

## OFF-TARGET EFFECT

The results obtained by AmiRNA Designer can be analysed for the off-target effect, using Perl scripts written for this purpose. The method to determine the direct interaction between the mRNA and amiRNA or the mRNA and amiRNA\* is based on the Perl regular expressions. For each miRNA and miRNA\*, the search for the binding site is performed along the entire transcripts with the shift of one nucleotide.

### We have prepared the following scripts:

1. Responsible for the detection of off-target effect (it takes quite a long time to calculate the results):

USAGE:

#### **amick.pl**

*amick.pl file\_with\_targets.fasta file\_with\_artificial\_miRNA.csv output\_filename.csv' with one of the following parameters +a or -a'*

where:

- *file\_with\_targets.fasta* - is the fasta file containing all sequences of mRNA of the organism of interest
  - *file\_with\_artificial\_miRNA.csv* - contains list of amiRNA/amiRNA\* sequences obtained from AmiRNA Designer
  - *output\_filename.csv* - output file name
  - *+a* - both amiRNA and amiRNA\* sequences will be analysed for the off-target effect
  - *-a* - only amiRNA sequences will be analysed for the off-target effect
2. It adds to the results those amiRNA and amiRNA\* sequences, that did not bind to any target according to the script described above ('no results' is assigned to such sequences).

USAGE:

#### **amick\_add.pl**

*amick\_add.pl file\_with\_results.csv file\_with\_artificial\_miRNA.csv* with one of the following parameters *+a* or *-a*

where:

- *file\_with\_results.csv* - the file being the result of the analysis conducted by *amick.pl*
  - *file\_with\_artificial\_miRNA.csv* - contains list of amiRNA/amiRNA\* sequences obtained from AmiRNA Designer
  - *+a* - both amiRNA and amiRNA\* sequences will be analysed for the off-target effect
  - *-a* - only amiRNA sequences will be analysed for the off-target effect
3. This script is responsible for filtering the results obtained from *amick\_add.pl* or *amick.pl*. It divides the file containing results into two separate csv sheets: one consisting of potential target sites within the gene of interest while the second one containing potential target sites within other genes.

USAGE:

#### **amick\_filter.pl**

*amick\_filter.pl file\_with\_results.csv output\_file\_targets\_results.csv*  
*output\_file\_gene\_results.csv file\_with\_keys.txt*

where:

- *file\_with\_results.csv* - the file being the result of the analysis conducted by *amick.pl* or *amick\_add.pl*
- *output\_file\_targets\_results.csv* - file consisting of potential target sites within the gene of interest
- *output\_file\_gene\_results.csv* - file containing potential target sites within other genes then the gene of interest
- *file\_with\_keys.txt* - file containing the ID numbers and names commonly used for the gene of interest