RNA extraction from *N. furzeri* embryos

Materials

RNeasy Mini Kit (Qiagen)
Disruption beads (0.5mm, 3.7g/cc): Research Products International Corp. #9834.

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. Replace PBS with 300 ul Buffer RLT
4. Break embryos and cells with disruption beads, centrifuge the lysate for 3 min at full speed and transfer supernatant to gDNA elimination spin column. Centrifuge for 15 s at >8000 g. **Save/Transfer** the flow-through to a new Eppendorf tube.
5. Add 1 volume of 70% EtOH to the cleared lysate, and mix immediately by pipetting. DO NOT centrifuge. Proceed immediately to the next step.
6. Transfer up to 700 ul of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2ml collection tube. Centrifuge for 15 s at >8000 g. Discard the flow-through.
7. Add 700 ul Buffer RW1 to the RNeasy spin column. Centrifuge for 15 s at >8000 g. Discard the flow-through.
8. Add 500 ul Buffer RPE to the RNeasy spin column. Centrifuge for 15 s at >8000 g. Discard the flow-through
9. Add another 500 ul Buffer RPE to the RNeasy spin column. Centrifuge for 2 min at 8000 g.
10. Pace RNeasy spin column in a new low-bind tube (or supplied 1.5 ml collection tube). Add 30-50 ul RNase-free water directly to the spin column membrane. Centrifuge for 1 min at >8000g to elute the RNA
11. If the expected RNA yield is >30 ug, repeat step 10 with the same eluate and tube
12. Qubit RNA concentration and run Bioanalyzer.

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