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miRprimerdesign3

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miR primer design

The tool for designing primers for miR-specific qPCR consist of the executable file "miRprimerdesign3_pub.exe".

The tool was written for Windows and has been tested under Windows XP and Windows 7.

Manual installation:

- 1) Download "miRprimerdesign3_pub.exe" to a folder on your computer.
- 2) Download the input file "input_miRs.txt".
- 3) Optional: Download this manual called "READ ME" to the same folder.

Automatic installation on the C: drive depending on your browser:

Chrome:

- 1) Click on the document called "installer".
- 2) Double click on the downloaded file called "installer .exe" at the bottom of the browser window.
- 3) Allow the program to make changes to your computer.
- 4) Follow the instructions.

Firefox:

- 1) Click on the document called "installer".
- 2) Save the file.
- 3) Double click on the downloaded file called "installer .exe" in the "Downloads" window.
- 4) Allow the program to make changes to your computer.
- 5) Follow the instructions.

Internet Explorer:

- 1) Click on the document called "installer".
- 2) Click on "Run" at the bottom of the browser window.
- 3) Click on "Run" once more at the bottom of the browser window or, if your computer comes up with some security warnings, grant the necessary permissions to run the installer.
- 4) Follow the instructions.

To design primers:

1) Place the miR sequences you want to design primers to in the file "input_miRs.txt" in the same folder as "miRprimerdesign3_pub.exe".

The sequences should be in strict fasta format:

```
>miR name  
sequence
```

Please see the examples that are already in the file "input_miRs.txt".

2) When the input is ready the PPR analysis can be started by double clicking the "miRprimerdesign3_pub.exe" icon.

This will open a small dos window that will indicate the progress of the primer design.

3) Press "enter" to close the dos window when the design program has finished.

4) The result are stored in the file "miR_primer_pairs.txt".

Interpretation of the result:

Open the result file "miR_primer_pairs.txt" in "MSexcel" or "OpenOffice calc".

The primer pair labeled "best pair" is the suggested pair.

However, if this primer pair does not perform well the program suggest four other primer pairs that can be tested. For each primer pair the score of the pair and the scores of the forward primer and the reverse primer are reported. The pairs are ranked according to the expected performance, which unfortunately is not always the same as the performance in test.

References:

Balcells I. Cirera S. and Busk P.K. (2011). Specific and sensitive quantitative RT-PCR of miRNAs with DNA primers. BMC Biotechnol. **11**(1), 70.

Busk P.K. (2010). Method for Quantification of Small RNA Species. Patent WO2010085966 (A2).

Good luck!

Peter Busk, 16.May.2013

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