Methods Article

Effects of Arabinose concentration on inducing fluorescence expression in transformed *Escherichia coli*

Aashi Agarwal, Owen Chen, Samuel Dillon, Adonai Haile, Eli Leav, Song Leav, Aryan Mirajkar, Anya Mittal, Siddharth Nirgudkar, Sophia Tang, Aprameya Tirupati, Henry Tshabalala, and Amanda Zhang

Acton-Boxborough Regional High School, Acton, Massachusetts, United States
Stanford University, California, United States

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Genetically modified bacteria can be used to efficiently detect chemical substances in the environment and report their presence through fluorescence. In this paper, we outline the procedures we used to transform *Escherichia coli* with the plasmid pARA-R, which allows *E. coli* to fluoresce in the presence of the signaling molecule arabinose. We tested *E. coli*'s fluorescence in response to multiple concentrations of arabinose and aimed to discover the minimum amount required to produce a noticeable amount of fluorescence. Our results show that the transformed *E. coli is noticeable* fluoresce at every arabinose concentration we tested—the lowest being 1mM. We also discovered that Luria Broth (LB) auto-fluoresces due to two of its ingredients: yeast extract and tryptone. We describe the procedure we created to effectively remove LB. In the future, we will test even lower arabinose concentrations. Additionally, we previously designed a *Legionella* detector, which we will test using the procedures detailed in this paper.

Keywords: Fluorescence, luria broth, Escherichia. coli, Legionella, arabinose, transformation

Mentors: Aaron Mathieu, and Kathryn Brink

Direct correspondence to 23dillons@abschools.org; amathieu@abschools.org

Background

This paper details the procedures we used to transform *E. coli* with the pARA-R plasmid, as well as observe the fluorescence of those *E. coli* in response to a signaling molecule. The paper also describes our arabinose testing procedures, which can be used to find the minimum amount of arabinose required to produce a noticeable amount of fluorescence.

We will use these methods to gain experience with transformation and fluorescence under different signaling molecule concentrations. This will allow for more accurate and efficient testing of our *Legionella* detector when we are able to obtain the necessary materials (Agarwal et al., 2021).

Planned *Legionella* detection system

To produce our *Legionella* detector, we will use the procedures detailed in this paper. Our Legionella detector seeks to counter outbreaks of *Legionella spp.*—waterborne opportunistic pathogens that contaminate drinking water and multiply in stagnant conditions. Certain *Legionella spp.* cause a deadly form of pneumonia known as Legionnaires' disease, as well as Pontiac Fever. There are 58 species of Legionella, causing 8,000 to 18,000 hospitalizations annually in the

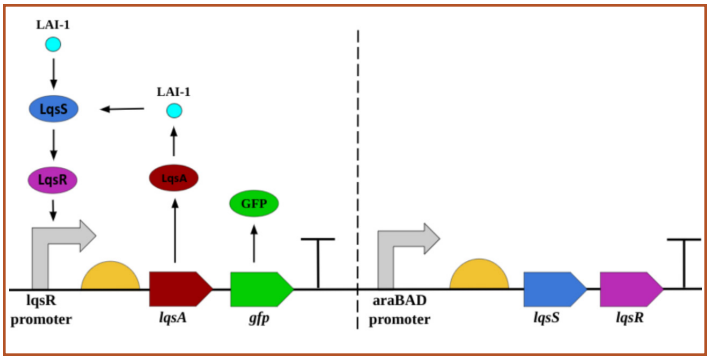

Figure 1: *Simplified plasmid we designed for the Legionella detector. Contains genes for LqsS (sensor kinase), LqsR (response regulator), LqsA (autoinducer synthase), and GFP (reporter). LqsS senses LAI-1 and then activates LqsR. (The genes coding for LqsS and LqsR are controlled by the araBAD promoter). LqsR will then activate the lqsR promoter, which controls expression of LqsA—which produces more LAI-1 to create a positive feedback loop—and GFP, which reports Legionella's presence.*

U.S. alone. Additionally, 5-10% of hospitalizations are fatal, hence making this vital to solve.

Our *Legionella* detector is a transformed E. coli chassis that can detect *Legionella spp.* in water through *Legionella*'s inter- and intra-species communication system called Legionella Quorum Sensing System (LQS) (Personnic, 2021). We have designed a plasmid that encodes a system that senses *Legionella*'s autoinducer LAI-1 (See plasmid overview in Figure 1.)

All *Legionella spp.* use LAI-1 as a signaling molecule in LQS, which is only released by *Legionella spp*. The plasmid we designed also contains a pathway to amplify the LAI-1 signal so that other *E. coli* in the environment can detect the LAI-1 easily and amplify the fluorescent signal. Thus, our system allows a user to detect *Legionella* despite it usually being present—and yet still harmful—at extremely low concentrations. This makes our system more efficient and more useful at detecting the presence of *Legionella*.

Additionally, we can easily interchange parts in the detection method we have developed (i.e., detecting waterborne pathogens by their species-specific, quorum-sensing signaling molecules) to detect other waterborne pathogens. Thus, in creating procedures detailed in this paper and testing the *Legionella* system in the future, we help push forward the detection of all ubiquitous waterborne pathogens to create clean water all over the world.

Trial detection system to develop procedures

Although we will later experiment with a plasmid we designed for our *Legionella* detector, we first wanted to assemble and test methods for transformation and fluorescence that can later be used in that project.

This would also allow us to gain experience with the methodology with more-accessible materials: the pARA-R plasmid in place of the plasmid we designed and arabinose in place of LAI-1.

Arabinose triggers the synthesis of RFP, which can be viewed under ultraviolet light. Our transformed *E. coli* will not fluoresce if arabinose is not present.

In developing our procedures, we transformed *E. coli* C2988J with the pARA-R plasmid—an arabinose-inducible, RFP-expressing plasmid. pARA-R contains a modified version of the L-arabinose operon under the pBAD promoter. The L-arabinose operon normally contains genes *araB*, *araA*, and *araD*, but in the pARA-R plasmid, these genes are replaced by the *rfp* gene, which codes for a protein that shines under UV light. The pARA-R plasmid also contains the *araC* gene, which codes for the AraC protein that binds to arabinose sugar, allowing RNA polymerase to bind to DNA and transcribe the genes under the pBAD promoter. The pARA-R plasmid also contains the ampicillin-resistance gene, allowing us to apply ampicillin to a plate of *E. coli*, which kills all *E. coli* that have not taken up the plasmid and leaves those that have.

The procedures outlined below can be used by other researchers with any similar pair of plasmid and signaling molecule

Requirements

Stocks requirements:

- Time: 45 minutes
- Equipment
  1. Mass balance
  2. 50mL conical tube
  3. Twenty 1mL aliquots
  4. Freezer capable of -20°C
  5. 50mL and 100mL graduated cylinders
- Materials
  1. 20 mL molecular-grade H2O
  2. 2 g ampicillin

Plates requirements:

- Time: 90 minutes
- Equipment
  1. Six agar plates
  2. Autoclave
  3. 2L flask
  4. Magnetic stirrer

- Materials
  1. 150g Luria Broth Miller
  2. 120g Bacto Agar
  3. 3L diH2O
  4. 4mL stock ampicillin
  5. 20mL stock arabinose

pARA-R Plasmid transformation requirements:

- Time: 45 minutes
- Equipment
  1. Ice bath
  2. Warm water bath
  3. Sterile cell spreaders
  4. Pre-made agar plates (made in "Agar Plate and Stock Preparation")
  5. Twelve 1.5mL microcentrifuge tubes
  6. Microcentrifuge tube holder rack
  7. 100µL - 1000µL pipette
  8. Pipette tips
  9. Incubator
  10. Vortex shaker
  11. UV light box
  12. Parafilm
- Materials
  1. 10µL pARA-R plasmid
  2. 100µL of New England BioLabs 5-alpha Competent E. coli cells (C2988)
  3. 300µL Luria broth

Arabinose Serial Dilution Requirements:

- Time: 20 minutes
- Equipment
  1. Mass balance
  2. Nine 50mL conical tubes
  3. Conical tube rack
  4. 100µL - 1000µL pipette
  5. Pipette tips
  6. Conical tube holder
- Materials
  1. 3g arabinose
  2. 82mL sterile water

Create LB Growing Solution for *E. coli*:

- Time: 15 minutes + 24 hour incubation period
- Equipment
  1. Sterile 10µL inoculating loop
  2. Sterile Luria Broth
  3. Incubator
- Materials
  1. pARA-R transformed *E. coli*

Create pure *E. coli* by removing LB

- Time: 30 minutes
- Equipment
  1. Four microcentrifuge tubes
  2. Micro centrifuge (capable of 14,000 rpm)
  3. 100µL - 1000µL pipette
  4. Pipette tips
- Materials
  1. 12mL LB + *E. coli* solution
  2. 13mL distilled water

Investigate fluorescence of individual LB ingredients

- Time: 45 minutes
- Equipment
  1. Six 15mL (or larger) tubes
  2. UV light box
  3. 20µL-200µL Micropipette
  4. 100µL-1000µL Micropipette
  5. Pipette tips
  6. Mass balance
- Materials
  1. 2g Yeast extract
  2. 2g Tryptone
  3. 2g NaCl
  4. 195mL sterile water

Procedures

1. Agar Plate and Stock Preparation (Note 1)

Stocks Procedure: (Note 2)

- Stock Ampicillin (Note 3)
  1. Dissolve 2.0g ampicillin in 20mL molecular-grade H2O.
  2. Filter with a sterile filter and then collect in a 50mL conical tube.
  3. Store in 1mL aliquots at -20°C
  4. Final concentration: 100mg/mL

Stock Arabinose (Note 4)

1. Create a stock concentration of 500mg/mL with arabinose in water.
2. Add 25g of L-(+)-Arabinose to 40mL of diH2O
3. Store in fridge

Plates Procedure:

- LB Plates:
  1. In a 2L flask add the following: 25g Luria Broth Miller, 20g Bacto Agar, 1L diH2O.
  2. Autoclave and mix with a magnetic stir bar
  3. Once temperature is below 50°C, you can start pouring plates
  4. Label the plates with one vertical line (I)
- LB/amp Plates
  1. In a 2L flask add the following: 25g Luria Broth Miller, 20g Bacto Agar, 1L diH2O, 1mL stock Ampicillin.
  2. Autoclave and mix with a magnetic stir bar
  3. Once temperature is below 50°C, you can start pouring plates
  4. Label the plates with two vertical lines (II)
- LB/amp/ara Plates
  1. In a 2L flask add the following: 25g Luria Broth Miller, 20g Bacto Agar, 1L diH2O, 1mL stock ampicillin, 10mL stock arabinose.
  2. Autoclave and mix with a magnetic stir bar
  3. Once temperature is below 50°C, you can start pouring plates
  4. Label the plates with three vertical lines (III)

2. pARA-R Plasmid Transformation (Note 5)

1. Pipette 10µL of pARA-R plasmid into P+ tube.
2. The recombinant plasmid (P+), competent cells, and Luria broth (LB) should each occupy a small tube, and a fourth tube should remain empty (P-). The tubes should be kept in an ice cup.
3. Pipette 50µL competent cells into each of the P+ and P- tubes after suspension, making sure to keep the tubes cold.
4. Leave the tubes on ice for 15 minutes.
5. Label plates with LB, LB/amp, and LB/amp/ara and label them on the agar side. (Draw a line on the outside of the LB and LB/amp plates that halves them. The halves should be labeled P-/P+. The LB/amp/ara plate should not be halved, and should have a single P+ label.)
6. Following the 15 minutes on ice, place the P+ and P- tubes in the warm water bath (42°C) for exactly 45 seconds. Afterwards, immediately place the tubes back on ice for 1 minute.
7. Add 150µL of LB into P- and P+ tubes and flick to mix them.
8. Incubate at room temperature for 15 minutes in a holding rack.
9. Using a new pipette set to 50µL, gently pipette a few times in the P- tube to mix the contents.
10. Pipette 50µL from the P- tube onto the P- sections of both the LB and LB/amp plates.
11. Use a cell spreader to spread the P- halves and close the lid.
12. With a new pipette tip, gently pipette a couple of times in the P+ tube.
13. Pipette 50µL of P+ onto the P+ sections of the LB and LB/amp plates, and 100 µL of P+ onto the entire LB/amp/ara plate.
14. Use a new spreader to spread P+ on the respective sections of the plates, and let them sit at room temperature for 5 minutes (with lids closed).
15. Parafilm plates and incubate at 37°C, upside down, for 24 hours.
16. Place on an orbital shaker at 90 rotations per minute.
17. After 24 hours, observe for E. coli growth.

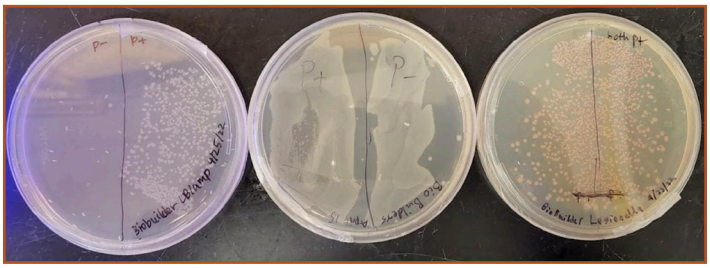

Figure 2: *(left to right): LB+amp, LB, LB+amp+ara. Plates from Procedure 2 after 24-hour incubation.*

3. Arabinose Serial Dilutions

1. Add 3g of Arabinose to 10mL of distilled water; makes a 100mM arabinose solution.
2. Serially dilute from 100mM to 10mM, 1mM (Note 6).

4. Create LB Growing Solution for E. coli

1. Use a sterile inoculating loop to collect about 10µL of E. coli.
2. Place in pure, sterile LB.
3. Incubate LB+E. coli for 24 hours at 37°C.
4. Test density of E. coli each day with a spectrophotometer at 600 nm (OD600).

5. Create pure E. coli by removing LB

We created this procedure due to the discovery that LB fluoresces, as explained in "Luria Broth Fluorescence" (below).

1. Add 1.5mL LB+E. coli solution to four microcentrifuge tubes (Note 7).
2. Centrifuge the tubes at 14000 rpm for 2 minutes.
3. Remove and discard the supernatant from each tube, leaving a pellet of E. coli.
4. Add another 1.5mL LB+E. coli to each tube.
5. Repeat steps 3-4 once more.
6. Add 1mL water to each tube, and shake vigorously to re-suspend the solutions.
7. Centrifuge at 14000 rpm for 1 minute.
8. Remove supernatant.
9. Repeat steps 6-8 twice more.
10. Add 100µL of distilled water to each tube. Re-suspend.

6. Test Fluorescence of Pure E. coli with Arabinose

1. First, place all tubes from Procedure 6 in a UV light box (we used blueBox by miniPCR) to check for fluorescence without adding arabinose.
2. If there is significant fluorescence, perform another round of washing and supernatant removal like in the previous procedure.
3. If there is no fluorescence, add arabinose concentrations of 0mM, 1mM, 10mM, 100mM.
4. Then, leave tubes under UV light for 1 hour.
5. Observe fluorescence.

Luria Broth Fluorescence

Initially, we tested our *E. coli*'s fluorescence after scraping them directly off agar plates prepared in procedure 1 and 2. After testing some combinations of arabinose concentrations and *E. coli*, all combinations fluoresced equally. Next, we wanted to test higher amounts of *E. coli* to produce a more noticeable difference in fluorescence. So, we grew the E. coli in LB to produce larger amounts (Procedure 4). Testing showed that again, all arabinose and *E. coli* solutions fluoresced equally. Sensing something was not right, we tested the fluorescence of LB's ingredients in procedure α (below). We found that yeast extract and tryptone fluoresce brightly (Figures 4 and 5).

Because LB fluoresces and thus interferes with viewing *E. coli* fluorescence, we decided to remove all LB from our LB and *E. coli* solution before testing it with arabinose. After a few trials, we created procedures 5 and 6 (above). These procedures successfully removed LB, as seen in Figure 3, where the negative control (pure water andE. coli) does not fluoresce at all, while all *E. coli* and arabinose solutions fluoresce noticeably.

Procedure α: Investigate Fluorescence of Individual LB Ingredients

1. Make the following substance:water ratios by volume in 15mL or 50mL tubes:
   a. 1:100 tryptone
   b. 1:1,000 tryptone (Note 8)
   c. 1:100 Yeast extract
   d. 1:2,000 Yeast extract (Note 8)
   e. 1:100 NaCl
   f. 1:20,000 NaCl (Note 8)
2. Put tubes in a UV light box to test for fluorescence. Fluorescence observable immediately.

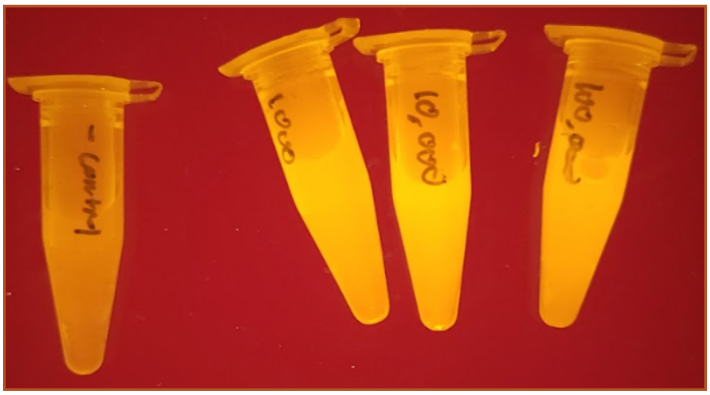

Figure 3: *(left to right): Pure E. coli and 0mM (negative control), 1mM, 10mM, and 100mM in miniPCR blueBox. There is an observable difference in fluorescence after 15 minutes, but fluorescence strengthens with time.*

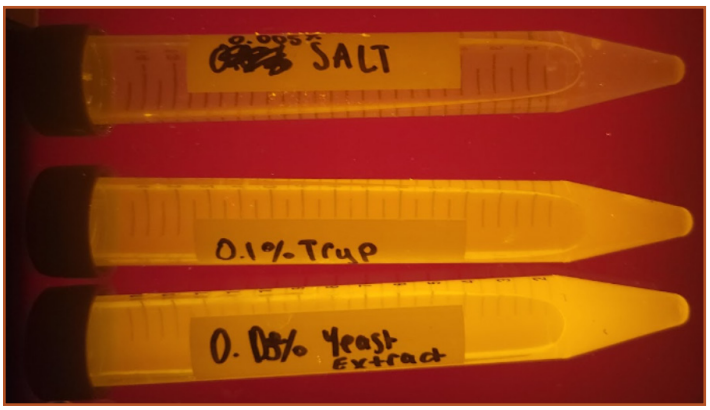

Figure 4: *0.1% tryptone, 0.05% yeast extract, 0.005% NaCl solutions observed under UV light in the miniPCR blueBox (observable immediately). These are the proper concentrations used in the Luria Broth.*

Figure 5: *1% tryptone, 1% yeast extract, 1% NaCl solutions observed under the miniPCR blueBox (observable immediately).*

Notes

1. We received these plates from Amgen Biotech Experience, so we did not perform this procedure ourselves. However, we included it because it would be otherwise necessary.
2. At this concentration, add 1mL stock ampicillin to 1L of LB broth. Stock solution can be used for preparation of LB/amp broth or LB/amp plates. Remember, Amp is heat sensitive.
3. This prepares 20mL of ampicillin stock
4. L-(+)-Arabinose is used to make plates, as well as a LB/amp/ARA flask.
5. We ran this procedure twice (but concurrently) so we would have more *E. coli* later on if one plate of each treatment did not provide enough.
6. You can use serial dilutions to create very small concentrations of arabinose without a very accurate mass balance. To perform serial dilutions, pipette 1mL of the previously created solution into 9mL of distilled water (ex. 1mL 100mM solution into 9mL water to create a 10mM solution). Repeat with subsequent solutions to create smaller solutions.
7. Spectrophotometer OD600 value: 0.550.
8. These are the ingredient ratios in actual LB. The 1:100 ratios in the other tubes were simply to see a larger effect.

Discussions

Our experiments accomplished three main objectives: 1. Discovering that Luria Broth (LB) fluoresces and which of its ingredients fluoresce, 2. Creating a procedure that successfully removes LB from LB and *E. coli* growing solution, and 3. Inducing fluorescence with as low as 1mM of a signaling molecule (arabinose).

Our results show that our pARA-R *E. coli* fluoresce noticeably at every arabinose concentration we tested—the lowest being 1mM—in comparison to the negative control (pure water andE. coli) does not fluoresce at all (Figure 3).

We also discovered that LB fluoresces, specifically two of its ingredients fluoresce: yeast extract and tryptone (See "Luria Broth Fluorescence" section in procedures for more information and analysis). Knowing this could help researchers choose or develop non-fluorescent broths by omitting yeast extract and tryptone, or by testing any new broth and its ingredients for fluorescence.

Although some labs might know about LB's fluorescence, certainly not all do, we have found some chatter online as recently as 2022 by researchers above the high-school level about media fluorescence and how to remove it (Pillai, 2022). And after much research, we have only found one source that touches on growth media fluorescence and describes a procedure to wash cells to remove the media (Bhatta et al., 2006).

It took so much searching to find just one paper of this kind—a paper that is not even focused on media fluorescence; this indicates that this knowledge is not as common in published works as it should be. Our paper can help disseminate the idea that some media fluoresce and provide procedures for removing media so that each lab does not have to rediscover it for themselves, which wastes time and resources, and could lead researchers to publish false results unknowingly.

This discovery was crucial for our *Legionella* biosensor project; without these experiments, we would have observed fluorescence in every trial with our *Legionella* system, and we would have assumed that we were getting positive results even with extremely small concentrations of our signaling molecule. Now, we have procedures to follow once we access our *Legionella* biosensor materials, allowing us to obtain accurate results.

Also, because our *Legionella* detection method could be expanded to detect all ubiquitous waterborne pathogens, teams in the future might implement our experimental process. If we had not discovered LB's fluorescence, each detector would falsely be seen as extremely effective. And any other project that uses fluorescence, other BioTreks teams, or other research teams might have been led down false paths. So, this project allows for more accurate fluorescence testing in other BioTreks projects and potentially professional labs.

Next Steps

We will utilize the data gathered and procedures created in this experiment to implement our *Legionella* biosensor project (Agarwal et al., 2021).

We had hoped that our transformed *E. coli* would detect arabinose in concentrations as low as *Legionella*'s threshold for harming humans, ranging from 10 CFU/ml to 1500 CFU/ml (State of Hawaii, 2019). We got positive results at 1mM of arabinose, but unfortunately, we did not have time to test even lower concentrations. Though 1mM is a low concentration, we would like to be able to detect a signaling molecule at concentrations as low as 10-40μM to also be confident in our *Legionella* biosensor. So, we will use our procedures to test even lower arabinose concentrations.

Due to our discovery and procedures about LB's fluorescence, we would also like to explore more effective methods to remove LB, or perhaps to avoid fluorescent media altogether and try using a non-fluorescent media.

Author contributions

O.C., S.D., Ar.M., An.M., S.N., S.T., A.T., and H.T. helped design experiments. A.A., O.C., S.D., E.L., S.L., Ar.M., An.M., S.N., S.T., A.T., H.T., and A.Z. contributed to experimentation. A.A., S.D., E.L., and An.M. wrote the Abstract. O.C., S.D., A.M., and S.N. wrote the Background. O.C., and S.D. wrote the Requirements. O.C., S.D., S.T., A.T., An.M., and H.T. wrote the Procedures. S.D., and A.T. wrote the Notes. S.D., S.T., and An.M. wrote the Discussion. S.D., S.N., and H.T. wrote the Next Steps. S.D., and S.N. wrote the Acknowledgements. S.D., and H.T. wrote the References. S.D. led experimentation, revised all sections, and is corresponding author.

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