SDM Assist Help

SDM-Assist is unique software that helps design site-directed mutagenesis primers introducing “silent” restriction sites

What is SDM?

Site-directed mutagenesis (SDM) is an indispensable tool for generating mutants in biological structure-function studies. The method is based on PCR using modified primers that introduce the required mutation directly into a plasmid-based gene. Following amplification, template DNA can be selectively digested using the restriction enzyme Dpn1. This process however, is often inefficient resulting in the recovery of both mutated and un-mutated clones when the Dpn1 digested PCR is transformed into E.coli to isolate individual constructs. Screening these constructs using sequencing requires money and time. This drawback can be overcome by enabling an easy distinction between mutated and un-mutated clones. In addition to the desired mutation, one could include an additional mutation; a silent one that causes no amino acid change but introduces a restriction site. Primer design software does indeed exist, but there was no single programme available that takes into account all the desirable features until now.

What is ‘SDM-Assist’?

SDM-Assist is a stand-alone application which allows SDM primer design in just 3 clicks without the need for an internet connection.

Key features:

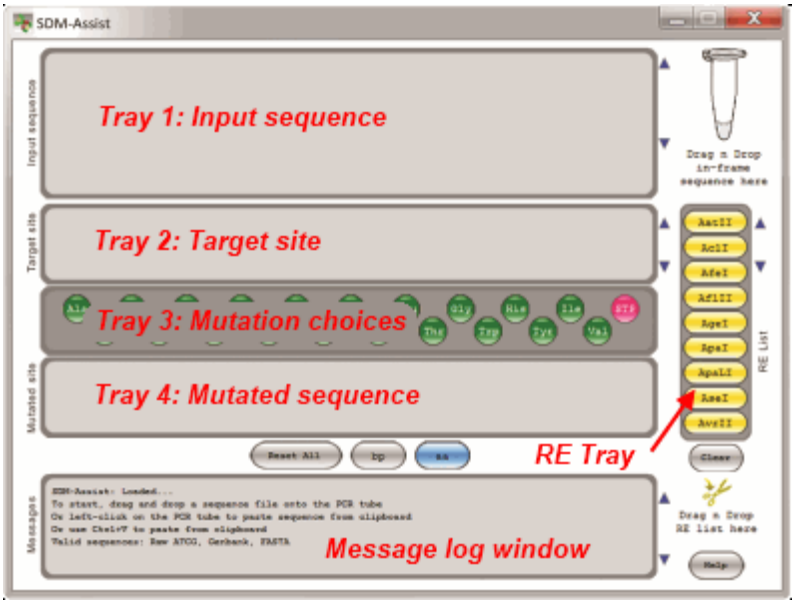
- Software can be easily downloaded by visiting the download link and installed on a laptop/PC/tablet (see requirements)
- Allows the user to generate and choose primers for SDM that contain a unique restriction site identity allowing for highly efficient identification of ‘mutated clones’ by a simple restriction digest.
- Suggested primer pairs are scored based on factors such as Tm, GC content, 5 prime and 3 prime-stability and secondary structure.
- Ability to custom choose and feed restriction enzymes to SDM Assist for inserting silent restriction sites in the primers.
- All suggested primer sequences along with detailed information on them can be exported into an excel or text file.
- A useful reference tool in the display window of the programme, which logs the sequence of events and provides brief tips as you design the primer.

Step-by-step through a full workflow of designing a mutated primer pair using SDM-Assist

- Click on the SDM-Assist icon generated after installing the software on your device to open the SDM-Assist display panel.

The display panel has 4 horizontal 'trays': The top tray displays the input sequence. The next tray displays the target site (a short stretch of the sequence in which the mutation is being introduced). The third tray provides the choice of amino acids for the mutagenesis. The fourth tray shows the short stretch of mutated sequence.

At the bottom, the message log window shows contextual information relevant to the last action performed by the user.



- To begin, drag and drop a DNA sequence in the correct reading frame onto the icon as a gene bank, FASTA or text file (bp sequences only). Left-clicking the microfuge tube in the top right corner or “Ctrl+V” pastes a sequence on the clipboard into the application.

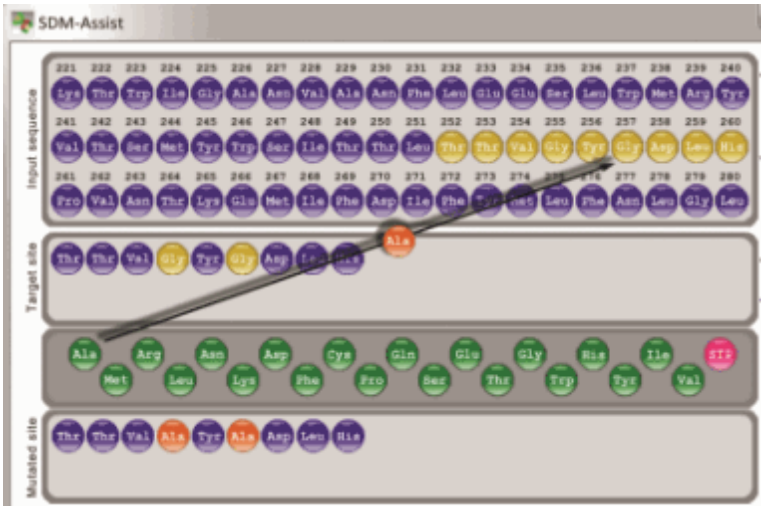
SDM assist automatically translates the DNA into a sequence of amino acids and indicates their position in the input sequence tray. Gene statistics will be listed in the log window at the bottom. You can change between base pairs and amino acids by clicking the 'aa' or the 'bp' button. This affects the input tray, the target site tray and the mutated site tray. These trays are also scrollable so that longer sequences can be viewed easily.

- Choice of Restriction enzymes for inserting with silent mutation.

The RE list tray on the right of the SDM-Assist panel contains commonly used restriction enzymes that can be selected for insertion in the primer through silent mutation. Right-clicking the enzyme shows the target sequence associated with it. By default all enzymes are selected. Reducing the number of restriction enzymes will decrease the chances of finding silent mutations that introduce restriction sites. The ability to filter the list however allows researchers to narrow down the options to the restriction enzymes they have access to. If you do not want SDM-Assist to consider all the restriction enzymes during primer design, you can simply deselect the unwanted enzymes with a left click. To narrow down the options to a very small number, press "Clear" and then select your preferred restriction enzymes. If you don't make any selections after pressing "Clear", SDM-Assist will use all the enzymes in the list.

Alternatively, if you need to add your own set of restriction enzymes to the list, drag and drop your list onto the  icon a file with the name and sequence for enzymes of your own choice. The file needs to contain the RE name and the target sequence separated by a space or comma or tab. Restriction enzymes that work on degenerate sequences need to be specified for each target sequence. A list of enzymes is available on the website for download. If the import succeeds, SDM-Assist will display them in the RE List tray. All the filtering options will work similarly for the imported list.

- To begin the next stage of the process, simply drag and drop the mutant amino acid from the amino acid tray into the desired position within the sequence in the Input sequence tray.



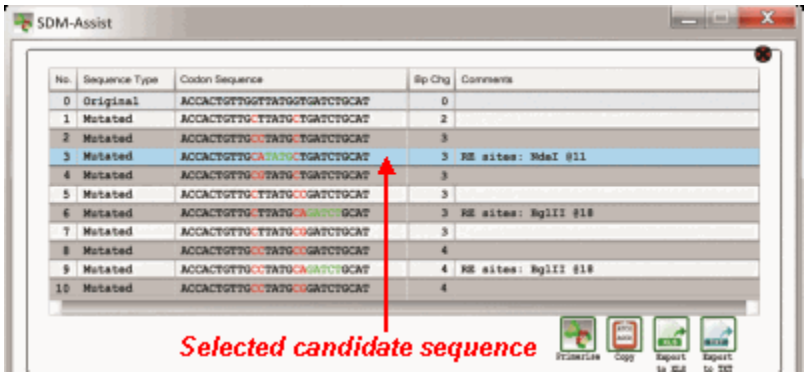
If you would like to choose a specific codon for the mutant amino acid, right clicking on the amino acid will cycle through all the available codons for use in the SDM primer design. Right-click till you see the desired codon sequence and then drag as before.

SDM assist will extract a small stretch of sequence from either side of the mutated amino acid. The original sequence will appear in the target site tray. The mutated sequence will appear in the mutated site tray with the mutated amino acid highlighted in red. Clicking left or right of that region within the parent molecule will extend the sequence. At this point, additional mutations can be introduced but keep in mind this will create longer primers, which may work less efficiently.

- After the desired mutated sequence is displayed in the mutated site tray, the "Mutagenize" button becomes visible. Clicking this button opens a new window that contains an evaluation of mutated sequences.

The mutated sequences highlight potential restriction sites that were present or new ones that can be introduced. Restriction sites or silent mutations are marked in green within the sequence, mutations that alter the coded amino acid in red.

- You have to select one of the sequences on which the primer pair will be based.



- After selecting one of the sequence, clicking the primerise button. The resulting window will present a list of 21 forward and reverse primers each.

SDM-Assist scores these primers on a number of factors including Tm, bp changes, and GC. An ideal primer would score 100 points. Clicking on the column headings allows alphabetical and/or numerical sorting for ease of use. We recommend using the primer pair with the highest scores.

The complete output list can also be exported as an Excel file (“xls”) or text file (“txt”). By selecting either of these two export buttons a file is saved to the desktop and automatically opened. Alternatively, you can also copy the primer using the copy button.

- At this point SDM-Assist resets to the opening display. Another mutation can now be introduced or a new sequence added to the microfuge tube for another round of primer design.

The step-by-step process of generating a SDM primer described in this help section can also be viewed in the Tutorial video that can be accessed via the download link given at the beginning of this help text.

We hope that SDM-Assist will enable you to design easier and more reliable site-directed mutagenesis primers.

Requirements:

Operating system(s): Platform independent (Tested for Mac OS 10.6, Windows XP and above)

Other requirements: Adobe AIR 2 or higher (http://get.adobe.com/air/)

Citation:

Karnik A, Karnik R and Grefen C: SDM-assist software to design site-directed mutagenesis primers introducing "silent" restriction sites. BMC Bioinformatics 2013, 14:105; DOI: 10.1186/1471-2105-14-105

For a detailed description please refer to our paper : (Journal Page)