Lab Recap: PCR Gels (PCRG)

The Moment of Truth: Part I

Purpose: To run PCR samples through a gel in order to obtain information about your duckweed insert.

Vocabulary/Terms:

Gel Electrophoresis - Running a current through a gel that is loaded with DNA samples in order to separate DNA fragments based on size.

Standard (also known as Marker or Ladder) - A collection of DNA fragments of known sizes. You will compare this to your own duckweed DNA sample to approximate the size of your duckweed insert. $tandard is very expen$ive so handle it with care. Color = dark blue.

Loading Dye - What you put in your PCR sample before you put it into the gel. It helps you see the sample in the gel (gives it blue color) and ensures that your DNA will not flow out of the well. This looks a lot like standard, so don't confuse the two. Color = dark blue.

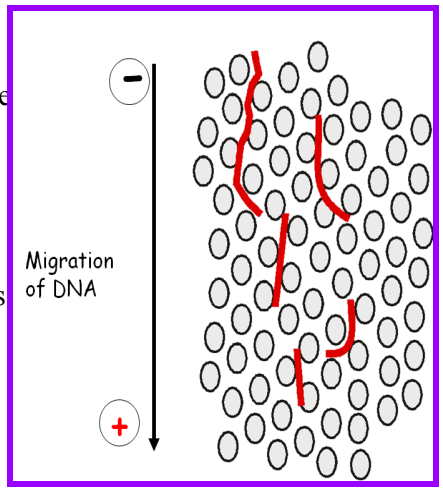
Agarose - The sugar molecules that make up the gels we are using. Smaller DNA fragments can pass through them in a gel relatively quickly. Large DNA fragments have trouble.

Stain - We we put in your gels so that you can see the bands under UV light. The stain adds radioactive tags to DNA and the tags glow under UV light. Since the stain adds radioactive things to DNA, it is considered a carcinogen. If the stain can add radioactive things to duckweed DNA, then it can add radioactive things to you as well--which is why you should never touch a gel without gloves. The stain we used it called Ethidium Bromide (EtBr) and you will most likely never see it unless you come in early to make gels. Color = red

Buffer - Something that regulates pH. Your gels are sitting in buffer.

Concept: Gel Electrophoresis

- We create an electric current by putting a negative (black) electrode on the top and a positive (red) electrode at the bottom
- DNA is negatively charged
- DNA is repelled by the negative electrode and attracted to the positive electrode, therefore it migrates down the gel
- Shorter fragments move farther because it is easier for them to pass through the agarose. Larger fragments do not move as much because it is harder to pass through the agarose.



What you will be doing and why you will be doing it

Before this lab:

- You set up your PCR reactions and z00k3 initiated the PCR reactions.
- You have a ton of duckweed DNA plus some extra nucleotides (remember the primers allowed us to amplify only that specific region). That DNA sample is also called your PCR sample.

During this lab:

- You will adding loading dye to the PCR samples to make sure they stay in the wells of the gel and

to make sure you can see the PCR samples go into the well

- You will then be pipetting the PCR samples + loading dye into wells (we call this loading a gel). You will also pipet standard into one of the wells.
- You will run a current through it and then as a class, we will look at all of the gels under UV light. We will take pictures of the gels and you will further analyze them later.

Why you are doing it:

- Once we have the pictures of the gel, you will be able to analyze it to find out:
  - Is your prep contaminated?
  - What is the approximate size of your duckweed insert?
  - Do you have an insert?
  - Did you mess up the labs?
- In general, we use gels to predict the (1) size and (2) quality of our DNA sample

PCRG is the moment of truth (Part I)

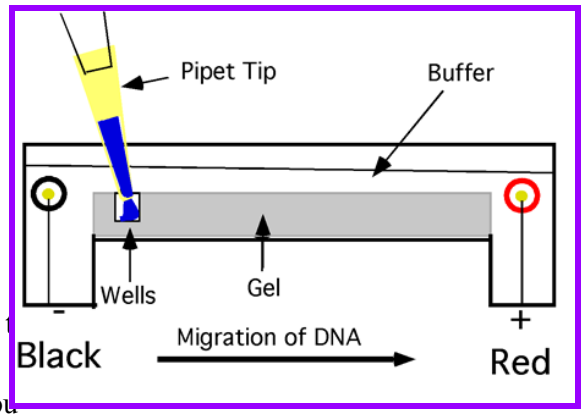
- If you messed up the ON lab, it will be obvious.
- If you messed up the PCR lab, it will be obvious.
- This is your first chance to find out some information about the duckweed DNA that was originally locked inside bacteria. Exciting, right?
- Part II = Restriction Digest Gels

Q&A:

Q: Any tips for loading a gel?

A: Make sure to:

- Not confuse standard with loading dye
- Pipet out your sample when you are near the top of the well so you do not puncture the gel
- Put a finger on your pipet or put your elbows on the table to help stabilize yourself
- Go down to the first stop when you are pipetting liquid into the well. This is the only time you go down to the first stop when you are removing something from your pipet (you usually go down t the second stop). Then, make sure you let go of the pipet when the tip is outside the gel so that you do not suck up any PCR samples in the well



Video (use DSAP login to access)

How to load a gel (skip to 2:24): https://wssp.rutgers.edu/WSSP-files/Videos/Gel_Loading.mov

*ignore comments about ethanol because that does not apply to this lab