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Code - 00056763

BASIC BIOTECHNOLOGY (SET I)

Semester 1 Answer Key **PAPER 2**

Q. I] Do as directed: (Any Fifteen) 15

1. It separates DNA strands during replication
2. Any agent may be chemical, physical or biological leads to mutation.
3. Bolivar and Rodriguez.
4. Topoisomerse / DNA gyrase
5. *E.coli*
6. Semi-conservative
7. T4 and *E.coli.*
8. Intercalating mutagen eg. Ethidium bromide,acridine etc.
9. False
10. True.
11. True
12. *E. coli, B. subtilis*
13. Any chemical mutagen example can be given..5bromouracil,intercalating agent….
14. S phase of cell cycle.
15. amp +
16. Photolyase involved in photoreactivation can remove UV induced thymine dimers.
17. ^{32}P and ^{35}S
18. Type of point mutation which results in stop codon thus resulting termination of protein synthesis
19. DNA ligase.
20. Transformation, Conjugation, Transduction, Transposition.

Q.2 A] Initiation of replication in *Escherichia Coli.*–Description-4 Marks

DNA replication begins from origin. In E coli, replication origin is called OriC. About 20 molecules of Dna A proteins binds with 9 mer repeats along with ATP which causes DNA to wraps around dnaA protein forming initial complex. The dna A protein and ATP trigger the opening of 13 mer repeats froming open complex.Two copies of dnaB proteins (helicase) binds to 13 mer repeats. This binding is facilitated by another molecule called dnaC The binding of helicase is key step in replication initiation. dnaB migrates along the single stranded DNA in 5'-3' direction causing unwinding of the DNA.The activity of helicase causes the topological stress to the unwinded strand forming supercoiled DNA. This stress is relieved by the DNA topoisomerase (DNA gyrase) by negative supercoiling.The DNA polymerase cannot initiate DNA replication. So, at first primase synthesize 10±1 nucleotide (RNA in nature) along the 5'-3' direction. Primer is closely associated with dnaB helicase so that it is positioned to make RNA primer as ssDNA of lagging strand. (Diagram- 4 Marks)

Q.2 B] Meselson-Stahl experiment:Meselson-Stahl conducted their famous experiments on DNA replication using *E.coli* bacteria as a model system. Cells were grown in a ^{15}N-containing medium for several replication cycles and then were transferred to a ^{14}N-containing medium.At various times over several replication cycles, samples were taken, the DNA was extracted and analyzed by CsCl equilibrium density gradient centrifugation.After one replication cycle(generation) in the ^{14}N medium, all of the DNA had a density that was exactly intermediate between that of ^{15}N DNA and that of ^{14}NDNA.After two replication cycles, half the DNA was of the intermediate density and half was of the density of DNA containing entirely ^{14}N.These observations were exactly what the semiconservative model predicted.

Explanation- 4Marks and diagrammatic presentation- 3Marks

OR

Q.2 C] Two DNA molecules with nicks induced by an endonuclease in strands of the same polarity can invade each other with free single strands.A Holliday junction, or Chi structure, is formed as a recombination intermediate, and is solidified by ligase sealing the nicks, uniting the two homologues.Branch migration allows exchange of material, since the loose area around the junction allows unzipping/rezipping.New nicks allow separation of recombined DNA and resolution of the recombination intermediate.The Holliday model assumes reciprocal and equal exchange of genetic material between DNA molecules.—4 Marks, Diagram- 4 Marks

Q.2 D] The process of DNA is initiated by initiator protein which nicks at the site called the double stranded origin on one strand of the double strand. The initiator protein remains on the 5' phosphate nick strand, and the 3' hydroxyl end of the nicked strand is elongated by DNA polymerase III. The unnicked strand acts as the template strand for replication and the 5' phosphate nick strand is displace by helicase. Eventually, the nick strand is completely displaced by newly synthesized strand and will remove itself from the original circular DNA by the same initiator protein nicking at the terminating sequence on the nicked strand. The nicked strand then form a new single stranded circular DNA .DNA polymerase III then use the single strand as atemplate to form new double-stranded circular DNA molecule.

Description- 4 Marks and Diagram- 3 Marks

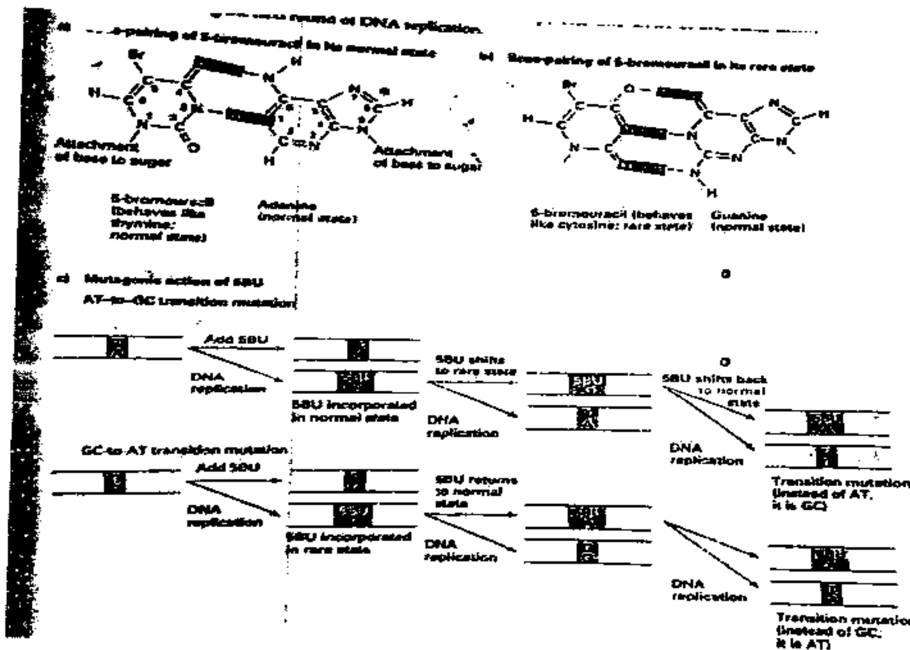
Q.3 A] Explain the term mutation 2 marks

Types of mutation can be explain in the form of table which should include Somatic, germline ,Spontaneous, induced, point,forword, backword,lethal,nutritional etc.(Types 6 marks)

Q.3 B] Chemical mutagen- 5-bromouracil as mutagenic agent. 07

5BU has bromine residue instead of the methyl group of thymine .In normal state 5BU resembles thymine and will pair with adenine in DNA,and in rare state it pairs with only guanine....

Diagrammatical explanation and brief explainations 3+4 marks



OR

Q.3 C] base pair substitution is change in a gene such that one base pair is replaced by another base pair e.g.AT to GC...(1 Marks)

Explain types Transversion, Transition, Missense, nonsense, neutral, silent and frameshift with examples 07 marks

Q.3 D] Explaination of Light repair 1 marks,Role of enzyme photolyase 1marks

Mechanism of photoreversal 5 marks

07

Q.4 A] *E. coli* was grown in medium containing either radioactive isotopes of phosphate and ~~sulphur. These were then infected by T2 phage and the progeny was collected. Two sets of T2~~ phage, one had proteins radioactively labelled with ^{35}S and the other had DNA labelled with ^{32}P. *E. coli* was then infected separately with both the types. When the ^{32}P phage was used, the radioactivity was found inside the cell. When the ^{35}S phage was used, radioactivity was found in

the phage ghosts. Since DNA entered the cells it meant that it carried the genetic information for new phage particles. This is how Hershey and Chase proved that DNA is the genetic material of the cell

Q.4 B] Two separate enzymes. one for cleaving and one for methylation. requires Mg^{2+} as a cofactor. Theses enzymes cut within the restriction sites to give either staggered ends or blunt ends. The restriction sites are 4 to 8bp in length. some restriction enzymes are isoenzymes. These enzymes can alter their specificity for the recognition sites under conditions like increasing temperature, increasing NaCl concentration.. 07

OR

Q.4 C] Griffith worked with two strains of *Streptococcus pneumonia*, the S strain produces smooth colonies and is highly virulent. It had a capsule. The R strain produces rough colonies and is avirulent. He worked with two varieties – II S and IIIS. The S type could mutate to R type and vice versa but variety II could not mutate to variety III. He injected mice with various strains. When mice were injected with II R bactertia they survived. When the mice were injected with III S bacteria they died. When the mice were injected with Idead II S bacteria they did not die. When mice were injected with a mixture of heat killed III S and live II R mice, the mice died and III S bacteria were recovered from the mice. Griffith concluded that the II R bacteria had been transformed by something from the dead III S bacteria and called it transforming principle.
Q.4 D] plasmid Bolivar and Rodriguez 322. it is 4362 bp long. It has Ori C of Col E1 plasmid. It contains two antibiotic resistant genes as markers, amp^r and tet^r. It contains unique restriction sites for 6 restriction endonucleases within the tet^r gene and two in its promoter. Three sites are present in the amp^r gene. If foreign DNA is cloned in any of these sites, insertional inactivation of the antibiotic resistant gene takes place. If a foreign DNA is inserted in the tetr gene, the recombinant plasmid will allow the cells to grow in presence of ampicillin only.

Q.5. Short notes (Any 3) 15

a. Expression vectors.
The vector which expresses the gene to give the gene product is an expression vector and to do so they contain the signal for protein synthesis. Expression of cloned gene is carried out by inserting a promoter sequence and terminator sequence.

b. DNA ligase.

To seal single stranded nicks in DNA which has 3' OH group termini or to seal two fragments of double stranded DNA. The two common sources are *E. coli* and T4 phage and the enzyme requires NAD and ATP as cofactors respectively. T4 enzyme can ligate both blunt and cohesive ends, the E. coli enzyme ligates only cohesive ends.

C. Role of Telomerase –DNA polymerase can synthesize new DNA only by extending a primer, there are special problems in replicating the ends- the telomere of eukaryotic chromosomes.Some cells have the ability to reverse telomere shortening by expressing telomerase, an enzyme that extends the telomeres of chromosomes. Telomerase is an RNA-

dependent DNA polymerase, meaning an enzyme that can make DNA using RNA as a templateThe enzyme binds to a special RNA molecule that contains a sequence complementary to the telomeric repeat. It extends (adds nucleotides to) the overhanging strand of the telomere DNA using this complementary RNA as a template. When the overhang is long enough, a matching strand can be made by the normal DNA replication machinery (that is, using an RNA primer and DNA polymerase), producing double-stranded DNA.The primer may not be positioned right at the chromosome end and cannot be replaced with DNA, so an overhang will still be present. However, the overall length of the telomere will be greater.

d. Intercalating agents: The intercalating mutagens including acridine , ethidium bromide, proflavin. Staining agents in DNA electrophoresis act by inserting (intercalating) between adjacent bases in one or both strands of DNA double helix.IF INTERCALETING AGENTS INSERTS BETWEEN ADJACENT BASE PAIRS OF DNA STRAND THAT IS THE TEMPLATE FOR NEW DNA SYNTHESIS,AN EXTRA BASE MUST BE INSERTED IN THE NEW DNA STRAND OPPOSITE THE INTERCALATING AGENT. After one more round of replication ,during which intercalating agent is lost,the overall result is a frameshift mutation due to the insertion of one base pair(CG) or deletion of one pair (TA).So an intercalating agent produces either a base pair addition or deletion.If this occurs in a protein coding gene,the result is frameshift mutation,this can be reverted by a second treatment with the same agents.

e. Biological mutagens

Any naturally occurring agent causing mutation are biological mutagen and mutations because of them are biological mutation 1mark.

Transposable agent explaination 2marks

Retroviruses and mechanisms 2 marks