

Making LB Broth, agar plats, and glycerol stocks (Yuan Lab)

Autoclave settings: LIQUID 40 minutes (sterilize 40 minutes, no dry time)

LB Broth

For culturing *E. coli*/agro (3 or 5ml cultures) & for *E. coli*/agro transformation:

Autoclave 150ml of LB broth in a 250ml bottle (follow directions on the LB broth bottle)

Once opened, store in the fridge

For floral spray/infiltration-mediated stable transformation:

Autoclave 300ml of LB broth in a 1L flask (cover with foil)

LB Agar Plate

For making plates:

1. Autoclave 500ml of LB agar in a 1L bottle with a stir bar to make ~20 plates
2. Cool with stirring on a stir plate until cool enough to hold the bottle (to speed the cooling, can put it in a Tupperware filled with water, but DO NOT let it start solidifying!) If starts solidifying, can microwave it until boiling to melt the agar, but only do this if you have NOT added antibiotics yet. However, Kanamycin can be briefly microwaved and still retain activity.
3. Label plates with appropriate colored lines to indicate what antibiotic is in the plates
 - Black = Kanamycin
 - Blue = Gentamycin
 - Red = Rifampicin
4. Add antibiotics when cooled enough according to table:

Antibiotics: keep at 4°C, store stock solutions at -20°C in aliquots

Antibiotic	Powder storage	Stock solution	Final concentration	µL stock/ mL media	Resistance
Kan	RT	50 mg/ml in water	50 µg/ml	1	Your plasmid
Gent	4°C	50 mg/ml in water	50 µg/ml	1	Ti plasmid marker (agro)
Rif	-20°C	12.5 mg/ml in methanol	25 µg/ml	2	Agro chromosomal marker

Glycerol stocks

Autoclave 50% v/v glycerol, aliquot 300ul into cryo tubes and store at 4°C

Add 700ul of dense culture to the cryo tube and invert to mix. Store at -80°C. Add to -80 inventory.