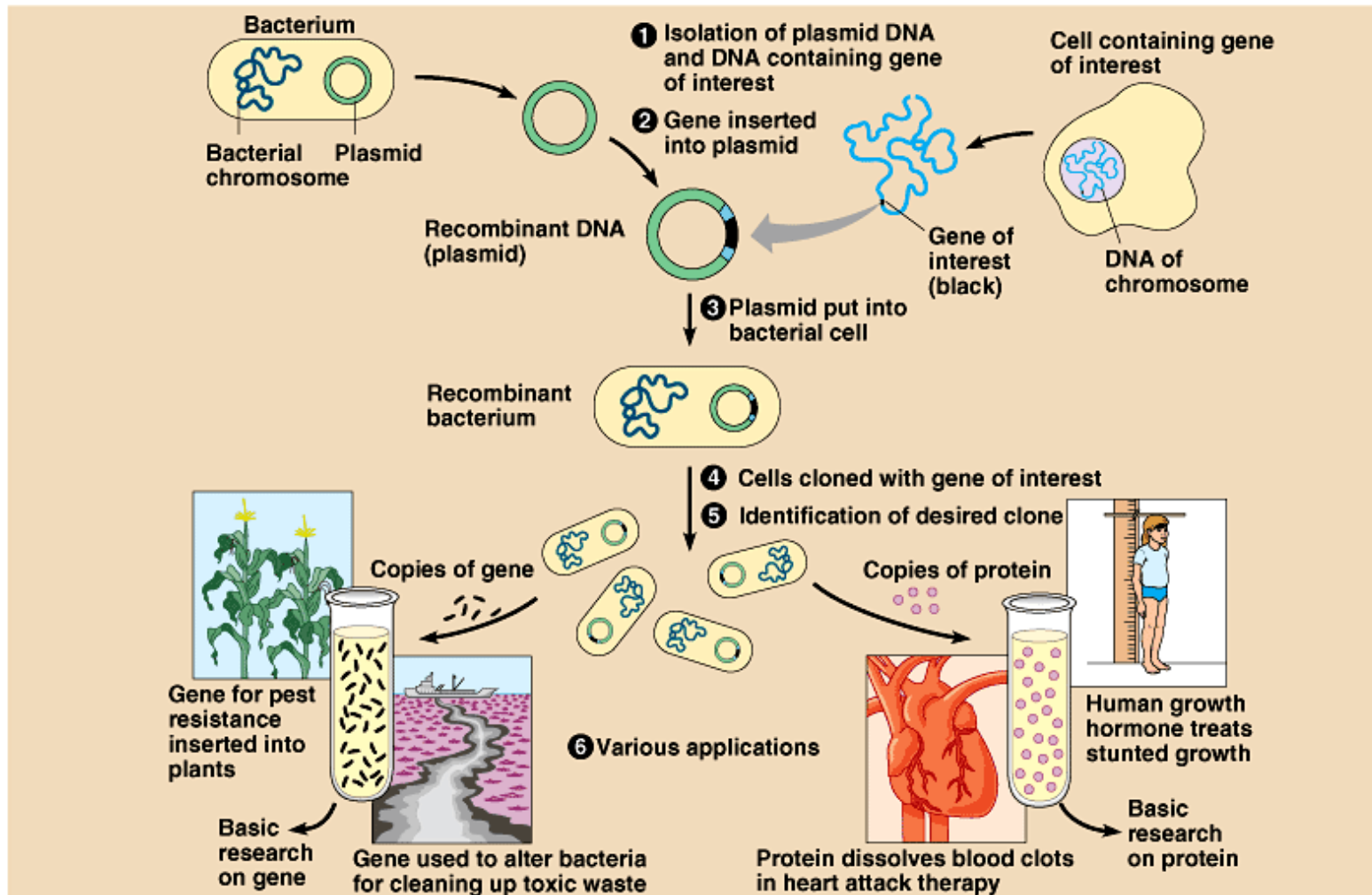




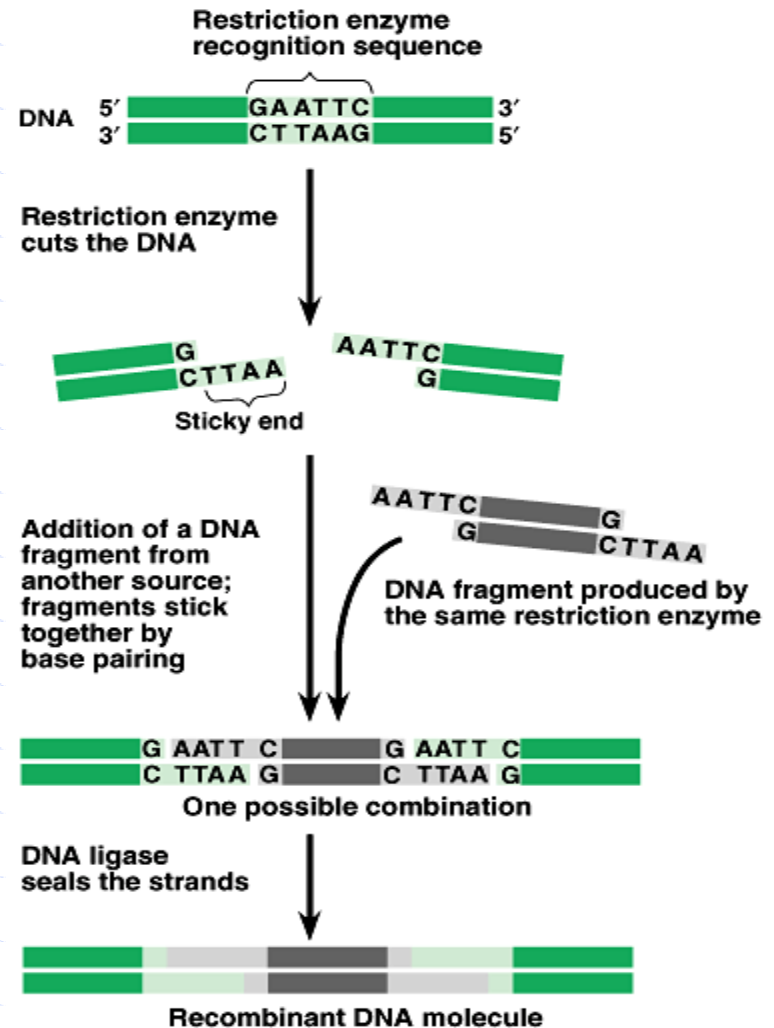
Aim: What is the process of DNA recombination?

DNA Cloning: an overview

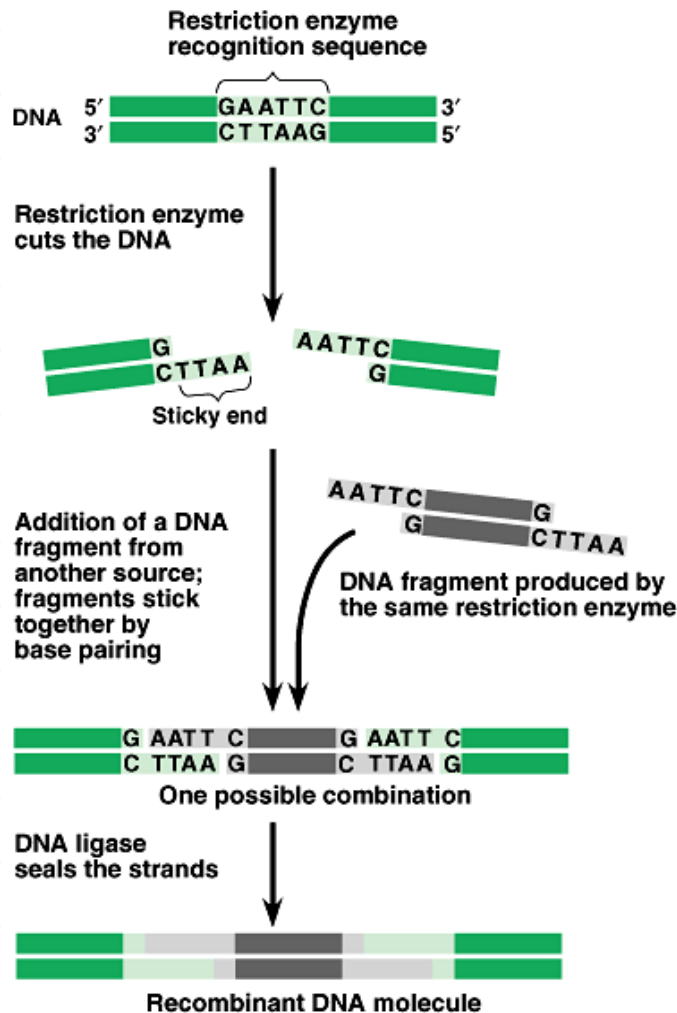


Restriction enzymes

- ◆ In nature, bacteria use restriction enzymes to cut foreign DNA, such as from phages or other bacteria.
- ◆ Most restriction enzymes are very specific, recognizing short DNA nucleotide sequences and cutting at specific point in these sequences.
 - Bacteria protect their own DNA by methylation.

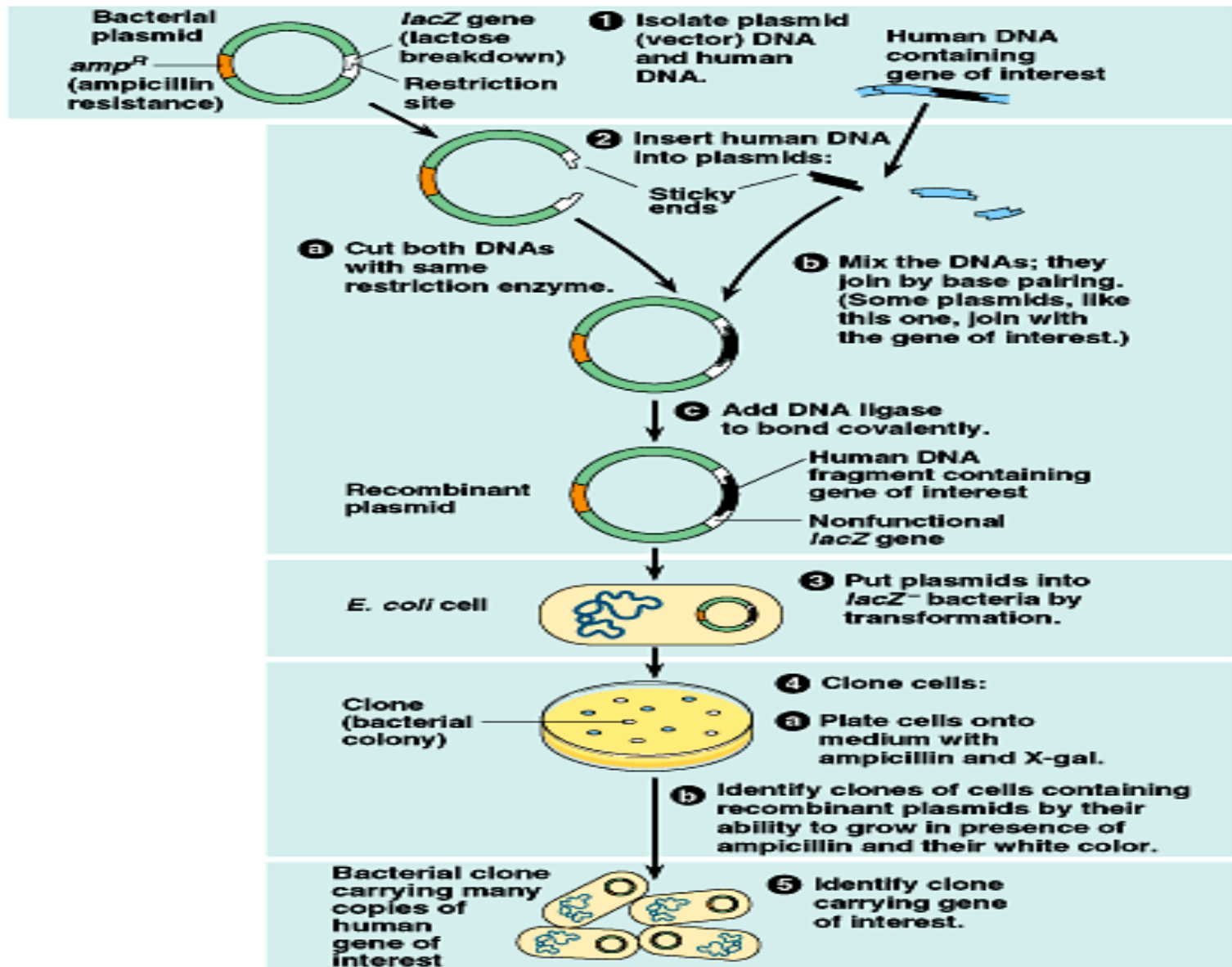


Restriction enzymes



- ◆ Restriction enzymes cut covalent phosphodiester bonds of both strands, often in a staggered way creating single-stranded ends, **sticky ends**
- ◆ These DNA fusions can be made permanent by **DNA ligase** which seals the strand by catalyzing the formation of phosphodiester bonds.

DNA Cloning: an overview



DNA Cloning Steps

1. Isolation of vector and gene-source DNA.

- ◆ The source DNA comes from human tissue cells.
- ◆ The source of the plasmid is typically *E. coli*.
 - This plasmid carries two useful genes, *amp^R*, conferring resistance to the antibiotic ampicillin and *lacZ*, encoding the enzyme beta-galactosidase which catalyzes the hydrolysis of sugar.

DNA Cloning Steps

2. Insertion of DNA into the vector.

- ◆ A specific restriction endonuclease cuts the source human DNA and the bacterial plasmid.
- ◆ The plasmid is cut somewhere inside the lacZ gene.
- ◆ DNA ligase is used to seal the two DNA fragments. It is now called ***recombinant DNA***.

DNA Cloning Steps

3. Introduction of the cloning vector into cells.

- ◆ The recombinant plasmids are added to the bacterial cells by transformation.
- ◆ Calcium salts help the recombinant plasmids enter the cell.

DNA Cloning Steps

4. Cloning of cells (and foreign genes).

- ◆ We can plate out the transformed bacteria on solid nutrient medium containing ampicillin and a sugar called X-gal.
 - Only bacteria that have the ampicillin-resistance plasmid will grow.
 - The X-gal in the medium is used to identify plasmids that carry foreign DNA.
 - ◆ Bacteria with plasmids lacking foreign DNA stain blue when beta-galactosidase hydrolyzes X-gal.
 - ◆ Bacteria with plasmids containing foreign DNA are white because they lack beta-galactosidase.

DNA Cloning Steps

5. Identifying cell clones with the right gene.

- ◆ In the final step, we will sort through the thousands of bacterial colonies with foreign DNA to find those containing our gene of interest.
- ◆ One technique, **nucleic acid hybridization**, depends on base pairing between our gene and a complementary sequence, a **nucleic acid probe**, on another nucleic acid molecule.
 - A radioactive or fluorescent tag labels the probe.

Bacterial colonies containing cloned segments of foreign DNA

