Green Fluorescent Protein Expression from pGLO plasmid within *Escherichia coli*

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Abstract

Over recent years, genetic modification of organisms to glow has become especially popular. In this study, the bacteria *Escherichia coli* will be genetically modified via Green Fluorescent Protein (GFP) from pGLO plasmid. To do this, a polymerase chain reaction (PCR) was used to assure the presence of GFP and agarose gel electrophoresis was then conducted to confirm the PCR reaction's success. The GFP produced via PCR was then purified and sequenced. After sequencing, the information was analyzed and the plasmid was added to the bacteria, *E. coli*. The bacteria was then spread on four agar gel plates containing different compounds and allowed to grow. Two of the four plates contained the pGLO plasmid, whereas the other two received water in place of the pGLO. Plate one contained Luria Broth (LB) and did not have the pGLO plasmid. In plate two, there was LB and ampicillin (amp), but no pGLO plasmid. Plate three was similar to plate two, as it also contained LB and amp, however it also had pGLO plasmid. Plate four contained LB, amp, and arabinose (ara), as well as pGLO plasmid. Plates one, three, and four showed bacterial growth, but only plate four showed GFP gene expression. The findings of this study disproved the hypothesis, as not all plates showed bacterial growth and only one plate showed GFP expression.

Introduction

Over the last 28 years there has been a significant increase in the use of Green Fluorescent Protein, also referred to as GFP, as a reporter for gene expression and a marker for genes within the genetic code of an organism (*Wordeman L. 1999*). Genetic modification using GFP has been used on a variety of organisms, ranging all the way from jellyfish to bacteria

(*Southward and Surette 2002*). The modification of bacteria using GFP in particular has become especially common. It has been done on a variety of bacteria, including but not limited to: *Enterococcus faecalis* and *Bacillus subtilis* (*Arends et al. 2012*). In this particular experiment the bacteria being modified will be the bacteria *Escherichia coli*. For the GFP amplicon to be expressed a plasmid is required. The plasmid being used in this experiment is pGLO, as it contains the β-lactamase gene for ampicillin resistance, which is necessary for expression of the GFP (*Bassiri EA. 2011*). The use of GFP in *E. coli* can be used to demonstrate specific interactions between components with the bacteria; specifically the interactions between sugars and the AraC protein (*Deutch CE. 2019*). In this experiment the focus will be the effects of *E. coli* growth and pGLO GFP expression within the bacteria based on the components of the agar gel plates. It has been hypothesized that there will be bacterial growth regardless of the contents of the plates, and that all samples containing pGLO will show the expression of the GFP gene.

Materials and Methods

Analysis of PCR Product through Agarose Gel Electrophoresis

A 1% agarose gel was prepared and placed into a gel electrophoresis chamber, with the well side of the gel on the black (negative) electrode side and the other end on the red (positive) electrode side. A 0.25 X TAE buffer was then added until the agarose gel was 3 mm under the liquid. Three µl of the pGLO DNA solution produced in the last lab was then measured out using a 20 µl micropipette and added to a tube with 7 µl of a loading buffer solution. The loading buffer solution consists of 2 µl of 6X loading buffer and 5 µl of water. The new solution was then pipetted in and out of the same tube 3 times to ensure the solution was completely mixed, and labeled with a sharpie. Next the tube was sealed and placed into a microfuge for 15

seconds to remove all bubbles from the mixture. All 10 µl of the solution was then pipetted (using the 20 µl micropipette) out of the tube and into one of the wells in the agarose gel. Other DNA solutions were pipetted into the other wells in the gel, along with the positive and negative controls, and the ladder. The lid to the electrophoresis chamber was then placed on the machine. Next, the electrical leads were then hooked up to the electrodes, with black on black and red on red. The power was then turned on and the voltage was adjusted to 300 V and allowed to run for 20 minutes. Once the time was up, the power was turned off, the leads unhooked, and the lid removed. The tray with the gel was then lifted out of the 0.25 X TAE buffer and allowed to drain the excess off. Next, the gel was then placed in a machine and viewed using UV light. Finally, an image of the gel was captured.

Purification of GFP Amplicon

Ninety Seven µl of the pGLO Polymerase Chain Reaction (PCR) sample was added to a 1.5 mL tube containing 500 µl of binding buffer using a 100 µl micropipette. To mix the new solution the same pipette was used to pipette the liquid up and down. Next, a 1000 µl micropipette was used to move the solution into a spin filter column that was placed inside a tube. The tube and column were then labeled using a sharpie, with the appropriate identifying number (7). The column was then sealed and the tube placed inside a centrifuge. The solution was then spun in the centrifuge at 13,000 rpm for one minute. Once that was complete, the tube was removed and the excess liquid in the bottom of the tube was poured out in the sink. Then 200 µl of DNA wash buffer was pipetted into the column using a 200 µl micropipette. The column was then sealed and the tube placed back into the centrifuge for one minute at 13,000 rpm. Once complete, the excess liquid in the tube was then dumped out into the sink. Another

200 µl of the DNA wash buffer was then pipetted into the column, which was then sealed. The tube was then placed back into the centrifuge for another minute at 13,000 rpm. Once complete, the tube was removed and the excess liquid in it was dumped out in the sink yet again. A new, clean 1.5 mL tube was then labeled using a sharpie with the identifying number, and the column was then placed in the new tube. Next, 20 µl of elution buffer was then pipetted into the center of the column using a 100 µl micropipette. The column was then sealed and allowed to sit, undisturbed, for one minute. Next, the tube was then placed into the centrifuge once more, and ran for one minute at 13,000 rpm. The column was then removed from the tube and disposed of.

Two µl of the remaining liquid in the tube was then pipetted out using a 20 µl micropipette and onto the bottom contact point of the nanodrop machine. The nanodrop machine was then run and the DNA concentration of the purified pGLO PCR sample was gathered, along with the A260/A280 ratio.

Two sequencing tubes were then gathered and 5 ul of a 4 µM primer (one for the forward primer and one for the reverse primer) for pGLO was added to it. The purified pGLO DNA sample gathered earlier was then diluted with water to 30ng/µl by mixing 7.1 µl of the solution with 7.9 µl of water. Five µl of the diluted solution was then placed in two sequencing tubes. Next, the tubes were then sent overnight to Louisville, Kentucky to be sequenced by Eurofins Genomics.

Sequencing and BLAST Analysis

Using one of the PDF files provided, the trace files within the GFP amplicon in pGLO are able to be viewed. A usable sequence from the PDF was then screenshot.

Next, using the two .seq files provided, the forward and reverse sequences of pGLO (in FATSA form) were gathered and put in a Word document. The website http://doua.prabi.fr/software/cap3 was then navigated to (on 2/24/2021), and the forward and reverse sequences were entered into the text box and run. The "contig" option was then selected and the contig for GFP was found and added to the Word document, in FATSA form. Then the website https://blast.ncbi.nlm.nih.gov/Blast.cgi was then navigated to (on 2/24/2021) and the "Nucleotide BLAST" option was chosen. The contig sequence was then entered into the textbox and the "BLAST" button was selected. Once the page was loaded, the top five matches within the sequence were then recorded, and a screenshot was taken.

The GFP sequence, which was the highest match, was then chosen. This sequence had an E-value of 0. The alignments of the GFP sequence and the contig were then found and recorded via screenshot (Figure 2.).

The "Accession" tab was then selected. The "FASTA" button was chosen and the format of the information changed to the FASTA format. The "Sent to" button was then clicked and the "File" option was chosen and the information was saved in a Word document.

The SnapGene application was then downloaded from the internet and the program was opened. Then the "New DNA File..." option was selected and the GFP contig DNA sequence was entered into the textbox. The "OK" button was then chosen and when prompted to add 3 features the "Cancel" option was clicked. Next, the DNA form was changed from "Map" to "Sequence." Once the page changed, the "Show Translations" tab (with the green and orange arrows) was opened, and the "ORF's Only" option was selected. A document containing the forward (F1) and reverse (R1) primers of the GFP amplicon that was used in a previous lab was then opened. The F1 primer was then copied and then found on the SnapGene file using the

control/command F function. This sequence was then highlighted in the contig on the Word document. Next, the R1 primer was translated for the bottom strand and found within the SnapGene file using control/command F once again. The R1 primer was then highlighted in the contig as well. Using the highlighted codon sequences the start and stop codons of the GFP sequence were found and also highlighted within the contig.

Transformation of Bacteria

Two microtubes containing 10 µl of thaw competent were provided and kept on ice. Using a sharpie, one was labed "-7" and the other labeled "+7" in order to keep track of the one containing pGLO and to who the sample belonged to. Five µl of pGLO plasmid DNA (30 ng/µl solution) was then pipetted, using a micropipette, into the "+7" tube. Then, 5 µl of sterile water was then pipetted to the "-7" tube. The tubes were then incubated on ice for 20 minutes. During this time, 4 aga plates were gathered and labeled based on their components. Once the time was up, the microtubes were then placed in a water bath at 42^{o}C for exactly 30 seconds, and then returned to ice and allowed to rest for 5 minutes. Next, 250 µl of SOC media was pipetted into each microtube. The tubes were then transferred to a water bath at 37^{o}C for incubation, where they remained for 40 minutes. After the time was up, the tubes were removed from the water bath and placed on a rack. Each tube was then inverted twice to ensure they were properly mixed. Then 100 µl of the solution in the "+7" tube was pipetted onto an agar plate labeled "+pGLO" and then spread across the gel using a sterile loop for a minute, and sealed. This process was repeated once more. Next, 100 µl of the solution in the "-7" tube was pipetted onto an agar plate that was labeled "-pGLO" and then spread among the gel in the same method, and sealed. This process was also repeated once more. The plates were then stacked, flipped upside

down, taped together, and then placed in an incubator set to 37ºC. They remained in the incubator for 24-48 hours in order for bacterial growth to occur.

The SnapGene viewer program was opened, and the "New DNA File…" option was selected. Next, the pGLO sequence gathered from the pGLO document was then copied and pasted into the text box. The "OK" button was then chosen. A features menu then popped up and the 12 features and their location were then recorded. Seven of the features were then found to have a direction and it was recorded as well. The 5 without a direction were then unselected and the "Add Features" option was clicked. On the new screen, the "Choose enzyme set" tab on the left was opened and the "choose enzyme" option was selected. A new window then popped up and the "remove all" button on the right was clicked, and then followed by the "OK" option. Then the backbone of the plasmid at the 5' end of the GFP was clicked, and the "Primers" menu was opened and the "add primer" option was chosen. On the new window, the forward primer, gathered from the pGLO document, was pasted into the textbox. The "add primer to template" button was then clicked. This process was repeated on the 3' end of the GFP and using the reverse primer. Once this was complete, a screenshot of the Map was taken. Next, the sequences of the araC gene, AmpR gene, and Fori gene were then copied and a BLAST was run for each. The results of the BLAST were then recorded via screenshots.

The plates were incubated for 24 hours, after which they were stored at 4 degrees Celsius. They were then retrieved and placed upside down on a UV trans-illuminator box. The lid of the box was then shut and the UV light was switched on. Doing this allowed one to see whether or not the bacteria would glow. A photo of the plates in the UV trans-illuminator box was then taken. The light was then shut off and the plates were removed. Next, the plates were sealed with parafilm paper and the colonies of bacteria in each cell were counted and recorded.

Lastly, the plates were taped together and returned to the refrigerator.

Results

Analysis of PCR Product through Agarose Gel Electrophoresis

The GFP product of a polymerase chain reaction (PCR) was run, which produced a complete agarose gel visible in Figure 1. The GFP amplicon size within pGLO is shown (Figure 1) to be 912 base pairs long. This proves that the PCR solution is accurate in accordance to the Ladder, as sample 7 is correctly placed between 900 and 1000 base pairs.

GFP Amplicon

After purification of the GFP amplicon, the concentration of the purified pGLO DNA sample was found to be 63.4 ng/µl with an A260/A280 ratio of 1.79. The necessary quantity of the solution to be added to the sequencing tube was found to be 7.1 µl, using the $C_1V_1=C_2V_2$ formula. The amount of water required to dilute the sample to the correct ratio of 30 ng/µl was then established to be 7.9 µl.

DNA Sequencing Set-up

A usable sequence of the trace files within the GFP amplicon in pGLO was found (Figure 2). The GFP sequence in pGLO from the PCR reaction was determined and the alignment found in FASTA format (Figure 3). This figure highlights both primers, along with the start and stop codons within the GFP contig.

DNA Sequencing Analysis

From the contig, the E-value of the GFP gene was determined to be 0 and it had a percentage match of 96.89% (Figure 4). The GFP sequence in pGLO from the PCR reaction was determined and the alignment found (Figure 5). This shows how all of the nucleotide bases line up in reference to the contig and the GFP sequence. The alignments of AmpR (bla) (Figure 6), fori (coli) (Figure 7), and araC (pBAD) (Figure 8) were also found. A MAP sequence of the pGLO was produced and the locations of the Forward (F1) and Reverse (R1) primers of GFP were made visible, along with the gene's direction (Figure 9). This figure makes the presence of other features within pGLO and their direction viewable. The features, other than GFP, being shown are araBAD, AmpR promoter, AmpR, f1 ori, and ori.

Transformation of Bacteria

The bacteria within the agar plates was transformed and the overall results of the experiment were found. Only under a UV light could the results be found, an image of which is viewable as Figure 10. The specificity of the results and the image can be seen in Table 1, where the contents of the agar gel and the overall plates are recorded.

Discussion

The effects of *E. coli* growth and pGLO GFP expression within the bacteria based on the components of the agar gel plates were found. The hypothesis was disproved as not all plates showed bacterial growth and not all containing pGLO showed GFP expression. Plate 2 showed no bacterial growth, this was due to the plate containing an antibacterial agent, ampicillin, and the bacteria did not possess the plasmid which contains the antibacterial resistance. This proved the efficiency of the antibacterial agent and that the plasmid does indeed work. On the other hand, Plate 1 had an infinite amount of growth even though it did not possess the plasmid, this was due to the lack of an antibacterial agent within the agar gel. Plate 3 did grow but was unable

to glow. It had the ability to grow as it possessed the pGLO plasmid necessary to resist the antibacterial agent, however it lacked the necessary compound for the GFP gene to be expressed. Plate 4 contained both the pGLO plasmid and the necessary compound, arabinose, for GFP gene expression. This allowed it to grow and glow. The findings of this experiment are in alignment with those of *Deutch CE. 2019*, and *Southward and Surette 2002*.

This experiment could be improved upon by completing more trails; doing so would provide the most accurate data, specifically how much growth there was on each plate. Another improvement that could be made is testing other compounds within the agar gel. This would provide a more detailed answer as to why the bacteria was or was not able to grow and glow.

Given all the possible improvements, the results of this version of the experiment are still relevant and important. It leads the way for further research on this topic, as it provides known information that will allow for new experiments to be conducted off of. It also demonstrates the success of antibacterial agents and helps prove the effect of antibacterial resistance. This information could be used for further experiments on such components and their usefulness for the future.

Figures and Tables

Figure 1.

This is the agarose gel after gel electrophoresis was performed. The Ladder, Quick-load 2-log DNA ladder (0.1-10kb) from Neb England BioLabs, is shown in the middle and labeled "L." The "pos" label represents the positive control and the "neg" represents the negative control. Samples 7 and 5 contain the pGLO sample.

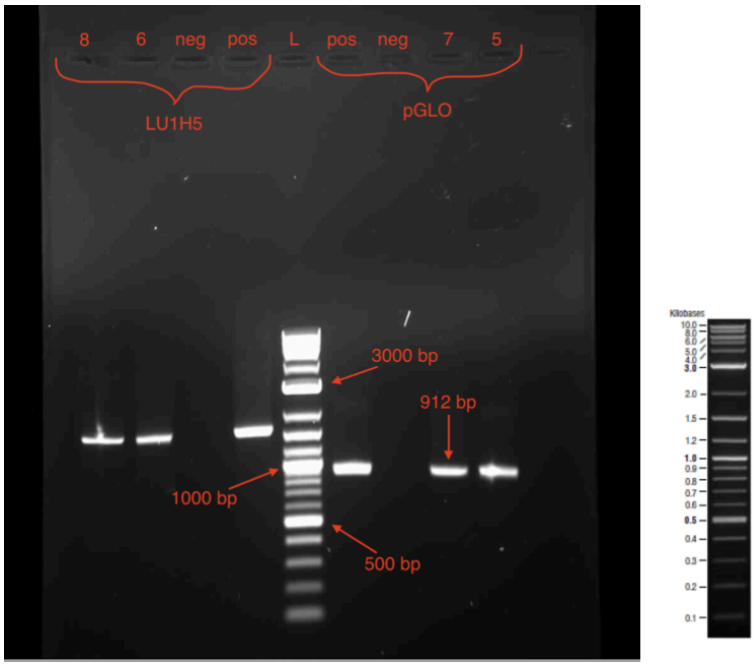


Figure 2.

Below is a chromatogram of a section of the pGLO plasmid. This shows that our pGLO sample was a clean one.

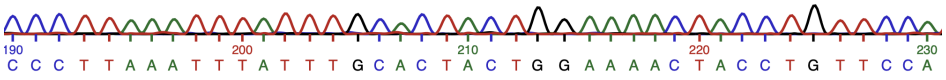


Figure 3.

This is a FASTA format of the pGLO sequence with the forward and reverse primers for the GFP highlighted, as well as the start and stop codons.

>Contig1
NNNNNNTNNACCNAACCNGGTNNCCCCCGNTNNTAAAAGCATTNNNNNNACNAAGNGGGNN
CNANNCCATGACAAAAACGCGNANNAAAAAGNNNCTANATCNCGGCNGAAAAGTCCACAT
TGATTATTTGCNNGGCGTCACACTTTGCTATGCCATAGCATTTTTATCCATAAGATNAGC
GGATCCTANNGACGCTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTTTTGGG
CTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCTAGCAAAGGAGAAG
AACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACA
AATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCTACATACGGAAAGCTTACCCTTAAAT
TTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCTCTT
ATGGTGTTCAATGCTTTTCCCGTTATCCGGATCATATGAAACGGCATGACTTTTTCAAGA
GTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGGGAACT
ACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAA
AAGGTATTGATTTTAAAGAAGATGGAAACATTCTCGGACACAAACTCGAGTACAACTATA
ACTCACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTTCA
AAATTCGCCACAACATTGAAGATGGATCCGTTCAACTAGCAGACCATTATCAACAAAATA
CTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAATCTG
CCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTG
CTGCTGGGATTACACATGGCATGGATGAGCTCTACAAATAATGAATTCGAGCTCGGTACC
CGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCTGTTTTGGCGGATGAGAG
AAGATTTTCAGCCTGATACAGATTAAATCAGAACGCAGAAGCGGTCTGATAAAACAGAAT
TTGCCTGGCGGCAGTANCGCGGNGGTCCCACCNGACCCCNATGCCGAANCTCANAAANTG
AAACNCCGTANCNNCGATGGTAGNGNNGGGGNCNTCCCCCATGCGAGANTAGNNNNGNCN
NGNNATCAAATAAAANNNAANNNNNNNNNCNAANGANTGNNNNNNNTTTTNNTCNNNNNN
TTNGNCGGNGNANN
– Forward Primer
– Start Codon
– Stop Codon
– Reverse Primer

Figure 4.

This is the BLAST search results, which show the top 5 matches to the FASTA sequence.

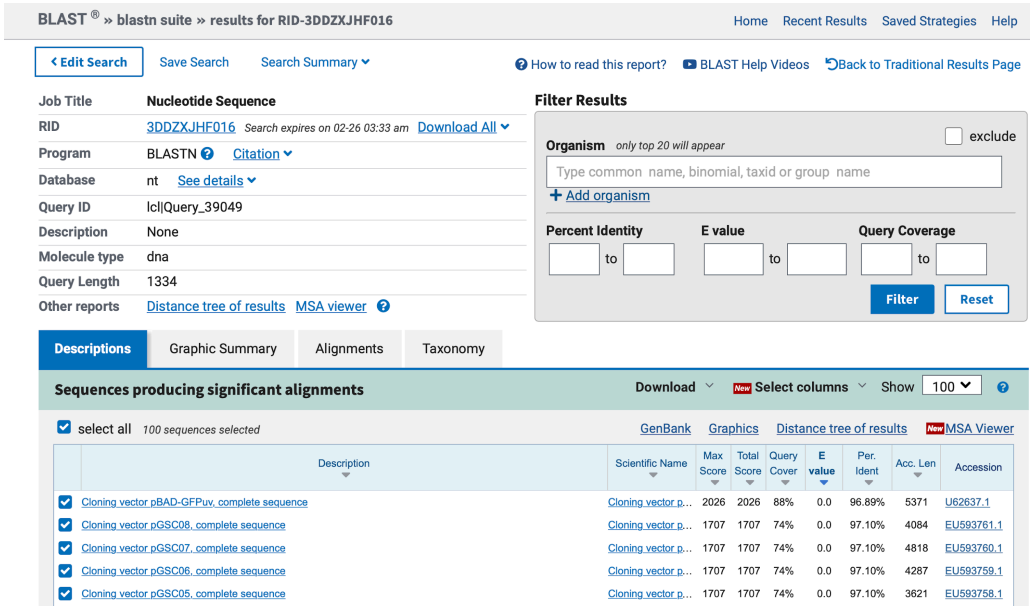
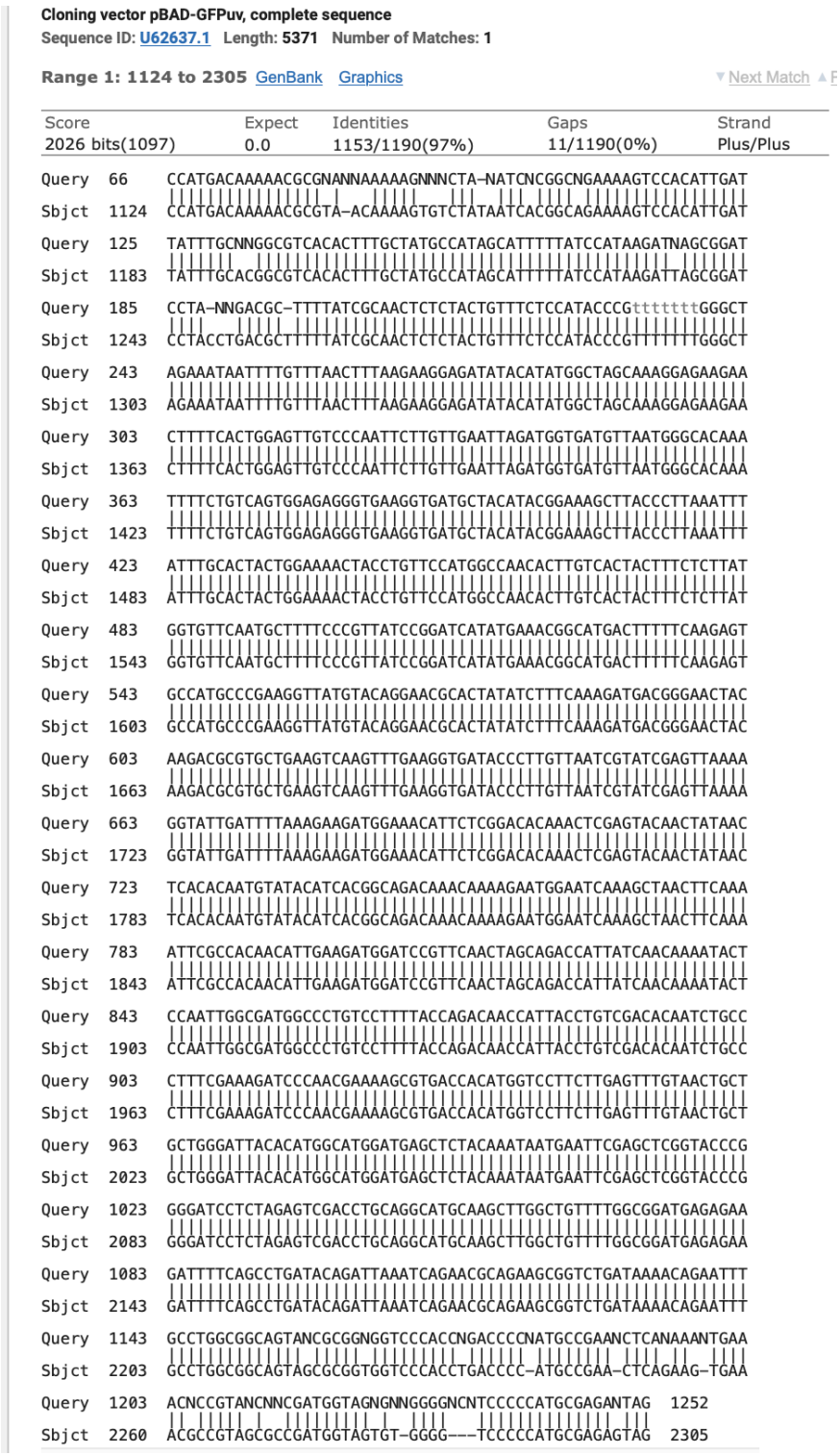


Figure 5.

This is the sequence line up of the GFP gene and a section of the FASTA sequence, gathered using a BLAST analysis.



Cloning vector pBAD-GFPuv, complete sequence
Sequence ID: U62637.1 Length: 5371 Number of Matches: 1

Range 1: 1124 to 2305 GenBank Graphics ▼ Next Match ▲ P

Score	Expect	Identities	Gaps	Strand
2026 bits(1097)	0.0	1153/1190(97%)	11/1190(0%)	Plus/Plus

```
Query  66    CCATGACAAAAACGCGNANNAAAAAGNNNCTA-NATCNCGGCNGAAAAGTCCACATTGAT
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Sbjct  1124  CCATGACAAAAACGCGTA-ACAAAAGTGTCTATAATCACGGCAGAAAAGTCCACATTGAT

Query  125   TATTTGCNNGGCGTCACACTTTGCTATGCCATAGCATTTTTATCCATAAGATNAGCGGAT
             |||||||  ||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  1183  TATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTTTTATCCATAAGATTAGCGGAT

Query  185   CCTA-NNGACGC-TTTTATCGCAACTCTCTACTGTTTCTCCATACCCGttttttGGGCT
             ||||  |||||  ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1243  CCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTTTGGGCT

Query  243   AGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCTAGCAAAGGAGAAGAA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1303  AGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCTAGCAAAGGAGAAGAA

Query  303   CTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAA
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Sbjct  1363  CTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAA

Query  363   TTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCTACATACGGAAAGCTTACCCTTAAATTT
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Sbjct  1423  TTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCTACATACGGAAAGCTTACCCTTAAATTT

Query  423   ATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCTCTTAT
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Sbjct  1483  ATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCTCTTAT

Query  483   GGTGTTCAATGCTTTTCCCGTTATCCGGATCATATGAAACGGCATGACTTTTTCAAGAGT
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Sbjct  1543  GGTGTTCAATGCTTTTCCCGTTATCCGGATCATATGAAACGGCATGACTTTTTCAAGAGT

Query  543   GCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGGGAACTAC
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Sbjct  1603  GCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGGGAACTAC

Query  603   AAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1663  AAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAA

Query  663   GGTATTGATTTTAAAGAAGATGGAAACATTCTCGGACACAAACTCGAGTACAACTATAAC
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1723  GGTATTGATTTTAAAGAAGATGGAAACATTCTCGGACACAAACTCGAGTACAACTATAAC

Query  723   TCACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTTCAAA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1783  TCACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTTCAAA

Query  783   ATTCGCCACAACATTGAAGATGGATCCGTTCAACTAGCAGACCATTATCAACAAAATACT
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Sbjct  1843  ATTCGCCACAACATTGAAGATGGATCCGTTCAACTAGCAGACCATTATCAACAAAATACT

Query  843   CCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAATCTGCC
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Query  903   CTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTGCT
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query  963   GCTGGGATTACACATGGCATGGATGAGCTCTACAAATAATGAATTCGAGCTCGGTACCCG
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Query  1023  GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCTGTTTTGGCGGATGAGAGAA
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Query  1143  GCCTGGCGGCAGTANCGCGGNGGTCCCACCNGACCCCNATGCCGAANCTCANAAANTGAA
             |||||||||||||| |||||| |||||||||| ||||||| |||||| |||| ||  ||||
Sbjct  2203  GCCTGGCGGCAGTAGCGCGGTGGTCCCACCTGACCCC-ATGCCGAA-CTCAGAAG-TGAA

Query  1203  ACNCCGTANCNNCGATGGTAGNGNNGGGGNCNTCCCCCATGCGAGAGTAG  1252
             || |||||  |  |||||||||  |  ||||   ||||||||||||||| |||
Sbjct  2260  ACGCCGTAGCGCCGATGGTAGTGT-GGGG---TCCCCCATGCGAGAGTAG  2305
```
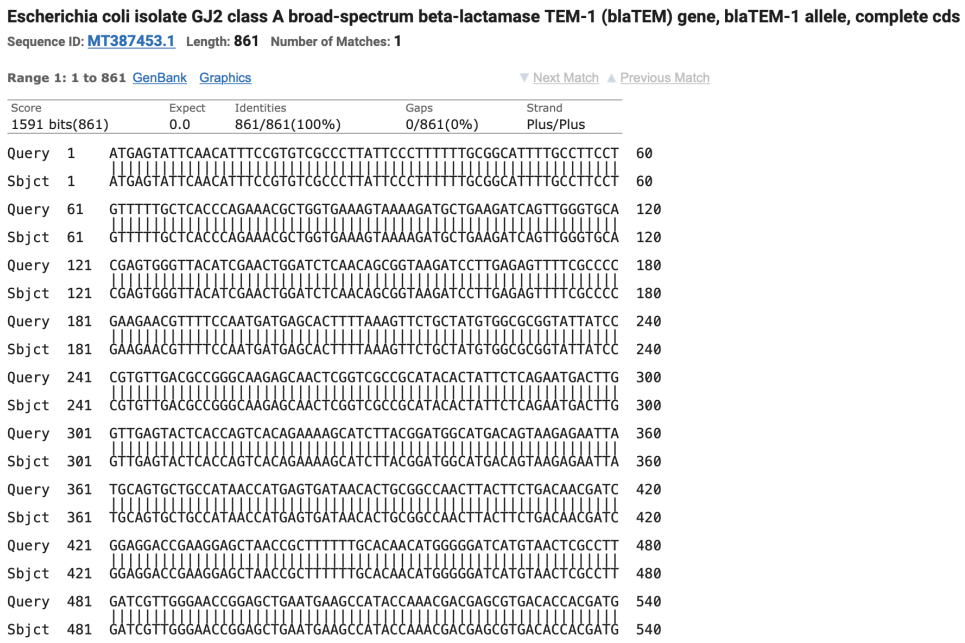

Figure 6.

Below is the sequence line up of the ampicillin resistance (AmpR) and a section of the FASTA, which was found using a BLAST analysis.



Escherichia coli isolate GJ2 class A broad-spectrum beta-lactamase TEM-1 (blaTEM) gene, blaTEM-1 allele, complete cds

Sequence ID: MT387453.1 Length: **861** Number of Matches: **1**

Range 1: 1 to 861 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1591 bits(861)	0.0	861/861(100%)	0/861(0%)	Plus/Plus

```
Query  1    ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCT  60
Sbjct  1    ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTGCGGCATTTTGCCTTCCT  60

Query  61   GTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCA  120
Sbjct  61   GTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCA  120

Query  121  CGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCC  180
Sbjct  121  CGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCC  180

Query  181  GAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCC  240
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Query  241  CGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTG  300
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Query  301  GTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTA  360
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Query  361  TGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATC  420
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Query  421  GGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTT  480
Sbjct  421  GGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTT  480

Query  481  GATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATG  540
Sbjct  481  GATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATG  540
```
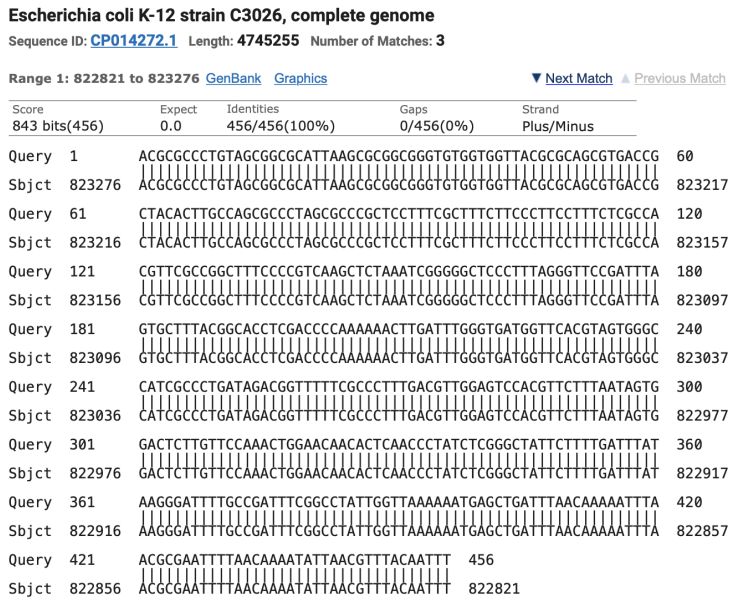

Figure 7.

This shows the alignment of E. coli (fori) and the FASTA sequence, gathered from a BLAST analysis.



Escherichia coli K-12 strain C3026, complete genome

Sequence ID: CP014272.1 Length: **4745255** Number of Matches: **3**

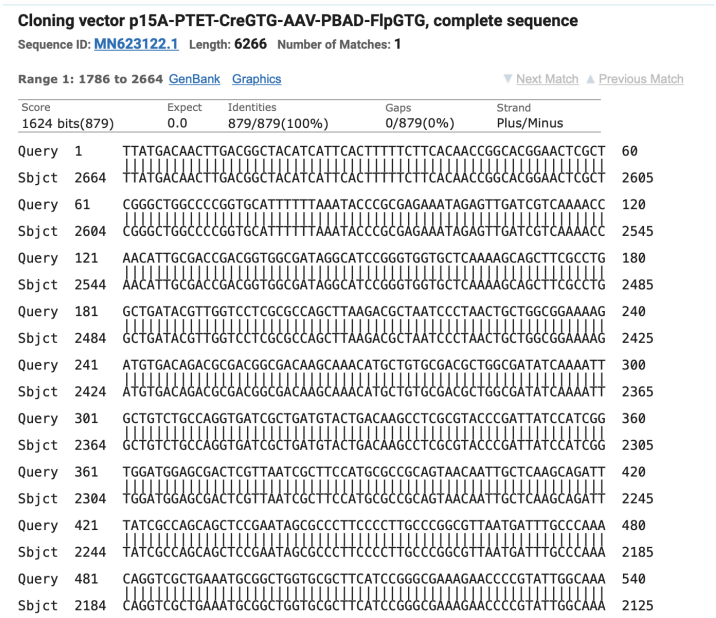
Range 1: 822821 to 823276 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
843 bits(456)	0.0	456/456(100%)	0/456(0%)	Plus/Minus

```
Query  1       ACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG  60
Sbjct  823276  ACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG  823217

Query  61      CTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCA  120
Sbjct  823216  CTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCA  823157

Query  121     CGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTA  180
Sbjct  823156  CGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTA  823097

Query  181     GTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGC  240
Sbjct  823096  GTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGC  823037

Query  241     CATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTG  300
Sbjct  823036  CATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTG  822977

Query  301     GACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGGCTATTCTTTTGATTTAT  360
Sbjct  822976  GACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGGCTATTCTTTTGATTTAT  822917

Query  361     AAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTA  420
Sbjct  822916  AAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTA  822857

Query  421     ACGCGAATTTTAACAAAATATTAACGTTTACAATTT  456
Sbjct  822856  ACGCGAATTTTAACAAAATATTAACGTTTACAATTT  822821
```

Figure 8.

Below is the alignment of arabinose (araC) and the FASTA sequence, which was found via a BLAST analysis.



Cloning vector p15A-PTET-CreGTG-AAV-PBAD-FlpGTG, complete sequence

Sequence ID: MN623122.1 Length: 6266 Number of Matches: 1

Range 1: 1786 to 2664 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1624 bits(879)	0.0	879/879(100%)	0/879(0%)	Plus/Minus

```
Query  1     TTATGACAACTTGACGGCTACATCATTCACTTTTTCTTCACAACCGGCACGGAACTCGCT  60
Sbjct  2664  TTATGACAACTTGACGGCTACATCATTCACTTTTTCTTCACAACCGGCACGGAACTCGCT  2605
Query  61    CGGGCTGGCCCCGGTGCATTTTTTAAATACCCGCGAGAAATAGAGTTGATCGTCAAAACC  120
Sbjct  2604  CGGGCTGGCCCCGGTGCATTTTTTAAATACCCGCGAGAAATAGAGTTGATCGTCAAAACC  2545
Query  121   AACATTGCGACCGACGGTGGCGATAGGCATCCGGGTGGTGCTCAAAAGCAGCTTCGCCTG  180
Sbjct  2544  AACATTGCGACCGACGGTGGCGATAGGCATCCGGGTGGTGCTCAAAAGCAGCTTCGCCTG  2485
Query  181   GCTGATACGTTGGTCCTCGCGCCAGCTTAAGACGCTAATCCCTAACTGCTGGCGGAAAAG  240
Sbjct  2484  GCTGATACGTTGGTCCTCGCGCCAGCTTAAGACGCTAATCCCTAACTGCTGGCGGAAAAG  2425
Query  241   ATGTGACAGACGCGACGGCGACAAGCAAACATGCTGTGCGACGCTGGCGATATCAAAATT  300
Sbjct  2424  ATGTGACAGACGCGACGGCGACAAGCAAACATGCTGTGCGACGCTGGCGATATCAAAATT  2365
Query  301   GCTGTCTGCCAGGTGATCGCTGATGTACTGACAAGCCTCGCGTACCCGATTATCCATCGG  360
Sbjct  2364  GCTGTCTGCCAGGTGATCGCTGATGTACTGACAAGCCTCGCGTACCCGATTATCCATCGG  2305
Query  361   TGGATGGAGCGACTCGTTAATCGCTTCCATGCGCCGCAGTAACAATTGCTCAAGCAGATT  420
Sbjct  2304  TGGATGGAGCGACTCGTTAATCGCTTCCATGCGCCGCAGTAACAATTGCTCAAGCAGATT  2245
Query  421   TATCGCCAGCAGCTCCGAATAGCGCCCTTCCCCTTGCCCGGCGTTAATGATTTGCCCAAA  480
Sbjct  2244  TATCGCCAGCAGCTCCGAATAGCGCCCTTCCCCTTGCCCGGCGTTAATGATTTGCCCAAA  2185
Query  481   CAGGTCGCTGAAATGCGGCTGGTGCGCTTCATCCGGGCGAAAGAACCCCGTATTGGCAAA  540
Sbjct  2184  CAGGTCGCTGAAATGCGGCTGGTGCGCTTCATCCGGGCGAAAGAACCCCGTATTGGCAAA  2125
```

Figure 9.

This is a gene map of the pGLO plasmid, which was found using the SnapGene Viewer application.

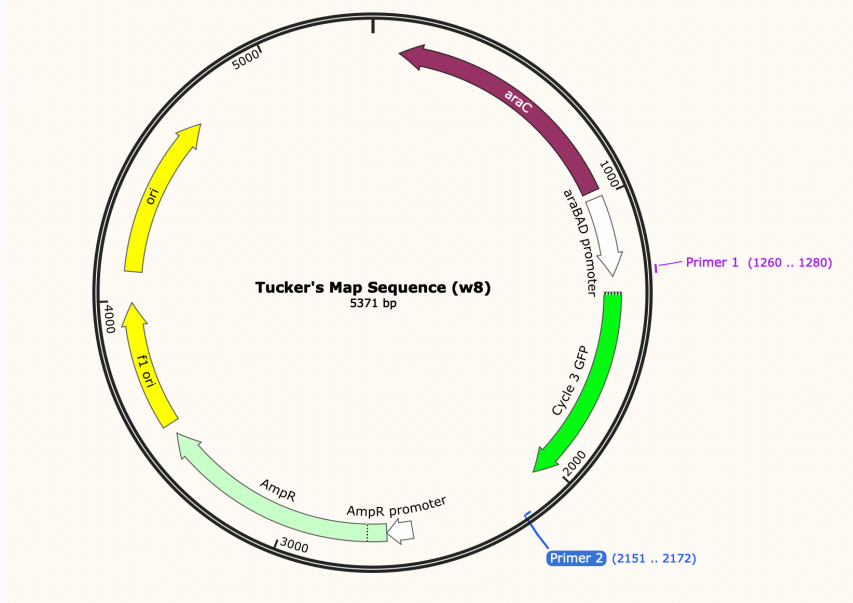
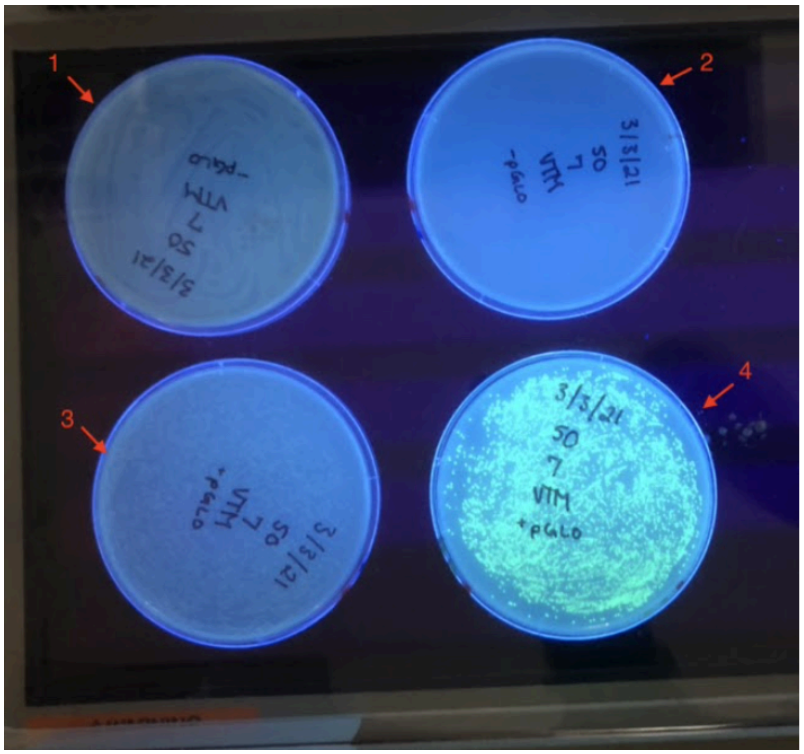


Figure 10.

Below is an image of the complete agar gel plates on top of a UV light box. Plate 1 has growth but doesn't glow, plate 2 has no growth, plate 3 has growth but no glow, and plate 4 has both growth and glow.



Legend	
1	LB
2	LB + amp
3	LB + amp
4	LB + amp + ara

The table below shows the contents of each agar plate, as well as it's results.

Table 1.

Plate Number	Plate Contents	pGLO	Growth	Amount of Colonies	Glow
1	LB	No	Yes	Infinite	No
2	LB + amp	No	No	N/A	N/A
3	LB + amp	Yes	Yes	2,440	No
4	LB + amp + ara	Yes	Yes	1,680	Yes

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