VIRsiRNAdb: a curated database of experimentally validated viral siRNA/shRNA

Nishant Thakur, Abid Qureshi and Manoj Kumar*

Bioinformatics Centre, Institute of Microbial Technology, Council of Scientific and Industrial Research (CSIR), Sector 39-A, Chandigarh-160036, India

ABSTRACT

RNAi technology has been emerging as a potential modality to inhibit viruses during past decade. In literature a few siRNA databases have been reported that focus on targeting human and mammalian genes but experimentally validated viral siRNA databases are lacking. We have developed VIRsiRNAdb, a manually curated database having comprehensive details of 1358 siRNA/shRNA targeting viral genome regions. Further, wherever available, information regarding alternative efficacies of above 300 siRNAs derived from different assays has also been incorporated. Important fields included in the database are siRNA sequence, virus subtype, target genome region, cell type, target object, experimental assay, efficacy, off-target and siRNA matching with reference viral sequences. Database also provides the users with facilities of advance search, browsing, data submission, linking to external databases and useful siRNA analysis tools especially siTarAlign which align the siRNA with reference viral genomes or user defined sequences. VIRsiRNAdb contains extensive details of siRNA/ shRNA targeting 42 important human viruses including influenza virus, hepatitis B virus, HPV and SARS Corona virus. VIRsiRNAdb would prove useful for researchers in picking up the best viral siRNA for antiviral therapeutics development and also for developing better viral siRNA design tools. The database is freely available at http://crdd.osdd .net/servers/virsirnadb.

INTRODUCTION

Viral diseases remain one of the public health problems due to emerging and reemerging nature of viruses such as influenza, hepatitis, Human Immunodeficiency Virus (HIV), Human Papillomavirus (HPV) & Severe Acute Respiratory Syndrome (SARS) etc. [1]. Combating majority of these viruses is compromised due to lack of effective vaccines and antiviral drugs [2]. Besides, development of new vaccines and antiviral drugs, there are continuous efforts to search for alternative therapeutic interventions. Lately, RNA interference has emerged as a potential approach in the battle against pathogenic viruses [3,4] and other human diseases [5,6].

RNAi was first reported by Fire *et al.* [7] when authors showed a potent gene silencing effect after injecting double stranded RNA into *C. elegans*. In RNA silencing pathway, long dsRNA is processed by RNase III family member, dicer, to a 19–21 nucleotide long double stranded siRNA, with 2-nucleotide unphosphorylated 3′ overhangs [8]. The double stranded siRNA is composed of a guide (antisense) strand and a passenger (sense) strand. Unwinding of the siRNA duplex is catalyzed by argonaute. After the unwinding step, the guide strand is incorporated into the RNA Induced Silencing Complex (RISC), while the passenger strand is released. Using the antisense strand RISC targets, the complementary mRNA resulting in the cleavage of the latter [9].

Using RNA silencing mechanism, researchers have reported considerable decrease in the expression of targeted viral genes [10,11]. For example, siRNAs directed against the influenza virus nucleocapsid (NP) and RNA transcriptase (PA) genes inhibited its transcription and replication [12]. Similarly, siRNAs against the hepatitis B virus polyadenylation (PA), precore (PreC) and surface (S) regions inhibited the viral replication [13]. In another study, siRNAs synthesized to target the E, M and N genes of SARS-CoV effectively down regulated the target genes expression by over 80% in a dose-dependent manner [14]. Inhibition of virus replication for several human viruses using RNAi strategy has been reviewed [3,15,16].

RNAi approach offers several advantages for antiviral therapeutics development. It has ability to target all types

of viral genomes [ssDNA, dsDNA, RNA(+), RNA(−) and dsRNA] which makes this versatile mechanism to be harnessed as broad-spectrum antiviral therapy (4). Further, RNAi targets a short stretch of viral nucleic acids instead of a functional domain of a viral protein, therefore, even a small viral genome offers many potential targets (11). Even more, multiple antiviral siRNAs can be expressed simultaneously or pooled in a way similar to current drug combination anti-viral therapy of infected individuals to sustain prolonged effect (17,18).

In the past decade, a number of RNAi therapeutic programs with focus on cancer, metabolic diseases, respiratory disorders, retinal degeneration, dominantly inherited brain and skin diseases and infectious diseases have entered the clinical practice (6,19,20). Simultaneously, several RNAi based antiviral therapeutic projects have also reached at clinical trial stages (21), for example, RSV (Phase II) (22), HBV (Phase I) (23), HCV (Phase II) (24) and HIV (Phase I) (25). Ongoing clinical trials further emphasize the need for development of the viral RNAi resources.

There is no dedicated viral siRNA database, except HIVsirDB, an HIV specific siRNA database (26). However, there are a few other siRNA databases reported in literature like HuSiDa (27) and siRNAdb (28) which provide sequences of published functional siRNA targeting human genes while siRecords (29) focused on siRNA data of mammalian RNAi experiments and DSTHO (30) on human oncogenes. VIRsiRNAdb is an attempt to provide comprehensive details of the experimentally validated viral siRNA targeting the diverse genome regions of as many as 42 important human viruses at one platform to help researchers working in the field of siRNA based antiviral therapeutic development.

DATABASE CONTENT

Data acquisition

Exhaustive literature search was carried out to extract the relevant articles from PubMed. This was accomplished by searching queries having combination of two keywords: (i) terms most commonly used for gene silencing viz: RNA interference, RNAi, silencing, siRNA(s), shRNA(s), small interfering RNA(s), short interfering RNA(s), small hairpin RNA(s) and short hairpin RNA(s) and (ii) virus names including their common names, aliases & abbreviations (like Severe acute respiratory syndrome, SARS, Corona Virus, SARS-CoV). Full text search using the above two keywords combinations was performed for each of the human viruses individually. The search results are given in Supplementary Table S1. Around 4000 abstracts were screened so as to select the articles likely to contain relevant viral siRNA information. Reviews, general methodological and non-English articles were not considered. After initial screening, around 1000 remaining potential articles were examined in detail to retrieve the viral siRNA information. Articles of siRNAs targeting the host genome regions were excluded. Further, articles that did not have individual siRNA sequence or its experimental efficacies were also not included. After this extensive filtering, 221 research articles were shortlisted to collect the siRNA data. In our database, complete siRNA data of almost all human viruses reported in the literature have been included.

Database architecture

The database provides comprehensive information of experimentally validated viral siRNAs which includes: (i) siRNA sequence, (ii) family of virus, (iii) virus subtype, (iv) target gene, (v) siRNA location, (vi) GenBank accession, (vii) design algorithm, (viii) production method, (ix) siRNA concentration, (x) cell type, (xi) transfection method, (xii) incubation time, (xiii) PubMed ID, (xiv) object used (i.e. mRNA protein, virus load etc), (xv) efficacy, (xvi) efficacy assay (e.g. Western blot, PCR, plaque number, ELISA) and (xvii) references. Further, wherever available, extended information regarding alternative efficacy assays has also been provided. Architecture of the database is depicted in the Figure 1. Structure of each siRNA predicted by Mfold (31) was also displayed in the data. In addition, we have also provided information of viral siRNA off-targets in human and the siRNA sequence matching with the reference viral genome sequences.

Database statistics

VIRsiRNAdb database provides information of 1358 experimentally validated siRNAs pertaining to 42 important human viruses belonging to 19 different virus families and targeting as many as 150 different viral genome regions. For HBV, HCV, SARS and Coxsackievirus many genome regions were being targeted by siRNAs as given in Table 1. The database entries contain siRNA experiments based on 71 different cell lines but Huh-7, 293T, MDCK, HepG2.2.15 and HeLa cell lines were mostly used (Figure 2a). In the database, 45% of the total siRNAs were highly effective with >70% inhibition efficacy and 9% siRNA have >90% efficacy. siRNAs (23%) have moderate efficacy of 50–70% whereas 32% of siRNAs were less effective with efficacy rating <50% (Figure 2b).

One of the major hindrances in RNAi based therapeutics is the lack of siRNA specificity. Besides, directly affecting the expression of the desired genes, a siRNA may affect regulation of unintended transcripts which possess complementarity to the siRNA sequence. siRNA off-target effect was initially reported in 2003 (32) and later Amanda Birmingham *et al.* (33) reported that off-targeting is associated with the presence of one or more perfect 3′ untranslated region (UTR) matches with the hexamer or heptamer seed region (positions 2–7 or 2–8) of the antisense strand of the siRNA. Seed based siRNA off-target was experimentally demonstrated by others also (34,35). The impact of non-specific siRNA off-target effect in therapeutic application was further reviewed (36).

We have predicted the off-targets in human for all the siRNAs present in our database, using three algorithms: (i) BLAST (37), (ii) Seed Locator (33) and (iii) SpecificityServer (38). Result outputs of each algorithm
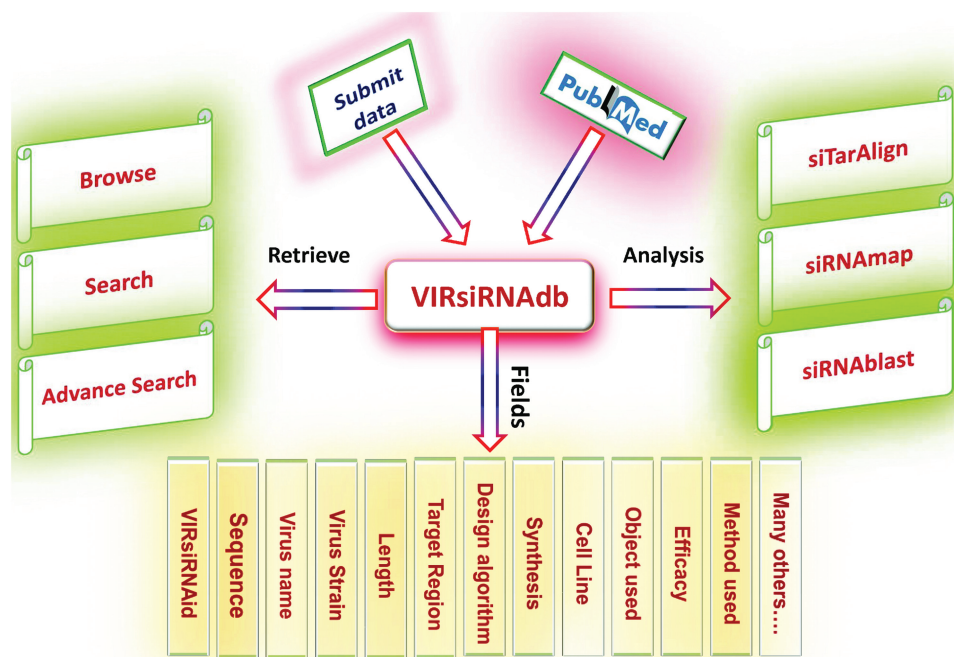
Figure 1. VIRsiRNAdb database architecture.

are given against respective siRNA record as link under off-target column. BLAST algorithm was commonly used to detect possible off-target effects of a siRNA by searching it against the human Unigene or transcriptome database (28). We have also used BLAST (37) with −e 1000; −q −4; −r 5 parameters and found that around 13% of siRNA having off-targets in the human genome. Seed Locator output include total genes with at least one seed match and multiple seed matches in the 3′-UTR. Finally, results of SpecificityServer which is designed to identify potential non-specific matches to siRNA showed that 113 siRNAs are not specific for both siRNA strands while 101 siRNA have off-targets for the sense strand and remaining does not have any off-target.

As we know that viruses exhibit greater genetic variability, therefore it is important to know that in how many viral genome sequences, siRNA sequence is matching. This analysis is helpful for users in selecting such siRNA which is having high matching with maximum reference viral strains. Significance of selection of conserved regions targeted by siRNA in HIV-1 has been discussed by Naito *et al.* (39,40). We have checked the siRNA sequence matching with the reference viral genome sequences available at NCBI. For this purpose, we have used ALIGN0 algorithm (41), which computes the alignment of two DNA sequences without penalizing for end-gaps. Pie chart result displayed the number of nucleotide differences or mismatches (0, 1, 2, 3, >3) between of each siRNA and respective viral reference genome sequences in the alignment. Cumulative results of all the siRNA showed that 2% of siRNAs were fully (100%) matching with respective viral genome sequences and 16% matched with 90–99% viral genomes while 61% were having <50% matching as shown in Figure 2c.

There are reports of escape mutants generated by the virus in the siRNA target site to overcome the effect of RNAi. These escape mutations in the target sequence decreases the potency of siRNA gene silencing (42). Wilson (43) observed maximum escape mutations at 12th and 18th residues for HCV NS5B while Konishi (44) reported appreciable mutation at the 15th residue for HCV NS5A gene. In another study, Jun (45) recorded changes in Coxsackie virus at positions 10 and 13. We have collected such 57 siRNA escape sequences having 52 substitutions; 2 deletions; 1 insertion and 2 substitution/deletion mutations. Position of these escape substitutions mutations among 57 escape sequences are shown in Figure 2d.

Tools

Viral siRNA database allows the users to take advantage of useful tools like siTarAlign, siRNAmap and siRNAblast. siTarAlign aligns the siRNA sequence with the respective virus/family reference genomes sequences using either BLAST (37) or Smith–Waterman algorithm from EMBOSS suite (46) The output shown below displays a list of flaviviruses and influenza A viruses targeted by respective siRNA (Figure 3). Viral/family reference genomes were taken from the NCBI viral genome resources as summarized in the Supplementary Table S2. In siTarAlign, user defined viral genome sequences can also be uploaded to align the siRNA sequence with user provided sequences also.

The 'siRNAmap' is a simple tool to display the perfectly matching siRNA available in our database to the user provided viral sequence. So, it helps the user to know that against the user provided viral sequence, how many siRNAs are available in VIRsiRNAdb. Additionally, the

Table 1. Number of siRNAs for 42 viruses and targeted genome regions

| Virus	Target gene	No. of siRNAs	Virus	Target gene	No. of siRNAs
BK polyomavirus	T-Ag(2)	2	Influenza A virus	M(33), NP(26), PB(19), PA(11), NS(6), C(1)	96
Chikungunya virus	E1(1), NSP3(1)	2	Influenza B virus	PB(33), NP(17), M(11), NS(10), PA(10)	81
Dengue virus [DENV]	E(6), NS5(6), 3'-UTR(5), 5'-UTR(2), C(1), NS3(1), PreM(1)	22	Japanese encephalitis virus [JE]	NS1(4), E(2)	6
Ebolavirus [EBOV]	ZNP(2), ZT(2), ZL(1)	5	John Cunningham virus [JCV]	T-Ag (1)	1
Encephalomyocarditis virus	EMCV-IRES(1)	1	Junín virus	Z(4)	4
Enterovirus [EV]	3D Pol(10), VP1(3), 2C(2), 3C pro(2), 5'NTR(2), 3'-UTR(1), MET-2C(1), VP2(1)	22	La Crosse virus	M(G2)(7), L(4), S(3)	14
Epstein–Barr virus [EBV]	EBNA1(25), LMP1(10), PR(4), BKRF3(2), LMP2A(1), Zta(1)	43	Lassa virus	GPC(1), L(1), NP(1), Z(1)	4
Hazara nairovirus	L(4), M(4), S(4)	12	Lymphocytic choriomeningitis virus	L(1), Z(1)	2
Hendra virus	N(2)	2	Marburg virus	VP30(2), NP(1)	3
Henipavirus	L(4), N(4)	8	Measles virus	N(16), L(8)	24
Hepatitis A virus [HAV]	2C(2), 3D(2), 3A(1), 3C(1)	6	Polio virus	Capsid(1), 5NC(1)	2
Hepatitis B virus [HBV]	S(60), X(48), C(25), P/S(24), P(17), C/P(11), ORF-C(3), ORF-S(3), P/X(3), NLS(2), PRE(2), preS/P(2), PA(1), PreS1(1), HBeAg(7)	227	Rabies	N(3)	3
Hepatitis C virus [HCV]	5'-UTR(38), 3'-UTR(26), NS5B(22), Core(19), E(11), NS3(8), E2(7), IRES (5'-UTR)(6), NS5A(3), NS4B(2), NAa(1), NS4A(1)	145	Reovirus	μNS(7), σNS(4), μ2(2)	13
Hepatitis delta virus [HDV]	Delta Ag(16)	16	Rotavirus	NSP5(2)	2
Hepatitis E virus [HEV]	ORF2(4), RdRp(4), Helicase(2), Replicase(2), 3CAE region(1)	13	SARS coronavirus	ORF9a, N-protein(31), ORF5, M-protein(23), ORF4, E-protein(22), ORF2, Spike(18), Replicase(16), RDRP(11), ORF1a(8), ORF1b(7), ORF3a(7), (6), 3A(3), NSP1(3), ORF7(3), 3'-UTR(2), 5'-UTR(1), Leader(1), TRS(1)	163
Herpes simplex virus [HSV]	U51(4), UL39(4), UL40(4), DNA polymer-ase(3), gD(3), UL29(3), UL5(3), Vp 16(3), gB(2), UL27(2), UL38(2), gE(1), K13(1), ORF75(1)	36	Semliki forest virus	Cold(7), Hot(7)	14
Human coxsackievirus [CV]	5'-UTR(29), 3D(25), 2A(6), VP1(6), 1B(5), 1D(5), 2C(5), 1C(4), 3C(3), Rev(3), 3'-UTR(2), RdRP(2), MET-2C(2), 5'NTR(2), 3A(1), AUG start Codon region(1), POL(1)	103	Sendai VIRUS	HN(5)	5
Human metapneumovirus	L(26), N(11), M(9), F(8), P(4)	58	St. Louis encephalitis [SLE]	E(1), C(2), NS5(2)	5
Human papillomavirus [HPV]	E6(42), E7(39), E6/E7(8)	89	Vaccinia	E3L(4)	4
Human respiratory syncytial virus [HRSV]	NS1(4), P(4), NS2(1)	9	West Nile virus [WNV]	E(18), NS5(17), Core(7), C(4), NS1(4), NS4B(4), PrM/M(3), 3'-UTR(2), NS3(2), CAP(1), NS2A(1), NS4A/B(1)	64
Human rhinovirus	3D(4), 2C3(3), 5-UTR(3), VP3(3), 3C(2), VP1(2), VP2(2), 2A(1), 3A(1), VP4(1)	22	Yellow fever virus	NS5(2), E(1), NS1(1)	4
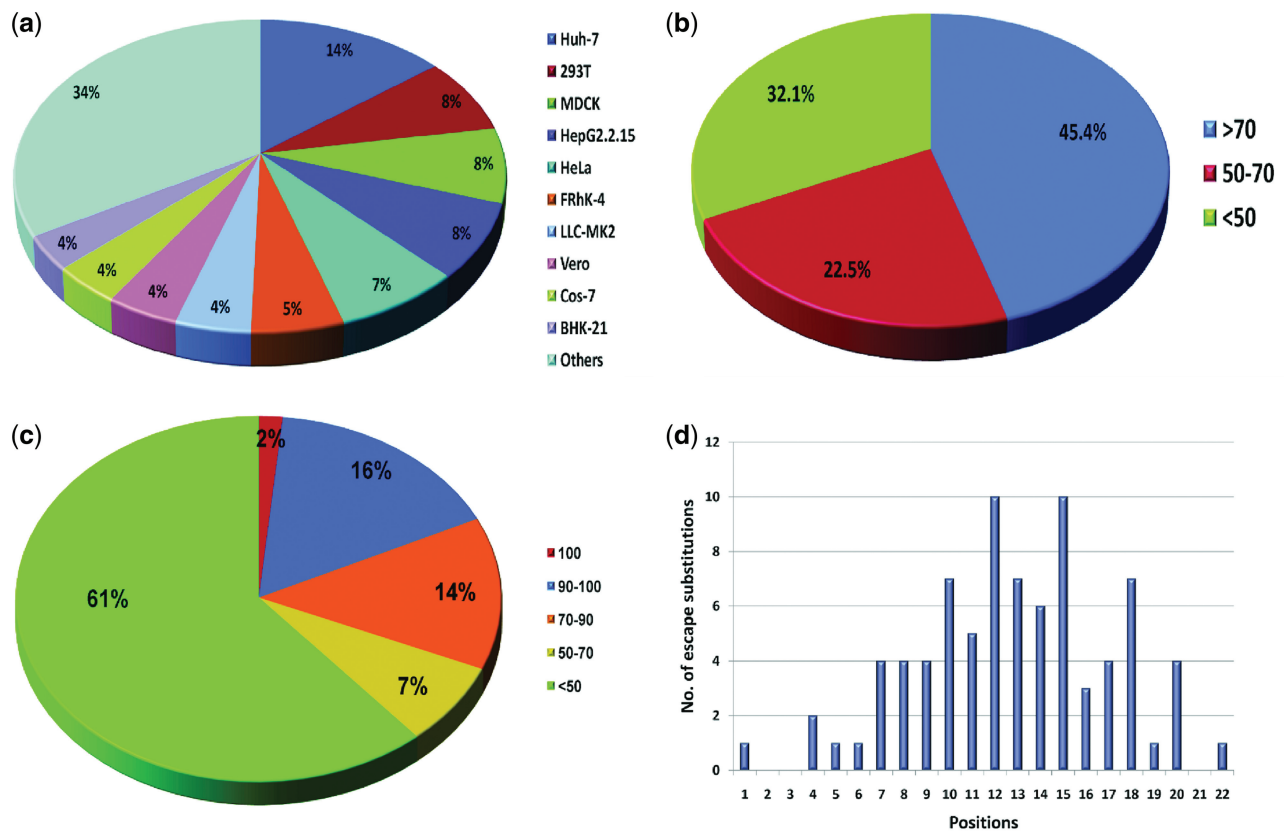
Figure 2. Database statistics (**a**) Cell line used (**b**) siRNA efficacy (**c**) siRNA sequence matching with reference viral genomes (**d**) Positions of the escape mutations.

siRNAblast allows alignment of a user provided siRNA sequence against all the siRNA sequences available in our database. This helps the user to confirm whether a given siRNA sequence or similar one has already been reported or not.

Data retrieval

It is possible to perform a quick search based on various database fields i.e. Virus name, siRNA sequence, target region, cell line and Pubmed ID. We have included a separate search option to retrieve siRNA with efficacy; greater than, equal to and lower than for a given value. Database also has qualitative efficacy of some siRNAs (where numerical values were not available) in three categories viz. 'High' (>70%), 'Medium' (50–70%) and 'Low' (<50%). The efficacy search will also fetch siRNAs with qualitative efficacies.

In the search output we have implemented the sorting and filtering functions. By clicking the heading of the given field, user can sort the displayed data. Simultaneously, by entering the desired keyword in the designated field, user can filter the siRNA data. Multiple filtering can be accomplished by entering desired keyword in different fields one after another. 'Advanced Search page' allows for more flexible queries using logical operators (AND, OR). These options enable the user to readily find appropriate siRNA data. External links pointing to the GenBank accession of the siRNA target sequence, Pubmed ID and International Committee on Taxonomy of Viruses (ICTV) are given for each siRNA record.

Data submission

Authors generating experimental viral siRNA data are encouraged to submit the data directly into viral siRNA database. For this purpose, a web form for data submission is provided. Submitted information will be included in the database update after ascertaining its authenticity.

Implementation

VIRsiRNAdb database is implemented on Red Hat Linux with MySQL (5.0.51b) and Apache (2.2.17) in back-end and front-end of web interface is implemented with PHP (5.2.14).

Future developments

As increasing number of articles are being published in the area of viral RNAi, therefore, in future our main priority would be to update the existing viral siRNA data as well as to include siRNA information for new viruses once appropriate data is available. We would also include virus specific siRNA design tool to further help the researchers.

(a)

Acc number	Strain name	Start	Alignment	End	% Identity
Query	Example	1	acagcatattgacacctgg	19	
NC_001437.1	Japanese encephalitis virus, genome	10862	. . . .	10880	100
NC_000943.1	Murray Valley encephalitis virus, complete genome	10900	. . . .	10918	100
NC_001563.2	West Nile virus (lineage II strain 956), complete genome	10854	. . . .	10872	100
NC_008718.1	Entebbe bat virus, complete genome	10473	. . . .	10491	100
NC_007580.2	St. Louis encephalitis virus, complete genome	10832	. . . .	10850	100
NC_009942.1	West Nile virus (lineage I strain NY99), complete genome	10921	. . . .	10939	100
NC_012533.1	Kedougou virus, complete genome	10613	. . . .	10631	100
NC_012534.1	Bagaza virus, complete genome	10833	. . . .	10851	100
NC_009026.2	Bussuquara virus, complete genome	10708	. . . .	10726	100
NC_006551.1	Usutu virus, complete genome	10951	. . . .	10969	100
NC_009029.2	Kokobera virus, complete genome	10770	. . . .	10788	100
NC_009028.2	Ilheus virus, complete genome	10647	. . . . g	10665	94
NC_010412.1	Simian enterovirus SV19, complete genome	3621	. . . c . . . g . . . . _	3604	84
NC_001477.1	Dengue virus type 1, complete genome	10629	. . . . g . tg . .	10647	84

(b)

Acc number	Strain name	Start	Alignment	End	% Identity
Query	virsi1480	1	gccgagatcgcacagagactt	21	
NC_007377.1	Influenza A virus (A/Korea/426/68(H2N2)) segment 7, complete sequence	89	. . . .	109	100
V01099.1	Influenza A virus (A/PR/8/1934(H1N1)) ORF1 and ORF2, genomic RNA	89	. . . .	109	100
NC_004907.1	Influenza A virus (A/Hong Kong/1073/99(H9N2)) segment 7, complete seq	96	. . . . g	116	95
NC_007367.1	Influenza A virus (A/New York/392/2004(H3N2)) segment 7, complete seq	89	. . . . g	109	95
NC_007363.1	Influenza A virus (A/Goose/Guangdong/1/96(H5N1)) strain A/Goose/Guang	89	. . . . g	109	95
NC_007376.1	Influenza A virus (A/Korea/426/68(H2N2)) segment 3, complete sequence	269	_____ . . . . _____	279	52
V01106.1	Influenza A virus (A/PR/8/1938(H1N1)) ORF1, genomic RNA	269	_____ . . . . _____	279	52

RED =100 % Complementary sequences ;   . = Identical residues ;   **Blue alphabets** = Mismatches ;   _ = Gaps

Figure 3. siTarAlign output screenshot showing the alignment of siRNA sequence with (**a**) family (**b**) virus reference genome sequences.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Tables 1 and 2.

FUNDING

Conflict of interest statement. None declared.

REFERENCES

1. Nichol,S.T., Arikawa,J. and Kawaoka,Y. (2000) Emerging viral diseases. *Proc. Natl Acad. Sci. USA*, **97**, 12411–12412.
2. Duffy,S., Shackelton,L.A. and Holmes,E.C. (2008) Rates of evolutionary change in viruses: patterns and determinants. *Nat. Rev. Genetics*, **9**, 267–276.
3. Arbuthnot,P. (2010) Harnessing RNA interference for the treatment of viral infections. *Drug News Perspect.*, **23**, 341–350.
4. Haasnoot,J., Westerhout,E.M. and Berkhout,B. (2007) RNA interference against viruses: strike and counterstrike. *Nat. Biotechnol.*, **25**, 1435–1443.
5. Angaji,S.A., Hedayati,S.S., Poor,R.H., Madani,S., Poor,S.S. and Panahi,S. (2010) Application of RNA interference in treating human diseases. *J. Genet.*, **89**, 527–537.
6. Davidson,B.L. and McCray,P.B. Jr (2011) Current prospects for RNA interference-based therapies. *Nat. Rev. Genet.*, **12**, 329–340.
7. Fire,A., Xu,S., Montgomery,M.K., Kostas,S.A., Driver,S.E. and Mello,C.C. (1998) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature*, **391**, 806–811.
8. Woessmann,W., Damm-Welk,C., Fuchs,U. and Borkhardt,A. (2003) RNA interference: new mechanisms for targeted treatment? *Rev. Clin. Exp. Hematol.*, **7**, 270–291.
9. Filipowicz,W. (2005) RNAi: the nuts and bolts of the RISC machine. *Cell*, **122**, 17–20.
10. Haasnoot,J. and Berkhout,B. (2009) Nucleic acids-based therapeutics in the battle against pathogenic viruses. *Handb. Exp. Pharmacol.*, **189**, 243–263.
11. Leonard,J.N. and Schaffer,D.V. (2006) Antiviral RNAi therapy: emerging approaches for hitting a moving target. *Gene Ther.*, **13**, 532–540.
12. Ge,Q., McManus,M.T., Nguyen,T., Shen,C.H., Sharp,P.A., Eisen,H.N. and Chen,J. (2003) RNA interference of influenza virus production by directly targeting mRNA for degradation and indirectly inhibiting all viral RNA transcription. *Proc. Natl Acad. Sci. USA*, **100**, 2718–2723.
13. Konishi,M., Wu,C.H. and Wu,G.Y. (2003) Inhibition of HBV replication by siRNA in a stable HBV-producing cell line. *Hepatology*, **38**, 842–850.

14. Shi,Y., Yang,D.H., Xiong,J., Jia,J., Huang,B. and Jin,Y.X. (2005) Inhibition of genes expression of SARS coronavirus by synthetic small interfering RNAs. *Cell Res.*, **15**, 193–200.
15. Stevenson,M. (2004) Therapeutic potential of RNA interference. *N. Engl. J. Med.*, **351**, 1772–1777.
16. Tripp,R.A. and Tompkins,S.M. (2009) Therapeutic applications of RNAi for silencing virus replication. *Methods Mol. Biol.*, **555**, 43–61.
17. Chen,Y. and Mahato,R.I. (2008) siRNA pool targeting different sites of human hepatitis B surface antigen efficiently inhibits HBV infection. *J. Drug Target.*, **16**, 140–148.
18. ter Brake,O., t Hooft,K., Liu,Y.P., Centlivre,M., von Eije,K.J. and Berkhout,B. (2008) Lentiviral vector design for multiple shRNA expression and durable HIV-1 inhibition. *Mol. Ther.*, **16**, 557–564.
19. Vaishnaw,A.K., Gollob,J., Gamba-Vitalo,C., Hutabarat,R., Sah,D., Meyers,R., de Fougerolles,T. and Maraganore,J. (2010) A status report on RNAi therapeutics. *Silence*, **1**, 14.
20. Lopez-Fraga,M., Martinez,T. and Jimenez,A. (2009) RNA interference technologies and therapeutics: from basic research to products. *Biodrugs*, **23**, 305–332.
21. Shah,P.S. and Schaffer,D.V. (2011) Antiviral RNAi: translating science towards therapeutic success. *Pharm. Res*, August 9 (doi:10.1007/s11095-011-0549-8; epub ahead of print).
22. DeVincenzo,J., Lambkin-Williams,R., Wilkinson,T., Cehelsky,J., Nochur,S., Walsh,E., Meyers,R., Gollob,J. and Vaishnaw,A. (2010) A randomized, double-blind, placebo-controlled study of an RNAi-based therapy directed against respiratory syncytial virus. *Proc. Natl Acad. Sci. USA*, **107**, 8800–8805.
23. Haussecker,D. (2008) The business of RNAi therapeutics. *Hum. Gene Ther.*, **19**, 451–462.
24. Lanford,R.E., Hildebrandt-Eriksen,E.S., Petri,A., Persson,R., Lindow,M., Munk,M.E., Kauppinen,S. and Orum,H. (2010) Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science*, **327**, 198–201.
25. DiGiusto,D.L., Krishnan,A., Li,L., Li,H., Li,S., Rao,A., Mi,S., Yam,P., Stinson,S., Kalos,M. *et al.* (2010) RNA-based gene therapy for HIV with lentiviral vector-modified CD34(+) cells in patients undergoing transplantation for AIDS-related lymphoma. *Sci. Transl. Med.*, **2**, 36ra43.
26. Tyagi,A., Ahmed,F., Thakur,N., Sharma,A., Raghava,G.P.S. and Kumar,M. (2011) HIVsirDB: A Database of HIV Inhibiting siRNAs. *PLoS One*, **6**, e25917.
27. Truss,M., Swat,M., Kielbasa,S.M., Schafer,R., Herzel,H. and Hagemeier,C. (2005) HuSiDa–the human siRNA database: an open-access database for published functional siRNA sequences and technical details of efficient transfer into recipient cells. *Nucleic Acids Res.*, **33**, D108–D111.
28. Chalk,A.M., Warfinge,R.E., Georgii-Hemming,P. and Sonnhammer,E.L. (2005) siRNAdb: a database of siRNA sequences. *Nucleic Acids Res.*, **33**, D131–D134.
29. Ren,Y., Gong,W., Xu,Q., Zheng,X., Lin,D., Wang,Y. and Li,T. (2006) siRecords: an extensive database of mammalian siRNAs with efficacy ratings. *Bioinformatics*, **22**, 1027–1028.
30. Dash,R., Moharana,S.S., Reddy,A.S., Sastry,G.M. and Sastry,G.N. (2006) DSTHO: database of siRNAs targeted at human oncogenes: a statistical analysis. *Int. J. Biol. Macromol.*, **38**, 65–69.
31. Zuker,M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.*, **31**, 3406–3415.
32. Jackson,A.L., Bartz,S.R., Schelter,J., Kobayashi,S.V., Burchard,J., Mao,M., Li,B., Cavet,G. and Linsley,P.S. (2003) Expression profiling reveals off-target gene regulation by RNAi. *Nat. Biotechnol.*, **21**, 635–637.
33. Birmingham,A., Anderson,E.M., Reynolds,A., Ilsley-Tyree,D., Leake,D., Fedorov,Y., Baskerville,S., Maksimova,E., Robinson,K., Karpilow,J. *et al.* (2006) 3′ UTR seed matches, but not overall identity, are associated with RNAi off-targets. *Nat. Methods*, **3**, 199–204.
34. Anderson,E.M., Birmingham,A., Baskerville,S., Reynolds,A., Maksimova,E., Leake,D., Fedorov,Y., Karpilow,J. and Khvorova,A. (2008) Experimental validation of the importance of seed complement frequency to siRNA specificity. *RNA*, **14**, 853–861.
35. Ui-Tei,K., Naito,Y., Nishi,K., Juni,A. and Saigo,K. (2008) Thermodynamic stability and Watson-Crick base pairing in the seed duplex are major determinants of the efficiency of the siRNA-based off-target effect. *Nucleic Acids Res.*, **36**, 7100–7109.
36. Jackson,A.L. and Linsley,P.S. (2010) Recognizing and avoiding siRNA off-target effects for target identification and therapeutic application. *Nature reviews. Drug Discov.*, **9**, 57–67.
37. Altschul,S.F., Gish,W., Miller,W., Myers,E.W. and Lipman,D.J. (1990) Basic local alignment search tool. *J. Mol. Biol.*, **215**, 403–410.
38. Chalk,A.M. and Sonnhammer,E.L. (2008) siRNA specificity searching incorporating mismatch tolerance data. *Bioinformatics*, **24**, 1316–1317.
39. Naito,Y., Ui-Tei,K., Nishikawa,T., Takebe,Y. and Saigo,K. (2006) siVirus: web-based antiviral siRNA design software for highly divergent viral sequences. *Nucleic Acids Res.*, **34**, W448–W450.
40. Naito,Y., Nohtomi,K., Onogi,T., Uenishi,R., Ui-Tei,K., Saigo,K. and Takebe,Y. (2007) Optimal design and validation of antiviral siRNA for targeting HIV-1. *Retrovirology*, **4**, 80.
41. Myers,E.W. and Miller,W. (1988) Optimal alignments in linear space. *Comput. Appl. Biosci.*, **4**, 11–17.
42. Das,A.T., Brummelkamp,T.R., Westerhout,E.M., Vink,M., Madiredjo,M., Bernards,R. and Berkhout,B. (2004) Human immunodeficiency virus type 1 escapes from RNA interference-mediated inhibition. *J. Virol.*, **78**, 2601–2605.
43. Wilson,J.A. and Richardson,C.D. (2005) Hepatitis C virus replicons escape RNA interference induced by a short interfering RNA directed against the NS5b coding region. *J. Virol.*, **79**, 7050–7058.
44. Konishi,M., Wu,C.H., Kaito,M., Hayashi,K., Watanabe,S., Adachi,Y. and Wu,G.Y. (2006) siRNA-resistance in treated HCV replicon cells is correlated with the development of specific HCV mutations. *J. Viral. Hepat.*, **13**, 756–761.
45. Jun,E.J., Nam,Y.R., Ahn,J., Tchah,H., Joo,C.H., Jee,Y., Kim,Y.K. and Lee,H. (2008) Antiviral potency of a siRNA targeting a conserved region of coxsackievirus A24. *Biochem. Biophys. Res. Commun.*, **376**, 389–394.
46. Rice,P., Longden,I. and Bleasby,A. (2000) EMBOSS: the European Molecular Biology Open Software Suite. *Trends Genet.*, **16**, 276–277.