**Easy UCD DNA digests** Sean O’Rourke, Mike Miller Updated: April 2017, Comments by SB 1/10/18

**AMPURE MAGNETIC BEAD DNA EXTRACTION PROTOCOL**

**Quality Control:**

* Wear latex or nitrile gloves throughout extraction protocol.
* Always use a new razor blade, forceps, and weighing dish when cutting up subsequent tissue and rinse each forceps with bleach and water after each use.
* Always use filtered tips and change them each time when adding reagents to samples if tissue is already inside the wells.
* Include a negative control for each batch of extractions (1 negative per 8 samples extracted). Be sure to keep the negative in the same order amongst other samples throughout the extraction process.

**Day 1 (Time ~2 hours)**

1. Make Liftons buffer (100 mM EDTA, 25 mM tris-HCl pH 7.5, 1% SDS). 500 ml: 100 ml 0.5 M EDTA pH 8.0, 12.5 ml 1 M tris-HCl pH 7.5, 25 ml 20% SDS, H2O to 500 ml.
2. Into each 96 plate well, pipette 80 ul Liftons buffer. Use fully skirted plates.
3. Place strip caps on wells and place in freezer until needed.
4. Add fin clip sample to each well, we use a piece 2-25 mm2. Open one strip cap at a time and reseal when all eight wells are filled. This helps prevent cross contamination of samples.
5. Place in freezer until the next step.
6. Make digestion master mix (Liftons buffer + 0.075 M DTT + 4.2 mg/ml Proteinase K).

One plate: 3.146 ml Liftons buffer, 0.924 ml 20 mg/ml Proteinase K, 330 ul 1 M DTT

1. Into each 96 plate well, pipette 40 ul of digestion master mix.

**Save tips at -20ºC for next use.**

1. Seal plate with sealing foil and vortex to mix.
2. Incubate plate at 55°C overnight

**Day 2 (Time ~ 2 hours)**

1. Spin the plate (1800 rpm, 1 sec) to collect any condensation, vortex the plate for 30 sec, spin the plate quickly again.
2. Make Hybridization buffer in advance (2.5 M NaCl, 20% PEG 8000, 0.025 M DTT) 250 ml: 1 g DTT, 29 g NaCl, 50 g PEG 8000, water to 250 ml (store at 4°C).
3. Into a new 96 well plate, pipette 60 ul Hybridization buffer and 15 ul resuspended Ampure XP beads.
4. **Using the saved tips,** transfer 60 ul lysate from the top of the wells to the Hyb buffer andAmpure XP plate. Leave any solids behind. Save residual digest for future extractions.
5. **Using the saved tips,** mix by pipetting up and down 10X.
6. Incubate plate at room temperature for 5 minutes.
7. Place the plate on a magnet.
8. **Reuse tips from last use** to aspirate and discard the supernatant.

**Discard tips.**

1. Remove the plate from the magnet and **use new tips** to add 150 ul freshly prepared 80% ethanol, pipette up and down 10X to resuspend the Ampure beads.

**Save the tips.**

1. Place the plate on a magnet
2. **Reuse tips** from last use to aspirate and discard the supernatant.
3. Repeat steps 18-20 for a total of **two** washes.
4. Remove residual 80% ethanol with 20 ul pipette tips.
5. Allow the beads to air dry while on the magnet (~5-10 min.).
6. Into each 96 plate well, **use new tips** to pipette 20-100 ul of low TE (10 mM tris-HCl pH 7.5, 0.1 mM EDTA).**Save the tips.**
7. Remove plate from magnet and **reuse tips** from last use to resuspend beads.
8. Place a kimwipe on the plate and heat 1 min. at 55°C in a thermocycler.
9. Place the plate on a magnet and allow the beads to collect, **reuse tips** from last use to pipette up and down twice and then remove the supernatant containing the DNA to a new plate.

**Health and Safety Warnings:**

* Wear latex or nitrile gloves, closed-toe shoes and a lab coat.
* Be careful handling razor blades.
* This procedure uses chemicals that may be harmful. Please refer to Safety Data Sheets before performing this protocol
* Proteinase K: May cause allergy or asthma symptoms or breathing difficulties if inhaled.
* Ethanol: Highly flammable. Causes moderate skin irritation. Inhalation may cause respiratory tract irritation, nausea, headaches, dizziness and suffocation
* Sodium Dodecyl Sulfate: Causes skin irritation Causes serious eye damage
* DTT: May form combustible dust concentrations in air Harmful if swallowed Causes skin irritation Causes serious eye irritation May cause respiratory irritation

**Equipment**

Magnet plate

Razors/cutting tools

Troughs

Conical falcon tube

P200 multichannel pipette

200ul pipette tips + empty box

Plate foil

**Reagents**

Eppendorf 96-Well twin.tec PCR Plates Clear; Skirted; 150µL, E9-510-20401

Thermo Scientific Flat PCR Cap Strips AB-0783

Water, DNA Grade, DNASE, Protease free, Fisher BioReagents BP24701

EDTA, Ethylenediaminetetraacetic Acid (0.5M Solution/pH 8.0), Fisher BioReagents, BP2482-500

Tris Hydrochloride, 1M Solution (pH 7.0, pH 7.5, and pH 8.0/Mol. Biol.), Fisher BioReagents, BP1758-500

SDS, Sodium Dodecyl Sulfate, 20% Solution (Electrophoresis/Molecular Biology), Fisher BioReagents, BP1311-200

Dithiothreitol (White Crystals or Powder/Electrophoresis), Fisher BioReagents, BP172-25

ProK, Fisher BioReagents Proteinase K (Tritirachium album/Molecular Biology), BP1700-500

NaCl, Sodium Chloride, Fisher BioReagents, BP3581

Carbowax PEG 8000 (Powder), Fisher Chemical, P156-500

Ethanol, Absolute (200 Proof), Molecular Biology Grade, Fisher BioReagents, BP2818-500

Agencourt AMPure XP Beads- PCR Purification A63881