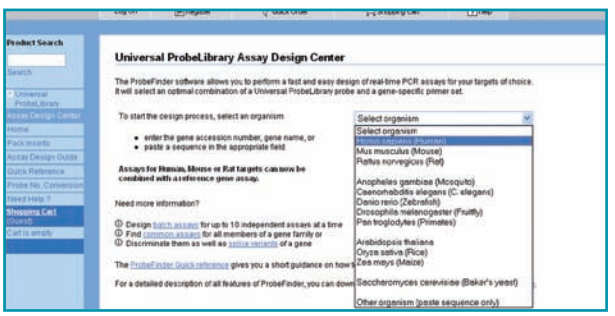
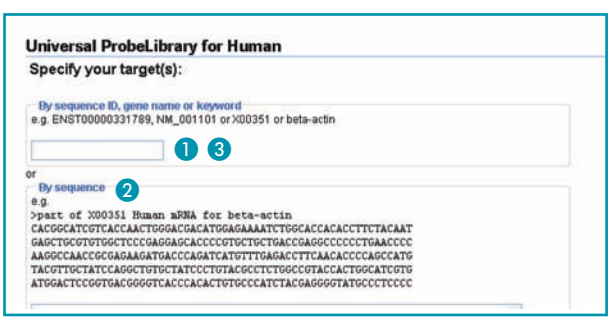
ProbeFinder Quick Reference

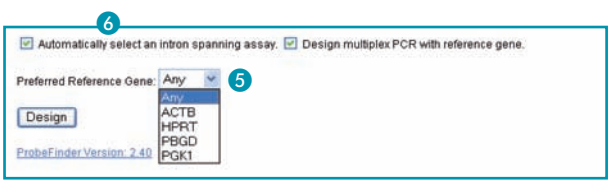
1. Choose an Organism

On the *Assay Design Center* screen select your **organism** of interest from the drop down menu to get to the target input screen. When your organism of interest is not available in the drop down menue, you can still design an assay by selecting “Other Organism”. In this case, you are asked to provide the sequence of the transcript, for which you want to design an assay (please refer to the option “Paste a Sequence”.)



2. Specify your Target





1 Option 1: Enter a sequence ID

The “By sequence ID” field accepts sequence identifiers from **Ensembl**, **RefSeq** and **EMBL** databases.

2 Option 2: Paste a sequence

The “By sequence field” accepts sequences in either the **FastA** or **Simple sequence** format.

3 Option 3: Enter a gene name

Type a gene name into the “By sequence ID” field. Probe Finder will fetch all sequence entries from the database which have this name in their description. From the retrieved sequences, select the one for which you want to design an assay.

4 Option 4: Enter multiple sequences

Available assay types are: **Batch**; **Differentiating**; **Common** (up to 10 sequences are accepted). (for details please refer to Universal ProbeLibrary Guide to Successful of PCR Assays).

5 Option 5: Design multiplex assays

When you want to design a multiplex assay with one of the UPL reference gene assays for human, mouse and rat, check the respective box. Choose one of the assays or choose “Any” when you want to leave it to ProbeFinder to find the most suitable reference gene assay for your gene of interest or select one of the provided assays in the drop-down menue.

6 Design intron-spanning assay

Choose the “Automatically select an intron spanning assay” option, to let ProbeFinder determine the locations of introns and exons in the input sequence. By default, ProbeFinder software will show probes that bind close to exon-exon splice junctions, thereby facilitating amplification of intron-spanning targets (which eliminates false positive signals from residual genomic DNA).

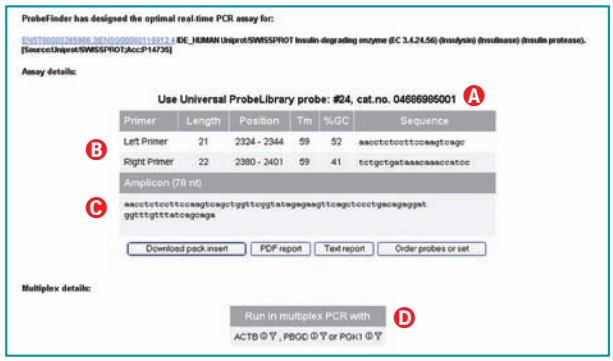
7 Press “Design” for Primer Design and Probe Selection.

3. Examine the Result

The highest ranked real-time PCR assay is presented on the result screen. The result screen has four parts: **Assay details**, **Multiplex details**, **Transcript overview** and **Detailed view**.

The **Assay details** section shows (Ⓐ) the number and catalog number of the Universal ProbeLibrary probe; (Ⓑ) sequences of the forward and reverse primers, along with additional primer parameters such as length, position in the target sequence, melting temperature and GC content; and (Ⓒ) the length and sequence of the amplicon.

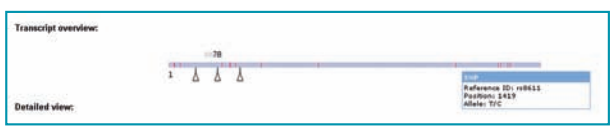
You can save all the assay details either as "**Text report**" or "**PDF report**".



Multiplex details Ⓓ
Information about the multiplex assay that you have choosen or which is recommended by ProbeFinder are shown. When you click on the small Information Icon ⓘ information about the respective reference gene assay is displayed.

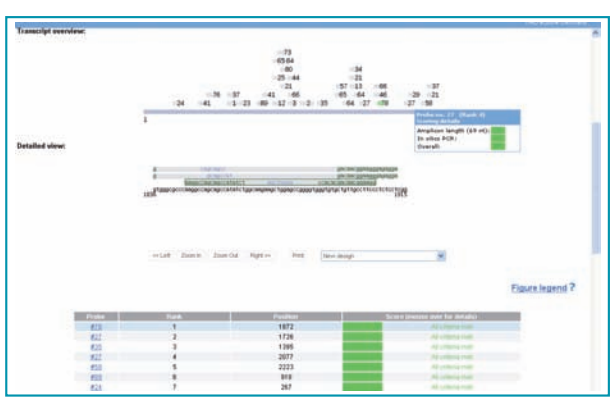
The **Order probes or set** button takes you to the *Roche Applied Science* e-Shop. Simply type in the name or catalog number of the probe in the e-Shop *Search* field to find the probe in the online catalog.

Transcript overview is a graphical overview of the complete target transcript. The numbers below the transcript refer to nucleotide positions in the input sequence. Wedges indicate exon-exon splice junctions . The number of the Universal ProbeLibrary probe appears above the transcript.

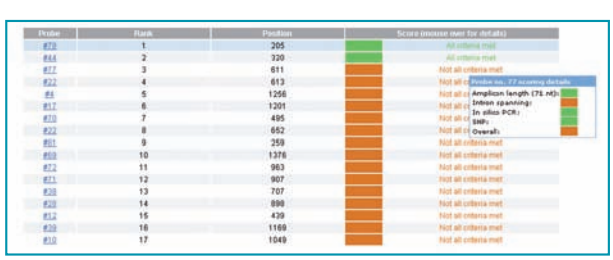


Detailed view is an enlarged graphical overview of the target transcript region. Details on the different information are obtained when using the mouse over function.

4. View additional assays



1. To see a table of alternative real-time PCR assays, click the "More assays" button on the Result screen. ProbeLibrary probes for additional assays are listed in a table and their position is shown in the "Detailed view". Assays are ranked according to the degree of fulfillment of scoring parameters and marked in decreasing shades of green. All multiplex options are shown for each assay.



2. A graphical overview of the complete target transcript appears above the assay table. In this graphic, the shaded areas above the transcript indicate the position and length of all amplicons targeted by the assays in the table, along with the number of the Universal ProbeLibrary probe that binds each amplicon. If you click on any amplicon in the graphic, the software will display a Details screen describing the corresponding assay. You can also use the "Zoom In" button below the graphic to enlarge the target display. Clicking other buttons will move the displayed sector left or right.

3. **See All Assays**
Click the "see all assays" button to display all assays found by the software, including those that do not meet all design criteria as amplicon size, intron spanning assay *in silico* PCR and (for human Ensembl) absence of SNPs in primer or probe binding regions. These assays are marked with a brownish color. If you decide to use such an assay, check the "not met" criteria using mouse over function to assure, that these are not critical for your experiment.

Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our Online Technical Support Site at: *www.roche-applied-science.com/support*