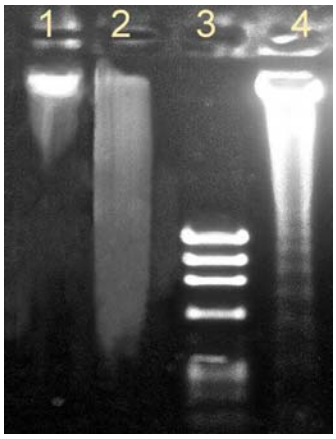
END-OF-COURSE ASSIGNMENTS

Topic 1. CHEMISTRY OF THE CELL

1. During a process called apoptosis that leads to natural cell death, genomic DNA is hydrolyzed into multiple fragments having 180 bp and n x 180 bp-length. The results could be analyzed by agarose gel electrophoresis (Figure 1).



← n x 180 bp

Fig. 1 Agarose gel electrophoresis of DNA extracted from cultured cells
1. Non treated cells, 2. Heat-killed cells, 3. DNA molecular size marker, 4. Cells collected after 24 h induction to apoptosis

1.1. How could you explain these results?
1.2. If you collect and analyze cells induced to apoptosis at 48 h after induction, what image do you expect to obtain after electrophoresis? Why?

Solution

1.1. Genomic DNA is fragmented into 180-bp and multiple of 180-bp corresponding to nucleosome size by caspases (enzymes which cut genomic DNA during apoptosis process). These fragmented products are expressed in agarose gel electrophoresis as "DNA laddering" (lane 4)
1.2. A clearer profile of "DNA laddering" with products having smaller molecular weight (bands migrating further) due to increased fragmentation of genomic DNA.

2. Compared to DNA extraction from cell, RNA extraction requires much more care to obtain intact RNA. What could be the reasons of this difference?

Solution

- RNA is a single-stranded nucleic acid and can be easily digested by endonucleases
- The 2'OH group of RNA is chemically labile and can be easily attacked by many environmental chemical factors leading to phosphodiester bond break.
- RNases are omnipresent and very hard to be eliminated (needs of special chemicals to destroy these enzymes)

3. Before a RNA solution is loaded in an agarose gel for electrophoresis, it must be heated 10 minutes at 65°C. What could be the reason?

Solution

RNA in solution can form secondary structures by weak chemical bonds. These secondary structures could interfere with RNA migration in the gel and give erroneous results. Heating RNA will break these bonds producing linear molecules.

4. You need two proteins having the capacity to bind to the same region in genomic DNA but one is regulated by thyroid hormone whereas the other is regulated by glucocorticoid hormone. The protein regulated by thyroid hormone represses the expression of genes it binds to whereas the other activates these same genes. How could you produce genes encoding these proteins?

Solution

Due to modular nature of many proteins, I can produce these proteins as follows:
Protein 1 : combination of a ligand binding domain which can binds thyroid hormone with a DNA binding domain which can recognize and bind to region A of the genome and finally a domain which represses transcription of genes having region A as regulatory sequence
Protein 2 : combination of a ligand binding domain which can binds glucocorticoid hormone with a DNA binding domain which can recognize and bind to region A of the genome and finally a domain which activates transcription of genes having region A as regulatory sequence.

5. Nucleic acids absorb UV radiation with maximum absorption at 260 nm wavelength. This absorption is mainly due to peripheral electrons of purines and pyrimidines and can be measured as optical density values.
A student measures the optical density of a nucleic acid solution before and after boiling and obtains the following values:

OD_{260} before boiling = 0.846
OD_{260} after boiling and rapid re-cooling (placing on ice) = 0.123
How can you explain these results?

Solution

This phenomenon is called hyperchromicity – an increased light absorption when double-stranded nucleic acids are denatured. The produced single strands with no hidden regions will absorb more, showed by increasing value of OD_{260}

6. Analysis of base percentage of a nucleic acid extracted from an unidentified organism shows that this nucleic acid is composed of 30% A, 15% C, 35% G and 20% T. What conclusion can you make about this organism?

Solution

That is an organism having single-stranded genomic DNA

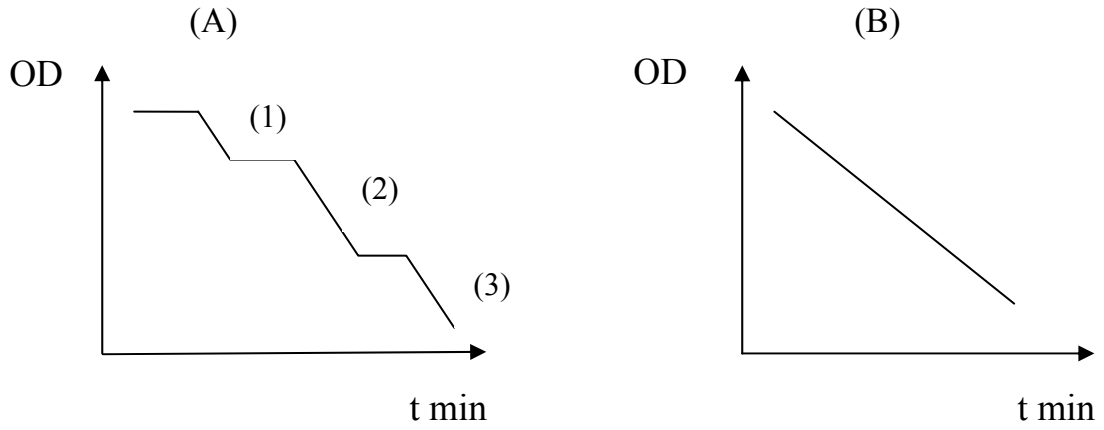
7. You are assigned the analysis of two samples, DNA and RNA extracts. The labels are unfortunately lost. How could you distinguish them?

Solution

- Measure of OD260 and hyperchromocity detection
- Use of DNase and RNase followed by electrophoretic analysis of digested products
- Use of uracyl-N-glycosylase followed by electrophoretic analysis of digested products

….

8. The following graphs show the reassociation rate of two genomic DNA samples, one from a bacteria and the other from a mammal. The genomic DNAs are denatured by boiling and are let to renature by slow cooling.



How could you explain these results?

Solution

(A) is the mammalian genomic DNA ; the dissociation profile is due to the different dissociation rate of three types of eukaryotic DNA sequences : (1) highly repetitive DNA sequences (microsatellite DNAs, …), (2) medium repetitive sequences (dispersed repeats, …), (3) unique sequences (protein-coding genes)

(B) is the bacterial DNA extract. Bacterial genome is nearly composed of unique sequences with little repetitive sequences present.

Topic 2. DNA REPLICATION

9. A student set up two reactions:

- Reaction I: enzyme buffer + DNA polymerase + 350 bp-DNA fragment + dNTP + a non-identified solution
- Reaction II: enzyme buffer + DNA polymerase + 350 bp-DNA fragment + dNTP

After 1h-incubation at 37°C, agarose gel electrophoresis analysis shows:

- A 350 bp-band from the reaction I
- No band observed from the reaction II

How could you explain these results?

Solution

Lacking of primers in the reaction II. In the reaction I, primers are present in cell extract

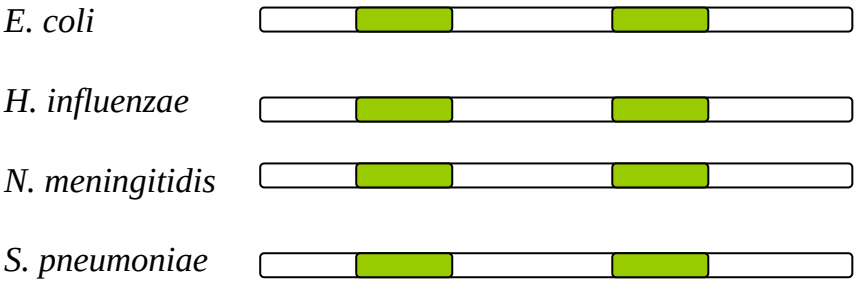
10. Cloning techniques aim at amplifying a specific gene ligated to a vector. The vector containing the inserted gene, called recombinant vector, is introduced into a host cell. Inside the cell, recombinant vectors replicate to give rise to multiple copies.

What is (are) the structural characteristic(s) of these vectors which allow this amplification?

Solution

Origin of replication

11. The structure of 16S rRNA gene of some eubacteria is shown in the following schema:



Conserved regions: similar sequences between different species

Varied regions: sequences specific to each species and different between different species

A scientist wants to: (1) detect all these eubacteria in the first step, and (2) specifically detect each of these species in clinical samples in the second step. He uses PCR (Polymerase Chain Reaction) technique which allows the *in vitro* amplification of DNA fragments.

How could he manage to do the first step? the second step?

Solution

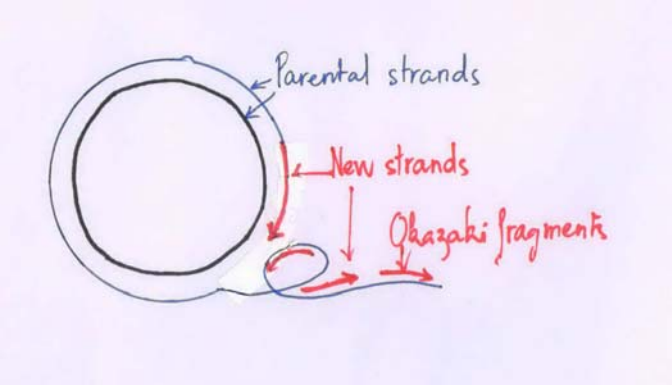
(1) To detect all eubacteria, he can design consensus primers in the conserved regions of all genomes. PCR with these consensus primers will amplify a region in all eubacteria having similar ends

2) In the second step, he can design two specific primers in varied regions which only amplify a species-specific DNA fragment, or he can design only one specific primer and reuse one of the consensus primer. In any way, he will detect a determined species.

12. Draw two replicons in the process of being replicated

13. Draw a scheme representing DNA replication by rolling circle to give rise to double-stranded DNA

Solution



Topic 3. DNA VARIATIONS

14. Sickle cell disease is expressed as abnormality of red blood cell shape (sickled-shape) due to abnormality of β-globin (a constituent of haemoglobin). Thalassemias are diseases caused by an abnormal low amount of globin proteins.

How can you tell about the possible causes of these diseases?

Solution

Sickle cell disease is due to a point mutation in the coding region of β-globin gene producing mutated β-globin proteins whereas thalassemias are caused by mutations in regulatory region of the genes leading to a decreased production of globin proteins

15. Explain how the Ames test can be used to detect mutagens

Solution

The Ames test uses a mutant strain of *Salmonella typhimurium*. This strain bears a mutation in the Histidine operon and can not survive in medium lacking histidine. When submitted this strain to mutagens, mutagens can induce new mutations which revert the mutated phenotype. In this case, the strain does not need histidine to grow. More the mutagens are powerful, more there are reverted colonies.

16. *Taq* polymerase used in PCR is a thermostable DNA polymerase which synthesizes DNA with a high error rate whereas *Pfu* polymerase has a low error rate. What makes this difference between the two enzymes?

Solution

Pfu polymerase has 3'5' exonuclease activities (proofreading activities) which *Taq* polymerase lacks.

17. Multiresistance to antibiotics in bacteria is due to transposition rather than mutations. Explain how a bacterium can become resistant to many antibiotics.

Solution

Multiresistance to antibiotics in bacteria easily occurs in environments where many bacterial species coexist, such as in hospitals or intestinal tracts. When a bacterium becomes resistant to many antibiotics, it could be caused by a combination of transposition of many genes responsible for antibiotic resistance through transformation as well as through conjugation between different individuals.

Topic 4. TRANSCRIPTION

18. Present a scheme showing the structure of a protein encoding DNA fragment, the related mRNA and polypeptide

19. Explain how the consensus sequences within bacterial promoters are established and how can one demonstrate that the promoter sequence is necessary for transcription initiation?

Solution

Multiple strong promoters, promoters that allow high expression of the downstream genes, are aligned and compared for each residue. The residue having the highest frequency among these promoters is retained for each position.

Point mutations as well as deletion can be made in the promoter sequence. Each combination is tested for transcription efficiency of a reporter gene.

20. To fight against some pathogens, people try to inhibit the production of pathogenic proteins inside the cell. Explain how could it be done?

Solution

Synthetic miRNAs and siRNAs can be designed to basepair with a region in the pathogenic mRNAs. Inside the cell, these short RNAs form double-stranded RNAs with the target RNAs and induce their degradation. They can inhibit translation by masking the ribosome binding site of the pathogenic mRNAs.

Topic 5. PROTEIN SYNTHESIS

21. What are the peptides that could be synthesized from the following DNA strand *in vitro* under nonstringent conditions: 5' TTGACGAGTAA 3'

Solution

The RNA synthesized from this template is 3' AACUGCUCAUU 5' or 5'UUACUCGUCAA 3'

Because there is no start codon, this RNA can be read in three reading frames:

(1) UUA CUC GUC → Leu Leu Val

(2) UAC UCG UCA → Tyr Ser Ser

(3) ACU CUG CAA → Thr Leu Gln

22. Puromycin is an antibiotic which inhibits the translational process. It can bind to the A site of the ribosome. Explain the mechanism of action of this antibiotic.

Solution

Puromycin binds to the A site and is considered as an aa-tRNA. This induces the transfer of peptide chain from the P site to puromycin, thus, terminates the translation.

Topic 6. REGULATION OF GENE EXPRESSION

23. What will happen if a mutation occurs in the coding region of the inhibitor gene of the lac operon leading to incapacity to bind lactose of the repressor, in the presence and absence of lactose?

Solution

The operon is always "closed" whether lactose is present or not because the repressor always binds to the operator and blocks the promoter from RNA polymerase binding.

24. An *E. coli* becomes insensitive to catabolite repression for lac operon. Give possible reasons for this.

Solution

- The binding site for CAP-cAMP is mutated and does not accept the complex
- The promoter is mutated and becomes a strong promoter whether or not the complex binds to its binding site

- A mutation occurs to the CAP protein that prevents its binding to the cAMP-CAP recognition site or its association to cAMP.

25. Could regulation by attenuation occur in a eukaryote? Why?

Solution

Attenuation can not occur in eukaryotes, because the transcription and the translation processes do not occur at the same time, due to the nucleus.