siRNA design including secondary structure target site prediction

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Abstract

When performing RNAi experiments using siRNA duplexes, the first critical challenge is the design of efficient siRNA. Recent studies concerning the importance of mRNA accessibility in relation to the efficiency of siRNA silencing has led MWG Biotech to create an online design tool allowing researchers to view the mRNA target site.

Preliminary data underlines the correlation between the siRNA knockdown efficiency and the local free energy of the mRNA target site. There is a strong evidence that considering the mRNA secondary structure within the siRNA design algorithm will lead to more efficient siRNAs.

The MWG online siRNA design tool

MWG's web-based siRNA design tool is free to use, has a full transparency, and features a flexible design process, in which all design parameters can be customized (Fig. 1).

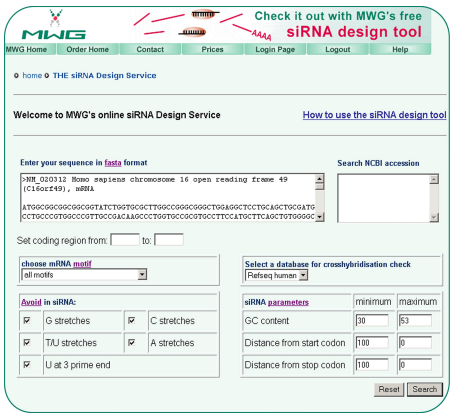

Figure 1 | MWG siRNA design tool

One or several genes can be input either in FASTA format or as NCBI accession numbers. The design algorithm then searches the DNA coding sequence (cds) for potential target sites, using the Tuschl[1] motifs. For each potential target site siRNAs are designed avoiding stretches with more than 3 identical nucleotides or a uracil at the 3' end (Fig. 1, bottom left). Other factors influencing siRNA design are, the G-C content and the distance of the siRNA from the start codon and the stop codon respectively (Fig. 1, bottom right). The resulting output list of all found siRNAs is sorted by the Reynolds[2] scores.

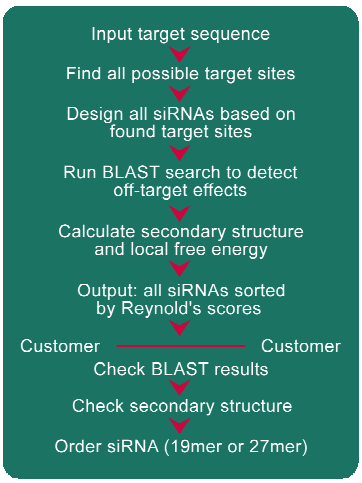

Figure 2 | Workflow of the MWG on-line siRNA design tool

A BLAST search can be performed using databases of human, mouse or rat mRNAs. The BLAST search detects possible off-target effects and can be reviewed on a separate BLAST output page. For each siRNA, all BLAST hits are shown with direct links to the NCBI web page.

The secondary structure view

The secondary structure of the mRNA target site plays an important role in siRNA efficiency[3,4,5]. Due to this fact, we have included a secondary structure view of the mRNA with the target site colored red, as shown in Fig. 3, using the RNAfold program of the Vienna RNA package. (http://www.tbi.univie.ac.at/~ivo/RNA/RNAfold.html)

As additional useful parameters, we calculate the local free energy and we count the number of bound bases at the target site. There is strong evidence that the local free energy and the number of bonds are correlated with the knockdown efficiency. A low number of hydrogen bonds and/or a low negative local free energy indicates a highly accessible target site which corresponds with a higher knockdown efficiency.

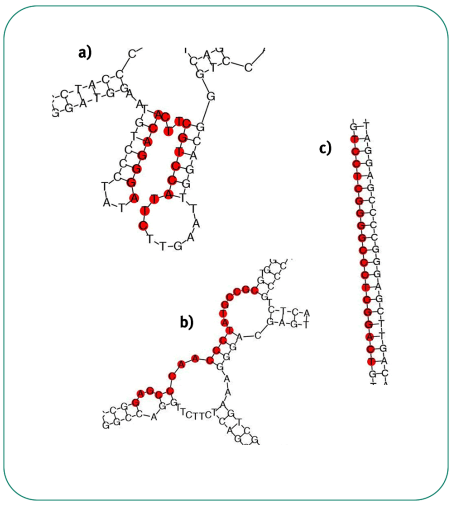

Figure 3 | Examples of siRNA target sites (red) on the corresponding mRNA secondary structure predicted using RNAfold. **(a)** Target site enclosed by two neighboring hairpin structures. **(b)** Target site on a region with three internal loops. **(c)** Target site on a stack region.

Influence of the secondary structure

It is not fully understood how the siRNA-mRNA target hybrid is formed but recent studies[3,4,5] showed the high potential of secondary structure for the *in silico* design of effective siRNAs. Nevertheless a larger screening on the influence of the mRNA secondary structure still remains.

Yiu et al[5] found target sites enclosed by neighboring branches to be less effective (Fig. 3a). In contrast to all other publications reviewed, their filtering algorithm removes target sites on or near to bigger loops ignoring the local free energy. Interestingly, they have found that applying their filtering algorithm to various design tools, including those with the Tuschl/Reynolds combination, decreases the output of ineffective siRNAs up to 53%.

Luo and Chang[4] found the number of hydrogen bonds formed by the target region to be a useful parameter in siRNA design. The presence of a large number of unpaired nucleotides (Fig. 3b) seems to be a marker for a highly accessible target site. In contrast a high number of bound bases (Fig. 3c) results in a stable target site which is less accessible for the siRNA and therefore lowers the knockdown efficiency.

Schubert et al[3] confirmed the influence of base-pairing on the silencing efficiency. Additionally they computed the local free energy of the mRNA target site and found it directly correlated with the relative protein expression (Fig. 4). A low negative local free energy, which correlates to an open region with a high number of unpaired nucleotides (Fig. 3a) results in improved potency.

Conclusions

- **The secondary structure view of the mRNA target sites including the number of bonds and the local free energy improves the selection of highly efficient siRNA**
- **The design algorithm is based on the Tuschl/Reynolds combination and will be extended by the local free energy parameter**
- **An optional BLAST search can identify potential off-targets**
- **The design tool is directly linked to the MWG Biotech's online ordering system, is free to use and the design parameters can be customized**

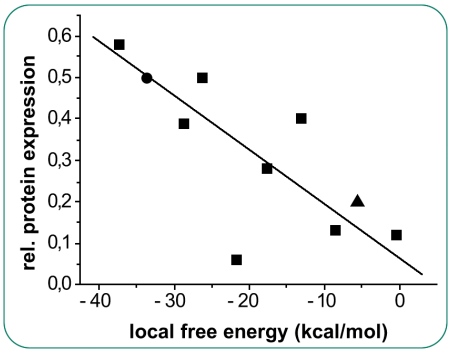

Figure 4 | Dependency of reporter gene expression on local free energy at target site. ■ siRNA of the rat vanilloid receptor subtype 1 acting on different target structures; ● siRNA directed against RNA-dependent RNA polymerase (RdRP) of coxsackievirus B3 against a fully base-paired target sequence; ▲ siRNA directed against serine/threonine kinase Pim-1 against an accessible target site. This Figure is kindly provided by Schubert et al.[3]

Outlook and Future Work

To verify the linear correlation between gene silencing and the local free energy (Fig. 4) more data is needed. Currently we are working on an in silico approach, where a dataset of validated siRNAs is analysed in respect to the corresponding local free energy of the target secondary structure. In parallel further data from transfection studies is generated comparing knockdown efficiency with the secondary structure classified in two groups of high and low negative local free energy.

These data will be used to implement the local free energy as an additional parameter into our siRNA design algoritm.

Acknowledgement

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Literature

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