

1. 6 points. You seek use CRISPR-Cas9 from *Streptococcus pyogenes* to cleave within the blue colored region of this 100 bp DNA sequence:

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5' ggcaatcatggggatcattatcatgatccagactccccaagatcttctgt
3' ccgttagtaccctagtaatagtactaggtctgaggggttctagaagaca
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tgtgtcattaccgcaactaattcataactcagctaccaccattttaaag 3'
acacagtaatggcggttgattaagtattgagtcgatgggtggttaaattttc 5'
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List all of the 20-base variable regions of possible guide RNAs that would direct cleavage within this sequence. Use a caret mark (^) to indicate where the double strand break would occur when directed by each guide RNA.

2. 6 points.

- (a) What is a "spacer" in the CRISPR-Cas9 system?
- (b) What is a "protospacer" in the CRISPR-Cas9 system?
- (c) How could you tell from the three nucleotides nucleotides on each side of a 20 bp sequence whether it may represent a spacer or a protospacer?

3. 12 points. Brouns et al. (PMID: 18703739) engineered bacteria that were resistant to phage lambda infection by introducing artificial CRISPR spacers that were complementary to phage sequences, as discussed in lecture 2. The "C" series of spacers were complementary to the coding strand of phage DNA, which is the strand that is not transcribed. The "T" series of spacers were complementary to the template (noncoding) strand, which is the strand that serves as template for synthesis of mRNA. (Please see the textbook diagram below. Assume that the corresponding protospacers were adjacent to PAM sites in the pahge.) Although most C and T spacers conferred phage resistance, the C spacers were overall much less effective than T spacers.

- (a) Propose a model for the difference in efficiency of C and T spacers.
- (b) If you like genetics, then explain how you might test your model genetically. If you like biochemistry, then explain how you might test your model biochemically. If you are not sure what you like, then propose any approach that makes sense to test your model.
- (c) Give the predicted outcome of your test in part (b) if your model is correct.

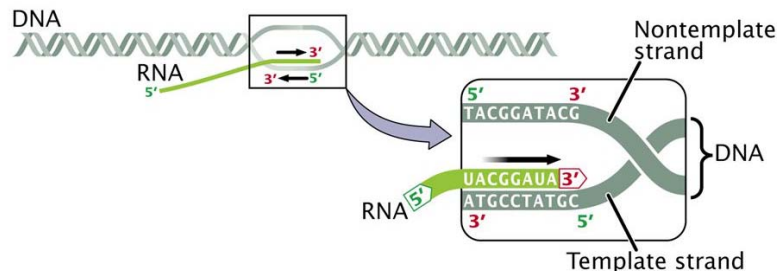


Fig. 13-04 Genetics, Second Edition © 2005 W.H. Freeman and Company