

To make Glycerol Stocks of Plasmids

** To be done in the hood and use RNase/DNase free tips**

1. In a 10 ml sterile tube add 3 ml autoclaved LB broth and 1.5 ul antibiotic (@ 100 ug/ul) or 3 ul antibiotic (@ 50 ug/ul) for a final concentration of 1:1000
2. Select one clone from the LB broth Plate and put into the 3 ml LB Broth and antibiotic solution.
3. Put in shaker incubator for 6-8 hours at ~ 225 rpm
4. After incubation time bring tube into hood and add 3 ml glycerol (autoclaved 60%), mix by pipetting
5. Aliquot at 500 ul/tube for later use, store at -80°C in box K14
6. When next use, thaw slowly on ice

Glycerol 60% stock solution

30 ml glycerol + 20 ml dH₂O = 50 ml total volume

Make sure mixture is very homogeneous by mixing by pipeting, shaking, vortexing

Autoclave on liquid cycle, store at room temperature

-or- filter with a 0.2 micron bacterial filter, store at room temperature

Transformation Protocol

Materials:

LB Agar Plates

DH5 α TM Competent Cells (Invitrogen Catalog # : 18265-017)

Antibiotic Media Preparation:

20 grams of LB Broth (Shelf 8)

15 grams of Agar (Shelf 8)

To a total volume of 1 Liter with the addition of distilled water.

Note: The top should be loosen to allow for pressure to be released during the autoclave process, LB Broth and Agar are located on Shelf 8 in the HPLC laboratory.

Autoclave:

Autoclave in a 60 minute liquid cycle, and cool media to 50°C.

Note: Do not allow the media to cool to room temperature because the solution will congeal.

Antibiotic:

After the solution has cooled the appropriate antibiotic is added and the LB Agar plates are poured in a sterile environment under the hood.

Next antibiotic preparation of stock solution is as follows:

	Concentration	1 ml	2 ml	5 ml	10 ml
Antibiotic	50mg/ml	50mg	0.1 g	250 mg	0.5 g
H ₂ O		1ml	2 ml	5 ml	10 ml
	Concentration	1 ml	2 ml	5 ml	10 ml
Antibiotic	100mg/ml	100mg	0.2 g	500 mg	1.0 g
H ₂ O		1ml	2 ml	5 ml	10 ml

Note: The final concentration of the antibiotic should be 50µg/ml or 100 µg/ml. Therefore this is 1:1,000 dilution.

$$C1 \quad \times \quad V1 = C2 \quad \times \quad V2$$

$$50 \text{ mg/mL} \quad \times \quad V1 = 50\mu\text{g/mL} \quad \times \quad 100 \text{ mL}$$

$$\text{Stock Concentration} \times V1 = \text{Working Concentration} \times 100 \text{ mL}$$

V1 = 0.1 µl of stock antibiotic in 100ml of water

LB Agar Plate Preparation:

1. Pour LB Agar into 100 mm diameter sterile petri-dishes 20 ml approximately.
2. Allow to set and cool.
3. Label and store inverted at 4°C.

4. The agar plate might need drying for 10 to 15 minutes in a 60°C oven.
5. Label plate on the bottom.
6. Grow plate upside down over night at 37°C.
7. Seal plate with parafilm, and store upside down at 4°C.

Transformation:

1. Thaw on ice one tube of DH5 α TM cells located in A3 in the -80°C Freezer.

Note: Keep cells chilled on ice to ensure high transformation efficiency.

2. Mix cells by flicking the tube gently, then remove 50ul per transformation into a pre-chilled 1.5 ml tube.

3. Add 200 -500 ng of DNA (in a volume no greater than 10ul). Quickly flick the tube several times to ensure the even distribution of DNA.

4. Immediately incubate tubes on ice for at least 30 minutes.

5. Heat shock the cells for 1 minute in a water bath at **exactly** 42° C. Do not shake!

6. Immediately place tubes on ice for 2 minutes.

7. Add 950ul of Room Temperature S.O.C. medium, and incubate for 1 hour at 37° C with shaking at ~225 rpm.

8. Prior to plating transformation solution, place LB agar plates in the incubator for 10 minutes; to remove any condensation that has accumulated on plates

9. Take plates from incubator. Add 50 μ l of transformation mixture to first plate. Spread with sterile spreader. Add 100 μ l to 2nd plate. Spread with sterile spreader. Return to incubator, placing them inverted. Leave overnight. Plating is best done late in the afternoon so the plates do not incubate for more than 12-16 hours.

Note: We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies.

10. Store the remaining transformation reaction at +4°C. Additional cells may be plated out the next day, if desired.

11. Place plates in the 37° C incubator and grow overnight.
12. The next day you should see nice separate colonies and then you will be ready to pick a clone to inoculate a colony for plasmid purification.

Glycerol Stocks

1. Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic.

2. Grow at 37°C for 5–6 h with vigorous shaking (approx. 300 rpm).

Use a tube or flask with a volume of at least 4 times the volume of the culture.

3. Harvest the bacterial cells by centrifugation at 6,000 x g for 15 min at 4°C.

4. Use an autoclaved 60 % glycerol solution and add the centrifuged bacteria

1 volume + 1 volume:
Example: 1 ml (glycerol) + 1ml (bacteria) = 2 ml

5. Aliquot the glycerol/bacterial solution in 100 µL per tube.

Note: All glycerol stocks are kept in the –80 Freezer Box K14.

Restriction Digestion

Materials:

Enzyme I
Enzyme II
Buffer
DNA (1ug)
Ice
Rnase/DNase dH2O

Reaction Mixture

Volume	Substrate
20-x	Water
2 ul	Buffer
1 ul	Enzyme I
1 ul	Enzyme II
1-10 ul*	DNA
20 ul	Total Volume

* 1 ug of DNA, volume is determined directly from the concentration, volume should NOT exceed 10 ul

Procedure

Add water and buffer to tube, followed by Enzyme I the Enzyme II, Lastly adding DNA, mix by flicking with finger.

Quick spin to remove air bubbles (they interfere with DNA/Enzyme contact area)

Incubate @37°C for 2 hours, stop reaction by placing Tube in boiling water for 3 minutes, put sample back on ice until you load (do not load a hot sample into gel)

Analyze Data:

Run reaction mixture in agarose gel (percentage depends on molecular weight of DNA)

To reaction sample add 5 ul of loading dye

Load DNA ladder 10 ul (ladder choice depends on the molecular weight of the DNA)

Take picture using camera in animal lab

Agarose Gel (1%) preperation

0.5 g agarose

40 ml 1x TBE

EB (causes cancer, be careful)

Weigh out desired amount of agarose, put into clean beaker, add 40 ml TBE. Bring to a boil using the microwave, stopping and stirring, occasionally. When all agarose is dissolved, remove from microwave and add 10 ul of Ethidium Bromide (EB). Stir by swirling. Allow gel mixture to cool, but not harden by running under cool water. When cooled, pour into tray, and add desired comb. Be sure that there are no air bubbles in the gel, as they would interfere with the electrophoresis.

Be careful with the gel as it is now contaminated with EB, wear gloves at all times when handling gel.