Lab Recap: PCR and Solution I

Amplify and Store

Purpose

- *PCR* - Amplify your plasmid DNA so that you can run through a gel and approximate the size of the insert in the next lab.
- *Solution I* - Suspend bacterial cells in a buffer solution for storage.

Vocabulary/Terms:

PCR - Polymerase Chain Reaction; amplifies DNA sequence using Taq Polymerase (a special type of DNA polymerase that can function in extreme temperatures); we perform PCR to increase/amplify the DNA we have, so that we can put it in a gel and determine the approximate size of our inserts

Centrifuge - Spins our bacterial cultures at super high speeds to separate substances of different densities. In this lab, it will be used to separate bacterial cells (containing plasmids and duckweed DNA) from the Luria Broth that your cells have been growing in.

Resuspend - In the lab Solution I, after centrifuging the culture (they have been shaking for two days on the shaker), we remove the liquid (Luria Broth+Amp). There is a very important little pellet left behind in our tubes. We add Solution I to this tube and pipette up and down to resuspend, or to get all of our important stuff (the bacterial cell) back into a solution called Solution I.

Solution I - Solution that acts as a buffer for cell suspension (regulates the pH of the cell suspension); limits DNA degradation to avoid partial digestion. We use it as a storage solution.

Supernatant - The liquid above the pellet in your microfuge tubes after centrifugation. We sometimes keep the supernatant instead of the pellet. However, in the Solution I lab, it's waste.

Primer - A strand of nucleotides that provides a starting point for DNA synthesis

Primer Mix - A combination of primers and sterile water used for convenience. We use mixes because we are smart lazy and we do not have time for everyone to pipet small amounts separately. You will be making a 5X primer mix.

Important Context: Understand the PCR Reaction (cycle of heating and cooling to amplify DNA)

1. Isolate DNA

2. Denature DNA: raising temperature to break hydrogen bonds between the nucleotides of DNA, causing the DNA to unwind

3. Anneal (glue) primers to DNA: The primers will anneal to the sequence of the original DNA strand., providing a starting point for DNA synthesis

4. DNA synthesis (extension by adding complementary nucleotides using Taq DNA polymerase): Taq DNA polymerase catalyzes the reaction by adding nucleotides to the primer sequence, resulting in a complementary strand of DNA.

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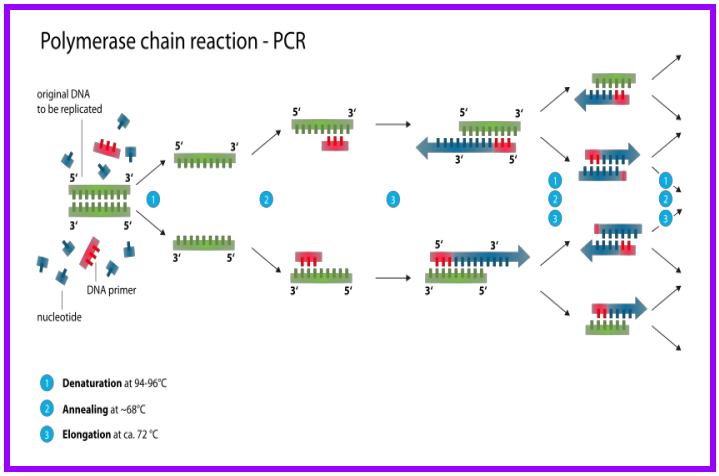

Video explaining a PCR reaction: https://www.youtube.com/watch?v=_YgXcJ4n-kQ

Understand this:

In the lab, you do not have to worry about any of the steps above. All of the stuff that's happening above will be happening in the PCR machine. *You are only responsible for adding all of the ingredients to the PCR tube.* The key is that you are not running a PCR reaction, but *setting up* a PCR reaction. Do your part correctly (putting things in a tube), everything that is supposed to happen with run smoothly.

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

PCR Lab:

- What you will be doing
  - Diluting your overnight culture samples
  - Making a 5X primer mix
  - Adding the primer mix and dilute cultures to a PCR tube containing a bead (the bead has polymerase, nucleotides, and buffer)
  - The PCR tube will have template DNA, primers, a type of DNA polymerase, nucleotides, and buffer at the end. Then you will give the tubes to us so we can start the PCR reaction.
- Why you are doing it
  - You will eventually be running your PCR samples through gels to approximate the size of your duckweed insert. In order to see the bands on the gel, you need to have a lot of DNA. We use PCR to multiply the DNA that you have so that there is enough to run gels.

Solution I Lab:

- What you will be doing
  - Centrifuging your bacterial cultures to isolate the bacterial cells
  - Suspending the bacterial cells in Solution I for storage
- Why you will be doing it
  - In order to safely store your bacterial cells for later. After this lab, you will take the cells suspended in solution I and extract plasmid DNA from them.

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Q&A:

Q: Why are primers important?

A:

- DNA Replication can only happen after primers
- They allow us to select a *specific* region to amplify.
- In this case, we are using primers to amplify only our duckweed insert (plus a few extra nucleotides). Remember you add primers when you add the 5X primer mix to your PCR tube.

Q: What's in the PCR bead?

A:

- Taq Polymerase
  - Special type of DNA polymerase that can survive at extreme temperatures (this is important because PCR requires rapid changes in temperature and normal DNA polymerase enzymes cannot survive those changes)
- Nucleotides
  - So that taq polymerase has bases to add to strands
- Buffer
  - Regulates pH so that the reaction can happen

Q: What does ddH_2O stand for?

A: It's just an abbreviation for distilled water.

Q: What does "ul" mean?

A: ul is an abbreviation for microliter. ***Make sure your pipette can handle the amount of microliters you are trying to suck up.*** In other words, if you need to collect 200 ul, don't use a pipette that only goes up to 20 ul (Ms. Sanelli will get angry if you break a pipette!).

Q: What is the difference between the 5X mix and the 6X mix?

A: The difference is the amount of ingredients. This depends on the number of people in your lab group. For example, if you have 4 people, you should probably make a 5X mix (*always make extra* just in case you mess up).

Q: Why do we use dilute cultures?

A:

- Too much original DNA overwhelms the PCR reaction (primers + nucleotides get used up)
- If primers get used up, there will not be as many copies of your insert and you will not have enough DNA to see in the gel

Q: Why do I need to keep tapping and resuspending the bacteria?

A: We tap to mix everything up. When we add new things to the tubes, you should mix because you want to have a uniform mixture. Some of the things we use are more dense than other ingredients, so they sink if you don't mix it well.