Sub-Zoology

Paper- CC11 (Molecular Biology)

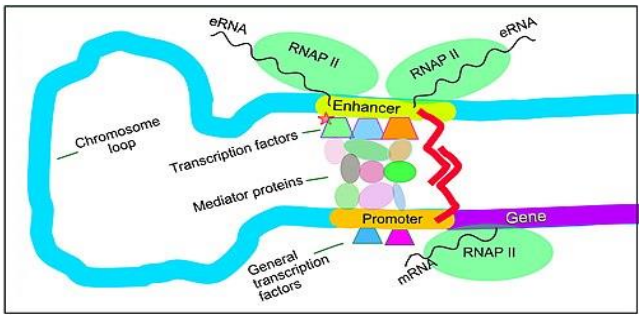
GROUP A

Q. 1. What is Replication Fork? **2*10=20**

Ans: The replication fork is a very active area where DNA replication takes place. It is created when DNA helicase unwinds the double helix structure of the DNA. The replication fork looks like a fork in the road that is composed of a leading strand and a lagging strand of DNA.

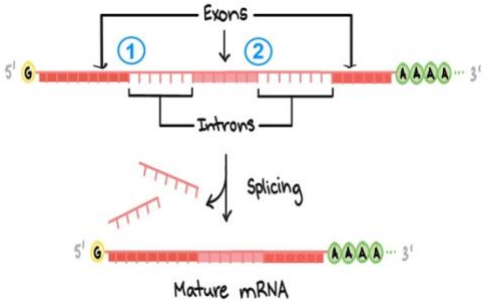
2.What is Enhancer?

Ans: In genetics, an enhancer is a short region of DNA that can be bound by proteins to increase the likelihood that transcription of a particular gene will occur. These proteins are usually referred to as transcription factors. Enhancers are cis-acting.



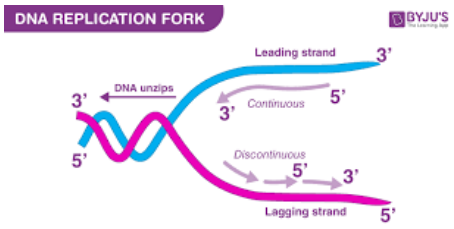
3. What is Splicing?

Ans:. Splicing is the process by which introns, the noncoding regions of genes, are excised out of the primary messenger RNA transcript, and the exons (i.e., coding regions) are joined together to generate mature messenger RNA. The latter serves as the template for synthesis of a specific protein.



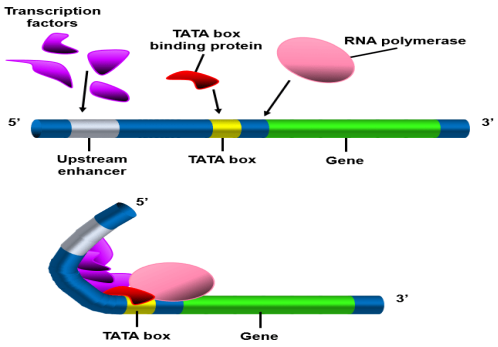
4**. . What is Leading strand and Lagging Strand?**

Ans: The strand that opens in the 3' to 5' direction towards the replication fork is referred to as the lagging strand. The strand that runs in the 5' to 3' direction in the replication fork is referred to as the leading strand Replication. The strand is replicated discontinuously.



5.What is TATA box?

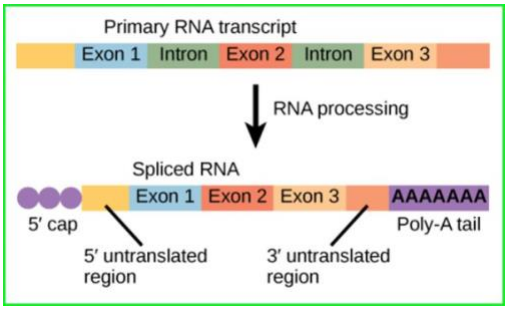
Ans: The TATA box is named for its conserved DNA sequence, which is most commonly TATAAA. Many eukaryotic genes have a conserved TATA box located 25-35 base pairs before the transcription start site of a gene. The TATA box is able to define the direction of transcription and also indicates the DNA stands to be read.



6. What is capping and Tailing?

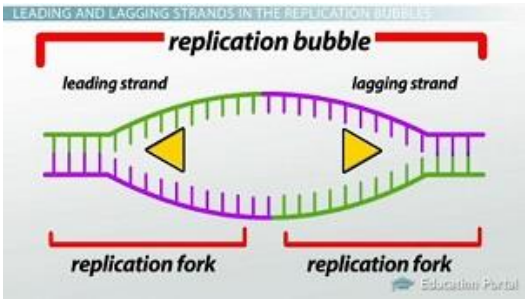
Ans : In capping an unusual nucleotide called methyl guanosine triphosphate is added to the 5- end of MRNA.

In tailing adenine is added to the 3-end of MRNA process of capping help recognising mRNA by the ribosomes.



7. What is Replication Bubble?

Ans: A replication bubble is an unwound and open region of a DNA helix where DNA replication occurs. Helicase unwinds only a small section of the DNA at a time in a place called the origin of replication. In eukaryotes, there are several origins of replication on each chromosome.



8. What is Activator?

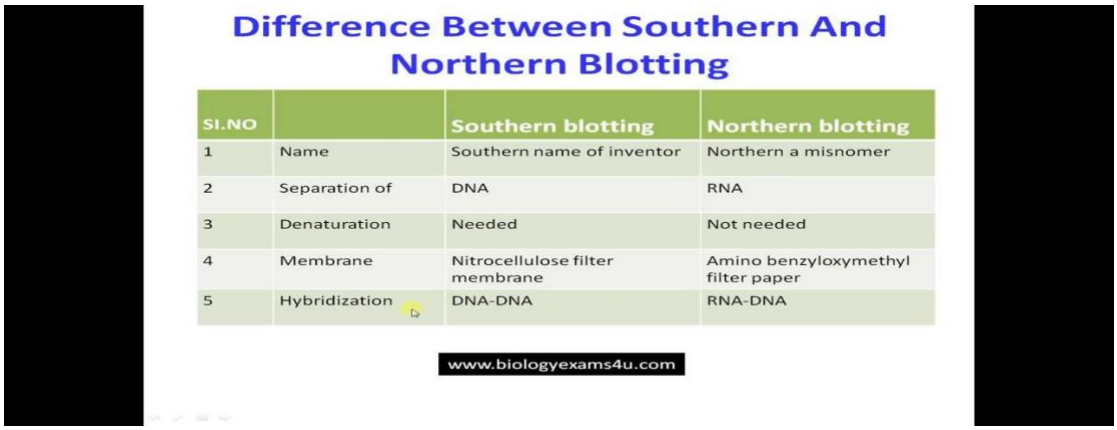
Ans: Transcriptional activators are required to turn on the expression of genes in a eukaryotic cell. Activators bound to the enhancer can facilitate either the recruitment of RNA polymerase II to the promoter or its elongation.

9. What is Chargaff's Rule?

Ans: **Chargaff's rules** state that in the DNA of any species and any organism, the amount of guanine should be equal to the amount of cytosine and the amount of adenine should be equal to the amount of thymine. Further, a 1:1 stoichiometric ratio of purine and pyrimidine bases should exist.

10. Write the difference between southern and northern blotting?

Ans:



GROUP-B

Q.1. What is Lac operon? Write the process of switch off and switch on condition of Lac Operon? 1+4=5

Ans:

⇩ **Defination**: "Lac operon is an operon or a group of genes with a single promoter that encode genes for the transport and metabolism of lactose in E.coli and other bacteria."

⇨ **Discover by:** F. Jacob and J.Monod.

⇩ **Switch off and Switch on Mechanism**:

(i)We divide the Lac Operon into two parts for easy understanding.

(ii)Switch off condition- when lactose sugar is absent in the cell.

(iii)Switch on condition- when lactose sugar is present in the cell.

⇨**Switch off condition**: When lactose sugar is present in the cell

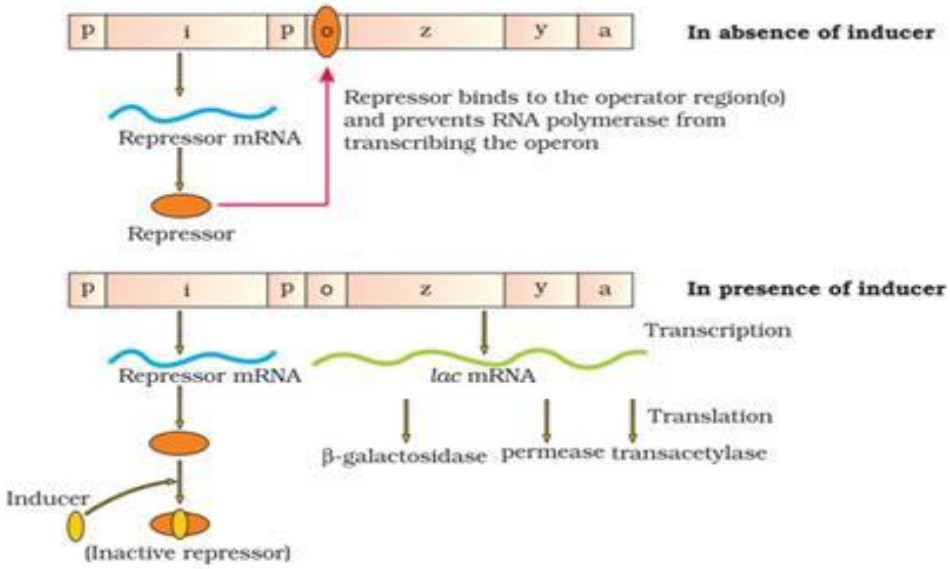
First, the transcription of the regulator gene produces the repressor m-RNA, which is then translated to form the repressor protein.

Now if the lactose sugar is absent in the cell, the repressor protein binds to the operator gene in the absence of lactose.

Due to which the operator gene becomes inactive, and there the switch off condition occurs.

Due to this, neither transcription nor translation takes place from the structural gene.

Due to which the synthesis of any kind of enzyme or protein does not take place from the structural gene.



Thus, if the lactose sugar is not present in the cell, the enzyme will not be formed from the structural gene.

Switch on condition: Switch on condition when lactose sugar is present in the cell-

The whole process starts as similar to earlier, but this time lactose sugar is present in the cell

It binds with the repressor protein, and the repressor-inducer complex is formed. This repressor cannot be attached to the inducer complex operator gene.

Due to which the operator gene is activated, and there the switch on condition occurs.

By which the coding of enzymes or proteins starts from the structural gene, that is to say, m-RNA is formed from the structural gene after transcription.

This m-RNA is called lac m-RNA. Upon translation of the Lac m-RNA (m-RNA), beta galactosidase, permease and trans-acetylase enzymes are formed.

The beta galactosidase enzyme will break down the lactose sugar, forming glucose and galactose.

And the *E.coli* bacteria cell will meet its energy requirement from this.

In this post, we learned how gene regulation is a mechanism in *E.coli* bacteria, and the synthesis of the beta galactosidase enzyme is regulated by structural genes being coded for.

Overall, if the *E.coli* cell contains lactose sugar, the beta galactosidase enzyme will form and if the cell does not contain lactose sugar, then the beta galactosidase enzyme formation will also not take place.

2:Write the advantage and disadvantage of PCR? What is Split gene? 4+1

Ans:

***Advantage:**

1. Highly specific: PCR can distinguish DNA sequences by just one nucleotide, making it a very accurate technique.

2. Sensitive: PCR is a very useful technique when the amount of DNA sample is limited because it allows the detection of even a single copy of a specific DNA template.

3. Versatile: The PCR technique can be used for various applications like genetic testing, criminal investigations, and paternity tests.

4. Rapid and efficient: PCR can efficiently and rapidly amplify a small amount of DNA sample to million copies in just a few hours.

***Disadvantage:**

1. Contamination: The PCR technique is very susceptible to contamination from other sources of DNA or RNA or the environment. This can mislead data interpretation.

2. Cost and complexity: PCR can be expensive and requires expert knowledge for high-throughput projects.

3. Lack of novel information: Since PCR can only amplify and target specific DNA sequences targeted by the primers, PCR provides limited information and cannot detect novel DNA sequences.

4. Inhibition from sample content: The whole PCR cycle can be disrupted by inhibitors that co-purify with DNA, such as heme from blood samples, reducing the sensitivity of the process.

5. Errors in amplification: Base substitutions, indels, and other alterations in DNA sequences can lead to inaccurate amplification and hence, false results.

Overall, PCR significantly impacts many research areas but careful quality measures should be performed while designing and interpreting PCR experiments.

***Split gene:**

A split or interrupted gene is defined as a gene consisting of introns (intervening sequences between exons) and exons (segments of an interrupted gene that are represented in the mRNA). Thus, a simple split gene has at least two exons and one intron.

3. Difference between Prokaryotic and Eukarytotic Transcription? What is Okazaki Fragment? 3+2

Ans:

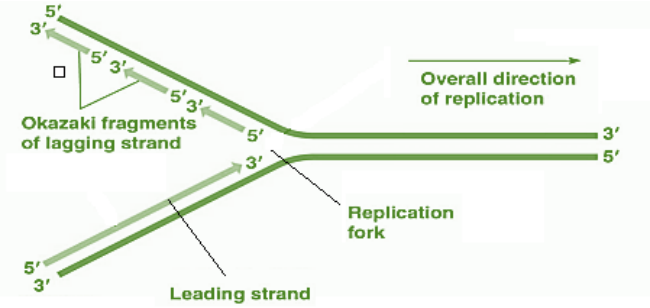
Prokaryotic transcription	Eukaryotic transcription
Occurs in the cytoplasm	Occurs in the nucleus
In the cytoplasm RNA are released and proceeds	In the nucleus RNA are released and proceeds
Five polypeptides constitute RNA polymerase	Ten to Fifteen polypeptides constitutes polymerases
Transcription and translation occurs at the same time	Transcription and translation do not occur simultaneously
It is not complex one	It is a complex one
One kind of RNA polymerase is released	Three kinds of RNA polymerase is released
Variation is less in promoters	Variation is more in promoters
Post-transcriptional modifications is absence	Post-transcriptional modifications is present
mRNA sequence is polycistronic	mRNA sequence is monocistronic.

***Okazaki Fragment:**

Ans:

***Defination:** Okazaki fragments are the short lengths of DNA that are produced by the discontinuous replication of the lagging strand.

The range of length of these fragments in the bacterial cells is about 1000-2000 nucleotides, while that in eukaryotic cells is approximately 100-200 nucleotides in length. The Okazaki fragments on the lagging strand are associated to generate a continuous new molecule of DNA.



4. Write down the function of DNA Polimarase I, DNA Helicase, DNA Ligase, SSBP, DNA Gyrase enzyme in DNA replication in Prokaryotes. **1×5= 5**

Ans:

***DNA Polimarase I:**

Function: DNA polymerase I of eubacteria functions in vivo to synthesize short stretches of DNA during excision repair and to remove RNA primers and fill the gaps between Okazaki fragments in lagging strand replication.

***DNA Helicase:**

Function: DNA helicases catalyze the disruption of the hydrogen bonds that hold the two strands of double-stranded DNA together. This energy-requiring unwinding reaction results in the formation of the single-stranded DNA required as a template or reaction intermediate in DNA replication, repair and recombination.

***DNA Ligase:**

Function: DNA ligase is a type of enzyme that facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond.

***SSBP:**

Function: SSBP or Single Strand DNA Binding Protein prevents the separated strands of DNA molecule from coiling back at the time of DNA replication process.

***DNA Gyrase enzyme:**

Function: DNA gyrase is an essential bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA. Gyrase belongs to a class of enzymes known as topoisomerases that are involved in the control of topological transitions of DNA.

GROUP C

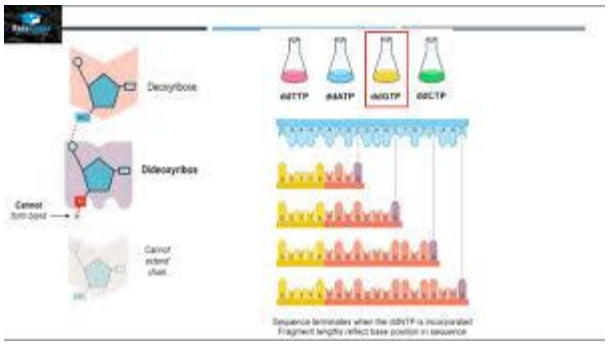
Q.1. Write the process of Sanger DNA Sequencing. Write the difference between rho-Dependent and termination in Transcription. What is Discontinuous replication? 5+3+2=10

Ans:

***Defination**: DNA sequencing refers to methods for determining the order of the nucleotides bases adenine, guanine, cytosine and thymine in a molecule of DNA. DNA sequencing can be performed by different methods, but there are two main methods are widely known to be used to sequence DNA.

Process: Sanger Method (Chain Termination Method) The chain terminator method is more efficient and uses fewer toxic chemicals and lower amount of radioactivity than the method of Maxam and Gilbert. The key principle of the Sanger method was the use of dideoxynucleotide triphosphates (ddNTPs) as DNA chain terminators, which are lack 3′OH.

(a) The DNA sample is divided into four separate reactions, containing all four of the standard deoxynucleotides (dATP, dGTP, dCTP, or dTTP) and the DNA polymerase and DNA primers.
(b) To each reaction, only one of the four dideoxynucleotide (ddATP, ddGTP, ddCTP, or ddTTP) is added, which represents the chain terminating nucleotide.
(c) When dideoxynucleotides are linked, the reaction in that piece of DNA will blocked because they lacking a 3'-OH group required for the formation of a phosphodiester bond between two nucleotides.
(d) The newly synthesized and labeled DNA fragments are heat denatured, and separated by size by gel electrophoresis with each of the four reactions run in individual lanes (lanes A, T, G, C).
(e) The DNA bands are then visualized by autoradiography or UV light and can be read.
(f) A dark band in a lane indicates a DNA fragment that is result of chain termination after combination of a dideoxynucleotide (ddATP, ddGTP, ddCTP, or ddTTP).
(g) The DNA sequence then will be easy to read from bottom to top.

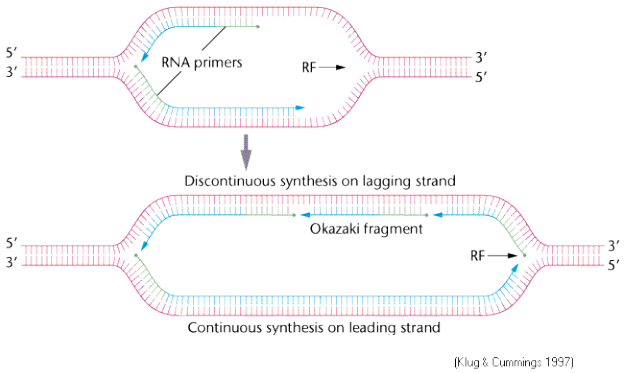


***Difference between Rho-Dependent and Rho-Independent Termination**

Rho-Dependent Termination	Rho-Independent Termination
Rho-dependent termination takes place when Rho binds to ribosome-free mRNA	Prokaryotes use intrinsic termination, also known as rho-independent termination, to indicate the termination of transcription and release the newly created RNA molecule.
Formation of hairpin loop structure does not take place	Formation of hairpin loop structure takes place
Rho factor utilises ATP	Rho factor does not utilise ATP
Uracil rich region in the transcript is absent	Uracil rich region in the transcript is present

***Discontinuous Replication**:

Defination: The synthesis of a new strand of a replicating DNA molecule as a series of short fragments that are subsequently joined together. Only one of the new strands, the so-called lagging strand, is synthesized in this way.



(Klug & Cummings 1997)