Lab Recap: Restriction Digest (RD)

Will cut your vector more than AP Physics will cut your GPA

Purpose: To use restriction enzymes to cut your plasmid so that you can separate your insert (plus 700 vector nucleotides) from your vector backbone via a restriction digest reaction. This lab prepares you for the Restriction Digest Gels (RDG) lab, in which you will run the products of this lab through a gel to once again get a glimpse at the size and quality of your duckweed DNA insert.

Vocabulary/Terms

Restriction Digest - using restriction enzymes to cleave specific parts of a DNA strand

Restriction Enzyme - protein that binds to a specific DNA sequence and cleaves (cuts) the DNA at or near a binding site

Restriction Site - specific DNA sequences that act as the binding site for restriction enzymes. The restriction enzymes cut at on or near these sites. We are cutting at the *Pvu*II site on the vector.

PvuII-HF Enzyme - the restriction enzyme that we are using in the lab. Notice that we are not just using PvuII, we are using PvuII-HF. Much like the normal PvuII enzyme, PvuII-HF still cuts at the two *Pvu*II sites on the vector. The only difference between PvuII and PvuII-HF is that PvuII-HF works faster.

Restriction Buffer - regulates the pH of the digest reactions so that the enzymes can function properly

5X Mix - contains water, buffer, and enzyme--all the requirements for the digest reaction. We use a 5X "master mix" in this lab because we're performing several digests. The 5X mix saves us a lot of time because instead of every member adding water, buffer, and enzyme individually, each member only has to add some of the 5X mix (#SmartLazy). A 5X mix is for 4 people in a group so that there is extra.

What you are doing and why you are doing it

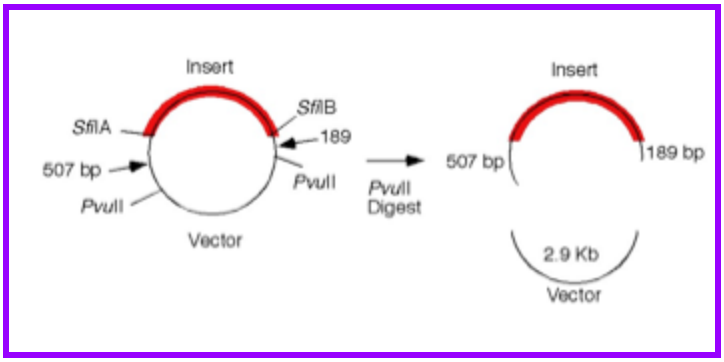
What you are doing:

- Making a 5X master mix containing water, buffer, and the PvuII-HF restriction enzyme
- Adding the 5X mix to your plasmid DNA
- Giving us the resulting tube to incubate in order to start the digest reaction, which will cut your vector in a way that physically separates the duckweed insert (plus 700 vector bases)

Why you are doing it:

- So that you can eventually run your digested DNA through a gel and verify the quality and size of your insert (first estimated in PCR)
- This is your final chance to determine whether your prep is worth sequencing.

Understanding the Context of the Digest Reaction



- The picture above shows the digest reaction that is happening in this lab
- Our insert is between the *Sfi*IA and *Sfi*IB sites
- Our enzyme cuts at PvuII sites, which are outside of the SfiIA/B sites
- The result is the insert+700bp and the vector backbone. That is important for the next lab.
- We use the PvuII-HF enzyme because it is cheaper than other enzymes and fast

Overview of *Restriction Digest* Lab (It's an EZ BREEZY lab so plz don't mess up)

1. Take your miniprep DNA out of the freezer and add a little bit of it to a microfuge tube so that you can prep a ***digest.*** Make sure you label this tube with a "D" for digest. Bring the yellow miniprep tubes back to the front to avoid confusion. We will be sending your miniprep DNA to Rutgers and the last thing we want is have something terrible happen to your hard work!

2. A major part of the digest reaction is the ***5X Mix***. This mix contains the ***restriction enzyme*** along with some sterile water and a restriction buffer to stabilize the reaction conditions. The restriction enzyme we are using is called PvuII-HF. Why do we use this specific enzyme? Because it is cheap and it gets the job done well! Make sure to **always add the enzyme last!**

3. All you have to do now is add 10 microliters of the 5X Mix to the clear microfuge tube that has some of your miniprep DNA in it and you're FINITO! Give us the "D" tubes so we can incubate them and let the mix do its magic. Then, you will hopefully be left with some digested DNA. You guys won't actually be able to see the reaction happen--it will be happening in the incubator. Sry :(

Q & A

Q: What is this "reaction" that you speak of?

A: We put our DNA and the restriction enzyme into the same tube. The restriction enzyme should recognize different cutting sites in your DNA sequence and cut at those sites. The rxn is on page 2.

Q: Why is it called restriction digest?

A: Because we are using a restriction enzyme to cleave your insert from plasmid DNA. Think of the *Pvu*II as Pac-Man: his nom-nom-nomming on your plasmid DNA helps to isolate your insert plus 700 base pairs. The ghosts in this case are lab errors, so try to avoid those!

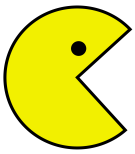
Q: Why does the insert have an extra 700 base pairs?

A: PvuII-HF cuts the plasmid outside of the *Sfi*IA and *Sfi*IB sites (these sites are closest to the ends of your insert). By cutting outside of these sites, this leaves about 700 bp at the ends of your insert.

Q: Why do we have to add restriction enzyme last to the 5X mix?

A: If you mess up while adding the other ingredients to the 5X mix and have to start over, you won't need to waste any more enzyme. The enzyme is VERY expensive!

Bonus: Comparing PCR and RD



	PCR	RD
Purpose	To *amplify* your insert from the vector and run it through a gel to analyze size and quality of your insert	To *cut* your insert from the vector and run it through a gel to analyze size and quality of your insert[1]
English plz	Making a bunch of copies of your insert	Nom-nomming your insert off your vector
What's in the 5X mix?	sterile water, FOR primer, REV primer	sterile water, restriction buffer, and PvuII-HF restriction enzyme
Do you see the reactions happening?	No - you end up giving us the tubes to put in the the thermocycler for the PCR reactions to happen	No - you end up giving us the tubes to incubate for the digest reaction to happen
Vector bases attached to insert	200	700

[1] You do not need to amplify your insert in this lab because you are using lots of your plasmid DNA, so you have enough DNA to see in your gel. Whereas in the PCR lab, you initially put in very little DNA.