Assignment-1 (BioInfo)

DATE
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Q1> What does our microbiome do?

Sol> Gut microbes help us in digesting food, especially when the stomach and intestine are unable to digest certain foods. There are also associations being found that the microbes help us in producing certain vitamins, specially vitamins B & vitamins K. Microbiome also provides protection against disease causing bacteria. There are also articles suggesting an association between gut microbiomes and obesity. Eg. the bacteria Christensenellaceae minuta is more common in people with low body weight

Q2> Why does DNA form a helical structure?

Sol> The twisted ladder/double helical structure of DNA is primarily due to the tendency of bases (A, G, T, C) to avoid water (hydrophobicity) while sugars & phosphates are hydrophilic so the DNA is will have bases to the insides and sugars & phosphates outside. But to further minimize water from sneaking into the inside rungs, the ladder ~~[illegible]~~ twists a bit on one side.

Q3> What is the role of Sugar & Phosphates in DNA?

Sol> The sugar involved in DNA is called deoxyribose. Deoxyribose, phosphates and nitrogenous bases (A, G, T & C) form a DNA. The sugar is called deoxy as it does not have a hydroxyl group at 2' position.

DNA a double helix looks like a twisted ladder and the sides of the ladder are known as the 'sugar-phosphate backbone': This backbone consists of alternating phosphate and sugar groups, with the sugar molecule of one nucleotide linking to the phosphate group of the adjacent one.

The sugar-phosphate backbone is hydrophilic as it has a (-ve) charge that allows DNA to easily dissolve in water and is also used by DNA binding proteins. These proteins have (+ve) charges that bind strongly to the (-ve) DNA backbone and then help in transcription regulation etc. It holds the DNA together, allows it to dissolve in water.

Q4) Briefly explain the similarities b/w transcription and DNA replication?

Sol) Similarities are :-

a) Both processes take place in the nucleus.
b) Both involve hydrolysis of phosphodiester bonds to initiate the process
c) Both involve addition of specific 3' endings:
   in replication → GTGGTTA is added, in
   transcription → poly-A tail is added
d) Both occur from 5' end to 3' end
e) Both start off by relieving supercoiling using DNA topoisomerases
f) Parental DNA strand is broken for initiation.
g) Both require a template strand called the parental DNA strand as foundation.

Q5) Atleast 3 differences b/w transcription & translation.

Sol)

DNA ——(Transcription)——→ RNA ——(Translation)——→ proteins.

So as we can see transcription makes RNA whereas

of genes while translation synthesizes proteins from these RNAs.

Transcription	Translation
a) Location → Nucleus	Endoplasmic Reticulum / Cytoplasm (prokaryotes)
b) Products → mRNA, tRNA, rRNA, noncoding RNA	Proteins
c) Template → DNA	mRNA
d) Controller → RNA polymerase	Ribosomes
e) Components → DNA, RNA polymerase, σ subunit	mRNA, Ribosome, initiation factors, elongation factors, t-RNA
f) Processing → 5' cap is added, 3' poly A tail & introns are spliced	Post Translation modifications occur, eg → phosphorylation, SUMOylation,

Q6) i) ~~GO AUGGUG~~

5' AUG | GUG | GCC | UAU | CAU | UAG | GGG | CUU 3'

↳ start codon ↳ stop codon

Met	Val	Ala	Tyr	His

or | MVAYH |

ii) a new sequence

5' AUG | GUG | GCC | UAA | CAU | CAG | GGG | CUU 3'

↳ Start ↳ Stop.

Met	Val	Ala

or | MVA |

a single point mutation at 12th base from

U to A causes a stop codon earlier resulting in a smaller ~~protein~~ amino acid sequence.

∴ so the new sequence is

5' AUG | CGU | AGC | CUA | UCA | UUA | GGG | GCU | U 3'

↳ start codon

As the sequence does not contain a stop codon the ribosome will get stalled and then ~~the~~ this aberrant transcript will get decayed by a process called non-stop decay.
The amino acid sequence for this incomplete mRNA is as follows.

| Met | Arg | Gly | Leu | Ser | Leu | Gly | Ala |

M R G L S L G A

7) BamHI has only one recognition site on the SARS-CoV-2 genome at position 25313. forming 2 fragments of length 25313 bp and 4590 bp.

As only 1 cut is present, this RE is not of much use as for sequencing we need manageable sizes to sequence using our sequencing machine. BamHI on alone is therefore not a good RE for SARS-CoV-2 and needs to be used in conjunction with Xho-1 to extract nsp13 gene etc.

Q) Total length → 10000 bp

Single Digest		
EcoRI	→	8000, 2000
BamHI	→	5000, 3000

Multi Digest		
EcoRI + BamHI	→	5000 (A), 3000 (B), 2000 (C)

The Multidigest ~~resources~~ fragments can add up to form single digest fragments.

EcoRI

8000 → A / (B+C)

5000 + 3000

= [A + B]

2000 = [C]

BamHI

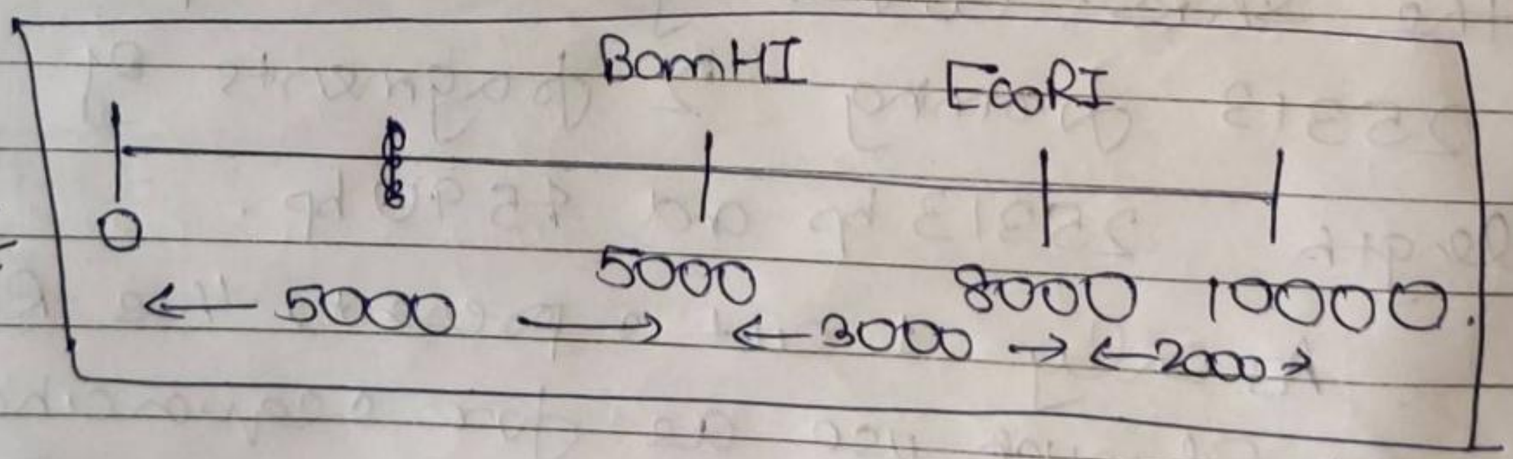
5000 → A / (B+C)

5000 → A / (B+C)

Q) aligning them together

[A A] + [B B] + [C C] → A+B+C.

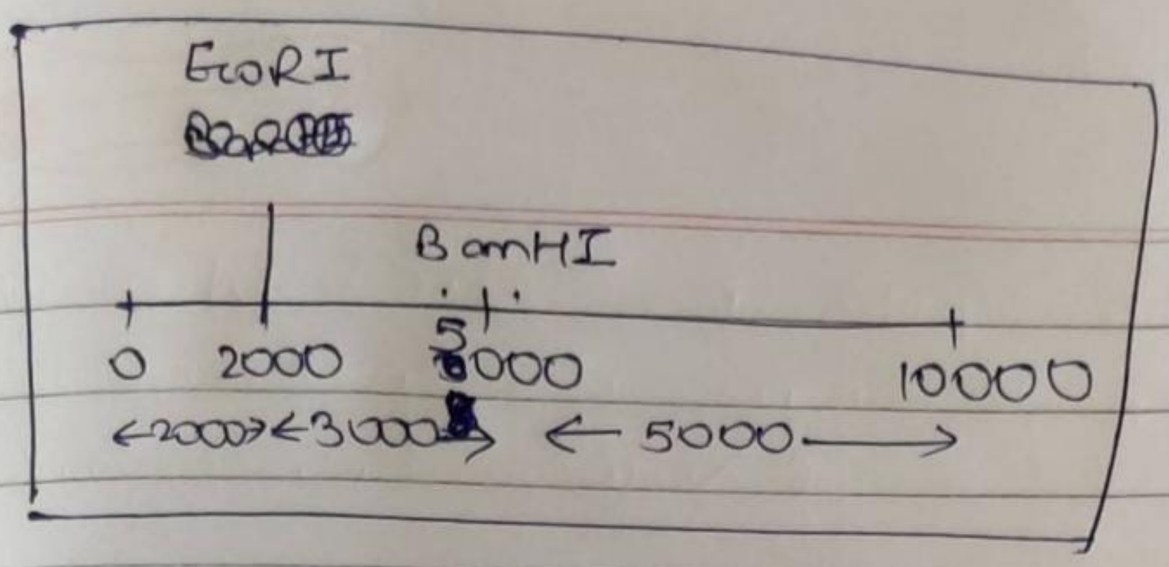
Ans 1



Q) but we can have another arrangement:

~~EcoRI~~

[C C] + [B B] + [A A] → C+B+A.



Ans 2

9) Our cloning vector (plasmid) has restriction sites for 2 restriction endonucleases (EcoRI & BamHI) but our DNA of interest does not have the recognition sites for these RE's therefore we need to cut our DNA using a RE whose recognition site is present. Once this is done, we can ligate it with linkers & adaptors using DNA ligase. ~~If we attach also which have~~. If we use a linker molecule, we need to use a linker molecule with an internal RE site for BamHI/ EcoRI whichever is used to digest our cloning vector. Then we can use the respective RE to form sticky ends in our linker molecule which is complementary to our cloning vector. If we use a adaptor, then we simple use the adaptor with EcoRI sticky end pattern and ligate it to our ~~DNA cut with~~ already cut DNA. Then on combination we can get our recombinant DNA.