EDVOTEK® INQUIRY GUIDE:

TRANSFORMATION

TEACHERS:
For the answer key, please contact us at **info@edvotek.com**

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Key Terms

DEFINE the following terms before you begin the guide then refer to them as needed. Use any resources you want!

Exogenous:

Plasmid:

Restriction Enzyme:

Sticky Ends:

SBOL:

Start Codon:

Stop Codon:

Promoter Region:

Terminator Region:

Competent Cell:

Recombinant DNA:

Recombinant Organisms:

IPTG:

Transformation Efficiency:

Transformation:

Transformation Worksheet

Transformation is a process where bacteria take up exogenous DNA. Scientists harness this ability to turn bacteria into protein production factories.

PREPARATION: DESIGNING PLASMIDS

1. First scientists must create DNA that contains desired genes. One way to do this is with restriction enzymes. Scientists digest a plasmid and a DNA segment containing the desired gene sequences with restriction enzymes to create matching sticky ends. These ends can then be coaxed to recombine, resulting in the incorporation of the gene into the plasmid.

   A popular restriction enzyme is *Eco*RI which recognizes and cuts DNA at a "GAATTC" target. **MARK** the recognition site where EcoRI would cut the following DNA segment.

***Eco*RI cut site:**

G|AATTC
CTTAA|G

DNA:

```
5'-  GAATTCGGATCGCGTACCGCATGAATTCCCGTACGCCGATCCGAATTG  -3'
     ||||||||||||||||||||||||||||||||||||||||||||||||
3'-  CTTAAGCCTAGCGCATGGCGTACTTAAGGGCATGCGGCTAGGCTTAAC  -5'
```

2. Scientists sometimes find a gene they need in nature such as the popular Green Fluorescent Protein (GFP) gene that was originally found in jellyfish. Other times a particular gene or gene sequence needs to be created. Synthetic biology allows scientists to design and build their own DNA using an open language called SBOL.

SYMBOL	DNA PART
EcoRI / AfeII	5′ *Eco*RI restriction site
	3′ *Afe*II restriction site
PFP	Purple Fluorescent Protein Coding Region
GFP	Green Fluorescent Protein Coding Region
AR	Ampicillin Resistance Coding Region
	Start Codon
	Stop Codon
	Promoter Region
	Terminator Region

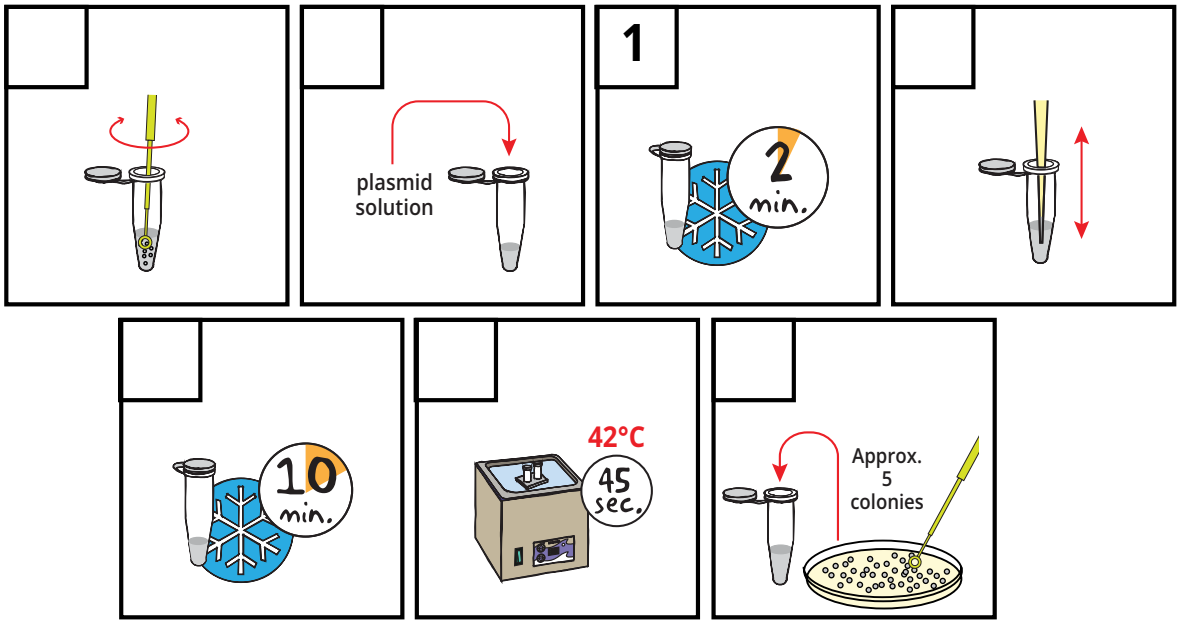
Example

CREATE a DNA construct (below) that could be combined with a plasmid using *Eco*RI and that would give an organism the ability to create a Purple Fluorescent Protein and grow on agar treated with ampicillin. Use the provided chart (at left) and reference the example (above) of a DNA construct that carries the GFP gene.

THE CONTINUING STORY: GETTING PLASMIDS INTO BACTERIA

3. Once a recombinant plasmid has been created it is inserted into a cell. However, many cells must first be primed to take in DNA. A cell that can take up foreign DNA from the surround environment is called competent.

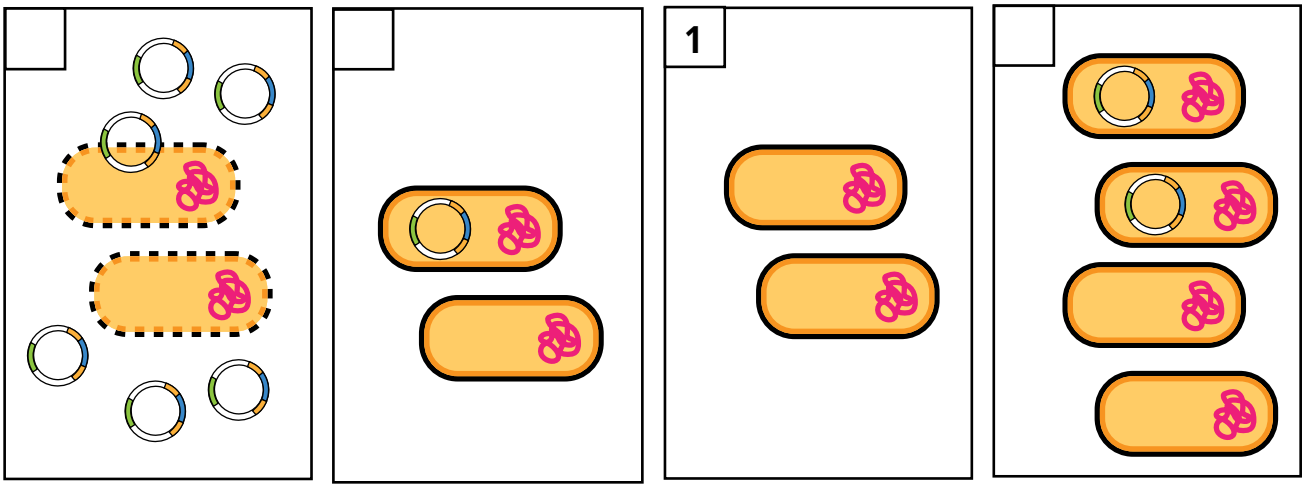
   Below are common instructions for the creation of competent *E. coli* cells. **MATCH** the steps to their illustrations by writing the step number in the upper left corner of the image. The first step has already been matched as an example.



1. **ADD** Calcium Chloride to a sterile test tube and place on ice for 2 minutes.
2. Using a sterile loop, **TRANSFER** 5 well isolated bacteria colonies to the test tube containing Calcium Chloride.
3. **TWIST** the loop between your fingers to free the cells.
4. **RESUSPEND** the bacteria cells by pipetting up and down.
5. **ADD** the plasmid solution to the tube.
6. **INCUBATE** on ice for 10 minutes. Just one tube.
7. **TRANSFER** to a warm water bath, incubate for 45 seconds.

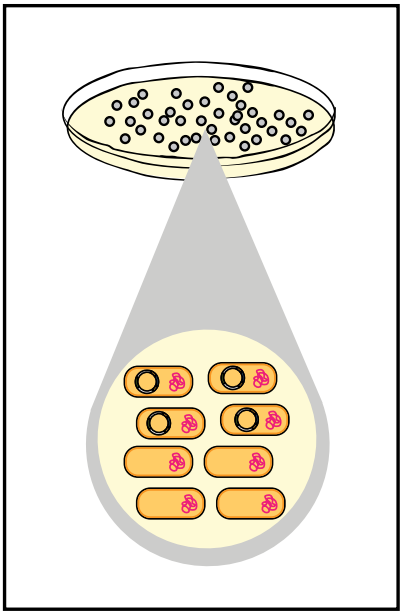
If you are struggling it can help to physically rearrange the tiles.
For a printable version, ask your teacher or visit:
http://edvotek.com/storytiles/transformationprotocol

4. Transformation can be summed up as Exogenous DNA + Competent Cell = Recombinant Organisms but is even better illustrated. Below are 4 tiles illustrating the key steps of transformation. **REORDER** and **NUMBER** them in the upper left corner to depict a full transformation experiment. The first tile in the sequence has already been labeled as an example.



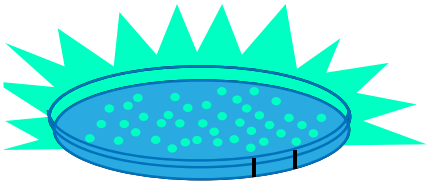
If you are struggling it can help to physically rearrange the tiles.
For a printable version, ask your teacher or visit:
http://edvotek.com/storytiles/transformation

5. Imagine that the plasmid in the above illustration contained a gene that created antibiotic resistance in bacteria. What would happen to each of the eight bacteria pictured below if they were plated on a petri plate containing both nutrients and the antibiotic ampicillin? **MARK** cells with an "X" that you think would not survive.

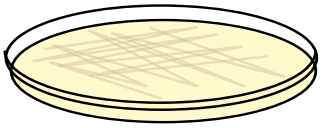


5. Below are the results from a transformation experiment involving a plasmid (pGFP) that contained an antibiotic resistant gene, a green fluorescent gene, and a lac promoter. The latter tells the bacteria to produce a green fluorescent protein (GFP) but only when a specific molecule (IPTG) is present. Three controls were included in this experiment.

   On the "Demonstrates" line, **WRITE** why you think the control was included.

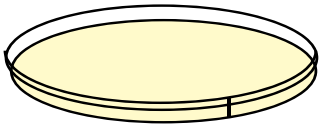


EXAMPLE:
Test Plate: Transformed bacteria grown on agar treated with ampicillin and IPTG.
Results: Colonies of fluorescent bacteria.
Demonstrates: Cells become resistant to ampicillin when transformed with the pGFP. Production of GFP protein is turned on in the presence of IPTG.



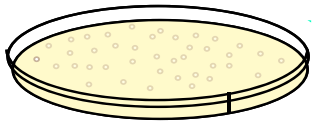
Control 1: Non-transformed bacteria grown on untreated agar.
Results: A lawn of non-fluorescent bacteria.
Demonstrates: _____

_____



Control 2: Non-transformed bacteria grown on agar treated with ampicillin.
Results: No bacteria growth.
Demonstrates: _____

_____



Control 3: Transformed bacteria grown on agar treated with ampicillin.
Results: Colonies of non-fluorescent bacteria.
Demonstrates: _____

_____

6. Transformation is a powerful and popular process because bacteria grow and multiply rapidly. This means that transformed bacteria can quickly be scaled to produce a large quantity of plasmids or proteins. Under ideal conditions the popular microbe *E. coli* doubles its population size every 20 minutes.

   FILL OUT the table below estimating *E.coli* growth following transformation.

Time after Transformation	Number of Transformed Bacteria Cells
0	10
20	
40	
60	
80	
100	
120	
140	
160	

7. Transformations are notoriously inefficient. Often only a few cells will be successfully transformed in an experiment involving thousands of cells and plasmids. Luckily that's often enough! Why is this?

8. The transformation efficiency is defined as the number of transformants generated per µg of supercoiled plasmid DNA used in the transformation reaction. It's a good measure of experimental success.

   CALCULATE the transformation efficiency of the following experiment using the information and formula below.

   Experiment:
   0.05 µg of plasmid DNA were added to competent cells.
   A final volume of 0.50 mL was recovered following the experiment.
   Half (0.25 mL) of this was plated onto antibacterial treated agar and the rest was saved for downstream applications.
   After overnight incubation 10 fluorescent colonies were discovered and counted.

$$\frac{\text{Number of transformants}}{\text{µg of DNA}} \times \frac{\text{Final vol. at recovery (mL)}}{\text{vol. plated (mL)}} = \text{Number of transformants per µg}$$

9. Your transformation experiment resulted in a low transformation efficiency and only a single colony on the plate! While you plan to grow this colony you also want to troubleshoot.

   BRAINSTORM a list of possible causes and tips that can improve your transformation efficiency next time. For some ideas go to **https://www.edvotek.com/transformation-troubleshooting-guide**.

10. Recombinant DNA products surround us and with new techniques like CRISPR the technology continues to grow. Like all powerful technologies, transformation and other forms of genetic engineering can have significant social implications both for good and bad.

    BRAINSTORM positive and negative effects of recombinant technology. **FILL OUT** the table below with your ideas. Include things that have already happened as well as possible future developments.

POSITIVES	NEGATIVES