**DNA Extraction Protocol for Snails**

CHAOS/Phenol-Chloroform/Ethanol Precipitation

Adapted from coral protocol (Fukami et al, 2004)

Buffers to prepare in advance:

**CHAOS buffer starting with For 100mL, add**

4M Guanidine Thiocyanate Salt FW = 118.2 g/mol, pwd 47.3 g

0.1% N-lauroyl sarcosin sodium (=Sarkosyl) FW = 293.38 g/mol, pwd 0.1 g

10mM Tris-HCl pH 8.0 FW = 121.14 g/mol; 1M liquid 1 mL

**0.1M 2-Mercaptoethanol USE IN FUME HOOD! FW = 78.13 g/mol; 14.3M liquid 700 uL**

Add all reagents to ~60ml of ddH2O. Mix well. Reaction will get very cold [may need to warm to mix fully]. Qs to 100ml.

\*\*\*2-Mercaptoethanol (“BME”) can lose its potency with time. If not all the CHAOS will be used immediately, make CHAOS omitting the BME, and add an appropriate amount of BME to an aliquot of CHAOS before use. Alternatively, spike the CHAOS with fresh BME each use.\*\*\*

**Phenol-Chloroform Extraction (PCE) Buffer starting with For 100mL, add**

100mM Tris-HCl pH 8.0 FW = 121.14 g/mol; 1M liquid 10 mL

10mM EDTA FW = 372.2 g/mol; 0.5M liquid 2 mL

0.1% Sodium Dodecyl Sulfate **WEAR A DUST MASK** FW = 288.38 g/mol; pwd 0.1 g

Add all ingredients to ~80ml of ddH2O. Mix well. Qs to 100ml.

**Other components needed [make sure to have molecular grade nuclease free reagents]**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1.5ml tubes and 2 ml tubes Ice cold 70% ethanol

Phenol-chloroform-isoamyl alcohol (25:24:1) Ice cold 100% ethanol

RNase A (10mg/ml) “T low E” buffer (10 mM Tris pH 8.0, 0.1 mM EDTA)

Chloroform Microcentrifuge at 4⁰C and at room temp

3M NaOAc (pH 5.8) [pH 5.5 is also appropriate] 5PRIME Phase Lock Gel (PLG) – Heavy [no needed by helps]

**Timeline (5-7 days total)**

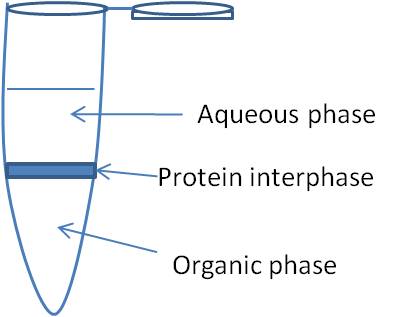
Day 1: Step 1 (lysis in CHAOS for 2-3 days typically)

Day 2: Steps 2-10 (leave in -20C overnight or over weekend)

Day 3: Steps 11-17

Day 4: 18-19

**DNA Extraction**

1. Place tissue for DNA extraction into a 1.5 ml polypropylene tube and add CHAOS buffer to ~4x the volume (minimally use 100 ul, 150 ul is usually good) of the snail tissue. Leave samples at room temperature for as long as it takes to fully digest the tissue, 2-10 days, depending on the amount of tissue. [2-3 days is usually fine]
2. After lysis, add a volume of PCE buffer equal to the amount of CHAOS buffer. Add 5uL of RNase A to each sample, then invert tube briefly to mix contents. Incubate at 37C for 10-15 minutes. For larger samples (200ul CHOAS and more), add more RNAse A to scale. **[If you do not have PGL tubes, follow DNA extractions steps on page 4]**
3. Transfer contents to a phase-lock gel (PLG) tube (centrifuge PLG tubes before at 12,000xg for 1 minute). For 2ml PLG tubes, ensure the volume of CHAOS + PCE buffer is at least 200ul. Add more to scale as needed.
4. PCIA step.  
   After incubation, add a volume of phenol-chloroform-isoamyl alcohol (25:24:1) equal to the volume of CHOAS (at least 100ml). Gently shake/invert for 30 seconds. Make sure emulsions forms and mixes with other contents, try to have the emulsions still present upon centrifugation (i.e. before the solution separates back to layers). Then, centrifuge samples at 12,000xg for 5 minutes.
5. [Generally skip] If necessary, add another volume of phenol-chloroform-isoamyl alcohol (25:24:1), gently shake until an emulsion forms in the top of the PLG tube. Then, centrifuge samples at 12,000xg for 5 minutes.
6. Chloroform step:
   1. If there is sufficient volume in the PLG tube, add 1 volume (usually 1.6-2 volumes is better) of chloroform to the aqueous phase. Gently shake/invert for 30 seconds. Make sure emulsions forms and mixes with other contents, try to have the emulsions still present upon centrifugation (i.e. before the solution separates back to layers). Then, centrifuge samples at 12,000xg for 5 minutes.

**Figure 1:** Distinct phases of PGL tube

* 1. If there is insufficient room in the PLG tube, carefully pipet off the aqueous layer into a clean tube and follow step 6a.

1. Pipet off the aqueous layer (Figure 1) and put into a clean 1.5 ml or 2 ml tube (determine minimum appropriate tube size based on calculations in steps 9 and 10; you’ll need at least a 1.5 ml tube for step 13). BE CAREFUL to NOT TOUCH THE GEL with the pipet tip, this will contaminate the sample.

Note: Phenol degrades DNA. Do not leave your sample in a step with phenol for too long!!!

**Ethanol precipitation & resuspension**

1. Determine the final volume of the aqueous product from step #8, and add NaOAc such that it constitutes 10% of the total final volume (i.e. ~200uL of DNA solution 🡪 add 22.2uL of NaOAc 🡪 total volume =222.2uL). Easy math: 1/9 x volume of aqueous product from step #8 = volume of NaOAc
2. Add double the total volume of ice cold 100% ethanol (ex: 222.2 ul DNA/NaOAc 🡪 add 444.4 uL Ethanol).

Invert tube to mix ethanol with DNA solution, then store in -20C freezer for 2 hours to overnight (or over weekend, longer time may yield more DNA).

1. Centrifuge samples at 13,000xg at 4C for 15 minutes.
2. Pipet off the supernatant without disturbing the pellet.
3. Add 1mL of ice cold 70% ethanol. Centrifuge at 13,000xg for 10 minutes at 4C.
4. Pipet off the supernatant without disturbing the pellet [may want to repeat the wash]
5. Centrifuge for 30 seconds [can be done at room temp in mini centrifuge or in the same]
6. Carefully pipet off the remaining supernatant (usually > 20 ul, use a P100). Let samples sit open at room temperature for 10-20 minutes, examine the tubes carefully to ensure that no liquid ethanol remains. DO NOT let the pellet get over dried.
7. Suspend samples in 30-60ul of “T low E” buffer. Gently move the pellet off of the tube wall into the liquid with the pipet tip to aid suspension. Leave samples at room temperature for 10-30 minutes, then move to refrigerator. Leave to suspend for a minimum of 4 hours, ideally overnight.

**Quality assessment**

1. To ensure DNA purity, nanodrop 1 ul or 1.5 ul of sample
   1. If necessary, clean samples with Zymo Clean & Concentrate (see page 3 for details). Pure DNA has a 260/280 absorbance ratio of 1.8 and 260/230 of 2.0. If the results of nanodrop result indicate contaminants, the sample should be cleaned.
2. To ensure DNA quality 100ng-300ng of sample should be run on a 0.8% agarose gel at 100V for ~70 minutes.
   1. Use a ladder with high molecular weight (HMW) markers, such as Lambda Phage.
   2. Alternative gel example: 0.7% gel of 1xTAE at 40V for 120 minutes (“low and slow” method).

Fukami et al (2004). Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature***427**, 832-835.

**Protocol for cleaning DNA sample with DNA Clean & Concentrator­TM-5**

1. Add 2 volumes of DNA Binding Buffer to each volume of DNA samples.

Example: 30 µl DNA, add 60 µl DNA Binding Buffer.

1. Load the mixture into a Zymo-Spin Column in a Collection Tube
2. Centrifuge at 12,000 x g for 30 seconds (set for 1 min. stop ~22 seconds). Discard the flow through.
3. Add 200 µl of DNA Wash Buffer (make sure ethanol has been added to it) to the column and centrifuge for 30 seconds.

Repeat and discard the flow through.

1. Add 200 µl of 80% ethanol (prepare day of) to the column and centrifuge for 30 seconds.

Repeat and discard the flow through.

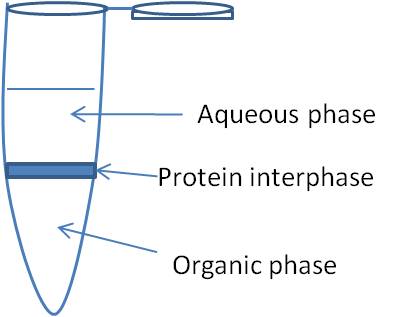
1. Centrifuge the column for 30 seconds.
2. Place the Zymo-Spin Column into a new 1.5 ml tube. Elute DNA in T low E (10mM Tris, 0.1mM EDTA). Elute twice into the same final 1.5 ml tube using half of the final volume for each spin.

Example for final volume of 20 µl:

Add 10 µl TlowE to column, sit 5 minutes then centrifuge for 30 seconds.

Repeat.

**DNA extraction without PLG tubes**

1. Add equal volumes of PCE buffer and phenol-chloroform-isoamyl alcohol (25:24:1) to the CHAOS buffer-snail tissue slurry. Also add 5uL of RNase A to each sample, then gently vortex or invert the tube briefly until an emulsion forms.
2. Centrifuge the samples at 10,000xg for 30 seconds, and pipet off the aqueous phase into a fresh tube. Save the organic phase until you’re done… just in case!!
3. Add an equal volume of phenol-chloroform-isoamyl alcohol to the aqueous phase in the clean tube. Gently shake/invert for 30 seconds. Make sure emulsions forms and mixes with other contents, try to have the emulsions still present upon centrifugation (i.e. before the solution separates back to layers). Then, centrifuge samples at 12,000xg for 5 minutes.
4. Pipet off the aqueous phase into a fresh tube. Repeat step 3 (2 cycles are usually sufficient with the snails to remove all residue from the interphase and organic phases, though if residue remained, steps 3 and 4 should be repeated until no protein interphase remains between the two layers and the organic phase appears clear.)
5. Add 2 volumes of chloroform to the aqueous layer in the clean tube.
6. Gently shake/invert for 30 seconds. Make sure emulsions forms and mixes with other contents, try to have the emulsions still present upon centrifugation (i.e. before the solution separates back to layers). Then, centrifuge samples at 12,000xg for 5 minutes.
7. Pipet off the aqueous layer and put into a clean 1.5 ml or 2 ml tube (determine minimum appropriate tube size based on calculations in steps 9 and 10 [in Ethanol precipitation & resuspension]; you will need at least a 1.5 ml tube for step 13.)

Move on to Ethanol precipitation & resuspension on page 2

Note: Phenol degrades DNA. Do not leave your sample in a step with phenol for too long!!!