RealTime ready
Universal ProbeLibrary – Assay Design

Utilize the free, online ProbeFinder assay design software at www.universalprobelibrary.com to generate qPCR assays for virtually any transcript of any organism

ProbeFinder Software

ProbeFinder is a web-based software tool, that is used in combination with the UPL probes. Based on the user-defined target information the software designs real-time PCR assays by combining a suitable UPL probe with a set of target specific PCR 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 as default, to give best results with UPL probes without any further optimization of assay conditions, as described in the product information. Experienced Primer3 users can modifiy these settings before they start assay design. (Details about used Primer3 settings can be found in the Glossary.)

In silico PCR

All primer pairs designed by ProbeFinder are checked by an in-house developed *in silico* PCR algorithm. The algorithm searches the relevant genome and transcriptome for possible mis-priming sites for either of the two PCR primers. 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 downgraded in the list of available assays and flagged as having failed the *in silico* PCR check.

The *in silico* PCR function minimizes:

- Risk of false assay signals from genomic DNA
- Risk of false assay signals from unrelated transcripts generated by splice variants or homologous genes/gene family members
- Detection of pseudogenes
- Targeting of genes with introns that are too short for effective intron-spanning assays

Design of Multiplex Assays with Human, Mouse or Rat Reference Genes

When the option "Design multiplex PCR with reference gene" is chosen, ProbeFinder will conduct assay design for your gene of interest, while at the same time subjecting each of these designs to an *in silico* test to evaluate its ability to be multiplexed with the selected UPL reference gene assays. The *in silico* PCR for multiplex assays, takes the following paramenter into consideration:

- Primer-primer interactions
- Primer-probe interactions
- Probe-probe interactions
- Probe-amplicon interactions (to prevent the probe from incorrectly generating signal on the amplicon)
- *In silico* PCR with all 4 primers (to prevent amplification of undesired cDNA fragments)

Assay Design Process

ProbeFinder performs a number of steps to select the optimal real-time PCR probe from the Universal ProbeLibrary in combination with a set of PCR primers. The following databases are available to ProbeFinder: h_sap_gene, h_sap_exon, h_sap_refseq, h_sap_embl, h_sap_genome. Database updates are done regularly.
(Details about data bases and sequence identifiers can be found in the Glossary.)

1 **Locate exon-exon junctions**	Introns are identified by one of following methods: • Look-up in Ensembl (if available) • Prediction by in house algorithm based on BLAST • User annotated in the input sequence ProbeFinder uses the following criteria when predicting introns: • The identity must be at least 95% certain • The exon must be at least 40 nucleotides long • The intron must be at least 30 nucleotides long
2 **Find appropriate UPL probe**	• Search input sequence for UPL probes target sites avoiding known human SNP's (only for Ensembl sequences). • The human, mouse and rat design relies on the 90 probes of the respective organism specific UPL sets where as the remaining are based on the complete 165 UPL probes
3 **Design PCR primer for each target site**	• Search the genome to ensure primer uniqueness • Search for gene family members and splice variants • Perform *in silico* PCR
4 **Rank the available assays to**	• Favour a unique assay without cross hybridizations to other areas of the genome (*in silico* PCR) • Favour intron spanning amplicons to remove false signals from contaminating genomic DNA • Favour a small amplicon size for reproducible and robust assays • Best multiplex combination with selected reference gene
5 **Display results**	• ProbeFinder always displays the best assay according to the above described ranking criteria. Assay details contain probe number, primer and amplicon sequence. In addition the "Multiplex PCR with Reference Gene" option is depicted. Results can be downloaded as pdf or text report. More assay details are shown in the"Transcript Overview" or the "Detailed View". When a sequence identifier from the GenBank/EMBL (*e.g.* ENST00000217133.1) is entered, SNPs of the whole transcript are displayed and details can be seen with the mouse over function. • When the "More Assays" option is selected, all possible assays for your gene of interest (with or without reference gene assays) are displayed in detail so that you select the best assay for your particular experiment.

Input Formats for Target Specification

The assay design process is startet by selecting the appropriate organism and entering target information in the depicted input windows.
Target information can be entered either by gene accession number, gene name or keyword, or the target nucleotide sequence. Acceptable entry formats include RefSeq, enBank/EMBL and Ensembl sequence IDs (details about the different sequence identifiers can be found in the Glossary). When gene names or keywords are entered, ProbeFinder provides results from a number of databases, containing your keyword, to help you select the gene ID or nucleotide sequence.

Other Organisms

When your organism of interest is not available in the drop down menue on the Assay Design Center, you can still use the ProbeFinder software to design assays. The UPL probes can be used to analyze any organism, and assays can be designed for any sequence from any organism (or any sequence from a non-natural source) provided that the sequence contains a probe binding site and corresponding acceptable primer sites. To design an assay for such a sequence select the button "Other Organisms" and paste the sequence into the "sequence"-field.

Entering Multiple Sequences

The "Batch processing" feature of ProbeFinder allows you to:

- Enter up to 10 different target genes at the same time
- See all results displayed on a single page
- Find assays that target splice variants or gene families

Please note: this feature is currently extended to process up to 200 design requests at a time.