

How to Design siRNA (Small Interfering RNA)

The sequence of the siRNA can be selected as follows:

1. Start 75-100 bases downstream from the start codon “ATG” of your gene of interest.
2. Locate the first “AA” dimer.
3. Record the next 19 nucleotides following the AA dimer.
4. Calculate the percentage of G/C content of the AA-N₁₉ 21-base sequence. It must be between 30% and 70% with 50% being ideal. If the sequence does not meet the criteria, the search continues downstream to the next “AA” dimer until this condition is met.
5. The 21-base sequence is subjected to a BLAST-search (NCBI database) against EST libraries of your organism to ensure that no other gene(s) is targeted. (The complement is automatically searched as well.)
6. If the conditions in either step 4 or 5 are not met, repeat steps 2 - 5.

The sequence selection process has no other constraints. It is important to note that structure within the targeted mRNA appears to have minimal effect on the availability of the mRNA target and efficacy of the siRNA silencing approach. To-date, successful silencing has been achieved using the above method to select the target sequence, although the method is essentially random with respect to accounting for mRNA structure.

Although siRNA silencing appears to be extremely effective by selecting a single target in the mRNA, it may be desirable to design and employ two independent siRNA duplexes to control for specificity of the silencing effect. This recommendation is only for specificity for it is yet unknown if the targeting of a gene by two different siRNA duplexes would be more effective than using a single siRNA duplex. It is believed that the rate-limiting component of the siRNA effect is the availability of cellular nuclease components and not mRNA target availability. Therefore, doubling the number of siRNA duplexes is not expected to double the rate or efficiency of silencing.

If the selected siRNA duplex(es) do not function for silencing, the following steps are recommended. First, a search is conducted for sequencing errors in the gene and possible polymorphisms. Initial studies on the specificity of target recognition by siRNA duplexes indicates that a single point mutation located in the paired region of an siRNA duplex is sufficient to abolish target mRNA degradation. Second, a re-examination is performed to confirm whether the cell line is from the expected species. Third, a second and/or third target are selected and the corresponding siRNA duplexes prepared.

References:

1. Elbashir, S. M, *et al.* (2001a) *Nature* **411**: 494-498.
2. Elbashir, S. M, *et al.* (2001b) *Genes & Dev.* **15**: 188-200.

