Diagnostic Restriction Digestion

Introduction

A diagnostic restriction digest helps identify correctly assembled clones from incorrect clones.

Materials

- 200 ul PCR strip tubes, one per reaction
- Restriction enzyme (chosen below), 1 ul per reaction.
- 10X restriction enzyme buffer (chosen below), 1 ul per reaction.
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Procedure

Choose A Good Restriction Enzyme

1. Using Benchling, choose a restriction enzyme that meets the following criteria:

   - Cuts at least once **in the insert** (for a pEXPR, either the promoter or the gene.)
   - Cuts at least once **in the backbone**.
   - Gives bands that aren't too large (> 8 kb) or too small (< 200 bp).
   - Gives a band pattern that is significantly different from the **expected error mode.**
     * For LR reactions, the most common error is a pDEST that slipped through the selection.
   - Begin in the "Brian's Favorites" list, then expand to the main Weiss lab list.
   - If you can't find a single enzyme that gives an acceptable band pattern, choose two enzymes that give a acceptable band pattern when used together.
     * This double-digest is subject to buffer compatibility, outlined below.

2. Record your enzyme choice on the plasmid's **Description** page.

3. Benchling will tell you the enzyme's **buffer compatibility and active temperature.** Record the buffer in which the enzyme is **most active**.

   If there are multiple bufers in which the enzyme is equally active, choose in this order: Buffer **3.1**, Buffer **CS**, Buffer **2.1**, Buffer **1.1**.

Set up the restriction digest

4. Retrieve the minipreps and the appropriate 10X buffer concentrate from the freezer. Thaw on the benchtop or in your fingers.

5. Label the PCR tubes with your initials and an incrementing number.

   ie: BT-1, -2, -3, -4

6. Vortex the minipreps and the 10X buffer concentrate briefly, then pulse down in the microfuge.

7. For each miniprep, set up a PCR tube containing the following **in order**:

   - 5 ul enzyme-quality H_2O
   - 1 ul enzyme buffer
   - 3 ul miniprep DNA

   - 1 ul enzyme

   Remove the enzyme from the freezer for as little time as possible.
   I have specified an "arbitrary" 3 μl volume of miniprep DNA; this should be fine as long as your miniprep concentration is >= 100 ng/ul.

8. Flick the strip tubes a few times to mix the reaction, then pulse down in the strip tube microfuge.

9. Incubate at the appropriate temperature for at least 1 hour and more more than 16 hours.

   If the enzyme's active temperature is 37°C, use the 37°C plate incubator.

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10. Stop the reaction by adding 2 ul of 6X NEB purple gel loading dye to each reacti on.

11. Flick the strip tubes a few times to mix the reaction, then pulse down in the strip tube microfuge.

    PAUSE POINT: The reaction can be stored almost indefinitely at room temperature once it's been stopped.

12. Proceed to gel electrophoresis.