

DNA extraction from *N. furzeri* embryos

Materials

DNeasy Blood & Tissue (Qiagen)

1. Remove chorion and yolk from embryos. Also, remove YSL/membrane as much as possible. Collect processed embryos in Eppendorf with PBS, on ice.
2. After all embryos are processed, wash x 2 with PBS
3. Remove PBS supernatant. Add 180 ul Buffer ATL. Vortex briefly.
(Optional) If RNA-free genomic DNA is required, add 4ul RNase A (100 mg/ml) here, vortex, and incubate for 2 min at RT.
4. Add 20 ul proteinase K. Mix thoroughly by vortexing, and incubate at 56°C overnight (or until embryos are completely lysed).
5. Vortex for 15 s. Add 200 ul Buffer AL and vortex. Then add 200 ul 100% EtOH, and vortex again.
6. Transfer the mixture from Step 5 (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at >6000 g for 1 min. Discard flow-through and collection tube.
7. Place the DNase Mini spin column in a new 2 ml collection tube, add 500 ul Buffer AW1, and centrifuge for 1 min at 6,000 g to dry the DNase membrane. Discard flow-through and collection tube.
8. Place the DNase Mini spin column in a new 2 ml collection tube, add 500 ul Buffer AW2, and centrifuge for 3 min at 20,000 g to dry the DNase membrane. Discard flow-through and collection tube.
9. Place the DNase Mini spin column in a clean 1.5 ml Eppendorf tube, and pipet 150 ul Buffer AE directly onto the DNase membrane. Incubate at RT for 1 min, and then centrifuge for 1 min at 6000 g to elute.
10. (Recommended & Optional) For maximum DNA yield, repeat elution once as described in Step 8 with the same elute and tube.
11. Determine DNA concentration by Nanodrop.

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