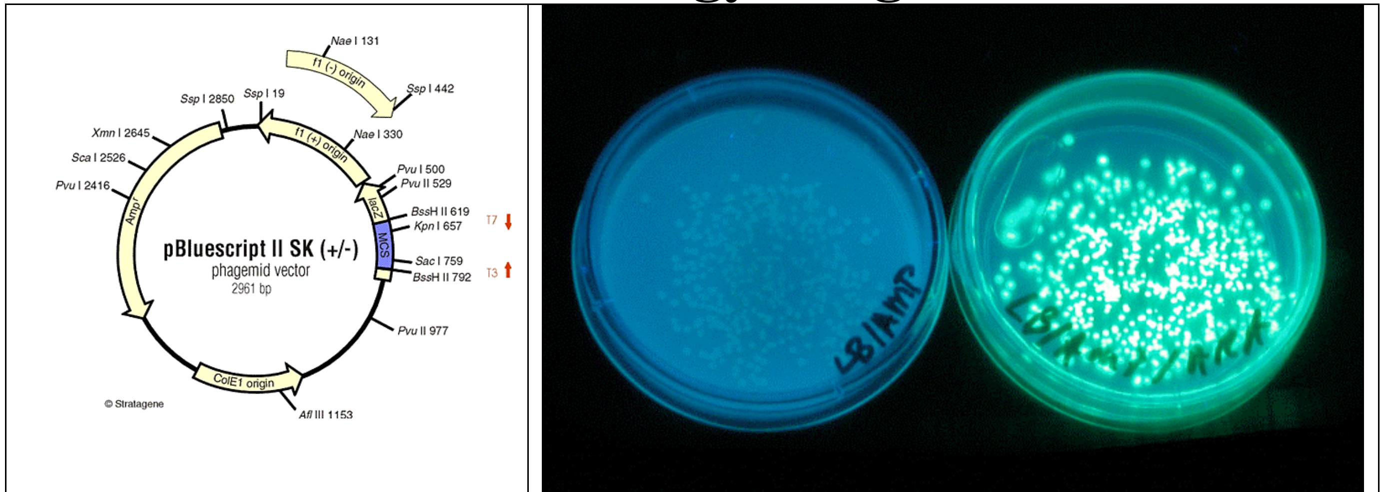
College of the Canyons
Biotechnology Program



Bacterial Transformation: Genetic Engineering w/ the pAMP Plasmid: Version A

- **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
Version A

OBJECTIVES:

1. To introduce fundamental techniques in microbiology and molecular biology.
2. To understand the principles of bacterial transformation.
3. To understand the mechanism of recombinant DNA or gene transfer using plasmid vectors.

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, however this is usually fatal since the foreign DNA could change the cell's ability to survive. Biologists have learned in the last 30 years to intentionally transform bacteria and yeast into medical "factories". Insulin, which was once purified from the pancreas of a pig can now be produced by genetically engineered bacteria. Transformation methods of putting foreign DNA into plant and animal cells are not as useful for mass production of medicines since these cells grow much more slowly than bacteria and fungi.

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 *genetic* code that "tells" the cell how to destroy ampicillin. Ampicillin-sensitive *E. coli* will be transformed into ampicillin-resistant cells during this lab period.

Transformation Lab
Version A Checklist

Be careful not to contaminate anything designated sterile!

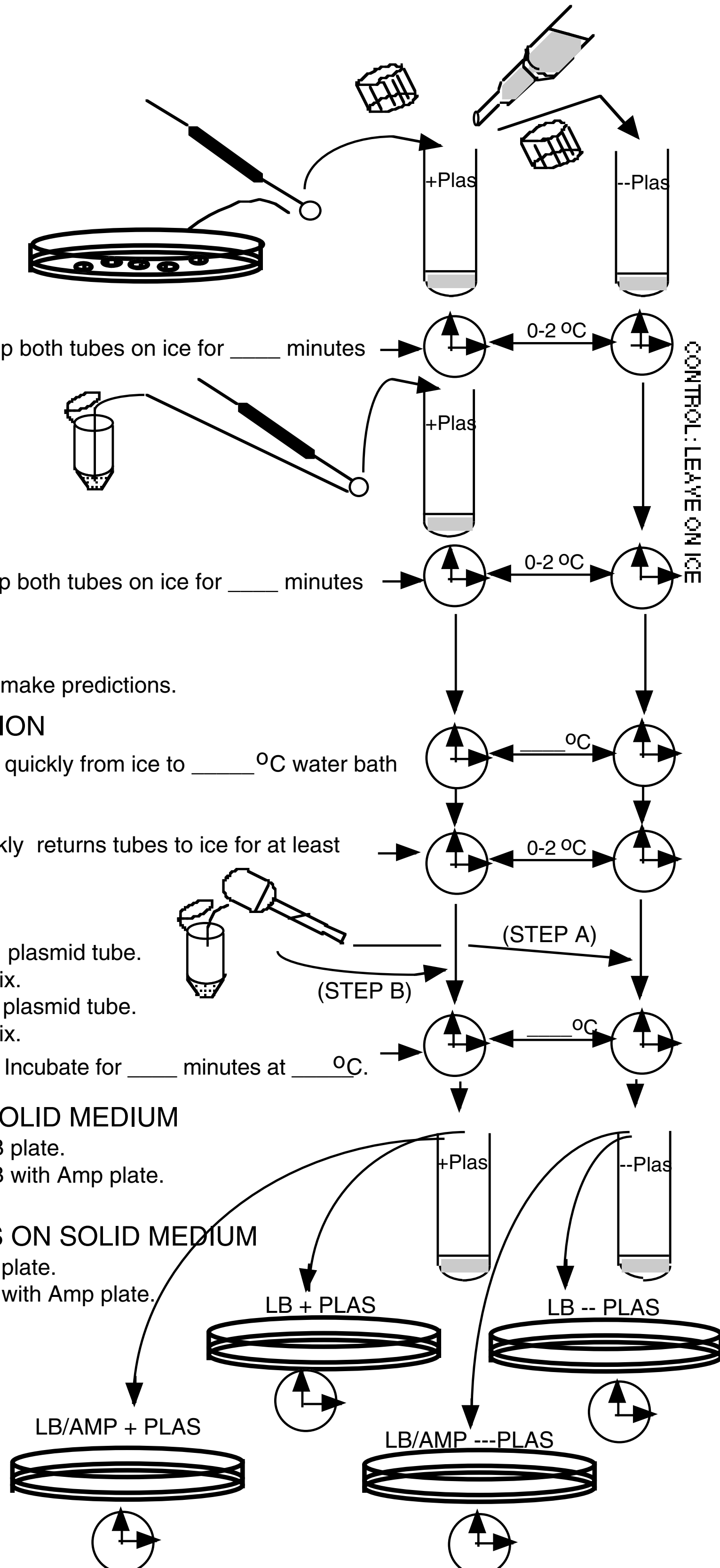
Materials for each station:

- ☐ 5 sterile plastic inoculating loops
- ☐ 8 sterile plastic transfer pipettes
- ☐ 2 sterile culture tubes with caps
- ☐ 2 sterile culture plates containing Luria broth agar marked "LB"
- ☐ 2 sterile culture plates containing Luria broth agar and ampicillin marked "LB+A"
- ☐ Vial of sterile Luria broth marked "L"
- ☐ Vial of 50 mM calcium chloride solution marked “Ca”
- ☐ Insulated cup 3/4 full of crushed ice
- ☐ Plastic beaker (used to collect loops, pipettes, tubes, and other bacteria-contaminated materials)
- ☐ Plastic beaker (used to collect paper trash)
- ☐ Waterproof marking pen

Materials and equipment at a central location in the classroom:

- ☐ Culture plate with colonies of ampicillin-sensitive *E. coli* marked "Starter culture".
- ☐ Vial of purified ampicillin-resistance plasmid solution (0.005 mg/μl) marked “pAMP”
- ☐ Water bath set at 42°C
- ☐ Water bath set at 37°C
- ☐ Incubator set at 37°C
- ☐ Biohazard bag

TRANSFORMATION



1. WEAKENING THE CELLS

A. Label the tubes.

B. Add _____ ul of cold $CaCl_2$ solution to each. Keep on ice.

C. Transfer colony to **+** plasmid tube. Suspend. Place on ice.

D. Transfer colony to --plasmid tube. Suspend. Place on ice.

E. ⟶ Keep both tubes on ice for ____ minutes

2. INTRODUCING THE PLASMID

A. Transfer plasmid Pamp to + plasmid tube. Mix. Place on ice.

B. ⟶ Keep both tubes on ice for ____ minutes

3. HOUSEKEEPING

Label petri dishes while you wait and make predictions.

4. EFFECTING THE TRANSFOMRATION

A. ---------->>**HEAT SHOCK** ! Transfer tubes quickly from ice to _____ ^oC water bath and incubate for _____ seconds.

B. --------->> After exactly ____ seconds: Quickly returns tubes to ice for at least ____ minutes.

5. REGAINING STRENGTH

A. ---------->> Add _____ul of Luria Broth to**--** plasmid tube. Gently tap tube to mix.

B. ---------->> Add _____ul of Luria Broth to**+** plasmid tube. Gently tap tube to mix.

C. --> Incubate for ____ minutes at _____ ^oC.

6. PLATING CONTROL CELLS ON SOLID MEDIUM

A. Transfer _____ ul of **---** plasmid cells to LB plate.

B. Transfer _____ ul of **---** plasmid cells to LB with Amp plate.

C. Spread cells.

7. PLATING TRANSFORMED CELLS ON SOLID MEDIUM

A. Transfer _____ ul of **+** plasmid cells to LB plate.

B. Transfer _____ ul of **+** plasmid cells to LB with Amp plate.

C. Spread cells.

8. PREPARING TO INCUBATE

A. Allow medium to set for ____ minutes.

B. Invert plates.

C. Stack and tape plates

D. Incubate plates overnight at _____ ^oC

Version A BIOLOGY TRANSFORMATION PROCEDURE

Check off each step as it is completed.

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

A. Label the two test tubes with team name. Label one tube "+ plasmid" and the other "- plasmid". ☐

B. Add 250 l ice-cold 50 mM $CaCl_2$ to each test tube using sterile transfer pipette. Replace caps. Put tubes on ice. ☐ Pipette 1

C. Transfer one or two 3mm diameter colonies of *E. coli* cells from starter culture to "+ plasmid" tube as follows:

i. Using sterile inoculating loop, gently scrape off the cells but be careful not to scrape off any agar. A 3mm diameter colony is the size of this capital O. ☐ Loop I

ii. Submerge the loop in the $CaCl_2$ solution in the "+ plasmid" tube and twirl and tap vigorously to dislodge the mass of cells. Hold 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 "+ plasmid" tube and return it to ice. ☐

D. Transfer *E. coli* cells to "- plasmid" tube using same steps as those described in C i-iv. ☐ Loop 2/ Pipette 3

E. Keep both test tubes on ice for at least 1 minute. ☐

Questions:

1. Why must sterile technique be used even after bacteria are introduced to the tube?

2. What makes the $CaCl_2$ solution quite different from the normal conditions inside the large intestine enjoyed by *E. coli*? Be specific about at least three differences.

3. In what ways could $CaCl_2$ interact with the cell membrane of the cells? Give at least two specific examples of possible interactions.

2. INTRODUCING THE PLASMID CONTAINING AMPICILLIN-RESISTANCE TO THE SUSPENDED BACTERIA

A. Add one loopful of Ampicillin Resistance Plasmid solution (0.005 mg/ml) to the "+ plasmid" tube using a sterile inoculating loop. One loopful (when the solution forms a "bubble" across loop opening) is approximately 10 μl of solution. Submerge ☐ Loop III

the loopful of plasmid solution directly into "+ plasmid" cell suspension and twirl and swish loop to mix. Return tube to ice.

B. Keep both tubes on ice for 15 minutes. Answer questions, then go to step 3 while you wait. ☐

Questions:

1. What negatively charged functional groups are plentiful on DNA?
2. If Ca^{++} interacts with the negatively charged phosphate groups on DNA, what change(s) might take place in the overall arrangement of the plasmid in the presence of plentiful Ca^{++} ions?

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" plates with group name and: ☐

i. On one: "+ plasmid cells on Luria broth agar" ☐

ii. On the other: "- plasmid cells on Luria broth agar" ☐

B. Label "LB+A" plates with group name and: ☐

i. On one: "+ plasmid cells on Luria broth agar with amp" ☐

ii. On the other: "- plasmid cells on Luria broth agar with amp" ☐

ALSO WHILE YOU WAIT:
Predict how many cell colonies will grow on each of the four plates.

a. Two plates will be inoculated with cells that we hope have been transformed with the Ampicillin-Resistance Plasmid. Predict colony number by circling:

On Luria broth agar plate: None Few Many

On Luria broth agar with Ampicillin plate: None Few Many

b. Two plates will be inoculated with cells that have not been transformed (although they have gone through all the same processes except for the addition of plasmid). Predict colony number:
On Luria broth agar plate: None Few Many

On Luria broth with ampicillin plate: None Few Many

4. EFFECTING THE TRANSFORMATION-- MAKING THE PLASMIDS ENTER THE CELLS

A. Transfer both test tubes <u>suddenly</u> from the ice bath to the 42°C water bath. Leave them at 42°C for 90 seconds. ☐

B. Transfer both test tubes <u>suddenly</u> 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 l sterile Luria broth to the "- plasmid" tube using sterile transfer ☐ Pipette 4

pipette. Shake and tap tube to mix.

B. Add 250 l sterile Luria broth to the "+ plasmid" tube using sterile transfer pipette. Shake and tap tube to mix. ☐ Pipette 5

C. Incubate both tubes in 37°C water bath for 5 minutes. ☐

6. RETURNING RECOVERED <u>CONTROL CELLS</u> TO SOLID MEDIUM WITH AND WITHOUT ADDED ANTIBIOTIC

A. Transfer 100 l of "- plasmid" cell suspension to plate marked "- plasmid cells on Luria broth". Use sterile transfer pipette. ☐ Pipette 6

B. Transfer 100 l of "- plasmid" cell suspension to plate marked "- plasmid cells on Luria broth plus Amp". Use the same sterile transfer pipette. ☐ Pipette 6 (again)

C. Spread cells over surface of plates without delay.

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)

7. PLATING RECOVERED <u>TRANSFORMED CELLS</u> ON SOLID MEDIA WITH AND WITHOUT ADDED ANTIBIOTIC

A. Transfer 100 l of "+ plasmid" tube to plate marked "+ plasmid cells on Luria broth agar". Use sterile transfer pipette. ☐ Pipette 7

B. Transfer 100 l of "+ plasmid" tube to plate marked "+ plasmid cells on Luria broth agar plus Amp". Use the same sterile transfer pipette. ☐ Pipette 7 (again)

C. Spread cells over surface of plates without delay (same method as in 6 C.) ☐ Loop 5

Question: Why did you make the transfer of control cells in step 6 before doing anything with the transformed cells in step 7?

8. PREPARING TO INCUBATE INOCULATED PLATES

A. Allow liquid on plates to set for 5 minutes before inverting them. ☐

B. Invert plates (so the medium side is up). ☐

C. Stack and tape (or rubber-band) your group's four plates. ☐

D. Incubate plates at 37°C for 24 hours. ☐

CLEAN UP:

BE SURE ALL USED LOOPS, PIPETTES, AND TUBES ARE IN THE BIOLOGICAL WASTE BEAKER.

PAPER WRAPPINGS NOT CONTAMINATED WITH BACTERIA GO IN TRASH BEAKER.

WASH DOWN LAB BENCH. THEN WASH YOUR HANDS THOROUGHLY!!

WASH HANDS AGAIN BEFORE EATING!!

OBSERVATIONS

Observe the plates (do not open the lids) and record number of colonies on each. If colonies are extremely plentiful, only count one quarter of the plate and quadruple. If cell growth is too dense to distinguish individual colonies, record "lawn".

CELLS	LURIA BROTH AGAR	LURIA BROTH AGAR WITH AMPICILLIN
+ PLASMID	# of colonies ____________ How do results compare to prediction (step 3)?	# of colonies ____________ How do results compare to prediction (step 3)?
- PLASMID	# of colonies ____________ How do results compare to prediction (step 3)?	# of colonies ____________ How do results compare to prediction (step 3)?

PUT THE PLATES AWAY, THEN WASH THE BENCH, THEN WASH YOUR HANDS!

Question: Each colony grew from how many cell(s) present at that location on the plate before incubation?

CONCLUSIONS AND CALCULATIONS

I. Compare the number of colonies that grew on each pair of plates listed below.
  a. Are the numbers about the same or quite different for the two plates being compared?
  b. Give an explanation for the similarity or difference in colony number

1. "- plasmid cells on Luria broth agar" compared to "+ plasmid cells on Luria broth agar"
   a.
   b.

2. "- plasmid cells on Luria broth agar with ampicillin" compared to "- plasmid cells on Luria broth agar"
   a.
   b.

3. "+ plasmid cells on Luria broth agar with ampicillin" compared to "- plasmid cells on Luria broth agar with ampicillin
   a.
   b.

4. "+ plasmid cells on Luria broth agar with ampicillin" compared to "+ plasmid cells on Luria broth agar"
   a.
   b.

CALCULATION OF TRANSFORMATION EFFICIENCY

Show all work. Include word equations, show substitutions, include units!

a. What was the total mass of plasmid DNA added to the cell suspension? (See list of materials for concentration of original solution.)

b. What was the volume of the cell suspension at the conclusion of the recovery step (step 5)?

c. What fraction of the cell suspension final volume (from b) was transferred to each plate for incubation on solid medium?

d. What was the mass of plasmid DNA that was transferred to one plate?

e. Calculate the number of colonies transformed per microgram of plasmid DNA. Use the colony count from your observations and the values computed above to solve this problem. You are calculating the transformation efficiency.

Final Discussion: List and discuss the numerous factors that could influence transformation efficiency.