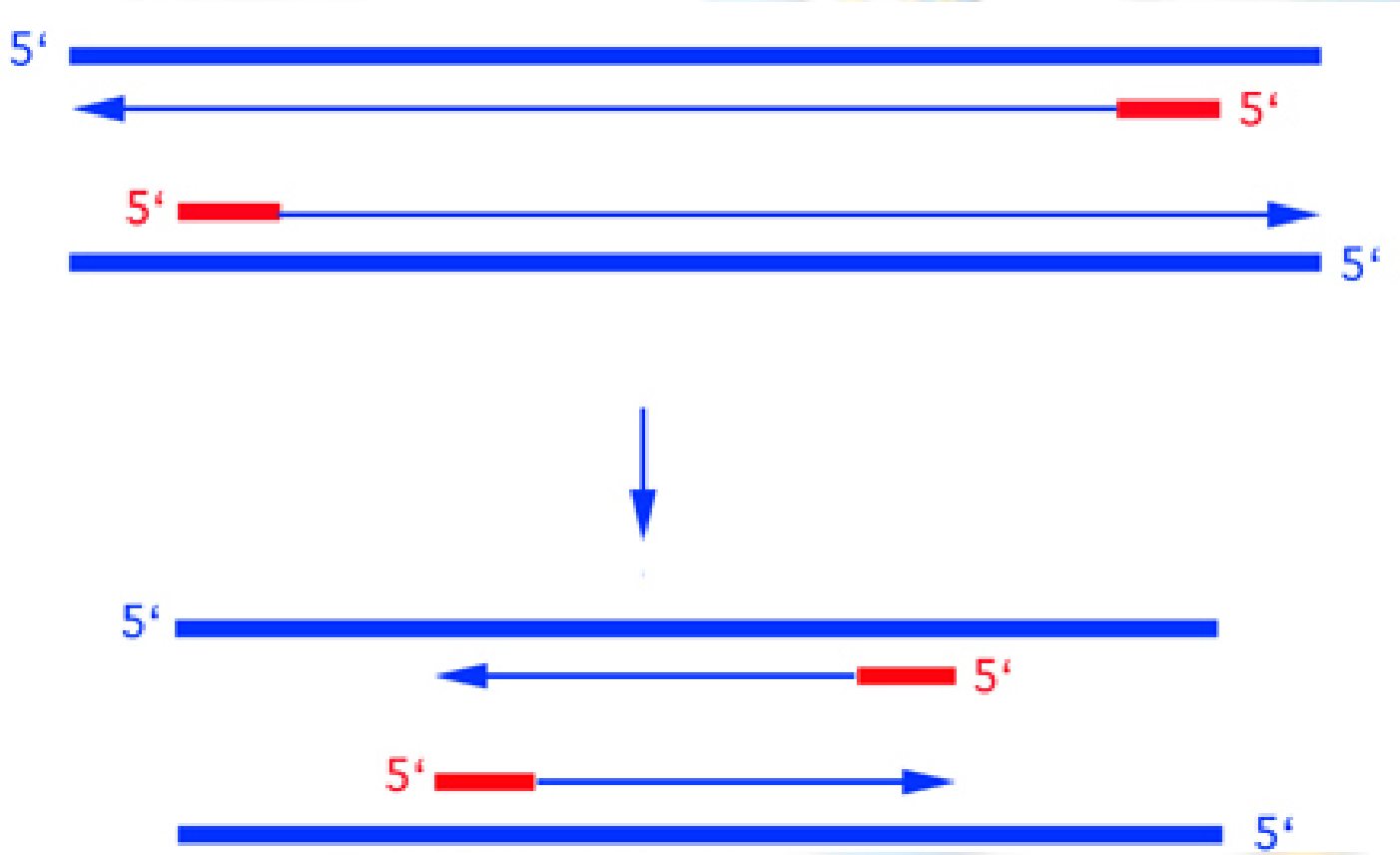
Nested PCR

NESTED PCR – SECOND PAIR OF PRIMERS

- Second pair of primers - nested primers (as they lie / are nested within the first fragment).

Nested PCR

NESTED PCR



Nested PCR

SECOND PCR PRODUCT IS SHORTER THAN FIRST ONE

- Second pair of primers bind inside the first PCR product fragment to allow amplification of a second PCR product.

Nested PCR

SECOND PCR PRODUCT IS SPECIFIC

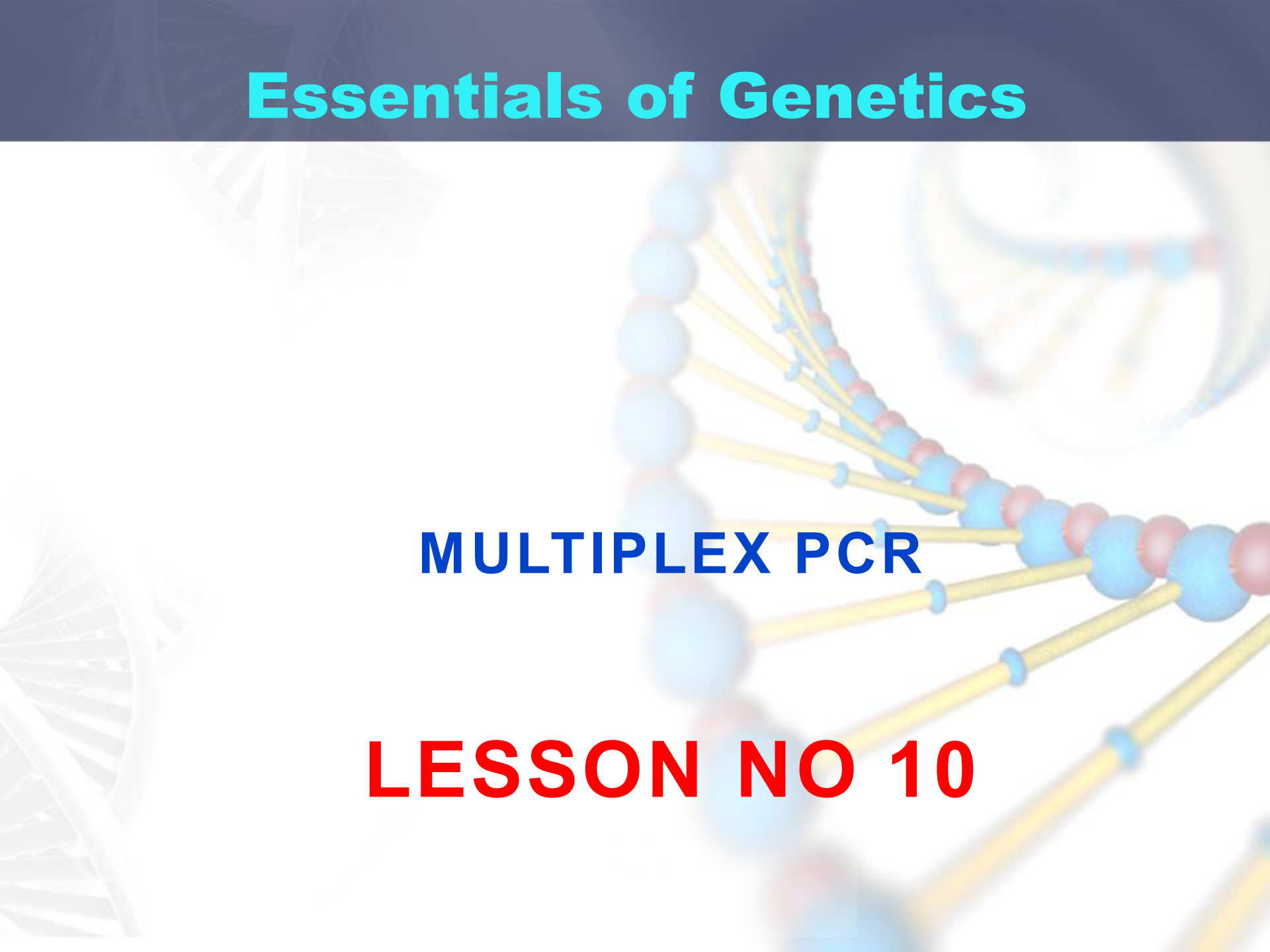
- increases the specificity of DNA amplification, by reducing background due to non-specific amplification of DNA.

Nested PCR

ADVANTAGE OF NESTED PCR

- Very low probability of nonspecific amplification.

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MULTIPLEX PCR

LESSON NO 10

Multiplex PCR

MULTIPLEX PCR

- Multiplex PCR is a variant of PCR which enables simultaneous amplification of many targets of interest in one reaction by using more than one pair of primers.

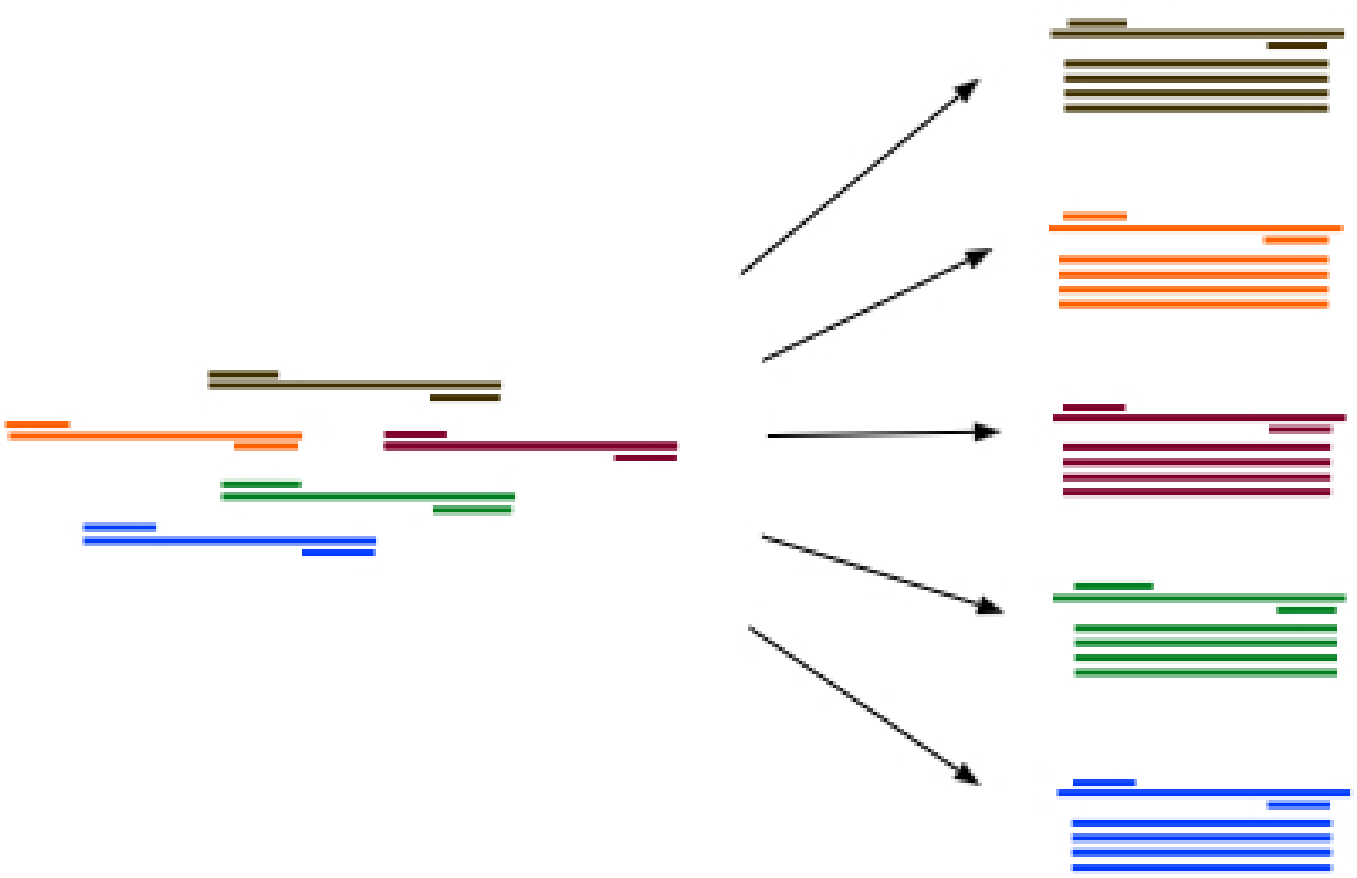
Multiplex PCR

AMPLICONS OF DIFFERENT SIZES ARE PRODUCED

- Multiplex-PCR consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences.

Multiplex PCR

MULTIPLEX PCR



Multiplex PCR

SINGLE REACTION, MANY AMPLICONS

- By targeting multiple sequences at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform.

Multiplex PCR

ANNEALING TEMPERATURES BE OPTIMIZED

- Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction.

Multiplex PCR

AMPLICON SIZE SHOULD BE DIFFERENT OR BE LABELED

- Amplicon sizes should be different enough to form distinct bands when visualized by gel electrophoresis.

Multiplex PCR

LABELED PRIMERS

- If amplicon sizes overlap, the different amplicons may be differentiated and visualised using primers that have been dyed with different color fluorescent dyes.

Multiplex PCR

PRIMERS PAIRS BE OPTIMIZED

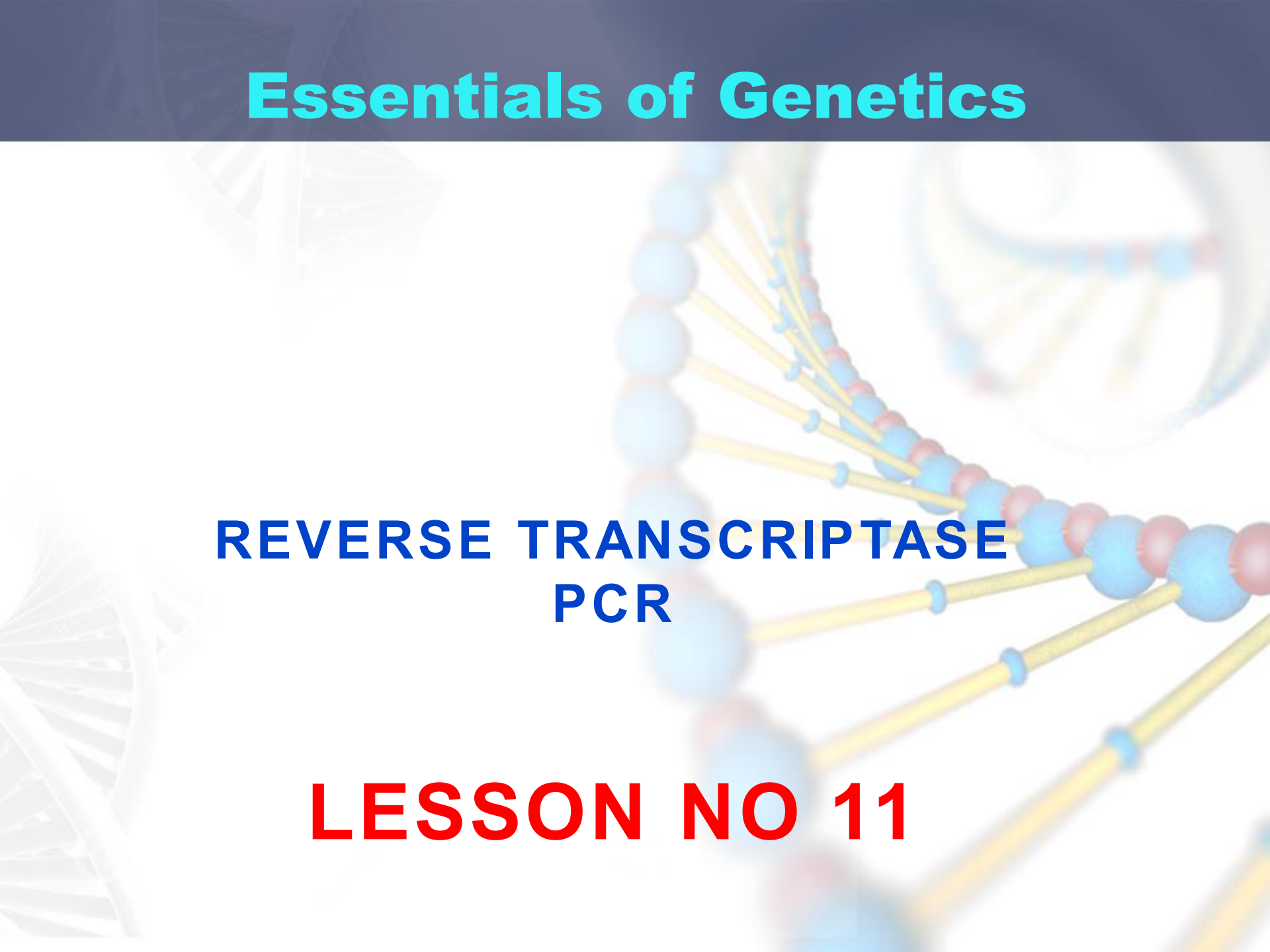
- The primer design for all primers pairs has to be optimized so that all primer pairs can work at the same annealing temperature during PCR.

Multiplex PCR

MULTIPLEX PCR

- The use of multiple, unique primer sets within a single PCR reaction to produce amplicons of varying sizes specific to different DNA sequences.

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REVERSE TRANSCRIPTASE PCR

LESSON NO 11

Reverse Transcriptase PCR

REVERSE TRANSCRIPTASE PCR

- Based on the process of reverse transcription, which reverse transcribes RNA into DNA.

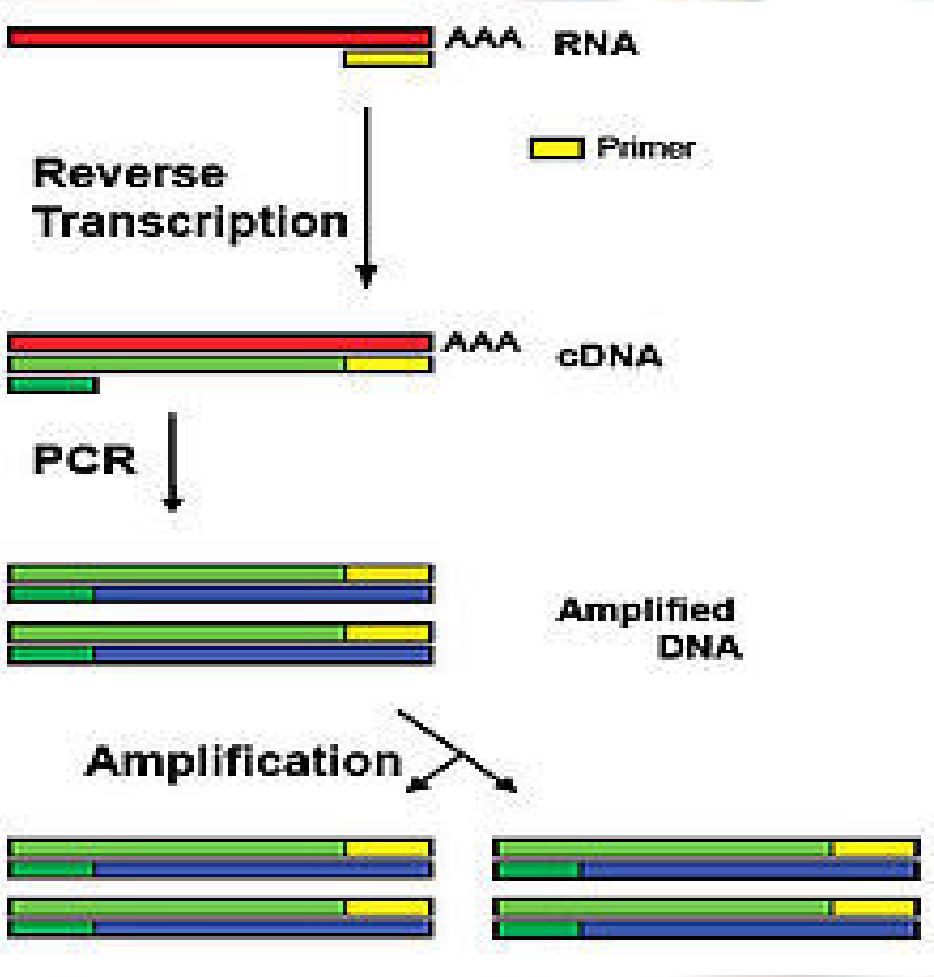
Reverse Transcriptase PCR

REVERSE TRANSCRIPTASE PCR

- First step of RT-PCR - first strand reaction
- Synthesis of cDNA using oligo dT primers (37°C) one hour.
- Second strand reaction - digestion of cDNA:RNA hybrid (RNaseH)-
- Standard PCR with DNA oligo primers .

Reverse Transcriptase PCR

REVERSE TRANSCRIPTASE PCR



Reverse Transcriptase PCR

ENZYME REVERSE TRANSCRIPTASE

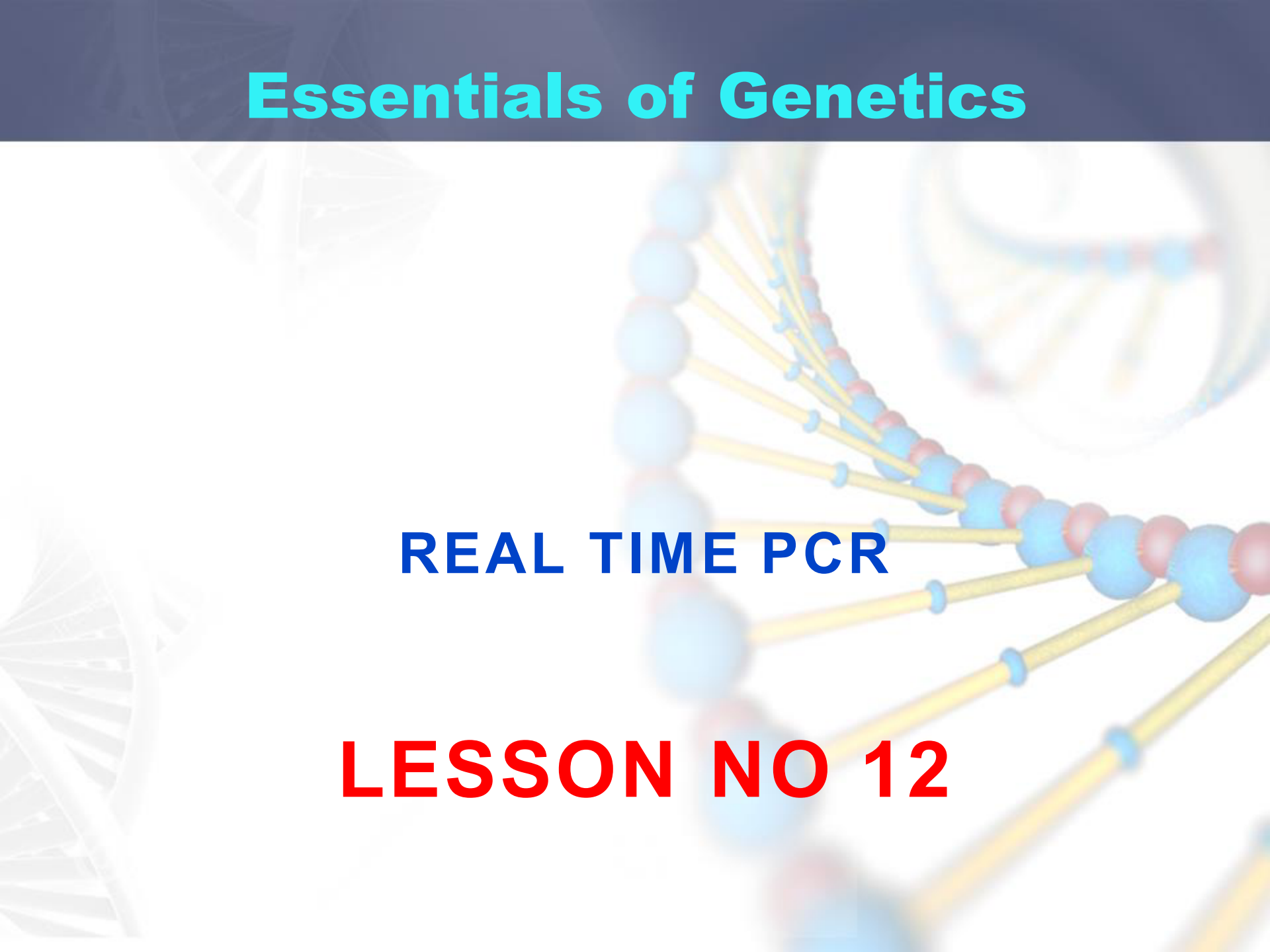
- The PCR is preceded by a reaction using reverse transcriptase to convert RNA to cDNA.

Reverse Transcriptase PCR

REVERSE TRANSCRIPTASE PCR

- RT-PCR is widely used in expression profiling, to determine the expression of a gene
- To identify the sequence of an RNA transcript.

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REAL TIME PCR

LESSON NO 12

Real Time PCR

REAL TIME PCR

- Real Time PCR is a technique in which fluoroprobes bind to specific target regions of amplicons to produce fluorescence during PCR.

Real Time PCR

REAL TIME PCR

- Real Time PCR is used to measure the quantity of a PCR product.
- The fluorescence, measured in Real Time, is detected in a PCR cycler with an inbuilt filter flurometer.
- It is the method of choice to quantitatively measure starting amounts of DNA, cDNA or RNA.

Real Time PCR

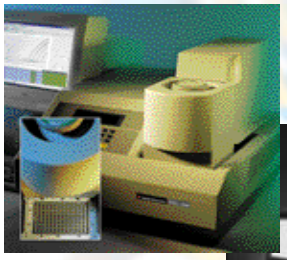
REAL TIME PCR



iCycler
BioRad



LightCycler
Roche



5700
Applied Biosystems



7700
Applied Biosystems



FluorTracker
Stratagene



FluorImager
Molecular Dynamics

Real Time PCR

REAL TIME PCR

- Quantitative real-time PCR is often confusingly known as RT-PCR (Real Time PCR) or RQ-PCR.
- QRT-PCR or RTQ-PCR are more appropriate contractions.
- QRT-PCR methods use fluorescent dyes, such as Sybr Green, or fluorophore-containing DNA probes, such as TaqMan, to measure the amount of amplified product in real time.

Real Time PCR

REAL TIME PCR

- Q-PCR is commonly used to determine whether a DNA sequence is present in a sample and the number of its copies in the sample.

Essentials of Genetics

HOT START PCR

LESSON NO 13

Hot Start PCR

HOT START PCR

- This is a technique that reduces non-specific amplification during the initial set up stages of the PCR.

Hot Start PCR

HOT START PCR

- The technique may be performed manually by heating the reaction components upto the melting temperature (e.g, 95°C).
- Before adding the polymerase, specialized enzyme systems have been developed that inhibit the polymerase's activity at ambient temperature.
- This is done either by the binding of an antibody or by the presence of covalently bound inhibitors that only dissociate after a high-temperature activation step.

Hot Start PCR

HOT START PCR

- DNA Polymerase- Eubacterial type I DNA polymerase, Pfu.
- These thermophilic DNA polymerases show a very small polymerase activity at room temperature .

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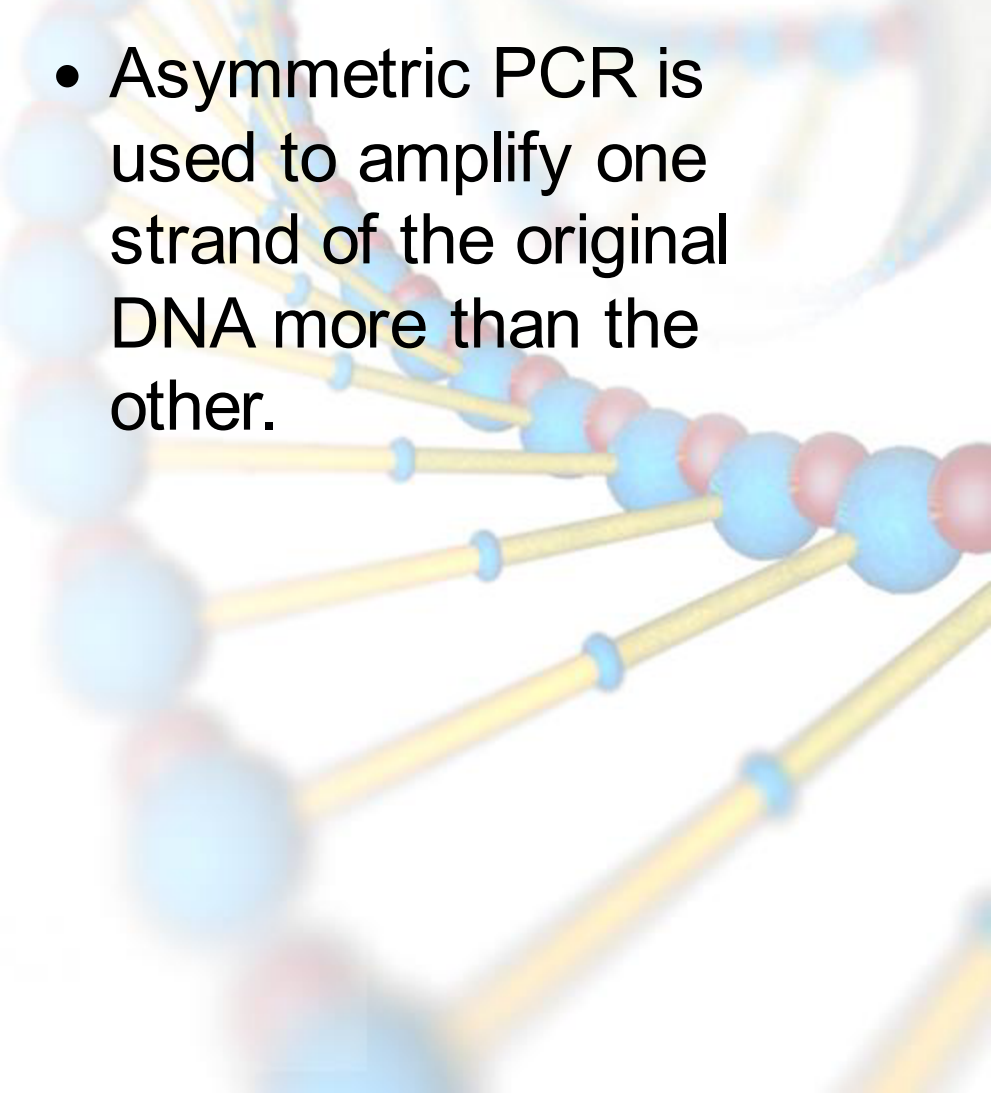
ASYMMETRIC PCR

LESSON NO 14

Asymmetric PCR

ASYMMETRIC PCR

- Asymmetric PCR is used to amplify one strand of the original DNA more than the other.



Asymmetric PCR

ASYMMETRIC PCR

- It is used in some types of sequencing and hybridization probing where having only one of the two complementary strands is ideal.

Asymmetric PCR

ASYMMETRIC PCR

- PCR is carried out as usual, but with a great excess of one primers for the chosen strand.

Asymmetric PCR

ASYMMETRIC PCR

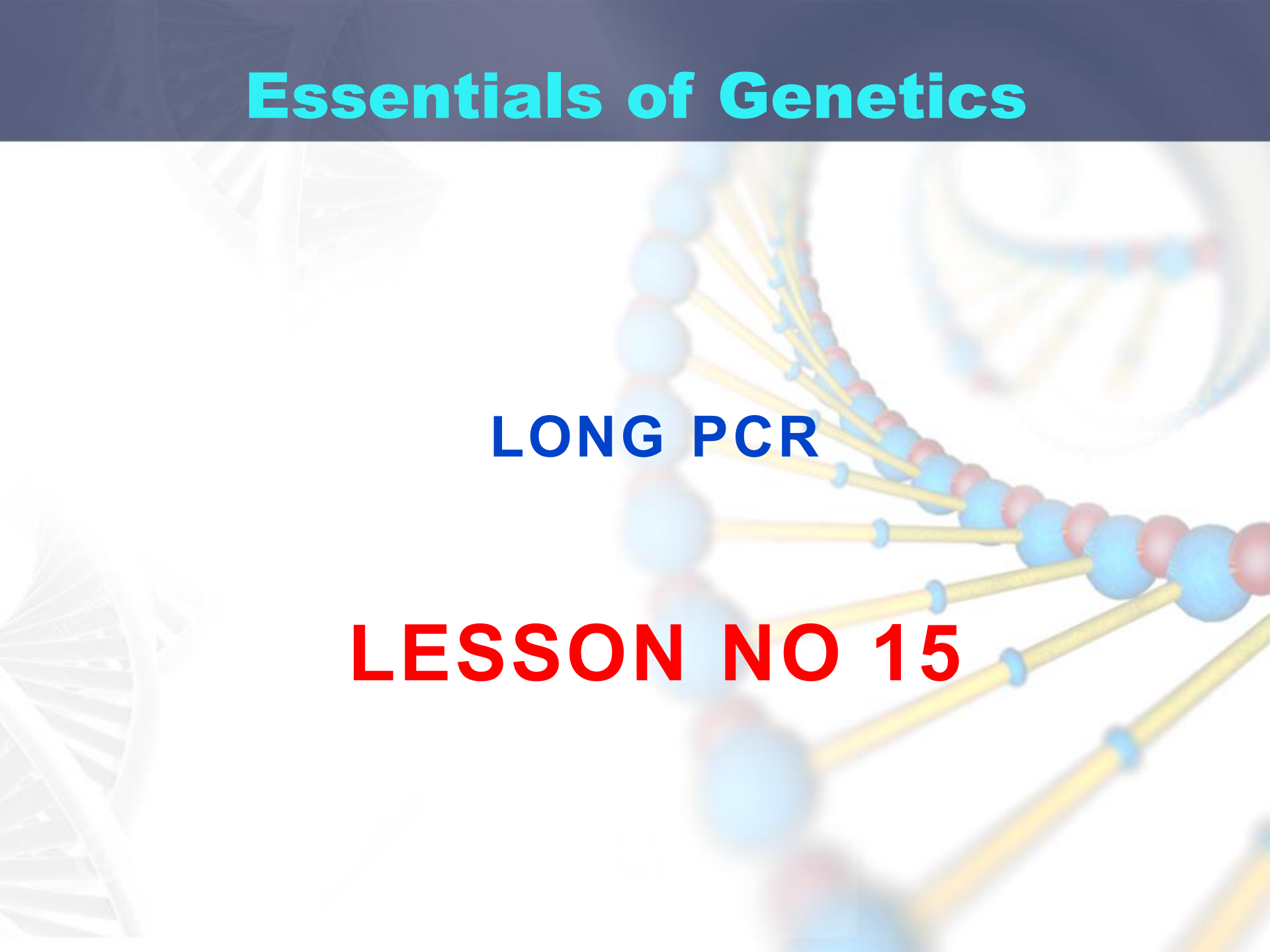
- Due to the slow amplification later in the reaction after the limiting primer has been used up, extra cycles of PCR are required.

Asymmetric PCR

ASYMMETRIC PCR

- It finds use in some types of sequencing and hybridization probing where having only one of the two complementary strands is required.

Essentials of Genetics



LONG PCR

LESSON NO 15

LONG PCR

LONG PCR

- Extended or longer than standard PCR, meaning over 5 kilobases (frequently over 10 kb).
- Long PCR is useful only if it is accurate.

LONG PCR

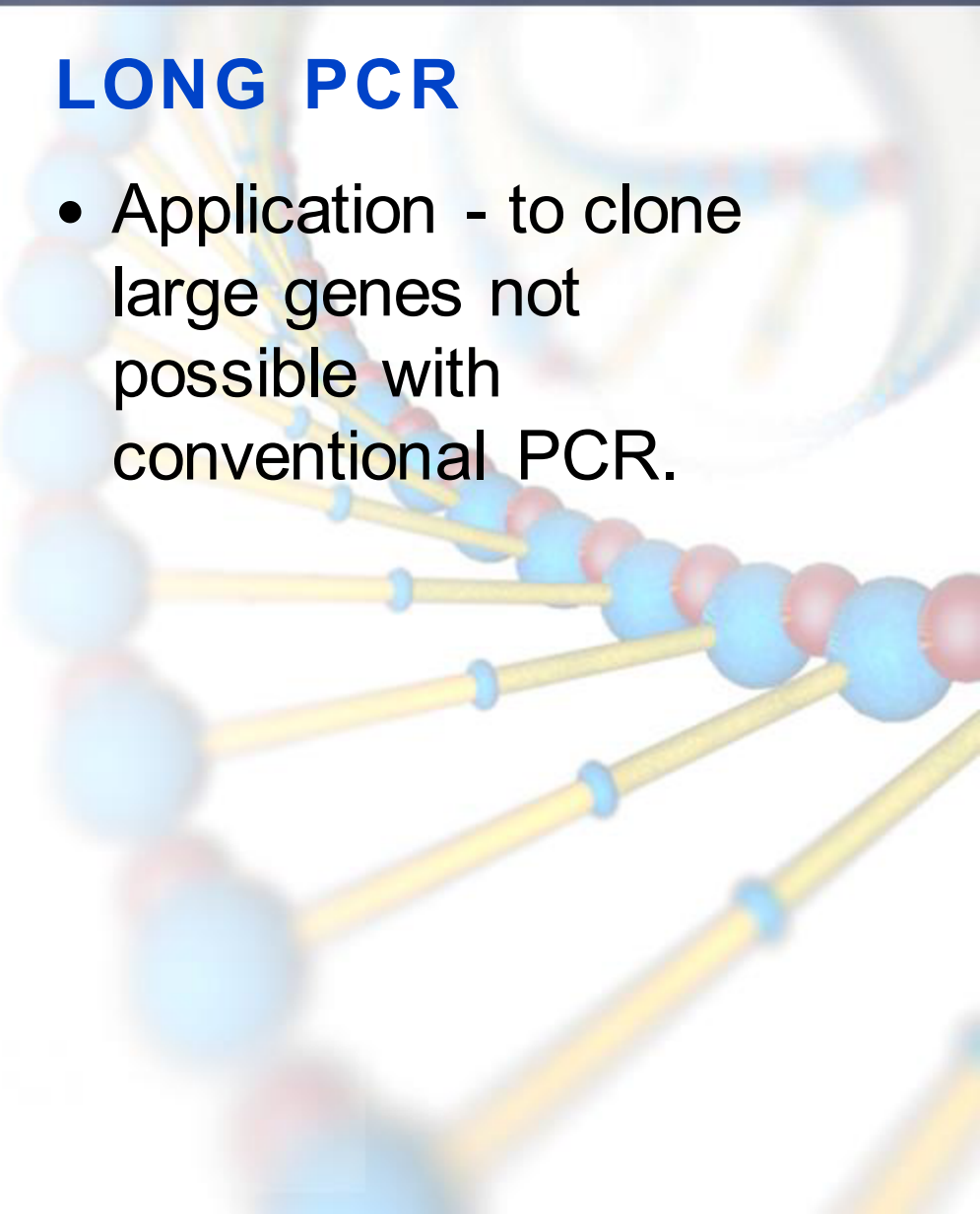
MIXTURES OF POLYMERASES

- Special mixtures of proficient polymerases along with accurate polymerases such as Pfu are often mixed together.

LONG PCR

LONG PCR

- Application - to clone large genes not possible with conventional PCR.



Essentials of Genetics

ALLELE SPECIFIC PCR

LESSON NO 16

Allele Specific PCR

ALLELE SPECIFIC PCR

- Allele-specific PCR used for identifying of SNPs.
- It requires prior knowledge of a DNA sequence, including differences between alleles.

Allele Specific PCR

ALLELE SPECIFIC PCR

- Uses primers whose 3' ends encompass the SNP.
- PCR amplification under stringent conditions is much less efficient in the presence of a mismatch between template and primer.

Allele Specific PCR

AMPLIFICATION WITH SNP SPECIFIC PRIMER

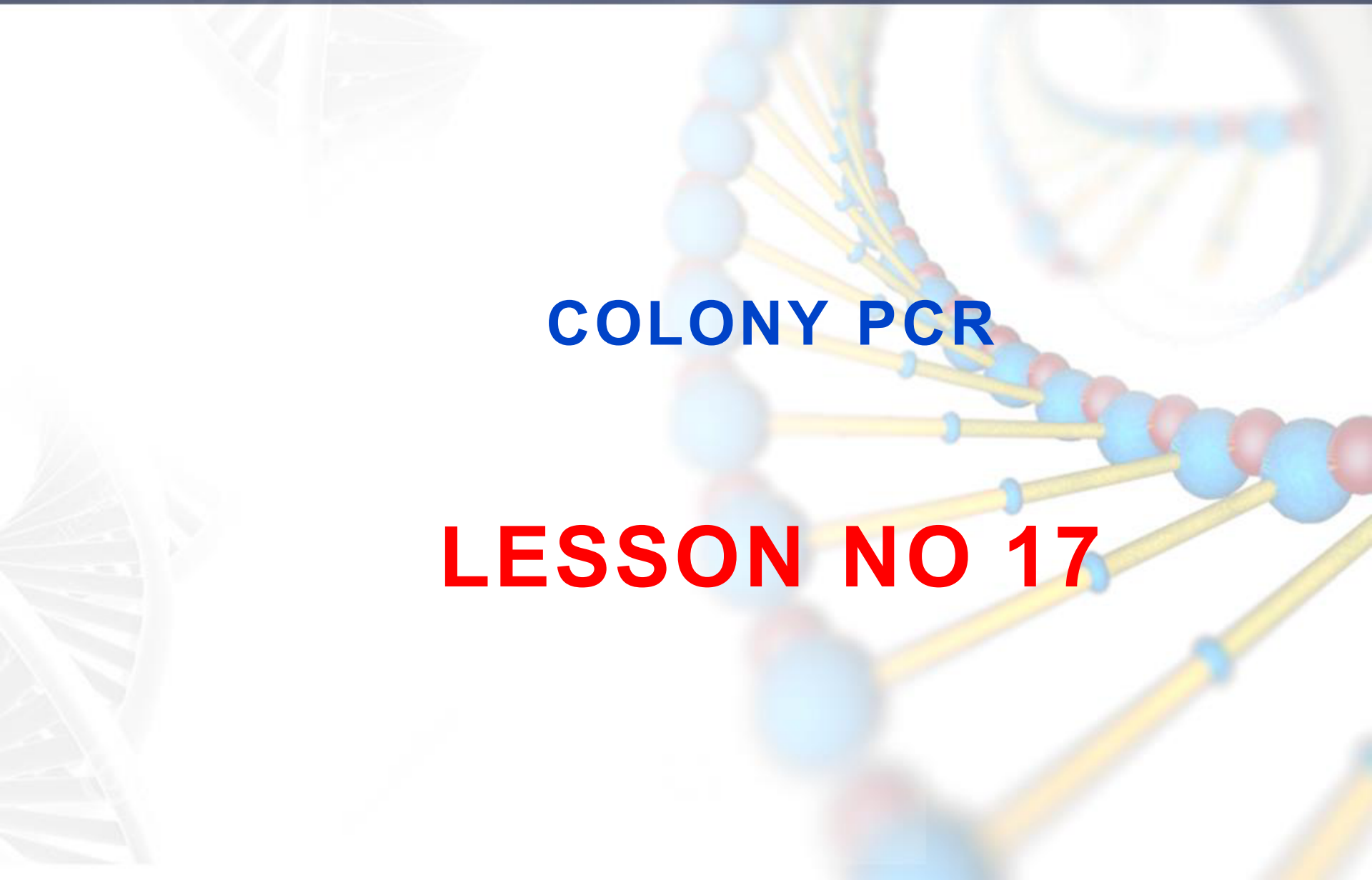
- Successful amplification with an SNP-specific primer signals presence of the specific SNP in a sequence.

Allele Specific PCR

ALLELE SPECIFIC PCR

- This diagnostic or cloning technique is used to identify or utilize single-nucleotide polymorphisms (SNPs).

Essentials of Genetics



COLONY PCR

LESSON NO 17

Colony PCR

COLONY PCR

- The screening of bacterial or yeast clones for correct ligation or plasmid products.

Colony PCR

COLONY PCR METHODOLOGY

- The screening of bacterial or yeast clones for correct ligation or plasmid products.
- Pick a bacterial colony with an autoclaved toothpick, swirl it into 25 µl of TE autoclaved dH2O in an microfuge tube.
- Heat the mix in a boiling water bath (90-100^{0}C) for 2 minutes
- Spin sample for 2 minutes high speed in centrifuge.

Colony PCR

COLONY PCR

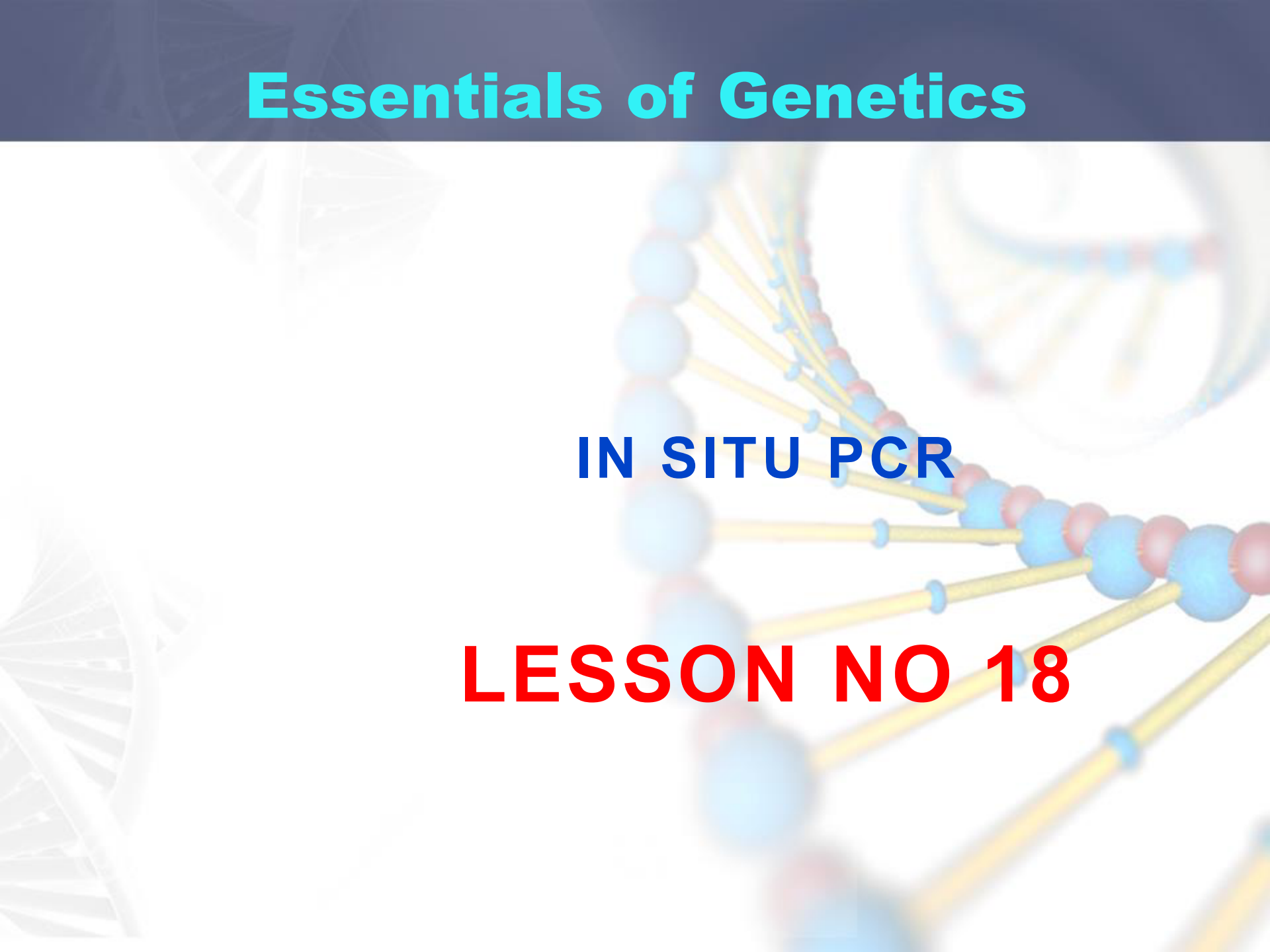
- Transfer 20 µl of the supernatant into a new microfuge tube.
- Take 1-2 µl of the supernatant as template in a 25 µl PCR standard PCR reaction.

Colony PCR

COLONY PCR

- The screening of bacterial or yeast clones for correct ligation or plasmid products.

Essentials of Genetics



IN SITU PCR

LESSON NO 18

In Situ PCR

IN SITU PCR

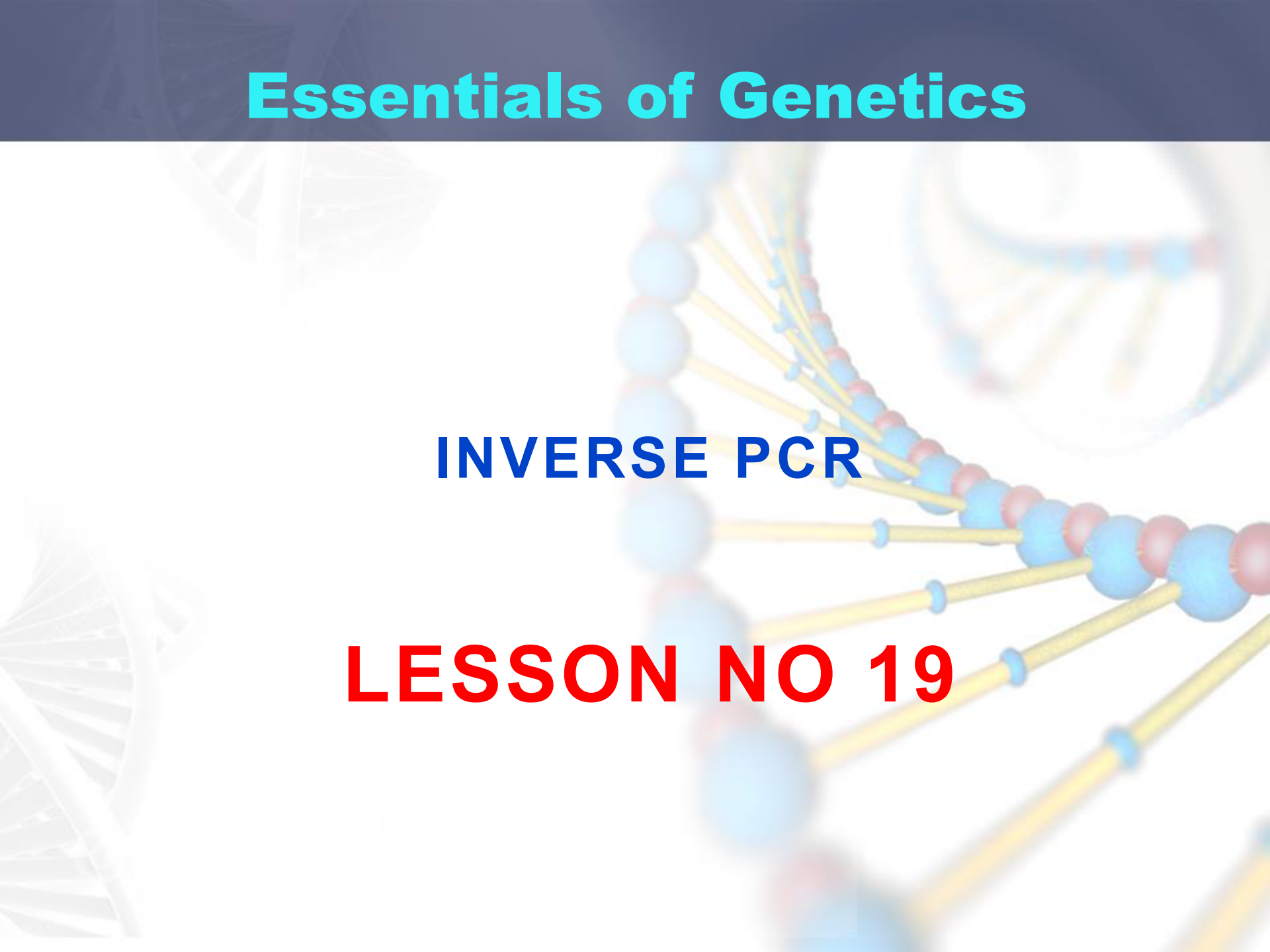
- In Situ PCR (ISH) is a polymerase chain reaction that actually takes place inside the cell on a slide.
- In situ PCR amplification can be performed on fixed tissue or cells .

In Situ PCR

IN SITU PCR

- Applies the methodology of hybridization of the nucleic acids.
- Allows identification of cellular markers
- Limited to detection of non-genomic material such as RNA.

Essentials of Genetics



INVERSE PCR

LESSON NO 19

Inverse PCR

INVERSE PCR

- Inverse PCR uses standard PCR primers oriented in the reverse direction of the usual orientation.

Inverse PCR

INVERSE PCR

- The template for the reverse primers is a restriction fragment that has been self-ligated
- Inverse PCR functions to clone sequences flanking a known sequence.
- Flanking DNA sequences are digested and then ligated to generate circular DNA.

Inverse PCR

IDENTIFICATION OF SEQUENCES FLANKING TRANSPOSABLE ELEMENTS

- Amplification and identification of sequences flanking transposable elements, and the identification of genomic inserts.

Inverse PCR

INVERSE PCR

- A method used to allow PCR when only one internal sequence is known.
- Especially useful in identifying flanking sequences to various genomic inserts.

Essentials of Genetics

AFLP PCR

LESSON NO 20

AFLP PCR

AFLP PCR - METHODOLOGY

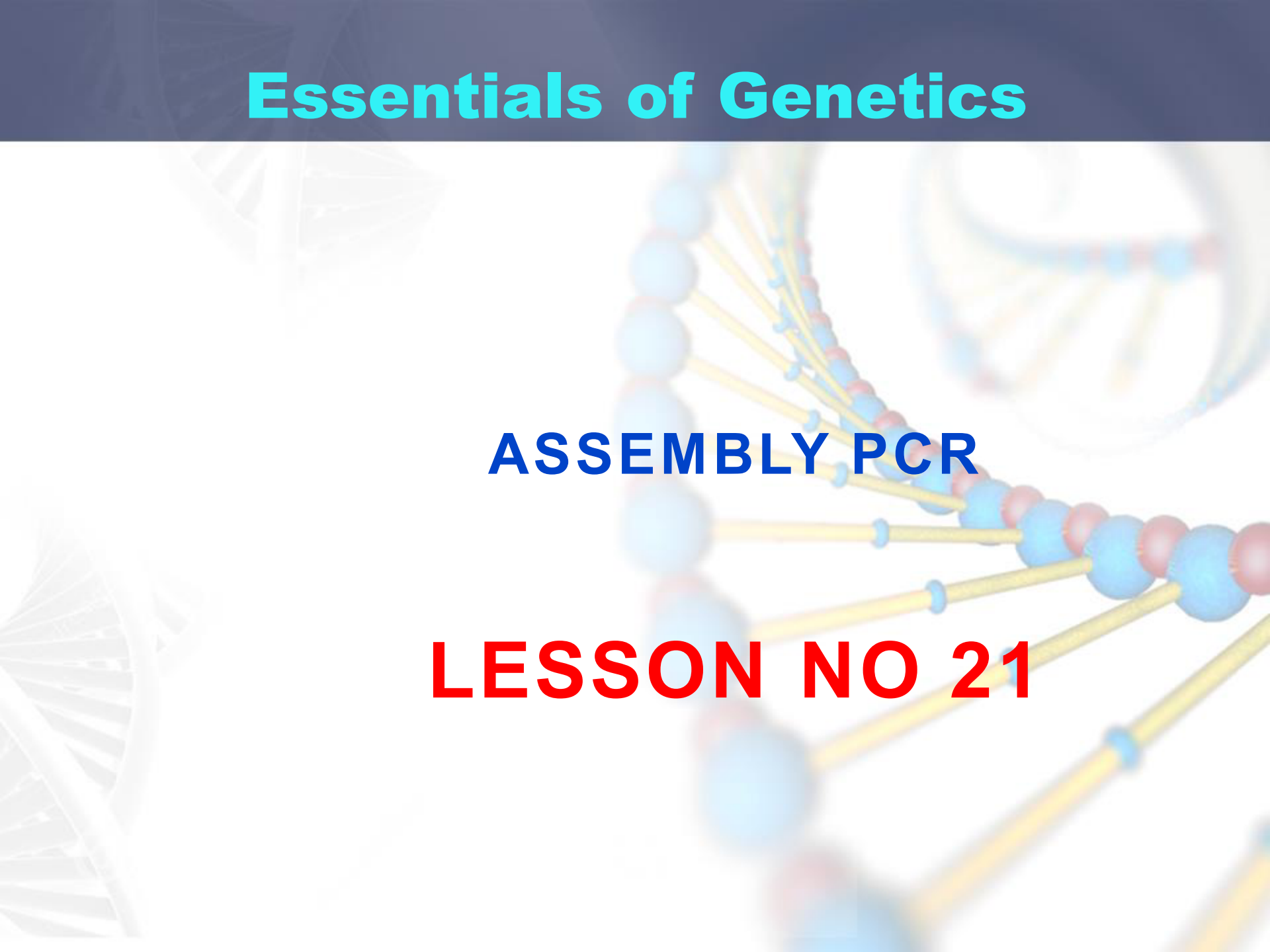
- Genomic DNA is digested with one or more restriction enzymes. tetracutter (MseI) and a hexacutter (EcoRI).
- Ligation of linkers to all restriction fragments. Pre-selective PCR is performed using primers which match the linkers and restriction site specific sequences.
- Electrophoretic separation and amplicons on a gel matrix, followed by visualization of the band pattern.

AFLP PCR

AFLP PCR

- AFLP is a highly sensitive PCR-based method for detecting polymorphisms in DNA.
- AFLP can be also used for genotyping individuals for a large number of loci.

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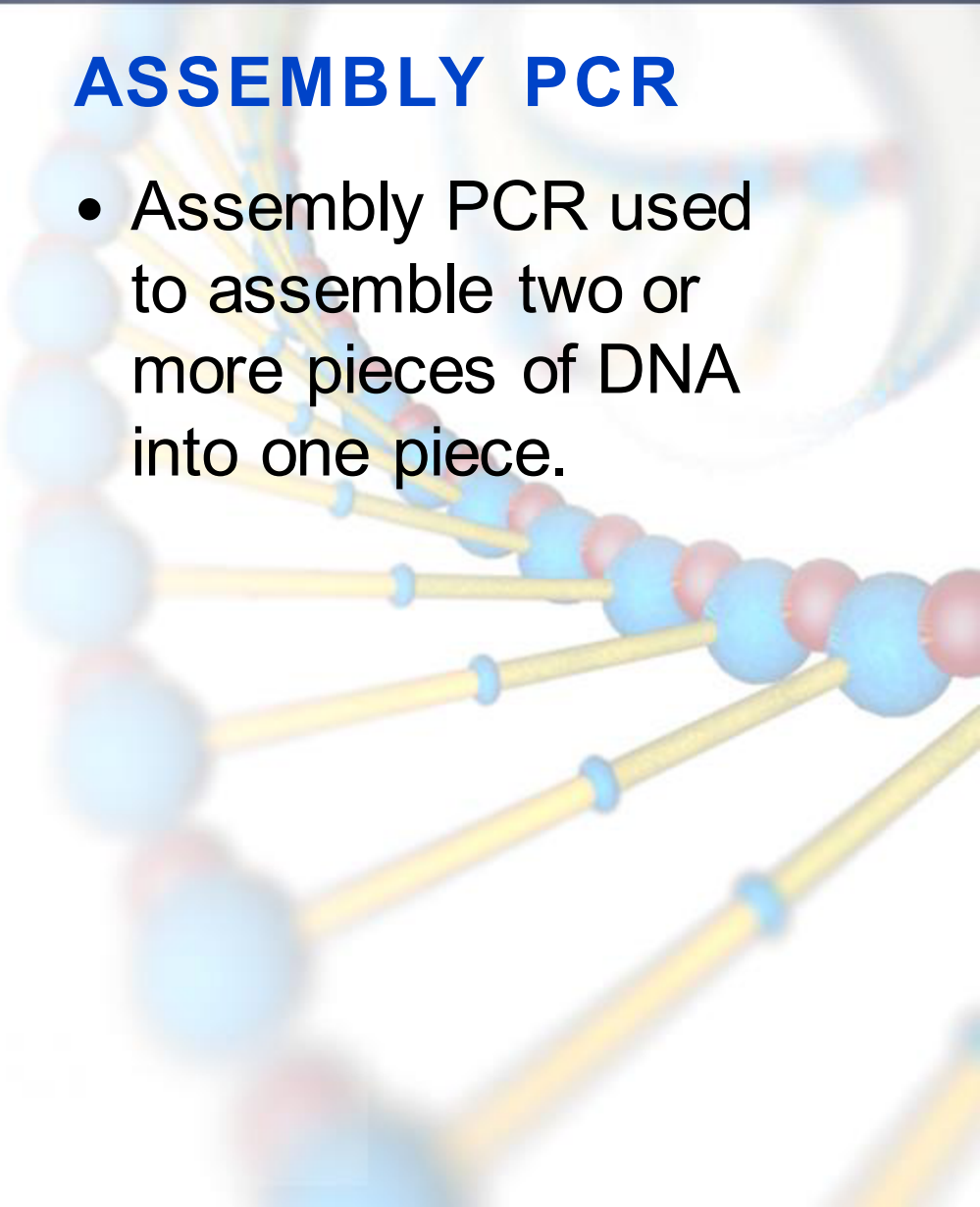
ASSEMBLY PCR

LESSON NO 21

Assembly PCR

ASSEMBLY PCR

- Assembly PCR used to assemble two or more pieces of DNA into one piece.



Assembly PCR

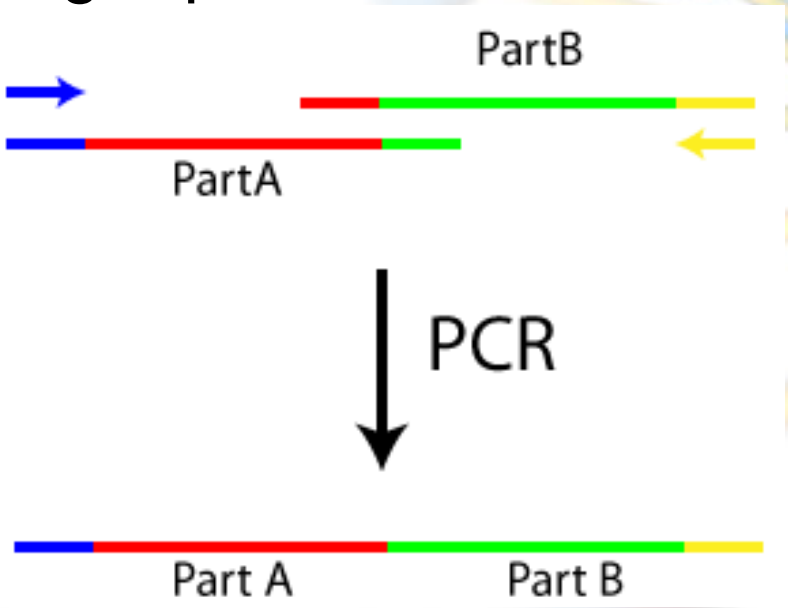
ASSEMBLY PCR - PRINCIPLE

- Assembly PCR is the synthesis of long DNA structures by performing PCR on a pool of long oligonucleotides with short overlapping segments, to assemble two or more pieces of DNA into one piece.

Assembly PCR

ASSEMBLY PCR - METHODOLOGY

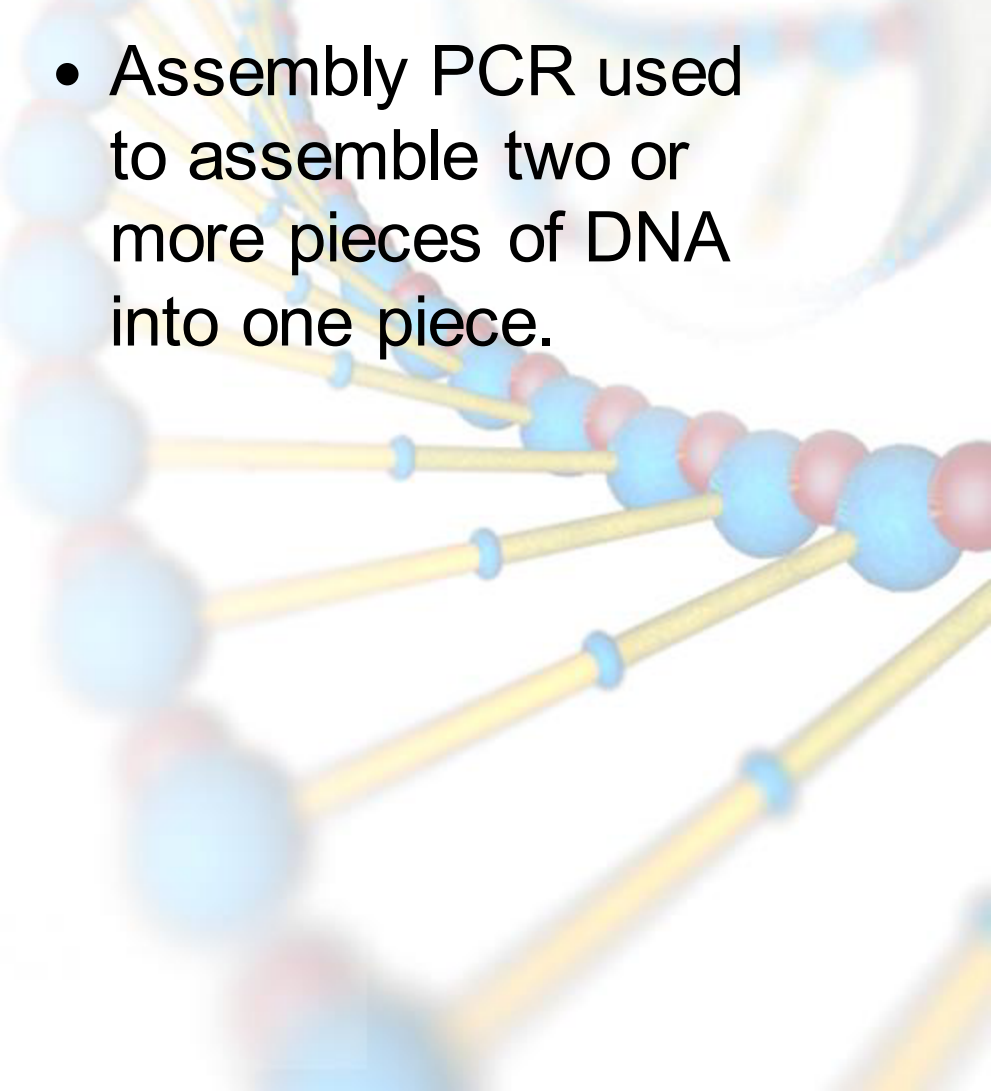
- It involves an initial PCR with primers that have an overlap and a second PCR using the products as the template that generates the final full-length product.



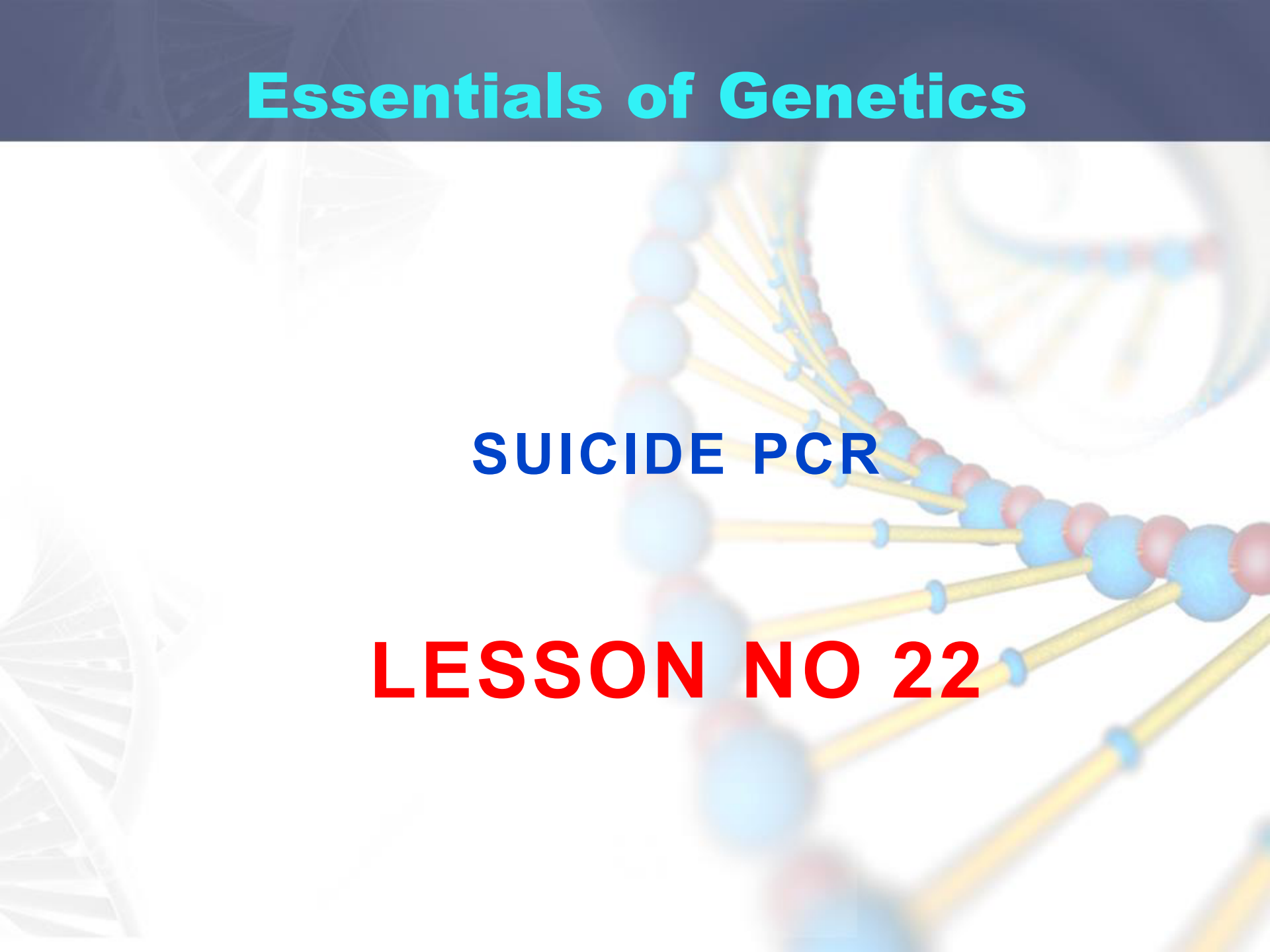
Assembly PCR

ASSEMBLY PCR

- Assembly PCR used to assemble two or more pieces of DNA into one piece.



Essentials of Genetics



SUICIDE PCR

LESSON NO 22

Suicide PCR

SUICIDE PCR - PRINCIPLE

- Suicide PCR is typically used in paleogenetics or other studies where avoiding false positives and ensuring the specificity of the amplified fragment is the highest priority.
- The method prescribes the use of any primer combination only once in a PCR, which should never have been used in any positive control PCR reaction.

Suicide PCR

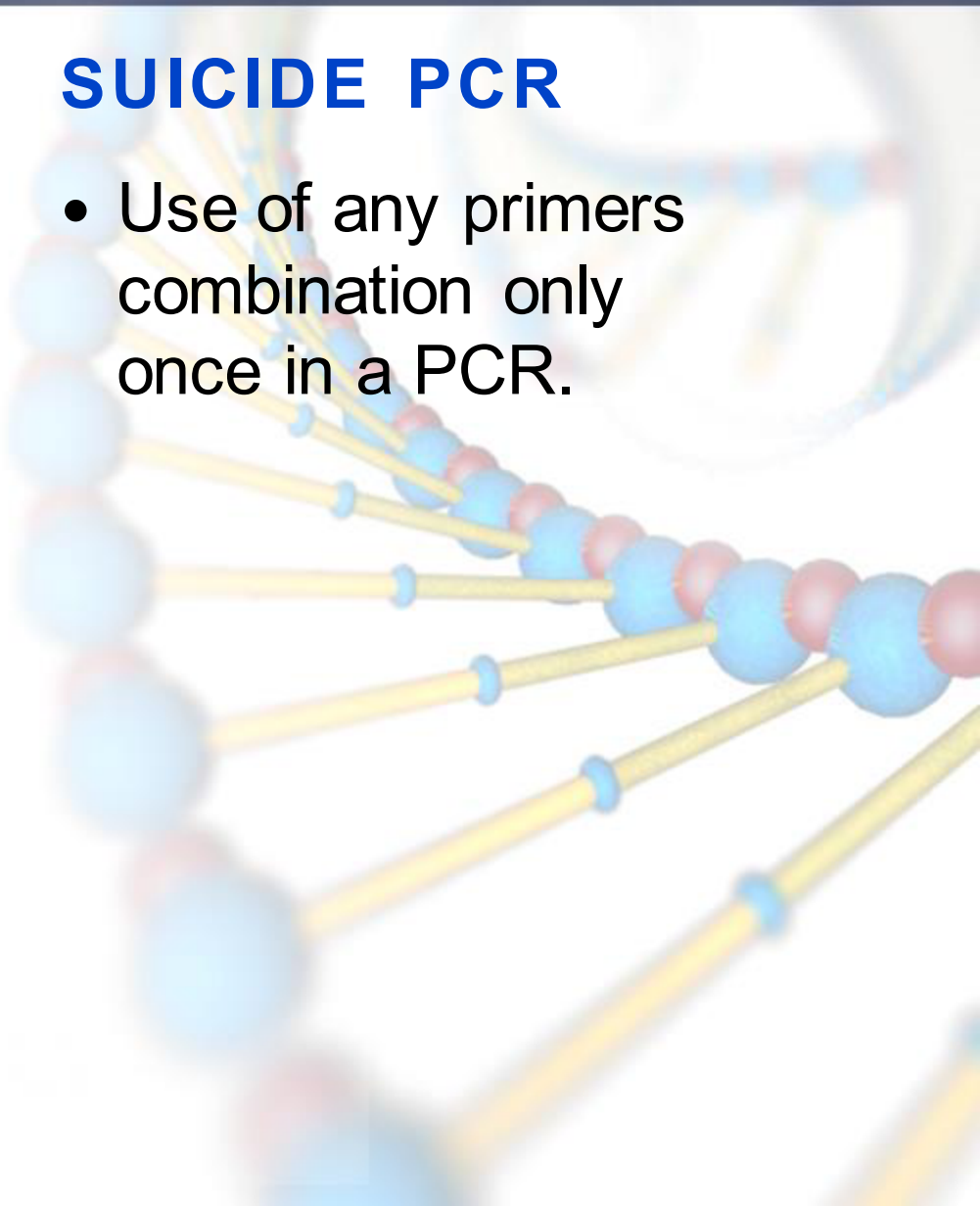
SUICIDE PCR

- Primers should always target a genomic region never amplified before in the lab using this or any other set of primers.
- This ensures that no contaminating DNA from previous PCR reactions is present in the lab, which could otherwise generate false positives.

Suicide PCR

SUICIDE PCR

- Use of any primers combination only once in a PCR.



Essentials of Genetics

METHYLATION SPECIFIC PCR

LESSON NO 23

Methylation Specific PCR

METHYLATION SPECIFIC PCR

- Methylation-specific PCR is used to identify patterns of DNA methylation at CpG islands in genomic DNA.

Methylation Specific PCR

METHYLATION SPECIFIC PCR

- Target DNA is first treated with sodium bisulfite, which converts unmethylated cytosine bases to uracil, which is complementary to adenosine in PCR primers.
- Two amplifications are then carried out on the bisulfite-treated DNA: One primer set anneals to DNA with cytosines (corresponding to methylated cytosine), and the other set anneals to DNA with uracil (corresponding to unmethylated cytosine).

Methylation Specific PCR

MSP

- MSP used in quantitative PCR provides information about methylation state of a given CpG island.

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INTER SEQUENCE SPECIFIC PCR

LESSON NO 24

InterSequence Specific PCR

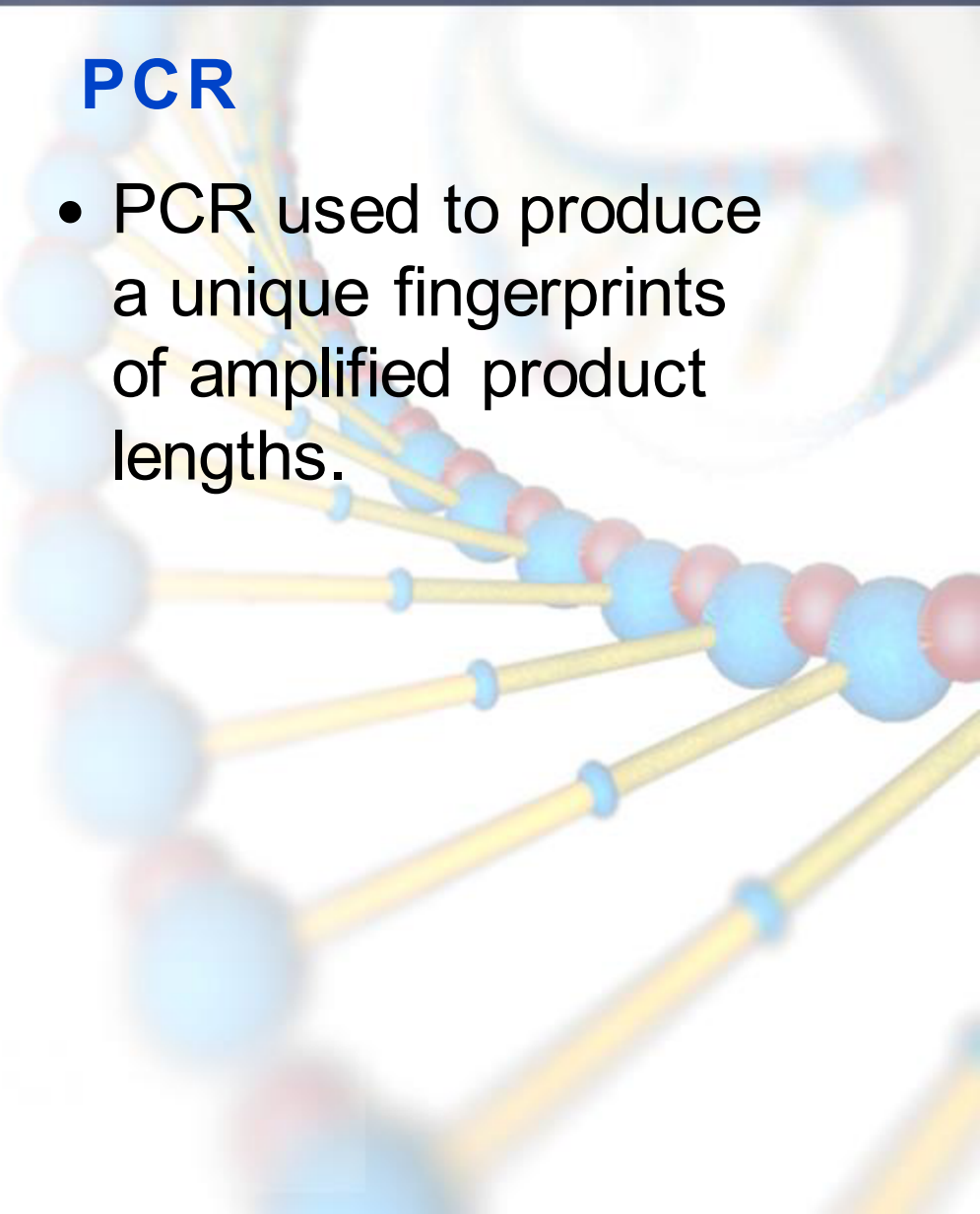
INTER SEQUENCE SPECIFIC PCR

- A method for DNA fingerprinting that uses primers selected from segments repeated throughout a genome to produce a unique fingerprint of amplified product lengths.
- The use of primers from a commonly repeated segment is called Alu-PCR, and can help to amplify sequences adjacent (or between) these repeats.

InterSequence Specific PCR

PCR

- PCR used to produce a unique fingerprints of amplified product lengths.



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LIGATION MEDIATED PCR

LESSON NO 25

Ligation-mediated PCR

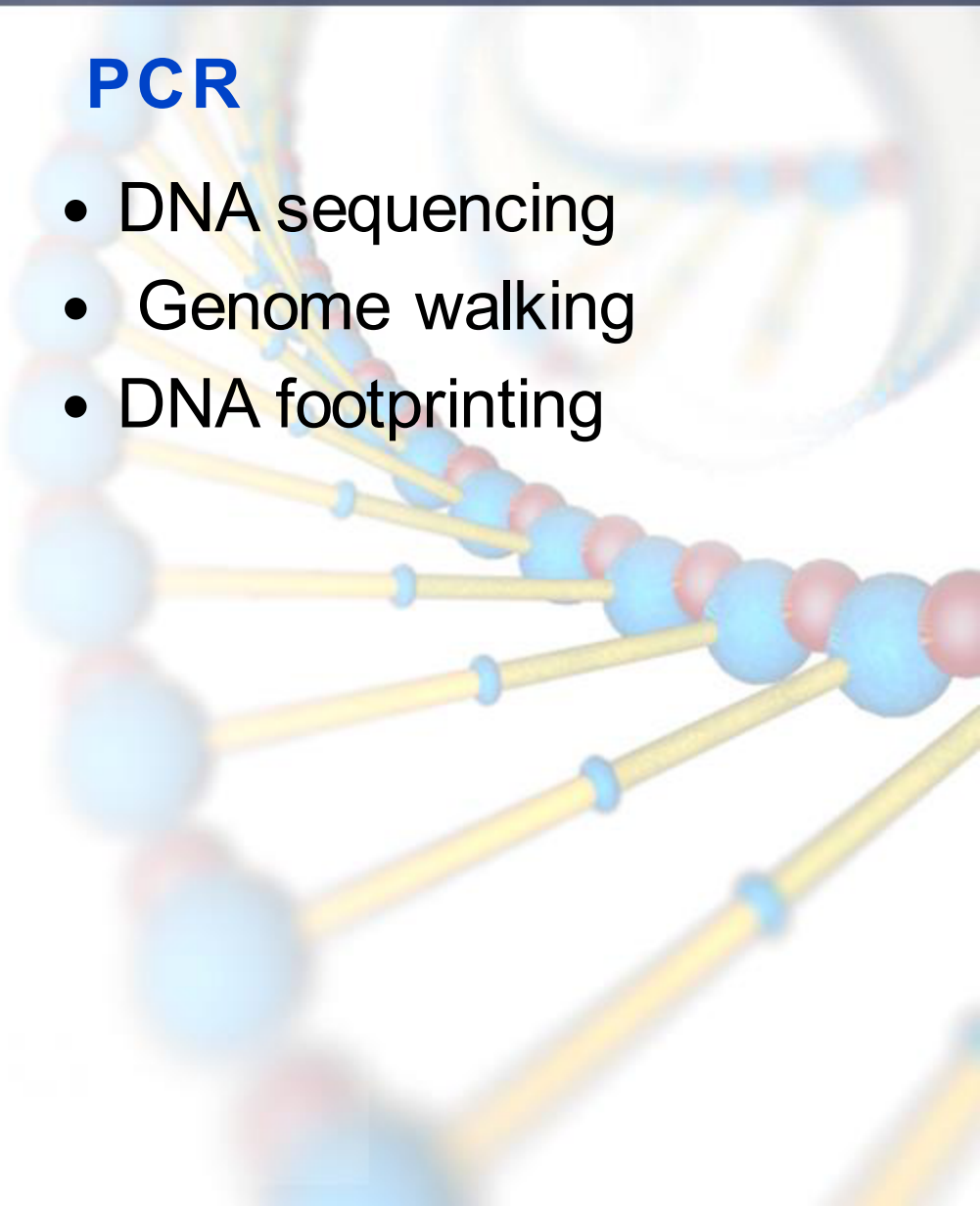
METHODOLOGY

- Uses small DNA oligonucleotide 'linkers' (or adaptors) that are first ligated to fragments of the target DNA.
- PCR primers that anneal to the linker sequences are then used to amplify the target fragments.

Ligation-mediated PCR

PCR

- DNA sequencing
- Genome walking
- DNA footprinting



Essentials of Genetics

WHOLE GENOME AMPLIFICATION PCR

LESSON NO 26

Whole Genome Amplification PCR

WHOLE GENOME AMPLIFICATION PCR

- Primers can be designed to be 'degenerate'

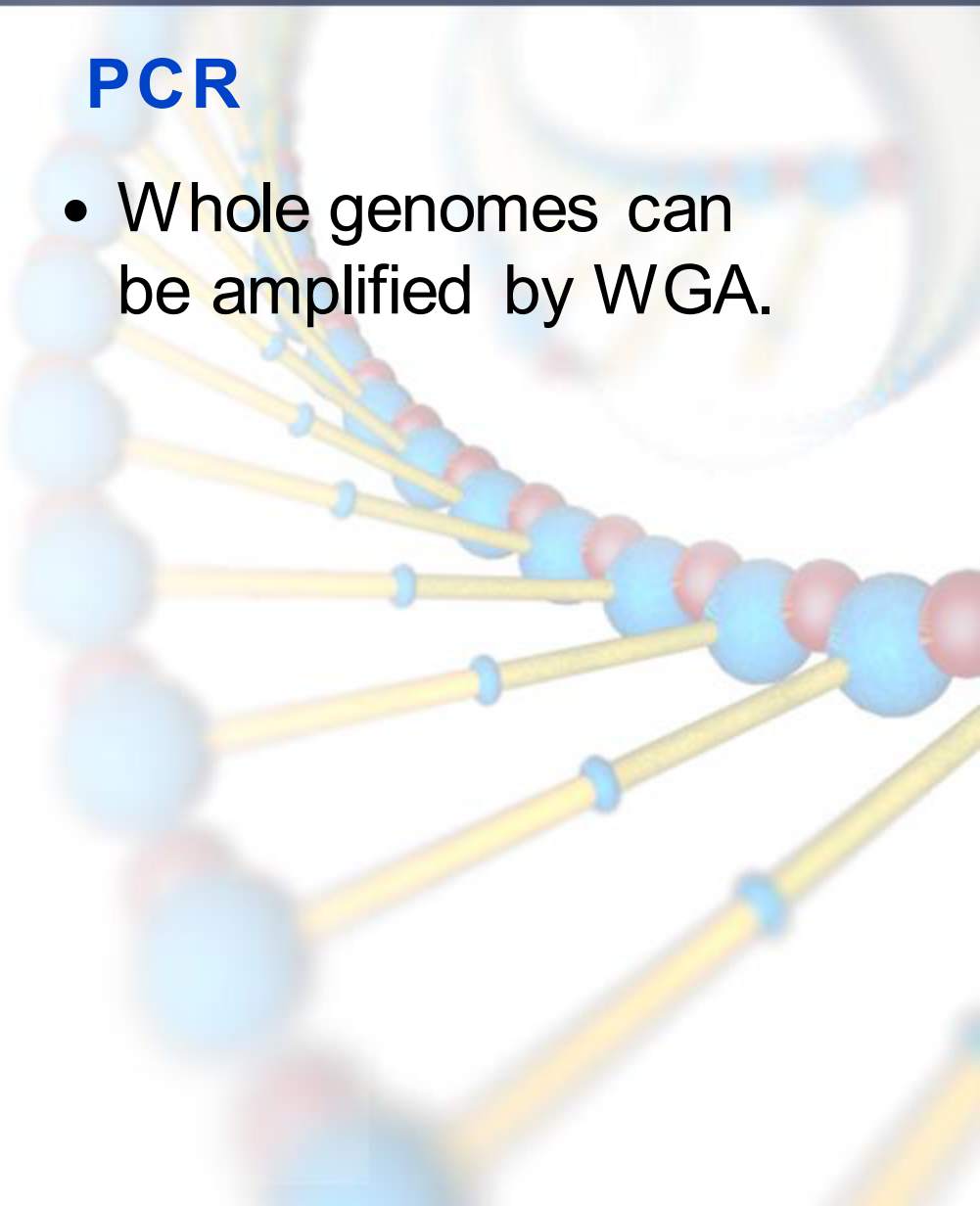
- able to initiate replication from a large number of target locations.

- Whole genome amplification (WGA) is a group of procedures that allow amplification to occur at many locations in a genome.

Whole Genome Amplification PCR

PCR

- Whole genomes can be amplified by WGA.



Essentials of Genetics

MINI-PRIMER PCR

LESSON NO 27

Mini Primer PCR

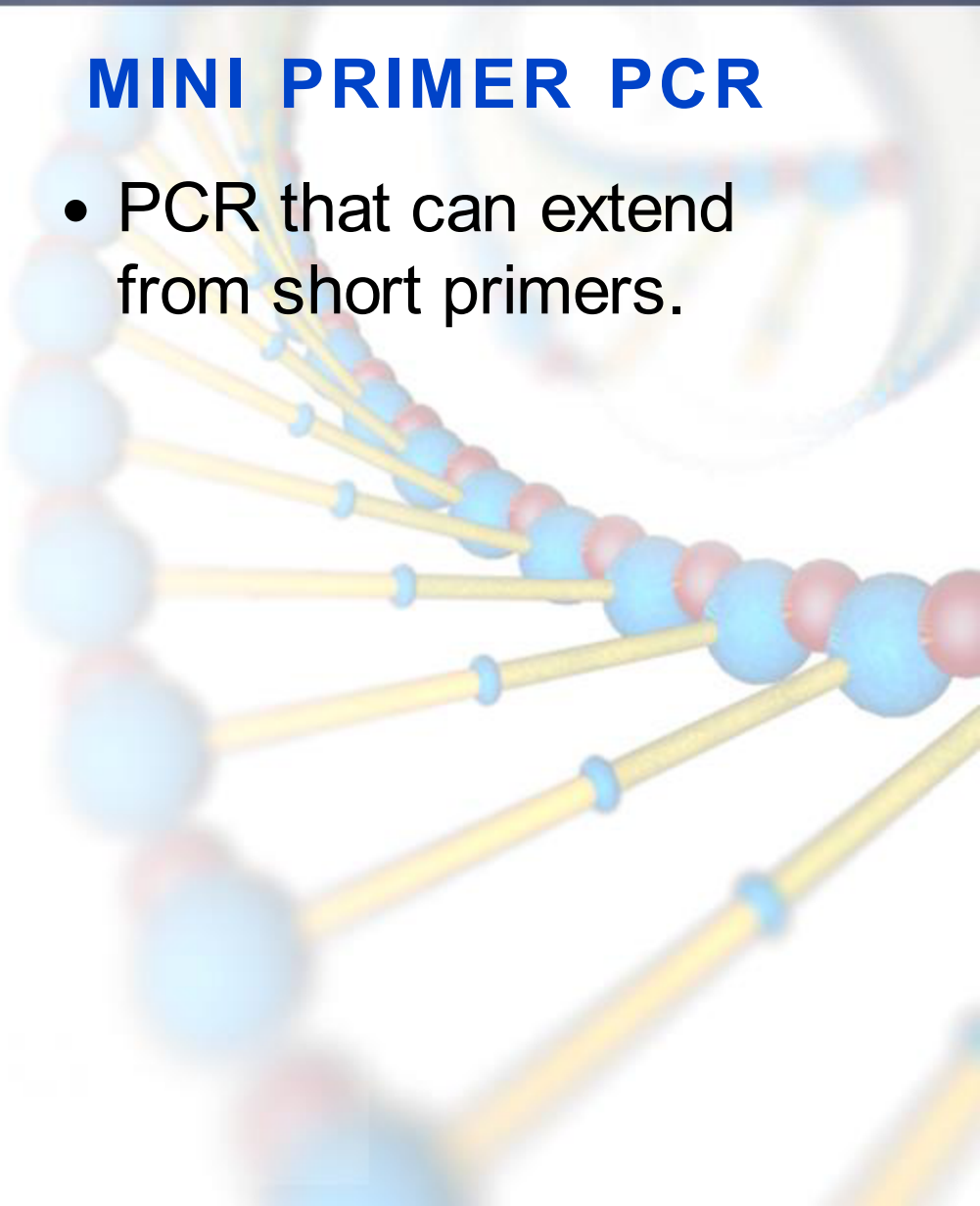
MINI PRIMER PCR

- Mini Primer PCR uses a thermostable polymerase (S-Tbr) that can extend from short primers as short as 9 or 10 nucleotides.
- This method permits PCR targeting to smaller primer binding regions, and is used to amplify conserved DNA sequences, such as the 16S (or eukaryotic 18S) rRNA gene.

Mini Primer PCR

MINI PRIMER PCR

- PCR that can extend from short primers.



Essentials of Genetics

ADVANTAGES AND LIMITATIONS OF PCR

LESSON NO 28

Advantages & Limitations

ADVANTAGES

- Specific
- Simple, rapid, relatively inexpensive
- Amplifies from low quantities
- Works on damaged DNA
- Sensitive
- Flexible

Advantages & Limitations

LIMITATIONS

- Contamination risk
- Primer complexities
- Primer-binding site complexities
- Amplifies rare species
- Detection methods

Essentials of Genetics

APPLICATIONS OF PCR

LESSON NO 29

Applications of PCR

APPLICATIONS

- Detection of Infectious diseases: AIDS, TB, CMV, H1N1, etc Viral, Bacterial and fungal infections,
- Diagnosis of latent viruses.
- Forensic applications: DNA finger printing

Applications of PCR

APPLICATIONS

- Detection of Mutations: Inherited disorders & carriers Track DNA abnormalities
- Prenatal diagnosis of genetic disorders.

Applications of PCR

APPLICATIONS

- Detection of pathogens Pre-natal diagnosis DNA fingerprinting Gene therapy Mutation screening
- Drug discovery
- Classification of organisms Genotyping

Applications of PCR

APPLICATIONS

- Molecular Archaeology
- Molecular Epidemiology
- Molecular Ecology
- Bioinformatics
- Genomic cloning
- Site-directed mutagenesis
- Gene expression studies

Applications of PCR

CLINICAL MICROBIOLY

- Identification of bacteria:
- Slow growing bacteria
- Bacterial antibiotic resistance genes.
- VIRUS: almost all viruses identification and viral load.

Applications of PCR

CLINICAL MICROBIOLY

- FUNGUS:
- Aspergillus spp
- Candida spp
- Plasmodium spp
- Trypanosoma spp
- Leishmania spp
- Babesia spp

Polymerase chain reaction (PCR)

Applications of PCR

- PCR has widespread applications in various fields of life sciences including genetic engineering, medical, forensic, agriculture, environment etc.

LESSON NO 30

Polymerase chain reaction (PCR)

PCR-Gene cloning and expression

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





Polymerase chain reaction (PCR)

PCR-Medicine

- PCR has major impact on medicine especially in the field of clinical microbiology or diagnosis
- Molecular tools have also allowed to perform prenatal genetic diagnosis

LESSON NO 31

Polymerase chain reaction (PCR)

PCR-Forensic sciences

- Forensic science is the application of scientific procedures to solve criminal and legal matters
- Molecular methods are used to established the filiations of a person or to obtain evidence from minimal samples of saliva, semen or other tissues

LESSON NO 32

Polymerase chain reaction (PCR)

PCR-DNA profiling

- DNA profiling or DNA fingerprinting is a forensic technique used to identify individuals by characteristics of their DNA





Polymerase chain reaction (PCR)

PCR-Agricultural sciences and environment

- PCR has also facilitated research in detection of pathogens in plants, animals and environment





LESSON NO 33

Polymerase chain reaction (PCR)

PCR-Molecular paleontology

- Molecular paleontology refers to the recovery and analysis of DNA and protein from ancient human, animal and plant remains





LESSON NO 34

Midterm Syalubus Finished
Lecture no 1 to 34

Effort By

Amaan Khan