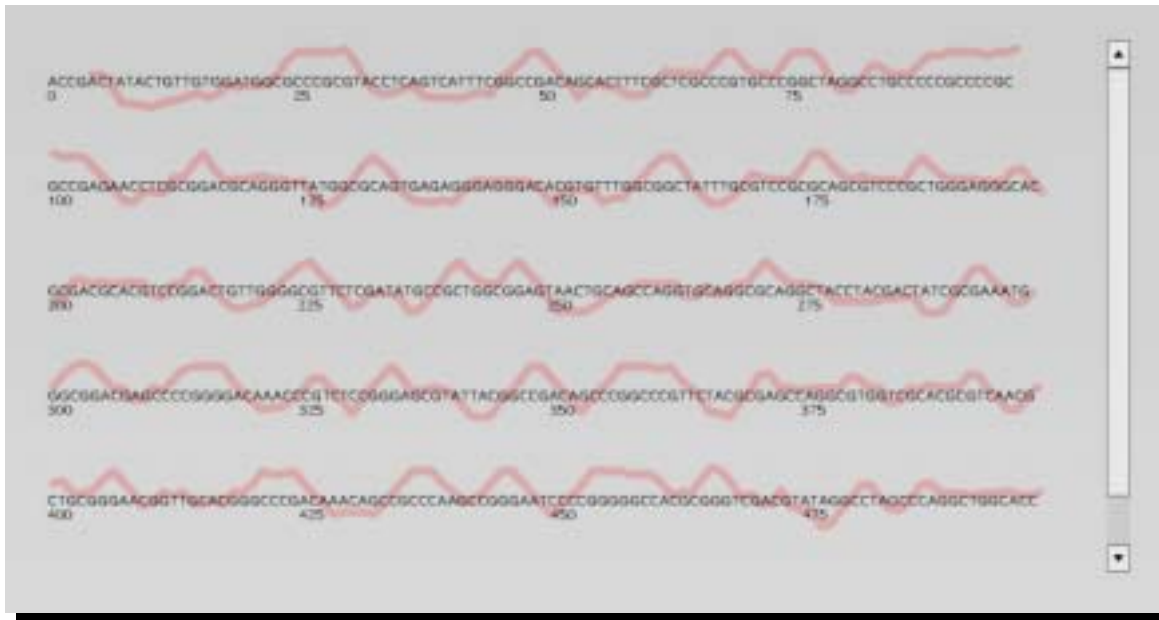


Designing Experiments

Evocyler™ software includes a sophisticated primer design page that automatically selects optimum primers within a given target sequence. Further, the software automatically generates the optimum thermal profile based on the identified primer sets and the specified amplicon.



The sequence plot shows the variation in duplex stability; see above.

Primer Design And Selection

Evocyler™ software recognises sequence information imported from a wide range of sequence sources; e.g. embl and swissprot. Alternatively sequences may be imported as text (.txt) files. To import a sequence file;

- select open from the file menu
- select sequence from the open as drop down box
- choose the sequence file

Once opened the software scans the sequence for optimum priming sites based on stability matching. Optimum priming occurs where there is a high five prime stability

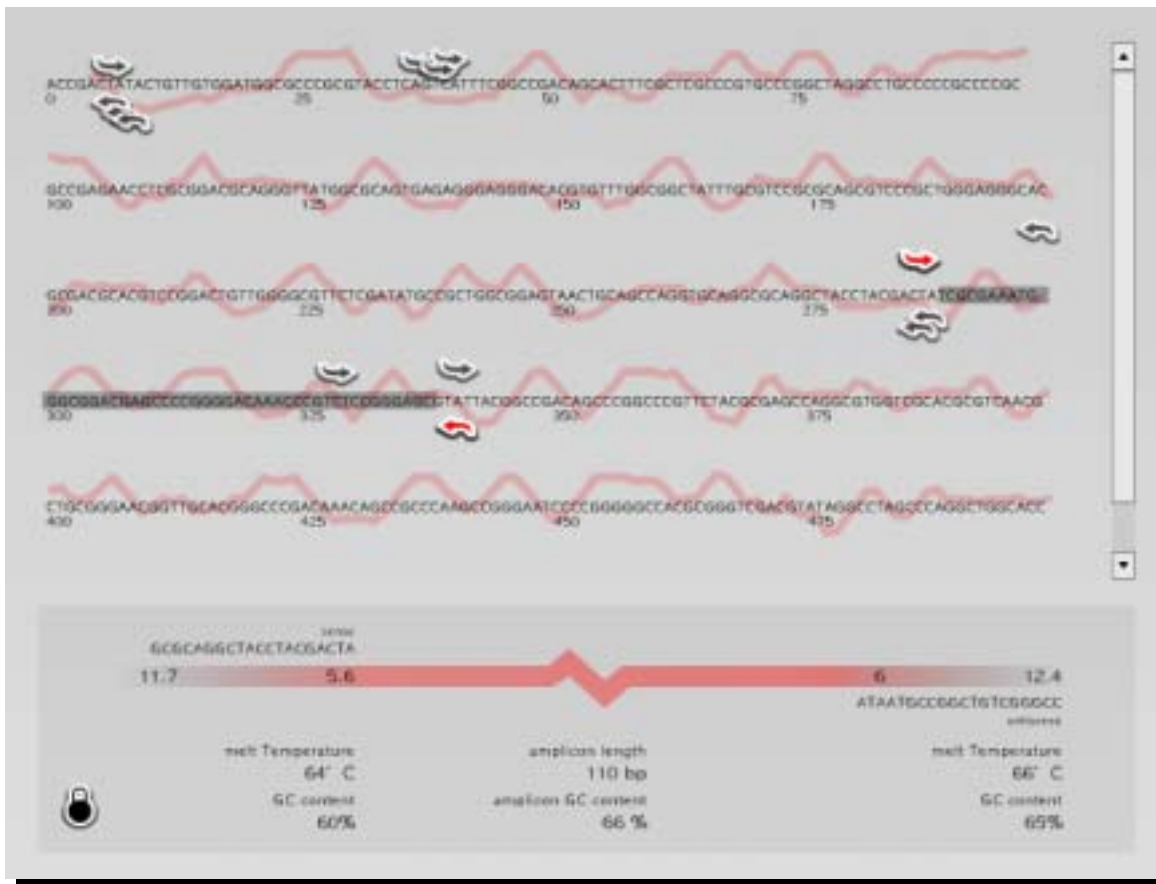


compared to a low three prime stability. These characteristics anchor the primer to the correct priming site (5'), while allowing for a high polymerisation efficiency (3').

To view the primers;

- select design from the menu
- select primers from the submenu

This will display a primer series according to annealing temperature; as the default the software automatically selects the annealing temperature that has the greatest density of primers.



The software automatically searches the amplicon for the most suitable primer pairing and displays the amplicon that will be generated from those priming sites.



You can switch between primer series with different annealing temperatures by:

- selecting design from the file menu
- double clicking on one of the primer icons to display primer subset graph
- clicking on the bar corresponding to the primer subset you wish to display and reselecting primers from the design submenu

User-defined primers may also be created from the imported sequence data. Using the mouse highlighting the section of sequence you wish to use as the priming sequence. Once selected identify whether you wish the highlighted sequence to be a sense or antisense primer.

Additional user annotations may be added to a particular highlighted sequence using the same methods for selecting user-defined primers but selecting the annotation icon from the popup menu.

Evocycler™ will automatically generate a thermal cycling profile based on the sequence and primer data. The software adjusts cycling conditions to reflect the new target sequence¹ taking into account;

- GC content of the target, amplicon, and primers
- annealing temperature of the amplicon and primers

If the autolink checkbox is checked the profile is automatically updated each time a new primer and amplicon pair are chosen. By unchecking the autolink option manual selection of these cycling conditions may be defined. In either case the user can manually adjust the number of cycles or add touch-up and touch-down gradients.

¹ It may be necessary to test a range of MgCl₂ concentrations depending on the source of genomic DNA; this is particularly true of large genomes where higher Mg²⁺ is normally required.