Practical activity (3)

Microbial Biosensor

(Teacher's Guide)

I. 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.

II. 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.

III. Teaching notes:

1. Introduce the task by going through the "Background" and "Scenario" with the students, or have the students read through these parts as well as the Guiding questions as a pre-class activity.
2. Discuss the design of the experiment with the students using the "Guiding questions about the design of the experiment".
3. Plan the laboratory work with reference to "Time allocation for the experimental activities" in section IV according to the lesson time of your school.
4. Always remind the students about the safety precautions of each part of the experiment.
5. Be aware that there will be 2 sessions of 30-min waiting time for incubation in lab session 2. You may have to plan for the teaching activity to be done during the incubation periods.
6. Go through the "Results" and "Discussion" with the students.

IV. Time allocation for the experimental activities:

Practical work		Duration	
		In class	**Out of class** (to be done by the laboratory technicians)
Lab session 1: Part (1) of the experiment			
1	Inoculation of *E.coli* bead and streak plate	30 min	
2	Incubation time		2 days
Lab session 2: Part (2) of the experiment			
1	Addition of plasmids	10 min	
2	Ice-cooling of *E. coli*	30 min	
3	Heat shock and recovery of *E. coli* cells	40 min	
4	Spread plates	10 min	
5	Incubation time		2–3 days
Lab session 3: Part (3) of the experiment			
1	Observation and analysis	30 min	
Total lesson time for the experimental activities		Lab session 1: 30 min Lab session 2: 1 hr 30 min Lab session 3: 30 min	

V. Equipment, materials, and preparatory work for the experiments

A commercial education kit (Edvotek™ Rainbow Transformation Kit; Edvotek; #224) will be needed for this experiment.

This kit has enough materials and reagents for 24 students (6 groups of 4 students). Some additional equipment or materials may be required.

Website: https://www.edvotek.com/224

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

Equipment (per group)

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

Materials (per group)

- 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

Preparatory work

Preparation of LB agar plates and aliquots of recovery broth **(One day before lab session; to be done by teacher/laboratory technicians)**

1. Dissolve LB agar powder in distilled water according to the manufacturer's instructions. Autoclave the LB agar solution, and pour it into Petri dishes (15–20 ml per dish). Wrap the agar plates with parafilm and store them properly at 4°C until use. Prepare 1 LB agar plate per group. If there is not enough time, purchase the ready-to-use LB agar plates from biotechnology companies.
2. Aliquot 15 µl recovery broth into micro-centrifuge tubes. Prepare 1 tube per group.

Sterilisation and disposal

After the experiment, some equipment and materials to discard (solid or liquid) should be sterilised by steam under pressure (autoclave) at 121ºC, 15 psi pressure for at least 30 minutes or immersed in 10% chlorine bleach for at least 2 hours.

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

Equipment (per group)

- 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 (per group)

- 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 × 1
- Parafilm
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach × 1

Preparatory work

Preparation of LB/ampicillin/IPTG agar plates and aliquots of experimental materials **(One day before lab session; to be done by teacher/laboratory technicians)**

1. According to the manufacturer's instructions, dissolve LB agar powder in distilled water and autoclave the LB agar solution. Upon cooling to ~50°C, add an appropriate amount of ampicillin (amp) and IPTG to the agar solution. Swirl it well, and pour it into Petri dishes (15–20 ml per dish). As soon as the agar solidifies, wrap the dishes with parafilm and store them properly at 4°C until use. Prepare 1 plate per group. If there is not enough time, purchase the ready-to-use LB/amp/IPTG agar plates from biotechnology companies.
2. Aliquot 1200 µl $CaCl_2$ solution into micro-centrifuge tubes. Prepare 1 tube per group.
3. Aliquot 1200 µl recovery broth into micro-centrifuge tubes. Prepare 1 tube per group.

Sterilisation and disposal

After the experiment, some equipment and materials to discard (solid or liquid) should be sterilised by steam under pressure (autoclave) at 121ºC, 15 psi pressure for at least 30 minutes or immersed in 10% chlorine bleach for at least 2 hours.

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

Equipment (per group)

- Mobile device × 1

Materials (per group)

- LB/amp/IPTG plate 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

Preparatory work

No specific preparation work is required.

Sterilisation and disposal

After the experiment, some equipment and materials to discard (solid or liquid) should be sterilised by steam under pressure (autoclave) at 121°C, 15 psi pressure for at least 30 minutes or immersed in 10% chlorine bleach for at least 2 hours.

Other teaching kits suitable for the experiment

1. pGLO™ Transformation and Inquiry Kit; Bio-rad; #10048976

   Website: https://www.bio-rad.com/zh-cn/product/pglo-transformation-inquiry-kit-for-ap-biology?ID=NEXYOKKG4

   Distributor in Hong Kong: Bio-Rad Pacific Limited, Quarry Bay, Hong Kong

   Contact number: 2789-3300 or 2272-8989

2. What a Colorful World Kit; Carolina; #217004P

   Website: https://www.carolina.com/gene-expression-advanced-topics/biobuilder-what-a-colorful-world-kit-with-perishables/217004P.pr?question=

VI. Suggested answers to the "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.

   LB agar should be used before the transformation. LB/ampicillin agar should be used after transformation.

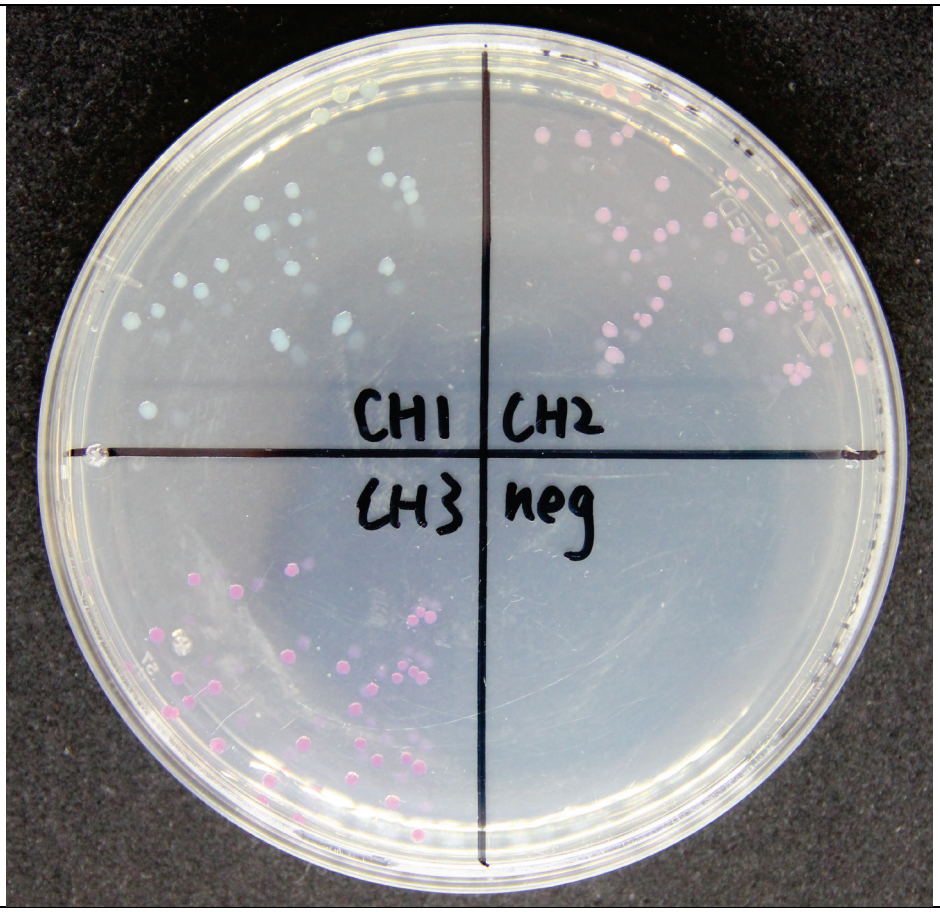
2. Suggest a molecular technique for introducing the external plasmid into the *E. coli* cells.

   Heat shock

VII. Results

1. Paste a photo or draw a picture of the agar plate in the box below.

   (For reference only)



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

(For reference only)

Plasmid	Number of CFU	Colour of colony
pCH1	*22*	*light blue*
pCH2	*43*	*pink*
pCH3	*34*	*purple*
Negative control	*0*	*N/A*

VIII. Discussion

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

   Calcium ions enhance the permeability of the bacterial cell membrane.
   This step weakens the cellular structure.
   Vortexing exerts shearing force to cells, which breaks the cells and causes cell death.
   It is necessary to mix the cells using mild methods.

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.

   During transformation, the cellular structure of bacteria was weakened. Prolonged heating damaged the cells and caused cell death.

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*.

   After the transformation process in the experiment, the successfully transformed E. coli should become resistant to ampicillin due to the incorporation of the ampicillin-resistant gene from the recombinant plasmids. The use of LB/amp agar allowed the selective growth of the transformed bacteria.

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

   E. coli cells in the "Negative control" tube were not transformed with any recombinant plasmid that carries the ampicillin-resistance gene. As a result, these cells could not survive in the culture agar that contains ampicillin.

IX. References

Froger, A., & Hall, J. E. (2007). Transformation of plasmid DNA into E. coli using the heat shock method. *Journal of Visualized Experiments*, *6*, e253. doi:10.3791/253

Video clip:
https://www.jove.com/t/253/transformation-of-plasmid-dna-into-e-coli-using-the-heat-shock-method

https://www.khanacademy.org/science/biology/bacteria-archaea/prokaryote-structure/a/prokaryote-reproduction-and-biotechnology

Video clip:
https://www.pbslearningmedia.org/resource/biot11.sci.life.gen.transbact/transforming-bacteria/