I. Abbreviations:

1. Write the full-form of the following:

a. Crossover hotspot instigator

b. Clustered Regularly Interspaced Short Palindromic Repeats

c. trans-activating crispr RNA

d. Transcription Activation Like Effector Nuclease

e. guide RNA

f. Zinc Finger Nuclease

g. Non-Homologous End Joining

h. Up regulated by AvrBs3

II. Multiple choice questions:

1. The step in which the bacteria generates "a historical genetic record of infection" is:

a. Acquisition

2. In order to acquire resistance to a certain strain of phage, what needs to happen to the CRISPR locus in the bacterial genome?

d. A and B

3. CRISPR is a _____ immune system:

d. DNA-encoded, RNA-mediated, DNA targeting

4. Which letter in the CRISPR acronym comes from a word that means the sequences involved in this can be read the same backward and forward?

d. P

5. Why is it so hard for even adaptive immune systems to fight viral infections over the long-term?

b. Viruses mutate rapidly

6. Which of these is a requirement of CRISPR-Cas9 genome editing?

a. 20 nucleotide editing site for your gene of interest with an adjacent PAM site on the 3' end

7. What parts of the CRISPR locus are homologous to viral DNA sequences?

a. Spacer

8. How does a genetic "knock out" alter the protein of the gene targeted?

a. **Inserting a piece of DNA that disrupts the coding sequence**

III. Short answer type questions:

1. **a.** Explain in brief, the four important structural features present in the avirulence gene AvrBs3: **Central repeat unit that can vary from 1.5 to 55.5 times with 34 to 35 aa, and 12th-13th aa make the Hypervariable region. C terminal has 3 NLSequence where 2nd and 3rd are critical for effector function, Acidic activation domain with bulky hydrophobic residues and left and right inverted repeats of 62 bases which confers horizontal gene transfer.**

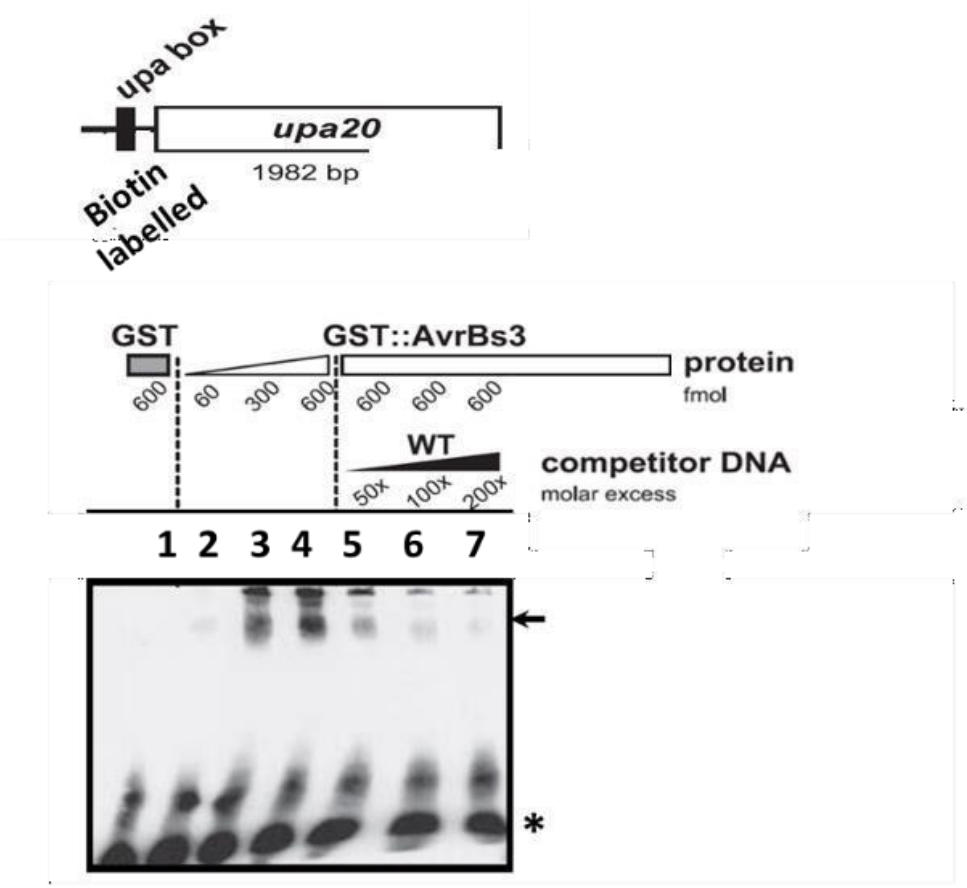
b. You have an avirulence gene "Y" that has two nuclear localization signals (NLS) at its C-terminal domain. How will you utilize the GUS reporter system to analyse the function of the NLS in onion epidermal tissues? **The idea is to generate individual mutants in the NLS or combined mutations in both NLS. These mutant proteins will be tested with GUS reporter to study the localization and effector significance of these sequences. If it is cytoplasm the NLS is not functional. If in the nucleus of the cell, then NLS is functional.**

c. Given below is the sequence of an UPA box. Write the Avr central repeat units that will bind to this box. (1 mark)

UPA Box – TATATAAACCTNNCCCT

T =0, A= NI/NS/NN, C= HD, T= NG, G= NK/NN/NH/NS

d. Explain all the lanes (1-7) in the provided figure of EMSA, where the promoter fragment of UPA20 is labelled with biotin; and AvrBs3 is purified over GST column. (2 marks)



Lane 1: Control where GST only is incubated with biotin labelled UPA box oligo

Lane 2: No complex formation can be observed in lane 2 GST-AVr.

Lane 3 and 4: When increased concentration of GST-AVrBs3 is used protein DN complex can be seen.

Lane 5,6 and 7: unlabelled UPA20 oligonucleotide is used as a competitor which binds strongly with the GST-AVr and the biotin labelled UPA20 is unable to form the complex.

e. When the TALEN was studied for scaffold optimization, it was found that a minimum of 127 amino acids preceding the central repeat units were critical. Explain the role of repeats: R0, R-1, R-2 and R-3 in the engineered TALEN scaffold. (1 mark)

Answer: Each repeats of TALEN scaffold optimization contained 2 alpha helices with an intertwining loop structure. It does not confer base pair binding specificity but it increases DNA-binding affinity in the engineered scaffold.

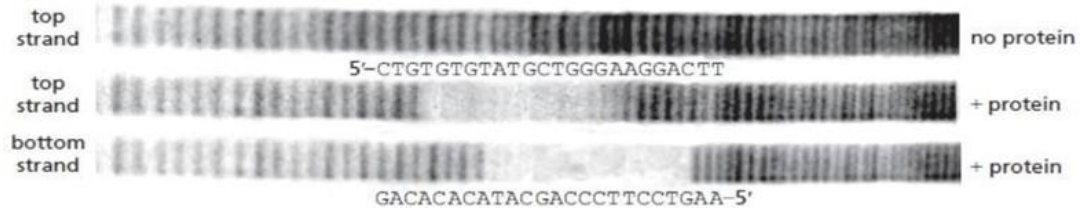
2. **a.** What do you understand by off-targets, in genome-editing? (1 mark)

Answer: Off-targets in genome editing refers to the non-specific binding and cleavage or modification of untargeted bases upon usage of nucleases for precise genome-editing.

b. What were the critical residues that were mutated to reduce off-targets and to generate obligate heterodimers of FOKI nucleases? (2 marks)

Answer: The essential mutations were (E490K; I538K and Q486E; I499L) for creating obligate heterodimers and reducing off-targets.

c. The image below represents the DNA sequences from the top and bottom strands of a DNA foot printing assay. As per the image, the footprints are slightly offset from one another, relative to the sequence of the DNA. Explain how can the footprints on the two strands be different? (2 marks)



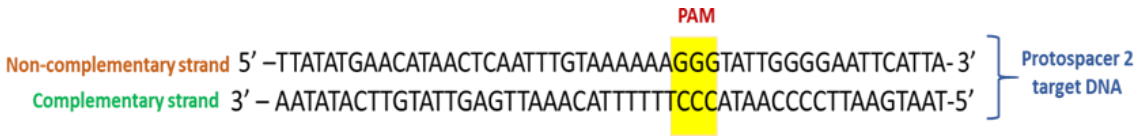
Answer: It is not surprising that the DNA foot printing on the two strand should be different. DNA is a three dimensional object, as is the protein. The interaction of the protein with the DNA need not protect the two strands identically. Especially at the margins of the footprint, one strand is likely to be more protected than the others.

Sec. IV. Descriptive type Questions:

1. Draw and briefly describe the three steps of adaptive acquired immunity in CRISPR-Cas system of the bacterial defense mechanism. (4 marks)

Answer: **Figure 1. Acquisition: cas-1 and 2 take part of protospacer and integrates into the array besides Leader sequence. Expression: During transcription these genes are transcribed and processed to generate mature RNA/gRNA. Interference: complementary base-pairing of guide RNA and target sequence allows nuclease activity of cas9 to degrade protospacer DNA.**

2. Following is the sequence where you have non-complementary strand and complementary strand in the protospacer target DNA. Now, design an experiment where you wish to make a nick only in the complementary sequence, exactly three nucleotides upstream of the PAM sequence; whereas there should **NOT** be any cleavage in the non-complementary sequence. Explain in terms of a schematic diagram. (4 marks)



Answer: NHN cleaves the complementary strand. And RuvCI nuclease would cleave non-complementary strand. Hence mutating RuvC1 (D10A) would curtail its nuclease activity. Therefore, only complementary strand would be cleaved by HNH and not he non-complementary strand.

3. **a.** Write in short, the role of RecBCD complex in Homology Directed Repair. (1 mark)

Answer: Role of RecBCD has both nuclease and helicase activity. This complex attaches the D-strand breaks and moves along DNA unwinding and degrading it. When the complex reaches the Chi sequence (which occurs at frequent intervals in E. coli), the nuclease activity decreases and

preferentially degrades the 5'end leaving a long 3'overhang which forms binding platform for RecA. Hence RecBCD is used in spacer acquisition process due to its ds-DNA break and also for creating long single stranded tails called pre-synaptic step.

b. What is NHEJ? Write two ways in which NHEJ repair occurs. (2 marks)

Answer: The NHEJ joins broken DNA ends together, for this no template is required. This is the only readily available pathway in G1. The NHEJ can occur via two methods: first, by simply ligation of the ends but during the process nuclease digestion can remove few nucleotides before ligation takes place; second, is resection which can expose single strand regions to align at a region of micro homology, the overhangs are trimmed and then ligated.

This nuclease digestion or resection means small section of DNA sequence is lost and therefore NHEJ is error-prone method of DNA repair.