Lab Recap: Overnight Cultures (ON)

Nickname: Picking Colonies

Purpose

To pick a bacterial colony containing a cDNA insert.

Vocabulary/Terms:

Lab Prep - A checklist or list of steps that you should write in your lab notebook before the lab. Lab preps are extremely important because not only do they guide you through your lab, but they also allow other members to finish your lab if your group has to leave when the bell rings.

Overnight Culture (ON) - The final contents in your tube in this lab will be called your Overnight Culture: "overnight" because your bacteria will be grown overnight and "culture" because that we are dealing with bacteria.

Inoculate Media - Placing your bacterial colony into Luria Broth to grow

Luria Broth - A substance you will put in your overnight culture tube. It is the food the bacteria use to grow.

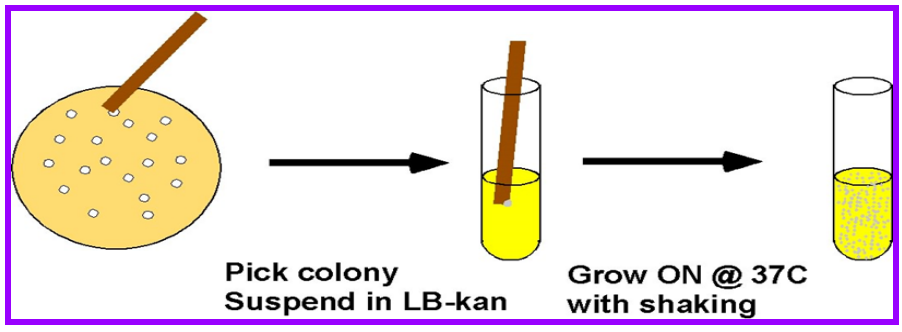
Ampicillin - Antibiotic that kills everything but the cells containing the pTriplEx plasmid vector.

Insert - The duckweed cDNA fragment that has been inserted into the pTripleEx plasmid vector. The vector containing the duckweed insert is inside bacteria (you will see bacteria on the plate)

Plate - A petri dish containing agar (gel-like substance) with the bacteria growing on it

Contamination - Picking two bacterial colonies and having two inserts. This mistake will be very obvious in the PCR gels and your waveform if they end up sequencing your prep. Try your best NOT to make this mistake because you will not be able to get published if you do.

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



You will be picking a white (or light blue), circular, and isolated bacterial colony on the plate. Using a wooden splint, you will transfer one colony from the plate to a culture tube with Luria Broth and Ampicillin. Then, you will give us the tubes to incubate your bacteria so they can grow.

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Q: Why should we pick white or light blue, circular, and isolated colonies?

A:

- White colonies have a duckweed cDNA insert. The insert disrupts the *LacZ gene*, which turns the colony white. In cells that do not have the insert, the LacZ gene is not disrupted and the colony is blue. You want to study duckweed DNA, so you need to pick the colonies with duckweed DNA
- Light blue colonies may have an insert. Sometimes, the insert may enter the vector in a way that only partially disrupts the *LacZ gene*, resulting in light blue color. Also, all white colonies will eventually turn blue after a couple weeks. If you are a new member, we suggest that you pick white colonies to be safe.
- Circular and isolated colonies reduce chance of contamination.
    - Circular - sometimes two colonies may fuse together and form an oval-ish looking colony. If you pick the oval-ish colony, you will have two colonies → contamination

Q: Why do we put the bacteria in Luria broth AND Ampicillin?

A:

- Together, they both allow the bacteria that we DO want (bacteria with duckweed DNA) to grow while at the same time killing the bacteria we DO NOT want.
- If we don't put Luria Broth in, the bacteria will not have food to grow
- If we don't put ampicillin into the culture tube, other bacteria that get into the tubes from the air, you sneezing, etc. will grow inside the culture tube. That means we will have an abundance of a bunch of different bacteria (AKA contamination → not get published → cry).
    - Remember, our vector has the ampicillin resistance gene (this is what we call the *"selectable marker"*). The ampicillin resistance gene codes for proteins that degrade the ampicillin. When we put the ampicillin in, it kills all bacteria that do not have the ampicillin resistance gene (anything other than the bacteria from the plate).

Q: What's the point of heating the cultures overnight and growing bacteria?

A:

- The culture tubes are heated to 37ºC (approximately our body temperature) which is the environment where the enzymes would work well for the bacteria.
- We have a *shaker,* which can heat the cells up to grow and shake them around to make sure nutrients are being distributed evenly in the tube
- Overall, the point of growing bacteria is that we need A LOT of plasmid DNA for what we are doing in the next couple of labs. By growing the bacteria now, we are increasing the amount of plasmid vectors, and the amount of duckweed DNA inside of them (remember duckweed DNA is inside the plasmid vector)

Video (use DSAP login to access)
https://wssp.rutgers.edu/WSSP-files/Videos/ONs.mov