

# Roche Universal ProbeLibrary (UPL)

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# Real Time PCR

## Quantitative Real Time PCR Chemistry Options:

- DNA Binding Dyes: SYBR Green I
- Probe-Based Chemistries:

Linear Probes: Hydrolysis or Taqman probes

Structured Probes: Molecular Beacons and Scorpions

# Universal ProbeLibrary

- 165 specific and prevalidated hydrolysis/Taqman probes that can quantify virtually any transcript of a large number of organisms.
- Labeled at 5'-end with FAM and 3'-end with a dark quencher dye.
- Organism specific sets of 90 probes are available for: Human, Mouse, Rat, Primates, Drosophila, C. elegans, and Arabidopsis.



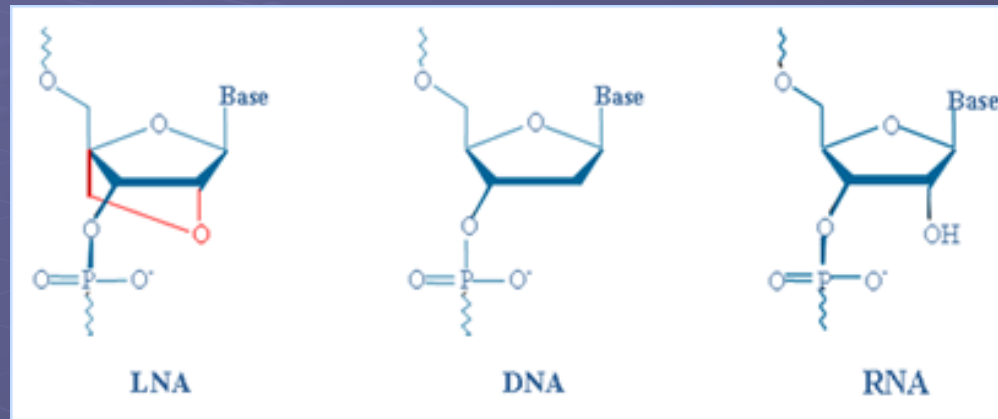
# How does UPL work?

- The versatility of the Universal ProbeLibrary probes has been achieved by shortening the length of the dual-labeled probes from 20-35 nucleotides to only 8-9.
- Probe sequences selected to hybridize to the most prevalent sequences in the transcriptomes. Each probe can hybridize to over 7000 transcripts.
- Specificity is attained by a defined combination of primers and the probe.
- High melting temperature and specificity of the short real-time PCR detection probes are retained by using a proprietary nucleotide chemistry, called Locked Nucleic Acids (LNA).

# Locked Nucleic Acids (LNAs).

- Locked nucleic acids (LNAs) is a class of nucleic acids analogues.
- The ribose moiety is “locked” with a methylene bridge connecting the 2'-O atom with the 4'-C atom.
- LNA nucleotides contain the six common nucleobases that appear in DNA and RNA and are able to form base-pairs with their complementary nucleosides according to the standard Watson-Crick base pairing rules.
- Therefore LNA nucleotides can be mixed with DNA or RNA bases in the oligonucleotide whenever desired.

- The locked ribose conformation enhances base stacking and backbone pre-organization. This significantly increases the thermal stability and discriminative power of duplexes.



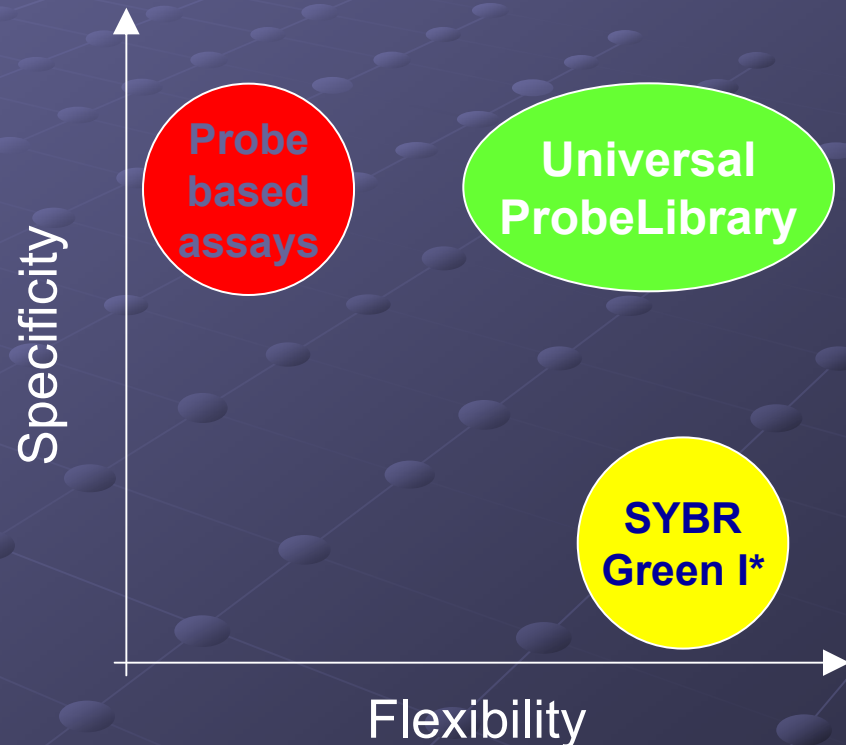
- LNA discriminates single base mismatches under conditions not possible with other nucleic acids.

	Perfect Match	Single Mismatch	$\Delta T_m$
 <p>LNA 8-mer 5'-TGC<b>I</b>GGTG-3'</p>	<p>3'-ACG<b>A</b>CCAC-5'</p> <p>71°C</p>	<p>3'-ACG<b>G</b>CCAC-5'</p> <p>45°C</p>	26°C
 <p>DNA 8-mer 5'-TGC<b>I</b>GGTG-3'</p>	<p>35°C</p>	<p>25°C</p>	10°C

# Universal ProbeLibrary

## Just 90 Probes but ...

- 99 % of mRNA transcripts in RefSeq Human are targeted
- 90 % of Ensembl mRNA transcripts are targeted with intron spanning assays
- Targeting 999 of the 1000 most cited human genes in RefSeq database with at least one assay



\* and other intercalating dye based assays

# Universal ProbeLibrary Procedure

Identify gene or target sequence of interest



Primers and probes for the specific intron spanning assay are designed in the web-based Assay Design Center



Order assay specific primers



Select appropriate probe from the Universal Probe Library



Perform Real-Time PCR

# Multiplex Assays with Universal ProbeLibrary Reference Gene Assays

- Universal ProbeLibrary Gene Assays quantify expression levels of a human, mouse or rat gene of interest in relation to an endogenous reference gene in a dual-colour assay.
- Specifically designed 12-mer UPL reference gene probe and gene-specific primer pair. Probe labelled with Lightcycler Yellow 555 at the 5'-end and dark quencher dye near the 3'-end.

# Assay Design

## Step 1: Choose an Organism

The screenshot shows the Roche Applied Science website's Assay Design Center. The top navigation bar includes links for 'At Your Service', 'Industry', 'Research Products', 'What's New', 'Online Ordering', 'Support', 'Printed Materials', 'Contact Us', and 'Roche Diagnostics'. The left sidebar contains a 'Product Search' box and a menu with options like 'Universal Probe Library', 'Assay Design Center', 'Home', 'Human', 'Mouse', 'Rat', 'C. elegans', 'Drosophila', 'Arabidopsis', 'Primates', 'XXX', and 'Need Help?'. The main content area is titled 'Universal ProbeLibrary Assay Design Center' and features the Roche Applied Science logo. It describes the ProbeFinder software and lists its benefits: Save Time, Simplicity, Design Assays You Can Trust, and Flexibility. A right-hand section titled 'Start the ProbeFinder software by choosing an organism' lists links for Human, Mouse, Rat, C. elegans, Drosophila, Arabidopsis, and Primates. The footer contains contact information for the webmaster and a link to the Roche Applied Science Home page.

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C. elegans  
Drosophila  
Arabidopsis  
Primates  
XXX  
Need Help ?

### Universal ProbeLibrary Assay Design Center

*Roche Applied Science*

The ProbeFinder software allows you to perform a fast and easy design of real-time PCR assays for your genes of choice.

- **Save Time**
  - Design your assays in seconds
  - Design up to ten assays at a time
- **Simplicity**
  - Just enter your target gene and you will get optimal design of your real-time PCR primers and a Universal ProbeLibrary probe selection
- **Design Assays You Can Trust**
  - 96% assay design success rate
  - Intron-spanning options
  - *In silico* PCR feature
- **Flexibility**
  - Assays for splice-variants, or splice-variant families
  - User-defined options

**Start the ProbeFinder software by choosing an organism**

- [Human](#)
- [Mouse](#)
- [Rat](#)
- [C. elegans](#)
- [Drosophila](#)
- [Arabidopsis](#)
- [Primates](#)

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ProbeFinder assay design software based on Primer3.

# Assay Design

## Step 2: Enter your Sequence/Gene of Interest

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- Rat
- C.elegans
- Drosophila
- Arabidopsis
- Primates
- Probe No. Conversion
- Need Help ?

**Universal ProbeLibrary for Human** *Roche Applied Science*

Enter your sequence(s) either by sequence ID or by pasting the sequence:

By sequence ID: e.g. ENST00000158302, NM\_001101 or AB062273

By sequence: e.g. >Human sequence in FASTA format or plain sequence format

```
CACGGCATCGTCACCAACTGGGACGACATGGAGAAAATCTGGCACCACACCTTCTACAAT
GAGCTGCGTGTGGCTCCCGAGGAGCACCCCGTGTGCTGACCCGAGGCCCCCTGAACCCC
AAGCCCAACCGCGAGATGACCCACATCATGTTTGAGACCTTCAACACCCAGCCATG
TACGTTGCTATCCAGGCTGTGCTATCCCTGTACGCCCTTGGCCGTACCCTGGCATCGTG
ATGGACTCCGGTGACGGGGTCACCCACACTGTGCCCATCTACGAGCGTATGCCCTCCCC
```

**Option 1: Enter sequence ID or gene name**

**Option 2: Paste sequence**

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# Assay Design

## Step 3: Receive Information on Optimal Assay

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**Universal ProbeLibrary for Human** *Roche Applied Science*

ProbeFinder has designed the optimal real-time PCR assay for:

[M33197.1](#) Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, complete cds.

You can increase sensitivity and obtain optimal performance of your qPCR assays by using the [FastStart TaqMan® ProbeMaster](#) on any block-based real-time PCR instrument.

**Assay details:**

**Use Universal ProbeLibrary probe: #60, cat.no. 04688589001**  
(Formerly Exiqon ProbeLibrary probe: Human#60)

Primer	Length	Position	Tm	%GC	Sequence
Left Primer	19	41 - 59	60	58	agccacatcgctcagacac
Right Primer	19	88 - 106	60	53	gcccaatcagaccaaattcc

**Amplicon (66 nt)**

```
agccacatcgctcagacacccatggggaaggtgaaggtcggagtcacggatttggtcgta
ttgggc
```

Download pack insert

Order probes or set

Download report

20\$ gift certificate drawing

[Figure legend ?](#)

# Assay Design

## Optional Step 4: Receive Additional Assays

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Figure legend ?

LibraryProbe	Rank	Position	Intron spanning	InSilicoPCR test	Score	Class
<a href="#">#60(Human#60)</a>	1	54	+	+	Perfect	Perfect
<a href="#">#45(Human#45)</a>	2	375	+	+	Perfect	Perfect
<a href="#">#87(Human#87)</a>	3	597	+	+	Good	Good
<a href="#">#45(Human#45)</a>	4	942	+	+	Good	Good
<a href="#">#25(Human#25)</a>	5	925	+	+	Good	Good
<a href="#">#39(Human#39)</a>	6	928	+	+	Good	Good
<a href="#">#9(Human#09)</a>	7	271	+	+	Good	Good

[All assays](#) or [return to best assay](#)

ProbeFinder Version: 2.04

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# *In silico* PCR

- Primer pairs designed by ProbeFinder are checked by *In silico* PCR. The algorithm searches the relevant genome and transcriptome for possible mis-priming sites for either of the two primer pairs.
- If any of the identified mis-priming sites are positioned in the genome or the transcriptome in a way that could potentially give rise to an unintended amplicon, the assay is down-graded and flagged as having failed the *In silico* PCR check.
- Also can choose “Design multiplex PCR with reference gene” ProbeFinder will conduct assay design for gene of interest and use *In silico* PCR test to validate its ability to be multiplexed with the selected UPL reference gene assay.
- Minimize risk of false assay signals from genomic DNA, unrelated transcripts generated by splice variants or homologous genes/gene family members, detection of pseudogenes, targeting genes with introns that are too short for effective intron-spanning assays

# Intron Spanning Options

- When pasting in a sequence can design intron-spanning assays by using opening and closing brackets to indicate the position of exon-exon boundaries.
- Or select “Automatically select an intron spanning option”

# Differentiating and Common Assay Options

- The “Differentiating Assay” option is useful to target splice variants of your gene. ProbeFinder tries to design assays that uniquely identify each of the input sequences by targeting areas of those sequences that are distinctly different.
- The ‘Common Assay’ option scans all the available assays and tries to identify one assay that targets an area found in all the input sequences. Useful when you wish to target all members of a particular gene family.

# Purchasing Probes

- Individually

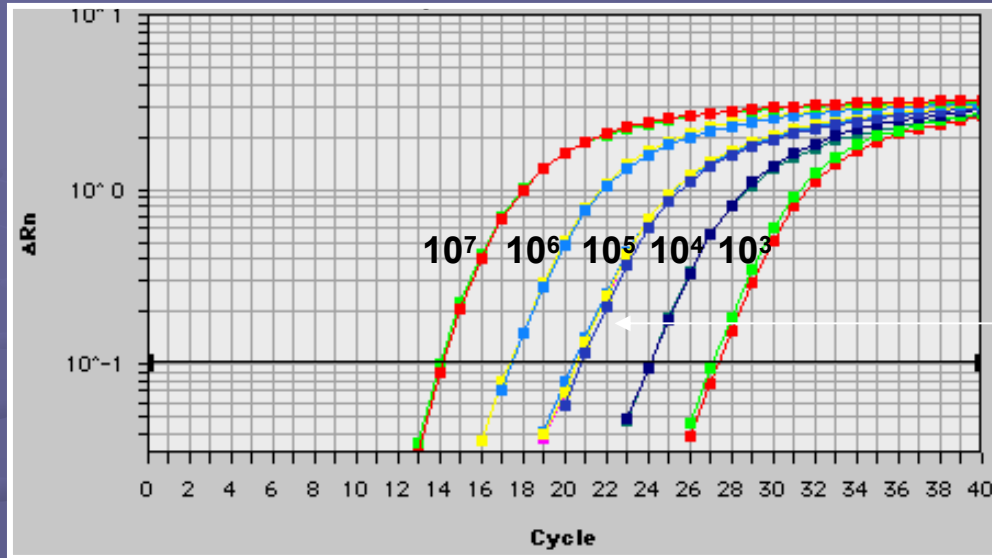
(2 vials 125ul of 10mM probe solution enough for 1000 25ul reactions)

- Universal ProbeLibrary Set human  
containing probes #1-#90

(90 vials 125ul of 10mM probe solution enough for 500 25ul reactions or 45,000 reactions for the full set)

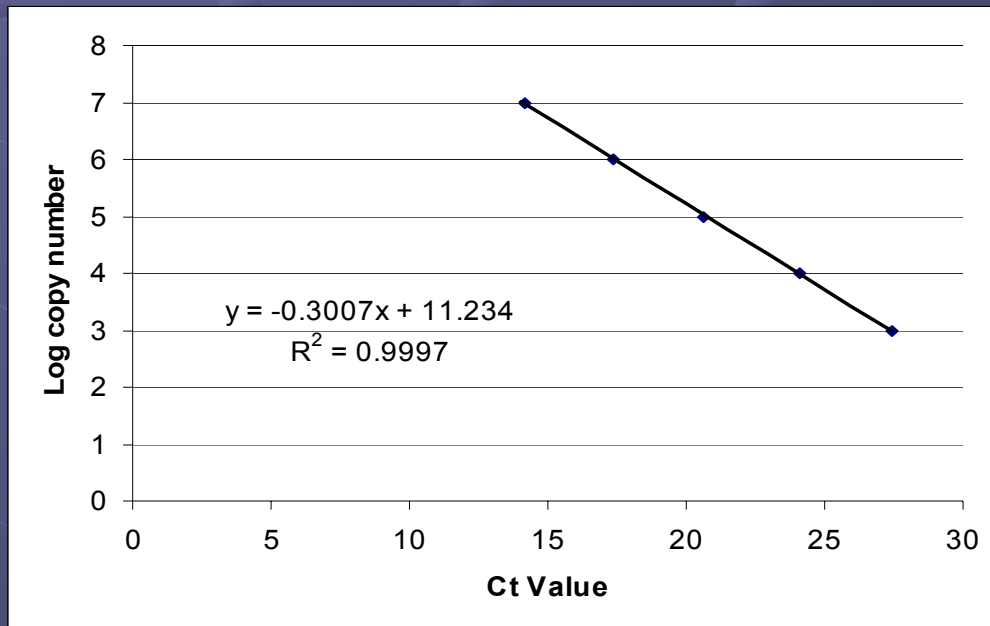
- Universal ProbeLibrary Extension Set (covers most species)  
containing probes #91-#165

# Results using ABI7700



Dilutions of vector:  
 $10^7$  to  $10^3$  copies

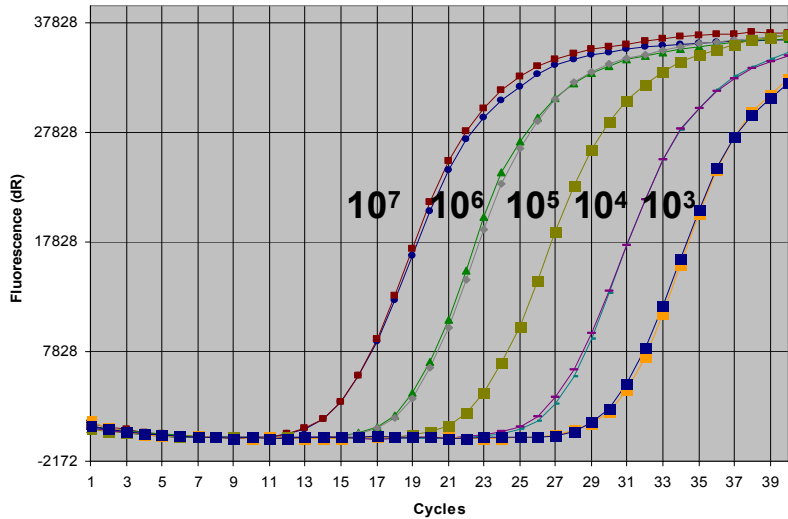
 Target Gene  
Ct Value = 20.525



Standard Curve of  
vector dilutions  
hu806 40A10

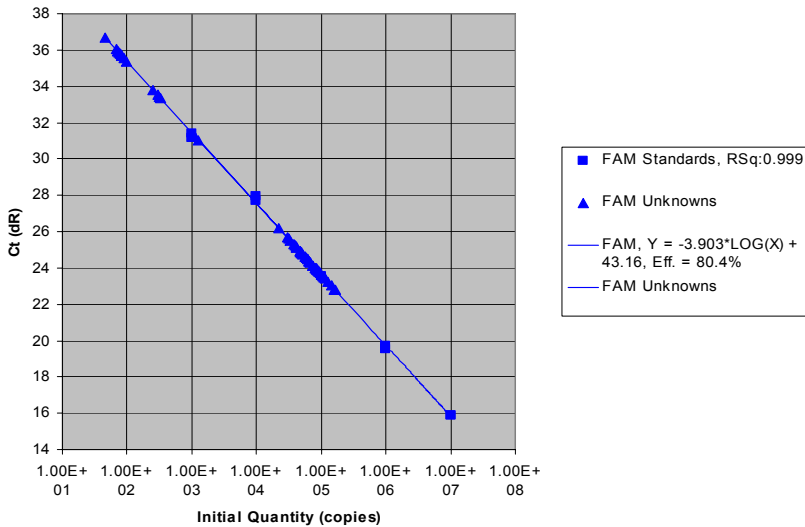
# Results using Mx3005P

Amplification Plots

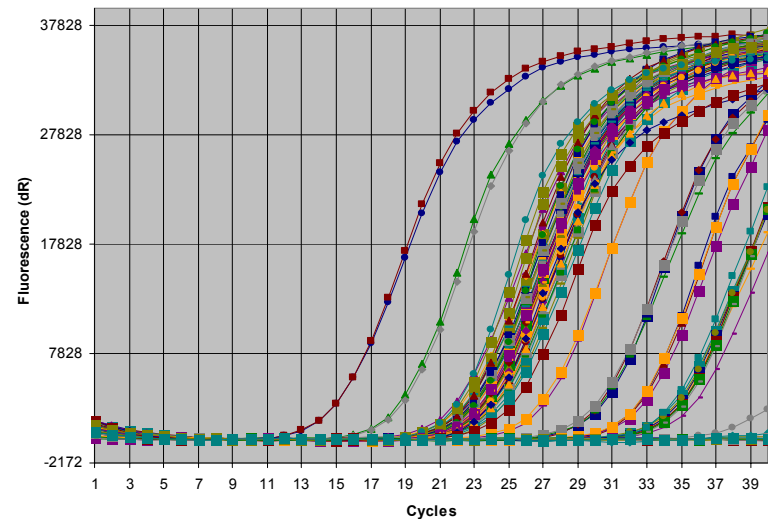


Standard Curve of  
vector dilutions  
hu806 40A10

Standard Curve



Amplification Plots



# Universal ProbeLibrary

- Flexible and specific assays
- Fast and easy assay design by web-based ProbeFinder software
- Faster time to result (Design one day, run PCR the next day)
- Assays are compatible with any real-time PCR instrument
- Cost sensitive assays in the range of SYBR Green I assays
- Target many different genes with real time PCR without ordering many different custom primers