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Restriction Enzyme Digest & Gel Electrophoresis

Protocol

Assemble Reaction

1. Each group should label 5 clear plastic tubes with a label indicating the enzyme used; **E**, **H**, **EH**, **C**, or **(-)** for negative control. Put tubes on ice.

2. If needed, vortex the pink lambda DNA tube (λ). Use a microcentrifuge to spin down all six colored tubes in your ice bucket. Spin these tubes for 3 - 5 seconds, returning the tubes to the ice bucket when done.
Be sure that your tubes are BALANCED in the microcentrifuge!!!

3. Add ingredients **IN ORDER.** Check off ingredients as you add them:

Reagent	Tube E	Tube H	Tube EH	Tube C	Tube (-)
Nuclease Free H_2O	3µl	3µl	3µl	3µl	4µl
10x Reaction Buffer B	1µl	1µl	1µl	1µl	1µl
Lambda DNA (60ng/µl)	5µl	5µl	5µl	5µl	5µl
Enzyme	1µl **E**	1µl **H**	1µl **EH**	1µl **C**	~~0 µl~~
Total Volume	10µl	10µl	10µl	10µl	10µl

4. Spin reaction tubes for 3 - 5 seconds in a microcentrifuge to bring the liquid down to the bottom of the tubes.

5. Incubate the reactions and the negative control at 37°C for 30-45 minutes. Time: _____

Tube Code	Enzyme	Organism	Recognition Site
E	**EcoRI**	***Escherichia coli***	**G↓AATTC**
H	**HindIII**	***Haemophilus influenzae***	**A↓AGCTT**
EH	**EcoRI & HindIII**		**G↓AATTC and A↓AGCTT**
C	**CfoI**	***Clostridium formicoaceticum***	**GCG↓C**
(-)	**Water** (neg control)		**none**

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Prepare Gel Box with Buffer and Gel

1. Obtain a 0.8% gel from an instructor.
2. Loosen the screws and lower the dams, or carefully remove the tape.
3. Hold the tray on the high side, with the comb closest to the black (negative) electrode, and slip it into the electrophoresis chamber on top of the platform. The dams should hang down over the ends of the platform.
4. Rock the comb very gently, back and forth in the gel. Gently remove the comb.
5. Add enough buffer to the electrophoresis chamber to just cover the gel, about 300-350ml.

Load Gel and Run

1. After the incubation, collect your reaction tubes. Time: _____
2. Add 2µl of the EZ-Vision DNA stain to each reaction tube.
3. Fill in the blanks (-----) on the table below, listing the order of the samples loaded on the gel. With the wells at the top of the gel, lane 1 is the well on the left side.

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
10µl **Negative Control** Sample	10µl --------	10µl --------	10µl --------	10µl EcoRI / HindIII Lambda **Marker**	10 µl --------

Controls for the gel:
10µl **negative control** sample of uncut DNA
10µl EcoRI/Hind III–Lambda DNA **Marker** in loading dye

4. Load the controls (10µl **negative control** and 10µl prepared EcoRI/HindIII lambda **marker**); load the 10µl of each digest into the appropriate wells.
5. Place the lid on the gel box and connect the electrodes into the power supply. Make sure that the black plug is in the black outlet and the red plug is in the red outlet.
6. Turn on the power supply and set it at 250V. Bubbles at the electrodes indicate that electric current is running through the gel. The gel will run for approximately 20 minutes.

View Gel

After the gel has run, turn down the voltage, turn off the power, and remove the gel from the gel box. Drain off as much buffer as possible. Place the gel on a UV light box to visualize the DNA and to photograph the gel.

Revised November 2023

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Analysis

The negative control for this experiment is _____ . This sample demonstrates

_____

_____

The molecular weight marker is used to _____

_____

My restriction enzyme was _____. It has ____ restriction sites on lambda DNA yielding ____ DNA fragments. On a 0.8% agarose gel, ____ of these bands resolved.

Cut Site	Fragment Size (bp)	Resolved on 0.8% gel? (Yes or No)

1) Why are smaller bands more difficult to see?

2) What are some real-life applications of restriction enzyme digests?

3) Why do bacteria make restriction enzymes?

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Appendix

Prepare Gel Tray & Pour Gel

To prepare a 0.8% agarose gel*, add 0.8g of agarose to 100ml of sodium borate buffer**. The agarose can be allowed to hydrate in the buffer before the mixture is microwaved to dissolve the agarose. The agarose is cooled to 55°C. (*For BTC Institute field trips, this is already done by the instructors*.)

1. Prepare the gel tray by bringing up the dams on the ends of the tray and carefully tightening the nylon screws snuggly, but not too tight, or firmly tape the ends.

2. Place the 6-well comb into the slots at the top of the gel.

3. Pour the agarose into the middle of the tray until it is about half way up the teeth of the comb and has filled the tray to the corners. Do not disturb the tray while the agarose is solidifying (about 20min).

*The gel can be prepared as a 0.8% - 1% agarose gel.

** Gels can be made with sodium borate buffer, TBE buffer, TAE buffer, etc. There are advantages and disadvantages to each. Sodium borate holds superior for this lab because it allows for the gel to run at an increased voltage of 250V. This means it takes less time to run the gel.