

RS puro - shID3 #1 (pg 1)
(or hyg)

Selection of target sequences:

Similar to selection of synthetic siRNAs:

Preferably, the 19 nt target sequence should be flanked in the mRNA with AA at the 5' and TT at the 3'. Giving good results are also sequences with flanking 5' AA only. Regions at the mRNA to select the 19 nt from, are preferably in the coding region: 100 bp from start and termination of translation.

GC richness of the 19 nt targeting sequence, preferably more than 30%.

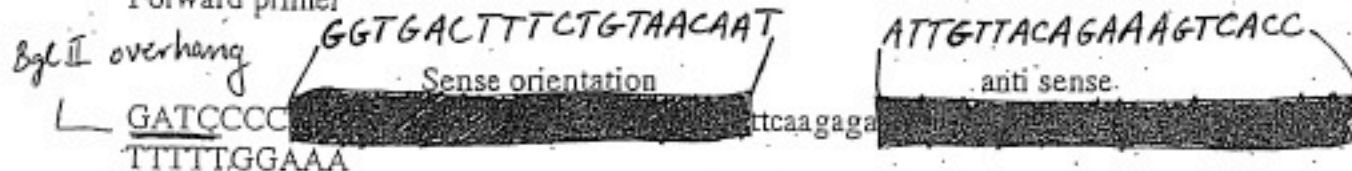
Special for pSUPER:

Take care that the 19 nt that you select does not contain a stretch of four or more Adenines or Thymidines, as this will give premature termination of the transcript.

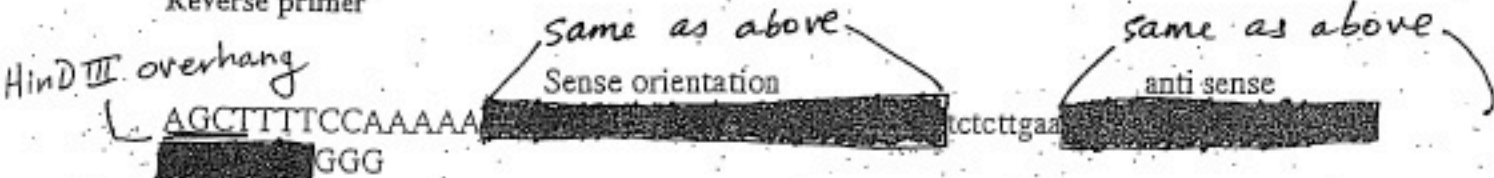
Design of oligos:

An example:

Forward primer



Reverse primer



Each oligo 64 nt.

Just replace your 19 nt sequence from target gene with the sequences in bold.