Fractionation of Nuclear and Cytoplasmic RNA

To COS-M6 cells grow in 6-well plates, 2ml media total
To CV1PD cells grow in 100mm plates.

1: Aspirate off the media from the cells and was the cell monolayers with 2x5ml (To 100mm dish) or 2x1ml (to 6-well plates) ice cold TD ($4^{o}C$ is OK). Be very gentle when washing the cells as transfected cells tend to come off the dish easily. You had better transfect 9 dishes or 18 wells at a same time, so that you will have 18 Eppendorff tubes that can be spun down in one centrifuge.

2: Add 1ml ice cold TD to the well. To COS-M6 cells, they can be washed off simply by pipetting 20 times per well. To CV1-PD cells, they should be scraped by rubber policeman. Handle the rubber policeman at the first 1/3 so that you will not spill the solution. Transfer the cells to a 1.5ml Eppendorff tube. Put each Eppendorff tube on ice until donw with all samples.

3: Spin cells at RT for 30sec at maxi speed

<u>From now on, keep tubes on ice as much as possible</u>

4: Remove supernatant with aspirator. Resuspend the cells with 100ul ice cold TD by pipetting up and down 10 times. Add 100ul VRC/1%NP-40/TD. Vortex 5 second and put on ice.
VRC/1%NP-40/TD:

2.4 ml 1%NP-40/TD	125ul VRC
4.8 ml 1%NP-40/TD	250ul VRC

5: Keep on ice for 5 min, vortex 5 seconds again. Quick spin 30 seconds at maxi speed at RT.

<u>6: Transfer cytoplasmic fraction to a new tube, put nuclear samples on ice now.</u>

7: To cytoplasmic fractions, add 0.9ml 1.2xTRIzol reagent. Vortex to mix completely, sit at RT for 5 minutes.

8: Add 200ul chloroform:IAA 200ul per 1ml TRIzol. Vortex at scale 6 for 1 min.

9: $4^{o}C$ spin 20min, less than 12000 g. The RNA should exist in supernatant exclusively

10. Transfer supernatant 3x200ul = 600 ul to a new tube carefully. The total volume of the supernatant should around 650-700ul, no need to transfer them all.

11: Add 0.25ml isopropanol
0.25ml RNA precipitation solution

Mix by vortex and sit at RT for 10 min

12: 4°C spin 10min
13: Aspirate off supernatant, wash with 70% EtOH, spin 5min at RT

14: Dry without heat in speed vac for 5 to 10 min

15: Resuspend in 50ul TE completely.

To nuclear fractions:

16: Resuspend nuclear samples in 200 ul 0.5%NP-40/TD by pipetting 10 times. Vortex 5 seconds and put on ice for 5 minutes.

16: Spin 30 seconds at RT, maxi speed.

17: Aspirate off supernatant and resuspend samples in 200 ul 0.5%NP-40/TD by pipetting 10 times again.

18: Follow the steps from 7 to 15 again.

Solutions:

TD: 2L

NaCl	16.0g
KCl	0.76g
Na_2HPO_4	0.20g
Tris-Cl	6g

Water to 2 L, pH 7.4-7.5

1xTRIzol: 200ml

	Final		Add
Guanidine thiocyanate	0.8 M	FW 118.2	18.912 g
Ammonium thiocyanate	0.4 M	FW 76.12	6.09 g
Sodium Acetate	0.1 M, pH 5	Stock 1M	20 ml
Glycerol	5%		10ml

Add water to 100ml
Mix well
Filter

Add phenol 76 ml and mix. Phenol can be solved in 60°C water bath.
The final concentration of phenol is 38%. Add water to 200 ml total volume.
pH ~ 4.5.

Wrap with aluminum foil.

1.2xTRIzol: 200ml

	Final	Add
Guanidine thiocyanate	0.8 M x 1.2 = 0.96 M	22.694 g
Ammonium thiocyanate	0.4 M x 1.2 = 0.48 M	7.308 g
Sodium Acetate	0.1 M x 1.2 = 0.12 M, pH 5	Stock 1M 24 ml
Glycerol	5% x 1.2 = 6%	12ml

Add water to 100ml
Mix well
Filter

Add phenol 76 ml x 1.2 = 91.2 and mix. Phenol can be solved in 60^{o}C water bath.

The final concentration of phenol is 38% x 1.2 = 45.6%. Add water to 200 ml total volume.

pH ~ 4.5.

Wrap with aluminum foil.

RNA precipitation solution: 200 ml

NaCl	1.2 M	14.026 g	FW 58.44
Sodium Citrate	0.8M	47.056 g	FW 294.10

Autoclaved (can't be filtered through the membrane)