sh-miR designer v1.0

a tool for construction of RNA interference reagents: sh-miRs

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1. Introduction

sh-miR Designer is a software aimed for fast and efficient design of effective RNA interference (RNAi) reagents - sh-miRs, also known as artificial miRNAs. sh-miRs are RNA particles whose structure is based on miRNA precursor pri-miRNA, but sequence interacting with transcript is changed depending on research purpose. Maintenance of structure of pri-miRNA is very important to enable cellular processing and therefore ensure functionality of artificial particles. sh-miRs delivered to cells on genetic vectors - plasmids or viral vectors - enter natural RNAi pathway and silence target mRNA. They can be used in genetic therapies and basic biomedical research.

2. Project Description

2.1. Biological background

RNA interference (RNAi) is a physiological process of posttranscriptional gene expression regulation caused by double stranded RNA molecules. This is one of the most willingly used experimental techniques. It is used in experiments designed to explore gene function, in creation cellular models of diseases or in genetic therapy design. Among RNAi technology reagents are siRNA (short interfering RNA) and vector reagents shRNA (short hairpin RNA) and sh-miR (sh- microRNA). RNAi reagents are integrated into endogenous RNAi pathway on different phases. sh-miR reagents are designed in the way to be similar to endogenous miRNA precursors (pri-miRNA), because it is important feature to be recognized by proteins involved in microRNA (miRNA) biogenesis.

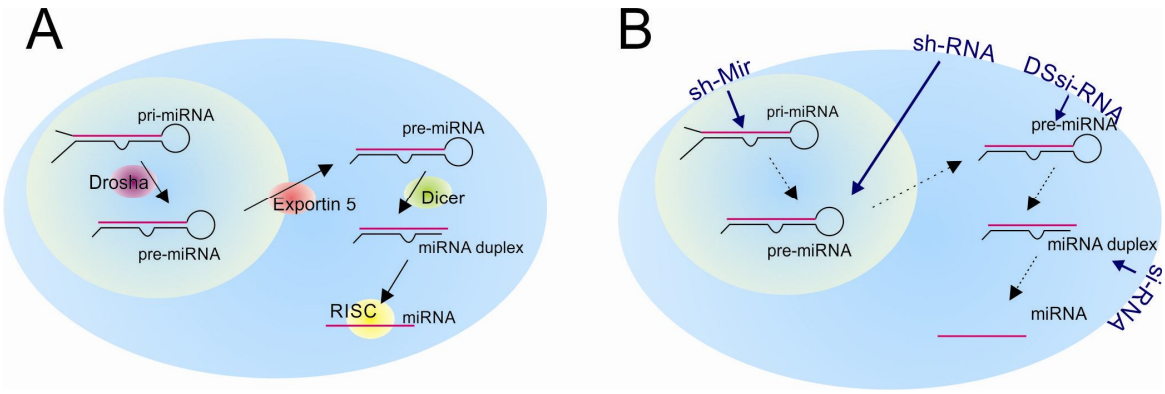

Fig. 1 (A) Biogenesis of miRNA and (B) stages where RNAi reagents are recognised by miRNA biogenesis proteins

shRNA or sh-miR reagents are introduced into cells with usage of genetic vectors – plasmids or viral vectors to ensure intracellular expression. They have huge therapeutic potential beacuse of stable reagent production in cells what enables one time therapeutic dosage. Usage of synthetic siRNA reagents requires often dosage, what makes therapy more difficult, especially in case of Central Nervous System Diseases.

It was recently shown that RNAi reagents introduces into cells can trigger unwanted non-specific effects, e.g. can interact with different transcripts besides the desired one (so called off-target effect) or induce immunological effect. Because in processing of sh-miR reagents endogenous endonucleasis Drosha and Dicer are involved, in case of high expression of reagent they can saturate those proteins as well as Exportin-5 transport protein responsible for export of sh-miR from nucleus. The saturation can influence endogenous RNAi mechanism. It was shown that sh-miR reagents influence less endogenous RNAi pathway, because of lower level of produced double-stranded RNA (dsRNA), preserving efficiency of translation silencing. They also show longer effect than different types of RNAi reagents. Because of described features sh-miR reagents seems to be good choice when designing efficient and non-toxic RNAi reagents.

2.2. Software background

sh-miR Designer is a software aimed for fast and efficient design of effective RNA interference (RNAi) reagents - sh-miRs, also known as artificial miRNAs. sh-miRs are RNA particles whose structure is based on miRNA precursor pri-miRNA, but sequence interacting with transcript is changed depending on research purpose. Until now scientists had to use multiple unconnected software to design a single sh-miR molecule. It is a time-consuming process, which limits the number of designed molecules for biological testing. There exists no software taking into consideration important features of these molecules, such as their spatial structure. Maintenance of structure of pri-miRNA is very important to enable cellular processing and therefore ensure functionality of artificial particles. This interdisciplinary project includes computer sciences with biomedical science. sh-miRs delivered to cells on genetic vectors - plasmids or viral vectors - enter natural RNAi pathway and silence target mRNA. They can be used in genetic therapies and basic biomedical research.

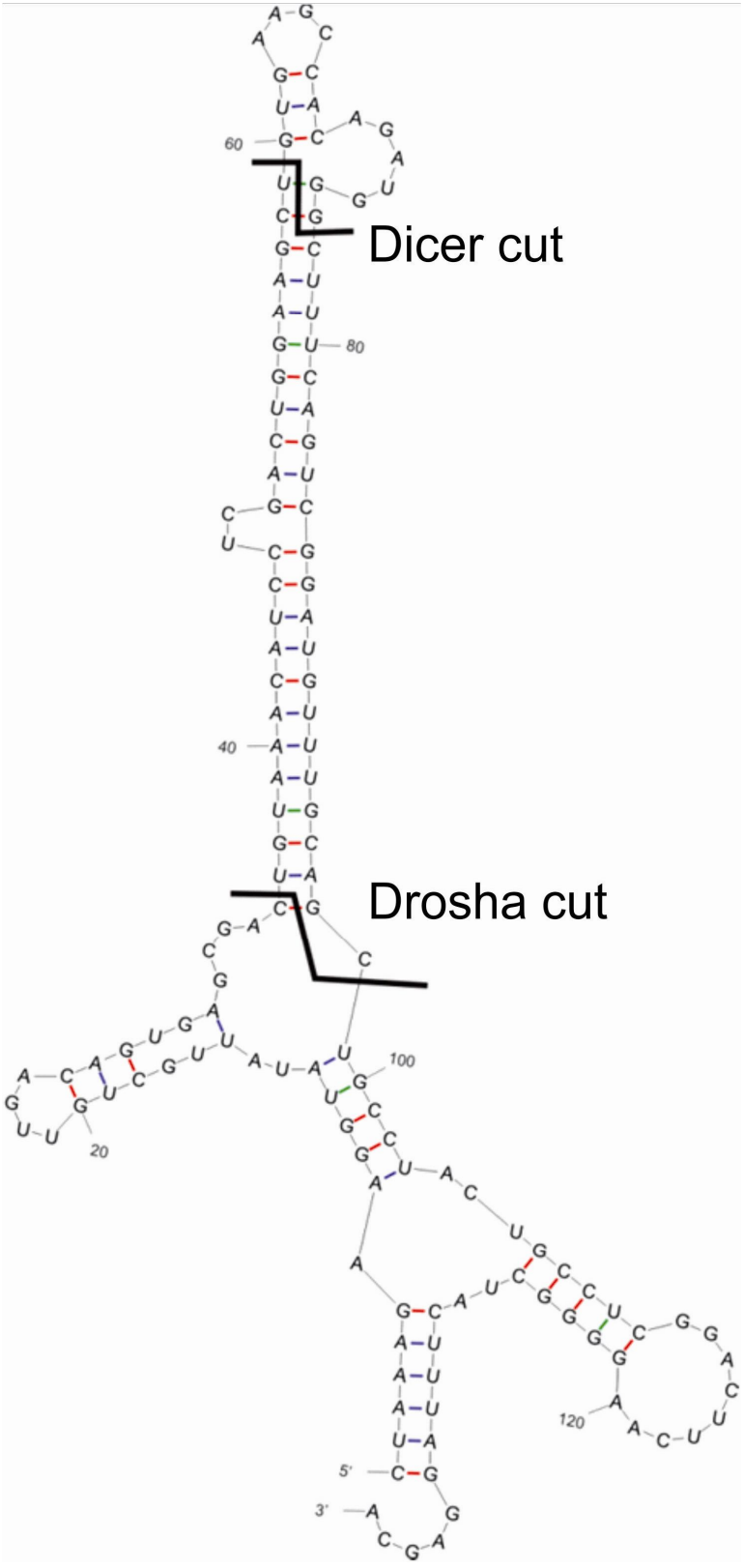

Fig. 2 Drosha and Dicer endonucleases cuts of pri-miRNA

3. Description of software functions

3.1. Input:

As input it is possible to use one strand of siRNA or both strands of siRNA separated by a space. Sequences have to be in 5'-3' orientation and be at least 80% complementary.

Our function checks:

- Whether the sequence has only actguACTGU letters
- Whether only one or two sequences are entered
- Whether a single sequence is 19-21 long
- If two strands given whether sequences are complementary in at least 80%

Function changes:

- Function cuts 'tt' and 'uu' ends, lowers all letters and changes all 'u' to 't'

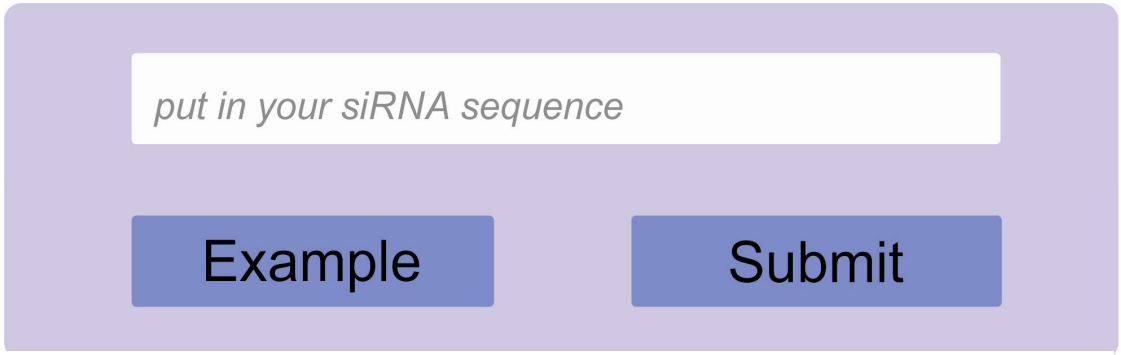

Fig. 3 Interface of input

3.2. Complementary check:

It is checked whether the two strands are complementary and whether they are in 0-0 position:

AACCTTGGAACCTTGG
TTGGAACCTTGGAACC

or are shifted:

e.g. +2, -2

AACCTTGGAACCTT
  GGAACCTTGGAACC

3.3. Insert siRNA sequence into flanking sequences:

The function takes all flanking sequences from our database (in sh-miR designer v1.0 there are five pri-miRNA flanking sequences).

Database structure:

```
id SERIAL PRIMARY KEY,
name varchar(10) NOT NULL,
flanks3_s varchar(80) NOT NULL,
flanks3_a varchar(80) NOT NULL,
flanks5_s varchar(80) NOT NULL,
flanks5_a varchar(80) NOT NULL,
loop_s varchar(30) NOT NULL,
loop_a varchar(30) NOT NULL,
miRNA_s varchar(30) NOT NULL,
miRNA_a varchar(30) NOT NULL,
miRNA_length integer NOT NULL,
miRNA_min integer NOT NULL,
miRNA_max integer NOT NULL,
miRNA_end_5 integer NOT NULL,
miRNA_end_3 integer NOT NULL,
structure varchar(200) NOT NULL,
homogeneity integer NOT NULL,
        /*homogeneity of products (length), 0 for very
         nonhomogenous, 5 for completely homogenous */
miRBase_link varchar(200) NOT NULL
```

pri-miRNA sequences used:

miRNA name	5’ flanking sequence	Loop sequence	3’ flanking sequence
miR-30a	CTAAAGAAGGTATATTGCTGTTGACAGTGAGCGAC	CTGTGAAGCCACAGATGGG	TGCCTACTGCCTCGGACTTCAAGGGGCTACTTTAGGAGCA
miR-155	AGGCTTGCTGTAGGTATGCTG	TTTTGCCTCCAACTGA	GTGTATGATGCCTGTTACTAGCATTCACATGGAACAAATTGCTGCTGCCGTGGGAGGATGACAAAGA
miR-122	TGGAGGTGAAGTTAACACCTTCGTGGCTACAGAGTTTCCTTAGCAGAGCTG	TGTCTAAACTATCA	GCTACTGCTAGGCAATCCTTCCCTCGATAAATGTCTTGGCATCGTTTGCTT
miR-21	TACCATCGTGACATCTCCATGGCTGTACCACCTTGTCGGG	CTGTTGAATCTCATGG	CTGACATTTTGGTATCTTTCATCTGACCATCCATATCCAATGTTCTCATT
miR-31	CATAACAACGAAGAGGGATGGTATTGCTCCTGTAACTCGGAACTGGAGAGG	GTTGAACTGGGAACC	CTTTCCTGTCTGACAGCAGCTTGGCTACCTCCGTCCTGTTCCTCCTTGTCTT

Insertion differs between flanking sequences because of miRNA sequence shift and some nucleotides have to be cut or added to retain proper length and folding.

3.4. Check structure:

First the function generates .ss files

Example of an .ss file fragment:

```
1       A       250.29  423.84  8       0
2       G       271.61  418.60  9       0
3       G       294.93  410.55  1       107
4       C       306.68  425.97  0       106
5       U       318.43  441.38  2       105
6       U       307.60  463.82  0       0
7       G       308.69  481.40  0       0
```

```
8        C        316.43   497.21   0        0

9        U        329.63   508.87   0        0
```

Then the file is parsed to contain only the first and last column:

```
1 0

2 0

3 107

4 106

5 105

6 0

7 0

8 0

9 0
```

The numbers assigned to the nucleotides have to be verified, because flanking sequences can be shortened or extended during insertion. Moreover, the length of the siRNA insert can differ from the natural one.

The modified file is compared with the structure file obtained from the .ss file for pri-miRNA. The third column represents scores if the structure is retained in the position

Example of a file fragment with scores:

```
1 0 0

2 0 0

3 107 1

4 106 1

5 105 1

6 0 2

7 0 2

8 0 2

9 0 2
```

The function scores the structure and returns a point value ranged from 0 to 100.

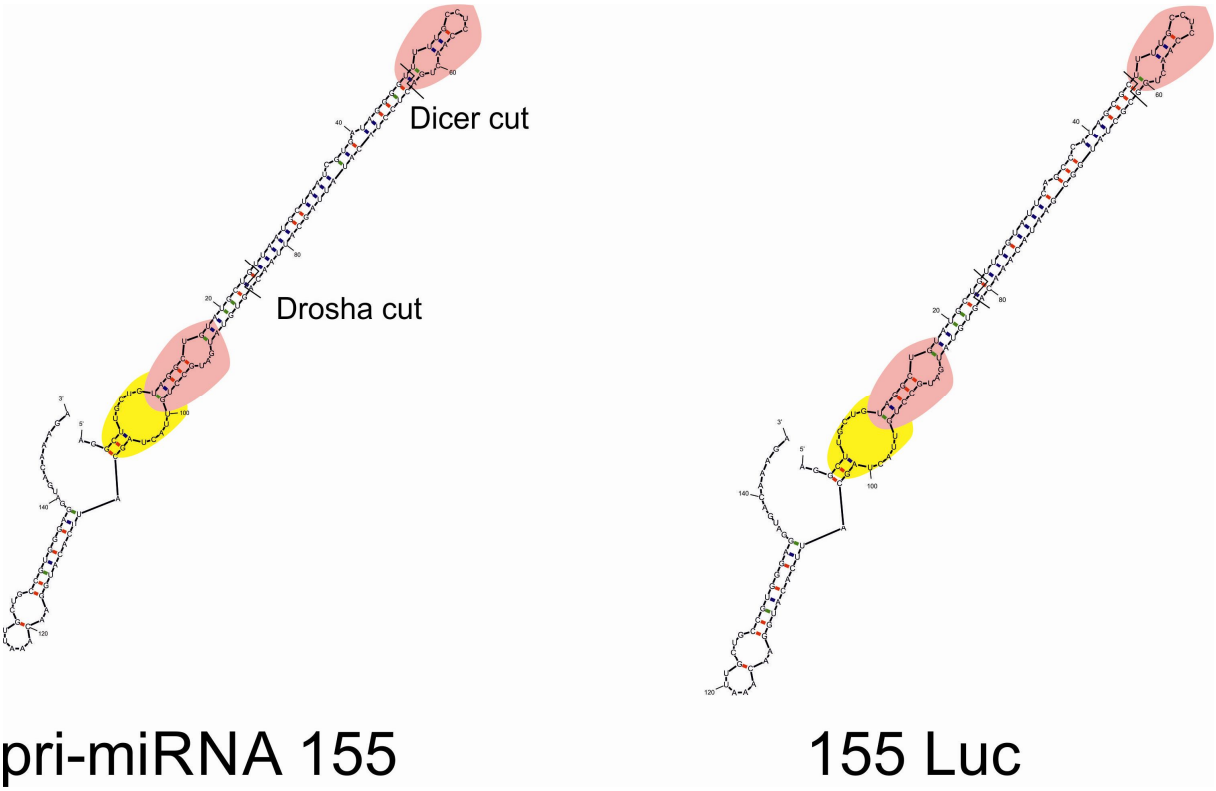

Fig. 4 Elements of structure which are most important for proper endonuclease processing (most important in scoring)

Final scoring:

The final scoring includes:

- points for structure (ranged 0-100)
- points for flanking sequences homogeneity (ranged 0-15)
- additional points if the first nucleotides of the siRNA and miRNA inserts are equal (0,4 or 10 points)

3.5. Output:

As output the user gets 3 the best results with a pdf file showing 2D structure.

4. Example

Example:

UUUGUAUUCGCCCUAGCGC (19)
CGCUAUGGCGAAUACAAACA (20)

Input:

'UUUGUAUUCGCCCUAGCGC **CGCUAUGGCGAAUACAAACA**'

Created sh-miRs (red are flanking sequences, black are siRNA strands and blue are added nucleotides):

155luc

AGGCTTGCTGTAGGCTGTATGCTGUUUGUAUUCGCCCUAGCGCTTTTGCCTCCAACTG**CGCUAUGGCGAAUACAAACA**
GTGTATGATGCCTGTTACTAGCATTCACATGGAACAAATTGCTGCTGCCGTGGGAGGATGACAAAGA

122luc

TGGAGGTGAAGTTAACACCTTCGTGGCTACAGAGTTTCCTTAGCAGAGCTGUUUGUAUUCGCCCUAGCGCGTGTCTAAACTATC
A**CGCUAUGGCGAAUACAAACA**GCTACTGCTAGGCAATCCTTCCCTCGATAAATGTCTTGGCATCGTTTGCTT

30luc

CTAAAGAAGGTATATTGCTGTTGACAGTGAGCGACTGUUUGUAUUCGCCCUAGCGCCTGTGAAGCCACAGATGGGGCGCUA
UGGCGAAUACAAACATGCCTACTGCCTCGGACTTCAAGGGGCTACTTTAGGAGCA

21luc

TACCATCGTGACATCTCCATGGCTGTACCACCTTGTCGGGGUUUGUAUUCGCCCUAGCGCCTGTTGAATCTCATGG**CGCUAU**
GGCGAAUACAAACACTGACATTTTGGTATCTTTCATCTGACCATCCATATCCAATGTTCTCATT

31luc

CATAACAACGAAGAGGGATGGTATTGCTCCTGTAACTCGGAACTGGAGAGGGUUUGUAUUCGCCCUAGCGCGTTGAACTGGGAA
CC**CGCUAUGGCGAAUACAAACA**CTTTCCTGTCTGACAGCAGCTTGGCTACCTCCGTCCTGTTCCTCCTTGTCTT

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