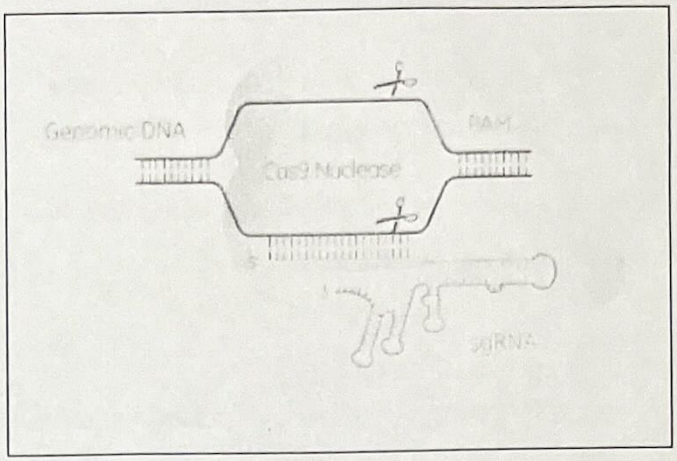
19-11-2024 BT-637 Genome Editing and Engineering Total = 30 marks

End-Semester Answer all the questions

Question 1. The CRISPR/Cas9 gene editing technology has emerged as a powerful technique with many applications in molecular biology and beyond. The illustration shows a general overview of the mechanism by which Cas9 functions together with a synthetic single-guide RNA (sgRNA).



(a) There have been other genome editing mechanisms used throughout the years such as zinc finger nucleases that showed promise. What is it about the CRISPR/Cas9 system that offers the potential for such exquisite site specific editing that was not a component of prior approaches to genome engineering (**2 marks**)?

(b) A modified version of the Cas9 protein exists where a single nuclease domain (indicated by the scissors) is inactivated. What would be the possible advantage of using such a form of Cas9 (**2 marks**)?

(c) Another modified version of the Cas9 protein exists where both nuclease domains (indicated by the scissors) are inactivated, so called dead Cas9 (dCas9). Suggest an application that this modified dCas9 protein could be used for (**2 marks**)?

(d) Name two specific challenges or concerns about using CRISPR/Cas9 as a potential therapeutic tool for genome editing. Why are these challenges/concerns (**4 marks**)?

(e) If you were treating patients with CRISPR/Cas9, would you choose to target a disease of the blood or a muscle disease? Provide the rationale for your decision (**4 marks**)?

The first attempt to use CRISPR/Cas9 genome editing for treating patients was carried out by Chinese scientists in the fall of 2016. These scientists isolated T-cells from a patient with cancer and used CRISPR/Cas9 genome editing to target the PD-1 gene and remove PD-1 protein in these cells. These cells, which are the patient's own cells, were re-injected into the patients. The PD-1 protein suppresses the immune response of the T-cells so these modified T cells should have the ability to mount a strong immune response against the cancer in these patients.

(f) Suggest a therapeutic approach where you could implement the power of CRISPR/Cas9. You need to provide details of what gene you would target, how you would edit the genome, and how you would actually introduce the modification to your patients. What cells are you targeting and why? Provide rationale for why you have opted to target this specific disease/condition. (**6 marks**)

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Question 2. Recently base editors are utilized more in clinics instead of CRISPR/Cas9.

a) Explain how does base editing differ from traditional CRISPR/Cas9-mediated genome editing in terms of its molecular mechanism? (**2 marks**)

b) What types of base editors are commonly used, and what chemical reactions do they catalyze to achieve base conversion? (**2 marks**)

Question 3. Provide and explain six key differences between Cas9 and Cas13 focusing on their structure, function and applications. (**6 marks**)

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