**DNA Extraction Protocol for Snails**

CHAOS/Phenol-Chloroform/Ethanol Precipitation

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

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 so I recommend adding 0.75µl of BME to each individual extraction, instead of adding into the CHAOS buffer.\*\*\* If you want to add BME to CHAOS buffer, BME should be 0.1M of the CHAOS buffer.

**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**

2-Mercaptoethanol (BME) **USE IN FUME HOOD!**

1.5ml tubes

RNase A (10mg/ml)

Phenol-chloroform-isoamyl alcohol (25:24:1) **FUME HOOD!**

Chloroform

“T low E” buffer (10 mM Tris pH 8.0, 0.1 mM EDTA)

3M NaOAc (pH 5.8) [pH 5.5 is also appropriate]

Ice cold 100% ethanol

Ice cold 70% ethanol

Heat block/water bath set to 65ºC

Microcentrifuge at 4⁰C and at room temp

**Timeline (Long CHAOS)**

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

Day 4: Steps 2-10 (leave in -20C overnight or over weekend)

Day 5: Steps 11-15

Day 6: 16-17

**Timeline (Rapid CHAOS)**

Day 1: Step 1R, steps 2-10 (leave in -20C overnight or over weekend)

Day 2: Steps 11-15

Day 3: Steps 16-17

**DNA Extraction (Pick either 1L or 1R)**

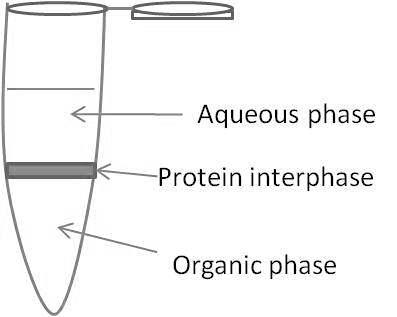
1L. **Long lysis:** Place tissue for DNA extraction into a 1.5 ml microcentrifuge tube and add CHAOS buffer to ~4x the

volume of the snail tissue (minimally use 100 ul, 150 ul is usually good for single snail). Add 0.75µl BME. Leave

samples at room temperature for 2-3 days (up to 7 days will be fine).

1R. **Rapid Lysis:** Place tissue for DNA extraction into a 1.5ml microcentrifuge tube. Using an ice-cold micropestle,

disrupt the tissue by hand until there are no large pieces of tissue. Add CHAOS buffer to ~4x the volume of the snail tissue (minimally use 100 ul, 150 ul is usually good for single snail). Add 0.75 µl BME. Incubate on a heat block or water bath at 65ºC for 10-30 minutes.

1. **PCE step.** 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 (if amount of CHAOS buffer added exceeds 200µl, add more RNase to scale), then gently vortex or invert the tube briefly until an emulsion forms.
2. **PCE Centrifuge step.** Centrifuge the samples at 10,000xg for 30 seconds, and pipet off the aqueous pm4phase into a fresh tube.
3. **PCIAA step.** 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. **Repeat PCIAA step.** 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. **Chloroform step.** Add chloroform to 2 volumes to the aqueous layer in the clean tube.
6. **Chloroform Centrifuge step.** 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. **Final aqueous product step.** Pipet off the aqueous layer and put into a clean 1.5 ml (may need 2ml tube for a large extraction – 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 11.)
8. **NaOAc step.** 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
9. **Ethanol precipitation step.** Add ice cold 100% ethanol to double the volume of the aqueous + NaOAc solution, 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).
   * (ex: 222.2 ul DNA/NaOAc 🡪 add 444.4 uL Ethanol)

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1. **Pellet DNA step.** Centrifuge samples at 13,000xg at 4C for 15 minutes. Pipet off the supernatant without disturbing the pellet.
2. **Wash DNA step.** Add 1mL of ice cold 70% ethanol. Centrifuge at 13,000xg for 10 minutes at 4C. Pipet off the supernatant without disturbing the pellet.
3. **Repeat DNA Wash step.** Repeat step 12, leave a small bit of liquid (~10-20µl) at bottom of tube.
4. **Remove Remaining EtOH step.** Quick centrifuge for 30 seconds. 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.
5. **Resuspend DNA step.** 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 to resuspend for a minimum of 4 hours, ideally overnight.

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1. **Quality assessment step.** Assess quality of the extraction for three measures: (1) quantity, (2) degradation, and (3) purity.
   * Quantity **–** Quantify 1 µl of sample using a Qubit (Nanodrop is not accurate for Phenol-Chloroform-based extractions. If you have to use a nanodrop, count on ~2 orders of magnitude over actual concentration).
   * Degradation – Run 100ng-300ng of sample on a 0.8% agarose gel at 100V for ~70 minutes. Use a ladder with high molecular weight (HMW) markers, such as Lambda Phage. Recommend Sodium Borate buffer over TAE buffer.
     1. Alternative method: 0.7% gel at 40V for 120 minutes (“low and slow” method).
   * Purity – Nanodrop 1 ul of sample, paying attention to the 260/230 ratio and the 260/280 ratios. Quantity is meaningless for a Phenol-Chloroform extraction as phenol emits light at a similar frequency to dsDNA.
     1. If preparing for NGS work, recommend cleaning with SPRI/AMPure beads at 1.4x or similar
2. **Storage step.** Store DNA at -20ºC for short-term use, -80ºC for long-term storage.

**References:**

Fukami et al (2004). Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals.

*Nature***427**, 832-835.