Protocol Name: DNA PBLs v1 – Isolation of DNA from PBLs (from QIAamp DNA Blood Mini Kit Handbook)

Notes:

- Equilibrate samples and all reagents to room temperature.
- Lyophilized QIAGEN Protease can be stored at room temperature for up to 12 months but should be stored at 2-8 °C for longer periods of time.
  Reconstituted QIAGEN Protease is stable for 2 months at 2-8 °C but should be aliquoted and stored at -20 °C for longer periods of time, avoid repeated freezing and thawing. Buffer AL should be mixed thoroughly before use. Buffers AW1 and AW2 are supplied as a concentrate and should have the appropriate amount of 100% ethanol added as indicated on the bottle.
- Turn on constant temperature incubators to 37 °C and 56 °C.
- If precipitate has formed in Buffer AL, dissolve by incubating at 56 °C.
- All centrifugation steps should be done at room temperature.
- If PBLs come resuspended in medium then start from step 1. If PBLs come already pelleted and not in medium then start with attempted resuspension in 200ul PBS and skip to step 2.

1. Thaw and transfer PBLs in medium to 1.5ml tube. Spin max speed for 5 minutes. Remove most of supernatant and resuspend pellet in 200ul PBS.
3. Add 200ul Buffer AL to the sample. Mix thoroughly by pulse vortexing for 15 seconds.
4. Incubate at 56 °C in constant temperature incubator for 10 minutes. Briefly centrifuge to remove drops from inside of lid.
5. Add 200ul 100% ETOH to the sample, and mix again by pulse vortexing for 15 seconds. Briefly centrifuge to remove drops from inside of lid.
6. Carefully transfer the mixture from step 5 to the QIAamp Spin Column. Avoid spilling and do not moisten the rim of the QIAamp Mini column. Close the cap and centrifuge for 1 minute at full speed. Place the QIAamp Spin Column in a clean 2ml collection tube and discard the tube containing the filtrate.
7. Carefully open the QIAamp Spin Column and add 500ul Buffer AW1. Close the cap and centrifuge for 1 minute at full speed. Place the QIAamp Spin Column in a clean 2ml collection tube and discard the tube containing the filtrate.
8. Carefully open the QIAamp Spin Column and add 500ul Buffer AW2. Close the cap and centrifuge for 3 minutes at full speed. Pour off filtrate and replace QIAamp Spin Column in collection tube. Centrifuge at full speed for 1 minute.
9. Place the QIAamp Spin Column in a clean 1.5ml tube and discard the collection tube containing the filtrate. Carefully open the QIAamp Spin Column and add 200ul water. Incubate at room temperature for 5 minutes and then centrifuge at full speed for 1 minute.
10. Dry down sample to approximately 50ul. Add 1ul RNase from ROCHE and incubate at 37 °C for 30 minutes.
11. Quantify on NanoDrop, blank instrument using water.