Quiz-1 BT-637 Genome Editing and Engineering Date 04-09-2024
(Total = 10 Marks)

1. a) What is the full form of FokI? Flavobacterium okeanokoites

b) Name the subtype of FokI R.E. TypeII Shifted cleavage

c) Write down the recognition site of FokI. GGATG

d) How many base pair overhangs are created at the 5' end upon FokI cleavage? 4bp

2. a) What is the function of Trypsin, how does it function? Digest after Lysine and Arginine

b) Explain why trypsin cleavage of FokI differs in the presence and absence of oligonucleotide. B'caz some sites are blocked/hindered by oligonucleotides

c) What are the functional domains of FokI? N-termial to bind oligo and Cter for cleavage

d) What do you understand by the modular nature of FokI? Independent functional unit

3. a) Name the vector and its features used to overproduce FokI enzyme. pRRSfokIR, pCBfokIR, lacZ, ori, AmpR, restriction enzyme, BamHI

b) Explain a reconstitution experiment performed on the FokI domain. 41 KDa binds DNA and 30+11 kDa mixed together were able to bind DNA.

c) What are the catalytic sites in FokI? D450A, D467A, K469A

d) Draw an imaginary footprinting gel showing protection of recognition domain but no protection at the cleavage site.

4. a) Write down the molecular weight and total amino acids of FokI. 64.5KDa, 578aa

b) Write down the eight amino acids of FokI that contact oligonucleotides from the major groove. Q12, N13, R-79, W105,K225,R228, E220, N217

c) Based on biochemical and crystal structure data, explain why the footprinting assay did not show protection at the cleavage site? Cdomain was piggybag on D3 domain

d) Write down the amino acids of FokI involved in creating the dimer interface. R487-D483

5. a) If an enzyme cuts 8 bp sequences, how often will it cut the human genome compared to an enzyme that cuts 6 bp sequence? $4^{\wedge 6} = 4096 < 4^{\wedge 8} = 65536$

b) What is the full form of Ubx? ultrabithorax

c) How large is the recognition sequence of the Ubx domain? 9bp

d) What do you understand by a chimeric enzyme? Mixing different domains from distinct enzyme

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6. a) What is “A factor”, and where was it purified from? Xenopus laevis

   b) How can CNBr be utilized in mapping a protein sequence? Digests after Methionine

   c) How many__9____ repeat units were revealed in TFIIA?

   d) Each unit contained ___30___residues.

7. Name the seven conserved amino acids in each unit that provide the framework for tertiary folding. Cys2, His2, Tyr/Phe6, Phe17, Leu 23

8. Name the three amino acids present in the zinc finger helix and their positions that form contacts with bases. -1 Arg, 2 Asp, 3 Glutamine, 6 Arg

9. Explain the role of Aspartic acid at the 2^{nd} position in the zinc finger protein. To orient Arginine at -1 position for contacting base pair

10. What do you understand by off-target? Binding of DNA at undesired location apart from the targeted location

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