Microbes in Agrobiotechnology

INTRODUCTION

- Based on recent advances in genetic engineering, molecular biology. This may revolutionize agriculture, as the green revolution did earlier.
- Increase in productivity – lead to environment friendly sustainable agricultural practices.
- Direct production of lysine rich wheat, edible vaccines etc.
- Others are insect resistant plants, golden rice, edible vaccines, delayed ripening of tomato, lysine rich cereals, herbicide tolerant plants, plant disease resistance etc.
- Great concern – safety of GM foods, environmental impact and moral issues.

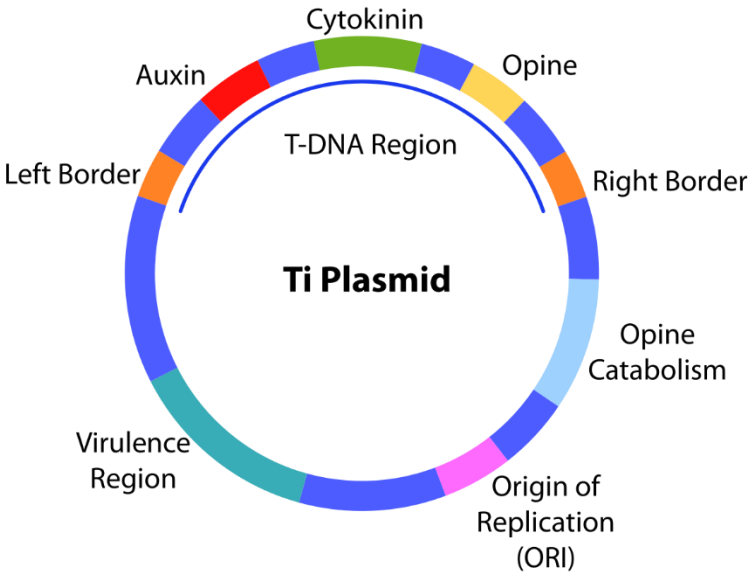
INTRODUCTION

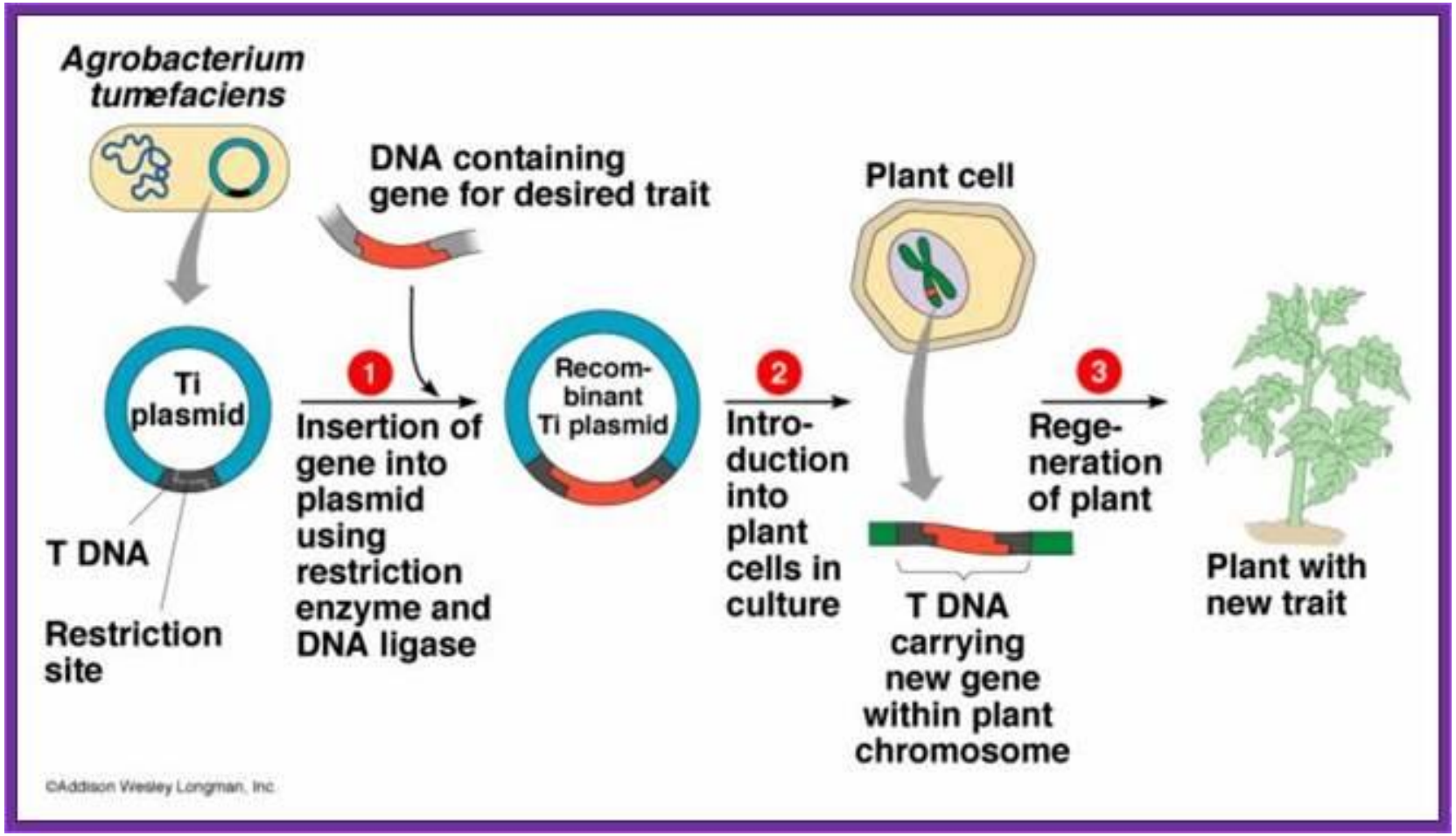
Challenges in the Post "Green Revolution" era:

- Human population increase – land available deterioting and decreasing – water supplies limiting – loss from weeds & insects etc.
- Post harvest losses – 10-30%. Demand for food – would double by 2025 and triple by 2050.
- Importance of agrobiotechnology – plant biotechnology would solve the crisis and problems faced – also new ways to produce human and animal health care products and industry raw materials.
- GE crops in the market. Predominant crops like maize, soybean cotton, potatoes etc.
- Studies and field trials of transgenic crops increasing with higher yields, improved quality and enhanced resistance against herbicides, diseases and pests.

Plant Transformation

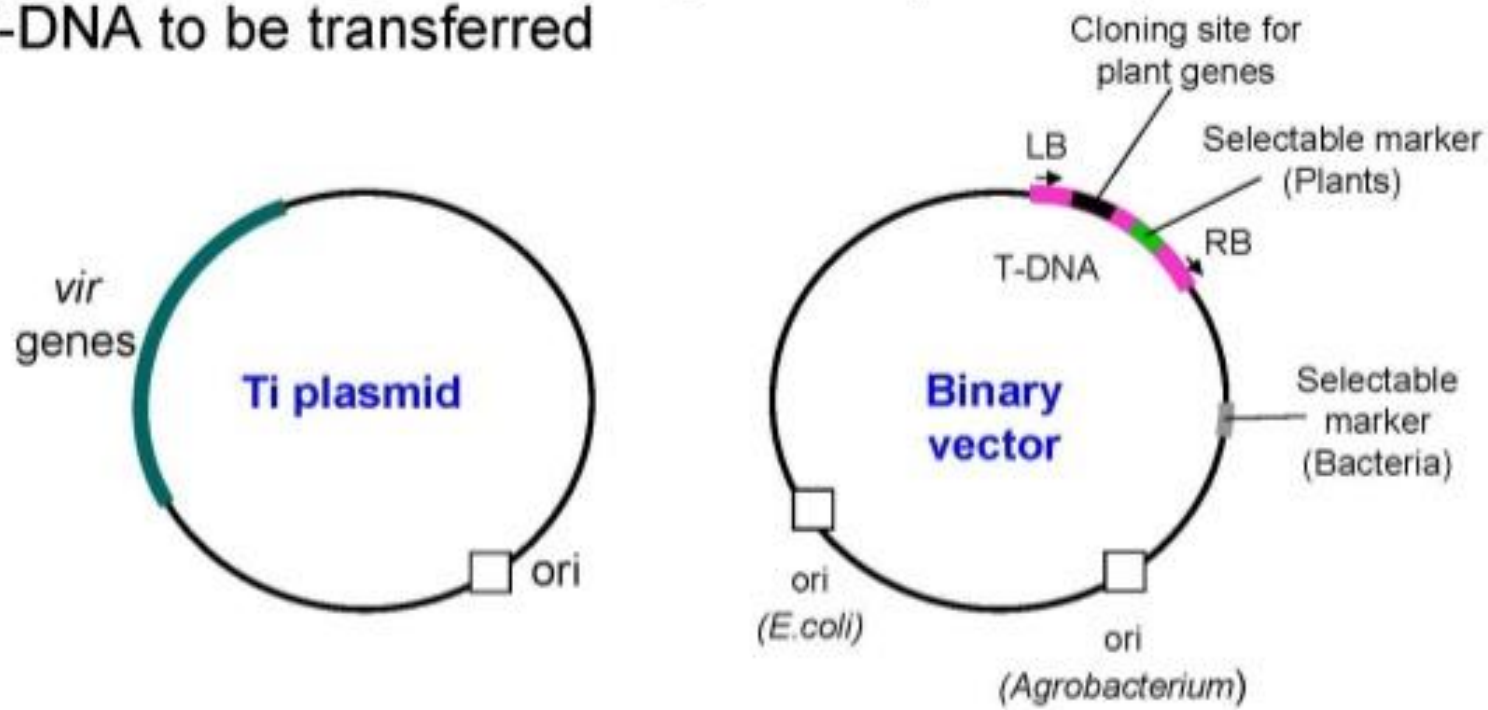
- Many ways – *Agrobacterium*, direct uptake of forieign DNA into protoplast, particle bombardment, electroporation etc.
- Agrobacterium mediated transformation – widely used – Ti plasmid used.

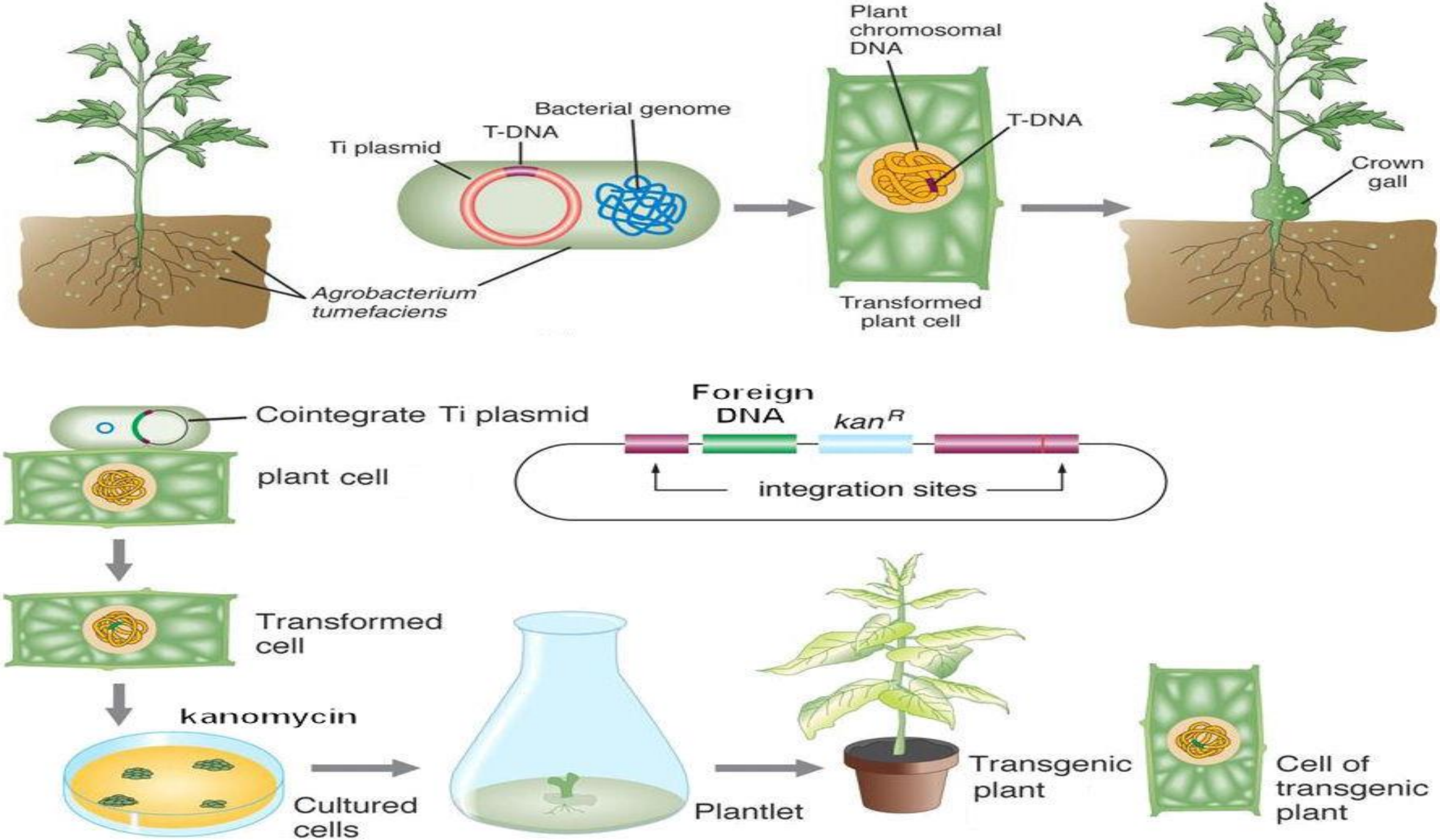




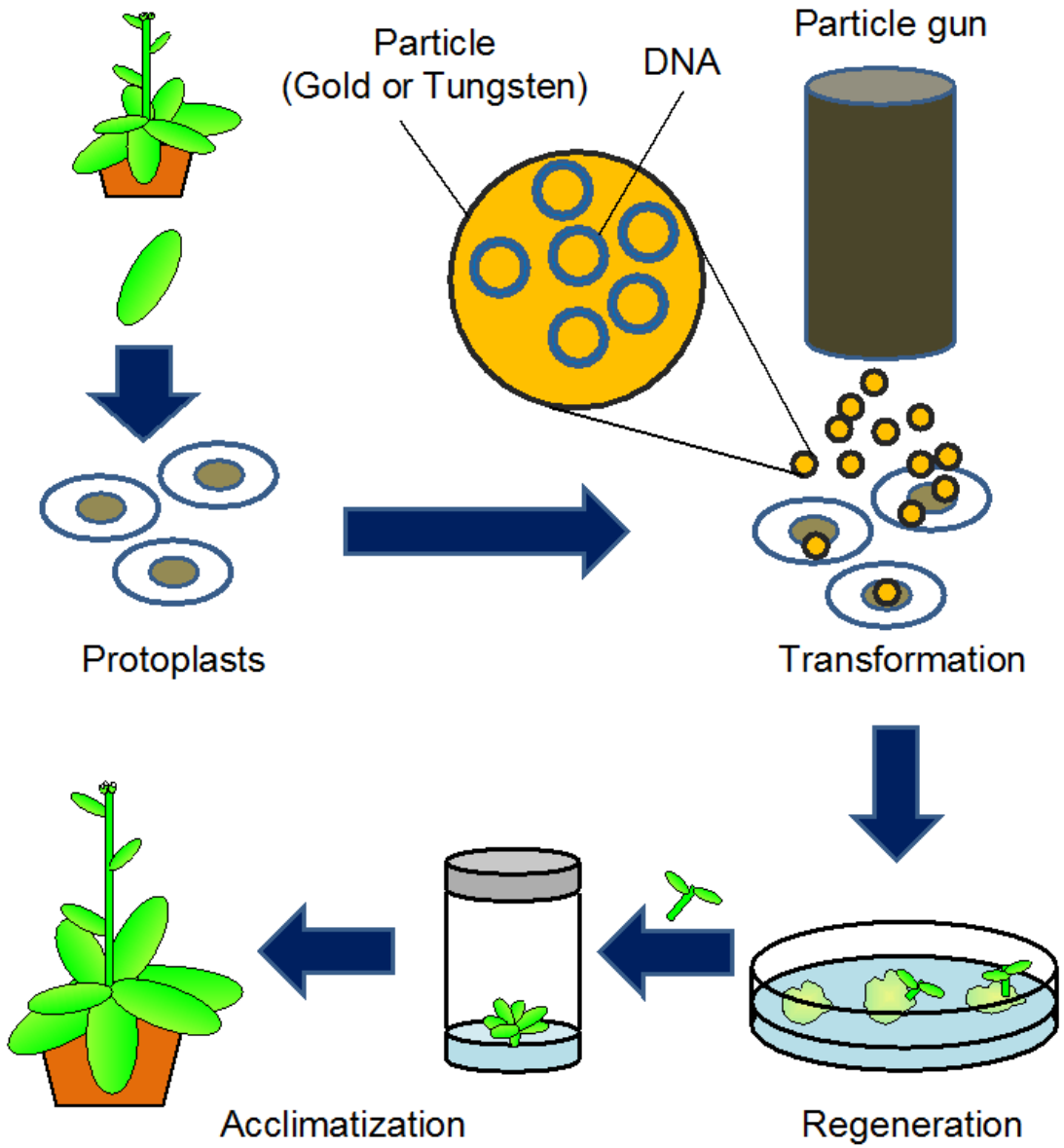
Binary vector system
for genetic engineering

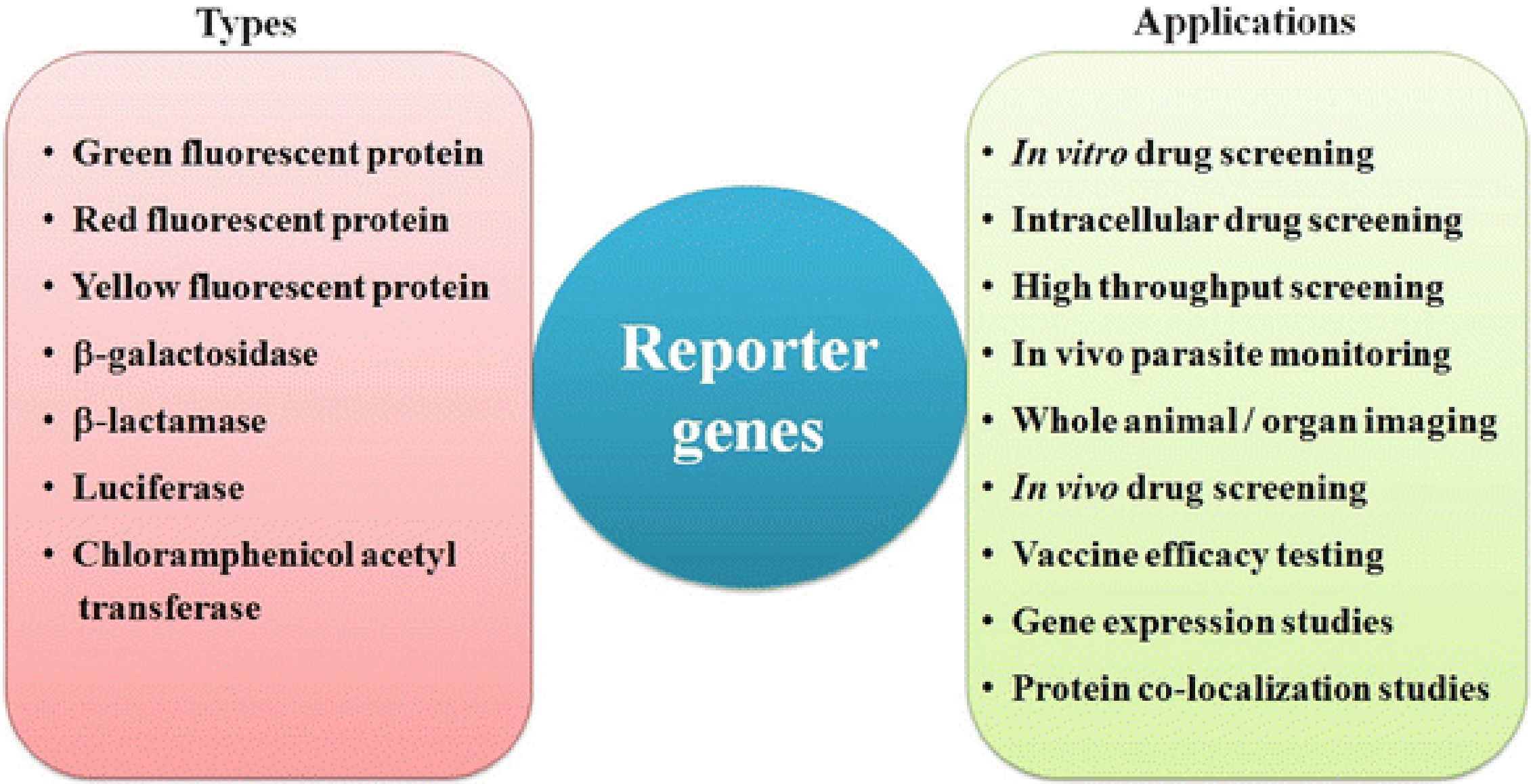
- *vir* genes and T-DNA can be on separate plasmids
- only left and right borders (LB & RB) are required for T-DNA to be transferred



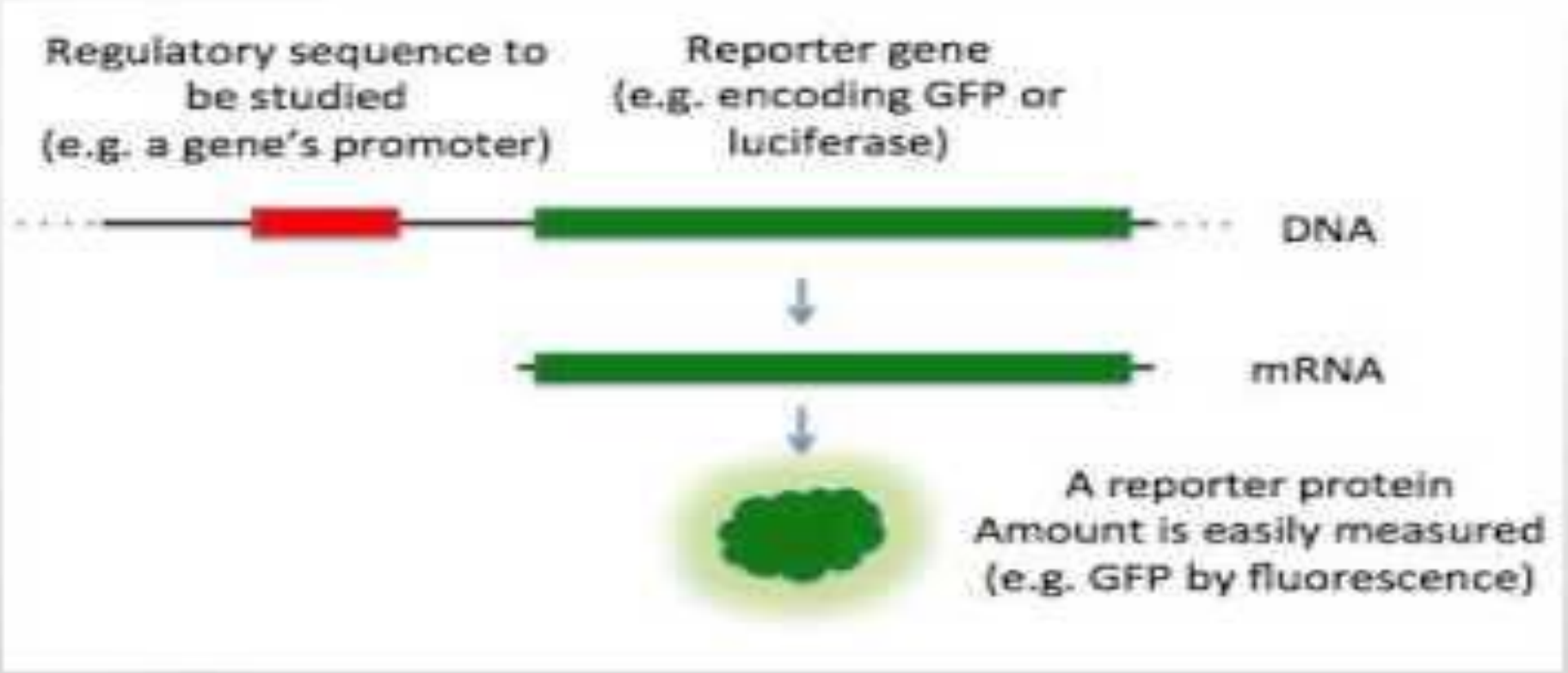

Plant chromosomal DNA
Bacterial genome
T-DNA
Ti plasmid
T-DNA
Crown gall
Agrobacterium tumefaciens
Transformed plant cell
Cointegrate Ti plasmid
plant cell
Foreign DNA
kanR
integration sites
Transformed cell
kanomycin
Cultured cells
Plantlet
Transgenic plant
Cell of transgenic plant

Particle bombardment or biolistics




Types
Green fluorescent protein
Red fluorescent protein
Yellow fluorescent protein
β-galactosidase
β-lactamase
Luciferase
Chloramphenicol acetyl transferase
Reporter genes
Applications
In vitro drug screening
Intracellular drug screening
High throughput screening
In vivo parasite monitoring
Whole animal / organ imaging
In vivo drug screening
Vaccine efficacy testing
Gene expression studies
Protein co-localization studies

Reporter gene



Selectable marker genes

- Selectable marker genes are conditionally dominant genes that confer an ability to grow in the presence of applied selective agents that are normally toxic to plant cells or inhibitory to plant growth, such as antibiotics and herbicides.
- A selectable marker helps to identify and delete **non**-transformants and allows processors to expand selectively. A selectable marker is a gene inserted into a cell, in particular a bacterium or a cultured cell, which confers a trait appropriate for artificial selection.
- Genes that are frequently used to select transformed plant tissues include *npt*II, *hpt*, *bar*, and *gox*, that confer resistance to kanamycin, hygromycin, phosphinothricin, and glyphosate, respectively.
- Commonly used selectable marker systems include neomycin phosphotransferase, hygromycin phosphotransferase, phosphoinothricin acetyltransferase, and glyphosate oxidoreductase.
- Hygromycin B has been used as a more favorable selective agent than kanamycin for transformation of a number of crop species including peanuts and soybean, in a somatic embryogenesis regeneration system

Promoters & Gene expression

- **Promoter**. A **promoter** is a sequence of DNA needed to turn a **gene** on or off. The process of transcription is initiated at the **promoter**. Usually found near the beginning of a **gene**, the **promoter** has a binding site for the enzyme used to make a messenger RNA (**mRNA**) molecule.
- The **Promoter** and the Transcription Machinery. **Genes** are organized to make the control of **gene expression** easier. ... The purpose of the **promoter** is to bind transcription factors that control the initiation of transcription. Within the **promoter region**, just upstream of the transcriptional start site, resides the TATA box.
- **CaMV 35S** is the most commonly used constitutive promoter for high levels of gene expression in dicot plants. **Maize Ubi** and rice Act-1 are the currently the most commonly used constitutive promoters for monocots.

Promoters & Gene expression

- **Chemically inducible promoters**
  Tetracycline and its derivatives serve as inducing agents to allow **promoter** activation. One of the most commonly used prokaryotic **promoters** is the negative **inducible** pLac **promoter**. This **promoter** requires removal of the lac repressor (lacI protein) for transcription to be activated.
- **Tissue specific promoters**
- A **promoter** whose activity is observed only in **specific tissues** of the **plant** is known as a **tissue-specific promoter**. Such **promoters** express the cistronic part of their gene in cells of **particular tissue** types, and their expression may also be induced in those **tissues** by internal or external factors.

Examples:

- Golden Rice
- Insect resistant plants
- Edible vaccines
- Delayed ripening in tomato
- Lysine rich cereals
- Herbicide tolerant plants
- Plant disease resistance