Easy UCD DNA digests Adapted from: Sean O'Rourke, Mike Miller

Last updated: September 2022 JDS/NF/LK

HIGH YIELD AMPURE MAGNETIC BEAD DNA EXTRACTION PROTOCOL

This is our "regular" DNA extraction protocol. Use this for fin clips, muscle, or any type of tissue that is not expected to be very low in DNA quantity. This is the protocol to use for most DNA extractions-talk to Lisa if questions on which to use for your project.

Quality Control:

- **BEFORE beginning protocol,** read through and check/confirm that you have all the reagents and supplies in the quantities needed to complete the entire procedure. Getting 1/2 way through and realizing we are short or out of a critical ingredient can cause ruined samples, etc. This includes checking the lab calendar to make sure the equipment will also be available (centrifuges, pipettes, magnet etc.)
- Wear nitrile gloves throughout the 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).
- Plan out extraction timing since the digestion overnight period should typically be only 12-15 hours.
- It is marked below the steps where it is possible to save and reuse tips to reduce waste and not have issues with contamination. Discuss if this should be done with your mentor/PI depending on comfort and skill level.
- Be careful removing caps from plates, it can cause solution/liquid to splash up if too forceful.
    - Spin down (w/ salad spinner or mini-centrifuge) to avoid liquid on the upper portion of the tubes that could splash when opening and cross contaminate. It's generally good practice to do this every time before opening, just a quick spin, not too vigorous, just enough to get all liquid in the bottom of the tube. If unsure of how much, have an experienced labmate/Lisa show you.
    - Pay attention to caps if reusing in between steps to make sure no contamination in between steps (number ends to ensure put in the same direction each time, put on clean chemwipe, etc.)

Day 1 (Time ~2 hours)

1) Remove DTT from freezer to thaw at room temperature
2) Set thermomixer to warm up to 55 degrees C
3) Into each 24 plate well, pipette 80 ul Liftons buffer. Use fully skirted plates.
   Note: Lifton's buffer is bubbly.
4) Add tissue sample to each well, we use a piece 2-25 mm_2. Open one strip cap at a time and reseal when all eight wells are filled. This helps prevent cross contamination of samples.
5) Make digestion master mix following the table below.
   N.B. If running smaller batches (~24 samples or less), a good rule of thumb is to estimate ~15-20% overage to account for pipetting error. When running higher batches, this becomes wasteful and better to either drop the percentage or include enough for 2-3 extra samples or such. Use best judgement and/or discuss with PI/mentor for best choice for your samples.

Buffer	vol (ul) per sample	no. samples	Percent overage multiplication factor	vol (ul) for MM total
Liftons	32.8			
Pk (20mg/ml)	9.6			
DTT 1M	3.4			

Example if running batch of 24 samples:

Buffer	vol (ul) per sample	no. samples	Percent overage multiplication factor	vol (ul) for MM total
Liftons	32.8	24	1.15	904.5
Pk (20mg/ml)	9.6	24	1.15	265.7
DTT 1M	3.4	24	1.15	94.9

6) Into each well, pipette 40 ul of digestion master mix.
7) Seal plate with tube caps (or sealing foil) and shake on thermomixer for 15 seconds at 1400rpm to mix.
8) Incubate plate at 55°C overnight

<u>Day 2</u> (Time ~ 2 hours)

9) Take out bead aliquot and bring to room temperature (~30 minutes) before use.
10) Shake plate on thermomixer for 30-60 sec at 1400rpm to further dissolve tissue. DO NOT VORTEX, lifton's buffer will dissolve the glue on your plate tape if vortexed too much.
11) Spin the plate for 1 minute.
12) Into a new 24 well plate, pipette 60 ul Hybridization buffer and 15 ul resuspended Ampure XP beads.
13) Transfer 60 ul lysate from the top of the wells to the Hyb buffer and Ampure XP plate. Leave any solids behind. Save residual digest for future extractions.
    Note: if you are getting solids in your tips, eject and re-spin plate
14) Mix by pipetting up and down 10X.
    Note: The color of the mixture should appear homogeneous after mixing.
15) Incubate plate at room temperature for 10 minutes.
16) Place the plate on a magnet until solution is clear (up to ~5 min).
17) Aspirate and discard the supernatant. **DNA is now on the beads.**
18) 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. When doing multiple rows, add ethanol to all rows before pipette mixing. **(optional: Save the tips.)**
19) Place the plate on a magnet until the solution is clear (up to ~5 min).
20) Aspirate and discard the supernatant **(optional: reusing tips from step 18).**
21) Repeat steps 18-20 for a total of **three** washes.
22) Cover plate, spin down for 1 min, remove residual 80% ethanol with 10 ul pipette tips.
23) Allow the beads to air dry while on the magnet (~2-3 min).
    Note: Be aware of over drying the beads, this can reduce the yield of DNA. Can adjust time if needed. Ideally, the beads will have a very slight sheen to them, but with no visible liquid otherwise.
24) Remove plate from magnet and into each well, pipette 20-100 ul of low TE (10 mM tris-HCl pH 7.5, 0.1 mM EDTA). When doing multiple rows, add lowTE to all rows before pipette mixing.
25) Resuspend beads by pipette mixing 10 times. **DNA is now in the solution.**
    Note: The color of the mixture should appear homogeneous after mixing.
26) Seal plate and heat 1 min. at 55°C in the thermomixer.
27) After 55°C incubation, incubate plate at room temperature for 10 min.
28) Salad spin, then place the plate on a magnet and allow the beads to collect (up to ~ 5 min), then transfer supernatant containing the DNA to a new plate.

Health and Safety Warnings:

- Wear 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

Buffer Recipes:

1. Liftons buffer (100 mM EDTA, 25 mM tris-HCl pH 7.5, 1% SDS).
    - 50 ml total volume: 10 ml 0.5 M EDTA pH 8.0, 1.25 ml 1 M tris-HCl pH 7.5, 2.5 ml 20% SDS, H_2O to 50 ml. (store at room temp)
    - 500 ml total volume: 100 ml 0.5 M EDTA pH 8.0, 12.5 ml 1 M tris-HCl pH 7.5, 25 ml 20% SDS, H_2O to 500 ml. (store at room temp)
2. Hybridization buffer (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. 1M DTT: add 0.77g of DTT powder and bring volume up to 5ml with MilliQ water. Aliquot 1000ul to sterile 1.5mL tubes. (store at -20°C)
4. Low TE: 20 ul 0.5M EDTA, 1000 ul of 1M Tris-HCl, 98.98 ml of milliQ water. (store at room temp)

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