

Assessing Quality of Nucleic Acids

CTRNet Standard Operating Procedure			
Assessing Quality of Nucleic Acids			
SOP Number:	05.002	Version:	e3.0
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Approved By:	CTRNet College of Advisors	
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1.0 PURPOSE

Quality assurance is fundamental to the successful operation of a tumour biobank offering tissue specimens and derivatives 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.

2.0 SCOPE

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

3.0 REFERENCE TO OTHER CTRNET SOPS OR POLICIES

- 3.1 *CTRNet Policy: POL 5 Records and Documentation*
- 3.2 *CTRNet Policy: POL 7 Material and Information Handling*
- 3.3 *CTRNet Standard Operating Procedure: SOP 5.001 Assessing Quality of Tissue Specimens*
- 3.4 *CTRNet Standard Operating Procedure: SOP 5.003 Assessing Quality of RNA*
- 3.5 *CTRNet Standard Operating Procedure: SOP 5.004 Assessing Quality of DNA*
- 3.6 *CTRNet Standard Operating Procedure: SOP 08.02.003 Blood Derivatives: Extraction of RNA*
- 3.7 *CTRNet Standard Operating Procedure: SOP 08.02.004 Blood Derivatives: Extraction of DNA*
- 3.8 *CTRNet Standard Operating Procedure: SOP 08.03.008 Tissue Derivatives - Extraction of DNA*
- 3.9 *CTRNet Standard Operating Procedure: SOP 08.03.009 Tissue Derivatives – Extraction of RNA*

Assessing Quality of Nucleic Acids

3.10 CTRNet Standard Operating Procedure: SOP 08.01.002 Biohazardous Material Waste Management

4.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from CTRNet member biobanks who are responsible for assessing the quality of nucleic acids.

Tumour Biobank Personnel	Responsibility/Role
Laboratory Technician/Technologist	Conducts and assists with quality assurance procedures. Records and documents outcomes.

5.0 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.

Materials and Equipment	Materials and Equipment (Site Specific)
Appropriate tubes	
UV Spectrophotometer and quartz cuvettes	
Agilent Bioanalyzer 2100	
RNA 6000 Nano Kit	
Thermocycler for PCR reaction	
Reagents for PCR reaction	
Reagents for Bioanalyzer	

6.0 DEFINITIONS

See the CTRNet Program Glossary: <http://www.ctrnet.ca/glossary>

7.0 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. These include, for example, physiological state of the tissue prior to harvesting, post-resection interval from collection to preservation and storage conditions.

The following procedures provide an example of steps that may be used by a biobank to assess the molecular calibre of the samples in the collection:

7.1 Quality Assessment – General Considerations for molecular assessment of nucleic acids

- 7.1.1 Assessment of molecular integrity of the samples in the collection must be done on a percentage of the stored samples as deemed suitable.
- 7.1.2 A designated laboratory using established procedures developed for this purpose must perform assessment of molecular integrity.
- 7.1.3 Use researcher feedback about sample quality to refine collection and storage practices and guide evolution of Quality Control procedures.
- 7.1.4 Develop and use a defined scoring system that allows for a 'quality score' to be assigned to a tissue or molecular sample that has undergone assessment at a designated quality control laboratory.
- 7.1.5 Use the score in the interpretation of the quality assessment results.

7.2 Participant File Creation and Maintenance

- 7.2.1 Extract/isolate DNA and document protocol used.
- 7.2.2 Take UV spectrophotometric measurements to determine the DNA concentration and OD 260/280 ratio.

7.3 Quality Assessment – DNA by Polymerase Chain Reaction (PCR)

The quality control centre of CTRNet uses the following procedure.

- 7.3.1 The method consists of amplifying different length fragments of the B-Globin gene (a “housekeeping” gene). The maximum amplicon size positively correlates with DNA quality.
- 7.3.2 The test and review must be performed by an individual, qualified by experience and training to do so.
- 7.3.3 Use the following primers:
 - B-Globin: GH20 GAAGAGCCAAGGACAGGTAC
 - B-Globin: PC04 CAACTTCATCCACGTTCCACC
 - B-Globin: RS42 GCTCACTCAGTGTGGCAAAG
 - B-Globin: KM29 GGTGGCCAATCTACTCCCAGG
 - 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
- 7.3.4 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

Assessing Quality of Nucleic Acids

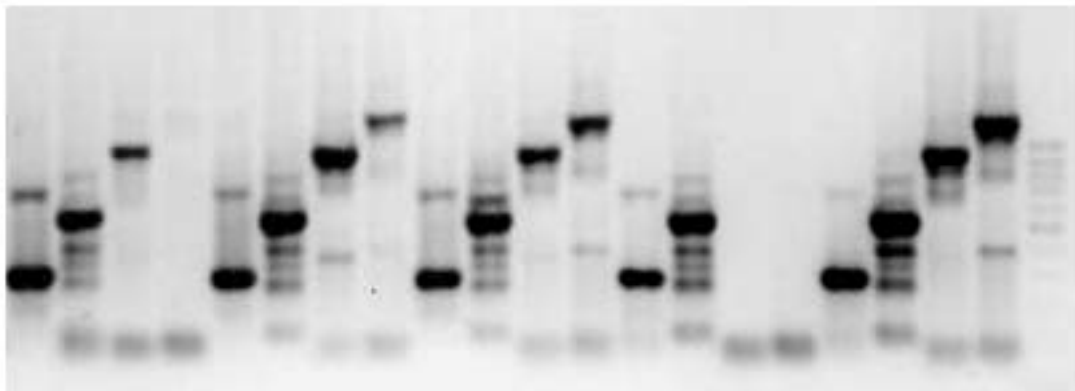
7.3.5 Use the following PCR reaction conditions:

- (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 # HBMSKIT2 adjust to suit alternate makes and model of thermocyclers)

7.3.6 Resolve on 1.2% agarose gel.

7.3.7 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



7.4 Quality Assessment – RNA by spectrophotometric measurements

7.4.1 Extract/isolate RNA and document protocol used.

7.4.2 Take UV spectrophotometric measurements to determine the RNA concentration and OD 260/280 ratio.

7.5 Quality Assessment – RNA by using the Agilent Bioanalyzer

The following procedure is used by the quality control centre of CTRNet and is based on the use of the Agilent 2100 bioanalyzer (with RNA 6000 Nanoassay Kit or suitable commercial kits) to determine the

Assessing Quality of Nucleic Acids

concentration and purity/integrity of RNA samples. It provides a read-out for sample quality and purity, has the added advantage of requiring small amounts of the sample and a quality score can be assigned based on the RNA integrity number value from the bioanalyzer.

7.5.1 Decontaminate Bioanalyzer Electrodes

- a. Fill wells of the electrode cleaner with 350 μ l of RNase ZAP and place in the bioanalyzer for 1 minute.
- b. Remove and replace with another electrode cleaner filled with RNase-free water for 10 seconds.
- c. Remove and wait 10 seconds for the water on the electrodes to evaporate before closing the lid of the bioanalyzer.

7.5.2 Prepare the gel

- a. Allow reagents to equilibrate to room temperature for 30 minutes before use.
- b. Place 550 μ l of gel matrix into a spin filter and spin for 10 minutes at 1500 g.
- c. Aliquot 65 μ l of the filtered gel into RNase-free microfuge tubes and store at 4° C until needed.

7.5.3 Prepare the gel-dye mix

- a. Allow reagents to equilibrate to room temperature for 30 minutes before use.
- b. Vortex dye concentrate for 10 seconds and spin down to the bottom of the tube.
- c. Add 1 μ l of the dye to a 65 μ l aliquot of the filtered gel and vortex thoroughly.
- d. Spin for 10 minutes at room temperature at 13000g in a microfuge.

7.5.4 Load the gel-dye-mix

- a. Place a new RNA nanochip on the chip priming station.
- b. Pipette 9 μ l of the gel-dye mix at the bottom of the well marked G in black.
- c. Close the chip priming station and press the plunger until it is held by the syringe clip.
- d. Wait for exactly 30 seconds and release the plunger.

Assessing Quality of Nucleic Acids

- e. Open the chip priming station and pipette 9 µl of the gel-dye into the other two wells marked G.

7.5.5 Load the marker

- a. Pipette 5 µl of the RNA Nano Marker into the well marked with the ladder symbol and each of the 12 sample wells.

7.5.6 Loading the ladder and samples

- a. Pipette 1µl of denatured ladder into the well marked with the ladder symbol.
- b. Pipette 1 µl of each of the denatured samples into each of the sample wells.
- c. Vortex the chip for 1 minute at 2400 rpm.
- d. Insert the chip in the bioanalyzer and start the instrument.

For more information about using the bioanalyzer to assess the quality of RNA refer to Section 8.9.

7.6 Quality Assessment – Records

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

8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

8.1 Declaration of Helsinki.

<http://www.wma.net/en/30publications/10policies/b3/index.html>

8.2 Tri-Council Policy Statement 2; Ethical Conduct for Research Involving Humans; Medical Research Council of Canada; Natural Sciences and Engineering Council of Canada; Social Sciences and Humanities Research Council of Canada, December 2010.

<http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/>

8.3 Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics

<http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420>

8.4 Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER).

http://www.isber.org/Search/search.asp?zoom_query=best+practices+for+repositories

Assessing Quality of Nucleic Acids

- 8.5** US National Biospecimen Network Blueprint
<http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp>
- 8.6** 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.
- 8.7** Alberta Research Tumor Bank, Best Practices Guide, Version 2. 2006.
- 8.8** 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>
- 8.9** Characterization of RNA quality using the Agilent 2100 Bioanalyzer. Application Note.
http://www.neurodiscovery.harvard.edu/images/stories/HND/for_research/atrc/atrc_downloads/online_library/PDFs/Character_of_RNA_quality_using_the_Agilent_2100_Bioanalyzer.pdf
- 8.10** RNA Quality Control with the Bioanalyzer. Fonds de la recherche en santé Québec Tissue Bank Protocol. Version 4. 27/01/2006.
- 8.11** DNA quality control using PCR. Fonds de la recherche en santé Québec Tissue Bank Protocol.
- 8.12** Interpreting Agilent Bioanalyzer Results. Version 1, November 2003, Oregon Health and Sciences University.
<http://www.ncbi.nlm.nih.gov/pubmed/16448564>

9.0 APPENDICES

- 9.1** Appendix A: Interpreting Agilent Bioanalyzer Results

10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions
QA 001.001	2005	JdSH	Initial document.
5.1.002 e1.0	2008	JdSH	Added quality assessment of DNA and RNA only
5.1.002 e1.0	May 2012	CMG	Grammatical and formatting throughout Definitions removed

Assessing Quality of Nucleic Acids

			<p>Revision History moved to bottom</p> <p>Reference links updates</p> <p>Updated SOP references</p> <p>Section 1: Revised Purpose. Second paragraph deleted.</p> <p>Section 2: Revised Scope. Second paragraph deleted.</p> <p>Section 3: Added Reference 3.8. Deleted reference to Generic SOP QA 001.001 (Assessing Quality of Tissue Samples).</p> <p>Sections 7.1-7.6 Procedures revised.</p> <p>Section 7.5 – Deleted “For interpretation of graphs generated by graphs see Appendix A.”</p> <p>Appendix: Included RIN numbers.</p>

Interpreting Agilent Bioanalyzer Results

Below are diagrams displaying High Quality RNA, Marginally degraded RNA, and Highly degraded RNA.

A. Electropherogram showing High Quality RNA (RIN 9)

High quality RNA is characterized by clear 28S and 18S peaks, low noise between the peaks and minimal low molecular weight contamination.

Interpreting Agilent Bioanalyzer Results

