CerviCare: A Point of Care Screening Device for Cervical Precancer

UMaryland iGEM Team

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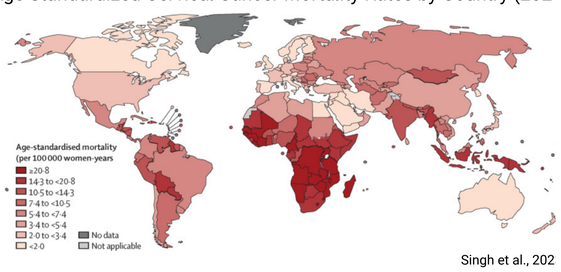
iGEM AND OUR TEAM

International Genetically Engineered Machine (iGEM) is a global competition that combines synthetic biology, wet lab research, entrepreneurship, and scientific collaboration. As an entirely undergraduate team, we develop skills in project design, bioengineering, computer modeling, and communication while tackling a new issue in health, sustainability, biotechnology, or equity each year.

INTRODUCTION

Lower- and middle- income countries (LMICs) endure 90% of worldwide cervical cancer deaths. Increased screening access would decrease cervical cancer mortality rates in LMICs; however, existing tests are hard to deploy in under-resourced areas.

Age Standardized Cervical Cancer Mortality Rates by Country (202(

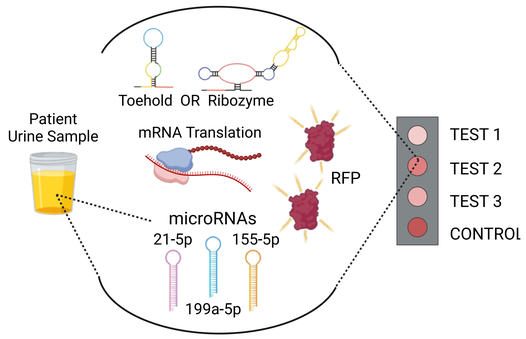


Singh et al., 202

We propose CerviCare, a point-of-care screening tool. CerviCare utilizes synthetic RNA devices to screen patients' urine for microRNAs (miRNAs) common in cervical precancer, increasing access and deployability.

PROPOSED METHODS

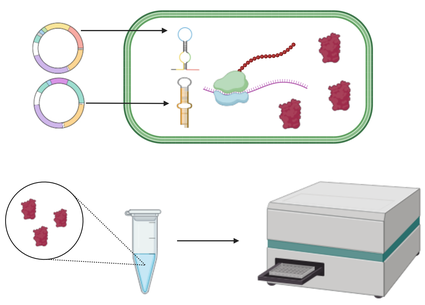
CerviCare Overview



CerviCare will detect upregulated biomarker miRNAs (miR-21-5p, miR-199a-5p, and miR-155-5p) from patient urine eventually using a paper-based assay. RNA devices called toehold switches and ribozymes were designed to bind to the miRNAs, causing a conformational change in the device and triggering translation of red fluorescent protein (RFP). RFP signals will be quantified with an optical reader to determine the patient's likelihood of developing cervical cancer.

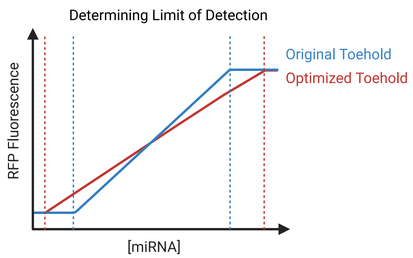
Toehold function is dependent on complementary base pairing and conformation change. Upon binding to a target nucleic acid sequence, the stem loop unfolds, allowing translation of RFP.

Confirming miRNA Toehold Binding in Vivo with RFP Fluorescence Intensity



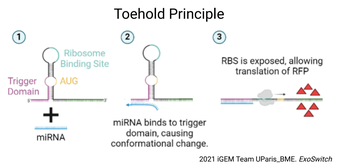
In order to validate each toehold's miRNA binding efficacy, we will transform E.coli with corresponding toehold/miRNA plasmids, extract cell contents, and quantify RFP fluorescnence intensity for a range of plasmid copy numbers.

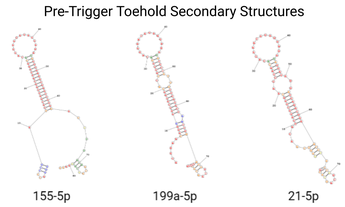
Defining Toehold Detection Limits

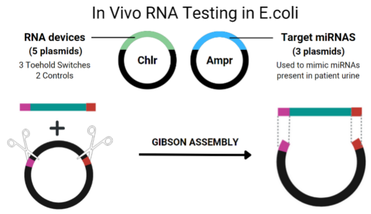


Using a cell-free protein synthesis kit, we will determine the relationship between miRNA concentration and resulting RFP fluorescence intensity. Toeholds will be optimized to adjust the assay's dynamic range.

Designing and Cloning miRNA/Toehold Sequences







Toeholds were designed according to guidelines published by Green et. al with a trigger sequence (complementary to miRNA biomarkers), a complementary stem region, a partial RFP coding region, and the necessary translational machinery [3]. Toehold secondary structure was confirmed using secondary structure prediction software RNAstructure. Toehold and miRNA sequences were ordered from IDT with vector overlap segments at the ends to facilitate insertion into plasmids via Gibson assembly. Toehold sequences will be cloned into chloramphenicol resistance plasmids and miRNA sequences will be cloned into ampicillin resistance plasmids.

Modeling miRNA and Toehold Binding Affinity

Differential Equations for Toehold Kinetics Modeling

$$\frac{d[CTS]}{dt} = k_{transcription}[DNA] - k_{open}[CTS][miRNA] - k_{decay}[CTS]$$

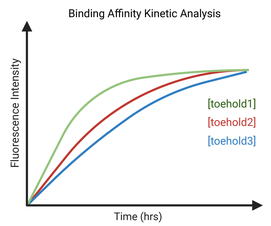
$$\frac{d[miRNA]}{dt} = -k_{open}[CTS][miRNA] - k_{decay}[miRNA]$$

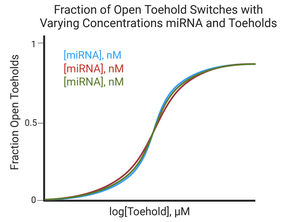
$$\frac{d[OTS]}{dt} = k_{open}[CTS][miRNA] - k_{decay}[OTS]$$

$$\frac{d[RFP]}{dt} = k_{translation}[OTS]$$

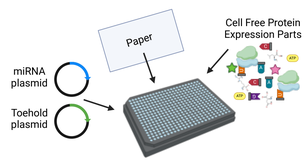
CTS = Closed Toehold Switch, OTS = Open Toehold Switch
2019 iGEM Team Sastra Thanjavur. *House of Toeholds.*

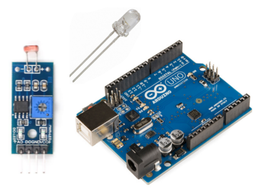
A mass action kinetics model was designed to predict output concentrations of RFP given input concentrations of miRNAs and toeholds. Rate constants for transcription, translation, and decay terms will be determined experimentally using a cell-free protein synthesis kit. Binding affinity assays will be performed to calculate the rate of conversion of closed toehold switches (CTS) to open toehold switches (OTS).





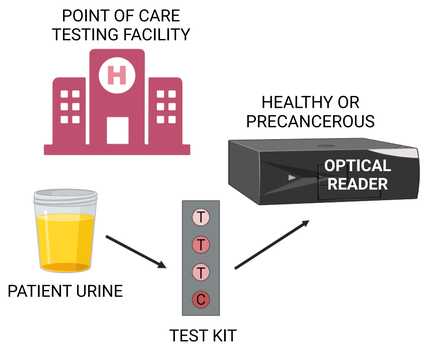
Paper-Based Assay and Optical Reader

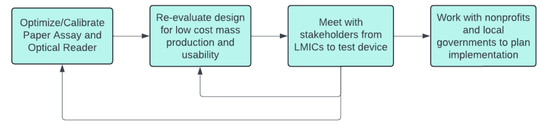




After confirming toehold function *in-vitro and in-vivo,* we plan to re-engineer the system into a paper-based assay for stable storage and simple POC deployment. The assay would incorporate cell-free expression components and our toehold plasmids and could be validated using synthetic urine. Additionally, we plan to design a low cost optical reader to quantify RFP fluorescence and a method to convert fluorescence to a + or - result.

DEPLOYMENT





Our paper-based assay and optical reader will first be optimized for accuracy, and then evaluated for mass production and usability. We will work with stakeholders to receive feedback on the device before meeting with nonprofits and local governments to discuss implementation in healthcare settings.

ACKNOWLEDGEMENTS

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