Standard Operating Procedure
Blood Processing for Next Gen Sequencing and Stable Nucleic Acid Purification

❖ Purpose

- To obtain stable nucleic acids, viable white blood cells, and plasma from whole blood.

❖ Materials

- Refrigerated Centrifuge
- Vortex Mixer
- Micropipettes and corresponding tips
- Ice
- Freshly collected blood with EDTA anticoagulant
- 1.5mL conical centrifuge tubes (or larger)
- Cryogenic storage tubes
- Buffer EL, Erythrocyte Lysis Solution (Qiagen, 79217)
- Phosphate Buffered Saline, pH 7.4, 1x (PBS) (Gibco, 10010023)
- Recovery Cell Culture Freezing Medium (Gibco, 12648010)
- **Buffer RLT Plus (Qiagen, cat # 1053393. Also included in Qiagen kit, cat #80224)
- Beta-mercaptoethanol
- **AllPrep DNA/RNA/miRNA Universal Kit (Qiagen, cat # 80224, includes RLT Plus)

❖ Procedure

1. Before beginning, mix beta-mercaptoethanol (B-Me) with Buffer RLT Plus. 10uL of B-Me per 1mL of RLT Plus. 350uL or 600uL of RLT Plus mixture will be required for each sample designated for DNA/RNA extraction.
2. Label a 1.5ml tube for plasma, 1.5mL (or larger) tubes for DNA/RNA extraction, and a 2mL cryogenic storage tube for viable cells for each blood sample. Label enough appropriate sized centrifuge tubes to accommodate the total blood volume x 6 (see step 6a below).
3. Obtain freshly collected blood on EDTA anticoagulant. Blood should be processed within a few hours of collection. **Do not use previously frozen blood.**
4. Keep blood tubes on ice (or refrigerated). Do not freeze.
5. Invert blood tube several times to resuspend settled cells
6. Transfer 200uL of whole blood into a chilled 1.5mL microcentrifuge tube.
   a. (If blood volume is larger than 200uL, larger centrifuge tubes can be used. Make certain tube can accommodate up to 6 x the total volume of blood. Alternatively, 200uL volumes of blood can be distributed between multiple 1.5mL microcentrifuge tubes and final cell pellets can be recombined later in the process.)
7. Centrifuge blood at 500 x g for 10 minutes at 4C.
8. Carefully remove the supernatant plasma without disturbing the cell layers.
9. Place plasma into a fresh tube and store at -80C. (Note: Plasma may be pink due to hemolysis if blood tubes have been sitting even a brief time following collection)
10. For every 200uL of whole blood, immediately add 1mL of Buffer EL to the separated whole blood (i.e. add 5 equivalent volumes of Buffer EL for every volume of blood).
11. Vortex briefly to mix.

12. Place tubes on ice and incubate 10-15 minutes (not greater than 20 minutes) to lyse red blood cells. Vortex tubes twice, briefly, during incubation period. Solution should become a transparent red.
13. Centrifuge at 500 x g for 10 minutes at 4C.
14. Carefully decant supernatant. Keep cell pellet on ice.
15. Add 400uL of Buffer EL to cell pellet (Or 2 volumes of Buffer EL equivalent to the original whole blood volume).
16. Vortex briefly to resuspend cells.
17. Centrifuge at 500 x g for 10 minutes at 4C.
18. Carefully pipette off all supernantant without disturbing the cell pellet. Keep cell pellet on ice.
19. Add 400uL of cold PBS to the cell pellet.
20. Vortex briefly to resuspend cell pellet.
    a. If blood sample was initially divided into multiple processing tubes for erythrocyte lysis, the white cell suspensions from that blood sample can now be recombined into a single tube. Vortex briefly to mix suspensions together.
21. Separate roughly 1/3 of the total volume of cell suspension into a fresh 1.5mL microcentrifuge tube for DNA/RNA extraction.
22. Centrifuge all cell suspensions at 500 x g for 10 minutes at 4C.
23. Carefully pipette off all supernatant.
24. Place pelleted cells on ice.
25. To each cell pellet designated for DNA/RNA extraction, add 350uL of Buffer RLT Plus with B-Me (600uL can be used for very large pellets).
26. Vortex for 1 minute until pellet fully dissolves.
27. Store crude cell lysate at -80C for later DNA/RNA extraction using Qiagen AllPrep DNA/RNA/miRNA Universal Kit # 80224.
28. To the larger cell pellet in the original processing tube, add 1mL of Recovery Cell Culture Freezing Medium.
29. Vortex briefly to resuspend cells.
30. Transfer live cell suspension to a cryogenic storage tube.
31. Slow freeze at -80C in an insulated styrofoam rack overnight.
32. The following day, cells can be transferred to liquid nitrogen cryostorage for later use.
33. Live cell recovery rate with proper handling is >70%.
34. Live white blood cell suspension can later be either sorted into individual subpopulations of cells or left unsorted (whole white cell population) for use in ATAC Seq, ChIP Seq, or any other elective application.

❖ **Additional Notes**

- This blood processing protocol is a variation on the protocol found in the Qiagen AllPrep DNA/RNA/miRNA Universal Kit (#80224), February 2016, p. 41.
- Kit #80224 will not work well with previously frozen blood.
- Red cell removal either by lysis or Ficoll gradient is required for DNA/RNA purification with kit #80224.
- Red cell removal is also required to prepare blood cells for ATAC Seq or ChIP related applications.
- A plasma removal step was added to the beginning of processing in order to preserve plasma for potential future use.
- A PBS wash step was added following RBC lysis to remove buffer EL from cells prior to cryopreservation.
- RNA yields with kit #80224 can be improved by:
    - Keeping blood and cell pellet chilled on ice until resuspension in RLT Plus Buffer with B-Me
    - Following kit recommendations including optional column drying steps and Appendix A.
    - Using a lower (30uL) water elution volume if you know your starting material input was small
    - Allowing the water to soak on the column membrane for up to 5 minutes prior to centrifugation during the elution step.
    - A second elution from the column membrane can be done in a separate sample collection tube. It may have a substantially lower concentration than the first elution. Recommend analysis by Nanodrop before pooling the 2 elutions
- DNA yields with kit #80224 can be improved by:
    - Allowing elution buffer to soak on the column membrane for 5-10 minutes prior to centrifugation during the elution step
    - Heating an aliquot of elution buffer to ~65C and applying it to the column membrane warm.
    - Second (or successive) elutions may be significantly different in concentration than the first elution. Recommend analysis by Nanodrop before pooling successive elutions.