Practical activity (3)

Microbial Biosensor

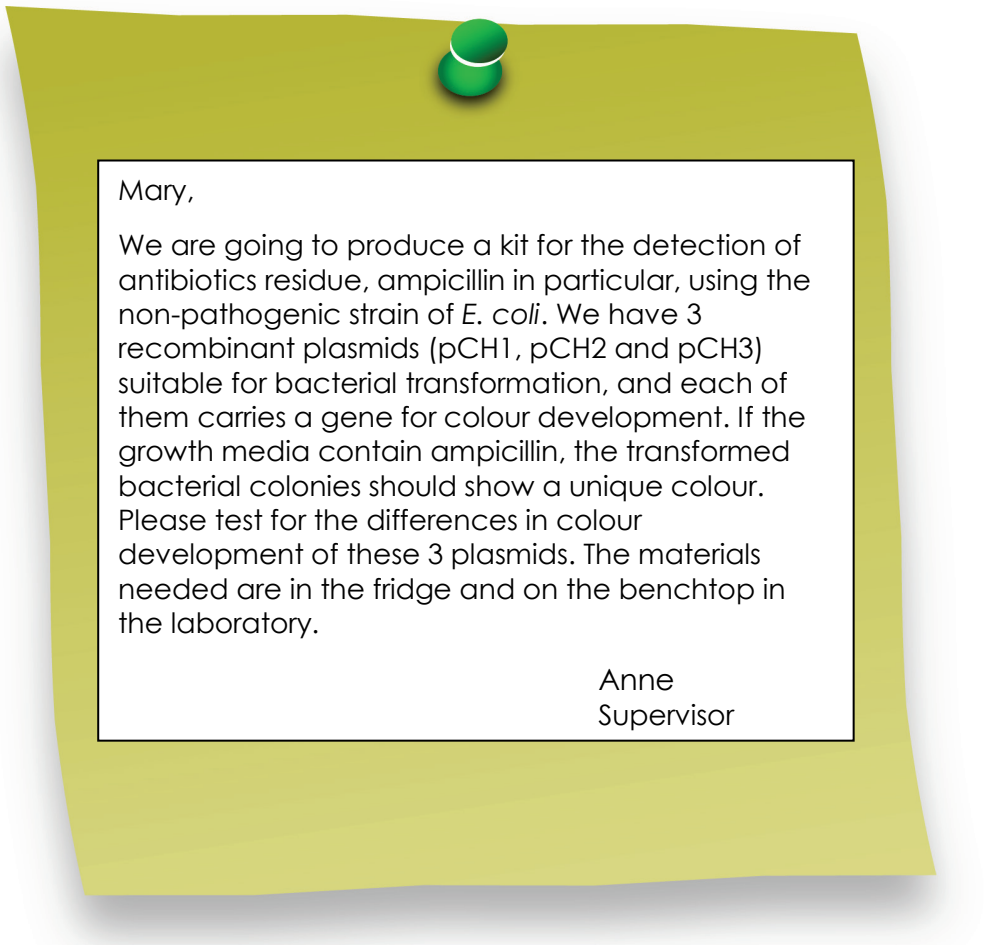
(Student version)

I. Background

Nowadays, some microorganisms, such as *Escherichia coli* (*E. coli*) and *Saccharomyces cerevisiae* (*S. cerevisiae*), are genetically modified as biosensors for the detection of heavy metals, antibiotics/drug residues and certain chemicals/molecules. The presence of these substances can trigger specific colour changes in these genetically modified microorganisms, mostly bacteria, which are visible to the naked eye. Such colour changes could provide us an easy, straightforward and reliable approach to detect the mentioned substances. Importantly, the application of genetically modified microorganisms is now widely used in the quality control of water and food samples.

Scenario

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



II. Guiding questions about the design of the experiment

1. Under normal conditions, the growth of *E. coli* can be inhibited by ampicillin. Upon successful transformation, the bacteria become resistant to ampicillin due to the incorporation of the ampicillin-resistant gene from the recombinant plasmids. Decide which type of agar plate (LB agar or LB/ampicillin agar) should be used before and after transformation respectively.
2. Suggest a molecular technique for introducing the external plasmid into the *E. coli* cells.

III. Objectives of the experiment

1. To transform the competent *E. coli* with recombinant plasmids "pCH1", "pCH2", and "pCH3" (each of which contains a gene for colour development and a gene for ampicillin resistance); and
2. To demonstrate the successfully transformed *E. coli* cells by observable colour changes.

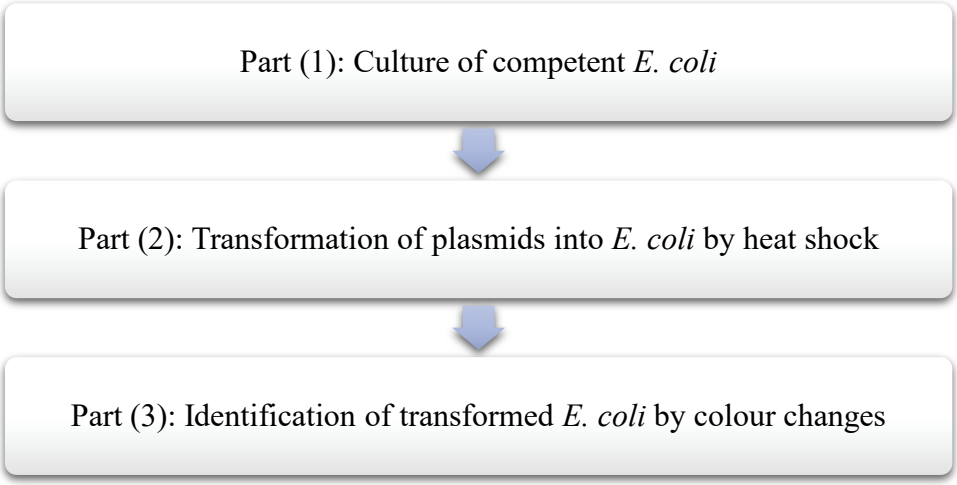
IV. Expected Learning Outcomes

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

1. outline the principle of producing genetically modified microorganisms;
2. recognise the potential application of biosensors;
3. apply aseptic techniques in transferring and culturing microbes; and
4. perform bacterial transformation.

V. The Experiment

A. Overview



B. Part (1) of the experiment: Culture of competent *E. coli*

a) Equipment and materials (per group)

Equipment

- Bunsen burner × 1
- Spark lighter × 1
- Micropipette (P20) and sterile pipette tips

Materials

- BactoBead™ (competent *E. coli**) × 1 vial (per class)
- Recovery broth (15 µl) × 1
- Forceps × 1
- Sterile disposable inoculating loop × 4
- LB agar plate × 1

*Note: The *E. coli* are treated so that they are more able to incorporate foreign DNA

- Permanent marker × 1
- 70% ethanol in spray bottle × 1
- Paper towel × 1 box
- Adhesive tape
- Parafilm
- 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.
- Keep ethanol away from fire.
- Make sure ethanol on the gloves 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 LB agar plate with the date of the experiment, your class, and the group number (Fig. 1).

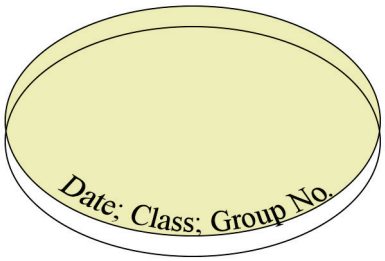


Fig. 1 LB agar plate

3. Light up a Bunsen burner.

4. Flame-sterilise the forceps and allow them to cool (Fig. 2).

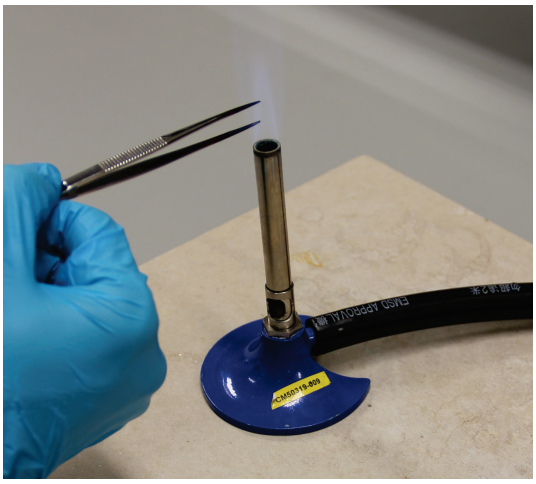


Fig. 2

5. Using a pair of sterile forceps, transfer 1 single bead of BactoBead™ (competent *E. coli*) from the container vial to the upper-left portion of the LB agar plate (Fig. 3).

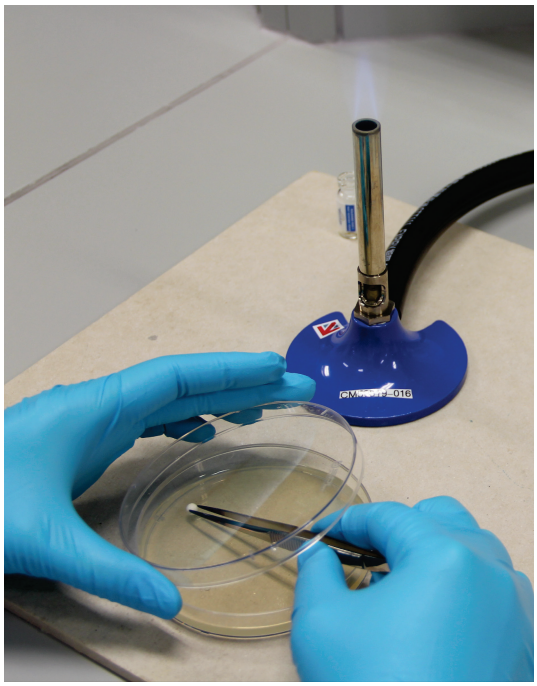


Fig. 3

6. Using a P20 micropipette and a sterile tip, transfer 10 µl recovery broth from the micro-centrifuge tube onto the bead (Fig. 4).

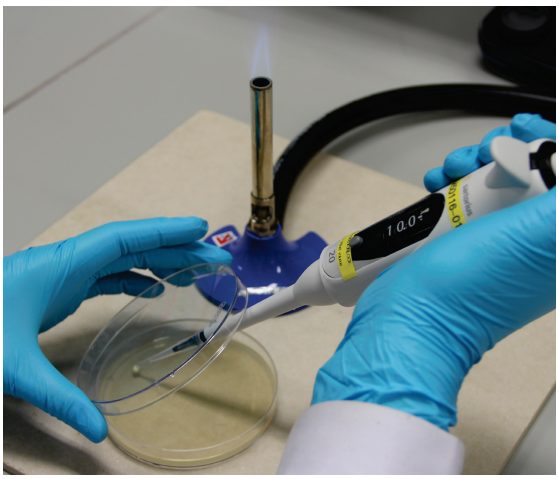


Fig. 4

7. As the *E. coli* bead gets dissolved by the recovery broth, use a new sterile inoculating loop* to streak the first quadrant (Fig. 6 part ⓐ) of the agar plate in a zigzag pattern as shown in Figs. 5 and 6a.

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

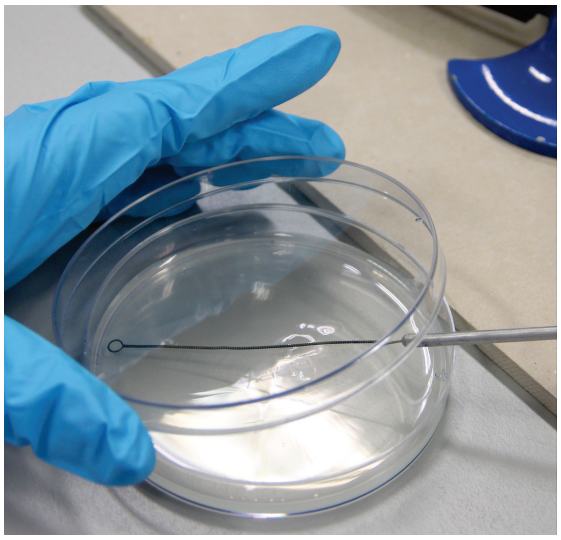


Fig. 5

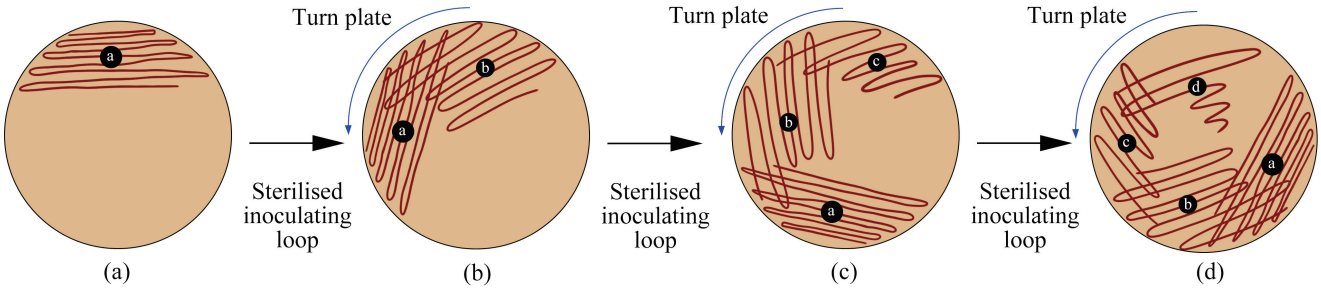


Fig. 6: Streak isolation pattern of *E. coli*

8. Replace the lid.
9. Rotate the plate 90°.
10. Using a new sterile inoculating loop, streak the overlapping part of the previous quadrant 2–3 times. Streak into the next quadrant (part ⓑ) in a zigzag pattern as shown in Fig. 6b.
11. Repeat Steps 8–10 for completing the streak isolation of *E. coli* in the third (part ⓒ) and fourth (part ⓓ) quadrants as shown in Fig. 6c and Fig. 6d respectively.
12. Replace the lid. Stick short strips of adhesive tape at opposite edges of the agar plate to hold the lid and base together as shown in Fig.7.

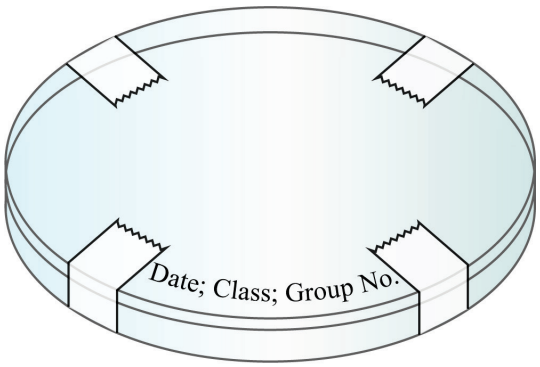


Fig. 7

Invert the plate and incubate it at room temperature* or in an incubator set at 25–30°C for at least 16 hours.

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

13. Discard the used tips in the designated disposal container with 10% chlorine bleach (no autoclave processing required) or in a biohazard bag for autoclave processing afterwards.
14. After the suggested incubation time, wrap the edge of the plate with parafilm and store the plate at 4°C until Part (2) of the experiment.

C. Part (2) of the experiment: Transformation of plasmids into *E. coli* by heat shock

a) Equipment and materials (per group)

Equipment

- Hot water bath (42°C) × 1 (per class)
- Hot water bath (37°C) × 1 (per class)
- Ice bath × 1
- Bunsen burner × 1
- Spark lighter × 1
- Timer × 1
- Micropipettes (P1000, P200 and P20) and sterile pipette tips

Materials

- LB agar plate from Part (1) × 1
- pCH1 plasmid (8 µl) × 1
- pCH2 plasmid (8 µl) × 1
- pCH3 plasmid (8 µl) × 1
- Distilled water (8 µl) × 1
- $CaCl_2$ solution (1200 µl) × 1
- Recovery broth (1200 µl) × 1
- Sterile micro-centrifuge tube × 5
- Micro-centrifuge tube rack × 1
- Sterile disposable inoculating loop × 5
- LB/ampicillin/IPTG agar plate × 1

- Permanent marker × 1
- 70% ethanol in spray bottle × 1
- Paper towel × 1 box
- Adhesive tape
- Parafilm
- 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.
- Keep ethanol away from fire.
- Make sure ethanol is fully vaporized 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*.

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

2. Light up a Bunsen burner.

3. Label the lid of 1 micro-centrifuge tube "S" and your group number (Fig. 8).

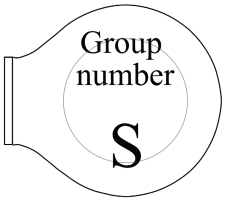


Fig. 8

4. Using the P1000 and P200 micropipettes, and sterile tips, add 1100 µl $CaCl_2$ solution to tube "S" (Fig. 9).

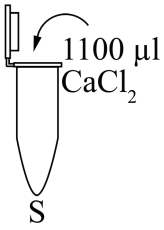


Fig. 9

5. Using a sterile inoculating loop, aseptically[#] transfer 10 colonies of *E. coli* from the LB agar plate (Fig. 10) to tube "S" (Fig. 11). Using a P1000 micropipette and a sterile tip, pipette the mixture up and down 10 times.

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

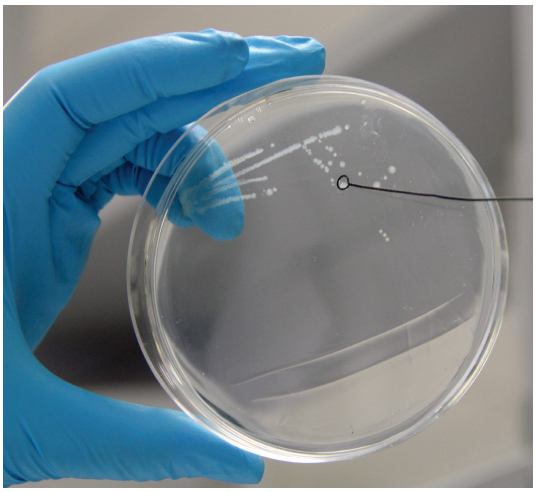


Fig. 10

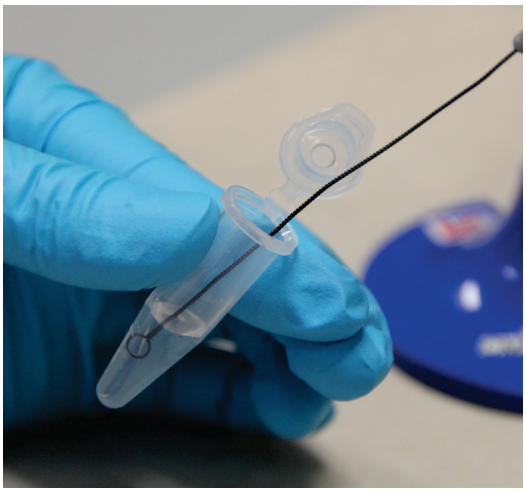


Fig. 11

6. Label the lid of 4 other micro-centrifuge tubes "CH1", "CH2", "CH3", and "neg" (for negative control) with your group number (Fig. 12).

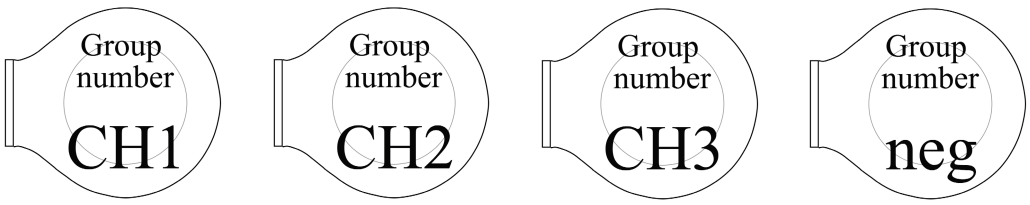


Fig. 12

7. Using a P1000 micropipette and sterile tips, transfer 250 µl solution from tube “S” to each of the tubes “CH1”, “CH2”, “CH3”, and “neg” (Fig. 13). Discard tube “S” in the designated disposal container.

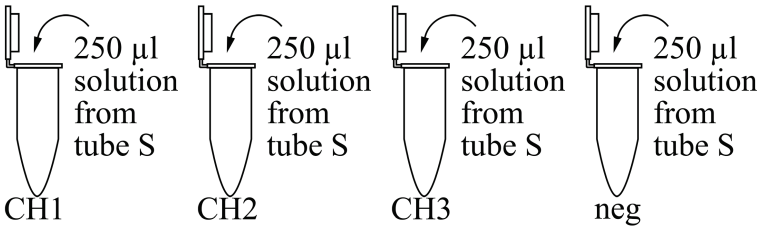


Fig. 13

8. Using a P20 micropipette and a sterile tip, add 5 µl pCH1 plasmid solution to tube “CH1” (Fig. 14).

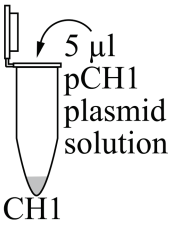


Fig. 14

9. Using a P20 micropipette and a sterile tip, add 5 µl pCH2 plasmid solution to tube “CH2” (Fig. 15).

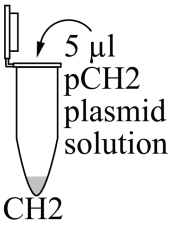


Fig. 15

10. Using a P20 micropipette and a sterile tip, add 5 µl pCH3 plasmid solution to tube “CH3” (Fig. 16).

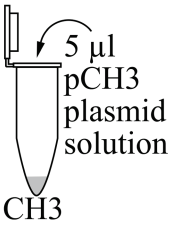


Fig. 16

11. Using a P20 micropipette and a sterile tip, add 5 µl sterile distilled water to tube “neg” (Fig. 17).

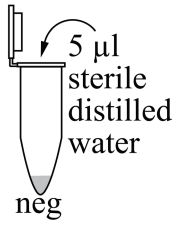


Fig. 17

12. Mix gently the contents of all the tubes by finger-flicking (Fig. 18).

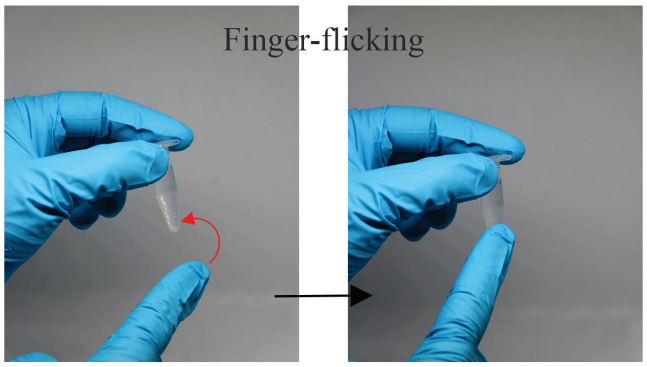


Fig. 18

13. Shut off the Bunsen burner.
14. Keep the tubes on ice for at least 30 minutes (Fig. 19).

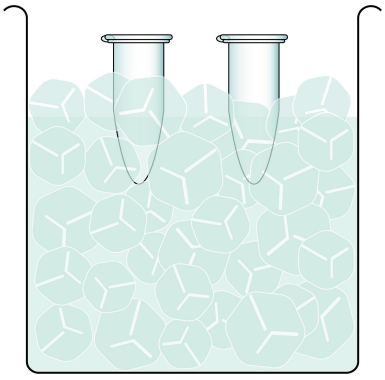


Fig. 19

15. After 30 minutes, move your ice bath containing the four tubes next to the hot water bath. Make sure the tubes are tightly closed.
16. As quickly as possible, transfer all the tubes from the ice bath to the 42°C water bath (Fig. 20) for a "heat shock" of exactly 90 seconds*.

*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.

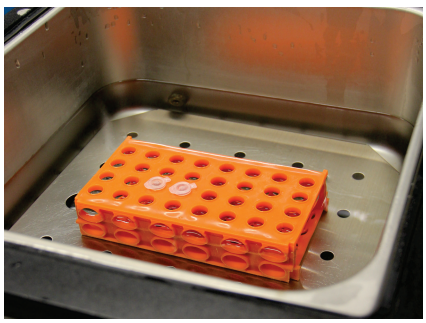


Fig. 20

17. After 90 seconds, quickly return the tubes to the ice bath for 2 minutes (Fig. 21).

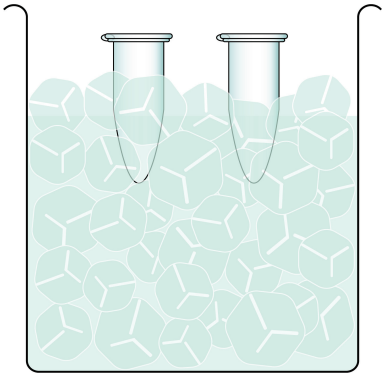


Fig. 21

18. Using a P1000 micropipette and sterile tips, add 250 µl recovery broth to each tube* (Fig. 22).

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

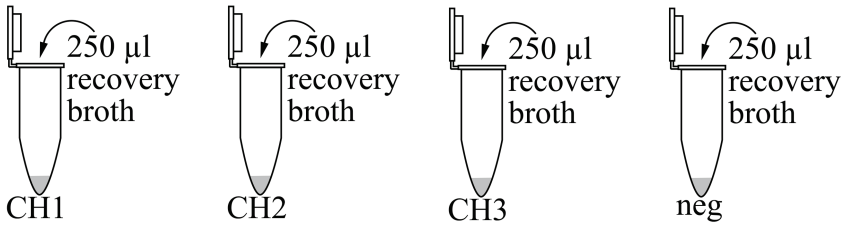


Fig. 22

19. Gently finger-flick (Fig. 23) or wrist-flick (Fig. 24) the tubes and allow them to incubate in a 37°C water bath for 30 minutes (Fig. 25). Finger-flick or wrist-flick the tubes every 10 minutes.

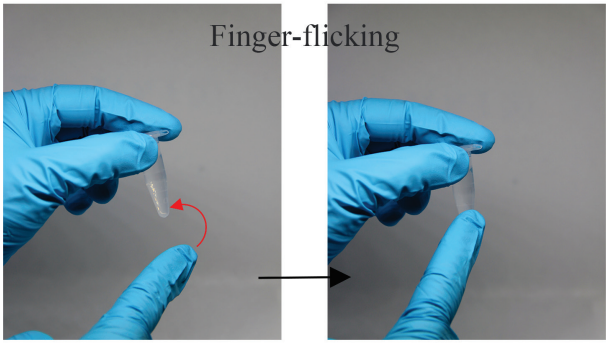


Fig. 23

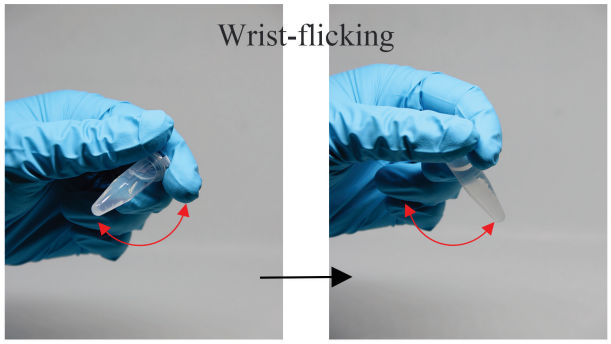


Fig. 24

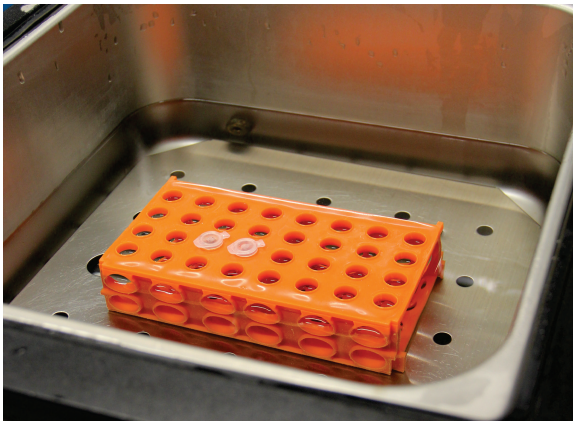


Fig. 25

20. Draw 2 lines to divide the bottom of the LB/amp/IPTG agar plate into 4 quadrants. Label the bottom of the plate “CH1”, “CH2”, “CH3”, and “neg” with the date of the experiment and your group number (Fig. 26).

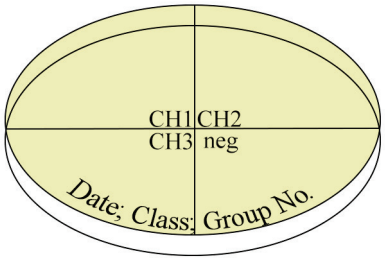


Fig. 26 LB / amp / IPTG agar plate

21. Using a P200 micropipette and a sterile tip, transfer 40 µl microbial broth from tube “CH1” to quadrant “CH1” of the agar plate (Fig. 27ⓐ). Spread the plate using a new sterile disposable inoculating loop.

22. Repeat Step 21 for transferring microbial broth from tubes “CH2”, “CH3”, and “neg” to the respective quadrants of the agar plate: quadrant “CH2” (Fig. 27ⓑ), quadrant “CH3” (Fig. 27ⓒ) and quadrant “neg” (Fig. 27ⓓ). Spread the plates using a new sterile disposable inoculating loop each time.

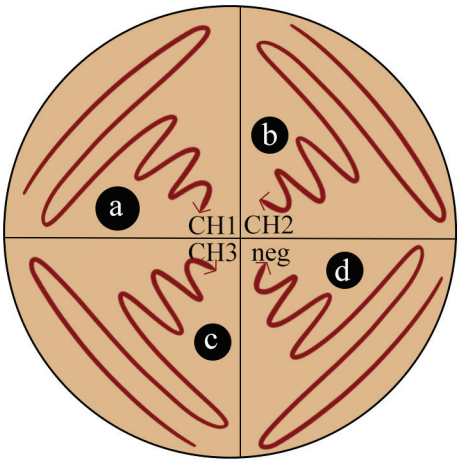


Fig. 27

23. Stick short strips of adhesive tape at opposite edges of the agar plate to hold the lid and base together as shown in Fig. 28. Invert the plate and incubate it at room temperature* or in an incubator set at 25−30°C for 2−3 days.

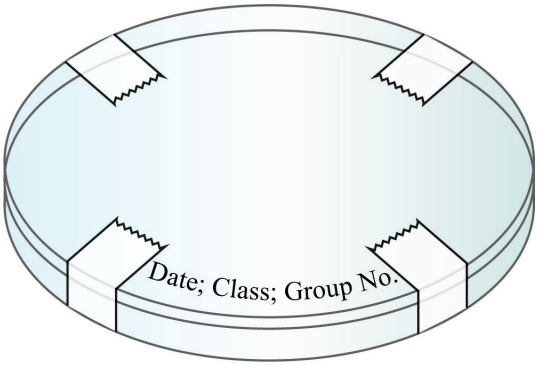


Fig. 28

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

24. Discard all used tips and tubes as well as the unwanted agar plate in the designated disposal container with 10% chlorine bleach (no autoclave processing required) or in a biohazard bag for autoclave processing afterwards.

25. After the suggested incubation time, wipe the edge of the plate with parafilm and store the plate at 4°C until Part (3) of the experiment.

D. Part (3) of the experiment: Identification of transformed *E.coli* by colour changes

a) Equipment and materials (per group)

Equipment

- Mobile device × 1

Materials

- LB/amp/IPTG agar plates from part (2) × 1
- 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

1. Tie up long hair.
2. Wear gloves during the experiment.
3. Wash hands to remove all possible contamination before and after the experiment.
4. Discard your plates to the designated container.

c) Procedure

1. Collect your plate. Using a permanent marker, count the colonies visually by marking dots on the back of the plate.
2. Record the number of colony-forming units (CFU)* and colour of colonies found on the plate. Mark TNTC (Too Numerous To Count) if the number of colonies on the plate is higher than 250 CFU.

> *Note: Only plates with a colony number between 25 and 250 can be used for counting.
>
> Using the term "colony-forming unit (CFU)" instead of the term "colony" is to reflect the fact that a single colony observed on an agar plate may not be formed by a single bacterium. In fact, such a colony can be formed by several bacteria (more than one bacterium) growing in proximity.
>
> If we present our data using the term "colony", then the actual number of bacteria in the test sample may be underestimated.

3. Take pictures of the plate using a mobile device.
4. Discard the unwanted agar plate 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 a photo or draw a picture of the agar plate in the box below.

2. Record the number of CFU and the colour of *E. coli* colonies in the following table.

Plasmid	Number of CFU	Colour of colony
pCH1		
pCH2		
pCH3		
Negative control		

VII. Discussion

1. After heat shock, the tubes were mixed by "finger-flicking" or "wrist-flicking" instead of vortexing. Suggest the reason for this.

2. During the heat shock step, a student accidentally heated the cells at 42°C for 5 minutes. Only a few colonies were found on the agar plates at the end of the experiment. Explain the results.

3. Suggest a reason why LB agar plates containing ampicillin were used for culturing the *E. coli* instead of LB agar plates after the transformation of *E. coli*.

4. Suggest why there is no bacterial colony found in the quadrant for "Negative control".