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Design of potential siRNA molecules for T antigen gene silencing of Merkel Cell Polyomavirus

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Abstract:
Merkel cell carcinoma (MCC) is the most aggressive skin cancer. Recently, it was demonstrated that human Merkel cell polyomavirus (MCV) is clonally integrated in 80% of MCC tumors. Genetic studies of MCV have shown that T antigen protein is responsible for replication of genome and play a foremost role in viral infection. Therefore, T antigen protein may be used as suitable target for disease diagnosis. Viral activity can be restrained through RNA interference (RNAi) technology, an influential method for post transcriptional gene silencing in a sequence specific manner. In current study four effective siRNA molecules for silencing of MCV were rationally designed and validated using computational methods, which may lead to knockdown the activity of virus. Thus, this approach may provide an insight for the chemical synthesis of antiviral RNA molecule for the treatment of MCC at genome level.

Keywords: MCC, MCV, T antigen, RNAi, siRNA, Thermodynamics

Background:
Merkel Cell Carcinoma (MCC) is a rare, aggressive cancer in which malignant cancer cells develop in hair follicles, or on or beneath the skin. Merkel cells are found in the top layer of the skin. These cells are very close to the nerve endings that receive the sensation of touch. Merkel cell carcinoma, also called neuroendocrine carcinoma of the skin or trabecular cancer, is a very rare type of skin cancer that forms when Merkel cells grow out of control. Merkel cell carcinoma starts most often in areas of skin exposed to the sun, especially the head and neck, as well as the arms, legs, and trunk. A newly discovered virus called Merkel cell polyomavirus (MCV) likely contributes to the development of the majority of MCC **[1]**. Approximately 80% of MCC have this virus integrated in a monoclonal pattern **[2, 3]**, indicating that the infection was present in a precursor cell before it became cancerous. MCV is the first polyomavirus strongly suspected to cause tumors in humans. Like other tumor viruses, most people who are infected with MCV probably do not develop MCC. It is currently unknown what other steps or co-factors are required for MCC-type cancers to develop **[4]**. MCC also occurs more frequently than would otherwise be expected among immuno suppressed patients, such astrans plant patients, AIDS patients, and the elderly persons, suggesting that the initiation and progression of the disease is modulated by the immune system **[5]**. Although rare, the incidence of MCC has tripled over the past 15 years to approximately 1500 new MCC cases in the U.S. each year. MCC is primarily seen in the elderly Caucasian population 65 years of age and above, and shows a slight predominance in males. Merkel cell carcinoma tends to grow quickly and to metastasize (spread) at an early stage. It usually spreads first to nearby lymph nodes and then may spread to lymph nodes or skin in distant parts of the body, lungs, brain, bones, or other organs. At present time there is no clinical treatment of Merkel cell carcinoma. Hence, there is an urgent need for novel therapeutics for the disease.

MCV is the fifth polyomavirus that infects humans to be discovered. It belongs to the murine polyomavirus group, one of the three mainclades of polyomaviruses **[1]**. MCV is genetically most closely related to the African green monkey lymphotropic polyomavirus **[1]**. The prototype sequence of MCV has a 5387 base pair genome, and encodes characteristic polyomavirus genes including a large T antigen, small T antigen, VP1 and VP2/3 genes **[1]**. MCV T antigen has similar features to the T antigens of other polyomaviruses, which are known oncoproteins, and is expressed in human tumors **[1]**. The MCV T antigen locus encodes for four differentially spliced mRNA transcripts corresponding to polyomavirus large T antigen (LT), and small T antigen (ST, encoded by two transcripts) as well as an additional isoform 57kT, which may represent an analogue to the SV40 17kT transcript **[6]**. Both large T and small T oncoproteins are needed to transform healthy cells into cancer cells, and they act by targeting tumor suppressor proteins, such asretinoblastoma protein **[7]**. The large T antigen possesses a helicase motif needed for virus replication that is deleted in MCC tumors. Unlike for other polyomaviruses, MCV small T antigen transforms cells in vitro **[8]** by activatingcap-dependent translation.

RNA interference (RNAi), an evolutionary conserved gene silencing mechanism, uses short double-stranded RNA (dsRNA) "trigger" is processed into siRNA and assembled with other components to degradation or translation repression of homologous RNA targets in a sequence-specific manner. This has been used as alternative antiviral therapy **[9]**. H Roland *et al* subsequently showed that the T antigen mRNA can be successfully targeted by siRNAs in cell culture **[7]**. Thus, T antigen protein coding mRNA of MCV is obligatory target to inhibit the RNA processing and may be suitable for antiviral therapy. Therefore, in the current study an attempt has been made to identify potential siRNA molecules for silencing of T antigen coding mRNA or gene in MCV using computational approach.

Methodology:

Data collection and analysis

Thirty seven complete cds of large T antigen and small T antigen gene sequences of MCV were retrieved from viral GenBank database, available at http://www.ncbi.nlm.nih.gov/. The viral database contains all experimentally identified widespread genome isolates of MCV which were further used for siRNA designing.

Target identification and rational siRNA molecule designing

siDirect 2.0 **[10]** (http://siDirect2.RNAi.jp/) tool, was used for target identification and designing of potential siRNA molecules. It utilized mixed rule approach of Ui-Tei, Amarzguioui and Reynolds rules **[11]** and melting temperature (Tm) below 21.5°C for siRNA duplex, as parameter. For further verification of predicted molecules GeneScript siRNA Target Finder (http://www.genescript.com/index.html), dharma siRNA technology (http://www.dharmacon.com/designcenter/) and siRNA at whitehead (http://sirna.wi.mit.edu/home.php) tool was also applied. Besides these other parameters were taken on the concept of algorithms given in **Table 1 (see supplementary material).**

Similarity search

Blast tool (http://www.ncbi.nlm.nih.gov/blast) **[12]** was used to identify any off target sequence similarity in other non targeted organism's genome against whole Genebank datasets by applying expected threshold value 10 and BLOSUM 62 matrix as parameter. The target sites having similarity of more than 16 adjoining base pair with any other organism were excluded from the consideration.

GC calculation and siRNA secondary structure prediction

GC calculator tool www.genomicsplace.com/gc_calc.html was used to calculate the GC content for selected siRNA molecule while secondary structure and free energy of folding was computed through Mfold server http://mfold.rna.albany.edu/?q=mfold/download-mfold.

Thermodynamics calculation of RNA-RNA interaction

RNAup program (www.tbi.univie.ac.at/~ulim/RNAup) at Vienna web suit **[13]** was used to study the thermodynamics of interactions between target gene and predicted siRNA molecules. It works on extension of the standard partition function approach to RNA secondary structures that compute energetic of RNA-RNA interactions **[14]**.

Result and Discussion:

Thirty five complete cds of large T antigen and small T antigen gene sequences of MCV are available in NCBI Genebank database which used in current study. siDirect 2.0 tool was used in current study to provide functional, target-specific siRNA molecules, which significantly reduces off-target silencing. To avoid offtarget effect, Tm for the seed-target duplex was calculated using the nearest neighbor model and the thermodynamic parameters for the formation of RNA duplex were also studied **[15]**. The formula for calculating Tm is:

$$Tm = \{(1000 \times \Delta H) / (A + \Delta S + R \ln (CT/4))\} - 273.15 + 16.6 \log [Na+] \quad \text{(Equation 1)}$$

Where ΔH (kcal/ mol) is the sum of the nearest neighbor enthalpy change, A is the helix initiation constant (-10.8), ΔS are the sum of the nearest neighbor entropy change **[16]**. R is the gas constant (1.987 cal/deg/mol), and CT is the total molecular concentration of the strand (100 µM). [Na+] was fixed at 100 mM. Apart from it, to check the accuracy of result Gene script target Finder was also applied and usage statistical modeling method.

In present study six hundred seventy one siRNA targets were identified for T antigen of MCV and potential siRNA molecules against these targets were obtained using mixed rule approach i.e. Ui-Tei, Amarzguioui and Reynolds rule. Out of six hundred seventy one predicted siRNA targets, three hundred thirty one were following all three rules. Hence, these three hundred thirty one siRNA targets were filtered out for further study and considered possible candidates. Consequently these three hundred thirty one targets were subjected to NCBI Blast tool. Out of these three hundred thirty one targets only 135 were selected on the basis of low off target similarity **Table 2 (see supplementary material)**. MSA of these selected 135 siRNA targets were depicted that these sequences divided in to four different groups which is shown in **(Figure 1)**.

However, there are the incompatible results regarding the effect of GC content and secondary structure on siRNA efficiency. Therefore, these parameters cannot be preferred as a primary determinant of siRNA efficiency. Still, it is recommended to choose sequences with low GC content (31- 58%) **[17-19]**, in the present study all predicted siRNA molecules having recommended range of GC content. Furthermore, the possible folding of predicted siRNA molecules for MCV was done with the online MFold package. Mfold follows most widely used algorithms for RNA secondary structure prediction, which are based on a search for the minimal free energy state **[19]**. Here, one siRNA molecule is having more than zero free energy of folding at 37^{0}C **Table 3 (see supplementary material)**. Earlier studies have recommended that an RNA molecule should have minimum free energy of folding for their stability. Therefore, the molecule with positive energy may be more accessible for target site and have high potential to bind with target and lead to in effective gene silencing. While other molecule is also having less than -1 kcal frees energy of folding.

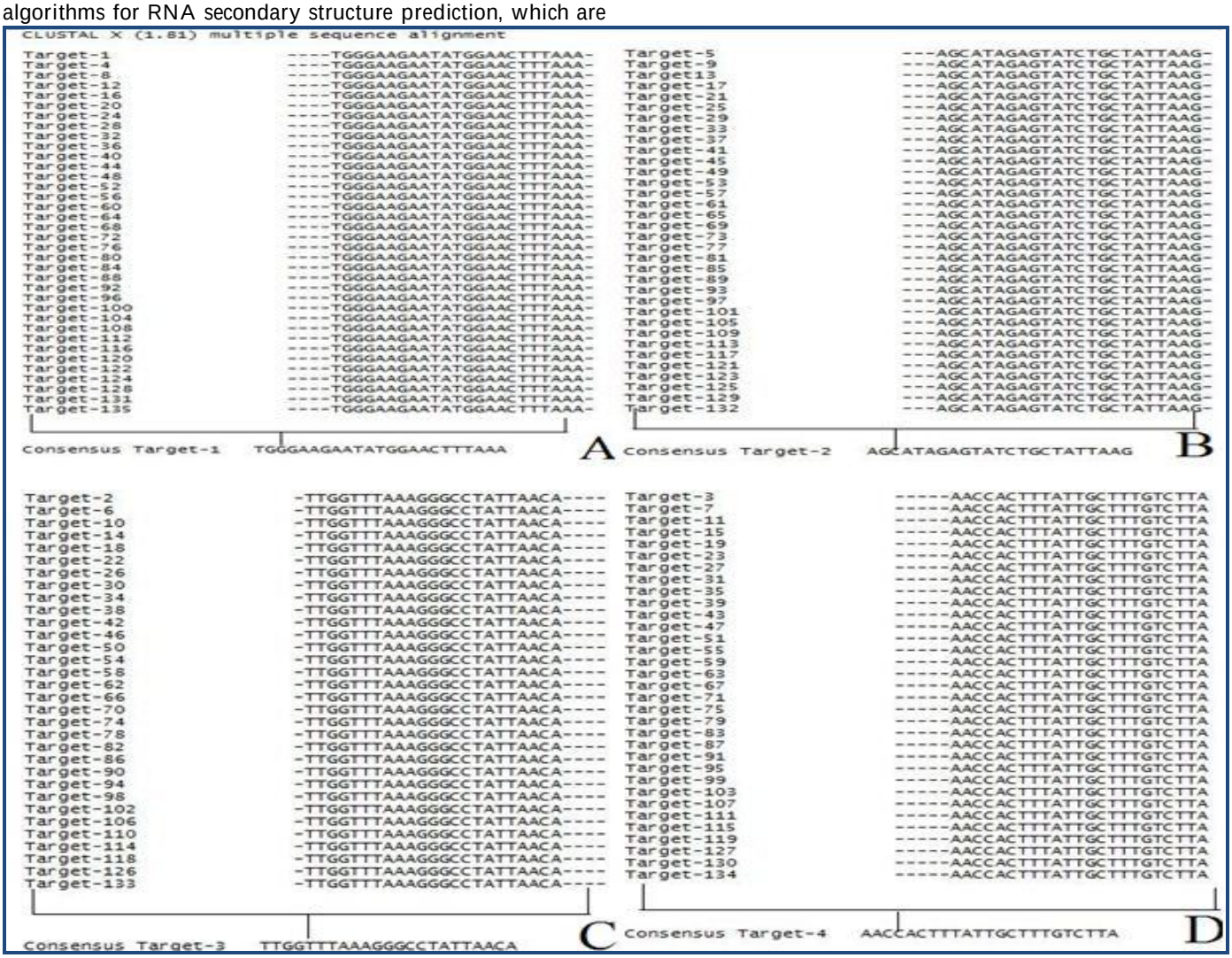

Figure 1: Multiple sequence alignment of predicted siRNA target sequences. **A)** Consensus Target-1; **B)** Consensus Target-2; **C)** Consensus Target-3; **D)** Consensus Target-4

Apart from this, a variety of biologically important RNAs were used for prediction of their function by interacting with other RNA molecules. Thus, thermodynamics study of RNA-RNA interactions may be an important aspect for siRNA molecule efficiency. The predicted siRNA molecules were subjected to RNA-RNA interaction study with their respective targets. The Vienna web site is a comprehensive collection of tool that offers state of the art algorithms for RNA folding, comparison and prediction of RNA–RNA interactions. RNAup one of the important tools of Vienna web site was used to predict free energy of RNA-RNA interactions. It models the binding energy for the interaction at a particular site as

(BE) $\Delta G_{binding} = \Delta G_{uA} + \Delta G_{uB} + \Delta G_h$ (Equation 2)

Where ΔG_{uAB} ($\Delta G_{uA} + \Delta G_{uB}$) is the free energy required to make the binding region in molecule A (target) or B (siRNA) accessible by removing intra-molecular structure. While ΔG_h denotes the free energy gained from forming the intermolecular duplex by the partition function over all structures where the short RNA binds to target region. Calculation of the free energy of interaction (binding) between a siRNA molecule and its target was performed by using (equation 2) **Table 3 (see supplementary material)**.

RNAi approach is successfully exploited in various cases such as hepatitis B infection **[20]** silencing of endonuclease Argonaute 2 in *Drosophila melanogaster* **[21]**. RNAi utilized in HIV-1 infection in human peripheral blood mononuclear cells

via best env-specific siRNAs, E7145 targeted to the central region of the V3 loop and E7490 targeted to the CD4 binding site of conserved regions on gp120, significantly inhibited the HIV-1 gene expression. Furthermore, E7145 and E7490 were effective against HIV-1NL4-3 replication in PBMCs for a relatively long time (14 days) **[22]**. In experimental brain cancer pegylated immunoliposomes (PIL) carrying short hairpin RNA expression plasmids driven by the U6 RNA polymerase promoter and directed to target EGFR expression by RNAi. The PIL is comprised of a mixture of known lipids containing polyethyleneglycol (PEG), which stabilizes the PIL structure *invivo* in circulation. The tissue target specificity of PILs is given by conjugation of ~1% of the PEG residues to monoclonal antibodies (mAbs) that bind to specific endogenous receptors (i.e., insulin and transferrin receptors) located in the brain vascular endothelium **[23]**. This approach was found to be successful in targeting bovine priongene PRNP in livestock **[24]**, carcinoma of the breast **[25]** and crown gall tumorigenesis in plants **[26]**. This technique was also used for silencing of capsid genes of Flavivirus using computational methods **[27]**. However, siRNA is the most influential means to control over gene expression in various organisms and showing antiviral activity too. Therefore, rational siRNA has provided the advancement in the development of experiment based approaches to prevent the MCV infections via gene silencing mechanism.

Conclusion:

Using RNAi technology a number of siRNA molecules may be designed for silencing of significant genes in various biological systems. Further their interactions with target can also be calculated, computationally. Therefore, in this study four siRNA molecules were predicted against T antigen protein as effective candidate using computational approaches. These molecules may lead to a novel antiviral therapy against MCV. Study outcome would also provide a basis to the researchers and pharma industry persons to develop the antiviral therapeutics at genomic level, experimentally.

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Supplementary material:

Table 1: Algorithms or rules for rational design of siRNA molecules

Ui-Tei Rules	Amarzguioui Rules	Reynolds Rules
A / U at the 5' terminus of the sense strand	Duplex End A / U differential > 0. Strong binding of 5'sense strand	Each rule is assigned a score which is summed up to a total duplex score to improve the efficacy of siRNA.
G / C at the 5' terminus of the antisense strand	No U at position 1. Presence of A at position 6.	
At least 4 A / U residues in the 5' terminal 7 bp of sense strand	Weak binding of 3'sense strand. No G at position 19	
No GC stretch longer than 9nt		

Table 2: Predicted siRNA target for T antigen gene of MCV

Accession No	Target	Location of target within gene	siRNA target sequence within gene
FJ173805.1	Target-1	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-2	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-3	2648-2670	AACCACTTTATTGCTTTGTCTTA
JN383833.1	Target-4	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-5	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-6	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-7	2648-2670	AACCACTTTATTGCTTTGTCTTA
FJ173813.1	Target-8	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-9	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-10	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-11	2648-2670	AACCACTTTATTGCTTTGTCTTA
FJ173804.1	Target-12	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-13	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-14	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-15	2648-2670	AACCACTTTATTGCTTTGTCTTA
FJ173803.1	Target-16	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-17	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-18	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-19	2648-2670	AACCACTTTATTGCTTTGTCTTA
JN383837.1	Target-20	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-21	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-22	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-23	2648-2670	AACCACTTTATTGCTTTGTCTTA
JN383823.1	Target-24	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-25	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-26	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-27	2648-2670	AACCACTTTATTGCTTTGTCTTA
FM955590.1	Target-28	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-29	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-30	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-31	2648-2670	AACCACTTTATTGCTTTGTCTTA
FM955587.1	Target-32	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-33	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-34	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-35	2648-2670	AACCACTTTATTGCTTTGTCTTA
JN383825.1	Target-36	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-37	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-38	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-39	2648-2670	AACCACTTTATTGCTTTGTCTTA
FJ173814.1	Target-40	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-41	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-42	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-43	2648-2670	AACCACTTTATTGCTTTGTCTTA
FM955588.1	Target-44	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-45	1564-1586	AGCATAGAGTATCTGCTATTAAG

	Target-46	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-47	2648-2670	AACCACTTTATTGCTTTGTCTTA
FJ173812.1	Target-48	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-49	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-50	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-51	2648-2670	AACCACTTTATTGCTTTGTCTTA
JN383834.1	Target-52	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-53	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-54	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-55	2648-2670	AACCACTTTATTGCTTTGTCTTA
FM955589.1	Target-56	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-57	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-58	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-59	2648-2670	AACCACTTTATTGCTTTGTCTTA
JN383832.1	Target-60	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-61	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-62	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-63	2648-2670	AACCACTTTATTGCTTTGTCTTA
JN383831.1	Target-64	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-65	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-66	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-67	2648-2670	AACCACTTTATTGCTTTGTCTTA
FM955593.1	Target-68	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-69	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-70	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-71	2648-2670	AACCACTTTATTGCTTTGTCTTA
FM955592.1	Target-72	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-73	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-74	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-75	2648-2670	AACCACTTTATTGCTTTGTCTTA
FM955586.1	Target-76	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-77	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-78	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-79	2648-2670	AACCACTTTATTGCTTTGTCTTA
JN383836.1	Target-80	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-81	1558-1580	AGCATAGAGTATCTGCTATTAAG
	Target-82	2192-2214	TTGGTTTAAAGGGCCTATTAACA
	Target-83	2642-2664	AACCACTTTATTGCTTTGTCTTA
JN383826.1	Target-84	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-85	1559-1581	AGCATAGAGTATCTGCTATTAAG
	Target-86	2193-2215	TTGGTTTAAAGGGCCTATTAACA
	Target-87	2643-2665	AACCACTTTATTGCTTTGTCTTA
JN383824.1	Target-88	252-274	TGGGAAGAATATGGAACTTTAAA
	Target-89	1556-1578	AGCATAGAGTATCTGCTATTAAG
	Target-90	2190-2212	TTGGTTTAAAGGGCCTATTAACA
	Target-91	2640-2662	AACCACTTTATTGCTTTGTCTTA
FM955591.1	Target-92	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-93	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-94	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-95	2648-2670	AACCACTTTATTGCTTTGTCTTA
FJ173807.1	Target-96	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-97	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-98	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-99	2648-2670	AACCACTTTATTGCTTTGTCTTA
JN383829.1	Target-100	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-101	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-102	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-103	2648-2670	AACCACTTTATTGCTTTGTCTTA
JN383828.1	Target-104	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-105	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-106	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-107	2648-2670	AACCACTTTATTGCTTTGTCTTA

JN383828.1	Target-108	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-109	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-110	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-111	2648-2670	AACCACTTTATTGCTTTGTCTTA
FJ173811.1	Target-112	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-113	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-114	2198-2220	TTGGTTTAAAGGGCCTATTAACA
	Target-115	2648-2670	AACCACTTTATTGCTTTGTCTTA
JN383827.1	Target-116	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-117	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-118	2199-2221	TTGGTTTAAAGGGCCTATTAACA
	Target-119	2649-2671	AACCACTTTATTGCTTTGTCTTA
FJ173810.1	Target-120	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-121	1564-1586	AGCATAGAGTATCTGCTATTAAG
FJ173809.1	Target-122	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-123	1564-1586	AGCATAGAGTATCTGCTATTAAG
FJ173806.1	Target-124	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-125	1564-1586	AGCATAGAGTATCTGCTATTAAG
	Target-126	2007-2029	TTGGTTTAAAGGGCCTATTAACA
	Target-127	2457-2479	AACCACTTTATTGCTTTGTCTTA
JN383830.1	Target-128	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-129	1491-1513	AGCATAGAGTATCTGCTATTAAG
	Target-130	2575-2597	AACCACTTTATTGCTTTGTCTTA
JN383835.1	Target-131	253-275	TGGGAAGAATATGGAACTTTAAA
	Target-132	1497-1519	AGCATAGAGTATCTGCTATTAAG
	Target-133	2131-2153	TTGGTTTAAAGGGCCTATTAACA
	Target-134	2581-2603	AACCACTTTATTGCTTTGTCTTA
FJ173808.1	Target-135	253-275	TGGGAAGAATATGGAACTTTAAA

Table 3: Four effective siRNA molecule with GC%, free energy of folding and free energy of binding with target

Target	Location of target within mRNA	siRNA target within in consensus target	Predicted siRNA duplex	GC%	Free energy of folding of siRNA candidate at 37^{0}C	Free energy of Binding with target
Consensus Target-1	253-275	TGGGAAGAATATG GAACTTTAAA	UAAAGUUCCAUAU UCUUCCCA GGAAGAAUAUGGA ACUUUAAA	53.8%	0.9	25.1
Consensus Target-2	1564-1586	AGCATAGAGTATC TGCTATTAAG	UAAUAGCAGAUAC UCUAUGCU CAUAGAGUAUCUG CUAUUAAG	50%	0.0	23.3
Consensus Target-3	2198-2220	TTGGTTTAAAGGG CCTATTAACA	UUAAUAGGCCCUU UAAACCAA GGUUUAAAGGGCC UAUUAACA	46.7%	1.0	23.1
Consensus Target-4	2648-2670	AACCACTTTATTGC TTTGTCTTA	AGACAAAGCAAUA AAGUGGUU CCACUUUAUUGCUU UGUCUUA	41.2%	0.6	26.2