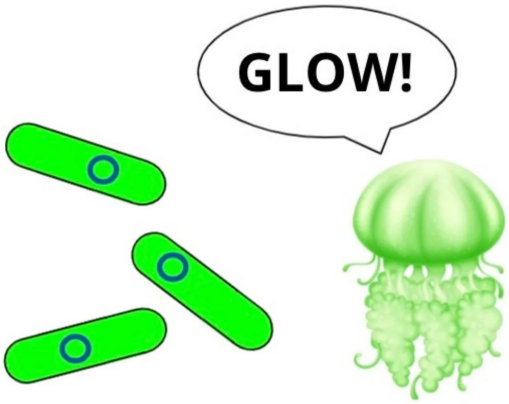
# Let it Glow Bacterial Transformation MiniLab Student FRQs

**Cat# M6300**

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This resource provides 3 total FRQs of the following types:

- Question 1: Interpreting and Evaluating Experimental Results (9 pts)
- Question 4: Conceptual Analysis (4 pts) - 2 Q4s provided

**Q1.** Molecular biologists use small, circular pieces of DNA called plasmids to introduce new DNA into prokaryotic cells through a process called bacterial transformation.

**Part A.**

**Describe** the cellular processes that would allow the DNA of a plasmid to result in a protein.

Researchers investigated the transformation of the bacteria *Escherichia coli* using a plasmid containing a gene for resistance to the antibiotic ampicillin ($Amp^r$). The plasmid also contains a gene that produces green fluorescent protein (eGFP) when the bacteria are grown in the presence of the sugar lactose.

Two cultures of bacteria were prepared:

- **Experimental group (+DNA):** Bacteria received the plasmid.
- **Control group (–DNA):** Bacteria did not receive the plasmid.

To investigate the effect of the plasmid on the growth of transformed bacteria, researchers plated equal volumes of each culture on four different types of agar plates. After the plates were incubated for 24 hours at 37°C, the number of colonies were counted (Table 1).

| Plate | Growth conditions | Number of colonies |
|---|---|---|
| A | LB (nutrient agar), –DNA | 310 |
| B | LB + ampicillin, –DNA | 0 |
| C | LB + ampicillin, +DNA | 68 |
| D | LB + ampicillin + lactose, +DNA | 65 (all fluorescent) |

TABLE 1. NUMBER OF COLONIES OBSERVED AFTER EXPOSURE TO DIFFERENT GROWTH CONDITIONS

**Part B.**

i. **Identify** the dependent variable in the first experiment.

ii. **Justify** the use of Plate A as a positive control in the experiment.

iii. **Describe** the effect on the rate of asexual reproduction when untransformed bacterial cells are grown in the presence of ampicillin.

**Q1 continued.** In a second experiment, researchers used the same strain of *E. coli* as in the first experiment to investigate the uptake of a different plasmid. The plasmid used in this second experiment contains two genes: a gene for resistance to the antibiotic kanamycin ($Kan^r$) and a gene that produces red fluorescent protein (RFP) when the bacteria are grown in the presence of IPTG, a chemical that activates the promoter controlling the gene.

The researchers used standard protocols to heat-shock and transform the bacteria. A second bacterial culture was treated identically but did not receive plasmid DNA. Equal volumes of each culture were plated on different media after recovery. After the plates were incubated for 24 hours at 37°C, the number of colonies were counted (Table 2).

| Plate | Growth conditions | Number of colonies | Red Fluorescence |
|---|---|---|---|
| E | LB (nutrient agar), –DNA | 245 | No |
| F | LB + kanamycin, –DNA | 0 | No |
| G | LB + kanamycin, +DNA | 96 | No |
| H | LB + kanamycin + IPTG, +DNA | 91 | Yes |

TABLE 2. NUMBER OF COLONIES OBSERVED AFTER EXPOSURE TO DIFFERENT GROWTH CONDITIONS

**Part C.**

i. **Identify** an independent variable in the second experiment.

ii. **Calculate** the difference in the number of colonies produced between the transformed bacteria grown on the LB + kanamycin plate and the untransformed bacteria grown on the LB + kanamycin plate.

iii. Based on Table 2, **support** the scientist's claim that the bacteria grown on Plates G and H are genetically identical.

**Part D.**

In a third experiment, researchers transformed bacteria with both the $Amp^r$/eGFP plasmid from the first experiment and grew the bacteria on a LB+kanamycin plate that contained lactose.

i. **Predict** the effect on the growth and color of the transformed bacteria grown on a plate containing LB, kanamycin, and lactose.

ii. **Justify** your prediction.

**Q4.** Bacterial transformation is a process by which prokaryotic, bacterial cells take up plasmids from their surroundings. Plasmids are not bacterial chromosomes. They are separate, smaller circular DNA molecules that exist freely within the cell and contain extra genetic information that isn't essential for the basic functions of the cell.

**Part A.**

**Describe** the structure and location of a prokaryotic chromosome.

The pRSET plasmid is a widely used plasmid vector in molecular biology designed for high-level expression of proteins in bacteria *Escherichia coli* (E.coli). The plasmid contains the gene for ampicillin resistance, $Amp^r$, and a T7 promoter region upstream of an enhanced green fluorescent protein (eGFP) gene.

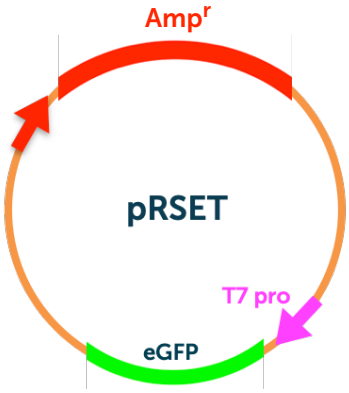



**Figure 1: A diagram of the pRSET plasmid.**

**Part B.**

**Explain how** the presence of the T7 promoter affects the gene expression of the eGFP gene.

A researcher investigated the effectiveness of the pRSET plasmid in transformed e.coli grown in other antibiotics besides ampicillin.

**Part C.**

**Predict** the effects of growing a transformed E.coli population containing the pRSET plasmid in a medium containing penicillin.

**Part D.**

**Justify** your prediction.

**Alternate Q4.** Bacterial transformation is a process by which prokaryotic, bacterial cells take up plasmids from their surroundings. Plasmids are not bacterial chromosomes. The pRSET plasmid, is a widely used plasmid vector in molecular biology designed for high-level expression of recombinant proteins in E. coli. The plasmid contains the gene for ampicillin resistance, $Amp^r$, and a modified eGFP gene controlled by a T7 promoter region as seen in Figure 1.

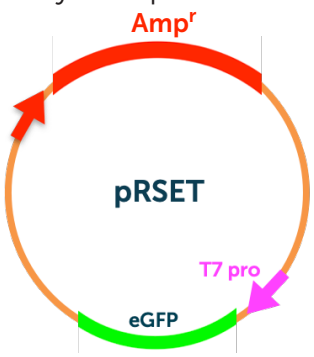



**Figure 1: A diagram of the pRSET plasmid.**

In a particular strain of bacteria the bacterial chromosome has been modified so the lac Z, lac Y, and lacA genes have been removed and replaced with a gene to create the T7promoter as seen in Figure 2.

Key:

- RP = RNA Polymerase
- LR = Lactose Repressor
- lacO = operator
- = Lactose

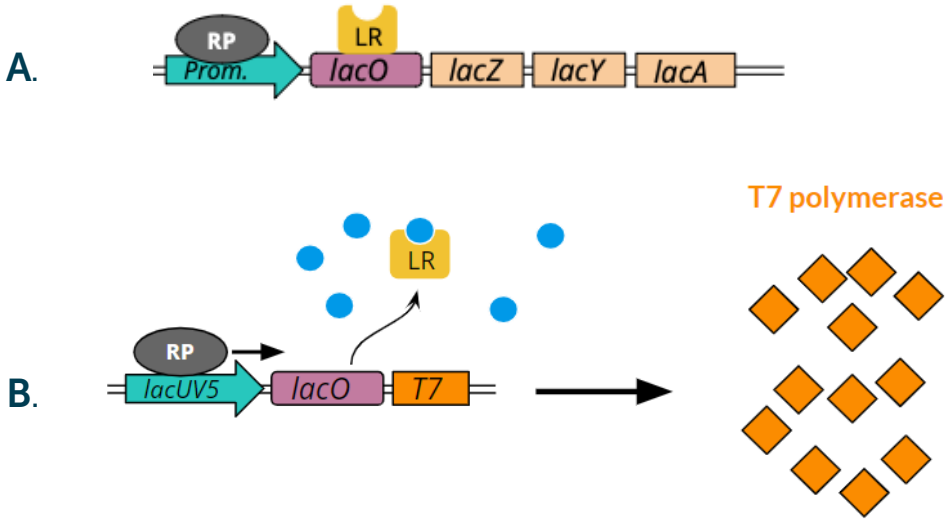



**Figure 2: (A) A simplified model of the lac operon and (B) the modified lac-controlled T7 RNA polymerase expression system.**

**Part A**

**Describe** the process by which genetic information is transferred from DNA to mRNA.

**Part B**

If the bacteria are grown in a media containing ampicillin, **explain how** the transformation of E.coli with the pRSET plasmid subjects the population to artificial selection.

**Part C**

To detect the fluorescent protein coded for by the eGFP gene, the bacteria must be observed using an orange hood and a fluorescent reader called a Winston. **Predict** the effects on the appearance of the E.coli colonies when transformed and grown the E.coli in a media containing ONLY ampicillin.

**Part D**

**Justify** your prediction.

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