I. PURPOSE:

The purpose of this procedure is to extract DNA from frozen PBMC samples.

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 #
Dulbecco's Phosphate Buffered Saline	Sigma	D8537
QIAGEN protease*	Qiagen	19157
Buffer AL*	Qiagen	19075
QIAamp Spin Column*	Qiagen	cannot be ordered separately from kit
100% ethanol	Pharmco-AAPER	111ACS200
Buffer AW1*	Qiagen	19081
Buffer AW2*	Qiagen	19072
Buffer AE*	Qiagen	19077

*Reagents also part of "QIAamp DNA Blood Mini Kit", Cat. # 51104 (50 samples), 51106 (250 samples)

IV. PROCEDURE:

1. Thaw sample. Using approximately 10M cells for the extraction should produce a good yield. Spin down and resuspend cells in **200ul PBS**.
2. Add **200ul PBS/PBMC** mixture to **20ul QIAGEN protease** aliquot (found in –20 in Room 5225).
3. Add **200ul buffer AL** to the tube.
4. Vortex for 15 sec and incubate at 56° for 10 minutes. Centrifuge to remove condensation from the lid.
5. Add **200ul ethanol** (96-100%). Vortex and centrifuge.
6. Add mixture (about 650ul) to QIAamp Spin Column, in 2ml collection tube. Close cap and centrifuge.
7. Place column in clean collection tube and add **500ul buffer AW1**. Close cap and centrifuge.
8. Place column in clean collection tube and add **500ul buffer AW2**. Close cap and centrifuge at full speed (20,000 x g/14,000 rpm) for 3 minutes.
9. Place column in a new 1.5ml microcentrifuge tube. Add **100ul buffer AE** and incubate at room temperature overnight.

10. The next day: centrifuge incubation sample.
11. Measure DNA concentration and aliquot samples for sequencing using nanodrop:
    - 50ng/ul in 100 ul (for HLA typing)
    - stock aliquot in 100 ul

V. REFERENCES/ ADDITIONS/ NOTES:

1. Set up 56° water bath prior to starting.
2. All centrifugation is done at 6,000 x g (8,000 rpm) for 1 min unless noted.