

Additional Table 1

A survey of hairpin stem lengths and loop sequences

We surveyed one hundred and one studies employing expressed shRNA (for mammalian applications) and scored the stem length and loop sequence for each hairpin. In some cases the actual stem length may differ from the designed (**Intended**) length due to a collapsed loop sequence, which results in a smaller loop and extended stem (**Alt.**).

Reference	Intended	Alt.	Loop
Arrighi et al., <i>J Virol</i> 78 , 10848 (Oct, 2004).	21	23	9 (UUCAAGAGA)^*
Babcock et al., <i>Mol Ther</i> 11 , 899 (Jun, 2005).	19	21	9 (UUCAAGAGA)^
Bernard et al., <i>Oncogene</i> (May 9, 2005).	19	21	9 (UUCAAGAGA)^*
Berns et al., <i>Nature</i> 428 , 431 (Mar 25, 2004).	19	-	not available**
Boden et al., <i>Mol Ther</i> 9 , 396 (Mar, 2004).	21	same	6
Boden et al., <i>Nucleic Acids Res</i> 32 , 1154 (2004).	21, other [#]	-	other [#]
Bot et al., <i>Blood</i> (May 10, 2005).	19	21	9 (UUCAAGAGA)^
Caldas et al., <i>J Med Genet</i> (May 20, 2005).	19*	21	9 (UUCAAGAGA)^*
Cao et al., <i>J Appl Genet</i> 46 , 217 (2005).	21	22	6 (AAGCUU)^
Chan et al., <i>Genes Dev</i> 19 , 196 (Jan 15, 2005).	19*	21	9 (UUCAAGAGA)^*
Chang et al., <i>Gene Ther</i> (Mar 3, 2005).	19	21	9 (UUCAAGAGA)^
Chen et al., <i>World J Gastroenterol</i> 11 , 831 (2005).	19	21	9 (UUCAAGAGA)^
Corydon et al., <i>Mol Genet Metab</i> (May 28, 2005).	19	21	9 (UUCAAGAGA)^
Das et al., <i>J Virol</i> 78 , 2601 (Mar, 2004).	19	21	9 (UUCAAGAGA)^
Denti et al., <i>Mol Ther</i> 10 , 191 (Jul, 2004).	24, other [#]	same	5 (UGUGU)
Einav et al., <i>FEBS Lett</i> 579 , 199 (Jan 3, 2005).	19	21	9 (UUCAAGAGA)^
Fish, Kruithof, <i>BMC Mol Biol</i> 5 , 9 (Aug 3, 2004).	19, 21, 23, 25	same	8 (CAAGCUUC)^
Fung, Demple, <i>Mol Cell</i> 17 , 463 (Feb 4, 2005).	21	23	9 (UUCAAGAGA)^*
Gewin et al., <i>Genes Dev</i> 18 , 2269 (Sep 15, 2004).	29	-	not available**
Gonzalez et al., <i>Mol Ther</i> 11 , 811 (May, 2005).	21	23	9 (UUUGUGUAG)
Guo et al., <i>World J Gastroenterol</i> 11 , 2912 (2005).	19	20	6 (CUCGAG)^
Hacker et al., <i>Gene</i> 341 , 227 (Oct 27, 2004).	21	23	9 (UUCAAGAGA)^
Hernandez-Munoz et al., <i>PNAS</i> . 102 , 7635 (2005).	21	23	9 (UUCAAGAGA)^*
Ho et al., <i>Exp Cell Res</i> 304 , 1 (Mar 10, 2005).	19	21	9 (UUCAAGAGA)^
Hosono et al., <i>Gene</i> 348 , 157 (Mar 28, 2005).	19	21	9 (UUCAAGAGA)^
Hua et al., <i>Gynecol Oncol</i> (May 24, 2005).	19	21	9 (UUCAAGAGA)^
Huang et al., <i>FEBS Lett</i> 558 , 69 (Jan 30, 2004).	25	same	4
Huang, Kochanek, <i>Hum Gene Ther</i> 16 , 618 (2005).	19	21	9 (UUCAAGAGA)^

Reference	Intended	Alt.	Loop
Hung, Kumar, <i>FEBS Lett</i> 560 , 210 (Feb 27, 2004).	19	21	9 (UUCAAGAGA)^
Hurtado et al., <i>J Mol Cell Cardiol</i> 38 , 647 (2005).	29	same	8 (GAAGCUUG)
Izeradjene et al., <i>Oncogene</i> 24 , 2050 (2005).	28	same	8
Jacobs, de Lange, <i>Curr Biol</i> 14 , 2302 (2004).	19*	21	9 (UUCAAGAGA)^*
Jenke et al., <i>Hum Gene Ther</i> 16 , 533 (Apr, 2005).	28, 29	same	8 (GAAGCUUG)
Kamradt et al., <i>J Biol Chem</i> 280 , 11059 (2005).	19	21	9 (UUCAAGAGA)^
Kanda et al., <i>Biochem Biophys Res Com</i> 330 , 1217 (2005)	19	21	9 (UUCAAGAGA)^
Kariko et al., <i>J Immunol</i> 172 , 6545 (Jun 1, 2004).	21	same	4 (UUAA)
Koper-Emde et al., <i>Biol Chem</i> 385 , 791 (2004).	19	same	3 (ACA)
Kudo, Sutou, <i>J Reprod Dev</i> (Apr 5, 2005).	21	23	9 (UUCAAGAGA)^
Kuninger et al., <i>Hum Gene Ther</i> 15 , 1287 (2004).	19	21	9 (UUCAAGAGA)^
Laatsch et al., <i>Eur J Cell Biol</i> 83 , 113 (Apr, 2004).	19	21	9 (UUCAAGAGA)^*
Lakka et al., <i>Oncogene</i> 23 , 4681 (Jun 10, 2004).	21	same	9
Lambeth et al., <i>BMC Biotechnol</i> 5 , 13 (2005).	19	21	9 (UUCAAGAGA)^
Langlois et al., <i>J Biol Chem</i> 280 , 16949 (2005).	21	same	4 (UGCG)
Lee et al., <i>Blood</i> (Apr 14, 2005).	21	same	9
Lefrancois et al., <i>Biol Proced Online</i> 7 , 17 (2005).	21	-	not available**
Li et al., <i>J Lipid Res</i> 46 , 220 (Feb, 2005).	21	same	9 (UUUGUGUAG)
Li et al., <i>Biochem Biophys Res Com.</i> 315 , 212 (2004).	19	same	4 (UUCG)
Li et al., <i>Oligonucleotides</i> 13 , 401 (2003).	21	23	9 (UUUGUGUAG)
Li et al., <i>FEBS Lett</i> (May 21, 2005).	19	21	9 (UUCAAGAGA)^
Li et al., <i>Molecular Therapy</i> . article in press (2005).	21	23	9 (UUCAAGAGA)^
Liu et al., <i>Biochem Biophys Res Com.</i> 324 , 1173 (2004).	19	21	9 (UUCAAGAGA)^
Lu et al., <i>Biochem Biophys Res Com.</i> 325 , 494 (2004).	19	21	9 (UUCAAGAGA)^
Lu et al., <i>Virology</i> 324 , 84 (Jun 20, 2004).	19	21	9 (UUCAAGAGA)^
Matthess et al., <i>Oncogene</i> 24 , 2973 (2005).	19	same	9 (UUCAUAUGG)
Mejillano et al., <i>Cell</i> 118 , 363 (Aug 6, 2004).	19	21	9 (UUCAAGAGA)^*
Miller et al., <i>Nucleic Acids Res</i> 32 , 661 (2004).	20, 21, 22	same	8 (GAAGCUUG)
Miyagawa et al., <i>J Biochem (Tokyo)</i> 137 , 503 (2005).	21	23	9 (UUCAAGAGA)^
Miyagishi et al., <i>J Gene Med</i> 6 , 715 (Jul, 2004).	19	20	11 (GUGUGCUGUCC)^
Modem et al., <i>Nucleic Acids Res</i> 33 , 873 (2005).	19	same	8 (GAGUACUG)
Moore et al., <i>J Gene Med</i> (Mar 9, 2005).	19	21	9 (UUCAAGAGA)^*
Nahreini et al., <i>Cell Mol Neurobiol</i> 24 , 781 (2004).	22, 27	-	other [#]
Nicholson et al., <i>Mol Ther</i> 11 , 638 (Apr, 2005).	22	same	8 (CAUCGAUA)
Paddison et al., <i>Nature</i> 428 , 427 (Mar 25, 2004).	29	same	4
Pebernard, Iggo, <i>Differentiation</i> 72 , 103 (Mar, 2004).	19	21	9 (UUCAAGAGA)^
Piva et al., <i>Blood</i> 105 , 1750 (Feb 15, 2005).	21	23	9 (UUCAAGAGA)^
Poltoratsky et al., <i>DNA Repair (Amst)</i> (Jun 8, 2005).	19	21	9 (UUCAAGAGA)^
Pruss et al., <i>Eur J Neurosci</i> 20 , 3184 (Dec, 2004).	19, 22	21, 24	9 (UUCAAGAGA)^
Ren et al., <i>J Viral Hepat</i> 12 , 236 (May, 2005).	19, 21, 23, 24, 25	same	9 (UUCAAGAGA)^
Rotolo et al., <i>Int J Cancer</i> 115 , 164 (May 20, 2005).	19	21	9 (UUCAAGAGA)^

Reference	Intended	Alt.	Loop
Scherer et al., <i>Mol Ther</i> 10 , 597 (Sep, 2004).	21	same	9
Scherr et al., <i>Gene Ther</i> 12 , 12 (Jan, 2005).	19	21	9 (UUCAAGAGA)^
Shah et al., <i>Biochem Biophys Res Com</i> 331 , 167 (2005).	21	22	6 (AAGCUU)^
Song et al., <i>Biochem Biophys Res Com</i> 323 , 573 (2004).	23	same	6 (UUUUGC)
Spankuch et al., <i>J Natl Cancer Inst</i> 96 , 862 (2004).	21	same	6
Takabatake et al., <i>Gene Ther</i> 12 , 965 (Jun, 2005).	19	21	9 (UUCAAGAGA)^
Takigawa et al., <i>Microbiol Immunol</i> 48 , 591 (2004).	19	21	9 (UUCAAGAGA)^
Taniai et al., <i>Cancer Res</i> 64 , 3517 (May 15, 2004).	19	21	9 (UUCAAGAGA)^
Tao et al., <i>Chin Med J (Engl)</i> 118 , 714 (2005).	20	same	4
Tatsuka et al., <i>Oncogene</i> 24 , 1122 (Feb 3, 2005).	19	21	9 (UUCAAGAGA)^
Tiscornia et al., <i>PNAS</i> 101 , 7347 (2004).	19	21	9 (UUCAAGAGA)^
Unwalla et al., <i>Nat Biotechnol</i> (Nov 28, 2004).	28	30	9 (UUCAAGAGA)^
Wakiyama et al., <i>Bioc Biophys Res Com</i> 331 , 1163 (2005)	21	-	other [#]
Wang et al., <i>Int J Cancer</i> 112 , 994 (Dec 20, 2004).	19	21	9 (UACAAGAGA)
Weiss-Haljiti et al., <i>J Biol Chem</i> 279 , 43273 (2004).	22	same	8 (CAAGCUUC)
Westerhout et al., <i>Nucleic Acids Res</i> 33 , 796 (2005).	19	-	not available**
Wu et al., <i>Biochem Biophys Res Com</i> 330 , 53 (2005).	19, 26	21, 28	9 (UUCAAGAGA)^, other [#]
Xu, Shrager, <i>J Neurosci Res</i> 79 , 428 (2005).	19	21	9 (UUCAAGAGA)^
Xu et al., <i>J Biol Chem</i> 279 , 41319 (Oct 1, 2004).	26	same	10 (GAAGCUUGAG)
Yang et al., <i>World J Gastroenterol</i> 11 , 498 (2005).	29	same	4, 8
Yang et al., <i>Mol Cell Biol</i> 25 , 4062 (May, 2005).	19	21	9 (UUCAAGAGA)^
Yang, Hecht, <i>FEBS Lett</i> 576 , 221 (Oct 8, 2004).	22	23	6 (CUCGAG)^
Yang et al., <i>Nucleic Acids Res</i> 33 , e57 (2005).	19	same	4 (UUCG)
Yogosawa et al., <i>J Biol Chem AOL</i> (2005).	19	20	12 (GTGTGCTGTCC) ^
Yoon et al., <i>Mol Cells</i> 18 , 127 (Aug 31, 2004).	19, 23	same	9*
Yoon et al., <i>Oncogene</i> (Apr 4, 2005).	19	21	9 (UUCAAGAGA)^
Yuan et al., <i>J Orthop Res</i> (May 28, 2005).	19	21	9 (UUCAAGAGA)^
Yuan et al., <i>Oncogene</i> 24 , 3657 (May 19, 2005).	19	21	9 (UUCAAGAGA)^
Zeng, Cullen, <i>Nucleic Acids Res</i> 32 , 4776 (2004).	19	21	9 (UUCAAGAGA)^
Zhang et al., <i>Gene Ther</i> (May 5, 2005).	21,23	same	6
Zhou et al., <i>Am J Pathol</i> 165 , 2033 (Dec, 2004).	19	-	not available**
Zhou et al., <i>Nucleic Acids Res</i> 33 , e62 (2005).	22, 23	24, 25	9 (UUCAAGAGA)^, other [#]

* Sequences or lengths were not specifically detailed but were implied by reference to previous publications.

** Sequence or length details were not available.

Non-specific lengths or sequences were evaluated, typically microRNA-derived stem or loop configurations.

^ Loop sequences that are at least partially self-complementary at their base yet were not acknowledged as such.

Additional Table 2

Sequences and features of the hairpins used

The name, transcript size (nt), and the sequence of the expected transcript (RNA, 5' to 3') are given for each vector. Hairpin names were generated using a general nomenclature according to the sequence they target (e.g. Tat), the position in the target of the first target / shRNA (guide strand) complementary pair (e.g. nucleotide 56 as measured from the ATG start site), the length of the stem or target complementary region (e.g. 29 bp), and any characteristic unique to the particular hairpin. Transcript sizes are estimates only due to the variable nature of pol III termination believed to incorporate between 1 and 6 'U' residues. Each shRNA incorporated an 8 – 9 nt loop sequence **NiCUCGAGNi** (shown in **bold**) and for some shRNA, an extra purine was included 5' to the first base of the sense stem strand to facilitate polymerase III transcriptional initiation (indicated in **bold**) or 3' to the final base of the anti-sense stem strand as required to prevent premature termination by an early run of 'T's (indicated in **bold**).

Name	Size	Sequence (5'-3')
Tat56-15	41	GAAACUGCUUGUACCA ACUCGAGA UGGUACAAGCAGUUUGU _x
Tat56-17	45	GAAACUGCUUGUACCAU ACUCGAGAA UUGGUACAAGCAGUUUGU _x
Tat56-18	47	GAAACUGCUUGUACCAU UACUCGAGAAA UUGGUACAAGCAGUUUGU _x
Tat56-19	49	GAAACUGCUUGUACCAUUG ACUCGAGACA AUUGGUACAAGCAGUUUGU _x
Tat56-20	51	GAAACUGCUUGUACCAUUGC ACUCGAGAG CAAUUGGUACAAGCAGUUUGU _x
Tat56-21	53	GAAACUGCUUGUACCAUUGCU ACUCGAGA AGCAAUUGGUACAAGCAGUUUGU _x
Tat56-22	55	GAAACUGCUUGUACCAUUGCU ACUCGAGAU AGCAAUUGGUACAAGCAGUUUGU _x
Tat56-23	57	GAAACUGCUUGUACCAUUGCUAU ACUCGAGAA UAGCAAUUGGUACAAGCAGUUUGU _x
Tat56-24	59	GAAACUGCUUGUACCAUUGCUAUU ACUCGAGAAA UAGCAAUUGGUACAAGCAGUUUGU _x
Tat56-25	61	GAAACUGCUUGUACCAUUGCUAUUG ACUCGAGACA AUAGCAAUUGGUACAAGCAGUUUGU _x
Tat56-26	63	GAAACUGCUUGUACCAUUGCUAUUGU ACUCGAGAA CAAUAGCAAUUGGUACAACAGUUUGU _x
Tat56-27	65	GAAACUGCUUGUACCAUUGCUAUUGUA ACUCGAGAU ACAAUAGCAAUUGGUACAAGCAGUUUGU _x
Tat56-28	67	GAAACUGCUUGUACCAUUGCUAUUGUAA ACUCGAGAUU ACAAUAGCAAUUGGUACAAGCAGUUUGU _x

Name	Size	Sequence (5'-3')
Tat56-29	69	GAAACUGCUUGUACCAAUUGCUAUUGUAAA ACUCGAGAUUU ACAAUAGCAAUUGGUACAAGCAGUUU GU_x
Tat56-33 ¹	77	GAAACUGCUUGUACCAAUUGCUAUUGUAAAGAGU ACUCGAGAA CUCU UUACAAUAGCAAUUGGUACAAGCAGUUU GU_x
Tat56-37 ^{1,2}	85	GAAACUGCUUGUACCAAUUGCUAUUGUAAAGAGUUUGC UCUCGAGU GCAAACUCUUUACAAUAGCAAUUGGUACAAGCAGUUU GU_x
Tat56-41 ¹	93	GAAACUGCUUGUACCAAUUGCUAUUGUAAAGAGUUUGCUUUC ACUC GAGAGAAAGCAAACU CUUUACAAUAGCAAUUGGUACAAGCAGUUU GU x
Vpu10-19	49	GAUAAUAGUAGCAAUAGUAG ACUCGAGACUACU AUUGCUACUAAUUAU GU_x
Vpu10-21	53	GAUAAUAGUAGCAAUAGUAGCA ACUCGAGAU GCUACUAAUUGCUACUAUUAU GU_x
Vpu10-23	57	GAUAAUAGUAGCAAUAGUAGCAU ACUCGAGAA UGCUACUAAUUGCUACUAAUUAU GU_x
Vpu10-25	61	GAUAAUAGUAGCAAUAGUAGCAU ACUCGAGACUAA UGCUACUAAUUGCUACUAAUUAU GU_x
Vpu10-27	65	GAUAAUAGUAGCAAUAGUAGCAU ACUCGAGAUACUAA UGCUACUAAUUGCUACUAAUUAU GU_x
Vpu10-29	69	GAUAAUAGUAGCAAUAGUAGCAU ACUCGAGAA CUACUAAUUGCUACUAAUUAU GU_x
Vpu51-19	49	GAGCAAUAGUUGUGUGGUCC ACUCGAGAGG ACCACACAACUAAUUGCUACUAAUUAU GU_x
Vpu51-21	53	GAGCAAUAGUUGUGUGGUCCAU ACUCGAGAA UGGACCACACAACUAAUUGCUACUAAUUAU GU_x
Vpu51-23	57	GAGCAAUAGUUGUGUGGUCCAU ACUCGAGACU AUGGACCACACAACUAAUUGCUACUAAUUAU GU_x
Vpu51-25	61	GAGCAAUAGUUGUGUGGUCCAUAGUA ACUCGAGAUACU AUGGACCACACAACUAAUUGCUACUAAUUAU GU_x
Vpu51-27	65	GAGCAAUAGUUGUGUGGUCCAUAGUAAU ACUCGAGAAU UACUAAUUGCUACUAAUUAU GU_x
Vpu51-29	69	GAGCAAUAGUUGUGUGGUCCAUAGUAAU ACUCGAGAU GAUUAUUGCUACUAAUUAU GU_x
Vpu127-19	49	GGAUAGACUAAUAGAAAGAG ACUCGAGACUCU UUUCUAAUAGUCUAAUUGCUACUAAUUAU GU_x
Vpu127-21	53	GGAUAGACUAAUAGAAAGAGCA ACUCGAGAU GCUCUUAUAGUCUAAUUAU GU_x
Vpu127-23	57	GGAUAGACUAAUAGAAAGAGCAGA ACUCGAGAU CUGCUCUUAUAGUCUAAUUAU GU_x
Vpu127-25	61	GGAUAGACUAAUAGAAAGAGCAGA ACUCGAGACU UUCGUCUUAUAGUCUAAUUAU GU_x
Vpu127-27	65	GGAUAGACUAAUAGAAAGAGCAGA ACUCGAGAGU CUUUCGUCUUAUAGUCUAAUUAU GU_x
Vpu127-29	69	GGAUAGACUAAUAGAAAGAGCAGA ACUCGAGACU GUCUUAUAGUCUAAUUAU GU_x
Vpu158-17	45	GGCAAUGAGAGUGAAGGA ACUCGAGAU CCUUCACUCUCAUUGCG GU_x
Vpu158-18	47	GGCAAUGAGAGUGAAGGAG ACUCGAGACU CCUUCACUCUCAUUGCG U_x

Name	Size	Sequence (5'-3')
Vpu158-19	49	GGCAAUGAGAGUGAAGGAGA ACUCGAG AUCUCCUUCACUCUCAUUGCGU _x
Vpu158-21	53	GGCAAUGAGAGUGAAGGAGAAG ACUCGAG ACUUCUCCUUCACUCUCAUUGCGU _x
Vpu158-23	57	GGCAAUGAGAGUGAAGGAGAAGUA ACUCGAG AUACUUCUCCUUCACUCUCAUUGCGU _x
Vpu158-25	61	GGCAAUGAGAGUGAAGGAGAAGUAUC ACUCGAG AGAUACUUCUCCUUCACUCUCAUUGCGU _x
Vpu158-27	65	GGCAAUGAGAGUGAAGGAGAAGUAUCAG ACUCGAG ACUGAUACUUCUCCUUCACUCUCAUUGCGU _x
Vpu158-29	69	GGCAAUGAGAGUGAAGGAGAAGUAUCAGCA ACUCGAG AUGCUGAUACUUCUCCUUCACUCUCAUUGCGU _x
Tat3-21	51	GGAGCCAGUAGAUCUAGACU ACUCGAG AAGUCUAGGAUCUACUGGCUCU _x
Tat3-29	68	GGAGCCAGUAGAUCUAGACUAGAGCCCU ACUCGAG AAGGGCUCUAGUCUAGGAUCUACUGGCUCU _x
Tat22-21	51	CUAGAGCCUGGAAGCAUCCA ACUCGAG AUGGAUGCUUCCAGGGCUUAGU _x
Tat22-29	68	CUAGAGCCUGGAAGCAUCCAGGAAGUCA ACUCGAG AUGACUCCUUGGAUGCUUCCAGGGCUCUAGU _x
Tat24-21	52	AGAGCCUGGAAGCAUCCAGG ACUCGAG ACCUGGAUGCUUCCAGGGCUCU AU _x
Tat24-29	69	AGAGCCUGGAAGCAUCCAGGAAGUCAGC ACUCGAG AGCUGACUUCUGGAUGCUUCCAGGGCUCU AU _x
Tat94-21	53	GUUUCAUUGCCAAGUUUGUUUC GCUCGAG GGAAACAAACUUGGCAUUGAA GU _x
Tat94-29	69	GUUUCAUUGCCAAGUUUGUUUCAUAACAAAG GCUCGAG GUUUUUUAUGAAACAAACUUGGCAUUGAA GU _x
Tat144-21	51	GCAGGAAGAAGCGGAGACAGC ACUCGAG AGCUGUCUCCGCUUCUUCUGCU _x
Tat144-29	68	GCAGGAAGAAGCGGAGACAGCGACGAAGA ACUCGAG AUCUUCGUCGUGUCUCCGCUUCUUCUGCU _x
Tat165-21	51	GACGAAGAGCUCAUCAGAACA ACUCGAG AUGUUCUGAUGAGCUCUUCGUCU _x
Tat165-29	67	GACGAAGAGCUCAUCAGAACAGUCAGACU ACUCGAG AAGUCUGACUGUUCUGAUGAGCUCUUCGUCU _x
Tat181-21	53	GAACAGUCAGACUCAUCAAGCU GCUCGAG GAGCUUGAUGAGUCUGACUGUUGU _x
Tat181-29	69	GAACAGUCAGACUCAUCAAGCUUCUCUAUC GCUCGAG GAUAGAGAAACUUGAUGAGUCUGACUGUUGU _x
Tat187-21	53	GCAGACUCAUCAAGCUUCUCUAUCAAGCAG GCUCGAG GUAGAGAAGCUUGAUGACUGUGU _x
Tat187-29	69	GCAGACUCAUCAAGCUUCUCUAUCAAGCAG GCUCGAG GUAGCUUUGAUAGAGAAGCUUGAUGAGUCUGU _x
(Tat56-)19+10	69	GAAACUGCUUGUACCAUUGGAUAACA UUUACUCGAG AAAUGUU AU CCAAUUGGUACAAGCAGUUUGU _x
(Tat56-)21+8	69	GAAACUGCUUGUACCAUUGCUUAACA UUUACUCGAG AAAUGUU AA GCAAUUGGUACAAGCAGUUUGU _x

Name	Size	Sequence (5'-3')
(Tat56-)23+6	69	GAAACUGCUUGUACCAAUUGCUAUACAUUUACUCGAGAAAAUGUAUA GCAAUUGGUACAAGCAGUUUGU _x
(Tat56-)25+4	69	GAAACUGCUUGUACCAAUUGCUAUUGAUUUACUCGAGAAAAUCAAUA GCAAUUGGUACAAGCAGUUUGU _x
(Tat56-)27+2	69	GAAACUGCUUGUACCAAUUGCUAUUGUAUUACUCGAGAAUACAAUA GCAAUUGGUACAAGCAGUUUGU _x
(Tat56-)2+27	69	GUUACUGCUUGUACCAAUUGCUAUUGUAAAAACUCGAGAUUUACAAUA GCAAUUGGUACAAGCAGUAAAGU _x
(Tat56-)4+25	69	GUUUGUGCUUGUACCAAUUGCUAUUGUAAAAACUCGAGAUUUACAAU AGCAAUUGGUACAAGCAGAAAGU _x
(Tat56-)6+23	69	GUUUGACCUUGUACCAAUUGCUAUUGUAAAAACUCGAGAUUUACAAUA GCAAUUGGUACAAGGUCAAAGU _x
(Tat56-)8+21	69	GUUUGACGAUGUACCAAUUGCUAUUGUAAAAACUCGAGAUUUACAAUA GCAAUUGGUACAUCGUCAAAGU _x
(Tat56-)10+19	69	GUUUGACGAACUACCAAUUGCUAUUGUAAAAACUCGAGAUUUACAAUA GCAAUUGGUAGUUCGUCAAAGU _x
Tat59-21	53	GCUGCUUGUACCAAUUGCUAUUACUCGAGAAUAGCAAUUGGUACAA GCAGGU _x
Tat59-21 (0-21-8)	69	GCUGCUUGUACCAAUUGCUAUUCAUUUGGCACUCGAGAGCCAAUUG AAUAGCAAUUGGUACAAGCAGGU _x
Tat59-21 (1-21-7)	69	GUUCUGCUUGUACCAAUUGCUAUUCAUUUGGACUCGAGACCAAUGA AUAGCAAUUGGUACAAGCAGAGU _x
Tat59-21 (2-21-6)	69	GUUCUGCUUGUACCAAUUGCUAUUCAUUUGACUCGAGACAAUGAA UAGCAAUUGGUACAAGCAGAAAGU _x
Tat59-21 (3-21-5)	69	GUUUCUGCUUGUACCAAUUGCUAUUCAUUUACUCGAGAAAAUGAAUA GCAAUUGGUACAAGCAGAAAGU _x
Tat59-21 (4-21-4)	69	GGUUUCUGCUUGUACCAAUUGCUAUUCAUUACUCGAGAAUUGAAUA GCAAUUGGUACAAGCAGAAACGU _x
Tat59-21 (5-21-3)	69	GAGUUUCUGCUUGUACCAAUUGCUAUUCAUACUCGAGAAUGAAUAG CAAUUGGUACAAGCAGAAACUGU _x
Tat59-21 (6-21-2)	69	GGAGUUUCUGCUUGUACCAAUUGCUAUUCAACUCGAGAUAAUAGC AAUUGGUACAAGCAGAAACUCGU _x
Tat59-21 (7-21-1)	69	GGGAGUUUCUGCUUGUACCAAUUGCUAUUCAACUCGAGAGAAUAGCA AUUGGUACAAGCAGAAACUCCGU _x
Tat59-21 (8-21-0)	69	GCGGAGUUUCUGCUUGUACCAAUUGCUAUUACUCGAGAAUAGCAA UUGGUACAAGCAGAAACUCCGU _x
Tat56-23+6 (v2)	69	GAAACUGCUUGUACCAAUUGCUAUUGGGGGACUCGAGACCCCCAU AGCAAUUGGUACAAGCAGUUUGU _x
Tat56-23+6 (v3)	69	GAAACUGCUUGUACCAAUUGCUAUUCCCCACUCGAGAGGGGGGAU AGCAAUUGGUACAAGCAGUUUGU _x
Tat56-23+6 (v4)	69	GAAACUGCUUGUACCAAUUGCUAUUAUAUUAUCUCGAGAAUAUAUA GCAAUUGGUACAAGCAGUUUGU _x
Tat56-23+6 (v5)	69	GAAACUGCUUGUACCAAUUGCUAUUGCGCGCACUCGAGAGCGCGCAU AGCAAUUGGUACAAGCAGUUUGU _x
Tat56-23+6 (v6)	69	GAAACUGCUUGUACCAAUUGCUAUUGACUGUACUCGAGACAGUCAUA GCAAUUGGUACAAGCAGUUUGU _x
Tat56-23+6 (v7)	69	GAAACUGCUUGUACCAAUUGCUAUACAGUCACUCGAGAGACUGUAUA GCAAUUGGUACAAGCAGUUUGU _x

Name	Size	Sequence (5'-3')
Tat56-24 (23+1)	59	GAAACUGCUUGUACCAAUUGCUAU ACUCGAGAU AUAGCAAUUGGUA CAAGCAGUUU GU _x
Tat56-25 (23+2)	61	GAAACUGCUUGUACCAAUUGCUAU ACUCGAGAGU AUAGCAAUUG GUACAAGCAGUUU GU _x
Tat56-26 (23+3)	63	GAAACUGCUUGUACCAAUUGCUAU ACAACUCGAGAU GUAGCAAUU GGUACAAGCAGUUU GU _x
Tat56-27 (23+4)	65	GAAACUGCUUGUACCAAUUGCUAU ACAUACUCGAGAAUGU AUAGCAA UUGGUACAAGCAGUUU GU _x
Tat56-28 (23+5)	67	GAAACUGCUUGUACCAAUUGCUAU ACAUUACUCGAGAAAUGU AUAGC AAUUGGUACAAGCAGUUU GU _x
Tat56-33 (23+10) ² 77		GAAACUGCUUGUACCAAUUGCUAU ACAUUUGUCAUCUCGAGU UAGC AAAUGU AUAGCAAUUGGUACAAGCAGUUU GU _x
Tat56-37 (23+14) ² 85		GAAACUGCUUGUACCAAUUGCUAU ACAUUUGUCAAAACGUUCUCGAGU CGUUUGACAAAUGU AUAGCAAUUGGUACAAGCAGUUU GU _x
Tat56-41 (23+18) 93		GAAACUGCUUGUACCAAUUGCUAU ACAUUUGUCAAAACGAAAGACUCG AGACUUUCGUUUGACAAAUGU AUAGCAAUUGGUACAAGCAGUUU GU _x

¹ These hairpins were not 100 % matched to the target. The nucleotide at stem position 30 was altered to a 'G' (in the upper strand, with a corresponding C in the lower strand) to prevent a run of 'T's that would have resulted in premature termination of transcription.

² Due to a cloning error these hairpins had 'U' splitter bases instead of the 'A' splitter bases.

[^] These transcripts may terminate prematurely due to an early run of 4 or more 'U' bases.

Ux: It was expected that pol III termination would add a variable number of 'U's to the 3' end of each hairpin, the exact number of which was unknown as there are conflicting reports of anywhere from 1 – 6 residues being added (2 U's [15, 16, 28], ≤ 4 U's [20], 4 U's [18, 22], ≤ 5 U's [21, 36], 4 – 6 U's [56]).

Target sequences

The fluorescent reporters used in this study were assembled with following target domains derived from the common HIV-1 laboratory strain, NL4-3 [Genbank:**AF324493**]. n.b. each of the following target domains was fused immediately downstream of GFP with several stop codons placed between the domains.

Tat x12 (both exons fused) (261 bp)

(positions **56** and **59** indicated in bold)

atggagccagtagatcctagactagagccctggaagcatccaggaagtcagccta**AaaCtgcttgta**
ccaattgctattgtaaaagtggtgctttcattgccaagttgtttcatgacaaaagccttaggcattctcctat
 ggcaggaagaagcggagacagcgacgaagagctcatcagaacagtcagactcatcaagcttctcta
 tcaaagcaaccacacctccaatcccgaggggacccgacaggcccggaaggaatag

Vpu (246 bp)

(positions **10**, **51**, **127**, and **158** indicated in bold)

atgcaacct**Ata**tagtagcaatagtagcattagtagtagcaataataat**Ag**caatagttgtgtggtccat
agtaatcatagaatataggaaaatattaagacaaagaaaaatagacaggtaatt**Gat**agactaataga
aagagcagaagacagtGcaatgagagtgaaggagaagtagcagcacttgaggatgggggtgg
 aaatggggcaccatgctccttggtatattgatctgtag

Vif (579 bp)

(used for the *AsRed-1* fused off-target control reporter)

atggaaaacagatggcaggtgatgattgtgtggcaagtagacaggatgaggattaacacatggaaaa
 gattagtaaaacaccatatgtatatattcaaggaaagctaaggactggttttatagacatcactatgaaagt
 actaatccaaaaataagttcagaagtacacatcccactaggggatgctaaattagtaataacaacatat
 ggggtctgcatacaggagaaagagactggcatttgggtcaggagctccatagaatggaggaaaaa
 gagatatagcacacaagtagaccctgacctagcagaccaactaattcatctgcactattttgattgttttc
 agaatctgtataagaaataccatattaggacgtatagttagtcctaggtgtgaatatcaagcaggacat
 aacaaggtaggatctctacagtacttggcactagcagcattaataaaacaaaacagataaaaccacc
 ttgcctagtgttaggaaactgacagaggacagatggaacaagccccagaagaccaagggccacag
 agggagccatacaatgaatggacactag