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iGEM CALGARY 2014: USING *BACILLIS SUBTILIS* TO DIAGNOSE MULTIPLE INFECTIOUS DISEASES IN PARALLEL

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INTRODUCTION

Infectious diseases including dengue fever, typhoid fever, and meningitis are symptomatically similar to malaria, and thus are often misdiagnosed in resource-poor developing countries lacking suitable medical diagnostic facilities. Failure to properly identify such diseases is concerning because it prevents medical professionals from administering appropriate treatments in a timely manner, resulting in economic costs and human suffering [1]. To address this issue, the iGEM Calgary 2014 team developed a genome-based, rapid point-of-care device to simultaneously diagnose multiple infectious diseases. *Bacillus subtilis* was engineered to generate a chromophoric reporter protein in response to pathogenic genetic markers indicative of these diseases. These organisms lie dormant as robust bacterial spores in a microfluidic device, enabling users to input blood samples and differentiate diseases based on the presence of output reporter proteins.

RESEARCH DESIGN AND METHODS

A highly specific, nucleotide-based detection mechanisms was explored for the purpose of detecting and reporting the presence of pathogenic DNA. This strategy employed the endogenous homologous recombination mechanism seen in *B. subtilis* [2] and to this end, a transcriptionally-repressed genetic circuit regulated by a repressor was designed. Disruption of the repressor gene via homologous recombination with the target DNA allowed expression of the reporter and produced a colorimetric signal (Figure 1). The engineered genetic circuits were first tested and cloned in *Escherichia coli* and then stably integrated into the *thrC* locus of *B. subtilis*. *B. subtilis* was chosen as the host organism due to its high level of competency and ability to form spores [3]. Spores offered the benefit of increasing the robustness of the device and eliminated the need for refrigeration during long term storage and transport. Amplification of the target sequences within the pathogens of interest was also considered in order to enhance the sensitivity of the final device. Methods of extracting DNA in a manner compatible with resource scarce environments were explored. Isothermal amplification methods were considered instead of traditional PCR methods in order to eliminate the necessity of a thermocycler and the appropriate training. This was ideal, as the final device was intended to be implemented in developing nations where such resources are not always available.

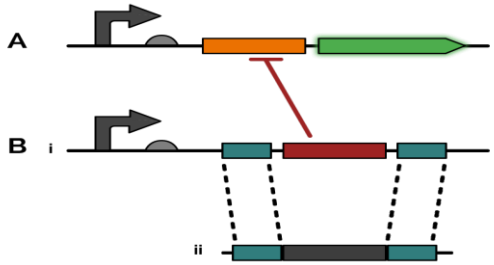

Figure 1. A) Reporter operon consisting of a constitutive promoter (grey arrow), ribosome-binding site (RBS; semicircle), operator (orange), and coding sequence of a reporter. Expression of the reporter is repressed by default. B) Repressor system for regulation of reporter expression. i) Repressor operon consisting of a repressor controlled by a constitutive promoter. Expression of the repressor specifically inhibits the operator shown in A). Blue regions indicate sequences that are homologous to the target DNA shown in ii). Upon homologous recombination (dotted lines), the disruption of the repressor operon leads to active reporter expression. ii) Amplified target DNA from pathogen of interest.

DISCUSSION AND CONCLUSIONS

The device offers a novel platform for the cost-effective detection of multiple infectious diseases. Its comprehensive simplicity offers a user friendliness and that is unmatched by current diagnostic methods. Future developments include the ability to quantify degrees of infection and the capacity to detect target sequences in other bodily fluids.

REFERENCES

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