

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 than the gene of interest
- *file_with_keys.txt* - file containing the ID numbers and names commonly used for the gene of interest