Universal ProbeLibrary: a new concept for streamlining gene expression analysis with qPCR

Roche Applied Science presents the Universal ProbeLibrary. This unique combination of open-access, online assay design with only 165 prevalidated probes allows users to analyze and quantify gene expression data with peak efficiency and high throughput.

Quantification of gene expression with real-time reverse transcription PCR (RT-PCR) has become a common and widely accepted technique, but the bottleneck for implementation of new assays is the time needed for assay design, optimization and validation. With the Universal ProbeLibrary, a novel, highly flexible concept for quantitative real-time RT-PCR assays, you can overcome these limitations and increase your laboratory’s productivity and throughput. The Universal ProbeLibrary concept is based on two components: probes and software.

Universal ProbeLibrary uses 165 prevalidated, dual-labeled detection probes that recognize the majority of transcripts in the human, mouse, rat, primates, Drosophila, Caenorhabditis elegans and Arabidopsis genomes (Fig. 1). Transcripts of many other organisms can be detected with high probability.

The ProbeFinder software quickly selects the optimal combination of probes and primers for intron-spanning assays. More than 155,000 different assays target 90% of all exon-exon splice junctions listed in the Ensembl human database.

How this concept works

Universal ProbeLibrary probes are only 8–9 nucleotides long, far shorter than the 20– to 25-nucleotide DNA probes ordinarily used for qPCR assays. These short probes recognize sequences that occur frequently in the transcriptome. Thus each probe can be used to detect and quantify many different transcripts. The short probes are substituted with the nucleotide analog, locked nucleic acid (LNA), which increases binding affinity of probes and elevates the melting temperature of the corresponding duplex. Therefore, Universal ProbeLibrary probes are compatible with all commonly used real-time RT-PCR protocols and instruments.

The web-based assay design software called ProbeFinder (accessible at http://www.universalprobelibrary.com) allows the design of specific customized assays in seconds. Simply select the organism of interest and enter the target name, accession number or sequence. ProbeFinder selects the optimal combination of primers and probes for at least one intron-spanning assay. You can order the primers at your preferred oligonucleotide supplier and either select the Universal ProbeLibrary probe from one of the organism-specific probe sets of 90 probes or order the individual probe.

How the Universal ProbeLibrary performs

The Universal ProbeLibrary offers a number of important benefits. First, there is no primer-dimer detection with Universal ProbeLibrary probes. Universal ProbeLibrary allows you to design and carry out RT-PCR assays with the same flexibility and speed as with SYBR Green I, yet provides the specificity only probe-based assays can deliver.

Universal ProbeLibrary probes produce results that are comparable to other assays in terms of linearity with HybProbe probes or SYBR Green I. Three different types of assays were used to detect three human mRNA targets: interleukin 8 (IL-8), interferon γ (IFN-γ) and interleukin β (IL-1β). Figure 2 shows the standard curves (that is, the
logarithm of template concentration versus crossing-point cycle values) obtained with the three assays. All assays were quite linear over four orders of magnitude (data provided by Thomas Giese, Institute of Immunology, University of Heidelberg, Germany).

In another set of tests, GAPDH was amplified from dilutions of a qPCR human reference cDNA conducting a Universal ProbeLibrary assay with probe #60 and a prevalidated competitor hydrolysis probe assay for GAPDH (Fig. 3). The Universal ProbeLibrary assay was done with the recommended standard concentrations of primers (200 nM) and probe (100 nM) and with the same (elevated) concentrations of primers (900 nM) and probe (250 nM) as used in the competitor assay. Both assay types were run on a LightCycler® 480 Instrument using LightCycler® 480 Probes Master for both assays. All three assays perform with optimal PCR efficiency of approximately $E = 2$. When conducting the Universal ProbeLibrary assay with the recommended standard concentration of probe (100 nM), a lower fluorescent signal is generated. With elevated probe concentration (250 nM) the signal height is the same as that of the competitor assay.

Conclusion
The Universal ProbeLibrary combines the flexibility and speed of a SYBR Green I assay with the high specificity of probe-based assays. Custom RT-PCR assays can be designed in seconds using the web-based ProbeFinder software, and the Universal ProbeLibrary probes can be readily available as organism-specific sets of 90 probes each, or they can be ordered individually.

Universal ProbeLibrary assays are compatible with all commonly used real-time PCR instruments and standard protocols. The Universal ProbeLibrary was successfully used in microarray validation$^{2,3}$ and gene knockdown quantification$^4$.

Additional information is available on our company website (http://www.universalprobelibrary.com).

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