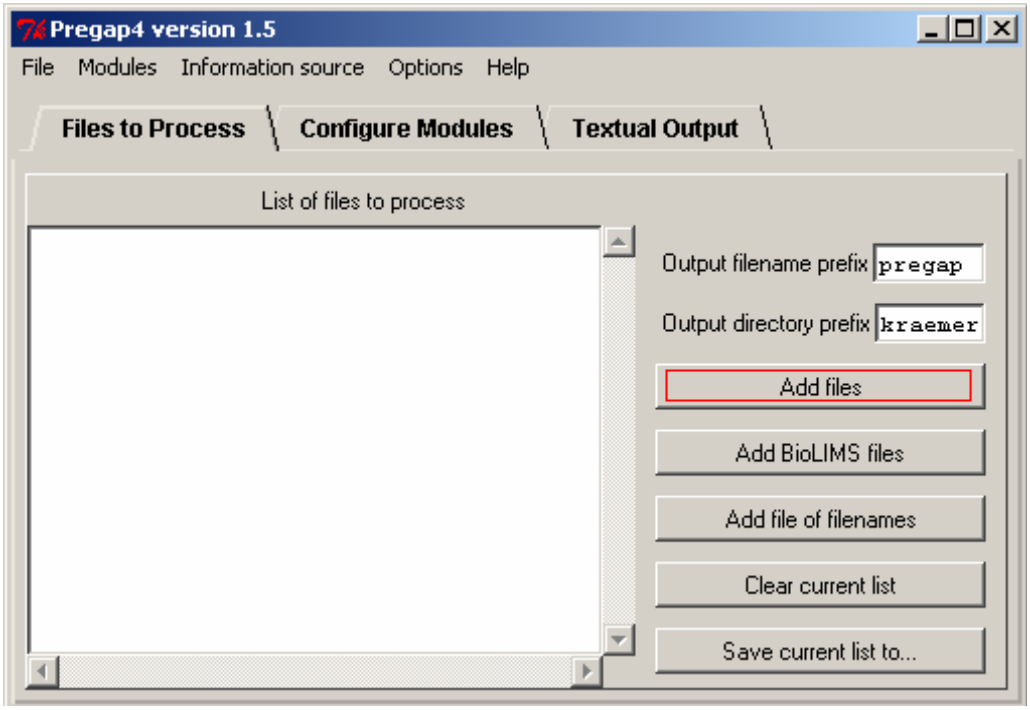
STAMP – Tutorial

From trace file to experiment file with SSR-tags

Before you start this tutorial you should read the “STAMP_Manual.pdf”, it can be found in the “doc”-folder of the Staden Package.

Loading the trace files

After starting Pregap4 click on the button “Add files” and mark all the trace files you want to process.

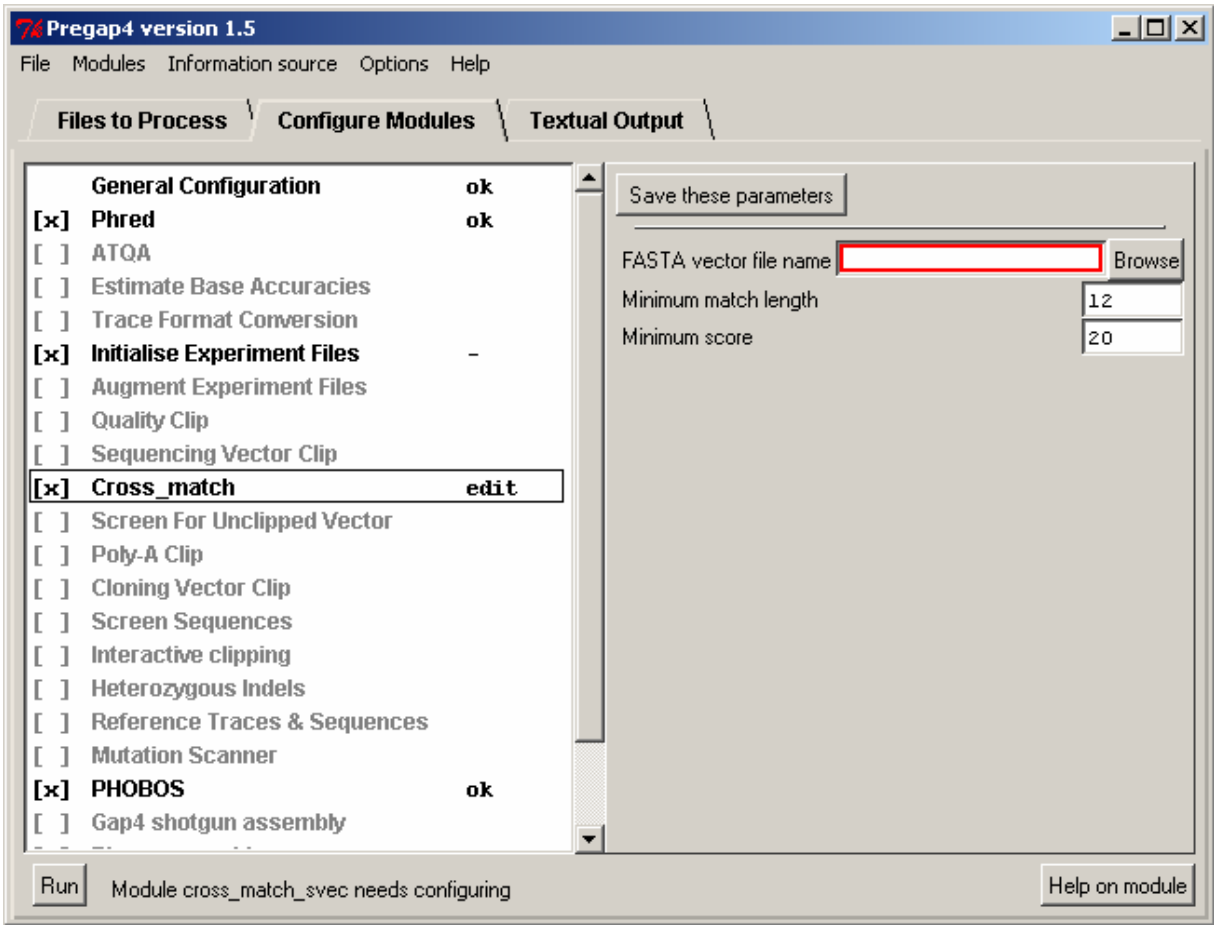


This done, you have to add the Phobos-module to Pregap4, like it is shown in the “STAMP_Manual.pdf”.

Choosing the modules

Now de-/select the modules like it is shown below. Maybe you have to install “Phred” and add it to your system path. To use “Cross match” you need a fasta file with your vector sequence and set its path in the red marked editbox.
You can use the standard parameters for Phobos; all you have to do now is press the “Run”-button on the left side of the Pregap4 window.

If you do not want to use “Phred”, you can activate the module “Estimate Base Accuracies”. Instead of “Cross_match” you could use the module “Cloning Vector Clip”.
It is possible to use other modules too; there should be no conflict with the Phobos module.



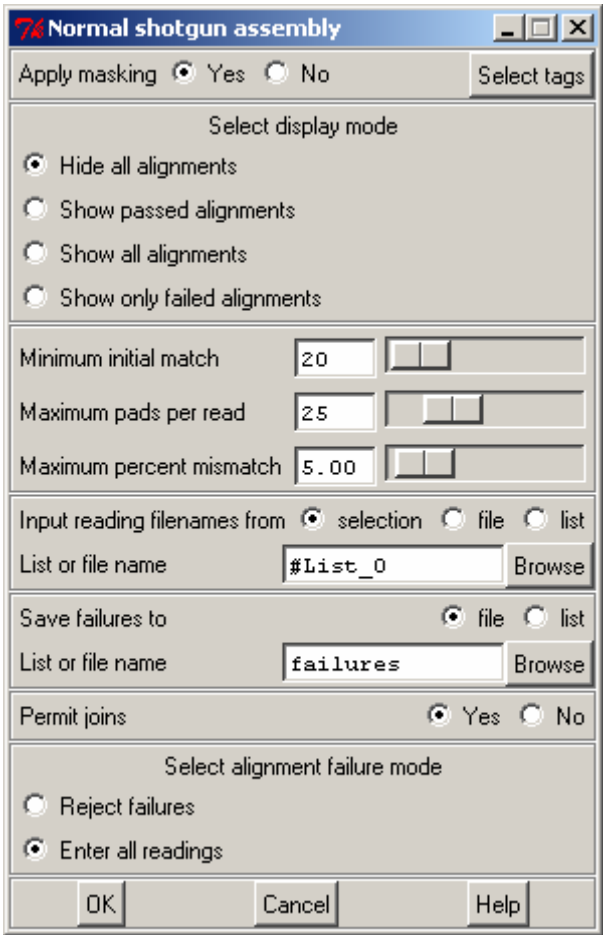
After Phobos has finished the created experiment files should be in the same folder as your trace files. Now you can start creating a database in Gap4 and try to find some flanking primer for the SSRs.

Tutorial for STAMP in Gap4

Before you start this tutorial you should read the “STAMP_Manual.pdf”, it can be found in the “doc”-folder of the Staden Package.

Creating the database

To create a new database you have to open the “File”-menu and click on “New”. A little window will open and you can click on “Browse” to choose the right folder or create a new one for the database. The database should be in the same folder as the experiment files which will be included in the database; otherwise it will not be possible to write something to the files, only to the database. After setting the directory and choosing a name press “OK”.



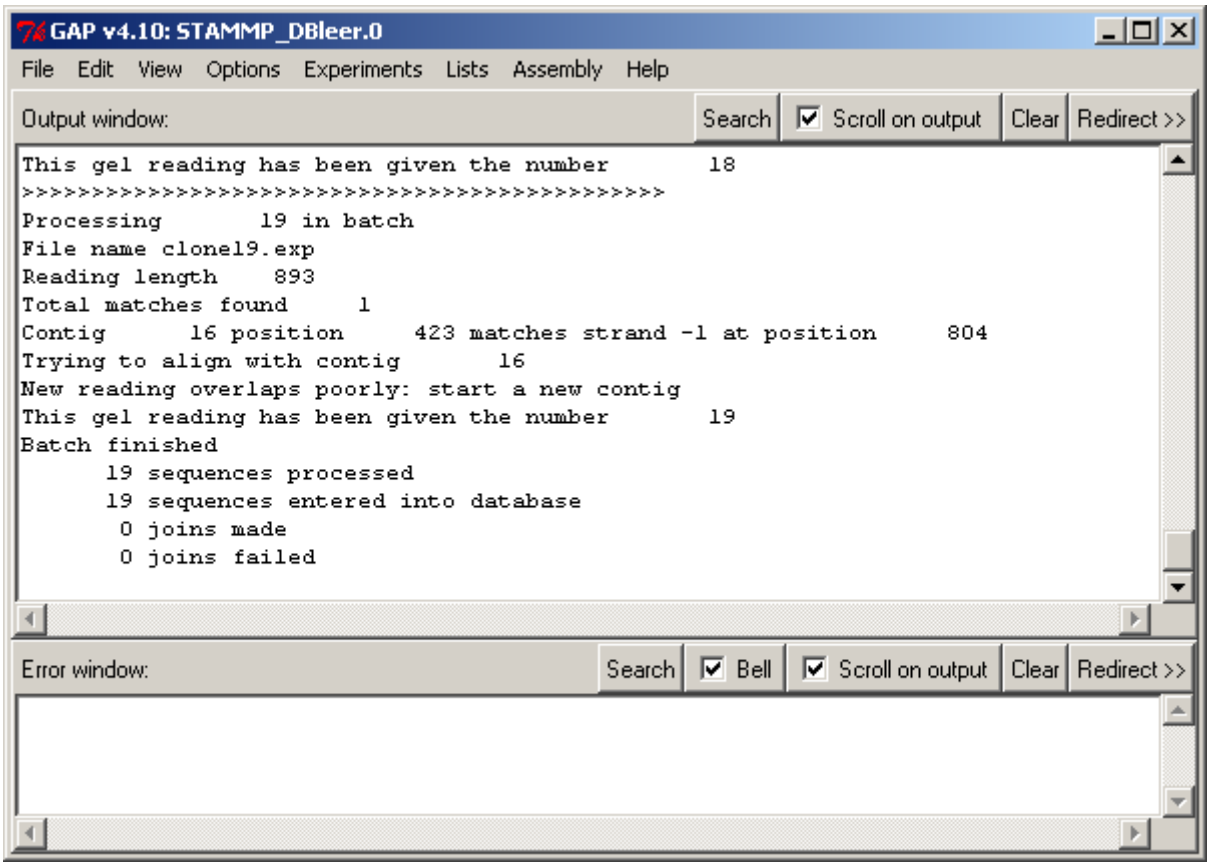
Now the database exists, but is empty. To fill it with some sequence data open the “Assembly”-menu and choose the kind of assembly you wish to perform. In this example we use “Normal shotgun assembly”.

Set the option “Apply masking” to “Yes”, with “Select tags” you can choose the tags of regions which should be ignored in the assembly. In this case “REPT” and “SVEC” should be selected. Under “Input reading filenames from” choose the experiment files for the database. If you have a file which contains the names of your experiment files take this one, otherwise choose the option “selection” and browse for the files you want to use. If Gap4 cannot assemble the files the problem could be the length of the filenames or the number of files added for the assembly. Under “Save failures to” write a filename, any that you want. After that, click on “OK” and the assembly begins.

An empty database already exists (STAMPDBleer.0.aux).

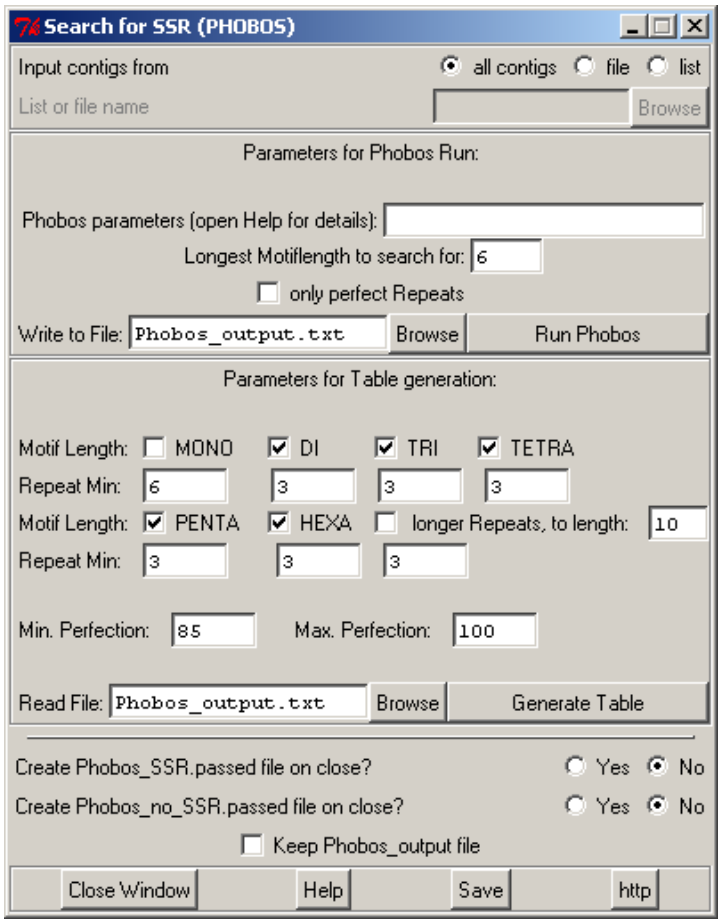
The database

Now we have a database which includes the data we want to examine. Through the “View”-menu and the “Database information”-option you get the current content of your database.



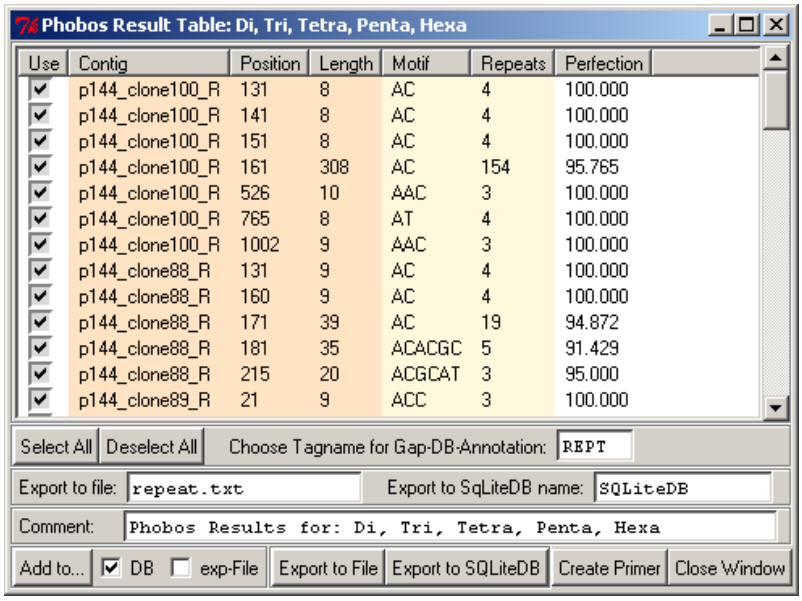
Search for microsatellites

To search for microsatellites (SSR) you open the “Experiments”-menu and then the “STAMP”-menu and click on “Search for SSR”. This will open the Phobos interface.



Through the “Help”-button will open a list and description of the Phobos parameters.

At first you should use the default parameters. Press on the “Run Phobos”-button. When Phobos created an output file you can start to generate tables. For this click on the “Generate Table”-button, it will create a list of all di, tri, tetra, penta and hexa repeats in the Phobos output.



To save the table in a SQLite Database click on “Export to SQLiteDB”.

The “Add to…”-button allows to write the repeats to the Gap4 database and the experiment files. Click also this button.

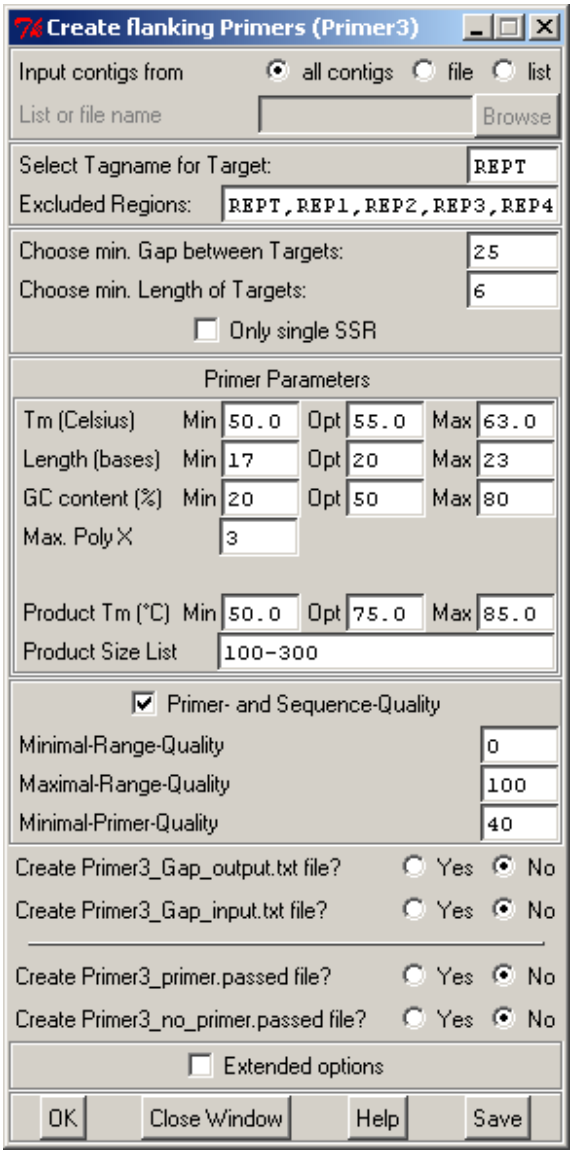
Create primer pairs for microsatellites

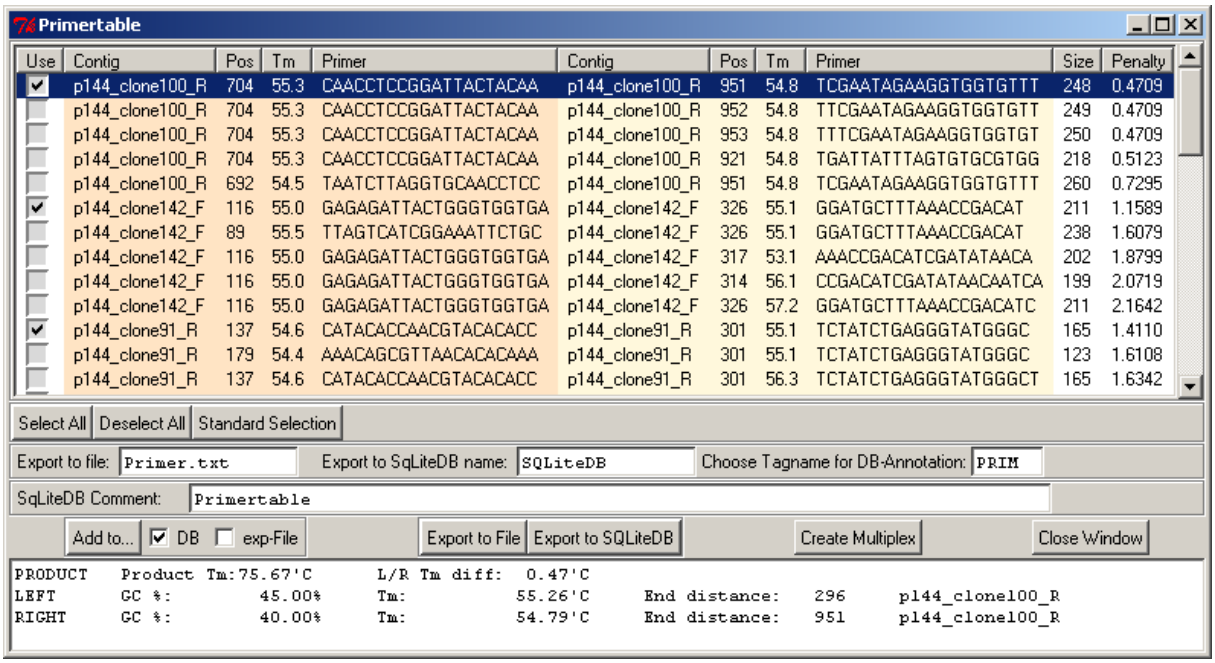
To create primers for microsatellites open the “Experiments”-menu and then the “STAMP”-menu and click on “Create flanking Primer” or on the “Create Primer”-button in an opened repeat table. This will open the Primer3 interface.

To find primers in the example data you have to deactivate the “Primer- and Sequence-Quality”-checkbutton. After that you click on “OK” and the primer table below should open.

A double click on a row will open a contig editor, which shows the product of the primer pair marked in yellow.

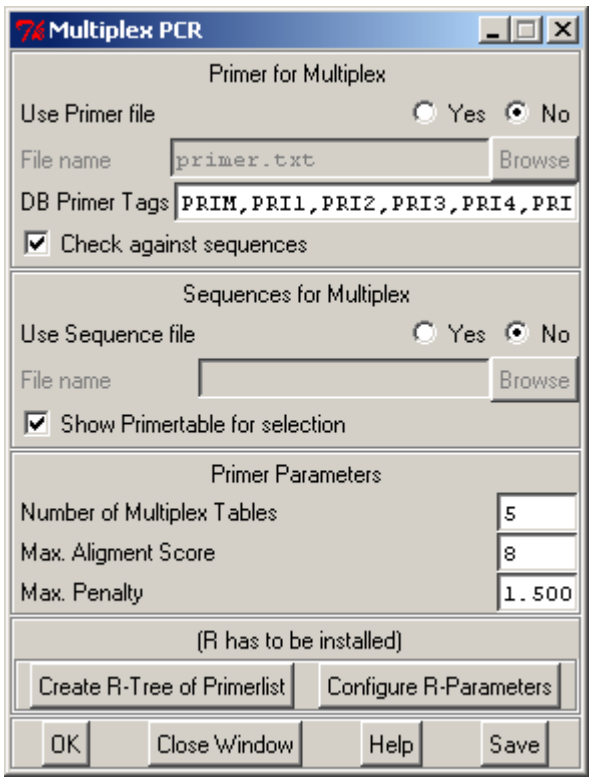
To write the primer list to the Gap4 and SQLite database press the buttons “Add to...” and “Export to SQLiteDB” like before.





Select primer pairs for a multiplex PCR

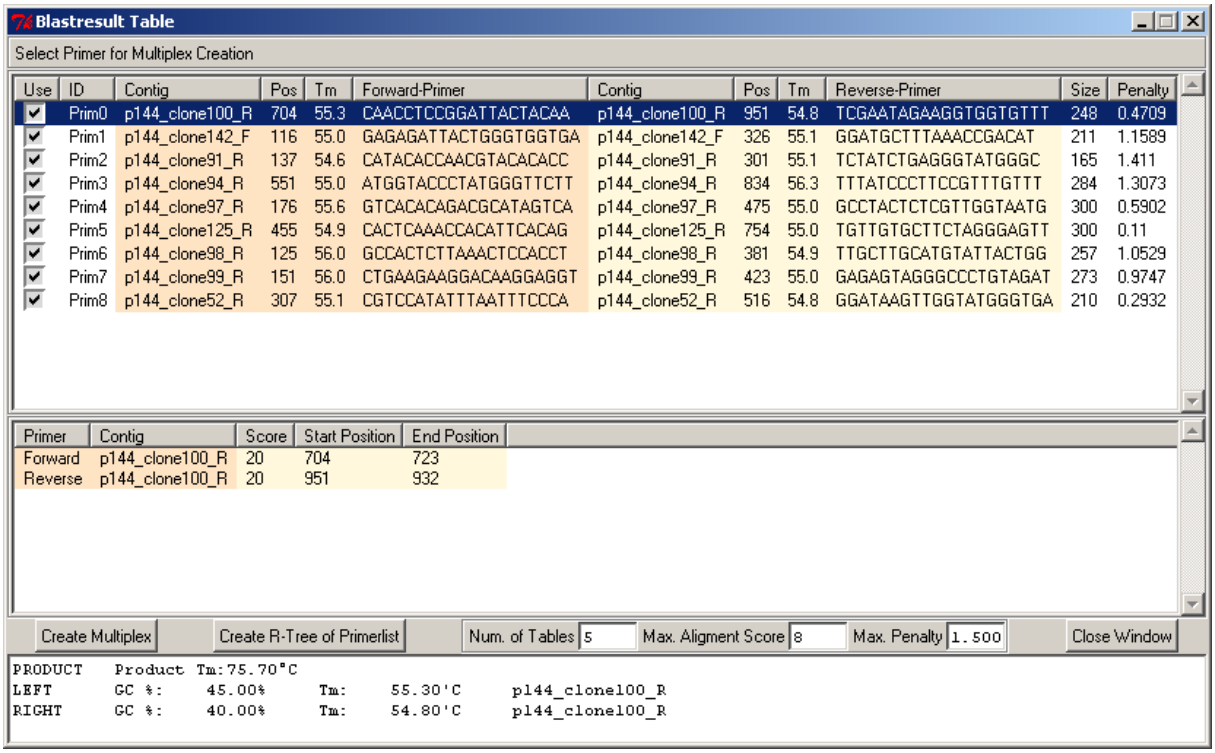
To select primers for a multiplex PCR open the “Experiments”-menu and then the “STAMP”-menu and click on “Create Multiplex” or on the “Create Multiplex”-button in an opened primer table. This will open the Multiplex interface.



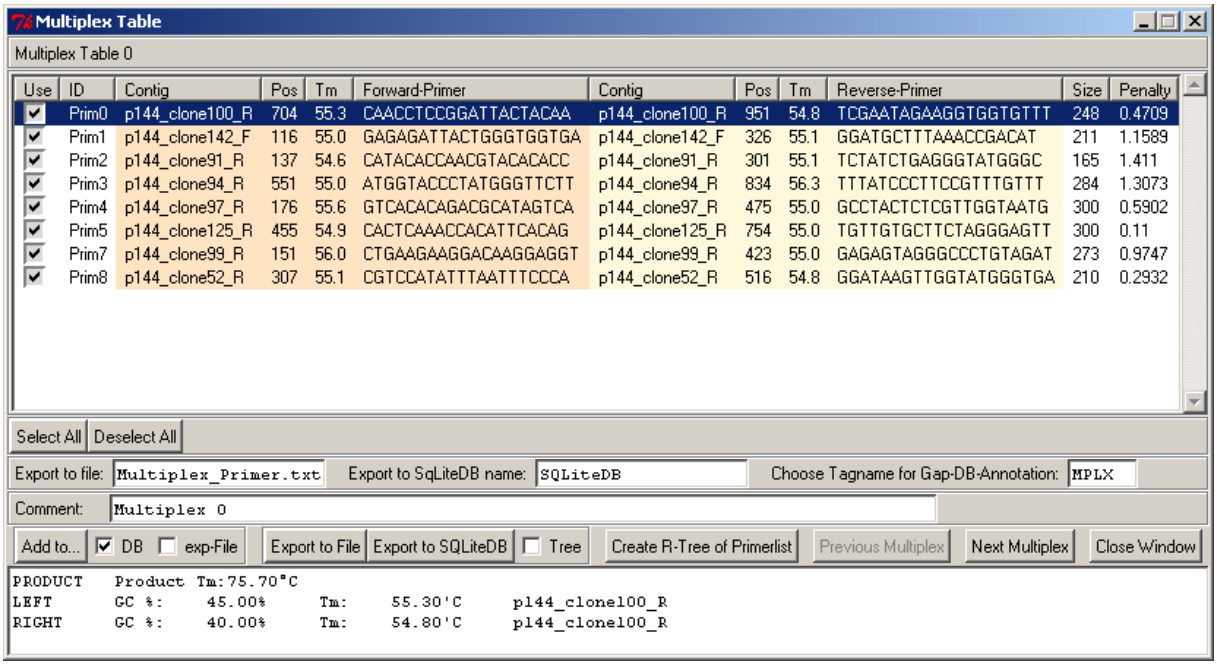
Set all parameters like on the picture and press “OK”. If you opened the interface through a primer table there will be no “Use Primer file”-option, instead all selected primer pairs of the table will be used.

By decreasing the “Max. Alignment Score” you will get more reliable primer combinations.

Now the “Blastresult Table” will be opened. It allows you to check the primers for multiple binding spots within the sequences of the Gap4 database. In this case all primer pairs are acceptable. Now click on “OK”.

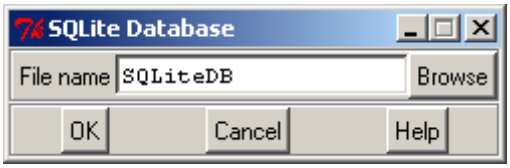


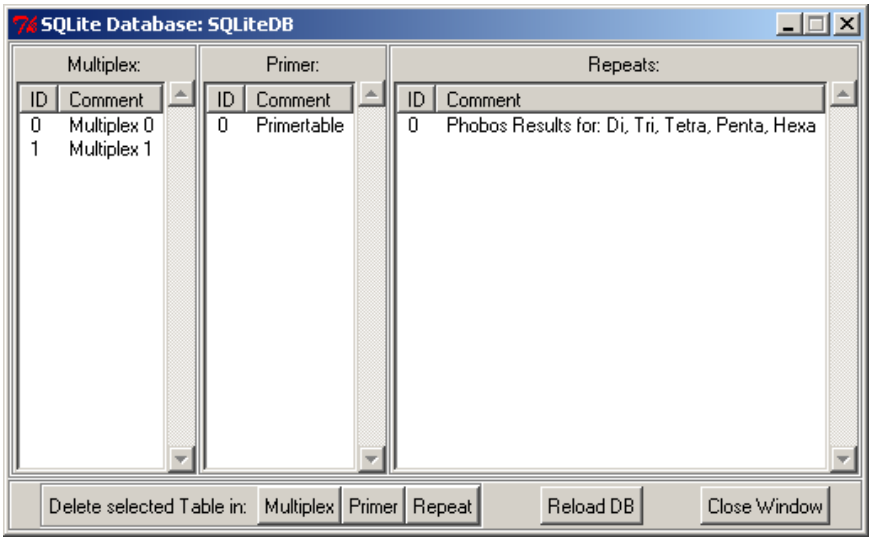
The “Mulitplex Table” contains lists of primer pairs useable together in a multiplex PCR experiment. By clicking the buttons “Previous Multiplex” or “Next Multiplex” you can switch between the different lists. Now export all lists (in this case 2) to the SQLite database by clicking the button “Export to SQLiteDB”.



The SQLite database

To open a SQLite database open the “Experiments”-menu and then the “STAMP”-menu and click on “SQLite Database”. A little window appears which asks for the name of the database you want to open. The name “SQLiteDB” should already be set, so you can click “OK”.





The database should now look like the one on the left picture.

A double click on a row will open the according table.

Double click on the repeat table.

Tables in a SQLite database contain an additional list field. In case of a repeat table the field shows a list of primers which are associated to the selected repeat. Primer and multiplex tables will show the associated repeats for a selected primer pair.

