Universal ProbeLibrary Set: One Transcriptome – One Kit

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Current Challenges in Quantitative Gene Expression Profiling

Using quantitative real-time RT-PCR for gene expression profiling is a common, widely accepted technique. Currently, a user wishing to do such profiling has two choices: either develop the RT-PCR assays in house or order a pre-validated assay from a commercial supplier. Neither, however, is very time-efficient.

Designing a quantitative RT-PCR assay in house is a cumbersome process. Initial results obtained with newly designed assays often lack the specificity, sensitivity, and reproducibility required for meaningful conclusions [1, 2]. Thus, valuable time is lost in repeating, optimizing, and validating each new assay.

ProbeLibrary - A Better, More Flexible Alternative

The ProbeLibrary system [3] can eliminate many of the time delays currently associated with developing quantitative real-time RT-PCR assays for gene expression analysis. The ProbeLibrary system has two components:

- The ProbeLibrary Kit with 90 pre-validated, dual-labeled detection probes recognizes at least 98% of the transcripts from a given genome.
- The ProbeFinder software quickly selects the optimal combination of human ProbeLibrary probes and target-specific PCR primers for more than 644,000 different assays of the human transcriptome. These assays target 90% of all exon-exon splice junctions listed in the Ensembl human database.

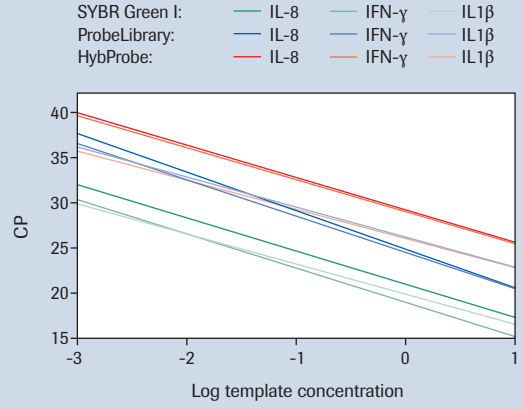

Figure 1: Comparison of ProbeLibrary real-time RT-PCR assays with HybProbe assays and SYBR Green I assays. Real-time PCR assays for human IL-8, IFN-γ and IL1β mRNA were performed in duplicate on a LightCycler® 1.2 Instrument. Each assay was performed with four different concentrations (undiluted, 1:100 dilution, 1:1000 dilution, and 1:10000 dilution) of the same human template cDNA pool. The graphs show the linear regression analysis of the results for SYBR Green I (green), ProbeLibrary (blue), and HybProbe assays (red).

The ProbeLibrary system was developed and launched in May 2004 by Exiqon, a highly innovative Danish company. Unique sets of ProbeLibrary probes are currently available for the human genome as well as the genomes of five other organisms (mouse, rat, *Arabidopsis*, *Drosophila*, and *Caenhorhabditis elegans*).

How the System Works

ProbeLibrary

ProbeLibrary probes are only 8–9 nucleotides in length, far shorter than the 20–25 nucleotide DNA probes ordinarily used for gene-expression profiling. These short probes recognize sequences that occur frequently in the transcriptome. Thus, each ProbeLibrary probe can be used to detect and quantify many different transcripts [3].

On average, each human ProbeLibrary probe recognizes more than 7,000 human transcripts listed in the NCBI RefSeq database. Conversely, each mRNA in the transcriptome contains recognition sites for 16 ProbeLibrary probes.

In addition, based on Ensembl gene structure predictions, ProbeLibrary probes often target sequences near exon-exon junctions. Thus, they can be used to develop assays that do not recognize the genomic DNA that may contaminate total RNA samples.

To ensure that the ProbeLibrary probes will remain hybridized to targets under the conditions of normal probe-based assays, ProbeLibrary probes contain the

high-affinity nucleotide analogue locked nucleic acid (LNA) [4, 5]. LNA is a chemical modification of the furanose ring of nucleotides that increases binding affinity of a ProbeLibrary probe against the template strand and therefore increases the melting temperature of the corresponding duplex. Thus, these probes are compatible with standard real-time RT-PCR methods and instruments.

ProbeFinder

The web-based assay design software called ProbeFinder (accessible at www.probelibrary.com) further simplifies the task of designing real-time PCR assays. Based on user-defined criteria, the software suggests appropriate sequence-specific primers and ProbeLibrary probes for a given assay. Per default, the ProbeFinder software will define an amplicon that spans an exon-exon splice junction.

Since each mRNA in a transcriptome usually contains recognition sites for several ProbeLibrary probes, the ProbeFinder software often suggests more than one primer-probe combination for each assay. The user can then choose the probe-primer combination that produces the best results in a particular analysis.

How the System Performs

To assess the performance of the ProbeLibrary system in real-time PCR, we compared RT-PCR results obtained with ProbeLibrary probes, HybProbe probes and SYBR Green I (Figure 1). The three different types of assays were used to detect three human mRNA targets (IL-8, IFN-γ and IL1β). Figure 1 shows the standard curves (*i.e.* the logarithm of template concentration versus crossing-point cycle values [CP]) obtained with the three assays. All three assays were quite linear over four orders of magnitude.

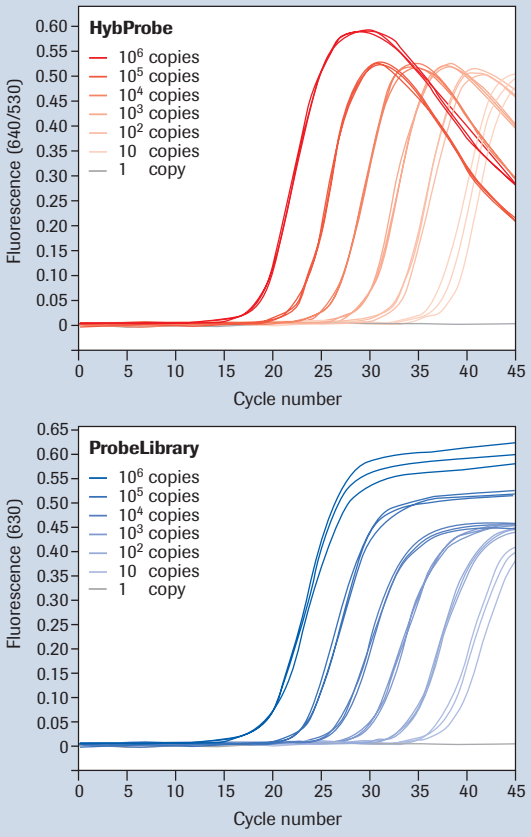

Figure 2: Comparison of ProbeLibrary real-time RT-PCR assays with HybProbe assays: LightCycler® results. Real-time PCR assays with β2-Microglobulin (β2M) were performed in seven tenfold serial dilutions of the same *in-vitro* transcribed human RNA. Each assay was performed in triplicate on a LightCycler® 2.0 Instrument. The LightCycler® FastStart DNA Master^{PLUS} Hybridization Probes was used for HybProbe assay. The LightCycler® TaqMan Master Kit was used for the ProbeLibrary assay.

CP values for the SYBR Green I assays are lower than those obtained in the ProbeLibrary and HybProbe assays. This is due to the fact that in SYBR Green I assays, a number of chromophores are incorporated into each amplicon, whereas in probe-based assays only one probe linked to one dye targets each amplicon. Due to their sequence-specific detection, however, probe-based assays generate signals only if the correct amplicon is amplified. In suboptimal SYBR Green I assays, on the other hand, a signal in addition to the signal for the correct amplicon can appear for every double stranded DNA such as primer dimers or by-products.

Figure 2 shows the amplification of the housekeeping gene β2-Microglobulin (β2M) with HybProbe probes and a ProbeLibrary probe. We used serial dilutions of *in vitro* transcribed human RNA as template, with template concentrations ranging from 10^6 copies down to one copy. In both assays, the amplification curves are homogenous and the PCR runs are very reproducible. The sensitivity for both assays is ten copies. Only the correct amplicon, free of by-products, is detected on analytical gels (Figure 3).

Conclusion

The results presented here clearly demonstrate that the ProbeLibrary system greatly simplifies and accelerates development of quantitative real-time RT-PCR assays for gene-expression profiling.

ProbeLibrary probes perform comparable to HybProbe probes and SYBR Green I in real-time PCR assays. Yet, ProbeLibrary probes offer a far more flexible assay

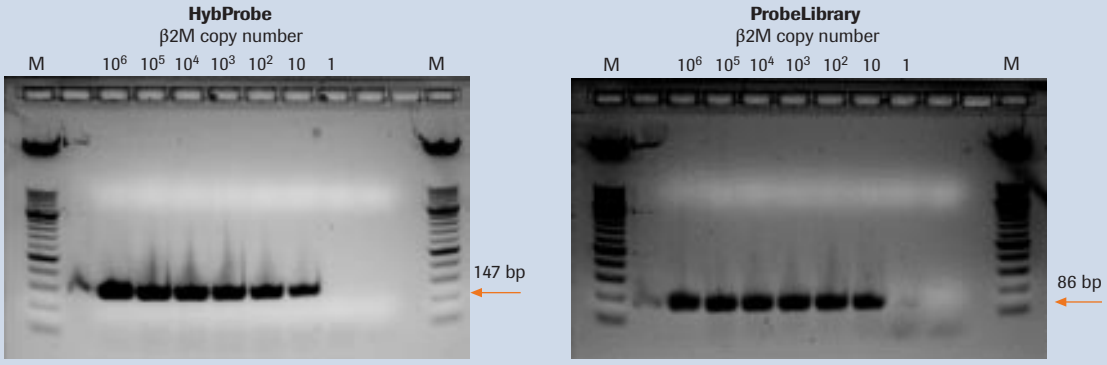

Figure 3: Comparison of ProbeLibrary real-time RT-PCR assays with HybProbe assays: Electrophoretic analysis of products. Products of the real-time PCR assays described in Figure 2 were analyzed by gel electrophoresis. The gels containing the β2M amplicons were then stained and photographed. The amplicon length was 147 base pairs (bp) for the HybProbe assay and 86 bp for the ProbeLibrary assay. No PCR by-products or primer dimers were seen in either assay.

development platform, since a single library of 90 probes can cover the entire transcriptome of one organism. Moreover, ProbeLibrary assays were significantly more specific than SYBR Green I assays, since the ProbeLibrary assays did not detect amplification signals derived from either primer dimers or nonspecific amplification.

Also, on average, each transcript from a given genome is recognized by more than one probe in the ProbeLibrary and the ProbeFinder assay design software can usually suggest more than one primer-probe combination for each target. If one primer-probe combination fails to give satisfactory real-time PCR assay results, the user can easily select one of the other combinations suggested by the software. The ability to select an alternative assay from a design list – rather than having to optimize a single, poorly performing assay – can save time and money.

Several ProbeLibraries have been developed so far, including libraries for human, mouse, rat, *Arabidopsis*, *Drosophila* and *C. elegans* genomes. The robust, flexible ProbeLibrary system is ideal for high-throughput expression analysis, rapid validation of microarray data and gene knockdown experiments. ■

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