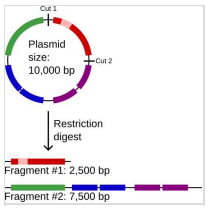

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Diagnostic Restriction Digest (Instructor Protocol)

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1 *Works for me* Share

This protocol is published without a DOI.

Yeast ORFans CURE

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ABSTRACT

This is the instructor protocol for

Diagnostic Restriction Digest
by Brian Teague,
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PREVIEW RUN

PROTOCOL CITATION

Brian Teague 2022. Diagnostic Restriction Digest (Instructor Protocol). **protocols.io** https://protocols.io/view/diagnostic-restriction-digest-instructor-protocol-cffftjjn

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MATERIALS TEXT

- **700 µL** or **1.7 mL** tubes for aliquots

  CutSmart® Buffer **New England**
- **Biolabs Catalog #B7204S** Step 1

  PvuII-HF - 5,000 units **New England**
- **Biolabs Catalog #R3151S** Step 2

  Diluent B - 5.0 ml **New England**
- **Biolabs Catalog #B8002S** Step 2

SAFETY WARNINGS

None of the materials are hazardous.

HOWEVER, we are shedding nucleases -- enzymes that degrade DNA -- all the time. Wear lab coats and gloves to keep your samples nuclease-free.

Setup

1 CutSmart® Buffer **New England**

Aliquot the **Biolabs Catalog #B7204S** : **20 µL** ul aliquots, 1 per 4 students

2 PvuII-HF - 5,000 units **New England**

Aliquot the **Biolabs Catalog #R3151S** enzyme: **4 µL** of enzyme in **16 µL** of Diluent B - 5.0 ml **New England** **Biolabs Catalog #B8002S** , 1 per 4 students.

Instructor Tips & Common Student Errors

3 **Instructor Tips**

- In a more advanced course, I would let students select which enzyme to use (chosen from the ones in the freezer.) In an intro course, I choose the enzyme for them. PvuII is a good choice because it has two cut sites in the backbone *and one in the GFP insert.* This makes it easy to distinguish between the correct plasmid and one with a GFP still present (maybe not glowing because of a mutation? Or some other error?)

- PvuII is a little on the expensive side -- but if you're giving the digest a full hour, diluting down to 2 units per ul gives two advantages: it decreases the amount used, and increases the volume of the pipetting step. Make sure you use the correct diluent, though.

- I used to include a heat-inactivation step. I don't any more because the SDS in the purple loading dye denatures the enzyme.

- Sometimes a student's miniprep isn't concentrated enough to get a full microgram of DNA into the digest. As long as they can get at least 500 ng in the digest, that should be enough to see on a gel. It's more acceptable to decrease the mass of DNA than it is to increase the volume -- contaminants in the miniprep often get in the way of the digest, particularly leftover ethanol.

- I don't have a positive control in this experiment as written. Maybe add one?

4 Common Student Errors

- Not mixing the reaction well.
- Not loading the entire reaction onto the gel. (Because the first one they did was a PCR, it's easy to assume that you just need 1-2 ul without reading the protocol carefully.)