

## 2011 Distribution

Source Plate \_\_\_\_\_

Date: \_\_\_\_\_

Name: \_\_\_\_\_

Name: \_\_\_\_\_

### Inoculating from Thawed Glycerol

#### Supplies Needed Today

- ☐ Thawed Glycerol Source Plate (takes 2hrs to thaw)
- ☐ (4) 96-deepwell square plates (MiniPrep)
- ☐ (2) 96-deepwell round plates (Glycerol)
- ☐ (4) 96-deepwell square plates (AB Test)
- ☐ LB Broth (Amp, Cm, Kan, Tet)
- ☐ Glycerol (80%)
- ☐ 1 box of 1.2ul MC pipette tips
- ☐ 1 box of 200ul MC pipette tips
- ☐ Labels for all plates
- ☐ VWR Reservoirs (8)
- ☐ Micropore tape
- ☐ Adhesive foil covers

#### Prepare Destination Plates

- ☐ Remove Glycerol Source Plate from -80C Freezer, let thaw for 1.5-2hrs
- ☐ Label Glycerol Plate with date of thaw

#### Clear off benchtop working area

- ☐ Clear and wipe down benchtop with 70% ethanol

#### Prepare Destination Plates

- ☐ Check Antibiotic layout before preparing destination plates
- ☐ Prepare MiniPrep plates (4) with 1.8ml of LB (w/ appropriate AB)
- ☐ Prepare Glycerol plates (2) with 37ul of 80% glycerol and 300ul of LB (w/ appropriate AB)
- ☐ Prepare AB Test Plates (4) with 500ul of Amp, Cm, Kan, and Tet, respectively

#### Cleaning the Pin Tool

1. Prepare 4 washes, 10% bleach solution, 1st dH<sub>2</sub>O, 2nd dH<sub>2</sub>O, 70% ethanol
2. Raise and lower pins through the meniscus in 10% Bleach (Wash 1) solution three times, dab on Kim Wipes to remove remainder
3. Raise and lower pins through the meniscus in 1st dH<sub>2</sub>O three times, dab on Kim Wipes to remove remainder
4. Raise and lower pins through the meniscus in 2nd dH<sub>2</sub>O three times, dab on Kim Wipes to remove remainder
5. Raise and lower pins through the meniscus in 70% Ethanol three times, let dry for 3min and shake any remainder to dry

#### Inoculate MiniPrep, Glycerols, and AB Test Plates

- ☐ Vortex thawed glycerol plate before use, spin down briefly if necessary, and carefully remove foil cover
- ☐ Remember to lift pin tool up and down 3x through the meniscus for proper loading
- ☐ Clean pin tool and let dry thoroughly prior to every inoculation for each plate
- ☐ Clean pin tool and let dry thoroughly after last wash with Ethanol (3min)

#### Store Plates

- ☐ Cover thawed Glycerol Source Plate with foil, vortex gently, and return to -80C freezer
- ☐ Cover all destination plates with Micropore film and lids
- ☐ Keep plates in 4C fridge until incubation

#### Incubation

- ☐ Place Miniprep plates according to copy letter on MacConnell shaker (37C) in incubator at 5pm (need 16 hours)
- ☐ Place AB Test plates in vertical holder in 37C shaker incubator at 5pm (need 16 hours)
- ☐ Place Glycerol plates on horizontal rack in 37C shaker (320rpm) incubator at \_\_\_\_\_ (need 12hrs)