High-Performance Reference RNA and cDNA

Human Universal Reference Total RNA and qPCR Human Reference cDNA

- **Broadest gene coverage of high- and low-abundance transcripts**
- **Made from a combination of human tissues—not just cell lines or a single tissue**
- **Virtually free of genomic DNA**

To precisely measure mRNA expression levels—whether by microarray analysis, ribonuclease protection assay, or quantitative PCR (qPCR)—the signal for each target gene is often standardized against an internal control or endogenous reference gene. Housekeeping genes such as β-actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are frequently used because their expression levels are expected to remain constant under different treatment conditions. Unfortunately, this assumption is not always valid and the expression levels of these genes can indeed vary in response to certain stimuli (1). Quantifying expression results based on housekeeping genes alone can therefore lead to biased results.

Superior Normalization Controls

One method of reliably controlling for signal variation across multiple experiments or instruments is to normalize the data using our **Human Universal Reference Total RNA** and **qPCR Human Reference cDNA**—two universal gene expression controls derived from total RNAs combined from a variety of human tissues (2). These products contain a comprehensive array of expressed genes that covers >90% of the 2,000 genes analyzed in a representative microarray (Figure 1, top panel). Including these controls in your experiments allows you to normalize the signal of your target gene to one or more external controls, all of which are likely to be represented in these universal reference standards. Human Universal Reference Total RNA is ideal for standardizing microarray results, while Human Reference cDNA is an excellent standard for qPCR.

Highly Complex RNA

Two key features of these high-quality reference standards are that they possess superior complexity and represent a multitude of gene targets. Our reference total RNA is collected and pooled from several different human tissues to ensure representation from genes that are expressed at very low levels. This RNA pool also serves as the starting material for our Reference cDNAs. The exceptionally broad gene coverage of the RNA is shown by microarray analysis, which demonstrates very consistent representation of the genome, much more so than either single tissue RNAs or a competitor reference RNA derived from only cell lines (Figure 1).

Complexity Preserved in cDNA

The superior gene coverage of our Universal RNA is also reflected in our Reference cDNA. We use our recently developed **SMART™ MMLV RT** (Cat. No. 639522), a highly purified, high-performance reverse transcriptase, to convert the Universal RNA into cDNA. SMART MMLV RT ensures efficient first-strand cDNA synthesis and produces a high-quality product ready for use in your experiments. In addition, we offer the cDNA in two different formats: one in which the RNA has been primed with oligo dT (Cat. No. 636692); and one that was primed using a mixture of random-sequence oligos (Cat. No. 639653). Regardless of your format preference, high-, medium-, and low-abundance gene transcripts are well-represented, allowing you to prepare a wide range of serially diluted standards for any qPCR assay (Figure 2). Indeed, we demonstrate the ability to generate a reproducible signal from as little as 6.4 pg of input template material.

Consistent Quality

A stringent PCR analysis revealed that our Universal Total RNA is virtually free of genomic DNA (3), allowing for highly accurate measurements of transcript copy number. The lot-to-lot variations of our Universal RNA and Reference cDNA are minimized because the source RNA is prepared on an industrial scale. For accurate and reliable results, use Universal Reference Total RNA and cDNA. They consistently outperform other controls.

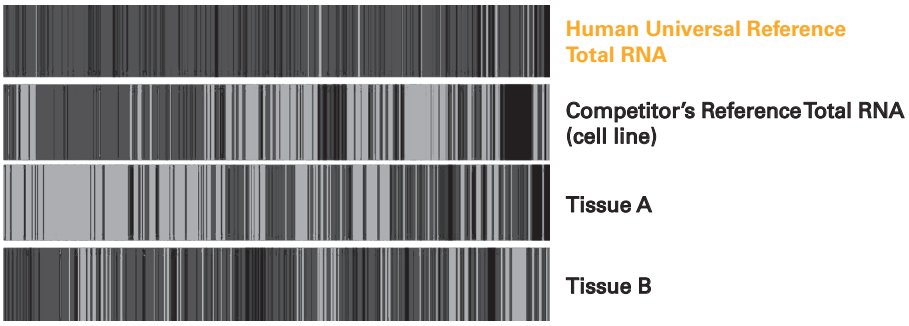

Figure 1. Human Universal Reference Total RNA, the starting material for qPCR Human Reference cDNA, demonstrates superior complexity. We generated Cy3™-labeled probes using either our Human Universal Total Reference RNA, a competitor's reference RNA, or RNA from single tissues—and hybridized the probes to a microarray containing 2,000 unique features. Expression levels were analyzed using GeneSpring® software. Among the genes detected by the Universal Reference RNA probe, 83% had intensities greater than or equal to the intensity obtained using the single tissue RNA probes. Nearly all of the genes expressed in individual tissues were detected using our Universal Reference RNA, which recognized greater than 92% of the sequences present in the array. Clontech Universal Reference RNA also outperformed a competitor's Reference RNA mixture which was prepared using RNA from cell lines only. The black and varying shades of gray indicate high and low expression levels, respectively.

High-Performance Reference RNA and cDNA...continued

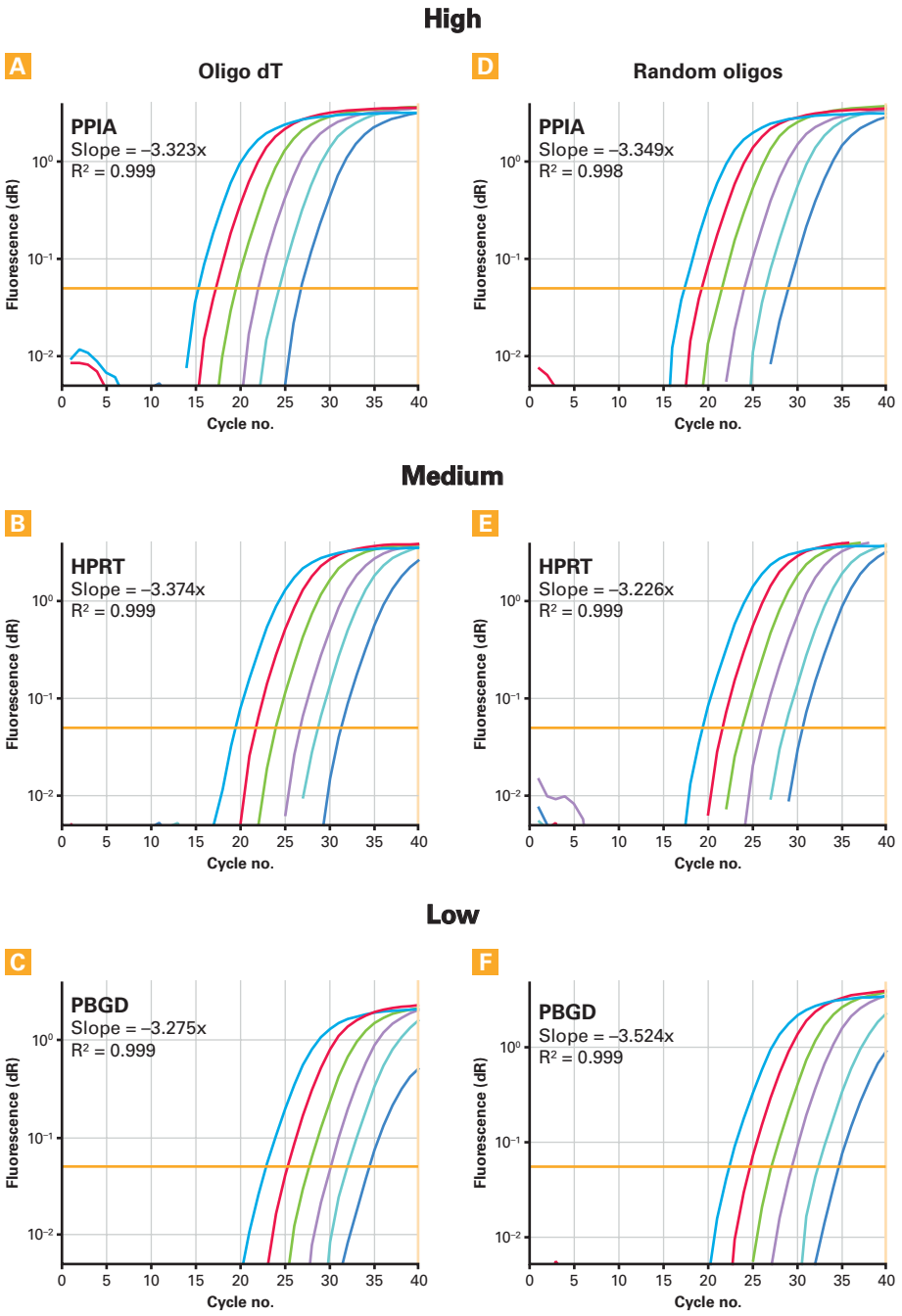

Figure 2. High-, medium- and low-abundance gene targets are easily detected in qPCR Human Reference cDNA. Oligo dT-primed (**Panels A–C**) and random oligo-primed (**Panels D–F**) qPCR Human Reference cDNA samples were each serially diluted fivefold such that the final quantities of cDNA template in the qPCR reactions were 20 ng, 4 ng, 800 pg, 160 pg, 32 pg, and 6.4 pg. Data were obtained on a Stratagene Mx3000P® real-time PCR instrument. The primer sets for all three genes demonstrate the ability to detect a wide range of signals from the serially diluted samples. PPIA = peptidylprolyl isomerase A (cyclophilin A; high-abundance); HPRT = hypoxanthine phosphoribosyltransferase I (medium-abundance); PBGD = porphobilinogen deaminase (low-abundance). Slope and R^2 values refer to the line determined by plotting C_t values versus template quantity.

Product	Size	Cat. No.
Human Universal Reference Total RNA	2 x 200 µg	636538
qPCR Human Reference cDNA, oligo dT	25 rxns	636692
	100 rxns	636693
qPCR Human Reference cDNA, random primed	25 rxns	639653
	100 rxns	639654
qPCR Human Reference Total RNA	25 µg	636690
SMART MMLV Reverse Transcriptase	2,000 units	639522

Related Products

- Mouse Universal Reference Total RNA (Cat. No. 636657)
- Rat Universal Reference Total RNA (Cat. No. 636658)
- SMART™ MMLV RT (Cat. Nos. 639522, 639523 & 639524)

References

1. Suzuki, T. *et al.* (2000) *BioTechniques* **29**(2):332–337.
2. Control RNA for Microarray Experiments. (April 2002) *Clontechniques* **XVII**(2):6.
3. Premium Total RNA Contains Virtually No Genomic DNA, An Important Factor in RNA Quality. (October 2002) *Clontechniques* **XVII**(4):8–9.