Mini-prep from *E. coli* culture

Start 4 ml cultures at approximately 5 pm in LB broth and ampicillin (80 mg/l final concentration) in PG's rotary incubator overnight (37° C and 240 rpm – tubes should be slanted to increase gas exchange)

Recipe for making starter culture LB:

1 g Bacto-tryptone
0.5 g Bacto-yeast extract
1 g NaCl
95 ml H_2O
pH to 7.0 with NaOH **very** slowly
Adjust to 100 ml
AUTOCLAVE
Add 80 µl ampicillin (100 mg/ml stock) and mix following cooling
Transfer 4 ml to each culture tube (avoid contamination)
Using sterile toothpicks, poke colony that you want to grow
Drop whole toothpick into LB
Place in incubator

You should also prepare at least 1 glycerol-frozen bacterial clone for future preps. For each clone to be stored at -70° C for the archive, prepare a sterile labeled cryovial. Pipet 112 µl sterile 80% glycerol into the cryovial. Add 0.5 ml of the overnight bacteria culture (frozen stock will be 15% glycerol). Mix well (vortex) and place tube at -70° C.

1) Spin down 1.5 ml of overnight culture in eppie for 1 minute on high.

2) Asprirate supernatant and resuspend cell pellet by vortexing in 100 μl Solution I.

3) Add 200 μl Solution II and mix gently by inversion. The cells should lyse and turn somewhat clear and viscous. Let stand ~3 min.

4) Add 150 μl Solution III and mix again by inversion. A white clot of DNA/protein/SDS should form. Incubate on ice 10-30 min. Spin for 5 minutes on high.

5) Transfer supernatant to fresh tube containing 500 μl phenol:chloroform, vortex and spin for 5 minutes on high. **This step can be skipped if extra clean-up is not required.**

6) Transfer aqueous layer to fresh tube containing 1 ml ethanol, mix well by inversion/vortexing, and spin for 5 minutes on high.

7) Remove supernatant and wash pellet with 100 μl 75% ethanol, spin for 1 minute.

8) Remove as much of ethanol as possible and dry tubes by leaving on bench with lids open for ~5 minutes or speed vac.

9) Resuspend DNA in 40 μl dH_2O or TE.

10) Use for transformation, restriction digestion, sequencing, etc. Stop here for zooxanthellae clade standards.

These preps contain RNA so if this is a problem a standard RNAse treatment will remove it.

<u>If further purification is needed, you can do the following:</u>

11) Precipitate the plasmid DNA by first adding 10 μl of 4 M NaCl, and then adding 50 μl of autoclaved 13% PEG8000

12) After thorough mixing, incubate the sample on ice for 20 min, and then pellet the plasmid DNA by centrifugation for 15 min at 4°C in a fixed-angle rotor.

Note 1: The temperature parameter here is very important; adhere to the recommended 4°C.

Note 2: If you use a horizontal rotor, do not aspirate the supernatant in Step 13 because the clear pellet adheres to the bottom of the tube and can be lost if you are not careful. Remove the supernatant by decanting. Either approach, decanting or aspirating, can be used to remove the supernatant from a tube spun in a non-horizontal rotor.

13) Carefully remove the supernatant and rinse the pellet with 500 μl of 70% ethanol. Then dry the pellet under vacuum for 3 min, resuspend in 20 μl of dH_20, and store at -20°C.

<u>Recipes for solutions required for mini-prep:</u>

<u>Solution I (100 ml)</u>	**<u>Solution II (10 ml)</u>**	**<u>Solution III (100 ml)</u>**
5 mM sucrose (0.17 g)	0.2 N NaOH (0.2 ml 10 N)	3 M sodium acetate (40.82 g)
10 mM EDTA (2 ml 0.5 M)	1% (w/v) SDS (1 ml 10%)	pH to 4.8 w/ acetic acid
25 mM Tris (0.3 g)	add H_2O to 10 ml	add H_2O to 100 ml
pH to 8.0 accordingly	**<u>prepare fresh</u>**	
add H_2O to 100 ml		
AUTOCLAVE		

NOTE: Sodium acetate in Solution III is NaAc-3 H_2O (trihydrate). If using NaAc anhydrous, the amount/weight must be adjusted accordingly.

References:

Birnboim, H. C. 1983. A rapid alkaline extraction method for the isolation of plasmid DNA. Methods Enzymol. 100, 243 - 255 [Abstract].