

Cloning and rDNA (II)

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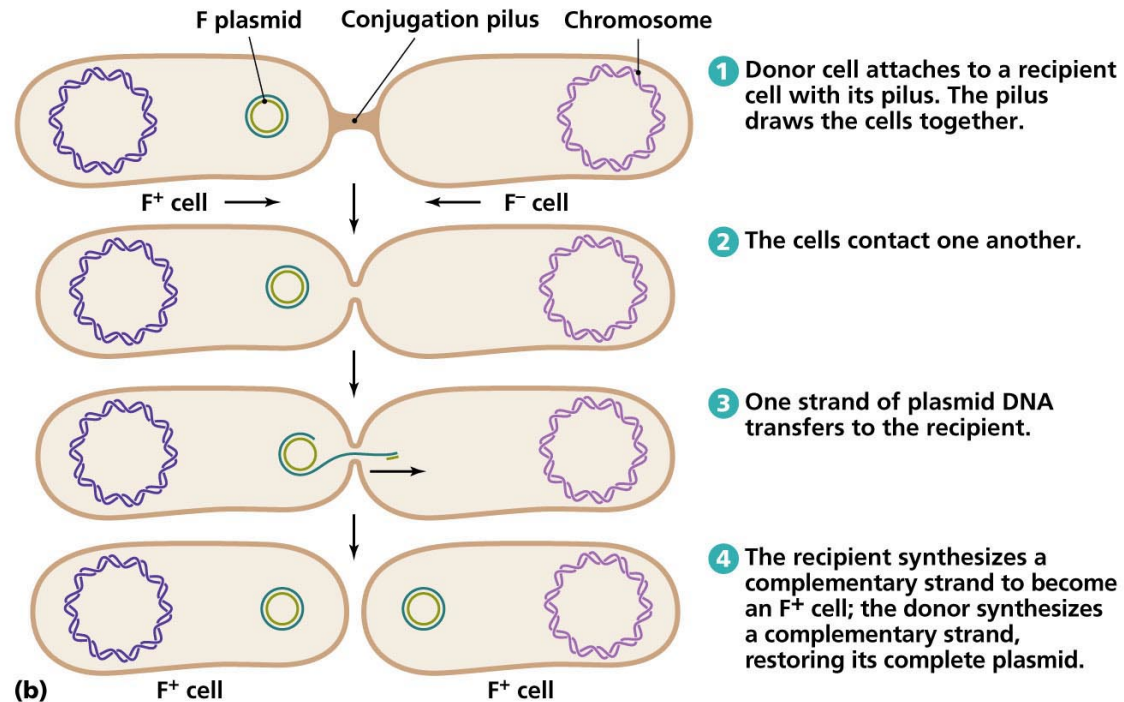
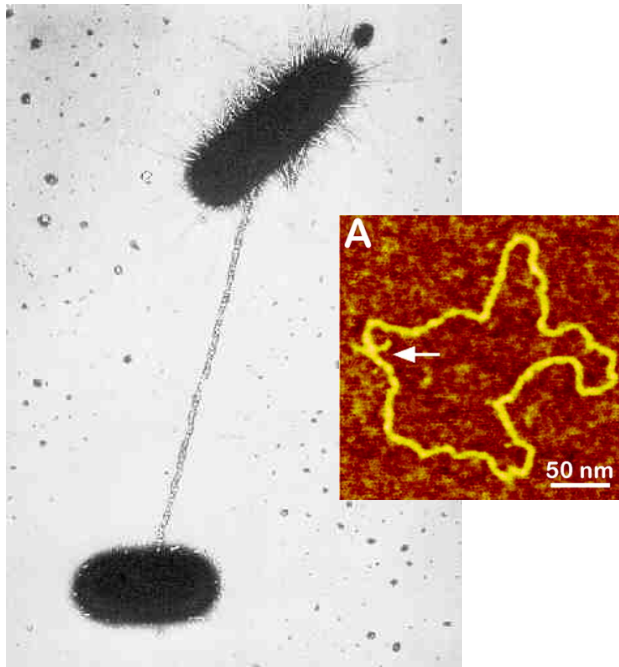
Objectives of this lecture

By the end of this lecture you will be able to:

1. Recognize the main elements of an expression vector
2. Understand the function of each element

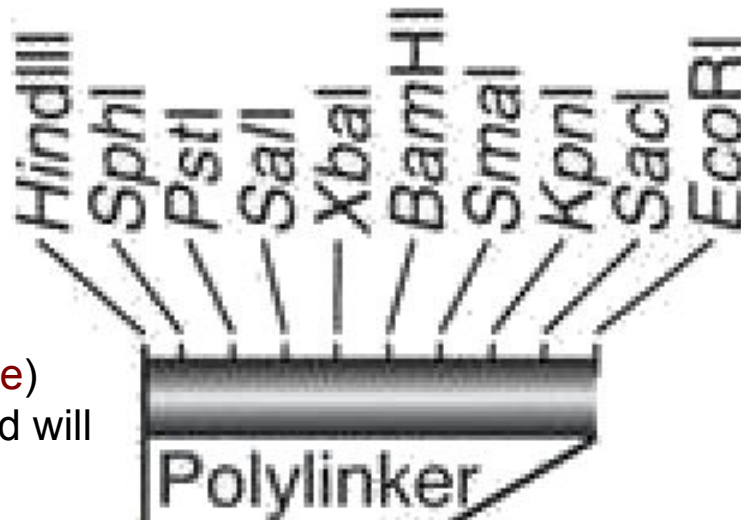
Plasmid

- Extrachromosomal DNA molecules
- Usually transfer antibiotic-resistance between bacteria



pUC18

Vector



Selectable marker

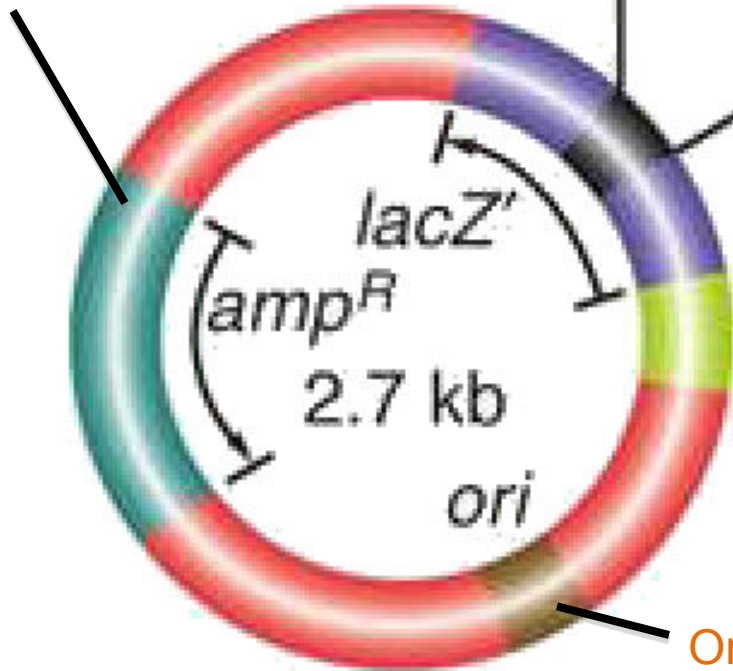
a gene (antibiotic resistance) when expressed on plasmid will allow host cells to survive

Multiple Cloning Site (MCS) also called **Polylinker** is a short segment of DNA which contains many (up to ~20) restriction sites

lac promoter

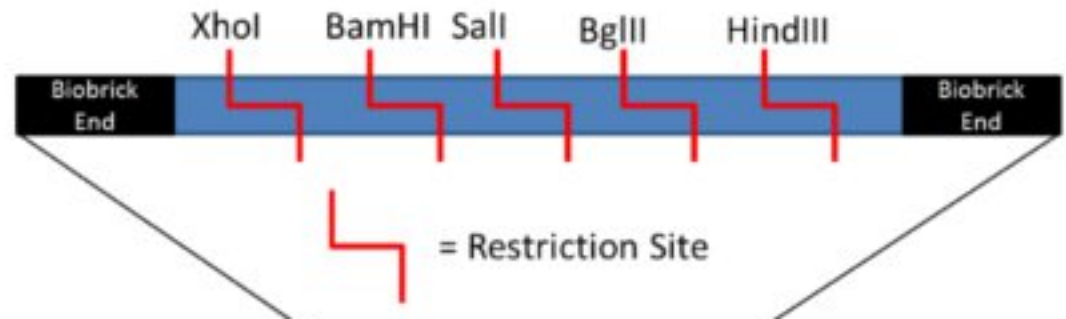
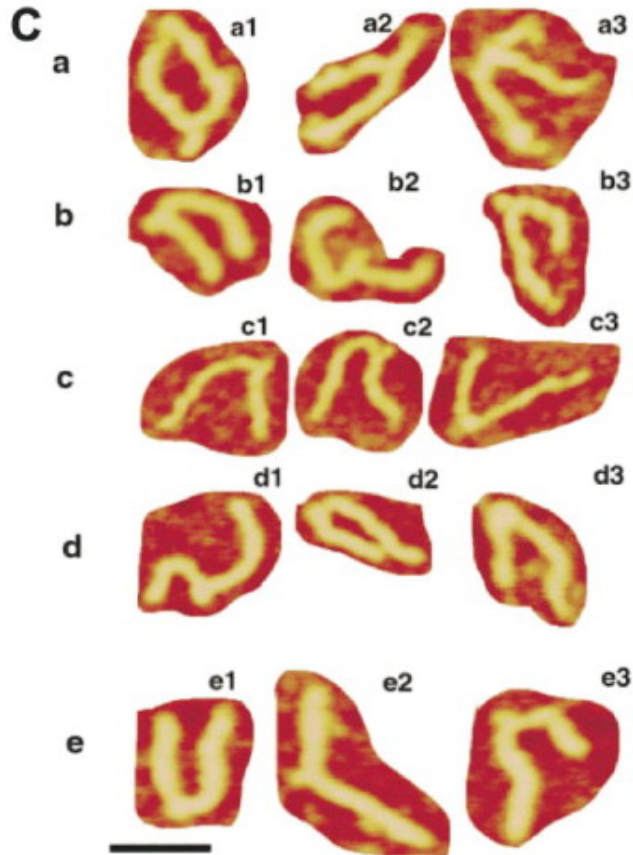
Promoter is a short DNA sequence which enhances expression of adjacent gene

Ori origin of replication is a particular sequence where replication is initiated



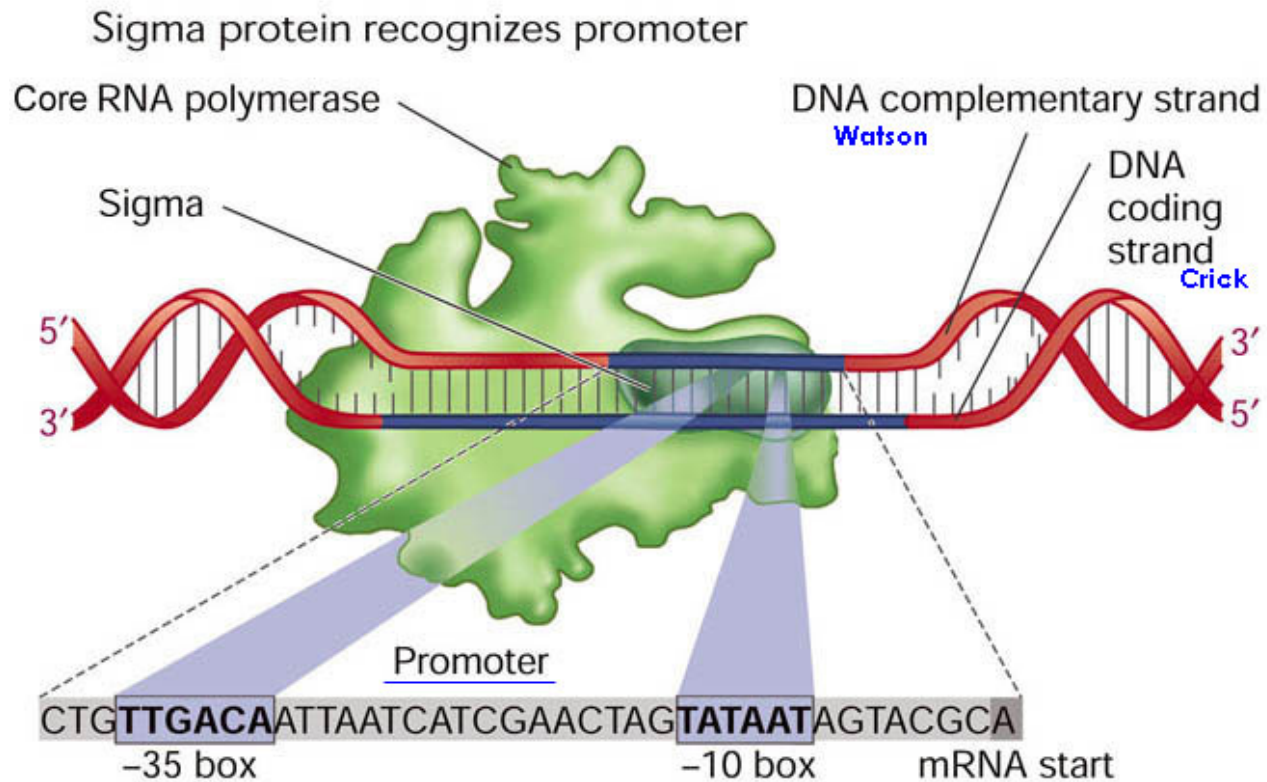
Multiple Cloning Site

- A short segment of DNA which contains many (up to ~20) restriction site for several restriction enzymes



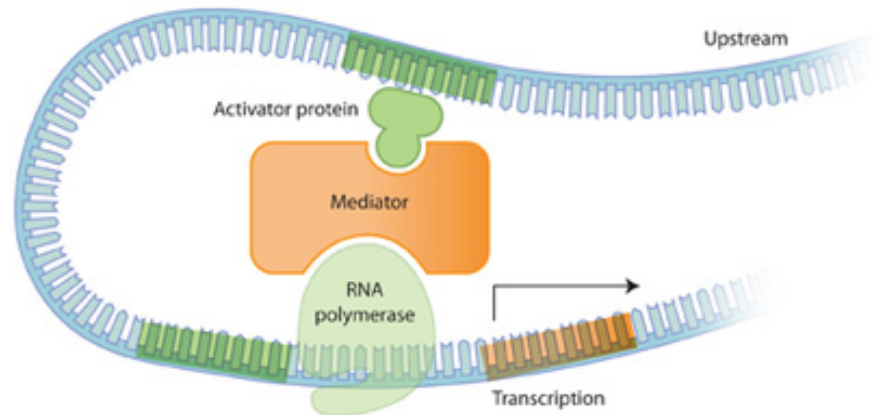
Promoter

- A region in the DNA that initiates the transcription of a particular gene



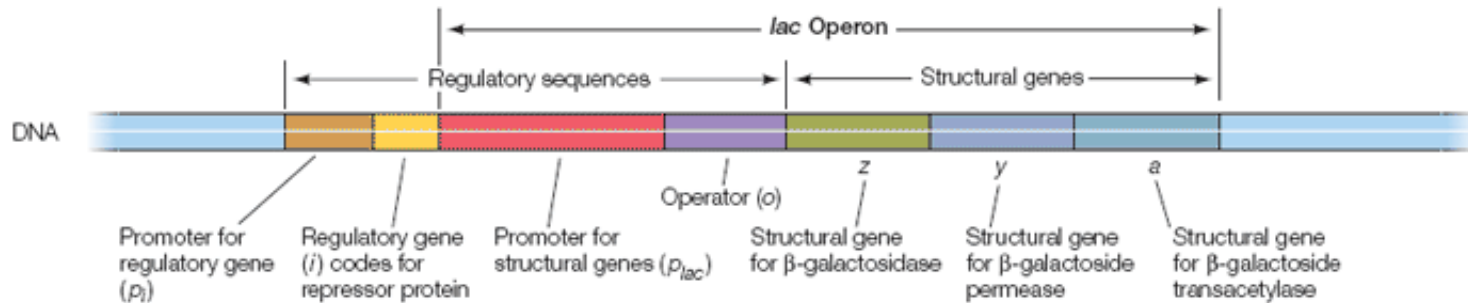
Promoter

- **Activator protein binds to enhancer sequence and attracts proteins to the promoter region that activate RNA polymerase II leading to transcription**



Lac Operon

- The lac operon of *E. coli* is a segment of DNA that includes a promoter, an operator, and the three structural genes that code for lactose-metabolizing enzymes



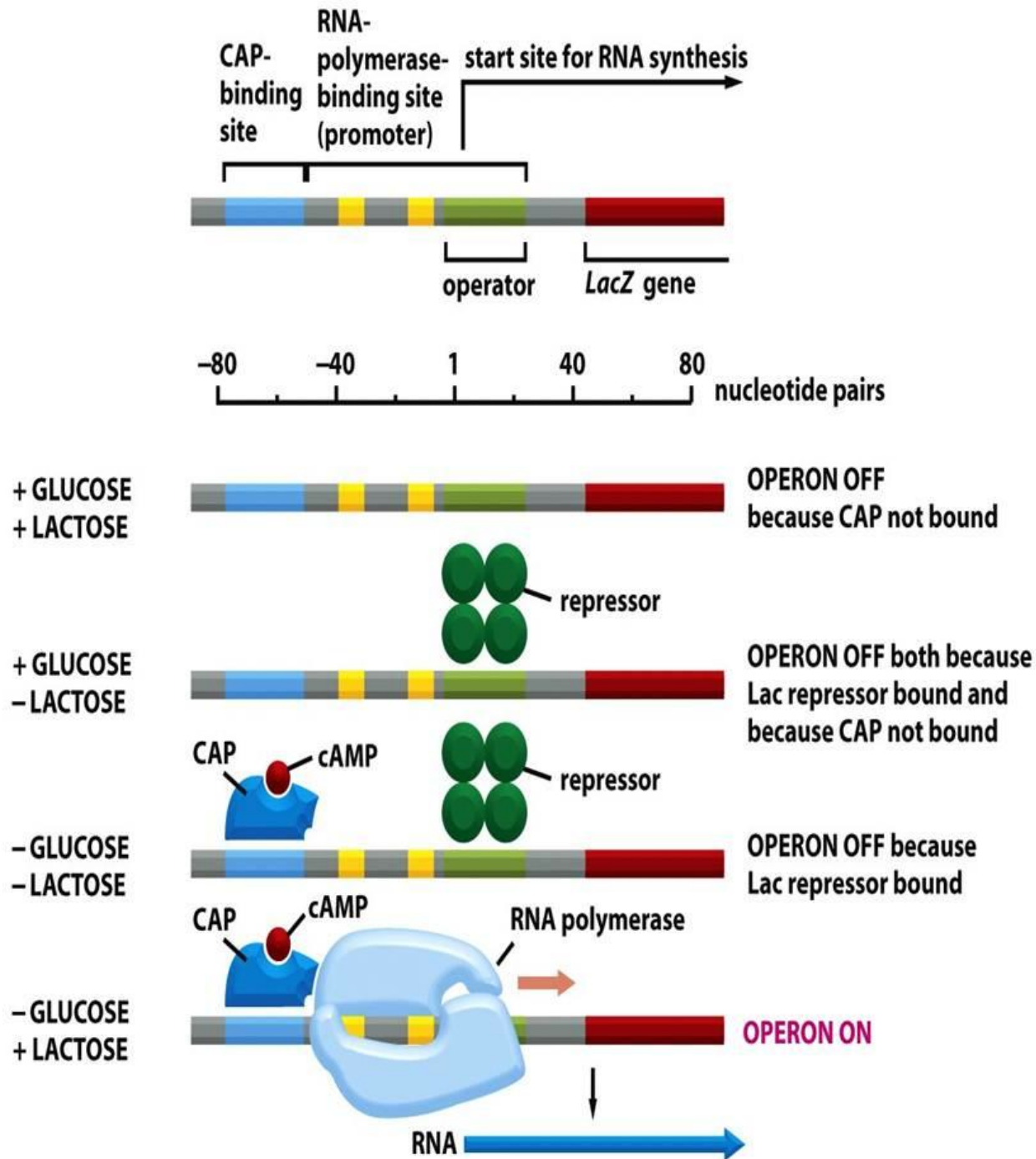
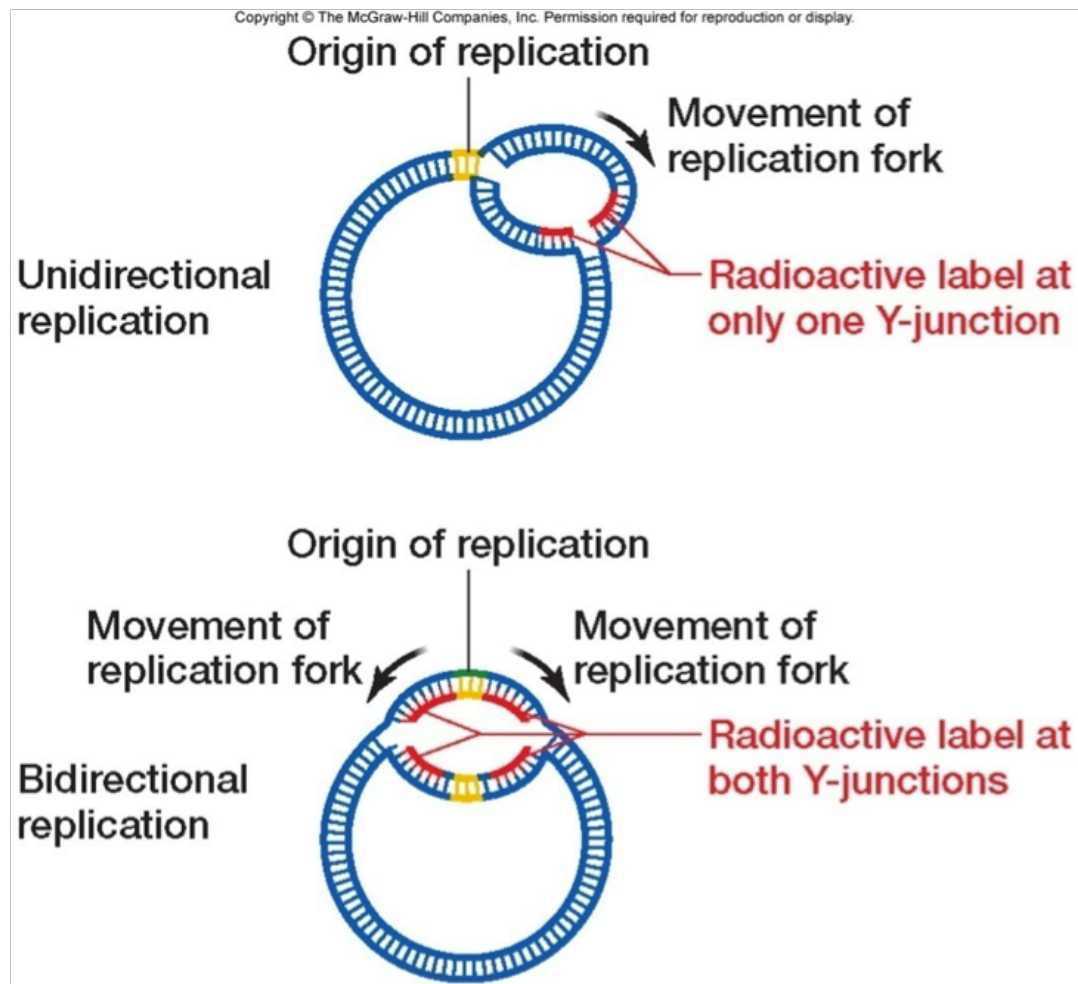
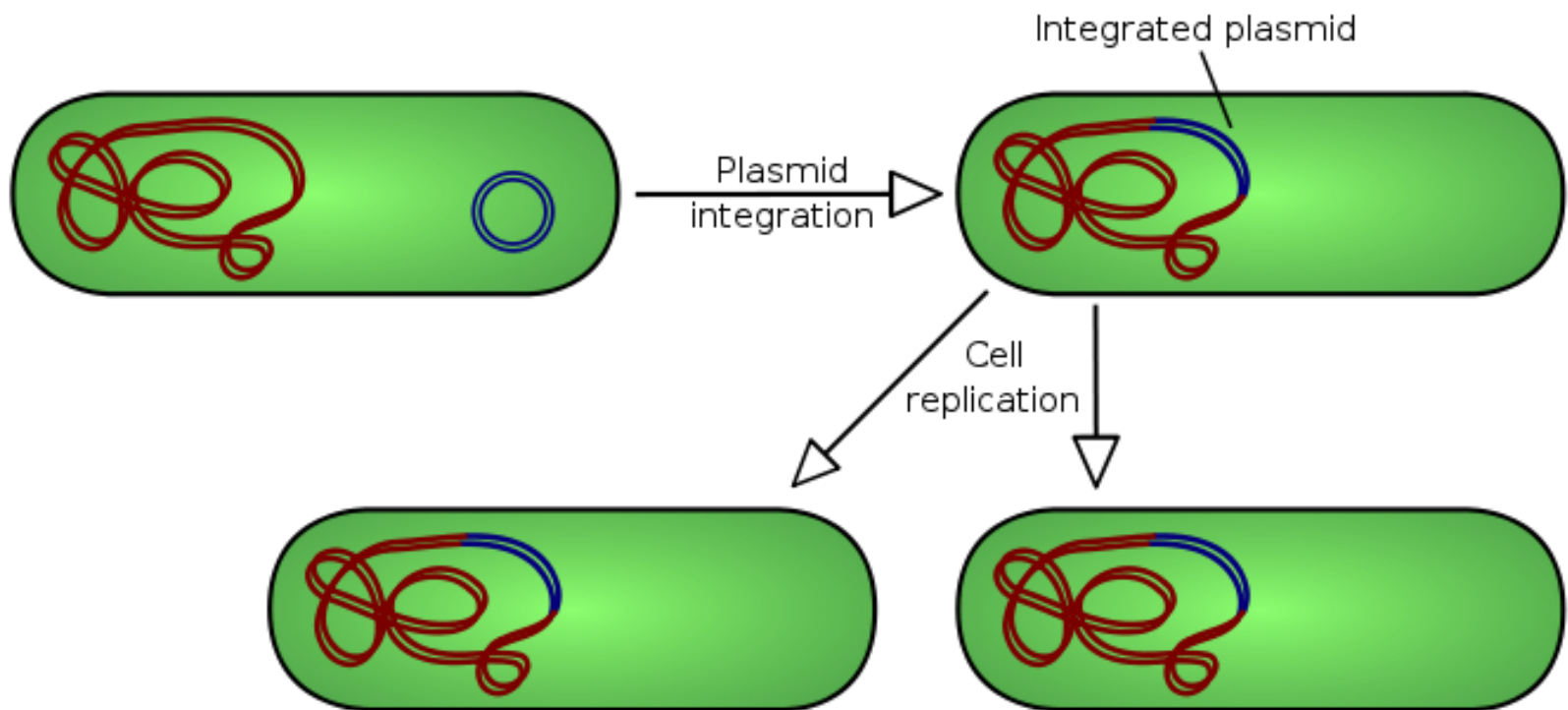
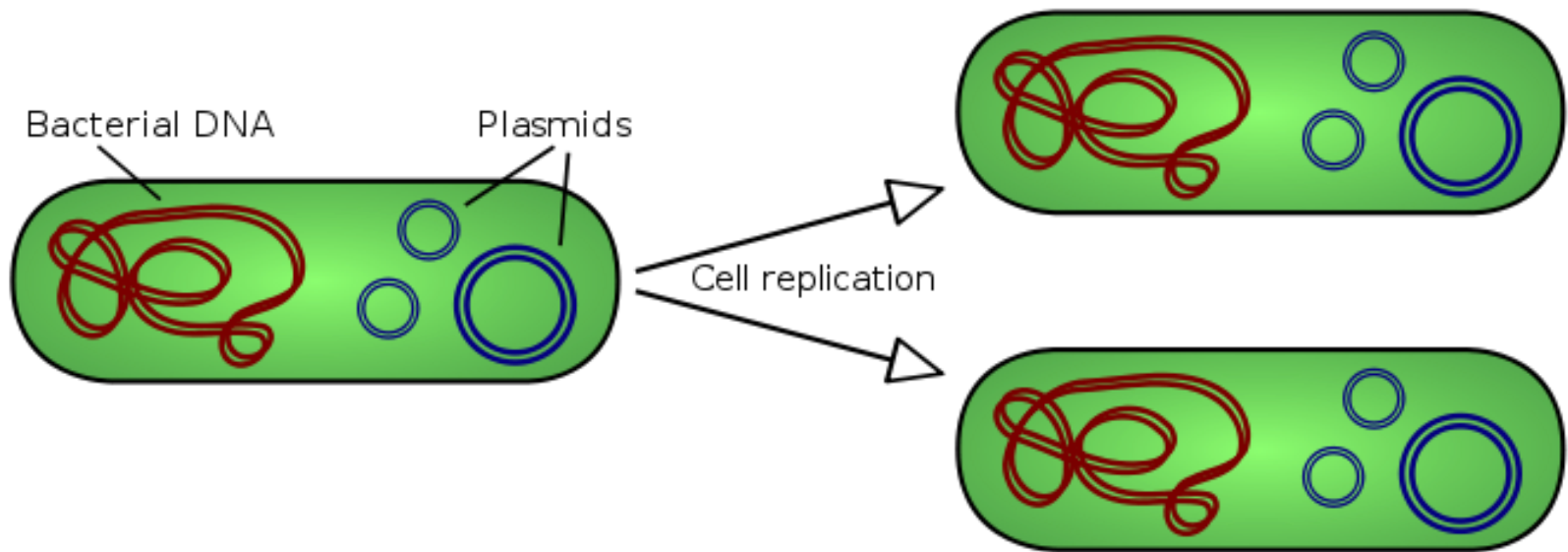


Figure 7-39 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Origin of Replication

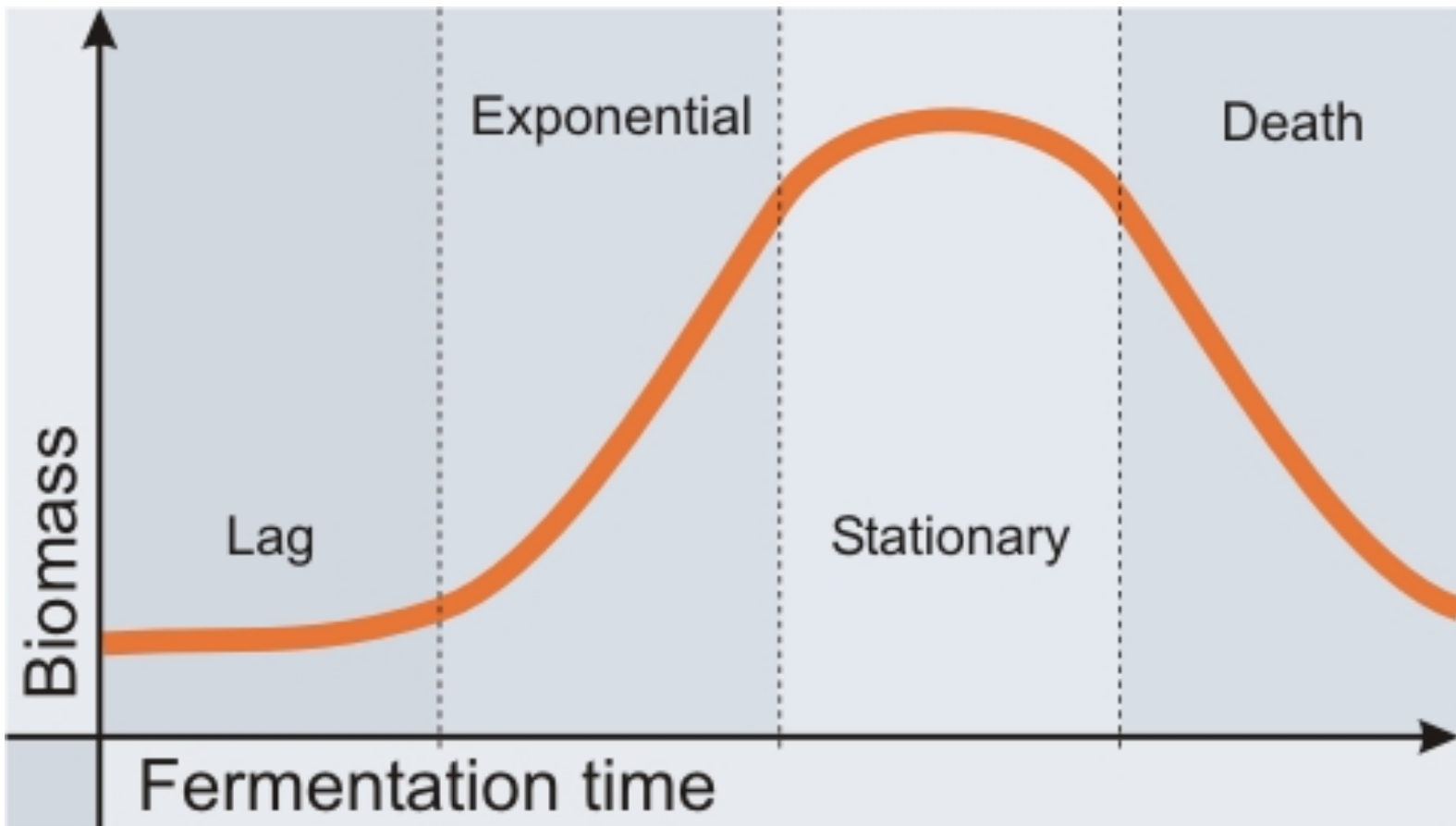
- A sequence of DNA at which replication is initiated





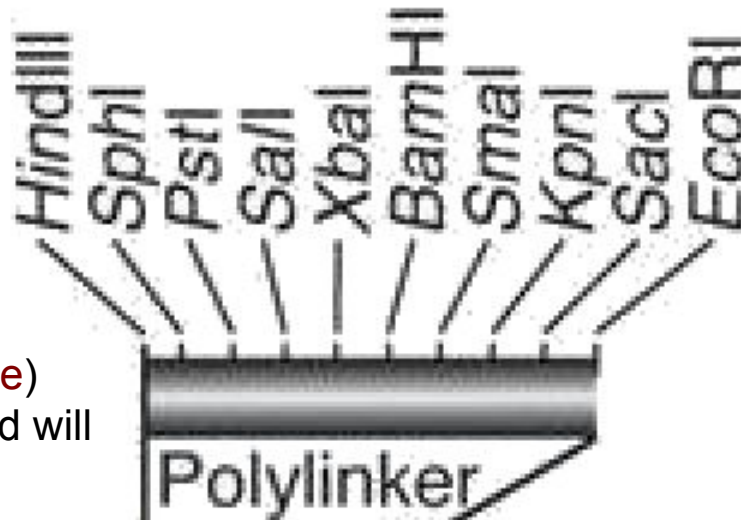
Selectable Marker

- A gene introduced to a cell that provides a property for artificial selection e.g. antibiotic resistance



pUC18

Vector



Selectable marker

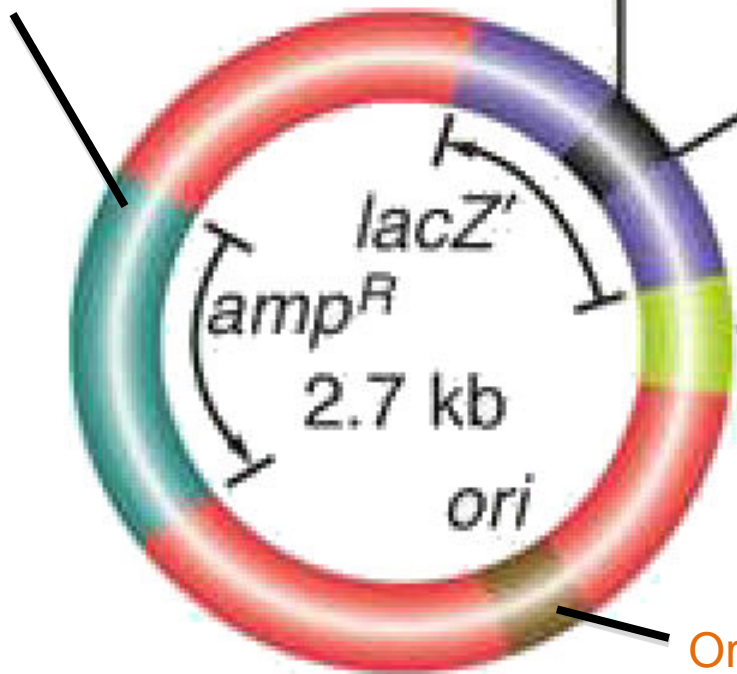
a gene (antibiotic resistance) when expressed on plasmid will allow host cells to survive

Multiple Cloning Site (MSC) also called **Polylinker** is a short segment of DNA which contains many (up to ~20) restriction sites

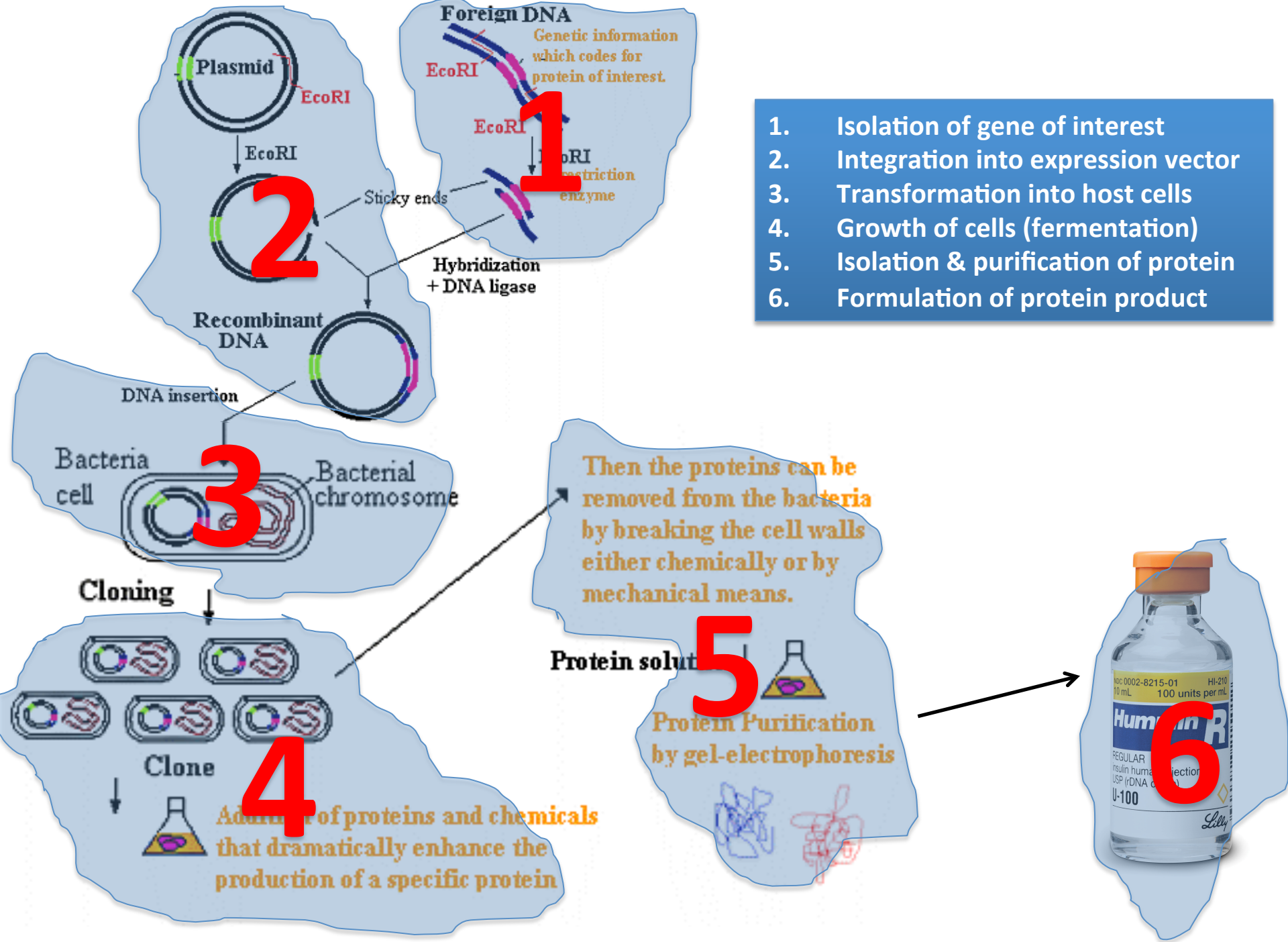
lac promoter

Promoter is a short DNA sequence which enhances expression of adjacent gene

Ori origin of replication is a particular sequence where replication is initiated



1. Isolation of gene of interest
2. Integration into expression vector
3. Transformation into host cells
4. Growth of cells (fermentation)
5. Isolation & purification of protein
6. Formulation of protein product



You are now able to:

- ✓ Recognize the main elements of an expression vector
- ✓ Understand the function of each element