NEET Important Questions with Solutions from Biotechnology - Principles and Processes

Q.1. Biolistics (gene-gun) is suitable for

A) Disarming pathogen vectors

B) Transformation of plant cells

C) Constructing recombinant DNA by joining with vectors

D) DNA fingerprinting

Answer: Transformation of plant cells

Solution: This method employs high-velocity gold or tungsten particles (0.2 to 0.4 micrometer) coated with foreign DNA. It is a vector-less method, also called particle bombardment or microprojectile bombardment, or biolistic transformation.

The particle gun accelerated particles penetrate even deep into the tissues. That's why it is used to transform plant cells which possesses cell wall. Because of the physical nature of the process, there is no biological limitation to the actual DNA delivery as compared to the Agrobacterium-mediated transformation, which can only be used in the case of dicot plants.

Q.2. The linking of the antibiotic resistance gene with the plasmid vector became possible with:

A) Exonuclease

B) DNA ligase

C) Endonuclease

D) DNA polymerase

Answer: DNA ligase

Solution: Several enzymes are needed for the recombinant DNA technology, like:

- Endonucleases or specifically Restriction endonucleases help in cutting the DNA.
- DNA ligases are needed for the joining of the sticky ends of gene to the vector.
- DNA polymerase helps in synthesis of nucleotide chain.

Q.3. Nucleic acid is fragmented by enzyme

A) ligases.

B) proteases.

C) nucleases.

D) polymerases.

Answer: nucleases.

Solution: Nucleases are class of enzymes that cleave the nucleic acid molecule i.e. RNA and DNA. They are of two types endonuclease and exonuclease.

Endonuclease cut DNA at internal sites while exonuclease cut at the ends.

E.g. RNase and DNase

Ligases are enzymes that carry out the joining of two fragments.

E.g. DNA ligase

Proteases are enzymes that carry out the function of cleaving the polypeptide chain in any protein.

Polymerases are enzymes that carry out polymerization reaction i.e. elongation of any chain.

All these enzymes are an integral part of the RDT.

Q.4. Which one of the following characteristics is generally not preferred for a cloning vector?

A) An origin of replication

B) An antibiotic resistance marker

C) Multiple restriction sites

D) A high copy number

Answer: Multiple restriction sites

Solution: Having multiple restriction sites in a single vector is not preferred as the plasmid/vector becomes prone to gets degraded by a variety of restriction enzymes.

Generally, an ideal vector possesses specific characteristics:
1) Origin of Replication (ori) should be present so that the vector is capable of autonomous replication inside the host organism.
2) Presence of multiple restriction sites for insertion of DNA molecule.
3) A selectable marker should be present to screen the recombinant organism (for example, antibiotic resistance gene).
4) The vector should be smaller in size for easy incorporation into the host machinery.

Q.5. Which of the following microbes transform normal plant and animal cells to cancerous cells, respectively?

A) Retroviruses and *Rhizobium*

B) *Escherichia coli* and *Agrobacterium tumefaciens*

C) *Agrobacterium tumefaciens* and Retroviruses

D) *Agrobacterium tumefaciens* and *A.rhizogenes*

Answer: *Agrobacterium tumefaciens* and Retroviruses

Solution: *Agrobacterium tumefaciens* is a plant pathogen and a natural genetic engineer. It integrates its plasmid genes into the plant genome and transforms it. When this bacteria infect a plant, a part of Ti plasmid called T-DNA is transferred to the plant chromosome. The tumour inducing gene (T-DNA) is replaced with a foreign DNA and this modified or disarmed plasmid is then used as a vector to infect and transfer our gene of interest into the plant cells.

Retroviral cloning vectors allows to clone the gene of interest and subsequently transfect into a retroviral packaging cell. Retroviral vectors are of particular interest because the genes they carry integrate into the target genome and can potentially provide permanent gene expression. Thus, these vectors are of choice in most of the clinical gene therapy trials to date.

Q.6. Agarose extracted from seaweeds is used in

A) spectrophotometry.

B) tissue culture.

C) PCR.

D) gel electrophoresis.

Answer: gel electrophoresis.

Solution: Gel electrophoresis this is a technique of separation of molecules such as DNA, RNA or protein. It occurs under the influence of an electrical field, so that they migrate in the direction of electrode bearing the opposite charge, since DNA fragments are negatively charged molecules, they can be separated by allowing them to move towards the anode (+ve electrode) under an electric field through a matrix of agarose gel.

Polymerase chain reaction (PCR): PCR is used in molecular biology to make many copies of (amplify) small sections of DNA or a gene (GOI). Using PCR it is possible for us to generate thousands to millions of copies of a particular section of DNA from a very small amount of DNA sample. PCR is a common tool used in medical and biological research labs for amplification.

Micropropagation is a technique of growing of tissues or cells in an artificial medium separate from the parent organism.

A spectrophotometer instrument used to quantitatively measure the transmission or reflection of visible light, UV light or infrared light.

Q.7. Which of the following is an incorrect statement?

A) Restriction enzymes are obtained from bacteria.

B) Restriction enzymes are called molecular scissors.

C) EcoRI was the first restriction enzyme to be isolated.

D) In 1978, Arber, Smith and Nathan were awarded the Nobel prize for the discovery of restriction endonuclease.

Answer: EcoRI was the first restriction enzyme to be isolated.

Solution: *HindII* was the first restriction enzyme isolated from the bacteria *Haemophilus influenzae.*

Arber, Smith, and Nathan were awarded in 1978 the Nobel Prize for Physiology or Medicine for the discovery of this restriction endonuclease.

Q.8. Restriction endonucleases

A) are used for in vitro DNA synthesis.

B) are synthesised by bacteria as a part of defence mechanism.

C) are present in mammalian cells for degradation of DNA when the cell dies.

D) are used in genetic engineering for ligating two DNA molecules.

Answer: are synthesised by bacteria as a part of defence mechanism.

Solution: Endonucleases perform their function by binding to the DNA at the recognition sequence, and then it cuts the two strands of DNA at specific points in their sugar-phosphate backbone. The restriction enzymes basically recognise specific palindromic nucleotide sequences in the DNA. Restriction endonucleases are synthesized by bacteria as a part of defence mechanism.

The DNA ligase enzymes are used in genetic engineering for ligating two DNA molecules. And all enzymes require Mg^{2+} as a cofactor for the endonuclease activity to be performed.

And Taq polymerase is an enzyme that extends the primer from its 3 end and generates newly synthesized strands in 3'-->5' end of the template strand during DNA replication. Caspase-Activated DNase (CAD) are enzymes that cut the DNA at inter-nucleosomal linker sites between nucleosomes. These are the only parts of the DNA strand that are exposed and thus easily accessible to CAD. These enzymes are present in mammalian cells for degradation of DNA when the cell dies.

Q.9. The endonucleases used in genetic engineering are

A) Type I

B) Type II

C) Type III

D) Type IV

Answer: Type II

Solution: *Restriction enzymes* are known as molecular scissors, and they cut at the internal position of the DNA at particular recognition site of $4-8$ Base pairs.

Type II Endonucleases: These are the simplest and the enzyme is made up of two identical subunits. They cut at the site of restriction sequence and the cutting is specific. Examples: EcoRI, Hind II.

Type II Enzymes consist of single, separate proteins for restriction and modification. One enzyme recognises and cuts DNA, the other enzyme recognises and methylates the DNA.

Type I: These are most complex. The enzyme is made up of three non-identical subunits. And they cut 1000 nucleotides away from the recognition site. The cutting is not specific. Examples: EcoR, EcoB.

Type III: These enzymes recognize short, non-palindromic sequences that can be methylated on only one strand.

Q.10. The term "competent" refers to

A) increasing the competition between cells

B) making cells impermeable for DNA

C) increasing the efficiency with which DNA enters the bacterium through pores in its cell wall

D) making cells permeable for divalent cations.

Answer: increasing the efficiency with which DNA enters the bacterium through pores in its cell wall

Solution: DNA cannot pass through the cell membranes as it is hydrophilic in nature. Hence, for the process of transformation, the bacterial cells must be made competent to take up DNA. In the calcium chloride method, the competency can be obtained by creating pores in the bacterial cells by suspending them in solution which is high in calcium concentration.

Calcium chloride helps in binding of the plasmid DNA to the cell membrane of bacteria and then be forced into the host cell by giving heat shock treatment at $42^{\circ}C$ for the transformation process.

Q.11. An extra free circular DNA material present in bacteria is

A) episome

B) plasmid

C) nucleosome

D) nucleoid

Answer: plasmid

Solution:

Plasmid:

A bacterial plasmid is an extrachromosomal circular DNA present independently in the cytoplasm of bacteria. A plasmid contains DNA as genetic material and, it is also present in the archaea and eukaryotic organisms.

They can replicate independently and usually contains certain specific genes which provide added advantage to the bacteria containing it. One such example is, it contains antibiotic-resistant genes.

A structural unit of a eukaryotic chromosome, here DNA is coiled around core of histones such is called nucleosome.

The region of undefined nuclear region in the cytoplasm of Prokaryotic cells is called nucleoid, and it contains chromatin which has the hereditary material of the cell.

One of a group of extrachromosomal genetic elements in bacteria is called episome and is capable of conferring a selective advantage upon the bacteria in which they occur.

Q.12. What will be the effect if pBR322, a cloning vector, does not carry 'ori' site?

A) Sticky, ends will not produce

B) Transformation will not take place

C) The cell will transform into a tumour cell

D) Replication will not take place

Answer: Replication will not take place

Solution: For the multiplication of any alien DNA in an organism, it needs to be a part of a chromosome with a certain sequence known as the 'origin of replication' (ori). If ori is not present in a cloning vector, replication will not be initiated.

pBR322 was the first artificial cloning vector constructed in the year 1977 by Boliver and Rodriguez.

p - plasmid

B - Boliver

R - Rodriguez

322 - the number assigned to the plasmid to distinguish this plasmid from other plasmids developed in the same laboratory.

Plasmid pBR322 has two resistance genes ampicillin resistance and tetracycline resistance genes. These genes are considered useful selectable markers that help select host cells containing the transformed vectors and eliminate the non-transformants.

Q.13. In biolistic method of gene transfer, the microparticles coated with foreign DNA are bombarded into target cells at a very high velocity. These microparticles are made up of

A) silver or tungsten

B) arsenic or silver

C) gold or tungsten

D) none of these

Answer: gold or tungsten

Solution: Plant genetic transformation is broadly achieved using biolistic microprojectile bombardment. Small particles of gold or tungsten coated with plasmid DNA is bombarded to pierce the cell wall and membranes without causing any damage to the plant cell. Because DNA delivery by particle bombardment is a physical process, that does not depend on bacteria, it has become a versatile and effective transformation method for many plants.

Q.14. *E. coli* cloning vector $\mathrm{pBR}\,322$ contains restriction sites (*Hind* III, *Eco*R I, *Bam*H I, *Sal* I, Pvu II, *Pst* I, *Cla* I)- ori, amp $\mathrm{amp}^{\mathrm{R}}$, $\mathrm{ret}^{\mathrm{R}}$, ret and rop. rop codes for the

A) antibiotic resistance genes

B) foreign DNA

C) selection of recombinants from non-recombinants

D) proteins involved in the replication of the plasmid

Answer: proteins involved in the replication of the plasmid

Solution: The Plasmids are small extranuclear, circular DNAs (a double-stranded, self-replicating) which carry extrachromosomal genes in bacteria and some fungi. These naturally occurring plasmids have been modified to serve as vectors in the laboratory.

The best-known plasmid vectors, which are also available commercially, are pBR322 and pUC-18. Both have been modified from natural plasmids of *Escherichia coli*. pBR322 vector has 'ori', rop, ampR, tetR and restriction sites of certain enzymes like *Hind* III, *Eco*R I, *Bam*H I, *Sal* I, *Pvu* I, *Pvu* II, *Cla* I etc. In this vector, term rop is used for proteins used in replication of plasmid.

Q.15. Which of the following steps should be performed by a person in order to visualise the bands of DNA fragments obtained from gel electrophoresis?

A) Exposure of DNA fragments to UV radiations

B) Staining with bromophenol blue followed by exposure to UV radiations

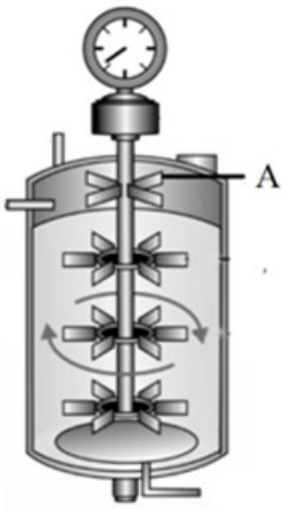
C) Staining with ethidium bromide followed by exposure to UV radiations

D) Person can see the bands without staining

Answer: Staining with ethidium bromide followed by exposure to UV radiations

Solution: EtBr (ethidium bromide) binds to the DNA and allows you to visualize the DNA under ultraviolet (UV) light. Ethidium binds by inserting itself between the stacked bases in double-stranded DNA. The intense fluorescence after binding of ethidium bromide with DNA is because of the hydrophobic environment found between the base pairs. Bromophenol blue is often used as a tracking dye gel electrophoresis.

Q.16. Study the given diagram below of the stirred tank fermenter. What is the function of part labeled A?



A) To maintain optimum pH.

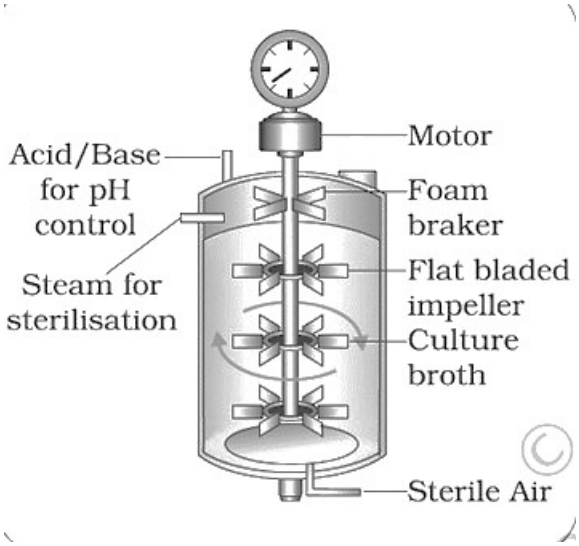
B) To control the foam within the reactor.

C) To maintain optimum oxygen concentration.

D) To facilitate even mixing.

Answer: To control the foam within the reactor.

Solution: A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents. The stirrer facilitates even mixing and oxygen availability throughout the bioreactor. The bioreactor has an agitator system, an oxygen delivery system, a foam control system, a temperature control system, a pH control system, and sampling ports.



Q.17. Which of the following is incorrect with respect to plasmids?

A) It is a circular DNA.

B) It carries antibiotic-resistance gene.

C) It has the ability of autonomous replication.

D) Its DNA is as long as chromosomal DNA.

Answer: Its DNA is as long as chromosomal DNA.

Solution: Plasmids are circular double-stranded DNA molecule that is physically separated from the chromosomal DNA and can replicate independently. The size of the plasmid is much smaller than the chromosomal DNA and varies from 1 to over 200 Kbp.

Q.18. In order to induce the bacterial uptake of plasmids, the bacteria are made competent by first treating with

A) Sodium chloride

B) Calcium chloride

C) Zinc chloride

D) Potassium chloride

Answer: Calcium chloride

Solution: In the calcium chloride method, the competency can be obtained by creating pores in the bacterial cells by suspending them in a solution which is high in calcium concentration.

Calcium chloride helps in binding of the plasmid DNA to the cell membrane of bacteria and then be forced into the host cell by giving heat shock treatment at $42^{o}C$ for the transformation process.

Q.19. If a person obtains transformants by inserting a recombinant DNA within the coding sequence of enzyme β-galactosidase, he will separate out recombinants from non-recombinants by which of the following observations?

A) Non-recombinant colonies do not produce any colour whereas recombinants give blue coloured colonies

B) Recombinant colonies do not produce any colour whereas non-recombinants give blue coloured colonies

C) Recombinants and non-recombinants both produce blue coloured colonies.

D) No colonies are formed due to insertional inactivation

Answer: Recombinant colonies do not produce any colour whereas non-recombinants give blue coloured colonies

Solution: Screenable markers have been developed as alternative selectable markers that help to differentiate recombinants and non-recombinants based on their ability to produce colour in the presence of a chromogenic substrate. In this, recombinant DNA is inserted within the coding sequence of an enzyme (3-galactosidase). This results in the inactivation of the enzyme, which is referred to as insertional inactivation. A chromogenic substrate's presence gives blue-coloured colonies if the plasmid in the bacteria does hot have an insert. The presence of insert results in insertional inactivation of the p-galactosidase, which results in no colouration of the colonies. These are identified as recombinant colonies.

Q.20. Restriction endonucleases are utilised in genetic engineering as

A) Molecular cement for combining DNA fragments into long chain.

B) Tools of recombinant DNA technology.

C) Molecular scalpels for cutting DNA at specific sites.

D) Both (B) and (C)

Answer: Both (B) and (C)

Solution: Restriction enzyme or restrictase or endonuclease is an enzyme that cleaves DNA into pieces or fragments at or near specific sites called restriction sites. GAATTC is the recognition sequence for the enzyme EcoRI.

They are extensively used in recombinant DNA technology and are often known as molecular scissors.

Q.21. Which of the following statements are correct for the enzyme Taq polymerase?
(i) It remains active during the high temperature induced denaturation of dsDNA.
(ii) It requires primers for carrying out the process of polymerisation.
(iii) It synthesises the RNA region between the primers, using dNTPs and Mg^{2+}.

A) (i) and (ii)

B) (ii) and (iii)

C) (i), (ii) and (iii)

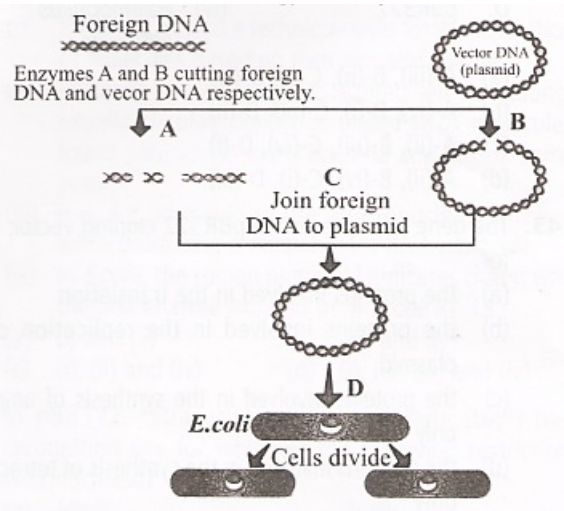
D) None of these

Answer: (i) and (ii)

Solution: The final step of PCR is the extension, wherein Taq DNA polymerase (isolated from a thermophilic bacterium *Thermus aquaticus*) synthesizes the DNA region between the primers, using dNTPs (deoxynucleoside triphosphates) and Mg^{2+}. The primers are extended towards each other so, that the DNA segment lying between the two primers is copied. The optimum temperature for this polymerisation step is $72^{o}C$. Taq polymerase remains active during high temperature-induced denaturation of double-stranded DNA.

So, the statements i and ii are true.

Q.22. The flow chart given below represents the process of recombinant DNA technology. Identify A, B, C and D.



A) A-Restriction endonuclease, B-Restriction exonuclease, C-DNA ligase, D-Transformation

B) A-Restriction endonuclease, B-Restriction endonuclease, C-DNA ligase, D-Transformation

C) A-Restriction endonuclease, B-Restriction endonuclease, C-Hydrolase, D-Transformation

D) A-Restriction endonuclease, B-Restriction endonuclease, C-Hydrolase, D-Transduction

Answer: A-Restriction endonuclease, B-Restriction endonuclease, C-DNA ligase, D-Transformation

Solution: A-Restriction endonuclease, B-Restriction endonuclease, C-DNA ligase, D-Transformation

Restriction enzymes are enzymes that have the ability to recognize specific DNA fragments called recognition sites and cleave at or near that specific recognition sites.

Ligase enzyme, also known as molecular glue, is an enzyme that can catalyze the joining of two large molecules by forming a new chemical bond such as C-O, C-S, C-N, or the linking together of two compounds.

Transformation is a method of horizontal gene transfer wherein short fragments of naked DNA or vectors are taken up by suitable host cells (usually bacteria).

Q.23. Identify the palindromic sequence among the following.

A) $\frac{GAATTC}{CTTUUG}$

B) $\frac{GGATCC}{CCTAGG}$

C) $\frac{CCTGG}{GGACC}$

D) $\frac{CGATA}{GCTAA}$

Answer: $\frac{GGATCC}{CCTAGG}$

Solution: A palindromic sequence is a double-stranded DNA or RNA sequence which, when read from 5' end to 3' end is the same as that on the complementary strand read from 3'end to 5' end.

Thus, $\frac{GGATCC}{CCTAGG}$ is a palindromic sequence.

Q.24. Which of the following statements is true about PCR?

A) *Taq* polymerase is isolated from a virus.

B) It is used for *in vivo* synthesis of DNA.

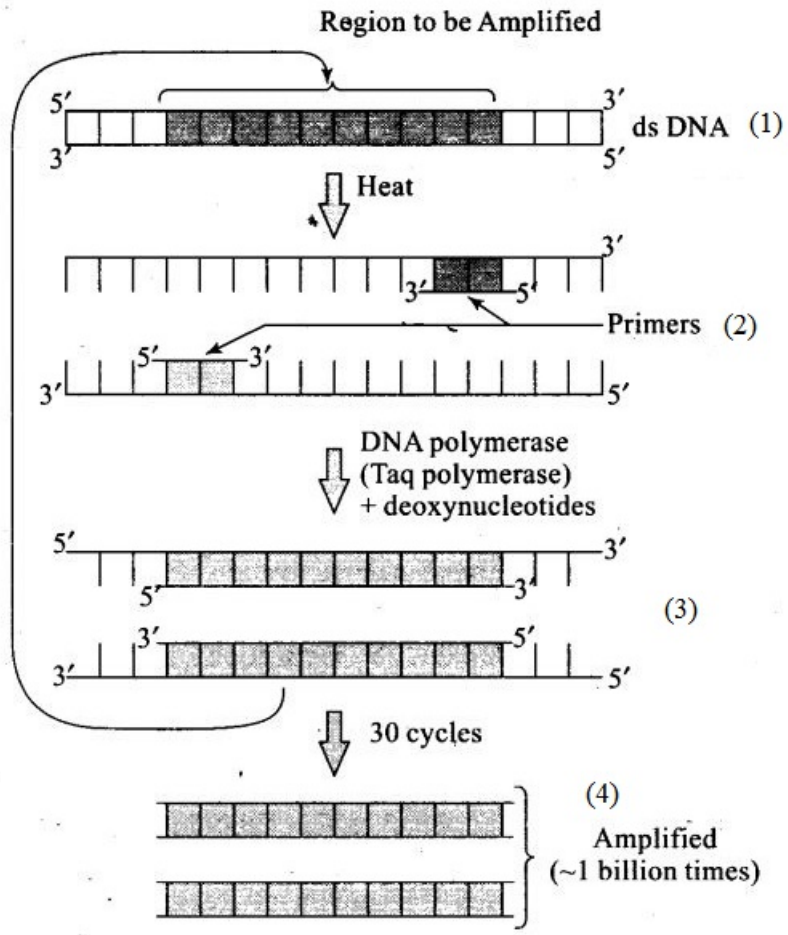
C) Primers act as selectable markers.

D) During amplification, *Taq* polymerase is used between annealing and extension.

Answer: During amplification, *Taq* polymerase is used between annealing and extension.

Solution: *Taq* polymerase is a thermostable DNA polymerase I, which is extracted from the thermophilic bacteria *Thermus aquaticus*. After annealing of primers to the two strands of DNA, T *aq* polymerase is added to one end of each primer to synthesize new strands of DNA, which is complementary to the template DNA molecules.

Q.25. The diagram given below refers to PCR. Identify the correct option.



A) 1 - Denaturation, 2 - Annealing, 3 - Extension, 4 - Amplification

B) 1 - Annealing, 2 - Denaturation, 3 - Extension, 4 - Amplification

C) 1 - Extension, 2 - Annealing, 3 - Denaturation, 4 - Amplification

D) 1 - Denaturation, 2 - Amplification, 3 - Annealing, 4 - Extension

Answer: 1 - Denaturation, 2 - Annealing, 3 - Extension, 4 - Amplification

Solution: The following are the steps in PCR:

1. **Denaturation** - In this step, the temperature of the reaction is raised, which leads to the melting of the two strands of DNA.
2. **Annealing** - In this step, the primers bind to the 3' end of the DNA strands.
3. **Extension** - This step involves the synthesis of a new strand of DNA by thermostable DNA polymerase.
4. **Amplification** - If the process of DNA replication is repeated continuously, the segment of DNA can be amplified to approximately a billion times, i.e., 1 billion copies are made at the end of 30 PCR cycles. It is possible to generate 2^n molecules after 'n' number of cycles.

Practice more on Biotechnology - Principles and Processes