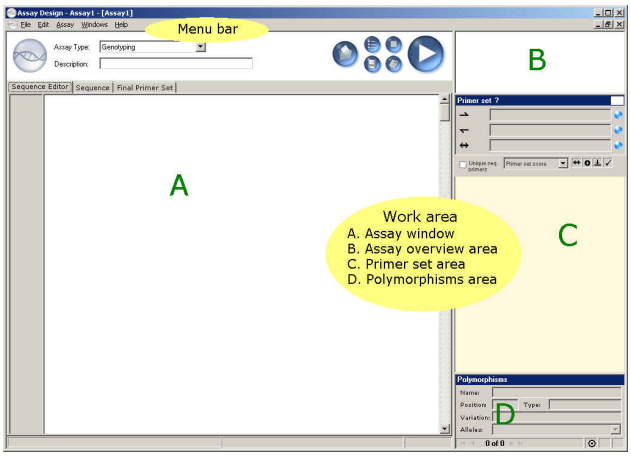
Quick Guide for Pyrosequencing™ Assay Design Software

Start Assay Design Software

Start the computer installed with Pyrosequencing™ Assay Design Software.

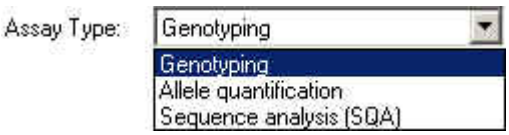
In Windows Start menu, choose **Programs | Biotage | PSQ Assay Design.** Alternatively, double-click the PSQ Assay Design icon on the Windows desktop.

The Assay Design Software start screen opens, displaying an empty assay file. The sequence can be entered or imported into the **Sequence Editor** tab in the **Assay** window.



Select assay type

From the **Assay Type** drop-down menu, select the desired assay type: genotyping, allele quantification (AQ), or sequence analysis (SQA).



The analysis steps and primer set scoring are automatically adjusted to the chosen application.

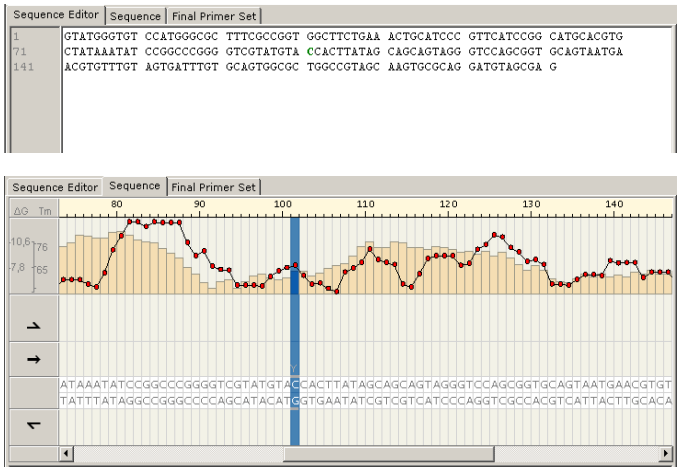
Enter DNA template sequence

On the **Sequence Editor** tab, enter a DNA sequence:

Import a sequence with the **Import** button *or* directly type in or copy/paste a sequence *or* open a previously saved assay file

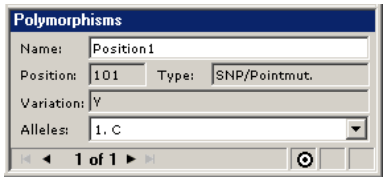
Enter SNPs with slash notation, e.g. A/T, or with IUPAC codes. Enter an unknown sequence stretch as a row of N, e.g. NNNNNNN. Position the cursor at the end of the sequence string and press Enter to finish.

The entered sequence is displayed on the **Sequence Editor** tab and on the **Sequence** tab. Polymorphisms are shown in the **Polymorphisms** area.



Enter position names

In the Polymorphisms area, the names of polymorphisms can be changed. These names (position names) are automatically transferred to the Entry at import of the assay into PSQ software.



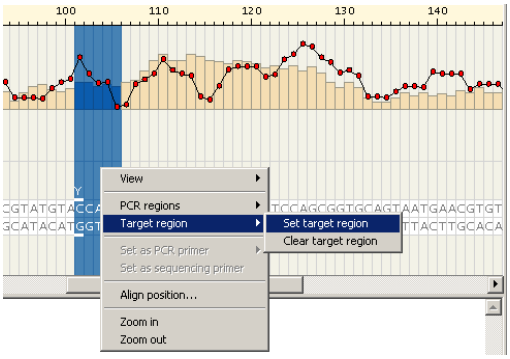
Biotage

Set target region

For genotyping and AQ, the default target region is the first polymorphism. For SQA, the default target region is the first unknown sequence, including 3 known bases flanking each side of the unknown region.

To change the target region:

Skip between polymorphisms/unknown regions in the **Polymorphisms** area and select the target by clicking the **Set target** button.
Alternatively, mark the desired polymorphism(s)/region on the **Sequence** tab and choose menu alternative **Set target region** (**Ctrl+T**).



The selected target region is highlighted in light blue on the **Sequence** tab. On the **Sequence Editor** tab, the target region is highlighted in yellow.

The software automatically sets PCR primer-annealing regions. However, it is possible to re-define the PCR regions to e.g. accommodate several SNPs within one amplicon. See Assay Design Software User Manual for more information.

Run assay design

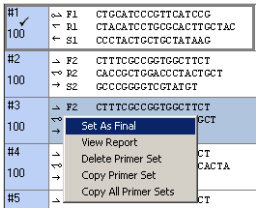
Click the Run Assay Design button to perform assay design.

View results and select primers

The results are displayed in a list in the **Primer Set** area. Primer sets are sorted by score (0-100, where 100 is the best score). By default, 100 primer sets are shown in the list. The top scoring primer set is automatically selected as final, which is indicated by a gray box surrounding the primer set.

Click on a primer set in the list. The highlighted primer set is displayed on the **Sequence** tab, where information about the assay can also be viewed. Double-click to open a report with detailed information about the assay and its primers. The report can be saved or printed. Four different report formats are available.

A different primer set can be selected as final by right-clicking on the set and choosing **Set as final**.



Save the assay

Click the **Save assay** button to save an .xml file.

Import into PSQ software

Open PSQ 96MA or PSQ HS 96A Software. Open the Simplex entries tree view. Right-click on a folder in which to save the imported assay and select **Import entries**. The **Entry import** dialog opens. Browse to locate the assay file (.xml) and click **Open**. The Entry ID will be the same as the name of the imported assay file.

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