

Bacteria expansion

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Note: This protocol is a translation of a protocol in Czech written by Jan Vrbsky and Helena Durikova download here

Preparation of cultivation dishes

1. Prepare LB agar (do one day ahead or use the stock already prepared)

- 35 g of LB agar in 1 l of dH₂O
- dissolve the agar properly
- distribute in 0.5 l bottles (1 l bottle will not fit into microwave when needed later)
- leave 1/4 of the volume free to avoid overflow during autoclaving
- sterilize in autoclave using a programme for liquids

2. Pour LB agar in dishes

- loosen the cap of the sterile LB agar bottle
- put sterile LB agar into microwave, 3-5 min. at max. intensity, watch for dissolution / overflow
- if it is not done, use thawing programme to dissolve it
- alternatively use water bath at 65 C

3. Antibiotic stocks (work in sterile hood)

Ampicilin from powder

- dissolve 1 g in 10 ml of sterile H₂O and aliquot by 1mL
- store frozen at -20 C
- when used, label with a dot and refreeze

Ampicilin from solution

- the solution comes 100 mg/ml
- aliquot in sterile conditions
- store at - 20 C

Kanamycine

- dissolve 100 mg in 10 ml in sterile H₂O and aliquot at 1 ml into 1.5 ml Epp.

4. Antibiotics mix (work in sterile hood)

Ampicillin Prep. (stable below 65 C), 1to1000, final conc.: 100 ug/ml

- add 40 ul of 100 mg/ml of Amp. to 40 ml of hot (below 65 C) LB agar
- mix by pipetting
- put 10 ml of mix into plates

Kanamycine Prep. (stable below 55 C), 1to200, final conc.: 50 ug/ml

- add 200 ul of 10 mg/ml of Kan. to 40 ml of hot LB agar
- mix by pipetting
- put 10 ml of mix into plates

5. Pour the LB agar into plates (work in sterile hood)

- pipette/pour 10 ml of LB agar into 100 mm dishes
- shake for agar to spread evenly
- let solidify for 20 min. (open plates)
- store the plates at 4 C for a week or so (check with literature)

Inoculation of LB agar plates

Work in Flow Hood

1A. From a liquid culture/frozen culture

Take 10 ul of culture and spread into four parts of the plate, take a new tool for each part

1B. From agar stab (as coming from addgene)

- Transfer the visible cultures (the stab in agar) onto the plate and make 3 lines
- Do three more lines across, then again, and finish by making a wave
- Use new green inoculation stick for each phase

2. Cultivation

- Cultivate for 24 h at 37 C without CO₂
- Transfer to 4 C in fridge
- Wrap with parafilm for storage

Inoculation of LB broth

Work without the laminar flow, using just the flame

Volumes

1. MINI kit - 12 ml of culture, in 50-100 ml Erlenmayer Flask
2. MAXI kit - 250 ml of culture 2x 125 ml in 250 ml Erlenmayer Flasks **NOTE:** Use the same concentration of antibody as before

Pick colony

- Choose a good colony, no microsatellites around, big and far away from others
- Pick using green inoculation loop and transfer to LB broth
- For MINI kit, direct transfer is okay
- For MAXI kit, transfer to 5 ml of LB broth in 50 ml falcon, let grow for 2 hours to overnight, then split in half to inoculate the big volumes

Cultivate

- Put in a shaker at 220 rpm 37 C for at least 16 hours

Storage

Work without the laminar flow, using just the flame * Mix 300 ul of 40% glycerol with 300 ul of bacterial culture into freezing vial * Label with * Plasmid Number * Addgene/Company number * Date * Initials * Antibody * Freeze in -80 C, put in the box with bacterial stocks

DNA isolation

MINI kit

- Work in an switched off hood using the burner till the step 4
- Then switch on the flow box without the burner working

MAXI kit

- Work in an switched off hood using the burner till the step 4
- Then switch on the flow box without the burner working
- split 250 ml into 7x50 ml tubes
- Go according to the protocol, optionally do centrifugation at 16k rpm for one minute before filtration to facilitate better filtration and avoid clogging
- At the end resuspend the pellet in 150-300 ul in TE buffer
- Once the plasmid concentration is measured, set to 1 ug/ul and split into aliquots
- Store isolated plasmid at -20 C, keep 10 ul separately for the concentration measurements
- Label with
 - Plasmid Number
 - Date
 - Concentration
 - Initials
 - MINI/MAXI

Material

dH₂O

LB agar (Ready to use stock in A.037, GMO II lab, RT)

Glycerol

Ampicillin (100 mg/ml; A5354-10mL; Lot# 056M486OV; A.050 20F1 7B; A.038 20F5 5A)

Kanamycine

MINI kit

MAXI kit

Inoculation loop

Plastic dish 100 mm (Ready to use stock in A.037, GMO II lab, RT)

Freezing Vial

Falcon Tube 50 ml

Erlenmayer Flask