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10/3

Week 7 Lab: PCR

Primer Dilutions

The primers are arrived at 10X concentration. We must make dilutions of them. If we make 100µL of primer, how much primer do we add and how much water do we add?

Polymerase Chain Reaction (PCR)

1. Thaw DNA extractions sample on ice.
2. Thaw EconoTaq PLUS 2X Master Mix in cold box.
3. Obtain 1.5mL microcentrifuge tube and prepare reaction.
4. Prepare and label two capped PCR tubes, one for reaction and one for negative control.
5. Prepare a 25µL reaction:
    a. Add 3µL 10µM forward primer
    b. Add 3µL 10µM reverse primer
    c. Add 2.5µL nuclease free water
    d. Add 4µL of DNA template **(not to negative control)**
    e. Add 12.5µL EconoTaq PLUS 2X.
6. Seal tubes with PCR tube sealer.
7. Gently mix the PCR components in a thin-walled reaction tube and spin briefly in a microcentrifuge in a minifuge to get the materials off side of the tubes and top of the cap.
8. Place in thermocycler and run program.
9. Remove from thermocycler and keep at 4°C until gel electrophoresis.

Questions

1. How do we determine what annealing temperature to use for the primers? How does the annealing temperature differ from the melting temperature? ↳ It is usually a few degrees lower than the melting temperature.

We consider that if too low, the primers may form hairpins or bind to sequences outside of the target, and if too high, that it may not sufficiently bind.

2. You can run a PCR with all of your extractions with the same set of primers. You can make a master mix of all of the ingredients (Econotaq, F primer, R primer, water) and then add this mixture to each tube. This makes it easy to simply add the DNA in an organized fashion. Use the table below to calculate your volumes to make the master mix of your reaction. Make sure to buffer for an extra sample or two in case you mess up. Don't forget about your negative control! Do not add your DNA template to this master mix!!

# of samples: 4

Ingredient	PCR tube volume	MM volume
dH_2O	2.5µL	12.5 µL
Forward primer	3µL	15 µL
Reverse primer	3µL	15 µL
EconoTaq PLUS 2X	12.5µL	62.5 µL
	Total Volume:	105 µL

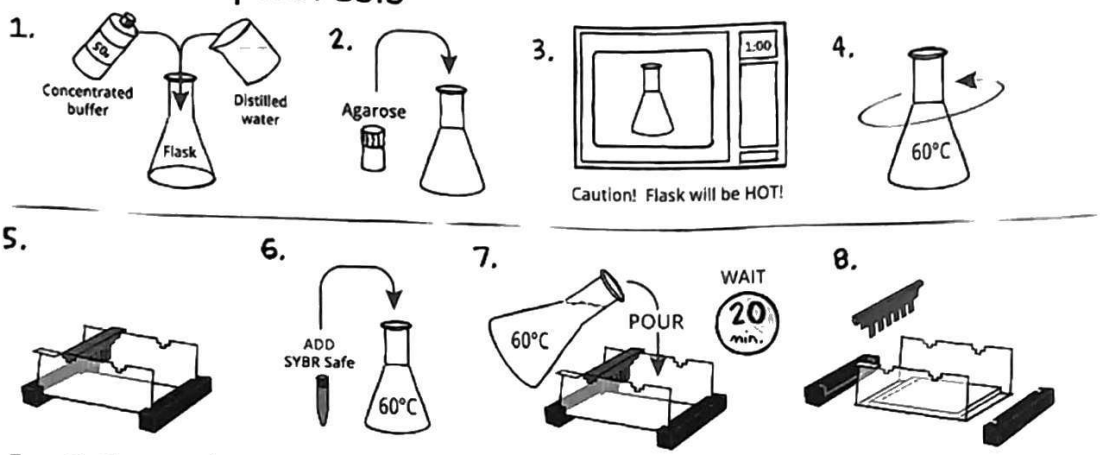
* Did x5 for MM.

3. What is the volume of your mastermix that you add to each tube?

2½ µL

25 µL − 4 µL =
total volume — DNA

Gel Electrophoresis



For 7x7 cm gel tray, Adapted from AddGene Protocol.

1. Prepare a standard 1% agarose gel.
    a. Measure 0.24g agarose on a weigh boat.
    b. Measure 30 mL 1X TBE buffer in microwavable flask
    c. Pour agarose powder into flask.
2. Microwave for 1 minute, stopping at 30 seconds to gently swirl flask and diffuse heat. Use heat resistant gloves. Glassware will be very hot. Do not overboil.
3. Add 30µL SYBR safe to gel mixture. Mix with pipette tip.
4. Pour the agarose into a gel tray. Pour slowly to avoid bubbles which will disrupt the gel. Any bubbles can be pushed away from the well comb or towards the sides/edges of the gel with a pipette tip.
5. Add the well combs...don't forget!
6. Let sit at room temperature for 20-30 mins, until it has completely solidified.
7. Once solidified, place the agarose gel into the gel box (electrophoresis unit).
8. Fill gel box with 1X TBE until the gel is covered.
    a. Remember, if you added SYBR safe to your gel, add some to the buffer as well. SYBR safe is positively charged and will run the opposite direction from the DNA. If you run the gel without SYBR safe in the buffer you will reach a point where the DNA will be in the bottom portion of the gel, but all the SYBR safe will be in the top portion and your bands will be differentially intense. If this happens, you can just soak the gel in SYBR safe solution and rinse with water to even out the staining after the

gel has been run, just as you would if you had not added SYBR safe to the gel in the first place.

9. Carefully load a molecular weight ladder into the first lane of the gel.
    a. *When loading the sample in the well, maintain positive pressure on the sample to prevent bubbles or buffer from entering the tip. Place the very top of the tip of the pipette into the buffer just above the well. Very slowly and steadily, push the sample out and watch as the sample fills the well. After all the sample is unloaded, push the pipettor to the second stop and carefully raise the pipette straight out of the buffer.*
10. Carefully load 3µL of sample to remaining wells, with negative control as last well. Make note of the well lanes in your lab notebook.
11. Run the gel at 80-150 V until the dye line is approximately 75-80% of the way down the gel. A typical run time is about 1-1.5 hours, depending on the gel concentration and voltage.
    a. *Black is negative, red is positive. The DNA is negatively charged and will run towards the positive electrode.* ***Always Run to Red.***
12. Using the gel doc that has UV light, visualize your DNA fragments. The fragments of DNA are usually referred to as 'bands' due to their appearance on the gel.

Q4. What does alcohol dehydrogenase do in plants?
Q5. How long is gene (bp) exons? etc.
Q6. What is the size of the human genome?

Q4A) It plays critical roles in anaerobic metabolism, plant growth, development, adaptation, fruit ripening, and aroma production. It is important in redox reactions and catalyzes the last step of the ethanol fermentation pathway.

Q5A) It is 2-3 kb in length w/ a ~1000 nucleotide coding sequence. It has 10 exons and 9 introns.

Q6A) It is approximately 3,200,000,000 base pairs long.
3.2 Gb