Genetics
& Cell biology

◉ Sheet ○ Slides

Number

2

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RNA:

RNA consists of long, unbranched chains of nucleotides joined by phosphodiester bonds between the 3'-OH of one pentose and the 5'-phosphate of the next. The pentose unit is β-D-ribose (it is 2-deoxy-D-ribose in DNA) and the pyrimidine bases are uracil and cytosine (they are thymine and cytosine in DNA).

In general, RNA is *single stranded*. As a result, it does not have a regular structure like DNA (which is double-helical); it can take different formations.

*Can RNA exist as a double stranded molecule? And where?

-YES, microRNA itself exists first as double stranded RNA as part of its maturation and processing. Some viruses also have double stranded RNA.

*Can you have a hybrid of DNA and RNA?

-YES, during the synthesis of retroviral genomic material. Also, during transcription, part of RNA will be complementary to DNA.

Side note:
Aptamers are small single-stranded DNA molecules which take specific 3D structure. They can be used as drugs; they can inhibit receptors like kinases and other enzymes.

Types of RNA

There are much more types of RNA than those listed in the table below, but these are the most common. We will take about some of them later on (the doctor just mentioned their names)

The Roles of Different Kinds of RNA

RNA Type	Size	Function
Transfer RNA	Small	Transports amino acids to site of protein synthesis
Ribosomal RNA	Several kinds—variable in size	Combines with proteins to form ribosomes, the site of protein synthesis
Messenger RNA	Variable	Directs amino acid sequence of proteins
Small nuclear RNA	Small	Processes initial mRNA to its mature form in eukaryotes
Small interfering RNA	Small	Affects gene expression; used by scientists to knock out a gene being studied
Micro RNA	Small	Affects gene expression; important in growth and development

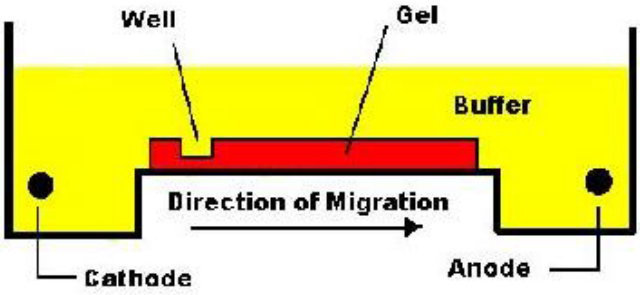
Gel electrophoresis:

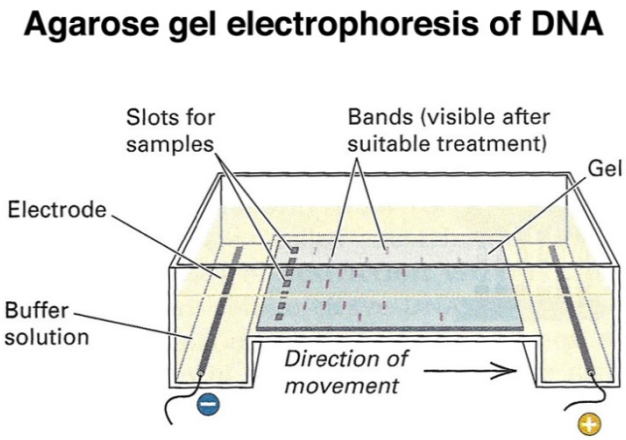
- The length and purity of DNA molecules can be accurately determined by the gel electrophoresis.
- It is very similar to gel electrophoresis of proteins. Difference: in proteins it is vertical but in DNA it is not.
- We use an ***agarose gel*** which is a type of sugar. It polymerizes and forms a solid gel. We can have holes (wells) in this gel to put the samples in. Then, when we apply an electrical current, the DNA will migrate from the cathode to the anode according to *size*.

  *Why does DNA move from the cathode (-) to the anode (+)?

  -DNA is highly negatively charged (because of the phosphate groups).

  So, the DNA *fragments* migrate according to size but NOT charge because they are all negatively charged. So when you have 2 DNA molecules in the same well, they will migrate and be separated according to size where the smaller one will move faster than the larger fragment.





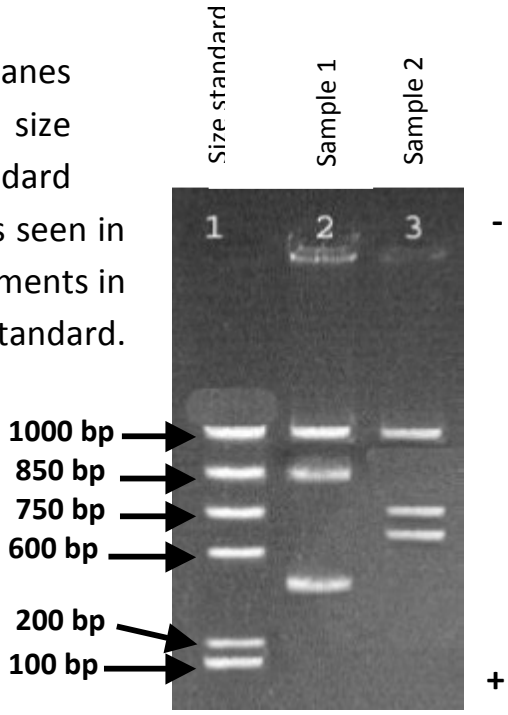
Detection: How can we detect DNA?

We can't see DNA because it absorbs light at the UV range. What do we do?

a) We *stain* the DNA by adding certain chemicals. Those chemicals will bind/intercalate with the DNA molecule giving us a color. One of these chemicals is ethidium bromide which is carcinogenic.
b) We can also use radioactive phosphorus (^{32}P) because DNA contains phosphate unlike proteins[1]. The radioactive phosphorus will give us a signal, and then we can observe DNA by exposing it to an x-ray film

[1]Proteins can contain phosphates if they're phosphorylated which is another story.

This is a real image of a DNA gel. We have 3 lanes and a sample in each lane. Usually, we run a size standard which we buy from a company. This standard contain different DNA fragments of known size (as seen in the picture). We can estimate the size of DNA fragments in these samples by comparing them to the size standard. For example, we can estimate that the 3rd band in sample 1 is approximately 400 bp (Base Pairs) because it came between the 600 and 200 bp bands. Also, it contains a DNA fragment that has a size of 850 bp. We can also compare between different samples. For example, we can see that both sample 1 and 2 contain a 1000 bp fragment.

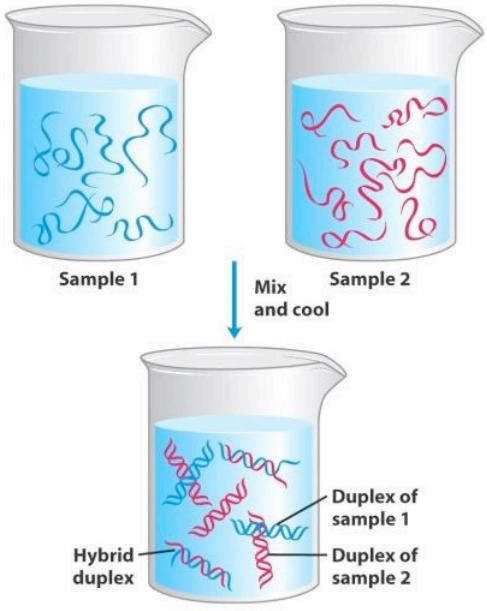


The DNA molecules of different lengths will run as bands. Each band contains thousands to millions of fragments (copies) of the DNA of the same size and length that can be of the same or different type (NOT one DNA molecule). So each band may contain *2 different* types of DNA fragments, but with the same size.

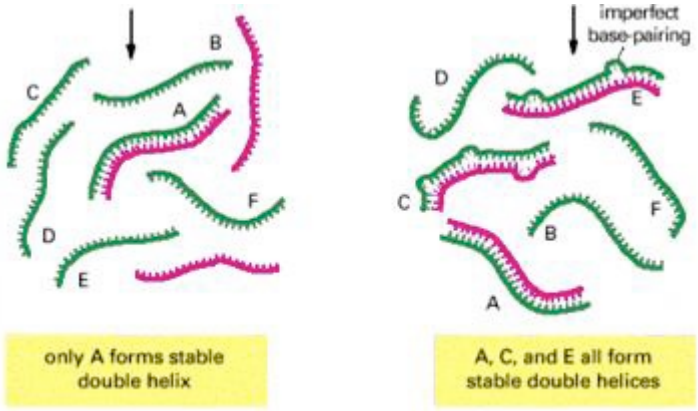
00:00-10:35

Hybridization

Hybrid is a thing made by combining two different elements together. Hybridization is a technique used in molecular biology whereby you can have, for example, 2 different ssDNA molecules from 2 different sources. Then, these 2 molecules can *hybridize* with each other forming dsDNA molecule (***hybrid***) ONLY *if* they are complementary (they can base-pair with each other, C-G and A- T). Thus, we can have 2 DNA strands from the same source or from 2 different sources as long as they are complementary to each other.



We can have what is called ***imperfect hybridization***. For example, if we have 2 DNA fragments that are 100 bp long, but they differ in 5% of their bases, they CAN still hybridize. However, their hybridization is *imperfect*. Meaning, there will be areas where there is NO hybridization, but as long as there are ENOUGH bases that are complementary with each other (can form hydrogen bonds with each other) imperfect DNA hybrids still can form.

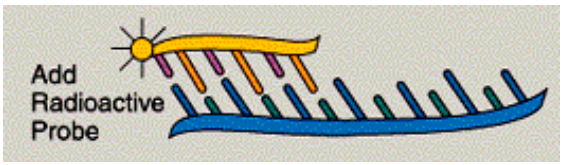


*Does it matter if the 2 DNA molecules come from different sources (ex: one from a human DNA and one from a bacterial DNA)? Does this mean that bacterial DNA will not hybridize with the human DNA?

-NO! As long as they are complementary, they CAN hybridize. Ex: I can hybridize a DNA fragment made in the lab with a natural DNA.

Hybridization techniques

Hybridization reactions can occur between any two single-stranded nucleic acid chains provided that they have complementary nucleotide sequences. Thus, we can use hybridization reactions (by techniques that are based on it) *to detect and characterize specific nucleotide sequences*.



1. Probes:

- To probe for something (as a verb) means to search for something.
- A probe is a short, small sequence of single stranded DNA (an oligonucleotide) that is complementary to a piece of a larger DNA molecule.
- When we add the probe to the DNA molecule, there will be a competition in binding to the DNA molecule between the probe and the complementary strand of this molecule. Because we have hydrogen bonds between the two strands of the molecule (which are weak), we

can easily break them and separate the two strands. So eventually, the probe can bind to part of the large DNA molecule.

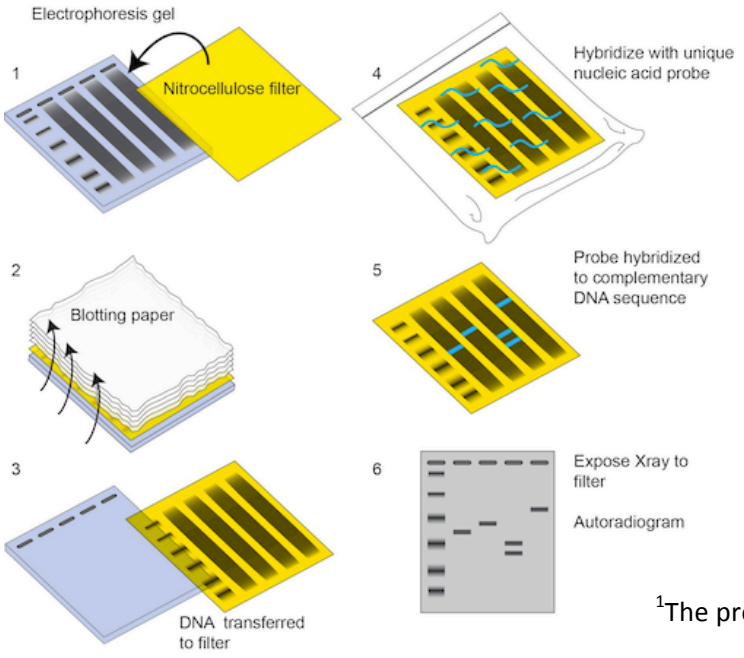
- What we now do is that we label the probe (by adding a dye or color that gives a signal, ex: radioactive phosphorus). If the probe binds to the large piece of DNA, a signal will be detected. Whenever you see the signal, you know that the probe has hybridized with the large DNA fragment. Meaning, This fragment has a sequence that is complementary to that of the probe.

2. Southern blotting:

- This technique is a combination of DNA gel electrophoresis and hybridization technique.
- Used to detect:
  - The presence of a DNA segment complementary to the probe.
  - The size of the DNA fragment.

10:15-20:00

- First, we use gel electrophoresis to separate DNA fragments according to size (smaller molecules will move faster). Then, we put a special piece of paper over the gel to transfer the DNA fragments to it. This piece of paper will be exactly like the gel (a replica/mirror image of it). We then take a probe (with a radioactive signal) and put it on the piece of paper. The probe will hybridize with the fragments that are complementary to it[1]. So the probe will not bind to any other fragment, and it will only bind to specific ones that are complementary to it. Finally, we expose the paper to an x-ray film and detect the bands.



*What information did this experiment provide?

a) It identified which sample has a DNA fragment with a sequence that is complementary to the probe.
b) It gave an estimate for the sizes of the fragments.

[1]The probe can bind to the whole fragment or just a part of

Endonucleases

We know that proteases degrade proteins, esterases cleave ester bonds and so on. **Nucleases** are enzymes that degrade nucleic acids. Nucleases that are specific for DNA and RNA are called **DNases** and **RNases** respectively.

Endonucleases are nucleases that cleave the phosphodiester bond between 2 nucleotides in the middle (within the DNA molecule). **Exonucleases** are nucleases that cleave the phosphodiester bond between terminal nucleotides (either at the 5' or the 3' end).

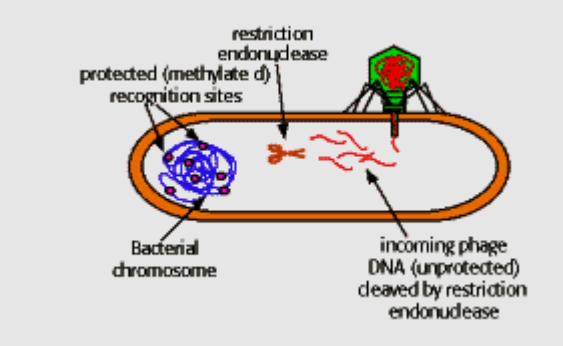
Restriction endonucleases are endonucleases that are *restricted* by a specific sequence. They do not cleave/break the phosphodiester bond randomly. When they cleave DNA they generate what we call **restriction fragments.** So restriction fragments are the DNA fragments that are produced by restriction endonucleases. The site that is recognized by the restriction endonuclease is known as a **restriction site** (4- to 8-bp).

RECAP

A restriction endonuclease is an endonuclease that recognizes a specific sequence in the DNA known as a restriction site. It cleaves the DNA (based on its recognition) generating restriction fragments.

20:00-29:35

Restriction endonucleases are present in bacteria as a protection mechanism. From what?? We have viruses which are called bacteriophages that infect bacteria and insert their DNA inside bacterial cells. Bacteria produce specific restriction endonuclease that recognizes the viral DNA and cleaves it protecting the bacterial cells from getting infected.



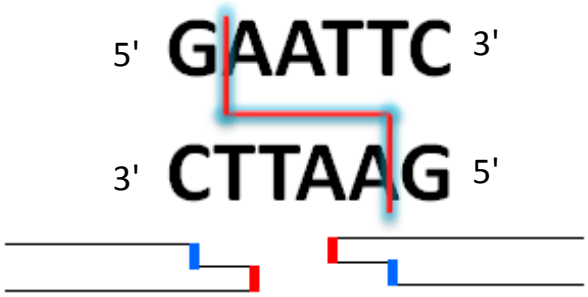
It turned out that there are hundreds of restriction endonucleases, and they are specific for the site that they recognize. One class of them is called **type II restriction endonucleases**; they recognize a restriction site and they cleave the DNA within this site. Other restriction endonucleases recognize a site and then cleave at another site.

Type II restriction endonucleases recognize certain restriction sites differently. For example, a restriction endonuclease was discovered in E.coli which is called **EcoR1**. This enzyme recognizes a DNA fragment that has the sequence *5'-GAATTC-3'*, and it cuts between G and A at the end. If I changed the sequence to 5'-GAATTG-3', it won't cut. Other enzymes can be more flexible. For example, an enzyme called **Hinf1** (from Haemophilus influenzae) recognizes *5'-ANTC-3'* where 'N' can be any base (A, G, C, or T).

Types of cleavages

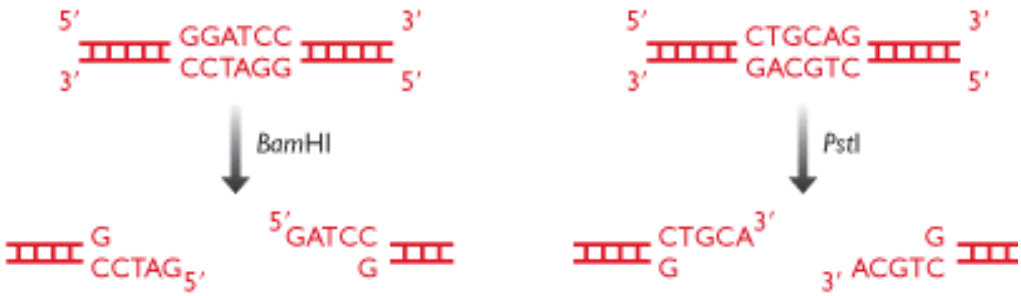
Restriction enzymes cut DNA in two different ways:

Staggered (off-center): enzymes cut the two DNA strands at different positions generating sticky or **cohesive ends**
If we flipped the sequence 5'-GAATTC-3' and looked at it from the complementary strand and read from the 5' end to the 3' end it will be 5'-GAATTC-3' (the same sequence). Meaning, EcoR1 will make a cut between G and A in the two strands generating two DNA fragments. Notice that the cut is **staggered**; it is not straight.



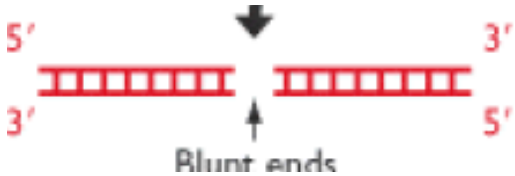
In this type of cut, there will be certain parts of the DNA that are single stranded at each end of the cut (**single-stranded overhangs**). Moreover, since the types of cuts are different for different enzymes (they cut at different sites), I can either have **5' overhang** or **3' overhang;** thus, the single-stranded part (the overhang) ends with 5' or 3' respectively.

(B) 5′ and 3′ overhangs



*How can we know if the endonuclease will make a 5' or a 3' overhang?
-We look at the cut. If it's closer to the 5'end (like EcoR1) it will make a 5' overhang. And if the cut is closer to the 3' end it will make a 3' overhang.

1. **Blunt-ended cut:** enzymes cut at the same position on both strands. Ex: between A and T in the previous sequence (5'-GAATTC-3').



Palindromic sequences

The sequences recognized by restriction endonucleases—their sites of action—and are read the same from left to right as they do from right to left (on the complementary strand). They are mirror images of each other (always read from the 5' to the 3' end). Usually the restriction sites of restriction endonucleases are **palindromic sequences**.

Examples:

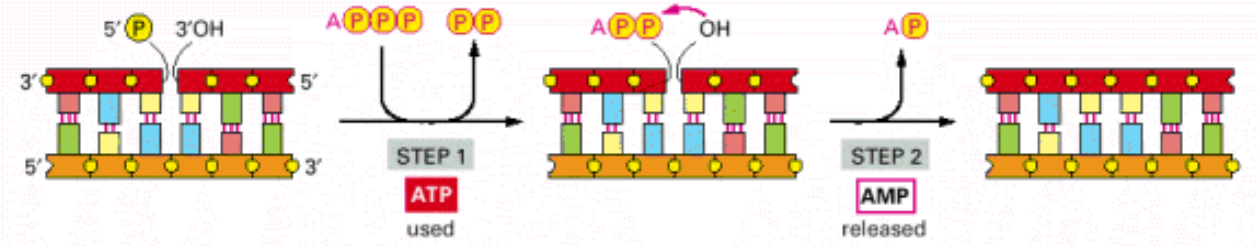
```
EcoRI       5'  GAATTC  3'
            3'  CTTAAG  5'

HindIII     5'  AAGCTT  3'
            3'  TTCGAA  5'

SmaI        5'  CCCGGG  3'
            3'  GGGCCC  5'

TaqI        5'  TCGA  3'
            3'  AGCT  5'
```

29:35-39:00

Notice that the 2 overhangs in the staggered cut are complementary to each other. So, when restriction endonucleases make a staggered cut generating the overhangs, these overhangs can come back and hybridize with each other (they are complementary and can form hydrogen bonds with each other), and that's why we also call them **cohesive ends**. That, however, does not mean that the cohesive-ended DNA fragments are stable when the ends bind to each other. This is because there are still no phosphodiester bonds between the two nucleotides, so the two strands can still be separated from each other. The two strands can be stabilized or connected to each other by an enzyme called **DNA ligase.**

DNA ligase

DNA ligase is an enzyme that can utilize ATP to make a phosphodiester bond between 2 nucleotides. It covalently joins DNA ends (example, restriction fragments) and catalyzes the formation of 3'→ 5' phosphodiester bonds between the 3'-hydroxyl end of one strand and the 5'-phosphate end of another strand.



NOTE:

The exam's material ends here. The rest of the sheet is NOT included in the **midterm Exam**.

Advantages of restriction endonucleases

The restriction endonucleases have revolutionized the field of molecular biology. We can use them in at least 2 techniques:

1. **Restriction fragment length polymorphism (RFLP)**
2. **Cloning**

DNA polymorphisms

- Poly means many and morph means shape.
- Our DNA is identical by 99.99%. This 0.01% difference is what makes us all different.

- We are diploids; we have 2 copies of the same DNA piece (2 copies of chromosome 1, and 2 copies of chromosome 2, etc.). We also have 2 copies of every single gene: 1 from your dad and 1 from your mom.

- **Allele** is the type of gene. Alleles can be homozygous or heterozygous.
  EX: I have 2 copies of the gene that determines the eye color. One gene indicates a blue eye color, and the 2nd indicates a black one, but one of them is dominant over the other. Same thing applies for the gene of hair color. There is an allele for black, brown, and blond hair.

39:00-46:34

"*Difficult roads lead to beautiful destinations*"

THE END