Medina Lab, Penn State University

DNA extraction from frozen coral sperm protocol (modified from Sambrook and Russell)

Solutions

Lysis Buffer (prepare 50 mL):

- 10 mM Tris-Cl (pH=8.0)

- 0.1 M EDTA (pH=8.0)

- 0.5% (w/v) SDS

Lysis Buffer Example (50 mL)

Tris-Cl (pH=8) stock 1 M:	*500 µL*
EDTA (pH=8) stock 0.5 M:	*10 mL*
SDS stock 10%:	*2.5 mL*
Nuclease free water:	*to 50 mL*

DNAse free RNAse (stock: 10 mg/mL)

Proteinase K (stock: 20mg/mL)

Ammonium acetate 7.5 M

EtOH (100%)

Phenol (buffered at pH=8.0)

TE (pH=8.0)

Materials

Frozen coral sperm sample

Mortar and pestle

Liquid nitrogen

Protocol

0. Before start, prepare 10 mL Lysis Buffer with RNAse

Example: to prepare 10 mL Lysis Buffer + RNAse

add 20 µL RNAse (stock: 10 mg/mL; final 20 mg*/mL) to 10 mL Lysis Buffer*

1. Pulverize coral sperm

- snap-freeze approx. 1 g of coral sperm into stainless-steel mortar filled with liquid N_2

- grind to fine powder

- allow liquid N_2 to evaporate, then add powder (little by litte) to 10 vol of lysis buffer (with RNAse) in a 50 mL Falcon tube

- shake the tube to allow powder to submerge

- when all powder is in solution, incubate tube for 1 h at 37°C

2. Gently add proteinase K to final concentration of 100 µg/mL

3. Incubate at 50°C overnight (or at least 3-4 h)

4a. Cool solution to room temperature, add equal volume of phenol (pH=8.0).

4b. Gently mix for 10 min (or until solution has formed an emulsion)

5. Spin at 5000 g (6500 rpm in Sorvall SS-34 rotor) at room temperature for 15 min

6. Use wide-bore pipette (3 mm diameter) to transfer viscous aqueous phase to fresh centrifuge tube

7. Re-extract aqueous phase with 1 vol. phenol until solution becomes clear (4-5 times) (Note: may need to precipitate protein and DNA clots by spinning without addition of phenol.)

8. Isolate DNA

- add 0.2666 volumes of 7.5 M ammonium acetate and 2 vol of EtOH (100%), and swirl tube

- remove precipitate using a Shepherd's crook

- wash pellet twice with 70% EtOH

- air-dry and resuspend in 1 mL TE (for 24 to 48 hours)