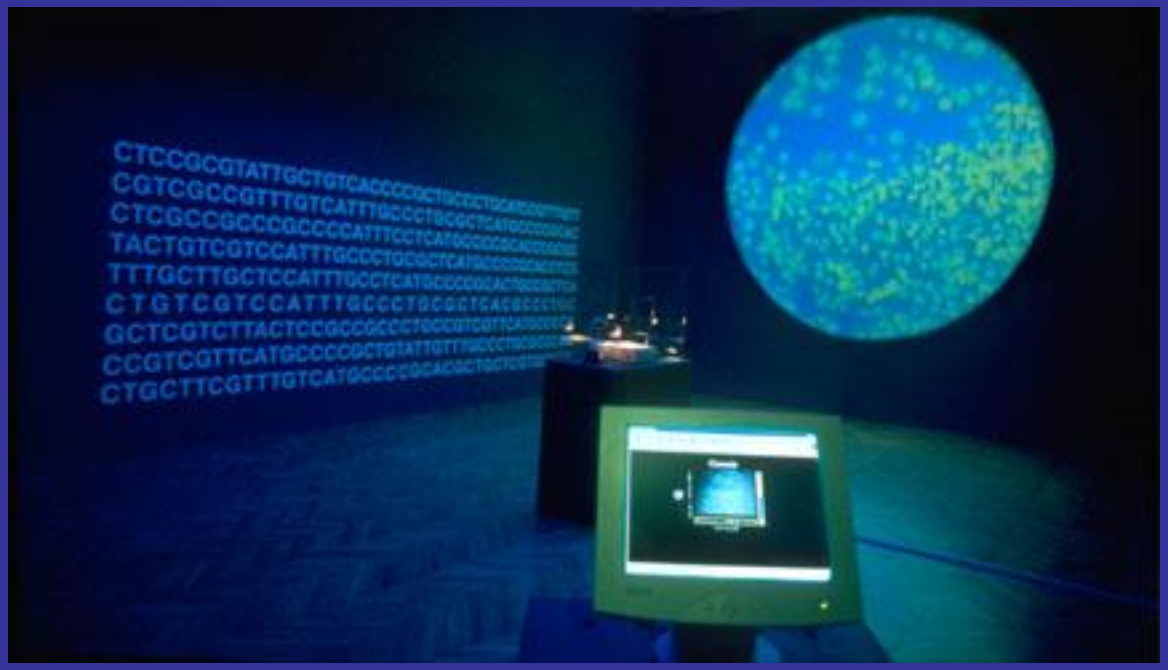
Bacterial Transformation

Green Fluorescent Protein



What is Transformation?

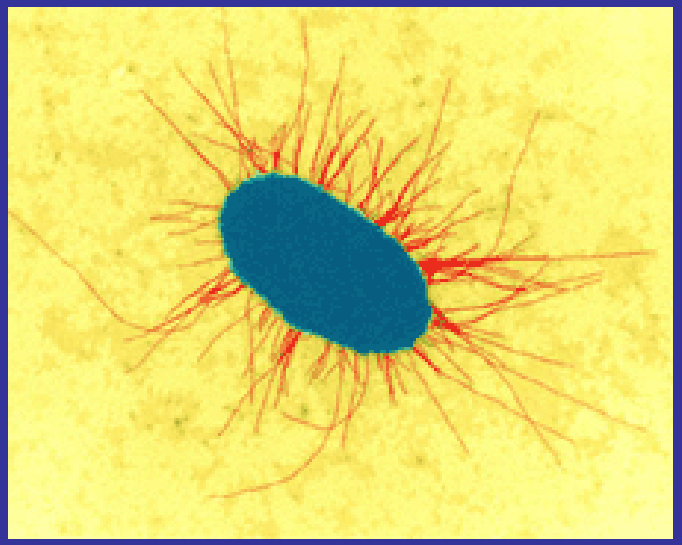
- Change…
- In molecular biology, change in which genetic material carried by an individual cell is altered by incorporation of foreign DNA

Applications

- Genes coding for traits such as disease or pest resistance may be transformed into plants
- Gene therapy replaces defective genes in a cell with functional copies
- Bacteria can be transformed with a gene for human hormone like insulin so it can be produced in mass quantities

Organism to be Transformed: *E. coli*

- *E. coli* bacteria are part of the normal flora in the human gut
- MM294 is a “K-12” strain that does not colonize the gut
- Proper decontamination is required nevertheless

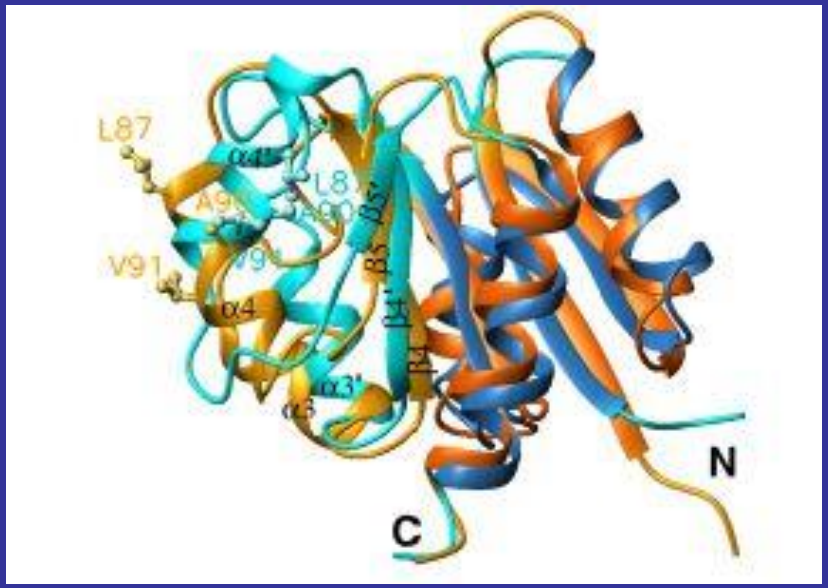


Gene to Insert:
Green Fluorescent Protein

- Comes from bioluminescent jellyfish - *Aequorea victoria*
- GFP is widely used as a marker to determine if transformation is effective

Gene to Insert: Antibiotic Resistance

- Beta-lactamase which provides resistance to ampicillin
- Inactivates ampicillin and allows bacterial growth
- Also used as a marker



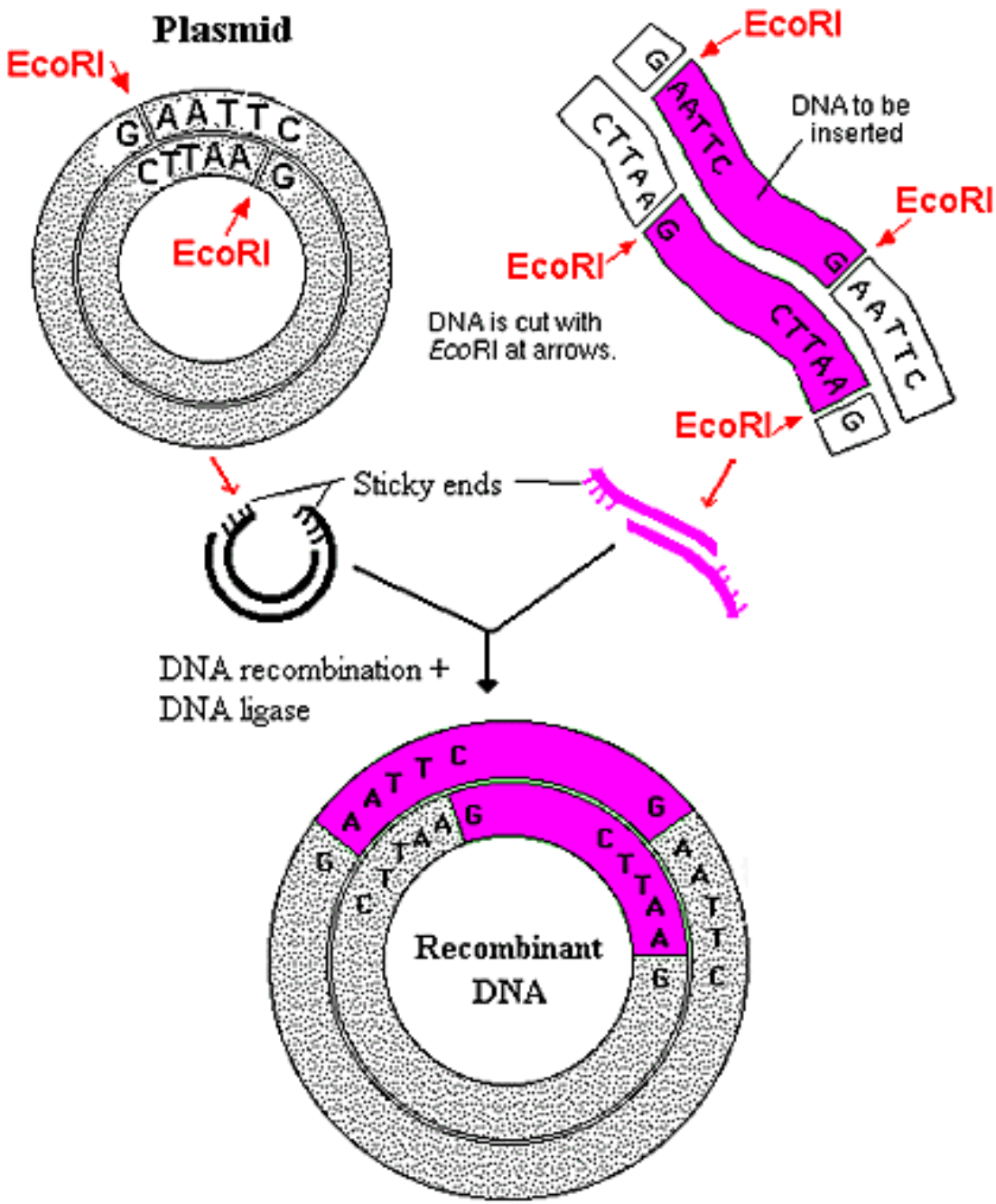
Plasmid

- A plasmid is a small piece of circular DNA in bacteria in addition to bacterial chromosome
- Typically contain genes for traits beneficial for survival such as antibiotic resistance
- In nature, bacteria transfer these plasmids back and forth
- Acts as a vector – a gene taxi to get new DNA into bacteria

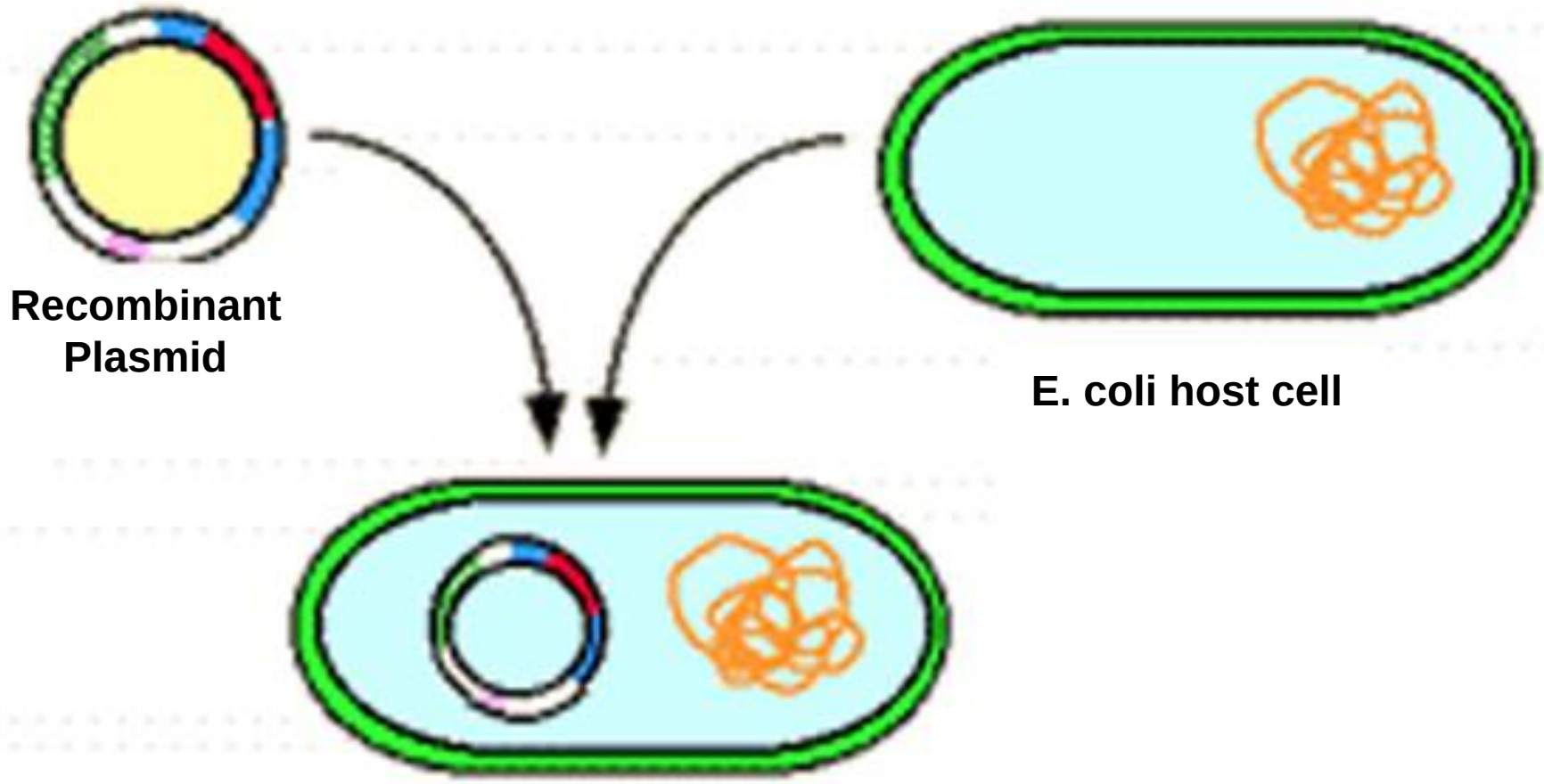
Constructing a Plasmid

- Specific fragments of DNA may be isolated, cut into discrete pieces by the action of restriction enzymes, and rejoined by the action of DNA ligase

http://www.dnai.org/b/index.html



Inserting a DNA Sample into a Plasmid


Recombinant
Plasmid
E. coli host cell
Transformed Bacteria

Transformation Methods

Agrobacterium tumefaciens

- *Naturally occurring soil bacteria that causes Crown Gall disease*
- *Ability to infect a plant cell and transfer its DNA*
- *Disease causing genes removed and gene of interest inserted*

Transformation Methods

Biolistics

- *“Gene gun”*
- *DNA stuck to microscopic gold or tungsten particles*
- *Blast of pressurized helium shoots DNA into plant tissue, some makes it through plant cell walls and membranes and into the nucleus*

Transformation Methods

Additional Techniques

- *Electroporation*
- *Heat Shock*

Steps in This Lab

- We will be using a plasmid construct that already contains our gene of interest
- Prepare cells to allow transfer of DNA through membrane
    - Add ++ ions
    - Heat shock
- Provide nutrients and allow cells to express newly inserted genes
- Grow transformed cells on agarose plates to isolate transformants

GFP Video