Cloning and rDNA (I)

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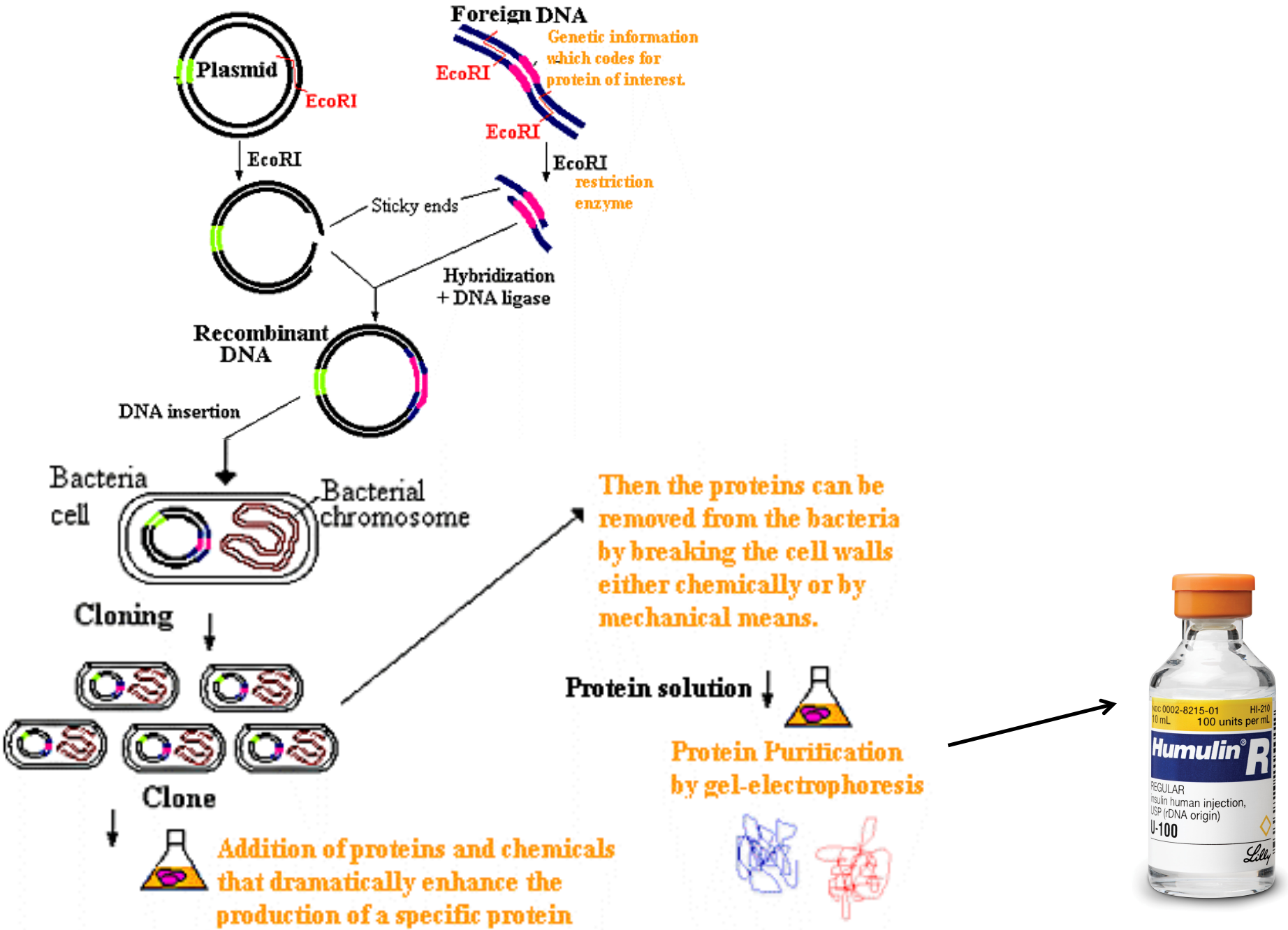
Objectives of this lecture

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

1. Understand the concept of recombinant DNA technology
2. Define terms related to cloning and DNA recombination
3. Realize the value of recombinant DNA technology


Foreign DNA
Genetic information which codes for protein of interest.
EcoRI
Plasmid
EcoRI
EcoRI
EcoRI
EcoRI
EcoRI restriction enzyme
Sticky ends
Hybridization + DNA ligase
Recombinant DNA
DNA insertion
Bacteria cell
Bacterial chromosome
Cloning
Clone
Addition of proteins and chemicals that dramatically enhance the production of a specific protein
Then the proteins can be removed from the bacteria by breaking the cell walls either chemically or by mechanical means.
Protein solution
Protein Purification by gel-electrophoresis
NDC 0002-8215-01
10 mL
HI-210
100 units per mL
Humulin® R
REGULAR
insulin human injection, USP (rDNA origin)
U-100
Lilly

Expression vector

Plasmid

EcoRI

Foreign DNA

Genetic information which codes for protein of interest.

EcoRI

EcoRI

Palindrome

DNA of interest

EcoRI

EcoRI

restriction enzyme

Restriction Enzyme

Sticky ends

Hybridization + DNA ligase

Recombinant DNA

Recombinant DNA

Delivery vector

Expressio system

Bacterial chromosome

Cloning

Clone

Expressio system

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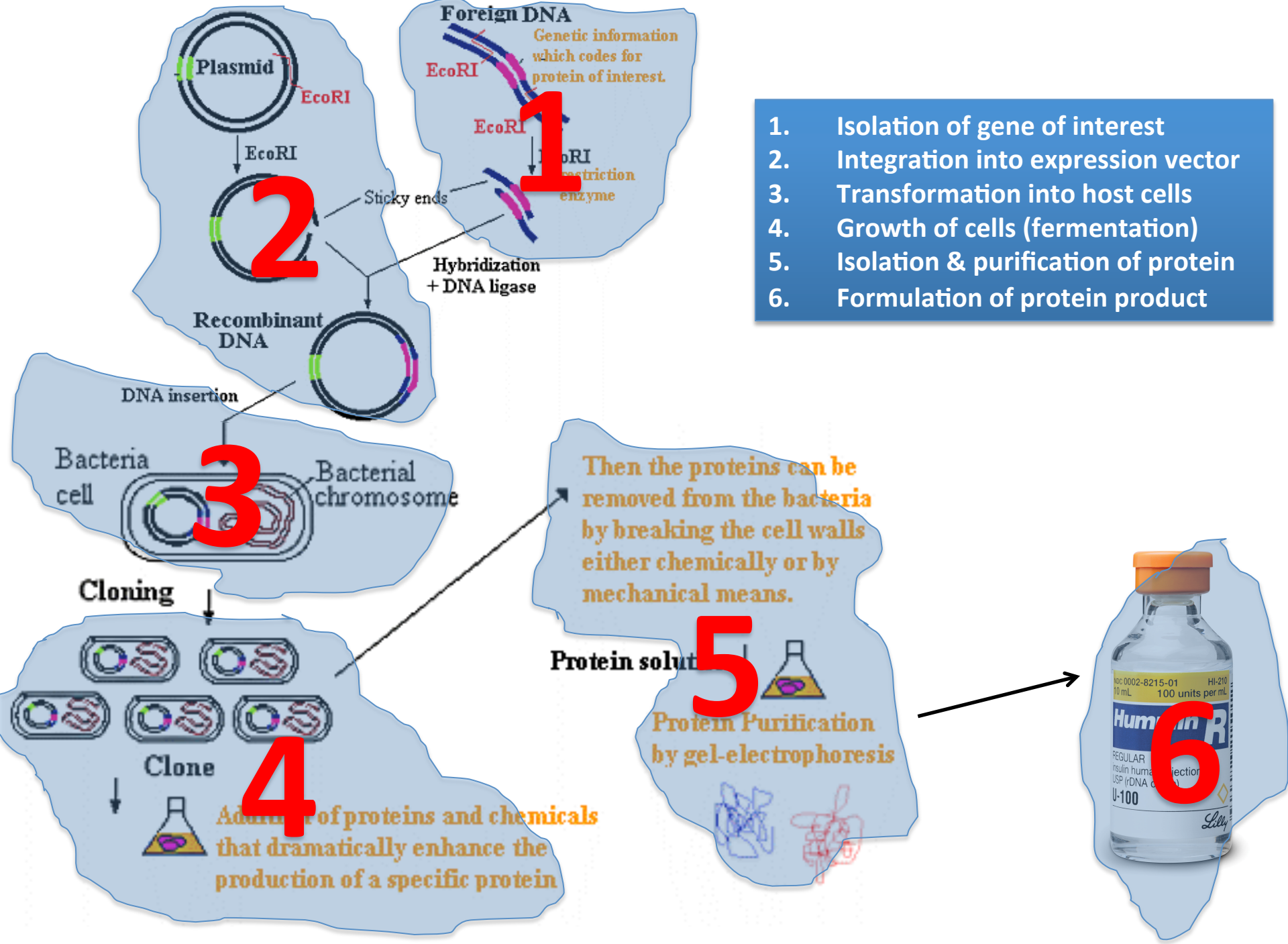
Protein solution

Protein Purification by gel-electrophoresis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Protein Purification by gel-electrophoresis
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1. Isolation of gene of interest
2. Integration into expression vector
3. Transformation into host cells
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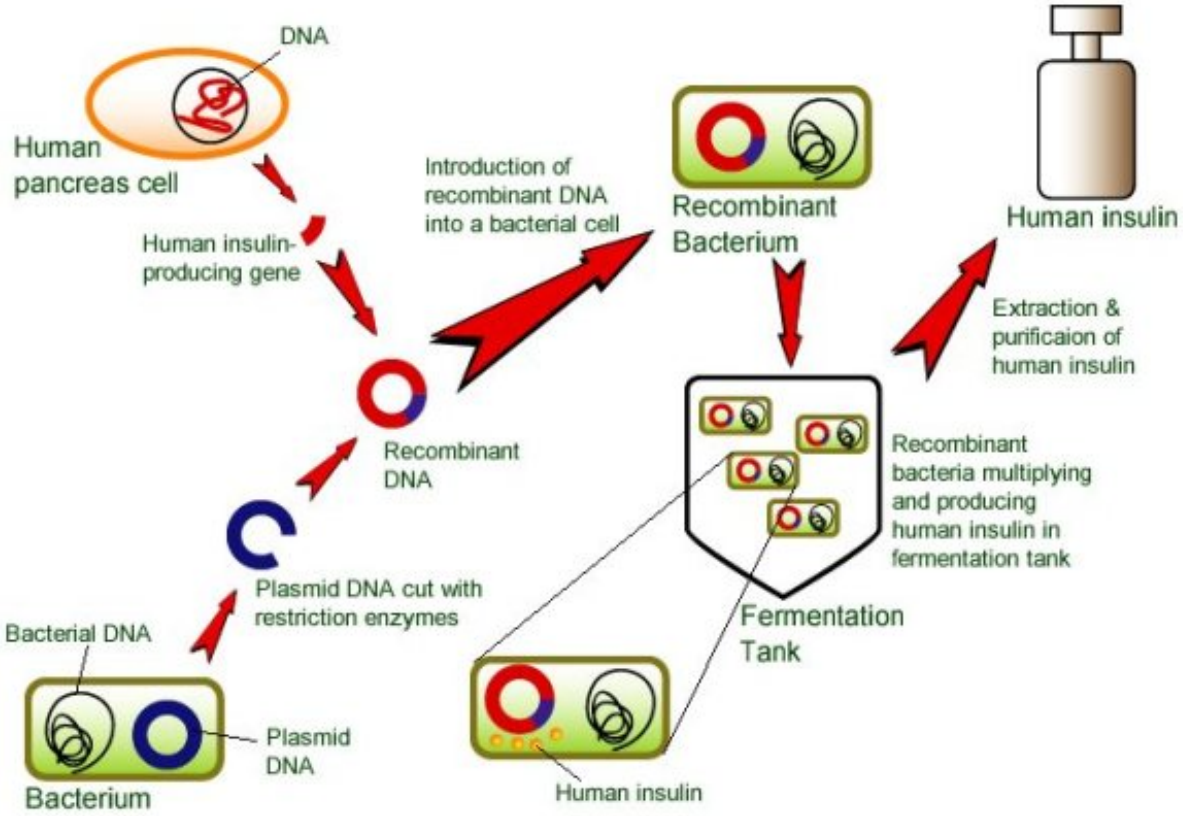
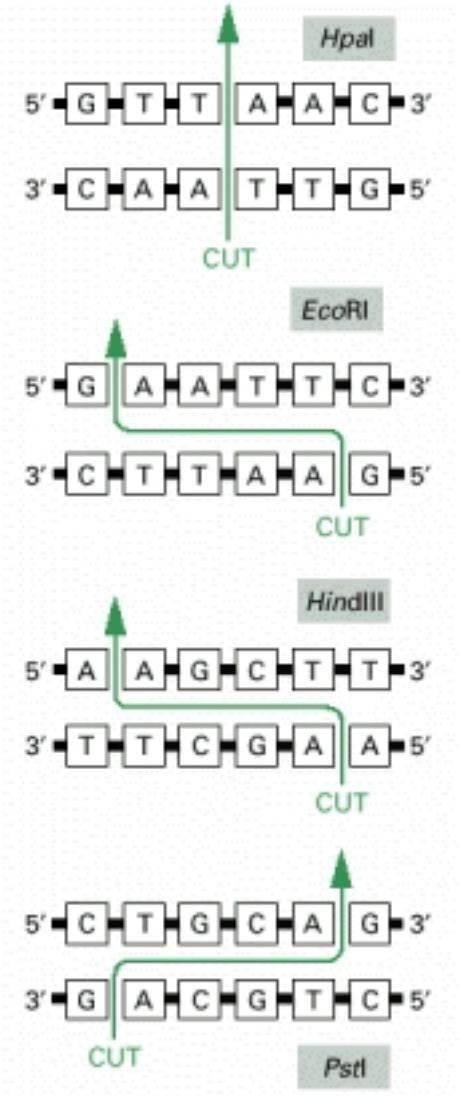
5. Isolation & purification of protein
6. Formulation of protein product

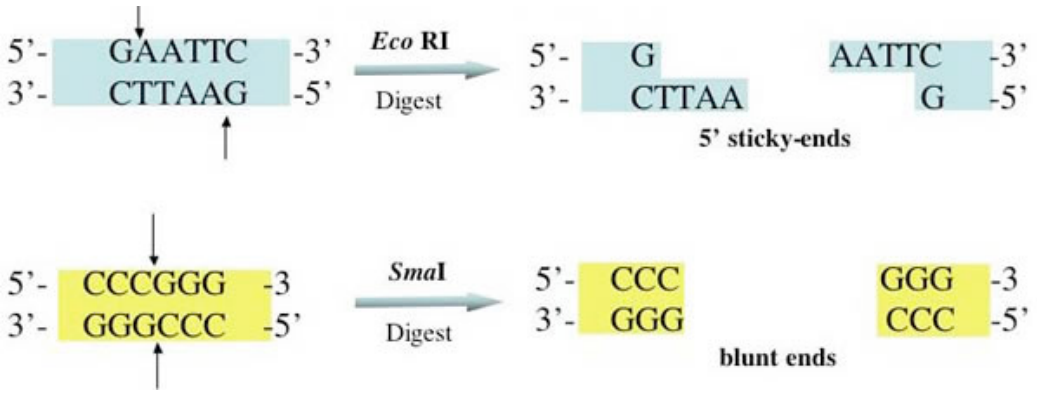
Human Insulin Production



Isolation of Gene of Interest

- **The gene of interest is a small segment in the large DNA molecule**
- **Restriction enzymes (endonucleases) cut the DNA double helix at specific sites**
- **Different restriction enzymes have different sequence specificities (palindrome)**





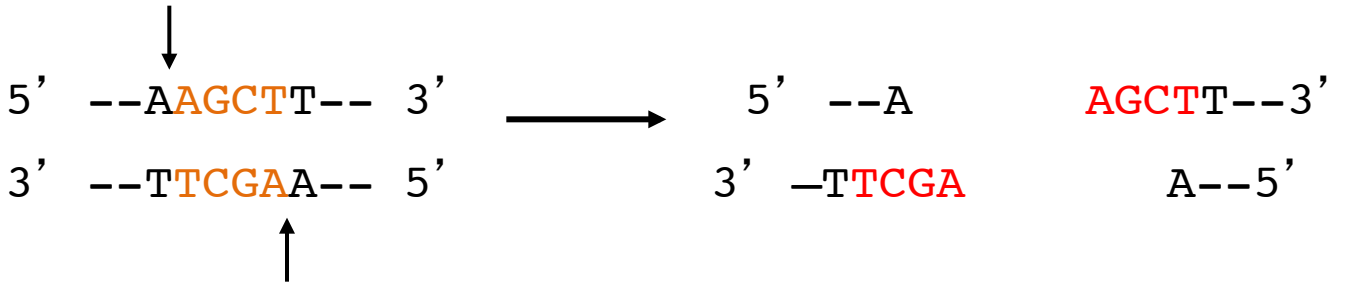
Examples of Palindromes

Noon

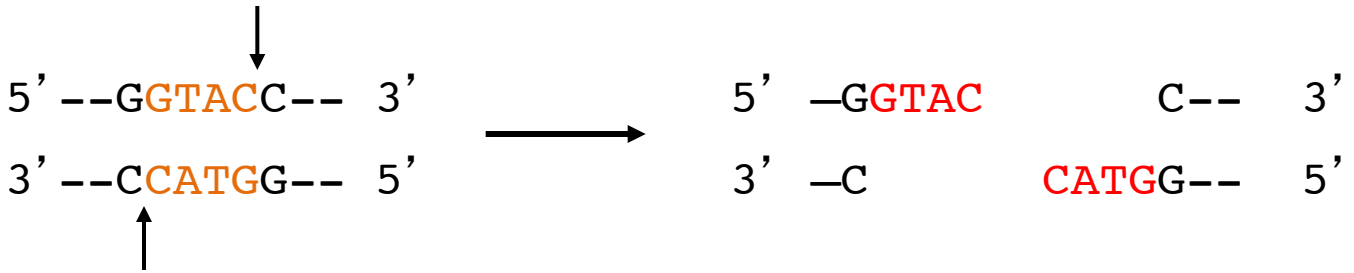
Murder for a jar of red rum

مودت هتدوم لك لهو *** لوه لكل مودت هتدوم

- **Enzymes with wobbling cuts give *sticky* ends**
- *Hin*dIII - leaves 5´ overhangs

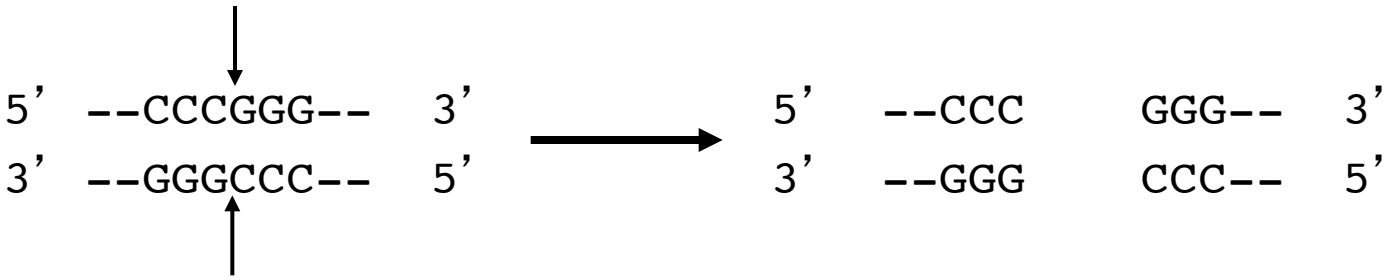


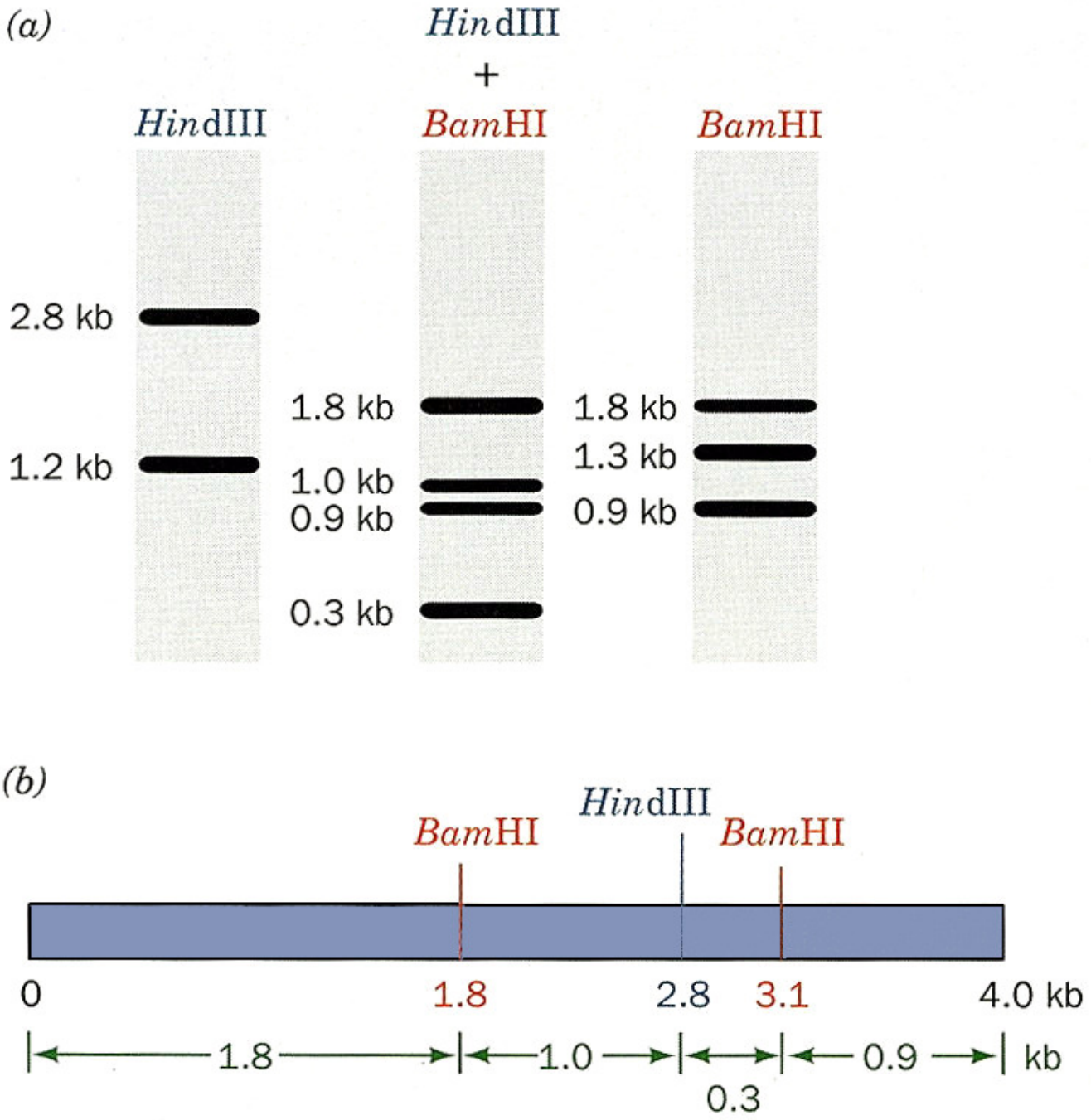
- *Kpn*I leaves 3´ overhangs



- **Enzymes that cut at same position on both strands give *blunt* ends**

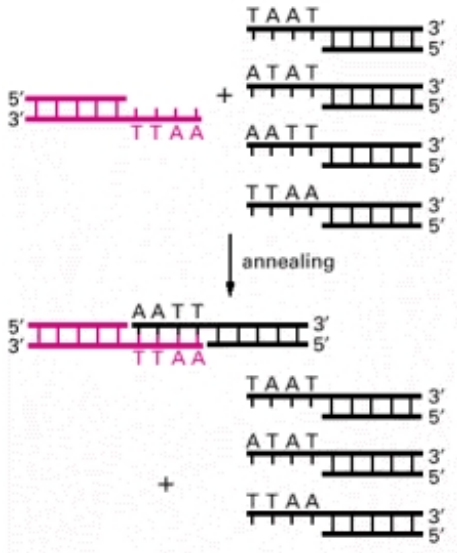
- *Sma*I

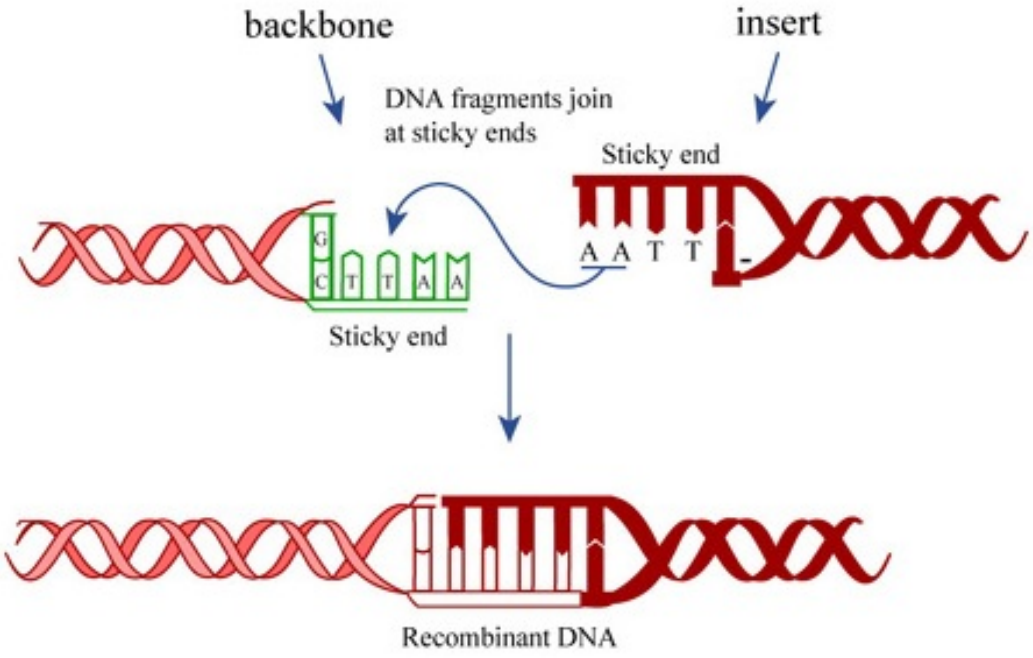



(a)
HindIII
+
HindIII
BamHI
BamHI
2.8 kb
1.2 kb
1.8 kb
1.0 kb
0.9 kb
0.3 kb
1.8 kb
1.3 kb
0.9 kb
(b)
HindIII
BamHI
BamHI
0
1.8
2.8
3.1
4.0 kb
1.8
1.0
0.9
kb
0.3

DNA Ligase

- DNA ligation is the act of joining together DNA strands with covalent bonds with the aim of making new viable DNA or plasmid
- T4 DNA ligase has the unique ability to join sticky and blunt ended fragments





DNA Ligase

- **Sticky ends that are complementary can be ligated together even if they are produced by different restriction enzymes.**
- **Sticky ends that are not complementary cannot be ligated together.**

- *Bam*HI

```
-G          GATCC-
-CCTAG          G-
```

- *Bgl*II

```
-A          GATCT-
-TCTAG          A-
```

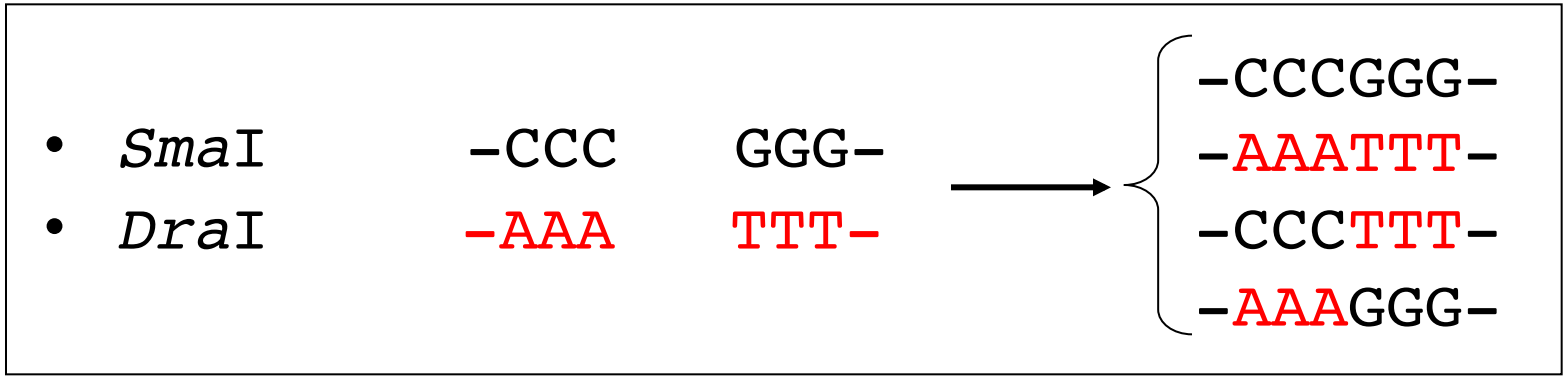
- Result

```
-GGATCT-
-CCTAGA-
```

No longer palindromic, so not cut by *Bam*HI or *Bgl*II

DNA Ligase can also join blunt ends

DNA fragments with blunt ends generated by different enzymes can be ligated together (with lower efficiency), but usually cannot be re-cut by either original restriction enzyme.



- Ligations that re-constitute a *Sma*I or *Dra*I site (CCCGGG or AAATTT) **can be re-cut by *Sma*I or *Dra*I.**

- Mixed ligation products (CCCTTT + AAAGGG) **cannot be re-cut by *Sma*I or *Dra*I.**

Expression vector

Plasmid

EcoRI

Foreign DNA

Genetic information which codes for protein of interest.

EcoRI

EcoRI

Palindrome

DNA of interest

EcoRI

EcoRI

restriction enzyme

Restriction Enzyme

Sticky ends

Hybridization + DNA ligase

Recombinant DNA

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Bacterial chromosome

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Protein solution

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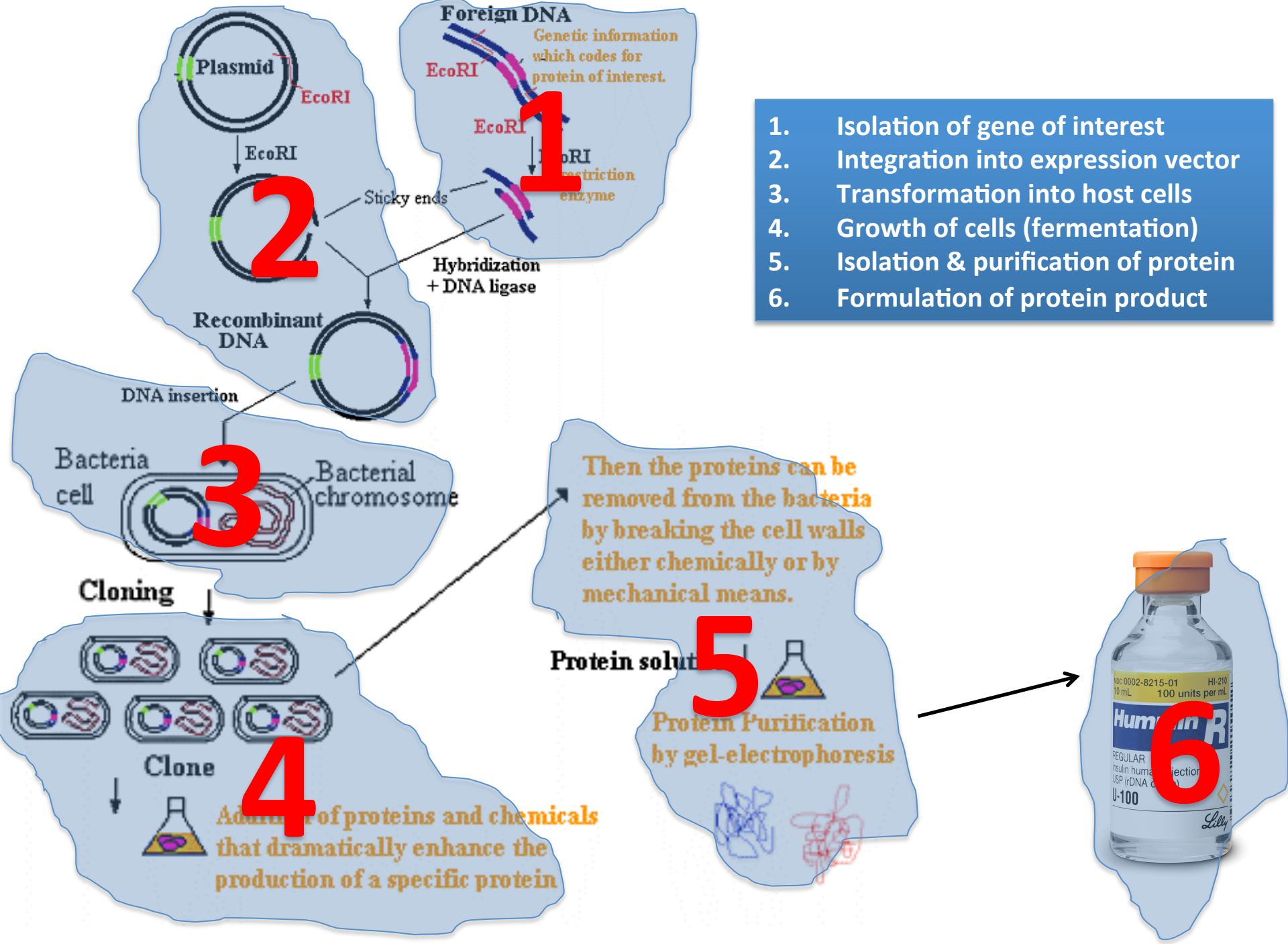
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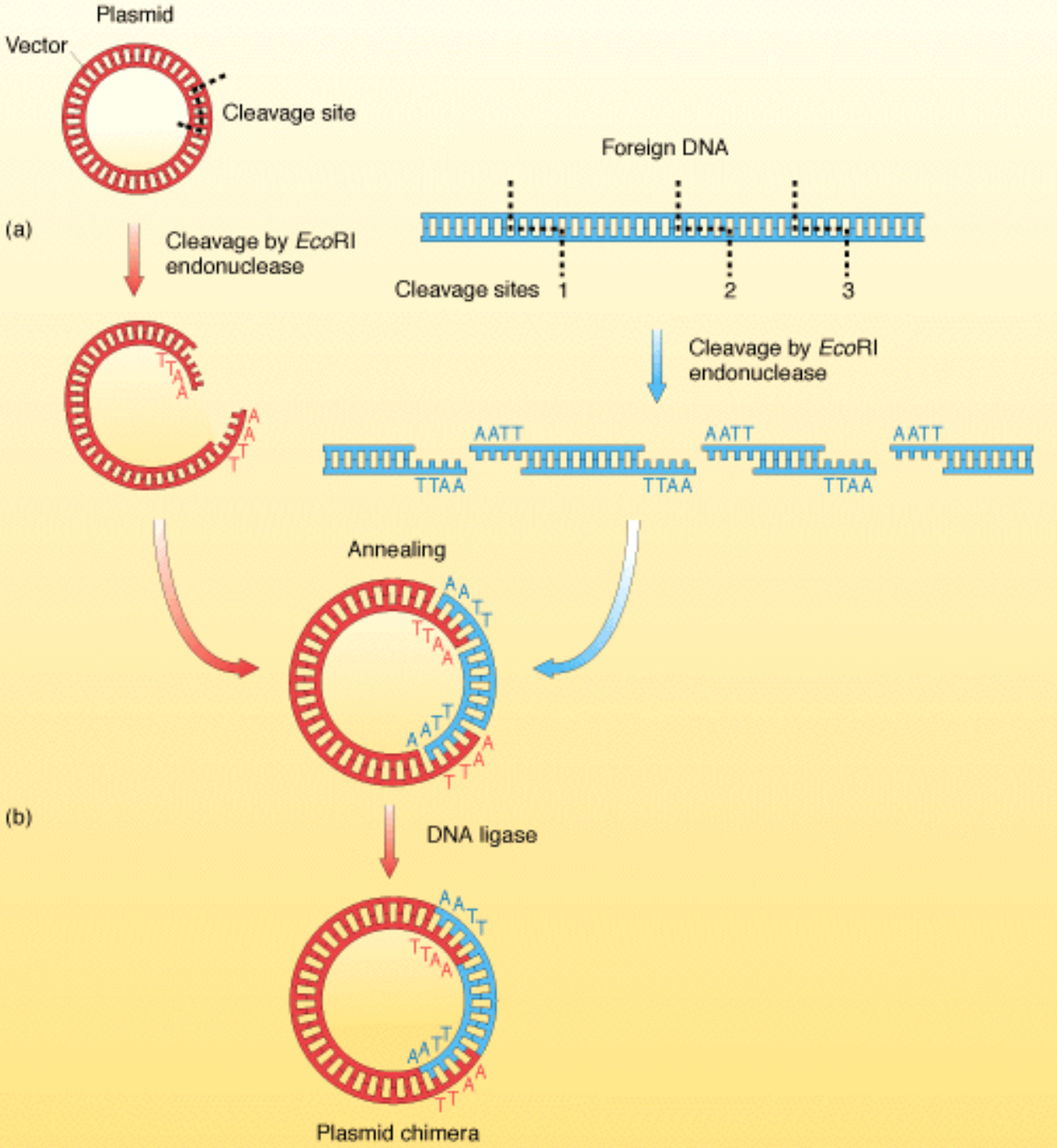
Plasmid
Vector
Cleavage site
(a)
Cleavage by EcoRI endonuclease
Foreign DNA
Cleavage sites 1 2 3
Cleavage by EcoRI endonuclease
AATT
TTAA
Annealing
(b)
DNA ligase
Plasmid chimera

You are now able to:

- ✓ Understand the concept of recombinant DNA technology
- ✓ Define terms related to cloning and DNA recombination
- ✓ Realize the value of recombinant DNA technology