From siRNA to shRNA

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Abstract

This brief protocol shows you how easy it is to get shRNA from demostrated siRNA sequence. With these self-designed shRNA, I've got more than 10 genes knockdown from mammalian cell lines, including mouse embryonic stem cells. Believe it or not, using siRNA sequence from Dharmacon will guarantee you the success.

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Protocol

Step 1.
Anchor the siRNA sequence (19nt) on your target gene.

Step 2.
Extract 2nt of upstream +19nt siRNA + 1nt of downstream, which is totally 22nt of nucleotides.

Step 3.
Antisense shRNA: Insert the reverse complement of the 22nt into shRNA backbone.

Step 4.
Sense shRNA: Insert the 22nt (from step 2) into shRNA backbone. Replace the 1st nt with other nucleotide (replace A with G and vice versa; replace C with T and vice versa).

Step 5.
Now your shRNA designing job is done. You see, how easy it is!

Step 6.
Synthesis the shRNA nucleotides and insert them into pTRIPZ vector. You may also translocate the shRNA from pTRIPZ vector into pGIPZ vector.