High throughput Drosophila DNA extraction

Materials needed

- Qiagen Puregene cell and tissue kit #158388
- PCR plates, strip-caps, plastic seals (Applied Biosystems 4306311)
- Plate centrifuge
- Hot chamber for 65*o*C and 37*o*C
- Glass beads (3.5 mm)[1]
- Bead machine

Tissue collection

Collect single or mass flies in 1.5 ml tubes.

Setup

1. Place cell lysis solution on ice until cloudy.
2. Turn on Hot chamber to 65°C.

Cell lysis

1. Add 100 µl **cell lysis solution** and 2 glass beads to each fly-containing tube[2].
2. Seal with parafilm[3].
3. Homogenise flies with bead machine[4].
4. Incubate samples at 65°C for 20 min.
5. Return samples to room temperature. Set incubator to 37°C. Set incubator to 37°C.

RNAse treatment

1. Mix 29.5 µl **H2O** and 0.5 µl **RNase A** (4 mg/ml) per sample.
2. Add 30 µl of **RNase mix** to each well of a 96-well plate.
3. Transfer 100 µl of each sample to the 96-well plate.
4. Dry plate with kimwipe, add new sealing film, whirlmix and incubate at 37°C for 40 min.

Protein precipitation

1. Add 33 µl **protein precipitation solution** to each well.
2. Dry plate with kimwipe, add new sealing film, whirlmix and incubate on ice for 10 min (5 min for tubes).
3. Centrifuge at 4,000 RPM for 20 min (14,000 RPM, 3 min for tubes).

DNA precipitation

1. Prepare a new plate with 100 ml **100% isopropanol** in each well.
2. Transfer 110 µl of each sample to the new plate with isopropanol. Mix each sample by pipettling.
3. Dry plate with kimwipe, add new sealing film, and centrifuge at 4,000 RPM for 30 min (14,000 RPM, 3 min for tubes).
4. Remove seal, place on folded paper towel and use hard plastic lid to safely invert plate. Slightly lift from paper towel to allow air so that solution drops to paper towel[5]. Without inverting the plate, transfer to fresh paper towel and spin inverted at 250 RPM for 1-2 sec to remove residual isopropanol.
5. Add 100 µl of freshly prepared **70% ethanol** to each well. Seal plate as before and centrifuge at 10,000 RPM for 10 min (14,000 RPM, 1 min for tubes).
6. Remove supernatent as before by inverting plate on paper towels and slow centrifugation.
7. Turn plate facing up and leave to air dry 5-15 min.

DNA resuspension

1. Add 20 µl **DNA hydration solution**[6] to each well.
2. Seal plate with 8-strip caps and incubate at RT overnight to resuspend the DNA.
3. Mix the plate by flicking and spin the solution down.

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[1] Can be cleaned by soaking in 10% bleach for 1 h, thoroughly rinsing with H2O, then with 95% ethanol, overnight drying and final autoclaving on dry cycle, followed by drying at 65oC.

[2] 1-10 flies -> 100 µl, 11-20 flies -> 200 µl etc.

[3] Can be done in parallel with the bead machine once enough samples have been sealed for one machine run.

[4] 1 fly with 2 beads in 100 µl needs 1-2 min. 100 flies with 3 beads in 1000 µl need 10 min.

[5] Light tapping on the plate to remove isopropanol is also allowed.

[6] Use **Low EDTA TE buffer** for the Swift library prep.