Isolation of DNA - Granulocytes

Equipment:

- Benchtop centrifuge (Allegra X-15R, Beckman Coulter)
- Pipette Gun (Drummond)

Materials:

- 1.8mL Cryotube vials (Fisher, #375418)
- 50mL conical vial (Fisher, #352070)
- 2mL and 50mL sterile, serological pipettes (Fisher, #356507, and #356550 respectively)

Reagents:

- 1x RBC Lysis Buffer (prepared from 10X solution) (BioLegend #420301)

Procedure:

1. Follow PBMC Isolation protocol to layer whole blood over Ficoll for differential centrifugation.
2. After centrifugation and removing the buffy coat into a 50mL conical, aspirate off the remaining plasma and Ficoll. Use caution to not bring up the granulocyte layer (dark red layer at bottom of conical)
3. Resuspend the whole blood in 1x RBC Lysis Buffer up to 50mL.
4. Allow the suspension to stand at room temperature for 10 minutes.
5. Centrifuge at 300 x g for 5 minutes at RT and aspirate off the supernatant.
6. Resuspend in 50mL of 1X RBC lysis buffer and centrifuge again at 300 x g for 5 minutes.
7. Aspirate the supernatant and resuspend the cells in 1.5mL of lysis buffer
8. Transfer entire suspension into a single 1.8mL cryovial.
9. Centrifuge at 300 x g for 5 minutes and aspirate the supernatant.
10. Freeze the remaining pellet at -80°C.