Processes of Recombinant DNA Technology

1 Mark Questions

1.Name the enzymes that are used for the isolation of DNA from bacterial and fungal cells for recombinant DNA technology.
[All India 2014; Foreign 2014]
Ans. The enzymes used for the isolation of DNA from bacterial cells is lysozyme and fungal cells is chitinase.

2.How can bacterial DNA be released from the bacterial cell for biotechnology experiments? [Delhi 2011]
Ans. Bacterial cells are treated with lysozyme to digest the cell wall for releasing DNA.

3. Why is the enzyme cellulase used for isolating genetic material from plant cells but not for animal cells? [Delhi 2010]
Ans. Cellulase is used for digesting the cellulosic cell wall of plant cells. Animal cells do not contain cell wall, so cellulase is not required.

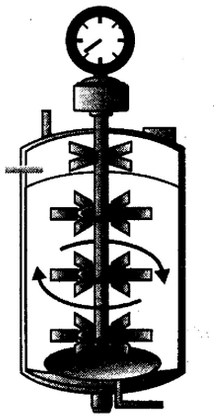
4. What is the host called that produce a foreign gene product? What is this product called? [Foreign 2010]
Ans. The host cells that produce foreign gene product are called transgenic organisms or Genetically Modified Organisms (GMOs). The product is called recombinant proteins.

2 Marks Questions

5. Name the source of the DNA polymerase used in PCR technique. Mention why it is used? [All India 2013,2012; Foreign 2011]

Ans. The DNA polymerase used in PCR is Tag polymerase extracted from Thermus aquaticus. It is a thermostable enzyme that can withstand high temperature used in the denaturation and separation of DNA strands. Hence, it can be used for a number of cycles in amplification.

6. Name the type of bioreactor shown. Write the purpose for which it is used? [All India 2011]

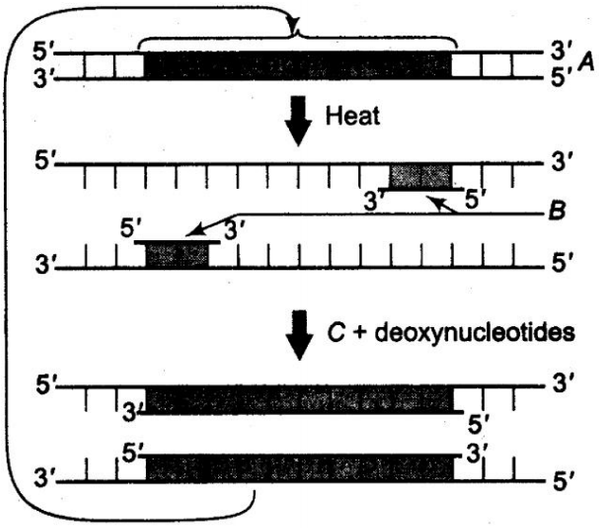


Ans. Figure is a simple stirred-tank bioreactor.
Bioreactors are used to produce large quantities of the desired gene products

7. (i) Mention the number of primers required in each cycle of Polymerase Chain Reaction (PCR). Write the role of primers and DNA polymerase in PCR.
(ii) Give the characteristic feature and source organism of the DNA polymerase used in PCR. [All India 2010]

Ans. (i) Two sets of primers are required. Primers are required for the addition of nucleotides to make multiple copies of the DNA of interest. The enzyme DNA polymerase extends the primers using the nucleotide provided.
(ii) The DNA polymerase is thermostable and withstands the high temperature treatment during denaturation of the DNA
it is obtained from a bacterium, Thermus aquaticus.

8. A schematic representation of Polymerase Chain Reaction (PCR) up to the extension stage is given below. Answer the questions that follow



(i) Name the process
(ii) Identify B.
(iii) Identify C and mention its importance in PCR. [Foreign 2010]
Ans. (i) A-Denaturation of the double stranded DNA.
(ii) B-Primers

(iii) C-DNA polymerase or Taq polymerase. **Importance in PCR**
It extends the primers using the nucleotides provided in the reaction medium and the genomic DNA as the template. Taq polymerase is thermostable and withstands the high temperature used in denaturation process.

9. Any recombinant DNA with a desired gene is required in billion copoies for commercial use. How is the amplification done? Explain. [Delhi 2010c]
Ans. Amplification of gene is done using polymerase Chain Reaction (PCR). it is carried out in the following steps:
(i) Denaturation The double stranded DNA is denatured by applying high temperature of 95°C for 15 seconds. Each separated strand acts as a template.
(ii) Annealing Two sets of primers are added, which anneal to the 3′ end of each separated strand.
(iii) Extension DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction. Taq polymerase is used in the reaction, which can tolerate heat. All these steps are repeated many times to get several copies of the desired DNA.

10. Explain the contribution of Thermus aquaticus in the amplification of a gene of interest. [Delhi 2009]
Ans. Thermus aquaticus provides thermostable DNA polymerase. It can withstand the high temperature used in denaturation and separation of DNA strands during Polymerase Chain Reaction (PCR). Hence, can be used for repeated amplification of DNA.

11. What are recombinant proteins? How do bioreactors help in their production? [All India 2009]
Ans. Recombinant proteins are produced by the expression of recombinant DNA in the transgenic organism. Bioreactors help in producing these proteins on a large scale as
(i) Large volumes of culture can be processed in bioreactors to produce desired quantity of

product.
(ii) These provide optimum conditions of pH, oxygen, salts, substrates, etc., to get the desired product.

12. Mention the three steps involved in each cycle of Polymerase Chain Reaction (PCR). How is repeated amplification of DNA made possible using PCR? [All India 2008C]

Ans. (i) A-Denaturation of the double stranded DNA.
(ii) B-Primers
(iii) C-DNA polymerase or Taq polymerase.

Importance in PCR

It extends the primers using the nucleotides provided in the reaction medium and the genomic DNA as the template. Taq polymerase is thermostable and withstands the high temperature used in denaturation process.

Repeated amplification of DNA in PCR is made possible by using thermostable DNA polymerase, which remain active during high temperature.

3 Marks Questions

13. (i) List the three steps involved in Polymerase Chain Reaction (PCR).
(ii) Name the source organism of Taq polymerase.
Explain the specific role of this enzyme in PCR. [Foreign 2014]

Ans. (I)Amplification of gene is done using polymerase Chain Reaction (PCR). it is carried out in the following steps:

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(ii) Annealing Two sets of primers are added, which anneal to the 3′ end of each separated strand.

(iii) Extension DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction. Taq polymerase is used in the reaction, which can tolerate heat. All these steps are repeated many times to get several copies of the desired DNA.

(II) (i) The DNA polymerase is thermostable and withstands the high temperature treatment during denaturation of the DNA
C-DNA polymerase or Taq polymerase. **Importance in PCR**

It extends the primers using the nucleotides provided in the reaction medium and the genomic DNA as the template. Taq polymerase is thermostable and withstands the high temperature used in denaturation process.

14. (i) What is a bioreactor? How does it work? (ii) Name two commonly used bioreactors. [Delhi 2014c]

Ans. (i) Bioreactors are large vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells or human cells.

The bioreactors work by providing optimal conditions to process the culture as well as the production of desired product by maintaining optimum pH, temperature, oxygen and other growth conditions required.

(ii) The two commonly used bioreactors are:
(a) Simple stirred-tank bioreactors
(ii) Sparged stirred-tank bioreactors.

15. What is a bioreactor used for? Name a- commonly used bioreactor and any two of its components? [All India 2014C]

Ans. A bioreactor is used for converting the raw materials into specific products biologically such as proteins, enzymes, etc., through the use of microbial, plant or animals cells.

The most commonly used bioreactors are of stirred type.
The two components of a stirred tank bioreactor are:
(i) In let for sterile air or oxygen
(ii) Agitator system
(iii) Temperature control system
(iv) pH control system
(v) Foam control system
(vii) Sampling ports (choose any two)

16. How is the amplification of a gene sample of interest carried out using Polymerase Chain Reaction (PCR)? [All India 2012]
or
Describe the process of gene amplification for rDNA technology experiments. [All India 201 ic]

Ans. Amplification of gene is done using polymerase Chain Reaction (PCR). it is carried out in the following steps:
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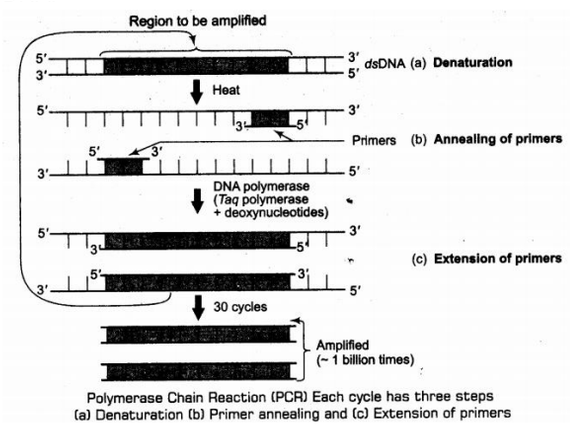
(ii) Annealing Two sets of primers are added, which anneal to the 3′ end of each separated strand.
(iii) Extension DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction. Taq polymerase is used in the reaction, which can tolerate heat. All these steps are repeated many times to get several copies of the desired DNA.



Polymerase Chain Reaction (PCR) Each cycle has three steps
(a) Denaturation (b) Primer annealing and (c) Extension of primers

17. How is the bacterium Thermus aquaticus employed in recombinant DNA technology? [All India 2008]

Ans. Thermus aquaticus, a bacterium yields DNA polymerase used in PCR in recombinant DNA technology.
(i) This enzyme remains active during the high temperature applied in the denaturation of double stranded DNA.
(ii) It extends the primers using the nucleotides provided in the reaction and the genomic DNA as template.
(iii) Repeated amplification is achieved by this enzyme. The amplified fragments, if desired can be used to ligate with a vector for further cloning.

18. What are bioreactors? List five growth conditions that a bioreactor provides for obtaining the desired product. [Delhi 2008C]

Ans. (i) Bioreactors are large vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells or human cells.
The bioreactors work by providing optimal conditions to process the culture as well as the production of desired product by maintaining optimum pH, temperature, oxygen and other

growth conditions required.
Growth conditions that a bioreactor provides for obtaining desired product are:
(i) Optimum temperature
(ii) Suitable pH
(iii) Salt
(iv) Vitamins
(v) Oxygen

5 Marks Questions

19. If a desired gene is identified in an organism for some experiments, explain the process of the following
(i) Cutting this desired gene at specific location.
(ii) Synthesis of multiple copies of this desired gene. [All India 2011]

Ans. (i) Cutting of desired gene at specific location is done by incubating the DNA with specific restriction endonuclease. Restriction
enzymes recognise a particular palindromic nucleotide sequence and cuts the DNA at that site.
(ii) Synthesis of multiple copies of desired gene is carried out by Polymerase Chain Reaction (PCR).
Amplification of gene is done using polymerase Chain Reaction (PCR). it is carried out in the following steps:
(i) Denaturation The double stranded DNA is denatured by applying high temperature of 95°C for 15 seconds. Each separated strand acts as a template.
(ii) Annealing Two sets of primers are added, which anneal to the 3′ end of each separated strand.
(iii) Extension DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction. Taq polymerase is used in the reaction, which can tolerate heat. All these steps are repeated many times to get several copies of the desired DNA.

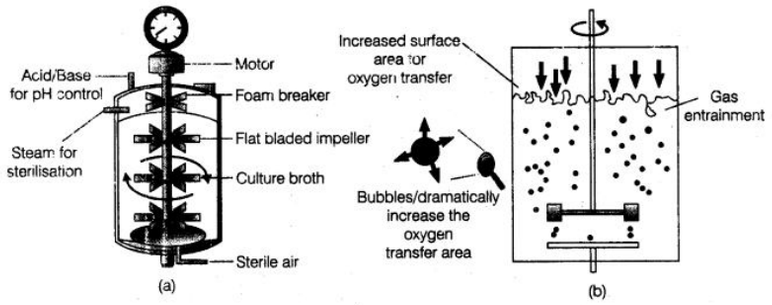
20. Name the source of taq polymerase.
Explain the advantage of its use in biotechnology. [All India 2009]

Ans. Taq polymerase is Pbtained from the bacterium Thermus aquaticus.
The enzyme is thermostable and can withstand the high temperature used for denaturation and separation of the two strands of DNA in PCR. Desired gene can be amplified to produce even a billion copy of DNA.
Thermus aquaticus, a bacterium yields DNA polymerase used in PCR in recombinant DNA technology.
(i) This enzyme remains active during the high temperature applied in the denaturation of double stranded DNA.
(ii) It extends the primers using the nucleotides provided in the reaction and the genomic DNA as template.
(iii) Repeated amplification is achieved by this enzyme. The amplified fragments, if desired can be used to ligate with a vector for further cloning.

21. What is a bioreactor? Draw a labelled diagram of a sparged stirred bioreactor. Explain its functioning. [All India 2009c]

Ans. (i) Bioreactors are large vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells or human cells.
The bioreactors work by providing optimal conditions to process the culture as well as the production of desired product by maintaining optimum pH, temperature, oxygen and other growth conditions required.

and



(a Simple stirred-tank bioreactor (b) Sparged stirred-tank bioreactor through, which sterile air bubbles are sparged

In the sparged stirred-tank bioreactor, sterile air bubbles are sparged. This increases the surface * sea for oxygen transfer, thus facilitating the growth and metabolism of cells and hence, productions of recombinant products as well.

Miscellaneous Questions

3 Marks Questions

1.Explain the basis on which the gel electrophoresis technique works. Write any two ways the products obtained through this technique can be utilised. [Delhi 2013C]

Ans. Gel electrophoresis technique works on the principle of separation of DNA fragment on the basis of electric charge.

Since, DNA is negatively charged molecule so, they can be forced to separate out according to their size towards anode under an electric field through a medium or matrix (commonly used is agarose). Shorter molecule moves faster and migrate further than the lohger one.

The products obtained through this technique can be utilised as follows:

(i) Construction of recombinant DNA by joining with cloning vectors.

(ii) Used in making multiple copies of same DNA by using PCR (Polymerase Chain Reaction).

2. How can the following be made possible for biotechnology experiments?

(i) Isolation of DNA from bacterial cell.

(ii) Reintroduction of the recombinant DNA into a bacterial cell.

[Foreign 2012]

Ans. (i) Isolation of DNA from bacterial cell can be done by:

(a) treating the bacterial cells with enzymes such as lysozyme to remove cell wall.

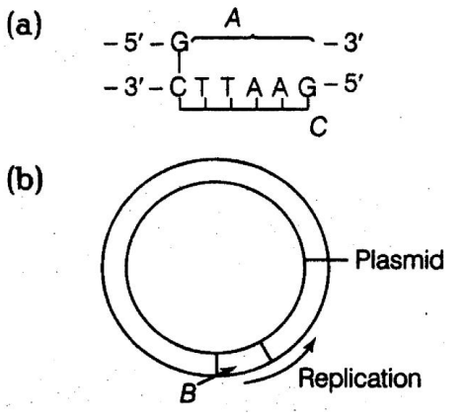
(b) the RNA associated with DNA can be removed by treatment with ribonuclease, whereas protein can be removed by treatment with protease. Similarly other molecules (if any) are removed by appropriate treatment.

(ii) Reintroduction of the recombinant DNA into bacterial cell can be done by the following methods:

(a) The recipient bacterial cell is made 'competent' to take up the recombinant DNA by treatment with a specific increase in concentration of calcium ions.

(b) the recombinant DNA is forced into the cells by heat shock treatment, i.e. by incubating the cells with rDNA followed by placing them at 42°C (heat shock) and then putting them back on ice. This enables bacteria to take up rDNA.

3. (i) Identify A and B illustrations in the following:



(ii) Write the term given to A and C and why?

(iii) Expand PCR. Mention its importance in biotechnology. [Delhi 2011]

Ans. (i) (a) A is recognition or restriction site (AATTC), which is recognised by restriction enzyme Eco

(b) B is rop gene protein involved in the replication of plasmid coded by this gene.

(ii) A and C are called palindromes. These are sequence of base pairs that reads same on the two strands of DNA, when orientation of reading is kept same.

(iii) PCR is polymerase chain reaction, multiple copies of the gene of interest can be synthesised in vitro by this technique. Thus, PCR can be utilised to amplify a single gene or fragment into thousands of copies to be used in cloning experiments.