Alanoud al-faghom
PLANT AND MICROBIOLOGY
DEPARTMENT

ا. العنود الفغم

Name of experiment	اسم التجربة	المعمل
Introduction to molecules biology	مقدمة	.1
DNA Extraction from fruit	استخلاص الدنا من الفواكهه	.2
DNA Extraction from plant leaves	استخلاص الدنا من الاوراق	.3
DNA Quantification	قياس كمية الدنا	.4
Gel electrophoresis	الفصل الكهربائي للدنا	.5
Protine isolation from plant tissue+ + SDS - PAGE	استخلاص البروتين من الانسجة النباتية.	.6
PCR and its application	جهاز البي سي ار وتطبيقاتة	.7
Application of molecules biology	تطبيقات في الاحياء الجزيئية	.8

LAB.1

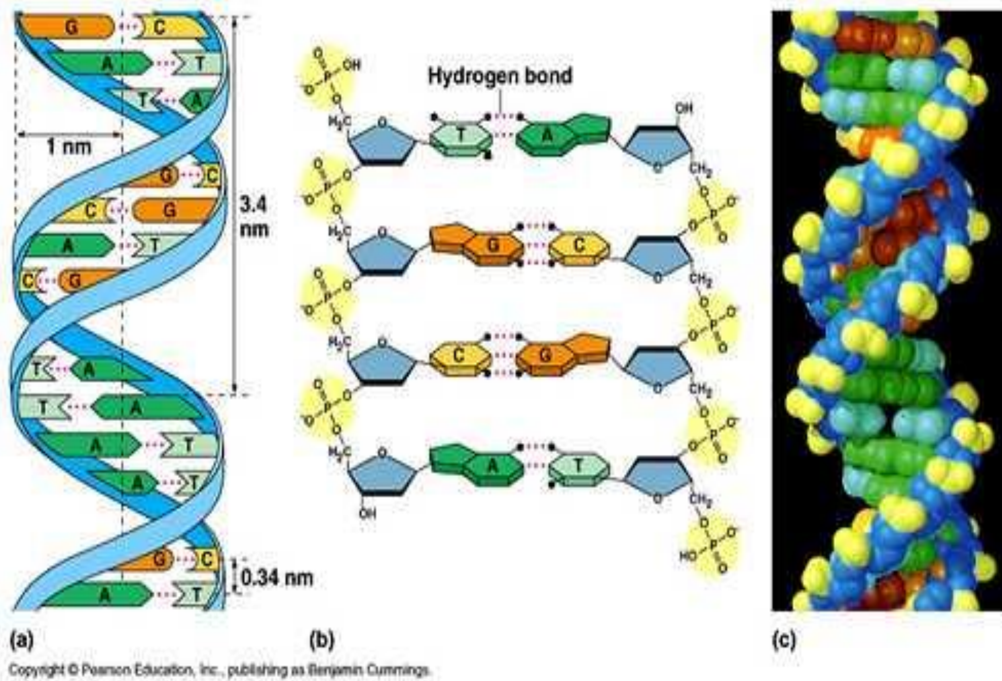
Introduction to molecules biology

Molecular genetics is the study of structure and function of genes at a molecular level.

The field studies how the genes are transferred from generation to generation. Molecular genetics employs the methods of genetics and molecular biology Components of DNA

DNA is the "Code of Life" .The DNA of eukaryotic cells is about 100,000 times as long as the cells themselves. However, it only takes up about 10% of the cells' volume. This is because DNA is highly convoluted (folded) and packaged as structures called chromosomes within cell nuclei. A chromosome is a bundle of tightly wound DNA coated with protein molecules. An organism's chromosomes bunch together within the nucleus like a ball of cotton, but during cell division (mitosis) they become individually distinct (human mitotic chromosomes are Xshaped) and can be observed as such with microscopes. **DNA is not visible to the eye** unless it is amassed in large quantity by extraction from a considerable number of cells. When chromosomal DNA is unfolded and the proteins coating it removed, the structure of DNA is exposed as a twisted ladder called a double helix. The sides of the ladder form the DNA backbone with alternating sugar and phosphate molecules linked by covalent bonds. The rungs of the ladder are comprised of pairs of nitrogenous bases [adenine (A) with thymine (T) and cytosine (C) with guanine (G)] joined by hydrogen bonds. Although the structure of DNA is well known and clearly

defined, even the most powerful microscopes cannot visualize the DNA double helix of chromosomes.



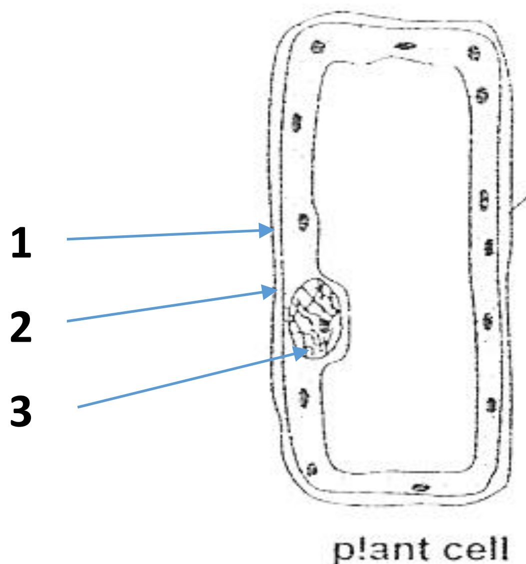
All living things are dependent on DNA, and the structure of DNA is consistent among all species. However, the particular sequence of nitrogenous bases within DNA molecules differs between organisms to create explicit "blueprints" that specify individual living things. This sequence of base pairs is what makes an organism an oak tree instead of a blue jay, a male instead of a female, and so forth.

DNA Extraction From Plant Cells

The DNA of a plant cell is located within the cell's nucleus. The nucleus is surrounded by a nuclear membrane and the entire cell is encased in both a cell membrane and a cell wall. These barriers protect and separate the cell and its organelles from the surrounding environment. Therefore, in order to extract

DNA from plant cells, the cell walls, cell membranes and nuclear membranes must first be broken. The process of breaking open a cell is called **cell lysis**.

Physical actions such as mashing, blending, or crushing the cells cause their cell walls to burst. The cell membranes and nuclear membranes may then be disrupted with a detergent-based extraction buffer. Just as a dishwashing detergent dissolves fats (lipids) to cleanse a frying pan, a detergent buffer dissolves the phospholipid bilayer of cell membranes. It separates the proteins from the phospholipids and forms water-soluble complexes with them. Once the cell wall and cell membranes are degraded the cell contents flow out, creating a soup of DNA, cell wall fragments, dissolved membranes, cellular proteins, and other contents. This “soup” is called the lysate or cell extract.



The DNA is surrounded by 3 briers:

1- A cell wall

2- A cell membrane and

3- .A nuclear membrane

DNA molecules are then isolated away from the cell debris in the lysate. For this purpose, the detergent-based extraction buffer also includes salt. The salt causes some of the cellular debris in the soup to precipitate out of solution while the DNA remains dissolved. This means that the cell debris become suspended

particles that can be seen. The cell extract is then filtered through layers of cheesecloth. The cheesecloth traps the precipitated cell debris while the soluble DNA passes through. DNA is soluble in the aqueous cellular environment and in the presence of the extraction buffer, but is insoluble in alcohol (such as ethanol and isopropanol). Applying a layer of ethanol on top of the filtered lysate causes the DNA to precipitate out of the solution, forming a translucent cloud of fine, stringy fibers at the point where the alcohol and cell extract meet. Cold ethanol works best to precipitate DNA to the fullest. DNA extracted from multiple cells is visible by eye and can be wound onto a wooden stick in a process known as “spooling” the DNA.

Importance of DNA Extraction

DNA extraction is a fundamental procedure in scientific laboratories around the world. By extracting DNA, scientists can learn how DNA encodes the instructions for all life processes. DNA extraction is important to the study of heredity and to the treatment of many diseases through the creation of gene therapy DNA molecules. Extracted DNA can also be used to create DNA fingerprints to help diagnose genetic diseases, solve criminal cases, identify victims of disaster and war, and establish paternity or maternity. Scientists can genetically engineer changes in DNA to create robust, disease-resistant genetically modified plants and animals. DNA extraction is also necessary in order to sequence the DNA code (order of base pairs) of different organisms (as in the Human Genome Project) and compare different species.

What does DNA look like? What will we see?

The structure of DNA is like a twisted ladder, forming what is called a double helix. The sides of the ladder are sugar-phosphate groups joined by covalent bonds and the rungs are nitrogenous bases joined by hydrogen bonds. However, in order to package DNA within the nucleus of eukaryotic cells, DNA is wound around protein molecules and tightly folded into chromosomes. Can we see DNA? Yes and no. Chromosomes have been studied using microscopes, but the double helix of unraveled chromosomes is so thin that even the most powerful microscopes cannot detect it. How will we see the DNA we extract? Chromosomal DNA from a single cell is not visible by eye, when DNA is extracted from multiple cells, the amassed quantity is visible and looks like strands of mucous-like, translucent cotton.

LAB.2

Strawberry DNA Extraction

Objectives

Understand how cell barriers are broken and how to extract DNA from strawberry cells.

Material

This kit accommodates up to 32 students working in pairs.

17 50-mL tubes with lids and bases

33 15-mL tubes with lids

16 resealable plastic bags

16 wooden sticks

16 funnels

1 pack of cheesecloth

16 transfer pipets

1 bottle of ethanol, 95% (100 mL)

1 bottle of liquid detergent

10 g salt (sodium chloride, NaCl)

Procedure

1. Obtain one fresh or one frozen and thawed strawberry. If you are using a fresh strawberry, remove the green sepals (tops) from the berry.
2. Place the strawberry in a resealable plastic bag.
3. Close the bag slowly, pushing all of the air out of the bag as you seal it.
4. Being careful not to break the bag, thoroughly mash the strawberry with your hands for two minutes.
5. Pour the 10-mL aliquot of extraction buffer into the bag with the mashed strawberry. Reseal the bag.
6. Mash the strawberry for one additional minute.
7. Place a funnel into a 50-mL centrifuge tube. Fold the cheesecloth in half along the longer side and place it in the funnel to create a filter. The cheesecloth will overlap the edge of the funnel.
8. Pour the strawberry mixture into the funnel, filtering the contents through the cheesecloth and into the 50-mL centrifuge tube.
9. Carefully pour 2 mL of the filtered contents from the 50-mL tube into a clean 15-mL tube. Use the lines on the side of the 15-mL tube to help measure the amount added.
10. Hold the 15-mL tube at an angle. Using a transfer pipet, carefully add 5 mL of cold 95% ethanol by running it down the inside of the tube. Add the 95% ethanol until the total volume is 7 mL (use the lines on the side of the tube to help you measure). You should have two distinct layers. Caution: Do not mix the strawberry extract and the ethanol!
11. Watch closely as translucent strands of DNA begin to clump together where the ethanol layer meets the strawberry extract layer. Tiny bubbles in the ethanol layer will appear where the DNA precipitates.

12. Slowly and carefully rotate the wooden stick in the ethanol directly above the extract layer to wind (or “spool”) the DNA. Remove the wooden stick from the tube and observe the DNA .

Notes

LAB.3

DNA isolation from plant materials

Collecting plant materials

DNA isolation using

Protocol

1. Collecting plant materials
2. Phase Separation
3. DNA Precipitation
4. DNA Wash
5. Redissolving the DNA

Collecting plant materials

1. The plant most be clean and free of soil, insect and microscopic fungi.
2. Keep the specimen inside clean container or zip lock bag.
3. Label information should be placed inside the zip lock bag with specimen which include: Taxon name, collection name, Date of collection.
4. If some time must elapse before shipping, refrigerate but do not freeze the plant.
5. Get a 2 sample from every specimen one for molecular work other to keep it in the university herbarium.
6. For extracted DAN you will need a small piece of plant.

7. Plant tissues may be efficiently powdered by first freezing in liquid nitrogen or dry ice/ethanol before DNA extraction.

Materials

CTAB buffer (Hexadecyl trimethy l-ammonium bromide)

Microfuge tubes, Mortar and Pestle, Microfuge

Absolute Ethanol (ice cold)

70 % Ethanol (ice cold)

7.5 M Ammonium Acetate

55o C water bath

Chloroform: Iso Amyl Alcohol (24:1)

Water (sterile)

Agarose

6x Loading Buffer

1x TBE solution (Tris base, boric acid, EDTA (pH 8.0)).

Agarose gel electrophoresis system

Ethidium Bromide solution

Procedure

I. Phase Separation

1. Grind 0.02 mg of plant tissue to a fine paste in approximately 500 µl of CTAB buffer.
2. Transfer CTAB/plant extract mixture to a microfuge tube.
3. Incubate the CTAB/plant extract mixture for about 15 min at 55o C in a recirculating water bath.

II. DNA Precipitation

4. After incubation, spin the CTAB/plant extract mixture at 12000 g for 5 min to spin down cell debris. Transfer the supernatant to clean microfuge tubes.
5. To each tube add 250 µl of Chloroform : Iso Amyl Alcohol (24:1) and mix the solution by inversion. After mixing, spin the tubes at 13000 rpm for 1 min.
6. Transfer the upper aqueous phase only (contains the DNA) to a clean microfuge tube.
7. To each tube add 50 µl of 7.5 M Ammonium Acetate followed by 500 µl of ice cold absolute ethanol.
8. Invert the tubes slowly several times to precipitate the DNA. Generally the DNA can be seen to precipitate out of solution. Alternatively the tubes can be placed for 1 hr at -20 o C after the addition of ethanol to precipitate the DNA.

III. DNA Wash

9. Following precipitation, the DNA can be pipetted off by slowly rotating/spinning a tip in the cold solution. The precipitated DNA sticks to the pipette and is visible as a clear thick precipitate. **To wash the DNA**, transfer the precipitate into a microfuge tube containing 500 µl of ice cold 70 % ethanol and slowly invert the tube. Repeat.
10. (alternatively the precipitate can be isolated by spinning the tube at 13000 rpm for a minute to form a pellet. Remove the supernatant and wash the DNA pellet by adding two changes of ice cold 70 % ethanol)).
11. After the wash, spin the DNA into a pellet by centrifuging at 13000 rpm fo 1 min.
12. Remove all the supernatant and allow the DNA pellet to dry (approximately 15 min).
13. Do not allow the DNA to over dry or it will be hard to re-dissolve.

IV. Redissolving the DNA

14. Resuspend the DNA in sterile DNase free water (approximately 50-400 µl H2O; the amount of water needed to dissolve the DNA can vary, depending on how much is isolated). RNaseA (10 µg/ml) can be added to the water prior to dissolving the DNA to remove any RNA in the preparation (10 µl RNaseA in 10ml H2O).
15. After resuspension, the DNA is incubated at 65o C for 20 min to destroy any DNases that may be present and store at 4o C.
16. Agarose gel electrophoresis of the DNA will show the integrity of the DNA, while spectrophotometry will give an indication of the concentration and cleanliness.

Notes

LAB.4

DNA Quantification

DNA yield can be assessed using various methods including absorbance (optical density), agarose gel electrophoresis, or use of fluorescent DNA-binding dyes. All three methods are convenient, but have varying requirements in terms of equipment needed, ease of use, and calculations to consider.

Checking the quality by agarose gel electrophoresis

Genomic DNA extraction reading at OD260 is equivalent to 50 µg/ml). A pure DNA solution has an OD260:OD280 ratio of 1.8 ± 1.The DNA concentration is calculated using the formula,

DNA concentration (µg /µl) = OD at 260 nm × dilution times × standard value

If OD260 is 1.00, it is equivalent to 50 µg DNA per ml (standard).

We are taking 50 µl of DNA in 1 ml TE buffer, which means it is diluted 20 times.

For example:

if the spectrophotometer reading is 0.112 (say), then the concentration of DNA is calculated as,

DNA concentration (µg/µl) = 0.112 × 20 × 50= 112 µg/ml.

Most DNA extracts are not reasonably pure and therefore estimates of concentration using spectrophotometric measurements of UV absorption may be misleading because of the interference by RNA or non-nucleic acids contaminants.

In this case, quantification of the DNA can be achieved by running the DNA samples on 1 % agarose gel stained with ethidium bromide (0.5 µg/ml)

(Ethidium bromide is a fluorescent chemical that intercalates between base pairs in a double stranded DNA molecule).

Aliquots of the DNA extracts are loaded [e.g. 7 µl of DNA mix (5 µl of each DNA sample and 2 µl of loading dye)] alongside a range [7 µl each of 5, 10, 25 and 50 ng/µl] of uncut lambda DNA standards.

High molecular weight DNA will appear as a well-resolved band alongside the lambda DNA bands whilst the smearing below the band indicates mechanical or chemical degradation.

A smeared band towards the bottom of the gel is an indication of the presence of RNA in the extract. A rough estimate of DNA content (±10 ng) may be obtained by comparing band intensities of the DNA extract and the standards by eye. Greater precision may be obtained with the aid of commercial gel imaging equipment and gel analysis software. However, most applications do not require that the amount of DNA be known exactly. Techniques such as RAPD analysis depend more upon ensuring a consistent amount of DNA from sample to sample.

Procedure for Spectrophotometre measurement

1. Switch on the spectrophotometer and allow warming up.
2. Turn on the mode to UV.
3. Set the wavelength to 280 nm.
4. Wash the cuvette with distilled H2O. Dry with tisuue.
5. using a micropipette, clean the inside of the cuvette by TE buffer.
6. Insert the cell containing 100 µl of TE into chamber.
7. Set the reading to zero.
8. Set wavelength to 260 and then set the reading to zero.

9. Remove the cuvette from its compartment and discard the TE.
10. Add 5.0 µI of the DNA sample in the cuvette.
11. Add 95 µl of TE. Mix solution thoroughly.
12. Insert the cuvette into the sample compartment and close the cover tightly.
13. Read the OD value directly from the screen. This gives the DNA concentration when multiplied by 1000. That is, if OD equals 0.200 then DNA concentration equals 0.200 x 50 x 20 = 200 µg/ml (50 because 1 unit OD corresponds to a concentration of 50 µg/ml and 20 because the DNA solution was diluted 20 X).

To assess purity of the samples:

14. Read the OD at 280 nm.
15. Compute for OD260/OD280. A ratio value of 1.8 suggests a highly pure preparation of DNA. Ratio values much less than that implies significant presence of contaminants (generally proteins) such that accurate quantitation of nulceic acids is not guaranteed.
16. Read the OD values for the other samples at 260 and 280 nm by repeating the steps described above. Make sure to wash the cuvette thoroughly with TE between DNA samples.

DNA fragments produced through amplification or through restriction enzyme digestion are separated by using electrophoresis. Either agarose and polyacrylamide gels are used which act as a selective filter so that DNA molecules having different molecular sizes are separated into specific bands as they move away from the one electrode to other.

DNA is negatively charged and will migrate to the positive electrode (anode) in an electric field. Because DNA molecules have a uniform charge:mass ratio, they exhibit similar electrophoretic migration properties in a resistance free medium. The migrated DNA is visualized under UV light with the help of an intercalating dye, ethidium bromide, which fluoresces when irradiated with UV.

The size of the fragment generated can be estimated by comparing the electrophoretic mobility (distance migrated through the gel per unit time) of an unknown DNA molecule to the electrophoretic mobility of DNA molecules for which the sizes are known. The standard method used to separate, identify and purify DNA fragments is electrophoresis through agarose gels. The technique is simple, rapid to perform and capable of resolving mixtures of DNA fragments. The migration rate of DNA through agarose gels is additionally dependent upon the molecular size of the DNA, the agarose concentration, the conformation of the DNA and the applied current. A DNA fragment of a given size migrates at different rates through gels containing different concentrations of agarose. Thus, by using gels of different concentrations, it is possible to resolve a wide size-range of DNA fragment.

Notes

LAB.5

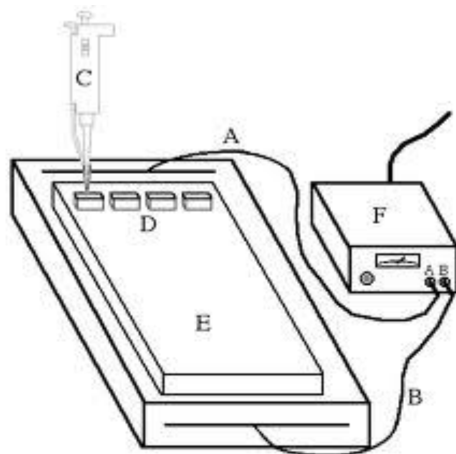
GEL ELECTROPHORESIS

Gel electrophoresis is a method used to separate nanoparticles by charge or size. **It is used to:**

1. Separate a mixed population of DNA and RNA fragments by length.
2. To estimate the size of DNA and RNA fragments or to separate proteins by charge.

Gel electrophoresis apparatus

An agarose gel is placed in this buffer-filled box and electrical field is applied via the power supply to the rear. The negative terminal is at the far end, so DNA migrates from A to B wair. There are 2 types of gel was commonly used in electrophoresis apparatus: Agarose gel and Polyacrylamide Gel.



A. positive (+) electrode (anode)
B. Negative (-) electrode (anode)
C. DNA sample
D. pore
E. gel
F. electronic source.

Agarose gel

- Agarose gels are easily cast and handled compared to other matrices. Samples are also easily recovered. After the experiment is finished, the resulting gel can be stored in a plastic bag in a refrigerator.
- Agarose gel electrophoresis can be used for the separation of DNA fragments ranging from 50 base pair to several megabases (millions of bases) using specialized apparatus.
- Most agarose gels are made with between 0.7% and 2% agarose dissolved in electrophoresis buffer.

Polyacrylamide Gel

- Polyacralamide gel electrophoresis (PAGE) is used for separating proteins ranging in size from 5 to 2,000 kDa due to the uniform pore size provided by the polyacrylamide gel.
- Pore size is controlled by controlling the concentrations of acrylamide and bis-acrylamide powder used in creating a gel.
- SDS-PAGE, Sodium Dodecyl Sulfate. Polyacrylamide Gel Electrophoresis: describes a technique used to separate proteins according to their electrophoretic mobility.

Buffers

- A **buffer solution** is an aqueous solution consisting of a mixture of a weak acid and its conjugate base or a weak base and its conjugate acid. It has the property that the pH of the solution changes very little when a small amount of strong acid or base is added to it.

- Buffer solutions are used as a means of keeping pH at a nearly constant value in a wide variety of chemical applications.
- There are a number of buffers used for electrophoresis. The most common being, for nucleic acids Tris/Acetate/EDTA (TAE), Tris/Borate/EDTA (TBE).

Visualization and analysis

- After the electrophoresis is complete, the molecules in the gel can be stained to make them visible.
- DNA may be visualized using ethidium bromide which, when intercalated into DNA, fluoresce under ultraviolet light.
- Protein may be visualized using silver stain or Coomassie Brilliant Blue dye.
- Visualization can also be achieved by transferring DNA to a nitrocellulose membrane followed by exposure to a hybridization probe. This process is termed **Southern Blotting.**
- After electrophoresis the gel is illuminated with an ultraviolet lamp (usually by placing it on a light box, while using protective gear to limit exposure to ultraviolet radiation). The illuminator apparatus mostly also contains imaging apparatus that takes an image of the gel, after illumination with UV radiation.
- The ethidium bromide fluoresces reddish-orange in the presence of DNA, since it has intercalated with the DNA. The DNA band can also be cut out of the gel, and can then be dissolved to retrieve the purified DNA.

Notes

LAB.6

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE) Analysis of Proteins

Materials & Equipment:

- Reagents:

  Acrylamide, N,N' Methylene-bis-acrylamide, Sodium dodecyl sulphate (SDS), Ammonium persulphate, Tetramethylethylenediamine (TEMED), Tris(hydroxymethyl)aminomethane (Tris), Hydrochloric acid (HCI), Glycerol, 2-Mercapthoethanol, Bromophenol Blue, Deionised water.
- Equipment:

  Latex gloves, Weighing balance, Graduated cylinder (200ml), Light-proof container (200ml), Fume cupboard, Electrophoresis Apparatus, Power Pack, Pipettes, Automatic Pipette, Aid Hamilton syringe, Eppendorf Tubes, Piercing Implement., Boiling water bath, Magnetic stirring bar and stirrer, Universal containers (25ml), Graduated cylinders, Beakers.
- Reagent Preparation:
  A. 1.5M Tris-HCI Buffer, pH 8.8 (100 ml) A.1 Dissolve 18.15g of Tris in 75ml deionised water using a magnetic stirrer. Adjust pH to 8.8 using 5M HCI and make up the total volume to 100ml using deionized water.
  B. 0.5M Tris-HCI Buffer, pH 6.8 (100ml) ,then Dissolve 6.05g of Tris in 75ml deionised water using a magnetic stirrer. Finally, Adjust to pH 6.8 using 5M HCI and make up the total volume to 100ml using deionised water.

C. 10% (w/v) Sodium Dodecyl Sulphate (10ml) C.1 Dissolve 1g of SDS in 8ml deionised water and make up to 10ml with deionised water. Store at room temperature for up to 6 months. If precipitation occurs during low temperature storage, incubate the mixture at 37oC until SDS is resolubilised.

D. 10% (w/v) Ammonium Persulphate (1ml) D.1 Dissolve 0.1g Ammonium Persulphate in 1ml deionised water in an eppendorf tube.

E. 30% (w/v) Acrylamide/Bis (200ml).

1. Weigh out 58.4g acrylamide and 1.6g N,N'-methylene-bis-acrylamide. Caution! Acrylamide is extremely toxic. Wear gloves and face mask. After that,
2. the two above reagents into a beaker and add 200ml deionised water.
3. Dissolve using magnetic stirring bar and stirrer.
4. Store the acrylamide/bis solution in a light proof container at 2 - 8oC.

F. 0.5% (w/v) Bromophenol Blue Solution (20ml) (Dissolve 0.1g bromophenol blue in 20ml deionised water).

G. Solubilisation Buffer 5X

1. Weigh out 10g (8ml) of glycerol into universal.
2. Add 4ml of deionised water, 1.6ml of 10%(w/v) SDS and 1ml of 0.5M Tris-HCI buffer, pH 6.8.
3. Add 0.4ml 2-mercapthothanol to the above solution (inside a fume cupboard) and finally add 0.2ml of bromophenol blue solution.

H. Electrode (Running) Buffer 5X (5 litre)

1. Add 75g Tris, 360g glycine and 25g SDS to 4000ml deionised water in a 5 litre graduated cylinder and dissolve using a magnetic stirrer. Once dissolved make up to 5 litres with deionised water. This is a 5X buffer concentrate.
2. Before electrophoresis, the running buffer is prepared by diluting 200ml of the above concentrate with 800ml deionised water.

I. Electrode (Running) Buffer 5X (1 litre)

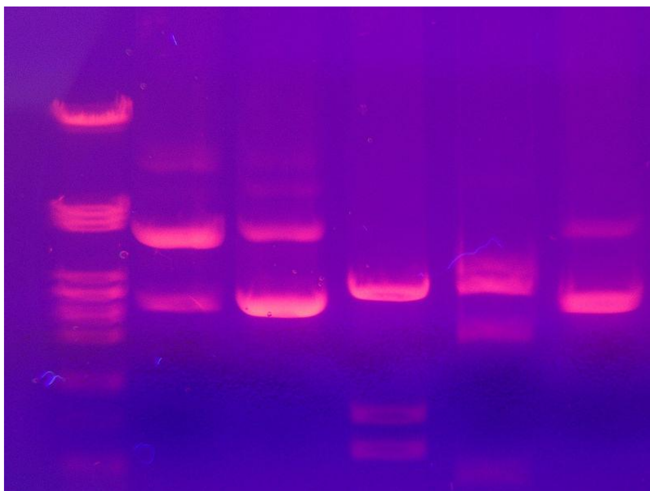
Note: 15g Tris, 72g glycine and 5g SDS to 1000ml deionised water in a 1 litre graduated cylinder and dissolve using a magnetic stirrer. Once dissolved make up to 1 litres with deionised water. This is a 5X buffer concentrate. Store at room temperature. Stable for up to 6 months. Before electrophoresis, the running buffer is prepared by diluting 200ml of the above concentrate with 800ml deionised water.

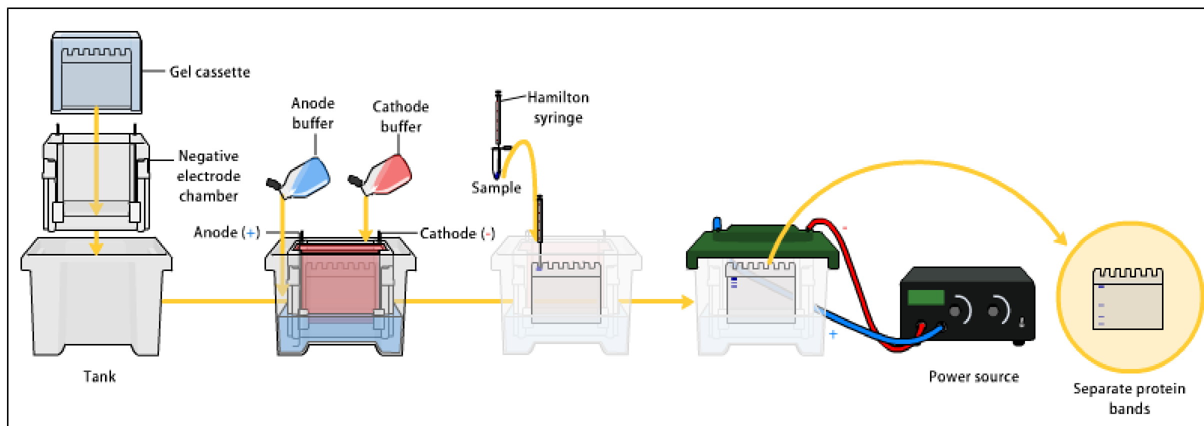
<u>Summary of SDS- PAGE procedure:</u>

1. **Sample preparation: Samples may be any material containing proteins**
2. **Mixing with SDS: The sample is mixed with SDS, anionic detergent.** To denatures secondary and non–disulfide–linked tertiary structures. To apply a negative charge to each protein.
3. **Heating: The samples are heated at 60°C.** To promote protein denaturation, helping SDS to bind.
4. **Addition of tracking dye:** A tracking dye may be added to the protein solution. As it has a higher electrophoretic mobility which allow the experimenter to track the progress of the protein solution through the gel.
5. **Preparing acrylamide gels:** Gels are usually polymerized between two glass plates in a gel caster, with a comb inserted at the top to create the

sample wells. After the gel is polymerized the comb can be removed and the gel is ready for electrophoresis

6. **Electrophoresis:** Various buffer systems are used in SDS-PAGE depending on the nature of the sample and the experimental objective. An electric field is applied across the gel, causing the negatively-charged proteins to migrate across the gel towards the positive (+) electrode (anode)





Picture show techniques' of separate the DNA in gel electrophoresis.

Notes

LAB.7

The polymerase chain reaction (PCR)

- The **polymerase chain reaction** (**PCR**) is a scientific technique in, molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
- Developed in 1983 by Kary Mullis

PCR Principles and Procedure

PCR is used to amplify a specific region of a DNA strand (the DNA target).

- A basic PCR set up requires several components and reagents. These components include:
- DNA template that contains the DNA region (target) to be amplified.
- Two primers that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strand of the DNA target.
- Taq polymerase or another DNA polymerase with a temperature optimum at around 70 °C.

Procedure

- PCR consists of a series of 20-40 repeated temperature cycles, with each cycle consisting of 2-3 discrete temperature steps.
- The cycling is often preceded by a single temperature step (called hold) at a high temperature (>90°C),

- The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters.

***Initialization step*:**

Temperature 94–96 °C,

Time required 1–9 minutes.

It is only required for DNA polymerases that require heat activation by hot-start PCR.

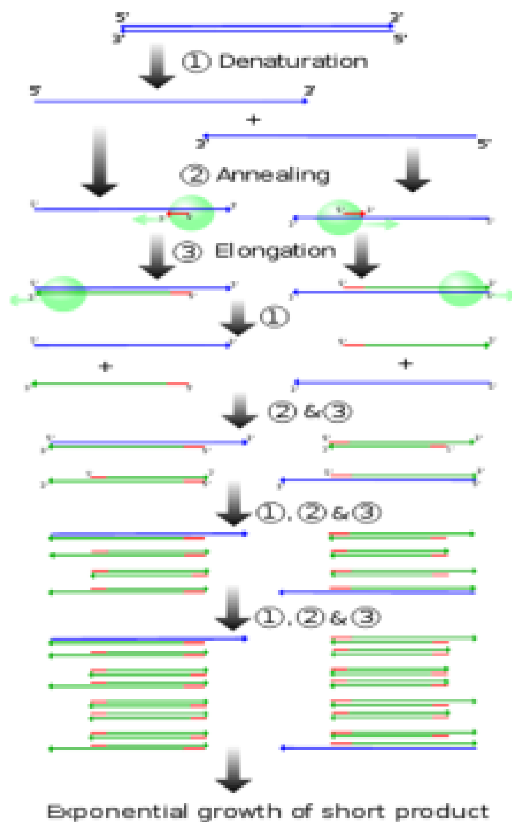
***Denaturation step*:**

First regular cycling event

Temperature 94–98 °C

Time required 20–30 seconds.

It causes melting of the DNA template by disrupting the hydrogen bonds, yielding single-stranded DNA molecules.



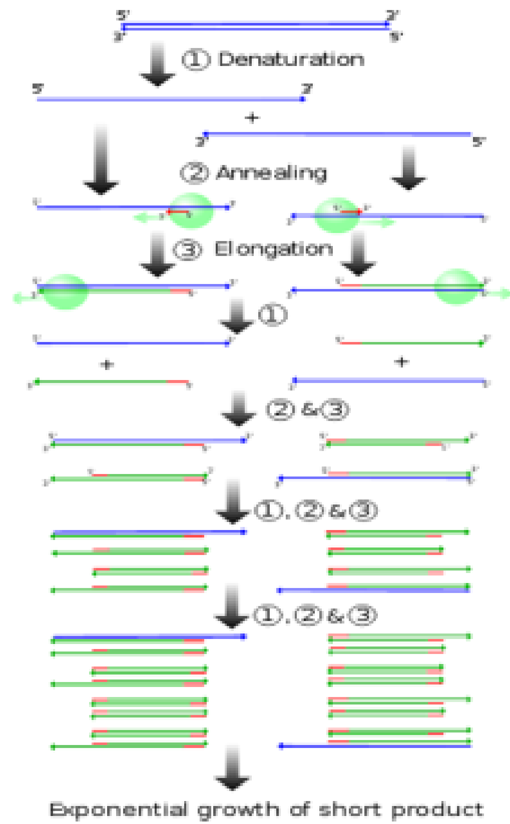
Annealing step:

Temperature 50–65 °C

Time required 20–40 seconds

allowing annealing/binding of the primers to the single-stranded DNA template.

The polymerase binds to the primer-template hybrid and begins DNA synthesis.



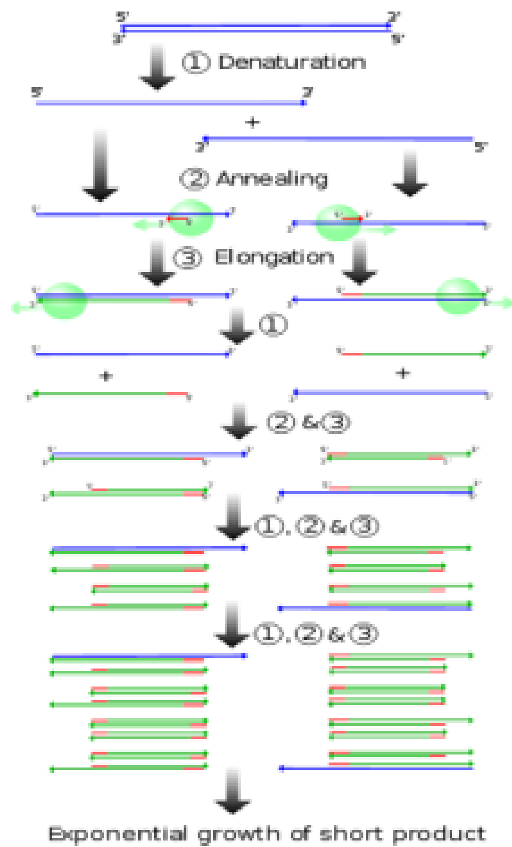
- –

***Extension/elongation step*:**

Temperature 72 °C (depending on the DNA polymerase used)

Taq polymerase has its optimum activity temperature at 75–80 °C,

DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs



- **The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified**
- **Under optimum conditions a thousand bases per minute can be amplified, if there are no limitations due to limiting substrates or reagents.**

Notes