Gel Protocol

Demos: parafilm, loading gel

1. Making Gel Mixture
2. Weigh 0.5 g agarose with weigh boat
3. Put into clean 125 ml flask
4. Add 50 ml of 1X TAE (fill to 50ml line in the flask) \*\*\*Toxic-don’t get on skin and clothes
5. Microwave (we will figure out time with 1st group, microwave until agarose is completely dissolved)
6. DO NOT BOIL OVER
7. Add the proper amount of invitrogen SYBR safe DNA gel stain (WE WILL TALK ABOUT THIS)

\*\*\*Don’t get this on your skin - contains DMSO, a solvent.

1. Swirl to mix
2. Pour gel into assembled casting tray (make sure comb is present)
3. Let cool until totally opaque and solid

II. Prep samples

1. Find your samples (i.e. 1-20, 21-40, …) → Follow Demo
2. Get a 6-inch strip of parafilm
3. For each sample, Add 2 ul loading dye to a spot on the parafilm
4. Pipette out 10 ul of sample onto parafilm with loading dye
5. Pipette up and down to mix

-make sure NO BUBBLES are present while mixing

1. Transferring gel

-Remove comb

-Slide gel from casting tray into gel box

III. Running the gel

1. Fill the gel box - completely covering gel - with 1X TAE (do NOT overfill)
2. Load samples with a new pipette tip for each sample (WITH EXTREME CARE)

-Tip of the pipette should be in the well but don’t stab the gel - this is easier said than done.

1. Load 5 ul of DNA ladder to first well on the gel
2. Add all 12 ul of DNA/dye mixture to well, 1 well per sample! Note in your lab notebook what samples are where - don’t lose track
3. Attach lid
4. Run at 150 volts until the dye is about 50% down the gel or when instructor says

-Ask instructor when to terminate/turn off gel - we will need to eyeball it for you :)

IV. Analyzing the gel

1. Take out gel
2. Visualize on the light box
3. Turn on lightbox - USE the Shield!!!!!! \*\*\*Directly looking at UV rays can damage eyes
4. Take a picture - add to lab notes
5. Record in lab notebook

* Was there a nice band? Talk about quality of the band
* Presence/Absence (David will talk about what faint bands mean)
* Indicate which samples will proceed to cleanup for next week’s class