Collect transfection and RNA extraction

1. Aspirate media from the cells
2. Add 1ml of 1x PBS to wash the cells. Aspirate
3. Add 1ml of Trizol reagent to each well
4. Pipette the Trizol to rinse the well and collect into eppitube. Can freeze samples at this point or proceed
5. Add 200ul of chloroform to each sample.
6. Vortex each tube until it turns milky pink (~30sec)
7. Spin in 4C centrifuge at 12,000rpm for 15 minutes
8. Meanwhile, prepare collection tubes
9. Carefully pipette 500ul of the clear aqueous phase into collection tubes. Do not pick up the white precipitate or the pink solution. Those contains DNA and protein
10. Add 500ul of isopropanol to precipitate the RNA. Mix by inverting. Can freeze samples at this point or proceed
11. Centrifuge in 4C centrifuge at 12,000rpm for 10 minutes
12. Carefully aspirate the liquid. Be very careful. The RNA pellet can get sucked up very easily. You do not need to aspirate absolutely all of the liquid. Can leave ~50ul
13. Add 500ul 70% ethanol to wash. Spin for 5 minutes. Aspirate
14. Add 500ul 70% ethanol to wash again. Spin for 5 minutes. Aspirate
15. Spin for 1 minute to collect any residual liquid to the bottom. Carefully use P200 pipette to remove as much liquid as possible without disturbing pellet
16. Let pellets air dry for 5 minutes. It will begin to turn from white to glassy/transparent
17. Add 25ul of water. Immediately place on ice. RNA is not stable at RT. Must be kept on ice at all times from now on

DNase I treatment and clean-up

1. Prepare a MM with the following reagents per well for a 50ul total reaction volume:
2. 5ul 10x Turbo Dnase buffer
3. 1ul Turbo Dnase I (add last)
4. 19ul NF water
5. Add all of the 25ul of the RNA into 8 strip reaction tubes
6. Add Dnase I to the MM and mix well by pipetting up and down several times
7. Add 25ul of the MM to the RNA and mix tubes by flicking them
8. Pulse briefly to collect liquid at the bottom of the tubes and run the DNase treatment program on the thermocycler for 1 hour at 37C
9. Once the run is finished, add 5ul of DNase Inactivation reagent to each reaction and mix well. Let sit for 5min. Flick 2-3 times during this time since the pellet will settle. This is meant to quench the DNase and other ions in solution
10. Spin down the samples in the centrifuge for 5min at 300rpm.
11. Transfer supernatant to another eppi, careful to not touch the white precipitate at the bottom
12. Clean samples with NEB Monarch RNA cleanup kit (I do this step, Yuma doesn’t. cDNA can be used for synthesis right after collecting the supernatant from step 8, but in my hands it doesn’t work as well so I do this additional clean-up step. Maybe you can skip this step if step 8 works well for you). Elute in 8ul NF water
13. Measure the RNA concentration. Be sure to select RNA setting on the nanodrop. Good RNA has 260/280 of 2.0 and 230/280 of greater than 2.0.
14. Continue with cDNA extraction or store RNA in -20C freezer.

cDNA synthesis

1. Mix:

2ug RNA

1ul reverse primer

Xul water to total v of 5ul

\*\*\* for 1 sample, add a no RT control to check for genomic contamination

1. In PCR machine, incubate at:

70C 5min

4C hold

1. Meanwhile, prepare master mix. Per sample contains:

6.6ul water

4ul 5x buffer

2.4ul 25mM MgCl2

1ul 10mM dNTP

1ul Improm II RT total v = 15ul

1. Add to samples
2. In PCR machine, incubate at:

25C 5min

42C 1hr

70C 15min

4C hold