DNA Isolation from Human Peripheral Blood
(QIAamp Blood Maxi Kit – Spin Protocol #51194)

Note: This protocol assumes the investigator is beginning this with one full Yellow-Top (type A) BD Vacutainer of human blood (5-8.5 mL). This should yield between 100 and 300 uG of HMW gDNA.

Things to do before starting:

- Equilibrate samples to room temperature (from frozen: 90 min or can place @ 4ºC the night before).
- Prepare a 70ºC water bath. (30 min)
- Ensure that Buffer AW1, Buffer AW2 and Qiagen Protease have been prepared according to manufacturer's instructions.
- Thoroughly mix AL Buffer before use. If a precipitate has formed, re-dissolve by incubating at 56ºC

Procedure:

1) Label the 50 mL conical tubes that the frozen vacutainers are in, 1 through 20. Twenty samples is the maximum number of samples the tabletop centrifuge will hold.
2) Either note the study ID# for each sample on the log sheet or transfer the sticky label from the tube to the log sheet.
3) Estimate volume of blood by comparing the blood tube to a calibrated vacutainer tube; note the volume on the log sheet.
4) Transfer contents of tube into the same 50 mL polypropylene conical centrifuge tube that the frozen vacutainer was stored in.
5) Bring volume to **9.5 mL** with 1X PBS.
6) Add **500 uL** Qiagen Protease, and ensure proper mixing after adding the enzyme by inverting 3X.
7) Add **12 mL** Buffer AL, and mix thoroughly by inverting the tube 15X, followed by vigorous shaking for at least 1 min over the sink. (Invert multiple tubes simultaneously by clamping them into a rack using another empty rack, grasping both racks and inverting them together). (**Note:** Do not add Qiagen Protease directly to Buffer AL.)
8) Incubate at 70ºC for 10 min. (**Note:** longer incubation times will not adversely affect yield).
9) Add **10 mL** 100% ethanol to the sample, and mix by inverting 10X, followed by additional vigorous shaking over the sink.
10) Carefully decant half of the solution from step 7 onto the QIAamp Maxi column placed in a 50 mL centrifuge tube (provided), taking care not to moisten the rim. (**Note:** Do not over tighten caps; Always hold the closed column in an upright position as liquid may pass through the ventilation slots on the rims of the columns even if caps are secure.)
11) Centrifuge at 1850xg for 3 min. (**Note:** If the solution has not completely passed through the membrane, centrifuge again at a slightly higher speed).
12) Remove column, discard the filtrate into properly labeled hazardous waste container, and place the column back into the same 50 mL centrifuge tube. Decant the remainder of the

solution from step 7 onto the column. Close cap and centrifuge again at 1850xg for 3 min. (**Note:** Wipe off any spillage from the thread of the 50 mL tube before re-inserting the column to ensure the rim of the column does not get wet; Again, if the solution has not completely passed through the membrane, centrifuge again at a slightly higher speed).

13) Remove column, discard filtrate into properly labeled hazardous waste container, and place the column back into the same 50 mL centrifuge tube. (**Note:** Wipe off any spillage from the thread of the 50 mL tube before re-inserting the column to ensure the rim of the column does not get wet).
14) Carefully, without moistening the rim, add **5 mL** Buffer AW1 to the column and centrifuge 4000 rpm (~3500xg) for 2 min. Do not discard flow through.
15) Carefully, without moistening the rim, add **5 mL** Buffer AW2 to the column and centrifuge 4000 rpm (~3500xg) for 30 min.
16) Place the column in a clean 50 mL tube (provided), and pour the filtrate into properly labeled hazardous waste container and discard tube into biohazard bag. (**Note:** Wipe any spillage off the column before inserting into tube).
17) Pipet **1.2 mL** Buffer AE directly onto the membrane of the column and close cap. Incubate at room temperature for 5 min, and centrifuge at 4000 rpm (~3500xg) for 4 min.
18) **Reload the eluate containing the DNA onto the membrane of the column. Close the cap and incubate at room temperature for 5 min, and centrifuge at 4000 rpm (~3500xg) for 10 min.** (**Note:** less than 1.2 mL will be eluted from the column, but this has no effect on DNA yield).
19) Keep samples on ice or at 4ºC for further analysis (Quantification and / or QC if necessary).

Other Reagents Needed

100% Ethanol
1X PBS