

Nucleus Developer Notes

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Correspondence to

Maram Naji
maram@U.northwestern.edu

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Theophylline-LacZ sensor validation in Nucleus Cytosol

Maram Naji¹  

¹Northwestern University

Abstract

We validated that a biosensor for theophylline switches on with the addition of 1.5 mM theophylline in Nucleus Cytosol, resulting in a faster conversion of CPRG from a yellow to red product through the production of LacZ from the biosensor.

1. OVERVIEW

The translational theophylline riboswitch controls expression of the downstream expression platform in response to theophylline, a xanthine derivative. This sensor uses a synthetic theophylline riboswitch designed by S. A. Lynch and J. P. Gallivan [1] and has since been demonstrated in several encapsulated systems.

In this work, the theophylline riboswitch has been encoded upstream of a LacZ reporter. The resulting biosensor is then added to a Nucleus Cytosol reaction supplemented with chlorophenol red- β -D-galactopyranoside (CPRG), which is converted from yellow into a red product by LacZ.

2. RESULTS

Comparing the absorbance at 570 nm over time demonstrates that the sensor is able to convert CPRG from yellow to red faster in the presence of 1.5 mM theophylline in standard Nucleus Cytosol conditions with 5 nM of sensor DNA. For each condition, a 10 μ l reaction was assembled with 0.6 mg/ml CPRG and incubated at 37 °C in a platereader.

3. CONCLUSION AND FUTURE STEPS

This preliminary test shows that the theophylline riboswitch with a LacZ reporter is compatible with Nucleus Cytosol. Encapsulation of the sensor will inform whether the DNA template concentration should be modified to improve extent of conversion or increase the time to leak in the - theophylline condition.

Table 1. *Experiment conditions*

Condition	Description
• theophylline	Control sensor reaction without theophylline
1. 1.5 mM theophylline	Sensor reaction with 1.5 mM theophylline

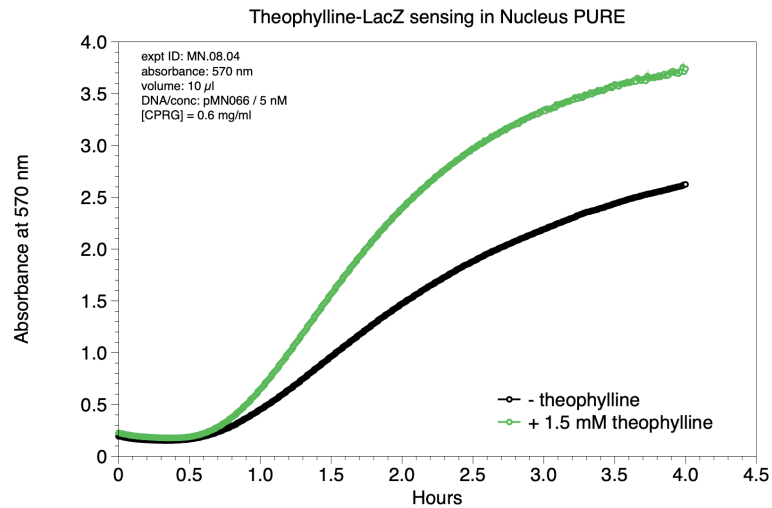
Table 2. *Reaction composition*

Component	Stock Concentration	Final concentration	• theophylline [μL]	1. 1.5 mM theophylline [μL]
SMix	3.33x	1x	3	3
PMix	15 mg/mL	1.80 mg/mL	1.2	1.2
Ribosomes	10 μM	1.8 μM	1.8	1.8
tRNA	35 mg/mL	3.5 mg/mL	1	1
pT7-theophylline-LacZ (pMN066)	49.55 nM	5 nM	1	1
DNA template				
CPRG	10 mg/mL	0.6 mg/mL	0.6	0.6
Theophylline	10 mM	1.5 mM	0.95	0.95
RNAse Inhibitor	40000 U/mL	2000 U/mL	0.5	0.5
Water			0.54	0

4. BILL OF MATERIALS

4.1. Critical Materials

Reagent	Product Name	Manufacturer	Part #	Price	Storage Conditions	Link
Cytosol	Nucleus Cytosol	b.next	N/A	N/A	-80C	N/A
CPRG	Chlorophenol red-β-D-galactopyranoside	Roche	10884308001	\$160	-20C in water at 10 mg/ml	https://www.sigmaaldrich.com/US/en/product/roche/10884308001


Figure 1. *Kinetics for colorimetric conversion of CPRG into a red product.*

Theophylline	Theophylline, ≥99% (HPLC), powder	Sigma-Aldrich	T1633	\$34.6	Prepared fresh at 10 mM in water	https://www.sigmaaldrich.com/US/en/product/sigma/t1633?srsId=AfmBOooXyXrNfmCDIKEsxtiHbE7P5z-FzHCoj2cL0aF2H_XgVCikS_0B
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REFERENCES

[1] S. A. Lynch and J. P. Gallivan, "A flow cytometry-based screen for synthetic riboswitches," *Nucleic Acids Research*, vol. 37, no. 1, pp. 184–192, Nov. 2008, doi: [10.1093/nar/gkn924](https://doi.org/10.1093/nar/gkn924).