Protocol for Freezing Bacterial Isolates:

This is a general protocol typically used with robust organisms (Listeria and Pseudomonas). If you are working with a new or unknown organism, you should confirm the viability of your frozen stock before throwing out your plates.

**Freezing bacterial isolates down requires good sterile technique to ensure you are freezing only the intended organism; if you have never been taught aseptic technique, please stop what you are doing and see a technician.

Begin with a fresh plate of a pure organism (i.e. with isolated colonies visible). Using a sterile stick, choose a single colony and inoculate 5ml of sterile broth. Grow bacteria "overnight" at its appropriate temperature. "Overnight" times are dependent on the organism. For most robust organisms 15-18 hours is adequate. Do not overgrow. The goal is to freeze down bacteria during late log or early stationary phase. Always inoculate a negative control when growing overnights.

- Each isolate must be frozen in duplicate. Label each sterile cryovial with the isolate's FSL number, the organism, your initials and the date. Each cryovial must also have the isolate # on the cap (use inserts or stickers). Organize all tubes within a cryovial rack before beginning.
- Loosen cap & microwave a small bottle of sterile glycerol for 10 seconds to make it easier to pipet.
- Working aseptically, aliquot 150ul of warm, sterile glycerol into each cryovial at an angle and replace cap. Do not remove cryovial caps and place on bench!
- After allowing glycerol to cool down for a few minutes, vortex your overnight culture briefly and aseptically add 850ul of overnight culture to its appropriate cryovial.
- Invert each cryovial until glycerol and overnight culture appear mixed.
- Immediately place cryovials into your –80 freezer box and log isolate locations onto sheets for the tower and box.

  Note: It is recommended that you freeze down no more than 20 isolates (in duplicate) at one time. If you do freeze down a large batch of isolates, place tubes on ice after mixing and invert again several times before placing in freezer. Do not let isolates sit in glycerol at room temperature.