CHAPTER:11 BIO TECHNOLOGY PRINCIPLES & PROCESSES

(KEY POINT)

S.No	Term	Explanation
1	rDNA	Recombinant DNA
2	Gene cloning	DNA technology used to produce multiple, exact copies of a single geneor other gene to obtain enough material for further study
3	Gene transfer	Incorporation of new DNA into an organism's cells, usually by a vector such As modified virus. Used in gene therapy See also: mutation, genetherapy, vector
4	Genetic Engineering	Altering the genetic material of cells or organisms to enable them to make new Substances or perform new functions
5	ORI	The specific sequence of bases in a DNA which initiates replication.
6	Restriction enzymes	They are molecular scissors capable of cutting DNA at specific site. These are enzymes present in bacteria which prevent the multiplication of bacteriophages in their cells
7	Plasmid	Autonomously replicating extra chromosomal circular DNA molecules, distinct from the normal bacterial genome and non essential for cell survival under non selective conditions. Some plasmids are capable of integrating into the host genome.A n umber of artificially constructed plasmids are used as cloning vectors
8	Cloning Vectors	Vectors that introduce foreign DNA into host cells, where the DNA can be reproduced in large quantities. Examples are plasmids, cosmids, and yeast artificial chromosomes
9	Endonuclease	Protein thatrecognizesspecific, shortnucleotidesequencesandcuts DNA atthosesites within the molecule
10	Nucleases	Enzymes that are specific to nucleic acids
11	Exo nucleases	An enzyme that cleaves nucleotides sequentially from free ends of a linear Nucleic acid substrate.
12	Palindromic sequences	The sequence of base pairs that reads the same on both the strands when read in the same orientation (ie 5' to 3' in both the strands)
13	Gel Electrophoresis	A method of separating large molecules (such as DNA fragments or

		proteins)from a mixture of similar molecules. An electric current is passed through a medium containing the mixture, and each kind of molecule travels through the medium at different rate, depending on its electrical charge and size Agarose and acrylamide gels are the media commonly used for electrophoresis of proteins and nucleic acids
14	Elution	The extraction of separated fragments of DNA from the electrophoresis gel
15	Auto radiography	A technique that uses X-Ray film to visualize radioactively labeled molecules or fragments of molecules, used in analyzing length and number of DNA fragments after they are separated by gel electrophoresis.
16	Transformation	Most common method to introduce DNA into living cells.In this procedure,bacterial cells take up DNA from the surrounding environment. Many hos t cell organisms such as, E.coli,yeast and mammalian cells do not readily take up foreign DNA and have to be chemically treated to become competent to do so
17	Selectable markers	A gene or other identifiable portion of DNA whose inheritance can be followed and used in the process of selection of transformed cells from non transformed ones
18	Insertional Inactivation	The process by which a gene encoding a protein is inactivated by the insertion of a foreign DNA within the coding sequence of the protein
19	Ti Plasmid	Tumour inducing Plasmid in Agrobacterumsp.causingtumour in plant cells
20	Tumour	Uncontrolled growth of cells in the body of plants or animals.
21	Microinjection	The process of introducing rDNA into animal cells using a micropipette
22	Biolistics/gene gun	A direct genetransfer method for delivering foreign genes into any tissues and cells or even seedlings. * The foreign DNA is coated or precipitated onto the surface of minute gold or tung stenp articles * It is bombardedors hotontothetargettissueorcellsusingthegenegun.
23	Embryonic stem(ES)cells	An embryonic cell having totipotency that can replicate indefinitely,transform into other types of cells ,and serve as a continuous source of new cells.These cells are derived from inner cell mass of the blastocyst or the 4-8cell stage of embryo

24	Lysozyme	The enzyme that digests the cell wall of bacteria
25	Cellulase	The enzyme that digests cellulose of plant cell walls
26	Chitinase	The enzyme that can digest cell walls of fungi containing chitin.
27	Polymerase Chain Reaction (PCR)	Polymerase Chain Reaction where DNA can be amplified in a short time to produce multiple copies of DNA (can be made in vitro)
28	Recombinant protein	Protein encoding gene expressed in a heterologous host
29	Bioreactors	Are vessels in which raw materials are Biologically converted into specific products using microbial,plant or animal ells
30	Downstream Processing	The process of formulation,separation and purification of rDNA products made in Bioreactors.
31	Spooling	The method of separating DNA precipitates in chilled ethanol ,after its isolation from the other cell contents
32	Disarmed pathogens	Some bacteria orviruses,whichareused to transfer recombinantDNA carrying the gene of interest the host'scells.
33	Retrovirus	RNA virus containing reverse transcriptase and can be used to transfer the gene of interest into the host chromosome
34	Ligases	Enzymes that can join fragments of DNA
35	Vector	NAmolecule,capableofreplicationinahostorganism,intowhichagene is insertedtoconstruct a recombinantDNAmolecule
36	Competent Host Cell	A cell which has been chemically treated to take up rDNA from its surroundings by causing pores in its cell wall
37	Amplification	An increase in the number of copies of a specific DNA fragment; can be in vivo or in vitro
38	Sticky ends	Single stranded overhanging ends of DNA formed by the restriction enzymes cutting the strands of DNA at specific palindromic sequences
39	Denaturation	Double stranded DNA is separated by applying high temperature of 95°C
40	Annealing	Primers bind to the 3'ends of the separated DNA strands
41	Extension	DNA polymerase extends the primers by adding complementary nucleotides .Taq polymerase is used here
42	Taq Polymerase	DNA polymerase obtained from bacteria Thermus aquaticus, which

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(FLOW CHART)

1 **CORE TECHNIQUES**

- Genetic Engineering
- Sterile conditions for culture of the host and collection of products

2 RECOMBINANT DNA TECHNOLOGY

- **Formation of Recombinant DNA .**

  (gene of interest + vector)
- **Gene Cloning in the vector**

  (in vivo)
- **Gene transfer to host cell for culturing and obtaining the product of the gene**

3 STEPS IN CREATION OF rDNA AND RECOMBINANT

1. Isolation of DNA from the organism by using enymes like lysozyme,chitinase,protease,RNAse and precipitating in chilled ethanol
2. Cutting of DNA at recognition sites by Restriction enzymes .The same enzyme cuts the cloning vector at a similar recognition site producing sticky ends
3. The cut fragment is separated using Gel Electrophoresis and amplified using PCR
4. The gene (DNA fragment)is joined with the cloning vector DNA using Ligase
5. The recombinant DNA thus formed is transferred into host cell using methods like Biolistics, microinjection, or pathogens like bacteria and retroviruses whose pathogenic properties have been removed
6. The host cell containing the rDNA is cultured in Bioreactors to produce the Product
7. The product is separated,purified,(downstream processing) formulated with preservatives followed by quality control testing.

4 ISOLATION OF GENE(DNA) OF INTEREST USING ENZYMES

- Lysozyme to break bacterial cell wall
- Chitinase to break ungal cell wall
- Cellulase to break plant cell wall
- Protease to digest proteins
- RNAse to digest RNA DNA is left

5 Characteristics of Restriction Enzymes :

They are Endonucleases → Have specific recognition sites which are palindromic → Are named after the genus, species and order of isolation from their host cell

6 Gel Electrophoresis:

Negatively charged DNA are forced to move through a gel ,towards an anode, in an electric field.

↓

The smaller fragments sieve through the gel faster and move faster to the anode

↓

The larger ones remain near the wells at the cathode end as they sieve slowly

↓

The separated fragments are stained with ethidium bromide and visualized under UV light

↓

The DNA fragments are cut out from the gel –a process known as **Elution**

7 PCR: POLYMERASE CHAIN

Denaturation of DNA (separation of DNA into single strands)by applying high temperature upto 95° C

↓

Annealing:Two sets of Primers (short stretches of RNA)attach to the single stranded DNA at complementary sites.

↓

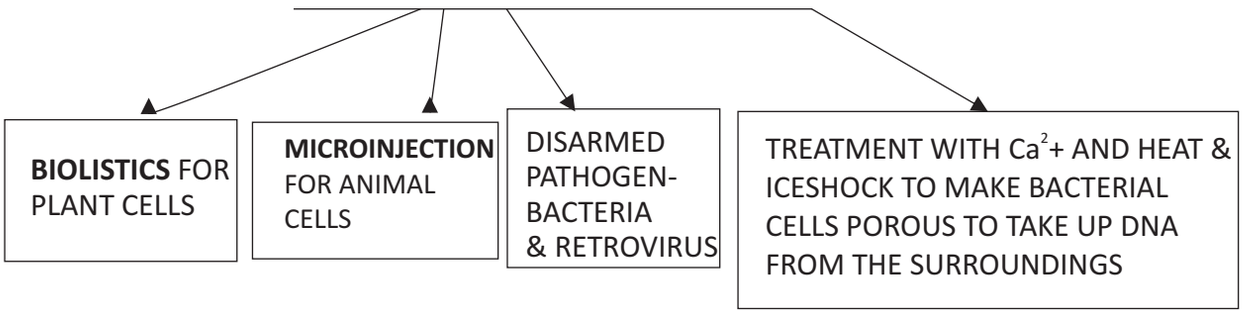
Taq Polymerase

Extension: The primers extend by addition of nucleotides in the presence of thermostable DNA polymerase complementary to the DNA strand .The Primers are removed .

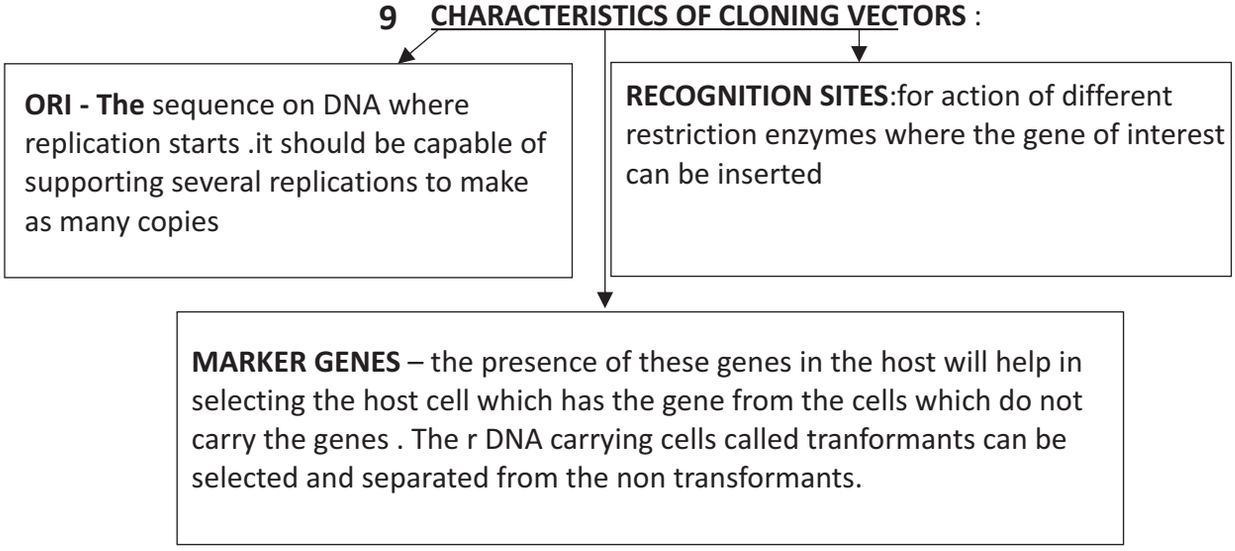
↓

Repeat:This cycle gets repeated 30 times and the DNA fragment gets amplified about I billion times

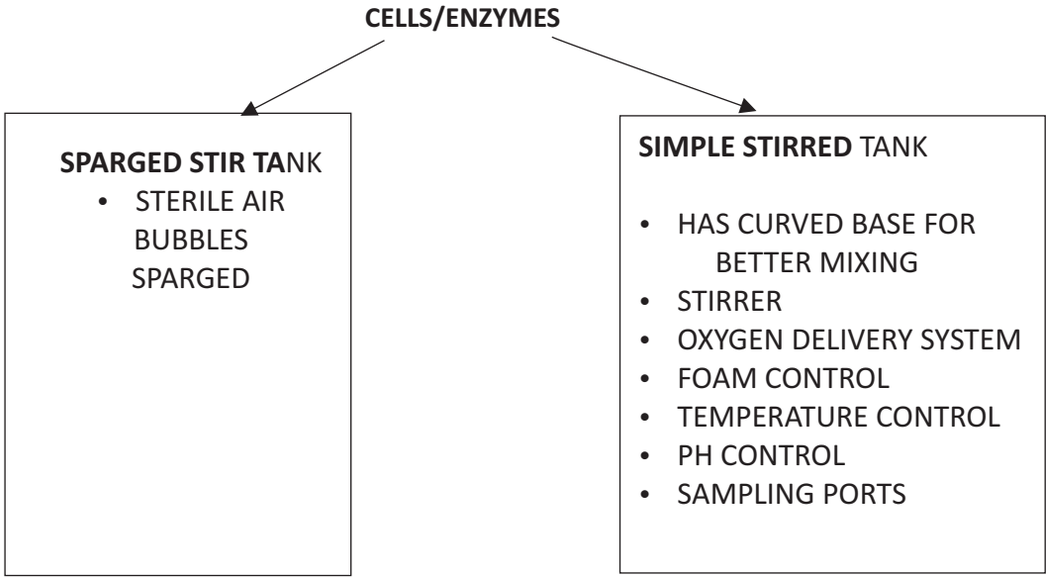
8 METHODS OF GENE TRANFER INTO HOST CELLS:



9 CHARACTERISTICS OF CLONING VECTORS :



10 BIOREACTORS: PRODUCE RECOMBINANT PROTEIN USING RAW MATERIALS AND LIVING CELLS/ENZYMES



DOWNSTREAM PROCESSING +QUALITY CONTROL TESTING before the product is made commercially available.

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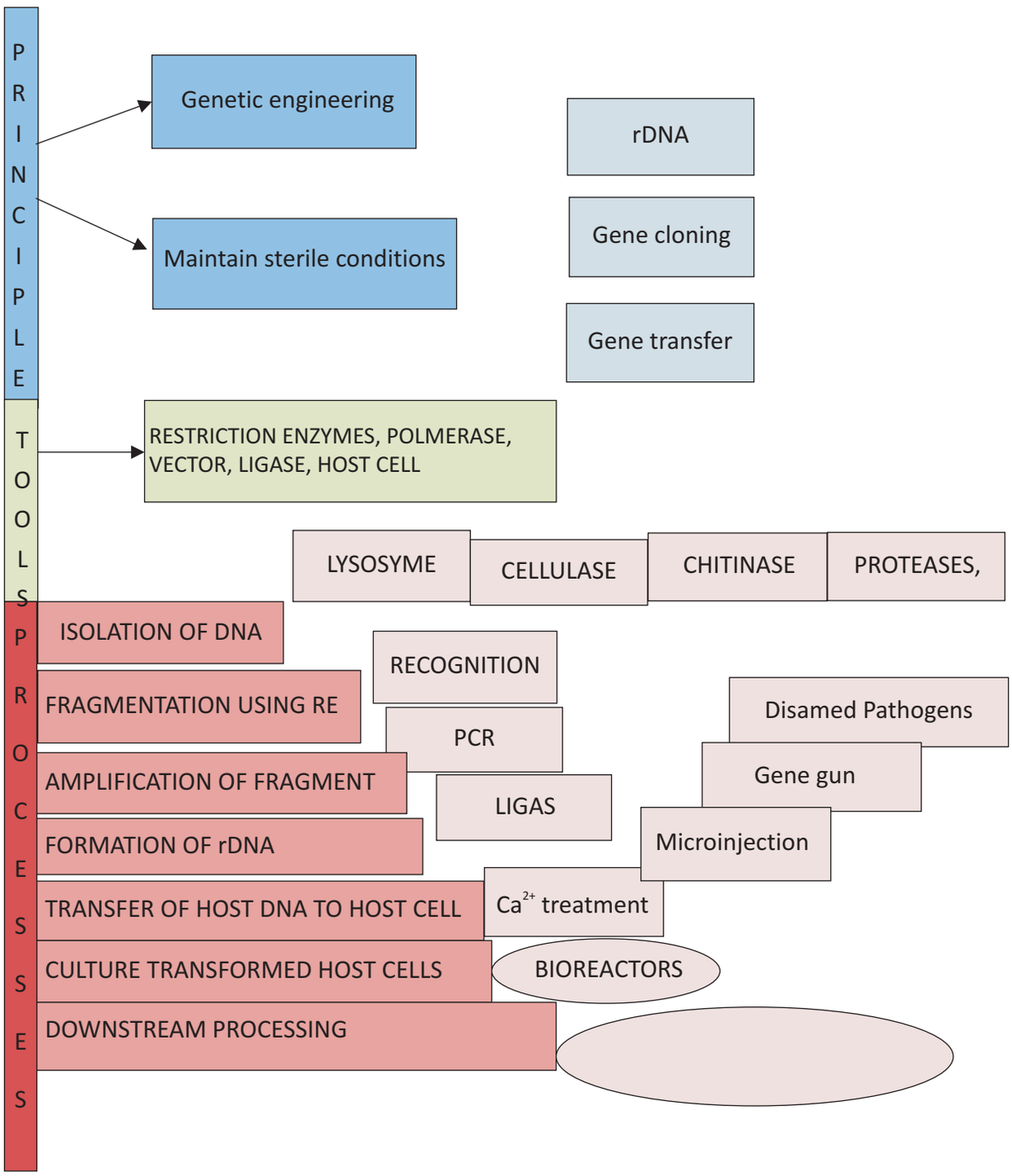
(CONCEPT MAP)



CHAPTER : 11 BIO TECHNOLOGY PRINCIPLES & PROCESSES
(QUESTION BANK)

1. What are the two core techniques that enabled the birth of Biotechnology?

2. How does genetic engineering help in overcoming the limitations of traditional hybridization procedures used in plants & animals?

3. How does the stickiness of cut ends of DNA help?

4. How can an alien piece of DNA made to multiply in a host cell?

5. What are the 'molecular scissors' in rDNA technology. What are they used for in rDNA technology?

6. List the major techniques in Genetic Engineering? Who was the first to construct an rDNA?

7. What are the three basic steps in genetically modifying an organism?

8. What are recognition sequences of endonucleases? Name the five key tools in rDNA technology.

9. What are nucleases. What are the two types ?

10. Show diagrammatically how endonucleases work?(3)

11. How can the fragments of DNA be separated? Explain

12. Explain the features required for a cloning vector(3)

13. How can the cloning vector pBR322 be used in separating the transformants and recombinants .Explain(3)

14. Do you agree with the construction of rDNA to give new traits in animals.How do you think it can be done .State any issue of concern in this?(4)

15. How is Agrabacterium tumifaciens used in rDNA technology.Explain ii)What and how are other pathogens are used for the purpose ?State two other methods by which host organism can be transformed?

16 Sequentially state the process you would adopt for getting a recombinant protein?(5)

CHAPTER : 11 BIO TECHNOLOGY PRINCIPLES & PROCESSES (ANSWER KEY)

.Q.No	HINTS	Marks
1	Genetic Engineering & Maintenance of sterile ambience	½+½
2	It allows use to isolate & introduce only the desirable gene/genes without Introducing the undesirable genes	1
3	Makes the joining easy ; Complimentary	1
4	Linked to ORI of the host genome	1
5	Restriction enzymes,DNA at specific sites	½+½
6	Creation of rDNA,gene cloning ,gene transfer,Stanley Cohen & Boyer	½+½+½+½
7	Identification of desirable DNA,introduction into host, maintenance and then transfer to its progeny	½+½+½+½
8	The sequence at which DNA is cut by a RE, RE, polymerases, ligase,vector,host	½+½+½+½
9	Enzymes that act on nuleic acids,Endonucleases ,Exnucleases	1+½+½
10	Fig 11.1	1+1+1
11	Gel electrophoresis-Explain Agarose gel	1+1+1
12	ORI,cloning sites,selectable markers	1+1+1
13	Tetracycline site can be cut ,to insert the desired insert ,the recombinants will lose resistance to tetracycline the transformants will have resistance to both tetracycline and ampicillin	1+1+1
14	Use of transgenic animals to serve several purposes. (chemical testing, drug safety, products for treatment,to study physiology.Not ethical when used for selfish greed.Not safe if done without caution	1+1+1+1
15	Used as vector ,by modifying tumour inducing Ti plasmid,it is not pathogenic ,transforms the host plant cell Retrovirus, disarmed	1+1+1 1+1
16	Isolation of DNA(enzymes used),cutting the DNA, separation of fragments , PCR, introducing into host cell, obtaining gene product in the Bioreactor, downstream processing	1+1+1+1+1