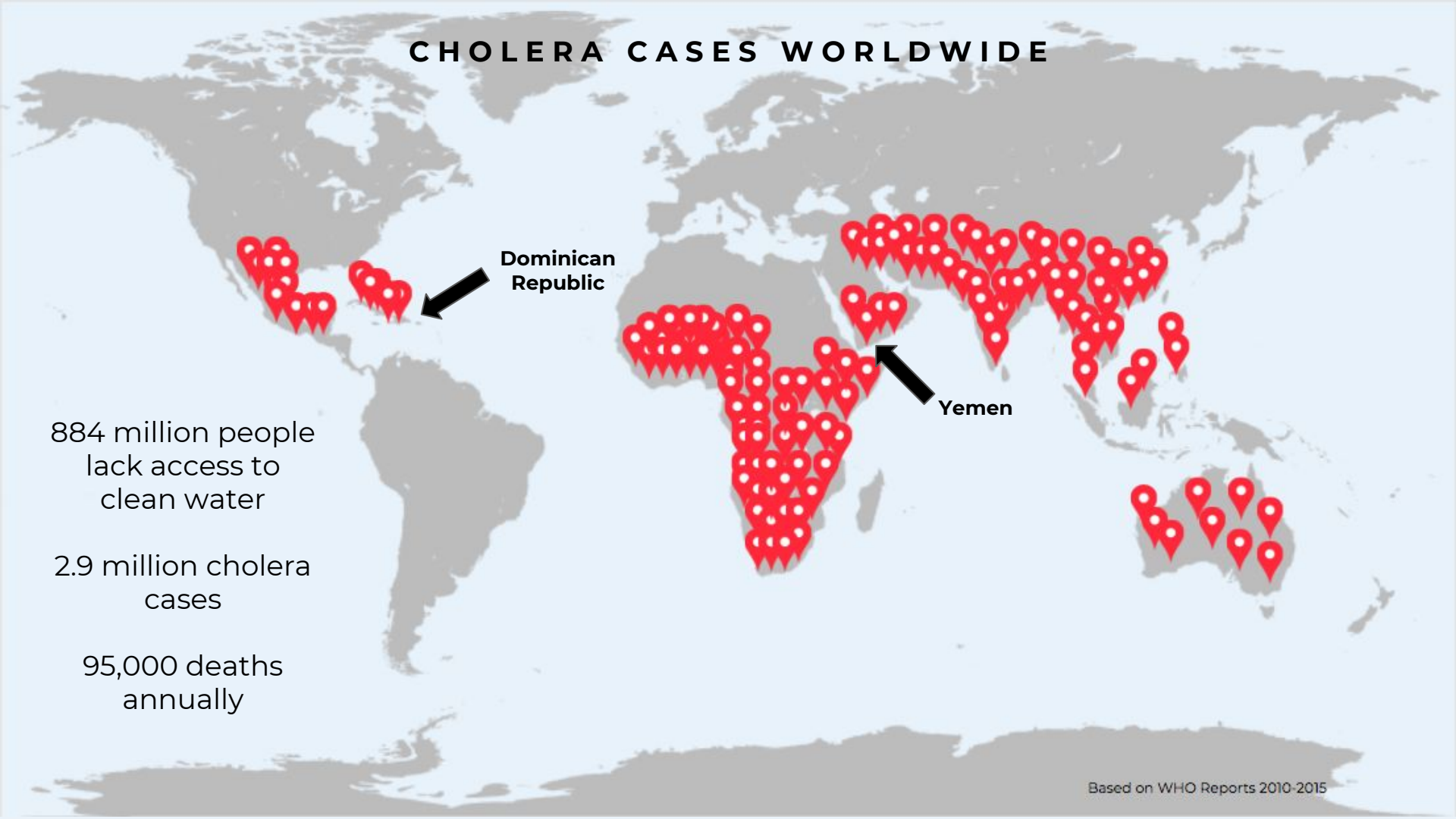
CAPTIVATE

Capture the Data | Activate the Response

2018 Lambert iGEM


w/w
winetowater
WARNING
AVISO
LANCO

CHOLERA CASES WORLDWIDE



884 million people lack access to clean water

2.9 million cholera cases

95,000 deaths annually


Claro

OUR SOLUTION

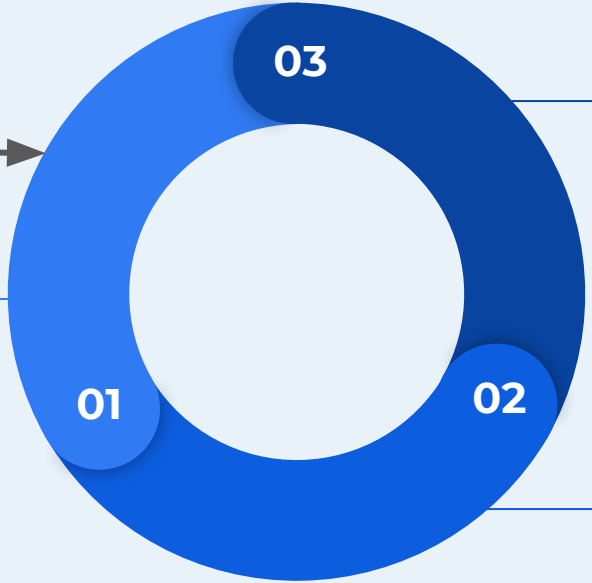
METHODOLOGY

Predict

Use CALM to predict cholera outbreaks and deploy kits

Detect

- Toehold Switch Biosensor Cells
- Infield electroporation using ElectroPen™

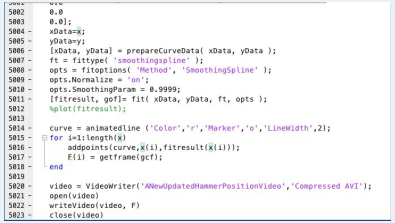


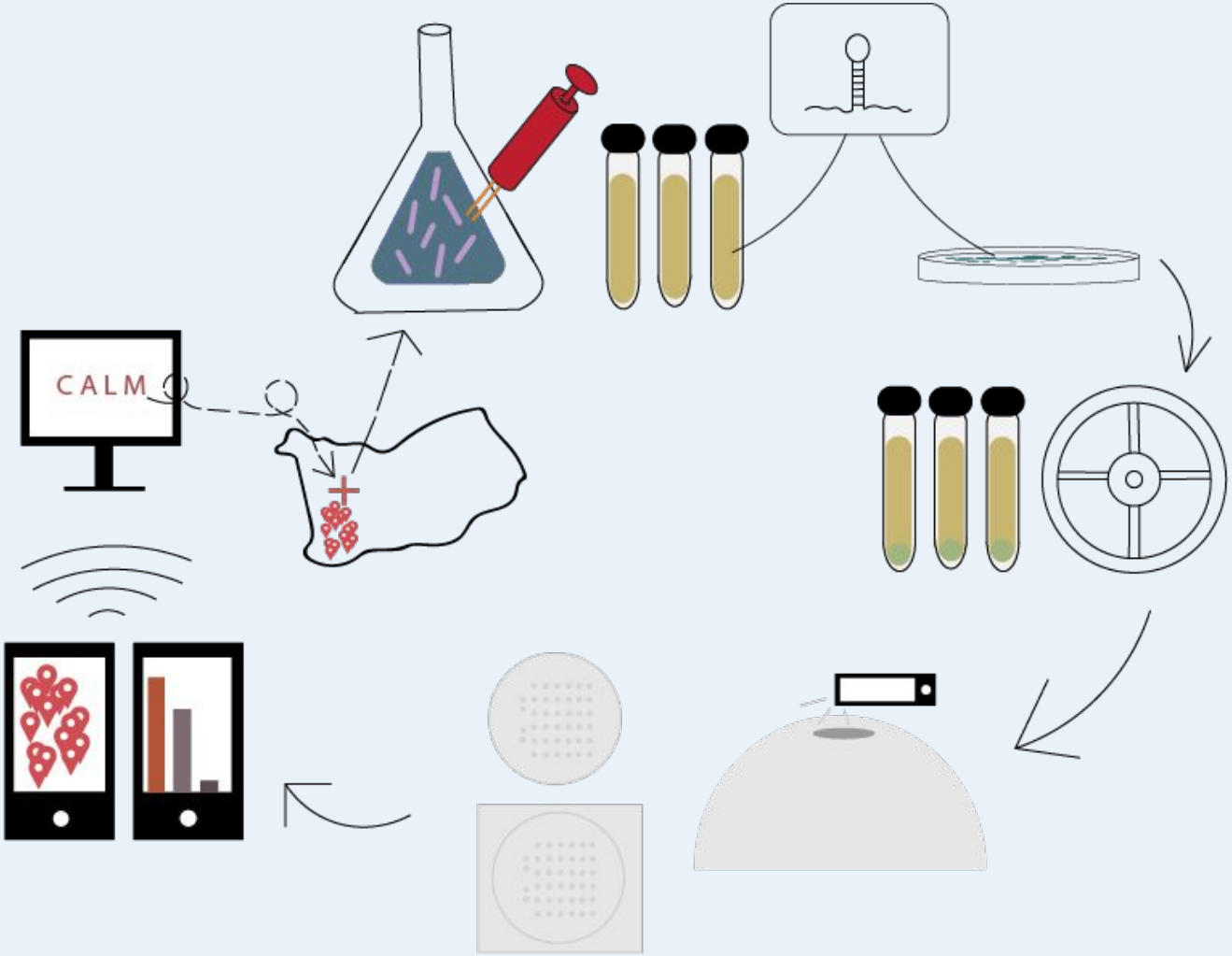
Distribute

- Inform healthcare organizations to mount responses
- Educate the public about the global water crisis

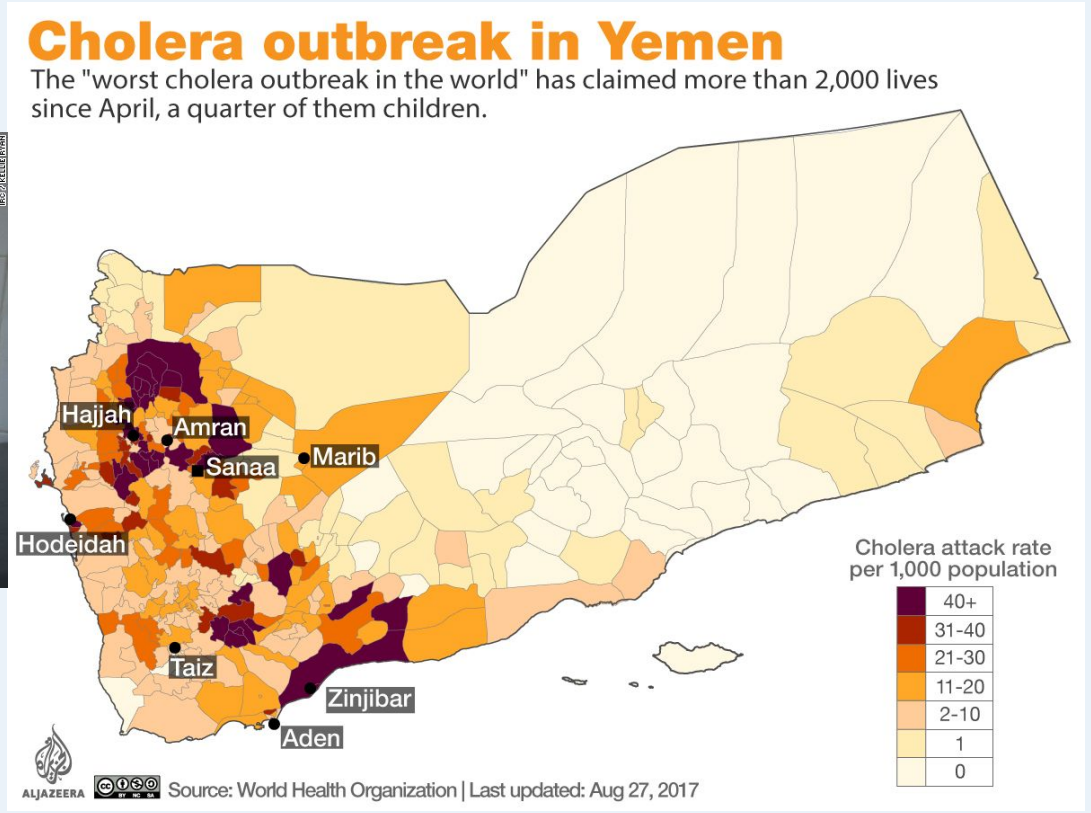
Analyze

- Capture image of samples in Chrome-Q chamber
- Sample analysis using Color Q App



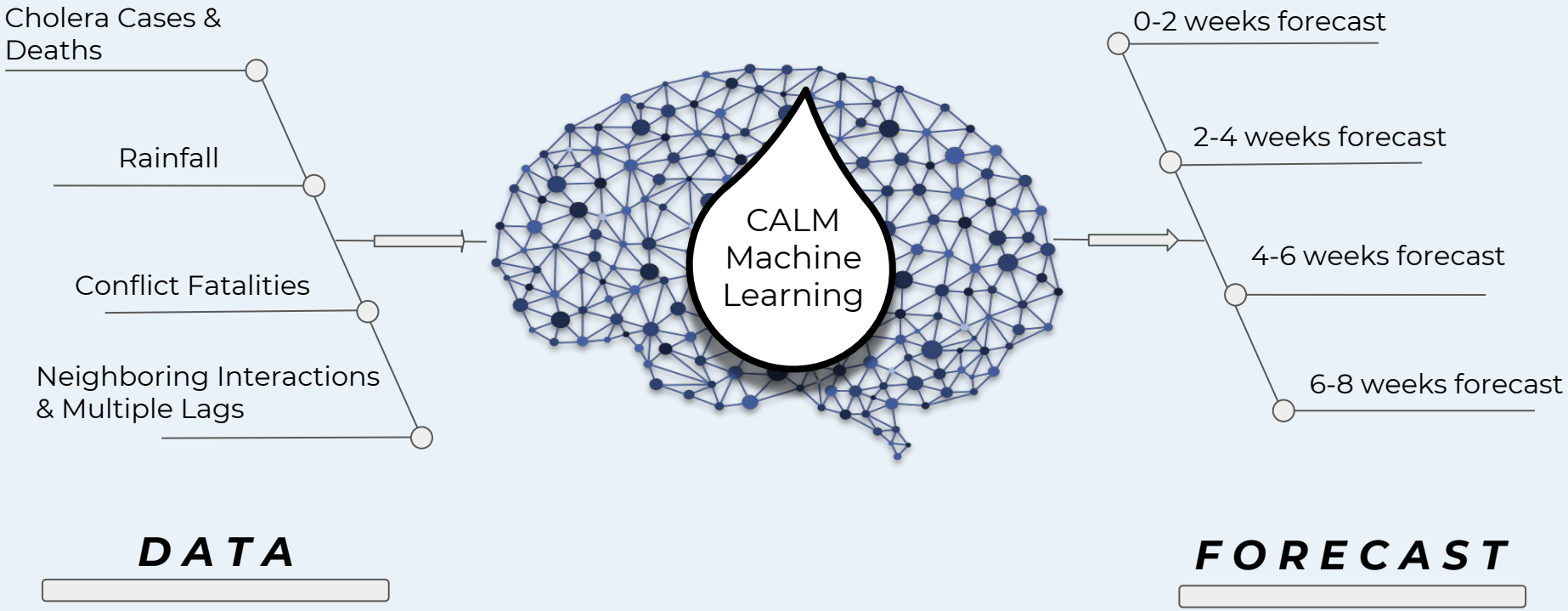

CALM

Yemen - one of the worst outbreaks in history



Map of cholera outbreak in Yemen in 2017 (Al Jazeera, 2017).

Cholera **A**rtificial **L**earning **M**odel



Paper pending

Other Models	CALM
Simpler Regression Tactics Inability to learn complex, linear relationships	**Extreme Gradient Boosting** Robust machine learning approach; able to learn complex, nonlinear relationships
Risk / Cumulative Case Prediction	**Forecast of the exact number of cases across four time ranges**
Use of Individual Datasets Only environmental factors, or only epidemiological factors	**Inclusion of Entire Span of Datasets** **Civil War Conflict Data (major nonseasonal influence)**, cholera incidence/mortality, & rainfall
Minimal Feature Engineering Simple/single mathematical representations of data	**Exhaustive Feature Engineering** Extracted ~45000 potential features and selected most useful few. Overall removed 99.9% of features to arrive at ~ best 50

Paper pending

RESULTS

Cross-validation and Holdout Error for four XGBoost forecasting models

Forecast Range	Cross-Validation Root Mean Error (new cases / 10000 people)	Hold-Out Set Root Mean Error (new cases / 10000 people)
0 - 2 weeks	6.867	3.921
2 - 4 weeks	5.765	4.034
4 - 6 weeks	4.123	4.737
6 - 8 weeks	3.904	4.607

Mean of Cases:

~19.148

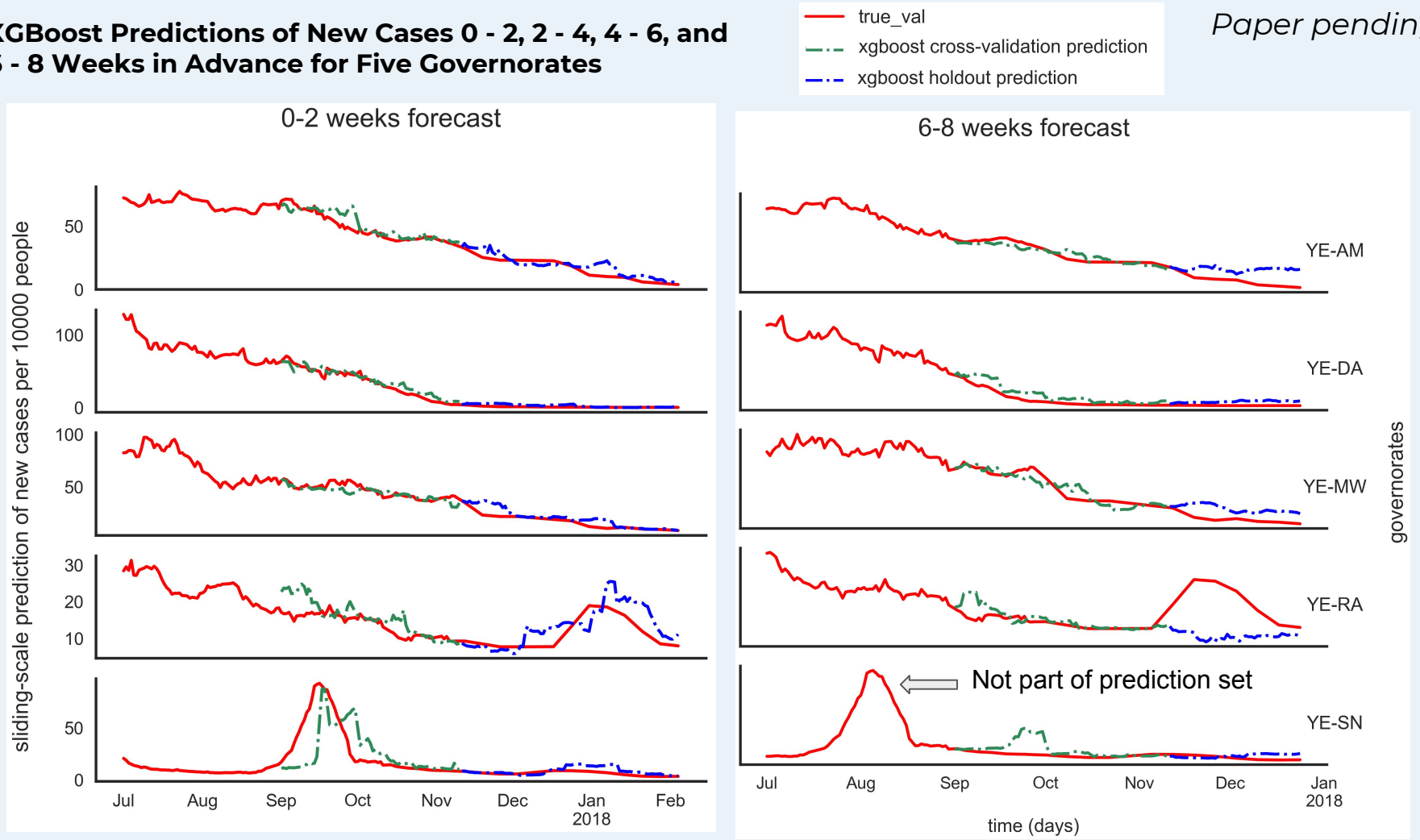
Standard Deviation of Cases:

~21.311

Paper pending

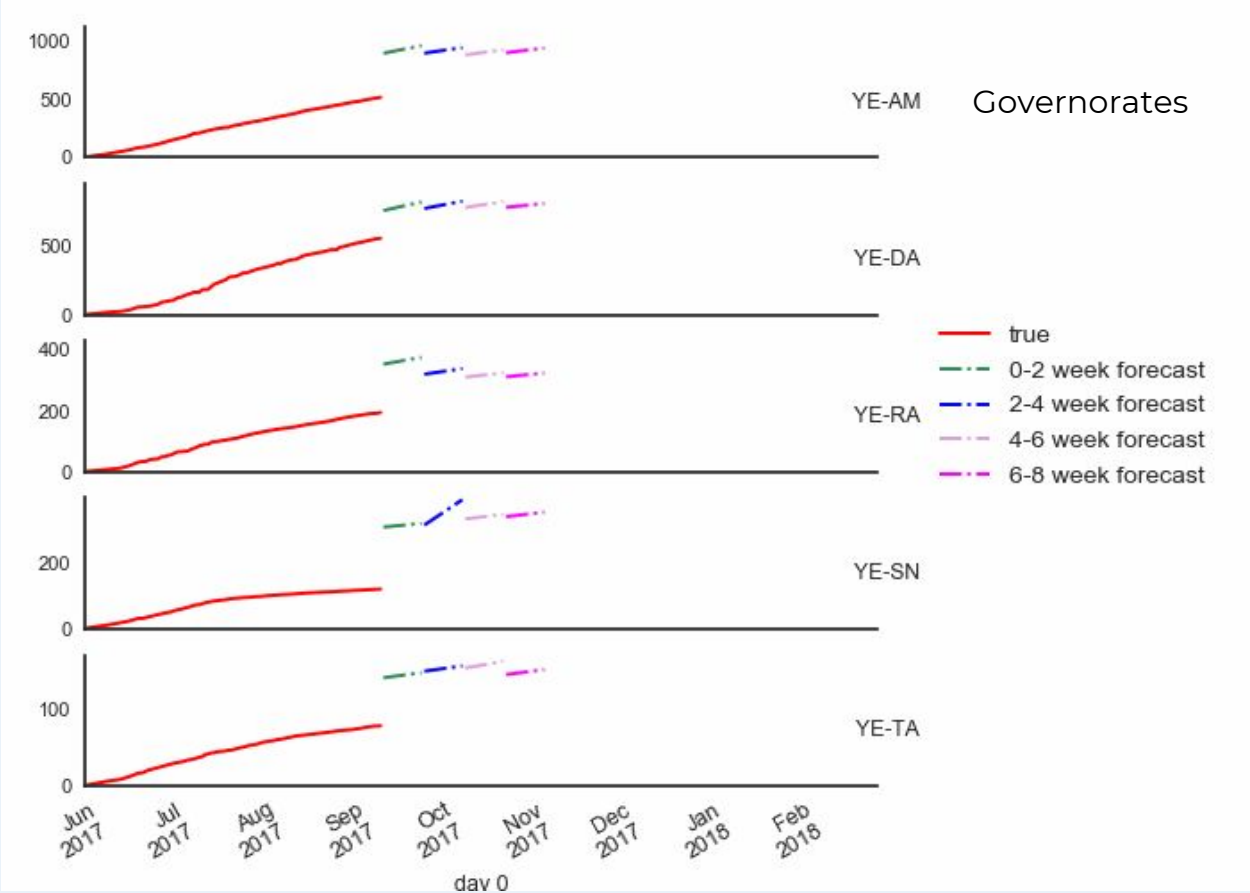
XGBoost Predictions of New Cases 0 - 2, 2 - 4, 4 - 6, and 6 - 8 Weeks in Advance for Five Governorates

Paper pending



Animation of our forecast vs cumulative cholera cases across representative sample of 5 governorates

Cumulative cholera cases
(normalized by population)



Time (days)

Paper pending

Yemen - one of the worst outbreaks in history

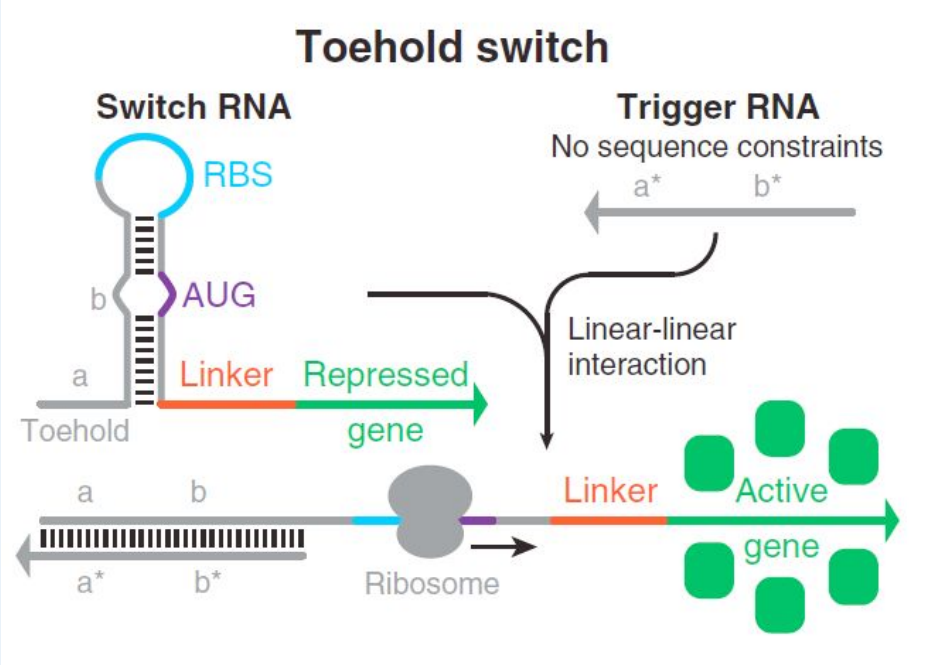
Souce - Al Jazeera

DETECTION:WET LAB

SPIRA SWAB

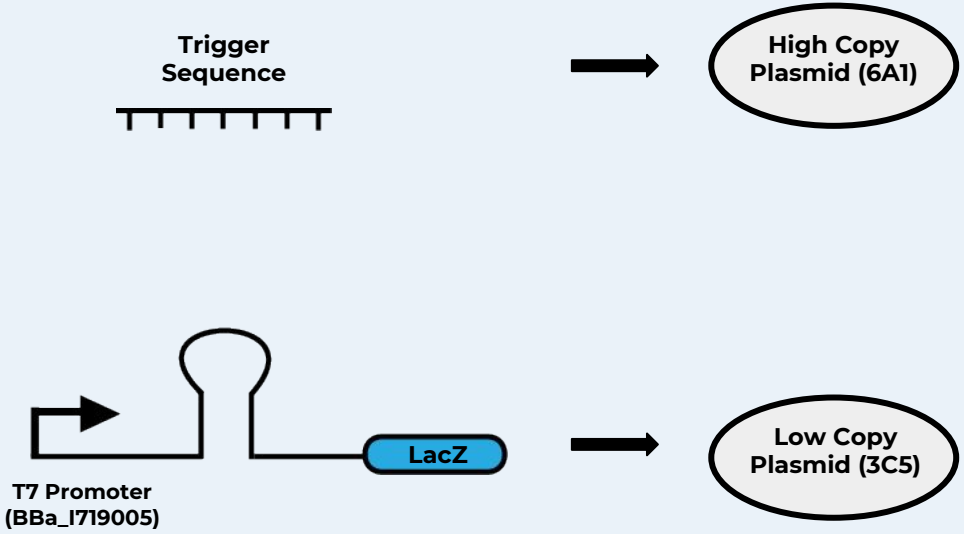
The filtration system used to isolate Cholera for our detection method.

TOEHOLD SWITCHES



Source: Alexander A. Green, Pamela A. Silver, James J. Collins, Peng Yin, Toehold Switches: De-Novo-Designed Regulators of Gene

PROOF OF CONCEPT



- Toehold Design from Dr. James Collins
- T7 Toehold LacZ
    - T7: Strong Constitutive Promoter
        - BBa_I719005
    - LacZ: Encodes Beta-galactosidase
        - BBa_I732005
- Performed Dual Plasmid Transformation in BL21 T7
    1. Dual Plasmid
    2. Single Switch
- 7 New Biobricks sent to iGEM HQ
    - Validated Part: BBa_K2550001

RESULTS

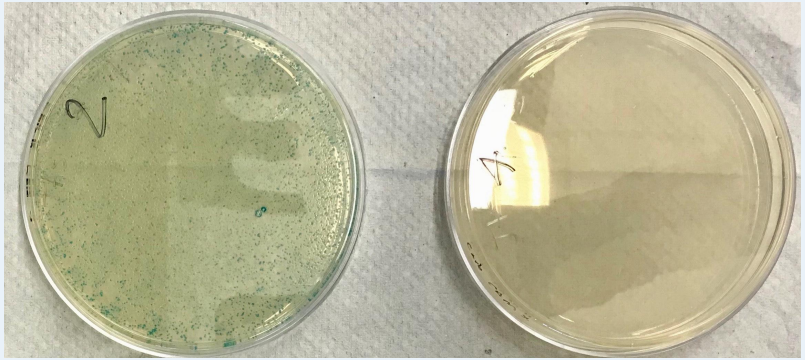


Figure 1. (Left) Dual Plasmid Transformation of LacZ Toehold Switch & Trigger. (Right) Single Transformation of LacZ Toehold Switch in BL21 E. coli.

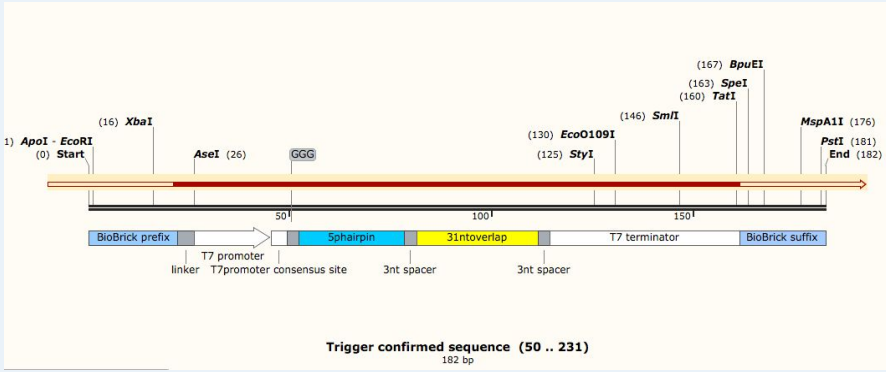
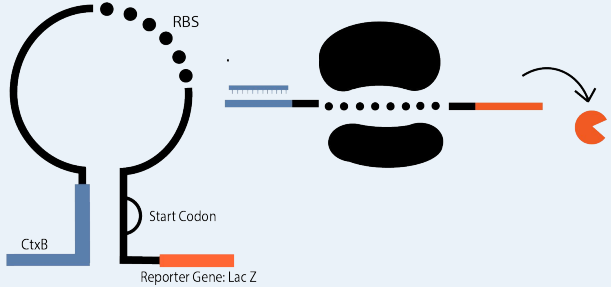


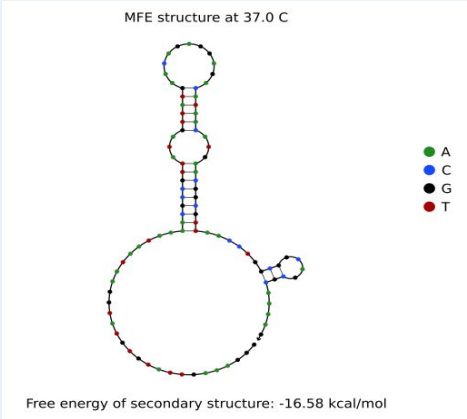
Figure 2. Sequencing results of the trigger sequence confirmed the part was correct.

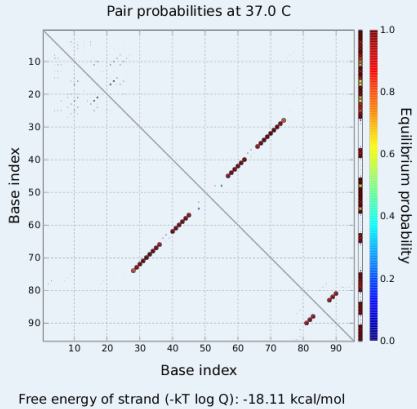
- **Validated Part (BBa_K2550001)**: T7 promoter with a trigger sequence to match the toehold sequence of part BBa_K2550000

CHOLERA SWITCH

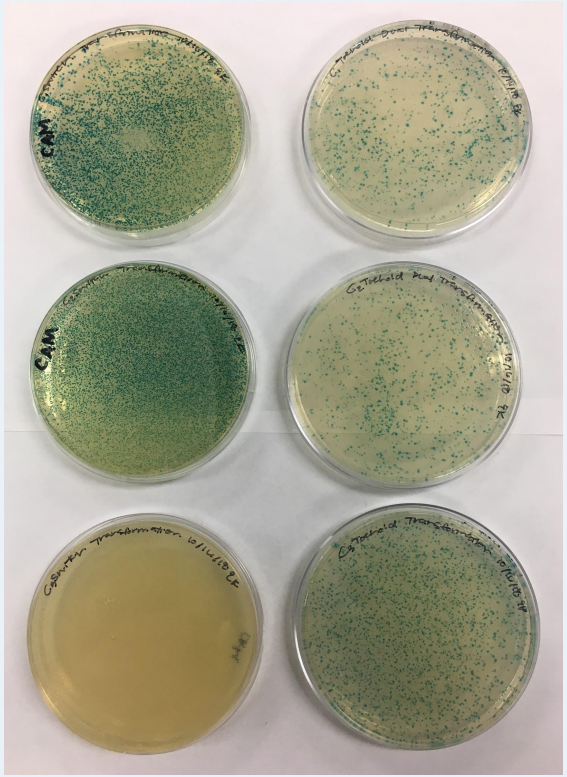


- Gene: ctxB (Cholera enterotoxin subunit B)
- Design Software from Chinese University of Hong Kong iGEM
- Nupack Software
  - Analyze Nucleic Acid structures
  - Thermodynamic Favorability
- Currently testing 3 Cholera Switches
  - Perform dual plasmid transformation
  - Submit 5 biobricks
- Focus on Sensitivity
  - Sensitivity= true positives/(true positives + false negatives) x 100
  - Specificity=true negatives/(true negatives + false positives) x 100



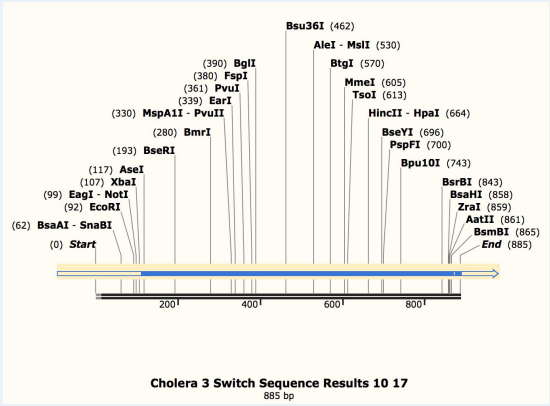


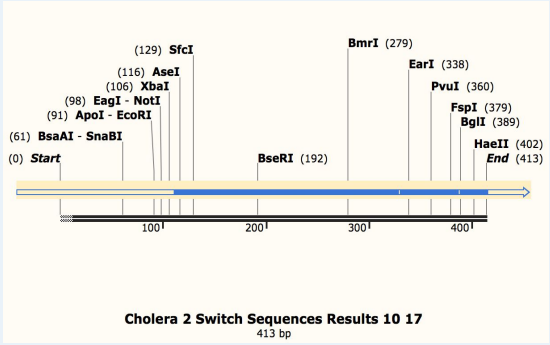
RESULTS



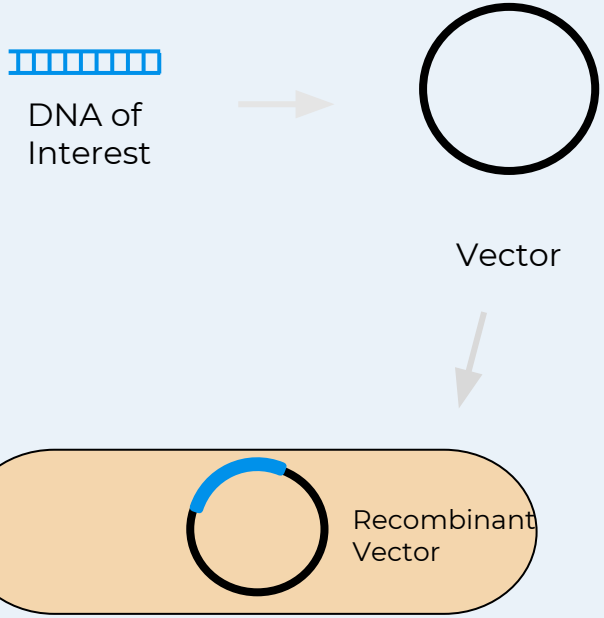
- (Left) Transformations of Toehold Switch presents leakiness. Dual Plasmid expressed lower blue expression in comparison to the single switch transformations.

- Sequencing results show that the Cholera switches did not contain the correct sequences.





ELECTROPORATION



Benefits:

- Exponentially more efficient, producing up to 10^10 cfu/ug
- Quick and easier to perform than chemical method
- Requires less resources for preparation of competent cells

Bottlenecks:

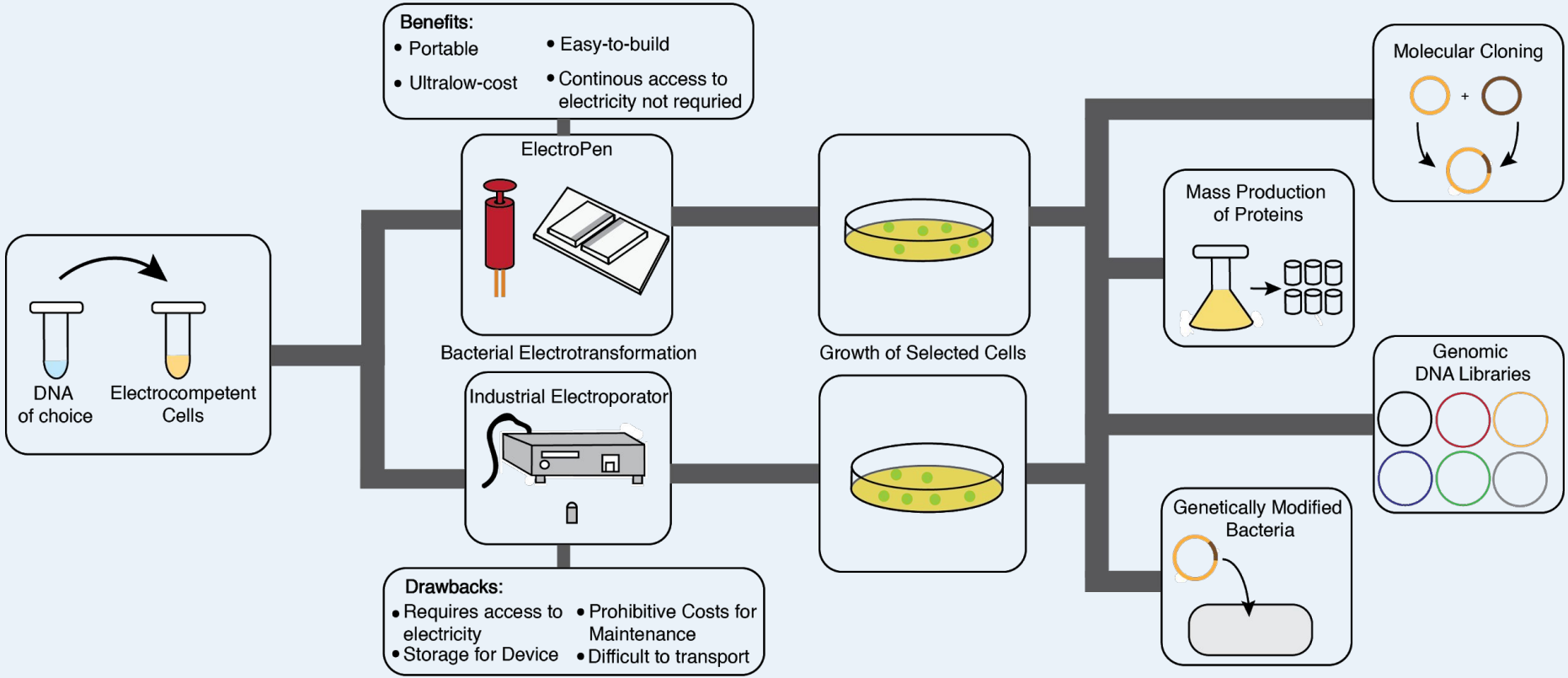
- Equipment is exceedingly expensive and out of reach for many research groups
- Requires access to electricity
- Difficult to transport

Patent and Publication Pending

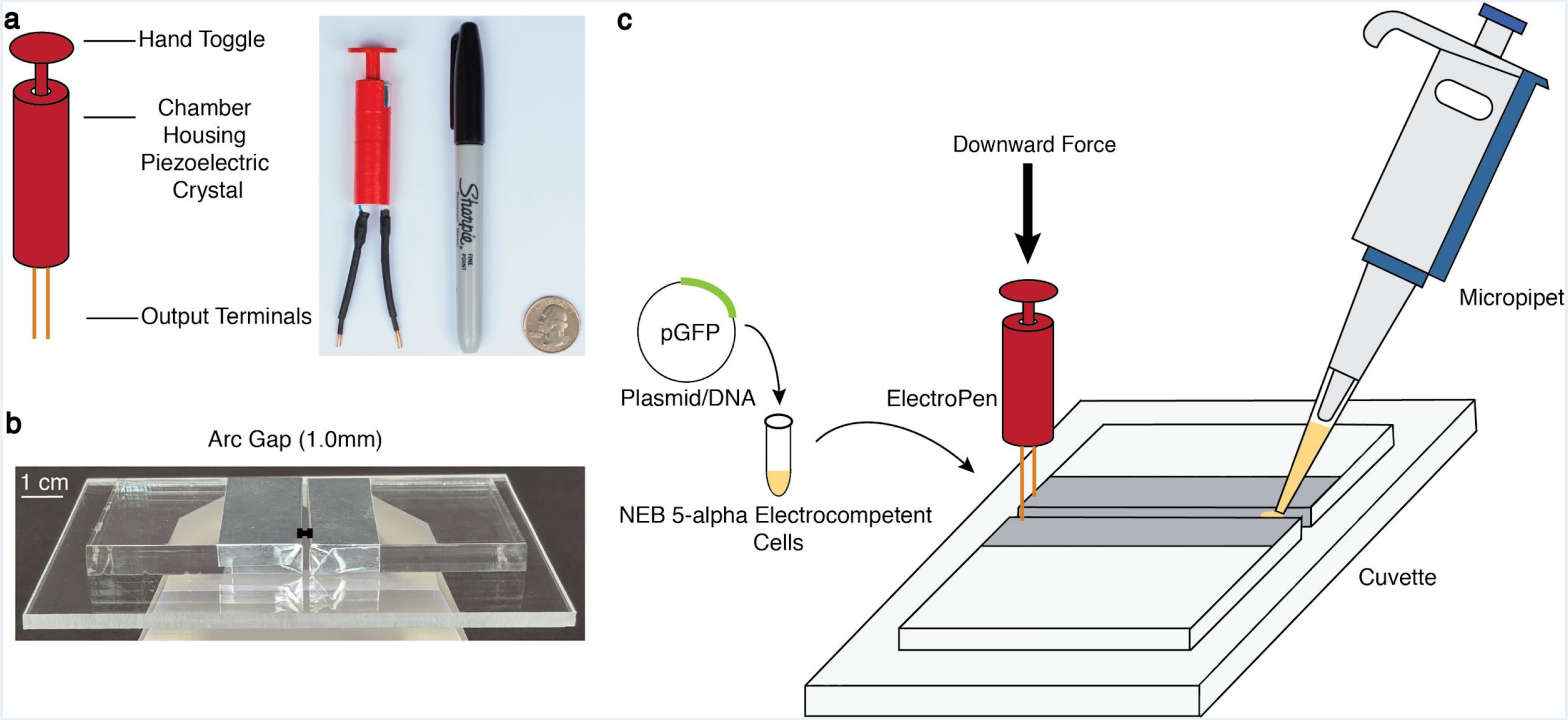
THE ELECTROPEN™

AN ULTRA-LOW COST PORTABLE ELECTROPORATOR

COMPARISON OF ELECTROPORATORS AND ELECTROPEN



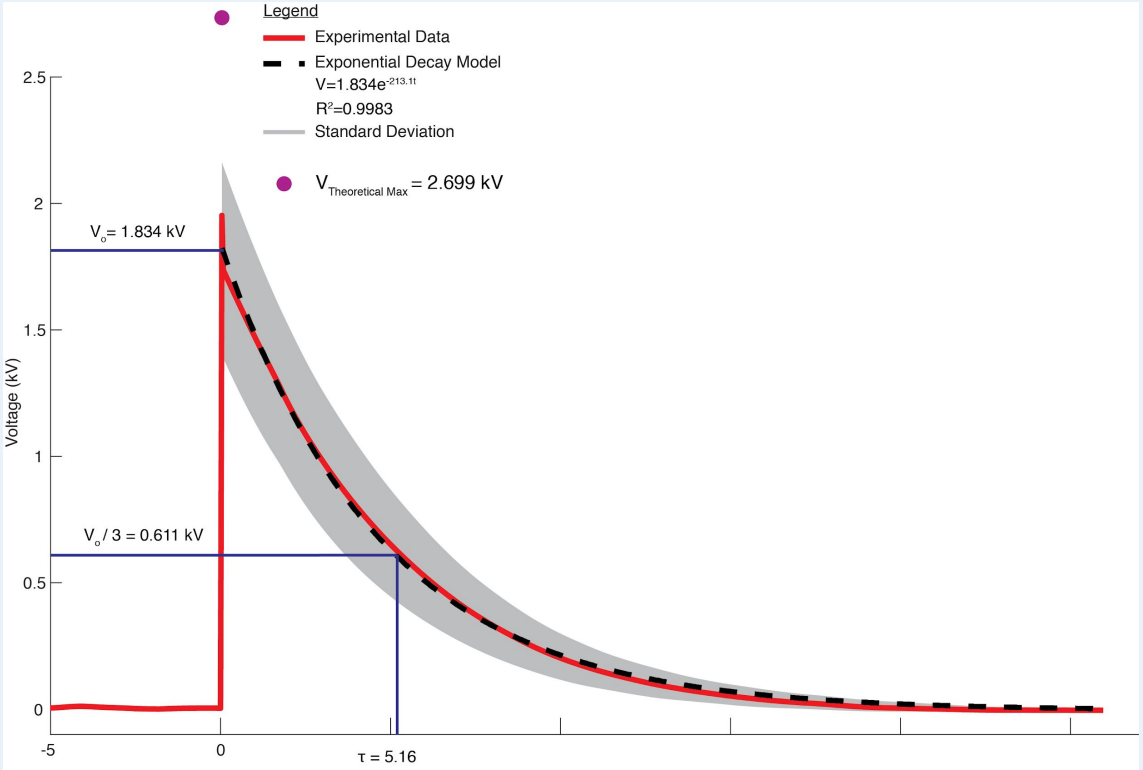
DESIGN LANDSCAPE OF THE ELECTROPEN™



a Structure of the ElectroPen device **b** Design of the custom fabricated cuvette **c** Illustration of overall workflow for using the ElectroPen

EXPONENTIALLY DECAYING WAVEFORM MODEL

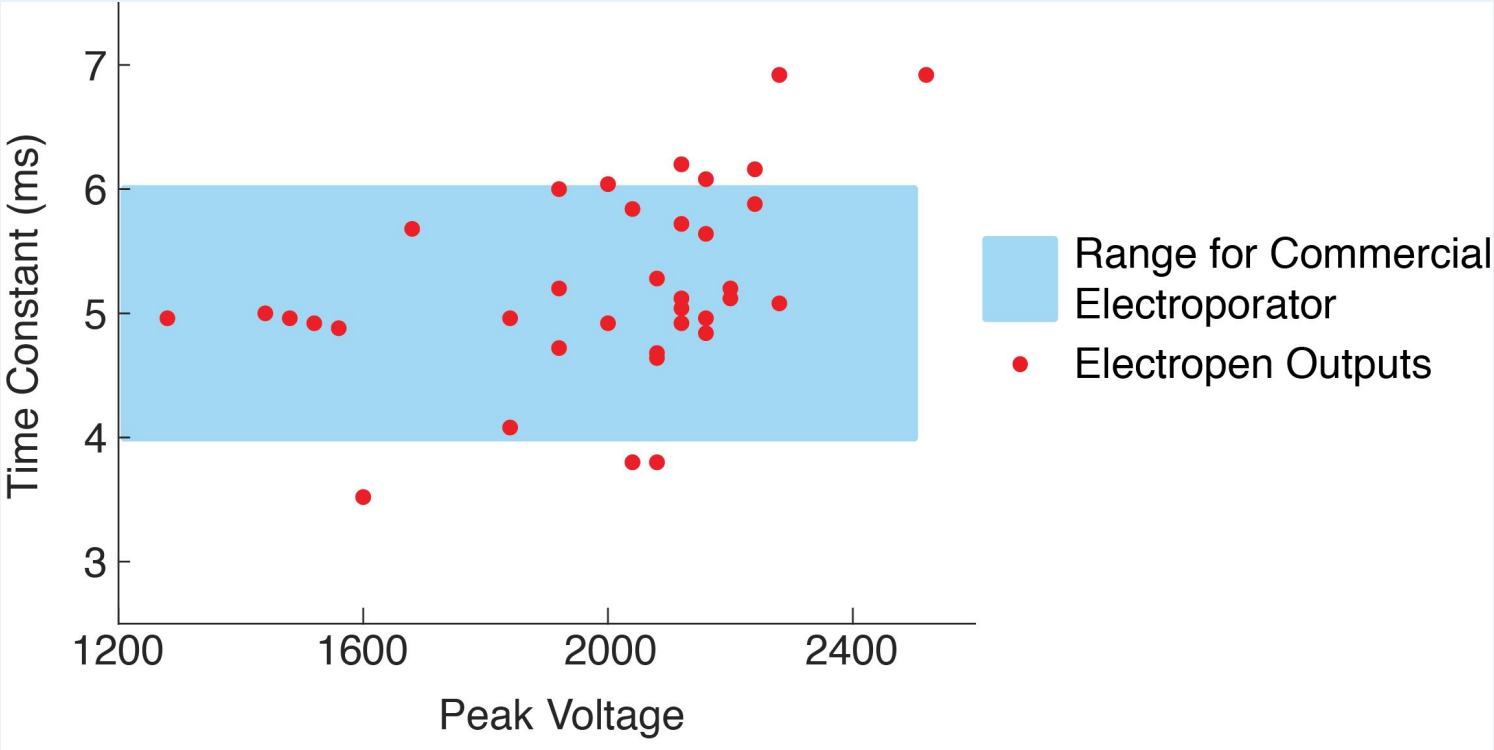
High-voltage pulses generated by the ElectroPen. Exponentially decaying pulses are optimized for electroporation of *E. coli*.



The ElectroPen achieves an average peak of 1.834kV and average time constant of 5.16ms.

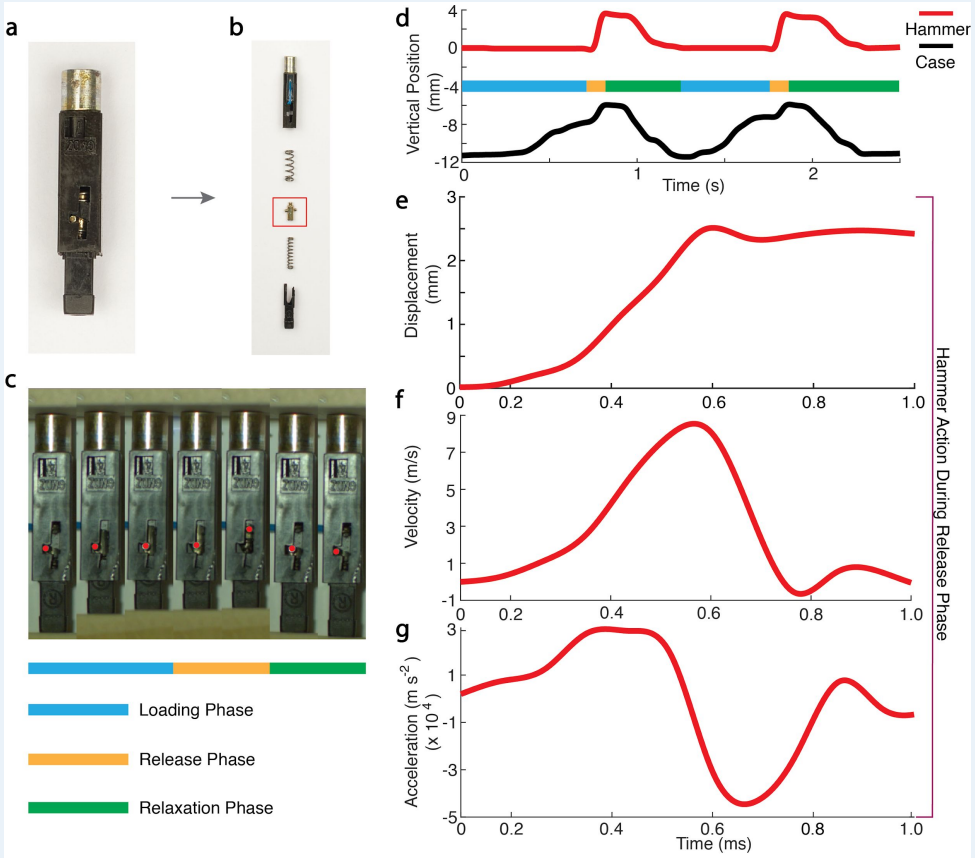
A critical component of electroporation is the waveform, often being exponentially decaying or square in nature. The ElectroPen utilizes the former.

CONSISTENCY OF HIGH-VOLTAGE OUTPUTS



The ElectroPen functions within the optimized parameters for E. coli and those produced by commercial electroporators. Values outside the range are still capable of allowing successful electroporation, although with less efficiency.

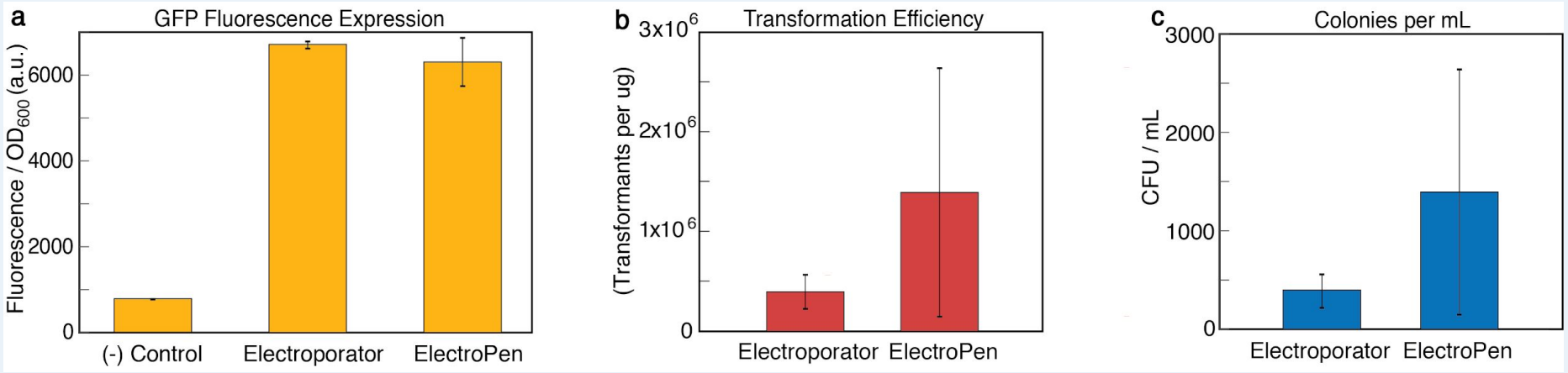
PIEZOELECTRIC THEORETICAL MODEL



a-b Parts of the hammer action found in the ElectroPen containing the lead zirconate titanate (PZT) piezoelectric crystal.
c Phases of hammer action as ElectroPen is pressed during electroporation, described as the loading phase (spring compression), release phase (spring extension projecting hammer onto crystal), and relaxation phase (return to origin).
d Movement of hammer and casing during electroporation
e-g Hammer achieves a peak acceleration of 30,000 m/s^2, producing a 10N force

Through this and piezoelectric theoretical modeling, we determined a theoretical maximum voltage of 2.699 kV, with expected resistance from our system's wires and casing, confirming our measured outputs.

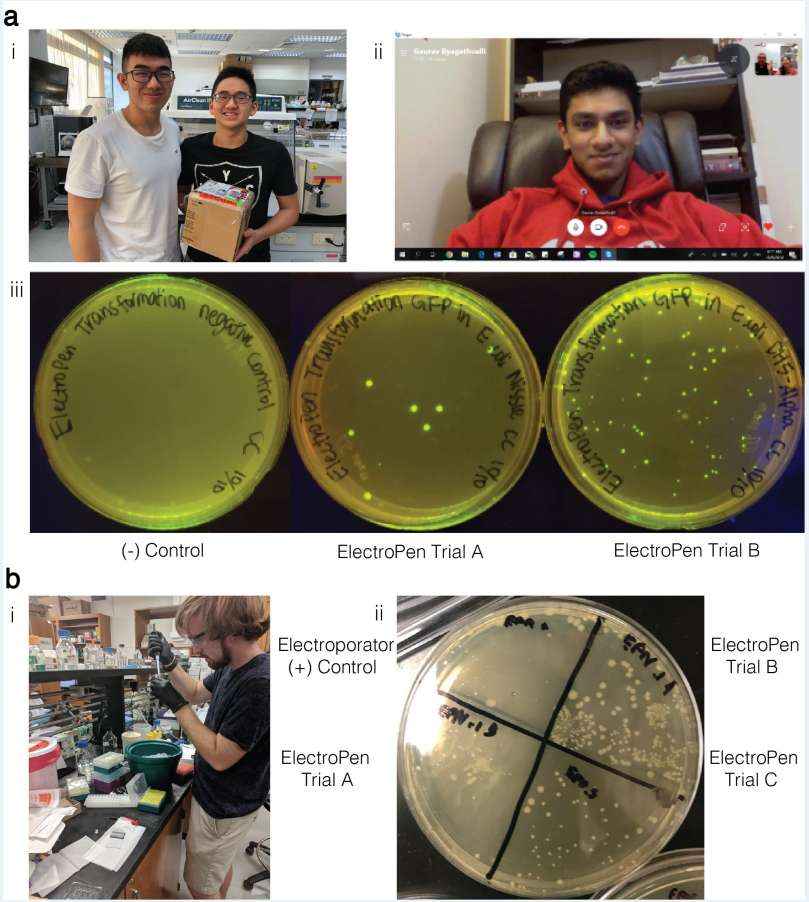
RESULTS FROM EXPERIMENTAL TRIALS



a Fluorescence expression from GFP trials conducted with each device **b-c** Efficiency of the device is comparable to commercial electroporators.
Error Bars = S.D., n=3.

Note: S.D. includes one trial where the ElectroPen was 5.5x more efficient that the standard electroporator

COLLABORATION STUDY WITH iGEM TEAMS



a Collaboration study with TAS Taipei iGEM where they tested the ElectroPen on DH5a and Nissle 1917 *E. coli* and validated the ElectroPen's functionality.

b Collaboration study with UGA iGEM, where 3 different trials validated previously obtained results, and even indicated higher efficiencies than the electroporator which we will examine in future studies.

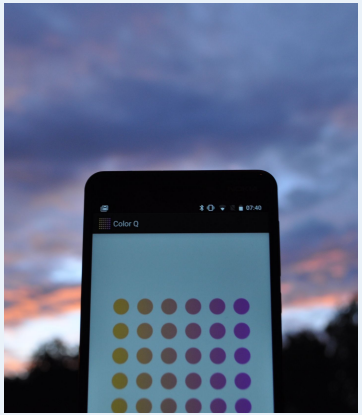
Through theoretical modeling and experimental evaluation, we have demonstrated that the 23-cent ElectroPen™ serves as a complete alternative for industrial electroporators

CHROME Q SYSTEM

- With the inaccessibility of plate readers in field biology, schools, and underfunded research groups, the Chrome Q represents a novel solution for reporter quantification.
- Based on RGB Color Theory, the Chrome Q represents values based on the hue and saturation of the particular color.
- Advantages:
  - Inexpensive (Costs $10)
  - Portable (Weighs 100g)
  - Operated by a battery
- Currently demonstrated to work with:
  - Chromoproteins: TsPurple, Scrooge Orange, Virginia Violet
  - Fluorescent Proteins: RFP
  - Other reporters: LacZ

Electroporate into Biosensor Cells → Capture Reporter Expression in Standardized System →

COLOR Q



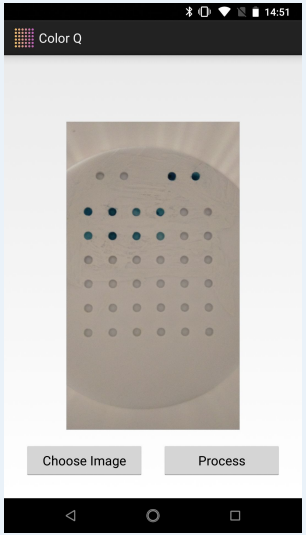
- With the prevalence of smartphones in developing nations, they act as an effective medium for capturing results from the diagnostic tests.
- With no need for a Wi-Fi connection for data interpretation, data can be stored on-the-go to allow for successive tests in different areas.
- Location data can also be stored to track where cases occur, and once WiFi is available, can be uploaded to a server to drive predictions and supply resources.

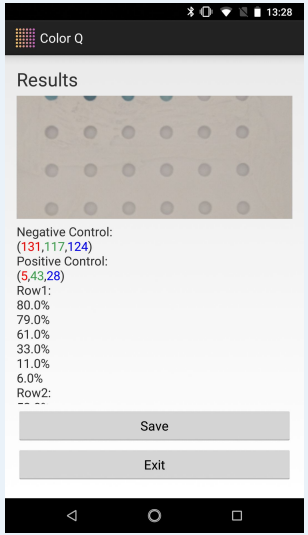
Electroporate into Biosensor Cells

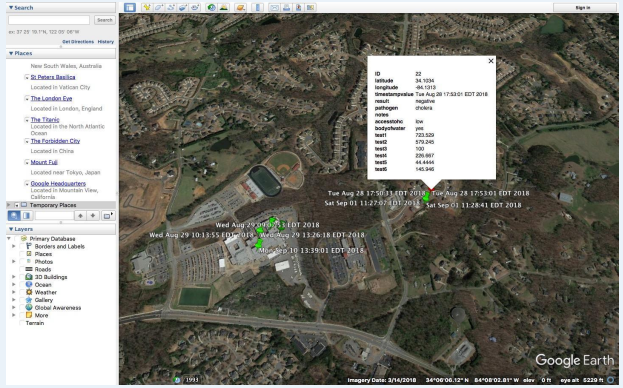
Capture Reporter Expression in Standardized System

Analyze Data from the Color Expression

HOW IT WORKS







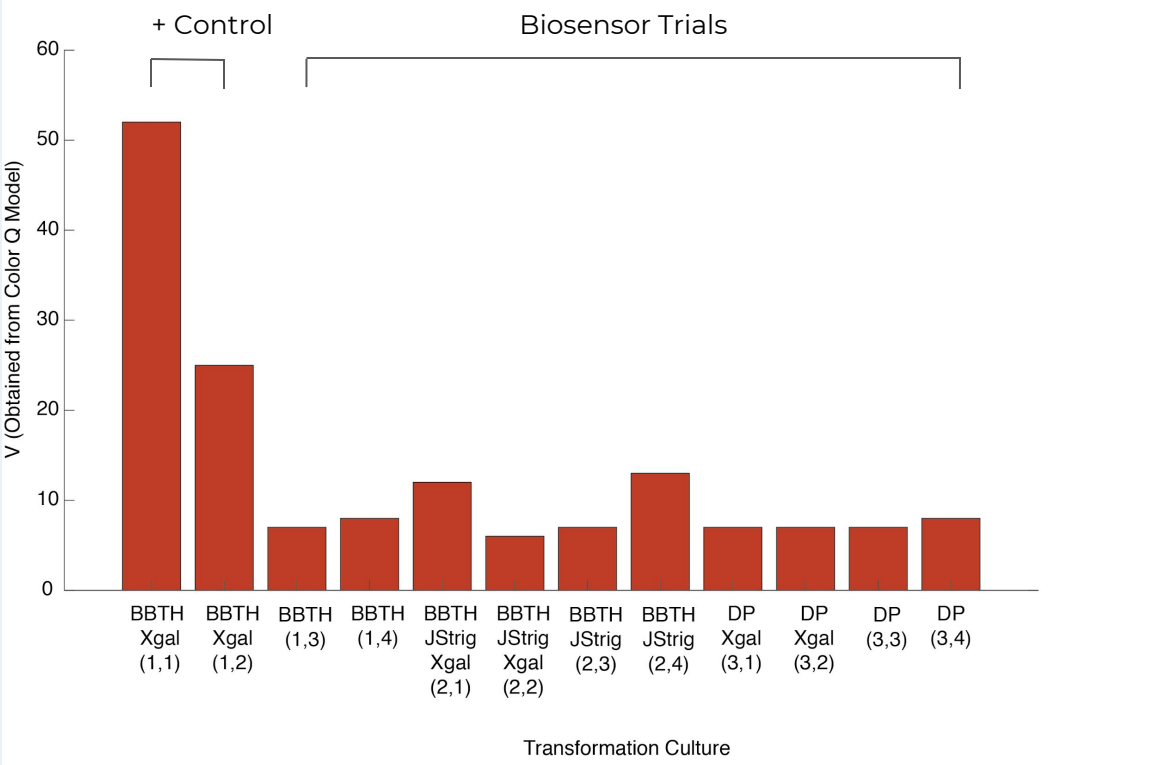
- Quantify color expression from reporters on the RGB scale to distinguish between positive and negative tests
- Combine location data to provide specific coordinates for these tests
- Distribute information to organizations to mount responses in areas potentially at-risk for a cholera outbreak

$$V = \frac{\sqrt{(R_e - R_n)^2 + (G_e - G_n)^2 + (B_e - B_n)^2}}{D}$$

Mathematical model for calculation of relative percentage values to distinguish between positive and negative tests

REPORTER EXPRESSION RESULTS

In comparison with the controls, toehold-induced LacZ expression can be easily distinguished from background expression



+ Control indicates expression of just the toehold switch

Biosensor trials refers to toehold+trigger combinations for activation of reporter expression

First two trials are negative and positive controls respectively, with remaining as experimental toehold biosensors. As can be seen, there is a clear distinction between control and positive biosensor values.

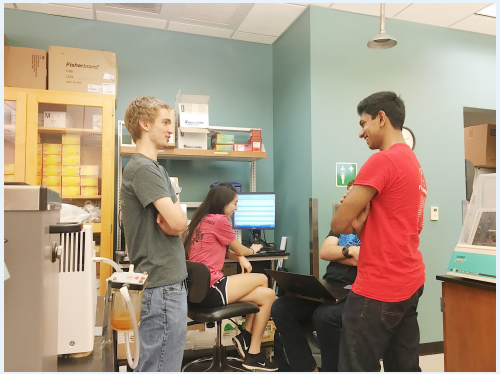
COLLABORATIONS & HUMAN PRACTICES

COLLABORATIONS

Atlanta Science Festival

GSU American Sign Language Camp

Synthetic Biology Summer Camp



Interlab Study at UGA

Georgia iGEM Meetup at Emory

TAS Taipei ElectroPen

HUMAN PRACTICES

Dominican Republic

Thirst Project

Summer Camp & Doll Kit

INTEGRATED HUMAN PRACTICES

Georgia Tech University Meeting

Dr. Rainyer from the Ministry of Health

State-wide Competition

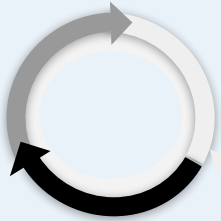
Girl Scouts Doll Kit Surveys

Presentation at Boehringer Ingelheim

Boehringer Ingelheim

Dr. Barr from Emory University

LEAN Methodology



UGA & TAS Taipei

ATTRIBUTIONS & ACKNOWLEDGEMENTS

FUTURE WORK

NEW DISEASES IN DIFFERENT COUNTRIES

This year, we focused on Yemen and Cholera, but in the future, we plan to detect other pathogens in different countries around the world.

CELL FREE

We plan to make the entire detection process cell-free, eliminating the need for sensitive materials such as competent cells.

DOMINICAN REPUBLIC

Next year's Lambert iGEM team is creating a water detection kit to distribute to the children living in the community. Using these kits, they will be able to detect what is in their water.

NON-PROFITS DISTRIBUTE PORTABLE KITS

In the future, we plan to distribute our portable detection kits to various non-profit organizations such as the Thirst Project.

CONCLUSION

- Bronze
  - Completed all requirements, including submitting InterLab study data
- Silver
  - Validated Part
  - Human Practices
  - Collaboration

CAPTIVATE

- Gold
  - Integrated Human Practices
  - Modeling
  - Demonstration of Work
- Acknowledgments: Special thanks to Georgia Tech, Emory University, & Boehringer Ingelheim

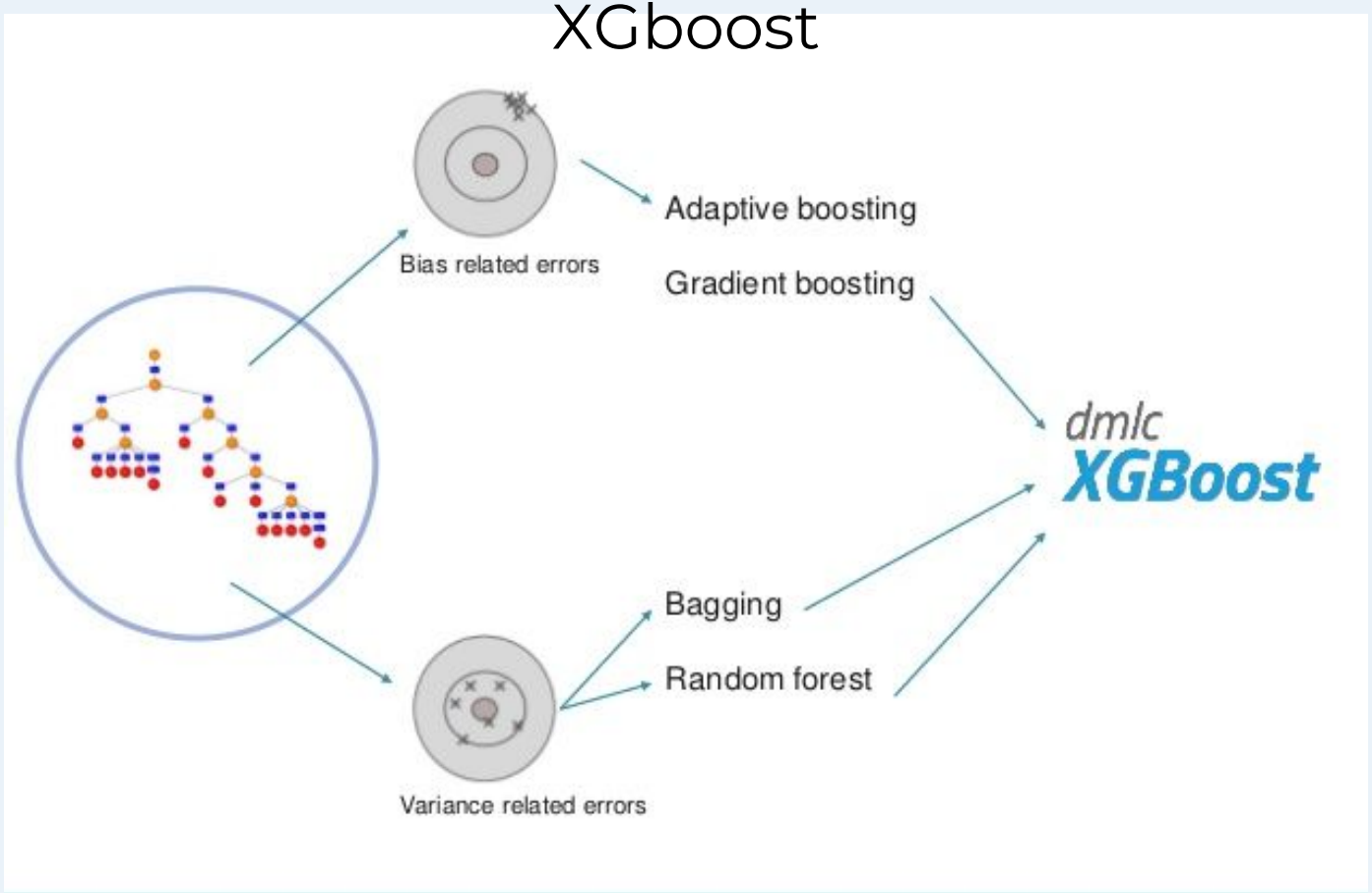

IBERIA

Q & A

REFERENCES

EXTRA SLIDES

eXtreme Gradient Boosting: XGboost



PROCESS

Feature Extraction

Rolled the time-series with varying time delays and calculated neighbor timeseries

Extracted **~45,000** potentially relevant features

Feature Selection

1. scalable hypothesis tests to identify ~10k relevant features

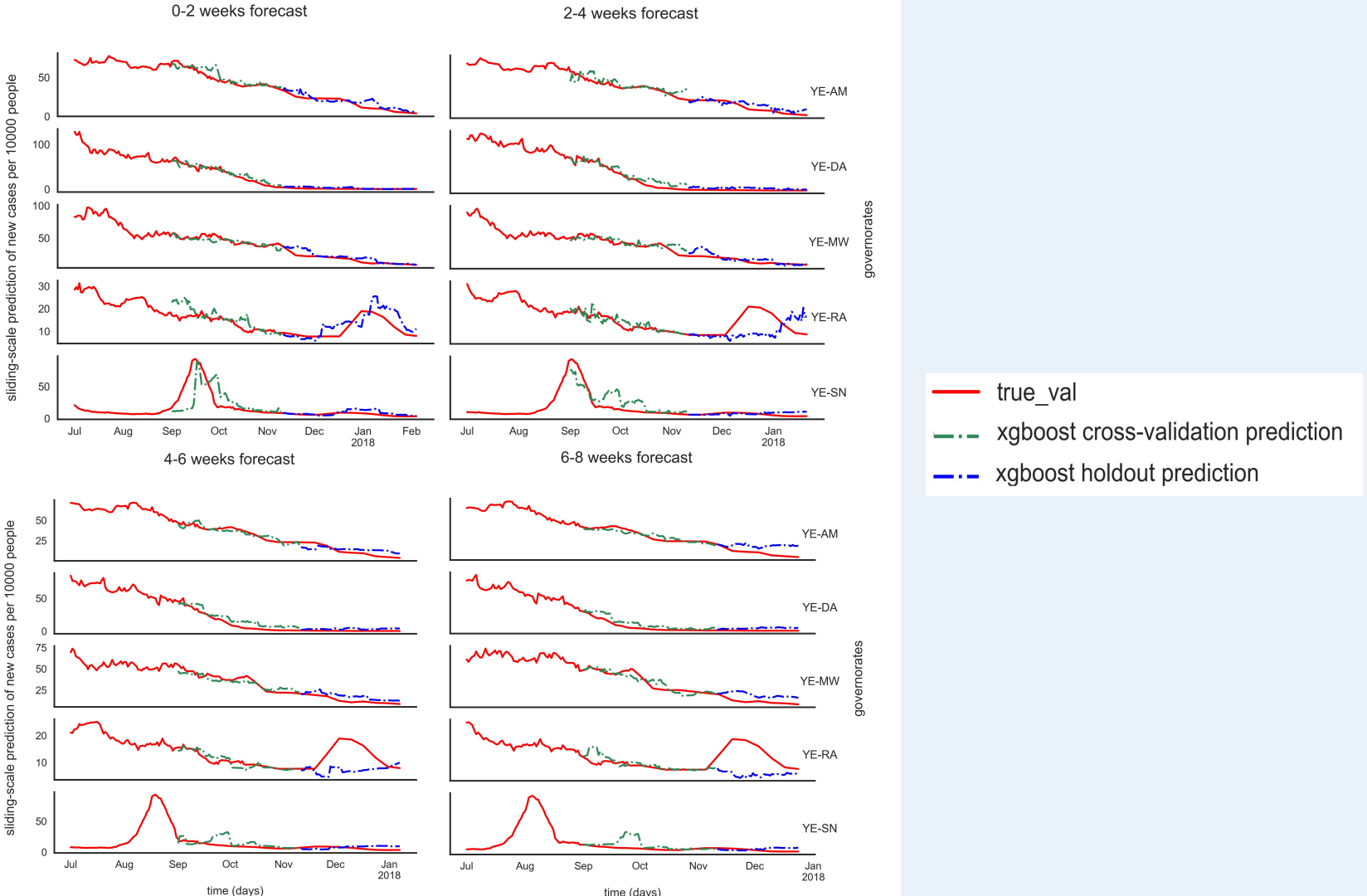
2. removal of collinear features

3. ranking of features through XGBoost & recursive feature addition

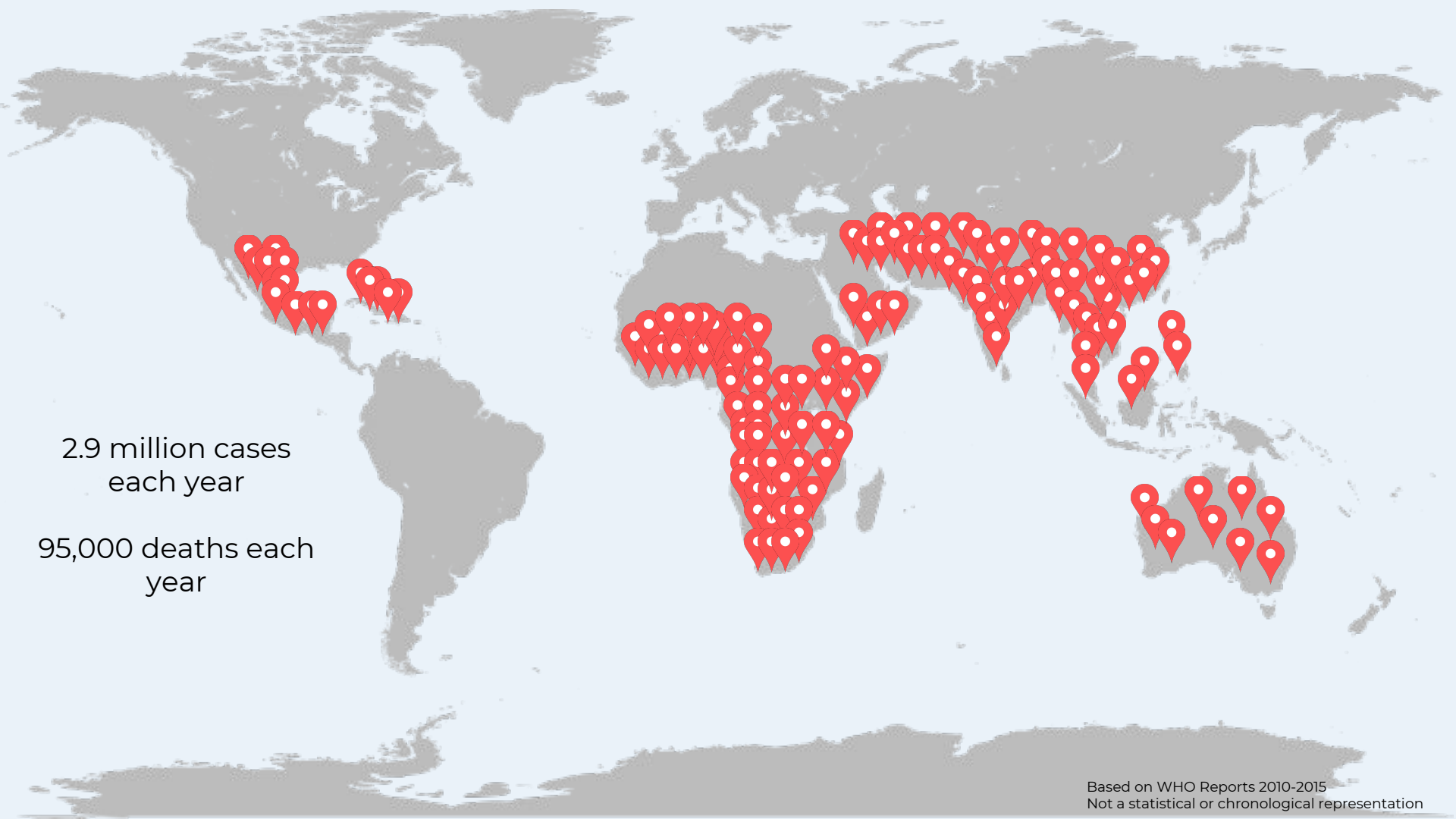
Narrowed down features **99.9%**

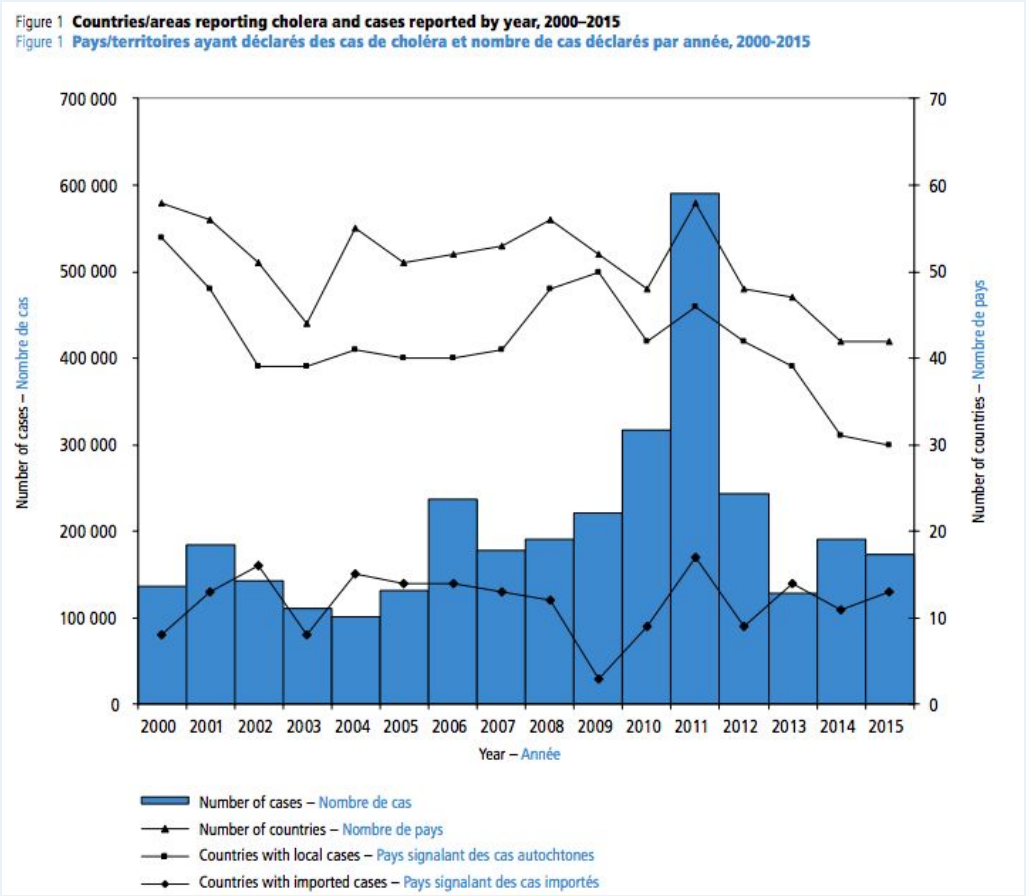
Tuning

Used **bayesian optimization** to tune models before feature rankings, as well as with the final feature set

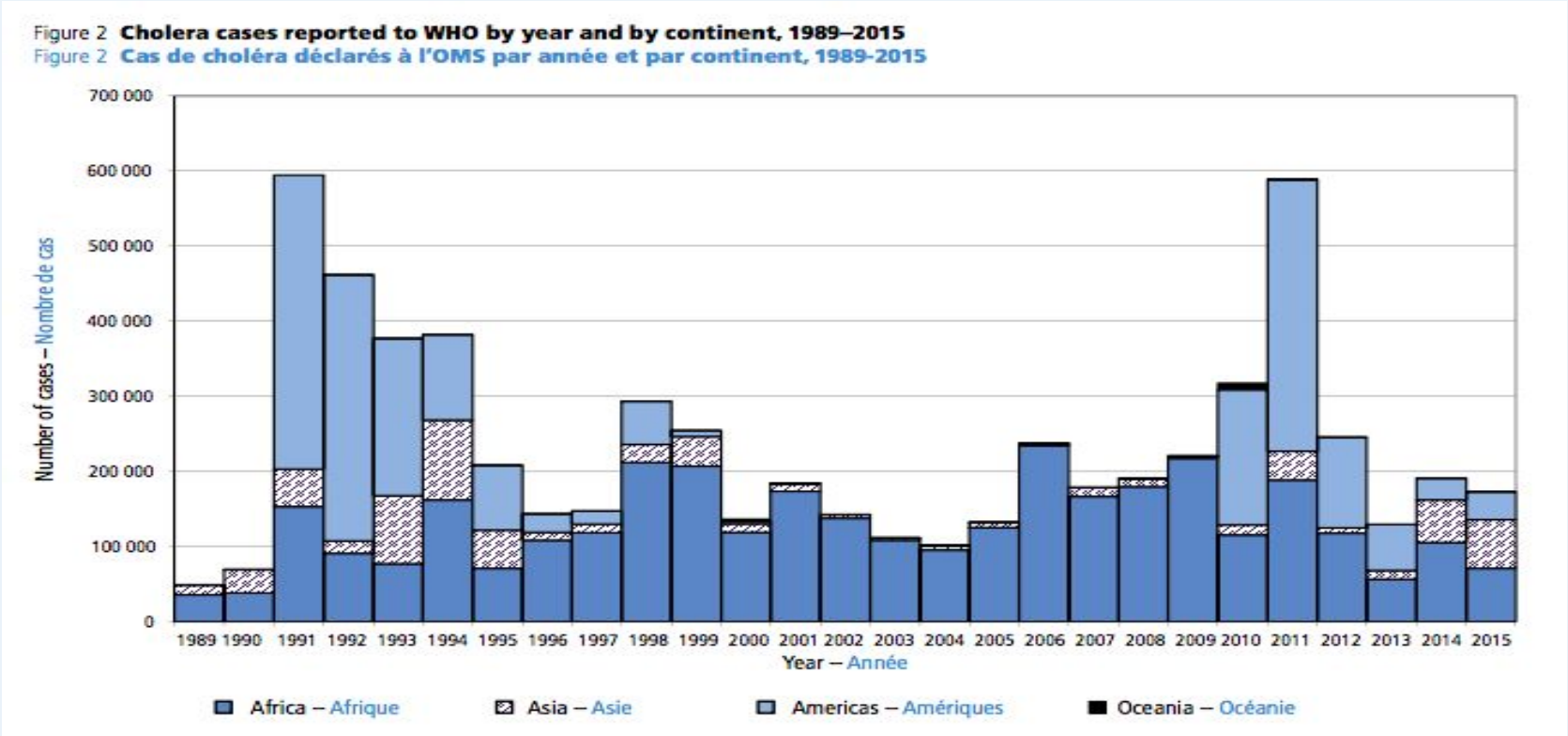

0-2 weeks forecast
2-4 weeks forecast
4-6 weeks forecast
6-8 weeks forecast
sliding-scale prediction of new cases per 10000 people
governorates
YE-AM
YE-DA
YE-MW
YE-RA
YE-SN
Jul
Aug
Sep
Oct
Nov
Dec
Jan
2018
Feb
time (days)
true_val
xgboost cross-validation prediction
xgboost holdout prediction


JEHOVA
W|W
inetowater™
Tel: 809-278-6266
829-708-7449
www.winetowater.org
dr@winetowater.org

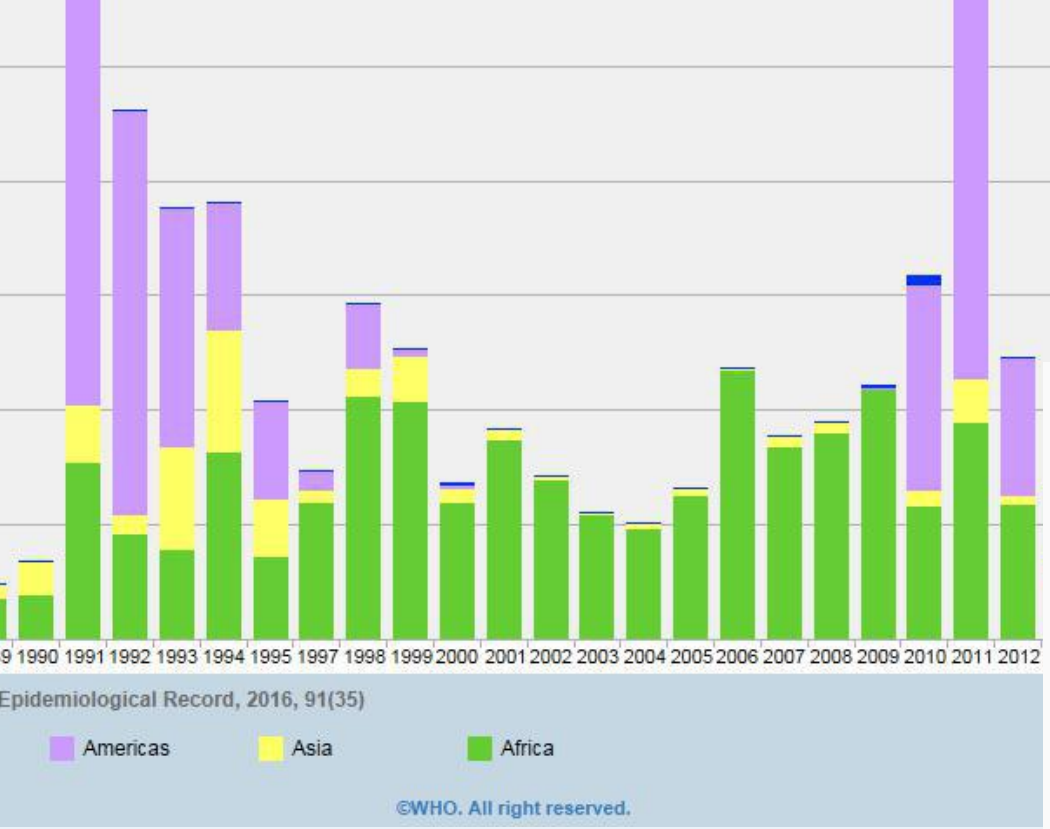

2.9 million cases each year
95,000 deaths each year
Based on WHO Reports 2010-2015
Not a statistical or chronological representation

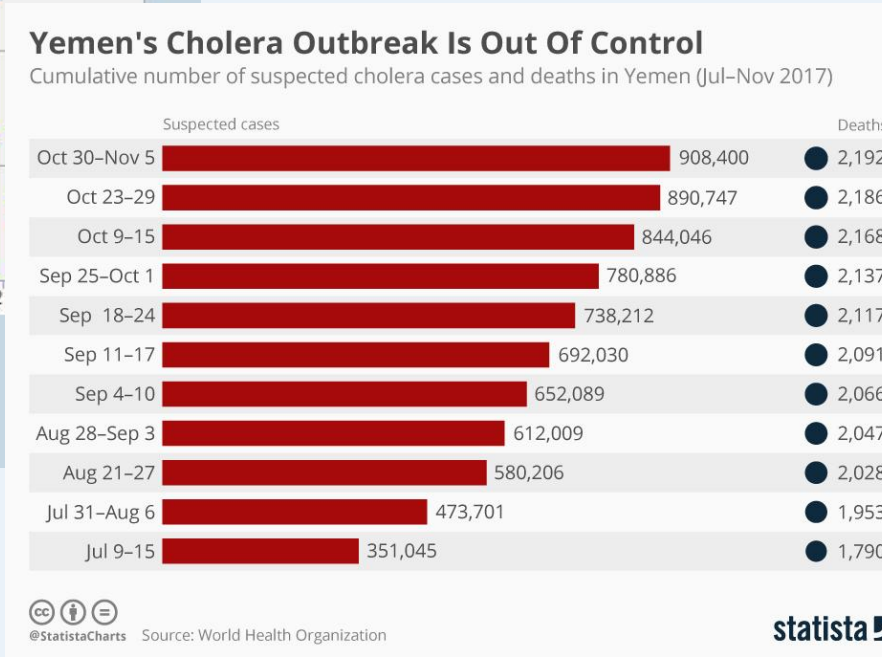


Countries/areas reporting cholera and cases reported by year, 2000-2015



Cholera cases reported by WHO by year and by continent, 1989-2015


9 1990 1991 1992 1993 1994 1995 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012
Epidemiological Record, 2016, 91(35)
Americas
Asia
Africa
©WHO. All right reserved.


Yemen's Cholera Outbreak Is Out Of Control
Cumulative number of suspected cholera cases and deaths in Yemen (Jul–Nov 2017)
Suspected cases
Deaths
Oct 30–Nov 5 908,400 2,192
Oct 23–29 890,747 2,186
Oct 9–15 844,046 2,168
Sep 25–Oct 1 780,886 2,137
Sep 18–24 738,212 2,117
Sep 11–17 692,030 2,091
Sep 4–10 652,089 2,066
Aug 28–Sep 3 612,009 2,047
Aug 21–27 580,206 2,028
Jul 31–Aug 6 473,701 1,953
Jul 9–15 351,045 1,790
@StatistaCharts
Source: World Health Organization
statista

Electroporate into Biosensor Cells

Using the ElectroPen, electroporate isolated DNA into biosensor cells for detection of potential cholera pathogen. Transformations will then be cultured for subsequent analysis.

Capture Reporter Expression in Standardized System

Using the Chrome-Q, pellets of the cultures obtained using the 3D-Fuge will be placed in the chamber and an image will be captured using a smartphone for processing.

Analyze Data from the Color Expression

Using the ColorQ app, the data from the color expression will distinguish between the positive and negative tests, and data will be stored with location on a global server.

SOLUTION

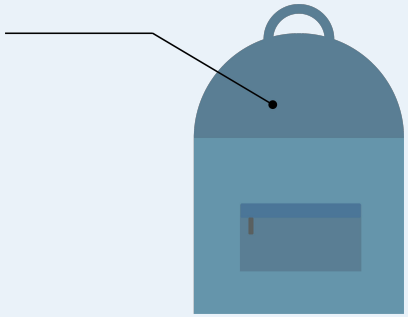
Utilize an inexpensive gene-based biosensor to detect cholera

Conduct water sampling tests with the help of inexpensive alternatives for lab equipment

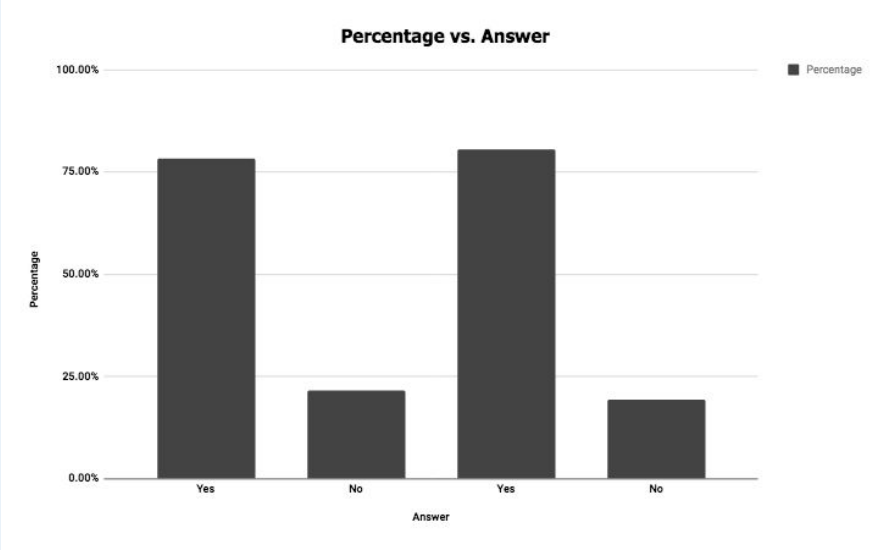
Contribute data and research to an open-source platform to advance the field of genetic engineering and disease detection

Predict the occurrence of cholera outbreaks using a machine learning model

Graphics

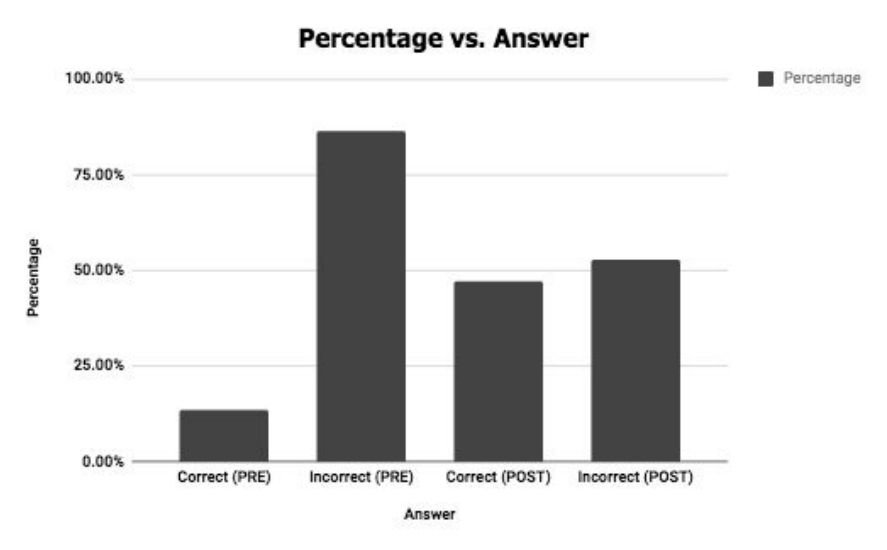


SUMMER CAMP SURVEYS



Question: Do you want to pursue a career in the STEM field?

'Yes' percent change: 2.18%



Question: What is the definition of biotech?

Correct answer percent change: 33.69%

CAMP GUIDE

STEP-TO-STEP GUIDE ON HOW TO RUN A SYNTHETIC BIOLOGY CAMP

LAMBERT IGEM

WHY DID LAMBERT IGEM PLAN A CAMP?

Lambert iGEM strived to empower the next generation of scientists and to expose them to the vast potential of synthetic biology. In an effort to instill a strong scientific foundation among 8th and 9th graders, Lambert iGEM ran a 3-day summer camp. During the camp, participants strengthened their knowledge of various scientific concepts and ran several different scientific experiments.

Science is about the future. In recognizing this, the team realized that this year's project was incomplete without an investment for the future.

WHY SHOULD YOU RUN A CAMP?

Synthetic biology camps, or day camps in general, are excellent ways to expose students to new information in an exciting and hands-on way. Engaging activities with interactive lectures can foster a camper's enthusiasm for learning! At the same time, running a camp can raise funds for your project.

Lambert iGEM created a step-to-step guide on how to run a camp for other iGEM teams. We hope that your team will find this guide useful.

REGISTRATION

- Lambert iGEM created a Google Form, which was linked to a Google Spreadsheet; all information went directly into the spreadsheet.
- Critical information to include: student's general information (first and last name, gender, upcoming grade), parent's name and email, emergency contact information, allergies, and t-shirt size.
- Payment in cash or check to be mailed to Lambert High School.
- Send mass emails to parents a month and a week before the camp date.

USEFUL PLATFORMS

Canva

Canva is a graphic-design tool website. This website is displayed in a drag and drop format and provides access to over a million photographs, graphics, and fonts.

With Canva, you can create promotional graphics and posters.

This step-to-step guide was made with Canva.

MailChimp

MailChimp is a marketing automation platform and an email marketing service. With MailChimp, you can send mass emails and newsletters.

SCHEDULES

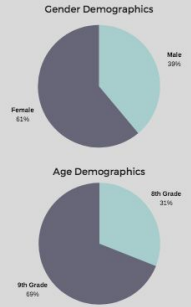
Create a detailed schedule, listing what activities and lectures will be running at certain times.

Make sure to leave room for unexpected occurrences. Also, leave room for bathroom and snack breaks.

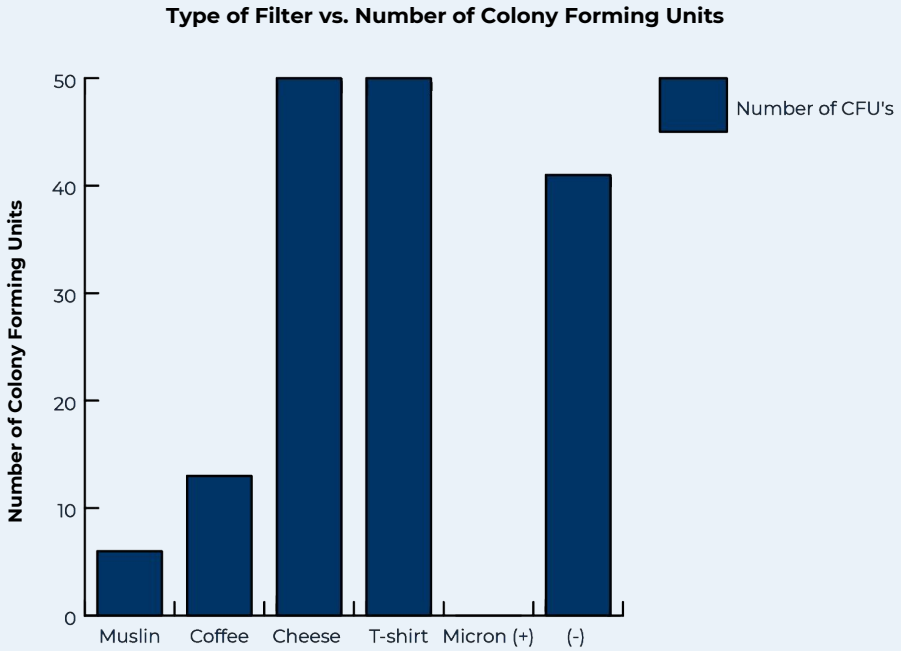
Here is a sample Lambert iGEM summer camp schedule:

https://docs.google.com/document/d/1-e5F6MYUedkWTuIprDfhEtZh0EkIkWq_5bhFzyY8eQs/edit?usp=sharing

LAMBERT SUMMER CAMP



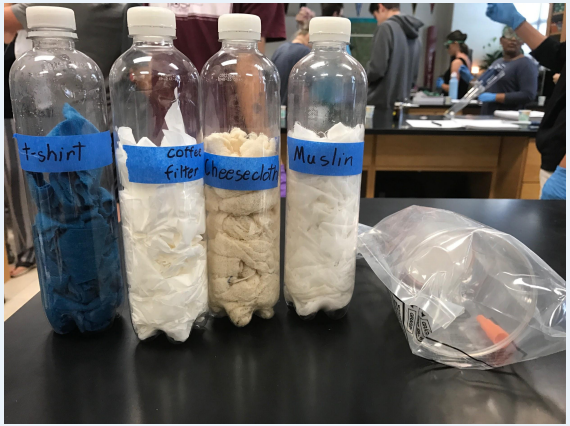
WATER COLLECTION



- Used 1000 mL of creek water spiked with 5 mL of *E. coli* culture
- Types of Filters
  - a. Muslin Filter
  - b. Coffee Filter
  - c. Cheese Filter
  - d. T-shirt Cloth
  - e. Positive Control: Micron Filter
  - f. Negative Control: no filter
- Number of Colony Forming Units were counted for each filter
- Cheese filter and T-shirt filter had a very high number of CFUs (too many to count)
- Muslin filter presents the least number of CFU's

WATER COLLECTION

Water sampling at a nearby creek



Plastic bottles with different types of filters

Filtered water samples for testing

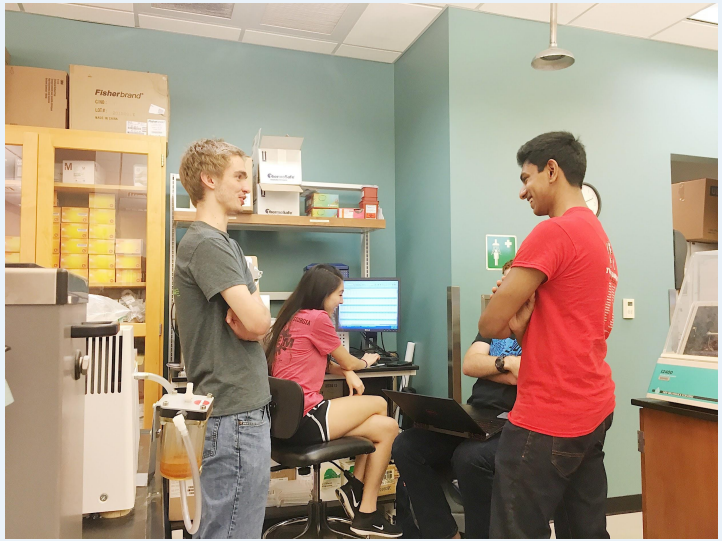
COLLABORATIONS

Lambert iGEM collaboration with the Georgia State iGEM team at the Atlanta Science Festival.

Lambert iGEM at the American Sign Language Camp held at Georgia State University.

COLLABORATIONS

University of Georgia iGEM members helped us with our synthetic biology summer camp experiments and lectures.



Lambert iGEM conducted the interlab study at UGA to have access to a plate reader.

COLLABORATIONS

Georgia iGEM Meetup at Emory University. iGEM teams from Georgia had the opportunity to share their projects, brainstorm future collaboration ideas, and meet new iGEM members.

INFIELD DIAGNOSTICS AND PREDICTIVE ANALYTICS

Gene-Based Biosensor	Inexpensive Hardware	Software Analysis	Machine Learning
Utilizing an inexpensive gene-based biosensor platform using RNA Toehold Switches to provide infield diagnostic results	Conduct water sampling tests with the incorporation of inexpensive alternatives for electroporators and plate readers that are suitable for field conditions	Analyzing biosensor samples for identification of positive and negative tests, and data can be uploaded on a global serve to track the spread of cholera	Utilizing predictive algorithms to forecast the occurrence of potential cholera outbreaks and mount effective responses and resources

Electroporate into Biosensor Cells

- Use the ElectroPen to transform isolated DNA into biosensor cells containing toehold switch
- Grow liquid cultures

Capture Reporter Expression in Standardized System

- Pellet the cell using low-cost centrifuge called the 3D-Fuge
- Add samples to Chrome-Q chamber
- Capture image for analysis

Analyze Data from the Color Expression

- Process image using Color Q app
- Results provided to distinguish + and - tests

INFIELD DIAGNOSTICS AND PREDICTIVE ANALYTICS

Gene-Based Biosensor	Inexpensive Hardware	Software Analysis	Machine Learning
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