

case the RNA looks undegraded according to their electrophoretic pattern, but cloning fails despite a control RNA yielding good results under the same protocol, the following procedure might help. Precipitate RNA with ethanol, pellet the RNA, and, after drying in a vacuum concentrator, redissolve the RNA in sterile cacodylate buffer (0.02M cacodylic acid, 0.002M sodium-EDTA, pH adjusted to 7.2 with 1N NaOH) at a concentration of 1 mg/mL. Add, per mL of RNA, 0.2 mL cacodylate buffer, 80 μ L mercaptoethanol, and 0.2 mL of a sterile 20% SDS solution in water. Mix well and incubate for 1–2 min at 60°C in a water bath. Thereafter, add 4 mL of TE-phenol and incubate, with occasional shaking, for 10 min in an ice bath. Break the emulsion by centrifugation in a low-speed centrifuge with swingout rotor at 8000g for 15 min at 20°C, remove the aqueous phase, and precipitate the RNA as described in **Subheading 3.7**. Two wash steps with 70% ethanol should be performed to remove phenol residues. **Caution:** Be careful with cacodylate buffer; it is toxic.

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