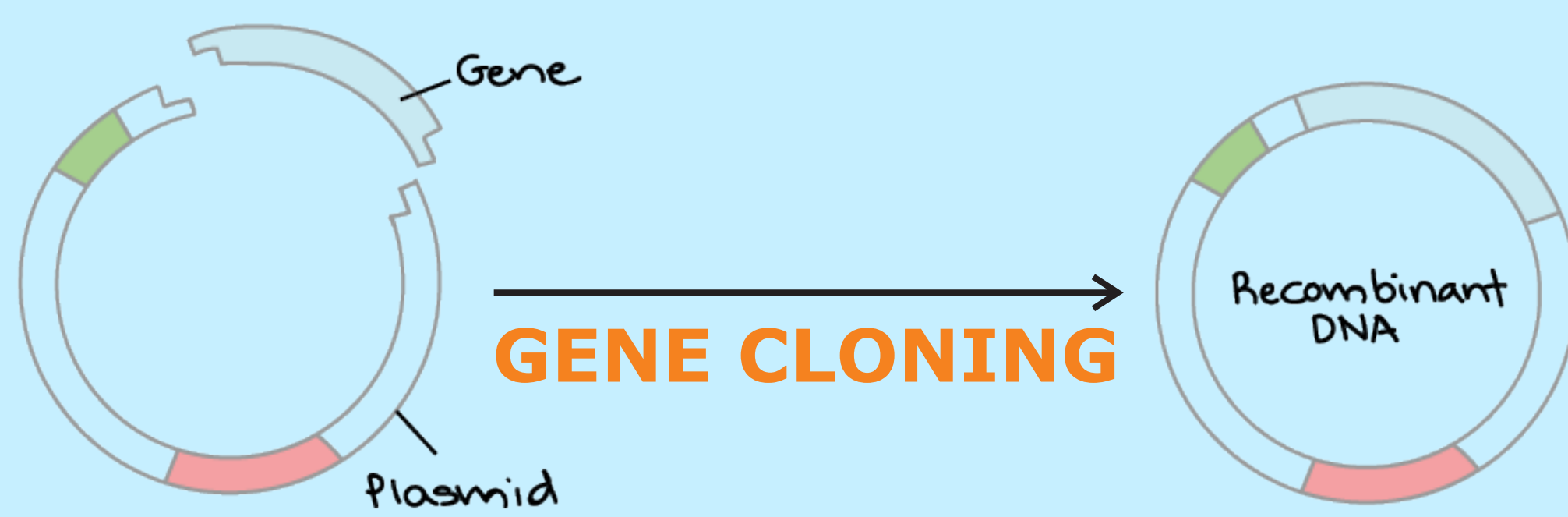


MIND MAP

GENE CLONING





GENE CLONING

Isolation and amplification of an individual gene sequence by insertion of that sequence into a bacterium where it can be replicated. Involves the construction of a novel DNA molecule, i.e., the product of gene cloning, Recombinant DNA (rDNA).

Components of Gene Cloning

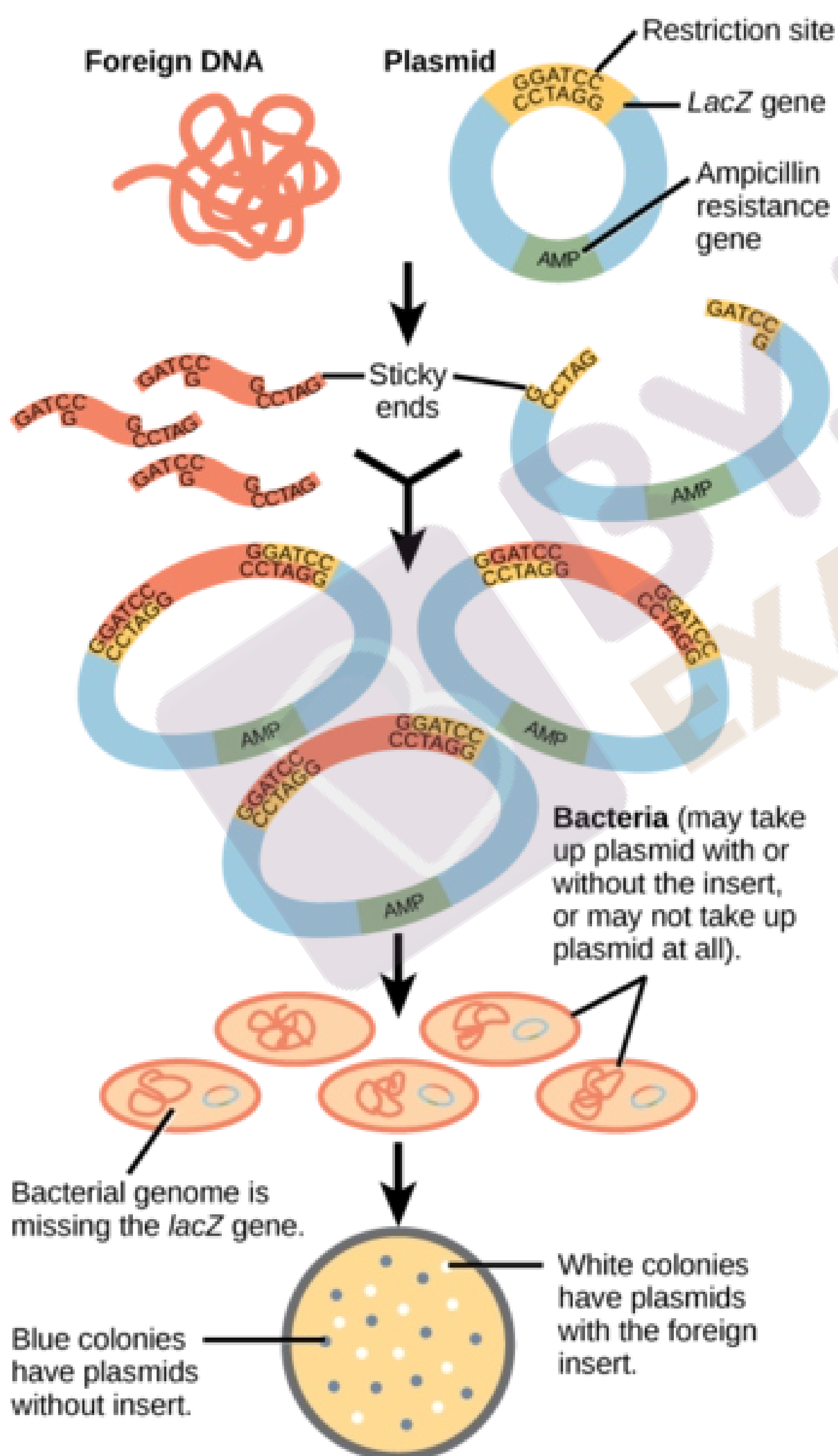
Enzymes for cutting and joining the DNA fragments

the DNA fragments (libraries)

Vectors (cloning vehicles)

Selection process

BASIC DESIGN OF A GENE CLONING EXPERIMENT



The foreign DNA and plasmid are cut with the same **restriction enzyme**, which recognizes a particular sequence of DNA called a *restriction site*. The restriction site occurs only once in the plasmid, and is located within the *lacZ* gene, a gene necessary for metabolizing lactose.

The restriction enzyme creates sticky ends that allow the foreign DNA and cloning vector to anneal. An enzyme called ligase glues the annealed fragments together.

The ligated cloning vector is transformed into a bacterial host strain that is ampicillin sensitive and is missing the *lacZ* gene from its genome.

Bacteria are grown on media containing ampicillin and X-gal, a chemical that is metabolized by the same pathway as lactose. The ampicillin kills bacteria without plasmid. Plasmids lacking the foreign insert have an intact *lacZ* gene and are able to metabolize X-gal, releasing a dye that turns the colony blue. Plasmids with an insert have a disrupted *lacZ* gene and produce white colonies.



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