Design Brief

Water treatment degradation of PFAS*

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Perfluoroalkyl compounds known as PFAS are a type of microplastic known as "forever chemicals." Exposure to PFAS can cause various adverse health effects. PFAS are found in several industrial products and the environment, including the drinking water supply. This project proposes constructing a transgenic bacterial system to degrade PFAS in the water supply into harmless organic material. The first step into engineering this biological system is to test its promoter, which is known to be activated in the presence of PFAS using a reporter green fluorescent protein (eGFP) gene. This will be followed by testing the ability of enzymes DeHa 1 and DeHa 2 to degrade the PFAS. After the PFAS are eliminated, the next step is to determine if the bacteria can be eliminated by activating the self-destruct gene RhaS under the arabinose promoter. The final step, after successfully removing PFAS and bacteria, is to integrate this system into the water processing treatment. The authors hope that, with their system, they can help alleviate the negative effects of PFAS exposure by eliminating them from the water system.

Keywords: PFAS, perfluoroalkyl compounds, microplastics, water supply, synthetic biology

Per- and polyfluoroalkyl substances (PFAS) are known as "forever chemicals" due to their resistance to breakdown under normal environmental conditions. PFAS are man-made chemicals consisting of carbon fluorine chains with bonds that are very difficult to break (Manojkumar et al., 2023). PFAS have been used since the 1940s and 1950s and have been used widely in many consumer products and industrial applications (Kowalska et al., 2023; Wisconsin Department of Health Services, 2018; U.S. EPA, 2024; Figure 1). There are over 5,000 PFAS of man-made products that are commonly used because of their oil and water resistant properties, making them both hydrophilic and hydrophobic (Illinois Environmental Protection Agency, n.d.; Kowalska et al., 2023). In addition, PFAS are so small that they can pass through the water filtration process system (Amen et al., 2023). PFAS

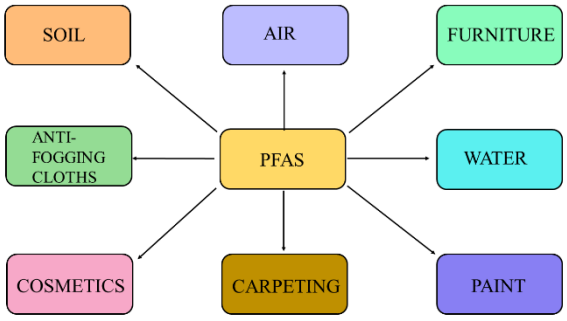

Figure 1. Some of the places where PFAS can be found. Otherwise, PFAS are ubiquitous in the environment.

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can cause adverse health effects, such as thyroid disruption, liver damage, and cardiovascular disease depending on individual factors and type of exposure (Fenton et al., 2020; Figure 2). Every day researchers are discovering more and more adverse effects to humans and animals linked to PFAS exposure.

Current filtration protocols, like reverse osmosis and ion exchange, have been shown to be effective at removing PFAS from the water (Amen et al., 2023). However, reverse osmosis has limits. The process is extremely slow while using lots of water and energy. Furthermore, reverse osmosis faces additional limitations such as removing healthy minerals like calcium, potassium, and magnesium when it comes to large-scale filtration (Pace, n.d.).

These microplastics have become prevalent in the environment. PFAS can be found in the air, water, and soil. Because of this, the authors' goal is to eliminate PFAS from the water supply. They have designed a bacterial system to degrade PFAS in water at a large scale and then induce the bacterial system to self-destruct.

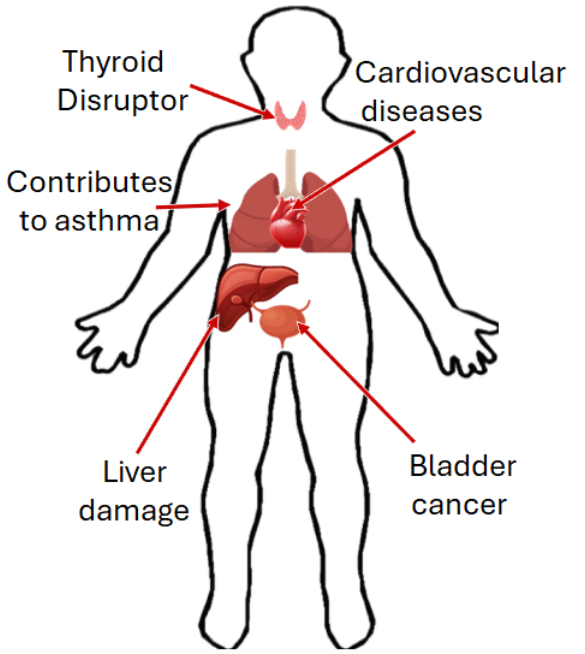

Figure 2. Some of the potential negative effects of PFAS in the human body. However, researchers are continuously identifying additional adverse effects from exposure to PFAS (Science, 2022).

Systems level

The overall objective is to develop a biological system to remove PFAS found in drinking water during water treatment. *Escherichia coli* (*E. coli*) is the chassis that will be engineered to respond to the presence of PFAS, activating the expression of enzymes that degrade them. Subsequently, they will be able to then activate the chassis self-destruction under the presence of arabinose. The first objective of this project is to develop a biological system using enzymes that are activated when exposed to PFAS that will result in their degradation. The second objective of this project is to design the biological system to self-destruct after the degradation of these compounds.

Device level

The purpose of this project is to design a system that eliminates PFAS from drinking water by engineering a bacterium *E. coli* DH5α that contains a plasmid activated by exposure to PFAS, thus expressing the enzymes and degrading PFAS into harmless organic compounds. The implementation of this system will be constructed and tested in three phases: testing the activity of the promoter, testing the efficacy of the enzymes (Figure 3A), and testing the destruction of this system by activating the kill-switch gene, *RhaS,* once the degradation of these forever chemicals in the water has been observed (Figure 3B).

The first objective is to test the efficacy of the promoter, prmA , which is known to be activated in the presence of PFAS (Young et al., 2021). The authors will insert their promoter in the EGFP-PBAD (Addgene plasmid # 54762) plasmid containing an enhanced green fluorescent protein (eGFP). The authors will use the T7 promoter that is already inserted into the plasmid to activate expression of the eGFP gene for the positive control for this assay. For the experimental construct, they replaced the T7 promoter with the prmA promoter **(**Figure 4**)**.

In the second step, the authors want to test their PFAS-degrading enzymes, dehalogenase 1 and 2, which are thought to

break the carbon-fluorine bonds. However, the exact pathway is currently being investigated (Harris et al., 2022;WP_011137954.1, BBaK3347002; Figure 5).. For this phase, the researchers will create three constructs: one to test the activity of dehalogenase 1 (Figure 5A), a second one to test the activity of dehalogenase 2 (Figure 5B), and a third construct to test the activity of both enzymes together (Figure 5C). The objective of this step is to measure the effectiveness of the enzymes in breaking down PFAS by quantifying the amount of PFAS that are left in the system versus the amount of PFAS inserted that are broken down.

The third step of constructing this system aims to initiate a self-destruct protocol to eliminate the bacteria from the water supply. For this construct, the researchers will add

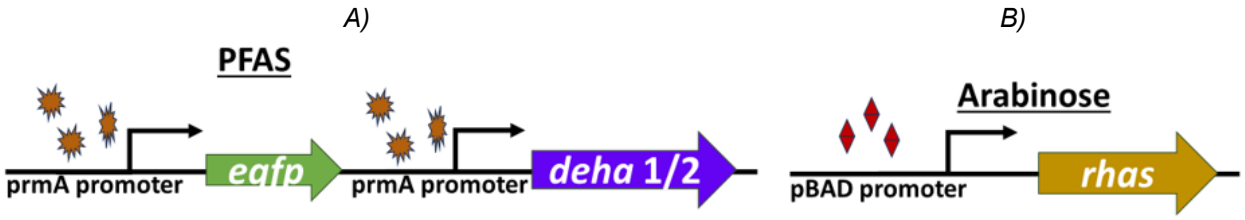

Figure 3. Overview of the PFAS degradation system. (A) Construct to measure activity of the prmA promoter by measuring the intensity of eGFP as a reporter, and the use of the prmA promoter to activate the Dehalogenase 1 and 2 enzymes required to break down PFAS. (B) After the PFAS degradation system has performed its job, the self-destruct gene will be activated by arabinose using the PBAD promoter.

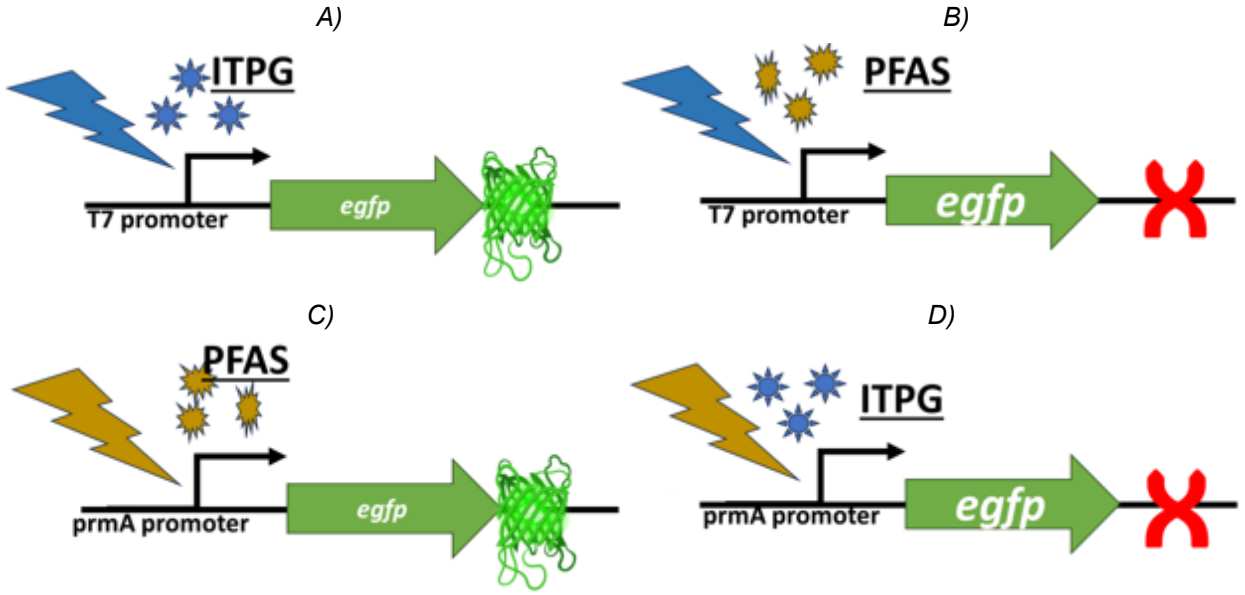

Figure 4. Testing the activity of the prmA promoter. (A) Positive control, expression of eGFP under the T7 promoter with the addition of ITPG and (B) the T7 promoter without ITPG. With the addition of PFAS, this should result in no GFP. (C) The experimental construct will be used to test the activity eGFP under the control of the prmA promoter. (D) The prmA promoter with ITPG instead of PFAS, which should result in no GFP.

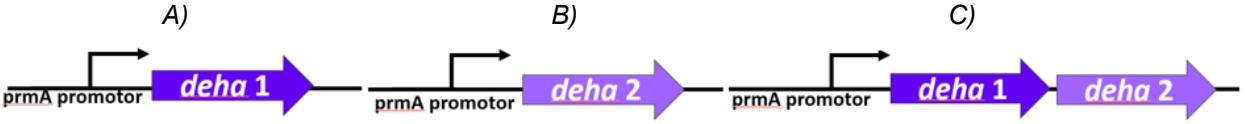

Figure 5. Testing the dehalogenase enzymes in the degradation of PFAS. (A) Construct testing the efficacy of dehalogenase 1, (B) construct testing the efficacy of dehalogenase 2, and (C) construct testing the efficacy of dehalogenase 1 and 2 together.

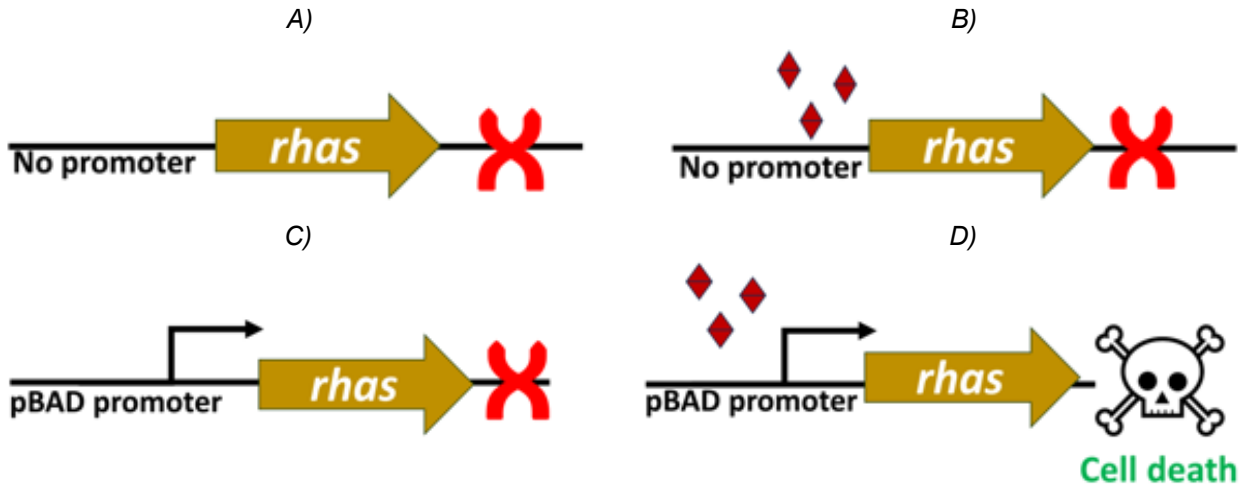

Figure 6. Activation of the self-destruct gene rhaS. Predicted outcomes: (A) Negative control construct with the kill-switch gene rhaS, but without the promoter and no arabinose should result in no cell death (B) no promoter with arabinose resulting in no cell death (C) construct with the arabinose promoter, PBAD and the rhaS gene, but no arabinose should result in no cell death (D) PBAD promoter and the rhaS gene with the addition of arabinose should result in cell death.

the kill-switch gene, *rhaS*, which ensures that the biological system will be inactivated after the degradation of PFAS (Meisner & Goldberg, 2016). The kill-switch gene will be under the pBAD promoter that can be activated in the presence of arabinose (Figure 6).

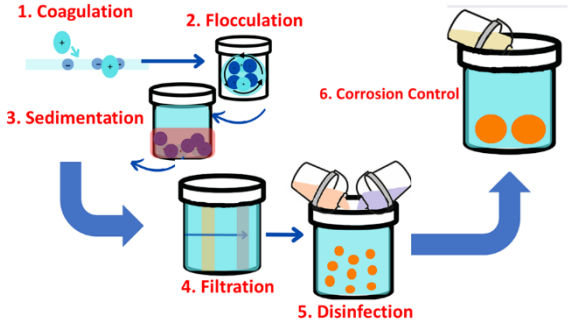

Figure 7. Integration of the proposed system into the water processing system 1. Coagulation: Positively charged coagulants are added to bond with negatively-charged particles in the water. 2. Flocculation: Results in small particles colliding and merging into larger particles. 3. Sedimentation: Heavier particles settle to the bottom; sludge is formed and removed during sedimentation. 4. Filtration: Materials such as sand and gravel are filtered out. 5. Disinfection: During this step, chemical disinfectants are added (e.g. chlorine or chlorine dioxide) killing the remaining bacteria, parasites, and viruses. 6. Corrosion Control: During the final step, the pH level is raised using caustic soda. It serves as a disinfectant and removes pollutants (CDC, 2022).

Once this biologically engineered system has successfully eliminated PFAS from the water supply, the authors aim to eliminate their biological system from the water supply. The first part of this step is to activate the kill-switch gene, which instructs the bacteria to self-destruct. Secondly, they propose to integrate their system into the water processing system after the coagulation process, which neutralizes fine particles by clumping them together. At this time, the system will degrade PFAS and then be induced to self-destruct. This step occurs before the flocculation process, in which flocculants added to the water bind smaller particles together and form larger, heavier particles that will settle to the bottom (ChemREADY, 2024). These will be easier to remove. The filtration and disinfection of the water processing treatment will further clear the water of any bacterial remnants (Figure 7).

Parts level

The proposed biological system aims to first validate a promoter that is known to be activated in the presence of PFAS

(BBa_K2911000). The second objective is to test enzymatic activity by measuring the degradation of PFAS. Lastly, in order to eliminate the bacterial system from the water, a self-destruct protocol will be activated using a promoter that responds to the presence of arabinose. This final step ensures that the bacterial system is then removed from the water processing. The final product showing how the plasmid will look is depicted in Figure 8. All constructs will be transformed into DH5α bacteria cells, which are a type *E. coli.* DH5α cells are genetically modified cells engineered by Douglas Hanahan in the 1980's that significantly increase transformation efficiency (Wert, 2024).

The first step is to engineer the EGFP-PBAD plasmid (Addgene plasmid # 54762) by inserting a promoter (prmA) that is known to be activated in the presence of PFAS (Young et al., 2021). The EGFP-PBAD plasmid contains a T7 promoter that, when activated by ITPG, will activate expression of a reporter gene known asenhanced green fluorescent protein (eGFP). This will be the positive control. Next the researchers will replace the T7 promoter with the prmA promoter to test the activation of the reporter gene in the presence of PFAS (BBa_K2911000). This will be the experimental construct.

For the experimental construct, the authors will genetically engineer the EGFP-PBAD plasmid as a vector by removing the T7 promoter and replacing it with their promoter, prmA, by double digest. Their prmA insert will be produced by PCR of a plasmid with the promoter, pSRKBB (Young et al., 2021). Their PCR primers are designed with flanking restriction enzymes BmtI and XhoI (Table 1). After double digestion of the PCR product 194 base pair (Table 2) and the vector EGFP-PBAD with the same restriction

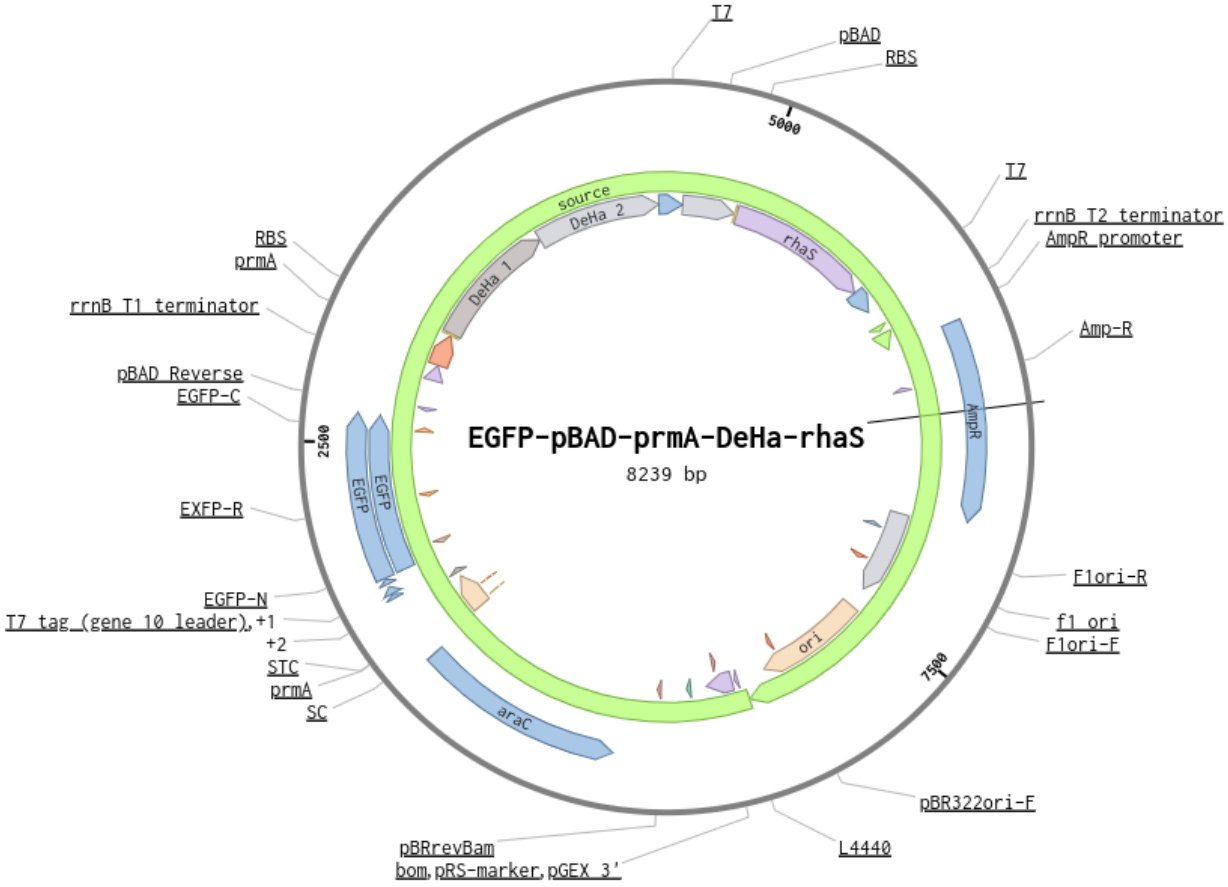

Figure 8. Components and map of the EGP-PBAD plasmid (Addgene plasmid # 54762) with the addition of prmA, DeHa 1/2, and rhaS. All vectors for the proposed plasmids were constructed using Benchling.

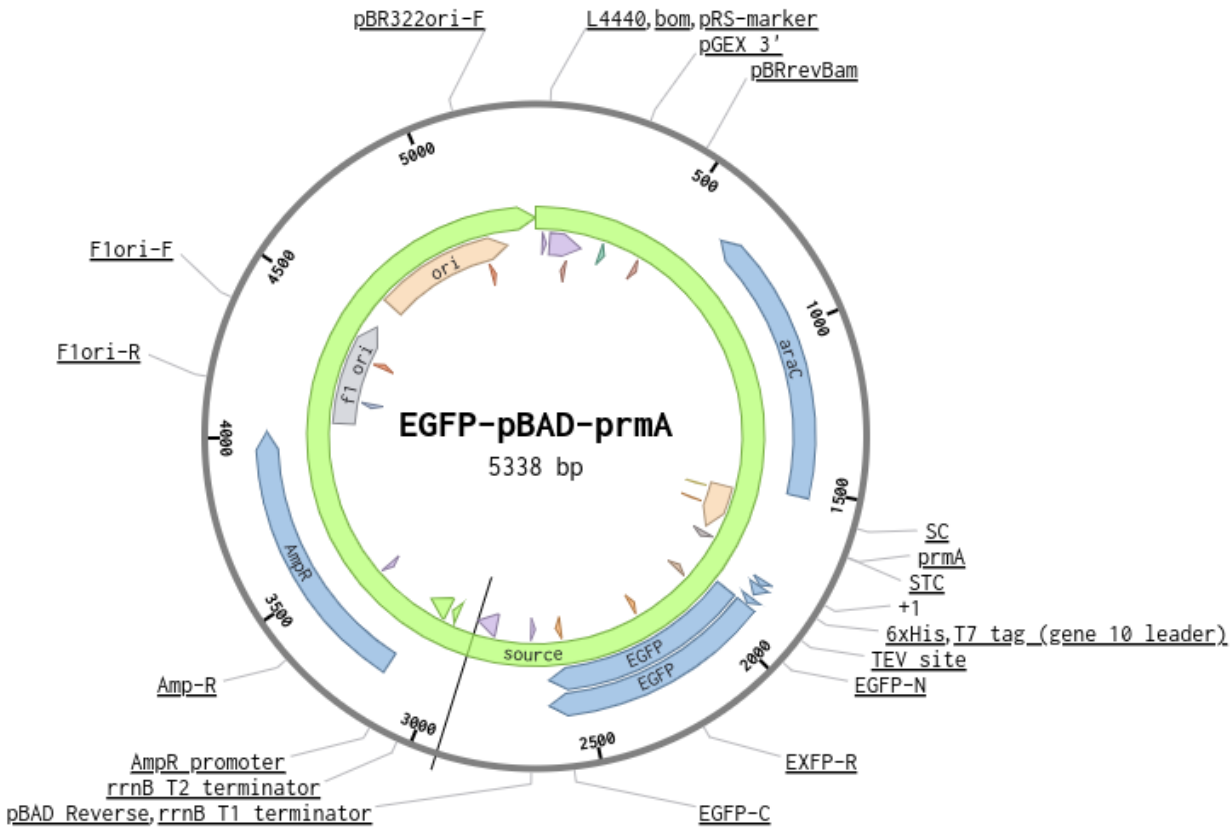

Figure 9. Proposed promoter, prmA ligated into EGFP-PBAD plasmid.

enzymes, the researchers will ligate them together creating their new construct, EGFP-PBAD-prmA plasmid (Figure 9). Construction and simulation of double digestion and PCR were carried out on Benchling.

The final step in engineering this system is to insert the rhaS gene, which is the kill-switch gene that instructs the bacterial system to self-destruct. The rhaS gene will be designed to activate the expression of the kill-switch gene in the presence of arabinose under the PBAD promoter. The kill-switch activation is the first step to eliminating the bacteria after its job of breaking down PFAS into harmless organic material has been completed.

Table 1. Modified primers for prmA with the addition of restriction enzymes flanking the promoter, BmtI at the 5' end and XhoI at the 3' end of the promoter (Young et al.,2021).

Primer Specification	*Sequence*
prmA F BmtI	*5' GCATGCTAGCgagggcgtgatggtctgc 3'*
prmA R XhoI	*5' GCATCTCGAGtgtgcgcctcctggatcgtg 3'*

Safety

Cell culture

E. coli is commonly used in scientific research. Protein expression in *E. coli* should be performed at Biosafety Level 1 (BSL1). To prevent contamination, before any lab experiment is performed, one should ensure that the workspace is clean and sterilized by wiping it down with 70% ethanol. Additionally, personal protective equipment (PPE) should be worn at all times and proper protocol should be followed according to the guidelines. Upon completion, all bacterial cultures must be properly disposed of. Bacteria is typically disposed of by autoclave, or if an autoclave is not available, with a 10% bleach solution to kill the bacteria (Addgene Protocols, n.d.)

Water treatment safety

The proposed PFAS-engineered system includes self-destruction of the bacteria after the degradation of PFAS. To further ensure that bacterial remnants are removed from the water supply, the authors propose the insertion of the PFAS system into the second stage of the water processing system (Figure 7). The researchers believe that the second stage is a good place to introduce their transgenic system because the system will have time to destroy PFAS before being introduced into the later stages of the water processing system (CDC, 2022). It is in the later stages in which all the remains of the system will be destroyed with chemicals and filters. Having the bioengineered system self-destruct is one scenario that the authors have been investigating in which the system would have to be replenished. Alternatively, one could find a way to harvest the system after degradation of PFAS and then reuse it. In conclusion, this PFAS bacterial system is first engineered to degrade PFAS into harmless organic materials, which can then be induced to self-destruct. Lastly, through the water treatment process, the bacteria will be safely removed from the water supply.

Table 2. Sequences of construction for the PFAS biological system. PrmA promoter (Young et al.,2021); Enzymes for the degradation of PFAS, dehalogenases 1 and 2 (DeHa 1 & DeHa 2; Harris et al. 2022); Kill-switch gene, rhaS for the destruction of the bacteria (Meisner J. et al. 2016).

Part	*Sequence*
prmA	gagggcgtgatggtctgccgcggcgcggggcaagtgtaggcagccaccgcccactcg gaagtgacctgcgtctcacactgagacgaatgagagctggctcacggggttcaatcaat ttccacaacgtccccccacagccggcacccggccggctcacgatccaggaggcgcac a
DeHa 1	atgctgcgcctgagcgattttgatgcggtgacctttgatgtgtatggcaccctgattgattgg gaaccgagcattgtggcgtttctgagcgaatgggcgggcgaactgggcgtgaaagcga gcggcgaagatctgattatgagctttgatcgcgcgcgcgcggaaattcagaaagaacg cccggcgcatctgtatccggaagtgctgcgcccgctgctttaaccgcattagcggcgaattt ggcgtggcggtggatgcggcgcgccgcgaagcgtttagcaaaaccccgcatcagtgg ccggcgtatagcgataccagcgcgggcctgaaagcgctgcaagcgcgcgcgcgcatt ggcgcgctgagcaacattgataacgcgagcctggcgagcagctgcaaaaacatggat attgaatttgatattgtggtgaccgcggaacgcgtgggcgcgtataaaccgagctgggaa cattttaacaccgcgattgcggaactggaaggcatgggcattccgcgcgaacgcattctg catgtgggccagagcctgcgcgcggatattaccccggcgaacaaactgggcctgaaa agcgcgtggattaaccgcccgcagcgcctgctgggcctgagcggcgaaggcgcggc ggaagcgcgcccggatctgaccgtggaaagcctggcggaactgctgaaagcgattgg ctaa
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rhaS	ttattgcagaaagccatcccgtccctggcgaatatcacgcggtgaccagttaaactctcg gcgaaaaagcgtcgaaaagtggttactgtcgctgaatccacagcgataggcgatgtca gtaacgctggcctcgctgtggcgtagcagatgtcgggctttcatcagtcgcaggcggttca ggtatcgctgaggcgtcagtcccgtttgctgcttaagctgccgatgtagcgtacgcagtga aagagaaaattgatccgccacggcatcccaattcacctcatcggcaaaatggtcctcca gccaggccagaagcaagttgagacgtgatgcgctgttttccaggttctcctgcaaactgct tttacgcagcaagagcagtaattgcataaacaagatctcgcgactggcggtcgagggta aatcattttcccctttcctgctgttccatctgtgcaaccagctgtcgcacctgctgcaatacgct gtggttaacgcgccagtgagacggatactgcccatccagctcttgtggcagcaactgatt cagcccggcgagaaactgaaatcgatccggcgagcgatacagcacattggtcagaca cagattatcggtatgttcatacagatgccgatcatgatcgcgtacgaaacagaccgtgcc accggtgatggtatagggctgcccattaaacacatgaataccgtgccatgttcgacaat cacaatttcatgaaaatcatgatgatgttcaggaaaatccgcctgcgggagccgggg

Discussions

Science-based research is constantly being updated with new reports describing health threats from exposure to these "forever chemicals." PFAS are difficult to eliminate due to their ubiquitous nature in the environment, and they bypass most cleaning stages of the water purification system. This makes humans more susceptible to PFAS intake, which impacts health.

PFAS are now considered public health risks. To protect the communities, the Biden-Harris administration with the Environmental Protection Agency (EPA) are now establishing rules and ways to regulate the amount of PFAS in the environment (U.S. EPA, 2024).

PFAS are a public health issue that needs to be addressed. Due to the current water treatment's inability to eliminate PFAS from the water supply, it is important for to develop a system that can safely remove PFAS. The authors are proposing a system that can be utilized in water treatment facilities.

Next steps

For their next steps, the researchers will test the promoter, prmA. For this step, they will have two constructs: EGFP-PBAD, their control construct that contains the T7 promoter with EGFP gene, and their experimental construct that replaces the T7 promoter with the prmA PFAS promoter. After transforming the construct into DH5α cells, their positive control bacteria will be activated with ITPG to see if the experiment will fluoresce. Then they will activate their experimental construct with PFAS.

One problem the authors foresee is an adequate way to measure PFAS. Currently, liquid chromatography with tandem mass spectrometry is the most accurate way to

measure the level of PFAS in the water system (Rehman et al., 2023). Although it is one of the most common ways to measure PFAS, one major problem with this is that it can be quite expensive. Due to financial constraints, the researchers will need to find an alternative method to measure PFAS.

Author contributions

This research project was conceived and developed by A.W.'s Advanced Placement Biology class, which was a group effort. E.C. was responsible for the plasmid designs, writing the systems level, and device level with the help of F.B. E.C. was also responsible for the writing of the parts level. Most of the background information was collected and written by J.M. with the help of K.C. The discussion was written primarily by B.G. with help from J.M. Both B.G. and J.M. wrote the safety section, while C.S. assisted in the research of the water treatment plants and the associated figure.

Acknowledgements

This project was accomplished through the participation of BioBuilderClub, which was embedded into our research class under the supervision of Ana White. We thank BioBuilderClub's Natalie Kuldell and Chloe Franklin for their patience and support throughout this process. We would also like to give special thanks to Janet Standeven and Dr. Saad Bhamla from Frugal Science at Georgia Tech, who assisted and supported the development of our project. Lastly, we acknowledge our BioBuilderClub mentor, Dr. Michael Sheets, Sunflower Therapeutics, for guiding us with our project design.

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