**Zymo Direct-zol RNA MiniPrep (R2051, R2053) Extraction Protocol**

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Updated: 01.13.20 M. Studivan

* All centrifugation steps are performed at **room temperature** and **16,000 x *g***, unless specified.
* Prior to first use, DNase I should be reconstituted per instructions on tube and stored as frozen aliquots to minimize freeze/thaw cycles.
* Prior to first use, add ethanol to buffer concentrates per instructions on bottles.

1. Prepare DNase prior to extraction. Add 5 µL DNase I and 5 µL DNase Digestion Buffer per sample (e.g. for 24 samples use 120 µL DNase I and 120 µL Digestion Buffer) to an RNase-free tube and mix thoroughly by gentle inversion. **Keep mixture on ice.**
2. Heat DNase/RNase-Free Water tube on a heat block at 55°C.
3. Scrape tissue from coral fragment and place into a 2 mL SafeLock tube with 0.1 mL (~ 0.075 g) of 0.5 mm glass beads.
4. Add 800 µL TRIzol.
5. Bead beat for 3 min (6 m/s, three 60 s intervals w/ 2 min cool down between).
6. Centrifuge lysate for 2 min at 20,000 x *g* to pellet beads and debris. Transfer supernatant (500 µL) to a 1.5 mL tube.
   1. Supernatant should be transparent, not murky or viscous. If not, increase TRIzol until solution clears.
7. Add equal volume of ethanol (500 µL) and mix thoroughly. Transfer to Zymo-Spin Column in a collection tube and centrifuge for 30 s. Transfer the column into a **new** collection tube.
8. Add 400 µL Direct-zol RNA PreWash to the column and centrifuge 30 s. Discard the flow-through and **repeat this step and discard flow-through**.
9. Add 700 μl RNA Wash Buffer to the column and centrifuge for 2 min to completely remove wash buffer. Transfer the column carefully into a **new** tube. Avoid contact of flow through and spin column.
10. To elute RNA, add 40 μL of heated DNase/RNase-Free Water directly to the column, incubate for 1–2 min, and centrifuge for 1 min. Repeat elution with the same aliquot.
11. Add 10 μL of prepared DNase I reaction mix to tube and mix gently by inversion.
12. Incubate at room temperature for 20 min, then proceed directly to RNA purification with Zymo RNA Clean & Concentrator-5 kit.