Dear Colleague,

Here is our T7 siRNA protocol. The main idea is to design primers that begin with GG so that transcription by T7 polymerase is efficient.

I) FIRST design oligos:

Using sense coding sequence of any gene....

(N1, N2, N22, N23, are numbered positions)

1) Find 5'-N1 N2 G/A G/A NNNNNNNNNNNNNNN C C N22 N23-3'

2) Drop N22 and N23

add 5'-TATAGTGAGTCGTATTA-3' to 3' END to get

5'-N1 N2 G/A G/A NNNNNNNNNNNNNNN C C TATAGTGAGTCGTATTA-3' = oligo A

3) From (1) 5'-N1 N2 G/A G/A NNNNNNNNNNNNNNN C C N22 N23-3'

drop N1 N2

convert G/A G/A to G G to get

5'-G G NNNNNNNNNNNNNNN C C N22 N23-3'

4) Add 5'-TAATACGACTCACTATA-3' to 5'END to get

5'-TAATACGACTCACTATA G G NNNNNNNNNNNNNNN C C N22 N23-3'

5) Get reverse complement of (4)

5'-N23' N22' G G NNNNNNNNNNNNNNN' C C TATAGTGAGTCGTATTA-3' = oligo B

6) Rank the initial target sequences with GG preferable to G G/A preferable to G/A G with A A not even considered unless absolutely necessary.

GG N15 CC > G G/A N15 CC > G/A G N15 CC >>>>>>>>> A A N15 CC

7) Order oligos

II) THEN make RNA in vitro

8) Anneal oligo A and oligo B separately to T7 primer (TAATACGACTCACTATAGG) or to primers that are complementary to oligoA and B

9) Use 2-3 ug annealed primer in a 20ul Ambion T7 Megashort script reaction Ambion Cat# 1354
(Follow Ambion's protocol Incubate 2-4 hrs)

10) Combine oligo A and B reactions

11) Anneal T7 transcribed RNAs using your favorite slow annealing protocol

e.g.
95C 5 min
70C 5min
50C 5min
37C 5min

12 A) Phenol-chloroform extract annealed, transcribed RNAs / Ethanol precipitate /

12 B) Or Purify on Ambion MegaClear columns

13) Resuspend in 50-250ul H_2O

14) Quantify yield

15) Transfect

use at least 0.5ug per well of a 6 well plate (3.0ug per 10 cm dish)