

siRNA Scanner - A Fuzzy Logic Based Tool for Small Interference RNA Design

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Abstract

The discovery of short interfering RNA has admitted the development of facile regulated methods for disruption of gene expression. However, this method continues to grow in popularity, designing effective siRNA can be demanding. So we have developed siRNA Scanner, a siRNA selection program that automatically selects Small Interfering RNA from the given RNA sequences. siRNA Scanner uses a fuzzy logic-based system to calculate siRNA qualities. This program is fully built in Practical Extract Report Language (PERL 5.8.8.6 Build 820) and accessible in a command line interface. siRNA Scanner's high performance, minimal user interaction, and its fast algorithm, make this program useful for selecting Small Interfering RNA for gene expression studies.

Availability: The program can be downloaded at <http://brsaran.googlepages.com/sirnascreener.exe>

Keywords: Fuzzy logic; siRNA; Design tool

Introduction

RNAi was initially observed in petunias, when Napoli et al. (1990) discovered that introducing a pigment-producing gene suppresses expression of both the introduced gene and the homologous endogenous gene, a phenomenon they called "cosuppression". RNA interference (RNAi) is the process of mRNA degradation that is induced by double-stranded RNA in a sequence-specific manner (Fire et al., 1998; Hannon, 2002). In all RNA silencing pathways, double-stranded RNA (dsRNA) is processed to a small RNA which is assembled with RISC into a silencing complex that specifically represses expression or function of a target gene or genomic region by cleaving the corresponding mRNA (McManus et al., 2002). However, introduction of long dsRNA into mammalian systems is more problematic. This is due to the fact that in most mammalian cells dsRNA, that is more than 40 nt, (Frank Buchholz, unpublished data) induces a nonspecific interferon response, leading to the general shutdown of transcription and/or cell death (Williams, 1999; Stark et al., 1998). In mammalian system the most standard protocols for RNAi therefore use chemically synthesized siRNAs of 19-22 base pair length for gene silencing. Yet this approach is limited by the fact that different sequences within a gene have dramatically varied inhibitory abilities (Holen et al., 2002). In essence, a large number of different synthetic siRNAs have to be screened for their efficacy at knocking down the gene of interest, which is a laborious and costly task.

Several siRNA design tool exists Ambion siRNA Target Finder (<http://www.ambion.com/techlib/misc/>

siRNA_finder.html), Jack Lin's siRNA Sequence Finder (<http://www.sinc.sunysb.edu/Stu/shilin/rnai.html>), siDESIGN Center (<http://www.dharmacon.com/sidesign>), siRNA Target Finder (<https://www.genscript.com/ssl-bin/app/rnai>), Imgenex sirna Designer (http://imgenex.com/sirna_tool.php), EMBOSS sirna (<http://bioweb.pasteur.fr/seqanal/interfaces/sirna.html>), IDT RNAi Design (SciTools) (<http://www.idtdna.com/Scitools/Applications/RNAi/RNAi.aspx>), BLOCK-iT RNAi Designer (<https://rnaidesigner.invitrogen.com/rnaiexpress>), siSearch (http://sonnhammer.cgb.ki.se/siSearch/siSearch_1.7.html), SiMAX (http://www.mwg-biotech.com/html/s_synthetic_acids/s_sirna_design.shtml), BIOPREDsi (<http://www.biopredsi.org/>), Promega siRNA Target Designer (<http://www.promega.com/siRNADesigner/program/>), QIAGEN siRNA Design Tool (<http://www1.qiagen.com/Products/GeneSilencing/CustomSiRna/SiRnaDesigner.aspx>), SDS/MPI (<http://i.cs.hku.hk/~sirna/software/sirna.php>), WI siRNA Selection Program (<http://jura.wi.mit.edu/bioc/siRNAext/>), which use a set of common criteria (e.g. GC%, Tm) for selecting the siRNA candidate in a specific target region selected by the user or in predefined region. Here, we describe a new tool for the designing small interference RNA based on various efficient criteria from the literature and these criteria are calculated for every siRNA candidate over the entire target sequence without masking. Using efficient fuzzy logic system, we assigned a quality grade to each of the candidate. To make the application as widely available as possible, the tool is programmed in PERL, which is multiplatform compatible.

Materials and Methods

siRNA Scanner for the design of functional siRNA's includes the rules based on the work recently published by different authors (Holen et al., 2002; Reynolds et al., 2004; Wuming et al., 2006), which have proven to be more efficient than the consensus rules accepted to date. The novelty of this tool is that it is the only tool till date that applies Fuzzy logic to select the efficient candidate for siRNA interference.

Physical Parameters for Fuzzy Construction

Direct Sequence features (Eight Features)

For each position in the sense strand of 19-mer siRNA sequence, 8 features were defined based on whether or not the nucleotide at the position is an adenine (A), a cytosine (C), a guanine (G), or a uracil (U), respectively (Holen et al., 2002). Eight features are listed below:

Feature 1	2 nd nucleotide	=	A
Feature 2	4 th nucleotide	=	C
Feature 3	6 th nucleotide	≠	C
Feature 4	7 th nucleotide	≠	U
Feature 5	9 th nucleotide	=	C
Feature 6	17 th nucleotide	=	A
Feature 7	18 th nucleotide	≠	C
Feature 8	19 th nucleotide	=	(A/U)

Percentage of GC Content

The GC content of the siRNA duplex is an obvious candidate for a parameter that might correlate with siRNA functionality. Too high GC content may slow down duplex unwinding by the putative helicase tentatively associated with the RISC complex (Reynolds et al., 2004) and might also be associated with a prohibitive secondary structure of the target mRNA. Too low GC content, on the other hand, may reduce the efficiency of target mRNA recognition and hybridization. The strongest correlation was observed for the GC range 31.6–57.9% (Reynolds et al., 2004) and this range is considered as optimum in our fuzzy based rule.

Thermodynamics

Three quality grades were defined based on whether or not the melting temperature (T_m) falls into following ranges 0°C – <20°C, >20°C – <60°C, and >60°C.

No Occurrences of Four or More Identical Nucleotides in a Row

This feature implies that a siRNA sequence have no internal repeated sequence of length ≥ 4 .

No Occurrences of G/C Stretches of Length 7 or Longer

This feature implies that a siRNA sequence have no GC stretch of length > 7 or longer.

At least 3(A/U)'s in the Seven Nucleotide at the 3' end

Based on the number of A/U in the seven nucleotide at the 3'-end, the feature is categorized into Least, At least, High. Least falls under <3 A/U, At least ≥ 3 A/U <5 , and High ≥ 6 .

Computational Methods

Fuzzy Logic

For the purpose of assigning relative weights to the parameters Simulink's Fuzzy logic Tool box (MATLAB®) was used. This tool takes a list of relevant parameters as input and their ranges are partitioned into categories according to specifications by the user. For example, the GC% content was categorized into Low, Optimum, High and Very High. These categories may be overlapping and we used specific membership functions to describe partial assignment of a value to a category. Table 1 lists a set of the rules used in this application. The fuzzy logic system designs a mathematical model that maps the input parameters to output parameters in accordance with the user's qualitative description. It is thus, not necessary for a user to design a mathematical description by himself. Instead, this description is generated by the fuzzy logic system based on only a verbal description of the relevant rules.

Data Storage

siRNA Scanner does not reject any siRNA candidate before having calculated all relevant parameters for each possible 19mer. For efficient handling of the huge amount of data generated during a siRNA candidate search we store all relevant information in a tree. This structure can be built up in a time proportional to the number of positions in the sequence using the on-line-construction algorithm of Ukkonen (Ukkonen, 1995).

Programming Language

siRNA Scanner V1.0 is entirely coded in PERL (Practical Extract Report Language) Version 5.8.8 Build 820. Three different modules namely, AI::FuzzyInference and Math::Fortran was downloaded from the CPAN archive and used in this application.

S.No	Fuzzy Rules
1	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=short & Atleast3AU=atleast & EightFunction=maximum' => 'RNAi=best',
2	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=short & Atleast3AU=more & EightFunction=maximum' => 'RNAi=best',
3	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=short & Atleast3AU=high & EightFunction=maximum' => 'RNAi=best',
4	'TM=optimum & GCPercent=optimum & GCStretch=medium & IdenticalBases=short & Atleast3AU=high & EightFunction=maximum' => 'RNAi=average',
5	'TM=optimum & GCPercent=optimum & GCStretch=medium & IdenticalBases=short & Atleast3AU=atleast & EightFunction=maximum' => 'RNAi=average',
6	'TM=optimum & GCPercent=optimum & GCStretch=medium & IdenticalBases=short & Atleast3AU=more & EightFunction=maximum' => 'RNAi=average',
7	'TM=optimum & GCPercent=optimum & GCStretch=long & IdenticalBases=short & Atleast3AU=high & EightFunction=maximum' => 'RNAi=worst',
8	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=medium & Atleast3AU=atleast & EightFunction=maximum' => 'RNAi=worst',
9	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=medium & Atleast3AU=more & EightFunction=maximum' => 'RNAi=worst',
10	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=medium & Atleast3AU=high & EightFunction=maximum' => 'RNAi=worst',
11	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=long & Atleast3AU=high & EightFunction=maximum' => 'RNAi=worst',
12	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=long & Atleast3AU=more & EightFunction=maximum' => 'RNAi=worst',
13	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=long & Atleast3AU=atleast & EightFunction=maximum' => 'RNAi=worst',
14	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=short & Atleast3AU=atleast & EightFunction=minimum' => 'RNAi=worst',
15	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=short & Atleast3AU=atleast & EightFunction=average' => 'RNAi=best',
16	'TM=optimum & GCPercent=optimum & GCStretch=medium & IdenticalBases=short & Atleast3AU=atleast & EightFunction=average' => 'RNAi=average',
17	'TM=optimum & GCPercent=optimum & GCStretch=medium & IdenticalBases=short & Atleast3AU=atleast & EightFunction=average' => 'RNAi=average',
18	'TM=optimum & GCPercent=optimum & GCStretch=long & IdenticalBases=medium & Atleast3AU=atleast & EightFunction=average' => 'RNAi=worst',
19	'TM=optimum & GCPercent=optimum & GCStretch=long & IdenticalBases=long & Atleast3AU=atleast & EightFunction=average' => 'RNAi=worst',
20	'TM=optimum & GCPercent=optimum & GCStretch=medium & IdenticalBases=long & Atleast3AU=atleast & EightFunction=average' => 'RNAi=worst',
21	'TM=optimum & GCPercent=optimum & GCStretch=medium & IdenticalBases=medium & Atleast3AU=atleast & EightFunction=average' => 'RNAi=worst',
22	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=medium & Atleast3AU=atleast & EightFunction=average' => 'RNAi=worst',
23	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=short & Atleast3AU=more & EightFunction=average' => 'RNAi=average',
24	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=short & Atleast3AU=high & EightFunction=average' => 'RNAi=average',
25	'TM=low & GCPercent=optimum & GCStretch=short & IdenticalBases=short & Atleast3AU=atleast & EightFunction=maximum' => 'RNAi=worst',

S.No	Fuzzy Rules
26	'TM=high & GCPercent=optimum & GCStretch=short & IdenticalBases=short & Atleast3AU=atleast & EightFunction=maximum' => 'RNAi=worst',
27	'TM=optimum & GCPercent=low & GCStretch=short & IdenticalBases=short & Atleast3AU=atleast & EightFunction=maximum' => 'RNAi=worst',
28	'TM=optimum & GCPercent=high & GCStretch=short & IdenticalBases=short & Atleast3AU=atleast & EightFunction=maximum' => 'RNAi=worst',
29	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=short & Atleast3AU=least & EightFunction=maximum' => 'RNAi=average',
30	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=short & Atleast3AU=least & EightFunction=maximum' => 'RNAi=average',
31	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=short & Atleast3AU=least & EightFunction=minimum' => 'RNAi=worst',
32	'TM=optimum & GCPercent=optimum & GCStretch=short & IdenticalBases=medium & Atleast3AU=least & EightFunction=maximum' => 'RNAi=worst',

Table 1 : Verbal rules for siRNA Scanner V1.0.

Results and Discussion

The procedure for siRNA selection has been implemented in PERL as a stand-alone tool. The use of this program is simple and one needs to input the template nucleotide sequence file alone as input. The file format is plain text. Initially the program splits all possible candidates for the calculation, then in the second phase it calculates the fuzzy rules, assigns weight based on the membership function and finally defuzzify and categorize based on the rule framed

(Table 1) each candidate as either Best or Average or Poor. Once the siRNA Scanner completes the task, it query the user for five different options for the filtering the result. It includes (a) Best SiRNAs Alone (b) Average SiRNAs Alone (c) Best & Average SiRNAs Alone (d) Poor SiRNAs Alone (e) All Possible SiRNAs (Figure1). The result produced from the tool is a hypertext file with user defined name and the result are presented as table (Figure 2).

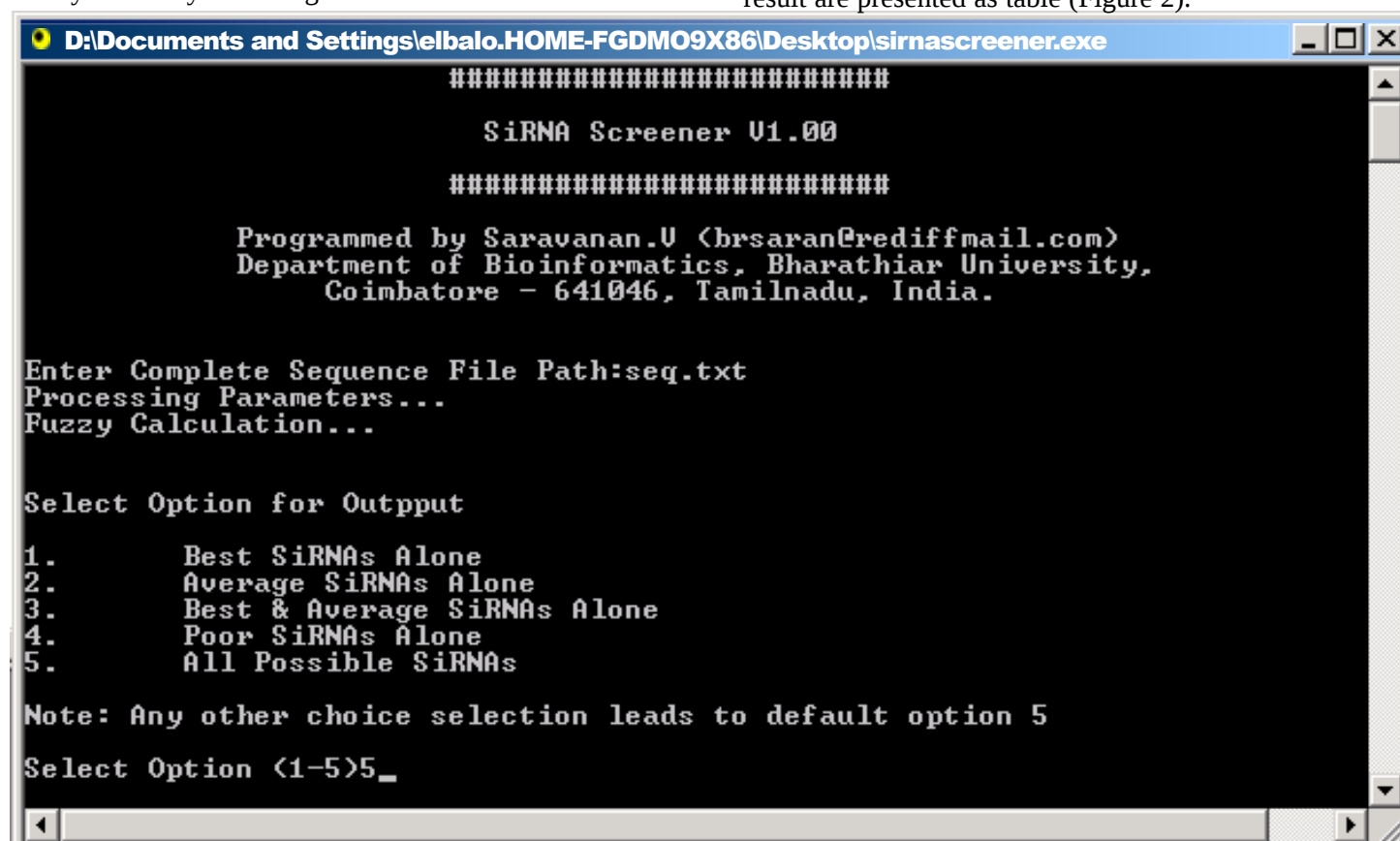


Figure 1 : Screen Shot of siRNA Scanner V1.0

SiRNA Screener V1.0

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S.No	Position	SiRNAs	Fuzzy Categeroy
1	1 - 19	GGCGCGUUAGAACAUUGCUCU	Poor
2	2 - 20	GCGCGUUAGAACAUUGCUC	Poor
3	3 - 21	CGCGUUAGAACAUUGCUCU	Poor
4	4 - 22	GCGUUAGAACAUUGCUCUAA	Average
5	5 - 23	CGUUAGAACAUUGCUCUAA	Average
6	6 - 24	GUUAGAACAUUGCUCUACA	Best
7	7 - 25	UUAGAACAUUGCUCUACA	Poor
8	8 - 26	UAGAACAUUGCUCUACAUC	Average
9	9 - 27	AGAACAUUGCUCUACAUC	Poor
10	10 - 28	GAACAUUGCUCUACAUC	Average
11	11 - 29	AACAUUGCUCUACAUC	Best
12	12 - 30	ACAUUGCUCUACAUC	Best

Figure 2 : Screen Shot of result generated from siRNA Scanner V1.0.

A test set of 79 genes, which already contains the reported active SiRNA's from literature, have been tested with the siRNA Scanner to analyze its success rate. Each gene sequences are collected from the NCBI website and fed into our tool for the prediction of possible efficient siRNA candidate. Out of 79 genes, for 69 gene set our tool predicted the published siRNA candidate as "Best", which is around 87.34% of the total input, for 6 gene set our tool reported the published siRNA candidate as "Average", which is around 7.25% of the total input; summing up siRNA Scan-

ner predicted the published siRNA as the possible candidate with the efficiency of 94.93%, that is 75 out of 79 siRNA candidate (Table 2), which is published as efficient gene silencer. Rest of the 4 candidate is predicted as "Poor" by our tool. Evaluation of test set shows an efficiency rate of 94.93% for siRNA candidate prediction. siRNA Scanner's high performance, minimal user interaction, and its fast algorithm, make this program useful for selecting Small Interfering RNA for gene expression studies.

S.No	Published Gene Target SiRNA	SiRNA Screener V1.0 Result	Gene Reference
1.	AATGAAGATCAAGATCATTGC	Best	DIP13-beta
2.	AAGAGATCGCCGCGCTGGTCA	Average	Dishevelled
3.	AAAGACUCCUUAGGACCGCUU	Best	hDv-2
4.	AATTGGTGCACCTTTTACGTGA	Average	Eg5
5.	AAGAATGAAGCTGATAGGACC	Best	emerin
6.	AAGGTCCACAGATTATTAGAT	Best	eve
7.	CTAGAGGAACAGAGAAGATCACA	Best	Fortilin
8.	AAGAAGAGGCCCAAGAAAAGCT	Best	GalTII
9.	AAGCAGAGUUCAAAAGCCCUU	Average	GalTII
10.	AAAGCAGAGUUCAAAAGCCCUU	Best	GAS41
11.	AAGACAGAGCCAAGUGGACUC	Best	HPV E6
12.	AAUUGGUAAAACGAUCAAGGAG	Best	HPV E7
13.	AAGGGAAAGAAGGAGGUUUC	Average	HtrA2
14.	AAGAAGCUUAAAACCGUGGAG	Best	HtrA2
15.	AACUGGGGAAGAAGGGCAACC	Best	HtrA2
16.	AAGGGGCACAGGCATGCCAAA	Best	keratin 18
17.	AAACAGCTGTTGTTGAGCGAA	Best	hKIS
18.	GCACAGTAAATACCCACTATT	Best	mKIS
19.	AAGGGGTTCTTAGTACTGCAA	Best	lamin A
20.	AAACTCTTACTGCTCTCCAGT	Best	lamin A
21.	AAGGUCAGUAAUACAACGGUG	Poor	lamin A/C
22.	AACCACCGCCCUCCGUGCA	Best	lamin B1
23.	CTCGTACAGCCTTGGGAGACA	Best	lamin B2
24.	ATGTTAAGACTAAGCATGTAC	Best	LAP2
25.	AAGGCCAAGGAGGCGCTGGAA	Best	MeCP2
26.	AAGUGCAGCAACACACCUGAC	Best	MAP2
27.	AAGCAGCACCUCUCCUCCCUU	Average	MAP2
28.	AACAAGATCACCTTCTCCGAG	Best	MAP2
29.	AACTTTGAGAACATGAGCAAC	Best	MCL1
30.	AACTGGATCGTAAGAAGGCAG	Best	Nicastrin [Nct]
31.	AACCGTGCTCCTGGGGCTGGG	Best	NuMA
32.	AACUCCUUGAACGGCAGCCGC	Best	Nup153
33.	AAGGUAACAUAUGACUCGC	Best	Orc6
34.	AAGCACGCGACGCGUGGCGCGC	Poor	Orc6
35.	AAGGCGGACGACGACUCCUUC	Best	hPlk1
36.	AAGACCTGTAACCCTGTATCA	Poor	PKR
37.	AAGAGGUUAUAUGACUUUGCUU	Best	hRad9
38.	AAAGGAGGAUGAAAUAGAUGG	Average	Rad17
39.	AAAACGGCUCAGGAUUCGUGG	Best	h/m Shc
40.	AAGGGGAGTTTGTGTTGCCA	Best	p66 ShcA
41.	AAGCTTTCCCGATGTTTCAGCA	Poor	Sphingosine kinase 1 [hSK1]
42.	AAGATGGCGAGGACTTTAATC	Best	SRC-1
43.	AAGCAGTTCTTGCCGCCAGGA	Poor	β -tubulin
44.	AAGCAGTTCCTGCCTCGGGGA	Best	Tissue Factor [hTF]
45.	GAAGCAGCUUCAGGAUGAGAU	Best	Tissue Factor [hTF]
46.	AAGGACCUGGAGGUCUGCUG	Best	β -TrCP, human
47.	AACTGGACTTCCAGAAGAACA	Best	TSG101
48.	AACGCGCTTGGTAGAGGTGGA	Best	VASP
49.	AAGAGGAGGAGGAAGCCGAGT	Best	vimentin
50.	AAAGACAAGTTGAAGAGTGAG	Best	vimentin
51.	AAGCAUGAGCCCGUGCAGCCA	Best	vimentin
52.	AAGAGGAUGGAUUGCCCGGCC	Best	vimentin

53	AAUCCUUCGUCUUUCCUCUCG	Average	vinculin, mouse
54	AACCCGUCCGUACUGGUGUUA	Best	zyxin, mouse
55	AAGGGCAAGTTTCCCGTGCAG	Best	DIP13-beta
56	AAGGCGTGGCAGGAGAAGTTC	Best	Dishevelled
57	AAGGCAGACTCTACCAAATGT	Best	hDv-2
58	AAUGGUAGCCACAUCGCGUGU	Best	Eg5
59	AAGATTGGACAGCAGGUCGAC	Best	emerin
60	AAGGGCGGCTTTGCCAAGTGC	Best	eve
61	AAGATCAAGTTTGGCCAATG	Poor	Fortilin
62	AAGUCUUUCCUGUCUGUCUUU	Best	GalTII
63	AACAGACUGGGUUGACCCAUC	Best	GalTII
64	TCCTACTTGGTTCGGTACATGGG	Best	GAS41
65	CGGAATGAGTCTCTGTCATCGTC	Best	HPV E6
66	AAGAGCUGCAAGGCCUUGCCC	Best	HPV E7
67	CCUCAGGGCAGAGAACCAUCUTT	Best	HtrA2
68	GACAGAGCCAAGUGGACUCAC	Best	HtrA2
69	AAGCGCUUCAGGCACUACAAA	Best	HtrA2
70	AAGAAGCAGACGUACUUGGCA	Best	keratin 18
71	GUGGAAUUUGUGGAACAUCTT	Best	hKIS
72	AACCUCCAGUCUUCUCUCGUC	Best	mKIS
73	AAAGAGGAAATCATTGAAGCC	Best	lamin A
74	AACTACATCGACAAGGTGCGC	Best	lamin A
75	AATACCAAGACCTGCTCAATG	Best	lamin A/C
76	AAGAATGGTACAAATCCAAGT	Best	lamin B1
77	AAATGGAAGAGAACTTTGCCG	Best	lamin B2
78	AAGCGGATTAGAACCAATCTC	Best	LAP2
79	AAAGCCTACCACCCACAGTGC	Average	MeCP2

Table 2 : Fuzzy Categorized result for the published siRNA by SiRNA Scanner V1.0

Three main objectives have been addressed in this work that makes siRNA Scanner a tool with efficient feature compared with other similar programs: speedy calculation; efficient prediction based on fuzzy logic; and considers all possible candidate without discarding any 19mers. In contrast to other siRNA design software, siRNA Scanner evaluates each possible siRNA candidate. The amount of computational effort here is comparatively best than other siRNA design program. siRNA Scanner is fast in calculating optimal siRNA's. It checks for all possible candidates from the given template sequence. Calculation time depends linearly on the length of sequence used for checking. On average, a siRNA calculation considering all possible candidates on a target sequence of length 5000 bases pairs takes ~ 21 seconds on an IBM Machine with Pentium 4 Processor. siRNA Scanner's high performance makes it feasible to efficiently search siRNA on large templates sequence.

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