INSTRUCTOR GUIDE

Experiment 1001. Anatomy and Evolution of the Genome.

I. Chemicals and Materials Provided

	Amount
Plasmid DNA - EcoR1 cut	130 µl
Phage Lambda DNA - Hind III cut	130 µl
Calf Thymus DNA - EcoR1 cut	130 µl
Calf Kidney DNA - EcoR1 cut	130 µl

The DNA is dissolved in electrophoresis sample buffer which contains glycerol and bromophenol blue.

II. Materials that are Needed but Not Provided

PROCELL Electrophoresis Unit and MB-170 Power Supply or equivalent
Accessory Kit or equivalent
Agarose, Electrophoresis Buffer and Gel Stain from Electrophoresis Package 3/4. Instructions for preparing the buffer and stain are provided with Electrophoresis Package 3/4.

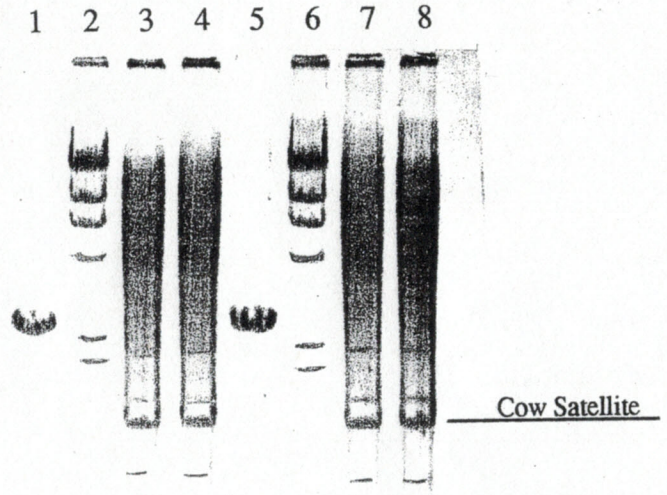
III. Laboratory Schedule

A few suggestions that can provide maximal utilization of laboratory time are given below.

Preparation of the agarose gels.

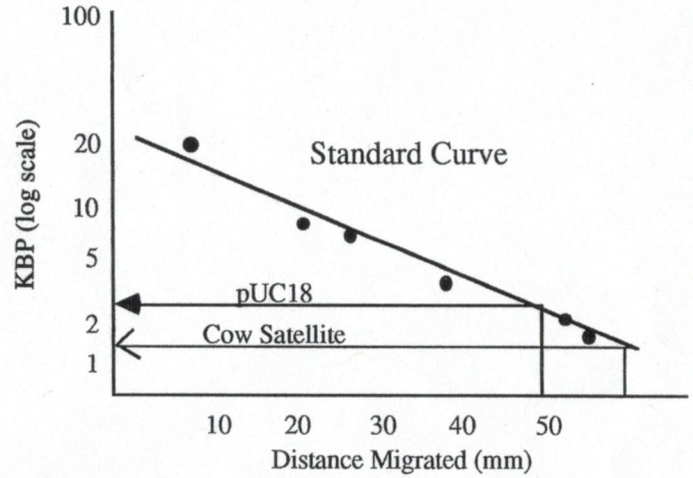
1. The agarose gels can be poured up to about one week before the laboratory session. If this option is chosen ,they should be wrapped in saran wrap with comb in place and stored in the refrigerator.
2. Agarose for the four 1.2% gels can be prepared in one operation by boiling 0.9 g of agarose with 75 ml of electrophoresis buffer in a 250 ml flask immersed in a beaker of water over a Bunsen burner. Alternatively, a microwave oven can be used. Fifteen ml of the agarose are then used to prepare each gel.

IV. Experimental Results: Picture of Stained Gel



V. Data Analysis and Answers to Study Questions

1.



2. Plasmid pUC18 contains about 2.69 KB (see the standard curve above).

3. The cow satellite is 1.40 KB in length.

4. 3.4 Å x 2,850 = 10.49 x 10^3 Å; 3.4Å x 1,400 = 4.76 x 10^3Å

5. 6.4 x 10^9 nucleotide pairs x 3.4 Å = 2.2 x 10^{10} Å = 2.2 meters DNA per cell x 10^{14} = 2.2 x 10^{14} meters (this distance is about 200 times greater than the distance from the earth to the sun).

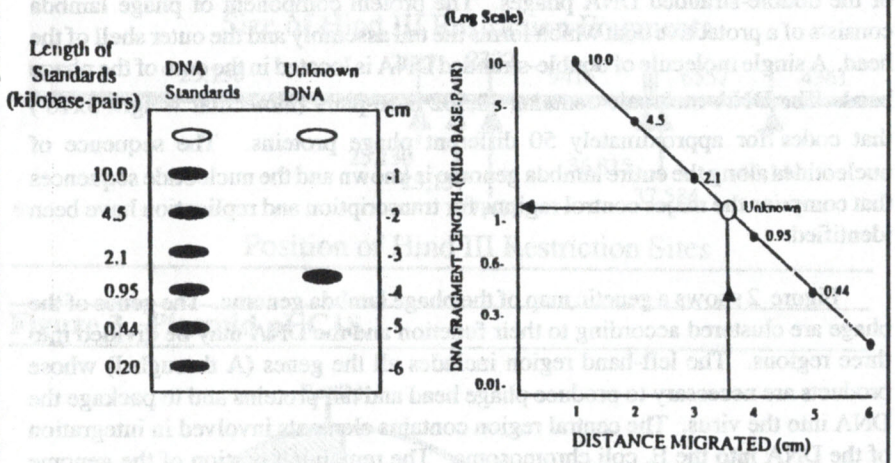
Experiment 1001. Anatomy and Evolution of the Genome.

Background Information

A. Determining the Length of DNA Molecules

A first step in the analysis of a DNA molecule in the molecular biology laboratory frequently involves the determination of its length in nucleotide pairs. Electrophoresis in agarose gels has proven to be an extremely useful tool for this purpose as the length of a given DNA fragment can be determined by comparing its electrophoretic mobility on agarose gels with DNA markers of known lengths. The smaller a DNA fragment, the more rapidly it moves during electrophoresis. As shown in Figure 1, a linear relationship is obtained if the logarithms of the sizes (in base-pair units) of the DNA fragments are plotted against their respective electrophoretic mobilities. The length of an unknown DNA fragment is then estimated from this calibration curve. In practice, DNA standards and unknown DNAs are electrophoresed on adjacent lanes of the same agarose gel. After electrophoresis, the positions of the standard and unknown DNA bands in the gel are determined and the size of the unknown calculated. The length of DNA is frequently given in base-pairs (BP) for small fragments and kilobase pairs (KB) for large ones. One kilobase-pair equals 1000 base-pairs. In this laboratory, you will use the procedure shown in Figure 1 to determine the size of DNA molecules.

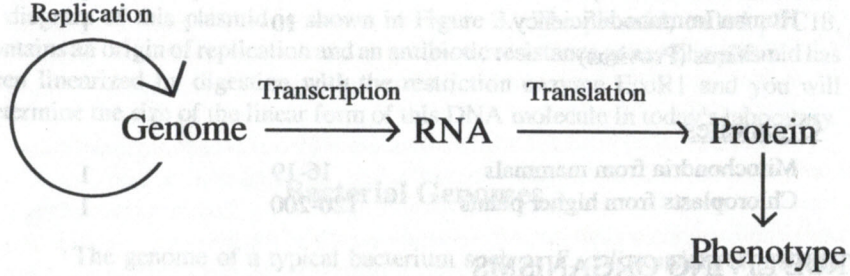
Figure 1. Determining the Length of DNA Molecules



Left: Six DNA standards and a DNA fragment of unknown length are electrophoresed on adjacent lanes of a gel and the distances (in cm) that each has migrated during the run are determined.
Right: The distances migrated by the standards are plotted against the logarithms of their lengths. The length of the unknown is determined by extrapolation from the standard graph. In the example, the unknown is about 1.5 KB.

B. The Genome

The genetic material of an organism is known as the genome. The genomes of all organisms direct two fundamental processes as diagrammed below. First, genes within the genome code for proteins and the proteins dictate phenotype by controlling cell structure and function . Second, the genome is self-replicating which provides genetic continuity through cell division and from one generation to the next.



Cellular organisms are frequently classified into two basic types: prokaryotic and eukaryotic. The prokaryotic plan is seen in bacteria and blue green algae. The prokaryotic cell lacks a discrete nucleus and the genome usually consists of a single circular DNA molecule that is compacted within the cell interior. The eukaryotic plan is observed in true algae, fungi, protozoans, and the cells of higher animals and plants. The genome of the eukaryotic cell is found in the nucleus where the DNA is portioned into a number of chromosomes. Genomes are also found in some groups of noncellular entities. These entities include viruses, plasmids, and certain cytoplasmic organelles that are found in eukaryotes including chloroplasts and mitochondria. This group shares with true living organisms the property that one generation gives rise to the next. Their genomes also contain genes which code for certain proteins that are responsible for their phenotype. However, these entities are dependent on true living organisms in that they can grow and reproduce only within a host prokaryotic or eukaryotic cell. Some examples of genome sizes are provided in Table 1 and a brief discussion of the genomes that you will study in this exercise is given below.

Eukaryotic Genomes

The genome of a higher eukaryote contains about 1,000 times more DNA than the genome of a bacterium (see Table 1). However, the actual number of different genes in the eukaryotic genome is only about 10-20 times more than is seen in the bacterial genome. Differences in the organization of the prokaryotic and eukaryotic genomes are responsible for the excess DNA that is observed in the eukaryotic nucleus. These differences are outlined in Table 2 and discussed below. First, most eukaryotic genes contain introns which are insertions of DNA that do not code for mRNA or protein. During transcription, these genes give rise to RNA species that are precursors to mRNAs. The intron sequences are then removed from the precursor and destroyed, and the remaining segments of the precursor are spliced together to form mRNA which can then be translated into protein. Genes in bacteria, viruses, and plasmids rarely contain introns. Thus , these genes are shorter than those seen in eukaryotes. Second, many protein coding genes are found in multiple copies within the eukaryotic genome. For example, there are approximately 10 - 20 growth hormone genes in humans. In contrast, nearly all genes in bacteria, viruses, and plasmids are present in only one copy per genome. Third, eukaryotic genes, unlike genes in the bacterial genome, are frequently separated by long stretches of nongenic DNA. Fourth, some of the nongenic DNA is comprised of DNA sequences that are repeated thousands or more times in the genome. Satellite DNA is one class of these repeated sequences and you will examine this class of sequences in today's laboratory.

Satellite DNA is not found in bacteria but is found in the genomes of nearly all eukaryotic organisms. In some vertebrates (e g., chickens and humans), satellite sequences occur in low concentrations. In other species (e.g., kangaroo rat and meal worm), these sequences can make up as much as 60% of the genome. The function of satellite DNA is not known. However, it is known that most satellites are not transcribed into RNA. In addition, most satellites are localized to discrete regions along the chromosomes. For example, many satellites are found in the centromeres of each chromosome. Centromeres are the sites of attachment of the chromosomes to the mitotic spindle and satellite DNA may play some rule in the attachment of the mitotic spindle to the chromosome. However, not all satellite DNA sequences are found within the centromere regions and there is no universally accepted hypothesis available for the function of these sequences.

Each differentiated cell in a multicellular organism contains the same number of chromosomes and the chromosome set appears identical in all cell types. As a general rule, the genome is also identical in all differential cells of the body. Thus, the genes that code for α and β polypeptide chains of hemoglobin are found in the same form in all cells including immature erythrocytes that produce hemoglobin and nonerythroid cells that do not. Likewise, the order of nucleotides or nucleotide sequence of satellite DNA is invariant in all cell types and this feature of the genome will be illustrated in today's laboratory exercise.

Table 2. Organization of Genomes

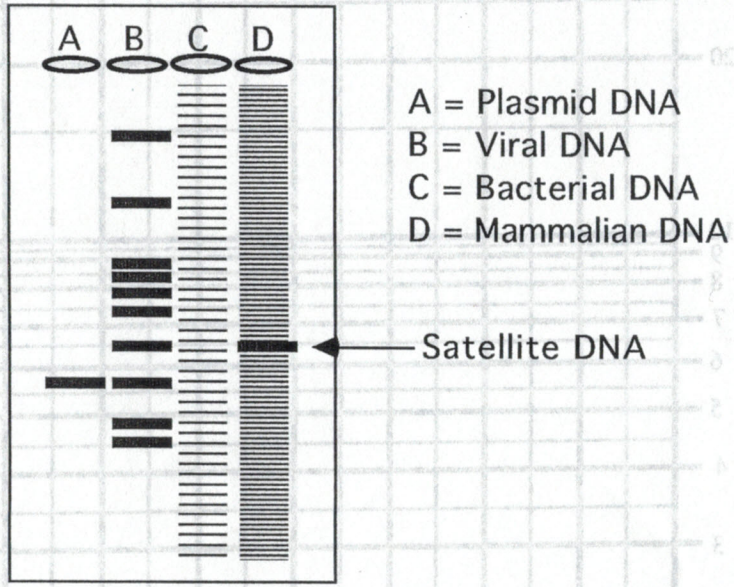
	Genome Type	
	Plasmid, Viral, and Prokaryotic Genomes	Eukaryotic Genomes
Genes		
Average Length	~ 1,000 bp	~5,000 bp
Introns	Rare	Common
Copies per Genome	Usually one	Often more than one
Spaces Between Genes	Usually small (less than 300 bp)	Usually large
Repeated Nongenic Sequences (e.g. DNA satellites)	Rare	Common

C. Description of this laboratory exercise

Figure 4 shows the results of an experiment similar to the one that you will perform in this laboratory. In the analysis, DNA from four sources was digested with EcoR1 and the fragments were then separated according to size by electrophoresis on an agarose gel. The DNA was obtained from A) a 4KB plasmid B) a 40 KB virus C) a bacterium with a genome size of 4×10^3 KB and D) a mammal with a genome size of 4×10^6 KB. The recognition site for EcoR1 is a specific sequence of 6 nucleotides. This recognition site is found, on average, once in every 4KB of genomic DNA. Consequently, one restriction fragment is derived from the genome of the plasmid, 10 from the genome of the virus, 10^3 from the bacterial genome, and 10^6 from the mammalian DNA. The small numbers of individual bands can clearly be distinguished in the digests of the plasmid and viral DNA's. However, the large number of fragments from the bacterial and especially mammalian genomes will

appear as smears on the gel lanes. Since there is essentially no repetitive DNA in the bacterial genome, each of the 1,000 bands will be of the same intensity. Thus, the smear on the gel lane will appear uniform. However, fragments derived from highly repeated sequences in the mammalian genome will form discrete bands which are superimposed on the background smear of the large number of different size fragments. Thus, highly repeated sequences like satellites are the only DNA sequences in the eucaryotic genome that can be detected readily by standard electrophoretic procedures.

Figure 4. Analysis of Genome Complexity by Electrophoresis.



The four DNA preparations were digested with EcoR1 prior to this electrophoretic separation.

Objectives

To compare the electrophoretic patterns of restriction digests of a plasmid (pUC18), phage lambda DNA, and cow DNA from thymus and kidney.

Materials Provided

Plasmid DNA - EcoR1 cut: The plasmid is known as pUC18 (see Figure 3)
Phage Lambda DNA - Hind III cut (see Figure 2)
Calf Thymus DNA - EcoR1 cut
Calf Kidney DNA - EcoR1 cut

Materials Needed but not Provided

Agarose, electrophoresis buffer, and gel stain

Procedure

The procedures for the preparation, electrophoresis and staining of the agarose gels are described in detail in the first part of this manual and are briefly outlined below. This experiment is designed so that the samples of two students will be analyzed on one agarose gel. If the students work in pairs, four students will share one gel. The samples of each student (or student pair) will be electrophoresed on four gel lanes.

1. Prepare the agarose gels as described in the first part of this manual. In this experiment, 1.2% gels will be used.

2. Load 15 µl of each sample into the wells as indicated below.

Sample Well Number	Sample
1	Plasmid DNA - EcoR1 cut
2	Phage Lambda DNA - Hind III cut
3	Calf Thymus DNA - EcoR1 cut
4	Calf Kidney DNA - EcoR1 cut
5	Plasmid DNA - EcoR1 cut
6	Phage Lambda DNA - Hind III cut
7	Calf Thymus DNA - EcoR1 cut
8	Calf Kidney DNA - EcoR1 cut

3. Electrophorese until the bromophenol blue (blue dye) in the DNA samples has migrated to within 1 mm of the positive electrode end of the gel. At 170 volts, this should take about 50 minutes. Remove the gels from the unit and stain them with methylene blue overnight in the refrigerator.

4. Destain the gels and place your gel over a light source. Measure the distance of all DNA bands (in mm) from the sample origin. Record these values below.

mm migrated

lanes 1, 5 - _____

lanes 2, 6 - _____

lanes 3, 7 - _____

lanes 4, 8 - _____

Data Analysis and Study Questions

1. On the semilog paper provided on the following page , plot the distance migrated by the DNA bands from phage lambda (lanes 2 and 6) as a function of the lengths of these fragments. The lengths of the fragments are 23.1 KB, 9.4 KB, 6.6 KB, 4.4 KB, 2.3 KB, and 2.0 KB (see Figure 2).

2. Determine the length, in KB, of plasmid pUC18 by the method described in Figure 1.

3. Estimate the length of the major satellite band from cow (lanes 3, 4, 7, 8).

4. The length of DNA helix occupied by one nucleotide pair is 3.4 A (angstrom). What is the length of pUC18?

5. A human has 10^{14} cells and each human cell has about 6.4×10^{9} nucleotide pairs of DNA. What is the length of double helix that could be formed from this amount of DNA in a human individual?