

Sample questions on DNA replication

- (a) The mechanism for initiating DNA synthesis by a DNA polymerase is fundamentally different from the mechanism for initiating RNA synthesis by a RNA polymerase. True or false? If true, identify the difference.

(b) Processivity is an inherent property of a DNA polymerase. Define processivity.

(c) What is the function of the 3' to 5' exonuclease activity associated with a DNA polymerase and what are the consequences in the cell of inactivating this function by a mutation?
- DNA synthesis by DNA polymerase III holoenzyme requires ATP. Identify the stage of DNA synthesis that depends on ATP and describe in detail this requirement.
- Describe the events leading up to and including the formation of bi-directional replication forks at *oriC*.
- Design a procedure to generate in a test tube negatively supercoiled DNA. Your starting material is relaxed circular DNA that is covalently closed in both strands. You have access to any reagent necessary, except for Topo I and Topo II enzymes.

5. A team of investigators carried out the following experiment: *oriC*-containing, superhelical plasmid DNA was incubated at 37°C in a reaction mixture containing the proteins dnaA, dnaC, dnaB, Hu, SSB (single stranded DNA-binding protein) and gyrase. The reaction also included ATP. At the end of incubation the reaction mixture was deproteinized by the use of a detergent and a protease. Following deproteinization the DNA was subjected to electrophoresis in agarose gels. It has been observed that the DNA now migrates faster than the original plasmid DNA.

(a) Identify the structure of the newly obtained DNA. How would you demonstrate that your identification is correct?

(b) What is the role of the proteins involved in generating this new DNA structure?

6. In *E. coli*, DNA polymease III holoenzyme is the enzyme responsible for replicating chromosomal DNA.

(a) What are the unique properties of this enzyme that make it distinct from other DNA polymerases in *E coli*?

(b) Describe the structure of DNA polymerase III holoenzyme and its relationship to function.