CTRNet Standard Operating Procedure Assessing Quality of RNA			
SOP Number:	05.003	Version:	e1.1
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1.0 PURPOSE

Quality assurance is fundamental to the successful operation of a 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. CTRNet-registered 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 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

Note: When adopting this SOP for local use please reference CTRNet.

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 5.002 Assessing Quality of Nucleic Acids
3.5 CTRNet Standard Operating Procedure: SOP 08.02.003 Blood Derivatives: Extraction of RNA
3.6 CTRNet Standard Operating Procedure: SOP 08.03.009 Tissue Derivatives – Extraction of RNA

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

4.0 ROLES AND RESPONSIBILITIES

This SOP applies to all biobank personnel involved in writing, revising, reviewing, approving and maintaining SOPs.

Biobank Personnel	**Responsibility/Role**
Lab technician	Conducts and assists with quality assurance procedures. Records and documents outcomes.

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)
Agilent Bioanalyzer 2100 Bioanalyzer system	
Vortex mixer IKA MS3 with chip adapter	
RNA 6000 Nano kit (5067-1511)	
Or RNA 6000 Pico kit (5067-1513)	
Thermocycler for PCR reaction	
Reagents for PCR reaction	
RNase ZAP for electrode decontamination	
Thermo cycler, heating block or water bath for heat denaturation	
RNase-free water	
Microcentrifuge (≥ 1300 g)	
1.5 ml RNase-free microcentrifuge tubes	
4200 or 4150 TapeStation System	
Loading tips (5067-5598, 1pk or 5067-5599, 10pk)	
Optical Tube 8x Strip (401428) and Optical Cap 8x Strip (401425)	
Vortex mixer IKA MS3 with 96-well sample plate adapter	
96-well sample plates (5042-8502) and 96-well Plate Foil Seal (5067-5154) (4200 TapeStation system only)	
Volumetric micropipettes for handling volumes from 1 to 15 µL	
Centrifuges for tube strips and 96-well sample plates	

5067-5576 RNA ScreenTape includes 7 ScreenTape devices • 5067-5577 RNA ScreenTape Sample Buffer • 5067-5578 RNA ScreenTape Ladder or	
5067-5579 High Sensitivity RNA ScreenTape includes 7 ScreenTape devices • 5067-5580 High Sensitivity RNA ScreenTape Sample Buffer • 5067-5581 High Sensitivity RNA ScreenTape Ladder	

6.0 DEFINITIONS

See the CTRNet Program Glossary: https://biobanking.org/webs/glossary

7.0 PROCEDURES

The following procedure is based on the use of the Agilent 2100 Bioanalyzer to determine the 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. The RNA 6000 Nano kit is suitable for total RNA samples with a concentration between 5 and 500 ng/µl (7.1.1). For RNA samples in lower concentration, the RNA 6000 Pico assay (5067-1513) can be used to estimate the concentration and to determine the integrity of RNA samples. It provides a read-out for sample concentration and quality, and a quality score can be assigned based on the RNA integrity number value from the Bioanalyzer. The RNA Pico assay offers high sensitivity and is suitable for samples from 50 to 5000 pg/µl total RNA (7.1.2).

7.1. Quality Assessment – RNA by using the Agilent 2100 Bioanalyzer System

7.1.1. RNA 6000 Nano assay

Essential Measurement Practices

- Replace the syringe at the chip priming station with each new kit.
- Use a new electrode cleaner with each new kit.
- Protect dye and dye mixtures from light.
- Handle and store all reagents according to the instructions on the label of the individual box or bag.

Heat denature the RNA 6000 Nano ladder upon arrival

- Spin ladder down.
- Heat denature the ladder for 2 min at 70 °C.
- Immediately cool the vial on ice.

- Prepare aliquots in RNase- free vials with the required amount for a typical daily use and store aliquots at -70 °C.

Prepare run

- Allow RNA 6000 Nano reagents to equilibrate at room temperature for 30 minutes.
- Thaw RNA Nano ladder aliquot and RNA samples on ice.
- Launch the 2100 Expert software and load the assay “Eukaryote Total RNA Nano Series II”.
- To minimize secondary structures, it is recommended to heat denature all RNA samples for 2 min at 70 °C.
- Immediately cool samples on ice. Briefly spin down sample to remove any condensation.
- Samples should be kept on ice until use.
- Vortex reagents and samples. Spin down before use.
- Adjust the base plate of the priming station to position C.
- Adjust the syringe clip to top position.

Decontaminate Bioanalyzer Electrodes

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

Prepare machine and the gel

- Turn on the Bioanalyzer so that the laser warms up as the reagents equilibrate.
- Allow RNA Nano reagents to equilibrate to room temperature for 30 minutes before use.
- Place 550 µl of RNA Nano gel matrix into a spin filter and spin for 10 minutes at 1500 g.
- Aliquot 65 µl of the filtered gel into RNase-free microfuge tubes and store at 4° C until needed. The filtered gel is stable up to 1 month after preparation.

Prepare the gel-dye mix

- Allow RNA Nano reagents to equilibrate to room temperature for 30 minutes before use.
- Vortex RNA Nano dye concentrate for 10 seconds and spin down to the bottom of the tube.
- Add 1 µl of the dye to a 65 µl aliquot of the filtered gel and vortex thoroughly.
- Spin for 10 minutes at room temperature at 13000g in a microfuge. Use prepared gel-dye mix within one day.

Load the gel-dye-mix

- Place a new RNA Nano chip on the chip priming station.
- Pipette 9 µl of the RNA Nano gel-dye mix at the bottom of the well marked G in black.
- Close the chip priming station and press the plunger until it is held by the syringe clip.
- Wait for exactly 30 seconds and release the plunger.
- Open the chip priming station and pipette 9 µl of the gel-dye into the other two wells marked G.

Load the marker

- Pipette 5 µl of the RNA Nano Marker into the well marked with the ladder symbol and each of the 12 sample wells. Do not leave any wells empty.

Loading the ladder and samples

- Pipette 1 µl of denatured RNA Nano ladder into the well marked with the ladder symbol.
- Pipette 1 µl of the denatured samples into each of the sample wells. Add 1 µl of the Marker to each unused sample well.
- Vortex the chip for 1 minute at 2400 rpm.
- Insert the chip in the Bioanalyzer and start the instrument. Ensure that the run is started within 5 minutes of loading the chip.

When the assay is complete, immediately remove the used chip from the Bioanalyzer instrument and clean the electrodes:

- Fill wells of the electrode cleaner with 350 µl of RNase-free water and place in the Bioanalyzer for 10 seconds.
- Remove and wait 10 seconds for the water on the electrodes to evaporate before closing the lid of the Bioanalyzer.

7.1.2. RNA 6000 Pico assay

Essential Measurement Practices

- Replace the syringe at the chip priming station with each new kit.
- Use a new electrode cleaner with each new kit.
- Protect dye and dye mixtures from light.
- Handle and store all reagents according to the instructions on the label of the individual box or bag.

Prepare and heat denature the RNA Pico ladder upon arrival

- Spin ladder down.
- Heat denature the ladder for 2 min at 70 °C.
- Immediately cool the vial on ice.
- Add 90 µl of RNase-free water and mix thoroughly.
- Prepare aliquots in RNase- free vials with the required amount for a typical daily use and store aliquots at -70 °C.

To minimize secondary structures, it is recommended to heat denature all RNA samples for 2 min at 70 °C.

Prepare run

- Allow RNA Pico reagents to equilibrate at room temperature for 30 minutes.
- Thaw RNA Pico ladder aliquot and RNA samples on ice.
- Launch the 2100 Expert software and load the assay “Eukaryote Total RNA Pico Series II”.
- Vortex reagents and samples. Spin down before use.
- Adjust the base plate of the priming station to position C.
- Adjust the syringe clip to top position.

Decontaminate Bioanalyzer Electrodes

- Fill wells of the electrode cleaner with 350 µl of fresh RNase-free water and place in the Bioanalyzer for 5 minutes.
- Remove and wait 30 seconds for the water on the electrodes to evaporate before closing the lid of the Bioanalyzer.

Prepare machine and the gel

- Turn on the Bioanalyzer so that the laser warms up as the reagents equilibrate.
- Allow RNA Pico reagents to equilibrate to room temperature for 30 minutes before use.
- Place 550 µl of RNA Pico gel matrix into a spin filter and spin for 10 minutes at 1500 g.
- Aliquot 65 µl of the filtered gel into RNase-free microfuge tubes and store at 4° C until needed. The filtered gel is stable up to 1 month after preparation.

Prepare the gel-dye mix

- Allow RNA Pico reagents to equilibrate to room temperature for 30 minutes before use.
- Vortex RNA Pico dye concentrate for 10 seconds and spin down to the bottom of the tube.
- Add 1 µl of the dye to a 65 µl aliquot of the filtered gel and vortex thoroughly.
- Spin for 10 minutes at room temperature at 13000g in a microfuge. Use prepared gel-dye mix within one day.

Load the gel-dye-mix

- Place a new RNA Pico chip on the chip priming station.
- Pipette 9 µl of the RNA Pico gel-dye mix at the bottom of the well marked G in black.
- Close the chip priming station and press the plunger until it is held by the syringe clip.
- Wait for exactly 30 seconds and release the plunger.
- Open the chip priming station and pipette 9 µl of the gel-dye into the other two wells marked G.
- Pipette 9 µl of the RNA Pico conditioning solution into the well marked CS.

Load the marker

- Pipette 5 µl of the RNA Pico Marker into the well marked with the ladder symbol and each of the 11 sample wells. Do not leave any wells empty.

Loading the ladder and samples

- Pipette 1 µl of denatured diluted RNA Pico ladder into the well marked with the ladder symbol.
- Pipette 1 µl of the denatured samples into each of the sample wells. Add 1 µl of RNA Pico Marker to each unused sample well.
- Vortex the chip for 1 minute at 2400 rpm.
- Insert the chip in the Bioanalyzer and start the instrument. Ensure that the run is started within 5 minutes of loading the chip.

When the assay is complete, immediately remove the used chip from the Bioanalyzer instrument and clean the electrodes:

- Fill wells of the electrode cleaner with 350 µl of RNase-free water and place in the Bioanalyzer for 30 seconds.

- Remove and wait 30 seconds for the water on the electrodes to evaporate before closing the lid of the Bioanalyzer.

For more information about using the Bioanalyzer to assess the quality of RNA refer to Section 8.1.

7.2 Quality Assessment – RNA by using the Agilent 4200 or 4150 TapeStation System

The following procedure is based on the use of The Agilent 4150 (G2992AA) and 4200 (G2991AA) TapeStation systems with RNA ScreenTape assay to determine the concentration and integrity of RNA samples. It provides a read-out for sample quantity and quality, has the added advantage of requiring small amounts of the sample. A quality score the RNA Integrity number equivalent (RIN^e) is automatically calculated and can be used to establish thresholds. Two RNA assays are available for the TapeStation systems for different concentration ranges. The RNA ScreenTape assay consists of 5067-5576 RNA ScreenTape, 5067-5577 RNA ScreenTape Sample Buffer and 5067-5578 RNA ScreenTape Ladder and is suitable for RNA samples between 25 and 500 ng/µl (see 7.2.1). For low concentrated RNA samples, the High Sensitivity RNA can be used which has a qualitative range from 1000 to 25000 pg/µl and consist of 5067-5579 High Sensitivity RNA ScreenTape, 5067-5580 High Sensitivity RNA ScreenTape Sample Buffer and 5067-5581 High Sensitivity RNA ScreenTape Ladder (see 7.2.2).

7.2.1 RNA Quality assessment with the RNA ScreenTape assay on a TapeStation system

Prepare run
- Allow RNA Sample Buffer to equilibrate at room temperature for 30 minutes.
- Thaw RNA Ladder and total RNA samples on ice.
- Launch the Agilent TapeStation Controller software.
- Flick the RNA ScreenTape device and insert it into the ScreenTape nest of the TapeStation instrument.
- Select required sample positions in the TapeStation Controller software.
- The required consumables (tips, further ScreenTape devices) are displayed in the TapeStation Controller software.
- Vortex reagents and samples. Spin down before use.

Prepare ladder in one well of a complete tube strip
- Pipette 5 µL RNA Sample Buffer and 1 µL RNA Ladder at position A1 in a tube strip.

Prepare samples
- For each sample, pipette 5 µL RNA Sample Buffer and 1 µL RNA sample in a tube strip or 96-well sample plate.
- Apply caps to tube strips and/or foil seals to 96-well sample plates.
- Mix liquids using the IKA MS3 vortexer at 2000 rpm for 1 min.
- Spin down sample and ladder for 1 min.
- Samples and ladder denaturation:
    - Heat samples and ladder at 72 °C (162 °F) for 3 min.

- Place samples and ladder on ice for 2 min.
- Spin down samples and ladder for 1 min.

Sample Analysis

- Load samples into the TapeStation instrument. Place ladder in position A1 on tube strip holder.
- Carefully remove caps of tube strips. Visually confirm that liquid is positioned at the bottom.
- Click Start.
- The TapeStation Analysis software opens automatically after the run and displays results.

7.2.2 RNA Quality assessment with the High Sensitivity RNA ScreenTape assay on a TapeStation system

Prepare run

- Allow High Sensitivity RNA Sample Buffer to equilibrate at room temperature for 30 minutes.
- Thaw High Sensitivity RNA Ladder and total RNA samples on ice.
- Launch the Agilent TapeStation Controller software.
- Flick the High Sensitivity RNA ScreenTape device and insert it into the ScreenTape nest of the TapeStation instrument.
- Select required sample positions in the TapeStation Controller software.
- The required consumables (tips, further ScreenTape devices) are displayed in the TapeStation Controller software.
- Vortex reagents and samples. Spin down before use.

Prepare ladder in one well of a complete tube strip

- Prepare diluted Ladder solution by adding 10 µL RNase free water to the High Sensitivity RNA Ladder vial and mix thoroughly.
- Pipette 1 µL High Sensitivity RNA Sample Buffer and 2 µL diluted High Sensitivity RNA Ladder at position A1 in a tube strip.

Prepare samples

- For each sample, pipette 1 µL High Sensitivity RNA Sample Buffer and 2 µL RNA sample in a tube strip or 96-well sample plate.
- Apply caps to tube strips and/or foil seals to 96-well sample plates.
- Mix liquids using the IKA MS3 vortexer at 2000 rpm for 1 min.
- Spin down sample and ladder for 1 min.
- Samples and ladder denaturation:
  - Heat samples and ladder at 72 °C (162 °F) for 3 min.
  - Place samples and ladder on ice for 2 min.
  - Spin down samples and ladder for 1 min.

Sample Analysis

- Load samples into the TapeStation instrument. Place ladder in position A1 on tube strip holder.

- Carefully remove caps of tube strips. Visually confirm that liquid is positioned at the bottom.
- Click Start.
- The TapeStation Analysis software opens automatically after the run and displays results.

For more information about using the 4200 & 4150 TapeStation systems to assess the quality of RNA refer to Section 8.2.

8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

8.1 Quality Assessment – RNA by using the Agilent 2100 Bioanalyzer System

- Agilent RNA 6000 Nano Quick Start Guide: https://www.agilent.com/cs/library/usermanuals/public/RNA-6000-Nano_QSG.pdf
- Agilent RNA 6000 Pico Quick Start Guide: https://www.agilent.com/cs/library/usermanuals/public/RNA-6000-Pico_QSG.pdf
- Simplified DV_{200} Evaluation with the Agilent 2100 Bioanalyzer System: https://www.agilent.com/cs/library/technicaloverviews/public/5991-8287EN.pdf

8.2 Quality Assessment – RNA by using the Agilent 4200 or 4150 TapeStation System

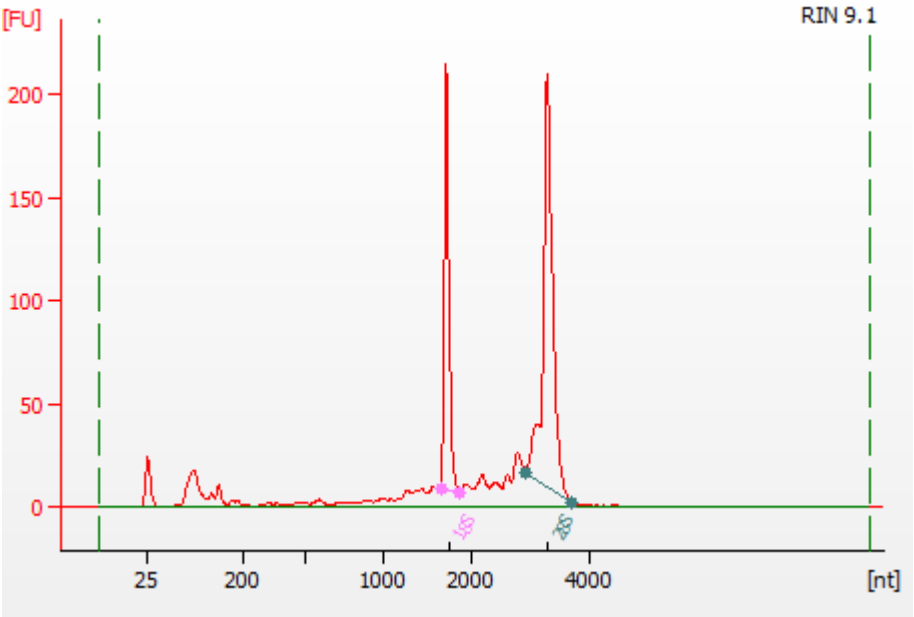
- Agilent RNA ScreenTape Quick Guide for TapeStation Systems: https://www.agilent.com/cs/library/usermanuals/public/RNA_QuickGuide.pdf
- Agilent High Sensitivity RNA ScreenTape Quick Guide for TapeStation Systems: https://www.agilent.com/cs/library/usermanuals/public/HS-RNA_QuickGuide.pdf
- Standardized DNA and RNA Sample Quality Control: https://www.agilent.com/cs/library/posters/public/Copy%20of%20Standardized_DNA_RNA_sample_quality_control_PR7000-1612_poster_Agilent.pdf
- Performance of the RNA and High Sensitivity RNA ScreenTape Assay for the 4150 TapeStation System: https://www.agilent.com/cs/library/technicaloverviews/public/technicaloverview-rna-hisens-rna-ScreenTape-performance_5994-1038en-agilent.pdf
- DV_{200} Evaluation with RNA ScreenTape Assays: https://www.agilent.com/cs/library/technicaloverviews/public/5991-8355EN.pdf

9.0 APPENDICES

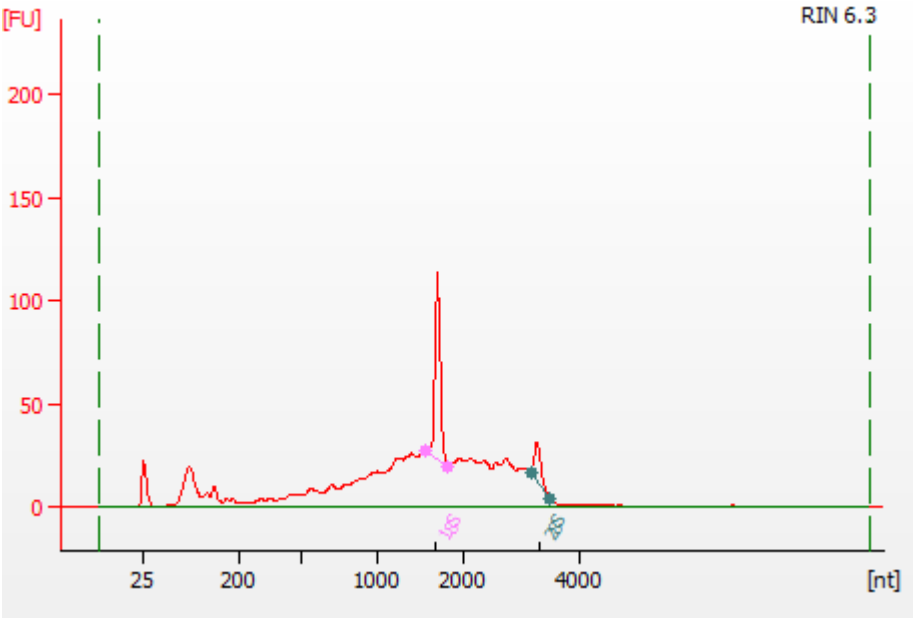
9.1 Appendix A – Interpreting RNA samples analyzed on a 2100 Bioanalyzer system with the RIN

Below are diagrams displaying high quality RNA, partially degraded RNA, and strongly degraded RNA.

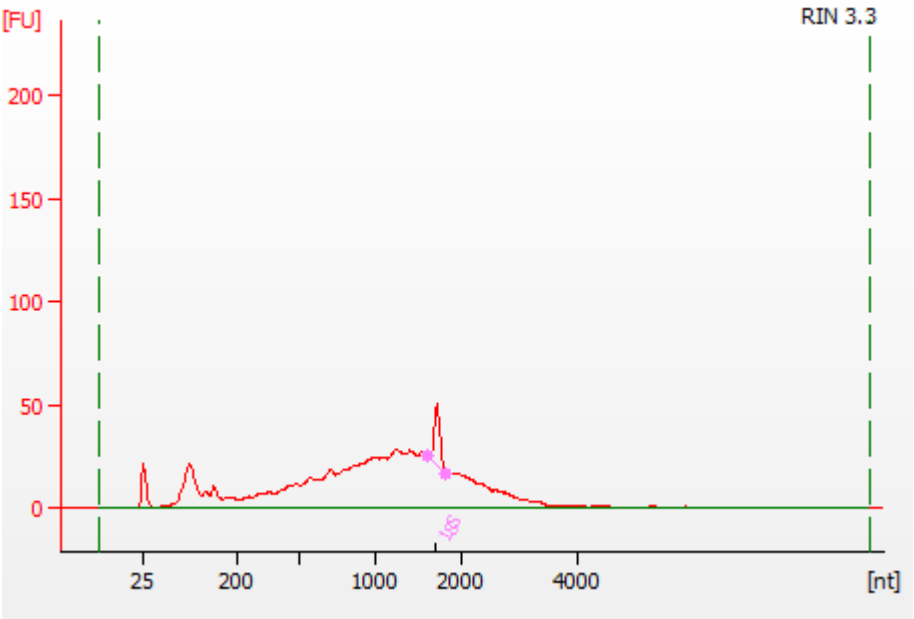
A. Electropherogram showing high quality RNA (RIN 9.1). High quality RNA is characterized by clear 28S and 18S peaks, low noise between the peaks and minimal amounts of low molecular weight material.



B. Electropherogram showing lower quality RNA (RIN 6.3). The 28S peak is almost fully degraded, and a broad hump with degraded RNA is present between the ribosomal peaks and below the 18S peak.



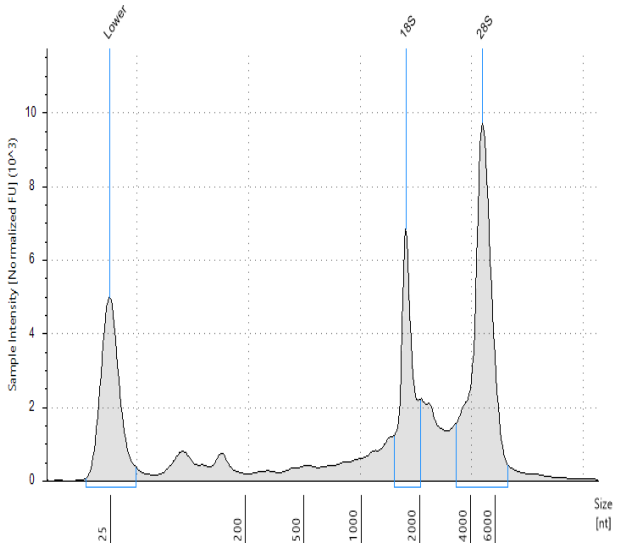
C. Electropherogram showing low quality RNA (RIN 3.3). Low RNA integrity is characterized by a lack of the 28S peak and degradation of the 18S peak. More fragmented RNA material is present below the 18S peak.



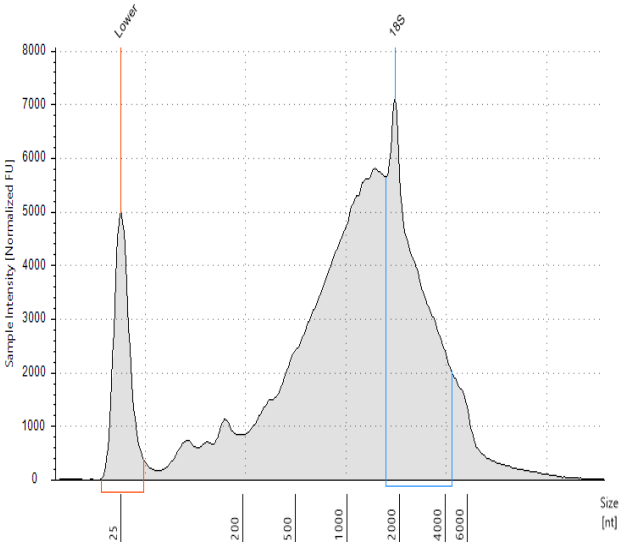
9.2 Appendix B – Interpreting RNA samples analyzed on a TapeStation system with the RIN^e

Below are diagrams displaying high quality RNA, and highly degraded RNA.

A. Electropherogram showing intact RNA with a RIN^e of 9.1
Intact RNA is characterized by clear 28S and 18S peaks and minimal amount of low molecular weight fragments.



B. Electropherogram showing degraded RNA with a RIN^e of 3.5
The 28S peak is degraded into smaller RNA fragments. Degraded RNA is characterized by an increase of low molecular weight fragments.



10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions