Practical activity (2)

Blue-white Screening

(Teacher's Guide)

I. Objectives of the experiment

1. To perform bacterial transformation using a recombinant plasmid with the *LacZα* gene for the production of β-galactosidase;
2. To perform the blue-white screening test; and
3. To demonstrate the successful transformation in the mutant strain of *E. coli* cells.

II. Expected Learning Outcomes

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

1. outline the principle of transformation;
2. apply aseptic techniques in bacterial culture;
3. carry out bacterial transformation;
4. understand the principle of blue-white screening; and
5. perform the blue-white screening test.

III. Teaching notes

1. Introduce the task by going through the "Background" with the students, or have the students read through this part 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 school.
4. Always remind the students about the safety precautions of each part of the experiment before they carry out the experiment.
5. Go through the "Results" and "Discussion" with the students.

IV. Time allocation for the experimental activities

Experimental activities		Duration	
		Within class	**Out of class** (To be done by the laboratory technicians)
Lab session 1: Part (1) of the experiment			
1	Insertion of DNA into plasmids		75 min
2	Ice-cooling and heat shock of *E. coli*	60 min	
Lab session 1: Part (2) of the experiment			
1	Plating of transformed *E. coli* cells on agar plate	20 min	
2	Incubation of microbes		1–2 days
Lab session 2: Part (3) of the experiment			
1	Plate count and analysis	30 min	
Total lesson time for the experimental activities		Lab session 1: 1 hr 20 min Lab session 2: 30 min	

V. Equipment, materials and preparatory work for the experiments

A commercial kit "Blue/White Cloning of a DNA fragment and Assay of β-galactosidase", EDVO-Kit #300 from EDVOTEK will be needed or 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/300

A. Part (1) of the experiment: Insertion of DNA fragments into a vector plasmid and transformation of *E. coli*

a) Equipment (per group)

- Water bath (42ºC) × 1 (per class)
- Water bath (37ºC) × 1 (per class)
- Centrifuge × 1 (per class)
- Bunsen burner × 1
- Spark lighter × 1
- Ice bath × 1
- Timer × 1
- Micropipettes (P1000, P200 and P20) and sterile tips

b) Materials (per group)

- Culture plate with *E. coli* cells × 1
- Sterile micro-centrifuge tube × 3
- Micro-centrifuge tube rack × 1
- Inoculating loop × 1

- Tube "D" containing DNA and plasmid mixture × 1
- Tube "P" containing plasmid solution × 1
- Tube "C" containing sterile distilled water × 1
- Reaction buffer (60 µl) × 1
- $CaCl_2$ solution (800 µl) × 1
- Recovery broth (800 µl) × 1

- 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 *E. coli* culture plates **(Two days before lab session; to be done by teacher/laboratory technicians)**

1. Remove a single BactoBead™ (provided in the education kit) from the vial, using forceps to transfer it to a regular source agar plate (e.g. LB agar plate).
2. Add 10 µl sterile distilled water to dissolve the BactoBead™.
3. Using a sterile inoculating loop, streak the dissolved bead liquid into 4 quadrants of the agar plate.
4. Invert the plate and incubate it at room temperature for 24–48 hours.
5. Prepare 1 plate per group.

Preparation of samples **(To be done by teacher/laboratory technicians before lab session)**

1. Tube "D": add 15 µl Reaction Buffer and 20 µl DNA fragment and plasmid mixture. Incubate the tube at room temperature for 1 hour. Prepare 1 tube "D" per group.
2. Tube "P": add 15 µl Reaction Buffer and 20 µl plasmid solution incubate the tube at room temperature for 1 hour. Prepare 1 tube "P" per group.
3. Tube "C": add 15 µl Reaction Buffer and 20 µl sterile distilled water. Incubate the tube at room temperature for 1 hour. Prepare 1 tube "C" per group.

Aliquot of experimental materials **(To be done by teacher/laboratory technicians before lab session)**

1. Aliquot 800 µl $CaCl_2$ solution into micro-centrifuge tubes. Prepare 1 tube per group.
2. Aliquot 800 µl Recovery Broth 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.

B. Part (2) of the experiment: Plating and incubation of *E. coli* on agar plates

a) Equipment

- Bunsen burner × 1
- Spark lighter × 1

b) Materials

- X-gal/IPTG/ampicillin agar plate × 1
- Inoculating loop × 1
- Permanent marker × 1
- Adhesive tape × 1
- Parafilm

- 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 X-gal/IPTG/ampicillin agar plates **(One day before lab session; to be done by teacher/laboratory technicians)**

1. Heat the bottle of ReadyPour medium (provided in the education kit) using a microwave oven or hot plate, with occasional swirling, until the medium is completely melted.
2. Allow the melted medium to cool to ~50°C. Add 0.3 ml ampicillin, 0.3 ml IPTG and all X-gal. (All reagents are provided in the education kit.) Swirl it well, and pour it into Petri dishes (12 ml per dish). Alternatively, purchase the ready-to-use X-gal/IPTG/ampicillin agar plates from biotechnology companies.
3. Prepare 1 X-gal/IPTG/ampicillin agar plate for each 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: Observation and analysis of experimental results

a) Equipment

- Mobile device × 1

b) Materials

- Agar 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

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.

D. Other teaching kits suitable for the experiment

1. HiPer Cloning Teaching Kit (Blue-White Selection); HiMediaLaboratories; #HTBM036

   Website: http://www.himedialabs.com/intl/en/products/Molecular-Biology/HiPer%C2%AE-Teaching-Kits-Molecular-Biology/HiPer%C2%AE-Cloning-Teaching-Kit-Blue-White-selection-HTBM036

2. Genetic defect correction with bacteria transformation; G-Biosciences; #BE-313

   Website: https://www.gbiosciences.com/Educational-Products/Genetic-Defect-Correction-with-Bacterial-Transformation

VI. Suggested answers to the "guiding questions about the design of the experiment"

1. Suggest a molecular technique for introducing external plasmids into *E. coli* cells.

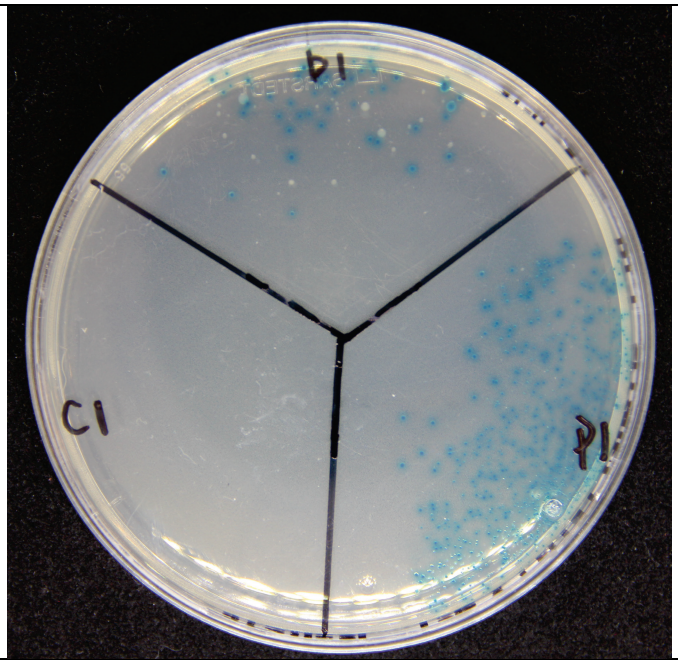
   Heat shock

2. Which chemical(s) should be added into the agar plates in order to induce the development of blue colour in the colonies?

   X-gal and IPTG

VII. Results

1. Paste the photo or draw a picture of your agar plate in the space provided below.



2. Record the number of blue colonies and white colonies in the following table.

   [Hint:
   Blue colonies: pale blue in the centre with a dense blue periphery.
   White colonies: faint blue in the centre but colourless at the periphery.]

   [For reference only]

Section	Number of blue colonies	Number of white colonies
D1	*48*	*22*
P1	*TNTC*	*1*
C1	*Nil*	*Nil*

3. Do you observe any colonies in section C1? Give a brief explanation of your observation.

   No colonies are found in section C1. The E. coli cells are not transformed with the given plasmid; thus they cannot grow in the presence of ampicillin without the ampicillin-resistance gene.

4. What do you observe in section P1 of the agar plate? Describe and explain your observation.

   There are only some blue colonies found in section P1 of the agar plate. This implies that these colonies have been transformed by the plasmids because the plasmids have an ampicillin-resistant gene. Also, these colonies turn blue. This implies that the colonies produced functional beta-galactosidase, and hence the substrate X-gal was metabolized and gave rise to products with blue pigments.

5. What do you observe in section D1 of the agar plate? Describe and explain your observation.

There are some blue colonies and some white colonies in section D1 of the agar plate. These bacterial colonies can grow on the agar plates with ampicillin. This implies that these colonies have been transformed by those plasmids because the plasmids have an ampicillin-resistant gene.

Also, some colonies are blue. This implies that the colonies produced functional beta-galactosidase, and hence the substrate X-gal was metabolised and gave rise to products with blue pigments. Hence, these blue colonies should have been transformed with the plasmids which have no target gene inserted. The plasmids have just replicated themselves.

In addition, some colonies remain white. This implies that they did not produce functional beta-galactosidase and hence did not metabolise the substrate X-gal and no blue precipitates/pigments are formed. Hence, these white colonies should have been transformed with the plasmids which have the target gene inserted and hence its LacZ gene is disrupted.

VIII. Discussion

1. After heat shock [Part (1) of the experiment, Step 21] the tubes were mixed by using "finger-flicking" or "wrist-flicking" instead of vortexing. Suggest the reason for this.

   Calcium ions enhance the permeability of the bacterial cell membrane.
   The heat shock step further weakens the cell structure.
   Vortexing exerts a shearing force to cells, which breaks the cells and causes cell death.
   It is necessary to mix the cells using milder 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 the bacteria was weakened. Prolonged heating damaged the cells and caused cell death.

3. What are the functions of X-gal and IPTG in blue-white screening?

   X-gal is actually the substrate of the enzyme β-galactosidase. Upon enzymatic metabolism, X-gal is degraded into products with blue pigments. IPTG is used to induce a strong expression of the LacZ gene in bacteria.

IX. References

Julin, D. A. (2018). Blue-white selection. In R. D. Wells, J. S. Bond, J. Klinman, & B. S. S. Masters (Eds.), *Molecular life sciences*. NY: Springer.

Ullmann, A., Jacob, F., & Monod, J. (1967). Characterization by in vitro complementation of a peptide corresponding to an operator-proximal segment of the beta-galactosidase structural gene of *Escherichia coli*. *Journal of Molecular Biology, 24*(2), 339–343.