RNA extraction protocol
(Always wear lab coat and gloves when operate RNA samples)

1. Collect $5x10^7$ -$1x10^8$ cells
2. ice-cold PBS wash 2 times
3. resuspend cell pellet in 1ml TRIzol and vortex
4. transfer to 1.5 ml Eppendorf tube and sit on ice for 10 min
5. add 0.2 ml chloroform and vortex
6. sit on ice for 10 min
7. spin for 10 min at maximal speed at 4℃
8. transfer 700-800 ul of aqueous phase to an new Eppendorf tube
9. add 350-400 ul iso-propanol and mix well
10. store at -80℃ for further purification

FOR QIAGEN RNeasy mini kit purification

11. spin RNA samples at max speed for 30 min at 4℃
12. remove supernatant and wash pellet in 70% EtOH (DEPC H_2O) for 2 times
13. re-suspend pellet in 100 ul DEPC H_2O
14. QIAGEN Clean-up (100ml/column)
    (1) 100 ul sample (<100 ug RNA) + 350 ul RLT mix well
    (2) add 250ml (96-100％ EtOH). Mix well
    (3) Apply sample (~700 ul) to column, spin at max speed rpm for 15 second.
    (4) Add 350 ul RW1 into column, spin max speed rpm for 15s
    (5) mix 10 ul RNase free DNase I and 70 ul RDD
    (6) pipet 80 ul DNase I solution directly on the membrane, place at RT for 15 min
    (7) pipet 350 ul RW1 into column and spin 15 sec
    (8) change a new collect tube.
    (9) Add 500 ul RPE into column, spin for 15 sec
    (10) Add another 500 ul RPE, spin at max speed for 2 min
    (11) Transfer the column to a new 1.5 ml tube, add 35-50 DEPC H_2O onto membrane, wait for 5-10 min, spin at max sped for 1 min
    (12) If RNA yield is < 30 ug, repeat elution step .