TRANSFECTION METHODS, PROMOTERS AND EXPRESSION VECTORS - II

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Biotechnology

ADVANTAGES OF LIPOFECTION

i. Yields high transfection efficiencies
ii. Works in a wide variety of eukaryotic cells
iii. Is simple to perform
iv. Ensures consistently reproducible results
v. Moreover, a number of cell lines normally resistant to transfection by other methods transfect successfully with cationic lipid reagents

DISADVANTAGES

i. Quite low transfection efficiency in suspension cells
ii. Dependence on cell division
iii. As well as on high rate of endocytosis
iv. Not applicable to all cell types

ELECTROPORATION

- Method used to apply an electric current across a cell membrane resulting in temporary “pore” formation enabling the uptake of exogenous molecules found in the medium to either cytoplasm or into the nucleus, thereby transfecting the cell.
- Used to introduce foreign genes into a host cell
- Electric shocks are used as a mechanism for introducing new DNA into a host cell by creating new pores in the plasma membrane of the host cell.
- The new DNA enters the host cell through new pores and is incorporated into the genome of the new cell.

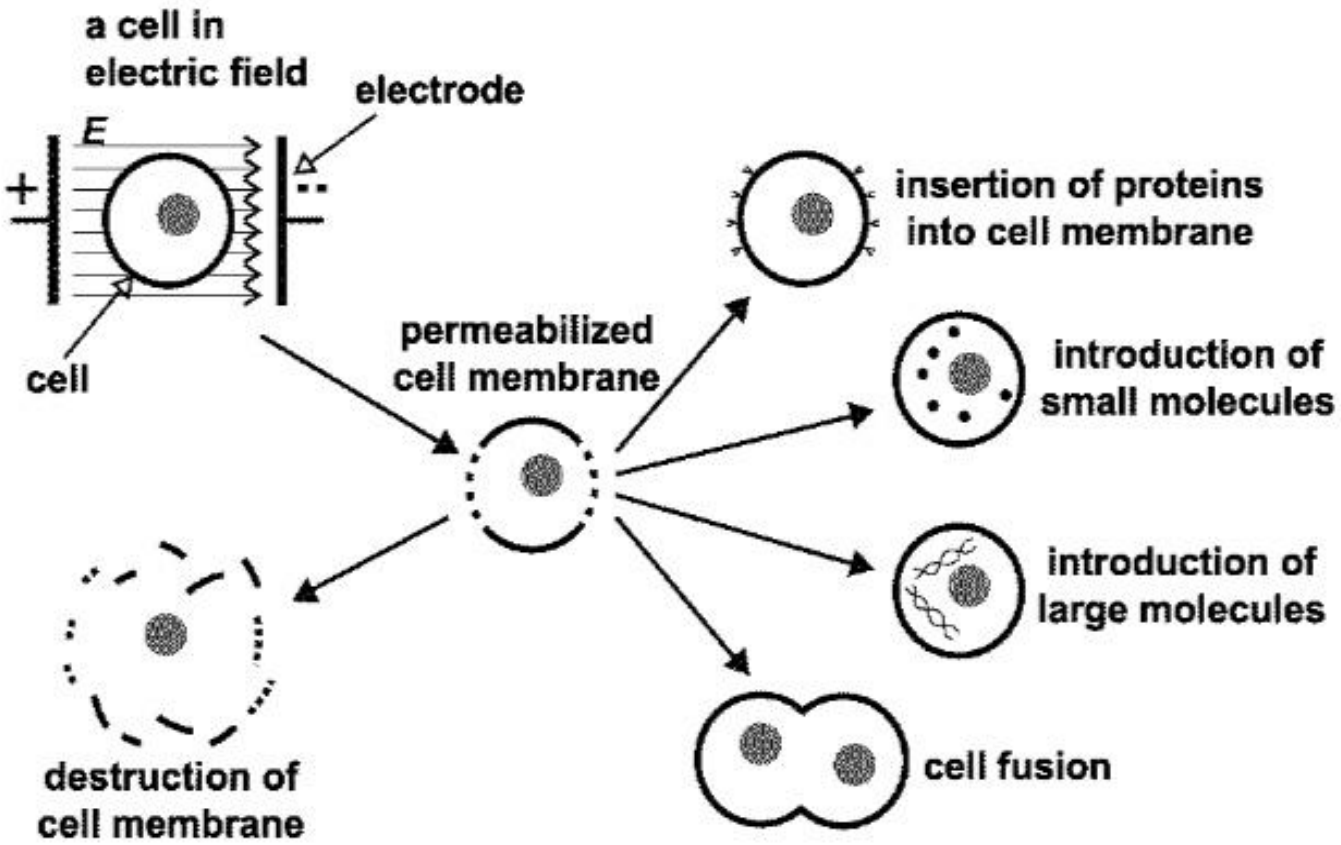
- **PHASES INVOLVED :**

i. Pore Formation – It is the response shown by the cell membrane by forming pore due to the induced threshold potential. Lasts for micro-seconds.

ii. Pore Expansion – Expansion of pore size for micro-milli second depending on the pulse duration.

iii. Membrane Recovery – The membrane is recovered and the pores are resealed that lasts for several minutes.

MECHANISM OF ELECTROPORATION



ADVANTAGES

i. **VERSATILITY** – Electroporation is effective with nearly all cell {monocot plant cells also } and species types.

ii. **EFFICIENCY-** A large majority of cells take in the target DNA or molecule. In a study on electro transformation of *E.coli*, for example, 80% of the cells received the foreign DNA.

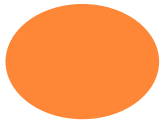
iii. **SMALL SCALE-** The amount of DNA required is smaller than for other methods .

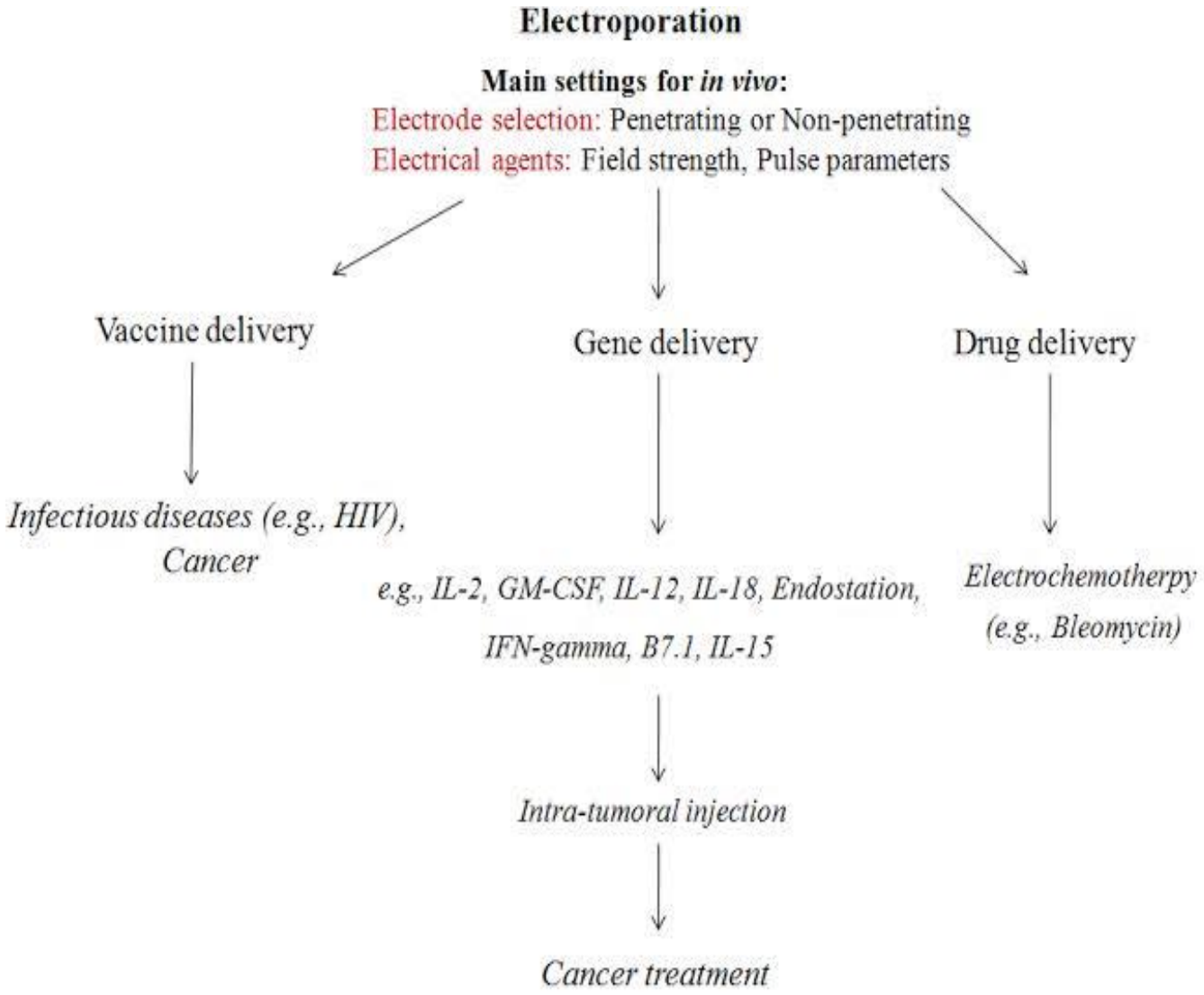
iv. *In-vivo*- Procedure may be performed with the intact tissue .

DISADVANTAGES

i. **CELL DAMAGE-** If the pulse are of the wrong length or intensity, some pores may become too large or fail to close after membrane discharge causing cell damage or rupture.

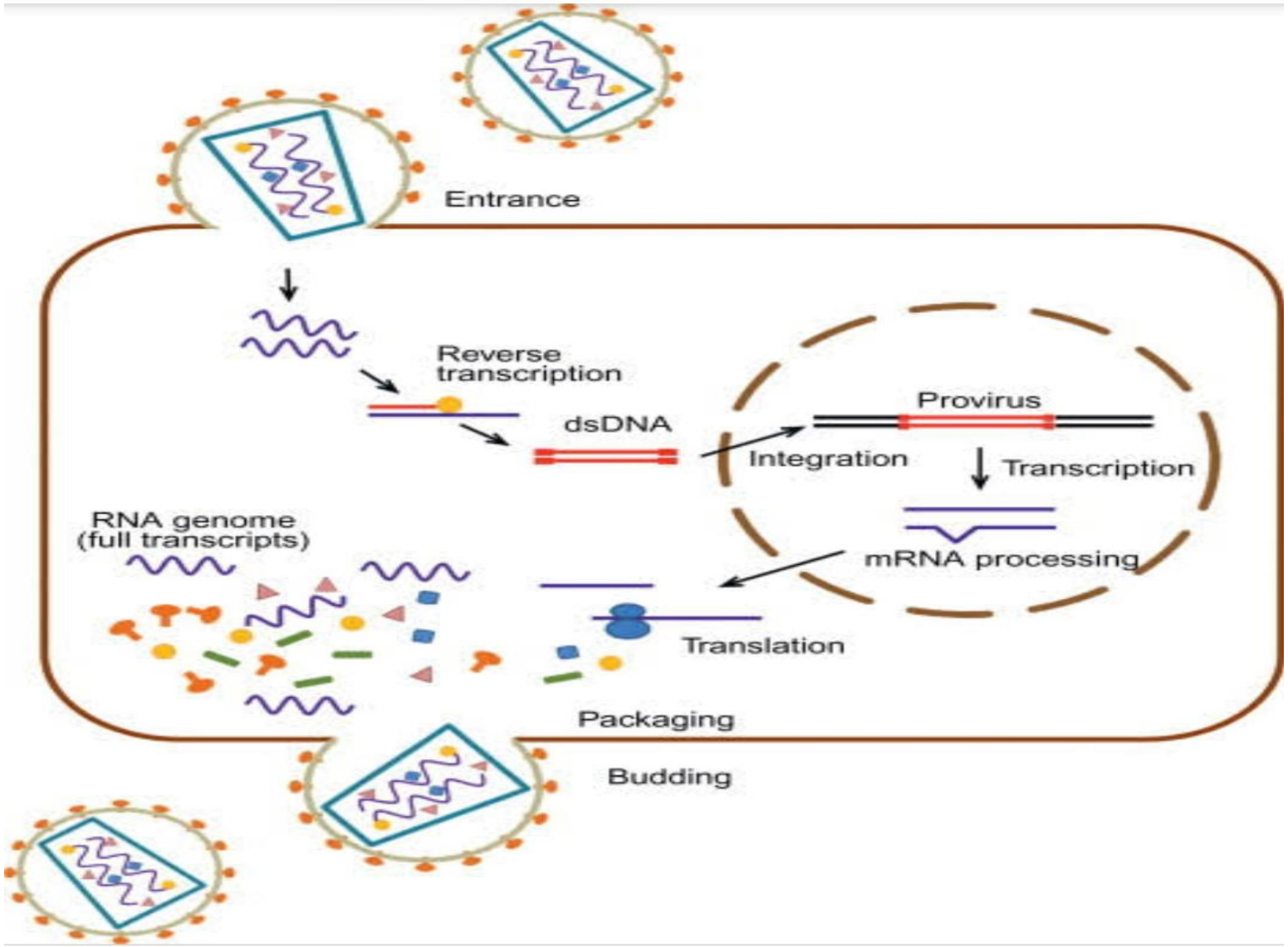
ii. **NON-SPECIFIC TECHNIQUE** : The transport of material into and out of the cell during the time of electro permeability is relatively non-specific. This may result in an ion imbalance that could later lead to improper cell function and cell death.




Electroporation
Main settings for *in vivo*:
Electrode selection: Penetrating or Non-penetrating
Electrical agents: Field strength, Pulse parameters
Vaccine delivery
Gene delivery
Drug delivery
Infectious diseases (e.g., HIV), Cancer
e.g., IL-2, GM-CSF, IL-12, IL-18, Endostation, IFN-gamma, B7.1, IL-15
Electrochemotherpy (e.g., Bleomycin)
Intra-tumoral injection
Cancer treatment

RETROVIRAL INFECTION

- Generation of high- titer retrovirus by transient production not only is less laborious than production of stable retroviral producer cell lines, but also has allowed the production of the high- titer retroviral supernatants from cDNAs that cannot be achieved by stable producer cell lines.
- Transient transfection has also increased the versatility of retrovirus-mediated gene transfer to include the rapid testing of different constructs, viral pseudo typing, and construction of retroviral cDNA libraries.
- Virus produced by transiently transfected cells can be used to infect the cells.
- This infection method are applicable to retrovirus produced by any of the stable producer cell lines.


Entrance
Reverse transcription
dsDNA
Provirus
Integration
Transcription
RNA genome (full transcripts)
mRNA processing
Translation
Packaging
Budding

DRAWBACKS OF RETROVIRAL VECTOR USAGE

- Vector derived from this viruses can transfer only small pieces (~8kb) of DNA.
- Although these vectors are designed to be replication defective, the genome of the retroviral strain (helper virus) that is needed to create a large quantities of the vector DNA can be integrated into the same nucleus as transgene.
- It is absolutely necessary that there should not be any retroviral contamination for applications in which either a commercial product is to be synthesized by the transgenic organism or the transgenic organism is used s food.
- In addition, transgene introduced on some retroviral vectors are silenced in mouse embryos.

ADVANTGES OF RETROVIRAL INFECTION

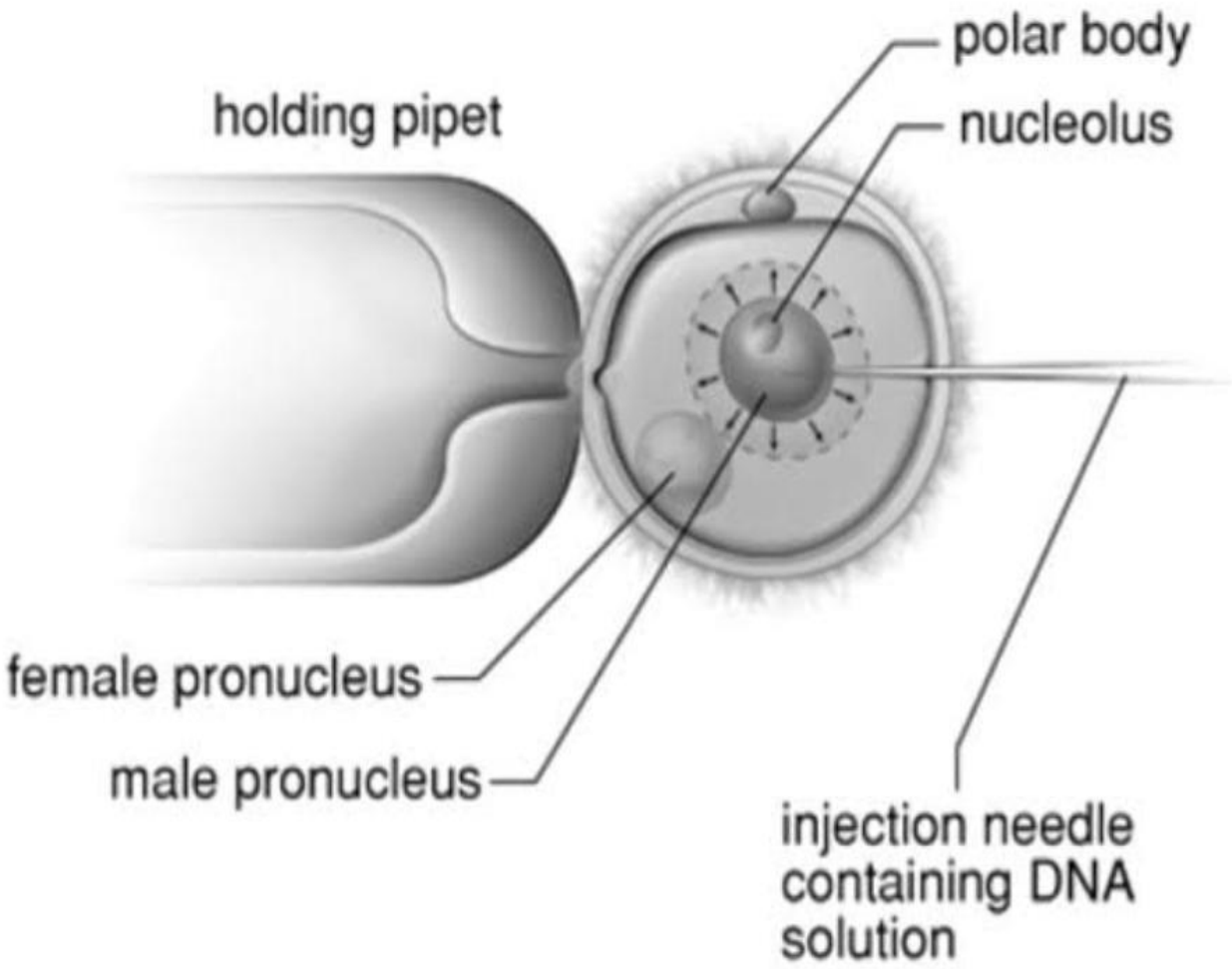
I. High transduction efficiency.
II. Insert up to size of approx. 8kb.
III. Integrated into host genome resulting in sustained expression of vectors.
IV. Extremely well suited system.
V. Vectors proteins are not expressed in the host.

DISADVANTAGES OF RETROVIRAL INFECTION

I. Requires dividing cells for infectivity.
II. Low titers(106-107).
III. Integration is random.
IV. In vivo delivery remains always poor.
V. Effective only when infecting helper cell lines.

MICROINJECTION

- Microinjection is a method of gene transfer which is used to introduce DNA into large cells, normally performed under a specialized optical microscope setup called a micromanipulator.
- It is considered as physical method of the gene transfer.
- Glass micropipette is usually of 0.5 to 5 micrometer, that easily penetrates into the host cell.
- The desired gene is then injected into the sub cellular compartment and the needle is removed.


polar body
holding pipet
nucleolus
female pronucleus
male pronucleus
injection needle
containing DNA
solution

APPLICATIONS OF MICROINJECTION

I. Process is applicable for plant as well as animal cell but it is more common to the animal cells.
II. Technique is ideally useful for producing transgenic animal quickly.
III. Procedure is important for gene transfer to embryonic cells.
IV. Applied to inject DNA into the plant nuclei.

LIMITATIONS OF MICROINJECTION

I. It is very costly as compared to the other methods of the gene transfer.
II. Skilled personal are required.
III. More useful for animal cells rather than plant cells.
IV. Embryonic cell preferred for manipulation.
V. Knowledge of mating, timing, oocyte recovery is essential.
VI. method is useful for protoplasts and not for the walled cells in case of plants.

PROMOTERS

- A promoter is a region of DNA that initiates transcription of a particular gene.
- Promoters are located near the transcription start sites of genes, on the same strand and upstream on the DNA (towards the 5’ region of the sense strand).
- Promoters can be about 10-1000 base pairs long.

TYPES OF PROMOTERS

I. **CONSTITUTE PROMOTER**:- these promoters are always active for gene expression and environment independent.

II. **TISSUE SPECIFIC**:- these promoters direct the gene expression in a specific tissues or at certain stage of development.

III. **SYNTHETIC PROMOTER**:- these promoters are made by primary element of a particular region from diverse origin.

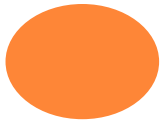
IV. **INDUCIBLE PROMOTER**:- these promoters dependent upon the external stimuli and environmental factors for gene expression.

EXPRESSION VECTOR

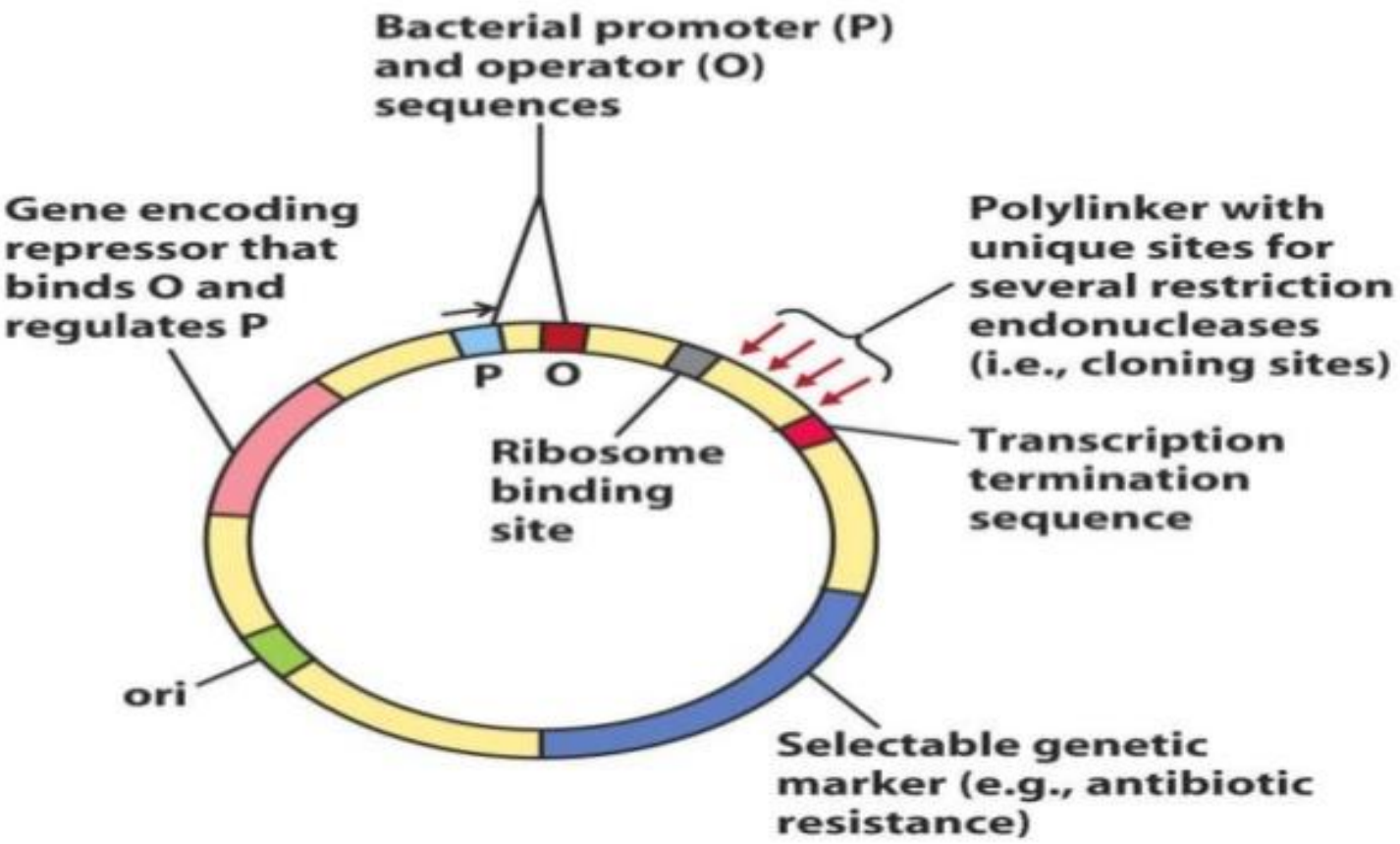
- The expression vector is usually a plasmid or virus designed for protein expression in cells.
- The expression vector is a plasmid engineered to introduce a particular gene into the target cell.
- Vectors that can yield the protein products of the cloned genes.
- Two elements that are required for active gene expression: a strong promoter and ribosome binding site near an initiating ATG codon.
- The main function of an expression vector is to yield the product of a gene, therefore a strong promoter is necessary. The more mRNA is produced, the more protein product is made.

GENETIC ELEMENTS REQUIREMENTS

I. Origin of replication
II. Selectable marker
III. Transcriptional promoter
IV. Unique multiple cloning sites
V. Translational initiation region translational terminator

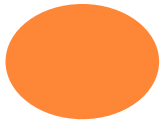


Expression vector



S. NO	HOST VECTOR SYSTEM	INDUCIBLE EXPRESSION/ PROMOTER
1.	**E.coli**	a) Lac promoter b) Tac promoter c) λPL promoter d) T7 expression system
2.	**Yeast** ▪ *Saccharomyces cerevisiae*	a) GAL system b) CUPI System
	▪ *Pichia pastoris*	a) Alcohol oxidase
	▪ *Schizosaccharomyces pombe*	a) Nmt 1

Thank You



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- Animal Biotechnology by Manjula **Shenoy**.
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