

# Discover the Differences – Gene Expression Analysis with Universal ProbeLibrary

## Now available for Dual Color Assays and New Organisms



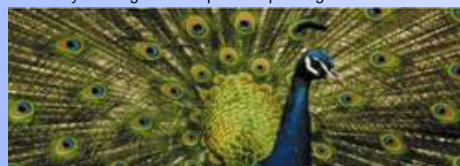
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### Introduction

Although quantitative real-time PCR is in principle a simple technique, assay design remains fairly complex, and assays designed for specific applications often lack sensitivity and reproducibility. The time spent on assay design, optimization, and validation often becomes a bottleneck, delaying implementation of new assays for large-scale expression profiling.



### Principle

The Universal ProbeLibrary (UPL) is a fast, specific, and flexible format for quantitative real-time PCR experiments. Just 165 short UPL probes, modified with locked nucleic acids (LNA), provide transcriptome-wide coverage in many organisms (including human, mouse, rat, arabidopsis, drosophila, primates, and *C. elegans*). Universal ProbeLibrary probes can be selected with the help of ProbeFinder, a free, web-based assay design software. In seconds, ProbeFinder can design highly specific intron-spanning assays for a target transcript. The system can meet a variety of design needs, including designs for transcript variants and gene family members, as well as designs for dual color reference gene assays.

### Technical Background

Advantages of Universal ProbeLibrary (UPL) over SYBR Green I:

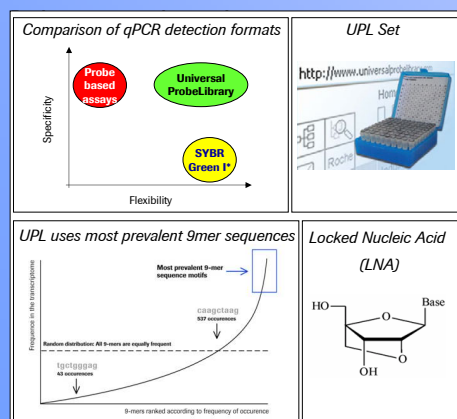
- No primer dimers or side products detected, due to use of specific probes

Advantages over other probe-based assays:

- Results available within 2 days (instead of 1 week)
- Less expensive than regular probe-based assays

Additional advantages of UPL Assays:

- Universal PCR conditions for all UPL assays eliminate the need for optimizing cycling conditions



### Summary

The results presented here demonstrate that:

- Universal ProbeLibrary (UPL) assays are now suitable for relative quantification experiments, since they can be used in reference gene assays that are compatible with many real-time instruments.
- The latest version of ProbeFinder software integrates the positions of known SNPs into the assay design process and shows the SNP positions on the screen.
- UPL assays can now be used for new organisms (maize, rice, zebrafish, anopheles, yeast); these deliver the same performance and success rates as assays for previously supported organisms.

**For more information  
please visit us at our booth!**

### Dual Color Assays

Established methods of relative quantification use a reference gene, e.g., a housekeeping gene, to compare the expression level of the target of interest to a non-regulated gene.

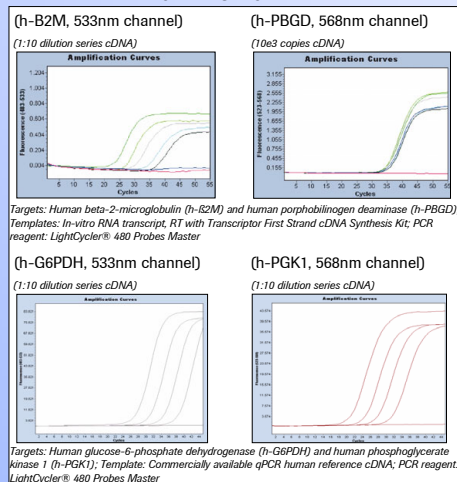
The following genes were selected for UPL Dual Color Reference Gene Assays:

- **Human:** h-G6PDH, h-PBGD, h-HPRT, h-ACTB, h-PGK1
- **Mouse:** m-ACTB, m-GAPD
- **Rat:** r-ACTB, r-GAPD
- **Eukaryotic 18s rRNA**

### Experimental Data

UPL Dual Color Reference Gene Assays are compatible with many real-time PCR instruments. Here we show some examples performed on the LightCycler® 480 Instrument.

#### UPL Dual Color Assays on LightCycler® 480 Instrument

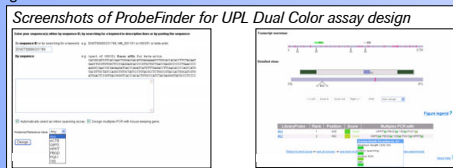


#### New ProbeFinder to design UPL Dual Color Assays

The new ProbeFinder software for designing UPL Dual Color Reference Gene Assays has two design options:

1. Select a reference gene from the list for your target of interest
2. Let ProbeFinder identify the best suitable reference gene for your target of interest

ProbeFinder designs the UPL Dual Color Reference Gene Assays by identifying the best match between the reference gene and the gene of interest.



### ProbeFinder Now Includes SNPs

Currently ProbeFinder does not take into account SNP positions when designing UPL assays. Here we describe the new version of ProbeFinder, which uses SNP information from the Ensembl database to avoid selecting primers or probes of UPL assays that span a polymorphism.

On the result screen, SNP positions are shown in magenta. Moving the mouse over these positions reveals more details on the selected SNP.



### New Organisms

In the past, Universal ProbeLibrary supported these organisms:

- **Human**
- **Mouse**
- **Rat**
- **Arabidopsis**
- **Drosophila**
- **C. elegans**
- **Primates**

For each of these organisms, ProbeFinder used a separate Assay Design Software and database to design customized real-time qPCR assays.

Now the UPL system covers the following new organisms:

- **Maize**
- **Rice**
- **Zebrafish**
- **Anopheles**
- **Yeast**

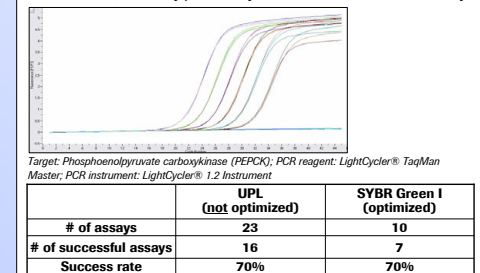
Databases for designing specific intron-spanning qPCR assays for these organisms are now available on the Assay Design Center. Other organisms, not mentioned in the organism selection chart, are supported by the "Other Organism" database. For all organisms, 165 UPL probes give successful assay designs with a high coverage rate.

#### Number of assays and coverage rates for UPL organisms:

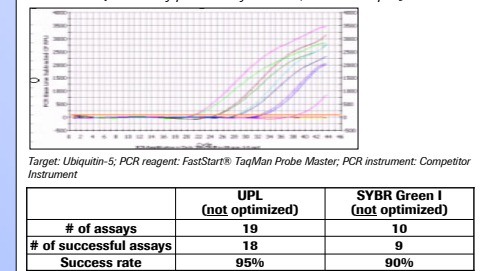
Organism	Species Name	Assays Available	Coverage Rate
Human	Homo sapiens	> 639 500	99%
Mouse	Mus musculus	> 509 500	99%
Rat	Rattus norvegicus	> 364 000	98%
Primates	Pan troglodytes	> 519 500	96%
Drosophila	Drosophila melanogaster	> 253 500	99%
Arabidopsis	Arabidopsis thaliana	> 199 000	98%
C. elegans	Caenorhabditis elegans	> 134 000	95%
Maize	Zea mays	> 61 500	94%
Rice	Oryza sativa	> 898 500	98%
Zebrafish	Danio rerio	> 630 000	98%
Anopheles	Anopheles gambiae	> 193 000	98%
Yeast	Saccharomyces cerevisiae	> 42 000	95%
Total		> 5 000 000	

### Experimental Data

#### UPL Maize (data kindly provided by Dr. Peterhansel, Aachen/Germany)



#### UPL Rice (data kindly provided by Dr. Mori, Tsukuba/Japan)



#### UPL Zebrafish (data kindly provided by Dr. Bally-Cuif, Munich/Germany)

