Working with DNA - Module 2
Booklet 2:
The SPARQ-ed Workshop – Cloning
GFP
# Table of Contents

- The Gene of Interest- GFP ................................................................. 3
- The pGLO Plasmid ........................................................................ 4
- The SPARQ-ed Workshop Overview ............................................. 5
- Questions ..................................................................................... 6
The Gene of Interest- GFP

A gene called Green Fluorescent Protein (GFP) has previously been obtained from a species of jellyfish called Aequorea victoria. It has been modified for use in research and is now widely used in laboratories around the world. GFP (the protein) fluoresces (glows) under UV light. When “pasted” together with other genes using DNA ligase, researchers can visualise their genes more easily under the microscope. Figure 3 shows how the GFP gene is expressed to make a green fluorescent protein.

![Diagram of gene expression](image)

Figure 1: Information to make Green Fluorescent Protein is encoded in a gene. Genes are made of DNA.

1. Label figure 1 with the following:
   - DNA
   - protein
   - mRNA

2. What colour is GFP when it fluoresces?
The pGLO Plasmid

pGLO is a plasmid used as a vector for the GFP _______. This was _______ into *E. coli* bacteria through a process called **transformation**. A diagram of the pGLO plasmid is shown in figure 2. There are three genes in the plasmid: *GFP*, *araC* (arabinose C) and *ampR* (ampicillin resistance). The marks around the outside indicate sites in the DNA at which specific restriction enzymes cut. There are many other restriction enzyme sites not shown in this diagram.

Figure 2: pGLO plasmid map created in Snap Gene Viewer.

Word Bank for pages 5 & 6:

*GFP, inserted, gene*
### The SPARQ-ed Workshop Overview

#### Pre-workshop

1. The pGLO plasmid containing the GFP gene was inserted into *E. coli* bacteria.
2. The *E. coli* containing the pGLO plasmid were grown in nutrient broth overnight; the cells approximately doubled in number every hour.

#### Part A - DNA Extraction (Mini-prep)

1. Resuspend the cells in a buffer.  
2. Lyse (break apart) the cells with an alkali solution.  
3. Neutralise the lysed cells  
4. Bind the DNA to a column  
5. Wash out the lysate that didn't bind  
6. Elute (collect) the DNA from the column.

#### Part B - Restriction Enzyme Digestion

1. Cut the DNA (from part A) using specific restriction enzymes.  
2. Incubate the DNA with the restriction enzymes so that the DNA becomes cut into fragments.

#### Part C - Gel Electrophoresis

1. Add digested DNA and whole DNA to an agarose gel.  
2. Apply electricity and separate DNA by size.
Questions

1. Define the term “recombinant”

2. Suggest why creating recombinant DNA with the green fluorescent protein (GFP) gene is so useful for researchers.

3. Define the term “transformation” in relation to working with DNA.

4. From the UV light image on slide 10, predict whether the pGLO plasmid was successfully transformed into the bacteria. Give a reason for your answer.

5. Explain why the bacteria need time to grow before DNA extraction.

6. Identify the three steps in the SPARQ-ed workshop. For each step, describe its purpose.