RNA Isolation

Danna Eickbush

Special Cautions:

Steps 3 and 6

Phenol (corrosive, toxic), chloroform/sevag (anesthetic, toxic)- work in hood, wear proper PPE, dispose waste in proper hazardous waste containers.

Step 8

Ethidium bromide (mutagen)- wear gloves, dispose waste in proper hazardous waste containers. UV radiation (skin burns, eye damage)- cover all exposed skin, wear eye goggles.

1. 25 females or 40 males.

2. Grind flies in 200 ul Buffer A.
   Add 200 ul Buffer B and 10 ul proteinase K (10 mg/ml).

   Incubate 1.5 hours at 37^0C. Add another 10 ul of proteinase K and continue incubation for another 1.5 hours.

3. Phenol/sevag extract (400 ul) 2X.
   Sevag extract (400 ul) 1X.

   Add 40 ul 3M NaAcetate and 900 ul cold 95% ethanol.
   Gently mix. Place at -20^0C for 25 minutes. Centrifuge 30 minutes, 13K in coldroom.

4. Dump off ethanol, rinse with 70% ethanol, quick spin, pipette off excess ethanol. Air dry for a few minutes. DO NOT OVER DRY.

5. Resuspend in 200 ul DNase I buffer (40 mM Tris 7.9, 5 mM MgCl2, 5 mM CaCl2).
   Add 20 units of DNase I (Ambion). Incubate at 37^0C for 40 minutes.

6. Phenol/sevag extract (200 ul) 2X.
   Sevag extract (200 ul) 1X.

   Add 20 ul 3M NaAcetate, 600 ul ethanol. -20^0C overnight. Centrifuge 25 minutes in cold. Rinse pellet with 70% ethanol, quick spin, pipette off excess ethanol, air dry a few minutes.

7. Resuspend 25 ul water.

8. Determine concentration, check integrity on agarose/ethidium bromide gel. The yield is usually between 75 and 100 ug total RNA.

Buffer A

- 10 mM Tris 8.0
- 60 mM NaCl
- 10 mM EDTA
- 0.15 mM spermidine
- 0.15 mM spermine
- 5% sucrose

Buffer B

- 200 mM Tris 9.0
- 30 mM EDTA
- 2% SDS
- 5% sucrose