BCHM 270: Module 1

MOLECULAR BASIS OF HEALTH AND DISEASE

Content Outline

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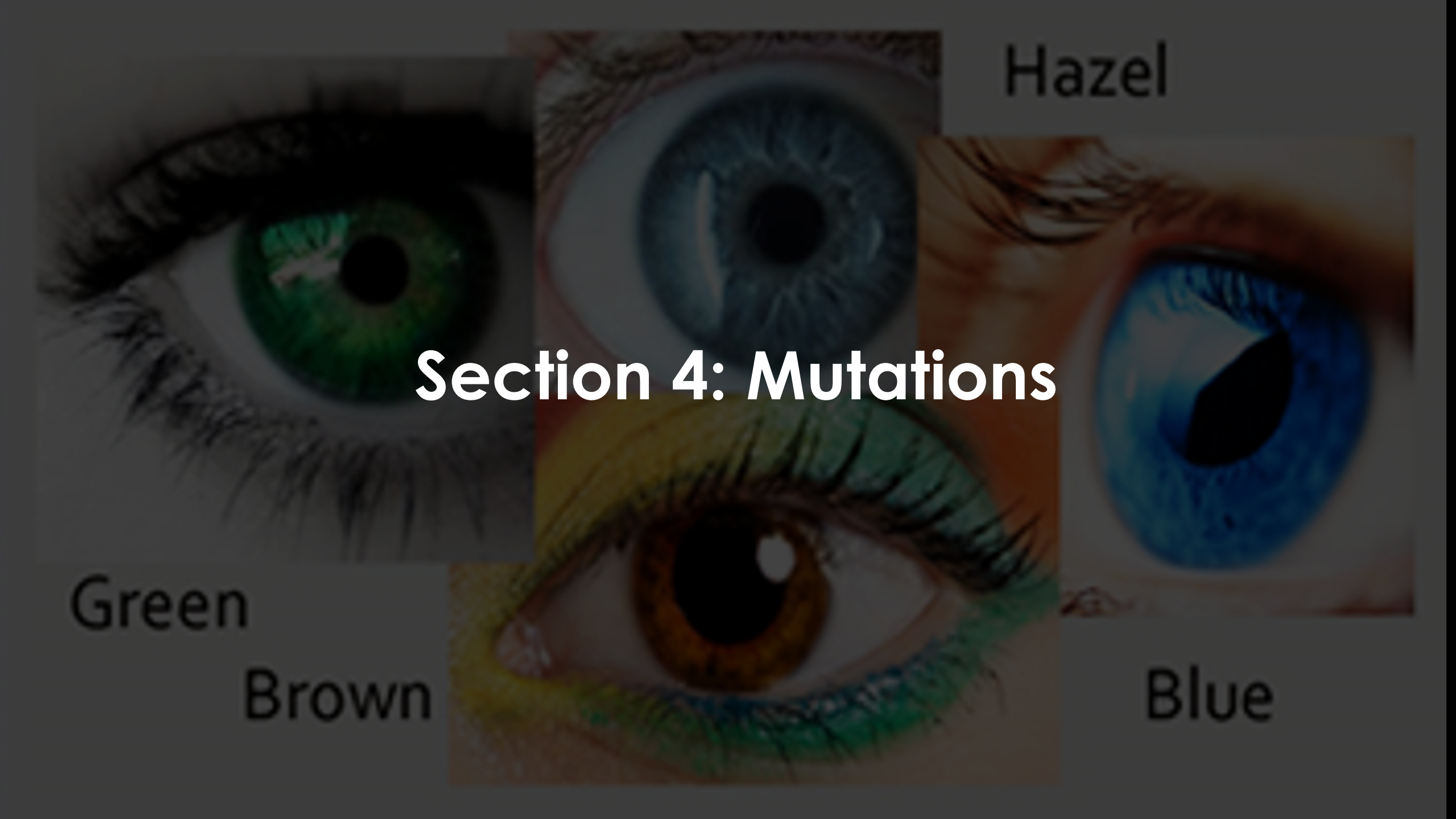
Section 5..... Biotechnology and Molecular Diagnostics



Section 4: Mutations

Concept 4.1: Point Mutations

Silent Mutations: A single nucleotide change that does not affect the amino acid sequence.

Missense Mutations: A single nucleotide change that results in a different amino acid being incorporated into the protein.

Nonsense Mutations: A single nucleotide change that results in a premature stop codon being inserted into the protein sequence.

Frameshift Mutations: One or more nucleotides are inserted or deleted, causing a shift in the reading frame of the codons, resulting in a non-functional or truncated protein.

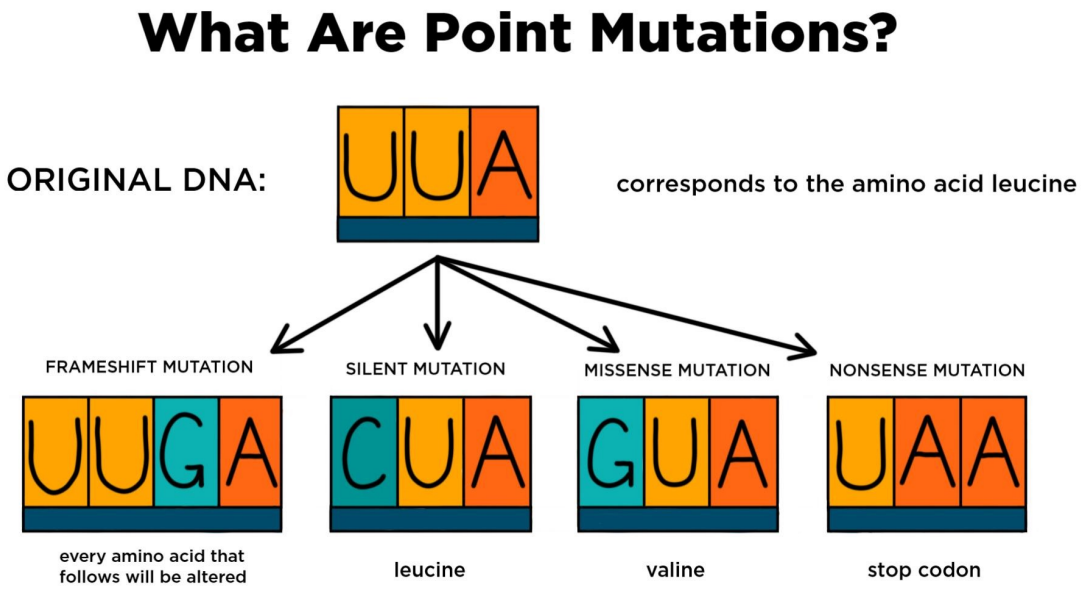


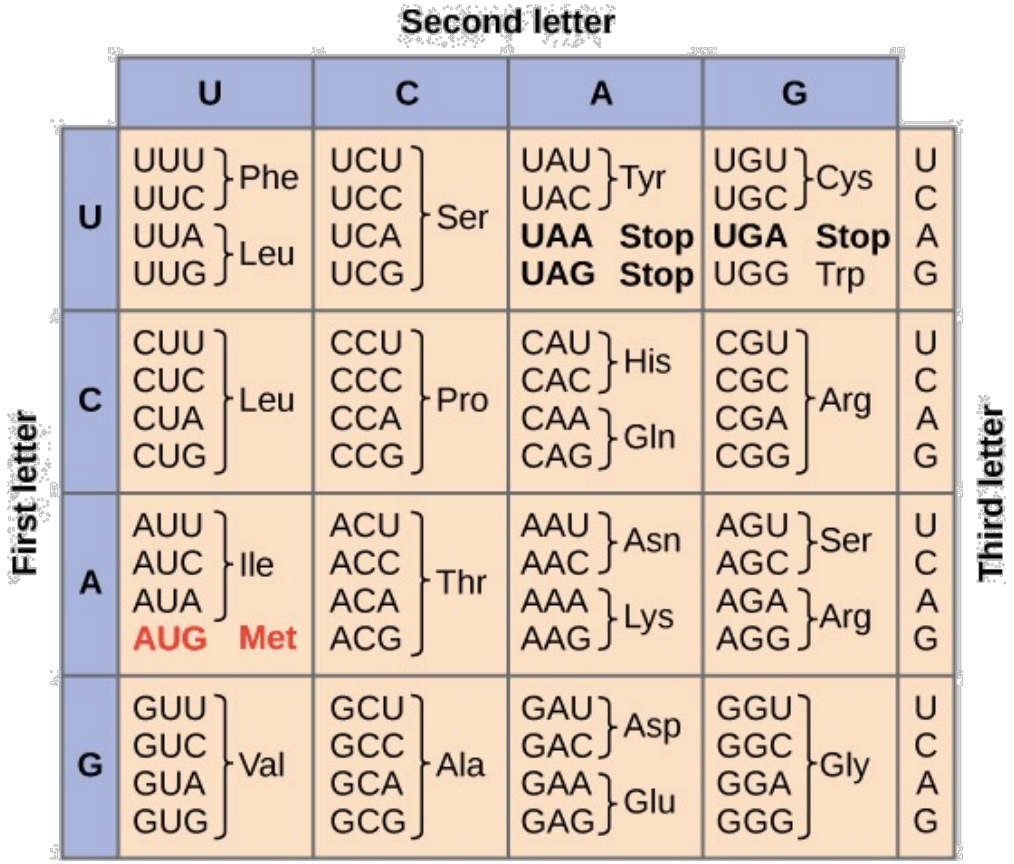
Figure 1. Point Mutation Examples

Section 4 Quiz: MCQ 1

Which type of mutation is the following example: AGC → TAG?

a) Silent mutation
b) Missense mutation
c) Nonsense mutation
d) Frameshift mutation

Answer: c) Nonsense mutation



Section 4 Quiz: MCQ 2

DM1 results from an increase in the number of 'CTG' triplet repeats in the *DMPK* gene, which encodes for a protein kinase, with a larger number of inserted repeats leading to more significant symptoms. Which mutation classification seen in DM1 would be the most applicable?

a. Point mutation
b. Frameshift mutation
c. Missense mutation
d. None of the above

Answer: d) none of the above

Section 5: Biotechnology and Molecular Diagnostics

Concept 5.1: Overview of Biotechnological Methods

- Many biotechnological tools used in biochemistry can be applied to each level of the central dogma (DNA, RNA, and proteins)
- DNA techniques are important to analyze the entire genetic code of an organism
- RNA is important to analyze gene expression
- Protein techniques observe a protein of interest or enzymatic activity

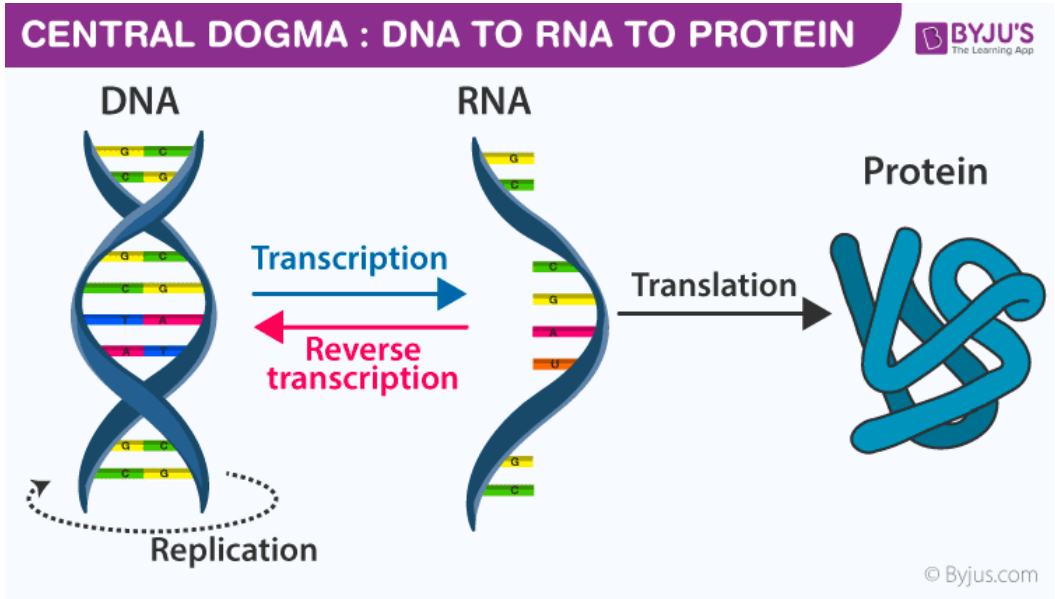


Figure 2. Central Dogma of Molecular Biology.

DNA Biotechnology Methods

Concept 5.2: Restriction Endonucleases

- Bacterial enzymes that cleave dsDNA from pathogens at specific sequences to produce restriction fragments which can be used to combine new DNA sequences together (DNA cloning)
- Naming convention:
  - 1st letter is the genus of bacterium, the next 2 letters are the species, and the number is the order in which the enzyme was discovered in each organism
- Recognize 4-6 bp stretches of DNA with specific sequences **(palindrome)**
- Restriction endonucleases cleave the DNA backbone to generate a 3'- 5' strand
  - Some form **"sticky"** ends that are single stranded sequences that are complementary to each other (Forms H bonds); others form **"blunt"** ends

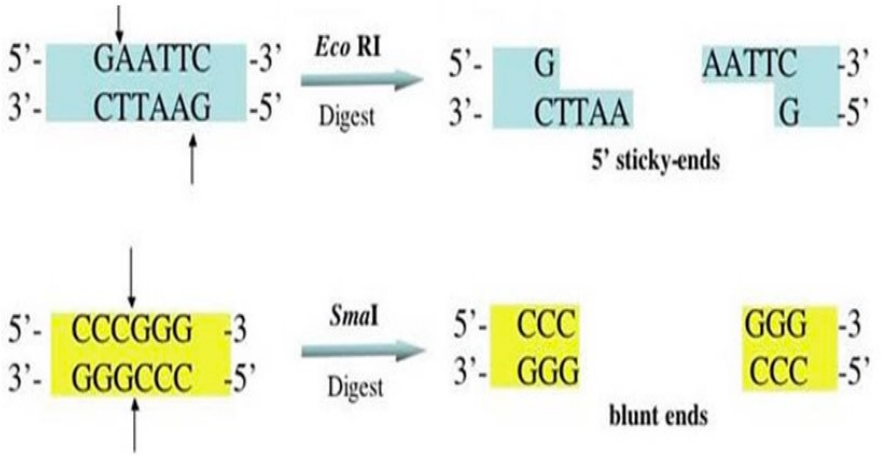


Figure 3. Restriction endonucleases sticky vs blunt ends.

Concept 5.3: Gel Electrophoresis

- Method to separate molecules by charge based on their size due to the molecules features, chemicals in the solutions and gels used
- DNA and RNA can be separated by size using agarose or polyacrylamide gels and the application of an electric field
- Restriction digest fragments and PCR products can be visualized using this method

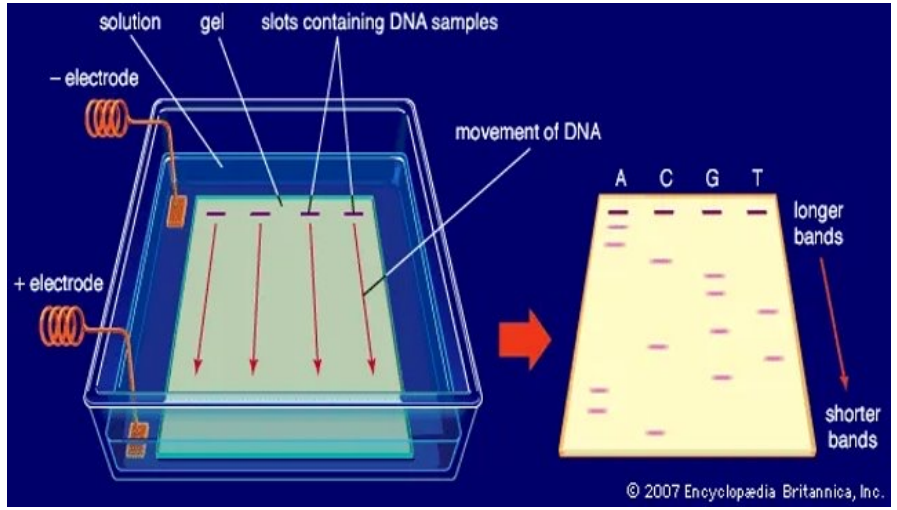


Figure 4. Gel Electrophoresis.

Concept 5.4: DNA Cloning

- Method used to isolate and then amplify a DNA sequence by introducing it to a cell
- The DNA of interest is **cleaved** with specific restriction endonuclease enzymes
- Fragments are joined with **DNA ligase** to a DNA cloning vector cleaved with the **same restriction endonuclease** to produce hybrid molecules
  - Each hybrid molecule of DNA is introduced into a single bacterial cell
  - As each bacteria divides, it forms a **'clone'** in which every bacterium carrier a copy of the same inserted DNA fragment in the cloning vector
- Cloned DNA can be expressed as proteins or used for other DNA cloning experiments, isolated, and analyzed

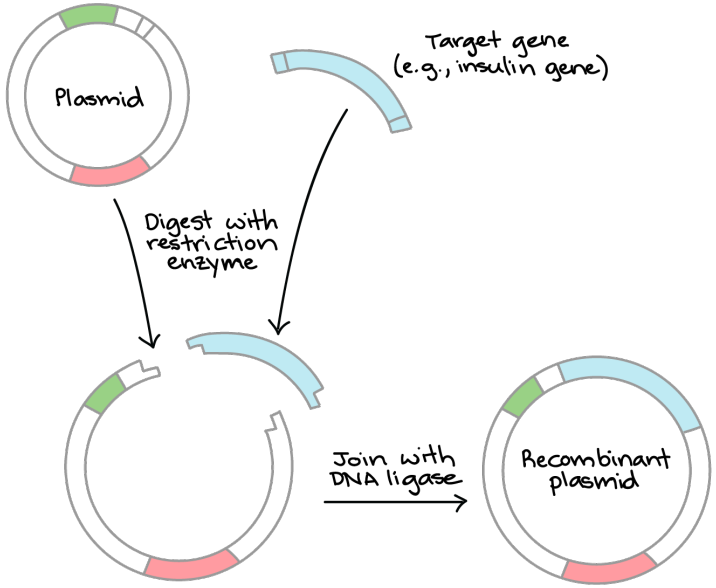


Figure 5. DNA Cloning

Concept 5.5: DNA Probes

Description:

- Single-stranded DNA molecules that are labelled with radioisotopes or non-radioactive labels
- Complementary sequence to the DNA of interest (target DNA)

Mechanism:

- Binding, or hybridization, of the labelled DNA probe to the target DNA is used to identify a sequence of interest from other sequences
- Since the target DNA sequence is known, single-stranded oligonucleotide probes complementary to a small region of the gene of interest can be made

Use:

- The amino acid sequence of the protein may be used to construct a probe using the genetic code as a guide

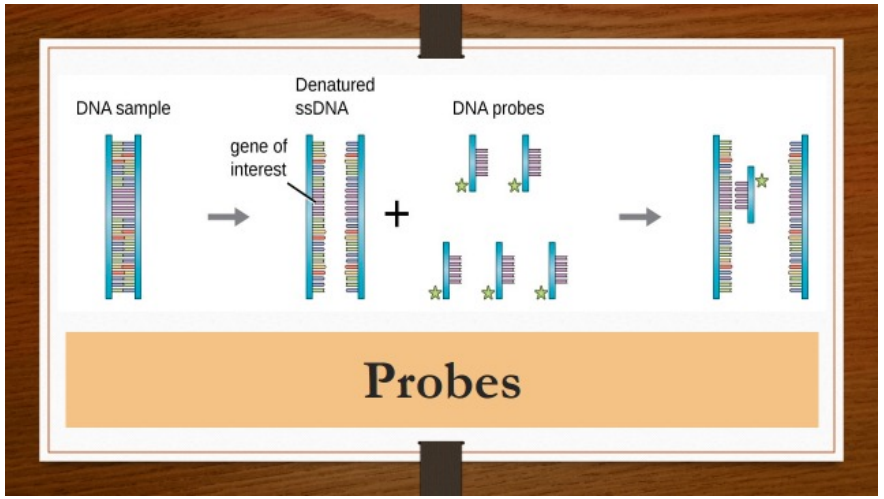


Figure 6. Mechanism of action of DNA probes.

Concept 5.6: Allele Specific Oligonucleotides

Function: To detect polymorphisms or mutations in a DNA sample.

How it Works: ASO probe for a specific DNA mutation is added to a DNA sample.

If the mutation is present in the DNA sample, it will bind to the ASO probe.

PCR can then be used to visualize the results.

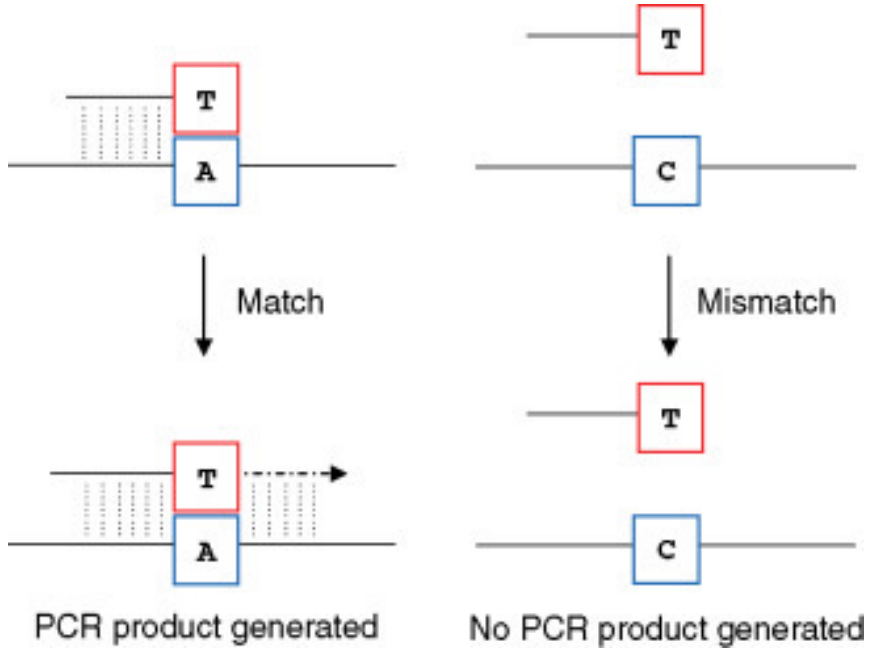


Figure 7. ASO Being used for PCR

Concept 5.7: Southern Blotting

Function: Southern blotting is used to separate DNA samples by size and determine the presence of specific DNA sequence

How it works:

DNA fragments are separated using **gel electrophoresis**

Samples are then moved to a nylon/PVDF membrane.

Sample is washed with blocking agents and radio-labelled DNA probes are used to detect specific DNA sequences.

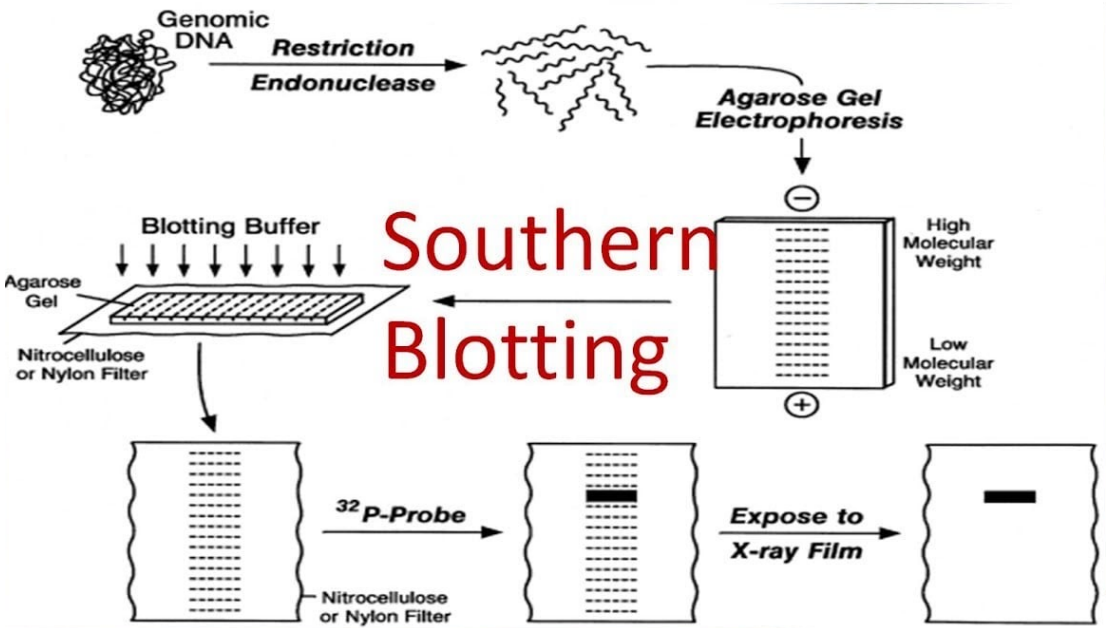


Figure 8. The Process of Southern Blotting

Concept 5.8: Polymerase Chain Reaction

Function: PCR is used for amplification of a DNA sequence

How it Works: DNA is added with DNA polymerase, nucleotides, magnesium and single-stranded DNA primers that are specific for the DNA sequence that is being amplified.

These are incubated and replicated to create millions of copies of the DNA sequence of interest.

Example: PCR tests were used to detect COVID-19 virus in samples by amplifying the virus' genome.

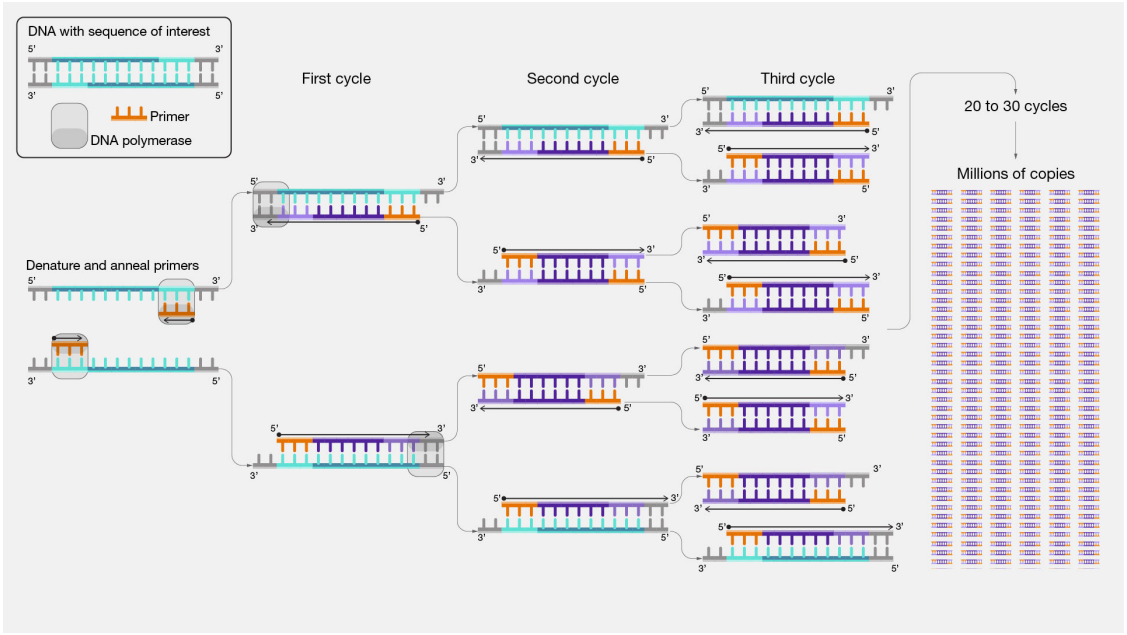


Figure 10. Overview of PCR

Concept 5.8: PCR Cycle

PCR Cycle Steps:

Step 1 (Denaturing the DNA): DNA sample is heated to be separated and create single-stranded DNA.

Step 2 (Attaching Primers): The sample is cooled to allow primers to attach to the DNA sequence it is complimentary to.

Step 3 (DNA Extension): The temperature is increased slightly and the Taq polymerases use the attached DNA primers to attach free nucleotides, synthesizing complimentary strands.

Step 4 (Repeat): These steps are repeated, which causes the DNA sequence of interest to be amplified

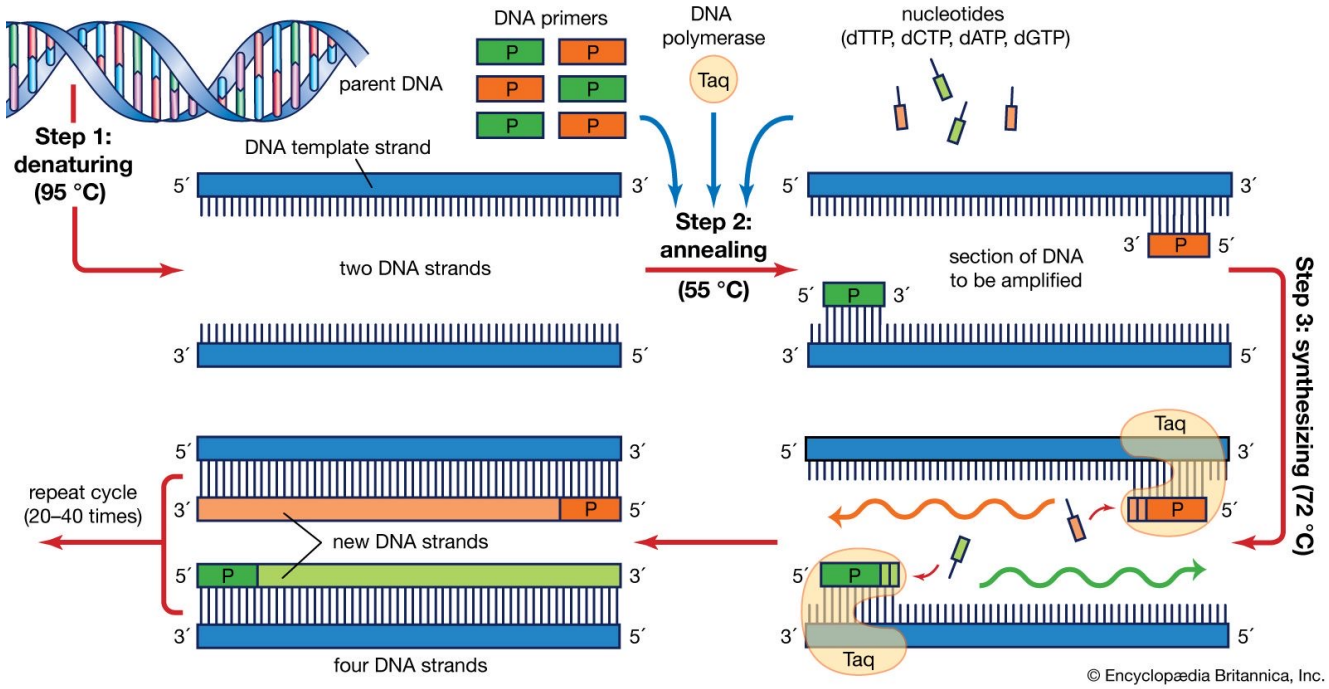


Figure 10. The Process of PCR

Concept 5.9: DNA microarrays

Function: DNA microarrays are used for genotyping.

Also can be used for locating mutations and multiple SNP in a genome.

How it Works:

- Normal genes and mutant genes are probed with different fluorescent compounds and the mixture is exposed to a gene chip, containing thousands of tiny DNA spots
- Each spot corresponds to a specific DNA sequence
- The amount of fluorescence in each sample indicates how much DNA is present

Example:

- Identifying mutations in genes compared to a control, such as in cancer research

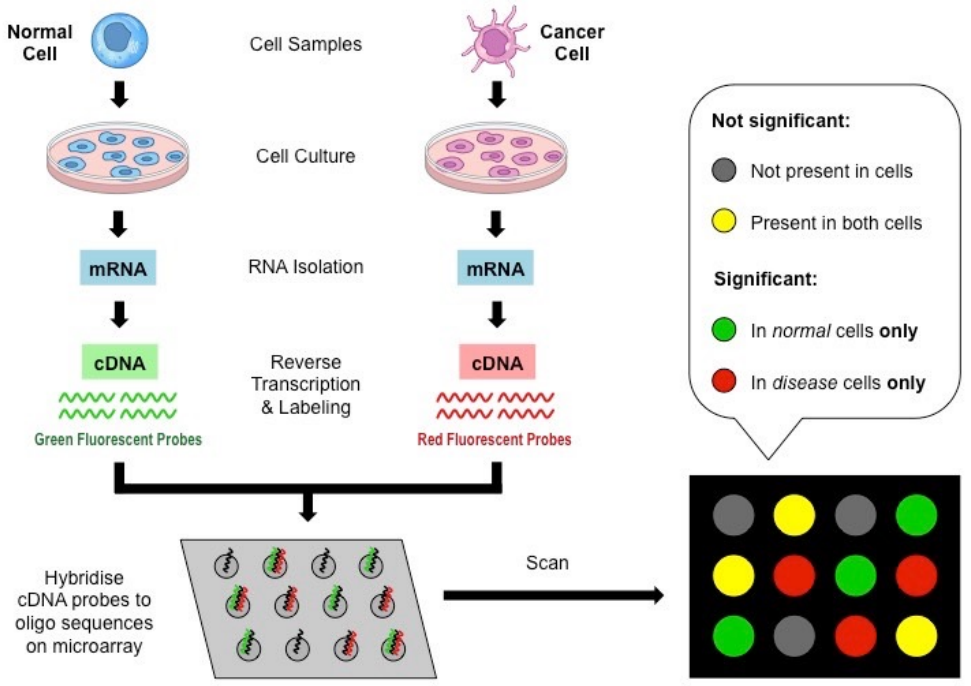


Figure 11. DNA Microarray Process

Concept 5.10: CRISPR-Cas9

Function

- Allows for the editing of cellular genome sequences

How it works

- Cas9 is an RNA-guided DNA endonuclease
  - Unwinds foreign DNA, compares it with the guide RNA, then cleaves it if there is a match
- Custom guide RNA sequences can be made to cause specific DNA double-stranded breaks

Example of its use

- Potential treatment for sickle cell anemia (recessive)
  - Used to repair one of the pathogenic disease alleles

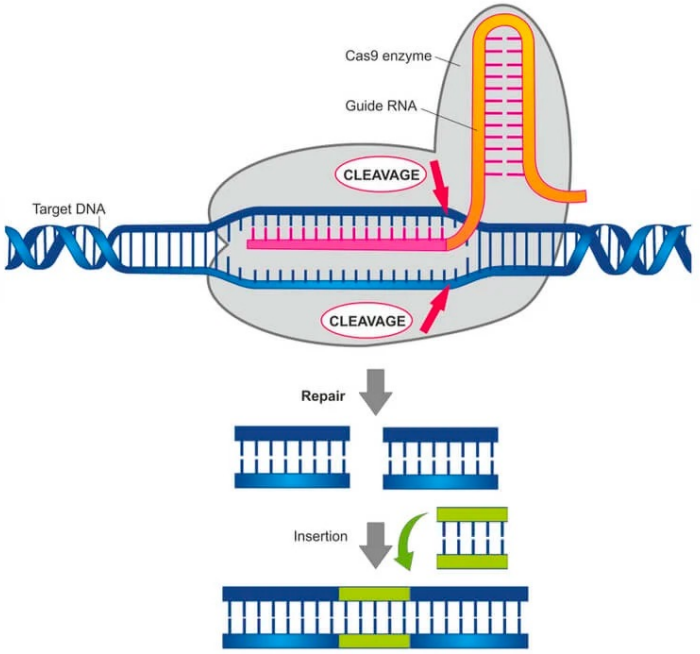


Figure 12. Mechanism of CRISPR-Cas9.

Section 5 Quiz: Biotechnology MC

What is the proper procedure for PCR?

A) Annealing, denaturation, extension
B) Extension, annealing, denaturation
C) Denaturation, extension, annealing
D) Denaturation, annealing, extension

Answer: D Denaturation, annealing, extension

Section 5 Quiz: DNA Biotechnology SA

Explain the mechanisms behind CRISPR-cas9 and the potential benefits and concerns of the biotechnology.

Answer:

- An RNA-guided DNA endonuclease enzyme system associated with the CRISPR adaptive immunity system
- Allows for cleavage of foreign DNA, such as invading viral or plasmid DNA
- Can be manipulated to allow for editing of cellular genome sequences
- Concerns: Ethical concerns on if gene editing should occur and what types of genes should be modified (e.g., germline vs somatic modifications, non-detrimental mutations etc.)

RNA Biotechnology Methods

Concept 5.11: Northern Blotting

Function

- Is used probe for a gene that is being expressed in a tissue or cell

How it works

- Uses the same process as Southern blotting, but uses mRNA instead of DNA

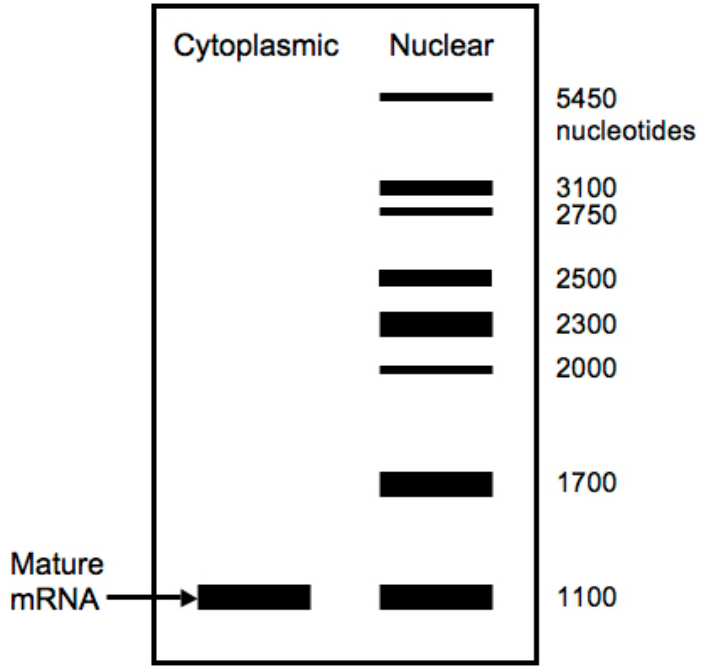


Figure 13. An example Northern blot result.

Concept 5.12: Quantitative PCR

Function

- Determines gene expression in cells

How it works

- Target mRNA is reverse transcribed by reverse transcriptase to form cDNA (complementary DNA)
- Fluorescent DNA probes and the cDNA is placed into a thermocycler that can measure fluorescence
- Standardized DNA templates are also added to the thermocycler to act as controls
- Thermocycler turns on, which measures how much cDNA is in the sample which then correlates to how much target mRNA was in the sample

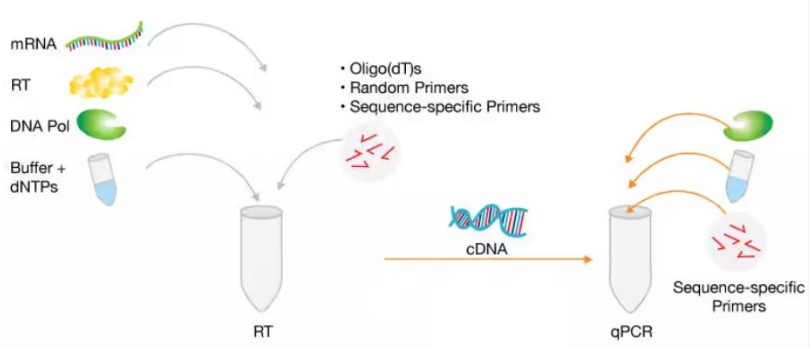


Figure 14. The process of conducting a qPCR.

Concept 5.13: RNA-Based cDNA Microarrays

Function

- Determines gene expression in cells

How it works

- mRNA is reverse transcribed by reverse transcriptase to form cDNA (complementary DNA)
- The cDNA from the different tissues being tested are fluorescently labelled with different colours
- The cDNAs are mixed and exposed to the gene microarray chip containing oligonucleotide spots
- The amount and colour of fluorescence on each spot correlates to how much cDNA and thus mRNA binds there

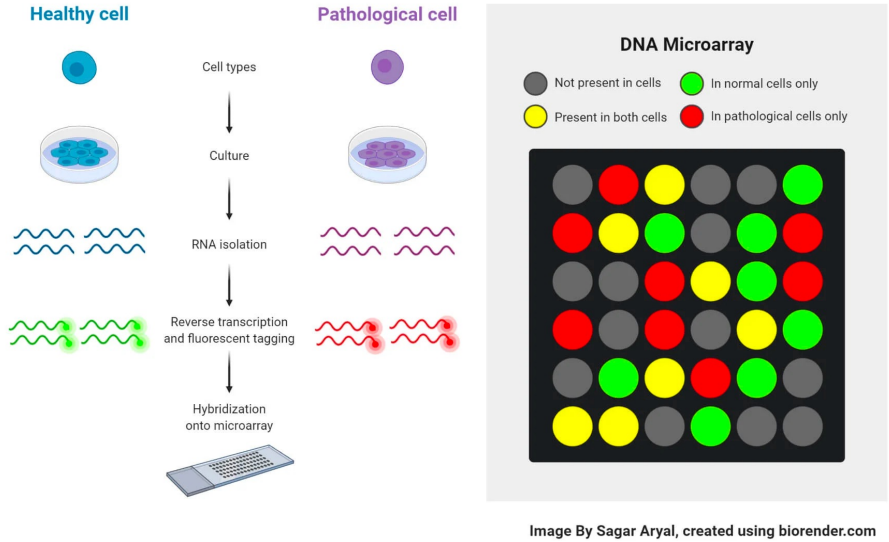


Figure 15: The process and result of a cDNA microarray.

Concept 5.14: SDS-PAGE

Function

- Measures protein size

How it works

- Is a gel electrophoresis conducted in polyacrylamide gels with detergent called sodium dodecyl sulfate (SDS)
  - Keeps proteins linear and unfolded
- Uses a dye front to keep track of the proteins in the gel electrophoresis
- Proteins of known size added to determine relative sizes of proteins of interest

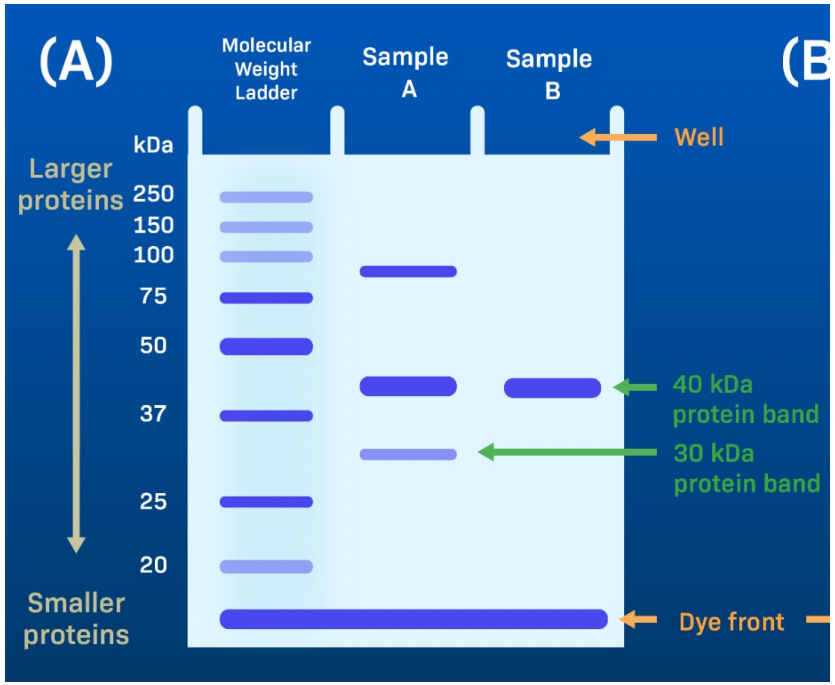


Figure 16. How to read an SDS-PAGE.

Protein Biotechnology Methods

Concept 5.15: Antibodies and Western Blotting

Function:

To separate proteins by size and identify their presence in a sample

How it Works:

Similar to other blotting techniques, but use proteins instead of DNA and RNA

After gel electrophoresis and transferred to a nylon membrane, antibodies are used to identify the protein

By using antigen-specific enzyme-labelled antibodies, a band is formed where the enzyme/protein is, which helps determine whether it is present in the sample and its size.

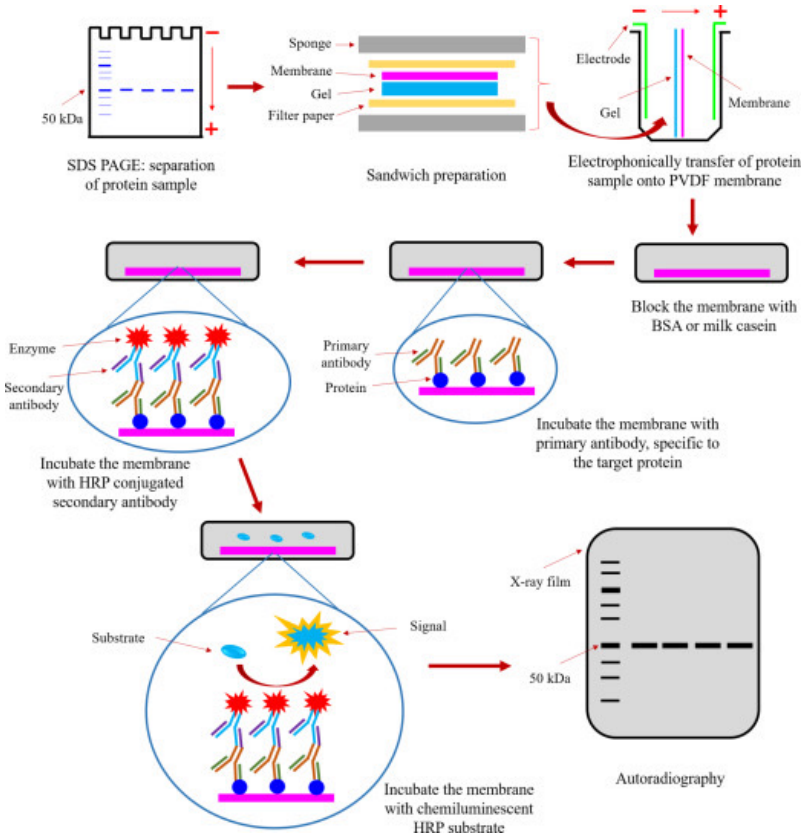


Figure 17. The Western Blotting Process

Concept 5.16: ELISA

Function: Use to quantify how much a protein is expressed and also look at interactions of proteins with antigens or other proteins.

Example: can be used to determine whether an individual has antibodies for a certain illness or if they have received a certain vaccine.

Direct

Wells of assay contain the antigen of interest. The sample is then added to the wells to test if it contains the antibody for the antigen

Sandwich

The wells of the assay contain the antibody that the antigen of interest binds to. If the sample added to the wells contains the antigen, it will bind to the antibodies in the wells. A second antibody is used visualize the antibody-antigen interaction.

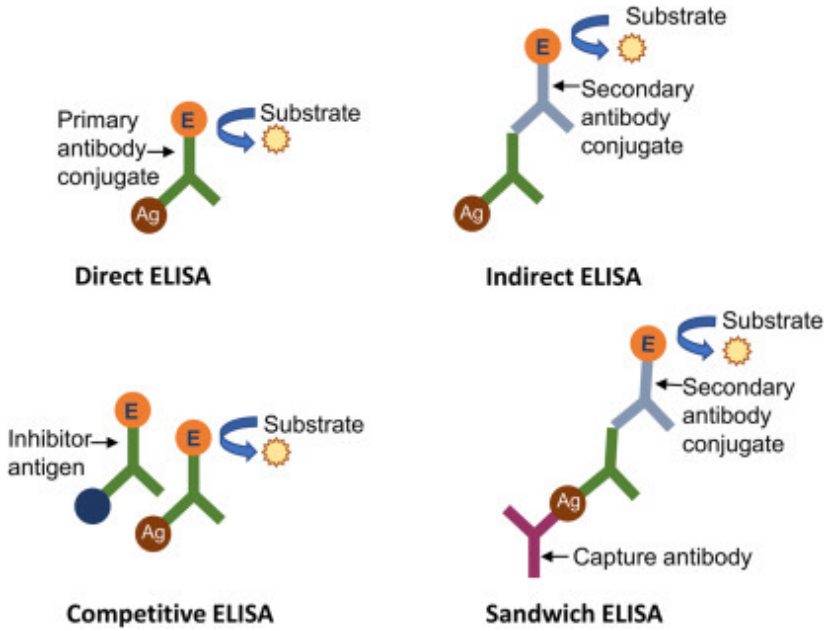


Figure 18. The Different Types of ELISA's

Questions

Section 5 Quiz: Biotechnology MC

A researcher is studying the effects of ischemic stroke on the interstitial fluid composition of the brain. The researcher hypothesizes that the concentration of albumin, which is normally found at much lower concentrations in the brain than the blood, will increase. Which biotechnology should the researcher use to answer this question?

a. Western Blot
b. Northern blot
c. Southern blot
d. Eastern blot

Answer: A

Albumin is a protein. Northern and southern blots measure nucleotide concentration. Since western blots measure protein concentration, it would be highly useful to answer this researcher's question

Section 5 Quiz: Biotechnology MC

A single point mutation in a gene results in a nonfunctional protein. Individuals heterozygous for this mutation were identified using a Southern blot. Which pair of wild-type (WT) and mutant alleles most likely contains the mutation?

A.
WT 5′–TAGTCGAAGCTTAGGCATCT–3′
Mutant 5′–TAGTCGATGCTTAGGCATCT–3′

B.
WT 5′–TAGTCGAAGCTTAGGCATCT–3′
Mutant 5′–TAGTCGAAGCTTAGGCATAT–3′

C.
WT 5′–TAGTCGAAGCTTAGGCATCT–3′
Mutant 5′–TAGTTGAAGCTTAGGCATCT–3′

D.
WT 5′–TAGTCGAAGCTTAGGCATCT–3′
Mutant 5′–TAGTCCAAGCTTAGGCATCT–3′

Answer: A

A Southern blot uses a restriction digest to differentiate between mutant and wild-type alleles. In order for a Southern Blot to be useful, the mutation should either eliminate or create a restriction site, most of which are palindromes and 4-6 base pairs long. The mutation shown in this option is the only one that disrupts a palindromic sequence, AAGCTT. This sequence is the recognition sequence for HindIII.

Section 5 Quiz: Biotechnology MC

Which of the following is a technique used to determine the expression level of a gene?

a. PCR
b. Gel electrophoresis
c. Allele-specific oligonucleotides
d. Microarray analysis

Answer: D