Revised Protocol for FastTrack 2.0 mRNA Extraction from Human Cells

Preparation of Cells

1. Prepare or collect between $2x10^7$ cells for each mRNA prep (will yield about 10-20μg of mRNA). If PBMCs from a whole blood sample are to be used, the sample should be prepared with Ficoll-Paque according to the manufacturer's protocol. The isolated PBMCs can then be used in this protocol.
2. If cells are adherent, gently agitate and scrape cells from the surface of the flask with a cell scraper to suspend in the media. If cells are non-adherent, proceed to next step.
3. Place the suspension of cells in a 50ml conical tube, pellet at ~400xg for 5 minutes.
4. Discard the supernatant, leave the pellet intact.
5. Freeze the pellet in a dry ice/ethanol bath (or on liquid N2) until solid.
6. Store at -70ºC until ready for mRNA preparation.

Modified FastTrack 2.0 Protocol
Isolation of mRNA

1. Add 15ml of Lysis Buffer (15ml Stock Buffer + 300 μl RNase/Protein Degrader) to the frozen pellet.
2. The tissue homogenizer should be cleaned so that it is RNase-free. At a minimum will require immersion and homogenization in ddH_2O and ethanol 3 times sequentially, may require boiling H_2O, SDS, or 10% bleach in addition. An RNAse degrader such as RNAse Zap can also be used.
3. Shear each sample with a 3-5 second run of the tissue homogenizer, essentially until the cell pellet is no longer visible. Clean homogenizer tip between runs.
4. Incubate the sheared samples in Lysis Buffer at 45ºC for 45 minutes.
5. Add 950 μl 5M NaCl stock solution and mix.
6. Shear DNA using a 21 gauge needle attached to a sterile 20cc syringe. This step involves drawing the lysate into the syringe through the needle and expelling it from the syringe, again through the needle. This step should be repeated 3 times.
7. Add one tube of oligo(dT) cellulose to each sample.
8. Incubate for 2 minutes at room temperature. Vortex the sample to resuspend the cellulose completely.
9. Rock the tube gently in a horizontal position for 60-90 minutes at room temperature.
10. Centrifuge the oligo(dT) slurry at 3,000 x g for 5 minutes at room temperature. Make sure the brake on the centrifuge is set at low for all centrifugation steps, as a high brake may disturb the pellet.
11. Carefully aspirate and discard the supernatant, trying not to disturb the easily dispersed pellet.

Washing Oligo(dT) Cellulose

1. Resuspend oligo(dT) in 20ml Binding Buffer by vortexing.
2. Centrifuge at 3,000 x g for 5 minutes at room temperature.
3. Resuspend oligo(dT) in 10ml Binding Buffer by vortexing.

4. Centrifuge at 3,000 x g for 5 minutes at room temperature.
5. Resuspend oligo(dT) in 10ml Low Salt Wash Buffer by vortexing.
6. Centrifuge at 3,000 x g for 5 minutes at room temperature.
7. Repeat Low Salt Wash Buffer wash two more times.
8. Resuspend oligo(dT) in 800µl Low Salt Wash Buffer using a 1-2ml serological pipette.
9. Transfer about 800µl of the oligo(dT) slurry to a spin column seated in the included microcentrifuge tube.
10. Centrifuge at 5,000 x g for 10 seconds at room temperature.
11. Aspirate and discard the flow-through liquid in the microcentrifuge tube.
12. Repeat the transfer and centrifugation steps until ALL of the oligo(dT) cellulose is in the spin column.

Elution and Precipitation of the mRNA

1. Place the spin column containing the oligo(dT) into one of the included clean microcentrifuge tubes.
2. Heat elution buffer to 65°C in a bath (or about 10-15 seconds in a microwave).
3. Resuspend the oligo(dT) in 200µl Elution Buffer (heated), using the pipette tip to gently swirl the cellulose, without puncturing the underlying spin column membrane.
4. Centrifuge at 5,000 x g for 30 seconds at room temperature. Do not decant. Keep the eluent, it contains your mRNA.
5. Again resuspend the oligo(dT) in 200µl Elution Buffer (heated).
6. Centrifuge at 5,000 x g for 30 seconds at room temperature.
7. Save the combined 400µl eluent in the microcentrifuge tube.
8. Add 60µl of 2M sodium acetate and mix.
9. Add 1ml of 95% ethanol (Do not use 100% ethanol, it contains fluorescent contaminants).
10. Freeze at -70°C overnight.
11. Thaw and centrifuge at 16,000 x g for 15 minutes at 4°C.
12. Carefully aspirate all the ethanol from the mRNA pellet, taking care not to disturb the pellet.
13. Resuspend the mRNA pellet in 20µl of Elution Buffer (heated as before).
14. Determine the concentration of the mRNA by OD or "eyeballing" on an agarose minigel.
15. Store mRNA at -70°C.

The standard FastTrack protocol can be found on the Invitrogen website.

ceb 4/21/99
revised jcb 7/13/99