Expression Analysis of Amplified RNA from Formalin-Fixed, Paraffin-Embedded (FFPE) Tissues

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Introduction

Most clinical tissue samples are preserved using formalin fixation followed by embedding in paraffin. By some estimates, almost 90% of tissue sections are prepared using this methodology. Although this process is excellent for maintaining morphological features, it does not preserve RNA well. In fact, RNA integrity in formalin-fixed, paraffin-embedded (FFPE) tissue is very poor. As a result, it is very difficult to extract sufficient intact RNA for analysis. This is unfortunate since there is much interest in analyzing gene expression in these clinically important samples. For these reasons, there is a strong need to have a reliable and robust approach to extract and prepare for expression analysis the small amount of intact RNA from FFPE-prepared tissues.

Some approaches to extract and amplify RNA from FFPE samples have been tried. Although there are ways to extract RNA from these samples, the degraded character of this RNA makes it difficult to reliably amplify it to produce enough usable material for expression analysis. Most amplification procedures, such as T7 *in vitro* transcription and Ribo-SPIA™ RNA amplification, rely on poly-A priming and produce product that is highly-biased toward the 3'-end of transcripts. These amplification methods do not typically amplify anything beyond the last 600 to 1000 bases of the transcript. In addition to leaving much of the RNA inaccessible to analysis, these 3'-biased approaches are particularly problematic with the degraded RNA derived from FFPE tissues, where they typically cannot produce usable product.

System Biosciences' (SBI's) **Full Spectrum™ Complete Transcriptome RNA Amplification Kit** provides an easy one-tube approach that makes use of a specially developed Universal Primer that robustly and uniformly amplifies all regions of gene transcripts using low-cycle PCR. The Universal Primer is composed of a proprietary, non-degenerate primer that binds to, and primes synthesis from numerous specific sites found throughout mRNA sequences. The combination of this primer mix and low-cycle PCR (typically < 20 cycles) produces uniform amplification of gene transcripts so the relative levels of each transcript in the starting mRNA sample are maintained—even when using starting amounts of RNA as low as 5 ng. This unique approach is particularly robust for amplifying degraded RNA making the Full Spectrum™ approach the obvious choice to use for amplifying RNA from FFPE tissues.

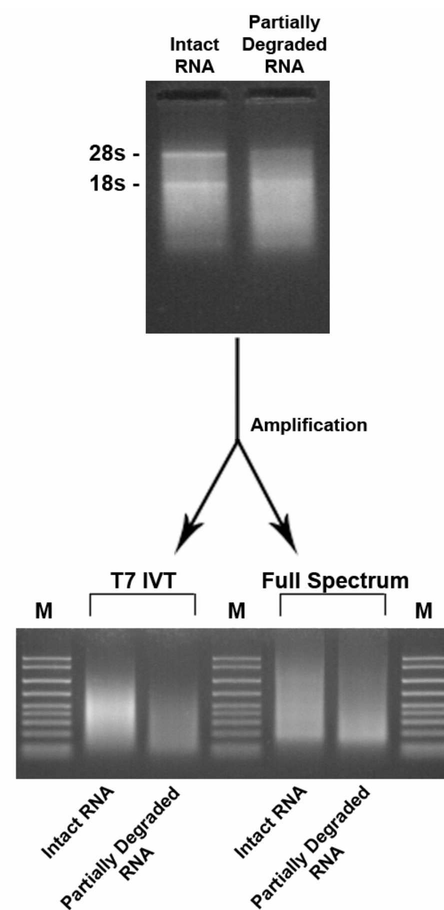

Figure 1A. Gel electrophoresis of intact and partially degraded RNA. The positions of the 18s and 28s ribosomal RNA bands are indicated.

Figure 1B. Size distribution of amplified RNA/cDNA generated by the T7 IVT and Full Spectrum™ methods with RNA shown in Figure 1A. 100bp size markers (M).

Amplify transcript 5' ends from Degraded RNA: Full Spectrum™ vs. T7 IVT

To demonstrate the power of the Full Spectrum™ amplification method on degraded RNA, a partially degraded sample of human universal RNA and a control intact RNA sample were subjected to amplification with both the Full Spectrum™ method and a competitor's T7 IVT kit (**Figure 1A,B**). As expected for both amplification methods, the size distribution of the amplified RNA/cDNA was smaller for the partially degraded RNA.

The amplified cRNA/cDNA was then interrogated by RT-PCR. For the T7 IVT method, the DNA template was generated by reverse transcription with random 9-mer primers. The amplified cDNA obtained by the SBI method was directly taken for PCR analysis. Sets of PCR primers were designed to amplify 3 regions of the human transferrin receptor (5.0 kb) and 2 regions of the human phospholipase A1 mRNA (1.75 kb). A map indicating the locations of the PCR primers is shown in **Figure 1C**. As a positive control, a primer pair was designed toward the 3'-end of the human β-actin mRNA (1.2 kb).

Amplified cDNA obtained using the Full Spectrum™ method with intact RNA generated PCR products representing all 3 regions of the transferrin receptor, both regions of the phospholipase A1 and the β-actin control mRNAs. Identical results were obtained with partially degraded RNA, demonstrating the amplification of transcript 5' ends by the Full Spectrum™ method (**Figure 1D**).

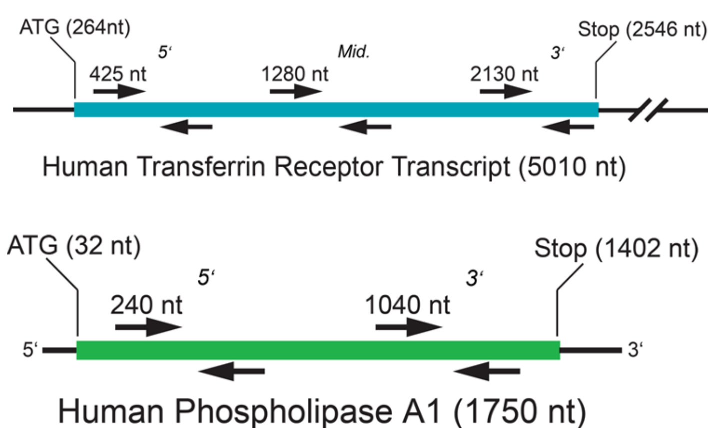

Figure 1C. Maps of the human transferrin receptor and phospholipase A1 mRNAs indicating the positions of the PCR primers.

The results obtained with the T7 IVT method were quite different. In this case, strong bands were produced only with the control mRNA and the 3'-most regions of the test mRNAs with intact RNA. This also held true for partially degraded RNA (**Figure 1D**). It is clear from these experiments that the Full Spectrum™ method maintains mRNA sequence information all along the human transferrin receptor and phospholipase A1 mRNAs. The results illustrate the extreme 3'-end bias of the T7 IVT method, a flaw eliminated by the unique priming method used in SBI's Full Spectrum™ RNA Amplification Kit.

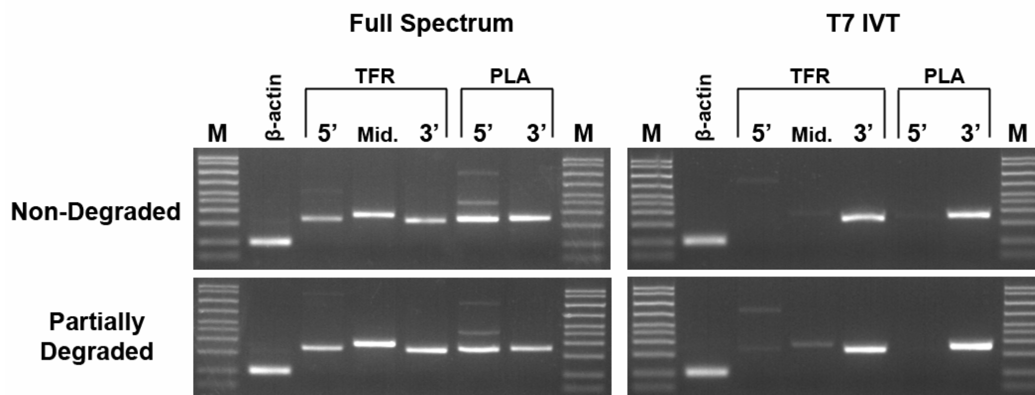

Figure 1D. PCR analysis of the amplified RNA/cDNA shown in Figure 1B utilizing primers indicated in Figure 1C. 100bp size markers (M).

mRNA Amplification from FFPE Tissue

To test the ability of the Full Spectrum™ Kit to amplify RNA derived from FFPE tissues, we isolated RNA from two 20μm sections of mouse liver. As can be see in **Figure 2**, the RNA from this tissue is substantially degraded. Following amplification, we ran samples of the cDNA product on a gel. The results show a range of cDNA products which are all relatively small in size. We also amplified a similar amount of RNA using T7-based *in vitro* transcription and, as anticipated, the amplified products look similar. However, since the Full Spectrum™ method does not rely on poly-A priming like the T7 approach does, the Universal Primer should amplify all regions of the transcripts relatively equally.

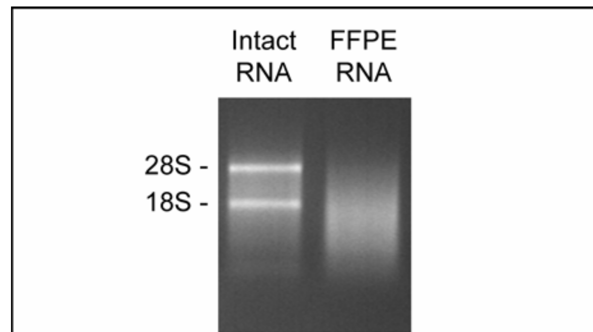

Figure 2. Gel electrophoresis of intact RNA and RNA obtained from mouse liver FFPE tissue. The positions of the 28S and 18S ribosomal bands are indicated. Absence of 28S and 18S bands indicates that the FFPE sample is severely degraded.

Thus, 5'-portions of the transcripts should be present in the Full Spectrum™ product. To test this, we amplified both samples with the transferrin receptor primers whose locations are shown in **Figure 3 (top)**, along with β-actin control primers. The results of this analysis are shown in **Figure 3 (bottom)** and support the conclusion that all regions of the transcripts are amplified relatively equally using the SBI Full Spectrum™ RNA Amplification Kit.

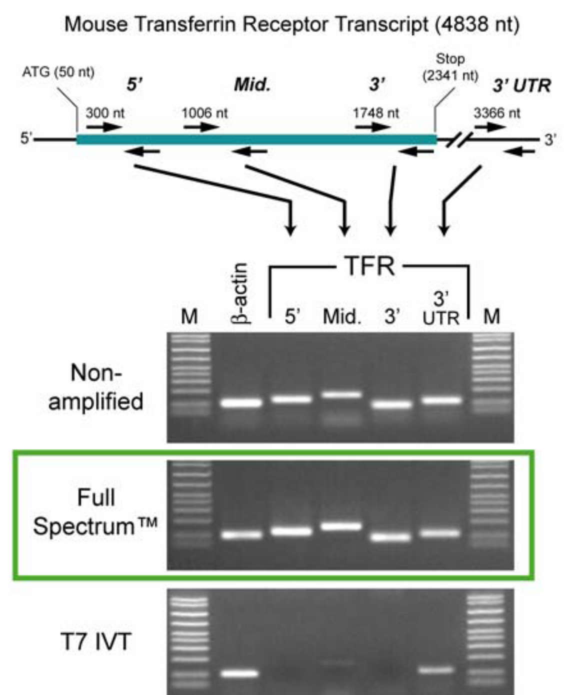

Figure 3. PCR analysis of the amplified RNA/cDNA shown in Figure 3 utilizing primers indicated in the map above the gel. SBI's Full Spectrum™ RNA Amplification Kit enabled analysis of the entire ~5kb transcript, where the competitor's T7 IVT based method fails.

Conclusions

These results demonstrate that Full Spectrum™ Complete Transcriptome RNA Amplification provides a superior approach to amplify RNA derived from FFPE tissues for expression analysis. In addition to robustly and reliably amplifying this notoriously difficult RNA from these valuable samples in a way that maintains the relative levels of each transcript, use of the Full Spectrum™ approach ensures that all regions of the transcripts are present in the amplified products. While producing a more representative population, this unbiased amplification also means that Full Spectrum™ samples can be used for analysis of gene families and alternate transcripts of the same gene. This feature also makes Full Spectrum™ amplification particularly suitable for the preparation of samples for microarray analysis.

Full Spectrum™ Complete Transcriptome RNA Amplification Kit (10 amplifications)
Cat. # RA101A-1

(pricing for U.S. customers only)