Design Brief

Visual quantification of PVC levels via luciferase and the catalase-peroxidase promoter: A unique solution to an under discussed issue*

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Polyvinyl chloride – PVC – is one of the most commonly used plastics, with more than 40 million tons produced annually. Microplastics are very small pieces of plastic debris that can easily pass through water filtration systems and enter our oceans and drinking water. Due to their small size, it is difficult to ensure that every bit of plastic has been detected or filtered out. However, despite its negative impact on human health and the environment, there is a lack of current research dedicated to detecting PVC. Therefore, discovering innovative methods to detect and degrade PVC is essential to minimizing the adverse effects of plastic waste. Our project aims to create a biosensor that can detect PVC microplastics using luciferase as a reporter gene under the control of a PVC-sensitive inducible promoter. We have chosen the promoter for the enzyme catalase-peroxidase because studies have shown that its expression can be induced by PVC. Once the relevant genomic sequences are inserted into a plasmid and expressed in Escherichia coli (E. coli), the biosensor will produce bioluminescence in the presence of PVC, which can then be detected using a microplate luminometer. We aim to conduct experiments under varying conditions, such as temperature and substrate concentration, to observe the optimal environment for our biosensor and understand its potential limitations. We plan to conduct field testing to validate the effectiveness of our biosensor and investigate its capabilities for detecting other plastics. This biosensor would be a valuable tool for measuring PVC contamination in water, raising awareness of potential contamination, and driving solutions to the significant health and environmental issues it causes.

Keywords: Polyvinyl chloride, biosensor, PVC microplastic, catalase-peroxidase, bioluminescence

Polyvinyl chloride (PVC) is one of the most commonly used plastics, with more than 40 million tons produced worldwide, each year ("PVC applications", 2023). PVC production emits the greenhouse gas methane, which, when inhaled in large amounts, can lead to many health consequences, such as suffocation ("Poison Plastic," 2024). Furthermore, PVC production also releases "hundreds of thousands of pounds of carcinogenic vinyl chloride into the air every year," ("PVC

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Poison Plastic," 2023). PVC plastic is unable to decompose naturally due to its high molecular weight, stable covalent bonds, and hydrophobic surface properties (Kudzin et al., 2023). Because of this durability, PVC is commonly used in construction, automotive, pipes, cables, and household goods in all communities (Kudzin et al., 2023). Therefore, a staggering 40 million tons of PVC plastic are produced annually. In the United States alone, 7 billion pounds of PVC are disposed of annually, depicting just how much of the material is produced ("PVC under Scrutiny as Hazardous Waste," 2023).

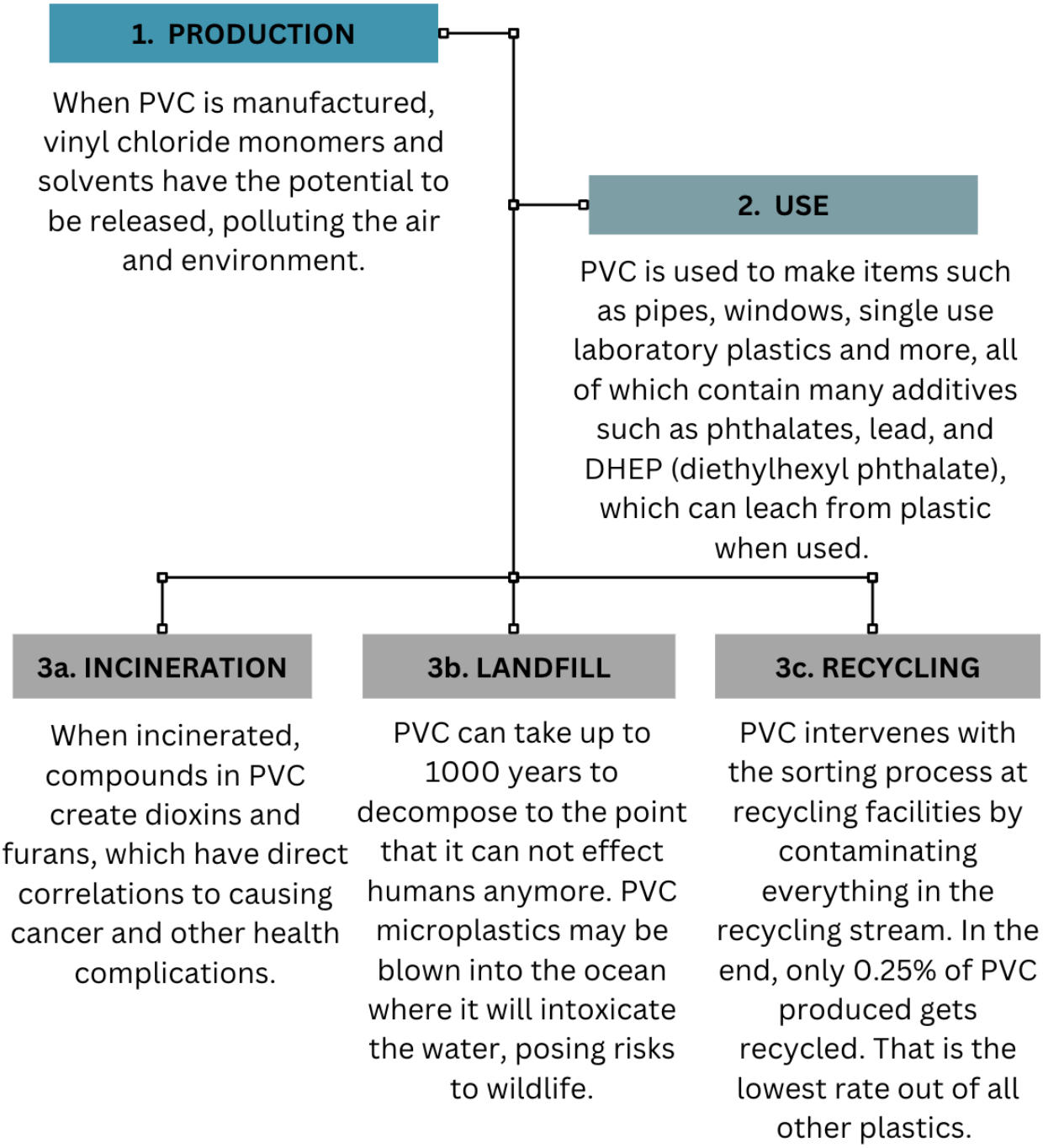

Figure 1. Lifecycle Impact of PVC. At every stage of the lifecycle outlined above, PVC has potential to release hazardous chemicals, creating significant environmental challenges and concerning human health risks.

A small percentage of this plastic is properly recycled, while the bulk of it is incinerated or ends up in landfills (Figure 1) ("PVC applications," 2023).

The widespread dispersal of PVC results in microplastics that, upon disposal and degradation, spread throughout the environment. These microplastics can become airborne and eventually reach the ocean, releasing toxic chemicals such as phthalates, lead, cadmium, and organotins ("Department of Human Services | PVC – a Major Source of Phthalates", n.d.). These released chemicals get into the water and also get ingested by animals, harming wildlife (Smith et al., 2018). A study was done with the aquatic animal *Daphnia magna* and it was found that the controls containing PVC microplastics had disruptions in their "reproduction and detoxification-related genes expression" in addition to oxidative stress (Liu et al., 2022).

Additionally, PVC was one of the most commonly found polymers in the benthic flatfish *Solea solea*. This presents a huge concern because these fish are edible, which means that microplastics ingested by fish make their way into people who eat them, potentially risking someone's health if consumed in large amounts (Bajt, 2021). In addition, PVC-containing products in landfills exposed to sunlight release carcinogens and hazardous gasses (Figure 1) (Kudzin et al., 2023). Regular exposure to vinyl chloride and these chemicals has been found to "increase the risk of a rare form of liver cancer (hepatic angiosarcoma), as well as primary liver cancer (hepatocellular carcinoma), brain and lung cancers, lymphoma, and leukemia" (National Cancer Institute, 2018). Moreover, many low-income communities have no choice but to use drinking pipes made of PVC because of their affordable price point and easy access, putting them at greater susceptibility to health problems associated with this plastic (Coz, 2023). Safer alternatives, such as high-density polyethylene (HDPE) pipes, cost more, making PVC a better option on a tighter budget. This also means there is much more PVC pollution in these low-income areas as pipes and other items made of PVC are disposed of improperly at higher rates compared to areas that may use less PVC-containing materials. It was discovered that residents of underfunded towns like Reveille Town and Mossville in Louisiana were fifty times more likely than the average American to develop cancer from air pollution due to regular exposure to vinyl chloride and plastic production ("Poison Plastic," 2024).

Because it is evident in each stage of the diagram above that PVC has many negative environmental and health impacts, our ability to detect it will be valuable for improving both the environment and the health of those affected. Our ultimate goal is to create a biosensor tailored for human and environmental health: a tool to test water quality in aquatic conservation areas, water access systems and industrial production plants to ensure toxic chemicals from any of the life stages of PVC above have not infiltrated water sources. This would allow companies and communities to take action in removing chemicals through degradation or water treatment methods. These actions can help eliminate chemicals that could pose significant health concerns for people and the environment. Detecting the presence of PVC is an essential first step before eventually moving toward removal. Our biosensor could also be used as a scientific tool to help scientists gauge whether or not their PVC plastic degradation efforts are effective.

Systems level

To create a PVC biosensor, we will utilize luciferase as a reporter gene, which will be linked to an inducible promoter that reacts to the presence of PVC. The promoter sequence must be cloned in front of the luciferase gene. The plasmid containing these genomic sequences will then be inserted into the *E. coli* bacteria using heat shock transformation methods. This process will allow *E. coli* to undergo bacterial transformation and acquire the necessary foreign DNA to later produce a bioluminescent output when exposed to a sample containing PVC. After bacterial transformation, the sample of *E. coli* bacteria will be cultured before undergoing DNA preparation. During DNA preparation, the DNA will be removed from the bacteria to

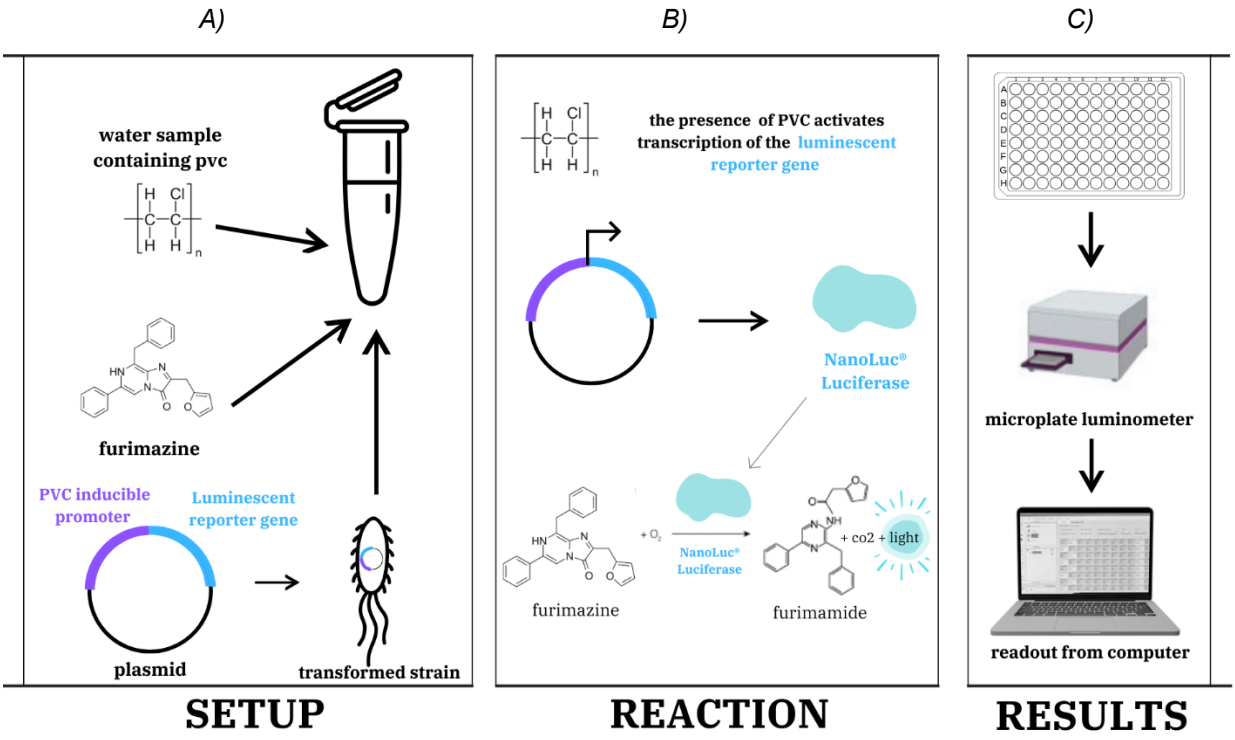

Figure 2. (A) Transformed E. coli and furimazine placed in a cuvette containing a water sample. (B) Depending on whether PVC is present in the sample, the transcription of the luminescent reporter gene may or may not be activated. (C) The resulting sample is placed in a microplate luminometer to accurately measure PVC concentration.

undergo cell transfection. The plasmid will then be placed into the cell, creating the biosensor.

For our experimental group, the biosensor (i.e. the transformed *E. coli*) will be placed in a cuvette, along with furimazine (the substrate for the reaction), as well as PVC microplastics (Figure 2A). The presence of PVC will lead to the production of the luciferase protein. This protein will then catalyze the reaction of furimazine to formamide, leading to the generation of luminescence. Therefore, in the experimental group, bioluminescence should be produced, signifying the presence of PVC in the original sample. Essentially, the reaction produces the NLuc luciferase enzyme, which acts as a catalyst for the reaction of furmazine to formamide, producing light as a product (Figure 2B). We will then place these samples in a microplate luminometer, and the readout from the computer will quantify the level of PVC present in the sample (Figure 2C).

In order to create a positive control, we will need to program the *E. coli* to consistently express luciferase, even under the condition that PVC is absent in the original sample. A positive control *E. coli* will always produce a bioluminescent output. This control will allow us to prove that our bacterial transformation, growth, transfection, and measurement protocols are all functioning appropriately, and that the *E. coli* is able to withstand the presence of PVC in a sample.

To curate a subsequent negative control, the experiment would be performed with *E. coli* that do not have the luciferase gene at all, and should not generate any bioluminescence. The negative control allows us to ensure that PVC is not itself luminescent under the conditions of our measurement protocol. Essentially, this control will confirm that the biosensor does not generate any luminescence when PVC is not present, or at least, detect the level of background noise from the environment.

Device level

Our team decided to utilize *Escherichia coli* (E. Coli) K-12 as the chassis for our biosensor because of its reliability, safety, and inability to manifest and develop in the human gut (Browning et al., 2023).

We decided to separate our biosensor into two devices: one for PVC detection and another for luminescence emission (Figure 3). This will result in a more flexible and easily adjustable sensor, should our experiments demonstrate the possibility of utilizing the biosensor for purposes other than strictly PVC detection.

Each device represents a group of genetic elements which will together complete one general action. Device One serves as the PVC detector. The presence of PVC will initiate a signal transduction pathway and activate device one. This will then trigger Device Two, the luminessence generator. Device Two produces luciferase protein in the sample, which catalyzes the reaction of furimazine to formamide to produce luminescence. The luminescence emitted in the presence of PVC can then be detected using a microplate luminometer connected to a computer to quantify the varying amounts of PVC present in a sample (Smale, 2010).

Parts level

To create a PVC biosensor, we needed to identify and select a promoter that reacts to the presence of PVC and attached it to our reporter gene, luciferase.

Previous research conducted by a group in Zhejiang Province, China, found that in the larvae of *Spodoptera frugiperda*, an insect pest, a gut bacterial strain (Klebsiella sp. EMBL-1) depolymerizes PVC. The group found that when the strain EMBL-1 was grown in the presence of PVC, catalase-peroxidase was one of the most highly expressed proteins (Zhang et al., 2022). Additionally, a group in Lahti, Finland researched the effects of different microplastics on *Nelumbo nucifera*, a lotus plant (Esterhuizen, 2021). Their research showed that catalase activity also increased with exposure to PVC. Since PVC led to a significant increase in catalase-peroxidase, we can infer that it induces transcription of the *KatG* gene, which encodes catalase-peroxidase.

These observations support that the promoter sequence for the *KatG* gene is responsive to PVC. To find this promoter sequence, a research group in Brazil mapped the *KatG* regulatory region through serial deletions. This promoter region can be found upstream of the *KatG* transcription site, and is responsible for increased expression of the *KatG* gene (Valéria, 2011). This promoter is activated by OxyR, which is a transcription factor (Esterhuizen, 2021). In *E. coli*, the OxyR protein is also present, and has been shown to activate the transcription of the *KatG* gene (Tao, 1991).

The promoter sequence for the *KatG* gene will be cloned in front of the luciferase gene and established in a plasmid. We aim to utilize the luciferase enzyme due to its unique sensitivity, stability, and enhanced luminescence compared to other, more traditionally used bioluminescent proteins such as the Firefly and *Renilla* luciferases (England, 2016). The next step is to transform *E. coli* with the plasmid containing the promoter and reporter gene (Figure 4).
We infer that the addition of PVC will result

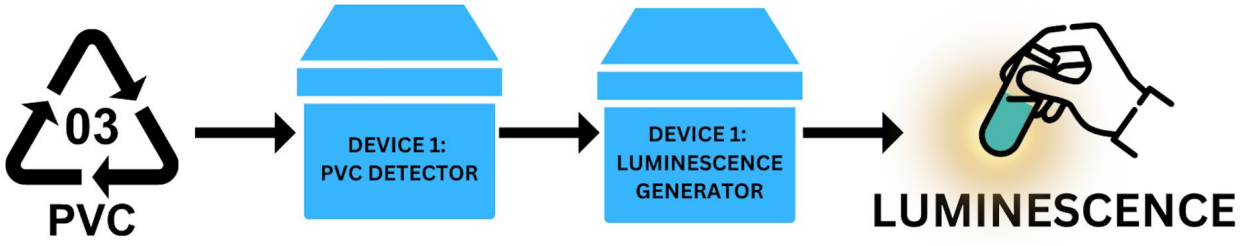

Figure 3. Device One and Device Two. Device One (PVC detector) and Device Two (Luminescence generator) shown. PVC's presence initiates a signal transduction pathway leading to activation of Device One, which will trigger Device Two. Device Two will produce luciferase and measurable luminescence.

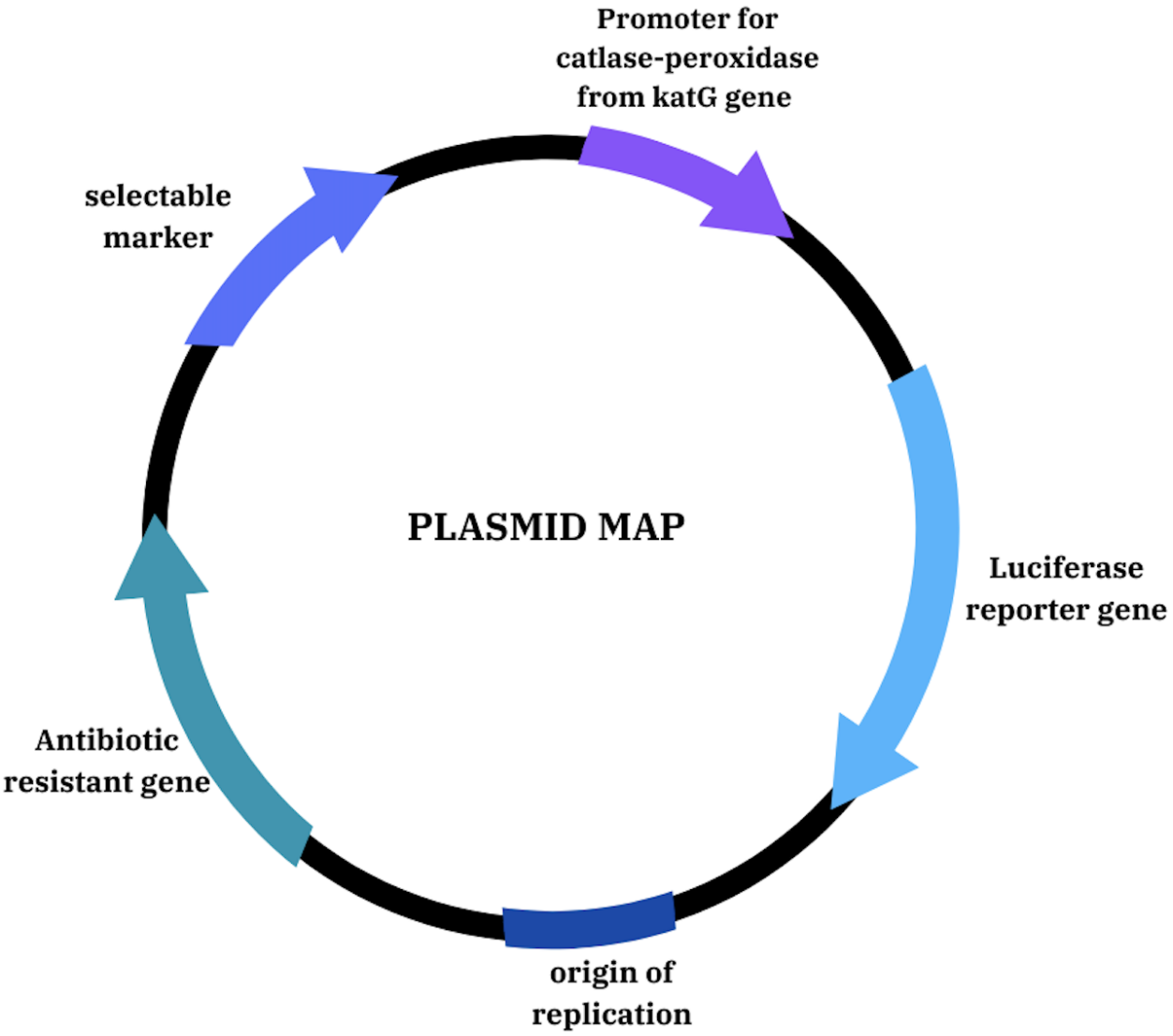

Figure 4. Proposed plasmid for biosensor. In the presence of PVC, the promoter for catalase-peroxidase is activated, initiating the transcription of the downstream luciferase reporter gene. The plasmid contains an origin of replication to ensure it replicates as the bacteria cells divide. Additionally, an antibiotic resistance gene and selectable marker are included to identify successfully transformed bacteria, allowing the removal of those that have not taken up the plasmid.

in an increase in OxyR (Figure 5). Although the exact signal transduction pathway still needs to be elucidated, we believe this pathway is evolutionarily conserved. This is because PVC has been shown to result in increased catalase-peroxidase in two very different species, bacterial strain EMBL-1, and the lotus plant. Hence, we believe it will be present in *E. coli* as well.

If our assumption is correct, the presence of PVC will lead to an increase in OxyR. This transcription factor will then bind to the *KatG* promoter sequence (Figure 6, step 1). Then RNA polymerase will bind to the transcription factor (Figure 6, step 2) and start transcription of the downstream luciferase gene (Figure 6, step 3). RNA will then be translated into luciferase protein (Figure 6, step 4). This protein will catalyze the reaction of furimazine and produce detectable luminescence, which can then be measured using a microplate luminometer. Therefore, the presence of PVC will be converted into a measurable luminescent

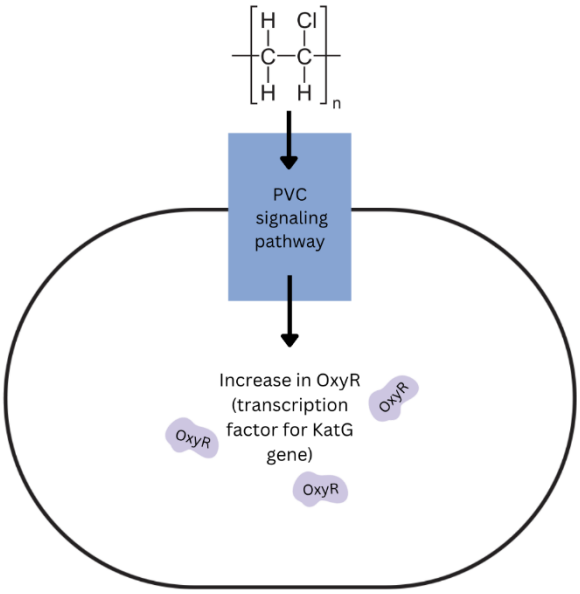

Figure 5. PVC signal transduction pathway. PVC signal transduction pathway leads to increase in OxyR protein.

signal.

Safety

To prevent potential accidents and injuries, all experiments will be performed in a laboratory setting. Proper personal protective equipment (PPE), which includes laboratory coats, goggles, and gloves, will be worn at all times, especially when handling hazardous bacteria or performing any experimentation. Surfaces will be properly disinfected before and after the use of the *E. coli* bacteria. Though researchers work with *E. coli* routinely, and it is not known to cause adverse health effects in healthy humans, it is still important that all lab safety protocols are followed when handling the bacteria. This includes ensuring the lab is appropriately equipped for working at Biosafety Level 1 (BSL-1). Luciferase is not toxic to humans, so with standard safety precautions, it does not pose serious safety concerns (Tiffen et al., 2010). Our bacterial cultures will also be regularly monitored for unexpected behavior.

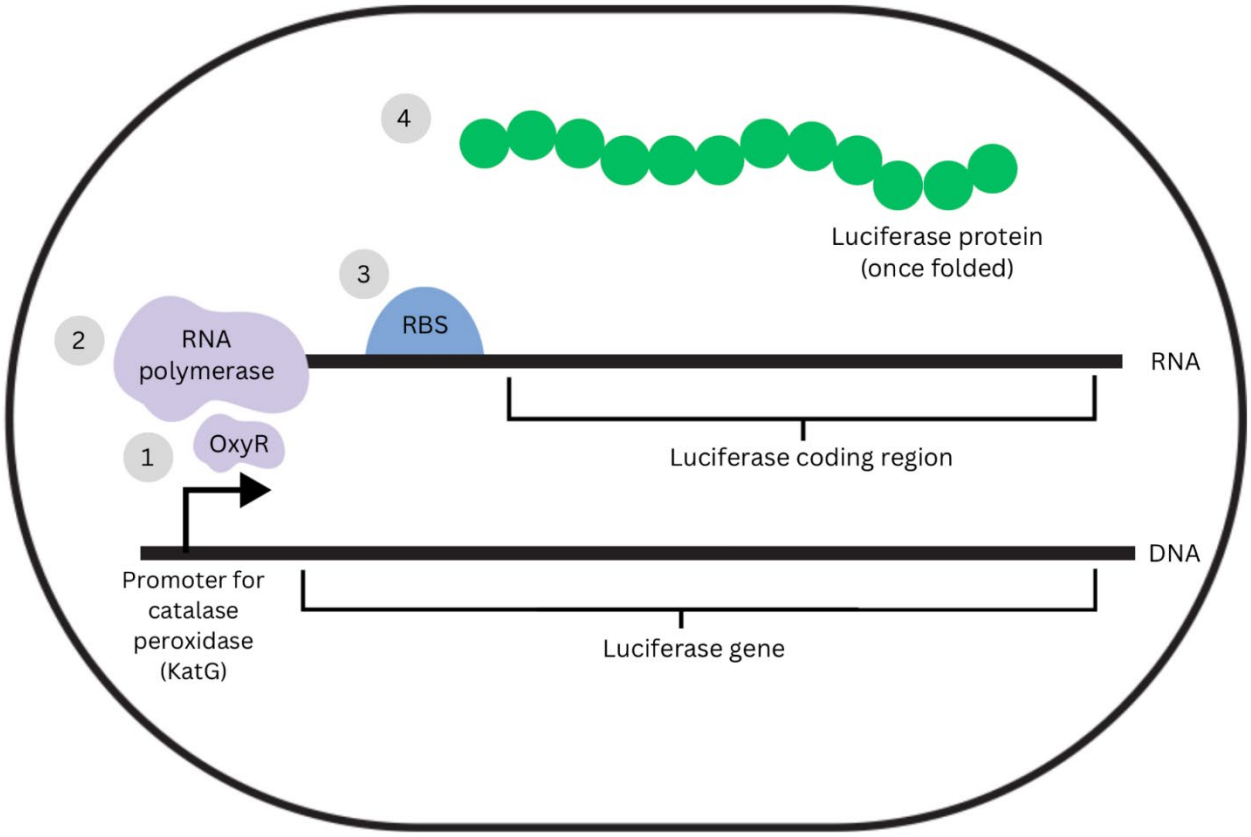

Figure 6. Pathway for production of luciferase protein. The OxyR protein binds to the catalase-peroxidase promoter region, facilitating RNA polymerase binding and subsequent transcription of the luciferase gene. The ribosome then attaches to the ribosome binding site on the RNA, translating it into luciferase protein.

After engineering the PVC biosensor, many tests under various conditions (temperature, pH, biosensor/enzyme concentration, etc.) will be performed to ensure its safety and productivity in various conditions. If any abnormalities arise in said experiments, adjustments will be made to the procedure to ensure the elimination of possible safety hazards.

Discussions

If PVC can indeed induce the promoter, our proposal not only serves as an important first step for microplastic biodegradation processes but can also become an important tool to detect the safety of widely distributed substances, such as drinking water. By granting scientists an efficient method to detect PVC microplastics, our proposal hopes to prevent the potential health risks that are correlated to PVC ingestion from drinking water. Many low-income communities have opted to install PVC drinking water pipes as a cost-effective alternative to older lead pipes, therefore, being able to identify contaminated water would be a step towards raising awareness of the potential health risks associated with ingesting PVC particles in said communities. Being able to test the microplastic contamination in drinking water from these new, popular PVC pipes can ideally urge communities towards non-PVC pipage options and determine the safety of drinking water for communities that may already have PVC pipes installed.

This proposal also provides a comprehensible means of interpreting test results. PVC detection will be signified visually through luminescence, giving the solution the potential to be utilized on a more universal scale. With our current measurement procedures, the connected computer will allow us to detect the presence of PVC in a quantitative manner. Eventually, as we begin to think about how our solution can be implemented for a larger audience, the luminescence aspect will be helpful for people who may not have the specific scientific knowledge to detect PVC in a laboratory setting. However, the necessity of a luminometer, *E. coli* cultures, and the skills to carry out the experiment, which are all rather expensive and difficult to acquire, makes the current setup of the project difficult to make mainstream. Potentially, the development of a less advanced method to measure luminescence, such as a lateral flow strip, could make usage of the product more accessible.

One of the shortcomings of our solution is that our techniques cannot yet be as safely replicated outside of the lab, especially due to the involvement of the *E. coli* bacteria species. Our proposal also requires the use of a microplate luminometer. Although the microplate luminometer is effective in measuring the luminescent output, it is not an easily accessible device outside of a laboratory. Thus, our current proposal is not an adequate solution ready for field-based analysis outside of a laboratory setting. Though it still has the potential to detect contamination in a more general sense for an entire community through laboratory testing, our device does not have the capacity to be utilized efficiently or safely in a home setting.

Additionally, due to a lack of testing, there are still a few aspects of our solution that pose challenges in large-scale implementation. In order for our proposal to be successful to a more universal audience, we have yet to determine the duration for which the reaction needs to occur in order for the bioluminescence to represent the PVC level in the sample adequately. If a minimum wait time for the reaction is not determined, we risk the device leading to faulty results as a result of the user not knowing how long it takes for the full-color change to occur. The time needed to culture the *E. coli* in PVC may also prove to be a hindrance in the time component of the proposal, as it could take a significant amount of time to induce Nluc synthesis and then add our substrate at a certain endpoint. We also have not yet determined how our proposal will yield results in varying conditions such as pH level or temperature and whether contamination of unrelated substances in the sample will have an impact on bioluminescent output in our results. Other variables such as concentration of PVC, duration of exposure to PVC, the buffer in which the PVC is dissolved, and more, in order to ensure that all potential outcomes are accounted for.

We face additional challenges when considering the role of transcription factors in the *E. coli* bacterium. We have yet to determine the specific mechanisms behind how transcription factors will bind to the catalase peroxidase promoter in the presence of PVC. If the protein binds to a repressor, therefore removing it from the promoter, or potentially an activator, activating the protein itself rather than initiating a binding, we would receive an inadequate end result. Thus, it is difficult to assume that simply utilizing the promoter sequence will facilitate the necessary reaction for our solution.

However, more extensive testing will target these challenges. Experimentation with PVC samples of varying pH and temperature may be utilized to determine the strength and duration required for the device to perform to maximum accuracy, for instance.

Next steps

Before putting our biosensor to use, we would first like to observe it under different environments. There are five possible experiments we would like to perform in order to better understand our biosensor's capabilities and possible setbacks:

1. Our first steps are to confirm our assumption that the presence of PVC causes an increase in OxyR in E. coli. To do this, we would measure OxyR protein in E. coli both in the presence and absence of PVC and observe if there is a large increase in OxyR with PVC. To measure an increase in transcript, we would also utilize a real time RT-PCR.
2. To test for precision we would take the same sample and run it on different machines and days to see how much the number varies. To test for sensitivity, we would conduct a titration curve with different concentrations of PVC.
3. Experiment under different conditions such as temperature, pH, and substrate concentration to observe the desirable activity of our biosensor. By discovering optimal conditions for luciferase and furimazine individually and also in conjunction with each other in our biosensor, we would be able to determine the best possible conditions that our biosensor would work in to maximize luminescence output and also possible environments that render it unviable.
4. Conduct field testing to validate the effectiveness of our biosensor for detecting PVC in natural environments. Collecting samples from varying environments would help us better understand our biosensor's capacity under different conditions. For example, conducting experiments under varying cleanliness levels (such as using muddy water) could help us determine the effectiveness of our biosensor outside of strictly laboratory conditions.
5. Test to see whether our biosensor generates luminescence in the presence of other plastics (e.g., PET HDPE, LDPE).

Additionally, if the biosensor does not work, another strategy is to identify an alternative promoter sequence which is sensitive to PVC in *E. coli*. To do this, we would conduct transcriptomics of *E. coli* both in the presence and absence of PVC. We would isolate the RNA, sequence it, and determine which transcript has the highest change between the two conditions. Then, we would identify the location of that gene and replace it with luciferase, keeping its promoter intact. Unlike the first strategy where we are introducing a known promoter in the plasmid, this second strategy involves replacing a gene in the genome of the *E. coli* with luciferase.

Author contributions

S.H.P. led the research project and the writing of the manuscript. S.H.P. and C.C. conducted extensive research on the project, with key assistance from S.W., H.K., and R.H. The writing and proofreading of the manuscript were carried out by H.K., S.H.P., C.C., and R.H. The graphics were created by S.W. with help from S.H.P.

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References

Bajt O. (2021). From plastics to microplastics and organisms. FEBS open bio, 11(4), 954–966. https://doi.org/10.1002/2211-5463.13120

Cade, C. E., Dlouhy, A. C., Medzihradszky, K. F., Salas-Castillo, S. P., & Ghiladi, R. A. (2010). Isoniazid-resistance conferring mutations in Mycobacterium tuberculosis KatG: catalase, peroxidase, and INH-NADH adduct formation activities. Protein science : a publication of the Protein Society, 19(3), 458–474. https://doi.org/10.1002/pro.324

Coz, E. L. (2023, April 20). New report warns about human health risks from PVC pipes used in drinking water systems. USA Today. https://www.usatoday.com/story/news/investigations/2023/04/18/new-report-warns-against-using-pvc-pipes-drinking-water-systems/11688737002/

Department of Human Services | PVC – a major source of phthalates. (n.d.). Www.nj.gov. https://www.nj.gov/humanservices/opmrdd/health/pvc.html#:~:text=PVC%20contains%20dangerous%20chemical%20additives

England, C. G., Ehlerding, E. B., & Cai, W. (2016). NanoLuc: A Small Luciferase Is Brightening Up the Field of Bioluminescence. Bioconjugate chemistry, 27(5), 1175–1187. https://doi.org/10.1021/acs.bioconjchem.6b00112

Esterhuizen, Maranda, and Young Jun Kim. (2021). “Effects of Polypropylene, Polyvinyl Chloride, Polyethylene Terephthalate, Polyurethane, High-Density Polyethylene, and Polystyrene Microplastic on Nelumbo Nucifera (Lotus) in Water and Sediment.” *Environmental Science and Pollution Research*, vol. 29, no. 12, pp. 17580–90, https://doi.org/10.1007/s11356-021-17033-0.

Forkl, H., Vandekerckhove, J., Drews, G., & Tadros, M. H. (1993). Molecular cloning, sequence analysis and expression of the gene for catalase-peroxidase (cpeA) from the photosynthetic bacterium Rhodobacter capsulatus B10. European journal of biochemistry, 214(1), 251–258. https://doi.org/10.1111/j.1432-1033.1993.tb17918.x

Kudzin, M. H., Piwowarska, D., Festinger, N., & Chruściel, J. J. (2023). Risks Associated with the Presence of Polyvinyl Chloride in the Environment and Methods for Its Disposal and Utilization. Materials (Basel, Switzerland), 17(1), 173. https://doi.org/10.3390/ma17010173

Liu, Y., Zhang, J., Zhao, H., Cai, J., Sultan, Y., Fang, H., Zhang, B., & Ma, J. (2022). Effects of polyvinyl chloride microplastics on reproduction, oxidative stress and reproduction and detoxification-related genes in Daphnia magna. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 254, 109269. https://doi.org/10.1016/j.cbpc.2022.109269

National Cancer Institute. (2018, December 28). Vinyl Chloride - Cancer-Causing Substances. National Cancer Institute; Cancer.gov. https://www.cancer.gov/about-cancer/causes-prevention/risk/substances/vinyl-chloride

National Oceanic and Atmospheric Administration. (2021, February 26). What Are microplastics? Noaa.gov; National Ocean Service. https://oceanservice.noaa.gov/facts/microplastics.html

PVC applications. ECVM. (2023, January 10). https://pvc.org/pvc-applications/

PVC Poison Plastic. (2023, April 13). Toxic-Free Future. https://toxicfreefuture.org/research/pvc-poison-plastic/

PVC Under Scrutiny as Hazardous Waste. (2022, May 24). EHS Daily Advisor. https://ehsdailyadvisor.blr.com/2022/05/pvc-under-scrutiny-as-hazardous-waste/

Poison Plastic: The Toxic Life Cycle of PVC. Ecology Center. (2024). https://www.ecocenter.org/plastic-pollution-and-zero-waste/poison-plastic-toxic-life-cycle-pvc#:~:text=Six%20percent%20of%20global%20coal,tons%20of%20CO2%20in%202020.

Polyvinyl chloride degradation by a bacterium isolated from the gut of insect larvae. (2022, September 12). NCBI. Retrieved April 18, 2024, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9468159/

Smale, Stephen T. (2010). "Luciferase assay." Cold Spring Harbor protocols vol. 2010,5: pdb.prot5421. doi:10.1101/pdb.prot5421. https://pubmed.ncbi.nlm.nih.gov/20439408/#:~:text=Cell%20debris%20is%20removed%20by,the%20consistency%20of%20the%20results

Smith, M., Love, D. C., Rochman, C. M., & Neff, R. A. (2018). Microplastics in Seafood and the Implications for Human Health. Current environmental health reports, 5(3), 375–386. https://doi.org/10.1007/s40572-018-0206-z

Tao, K., et al. (1991). "Purification and Characterization of the Escherichia Coli OxyR Protein, the Positive Regulator for a Hydrogen Peroxide-Inducible Regulon." *Journal of Biochemistry*, vol. 109, no. 2, pp. 262–66, pubmed.ncbi.nlm.nih.gov/1864839/. Accessed 20 May 2024.

Tiffen, J. C., Bailey, C. G., Ng, C., Rasko, J. E., & Holst, J. (2010). Luciferase expression and bioluminescence does not affect tumor cell growth in vitro or in vivo. Molecular cancer, 9, 299. https://doi.org/10.1186/1476-4598-9-299

Toxic Chemistry: PVC (Vinyl): Toxicity, Health and the Environment. (n.d.).https://www.greenspec.co.uk/building-design/polyvinyl-chloride-pvc-environment-health/

U.S. Plastics Pact's Problematic and Unnecessary Materials List - The U.S. Plastics Pact. (2022, January 24). https://usplasticspact.org/problematic-materials/

Valéria C. S. Italiani, et al. (2011). "Regulation of Catalase-Peroxidase KatG Is OxyR Dependent and Fur Independent in *Caulobacter Crescentus*." *Journal of Bacteriology*, vol. 193, no. 7, American Society for Microbiology, pp. 1734–44. https://doi.org/10.1128/jb.01339-10. Accessed 22 Jan. 2024.

Which Plastic Can Be Recycled? (2021, May 20). Plastics for Change. https://www.plasticsforchange.org/blog/which-plastic-can-be-recycled#:~:text=Low%2Ddensity%20polyethylene%2C%20also%20known

Zhang, Z., Peng, H., Yang, D. et al. (2022). Polyvinyl chloride degradation by a bacterium isolated from the gut of insect larvae. Nat Commun 13, 5360.https://doi.org/10.1038/s41467-022-32903-y