Aquaculture Biotechnology

Recombinant DNA technology
Gene Cloning

Recombinant DNA technology

- ✓ DNA molecules that are extracted from different sources and chemically joined together;

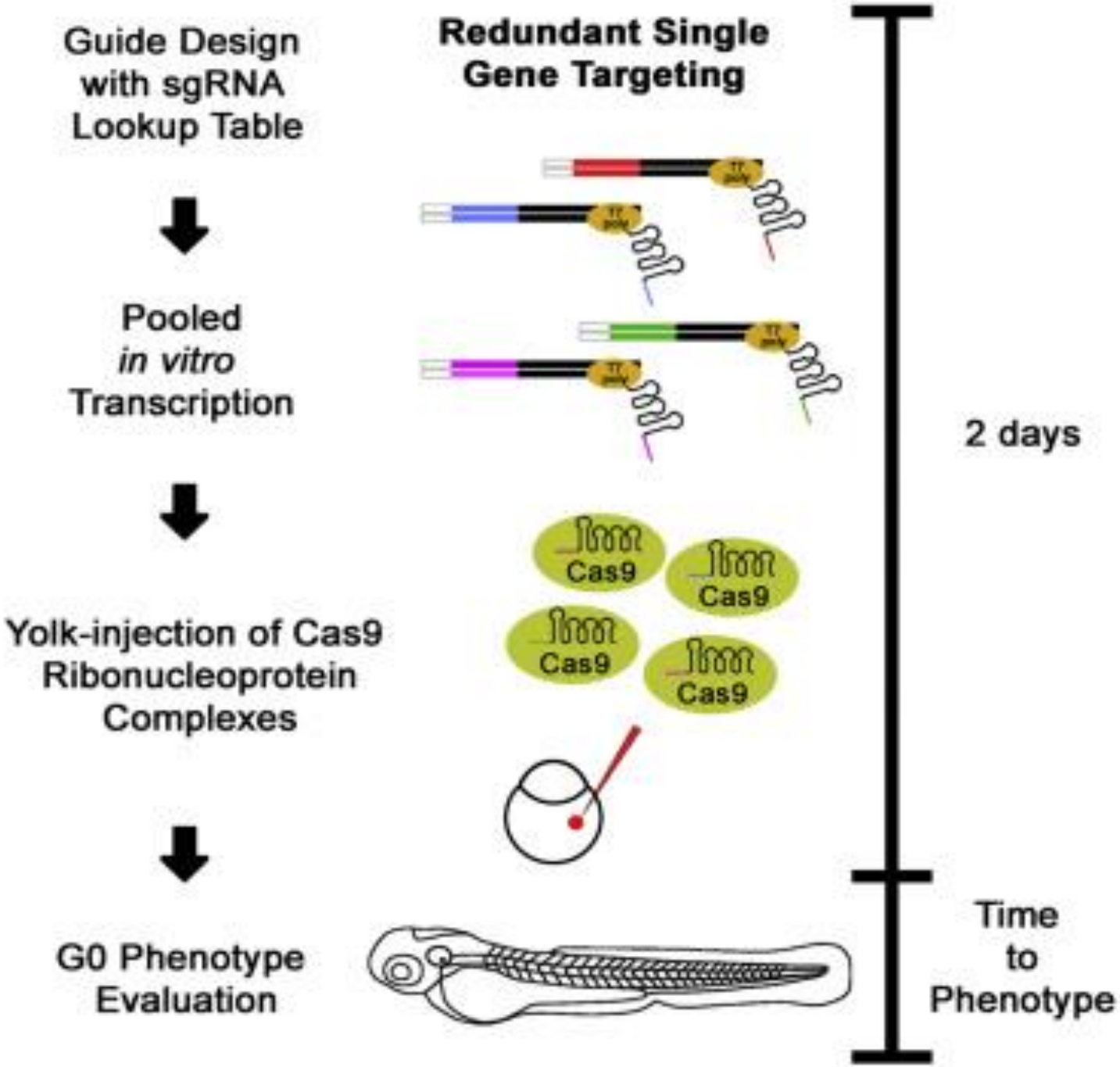
- ✓ for example DNA comprising an animal gene may be recombined with DNA from a bacterium

Discovery of recombinant DNA technology

- ✓ Discovery of DNA structure Watson & Crick in 1953
- ✓ Isolation of DNA ligase in 1967
- ✓ Isolation of REase in 1970
- ✓ Paul Berg generated rDNA technology in 1972
- ✓ Cohen & Boyer in 1973 produced first plasmid vector capable of being replicated within a bacterial host

Goals of recombinant DNA technology in Aquaculture

- To isolate and characterize a gene of interest
- To make desired alterations in one or more isolated genes
- To return altered genes to living cells
- Artificially synthesize new gene
- Alternating the genome of an aquatic organism
- Understanding the hereditary diseases and their cure in aquaculture species
- Improving genome



Directed Gene Knockout in fish

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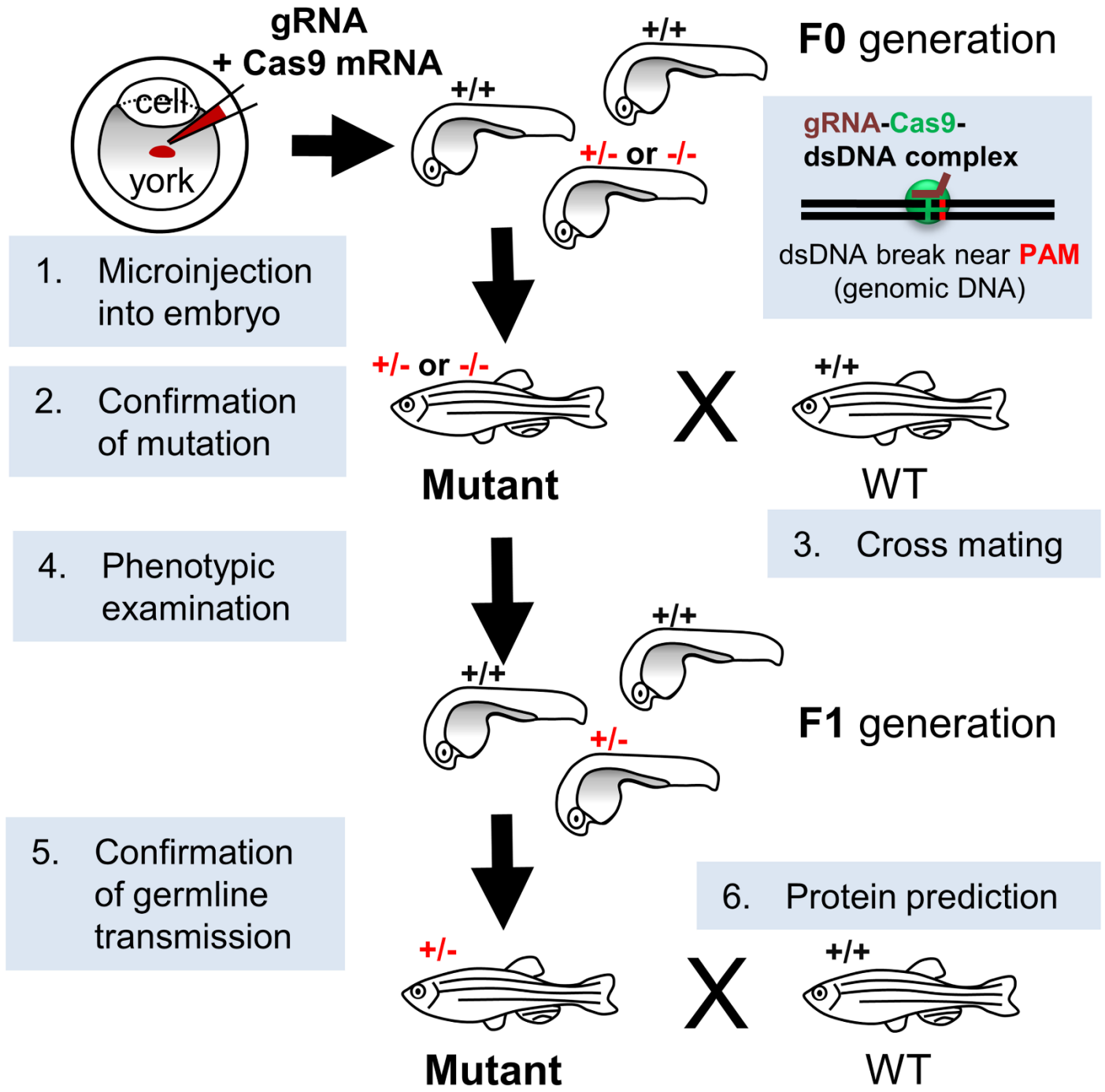
Gene Knockout in fish



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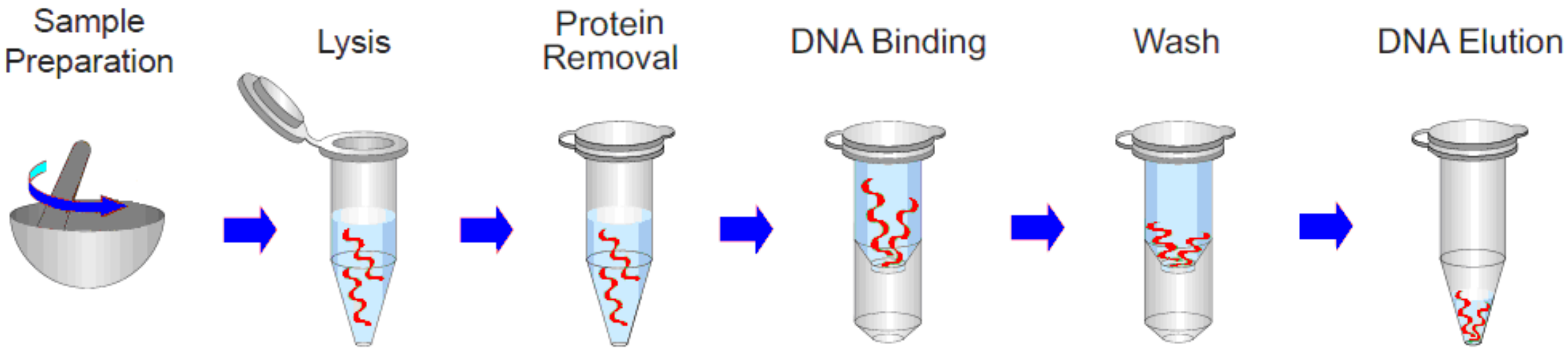
Procedure of making rDNA/ cloning

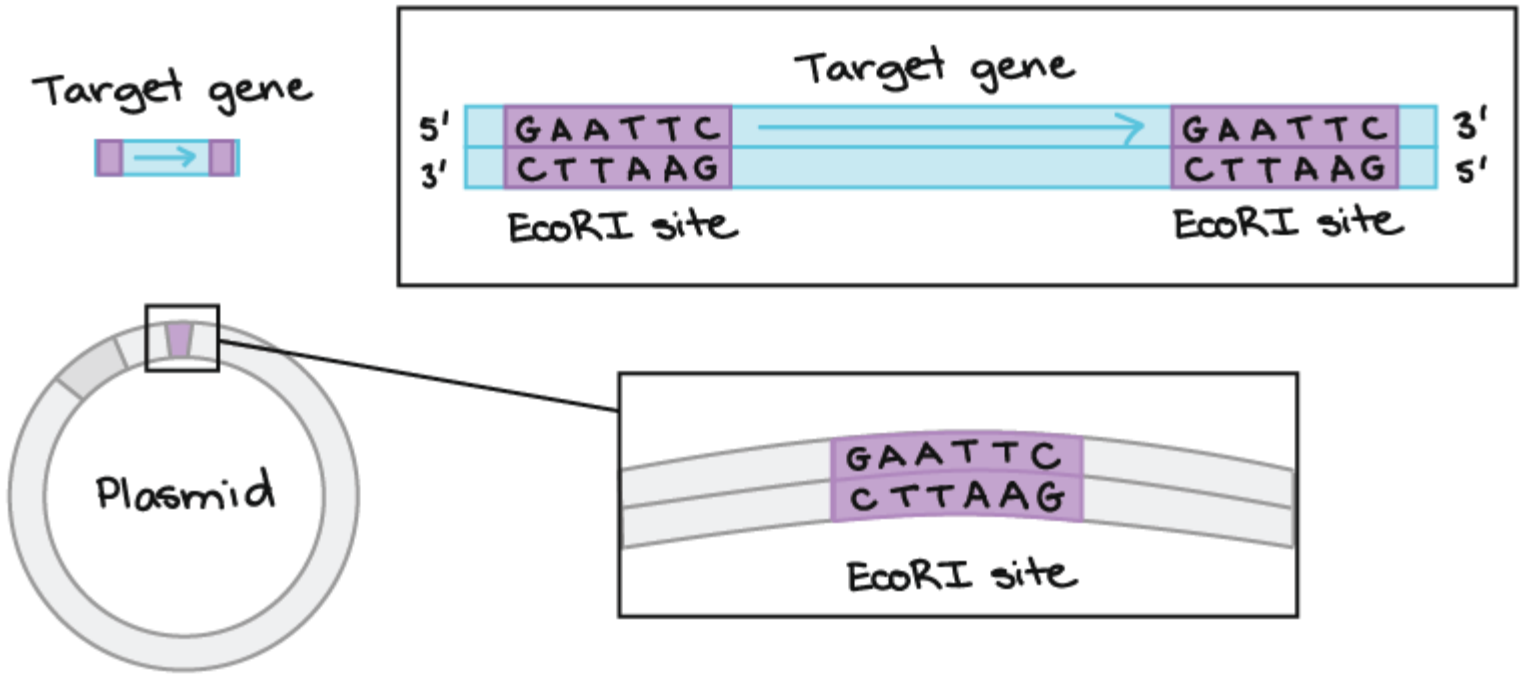
1. *Isolating of DNA*
2. *Cutting of DNA*
3. *Joining of DNA*
4. *Amplifying of DNA*

Isolating of DNA



Cutting of DNA

- DNA can be cut into large fragments by mechanical shearing.
- Restriction enzymes are the scissors of molecular genetics.

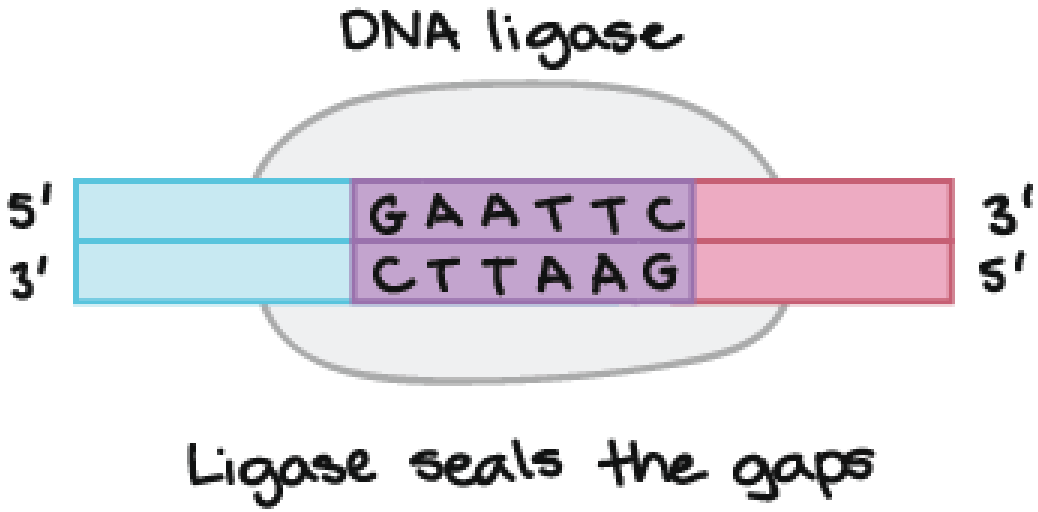


Restriction enzyme

- ✓ A special class of sequence-specific enzyme
- ✓ Found in bacteria
- ✓ Site-specific-cleave DNA molecules only at specific nucleotide sequence
- ✓ REases recognize DNA base sequence that are palindrome
- ✓ REase make staggered cuts with complementary base sequences for easy circulization

Joining DNA

DNA ligase help to join DNA fragments



Amplifying the recombinant DNA

- ✓ Transforming the recombinant DNA into a bacterial host strain.
- ✓ The cells are treated with $CaCl_2$
- ✓ DNA is added
- ✓ Cells are heat shocked at 42 C
- ✓ DNA goes into cell by a somewhat unknown mechanism.
- ✓ Once in a cell, the recombinant DNA will be replicated.
- ✓ When the cell divides, the replicated recombinant molecules go to both daughter cells which themselves will divide later. Thus, the DNA is amplified

Enzymes of *in vitro* DNA recombination

- ✓ DNA ligase • Bind to DNA molecules
- ✓ Type II restriction endonuclease • Cleaves DNA at specific sites
- ✓ Reverse transcriptase • Make a DNA copy of RNA molecule
- ✓ DNA polymerase I • Fill single stranded gapes of DNA duplex
- ✓ Polynycleotide Kinase • Adds a phosephate to the 5'-OH end of a polynucleotide
- ✓ Terminal transferase • Adds homopolymer tails to the 3'-OH ends
- ✓ Exonuclease III • Removes nucleotide residues from the 3' ends
- ✓ Bacteriophage {lambda) exonuclease • removes nucleotides from the 5' ends
- ✓ Alkaline phosphatase • Removes terminal phosphates

Vectors used in cloning

- ✓ A vector is an area of DNA that can join another DNA part without losing the limit for self-replication
- ✓ Should be capable of replicating in host cell
- ✓ Should have convenient RE sites for inserting DNA of interest
- ✓ Should have a selectable marker to indicate which host cells received recombinant DNA molecule
- ✓ Should be small and easy to isolate

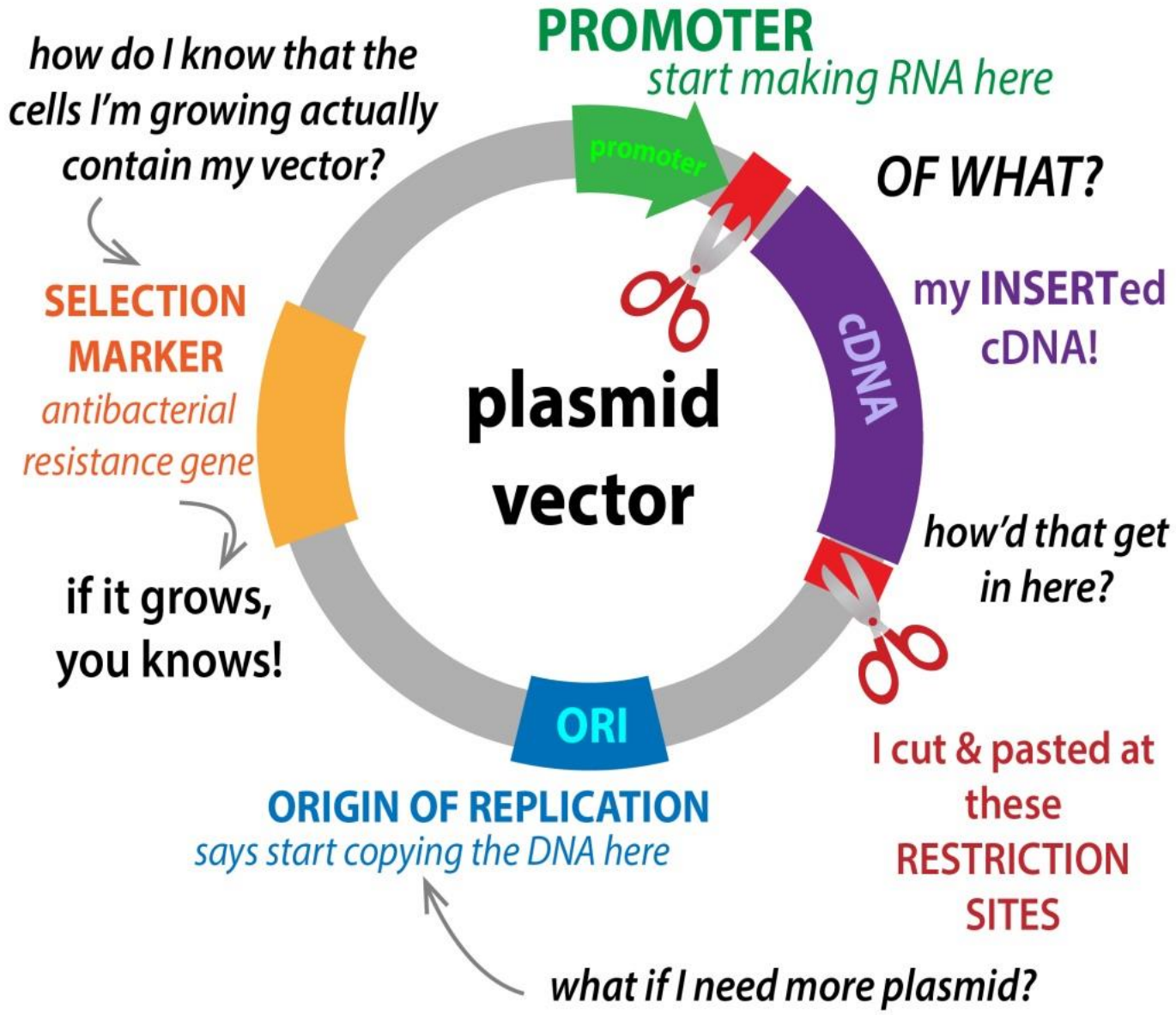
Plasmid vector

Plasmids are small, circular DNA molecules that are separate from the rest of the chromosome.

They replicate independently of the bacterial chromosome.

Useful for cloning DNA inserts less that 20 kb (kilobase pairs).

Inserts larger than 20 kb are lost easily in the bacterial cell.



Lamda phage vector

Lamda phage vectors are recombinant infections, containing the phage chromosome in addition to embedded "outside" DNA.

All in all, phage vectors can convey bigger DNA groupings than plasmid vectors.

Cosmid vector

- ✓ Cosmids are hybrids of phages and plasmids that can carry DNA fragments up to 45 kb.
- ✓ They can replicate like plasmids but can be packaged like phage lambda