LB BROTH

For 1 liter:

To 950 ml deionized water add:

10 g bacto-tryptone  
5 g bacto-yeast extract  
10 g NaCl

Mix and pH to 7.0 using NaOH solution. Pour media into 1 liter graduated cylinder and adjust the volume to 1 liter with water. Pour solution back into beaker and mix.

Aliquot the mixture into ten 100 ml bottles. Autoclave for 30 min on liquid cycle and store on shelf. Be sure to label the autoclave tape LB with the date.

LB PLATES

Make solution as described above.

Weigh out 15 g AGAR (this is not agarose) and pour into dry 1 liter bottle (orange cap). Pour the LB media (1 liter) into the bottle containing the AGAR. Autoclave for 45 min on liquid cycle.

While autoclaving, turn on water bath to 50°C in back of lab by incubators. Make sure water level is about 3/4 full. Once autoclave cycle is complete, screw down cap but not too tight, and place bottle in the water bath.

Prepare carbenicillin or kanamycin antibiotic (50mg/ml) in water. You will need 2 mls to add to 1 liter of the cooled broth. (see Dr. Rowland if you need to make the antibiotic). Go down to our small lab and turn on the sterile laminar flow hood and clean surface with 70% ethanol. Bring 2 sleeves Petri dishes down to the lab.

Once the broth has reached 50°C (can hold bottle without burning hand, but not too cool), take bottle down to sterile hood. Lay out several Petri dishes. Add antibiotic solution (2 ml) to the media and gently swirl bottle to mix thoroughly. Be sure to open the bottle only in the hood. Don’t swirl the media too vigorously - you don’t want to introduce bubbles at this point. This and all subsequent steps should be done in the laminar flow hood.

Pour media into the plates in the hood and allow to cool. Start with plated furthest from you and work toward you to minimize chances of contaminating the plates. Put enough media in the plate to just cover the bottom. Once all plates poured (they will be stacked in a pyramid) – turn off the hood. Allow to cool and set overnight in the hood. Next day seal plates with parafilm, two plates in each pack, and mark with date and antibiotic. Store at 4°C in lab refrigerator.
**2 X YT Medium**
Use this medium to grow difficult to grow clones. It is rich in glucose and nutrients.

For 1 liter:

To 950 ml deionized water add:

- 16 g bacto-tryptone
- 10 g bacto-yeast extract
- 5 g NaCl

Mix and pH to 7.0 using NaOH solution. Pour media into 1 liter graduated cylinder and adjust the volume to 1 liter with water. Pour solution back into beaker and mix.

Aliquot the mixture into ten 100 ml bottles. Autoclave for 30 min on liquid cycle and store on shelf. Be sure to label the autoclave tape with 2xYT and the date.

For 200 mls:

- 3.2 g bacto-tryptone
- 2 g bacto-yeast extract
- 1 g NaCl

**BEFORE USE: You may add 2 M glucose (to final 20 mM) to media.**
Sterile 2M glucose is in the BSL-2 hood. Open it only in the hood.
Add 10 ul glucose solution to each 1 ml media to obtain 20mM final glucose concentration.