VOColi

St. Mark's School of Texas iGEM Team

Detecting certain exhaled VOC biomarkers through genetically modified *E. Coli*, which has the potential to become a streamlined and sensitive preemptive diagnostic for lung cancer.

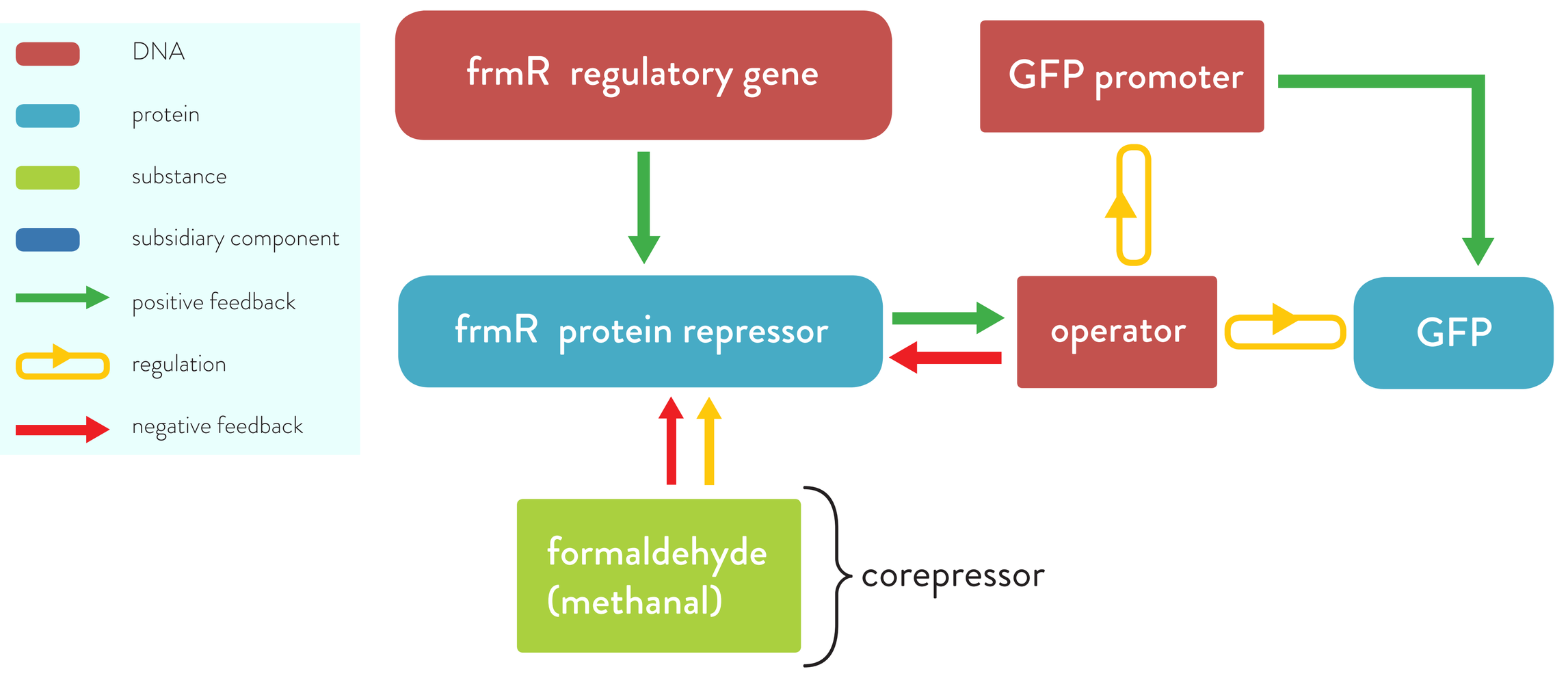
INTRODUCTION

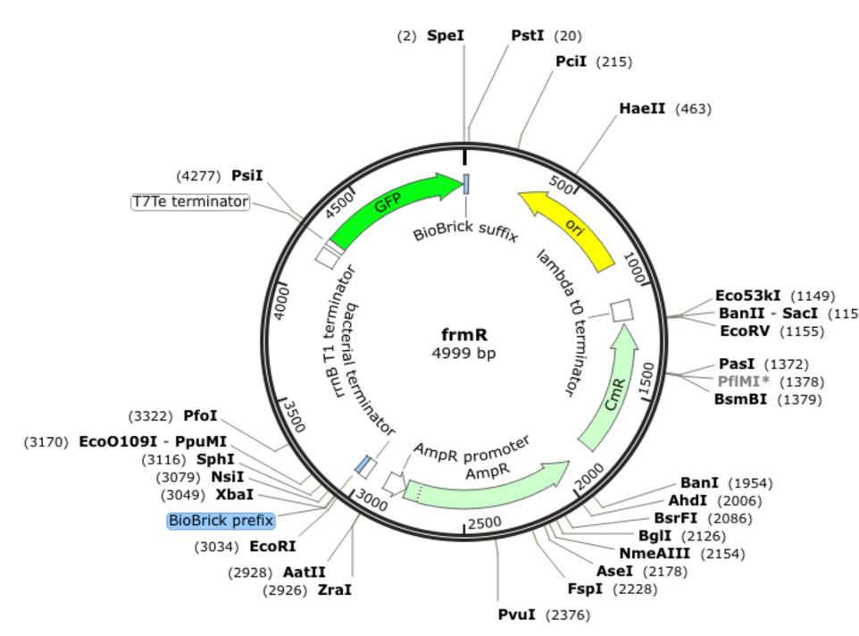
Lung cancer is the leading cause of cancer-related death around the world. Early detection systems would enable us to diagnose the disease at a time when therapeutic intervention would be most effective. Noninvasive breath sampling has the potential to save lives as approximately 86% of lung cancer patients die within five years of diagnosis. Early detection improves survival rate by 50% from stage III to stage I of the cancer. Breath biochemical sensors also have important applications for other diseases. According to the World Health Organization's latest report on noncommunicable diseases (NCDs), these "diseases are the leading global causes of death, causing more deaths than all other causes combined." Further research into these noninvasive tests while maintaining the tests' accuracy and sensitivity could have a major impact on survival rates of other NCDs. Tests of this nature would reduce inequity and provide improved means of treatment for affected individuals regardless of nationality, income, or access to healthcare.

OVERVIEW

Inspired by the olfactory ability of canines to detect diseases, we will pursue a long-term project that revolves around the creation of a minimally invasive, sensitive, and inexpensive detection system for lung cancer, through the identification of exhaled biomarkers. Twenty-two volatile organic compounds (VOCs) have been distinctly found in the breath of affected patients, creating a viable "fingerprint" for reliable detection (Horvath et al.). This year we focused on creating biosensors for three VOCs: ethanol, formaldehyde, and xylene. We created genetic circuit systems for aldB induced by ethanol, frmR recognition of formaldehyde, and xylR activated by xylene. Our current device will utilize three reporter proteins (Cyan Fluorescent Protein, Green Fluorescent Protein, Red Fluorescent Protein) to indicate the concentrations of the three VOCs present in an exhaled sample. This research will eventually go into creating a conclusive test for use in the developing world.

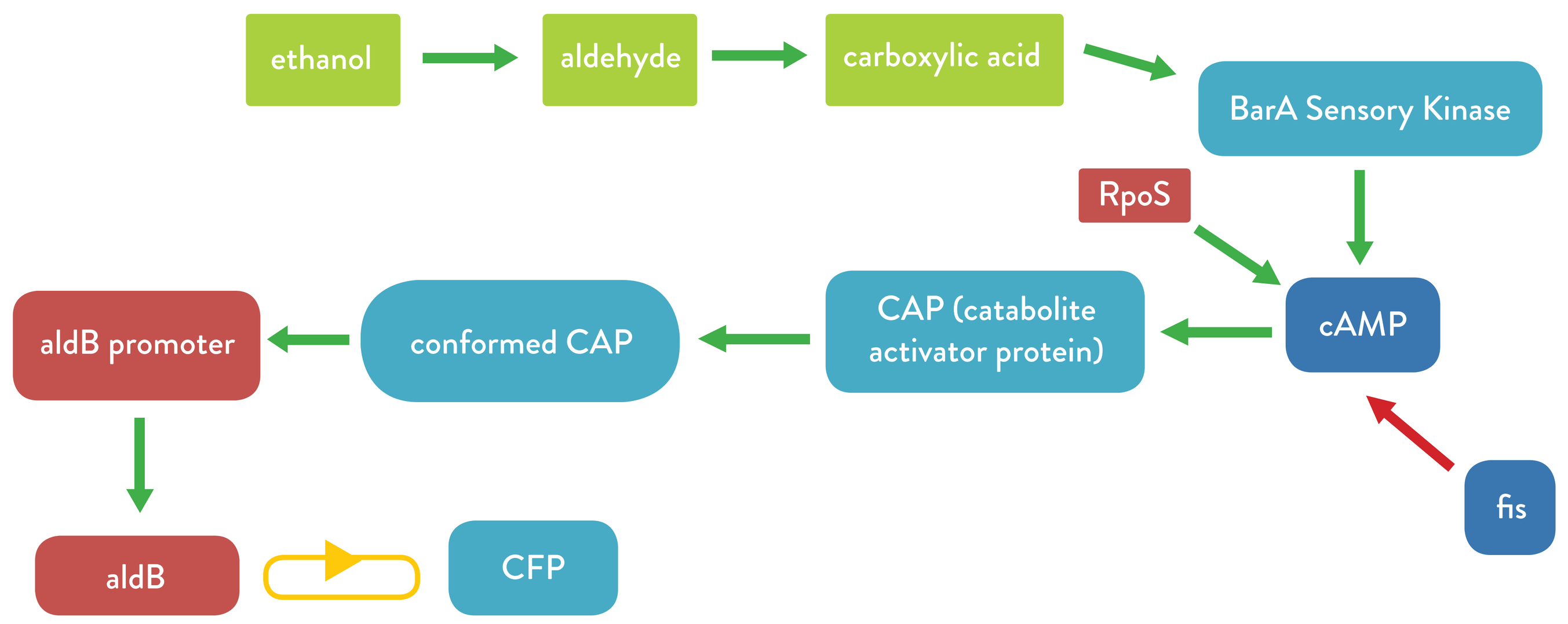
FORMALDEHYDE DETECTION

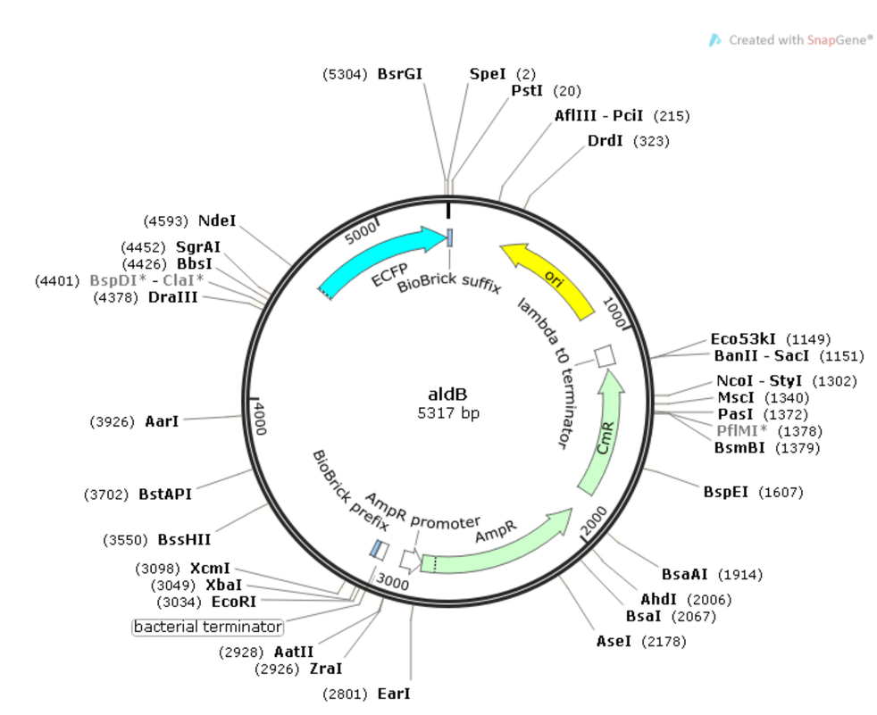




Formaldehyde induces the frmR gene, functioning as a regulatory gene of Green Fluorescent protein. When transcribed, it expresses a regulatory protein that binds to an downstream operator that prevents the movement of RNA polymerase. Under typical conditions, the promoter downstream of the regulatory gene increases the affinity of RNA polymerase to the DNA strand, but the transcription enzyme cannot bypass the operator and transcribe GFP, the gene that is ultimately under regulation. In such a scenario, the regulatory protein that frmR expresses functions as a repressor and effectively inhibits transcription of the coding sequence. Formaldehyde, on the other hand, induces the transcription of GFP and ultimately causes bacterial fluorescence. Acting as a corepressor, the VOC binds to the regulatory protein and conforms it into an inactive shape, allowing for the passage of RNA polymerase through the operator and transcribe the GFP protein.

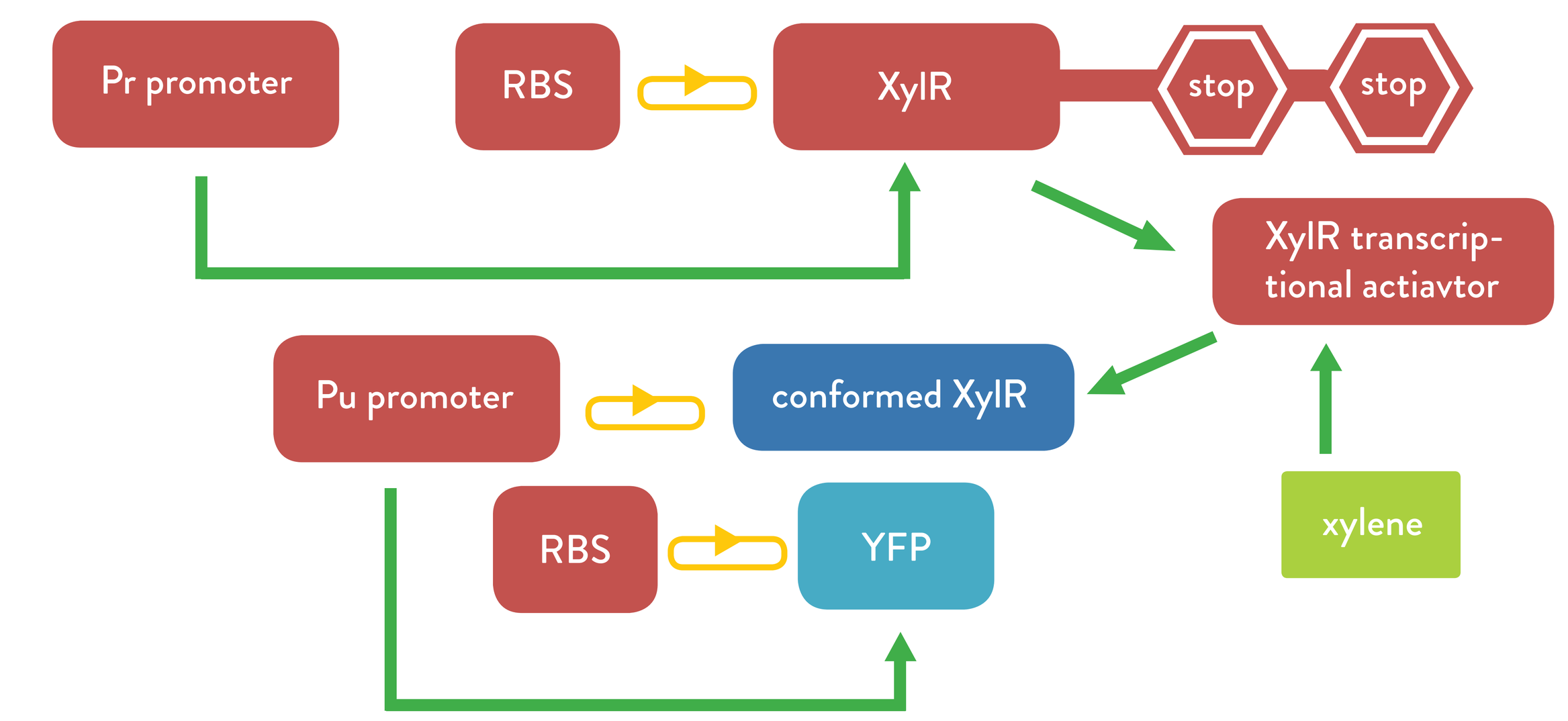
ETHANOL DETECTION

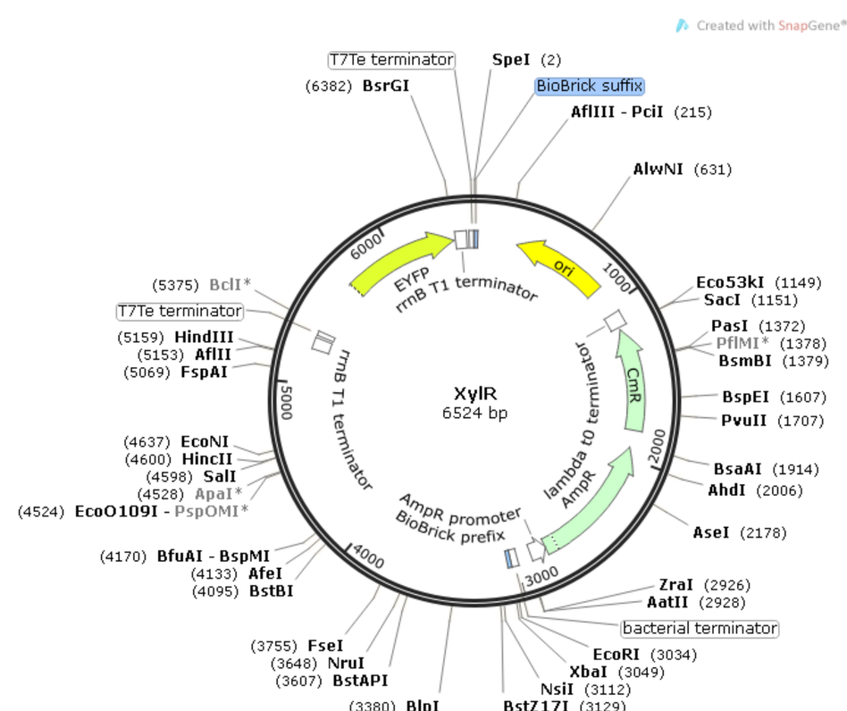




The aldB gene codes for a functional aldehyde dehydrogenase, which is directly induced by ethanol. Metabolism of the alcohol consists of its transformation into an aldehyde and then into a carboxylic acid in activating several related pathways. The acid reacts with the BarA histidine sensory kinase, a signalling enzyme involved in a two component signal transduction system present in *E. coli*, to catalyze the breakdown of various carboxylic acids. The kinase is additionally responsible for the induction of RpoS, a regulatory gene of aldB that directly opposes fis during the activation of the aldB operon. Eventually, BarA triggers a series of vital reactions that affect the Crp-cAMP regulatory mechanism, a dual complex that controls the expression of the aldB coding sequence. In the complex, cAMP conforms the shape of Crp, also known as CAP (catabolite activator protein). This newly conformed Crp then attaches to the promoter and contributes to the initiation of transcription of the aldB operon. Downstream of aldB, CFP (cyan fluorescent protein) is expressed and the bacteria exhibits cyan fluorescence.

XYLENE DETECTION





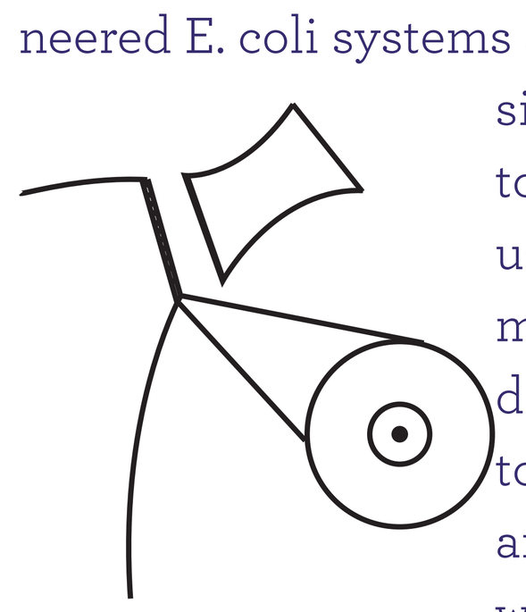
The genetically-related expression of the XylR gene consists of promoters, regulator complexes, and proteins that all aid in the expression of fluorescent proteins. The initial DNA sequence Pr promotes the expression of XylR gene exon itself. Shortly after, it is followed by a ribosomal binding site that orchestrates the timing and efficiency of translation. The naturally expressing XylR sequence succeeds the RBS and undergoes strict regulation of the Pr promoter. Because this protein triggers a secondary response in the bacterium which is vital to Xylene detection, a double termination sequence is essential to the discontinuation of sequences downstream of the XylR coding region is not expressed which can disrupt the reactions involved in the detection system. These stop codons, which are short and effective, operate with a stem-loop that possesses both forward and reverse termination mechanisms. The expressed XylR protein then reacts with xylene and is conformed to accommodate a secondary gene sequence. This newly conformed version of the protein can then bind to the Pu promoter. After a second ribosomal binding site (strong) is subsequently intiated and YFP is expressed, which supersedes the RBS, the bacteria will exhibit yellow fluorescence to indicate a positive test.

THE CONCEPT

Early detection of lung cancer is vital to increasing the success rate of current treatment options. Current diagnostic methods, including chest radiographs and CT scanning, are expensive, unreliable, and potentially dangerous. Our proposed alternative was inspired by observing canines, and their ability to detect noncommunicable diseases quickly and accurately without harm to the patient. According to leading research Rainer Ehmann's study on canine olfactory screening of lung cancer, "[Lung cancer] was identified with a sensitivity of 90% and a specificity of 72%" (3). Training dogs for this purpose, however, is expensive and time-consuming.

THE IMPLEMENTATION

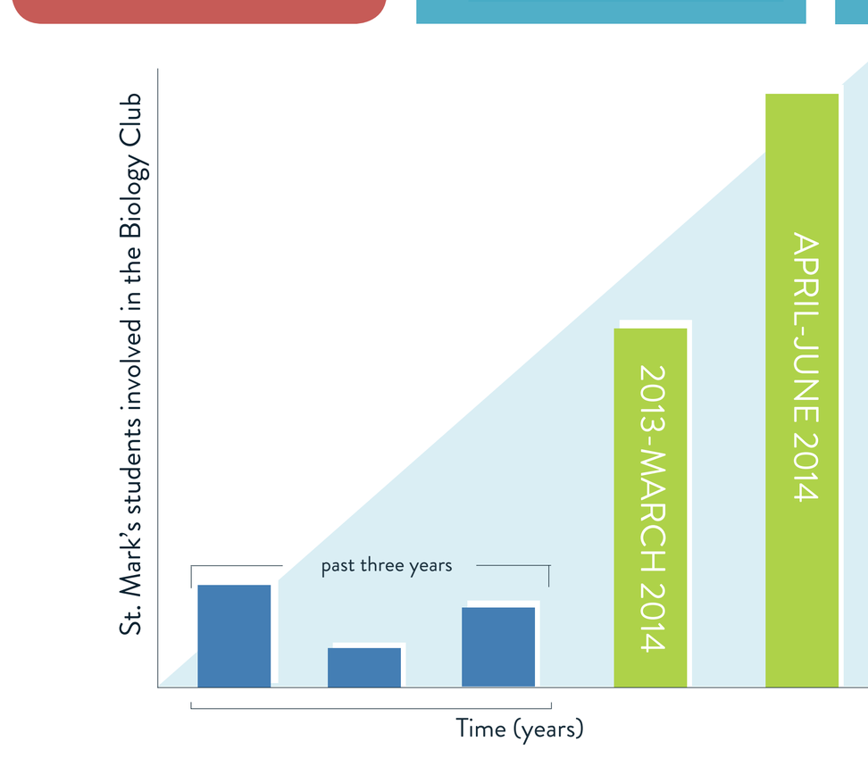
While the development of mechanical and electronic noses have been in progress for many decades, we propose that a circuit of genetically-modified E. coli used in conjunction with a mechanical contraption has the potential to be used a preliminary diagnostic for lung cancer. We propose a device similar to a breathalyzer that utilizes a cylindrical container with our genetically engineered E. coli systems at the bottom of the aluminum shell. To prevent the possibility of contaminants, an adapter would attach to the top of the cylindrical shell for the user to breath into. The user would fasten the adapter onto the shell and exhale as much as possible. Upon exhalation, the user would immediately detach the adapter to secure the system designed to automatically shut off after the removal of the adapter and the shell. The genetic systems would be underlain with a sensitive paper that would be dyed upon the expression of the fluorescent proteins. We would utilize Cambridge 2009 iGEM Team's E. Chromi color generator to verify and differentiate the identities of the VOCs present in the user's breath sample. Currently under development by our team, this device has the potential to be an inexpensive, sensitive, and noninvasive solution to early screening of lung cancer.



BIOTECHNOLOGY INITIATIVE

To improve the community by pioneering investigations into biotechnology by solving real world problems in order to promote scientific inquiry and educate the future leaders of biological engineering.
— St. Mark's School of Texas iGEM Team Mission Statement

COMMITTEES | PURE RESEARCH | APPLIED RESEARCH | OUTREACH | WEB DEVELOPMENT



The first iGEM high school team from the state of Texas, the St. Mark's iGEM Team was established as part of a Biotechnology Initiative begun in September of 2013 by our school's Biology Club. This Initiative, spurred on by iGEM, revitalized our Club and helped generate significant interest in synthetic biology from the student body, faculty, and school administration. In our first year we achieved regular access to a radiation oncology lab at the University of Texas Southwestern Medical Center, $1500 in funding from the school to cover the registration fee for the iGEM Competition, exlcusive laboratory space at the school, and a more than four-fold increase in membership of the Biology Club to twenty-two members.

Our iGEM Team blends the setup of a traditional club with the highly efficient structure of a professional research laboratory. The Team is divided into four Committees: Fundamental Research, Applied Research, Outreach, and Web Development. This allows us to work more efficiently and cohesively. We have had to lay the foundation of our iGEM Team this year by informing the school community on synthetic biology, seeking financial support, and establishing laboratory resources for our project. But it was our infectious enthusiasm and indefatigable determination for synthetic biology that allowed us to overcome administrative hurdles and a steep learning curve. Through our journey this year, we have proven that a school with limited resources in biotechnology can make significant strides, effectively pioneer investigations in the field, and shown our community the tremendous potential we have in the future.

HUMAN PRACTICES

The St. Mark's iGEM Team is commited to serving the community through innovative research and engaging outreach. Our mission is in line with that of iGEM's by promoting and fostering scientific research in our school and beyond. We strive to engage with social, cultural, ethical, philosophical, environmental, political, legal, and economic dimensions of synthetic biology. In our first year, we have achieved the following.

BRENDAN COURT | COMMUNITY OUTREACH

Brendan Court is an enrichment program in which underprivileged students who are entering the seventh and eighth grades are given the opportunity to study mathematics, science, humanities, and the arts at St. Mark's School of Texas during summer break. As an iGEM Team, we sought to teach and inspire the students in synthetic biology. We presented informative and engaging presentations that explained basic biotechnology concepts along with compelling and visual demonstrations that captivated the students.

COMICS | YOUTH OUTREACH

To bring youth awareness to our Biotechnology Initiative, we developed comics that would integrate synthetic biology concepts into the plot and artwork.

TUTORIAL VIDEOS | GLOBAL OUTREACH

Through the creation of engaging YouTube biotechnology tutorial videos, we strove to give a global online audience access to quality instructional resources in synthetic biology.

#INSPIRINGTOMORROW | GLOBAL OUTREACH

We launched our school's greatest social media campaign through Facebook, Twitter, Instagram, and YouTube. We strove to inspire students to become passionate scientists and good leaders in tomorrow's world. Through social media, we were able to incite a global audience to action by showing them the potential synthetic biology has to solve some of the world's most pressing problems.

BIOTECHNOLOGY FILM | GLOBAL OUTREACH

Instead of creating a traditional slideshow and presentation of our work this year, we sought to utilize this opportunity as a virutally participating team to create a professional film to showcase our Biotechnology Initiative. We utilized YouTube and Vimeo to reach out to a global audience.

FUTURE DIRECTIONS

We intend to continue the expansion of our iGEM Team and the inclusion of all those who are interested in synthetic biology. This year we worked extensively through the month of June from 9 AM to 3 PM every weekday. We hope to establish an official summer camp for St. Mark's iGEMers and interested students in our local community. Besides continuing to serve local and global communities both directly and online, we hope to make more significant strides in the creation of our genetic constructs combined with our mechanical device. Since synthetic biology fuses Science, Technology, Engineering, and Math (STEM), our iGEM Team also intends to promote an even more interdisciplinary approach to solving problems at St. Mark's. By combining the talents and interests of team members, we can research and innovate with an open mind to new possibilities. The collaboration, curiosity, and consideration of ethics necessary for iGEM present St. Mark's students with real world problems and opportunities for growth both intellectually and personally.

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