BioSpotlight

Stranded

Recently, it has become clear that bidirectional transcription plays an important functional role in eukaryotic gene silencing. When studying bidirectional transcription, one of the most sensitive methods to quantify strand-specific transcripts is reverse transcription qPCR using strand-specific reverse transcription primers. However, the accuracy of this technique can be compromised by primer-independent reverse transcription, which generates undesired cDNA from the opposite strand that can be amplified during the subsequent qPCR step. This problem was first reported by researchers analyzing strand-specific viral transcripts, but is now complicating analyses in eukaryotic cells as well. Primer-independent reverse transcription is believed to be the result of various factors, including RNA hairpins and non-specific priming by exogenous nucleic acid fragments. In this issue, J. Stenman and colleagues in Helsinki, Finland, describe their method for strand-specific reverse transcription qPCR using sequence-modifying reverse transcription primers and post-qPCR melting curve analysis to accurately distinguish primer-initiated cDNAs from non-specifically primed cDNAs. Here, reverse transcription primers are designed to alter the sequence of specifically primed cDNAs by changing several A/T nucleotides to C/G, or vice versa, resulting in a 3°-5°C

shift in the melting temperature of the resulting PCR amplicon compared to the cDNA amplicon derived from nonspecific reverse transcription of the opposite strand. After thoroughly removing the sequence-modifying reverse transcription primer by exonuclease treatment, cDNAs are qPCR amplified and the relative amounts of specifically primed vs. nonspecifically primed cDNA can be accurately determined by post-qPCR melting curve analysis. Using this new approach, the authors initially analyzed the amount of primer-independent reverse transcription under different reaction conditions in order to optimize reverse transcription specificity. They then demonstrated the utility of the method to simultaneously quantify the expression of both the plus and negative strands of the PB2 gene during flu virus replication in cell culture. This new technique should prove a powerful tool for the accurate quantitation of strand-specific mRNA expression, especially in studies of bidirectional transcription in eukaryotic cells.

See “Technique for strand-specific gene-expression analysis and monitoring of primer-independent cDNA synthesis in reverse transcription” on page 263

miRNA Matchmaker

Real-time PCR has made it possible to quantitate numbers of biomolecules, including microRNAs (miRNA), from a wide variety of samples. Although quantitative, such PCR methods lack the spatial resolution to localize miRNA expression within specific cells or tissues. In situ hybridization (ISH), on the other hand, allows for localization of miRNA species, but, to date, has only produced semi-quantitative results when it comes to looking at numbers of miRNAs present in a sample. A marriage between the two approaches could supply both the spatial and quantitative information needed to better understand how miRNAs function in cells. Now in a report in the current issue of *BioTechniques*, D. Rimm and his colleagues at Yale University describe the application of the automated quantitative analysis (AQUA) system to spatial quantitation of miRNAs in tissue epithelia. The authors took advantage of the ability of the AQUA system to determine fluorescent signals in subcellular compartments using DAPI and cytokeratin immunofluorescence in order to measure miRNA expression levels in these compartments. In the end, a numerical score that is directly proportional to the number of molecules per unit area can be obtained using the new

miRNA qISH assay validation: miR-221 ISH (red) performed on MCF-7 cells transfected with 30 nM control (anti-miR negative control) or anti-miR-221 inhibitor and merged with DAPI (blue)

technique. In a proof-of-principle experiment, the authors assessed the expression of four miRNAs (miR-21, miR-92a, miR-34a, and miR-221) in 473 breast cancer samples on tissue microarrays. The results obtained from these experiments clearly demonstrate that the approach developed by Rimm and colleagues for spatial quantitation of miRNAs is both high-throughput and leads to new insights on the various roles that miRNAs play in cellular biology and disease. One especially intriguing finding made by

the authors using their new methodology was that the inverse relationship previously suggested to exist between miRNAs and their suspected protein targets did not seem to apply in the large breast cancer sample set examined. This observation, and its biological relevance, is likely to be examined in greater detail in the coming months.

See “Quantitative analysis of microRNAs in tissue microarrays by in situ hybridization” on page 235.

Written by Patrick C.H. Lo, Ph.D. and Nathan Blow, Ph.D.

BioTechniques 52:221 (April 2012)
doi 10.2144/000113832

To purchase reprints of this article, contact:
biotechniques@fosterprinting.com