

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 tube of human blood (5-10 ml). This should yield between ____ and ____ ug of high quality genomic DNA.

Things to do before starting:

- Equilibrate samples to room temperature before starting. (from frozen: 90 min)
- Prepare a 70°C 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, redissolve by incubating at 56°C

Procedure:

- 1) Transfer contents of tube into a 50 ml polypropylene conical centrifuge tube (not provided; note volume).
- 2) Bring volume to 10 ml with PBS.
- 3) Add 500 uL Qiagen Protease, and ensure proper mixing after adding the enzyme by inverting 3X.
- 4) Add 12 mL Buffer AL, and mix thoroughly by inverting the tube 15X, followed by vigorous shaking for at least 1 min. (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.)
- 5) Incubate at 70°C for 10 min. (**Note:** longer incubation times will not adversely affect yield).
- 6) Add 10 mL 100% ethanol to the sample, and mix by inverting 10X, followed by additional vigorous shaking.
- 7) Carefully transfer half of the solution from step 6 onto the QIAamp Maxi column placed in a 50 mL centrifuge tube (provided), taking care not to moisten the rim. (**Note:** Do not overtighten 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.)
- 8) Centrifuge at 1850xg for 3 min. (**Note:** If the solution has not completely passed through the membrane, centrifuge again at a slightly higher speed).
- 9) Remove column, discard the filtrate into hazardous waste container, and place the column back into the 50 mL centrifuge tube. Load the remainder of the solution from step 5 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).
- 10) Remove column, discard filtrate into hazardous waste container, and place the column back into the 50 mL centrifuge tube. (**Note:** Wipe off any spillage from the thread of the

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50 mL tube before re-inserting the column to ensure the rim of the column does not get wet).

- 11) Carefully, without moistening the rim, add 5 mL Buffer AW1 to the column and centrifuge 4000 rpm (~3500xg) for two min. Do not discard flow through.
- 12) Carefully, without moistening the rim, add 5 mL Buffer AW2 to the column and centrifuge 4000 rpm (~3500xg) for 30 min.
- 13) Place the column in a clean 50 mL tube (provided), and pour the filtrate into hazardous waste container and discard tube into biohazard bag. (**Note:** Wipe any spillage off the column before inserting into tube).
- 14) 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 four min.
- 15) 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).
- 16) Keep samples on ice or at 4°C for further analysis (Quantify and QC if necessary).

Other Reagents Needed

100% Ethanol

PBS