

Nucleus Developer Notes

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Toehold switch-enabled translation regulation verified in Nucleus Cytosol

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Abstract

These results are part of preliminary testing for the pH-responsive DevCell module conducted during the DevCell Kickoff workshop. Using Nucleus Cytosol, the toehold switch enabled translation regulation of pHtdGFP in the presence of trigger ssDNA.

Keywords Cytosol, Module, translation regulation, trigger ssDNA, toehold switch

1. INTRODUCTION

As part of the development for the pH-responsive DevCell module for the Chicago node, the Liu lab conducted a brief test to validate the toehold switch system in Nucleus Cytosol. The specific toehold switch used in this test is published in S.-W. Hwang, Y. Li, A. A. Green, and A. P. Liu [1], and is originally developed for PUREfrex2.0. This preliminary test was conducted at neutral pH, and pH-responsive protein expression was not tested.

2. RESULTS

This preliminary test demonstrated that, using Nucleus Cytosol, the toehold-pHtdGFP linear DNA template was able to produce toehold pHtdGFP RNA and induce pHtdGFP expression gated by the presence of the trigger ssDNA. This preliminary test followed standard protocol for bulk reactions using Nucleus Cytosol (p0pen-deGFP plasmid concentration is 3 nM), and the toehold-pHtdGFP linear DNA template and trigger ssDNA concentrations are 2 nM and 4.8 nM, respectively, following those used in S.-W. Hwang, Y. Li, A. A. Green, and A. P. Liu [2]. For each condition, three identical 10- μ L reactions were incubated for 6 hours at 37 °C in a plate reader. The composition for each reaction is provided in [Table 2](#).

*SMix contains Mg-acetate and provides 8 mM final Mg-acetate concentration in the reaction.

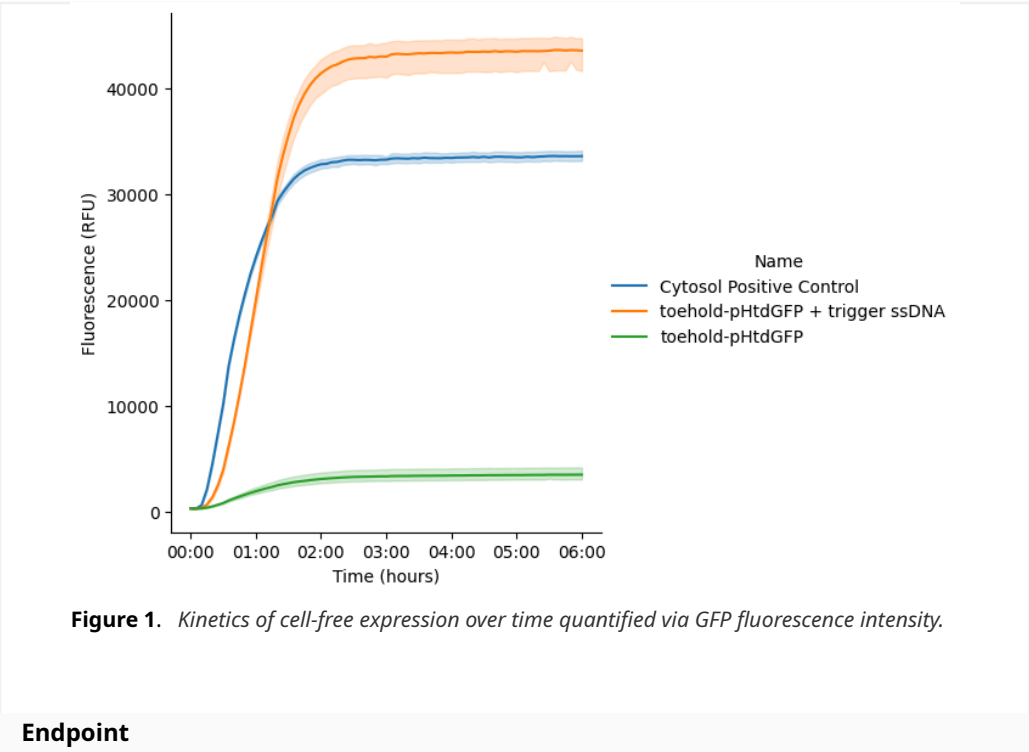
Kinetics

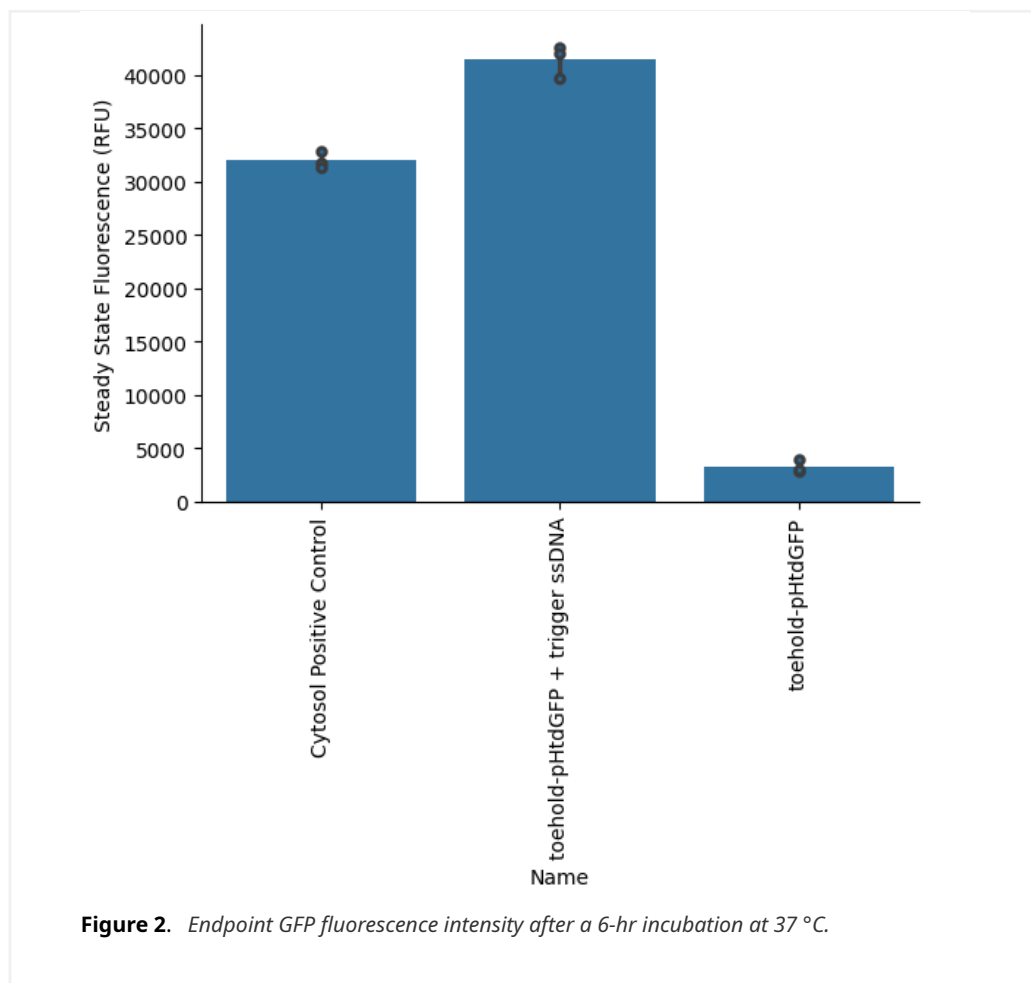
Table 1. *Experiment conditions*

Name	Description
Cytosol Positive Control	Control Cytosol reaction expressing deGFP using p0pen-deGFP construct
toehold-pHtdGFP + trigger ssDNA	DNA template producing toehold-pHtdGFP RNA mixed with trigger ssDNA
toehold-pHtdGFP	DNA template producing toehold-pHtdGFP RNA without trigger ssDNA

Table 2. *Reaction composition*

Component	Stock Concentration	Final concentration	Cytosol Positive Control [μL]	toehold-pHtdGFP + trigger ssDNA [μL]	toehold-pHtdGFP [μL]
SMix*	3.33x	1x	10.5	10.5	10.5
PMix	15 mg/mL	1.80 mg/mL	4.2	4.2	4.2
Ribosomes	10 μM	1.8 μM	6.3	6.3	6.3
tRNA	35 mg/mL	3.5 mg/mL	3.5	3.5	3.5
pOpen-deGFP DNA template	124 nM	3 nM	0.85	0	0
toehold-pHtdGFP DNA	40 nM	2 nM	0	1.75	1.75
trigger ssDNA	100 nM	4.8 nM	0	1.68	0
RNAse Inhibitor	40000 U/mL	2000 U/mL	1.75	1.75	1.75
Water			7.9	5.32	7
Total mastermix volume [μL]			35	35	35
Reaction Volume × No. of replicates			10 μL × 3	10 μL × 3	10 μL × 3





3. CONCLUSION AND FUTURE STEPS

This preliminary test shows that the toehold switch system is compatible with Nucleus Cytosol. Further testing of Nucleus Cytosol at acidic pH levels (~pH 6-6.5) would be needed to fully validate the compatibility of Nucleus Cytosol for the proposed pH-responsive DevCell module.

REFERENCES

- [1] S.-W. Hwang, Y. Li, A. A. Green, and A. P. Liu, "pH-Responsive Synthetic Cells for Programmable *In Situ* Protein Synthesis and Release," Nov. 16, 2025. doi: [10.1101/2025.11.16.688650](https://doi.org/10.1101/2025.11.16.688650).
- [2] S.-W. Hwang, Y. Li, A. A. Green, and A. P. Liu, "pH-Responsive Synthetic Cells for Programmable *In Situ* Protein Synthesis and Release," Nov. 2025, doi: [10.1101/2025.11.16.688650](https://doi.org/10.1101/2025.11.16.688650).