



# Ask Frank

By Fred and Hank

## RNA Purification



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### **Q. Why are spin columns considered to be suboptimal for RNA purification?**

A. While spin columns are convenient and easy to use, they have a number of limitations. The primary concern is that RNA less than 200 nucleotides in length will not be recovered by the matrix in the spin column. This means that microRNAs and other important regulatory species of small RNA will be lost during the purification. Small mRNA may be lost as well.

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### **Q. What is the best way to check for high-quality RNA after the purification step?**

A. Quality checks for RNA start with examination of purity by spectrophotometer measurements ( $A_{260}/A_{280}$ ). Checking for organic contaminants is also important (using  $A_{260}/A_{230}$ ). An  $A_{260}/A_{280}$  value greater than 1.8 is desired for best results when amplifying RNA for use on microarrays. Absorption at 230 nm reflects impurities of carbohydrates, peptides, phenols, or aromatic compounds. The  $A_{260}/A_{230}$  should be above 2.0 for pure samples. Another excellent RNA quality measurement is by gel electrophoresis or evaluation of the electrophoretic profile of an RNA preparation using an Agilent® Bioanalyzer. This device calculates the RNA Integrity Number (RIN), using an algorithm that analyzes several parameters associated with RNA quality.<sup>1</sup>

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### **Q. Does the input RNA need to be really pure for use of RNA amplification kits like the TargetAmp™ or MessageBOOSTER™ Kits?**

A. For RNA amplification work, high-quality purified RNA will provide the best RNA amplification results: longest amplified message, low 3'/5' ratios, and best base calls. While an excellent RNA purification system can produce clean, pure RNA from any sample, the quality of the sample is just as important a consideration as is the method of purification. Tissue or cell samples that

are fresh or stored with care will be the best RNA source for amplifying RNA for microarray and qRT-PCR applications. Stored samples should use an additive that prevents endogenous RNase activity from degrading the RNA in a sample prior to purification.

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### **Q. Why is getting high-quality RNA from formalin-fixed, paraffin-embedded (FFPE) tissues so difficult?**

A. Preparing tissues for embedding into paraffin involves the use of organic reagents that can dehydrate the tissue, and fracture and degrade RNA. In addition, removal of paraffin using hexanes or other organic solvents, followed by washing with ethanol/methanol, can also degrade RNA. Other factors that affect RNA quality from FFPE tissues include the age of the paraffin block, how the tissue were originally fixed, and the quality of the reagents used in original tissue fixing and removing paraffin. Typical RIN values of total RNA purified from FFPE tissue, using almost any method, are 2.0-3.0.

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### **Q. I have an Agilent Bioanalyzer. What is the minimal RIN needed for best results with the ExactSTART™, TargetAmp, or MessageBOOSTER Kits?**

A. In general, for best amplification results, we suggest using RNA that has a RIN no lower than 7.0. This level of RNA quality will provide good 3'/5' ratios, better reproducibility, and better concordance between amplified and unamplified RNA samples.

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### **Q. What is the smallest RNA species that can be purified using the MasterPure™ RNA Purification Kit?**

A. Using radiolabeled RNA, we have observed that the smallest RNA species recovered using the MasterPure RNA Purification Kit is approximately 20 nucleotides. The efficiency of recovery of these small RNAs is approximately 20%.

While this efficiency is low, the amount of small RNA obtained using the MasterPure Kits is superior compared to standard spin-column methods of RNA purification.

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### **Q. Why is a good RNA preparation needed for amplification and subsequent cloning?**

A. For RNA amplification, high-quality RNA ( $A_{260}/A_{280} > 2.0$ ) ensures the best "fidelity" in amplification when comparing amplified vs. unamplified RNA expression profiles, the best 3'/5' ratios, and the best reproducibility in microarray studies. A high-quality RNA preparation will provide a greater amount of full-length mRNA for cDNA cloning, and will also preserve the relative expression profile and gene representation in a cDNA library as compared to a lower-quality sample. These factors are critical when generating cDNA libraries and performing high-throughput sequencing (RNA-seq) using EPICENTRE's new ExactSTART™ products.

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### **Q. What is the difference between the RNA obtained from the MasterPure RNA Purification Kit and the QuickExtract FFPE RNA Extraction Kit?**

A. The main difference between the two products for RNA isolation/purification is the quality of the output RNA. The MasterPure RNA Purification Kit is able to produce very clean RNA from FFPE tissues that is ideal for end-point or real-time RT-PCR, and for microarray analysis. The QuickExtract FFPE RNA Extraction Kit is, as the kit name denotes, an extraction procedure that releases RNA from the sample but does not produce fully purified RNA with  $A_{260}/A_{280}$  ratio of greater than 2.0. The RNA obtained from samples using the QuickExtract FFPE RNA Extraction Kit may be further purified using additional downstream steps.

### **References**

1. Schroeder, A. *et al.* (2006) *BMC Mol. Biol.* 7:3.