Frozen Tissue Core Study: Analysis of RNA Extracted from Frozen Uterine Tissue Cores with the CryoXtract™ CXT 350 Frozen Sample Aliquotter

Promega Corporation

Materials Required:
- CyroXtract™ CXT 350 Frozen Sample Aliquotter
- Maxwell® 16 Instrument (Cat.# AS2000[1])
- Maxwell® 16 LEV simplyRNA Tissue Kit (Cat.# AS1280[2])
- Pellet Pestle™ Cordless Motor (Fisher Cat.# 12-141-361)
- QuantiFluor® RNA System (Cat.# E3310[1])
- NanoDrop® 1000 Spectrophotometer
- Agilent 2100 Bioanalyzer (Cat.# G2939AA)
- Agilent RNA 6000 Pico Kit (Cat.# 5067-1511)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *Maxwell® 16 Instrument Operating Manual,* #TM295, and *Maxwell® 16 LEV simplyRNA Cells Kit and Maxwell® simplyRNA Tissue Kit* #TM351.

[1]For Research Use Only. Not for use in diagnostic procedures.
[2]For Laboratory Use.

Introduction

Limiting pre-analytical variability in biological samples is crucial for scientific and translational medicine research. Inconsistent and improper handling of frozen biological samples is often a significant source of such variability and can result in the obscuring of data sets and scientific outcomes. CryoXtract's frozen dispensing technology presents a novel method for targeted and repeated access to frozen tissue samples without the need for freeze-thaw sampling. Labile biomolecules, such as RNA, are better preserved over repeated samplings when the parent sample is maintained in an ultra-cold state. In this study, total RNA was extracted from frozen normal uterine tissue samples derived from a single individual. Two sample-handling scenarios were compared and evaluated by measuring standard RNA quality attributes (i.e., yield, A_{260}/A_{280} ratios and RIN scores).

Methods

CryoXtract™ Tissue Handling and Coring Procedures

Fresh frozen normal human uterine tissue was obtained from the Cooperative Human Tissue Network (#60762T). As defined by the procurement protocol, the tissue was subdivided into four portions. Each portion was treated with RNA*later*® Solution for 24 hours, flash frozen in vapor phase liquid nitrogen, and placed into –80°C, storage for 10 days prior to this study. Two of the four portions stored (referred to as parent tissues A and B in Figure 1) were used in the following study. A slice was taken from each parent tissue as a precoring control (Samples AS1 and BS1). While frozen, five cores were taken from each parent tissue (Samples A1–A5 and B1–B5) using the CXT 350 Frozen Sample Aliquotter. Tissue A was then stored for two weeks at –80°C while tissue B was stored for two weeks at 4°C. After two weeks of storage, tissue B was refrozen, and five cores were taken from each tissue (samples A6–A10 and B6–B10). Samples taken as slices or as cores were immediately stored at –80°C until completion of sample gathering. All cores and remaining parent tissues were shipped to Promega for RNA extraction and analysis. Upon receipt, a post-coring control slice was taken from each parent tissue while frozen.

RNA Extraction and Analysis

RNA was purified from frozen cores of uterine tissue ranging from 15.1mg–34.4mg using the Maxwell® 16 LEV simplyRNA Tissue Kit. The cores were homogenized on ice with a Fisherbrand® Pellet Pestle (Cat. #12-141-361) in 400µl of Homogenization Solution/1-thioglycerol. 400µl of homogenate was added to

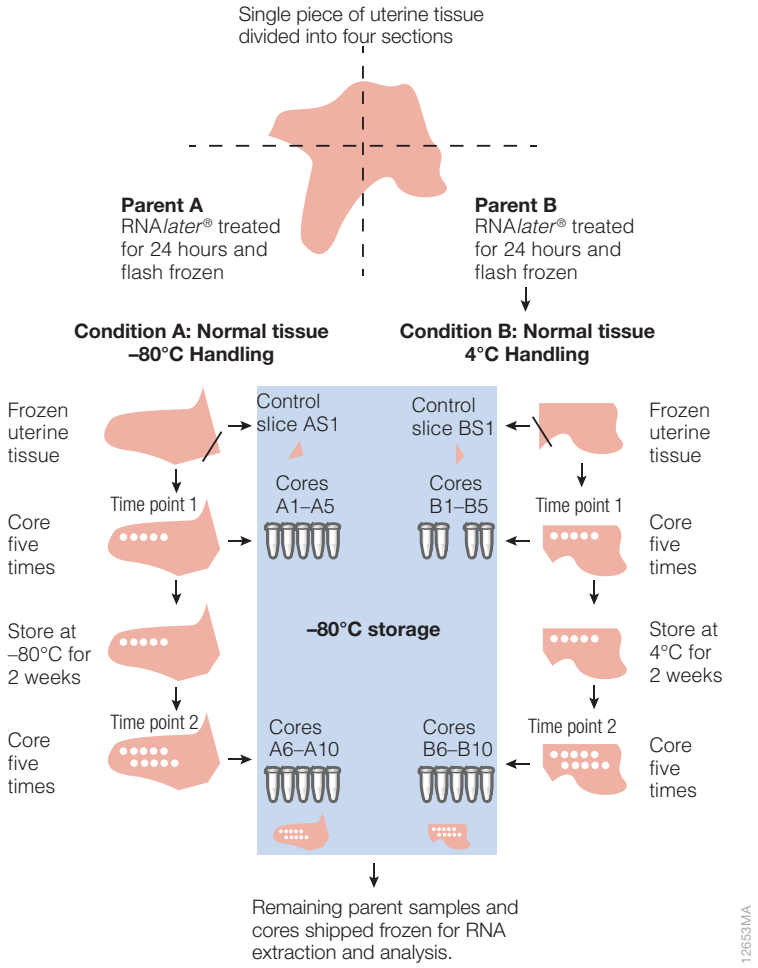

Figure 1. Tissue handling and coring procedures using the CXT 350 Frozen Sample Aliquotter.

400µl of Lysis Buffer and vortexed for 15 seconds. The 800µl suspension was split into two replicates. Both 400µl replicate samples were processed on the Maxwell® 16 instrument (Cat.# AS2000) using the Maxwell® 16 LEV simplyRNA Tissue Kit according to the Technical Manual (#TM351). The purified RNA was eluted into 50µl of Nuclease-Free Water. Each sample was quantitated using the Quantifluor® RNA System (Cat.# E3310), and RNA purity ratios were determined using the NanoDrop® 1000 spectrophotometer. RNA integrity was assessed using the Agilent 2100 Bioanalyzer and the Agilent RNA 6000 Pico Kit according to the Agilent RNA 6000 Pico Kit Guide (G2938-90046).

Results

Samples cored after storage at –80°C and again two weeks later (Condition A) yielded an average of 1.29 and 3.29µg of RNA, respectively. RNA yield was calculated by combining the total RNA obtained from the two replicate purifications from a single core (Table 1). Additionally, the total yield was normalized to the weight of the original core (Figure 2). RNA yields were somewhat variable between cores taken from each section of the tissue, but variability was independent of handling, which suggests that differences in yield are a result of tissue morphology and not a consequence of the coring procedure.

RNA purified from samples was pure as indicated by average A_{260}/A_{280} ratios of 1.92 for RNA from the initial cores and 2.01 for RNA from the cores taken after storage for two weeks at –80°. The A_{260}/A_{230} ratios averaged 1.74 for RNA from the initial cores and 1.79 for RNA prepared from cores taken after

Table 1. Condition A (Normal Tissue /–80°C Handling) Sample Results

Sample ID	Description	Weight of Core (mg)	QuantiFluor Total µg RNA	A_{260}/A_{280}	A_{260}/A_{230}
AS1	Precore Slice	21.6	0.31	n.d.	n.d.
A1	Initial Core	100	0.76	n.d.	n.d.
A2		25.3	0.61	n.d.	n.d.
A3		26.3	2.08	1.92	1.73
A4		27.4	0.94	1.83	1.61
A5		22.3	2.08	2.01	1.89
A6	Cores after 2 weeks at –80°C	30.7	2.60	2.02	1.90
A7		27.8	5.65	2.05	2.09
A8		30.6	3.71	2.00	1.81
A9		24.5	1.97	2.00	1.21
A10		15.1	2.51	1.97	1.94
AS2	Post-core Slice	26.5	1.28	1.89	2.56

The total yield and average purity ratios from replicate samples are shown. n.d. = not determined due to sample concentration near or below the lower limit of detection for the NanoDrop® 1000 Spectrophotometer.

Table 2. Condition B (Normal Tissue/ 4°C Handling) Sample Results.

Sample ID	Description	Weight of Core (mg)	QuantiFluor Total µg RNA	A_{260}/A_{280}	A_{260}/A_{230}
BS1	Precore Slice	34.2	0.10	n.d.	n.d.
B1	Initial Core	23.9	5.52	2.06	1.90
B2		28.6	6.93	2.06	2.00
B3		26.9	2.57	1.97	1.63
B4		34.4	0.50	n.d.	n.d.
B5		26.5	0.30	n.d.	n.d.
B6	Cores after 2 weeks at 4°C	26.3	0.08	n.d.	n.d.
B7		20.4	1.46	1.76	1.26
B8		20.2	1.01	1.95	1.43
B9		23.4	1.34	1.83	1.42
B10		20.8	0.07	n.d.	n.d.
BS2	Post Core Slice	39	0.72	n.d.	n.d.

The total yield and average purity ratios from replicate samples are shown. n.d. = not determined due to sample concentration near or below the lower limit of detection for the NanoDrop® 1000 Spectrophotometer.

storage for two weeks at –80°C . Purity ratios for samples near or below the lower limit of detection for the NanoDrop® 1000 Spectrophotometer could not be accurately assessed (samples <10ng/µl). Overall, no decrease in yield or purity was observed as a result of the coring process or storage at –80°C.

Samples cored after storage at 4°C yielded an average of 0.79µg of RNA, compared to an average of 3.16µg before 4°C storage (Figure 2). Initial cores taken resulted in good purity ratios, with average A_{260}/A_{280} and A_{260}/A_{230} purity ratios of 2.03 and 1.84, respectively. However, after the tissue was stored for two weeks at 4°C, lower purity ratios were observed with average A_{260}/A_{280} and A_{260}/A_{230} purity ratios of 1.85 and 1.37, respectively (Table 2).

RNA Integrity Numbers, or RINs, were measured using the Agilent 2100 Bioanalyzer and the Agilent RNA 6000 Pico Kit. RNA from the initial cores taken had high RIN values (averaging 8.7), demonstrating that the coring process had no detectable impact on RNA integrity. For samples cored after the two-week storage at –80°C (Condition A), RIN values averaged 9.2, with no decrease in average RIN values observed (Figure 2). Conversely, RNA from samples cored after storage for two weeks at 4°C showed a significant decrease in RIN values, with an average of 6.3, when compared to the initial cores that averaged 8.3 (Condition B). This RIN data indicates that the freeze/thaw process and 4°C storage had a negative impact on RNA integrity even in the presence of RNA*later*® Solution.

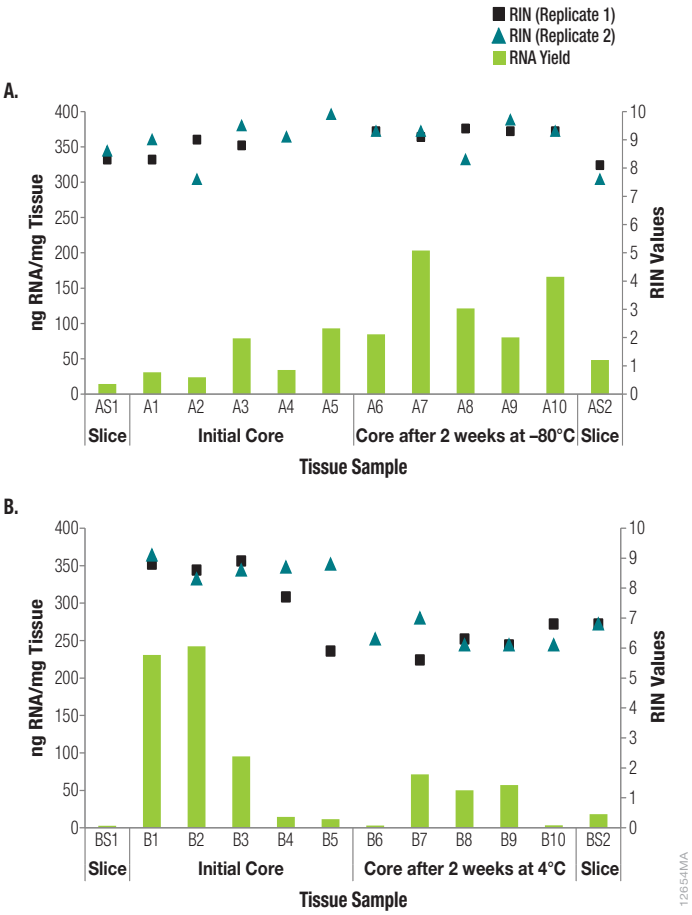

Figure 2. RNA yield per mg of core tissue. Panel A. Normal Tissue/–80°C Storage. **Panel B.** Normal Tissue/4°C Storage. RNA yield from two extractions was used to calculate amount of RNA per tissue core, and this value was normalized to the weight of each tissue core. RIN values indicating RNA integrity for each replicate are shown.

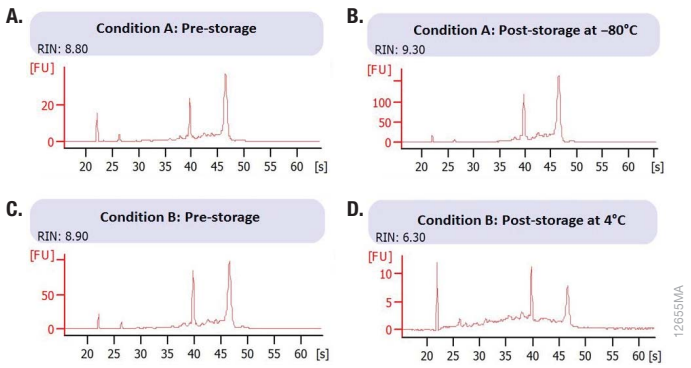

Figure 3. Example of electropherograms produced by the Bioanalyzer. Panel A. Condition A, prestorage at –80°C. Panel B. Condition A, post-storage at –80°C. Panel C. Condition B, prestorage at 4°C. Panel D. Condition B, post-storage at 4°C. Only one replicate is shown for each set.

Conclusion

Multiple freeze-thaw cycles can damage the integrity of tissue and labile biomolecules like RNA, even when actively stabilized, making a frozen-handling alternative appealing. Uterine tissue stored at –80°C, cored using the CryoXtract ™ CXT 350 Frozen Sample Aliquotter, provided tissue cores with RNA that was pure and of high integrity. The same tissue cored again after –80°C storage for two weeks showed no apparent decrease in quality. Tissue stored at 4°C showed a decrease in purity as measured by the A_{260}/A_{280} and A_{260}/A_{230} ratios, as well as a decrease in integrity as measure by RIN values. This highlights the importance of limiting freeze-thaw cycles when handling tissue samples and the benefit of the CryoXtract™ CXT 350 Frozen Sample Aliquotter, which allows frozen sample core collection.