FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

MOLECULAR DIAGNOSTICS

Accessible, In-House Cell-Free Expression System for Biosensor Development

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Intent & Motivation

Objectives: To develop and optimize in-house *E. coli* based cell-free lysate for expressing riboswitch and toehold switch fluorescent reporters for use in biosensing applications.

Significance: Cell-free expression systems (CFEs) are crucial for biosensor deployment outside of a cell. Commercial options can be prohibitively expensive which limits use in low-cost labs. Additionally, these costs prohibit uses of CFE for common biosensor applications such as affordable point-of-care diagnostic and screen tools. As such, an affordable option is highly desirable.

Background

Cell-Free Transcription/Translation Systems (TXTLs)

Enable faster, safer, and more controllable detection by eliminating the need for living cells.[1] Used in synthetic biology and diagnostics to produce proteins or detect biomolecules.[2]

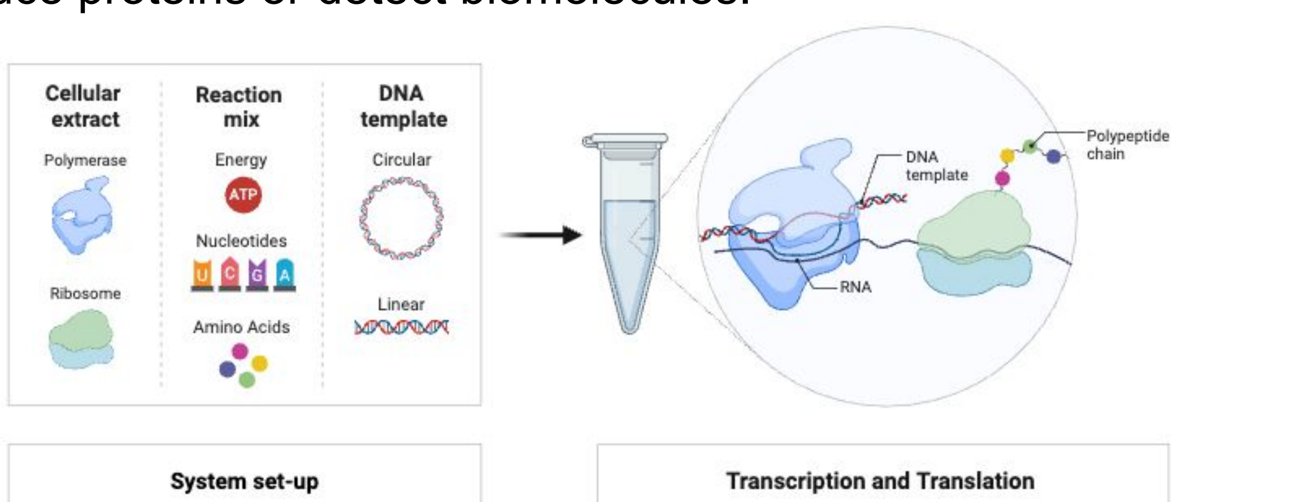


Figure 1: Overview of TXTL (Transcription and Translation) systems with cellular extract, energy mix, and DNA templates.[3]

Riboswitch & Toehold Switch Biosensors

RNA elements that bind to specific molecules, causing a conformational change that activates or represses gene expression.[2]

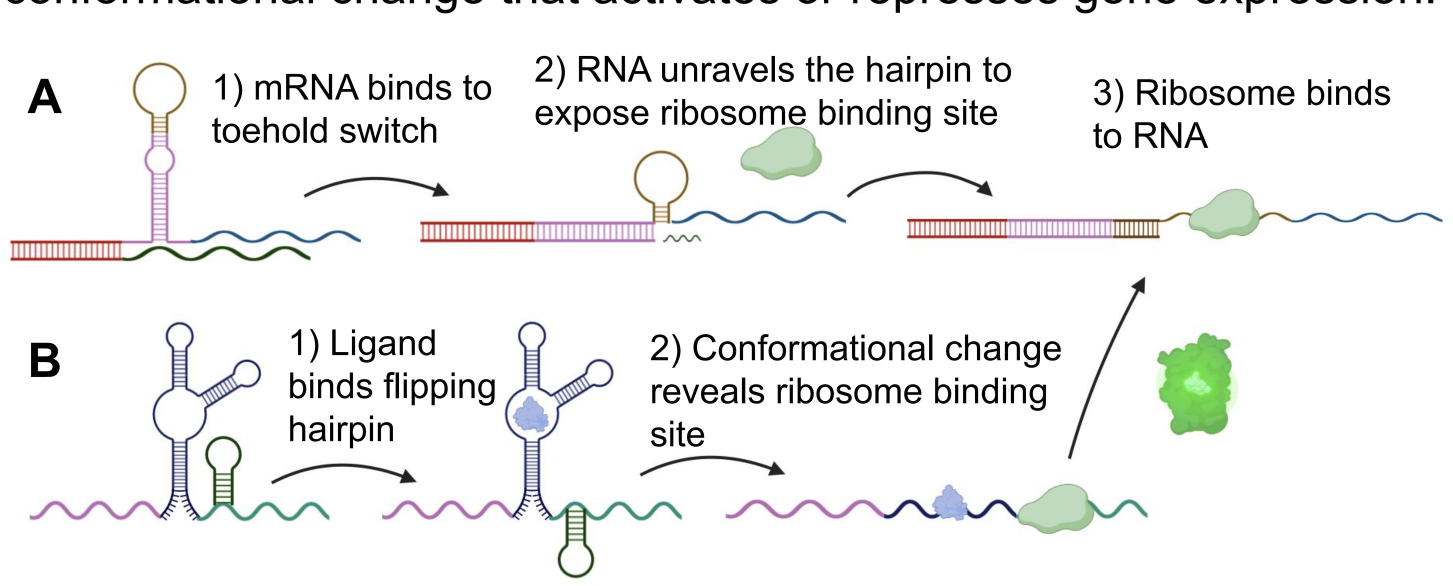
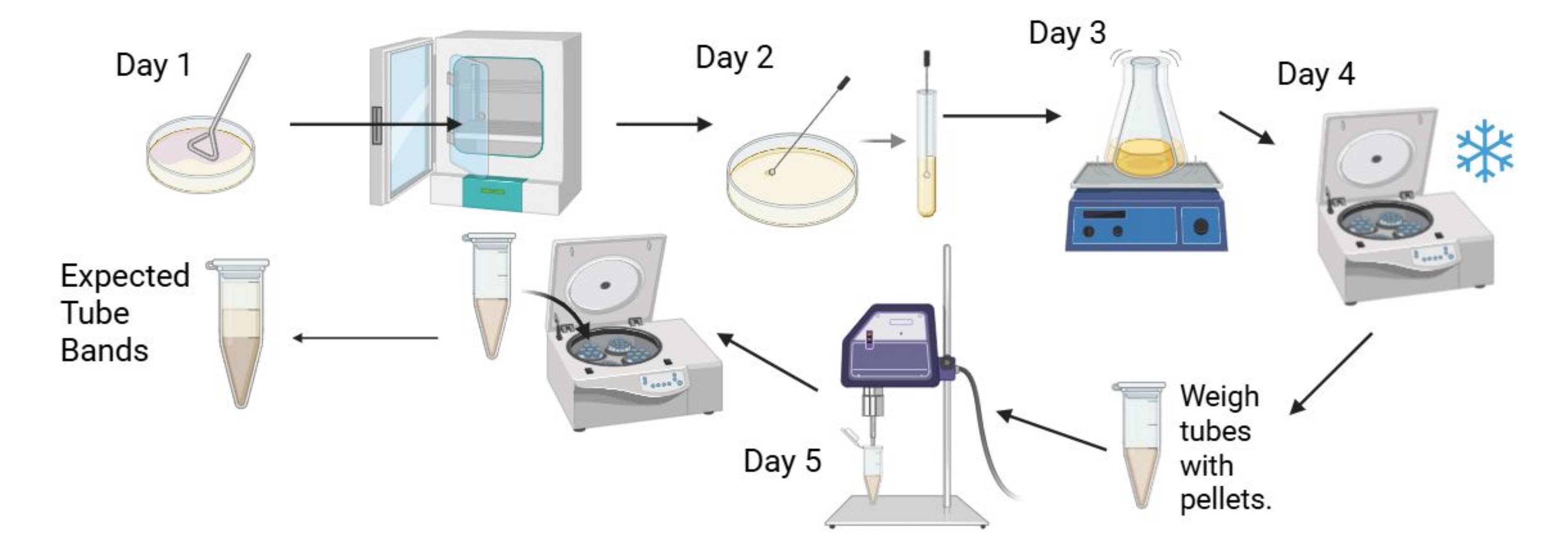


Figure 2: (A) Toehold Switch: Target mRNA binds to a toehold region, displacing a hairpin sequence and exposing a ribosome-binding site, enabling expression of the fluorescent reporter (B) Riboswitch: Ligand binding changes the conformation of the hairpin sequence and allowing for ribosome to attach to its binding site, activating the translation of the fluorescence reporter.[2]

Methods



1) **Preparation of Cell-free E. Coli Lysate: Figure 1 (above)**[4]
- Day 0: Preparation of Media, Buffer, and Plates
- Day 1: Streak Plates and Incubate
- Day 2: Complete Overnight Starter Culture
- Day 3: Growth Phase
- Day 4: Cold Centrifuge
- Day 5: Lysate Sonication

2) **Lysate Expression Optimization**

Variables were controlled to evaluate effects on T7p14-deGFP expression:
1. Range of plasmid concentrations from 0 nM to 10 nM
2. Basal expression of T7p14-deGFP at 5 nM for each lysate
3. Addition of SUPERase·In™[5] RNase Inhibitor to prohibit potential T7p14-deGFP RNA degradation

Compared Lysates:
- Aberdeen Proving Grounds Bacterial Lysate
- myTXTL T7 Expression Kit (505024) from Arbor Biosciences[6]

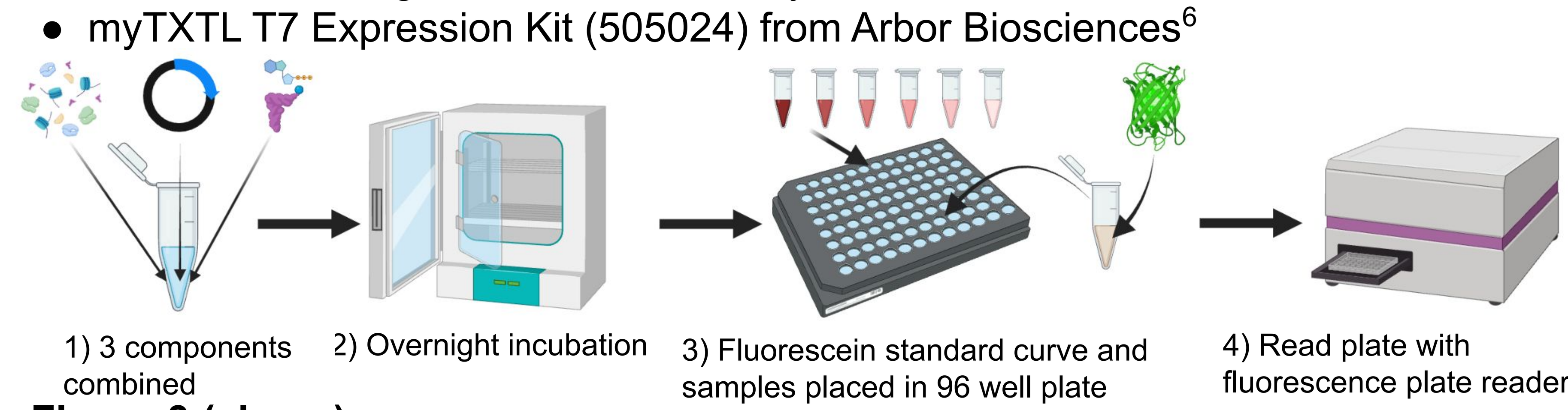


Figure 3 (above)

Rosetta 2(DE3)pLysS *E. coli* lysate, Panox Energy Mix[6], and T7p14-deGFP plasmid are combined in an eppendorf tube and incubated overnight at 29°C. Preparation of the 96-well plate involves creating a fluorescein standard curve and diluting overnight reactions in PBS buffer. Plate is then read by a fluorescence plate reader.[7]

3) **Riboswitch Design**

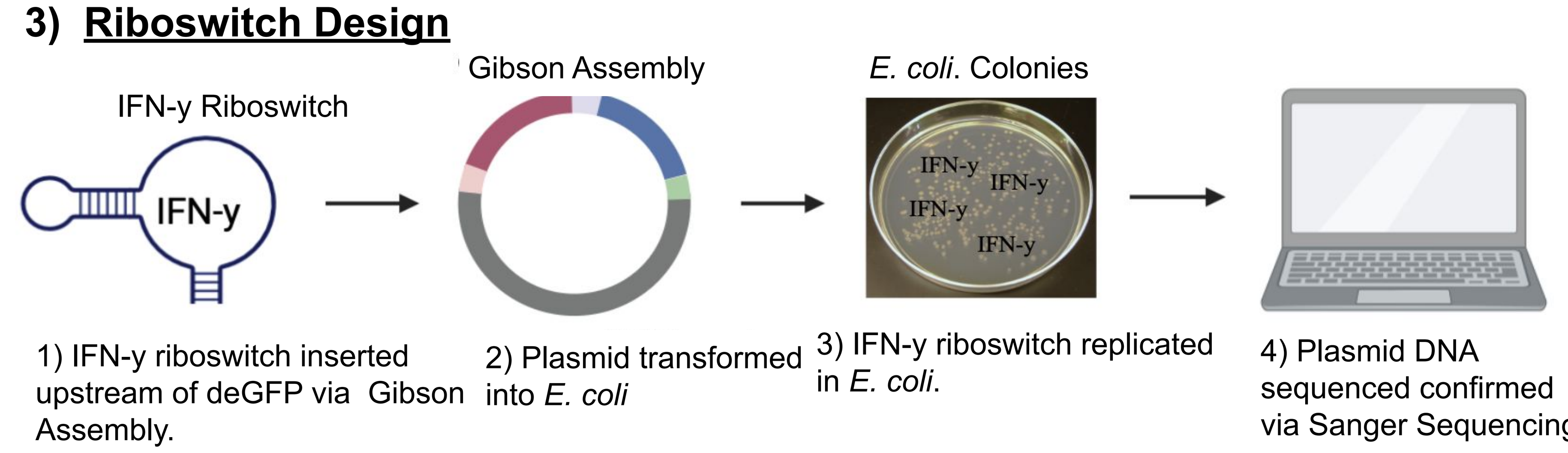
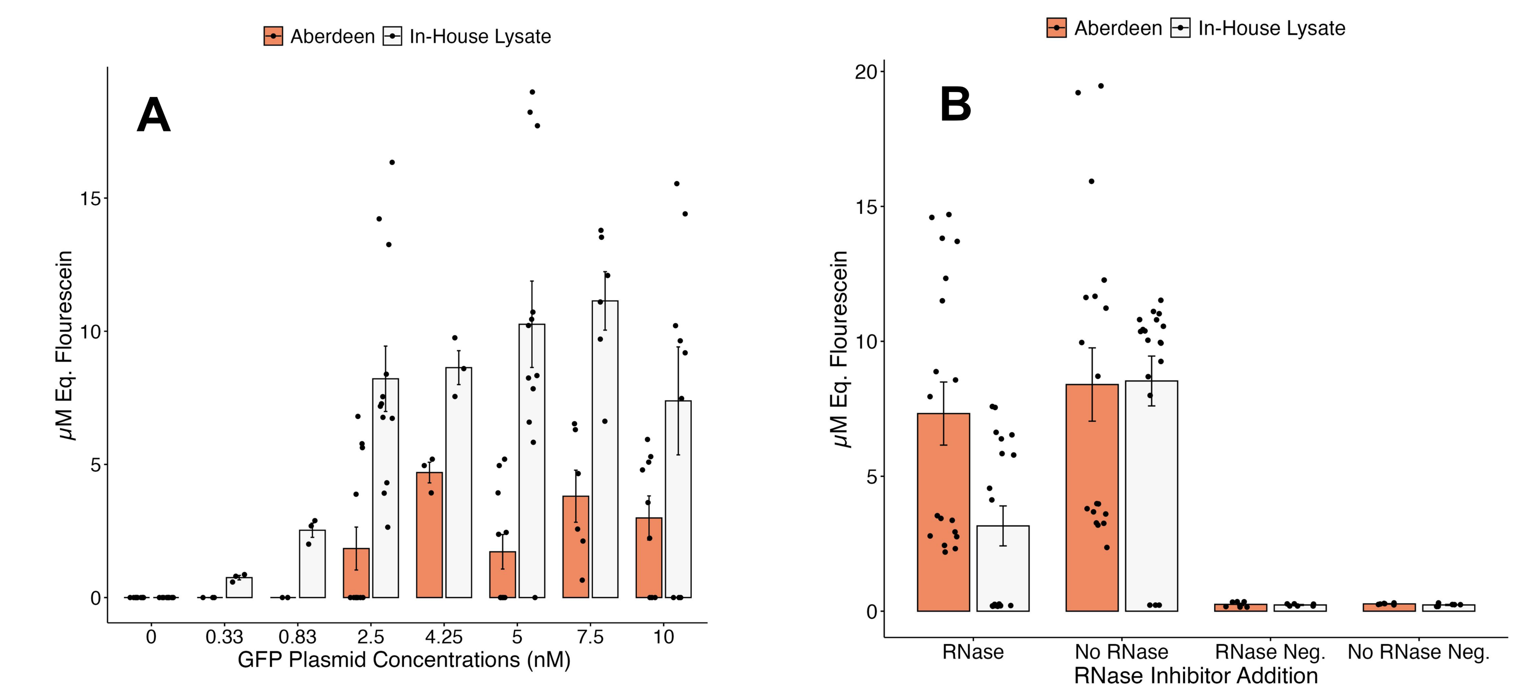


Figure 4 (above).

Using Gibson assembly, the IFN-γ riboswitch reporter (using an existing aptamer) is placed upstream of deGFP. The riboswitch controls expression of deGFP. The assembly is then transformed into *E.coli* colonies for replication. Sanger sequencing is used to confirm the sequence of our new plasmid DNA.[8]

Results & Discussion



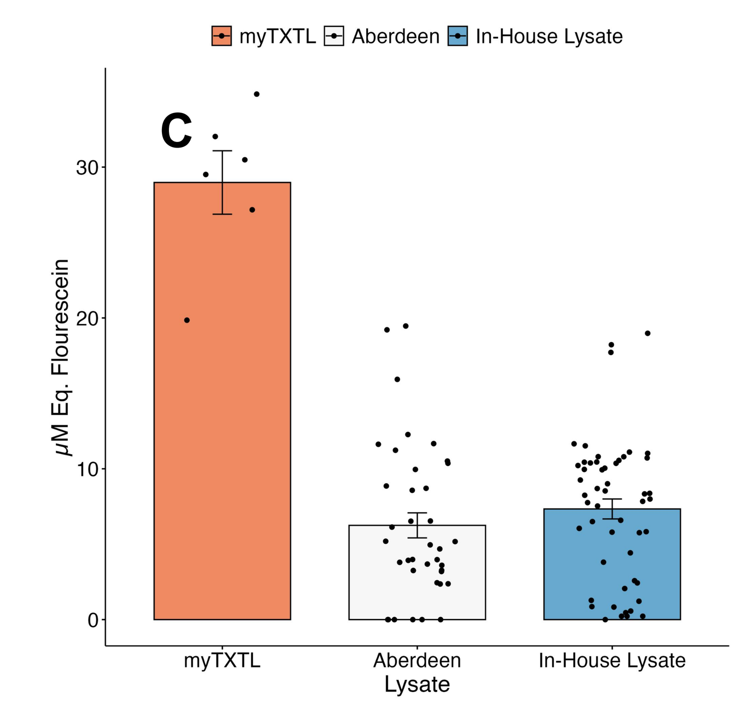


Figure 5: Comparison of T7p14-deGFP expression in our (CellEx) vs Aberdeen in-house cell-free lysate at different plasmid DNA concentrations: 0nM, 0.33nM, 0.83nM, 2.5nM, 4.25nM, 5nM, 7.5nM, and 10nM (**A**). Comparison of T7p14-deGFP expression with and without Superase RNase Inhibitor and negative controls (right) at 5nM (**B**). Comparison of MyTXTL, CellEx, and Aberdeen GFP expression at 5 nM (**C**)

Discussion:
- Optimal plasmid DNA concentrations for GFP expression were between 4.5 and 7.5 nM. As concentrations increased past 7.5 nM, expression plateaued (**A**).
- RNase inhibitor had no effect on expression in the Aberdeen lysate and inhibited the CellEx lysate (**B**).
- Our (CellEx) lysate underperformed as compared to the commercially available myTXTL lysate. However its performance was comparable to that of the other in-house lysate from Aberdeen (**C**).

Future Work

- Troubleshoot issues with expressing RFP in our lysates and optimize its performance in our system
- Evaluate effect of storage time on lysate performance by preparing a new lysate batch and comparing expression
- Test riboswitch designs in the cell-free lysate to confirm functionality

References & Acknowledgements

References

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