Genome-wide miRNA Analyses

MicroRNAs (miRNAs) are an evolutionarily conserved class of small 19-22 nucleotide RNAs that are present in the cell and perform a variety of functions including the regulation of gene expression. Traditionally, miRNAs are known to regulate gene expression by binding to the 3’ untranslated region of an mRNA and either targeting that mRNA for degradation or blocking translation to protein. More recently, additional functions of miRNAs have been elucidated including their use as biomarkers of disease pathology, and their putative role in intercellular signaling.

Analysis of miRNAs from cells, tissues and body fluids using a next generation sequencing platform is termed miRNA-seq. MiRNA-seq is useful for identifying the miRNAs (both known and unknown) present in any given sample and for determining how miRNAs change in response to disease pathology. For example, the miRNA profile determined from a colon cancer tissue sample is very different than that of normal colonic epithelial tissue\(^1\). Furthermore, miRNAs have been found in the blood and the profile of these circulating miRNAs changes when the patient develops cancer\(^2\) or is infected with a virus\(^3\), suggesting that these molecules may be useful biomarkers.

How does miRNA-seq work?
The initial starting material is either total RNA or purified small RNA fragments. If choosing to purify small RNA fragments from your sample, be sure to use a kit that is specifically designed for this purpose, such as the mirVana kit by Life Technologies or the miRNeasy kit from Qiagen. Many of the standard kits commonly used to isolate total RNA are designed to isolate larger mRNA species and wash away the smaller miRNAs. Since most mature miRNAs have a 5’-phosphate and a 3’-hydroxyl group as a...
result of the cellular pathway used to create them, 3’ and 5’ adapter molecules were specifically designed to target microRNAs and other small RNAs. These miRNA-specific adapters are ligated to the ends of the miRNA molecule and an RT reaction is used to create cDNA. The cDNA template is then PCR amplified using a common primer (homologous to the 5’ adapter sequence) and a primer containing one of 48 index sequences (homologous to the 3’ adapter sequence, plus index sequence). The index sequence, or barcode, is a known nucleotide sequence that allows many samples to be pooled for the sequencing analysis. Once the miRNAs have been amplified by PCR, they are gel purified and used as the template for next generation sequencing (See Gennovations, Volume 1, Issue 1) to identify all of the miRNAs present in the sample.

References and for more information:

