

## Moving Genes Part I

# Steps in Bacterial Transformation

### Student Instructions:

These cards contain the basic steps in using plasmid vectors to transform bacteria. Sort them into the correct order.

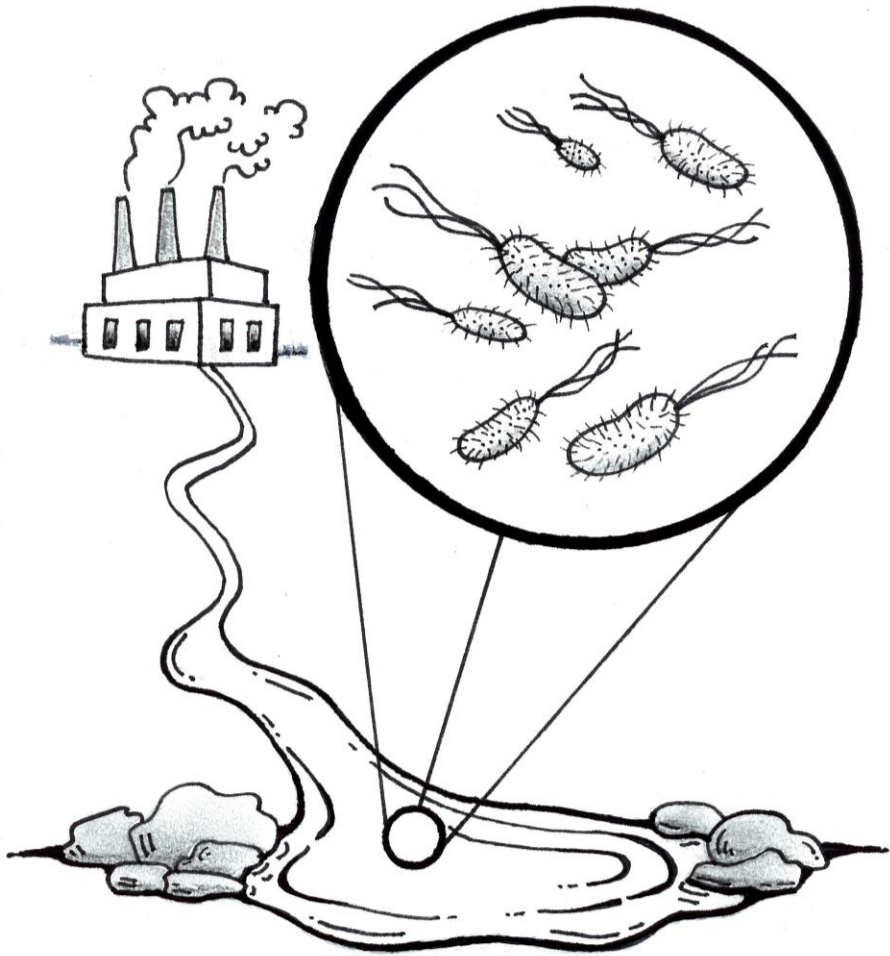
Be sure to learn and understand the meanings of any words that are unfamiliar to you.

### Teacher Instructions:

1. Copy these cards onto card stock, laminate, and cut.
2. Pass out to students so that the cards are in random order.
3. This activity may be used to introduce and/or review the topic of "bacterial transformation."
4. Ask students to place the cards into an order that "makes sense." Students may work singly, in pairs, or in small groups.
5. Ask questions as you circulate around the room.
  - What do you need to start?
  - What is the final product you want?
  - Why did you put this card in this place?
  - What made you think that?
  - Which would come first: a few, or a lot?
6. Remind students to use the vocabulary words as they ask questions to you and discuss the cards with their classmates. Encourage them to make a list of unfamiliar terminology and use favorite techniques to learn this new vocabulary.
7. Review the correct order with them.
8. Students may then take notes from the cards.

Identify a phenotype  
of interest

**B**



Example: Bacteria that grew in the presence of glyphosate (Roundup) were found in a waste pond.

Identify a gene that codes  
for the desired phenotype

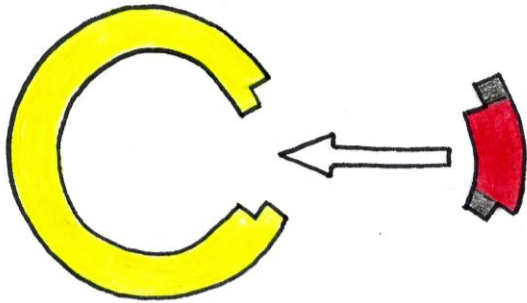
**D**



Example: Through experimental research, the gene that gave resistance to glyphosate was identified.

Gene of interest is engineered into a plasmid vector

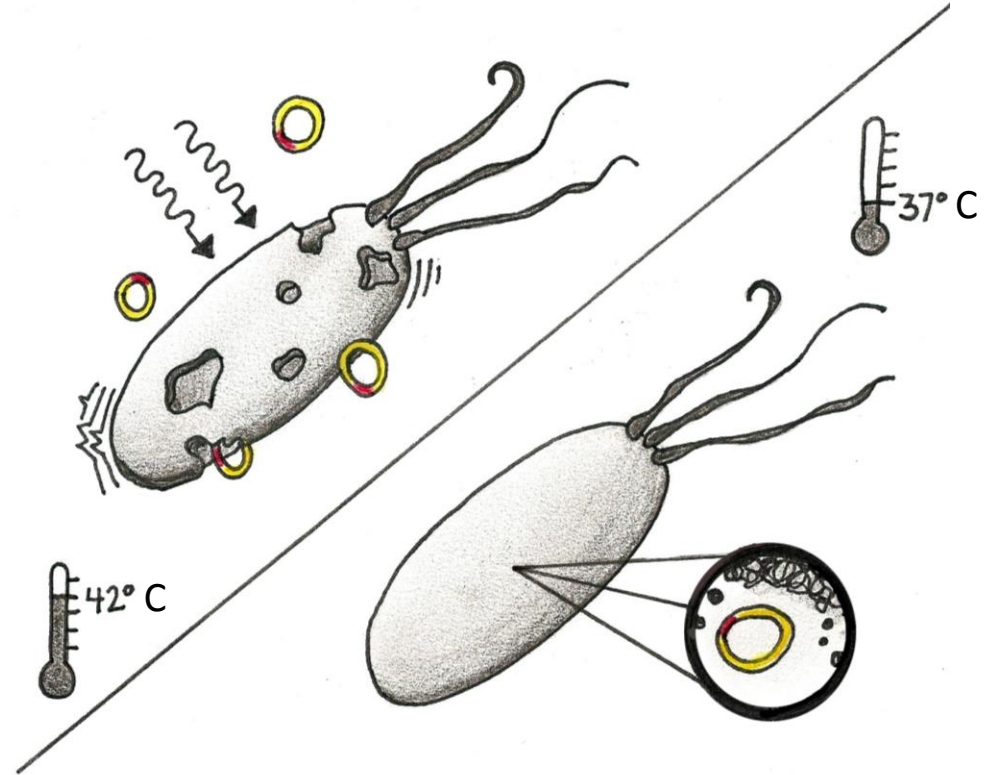
C



- Plasmid vector may be engineered by an individual lab or purchased from a supplier
- Engineering of the plasmid occurs outside of a cell (in vitro)
- Restriction enzymes cleave DNA, opening the plasmid
- Gene of interest is mixed with open plasmids
- Ligation reaction seals gene of interest in plasmid

*E. coli* are transformed by the plasmid vector

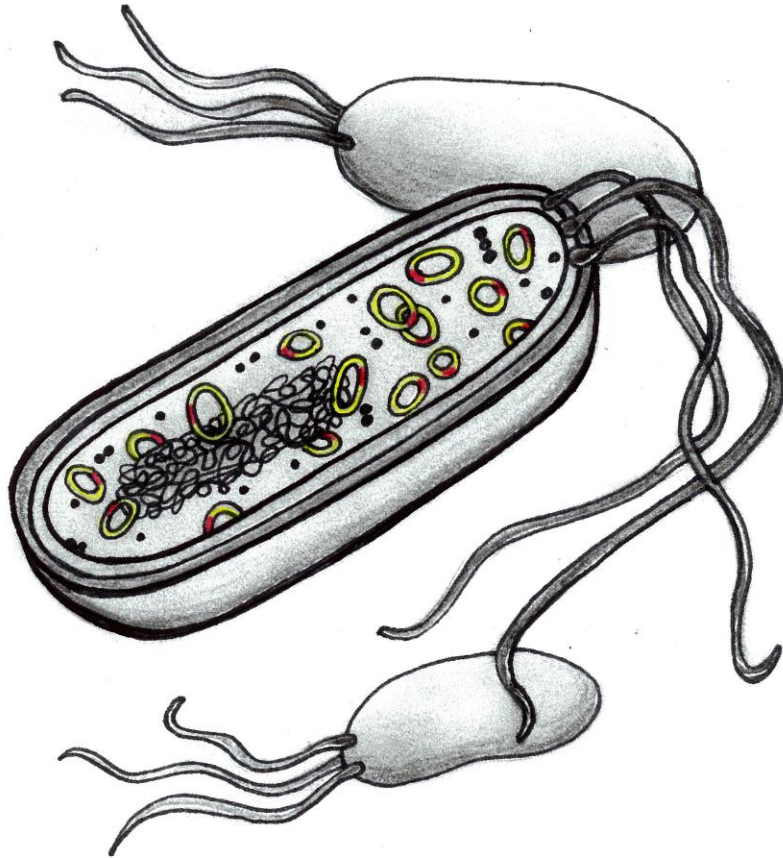
E



*E. coli* are made **competent** through heat shock. Placing bacterial cells in a hot water bath disrupts the cell membranes and makes them more permeable. The plasmids are now able to cross the disrupted membrane.

Replication of plasmid vector  
by *E. coli*

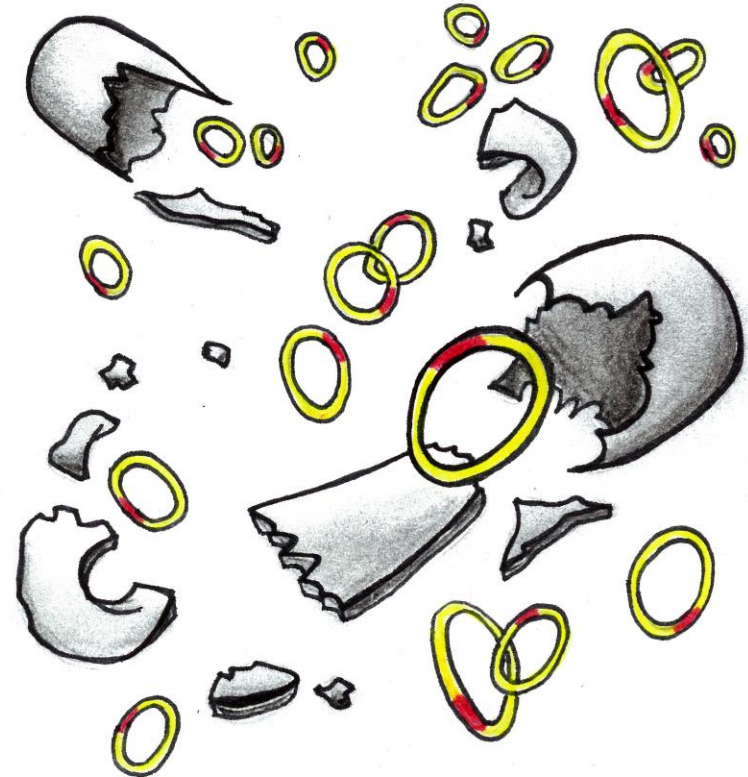
**A**



*E. coli* cell machinery is harnessed as a way to multiply the plasmid vector containing the desired genes.

Plasmid vector extracted  
from *E. coli*

**F**



Following successful transformation, the plasmid vector can again be isolated from the recipient cell. Detergents are used to break down the cell wall and membrane.