Restriction Enzyme Cleavage of DNA

Laboratory BIOT 3250

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Experiment Objective

The objective of this experiment is to develop an understanding of the use of restriction endonucleases as tools to cut DNA at specific sequences.

Materials

READY-TO-LOAD™ DNA SAMPLES FOR ELECTROPHORESIS

A	Plasmid DNA (uncut)
B	Plasmid cut with *Bgl* I
C	Plasmid cut with *Eco* RI
D	Lambda DNA (uncut)
E	Lambda DNA cut with *Eco* RI
F	Lambda DNA cut with *Bgl* I

REAGENTS & SUPPLIES

- UltraSpec-Agarose™ powder
- Concentrated electrophoresis buffer
- FlashBlue™ DNA Stain
- InstaStain® Blue cards
- Practice Gel Loading Solution
- 1 ml pipet
- Microtipped Transfer Pipets

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Small plastic trays or large weigh boats (for gel destaining)
- DNA visualization system (white light)
- Distilled or deionized water

Concepts

The discovery of restriction enzymes ushered in a new era of molecular genetics. These enzymes cut the DNA molecule in a highly specific and reproducible way. This, in turn, has lead to the development of molecular cloning and the mapping of genes.

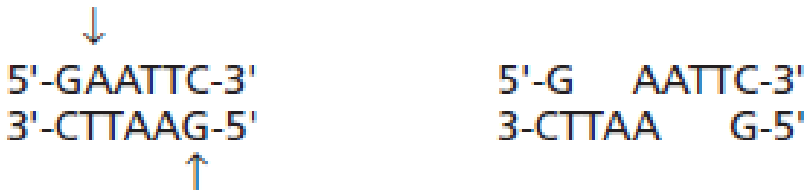
Restriction enzymes are endonucleases which catalyze the cleavage of the phosphodiester bonds within both strands of DNA. They require Mg^{+2} for activity and generate a 5 prime (5') phosphate and a 3 prime (3') hydroxyl group at the point of cleavage. The distinguishing feature of restriction enzymes is that they only cut at very specific sequences of bases. Restriction enzymes are obtained from many different species of bacteria (including blue-green algae). To date, over 3,000 restriction enzymes have been discovered and catalogued.

Concepts (cont…)

Restriction enzymes are named according to the organism from which they are isolated. This is done by using the first letter of the genus followed by the first two letters of the species. Only certain strains or sub-strains of a particular species may produce restriction enzymes. The type of strain or substrain sometimes follows the species designation in the name. Finally, a Roman numeral is always used to designate one out of possibly several different restriction enzymes produced by the **same** organism or by different substrains of the **same** strain.

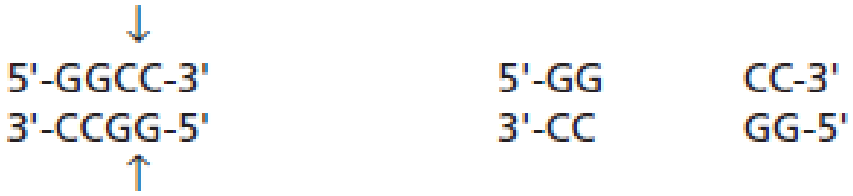
A restriction enzyme requires a specific double stranded recognition sequence of nucleotides to cut DNA. Recognition sites are usually 4 to 8 base pairs in length. Cleavage occurs within or near the site. The cleavage positions are indicated by arrows. Recognition sites are frequently symmetrical, i.e., both DNA strands in the site have the same base sequence when read 5' to 3'. Such sequences are called palindromes. Consider the recognition site and cleavage pattern of *Eco* RI as an example.

Concepts (cont…)



As shown above, *Eco* RI causes staggered cleavage of its site. The ends of the DNA fragments are called "sticky" or "cohesive" ends because the single-stranded regions of the ends are complementary.

Some restriction enzymes, such as *Hae* III, introduce cuts that are opposite each other. This type of cleavage generates "blunt" ends.



Concepts (cont…)

Restriction Enzyme	Organism
Bgl I	Bacillus globigii
Bam HI	Bacillus amyloliquefaciens H
Eco RI	Escherichia coli, strain RY 13
Eco RII	Escherichia coli, strain R 245
Hae III	Haemophilus aegyptius
Hind III	Haemophilus influenzae R_d

Concepts (cont…)

The recognition sites of some restriction enzymes contain variable base positions. For example, *Ava* I recognizes:

```
     ↓
5'-CPyCGPuG-3'      (Py = pyrimidine = C or T and
3'-GPuGCPyC-5'   Pu = purine = G or A)
          ↑
```

Keep in mind that A pairs with T and G pairs with C. Consequently, there are four possible sequences *Ava* I recognizes. Recognition sites of this type are called degenerate.

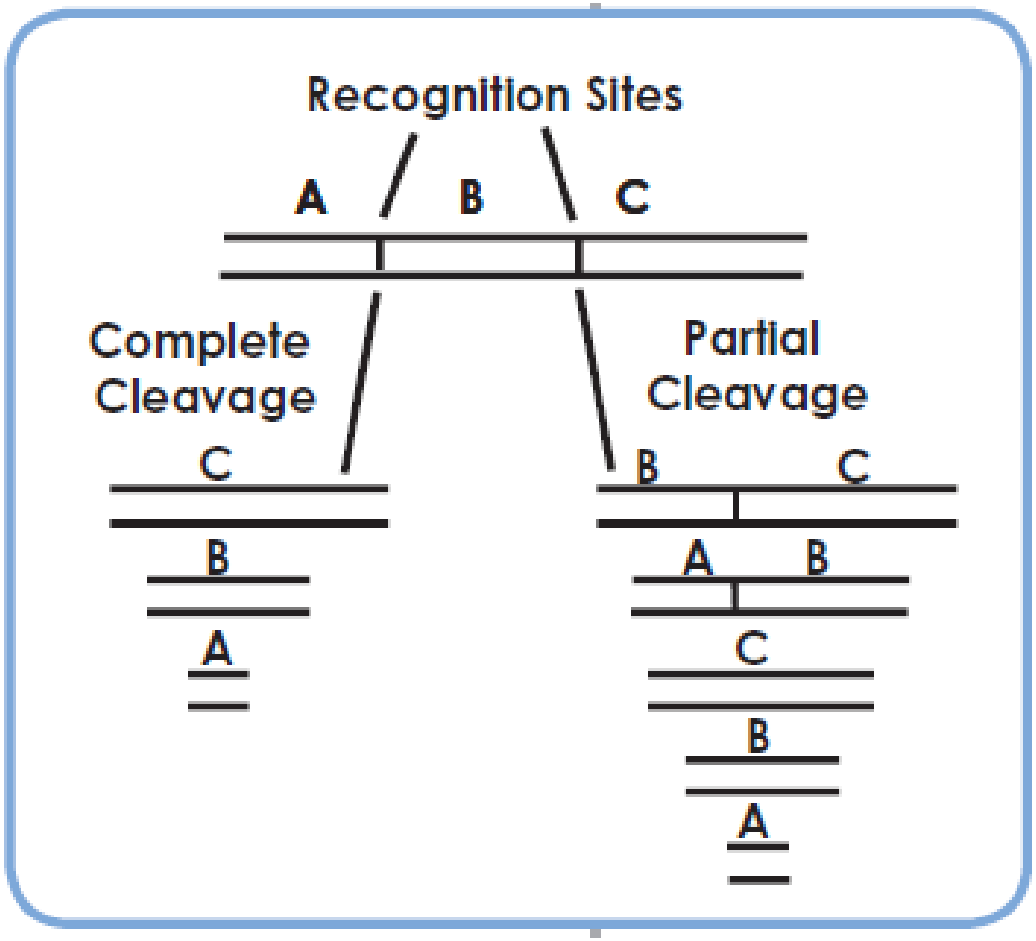
There are some recognition sites that are divided by a certain number of totally variable bases. For example, *Bgl* I recognizes:

```
          ↓
5'-GCCNNNNNGGC-3'      (N = A, G, C or T)
3'-CGGNNNNNCCG-5'
          ↑
```

Concepts (cont…)

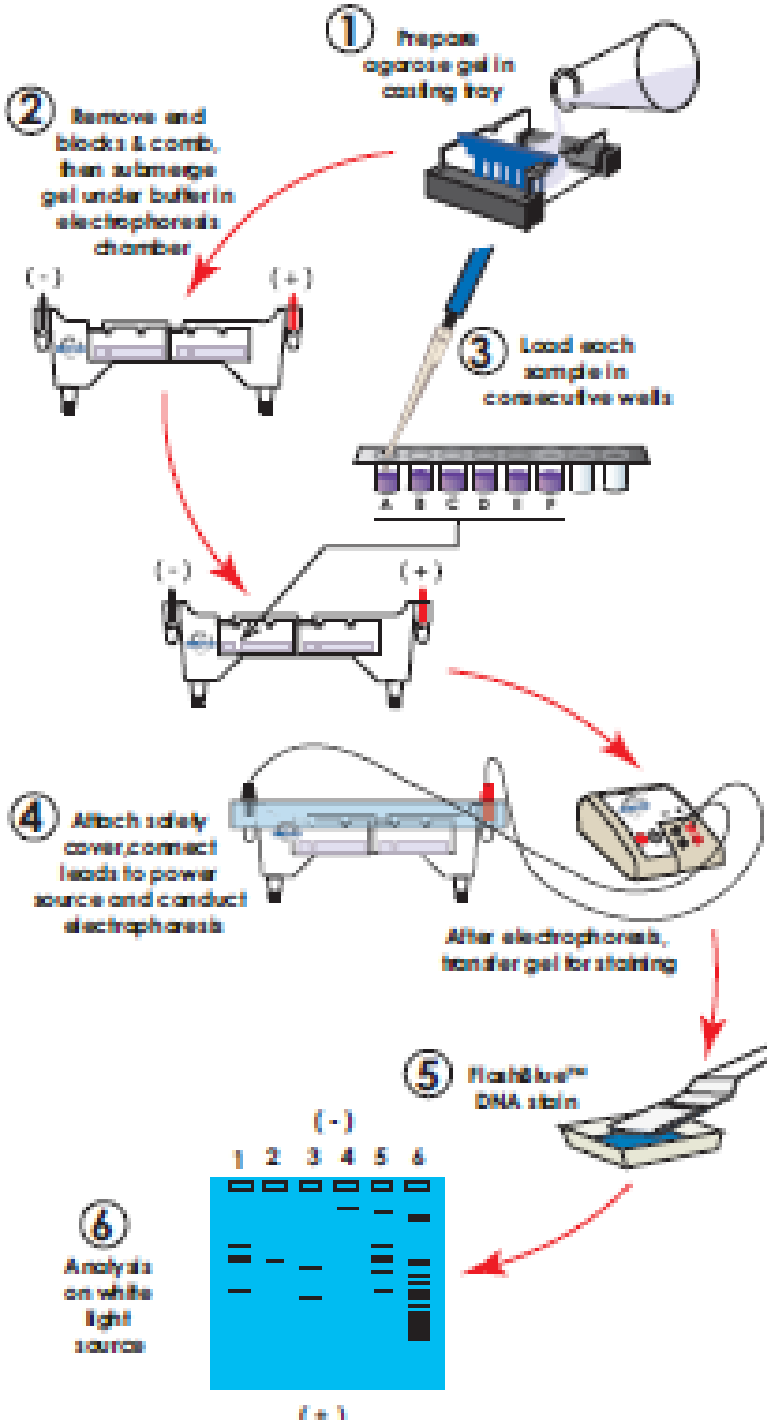
Plasmids and many viral DNAs are circular molecules. If circular DNA contains one recognition site for a restriction enzyme, then it will open up to form a linear molecule when cleaved. By contrast, if a linear DNA molecule contains a single recognition site, when cleaved once it will generate two fragments. The size of the fragments produced depends on how far the sites are from each other. If a DNA molecule contains several recognition sites for a restriction enzyme, then under certain experimental conditions, it is possible that certain sites are cleaved and not others. These incompletely cleaved fragments of DNA are called partials. Partials can arise if low amounts of enzyme are used or the reaction is stopped after a short time. In reality, reactions containing partials also contain some molecules that have been completely cleaved.

Concepts (cont...)

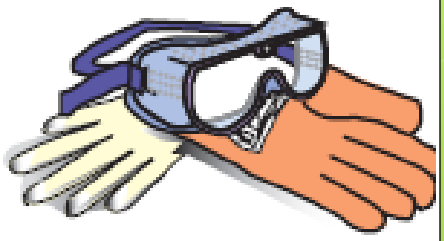


Concepts (cont…)

Flow Chart


1
Prepare agarose gel in casting tray
2
Remove end blocks & comb, then submerge gel under buffer in electrophoresis chamber
(-)
(+)
3
Load each sample in consecutive wells
A B C D E F
(-)
(+)
4
Attach safety cover, connect leads to power source and conduct electrophoresis
After electrophoresis, transfer gel for staining
5
FlashBlue™ DNA stain
(-)
1 2 3 4 5 6
6
Analysis on white light source
(+)

LABORATORY SAFETY



1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOK RECORDINGS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:

- Record (draw) your observations, or photograph the results.

Following the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

EXPERIMENT

Agarose Gel Electrophoresis

Prepare the Gel

1. Prepare an agarose gel with specifications summarized below.

• Agarose gel concentration required:	0.8%
• Recommended gel size:	7 x 10 cm or 7 x 14 cm
• Number of sample wells required:	6
• Placement of well-former template:	Middle set of notches (7 x 10 cm) Middle set of notches (7 x 14 cm)

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Load the Samples

2. Load the DNA samples in tubes A - F into the wells in consecutive order.

   - For gels to be stained with FlashBlue™ or InstaStain® Blue, fill wells with 35 - 38 µl.
   - For gels to be stained with InstaStain® Ethidium Bromide, fill wells with 18 - 20 µl.

Lane	Tube	
1	A	Plasmid DNA (uncut)
2	B	Plasmid cut with Bgl I
3	C	Plasmid cut with Eco RI
4	D	Lambda DNA (uncut)
5	E	Lambda DNA cut with Eco RI
6	F	Lambda DNA cut with Bgl I

Run the Gel

3. After DNA samples are loaded, connect the apparatus to the D.C. power source and set the power source at the required voltage.

4. Check that current is flowing properly - you should see bubbles forming on the two platinum electrodes. Conduct electrophoresis for the length of time specified by your instructor.

5. After electrophoresis is completed, proceed to DNA staining and visualization. Refer to Appendix E, F, G, or H for the appropriate staining instructions.

6. Document the results of the gel by photodocumentation.

   Alternatively, place transparency film on the gel and trace it with a permanent marking pen. Remember to include the outline of the gel and the sample wells in addition to the migration pattern of the DNA bands.

Results

Conclusions

Questions

Reference

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