

AIDS and Cancer Specimen Resource (ACSR)	Effective Date:
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1.0 PURPOSE

The purpose of this document is to establish the procedure of quality testing of nucleic acids to monitor and assess the quality of the specimens prior to cryopreservation and distribution for the AIDS and Cancer Specimen Resource (ACSR).

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 order to provide investigators with a quality product. This SOP applies to all personnel from ACSR Regional Biospecimen Repositories (RBRs) and affiliates that perform quality testing of nucleic acids specifically for the ACSR. The SOP does not cover detailed safety procedures for handling biohazardous material and it is recommended that personnel follow institutional guidelines.

3.0 REFERENCE TO OTHER ACSR SOPS OR POLICIES

3.1 Live Tissue Collection (Tech005) and Solid Tissue Collection (Tech002)

4.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel from ACSR RBRs and affiliates that are responsible for performing nucleic acid quality assessment.

ACSR Personnel	Responsibility/Role
ACSR staff member	Conducts quality retrieval and testing of nucleic acids and results.

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5.0 MATERIALS, EQUIPMENT AND FORMS

The materials, 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)
Bioanalyzer	Agilent Bioanalyzer - 2100 or Tape Station
Spectrophotometer	NanoDrop – 1000, 2000, 2000C, Lite, or 8000
Agilent RNA 6000 Pico kit	#5067-1513
Agilent small RNA kit	#5067-1548
Agilent DNA 7500 kit	#5067-1506
Reagents for Bioanalyzer from kits	
Bayonet electrode cartridge	Agilent #5065-4413
RNase ZAP	Ambion # 9780
Molecular Grade Water	Sigma #W4502
0.5mL and 1.5mL molecular grade tubes, and PCR tubes	Eppendorf PCR 0.2ml strip tubes (951010022), 0.2ml tubes (951010006), 0.5 ml tubes (30124537; Eppendorf microcentrifuge tubes 0.5ml (022431064), 1.5ml tubes (022431081)
Microcentrifuge ($\geq 13,000$ g)	Eppendorf, ThermoScientific
Heating block or water bath to 70°C	

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Pipettes (10uL, 100uL, 1000uL) with molecular grade pre-sterilized non-filter tips	
Thermocycler for PCR reaction	Bio-Rad, Roche LightCycler or Pro-Flex PCR system
Reagents for PCR reaction	Life Technologies, Roche, Bio-Rad
Agarose gel box, comb, tray and power supply	
UV light box	
Digital Image capture system to document gel	
SYBR Green II RNA Gel Stain	Life Technologies #E S-7564
SYBR Green I for DNA Gel Stain	Life Technologies # S-7567
Ethidium Bromide 10mg/mL	(Sigma E-7637)
Ice bucket	
Racks for Eppendorf tubes	
Trays for staining gels	Pyrex 9x9 or tupperware container.
Agarose – molecular grade	Life Technologies # 16500-100
DNA/RNA Mini Kit	Qiagen AllPrep # 80204 for 10 million cells or 1 - 10micron cut of 1x1cm tissue
DNA/RNA FFPE Kit	Qiagen AllPrep # 80234 for 1 - 10micron cut of 1x1cm tissue

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Roche High Pure miRNA/ total RNA isolation Kit	# 05080576001 for 10 million cells or 1 – 10 micron cut of 1x1cm tissue
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6.0 DEFINITIONS

See ACSR Glossary

7.0 PROCEDURES

This procedure is intended to ensure that biospecimens obtained from consented participants are of good molecular integrity and quality.

7.1 Special Safety Precautions

7.1.1 Comply with “Universal Precautions” when collecting and handling all specimens.

7.1.2 Use PPE (personal protective equipment) in accordance with collecting institution’s guidelines.

7.1.2 Standard best-practice working procedures include careful manipulation of the patient samples, disinfection of countertops and equipment used during testing and disposal of biohazard waste into appropriate receptacles.

7.2 Verification of Identifying Information

As applicable, verify the accuracy of patient information (in keeping with privacy and ethical policies) and ensure that it corresponds with the information on labels on collection tubes. Ensure that all personnel are trained in the use of the electronic information system(s).

7.3 General Considerations and Quantification of DNA or RNA using the NanoDrop

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/ pre-analytic variables such as: physiological state of the tissue prior to harvesting, post-resection interval from collection to preservation and storage conditions; influence the quality of the extracted nucleic acids.

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Isolate total RNA, miRNA and/or DNA using quality commercial kits that have been recommended by vendor for end product procedures. Some kits require modification, as indicated by the manufacturer to recover total RNA that includes miRNA.

- 7.3.1 Clean the spectrophotometer eye on the NanoDrop with 70% ethanol.
- 7.3.2 Add 2ul of diluent to the eye, lower the arm and blank the machine.
- 7.3.3 Wipe, apply sample, read. Repeat for all samples
- 7.3.4 Export data file for concentration calculation, total recovery calculation, database entry and archive data. Note: A 260/280 ratio between 1.8 - 2.0 and a 260/230 ratio between 1.8 - 2.2, indicate good quality and nucleic acid purity.

7.4 Quality Assessment of RNA using the Agilent Bioanalyzer

The following procedure is for analyzing total RNA using the Agilent RNA Pico 6000 kit or small RNA using the Agilent Small RNA kit in conjunction with the Agilent Bioanalyzer 2100. This system provides data which indicates sample quality and gives a quality score based on the RNA integrity number determined by the bioanalyzer. The system generates a tracing which indicates the length of the nucleic acids and defines this in comparison to a control ladder.

- 7.4.1 Decontaminate Bioanalyzer Electrodes
 - 7.4.1.1 Fill wells of the electrode cleaner with 350 µl of RNase-free water.
 - 7.4.1.2 Open lid and place electrode cleaner in the bioanalyzer.
 - 7.4.1.3 Close lid for 5 minutes.
 - 7.4.1.4 Remove electrode cleaner and wait 30 seconds for the water on the electrodes to evaporate before closing the lid of the bioanalyzer.
- 7.4.2 Prepare the gel for Agilent **Small** RNA Assay
 - 7.4.2.1 Allow reagents to equilibrate to room temperature for 30 minutes before use.
 - 7.4.2.2 Transfer complete volume (approx. 650 µl) of Small RNA gel matrix into the top of the spin filter.

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7.4.2.3 Place spin filter in a microcentrifuge and spin for 15 minutes at 10000 g.

7.4.2.4 Remove filter and label the filtered gel with the filtration date.

7.4.2.5 Store filtered gel at 4° C until needed (within one month of preparation).

7.4.3 Prepare the gel for Agilent RNA 6000 **Pico** Assay

7.4.3.1 Allow reagents to equilibrate to room temperature for 30 minutes before use.

7.4.3.2 Place 550 µl of RNA 6000 Pico gel into the top of a spin filter.

7.4.3.3 Place spin filter in a microcentrifuge and spin for 10 minutes at 1500 g.

7.4.3.4 Aliquot 65 µl filtered gel into 0.5 ml RNase-free microcentrifuge tubes provided.

7.4.3.5 Store aliquots at 4° C until needed (within one month of preparation).

7.4.4 Prepare the gel-dye mix for Agilent **Small** RNA Assay

7.4.4.1 Allow reagents to equilibrate to room temperature for 30 minutes before use.

7.4.4.2 Vortex Small RNA dye concentrate for 10 seconds and spin down to the bottom of the tube.

7.4.4.3 Add 2 µl of the dye into 0.5 ml RNase free microtube provided.

7.4.4.4 Add 40 µl of filtered gel and mix until mixture is homogenous.

7.4.4.5 Spin tube at 13000 g for 10 minutes at room temperature.

7.4.5 Prepare the gel-dye mix for Agilent RNA 6000 **Pico** Assay

7.4.5.1 Allow reagents to equilibrate to room temperature for 30 minutes before use.

7.4.5.2 Vortex RNA 6000 Pico dye for 10 seconds and spin down to the bottom of the tube.

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7.4.5.3 Add 1 μ l of RNA 6000 Pico dye to a 65 μ l aliquot of the filtered gel and vortex thoroughly.

7.4.5.4 Spin for 10 minutes at room temperature at 13000 g (use within 1 day).

7.4.6 Load the gel-dye-mix for Agilent **Small** RNA Assay

7.4.6.1 Allow gel-dye mix to equilibrate to room temperature for 30 minutes and protect from light.

7.4.6.2 Place a new Small RNA chip on the chip priming station.

7.4.6.3 Pipette 9 μ l of the gel-dye mix at the bottom of the well-marked G in black.

7.4.6.4 Make sure plunger is position at 1 ml and close the chip priming station and press the plunger until it is held by the syringe clip.

7.4.6.5 Wait for exactly 60 seconds and release the plunger.

7.4.6.6 Visually inspect that the plunger moves back at least the 0.3 ml mark.

7.4.7 Load the gel-dye-mix for Agilent RNA **6000** Pico Assay

7.4.7.1 Allow gel-dye mix to equilibrate to room temperature for 30 minutes and protect from light.

7.4.7.2 Place a new Small RNA chip on the chip priming station.

7.4.7.3 Pipette 9 μ l of the gel-dye mix at the bottom of the well-marked G in black.

7.4.7.4 Make sure plunger is position at 1 ml and close the chip priming station and press the plunger until it is held by the syringe clip.

7.4.7.5 Wait for exactly 30 seconds and release the plunger.

7.4.7.6 Visually inspect that the plunger moves back at least the 0.3 ml mark.

7.4.7.7 Wait for 5 seconds then pull back the plunger to the 1 ml position.

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7.4.8 Load the marker

7.4.8.1 Pipette 9 µl of the RNA conditioning solution into the well-marked CS.

7.4.8.2 Pipette 5 µl of RNA marker into the well-marked with a ladder and each of the 11 sample wells.

7.4.9 Load the ladder and samples

7.4.9.1 Thaw ladder aliquots on ice. To minimize secondary structure, the samples may be denatured at 70° C for 2 minutes.

7.4.9.2 Pipette 1µl of denatured ladder into the well-marked with the ladder symbol.

7.4.9.3 Pipette 1 µl of each of the denatured samples into each of the sample wells.

7.4.9.4 Place the chip horizontally in the adapter of the IKA vortex mixer.

7.4.9.5 Vortex the chip for 1 minute at 2400 rpm.

7.4.9.6 Insert the chip in the bioanalyzer and start the run within 5 minutes.

7.5 Quality Assessment of DNA using the Agilent Bioanalyzer

The following procedure is for analyzing DNA using the Agilent DNA 7500 and DNA 12000 kit with the Agilent Bioanalyzer 2100. This system provides data which indicates sample quality and gives a quality score based on the DNA integrity number determined by the bioanalyzer. The system generates a tracing which indicates the length of the nucleic acids and defines this in comparison to a control ladder.

7.5.1 Prepare the Gel-Dye Mix

7.5.1.1 Allow the DNA dye concentration and DNA gel matrix to equilibrate to room temperature for 30 minutes.

7.5.1.2 Vortex blue-capped vial with DNA dye concentrate for 10 seconds and spin down, make sure DMSO is completely thawed.

7.5.1.3 Pipette 25 µl of dye concentrate into a DNA gel matrix vial. Store dye concentrate at 4° C in the dark.

7.5.1.4 Cap the tube, vortex for 10 seconds.

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7.5.1.5 Transfer the gel-dye mix to a spin filter.

7.5.1.6 Place the spin filter in a microcentrifuge and spin for 10 minutes at 1500 g at room temperature.

7.5.1.7 Discard the filter and label and date the tube.

7.5.2 Load the Gel-Dye Mix

7.5.2.1 Allow reagents to equilibrate to room temperature for 30 minutes before use and protect from light.

7.5.2.2 Take a new DNA chip and place the chip on the chip priming station.

7.5.2.3 Pipette 9 μ l of the gel-dye mix at the bottom of the well-marked with a black G and dispense the gel-dye mix.

7.5.2.4 Close the chip priming state and make sure that the plunger is positioned at 1 ml.

7.5.2.5 Press the plunger of the syringe down until it is held by the clip.

7.5.2.6 Wait for exactly 30 seconds and then release the plunger with the clip release mechanism.

7.5.2.7 Make sure that the plunger moves back at least to the 0.3 ml marker.

7.5.2.8 Wait for 5 seconds and slowly pull back the plunger to the 1 ml position.

7.5.2.9 Open the chip priming station.

7.5.2.10 Pipette 9.0 μ l of the gel-dye mix in each of the wells marked.

7.5.3 Load the Marker

7.5.3.1 Pipette 5 μ l of green-capped DNA marker vial into the well-marked with the ladder symbol and into each of the 12 sample wells.

7.5.4 Load the Ladder and the Samples

7.5.4.1 Pipette 1 μ l of the yellow-capped DNA ladder vial in the well-marked with the ladder symbol.

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7.5.4.2 Pipette 1 µl of sample into each of the 12 sample wells or 1 µl of deionized water in the unused wells.

7.5.4.3 Place the chip horizontally in the adapter of the IKA vortex mixer and vortex for 60 seconds at 2400 rpm.

7.5.4.4 Insert the chip in the bioanalyzer and start the run within 5 minutes.

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

Ethidium Bromide (EtBr) is a known carcinogen and exposure to skin or inhalation must be avoided. The staining solution must be filtered through the Whatman Extractor system (VWR # 28165-502), a one-step filtration method for the rapid removal of ethidium bromide from gel staining solutions. This limited reuse disposable unit removes >99% of EtBr from a liter of electrophoretic buffer in about one minute. The Extractor device will maintain this removal efficiency for up to 10 successive liters. The stained gel must be disposed of in chemical waste and gloves must be worn. EtBr is visualized with a UV light box – wear a UV protective face shield and tape sleeves of lab coat so the skin at the wrist is not exposed.

Quality control assessment of DNA by PCR can be done using a standard housekeeping gene such as beta actin, TATA box binding protein or as used below – B-Globin gene.

7.6.1 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.

7.6.2 The test and review must be performed by an individual, qualified by experience and training to do so.

7.6.3 Use the following primers:

- B-Globin: GH20 GAAGAGCCAAGGACAGGTAC
- B-Globin: PC04 CAACTTCATCCACGTTACAC
- B-Globin: RS42 GCTCACTCAGTGTGGCAAAG

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- B-Globin:KM29 GGTGGCCAATCTACTCCCA
 GG
- 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

7.6.4 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

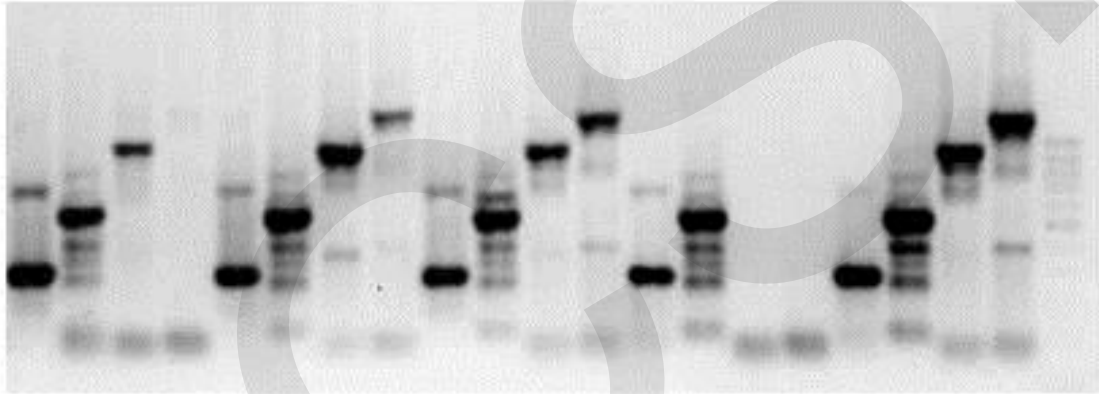
7.6.5 Use the following PCR reaction conditions:

- (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 # HBMBKIT2 adjust to suit alternate makes and model of thermocyclers)

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7.6.6 Resolve on 1.2% agarose gel stained with ethidium bromide or SYBR® Green I

7.6.7 Sample results and scoring system for 4 primer pairs.

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

8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

- 8.1 NCI Best Practices for Biospecimen Resources
<http://biospecimens.cancer.gov/practices/default.asp>
- 8.2 Declaration of Helsinki.
<http://www.wma.net/en/30publications/10policies/b3/index.html>
- 8.3 Best Practices for Repositories: Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER).
http://c.ymcdn.com/sites/www.isber.org/resource/resmgr/Files/ISBER_Best_Practices_3rd_Edi.pdf

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- 8.4 US National Biospecimen Network Blueprint
<http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp>
- 8.5 National Bioethics Advisory Commission: Research involving human biological materials: Ethical issues and policy guidance, Vol. I: Report and recommendations of the National Bioethics Advisory Committee. August 1999.
<http://bioethics.georgetown.edu/nbac/hbm.pdf>
- 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 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>
- 8.8 Characterization of RNA quality using the Agilent 2100 Bioanalyzer. Application Note.
http://neurodiscovery.harvard.edu/images/stories/HND/for_research/atrc/atrc_downloads/online%20library%20PDFs/Character%20of%20RNA%20quality%20using%20the%20Agilent%202100%20Bioanalyzer.pdf
- 8.9 Interpreting Agilent Bioanalyzer Results. Version 1, November 2003, Oregon Health and Sciences University.
<http://www.ncbi.nlm.nih.gov/pubmed/16448564>
- 8.10 Agilent Small RNA Kit Guide
http://www.chem.agilent.com/library/usermanuals/Public/G2938-90093_SmallRNA_KG_EN.pdf
- 8.11 Agilent RNA 6000 Pico Kit Guide
http://www.chem.agilent.com/library/usermanuals/Public/G2938-90046_RNA600Pico_KG_EN.pdf

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8.12 Agilent DNA 7500 Kit Guide
http://www.chem.agilent.com/library/usermanuals/Public/G2938-90024_DNA7500-12000_KG.pdf

9.0 APPENDICES

10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions