MID-SEM Exam BT-637 Genome Editing and Engineering Total marks = 40

I. Abbreviations:

1. Write the full-form of the following:

a. Chi	c. tracrRNA	e. gRNA	g. NHEJ
b. CRISPR	d. TALEN	f. ZFN	h. UPA

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II. Multiple choice questions:

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

a. Acquisition
b. Expression
c. Interference
d. Transcription

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

a. New repeats need to be added
b. New spacers need to be added
c. A new PAM needs to be added
d. A and B

3. CRISPR is a _____ immune system:

a. RNA-encoded, DNA-mediated, RNA targeting
b. DNA-encoded, DNA-mediated, DNA targeting
c. DNA-encoded, RNA-mediated, RNA targeting
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?

a. C
b. I
c. S
d. P

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

a. Viruses have an immune system of their own that helps them stay alive
b. Viruses mutate rapidly
c. Viruses are small enough that they can go "unnoticed" by the immune system over the long-term
d. The immune system can only remember certain diseases for a finite period of time

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
b. 20 nucleotide editing site for your gene of interest with an adjacent spacer on the 3' end
c. 20 nucleotide editing site for your gene of interest with an adjacent PAM site on the 5' end
d. 20 nucleotide editing site for your gene of interest with an adjacent spacer on the 5' end

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

a. Repeat
b. Cas gene
c. Spacer
d. None of the above

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
b. Inserting a piece of DNA that disrupts the primer region of the gene
c. Removing a piece of DNA that is essential for transcription of the gene
d. Removing a piece of DNA that is essential for the translation of the gene

III. Short answer type questions:

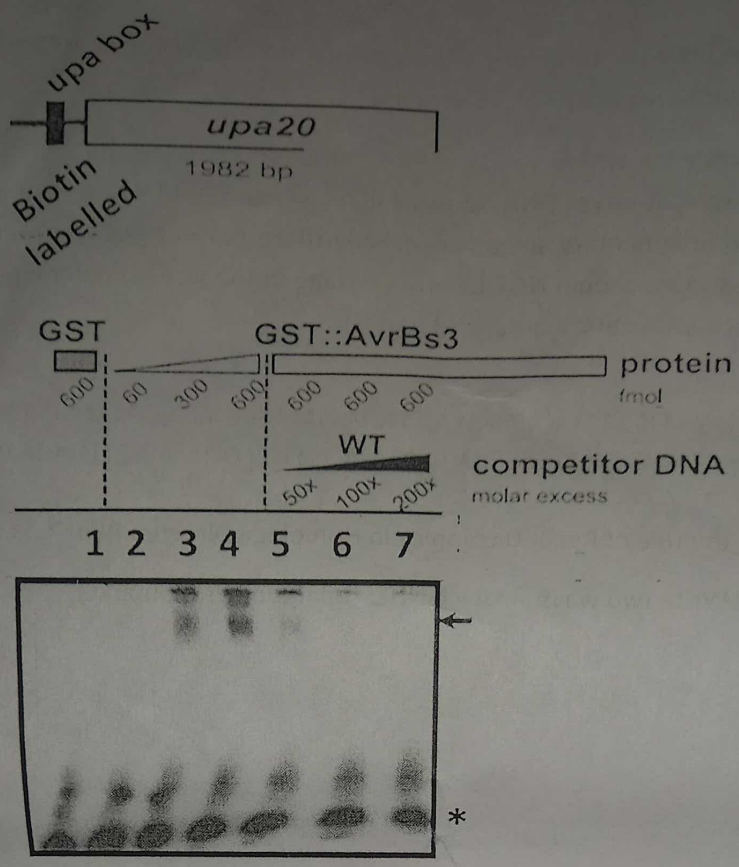
1. **a.** Explain in brief, the four important structural features present in the avirulence gene AvrBs3. (2 marks)

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? (2 marks)

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

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)

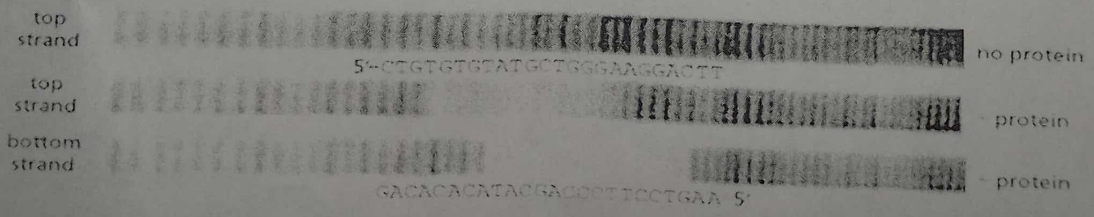


e. When the TALEN was studied for scaffold optimization, it was found that a minimum of 127 amino acids preceding the central repeat units were critical. Explain the role of repeats: R0, R-1, R-2 and R-3 in the engineered TALEN scaffold.(1 mark)

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

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

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)



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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)

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)

PAM

Non-complementary strand 5' –TTATATGAACATAACTCAATTTGTAAAAAAGGGTATTGGGGAATTCATTA-3' } Protospacer 2 target DNA

Complementary strand 3' – AATATACTTGTATTGAGTTAAACATTTTTTCCCATAACCCCTTAAGTAAT-5' }

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

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