Practical activity (1)

# Bacterial Transformation

## (Student version)

## I. Background

Nowadays, many biomolecules can be produced by using transformed microorganisms. For example, patients suffering from diabetes mellitus require injections of insulin to control their blood glucose levels. As a large amount of insulin is needed by these patients all over the world, insulin is mass-produced from bacteria, such as *Escherichia coli* (*E. coli*) and *Saccharomyces cerevisiae* (*S. cerevisiae*), that are transformed with the human insulin gene. Hence, bacterial transformation is an important and useful technique in the field of biotechnology.

### Scenario

Suppose you are a student intern working in a biotechnology company. Your supervisor left some materials and a memo on your desk:

Mary,

We are going to mass produce amylase for our new "DIY fermentation startup kit", which will be available on the market in the coming autumn. However, we have just found out that we do not have enough amylase for the new product. To lower the cost and maintain a stable supply of amylase, we plan to produce amylase ourselves. Please follow the instructions below to transform the bacterium *E. coli* with the amylase gene to produce amylase. The materials needed are in the fridge and on the bench in the laboratory.

Anne
Supervisor

## II. Guiding questions about the design of the experiment

1. Naturally, the bacterium *E. coli* does not produce amylase. How can we turn it into an amylase-producing microbe?
2. A recombinant plasmid called "pAmylase" will be used in this experiment. Which types of genes should be found in this recombinant plasmid?
3. How do you know whether the bacteria have successfully picked up the recombinant plasmid? What kind of nutrient agar plate should be used?

## III. Objectives of the experiment

1. To transform the non-pathogenic *E. coli* with the recombinant plasmid "pAmylase", which contains a gene for amylase production and a gene for ampicillin resistance; and
2. To demonstrate the successful transformation of the *E. coli* cells.

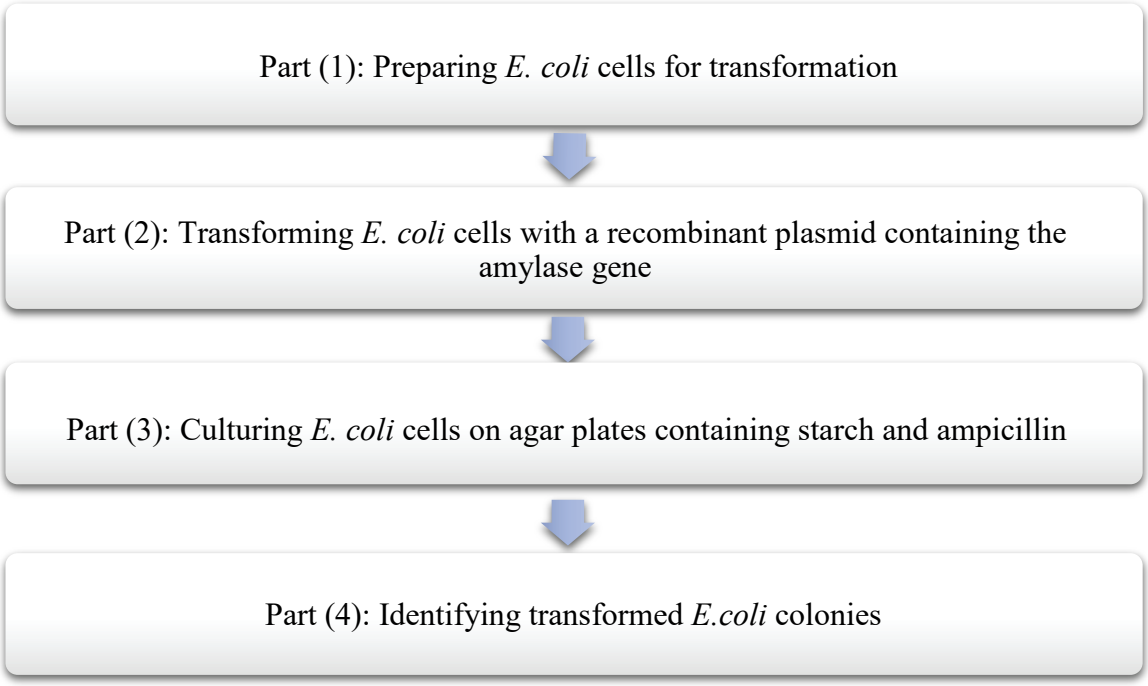
## IV. Expected Learning Outcomes

Upon completion of the activities, students should be able to:

1. recognise the potential applications of bacterial transformation;
2. apply aseptic techniques in bacterial culture;
3. perform fundamental bacterial transformation; and
4. identify the transformed bacteria.

## V. The experiment

### A. Overview



## B. Part (1) of the experiment: Preparing *E. coli* cells for transformation

### a) Equipment and materials (per group)

Equipment

- Bunsen burner × 1
- Spark lighter × 1

Materials

- Agar plate with *E. coli* colonies × 1 (per class)
- Culture tube with 5 ml LB broth* × 1
- Inoculating loop × 1
- Permanent marker × 1
- 70% ethanol in spray bottle × 1
- Paper towel × 1 box
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach × 1

*Note: Luria Bertani (LB) broth is a kind of nutrient broth with salt content more suitable for the growth of *E. coli* than the conventional media.

### b) Safety precautions

- Wear a laboratory gown.
- Tie up long hair.
- Wear gloves during the experiment.
- Wash hands to remove all possible contamination before and after the experiment.
- Make sure ethanol is fully vaporised before approaching any flame.
- Dispose of or disinfect all materials properly after each experiment.

### c) Procedure

1. Disinfect the benchtop and gloved hands with 70% ethanol#.

**#Caution: Keep away from fire! DO NOT light the flame until the ethanol has been completely vaporised!**

2. Label the 5 ml LB broth culture tube "*E. coli*", with the date of the experiment, your class, and group number (Fig. 1).

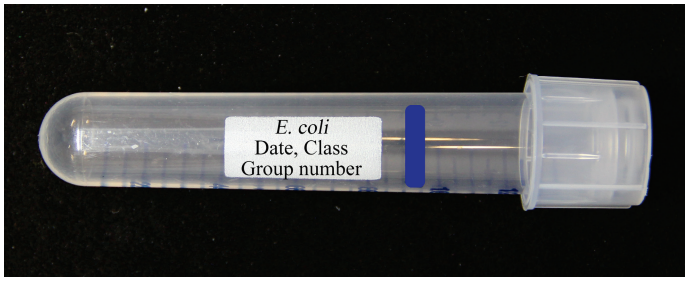



Fig. 1

3. Light a Bunsen burner.
4. Flame the inoculating loop to red hot (Fig. 2) and allow it to cool down*.

*It is essential to flame the metal inoculating loop to red hot, to eliminate any previous bacterial residue and to prevent contamination of the current sample.

It is also important to cool the loop, as insufficient cooling kills the inoculated bacteria.

If a sterile plastic disposable inoculating loop is provided, flaming is not required.

Use a new inoculating loop when handling different microbial samples.

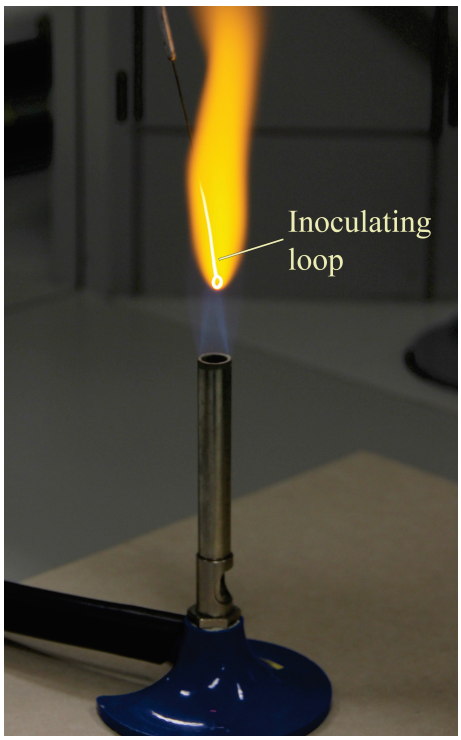



Fig. 2

5. Using the aseptic technique#, pick a single colony with the sterilised inoculating loop from the *E. coli* agar plate.

#Note: Please refer to Steps 3–4 of Appendix 1 for details of the aseptic technique.

6. Raise the lid of the agar plate at about 45°^ (Fig. 3). Put the inoculating loop onto the agar.

^Note: This is to minimize the exposure of agar to the atmosphere.

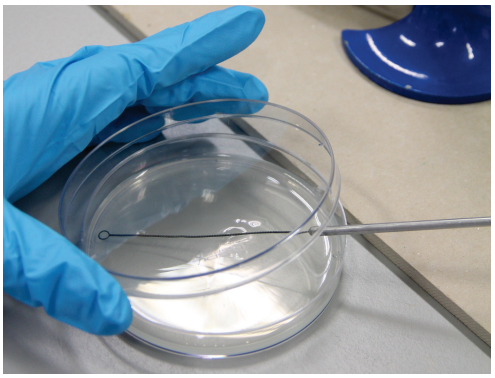


Fig. 3

7. Transfer the loopful of the *E. coli* colony directly into the 5 ml culture tube labelled "*E. coli*" (Fig. 4).

Fig. 4

8. Cap the culture tube. Finger-flick the broth 2–3 times for a proper re-suspension.
9. Switch off the Bunsen burner.
10. Incubate the culture tube at room temperature* or in an incubator set at 25−30°C for 48 hours.

> *Note: Room temperature may vary, depending on the ambient conditions of the laboratory environment. Hence, the incubation time may also vary.

11. After the suggested incubation time, store the tube at 4°C until lab session 2.

## C. Part (2) of the experiment: Transforming *E. coli* cells with a recombinant plasmid containing the amylase gene

### a) Equipment and materials (per group)

Equipment

- Micro-centrifuge for 2.0 ml micro-centrifuge tubes × 1 (per class)
- Water bath (42°C) × 1 (per class)
- Ice bath × 1
- Timer × 1
- Micropipettes (P1000, P200, and P20) and sterile tips

Materials

- *E. coli* broth culture from Part (1) × 1
- Competent buffer (450 µl) × 1
- pAmylase plasmid (0.08 µg/µl, 7 µl) × 1
- Sterile distilled water (20 µl) × 1
- LB broth (550 µl) × 1
- 1.75 ml micro-centrifuge tube × 2

- Permanent marker × 1
- 70% ethanol in spray bottle × 1
- Paper towel × 1 box
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach × 1

## b) Safety precautions

- Wear a laboratory gown.
- Tie up long hair.
- Wear gloves during the experiment.
- Wash hands to remove all possible contamination before and after the experiment.
- Make sure ethanol is fully vaporised before approaching any flame.
- Dispose of or disinfect all materials properly after each experiment.

## c) Procedures

*Replace LB broth with competent buffer*

1. Disinfect the benchtop and gloved hands with 70% ethanol*.

***Caution: Keep away from fire! DO NOT light the flame until the ethanol has been completely vaporised!**

2. Label 2 micro-centrifuge tubes with your group number. Label one of them "pAmy" and the other one "-C" (for the negative control) (Fig. 5).

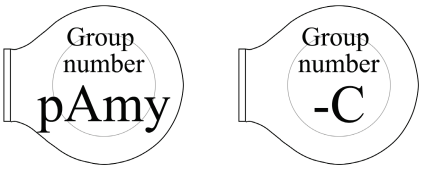



Fig. 5

3. Finger-flick (Fig. 6) the *E. coli* broth culture tube to re-suspend the cells. Use a P1000 micropipette# and a new sterile tip to transfer 1.5 ml of this culture to each of the labelled micro-centrifuge tubes.

#Note: The maximum volume of P1000 is 1000 µl or 1 ml. To transfer a volume of 1.5 ml, 2 draws of 750 µl are needed.

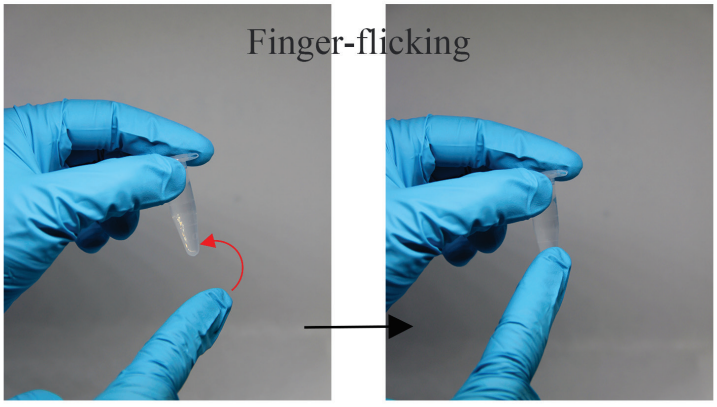



Fig. 6

4. Centrifuge the 2 tubes at 5,000 rpm for 3 minutes to pellet the *E. coli* cells to the bottom (Fig. 7). Carefully pour off and discard the supernatant in the designated disposal container with 10% chlorine bleach. **Save the cell pellet,** which is located at the bottom of the tube**.**

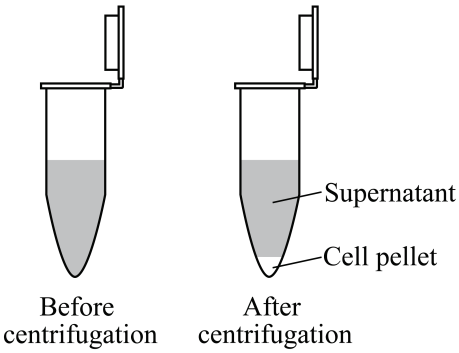



Fig. 7

5. Using a P200 micropipette and sterile tips*, add 100 µl ice-cold competent buffer** to each pellet as a wash (Fig. 8). Gently pipette up and down a few times to suspend the pellet completely. Proceed to step 6 quickly.

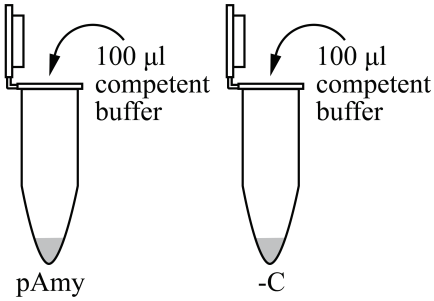



Fig. 8

*Note: Use a new pipette tip for each sample.

**Note: Under normal conditions, DNA cannot pass through the cell membrane of bacteria. Calcium ions in the competent buffer help increase the permeability of the bacterial cell membrane, so that the plasmids can be incorporated.

6. Centrifuge the 2 tubes at 5,000 rpm for 3 minutes to pellet the *E. coli* cells (Fig. 7). Carefully pour off and discard the supernatant in the designated disposal container with 10% chlorine bleach. **Save the cell pellet.**

7. Using a P200 micropipette and sterile tips#, add 100 µl ice-cold competent buffer to each pellet (Fig. 8). Gently pipette up and down a few times to suspend the pellet completely.

#Note: Use a new pipette tip for each sample.

8. Gently finger-flick the tubes^ (Fig. 6) to avoid any cell sediment at the bottom of the tubes.

^Note: Be gentle to these cells and do not vortex. They are fragile and can burst and die easily.

*Transform plasmids into E. coli by cold shock and heat shock*

9. Keep all tubes on ice until Step 12.

10. Using a P20 micropipette and a new sterile tip, add 5 µl sterile distilled water to tube "-C" (Fig. 9). Wrist-flick the sample to pool the reagents (Fig. 10). **Return tube "-C" to the ice bath.**

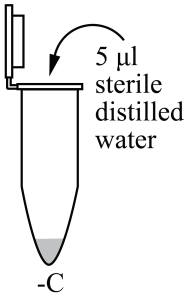



Fig. 9

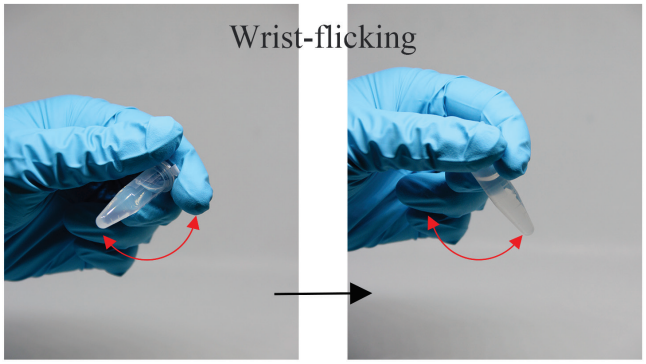



Fig. 10

11. Using a P20 micropipette and a new sterile tip, add 5 µl plasmid to tube "pAmy". Wrist-flick the sample to pool the reagents. **Return tube "pAmy" to the ice bath**.

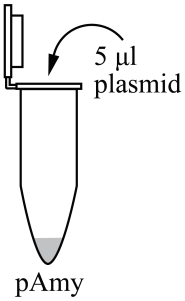



Fig. 11

12. Leave the tubes on ice for a minimum of 30 minutes (Fig. 12).

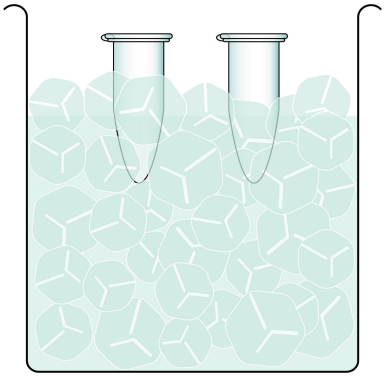


Fig. 12

13. After 30 minutes, move the ice bath containing the tubes next to the hot water bath. Make sure the tubes are tightly closed. As quickly as possible, transfer both tubes from the ice to the 42°C water bath for a "heat shock" of exactly 90 seconds (Fig. 13).

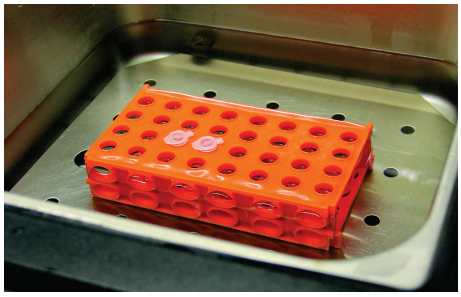


Fig. 13

14. After 90 seconds, quickly return the tubes to the ice bath for 2 minutes*.

> *Note: A sudden increase in temperature creates pores on the plasma membrane of *E. coli* and allows plasmid DNA to enter the bacterial cells. Transfer the tubes as quickly as possible, since the more distinct the temperature change in the heat shock, the greater the transformation efficiency.

15. Using a P1000 micropipette and new sterile tips#, add 250 µl sterile LB broth to each tube (Fig. 14). Mix the contents by finger flicking.

> #Note: Use a new pipette tip for each sample.

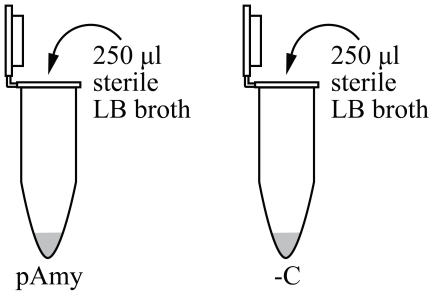



Fig. 14

16. Incubate the tubes at room temperature^ or in an incubator set at 25–30°C for 45 minutes.

> ^Note: Room temperature may vary, depending on the ambient conditions of the laboratory environment. Hence, the incubation time may also vary.

17. Discard the used tubes and tips in the designated disposal container with 10% chlorine bleach (no autoclave processing required) or in a biohazard bag for autoclave processing afterwards.

## D. Part (3) of the experiment: Culturing *E. coli* cells on agar plates containing starch and ampicillin

### a) Equipment and materials (per group)

Equipment

- Micropipette (P200) and sterile tips

Materials

| | |
|---|---|
| - Transformed *E. coli* culture from Part (2) | × 2 tubes |
| - LB agar plate | × 1 |
| - LB agar plate with ampicillin and 2% starch | × 1 |
| - Disposable inoculating loop | × 4 |
| - Adhesive tape | |
| - Permanent marker | × 1 |
| - 70% ethanol in spray bottle | × 1 |
| - Paper towel | × 1 box |
| - Biohazard bag | × 1 |
| - Disposal container with 10% chlorine bleach | × 1 |

### b) Safety precautions

- Wear a laboratory gown.
- Tie up long hair.
- Wear gloves during the experiment.
- Wash hands to remove all possible contamination before and after the experiment.
- Make sure ethanol is fully vaporised before approaching any flame.
- Dispose of or disinfect all materials properly after each experiment.

### c) Procedures

1. Disinfect the benchtop and gloved hands with 70% ethanol*. Use a laminar flow hood, if available.

***Caution: Keep away from fire! DO NOT light the flame until the ethanol has been completely vaporized!**

2. Obtain one LB/amp/starch agar plate (selective agar plate) and one LB agar plate (control agar plate). Label the bottom of the plates with the date of the experiment, your class, and group number. On the bottom of each plate, draw a line down the centre. Label one side "pAmy" and the other side "-C" (Fig. 15).

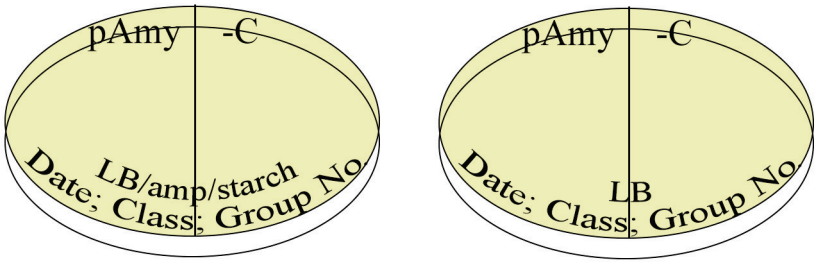



Fig. 15

3. Collect the LB/amp/starch agar plate. Raise the lid of the agar plate at about 45°.

4. Using a P200 micropipette and a new sterile tip, transfer 30 µl *E. coli* culture from the tube "pAmy" onto the "pAmy" section of the LB/amp/starch agar plate (Figs. 16 and 17). Discard the used tip in the designated disposal container with 10% chlorine bleach.

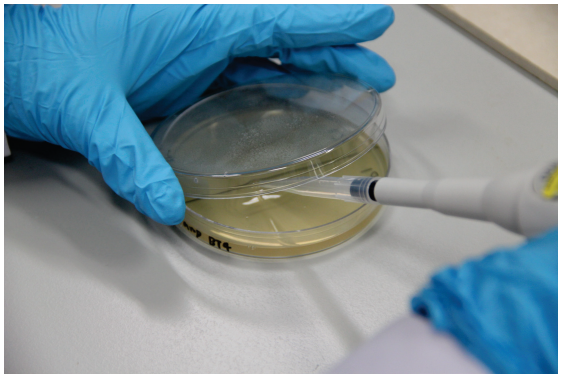
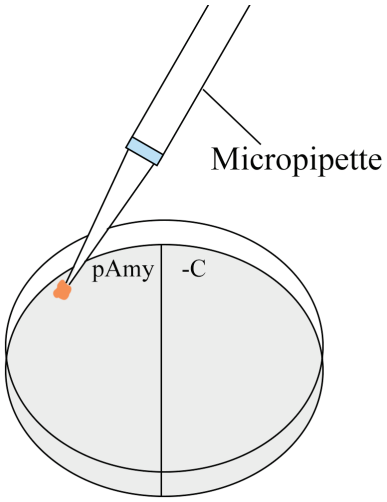


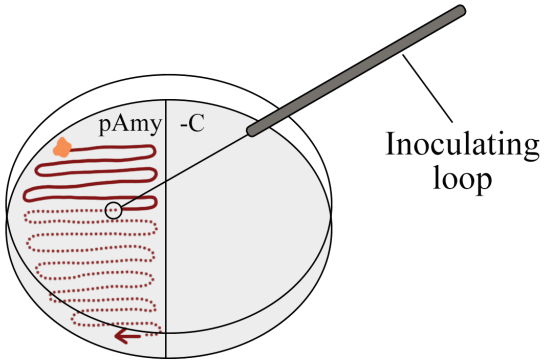
Fig. 16




LB/amp/starch agar plate
Fig. 17

5. Streak the "pAmy" section with a sterile inoculating loop (Fig. 18). Discard the disposable inoculating loop* in the designated disposal container with 10% chlorine bleach.

*Note: If a reusable inoculating loop is used, please refer to Steps 3–4 of Appendix 1 of for details of the aseptic technique.




LB/amp/starch agar plate
Fig. 18

6. Collect the LB agar plate. Raise the lid of the agar plate at about 45°.

7. Using a P200 micropipette and a new sterile tip, transfer 30 µl *E. coli* culture from the tube "pAmy" onto the "pAmy" section of the LB agar plate (Figs. 19 and 20). Discard the used tip in the designated disposal container with 10% chlorine bleach.

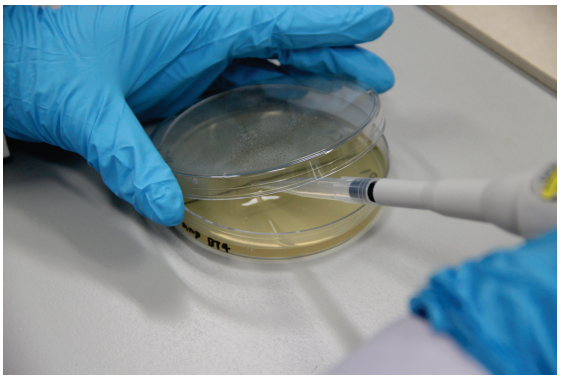
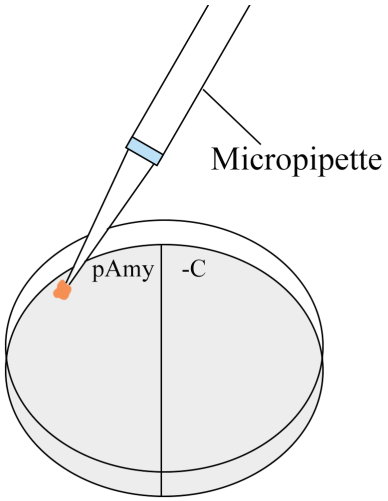


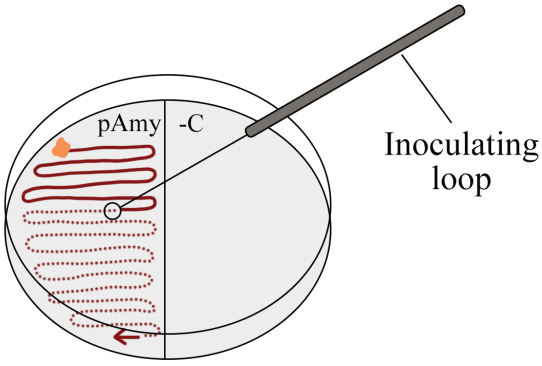
Fig. 19




LB agar plate
Fig. 20

8. Streak the "pAmy" section with a sterile inoculating loop (Fig. 21). Discard the disposable inoculating loop[#] in the designated disposal container with 10% chlorine bleach.

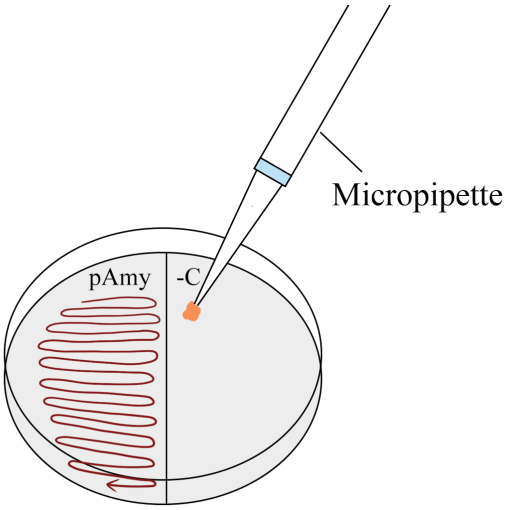
[#]Note: If a reusable inoculating loop is used, please refer to Steps 3–4 of Appendix 1 for details of the aseptic technique.




LB agar plate
Fig. 21

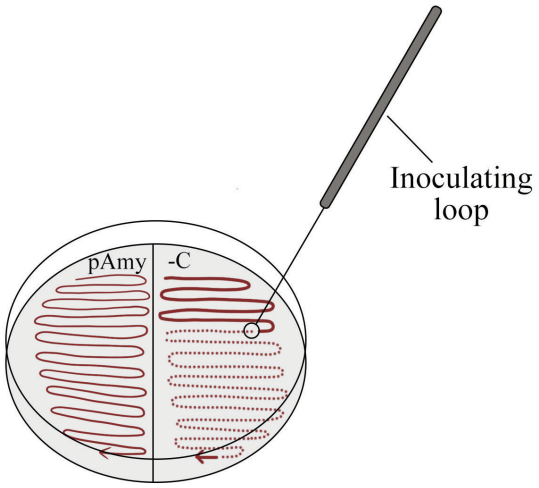
9. Collect the LB/amp/starch agar plate. Raise the lid of the agar plate at about 45°.

10. Using a P200 micropipette and a new sterile tip, pipette 30 µl *E. coli* culture from tube "-C" onto the "-C" section of the LB/amp/starch agar plate (Fig. 22). Discard the used tip in the designated disposal container with 10% chlorine bleach.




LB/amp/starch agar plate
Fig. 22

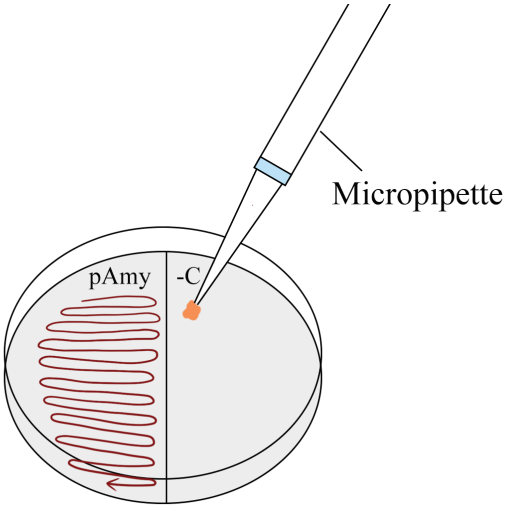
11. Streak the "-C" section with a sterile inoculating loop (Fig. 23). Discard the disposable inoculating loop in the designated disposal container with 10% chlorine bleach.




LB/amp/starch agar plate
Fig. 23

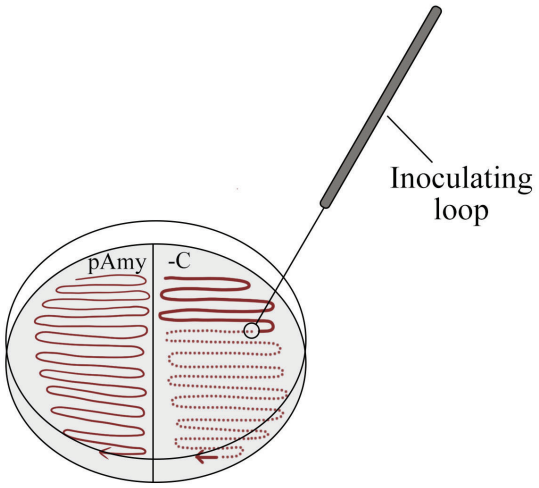
12. Collect the LB agar plate. Raise the lid of the agar plate at about 45°.

13. Using a P200 micropipette and a new sterile tip, pipette 30 µl *E. coli* culture from tube "-C" onto the "-C" section of the LB agar plate (Fig. 24). Discard the used tip in the designated disposal container with 10% chlorine bleach.




LB agar plate
Fig. 24

14. Streak the "-C" section with a sterile inoculating loop (Fig. 25). Discard the disposable inoculating loop in the designated disposal container with 10% chlorine bleach.




LB agar plate
Fig. 25

15. Allow the culture suspension to be absorbed into the agar, by resting the plates on the bench undisturbed for 5 minutes, the lids facing up.

16. Stack the plates. Place short strips of adhesive tape at opposite edges of LB agar plates (Fig. 26) to prevent the plates from being accidentally opened.

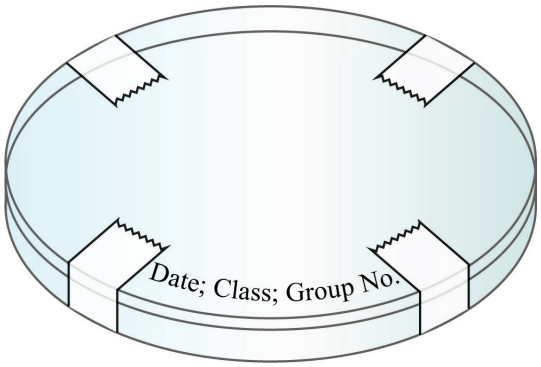



Fig. 26

17. Invert the plates. Incubate them at room temperature* or in an incubator set at 25−30°C for 3 days.

> *Note: Room temperature may vary, depending on the ambient conditions of the laboratory environment. Hence, the incubation time may also vary.

18. Incubate the plates in an upside-down position at 4°C for 24 hours. Observe the results in Part (4) of the experiment within 3−4 days.

## E. Part (4) of the experiment: Identifying transformed *E.coli* colonies

### a) Equipment and materials (per group)

Equipment

- Mobile device × 1

Materials

- Agar plate from Part (3) × 2
- 70% ethanol in spray bottle × 1
- Paper towel × 1 box
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach × 1

### b) Safety precautions

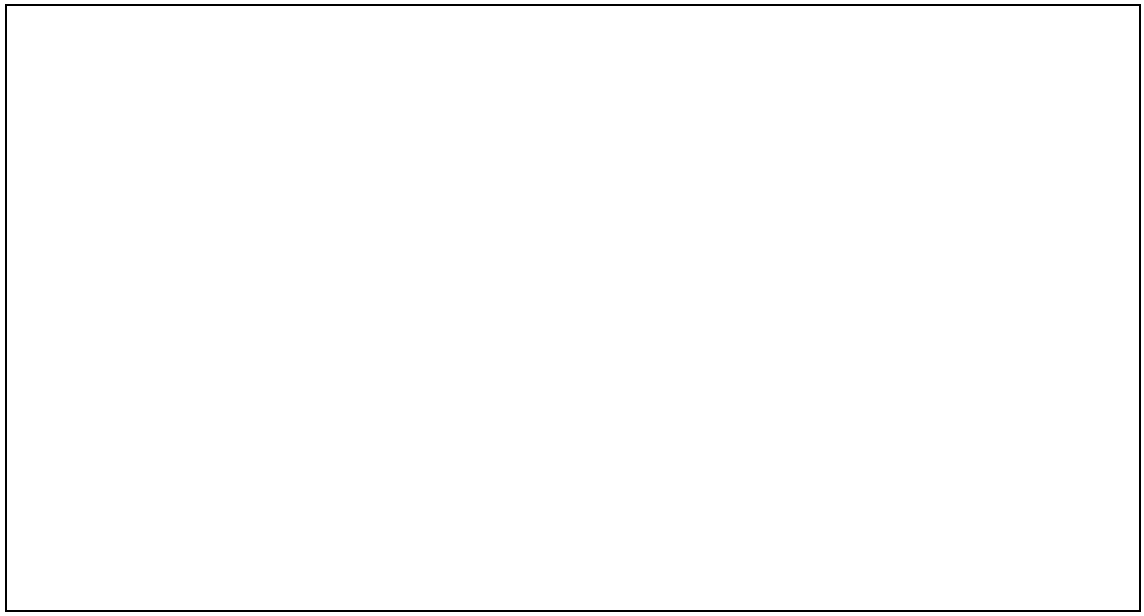
- Wear a laboratory gown.
- Tie up long hair.
- Wear gloves during the experiment.
- Wash hands to remove all possible contamination before and after the experiment.

### c) Procedure

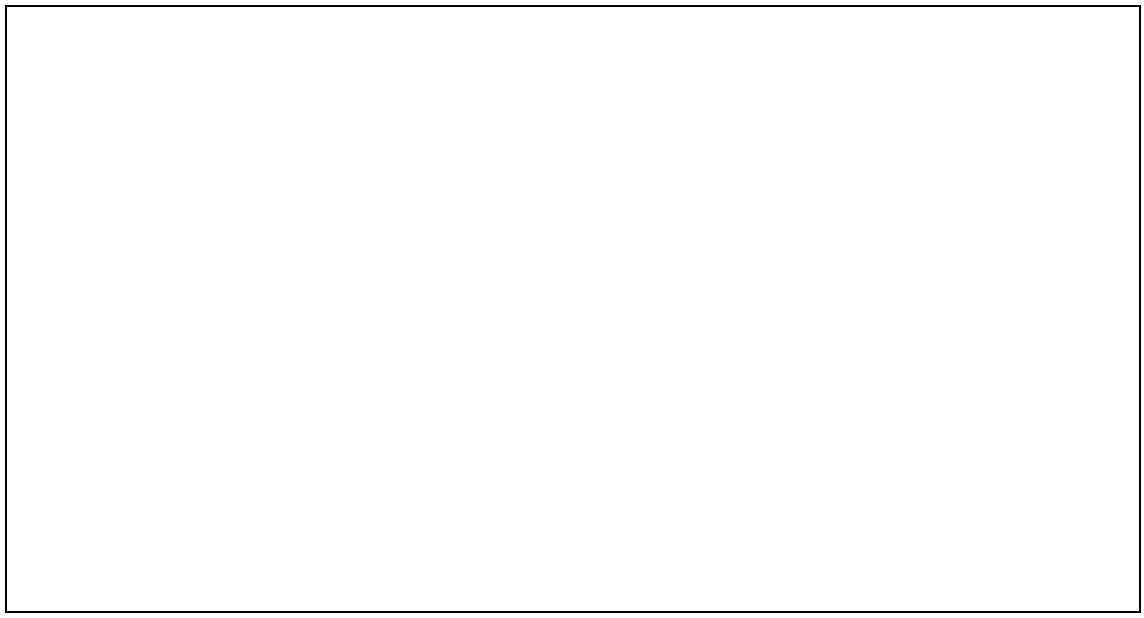
1. Collect your group's plates.
2. Observe the colonies on different agar plates.
3. Take pictures of the plates, using a camera or mobile device.
4. After observation and analysis, discard all unwanted culture plates in the designated disposal container with 10% chlorine bleach (no autoclave processing required) or in a biohazard bag for autoclave processing afterwards.

## VI. Results

1. Paste the photo or draw a picture of the LB agar plate (control agar plate):



2. Paste the photo or draw a picture of the LB/amp/starch agar plate (selective agar plate):



3. Describe and briefly explain what you have observed in the pAmy section of the LB/amp/starch agar plate.

## VII. Discussion

1. Why have we used an agar plate with starch in this experiment?

2. Suggest one method to show that the starch is digested by *E. coli*.

3. After the cold shock and heat shock, the tubes were mixed by “finger-flicking” and “wrist-flicking” instead of vortexing. Suggest the reason for this.

4. Suggest the function of adding ampicillin in the growth agar in this experiment.