



Developer Note: deGFP expression using Nucleus Cytosol

Status	Shipped
Date	@November 3, 2025
Tags	© Nucleus
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Keywords	
Projects	 Nucleus Distribution v2.0
Project Status	Planning
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Summary	Research on deGFP expression using Nucleus Cytosol was conducted, comparing it with PURExpress. Experiments included varying Mg ²⁺ concentrations to optimize protein yield, with results indicating comparable activity at 8 mM Mg ²⁺ . Detailed compositions and setups for each reaction were provided.

Overview

This research notebook documents testing of Nucleus Cytosol by expressing deGFP using the pOpen-deGFP construct.

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 Notebook

▼ **4X SMix Composition**

Component	Stock concentration [mM]	Concentration of components in reaction [mM]	Concentration in SMix [mM]	Final volume to add [μL]
HEPES	1000	50	200	40.0
Potassium glutamate	2500	100	400	32.0
NTP	100	2	8	16.0
Creatine phosphate	1000	20	80	16.0
TCEP	500	1	4	1.6
Folinic acid	5	0.02	0.08	3.2
Spermidine	200	2	8	8.0
Amino Acid solution	3.25	0.3	1.2	73.8
Water				9.4
SMix total		Final concentration [fold]		Final volume
		4		200

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Experiment 1: pOpen-deGFP expression in Nucleus Cytosol and PURExpress

To test and compare the activity of Nucleus Cytosol, we expressed the pOpen-deGFP construct in both Nucleus Cytosol and PURExpress.

Five reactions were set up to test the activity:

1. Nucleus Cytosol (Pmix 08-02) + pOpen-deGFP (maxiprep + ethanol precipitation)
2. Nucleus Cytosol (Pmix 08-02) + pOpen-deGFP (maxiprep only)

3. Nucleus Cytosol (Pmix 08-02) without pOpen-deGFP template—*Negative Control*

4. PURExpress + pOpen-deGFP (maxiprep + ethanol precipitation)

5. PURExpress + pOpen-deGFP (maxiprep only)

The reaction setup for each condition is provided below. We prepared 35 μL of mastermix for each reaction and aliquoted $3 \times 10 \mu\text{L}$ into a 384-well plate for fluorescence measurement.

▼ Nucleus Cytosol (Pmix 08-02) + pOpen-deGFP (maxiprep + ethanol precipitation)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [μL]
4X SMix	4.00	x	1	x	8.75
Pmix (08-02)	15	mg/mL	1.80	mg/mL	4.20
Nucleus Ribosome	10	μM	1.8	μM	6.30
pOpen-deGFP DNA	231	ng/ μL	3	nM	0.83
Nucleus tRNA	35	mg/ml	3.5	mg/ml	3.50
Mg-Acetate	200	mM	8	mM	1.40
Water					10.02
Total volume [μL]					
35					
Calculation for DNA concentration	DNA length [bp]	Unit	Avg. MW of bp	Unit	
pOpen-deGFP DNA	2812	bp	650	g/mol	
		bp	650	g/mol	

▼ Nucleus Cytosol (Pmix 08-02) + pOpen-deGFP (maxiprep only)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [μL]
4X SMix	4.00	x	1	x	8.75
Pmix (08-02)	15	mg/mL	1.80	mg/mL	4.20
Nucleus Ribosome	10	μM	1.8	μM	6.30
pOpen-deGFP DNA	155	ng/ μL	3	nM	1.24

Nucleus tRNA	35	mg/ml	3.5	mg/ml	3.50
Mg-Acetate	200	mM	8	mM	1.40
Water					9.61
Total volume [μL]					
35					
Calculation for DNA concentration	DNA length [bp]	Unit	Avg. MW of bp	Unit	
pOpen-deGFP DNA	2812	bp	650	g/mol	
		bp	650	g/mol	

▼ Nucleus Cytosol (Pmix 08-02) without pOpen-deGFP template—*Negative Control*

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [μL]
4X SMix	4.00	x	1	x	8.75
Pmix (08-02)	15	mg/mL	1.80	mg/mL	4.20
Nucleus Ribosome	10	μM	1.8	μM	6.30
pOpen-deGFP DNA	115	ng/μL	0	nM	0.00
Nucleus tRNA	35	mg/ml	3.5	mg/ml	3.50
Mg-Acetate	200	mM	8	mM	1.40
Water					10.85
Total volume [μL]					
35					
Calculation for DNA concentration	DNA length [bp]	Unit	Avg. MW of bp	Unit	
pOpen-deGFP DNA	2812	bp	650	g/mol	
		bp	650	g/mol	

▼ PURExpress + pOpen-deGFP (maxiprep + ethanol precipitation)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [μL]
SolA	2.50	x	1	x	14.00
Sol B	8	mg/mL	2.40	mg/mL	10.50
pOpen-deGFP DNA	231	ng/μL	3	nM	0.83
Water					9.67
Total volume [μL]					
35					
Calculation for DNA concentration	DNA length [bp]	Unit	Avg. MW of bp	Unit	
pOpen-deGFP DNA	2812	bp	650	g/mol	
		bp	650	g/mol	

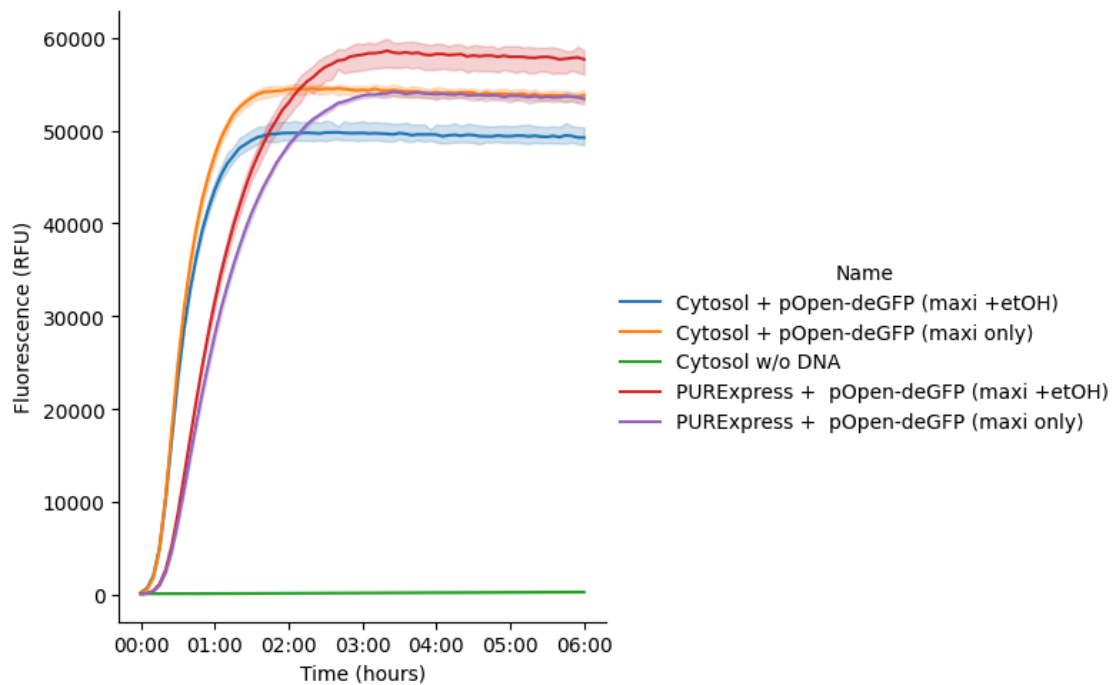
▼ PURExpress + pOpen-deGFP (maxiprep only)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [μL]
SolA	2.50	x	1	x	14.00
Sol B	8	mg/mL	2.40	mg/mL	10.50
pOpen-deGFP DNA	155	ng/μL	3	nM	1.24
Water					9.26
Total volume [μL]					
35					
Calculation for DNA concentration	DNA length [bp]	Unit	Avg. MW of bp	Unit	

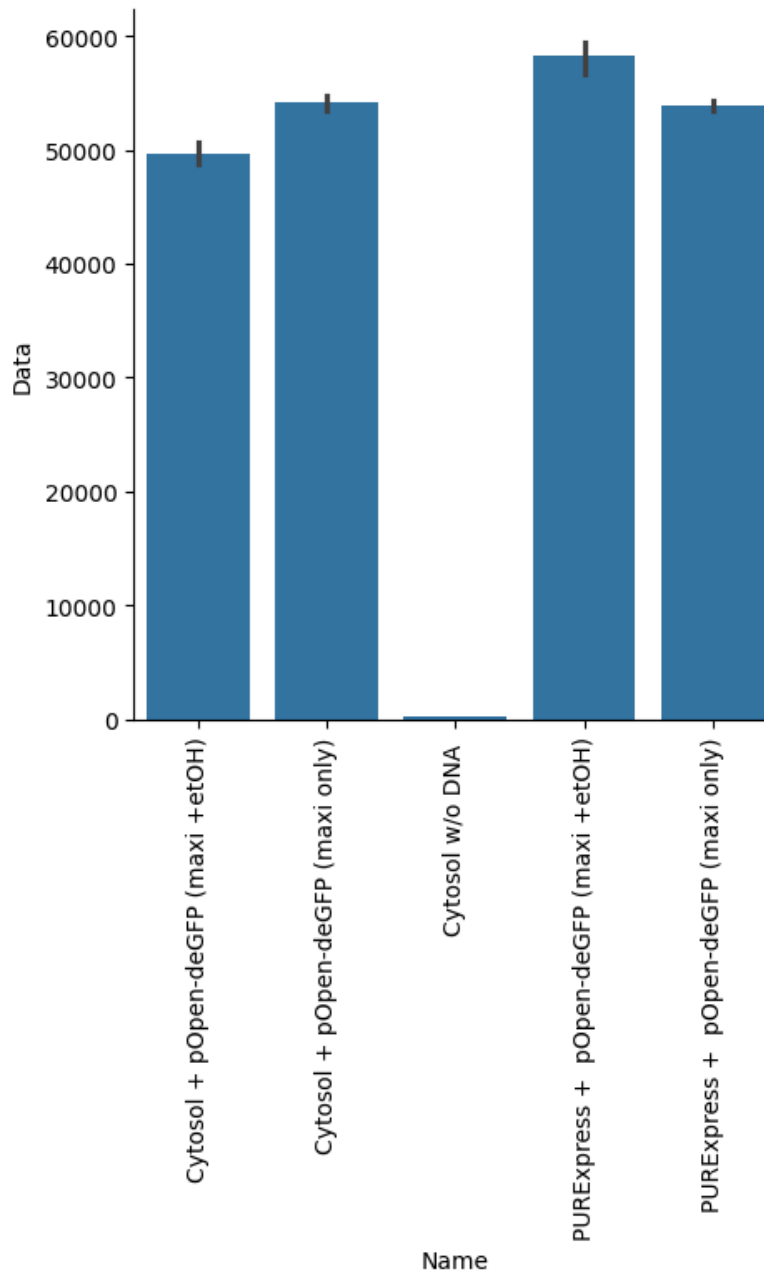
pOpen-deGFP DNA	2812	bp	650	g/mol	
		bp	650	g/mol	

Results:

▼ Kinetics



▼ Steady state



@November 7, 2025

Experiment 2: Mg²⁺ sweep in Nucleus Cytosol

To optimize protein expression yield in Nucleus Cytosol, we performed a Mg-acetate titration across a range of 4–12 mM in 2 mM increments. The reaction setup for each condition is provided below. We prepared 35 μ L of mastermix for each reaction and aliquoted $3 \times 10 \mu$ L into a 384-well plate for fluorescence measurement. All subsequent experiments used maxiprepmed pOpen-deGFP cleaned up with ethanol precipitation.

▼ 4 mM

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [μ L]
4X SMix	4.00	x	1	x	8.75
Pmix (08-02)	15	mg/mL	1.80	mg/mL	4.20
Nucleus Ribosome	10	μ M	1.8	μ M	6.30
pOpen-deGFP DNA	231	ng/ μ L	3	nM	0.83
Nucleus tRNA	29.6	mg/ml	3.5	mg/ml	4.14
Mg-Acetate	200	mM	4	mM	0.70
Water					10.08
Total volume [μ L]					
35					
Calculation for DNA concentration	DNA length [bp]	Unit	Avg. MW of bp	Unit	
pOpen-deGFP DNA	2812	bp	650	g/mol	
		bp	650	g/mol	

▼ 6 mM

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [μ L]
4X SMix	4.00	x	1	x	8.75
Pmix (08-02)	15	mg/mL	1.80	mg/mL	4.20
Nucleus Ribosome	10	μ M	1.8	μ M	6.30
pOpen-deGFP DNA	231	ng/ μ L	3	nM	0.83
Nucleus tRNA	29.6	mg/ml	3.5	mg/ml	4.14
Mg-Acetate	200	mM	6	mM	1.05
Water					9.73

Total volume [μL]					
35					
Calculation for DNA concentration	DNA length [bp]	Unit	Avg. MW of bp	Unit	
pOpen-deGFP DNA	2812	bp	650	g/mol	
		bp	650	g/mol	

▼ 8 mM

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [μL]
4X SMix	4.00	x	1	x	8.75
Pmix (08-02)	15	mg/mL	1.80	mg/mL	4.20
Nucleus Ribosome	10	μM	1.8	μM	6.30
pOpen-deGFP DNA	231	ng/μL	3	nM	0.83
Nucleus tRNA	29.6	mg/ml	3.5	mg/ml	4.14
Mg-Acetate	200	mM	8	mM	1.40
Water					9.38
Total volume [μL]					
35					
Calculation for DNA concentration	DNA length [bp]	Unit	Avg. MW of bp	Unit	
pOpen-deGFP DNA	2812	bp	650	g/mol	
		bp	650	g/mol	

▼ 10 mM

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [μL]
4X SMix	4.00	x	1	x	8.75

Pmix (08-02)	15	mg/mL	1.80	mg/mL	4.20
Nucleus Ribosome	10	μM	1.8	μM	6.30
pOpen-deGFP DNA	231	ng/μL	3	nM	0.83
Nucleus tRNA	29.6	mg/ml	3.5	mg/ml	4.14
Mg-Acetate	200	mM	10	mM	1.75
Water					9.03
Total volume [μL]					
35					
Calculation for DNA concentration	DNA length [bp]	Unit	Avg. MW of bp	Unit	
pOpen-deGFP DNA	2812	bp	650	g/mol	
		bp	650	g/mol	

▼ 12 mM

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [μL]
4X SMix	4.00	×	1	×	8.75
Pmix (08-02)	15	mg/mL	1.80	mg/mL	4.20
Nucleus Ribosome	10	μM	1.8	μM	6.30
pOpen-deGFP DNA	231	ng/μL	3	nM	0.83
Nucleus tRNA	29.6	mg/ml	3.5	mg/ml	4.14
Mg-Acetate	200	mM	12	mM	2.10
Water					8.68
Total volume [μL]					
35					
Calculation for DNA	DNA length [bp]	Unit	Avg. MW of bp	Unit	

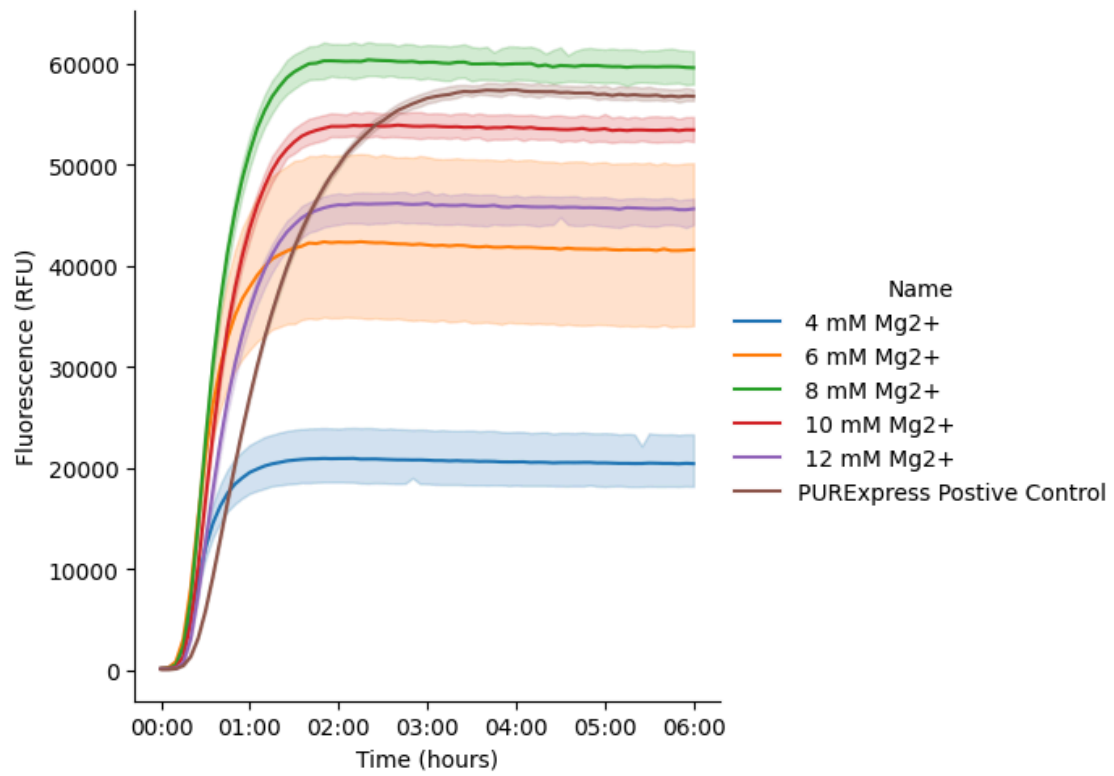
concentration					
pOpen-deGFP DNA	2812	bp	650	g/mol	
		bp	650	g/mol	

▼ PURExpress Positive Control

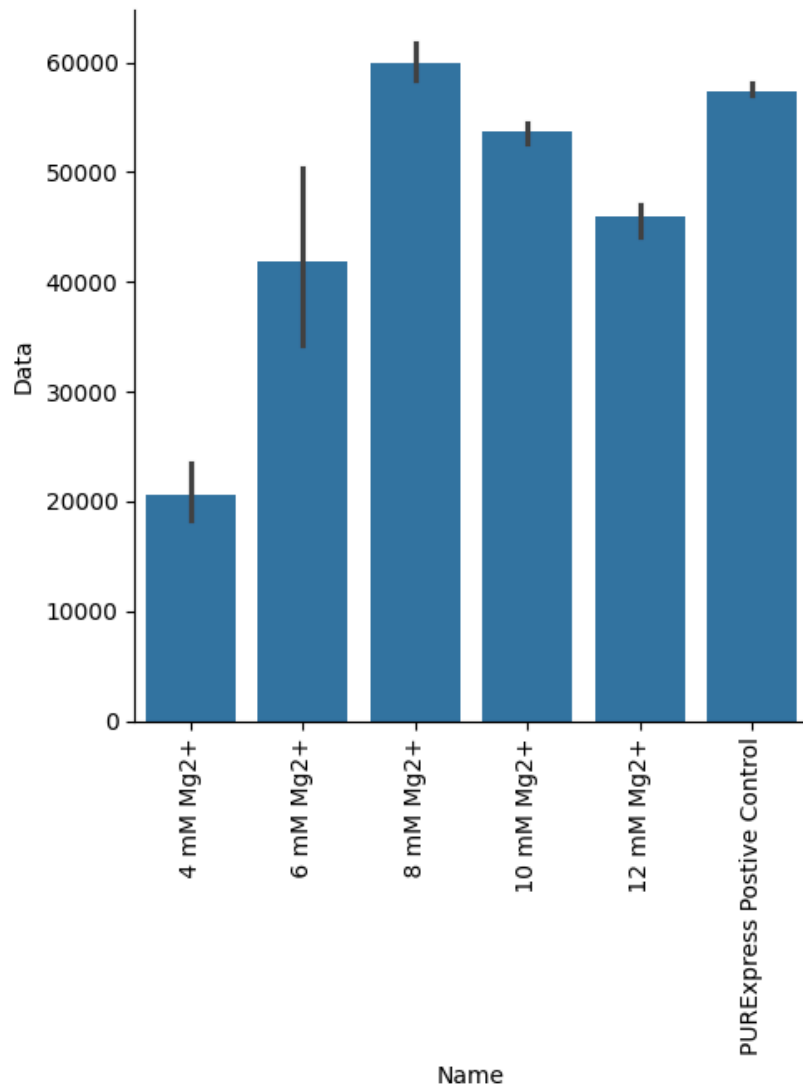
Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [μL]
SolA	2.50	x	1	x	14.00
Sol B	8	mg/mL	2.40	mg/mL	10.50
pOpen-deGFP DNA	231	ng/μL	3	nM	0.83
Water					9.67
Total volume [μL]					
35					
Calculation for DNA concentration	DNA length [bp]	Unit	Avg. MW of bp	Unit	
pOpen-deGFP DNA	2812	bp	650	g/mol	
		bp	650	g/mol	

Results

▼ Kinetics



▼ Steady state



Conclusion

Nucleus Cytosol shows activity comparable to PURExpress for deGFP expression at a final Mg2+ concentration of 8 mM.