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

Answer all the questions

I. Abbreviations: **3 marks**

1. Write the full forms of the following:

a) FokI **Flavobacterium okeanokoites** b) ZFN **Zinc Finger Nuclease** c) Avr **Avirulence** d) UPA **Up regulated by AvrBs3** e) TALEN **Transcription Activation Like Effector Nuclease** f) CRISPR **Clustered Regularly Interspaced Short Palindromic Repeats**

II. Multiple choice questions: **5 marks**

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

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

3. 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 Protospacer Adjacent Motif (PAM) needs to be added
d. A and B

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

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

III. Short answer type questions:

7. a. Explain in brief, the four important structural features present in the avirulence gene AvrBs3. 2 marks

Answer: Central repeat unit that can vary from 1.5 to 55.5times with 34 to 35 aa, and 12th-13thaa make the Hypervariable region. C terminal has 3 NLSequence where 2ndand 3rdare 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 2 marks

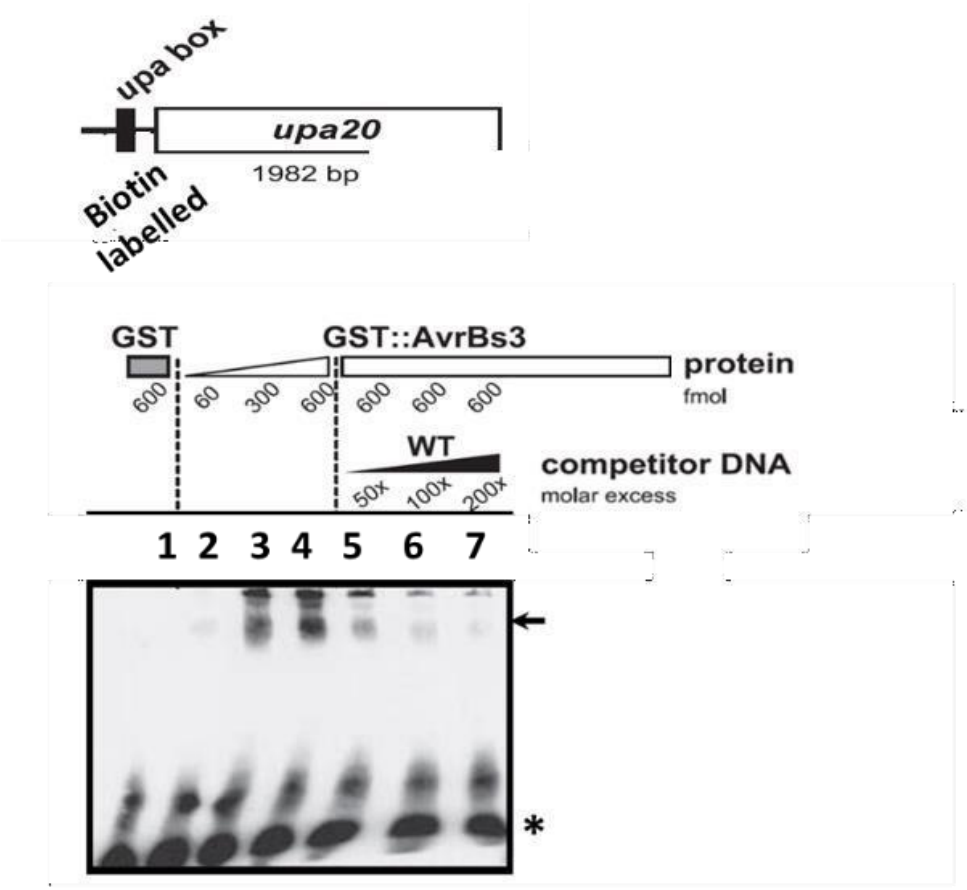
Answer: The idea is to generate individual mutants in the NLS or combined mutations in both NLS and compare it with the control sample where all NLS are functional. These mutant proteins will be tested with GUS reporter to study the localization and effector significance of these sequences. If it is 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. 3 marks

UPA Box – TATATAAACCTNNCCCT

Answer= 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. 3 marks



Lane 1: Control where GST only is incubated with biotin labelled UPA box oligo
Lane 2: No complex formation can be observed in lane2GST-AVr.
Lane 3 and 4: When increased concentration of GST-AVrBs3 is used protein DNa 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. 2 marks

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.

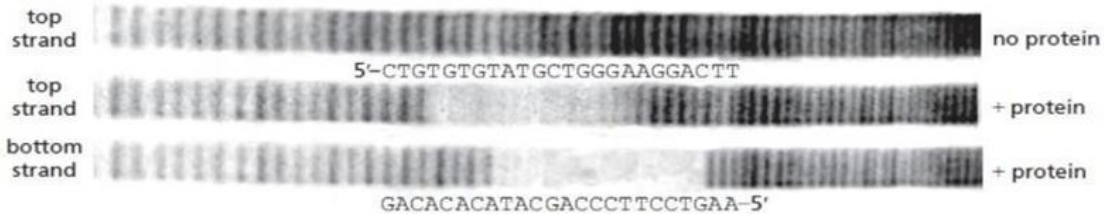
8. a. What do you understand by off-targets, in genome-editing? 2 marks

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? 3 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? 3 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.

IV. Descriptive type Questions:

9. Why do you suppose the zinc finger motif is thought to enjoy a particular advantage over other DNA-binding motifs when the strength and specificity of the DNA–protein interaction needs to be adjusted during evolution? 4 marks

Ans: The zinc finger motif is considered advantageous over other DNA-binding motifs when the strength and specificity of the DNA-protein interaction need to be adjusted during evolution for several reasons:

Modularity: Zinc finger domains are modular, meaning that they can be combined in various arrangements to recognize specific DNA sequences. Each zinc finger typically recognizes three DNA bases, and by combining multiple zinc fingers, a protein can target longer and more specific DNA sequences. This modularity allows for flexibility in DNA binding and target site recognition.

DNA Recognition Code: The zinc finger motif follows a relatively well-understood DNA recognition code. This code is based on the interactions between amino acid residues within the zinc finger domain and the DNA bases. This predictability makes it easier to design or evolve zinc finger proteins to bind to specific DNA sequences.

Engineering Versatility: Zinc finger domains can be engineered or evolved in the laboratory to bind to virtually any desired DNA sequence. This versatility has led to the development of zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) for genome editing applications. Researchers can customize zinc finger proteins to target specific genes or genomic regions.

Natural Occurrence: Zinc finger motifs are naturally occurring and are found in a wide range of organisms. This natural prevalence suggests that they have evolved to provide a balance between specificity and adaptability in DNA-protein interactions.

Historical Success: Zinc finger domains have been successfully used in various biotechnological and therapeutic applications, including gene editing, gene regulation, and gene therapy. Their track record of success in these applications has contributed to their continued use and development.

Overall, the zinc finger motif's combination of modularity, predictability, versatility, and historical success makes it a preferred choice when fine-tuning the strength and specificity of DNA-protein interactions during evolution or in laboratory applications.

However, other DNA binding motifs, which function primarily as head to head dimers like homeodomain cannot be so readily formed into repeating units.

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

Answer: Figure1. 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.

11. List four key differences between ZFN and TALEN genome editing tools. 4 marks

Ans: ZFN (Zinc Finger Nucleases) and TALEN (Transcription Activator-Like Effector Nucleases) are both genome editing technologies, but they have some key differences:

1. DNA-Binding Domains:

- **ZFNs use zinc finger proteins as their DNA-binding domains. Each zinc finger recognizes a specific DNA triplet.**
- **TALENs use transcription activator-like effectors (TALEs) as their DNA-binding domains. TALEs have a modular structure, and each repeat recognizes a single DNA base.**

2. Specificity:

- **TALENs are often considered more specific than ZFNs because TALEs recognize single DNA bases, allowing for precise targeting.**
- **ZFNs recognize DNA triplets, which may have some limitations in terms of specificity.**

3. Design Complexity:

- **Designing ZFNs can be more challenging due to the need to engineer multiple zinc finger domains with high specificity.**
- **TALENs are considered easier to design because the TALE domains have a consistent one-to-one relationship with DNA bases.**

4. Flexibility:

- **TALENs are more flexible when it comes to changing their DNA-binding specificity. You can easily customize the TALE repeat sequence to target different DNA sequences.**
- **ZFNs may require more significant engineering efforts to retarget them to different DNA sequences.**

Both technologies have been used for genome editing, and the choice between them may depend on the specific application and the level of customization required.

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