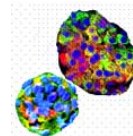


# SOP



Title:	<b>siRNA design rules</b>				
Protocol #:	1.2	Submitted:	050610	Approved:	200610
Category:	MB	Author(s): <sup>1</sup>	SNS	Checked by:	AAH

## Procedure:

### RNAi target selection rules:

1. Targeted regions on the cDNA sequence of a targeted gene should be located 50-100 nt downstream of the start codon (ATG).
2. Search for sequence motif AA(N<sub>19</sub>)TT or NA(N<sub>21</sub>), or NAR(N<sub>17</sub>)YNN, where N is any nucleotide, R is purine (A, G) and Y is pyrimidine (C, U).
3. Avoid targeting introns, since RNAi only works in the cytoplasm and not within the nucleus.
4. Avoid sequences with > 50% G+C content.
5. Avoid stretches of 4 or more nucleotide repeats.
6. Avoid 5URT and 3UTR, although siRNAs targeting UTRs have successfully induced gene inhibition.
7. Avoid sequences that share a certain degree of homology with other related or unrelated genes.

### How to obtain a cDNA sequence for target selection

Before finding a RNAi target on the gene of your interest, you need to get its mRNA sequence or sequence accession number as some siRNA design tools can take accession number as input. It is recommended to use the gene's [RefSeq](#) from [NCBI](#), since the RefSeq represents non-redundant, curated and validated sequences. RefSeq mRNA sequences have unique accession numbers which start with NM or XM, followed by 6 digits. For example, NM\_123456 (curated mRNA sequence) or XM\_0123456 (model mRNA sequence predicted by genome sequence analysis). There are several ways of querying RefSeq.

1. Search LocusLink by gene name or symbol at <http://www.ncbi.nlm.nih.gov/LocusLink/>. Once the locus of your gene is found, scroll down to the "NCBI Reference Sequence (RefSeq)" section and look for mRNA.
2. Search Entrez Gene at <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>, and select the right gene of desired organism. Once the page for the gene is shown, scroll down to the "NCBI Reference Sequence (RefSeq)" and look for mRNA.
3. Search Nucleotide database using Entrez query tool at <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide> and use Entrez Limits settings to restrict your query to the RefSeq database only
  - o select "RefSeq" from the "Only from" menu, this restricts the query to the RefSeq collection

# SOP

- select "mRNA" from the "Molecule" menu, this restricts the query to mRNA RefSeq records

## Homology search

The RNAi targeted region on the mRNA sequence of a gene should not share significant homology with other genes or sequences in the genome, therefore, homology search is essential to minimize off-target effects. Although most siRNA design tools provide BLAST option, some simply use NCBI BLAST tools which sometimes are quite slow. Here are some BLAST tools for homology search.

- NCBI Blast tool: [Nucleotide-nucleotide BLAST \(blastn\)](#) or [Search for short, nearly exact matches](#)
- Blat tool on UCSC Genome Website <http://genome.ucsc.edu/cgi-bin/hgBlat>
- Ensembl Blast <http://www.ensembl.org/Multi/blastview>

## Examples of RNAi target selection

```
Homo sapiens vimentin (VIM), mRNA, NM_003380
1  GGGCGCGCCA GAGACGCAGC CGCGCTCCCA CCACCCACAC CCACCGCGCC CTCGTTTCGCC
61 TCTTCTCCGG GAGCCAGTCC GCGCCACCGC CGCCGCCAG GCCATCGCCA CCTCCGCAG
121 CCATGTCAC CAGGTCCGTG TCCTCGTCCT CCTACCGCAG GATGTTCCGC GGCCCGGGCA
181 CCGCGAGCCG GCCGAGCTCC AGCCGGAGCT ACGTGACTAC GTCCACCCGC ACCTACAGCC
241 TGGGCAGCGC GCTGCGCCCC AGCACCAGCC GCAGCCTCTA CGCCTCGTCC CCGGGCGGGC
301 TGATATGCCAC GCGTCTCTCT GCCGTGCGCC TCGGAGCAG CGTGCCCGGG GTGCGGCTCC
361 TGCAGGACTC GGTGGACTTC TCGCTGGCCG ACGCCATCAA CACCGAGTTC AAGAACACCC
421 GCACCAACGA GAAGGTGGAG CTGCAGGAGC TGAATGACCG CTTGCGCCAAC TACATCGACA
481 AGGTGCGCTT CCTGGAGCAG CAGAATAAGA TCCTGTGGC CGAGCTCGAG CAGCTCAAGG
541 GCCAAGGCAA GTCGCGCCTA GGGGACCTCT ACGAGGAGGA GATGCGGGAG CTGCGCCGGC
601 AGTTGGACCA GCTAACCAAC GACAAAGCCC GCGTCGAGGT GGAGCGCGAC AACCTGGCCG

AA(N19)TT

Vimentin cDNA: 5' AACTACATCGACAAGGTGCGCTT
sense siRNA: 5' CUACAUCGACAAGGUGCGC-dTdT
antisense siRNA:3' dTdT-GAUGUAGCUGUCCACGCG 5'
```

## References:

1. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature*. 2001 May 24;411(6836):494-8.
2. Elbashir SM, Lendeckel W, Tuschl T. RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev*. 2001 Jan 15;15(2):188-200