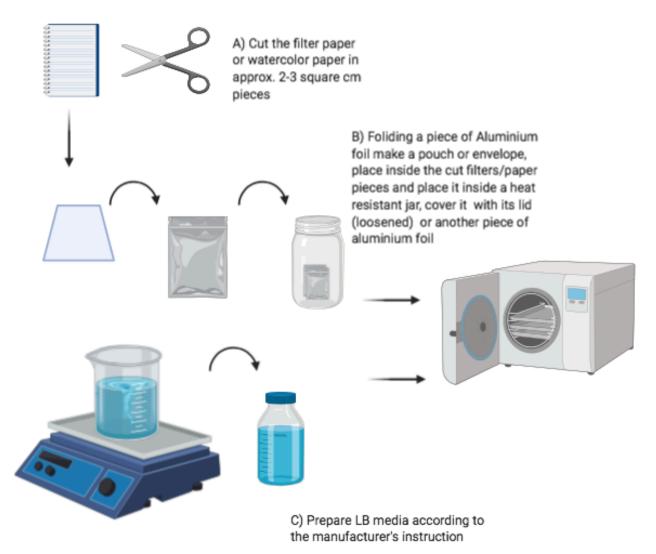
Recovery of cells from glycerol stock

1) Preparation of materials



 D) Autoclave (120°C, 15 psi, 25-35 min) or a pressure cooker for about 25mins (120°C/25min or the "sterilization" program)

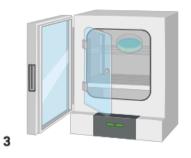
2) Cell culture Two options:

A) LB-Agar Plates

Preparare steriized LB+Agar pour in Petri dishes+ antibiotics (*Ampicilin). Add Amp. when media is cooled to touch. Let plates to

harden and cool down at R.T.

With an sterile loop or micropipette tip, scrap the distro plate of the desired well. (one tip per well) Streak a fresh LB-Agar+Amp plate (one plate per well)

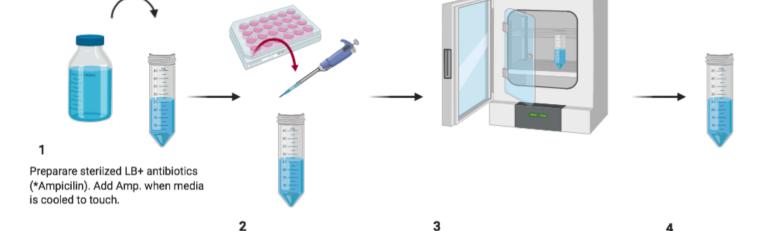


Incubate the plates at 37°C O/N ,or at R.T. for 2 days.



Petridish with isolated colonies.

B) Direct LB culture



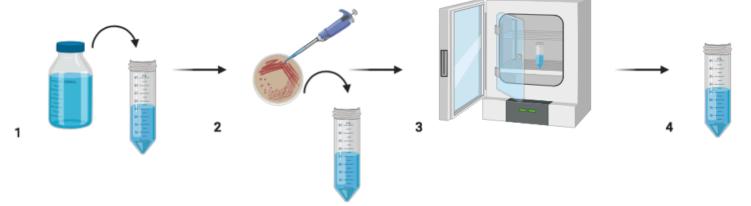
*Ampicillin:

Normal concentrations for plasmid selection are 100ug / mL. 1 gram makes 10 mL(mililiter) enough for 10 Liters of media. A 1000x stock is made by adding 100mg per mL of distilled water. Then add 1uL(microliter) stock per mL of culture media.

With an sterile loop or micropipette tip, scrap the distro plate of the desired well. (one tip per well) Inoculate a fresh LB-Agar+Amp sterile tube. Incubate the culture at 37°C O/N ,or at R.T. for 1 day, if possible on mild agitation.

Liquid culture with grown bacteria.

3) Filter paper preparation

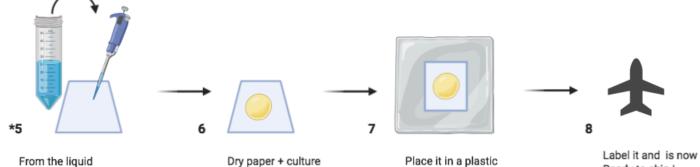


Pour some fresh sterile rLB +Amp in a sterile conical tube. (2 to 5ml)

With an sterile loop or micropipette tip, pick up one colony (one tip per colony) Innoculate into fresh LB-Amp in a sterile conical tube.

Incubate the tube at 37°C for 6-8 hrs or at R.T. for 1 day, if possible with miild agitation

Liquid culture with grown bacteria.



*5 If using a direct culture (Page 2) you can start from here, but is preferable to use a culture from an isolated colony.

culture with grown bacteria. Take about 100 to 200uL of the culture and place it in sterile filter /artist paper. Tips should be sterile as well.

at R.T. in a covered plate or clean plastic container, an unused clean or sterile disposibale Petri dish can be fine.

wrap or envelope, those for electronic components can work. If not, cover in Saran wrap.

Ready to ship!