

## Media

### *5X M9 Salts*

- 24 g Na<sub>2</sub>HPO<sub>4</sub>
- 12 g KH<sub>2</sub>PO<sub>4</sub>
- 4 g NH<sub>4</sub>Cl
- 2 g NaCl
- DI H<sub>2</sub>O to 800 mL
- 107 µL of sterile 1M CaCl<sub>2</sub> (after autoclaving)

Mix ingredients in a 1L glass jar. Lightly screw cap on (so the jar does not explode in the autoclave) cover the cap with aluminum foil, put a piece of autoclave tape on the cap and autoclave the media. Once the media has completely cooled overnight (other wise it will precipitate out and the media will be ruined), add 107 µL of 1M CaCl<sub>2</sub>.

### *Liquid LB*

- 16 g of LB Broth Lennox (does not contain agar)
- DI H<sub>2</sub>O to 800 mL

Mix ingredients in a 1L glass jar. Lightly screw cap on (so the jar does not explode in the autoclave) cover the cap with aluminum foil, put a piece of autoclave tape on the cap and autoclave the media.

### *Antibiotic Stock Solutions*

- 35 mg/mL Chloramphenicol Stock solution
  - 350 mg chloramphenicol
  - 10mL of ethanol
- 50 mg/mL Kanamycin Stock solution
  - 500mg of Kanamycin
  - 10mL of DI H<sub>2</sub>O
- 20 mg/mL Tetracycline Stock solution (Did not make yet)
  - 200 mg of tetracycline
  - 3mL of DI H<sub>2</sub>O
  - 7mL ethanol
- 100 mg/mL Ampicillin Stock solution
  - 1000 mg of Ampicillin
  - 5 mL DI H<sub>2</sub>O
  - 5 mL of ethanol

After making stock solution, filter sterilize all the stock that are dissolved in water with a 22 µm filter. For each stock, 1 mL aliquots were made and placed in Jeremy's T. reecei box in the -20C fridge: KAN #63-71, CHL #72-81, AMP # 53-70

### *LB for plates*

- 3 x 18 g of LB Agar Miller (contains agar)
- DI H<sub>2</sub>O to 450 mL
- Antibiotics (after autoclaving)

- 450  $\mu$ L of 35 mg/mL stock of chloramphenicol in ethanol (35 $\mu$ g/mL conc)
- 450 mL of 50 mg/mL stock of kanamycin (50  $\mu$ g/mL conc)
- 450  $\mu$ L of 20 mg/mL stock of tetracycline in 70% ethanol (20 $\mu$ g/mL conc)

Have three different replicates, one replicate will contain one of each antibiotic listed above. Mix ingredients in a 1L flask. Place aluminum foil over the top, put a piece of autoclave tape on the cap and autoclave the media. After autoclaving add antibiotic resistance when cooled below 50C (otherwise antibiotic will degrade). Mix in the antibiotic and pore the plates before the agar starts to solidify.