Restriction analysis of Plasmid DNA

In this exercise, you will digest the plasmid pBR322 with 5 different restriction enzymes and resolve the fragments by agarose gel electrophoresis. pBR322 is a small cloning vector with several unique restriction sites; it forms the backbone of many larger, more sophisticated plasmids in use today.

Procedure

1. Set up the following 6 restriction digests in 1.5 mL microcentrifuge (eppendorf) tubes A-F. All reagents and tubes should be stored on ice.

The reactions should be set up as follows: First, add water to each tube (you may use the same tip); next, add 2x buffer; then DNA. **Always add enzymes last.**

All volumes are in **microliters (µL).**

<table>
<thead>
<tr>
<th>Tube</th>
<th>DNA</th>
<th>2X restriction buffer</th>
<th>BglII</th>
<th>DraI</th>
<th>EcoRI</th>
<th>HaeIII</th>
<th>HindIII</th>
<th>H2O (distilled)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>5</td>
<td>1</td>
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<td>--</td>
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<td>3</td>
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<tr>
<td>B</td>
<td>1</td>
<td>5</td>
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<td>C</td>
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<td>D</td>
<td>1</td>
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<td>E</td>
<td>1</td>
<td>5</td>
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<td>1</td>
<td>3</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>5</td>
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<td>4</td>
</tr>
</tbody>
</table>

Total reaction volume: 10 µL

Note DNA concentration.

2. Tightly close the tubes and mix reactants by tapping. Spin for 1-2 seconds in microcentrifuge to mix & collect at the bottom.

3. Incubate in 37°C water bath for 35-45 minutes. Make sure lids are tightly closed to prevent evaporation (a problem with such tiny volumes).

4. Prepare 1% agarose gel.

5. Remove the tubes from water bath and spin 1-2 seconds to collect condensate. Add loading dye to each tube (if 10X loading dye, 1 µL; if 6X loading dye, 2 µL). Be careful to change tips each time!

6. Tap to mix & spin.

7. Load entire contents of each sample in wells from left to right, A-F.

8. Obtain a DNA size marker/standard (e.g., λHindIII). Load appropriate amount in next well. (Instructor will tell you.)

9. Electrophorese at 100-150V until the bromophenol blue has migrated to within 3 cm of the end of the gel. Make sure wells are nearest to the cathode (black, negative).

10. Stain, view, and photograph gel.
For your restriction digestion experiment:

The enzymes you used cut pBR322 at the following locations:

- **Bgl II**: no sites
- **DraI**: 3230, 3249, 3941
- **EcoRI**: 4359
- **HindIII**: 29
- **HaeIII**: more than 6 cut sites

In your lab notebook, be sure to include the following elements:

1. Draw a simple pBR322 restriction map showing the sites for enzymes used, in relative locations;
2. Predict the expected number of linear DNA bands following complete digestion with each enzyme;
3. Predict the expected sizes of those bands and use the size marker to help identify them on your gel;
4. Explain what you see when pBR322 is digested with BglII;
5. Explain why HaeIII is expected to have many more restriction sites in pBR322 than the other enzymes;
6. Comment on any unexpected bands in your gel.