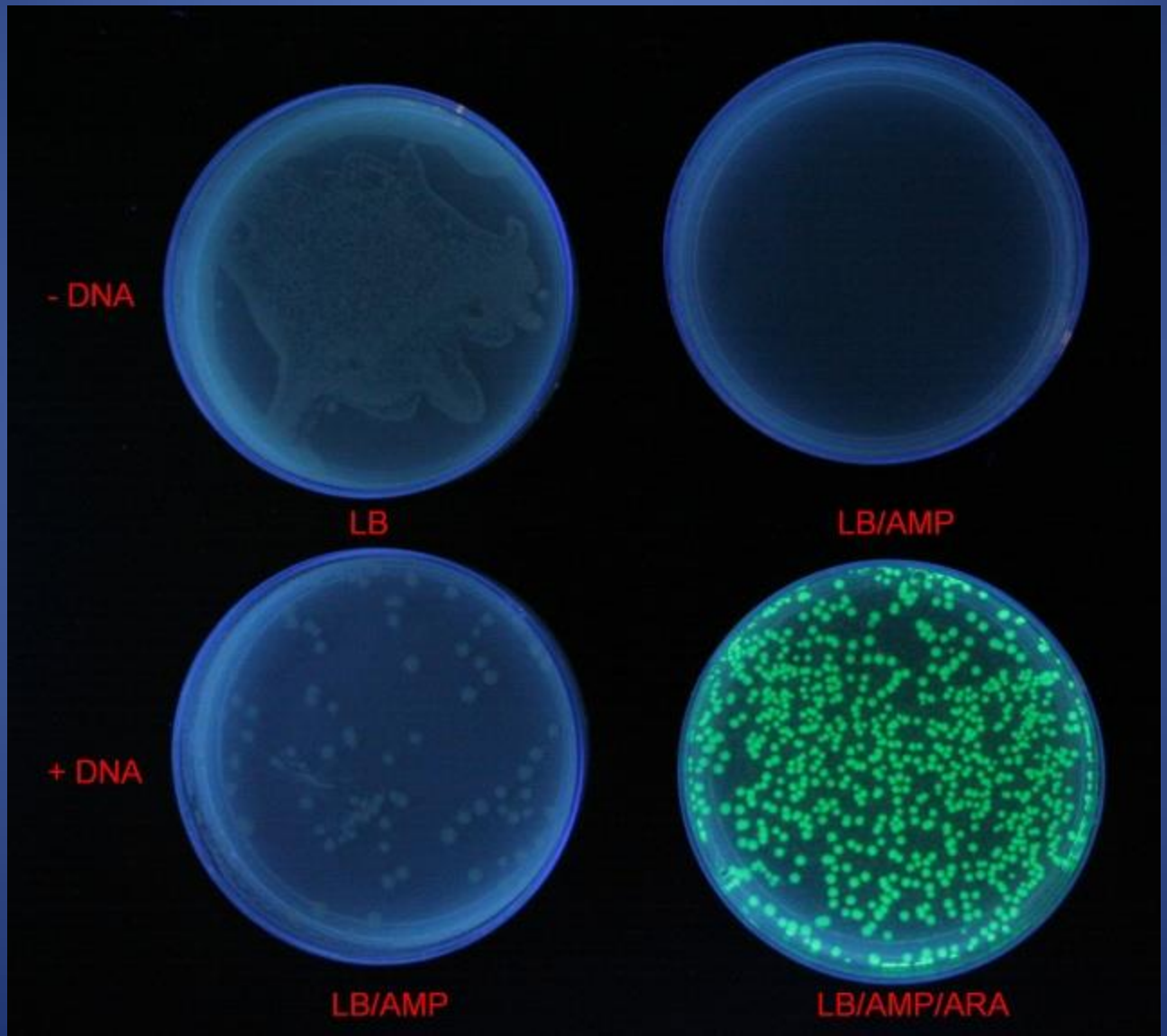
Bacterial Transformation



Bacterial Transformation

- Genetic Engineering: Any manipulation of _____ within a cell or organism.

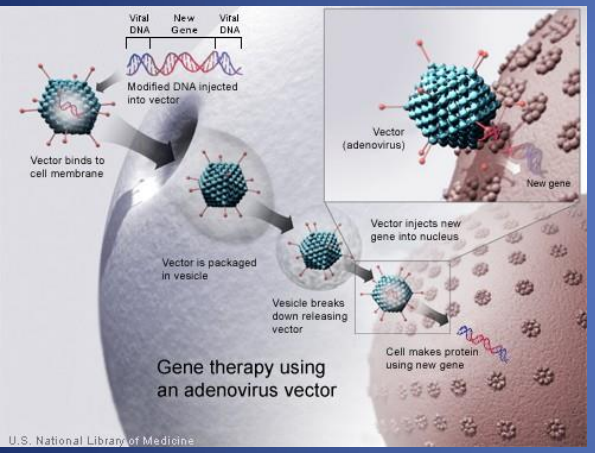
- Transformation: Splicing gene(s) from_____ and inserting them_____ _____.

- Recombinant DNA (rDNA): DNA made _____ from different sources.

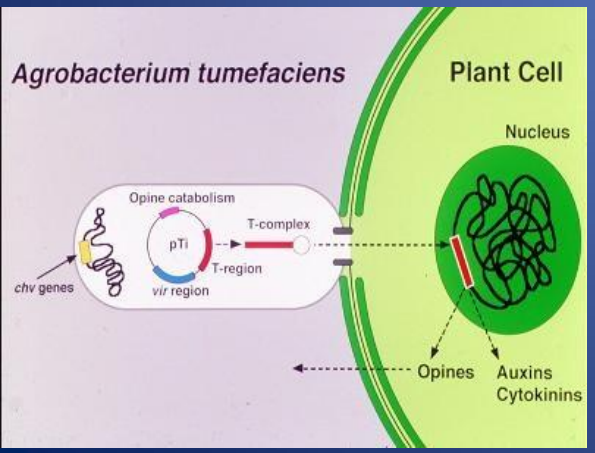
- Transgenic organism: An organism _____ which it expresses.

Bacterial Transformation

- Transformation Vector: The method used to insert _____ of interest into _____.
  - Examples:



- Altered Viruses: Genetic material inside viruses are altered to contain the gene(s) of interest. Used in _____.



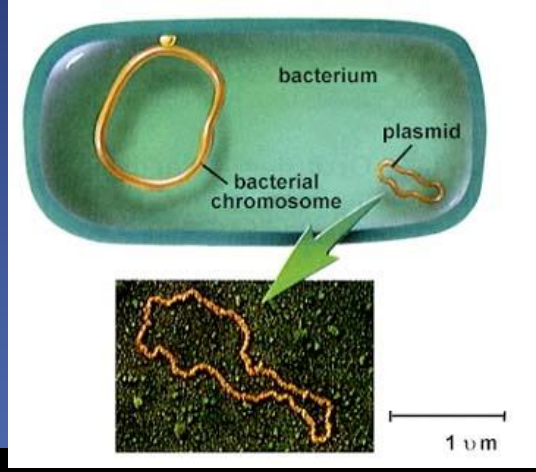
- Agrobacterium: A bacteria that usually causes tumors in plant cells is altered to insert the gene(s) of interest. Used in _____.

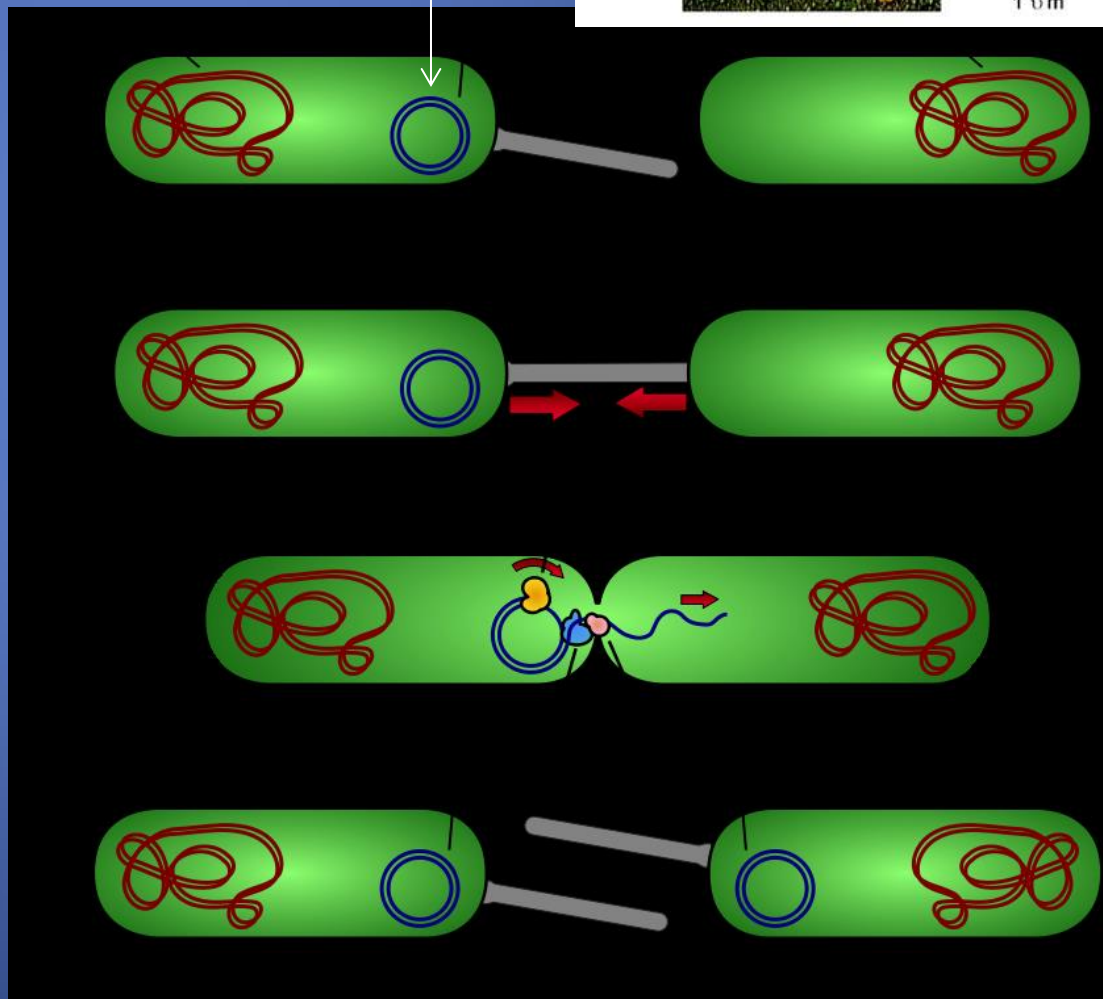
- Plasmids: Small, _____ circular pieces of DNA that is altered with the gene(s) of interest. Used in _____.

Plasmids

- Plasmids are shared by bacteria and can contain genes such as antibiotic resistance.

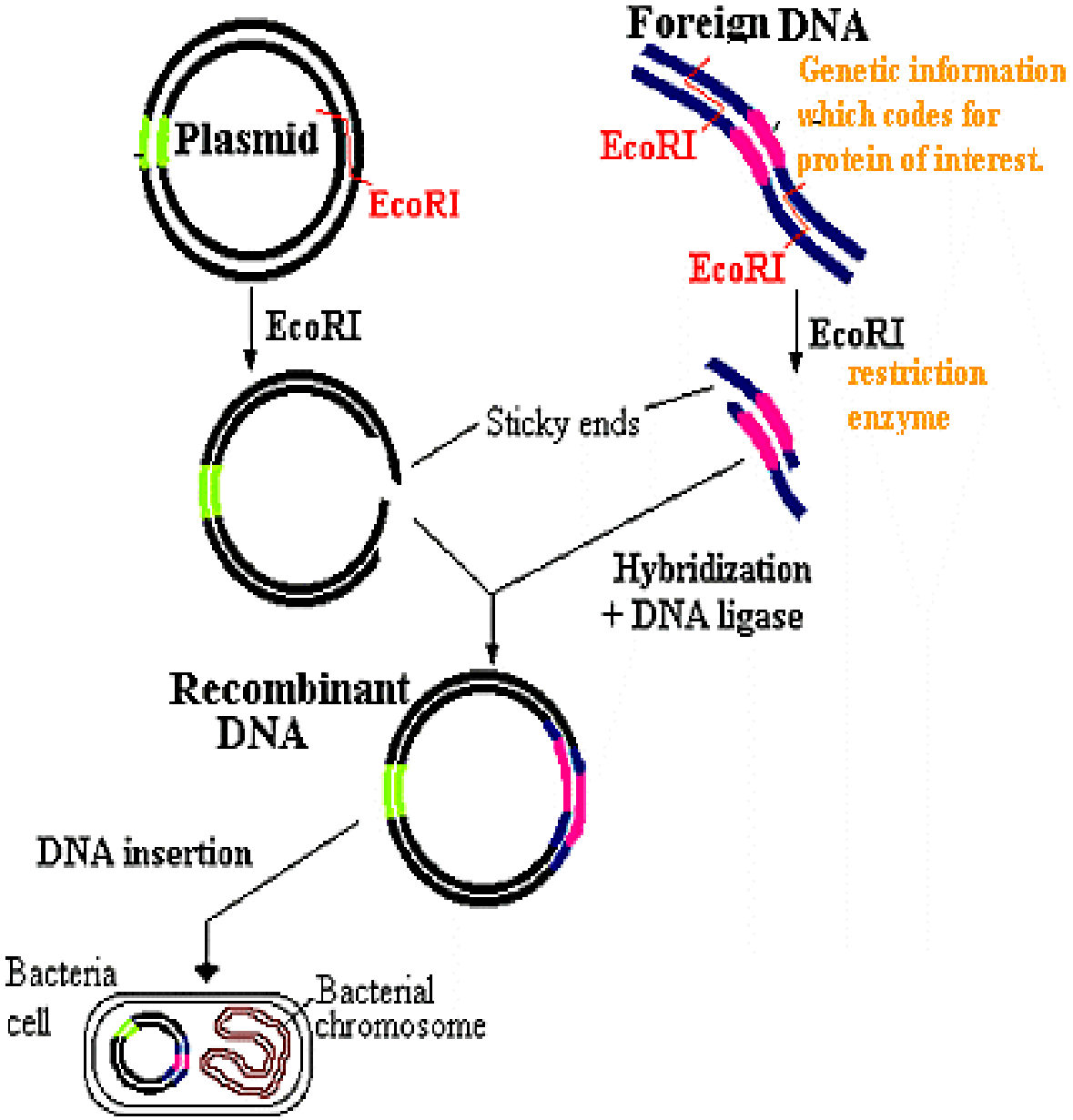
- Sharing plasmids is a way for bacteria to be _____.





Creating the Plasmid Vector

1. Cut plasmid with _____.
2. Cut gene of interest with the same restriction enzyme to create _____.
3. Insert gene into plasmid with DNA _____.
4. Insert plasmid into host bacteria by _____them with either heat or electrical current (this opens their pores).
5. Grow host bacteria on plates of _____

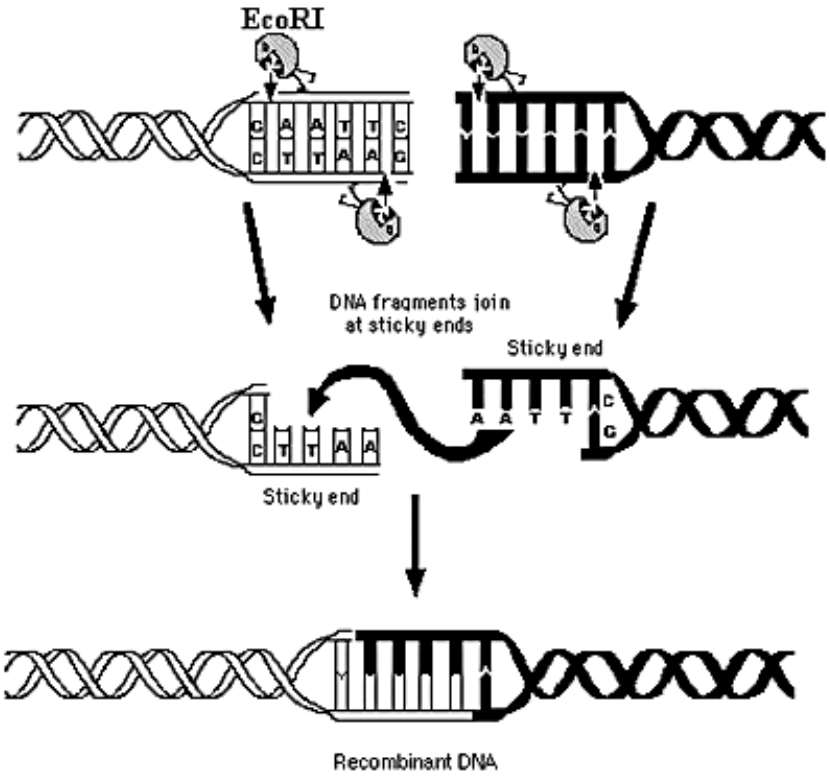


Cutting DNA and splicing

- Restriction enzymes are used to _____at a specific palindrome sequence (read forward and backward the same)
  - Word Example: Race Car
  - DNA example: _____

- "Sticky ends" (complementary sequences) are created when host DNA is cut with the _____.

- DNA ligase enzymes act as chemical _____ and covalently bond (sugar & phosphate) the DNA together.

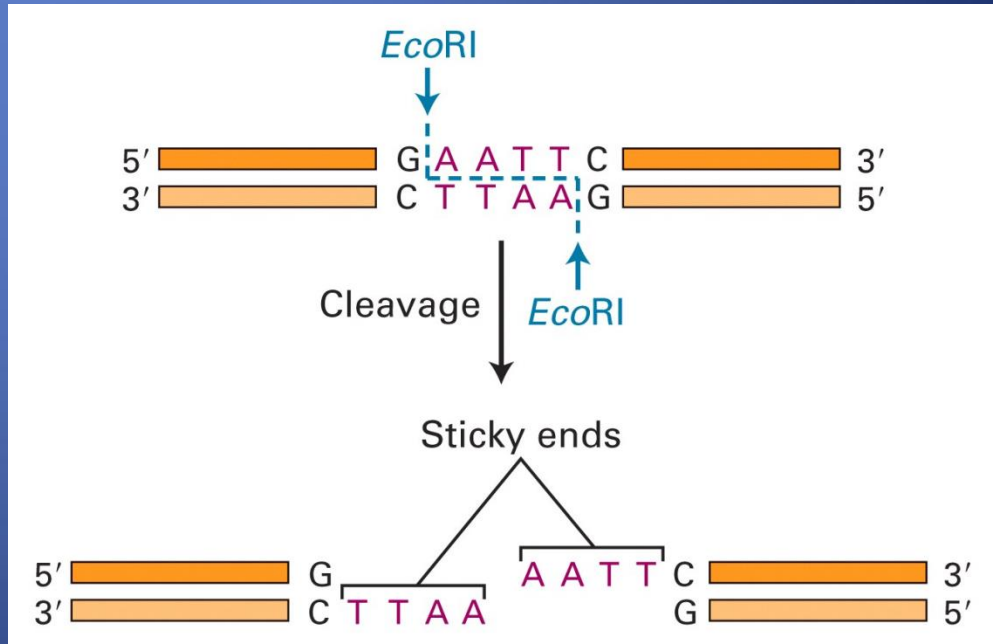


Restriction Enzyme Action of EcoRI

Sticky Ends

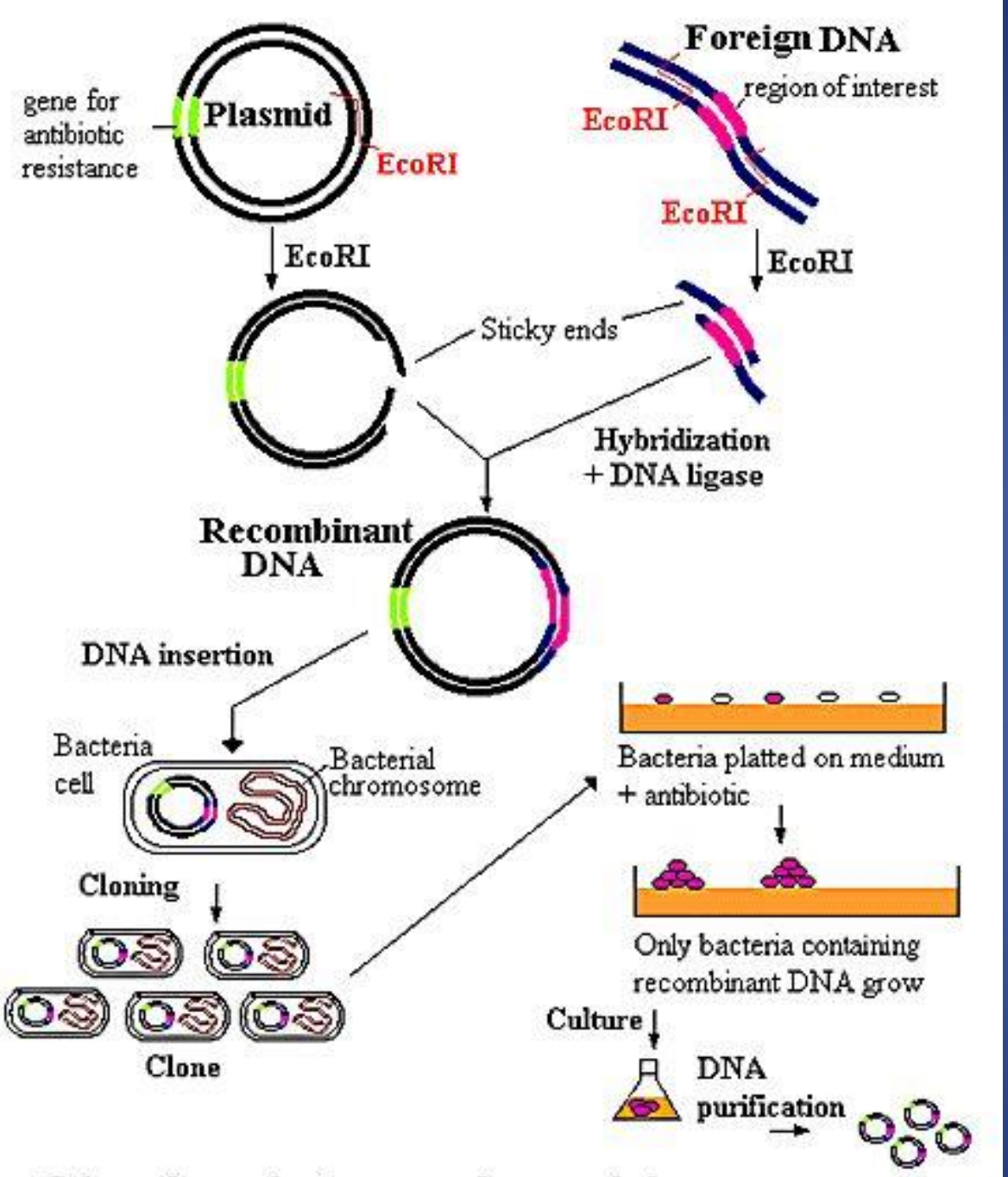
Many restriction enzymes cut sequence in a way that produces single-stranded "sticky ends".

Any two DNA strands cut with the SAME restriction enzyme can bind together due to complementary pairing of sticky ends.



Bacterial Transformation

http://www.youtube.com/watch?v=8rXizmLjegI

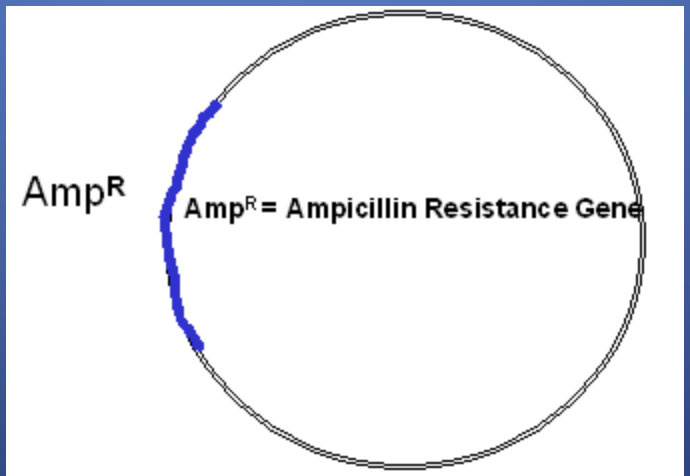


Cloning into a plasmid

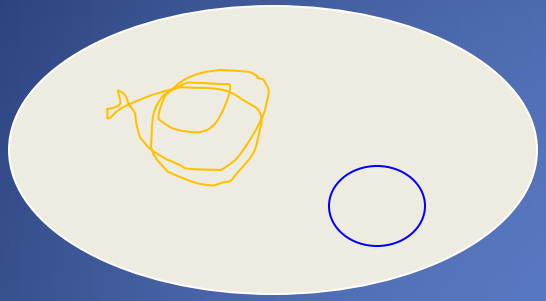
Screening for successful Transformation

Not all the bacterial cells will successfully take in the foreign DNA with the desired gene.

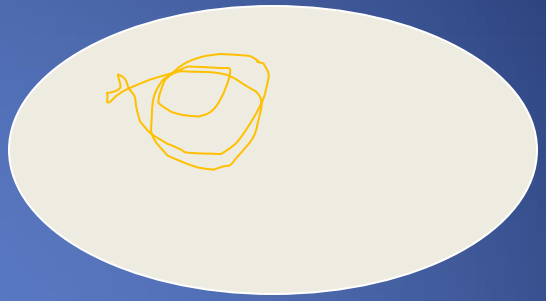
In order to be able to screen for successfully transformed cells, scientists often chose a plasmid vector with an _____gene and a host cell that is susceptible to the antibiotic.



Transformed bacteria cell with antibiotic resistance gene on plasmid

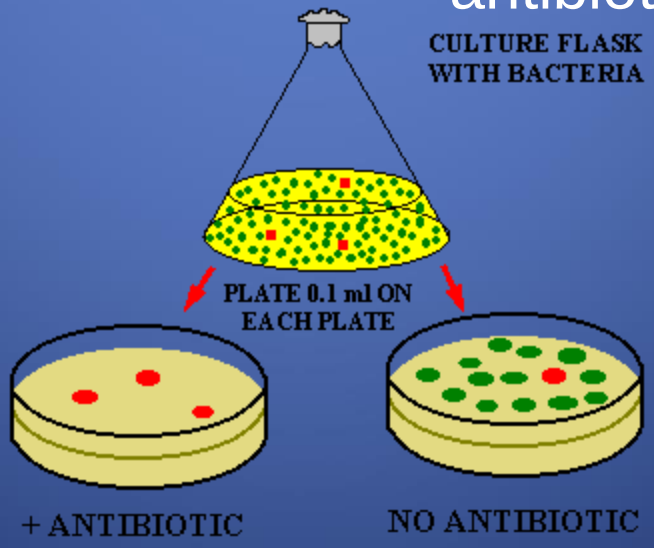


Bacteria cell that did NOT receive plasmid



Will be able to _____in the presence of the antibiotic as well as on regular petri dishes.

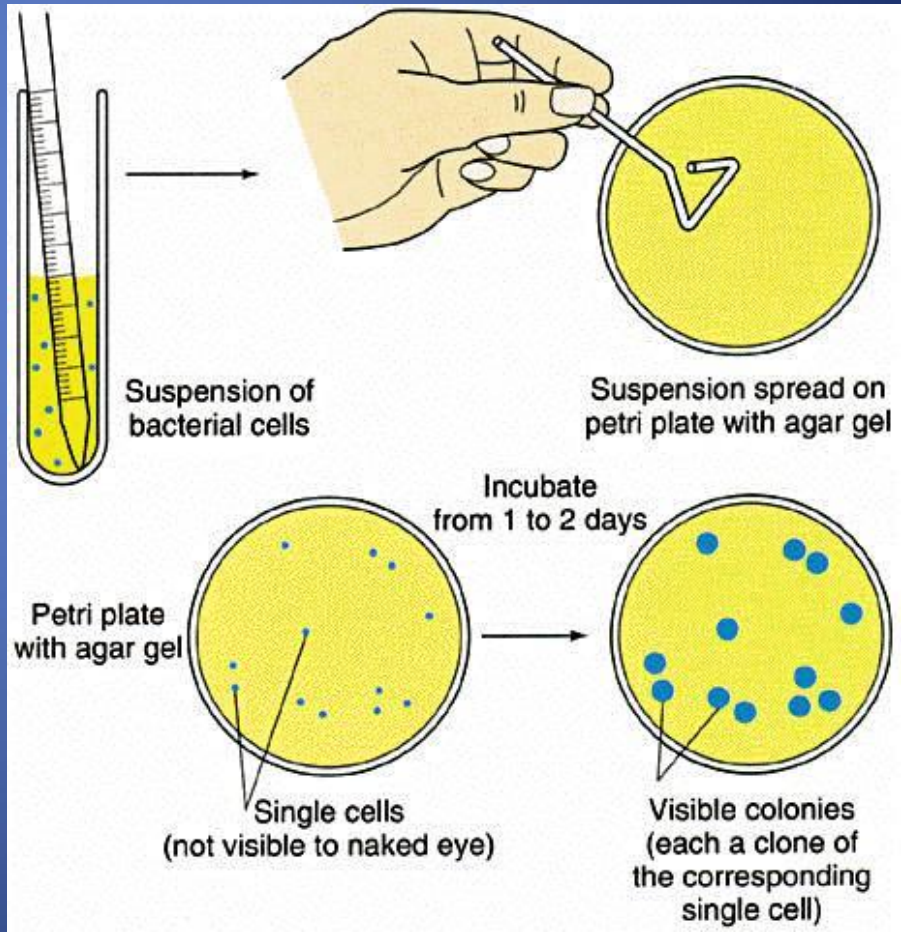
Will only be able to grow on petri dishes that do _____ the antibiotic.



Plating and selecting Transformed Bacteria

- Growing bacteria on an agar plate containing the antibiotic will only allow bacteria with _____(that has the gene for resistance) to grow.

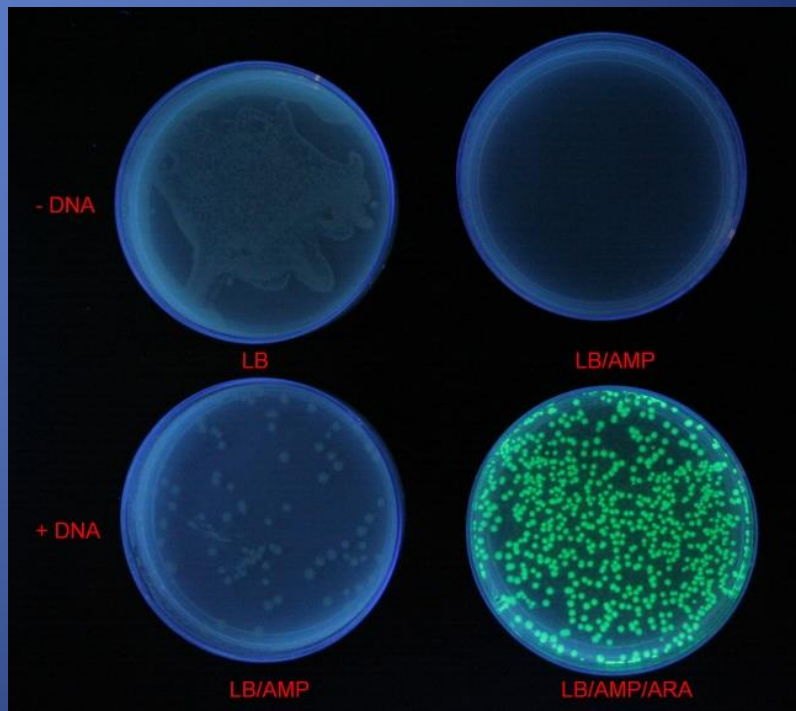
- Colonies: single bacteria (not visibile) that have multiplied creating a _____. They are all _____ of the original bacteria.
- Lawn: So many bacteria grow on a plate that they _____.

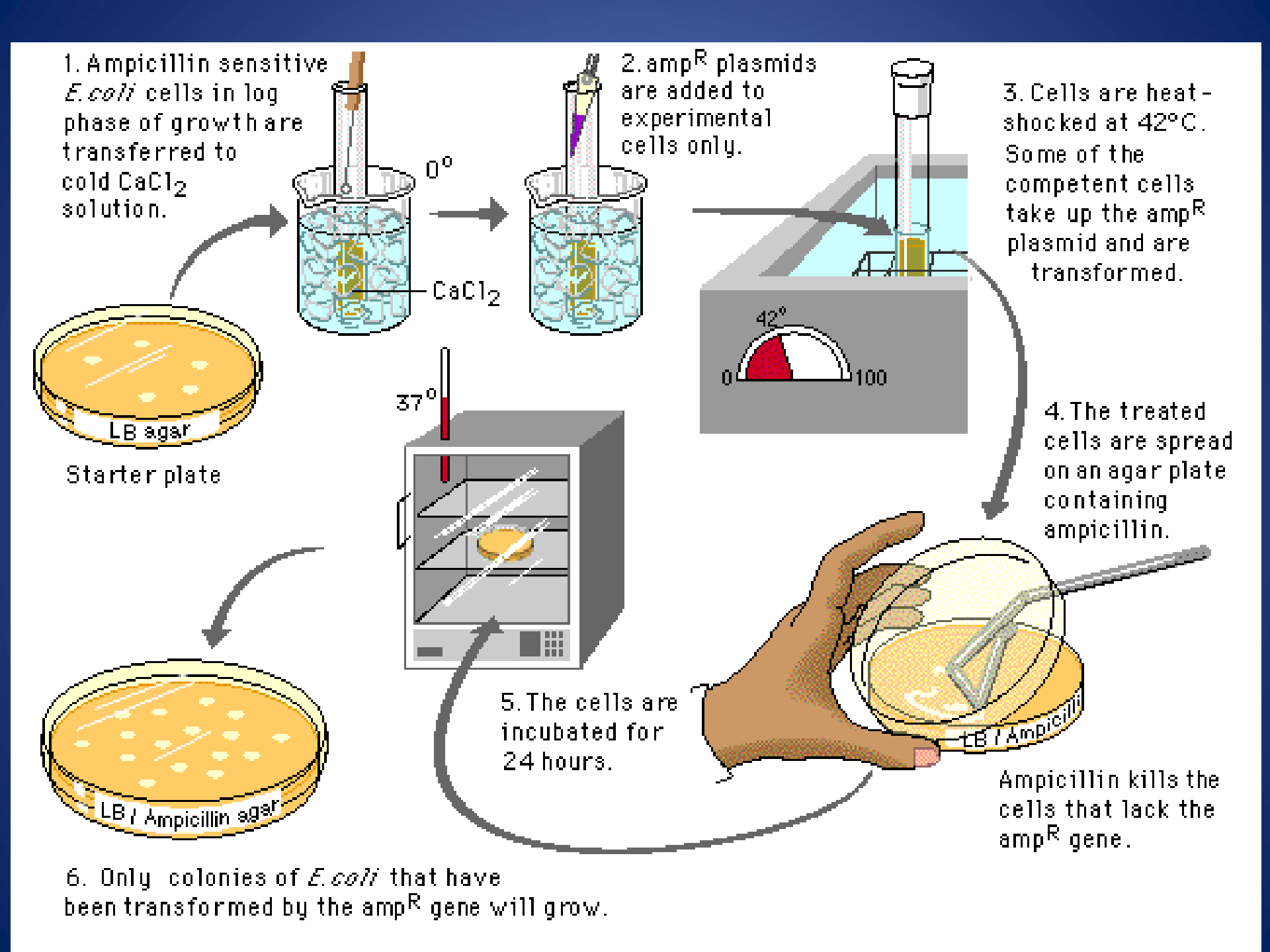


Heat Shock and growth

- Heat shocking: By increasing the temperature of the bacteria, their _____allowing plasmids from the outside to enter. This can also be done with electricity.

- Results: Note the growth of the plates after they were incubated at 37 degrees C. The plasmid contained a gene that produced a glowing color and also a resistance to ampicillin.




1. Ampicillin sensitive *E. coli* cells in log phase of growth are transferred to cold $CaCl_2$ solution.
0°
$CaCl_2$
2. amp^R plasmids are added to experimental cells only.
3. Cells are heat-shocked at 42°C. Some of the competent cells take up the amp^R plasmid and are transformed.
42°
0
100
4. The treated cells are spread on an agar plate containing ampicillin.
LB agar
Starter plate
37°
5. The cells are incubated for 24 hours.
LB / Ampicillin
Ampicillin kills the cells that lack the amp^R gene.
LB / Ampicillin agar
6. Only colonies of *E. coli* that have been transformed by the amp^R gene will grow.