BACTERIAL CULTURING

Stocker Lab Protocol

Effective Date: 23 December 2013

1. Scope

This protocol describes the steps and materials required to start bacterial cultures from freezer stocks. Freezer stocks are the original archived culture stored in 50% glycerol at -80C, and should be handled aseptically to avoid contamination of these precious archives. A small amount of archived sample is streaked onto a fresh agar plate to allow individual colonies to grow from a single starting cell. These individual colonies can then be transferred to liquid media to grow liquid cultures from a single starting clonal population of the cell line.

2. Definitions/Acronyms

BSC	Biosafety Cabinet
EtOH	Ethanol
-80	Ultra low temperature freezer- located in the basement

3. Personal Protective Equipment

Lab coat, gloves and glasses shall be worn at all times. If using a BL2 organism, please notify the lab manager (Becky) and pay particular attention to disinfecting all surfaces and equipment you use before and after culturing (i.e. wipe everything with 10% bleach or 70% EtOH, including pipets, tip boxes, counters, etc.).

4. Materials and Equipment

4.1. Streaking Bacterial Plates from Freezer/Glycerol Stocks

- Biosafety Cabinet
- Agar Plates
- Frozen Tube Rack (Cryo Box)
- Frozen (-80C) Glycerol Stock of Culture
- Sterile Inoculation loop (VWR Cat# 82051-144)
- Parafilm
- Sharpie

4.2. Starting Liquid Culture from a Single Colony (taken from agar plates)

- Liquid Media
- Plate of Colonies
- 14ml Culture Tubes (VWR Cat# 60819-524) (or any other sterile growing vessel)
- Seriological pipette
- Automatic pipette
- Sterile Inoculation loop/ Autoclaved Pipette tip
- Shaker/Incubator

4.3. Preparing Freezer Stock's

5. Preparation

5.1. Starting bacterial plates from glycerol stocks

5.1.1. Turn on the Biosafety cabinet fan and light at least 15 minutes prior to use to establish laminar flow.
5.1.2. Open the BSC and use a paper towel to wipe down the surface with 70% EtOH.
5.1.3. Label the bottom (agar side) of the plates with the culture you are inoculating, your initials, the date, and the type of media. Plates are stored lid side down so that any condensation that collects on the lid will not drip onto your sample (i.e. the agar).

NOTE: For almost all things in the lab, you want to avoid labeling lids since they can become separated from the sample itself (say if you were carry a few plates and dropped them). It is always best practice to label the body of the tube/plate where the sample is actually in contact with the vessel rather than lids.

5.2. Starting Liquid Culture from Plates

5.2.1. Turn on the Biosafety cabinet fan and light at least 15 minutes prior to use to establish laminar flow.
5.2.2. Open the BSC and use a paper towel to wipe down the surface with 70% EtOH.
5.2.3. Label the sides of your tubes with the culture you are inoculating, your initials, the date, and the type of media.

6. Procedure

6.1. Starting bacterial plates from freezer stocks

6.1.1. Ensure the BSC has been on for at least 15 minutes to establish laminar flow and the plates are labeled.
6.1.2. Remove the frozen culture from the -80 and place it in a blue cryo box, which will help keep it cold. Place the cryo box in a secondary container and transport it to the lab.
6.1.3. Place the cryo box in the BSC with your labeled plates.
6.1.4. Wipe off the cryo tube to make sure there is no ice around the lid that could fall in and contaminate the culture.
6.1.5. Carefully remove the lid and scrape the surface of the culture with the inoculation loop.
6.1.6. Place the lid back on the cryo tube and put it back in the cryo box.
6.1.7. Remove the lid on the petri dish and streak your frozen sample across the top.
6.1.8. Using a clean side of the loop (or use a new loop), turn the petri dish 90^{o}
6.1.9. Pull through your original streak and make 3 streaks in a serpentine pattern.

6.1.10. Using a clean side of your loop, turn the petri dish 90^{o}. Streak another serpentine of 3-4 lines Start by streaking through the last line of your previous serpentine.
6.1.11. Repeat 6.1.10 one more time to finish the 4th side of the plate.
6.1.12. Replace the lid on the petri dish and wrap with parafilm. Store, lid side down at the appropriate growth temperature.

NOTE: These plates will remain reliably viable for ~1-2 weeks. After this time frame, the cells are likely to have incorporated mutations and should not be used for experiments. Streak a new plate from the same glycerol stock as often as needed.

6.2. Starting a Liquid Culture from a single clonal colony

6.2.1. Ensure the BSC has been on for at least 15 min to establish laminar flow.
6.2.2. Put your labeled tubes in a rack and place in the BSC along with your media.
6.2.3. Using a Steripipette, pipette 4 or 5 ml of media into your tubes using good asceptic techinque.
6.2.3.1. Try to minimize opening your sterile media bottle. If you are filling multiple tubes then use a pipette that can hold the total volume you will need (i.e. You want to fill 4 tubes with 5 ml of media. Use a 25ml steripipette, suck up 20ml of media, then distribute 5ml increments to each tube.) That way you only go into your sterile media bottle 1 time.

NOTE: Your growth vessel should only be 1/3 full (never more than ½ full) so the volume of media you add depends on the total volume of your vessel.

6.2.4. Find a single colony on your plate. Using an inoculation loop or pipette tip, lightly scrape the colony. Then place it in your culture tube and swirl (or pipet up and down) to mix.
6.2.5. Put the cap on the tube to the 1st stop, to allow for aeration and place the tube in one of the racks in the incubator.

NOTE: The 14ml tubes have 2-stop caps—the 1st stop protects the culture from contamination, but allows gas exchange to continue; the 2nd stop if fully sealed and does not permit gas exchange.

6.2.6. Wrap your plate with parafilm for storage.

NOTE: The culture should be prepared ~6-24 hrs. before use, depending on the species and what attributes you are studying. During this time most bacterial strains are in the Exponential Growth Phase, which will eventually plateau and they will enter the Stationary Phase. During Stationary Phase different mutations and behaviors may occur. It is recommended that you perform a growth curve before beginning work with a new strain to be sure you know its growth behavior and thus the appropriate timing for use in your experiments.