

# Analysis of the Molecular Quality of Human Tissues

## An Experience From the Cooperative Human Tissue Network

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### Abstract

*The scientific usefulness of the data obtained from tissue analysis is related to specimen quality, which may be affected by conditions that may contribute to the degradation of the specimen before processing and analysis.*

*We determined the usability of nucleic acids extracted from banked human tissues for further molecular analyses. We assayed 151 tissue specimens, stored for various times at 4 divisions of the Cooperative Human Tissue Network, National Cancer Institute, Bethesda, MD, for DNA and RNA degradation. Simple electrophoresis, polymerase chain reaction (PCR), reverse-transcriptase (RT)-PCR, and Northern blot analysis were compared to determine the optimal quality control procedure. In addition, a time course degradation procedure was performed on human lung tissue.*

*Gel electrophoresis was as informative as PCR, RT-PCR, and Northern blot analysis in determining the molecular usefulness of the human tissues. Overall, 80% of the stored human tissues had good-quality DNA, and 60% had good-quality RNA. Electrophoresis procedures for DNA and RNA offer a quick and valuable measure of the molecular quality of stored human tissues. The DNA and RNA degradation of one tissue type (lung) was stable for both nucleic acids for up to 5 hours after excision.*

Clinical and molecular pathology are undergoing a revolution based on the accelerated advances in biotechnology such as DNA microarrays and proteomics. Answers to fundamental questions such as “How does the DNA sequence differ between individuals?” and “What makes one individual more prone for a certain disease?” are eagerly sought in this postgenomic era. Several novel genes and their products have been identified in human cancers by screening archival tissue samples using molecular methods.<sup>1,2</sup> More important, molecular diagnostic testing is requested more often in certain clinical conditions, eg, testing for T- or B-cell clonality in early cutaneous lymphomas.<sup>3</sup>

The scientific usefulness of the data obtained from the analysis of tissues is related directly to the quality of the tissue specimen. The most ideal tissue specimen is one that carries a complete and unaltered representation of the tissue in vivo. While this may be close to impossible ex vivo, it is critical that we understand and attempt to improve on the quality of research specimens. Unlike animal tissues, which can be controlled to maximize the quality of the specimen, human tissues used for research often are subjected to conditions that may contribute to the degradation of the specimen before they can be appropriately processed and analyzed. For example, factors such as the time the vascular system is compromised before surgical removal of a tissue may affect the quality of the tissue. With the growing number of investigators requesting human tissues for research and an increasing use of tissue microarrays and high-throughput molecular profiling to evaluate diseased tissue, it is important to provide investigators with information about the molecular quality of the tissue.<sup>1,2</sup>

An often-used method for rapid evaluation of the yield of the nucleic acid extracted from cells or tissue is to determine

the ratio of optical absorbance at 260 and 280 nm. However, the ratio suggests degree of protein contamination and does not necessarily reflect the quality of nucleic acid.<sup>4,5</sup> The expression of 28S ribosomal RNA (rRNA) expression is considered the best marker for assessing the integrity of the nucleic acid in the tissue because it remains stable in stimulated and nonstimulated conditions.<sup>6</sup> Several factors, including the specimen type, preexcision hypoxia, preservation treatment of the tissue, extraction method, type and length of storage, and freeze and thaw factors affect the molecular quality of the tissue.<sup>3,7</sup>

General dogma suggests the following<sup>5-14</sup>: (1) Acellular and pancellular tissues are less labile than densely cellular tissue.<sup>6,14</sup> (2) Hypoxic conditions initiate cell death mechanisms and subsequent degradation.<sup>10</sup> (3) Quick treatment to inactivate degrading enzymes is important for preserving tissue and nucleic acid integrity.<sup>9</sup> (4) Fresh, unfixed tissue is better than chemically fixed tissue for extracting nucleic acids.<sup>11</sup> (5) Short periods of storage yield better quantity and quality of nucleic acid than longer periods.<sup>7,12</sup> (6) With frozen tissue, nucleic acid preservation is enhanced by thawing the tissue as quickly as possible.<sup>8</sup>

Human tissues procured for research are frozen and stored for variable periods. Conventionally, it is thought that the longer the period of storage, the lower the yield or quality of the nucleic acid. Preliminary data from a study at the University of Alabama at Birmingham (W.H.G., oral communication, November 2000) and a recent survey by us (unpublished data) suggested that the vast majority of the investigators rated tissues procured from the Cooperative Human Tissue Network (CHTN) as a good source of DNA and RNA usable for further molecular analysis. However, there is a lack of a controlled study to validate these observations. The purpose of the present study was to determine the usability of nucleic acids extracted from banked human tissues for further molecular analyses. We suggest a quality control method for evaluating the molecular integrity of the tissue before distribution.

Materials and Methods

Human Tissues

We obtained 151 stored human tissue specimens from the 4 adult (tissue) divisions of the CHTN **Table 1**. The CHTN, a National Institutes of Health–funded organization, specializes in the prospective procurement, preservation, and distribution of various human tissues, including normal, diseased, benign, and malignant tissues for institutional review board–approved investigators.<sup>8</sup> The tissues supplied to researchers are not tissues removed specifically for

**Table 1**  
Cohort of Human Tissues Assayed for Nucleic Acid Quality and Integrity\*

Tissue Type	Months of Storage				Total
	0-1	3-6	6-12	≥12	
Breast	13	8	8	4	33
Colon	33	6	14	14	67
Liver	6	4	0	2	12
Lung	7	4	6	6	23
Ovary	10	0	0	2	12
Endometrium	1	0	0	2	3
Cervix	1	0	0	0	1
Total	71	22	28	30	151

\* Data are given as number of tissue samples.

research but are “excess” tissues not essential for routine diagnosis and staging. Patient confidentiality is maintained.<sup>9</sup>

Genomic DNA Isolation

The PUREGENE DNA Isolation Kit (Gentra Systems, Minneapolis, MN) was used to isolate genomic DNA from snap-frozen tissue. Samples weighing between 70 and 100 mg were prepared for RNA isolation. Each specimen was placed in a chilled bag and immediately immersed in liquid nitrogen. The bag was removed briefly to the chilled platform, where the sample was pulverized with a mallet. The sample was returned occasionally to the liquid nitrogen to ensure that it remained frozen. After the tissues were ground to a powder, each was homogenized in 12 volumes of lysis/binding solution. The lysate was incubated in Proteinase K solution (20 mg/mL) overnight at 55°C. After washing, the contaminating RNA was removed with a 30-minute incubation with ribonuclease (RNase) A solution (4 mg/mL). After heat inactivating the RNase, the contaminating proteins were removed by precipitating with the protein precipitation solution. DNA in the supernatant was precipitated with isopropanol, washed with 70% ethanol, and hydrated in 150 µL of DNA hydration solution. Spectrophotometric measurements at 260 and 280 nm were taken to determine the concentrations of RNA and DNA.

DNA Electrophoresis

For electrophoresis, 100 ng of genomic DNA was loaded onto 0.7% agarose minigels stained with ethidium bromide. Gels were electrophoresed for 3 hours at 30 V. A 1-kilobase (kb) DNA ladder was run as a marker in each gel.

Polymerase Chain Reaction

Genomic DNA polymerase chain reaction (PCR) was performed with 1 µg of DNA as the template in a 50-µL reaction volume consisting of a 20-mmol/L concentration of tris(hydroxymethyl)aminomethane-hydrochloride, a

50-mmol/L concentration of potassium chloride, a 1.5-mmol/L concentration of magnesium chloride, a 200-nmol/L concentration of each primer (hypoxanthine-guanine phosphoribosyltransferase [HPRT]: 5'-gTc TcT cTg TAT gTT ATA TgT cAc-3' and 5'-Tgc gTg TTT TgA AAA ATg AgT gAg-3')<sup>8</sup> for a 379-base-pair (bp) genomic HPRT, a 200- $\mu$ mol/L concentration of deoxynucleoside triphosphates, and 1.25 U of *Taq* DNA Polymerase (GibcoBRL Life Technologies, Gaithersburg, MD). Samples were denatured initially for 5 minutes at 95°C, then cycled through 30 cycles of 94°C for 1 minute, 56°C for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 5 minutes. Products were visualized on 1% agarose gels stained with ethidium bromide.

### Total RNA Isolation

Total RNA was isolated from 50- to 75-mg specimens using the RNAqueous kit (Ambion, Austin, TX). Before isolation, a stainless steel platform (1  $\times$  4-in diameter) was chilled to -80°C. The 4  $\times$  6-in KAPAK/SCOTCHPAK heat sealable pouches (Kapak, Minneapolis, MN) were submerged in liquid nitrogen. Tissues were powdered as described above and homogenized in cell lysis solution. The lysate was passed initially through a 21-gauge needle 10 times, then spun through a QIA shredder column (QIAGEN, Valencia, CA). The homogenized lysate mixed with an equal volume of 64% ethanol was applied to an RNAqueous filter cartridge (QIAGEN). The bound RNA was washed, dried, and eluted.

### RNA Electrophoresis

For RNA electrophoresis, 8 ng of total RNA mixed with an equal volume of glyoxal loading dye (NorthernMax-Gly Kit, Ambion) was denatured by heating at 50°C for 30 minutes and loaded onto 1% agarose minigels stained with ethidium bromide. Gels were electrophoresed for 1 hour at 60 V. An RNA ladder was run as a marker in each gel.

### Northern Blot Analysis

The RNA gels were photographed under UV light and transferred to positively charged nylon membranes (Nytran SuPerCharge, Schleicher & Schuell, Keene, NH) using downward transfer for 1 hour with transfer buffer (Ambion). Overnight hybridization with a 0.1-mmol/L concentration of psoralen-biotin-labeled riboprobe, proceeded by prehybridization, was performed in ULTRAhyb (Ambion) at 68°C according to the manufacturer's instructions. An initial low-stringency wash was done at room temperature for 10 minutes, followed by 2 high-stringency washes at 68°C. The Southern-Star Chemiluminescent Detection System (Tropix, Bedford, MA) was used to detect bound probe. Blocking, conjugate incubation, and detection were done according to the manufacturer's instructions. Blots were exposed to BioMax Light Imaging Film (Eastman Kodak, Rochester,

NY) for 2 to 20 minutes, and films were developed in an automatic processor.

For Northern hybridization, a human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) complementary DNA (cDNA) control probe was purchased from Clontech (Palo Alto, CA). This is a cDNA fragment, located from 71 to 1,053 bp, attached to a T7 RNA polymerase promoter.<sup>9</sup> The Strip-EZ RNA Kit (Ambion) was used to create a riboprobe that could be stripped completely from the blot. Equal volumes of 10-mmol/L concentrations of adenosine triphosphate, guanosine triphosphate, and uridine triphosphate and a 2-mmol/L concentration of modified cytidine triphosphate were added to the template probe in the transcription reactions. The reactions were incubated for 2 hours at 37°C, followed by a 20-minute deoxyribonuclease (DNase) treatment at 37°C. An ammonium acetate precipitation removed free nucleotides from the reaction. Psoralen-biotin was intercalated into the riboprobe using the BrightStar Psoralen-Biotin Labeling Kit (Ambion) according to the manufacturer's instructions. Labeling was verified with the detection of dot blots of serially diluted probe. As little as 1 pg could be detected.

For RT-PCR, 1  $\mu$ g of RNA was reverse transcribed in a 20- $\mu$ L reaction volume using 50 ng of random hexamers, then RNase treated according to the manufacturer's instructions (SUPERScript Preamplification System for First Strand cDNA Synthesis, GibcoBRL Life Technologies). RT-PCR was performed in a 50- $\mu$ L reaction volume consisting of a 5- $\mu$ L aliquot of the RT reaction and a 200-nmol/L concentration of each primer (HPRT, 5'-gTA ATg Acc AgT cAA cAg ggg Ac-3' and 5'-ccA gcA Agc TTg cgA ccT TgA ccA-3')<sup>8</sup> for a 177-bp HPRT messenger RNA (mRNA) fragment under the same conditions as PCR. Products were visualized on 1% agarose gels stained with ethidium bromide.

### Time Course Degradation Experiment

Fresh lung tissue was procured and immediately dissected into 0.2-gm aliquots. The aliquots were snap frozen at definite periods beginning 15 minutes after procurement to 30 minutes and every hour for 5 hours. The DNA and RNA were extracted at each time point and evaluated by RT-PCR or PCR as described.

## Results

Several assays were used to evaluate the molecular quality of DNA and RNA isolated from stored human tissues.

### DNA and RNA Electrophoresis

All tissue specimens were assayed by electrophoresis. To assess the degree of degradation in the tissue samples, a standard of measurement was developed. ■Image 1A■ shows

that treatment of 1  $\mu$ g of human placental genomic DNA with 0.25 U of DNase resulted in partial degradation with detection of 1 kb of DNA intact but complete degradation at higher concentrations of DNase. Similarly, treatment with 5 ng and higher concentrations of RNase resulted in complete degradation of total RNA isolated from normal liver tissue (Image 2A). Lesser degrees of degradation in RNA resulted in several bands between the 28S and 18S bands. Scoring of the degradation in each tissue was standardized to the relative degradation of a pure DNA or RNA standard. Usable quality of DNA was considered present if the electrophoresis of tissue DNA showed discrete banding at the top of the DNA ladder (12 kb). The DNA quality was graded 1+ (no visual degradation, lane 1, Image 1A), 2+ (mild to moderate degradation, lane 2, Image 1A), or 3+ (excessive degradation, lanes 3 and 4, Image 1A). In Image 1B, the DNA integrity from 7 of 9 specimens shown was graded usable, and the DNA from specimens 26 and 32 was partially degraded and graded as +2.

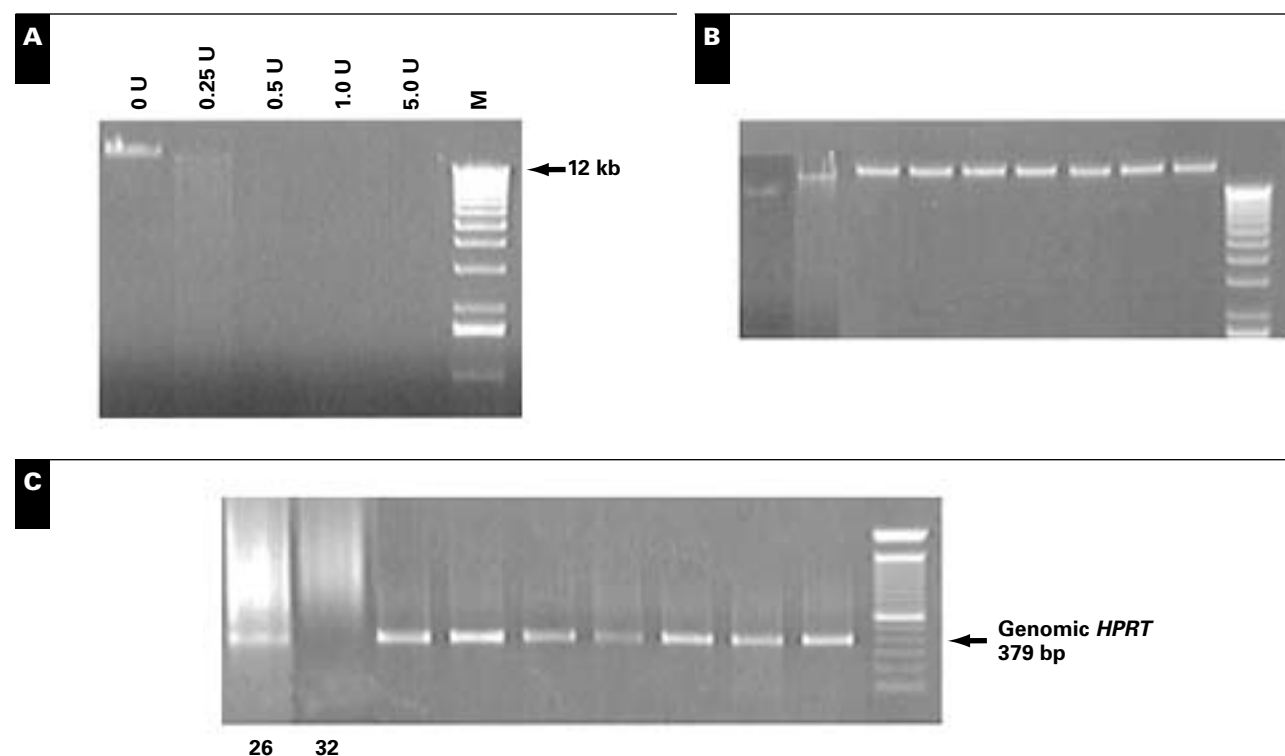
Good-quality RNA was represented by clearly detectable rRNA 28S and 18S bands. The RNA in 6 of the 8 specimens shown was graded usable (Image 2B). Although specimen 47 appears degraded, we repeated the electrophoresis and graded it as +1 (data not shown).

### Genomic DNA PCR

PCRs were performed to evaluate the usability of the DNA. Image 1C shows a distinct 379-bp PCR product specific for the *HPRT* gene in the tissues analyzed. Comparison of Image 1B and Image 2B reveals that tissues exhibiting good-quality DNA by electrophoresis also have good DNA integrity as evidenced by good amplification of the PCR product.

### RT-PCR for *HPRT* mRNA

RT-PCR was performed from the total RNA isolated from all tissues. Image 2C shows a distinct 177-bp RT-PCR product specific for the *HPRT* mRNA in all the tissues. Good



**Image 1** DNA degradation standardization, gel electrophoresis and polymerase chain reaction. **A**, Human placental genomic DNA, 1  $\mu$ g, was treated with increasing concentrations of deoxyribonuclease (DNase) as indicated. Untreated DNA (lane 1) is graded as +1, DNA treated with 0.25 U of DNase exhibiting degrading DNA as a 1-kilobase (kb) band is graded as +2. At 0.5 U and greater DNase concentrations, complete degradation of DNA is indicated by a smear and graded as +3. M, DNA ladder with the largest DNA band at 12 kb. **B**, Genomic DNA of the indicated specimen (100 ng) and a 1-kb DNA ladder were electrophoresed on 0.7% agarose minigels for 3 hours at 30 V. Good-quality DNA (score = 1), is seen at the top of the gel in all specimens except specimens 26 (score = 2) and 32 (score = 3) (see the "Materials and Methods" section). **C**, Ten-microliter aliquots of polymerase chain reaction products and a 100-base-pair (bp) DNA ladder were electrophoresed on 1% agarose minigels for 30 minutes at 70 V. A 379-bp product specific for the *HPRT* gene is visualized in all specimens.

correlation is observed between the RNA gel electrophoresis and RT-PCR (Images 2B and 2C).

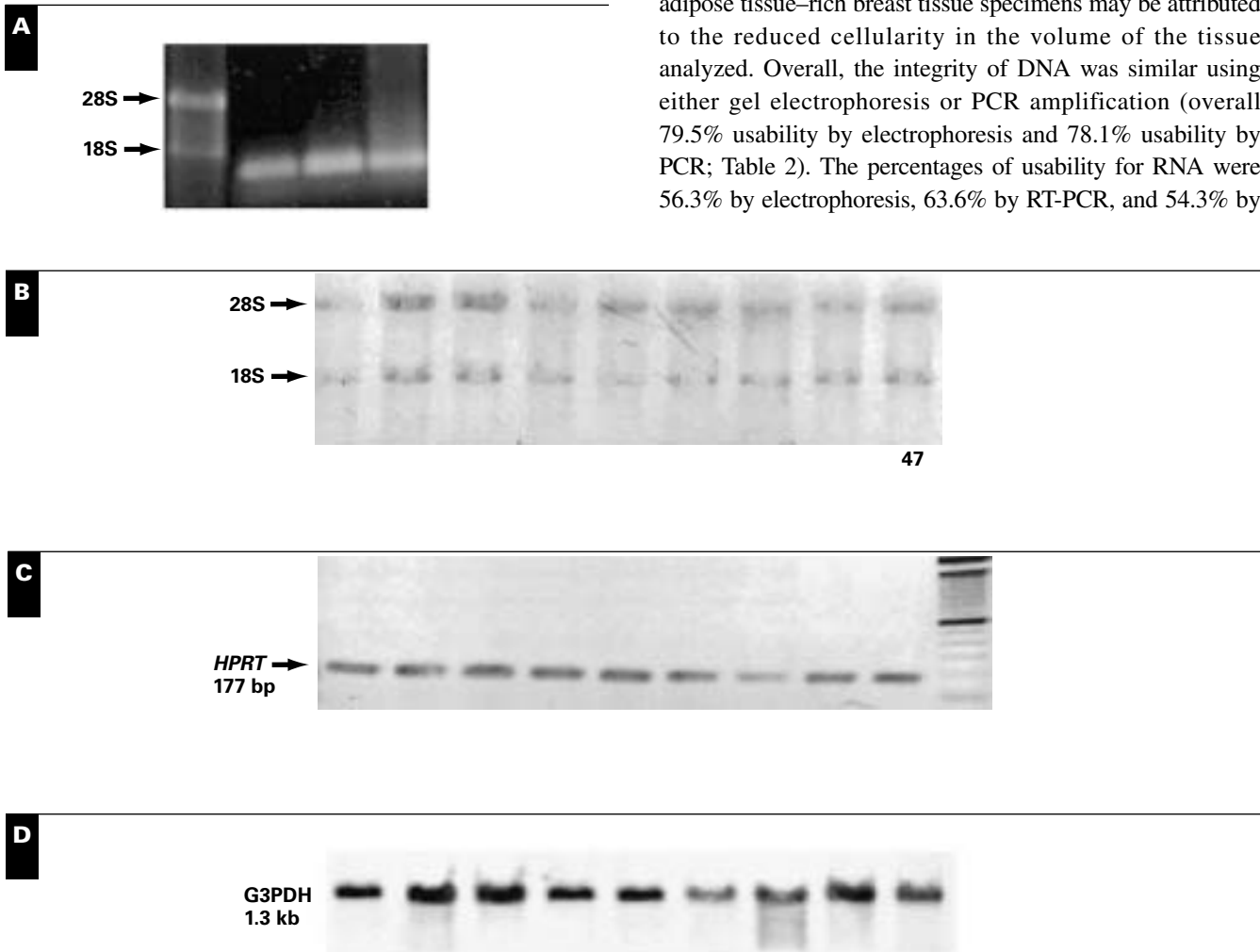
### Northern Blot Assays for G3PDH

The Northern blot procedure for RNA was carried out using a psoralen-biotin-labeled G3PDH riboprobe as described. **Image 2D** shows the 1.3-kb gene product specific for the G3PDH, suggesting that all tissues that

exhibited undegraded total RNA (28S rRNA band in Image 2B) are a source of good-quality, nondegraded mRNA. The data for all tissues are summarized in **Table 2**.

### Comparative Analysis of Experimental Methods to Assess the Molecular Quality of Tissues

DNA was detectable in 79.5% of tissues by electrophoresis and 78.1% by PCR (Table 2). Most of the tissue types had between 90% and 100% usable DNA, except for breast tissue (52% usable DNA). The lower yield in the adipose tissue-rich breast tissue specimens may be attributed to the reduced cellularity in the volume of the tissue analyzed. Overall, the integrity of DNA was similar using either gel electrophoresis or PCR amplification (overall 79.5% usability by electrophoresis and 78.1% usability by PCR; Table 2). The percentages of usability for RNA were 56.3% by electrophoresis, 63.6% by RT-PCR, and 54.3% by



**Image 2** Quality of RNA by electrophoresis, Northern blot, and reverse transcriptase-polymerase chain reaction (RT-PCR). **A**, Two-microgram aliquots of total RNA extracted from normal adjacent liver tissue were incubated with increasing concentrations of ribonuclease (RNase) as indicated. RNase was neutralized with 40 U of RNase inhibitor before gel electrophoresis. The complete loss of 18S and 28S rRNA at 5.0 ng and greater concentrations indicates complete degradation. **B**, Eight micrograms of total RNA extracted from the indicated tissue specimens and an RNA ladder mixed with an equal amount of glyoxal dye were denatured at 50°C for 30 minutes and electrophoresed on 1% agarose minigels for 1 hour at 60 V. Good-quality RNA as evidenced by the 18S and 28S bands is seen in all specimens. **C**, Ten microliters of the RT-PCR products and a 100-base-pair (bp) DNA ladder were electrophoresed on 1% agarose minigels for 30 minutes at 70 V. A 177-bp product specific for the hypoxanthine-guanine phosphoribosyltransferase (HPRT) messenger RNA is visualized in all specimens. **D**, Eight micrograms of total RNA was transferred onto a positively charged nylon membrane and probed with a psoralen-biotin-labeled glyceraldehyde-3-phosphate dehydrogenase (G3PDH) riboprobe. A 1.3-kb gene product specific for G3PDH is observed in all specimens.

**Table 2**  
DNA and RNA Usability Determined by Electrophoresis, PCR, RT-PCR, and Northern Blot Analysis\*

Tissue Type	DNA				RNA					
	Electrophoresis		PCR (379-bp Product)		Electrophoresis		RNA RT-PCR (177-bp Product)		Northern Blot Analysis (1.27 kb)	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Breast (n = 33)	17	16	17	16	9	24	9	24	6	27
Cervix (n = 1)	0	1	0	1	0	1	0	1	0	1
Colon (n = 67)	56	11	56	11	44	23	52	15	44	23
Endometrium (n = 3)	3	0	3	0	1	2	1	2	1	2
Liver (n = 12)	12	0	11	1	10	2	9	3	10	2
Lung (n = 23)	21	2	19	4	12	11	16	7	12	11
Ovary (n = 12)	11	1	12	0	9	3	9	3	9	3
Total (n = 151)	120	31	118	33	85	66	96	55	82	69

PCR, polymerase chain reaction; RT-PCR, reverse transcriptase–polymerase chain reaction.  
\* Yes and No indicate usable and unusable, respectively. See the “Results” section for an explanation of the scoring.

Northern blot analysis (Table 2). Similar to DNA, RNA usability was lower in breast tissue (18%-27%).

**The Effect of Storage on the Molecular Quality of Tissues**

Most of the tissues analyzed had been stored at –80°C for variable times (Table 1). The overall stability of the DNA was fairly consistent during the entire time for all tissue types. We observed an initial drop in the graded usability of the DNA after the first month of storage in some tissues (data not shown).

**Time Course Degradation Experiment**

Molecular analyses were performed to determine the effect of delay in fixing the tissues after procurement. **Image 3A** shows that high-quality, high-molecular-weight DNA was obtained from the human lung tissue even after a time lapse of 5 hours. The integrity of DNA at all time points was good, as evidenced by the 379-bp PCR product of the *HPRT* gene **Image 3B**. Intact RNA was isolated at all time points, as indicated by the 28S and 18S ribosomal units **Image 3C**. The usability of the RNA was confirmed by the RT-PCR amplification of a 177-bp *HPRT* mRNA fragment **Image 3D** and Northern blot hybridization of a psoralen-biotin–labeled G3PDH riboprobe to immobilized and denatured RNA **Image 3E**.

**Discussion**

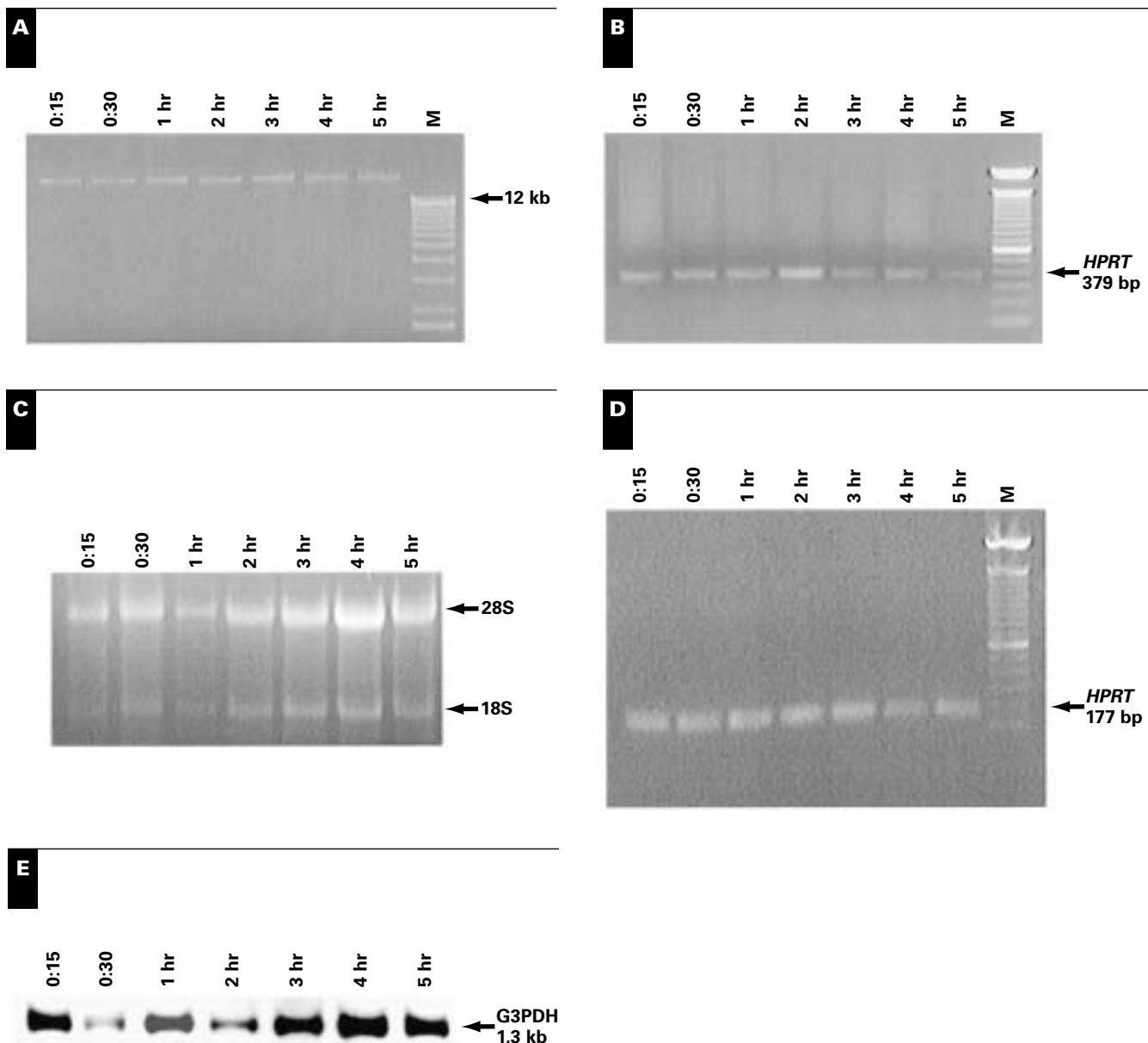
There is a general assumption that tissues procured for research represent a valid source of good-quality DNA and RNA. We tested this assumption by evaluating 151 human tissue samples procured from 4 divisions of the CHTN for their nucleic acid content and quality. A consistent electrophoretic

pattern for DNA and RNA and the presence of specific amplified DNA or RNA products were used as parameters for assessing the quality and integrity of the nucleic acids.

DNA and RNA electrophoresis primarily helps in the identification of undegraded and degraded nucleic acids.<sup>4,5</sup> However, the degree of degradation, if present, is not known. To objectively evaluate the quality of DNA and RNA from the tissues procured for research, we used standardized enzymatic degradation assays. The total DNA and RNA from each tissue were graded against the electrophoretic pattern of a constant quantity of human placental DNA or liver RNA subjected to increasing concentrations of DNase or RNase. In addition to providing a semiquantitative analysis of DNA and RNA degradation, this method also provided a comparative visual confirmation of tissues with poor molecular integrity. Diminished nucleic acid isolation usually indicates tissue degradation.

The integrity of the DNA isolated from each tissue was assessed by amplifying a 379-bp product of the common housekeeping gene, *HPRT*, by PCR. Significantly, all tissues that had a high molecular weight DNA by electrophoresis also exhibited high genomic integrity. The DNA from 79.5% and 78.1% of the tissues was graded as usable by electrophoresis and PCR, respectively.

The integrity of the RNA extracted from each tissue was evaluated by Northern blot analysis using a specific nucleic acid riboprobe of *G3PDH*, a common housekeeping gene. The results were further confirmed by RT-PCR amplifying a 177-bp fragment of the *HPRT* mRNA. Once again, it was observed that the tissues that exhibited a high level of RNA integrity also exhibited good-quality 28S rRNA by electrophoresis. Taken together, these results suggest that in general, the integrity of DNA and RNA extracted from human tissues can be assessed with a reasonable degree of confidence by simple electrophoresis. Minor causes of degradation that



**Image 3** Time course degradation. Lung tissue obtained from the tissue procurement services at Ohio State University, Columbus, was divided into specimens of equal volume and frozen at 15 and 30 minutes and at 1, 2, 3, 4, and 5 hours after procurement. RNA and DNA were isolated at each time point. **A**, Gel electrophoresis indicates that high-quality, high-molecular-weight DNA was obtained at all time points. **B**, Good integrity of the DNA at all points is evidenced by the intact 379-base-pair polymerase chain reaction product specific for the *HPRT* gene. **C**, RNA gel electrophoresis shows the presence of 28S and 18S ribosomal units, indicating good, intact RNA at all time points. The integrity of the RNA is confirmed by the 177-bp hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) messenger RNA reverse transcriptase–polymerase chain reaction product (**D**) and the specific 1.3-kilobase *G3PDH* gene product by Northern blot analysis (**E**).

could lower the usability of the nucleic acids and that may not be visualized using electrophoresis include chemical fixatives, shearing forces, and oxidation.<sup>6,7,14</sup> However, these causes can be minimized because they are implemented after the initial stabilization of the tissue and, thus, are not the immediate causes for the degradation of tissue integrity.

The tissues that yielded DNA with no or reduced PCR product for genomic *HPRT* or RNA with undetectable product in Northern blot or RT-PCR were graded as poor quality. While diminished nucleic acid isolation may indicate tissue degradation, it is likely that the tissues assessed may be predominantly acellular. The moderate success in

isolating usable nucleic acid from breast tissue in the present study may be attributed to the decreased cellularity in these tissues that generally consist of a large amount of adipose tissue. Previously it has been shown that mucinous tumors are poor specimens for the isolation of RNA because of reduced cellularity.<sup>15</sup> We suggest that in such situations, greater care must be taken to obtain a sufficient quantity of tissue for molecular analyses.

Immediate freezing of tumor biopsy samples in liquid nitrogen and storage at  $-70^{\circ}\text{C}$  or in liquid nitrogen vapor are the most commonly used methods of tissue preservation for the future analysis of RNA. In a time course degradation analysis of lung tissue, we found that good nucleic acid stability was maintained up to 5 hours after excision at room temperature. Others also have shown that RNA is stable up to 3 hours or longer from both routinely processed and microdissected specimens.<sup>15-19</sup> Human tissue mRNA also has been shown to be measurable by RT-PCR under conditions of up to 12 hours before fixation of the tissue.<sup>10,20-23</sup> However, we caution that comparative analysis of nucleic acids from different tissue types with inherent differences in the nuclease content and lability should be performed before reaching a conclusion on the effect of prefixation time on the molecular integrity of the tissue samples.

Measuring the molecular quality of tissue offers the opportunity to improve procurement processes.<sup>11,17,18,23</sup> It also provides a reasonable way to evaluate the molecular usefulness of the tissue before distribution to investigators on isolation of nucleic acids. While snap freezing tissue immediately after it is removed from the body is the accepted ideal procedure for maintaining tissue quality, this procedure would not be practical for most remnant human tissues because pathologic diagnostic review is required before freezing. In the future, tissue banks serving research investigators may offer isolated DNA and RNA from tissue specimens. This might be advantageous for investigators because the DNA or RNA would be isolated at the time of procurement to minimize storage- and processing-related degradation. In addition, the nucleic acids would be immediately usable, which would improve the representation of the tissue and the associated findings. New reagents and methods designed to retard the degradation and to aid in immediate isolation of nucleic acids should be considered as procedures to improve the molecular quality of prospectively procured research specimens. However, it must be cautioned that the quality control tests for the molecular integrity of the tissues at the procurement banks do not substitute for the internal controls at the research laboratory.

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## References

- Schraml P, Kononen J, Bubendorf L, et al. Tissue microarrays for gene amplification surveys in many different tumor types. *Clin Cancer Res*. 1999;5:1966-1975.
- Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor tissue specimens. *Nat Med*. 1998;4:844-847.
- Visvikis S, Schlenck A, Maurice M. DNA extraction and stability for epidemiological studies. *Clin Chem Lab Med*. 1998;36:551-555.
- Glaser JA. Validity of nucleic acid purities monitored by 260nm/280nm absorbance ratios. *Biotechniques*. 1995;18:62-63.
- Manchester KL. Value of A260/A280 ratios for measurement of purity of nucleic acids. *Biotechniques*. 1995;19:208-209.
- Ivell R. A question of faith: or the philosophy of RNA controls. *J Endocrinol*. 1998;159:197-200.
- Berger A, Bruschek M, Grethen C, et al. Poor storage and handling of tissues mimics mitochondrial DNA depletion. *Diagn Mol Pathol*. 2001;10:55-59.
- Caggana M, Benjamin MB, Little JB, et al. Single-strand conformation polymorphisms can be used to detect T cell receptor gene rearrangements: an application to the in vivo hprt mutation assay. *Mutagenesis*. 1991;6:375-379.
- Tso JY, Sun XH, Kao TH, et al. Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: genomic complexity and molecular evolution of the gene. *Nucleic Acids Res*. 1985;13:2485-2502.
- Zhong H, Simons JW. Direct comparison of GAPDH, beta-actin, cyclophilin, and 28S rRNA as internal standards for quantifying RNA levels under hypoxia. *Biochem Biophys Res Commun*. 1999;259:523-526.
- Margan SH, Handelsman DJ, Mann S, et al. Quality of nucleic acids extracted from fresh prostatic tissue obtained from TURP procedures. *J Urol*. 2000;163:613-615.
- Godfrey TE, Kim SH, Chivira M, et al. Quantitative mRNA expression analysis from formalin-fixed, paraffin-embedded tissues using 5' nuclease quantitative reverse transcription-polymerase chain reaction. *J Mol Diagn*. 2000;2:84-91.
- Yoshii A, Koji T, Ohsawa N, et al. In situ localization of ribosomal RNAs is a reliable reference for hybridizable RNA in tissue sections. *J Histochem Cytochem*. 1995;43:321-327.

14. Marchuk L, Sciore P, Reno C, et al. Postmortem stability of total RNA isolated from rabbit ligament, tendon and cartilage. *Biochim Biophys Acta*. 1998;1379:171-177.
15. Magdinier F, Ribieras S, Lenoir GM, et al. Down-regulation of BRCA1 in human sporadic breast cancer: analysis of DNA methylation patterns of the putative promoter region. *Oncogene*. 1998;17:3169-3176.
16. Chuaqui R, Cole K, Cuello M, et al. Analysis of mRNA quality in freshly prepared and archival Papanicolaou samples. *Acta Cytol*. 1999;43:831-836.
17. Taylor GP, Troyer DA, Giambernadi TA, et al. Extraction of RNA from single frozen sections. *J Pathol*. 1998;184:332-335.
18. Eikmans M, Baelde HJ, De Heer E, et al. Processing renal biopsies for diagnostic mRNA quantification: improvement of RNA extraction and storage conditions. *J Am Soc Nephrol*. 2000;11:868-873.
19. Striker LJ, Esposito C, Striker GE. Molecular biology of human glomerular diseases. *Kidney Int*. 1997;51(suppl 58):S62-S65.
20. Klebe RJ, Grant GM, Grant AM, et al. RT-PCR without RNA isolation. *Biotechniques*. 1996;21:1094-1100.
21. Grotzer MA, Patti R, Geoerger B, et al. Biological stability of RNA isolated from RNALater-treated brain tumor and neuroblastoma xenografts. *Med Pediatr Oncol*. 2000;34:438-442.
22. Carpousis AJ, Vanzo NE, Raynal LC. mRNA degradation: a tale of poly(A) and multiprotein machines. *Trends Genet*. 1999;15:24-28.
23. Schultz CL, Akker Y, Du J, et al. A lysis, storage, and transportation buffer for long-term, room temperature preservation of human clinical lymphoid tissue samples yielding high molecular weight genomic DNA suitable for molecular diagnosis. *Am J Clin Pathol*. 1999;111:748-752.