

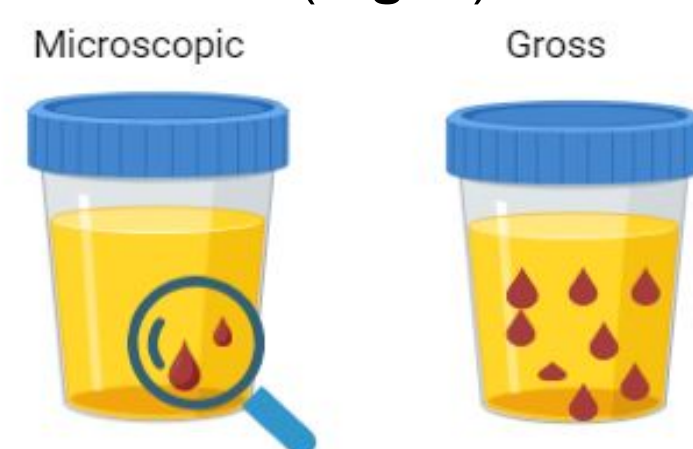
## BACKGROUND

### Significance

- Hematuria can indicate significant health issues, including diseases and cancers.
- Microscopic hematuria is harder to detect than gross hematuria (Fig. 1).

### Existing Methods

- Several existing diagnostic tools.
- Urinalysis strips detect microscopic hematuria but have high false-positive rates.<sup>1</sup>
- Cytological examination is effective, but inaccessible when cytologist is not present.



**Figure 1:** Visual representation of the two main types of hematuria.

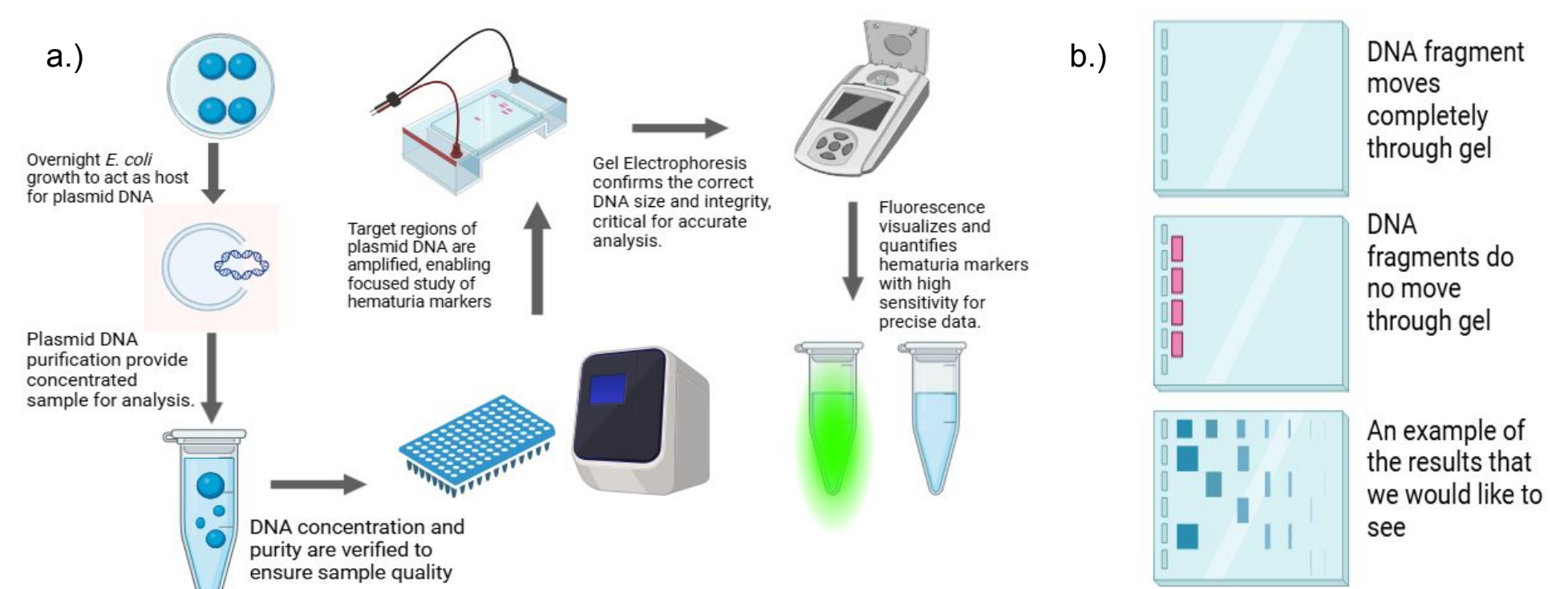
### Cell-Free Expression

- Biosensors allow for targeted and controllable detection of biological targets.
- Cell-free expression (CFE) systems allow point-of-care biosensor deployment.
- Whole-cell biosensors and aptamers for heme have not been translated to CFE.

## INTENT & MOTIVATION

- Adapt existing GFP-producing riboswitch design<sup>2</sup> with heme aptamers to create a heme biosensor.
- Assemble riboswitch in plasmid DNA for cell-free expression testing.
- Assess viability, accuracy, and point-of-care effectiveness of riboswitch-based sensor compared to existing methods.

## METHODS



**Figure 2:** a.) Experimental workflow flowchart. b.) Workflow of PCR analysis via agarose gel electrophoresis.

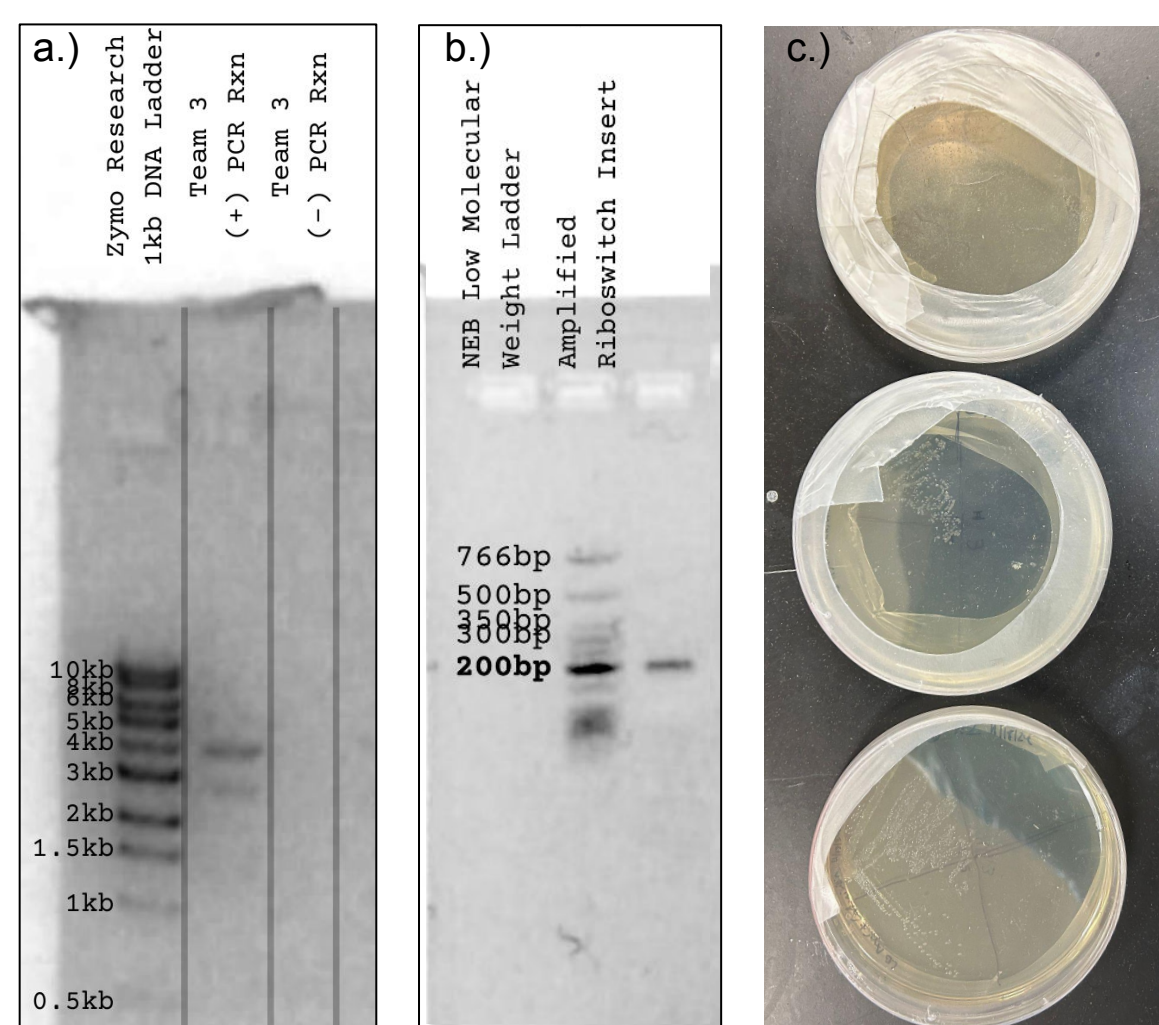
### Acquisition of Biological Parts

- Modified Harbaugh et al. riboswitch with heme aptamer 6-3<sup>3</sup> from IDT.
- Culture existing *Escherichia coli* DH5 $\alpha$  line with GFP plasmid to create stock.
- Isolate GFP gene-containing plasmid via miniprep and confirm purity.
- Linearize plasmid DNA and amplify riboswitch insert using PCR.
- Ensure correct PCR product lengths via gel electrophoresis and purify.

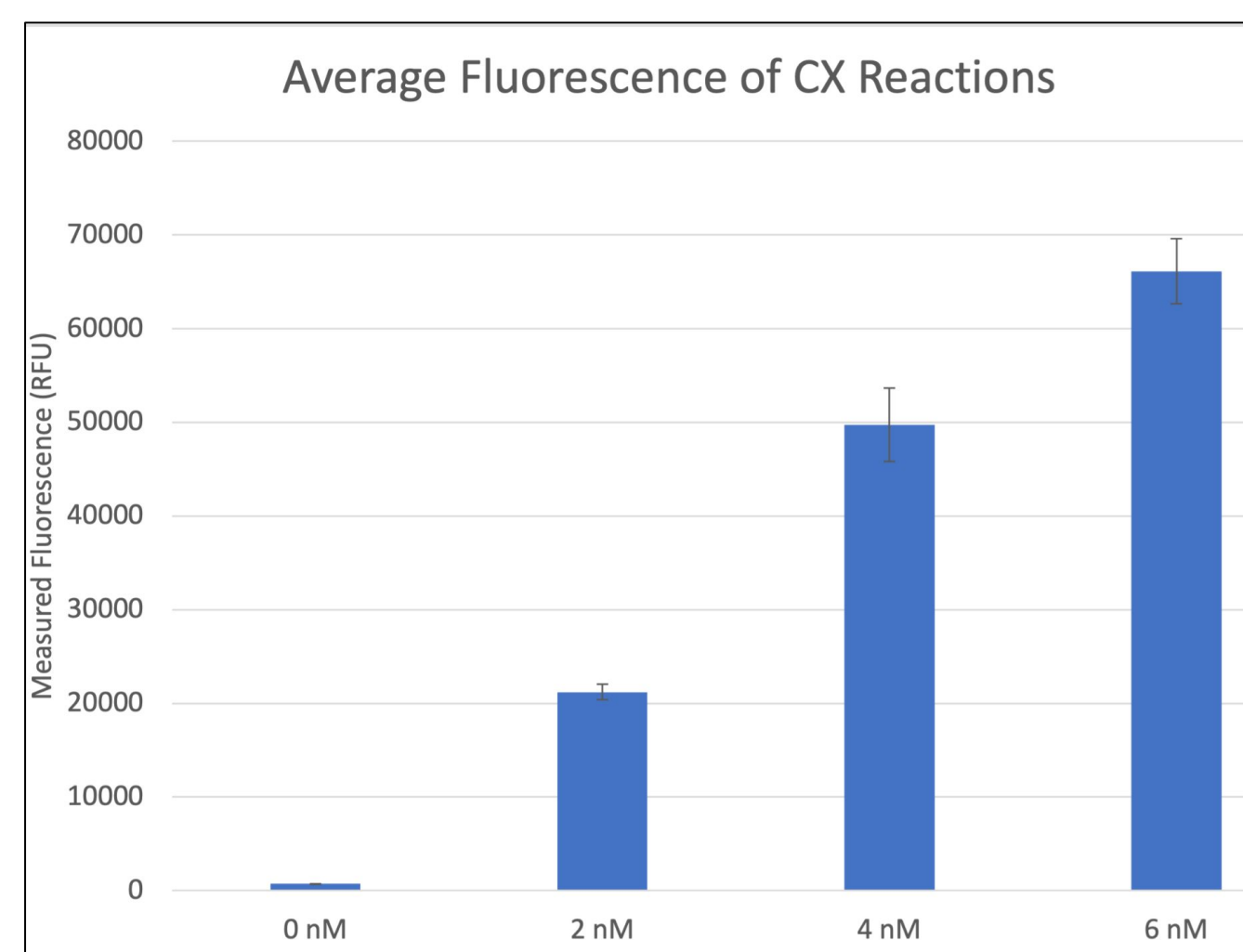
### Modification and Testing

- Insert riboswitch upstream of GFP gene via Gibson Assembly.
- Transform into *E. coli* DH5 $\alpha$
- Isolate modified riboswitch plasmid via plasmid miniprep.
- Test riboswitch using myTXTL Cell-Free Expression Kit and in house lysate.

## RESULTS



**Figure 3:** 0.8% gel of linearized plasmid DNA (a) and 2% gel of insert (b). Plated *E. coli* colonies on LB agar + carbenicillin plates with riboswitch plasmid (c).



**Figure 4:** Analysis of GFP production based on variations in GFP plasmid concentration. FIRE CellEx Lab cell-free lysate was used for these tests. All samples showed statistically significant difference.

### Preparation of Insert and Linearized Plasmid

- Multiple iterative PCR reactions conducted to amplify DNA. Gel electrophoresis conducted to verify DNA fragment size (Fig. 3).
- Initial gel showed unexpected secondary PCR product that was subsequently rectified in future reactions.

### Transformation and Plating

- Positive gel results prompted Gibson Assembly and transformation into *E. coli* DH5 $\alpha$  for culturing and storage.
- Carbenicillin selection was employed, indicating that colonies in Fig. 3 contain our modified plasmid.

### Cell-Free Expression Testing

- GFP plasmid without riboswitch sequence was employed for initial cell-free expression testing.
- Tests using in-house cell-free lysate indicated that concentrations around 6 nM could be feasibly used for future tests (Fig. 4).

## FUTURE WORK

### Future Lysate Testing

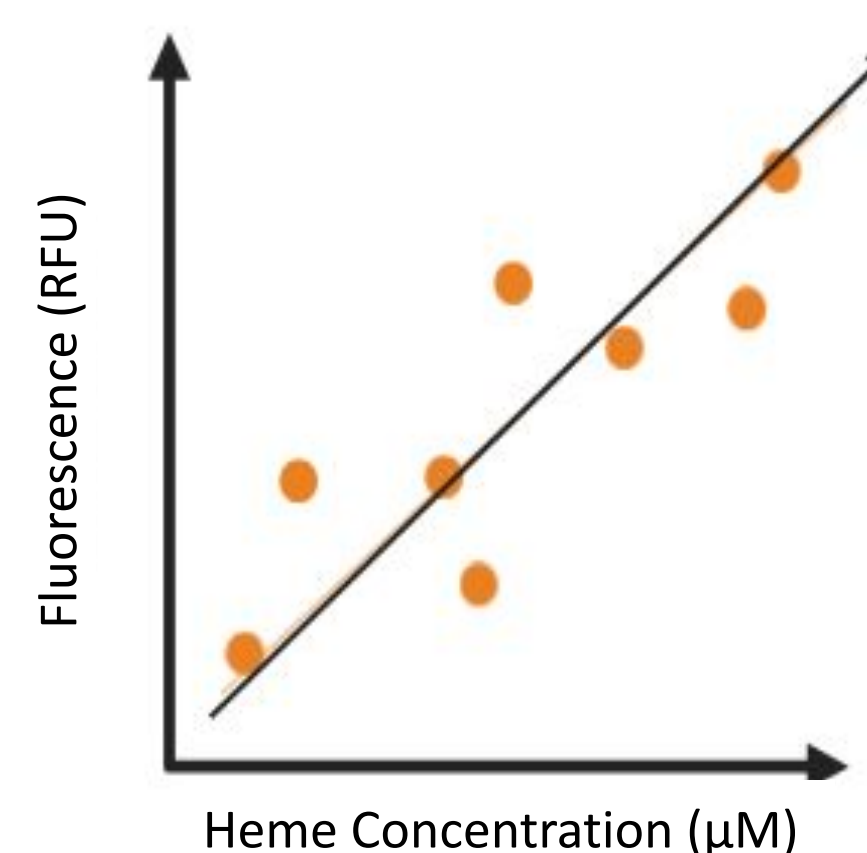
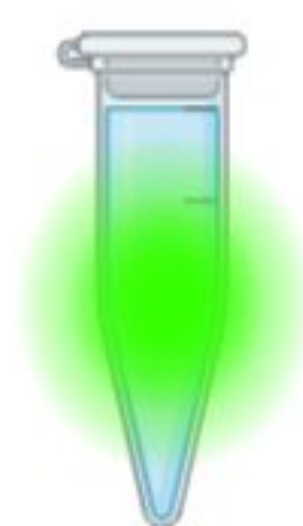
- Future testing with cell-free lysate will be required to assess the performance of the modified riboswitch.
- Testing could be conducted with commercial lysate as well as in-house cell-free lysate to compare performance (measured in RFU).
- Optimal plasmid concentration could also be determined via additional testing.

### Assay Translation

- Translation to a one-pot or paper-based assay could be explored to demonstrate clinical relevance.
- Output with different concentrations of plasmid and lysate compared to traditional CFE reactions could also be documented.

### Point-of-Care Assessment

- Assay time of deployment, translatability to accessible platform, and safety for use in a clinical setting could all be evaluated.



**Figure 5:** An example of future results from cell-free expression lysate testing.

## REFERENCES



**Figure 6:** QR code linking to poster references and acknowledgements.