CHAPTER I

BIOMOLECULES

Living organisms are enormously complex. Higher organisms correspondingly have a greater complexity. Human beings (*Homo sapiens*), for example, may contain 100,000 different types of molecules, although only a minor fraction of them have been characterized. Living organisms have an underlying regularity that derives from their being constructed in a hierarchical manner. **Anatomical** and **histological** studies have shown that multicellular organisms are organizations of **organs**, which are made of **tissues** consisting of **cells**, composed of subcellular **organelles**. At this point in our hierarchical descent, we enter the **biochemical** realm since organelles consist of **supramolecular** assemblies, such as **membranes** or **fibers**, which are organized clusters of **macromolecules** (**polymeric molecules** with molecular masses from thousands of dalton on up).

Living things in general, contain different types of macromolecules: **protein** (Greek: *proteios,* of first importance), **nucleic acids**, and **polysaccharides** (Greek: *sakcharon,* sugar). All of these substances have a modular construction; they consist of linked monomeric units that occupy the lowest level of our structural hierarchy. Thus proteins are polymers of amino acids, nucleic acids are polymers of nucleotides, and polysaccharides are polymers of sugars. **Lipids** (Greek; *lipos*, fat) the fourth major class of biological molecules, are too small to be classified as macro molecules but also have a modular construction. Proteins are all synthesized from the same 20 species of amino acids, nucleic acids are made from 8 types of nucleotides (4 each in **DNA** and **RNA**), and there are ~ 8 commonly occurring types of sugars in polysaccharides. The great variation in properties observed among macromolecules of each type largely arises from the enormous number of ways its monomeric units can be

arranged and, in many cases, derivatized. Besides the existing macromolecules, living cells also contain **minerals** in form of kations and anions, such as **Na^{+}, Ca^{+2}, Cl^{-}, SO_4^{-2}** and **PO_4^{-3}.**

The aim of studying biochemistry is to understand the biological structure and function of living organisms in chemical terms. One of the most excellent approaches to understand biological phenomena was to purify an individual chemical component, the biomolecules, such as protein, and to characterize its chemical structure or catalytic activity.

In this chapter of the laboratory exercises, students will learn more about molecules and macromolecules that build the living cells. A clinical case of patient with chronic renal failure post streptococcal glomerulonephitis, which presents several sign and symptoms due to biomolecular changes of the body, will be presented as an entry to discuss the functional related structure of biomolecules. The students will conduct plasma protein electrophoresis, the assays of plasma sodium, blood urea nitrogen and creatinine using spectrophotoscopic principle, and determine several minerals in the urine. Since dietary management is one of the modality of treatment in this patient, the students have also to take biomolecular aspect of dietary resources into their consideration during the discussion.

In this chapter the students have to learn about hierarchy, structure and function of biomolecule, and metabolism of nucleotide.

LABORATORY ACTIVITY 1

Resource Person : Dr.Lelly Yuniarti, S.Si.,M.Kes
Subject : Biomolecules
Department : Biochemistry

A	Sequent			
	I	Introduction	:	40 menit
	II	Pretest	:	5 menit
	III	Laboratory activity	:	120 menit
	IV	Post test	:	5 menit
B	Topic			
	Date:			
	1. Electrophoresis of plasma proteins 2. Determination of albumin urine 3. Determination of uric acid		: : :	40 menit 40 menit 40 menit
C	Venue			
	Biomedical Laboratory, Faculty of Medicine, Unisba, Jl. Tamansari No.22 Bandung 40116			
D	Equipment			
	1	Electrophoresis of plasma proteins	1. Horizontal electrophoresis apparatus 2. Power pack 3. TAE buffer (Tris Acetate EDTA) pH 8,5 4. Ponceau S protein stain (2g/L in 30 g/L TCA) 5. Metanol absolute 6. Serum/plasma 7. Agarose gel (Merck) 8. Whatman 3 MM filter paper 9. Methanol : water (8:2)	
	2	Determination of albumin urine	1. Urine of acute glomerulonephritis patient 2. Acetic acid 0.1M 3. Sentrifuge 4. Reaction tube 5. Pipette 6. Bunsen burner 7. Clamp	
	3	Determination of uric acid	1. Uric acid strip rapid test 2. Blood lancet 3. Alcohol swab	
E	Implementation			
	1. Students are divided into 18-20 groups 2. Each group is supervised by one tutor			

Activity 1 : Electrophoresis of plasma protein

Introduction

Electrophoresis is a method widely used in the analysis of biological macromolecules (serum of proteins, membrane of proteins, hemoglobin, DNA/RNA,etc), adapted for serial investigations, e.g. in the clinical laboratory. Many biological molecules carry an electrical charge, the magnitude of which depends on the particular molecule and also the pH and composition of the suspending medium. These charged molecules migrate in solution to the electrodes of opposite polarity when an electric fields is applied, and this principle is used in electrophoresis to separate molecules of

differing charges.

The electrophoresis mobility depends mainly on the ignitable groups present on the surface of the particle, and the sign and magnitude of the charge carried by the ionizing groups varies according to the ionic strength and pH of the medium in a characteristic manner. Separation of molecules can therefore be affected by selecting the appropriate medium.

Some of electrophoresis methods are:

1. Moving boundary electrophoresis.
2. Zone electrophoresis.

The effects of confection can be reduced to a minimum if electrophoresis is carried out on supporting medium impregnated with buffer solution. Complete separation of a maximum can be effected into distinct zones, and this technique has replaced the classical method of free boundary electrophoresis. Some commonly used supporting media are now considered.

Principle

There are numerous applications of electrophoresis in clinical and biochemical fields, and one test frequently carried out is the analysis of serum/plasma for changes in proteins during disease. A rapid and convenient method for this is cellulose acetate electrophoresis illustrated in the following experiment.

Cellulose acetate membranes have been used advantageously for the electrophoresis separation and study of numerous protein mixtures. They are thin (130 μm) and micro porous which, along with the fact that most of their constituent hydroxyl groups contain derivative groups, makes them practically non-adsorptive. Since adsorption is minimal, tailing is eliminated and solutes can be separated as sharply defined bands. The membranes are opaque and brittle when dry but become quite strong and pliable when wet.

In addition to the elimination of tailing, cellulose acetate also offers the following advantages over paper electrophoresis.

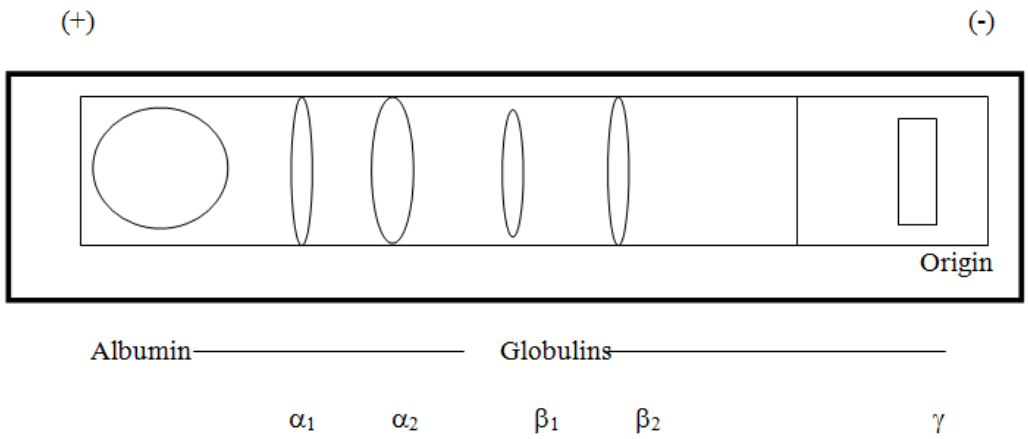
1. Separation is much more rapid
2. Very small quantities can be separated
3. The membranes can be made transparent, which decreases the back-ground error in quantitative scanning.
4. Proteins can be separated more readily.
5. Immune diffusion techniques can be applied.
6. Solutes can be easily eluted the membrane is soluble in certain solvents
7. Many proteins stain much better.
8. Low background radioactivity enables effective isotope counting.

Method

1. Put the agarose gel on the bridge of electrophoresis apparatus.
2. Lightly put plasma into the well of agarose gel, and connect it to the buffer compartments with filter paper wicks.
3. Switch on the current and adjust to 0,4 am per centimeter width of strip.
4. Apply a streak of serum from a micro liter pipette or melting point tube about one-third of the length of the strip from cathode. This is best carried out by guiding the application with a ruler placed across the tank.
5. Carry out electrophoresis for 1.5 – 2 h.
6. Remove the agarose gel, and stain with Ponceau S for 5 min.
7. Prior heating is not needed here since the TCA fixes the proteins for staining.
8. Remove excess dye from the agarose gel by washing repeatedly in metanol

absolute and destaining solution methanol : water (8:2).

9. Compare your electrophoresis pattern with that shown in the following Figure.



The separation of human serum / plasma proteins by electrophoresis on cellulose Acetate at pH 8,6

Student task :

1. perform the electrophoresis procedure in group
2. conclude the result

Activity 2: Determination of Albumin urine

Principle:

White sediment would formed if urine containing albumin were heated

In base condition, phosphat and carbonate would also form sediment, to distinguish albumin and those compound, add acetic acid. Albumin still reveal to be sediment while phosphat and carbonate dissolve.

Method

1. Sentrifuge urine – use the supernatan
2. Add each 3 ml urine in 2 reaction tubes
3. Heat 1 tube, the other used as control
4. White sedimentation will reveal in heated tube
5. Add 2 drop acetic acid, the sediment will remain visible

Student task:

1. perform the determination of albumin urine procedure in group simultaneous with the electrophoresis
2. conclude the result

Activity 3: Determination of Uric Acid

Principle

The Uric acid test strip uses electrochemical sensor technology. A whole blood sample is drawn by capillary action into a reaction zone on the strip automatically that will achieve a stable sample volume. When the uric acid in whole blood sample is oxidized by the electrodes, a current proportional to the concentration of uric acid can be detected by the meter when applying a fixed potential across the electrodes. The current is then converted into a reading of uric acid concentration.

Method

1. Prepare the equipment, insert the strip into the strip reader
2. Choose a finger to draw blood sample
3. Swab with alcohol

	4. Apply blood lancet to the chosen finger perpendicular to skin surface 5. Take the blood sample by applying the strip test attached to reader 6. Read the result
	Student Task: 1. Perform the procedure 2. Conclude the result
F	**Home work** 1. What is the principle of electrophoresis? 2. In what condition could we find albumin in urine? 3. What is the importance of heating and acetic acid administration in albumin examination? 4. Range of uric acid normal value is between.... and .... in male, and between.... and .... in female 5. Why is there a difference in range of uric acid normal value between male and female?
G	**References:** **1. Harper's Biochemistry 28th ed.** **2. Devlin Biochemistry** **3. Lippincott's Biochemistry**

CHAPTER II
BIOMOLECULES AND NUTRITION

Introduction

We have all witnessed through the medium of television and the newspapers harrowing pictures of starving children dying in various areas in the country due to the prolonged multi dimension crisis. Our immediate reactions are of compassion and we feel compelled by our common humanity to contribute what we can to charitable agencies engaged in famine relief. Almost certainly we will also feel anger and helplessness in the face of the apparently insuperable problems of drought, wars, foreign debt, local corruption and adverse trading arrangements with Western countries which are at the root of such calamities.

Food aid, channeled through relief agencies, is of course an immensely important immediate response. However, it has to be recognized that the affected individuals are likely to have multiple nutritional deficiencies, not only of protein and calories, but also of vitamins, minerals and other dietary components. Moreover, the nutritional needs of different groups within the population, such as young children, pregnant women, nursing mother, manual workers and elderly, are quite different. Local conditions also vary enormously; parasitic infections are usually rife and resistance to even minor infectious diseases may be very low. Food storage conditions are usually far from ideal, resulting in spoiling of locally-produced and imported foodstuff alike. Consequently, relief schemes must be devised and coordinated by experts well acquainted with local conditions. Famine and its consequences are not intractable. Relatively simple measures would make an immense difference if only there was the political and humanitarian will to do it!

In this chapter the students have to learn about nutrition, metabolism of nutrition (carbohydrate, lipid, protein), interrelated metabolism in undernourished condition and malnutrition.

LABORATORY ACTIVITY 2

Resource Person : Dr.Lelly Yuniarti, S.Si.,M.Kes
Subject : 1.Qualitative Test for Ketone Bodies in Urine
2.BUN
Department : Biochemistry

A	Sequent			
	I	Introduction	:	40 menit
	II	Pretest	:	5 menit
	III	Laboratory activity	:	120 menit
	IV	Post test	:	5 menit
B	Topic			
	Date:			
	1. Gerhardt's Ferric Chloride Test 2. Rothera'a Nitroprusside Test 3. Determination of BUN		: : :	40 menit 40 menit 40 menit
C	Venue			
	Biomedical Laboratory, Faculty of Medicine, Unisba, Jl. Tamansari No.22 Bandung 40116			
D	Equipment			
	1	Gerhardt's Ferric Chloride Test	1. Ferric chloride solution (100 g/l) 2. Filter paper 3. Bunsen burner 4. Test tube 5. Pipette	
	2	Rothera's Nitroprusside Test	1. Sodium Nitroprusside 20 g/l 2. Solid ammonium sulphate	
	3	Determination of BUN	1. Blood lancet 2. Alcohol swab	
E	Implementation			
	1. Students are divided into 18-20 groups 2. Each group is supervised by one tutor			

Activity 1 : Gerhardt's Ferric Chloride Test

Introduction:

The most important consequence of the increased metabolism of fat in starvation or diabetic patients is the accumulation in blood of the following substances:

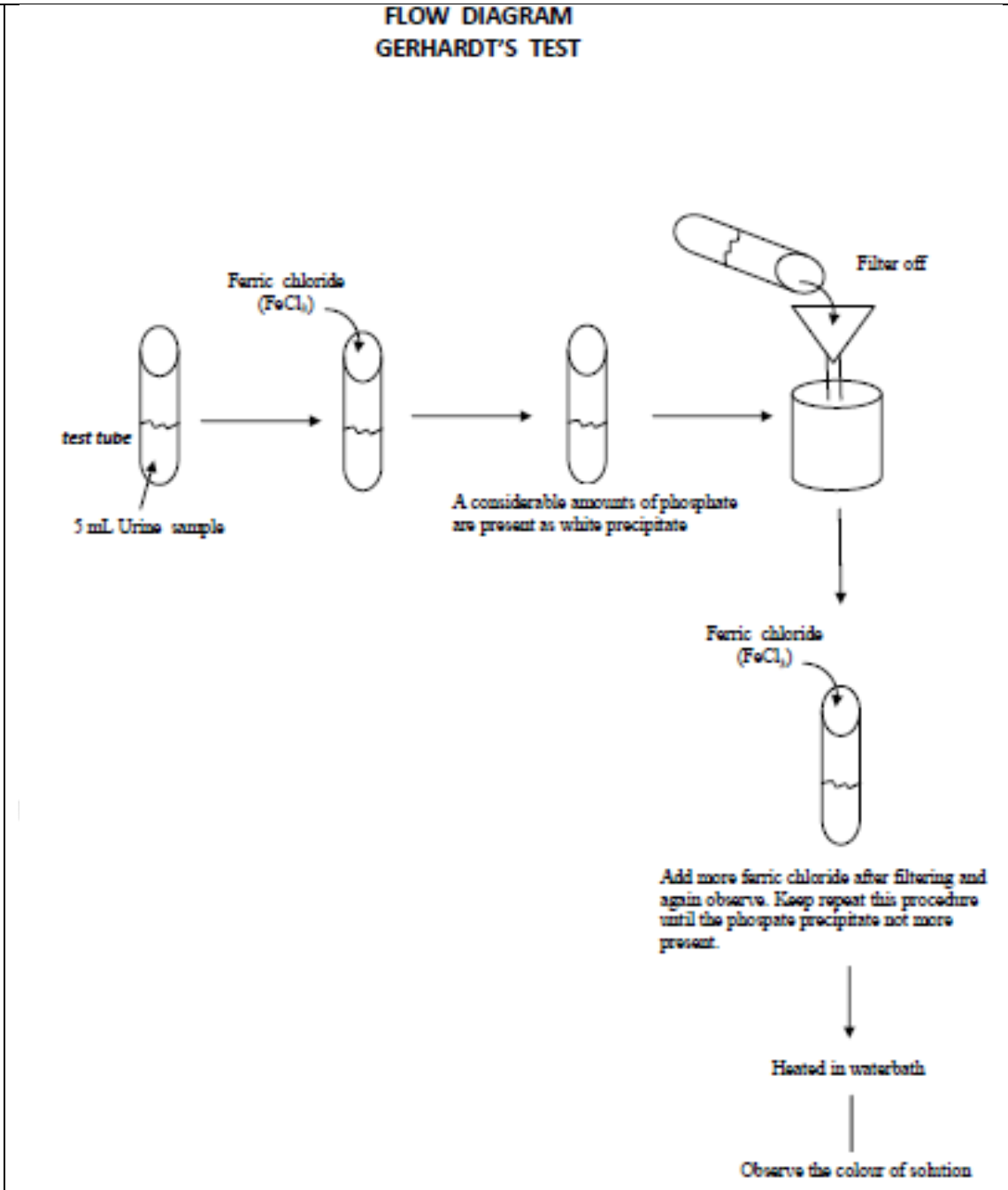
- acetoacetic acid ($CH_3.CO.CH_2.COOH$).
- β-hydroxybutyric acid ($CH_3.CHOH.CH_2.COOH$).
- acetone ($CH_3.CO.CH_3$).

It is now held that the liver is the main site of formation of the two acids given above. Unless adequate glucose is available, the liver is compelled to use an increased amount of fat, so that these acids are formed in quantities too great for the tissues to metabolise them. Then they begin to accumulate in the blood and to be excreted in the urine. It has been shown that diabetics are able to metabolise them normally. It is the increased production which is significant. In addition to the ketosis of diabetes, starvation ketosis arises in the same way. Testing the urine for these substances is an important part of the routine examination of diabetic or starved patients.

Principle:

The definite reaction is not yet unknown, probably the oxidation of acetoacetic acid by $FeCl_3$

	produces a red brown colour substance.
	Method **Procedure:** 1. Add ferric chloride solution (100 g/l), drop by drop, to a few ml of urine in a test tube. A red brown colour is given by acetoacetic acid. 2. If considerable amounts of phosphate are present a precipitate of ferric phosphate is produced. It may be necessary to filter this off. 3. Add more ferric chloride after filtering and again observe. Similar colour is given by salicylic acid and salycilates as well. This can be differentiated from acetoacetic acid by their different behaviour towards heat. 4. On thoroughly boiling the urine, acetoaccetic acid loses carbon dioxide, and is converted into acetone, which no longer gives the test. 5. Repeat the test on the boiled sample and if the test is still positive, then acetoacetic acid is not present but the color is from an interfering substance. 6. If the repeated test is negative, then the color in the original test was due to acetoacetic acid.



Student task :

3. perform the procedure in group
4. conclude the result

Activity 2: Rothera'a Nitroprusside Test

Principle:

Sodium nitroprusside (sodium nitroferricyanide) in a acid condition will split to $Na_4Fe(CN)_6$-$NaNO_2$ and $Fe(OH)_3$, a strong oxidating agent. Acetoacetic acid and acetone will be oxidized and produces a red purple colour mix of substances. To stabilize these substances, a buffer solution $(NH_4)_2SO_4$ is needed.

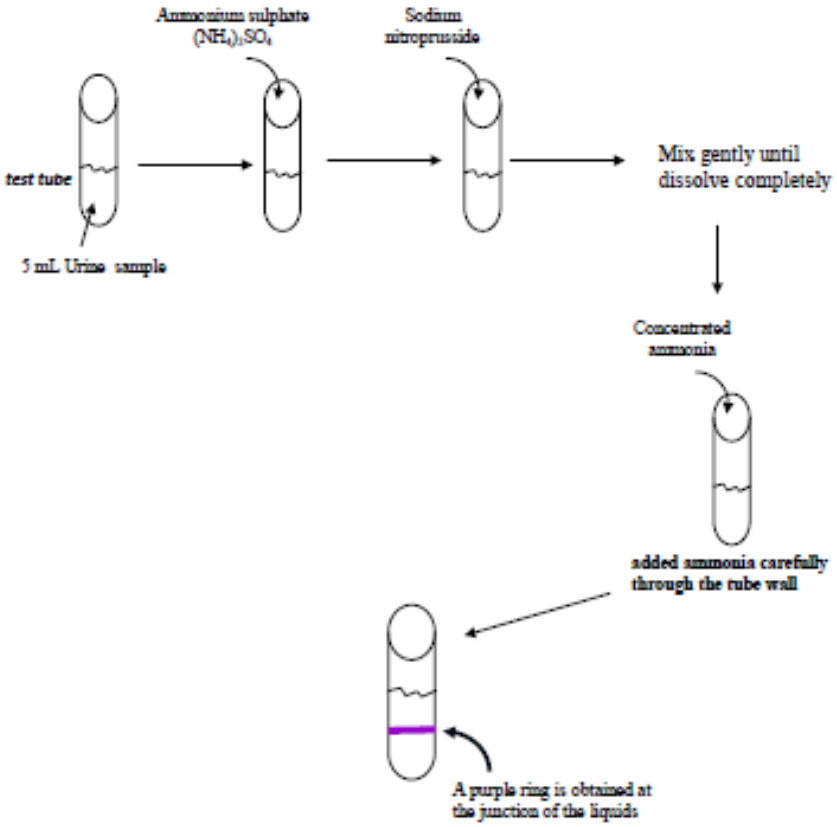
Method

1. Saturate about 5 ml urine with solid ammonium sulphate and add a little sodium nitroprusside, either about 0.5 ml 20 g/l solution or a small quantity of powdered solid.
2. Mix and add about 0.5 ml concentrated ammonia. A purple color is given by

acetoacetic acid and acetone which is maximal in 15 min.

3. If the ammonia is layered on, a purple ring is obtained at the junction of the liquids. This test is not given by salicylates.
4. Rothera's test is much more sensitive than Gerhardt's, being positive at about one part in 40 000 compared with one in a few thousands for the Gerhardt test.

FLOW DIAGRAM
ROTHERA'S TEST



Student task:

3. perform the procedure in group
4. conclude the result

Activity 3: Determination of BUN

Principle of Berthelot method (colorimetric method)

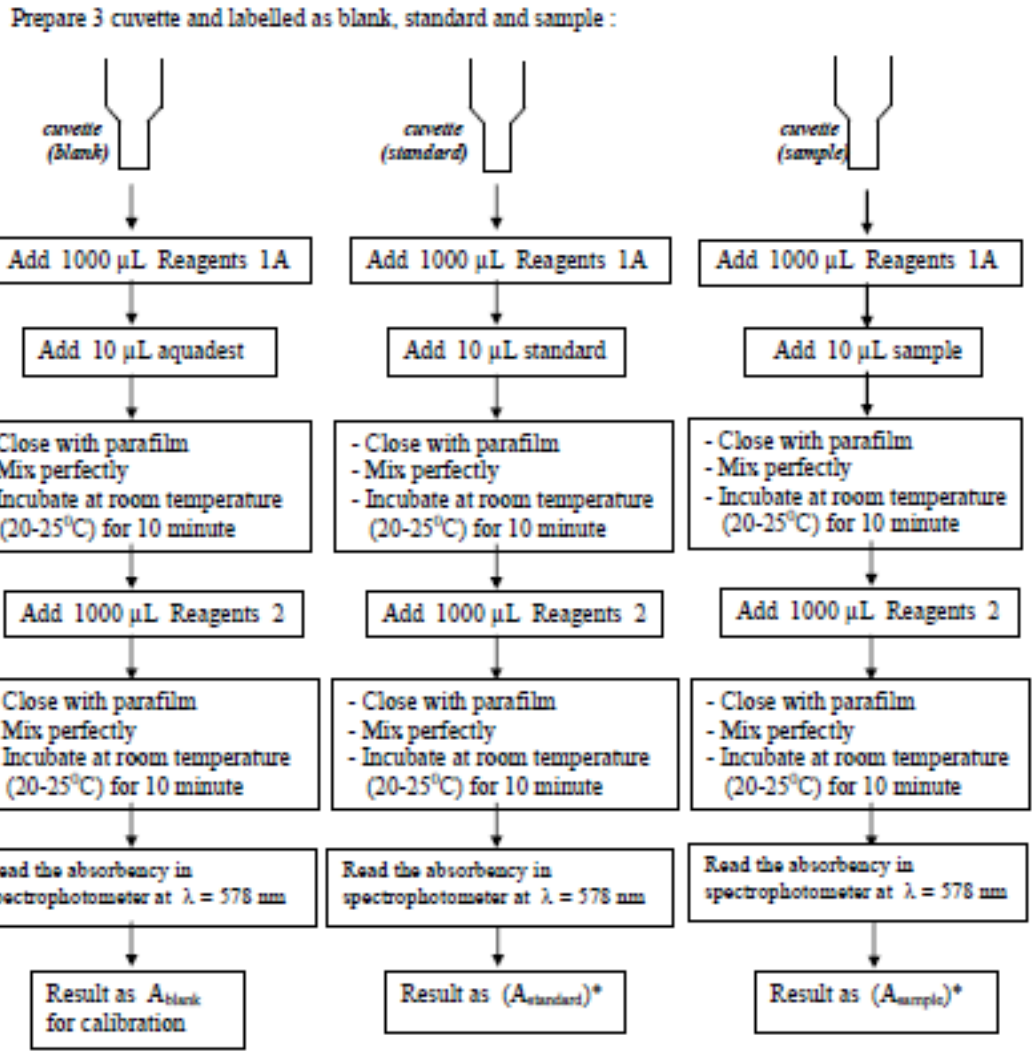
In this analysis, urease catalyzes the hydrolysis of urea to NH_3 and CO_2. The ammonium ions react with hypochlorite and salicylate to give a green dye. The color of the unknown is read against a blank at A_{578nm} and calculations are made by comparison with absorbency values of a standard of known concentration. Follow the procedure shown in the following table.

Procedure

Additions	Blank	Standard	Sample
Reagent 1A*	1000 µL	1000 µL	1000 µL
Akuadest	10 µL	-	-
Standard	-	10 µL	-
Sample (serum)	-	-	10 µL
Mix and incubate at room temperature (20-25^{0}C) for 10 minute			
Reagent 2**	1000 µL	1000 µL	1000 µL
Mix and incubate at room temperature (20-25^{0}C) for 10 minute			
Read absorbance of sample and standard against the blank at 578 nm in a spectrophotometer			

**Reagent 1A containing sodium salicylate & sodium nitroprusside (color reagent), and urease (enzyme reagent)*

***Reagent 2 containing sodium hypochlorite & sodium hydroxide (base reagent)*



$$A_{sample} = (A_{sample})^* - A_{blank}$$

$$A_{standard} = (A_{standard})^* - A_{blank}$$

	Calculation : Urea (mg/dL) = A.sample / A.standard X Standard (mg/dL) Note : concentration of standard = 50 mg/dL **Conversion factor :** Urea (mg/dL) x 0.467 = BUN (mg/dL)
	Student Task: 3. Perform the procedure 4. Conclude the result
G	**Homework:** 1. What is the principle of Gerhardt's Test? 2. What is the principle of Rothera's Test? 3. What is the difference between ureum and BUN? 4. What is the principle of spectrophotometer? 5. Mention conditions when we could find keton bodies in urine! 6. Explain the urea cycle!
H	**References** **1. Harper's Biochemistry 28th ed.** **2. Devlin Biochemistry** **3. Lippincott's Biochemistry**

Chapter III
Genetic Information

There has been a growing recognition of the influence of genetic factors in human disease. The appreciation of the importance of inherited components of common diseases, congenital malformations, and cancer has increased substantially in recent years. At the same time, revolutionary developments have occurred in the basic science of genetics. Major efforts have focused on the application of molecular genetics to understanding heritable disease, and extraordinary progress has been made to use these advances in the practice of medicine. The approaches to diagnosis, genetic counseling, and screening of individuals at risk for genetic disease have been revolutionized by the application of molecular genetics.

The scope of molecular genetics extends from the structure of genes to the functioning of their products in a cell. This field is dominated by powerful and rapidly changing technology involving the manipulation of DNA, RNA, and protein, resulting in a constant interchange between new insights in basic science and application to medical problems. A fundamental goal of molecular genetics is to identify a heritable disease at the level of the affected gene and to chemically define the precise mutation. Once the mutation has been identified, efforts are made to understand what impact it has on the functioning of the cell, tissue, organ, and organism. The mutation is traced from DNA to the corresponding RNA copies of the gene, to the protein translated from the RNA. Studies at this level of the effects of mutations generally provide novel insights into the biologic design of the normal cellular constituents.

In this laboratory activity it is expected that students can improve their understanding on genetic information and implement it in clinical setting using clinical problem of a major thalassemic patient. Some techniques in molecular biology will also be practiced and introduced to give the students more insight into the advancement of biomolecular technology.

In this chapter students have to learn about DNA structure and packaging, central dogma and mutation

LABORATORY ACTIVITY 3

Resource Person : Dr.Lelly Yuniarti, S.Si.,M.Kes
Subject : DNA isolation
Department : Biochemistry

A	Sequent			
	I	Introduction	:	40 menit
	II	Pretest	:	5 menit
	III	Laboratory activity	:	120 menit
	IV	Post test	:	5 menit
B	Topic			
	Date:			
	4. DNA Isolation 5. Polymerase Chain Reaction (PCR)		: : :	80 menit 40 menit
C	Venue			
	Biomedical Laboratory, Faculty of Medicine, Unisba, Jl. Tamansari No.22 Bandung 40116			
D	Equipment			
	1	DNA isolation	1. 1 ml EDTA blood 2. RBC (red blood cell) lysis solution (150 mM NH_4Cl, 0.5 mM EDTA, 10 mM $KHCO_3$, KOH) 3. Cell lysis solution (10 mM Tris-HCl pH 8.0, 25 mM EDTA, 0.5 % SDS) 4. RNAse (10 mg/ml) 5. Ammonium acetate 5M 6. Isopropanol 7. Ethanol 70 % 8. TE buffer **9.** Eppendorf tube	
	2	Polymerase Chain Reaction	- Animation of PCR video	
E	Pre-requisite/ Pretest			
	Before laboratory activity students should have initial knowledge about: 1.			
	Note: If the pre-test score less than 50, the student won't be allowed to follow laboratory activity			
F	Implementation			
	3. Students are divided into 18-20 groups 4. Each group is supervised by one tutor			

Activity 1 : DNA Isolation

Introduction

Extraction of chromosomal DNA from whole blood

DNA, the genetic substance in biological cells, is large polymer of the de-oxyribonucleotide monomers. This experiment introduces the student to a general method for isolation and partial purification of nucleic acids from white blood cells. The procedure consists of disrupting the cell wall or membrane, dissociating bound proteins, and separating the DNA from other soluble compounds. The isolated DNA is characterized and quantified by ultraviolet spectroscopy under native and denaturing conditions.

Isolation of DNA

Because of the large size and the fragile nature of chromosomal DNA, it is very difficult to isolate in an intact, undamaged form. Several isolation procedures have been developed that provide DNA in biologically active form, but this does not mean it is completely undamaged. These DNA preparations are stable, of high molecular weight, and relatively free of RNA and protein. Here, a general method will be described for the isolation of DNA in a stable, biologically active form from microorganisms. The procedure outlined is applicable to many microorganisms and can be modified as necessary.

Designing an isolation procedure for DNA requires extensive knowledge of the chemical stability of DNA as well as its condition in the cellular environment. The experimental factors that must be considered and their effects on various structural aspects of intact DNA are outlined below.

1. pH

(a) Hydrogen bonding between the complementary strands is stable between pH 4 and 10
(b) The phosphodiester linkages in the DNA backbone are stable between pH 3 and 12
(c) N-glycosyl bonds to purine bases (adenine and guanine) are hydrolyzed at pH values of 3 and less.

2. Temperature

(a) There is considerable variation in the temperature stability of the hydrogen bonds in the double helix, but most DNA begins to unwind in the range 80-90^{0}C.
(b) Phosphodiester linkages and N-glycosyl bonds are stable up to 100^{0}C.

3. Ionic Strength

(a) DNA is most stable and soluble in salt solutions. Salt concentrations of less than 0.1 *M* weaken the hydrogen bonding between complementary strands.

4. Cellular Conditions

(a) Before the DNA can be released, the bacterial cell wall must be lysed. The ease with which the cell wall is disrupted varies from organism to organism. In some cases (yeast), extensive grinding or sonic treatment is required, whereas in others (*B.subtilis)*, enzymatic hydrolysis of the cell wall is possible.
(b) Several enzymes are present in the cell that may act to degrade DNA, but the most serious damage is caused by the deoxyribonucleases. These enzymes catalyze the hydrolysis of phospodiester linkages
(c) Native DNA is present in the cell as DNA-protein complexes. The proteins (basic proteins called **histones**) must be dissociated during the extraction process.

5. Mechanical Stress on the DNA

(a) Gentle manipulations may not always be possible during the isolation process. Grinding, shaking, stirring, and other disruptive procedures may cause cleavage (shearing or scission) of the DNA chains. This usually does not cause damage to the secondary structure of the DNA, but it does reduce the length of the molecules.

Principle

Step 1. Disruption of the cell membrane and release of the DNA into a medium in which it is soluble and protected from degradation

The isolation procedure described here calls for the use of an enzyme, lysozyme, to disrupt the cell membrane. Lysozyme catalyzes the hydrolysis of glycosidic bonds in cell

wall peptidoglycans, thus causing destruction of the cell wall and release of DNA and other cellular components. The medium for solution of DNA is a buffered saline solution containing EDTA. DNA, because it is ionic, is more soluble and stable in salt solution that in distilled water. The EDTA serves at least two purposes. First, it binds divalent metal ions (Cd^{2+}, Mg^{2+}, Mn^{2+}) that could form salts with the anionic phosphate groups of the DNA. Second, it inhibits deoxyibonucleases that have a requirement for Mg^{2+} or Mn^{2+}. Citrate has occasionally been used as a chelating agent for DNA extraction; however, it is not an effective agent for binding Mn^{2+}. The mildly alkaline medium (pH 8) acts to reduce electrostatic interaction between DNA and the basic histones and the polycationic amines, spermine and spermidine. The relatively high pH also tends to diminish nuclease activity and denature other proteins.

Step 2. Dissociation of the protein-DNA complexes

Detergents are used at this stage to disrupt the ionic interactions between positively charged histones and the negatively charged backbone of DNA. Sodium decocyl sulfate (SDS), an anionic detergent binds to proteins and gives them extensive anionic character. A secondary action of SDS is to act as a denaturant of deoxyribonucleases and other proteins. Also favoring dissociation of protein DNA complexes is the alkaline pH, which reduces the positive character of the histones. To ensure complete dissociation of the DNA-protein complex and to remove bound cationic amines, a high concentration of a salt (NaCl or sodium perchlorate) is added. The salt acts by diminishing the ionic interactions between DNA and cations.

Step 3. Separation of the DNA from other soluble cellular components

Before DNA is precipitated, the solution must be deproteinized. This is brought about by treatment with choloform-isoamyl alcohol and followed by centrifugation. Upon centrifugation, three layers are produced: an upper aqueous phase, a lower organic layer, and a compact band of denatured protein at the interface between the aqueous and organic phases. Chloroform causes surface denaturation of proteins. Isoamyl alcohol reduces foaming and stabilizes the interface between the aqueous phase and the organic phases where the protein collects.

The upper aqueous phase containing nucleic acids is then separated and the DNA precipitated by addition of ethanol. Because of the ionic nature of DNA, it becomes insoluble if the aqueous medium is made less polar by addition of an organic solvent. The DNA forms a threadlike precipitate that can be collected by "spooling" onto a glass rod. The isolated DNA may still be contaminated with protein and RNA. Protein can be removed by dissolving the spooled DNA in saline medium and repeating the chloroform-isoamyl alcohol treatment until no more denatured protein collects at the interface.
RNA does not normally precipitate like DNA, but it could still be a minor contaminant. RNA may be degraded during the procedure by treatment with ribonuclease after the first or second deproteinization steps. Alternatively, DNA may be precipitated with isopropanol, which leaves RNA in solution. Removal of RNA sometimes makes it possible to denature more protein using chloroform-isoamyl alcohol. If DNA in a highly purified state is required, several deproteinization and alcohol precipitation steps may be carried out. It is estimated that up to 50% of the cellular DNA is isolated by this procedure. The average yield is 1 to 2 mg per gram of wet packed cells. The isolated DNA has a molecular weight on the order of 10×10^6.

Method

1. Add 300 ul blood + 900 ul (3 x volume blood) RBC Lysis Solution to Eppendorf tube. Invert the tube 2-3 times during the preparation. Incubate the sample for at least 10 minutes at room temperature. Do not allow the sample to sit in the RBC Lysis Solution for extended periods of time, this can be detrimental to the sample.
2. Centrifuge at room temperature at 13.000 – 16.000 rpm for 20 seconds. Remove the supernatant leaving behind the Visible layer of white blood cells pellet. Then repeat step 1 until virtually all of the red blood cells are gone. Then vortex the pellet to spread the cells into the remaining drops of supernatant.
3. Add 300 ul of Cell Lysis Solution to the centrifuge tube and right away pipette up and down to lyse the cells (make sure the solution is homogenous).
4. Add 1,5 ul of RNAse A (10 mg/ml) to the centrifuge tube. Mix the solution by inverting it several times and then incubate in a 37^0C water bath for 15 minutes. This can be run longer.
5. To precipitate protein, add 100 ul of Protein Precipitation Solution (5 M ammonium acetate) to the centrifuge tube and then vortex until the solution looks milky.
6. Centrifuge at 13.000 – 16.000 for 3 minutes. The precipitated proteins will form a light brown pellet. If a protein pellet is not visible, repeat step 5.
7. Then pour the supernatant containing the DNA into a clean eppendorf tube containing 300 ul ml of isopropanol, do this at room temperature.
8. Mix the tube by inverting 25-30 times until white DNA pellet becomes visible (make sure all the pellet becomes visible).
9. Centrifuge at 13.000 – 16.000 rpm for 1 minute. The DNA should be visible as a small white pellet.
10. Pour of the supernatant and add 300 uL ethanol 70%. Invert several times to wash the DNA
11. Spin again and pour off the 70% ethanol. Then let the DNA air dry. Use a cotton swab to remove the excess supernatant on the sides of the tube.
12. Rehydrate the DNA in 100 ul of TE. Place in 37 ^{0}C Water bath for 2 hours to get the DNA in solution. Make sure that it is a homogenous solution.
13. Store at -20^{0}C

Characterization of DNA

The DNA isolated in this experiment is of sufficient purity for characterization studies. DNA has significant absorption in the UV range because of the presence of the aromatic bases, adenine, guanine, cytosine, and thymine. This provides a useful probe into DNA structure because structural changes such as helix unwinding affect the extent of absorption. In addition, absorption measurements are used as indication of DNA purity. The major absorption band for purified DNA peaks at about 260 nm. Protein material, the primary contaminant in DNA, has a peak absorption at 280 nm. The ratio A_{260}/ A_{280} is often used as a relative measure of the nucleic acid/protein content of a DNA sample. The typical A_{260}/ A_{280} for isolated DNA is 1.9 A smaller ratio indicates increased contamination by protein.

Student task :

5. perform the electrophoresis procedure in group
6. conclude the result

Activity 2: Polymerase Chain Reaction

Introduction and Theory

The polymerase chain reaction (PCR) is an in vitro technique, which allows the amplification of a specific deoxyribonucleic acid (DNA) region that lies between two regions of known DNA sequence. The PCR is a tube system for DNA replication that allows a "target" DNA sequence to be selectively amplified, or enriched, several million-fold in just a few hours. Within a dividing cell, DNA replication involves a series of enzyme-mediated reactions, whose end result is a faithful copy of the entire genome. Within a test tube, PCR uses just one indispensable enzyme – DNA polymerase – to amplify a specific fraction of the genome.

During cellular DNA replication, enzymes first unwind and denature the DNA double helix into single strands. Then, RNA polymerase synthesizes a short stretch of RNA complementary to one of the DNA strands at the start site of replication. This DNA /RNA heteroduplex acts as a "priming site" for the attachment of DNA polymerase, which the produces the complementary DNA strand. During PCR, high temperature is used to separate the DNA molecules into single strands, and synthetic sequences of single-stranded DNA (20-30 nucleotides) serve as primers. Two different primer sequences are used to bracket the target region to be amplified. One primer is complementary to one DNA strand at the beginning of the target region: a second primer is complementary to a sequence on the opposite DNA strand at the end of the target region.

To perform a PCR reaction, a small quantity of the target DNA is added to a test tube with buffered solution containing DNA polymerase, the four deoxyriobonucleotides building blocks of DNA, the buffer consists of KCl, Tris-$MgCl_2$, and oligonucleotide primers (left primer and right primer). The polymerase is the *Taq* polymerase, named for Thermus aquaticus, from which it was isolated. This enzyme remains stable at high temperatures.

The PCR mixture is taken through replication cycles consisting of:

1. **Denaturation.** One to several minutes at 94-96^{o} C, during which the DNA is denatured into single strands
2. **Annealing.** One to several minutes at 50-65^{o} C, during which the primers hybridize or "anneal" (by the way of hydrogen bonds) to their complementary sequences on either side of the target sequence; and
3. **Extension.** One to several minutes at 72^{o} C, during which the polymerase binds and extends a complementary DNA strand from each primer.

This replication cycle is taken through 20-30 cycles of amplification.

Amplification Refractory Mutation System (ARMS)

There are several PCR-based approaches to the analysis of known mutation, including amplification refractory mutation system (ARMS). ARMS is devised for rapid detection of the point site mutation in the gene. A typical ARMS assay comprises two PCRs, each conducted using the same substrate DNA, using 2 pairs of primers (4 specific primers) i.e., 2 control primers, 1 common primer and ARMS primer that consists of mutant type and wild type (normal). The amplification is same with other PCR (denaturation: 92^{o} C I min; annealing: 65 ^{o} C 1 min; extension: 72 ^{o}C 1.5 min) x 25 cycles and extra extension: 72 ^{o} C 3 min. The superiority of ARMS are:

1. More simple because radioactive labeled was not required
2. Point mutation, small insertion or deletion can be directly identified

3. Heterozygous and homozygous form can be distinguished directly without DNA parents' identification.

Polymerase Chain Reaction Techniques

An amplification refractory mutation system (ARMS) was devised for rapid detection of the point mutation in the globin gene, using 4 oligonucleotide primers in the same time to detect the suspicious site of mutation, i.e. IVS1-nt5.

Reagents:

a. Oligonucleotide primer

Control A primer contents of 24 nucleotides: 5'CAA TGT ACT ATG ACCT CTT TGC ACC'3

Control B primer contents of 25 nucleotides: 5'GAG TCA AGG CTG AGA AGA TGC AGG A3'

Common C primer contets of 20 nucleotides: 5'ACC TCA CCC TGT GGA GCC AC3'

IVS1-nt5 mutant primer contents of 30 nuclotide: 5'CTC CTT AAA CCT GTC TTG TAA CCT TGT TAG3'

IVS1-nt5 normal primer contents of 30 nuleotides: 5'CTC CTT AAA CCT GTC TTG TAA CCT TGT TAC3'

Variation of primers were used to detect mutant gene area, they were:

(Stock solution concentration for each primer: 1 μM)

IVS1-nt5 mutant: Control A primer
Control B primer
Control C primer
IVS1-nt5 mutant primer

IVS1-nt5 normal: Control A primer
Control B primer
Control C primer
IVS1-nt5 normal primer

b. PCR Buffer 10X

10mM Tris pH 8.3
50 mM KCl
1.5 mM $MgCl_2$
0.01% Gelatin

dNTP stock solution 10 X

Deoxynucleotide Triphosphates solution (Perkin Elmer-Cetus), neutralized by NaOH to pH 7.0

dNTP stock solution consists of :
50 mM dATP (deoxyadenin triphosphates)
50 mM dTTP (deoxytimin triphosphates)
50 mM dGTP (deoxyguanin triphosphates)
50 mM dCTP (deoxycytosin triphosphates)

c. *Taq* Polymerase (Perkin Elmer-Cetus)

Stock solution: 0.50 U

d. ddH_2O

All solution (1a- 1e) were stored at –20^oC

e. Mineral oil (Paraffin Liquid)

Procedure:

1. Make PCR mix for (in cold condition):

a. Mutant

PCR mix in eppendorf tube 0.5 ml consists of:

PCR buffer solution 10 X	= 2.5 µl
dNTP solution 10 X	= 2.0 µl
Primer: control A primer	= 1.0 µl
control B primer	= 1.0 µl
control C primer	= 1.0 µl
IVS1-nt5 mutant	= 1.0 µl
Taq polymerase 0.5 U	= 0.2 µl
Genomic DNA	= 1.0 µl
Sterilized ddH_2O	= 15.0 µl
Total	= 25.0 µl

Normal

PCR mix in eppendorf tube 0.5 ml consists of:

PCR buffer solution 10 X	= 2.5 µl
dNTP solution 10 X	= 2.0 µl
Primer: control A primer	= 1.0 µl
control B primer	= 1.0 µl
control C primer	= 1.0 µl
IVS1-nt5 normal	= 1.0 µl
Taq polymerase 0.5 U	= 0.2 µl
Genomic DNa	= 1.0 µl
Sterilized ddH_2O	= 15.0 µl
Total	= 25.0 µl

For each detection make blank PCR mix by changing genomic DNA with sterilized ddH_2O, while other reagents composition are same.

2. Add 25 µl paraffin liquid or mineral oil to 25 µl PCR mix, in order to avoid evaporation while incubate in high temperature
3. Centrifuge the solution at 5000 rpm in 30 seconds
4. Put all eppendorf tubes that filled by PCR mix into PCR machine or *Programmable Thermal Controller*. The amplification program is:

Denaturation : 93^o C in 1 minute
Annealing : 65^o C in 1 minute 25 cycles
Extension : 72^o C in 1.5 minutes

Extra extension: 72^o C in 3 minutes, than 4^o C to store the PCR product.

Agarose gel electrophoresis

Resolution of large DNA fragments on agarose gels.

Materials

1. Electrophoresis buffer TAE or TBE (50 x stock solution, pH ~ 8.5: 242 g Tris base, 57.1 ml glacial acetic acid, 37.2 g $Na_2EDTA.2H_2O$, and H_2O to 1 liter)

2. Ethidium bromide solution (1000 x stock solution, 0.5 mg/ml: 50 mg ethidium bromide, 100 ml H_2O. Working solution, 0.5 μg/ml: dilute stock 1:1000 for gels or stain solution)
3. Agarose gel (~1 % agarose in 1x TAE or TBE buffer)
4. 10 x loading buffer (20% (w/V) Ficoll 400, 0.1 M Na_2EDTA, pH 8.0, 1.0% (w/v) SDS, 0.25% (w/v) bromphenol blue and 0.25% (w/v) xylene cyanol)
5. Horizontal gel electrophoresis apparatus
6. Gel casting platform
7. Gel combs (slot formers)
8. DC power supply

Method

1. Prepare the gel, using electrophoresis buffer and electrophoresis-grade agarose (see the Table below) by melting in a microwave oven or autoclave, mixing, cooling to 55^0C pouring into a sealed gel casting platform, and inserting the gel comb. Ethidium bromide can be added to the gel and electrophoresis buffer at 0.5 μg/ml.

CAUTION: Ethidium bromide is a potential carcinogen. Wear gloves when handling.

2. After the gel has hardened, remove the seal from the gel casting platform and withdraw the gel comb. Place into an electrophoresis tank containing sufficient electrophoresis buffer to cover the gel ~ 1 mm

Table: Appropriate Agarose Concentrations for Separating DNA Fragments of Various Sizes

Agarose (%)	Effective range of resolution of linear DNA fragments (kb)
0.5	30 to 1
0.7	12 to 0.8
1.0	10 to 5
1.2	7 to 0.4
1.5	3 to 0.2

3. Prepare DNA sample with an appropriate amount of 10x loading buffer and load samples into wells with a pipettor. Be sure to include appropriate DNA molecular weight markers.
4. Attach the leads so that the DNA migrates to the anode or positive lead and electrophoresis at 1 to 10 V/cm of gel
5. Turn off the power supply when the bromphenol blue dye from the loading buffer has migrated a distance judged sufficient for separation of the DNA fragments
6. Photograph a stained gel directly on an UV transilluminator or first stain with 0.5 μg/ml ethidium bromide 10 to 30 min, destaining 30 min in water, if necessary.

Student task: Student watch the animation video of PCR and tutor leads the discussion

F **Homework:**

1. What tissue could be the source for DNA analysis?
2. What is the purpose of DNA isolation?
3. What is the function of PCR? How are the steps?
4. What are the functions of DNA polymerase enzyme and dNTP?

G	**References** 7. ..... 8. ..... 9. .....

Chapter IV
Basic Principles of Hereditary

This chapter is about the principles of heredity: how genes are passed from generation to generation. These principles were first put forth by Gregor Mendel, so we begin by examining his scientific achievements. We then turn to simple genetic crosses, those in which a single characteristic is examined. We learn some techniques for predicting the outcome of genetic crosses and then turn to crosses in which two or more characteristics are examined. We will see how the principles applied to simple genetic crosses and the ratios of offspring that they produce serve as the key for understanding more complicated crosses. The end of the chapter by considering statistical tests for analyzing crosses and factors that vary their outcome. Throughout this chapter, several concepts are interwoven: Mendel's principles of segregation and independent assortment, probability, and the behavior of chromosomes. These might at first appear to be unrelated, but they are different views of the same phenomenon, because the genes that undergo segregation and independent assortment are located on chromosomes.

Mendel: The Father of Genetics

In 1902, the basic principles of genetics, which Archibald Garrod successfully applied to the inheritance of alkaptonuria, had just become widely known among biologists. Surprisingly, these principles had been discovered some 35 years earlier by Johann Gregor Mendel (1822–1884). Mendel was born in what is now part of the Czech Republic. Although his parents were simple farmers with little money, he was able to achieve a sound education and was admitted to the Augustinian monastery in Brno in September 1843. After graduating from seminary, Mendel was ordained a priest and appointed to a teaching position in a local school. He excelled at teaching, and the abbot of the monastery recommended him for further study at the University of Vienna, which he attended from 1851 to 1853. There, Mendel enrolled in the newly opened Physics Institute and took courses in mathematics, chemistry, entomology, paleontology, botany, and plant physiology. It was probably here that Mendel acquired the scientific method, which he later applied so successfully to his genetics experiments. After 2 years of study in Vienna, Mendel returned to Brno, where he taught school and began his experimental work with pea plants. He conducted breeding experiments from 1856 to 1863 and presented his results publicly at meetings of the Brno Natural Science Society in 1865. Mendel's paper from these lectures was published in 1866. Despite widespread interest in heredity, the effect of his research on the scientific community was minimal.

Mendel's Success

Mendel's approach to the study of heredity was effective for several reasons. Foremost was his choice of experimental subject, the pea plant *Pisum sativum* which offered clear

advantages for genetic investigation. It is easy to cultivate, and Mendel had the monastery garden and greenhouse at his disposal. Peas grow relatively rapidly, completing an entire generation in a single growing season. By today's standards, one generation per year seems frightfully slow fruit flies complete a generation in 2 weeks and bacteria in 20 minutes but Mendel was under no pressure to publish quickly and could follow the inheritance of individual characteristics for several generations. Had he chosen to work on an organism with a longer generation time horses, for example, he might never have discovered the basis of inheritance. Pea plants also produce many offspring their seeds which allowed Mendel to detect meaningful mathematical ratios in the traits that he observed in the progeny. The large number of varieties of peas that were available to Mendel was also crucial, because these varieties differed in various traits and were genetically pure. Mendel was therefore able to begin with plants of variable, known genetic makeup able to begin with plants of variable, known genetic time, no one seems to have noticed that Mendel had discovered the basic principles of inheritance. In 1868, Mendel was elected abbot of his monastery, and increasing administrative duties brought an end to his teaching and eventually to his genetics experiments.

He died at the age of 61 on January 6, 1884, unrecognized for his contribution to genetics. The significance of Mendel's discovery was unappreciated until 1900, when three botanists Hugo de Vries, Erich von Tschermak, and Carl Correns began independently conducting similar experiments with plants and arrived at conclusions like those of Mendel. Coming across Mendel's paper, they interpreted their results in terms of his principles and drew attention to his pioneering work.

In this chapter students, must learn about principle of hereditary, gene, chromosome, Mendel law, pedigree, and population genetics

LABORATORY ACTIVITY

Recourse Person : Arief Budi Yulianti

Subject : The principles of Hereditary

Department : Medical Biology and Histology

A	Sequent			
	I	Introduction	:	30 Minutes
	II	Pre-test	:	10 Minutes
	III	Lab. Activities	:	100 Minutes
	IV	Post test	:	10 Minutes
B	Topic			
	December, 2016			
		1. Blood type examination	:	40 Minutes
		2. Karyotype	:	15 Minutes
		3. Pedigree	:	15 Minutes
		4. Allele frequency	:	30 Minutes
C	Venue			
	Biomedical Laboratory, Faculty of Medicine, Unisba, Jl. Tamansari No.22 Bandung 40116			
D	Equipment			
1	Blood type examination	Blood type Kits		
2	Karyotype	Pictures of normal karyotype (man and women)		
3	Pedigree	1. Autosomal (dominant, recessive) 2. X-link (dominant, recessive)		
4	Population genetics	1. Colored buttons 2. Box/Baker glass		
E	Home work			
	Before laboratory activities students must prepare: 1. Describe about blood type examination?			

	2. Explain about karyotype 3. Explain about pedigree 4. Describe about allele frequency
F	Lab. Activities
	1. The Students were divided into six group 2. Each group do lab. Activities accompanied by tutor
	Lab. Ac1:

Blood type examination:

- Wash your hand according WHO step wash hand
- Take hand glove
- Card blood group test lay in flat surface
- Take the tiny lancet
- Make sure the students whit cleanly hands
- Take a alcohol swab and place the lancet against the end of the finger and press the green body against your finger to release the needle
- Massage the finger from the bottom to the top to encourage blood flow and press the blood towards fingertip. Repeat pressing until a drop with a 3 to 4 mm (1/8 inch) diameter is seen
- Then follow the steps indicated on Kit
- Determination the students blood group type

Student task:

- Collect all of blood type in your group members
- Calculate the allele A, B, and O frequency in your groups
- Calculate the allele A, B, and O frequency in your class

Lab. Ac2

Karyotype

Somatic (body) cells in humans have 46 chromosomes. Both males and females normally have 23 pairs of chromosomes, known as **autosomes**, and a pair of unequal length in males. The larger chromosome of this pair is the **X chromosome** and the smaller is the **Y chromosome**. These are called the **sex chromosomes** because they contain the genes that determine sex. Thus, the sex chromosomes are either XY (males) or XX (females). The members of a pair have the same size, shape, and constriction (location of the centromere), and they also have the same characteristic banding pattern upon staining (except in males). The resulting display of pairs of chromosomes is called a **karyotype**. To view the chromosomes, a cell can be photographed just prior to division so that a picture of the chromosomes is obtained.

Principle:

A comparatively simple, rapid, and reliable method for chromosome analysis has been

developed by culturing leukocytes obtained from peripheral blood.

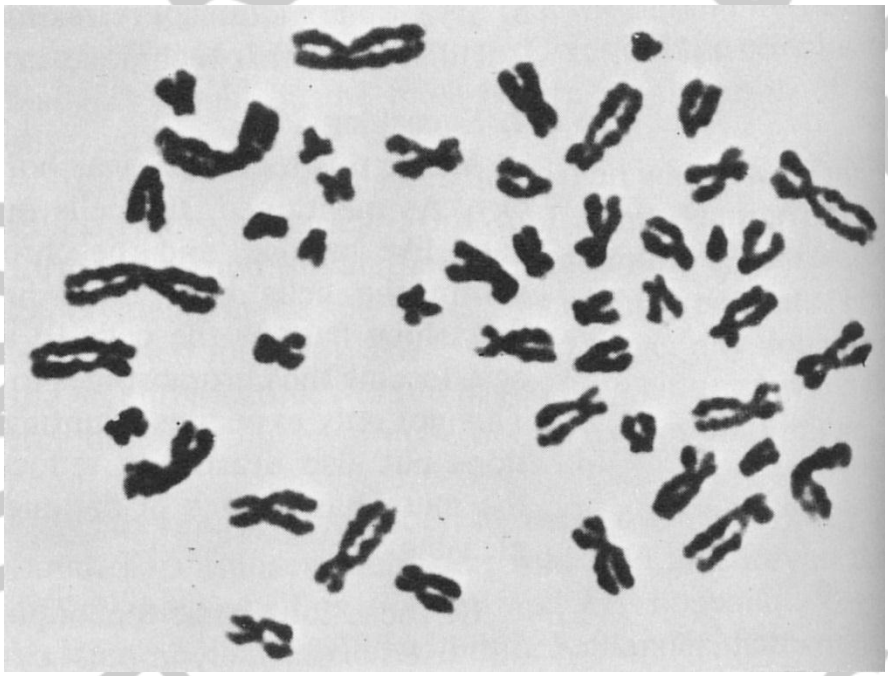
Normally, these blood cells do not undergo mitotic activity in circulation nor do they do so readily in vitro. Nowell, observed mitotic activity in mononuclear cells from peripheral blood of normal subjects after a lag period of 48 to 72 hours in culture. It soon became apparent that an important factor responsible for the high degree of mitotic activity attained was the presence of a phytohemagglutinin (PHA) extracted from the red kidney bean, *Phaseolus vulgaris*, in the culture system. When PHA, a mucoprotein, is added to a heparinized blood specimen, many of the leukocytes separated from the agglutinated erythrocytes will enter mitosis in culture.

Under normal circumstances dividing cells in peripheral blood cultures are entirely lymphocytes, which apparently are the only cells responsive to the mitogenic activity of PHA.

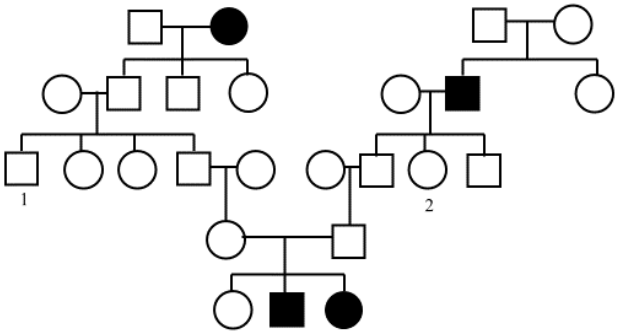
In essence, lymphocytes from a 10 ml sample of heparinized venous blood are separated from erythrocytes by hemagglutination with PHA and centrifugation and incubated for 3 days in appropriate culture medium. Following the addition of colchicine and hypotonic treatment, a sufficient number of mitotic mononuclear cells arrested in metaphase are then available for chromosome analysis. To spread the chromosomes in a single plane on glass object.

Students task:

1. Prepared the photography of the spreading of chromosomes from the single lymphocyte.
2. Cut with scissors every chromosome and arrange according to Denver Classification of Human Chromosomes on the karyotype card.
3. Autosomes were numbered from 1 to 22 in descending order, the longest in length being no.1 and the shortest no. 22. Sex chromosomes, it was decided, would retain the designations X and Y instead of being given a number.
4. Autosomes were classified into seven groups by the criteria given below:
   - Group A (1-3): Large chromosomes with approximately median centromeres; readily distinguished from each other by size and centromere position.
   - Group B (4-5): Large chromosomes with submedian centromeres; difficult to distinguish, but 4 is slightly longer.
   - Group C (6-12): Medium-sized chromosomes with submedian centromeres; X chromosome resembles the longer chromosomes in this group, especially chromosome 6, from which it is difficult to distinguish.

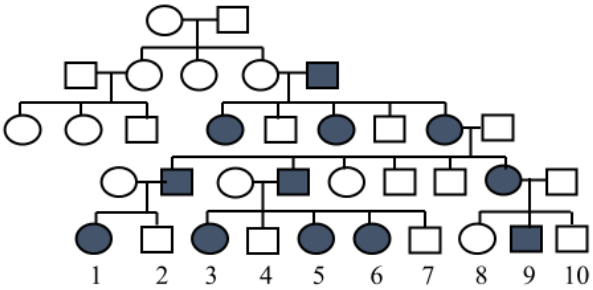
	– Group D (13-15): Medium-sized chromosomes with nearly terminal centromeres ("acrocentric" chromoosome) – chromosome 13 has prominent satellites on the short arms; chromosome 14 has small satellites on the short arms; no satellites have been detected on chromosome 15. – Group E (16-18): Rather short chromosomes with approximately median (in chromosome 16) or submedian centromeres. – Group F (19-20): Short chromosomes with approximately median centromeres. – Group G (21-22): Very short, acrocentric chromosomes – chromosome 21 has satellites on its short arms; Y chromosome is similar to this chromosomes. 
	Lab. Ac3. Pedigree

Student's task



Write the genotypes in every possible place.
If individuals 1 and 2 marry, what is the probability that their first child will be albinism?

Student's task



a. What is the most likely mode of inheritance ?

b. What would be the outcomes of the cousin marriages 1 x 9, 1 x 4, 2 x 3, and 2 x 8 ?

Population genetics

Natural selection deals with the idea that those individuals within a population with genes that make them better adapted to their surroundings are more likely to survive, reproduce, and pass their genes on the next generation. In biological terms, *nature selects* those individuals *most fit* to survive. Over time, the process of natural selection leads to an increase in the frequency of "good," or beneficial, genes in a population and a decrease in the frequency of "bad," or harmful, genes. New alleles for a gene come into being because of **mutation**, which is a change in the DNA of an individual. Mutations may have no effect, be beneficial, or cause harm.

There are many degrees of injury caused by harmful alleles. Some such as nearsightedness, may cause minor inconveniences but could shorten life because of an inability to see well (auto accidents). Others, such as the allele for sickle-cell anemia, may cause severe symptoms and shorten life for many people. Some harmful alleles, such as the allele that causes Tay-Sachs disease, are lethal in the homozygous recessive condition. **Homozygous recessive** individuals contain 2 recessive alleles for a characteristic. All homozygous recessive individuals with 2 Tay-Sachs alleles die. Severely harmful alleles are always rare in a population; they have a low allele frequency. The **allele frequency** is a mathematical statement about how common a specific allele is in the population. The study of the change in allele frequency in populations is known as **population genetics.**

In this exercise, we will use an artificial population to follow the frequency of a harmful allele over several generations. We will assume that there are two alleles present in the population: a dominant normal allele and a **recessive lethal allele**. Those individuals who inherit

2 recessive alleles must get 1 from each of its parents. When organisms receive 2 recessive lethal alleles, they die without reproducing and do not pass on their alleles to the next generation. Therefore, you might expect the number of recessive lethal alleles in the population to decrease over several generations.

The allele frequency of the recessive lethal allele should decrease as the recessive lethal allele becomes less common and the dominant normal allele becomes more common. In this exercise, we will conduct a **simulation** (an artificial setup that approximates the real situation) to determine how natural selection will affect the allele frequency of a recessive lethal allele in a population.

During this exercise, you will:

1. Use black buttons to represent the dominant normal alleles and white buttons to represent the recessive lethal alleles in a population.

2. Simulate sexual reproduction and natural selection over 10 generations.

Procedure

Generation
1

Use black buttons to represent the normal dominant allele (one form of a gene) and white buttons to represent the recessive lethal allele. Black = *A*; white = *a*

1. Set up a gene pool of 100 genes (80 black alleles and 20 white alleles). The **gene pool** is all the genes in the population of a species. Use the box/bag provided as a container for your buttons
2. Shake the buttons in the container and randomly draw two buttons at a time (to simulate

the allele combinations in the individuals) and set out the pairs of buttons on your bench top. The mixing of the buttons simulates the mixing of genes that occurs during sexual reproduction because of meiosis and random mating. The buttons "pairs" you've just drawn from the box/bag represent the individual organisms of the first generation of organisms.

3. Tally the "allelic" combinations you just drew out of the container. We count the buttons two- at-a-time because diploid individuals inherit 2 alleles, 1 from each parent. As you count, you will have three possible combinations: two black buttons (*AA*), black and a white buttons (*Aa*), or two white buttons (*aa*). Also tally the total number of "A" and "a" alleles.

Genotype	**Phenotype**
AA	Organism lives
Aa	Organism lives
aa	Lethal; organism dies

4. Two white buttons represent a lethal combination. This organism will die. Simulate the deaths of these homozygous recessive individuals by removing them from the population. (Take the two white buttons out of your population and separate them from the remaining buttons). After you have examined all the pairs of beans,
   a. determine the number of red beans remaining.
   b. determine the total number of beans remaining.
   c. record your results in Tables follows

Allele Frequency Data

Generation	**% white buttons at Start of Run**	**Number of white buttons at End of Run**	**Total Number of buttons at End of Run**	**New Allele Frequency** **% white buttons at End of Run** **White ÷ Total**
1				
2				
3				
4				

5				
6				
7				
8				
9				
10				

d. calculate the percentage of white buttons remaining in your population (number of **white** buttons remaining divided by the total number of buttons remaining).

% white buttons remaining = total white buttons in population/total buttons in population

e. record this new allele frequency in the last column of above Table
f. Prepare to generations 2-10
   a) To begin the next generation, we will assume that the survivors reproduce and thus the population will grow. In many populations of organisms, the number of individuals being born and the number of those dying are about the same and the population is considered stable. We will assume that in our hypothetical "button" population the reproductive capability of the organisms keeps the population of total alleles at 100 for each generation and that the surviving alleles (black and white buttons) reproduce equally well. You have already calculated the percentage of white buttons remaining after the first generation (percentage means number out of 100). Therefore, your second generation starts with 100 buttons but with the ***new percentage of white buttons you have just calculated***. To bring the total number of beans back up to 100, while maintaining the new percentage of black buttons:
   b) do the following:
      a) Count the white buttons remaining in the container.
      b) Add enough white buttons to the container so that the ***total number of white buttons*** is equal to the ***percentage of white buttons you calculated in Table***

c) After you have added the black buttons, add enough black buttons have a total population of black and white buttons equal 100 as total buttons.
d) Starting with the newly reproduced population which is the new generation 2
   - Shake the buttons and count off the buttons, two by two.
   - Remove the homozygous recessive lethal organisms (those with two white buttons), as you did for the first generation.
   - Calculate the percentage of white buttons remaining among the survivors, as you did after generation 1.
   - Record your data in Table above
   - Repeat this process until you have completed 10 generations.
   - Graph the results of your simulation on the graph paper provided (Generation time vs. Allelic frequency at the end of each generation

References:

1. Lewis R, Human Genetics 10th Edition, Textbook PDF download, 2014
2. Pierce B, Genetics Conceptual approach 2nd Edition, Textbook PDF download, 2005
3. Dean L, Blood Groups and Red Cell Antigens, Bethesda (MD): National Center for Biotechnology, NCBI Bookshelf. A service of the National Library of Medicine, National Institutes of Health, Information (US), 2005.