THE FUNCTION OF RESTRICTION ENZYMES

BACKROUND

Genome of all the living creatures consists of DNA, deoxyribonucleic acid. DNA is made of two strands of DNA that are connected to each other in a double helix. The two strands are composed of simpler units called nucleotides. Each nucleotide has three parts: nitrogen (containing nucleobase), sugar called deoxyribose and a phosphate group. In the nitrogen part there are four different possible nucleobases: cytosine (C), guanine (G), adenine (A) or thymine (T). These different bases code all information needed in cell in different situations and these code is also the way this information is passed to future generations.

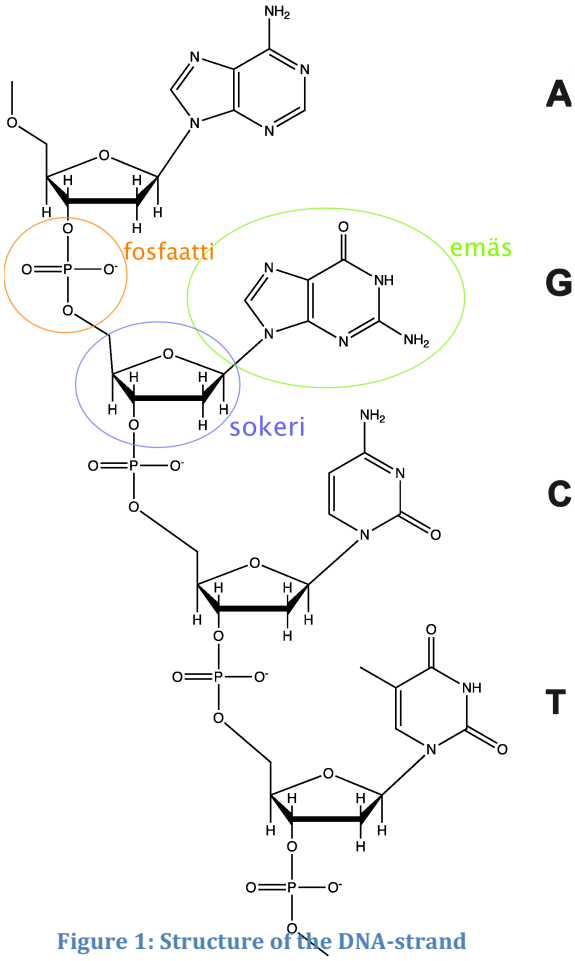

Figure 1: Structure of the DNA-strand

In this lab experiment DNA is cut to smaller pieces with restriction enzymes. Restriction enzymes cut DNA at or near specific nucleotide sequences. For example EcoRI -enzyme looks for DNA sequense 5'5'GAATTC3´ and cuts DNA after first G-base:

G|AATTC
CTTAA|G

In this experiment you will use DNA from lambda phage. Lambda phage is a bacterial virus or pacterophage that infects bacterial species *Escherichia coli*. Lambda phage has a small genome consisting 48 000 base pairs long DNA strand. That DNA codes for 29 different proteins, that are needed for building and keeping virus structure, infecting bacteria, lysing their cell structure and making DNA copies.

In this experiment phage DNA is cut with three different restriction enzymes.

Your task is to find out in how many different parts restriction enzyme cuts the phage DNA and how long are these pieces of DNA.

Lambda phage DNA is double-stranded linear DNA molecule, that each restriction enzyme is able to cut at one or multible restriction sites. Different lenght DNA pieces will form as a result. In Figure 2 you can see what happens if a 4500 basepair (bp) long linear DNA

is cut with enzyme that cuts DNA in two sites. As original DNA forms a single band in agarose gel electrophoresis (band length being about 4500 bp) will restricted DNA form three visible bands (lenghts of those bands being 1000, 1500 and 2000 bp).

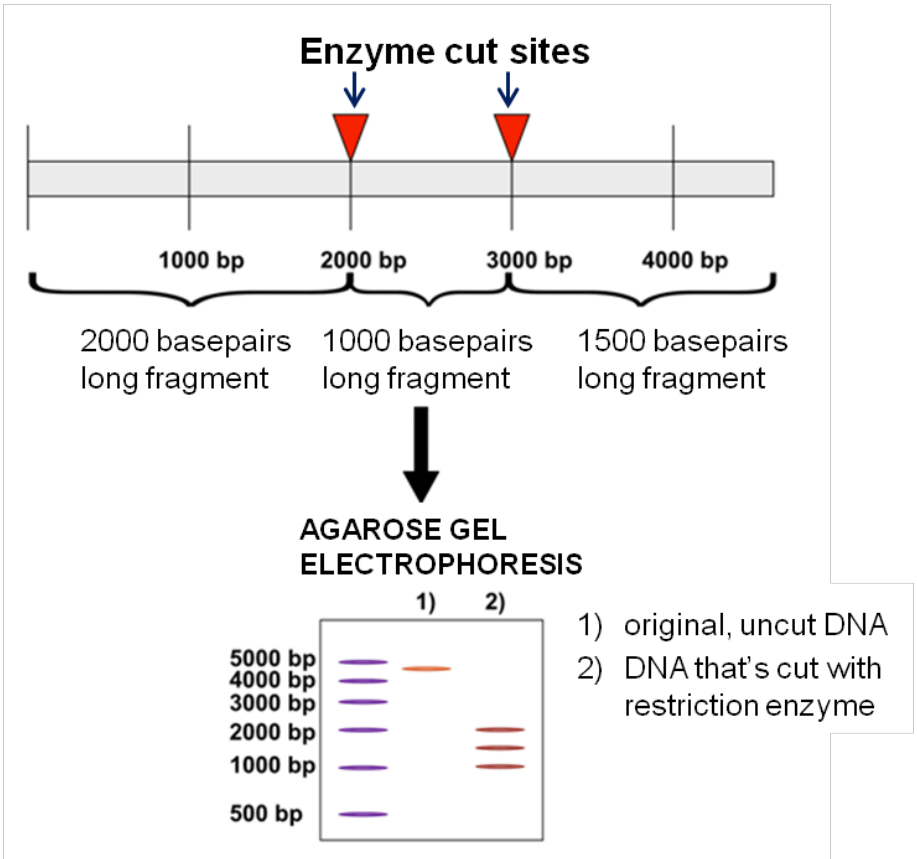

Figure 2. Principle of the experiment. Linear DNA is cut with restriction enzymes in the experiment. The number of the enzyme cutting sites can be determined based on the number of the fragments that are seen in the gel.

In this experiment DNA fragments are separated based on size using agarose gel electrophoresis. In electrophoresis the substances with electrical charge are separated using electric field. Substances are drawn towards the opposite electric charge. For example negatively charged DNA molecule runs inside the gel towards positively charged electrod.

Electrophoresis apparatus reminds electrolytic cell. It consists of power suply, electrods, gel box and samples. usually samples are run in gel-like matter to separate them. In this experiment an agarose gel is used so the method is called agarose gel electrophoresis (AGE).

In gel electrophoresis the running speed of the samples is affected by the the charge and the size of the molecules. Bigger molecules move more slowly than the small, because gel resists more the movement of the big molecules. This is why the biggest molecules move most slowly and the smallest molecules are fastest. This is the method for separating DNA fragments of different sizes.

CONSIDER BEFORE THE WORK

- How many bands (DNA fragments) you see on a gel if enzyme cuts (digests) the lambda phage DNA from three separate sites?
- What is restriction enzyme and how and where they can be used?
- Make sure you know how to use micropipette

EQUIPMENT

- Bio-Rad reagent kit: *Restriction Digestion and Analysis of Lambda DNA Kit*
  - DNA size marker
  - HindIII-enzyme
  - PstI-enzyme
  - EcoRI-enzyme
  - Restriction buffer (2x solution)
  - Lambda phage DNA
  - Loading dye (5x solution)
  - DNA-stain (500x solution)
  - Microtubes
  - Racks for tubes
  - Elektrophoresis buffer (50x solution)
- Micropipettes and tips
- Agarose gel electroforesis apparatus and power suply
- Ready-made agarose gel with 8 (x2) wells
- Markers

PROTOCOL

Työohje perustuu Bio-Radin reagenssisarjaan: *Restriction Digestion and Analysis of Lambda DNA Kit.*

1. See where you can find the enzymes, DNA and restriction enzyme buffer. Keep the solution tubes on ice.
2. Take four microtubes (Eppendorf tubes) and name them in a following way:
   a. **L** (Lambda DNA)
   b. **P** (PstI enzyme digestion)
   c. **E** (EcoRI enzyme digestion)
   d. **H** (HindIII enzyme digestion)
3. Pipette one enzyme reaction working as a pair or group of 3 people. Instructor will tell you which one. Pipette each solution (enzyme last) of your reaction according to the following table:

Microtube	DNA	Puskuriliuos	PstI	EcoRI	HindIII
L	4 µl	6 µl	–	–	–
P	4 µl	5 µl	1 µl	–	–
E	4 µl	5 µl	–	1 µl	–
H	4 µl	5 µl	–	–	1 µl

4. Pipette each solution to the bottom of the tube and in the previous solution
5. Keep the tube in +37 °C 10–30 minutes (mark how many minutes = this is your restriction enzyme digestion reaction time).
6. Add 2 µl loading dye to your reaction tube. Mix by pipetting up and down the solution mixture a few times.
7. (Agarose gel electrophoresis apparatus is put together. Teacher helps.)
8. Pipette 10 µl of your reaction mixture to one well of the agarose gel. Mark down in which well your sample is in. Pipette only one sample in one well. For example in a following way:
    a. Well 1 = DNA-sizemarker (teacher)
    b. Well 2 = L reaction
    c. Well 3 = P reaction
    d. Well 4 = E reaction
    e. Well 5 = H reaction
9. Run the gel on 200 V voltage for 20 min or on 100 V 30 min.
10. Stain the agarose gel in a following way:
    - Add 120 ml 100x DNA-stain solution to a plastic chamber.
    - Stain gel for 2 min mixing once in a while. Collect the stain back to flask.
    - Put the gel in a new chamber and rinse with warm water (c. 50 °C) about 10 seconds.Wave the chambet and gel every now and then.
    - Wash the gel in warm water 2–3 times in warm water for about 5-15 minutes or until you can see the DNA fragments. Wave gel gently.
11. Analyze the results from the gel. How many fragments can you find from your sample? How many times the enzyme digested the lambda DNA? What is the size of the fragments of lambda DNA in basepairs?

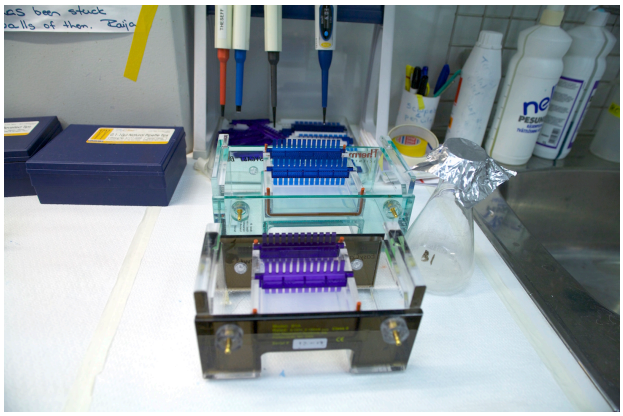

Figure 3. Agarose gel electrophoresis apparatus

CONSIDER AFTER EXPERIMENT

- How could you figure out on what site the restriction enzyme cuts the lambda DNA?
- Enzyme cut sites are usually palindromic. What does this mean?
- Restriction enzymes can leave cohesive or blunt ends to DNA. What does this mean?
- How could you put back together the whole labbda phage DNA from the digested fragments?
- Why does the restriction enzyme reaction take 10–30 minutes in +37 °C when it takes more than 8 hours in room temperature?