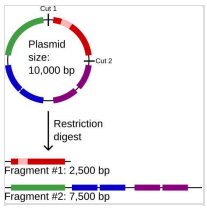

Aug 11, 2022

Diagnostic Restriction Digest

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1 *Works for me* Share

This protocol is published without a DOI.

Yeast ORFans CURE

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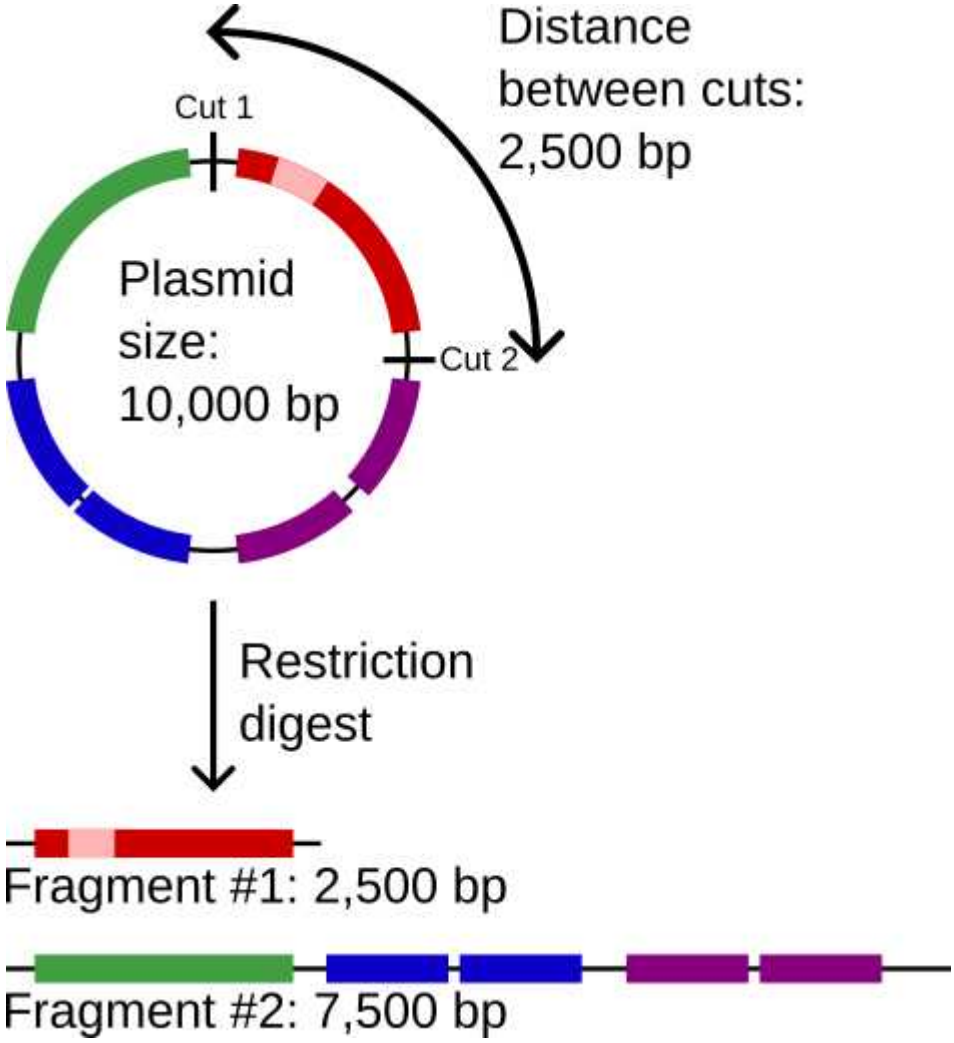
ABSTRACT

In this restriction digest, you'll use an enzyme that cuts DNA to cut your miniprepped plasmid. This can give you some evidence as to whether your plasmid is what you expected or not.



You'll also use Benchling to *predict* the result of your digest -- that way, you can compare your prediction to your actual result.

PROTOCOL CITATION

Brian Teague 2022. Diagnostic Restriction Digest. **protocols.io** https://protocols.io/view/diagnostic-restriction-digest-ce58tg9w

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MATERIALS TEXT

- Miniprep DNA
- PvuII-HF - 5,000 units **New England**
- **Biolabs Catalog #R3151S**
- CutSmart® Buffer **New England**
- **Biolabs Catalog #B7204S**
- Nuclease-free water
- A 200 ul PCR tube

SAFETY WARNINGS

None of the materials we're using today 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.

Perform the diagnostic digest 1h

1 For each miniprep, compute the volume that contains **1 µg** of DNA.

2 In the PCR tube, mix:

- The volume of DNA you computed in step 1, *up to a maximum of* **5 µL**
- **2 µL** of CutSmart enzyme buffer
- **2 µL** of PvuII enzyme
- Enough nuclease-free water for a total volume of **20 µL**

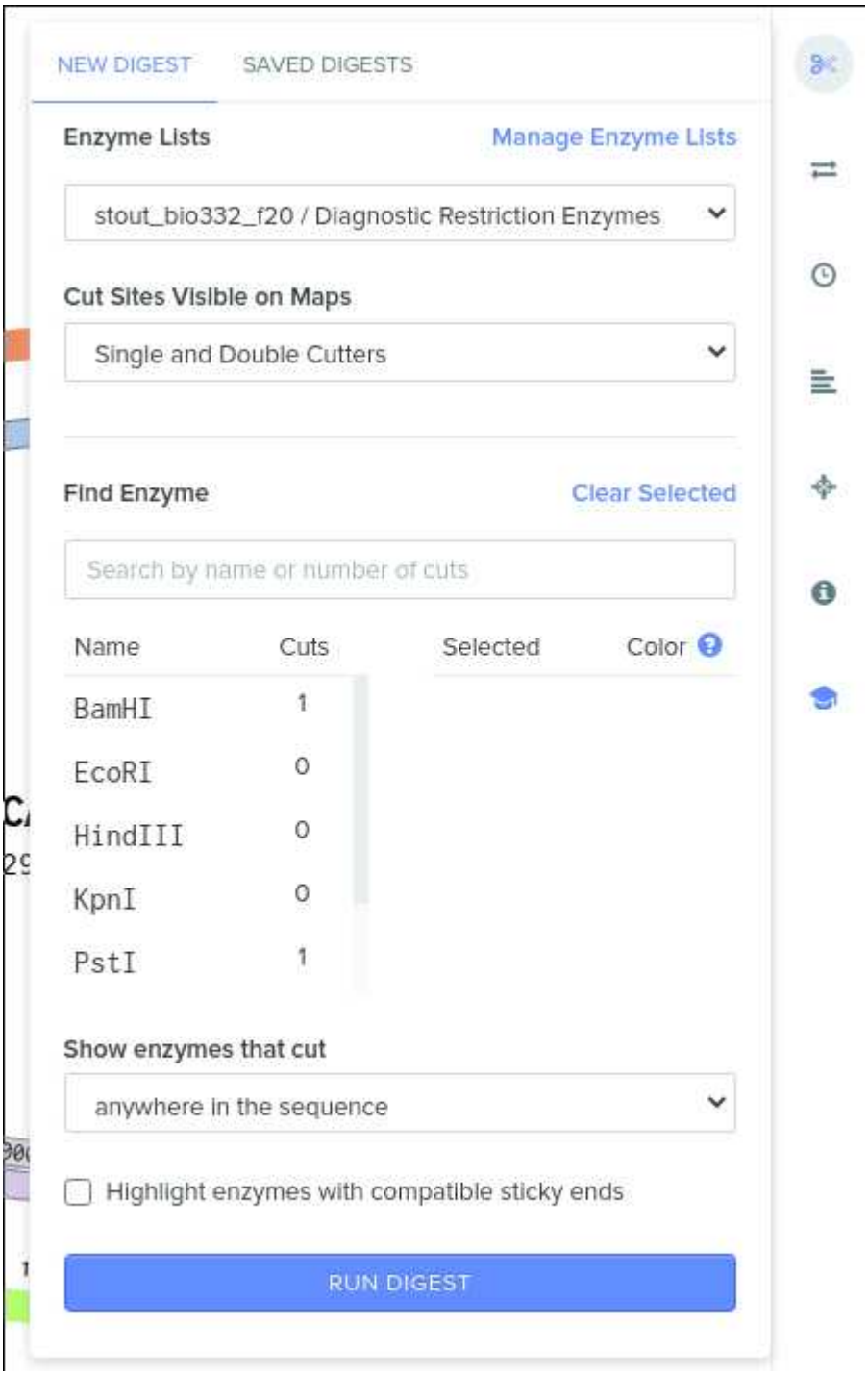
3 Mix by flicking the tube gently, then spin down briefly.

1h

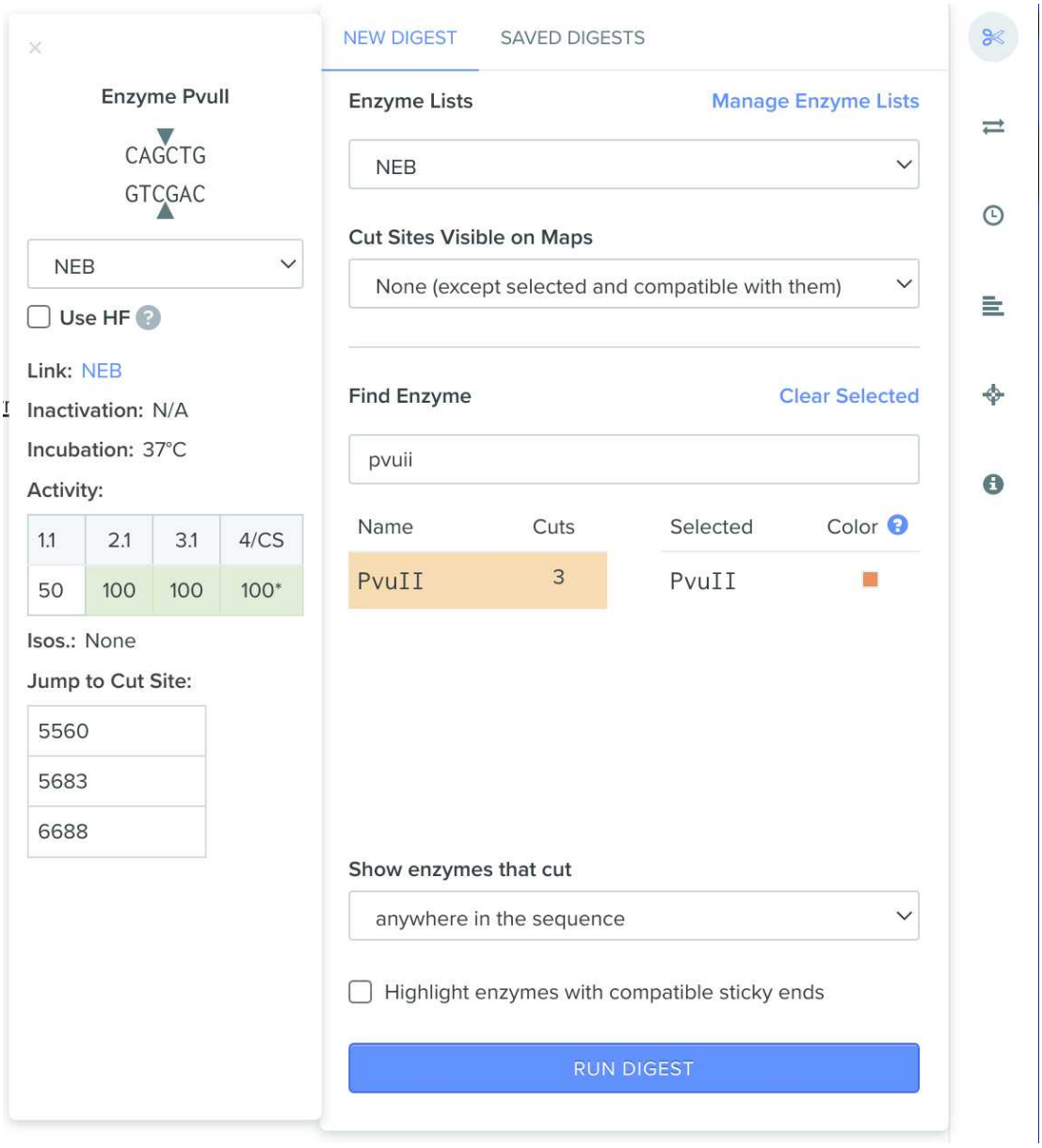
4 Incubate your reaction for **01:00:00** at **37 °C**

Simulate the diagnostic digest on Benchling 1h

5 While your digest is running, you can use Benchling to simulate your digest. Begin by opening the "Cas9 Plasmid" document (or whichever document your instructor indicates). Then, click the "scissors" icon on the right to open the restriction digest panel.

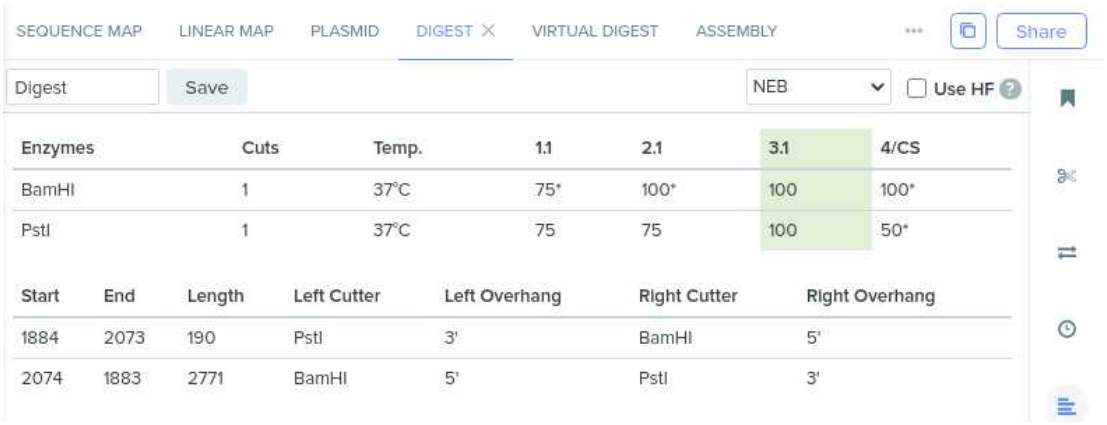


6 Pull down the "Enzyme Lists" drop-down and choose "NEB". In the "Find Enzyme" box, type "PvuII" (that's the letters "Pvu" and two upper-case i's). The enzyme list should just show "PvuII" -- click it.



Helpful hint: Once you have chosen a list, you can see where on the plasmid each of them cuts by closing the restriction digest panel and selecting the "PLASMID" tab at the top.

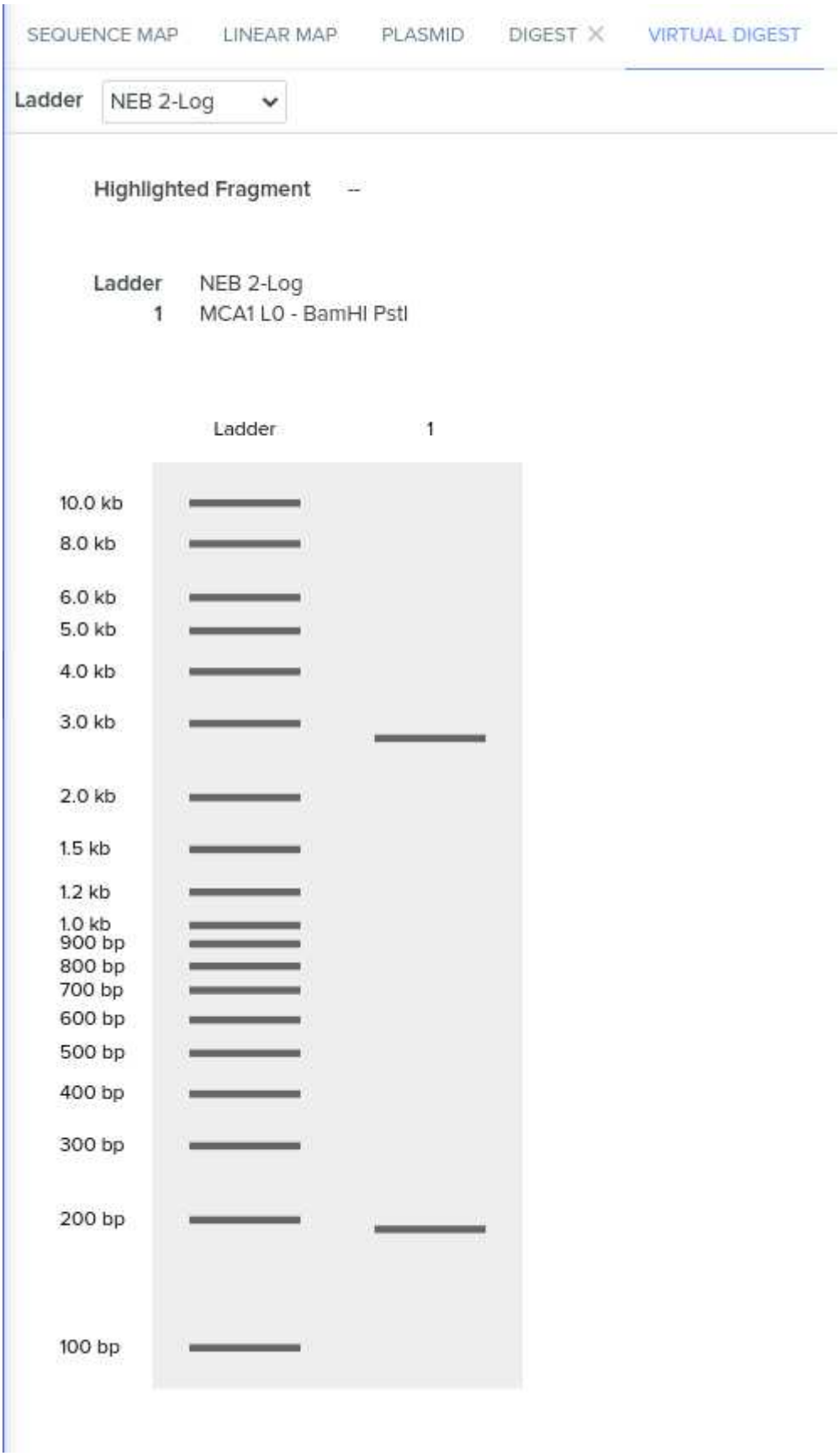
7 Click the blue RUN DIGEST button at the bottom. The new tab that opens tells you the sizes of the DNA fragments were you to digest this plasmid with those enzyme(s).



Enzymes	Cuts	Temp.	1.1	2.1	3.1	4/CS
BamHI	1	37°C	75*	100*	100	100*
PstI	1	37°C	75	75	100	50*

Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
1884	2073	190	PstI	3'	BamHI	5'
2074	1883	2771	BamHI	5'	PstI	3'

8 There's also a new tab called VIRTUAL DIGEST. Click it. Change **Ladder** option to "NEB 2-Log", which is the ladder we are using in the lab. Voila, a simulation of the gel you can expect to see when you run the digest for real.



After your ditest is complete, proceed to analyze your restriction digest using gel

9 electrophoresis.

When you prepare your samples for the gel, mix **5 µL** of loading dye with your entire digest and load the whole thing on the gel. There's no need to keep any around for future use.