UNIT 8: *DNA and Human Genetics*

Laboratory Objectives

After completing this exercise, you should be able to describe or answer:

1. What DNA stands for and what is the building block of DNA?
2. Composition of a Nucleotide.
3. Four nucleotides and complementary base pairing
4. Gene, genome, allele, heterozygous, homozygous
5. Genotype, phenotype, dominant, recessive
6. Where DNA is located in the cell.
7. What is the purpose of using detergent for DNA extraction?
8. What is the purpose of using alcohol for DNA extraction?
9. Principle of DNA profiling
10. Interpret the results of the DNA profile.

Introduction

DNA & NUCLEOTIDES

All living organisms contain thread-like strands of **deoxyribonucleic acid (DNA)**, which carry and transmit heredity information. All DNA is composed of repeating units called **nucleotides** which consist of a **sugar** (deoxyribose), a **phosphate** group, and a nitrogen-containing **base** (either **adenine**, **thymine**, **cytosine**, or **guanine**; See Figure 8-1, A).

COMPLEMENTARY BASE PAIRING

Notice that guanine only pairs cytosine, and that thymine only pairs with adenine – this is referred to as **complementary base pairing** and make DNA in double-helix shape that was elucidated by **Watson and Crick** in the 1950s.

CHROMOSOME

Chromosomes are consists of the strands of DNA and associated proteins. In humans, each cell normally

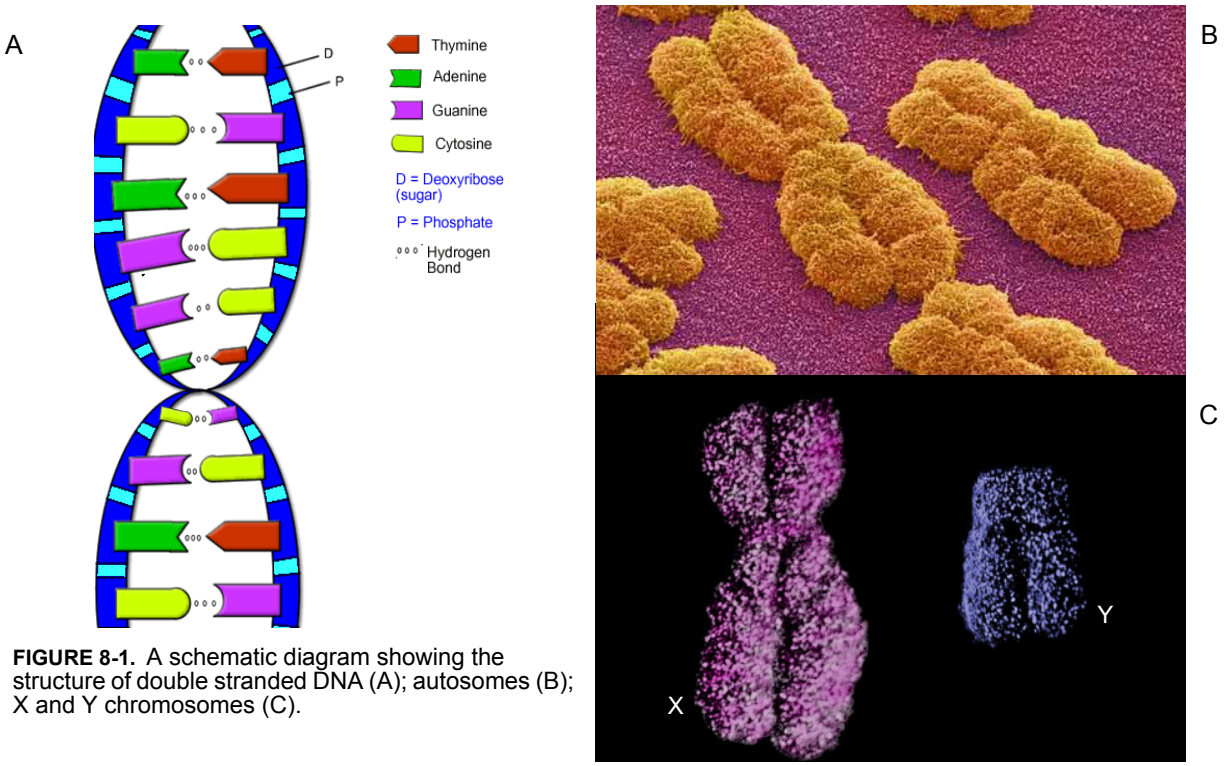


FIGURE 8-1. A schematic diagram showing the structure of double stranded DNA (A); autosomes (B); X and Y chromosomes (C).

contains 23 pairs of chromosomes, for a total of 46, except for the germ line cells (sperms and eggs). Twenty-two of these pairs, called autosomes, look the same in both males and females (See Figure 8-1, B). The 23rd pair, the sex chromosomes, differ between males and females (See Figure 8-1, C). Half of each chromosomal pair is inherited from the mother; the other half, from the father.

HOMOLOGOUS CHROMOSOME

The chromosome pairs that look same in both male and female are called homologous chromosomes (also called homologs or homologues). As one homologous chromosome is inherited from mother, and the other from father, they are not usually identical.

GENES & LOCUS

Each chromosome carries thousands of **genes** (units of heredity). **Genes are specific segments of DNA located on specific chromosomes**. For example, the gene for Alzheimer's disease is found on chromosome 21 and the gene for cystic fibrous is on chromosome 7. The specific locations of a genes (or DNA sequences) on a chromosome is called **loci** (singular, **locus**).

ALLELES, HOMOZYGOUS, HETEROZYGOUS

Each member of a gene pair are at the same loci on each of the homologous chromosomes, and the members can be alike or different. These different forms a gene can take are called **alleles** and can be either **homozygous,** if identical (*e.g.,* both genes are for blue eyes) or **heterozygous** if different (*e.g.,* one gene for blue eyes and the other for brown eyes).

GENOME

A **genome** is all of the genes that make up an organism's genetic information, and in a typical human that is nearly 30,000 genes that are responsible for producing our human traits.

Exercise 1. *Extraction And Observation of DNA*

DNA is located in the nucleus of all body cells. Therefore, DNA can be extracted from any tissue. Common sources include white blood cells, hair follicles, semen, saliva (which contains epithelial cells), and other body tissues.

DNA extraction from epithelial cells contained in a mouth wash is fairly simple. **Detergent** will break open the cells by destroying the **cellular membranes** as well as the **nuclear membranes** within the cells. The detergent also helps to strip away the associated proteins. Because **DNA is not soluble in alcohol**, whereas the rest of the cellular components are alcohol soluble, DNA **precipitates out of the solution** and collects at the interface of the soap and alcohol layer. Colder the alcohol, the less soluble DNA is in it. Individual strands of DNA are too small to be visible to the human eye (about 1 million strands will fit in the period at the end of this sentence).

NOTE: This experiment is to be carried out by each student.

1. Obtain one large glass tubes, label with your initials, and dispense 5 ml detergent solution into the glass tube from the dispenser bottle on the front supply station.
2. Obtain one (1) disposable plastic cup and, using the dispenser provided on the supply station, dispense 10 ml of 0.9% sterile saline solution into the cup.

NOTE: If you have just eaten, rinse your mouth with water before starting. Food chunks will not make your experiment go well.

3. Pour the saline solution from the cup into your mouth. Do not swallow, but rinse vigorously for 45 seconds. The vigorous swirling will allow a large number of cheek cells to slough off.
4. Carefully expel the contents from your mouth back into the plastic cup.
5. Pour the contents from the cup into the glass test tube containing the detergent solution for DNA extraction.
6. Using a coffee stirrer or wooden stick, stir the contents of the tube for three (3) minutes. Use a gentle motion to avoid forming bubbles.
7. After finishing 3-minute stirring, remove the stirrer, and incubate the tube in a water bath at 42°C for 30 min. Save the stirrer for later use.
8. When instructed to, retrieve your tube from the water bath.
9. Stir and mix the contents in the tube for more than 2 minutes, and let it stand for 30 minutes to cool down to room temperature.
10. When instructed to, obtain a squeeze bottle (one bottle per group) containing cold ethanol from -20°C freezer.
11. Carefully tilt the test tube at a 45° angle. Using the squeeze bottle, **slowly** add approximately 15 ml (equal volume of the contents in the tube) of ice cold ethanol by letting it **run down the inside wall of the test**

tube.

NOTE: ***Do not shake or mix the ethanol with the contents in the test tube.*** *The alcohol should form a layer on top of the DNA extraction solutions.*

12. Slowly return the tube in vertical position, place it in a test tube rack, and allow it to stand for 1 minute. Observe to see a white fluffy or stringy mass of DNA precipitate out of the solution in test tube. You will see bubbles formed by the vaporized ethanol.
13. Using a long needle probe with hooked end, slowly and gently retrieve the DNA. DNA mostly precipitates at the interface between DNA extraction solution and the alcohol.
14. Place the retrieved DNA on a clean slide and add a drop of Fast DNA Staining solution.
15. Place a coverslip and remove excess solution using a piece of Kimwipe.
16. Observe stained DNA under microscope (4X→10X→40X). DNA will be seen like a bundle of thread stained blue. Individual strands of DNA are too small to be visible under a compound light microscope.
17. Thoroughly wash the glass tube and place it up side down on the test tube rack. Rinse the used glass slide in a designated container. Dispose of the used coverslip in the broken glass container. **DO NOT discard coverslips in the trash can.**

Exercise 2. *Exploring Human Genetics*

GENOTYPE & PHENOTYPE

An individual's genetic makeup (**genotype**) influences that individual's physical characteristics (**phenotype** – what an individual looks like). A trait may be controlled by one pair of genes, or it may be controlled by more than one pair of genes. In many cases, one allele (**dominant allele** – represented by a capital letter 'A') will prevent or mask the expression of another allele (**recessive allele** – represented by a small letter 'a'). Recessive alleles are often only expressed when they are homozygous.

The following exercise has been designed to consider traits that are controlled by only one pair of genes, whereas, the majority of human traits are controlled by more than one gene.

Working with your lab partner(s), determine your phenotype for the traits discussed in the exercise. Record your phenotype and possible genotype in Table 8-1 on page 6.

1) Bent little finger

Examine your little finger on each hand. If the last joint of your little finger bends towards your ring finger, you have the dominant gene 'C' for bent little finger. A straight little finger gene 'c' is recessive. The condition of bent little finger is called *clinodactyly*.

2) Tongue rolling

Extend your tongue and attempt to roll it into a U-shape. The gene for tongue-rolling 'R' is dominant to the gene for non-tongue-rolling 'r'.

3) Widow's peak

Individuals with a V-shaped hairline in the middle of the forehead have a widow' peak which is dominant 'W' while a straight hairline is recessive 'w'.

4) Dimpled chin

The presence of a dimpled chin 'D' is dominant to the gene for a non-dimpled chin 'd'.

5) Free earlobe

If a portion of the earlobe remains unattached below the point of attachment to the head, you have a dominant gene 'E' for a free earlobe. The recessive gene 'e' results in attached earlobes.

6) Thumb-crossing

Swing your hands freely, and suddenly clasp your hands, interlocking your fingers. If your left thumb is uppermost, you possess the dominant allele 'T'. If your right thumb is uppermost, that is the result of the recessive allele 't'.

7) Hitchhiker's thumb

If you don't have the ability to bend your thumb back at a 60-degree angle, you are dominant for a straight thumb 'S'. If you can bend your thumb, you possess the recessive allele 's' and have hitchhiker's thumb.

8) Finger hair

If you possess hair on the middle segment of your fingers, you have the dominant allele 'H' for mid-digit hair. If you do not have hair, you are recessive for the 'h' allele.

9) Dimpled cheeks

The presence of dimples in one or both cheeks, 'D', is dominant to the absence of dimples 'd'.

10) Eyebrow raising

If you can raise your eyebrows you have the dominant allele 'Y'. If not, you have the recessive allele 'y'.

11) Ear wiggling

The gene for having the ability to wiggle your ears, 'W', is dominant to the non-wiggling, 'w'.

12) Long toe

Check your second toe. If it is longer than your big toe, it is a result of a dominant allele 'L'. A shorter toe is recessive 'l'.

13) Curly hair

Curly hair 'A' is dominant over straight hair 'a'.

14) Freckles

The presence of freckles results from the dominant allele 'Z'. The allele 'z' is recessive.

15)PTC tasting

Approximately 70% of the U.S. population has the ability to taste PTC (phenylthiocarbamide) impregnated in paper. Place a piece of PTC paper on your tongue. Record your results. PTC tasting comes from the dominant allele 'P'. Not tasting the PTC paper indicates a recessive allele.

TABLE 8-1. A Survey of some common human characteristics.

Total number of students in class: _____						
Traits	**Your phenotype**	**Possible genotypes**	**Number of Class phenotypes**		**% Class phenotypes[a]**	
			Dominant	**Recessive**	**Dominant**	**Recessive**
1) Bent little finger						
2) Tongue rolling						
3) Widow's peak						
4) Dimpled chin						
5) Free ear lobe						
6) Thumb crossing						
7) Hitchhiker's thumb						
8) Finger hair						
9) Dimpled cheeks						
10)Freckles						
11)Curly hair						
12)Eyebrow raising						
13)Ear wiggling						
14)Long toe						
15)PTC tasting						

a. % Class phenotypes = Number of Class phenotypes divided by the total number of students in class.

Exercise 3. *DNA Profile (DNA Fingerprint)*

Every human being has a **unique DNA pattern** (except for identical twins). Tissue samples containing DNA can be used for identification in criminal cases, in paternity suits, and in cases where visual identification is not possible. The technique used to make these genetic comparisons produces a **DNA profile** (also called a **DNA finger printing**). DNA for examination is isolated with a process similar to the method you used in the DNA spooling exercise.

Various genetic traits are produced on the basis of the genetic blueprint (DNA). However, not all of a person's DNA codes for synthesis of proteins. DNA molecules also have regions that do not serve as codes for genes. This **noncoding DNA** was once called "junk DNA" because scientists didn't understand or recognize the function for these sections of the chromosomes (see Figure 8-2). They originally believed that these sequences were present to simply fill the gaps between the genes. Although the functions for most noncoding regions of DNA have not yet been discovered, scientists are beginning to find evidence that some of these DNA sequences play important roles in cellular metabolism and inherited diseases.

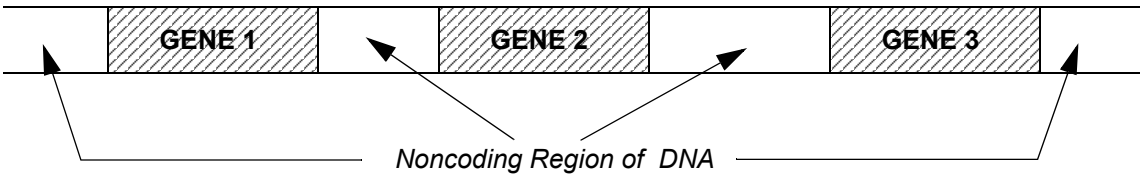


FIGURE 8-2. A schematic diagram showing genes (coding regions) and noncoding regions of DNA.

Regions of noncoding DNA vary a great deal among individuals, and when specific DNA regions are studied, scientists can use this information to establish human identity, analyze evolutionary trends, and determine predispositions to certain diseases.

The pattern of noncoding regions between any given two genes is unique and always has the same repeating pattern of nucleotides (for example **C A T**). *The number of times these nucleotides are repeated, however, is highly variable among people*. For example, as you can see in Figure 8-3, Individual A has nine repeating C A T segments, while Individual B has only five. Some people have dozens of repeats of the same pattern. This varying pattern among individuals is called **DNA profile** (or DNA fingerprint).

One of the original processes used to generate a DNA profile is called **RFLP analysis**, which stands for **restriction fragment length polymorphism**.

This technique is used to create a DNA profile (**restriction fragment**) and based on the fact that the lengths of fragments of noncoding regions are different in different people (This phenomenon is called **length polymorphism**). To make a reliable "RFLP match," molecular geneticists use several different noncoding DNA regions. When several different regions are compared, the odds against a coincidental match can be more than a billion to one.

To produce fragments of DNA, enzymes (called *restriction endonucleases*) are used to cut the DNA molecule at specific sites. These fragments vary in length from individual to individual due to the different number of times that a core sequence (CAT in Figure 8-3) is repeated.

A DNA segment of Individual A.

GENE 2	C A T C A T C A T C A T C A T C A T C A T C A T C A T C A T	GENE 3

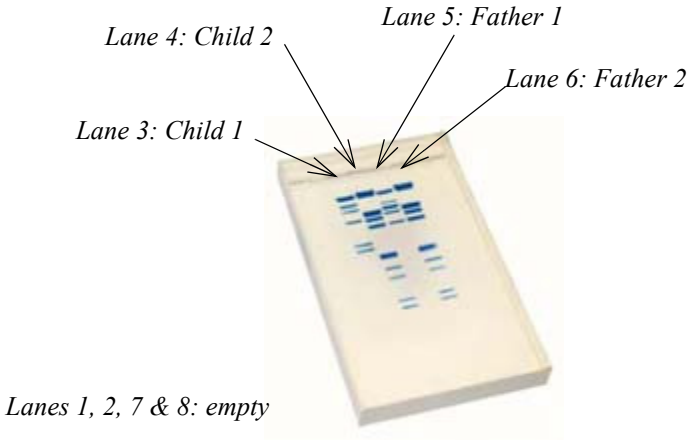
A DNA segment of Individual B.

GENE 2	C A T C A T C A T C A T C A T	GENE 3

FIGURE 8-3. Repeating noncoding DNA segments.

Fragments of DNA cut by restriction enzymes are separated using a technique called **gel electrophoresis**, in which the DNA pieces are separated based on their sizes. Smaller, lighter fragments migrate more easily through the gel and therefore travel farther in a given time period than larger, heavier fragments, forming separate bands across the gel, as you can see the molded gel on your lab bench.

In this simulation experiment, DNA was extracted from samples obtained from two children and two possible fathers. After the restriction fragments were prepared as described above, agarose gel electrophoresis was carried out and the resulting gel was permanently molded in silicon rubber as provided on your lab bench (Figure 8-4).



Lane No (from left to right)	Restriction Fragments of DNA obtained from:
1	Empty
2	Empty
3	Child 1
4	Child 2
5	Father 1;
6	Father 2
7	Empty
8	Empty

FIGURE 8-4. A simulation gel showing restriction fragments from 4 individuals for a paternity test.

The objective is to analyze and match the DNA fragment patterns and determine if Father 1 or Father 2 is the biological parent of the children. Each lane on the gel contains the following DNA fragments:

NOTE: When determining whether two DNA profiles are a match, all of the bands have to be the same. If even one band doesn't match, the profiles are not from the same individual.

Q 8-1. *How many unique DNA profiles were found on the gel?*

Q 8-2. *How many DNA bands are present in Lane 3 (Child 1).*

Q 8-3. *Did any of the DNA profiles match each other? If so, list them:*

Q 8-4. *Did Child 1 have a DNA profile matching either one of the fathers?*

Q 8-5. *Did Child 2 have a DNA profile matching either one of the fathers?*

Q 8-6. *What conclusion could you make on the paternal relationships among two children and two possible fathers?*

Q 8-7. *Suppose the two matching DNA profiles are from Child 1 and Child 2, respectively. What conclusion could you make on the relationship between two children? How the children are related two possible fathers?*

UNIT 8: DNA and Human Genetics

Lab Section #:_____ Lab Group #:_____

Name:_____

Q 8-1. How many unique DNA profiles were found on the gel? (p. 9)

Q 8-2. How many DNA bands are present in Lane 3 (Child 1). (p. 9)

Q 8-3. Did any of the DNA profiles match each other? If so, list them: (p. 9)

Q 8-4. Did Child 1 have a DNA profile matching either one of the fathers? (p. 9)

Q 8-5. Did Child 2 have a DNA profile matching either one of the fathers? (p. 9)

Q 8-6. What conclusion could you make on the paternal relationships among two children and two possible fathers? (p. 9)

Q 8-7. Suppose the two matching DNA profiles are from Child 1 and Child 2, respectively. What conclusion could you make on the relationship between two children? How the children are related two possible fathers? (p. 9)