

Nucleic Acid Extraction Protocol

Optimized for *Aurelia* jellyfish, successfully used on various plants and animals

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- 1. Create working L1 solution by adding 10 μ l of β -mercaptoethanol to 990 μ l of L1 stock.
- 2. Add 200 μ l L1 solution to tissue. Homogenize sample.
 - *Good homogenization of the tissue is critically important. I recommend using an electric pestle.*
- 3. Add 2.5 μ l Proteinase K (20 mg/mL), vortex, and incubate 10 min @ 55°C
 - *Occasionally vortex samples during incubation, or use a shaking incubator.*
 - **Optional:** *To protect RNA, add 1 μ l of RNase inhibitor along with Proteinase K*
 - **Optional:** *To isolate DNA only, add 1 μ l of RNase A along with Proteinase K*
- 4. Proceed to Trizol / TRI Reagent protocol or Phenol-chloroform protocol:

Trizol / TRI Reagent protocol (for RNA)

- 5A. Add 1 mL of Trizol or TRI Reagent, and let stand for 5 minutes at room temperature (RT)
- 6A. Add 0.2 mL of chloroform, shake vigorously for 15 seconds, and let stand for 2–15 minutes at RT

Phenol-chloroform protocol (for RNA and DNA)

- 5B. Put on ice, add 11 μ l 0.2M Sodium Acetate (pH \approx 4), and 250 μ l phenol:chloroform:isoamyl alcohol (25:24:1, pH \approx 7.5).
- 6B. Vortex for 10 seconds and keep on ice for 15 min.

- 6. Centrifuge for 15 min at 12,000 g at 4°C.
- 7. Put upper phase in new tube, and add 1 volume isopropanol and 1 μ l GlycoBlue. Mix well and let stand for 10 minutes at room temperature, or freeze at -80°C overnight.
- 8. Centrifuge for 15 min at 12,000 g at 4°C.
- 9. Wash pellet in 1 ml of 70% ethanol, mix by vortexing.
- 10. Centrifuge for 5 min at 4°C at 7,500 g.
 - *For long-term storage, add 1mL of ethanol to the pellet, and store at -80°C.*
- 11. Remove the supernatant and air-dry the pellet in a fume hood for approx. 10 min.
- 13. Resuspend pellet in 20-40 μ l of water or preferred buffer.

L1 Solution

	<u>Stock</u>	<u>Final Concentration</u>	<u>50mL</u>
Tris/HCL (pH \approx 5.5)	1 M	100 mM	5 mL
EDTA (pH \approx 8)	0.5 M	10 mM	1 mL
NACl	dry	0.1 M	292 mg
SDS	dry	1%	500 mg