Molecular Biology

2025-2024

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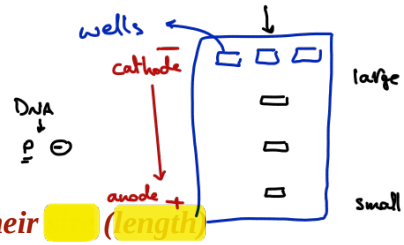
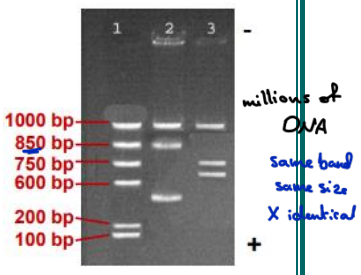
Basic Techniques

- DNA can be observed and detected by 2 methods:
  - ***DNA Staining:*** Using pigments non-covalently interacts with DNA giving a *certain color*
  - ***DNA Labeling:*** Using specific molecules that bind specific parts of the DNA covalently, and then *emit signals* at certain wavelength (more sensitive)

Gel Electrophoresis



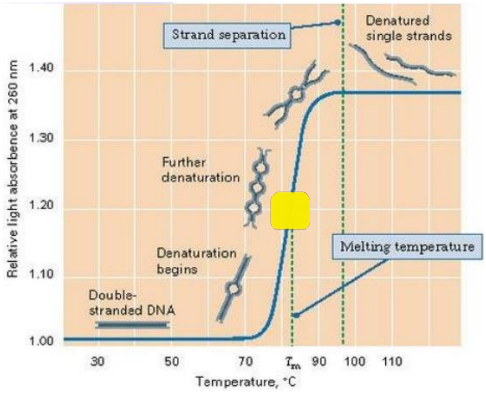
- It is a technique used for the ***separation of DNA fragments according to their*** ***(length)***
- It consists of a container containing a gel under an electrical field
  - *Wells:* Spaces in the beginning of the gel, where the DNA samples are loaded (added)
- *DNA is negatively* charged due to the presence of *phosphate*, and that causes DNA fragments to move from the ***cathode (– side) to the anode (+ side)***
  - *Smaller fragments can run faster* and move far away from the cathode
- DNA fragments appear on the gel in the form of bands
  - Each band contain millions of DNA fragments with the *same size* (same number of base pairs)
  - DNA fragments *do not have to be identical*



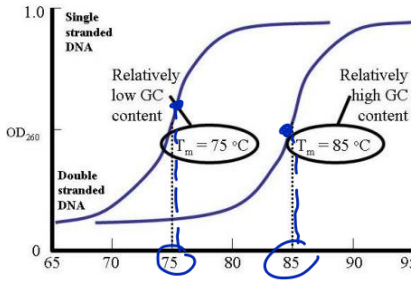
- DNA fragments are then stained by *ethidium bromide* or labeled by *radioactive Phosphorus (^{32}P)*

Light Absorbance of Nucleic Acids

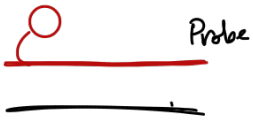
- The maximum (peak) light absorbance of nucleic acids is for *UV light* at wavelength of *260 nm*
  - It is caused by the aromatic structure of *purines and pyrimidines*
  - It is measured by spectrophotometry
- Light absorbance is used as a measurement for nucleic acid concentration in a sample
  - DNA Concentration = Light absorbed × Concentration per light unit × Dilution factor
    - ✓ Concentration per light unit in dsDNA: ***50*** µg/mL
- *ssDNA absorbs more* light than dsDNA due to the exposure of purines and pyrimidines
- As *dsDNA denatures*, *absorption of light increases* due to the increased fraction of ssDNA
  - ***Denaturation:*** The *separation* and disruption of the double stranded structure of the DNA, by *breaking H-bonds* between strands
  - Denaturation is a reversible process can be reversed by removing the denaturing factor causing ***renaturation***



- ***Melting Temperature (T_m):*** It is the temperature at which 50% of the DNA sample is denatured
  - It increases by increasing stability of H-bonds between the strands, such as increasing C-G content, length, salts and ions (cations)
  - T_m **decreases** by the presence of *destabilizing agents*, *extreme pH* and A-T content
    - ✓ Destabilizing agents: alkaline solutions, formamide and urea



- Explanation:
  - ↑ length, ↑ H-bonds, ↑ strength and stability, ↑ T_m
  - ↑ C-G content, ↑ H-bonds, ↑ strength and stability, ↑ T_m
  - ↑ salts and cations, hide negatively charged phosphates, ↓ repulsion, ↑ strength and stability, ↑ T_m
  - Extreme pH causes disruption of H-bonds, ↓ H-bonds, ↓ strength and stability, ↓ T_m
  - Destabilizing agents cause disruption of H-bonds, ↓ H-bonds, ↓ strength and stability, ↓ T_m



Hybridization and Blotting

- ***Hybridization:*** complementary pairing between 2 nucleic acid segments from *different sources*
  - Occur between any two single-stranded nucleic acid chains that have complementary sequences
  - Hybridization can occur between DNA and RNA fragments if they are complementary
  - It can be perfect or imperfect hybridization
  - ✓✓ *Perfect hybridization:* the 2 segments are totally complementary, so it can occur at any condition
  - ✗✓ *Imperfect hybridization:* the 2 segments are somewhat complementary
    - It can't occur at strict conditions (↓ stability; ↑ T, pH extreme, destabilizing some diff.)
    - Occurs only at favorable conditions with stabilizing factors such as ↓ *temperature*, ↑ *salts* and cations and ↑ *C-G content*

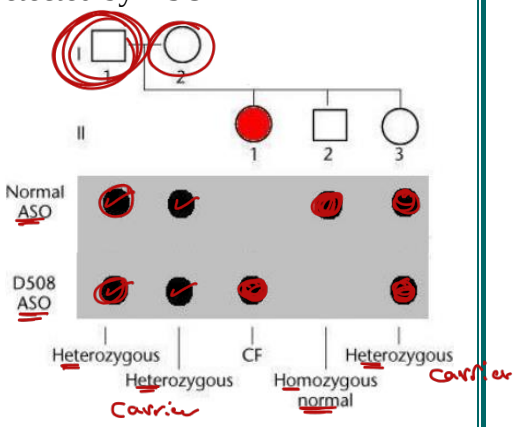
How to perform specific hybridization? by introducing strict conditions

- Hybridization is used to ***detect the presence of a specify nucleotide sequence*** using probes
  - ***Probe:*** A short sequence of single stranded DNA (oligonucleotide) that is complementary to a small part of a larger DNA sequence
  - Hybridization reactions use labeled DNA probes to detect larger DNA fragments
  - Probes are usually used under strict conditions preventing imperfect binding to ***increase specificity*** (↑ perfect → specific)
- ***Dot Blot:*** It is a technique where DNA fragments are attached to a solid membrane the labeled probes are added to detect the presence of a specific sequence is a larger DNA fragment
  - If the sequence is *present*, a *signal is emitted* from the labeled probe

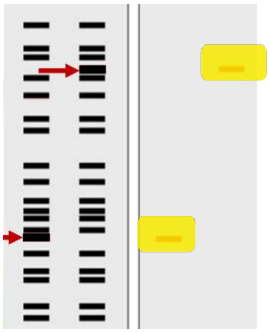
- Gene: A part of the DNA that determines a specific characteristic (phenotype)
- Allele: A form (variant) of the gene
- Homozygous: 2 similar alleles
- Heterozygous: 2 different alleles

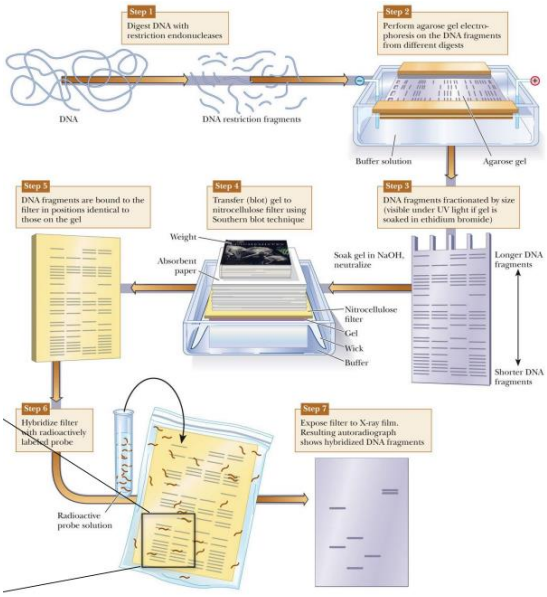
- Dominant: Requires only one allele to appear in the phenotype
- Recessive: Requires 2 alleles to appear in the phenotype
- Pedigree: شجرة العائلة

- Hybridization is important in detecting diseases by using ASO probes (Allele specific oligonucleotides)
- *Cystic fibrosis* gene is ***Δ508*** has 3 bp deletions (AGA) which can be detected by ASO
  - 2 probes are used, one for the normal allele, and the other for the mutated allele
  - If only the normal allele emitted a signal, the individual is homozygous normal
  - If only the mutated allele emitted a signal, the individual is homozygous affected
  - If both alleles emitted a signal, the individual is heterozygous (carrier)



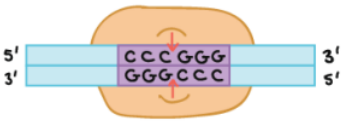
- *Southern blotting*: This technique is a combination of DNA gel electrophoresis and dot blotting
  - It is used to detect the presence of a *specific DNA* ***segment*** and its *size*
  - DNA fragments are separated by gel electrophoresis, then they are transferred into a membrane (replica) and probes are added to detect the presence of the needed sequence





Restriction endonucleases

- *Endonucleases*: Enzymes that degrade DNA within the molecule
- *Restriction endonucleases*: Bacterial enzymes that *recognize and cut* (break) the phosphodiester bond between nucleotides at *specific sequences* generating restriction fragments



- Restriction site (recognition site) must be:
  - *4 – 8* bp sequences
  - *Palindromic sequences*: Sequences read the same from both strands (left to right, or right to left)

```
5'  GAATTC  3'
3'  CTTAAG  5'
```

- Restriction endonucleases can cut the same DNA strand at ***several locations*** generating multiple restriction fragments of different lengths
  - Individual *variations* in DNA sequence (genetic variants) may *create or remove restriction sites* generating different restriction fragments

Our cells are diploid having 2 alleles for each gene which can be homozygous or heterozygous

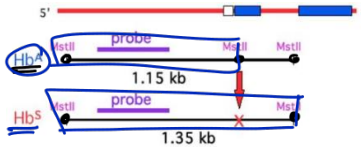
- Restriction Fragment Length Polymorphism (***RFLP***): Generation of fragments of different lengths due to the variation in DNA sequences
  - Individuals can generate restriction fragments of variable lengths known as ***molecular fingerprinting***

- After fragmenting the DNA molecule into smaller fragments, they must be separated by:
  - Gel Electrophoresis (all the fragments appear, arranged according to their lengths)
  - Southern blotting (Only fragments complementary to probes will appear)
    - ✓ The position of the band on the gel reflects the size of the fragment itself not the size of the probe

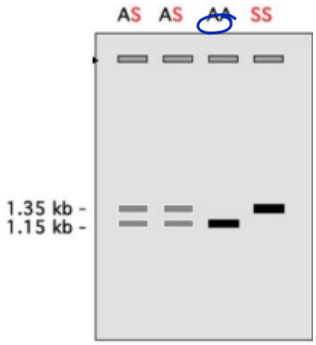
- RFLP can be used as diagnostic tools

1) Disease detection by RFLP

- RFLP detects whether the person is diseased as a result of this mutation and from which parent this allele was inherited
- Example: ***sickle cell anemia*** which is caused by a mutation in one nucleotide (base) in the globin gene that is responsible for making hemoglobin

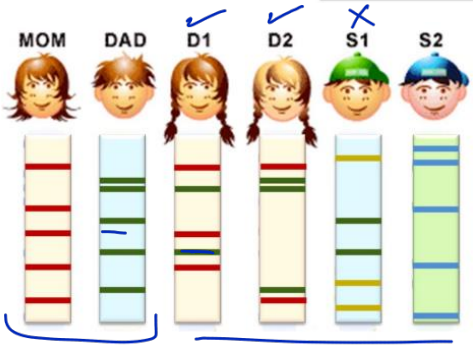


  - The Normal allele (GAG) produces glutamate, and the mutated allele (GTG) produces valine
  - Homozygous with two normal alleles (AA)
  - Heterozygous or carriers of one normal allele and one mutated allele (AS)
  - Homozygous for the mutated allele (SS)



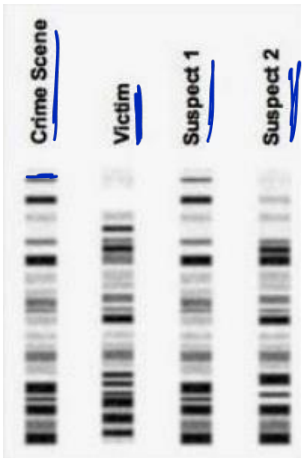
2) Paternity testing

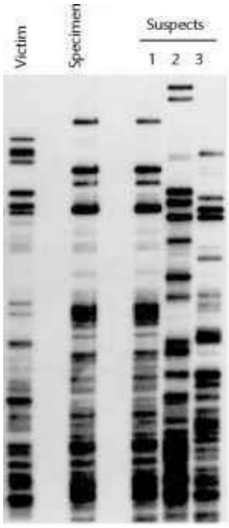
- All the fragments of the child must be present in the profile of one parent at least



3) Forensics

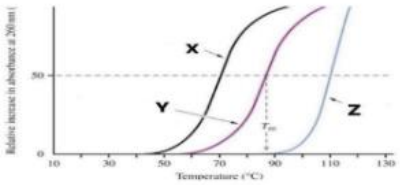
- Usually used to detect criminals using a sample that presents in the place of the crime





Past Papers

1) The plot shown illustrates the denaturation curve of three DNA samples, (X, Y and Z). One of the following is or can be a true statement:



A) Sample Y has higher CT content than sample Z
B) At 50% absorbance, half of the DNA molecules are FULLY denatured and half are FULLY double-stranded
C) At 80°C, the majority of DNA in samples X and Y are denatured
D) The melting temperature of all samples is approximately 120°c
E) The increase in the absorbance at higher temperature is because DNA becomes single stranded

2) When using gel electrophoresis, 1000 bp fragment size indicates:

A) A total of 1000 bases each DNA molecule
B) A total of 1000 base pairs each DNA molecule. A total of 2000 bases each DNA molecule
C) A total of 1000 bases each DNA strand
D) B &C and D
E) B&C only

diluted (X) (÷)

3) A DNA sample has a concentration of 0.1 µg/ml. It was CONCENTRATED 1:50. What do you expect the absorbance of the CONCENTRATED SAMPLE to be at 260 nm of light?

A) 5
B) 50
C) 1
D) 0.1
E) 0.5

$0.1 = ? \times 50 \times \frac{1}{50}$

$? = 0.1$

4) All of the following regarding gel electrophoresis are true EXCEPT:

A) Agarose gel is used
B) Smaller molecules move faster than larger ones
C) Molecules move towards the positive electrode
D) The higher the density of the gel, the higher the resolution

5) what would the ABSORBANCE an ORIGINAL DNA sample be if the concentration of the sample, when diluted 1:5, is 2 µg/ml?

A) 0.1
B) 1
C) 0.2
D) 0.5
E) 5

6) Melting temperature of DNA is:

A) The temperature at which the DNA strands are denatured completely
B) The temperature at which the DNA strands are half denatured.
C) The temperature at which the DNA strands renatured.
D) None of the above

7) The melting temperature of DNA fragment (X) is 60oC, whereas it is 75°C for fragment (Y). This SURELY informs us that

A) fragment (X) is shorter than fragment (Y)
B) fragment (X) exists in an alkaline solution but not fragment (Y)
C) The sources of both fragments are different
D) fragment (X) has less GC content than fragment (Y)
E) fragment (X) has weaker hydrogen bonding between the 2 strands than fragment (Y)

8) Denaturation of DNA molecules is a necessity in southern blotting in order to allow binding between the probs and the separated DNA strands, which of the following pH values allow this to occur and specifically in southern blotting:

A) 6.5
B) 7
C) 13
D) A & C
E) None of the above

9) A DNA sample has a concentration of 250 µg/ml. It was diluted 1:50. What do you expect the absorbance of the diluted sample to be at 260 nm of light?

A) 5
B) 50
C) 1
D) 0.1
E) 0.5

$250 = ? \times 50 \times 50$

$\frac{250}{2500} = ? \times \frac{2500}{2500}$

$? = 0.1$

10) Which of the following double stranded DNA sequences needs higher temperature to separate into single-stranded DNA?

A) 5'-GGGCCATTGC-3'
B) 5'-ATTATTCTGC-3'
C) 5'-GGGCCATTTC-3'
D) 5'-GGGCCGTTGC-3'
E) 5'-GGGCCCCTGC-3'

11) Calculate the the concentration of double stranded DNA molecules if a concentrated solution of which (by a factor of 5) absorbed 2 units of light with a length of 260 nm

A) 10
B) 20
C) 500
D) 50

12) One of the following is a feature of gel electrophoresis of DNA

A) the migration of DNA fragments is influenced by chromatin structure and total charge.
B) movement of DNA fragments is dependent of their length only.
C) DNA fragments appear as band because of the way they interact with each other.
D) the distinct color of DNA makes them observable.
E) (GC) content is an important factor in separation of DNA fragments.

13) Which of the following about ASO is incorrect :

A) Two types of probes are used
B) It's used in the detection of cystic fibrosis
C) When a signal is produced on both membranes after dna hybridization this indicates heterozygous person where only the dominant allele is expressed
D) The defection in cystic fibrosis is the deletion of 2 nucleotides in a specific gene
E) All of the above

14) one of the following is NOT true in regards to this DNA fragment AGCTGGCTCGAG:

A) all nucleotides are in the deoxysugar form
B) if transcribed, the RNA produced will be CUCGAGCCAGCU
C) the terminal A is located at 5'- end
D) its complementary strand is TCGACCGAGCTC
E) it has a higher melting point than TTAGCTACAATT

15) you have three individuals (A, B, and C) where A is homozygous for a normal allele, B is homozygous for a mutated allele, and C is heterozygous. You perform dot blotting using allele-specific oligonucleotides (ASO) for the normal (ASOX) and mutated (ASOY) alleles. One of the following is TRUE

A) signals will be seen for individual C by dot blotting when using either ASO
B) a signal will be seen for individuals A and B by dot blotting when using ASOX
C) ASOX cannot differentiate individuals A and B from each other by dot blotting
D) a signal will be seen for individual A only when using ASOY
E) ASOY is more specific than ASOX

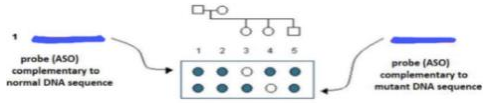
16) Which of the following features of DNA is primarily responsible for movement of DNA molecules in an electric field?

A) Nitrogenous base
B) Deoxyribose sugar
C) Phosphate
D) Complementary base pairing
E) Antiparallel orientation

17) A human genomic DNA is cut by a restriction endonuclease and then analyzed by Southern blotting, you can know the following:

A) The numbers, but not sizes and sequences, of the fragments
B) The sizes and numbers, but not sequences, of the fragments
C) The sequences, but not the numbers or sizes, of the fragments
D) The sizes, but not the numbers or sequences, of the fragments
E) The sequences, sizes and numbers of the fragments

18) A dot plot hybridiation is carried out for the family shown this the pedigree which of the following statement is True?



A) Both daughters are disease affected
B) Both daughters are heterozygous
C) The son is Homozygous for the mutant DNA sequence
D) Both parents are disease affected
E) Both daughters are Homozygous

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