**Practical 10. Study of transformed bacteria on the basis of antibiotic resistance**

You will provide with a plasmid containing one or more genes. The plasmid likely will contain the gene for resistance to ampicillin (pAMP), an antibiotic that is lethal to many bacteria, including *E. coli* cells. This transformation procedure involves the following three main steps to introduce the plasmid DNA into the *E. coli* cells and to provide an environment for the cells to express their newly acquired genes:

**1.** Adding CaCl2

**2.** “Heat shocking” the cells

**3.** Incubating the cells in nutrient broth for a short time before plating them on agar

Materials required

**Your Workstation**

* *E. coli* starter plate
* Poured agar plates most likely the following:
* 2 LB agar plates
* 2 LB/amp agar (LB agar containing ampicillin) plates
* Transformation solution (CaCl2, pH 6.1) kept *ice cold*
* LB nutrient broth
* Sterile inoculation loops
* 100–1000 μL sterile bulb pipettes
* 1–10 μL micropipettes with sterile tips
* Microcentrifuge tubes
* Microcentrifuge tube holder/float
* Container full of crushed ice
* Marking pen

**Common Workstation**

**•** DNA plasmid (most likely pAMP at 0.005 μg/μL)

**•** 42°C water bath and thermometer

**•** 37°C incubator

**•** 20 μL adjustable-volume micropipettes and tips (optional)

**•** 10% household bleach

**•** Biohazardous waste disposal bags

**•** Masking or lab tape

**Procedure**

In your lab notebook, record data, answers to questions, and any questions that arise during this part of the activity.

**Step 1**

Form lab teams, as instructed by your teacher. Familiarize yourself with sterile technique, materials and lab equipment, and safety procedures for handling bacteria and decontaminating the work area.

**Step 2** Label one closed microcentrifuge tube (micro test tube) “+ plasmid” and one tube “-plasmid.” (What do the “+” and “-” symbols mean?) Label both tubes with your group’s number (e.g., G2), and place them in the microcentrifuge tube holder/float.

**Step 3** Carefully open the tubes and, using a 100–1000 μL bulb pipette with a sterile tip, transfer 250 μL of the ice cold transformation solution (CaCl2) into each tube



**Step 4** Place both tubes on (into) the ice.

**Step 5** Use a sterile inoculation loop to pick up a single colony of bacteria from your

starter plate. Be careful not to scrape off any agar from the plate. Pick up the “+ plasmid”

tube and immerse the loop into the CaCl2 solution (transforming solution) at the bottom

of the tube. Spin the loop between your index finger and thumb until the entire colony is

dispersed in the solution. Appropriately discard the loop.

**Step 6** Use a new sterile 100–1,000 μL micropipette to repeatedly pulse the cells in

solution to thoroughly resuspend the cells. (Note that the clear transformation solution

will become cloudy as the *E. coli* cells are suspended.) Place the tube back on the ice.

**Step 7** Using a new sterile inoculation loop, repeat Steps 5 and 6 for the “- plasmid” tube.

**CAUTION: Keep your nose and mouth away from the tip end when pipetting**

**suspension culture to avoid inhaling any aerosol!**

**Step 8** Using a 1–10 μL micropipette with a sterile tip, transfer 10 μL of the plasmid

solution *directly into the* E. coli *suspension* in the “+ plasmid” tube. Tap tube with a finger

to mix, but avoid making bubbles in the suspension or splashing the suspension up the

sides of the tube. Do not add the plasmid solution into the “- plasmid” tube! (Why not?)

**Step 9** Incubate both tubes (“+ plasmid” and “- plasmid”) on ice for 10 minutes. Make

sure the bottom of the tubes make contact with the ice.

**Step 10** While the tubes are sitting on ice, label each of your agar plates on the bottom

(not the lid) as directed by your teacher.

**Step 11** Following the 10-minute incubation at 0°C, remove the tubes from the ice and

“heat shock” the cells in the tubes. It is critical that the cells receive a sharp and distinct

shock! Make sure the tubes are closed tightly! Place the tubes into a test tube holder/

float, and dunk the tubes into the water bath, set at 42°C, for exactly 50 seconds. Make

sure to push the tubes all the way down in the holder so that the bottom of the tubes

with the suspension makes contact with the warm water.

**Step 12** When the 50 seconds have passed, place both tubes back on ice. For best

transformation results, the change from 0°C to 42°C and then back to 0°C must be rapid.

Incubate the tubes on ice for an additional two minutes.

**Step 13** Remove the holder containing the tubes from the ice and place on the lab counter.

Using a 100–1,000 μL micropipette with sterile tip, transfer 250 μL of LB nutrient broth

to the “+ plasmid” tube. Close the tube and gently tap with your finger to mix. Repeat

with a new sterile micropipette for the “- plasmid” tube.

**Step 14** Incubate each tube for 10 minutes at room temperature.

**Step 15** Use a 10–1,000 μL micropipette with sterile tip to transfer 100 μL of the

transformation (“+ plasmid”) and control (“- plasmid”) suspensions onto the appropriate

LB and LB/Amp plates. Be sure to use a separate pipette for each of the four transfers.

**Step 16** Using a new sterile inoculation loop for each plate, spread the suspensions evenly

around the surface of the agar by quickly “skating” the flat surface of the sterile loop

back and forth across the plate surface (Figure 3). Do not poke or make gashes in the

agar! Your teacher might suggest that you use small sterile glass beads to spread the

suspensions by gently rocking the beads across the surface of the agar. Allow the plates to

set for 10 minutes.



**Step 17** Stack your plates and tape them together. Place the stack upside down in the 37°C

incubator for 24 hours or as per instructed by your teacher.

**Calculating Transformation Efficiency**

**Transformation efficiency** = Total number of colonies growing on the agar plate

 Amount of DNA spread on the agar plate (in ug)