Practical 5 - R(a) Bacterial Transformation

This practical focuses on- **Using complex apparatus, analysis and evaluation**

Intended learning outcomes

By the end of this practical and its write-up you should be able to:

- Learn and apply the steps involved in the transformation process
- Understand transformation and its applications in biology
- Analyse and interpret experimental results
- Understand regulation of gene expression by the arabinose operon

Safety Information

The safety instructions provided with the Bio-Rad Laboratories kit should be followed.

You should avoid exposing eyes or skin to UV light. Care should be taken as the UV light may not be visible to human eyes.

Background information

- Genetic transformation occurs when a cell takes up and expresses a new piece of genetic information (DNA).
- Genetic transformation has many uses ranging from genetic modification of crops to give them more desirable qualities e.g. frost or drought resistance.
- The desirable gene is cut from human, plant or animal DNA and placed inside bacteria, which then reproduces, replicating the new gene and synthesising the protein coded by the gene.
- You will introduce a gene that codes for green fluorescent protein (GFP) into bacteria.
- The GFP gene is present in jellyfish and codes for the production of GFP, so the jellyfish glows in the dark.
- After the transformation, the bacteria containing the GFP gene should also glow in the dark.

You will introduce the GFP gene into the bacterium *Escherichia coli* using the pGLO Bacterial Transformation Kit from Bio-Rad Laboratories.

Method

Preliminary Study

The goal of genetic transformation is to change an organism's phenotype. Before any change in the phenotype can be detected, an examination of the organism's natural (pre-transformed) phenotype must be made.

1 Look at the colonies of *E. coli* on the starter plate. List all observable traits or characteristics that can be described.

Day One – Transformation Procedure

1 Label one micro test tube **+pGLO** and a second test tube **–pGLO**.

2 Put your initials on the lid of both tubes then place the tubes in a foam test tube rack.

3 Open the tubes and using a sterile transfer pipette, transfer 250μl of transformation solution ($CaCl_2$) into each tube.

4 Put both tubes on ice.

5 Using a sterile loop, pick up **one single colony** of bacteria from your starter plate.

6 Immerse the loop in the liquid in the tube labelled **+pGLO**. Agitate the loop in the liquid until the colony is dispersed in the solution.

7 Place the tube back in the ice and repeat using the other tube labelled **–pGLO**.

8 Examine the solution in the pGLO DNA tube provided using a UV lamp and note any observations.

9 Immerse a new sterile loop into the pGLO DNA tube, when you remove the loop there should be a film of solution across the ring. Mix this into the contents of the **+pGLO** tube then put it back on ice.

10 Leave the tubes on ice for 10 minutes.

11 While you wait, label your 4 nutrient agar plates on the **bottom** as follows:

Label one LB/amp plate **+pGLO**

Label the LB/amp/ara plate **+pGLO**

Label the other LB/amp plate **–pGLO**

Label the LB plate **–pGLO**

12 Using the foam rack as a float put the **+pGLO** and **–pGLO** tubes into the water bath (42°C) for 50 seconds.

13 After 50 seconds put the tubes back on ice for 2 minutes.

14 After 2 minutes remove the tubes from the ice. Using a transfer pipette, add 250μl of LB nutrient broth (provided) to each tube. Allow the tubes to stand at room temperature for 10 minutes.

15 After 10 minutes, flick the tubes to ensure the contents are mixed. Using a clean transfer pipette each time, add 100μl of **+pGLO** suspension to the plates you labelled **+pGLO** and 100μl of **–pGLO** suspension to the plates you labelled **–pGLO**.

16 Using a new sterile loop for each plate gently spread the liquid across the surface of the agar plates. **DO NOT put the lid down on the bench and DO NOT press into the agar**.

17 Stack your plates and tape them together. By tomorrow you should be able to determine whether the bacteria have taken up the GFP gene and expressed the protein.

Day One - Review considerations

1 Identify the plates you would expect to find bacteria most like the original non-transformed *E. coli* colonies you observed. Explain your predictions.

2 Predict which plate(s) are most likely to have any genetically transformed bacterial cells. Explain your predictions.

3 Identify which plates should be compared to determine if any genetic transformation has occurred. Explain why.

4 What is meant by a control plate? What purpose does a control serve?

Day Two – Data Collection

1 Observe the results you obtain from the transformation procedure under normal lighting conditions. Then hold the UV lamp over the plates.

2 Carefully observe and draw what you see on each of the four plates.

3 Write down the following observations for each plate:

How much bacterial growth do you see on each plate?

What colour are the bacteria?

How many bacterial colonies are on each plate (count the spots you see).

Day Two – Analysis of Results

1 Which of the traits that you originally observed for *E. coli* did not alter?

2 Which of the traits did alter?

3 If the genetically transformed cells have acquired the ability to live in the presence of the antibiotic ampicillin, then what might be concluded about the other genes on the plasmid that you used in your transformation procedure?

4 From the results that you obtained, how could you prove that the changes that the changes were due to the procedure that you performed?

Lesson Plan

Bacterial Transformation

Context

A practical investigation set in the context of 9700 Syllabus – a bacterial transformation procedure.

It is anticipated that students will have completed an AS practical course so that they will have good basic practical skills. It is also anticipated that they will have been given learning opportunities before this so that they know how to identify transformed bacteria.

The GFP gene will be introduced into the bacterium *Escherichia coli* using the pGLO Bacterial Transformation Kit from Bio-Rad Laboratories

Key aims of lesson

This practical is designed to develop the practical, observational, data handling and analysis skills.

Intended learning outcomes

By the end of this practical the student should be able to

- Learn and apply the steps involved in the transformation process
- Understand transformation and its applications in biology
- Analyse and interpret experimental results
- Understand regulation of gene expression by the arabinose operon

Resources required

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

Planned activities (timings can be altered to suit shorter or longer lessons)

Timings/ minutes	**Teacher / Student Activities**
previous lesson	**Preliminary study** - The goal of genetic transformation is to change an organism's phonotype. Before any change in the phenotype can be detected, an examination of the organism's natural (pre-transformed) phenotype must be made. Students will need to look at the colonies of *E. coli* on the starter plate and list all observable traits or characteristics that can be described.
Day one 0-4	**Introduction** to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	**Context** - review of bacterial transformation. Teacher-led questioning, student responses / discussion, students building a multicoloured learning outline on the board.

8-12	**Introduction to method** - teacher demonstration of transformation procedure.
12-50	**Carrying out the practical** - students carry out the practical work, entering their results into a table on the board and tidying away apparatus as soon as they have finished.
50-60	**Drawing together the threads** - teacher-led class discussion on the procedure as well as a look at the review questions. Review questions to be completed prior to day two of practical.
Day Two 0-4	**Introduction** to the aims, intended outcomes and shape of the lesson - teacher led oral presentation – review of day one.
4-8	**Day One Review Questions** – teacher led discussion of answers to review questions.
8-12	**Introduction to day two** - teacher to introduce analysis of results session.
12-25	**Observations** – students make and record observations and compare these with those made in the preliminary study.
25-35	**Drawing together the observations** - teacher-led class discussion on the observations.
35-50	**Analysis** – students complete an analysis of the results and draw conclusions.
50-60	**Drawing together the threads** - teacher-led class discussion on the student analysis.

Useful Information

- The pGLO kit can be used to introduce students to the concept of genes and their basic function of coding for proteins.
- The kit can be used for independent study projects.
- Transformation is commonly used in biotechnology research and industry to study and manufacture proteins so a link to the real world can be made.
- A flow chart can be used to show the transformation procedure.
- Each kit is supplied with a Teacher's Guide, Student Guide and graphic quick guide.
- There is an advanced preparation step which needs to be performed 3 to 7 days before the transformation procedure is undertaken.
- The regulations about investigations using genetic transformation vary between countries. Teachers will need to check the regulations in their countries before embarking on these investigations
- Suppliers may be limited by import regulations and extra taxation in some countries.

Technical Information

Bacterial Transformation

The **apparatus and materials** required for this are listed below. The kit provides materials for 32 students or 8 complete student workstations.

Apparatus and materials per group:

1 1 pGLO Bacterial Transformation Kit (Bio-Rad Laboratories)

2 1 UV lamp

3 500 ml distilled water

4 1 Beaker of crushed ice

Apparatus to be available in laboratory:

1 Water bath at 42 °C

2 1 paper towel

3 Rubber gloves

GMO's

- Countries have different regulations with regard to the use of genetically modified organisms.

Teachers will need to be aware of these.

Safety Precautions/Risks.

When using the Bio-Rad Laboratories product the manufacturers instructions should be followed.

Due care should be taken with regard to exposure to UV light.

A risk assessment should be carried out as a matter of course.