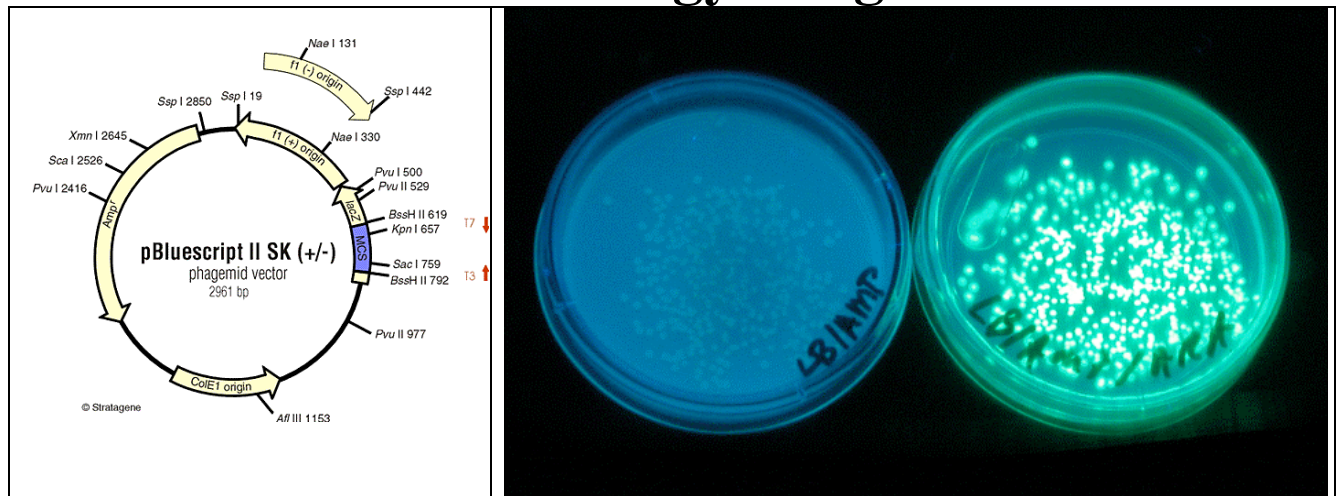


College of the Canyons Biotechnology Program



Bacterial Transformation: Genetic Engineering w/ the pAMP Plasmid: Version B (Transformation)

- ∞ Genetic engineers put new genes into cells. You will insert a gene for drug resistance into bacteria.
- ∞ Chemical and heat treatment helps the gene enter the bacteria.
- ∞ Genetic engineers also design genes such as the gene you are using today.
- ∞ A few selected uses for genetic engineering include: producing hormones such as insulin and human growth hormone, making plants frost resistant, developing plants that do not need fertilizer and making bacteria that “eat” oil slicks.
- ∞ **FLESH EATING** bacteria have acquired their resistance to antibiotic through transformation occurring in hospital and even in patients!

Read the SOP prior to coming to lab & complete the flowchart on page 4. This will permit you to use this flowchart and conduct an expedient/efficient lab.

Did you know that rigorous science training will make you more competitive for ANY type of job? Employers know that students who can tackle hard science will do well with almost ANY challenges presented them. For information on biotechnology and other robust science courses, contact: Jim Wolf, College of the Canyons Biotechnology Director at (661)362-3092 or email:

jim.wolf@canyons.edu

GOT SCIENCE? GET AHEAD!

LAB UNIT 3
TRANSFORMATION LAB
TRANSFORMATION TEAM
Version B

OBJECTIVES:

1. To learn some fundamental techniques in microbiology and molecular biology.
2. To understand what caused the bacteria to become drug resistant.
3. To understand the purpose of using controls in experiments.

Putting new DNA into a cell changes that cell's characteristics and those of its offspring, hence the name transformation. Transformation takes place occasionally by chance in nature when a cell happens to take up and use a piece of DNA that is adrift in the cell's environment, but this is usually a fatal event since foreign DNA could change the cell's ability to survive or immediately kill it. Biologists have learned in the last 30 years to intentionally transform bacteria and yeast into medical "factories". Insulin, for example, no longer must be purified from pig pancreas, and pituitary glands from fresh human brains are no longer required to obtain Human Growth Hormone.

This laboratory exercise involves the transformation of *Escherichia coli* (*E. coli*, a common bacterium in the human large intestine). Normal *E. coli* are killed by the antibiotic ampicillin, one of many penicillin-like drugs that prevent certain bacteria from making cell walls. In contrast, ampicillin has no effect on *E. coli* cells that have previously taken in a relatively small ring of DNA (called a plasmid) containing the code that "tells" the cell how to destroy ampicillin. Ampicillin-sensitive *E. coli* will be transformed into ampicillin-resistant cells during this lab period.

**Biology: Transformation Team
Transformation Lab Materials**

Be careful not to contaminate anything designated sterile!

Materials for each station:

- 3 sterile plastic inoculating loops
- 4 sterile plastic transfer pipettes
- Sterile culture tube with cap
- Sterile culture plate containing Luria Broth agar marked LB. "LB plate"
- Sterile culture plate containing Luria Broth agar and Ampicillin marked LB+A. "LB+A plate"
- Vial of sterile Luria Broth marked "L"
- Vial of Calcium chloride solution marked "Ca"
- Plastic beaker (used to collect loop, pipettes, tubes, and other bacteria-contaminated materials)
- Plastic beaker (used to collect paper trash)
- Insulated cup 3/4 full of crushed ice
- Waterproof marking pen

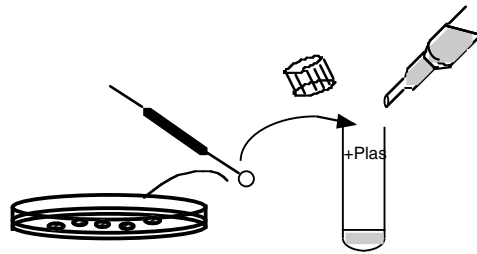
Materials and equipment at a central location in the classroom:

- Culture plate with colonies of ampicillin-sensitive *E. coli* ("Starter culture".)
- Vial of purified ampicillin-resistance plasmid solution marked "pAMP"
- Water bath set at 42°C
- Water bath set at 37°C
- Incubator set at 37°C
- Biohazard bag

TRANSFORMATION LAB: TRANSFORMATION TEAM

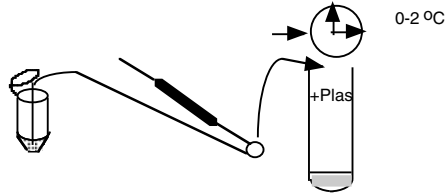
1. WEAKENING THE CELLS

- A. Label the tubes.
- B. Add 250 ul of cold CaCl₂ solution to each. Keep on ice.
- C. Transfer cells to tube. Suspend. Keep on ice.



2. INTRODUCING THE PLASMID

- A. Transfer plasmid "pAMP" to + plasmid tube. Mix.
- B. Keep tube on ice for 15 minutes. Go to step 3 while you wait.

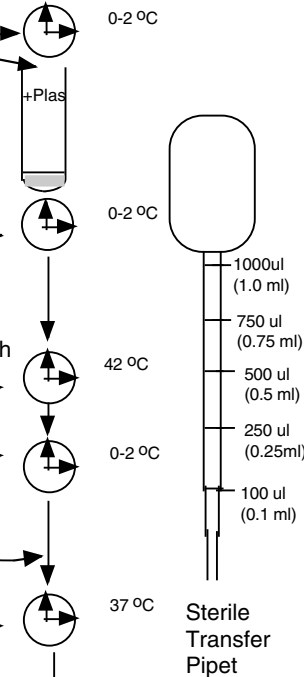


3. HOUSEKEEPING

- a. Label culture plates and make predictions

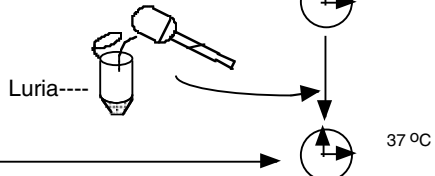
4. EFFECTING THE TRANSFORMATION

- A. **HEAT SHOCK** ! Transfer tubes quickly from ice to 42 °C water bath and incubate for 90 seconds.
- B. - After exactly 90 seconds; Quickly return tubes to ice for at least 1 minute.



5. REGAINING STRENGTH

- A. Add 250 ul of Luria Broth to tube. Gently tap tube to mix.
- B. Incubate for 5 minutes at 37°C

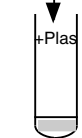
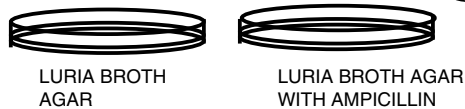


6. PLATING CONTROL CELLS ON SOLID MEDIUM

- A. Transfer 100 ul to LB plate.
- B. Transfer 100 ul LB / Amp plate.
- C. Spread cells.

7. PREPARING TO INCUBATE

- A. Allow medium to set for 5 minutes.
- B. Invert plates.
- C. Stack and tape plates
- D. Incubate plates overnight at 37 °C



TRANSFORMATION TEAM PROCEDURE

Check off each step as it is completed.

1. WEAKENING THE CELLS SO THEY MORE READILY TAKE IN "FOREIGN" DNA

- A. Label the test tube with group name and "+ plasmid". (pronounced "plus plasmid")
- B. Add 250 μ l ice-cold calcium chloride solution (CaCl_2) to test tube using sterile Pipette 1 transfer pipette.
Replace cap. Put tube on ice.
- C. Transfer *E. coli* cells from starter culture to test tube as follows:
 - i. Using sterile inoculating loop, gently scrape off one or two 3 mm diameter colonies but be careful not to scrape off any any agar. (Loop I)
 - ii. Submerge the loop in the CaCl_2 solution in the test tube and twirl and tap vigorously to dislodge the mass of cells. Hold test tube up to the light to make sure cells come off loop.
 - iii. Quickly suspend cells by pipetting solution in and out several times with sterile transfer pipet. Hold tube up to light to check for clumps. (Pipette 2)
 - iv. Replace cap on test tube and return it to ice.

Question: Why must sterile technique be used even after bacteria are introduced to the tube?

2. INTRODUCING THE PLASMID (ring of DNA) CONTAINING AMPICILLIN-RESISTANCE TO THE SUSPENDED BACTERIA

- A. Add one loopful of ampicillin resistance plasmid solution (0.005 g/l) Loop II
to the "+ plasmid" tube using a sterile inoculating loop. One loopful (when the solution forms a "bubble" across loop opening) is approximately 10 μ l of solution. Submerge loopful of plasmid solution directly into "+ plasmid" cell suspension and twirl and swish loop to mix. Return tube to ice.
- B. Keep tube on ice for 15 minutes. Go on to step 3 below while you wait.

Questions:

- 1. *Escherichia coli* (*E. coli*) is part of the normal symbiotic bacterial "ecosystem" in the human large intestine. Is a test tube containing ice cold calcium chloride solution similar to the normal environment for this bacterium? _____ List at least three conditions that are different from the large intestine.

- 2. Other students in the room are doing the control experiment. No DNA will be added to their cells, yet they are soaking the cells in cold CaCl_2 solution. Why do their cells need to go through this "weakening" step?

3. **GENERAL HOUSEKEEPING: Label the culture plates while you wait:**
(Write labels on plate bottom so they do not obscure the center area of plate.)

A. Label "LB" plate as shown on page 4: Group name and "+ plasmid cells on Luria broth agar"

B. Label "LB+A" plate as shown on page 4: Group name and "+ plasmid cells on Luria broth agar with Amp"

ALSO WHILE YOU WAIT:

Predict how many cell colonies will grow on each of the plates.

Your two plates will be inoculated with cells that we hope have been transformed with the ampicillin resistance plasmid.

Predict colony number:

On Luria broth agar plate: None Few Many

On Luria broth agar with ampicillin plate: None Few Many

Explain your predictions.

4. **CREATING THE CONDITIONS FOR MAKING THE PLASMIDS ENTER THE CELLS**

A. Move test tube suddenly from the ice bath to the 42°C water bath. Leave it at 42°C for 90 seconds.

B. Move test tube suddenly from the water bath back to the ice. Keep on ice for at least one minute.

5. **ALLOWING THE CELLS TO REGAIN THEIR STRENGTH AND START TO MULTIPLY**

A. Add 250 μ l sterile Luria Broth to the test tube using sterile transfer pipette. (Pipette 3)

Shake and tap test tube to mix.

B. Move test tube to 37°C water bath and incubate for 5 minutes.

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6. **RETURNING THE CELLS TO SOLID MEDIA WITH AND WITHOUT ADDED ANTIBIOTIC**

A. Transfer 100 μ l of cell suspension to plate marked "+ plasmid cells on Luria (Pipette 4)

Broth agar". Use sterile transfer pipette.

B. Transfer 100 μ l of cell suspension to plate marked "+ plasmid cells on Luria Broth agar plus Amp". Use the same sterile transfer pipette. (Pipette 4)

- i. Lifting the lid of the "+ plasmid cells on Luria Broth agar" plate, sweep the drop of cell suspension with a loop to distribute it over the surface of the plate. (Loop 4)
- ii. Repeat to spread cells on "+ plasmid on Luria broth agar with Ampicillin" plate. Use the same loop. (Loop 4)
- (again)

Question: What is the advantage of adding Luria broth and incubating the cell suspension at 37°C before spreading it on the Luria broth agar?

7. PREPARING TO INCUBATE INOCULATED PLATES

- A. Allow liquid on plates to set for five minutes before moving them.
- B. Invert plates (so the agar side is up).
- C. Stack your group's plates and use tape to keep them together.
- D. Place plates in incubator at 37°C for 24 hours.

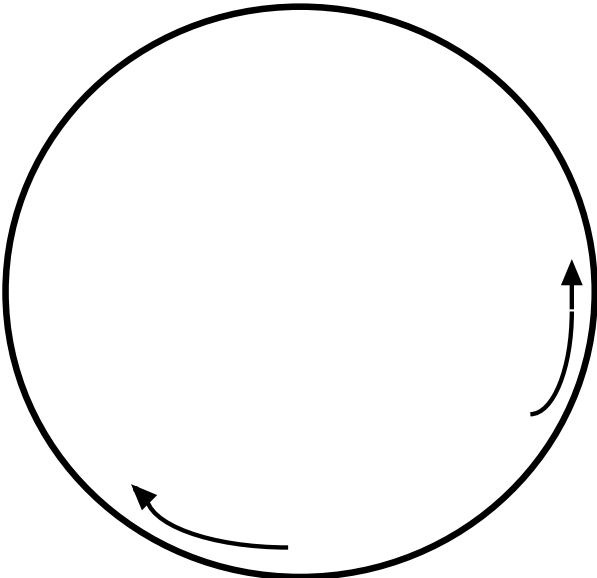
DRAW IN COLONIES

Number of colonies _____

How do results compare to predictions made in step 3?

Explanation _____

OBSERVATIONS



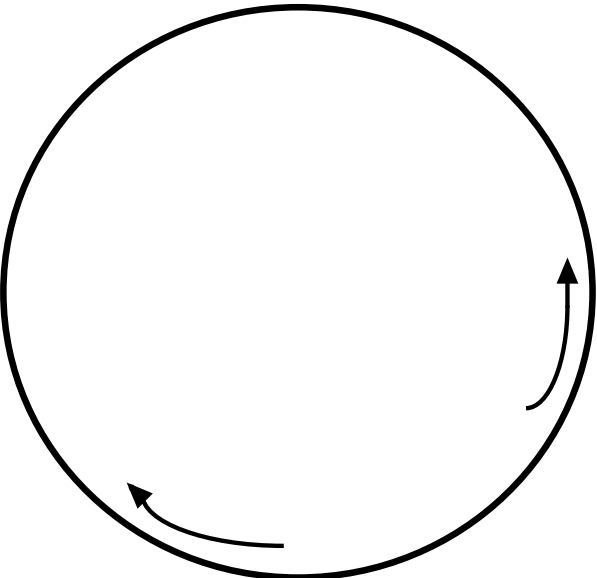
On the above Petri dish write the following information in a circle around the perimeter of the bottom: +plamsid cells on Luria broth agar and your names.

DRAW IN COLONIES

Number of colonies _____

How do results compare to predictions made in step 3?

Explanation _____



On the above Petri dish write the following information in a circle around the perimeter of the bottom: +plamsid cells on Luria broth agar with ampicillin and your names.

OBTAIN COLONY NUMBERS FROM YOUR "PARTNER CONTROL TEAM" AND ENTER BELOW. Then make a comparison for each pair shown below.

- a. Is number of colonies on the two plates similar or different?
- b. Give reason(s) for similarity or difference.

