

Name _____

** Graphics from Lab Bench

A more technical term used to describe this process is **transformation**, which is the uptake of DNA by an organism. Bacterial cells are considered **competent** when they are capable of uptaking DNA.

One way of completing bacterial transformation in the lab is to introduce plasmids into *E. coli*. Plasmids are small circular DNA molecules found in many bacteria. Plasmids can carry one or more genes that encode proteins that confer resistance to certain antibiotics.

Transformation Procedure

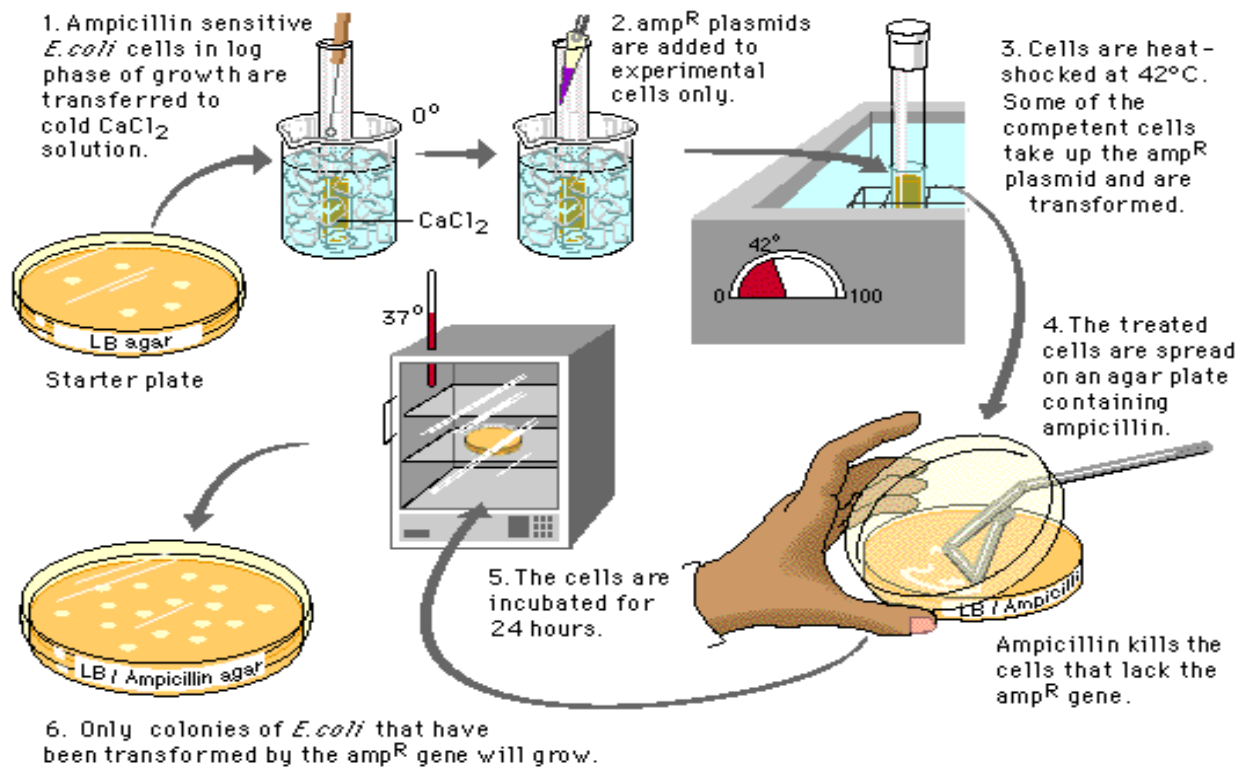
Plasmids that carry the amp^R gene can be used to transform *E. coli* cells that lack this gene. The illustration below outlines the major steps in this procedure.

In this procedure a second group of *E. coli* cells is usually prepared as a control to verify that *E. coli* will not grow on agar with ampicillin unless it is transformed, and that nothing in the procedure itself affects the survival of *E. coli*. The procedure is the same for both groups of cells except in step 2, where you add amp^R plasmids to the experimental cells but not to the control cells.

The purpose of suspending these cells in cold calcium chloride solution is to slow or stop the growth of the bacteria. The subsequent "**heat shock**" incubation period that encourages the uptake of plasmids by the competent cells.

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If there is no ampicillin in the agar, *E. coli* will cover the plate with so many cells it is called a "lawn" of cells. The bacteria require warm, moist conditions with sufficient nutrients in order for this to occur.

Only transformed cells can grow on agar with ampicillin. Since only some of the cells exposed to the amp^R plasmids will actually take them in, only some cells will be transformed. Thus it is possible to see only individual colonies on the plate with the ampicillin that are resistant to the ampicillin.

If none of the sensitive *E. coli* cells have been transformed, nothing will grow on the agar with ampicillin.

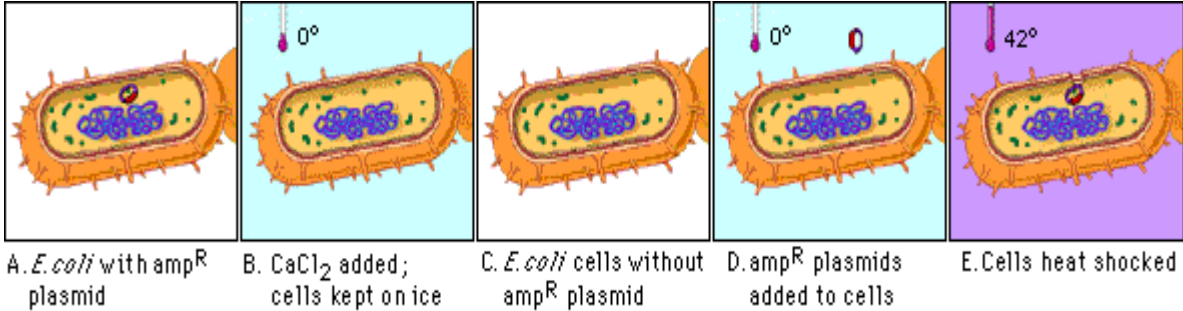
Please place the answers to these using complete sentences on your own paper.

1. What is a plasmid?
2. Identify the control and the independent variable in this described investigation.
3. Ampicillin resistance is the dependent variable in this investigation. Explain why this is the case.

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4. What is the purpose of the cold CaCl₂ and heat shocking at 42 C in this experiment?



5. Place in the correct order the sequence of the above pictured steps in this experiment.
6. What is meant by a “lawn” of cells? Why is it necessary to incubate the cultures to produce this?
7. Transformation efficiency refers in the case to the number of colonies transformed to be ampicillin resistant. List two factors which would influence transformation efficiency.
8. What is an autoclave and why is it used with this experiment?
- 9-10. Explain the experimental results pictured below.

