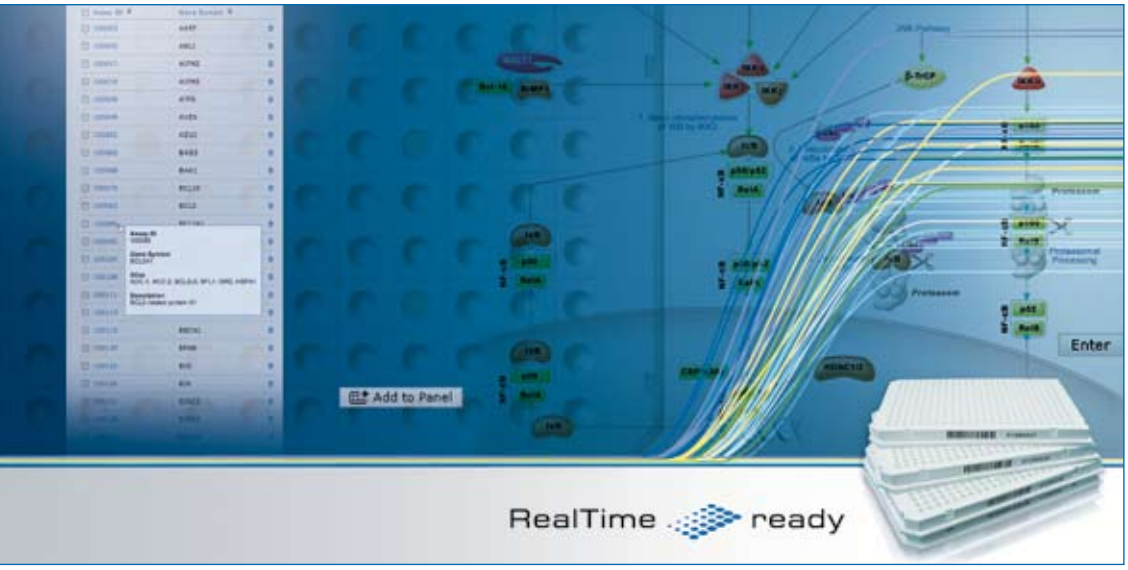
New: RealTime ready – Custom qPCR Assays and Panels for Human Targets

Gene expression profiling of the human genome on the LightCycler® 480 System has never been so easy.

Function tested qPCR assays for human targets are now only a few clicks away, with the new RealTime ready Configurator. This free online assay selection and shopping tool can be used to order single assays (for up to 300 20-µl reactions) or to configure customized panels on LightCycler® 480 Multiwell plates, with ready-to-use assays for one PCR reaction.



RealTime ready Configurator

Convenient online assay search

Assay selection or custom panel configuration is easy with the comprehensive search functions and intuitive graphical design of the RealTime ready Configurator. The four different search functions, in combination with detailed assay information and bioinformatic background on the targets, help to define relevant targets and find the right assays for individual research needs.

- To order a few single assays or configure layouts with a small number of assays, the "Search by Keyword" option is used to enter up to five search terms at a time.
- In order to search for a large number of already selected targets, the "Batch Upload" option is used to upload up to 384 different assay or accession IDs.
- To study regulation of larger gene groups after knockouts or after cell-line treatments that influence certain pathways or signaling cascades, one of two convenient functions, "Search by Pathway" or "Search by Focus Panel" can be used.
  - The "Search by Pathway" function is used to select your targets from a variety of interactive maps of cellular or signaling pathways.
  - With the "Search by Focus Panel" option individual Focus Panels are configured by searching a large number of pre-selected targets of relevant gene superfamilies, such as GPCR, protein kinases, or functionally related groups such as transcription factors and ion channels.

Graphical panel configuration

The RealTime ready Configurator features an intuitive graphical interface. A large variety of layouts (with or without controls) can be simply filled with assays. Changes and edits during the configuration process are automatically stored in a password-protected account at every step; the panel content will be available for download after the purchase is complete. Panel content is downloaded as a text file (.txt) for use with the LightCycler® 480 Instrument Software.

After making a final review of your configurations and price totals, you can go straight to the shopping cart and order online, or print out a hard-copy form to order via normal mail. For detailed information, please visit www.realtimeready.roche.com to view a short tutorial video or to go directly to the RealTime ready Configurator. ■

RealTime ready – Function Tested qPCR Assays Based on the Universal ProbeLibrary Technology

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RealTime ready qPCR Assays redefine real-time qPCR because each assay is function-tested in PCR and qualified under strict criteria to assure superior performance. The RealTime ready Assays are based on the well-known Universal ProbeLibrary (UPL) technology and are available in a ready-to-use formulation as dried-down assays in LightCycler® 480 Multiwell Plates 96 and 384, or as a primer probe mix for single assays in a liquid formulation for all real-time PCR platforms available in the market.

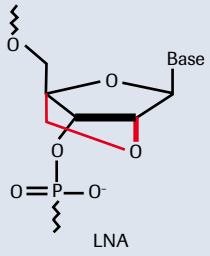


Figure 1: UPL probes incorporate LNA nucleosides. LNA is a class of nucleic acid analogues where the ribose ring is "locked" with a methylene bridge connecting the 2'-O atom with the 4'-C atom. The locked ribose conformation enhances base stacking and backbone pre-organization, increasing thermal stability and discriminative power of duplexes. LNA discriminates single base mismatches under conditions not possible with other nucleic acids.

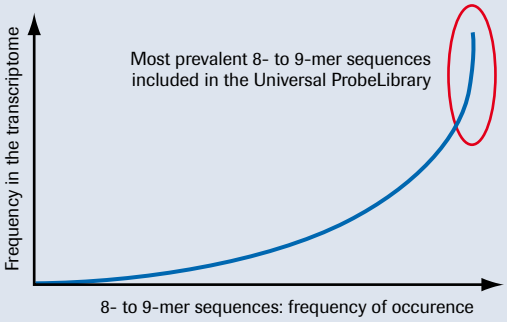


Figure 2: UPL probes detect highly prevalent 8- to 9-mer sequences.

Introduction

The Universal ProbeLibrary is based on only 165 short hydrolysis probes, labeled at the 5'-end with fluorescein (FAM) and at the 3'-end with a dark quencher dye. UPL assays are compatible with all real-time PCR instruments capable of detecting fluorescein (FAM) or SYBR Green I. The extensive transcript coverage rate of the UPL probes is due to their short length of just 8-9 nucleotides and the selected sequences. In order to maintain the specificity and melting temperature that hybridizing qPCR probes require, locked nucleic acid (LNA; Figure 1) are incorporated into the sequence of each UPL probe. LNAs is a DNA analogue with increased binding strengths compared with standard DNA nucleotides. The sequences of all existing 165 UPL probes have been carefully selected to detect 8- to 9-mer motifs (Figure 2) that are highly prevalent in the transcriptomes, ensuring optimal coverage of all transcripts in a given transcriptome. Within the human transcriptome, each probe binds to approximately 7.000 transcripts, while each transcript is detected by approximately 16 different probes. Only one specific transcript is detected at a time in a given PCR assay, as defined by the set of specific PCR primers.

The major advantage of the UPL technology is the fast turnaround time from assay design to PCR result. With the assay design software ProbeFinder it is possible to design specific, intron-spanning assays for gene expression profiling applications in seconds. For each transcript of the human genome one RealTime ready qPCR Assay is designed and tested and can be ordered from Roche Applied Science. All assays are developed for single-color mode only and can be used in two-step real-time PCR applications by using cDNA as sample material.

Design Process for RealTime ready Assays

RealTime ready Assays are designed using the established Universal ProbeLibrary ProbeFinder Software, which is based on verified design algorithms. For a particular gene, one unique transcript is chosen for assay design. The selection of this representative transcript is based on annotation and cross-references within two of the main public gene/transcript annotation resources, Ensembl (www.ensembl.org) and Entrez Gene (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene). If possible, an intron-spanning assay is designed. This procedure is thought to detect a broad range of splice variants of a certain gene.

To find your assay or gene of interest, almost any gene, protein, transcript, sequence ID, or probe ID may be

used as a search term. The annotations are based on the GRCh37 gene build released by the Genome Reference Consortium (http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/index.shtml).

Based on the selected reference gene ID, ProbeFinder software designs real-time PCR assays by combining a suitable UPL probe with a set of target-specific primer pairs. Together, the probe and PCR primers constitute a specific real-time PCR assay for a given target. ProbeFinder assay design software is based on Primer3 software using optimized settings to adapt the design algorithm to the specific needs when LNA probes are used. The design process takes into account the target sequence and designs preferred intron-spanning assays with a very high success rate and superior quality. The *in silico* process at the end of the design process calculates possible primer dimer formation, primer/primer and primer/probe interaction, self-assembly of the primer, and checks for cross-hybridization, favoring intron-spanning assays with short introns and short amplicon lengths. At the end of the design process ProbeFinder software ranks all possible assays according to the above-mentioned criteria to identify the best-performing assay in respect to the expected PCR performance.

Although the ProbeFinder design process yields assays with a very high success rate in PCR, all designed assays are pre-tested with a commercial cDNA to ensure that all assays have a guaranteed high-quality performance.

RealTime ready Assay Performance and Application Data

Each RealTime ready Assay is selected and function tested according to stringent criteria to ensure optimal performance: assays are tested in qPCR using the LightCycler® 480 System. A universal human cDNA sample is tested in a dilution series (50 ng, 5 ng, 500 pg, 50 pg, 5 pg equivalents of total RNA), and with a no-template control. Each RealTime ready Assay meets the following criteria:

- PCR efficiency 2.0 ± 0.2
- Cp of highest cDNA concentration ≤ 34
- Linear dynamic range of at least 3 logs
- R2 value of standard curve between 0.99 and 1.00
- High amplification specificity, no side products in gel analysis

Figure 3 displays the amplification curves on a LightCycler® 480 Instrument II. The replicates show almost identical Cp values (crossing points; Table 1). The distances between the different dilutions are consistent and the steepness of all individual curves is almost identical.

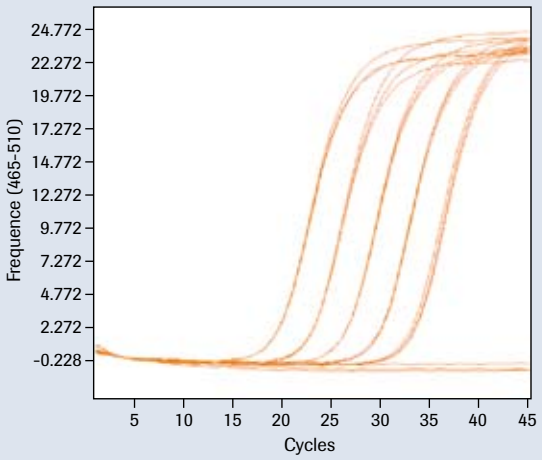


Figure 3: Human RAN/RANP1 gene. Dilution series: 50 ng, 5 ng, 500 pg, 50 pg, 5 pg, no template control, each in triplicates.

Table 1: Mean Cp and standard deviation of the different dilutions.

Concentration	Mean CP	SD CP
50 ng	19.31	0.020
5 ng	22.78	0.035
500 pg	26.19	0.015
50 pg	29.71	0.030
5 pg	33.06	0.191
NTC	-	-

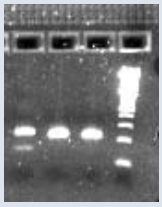
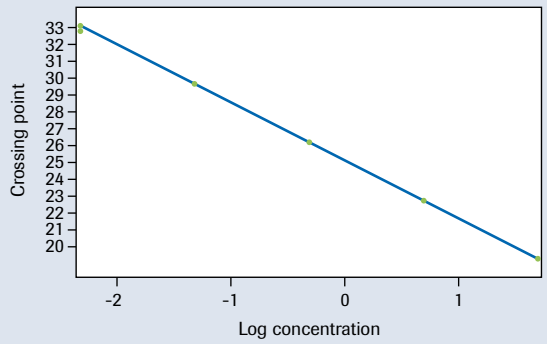


Figure 4: Gel analysis of above PCR (human RAN/RANP1 gene). 4% Agarose gel of human RAN/RANP1 gene. In the left lane an aliquot of the 50 ng dilution is applied on the gel; middle lane: 5 pg; right lane: 50-bp ladder. There are no side products detectable in gel analysis.

The standard curve shows that the replicates are all on a line and that efficiency meets the above-mentioned criteria (Figure 5).

The results with RealTime ready Assays are highly reproducible, as shown for example by Zhang et al [1]. They tested the correlation between two biological replicates on a) the Reference Gene Panel, b) the Cell Cycle Regulation Panel and c) the Apoptosis Panel. An almost ideal straight line is found and R^2 values range from 0.9996 to 0.9875 respectively.

They also investigated the variances of biological replicates over a range of different expression levels, and



Slope	R	Efficiency
-	-	
3,44	0,9999	1,95

Mean Cp Conc1	Mean Fluo hub Conc1
19,31	23,3120192

Overall score	Error
pass	0,00341

Figure 5: Standard curve of human RAN/RANP1 gene. 10-fold dilution series from 50 ng to 5 pg, each in triplicates.

determined the relative expression level with the ΔΔCT method over all three RealTime ready Panels (947 duplicate measurements). The coefficient of variance (CV) is very stable within the range of 0.1- to 2.5-fold expression and is around 10%. This makes it easy to determine "true" variations. ■

References

1. Zhang et al (2009) Biochemica 2:22

Further reading

Jori B et al. (2009) Biochemica 4, in press
Zhang J et al. (2009) Biochemica 2:21–24
Scheuermann T et al. (2009) BioTechniques 46:557–558
Sun Y et al. (2009) Roche Diagnostics GmbH, Gene Expression Application Note No. 2
Scheuermann T et al. (2009) Roche Diagnostics GmbH Cellular Analysis Application Note No. 5
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Order INFO

Product	Pack Size	Cat. No.
Transcriptor First Strand cDNA Synthesis Kit	1 kit (50 reactions, including 10 control reactions)	04 379 012 001
	1 kit (100 reactions)	04 896 866 001
	1 kit (200 reactions)	04 897 030 001
Transcriptor High Fidelity cDNA Synthesis Kit	1 kit (50 reactions, including 10 control reactions)	05 081 955 001
	1 kit (100 reactions)	05 091 284 001
	1 kit (200 reactions)	05 081 963 001
LightCycler® 480 Instrument II	1 instrument (96-well)	05 015 278 001
	1 instrument (384-well)	05 015 243 001
LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
LightCycler® 480 Software, Version1.5	1 software package	04 994 884 001
LightCycler® 480 Multiple Plate Analysis Software	1 software package	05 075 122 001
LightCycler® 480 Probes Master	5 × 1 ml (5 × 100 reactions, 20 µl each)	04 707 494 001
	10 × 5 ml (10 × 500 reactions, 20 µl each)	04 887 301 001
	1 × 50 ml (5,000 reactions, 20 µl each)	04 902 343 001
RealTime ready DNA Probes Master	5 x 1 ml (12,500 x 2 µl reactions, or 1,250 x 20 µl reactions)	05 502 381 001