# Chapter 8: Techniques in Molecular Biology

- PCR
- Restriction digestion
- Gel electrophoresis
- Gene cloning

# Basic Tools and Techniques

- **Gene cloning**

  Insertion of a fragment of DNA, containing a gene, into a cloning vector, and subsequent propagation of the recombinant DNA molecule in a host organism.

- **Recombinant DNA technology**

  The techniques involved in the construction, study and use of recombinant DNA molecules.

- **Nuclease**

  An enzyme that degrades a nucleic acid molecule.

  - Exonuclease: An enzyme that removes nucleotides from the ends of a nucleic acid molecule
  - Endonuclease: An enzyme that breaks phosphodiester bonds within a nucleic acid molecule.

# Basic Tools and Techniques

- **Restriction endonuclease**

  An enzyme that cuts DNA molecules at a limited number of specific nucleotide sequences.

- **DNA ligase**

  An enzyme that synthesizes phosphodiester bonds as part of DNA replication, repair and recombination processes.

- **Cloning vector**

  A DNA molecule that is able to replicate inside a host cell and therefore can be used to clone other fragments of DNA.

# Basic Tools and Techniques

- **Selectable marker**

  A gene carried by a vector and conferring a recognizable characteristic on a cell containing the vector or a recombinant DNA molecule derived from the vector.

- **Transformation**

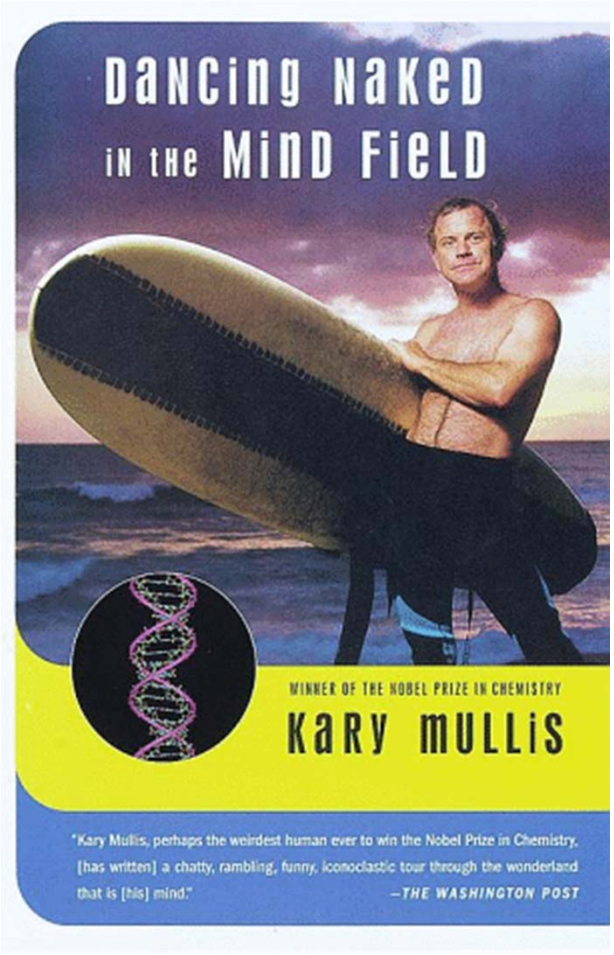
  The acquisition by a cell of new genes by the uptake of naked DNA

- **PCR**

  Polymerase chain reaction

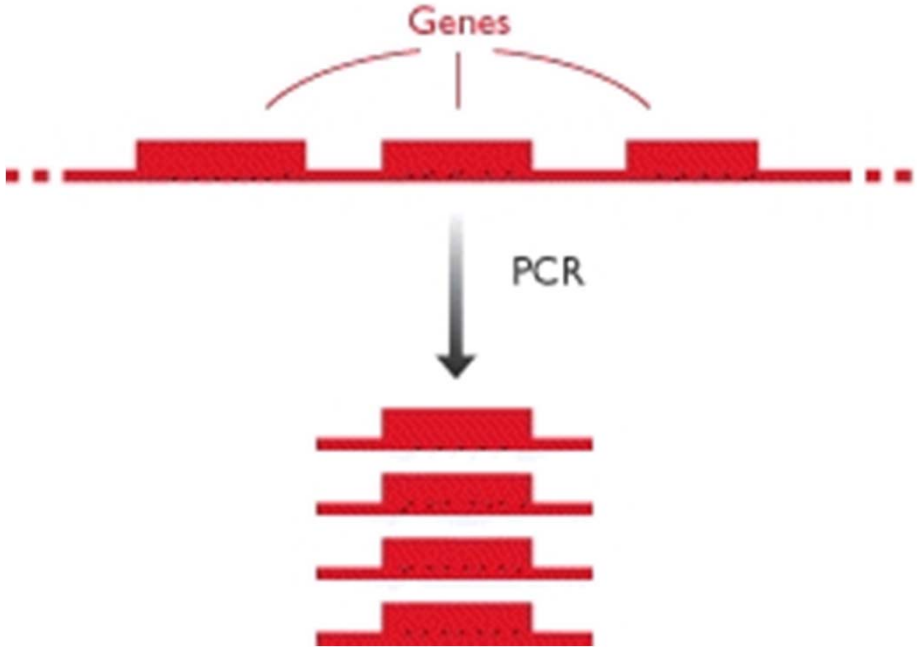
# The Synthesis of ‘short’ products in a PCR (Polymerase Chain Reaction)

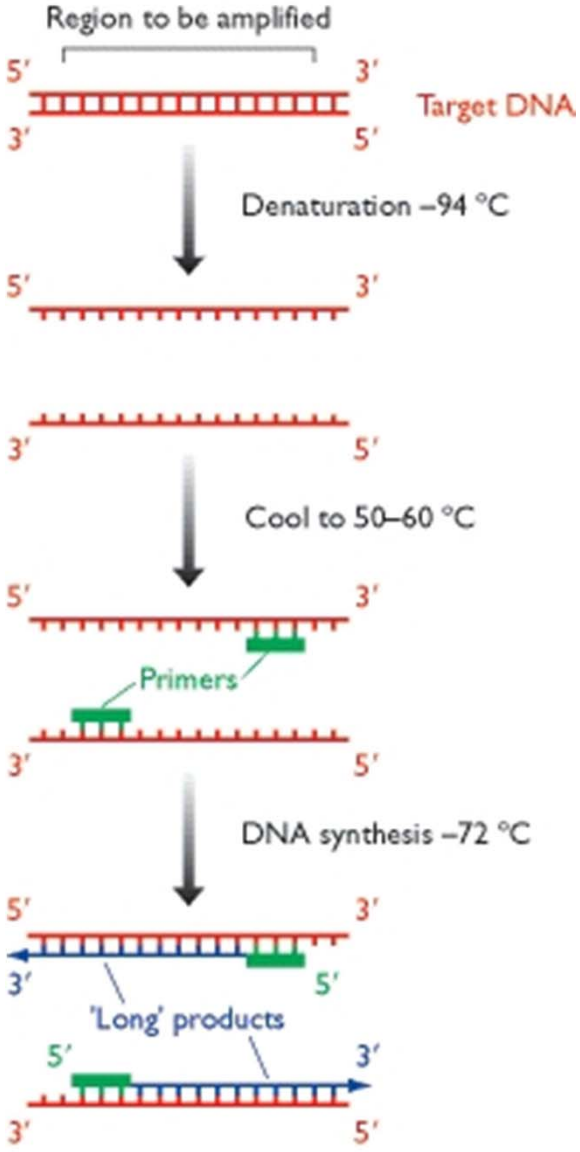
Kary Mullis



# The Synthesis of 'short' products in a PCR (Polymerase Chain Reaction)

**Make copies of a selected segment of a DNA molecule**

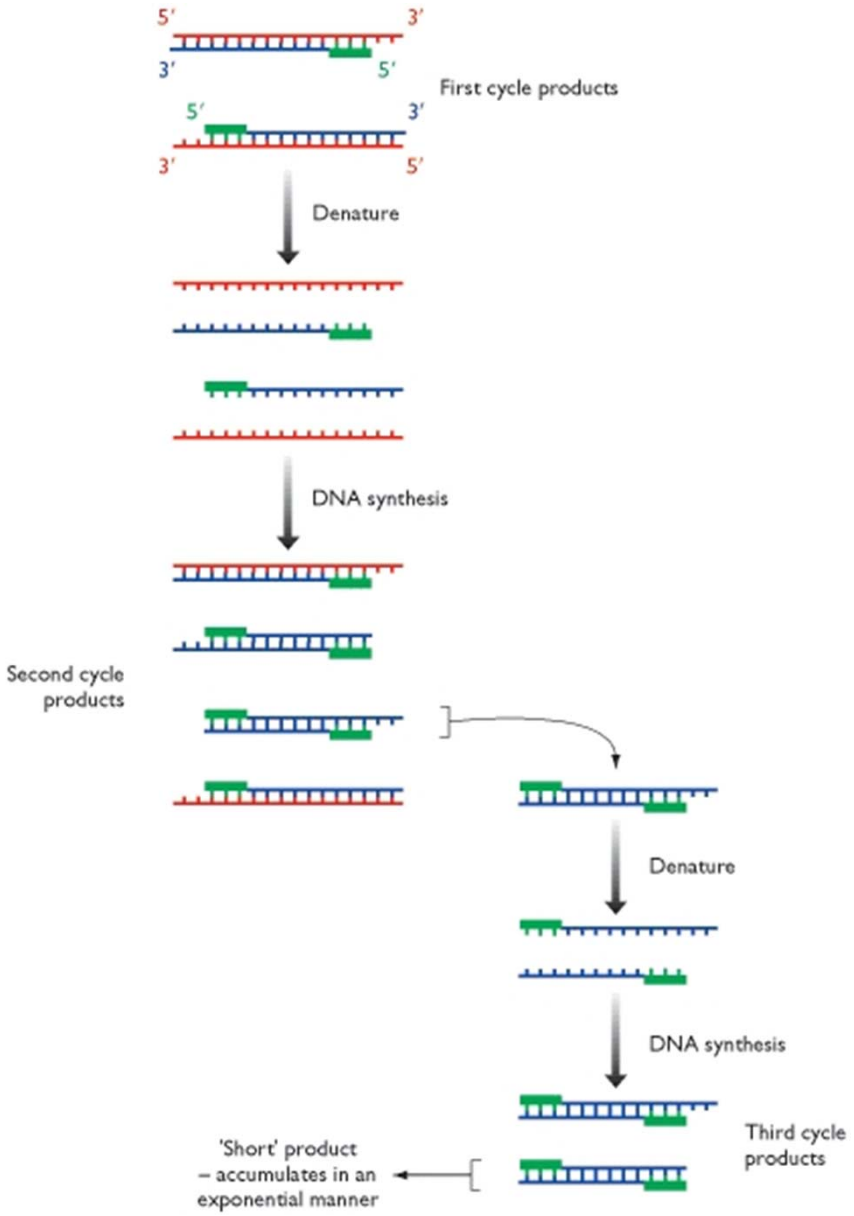



Kary Mullis

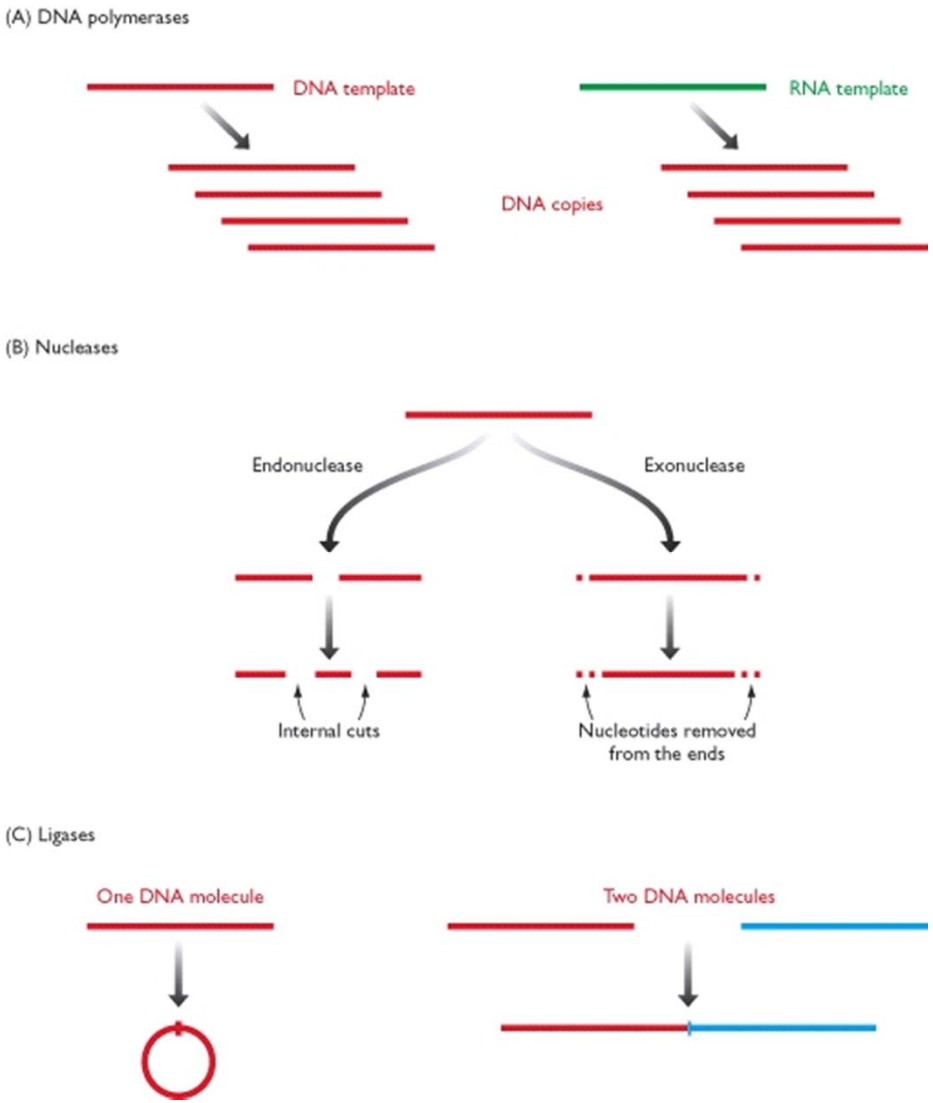
# The Synthesis of 'short' products in a PCR (Polymerase Chain Reaction)

The next cycle of denaturation-annealing-synthesis leads to four products, two of which are identical to the first cycle products and two of which are made entirely of new DNA. During the third cycle, the lattergive rise to 'short' products which, in subsequent cycles, accumulate in an exponential fashion.



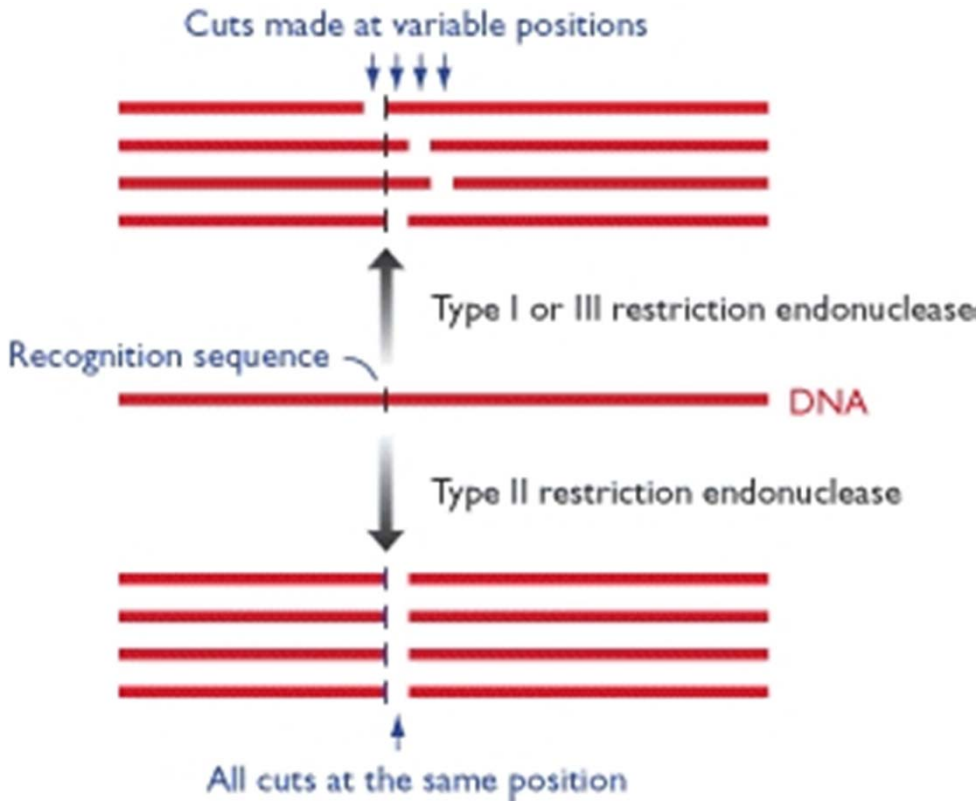
# The activities of DNA polymerases, Nucleases, and Ligases

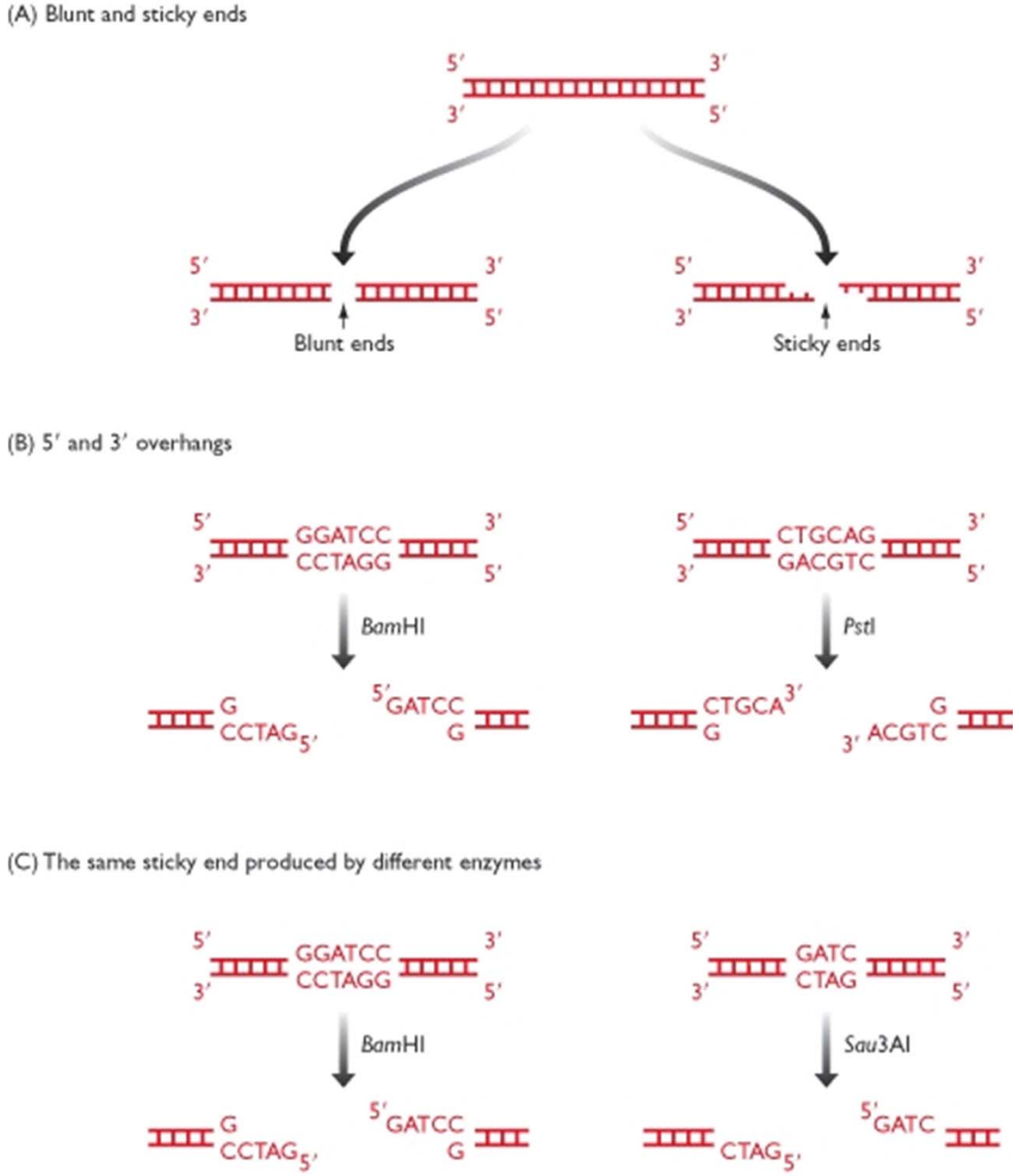
In (A), the activity of a DNA-dependent DNA polymerase is shown on the left and that of an RNA-dependent DNA polymerase on the right. In (B), the activities of endonucleases and exonucleases are shown. In (C) the red DNA molecule is ligated to itself on the left, and to a second molecule on the right.



# Cuts produced by restriction endonucleases

In the top part of the diagram, the DNA is cut by a Type I or Type III restriction endonuclease. The cuts are made in slightly different positions relative to the recognition sequence, so the resulting fragments have different lengths. In the lower part, a Type II enzyme is used. Each molecule is cut at exactly the same position to give exactly the same pair of fragments






**The results of digestion of DNA with different restriction endonucleases**

(A) Blunt ends and sticky ends.

(B) Different types of sticky end: the 5′ overhangs produced by *Bam*HI and the 3′ overhangs produced by *Pst*I.

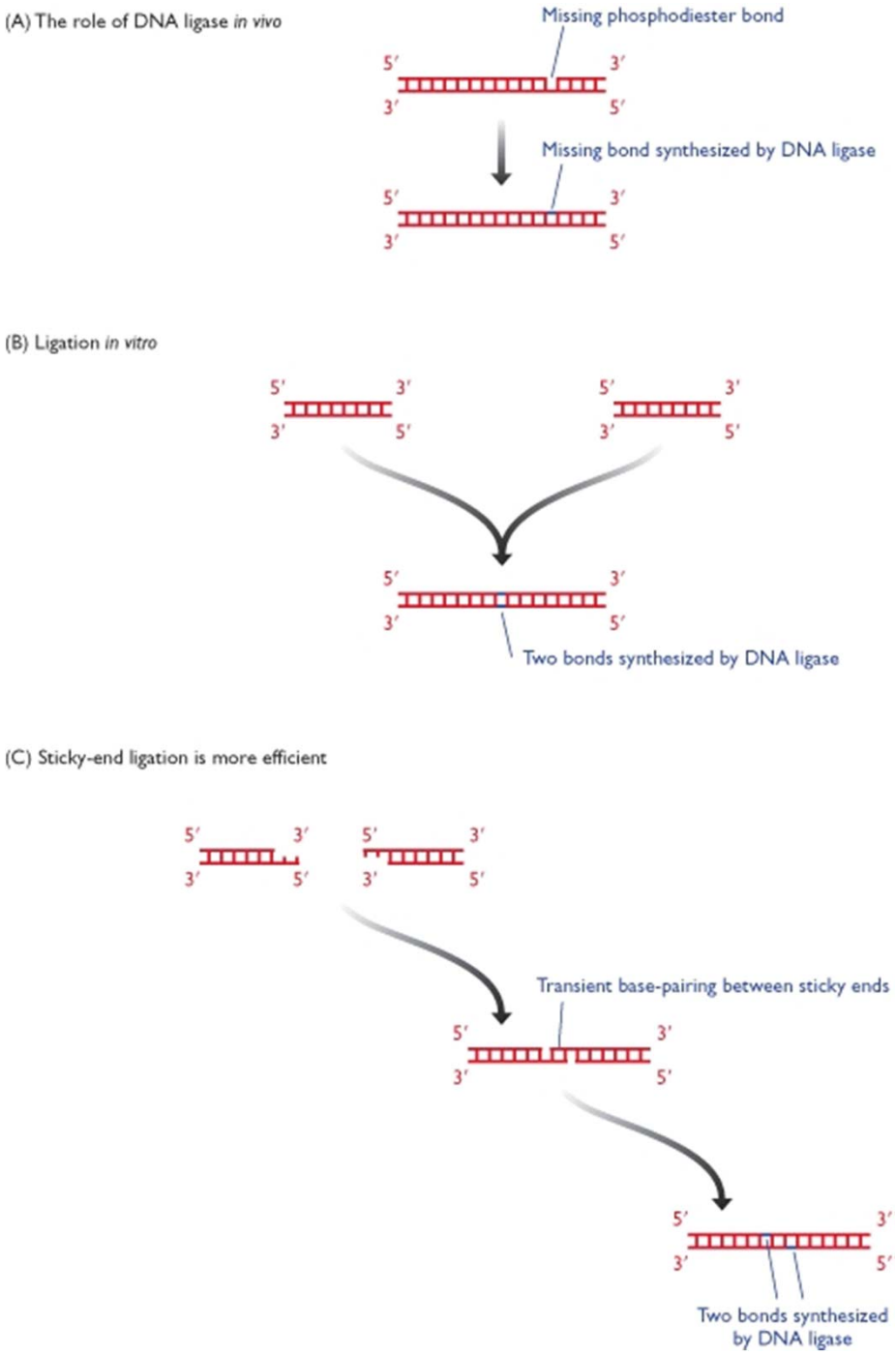
(C) The same sticky ends produced by two different restriction endonucleases: a 5′ overhang with the sequence 5′-GATC-3′ is produced by both *Bam*HI (recognizes 5′-GGATCC-3′) and *Sau*3AI (recognizes 5′-GATC-3′).

# Some examples of restriction endonucleases

N = any nucleotide.

Note that most, but not all, recognition sequences have inverted symmetry: when read in the 5′→3′ direction, the sequence is the same in both strands.

| Enzyme | Recognition sequence | Type of ends | End sequences |
|---|---|---|---|
| *Alu*I | 5′-AGCT-3′<br>3′-TCGA-5′ | Blunt | 5′-AG CT-3′<br>3′-TC GA-5′ |
| *Sau*3AI | 5′-GATC-3′<br>3′-CTAG-5′ | Sticky, 5′ overhang | 5′- GATC-3′<br>3′-CTAG -5′ |
| *Hin*fI | 5′-GANTC-3′<br>3′-CTNAG-5′ | Sticky, 5′ overhang | 5′-G ANTC-3′<br>3′-CTNA G-5′ |
| *Bam*HI | 5′-GGATCC-3′<br>3′-CCTAGG-5′ | Sticky, 5′ overhang | 5′-G GATCC-3′<br>3′-CCTAG G-5′ |
| *Bsr*BI | 5′-CCGCTC-3′<br>3′-GGCGAG-5′ | Blunt | 5′- NNNCCGCTC-3′<br>3′- NNNGGCGAG-5′ |
| *Eco*RI | 5′-GAATTC-3′<br>3′-CTTAAG-5′ | Sticky, 5′ overhang | 5′-G AATTC-3′<br>3′-CTTAA G-5′ |
| *Pst*I | 5′-CTGCAG-3′<br>3′-GACGTC-5′ | Sticky, 3′ overhang | 5′-CTGCA G-3′<br>3′-G ACGTC-5′ |
| *Not*I | 5′-GCGGCCGC-3′<br>3′-CGCCGGCG-5′ | Sticky, 5′ overhang | 5′-GC GGCCGC-3′<br>3′-CGCCGG CG-5′ |
| *Bgl*I | 5′-GCCNNNNNGGC-3′<br>3′-CGGNNNNNCCG-5′ | Sticky, 3′ overhang | 5′-GCCNNNN NGGC-3′<br>3′-CGGN NNNNCCG-5′ |




## Ligation of DNA molecules with DNA ligase

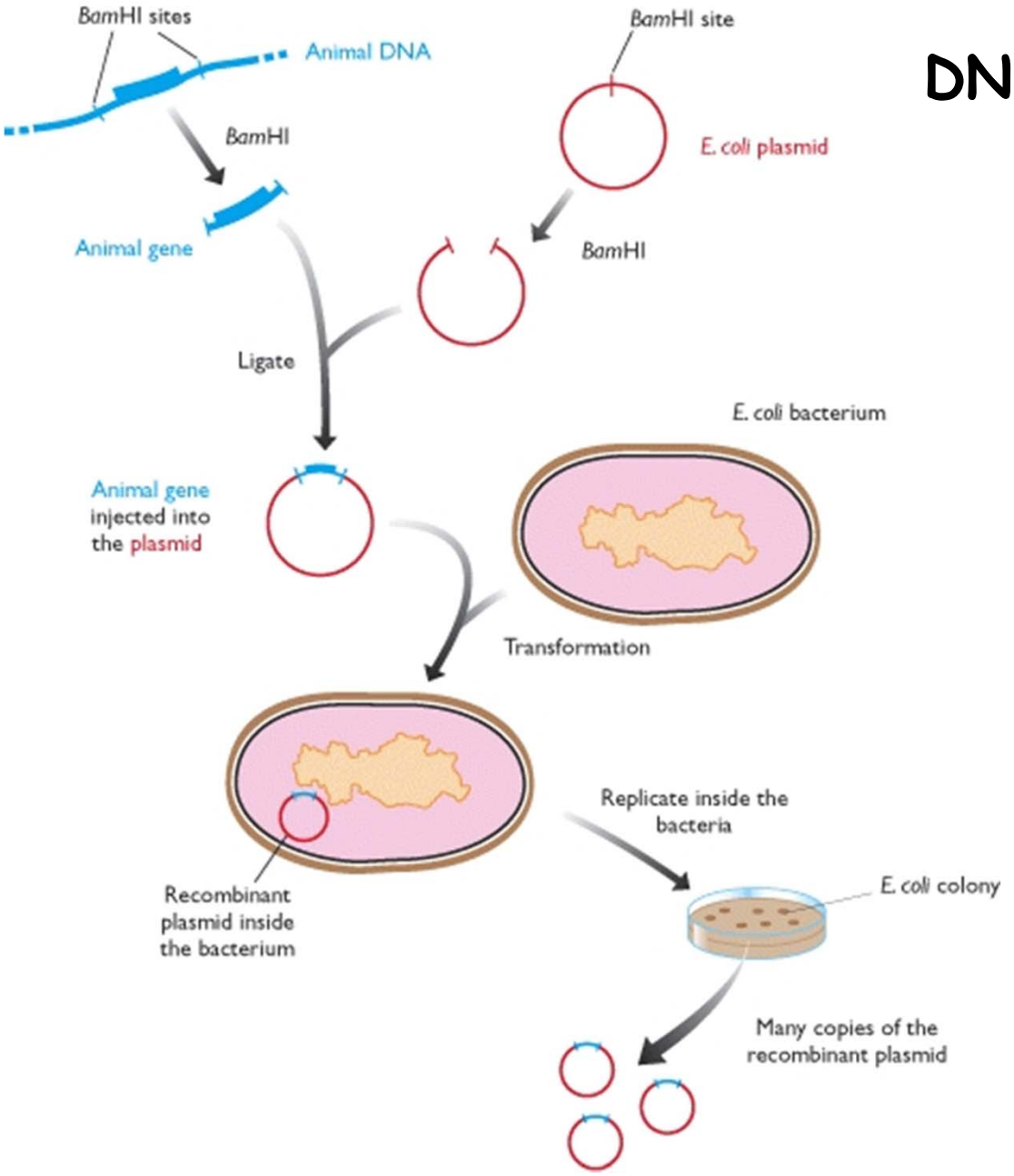
(A) In living cells, DNA ligase synthesizes a missing phosphodiester bond in one strand of a double-stranded DNA molecule.

(B) To link two DNA molecules *in vitro*, DNA ligase must make two phosphodiester bonds, one in each strand.

(C) Ligation *in vitro* is more efficient when the molecules have compatible sticky ends, because transient base-pairing between these ends holds the molecules together and so increases the opportunity for DNA ligase to attach and synthesize the new phosphodiester bonds.

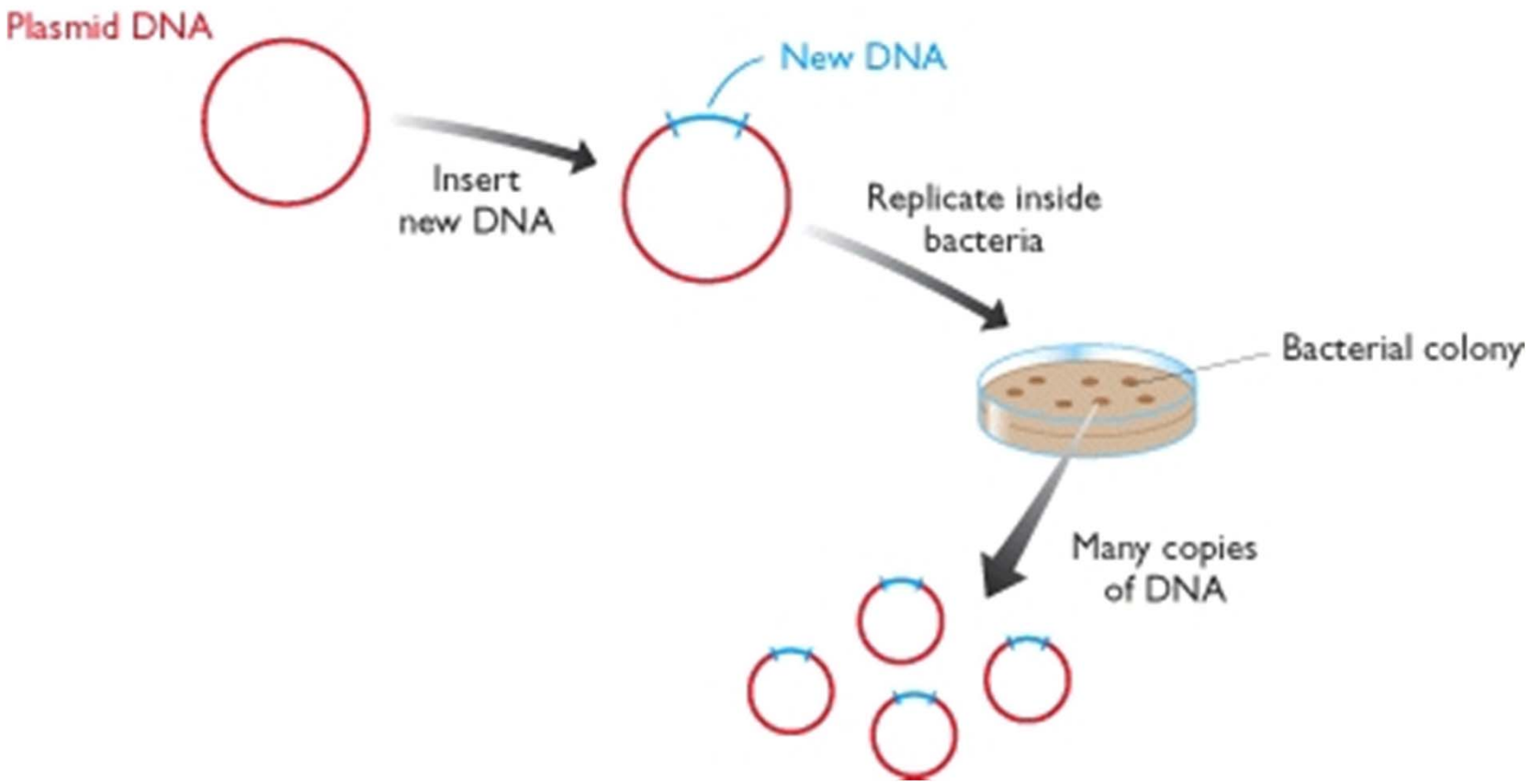
# DNA (gene) Cloning

Outline



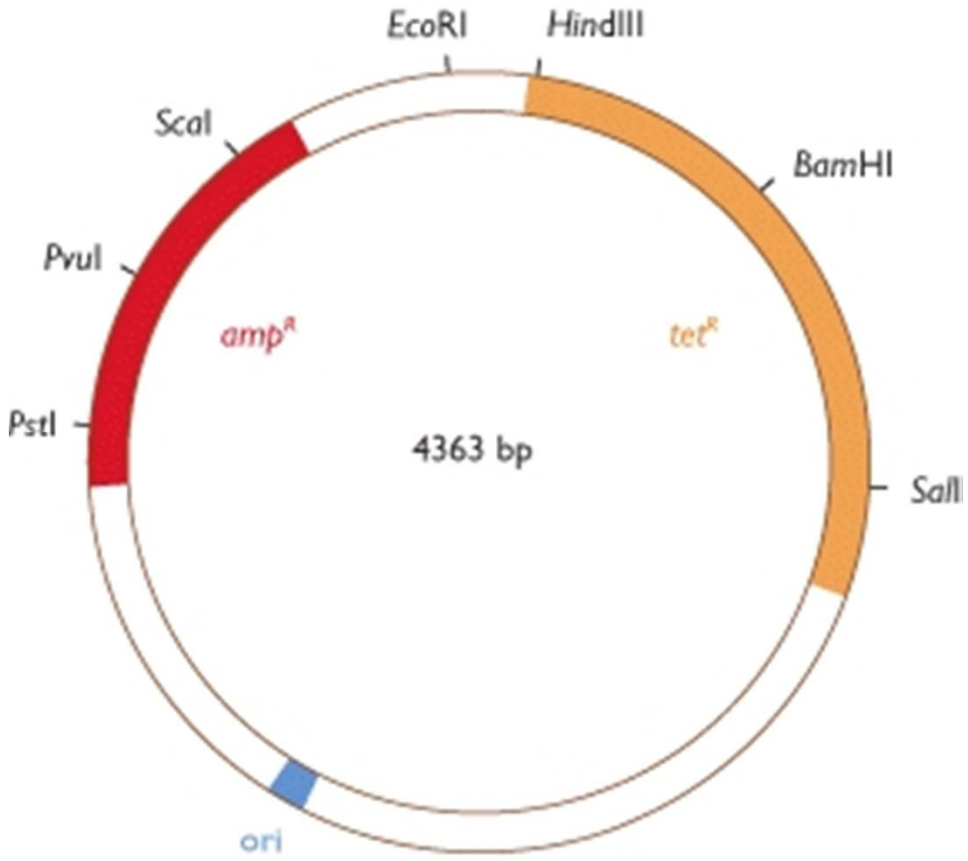
# DNA(gene) Cloning

In this example, the fragment of DNA to be cloned is inserted into a plasmid vector which is subsequently replicated inside a bacterial host.

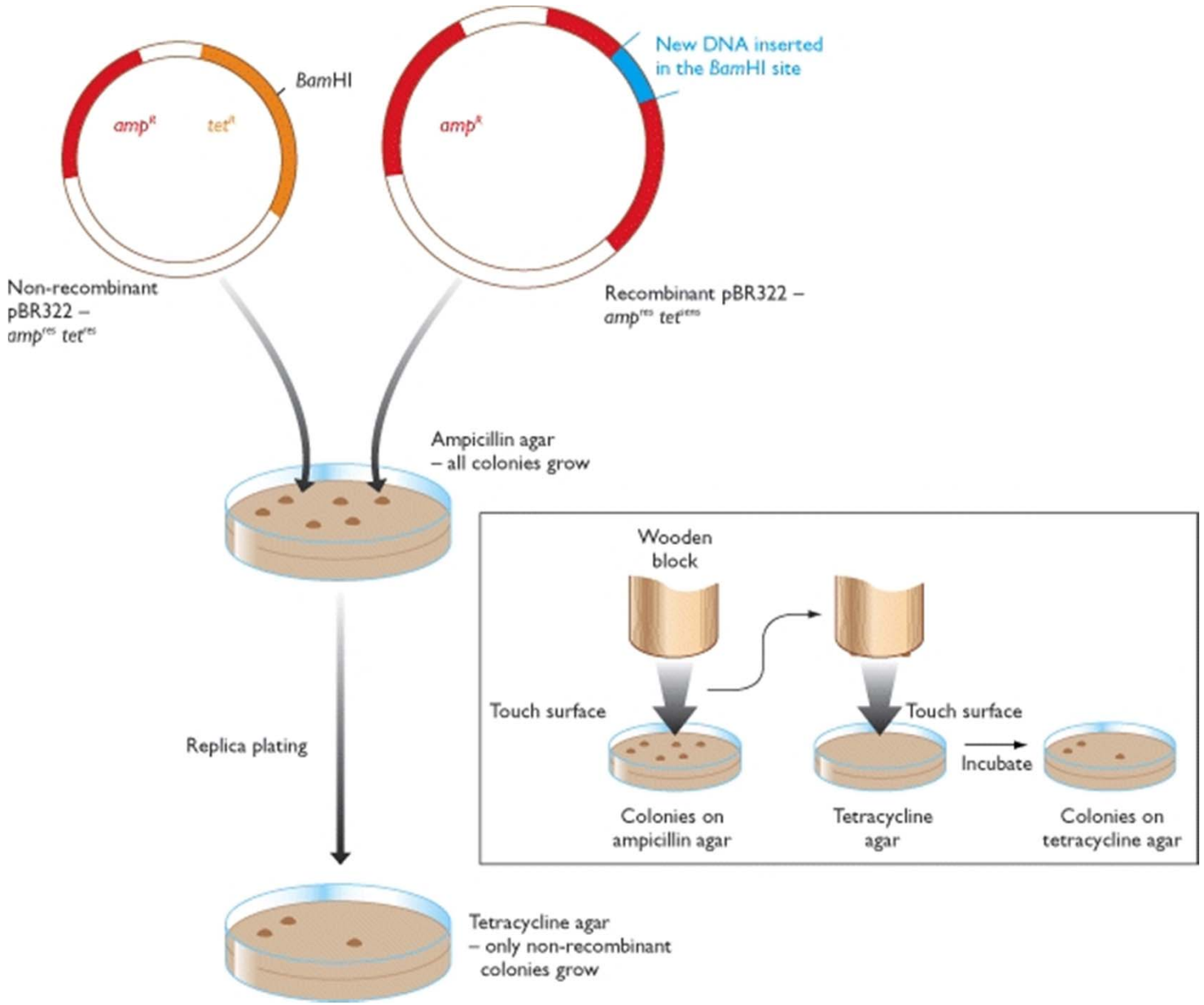


## Plasmid vector pBR322

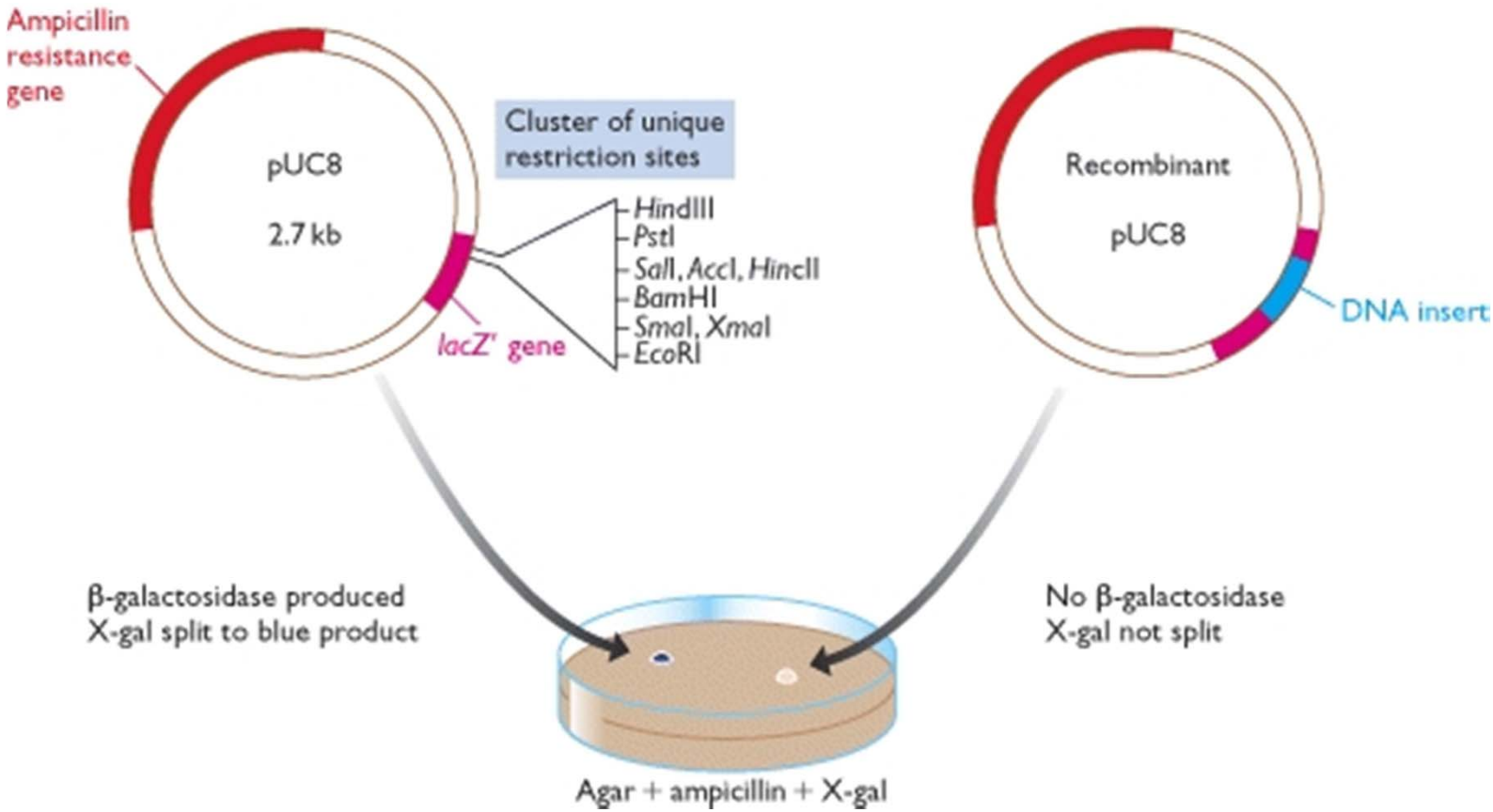
The map shows the positions of the ampicillin-resistance gene (*amp* $^{R}$), the tetracycline-resistance gene (*tet* $^{R}$), the origin of replication (ori) and the recognition sequences for seven restriction endonucleases.



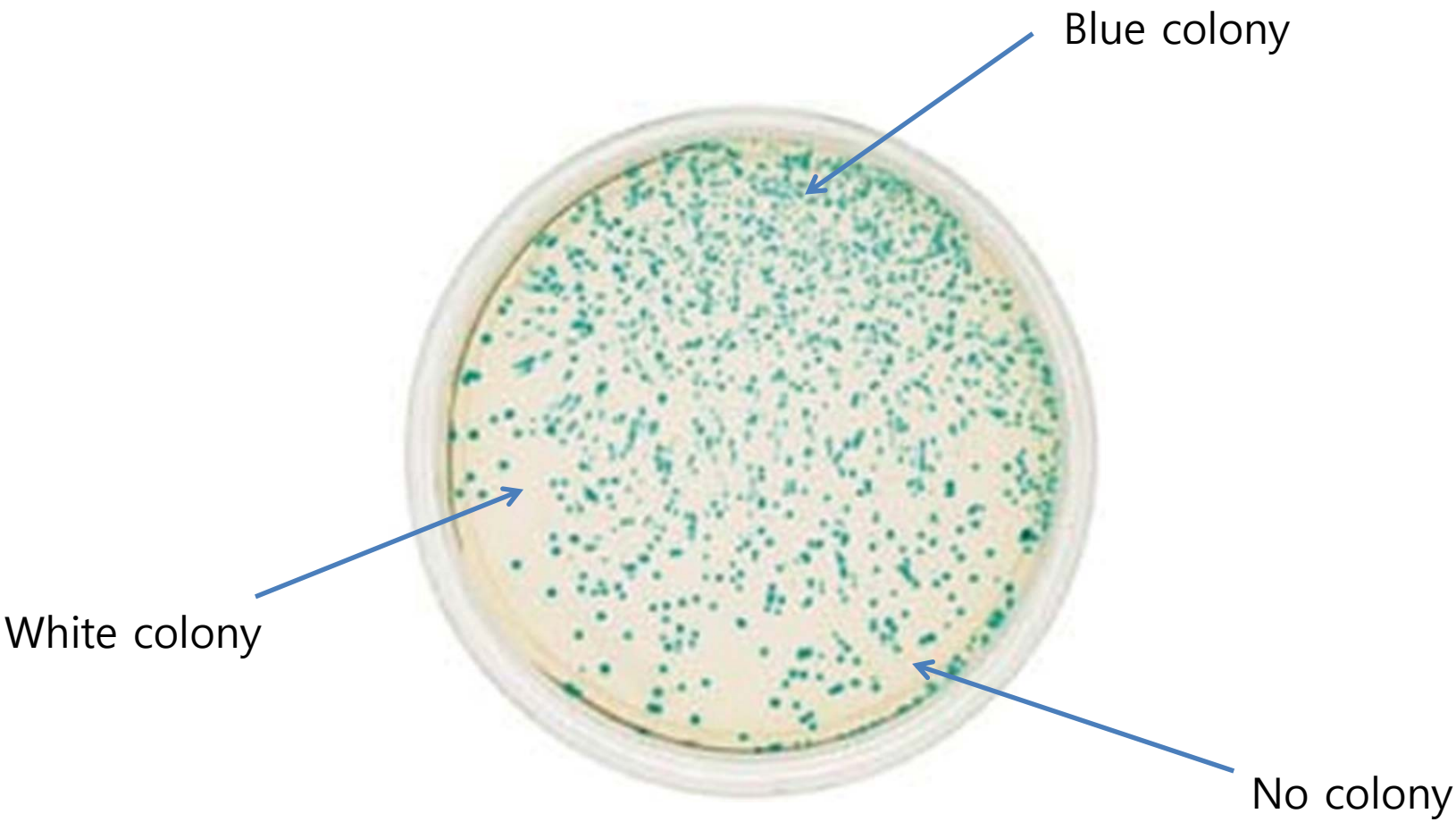
# Recombinant selection with pBR322



# Recombinant selection with pUC8



# Blue/white colony selection



## Analyzing the Results of a PCR by Agarose Gel Electrophoresis

The PCR has been carried out in a microfuge tube. A sample is loaded into lane 2 of an agarose gel. Lane 1 contains DNA size markers, and lane 3 contains a sample of a PCR done by a colleague. After electrophoresis, the gel is stained with ethidium bromide Lane 2 contains a single band of the expected size, showing that the PCR has been successful. In lane 3 there is no band - this PCR has not worked.