Practical activity (1)

Bacterial Transformation

(Teacher's Guide)

I. 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.

II. 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.

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 and the "Guiding questions about the design of the experiment" 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 the "Time allocation for the experimental activities" in section IV according to the lesson time of your schools.
4. Always remind the students about the safety precautions of each part of the experiment.
5. Be aware that there will be a 45-min waiting time for incubation in lab session 2. You may have to plan for the teaching activity to be done during this incubation period.
6. Go through the "Results" and "Discussion" with the students.

IV. Time allocation for the experimental activities:

Experimental activities		Duration	
		In class	**Out of class** (To be done by laboratory technicians)
Lab session 1: Part (1) of the experiment			
1	Preparation of *E. coli* broth culture	30 min	
2	Incubation of microbes		48 hr
Lab session 2: Part (2) of the experiment			
1	Ice cooling of *E. coli*		40 min
2	Heat shock and cold shock	10 min	
3	Incubation in LB broth	45 min	
Lab session 2: Part (3) of the experiment			
1	Streak plate	25 min	
2	Incubation of microbes		Room temperature 3 days, followed by 4°C for 1 day; optimal observation time: not more than 4 days at 4°C
Lab session 3: Part (4) of the experiment			
1	Plate count and analysis	30 min	
Total lesson time for the experimental activities		Lab session 1: 30 min Lab session 2: 1 hr 20 min Lab session 3: 30 min	

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

A commercial kit "Transformation of *E. coli* with p-Amylase #BTNM-8C" from G-Bioscience 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.

Local distributor: Medikonia Limited

Email: cs@medikonia.com

Website: https://www.gbiosciences.com/Educational-Products/Transformation-of-E-coli-with-pAmylase-Lab-8C

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

a) Equipment (per group)

- Bunsen burner × 1
- Spark lighter × 1

b) Materials (per group)

- 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.

c) Preparatory work

Preparation of *E. coli* culture plate and LB broth **(One day before lab session; to be done by the teacher/laboratory technicians)**

1. Streak *E. coli* culture (a non-pathogenic strain, i.e. DH5α, provided in the teaching kit) onto an LB agar plate. Incubate it at room temperature for 24 hours. Prepare 1 plate per class.

2. Dissolve LB broth powder in distilled water according to the manufacturer's instructions. Autoclave the solution and store it properly until use. Before the lab session, aliquot 5 ml LB broth into sterile tubes. Prepare 1 tube per group. If there is not enough time, purchase the ready-to-use LB broth from biotechnology companies.

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

a) Equipment (per group)

- 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	

b) Materials (per group)

- *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

c) Preparatory work

Preparation of LB broth and aliquot of experimental materials **(To be done by the teacher/laboratory technicians)**

1. One day before lab session, dissolve LB broth powder in distilled water according to the manufacturer's instructions. Autoclave the solution, and store it properly until use. Before the lab session, aliquot LB broth into sterile micro-centrifuge tubes (550 µl per tube). Prepare 1 tube per group. If there is not enough time, purchase the ready-to-use LB broth from biotechnology companies.
2. Aliquot 450 µl competent buffer into micro-centrifuge tubes. Prepare 1 tube per group.
3. Aliquot 7 µl pAmylase plasmid solution into micro-centrifuge tubes. Prepare 1 tube per group.
4. Aliquot 20 µl sterile distilled water into micro-centrifuge tubes. Prepare 1 tube per group.

d) 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.

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

a) Equipment (per group)

- Micropipette (P200) and sterile tips

b) Materials (per group)

- Transformed *E. coli* culture from Part (2)	× 2 tubes
- LB agar plate	× 1
- LB agar plate with ampicillin and 2% starch	× 1
- Disposable inoculation 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

c) Preparatory work

Preparation of LB agar plates and LB agar plates with ampicillin and 2% starch **(to be done by the teacher/laboratory technicians)**

1. One day before the lab session, 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). As soon as the agar is solidified, wrap the dishes with parafilm and store them properly at 4°C until use. If there is not enough time, purchase the ready-to-use LB agar plates from biotechnology companies. Prepare 1 plate per group.
2. One day before the lab session, dissolve LB/starch agar powder in distilled water according to the manufacturer's instructions. Autoclave the LB/starch agar solution. Upon cooling to ~50°C, add an appropriate amount of ampicillin to the solution. Swirl the agar solution well and pour it into Petri dishes (15–20 ml per dish). As soon as the agar is solidified, 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/starch agar plates from biotechnology companies.

d) 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.

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

a) Equipment (per group)

- Mobile device × 1

b) Materials (per group)

- 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

c) Preparatory work

No specific preparation work is required.

d) 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.

E. Other teaching kits suitable for the experiment:

1. HiPer® Transformation Teaching Kit; HiMedia Laboratories; #HTBM017

   Website: http://www.himedialabs.com/intl/en/products/Molecular-Biology/HiPer%C2%AE-Teaching-Kits-Molecular-Biology/Transformation-Teaching-Kit-HTBM017

   Email: info@himedialabs.com

2. GeNei Transformation Teaching Kit; BioMall.in; #6100700021730

   Website: https://www.biomall.in/product/genei-transformation-teaching-kit-6100700021730-6100700021730

   Email: info@biomall.in

VI. Suggested answers to the "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?

   Plasmids carrying the amylase gene can be incorporated into the E. coli genome by transformation. The transformed E. coli can then produce the enzyme amylase.

2. A recombinant plasmid called "pAmylase" will be used in this experiment. Which types of genes should be found in this recombinant plasmid?

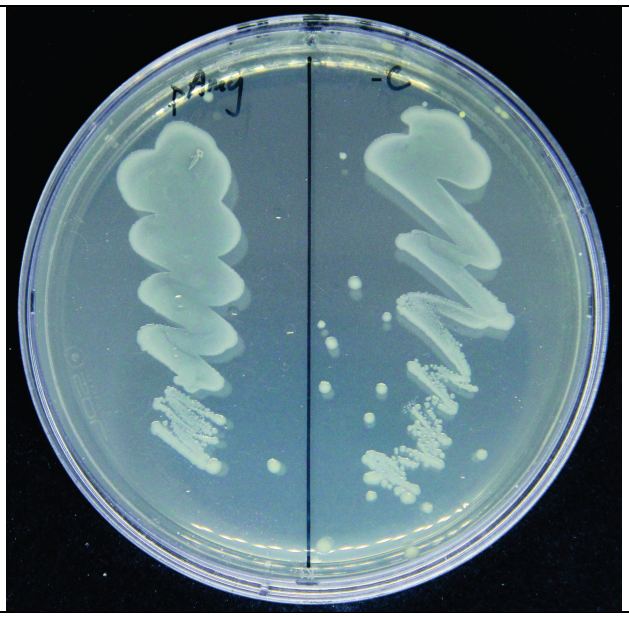
   The recombinant plasmid should contain a gene coding for amylase and a gene coding for ampicillin resistance. The gene coding for amylase is the target gene of the current transformation. The gene for ampicillin resistance helps distinguish the successfully transformed E. coli.

3. How do you know whether the bacteria have successfully picked up the recombinant plasmid? What kind of nutrient agar plate should be used?

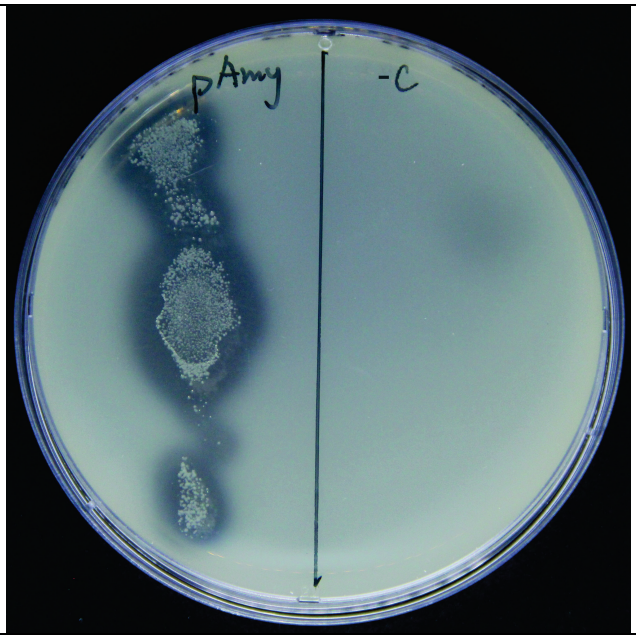
   For identifying those bacteria which have successfully picked up the recombinant plasmid, selective agar plates can be used. The growth of bacteria on the LB agar that contains starch and ampicillin indicates these bacteria have successfully taken up the recombinant plasmid. The transformed bacteria can produce amylase. Starch in the agar will be digested; thus, the agar surrounding these colonies become more transparent. The transformed bacteria have the ampicillin resistant gene. They can grow properly on the agar containing ampicillin; otherwise, growth is inhibited.

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.

A clear zone was found around the E. coli colonies in the pAmy section of the agar plate. This suggests the white-colour starch was digested by the amylase produced in the transformed bacteria.

VII. Discussion

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

   - *Starch is a large biomolecule that is insoluble in water and forms colloidal in the agar plate.*
   - *Starch can be digested by the enzyme amylase, which is only produced by the transformed E. coli cells.*
   - *The digestion of starch provides evidence of a successful transformation of the E. coli cells.*

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

   - *We can add some iodine solution to the agar plates.*
   - *The iodine solution turns from brown to blue, purple, or black (depending on the concentration of iodine) in the presence of starch.*
   - *A clear zone around the bacterial colonies indicates that the bacteria have digested the starch.*

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.

   - *Calcium ions in the competent buffer enhance the permeability of the cell membrane.*
   - *The heat shock step weakens the cell structure of the bacteria.*
   - *Vortexing exerts shearing force to the cells, which breaks the cells and make them die.*
   - *It is necessary to mix the cell suspension using mild methods.*

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

   Under normal conditions, the growth of most bacteria can be inhibited by ampicillin. In this experiment, upon successful transformation, E. coli should become resistant to ampicillin due to the incorporation of the ampicillin-resistant gene from the given recombinant plasmid. Therefore, the addition of ampicillin to the growth agar in this experiment is aimed to distinguish the transformed E. coli.

VIII. References

Panja, S., Aich, P., Jana, B., & Basu, T. (2008). How does plasmid DNA penetrate cell membranes in artificial transformation process of Escherichia coli? *Molecular Membrane Biology, 25*, 411–422.

https://www.sciencelearn.org.nz/resources/2032-bacterial-transformation

https://www.sigmaaldrich.com/technical-documents/protocols/biology/transformation.html