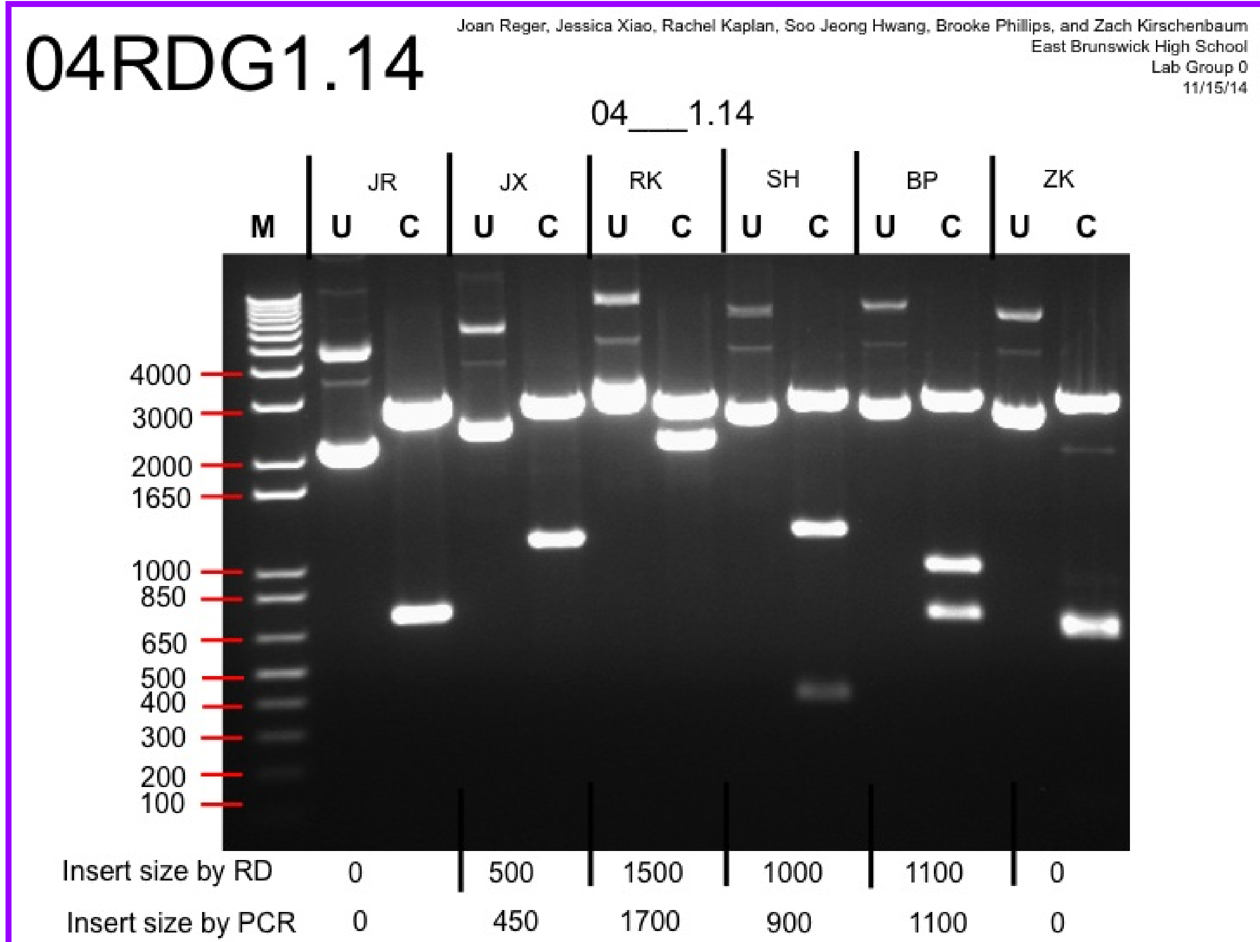
TEMPORARY RDG MOCKUP RECAP--WILL BE UPDATED BEFORE THE NEXT RDG MOCKUP (DECEMBER OR JANUARY)

Sample Mockup



Directions

1. First you need to download the RDG mockup template from the website
2. Just like PCR gels, you need to import your picture from the weebly website
3. Align the standard markers
4. Align your unknown lanes. Remember each person has uncut, THEN cut
5. Draw an imaginary line from your CUT to your standard, then subtract **700.** Record the value under insert size by RD.
6. Go to Clone Report Sheet (CRS) and use it to type in insert size by PCR.
7. Save mockup as a .jpeg, upload to google drive, post link and insert size on CRS
8. Indicate on CRS whether the WSSP should send your clone to be sequenced

The Uncut Lane:

- Functions as *negative control*
- You can compare it to your cut/digest lane to see if the enzyme cut
- If your uncut lane looks exactly like your cut lane, then your enzyme did not cut and you cannot use RDG to approximate the length of your insert
- If your uncut lane looks kind of like your cut lane, then your enzyme may have only partially cut (partial digest) and you cannot use RDG to approximate the size of your insert
- The uncut DNA did not have buffer in it, so the physical act of pipetting sheared it into 3 separate bands
    - Open circle (top)
    - Linear (middle)
    - Supercoiled (bottom)
- Do not worry about those bands for now. Just know that it's normal to have 3 bands in your uncut lane.

The Cut/Digest Lane:

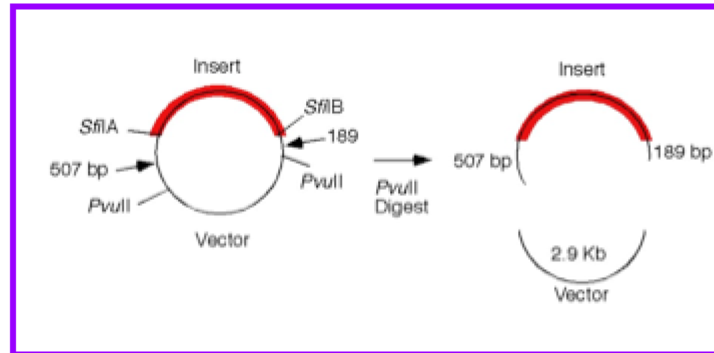
- You added restriction enzymes to cut your plasmid DNA, separating your insert+some bases from your vector backbone
- You use this lane to determine insert size
- Normally, you should see TWO bands (insert and vector backbone). However, sometimes there may be more bands and it could not be an issue. That will be discussed at the bottom so scroll down a little bit.

Q&A

What if I have more than two bands in my cut lane?

- IF THEY ARE THE SAME INTENSITY → contamination.
    - You can confirm that from the PCR gel. You can even look to see if the size of the two inserts are the same between PCR and RD gels.
- IF THEY AREN'T THE SAME INTENSITY...read on
- The digest reaction: the restriction enzyme recognizes a short DNA sequence on the vector (restriction sites) and then cuts
- However, sometimes those sites on the vector can be in your insert
- If your insert has one restriction site, then you will see 3 bands. If your insert has 2, you will see 4 bands.
    - If this is the case, add all bands together (other than the vector backbone of course) and subtract 700 base pairs from the total

Why do we subtract 700 bp?



- Our insert is between the *Sfi* sites
- The enzyme cuts at *Pvu*II sites, which are outside of the *Sfi* sites
- Therefore, there is about 700 bp combined on both sides between your insert and the places the enzyme cut. So you must subtract the 700 vector bases out.

What are some gel loading or loading dye mistakes?

All of these mistakes mean that you did not properly get DNA into your well. The biggest sign of this is that you do not see the dark blue DNA in the well after you pipet. If your samples looked very light in the gel, then this is your error.

- You punctured the gel when you were pipetting. That is something that you would have noticed because you would have realized that you were trying to release liquid but nothing was initially coming out.
- You missed the well when you were releasing your PCR samples into the well. For example, you could have released when you were not inside the well, therefore the DNA floated away into your buffer.
- You released too quickly, so the DNA bounced out of your wells and floated away out of the wells and into the buffer.
- You did not add any or enough loading dye. Since loading dye allows your samples to stay into the well without floating away, if you do not have enough loading dye, then your samples will simply float away. If your samples were dark blue before you loaded, then this is probably not the problem.

How do I know if I should sequence my clone:

- You SHOULD sequence your clone if

    - Your gels show your sequence is >300 bp (if it's close like 250, ask ZK or BP)
    - You have shown that your sample is not contaminated
    - Your uncut lane looks good
- You SHOULD NOT sequence your clone if
    - Both RD and PCR gels show your prep is contaminated
    - Your sequence is significantly less than 300bp
    - Your uncut DNA bands was too faint
        - If the band is faint because of pipetting issues, then disregard this
- If you have ANY QUESTIONS, ask ZK or BP

IF YOU HAVE ANY QUESTIONS, ASK BP OR ZK.

IF YOU CANNOT DETERMINE THE SIZE OF YOUR INSERT FROM RD GELS, PUT EITHER CONTAM OR N/A, AND EXPLAIN WHY IN A COMMENT

IF YOU DO NOT THINK YOU SHOULD SEQUENCE YOUR PREP, INDICATE WHY IN A COMMENT.

IF YOU THINK YOU NEED TO JUSTIFY SEQUENCING YOUR PREP, INDICATE SO IN A COMMENT