King Abdulaziz University
Faculty of Science
Department of Biochemistry
Girls Section

Molecular Biochemistry Lab
BIOC 432

Organized by a collaborative work with lecturers:
Omima Niazy, Nouf Alshereef, Shereen bakhashab, and
Wedam Alghazzawi

Lab Schedule

Week #	Topic
1	Instruments and equipment Introduction to nucleic acids
2	Chemical and physical properties of nucleic acids
3	Isolation of RNA from yeast
4	Estimation of RNA by orcinol
5	The Folin method of protein assay
6	Isolation of DNA from blood
7	Isolation of DNA from spleen
8	Estimation of DNA by diphenylamine
9	Polymersae Chain Reaction
10	Restriction endonucleases and ligases
11	Agarose gel electrophoresis
12	Bioinformatics and the internet

Instrumnets

1. Thermocycler Machine

2. Centrifuge machine

3. Vortex Mixer

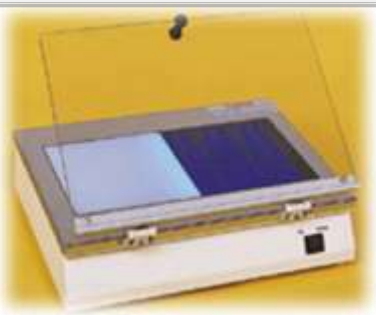
4. Spectrophotometer

5. The GeneQuant RNA/DNA calculator

6. Autoclave Machine

7. HE 33 Mini horizontal submarine

8. Ultra-Violet (UV) light machine



Equipment

1. Microcentrifuge tubes (1.5 ml)

3. Sterile Tips (20-200 ul)

2. Micropipettes

4. PCR tubes (0.2 ml & 0.5 ml)

5. Pathlength cell

Introduction to nucleic acids: structural properties

Molecularar Biochemistry

DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) are polymers of nucleotides linked in a chain through phosphodiester bonds. In biological systems, they serve as information-carrying molecules. As DNA and RNA are the major molecules of molecular biology, understanding their structure is critical to understand the mechanisms of gene replication and protein synthesis.

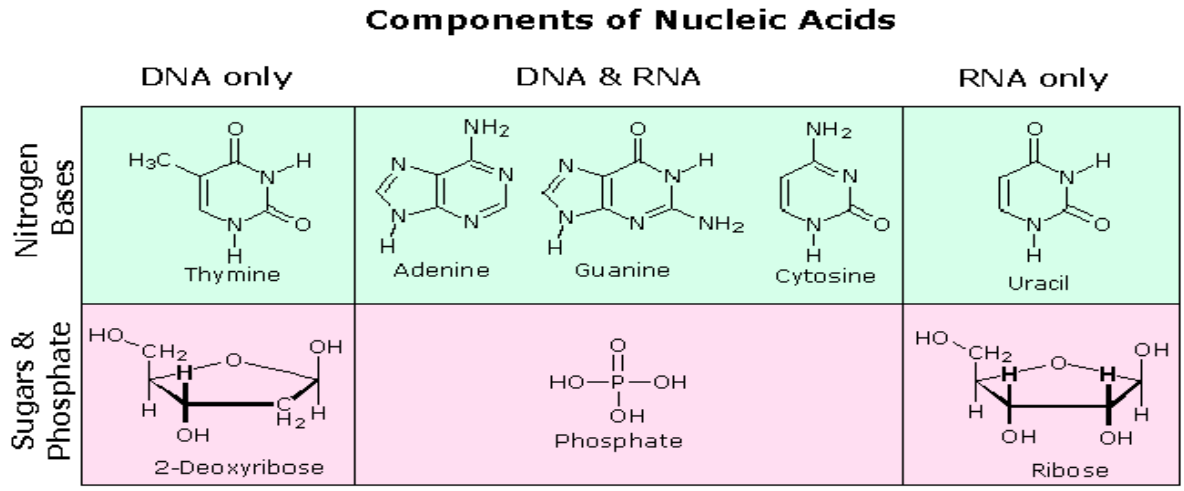
Structure of nucleic acids:

Nucleotides are the building blocks of all nucleic acids. Nucleotides have a distinctive structure composed of three components covalently bound together:

- **Nitrogen-containing "base"** - either a pyrimidine (one ring) or purine (two rings)
- **Five-carbon sugar** - ribose or deoxyribose
- **Phosphate group**

The combination of a base and sugar is called a *nucleoside*. Nucleotides also exist in activated forms containing two or three phosphates, called nucleotide diphosphates or triphosphates. If the sugar in a nucleotide is deoxyribose, the nucleotide is called a deoxynucleotide; if the sugar is ribose, the term ribonucleotide is used.

The bases of DNA and RNA are heterocyclic (carbon and nitrogen-containing) aromatic rings. Adenine (A) and guanine (G) are purines, bicyclic structures (two rings), whereas cytosine (C), thymine (T) and uracil (U) are monocyclic pyrimidines. In RNA, the thymine base is replaced by uracil.



In nucleic acids, the bases are covalently attached to the 1'- position of a pentose sugar ring, to form a nucleoside. In RNA, the sugar is ribose and in DNA is 2' - deoxyribose, in which the hydroxyl group at the 2'- position is replaced by hydrogen. The bond between the bases and the sugars is the glycosidic bond.

A nucleotide is a nucleoside with one or more phosphate groups bond covalently to the 3'-, 5'- in deoxyribonucleotides only or the 2'- position. Nucleoside 5'- triphosphate (NTPs) or deoxynucleosides 5'- triphosphates (dNTPs) are the building blocks of the polymeric nucleic acids.

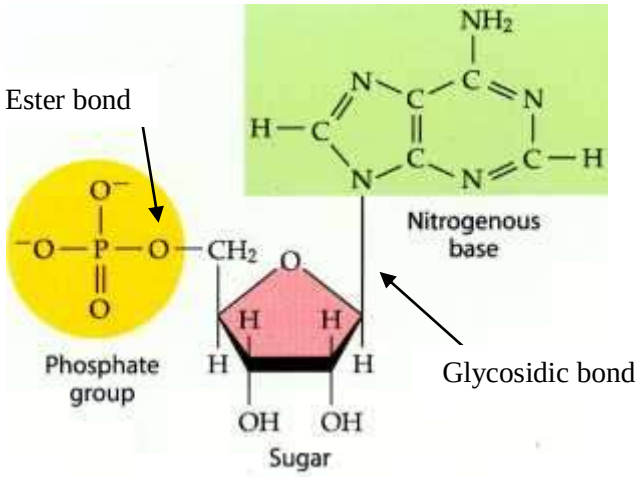


Figure 1: Structure of nucleotide

DNA double helix: (B-DNA)

- DNA most commonly occurs in nature as double helix.
- Two separate chains of DNA are wound around each other, each following a helical path, resulting in a right-handed double helix.
- The negatively charged sugar-phosphate backbones of the molecules are on the outside, and the bases in the center of the helix.
- The strands are joined by hydrogen bonds between the bases on opposite strands to form base pairs.
- There are around 10 base pairs per turn in the DNA double helix. The two strands are oriented in opposite directions (anti-parallel) in terms of their 5'→3' direction and the two strands are

complementary in terms of sequence. This feature arises because the nitrogenous bases hydrogen bonds to each other as purine-pyrimidine pairs. Guanine pairs with cytosine (three H-bonds) and adenine pairs with thymine (two H-bonds) (law of complementary base pairing).

- The sequence of one strand specifies the sequence of the other.

Location and function of DNA:

Most DNA is located in the cell nucleus where it is called **nuclear DNA**, but a small amount of DNA can also be found in the mitochondria where it is called **mitochondrial DNA** or mtDNA. DNA serves as code for protein synthesis, cell replication and reproduction.

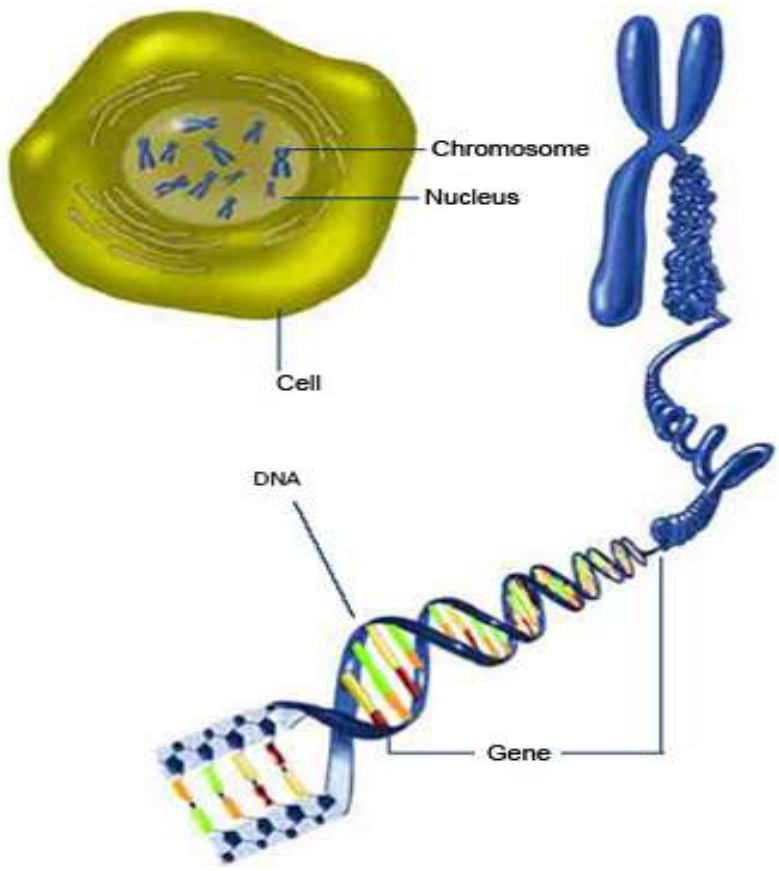


Figure 2: The long DNA that makes up genes is spooled within chromosomes inside the nucleus of a cell.

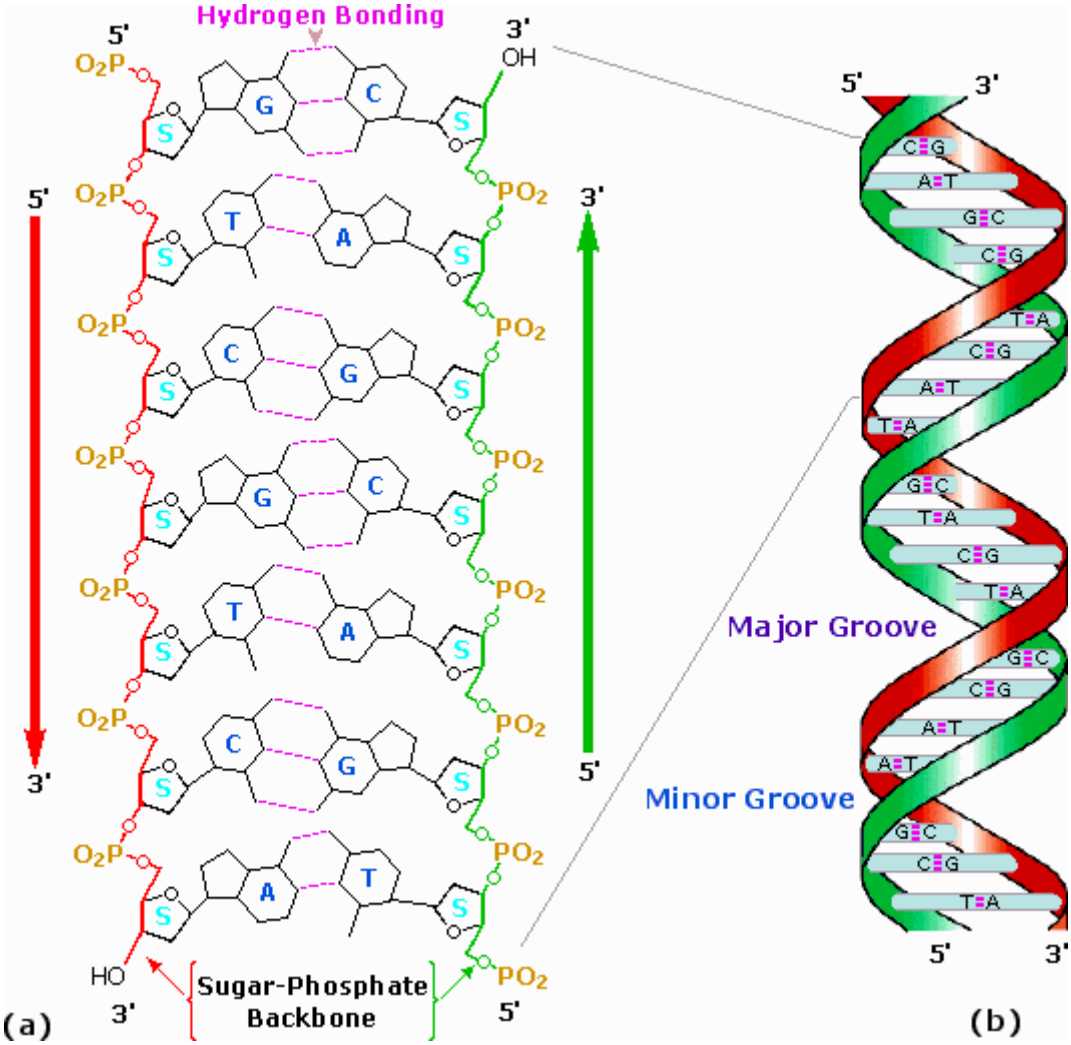
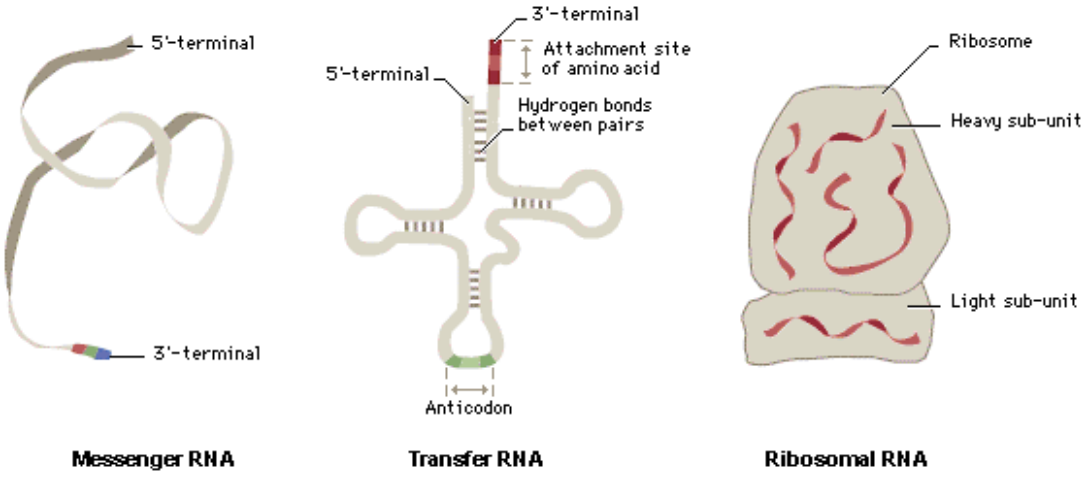


Figure 3: DNA consists of two long, twisted chains made up of nucleotides. Each nucleotide contains one base, one phosphate molecule, and the sugar molecule deoxyribose. The bases in DNA nucleotides are adenine, thymine, cytosine, and guanine.

The structure and function of RNA:

- RNA normally occurs as a single-stranded molecule.
- RNA forms relatively globular conformations, in which local regions of helical structure are formed by intramolecular hydrogen bonding and base stacking within the single nucleic acid chain. These regions can form where one part of the RNA chain is complementary to another (RNA secondary structure).
- Essential function is to interpret DNA code and direct protein synthesis.

- **There are four types of RNA:**
  1) Transfer RNA (tRNA): carries amino acids in the cytoplasm to the ribosomes.
  2) Messenger RNA (mRNA): re-writes DNA and takes it out of the nucleus to the ribosome.
  3) Ribosomal RNA (rRNA): building blocks of ribosomes. Eukaryotic ribosomes contain four different rRNA molecules: 18 s, 5.8 s, 28 s, and 5 s rRNA. Three of the rRNA molecules are synthesized in the nucleolus and one is synthesized elsewhere. rRNA molecules are extremely abundant, they make up at least 80% of the RNA molecules found in a typical eukaryotic cell.
  4) Small nuclear RNA (snRNA): refer to a number of small RNA molecules found in the nucleus. These RNA molecules are important in number of processes including the maintenance of the telomeres or chromosome ends.



Chemical and physical properties of nucleic acids

Molecular Biochemistry

Background:

DNA is generally stable than RNA, but there are many chemical and physical factors affect the nucleic acids. The chemical factors that affect nucleic acids are such as hydrolysis by acids, alkali, enzymes, and mutagenic factors of the DNA bases. The physical factors are heat, pH, salt concentration, and base composition.

The ultraviolet absorption of nucleic acid:

Nucleic acids absorb in the ultraviolet region of the spectrum due to the conjugated double bond and ring systems of the constituent purines and pyrimidines. The maximum absorbance is at the wavelength 260 nm and minimum at 230 nm.

DNA hyperchromic & hypochromic effect:

The absorption of single strand DNA (ssDNA) is higher than the absorbance of double strand DNA (dsDNA) this is known as a **hyperchromic effect** (means: "more color"). The hydrogen bonds between the paired bases in the double helix limits the resonance behavior of the aromatic ring of the bases which results in decrease in the UV absorbance of dsDNA (**hypochromic effect**), while in ssDNA the bases are in free form and don't form hydrogen bonds with complementary bases which results in 40% higher absorbance in ssDNA (hyperchromic) at the same concentration.

The stability of DNA structure:

The stability of DNA structure depends on the integrity of two type bonds: phoshodiester bonds (which link between the sugar and phosphate groups in the DNA backbone), this bond is very strong and can't be broken by conventional methods, it can be broken by specific nucleases enzyme and hydrogen bond (which links between the complementary bases of the two polynucleotide strands) are relatively weak and can be disrupted by different factors such as heat.

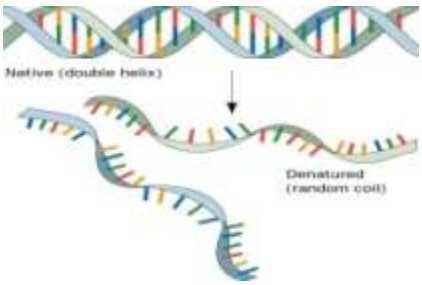


Figure 1: DNA denaturation (breaking hydrogen bonds) results in two separate strands.

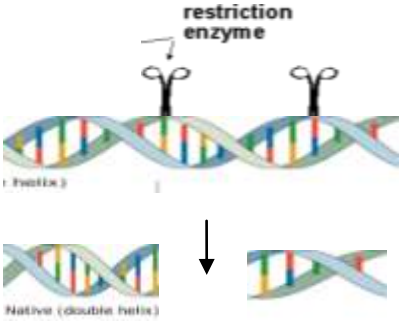
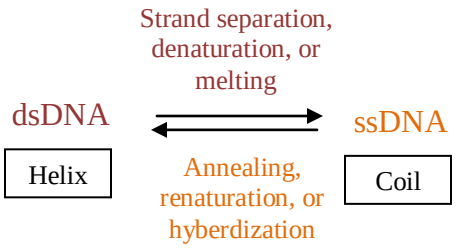


Figure 2: DNA digestion (breaking phoshodiester bonds) results in DNA fragments.

DNA denaturation & renaturation:

DNA denaturation, or DNA melting, is the process by which double-stranded DNA unwinds and separates into single-stranded strands through the breaking of hydrogen bonds between the bases. Complementary DNA reform is called annealing or renaturation. Disruption occurs in lab by different methods such as: heating to high degree, change salt conc., adding alkali or change pH.



DNA denaturation by heating:

When DNA is heated, the temperature at which half of helix structure is lost is known as **melting temperature (T_m)**. The melting temperature depends on both the **length of the DNA**, and **the nucleotide sequence composition**, higher GC content higher Tm. This is because the triple hydrogen bonds between G and C need more energy to disrupt than the bouble bonds between A and T.

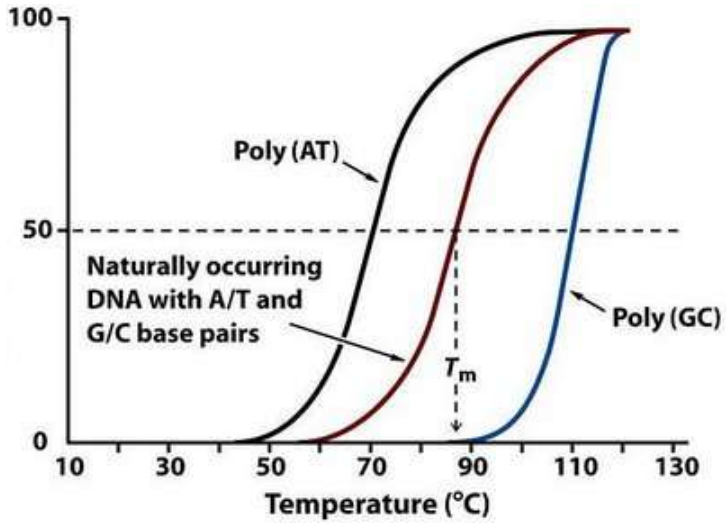


Figure 4: the effect of DNA structure on its UV absorption

Monitoring the DNA denaturation and recombination by UV absorbance:

When a solution of double-stranded DNA is slowly heated, the absorbance increases rapidly to a higher value, which is not significantly changed by further heating. If the hot DNA solution is then cooled slowly, the two threads recombine and the "cooling curve" should be superimposed on the "melting curve". If the DNA is cooled rapidly then some recombination of the two strands takes place in a random manner so that the extinction of the solution at room temperature is higher than the of the original DNA solution before heating.

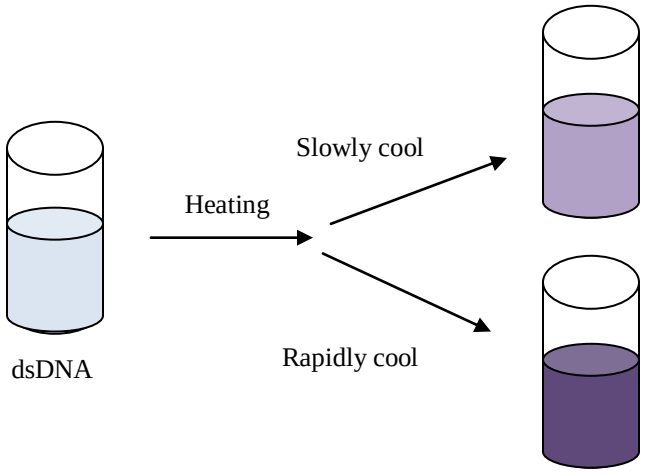


Figure 3: The effect of nucleotide composition on DNA melting temperature.

The effect of heating and cooling on ultraviolet absorption of the DNA

Instruments & Equipment:

- Ultraviolet spectrophotometer with thermostatically.
- Controlled cell housing.
- Water bath for use at temperatures up to 95°C.

Materials:

- Deoxyribonucleic acid solution
- Ice

Method:

1. Place the solutions (5 ml) in cuvette in a spectrophotometer and record the absorbance at room temperature. Use distilled water as blank in this experiment.
2. Transfer the DNA solution to test tube with thermometer and place in water bath, then record the change in UV absorption every 10°C elevation in temperature.

   Note: The temperature of the water bath is usually higher than the temperature of the test tube contents, so the temperature of the DNA solutions should be measured directly if possible.
3. Every time record the temperature and absorbance and continue until no change in absorbance is recorded (around 95°C.)
4. **Slowly cool** the DNA solution from 95°C until room temperature and measure the extension every 10°C decrease.
5. Repeat the experiment but this time **rapidly cools** the solution in ice and record the change in absorbance every 10°C decrease.

Result sheet

1. Record your results in table (Temp. & U.V. absorbance).
2. Each group must construct DNA melting curve and calculate Tm.
3. Construct DNA rapid cooling curve and other group construct DNA slow cooling curve by drawing a graph using the absorbance readings against the temperature for each experiment.
4. Compare the cooling and melting curves for the two experiments and comment on the effect of temperature.

For each group

DNA melting temp.	Absorbance
25°C	
35°C	
45°C	
55°C	
65°C	
75°C	
85°C	
95°C	

Groups are dividing for taking one of these experiments

Temp. of rapid cooling on ice	Absorbance
95°C	
25°C	

Temp. of slow cooling	Absorbance
95°C	
85°C	
75°C	
65°C	
55°C	
45°C	
35°C	
25°C	

Tm is calculated from curve =_____.

Isolation RNA from yeast

Molecular Biochemistry

Principle:

Yeast is eukaryotic microorganisms that belong to the kingdom of fungi; there are 100,000 species or more. This experiment Saccharomyces cerevisiae (Baker's yeast) is used in baking. It is one of the most studied eukaryotic model. It reproduces by division process known as budding. Many proteins in human biology were first discovered by studying their homologs in yeast. S. cerevisiae was the first eukaryotic genome to be completely sequenced. The genome composed of 13,000,000 base pairs, 6275 genes only 5,800 genes are functional. It was estimated that yeast shares 23% of its genome with humans.

Total yeast RNA is obtained by extracting a whole cell homogenate with phenol. The concentrated solution of phenol disrupts hydrogen bonding in the macromolecules, causing denaturation of the protein. The turbid suspension is centrifuged and two phases appear: the lower phenol phase contains DNA, and the upper aqueous phase contains carbohydrate and RNA. Denatured protein, which is present in both phases, is removed by centrifugation. The RNA is then precipitated with alcohol. The product obtained is free of DNA but usually contaminated with polysaccharide. Further purification can be made by treating the preparation with amylase.

Materials:

- Dried yeast (200 g)
- Phenol solution (900 g/liter)
- Potassium acetate (200 g/litre, pH = 5)
- Absolute ethanol
- Diethyl ether (500 ml)

Equipment:

- Test tubes
- Water bath adjusted at 37°C

Procedure:

1. Suspend 2.5 g of dried yeast in 15 ml of water previously heated to 37°C. Leave for 15 min at this temperature and add 12.5 ml of concentrated phenol solution (Care: corrosive).
2. Stir the suspension mechanically for 30 min at room temperature, then centrifuge at 5000 rpm for 15 min in the cold to break the emulsion.
3. Carefully remove the upper aqueous layer with a Pasteur pipette and centrifuge at 5000 rpm for 7 min in a refrigerated centrifuge to sediment denatured protein.
4. Add potassium acetate to the supernatant to a final concentration of 20 g/litre (Note: every 1 ml of supernatant adds 9 ml of potassium acetate) and precipitate the RNA by adding 2 volumes of ethanol.
5. Cool the solution in ice and leave to stand for 1 h.
6. Collect the precipitate by centrifuging at 5000 rpm for 7 min in the cold.
7. Wash the RNA with ethanol-water (3:1) depend on the amount of precipitate.
8. Filter the solution and then add 3 ml of ethanol to the filter paper.
9. Finally, wash with 3 ml ether; air dry, and weight. (Note: Yeast contains about 4 per cent RNA by dry weight).
10. Dissolve RNA powder in 10 ml, 1% NaOH.
11. Compare your product with a commercial preparation by measuring the pentose, phosphorus, and DNA content and determining the absorption spectrum. Keep your preparation for use in later experiments.

Result sheet

Hint: Yeast contains about 4 percent RNA by dry weight

1. Calculate the weight of RNA?

_____

_____

_____

_____

2. What is the yield of RNA?

_____

_____

_____

_____

Estimation of RNA by orcinol

Molecular Biochemistry

Principle:

This is a general reaction for pentoses. The orcinol reagent reacts with pentose groups in the backbone of the RNA molecule and depends on the formation of furfural, when the pentose is heated with concentrated hydrochloric acid. Orcinol reacts with the furfural in the presence of ferric chloride act as a catalyst to give a green colour. Only the purine nucleotides give any significant reaction.

Materials:

- Orcinol reagent (6% orcinol reagent in 100 ml ethanol).
- Ferric chloride + hydrochloric acid solution (0.5 ml of 10% ferric chloride solution was added to 99.5 ml of conc. HC1.
- Std. RNA 250 µl/ml.
- All the reagents are made fresh
- Spectrophotometer
- Pipettes (1 ml & 5 ml).
- 12 x 10 ml test tubes and parafilm
- Water bath

Method:

1. Add the following amounts in four test tubes:

Reagents	Unk. 1	Unk. 2	Standard	Blank
RNA (250 µg/ml)	1 ml	1 ml	1 ml	---
Orcinol	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Ferric chloride+HCl	3 ml	3 ml	3 ml	3 ml
Total	4.2 ml	4.2 ml	4.2 ml	3.2 ml

2. Place the tubes in 100°C water bath for 30 min.
3. Cool the tubes and read at 660 nm.
4. Calculate the concentration of unknown in µg/ml and µg% by using Beer's law.

The Folin method of protein assay

Molecular Biochemistry

Principle:

Protein reacts with the Folin-Ciocalteau reagent to give a coloured complex. The colour so formed is due to the reaction of the alkaline copper with the protein as in the biuret test and the reduction of phosphomolybdate by tyrosine and tryptophan present in protein. The intensity of colour depends on the amount of these aromatic amino acids present and will thus vary for different proteins.

Materials:

- Reagent mix:

  A) alkaline sodium carbonate solution (20 g /L Na_2CO_3 in 0.l mol/l NaOH).

  B) Copper sulphate-Sodium potassium tartrate solution (5g/L $CuSO_4$. 5 H_2O in 10 g /L Na,K tartrate). Prepare fresh by mixing stock solutions. Mix 50 ml of (a) with 1 ml of (b) on day of use.
- Bovine serum albumin (standard protein solution (400 ng /ml).
- Folin-ciecalteau reagent: Sodium molybdate in phosphoric and hydrochloric acid, dilute with equal volume of water on the day of use.

Method:

1. Add the following amounts in four test tubes:

Reagents	Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Unk.	Blank
Protein (250 µg/ml)	0.1	0.2	0.3	0.4	0.5	0.3	
H_2O	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1.5
Reagent mixture (A & B)	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
Allow standing for 10 minutes							
diluted folin and ciocalteau reagent	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Note: Mixing immediately (this is very important) to each tube.							

2. Allow standing for at least 30 minutes and read at 750 nm.
3. Plot the OD at 750 nm versus the concentration of protein.
4. Calculate the concentration of the (Unknown) by (microgram /ml).

Isolation DNA from spleen

Molecular Biochemistry

Principle:

Although almost all cells contain DNA, the amount present in some tissues is quite small so that they are not a particularly convenient source. In addition, some tissues contain high deoxyribonuclease activity so that the DNA is broken down into smaller fragments. A convenient source for the isolation of DNA should therefore contain a high quantity of the material and have low deoxyribonuclease activity.

Lymphoid tissue is very good in these respects and thymus is probably the best source, with spleen as a good alternative. The tissue is homogenized **in isotonic saline buffered with sodium citrate** pH 7.4. At this ionic strength, the deoxyribonucleoprotein is insoluble and separates well from other proteins. **Sodium citrate** inhibits deoxyribonuclease activity by binding Ca^{++} and Mg^{++}, which are cofactors for this enzyme. The extraction procedure is carried out in the cold so that any residual DNA'ase activity is minimal. Glass or plastic vessels are used throughout to avoid degradation of the DNA.

The DNA is finally precipitated as a fibrous white mass by the addition of **ethanol**. After washing with ethanol, the DNA is dissolved in saline buffered with **sodium citrate** to pH 7.4.
The material is best stored frozen and does not undergo any demonstrable change for several months but drying of the DNA tends to lead to denaturation.

Materials:

- Spleen.
- Buffered saline ssc (0.15 mol/l Nail buffered with 0.015 mol/l Sodium citrate, pH 7).
- Sodium Chloride (2M)
- Ethanol and ether

Method:

1. Chop 5 g of calf spleen into small fragments and homogenize with 20 ml of buffered saline for 1 min.
2. Centrifuge the suspension at 5000 g for 15 min
3. Rehomogenize the precipitate in a further 40 ml of buffered saline.

4. Discard the supernatant and suspend the combined sediments uniformly in 2 mol/litre NaCl to a final volume of 100 ml when most of the material should dissolve.
5. Remove any sediment by centrifugation and stir the solution continuously with a stirring glass rod while adding an equal volume of ice-cold water.
6. Spool the fibrous precipitate on to a glass rod and leave it to stand in a beaker for 30 min. During this time the clot will shrink and the liquid expressed should be removed with filter paper.
7. Dissolve the deoxyribonucleoprotein in about 100 ml of 2 mol/litre NaCl.
8. Add an equal volume of the chloroform/amyl alcohol mixture (6:1), and blend for 30s.
9. Centrifuge the emulsion at 5000 g for 10-15 min and collect the upper (opalescent) aqueous layer containing the DNA. This is best carried out by gentle suction into a suitable container so that the denatured protein at the interface of the two liquids is not disturbed.
10. Repeat the treatment with organic solvent twice more and collect the supernatant in a 500 ml beaker.
11. Precipitate the DNA by slowly stirring 2 volumes of ice-cold ethanol with the supernatant and collect the mass of fibres on the glass stirring rod.
12. Carefully remove the rod and gently press the fibrous DNA against the side of the beaker to expel the solvent.
13. Finally, wash the precipitate by dipping the rod into a series of solvents and expelling the solvent as described. Four solvents are used: 70 per cent v/v ethanol, 80 per cent v/v ethanol, absolute ethanol and ether. Remove the last traces of ether by standing the DNA in a fume cupboard for about 10 min.
14. Weigh the dry DNA and dissolve by continuously stirring in buffered saline diluted one in ten with distilled water (2 mg/ml); store frozen until required.

.

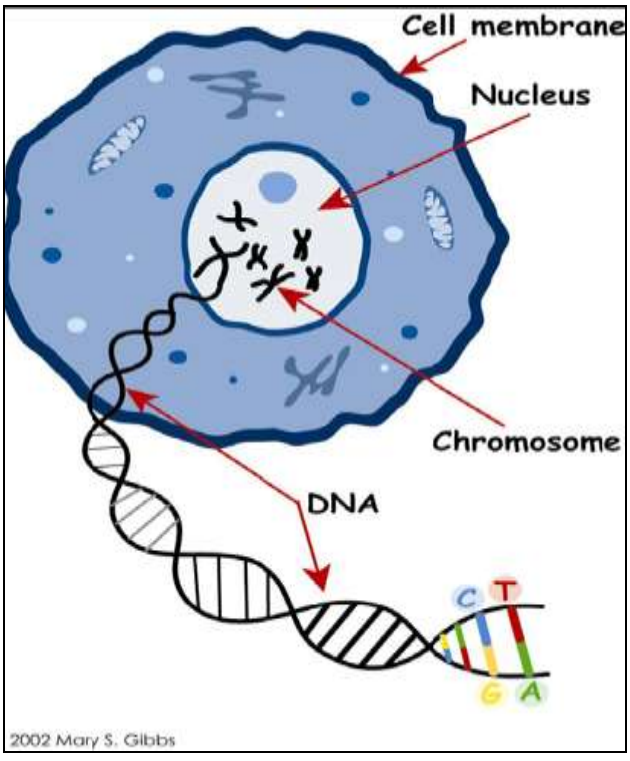
Isolation of DNA from blood

Molecular Biochemistry

Introduction:

All living things are made of cells. The three main parts of the cell are the ***nucleus***, which holds DNA, the ***cell membrane***, which surrounds and protects the cell, and the ***cytoplasm***, which is the jelly-like part of the cell between the membrane and the nucleus. All of the smaller organelles, such as mitochondria, are found in the cytoplasm (Figure 1).

Deoxyribonucleic acid or DNA is the molecule that controls everything that happens in the cell. DNA contains the genetic code or commands that direct the activities of cells and ultimately, the body. DNA is present in all living things from bacteria to animals. In animals, it is found in almost all cell types, except red blood cells. DNA found within a nucleus in the cells. In order to extract DNA it's necessary to denature and break down the cells to release DNA from these cells. Protein enzyme or protease can be used to break own proteins. Once DNA fragments are released into solution, the DNA can be spooled together by using ice-cold alcohol. The alcohol allows DNA fragments to stick together.



Extraction of DNA basically consists of four major steps:

1. Preparation of a cell extract
2. Purification of DNA from cell extract
3. Concentration of DNA samples
4. Measurement of purity and DNA concentration

Preparation of a cell extract

To extract DNA from tissue/cells of interest, in this experiment the blood, the cells have to be separated and the cell membranes have to be disrupted.

Reagents

Chemicals such as EDTA (Ethylenediaminetetraacetate) which removes Mg^{+2} ions that is essential for preserving the overall structure of the cell membrane and SDS (Sodiumdodecylsulfate), which aids in disrupting the cell membranes by removing the lipids of the cell membranes, are included in the extraction buffer which lysing the cells, the final step in the preparation of a cell extract is removal of insoluble cell debris .Cell debris and partially digested organelles etc. can be pelleted by centrifugation leaving the cell extract as a reasonably clear supernatant.

Purification of DNA from cell extract

In addition to DNA the cell extract will contain significant quantities of protein and RNA. A variety of procedures can be used to remove these contaminants, leaving the DNA in a pure form.

Reagents

Phenol:

The standard way to de-proteinize a cell extract is to add phenol or a ١:١ mixture of phenol: chloroform. These organic solvents precipitate proteins but leave the nucleic acids in aqueous solutions .The aqueous solution of nucleic acid can be removed with a pipette.

Ribonuclease enzyme:

Ribonuclease enzyme remove RNA by degrade these molecules into ribonucleotide subunits.

Concentration of DNA samples

The most frequently used method of concentration is ethanol precipitation. In the presence of salt and at a temperature of° ٢٠- C or less, absolute ethanol will efficiently precipitate polymeric nucleic acids .With a concentrated solution of DNA one can use a glass rod to pull out the adhering DNA strands while for dilute solutions precipitated DNA can be collected by centrifugation and redissolving in an appropriate volume of water.

Measurement of purity and DNA concentration

DNA concentrations can be accurately measured by UV absorbance spectrometer at 260 nm. The amount of UV radiation absorbed by a solution of DNA is directly proportional to the amount of DNA sample UV absorbance can also be used to check the purity of a DNA preparation .With a pure sample of DNA the ratio of the absorbencies at 260 nm and 280 nm (A_{260}/A_{280}) (is ratio of less than 1,8 indicate that the preparation is contaminated, either with protein or with phenol.

DNA preparation from blood 

Instruments & Equipment:

- Cold centrifuge
- Freeze (-80°)
- Water bath
- EDTA tube
- Test tube
- Micropipettes (20-200 ul) & Tips
- Centrifuge tubes
- Gloves

Materials:

- Human blood (5 ml)
- Proteinase K
- Ammonium chloride (NH_4Cl)
- Chloroform
- Ethanol, absolute
- HCl
- Isoamyl alcohol
- Isopropanol (2-Propanol)
- Phenol
- Potassium carbonate ($KHCO_3$)
- Sodium acetate
- Sodium chloride
- Sodium EDTA (Na_2EDTA)
- Sodium dodecyl sulfate (SDS 10%)
- Tris EDTA buffer, pH 8.0

Preparation:

Lysis Buffer

NH_4Cl 8.29 g f.c. [155 mM]

$KHCO_3$ 1 g f.c. [10 mM]

Na_2EDTA 0.034 g or 200 µl EDTA 0.5 M f.c. [0.1mM]

Fill to 1000 ml with distilled water

Adjust to pH 7.4 with 1 M HCl or NaOH for each use

Chloroform/Isoamyl alcohol 24:1

Chloroform 24 ml

Isoamylalcohol 1 ml

SE (Saline EDTA) Buffer

NaCl 4.39 g f.c. [75 mM]

Na_2EDTA 8.41 g or 50 ml EDTA 0.5 M f.c. [25 mM]

Fill to 1000 ml with distilled water

Adjust to pH 8.0 with 1 M NaOH for each use

Sodium acetate

3 M Sodium acetate 246 g/L

Adjust to pH 5.2 with CH3COOH

Proteinase K (10mg/ml)

Dissolve 100 mg Proteinase K in 10 ml TE for 30 min at room temperature

Aliquot and store at –20°C.

Procedure:

1. To 5 ml whole blood (EDTA, heparin, citrate) add 15 ml lysis buffer, shake gently, incubate for 30 min on ice, and centrifuge at 1200 rpm for 10 min at 4^o C.

2. Remove supernatant (blood waste), add 5 ml lysis buffer, resuspend the pellet, and centrifuge for 10 min at 4°C (1200 rpm).
3. Remove supernatant (blood waste), add 2.5 ml SE-buffer, resuspend the pellet, and centrifuge for 10 min at 4°C (1200 rpm).
4. Remove supernatant (blood waste).

   (It is possible to store the pellet at -80°C. To do so, add 1 ml SE-buffer and resuspend the pellet. Use a cryo-tube and centrifuge at 1200 rpm for 10 min at 4°C. Remove the supernatant and freeze the pellet.)
5. Add 2.5 ml SE-buffer and resuspend the pellet, add 20 µl proteinase K (10mg/ml) and 125 µl 20% SDS shake gently, and incubate overnight at 37°C in a water bath.
6. Add 2.5 ml SE-buffer and 5 ml phenol shake by hand for 10 min, and centrifuge at 3000 rpm for 5 min at 10°C.
7. Transfer the supernatant into a new tube, add 10 ml phenol/chloroform/isoamyl alcohol (25:24:1), shake by hand for 10 min, and centrifuge at 3000 rpm for 5 min at 10°C.
8. Again transfer the supernatant into a new tube, add 5 ml chloroform/isoamylalcohol (24:1), shake by hand for 10 min, and centrifuge at 3000 rpm for 5 min at 10°C.
9. Transfer the supernatant into a new tube, add 150 µl 3 M sodium acetate (pH 5.2) and 5 ml isopropanol, shake gently until the DNA precipitated, use a glass pipette, make a hook over a bunsen burner, and capture the DNA.
10. Wash the DNA in 70% ethanol and dissolve the DNA in 0.5-1 ml TE-buffer or in ddH_2O overnight at 4°C on a rotating shaker. (If the DNA is not dissolved leave it longer at 4°C on the rotating shaker).
11. Measure the DNA concentration in a spectrophotometer (Pharmacia, GeneQuant) and run 200 ng on a 1% agarose gel.

Estimation of DNA by diphenylamine

Molecular Biochemistry

Principle:

The DNA is treated with diphenylamine under acidic condition; a blue compound is formed with a sharp absorption maximum at 595 nm. This reaction is given by 2-deoxypentoses in general and not specific for DNA. In acid solution, the straight chain form of the deoxypentose is converted to highly reactive hydroxy levuinaldehyde which reacts with diphenylamine (D.P.A.) to give a blue complex. In DNA only the deoxyribose of the purine nucleotides reacts so that the value obtained represents half of the total deoxyribose present.

Materials:

- Calf thymus DNA standard 1000 ug/ml (2 ml) for every student.
- D.P.A. reagent (1g of pure diphenylamine + 100 ml glacial acetic acid + 2.5 ml conc. sulphuric acid). Note**:** D.P.A is a poisoning reagent.
- All the reagents are made fresh.
- Spectrophotometer
- Pipettes (1 ml & 5 ml).
- 5 x 10 ml test tubes.
- Water bath
- Parafilm

Procedure:

1. Add the following amounts in four test tubes:

Reagents	1	2	3	4	5
Standard DNA (1000 µg/ml)	0.2 ml	0.3 ml	0.4 ml	0.5 ml	---
Unknown	---	---	---	---	0.3 ml
H_2O	1.3 ml	1.2 ml	1.1 ml	1.0 ml	1.2 ml
D.P.A	2.5 ml	2.5 ml	2.5 ml	2.5 ml	2.5 ml

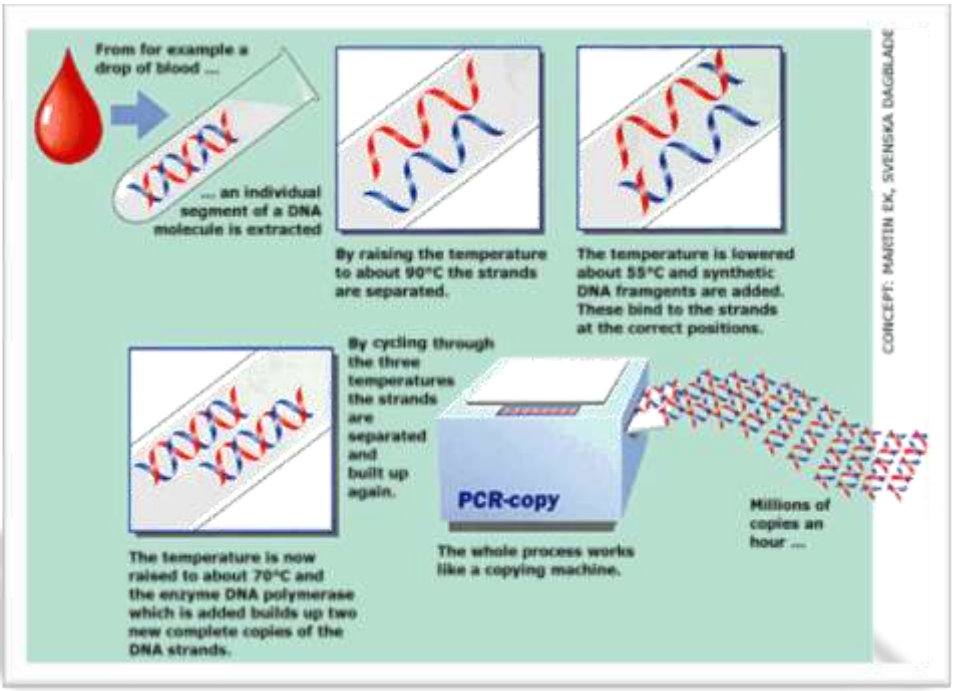
2. Place tubes in 100°C water bath for 10 min.
3. Cool the tubes and measure the Optical density (O.D.) at 595 nm.
4. Follow the below procedure in the next page for doing the experiment.
5. Drawing a standard curve using the absorbance readings against the conc. (µg/ml), then determine the conc. unknown of DNA, then the result multiple by 2.
6. Calculate conc. unknown (µg/ml and µg%).

Polymerase Chain Reaction

Molecular Biochemistry

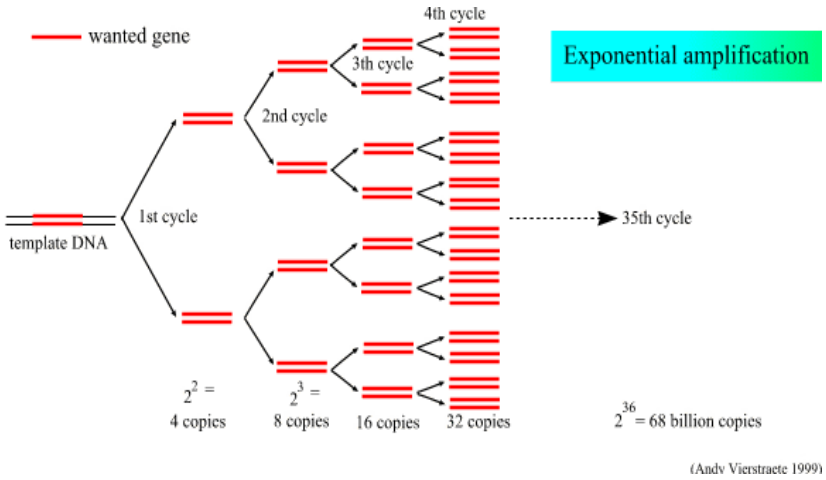
Background:

PCR (Polymerase Chain Reaction) is a molecular biological technique that is used to amplify specific fragment of DNA in vitro without using living organism such as bacteria, sometimes it is called "molecular photocopying or Xeroxing" since it allows the production of more than 10 million copies of a target DNA sequence from only a few molecules. The sensitivity of this technique depends on avoiding the contamination of the sample with any other DNA in the laboratory environment. The purpose of a PCR is to make a huge number of copies of a gene.



Principle:

The reaction depends on three incubation steps at different temperature denaturation, annealing and extension. At the end of the first cycle two copies of original DNA are produced, then the cycle is repeated and four copies are produced from the second cycle and so on. Usually the cycles are repeated for 30 to 40 cycles.



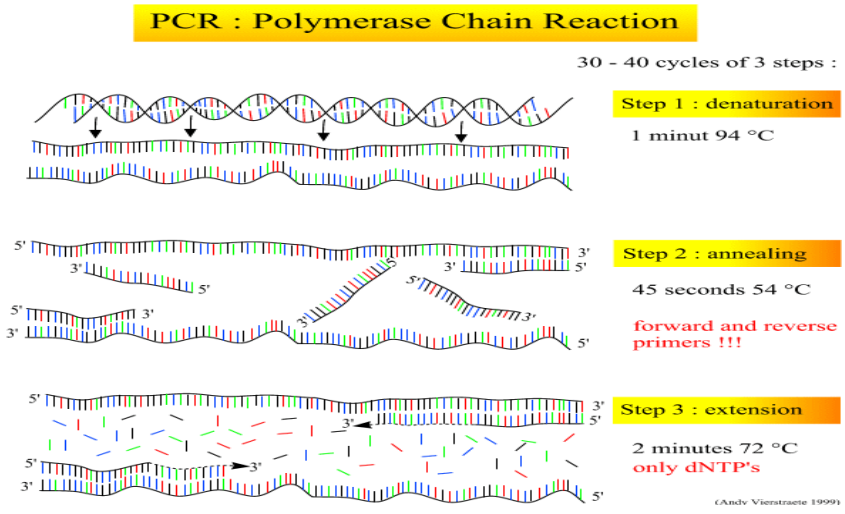
The amount of DNA copies that are produced from the reaction cycles is calculated by 2^n (n= number of cycles).

Initialization: The mixture is heated at 96°C for 5 minutes to ensure that the DNA strands as well as the primers have melted. The DNA Polymerase can be present at initialization, or it can be added after this step.

Denaturation: In this step DNA double strand is separated (or melted) to two single strands by heating to high temperature (94°C- 96°C) that disrupt the hydrogen bonds between the double strands.

Annealing: two primers are annealed to their complementary sequence in template DNA, this is done by lowering the temperature to (40°C-60°C).

Extension: DNA polymerase start its action to elongates DNA fragment by adding complementary nucleotides (dNTPs). The temperature in this step is depends on the DNA polymerase enzyme optimum temperature usually 72°C is used.



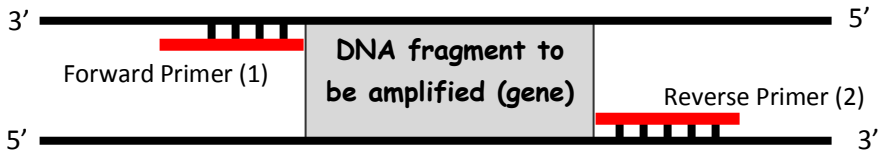
Components of PCR reaction:

1. Primers:

Primer is a short single strand DNA (less than 50 nucleotide usually between 18-25nt) which is complementary to the ends of the DNA fragment to be amplified. Primer is needed to start the replication by DNA polymerase at specific site on DNA template. Two primers are used:

Forward primer is needed to determine start point of replication.

Reverse primer is needed to determine the end point of replication.



2. DNA template:

It is the DNA fragment to be amplified.

3. dNTPs:

It is a mixture of four nucleotides triphosphate (dGTP, dATP, dCTP, and dTTP) that are used to elongate DNA strand.

4. Taq DNA polymerase:

It is a thermostable enzyme which is used to elongates DNA template. It is extracted from thermophilis bacteria called ***T**hermus **Aq**uaticus* that life in hot spring, so it doesn't affected or denaturated by high temperature that is used in PCR.

5. $MgCl_2$:

It is a cofactor for DNA polymerase.

6. Buffer:

It is provide suitable chemical environment for the enzyme by controlling the reaction pH.

7. Water:

It is used to complete the reaction volume to the required volume (25µl, 50µl, or 100µl).

Thermocycler:

It is a machine that is used for PCR reaction, it allows changing the temperature automatically at each cycle. It has a thermal plate with holes where the PCR tubes are inserted. The thermocycler rises and lowers the temperature of the plate as it is programmed before, (temperature range from 4°C to 96°C). Thermocycler has a lid on the top of reaction tubes that is heated to 105°C to prevent the evaporation of the reaction mixture.

Thermocycler Programming:

The temperature and time for each step of the reaction cycle and the number of cycles is programmed.

1. **Denaturation temperature:** 94°C for 1 min.

2. **Annealing temperature:** depends on the primer sequence, the annealing temperature is calculated by the law 2(A+T) + 4(G+C) for each primer, then the average temperature is taken and subtracting from 5 (i.e. average temp-5).

3. **Extension temperature:** 72°C and time depends on the DNA fragment to be amplified. Recommended extending time is 1 min for the synthesis of DNA fragments up to 2 kb. For larger DNA fragments, the extending time is usually increased by 1 min for each 1000 bp.

That is used

Detection and analysis of the reaction product 

The PCR product should be DNA fragments at defined size. The simplest way to check for the presence of these fragments is to load a sample taken from the reaction product, along with appropriate molecular-weight markers, onto an agarose gel (0.8-4.0%) which contains ethidium bromide. PCR products can then be visualized on the gel under ultraviolet trans-illumination, then comparing product (bands) with the molecular-weight marker (band).

Aim:

In this lab you are going to learn how to set up a PCR reaction using control DNA template. On the next lab you will run your PCR product on agarose gel electrophoresis.

Instruments & Equipment:

- Micropipettes rang 0.2-2 µl, 0.5-20 µl and 20-200 µl with their tips (autoclaved)
- Autoclaved PCR tubes (Two sizes are available: 0.2 ml for total 25-50 µl or 0.5 ml for total 100 µl)
- Gloves
- Ice bucket and reaction tube holder
- Microcentrifuge
- Thermocycler machine

Takara* PCR Amplification Kit:

The components are:

1. **Control DNA template λ-DNA**
2. **Control primer 1 :** 5' GATGAGTTCGTGTCCGTACAACT 3'
3. **Control primer 2 :** 5' CCACATCCATACCGGGTTTCAC 3'
4. **Control primer 3 :** 5' GGTTATCGAAATCAGCCACAGCGCC 3'

   Control primer 1 and 2 will results 6012bp from control DNA template.

   Control primer 1 and 3 will results 500bp from control DNA template.
5. **dNTPs mixture**

6. **Taq DNA polymerase**
7. **$MgCl_2$**
8. **10 × PCR buffer**

   Two buffer are supplied by the kit one contains $MgCl_2$ (buffer (+)) and the other without $MgCl_2$ (buffer -) any one of these buffers can be used. If buffer (-) was used so $MgCl_2$ will be added to the mixture.

Procedure:

1. Label PCR tube with a marker pen. The best place to label it is on the tube cap to avoid the rubbing off in the Thermocycler.
2. Follow the next table to prepare PCR reaction mixture in PCR tubes, pipette very carefully and accurately the required volume. Change the pipette tips between solutions to avoid contamination.
3. Close the lid of the PCR tube.
4. Mix the reaction on the tube by flicking with your finger or by vortex.
5. Quickly spin the reaction tube by microcentrifuge to be sure that all the reaction mixture is settled down of the tube.
6. Load the tubes in Thermocycler and run it. The thermocycler will take about two hours according to cycler time. Program the thermocycler as the following:

Temperature	Time	Reaction
96°C	5 min	Denaturation
96°C	35 sec	Denaturation
55°C	35 sec	Annealing
72°C	45 sec	Extension
Go to 2 rep	For 40 cycles	
72°C	4 min	Extension
Hold for 4°C		

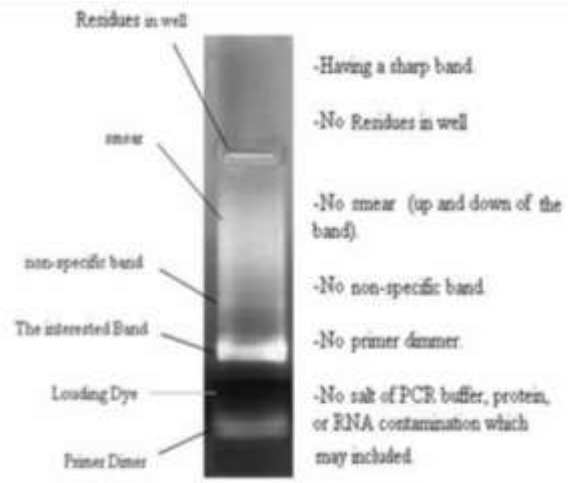
7. The reaction tube will be stored on the fridge (-20°C) until next week to be run on the agarose gel electrophoresis.

Components	Volume to be pipetted	Volume to be pipetted for *negative control	Concentration
10x PCR buffer	2.5 µl	2.5 µl	100 mM Tris-HCl, pH8.3, 500 mM KCl, 15 mM MgCl2)
dNTP Mixture	2.0 µl	2.0 µl	2.5 mM
Control Primer 1	0.2 µl	0.2 µl	20 pmol/µl
Control Primer 2 or 3	0.2 µl	0.2 µl	20 pmol/µl
Taq polymerase	0.5 µl	0.5 µl	5 units/µl
Control Template	2.0 µl	------	1 µg/ml λDNA
RNase or injection water	17.6 µl	19.6 µl	----
Total	25.0 µl	25.0 µl	

* Negative control doesn't contain a DNA template so it is expected no bands in gel electrophoresis, if any bands are appeared that does indicate for the contamination.

Notes:

- PCR is sensitive for contamination with any DNA in lab (that will appear in the result as the below figure), so the experiment must be performed in high sterile conditions to avoid contamination. All equipment must be autoclaved, fresh gloves should be worn gloves and working in hood.
- Micropipettes tips are used ones then dispose them and use new one.
- A Cabinet equipped with a UV lamp is recommended for preparing the reaction mixture.
- Autoclaving of all solutions.



Restriction endonucleases & Ligases

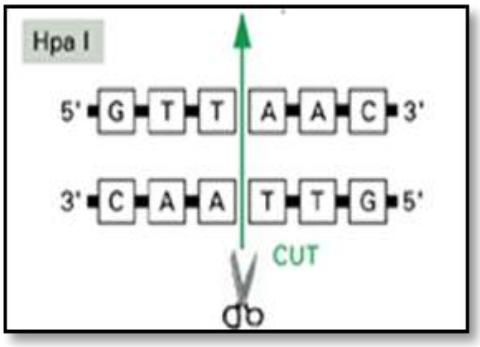
Molecular Biochemistry

Background:

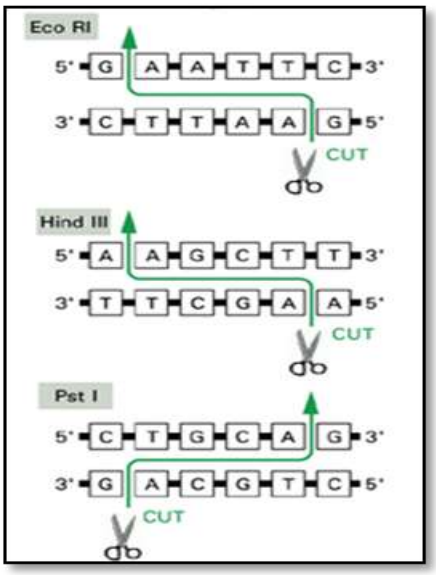
The discovery and characterization of a number of key enzymes have enabled the development of various techniques for analysis and manipulation of DNA.

Type II restriction endonucleases play a key role in molecular biochemistry. These enzymes recognize specific sequence of DNA, usually 4-8 bp in length and cleave them, by breaking phosphodiester bonds, in specific manner this sequence is usually known as recognition sequences. The sequence recognized is plaindromic (They read the same backwards and forwards on each strand). Two types of RE according to the types of ends which they generate:

- RE Cut dsDNA in straight manner to give blunt ends.
- RE Cut dsDNA in staggered cuts to give sticky ends.



Blunt ends



Sticky ends

Approximately 500 REs have been characterized, which can recognize over than 100 different target sequences. Hundreds of restriction enzymes available commercially, they are purified from bacteria and blue green alga. The names of restriction enzymes are derived from the first letter of the **genus** name and the first two letters of the **species** name of the source organism and then the number of RE which isolated from the same species. For example, the enzyme ***EcoRI*** was the first restriction enzyme isolated from **E**scherichia **co**li strain **R**Y13 and **BamHI** was the first restriction enzyme isolated from **B**acillus **am**yloliquefaciens strain **H**.

Ligase enzymes are used to covalent joining of two DNA fragments which cut be the same RE. This is used in molecular biochemistry to construct recombinant DNA "i.e. joining of DNA fragments from different sources.

Table (1): Examples of some REs and their reorganization sites.

Recognition site	Source	Restriction endonucleases
GGATCC	*Bacillus amyloliquefaciens* H	*Bam*HI
GAATTC	*Escherichia coli* RY13	*Eco*RI
GGCC	*Haemophilus aegyptius*	*Hae*III
AAGCTT	*Haemophilus influenzae* Rd	*Hin*dIII
GTTAAC	*Haemophilus parainfluenzae*	*Hpa*I
CCGG	*Haemophilus parainfluenzae*	*Hpa*II
GATC	*Moraxella bovis*	*Mbo*I
GCGGCCGC	*Nocardia otitidis-caviarum*	*Not*I
GGCCNNNNNGGCC	*Streptomyces fimbriatus*	*Sfi*I
TCGA	*Thermus aquaticus*	*Taq*I

Detection and analysis of the reaction product

Principle:

Cleavage of DNA by a RE is called digestion and is usually carried out at 37^{o}C in a small volume (e.g., 20 µl). Each RE has its specific buffer which provides the optimal conditions. RE digestion involves adding enough RE to the substrate DNA in the appropriate buffer and incubating the digest at the appropriate temperature for at least one to four hours or in some cases overnight and the new commercial restriction enzyme can be taken one hour known as fast digest. REs are measured functionally in units (u), which are defined as the amount of RE that will completely digest a defined amount of a specific substrate DNA in a defined time at a specific temperature.

Materials:

- Restriction enzymes kits
- Samples (DNA & Specific PCR product)
- Micropipetteor rang 0.5-20 µl and Yellow tips.
- Microcentrifuge tube (Eppendorf tube)
- Water bath

Restriction enzyme kits:

- *Msp*I enzyme: Recognition sequence (5'…C↓CG G…3')
  (3'…G GC↑C…5')
- RE 10X buffer **(**Contains 10 mM Tris-HCl pH 7.4, 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mg/ml BSA and 50% glycerol).
- Bovine Serum Albumin
- Multi-Core buffer
- Sterile, deionized water

Procedure:

1. Add 3-5 µl the specific PCR product special for this RE or DNA sample (1 µl, 1 µg/µl) was mixed with 0.5 µl (10 u/ µl) restriction enzyme, 0.5 µl Bovine Serum Albumin, 2.0 µl RE 10X buffer, and 12-14 µl of sterile water. The total reaction mixture is 20 µl. Mix gently by pipetting, close the tube and centrifuge for a few seconds in a microcentrifuge. Incubate at the 37ºC for 1-4 hours. The digestion products were separated on 2.60% agarose gel electrophoresis for identification. Note: The overnight digests may result in DNA degradation.
2. Add 4 µl of 6X loading buffer and running in the gel to analysis.

Agarose Gel Electrophoresis

Molecular Biochemistry

Is a technique that is used to separate charge molecules especially proteins and nucleic acids as DNA, RNA that differ in size, charge or conformation. It is one of the most widely-used techniques in biochemistry and molecular biology.

Principle:

Under the influence of electrical field, charged molecules will migrate toward the electrode that carry an opposite charge. A mixture of different charges molecules will migrate according to their charges, similar charge compounds will migrate according to their size. Larger molecules migrate more slowly than smaller ones, and so the distance of migration within a gel can be used to determine a molecule's size.

Factors that effect on the sample migration:

1. Charge, size, and shape of sample.
2. Gel concentration "pore size".
3. Strength of electrical field.
4. Buffer pH: effect on electrical conductivity.

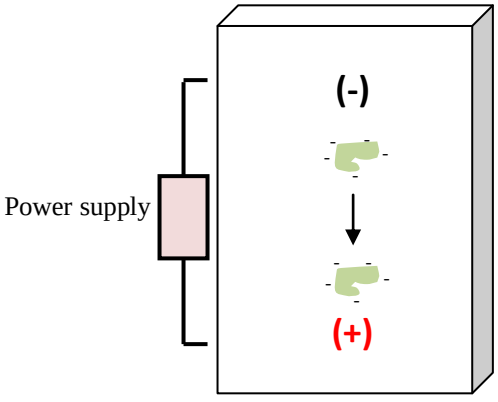


Figure 1: Sample is separated according to their charge.

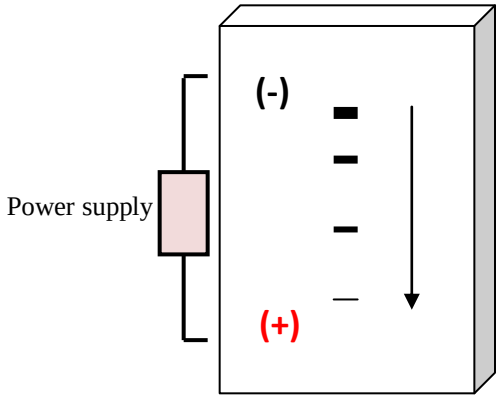


Figure 2: Similar charge mixture is separated according to their size.

Requirement of gel electrophoresis:

1. Gel "supporting media".
2. Buffer.
3. Fluorescent dye.
4. Samples
5. DNA Marker.
6. Electrophoresis apparatus: Tank, plate, electrodes, power supply, and combs.
7. Detection system.

1. Gel:

There are two types of gel: Agarose and polyacrylamide.

- **Agarose:**

  It is a polysaccharide extracted from seaweed and is typically used at concentrations of 0.5% to 3%. The higher concentration of agarose is the "stiffer" the gel. Polymerized agarose is porous, allowing the movement of DNA through it. Agarose gels have a large range of separation, but relatively low resolving power. DNA fragments from about 0.2 kbp to 50 kbp can be separated in agarose. Purified agarose is in powdered form, and insoluble in water or buffer at room temperature but it dissolve in boiling water. Different purities of agarose are commercially available as are agaroses with different melting properties.

- **Polyacrylamide:**

  It is a cross-linked polymer of acrylamide. A wide range of conc. can be used between 3.5% to 20% (Advantage to give higher resolution to separate very small DNA fragments that are differ in a one bp and so used for DNA sequencing). Polyacrylamide gels are more annoying to prepare than agarose gels and toxic (Disadvantage). Because oxygen inhibits the polymerization process, they must be poured between glass plates (or cylinders). Polyacrylamide gels have a small range of separation, but very high resolving power. DNA less than 0.5 kbp can be separated by polyacrylamide.

  Gel concentration is very important since it determine the pore size that the sample will migrates through.

High conc. → Small pore size → Separate small molecules

Low conc. → Large pore size → Separate large molecules

2. Buffers:

Two buffers are used together:

Electrophoresis buffer:

Provide ions to conduct the electricity and to maintain the pH at constant value. TBE buffer (Tris/Borate/Na_2EDTA) is usually used.

Loading buffer:

There are many names: Tracking buffer (Tracking dye) and blue juice.

It is used a color marker and density to the sample when load into wells. It is carries slight negative charges in neutral buffers and thus migrate in the same direction as the DNA during electrophoresis. For example, bromophenol blue, Xylene Glycerol, or Orange G.

3. Fluorescent dye:

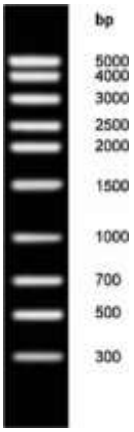
It is important to visualize the separated DNA bands, usually ethidium bromide (EtBr) is used. EtBr is a fluorescent that intercalating between the bases of DNA and glows pink when excited by UV dye. It is previously mixed with the gel or in tank containing buffer, or after electrophoresis the gel is soaked in a solution contains EtBr.

4. Samples:

It can be DNA, RNA and Protein.

5. DNA Molecular weight Marker:

There are many names: DNA Molecular weight Marker, DNA ladder, or DNA standard. It is a mixture of DNA fragments of known sizes. The size of a fragment is measured by base pairs (bp). Two common ladders are used the 100bp and the 1000bp (1kbp) ladders. Ladders are commercially available by different companies. The 100 bp ladder is composed of a mixture of small fragments (100 to 2000 bp). The 1000 bp ladder is composed of a mixture of larger fragments (500 to 12000 bp).



When ladder run in a gel electrophoresis, the fragments will be separated into distinct bands that can be used as size references.

6. Electrophoresis apparatus:

- **Tank:**

  It is the container which contain the buffer. It always has a cover to prevent the evaporation of buffer and for safety. There are two types of tanks: one is called the horizontal while the other is the vertical. In the horizontal tank we are using agarose or acrylamide gel as a support media and it is used to identify DNA and RNA. On the other hand, vertical tank only polyacrylamide gel is used. Vertical is used mainly in identifying protein and in DNA sequencing.

- **Tray:** Is the actual mold which provides a shape for the gel as it polymerizes (or solidify). After polymerization, the gel will move out of the mold and submerse it in a tank of buffer to run.

- **Support:** This is just a small piece of glass or plastic that rests snugly in the bottom of the tray. When the gel is finished polymerizing, the support is gently pushed upwards out of the tray.

- **Power supply:** It can be monitored and operated in current (amps), voltage (volts) or power (watts) mode. The black and red cords leading from the power supply are then attached to the tray in which the gel is run.

- **Combs:** It used to make wells on the gel to load different samples.

7. Detection system:

Transilluminator (Ultraviolet light box) and camera: to visualize the bands. After running the electrophoresis, the gel is placed on a UV light box. It's picture is either acquired by camera so fragments of sample can be detected.

Which DNA can be used for electrophoresis?

1. PCR product
2. DNA that cut with restriction enzyme. Then these fragments are separated according to their sizes.

Result analysis:

A. **Band location:** It is interested band that indicates the size of DNA fragment when it compare with the ladder or marker.

B. **Intensity of the band:** Depends on the DNA concentration.

C. **How many fragments:** According to the DNA sample and how is it treated (which restriction enzyme).

D. **Other bands:** It will be appeared other than interested band such as: primer dimer, non-specific bands, residue in well, salt PCR buffer, protein, or RNA contamination, and smear.

E.

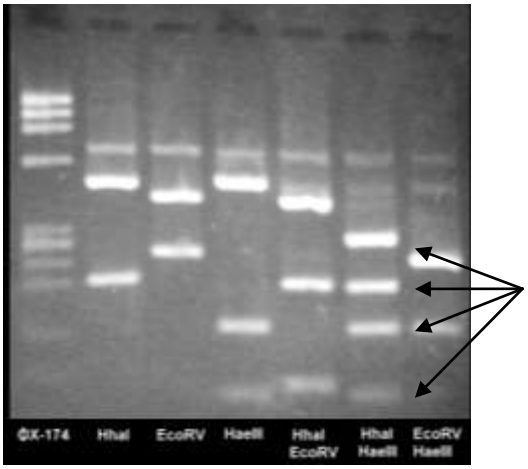
(A)

Band location as PCR product

(B)

Intensity of the band

(C)



Fragments cut with different restriction enzymes separated according to their size.

Detection and analysis of the reaction product

Aim:

We will use agarose gel electrophoresis to show DNA sample and determine the size of PCR products and after cut it with restriction enzyme. You will learn how to prepare the buffer and gel, pour the gel, sample loading and analyze the results.

Materials:

- Agarose powder.
- TBE (Tris/Borate/Na_2EDTA) buffer
- Ethidium bromide (10mg/ml)
- 6X Loading buffer (bromophenol blue 0.25% (w/v) and sucrose 40% (w/v)).
- Samples (DNA sample, PCR products, PCR products with cut it)
- Micropipetteor rang 0.5-20 µl and Yellow tips.
- Electrophoresis apparatus
- Transilluminator
- Digital camera
- Conical flask
- Boling water bath or Microwave oven
- Microcentrifuge tube (Eppendorf tube)
- Parafilm
- Aluminum foil

Procedure:

Buffer preparation:

10X TBE buffer

Make 0.89 M Tris-Base (108 g/L), 0.89 M Boric acid (55.0 g/L), and 0.02 M (7.44 g/L) of EDTA-Na2-salt, complete to 1000 ml with distilled water, and then adjust pH to 8.3 (with NaOH).

1X TBE buffer

Take 100 ml form 10X stock solution and complete to 1000 ml with distilled water. Then fill the tank of electrophoresis and dissolve agarose powder with this buffer.

Gel preparation:

A. Dissolve agarose powder (2%), i.e. 2 gm in 100 ml 1X TBE buffer.
B. Melt the gel in a microwave oven or in boiling water bath until completely melted.
C. Cool the solution to about 60°C.
D. Add 1 µl ethidium bromide stock per 10 ml gel solution and mix gently.

Pouring the gel:

A. Before pouring, insert the comb on the tray and pour the gel slowly without bubbles and allow the gel to solidify at room temperature.
B. After the gel solidify, remove the comb to make a wells on the gel.
C. Insert the tray on electrophoresis chamber and cover the gel with 250 ml 1X TBE buffer (The samples must be in a direction of the (–ve) electrode usually the black one).

Preparing the sample:

A. Mix 2-5 µl of the PCR products or restriction enzyme solution (20 µl) sample with 2-3 µl of loading buffer in eppendorf tube or mix on parafilm.
B. Mix Loading buffer with marker: mix 5 µl of DNA marker with 2 µl of loading buffer. But now some markers are already become mixed.

Loading the samples:

A. Write the sample and well numbers on you lab report.
B. First load the DNA marker in the first and last well and then load the other samples between them.
C. Turn on the power supply.
D. After finishing the electrophoresis, transfer the gel to transilluminator, visualize the bands and then take a photo with camera.

NOTE:

- Always wear protective gloves and eyewear when handle with preparation of gel and observing DNA on a transilluminator to prevent damage to the eyes from UV light.
- EtBr is carcinogenic (Take precautions)

Result sheet

Paste the photo here.

B. **Analyze** the results of the different types of samples that showed in the agarose gel.

Well #	Type	Resolution of band	Comment
1	DNA Ladder		
2	Whole DNA		
3	Cutting DNA		
4	PCR product 1		
5	PCR product 2		
6	PCR product 3		
7	PCR product 4		
8	PCR product 5		
9	PCR product 6		
10	PCR product 7		
11	Cutting PCR product 1		

Well #	Type	Resolution of band	Comment
12	Cutting PCR product 2		
13	Cutting PCR product 3		
14	Cutting PCR product 4		
15	Cutting PCR product 5		
16	Cutting PCR product 6		
17	Cutting PCR product 7		
18	DNA Ladder		

Bioinformatics and the Internet

Molecular Biochemistry

Overview:

Bioinformatics also known as computational molecular biology, computational genomics, biocomputing, or biological computing. It is a field concern with use of development techniques including computer science, statistics applied mathematics, information technology, biology, and biochemistry to analyze, understanding, and solve complexity biological, biochemical and biophysical data of biological data, usually on the molecular level.

The name of bioinformatics started in the mid-1980 and in 1988 the software tools were developed for analysis of nucleic acid sequence, and with the beginning of Human Genome Project in 1990, the efforts in bioinformatics field is intensified. It is one of the marvels of modern technology that can save you months of lab work. Use highly sophisticated programs over the Internet without installing anything new on your own computer.

Bioinformatics is the technology that allows to search, manages, biological databases, store, characterize the genetic code and the proteins linked to each gene and their associated functions, compare sequences between different genes, examine the structure of proteins, and understand the sequence of the human genome from large database as following:

1. NCBI (National Center for Biotechnology Information),
2. DDBJ (DNA Data Bank of Japan), and
3. EMBL (European Molecular Biology Laboratory)

The above websites contain information about genes sequence of plants and animals and proteins structure for use in discover new drugs by the use of computer science.

Bioinformatics tools:

Bioinformatics tools:

Many tools can be used to facilitate the analysis of genes and to get its information as following:

1. NCBI Map Viewer to know set of chromosomes for each organism,
2. FASTA to know the sequence of nucleotides and amino acids
3. BLAST to align sequence.

4. ClustalW to compare the sequence with others.

5. PubMed

6. ExPASy (Expert Protein Analysis System) is a complete set of protein-analysis tools, searching all the literature of the life sciences respectively.

There are now a large number of genetic databases that have sequence information about variety organisms. A number of these important databases on **W**orld **W**ide **W**eb resources are listed in table:

Database	Has data about	URL
Nucleotide sequence databases		
- NCBI (GeneBank)	US genetic database	http://www.ncbi.nlm.nih.gov
- EMBL	European genetic database	http://www.ebi.ac.uk/embl
- DDBJ	Japanese genetic database	http://www.ddbj.nig.ac.jp
Human/mouse genome	Ensemble	http://www.ensemble.org
PubMed	Literature references	www.ncbi.nlm.nih.gov/entrez/query.fcgi?DB=pubmed
Protein sequence databases		
- Swis-Prot	European protein sequence database	http://www.expasy.ch
- TREMBL	European protein sequence database	http://www.ebi.ac.uk/trembl
- PIR	US protein information resource	http://pir.georgetown.edu/
OMIM	Genetic diseases	www.ncbi.nlm.nih.gov/omim/
Enzymes	Enzymes	www.chem.qmul.ac.uk
KEGG	Metabolic pathways	www.genome.ad.jp
Protein structure databases - PDB	Protein data bank	http://www.rcsb.org/pdb/
Genome project databases		
- dbEST (cDNA and partial sequences)		http://www.ncbi.nlm.nih.gov
- Whitehead Institute (YAC and physical maps)		http://www-genome.wi.mit.edu/

Follow the below steps for analysis gene:

1. **Choose any gene and where is this gene in human genome.**

   By use **NCBI Map viewer**.

   Link: http://www.ncbi.nlm.nih.gov/mapview/static/MVstart.html

2. **How to known more information about this gene and what the articles associated with this** gene locus?

   By enter to **Entrez Gene** and click **MIM**. These are links to OMIM entries (Online Mendelian Inheritance in Man) databases and select **PubMed Links** from next to Display button.

   Link: http://www.ncbi.nlm.nih.gov/sites/entrez

3. **What are the nucleotides and amino acids sequence of this gene?**

   By enter to **Entrez Nucleotide** and **Entrez Protein databases**, then use **FASTA** tool from next to Display button.

   Links: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore&cmd=search&term=
   http://www.ncbi.nlm.nih.gov/sites/entrez

4. **What is the proteins sequence in human similar to sequence of this gene & where are the genes for other proteins found in chromosome?**

   By use a **FASTA** sequence as an input to **BLAST** tool.

   (Enter to **NCBI Map viewer** and click on **circled B (Blast symbol)** next to Homo sapiens (human).

   Link: http://blast.ncbi.nlm.nih.gov/Blast.cgi

5. **How are the specific genes related to each other?**

   By use a **ClustalW** tool in Expert Protein Analysis System (**ExPASy**) page in to make a multiple sequence alignment and then using it to make a phylogenetic tree.

   Link: http://us.expasy.org

6. **What is the structure of a gene product (Protein) by homology modeling?**

   By use **DeepView** (also known as Swiss-PdbViewer) and **PDB Jmol viewer** in PDB (Protein Data Bank) that is the database for macromolecular structures.

   Links: http://www.rcsb.org/pdb/

   http://spdbv.vital-it.ch/

7. **How to find gene homology in the PDB?**

   By click on the **FASTA** sequence in the **PDB** page and insert to **BLAST** tool.

   Links: http://www.rcsb.org/pdb/

References:

- http://www.vivo.colostate.edu/hbooks/
- http://www.encyclopedia.com/
- http://ghr.nlm.nih.gov/handbook/basics/dna
- http://publications.nigms.nih.gov/thenewgenetics/chapter1.html
- http://www.eisenlab.org/FunFly/?page_id=24
- Cooper GC, Hausman RE (2004). The Cell: A Molecular Approach (3rd ed.). Sinauer. pp. 261–76, 297, 339–44.
- Wirta W (2006). Mining the transcriptome – methods and applications. Stockholm: School of Biotechnology, Royal Institute of Technology
- http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/nucacids.htm
- Gueneau de Novoa P, Williams KP (2004). "The tmRNA website: reductive evolution of tmRNA in plastids and other endosymbionts". Nucleic Acids Res
- Plummer D.T., An introduction to Practical biochemistry, Tata McGraw – Hill
- Section of Cancer Genomics, Genetics Branch, NCI (National Institutes of Health).
- Plummer D.T., An introduction to Practical biochemistry,Tata McGraw – Hill.
- Dreisbach R.H., Handbook of poisoning, Lange.
- Agarose gel electrophoresis (basic method)". Biological Protocols. http://www.methodbook.net/dna/agarogel.html. Retrieved 23 August 2011.
- Schägger, Hermann (2006). "Tricine–SDS-PAGE". Nature protocols 1 (1): 16–22. doi:10.1038/nprot.2006.4. PMID 17406207.
- http://users.ugent.be/~avierstr/index.html
- Rychlik W, Spencer WJ, Rhoads RE (1990). "Optimization of the annealing temperature for DNA amplification in vitro". Nucl Acids Res 18 (21): 6409–6412. doi:10.1093/nar/18.21.6409. PMC 332522. PMID 2243783.

Useful links:

- http://www.chemistry.nmsu.edu/studntres/chem435/Lab4/intro.html
- http://oregonstate.edu/instruction/bb492/lectures/StructureI.html
- http://pcrworld.blogspot.com/2012_06_01_archive.html