4.3. GENETIC ENGINEERING

SYNOPSIS

- Natural recombination of genes occur during meiotic crossing over.
- Genetic engineering permits novel & desired combination of genes through rDNA technology.
- rDNA technology is a Laboratory technique of gene manipulation.

The important steps in recombinant DNA technology are performed through genetic engineering. They are given below.

1. Isolation of a desired gene
2. Insertion of the isolated gene into a suitable vector
3. Introduction of recombinant vector into the host
4. Selection of the transformed host cells

- **Isolation of a desired gene**

The first step in the isolation of desired gene from a cell is the digestion of the cell wall by **enzymatic action.**

Dissolution of all the biological membranes within a cell by **detergent lysis** (using high powered detergents).

The protoplasmic mass thus obtained is treated with phenols, proteases and suitable ribonucleases and further subjected to **gradient centrifugation**.

Ultimately purified DNA is precipitated out after the addition of chilled ethanol.

The purified DNA is cut into a number of fragments by enzymes called **restriction endonucleases.** The restriction endonucleases are a group of enzymes that recognise and cut DNA at specific locations.

Each restriction endonuclease functions by 'inspecting' the length of DNA and recognises a specific palindromic nucleotide sequence in that DNA.

Then it will bind to the DNA and cut each of the two strands of the double helix at specific points. These are also called **'molecular scissors'** and are obtained usually from bacteria.

They were first discovered by **Nathans** (1970)

Palindromic sequence of DNA is that which is the same in its two strands while reading in opposite directions.

The restriction enzymes cleave DNA molecules in two ways.

i) In one way they cut both strands of DNA at exactly opposite points to each other.

This results in DNA fragments with **blunt ends** or **flush ends**, where two strands end at the same point.

Such cut is generally termed as **even cut.**

```
                ↓
5' - AGCT - 3'  5'  AG  CT  3'
     | | | |   →    | |  | |
3' - TCGA - 5'  3'  TC  GA  5'
          ↑
     Event cut      Blunt ends
```

DNA cleavage by restriction endonucleases resulting in blunt ends. The vertical arrows indicate the site of cut in DNA strand

ii) But commonly, most enzymes cut the two strands of DNA double helix at different locations. Such a cleavage is generally termed as **staggered** cut.

Protruding complimentary strands readily pair with each other and such ends are called **cohesive** or **sticky ends.**

When cut by the same restriction enzyme, the resultant DNA fragments have the same kind of 'sticky ends' and these can be joined together readily by using **DNA ligases.**

This property of the restriction enzymes is of great value for the construction of recombinat DNA.

e.g :- The restriction enzyme *Eco* RI

E - The first letter, represents the name of genus *Escherichia.*

co- The next two letters, represent the species *Escherichia coli.*

These three letters are generally written in italics.

If the enzyme is coded by a plasmid, the plasmid name is written as a subsccript. eg :- Eco_{RI}

This enzyme specifically recognises GAA sites on the DNA and cuts it between G and A (G ↓ A).

(Eco_{RI} recognizes 5' G A A 3')

```
          ↓
5' A G A A T T C A C A C C  3'
   | | | | | | | | | | | | Eco RI cleavage
3' T C T T A A G T G T G G  5'
          ↑
 5' A G  A A T T C A C A C C 3'
    | |  | | | | | | | |
3' T C T T A A  G T G T G G 5'
```

Staggered cleavage of a double stranded DNA fragment, producing single stranded sticky ends.

Over 250 restriction enzymes have been isolated so far.

When the purified linear DNA is treated with a particular restriction enzyme, a large number of DNA fragments are formed.

The resultant fragments are separated from each other by a technique called **gel electrophoresis.**

Finally the desired DNA fragments are selected by a **southern blotting technique.**

- **Insertion of the isolated gene into suitable vector**

Once the desired fragment (or) fragments of DNA (genes) are obtained, they are inserted into a suitable vector (DNA) to produce indefinite number of copies of genes.

This is known as **'gene cloning'**.

A cloning vector acts as a vehicle to carry the desired gene.

An ideal cloning vector should have following properties.

i) It must have low molecular weight
ii) It must have a unique cleavage site for the activity of restriction enzymes at single point.
iii) It must be able to replicate truly inside a host cell after its introduction (through *ori* gene- origin of replication)
iv) It must contain genes which provide resistance to antibiotics (tet^R for tetracycline resistance, amp^R for ampicillin resistance etc). These help in selection of transformed cells from untransformed cells.

Several types of vectors are used in recombinant DNA technology like plasmids, phages etc.,

- **Plasmids :-** Plasmids are circular DNA molecules found in almost all bacterial species. They are inheritable and carry a few genes, which determine a variety of biological functions.

The advantage of a plasmid is that it is very easy to isolate and reintroduce into the bacterium (host).

Apart from natural vectors, artificially restructured / modified plasmids like pBR 322 (after Boliver & Rodriguez); pUC 19, 101 (after university of California) are popularly used

To isolate a plasmid, the bacterial cell is treated with EDTA (ethylene diamine tetra acetic acid) along with lysozyme (enzyme) to digest the cell wall.

Then the bacterial cell is subjected to centrifugation in sodium lauryl sulphate solution to separate the plasmid.

The plasmid DNA isolated is cut with same restriction endonuclease enzyme which was used to cut desired DNA in first step.

This enzymatic cleavage converts the circular plasmid into a linear molecule having sticky ends. The two sticky ends of this linear plasmid are now joined to the ends of the desired gene.

The enzyme **DNA ligase** joins the complementary ends of plasmid DNA with that of desired gene by covalent bonding to regenerate a circular hybrid called **Recombinant (r) DNA or chimeric DNA.**

- **Introduction of recombinant vector into the host**

The recombinant plasmid or vector is transferred into a suitable bacterial host cell (generally *E.coli*), by a method known as transformaton for the expression of the desired gene.

The cells into which the recombinant DNA are inserted are called **'transformed cells'**

Bacterial cell walls are not ordinarily permeable to such recombinant vectors, but keeping in dilute solution of calcium chloride renders the bacterial cell wall permeable to the recombinant vectors.

Inside the host cell, these recombinant DNA starts replicating.

The transformed cell will begin to grow (on medium) and divide as separate units.

The replicated vectors in each cell are passed on to daughter cells giving rise to clones.

- **Selection of the transformed host cells**

Depending upon the gene incorporated into the transformed cell, selection of recombinant clones can be done by two ways :-

a) Without using probes
b) By using probes (colony hybridization assay)

- **Without using probes :-** For example, if antibiotic resistant gene is being cloned, the transformed cells are first incubated in a medium without the antibiotic for about an hour, to allow the antibiotic resistant genes to be expressed.

Later, these cells are transferred to a medium containing antibiotic.

The cells which have expressed the gene will survive and the others die.

- **By using probes**

When transformed cells are cultured on the nutrient medium, several thousands of cells are produced.

All the cells in the culture may not contain the desired gene.

In order to select the cells containing the desired gene a method called **colony hybridization** is employed.

In this gene specific probes are used.

A **probe** is a small fragment of single stranded DNA or RNA which is tagged with a radioactive molecule and is complementary atleast on one part of desired DNA.

So that this can search out or locate complementary DNA sequences from an organism.

Eg:- Human insulin producing gene is isolated and incorporation of that gene into the vector, which is inserted into a bacterium *E. coli* to produce human insulin called **humilin.**

- **Transgenic plants**

Plants created through gene transfer method are called transgenic plants.

Ti plasmid of Agrobacterium tumefaciens has been widely used as effective vector for obtaining the transgenic plants.

Ex :- Transgenic papaya is resistant to papaya ring spot virus. Bt cotton is resistant to insects.

Round up ready soybean is herbicide tolerant.

Transgenic tomato plants are resistant to pseudomonas. Transgenic potato plants are resistant to phytophthora.

Flavr Savr tomato is bruise resistant, has higher shelf life due to delayed ripening.

Golden rice is rich in Vit- A. prevent blindness

Brassica napus male sterile plant can be directly used as female parent in hybridization.

By molecular forming we can express the gene of insulin interferon, human growth hormones, etc.

- **Genetically modified crops.**

Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms (GMO).

Genetically modified plants have been used in many ways.

A) Genetically modified crops are more tolerant to abiotic stresses like cold, drought, salt, heat etc. eg:- Basmati variety of rice made resistant against biotic & abiotic stresses.

B) Genetically modified crops are pest-resistant so their dependence on chemical pesticides is reduced. Eg. Bt cotton, Bt potato etc.

C) Post harvest losses are reduced in GM crops. eg :- Delayed ripening in Flavr Savr, a variety of tomato by antisense technology.

D) Genetically modified crops show increase efficiency of mineral usage, which prevents early loss of fertility in soil.

E) Genetically modified crops have enhanced nutritional value. eg:- Vitamin 'A' enriched rice

F) Besides these, genetically modified crops have evolved as an alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

- **Biosafety issues concerned with genetically modified crops.**

1. There is fear of transferring allergens from genetically modified food to humans and animals
2. Due to molecular farming, there is a risk of changing the fundamental nature of vegetables
3. These crops are not naturally evolved, they have been manipulated artificially. There is a risk whether they pose harmful effect on biodiversity (other living organisms) and overall impact on environment.
4. There is a risk of gene pollution, which may result in the development of super weeds.
5. Plants generally adapt the fluctuations occurring in nature and evolve gradually. GM plants may bring about changes in natural evolutionary pattern.

- **Applications of genetic engineering**

A) Cloned genes (r DNAs) are used in the production of growth hormones, vaccines and commercial chemicals.

B) rDNA technology has made it easier to detect. diagnose and cure genetic diseases.

Genetic disorders in plants can be treated by gene therapy using rDNA technology

C) Genes are the ultimate molecular switches that control various cellular processes.

Abnormal gene expression is due to specific genetic disorders.

With the help of rDNA technology it is possible to identify those disorders and transfer genes for treatment of human diseases by gene replacement therapy.

D) DNA finger printing has successfully helped the forensic science in the search of criminals and also solving parentage dispute tc.

E) Hundreds of transgenic plants and animals are produced which are beneficial to society

F) Overcoming pollution through genetically engineered microorganisms which detoxify and degrade toxic chemicals and help in environmental clean up.

EXERCISE

LEVEL-I

205. The gene cloning can also carried out in a computerized machine called themocycler by a method called
1) Carboxylic cycle 2) Citric acid cycle
3) Polymerase cyclic reaction
4) Polymerase chain reaction

206. One of the following technique is used to separate DNA fragments
1) Hybridoma technology
2) Gel Electrophoresis
3) Gene cloning 4)Molecular farming

207. Genetically engineered human insulin is manufactured in
1) E.coli 2) Rhizopus
3) Pseudomonas 4) Asparagus

208. When a cell synthesises new proteins after the intake of a foreign DNA, such a cell is called
1) Cloned 2) Mutated
3) Transformed 4) Modified

209. Production of numerous copies of desired fragments of DNA by inserting it into a suitable vector is called
1) Teminism 2) Transcription
3) Gene cloning 4) Gene therapy

210. The bacterium which is used as a biopesticide is
1) Agrobacterium tumefaciens
2) Bacillus vulgaris
3) Clostridium acetobutylicum
4) Bacillus thuringiensis

211. Ti plasmids are present in
1) E.Coli 2) Bacillus
3) Agrobacterium 4) Pseudomonas

212. Genes which confer antibiotic resistance on bacteria are located in
1) Chromosomal DNA 2) Plasmid
3) RNA 4) Polysome

213. Plasmids are ideal vectors for gene cloning because
1) They can be multiplied in culture medium
2)They replicate freely outside the bacterial cells
3) They self-replicating within the bacterial cells
4) 1, 2 & 3

214. The enzyme producing sticky end is
1) Restriction endonuclease
2) RNA dependent RNA polymerase
3) Nucleotide transferase
4) RNA dependent DNA polymerase

215. Which of the following is a recombinant DNA?
1)DNA of one bacterium within another bacterium
2) DNA of two viruses
3) DNA of bacteria and man
4) DNA of animals

216. Which of the following is associated with genetic engineering?
1) Plastid 2) Plasmid
3) Mutation 4) Hybrid vigour

217. The sites at which a restriction endonuclease recongnises the DNA molecules is known as
1) Palindrome 2) Sticky sites
3) Recognition sequences 4) Blunt ends

218. Sequences of the DNA molecule that are the same on both strands when read in the same direction (5'-3') are known as
1) Palindromes 2)Recognition sequences
3) Sticky sites 4) Blunt end sites

219. Restricted DNA fragments can be isolated by using the following technique
1) Gas chromatography 2) Gel electrophoresis
3) Liquid chromatography 4) Centrifugation

220. All of the following are descriptive of cloning vectors except
1) Plasmids 2) Cosmids
3) Bacteriophages 4) Genomic DNA

221. Which of the following enzymes join two pieces of DNA that have been cut by the same restriction endonuclease?
1) Ligase 2) Kinase
3) Polymerase 4) Lyase

222. Ligation involves the attachement of
1) Sticky ends 2) Blunt ends
3) Cloned DNA 4) Both 1 and 2

223. Uneven ends of a cleaved DNA are called
1) Blunt ends 2) Sticky ends
3)Slimy ends 4) Blind ends

224. The enzyme required for the formation of rDNA is
1) DNA-ase 2) DNA polymerase
3) DNA ligase 4)Reverse transcriptase

225. The process where by a section of DNA is inserted into a plasmid or bacteriophage and then replicated to numerous copies of insert is known as
1) Splicing 2) Cloning
3) Spooling 4) Transformation

226. Substances used to dissolve membranes during extraction of genomic DNA from cells are
1) Detergents 2) Phenol
3) Alcohol 4) Nucleases

227. Restriction enzymes were discovered by
1) Sumner 2) Nathans
3) Northrope 4) Buchner

228. The desired fragments of DNA can be selected by a technique called
1) Autoradiography 2) Electrophoresis
3) Southern blotting 4) Chromotography

229. DNA molecules employed to introduce foreign genes into host cells are called
1) Donors 2) Vectors 3) cDNA 4) rDNA

230. Which of the following is a palindromic sequence of Eco RI
1) G A A A A G / C T T T T C 2) G A A T T C / G A A A A G
3) G A A T T C / C T T A A G 4) G A A T T C / G A A A A G

231. Which enzyme are called as molecular scissores?
1) DNA ligases
2) Restriction Endonucleases
3) Reverse transcriptases
4) Polymerases

232. Recombinant DNA techonology is used for
1) Transfer of foreign genes between unrelated organisms
2) Transfer of prokaryotic gene to a eukaryotic cell
3) development of new strains in less time
4) All the above

233. Molecular farming is
1) Producing cultivated plants using radioactive isotopes
2) Commercial production of specialised medicines, chemicals and antibodies in transgenic plants
3) Artificial synthesis of DNA and RNA
4) Cloning of bacteria

234. pBR 322 is
1) A chemical to identify single stranded DNA
2) Artificial vector useful in genetic engineering
3) A specific restriction endonuclease enzyme to cut DNA
4) An antibody

235. *Eco* RI is
1) A recombinant bacterium
2) Restriction enzyme
3) Plasmid 4) Transposon

236. *Eco* RI recognises this base sequence
1) CAG 2) GCA 3)CCC 4) GAA

237. Isolated plasmids can be reintroduced into a bacterium by a process called
1) Conjugation 2) Transformation
3)Gradient centrifugation 4) Electrophoresis

238. The cloning vector pBR 322 is named after
1) Bhaskar and Rao 2) Bohr and Roden
3) Bolivar and Rodriguez
4) Bohr and Rodriguez

239. The bacterium widely used for transfer to foreign DNA into dicots is
1) Agrobacterim tumefaciens
2) Rhizobium radicicola
3)Clostridium butyricum
4) Escherichia coli

240. *Eco* RI will split polynucleotide chain of DNA between
1) A and A 2) G and A 3) T and T 4) T and G

241. A probe in genetic engineering is used to
1) Locate a protein 2) Isolate mRNA
3) Synthesise DNA
4) To detect the gene of interest

242. A probe is
1) A mutant RNA 2) A mutant DNA
3) A single stranded radio actively labeled RNA (or) DNA
4) RNA or DNA having rare base pairs

243. Colony hybridization is a method useful for
1) The detection of the presence of a gene in a colony
2) Producing recombinant DNA
3) Producing a progeny by conjugation between different bacteria
4) Producing new mutant varieties

244. Chimeric DNA is
1) cDNA 2) rDNA 3) zDNA 4) All

245. Transformed cells contain
1)cDNA 2)DNA clones 3)rDNA 4)both 1 and 3

246. Which of the following statements is correct?
1) Plasmid exists independent of bacterial chromosome
2) Plasmid can replicate
3) Plasmid is inherited 4) All the above

247. In making recombinant DNA, the most popular approach is
1) Using various types of restriction endonucleases to cut donor DNA and plasmid
2) To use the same restriction endonuclease to cut bits of donor DNA and plasmid
3) To use two different types of endonucleases along with two types of polymerases
4) To use different types of endonucleases to cut donor DNA and plasmid

248. Plants produced through genetic engineering are called
1) Transformed cells 2) Hybrid plants
3) Transgenic plants 4) Genetical plants

249. In genetic engineering it is required for the extraction of purified DNA
1) dil. HCl 2) Ethyl alcohol
3) Ultra centrifugation 4) Saline solution

250. Which of the following enzyme pairs are used in obtaining recombinant DNA?
1) DNA polymerase and R.E. enzyme
2) R.E. enzyme and DNA ligase
3) R.E. enzyme and DNA
4) R.E. enzyme and helicase

251. One of the following methods yield 'transgenic plants'
1) Mutation breeding
2) Intergeneric hybridization
3) Polyploid breeding 4) Genetic engineering

252. The widely used 'vector' to obtain transgenic plants by introducing cloned genes in plants is
1) Cosmid 2) pBR 322
3) Ti-plasmid 4) pUC 19

253. Donor DNA is attached to the cut ends of plasmid by
1) Restriction endonuclease 2) DNA ligase
3) Reverse transcriptase 4) Lyase

254. Other name for recombinant DNA (rDNA) is
1) cDNA 2) B-DNA
3) Z-DNA 4) Chimeric DNA

255. A gene carried by recombinant DNA is cloned when
1) Its host bacterium divides by binary fission
2) It is transcribed
3) It is cleaved by restriction enzymes
4) It is hybridized

256. A piece of nucleic acid used to find a gene, by forming a hybrid with it, is called a
1) Plasmid 2) Cosmid 3) Probe 4) Linker

257. A restriction enzyme break bonds between the
1) Adjacent bases of DNA
2) Base pairs of DNA-RNA hybrid molecule
3) Complimentary bases of DNA
4) Exons and introns of DNA molecule

258. A single stranded RNA or DNA segment which is radioactively labelled and used to locate complementary DNA sequence from an organism is called
1) Genomic DNA library 2) Plasmid
3) Cosmid 4) Probe

259. Purified DNA is precipitate out with the help of
1) Phenols 2) Chilled Ethanol
3) Chilled Methanol 4) Ribonucleases

260. The correct notation of the restriction enzyme that acts on "GAATTC" is
1) ECORI 2) EcoRI 3) *E*coRI 4) $E co_{RI}$

261. Which gene is responsible for plasmid replication
1) amp^2 2) tet^2 3) *Nif* 4) *ori*

262. *pBR* 322 has resistant genes for
I) Amphicillin II) Tetracycline III) Penicillin.
1) I II 2) II III 3) I II III 4) II only

263 A plasmid can be linearised by
1) minimum of single enzymatic cleavage
2) minimum of two enzymatic cleavages
3) Maximum of two enzymatic cleavages
4) Maximum single enzymatic cleavage

264. Generally which bacteria are used to introduce the r DNA.
1) Bacillus spp 2) E.coli
3) Agrobacterium 4) NPV

265. Plants with desirable characters created through gene transfer methods are called
1) Bioreactors 2) Transgenic plants
3) Exotic plants 4)Bonsai plants

266. Initially, transgenic plant production was restricted to this group
1) Gymnosperms 2) Agiosperms
3) Dicots 4) Monocots

267. Transgenic papaya is resistant to
1) Ring spot virus 2) Baculo virus
3) Mosaic virus 4) leafroll virus.

268 Roundup ready soybean is tolarent to
1) Insecticides 2) Herbicides
3) Pesticides 4) Bactericides

269. Transgenic tomato plants are resistant to this pathogen
1) Phytophthora 2) Pseudomonas
3) Bruise 4) Insects

270. Flavr Savr tomato is resistant to
1) Insects 2) Bruise 3) Bacteria 4) virus

271. Rice variety resistant to biotic & abiotic stresses is
1) Basmati 2) IR-8 3) Taipei 4) CO-4

272. Delayed ripening in Flavr Savr variety is developed by
1) P.C. R 2) Antisense technology
3) Gel electrophoresis 4) Southern bloting

273. Rice variety with enhanced nutritional value is
1) Golden Rice 2) IR-8
3) Co-4 4)Flavr savr

274. Gene pollution leads to the development of
1) Super weeds 2) Allergens
3) Biodiversity 4) Biosafety

275. Ultimate molecular switches that control various cellular processes are
1) Chromosomes 2) Genes
3) Nucleus 4) Introny

276. Parentage dispute can be solved by using
1) DNA finger printing 2) Gel electrophoresis
3) Centrifugation 4) cloning

277. Genetic disorder /diseases can be treated by
1) DNA finger printing
2) Gene replacement therapy
3) cloning
4) Tissueculture

EXCERSISE

LEVEL-II

278. The term 'Biotechnology' was first used by
1) Nathans 2) Carl Ericay
3) Watson and Crick 4) Pasteur

279. Gene transfer between unrelated organisms is possible by
1) Polyploidy breeding 2) Mutation breeding
3) Genetic engineering 4) Hybridization

280. Biotechnology is not based on the principles of
1) Molecular genetics 2) Microbiology
3) Biochemistry 4)Environmental biology

281. An antibiotic produced on a large scale during the second world war is
1) Neomycin 2) Streptomycin
3) Penicillin 4) Streptocyclin

282. Recombinant DNA technology progressed with the identification of
1) DNA polymerase 2) RNA polymerase
3) Restriction endonuclease
4) Reverse transcriptase

283. Mostly followed definition for biotechnology was given by
1) Asian Federation of Biotechnology
2) European Federation of Biotechnology
3) German Federation of Biotechnology
4) American Federation of Biotechnology

284. Butanol and acetone were produced by
1) Alga 2) Fungus 3) Bacterium 4) Yeast

285. Some micro organisms produce antibiotics except
1) all Algae 2) Actinomycetes
3) Bacteria 4) Fungi

286. Assertion (A): Restriction endonucleases recognize and cut the DNA
Reason (R): These are called molecular scissors

287. The food supplement agent for flavour produced by using biotechnology is
1) Aspertate 2) Nucleotides
3) Monosodium glutamate 4) Lysine

288. Cheese is produced by using
1) Protein 2) Fat 3) Rennet 4) Aspertate

289. Human insulin produced through genetically engineered E.coli is called
1) Rennet 2) Humulin 3) Colin 4) Colchicine

290. The first microorganism that has given an idea that biochemical substances can be produced at commercial level by using microbes
1) Rhizobium 2) Bacillus thuringiensis
3) Agrobacterium tumefaciens
4) Clostridium acetocbutylicum

291. Bacterium and a virus respectively used as biopesticides are
1) Chromatium and bacteriophage
2) Bacillus thuringiensis and NPV
3) Lactobacillus and adenovirus
4) Rhodospirullum and poliovirus

292. A laboratory technique of gene manipulation is
1) Gene cloning 2) Genetic engineering
3) Recombinant DNA technology
4) Biotechnology

293 .In the process of gene isolation cell membranes are disrupted by using
1) Cellulases 2) Pectinases
3) Ligases 4) Detergents

294. Pure DNA can be extracted from cellular protein DNA and RNAs by treating with
1) Calcium chloride 2) Phenol
3) Restriction enzymes 4) DNA polymerase

295. Pure DNA can be isolated by subjecting the protop lasmic mass for
1) Southern blotting 2) Electrophoresis
3) Gradient centrifugation 4) Transformation

296. Enzymes used for cutting donor DNA into fragments
1) Restriction endonuclease 2) DNA polymerase
3) Ligases 4) Reverse transcriptase

297. Restriction endonucleases are usually found in
1) Algae 2) Fungi 3) Bacteria 4) Viruses

298. Restriction endonucleases were first discovered by
1) Carl Ericay 2) Nathans
3) Pasteur 4) Robert Brown

299. The first part of name of restriction enzyme indicates
1) The generic name of donor
2) The species name of the donor
3) The variety of the donor
4) The character of the donor

300. Restriction endonucleases identify and cut DNA at specific sites at
1) Staggered ends 2) Palindromes
3) Non sense codons 4) Mutated regions

301. Identify the palindromic sequence for EcoRI
1) 5^1G A A T T C 3^1
3^1C T T A A G 5^1
2) 5^1G A T T G C 3^1
3^1C T A A C G 5^1
3) 5^1A G C G A T 3^1
3^1T C G C T C 5^1
4) 5^1A A C C A C 3
3^1 T T G G T G 5^1

302. EcoRI cuts DNA between
1) G and A 2) G and C 3) C and G 4) G and T

303. The enzyme that plays a role in annealing DNA fragments is
1) Endonuclease 2) Lysozyme
3) Ligase 4) Cytase

304. pBR – 322 is prepared from the plasmid of
1) Algae 2) Fungus 3) Virus 4)Bacterium

305. Plasmid can be reintroduced into a bacterium by
1) Conjugation 2) Transduction
3) Transformation 4) Binary fission

306. A small, circular, self replicating naked DNA found in bacterial cell other than chromosomal DNA is called
1) Plasmid 2) Cosmid 3) Gene 4) Endonuclese

307. Which of the following is not the property of ideal cloning vector
1) Low molecular weight
2) Single site for the activity of restriction enzyme
3) Ability to replicate truely
4) Bearing suscetibility to antibiotics

308. EDTA and lysozyme are useful in digesting
1) Bacterial cell wall 2) Plant cell wall
3) Fungal cell wall 4) All the above

309. To separate plasmid the naked bacterial cell is subjected to centrifugation in
1) Calcium chloride 2) Phenol
3) Sodium lauryl sulphate 4) Sodium carbonate

310. Desired donor DNA can be identified and isolated by
1) Colony hybridization 2) Southern blotting
3) Gel electrophoresis 4) Gene cloning

311. A technique used to isolate DNA fragments is
1) Colony hybridization 2) Southern blotting
3) Gel electrophoresis 4) Gene cloning

312. Vector DNA with gene of interest is called
1) r DNA 2) c DNA 3) Z-DNA 4) B-DNA

313. The bacterial cell with r DNA is treated as
1) Naked cell 2) Prokaryotic cell
3) Transformed cell 4) Infected cell

314. The method useful for identifying the colony with desired gene is
1) Southern blotting 2) Colony hybridization
3) Centrifugation 4) Electrophoresis

315. Even cut of DNA results in
1) flesh ends 2) Cohesive ends
3) sticky ends 4) Blunt ends

316. A probe is a radioactively laballed
1) Double stranded DNA
2) Double stranded RNA
3) Single stranded DNA or RNA
4) All the above

317. The correct sequncee of isolation of desired gene from a cell
I) detergent lysis II) enzymatic action
III) gradient centrifuyation
IV) southern blotting technique
1) II, I, III, IV 2) I, II, III, IV
3) I, III, II, IV 4) II, III, I, IV

318. Widely used vector for the production of transgenic plants are
1) pBR–322 2) pUC–19 3)Cosmid 4)Ti Plasmid

319. Ti Plasmids are present in
1) Bacillus thuringiensis
2) Agrobacterium tumefaciens
3) Erwinia amylovora 4) Bacillus mycoides

320. Initially transgenic plants are produced in
1) Gymnosperms 2) Monocots
3) Dicots 4) Pteridophytes

321. Bruise resistance and delayed ripening tomato plants are produced through
1) Mutation 2) Hybridization
3) Transgenics 4) Selection

322. Male sterile transgenic plants are produced in
1) Lycopersicon esculentum 2)Brassica napus
3) Triticum durum 4) Triticum aestivum

323. Insertion of a desired gene into a suitable vector to produce its copies is called
1) Colony hybridization 2) Southern blotting
3) Electrophoresis 4) Gene cloning

324. Gene cloning is done in a computerized machine called
1) Spectrophotometry 2) Autoclave
3) Centrifuge 4) Thermocycler

325. Polymerase chain reaction is useful in
1) Sterilizing the medium 2) Gene cloning
3) Electrophoresis 4) Southern blotting

326. Which of the following is not used for gene cloning
1) Plasmid 2)Cosmid 3) Algae 4) Bacteriophage

327. The method of using transgenic plants as bioreactors for production of medicines, chemicals and antibiotics on large scale is called
1) Micropropagation 2) Molecular farming
3) Transgenics 4) Gene cloning

328.

List – I	**List – II**
A. Digestion of cell wall	I) Gel electrophoresis
B. Lysis of cell membrane	II) Southern blotting
C.Separation of DNA fragments	III)Cellulase, pectinase
D. Selection of gene of interest	IV) Calcium chloride precipitation
	V) Detergent

The **correct** match is

A	B	C	D		A	B	C	D
1) III	V	I	II	2)	I	II	III	IV
3) V	IV	III	II	4)	IV	V	I	II

329. **List – I** **List – II**

A. Artificial sweetner I) Cheese

B. Monosodium glutamate II) NPV

C. Rennet III) glucose isomerase

D. Biopesticide IV) Antibiotics

V) Flavouring agent

The **correct** match is

A	B	C	D		A	B	C	D
1) I	II	III	IV	2)	I	III	IV	V
3) IV	III	II	I	4)	III	V	I	II

330. **List – I** **List – II**

A. PCR I) Restriction endonuclease

B. EcoRI II) Vector

C. pBR - 322 III) Bacterium

D. E.Coli IV) Virus

V) Gene cloning

The **correct** match is

A	B	C	D		A	B	C	D
1) IV	III	II	I	2)	I	II	III	IV
3) III	II	I	IV	4)	V	I	II	III

331. **List – I** **List – II**

A. Phage vector I) adioactively labelled single stranded DNA

B. Probe II) Agrobacterium tumefaciens

C. Ti Plasmid III) Male sterile transgenic plant

D. Brassica napus IV) SCP

V) Lambda phage

The **correct** match is

A	B	C	D		A	B	C	D
1) V	III	II	I	2)	V	VI	III	II
3] V	I	II	III	4)	I	II	III	V

332. Biopesticide

I) Escherichia coli II) Agrobacterium

III) Baculovirus IV) Bacillus thuringiensis

1) I and II are correct 2) II and III are correct

3) III and IV are correct 4) I and IV are correct

333. Transgenic tomato plant possesses

I) Bruise resistance II) Delayed ripening

III) Medicinal property IV) Broad leaves

1) I and II are correct 2) II and III are correct

3) III and IV are correct 4) II and IV are correct

334. Arrange sequence of steps involved in isolation of gene of interest

I) Differential centrifugation II) Electrophoresis

III) Southern blotting IV) Digestion of cell wall

1) IV, I, II, III 2) I, II, III, IV

3) I, III, II, IV 4) II, III, I, IV

LEVEL - III

335. Development of super weeds is a topic of concern about GM crops. What could be the reason for this.

1) Transfer of allergins

2) Change in nature of vegetables

3) Gene pollution

4) Reduction of postharvest losses.

336. Which one of the following has the power to permit large scale use of GMO's at commercial level?

1) IBCS 2) RCGM 3) GEAC 4) EFB

337. Identity the correct combinations.

I. Gene therapy—Curing genetic disorders.

II. Bioremediation --Study and design of genomes.

III. Gene transfer-- Production of transgenic plants.

IV. DNA finger printing—Helpful in forensic science

1) I only 2) I & II only

3) I, II & III only 4) I, III & IV only

338. An ideal cloning vector should have

I) low molecular weight II) origin of replication

III) Desired genes IV) resistance to antibiotics

1) III & IV 2) I ,II, IV 3) I & III 4) III & II

339. Assertion (A) : The cells into which the recombinant DNA are inserted are called trans formed cells

Reason (R) : Transfer of recombinant plasmid into a host cell is known as transformation

340. Genetically homogenous population of cells derived from a single cell called as

1) trans formed cell 2) clones

3) r DNA 4) CDNA

341. Transgenic monocotyledons plants

1) barley 2) rice 3) oats 4) 1, 2 & 3

342. Match the following

Transgenic plants	**Resistant to**
(A) Round up ready soya bean	(I) Phytopthora
(B) papaya	(II) herbicide tolerant
(C) potato	(III) to ring spot virus
(D) Bt cotton	(IV) Pseudomonas
	(V) Insects

1) A-III, B-II, C-I, D-V 2) A-II, B-III, C-I, D-V

3) A-I, B-II, C-III, D-IV 4) A-II, B-III ,C-I , D-IV

343. Trangenic tomato are bruise resistant due to

1) delayed ripening 2) herbicide tolerant

3) virus resistant 4) fungal resistant

344. The biochemicals which are produced by transgenic plants are better than those of bacteria

1) insulin 2) interferons

3) growth hormone 4) all the above

345. The genes of these are manipulated to produce GMO
1) plants , animals 2) Bacteria, fungi
3) 1,2 are correct 4) man and animals

346. The precursor rice variety responsible for the production of transgenic golden rice
1) Basmati 2) Taipei 3) kasturi 4) Samba

347. Environmental clean up through bioremediation by utilizing sewage & agro wastes for the production of
I) SCP. Mushrooms II) GM crops
III) Biogas & vermicompost IV) Transgenic plants
1) II, III 2) III, IV 3) I, III 4) II, IV

348. The steps in the isolation of desired gene from a cell are arranged in a sequence. The correct sequence is
I) Addition of chilled Ethanol
II) Cell wall digestion
III) Gradient centrifugation
IV) Gel electrophoresis
V) Detergent lysis
VI) Southern blotting technique
VII) Use of Molecular scissors
1) II → V → I → III → VII → IV → VI
2) II → V → III → I → VII → IV → VI
3) II → V → III → VI → VII → IV → I
4)II → V → III → I → VII → VI → IV

349. The *Eco* RI cuts the phosphodiester bond of a DNA strand in between
1) A purine and a pyrimidine
2) A pyrimidine and a purine
3) A purine and a purine
4) A pyrimidine and a pyrimidine

350. In the DNA fragments generated by even cuts the number of nitrogen bases found in both the strands of each fragment
1) are always in 1 : 1 ratio
2) are always not in 1 : 1 ratio
3) may are may not be in 1 : 1 ratio
4) cannot be determined

351. The minimum number of phosphodiesterbonds and the hydrogen bonds cleaved per the generation of five fragments from a linear DNA through even cuts respectively are
1) 8 and 32 2) 10 and 40
3) 10 and 0 4) 8 and 0

352. The property of the restriction enzymes that is of great value for the construction of recombinant DNA among the following is
1) Ability to cause staggered cut
2) Ability to cause even cut
3) Ability to cause staggered cut or even cut
4) Ability to cause staggered and even cut

353. Number of phosphodiester bonds and hydrogen bonds formed during the construction of rDNA (circular) by using linear plasmid and desired gene generated by using *Eco*RI respectively are
1) 8 and 32 2) 4 and 16
3) 2 and 16 4) 2 and 8

354. The computerised machine used in gene cloning is called as
1) Thermometer 2) Thermostat
3) Thermocycler 4) Thermoinsulater

355. The ratio between the number of purines and the number of pyrimidines found on a sticky end of a DNA fragment generated by using *Eco*RI is
1) 1 : 1 2) 1 : 3 3) 3 : 1 4) 4 : 0

356. The technique that is to be followed to produce multiple copies of a desired gene is
1) Gel electrophoresis
2) Southern blotting technique
3) Gene cloning 4) Colony hybridization

357. The method that is to be employed in the selection of transformed cells by using probe is
1) Gel electrophoresis
2) Southern blotting technique
3) Gene cloning
4) Colony hybridization

358. The human insulin produced by genetically engineered *E.coli* is called as
1) Colchicine 2) Interferon
3) Humilin 4) Asprin

359. The substance that makes the bacterial cell wall permiable to r DNA is
1) Sodium lauryl sulphate 2) Calcium chloride
3) Monosodium glutamate
4) Sodium hypochlorite

360. Identify the correct expression from the following statements:
1) All the vectors used in gene cloning experiments are bacterial in origin
2) The penicillin is an antibiotic produced from a prokaryote
3) Clone of E.coli is formed as a result of asexual reproduction.
4) All the probes are used in colony hybridization are eoxy ribonucleic acids

361. The recombinant plasmid is introduced in to a cell of *E.coli* by a method known as

1) Transformation 2) Transduction
3) Conjugation 4) Binary fission

362. In *Eco*RI, the R indicates

1) The name of genus 2) The name of the speices
3) The name of the plasmid
4) The name of auther

363. The latest technology that helps us in solving the parentage disputes among the following is

1) Genomics 2) Proteomics
3) Bioinformatics 4) DNA finger printing

364. The advantage in the use of a plasmid as a gene cloning vector is

1) It has high molecular weight
2) It has no *ori* - gene
3) It is very easy to isolate and reintroduce the plasmid into a bacaterial cell
4) It has no cleavage sites for the action of *Restriction endonucleases*

365. The correct sequence of the events that take place in the functioning of a restriction endonu clease is

I) Binding of restriction endonuclease to the DNA
II) Recognition of a specific palindromic nucleotide sequence of the DNA
III) Inspection of the length of the DNA
IV) Cleavage of the two strands of the DNA double helix at specific points

1) III $\rightarrow$ II $\rightarrow$ I $\rightarrow$ IV 2) IV $\rightarrow$ I $\rightarrow$ II $\rightarrow$ III
3) III $\rightarrow$ I $\rightarrow$ II $\rightarrow$ IV 4) II $\rightarrow$ III $\rightarrow$ IV $\rightarrow$ I

366. Microorganism used in the making of bread among the following is

1) *Lactobacillus* 2) *Penicillium notatum*
3) *Clostridium acetobutylicum* 4) Yeast

367. Study the following

A) $CaCl_2$ B) Sodium lauryl sulphate
C) Lysozyme D) EDTA
E) Centrifugation

Which of the above are used in isolation of vector during r-DNA technology?

1) A, B, C, D & E 2) B, D & E only
3) All except 'A' 4) A, C & E only

368. A molecular probe can be

1) ds DNA or ds RNA 2) ss DNA or ds RNA
3) ds DNA or ss RNA 4) ss DNA or ss RNA

369. **Statement - I :** During r-DNA technology, while selecting the transformed host cells using colony hybridization, the transformed cells are first incubated in a medium without antibiotic for about an hour.

Statement II : When the transformed cells are first incubated in a medium without antibiotic, the antibiotic resistant genes will be expressed.

1) Both Statements I & II are true.
2) Both Statements I & II are false.
3) Satement I is true but Statement II is false.
4) Statement II is true but Statement I is false.

370. Herbicidal tolerant transgenic plant is

1) Bt cotton 2) Flavr savr tomato
3) Taipei rice 4) Round up ready soybean

371. Transgenic tomato variety with high shelf life period is

1) Flavr Savr 2) IR 8 3) GEB 4) TNI

372. Genetically modified Basmati variety of rice is made resistant to

i) Biotic stress ii) Abiotic stress
iii) Fungal diseases iv) Viral diseases

1) i & ii only 2) ii & iii only
3) i & iv only 4) ii only

373. Transgenic papaya is resistant to disease caused by

1) Eukaryote 2) Prokaryote
3) Virus 4) Abiotic agents

374. Which of the following monitor the research on genetically modified crops at laboratory level

1) EPA 2) IBCS 3) RCGM 4) GEAC

375. Tomato produced by antisense technology shows

1) high yield 2) male sterility
3) pest resistance 4) delayed ripening

376. Assertion (A): Transgenic plants are used for hybrid seed production.

Reason (R): Male sterile Brassica napus used directly as male parent in hybridization.

377. The fragments of DNA formed after treatment with endonucleases are separated by this technique

(EAMCET - 2005)

1) Polymerase chain reaction
2) Southern blotting
3) Electrophoresis 4) Colony hybridization