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.
17. Add 50ul of water. Immediately place on ice. RNA is not stable at RT. Must be kept on ice at all times from now on.
18. 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.
19. Continue with cDNA extraction or store RNA in -20C freezer.