

## **DNA Extraction from Lost City Rock**

Modified 2015 by the Brazelton Lab from protocols by Rika Anderson, Colleen Kellogg, Julie Huber, and Byron Crump. Incorporated some recommendations from Lever et al. (2015) *Frontiers in Microbiology* doi: 10.3389/fmicb.2015.00476.

*Do ahead of time:*

Heat water bath or oven to 65°C

Prepare solutions:

3 M sodium acetate, pH 5.2	
DNA Extraction Buffer (DEB):	for 45 mL:
0.1M Tris-HCl (pH 8)	4.5 mL of 1.0 M
0.1M Na-EDTA (pH 8)	9 mL of 0.5M
0.1M KH <sub>2</sub> PO <sub>4</sub> (pH 8)	0.54 g
1.5M NaCl	13.5 ml of 5M
0.8M Guanidine HCl	3.44 g
0.5% Triton-X 100	0.225 mL (225 µL) of 100%

Add above ingredients to 50 mL tube.

Add milli-Q water to ~40 mL

Add NaOH to pH 10 (several drops at a time)

Add milli-Q water to 45 mL

Filter-sterilize to remove possible spores

Autoclave. Slightly loosen lid so that it is not air-tight. Recover from autoclave very soon after the autoclave cycle is completed.

Pour autoclaved solution into fresh 50 mL tube.

Aliquot into 1.5 mL tubes.

### **Sample Prep:**

1. Flame ceramic mortar and pestle
2. Wipe down mortar and pestle with dichloromethane (treat DCM as you would phenol)
3. Crush and homogenize sample
4. Divide sample into two 50mL falcon tubes-- half for chemistry and half for biology
5. Store homogenized sample at -80°C

### **Hot Lysis:**

1. Measure 0.25g of sample
2. In a 2mL tube, add 1.4 mL of DEB to 0.25g of sample
3. Freeze sample (Possible stopping point, store sample in -20°C freezer)
4. Incubate at 65°C for 30 mins in the ThermoMixer at 1500rpm

### **Bead Beating:**

1. Using a pipette, withdraw fluid and any undissolved sediment and eject into bead tube (glass 0.1 mm for bacteria).
2. Bead beat for 40 s.

3. Centrifuge for 2 min at 5000 g.
4. Transfer fluid - avoiding beads - into fresh Eppendorf tube. Add no more than 900  $\mu\text{L}$  in each tube (or no more than 750  $\mu\text{L}$  if using 1.5 mL tubes).

**Phenol / chloroform extraction:**

5. Add equal volume of phenol / chloroform / isoamyl alcohol (25:24:1, bought pre-mixed with alkaline buffer) to each tube.
6. Gently shake a few times and then centrifuge at 14,000g for 1 minute.
7. Remove supernatant to fresh tube.
8. Add equal volume of chloroform / isoamyl alcohol (24:1) to each tube.
9. Gently shake a few times and centrifuge.
10. Remove supernatant to fresh tube, carefully avoiding the bottom organic layer.

**Ethanol precipitation:**

11. Redistribute aqueous phase among 3 tubes so that each 2.0 mL tube has 550  $\mu\text{L}$  or less and each 1.6 mL tube has 450  $\mu\text{L}$  or less. *For some samples, additional salt is not necessary, and you can skip the sodium acetate. In this case, you can add up to 600  $\mu\text{L}$  in a 2.0 mL tube.*
12. Add 0.1 volumes sodium acetate (3M, pH 5.2). (*e.g.* add 55  $\mu\text{L}$  to 550  $\mu\text{L}$ .)
13. Add 2 volumes 100% ethanol. (*e.g.* add 1210  $\mu\text{L}$  to 605  $\mu\text{L}$ .)
14. [optional for low biomass samples] Add 1.2  $\mu\text{L}$  of glycogen (20  $\mu\text{g}/\mu\text{L}$ ).
15. Invert a few times to mix.
16. **Incubate at -20°C for at least 1 hr. or overnight.** Incubation on ice might work just as well and yield a cleaner pellet.
17. Centrifuge for 40 minutes at 16,000g. (Optional: used cooled centrifuge at 0°C)
18. Pour out supernatant. Do not completely invert tube; keep at a gentle angle to minimize the chance of the pellet falling out.
19. Add 500  $\mu\text{L}$  of cold 70% ethanol to each tube.
20. Invert the tube to mix. Make sure the pellet is dislodged from the bottom so that it is properly washed.
21. Centrifuge at 16,000g for 10 minutes.
22. Remove liquid again with pipettor. Be careful to avoid pellet.
23. Place tubes with open lids in the Vacufuge. Spin for 7 minutes at 30°C on the V-AL setting. If you can see ethanol in the tube, spin for another 2-5 minutes. If the pellets become powdery, they are too dry.
24. Resuspend in 100  $\mu\text{L}$  of low EDTA TE. Heat to 55°C for 10 or more minutes to dissolve pellet and store at 4°C. For long-term storage, place at -20 or -80°C, but avoid repeated freezing and thawing of the DNA. One strategy is to keep half at 4°C for the working sample and store the other half at -80°C as the archive sample.

**Recipe for low EDTA TE:**

10 mM Tris-HCl  
0.1 mM EDTA

For 50 ml:

500  $\mu$ l 1 M Tris-HCl (pH 8.0) autoclaved  
10  $\mu$ l 0.5 M EDTA (pH 8.0) autoclaved  
→ to 50 ml with milliQ H<sub>2</sub>O  
→ filter sterilize with 0.22  $\mu$ m syringe filter

TE is good for DNA storage, but EDTA inhibits PCR. So this low EDTA TE buffer is a good compromise for storing DNA for later PCR amplification. You can also just use EB (10 mM Tris-HCl, pH 8 or 8.5).