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SiDESIGN CENTER SOFTWARE FOR DESIGNING EFFICIENT siRNAs

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ABSTRACT: Small interfering RNA (siRNA) has verified to be a robust tool for target-specific gene silencing via RNA interference (RNAi). Its ability to regulate targeted organic phenomenon provides new hope to gene therapy as a treatment for cancers and genetic diseases [1]. The siRNAs designed using Reynolds rules were very efficient in producing RNA interference. They developed eight criteria to evaluate the siRNA test panel. To minimize potential off-target silencing effects, only sequences with more than mismatches against un-related sequences were selected. A web-based design center was developed to facilitate the designing process of siRNA. The program 'siRNA target filter' automates homology filtering, minimizes non-specific cross-reactions and filters target sites based on internal stability (of siRNA duplexes) and secondary structure. This programme also takes into account single nucleotide polymorphism (the regions of the target sequence showing single nucleotide polymorphism should be avoided). Online tools for designing siRNAs provides useful guidelines which are experimentally analyzed by others. This guidelines are helpful for selection of target siRNAs.

KEYWORDS: siRNA, hTERT, RNAi, Reynolds rules.

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1.INTRODUCTION

Ribonucleic acid interference (RNAi) a fast methodology for sequence-specific post-transcriptional gene silencing mechanism, that is triggered by double-stranded ribonucleic acid (dsRNA), ends up

in the degradation of mRNAs homologous in sequence to the dsRNA. Thereby, inhibiting the particular organic phenomenon with success [2]. Similarly, small Interference RNAs (siRNAs) could be a valuable tool for suppressing the expression of specific gene exhibit a high degree of specificity and have necessary medical implication like selective gene repression in cancer [3]. The role of RNAi against cancer therapy is to knock out the expression of a cell cycle gene associate degreed/or an anti-apoptotic gene gift within the cancer cells thereby inhibiting the tumor growth and killing the cancer cells. Therefore, RNAi by eliminates the cancer cells while not damaging traditional cells. The RNAi would be targeted to a selected gene that is concerned with the growth or survival of the neoplastic cell. Designing of siRNA sequences for a target gene is that the initial important step in its therapeutic applications. Bearing in mind the designing siRNA should be conducted correctly to attain potent silencing result whereas minimizing off-target effects in complexities of the RNAi mechanism. siRNAs have become a core interest of many biological research laboratories in the last decade. Several efforts have been made to rationalize siRNA design, starting with Tuschl principles [4], Reynolds [5], Amarzguioui [6], Takasaki [7], Katoh [8], Ui-Tei [9], and Hsieh [10] who developed some of the first-generation position dependant tools for siRNA design that had a relatively low correlation to actual siRNA activity [11].The second aspect is target accessibility and thermodynamic features of both siRNAs and targeted mRNAs, for which several studies have been performed to investigate thermodynamic features affecting siRNA functionality. These features include thermodynamic differential end instability as a key feature reported in different studies [12,13], unstructured guide strands (unstable siRNA secondary structure) [14], and high probability of siRNA terminal-ends to single-stranded (unpaired) nucleotides [15,16]. All of these affect siRNA and mRNA binding, and are correlated with their silencing efficiency [14,17]. So far, there are so many academic institution and commercial siRNA suppliers to provide various online siRNA design tools. However, there are not any universally accepted rules or computer code to style siRNAs. Each computer code uses various style algorithms and also the optimum sequence has to be determined by experimentation, by comparison, the silencing result of various candidates [3].

Selection of functional siRNA sequences

Cells transfected with an extremely effective siRNA, RISC with the antisense strand might predominate. In 2004, practical guidelines for choosing extremely effective siRNA sequences for class RNAi are projected. Based on the rules the extremely effective RNAi happens in mammalian cells and chick embryos by siRNA that satisfies the subsequent four conditions at constant time: (i) A/U at the 5’ end of the antisense strand, (ii) G/C at the 5’ finish of the sense strand, (iii) AU-richness within the 5’ terminal third of the antisense strand and (iv) the absence of any Gc rate stretch over nine bp long (Figure 1). These guidelines were almost like the molecular mechanism of RISC assembly as already expressed. The first 3 guidelines might guarantee that the 5' end of the antisense

strand of siRNA is located at or close to the thermodynamically less stable siRNA duplex end [18].

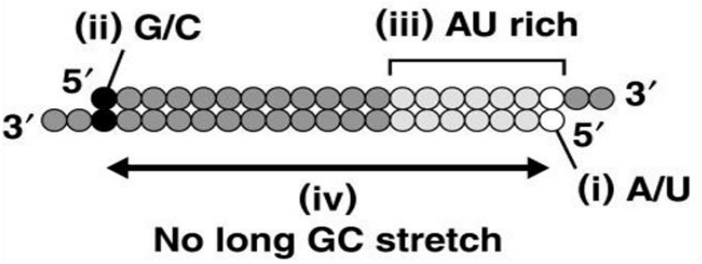


Figure 1: Structure of extremely effective siRNA

Selection of siRNA sequence with reduced off-target results

To avoid siRNA primarily based seed-dependent off-target effect is to pick out the siRNA guide strand whose seed sequence isn't complementary to any sequences within the 3' UTR of all non-targeted genes [4]. Therefore, the potency of an off-target result is extremely related to the physical stability of the duplex shaped between the seed region of the siRNA guide strand and it's target mRNA [19]. The Tm of 21.5˚C may serve as the standard, that differentiates the just about off-target-free seed sequences from the off-target-positive ones. Furthermore, the off-target result may be caused not only by the guide strand but also by the passenger strand, siRNAs whose seed-target Tm is sufficiently low for both strands are favorable.

Elimination of near-perfect matched off-target genes

Even though the Tm value of the seed-target duplex is sufficiently low, still the target gene silencing can take place if the non-seed region is completely complementary. Therefore, siRNAs that have similarity to any other non-targeted transcripts were eliminated. Previous and up to date studies have tried to search out a correlation between the secondary structure of sequences and their efficiency on siRNA activity. The previous report showed that there was no correlation between the M-Fold expected secondary structures and siRNA potency [20]. Though, some recent studies showed that such a correlation might exist. Holen *et al.* (2002). Furthermore, it absolutely was reportable that the energy needed for gap the siRNA duplex and RNA ought to be lesser than the interbreeding energy between siRNA and also the RNA. Recent proof proved the correlation between siRNA inhibition potency and siRNA-mRNA energy [21]. Moreover, the off-target filtration, as single siRNA can be targeting many RNA targets by either sense or antisense [22]. ‘‘Preferably, the siRNA might not cause any effects aside from those associated with the knockdown of the target gene" [23]. The accuracy of automated siRNA design tools in exceedingly realistic experimental surroundings, we tend to center on the Dharmacon online siRNA Target Finder and consistently investigated however well it behaves in ‘‘real-life" by measurement mRNA knockdown in an exceedingly standardized cell-based assay. We assessed the silencing efficiency of 21-nt siRNA duplex sequences directed against 2 human enzyme polymerase (hTERT) genes. we tend to conjointly quantify the prophetical power of the siRNA style formula by Dharmacon online siRNA Target

Finder. It helps to any investigate the factors that doubtless improve the siRNA targets [24].

2. MATERIALS AND METHODS

Data collection and analysis

4,015 genome isolate sequences of hTERT were retrieved from the GenBank database: AF015950.1, available at http://www.ncbi.nlm.nih.gov/. The database contains all experimentally identified widespread genome isolates of hTERT which were further used for siRNA designing.

Target identification and rational siRNA molecule designing

Dharmacon siRNA Design Center tool was used for target identification and designing of potential siRNA molecules. It utilized the rule approach of Reynolds rules [25] and melting temperature (Tm) below 21.5°C for siRNA duplex, as the parameter. Besides these other parameters were taken on the concept of algorithms given in Table 1.

Similarity search

Blast tool (http://www.ncbi.nlm.nih.gov/blast) [26] was used To assure that the siRNAs were specific for the relevant genome region; we performed a BLAST analysis against homo sapiens database by applying expected threshold value 20 and BLOSUM 62 matrix as a parameter. Prediction of the genome's secondary structure was used to demonstrate that the predicted siRNAs were complementary to the target site in the genome. The target sites having a 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 were computed through Mfold server http://mfold.rna.albany.edu/?q=mfold/download-mfold.

Thermodynamics calculation of RNA-RNA interaction

RNA up program (www.tbi.univie.ac.at/~ulim/RNAup) at Vienna web suit [27] was used to study the thermodynamics of interactions between a target gene and predicted siRNA molecules. It works on an extension of the standard partition.

3. RESULTS AND DISCUSSION

siRNA design

The result of silencing of hTERT sequence at the transcriptional level on the neoplastic cell proliferation was studied by RNAi technology. The crucial factors in estimating the target sequence knockdown are the sequence and position of specific nucleotides within the siRNA molecules (Reynolds *et al*., 2004). The predicted sequences of the human hTERT sequence were retrieved from NCBI (National Centre for Biotechnology Information) and also the sequences were fed in Dharmacon online siRNA Target Finder tool to come up with all the doable 21 base sequences. The parameters within the tool were any refined to match the properties of effective siRNA as ascertained by others. The obtained sequences were scored supported the factors of rational siRNA style

(Table.1). The remaining sequences were subjected to similarity search victimization BLAST to confirm target specificity and ward-off any doable off-target effects. The selected sequences are noncommissioned.

Table 1. Criteria for rational siRNA design

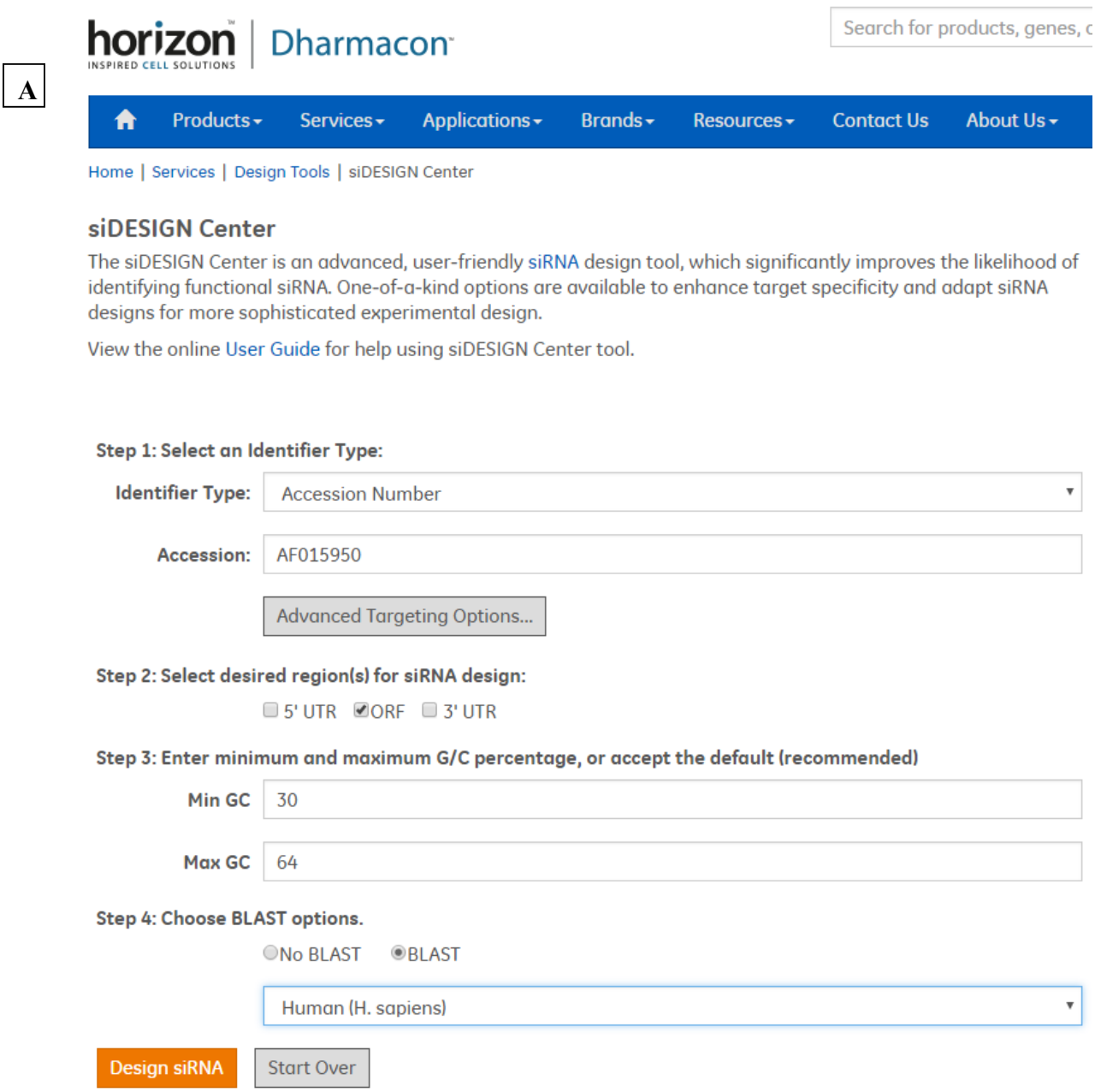
S.No.	Description	Score
1.	GC Content (30-52%)	1 point
2.	No internal repeats	1 point
3.	An at position 19	1 point
4.	U at position 10	1 point
5.	An at position 3	1 point
6.	G/C position at 19	- 1 point
7.	G at position 13	- 1 point
8.	A/U at position 15-19	1 point each

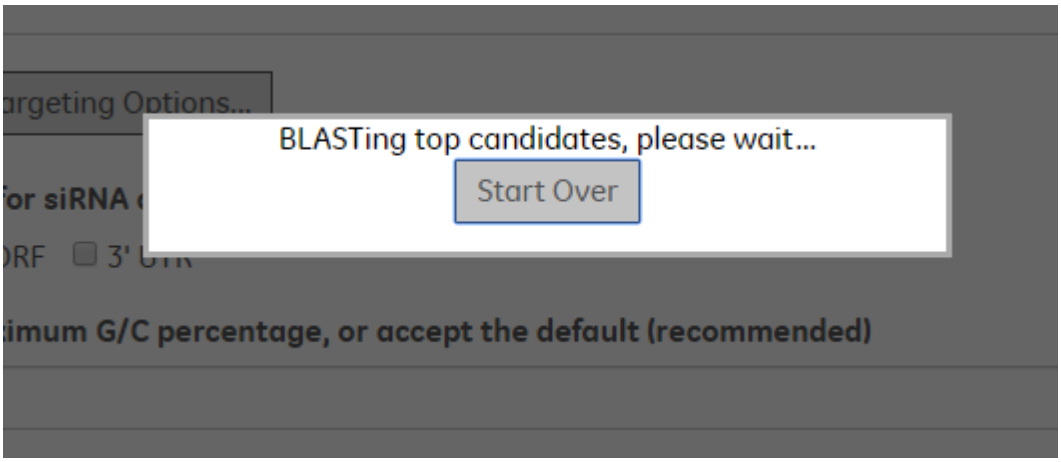
Table 2: Sequence of siRNA utilized in the study

S.No.	TARGET	SENCE SEQUENCES
1.	hTERT- 1	5'-GGAGCAAGUUGCAAAGCAUUG-3
2.	hTERT- 2	5' GGAACACCAAGAAGUUCAUCU-3
3.	hTERT- 3	SC-36641(SANTA CRUZE BIOTECHNOLOGY, ING Europe)

The most goal of the current study was to assess the effectualness of siRNA sequences designed by Dharmacon online siRNA Target Finder. We tend to conferred Dharmacon online siRNA Target Finder, which provides practical, target-specific siRNA style software system (siDESIGN center).The siRNA target sequence that is the chemical process monetary unit of the telomerase. Telomeres are maintained by a telomerase enzyme, a multi-subunit protein comprised of associate degree RNA part, that acts as a model for creating telomeric repeats and a protein part, hTERT, that catalyzes the synthesis of hexameric repeats TTAGGG (7). These genes are expressed naturally in all human cancer cells. The capability of 3 freelance siRNAs in Table.2 that downregulates the hTERT mRNA expression within the HeLa cell line was analyzed. In our previous study, one of the economical hTERTsiRNA among 3 was selected from the results of RT-PCR. The knockdown potency of hTERT siRNA-2 is larger than hTERTsiRNA-1 and hTERT siRNA-3. Hence, hTERT siRNA-2 with the cy3 tag was selected for the nanoencapsulation, characterization and for the silencing of hTERTmRNA.

A





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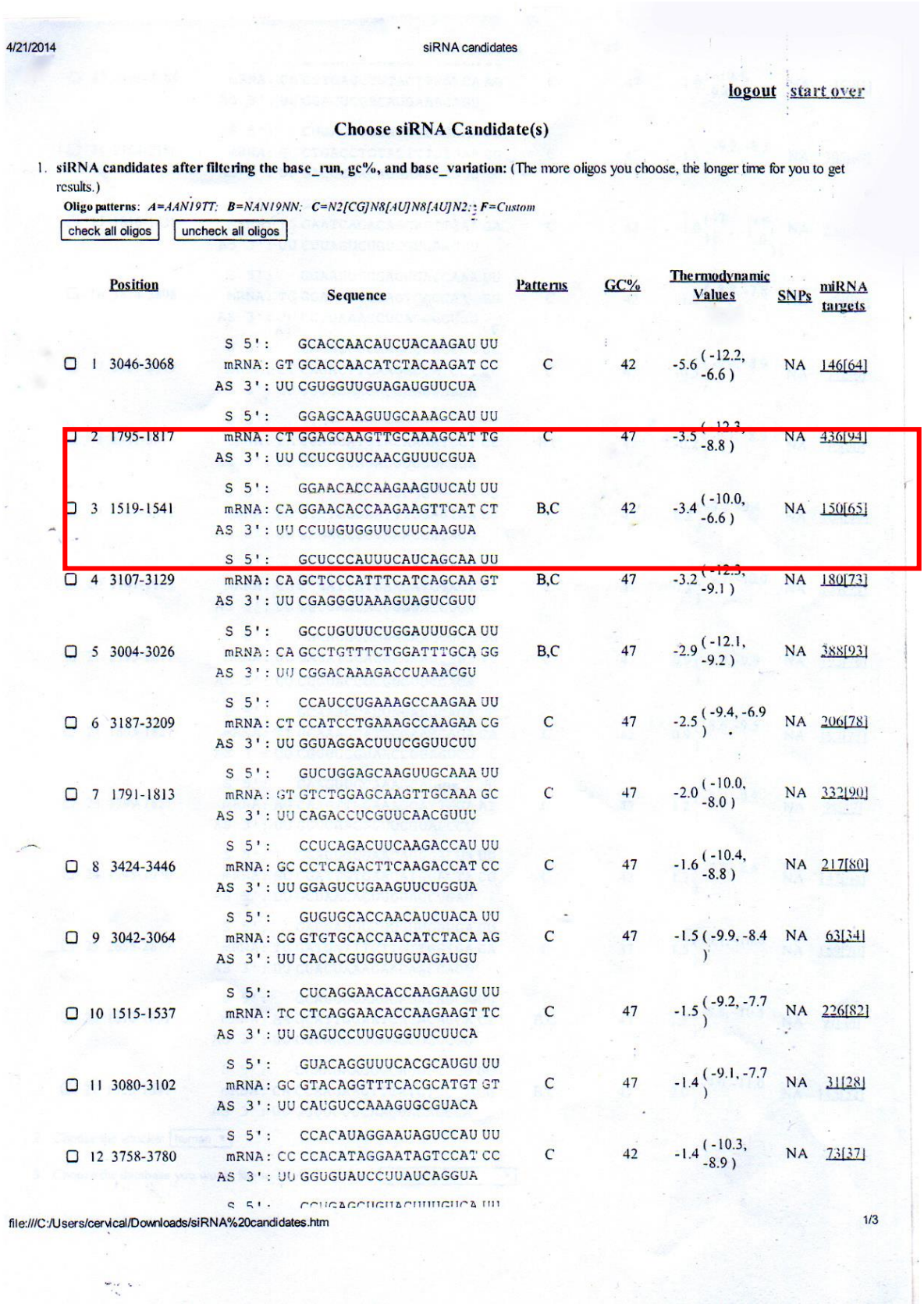

4/21/2014 siRNA candidates

logout start over

Choose siRNA Candidate(s)

1. **siRNA candidates after filtering the base_run, gc%, and base_variation:** (The more oligos you choose, the longer time for you to get results.)

Oligo patterns: *A=AAN19TT; B=NAN19NN; C=N2[CG]N8[AU]N8[AU]N2; F=Custom*

check all oligos | uncheck all oligos

	Position	Sequence	Patterns	GC%	Thermodynamic Values	SNPs	miRNA targets
☐ 1	3046-3068	S 5': GCACCAACAUCUACAAGAU UU mRNA: GT GCACCAACATCTACAAGAT CC AS 3': UU CGUGGUUGUAGAUGUUCUA	C	42	-5.6 (-12.2, -6.6)	NA	146[64]
☐ 2	1795-1817	S 5': GGAGCAAGUUGCAAAGCAU UU mRNA: CT GGAGCAAGTTGCAAAGCAT TG AS 3': UU CCUCGUUCAACGUUUCGUA	C	47	-3.5 (-12.3, -8.8)	NA	436[94]
☐ 3	1519-1541	S 5': GGAACACCAAGAAGUUCAU UU mRNA: CA GGAACACCAAGAAGTTCAT CT AS 3': UU CCUUGUGGUUCUUCAAGUA	B,C	42	-3.4 (-10.0, -6.6)	NA	150[65]
☐ 4	3107-3129	S 5': GCUCCCAUUUCAUCAGCAA UU mRNA: CA GCTCCCATTTCATCAGCAA GT AS 3': UU CGAGGGUAAAGUAGUCGUU	B,C	47	-3.2 (-12.3, -9.1)	NA	180[73]
☐ 5	3004-3026	S 5': GCCUGUUUCUGGAUUUGCA UU mRNA: CA GCCTGTTTCTGGATTTGCA GG AS 3': UU CGGACAAAGACCUAAACGU	B,C	47	-2.9 (-12.1, -9.2)	NA	388[93]
☐ 6	3187-3209	S 5': CCAUCCUGAAAGCCAAGAA UU mRNA: CT CCATCCTGAAAGCCAAGAA CG AS 3': UU GGUAGGACUUUCGGUUCUU	C	47	-2.5 (-9.4, -6.9)	NA	206[78]
☐ 7	1791-1813	S 5': GUCUGGAGCAAGUUGCAAA UU mRNA: GT GTCTGGAGCAAGTTGCAAA GC AS 3': UU CAGACCUCGUUCAACGUUU	C	47	-2.0 (-10.0, -8.0)	NA	332[90]
☐ 8	3424-3446	S 5': CCUCAGACUUCAAGACCAU UU mRNA: GC CCTCAGACTTCAAGACCAT CC AS 3': UU GGAGUCUGAAGUUCUGGUA	C	47	-1.6 (-10.4, -8.8)	NA	217[80]
☐ 9	3042-3064	S 5': GUGUGCACCAACAUCUACA UU mRNA: CG GTGTGCACCAACATCTACA AG AS 3': UU CACACGUGGUUGUAGAUGU	C	47	-1.5 (-9.9, -8.4)	NA	63[34]
☐ 10	1515-1537	S 5': CUCAGGAACACCAAGAAGU UU mRNA: TC CTCAGGAACACCAAGAAGT TC AS 3': UU GAGUCCUUGUGGUUCUUCA	C	47	-1.5 (-9.2, -7.7)	NA	226[82]
☐ 11	3080-3102	S 5': GUACAGGUUUCACGCAUGU UU mRNA: GC GTACAGGTTTCACGCATGT GT AS 3': UU CAUGUCCAAAGUGCGUACA	C	47	-1.4 (-9.1, -7.7)	NA	31[28]
☐ 12	3758-3780	S 5': CCACAUAGGAAUAGUCCAU UU mRNA: CC CCACATAGGAATAGTCCAT CC AS 3': UU GGUGUAUCCUUAUCAGGUA	C	42	-1.4 (-10.3, -8.9)	NA	73[37]

S 5': CCUGAGCUGUACUUUGUCA UU

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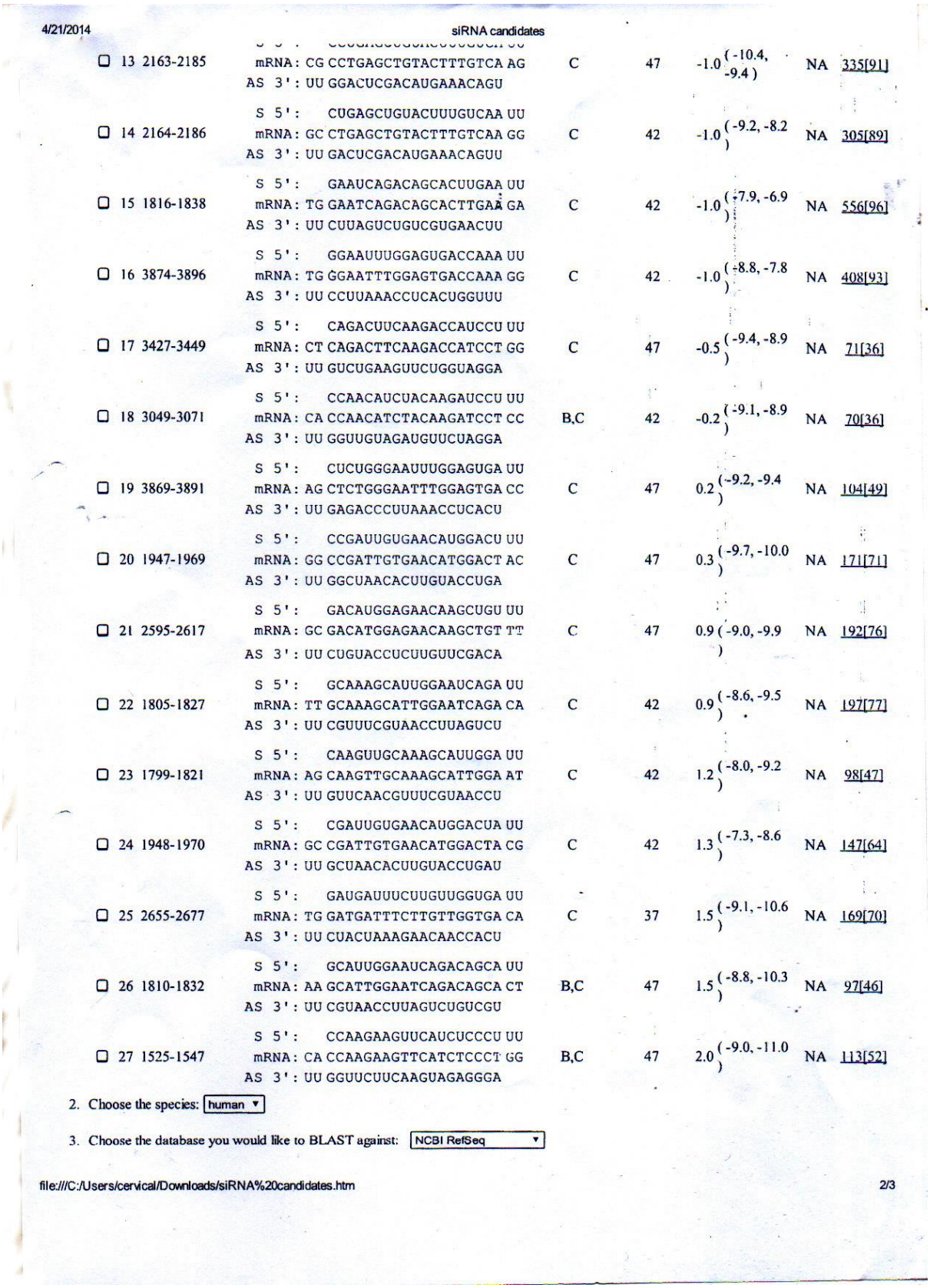

4/21/2014 siRNA candidates

☐ 13 2163-2185	S 5': CUGAGCUGUACUUUGUCAA UU mRNA: CG CCTGAGCTGTACTTTGTCA AG AS 3': UU GGACUCGACAUGAAACAGU	C	47	-1.0	(-10.4, -9.4)	NA	335[91]
☐ 14 2164-2186	S 5': CUGAGCUGUACUUUGUCAA UU mRNA: GC CTGAGCTGTACTTTGTCAA GG AS 3': UU GACUCGACAUGAAACAGUU	C	42	-1.0	(-9.2, -8.2)	NA	305[89]
☐ 15 1816-1838	S 5': GAAUCAGACAGCACUUGAA UU mRNA: TG GAATCAGACAGCACTTGAA GA AS 3': UU CUUAGUCUGUCGUGAACUU	C	42	-1.0	(-7.9, -6.9)	NA	556[96]
☐ 16 3874-3896	S 5': GGAAUUUGGAGUGACCAAA UU mRNA: TG GGAATTTGGAGTGACCAAA GG AS 3': UU CCUUAAACCUCACUGGUUU	C	42	-1.0	(-8.8, -7.8)	NA	408[93]
☐ 17 3427-3449	S 5': CAGACUUCAAGACCAUCCU UU mRNA: CT CAGACTTCAAGACCATCCT GG AS 3': UU GUCUGAAGUUCUGGUAGGA	C	47	-0.5	(-9.4, -8.9)	NA	71[36]
☐ 18 3049-3071	S 5': CCAACAUCUACAAGAUCCU UU mRNA: CA CCAACATCTACAAGATCCT CC AS 3': UU GGUUGUAGAUGUUCUAGGA	B,C	42	-0.2	(-9.1, -8.9)	NA	70[36]
☐ 19 3869-3891	S 5': CUCUGGGAAUUUGGAGUGA UU mRNA: AG CTCTGGGAATTTGGAGTGA CC AS 3': UU GAGACCCUUAAACCUCACU	C	47	0.2	(-9.2, -9.4)	NA	104[49]
☐ 20 1947-1969	S 5': CCGAUUGUGAACAUGGACU UU mRNA: GG CCGATTGTGAACATGGACT AC AS 3': UU GGCUAACACUUGUACCUGA	C	47	0.3	(-9.7, -10.0)	NA	171[71]
☐ 21 2595-2617	S 5': GACAUGGAGAACAAGCUGU UU mRNA: GC GACATGGAGAACAAGCTGT TT AS 3': UU CUGUACCUCUUGUUCGACA	C	47	0.9	(-9.0, -9.9)	NA	192[76]
☐ 22 1805-1827	S 5': GCAAAGCAUUGGAAUCAGA UU mRNA: TT GCAAAGCATTGGAATCAGA CA AS 3': UU CGUUUCGUAACCUUAGUCU	C	42	0.9	(-8.6, -9.5)	NA	197[77]
☐ 23 1799-1821	S 5': CAAGUUGCAAAGCAUUGGA UU mRNA: AG CAAGTTGCAAAGCATTGGA AT AS 3': UU GUUCAACGUUUCGUAACCU	C	42	1.2	(-8.0, -9.2)	NA	98[47]
☐ 24 1948-1970	S 5': CGAUUGUGAACAUGGACUA UU mRNA: GC CGATTGTGAACATGGACTA CG AS 3': UU GCUAACACUUGUACCUGAU	C	42	1.3	(-7.3, -8.6)	NA	147[64]
☐ 25 2655-2677	S 5': GAUGAUUUCUUGUUGGUGA UU mRNA: TG GATGATTTCTTGTTGGTGA CA AS 3': UU CUACUAAAGAACAACCACU	C	37	1.5	(-9.1, -10.6)	NA	169[70]
☐ 26 1810-1832	S 5': GCAUUGGAAUCAGACAGCA UU mRNA: AA GCATTGGAATCAGACAGCA CT AS 3': UU CGUAACCUUAGUCUGUCGU	B,C	47	1.5	(-8.8, -10.3)	NA	97[46]
☐ 27 1525-1547	S 5': CCAAGAAGUUCAUCUCCCU UU mRNA: CA CCAAGAAGTTCATCTCCCT GG AS 3': UU GGUUCUUCAAGUAGAGGGA	B,C	47	2.0	(-9.0, -11.0)	NA	113[52]

2. Choose the species: human

3. Choose the database you would like to BLAST against: NCBI RefSeq

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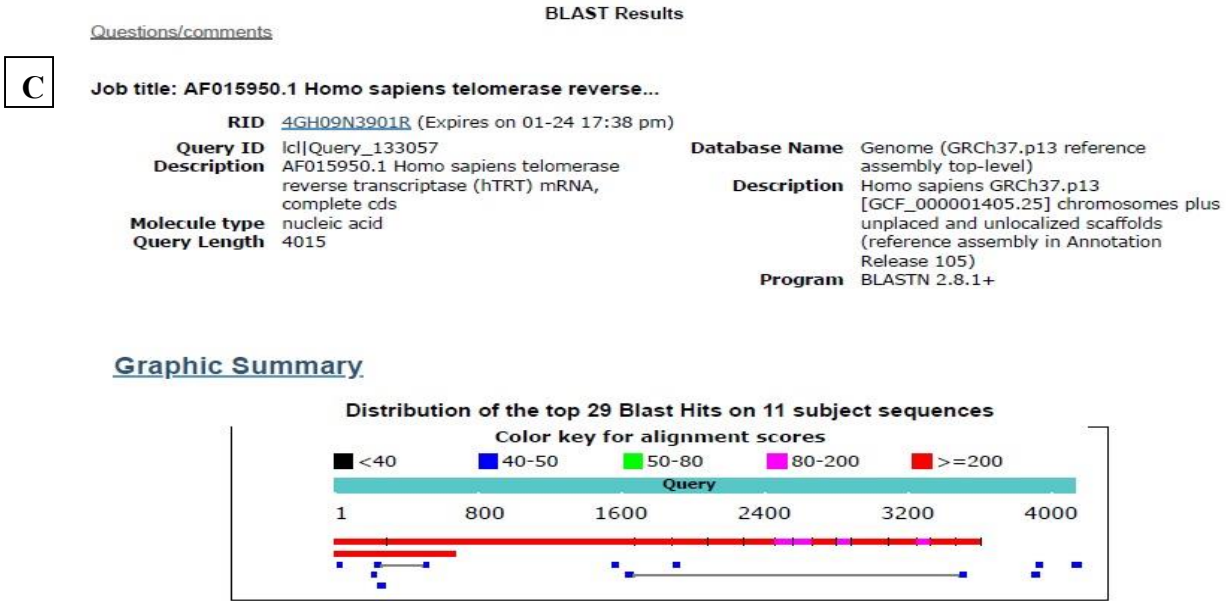

Figure 2: Screen views of the siDESIGN center software (A)Input Gene ID or gene symbol, specify the organism and a gene target (B) Effective siRNA candidates (C) Graphical view of BLAST result.

DISCUSSION

The experimental validation is, however, to be overcome for siRNA planning ways facilitating the choice of practical siRNA despite significant and regular progress. victimization heterogeneous datasets, many machine models of siRNA styles were developed, that makes it tough to assess however these prophetical models perform in real-life. Estimating every siRNA for his or her precise level of sequence silencing is high-cost and long. Therefore, these assays got to be finely tuned for fresh targeted genes, reporter-based assays are developed to hurry up the identification of the foremost potent siRNA sequences [28,29]. These results ought to be by experimentation verified that reporter-based activity correlates well with the effectualness of endogenous target depletion [30]. In this study, Dharmacon online siRNA Target Finder is employed to style 21-nt siRNA sequences, and so evaluated the effectiveness of those siRNA sequences in an exceedingly cell-based assay victimization HeLa cells. we tend to center on human enzyme polymerase (hTERT) sequence, that is expressed at low levels was chosen, that has reportable that low abundance sequence product is less amenable to siRNA-mediated knockdown. Additionally, a number of these genes might code for enzymes, knockdown should be terribly economical to yield a transparent makeup. However, these are contrasted to structural protein targets, that even slight knockdown may result in an exceedingly constitution result. To avoid bias because of measurement systems, the potency of siRNA knockdown was consistently measured on endogenously expressed genes employing a standardized validation procedure. From the study, our information reflects that natural characteristics like structure and localization of a target mRNA within the cellular surroundings [31]. The experiments incontestable here used ten-fold less siRNA and achieved an analogous decrease

in hTERT expression with the 3 siRNA designed by Dharmacon online siRNA Target Finder software system. This result suggests that victimization Dharmacon online siRNA Target Finder software system may stop OTE (off target effect). Since these entire target genes are expressed at low levels, these results counsel that this property alone isn't associate degree indicator of prosperous silencing potential was antecedently ascertained. Moreover, if siRNA sequences with well-known negative criteria like targeting the 5'UTR region of the target mRNA are discarded. The success rate for siRNA designed with Dharmacon online siRNA Target Finder reaches a really satisfactory level for a minimum of 3 of our target genes (hTERT siRNA-1, hTERTsiRNA-2 and hTERT siRNA-3). The foremost acceptable sequence silencing was achieved with hTERTsiRNA-2, that all siRNA expeditiously knockdown expression. Meister and Rossire reportable that the planning of 5 siRNA sequences per target might facilitate to urge a minimum of one economical siRNA [17].Supported the results conferred here, this limit is often reduced. this can permit synthesis of a smaller range of reagents targeting most transcripts. However, this should be qualified by the success rate ascertained for hTERTsiRNA-2. Results for these genes counsel that vital options that are concerned in siRNA-mediated silencing got to be known. This study provides a very new dataset combining the siRNA sequences, and their foretold and by experimentation measured efficiencies. This dataset constitutes a strong tool to spot, analyze and validate potential options contributory to siRNA potency. Since the Dharmacon online siRNA Target Finder formula was designed supported analysis of intrinsic guide-strand sequence determinants on the siRNA, we tend to any checked all determinants and descriptors that are shown or are suspected to be concerned within the RNAi pathway.

4. CONCLUSION

During this study, we tend to by experimentation designed hTERT siRNA victimization Dharmacon online siRNA Target Finder software system by measurement of actual knockdown efficiencies for siRNA with high foretold efficiencies. Dhamacon online siRNA Target Finder estimates within the analysis agree well with experimental silencing, confirming that Dhamacon online siRNA Target Finder is one of the most effective predictors of active siRNA. Moreover, Dhamacon online siRNA Target Finder was conjointly verified to dependably predict shRNAs for an efficient knockdown in transgenic flies. Besides, Dhamacon online siRNA Target Finder siRNA potency has been extensively valid on long silenced human cells. The new dataset conferred here, containing over 100 qualified siRNA sequences, are useful for the community operating toward improved siRNA style that is in constant progress. This internet tool and also the list of valid siRNA directed against terribly relevant cancer-related targets are freely accessible. In our previews study, we tend to found that the knockdown potency of hTERT siRNA-2 is larger than hTERTsiRNA-1 and hTERT siRNA-3 using the RT-PCR. Hence, hTERT siRNA-2 with the cy3 tag was selected for the nanoencapsulation, characterization and for the silencing of hTERTmRNA.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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