

Molecular Biology Questions and Answers – General Principles for Chain Termination Sequencing

1. Which of the following is not required for DNA sequencing?
 - a) Restriction digestion
 - b) Electrophoresis
 - c) Cloning
 - d) Polymerase chain reaction

Answer: c

Explanation: DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes the methods and technologies of restriction endonuclease, electrophoretic techniques and Polymerase Chain Reaction.

2. End labeled DNA sequencing is known as dideoxy method of sequencing
 - a) True
 - b) False

Answer: b

Explanation: The end labeled DNA sequencing is known as Maxam and Gilbert method. This procedure involves either 3' or 5' end labeling thus is also known as end labeled DNA sequencing.

3. The ^{32}P is added at the 3' end by polynucleotidyl kinase
 - a) True
 - b) False

Answer: b

Explanation: The ^{32}P dNTP is added at the 5' end by polynucleotidyl kinase. The ^{32}P dNTP is added at the 3' end by deoxynucleotidyl transferase. The end labeling is done in either one of the two ends.

4. The end labeled fragment is cleaved in how many pieces?
 - a) 2
 - b) 3
 - c) 4
 - d) 5

Answer: a

Explanation: The end labeled fragment is digested with a restriction

endonuclease which cleaves it into two unequal lengths of fragments. As a result, only one end of each of the two fragments thus produced will be labeled.

5. Restriction digestion is the only process to achieve sequencing by the Maxem and Gilbert method.
- a) True
 - b) False

Answer: b

Explanation: Restriction digestion is the one of the two processes to achieve sequencing by the Maxem and Gilbert method. The alternative method includes denaturation of its two complementary strands separated by gel electrophoresis.

6. The denatured strands cannot be separated.
- a) True
 - b) False

Answer: b

Explanation: The two complementary strands of DNA generally show different mobility during electrophoresis. This is because of their different molecular weight as one of the strands has a higher number of purines and the other pyrimidines.

7. What are the basic base – specific cleavage sites used in Maxem and Gilbert method?
- a) A, T, G, C
 - b) C, T, A+G, T+C
 - c) A, G, A+T, G+C
 - d) G, C, A+G, C+T

Answer: d

Explanation: The single end labeled double or single stranded DNA samples produced is subjected to base – specific cleavage. The bases – specific cutters are used to cut at one of the four sites G, C, A+G and C+T.

8. Which step is not involved in base – specific cleavage of DNA fragment?
- a) Modification of concerned base
 - b) Removal of modified base from DNA strand
 - c) Induction of random strand break
 - d) End labeled DNA fragments of variable lengths produced

Answer: c

Explanation: The strand breaks is induced in a specific position. This type of break is induced in the position from which a modified base is removed.

9. The process of electrophoresis is the key to sequencing.

- a) True
- b) False

Answer: a

Explanation: The digests from the four reaction mixtures, that is, G, C, A+G, C+T, are separately subjected to electrophoresis. This separates the fragments according to their sizes. The base sequence is thus determined by the sequential reading of the bands developed in the four lanes of the gel through electrophoresis.

10. The dideoxy method is also known as

- a) Maxam and Gilbert method
- b) Autosequencing
- c) Sanger's enzymatic sequencing
- d) Pyrosequencing

Answer: c

Explanation: The dideoxy method is also known as Sanger's enzymatic sequencing. It was developed by Fred Sanger and his co-workers in 1970s. It is also known as the chain termination sequencing.

11. Which enzyme is used for the replication in case of Sanger's method of sequencing?

- a) Polymerase I
- b) Smaller subunit polymerase I
- c) Polymerase III
- d) Larger subunit polymerase I

Answer: d

Explanation: One of the two complementary strands is used as a template for DNA replication in the dideoxy method of sequencing. DNA replication is catalyzed by the larger subunit known as the klenow fragment.

12. With respect to Sanger's enzymatic method of sequencing pick the odd one out.

- a) Radioactive dideoxynucleotides
- b) Primers
- c) Klenow fragment
- d) Restriction digestion

Answer: d

Explanation: In the reaction system for DNA replication, at least one of the four deoxyribonucleotides is radioactive in order to permit the autoradiographic development of bands after gel electrophoresis. A small primer sequence with a free 3' – OH group must be provided with the template strand of DNA replication to proceed, since a free 3'

– OH is absolutely essential for the larger subunit of DNA polymerase I to catalyze DNA replication.

13. The four reaction mixtures that were prepared, contained

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- a) dCTP, dTTP, dGTP, dATP
 - b) dCTP, ddTTP, dGTP, ddATP
 - c) ddCTP, dTTP, ddGTP, dATP
 - d) dCTP, ddTTP, dGTP, dATP

Answer: d

Explanation: Four different reaction mixtures are prepared for the replication of each DNA strand to be sequences. One of the systems contains 2', 3' – dideoxycytidine triphosphate in a concentration of about 1/100th of the normal amount of 2' – dideoxycytidine triphosphate present in the mixture. In each one of the other 3 reaction mixtures using the same DNA an template, 2', 3' – dideoxythymidine triphosphate (), 2', 3' – dideoxyadenosine triphosphate () and 2', 3' – dideoxyguanosine triphosphate () is used.

14. ddNTP acts as the chain radioactive markers.

- a) True
- b) False

Answer: b

Explanation: ddNTP acts as a terminator of the polynucleotide chain being newly synthesized on the template strand. Chain termination by ddNTP is achieved due to the fact that it does not have a 3' – OH group, as a result of which further nucleotides cannot be added to the new chain.

15. Strand separation for DNA sequencing is done to

- a) sort the fragments according to their size
- b) sort the fragments according to their molecular weight
- c) obtain consecutive sequence as per termination
- d) obtain specific lanes of sequencing

Answer: c

Explanation: The four single stranded sample reaction mixtures are subjected to electrophoresis. This separates the strands according to their size. The smallest is the fastest moving strand that migrates furthest from the negative terminal, and vice versa. Therefore, by comparing the bands of the four gels thus obtained, nucleotide sequence of the DNA fragment can be determined.

16. The position of band during electrophoresis indicates the dNTP used as chain terminator in that mixture.

- a) True
- b) False

Answer: b

Explanation: The position of a band during electrophoresis indicates the ddNTP used as chain terminator in that mixture. ddNTP is used as the chain terminator, dNTP is the normal nucleotide base.