FSL Isolate Designation, ID Assignment & Glycerol Stock Preparation

FSL Isolate Designations:
FSL isolate designations are assigned to staff & students freezing strains for permanent addition to the FSL isolate collection, which is maintained at –80°C. The FSL isolate numbers are assigned by either the PI or person in charge of the –80°C freezer organization. Designations consist of the first letter of an individual's last name and a pre-determined number.

Example: Joe Schmoe's FSL isolate designation will be S7. The first isolate that Joe assigns a number to will be assigned FSL S7-0001. Joe's isolates will all have S7 designations regardless of the project they are associated with until Joe reaches FSL S7-9999. At that time, he will receive a new designation due to database constraints.

There is a list of current FSL isolate designations posted on the -80C freezers that is periodically updated.

FSL ID Number Assignment:
In general, whenever a new isolate or strain enters the lab or is created within the lab, it should be given an FSL ID number. For clarification purposes, the following is a list of specific examples of isolates or strains that need to be given FSL numbers:

- An isolate is received from an external source.
- A new isolate is recovered from a sample or enrichment.
- *E. col*i containing a plasmid constructed with an insert or deletion or reporter gene.
- *Listeria* containing a plasmid constructed with an insert or deletion or reporter gene.
- *Listeria* mutants.

Do not re-assign internal FSL isolates with a new FSL number once you begin working with it (i.e. 10403S is FSL X1-001…*forever*).

FSL Protocol for Freezing Bacterial Isolates:

This is a general protocol typically used with robust organisms (Listeria, Salmonella, E. coli 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.

All FSL isolates become part of a permanent collection that is maintained at -80°C. Every isolate or strain that is assigned an FSL ID # must be frozen and entered into the Food Microbe Tracker database, complete with storage information.

- RNA collection boxes do not need to be logged because they are only temporary storage

Begin with a fresh plate of a pure organism (i.e. with isolated visible colonies). 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.

- Disinfect your bench, pipetman, and rack with 70% ETOH before you begin.
- 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.