FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

MOLECULAR DIAGNOSTICS

Fluorescent Cell-Free Biosensor to Detect Tuberculosis

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BACKGROUND

Tuberculosis

- An infectious disease that can be treated if caught early.[1]

Interferon-gamma (IFN-γ)

- It is secreted as a host immune response to counteract microbial infection,[2] thus it is elevated in the blood of patients infected with tuberculosis.

Cell-free Biosensors

- Cell-free systems eliminate the need for living cells, allowing for faster, more controllable, and safer detection.
- A riboswitch acts as a molecular switch to detect specific molecules - such as nucleic acid targets, proteins, and more - and trigger a response.[3]

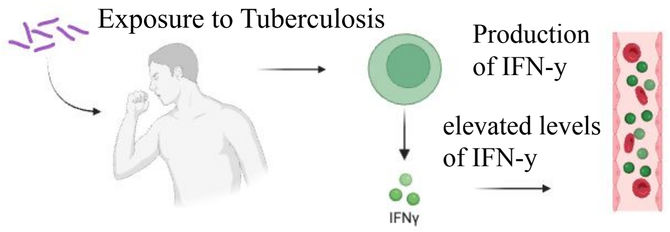

Figure 1.
Exposure to Tuberculosis causes IFN-y to be produced as an immune response, leading to elevated levels of IFN-y in the bloodstream

OBJECTIVE AND SIGNIFICANCE

- Our objective is to create a cell free fluorescent riboswitch based biosensor to detect the biomarker interferon gamma for *Mycobacterium tuberculosis*.
- Current tuberculosis diagnostic tests are expensive, ineffective, invasive, and slow, thus making them inaccessible to many people.[4]

METHODS

Design of Riboswitch Reporter

- A riboswitch reporter[5] was designed with an RNA aptamer for interferon-gamma controlling expression of a deGFP reporter protein[6].

***Escherichia coli* Cultures**

- Grew up *E. coli* containing GFP plasmid from glycerol stock.
- Did plasmid miniprep and DNA cleanup to get purified plasmid DNA.

PCR

- Completion of PCR of T7p14-deGFP (destabilized green fluorescent protein driven by T7 promoter) plasmid DNA and IFN-y riboswitch.

Gibson Assembly

- Assembled plasmids to contain IFN-y riboswitch upstream of GFP reporter. Transformed plasmids into *E. Coli*. Screened colonies to confirm Gibson Assembly was successful and riboswitch was inserted.

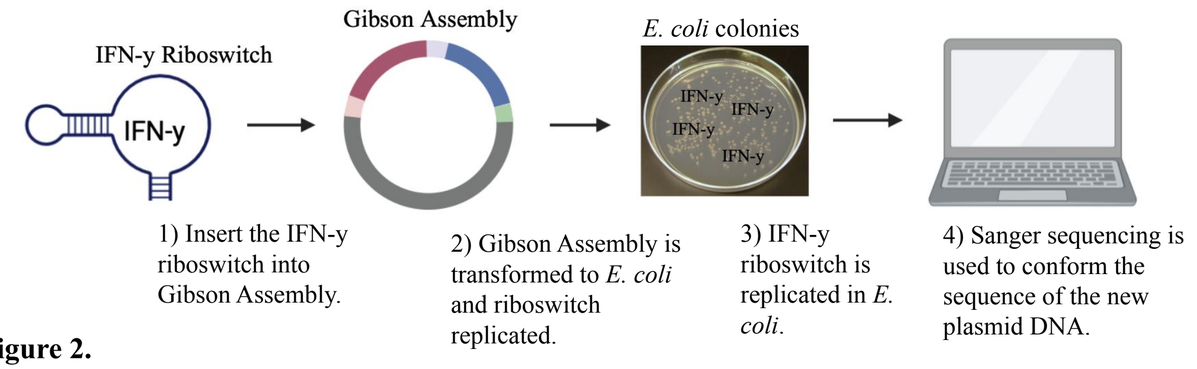

Figure 2.
The new riboswitch reporter with the IFN-y riboswitch upstream of deGFP is assembled using Gibson assembly. The riboswitch controls expression of deGFP. The assembly is then transformed into *E.coli* colonies to allow for the riboswitch to be replicated. Gibson assembly can be checked for success using Sanger sequencing to confirm the sequence of our new plasmid DNA.

RESULTS

- Created T7p14-deGFP plasmid DNA by inoculating an overnight culture from an *E. coli* glycerol stock containing the plasmid
- Tested the plasmid DNA in the *E. coli* cell-free lysate from Aberdeen & measured the fluorescent output at different plasmid DNA concentrations
- Completed PCR using the Q5 DNA polymerase
- Completed Gibson assembly of plasmid containing interferon gamma riboswitch.
- Completed plasmid miniprep and measured A260/A280 and concentration (Nanodrop).
  - A260/A280: 1.90 ± 0.06
  - Concentration: 21.4 ± 8.4 ng/µL

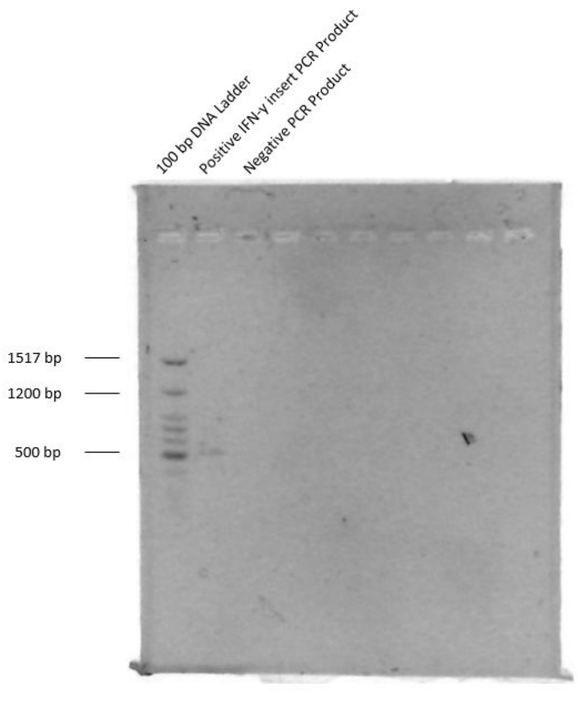

Figure 3.
Gel of PCR product of IFN-y riboswitch insert.This gel was successful with the positive PCR product developing a band at the correct numerical value.

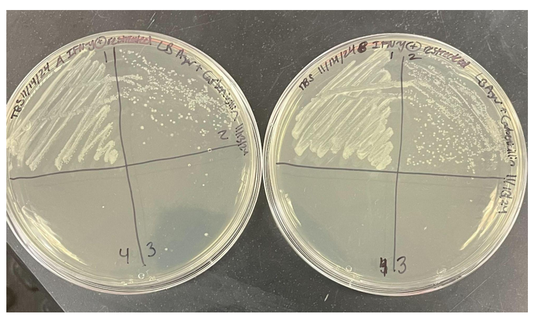

Figure 4.
Plates of *E. coli* transformants with riboswitch reporter post Gibson assembly

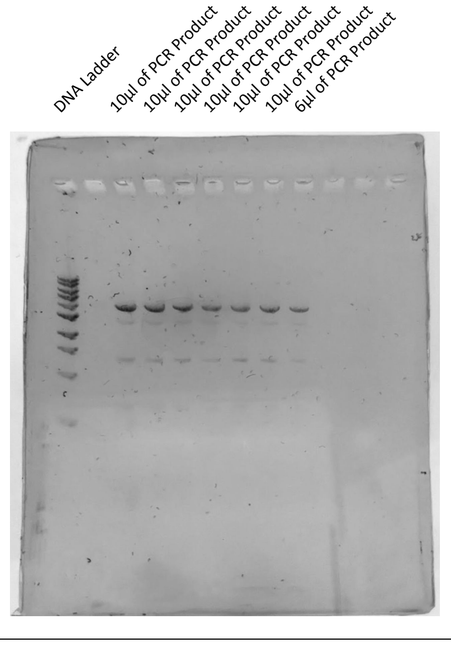

Figure 5.
Gel of GFP PCR product with clear bands at the expected size. Gel extraction resulted in low yields.

FUTURE WORK

- Sequence the plasmid DNA to confirm that the gibson assembly was successful.
- Optimize the performance of the cell-free biosensor in cell-free lysate.
- Test the specificity and sensitivity of the biosensor.
- Optimize the performance of the biosensor in the target sample matrix, which is blood.

REFERENCES & ACKNOWLEDGEMENTS

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Figures created in BioRender.