INTEGRATED BIOREPOSITORY OF H3AFRICA UGANDA

MAKERERE UNIVERSITY
COLLEGE OF HEALTH SCIENCES

STANDARD OPERATING PROCEDURE

TITLE: **QUALITY CONTROL OF NUCLEIC ACIDS**		**PAGE 1 of 11**
SOP #: **IBRH$_3$AU-SOP-BSP-008**	Effective Date: **09/01/2014**	Next Rev: **DEC 2014**

Prepared by:	Reviewed by:	Approved by:
(Signature & Date) NAME: Musinguzi Henry TITLE: Lab Manager	(Signature & Date) NAME: Dr. Samuel Kyobe TITLE: Coordinator	(Signature & Date) NAME: Prof. Moses Joloba TITLE: Principal Investigator

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
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			

Table of Contents

1 INTRODUCTION

Quality assurance is fundamental to the successful operation of a biorepository providing samples for research purposes. A high level of molecular integrity is essential for avoiding inconsistencies and variables in research studies. Nucleic acid quality is critically important for many techniques utilized in genomic analysis, for the meaningful interpretation of results and for the facilitation in the comparison of results across independent laboratories. All biobanks should be confident that they are providing adequate samples for the specified research purpose. Ideally, testing procedures should be in place to monitor and assess the quality of the samples in the collection.

2 PURPOSE

This standard operating procedure (SOP) outlines minimum assessment and testing that must be routinely performed to evaluate the quality of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) extracted in laboratories and biorepositories to provide investigators with a product that is consistent with their needs.

3 SCOPE

The SOP is applicable to all DNA and RNA samples, regardless of extraction method, unless otherwise specified.

4 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from the IBRH3AU who are responsible for assessing the quality of nucleic acids

Biorepository Personnel	**Responsibility/Role**
PI/Scientific director	1. Determine if the procedures outlined in this SOP for nucleic acid QC are appropriate for their study in consideration of sample collection supplies, downstream assays and logistics. 2. Inform the Study Investigator if deviations to the protocol are required.
Coordinator	1. Ensures that laboratory personnel have been trained in accordance with this SOP before isolating DNA/RNA to ensure that the training is documented. 2. Modify the current document or draft a new protocol when deviations are required and ensure all staff are informed and review the revised or new SOP.
Laboratory Technician/Trainee	1. Ensures he/she has read, understands and follows the SOP while assessing DNA and RNA quality. 2. Conducts and assists with quality assurance procedures. 3. Records and documents outcomes. 4. Record and notify the Study Investigator of any deviations from this procedure, which are not accounted for in study specific procedures.

5 MATERIALS, REAGENTS, EQUIPMENT AND FORMS

The materials, reagents, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

- Appropriate tubes
- UV Spectrophotometer and quartz cuvettes or a Nanodrop (Thermo Scientific)
- Gel electrophoresis apparatus
- Reagents for running RNA gel
- RNA 6000 Nano Kit
- Thermocycler for PCR reaction
- Reagents for PCR reaction

6 PROCEDURES

The research and scientific utility of the data obtained from the analysis of nucleic acids correlates specifically with the molecular integrity of the extracted DNA or RNA. Degraded or contaminated nucleic acid samples will lead to inconsistent or unreliable results. Confounding factors (termed pre-analytic variables) influence the quality of the extracted nucleic acids. The following procedures provide an example of steps that may be used to assess the molecular calibre of the samples in the collection:

Quality Assessment – General Considerations for Molecular Assessment of Nucleic Acids

- Assessment of molecular integrity of the samples in the collection must be performed on a percentage of the stored samples as deemed suitable and all samples that will be distributed from one facility to another for testing/storage.
- A designated laboratory using established procedures developed for this purpose must perform assessment of molecular integrity.
- Use researcher feedback about sample quality to refine collection and storage practices and guide evolution of Quality Control procedures.
- Develop and use a defined scoring system that allows for a ‘quality score' to be assigned to a molecular sample that has undergone assessment at a designated quality control laboratory.
- Use the score in the interpretation of the quality assessment results.

Quality Assessment – DNA by Polymerase Chain Reaction (PCR)

Optional quality control procedure:

- The method consists of amplifying different length fragments of the B-Globin gene (a “housekeeping” gene for human samples). The maximum amplicon size positively correlates with DNA quality.

- The test and review must be performed by an individual, qualified by experience and training to do so.
- Use the following primers:
    - B-Globin: GH20 GAAGAGCCAAGGACAGGTAC
    - B-Globin: PC04 CAACTTCATCCACGTTCACC
    - B-Globin: RS42 GCTCACTCAGTGTGGCAAAG
    - B-Globin: KM29 GGTTGGCCAATCTACTCCCAGG
    - B-Globin: RS40 ATTTTCCCACCCTTAGGCTG
    - B-Globin: RS80 TGGTAGCTGGATTGTAGCTG

  Primer pairs and expected amplicon lengths:
    - GH20 + PC04 = 268 base pairs (bp)
    - RS42 + KM29 = 536 bp
    - RS40 + RS80 = 989 bp
    - KM29 + RS80 = 1327 bp

  Use the following reagents for the PCR reaction master mix (adjust total volume to accommodate the total number of samples being tested):

  Master Mix:

  2.5 µL 10X Taq Buffer (such as Amersham #27-0799-05)

  4.0 µL dNTP (1.25 mM of each, such as Amersham # 27-2035-01)

  1.0 µL Primer pairs (diluted at 20pM each)

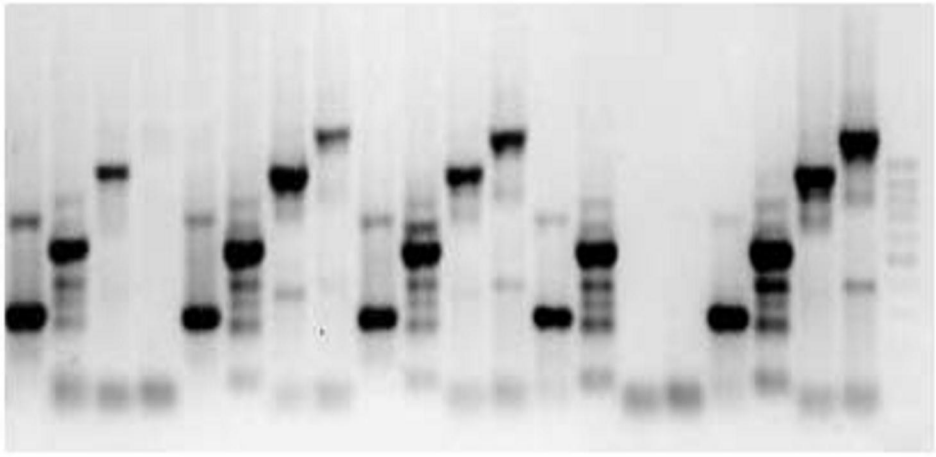
  15.0 µL H2O

  0.5 µL Taq DNA polymerase 5X (such as Amersham #27-0799-05)

  23.0 µL Total of the master mix + 2 µL of DNA (50-100 ng/µL) = 25µL per reaction

- Use the following PCR reaction conditions:
  - (3 min at 95°) 1 cycle
  - (1 min at 95°, 2 min at 55°, 1 min at 72°) 40 cycles
  - (5 min at 72°) 1 cycle
  - (Optimized for PCR Thermo Hybaid MBS # HBMBSKIT2 adjust to suit alternate makes and model of thermocyclers)
- Resolve on 1.2% agarose gel.
- Sample results and scoring system for 4 primer pairs

Good	**Very good**	**Very good**	**Poor**	**Very good**
3 bands	4 bands	4 bands	2 bands	4 bands



Quality Assessment – DNA by Spectrophotometric Measurement/Nanodrop

- Take UV spectrophotometric measurements to determine the DNA concentration and OD 260/280 ratio.
- A 260/280 ratio of ~1.8 is generally accepted as "pure" for DNA and the range of acceptability is 1.7 – 1.9. Very low ratio (~1.6) may indicate

significant protein contamination and very high ratio (~ 2.0) may indicate significant RNA contamination.

Quality Assessment – Nucleic acid concentration by Spectrophotometric Measurements/Nanodrop

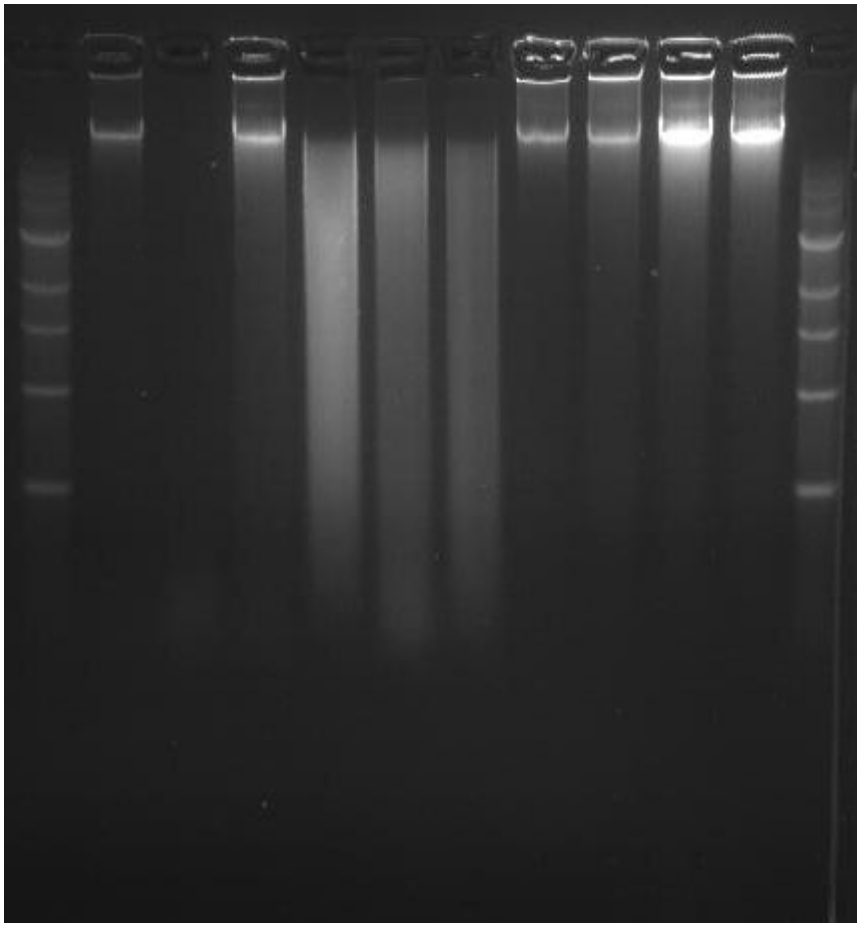
- Extract/isolate nucleic acid and document protocol used.
- Take UV spectrophotometric measurements to determine the nucleic acid concentration and OD 260/280 ratio.
- A 260/280 ratio of ~1.8 is generally accepted as "pure" for DNA and the range of acceptability is 1.7 – 1.9. Very low ratio (~1.6) may indicate significant protein contamination and very high ratio (~ 2.0) may indicate significant RNA contamination.

Quality Assessment – Records

- Record test results for each quality assurance tested sample in the institution database or informatics system.
- Include in the test results the SOP used.

Quality assessment –gel electrophoresis

Check the quality/quantity of the DNA by using a Nanodrop and/or by agarose-electrophoresis of 4ul of the DNA in 0.85% gel with known ladders/markers. Stain with ethidium bromide. On the gel, good genomic DNA should be a single high-molecular weight band. Poor genomic DNA will show a smear, an evidence of shearing [which could result from harsh treatments throughout the procedure]. Degraded DNA tends balloon-out upon agarose gel electrophoresis, and this could mostly result from prolonged incubations [DNAses activated]. Degraded genomic DNA may show curved bands on RFLP/Southern blot



Gel image showing Markers/ladders, Good quality DNA bands and sheered DNA bands

Mixture preparation

1microliter of DNA + 4microliters water (free of nucleic acid and nucleases)+1microliter of 6x loading dye run on 0.85% agarose gel stained with 0.5ug/ml ethidium bromide alongside 1kb ladder similarly diluted in 1x TBE or TAE buffer at 120V for 30 minutes to one hour

7 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

8.1 H3Africa Standard Operating Procedures on Assessing Quality of Nucleic Acids SOP 02.002, v 1.0

8.2 Declaration of Helsinki. http://www.wma.net/en/30publications/10policies/b3/index.html

8.3 Jewell, S. et al. 2002, Analysis of the Molecular Quality of Human Tissues, an experience from the Cooperative Human Tissue Network. Am. J. Clin. Pathol. 118:733-741.

8.4 Alberta Research Tumor Bank, Best Practices Guide, Version 2. 2006.

8.5 Thermo Scientific Technical Bulletin T042 Assessment of nucleic acid purity http://www.nanodrop.com/Library/T042-NanoDrop-Spectrophotometers-Nucleic-Acid-Purity-Ratios.pdf

8.6 Ambion TechNotes 11(1) Assessing RNA quality. http://www.invitrogen.com/site/us/en/home/References/Ambion-Tech-Support/rna-isolation/tech-notes/assessing-rna-quality.html.

8 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