**Noor Lab Single Fly Phenol-Chloroform DNA Extraction Protocol**

Compiled by Kieran Samuk, Sept 28-2016

Based on the PCl protocols from:

Glenn Lab (University of South Carolina, modified by S. Sander and K. Korunes)

Schluter Lab (University of British Columbia, Stickleback Molecular Manual)

**Materials**

3 labeled 1.5 – 2.0 mL microcentrifuge tubes per sample.

Plastic mini pestles

Digest Buffer (10 mM Tris, 10 mM EDTA, 100 mM NaCl, 1% SDS)

Proteinase K (20 mg/mL)

PCI (Phenol:Chloroform:Isoamyl Alcohol; 25:24:1)

CI (Chloroform:Isoamyl Alcohol; 24:1)

5M NaCl (autoclave to speed saturation)

Ethanol (95%, 70%), @ -20°C

**Safety & PPE**

Fume hood

Bench liner (in hood, for spills)

Nitrile gloves

Lab coat

40% PEG (polyethylene glycol solution) in spray bottle.

Double-bagged (phenol-safe bags) phenol waste containers for tips + tubes (in hood)

**Safety Tips**

- Double glove when working with PCl and Cl.

- Work with PCl and Cl only in fume hood.

- Wear PPE or you will probably die or least become a living skeleton

- If you get phenol on your skin, use PEG to clean it off, followed by soap + water (15 mins)

- Wear close-toed shoes; pants.

**A. Grind and Digest (bench)**

1. Place one whole fly in a 1.5 mL microcentrifuge tube.
2. P1000: Add 200 uL digest buffer to each tube.
3. Grind each fly with a separate plastic pestle. The more obliterated, the better. Leave the pestle in the tube after grinding.
4. P1000: Rise each pestle with an additional 200uL of digest buffer. Dispose of pestles after rinse.
5. P10: Add 10 uL proteinase K to each tube (add a little more if the proteinase K is old).
6. Incubate overnight at 55°C.
7. P2: Add 1 uL of 4mg/mL RNase-A to the sample and allow to incubate for 20 min at 50°C. Now is a good time to make the remaining two sets of tubes.
8. P200: Remove tubes from the incubator and adjust the volume to approximately 500 uL with fresh digest buffer.

**B. Phase Separation (fume hood)**

1. P1000: Add 500µL of PCI to each tube. Close the tube and vortex for 2-3 second. Hold the top of the tube Contents should be milky white
2. Spin the tubes at 12000 X g for 8 min. You might need to leave the hood.
3. P200: pull off the top aqueous layer (leaving the interface) and place it in the appropriate fresh tube. You should be able to get 350-400uL without disturbing the interface.
4. P1000: Repeat 9-11 using CI rather than PCI.

**E. DNA Precipitation (bench)**

1. P10: Add 6.4µL of 5M NaCl. This assumes recovery of ~370µL of aqueous phase, if you are different from that, you need to scale appropriately (1.7µL of 5M NaCl per 100µL of aqueous sample).
2. P1000: Add 780 µL of chilled (-20C) 95% ethanol to the samples and vortex briefly at setting 7ish. Again, this assumes recovery of ~370µL of aqueous phase, if you are different from that, you need to scale appropriately (2.1 volumes of 95% EtOH per volume of aqueous sample).
3. Put tubes in -20C freezer for 2 hours.
4. Spin the tubes at 12,000 X g (RCF) for 20 minutes.

**F. DNA Wash (bench)**

1. P1000 + P20: Pull off the 95% ethanol and dispose in Falcon tube
2. P1000: add 1 mL of chilled 70% EtOH.
3. Spin the tubes at 12,000 X g (RCF) for 10 minutes.
4. P1000 + P20: Pull off as much of the EtOH as possible with a pipet.
5. Air-dry in fume hood until no liquid ethanol is present. Could also speed vac.

**G. Elution (bench)**

1. P200: Add 20uL nuclease-free H2O or TE. Pipette up and down to ensure suspension. Store tubes at -20C or -80C (long term).