BioBuilderClub

The Final Assembly

MIT WELCOME CENTER

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3.14.24

Abstract Book

BioBuilder Educational Foundation

Welcome

EMPOWERING THE NEXT GENERATION TO ADDRESS GLOBAL CHALLENGES WITH BIOSCIENCE.

47 TEAMS.

4 COUNTRIES.

11 STATES.

Thank you to the wonderful teachers, mentors, and students who generously gave their time and energy to these projects.

Many of our teams are participating remotely today. Scan these QR codes to view the Virtual Poster Gallery, join our Discord server to engage directly with teams, and download this abstract book.

POSTER GALLERY

BIOBUILDER DISCORD

ABSTRACT BOOK

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Environment and Sustainability

This year, environment and sustainability was the most popular project topic area as these teams used synthetic biology to take on challenges ranging from the impact of global warming on diverse ecosystems to the toxic accumulation of microplastics in the environment.

Teams addressed pollution caused by a variety of plastics including PET, PVC, and PFAS. Strategies ranged from designing transgenic organisms that produce plastic-degrading enzymes to developing a biosensor to locate a specific type of plastic to promote its recycling.

Some teams took on global challenges such as how to protect coral reefs from bleaching caused by ocean acidification and chemicals found in sunscreen. Others focused on issues of significant local concern such as how to enrich the soil or protect the staple crop of theirgeographic region.

Teams thought deeply about the environmental impact of chemicals in commonly employed solutions to challenges ranging from icy winter roads to disease-carrying tick bites and proposed sustainable alternatives employing synthetic biology.

Students envision a future where synthetic biology is used to increase the safety of the food we eat and the water we drink by reducing mercury levels in fish or decreasing BPA contamination in water. Students proposed more sustainable ways to remediate pesticide runoff or increase the nutritional content of crops.

Regardless of the specific project, students approached significant challenges relating to the environment and sustainability with careful consideration and creativity resulting in the kinds of solutions we need to tackle some of the most pressing issues faced globally.

Evaluating the Efficacy of the PETase and Leaf Compost Cutinase Enzymes in Breaking Down Polyethylene Terephthalate Plastic in a Cell-Free System

Andover High School, Andover, MA, US

Vismay Ravikumar, Evelyn Wheel, Hannah Lehmann, Megan Zhang, Aarit Chauhan, Miley Arora, Sanjith Kalpat

Teacher: Lindsey L'Ecuyer
Mentor: Nathan Crook and Tianyu Li (NCSU)

Plastic water bottles currently are made primarily from PET (polyethylene terephthalate), a plastic that does not degrade on its own and is polluting our oceans. The enzyme PETase can break down PET into its monomer, mono ethylene terephthalate, and then further into ethylene glycol(EG) and terephthalic acid(TPA) by the enzyme MHETase. Leaf Compost Cutinase(LCC) is an alternative enzyme that hydrolyzes PET into its monomeric parts, functioning at temperatures from 60 to 70 degrees Celsius. Using an engineered plasmid containing the DNA sequences for either PETase or LLC in a cell-free system, our project is a proof of concept analyzing the efficacy of these enzymes on PET substrate in powder and film form.

Project PVC: Detecting Polyvinyl Chloride

Brookline High School, Brookline, MA, US

Soleil Hayes-Pollard, Chelsy Co, Sophie Wesemann Isabella Wong, Haley Kim, Simrah Bawa, Roman Horowitz, Suvi Carlile

Teacher: Sarah Hemphill
Mentor: Tarik Hunt (Lanza Tech)

Polyvinyl chloride (PVC) is one of the most commonly used plastics, with more than 40 million tons produced annually. Despite its negative impact on human health and the environment, there has been a lack of extensive research conducted to detect PVC and find ways to recycle it. Therefore, working towards innovative methods to detect and degrade PVC is essential. This project aims to create a PVC biosensor that can detect PVC by using luciferase as a reporter gene and a suitable promoter, which will be inserted into *Escherichia coli (E. coli)*. A group in Zhejiang Province, China, researched a bacterial strain in the gut of *Spodoptera frugiperda* (*Klebsiella* sp. EMBL-1), which can depolymerize and degrade PVC. Through conducting proteomics, the group characterized several enzymes as part of the process. Specifically, we focus on catalase-peroxidase, an enzyme within the larvae that is critical in the degradation process. To find the promoter sequence, we will clone various lengths of DNA regions upstream of catalase-peroxidase within the genome. We will then clone these sequences in front of luciferase in a plasmid, which will then be transformed into a bacterial host. Control experiments and quantitative analysis methods will be used to determine the level of luminescence, ultimately providing insight into a functioning promoter's DNA sequence. This research will be a step forward in understanding how we can detect PVC.

Water Treatment Degradation of PFAS

PFAS are a type of microplastic known as forever chemicals. Exposure to PFAS can cause various adverse health effects and are found in a variety of industrial products and throughout the environment including our drinking water supply. Our project proposes constructing a transgenic bacterium to degrade PFAS in the water supply into harmless organic material. Our initial step is to test our promoter, which is known to be activated in the presence of PFAS using a reporter GFP gene. The next step is to test the ability of enzymes DeHa 1 and DeHa 2 to degrade the PFAS. After the PFAS is eliminated, our next step is to determine if the bacteria can be eliminated by activating the self-destruct gene rhaS under the arabinose promoter. If our system successfully removes the PFAS and bacteria, we will integrate it into the water processing system.

Cross Keys High School, Atlanta, GA, US

Exahel Castaneda, JoAnna Chaclan, Floricela Bravo, Juan Mendoza, Carolina Torres, Camillo Samano, Brandon Gonzalez, Kevin Cruz,

Teacher: Ana White
Mentor: Dr. Michael Sheets (Sunflower Therapeutics)

Protectinga Midsouth Staple: Using Genetically Modified Bacteria as a Pesticide to Protect Cotton

The objective of our project is to develop a safe pesticide that can reliably deter common pests that target cotton, particularly boll weevils.

Crosstown High School, Memphis, TN, US

Santiago Arbelaez, Freya Donati, Alyssa Gray, Rowan Heeter, Zoe Ingram, Lily Little, Mason Owens, M Pfeiffer, Sam Sailors, Jaxon Treadwell, Tyler Vego

Teachers: Nikki Wallace, Jacob Weaver
Mentor: Chris Kuffner (Boston University)

NitriAlert

Fish in tanks can easily die from high concentrations of nitrate and nitrites in water. Water quality testing contains harsh chemicals and is time-consuming. To address this issue, a plasmid was designed to combine the PyeaR promoter, a ribosome binding site, and the tspurple gene. This plasmid is intended for use in transforming *E. coli,* which will be housed in a chamber attached to the side of the fish tank. This system, named "NitriAlert," functions by inducing a visual color change in response to excessive levels of nitrate/nitrites in the tank, thereby alerting users to the need for a water change before harm is caused to the fish. By incorporating "NitriAlert" into the tank, fewer fish will be endangered by sudden spikes in nitrate/nitrite levels.

Dobyns-Bennett Excel High School, Kingsport, TN, US

The BioBuds

Chase Howard, Tomidylan Klepper, Eric Thomas, Natalie Baker, Brylee Jones, and Tucker McLain,

Teacher: Amanda Dunham
Mentor: Hia Ming (Boston University)

PRJCT SCRaPES: Save Coral Reefs and Promoting Ecosystem Sustainability

An ever-growing issue, global warming has haunted more than just surface temperatures. In the ocean, a process called ocean acidification is driven by the proliferation of greenhouse emissions in the atmosphere. The raised temperatures in the ocean have a direct, adverse impact on ocean life, including coral. Coral holds a symbiotic relationship with the microalgae Zooxanthellae. Ideally, coral and Zooxanthellae benefit from each other by providing safe habitats and nutrients for the other organism to live. However, when temperatures rise too high, Zooxanthellae produce reactive oxygen species which are harmful to the coral. As a direct result, the Zooxanthellae is expelled from the coral to prevent any harm to the coral, causing coral bleaching, a worldwide plague. This project, in its future ambitions, aims to find the optimal way to genetically modify Zooxanthellae to promote a healthy relationship with the coral in which no harm occurs to either species. This is important as a multitude of ecosystems are heavily reliant on coral reefs.

Dobyns-Bennett High School, Kingsport, TN, US

The Synthesizers

Mia McVey, Liam Klug, Lyle Musesengwa

Supervised by Ellen Klug
Mentor: Dr. Donald Lorimer (UCB)

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Plastic-Eating Algae, brought to you by the GENEIES!

International School of Boston, Cambridge, MA, US

Josh Beresford, Oliver Beresford, Elizabeth Hossack, Samantha Botti, Nina Minsky, Nyla Tait, Roma Hatton-Rueda, Arezu Kabuli, Jasmine Rancourt, Shreya Desai, Karin Schinkmann, Mark Cherepashensky (Mentor-Boston University)

Plastic pollution is a pressing environmental issue especially affecting the health of marine animals. There are ongoing efforts to mitigate the impact of this marine debris by developing novel organisms that have enhanced abilities to degrade plastics. In a particular study by Cell Factory Research has described experiments to insert a PETase gene into the pBR9_mCherry_Cre plasmid and introduce it into a cell wall deficient strain of *Chlamydomonas reinhardtii*.

Our research goals are to integrate a PETase gene containing plasmid into a strain of *Chlamydomonas reinhardtii* that has a naturally occurring cell wall. In order to do this we will adapt our methods to integrate the plasmid into the target organism using a glass bead method. This experiment will allows us to explore the potential of plastic eating algae's ability to reduce polyethylene waste in the ocean.

DETOX-I-FISH

Lambert High School: Team GA1, Suwanee, GA, US

Tessica Selvaganesan, Aditya Mukker, Ayan Ragunathan, Aditi Pabidi, Daksh Reddy, David Park, Keerthana Anumukonda, Julia Crossen, and Kensley Burke

Supervised by Catherine Sharer and Pam Seeley
Mentor: Dr. Kristin Jenkins (BioMADE)

We are working on developing an *E. coli* that uses different Mer proteins to break down the methylmercury found in fish into a less toxic form of mercury. Fish are soaked in this *E. coli* solution in order to break down the methylmercury and then cooked to remove all the *E. coli,* as the bacteria are unable to survive in higher temperatures. Our project is used to prevent mercury contamination and mercury poisoning in food. Mercury poisoning is a lethal condition when it isn't addressed, especially in shoreline and fish reliant countries. This plan is a potential efficient treatment process to ensure food safety.

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Degradation of Bisphenol-A Using Germinating Schizachyrium scoparium Seeds

Bisphenol A (BPA) is a common material used in plastic manufacturing. Plastic products, such as water bottles made with BPA can leach BPA into drinking water and food products, which can cause physiological disruptions in the body once ingested. To counter this problem, our team modified a procedure performed by Putnam and her team (2017) to decrease BPA concentrations in sources of water using germinating *Schizachyrium scoparium* seeds. This research supports that germinating *S. scoparium* seeds decrease the concentration of BPA present in a solution. Further trials and an improved procedure would be necessary to validate these results. Bisphenol A (BPA) is a common material used in plastic manufacturing. Plastic products, such as water bottles made with BPA can leach BPA into drinking water and food products, which can cause physiological disruptions in the body once ingested. To counter this problem, our team modified a procedure performed by Putnam and her team (2017) to decrease BPA concentrations in sources of water using germinating *Schizachyrium scoparium* seeds. This research supports that germinating *S. scoparium* seeds decrease the concentration of BPA present in a solution. Further trials and an improved procedure would be necessary to validate these results.

North Quincy High School, Quincy, MA, US

Nancy Chieu, Aakanksha Deshmukh, Diana Dang, Tabitha Lee, Christine Yan Lin, Isabelle Ng, Huy Nguyen, Kimy Ong, Irena Shi, Chloe Wu, Julia Buben (Teacher), Dr. SitaRam Meena (UCB)

Combating Oxybenzone with Laccase Enzyme

Oak Park and River Forest High School: Environment Team, Oak Park, IL, US

Ally Sugita, Cael Walicki, Ellie Smith, Linnea Nam, Maggie Herman, Marin Chalmers, Sadie Parkinson, Will Kaegi, Matthew Kirkpatrick (teacher), Dr. Alberto J. Donayre Torres (Mentor-UTEC)

Issues such as coral bleaching and death being addressed through synthetic biology is important for broadening and improving methods to mitigate anthropogenic consequences on marine wildlife. This project focused on using synthetic biology to combat the negative effects of the common sunscreen ingredient, Oxybenzone, on corals without harming other organisms found in reef environments. Previous studies have used the Oxidase enzyme, Laccase, as a means of preventing the metabolic reaction which turns Oxybenzone phototoxic and therefore harmful towards corals (Luisa, n.d.). We propose using an alginate matrix with immobilized Laccase from the fungi species *Trametes Versicolor,* to release into environments affected by Oxybenzone. The Laccase enzyme will be produced by inserting *Trametes versicolor* strain DNA into a T7 plasmid vector, which when expressed, will produce the enzyme. Once isolated, the enzyme will be immobilized and ready for use.

Bio-Char(ming) New England's Soil Fertilizer

Shrewsbury High School, Shrewsbury, MA, US

Aadrishma Dahal, Aashi Gupta, Aleena Shaikh, Amal Nadaf, Ayesha Naveed, and Rachael Laikangbam, Dr. Duggan (teacher), Susana Donkor (Mentor-Aurion Biotech)

This study researches the impact of Plant Growth-Promoting Bacteria (PGPB) and nutrient-infused biochar on plant growth in New England soil. New England's soil is notorious for its lack of certain nutrients, rocky structure, and other problems. When carried out, the experiment will soak biochar in water and infuse it with nutrient rich substances and PGPB. Then, it will be mixed in soil to grow plants, compared against a control group grown in regular soil. Over five months, growth metrics—height, stem diameter, root length, and leaf count— are expected to be significantly better in plants grown in the biochar-amended soil. The results will highlight biochar and PGPB's potential in improving agricultural productivity through enhanced nutrient availability; furthermore, results will suggest BioChar's potential as a sustainable and non-toxic alternative to fertilizer.

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Maximizing Nutrient Content in *Brassica oleracea*: The Ultimate Broccoli

Sinarmas World Academy, Tangerang, Banten, Indonesia

Jaeyi Kim, Chelsea Djajaria, Jing xiong, Yu Ju Chao, Andrew Tan, Avni Tyagi, Haoken Huoermaiti, Michelle Cheach, Gitanshu Bhatia (Mentor-LanzaTech)

The aim of this project is to enhance the nutritional content and yield of *Brassica oleracea* (broccoli) through genetic engineering. Broccoli is known for its large quantity of essential vitamins such as C, K, and A and minerals such as potassium, calcium, and iron as well as antioxidants such as vitamins C and E, and β-carotene. Our focus is on arranging the makeup of broccoli to increase its protein and glucosinolate content. We intend on using CRISPR genetic editing to maximize two genes, MYP34 and CYP79A2, and conduct cross-plant genetic engineering with Chinese kale to introduce the BoMYB29 gene and Serine/threonine protein kinase SRK2n (SnRK2) genes. Techniques such as gel electrophoresis and colorimetry will be utilized to check the modified nutrient levels. This project uses biotechnology to create a healthier and more resilient crop which is important for both agriculture and human nutrition.

Pond Data Collection Device

The Rivers School, Weston, MA,US

Tyler Karp, Christopher Kim, Chelsea Yan, Evan Canty

Teacher: Stewart Pierson
Mentor: Thomas Hyde

Our group started off by thinking of different ways that we could collect data and how that would impact how our device is built, such as a net of sensors or just a buoy that would sit out on the lake. Going off our general idea, we then had to figure out how to not only make our device float but also make sure none of the tech gets damaged. We also needed to conduct some research on our own pond to decide what data to collect using our device. Then, all that was left was to program.

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Runoff Remediation of Atrazine and Chlorobenzene via a Novel *Pseudomonas putida* Construct

Western Reserve Academy: Runoff WRAnglers, Hudson, OH, US

Zhongchen Cai, Nana Kyei, Chongming Liu, Anya Mathur, Lingzhen Wang

Teacher: Dr. Beth Pethel
Mentor: Scott Gaines (Boston University)

Atrazine and chlorobenzene are chemicals commonly found in pesticides and herbicides that pose a threat to both humans and the environment as endocrine-disrupting pollutants. Our BioBuilder design targets the challenge of chemical contamination in water and soil by implementing an innovative and sustainable bacteria-based remediator of these two chemicals. Utilizing *Pseudomonas aeruginosa* strain ADP's exceptional ability to consume atrazine as its nitrogen source, we intend to integrate atzA, atzB, and atzC and DEF genes into a *Pseudomonas putida* strain, a compatible early colonizer in water and soil sources. P. putida strain GJ31 has been identified to possess a chlorobenzene degradation pathway, with genes encoded on plasmid pKW1's clusters and chromosome. By introducing atrazine catabolic plasmid pADP-1 into P. putida GJ31, we can apply a cost-effective and environmentally friendly approach to alleviate the hazards of two common toxins. Evaluating the effectiveness and safety of this design will demonstrate the design's potential for atrazine and chlorobenzene degradation.

The Application of Cyanobacterial Pigments to Construct Environmentally Friendly Sunscreen

Western Reserve Academy: Cyano-Sunscreen Team, Hudson, OH, US

Yihan (Mariana) Ma

Teacher: Dr. Beth Pethel
Mentor: Jackie Thompson (Asimov)

Ultraviolet radiation plays a crucial role in the development of skin cancer. The sun emits electromagnetic waves in the form of ultraviolet radiation, penetrating the human skin layers and contributing to skin burning, wrinkling, and aging. Overexposure to UV radiation can also produce reactive oxygen species (ROS), byproducts of oxidative metabolism, which cause untreatable cell damage and increase the risk of skin cancer. Sunscreen use blocks UV rays from the skin. Even low-protection sunscreen reduces non-melanoma skin cancer by about 40% and lowers the risk of melanoma skin cancer by 50%. However, many of the active ingredients found in sunscreen are harsh chemicals that further skin damage. In 2021, fourteen of the sixteen FDA-allowed sunscreen chemicals underwent scrutinization for their lack of effectiveness and safety concerns. For example, two chemicals, oxybenzone and octinoxate, which cause coral reef bleaching, ultimately kill the coral. Dramatic reduction in coral reef populations can disrupt the ecological balance and seriously impact the ecosystem.
(Continued on next page)

Our project is an innovative method to extract two sunscreen pigments from a marine bacteria, cyanobacteria, offering the opportunity to replace the traditional active ingredients in chemical sunscreens with nonhazardous pigments. This photosynthetic microbe produces two environmentally friendly pigments, scytonemin and mycosporine-like amino acids (MAAs), which shield the cell from 90% UV-A light and absorb UV-B radiation, respectively. We will directly induce the production of scytonemin in the cyanobacterium *Nostoc punctiforme* and engineer *Escherichia coli* to overexpress the gene required for the biosynthesis of MAAs, synthesized mycosporine-glycine gene (MysC). Combining these pigments with inactive but beneficial ingredients commonly found in other sunscreens produces an environmentally friendly sunscreen that will benefit both the ecosystem and humans.

An *Escherichia coli*-based Formaldehyde Detector: An Economical and Effective Solution for Safe Air Monitoring

Western Reserve Academy: FormalDetector Team, Hudson, OH, US

Lujiong Danzhen, Ethan Ta

Teacher: Dr. Beth Pethel
Mentor: Mr. Michael Stark

Formaldehyde is a common colorless gas found in the production of preservatives, building materials, and vaccines. Prolonged exposure to the gas causes severe eye and respiratory irritation and increases various cancer risks. Therefore, determining formaldehyde concentration in the air and regulating it to a safe level becomes a priority for places with potential formaldehyde exposure risks. Current detection methods, including using a mechanical detector, conducting lab samples, and smelling, are often unreliable, expensive, and dangerous. Our previous design is an economical and effective detector based on the ΔfrmR strain of *Escherichia coli*. The detector will be yellow by default and turns red when formaldehyde is present. We will construct the formaldehyde-detecting plasmid based on the R0010 (pLacI)_AB plasmid backbone and transform it into cells. Using a Pfrm promoter, the detector reacts to formaldehyde at levels around 100 micromolar.
(Continued on next page)

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We then utilize the Plac-lacI repressor system as a genetic switch: with the presence of formaldehyde, lacI, a Plac repressor, is expressed to deactivate the yellow pigment and turn the cell red. The bacteria will be cultured in lactose-rich media to ensure the constitutive expression of the yellow pigment under Plac. Moreover, the frmA and frmB genes in the construct remediate formaldehyde in the solution. As the concentration of formaldehyde decreases through frmAB in the detector solution, the detector reverts to yellow, making the detector system reusable when provided with sufficient nutrients.

PolyWreck-Degrading Polymers by Inserting Enzymes

Western Reserve Academy: PolyWreck Team, Hudson, OH, US

Amanda Blasberg, Jacob Wells, James Vacca, Brennan Christian, Arsh Goyal, Dr. Beth Pethel (teacher), Maegan Plank (Mentor-UCB)

Plastic pollution plagues the world, lacking a sustainable and practical solution. Current solutions such as recycling and incineration can be effective. However, they often pose problems that either threaten the environment's health or are generally inefficient. This project aims to develop an efficient way to degrade polyethylene terephthalate (PET), one of the most common single-use thermoplastic polymers. To do this, we propose using the bacteria *Comamonas testosteroni* as a chassis to host genes from *Ideonella sakaiensis* with the objective of producing a super bacteria with an enhanced ability to degrade plastics. The design aims to utilize *C. testosteroni's* ability to target and break down benzene rings that hold PET together, which will be aided by enzymes PETase and MHETase, which *I. sakaiensis* produces. These enzymes, which help in plastic degradation, leave monomers that pose no environmental harm, and, since *C. testosteroni* can be grown in standard laboratory conditions, we propose the PETase and MHETase genes from *I. sakaiensisare* transformed into chassis *C. testosteroni*. The development of a new, more efficient, plastic degrading microbe would be helpful in creating a new waste management process for PET and can positively impact the environment and the world humans inhabit.

A Fungal-Derived Enzymatic Anti-Tick Spray: Targeting the *Ixodes scapularis* Population

Western Reserve Academy: Ticked Off Team, Hudson, OH, US

Evangeline Campbell, Anna Childs, Celine Omega, Natalia Ortiz, Dr. Beth Pethel (teacher), Maegan Plank (Mentor-Union Chimique Belge)

Ticks are a type of arachnid with species found on six continents around the world. There are over 800 species of ticks worldwide, but only a few of those species are capable of transmitting disease to humans. One of those species is *Ixodes scapularis,* the black-legged tick, which is native to the eastern half of the United States. The species is responsible for the transmission of several severe illnesses, including Lyme disease. The Centers for Disease Control estimates that over 400,000 people are diagnosed and treated for Lyme disease each year in the United States. The ticks attach themselves to a host to consume and store blood using a tube-like mouth; this allows ticks to spread disease to a new host from a previous host's blood. In the past decade, tick populations and tick-borne diseases have spiked around the world due to increasing global temperatures and precipitation from climate change. Our goal for this project is to create a bio-friendly super-spray that includes the fungal enzymes from *Beauveria bassiana*. The fungal enzymes allow a breakdown of the *I. scapularis*'s cuticle, and toxins are subsequently released by the fungus which increases the tick's mortality rate. People can then spray their clothes or other areas that are susceptible to ticks to control the *I. scapularis* population and reduce the spread of tick-borne diseases.

Road Salt Alternative Using AFPs

Every winter, drivers are threatened by hazardous icy roads. Among methods to prevent snow-related traffic accidents, the most common measure is road salt. However, the application of road salt causes many problems, such as its corrosion, as well as its harmful impacts on the environment. The negative effects of road salt necessitate an alternative solution to treating winter roads. Our plan involves inserting three AFP gene sequences into plasmids, transforming the plasmids into *Pichia pastoris*, and harvesting three AFPs from mealworms, plants, and arctic yeast genes. These AFPs adhere to the surface of newly formed ice crystals, inhibiting ice crystal formation in sub-zero temperatures. By harvesting the AFPs from yeast, we can apply the product as a cost-effective road salt alternative. This innovative solution addresses environmental concerns and uses a more eco-friendly approach. Testing this design's efficacy and safety will prove its feasibility as a replacement for road salt.

Western Reserve Academy: Salty Squirmerzzz Team, Hudson, OH, US

Loretta Wang, Paige Dix, Nicole Pae, Taylor Strilesky, Min Roh, Dr. Beth Pethel (teacher), Vicki Liu (mentor-Lanza Tech)

Manufacturing

Synthetic biology has proven to be a successful direction for manufacturing as an increasing number of companies use biological systems in their processes. Getting novel technologies from the research stage to consumers and clinicians requires innovation in manufacturing techniques. This team considered the challenges faced in biotech and proposed a more heat robust fluorescent protein that can be used to determine gene expression in a greater range of conditions. It is innovation in protein design and other aspects of the use of biological systems that we need to bring about the next industrial revolution, powered by biotechnology.

“Let it Glow:” The Heat Never Bothered Me Anyway- Fluorescent Heat-Stable Proteins

Fluorescent proteins are widely used in biotechnology to determine levels of gene expression and protein localization in cells. One major drawback of GFP is that it denatures at temperatures higher than 70 degrees Celsius. Our goal is to analyze what motifs and domains could be used to help stabilize GFP and other fluorescent proteins at higher temperatures, and to genetically engineer the coding sequence of DNA to create heat stable fluorescent proteins.

Castro Valley High School, Castro Valley, CA, US

Ariadna Estebanez, Samantha O’Brien, Lillian Sequeira, Jishnu Sanyal, Jad Yehia, Tomi Chen, Terry Deng, Luchezar Pirogov

Teacher: Laura O’Brien
Mentor: Mary Lerner (UCB)

Food

To grow the future, we need to feed the future. These teams took on the challenge of safely, efficiently, and sustainably feeding the world's growing population.

Some teams are working on innovative ways to replicate qualities of specific foods using bacteria such as how to produce more meat-like meat substitutes or more cost-effective vanilla. Others are working to protect crops susceptible to disease or pests.

Safety is top of mind for other teams tackling food-borne illness and vitamin deficiency. One team is even working on using algae to ensure sufficient food production given the increased risk of famine and food shortage posed by climate change and population growth.

While ensuring that the basic needs of a growing world population are met is a serious challenge, these teams are getting dinner on the table.

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Producing a Meat-Like Aroma Protein Using Genetically Engineered *E. coli*

Academy of Notre Dame, Tyngsboro, MA, US

Katherine Duanmu, Jomana Elghamrawy, Abi Nunes, Ian Walters, Alvin Dzoung

Teacher: Dr. Enhua Wang
Mentor: Dr. Nathan Gardner (Arbor Biosciences)

We propose to have the bacteria *E. Coli* to express the gene aminolevulinic acid. This gene expression product-protein heme would be used as a key ingredient to make plant-based burger that smells and taste like meat.

Photosynthetic Enhancement of Algae to Increase Food Production

Acton-Boxborough Regional High School, Acton, MA, US

Alisha Raiker, Henry Tshabalala, Zachary Kendall, Amelia Landry, Katie Dong, Arjun Macha, Mihir Vidhate, Isaac Childers, Espen Wellner, Zayd Dhasthageer, Aaron Mathieu (Teacher), Prasanna Neti (mentor-NECI)

Famines and food shortages can be deadly, causing an estimated 9 million deaths a year; the threat of these crises are only exacerbated by global warming, limited space, and many more factors of the modern world. Much of the attempt to address the phenomenon through synthetic biology has focused on the enhancement of traditional crops. We aspired to build on and recontextualize these pursuits by enhancing algae — which has many advantages over traditional crops — with components native to diverse fields of study. The systems designed tentatively promise improved light capture, carbon fixation, and starch production, through three distinct but interdependent mechanisms. Phycobilisomes — large light harvesting structures found in red algae and cyanobacteria — will be expressed for their incredibly efficient light capture. A multicopper oxidase will be leveraged to reduce O2 into water, freeing RuBisCO to operate more efficiently. Finally, overexpression of CmGLG1 — which initiates starch and glycogen synthesis — and its regulator, target of rapamycin, is predicted to leverage the augmented resources. With development, such systems may be able to circumvent the causes of famine that arise from traditional farming methods or contribute to famine relief efforts.

Detecting *S. typhi* with Engineered Yeast

Salmonella typhimurium (S. typhi) is a prevalent bacterial species, and is estimated to cause around 33% of food-borne illness in Sub-Saharan Africa, South Asia, East Asia, and Pacific regions, affecting 762 per 100,000 person every year. To decrease food contamination in these areas, a portable and self-regenerating detection system is needed. To fulfill these needs, we designed a yeast-based detection system using synthetic biology. Our system will express an antibody to S. typhi via a-agglutinin complex subunits (Aga1p and Aga2p) on the surface of *Saccharomyces cerevisiae (S. cerevisiae),* a yeast that is both engineerable in the lab and safe to provide to households in affected areas. When the antibody presented on the surface of these engineered yeast binds to a lipopolysaccharide of *S. typhi*, it can activate the Hog1 pathway. This pathway is naturally present in *S. cerevisiae,* and will be engineered to generate a detectable signal that will provide an early warning for food contamination. Our system, if successful, will create a low-cost, self-regenerating detector for *S. typhi.* Its low cost and its capacity to reproduce will ensure greater access to our system, making food and water in third-world countries safer to consume and reducing disease.

Montrose School: Team One, Medfield, MA, US

Hana Shinzawa, Cate Lynch, Leslie Baker, Rosalia Reale, Sarah Hanna (teacher), Isabelle Ortiz (teacher), Dr. Natalie Kuldell (mentor-BioBuilder)

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Deterring Fruit Flies Through Genetic Modification

North Forsyth High School, Cumming, GA, US

Ava Bingaman, Lyla Bingaman, Sritan Chiluka, Samved Gazzala, Arianna Hagen, Hannah Hagen, Kelly Jordan, Drew Landers, Jaxxon Otteson, Vibhi Rathor, Stephen Nelson, (teacher), Rebecca C. Meyer, PhD (Mentor-MIT)

Over 400 fruits and vegetable species are impacted by the infestation of *Drosophila melanogaster*. Fruit flies are drawn to fruits by the presence of ethylene, a gas that is released as fruits ripen. Studies have shown that fruit flies can be deterred by scents such as peppermint, lavender, basil, and lemongrass. Through CRISPR editing, our goal is to genetically modify the scent production pathways of highbush blueberries to produce these scents to repel *Drosophila melanogaster* despite the presence of Ethylene during ripening. We plan to approach this genetic modification by utilizing guide RNA to target all alleles coding for scent production. Our research serves as the foundation for a two-year undertaking to later implement in the CRISPR editing of highbush blueberries. Our team is looking into ways to effectively modify blueberry plants over a two-year period through CRISPR editing using Agrobacterium-mediated transformation. By targeting the Vcpds endogenous reporter gene, we are able to locate and alter the genetic makeup to deter fruit flies, Drosophila melanogaster. The genetic manipulation of fruit ripening using antisense technology shows promise in specifically modifying biochemical pathways to alter fruit without affecting other aspects of ripening.

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Fusarium Wilt Resistance in Cavendish Bananas

Western Reserve Academy: Fusarium Fighters, Hudson, OH, US

Peter McGinnes, Josh Li, Andrew Parrish, Kate McMullin, Bob Wang, Dr. Beth Pethel (teacher), Dr. Michael Sheets (Mentor-Boston University)

Fusarium wilt is an infection caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (FoC) that targets Cavendish banana plants. The fungus enters the plant through the roots and then blocks the plant's ability to absorb necessary nutrients. The bananas' inability to ward off this infection results in plant death. Further, Cavendish bananas' vulnerability to this disease has prompted billions of dollars in economic losses to countries that rely on banana production and exportation. While previous trials have attempted to eradicate the disease, an effective solution to eliminate FoC is yet to be discovered. Our proposed solution highlights the usage of allicin, a compound found in garlic that has demonstrated effectiveness in stopping the growth and proliferation of fungal infections. Our design is an allicin expression system for *Enterobacter cloacae* that works by inserting the gene encoding allicin into a bacterial endophyte (*E. cloacae*) and implementing that endophyte into the soil. As a result, we hypothesize Cavendish banana plants can gain resistance to FoC.

Nature's Dose of Sunshine: Engineering Vitamin D in Peppers

Western Reserve Academy: The Pepper Alchemists, Hudson, OH, US

Junning Hu, Fernando Bermudez, Beth Pethel (teacher), Michael Stark (mentor-University of Pretoria)

Vitamin D deficiency affected more than 15% of the worldwide population from 2000 to 2022. Vitamin D deficiency is often linked to a wide range of chronic conditions such as osteoporosis, muscle weakness, and fatigue. Hardly any foods naturally produce adequate vitamin D concentrations and vitamin D supplements do not fully alleviate the symptoms of the deficiency. In recent years, scientists have used biofortification to enhance vitamin D levels in various vegetables such as tomatoes. Last year a team of WRA students proposed a CRISPR-based knockout method to produce vitamin D in Bell Peppers emulating a similar study done in tomatoes, which belong to the same Solanaceae family. Our team now has focused on the experimentation of the mentioned methods. We began by extracting the plasmid pHEE401E from the E. coli bacteria through the Qiagen plasmid miniprep. Our team also designed the gRNA targeting the 7-DR2 gene of bell peppers. Using Golden-gate cloning, the gRNA sequence is inserted into the pHEE401E plasmid. Three different bell pepper cultivars are used and grown in the lab to determine the vitamin D level before the CRISPR knock-out experiment. Finally, a Vitamin D ELISA kit is used to quantify vitamin D levels in the fruit of bell peppers.

Vanillin Compound Production in *Amycolatopsis* sp. ATCC 39116 Bacteria

Prices of vanilla plants are high due to the necessity of labor, resources, as well as their need to be in a particular environment. Through this project, we hope to work towards a bacterial alternative with the ability to create the same taste at a lower cost. The taste of vanilla comes from an aromatic compound called vanillin ($C8H8O3$), which is being attempted to be mimicked by the increased expression of fcs and ech genes through the repression of the vdh gene in *Amycolatopsis* sp. ATCC 39116. Additionally, looking at the prokaryote's metabolic system, we also hope to increase the synthesis of ferulic acid which is a primary substrate of vanillin producing amino acids.

Westwood High School, Westwood, MA, US

Lucy Park, Jeffrey Pan, Nina Pappas, Tyler Dong, Nicole Lu, Ethan Lee, Andrew Gong, Rayaan Padria, Ivan Cai, Ellie Wu, Dr. Miller, Mr. Mullins, Dr. Anna Li (Mentor-Ginkgo Bioworks)

Medicine

Medicine was a popular choice of topic area this year. Teams recognized the enormous potential of using a synthetic biology approach like biosensors to monitor a variety of health conditions.

Teams proposed designs for biosensors to detect everything from peanut allergens to contaminated water to STIs to brain tumors. Such biosensors have the potential to be more cost-effective and safer than traditional approaches.

Several teams proposed biological systems as novel approaches to regulating gut neurotransmitters for improved mental health, increasing prebiotic production, and relieving symptoms of irritable bowel syndrome.

Two projects stress the importance of oral health by creating synthetic tooth enamel and reducing the production of cavity-causing lactic acid in oral bacteria.

The variety of these projects addressing human health and medicine reflect the broad potential synthetic biology has for transforming medicine. These teams are bringing about the biosensor revolution.

BioBuilderClub

SynthMind Meals: Creating Meal Plans with Engineered Gut Microbes for Neurotransmitter Production to Improve Mental Health

Lambert High School: Team GA2, Suwanee, GA, US

Shashank Ramireddy, Yun Xin Lin, Eshawnive Kallu, Veda Vudithyala, Kanishq Palla, Harrison Turner, Vedant Kalipatnapu, Amey Saokar, Korah Kenyatta-Simmons, Sarah Verma
Mentor: Dr. Kristin Jenkins (BioMADE)

Mental and substance use disorders have become the leading cause of disability, and over 70% of those suffering from a mental illness worldwide lack access to the necessary treatment options. Through SynthMind Meals, we plan to manipulate and regulate the biosynthesis, release, and reuptake of neurotransmitters by using naturally occurring microbes in the gut, which can be used to help those with mental health illnesses. Specifically, we will extract plasmid DNA of a lactobacillus bacteria and insert neurotransmitter-producing genes. We will then introduce the modified plasmid back into Lactobacillus cells through transformation and use an *E. coli* culture to replicate probiotics. Lastly, we can implement these microbes into meal plans that consumers can purchase from the grocery store, increasing accessibility of treatment. This way people can simultaneously find nourishment and improvement to their mental health.

Using Augmented BCI Technology to Detect the Presence and Progression of Glioblastoma Tumors

Lambert High School: Team GA3, Suwanee, GA, US

Naga Sahithi Rajuladevi, Jagruti Lokam, Aarya Trivedi, Chaithra Jogiraju, Vaibhavee Shirkhedkar, Arav Raghunathan, Sujaal Gelle, Nimal Murugan, Abhay Kumar Prashanth, Janahan Mural, Kate Sharer and Pam Seeley (teachers), Dr. Kaitlin Dailey (University of Nebraska Medical Center)

Our research project involves the use of augmented BCI to create a dry biosensor (photonic chip) that is used to detect both EEGs and MGMT presence. The problem we've targeted is tracking glioblastoma after it's been diagnosed. MGMT is a biomarker associated with the presence of glioblastoma where the less frequent it's been detected, chemotherapy for glioblastoma is working. In our augmented BCI technology, we've included a chemosensor to create fluorescence specifically for MGMT and a fluorometer is implemented to create a quantitative value for the fluorescence being detected. This synthetic biology project is cost-effective and efficient to make sure glioblastoma is kept stabilized. Brain tumors are known to be unpredictable, our goal was to create a way for doctors and patients to be aware of their tumor status, particularly glioblastoma which is one of the most common brain tumors.

Synthetic Biology-Based Dihydrotestosterone (DHT) Biosensor for Accessible Health Monitoring

Maimonides School, Brookline, MA, US

Hayim Sims1, Shlomo Vedol, Aaron Missaghi, Leah Kaplan, Shaindle Kaplan and Avi Abbett, Maria Lazebnik (advisor), Scott Gaines, (Mentor-Boston University)

This research presents a novel biosensor for dihydrotestosterone (DHT), an androgen linked to various health concerns like Androgenic Alopecia and an increased risk of prostate cancer, as well as 5-alpha-Reductase Deficiency leading to intersex conditions. Traditionally, DHT levels are measured using High-Performance Liquid Chromatography, a method that is expensive, time-consuming, and inaccessible to many. Our study introduces a more affordable, rapid, and accessible alternative using a synthetic biology approach. We have developed a biosensor employing Transcription Factors (TFs) from Pimelobacter simplex, known for their responsiveness to hormones. These STRF1 transcription factors (TF), previously identified to respond to progesterone (by Galagan Lab), are hypothesized to also sense DHT. The presence of DHT influences the TF's binding to the STRF1 site within the T7 promoter, consequently regulating the visual output of GFP production. The fluorescent output varies according to the DHT concentration, offering an easily interpretable measurement of DHT levels. Our biosensor demonstrates the potential to significantly reduce the cost and increase the accessibility of DHT testing. It is particularly beneficial for communities with limited access to advanced medical facilities. This innovative approach not only aids in early diagnosis and treatment of DHT-related health issues but also contributes to the broader field of medical diagnostics and hormone sensing technologies.

Prebiotics in *Lactobacillus E. coli* Co-cultures

Our research aims to examine the impact of euglena powder prebiotics on *Lactobacillus* growth, both individually and in co-culture with *E. coli* K12. While prior research indicates that euglena powder enhances the growth of various *Lactobacillus* species, its effects on *Lactobacillus*-*E. coli* co-cultures remain unexplored (Dai et al., 2022). *Lactobacillus*, a gram-positive bacterium, plays a key role in lactose breakdown, with lactic acid production contributing to lactose intolerance symptoms (Pakadaman et al, 2016). Our study aims to assess the efficacy of euglena powder as a natural prebiotic in promoting *Lactobacillus* growth, potentially offering new strategies for alleviating lactose intolerance symptoms. The experimental design involves preparing MRS broths with different euglena powder concentrations, inoculating them with *Lactobacillus* and *E. coli* or *Lactobacillus* alone, and incubating them before plating on MRS petri dishes. We anticipate that higher euglena powder concentrations will stimulate *Lactobacillus* growth, although our design does not address the underlying mechanisms of *Lactobacillus*-*E. coli* interactions. Future research could investigate these interactions and explore the effects of other prebiotics and specific nutrients present in euglena powder.

Marietta High School, Marietta, GA, US

Makayla Dunn, Ty Heinlen, Amanda Barrett (teacher), Dr. Benjamin Steil (Mentor-Arbor Biosciences)

BioBuilderClub

Developing a Whole-Cell Biosensor and Bioreporter to Detect Peanut Allergens

Montrose School: Team Two,Medfield, MA,US

Rachel Dunn, Siena Elliffe, Aneesa Maity, Tvesha Patel, Ruby Quintiliani, Liesl-Ann Vaz, Sarah Hanna and Isabelle Ortiz (teachers), Ming Hia (mentor-Boston University)

To provide certainty to protect this vulnerable population, our project intends to create a portable biosynthetic biosensor and bioreporter for the detection of peanut traces in food samples by detecting the allergenic protein in peanuts: Ara-H2. Using a yeast cell because of its compatible cell membrane with our synthetic receptor, our team engineered a yeast cell modified to produce green fluorescent protein (GFP) upon detection of the antigen Ara-H2. This extracellular detection would then signal the gene circuit, including the activator, promoter, and ribosome binding site, to begin transcription to create GFP. The yeast cell would then output GFP as a visual indication of the detection of peanuts. Because the gene circuit for the production of GFP is only activated when the engineered receptor detects the allergen, the visual signal will only be produced if peanuts are present, making this an effective solution to detect peanut allergens.

Utilization of Chitinase for Digestion of Indigestible fibers like Chitin to Alleviate Symptoms and Inflammation in Irritable Bowel Disease

Oak Park and River Forest High School: Medical Team, Oak Park, IL, US

Dee Epshtein, Charlie Scott, River Scott, Juliette Bezaire, Rafi Butt, Jordan Alioto, Jeremy Miere, Gabe Shaye Almanzar, Evan O'Brien, and Kavi Whyte. Mr. Matthew Kirkpatrick (teacher), and mentors Mr. Eric Jorgenson (ETSU), and Dr. Jennifer Watson (Limuless)

Irritable Bowel Diseases (IBD), such as Irritable Bowel Syndrome (IBS), are chronic health conditions that cause disruptive and challenging symptoms. Chitin, a type of indigestible fiber, often exacerbates IBD symptoms. Studies suggest that the ability to digest chitin could lessen IBD symptoms. This experiment will utilize chitinase to break down chitin, thus alleviating symptoms of IBD. This project aims to generate a probiotic bacteria that is able to digest chitin.

Pan-Cancer Diagnostic Using Activity-Based Nanoparticles

Sage Hill School, Newport Coast, CA, US

Harriet Lai, Jayla Chan, Sarah Huang, Nancy Sun Derek Shapiro (teacher), Dr. Peter Horanyi (mentor-UCB)

Many cancers have been associated with the dysregulation of extracellular proteases[2].One such family of proteases is Matrix metalloproteinases, or MMPs, which have shown similar patterns of upregulation across various cancers[2]. This project aims to create a method of detecting upregulated matrix metalloproteinases using inhalable nanosensors with PATROL (point-of-care aerosolizablenanosensors with tumor-responsive oligonucleotide barcodes) technology. Using reporter DNA barcodes bound to 8-arm PEG nanoparticles by MMP substrates, upregulation of MMPs can be indicated when arms are cleaved, releasing barcodes into the bloodstream and eventually the urine, which can be detected with a lateral flow assay urine test. This method, which requires a simple urine strip to test, can be a cheaper and more accessible alternative to scans and other costly cancer screening methods requiring heavy equipment/technology within low-income or developing areas.

Bon Apatite! Synthetically Fabricated Human Enamel

Taipei American School: Team A5, Taipei, Taiwan

Angela Chu, Isabelle Lee, Evelyn Lee, Matthew Chuang, Sohoon Park, Jessie Chiang, Thomas Sung, Cindy Kim, Judy Yen, Andrea Wu, Victor Chao, Dr. Jonathan Hsu, Mr. Alex Dezieck, University of Chicago iGEM team (mentors)

Our research aims to synthetically fabricate enamel, the hardest tissue in our body. Our goal is to mimic natural enamel formation using a 56 amino-acid product of amelogenin, leucine-rich amelogenin peptide (LRAP), to regulate hydroxyapatite (HAp) crystal shape and orientation. Here, we present a preliminary finding of our synthetic HAp product compared to porcine enamel samples. While the ratios of Ca: PO_4 in our product and the porcine enamel are similar, the patterns by scanning electron microscopy are quite different, likely due to the absence of LRAP. Furthermore, we plan to produce and purify LRAP in bacteria, hoping to enhance the HAp structure and make it more similar to natural enamel. Our ultimate goal is to combine LRAP and HAp and implement it in a user-friendly product such as toothpaste or mouthwash.

BioBuilderClub

Lack-Tis-Acid: Engineering a Lactic-Acid Free Oral Bacteria

Taipei American School: Team B5, Taipei, Taiwan

Nikki Huang, Annie Chang, Audrey Leu, Steven Kuo, Elliot Brennan, Kenneth Miao, Ethan Lin, Tinsley Wu, Aaron Liu, Junui Song, Emma Shih, Dr. Jonathan Hsu (teacher), University of Chicago iGEM team (mentors)

Lactic acid, the cause of dental caries, is largely produced by *Streptococcus mutans*, a strain of lactic acid (LA) bacteria in the mouth. Using *Lactococcus lactis* as a model for *S. mutans,* we aim to remove the gene, ldh, that directs LA production from its genome, resulting in a strain of mutant bacteria with hindered lactic-acid production. We plan on utilizing molecular cloning techniques like cross recombination for gene deletion, and have found a preliminary method for testing the pH of bacteria. In the future, we plan on researching possible implementation methods of our bacterial strain.

Sensor-Yeast: Creating a Widely Accessible Yeast Biosensor

Troy High School, Troy, MI, US

JoAnn Tu, Jessica Wu, Aishwarya Viswanathan, Akshara Palanisamy, Anaïs Ndione, Angelina Kodudhula, Ella Rahimo, Jons Jaison, Rishi Rao, Samiksha Saravanan Mrs. Rebecca Brewer, Dr. Ben Steil (Mentor-Arbor Biosciences)

The presence of harmful bacteria in drinking water suggests that water often contains pathogens that can lead to disease. It is important to be able to identify unsafe drinking water to determine particular substances that may lead to illness. Our purpose is to create a functional and self renewing biosensor that can be made to become accessible and cost-effective. We plan to transform yeast to express an antibody that binds to *E. coli.* To analyze its results, we will perform an ELISA assay to determine its effectiveness. Understanding the capability of producing a biosensor is essential to ensure safe drinking water and effective water filtration methods. This critical first step is also needed to better understand waterborne pathogens.

Slowing Parkinson's Disease Progression with Biomimicry-Inspired Therapeutics

Weston High School, Weston, MA, US

Ellie Tillman-Schwartz, Eva Odio, Mary Liu, Peter Horanyi, Ph.D. (mentor-UCB)

Many current Parkinson's Disease therapies target the dopamine regulation. However, we plan to develop a therapeutic that instead targets the aggregation of Lewy body formation. We will slow Parkinson's disease progression through antibody-driven immune clearance of aggregates.

A Novel Parasitic STI Detection System Utilizing *Trichomonas vaginalis* Specific Gene Expression

Western Reserve Academy: Team P&C, Hudson, OH, US

Suhaan Yadama, Mira Zamarro, Dr. Beth Pethel (teacher), Dr. Becky Meyer (Mentor-MIT)

Sexually Transmitted Infections (STIs) are a widespread healthcare concern, affecting 1 in 5 individuals worldwide. Diagnosing STIs is challenging due to their asymptomatic nature and the limitations of current testing methodologies, which makes an accessible testing method a priority in modern healthcare. Trichomoniasis, caused by the parasite *Trichomonas vaginalis*, is especially difficult to detect. We designed an at-home urinary strip test for Trichomoniasis, utilizing *T. vaginalis* transcription factors to trigger a color change in responsive genes. Using the T. vaginalis-specific protein IBP39, we aim to bind it to the DNA initiator and M5 motif for transcription activation. The IBP39 protein should be present and available to transcribe due to lysed *T. vaginalis* appearing in urine. The genetic construct will be subsequently cloned into a plasmid and introduced into *Escherichia coli* through transformation. The project's modified *E. coli* and supportive growth media will be in a test tube, to be used with a urine sample. If the media, originally a pale-yellow color, undergoes a change to a blue color, that is an indication of the parasitic infection. In the case of this, it is strongly recommended to reach out to a doctor for further evaluation and treatment. Offering a private and accessible alternative to conventional testing, this method aims to streamline sexual health screenings, ensuring earlier detection and treatment.

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