Fast-Trackprotocol for DLCL tissue samples

I. Homogenization (Day 1)

Set water bath to 45 C.

1. Prepare tissue homogenizer. Clean generator with RNAseAway,then rinse with DDW, EtOH, and DDW. Dry with a Kimwipe.
2. Check Stock Buffer for SDS ppt; heat or microwave to dissolve.Add 300 µ L RNAse/Protein Degrader to each 15 mL of Stock Buffer(2 mL per 100 mL). For each sample, place 15+ mL into a 50 mLconical tube.
3. Process frozen samples one at a time. Divide the frozen sampleinto pieces <5 mm across, using a razor blade and minimal thawing,and drop into Lysis Buffer.
4. Homogenize sample. Hold tube so that generator tip is justabove sample pieces at bottom of tube. Start rotation, and allowvortex to draw pieces into generator tip. Vortex until completelyhomogenized, although there may be some fragments of fascia. Minimizebubble formation.
5. Set homogenized samples aside until all are processed. Cleangenerator tip between samples by rinsing sequentially with DDW,100% EtOH, and DDW for a final time. Dry with a Kimwipe.

II. mRNA isolation (Day 1 continued)

1. Place homogenates in 45 C. water bath for 30-45 min. If insolublematerial persists, centrifuge at 4,000 x g for 5 min at RT andtransfer the supernatant to a new tube.
2. Adjust NaCl concentration to 0.5 M by adding 950 µ L of5 M NaCl to each 15 mL lysate. Mix by vortexing.
3. Shear remaining DNA in lysate 3-4X with an 18-21 Ga needle.If lysate is still too viscous, add 15 mL more Lysis Buffer mixand treat as 2 samples.
4. To each 15 mL lysate, add a vial of oligo(dT) cellulose. Mixby gentle vortexing. Oligo(dT) cellulose is hygroscopic, and shouldbe stored at -20 C.
5. Rock lysates for 1.5 hr-ON at RT.

III. Washing oligo(dT) cellulose (Day 2)

Warm Elution Buffer to 55 C, in preparation for IV below.

1. Pellet the oligo(dT) cellulose at 3,000 x g for 5 min at RT.Transfer the supernatants carefully to new tubes for DNA recovery(see V below; convenient to do during steps 2-5 below).
   Note: During steps 1-5, oligo(dT) cellulose will become increasingly better packed after spinning (as well as easier to resuspend). Therefore, the removal of supernatants should be gentleat first, so as to avoid losing oligo(dT) cellulose, but can becomemore complete at later steps.
2. Gently resuspend each pellet in 20 mL of Binding Buffer. Pelletthe oligo(dT) cellulose at 3,000 x g for 5 min at RT. Discardsupernatants.
3. Repeat step 2, using 10 mL of Binding Buffer.
4. Repeat step 2, using 10 mL of Low Salt Wash Buffer.
5. Repeat step 4 for 2-4X, until supernatant is free of bubbles.
6. Place 50 mL tubes into a rack or holder. Place spincolumns into labeled Eppendorf tubes and place tubes in a microfuge,with the lids open.
7. Transfer cellulose to spin columns in the following manner,paying careful attention to detail. Add 400 µ L of Low SaltWash Buffer to each pellet-containing 50 mL tube. Resuspend pelletinto a slurry by tilting and swirling the tube with one hand,and at the same time aspirate the slurry with a 2 mL pipet. Youmay need to repeat, or pipet up and down, to minimize leavingcellulose in the tube or pipet. Gently express the slurry,

whichmay contain bubbles, into the top of a spin-column in a microfugetube. Don't worry if you have not transferred all cellulose,or there is a plug left in the pipet, but save the pipet and the50 mL tube! Place each pipet back in the same 50 mL tube for step9. If it appears that there is too much slurry to close the Eppendorftube lid, try to disrupt bubbles and/or let the slurry settle;you can then close the tube lid, and any liquid that spills willcontain little suspended cellulose.

8. Spin Eppendorf tubes at 7500 rpm for 30 sec at RT. Remove spin-column,discard eluate from the tube, and restore spin-column to the tubeand the tube to the microfuge.
9. Add 600 μ L of Low Salt Wash Buffer to the 50 mL tube. Usingthe same pipet and swirling, draw up dilute slurry and mobilizeany cellulose left in the pipet. Express slurry to top of spin-column.Spin again.
10. Optional: Discard eluate, and wash cellulose once more with500 μ L Low Salt Wash Buffer.

IV. Elution and precipitation of the mRNA

Day 2, continued

1. Transfer spin-columns to new microfuge tubes in kit. Discardold tubes and eluate. With a yellow pipet tip, add 200 μL of 55 C Elution Buffer to tops of spin-columns. Use the pipettip as a mixer to ensure even distribution of Elution Buffer overthe surface of the cellulose.
2. Centrifuge for 30 sec to elute mRNA, and leave eluate in themicrofuge tube. DO NOT DISCARD ELUATE! Add a second 200μ L of 55 C Elution Buffer to tops of spin-columns, mix withcellulose, and spin again.
3. Discard the spin-column. To the eluate (400 μ L), add 0.15volume (60 μ L) of 2 M NaOAc and 2.5 volumes (1 mL) of 100%EtOH. Mix and place at -80 C. and leave ON or longer.

Day 3

4. Thaw eluates and microfuge at maximum speed for 30 min at 4C.
5. Remove supernatant by careful aspiration. Airdry briefly (5-10min) at RT. Resuspend pellet (sometimes visible, but not always)in 20-50 μ L of Elution Buffer (10 mM Tris, pH 7.5). For now,use 22 μ L and aliquot as follows:
   - 2 aliquots of 10 μ L each, for chip analysis.
   - 1 μ L for later SMART amplification.
   - 1 μ L for immediate gel analysis. Run on a 1% TAE/EtBr gel,with 1 Kb ladder. Also use Jurkat mRNA from Ash, 1 μ L as isand diluted 1:10. Photograph with IP Lab program.

V. DNA recovery from Fast-Track lysate (approximately 15mL)

1. Add 3.5 mL of saturated NaCl. Shake vigorously for 30 sec,then spin at 2500 x g for 5 min at RT to precipitate proteins.
2. Transfer supernatant to another 50 mL tube, and discard pellet.Will probably need to spin and transfer again, to avoid transferof some particulate. When supernatant is clear, measure the volume.Add 2 volumes of 100% EtOH, and record whether a visible spoolof DNA is formed. Leave at -20 C. ON to precipitate further. Canstore in EtOH -20 C indefinitely.
3. Spin at 2500 x g for 10 min at 4 C to precipitate DNA. Washcarefully with 70% EtOH, then airdry briefly.
4. Resuspend in a minimum volume of TE, e.g. 1 mL. If needed,rock centripetally at 4 C. Store at -20 C.