horizontal line

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Waterborne Polio Biodetection System

**ABE 59100 Fall 2018**

# OVERVIEW

Polio is a crippling disease which attacks the nervous system and is spread through contact with the feces of an infected person or drinking water or eating food contaminated with infected feces [1]. Despite the development of the polio vaccine in 1955, three countries are still fighting a polio endemic to this day: Afghanistan, Nigeria, and Pakistan. This puts neighboring countries at-risk of seeing polio return to their populations [2]. Nigeria specifically has been categorized as a Level 2 hazard to travelers by the Centers for Disease Control and Prevention and it is recommended to receive a booster dose of the polio vaccine before traveling to the country [1].

Nigeria, in need of innovative solutions to increase food production for its growing population, signed a biosafety bill into law in April of 2015 to bring biotechnology companies and investments into the country [3]. With the country’s open-mindedness toward biotechnology solutions taken into consideration, our team plans to use synthetic biology to tackle the problem of the Nigerian polio endemic by creating a user-friendly home water test for Nigerians to protect themselves from contracting polio via their drinking water.

# DESIGN

## SPECIFICATIONS

1. User-friendly: the test kit must be able to be used and understood by people without any training and can be performed at home without uncommon equipment.
2. Fast: the test must report results in under one hour.
3. Positive and negative results: the test must create some form of signal for both the detection of polio and the absence of polio.
4. Accessible to the majority of the population: results must be easy to understand and accessible to the color blind.
5. Cheap: the test must be produced and sold for under $10 to make it accessible to all those affected by polio.

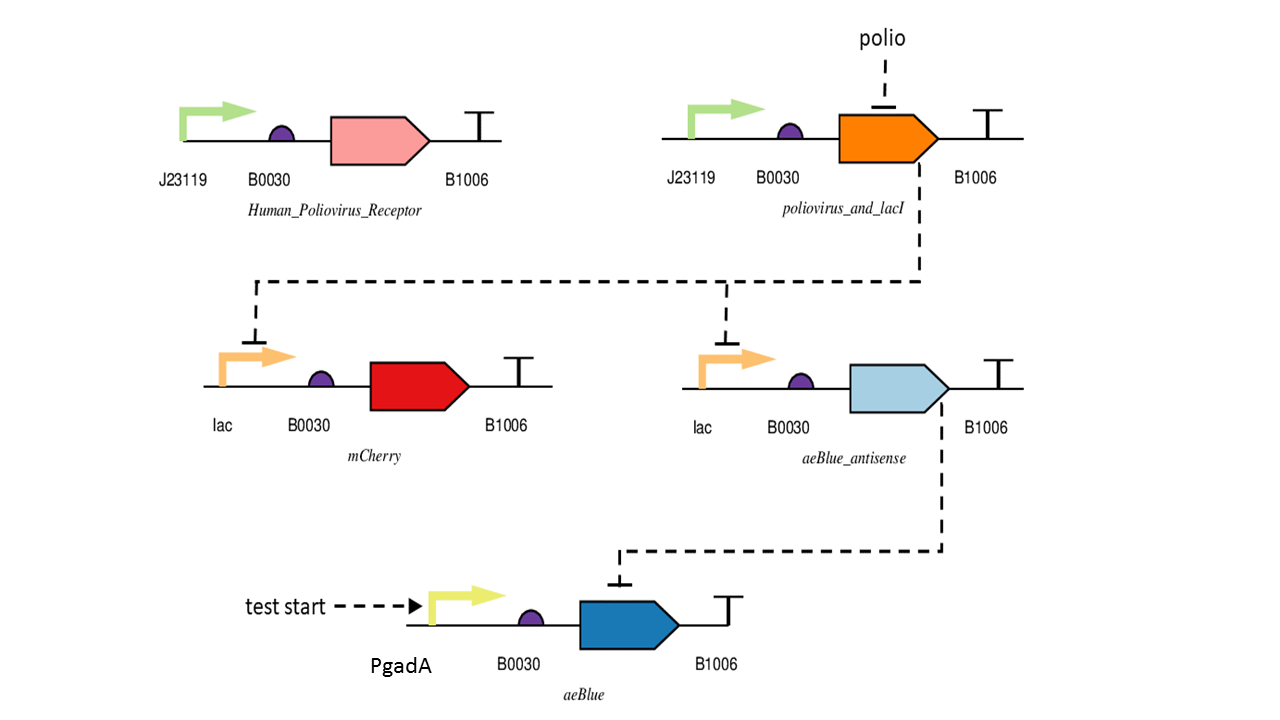
## HIERARCHICAL DESIGN

### CHASSIS

*E. coli* was chosen for the chassis of this device as it has been studied in-depth and is the standard for genetic engineering design. It is known that it can survive in a variety of pHs and temperatures that make it ideal for transportation with a relatively long shelf life [4]. This chassis can be engineered to be equipped with a membrane protein receptor for the polio virus. The frequency of PAM sites in *E. coli* is high such that CRISPR is feasible.

### DEVICES

The chassis will contain one engineered device made up of five systems. Together, the device will serve as a bioditector for the presence of poliovirus, as described by Figure 1.



*Figure 1: Genetic circuits of the polio detection device.*

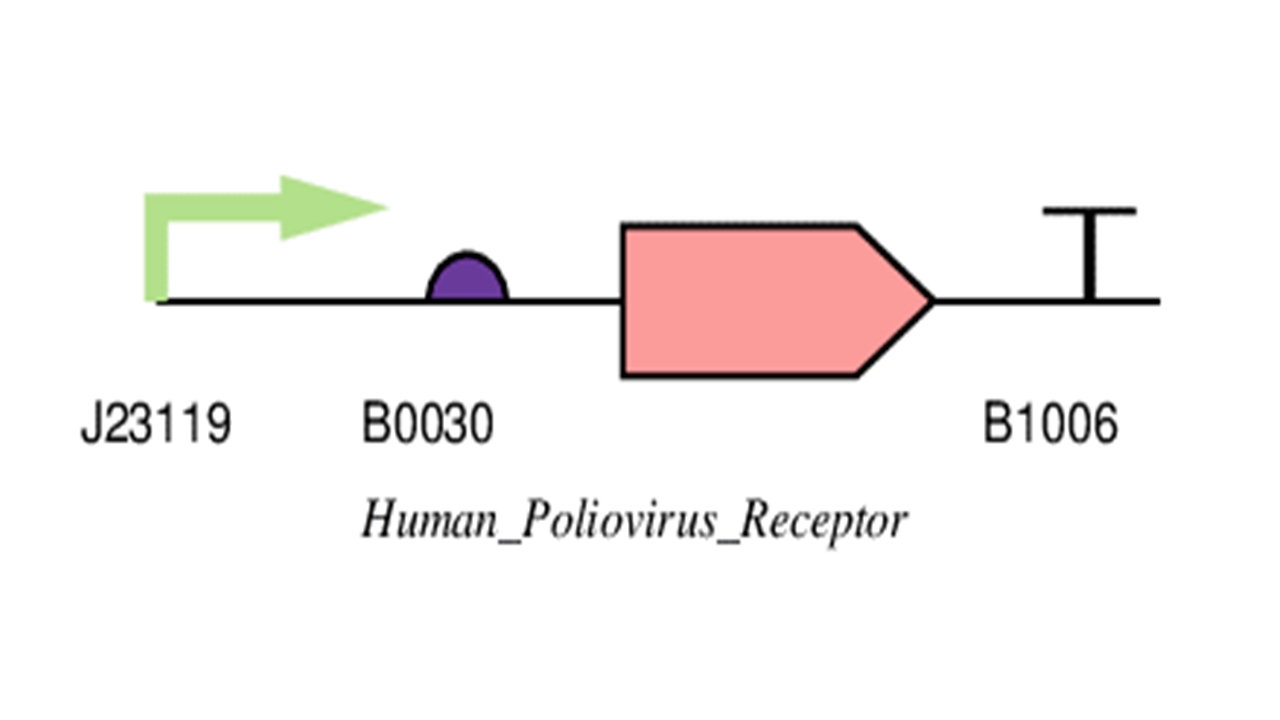
The device works to produce AND logic, meaning that if both the test has been started AND the poliovirus RNA has been detected, the red fluorescent protein will be produced. This can be seen in the logic gate in Figure 2.

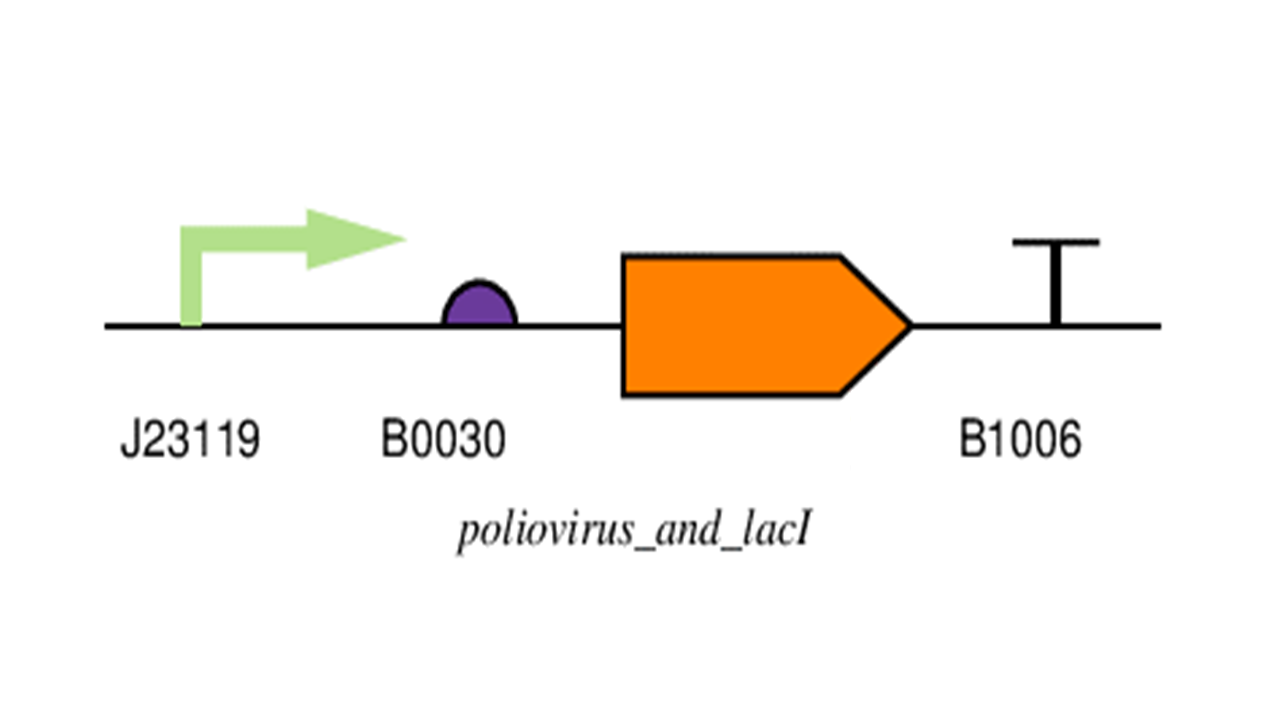


*Figure 2: Logic gate for the polio detection device*

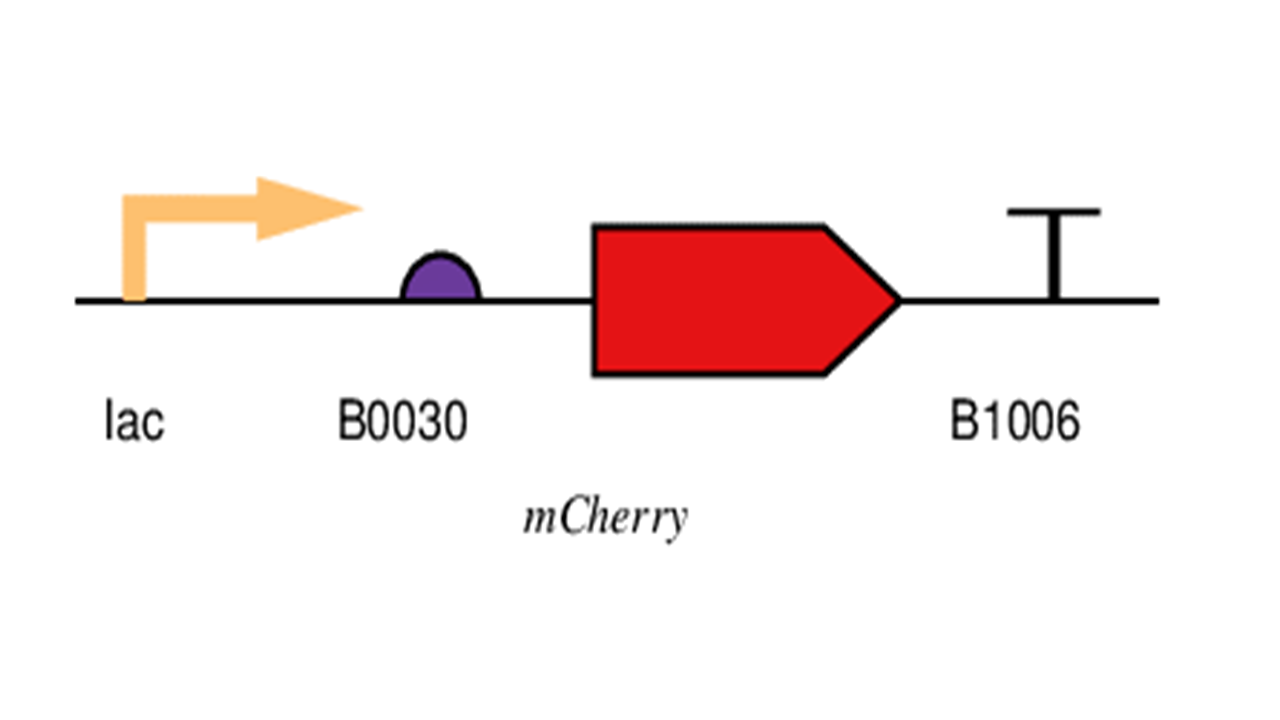
### SYSTEMS

Our project requires five systems, as described by Figures 3 - 8.

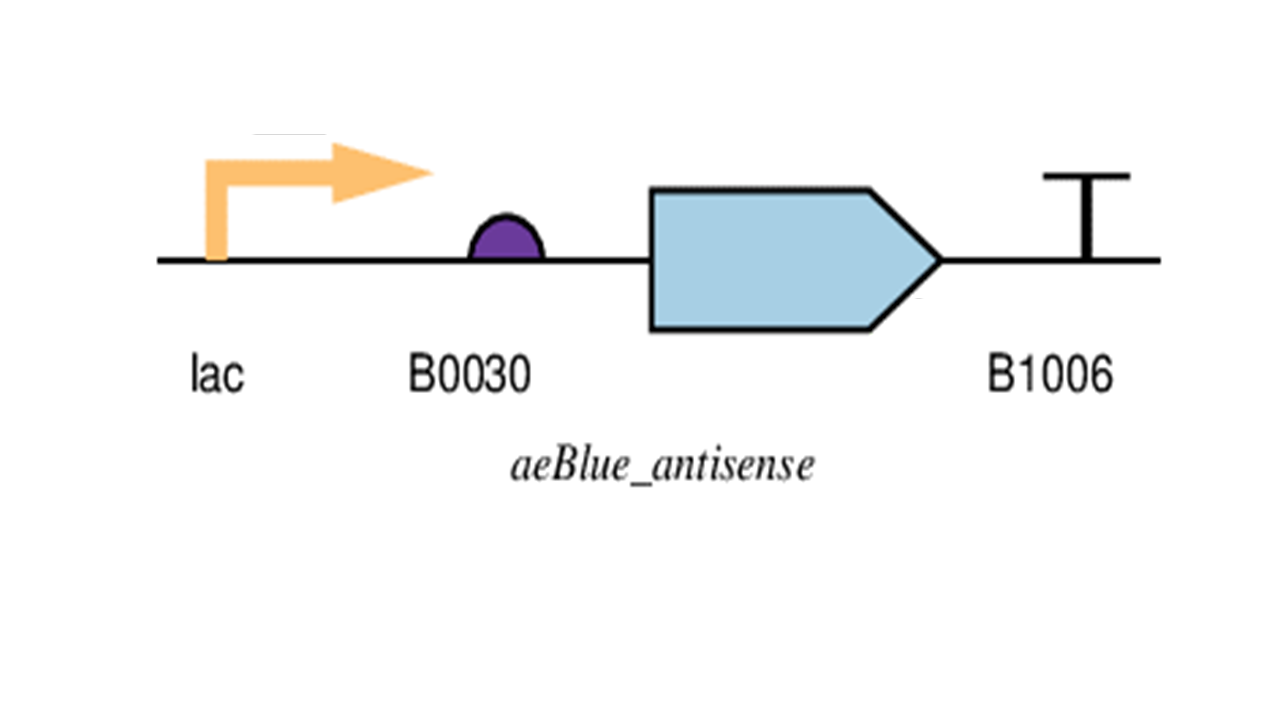


*Figure 3: System 1 produces the human poliovirus receptor on the membrane of the* E. coli *cell.* 

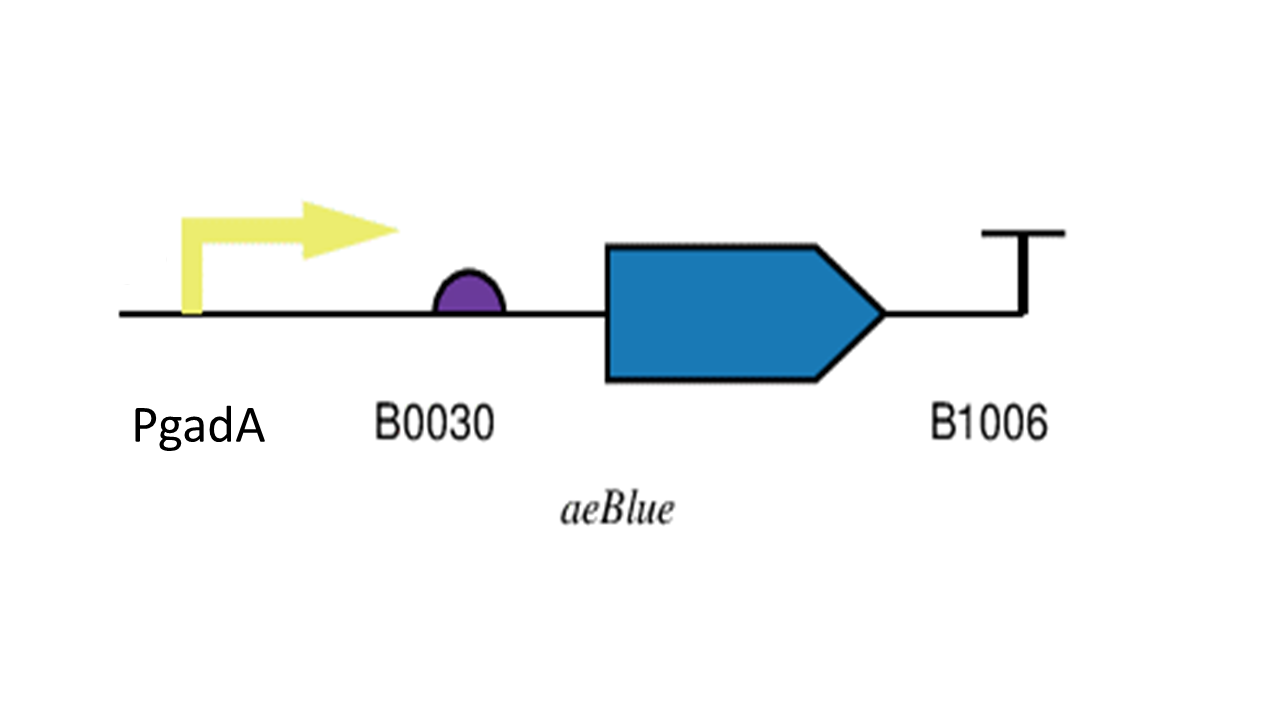
*Figure 4: System 2 is inhibited by polio which is allowed into the cell by System 1 and produces* lacI*.*



*Figure 5: System 3 is inhibited by* lacI *from System 2 and produces red fluorescent protein.*



*Figure 6: System 4 is inhibited by* lacI *produced by System 2 and produces the antisense of the blue protein produced in System 5.*



*Figure 7: System 5 is activated by the test start but inhibited by the antisense sequence produced in System 4 and produces a blue protein.*

### PARTS

All systems utilize the ribosome binding site Ba\_B0030 from the iGEM Registry of Standard Biological Parts because it is a strong RBS with Golden Gate compatibility [5]. The efficiency for the RBS is 0.6. All systems incorporate the same terminator BBa\_B1006, also from the iGEM Registry of Standard Biological Parts, because it has an 8 base pair stem and 6 nucleotide loop which terminates with 99% efficiency [6].

For Systems 1 and 2, promoter BBa\_J23119 from the iGEM Registry of Standard Biological Parts was selected because it is a strong constitutive promoter [7].

Systems 3 and 4 use the *lac* operon to allow inhibition by *lacI* production from System 2 [8]. This was selected because it is a common inhibition sequence with a relatively large Hill coefficient which, in combination with the cascade created between the interaction between Systems 2 and 3 and 4, will make the device more binary in on versus off states.

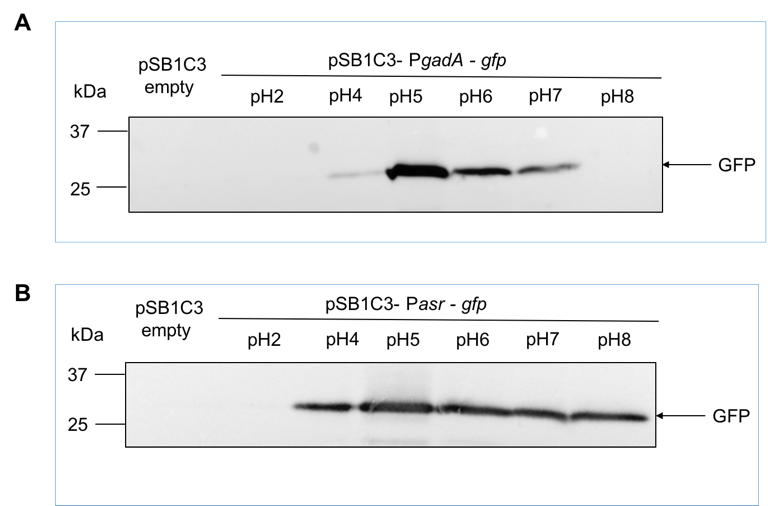
The coding region for System 1 is the Human Poliovirus Receptor gene [9]. This region will allow the receptor that polio recognizes to be expressed on the surface of our chassis. The virus will then bind to the receptor and infect cells with its RNA, which is the molecule System 2 recognizes to determine the presence of polio.

System 2’s coding region includes the first fifteen base pairs of the poliovirus before the *lacI* gene [10, 11]. The poliovirus sequence acts as an inhibitor on the system, binding to the RNA of the polio infection and preventing translation of the *lacI* gene. The *lacI* gene was selected as, again, it is a part of a common inhibition system for *E. coli* and produces a tetramer. The four subunits of the protein create a relatively high number of binding sites to increase the Hill coefficient of the system, again, making the device more definitive in determining the on or off state. The combination of the two sequences is possible because *lacI* is a relatively stable protein.

System 3 codes for the red fluorescent protein mCherry, which was selected because it is fast-folding and can be seen with the naked-eye [12, 13]. This will satisfy our requirements that the test can be performed in under one hour and does not require uncommon or expensive materials to perform.

The coding region of System 4 produces the antisense of the coding region of System 5 [14]. When it is transcribed, it will act as an inhibitor to System 5 such that the negative test result is not produced.

System 5’s promoter PgadA was selected from the iGEM Registry of Standard Biological Parts because it is sensitive to a change in pH [15]. Transcription occurs to a greater extent when the environment is between a pH of 5 and 7 (See Figure 3). The test kit will be stored in a pH of 4.5 prior to the test start as *E. coli* cells can survive in acidic conditions above a pH of 4 for at least 56 days [4]. When the prescribed volume of water is added to produce an environment around pH 5, the promoter will be activated and the “test start” condition will be satisfied. The system codes for the protein aeBlue [16]. The blue color was selected as it will not be easily confused with the red signal produced by System 3 by those affected by color blindness. This is important because about 4% of the Nigerian population is color blind [17]. Additionally, blue is a color associated with clean water while red is a universal signal for “stop” or “warning.” The protein itself was selected because it has a half life of 24 minutes, which satisfies our requirement of producing a signal in under an hour, and it can be seen with the naked eye.



*Figure 8: Dundee iGEM team 2016 characterization of PgadA promoter under different pH conditions [16]. The promoters were cloned with GFP downstream and transformed into* E. coli *cells and incubated for 16 hours at 37oC. The cells were then put into LB broth adjusted to the respective pHs above and after 20 minutes, the cells were pelleted. Pellets were resuspended in 100 uL Laemmli buffer and then separated by SDS PAGE and transferred to PVDF membrane and then probed with anti-GFP antibody.*

### SEQUENCES

See Appendix C for sequences.

# BUILD

## CLONING

In order to clone the genetic cassettes into the *E. coli* cells, we plan to use Golden Gate assembly. We will create fifteen parts total by combining the promoters and ribosome binding sites for each system into one part while the coding regions and terminators serve as standalone parts. As such, we will perform Golden Gate twice.

## PRE-CLONING SEQUENCE ANALYSIS

### CODON FREQUENCY ANALYSIS

All coding sequences that did not come from *E. coli* were run through the IDT Codon Optimization Tool to ensure that they would most efficiently be expressed in our chassis [18]. Changes made to the sequences are highlighted in Appendix C.

### RESTRICTION ENZYME RECOGNITION SITES

Following codon optimization, the sequences were entered into Benchling, and a BsaI digest was run on the systems to ensure that the sequences did not contain undesired BsaI recognition sites. None were found, so each Golden Gate part was then edited to contain the desired recognition sites in order to perform the assembly. See Appendix C for the BsaI recognition site additions.

# TEST

## SYSTEM ODE

The ODE defining the system is given by equations representing activation, repression, decay, and simplified hybridization kinetics. Activation and repression of mRNA and protein are expressed with Hill equations. Decay kinetics follow first order. Hybridization is assumed to bind non-reversibly by first order kinetics.

### ASSUMPTIONS

* Hybridization events bind tightly and irreversibly
* First order kinetics
  + Protein Synthesis
  + Decay rates
* Hill kinetics
  + LacO high affinity to DNA
  + LacO is a dimer
    - N = 2

## ODE NORMALIZATION AND JACOBIAN

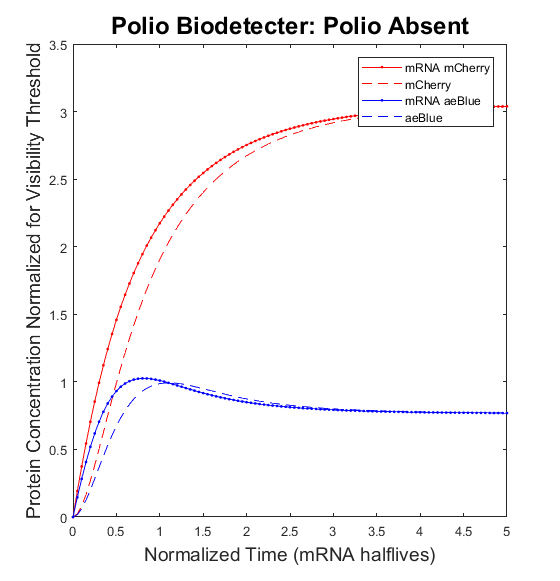
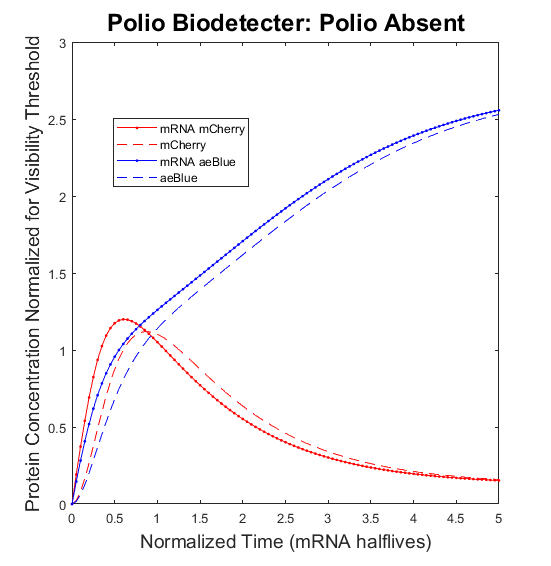
The system ODE was normalized using relationships shown in Appendix A. The normalized ODE is shown below as well as assumptions needed. This was used to find regions of stable parameter choices by finding the system parameters that yielded negative real eigenvalues. The Jacobian used is found in Appendix B, and its determinant for eigenvalue calculation can be found in Appendix B. Using MATLAB to calculate eigenvalues symbolically, all positive values for ,, andyield negative eigenvalues (Appendix B). Given negative eigenvalues, the system will converge to a steady state signal. No further information can be obtained by the Jacobian.

ASSUMPTIONS

* Lac protein expression is high
* No secondary structure by the RNA
* 𝜇 = 20 min; kPROTd = 20 min ; kmRNAd = 4 min; klacI d = 10 min
* Strong RBS
* Colorimetric proteins normalized to their visible threshold concentration

## FINAL DEVICE SIMULATION

For detection design, kinetic parameters could be freely chosen given the Jacobian analysis from above. Parameters(Appendix A) were manually adjusted until output signal was >2x above predetermined threshold. This predetermined threshold was used to normalize mCherry and aeBlue signals for their visibility threshold concentration. These signal modeled in Figure 9 and 10 would be visible once on the signal climbs over 1. Showing proper signal delay, a determined signal is produced at least 2 mRNA half lives or at least 8 min while reaching a confident steady state signal when approaching 20 min. Table with full signal dynamic characteristics in Appendix A.



*Figure 9: Simulated polio biodetector behaviour. a) Polio absent and aeBlue is expressed. b) Polio present and mCherry is expressed*

# LEARN

All experiments would be performed with water similar to water found in target areas in Nigeria such that results would show effects of the dissolved materials in that water on the system.

Initial testing of the genetically engineered *E. coli* would gather and confirm simulated quantitative parameters for that strain such as the translation/transcription rates and Hill equation parameters. Lab testing would include L\*A\*B colorimetric measurements to quantify the appearance of fluorescent proteins. Expression of the proteins would be adjusted to meet quantitative L\*A\*B values such that the colors are qualitatively easy to observe in the recommended amount of time. Further tests on the biodetection system would be conducted for a range of water sample volumes around the suggested fill volume. Varying water volume also changes the pH of the sample. The design would be optimized for a reasonable range of water volume/pH conditions.

In-country preliminary testing would include testing water samples with varying amount of sediment or other commonly present materials to ensure any effects from foreign materials can be mitigated if possible. Behavioral studies of Nigerians piloting the system with the given test kit and instructions may show additional problems to consider in future iterations of the design.

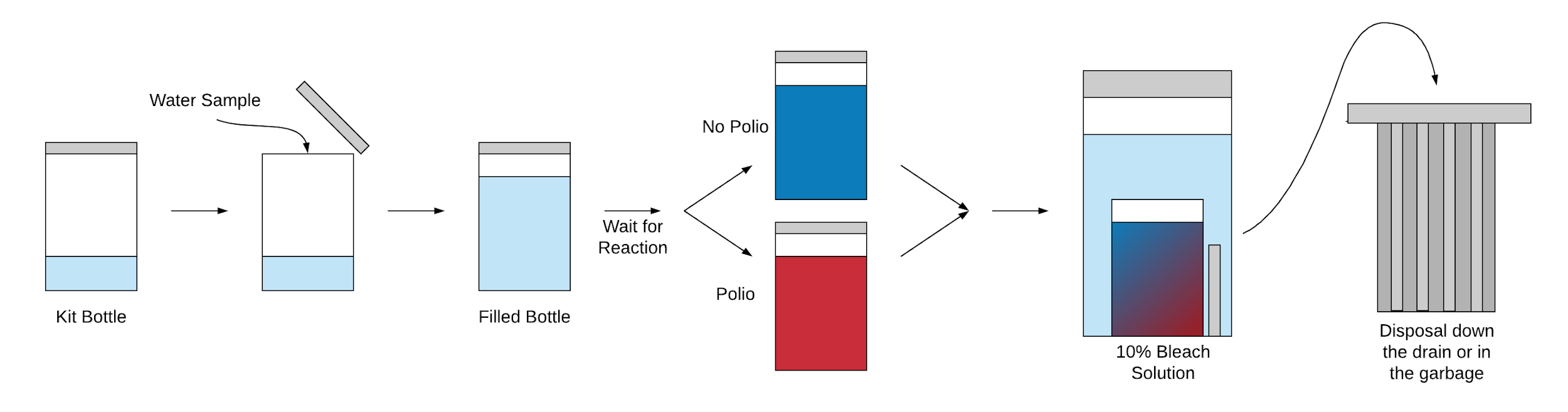
# AT-HOME KIT DESIGN

## PACKAGING

Conner & Kotrola (1994) found that *E. coli* populations in acidic conditions, established by six individual organic acids, remained essentially constant with lactic acid at three tested temperatures (4, 10, and 25C) [4]. As that temperature range is indicative of environments the kits may experience during the supply chain, using lactic acid, a natural product of *E. coli*, to produce an environment of 4.5 ensures the intended population of *E. coli* biodetectors reaches the consumer.

All recommended packaging would be cost-efficient. Primary packaging would be a plastic bottle and lid resistant to breakage and spillage. Secondary packaging would contain all items for a complete test kit and would not allow any materials to leak out if primary packaging were compromised. Tertiary packaging (cardboard boxes, styrofoam, bubble wrap, other types of insulation) would be designed with heat transfer principles such that the test kits would remain in an appropriate temperature range even in the most extreme environments found throughout the supply chain.

## USAGE



*Figure 11: Kit Usage Flow Chart*

A paper of instructions in the vernacular would be included with each kit.

The primary test container will be equipped with a fill line such that the volume of water added to the pH 4.5 concentrated biodector establishes a pH of 5. Then, the pH-sensitive promoter will induce a high rate of transcription.

Intended for use after every water testing, the provided container of 10% bleach will be of the appropriate volume to kill all *E.coli* and polio in the test kit. Additionally, the amount of bleach would ensure that the pH of the final solution would be appropriate to pour down a drain. The entire water sample container will be placed inside the bleach container so that no residual material would be left in the bottom. The kit will then be safe for disposal like any other non-hazardous trash or liquid. If the consumer intends to put the materials down the drain, a time would be specified for the bleach solution to have effectively killed all organisms before pouring.

# RESPONSIBLE SCIENCE AND ETHICAL CONSIDERATIONS

* **Biosafety:** to prevent the engineered bacteria from entering the natural environment, the test kit is equipped with instructions for safe disposal.
* **Biosecurity:** we do not envision an unsafe dual-use of this product at the moment. However, it could be repurposed in a beneficial manner to detect other waterborne diseases.
* **Justice and Fairness:** the product will ideally be purchased by a government (local or national) and distributed to constituents for free so that anyone can test water regardless of income.
* **Socioeconomics:** we do not envision this product disrupting any industries at the moment, but we intend to patent the intellectual property.
* **Eugenics/Playing God:** we do not envision any major moral backlash to this product since *E. coli* is a commonly engineered cell type.

# 

# APPENDIX A: MODELING MATLAB CODE

metabini = [1 % LacI mRNA

1 % LacI

0 % mCherry mRNA

0 % mCherry

0 % aeBlue mRNA

0 % aeBlue

0]; % aeBlue mRNA antisense

timespan = 0:0.05:5; %0:~20min

[tv, Yt] = ode45(@ABE591Model,timespan, metabini);

figure

plot(tv,Yt(:,3),'-r.',tv,Yt(:,4),'--r',tv,Yt(:,5),'-b.',tv,Yt(:,6),'--b')

title('Polio Biodetecter: Polio Absent','fontsize',18);

xlabel('Normailized Time (mRNA halflives)','fontsize',14);

ylabel('Protein Concentration Normalized for Visibility Threshold','fontsize',14);

legend('mRNA mCherry','mCherry','mRNA aeBlue','aeBlue');

function f = ABE591Model(t,y);

%Parameters

m\_polio = 10; %0 or 10

alpha\_lac = 50;

b\_lac = 10/4;

alpha\_ofp = 4;

b\_ofp = 20/4;

alpha\_bfp = 3;

b\_bfp = b\_ofp;

K\_ofp = .015;

trns\_eff = 10;

gamma = 0.06;

n\_lacO = 2;

%component mass balances

f(1,1) = alpha\_lac - y(1) - y(1)\*m\_polio;

f(2,1) = b\_lac \* y(1) - b\_lac \* y(2);

f(3,1) = alpha\_ofp / (1 + (K\_ofp \* y(2)^ n\_lacO)) - y(3);

f(4,1) = b\_ofp \* y(3) - b\_ofp \* y(4);

f(5,1) = alpha\_bfp - y(5) - y(5)\*y(7);

f(6,1) = b\_bfp\*y(5) -b\_bfp\*y(6);

f(7,1) = alpha\_ofp / (1 + (K\_ofp \* y(2)^ n\_lacO)) - y(7) - gamma\*y(7)\*y(5);

### SIMULATION NORMALIZED PARAMETERS

|  |  |
| --- | --- |
| Parameter | Value [unitless] |
| blac | 2.5 |
| bmCherry = baeBlue | 5 |
| kmCherry | 0.015 molar LacI |
| 𝛼lac | 50 |
| 𝛼mCherry | 4 |
| 𝛼aeBlue | 3 |
| ℽ | 0.06 |
| nlacI | 2 |

### SIMULATED SIGNAL DYNAMICS

|  |  |  |
| --- | --- | --- |
|  | Polio Present | Polio absent |
| Min Confirmation | ~30min | ~16min |
| Strength of signal at end of test | 3X | 2.5X |

# 

# APPENDIX B: MODELING SUPPLEMENT

### NORMALIZATION RELATIONSHIPS

### EIGENVALUE CALCULATIONS

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | mRNA  lac | lacI | mRNA  mCherry | mRNA  aeBlue  antisense | mCherry | mRNA | aeBlue |
|  | -1-mpolio | 0 | 0 | 0 | 0 | 0 | 0 |
|  | blac | -blac | 0 | 0 | 0 | 0 | 0 |
|  | 0 | Xlac | -1 | 0 | 0 | 0 | 0 |
|  | 0 | Xlac | 0 | -1-ˠ | 0 | -1 | 0 |
|  | 0 | 0 | bmCherry | 0 | -bmCherry | 0 | 0 |
|  | 0 | 0 | 0 | -1 | 0 | -2 | 0 |
|  | 0 | 0 | 0 | 0 | 0 | baeBlue | -baeBlue |

## 

# 

# APPENDIX C: SEQUENCES SUPPLEMENT

## RAW SEQUENCES

### PROMOTERS

#### SYSTEMS 1 & 2: BBa\_J23119

5’ TTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGC 3’

#### SYSTEMS 3 & 4: *lac*

5’ GGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGA 3’

#### SYSTEM 5: PgadA (BBa\_K1962014)

5’ CGCTGTAATTTATTCAGCGTTTGTACATATCGTTACACGCTGAAACCAACCACTCACGGAAGTCTGCCATTCCCAGGGATATAGTTATTTCAACGGCCCCGCAGTGGGGTTAAATGAAAAAACAAATTGAGGGTATGACA 3’

### RIBOSOME BINDING SITE

#### ALL SYSTEMS: BBa\_B0030

5’ ATTAAAGAGGAGAAA 3’

### CODING REGIONS

#### SYSTEM 1: Human Poliovirus Receptor (Amino Acid Sequence to remove introns)

MARAMAAAWPLLLVALLVLSWPPPGTGDVVVQAPTQVPGFLGDSVTLPCYLQVPNMEVTHVSQLTWARHGESGSMAVFHQTQGPSYSESKRLEFVAARLGAELRNASLRMFGLRVEDEGNYTCLFVTFPQGSRSVDIWLRVLAKPQNTAEVQKVQLTGEPVPMARCVSTGGRPPAQITWHSDLGGMPNTSQVPGFLSGTVTVTSLWILVPSSQVDGKNVTCKVEHESFEKPQLLTVNLTVYYPPEVSISGYDNNWYLGQNEATLTCDARSNPEPTGYNWSTTMGPLPPFAVAQGAQLLIRPVDKPINTTLICNVTNALGARQAELTVQVKEGPPSEHSGMSRNAIIFLVLGILVFLILLGIGIYFYWSKCSREVLWHCHLCPSSTEHASASANGHVSYSAVSRENSSSQDPQTEGTR

#### SYSTEM 2: Poliovirus Recognition Sequence (24 bp) + *lac*

5’ GTGTTAAAACAGCTCTGGGGTTGTTCCAAACCAGTAACGTTATACGATGTCGCAGAGTATGCC GGTGTCTCTTATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAACCGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCAGTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTAACTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGTTATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGACTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACTGCGATGCTGGTTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCCCGCCGTTAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGA 3’

#### SYSTEM 3: mCherry (Amino acid sequence)

MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK

#### SYSTEM 4: aeBlue Antisense (with added start and stop codons for transcription)

5’ ATGAATAAATATTTCGAGCAGGTAAGGTTCTCATTAGGGCCGTCGTCAGTGTTTAAGATTGTTT TGGTACACCAGTGCAAAAAGCAAGCCTAGAAACCTATTGTGTCTGACTCATCTGTCTATTACCGATAGTCCATTTTCCTGACCCGGCAGCGGTTATCCGCATAAGACGACCATCACCAGTCGGTCAACGTGCCTTGGCAGAAGTTGTAACACCGCTTAGAACTTCAAATGGAACTATGGTAAAAAGACGAACAGGCGGTATTATATGTGTAATACTCTTAATTTCAATATAAGATTAAATACAGGTTCCTACAAAGGTAGGAGGAACTTTAGTTACGGAAAATTGAGTTACGCCAAGTGATTCCACAGCGGAAGTTTGAACTGGAGACGCGCGCAAAACATTCACGGTAGCAGGAACTTTTTCTACCATGCAAGGACTTGTATCGGGAGCCCGTAACGACTGAATTTCTTCAGTACGACGAAGTACACCAGACCCATCGCACGCTTCGTAACTTGTGGTACACTATTCCAGCAGTGGTTGCAGCCGGTTCCTTGACCGTCGAAAGGGCAGCATGTTTATTTAAAGTTGCAATTGAATGGCAAACACCGCAGGGGAAGCGGGAGGGGTGCTTGGCTCTTGAACACAGGTAAATGTAGAGGTAGATTAAGGTGGTCCTAACCCTGCTGTGGCCATTTGTTGAGAAGCGGGAATGAGTGGTATAA 3’

#### SYSTEM 5: aeBlue (BBa\_K864401)

5’ ATGGCTTCACTGGTTAAAAAAGACATGTGCATCAAAATGACGATGGAAGGAACAGTAAACGG TCACCATTTCAAGTGTGTAGGAGAAGGCGAAGGCAAACCATTTGAAGGGACCCAGGTGGAAAAGATACGCATCACTGAAGGTGGGCCCTTACCATTTGCGTATGATATTTTGGCCCCTTGTTGCATGTATGGCAGTAAAACCTTCATTAAGCATGTGTCGGGTATTCCGGATTACTTTAAGGAGTCTTTTCCTGAGGGCTTTACCTGGGAAAGAACACAAATCTTCGAGGATGGCGGCTATCTCACCATACACCAGGACACGAGCCTTCAGGGTAATAATTTTATTTTCAAAGTTAATGTCATCGGTGCCAACTTCCCTGCAAACGGTCCCGTGATGCAGAAAAAAACAGCTGGATGGGAACCGTGCGTTGAGATGCTTTATCCGCGGGACGGCGTCCTGTGTGGTCAGAGCCTGATGGCCCTGAAATGCACTGATGGCAATCATCTGACGTCCCACCTGCGCACTACCTATCGTTCTCGCAAGCCATCCAATGCAGTTAACATGCCGGAATTTCATTTTGGGGATCATCGCATTGAGATTTTGAAAGCTGAACAAGGTAAATTTTATGAACAATACGAGTCAGCGGTGGCCCGTTACTGTGAGGCGGCACCGAGTAAATTAGGGCATCACTAATAA 3’

### TERMINATOR

#### ALL SYSTEMS: BBa\_B1006

5’ AAAAAAAAACCCCGCCCCTGACAGGGCGGGGTTTTTTTT 3’

## 

## CODON FREQUENCY OPTIMIZATION

#### SYSTEM 1: Human Poliovirus Receptor

The amino acid sequence was run through the IDT Codon Optimization Tool to produce the following DNA sequence optimized to be produced in *E. coli*:

5’ ATGGCTCGCGCGATGGCCGCTGCTTGGCCCCTTCTGCTTGTGGCGTTATTAGTCTTGAGTTGG CCTCCACCGGGGACAGGAGATGTAGTCGTTCAAGCCCCCACCCAAGTCCCGGGTTTCTTGGGTGATAGTGTAACTCTTCCTTGTTATCTGCAGGTTCCCAACATGGAAGTGACCCACGTCAGTCAATTGACGTGGGCACGTCATGGTGAGAGCGGTAGCATGGCTGTTTTTCATCAGACCCAGGGTCCCAGTTATTCAGAAAGTAAACGTTTAGAATTCGTAGCTGCCCGCTTAGGGGCTGAGTTACGTAACGCTAGCTTACGTATGTTTGGCCTGCGCGTCGAAGATGAAGGCAACTACACGTGCCTTTTCGTCACCTTTCCGCAGGGATCCCGTAGCGTGGATATCTGGTTACGTGTACTGGCAAAACCGCAAAACACAGCCGAGGTCCAGAAAGTGCAGTTGACGGGAGAACCAGTACCCATGGCACGCTGCGTGTCCACAGGCGGACGTCCACCTGCCCAAATCACATGGCATTCAGATCTGGGAGGTATGCCAAATACATCACAAGTTCCAGGGTTCCTGTCCGGCACAGTCACGGTGACCAGCCTTTGGATTCTGGTACCCTCGTCACAAGTGGATGGGAAGAATGTTACATGTAAAGTGGAACATGAATCCTTCGAAAAACCACAGCTTCTGACTGTGAATCTTACTGTGTATTATCCCCCCGAAGTTTCAATCAGTGGCTATGATAATAACTGGTACCTGGGACAAAATGAAGCCACTTTGACCTGTGATGCACGCAGCAATCCCGAGCCGACTGGTTACAACTGGTCTACAACGATGGGTCCATTACCCCCCTTTGCTGTGGCCCAAGGGGCGCAACTTTTAATCCGCCCGGTCGATAAACCGATCAACACTACGTTAATTTGTAACGTGACAAATGCTCTGGGCGCTCGTCAAGCCGAGCTTACTGTACAAGTGAAGGAGGGCCCTCCCTCTGAACATAGCGGTATGTCTCGCAATGCGATTATTTTCCTTGTGCTGGGGATCCTGGTATTTTTAATTTTGTTAGGAATCGGAATCTACTTCTATTGGAGTAAATGTTCTCGTGAGGTGCTGTGGCACTGCCATTTGTGTCCCAGTTCCACCGAACATGCCTCGGCCTCCGCCAATGGACATGTGAGTTATAGTGCGGTATCACGTGAGAACTCCTCTAGCCAAGACCCACAGACGGAGGGGACACGT 3’

#### SYSTEM 2: Poliovirus Recognition Sequence (24 bp) + *lac*

As the *lac* gene is native to *E. coli* and the poliovirus sequence is necessary to be the exact complement of the polio RNA, this sequence did not need to be optimized.

#### SYSTEM 3: mCherry (Amino acid sequence)

The amino acid sequence was run through the IDT Codon Optimization Tool to produce the following DNA sequence optimized to be produced in *E. coli*:

5’ ATGGTATCGAAAGGTGAAGAAGATAATATGGCAATCATTAAGGAGTTTATGCGCTTCAAAGTG CATATGGAGGGTTCGGTGAATGGTCACGAATTTGAGATCGAGGGTGAGGGGGAAGGTCGTCCTTATGAAGGAACTCAAACGGCAAAACTTAAGGTCACAAAGGGGGGGCCACTTCCGTTTGCTTGGGACATTTTAAGTCCACAGTTTATGTATGGCAGTAAGGCATACGTCAAACACCCAGCAGATATTCCGGATTACCTGAAGTTGTCATTCCCGGAAGGGTTCAAATGGGAACGCGTAATGAACTTTGAGGACGGGGGCGTGGTGACGGTTACTCAAGATAGTTCTCTTCAAGACGGGGAGTTCATTTACAAGGTTAAGTTGCGCGGTACCAATTTCCCCAGTGATGGGCCTGTTATGCAGAAGAAGACGATGGGTTGGGAGGCCTCCTCAGAGCGCATGTATCCTGAAGATGGGGCCCTGAAAGGCGAGATTAAGCAACGTCTTAAACTTAAAGATGGCGGTCACTATGACGCTGAAGTGAAGACCACCTACAAGGCGAAGAAGCCGGTGCAATTGCCGGGAGCTTACAATGTGAACATCAAACTTGACATTACCAGCCATAATGAAGATTATACGATTGTGGAACAGTACGAACGTGCAGAAGGCCGTCATAGCACAGGCGGAATGGACGAGTTGTATAAG 3’

#### SYSTEM 4: aeBlue Antisense (with added start and stop codons for transcription)

As this sequence needs to be the exact complement to the System 5 sequence, it was not optimized. Instead, it was produced following the optimization of the System

#### SYSTEM 5: aeBlue (BBa\_K864401)

This sequence, originally from a beadlet anemone *Actinia equina*, was run through the IDT Codon Optimization Tool. The following was produced:

5’ ATGGCTAGTCTGGTCAAGAAAGATATGTGTATTAAGATGACTATGGAGGGTACCGTAAACGGT CATCACTTCAAGTGCGTCGGGGAAGGTGAGGGAAAGCCCTTTGAGGGCACTCAGGTGGAAAAGATCCGCATTACCGAGGGCGGGCCACTGCCATTCGCATATGACATTCTTGCCCCTTGCTGCATGTATGGCTCTAAAACTTTCATTAAGCACGTGAGTGGGATCCCTGATTATTTCAAGGAATCCTTCCCGGAAGGGTTTACATGGGAGCGCACGCAAATTTTCGAGGACGGTGGATACCTGACTATCCACCAAGATACCTCCTTACAGGGAAACAATTTCATCTTCAAGGTGAACGTAATTGGGGCTAACTTTCCGGCCAACGGGCCGGTCATGCAAAAGAAGACAGCGGGGTGGGAGCCATGCGTTGAAATGCTTTATCCCCGCGATGGGGTCTTATGTGGACAGTCTCTGATGGCCCTGAAGTGTACGGACGGTAATCATCTGACATCACACTTGCGCACCACCTACCGTTCGCGTAAGCCTAGCAATGCCGTGAATATGCCGGAGTTTCACTTTGGCGACCACCGCATTGAGATCCTGAAAGCTGAACAAGGCAAATTCTACGAACAATATGAGTCCGCAGTCGCACGCTACTGCGAGGCTGCGCCATCCAAATTAGGACACCATTGATAA 3’

## PLAN OF ASSEMBLY

Restriction enzyme recognition sites were added to the beginning and end of each part mentioned in the cloning section. Added sites are shown in italics. Cut sites are shown in bold.

### SYSTEM 1:

#### PROMOTER AND RIBOSOME BINDING SITE:

5’ TTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGCATTAAAGAGGAGAAA***ATGG****CGAGACC* 3’

#### CODING REGION:

5’ *GGTCTCA***ATGG**CTCGCGCGATGGCCGCTGCTTGGCCCCTTCTGCTTGTGGCGTTATTAGTCTT GAGTTGGCCTCCACCGGGGACAGGAGATGTAGTCGTTCAAGCCCCCACCCAAGTCCCGGGTTTCTTGGGTGATAGTGTAACTCTTCCTTGTTATCTGCAGGTTCCCAACATGGAAGTGACCCACGTCAGTCAATTGACGTGGGCACGTCATGGTGAGAGCGGTAGCATGGCTGTTTTTCATCAGACCCAGGGTCCCAGTTATTCAGAAAGTAAACGTTTAGAATTCGTAGCTGCCCGCTTAGGGGCTGAGTTACGTAACGCTAGCTTACGTATGTTTGGCCTGCGCGTCGAAGATGAAGGCAACTACACGTGCCTTTTCGTCACCTTTCCGCAGGGATCCCGTAGCGTGGATATCTGGTTACGTGTACTGGCAAAACCGCAAAACACAGCCGAGGTCCAGAAAGTGCAGTTGACGGGAGAACCAGTACCCATGGCACGCTGCGTGTCCACAGGCGGACGTCCACCTGCCCAAATCACATGGCATTCAGATCTGGGAGGTATGCCAAATACATCACAAGTTCCAGGGTTCCTGTCCGGCACAGTCACGGTGACCAGCCTTTGGATTCTGGTACCCTCGTCACAAGTGGATGGGAAGAATGTTACATGTAAAGTGGAACATGAATCCTTCGAAAAACCACAGCTTCTGACTGTGAATCTTACTGTGTATTATCCCCCCGAAGTTTCAATCAGTGGCTATGATAATAACTGGTACCTGGGACAAAATGAAGCCACTTTGACCTGTGATGCACGCAGCAATCCCGAGCCGACTGGTTACAACTGGTCTACAACGATGGGTCCATTACCCCCCTTTGCTGTGGCCCAAGGGGCGCAACTTTTAATCCGCCCGGTCGATAAACCGATCAACACTACGTTAATTTGTAACGTGACAAATGCTCTGGGCGCTCGTCAAGCCGAGCTTACTGTACAAGTGAAGGAGGGCCCTCCCTCTGAACATAGCGGTATGTCTCGCAATGCGATTATTTTCCTTGTGCTGGGGATCCTGGTATTTTTAATTTTGTTAGGAATCGGAATCTACTTCTATTGGAGTAAATGTTCTCGTGAGGTGCTGTGGCACTGCCATTTGTGTCCCAGTTCCACCGAACATGCCTCGGCCTCCGCCAATGGACATGTGAGTTATAGTGCGGTATCACGTGAGAACTCCTCTAGCCAAGACCCACAGACGGAGGGGAC**ACGT***AGAGACC* 3’

#### TERMINATOR:

5’ *GGTCTCG****ACTG***AAAAAAAAACCCCGCCCCTGACAGGGCGGGGTTTTTTTT***TTGA****TGAGACC* 3’

### SYSTEM 2:

#### PROMOTER AND RIBOSOME BINDING SITE:

5’ *GGTCTCA***TTGA**CAGCTAGCTCAGTCCTAGGTATAATGCTAGCATTAAAGAGGAGAAA***GTGT****TG AGACC* 3’

#### CODING REGION:

5’ *GGTCTCA***GTGT**TAAAACAGCTCTGGGGTTGTTCCAAACCAGTAACGTTATACGATGTCGCAGA GTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAACCGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCAGTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTAACTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGTTATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGACTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACTGCGATGCTGGTTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCCCGCCGTTAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCA**GTGA***AGAGACC* 3’

#### TERMINATOR:

5’ *GGTCTCA****GTGA***AAAAAAAAACCCCGCCCCTGACAGGGCGGGGTTTTTTTT***GGCT****AGAGACC* 3’

### SYSTEM 3:

#### PROMOTER AND RIBOSOME BINDING SITE:

5’ *GGTCTCA***GGCT**TTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTAAAGAGGA**GAAA***A GAGACC* 3’

#### CODING REGION:

5’ *GGTCTCA****GAAA***ATGGTATCGAAAGGTGAAGAAGATAATATGGCAATCATTAAGGAGTTTATGC GCTTCAAAGTGCATATGGAGGGTTCGGTGAATGGTCACGAATTTGAGATCGAGGGTGAGGGGGAAGGTCGTCCTTATGAAGGAACTCAAACGGCAAAACTTAAGGTCACAAAGGGGGGGCCACTTCCGTTTGCTTGGGACATTTTAAGTCCACAGTTTATGTATGGCAGTAAGGCATACGTCAAACACCCAGCAGATATTCCGGATTACCTGAAGTTGTCATTCCCGGAAGGGTTCAAATGGGAACGCGTAATGAACTTTGAGGACGGGGGCGTGGTGACGGTTACTCAAGATAGTTCTCTTCAAGACGGGGAGTTCATTTACAAGGTTAAGTTGCGCGGTACCAATTTCCCCAGTGATGGGCCTGTTATGCAGAAGAAGACGATGGGTTGGGAGGCCTCCTCAGAGCGCATGTATCCTGAAGATGGGGCCCTGAAAGGCGAGATTAAGCAACGTCTTAAACTTAAAGATGGCGGTCACTATGACGCTGAAGTGAAGACCACCTACAAGGCGAAGAAGCCGGTGCAATTGCCGGGAGCTTACAATGTGAACATCAAACTTGACATTACCAGCCATAATGAAGATTATACGATTGTGGAACAGTACGAACGTGCAGAAGGCCGTCATAGCACAGGCGGAATGGACGAGTTGTA**TAAG***AGAGACC* 3’

#### TERMINATOR:

5’ *GGTCTCA****TAAG***AAAAAAAAACCCCGCCCCTGACAGGGCGGGGTTTT**TTTT***TGAGACC* 3’

### SYSTEM 4:

#### PROMOTER AND RIBOSOME BINDING SITE:

5’ *GGTCTCT****TTTT***GGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTAAAGAGGAG AAA***AGTA****AGAGACC* 3’

#### CODING REGION:

5’ *GGTCTCA***ATGA**ATAAATATTTCGAGCAGGTAAGGTTCTCATTAGGGCCGTCGTCAGTGTTTAA GATTGTTTTGGTACACCAGTGCAAAAAGCAAGCCTAGAAACCTATTGTGTCTGACTCATCTGTCTATTACCGATAGTCCATTTTCCTGACCCGGCAGCGGTTATCCGCATAAGACGACCATCACCAGTCGGTCAACGTGCCTTGGCAGAAGTTGTAACACCGCTTAGAACTTCAAATGGAACTATGGTAAAAAGACGAACAGGCGGTATTATATGTGTAATACTCTTAATTTCAATATAAGATTAAATACAGGTTCCTACAAAGGTAGGAGGAACTTTAGTTACGGAAAATTGAGTTACGCCAAGTGATTCCACAGCGGAAGTTTGAACTGGAGACGCGCGCAAAACATTCACGGTAGCAGGAACTTTTTCTACCATGCAAGGACTTGTATCGGGAGCCCGTAACGACTGAATTTCTTCAGTACGACGAAGTACACCAGACCCATCGCACGCTTCGTAACTTGTGGTACACTATTCCAGCAGTGGTTGCAGCCGGTTCCTTGACCGTCGAAAGGGCAGCATGTTTATTTAAAGTTGCAATTGAATGGCAAACACCGCAGGGGAAGCGGGAGGGGTGCTTGGCTCTTGAACACAGGTAAATGTAGAGGTAGATTAAGGTGGTCCTAACCCTGCTGTGGCCATTTGTTGAGAAGCGGGAATGAGTGGTA**TAA*T****AGAGACC* 3’

#### TERMINATOR:

5’ *GGTCTCA****TAAT***AAAAAAAAACCCCGCCCCTGACAGGGCGGGGTTTTTTTT***CCGC****TGAGACC* 3’

### SYSTEM 5:

#### PROMOTER AND RIBOSOME BINDING SITE:

5’ *GGTCTCC****C*CGC**TGTAATTTATTCAGCGTTTGTACATATCGTTACACGCTGAAACCAACCACTC ACGGAAGTCTGCCATTCCCAGGGATATAGTTATTTCAACGGCCCCGCAGTGGGGTTAAATGAAAAAACAAATTGAGGGTATGACAATTAAAGAGGAG**AAA*G****GGAGACC* 3’

#### CODING REGION:

5’ *GGTCTCA****AAAG***ATGGCTAGTCTGGTCAAGAAAGATATGTGTATTAAGATGACTATGGAGGGTA CCGTAAACGGTCATCACTTCAAGTGCGTCGGGGAAGGTGAGGGAAAGCCCTTTGAGGGCACTCAGGTGGAAAAGATCCGCATTACCGAGGGCGGGCCACTGCCATTCGCATATGACATTCTTGCCCCTTGCTGCATGTATGGCTCTAAAACTTTCATTAAGCACGTGAGTGGGATCCCTGATTATTTCAAGGAATCCTTCCCGGAAGGGTTTACATGGGAGCGCACGCAAATTTTCGAGGACGGTGGATACCTGACTATCCACCAAGATACCTCCTTACAGGGAAACAATTTCATCTTCAAGGTGAACGTAATTGGGGCTAACTTTCCGGCCAACGGGCCGGTCATGCAAAAGAAGACAGCGGGGTGGGAGCCATGCGTTGAAATGCTTTATCCCCGCGATGGGGTCTTATGTGGACAGTCTCTGATGGCCCTGAAGTGTACGGACGGTAATCATCTGACATCACACTTGCGCACCACCTACCGTTCGCGTAAGCCTAGCAATGCCGTGAATATGCCGGAGTTTCACTTTGGCGACCACCGCATTGAGATCCTGAAAGCTGAACAAGGCAAATTCTACGAACAATATGAGTCCGCAGTCGCACGCTACTGCGAGGCTGCGCCATCCAAATTAGGACACCATTG**ATAA***GGAGACC* 3’

#### TERMINATOR:

5’ *GGTCTCA****ATAA***AAAAAAAAACCCCGCCCCTGACAGGGCGGGGTTTTTTTT 3’

# 

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