INTEGRATED BIOREPOSITORY OF H3AFRICA UGANDA

MAKERERE UNIVERSITY

COLLEGE OF HEALTH SCIENCES

STANDARD OPERATING PROCEDURE

TITLE: **PAXGENE BLOOD DNA ISOLATION**		**PAGE 1 of 8**
SOP #: **IBRH$_3$AU-SOP-BSP-004.1**	Effective Date:**09/01/2013**	Next Rev: **DEC 2015**
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VALIDATION AND RETIREMENT

	NAME	DATE
Validated by:		
Retired by:		

ACKNOWLEDGEMENT OF READING AND UNDERSTANDING

I have received and understood the training on this SOP. If I have not understood the training I have asked the trainer to retrain me to ensure that I completely understand all the requirements.

	NAME	**SIGNATURE**	**DATE**
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1 PURPOSE

To describe the procedures required for the isolation of genomic DNA using whole blood collected in PAXgene blood DNA tubes and PAX-gene blood DNA kit.

2 LIMITATIONS

This procedure is for research use only, not for use in diagnostic procedures.

3 SCOPE

This protocol describes the purification of genomic DNA from 8.5 mls whole blood using PAXgene blood DNA kit. The PAXgene system typically results in DNA yield of 150-500µg DNA and A260/A280 RATIO OF 1.7-1.9.

4 ABBREVIATIONS

4.1 DNA – deoxyribonucleic acid

4.2 µg – microgram

4.3 µl – microliter

4.4 ml- milliliter

4.5 ng- nanogram

5 SOP SPECIFIC NEEDS AND REQUIREMENTS

5.1 Supplies/Equipment required.

5.2 Hand gloves

5.3 Lab coat

5.4 PAXgene Blood DNA tube containing sample

5.5 Ethanol (70%)

5.6 Pipets and pipette tips

5.7 Graduated cylinder

5.8 Centrifuge capable of attaining 2000-8000g and equipped with a swing out rotor

5.9 Buckets with adaptors to hold 15ml and 50ml falcon tubes

5.10 Vortex mixer

5.11 Crushed ice.

5.12 Permanent pen for labeling.

5.13 Shaker-incubator capable of incubating at 55C and 65C and shaking at 400rpm, not exceeding 1400rpm (e.g. Eppendorf thermo mixer compact or equivalent).

5.14 **Kit contents**

5.14.1 Processing tubes containing 25ml Buffer BG1(Lysis buffer) 25

5.14.2 Buffer BG2 (Wash buffer) 140 ml

5.14.3 Buffer BG3 (Digestion buffer) 140 ml

5.14.4 Buffer BG4 (Re-suspension buffer) 50 ml

5.14.5 PreAnalytiX Protease (1 vial)

5.14.6 Handbook (1)

6 SAMPLE INFORMATION

6.1 Required sample type is whole blood.

6.2 Samples collected in PAXgene Blood DNA Tubes must be processed within 14 days if stored at ambient temperature (15–25°C) or within 28 days if refrigerated (2–8°C), up to 3 months (20°C).

7 SPECIAL SAFETY PRECAUTIONS

7.1 To avoid the risk of infection (e.g., from HIV or hepatitis B viruses) or injury when working with biological and chemical materials, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). Available at:www.preanalytix.com/dna msds.asp.

7.2 Buffers BG3 contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. Therefore, do not clean sample-preparation waste, or BG3 spills directly with bleach, use detergent and water. If the spill contains potentially infectious agents clean with 1% Sodium Hypochlorite after detergent and water.

7.3 Protease: Contains proteinase K (Tritirachium album): sensitizer, irritant.

8 MAINTENANCE & CALIBRATION

8.1 Equipment should be maintained and calibrated in accordance with Manufacturers Manual and Equipment SOP. Reference can be made to other SOPs if maintenance is general indicating the name of the SOP(s) and document control number(s).

9 QUALITY CONTROL

9.1 All purified DNA samples should be subjected to Quality Control criteria specified in the DNA Quality Control SOP and in accordance to DNA QC procedures established.

10 PREPARATIONS

10.1 Thaw frozen PAXgene Blood DNA tubes at ambient temperature (18-25°C) for at least 2 hours or at 37°C in a water bath for at least 15 minutes.

10.2 Set a heating block or water bath to 65°C.

10.3 This procedure consists of centrifugation to pellet nucleic acid, washing, re-suspension and purification.

10.4 All centrifugation steps must be carried out at room temperature (15-25°C) in a swing-out rotor centrifuge.

10.5 For every sample, mix 5ml Buffer BG3 50 ul reconstituted PreAnalytiX protease. E.g. to process 10 samples mix 50ml Buffer BG3 and 500 ul PreAnalytiX protease. Ensure to do this before start of the procedure.

11 PROCEDURE

11.1 Pour all the blood from one PAXgene Blood DNA Tube into a Processing Tube containing 25 ml Buffer BG1. Close the tube. To avoid cracking the blue lids of the Processing Tubes, do not over tighten them. Tighten the lid only until the first sign of resistance is felt. Mix by inverting the tube 5 times. If the blood in the PAXgene blood DNA tube has been separated into plasma and red blood, invert the tube carefully 10 times to homogenize the sample.

11.2 Centrifuge for 5 minutes at 2500g in a swing out rotor centrifuge.

11.3 Carefully discard the supernatant and place the tube in a rack. In rare cases the pellet maybe lost, so pour carefully.

11.4 Add 5 ml Buffer BG2, close the tube, and wash the pellet by vortexing vigorously for 5 seconds.

11.5 Centrifuge for 3 minutes for 2500 x g using a swing-out rotor.

11.6 Carefully discard the supernatant and place the tube back in the rack.

11.7 Ensure precaution when doing this to avoid loss of cells.

11.8 Add 5 ml of Buffer BG3/PreAnalytiX protease; close the tube, vortex for 20 seconds at high speed. Vortexing for 20 seconds is essentially to dissolve pellet completely. Shorter vortexing times may result in incomplete re-suspension of pellet and reduced DNA yield or purity. After this step, sample can be stored for at least 7 days at 2-8°C.

11.9 Place the tube in a heat block or a water bath and incubate at 65°C for 10 minutes. The sample changes color from light red to light green, indicating that protein digestion has occurred.

11.10 Vortex again for 5 seconds at high speed.

11.11 Add 5 ml of isopropanol (100%) and mix by inverting the tube 20 times until the white DNA strands clumps visibly. This is essential to precipitate DNA; only clumped DNA strands can be efficiently pelleted by centrifugation. Do not vortex as this might reduce DNA yield.

11.12 Centrifuge for 3 minutes at 2500 x g.

11.13 Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for one minute. This is done to minimize backflow of isopropanol from the rim and sides of the tube onto the pellet. This should be done slowly and carefully.

11.14 Add 5 ml 70% (v/v) ethanol and vortex for 1 second on high speed.

11.15 Centrifuge for 3 minutes at 2500 x g.

11.16 Discard the supernatant and leave the tube inverted on a clean absorbent paper for at least 5 minutes. This is done to minimize backflow of ethanol from the rim and sides of the tubes into the pellet.

11.17 Carefully dab the tube onto absorbent paper to remove ethanol from the rim, and leave it inverted for a further 5 minutes to allow the DNA pellet to dry. Avoid over drying the pellet, since over dried DNA is very difficult to dissolve.

11.18 Add 1 ml Buffer BG4 and dissolve the DNA by incubating for 1 hour at 65°C in a heating block or water bath, followed by incubation overnight at room

temperature. Highly concentrated, high-molecular weight genomic DNA samples may not re-dissolve completely after an incubation of 1 hour at 65°C therefore an additional overnight incubation is recommended.

11.19 Aliquote the DNA into appropriately labeled tubes

12 APPLICABLE REFERENCES

12.1 Qiagen maintains a large, up-to-date online database of scientific publications utilizing qiagen and PreAnalytiX products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

12.2 For a complete list of references, visit the qiagen reference database online at www.qiagen.com/refdb/search.asp or contact qiagen technical services.

13 REVISION HISTORY

Revision No	Effective Date	Description of Changes Made from Preceding Revision	Approved by/ Date

ANNEX 1: DOCUMENTATION OF SUGGESTED CHANGES TO THIS SOP

CLAUSE	SUGGESTION	BY	DATE