RNA EXTRACTION

Sample Preparation:

1. Transfer collected cells into a 0.6ml tube, with minimal media. Get rid of excess media by briefly centrifuging the tube and then removing the media with mouth pipette (while looking at the tube under the microscope, to make sure not to take out any cells).

2. Tube can be stored at -80^{o}C until RNA extraction.

Extraction:

Kit used: ARCTURUS® PicoPure® RNA Isolation Kit from Life Technologies Applied Biosystems (ca. KIT0202, KIT0204)

Follow the kit protocol, briefly summarized below:

1. Add 50 µL of Extraction Buffer (XB) to tube containing cells. Incubate at 42 ^{o}C for 30 minutes.

2. 10 minutes before the end of that incubation, add 250 µL of Conditioning Buffer (CB) onto the column (placed inside the collection tubes provided in the kit). Incubate for 5 min at RT. Centrifuge at 13,000 rpm for 1 min.

3. At the end of the 30 min incubation, add 50 µL of 70% ethanol (EtOH) to the sample tubes and mix well by pipetting. Do not centrifuge.

4. Transfer contents of tube (≈100 µL) onto the column without wetting the rim. Centrifuge for 3 min at 3000 rpm. When the time on the centrifuge reaches 1 min, increase speed to 13000 rpm.

5. Add 100 µL of Wash Buffer 1 (W1) onto the column. Then centrifuge at 8000 rpm for 1 min.

6. (OPTIONAL) Remove DNA by DNase treatment: mix 5 µL of DNaseI for RT (Qiagen RNase-free DNase Kit, ca: 79254) and 35 µL of buffer RDD, pipette the 40 µL onto the column and incubate at RT for 15 min.

7. Add 100 µL of Wash Buffer 2 (W2). Then centrifuge at 8000 rpm for 1 min.

8. Add 100 µL of Wash Buffer 2 (W2). Then centrifuge at 13,000 rpm for 2 min.

9. Remove column from collection tube and place onto new 0.6 ml tube provided in the kit.

10. Add 10 µL (maximum 30 µL) of Elution Buffer (EB) directly onto the membrane of the column. Incubate for 1 min at RT.

11. Centrifuge at 2000 rpm for 1 min.

12. Centrifuge at 13,000 rpm for 1 min.

13. Store tube containing RNA at -80 ^{o}C until needed for cDNA synthesis.