STANDARD OPERATING PROCEDURE				
Ragon Institute of MGH, MIT and Harvard	**DNA Extraction from Ficoll (Gentra Kit)**		**SOP #**	PL-SOP-001
	Originated by:	Ildiko Toth	Date:	25 April 17
	Laboratory:	Processing Laboratory	Pages:	1 of 2
	Approved by:	Alicja Trocha		

I. PURPOSE:

The purpose of this procedure is to extract DNA from granulocytes, separated from whole blood during a Ficoll isolation of PBMCs. It is done to preserve PBMCs for research and use discarded granulocytes for DNA extraction.

II. SAFETY:

This protocol needs to be carried out in a biosafety cabinet in BSL 2+ Lab. Personnel are required to wear safety glasses, gloves and a disposable gown before initiating any work and a secondary pair of gloves over the first before entering the biosafety cabinet. Secondary pair of gloves MUST be removed every time while exiting from the hood even if it is for a short time.

III. REAGENTS:

Reagent	Vendor	Catalogue #
Hanks Balanced Salt Solution	Sigma	H-9394
RBC Lysis Solution	Gentra/Qiagen	D-50K1/158904
Cell Lysis Solution	Gentra/Qiagen	D-50K2/158908
Protein Precipitation Solution	Gentra/Qiagen	D-50K3/158912
100% isopropanol	Fischer Scientific	A415-4
70% ethanol		
1X Tris/EDTA Buffer Solution (TE buffer)	Fischer Scientific	BP2473-1

IV. PROCEDURE:

1. After harvesting PBMCs from Ficoll layer, aspirate down to RBC layer.
2. Wash pellet once using **Hanks solution.** (Add Hanks up to 45ml, vortex to mix, spin @ 1500 rpm for 10 minutes, aspirate down to pellet).
3. Add ~ 25ml of Gentra Kit **RBC Lysis Solution** (enough to bring total volume to 30ml). Vortex to mix. Allow solution to sit for one hour in the hood. Vortex again to mix.
4. Spin @ 3000 rpm (2000g) for 10 minutes at room temperature.
5. A white pellet of granulocytes should form at the bottom of the tube. If lysis was performed incorrectly or if blood was old, pellet may be red.
6. Aspirate down to pellet. Resuspend pellet in 5 ml of Gentra Kit **Cell Lysis Solution**.

** DNA extraction can be stopped at this point and continued later. DNA is stable for up to 18 months in this solution.

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7. Add 2 ml of **Protein Precipitation Solution**. Vortex well.
8. Put conical on **wet** ice for 5-10 minutes to stimulate protein precipitation.
9. Spin @ 3000 rpm for 10 minutes at room temperature.

Carefully pour supernatant into new 50 ml conical and add an **equal volume** of **100% isopropanol**.

10. Invert conical 50 times. Small white strands of DNA should be visible at this point.
11. Spin @ 3000 rpm for 3 minutes at room temperature.
12. Pour off supernatant and blot on gauze. Be careful not to lose the pellet, as it can detach from the conical wall.
13. Add 3ml of **70% ethanol** to wash pellet. Invert conical gently several times to mix.
14. Spin @ 3000 rpm for 3 minute at room temperature.
15. Pour off supernatant and blot on gauze. Dry in hood for 15 minutes.
16. Add 200 ul of **TE buffer** to dissolve DNA pellet. Depending on the size of the DNA pellet, you may need to add more buffer to completely dissolve DNA. Allow DNA to hydrate overnight or at least 4 hours before measuring concentration.
17. Measure DNA concentration using spectrophotometer and aliquot samples for sequencing:
    a. 50ng/ul in 100 ul (for HLA typing)
    b. Stock aliquot in 100-400 ul

V. REFERENCES/ ADDITIONS/ NOTES:

N/A