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DNA, Chromosomes, and the Nucleus,” Part 3

JOHNS HOPKINS
WHITING SCHOOL
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Information: DNA, Chromosomes, and the Nucleus
Part 3

DNA in the Genome

- The **genome** of an organism or virus consists of the DNA (or for some viruses, RNA) that contains one complete copy of **all the** genetic information of that organism or virus.
- For many **viruses** and **prokaryotes**, the genome resides in a single DNA molecule.
- **Eukaryotic** cells have a **nuclear** genome, a **mitochondrial** genome, and, in the case of plants and algae, a **chloroplast** genome as well.
- The nuclear genome generally consists of multiple DNA molecules dispersed among a haploid set of chromosomes.
- Sperm and egg cells each have a haploid set of chromosomes.

DNA Packaging

- The typical E. coli cell measures about 1 microns in diameter and 2 microns in length, yet it must accommodate a (circular) DNA molecule with a length of about 1600 microns.

- A human cell of average size contains enough DNA to wrap around the cell more than 15,000 times.

- DNA must be efficiently packaged into cells and yet remain accessible to the cellular machinery for both DNA replication and the transcription.

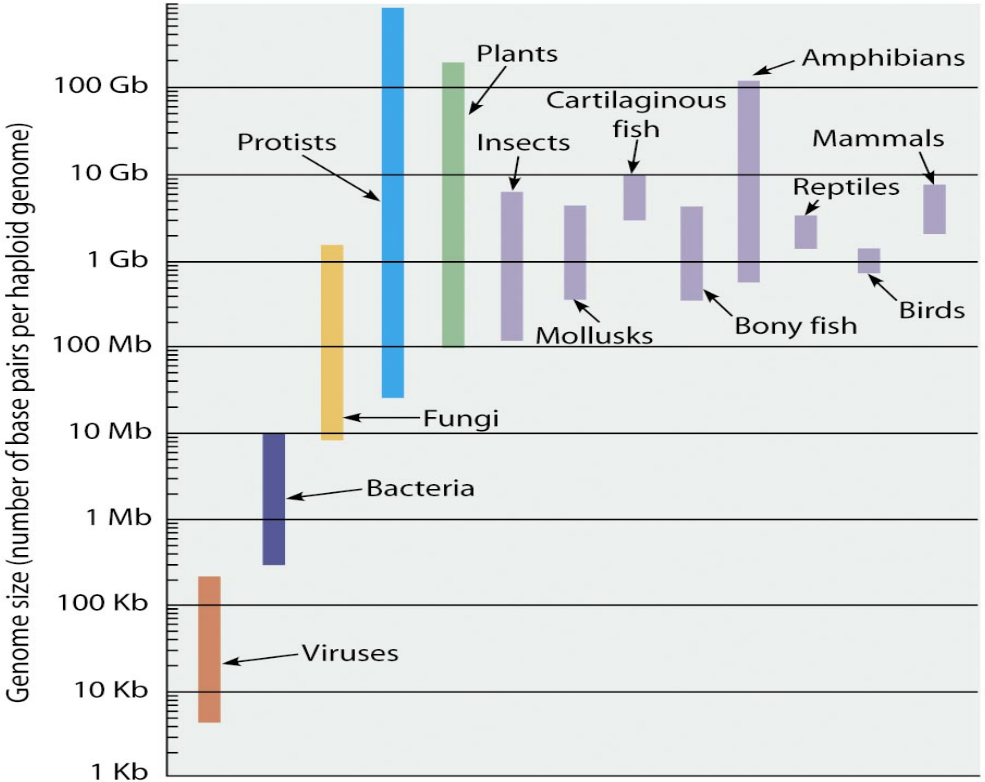

Genome size (number of base pairs per haploid genome)
100 Gb
10 Gb
1 Gb
100 Mb
10 Mb
1 Mb
100 Kb
10 Kb
1 Kb
Plants
Amphibians
Cartilaginous fish
Protists
Insects
Mammals
Reptiles
Birds
Bony fish
Mollusks
Fungi
Bacteria
Viruses

Figure 18-11

Genome size

- Genome size increases with complexity of the organism.

- There are great variations in genome size among eukaryotic species that do not seem to correlate with any known differences in organismal complexity.

- Trillium is a member of the lily family, with no obvious need for exceptional amounts of genetic information. Its genome size is more than **20 x** that of peas and **30 x** that of humans. We have no idea what it does with all that DNA.

- Genome size is less important than the number and identity of functional genes.

Restriction enzymes cleave DNA molecules at specific sites

- Most DNA molecules are far too large to be studied intact. Until the early 1970s, DNA was the most difficult biological molecule to analyze biochemically.

- Eukaryotic DNA seemed especially intimidating, given the size of most eukaryotic genomes, and no method was known for cutting DNA at specific sites to yield reproducible fragments.

Restriction enzymes

- **Restriction enzymes** have made it possible to cut DNA molecules at specific sites.

- Restriction enzyme generates a specific set of DNA pieces called restriction fragments. Each restriction enzyme cleaves double stranded DNA only in places where it encounters a specific recognition sequence, called a **restriction site**.

5′ G↓A—A—T—T—C 3′
3′ C—T—T—A—A↑G 5′

Restriction enzymes

- Arrows indicate where EcoRI cuts the DNA.
- Restriction enzymes cleave DNA into fragments ranging from a few hundred to a few thousand base pairs in length.
- Fragments are more amenable to further manipulation than the enormously long DNA molecules.

Table 18B-1	Some Common Restriction Enzymes and Their Recognition Sequences	
Enzyme	**Source Organism**	**Recognition Sequence***
*Bam*HI	*Bacillus amyloliquefaciens*	5′ G↓G—A—T—C—C 3′ 3′ C—C—T—A—G↑G 5′
*Eco*RI	*Escherichia coli*	5′ G↓A—A—T—T—C 3′ 3′ C—T—T—A—A↑G 5′
*Hae*III	*Hemophilus aegyptius*	5′ G—G↓C—C 3′ 3′ C—C↑G—G 5′
*Hin*dIII	*Hemophilus influenzae*	5′ A↓A—G—C—T—T 3′ 3′ T—T—C—G—A↑A 5′
*Pst*I	*Providencia stuartii* 164	5′ C—T—G—C—A↓G 3′ 3′ G↑A—C—G—T—C 5′
*Pvu*I	*Proteus vulgaris*	5′ C—G—A—T↓C—G 3′ 3′ G—C↑T—A—G—C 5′
*Pvu*II	*Proteus vulgaris*	5′ C—A—G↓C—T—G 3′ 3′ G—T—C↑G—A—C 5′
*Sal*I	*Streptomyces albus* G	5′ C↓T—C—G—A—C 3′ 3′ C—A—G—C—T↑G 5′

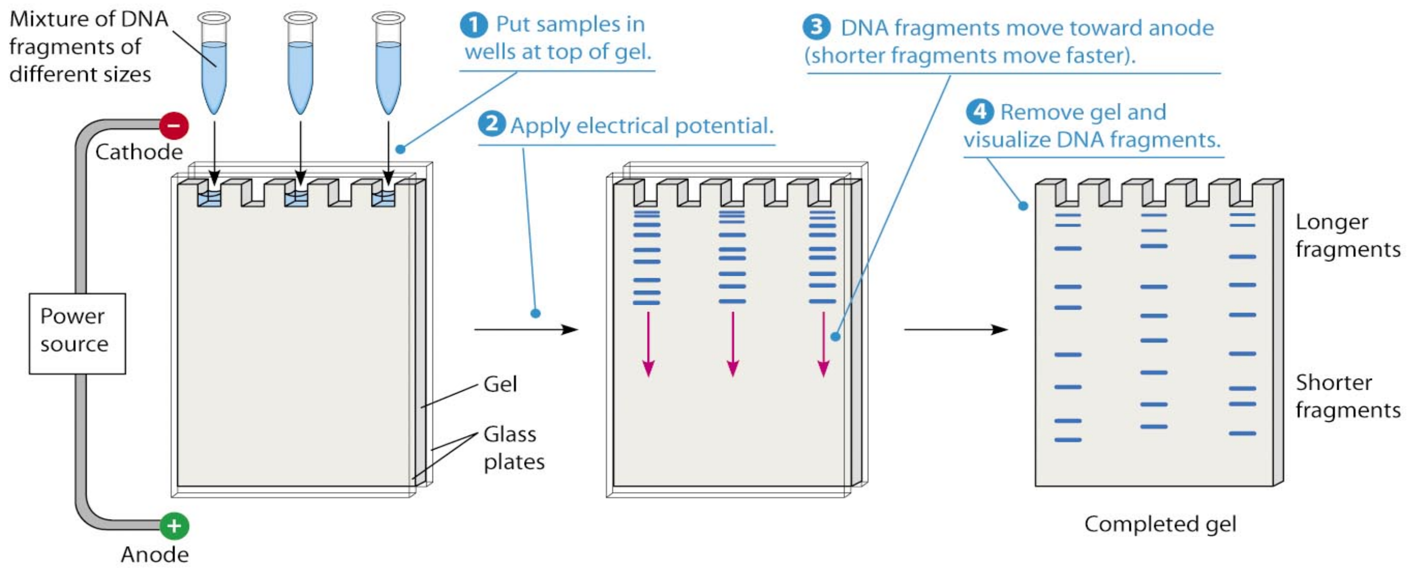
*The arrows within the recognition sequence indicate the points at which each restriction enzyme cuts the two strands of the DNA molecule.

Table 18B-1

Separation of restriction fragments by gel electrophoresis

- To determine the number and lengths of DNA fragments and to isolate individual fragments for further study, one must be able to separate the fragments from each other.

- **Gel electrophores** is the technique of choice to determine the number and lengths of DNA fragments and to isolate individual fragments for further study.



Mixture of DNA fragments of different sizes
1 Put samples in wells at top of gel.
2 Apply electrical potential.
3 DNA fragments move toward anode (shorter fragments move faster).
4 Remove gel and visualize DNA fragments.
Cathode
Power source
Anode
Gel
Glass plates
Longer fragments
Shorter fragments
Completed gel

Figure 18-12

The nucleus

- The **nucleus** is the site within the eukaryotic cell where the chromosomes are localized and replicated and where the DNA they contain is transcribed.

- The nucleus is both the **repository** of most of the cell's genetic information and the **control center** for expression of information.