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Restriction Enzyme Digest & Gel Electrophoresis

Plasmid DNA Protocol

Assemble Reaction

1. Each group should label the caps of five clear 1.5mL tubes with a label indicating the enzyme to be used: **HC**, **HF**, **R**, **NX, and –** (for the negative control). Put tubes on ice.

2. 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 HC	Tube HF	Tube R	Tube NX	Tube (-)
Nuclease Free H_2O	6µl	6µl	6µl	6µl	**8µl**
10x Reaction Buffer	2µl	2µl	2µl	2µl	2µl
Plasmid DNA (50ng/µl)	10µl	10µl	10µl	10µl	10µl
Enzyme	2µl **HC**	2µl **HF**	2µl **R**	2µl **NX**	~~0µl~~
Total Volume	20µl	20µl	20µl	20µl	20µl

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 at 37°C for 30-45 minutes or longer. Instructor may heat-inactivate the enzymes after this incubation.

Time: _____

Tube Code	Enzyme	Organism	Recognition Site
HC	**HincII**	***Haemophilus influenzae***	**GTY↓RAC**
HF	**HinfI**	***Haemophilus influenzae***	**G↓ANTC**
R	**RsaI**	***Rhodopseudomonas sphaeroides***	**GT↓AC**
NX	**NcoI & XbaI**	***Nocardia corallina & Xanthomonas badrii***	**C↓CATGG & GATC↓T**

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Sample Preparation

1. After the incubation, collect your reaction tubes. Time: _____
2. Add 4µl of 6x loading dye to each restriction enzyme digest reaction and control tubes at your table.

Agarose Gel Electrophoresis

1. Obtain a 1% gel from an instructor and set up your MiniOne electrophoresis system.
2. Place the gel tray with the gel in the clear buffer tank.
3. Add ~135ml of 1x sodium borate buffer to the buffer tank. There is a marking on the buffer tank to help guide you. The buffer should just cover the gel. Do not overflow!
4. With the wells at the top of the gel, Lane 1 is the well on the left side. Fill in the blanks (--------) on the table below with the label of sample that you plan to load in each lane of the gel. If you make a mistake, you can make use of the two extra lanes.

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
10 µl 1kb **Ladder**	**10 µl** _____	**10 µl** _____	**10 µl** _____	**10 µl** _____	**10 µl** _____

5. Load 10µl of the DNA ladder as is and 10µl of each sample with dye into the appropriate wells of the gel.
6. Place the amber filter photo hood on top of the MiniOne gel box and push the power button. To see the DNA bands in the gel, push the light bulb button along the right side of the MiniOne unit. Your gel will run for approximately 30 minutes.
7. When the gel has separated far enough to see your bands relative to the DNA ladder, push the power button to stop the electrophoresis. Use a camera to take a picture of your gel through the window at the top of the photo hood to analyze.

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Optional Analysis

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

_____.

The DNA Ladder is used to _____.

The restriction enzyme I selected was _____. It has ____ restriction sites on Plasmid DNA and should yield ____ DNA fragments. On a 1% agarose gel, ____ of these bands resolved.

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

Why do you think some bands are more difficult to see?

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Appendix

Prepare Gel Tray & Pour Gel

To prepare a 1% agarose gel*, add 1g of agarose to 100ml of 1x 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. 10ul of 10,000x GelGreen DNA Stain is added (*For BTC Institute field trips, this is already done by the instructors*.)

1. Prepare the gel tray by bringing placing the gel trays into the casting system with the appropriate combs.

2. 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. For a MiniOne gel, this is approximately 10ml. Do not disturb the tray while the agarose is solidifying (about 20min).

*The gel can be prepared as a 0.7% - 1% agarose gel.

** Gels can be made with sodium borate buffer, TBE buffer, TAE buffer, etc. There are advantages and disadvantages to each.