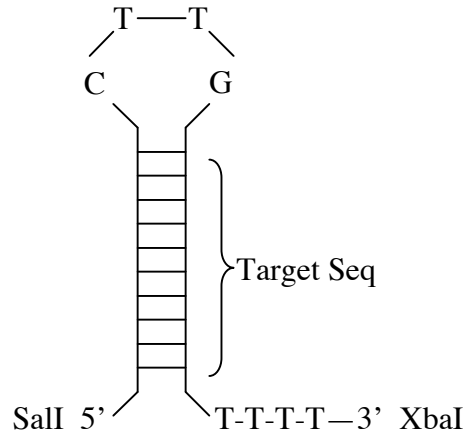


pAVU6+27 RNAi

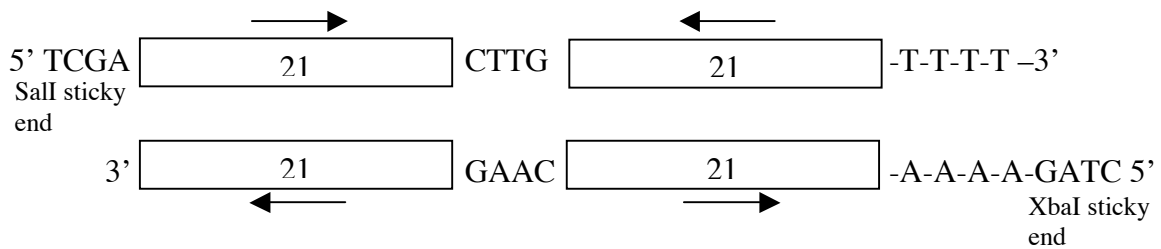
pAVU6+27 for mammalian expression of shRNA.

Clone an oligo sequence into Sall/XbaI sites.
Use 21bp of target sequence.



Primer Design

MAKE SURE TO HAVE PHOSPHATES PUT ON 5' ENDS!!



Vector Construction

Dilute primers to 50µM. Use 6µL of each oligo (1pair per rxn) in a total of 20µL ligation buffer. Heat oligos @ 95°C for 3min and cool slowly to RT. (simply take heat blocks out and put one on top of tubes and let cool to RT o/n.

Cut the pAVU6+27 with Sall and XbaI o/n, CIP, and then gel purify.

Use 50ng vector with 3µL of annealed oligos, ligate, and transform.

Test for ligation using U6F and U6R primers using 10ng of plasmid DNA.

U6F CCGTAACTTGAAAGTATTTTCG
U6R GCGGCTCGAGATCGATGCGGC

Cycling Parameters

Denature	95°C	15m	} 30 cycles
Denature	95°C	0.5m	
Anneal	55°C	0.5m	
Elongate	72°C	1m	
Final	72°C	10m	