FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

CELLEX

Optimization of an in-house bacterial cell-free expression system to evaluate the design of toehold switch sensors for selected cervical precancer miRNA biomarkers

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Introduction

- Cell-Free Transcription/Translation Systems (TXTLs) are essential for cell free reactions and their implementation in point of care testing devices. However, most commercial systems are expensive which increase development and consumer costs[1].

- **Objectives:**
  - To compare the efficiency of an in-house bacterial lysate[3] (CX) with an established cell-free system (Ab), by testing various volume ratios of lysate, energy mix, and template plasmid DNA (T7 p14-deGFP HP) to determine an optimal reagent composition

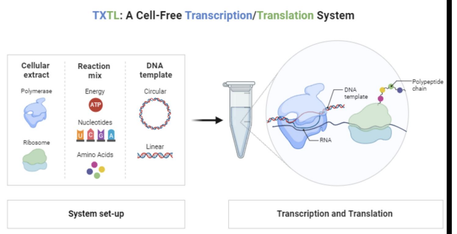

Figure 1: Overview of TXTL systems in which cellular extract, reaction mixtures, and DNA templates are mixed to enable transcription and translation[2].

Methods

- Conducted TXTL cell-free reactions (rxns) combining the following reagents:

Number of rxns	Bacterial Lysate	Template DNA (T7p14-deGFP HP)	Panox Energy Mix	Inhibitor Added	Total rxn volume
1	9µL Sigma 70 Master Mix (myTXTL)	2.5µL (5 nM final) + 0.5µL P70a-T7rnap HP	-	-	12 µL
2	X µL Ab or CX (33%)	X µL (5 nM final, 33%)	X µL (33%)	-	12 µL
2	X µL Ab or CX (50%)	X µL (1.2 nM final, 5%)	X µL (45%)	-	50µL
4	X µL Ab or CX (33% or 50%)	X µL (1.2 nM final, 33% or 5%)	X µL (33% or 45%)	Protease	12 µL
4	X µL Ab or CX (33% or 50%)	X µL (1.2 nM final, 33% or 5%)	X µL (33% or 45%)	RNAse	12 µL
4	X µL Ab or CX (33% or 50%)	X µL (1.2 nM final, 33% or 5%)	X µL (33% or 45%)	Protease + RNAse	12µL

- Incubated reactions overnight in a 30°C incubator (constant atmospheric T)
- Calculated a fluorescein standard curve through performing 1:1 serial dilutions with 10µM fluorescein stock solution and PBS buffer
- For each reaction, split total reaction volume between three wells and filled remaining volume with PBS buffer up to 100µL in a 96-well plate
- Recorded fluorescence measurements on a plate reader using an excitation wavelength of 490 nm

Results

Fluorescein Standard Curve:

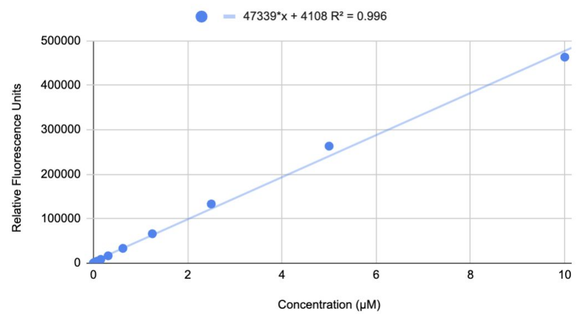

Figure 2: Standard curve generated from serial dilutions

Inhibitor Addition Comparison Graph:

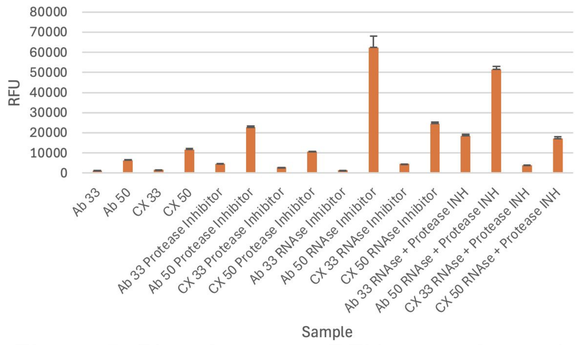

Figure 5: Plot of average RFU output for each cell free reaction volume composition while varying the addition of RNAse inhibitor and Protease inhibitor. 4 groups of reactions were performed: one with no RNAse or Protease added, one with RNAse added, one with Protease added, and one with both RNAse and Protease added. Plasmid concentration held constant at 1.2nM for all reactions

Lysate Comparison Numerical Results:

Sample	Average µM Equivalent Fluorescein	Standard Deviation	Standard Error
myTXTL	27.78810811	6.145125171	1.536281293
Ab 33	10.41348043	3.131198218	0.521866369 7
Ab 50	3.965401192	2.708452882	0.4514088136
CX 33	11.14980775	6.971190699	1.161865116
CX 50	2.423127139	1.384479764	0.2307466273

Figure 3: Computations for the average µM fluorescein equivalents and standard error.

Lysate Comparison Graph:

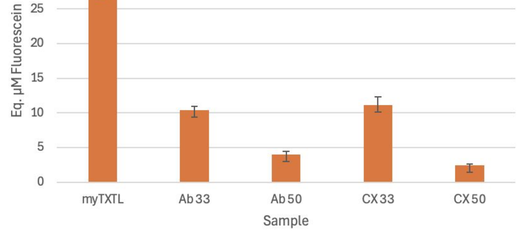

Figure 4: Plot of the average µM equivalent fluorescein value for each cell-free volume composition reaction. Linear regression from fluorescein standard curve used to generate a 10 µM equivalent fluorescein value which is then multiplied by a dilution factor, total volume divided by reaction volume, to obtain final values. Plasmid concentrations not held constant and instead, % DNA for each reaction volume ratio is held constant.

Discussion

- Fluorescence readings show that the 33:33:33 CellEx System and 33:33:33 Aberdeen System had the highest relative fluorescence aside from the myTXTL reaction.
- This indicates that the reaction mixture of 33% Energy Mix, 33% CellEx and Aberdeen lysate, and 33% DNA perform at similar levels
- Additionally, this indicates that the 33:33:33 ratio may be more ideal for a cell free expression system than the 45:50:5 ratio

Future Directions

- Test additional lysate, energy mix, and plasmid DNA ratios for further optimization
- Perform a cost-benefit analysis for several cell-free expression systems
- Implement an ideal in-house lysate onto a paper based assay to express toehold switches designed to recognize cervical precancer miRNAs[4,5]

References

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Acknowledgements

Financial support for this project was provided by The First-Year Innovation and Research Experience program at the University of Maryland. We would like to thank Aberdeen Proving Ground, FIRE-HPI, and FIRE-FS for their generosity with equipment and reagents. Thanks to the organizers of the Summer Undergraduate Research Conference for the opportunity to present our work. Additional thanks to UMaryland iGEM, Senanga Perera, and FIRE Peer Research Mentors Audrey Heber, Blaise Ryan, Maya Murthy, and June Philip for their assistance and cooperation with this collaboration.