CTRNet Standard Operating Procedure Assesing Quality of Nucleic Acids			
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REVISION HISTORY

SOP Number	Date Issued	Author (Initials)	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

1.0 PURPOSE

Quality control is fundamental to the successful operation of a tissue bank offering tissue specimens and derivatives for research purposes. A high level of molecular integrity is essential for avoiding inconsistencies and variables into research studies. Nucleic acid quality is critically important for many techniques utilized in genomic analysis. CTRNet repositories should be confident that they are providing nucleic acids that are suitable for meaningful gene expression data. Testing procedures should be in place to monitor and assess the quality of the samples in collection.

The purpose of this procedure is to outline a minimum of testing and assessment that should be carried on tissue derivatives such as nucleic acids (DNA and RNA) to maintain quality and standardization of material.

2.0 SCOPE

This standard operating procedure (SOP) outlines minimum assessment and testing that that should be in place to evaluate the quality of DNA and RNA extracted in the repository; in order to provide investigators with a product that is consistent with their needs. The same metrics may also be used to assess the molecular integrity of selected tissue samples.

The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 REFERENCE TO OTHER POLICIES and SOPS

1. CTRNet Policy: POL 005.001 Records and Documentation
2. CTRNet Policy: POL 007.001 Material and Information Handling Policy
3. CTRNet Generic SOP # QA 001.001 Assessing Quality of Tissue Samples
4. CTRNet SOP# 8.2.003 Creating Derivatives RNA extraction from Blood samples
5. CTRNet SOP# 8.2.004 Creating Derivatives DNA extraction from Blood samples
6. CTRNet SOP# 8.2.008 Creating Derivatives DNA extraction from Tissue
7. CTRNet SOP# 8.2.009 Creating Derivatives RNA extraction from Tissue

4.0 ROLES AND RESPONSIBILITY

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

Tumour Bank Personnel	**Responsibility/Role**	**Site Specific Personnel and Contact Information**
Lab Technician	Conducts and Assists with quality assurance procedures. Records and documents outcomes.	

5.0 MATERIALS, REAGENTS, 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)
Appropriate tubes	
UV Spectrophotometer and quartz cuvettes	
Agilent Bioanalyser 2100	
RNA 6000 Nano Kit	
Thermocycler for PCR reaction	
Reagents for PCR reaction	
Reagents for Bioanalyzer	

6.0 DEFINITIONS

DNA: Deoxyribonucleic acid

Electropherogram: The Agilent 2100 Bioanalyzer provides a platform that uses a fluorescent assay involving electrophoretic separation to evaluate RNA samples qualitatively. The Agilent Bioanalyzer software creates a graph called an electropherogram, which diagrams fluorescence over time.

PCR: Polymerase Chain Reaction

Quality: Conformance of a specimen or process with pre-established specifications or standards.

Quality Assurance (QA): All those planned and systematic actions that are established to ensure that the Tumour Repository Program is performed and the data are generated, documented (recorded), and reported in compliance with applicable regulatory requirement(s).

Quality Control: Quality control is the system of technical activities that measures the attributes and performance of a process, or item, against defined standards, to verify that the stated requirements are fully met.

QMS: Same as QA above

RNA: Ribonucleic acid

UV: Ultraviolet

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 influence the quality of the extracted nucleic acids. These include: physiological state of the tissue prior to harvesting, post-resection intervals from collection to preservation, storage conditions etc.

These procedures outline minimum steps that should be followed to assess the molecular caliber of the samples in the collection.

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

1. Assessment of molecular integrity of the samples in the collection must be done on a percentage of the stored samples as deemed suitable.
2. Assessment of molecular integrity must be performed by a designated laboratory using established procedures developed for this purpose.
3. Use researcher feedback about sample quality to refine collection and storage practices and guide evolution of Quality Control procedures.
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.
5. Use the score in the interpretation of the quality assessment results.

7.2 Quality Assessment - DNA by spectrophotometric measurements, enzymatic digestion and gel electrophoresis.

1. DNA quality must be assessed in 1% percentage of the blood and tumour tissue samples stored as well as in 1% of the DNA extracted from the blood and tumour samples (using the CTRNet SOPs for the extraction of DNA).
2. Take UV spectrophotometric measurements to determine the DNA concentration.
3. Develop a standard of measurement against which relative DNA degradation can be compared. Use a standard DNA such as commercially available human genomic DNA that has been aliquoted and appropriately stored (to prevent DNA degradation from freeze/thaw cycles).
4. The quality assessment includes testing the suitability of the test sample DNA for use in enzymatic reactions (such as Hind III digestion) and visual assessment following agarose gel electrophoresis. Use a control sample as a reference.

5. Assign a quality score to the tested sample based on DNA quantification analysis (OD 260/280 ratio, agarose gel electrophoresis and restriction digestion):

DNA quality based on UV spectrophotometry

OD 260/280 ratio	**Assigned score**
1.8-2	4
1.6-1.8	3
1.4-1.6	2
1.2-1.4	1
Less than 1.2	0

DNA Quality based on electrophoresis of genomic DNA

Electrophoretic Integrity	**Assigned score**
Discrete high MW band	4
10kb smear	3
5kb smear	2
2kb smear	1
Less than 2kb smear	0

DNA Quality based on enzymatic digestion of genomic DNA

Electrophoretic Integrity	**Assigned score**
Discrete digestion band pattern	2
No band pattern	0

6. Add up the score. A score of 10 is indicative of high quality DNA, A score below 7 is indicative of poor quality DNA.

7.3 Quality Assessment - DNA by PCR

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.
2. The test and review must be performed by an individual, qualified by experience and training to do so.
3. 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 lenghts:

GH20 + PC04 = 268 base pairs (bp)
RS42 + KM29 = 536 bp
RS40 + RS80 = 989 bp
KM29 + RS80 = 1327 bp

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

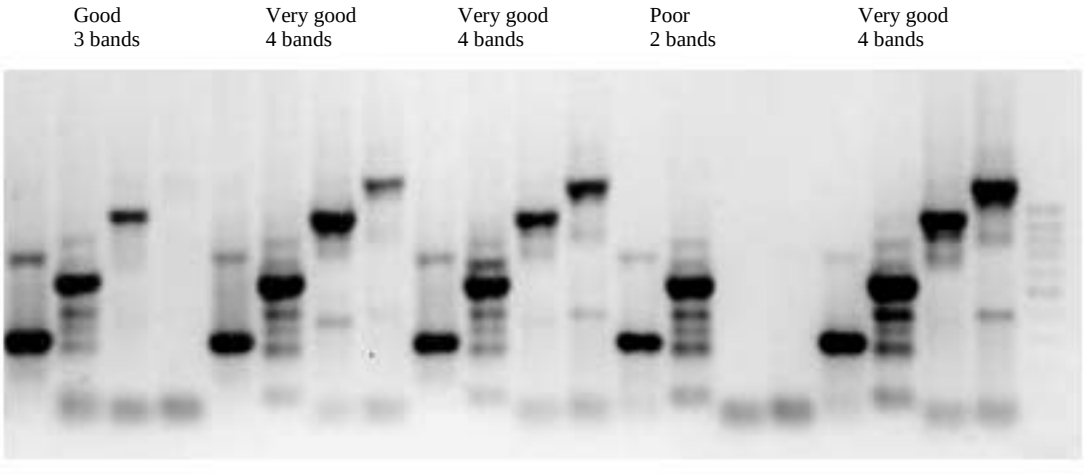
Master Mix:

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	H_2O
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

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 # HBMBSKIT2 adjust to suit alternate makes and model of thermocyclers)

6. Resolve on 1.2% agarose gel.
7. Sample results and scoring system for 4 primer pairs



7.4 Quality Assessment - RNA by spectrophotometric measurements and gel electrophoresis.

1. RNA quality must be assessed in 1% percent of the blood and tumour tissue samples stored as well as in the RNA extracted from the blood and tumour samples (using the CTRNet SOPs for the extraction of RNA).
2. Use a UV spectrophotometer measure the OD at 260 to determine RNA concentration.
3. Use the OD at 260/280 ratio to assess purity.
4. Run a small amount of the sample on a denaturing agarose gel to visualize the ribosomal RNA bands (28s and 18s). Crisp 28s and 18s bands are indicative of intact RNA. Also, a 2:1 ratio of 28s:18s species has been considered the benchmark for intact RNA (for more details on Assessing RNA quality see Reference # 8). This method consumes a large proportion of the sample so the method described under Section 7.5 is recommended.

7.5 Quality Assessment - RNA by using the Agilent Bioanalyzer.

The visual assessment described above is subjective and it is optimal to use an analytical tool such as the Agilent 2100 bioanalyzer (with RNA 6000 Nanoassay Kit) to determine the concentration and purity/integrity of RNA samples. It provides a readout 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.

1.0 Decontaminate Bioanalyzer Electrodes

1.1 Fill wells of the electrode cleaner with 350 µl of RNase ZAP and place in the bioanalyser for 1 minute.

1.2 Remove and replace with another electrode cleaner filled with RNase-free water for 10 seconds.
1.3 Remove and wait 10 seconds for the water on the electrodes to evapourate before closing the lid of the bioanalyzer

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

3.0 Prepare the gel-dye mix
3.1 Allow reagents to equilibrate to room temperature for 30 minutes before use.
3.2 Vortex dye concentrate for 10 seconds and spin down to the bottom of the tube.
3.3 Add 1 µl of the dye to a 65 µl aliquot of the filtered gel and vortex thoroughly.
3.4 Spin for 10 minutes at room temperature at 13000g in a microfuge.

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

5.0 Load the marker
5.1 Pipette 5 µl of the RNA Nano Marker into the well marked with the ladder `symbol and each of the 12 sample wells.

6.0 Loading the ladder and samples
6.1 Pipette 1µl of denatured ladder into the well marked with the ladder symbol.
6.2 Pipette 1 µl of each of the denatured samples into each of the sample wells.
6.3 Vortex the chip for 1 minute at 2400 rpm.
6.4 Insert the chip in the bioanalyzer and start the instrument.

For more information about using the bioanalyzer to assess the quality of RNA see the Application Note at:

http://www.hcnr.med.harvard.edu/programs/atrc/online%20library%20PDFs/Character%20of%20RNA%20quality%20using%20the%20Agilent%202100%20Bioanalyzer.pdf

For interpretation of graphs generated by graphs see Appendix 1

7.6 Quality Assessment - Records

1. Record test results for each quality assurance tested sample in the institution database or informatics system.
2. Use Ribosomal RNA bands (by electrophoresis and bioanalyser trace) to indicate RNA quality (See Section 7.4.4 above and Appendix 1). Present these results to researchers to allow them to determine if the sample quality is adequate for their particular research study.

8.0 APPLICABLE REferences, REGULATONS AND GUIDELINES

1. Declaration of Helsinki. http://ohsr.od.nih.gov/helsinki.php3 http://www.wma.net/e/policy/b3.htm
2. Tri-Council Policy Statement; 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, August 1998. http://www.pre.ethics.gc.ca/english/policystatement/policystatement.cfm
3. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics Series. http://www.mrc.ac.uk/pdf-tissue_guide_fin.pdf
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
5. US National Biospecimen Network Blueprint http://www.ndoc.org/about_ndc/reports/NBN_comment.asp
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.
7. Alberta Research Tumor Bank, Best Practices Guide, Version 2. 2006
8. Ambion TechNotes 11(1) Assessing RNA quality. http://www.ambion.com/techlib/tn/111/8.html
9. Character of RNA quality using the Agilent 2100 Bioanalyzer. Application notes. Agilent technologies. Accessed at: http://www.hcnr.med.harvard.edu/programs/atrc/online%20library%20PDFs/Charact

er%20of%20RNA%20quality%20using%20the%20Agilent%202100%20Bioanalyzer.pdf

10. RNA Quality Control with the Bioanalyzer. Fonds de la recherché en santé Quebec Tissue Bank Protocol. Version 4. 27/01/2006.

11. DNA quality control using PCR. Fonds de la recherché en santé Quebec Tissue Bank Protocol.

12. Interpreting Agilent Bioanalyzer Results. Version 1, November 2003, Oregon Health and Sciences University. http://www.ohsu.edu/gmsr/amc/info_docs/AMC_Bioanalyzer_Interpret.pdf

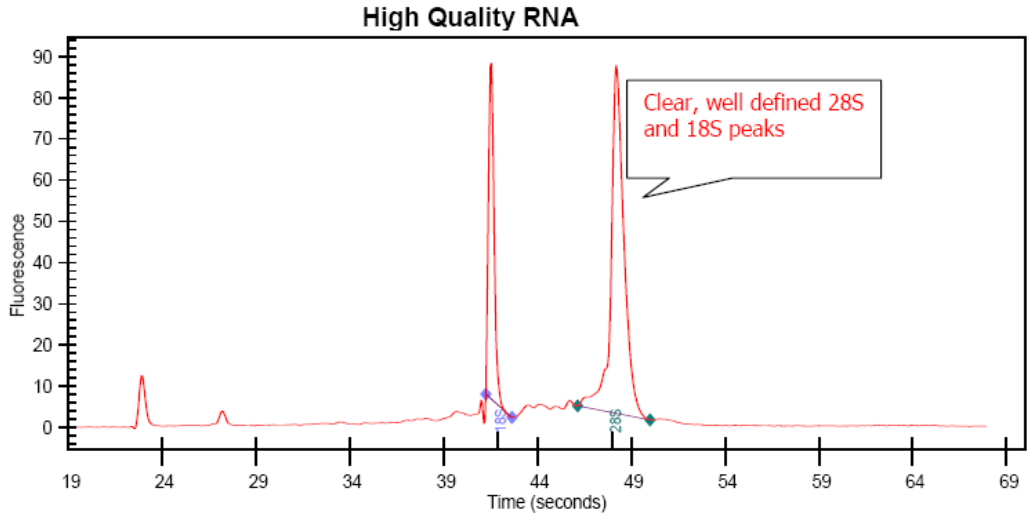
Appendix 1.

Interpreting Agilent Bioanalyzer Results

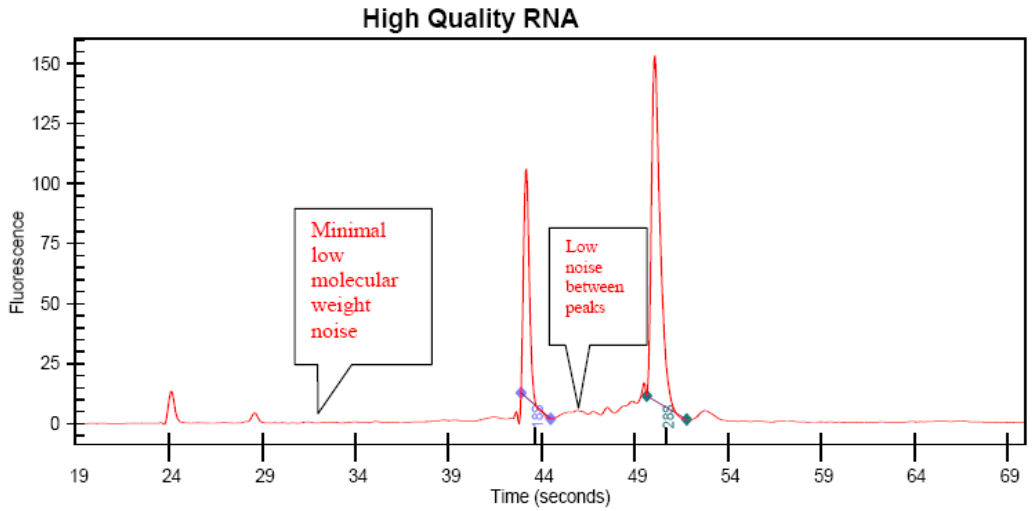
Below are diagrams displaying High Quality RNA, Marginally degraded RNA, and Highly degraded RNA.

A. Electropherogram showing High Quality RNA

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



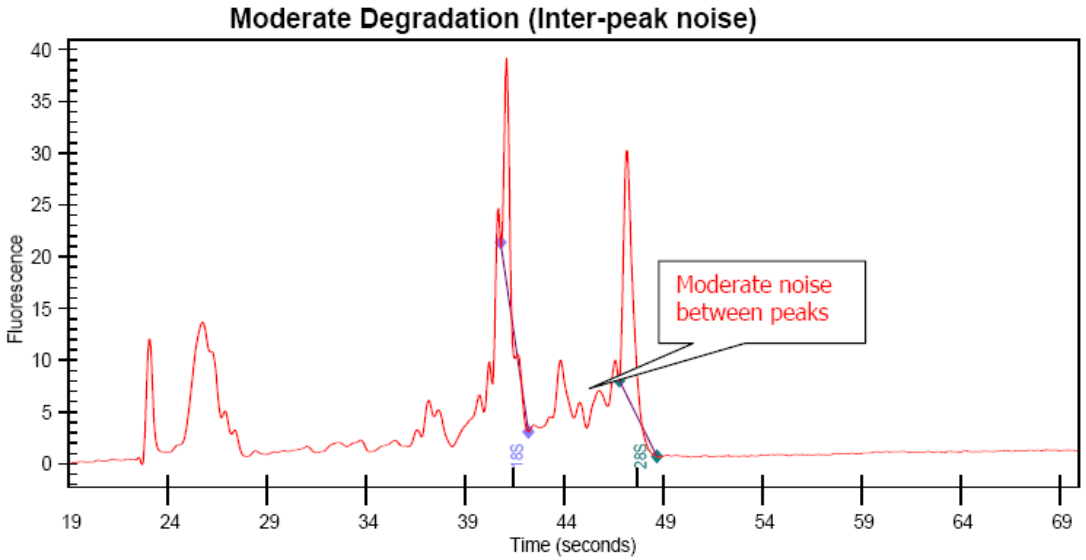
RNA Area 360.81
rRNA Ratio [28S / 18S] 1.92



RNA Area 398.29
rRNA Ratio [28S / 18S] 1.71

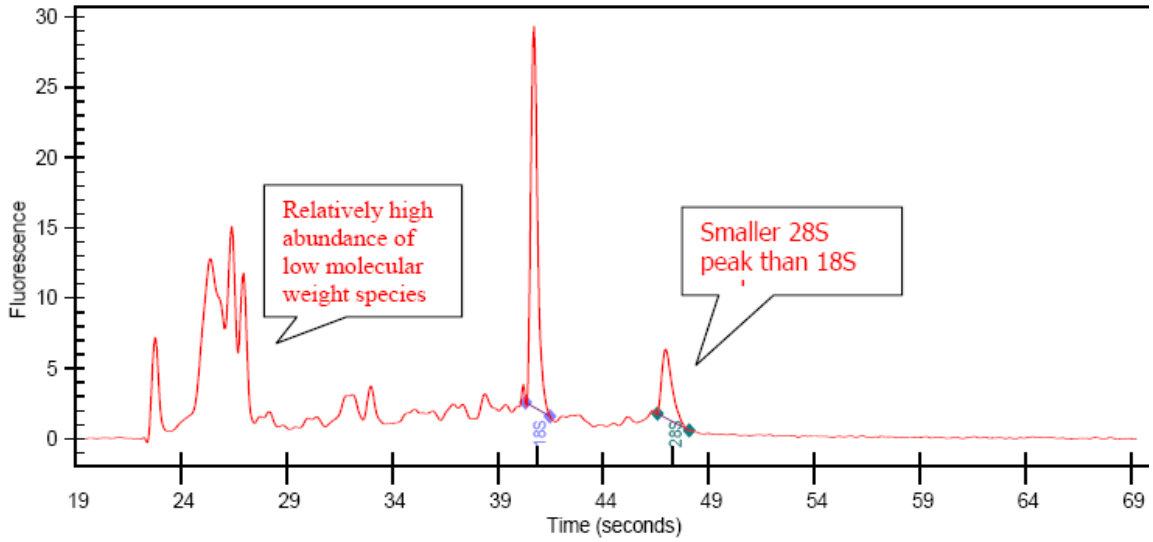
B. Electropherogram showing Marginally degraded RNA

Characterized by the presence of low molecular weight species, noise between 28S and 18S peaks, and a smaller 28S than 18S peak.



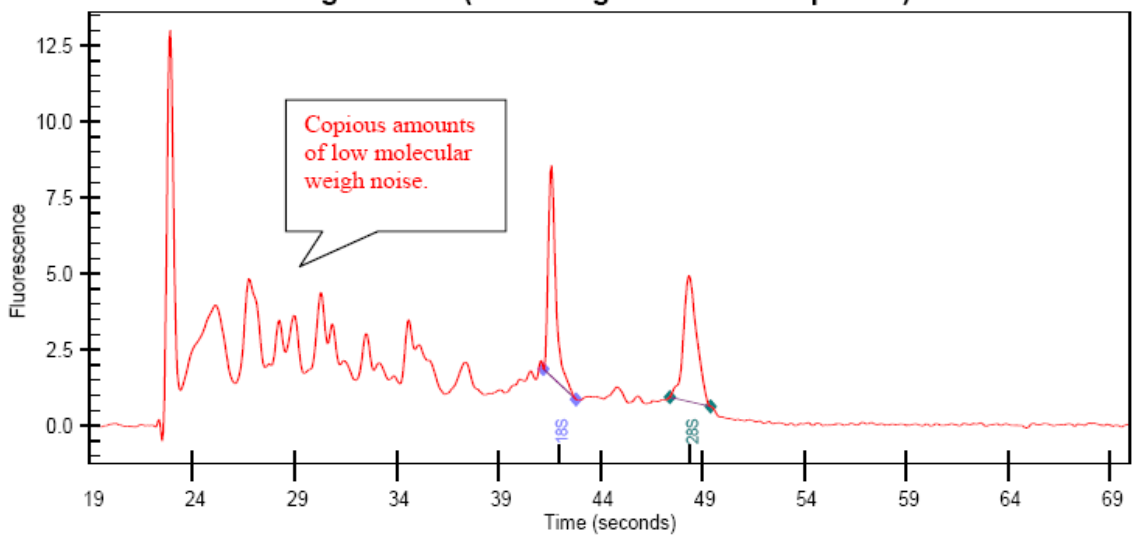
RNA Area 309.05
rRNA Ratio [28S / 18S] 1.70

Moderate Degradation (disproportionate 28S/18S ratio)



RNA Area 227.06
rRNA Ratio [28S / 18S] 0.24

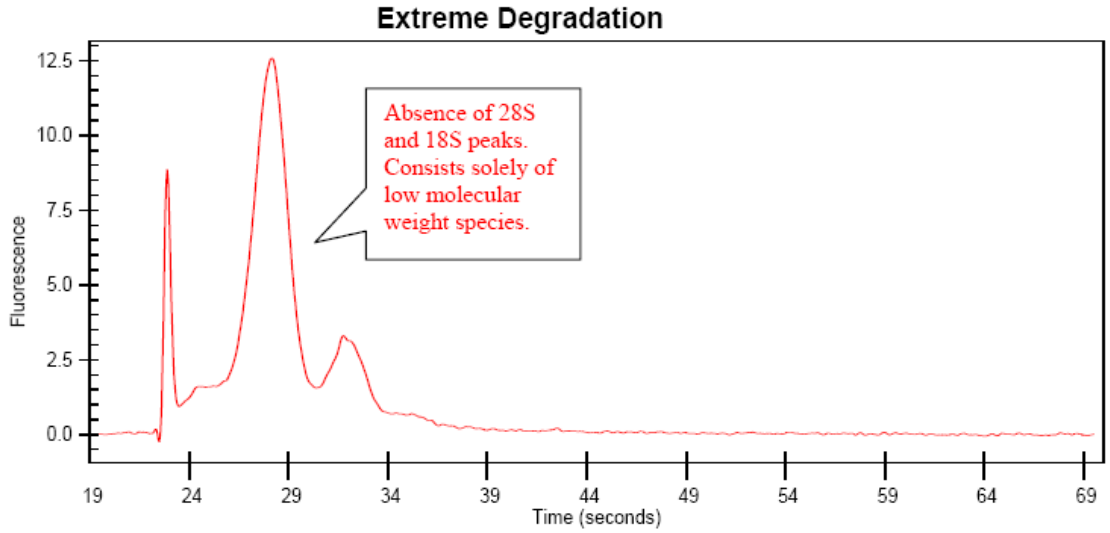
Moderate Degradation (Low Weight Molecular Species)



RNA Area 161.17
rRNA Ratio [28S / 18S] 0.91

C. Electropherogram showing highly degraded RNA

Characterized by the lack of the 28S and 18S peaks altogether and consists solely of low molecular weight species.



RNA Area 147.22
rRNA Ratio [28S / 18S] 0.00