7.003 Spring 2022
Day 19 In-Lab Questions

1) Today, you will choose a SARS-CoV-2 viral protein to use as a target protein for engineering antibody binders against. What are some considerations to make when deciding on which protein to use as a target and on which particular regions of that protein to focus as a target?

2) What are some considerations to make when designing PCR primers (e.g. in terms of overall base composition and primer length)?

3) You have a short double-stranded DNA fragment (Fragment X) shown below:

```
Sense strand        5′ – CAG CAG CAG CAG CAG CAG TCA TCA TCA TCA TCA TCA – 3′
Anti-sense strand   3′ - GTC GTC GTC GTC GTC GTC AGT AGT AGT AGT AGT AGT – 5′
```

You want to design PCR primers to amplify Fragment X.

A) In the diagram below, write in an appropriate forward and reverse primer to amplify Fragment X. Label the 5' and 3' ends of your primers.

```
Sense strand        5′ – CAG CAG CAG CAG CAG CAG TCA TCA TCA TCA TCA TCA – 3′


Anti-sense strand   3′ - GTC GTC GTC GTC GTC GTC AGT AGT AGT AGT AGT AGT – 5′
```

B) Fill in the blanks:

The **forward primer** binds to the _____ strand and has the same sequence as the _____ strand, reading in the 5' → 3' direction.

The **reverse primer** binds to the _____ strand and has the same sequence as the _____ strand, reading in the 5' → 3' direction.

4) Suppose you wanted to amplify Fragment X with restriction sites flanking each end of the fragment. In your diagram from Question 3A, modify your forward and reverse primers to achieve this goal.

5) If you are cloning a gene into a plasmid with the intent of expressing that gene and producing functional recombinant protein, what are some additional considerations to make when designing your cloning strategy (e.g. in terms of required DNA elements and their locations, etc.)?

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