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Techniques learned by students to determine which agar coated plates will show bacterial growth and glow.

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Background

- The pGLO plasmid used for this experiment includes a reporter gene called green fluorescent protein, also known as GFP (Bassiri, 2011).
- The GFP gene is found in a jellyfish known as *Aequorea victoria* (Deutch, 2019) .
- This gene can only be expressed when in the presence of arabinose which is what helps the fluorescence when put under a UV light.

Specific Aim

Scientific Aim: Will both the positive and negative pGlO plates show bacterial growth?
Hypothesis: If four agar coated plates are compared on bacterial growth and glow, then the positive plates will show the most growth and glow because they were treated with pGlO plasmid.

Methods

- Creation of PCR in order to replicate DNA containing GFP
- PCR analysis product using agarose gel electrophoresis
- PCR amplicon purification
- DNA insertion containing the GFP gene into the E. *coli* of Agar plates
- Analysis of sequences present within pGLO plasmid using the SnapGene Viewer application
- Plate analysis

Results

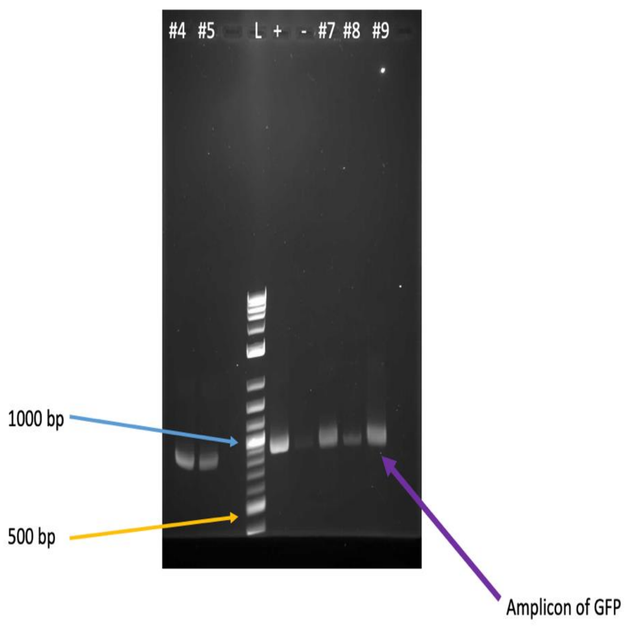

Figure 1. Size of the amplicon of GFP number nine after comparing it to the DNA ladder.

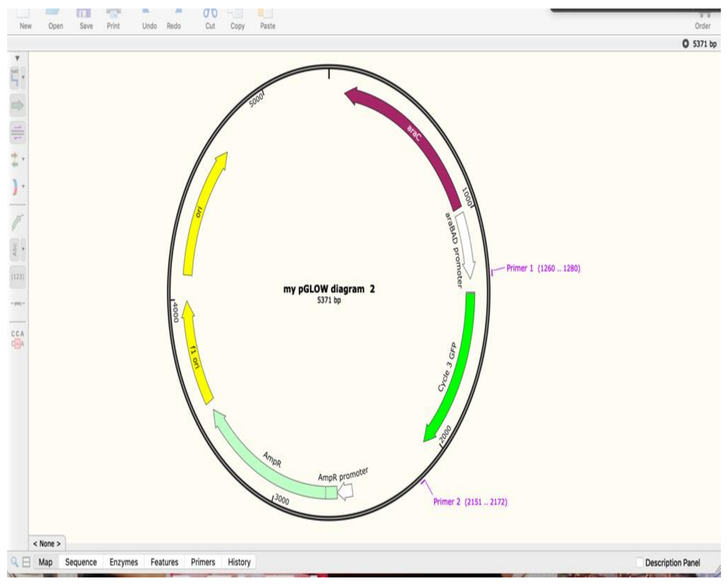

Figure 2. pGLO map including both forward and reverse primer sequences from the SnapGene Viewer application.

Table 1. Results on agar plates

Media on plate	Contains pGLO (yes or no)	Does the plate shown have growth?	If growth is present, how many colonies are there?	If growth is present, do the colonies glow or not?
+pGLO LB/amp	Yes	Yes	672 colonies	No
+pGLO LB/amp/ara	yes	yes	824 colonies	Yes
-pGLO LB	No	Yes	Infinite	No
-pGLO LBamp	no	no	n/a	No

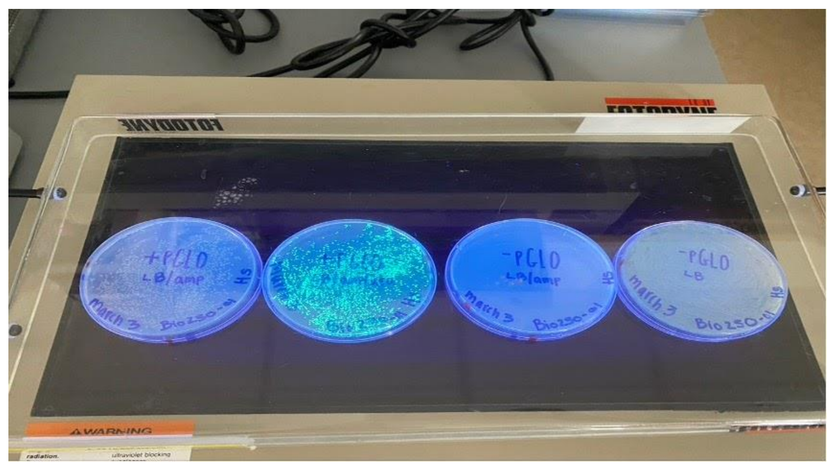

Figure 3. Bacteria growth and glow on +pGLO and -pGLO plates.

Conclusions

- The plate labeled +pGLO LB/amp/ara contained pGLO and had growth shown on the plate and glowed under UV light. The plate labeled +pGLO LB/amp also showed bacterial growth in the form of colonies (Figure 3). In this figure it was also determined that both of the negative plates did have any bacteria growth or glow.
- The amplicon of GFP after comparing it to the DNA ladder and positive and negative charges showed that it 912 bp (T.Vintila, D.Vintila, Dragomirescu, Igna, 2009).
- The pGLO map showed both the forward and reverse primers using the SnapGene Viewer application (Figure2.)
- After reminding ourselves of the hypothesis, that the positively labeled plates would contain the most bacterial growth and after examination, it was concluded that our hypothesis was supported.

References

Bassiri EA. Pglo mutagenesis: a laboratory procedure in molecular biology for biology students. Biochemistry and Molecular Biology Education. 2011;39(6):432–439. doi:10.1002/bmb.20538
DEUTCH CHARLESE. Transformation of escherichia coli with the pglo plasmid: going beyond the kit. American Biology Teacher (university of California Press). 2019;81(1).
T. VINTILA, D. VINTILA, M. DRAGOMIRESCU, V. IGNA. Entrapment of fluorescent e. coli cells in alginate gel. Scientific Papers Animal Science and Biotechnologies. 2009 [accessed 2021 Apr 12];42(1):130–135. INSERT-MISSING-URL