RNA Extraction from Embryos, Larvae and Flies

Using Amersham Biosciences "QuickPrep Micro mRNA Purification Kit"

Notes: - spinning times stated are started from the time the centrifuge reaches top speed!
(e.g. not from the time the centrifuge is started!)
- it is more convenient to do two extractions in parallel

Preparation (per prep):

- Fill container with liquid nitrogen.
- Pipette 250 □ elution buffer into a 1.5 ml eppendorf tube and heat at 65 °C until use.
- Pipette 1.2 ml elution buffer into a 1.5 ml eppendorf tube and set aside
- Pipette 600 □l extraction buffer into a 2 ml eppendorf tube and set aside.
- Pipette 1 ml of dT-cellulose solution into a 1.5 ml eppendorf tube
  (be sure to swirl the dT-cellulose tube prior to pipetting, to resuspend beads) and set aside.

Protocol (per prep):

1. Collect larvae:
   e.g. add water to fly bottle and pour larvae suspension onto mesh or screen, keep amount of food collected to a minimum, and wash away food with tap water, larvae can then be transferred to the mortar on ice (try to get 2 ml larvae).
2. Collect embryos by bleaching overnight embryo collection plates for 2 min, then pouring the embryo/bleach mixture onto a sieve. Wash embryos and transfer to the mortar on ice.
3. Collect 1 ml of adult flies into the mortar on ice.
4. CAREFULLY and SLOWLY add liquid nitrogen to the mortar.
5. Grind the mixture with the pestle.
6. Repeat steps 4 and 5 until mixture resembles a fine homogenous powder.

7. Transfer powder using a small spatula to the prepared eppendorf with 600 □ extraction buffer (to add up to 1 ml final volume).
8. Vortex thoroughly for 20 sec and add 1.2 ml room temperature elution buffer.
9. Vortex gently and spin for 1 min at top speed (e.g. 13.000 rpm). (Spin prepared eppendorf tube containing dT-cellulose solution at the same time as well).
10. Carefully remove the supernatant from the dT-cellulose beads.
11. Transfer the supernatant (extraction buffer plus RNA) from the 2 ml eppendorf to the eppendorf containing the dT-cellulose beads pellet.
12. Resuspend dT-cellulose pellet and keep it in suspension by inverting the tube for 3 min (give RNA enough time to bind to dT-cellulose).
13. Spin this mixture at top speed for 10 seconds (from the time top speed is reached).
14. Remove supernatant.
15. High Salt Buffer Washes (to be done 5 times!): add 1 ml High Salt Buffer to pellet, invert tube until pellet is resuspended, spin 10 seconds, remove supernatant.
16. Low Salt Buffer Washes (to be done 2 times!): add 1 ml Low Salt Buffer to pellet, invert tube until pellet is resuspended, spin 10 seconds, remove supernatant.
17. Snap the cap off the bottom of a spin column and place it into an autoclaved 2 ml eppendorf with the lid removed.
18. Add 300 □l of Low Salt buffer to the washed pellet, resuspend the slurry and transfer it to the spin column prepared in step 17. (use a blue tip)
19. Centrifuge the column using the 2 ml eppendorf as a collection tube, at high speed for 5 seconds.
20. Discard the flow through.
21. Add 500 □l Low Salt Buffer to the column, careful not to disturb the cellulose beads, and spin through at high speed for 5 seconds.
22. Discard the flow through and repeat step 21 one time.
23. Place the column into a clean screw-cap tube, and pipette 200 □ of the pre-warmed elution buffer into the column. Elute RNA to the screw-cap tube by centrifugation at top speed for 5 seconds.
24. Close the screw-cap tube containing the RNA and store it at -20 degrees Celsius (not for too long! some days should be fine) until use.
25. Control gel (1%) load 5 □ RNA □ smear should be visible