**DNA Extraction Protocol 11/12/19**

**QIAamp DNA micro kit**

Materials:

* Ethanol (96-100%) \*\*NOT denatured alcohol
* 1.5mL microcentrifuge tubes
* Pipets and pipet tips
* Heat block (capable of settings of 56-70°C)
* Microcentrifuge
* Vortex

Preparations:

**Preparing carrier RNA**

1. Add 310µL Buffer AE to the tube containing 310µg lyophilized carrier RNA to obtain a stock solution of 1µg/µL.
2. Once dissolved, divide it into conveniently sized aliquots and store at -20°C (15µL aliquots).
3. Thaw when needed; but do not thaw one aliquot more than 3x.

**Preparing buffers**

*Buffer ATL:* Check for precipitate before using. If present, dissolve by heating to 70°C with gentle agitation.

*Buffer AL:* Check for precipitate before using. If present, dissolve by heating to 70°C with gentle agitation.

*Buffer AW1*: add 25mL ethanol (96-100%) to the bottle containing 19mL bugger AW1 concentrate. Tick the check box on the bottle to indicate that ethanol has been added. Buffer AW1 can be stored at room temp for up to a year. Reconstitute by shaking before procedure.

*Buffer AW2:* add 30mL ethanol (96-100%) to the bottle containing 13mL bugger AW2 concentrate. Tick the check box on the bottle to indicate that ethanol has been added. Buffer AW2 can be stored at room temp for up to a year. Reconstitute by shaking before procedure.

Steps:

1. Turn the heat block on and set to 56°C.
2. Pipet 1-100µL media containing the cells into a 1.5mL microcentrifuge tube.
3. *Add buffer ATL to a final volume of 100µL.*
4. Add 10µL proteinase K.
5. In a separate microcentrifuge tube, aliqout 100µL Buffer AL and add 1µL carrier RNA solution.
6. Add this resulting mixture to the tube containing cells, close the lid and vortex for 15 seconds.
7. Incubate at 56°C for 10 min. *\*\*if shaken during this period, DNA yields can be increased.*
8. Pulse-centrifuge the 1.5mL tube to remove drops from inside the lid.
9. Add 50uL ethanol (96-100%), close the lid, and mix thoroughly by pulse-vortexing for 15s. Incubate at RT for 3 min.
10. Briefly centrifuge the 1.5ml tube to remove drops from the inside of the lid.
11. Transfer the entire lysate from step 8 into the QIAamp MinElute column without wetting the rim. Close the lid, and centrifuge at 6,000 x g (8,000 RPM) for 1 min. Discard the collection tube containing the flow-through. Place within a clean 2mL microcentrifuge tube.
12. Open the QIAamp MinElute column and add 500µL Buffer AW2 without wetting the rim. Close the lid and centrifuge at 6,000 x g (8,000 RPM) for 1 min. Place the column in a clean 2mL collection tube and discard the collection tube containing flow-through.
13. Centrifuge at full speed (20,000 x g; 14,000 RPM) for 3 min to dry the membrane completely.
14. Place the QIAamp MinElute column in a clean 1.5mL microcentrifuge tube and discard the collection tube containing flow-through. Carefully open the lid of the QIAamp MinElute column and apply 20-100µL Buffer AE *or distilled water* to the center of the membrane.
15. Close the lid and incubate at room temperature for 5 min. Centrifuge at full speed (20,000 x g; 14,000 RPM) for 1 min. The resulting elution contains your DNA.

**NOTES:**