Qiagen DNeasy 96 fin clip DNA extraction
Plate xx, 20xx xx xx

Points before starting

- Centrifugation steps are done at room temperature.
- Transferring lysate requires extended-length tips (ELT). VWR P1000s work here.
- Supplies required:

  5 columns Eppendorf green P1000, 1 box ELT P1000, 2 boxes P200

  2 new or autoclaved S-blocks, DNeasy plate, 1.2 ml collection microtube rack, elution microtube rack, 3 Airpore Tape Sheets.

  Empty collection microtube rack with end removed.

  Balance collection microtube rack, S-block, DNeasy plate and elution microtube rack

  19 ml ATL (1.1x), 2 ml pro-K (1.04x), 43 ml AL/E (1.09x), 52 ml AW1 and AW2 (1.08x), 13 ml AE (1.08x).

Things to do before starting

- AL, AW1, and AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of 100% ethanol and shake well.
- ATL and AL/E may form precipitates in storage. Can pour off into beaker to inspect. Warm to 56C as needed.
- Preheat thermomixer to 56C.

Lysing with ATL:proteinase-K

1. add 180 ul lysis buffer ATL to each of racked collection microtubes.
2. submerge ≈4 mm triangular snips ($\leq$ 20 mg) of fin clip samples in tubes.
3. add 20 ul pro-K to each sample. Cap tubes.
4. vortex or shake vigorously 15 s. Spin down briefly at 3000 rpm.
5. check that samples are submerged. Tape lid down **securely**.
6. incubate in thermomixer at 56C for 12+ hr., with 1 min. of 1200 rpm agitation every 20 min.
7. At conclusion, lysate may be viscous, but check for gelatinous masses by tipping. If present, extend lysis with an additional 100 ul ATL and 15 ul pro-K.
8. Briefly spin down condensate at 3000 rpm.

Binding with AL/E

prep: label DNeasy 96 plate, S-block, and elution microtube rack. Position plate on top of S-block. Do same for balance plate and block. Load balance plate. Preheat incubator to 56C.

1. remove caps and add 410 ul AL/E to each sample. Seal with new caps. If additional ATL:K was added, use 615 ul AL/E.
2. vigorously shake samples for 15 s. Briefly spin down at 3000 rpm. A white precipitate is ok, but vortex any gelatin formed.
3. working with 1 strip at a time in the separate rack, use ELT to transfer the 610 ul of lysate to the DNeasy plate. Avoid sample overflow. Avoid taking up undigested tissue, taking up only 600 ul lysate as necessary. Dispense directly to membrane.
4. seal with Airpore Tape Sheet.
5. centrifuge **10** min at 6000 rpm (5796 x g). Check that all lysate has passed through.

First wash with AW1

prep: check that incubator is at 56C, adjust balance block weight.

1. remove sheet, add 500 ul wash buffer AW1.
2. seal with new tape sheet.
3. spin for **5** min at 6000 rpm.

Second wash with AW2

prep: warm AE in thermomixer, adjust balance block.

1. replace S-block with clean, dry block.
2. remove tape sheet, add 500 ul wash buffer AW2.
3. **do not seal** with tape sheet.
4. spin for **15** min at 6000 rpm .

Additional time and heat from centrifuge dries ethanol from membrane.

Single elution with AE

1. place DNeasy plate on elution microtube rack.
2. dispense 125 ul elution buffer AE directly to membrane.
3. seal with new tape sheet.
4. tap assembly on table to distribute AE over membrane.
5. incubate for 5 min at RT, centrifuge for 2 min at 6000 rpm.
6. from elution microtubes, aliquot 30 ul working stock. Spin down aliquot, store -20C.
7. seal elution microtubes with special purpose caps. store -80C.

Target concentration, single elution

For fin clips, Qiagen gives an expected yield of 10–20 ug given a double elution with 200 ul. (We do a single.)

$$\frac{15\ ug\ DNA}{125\ ul\ eluate} \cdot \frac{1000\ ng}{ug} = \frac{120\ ng}{ul}$$