1. RNA isolation from low cell numbers using RNAzol RT

For cells FACS-sorted in Lonza freeze media (10 ul, Lonza Cat#12-769E, to use only with 100 cells or less) or PBS containing RNAse inhibitor:

- Spin down the cells (2500 rpm, 4 degrees, 5 minutes) in siliconized tubes
- Remove most of the supernatant without disturbing cell pellet.
- Wash once with DEPC treated/nuclease free PBS and repeat the centrifugation
- Remove supernatant leaving no more than 100ul (400ul aqueous/ 1ml RNAzol)
- Add 250ul of room temp RNAzol reagent (1ml per 10^7 cells)
    - Stopping point Samples can be frozen in -80 once lysed.
- *Add 100-200ul of nuclease free H20* (400-800ul aqueous/ 1ml RNAzol)
- Vortex vigorously and store for 5-15min @ RT **Should be Cloudy blue!!! Not at all clear**
- Centrifuge samples at 12,000g for 15min (between 4-28C
- Carefully save up to 85% or 300-400ul of the aqueous supernatant making sure not to disturb the 2mm pellet of phenol/interphase at bottom. **Should discard dark blue protein/DNA pellet**
    - If no blue pellet add more h20 vortex till cloudy and re-spin
- **Add 1 ul LPA (GeneElute LPA, Sigma, Cat#56575) to collected supernatant and mix well**
- Precipitate using an equal volume (~500 ul) of cold isopropanol. Precipitate at -20 30min.
    - Stopping point: Samples can be left at -20 O/N or O/weekend
- Spin at >12000xg (max speed of 20,000-30,000g for best yield) for 15-30min @4C
- Remove sup (The LPA addition makes pellet less sticky, Careful!)
- Wash with clean 70% ethanol (use DEPC-treated or nuclease free water).
- Spin at max speed for 4min @4C
- Remove as much Ethanol as possible
- Repeat 70% EtOH wash and spin and remove Ethanol
- Air dry for at least 5min. You should see the edges of the RNA pellet begin to go clear
- Resuspend in 13-30ul nuclease free water with 1:20 Superase In. If possible use enough water to approximate 200-500ng/ul.

Samples can be stored at -80 or taken directly to QC or cDNA librarying.

notes: This protocol can also be used with Trizol/chloroform isolation if preferred.

Well homogenized and flash frozen samples can be stored in the lysis for years.

DEPC wash, longer incubation times, and longer/faster spins are for lower yield samples to ensure complete recovery of samples under 100ng.

LPA only needs to be added to samples that are thought to have less than 250ng total yield.

http://www.genecopoeia.com/wp-content/uploads/2013/06/RNAzol_RT_RNA_Isolation_Reagent_User_Manual.pdf

http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/1/r4533bul.pdf

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