

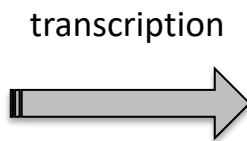
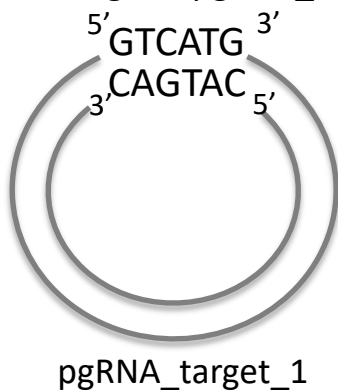
gRNA (DNA sequence) Design for CRISPRi (fill in the blanks)

Target gene sequence: $5' \text{GTCATG} 3'$ ← _____(coding) strand
 Complementary strand: _____ ← Template (noncoding) strand
 $3' \text{ } 5'$

Scenario 1

gRNA (DNA sequence) is the same as the nontemplate (NT) strand: $5' \text{GTCATG} 3'$

The plasmid you generate after inserting the sequence would look something like pgRNA_target_1:



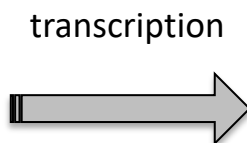
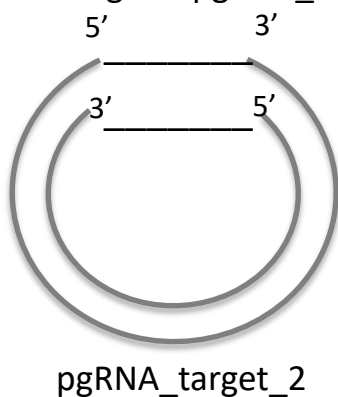
gRNA produced: $5' \text{ } 3'$

This gRNA binds to the nontemplate/template (circle one) strand of the target gene.

Scenario 2

gRNA (DNA sequence) is the same as the template (T) strand: $5' \text{ } 3'$

The plasmid you generate after inserting the sequence would look something like pgRNA_target_2:



gRNA produced: $5' \text{ } 3'$

This gRNA binds to the nontemplate/template (circle one) strand of the target gene.

Design rules:

- (1) If you target the template DNA strand, the gRNA sequence will be the same as the transcribed (nontemplate) sequence.
- (2) If you target the nontemplate strand, the gRNA sequence will be the reverse-complement of the transcribed sequence.
- (3) If targeting the 5' UTR or Coding region, you must design the gRNA sequence to target the nontemplate strand
- (4) If targeting the operator or promoter region, you may design the gRNA to target either the template or nontemplate strand.