RNA extraction technique from tissue

Prepared by Ms Alex Aitken

You will need:
- Trizol reagent
- Chloroform
- Acid phenol/chloroform (ph 4.7)
- Propan-2-ol
- 75% EtOH (DEPC treated)
- DEPC treated water
- Baked mortar and pestle/ glass homogenisers/ implements for scraping
- RNasin
- DNase I (Turbo from Ambion)
- RNase free tips and microcentrifuge tubes

1) Grind tissue to fine powder with mortar and pestle under liquid nitrogen.
2) Transfer to glass homogeniser with 1ml trizol already added
3) Weigh tissue sample and adjust amount of trizol accordingly. DO NOT exceed more than 100mg tissue/ml trizol
4) Homogenise trizol and tissue slurry and transfer 1ml to micro-centrifuge tubes

(Trizol/tissue slurry can be stored at -80°C indefinitely.)

5) Spin 1ml trizol/tissue slurry 12 000 x g 10 min
6) Remove supernatant to fresh tube avoiding pelleted debris
7) Add 200ul of chloroform to supernatant, shake vigorously 30 seconds, and stand for 2-3 min
8) Spin 12 000 x g 15 min
9) Take aqueous phase (clear) avoiding interface and remove to fresh tube
10) Add 550ul of acid phenol chloroform (pH 4.7), shake vigorously (or vortex) 1 min.
11) Spin 12 000 x g 5 min
12) Take aqueous phase into fresh tube, and add 500ul of propan-2-ol. Invert to mix and let stand 10min
13) Spin 12 000 x g 10min
14) Remove supernatant, leaving RNA pellet intact
15) Add 1ml of 75% ethanol (DEPC treated) (this stage can be stopped here and the pellet/ethanol mix stored -20°C indefinitely). Dislodge pellet from side of tube.
16) Spin max speed 5 min. Remove ethanol, spin briefly to bring residual ethanol to bottom of tube, and remove as well.
17) Air dry 5 min… DO NOT OVER DRY as the pellet becomes difficult to dissolve.
18) Resuspend in 84ul of DEPC treated water. Heat pellet to 55°C to resuspend.
19) Add 1ul RNasin, 10ul of 10x DNase buffer and 5ul of turbo DNase. Incubate at 37°C for 40min, and add another 10ul of DNase (I use Roche for this second step). Incubate for another 30min and add 100ul of DEPC water.
20) Add 200ul of acid phenol chloroform, shake/vortex vigorously for 1-2min and centrifuge max speed 5 min.
21) Take aqueous phase making sure not to take any interface, and add $1/10^{th}$ volume of 5M NH_4Oac (20ul) and 2.5 volumes of absolute ethanol (500ul).
22) Store -20°C for 1hour (minimum), and spin down max speed 10min.
23) Remove ethanol and add 1ml of 75% ethanol (DEPC treated). Spin max speed 5 min. Remove ethanol, spin briefly to bring residual ethanol to bottom of tube, and remove as well.

24) Air dry 5 min… DO NOT OVER DRY as the pellet becomes difficult to dissolve.
25) Resuspend in around 50ul (depends on pellet size) of DEPC treated water. Heat pellet to 55°C to resuspend.
26) GAPDH PCR to confirm no DNA
27) 260/280 reading to quantitate amount of RNA.

Kerri's foolproof RNA extraction technique from cells

You will need:
Trizol reagent
Chloroform
Acid phenol/chloroform (ph 4.7)
Propan-2-ol
75% EtOH (DEPC treated)
DEPC treated water
Baked mortar and pestle/ glass homogenisers/ implements for scraping
RNasin
DNase I (Turbo from Ambion)
RNase free tips and microcentrifuge tubes

1) Attached cells are removed from the vessel by trypsinising. The trypsinised cells are pelleted and the supernatant removed, and 1ml of trizol added. The cells are lysed by pipetting repeatedly in trizol.
2) Add 200ul of chloroform to trizol, shake vigorously 30 seconds, and stand for 2-3 min
3) Spin 12 000 x g 15 min
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