

Nucleus Developer Notes

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TetO-Catecholase sensor validation in Nucleus Cytosol

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Abstract

We validated that a sensor for anhydrotetracycline (aTc) switches on in Nucleus Cytosol with the addition of 10 μ M aTc. aTc sensing results in the production of catecholase which converts colorless catechol into a yellow product that is observed at absorbance 385 nm.

1. OVERVIEW

The TetR protein binds to the Tet operator in DNA to repress transcription of the downstream gene. When bound to tetracycline or tetracycline derivatives, such as anhydrotetracycline, TetR no longer binds the operator DNA, allowing transcription to occur.

In this work, the Tet operator has been encoded upstream of a catecholase reporter (C23DO). The resulting biosensor is then added to a Nucleus Cytosol reaction supplemented with catechol, which is converted from a colorless substrate to a yellow product by catecholase. In this preliminary work, a homemade stock of TetR in glycerol was used. Glycerol may cause reaction poisoning in Nucleus Cytosol and is not an optimal storage buffer for TetR.

2. RESULTS

Comparing the absorbance at 385 nm over time demonstrates that the sensor is able to convert catechol from colorless to yellow faster to visual levels only in the presence of 10 μ M anhydrotetracycline in standard Nucleus Cytosol conditions with 20 nM of sensor DNA. Prior visual calibrations determined the visual threshold to be an absorbance level of 1.0. For each condition, a 10 μ l reaction was assembled with 1 mM catechol and incubated at 37 °C in a platereader.

3. CONCLUSION AND FUTURE STEPS

This preliminary test shows that the TetR sensor with a LacZ reporter is compatible with Nucleus Cytosol. Encapsulation of the sensor will inform whether 10 μ M aTc is sufficient for derepression and whether DNA template should be tuned to control leak.

Table 1. *Experiment conditions*

Condition	Description
Unregulated	Control sensor reaction without TetR
Regulated	Sensor reaction with TetR
Derepressed	Sensor reaction with TetR and aTc

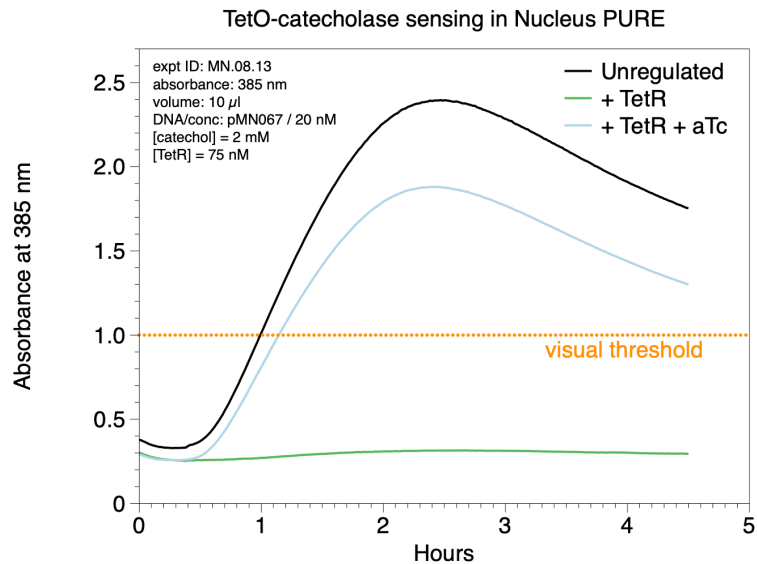
Table 2. *Reaction composition*

Component	Stock Concentration	Final concentration	Unregulated [μL]	Regulated [μL]	Derepressed [μL]
SMix	3.33x	1x	3	3	3
PMix	15 mg/mL	1.80 mg/mL	1.2	1.2	1.2
Ribosomes	10 μM	1.8 μM	1.8	1.8	1.8
tRNA	35 mg/mL	3.5 mg/mL	1	1	1
pT7-TetO-catecholase (pMN067)	275.7 nM	20 nM	0.73	0.73	0.73
DNA template					
Catechol	100 mM	1 mM	0.2	0.2	0.2
TetR	1305.6 nM	75 nM	0	0.57	0.57
anhydrotetracycline	100 μM	10 μM	0	0	1
RNAse Inhibitor	40000 U/mL	2000 U/mL	0.5	0.5	0.5
Water			1.57	1	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
TetR	Homemade*	Lucks Lab	N/A	N/A	-80C in glycerol	N/A


Figure 1. *Kinetics for colorimetric conversion of catechol into a yellow product.*

Catechol	Catechol 120-80-9	Tokyo Chemical Industry	P0567	\$36	4C in water at 100 mM	https://www. tcchemicals. com/MX/en/p/ P0567
Anhydrotetracycline	Anhydrotetracycline VETRANAL analytical standard	Sigma-Aldrich	37919	\$275	-20C in water at 5 mM	https://www. sigmaaldrich. com/US/en/ product/sial/ 37919?srltid= AfmBOor- kWLQUYIchxiC- ZxD-- uDKjnDc- uSqG_UDo6 Q_e35uec- Qnid

*Homemade TetR used as a temporary solution, though this preparation stored in glycerol is not ideal given glycerol poisoning effects against the Cytosol reaction