Bacterial culturing from -80°C freezer stock

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- Prepare LB agar media following the instruction on media bottle.
  - Loosely close the cap on the bottle (do NOT close all the way or the bottle may explode!) Autoclave and allow to cool to 55 C.
- Add the appropriate antibiotic (if you need to grow an antibiotic resistant species) (Table.1)
- Now pour the media in petri plates and keep them stacked inside the biosafety cabinet till they get solidified.
- Using a sterile pipette tip or toothpick, get a small part of frozen culture in -80 (find the information of bacterial stocks on Quartzy)
- Streak the tip or toothpick into the LB agar
- Cover the culture with the lid
- Incubate bacterial culture at 37°C for 18-24 hr in incubator.
- When ready to grow your culture, add liquid LB to a tube or flask and add the appropriate antibiotic (if you need to grow an antibiotic resistant species) to the correct concentration (Table.1)
- Using a sterile pipette tip or toothpick, select a single colony from your LB agar plate.
- Drop the tip or toothpick into the liquid LB + antibiotic and swirl.
- Loosely cover the culture with sterile aluminum foil or a cap that is not air tight.
- Incubate bacterial culture at 37°C for 12-18 hr in a shaking incubator.

  Note: *Some plasmids or strains require growth at 30°C. If so, you will likely need to grow for a longer time to get the correct density of bacteria since they will grow more slowly at lower temperatures.*

- After incubation, check for growth, which is characterized by a cloudy haze in the media

Media without growth (right) and with growth (left)

Note: *Some protocols require bacteria to be in the log phase of growth. Check the instructions for your specific protocol and conduct an OD600 to measure the density of your culture if needed.*

- Log Phase culture

  Transfer 100 µl of overnight liquid culture to 5ml LB broth and incubate for ~ 3-4 hr

Note: *A good negative control is LB media + antibiotic without any bacteria inoculated. You should see no growth in this culture after overnight incubation.*

- *(Optional)* For long term storage at -80 of the bacteria, you can proceed with creating a Glycerol Stock.

Table 1. Antibiotic Concentrations

Commonly Used Antibiotics	Recommended Concentration
Ampicillin	100 µg/mL
Chloramphenicol	20 µg/mL
Gentamycin	15 µg/mL
Kanamycin	30 µg/mL
Spectinomycin	50 µg/mL
Tetracycline	10 µg/mL

Bacterial competent cell preparation

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TSS Buffer (see recipe below)

- 5 g PEG (polyethylene glycol) 8000 (or 3350)
- 1.5 ml 1M $MgCl_2$
- 2.5 ml DMSO
- Add LB to 50 ml
- Filter sterilize via 0.22 µm filter and store at 4°C

- Streak bacteria from a glycerol stock on an LB agar plate and incubate the plate at 37°C overnight.

- Inoculate a 5 mL LB broth culture (no antibiotic) using a single colony from the plate in step 1. Incubate the culture overnight at 37°C.

- Dilute the culture 1:100 by inoculating four 50 mL conical tubes with 50 mL LB broth and 500 µL of overnight bacterial culture.
- Grow the cultures at 37°C until the OD600 is between 0.2 and 0.5.
- Chill the culture, 1.5 mL microcentrifuge tubes and TSS on ice 10 min. Meanwhile, cool the centrifuge to 4°C for the next step.
- Pellet the bacterial cells at >3000 RCF for 10 min at 4°C.

Important: The cells must remain on ice and stay cold from this point forward in order to maintain a high transformation efficiency.

- Remove the supernatant and resuspend in 10% volume chilled TSS buffer (ex. resuspend a 50 mL culture in 5 mL TSS buffer)
- Aliquot 100 µL cells in TSS buffer into the chilled 1.5 mL microcentrifuge tubes.
- Flash freeze the aliquots in liquid N2 and store at -80°C.

The transformation efficiency should be tested for every new batch of chemically competent cells

Transformation Efficiency (TE)= (number of colonies /ug of DNA) * (Vol recovery (ml)/ Vol plated (ml))

Plasmid purification

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- Order required plasmid from Addgene in the form of DH5 alpha bacterial stab culture
- Streak out the bacterial stab in an appropriate LB agar media and incubate overnight
- Prepare an overnight culture of a single colony of bacteria from the above agar plate into a LB broth tub for following plasmid prep steps
- Use 1 ml bacterial cells for plasmid purification
- Follow ZymoPURE™ Plasmid Miniprep Kit Catalog No. D4210 protocol
- Use NanoDrop 1000 to examine the extraction efficacy (Read the concentration)
- Use the extracted DNA/Plasmid for plasmid transformation.

Plasmid transformation

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- Take competent cells out of -80°C and thaw on ice.
- Add 1 - 5 µL of plasmid (usually 10 pg - 100 ng) into ~100 µL of competent cells in a cold 1.5 mL tube. Gently mix by flicking. Best result for low efficacy cells is 4ul = 36 ng DNA
- Place the competent cell and DNA mixture on ice for 20-30 min.
- Heat-shock in a 42°C water bath for 30-60 s. (80 s for low efficacy cells in microcentrifuge tubes). Do Not Shake.
- Place on ice for 2 min.
- Add 900 µL of SOC or LB+ appropriate additives for your target cell same as DAP
- Incubate at 37°C with shaking (225 RPM) for 45 min.
- Plate bacteria on agar plates (with appropriate antibiotic), typically 3 plates with 100 uL, 200 uL, and 300 uL each. Pipette bacteria onto the plate, add ~10 glass beads, put the lid on the plate and slowly roll the beads through the liquid and around the entire surface area of the plate.
- Dump used beads into a glass bottle with 100% ethanol for later re-use.
- Incubate the plates at 37°C overnight.
- After 24hr incubation: Observe bacterial growth on plates (Control V.S. Transformed)
- Pick 5 colonies (candidates) and transfer each to a separate LB broth antibiotic amended media
- Take the overnight culture and centrifuge at 4000 rpm for 5 minutes
- keep 1 ml pellet for plasmid purification (QC of transformation)

Note: Negative and positive controls should be included in the transformation step to evaluate the success of the experimental procedure.

Control	Sample components	Assessment
Negative	• Cells alone/ No DNA	• Background colony formation • Stability of antibiotic(s) in plate
Positive	• Cells with Intact plasmid carrying the desired selectable marker (e.g., antibiotic resistance)	• Cell viability • Transformation efficiency • Protocol success