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Restriction Enzyme Digest & Gel Electrophoresis Inquiry

Partner Names (& Initials): _____

_____

For this experiment, you and your laboratory group will perform two procedures as outlined below:

- The **control procedure** is a method for digesting lambda bacteriophage DNA with restriction enzymes.

  As your team performs the control procedure:

  - Read each step carefully.
  - Pay attention to how each step is done and think about how that particular step effects all of the other steps, and the outcome of the procedure.
  - Share your observations and questions with your lab team. This should help your team to identify something to change in the experimental procedure that you do next.

- The **experimental procedure** is a method that you will perform that is exactly like the control procedure except that you will **change** one detail. You can choose to change any single detail that appeals to you. You could alter the amount or alter the type of an ingredient in the digestion, such as:

  - Use different restriction enzymes
  - Use a different digestion temperature
  - Use a different length of digestion time

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Restriction Enzyme Digest & Gel Electrophoresis Inquiry

Control Procedure

Partner Initials: _____

I. Reaction Set-up

Procedure
1. Each group should label 5 clear plastic tubes with their initials and and indicator for the enzyme used: **E, C, H or EH**; or **(-)** for 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 to 5 seconds, returning the tubes to the ice bucket when done. **Be sure that your tubes are BALANCED in the centrifuge!!!**
3. Add ingredients **IN ORDER**, according to the following table.

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 the reaction tubes for 5 seconds in a microcentrifuge tube to bring the liquid down to the bottom of the tube.
5. Incubate the reactions and the negative control at 37°C for 15 minutes. Record the start time and the total incubation time below: *Start Time:* _____ *End Time:* _____ *Total Incubation Time:* _____ *minutes*

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Restriction Enzyme Digest & Gel Electrophoresis Inquiry

Control Procedure

Partner Initials: _____

II. Prepare Gel Box with Buffer and Gel

Procedure
1. Obtain a 0.8% agarose gel from your instructor.
2. Loosen the screws and lower the dams on both sides of the gel casting tray.
3. Place the agarose gel tray into the electrophoresis cell with the comb closest to the negative (black) electrode. It should slide in easily. Ensure that the gel tray dams are still down.
4. Add enough buffer to the electrophoresis chamber to just cover the gel, about 325-350ml.
5. Rock the comb very gently, back and forth, in the gel. Gently remove the comb. Set the comb on top of the power supply (please **do not** throw the comb away).

III. Load Gel and Run

1. Retrieve your samples from the heat block.
2. Add 2µl of the EZ-Vision DNA stain to each reaction and the negative control.
3. Fill in the blanks (-----) in the loading diagram 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** Uncut Lambda DNA	10µl --------	10µl --------	10µl --------	10µl EcoRI/ HindIII Lambda **Marker**	10 µl --------

4. Load 10ul **negative control,** 10ul prepared EcoRI/HindIII lambda **marker**), and load the 10ul of each digest into the appropriate wells as described in the table above.

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I N S T I T U T E

Restriction Enzyme Digest & Gel Electrophoresis Inquiry

Control Procedure

Partner Initials: _____

III. Load Gel and Run (continued)

Procedure
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 into the red.
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 should run for approximately 20 minutes. Record the start time and the total run time below: *Start Time: _____* *End Time: _____* *Total Run Time: _____ minutes*

IV. Results & Observations

1. Tape a picture of each of your gels or draw your gel results below. Be sure to annotate your gel picture or diagram with the following information: ✓ Agarose gel % ✓ Name of the running buffer ✓ A legend detailing the wells/lanes and you put in them ✓ Type of DNA stain (EZ-Vision) ✓ Run Time in minutes ✓ Voltage at which the gel was run
2. Record any observations from your results that may be helpful when you go on to perform the experimental procedure.

BioPharmaceutical Technology Center
I N S T I T U T E

Restriction Enzyme Digest & Gel Electrophoresis Inquiry

Experimental Procedure

Partner Initials: _____

After performing the control procedure, reflect on how what you did affected your results. Talk to your laboratory team to identify a detail of the control procedure that you will change in the experimental procedure. An **experimental procedure** is exactly like the control procedure except that you will **change** one detail.

Design an experiment according to the following rules:

- Change **only one variable** between your control and experimental procedures. You can choose to change any single detail that appeals to you. Your group may decide to alter the amount or alter the type of an ingredient in the digestion, such as:
    - Use a different restriction enzymes
    - Use a different digestion temperature
    - Use a different length of digestion time
- You will be limited to **seven** samples including your control samples.

1. Write down the experimental variable that you've chosen: _____

2. Describe the negative and positive controls that you will prepare given the variable you've chosen:

Positive Control(s)	Negative Control(s)

As you and your partner proceed to run the experiment, think about the following:

- **Follow the instructions** to the best of your ability.
- **Write down** any observations that you or your partner make, such as anything that is different than, or in addition to, the instructions as they are written.

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I N S T I T U T E

Restriction Enzyme Digest & Gel Electrophoresis Inquiry

Experimental Procedure

Partner Initials: _____

I. Reaction Set-up

Procedure	Observations
1. Write down the experimental variable you have chosen.	
2. Describe the controls that you will run for the experimental variable you have chosen. **Positive (+):** **Negative (-):**	
3. Devise a way to label your tubes so that initials or symbols can represent what will be in each tube. Record your labelling scheme below:	

Tube Label							
Tube contents							

4. Use a microcentrifuge to spin down all the tubes you will use from your ice bucket. Spin tubes for 5 seconds, returning them to the ice bucket when done. **Remember to BALANCE your centrifuge!!!**

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Restriction Enzyme Digest & Gel Electrophoresis Inquiry

Experimental Procedure

Partner Initials: _____

I. Reaction Set-up (continued)

Procedure	Observations
5. Below is a recipe for a single 10μl restriction enzyme digest. If you use the recipe for some or all of your reactions, then circle the recipe and annotate it appropriately. If you modify the recipe, record in the open space what your adjusted recipe(s) are.	

3μl Nuclease Free H_2O **1μl 10x reaction buffer B** **5μl Lambda DNA (60ng/μl)** **1μl Enzyme(s)** (or water for (-) control) **----------** **10μl total volume (300ng DNA)**	

6. Spin the reaction tubes for 5 seconds in a microcentrifuge tube to bring the liquid down to the bottom of the tube.

7. Incubate the reactions and the controls. Record the temperature and time of incubation below:

Temperature: _____ *°C*

Start Time: _____ *End Time:* _____

Total Incubation Time: _____ *minutes*

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Restriction Enzyme Digest & Gel Electrophoresis Inquiry

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Partner Initials: _____

II. Prepare Gel Box with Buffer and Gel

Procedure
1. Obtain a 0.8% agarose gel from your instructor.
2. Loosen the screws and lower the dams on both sides of the gel casting tray.
3. Place the agarose gel tray into the electrophoresis cell with the comb closest to the negative (black) electrode. It should slide in easily. Ensure that the gel tray dams are still down.
4. Add enough buffer to the electrophoresis chamber to just cover the gel, about 325-350ml.
5. Rock the comb very gently, back and forth, in the gel. Gently remove the comb. Set the comb on top of the power supply (please **do not** throw the comb away).

III. Load Gel and Run

Procedure	Observations
1. Retrieve your samples from the heat block.	
2. Add EZ-Vision DNA stain to each reaction and the controls at a proportion of 2µl EZ-Vision / 10µl of reaction.	
3. Fill in the blanks (-----) in the loading diagram below, listing the order of the samples loaded on the gel.	

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
10µl --------	10µl --------	10µl --------	10µl --------	10µl --------	10 µl --------	10 µl --------	10 µl --------

4. Remember to reserve space in your gel for one well to hold 10µl EcoRI/HindIII Lambda DNA Marker for a size standard, and load 10µl of your remaining samples into the gel as described in the table above.	

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Restriction Enzyme Digest & Gel Electrophoresis Inquiry

Experimental Procedure

Partner Initials: _____

III. Load Gel and Run (continued)

Procedure
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 in the red.
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. *Start Time:* _____ *End Time:* _____ *Total Run Time:* _____ *minutes*

IV. Results & Observations

Tape a picture of each of your gel(s) or draw your gel results below or on a separate page and annotate appropriately.

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Restriction Enzyme Digest & Gel Electrophoresis Inquiry

Experimental Procedure

Partner Initials: _____

V. Analysis

Procedure
1. Describe the differences between how you set up your experimental samples and your control samples.
2. Describe all of the differences that you observed between your control and experimental procedures. This should include the experimental variable or change that you selected, plus any other differences that you observed.
3. Did the gel look the way you expected? Explain why or why not.

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Restriction Enzyme Digest & Gel Electrophoresis Inquiry

Experimental Procedure

Partner Initials: _____

V. Analysis (continued)

Procedure
4. Based on the results of your experiment, describe how your chosen experimental variable affected your result.
5. Explain what part of your experiment surprised you the most.
6. If you could run a new experiment, what would you try next?