BioSpotlight

Lighting the Way with Multiple LAMPs

Molecular diagnostic assays based on isothermal amplification of nucleic acids are well suited to point-of-care pathogen detection, owing to the rapidity, simplicity, and minimal equipment requirements of the technique. Among isothermal amplification methods, loop-mediated isothermal amplification (LAMP) is relatively simple, requiring only a strand-displacing DNA polymerase and six primers to achieve amplification of a target sequence. While real-time measurement of LAMP products is possible, in the past, this has been restricted to the detection of a single target. In order to broaden the utility of LAMP, simultaneous real-time measurement of multiple targets is necessary. In this issue, Evans, Jr. and colleagues at New England Biolabs (Ipswich, MA) describe a real-time multiplex LAMP assay named “detection of amplification by release of quenching” (DARQ). The detection probe for this assay is a modified version of the LAMP forward internal primer (FIP), where a dark quencher is attached to the 5′ end (Q-FIP), while a shorter oligonucleotide with a fluorophore at its 3′ end (Fd) is annealed to the 5′ half of Q-FIP. Juxtaposition of the dark quencher and fluorophore at the end of the Q-FIP:Fd probe duplex quenches fluorophore emission. Quenching is subsequently released after extension

of the Q-FIP primer by *Bst* polymerase during LAMP amplification and DNA polymerization from the reverse direction, which disrupts the probe duplex, displacing Fd and separating the fluorophore from the quencher, resulting in a fluorescence signal that can be measured in real time. Initial testing of the LAMP reaction with Q-FIP:Fd indicated an equimolar mixture of FIP and Q-FIP:Fd was ideal for maximal reaction efficiency and that the detection probe worked equally well if the quencher was attached to Fd and the fluorophore to FIP. For multiplexing, probes specific for target sequences on different genomic DNAs were labeled with different fluorophores. In a duplex reaction, the dynamic range of detection was $\sim 10^{-10}$ copies, and as little as three copies of a target could be detected in a reaction containing robust amplification of $\sim 8 \times 10^5$ copies of another target. DARQ was easily extended to the simultaneous detection of three and four targets, with accurate and specific detection of intended targets even in the presence of large amounts of the other genomic DNAs.

See “Simultaneous multiple target detection in real-time loop-mediated isothermal amplification” on page 81.

Persistently Positive Probes

Following its introduction in 1995, microarray analysis quickly became the standard for detecting expression levels from thousands of RNA transcripts simultaneously. More recently, ultra high throughput RNA sequencing (RNA-Seq) has emerged as an alternative to microarray transcription analysis. Comparisons between RNA-Seq and microarray datasets have shown correlation, except in some cases where genes are expressed at extreme high and low levels. In this issue of *BioTechniques*, Mao et al. identify a series of discordant results between these techniques, highlighting the need for caution when analyzing expression data. In experiments comparing sperm and testes RNA profiles obtained using both microarray analysis and RNA-Seq, the authors identified a set of 195 and 2391 probes, respectively, on the Illumina HumanHT-12 bead array that showed high signal intensity, but were undetected by RNA-Seq. To further investigate the disparate results, the authors designed primer pairs that corresponded to microarray probe locations on the genome and quantified expression of seven discordant genes using qRT-PCR. Concurring with the RNA-Seq data, no expression was observed for any of the tested genes. The authors then analyzed additional available

Four-way Venn diagram of discordant probes from four tissues/cell lines: sperm, testes, placenta, and fibroblast.

datasets to look for correlations between the discordant probes and specific tissues, procedures, or individual laboratories, and found several discordant probes in human skin fibroblasts and placenta samples as well. A comparison of these data with the sperm and testes datasets revealed 99 probes that were consistently identified as present on microarrays, but absent in RNA-Seq data in all four tissues or cell lines. An expanded study of 713 samples retrieved from the Gene Expression Omnibus repository for high-throughput gene expression data showed significant signal levels for all 99 probes in 95% of the samples and for 70 probes in all of the samples, regardless of tissue source or

cell type. These probes did not correspond to ribosomal RNA or known microRNA sequences, and no common biological functions or pathways were present among them. While an explanation for the high microarray signals at these particular probes remains unclear at the moment, researchers can now adjust for these false positive signals to avoid any potential biases when analyzing gene expression data and be aware of such potentially confounding artifacts moving forward.

See “Identification of artifactual microarray probe signals constantly present in multiple sample types” on page 91.

Written by Patrick Lo, Ph.D., and Kristie Nybo, Ph.D.

BioTechniques 53:68 (August 2012)
doi 10.2144/000113896

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