The Insertion of PpbrA into *E. coli* to Detect Lead in Gunshot Residue

William Kish

Department of Environmental and Biological Sciences

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Abstract

Synthetic biological systems are created through genetically modifying natural biological systems to perform certain tasks. One useful way synthetic biology can be used for real world implications would be for the detection of gunshot residue (GSR). Lead is a common metal found in GSR and the PpbrA promoter is able to detect lead. Through the transformation of *E. coli* and insertion of the PpbrA promoter, *E. coli* would be able to detect the lead in GSR. Once detected, our modified *E. coli* would expressed red fluorescent protein (RFP) which turned the *E. coli* reddish orange. This mechanism would be useful in the forensic field by providing quick and accurate tests for GSR in the field. This project concluded the third part MicRobo Cop research project which now allows for all three parts to be combined and field tested.

Introduction

Synthetic biology is the creation of biological systems to perform a certain task that otherwise would not occur in a nature setting (Serrano, 2007). One way this is done is by using bacteria and genetically adding mechanisms not commonly found in bacteria. For example, Levskaya et al. (2005) engineered *Escherichia coli* (*E. coli)* to signal in the presences of red light by adding in phycocyanobilin to the *E. coli*.

An application of synthetic biology would be to advance the detection of gunshot residue (GSR). GSR consists of particles produced by the firearm upon firing and is composed of burnt propellant, grease, smoke, ammunition primers, lubricants, and metals (Dalby et al. 2010). A popular way to test for GSR is to use electron microscopy/energy-dispersive X-ray (SEM/ EDX) (Zeichner et al. 1991). This is a popular method because it can detect multiple compounds like lead (Pb), antimony (Sb), and barium (Ba) from the GSR (Weber et al. 2014). Another method for detection would be laser- induced breakdown spectroscopy, which is able to detect Pb, Ba, and Sb with the drawback of error in positive and negative tests (Dockery and Goode, 2003). Drawbacks for these methods involve performing the tests in the lab which could take time.

A way synthetic biology would be able to aid in the detection of GSR would be to develop a mechanism that can detect GSR on the body of the shooter. For this to happen, bacteria would need to be genetically modified to be able to detect the lead in GSR (Porter et al.

2018). The plasmid J10060 has been found to have red fluorescent protein in it which will aid in the detection of GSR. The promoter for a lead binding protein, PpbrA, needed to be inserted into *E. coli* for it to have the specific lead resistant mechanism (Borremans et al. 2001). When Ppbra is inserted into *E. coli*, it has been shown to express the lead binding promoter (Hobman et al. 2012). Once the lead binding promoter is switched on in the presence of lead, the red fluorescent protein will be expressed. Once the red fluorescent protein is expressed, that would signify that there is lead.

In this project, the arabinose sensitive promoter was replaced for a lead detecting promoter inside of *E. coli.* With this new promoter, the *E. coli* would be able to glow red in the presence of lead inside of GSR with the red fluorescent protein. This synthetic mechanism would aid in the detection of GSR on suspects and increase the detection period of GSR.

Methodology

Plasmid Isolation

To isolate the J10060 plasmid, 1.5 mL of cultured *E. coli* was centrifuged for 1 minute and the supernatant was discarded. Two hundred microliters of PD1 Buffer was added and resuspended followed by 200 µL of PD2 Buffer and was let to incubate at room temperature for 2 minutes. Three hundred microliters of PD3 Buffer was added and mixed immediately by inversion and centrifuged for 3 minutes. A PD column was placed into a 2 mL collection tube, the supernatant from earlier was added to the PD column and centrifuge for 30 seconds. The flow through was discarded and the PD column was placed into the 2 mL collection tube. Four hundred microliters of W1 Buffer was added into the PD column and microcentrifuged for 30 seconds. The flow through was discarded and the PD column was placed back into the 2 mL collection tube. Six hundred microliters of wash buffer was added to the PD column and

microcentrifuged for 30 seconds. The flow through was discarded and the PD column was placed back into the 2 mL collection tube and microcentrifuged for 3 minutes. The dried PD column was transferred to a new microcentrifuge tube and then 50 µL of Elution Buffer was added. The column was let to sit for 2 minutes and then microcentrifuge for 2 minutes.

Ligation and Transformation

The following reaction was set up in a microcentrifuge tube on ice: 2 µL of 10X T4 DNA Ligase Buffer, 3 µL of Plasmid DNA (3 kb), 10 µL PpbrA DNA (0.7 kb), 4 µL Nuclease-free water, and 1 µL of T4 DNA Ligase. The reaction was then gently mixed and incubated at room temperature for 10 minutes and heat inactivated at 65°C for 10 minutes.

A tube of 20 µL NEB 5-alpha Competent *E. coli* cells was thawed on ice. Five microliters of plasmid DNA was added to the cell mixture and was made sure not to be vortexed. The mixture was then placed on ice for 2 minutes and then heat shocked at exactly 42°C for exactly 30 seconds. Mixture was then placed on ice for 2 minutes then 380 µL of room temperature SOC was pipetted into the mixture. The mixture was then immediately spread onto a Luria Broth agar plate containing ampicillin and incubated overnight at 37°C.

Colony Polymerase Chain Reaction (PCR)

The following reaction was set up into 4 separate PCR tubes: 0.5 µL of 10 µM Forward Primer (5'- GCCGCTTGAATTCGTCATATAT-3'), 0.5 µL of 10 µM Reverse Primer (5'- GCCGCTTGAATTCGTCTAGACT- 3'), 12.5 µL One*Taq* 2X Master Mix, and 11 µL Nuclease-free water. A colony from the transformed *E. coli* was scraped onto a new Luria Broth agar plate containing ampicillin as well as swirled into the PCR mix. These steps were repeated with all 4 PCR tubes. The tubes were then transferred to the PCR machine and thermocycling began with the following steps: the initial denaturation was set at 94°C for 30 seconds, then 30 cycles at

94°C, 55°C, and 68°C respectively for 30 seconds, 45 seconds, and 60 seconds respectively, then the final extension was at 68°C for 5 minutes, then left to hold at 4°C.

Gel Electrophoresis

Five microliters of loading buffer was added into each PCR tube. The electrophoresis chamber was filled with the 1X TAE buffer (about 275 mL of buffer) covering the gel. Ten microliters of each sample was loaded into separate wells of the gel. The gel was then ran at 120 V for 30 minutes using the NEB 100 bp DNA ladder. Once the gel was completed, the gel was then visualized under a UV camera.

Plasmid Isolation

A colony from the J10060 plasmid with the PpbrA promoter was selected for isolation using the same isolation methods as mentioned before.

Function Check

E. coli was streaked onto a LB/ampicillin agar plate containing no lead and one plate with LB/ampicillin/lead agar plate (100μM). The plates were then incubated at 37°C for 24 hours. The plates were then checked for expression of the Red Fluorescent Protein (RFP).

DNA Sequencing

To identify if there was a proper ligation of the PpbrA promoter upstream of the RFP gene in the J10060 plasmid, the DNA was sent out to sequencing to Eurofins Genomics. Our plasmids were mixed with a forward and reverse primer. The forward primer was 5'-GTAAAACGACGGCCAGTG-3' and the reverse primer was 5'-GGAAACAGCTATGACCATG-3'. Four microliters of the sequencing primer was placed into the sequencing tube along with 5μL of J10060 plasmid with the PpbrA promoter and 3μL of deionized water.

Results

After a successful transformation and PCR, the results from the gel electrophoresis show that the colonies chosen have the plasmid with the PpbrA promoter. There were three bands on the gel, the top band is the whole genomic DNA of the *E. coli*, the band at 300 bp is the PpbrA promoter. All four colonies are shown having the PpbrA promoter. The other bands past 100 bp are the left over primers (Figure 1). In order to test the effectiveness of our transformed *E. coli*, select colonies were grown in LB agar plates with and without lead. The *E. coli* grown in the LB plates without lead grew normal color (Fig 2A), but when grown in the LB plates with lead, the *E. coli* express the RFP gene and glow reddish orange (Fig 2B). To confirm that the PpbrA promoter sequence was placed into the plasmid in the correct place, the *E. coli* DNA was sent for sequencing. As seen in Table 1, the expected PpbrA promoter sequence is on top while the colony sequence is below it. The colony DNA showed the same PpbrA promoter sequence in the correct location.

Discussion

The purpose of the experiment was to modify *E. coli* to express RFP in the presence of lead. Based on the results from Figure 1, the PpbrA was successfully inserted into the J10060 plasmid. The effectiveness of the PpbrA was then tested to see if it would turn on the RFP gene. In figure 2, one can see that in the presence of no lead, the *E. coli* remain white (Fig 2A). But in the presence of lead, the *E. coli* turn a reddish orange color (Fig 2B), proving that our synthetic biological mechanism worked as expected. It can also be concluded that the PpbrA promoter was successfully ligated into the J10060 plasmid as expected (Table 1).

These results show that our modified *E. coli* can change color in the presence of lead. This was part three of a three part project which *E. coli* can now detect lead, antimony, and barium to confirm GSR (Porter et al. 2018). Domaille et al. (2008) had a similar system that detected metals and in response had a chemical signal. Fluorescent probes were used to activate in the presence of metal ions in the cells. This system glowed in the presence of excess zinc in brain cells (Domaille et al. 2008). This system and the modified *E. coli* are similar in that each could be specialized for detecting different kinds of heavy metals such as detecting lead in water sources.

Broader impacts for this project would be to implement this mechanism into the forensic field. This would be able to provide an accurate and quick method of detecting GSR. Some future directions for this work would be to create a biological system that would be able to respond to gunshot residue that doesn't have lead in them (Weber et al. 2014). If a biological system was able to detect gunshot residue with or without lead, then any type of gunshot residue would be able to be detected and would lead to quicker conclusions in gun cases. With the third part of the MicRobo Cop research project now completed, it can go onto field testing.

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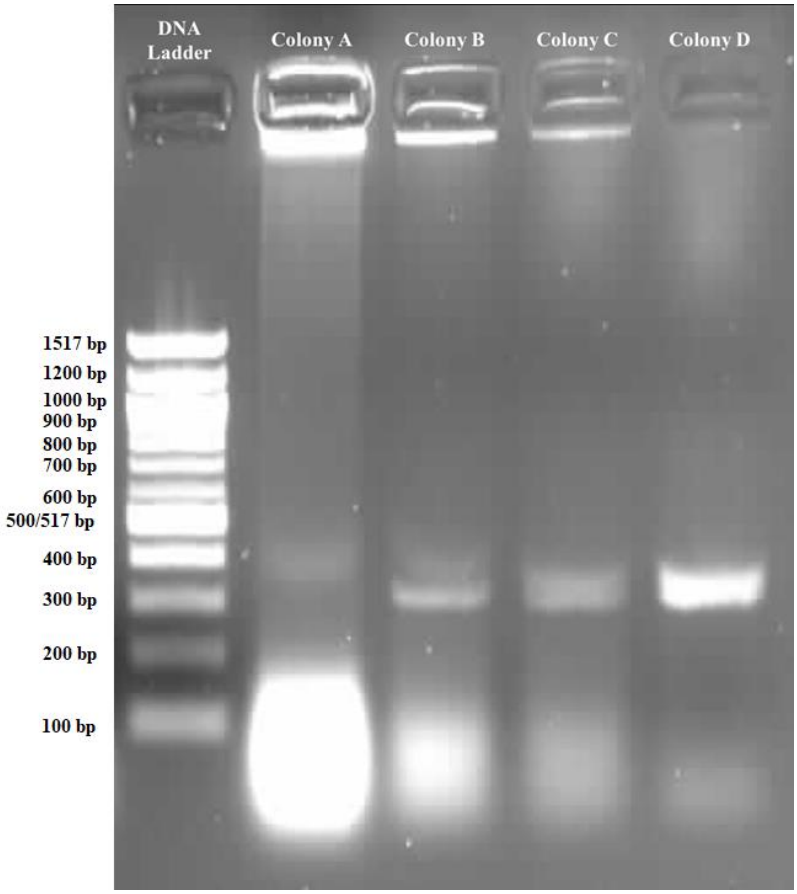

Figure 1. ***Gel Electrophoresis of PCR product for insertion of PpbrA promoter into J10060 plasmid.*** Well 1 is the DNA ladder while wells 2-5 are colonies A-D respectively. Top bands indicate the whole genomic DNA while the bands at 300 bp is the PpbrA promoter.

Figure 2. ***Test of effectiveness with successfully transformed E. coli.*** (A) Transformed *E. coli* without lead. (B) Transformed *E. coli* in the presence of lead.

Table 1. ***Confirming the PpbrA promoter sequence.*** The PpbrA expected promoter sequence was compared against the newly sequenced colony DNA.

J10060 with PpbrA promoter	5'-GTCTTGACTCTATAGTAACTAAGGGTGTATAATCGGCAACGCGA-3'
Colony Sequence	5'-GCCGCTTGAATTCGTCTTGACTCTATAGTAACTAAGGGTGTATAATCGGCAACGCGAGCTAGCGCAT-3'