Effect of microRNA GC Content on RNA Purification Efficiency

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Unlike messenger RNA and large RNA species, a slight change in base composition in microRNA (miRNA) will drastically alter the molecules GC content and its binding preference to targets. It has been commonly assumed that standard RNA isolation methods work equally well for all miRNAs without bias. In this study, we have shown that GC content does indeed bias purification efficiency in most phenol-based methods tested, and that the best non-biased isolation product is the Silicon Carbide-based technology from Norgen Biotek. Among the advantages of this technology are the following:
1) Simple and Fast Procedure with no lengthy phenol:chloroform extractions, filtration or precipitation
2) Minimal GC Content Bias of RNA Isolation from various inputs including cells, bodily fluids and tissues
3) Sensitive Isolation of RNA from biological inputs with low RNA content including plasma, serum or urine

Introduction

MicroRNAs (miRNAs) are small RNAs (~ 22 nucleotides) that play important roles in gene regulatory networks by binding to and repressing the activity of specific target messenger RNAs (mRNAs). A large volume of basic research has been done to characterize miRNAs biology in various tissues and cell lines of model organisms. Moreover, the potential utilization of miRNAs as biomarkers in molecular diagnostics has been extensively studied. Expression profiling of miRNAs has been tested with various clinical specimens including urine, blood, serum, plasma, tumor biopsies and formalin-fixed paraffin-embedded (FFPE) tissues.

A number of procedures have been developed to purify total RNA including miRNAs from these aforementioned samples. These protocols are generally divided into two main categories; 1) traditional, phenol:chloroform extraction followed by alcohol precipitation; and 2) column-based purification (with affinity resin including silica and silicon carbide). The majority of these methods are modifications or derivatives of existing protocols for large mRNA purification which assume that all biomolecules purified are highly homogenous.

However, in small molecules such as miRNAs, parameters such as GC content could vary drastically, with published data suggesting a range of 20 - 80%. The large variation of GC content could result in a diverse surface property of the molecule. Such diversity may not only affect endogenous function of the molecule but it may also impact sample preparation efficiency. In fact, it has been previously shown that there was an insufficient recovery of miRNA with low GC content using certain isolation procedures, including the traditional phenol:chloroform extraction.

As miRNA expression study has become an important part of many basic biological research areas, as well as biomarker discovery, the choice of a sample preparation method that has the best coverage (and hence least bias) is critical. Here, we evaluated the recovery of different miRNA by numerous commercially available RNA extraction methods from several important clinical sample types.

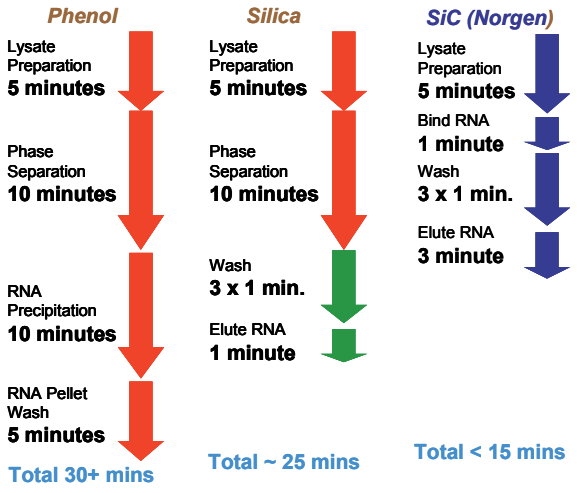

Figure 1. Outline of Different Isolation Methods for Total RNA (microRNA included)

Methods of Total RNA (including miRNA) Purification Vary Drastically in Processing Time and Complexity

MicroRNAs of different GC content that were previously studied (**ref. 1**) were synthesized. The synthesized microRNAs were reconstituted in water and mixed in equal molar fashion. An input of 50 fmoles of microRNA mix was used in various RNA isolation methods including standard phenol:chloroform extraction coupled with alcohol precipitation; silica column purification (which requires phenol:chloroform separation); or Norgen's silicon carbide column purification (no phenol required). As depicted in **Figure 1**, methods involving phenol are lengthy and require multiple steps while Norgen's method using silicon carbide is simple, does not require the use of phenol and can be completed in less than 15 minutes.

Intrinsic Variation in microRNA Recovery Efficiency and GC Bias by Different RNA Isolation Methods

In order to study how each purification method recovers microRNA under the best optimized conditions, without interference from components of the sample input (such as contaminants and other biomolecules), we tested the miRNA spike-in recovery using each isolation method without any biological input (Figure **2A**). Recovery of low amounts of miRNA, particularly with low GC%, is very poor using phenol:chloroform extraction coupled with alcohol precipitation. Given the low amount of miRNA spike-in used (50 fmoles), it was not surprising that the RNA recovery by phenol:chloroform extraction was very low as the effectiveness of RNA precipitation by alcohol is concentration-dependent. Recovery of miRNA extracted by phenol:chloroform could be improved by using either silicon carbide or silica columns in place of precipitation, however there was some bias towards the high GC content miRNAs. More importantly, non-phenol purification of miRNA spike-in using silicon carbide columns resulted in the best recovery with minimal bias by GC content.

Difference in Recovery Efficiency of microRNA with Different GC Content from Biological Fluids (Blood, Plasma and Urine)

A tremendous amount of research has been done in blood and plasma microRNA profiling. Urine, while not as frequently studied, could be a good non-invasive alternative source. Unlike other inputs such as tissues and cells, the challenge in these biological fluids is that the RNA content is usually very low (< 1 to 100 ng per 100 µL). Here, we tested the recovery of the various spiked-in miRNAs from human plasma (Figure **2B**), human urine (Figure **2C**) and leukocyte of human blood (Figure **2D**). Good recovery with minimal GC bias was observed when input RNA content was high (such as in leukocyte). Very poor isolation was observed with phenol:chloroform extraction coupled with alcohol precipitation, particularly in plasma and urine. In contrast, the best recovery of RNA from plasma and urine was achieved by using Norgen's silicon carbide column without phenol. All methods tested for plasma and urine showed relatively lower recovery of microRNA with lower GC content. However, the non-phenol method using silicon carbide columns showed slightly better low GC microRNA recovery, mainly due to the overall higher recovery.

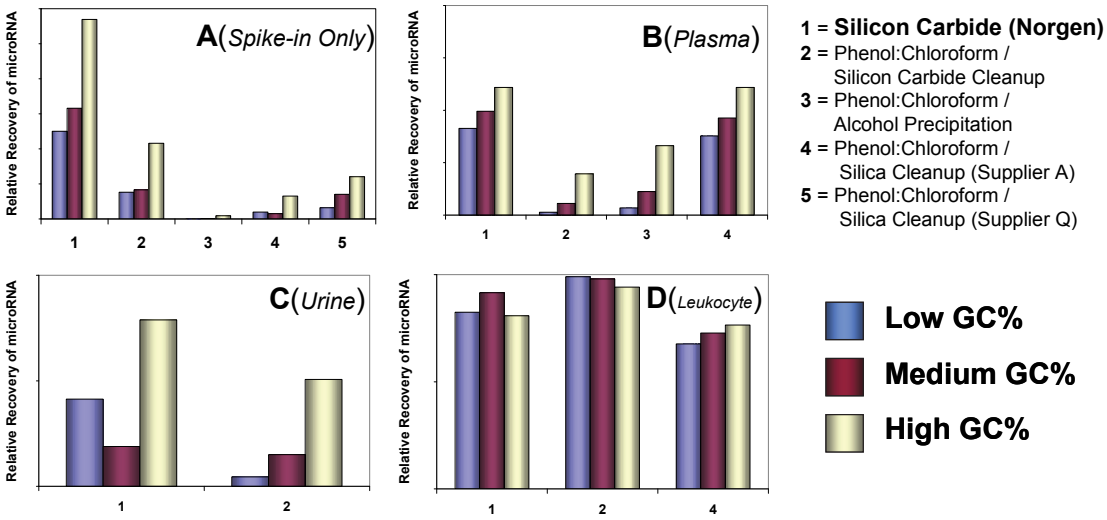

Figure 2. Recovery of microRNA of Different GC Content from Different Biological Fluids Input using Different Total RNA Isolation Methods

Reference

1. Davis et al. 2008. Computational Biology and Chemistry. **32**:222–226

Product	Catalogue #	Product Usage
Total RNA Purification Kit	**17200**	Total RNA Isolation for Plasma, Cells and Tissues
RNA Clean-Up and Concentration Kit	**23600**	Effective Cleanup including Phenol:Chloroform Extraction
Urine microRNA Purification Kit	**29000**	RNA Isolation for up to 1.5 mL of Urine
Plasma/Serum Circulating RNA Purification Kit	**42800**	RNA Isolation for 0.25 to 5 mL of Plasma/Serum

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