



DNA toolkit: T7 promoter testing

Status	Write Up
Tags	
Researchers	© Charlie Newell
glycine directory	bnex/experiments/20250819-DNA-toolkit-T7-promoter
Keywords	
Projects	📍 DNA Toolkit
Project Status	In Progress
Project Team	『ACJS』
Summary	The research focuses on quantifying the performance of the 'pURET7' promoter collection, which includes 10 T7 promoters designed for varying expression levels. The study aims to replicate and expand upon previous testing to enable better control of gene expression in synthetic biology applications. Experimental workflows involve plasmid preparation, sequencing, and testing promoter strength using fluorescence signals. The results indicate a dynamic range of expression across promoters, with deGFP showing higher sensitivity compared to plamGFP. Overall, the findings suggest that the number of mutations in promoter sequences correlates with expression strength, highlighting the importance of reporter choice in quantification.

Overview

This work seeks to quantify the performance of the 'pURET7' promoter collection. This will aid in the future development of DNA constructs which have defined and controllable rates of expression. This work sits under the broader scope of the DNA toolkit project.

Background

The 'pURET7' promoter collection curated by ACJS consists of 10 T7 promoters selected across two different libraries - the phage T7 phage genome engineered by Chan, Kosuri and Endy¹, and the T7 promoter variant library constructed and tested in vitro by Komura², et al. The pURET7 collection was designed to include promoters which express at low, medium, and high levels, and span a 100-fold dynamic range. This enables DNA constructs to be designed with a high-level of expression control to suit a range of synthetic biology applications.

This work seeks to replicate and build upon the work done by ACJS to repeat the testing of the entire pURET7 collection both by hand, and using the OpenTrons. Once characterised, the collection can be integrated into the nucleus distribution.

Contents

Experimental background

[AmajLime collection](#)

[pURE plasmid prep \(25.08.19\)](#)

[24-well colony plates \(25.08.18\)](#)

[Outgrowth cultures \(25.08.19\)](#)

[Miniprep \(25.08.21\)](#)

[Sequencing \(25.08.22\)](#)

[Miniprep \(25.08.23/24\)](#)

[Linearisation PCR #1 \(25.08.25\)](#)

[DNA cleanup \(25.08.28\)](#)

[Amplicon sequencing](#)

[PURE test](#)

[Linearisation PCR #2 \(25.08.29\)](#)

[PURE test \(25.08.29\)](#)

[Linearisation PCR #2 - Cleanup \(25.08.29\)](#)

[PURE test: Optimising gain \(25.09.02\)](#)

[@September 9, 2025: PURE test: Linear T7-1, T7-2 and T7-10 \(15 µL, 12 hours, endpoint\)](#)

[@September 11, 2025: PURE test: Linear T7-1:10](#)

[PlamGFP/deGFP collection](#)

References

1. Chan LY, Kosuri S, Endy D. Refactoring bacteriophage T7. Mol Syst Biol. 2005;1:2005.0018. doi: 10.1038/msb4100025. Epub 2005 Sep 13. PMID: 16729053; PMCID: PMC1681472.
2. Komura R, Aoki W, Motone K, Satomura A, Ueda M (2018) High-throughput evaluation of T7 promoter variants using biased randomization and DNA barcoding. PLOS ONE 13(5): e0196905. <https://doi.org/10.1371/journal.pone.0196905>

[@September 12, 2025: Designing pURET7 primers to amplify plamGFP/deGFP.](#)

[@September 17, 2025: Gradient PCR to find optimum Ta for pURET7-plamGFP/deGFP](#)

[@September 18, 2025: PCR scale-up for pURET7-1,3:10-plamGFP/deGFP](#)

[@September 22, 2025 → September 25, 2025: pURET7-plamGFP-deGFP w/ @Astrid Jørgensen](#)

[Final data analysis](#)

[Promoter recap](#)

[AmajLime](#)

[PlamGFP/deGFP](#)

[Summary data](#)

[Kinetic curves](#)

[Overall conclusions](#)

Experimental background

The pURET7 collection (1-10) are encoded in a pOpen plasmid upstream of 'amajLime' enabling the strength of each promoter to be tested by proxy of fluorescence signal measured in a fluorescence plate reader. The collection has been transformed into E.coli and stored as glycerol stocks within the [Nucleus v0.1.0-001 distribution plate](#). The location of each promoter construct is outlined below.

Promoter	Location
pURET7-1	G6
pURET7-2	C9
pURET7-3	B7
pURET7-4	E7
pURET7-5	D10
pURET7-6	B8
pURET7-7	A7
pURET7-8	C10
pURET7-9	F10
pURET7-10	D7

As a short summary of the experimental workflow for this element of the toolkit, purified plasmids will first need to be obtained from the above glycerol stocks and then sequence verified. This will be done by streaking out glycerol stocks, isolating individual colonies followed by outgrowth, miniprep, and plasmid sequencing. After sequence verification, T7pro-amajLime-T7term sequences will be linearised from the plasmids through PCR, generating stocks to be used in PURE reactions. PURE reactions will be prepared using both plasmid and linearised stocks at a variety of different concentrations both by hand and also by the OpenTrons. This will enable the strength of each promoter to be assessed, along with PURE performance when using plasmid compared to linearised DNA, and also the ability for the OpenTrons to robustly reproduce results generated from hand-prepared PURE reactions.

AmajLime collection

pPURE plasmid prep (25.08.19)

Aim

To inoculate 24-well colony plates of each of the 10 strains before outgrowth, mini prep, and sequence verification in the following days.

Notes: pURET7 glycerol stocks are located in the [Nucleus v0.1.0-001 distribution plate](#) in the left-hand side -80°C within the foundry. Glycerol stocks stored in 96-well plates sealed with foil.

24-well colony plates (25.08.18)

Colonies are isolated through inoculating serial dilutions of inoculum of each strain into the columns of a 24-well culture plate (1.5 % LB agar, 1x Carb). The serial dilutions (20:180 µL) are generated from an initial well which receives a pipette tip containing a small quantity of glycerol stock. This initial well, along with 3 other serial dilutions are plated (20 µL). The serial dilutions are prepared in 96-well plates with 1 column spacing between samples which enables the pipettor to use a multi-channel pipette in the same spatial arrangement as the plate. Once plated, the samples are incubated (37°C, ~ 16hrs).

Results

Colonies were present on all plates. Colonies derived from pURET7-1:6 were very small and required ~2hrs extra 37 °C. Colonies from pURET7-7:10 were large and easier to pick. Colonies picked from the most diluted sample well where possible.

Outgrowth cultures (25.08.19)

Single colonies were picked for each strain and deposited in 14 mL culture tubes (3 mL LB, 1x carb) and incubated (37°C, ON).

Notes: It was difficult to pick colonies from pURET7-1:6 due to their very small size. It is expected that at least one of these may not grow.

Results

All colonies except pURET7-10 were cultured successfully contrary to my previous assumption. pURET7-10 left in the incubator to see if it grows. pURET7-10 successfully grew after ~4hrs extra in the incubator. Whilst all strains grew, the density of the cultures was rather low, I suspect this was caused due to excessive fixing of the cap and not allowing the cultures to breathe.

- Pellets were stored at 4°C O/N. Next time they will instead be pelleted and stored at -20 °C.

Miniprep (25.08.21)

All cultures were mini prepped and eluted in 35 µL elution buffer EY.

Notes: After pelleting (4000xG / 1 min) the resultant pellets were very small due to the low density of cultures.

Results

Sample	Conc (ng/µL)
pURET7-1	33
pURET7-2	12
pURET7-3	17.4
pURET7-4	25.8
pURET7-5	24
pURET7-6	29
pURET7-7	26
pURET7-8	35
pURET7-9	30.4
pURET7-10	16.9

- DNA concentrations all low.
- Samples will be sent for sequencing regardless to check verify their sequence. The samples in which align correctly to their reference sequence will be used as stocks for gene linearisation.

Notes: A fresh set of overnight cultures will be prepared in 50 mL falcon tubes (5 mL LB, 1x carb) and mini prepped and sent for sequencing (25.08.22).

Sequencing (25.08.22)

All pURET7 plasmids (Miniprep round #1) were sent for plasmidsaurus WPS. Sequences were aligned to the nucleus v0.1.0-001 distribution reference sequences using SnapGene. All sequences displayed successful alignment. The only discrepancies were two conserved point mutations across all sequences, the first being a single base insertion upstream of the T7pro sequence and a C→T substitution in the 'upstream anneal' feature. These should not interfere with downstream experiments.

- These plasmids will be used as PCR templates to amplify linear DNA constructs (5' T7pro - amajLime - T7term 3').

Miniprep (25.08.23/24)

Results

Sample	Conc (ng/μL)	Vol DNA for 30 ng / μL (μL)	Vol H2O for 30 ng / μL (μL)
pURET7-1	69.4	6.5	8.5
pURET7-2 #1	122.2	3.7	11.3
pURET7-2 #2	132.4	3.4	11.6
pURET7-3	80.2	5.6	9.4
pURET7-4	77.9	5.8	9.2
pURET7-5	92.6	4.9	10.1
pURET7-6 #1	36.5	12.3	2.7
pURET7-6 #2	46.1	9.8	5.2
pURET7-7	127.2	3.5	11.5
pURET7-8	125.8	3.6	11.4
pURET7-9	134.7	3.3	11.7
pURET7-10	164.4	2.7	12.3

Note: All samples that took a long time to grow - primarily sample 2 and sample 6 (2 days) - were derived from teeny tiny colonies. To solve this problem I simply need to do a new set of 24 well colony plates to get large juicy colonies for all samples. For the time being, if these sequences come back correct, I should have a reasonable amount of plasmid to work with for a few PURE rxns.

- **Sequencing results** - All samples aligned correctly to their reference sequence. Just one recurring point mutation (C→>T substitution in the 'upstream anneal' feature).

Linearisation PCR #1 (25.08.25)

- Using the original purified plasmids, each pURET7 TU will be linearised through PCR using the M13fwd and the T7rev primer sequences using the NEB Q5 2x MM kit. As there are 10 sequences, for the first run I won't do any repeats and I won't do a gradient PCR. I will simply do a single 25 μL reaction for each sample and see how it goes. If it isn't successful, further optimisation will need to be done.
- Conditions: Denaturing - 98°C 30 secs. 30 cycles: denaturing - 98°C 5 secs, Annealing - 63°C 30 secs, extension 72°C, 25 secs. Final extension 72°C, 2 mins. Hold - 10°C, infinity.

Note: Each pURET7 template will need to be normalised to 1 ng/μL

Template prep (1 ng / μL)

Template dilutions (1 ng / μL)	Template	V DNA (μL)	V H2O (μL)	Total	Template Cl (ng / μL)
	pURET7-1	0.5	14.5	15.0	33
	pURET7-2	1.3	13.8	15.0	12

	pURET7-3	0.9	14.1	15.0	17.4
	pURET7-4	0.6	14.4	15.0	25.8
	pURET7-5	0.6	14.4	15.0	24
	pURET7-6	0.5	14.5	15.0	29
	pURET7-7	0.6	14.4	15.0	26
	pURET7-8	0.4	14.6	15.0	35
	pURET7-9	0.5	14.5	15.0	30.4
	pURET7-10	0.9	14.1	15.0	16.9

Reaction prep

Standard reaction					
	Component	Final Concentration	Vol (μL)		
	Q5 High-Fidelity 2X Master Mix	1X	12.5		
	10 μ M Forward Primer	0.5 μ M	1.25		
	10 μ M Reverse Primer	0.5 μ M	1.25		
	Template	0.04 ng/ μ L (1 ng total)	1		
	Nuclease-Free Water		9		
	Total		25		
	Total (11x)		275		
Standard reaction (- template)	Component	Final Concentration	Vol (μL)	Vol 11x (μL)	
	Q5 High-Fidelity 2X Master Mix	1X	12.5	137.5	
	10 μ M Forward Primer	0.5 μ M	1.25	13.75	
	10 μ M Reverse Primer	0.5 μ M	1.25	13.75	Distribute 24 μ L into 10x tubes
	Nuclease-Free Water		9	99	
	Total		24	264	
Template addition	Sample	V DNA	Total		
	pURET7-1	1	25		
	pURET7-3	1	25		
	pURET7-4	1	25		
	pURET7-5	1	25		
	pURET7-6	1	25		
	pURET7-7	1	25		
	pURET7-8	1	25		
	pURET7-9	1	25		
	pURET7-10	1	25		

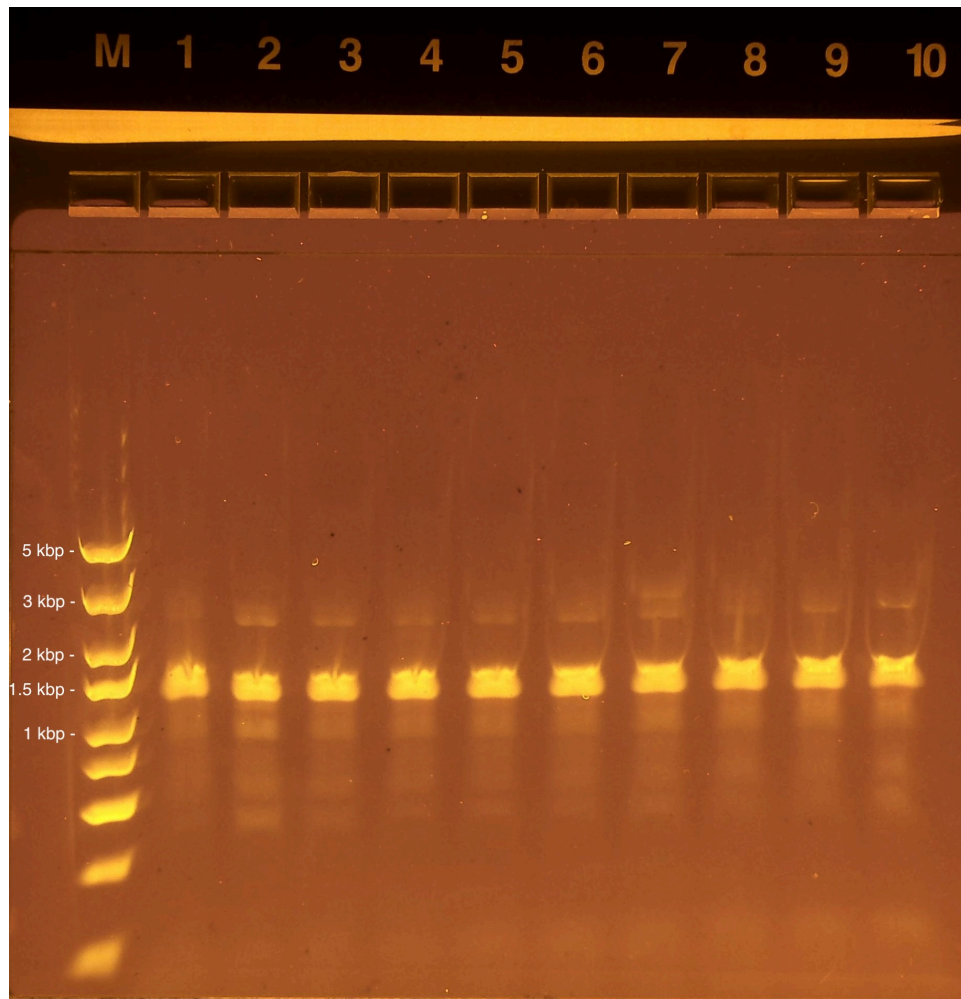
Results

- 20 μ L Samples (10 μ L 0.5x PCR product, 10 μ L Express running buffer) were run on an EX 1% gel for ~12.5 mins.
- Estimated amplicon size = 1.06 kbp

Lanes

M - Ex 1kb plus ladder

1-10 - Linear pURET7-1:10



- Amplicons of ~ 1.5 kbp present in all samples. hmm. ~500bp larger than estimated amplicon. Doesn't appear to be any secondary primer binding sites on the plasmid.
- Seems to be a reasonable amount of off-target amplification in all samples. 1 band present in all samples around the estimated amplicon size. Not familiar with E-gels though and they do use SYBR gold II which I know is highly sensitive so this may not be of great concern.

Plan

☒ ~~DpnI digest followed by PCR cleanup~~

Note: NEB states that 5 units of DpnI will cleave ~50% of 1 µg template in a 50 µL Q5 HF PCR rxn. This reaction contained 1ng of template, theoretically requiring = 0.01 units (0.005 units *2) at these conditions. DpnI is provided at 20 units/ µL. 0.2 µL (4 units / lowest pipettable volume) should easily suffice to degrade the 1 ng template in their current buffer.

☒ ~~Amplicon sequence~~

☒ ~~Test in PURE(triplicate)~~

☒ ~~linear pURET7-7~~

- ☒ linear pURET7-9
- ☒ Negative
- ☒ Positive pURET7-9

DNA cleanup (25.08.28)

- All Samples were subject to DpnI digest (0.2 μ L, 37°C, 1hr) prior to cleanup using the monarch spin PCR and DNA cleanup kit.
- Samples eluted in 15 μ L buffer EY

Results

Sample	Conc (ng/ μ L)
Linear pURET7-1	9.1
Linear pURET7-2	15.1
Linear pURET7-3	13.7
Linear pURET7-4	14.2
Linear pURET7-5	12.6
Linear pURET7-6	16
Linear pURET7-7	15.2
Linear pURET7-8	12.7
Linear pURET7-9	15.4
Linear pURET7-10	7.9

- Low concentration in all samples
 - Scale up inbound.
- Samples pURET7-4 and pURET7-6 will be sent for amplicon sequencing
- Samples pURET7-7 and pURET7-9 will be tested in PURE

Amplicon sequencing

pURET7-4

Expected size: 1063bp

- Size: 1247
- Amplicon contains ~200bp of the AmpR gene. Coverage plot indicates that the majority is the expected product. Additional sequence must result from parental plasmid carry over, or poor primer specificity.
- Fix (parental plasmid carry over): PCR cleanup prior to DpnI digest followed by 2nd round of cleanup / gel purification.**
- Fix (poor primer specificity): Design new fwd primer / gel purification.

pURET7-6

Expected size: 1063bp

- Size: 1031
- Expected amplicon was generated.

Summary

- Amplification will be scaled up to 75 μL with 35 cycles.

PURE test

- Linear pURET7-7 and pURET7-9 (4.2 ng / μL) were expressed in PURE reactions (10 μL , triplicate) to test their activity.
- pURET7-9 (plasmid) used as positive control.
- New protocol setup 'amajLime-PURE-timecourse' (Cytation 3/5)
 - Ex 458, Em 486
 - **Gain: 70** - Will this be enough? how does auto-adjust work? - **Need to understand cytations better.**
- Note: pLam GFP also tested for QC for Jon.

Standard reaction						
	Component	Starting concentration	Final Concentration	Vol (μL)		
	Solution A	2.5x	1X	12.0		
	Solution B	3.3x	1x	9.0		
	Murine RNase inhibitor	40 U / μL	1 U / μL	0.75		
	DNA Template (100 ng / μL)	—	5 ng / μL	1.5		
	Nuclease-Free Water	—	—	6.7		
	Total			30		
Standard reaction (-DNA, -H2O)	Component	Starting concentration	Final Concentration	Vol (μL)	Vol for 5.5x rxns (μL)	
	Solution A	2.5x	1X	12.0	66.0	
	Solution B	3.3x	1x	9.0	49.5	
	Murine RNase inhibitor	40 U / μL	1 U / μL	0.75	4.1	Distribute 21.8 μL in 5x tubes
	Total			30		
	Total (check)			21.8	119.7	
Template addition	Sample	V DNA	V H2O	Total		
	pURET7-9	8.2	0	30.0		
	pURET7-7	8.2	0	30.0		
	Negative	0	8.2	30.0		
	Positive	1.5	6.7	30		
	pLAM	1.5	6.7	30		
Template dilutions (100 ng / μL)	Template	V DNA (μL)	V H2O (μL)	Total	Template CI (ng / μL)	
	Positive (plasmid - pURET7-9)	3.7	1.3	5.0	134.7	

- Linearisation was successful at low yield. Purification was not.

- (+ ve) Plasmid pURET7-9 was active (high)
- Linear pURET7-9 was active (**low**)
- Linear pURET7-7 was inactive
- Inactivity of pURET7-7 is suspected to be due to salt carry over from poor cleanup and a result of constituting ~8% of total rxn volume.
 - **Fix: Make more.**
- End-point RFU of +ve control was low compared to plamGFP. **Gain settings need to be adjusted.**

Linearisation PCR #2 (25.08.29)

- Scale-up of linearisation PCR #1 - 35 cycles, 75 µL vols.
- Conditions: Denaturing - 98°C 30 secs. 35 cycles: denaturing - 98°C 5 secs, Annealing - 63°C 30 secs, extension 72°C, 25 secs. Final extension 72°C, 2 mins. Hold - 10°C, infinity.

Standard reaction					
	Component	Final Concentration	Vol for 25 µL (µL)	Vol for 75 µL (µL)	
	Q5 High-Fidelity 2X Master Mix	1X	12.5	37.5	
	10 µM Forward Primer	0.5 µM	1.25	3.75	
	10 µM Reverse Primer	0.5 µM	1.25	3.75	
	Template	0.04 ng/ µL (1 ng total)	1	1	
	Nuclease-Free Water		9	29	
	Total		25	75	
	Total (11x)		275	825	
Standard reaction (- template)	Component	Final Concentration	Vol (µL)	Vol 11x (µL)	
	Q5 High-Fidelity 2X Master Mix	1X	37.5	412.5	
	10 µM Forward Primer	0.5 µM	3.75	41.25	
	10 µM Reverse Primer	0.5 µM	3.75	41.25	Distribute 74 µL into 10x tubes
	Nuclease-Free Water		29	319	
	Total		74	814	
Template addition	Sample	V DNA	V H2O	Total	
	pURET7-1	1	0	75	
	pURET7-3	1	0	75	
	pURET7-4	1	0	75	
	pURET7-5	1	0	75	Split 75 µL rxns into 3x 25 uL
	pURET7-6	1	0	75	
	pURET7-7	1	0	75	
	pURET7-8	1	0	75	
	pURET7-9	1	0	75	

	pURET7-10	1	0	75	
--	-----------	---	---	----	--

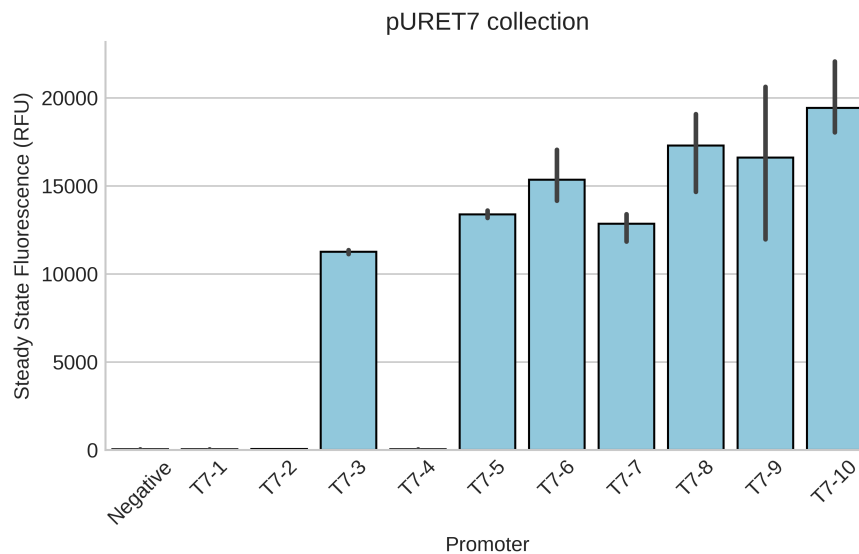
PURE test (25.08.29)

- Plasmid pURET7-1:10 (5 ng/μL) were expressed in PURE reactions (10 μL, triplicate) to test their activity.
- Gain increased to **80**.

Standard reaction						
	Component	Starting concentration	Final Concentration	Vol (μL)		
	Solution A	2.5x	1X	12.0		
	Solution B	3.3x	1x	9.0		
	Murine RNase inhibitor	40 U / μL	1 U / μL	0.75		
	DNA Template (100 ng / μL)	40	5 ng / μL	3.75		
	Nuclease-Free Water	—	—	4.5		
	Total			30		
Standard reaction (-DNA, -H2O)	Component	Starting concentration	Final Concentration	Vol (μL)	Vol for 10.5x rxns (μL)	
	Solution A	2.5x	1X	12.0	126.0	
Scale factor	Solution B	3.3x	1x	9.0	94.6	
10.5	Murine RNase inhibitor	40 U / μL	1 U / μL	0.75	7.9	Distribute 21.8 μL into 10x tubes.
	Total			30		Oops forgot the -ve control. Extra single reaction prepared.
	Total (check)			21.8	228.5	
Template addition	Sample	V DNA	V H2O	Total		
	Negative	0	8.2	30.0		
	pURET7-1	3.75	4.49	30.0		
	pURET7-2	3.75	4.49	30.0		
	pURET7-3	3.75	4.49	30.0		
	pURET7-4	3.75	4.49	30.0		
	pURET7-5	3.75	4.49	30.0		
	pURET7-6	3.75	4.49	30.0		
	pURET7-7	3.75	4.49	30.0		
	pURET7-8	3.75	4.49	30.0		
	pURET7-9	3.75	4.49	30.0		
	pURET7-10	3.75	4.49	30.0		
Template dilutions (40 ng/μL)	Sample	Conc (ng/μL)	V DNA	V H2O	Total	
	pURET7-1	69.4	5.76	4.24	10.00	
	pURET7-2 #1	122.2	3.27	6.73	10.00	

pURET7-3	80.2	4.99	5.01	10.00	
pURET7-4	77.9	5.13	4.87	10.00	
pURET7-5	92.6	4.32	5.68	10.00	
pURET7-6 #2	46.1	8.68	1.32	10.00	
pURET7-7	127.2	3.14	6.86	10.00	
pURET7-8	125.8	3.18	6.82	10.00	
pURET7-9	134.7	2.97	7.03	10.00	
pURET7-10	164.4	2.43	7.57	10.00	

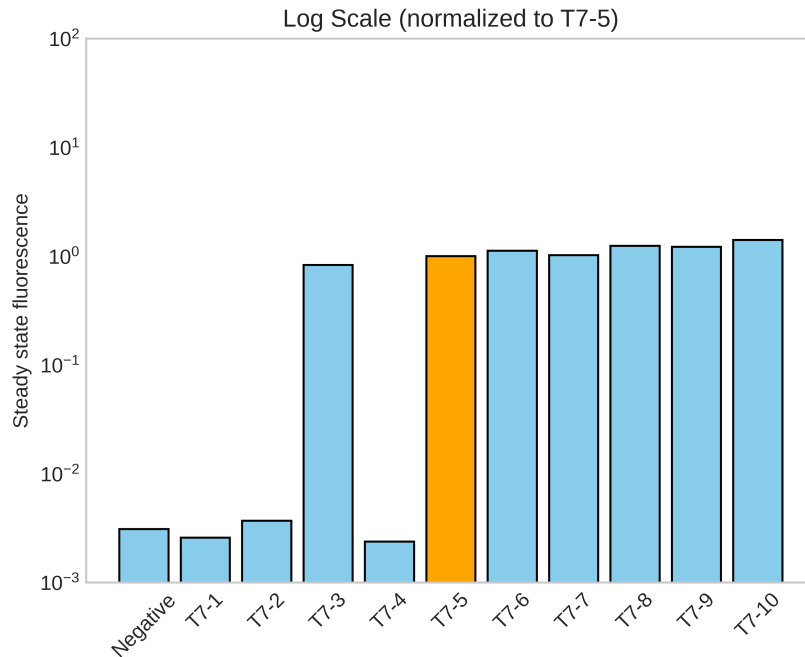
Results



- Active: pURET7 3, and pURET7-6:10
 - Samples 3 and 5-10 roughly showed trend of increasing fluorescent signal. good.
- Inactive: pURET7-1:2, and pURET7-4
 - Interesting that pURET7-4 was inactive. **Didn't add DNA? Signal too low?**

Problem: Gain setting too low.

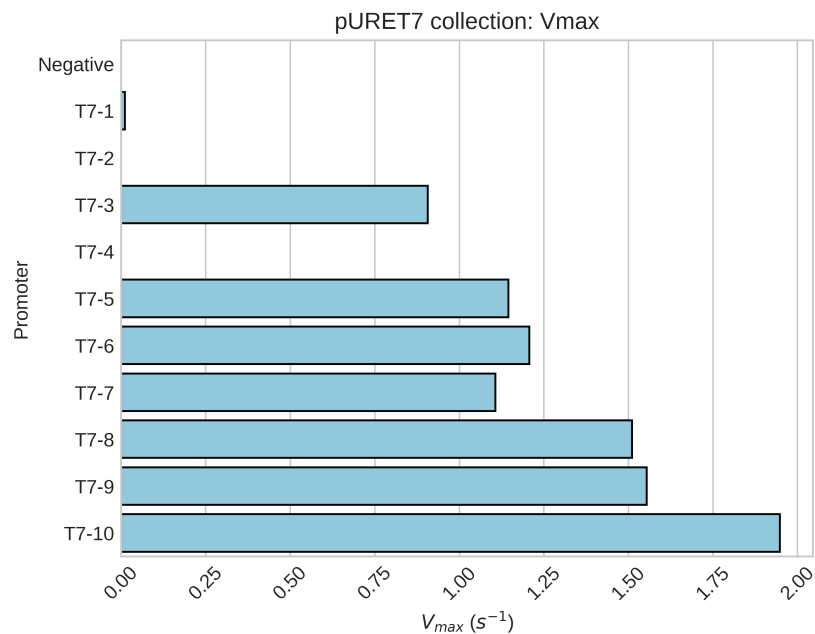
- Fix: Auto-adjust gain for pURET7-1. Use gain setting for all samples, highly unlikely that it will overexpose pURET7-10.
 - Gain 90 - 93 should satisfy entire dynamic range.



- Difficulty distinguishing steady state fluorescence values between promoters 3-10.
 - Perhaps this can't be represented using the log scale. Trade-off between resolution of high range promoters and signal detection of promoters 1 and 2.

Problem: no signal from T7-1 and T7-2, yet all other reactions gone to completion.

- **Fix:** Generate enough signal in T7-1 and T7-2 to choose steady state fluorescence values when the reaction is ~75% through completion.
 - Increase reaction volume
 - Further optimise gain settings



- Positive trend in Vmax across promoters
- Should be able to distinguish between promoters 3-10 with optimised settings.

Next steps

- ☒ ~~Test Linear/plasmid pURET7-1 and pURET7-10 on same plate with gain 90.~~
 - ☒ ~~Auto-adjust gain settings on pURET7-1 wells.~~
 - ☒ ~~Nanodrop pURET7-4 DNA dilution~~
 - pURET7-4 @ 42 ng / μ L - **Didn't add DNA then?**
- ☐ Test Linear pURET7-1:10
 - ☒ ~~Cleanup linearisation PCR #2~~
 - ☒ ~~DpnI digest~~
 - ☒ ~~Cleanup~~

Linearisation PCR #2 - Cleanup (25.08.29)

- All samples were cleaned up using the monarch spin PCR and DNA cleanup kit.
- Samples eluted in 20 μ L buffer EY (Rough DNA conc across samples : 90 ng / μ L)
- DpnI digest (0.2 μ L, 4 units) in 1x rcutsmart buffer. Total rxn vol: 50 μ L, incubated (1hr, 37°C, 350rpm).
- Final Monarch spin PCR and DNA cleanup kit.
- Samples eluted in 15 μ L buffer EY.

Sample	Conc (ng/ μ L)
Linear pURET7-1	92.2
Linear pURET7-2	103.8
Linear pURET7-3	105.7
Linear pURET7-4	102.5
Linear pURET7-5	106.8
Linear pURET7-6	99.9
Linear pURET7-7	110.6
Linear pURET7-8	102
Linear pURET7-9	101
Linear pURET7-10	102.8

- Consistent good quality, high concentration samples.

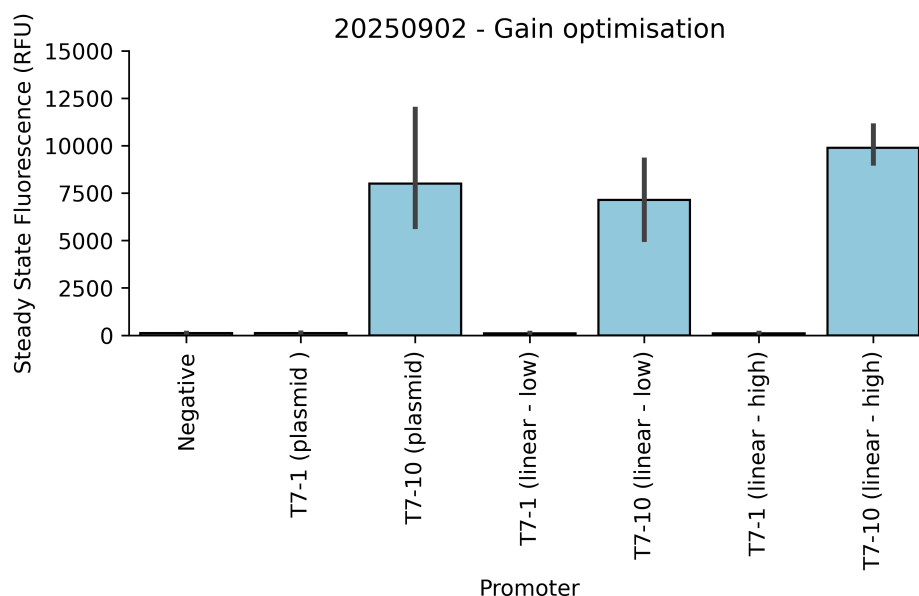
PURE test: Optimising gain (25.09.02)

- T7-1 and T7-10 expressed in PURE (10 μ L, triplicate)
- Plasmid and linear templates @ 5 ng/ μ L.
- Linear samples @ 1.7 ng/ μ L were also included as an equimolar equivalent to 5 ng/ μ L plasmid samples.

Standard reaction						
	Component	Starting concentration	Final Concentration	Vol (μ L)		
	Solution A	2.5x	1X	14.00		
	Solution B	3.3x	1x	10.51		

	Murine RNase inhibitor	40 U / μ L	1 U / μ L	0.88		
	DNA Template (100 ng / μ L)	40	5 ng / μ L	4.38		
	Nuclease-Free Water	—	—	5.24		
	Total			35		
Standard reaction (-DNA, -H ₂ O)	Component	Starting concentration	Final Concentration	Vol (μL)	Vol for 7.5x rxns (μL)	
	Solution A	2.5x	1X	14.0	105.0	
Scale factor	Solution B	3.3x	1x	10.5	78.8	
7.5	Murine RNase inhibitor	40 U / μ L	1 U / μ L	0.88	6.6	Distribute 25.4 μL into 7x tubes
	Total			30		
	Total (check)			25.4	190.4	
Template addition	Sample	V DNA	V H₂O	Total		
	Negative	0	9.6	35.0		
	pURET7-1 (5 ng / μ L)	4.38	5.24	35.0		
	pURET7-10 (5 ng / μ L)	4.38	5.24	35.0		
	Linear pURET7-1 (1.8 ng / μ L)	1.58	8.04	35.0		
	Linear pURET7-10 (1.8 ng / μ L)	1.58	8.04	35.0		
	Linear pURET7-1 (5 ng / μ L)	4.38	5.24	35.0		
	Linear pURET7-10 (5 ng / μ L)	4.38	5.24	35.0		
Template dilutions (40 ng/ μ L)	Sample	Conc (ng/μL)	V DNA	V H₂O	Total	
	Linear pURET7-1	92.2	4.34	5.66	10.00	
	Linear pURET7-10	102.8	3.89	6.11	10.00	

Results



- No signal detected in T7-1 samples.
- T7-10 (linear - low) performed similarly to T7-10 plasmid
 - Equimolar pT7-amajLime TUs perform equivalently. makes sense.
- **But/problem** - sample only ran for 1:30 hrs due to lack of signal in T7-1 samples and others needing to use platereader.
 - Anton ran samples for 12hrs. Weak promoters must need longer reaction times to generate enough signal.
 - **Fix: Increase reaction volume to 15 μ L, optimise platereader parameters, run for 12hrs.**
 - All previous experiments were performed on Cyt3 which has a lower detection sensitivity and less controllable monochromators. Cytation 5 has greater detection sensitivity and allows monochromator bandwidths to be defined.
 - Platereader params were optimised for Cytation 5. Bandwidth = 20, gain = 67, Read-height = ~ 11.25 mm.

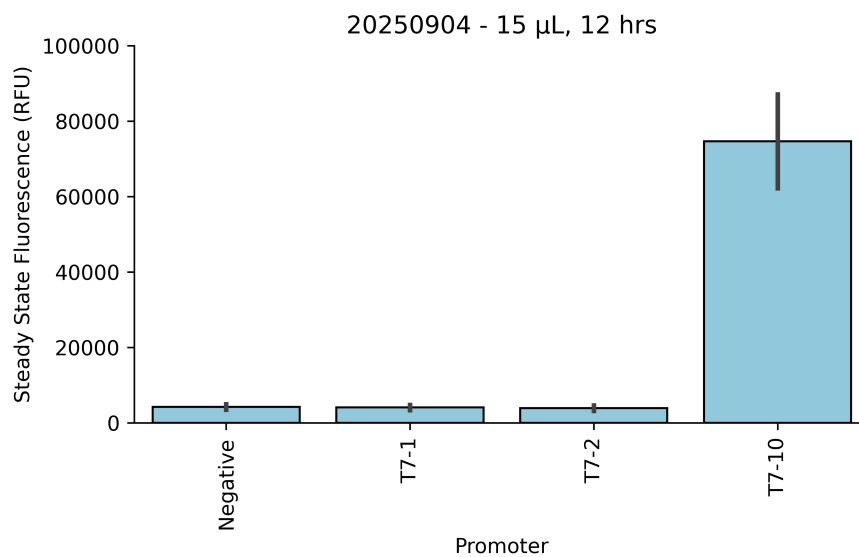
PURE test: High reaction vol, longer read time (25.09.04)

- T7-1, T7-2 and T7-10 expressed in PURE (5 ng/ μ L, 15 μ L, duplicate)
- Gain: 65 and 62, Bandwidth: 20, read-height = 12 mm (for 15 μ L)

Standard reaction						
	Component	Starting concentration	Final Concentration	Vol (μ L)		
	Solution A	2.5x	1X	14.00		
	Solution B	3.3x	1x	10.51		
	Murine RNase inhibitor	40 U / μ L	1 U / μ L	0.88		
	DNA Template (100 ng / μ L)	40	5 ng / μ L	4.38		
	Nuclease-Free Water	—	—	5.24		
	Total			35		

Standard reaction (-DNA, -H2O)	Component	Starting concentration	Final Concentration	Vol (μ L)	Vol for 4.5x rxns (μ L)	
	Solution A	2.5x	1X	14.0	63.0	
Scale factor	Solution B	3.3x	1x	10.5	47.3	
4.5	Murine RNase inhibitor	40 U / μ L	1 U / μ L	0.88	3.9	Distribute 25.4 μL into 4x tubes
	Total			30		
	Total (check)			25.4	114.2	
Template addition	Sample	V DNA	V H2O	Total		
	Negative	0	9.6	35.0		
	pURET7-1 (5 ng / μ L)	4.38	5.24	35.0		
	pURET7-2 (5 ng / μ L)	4.38	5.24	35.0		
	pURET7-10 (5 ng / μ L)	4.38	5.24	35.0		
Template dilutions (40 ng/ μ L)	Sample	Conc (ng/μL)	V DNA	V H2O	Total	
	pURET7-1	69.4	5.76	4.24	10.00	
	pURET7-2 #1	122.2	3.27	6.73	10.00	
	pURET7-10	164.4	2.43	7.57	10.00	

Results



- Still no activity in weak promoters. Signal cannot be discerned from negative noise.
- ACJS conditions: 5 ng/ μ L linear template, 10 μ L, 37°C, 12 hrs.
 - 5 ng/ μ L linear template is 2.7x more DNA than my current plasmid samples. **Why didn't I do this from the start?**
 - Try these conditions. If unsuccessful, continue w/o T7-1 and T7-2.

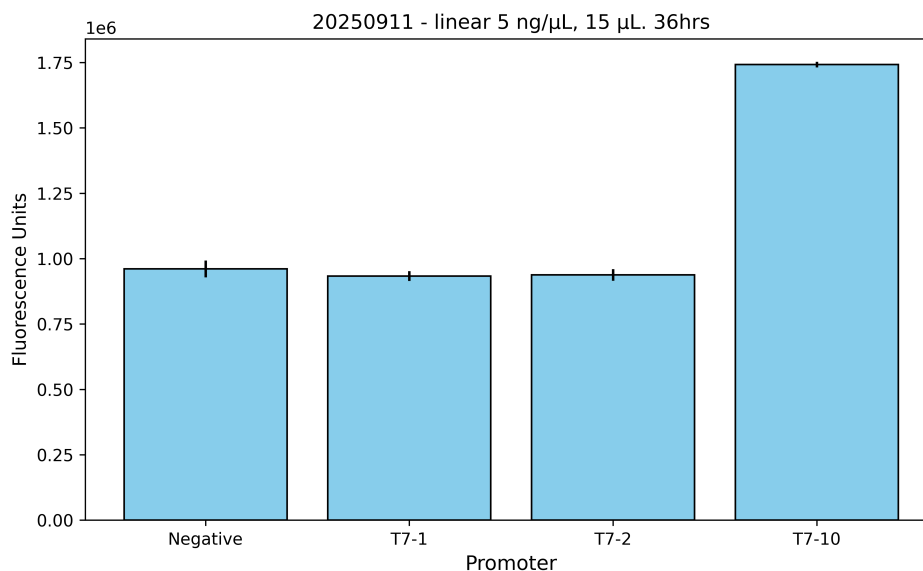
@September 9, 2025: PURE test: Linear T7-1, T7-2 and T7-10 (15 µL, 12 hours, endpoint)

- Linear T7-1, T7-2 and T7-10 expressed in PURE (5 ng/µL, 15 µL, Triplicate)
- High gain endpoint read to determine whether T7-1 and T7-2 produce signal.

Standard reaction						
	Component	Starting concentration	Final Concentration	Vol (µL)		
	Solution A	2.5x	1X	20.00		
	Solution B	3.3x	1x	15.02		
	Murine RNase inhibitor	40 U / µL	1 U / µL	1.25		
	DNA Template (100 ng / µL)	40	5 ng / µL	6.25		
	Nuclease-Free Water	—	—	7.48		
	Total			50		
Standard reaction (- DNA, -H2O)	Component	Starting concentration	Final Concentration	Vol (µL)	Vol for 4.5x rxns (µL)	
	Solution A	2.5x	1X	20.0	90.0	
Scale factor	Solution B	3.3x	1x	15.0	67.6	
4.5	Murine RNase inhibitor	40 U / µL	1 U / µL	1.25	5.6	Distribute 36.3 µL into 4x tubes
	Total			50		
	Total (check)			36.3	163.2	
Template addition	Sample	V DNA	V H2O	Total		
	Negative	0	13.7	50.0		
	pURET7-1 (5 ng / µL)	6.25	7.48	50.0		
	pURET7-2 (5 ng / µL)	6.25	7.48	50.0		
	pURET7-10 (5 ng / µL)	6.25	7.48	50.0		
Template dilutions (40 ng/µL)	Sample	Conc (ng/µL)	V DNA	V H2O	Total	
	Linear pURET7-1	92.2	4.34	5.66	10.00	
	Linear pURET7-2	103.8	3.85	6.15	10.00	
	Linear pURET7-10	102.8	3.89	6.11	10.00	

Results

- Prior to measuring this sample I conducted some amajLime research
 - AmajLime is primarily a **chromoprotein** which absorbs light at 458nm, but also emits light (fluoresces) at an excitation maxima of ~488nm. ([see here for more info](#))
 - Visible colour is generated in E.coli between 12-48hrs of incubation in LB broth or LB agar plates. This suggests that the maturation time is **slow** ([see here for more info](#))
 - The structural data on AmajLime is difficult to interpret, but this is what I understood ([See here for more info](#)). The chromophore exists in a 1:1 ratio between cis and trans states (fluorescent / non-fluorescent) which explains the low quantum yield of the protein. Blue chromoproteins tend to typically exist in a neutral rather than anionic ground state enabling them to absorb shorter wavelengths of light, but also meaning they are less primed for fluorescence.
 - AmajLime: maximal extinction coefficient = 40,000, Quantum yield = 0.24, maturation time: ~long.
 - PlamGFP: maximal extinction coefficient = 98,600, Quantum yield = 0.96, maturation time: ~ 30mins - 2 hours.
 - Based on this, I should **increase incubation times** for amajLime experiments, and **switch over to plamGFP** for future experiments. It will be interesting to compare the two as it is still unknown as to whether a brighter, faster maturing protein will be beneficial for this experiment. Logically yes, but who is to know.



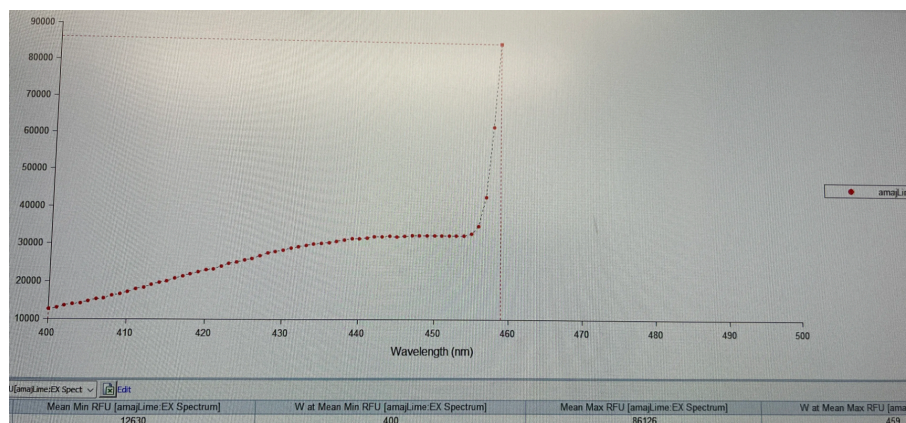
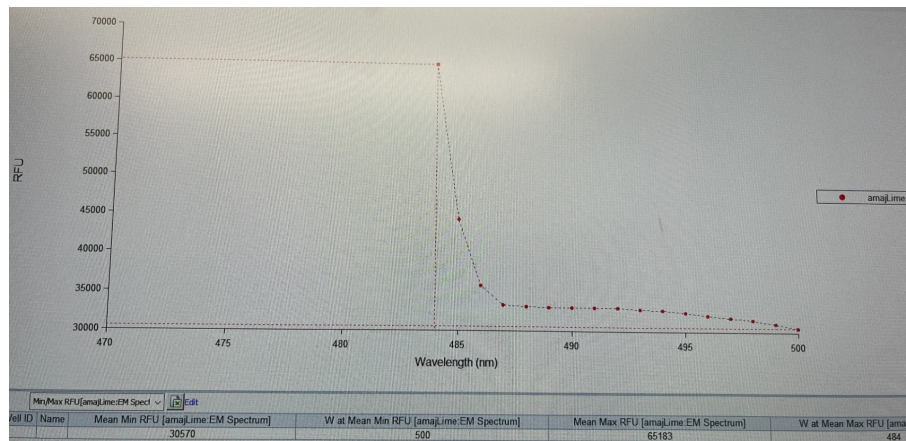
- Incubation: 37°C ~ 24 hrs, R/T ~ 12 hrs
- No activity in T7-1 or T7-2 @ 15μL + extended incubation time.
- **Next steps:** Do a full sweep of linear constructs @ 5 ng/μL, 10 μL, 12 hrs to complete the data and then move onto **plamGFP**.

@September 11, 2025: PURE test: Linear T7-1:10

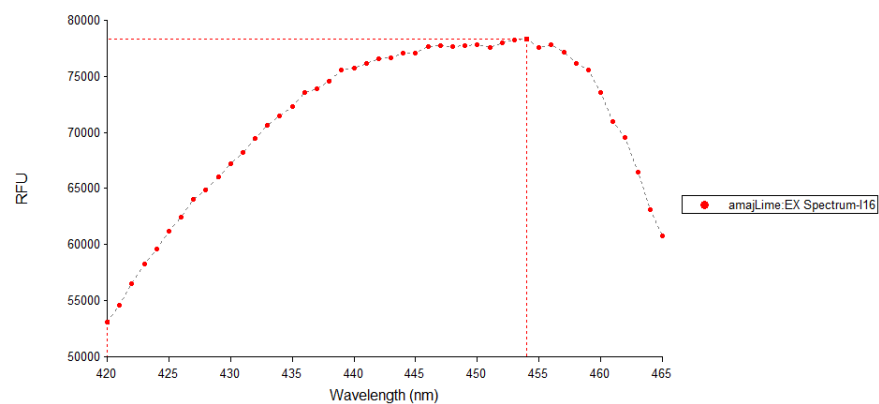
- Linear T7-1:10 expressed in PURE (5 ng/μL, 10 μL, Triplicate)
- **Note:** A kinetic read was setup using the following parameters Ex/Em 459nm / 484nm (20nm), Gain: **Extended**, Read height: 11.75.
 - Ex/Em parameters were determined from the following **inaccurate** fluorescence spectra in which the gain was set too high preventing a full spectra within the set limits to be produced. Whilst the peak values weren't too dissimilar from the subsequent **accurate** spectra, it appears that **Ex 459nm** produces significant autofluorescence in **blank**

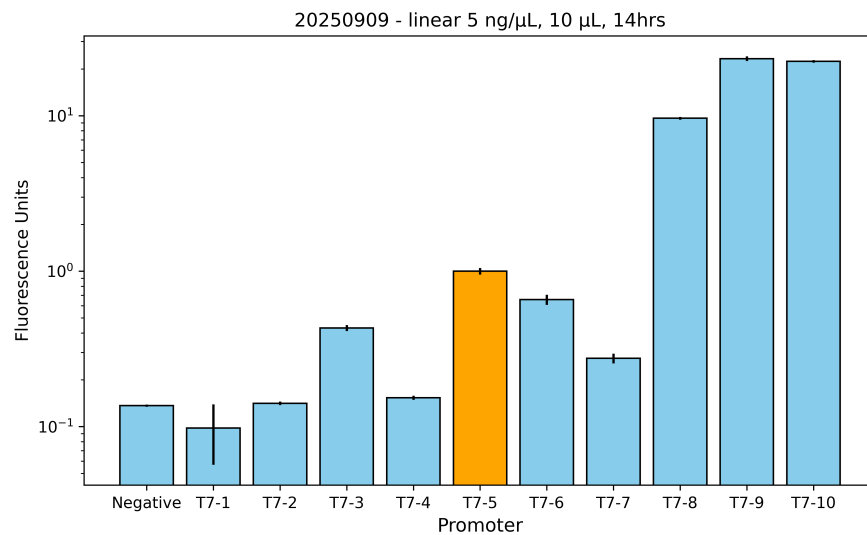
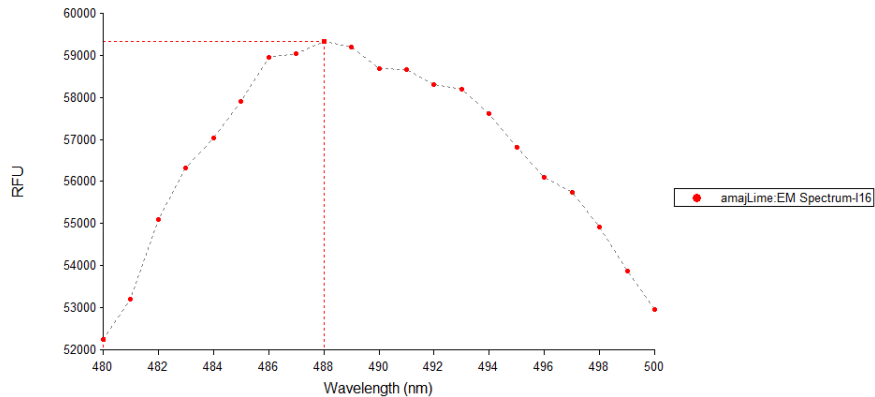
wells which prevented my samples from exceeding the background noise during the kinetic read - I need to follow up on this. Instead, I settled for an endpoint read using the **accurate** Ex/Em 454nm, 488nm.

▼ Excitation / Emission (**inaccurate**)



▼ Excitation / Emission (**accurate**)





- **Note:** Prior to analysing this data I noticed that **T7-1** and **T7-2** have the **identical sequence** of 'taatatcactgactaaagt'. This makes sense. Either promoter is not distinguishable from the negative control which is to be expected from a highly mutated promoter sequence.
- Overall the data isn't too dissimilar from ACJS's original data. The major outliers are **T7-4** which also failed to work in the original plasmid test and **T7-6**, and **T7-7** which both performed worse than expected. T7-7 performed equally badly in the original plasmid test, but T7-6 previously performed as expected.

Possible problems

- **T7-4:** 1. Promoter sequence is not functional, 2. Poor template quality - **Would be surprising as the sequence has been QC'd.**
- **T7-6/T7-7** 1. Inaccurate DNA concentration of templates, 2. Slow maturation of AmajLime decouples fluorescence signal from promoter strength resulting in inconsistencies within the data.

Next steps

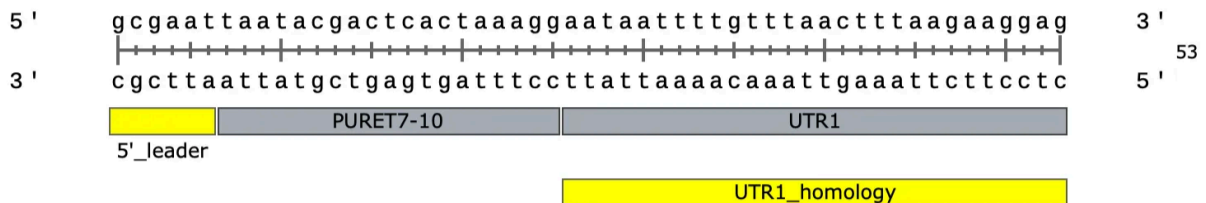
- ☐ Nanodrop T7-6
- ☐ Nanodrop T7-7
- ☐ Prepare pURET7-1:10-plamGFP/deGFP

PlamGFP/deGFP collection

@September 12, 2025: Designing pURET7 primers to amplify plamGFP/deGFP.

- Individual PCR primers were designed for each pURET7 promoter (excluding T7-2, designated T7-1, T7-3:10 to maintain consistency between experiments) to amplify both plamGFP/deGFP. This was done through utilising the UTR1 region upstream of the CDS which is conserved across both reporters.

▼ Primer design: 5' leader sequence ([NEB PURE recommendation](#)) - pURET7 promoter - UTR1_homology (28 bp)



- PCR will be performed the **T7rev primer** to maintain consistency between experiments.
 - Note:** T7rev bind upstream of the terminator on deGFP. **M13Rev** will be used for both plasmids instead.

@September 17, 2025: Gradient PCR to find optimum Ta for pURET7-plamGFP/deGFP

- Ta's: 55°C, 60°C, 65°C, 72°C.
- 25 µL rxns.

▼ PCR calculations / conditions

Standard reaction						
	Component	Final Concentration	Vol for 25 µL (µL)	Vol for 100 µL (µL)		
	Q5 High-Fidelity 2X Master Mix	1X	12.5	50		
	10 µM Forward Primer	0.5 µM	1.25	5		
	10 µM Reverse Primer (M13rev)	0.5 µM	1.25	5		
	Template	0.04 ng/ µL (1 ng total)	1	4		
	Nuclease-Free Water		9	36		
	Total		25	100		
	Total (6x)		225			
Standard reaction (- template, - Fwd primer)						
	Component	Final Concentration	Vol (µL)	Vol 9x (µL)		
Scale factor	Q5 High-Fidelity 2X Master Mix	1X	12.5	112.5		
9	10 µM Reverse Primer (M13rev)	0.5 µM	1.25	11.25	Distribute 91 µL in 2x tubes	

	Nuclease-Free Water		9	81		
	Total		22.75	204.75		
Template addition	Sample	V Fwd primer (T7-10_insert)	V Template	Total		
	DeGFP	5	4	100	Distribute 25 µL into 4x tubes	
	PlamGFP	5	4	100		
PCR conditions	Sample	Initial denaturation (secs)	Denaturation (secs)	Annealing (secs) (55°C, 60°C, 65°C, 72°C)	Extension (secs)	Final extension (secs)
	DeGFP	30	10	30	22 (860bp)	120
	PlamGFP	30	10	30	22 (964bp)	120
			35 cycles			

▼ Agarose gel

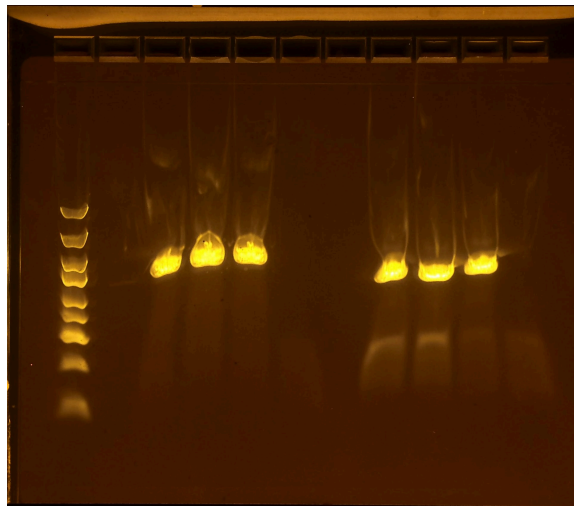
- 20 µL Samples (10 µL 0.5x PCR product, 10 µL Express running buffer) were run on an EX 1% gel.
- Estimated amplicon size - plamGFP = 964bp, deGFP = 860bp

Lanes

M - Ex 1kb plus ladder

2-5 - plamGFP (55°C, 60°C, 65°C, 72°C)

7-10 - deGFP (55°C, 60°C, 65°C, 72°C)



Results

- Amplicons generated at all Ta's except 72°C.
- Optimum Ta's (highest yield with lowest contamination)
 - plamGFP: 60°C

- deGFP: 65°C

@September 18, 2025: PCR scale-up for pURET7-1,3:10-plamGFP/deGFP

- 100 µL PCR rxns (2× 50µL) using optimum Ta's

▼ PCR calculations / conditions

PCR conditions	Sample	Initial denaturation (secs)	Denaturation (secs)	Annealing (secs) (55°C, 60°C, 65°C, 72°C)	Extension (secs)	Final extension (secs)
	DeGFP	30	10	30	22 (860bp)	120
	PlamGFP	30	10	30	22 (964bp)	120
			35 cycles			
Standard reaction						
	Component	Final Concentration	Vol for 25 µL (µL)	Vol for 100 µL (µL)		
	Q5 High-Fidelity 2X Master Mix	1X	12.5	50		
	10 µM Forward Primer	0.5 µM	1.25	5		
	10 µM Reverse Primer (M13rev)	0.5 µM	1.25	5		
	Template	0.04 ng/ µL (1 ng total)	1	4		
	Nuclease-Free Water		9	36		
	Total		25	100		
	Total (6x)		475			
Standard reaction (- template, - Fwd primer)	Component	Final Concentration	Vol (µL)	Vol 19x (µL)		
Scale factor	Q5 High-Fidelity 2X Master Mix	1X	50	950		
19	10 µM Reverse Primer (M13rev)	0.5 µM	5	95	Distribute 91 µL in 18x tubes	
	Nuclease-Free Water		36	684		
	Total		91	1729		
Template addition (plamGFP)	Sample	V Fwd primer	V Template (plamGFP)	Total		
	T7-1_insert	5	4	100		
	T7-3_insert	5	4	100		
	T7-4_insert	5	4	100		
	T7-5_insert	5	4	100		
	T7-6_insert	5	4	100		

	T7-7_insert	5	4	100		
	T7-8_insert	5	4	100		
	T7-9_insert	5	4	100		
	T7-10_insert	5	4	100		
Template addition (deGFP)	Sample	V Fwd primer	V Template (deGFP)	Total		
	T7-1_insert	5	4	100		
	T7-3_insert	5	4	100		
	T7-4_insert	5	4	100		
	T7-5_insert	5	4	100		
	T7-6_insert	5	4	100		
	T7-7_insert	5	4	100		
	T7-8_insert	5	4	100		
	T7-9_insert	5	4	100		
	T7-10_insert	5	4	100		

- Following steps to generate purified template:

✓ ~~PCR cleanup~~

- Eluted in 20 μ L buffer EY
- ~DNA conc across all samples: 200 ng/ μ L

✓ ~~DpnI digest~~

- 0.5 μ L DpnI (10 Units) - Significant excess, but accurately pipetable.
- 37°C, 1hr, 350 rpm shaking

▼ DpnI calculations

- Note: Realised half-way through that I could create a mastermix, so didn't waste all my time pipetting 🤖
- @September 19, 2025 samples stored @ -20°C.

Sample (plamGFP)	V sample (μ L)	V DpnI (20 U/ μ L) (μ L)	V rCutSmart buffer (10x) (μ L)	V H2O (μ L)	Total (μ L)
T7-1	20	0.5	5	24.5	50
T7-3	20	0.5	5	24.5	50
T7-4	20	0.5	5	24.5	50
T7-5	20	0.5	5	24.5	50
T7-6	20	0.5	5	24.5	50
T7-7	20	0.5	5	24.5	50
T7-8	20	0.5	5	24.5	50
T7-9	20	0.5	5	24.5	50
T7-10	20	0.5	5	24.5	50
Sample (deGFP)	V sample (μ L)	V DpnI (μ L)	V rCutSmart buffer (μ L)	V H2O (μ L)	Total (μ L)
T7-1	20	0.5	5	24.5	50
T7-3	20	0.5	5	24.5	50
T7-4	20	0.5	5	24.5	50
T7-5	20	0.5	5	24.5	50

T7-6	20	0.5	5	24.5	50
T7-7	20	0.5	5	24.5	50
T7-8	20	0.5	5	24.5	50
T7-9	20	0.5	5	24.5	50
T7-10	20	0.5	5	24.5	50

✓ PCR cleanup

@September 22, 2025 → September 25, 2025: pURET7-plamGFP-deGFP w/ @Astrid Jørgensen

▼ @September 22, 2025

1. Linear template DNA cleanup (@September 22, 2025 10:00 AM (PDT))
2. plamGFP/deGFP (T7-10) PURE rxns (@September 22, 2025 5:00 PM (PDT))
 - End-point rxns for fluorescence spectra.

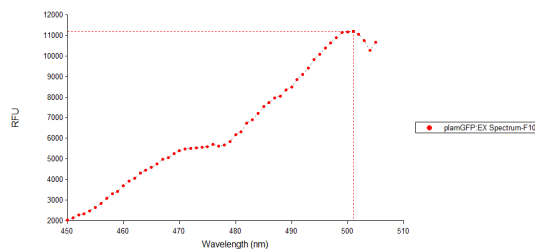
▼ @September 23, 2025

1. plamGFP/deGFP spectra acquisition (@September 23, 2025 9:30 AM (PDT))
 - Data analysis to determine Ex/Em parameters

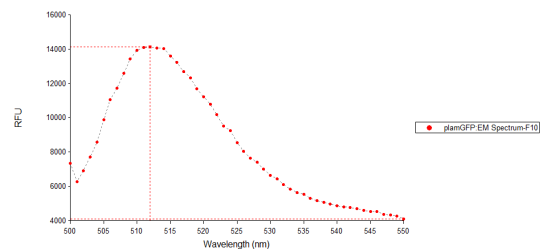
▼ Results

▼ plamGFP

Ex - 501nm

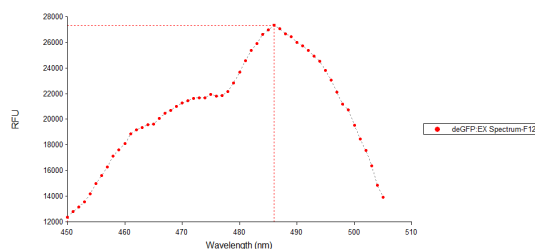


Em - 512nm

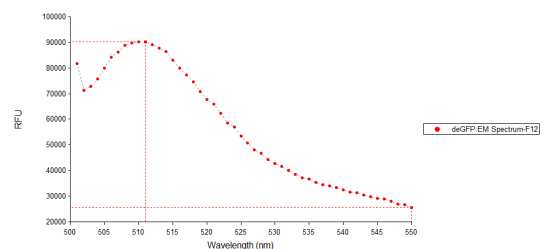


▼ deGFP

Ex - 485nm



Em - 511 nm



1. plamGFP/deGFP (T7-1:10) PURE rxns (@September 23, 2025 3:00 PM (PDT))

- Kinetic run to quantify promoter performance
- @Astrid Jørgensen: plamGFP, @Charlie Newell: deGFP
- **Note:** Using the monochromators on cytation 5, plamGFP is currently being measured using Ex/Em 485/528. This is because these parameters match the cytation 3 filter set. I suspect this is to maintain consistency across all reads. For this assay, plamGFP/deGFP were measured on the same plate under the following parameters - Ex/Em: 485/512

(17nm), gain: extended. This Ex/Em is perfect for deGFP and also provides an enhanced signal for plamGFP as the Em is closer to its peak Em of 501. 17nm bandwidth is required to ensure a low signal to noise ratio to prevent overlap between Ex/Em. This Signal to noise ratio is comparable to the signal to noise ratio of the current plamGFP Ex/Em parameters.

▼ Results (raw)

Spectrophotometer parameters

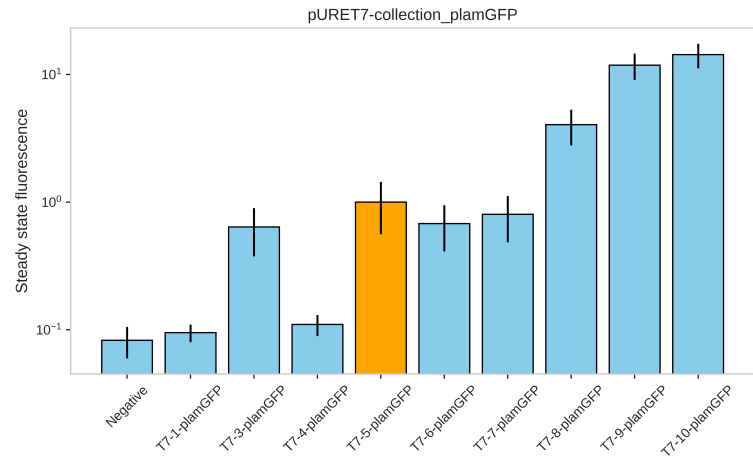
Ex/Em: 485/512 (17nm)

Gain: extended

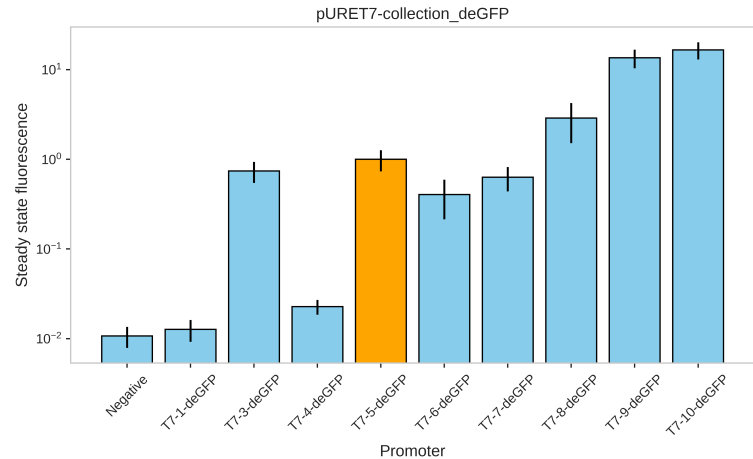
Time: 12 hours

Read intervals: 5 mins

▼ plamGFP

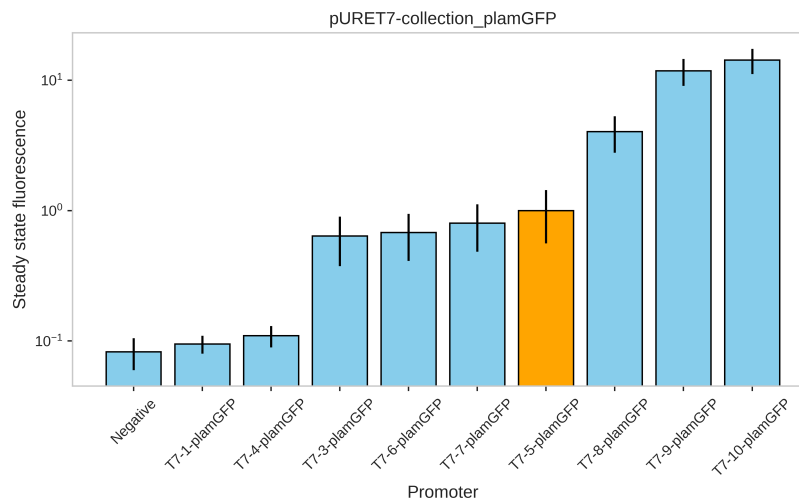


▼ deGFP

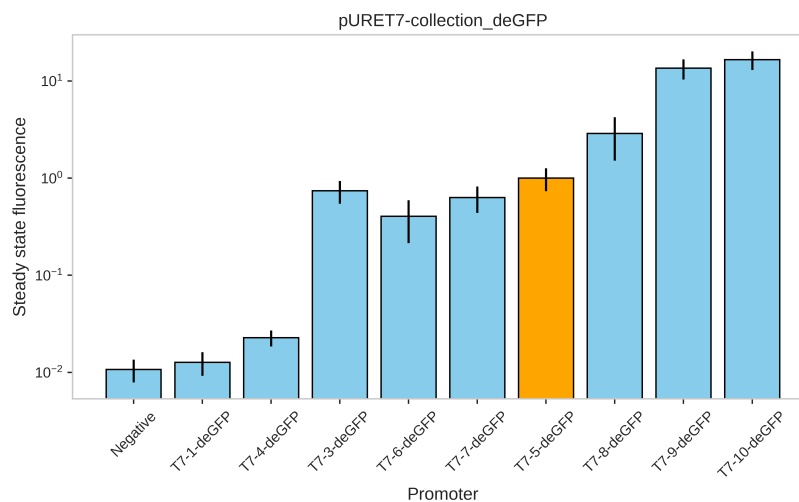


▼ Results (refined order)

▼ plamGFP



▼ deGFP



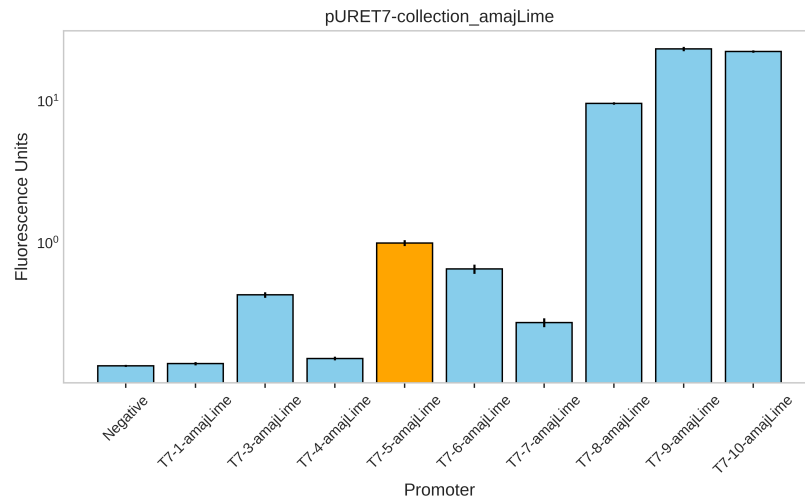
▼ @September 24, 2025

1. plamGFP/deGFP kinetic data analysis (@September 24, 2025 9:30 AM (PDT))
 - Optimise conditions if necessary
2. plamGFP/deGFP (T7-1:10) PURE rxns (@September 24, 2025 3:00 PM (PDT))
 - @Astrid Jørgensen: deGFP, @Charlie Newell: plamGFP.

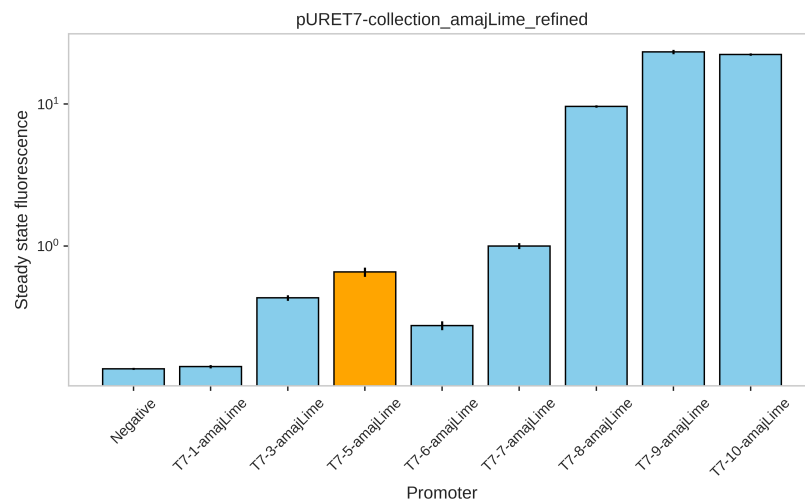
▼ @September 25, 2025

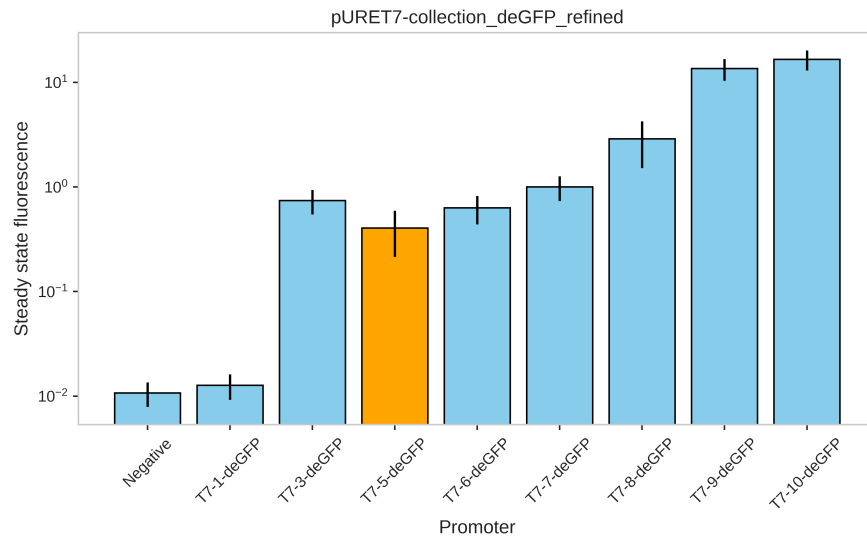
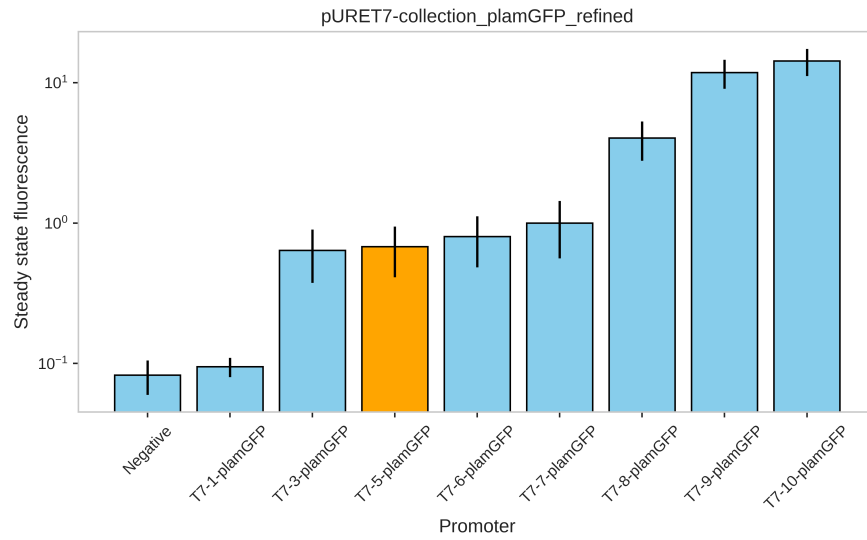
1. plamGFP/deGFP kinetic data analysis (@September 25, 2025 9:30 AM (PDT))
 - Determine whether extra data is required for DevNote.

▼ Results

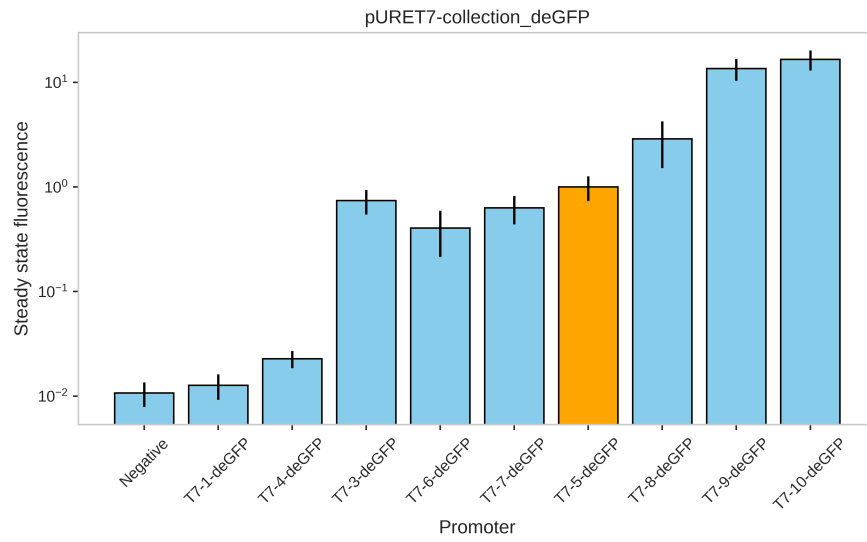
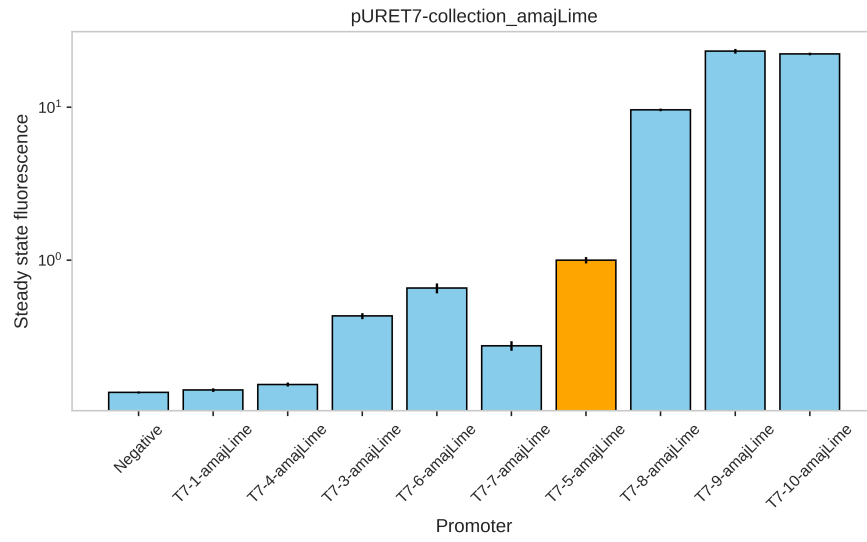


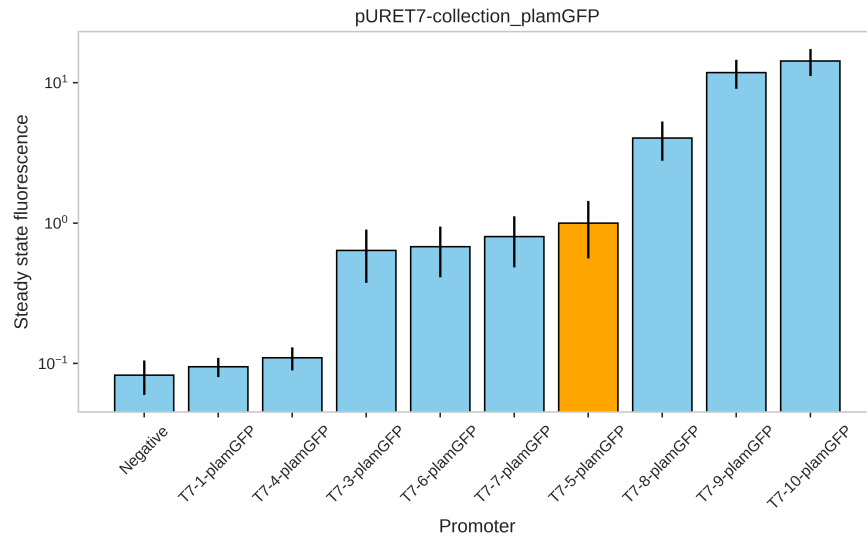
▼ Results refined



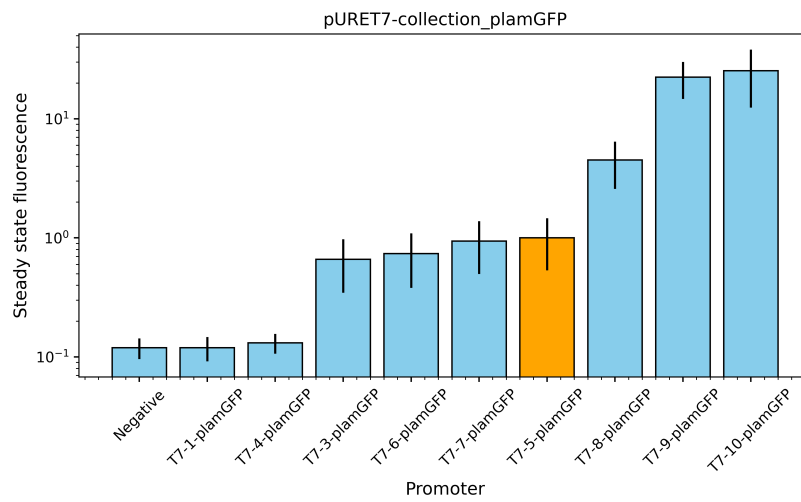
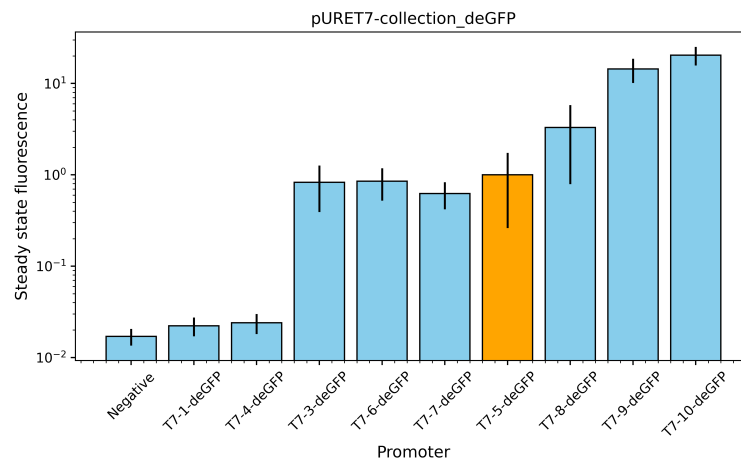


▼ @September 26, 2026: pURET7-collection AmajLime/plamGFP/deGFP comparison





▼ @September 29, 2025: pURET7-collection plamGFP/deGFP comparison #2 †



Final data analysis

This data analysis uses the data generated from the final amajLime experiment performed on @September 11, 2025 and the plamGFP/deGFP experiments performed @September 22, 2025 → September 25, 2025. All data was background subtracted prior to plotting by subtracting the mean negative control value from all data points. Data was plotted using the CDK and the analysis files can be found in the “DNA-toolkit-T7-promoters” directory within the nucleus jupyterhub. Data points assigned to each promoter sequence have been color coded to represent low, medium, and high level expressing promoters.

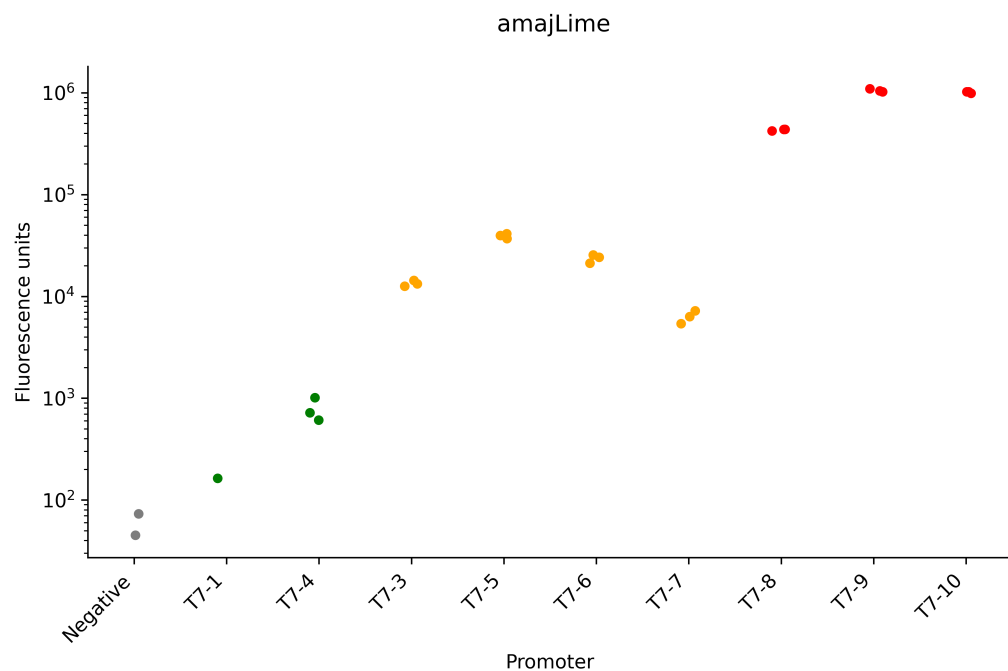
Promoter recap

Promoter	Relative expression level	Sequence 5'-3'	No. of mutations
T7-1	low	TAATAT CT ACT G ACTAAAGT	5
T7-3	mid	TGAA ACGACTCACTTTAGG	3
T7-4	low	TTATACG TG TCCCT G TAGG	5
T7-5	mid	GA ATACG CC TCACTTTAGG	3
T7-6	mid	CA ATACG TCT CACTATAG C	3
T7-7	mid	TTATACGACTCA G TATAGT	3
T7-8	high	CG ATACGACTCACTATAGG	2
T7-9	high	GA ATACGACTCACTATAGG	1
T7-10	high	TAATACGACTCACTAAAGG	1

AmajLime

Experimental conditions

10 μ L PURExpress rxns (triplicate), 37°C, 14hrs. Ex/Em, bandwidth: 454nm/488nm, 20nm. **Endpoint.**



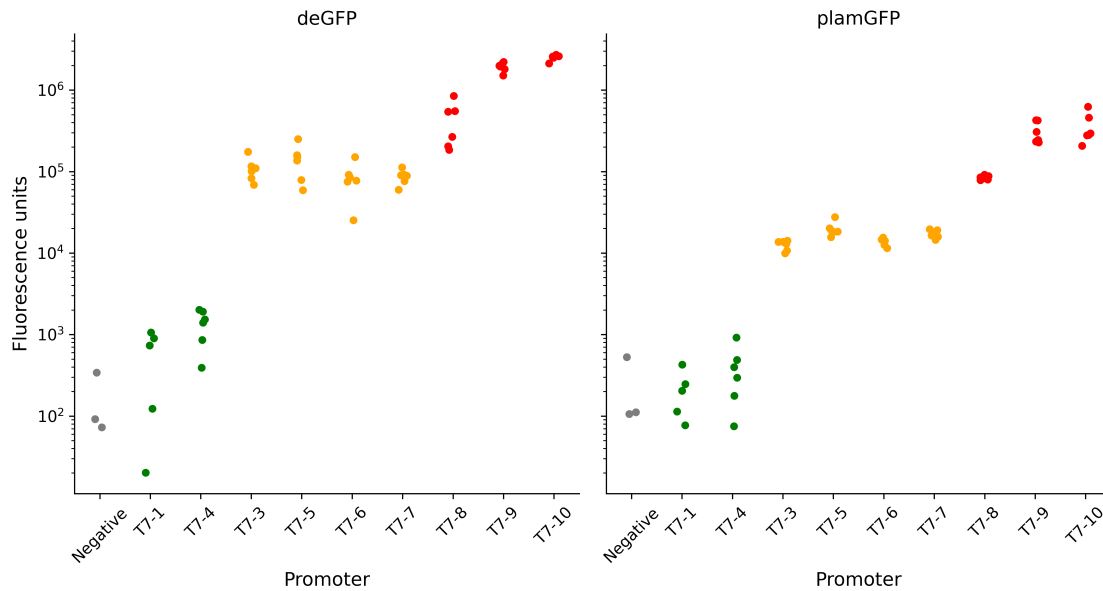
- Dynamic range: ~ 1000 fold dynamic range across promoters.
- Low promoters: T7-1: Only a single point exceeds background. T7-4: Comfortably exceeds background.

- Mid promoters: No discernible trend in mid promoter performance
- High promoters: T7-8 isolated with T7-9 and T7-10 showing higher levels of expression.

PlamGFP/deGFP

10 μ L PURExpress rxns (triplicate), 37°C, 12hrs. Ex/Em, bandwidth: 485nm/512nm, 17nm. **Kinetic.**

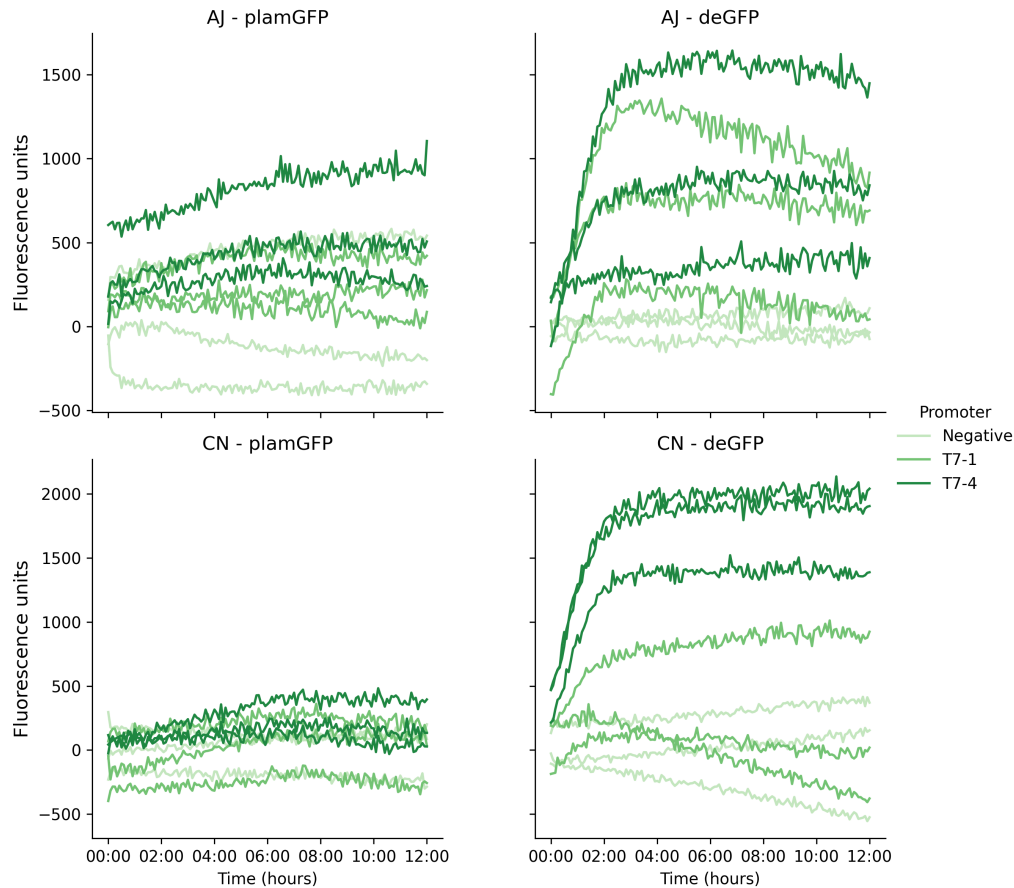
Summary data



- Dynamic range: plamGFP - <1000 fold dynamic range across promoters. deGFP - 1000 fold dynamic range across promoters
- Low promoters: plamGFP - T7-1 and T7-1 fail to exceed negative noise. deGFP - T7-1 and T7-4 exceed negative noise.
- Mid promoters: No discernible trend in mid promoter performance
- High promoters: T7-8 isolated with T7-9 and T7-10 showing higher levels of expression.

Kinetic curves

Low promoters



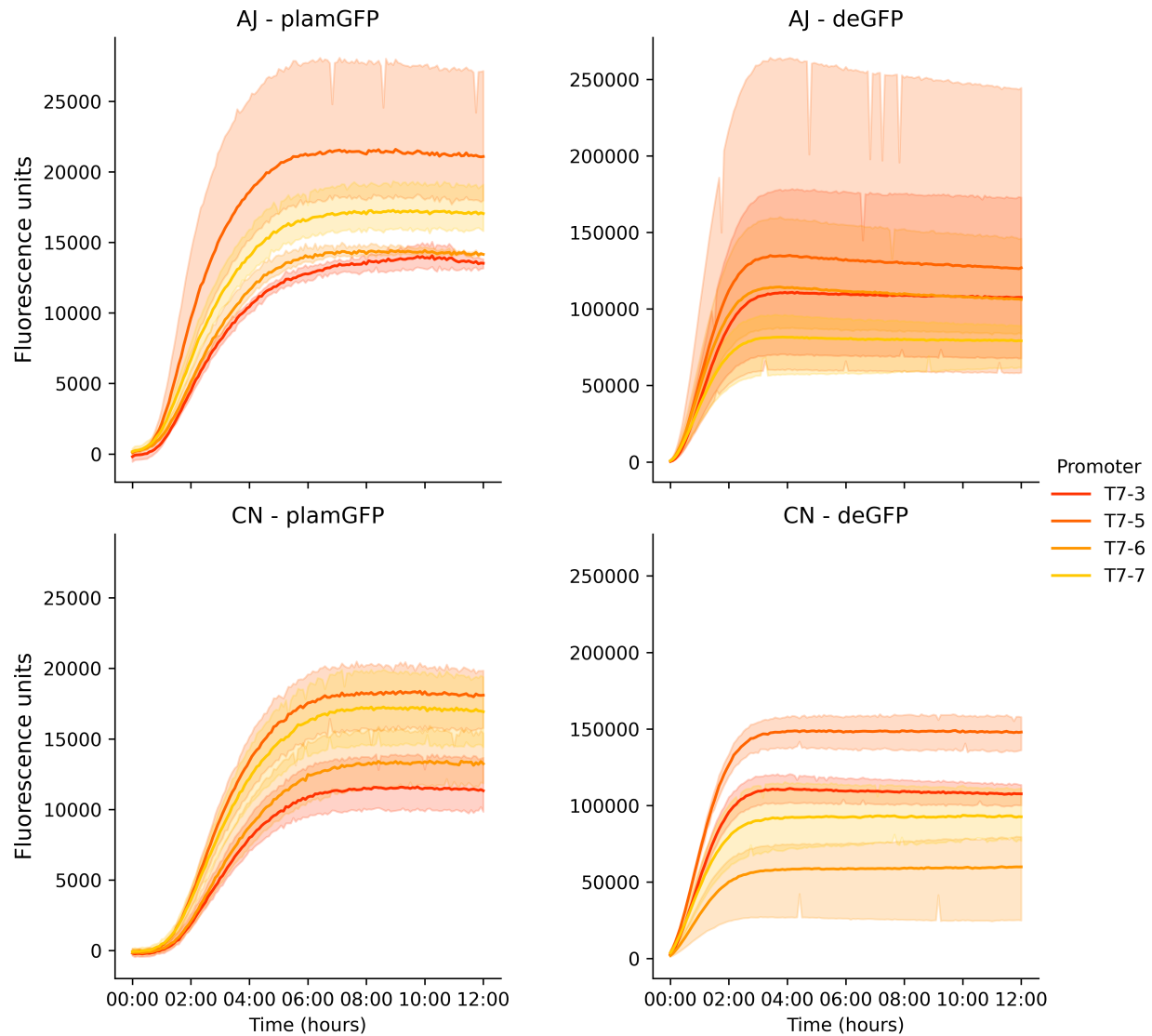
plamGFP - very little presence of sigmoidal curve in plamGFP samples for either experimentalist. Unable to discern T7-1 and T7-4 from negative noise. May be hints of S-curve in AJ's samples. Definitely more so than CN. **Important to note here that measurements were recorded ~ 15 minutes after plates were placed at 37°C. This means we miss the lag phase of the S-curve.**

deGFP - Presence of S-curve across both T7-1 and T7-4 for both experimentalists. Moreso in AJ's samples rather than CN for T7-1. Yet overall signal for CN is higher than AJ.

Conclusions

DeGFP more sensitive reporter under these pure quantification conditions. Indicates reporter choice is important to reveal expression characteristics of DNA regulatory elements.

Mid promoters



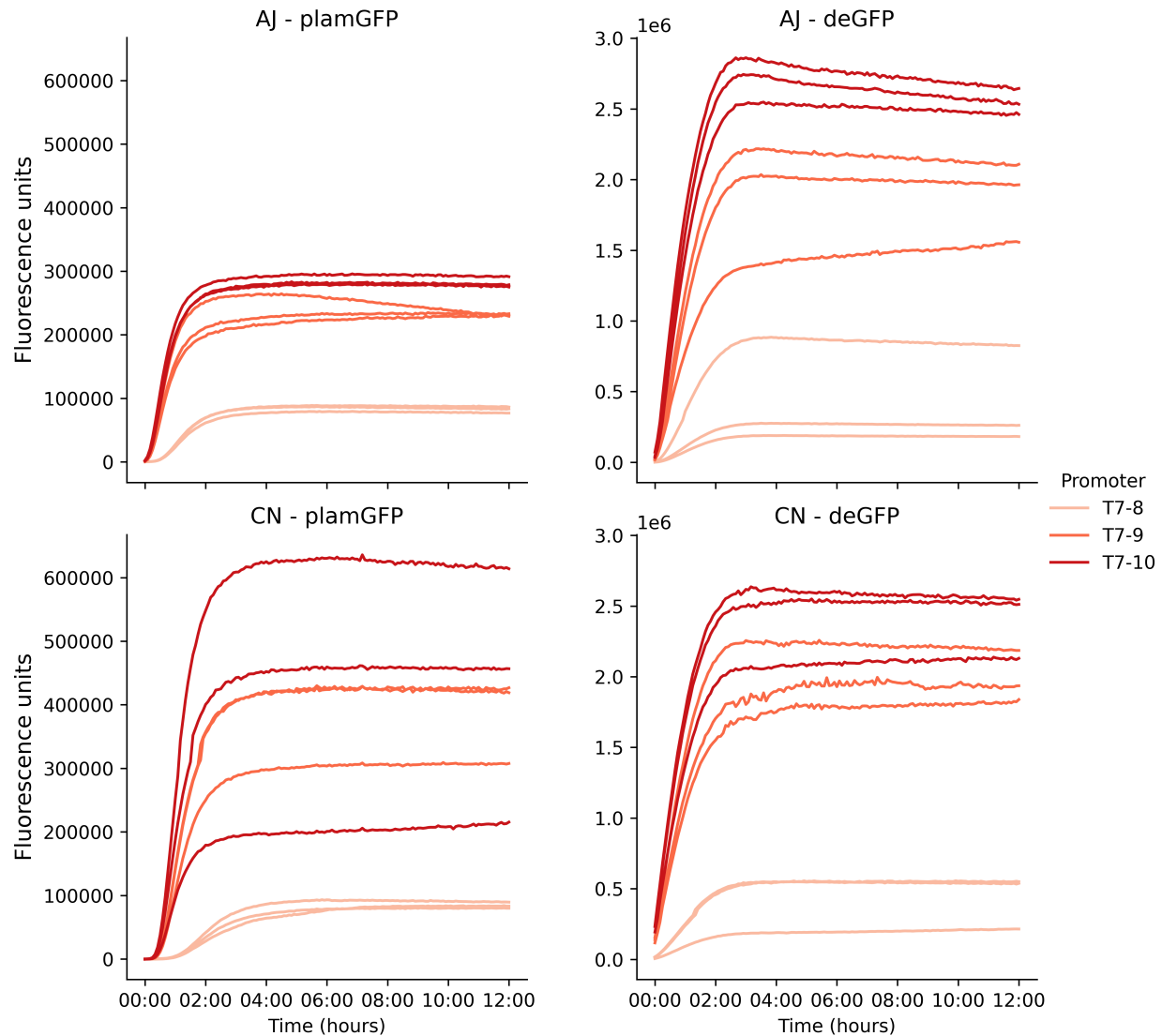
plamGFP - STD of promoters overlap for majority of promoters. Appears to be a loose pattern that T7-3/T7-6 and T7-5/T7-7 are clustered together which can be observed in the summary data. **Trend not mirrored in deGFP.** AJ seems to have higher average expression in samples but with larger STD.

deGFP - Again STD of promoters overlap for the majority of promoter sequences. Large STD in AJ samples.

Conclusion

No discernible difference in promoter strength between mid-range promoters. deGFP significantly more sensitive for reporting under these conditions shown by order of magnitude difference in fluorescence units.

High promoters



plamGFP - Discernible difference between promoter expression levels. T7-8 low, T7-9 and T7-10 in that order. More easily noticeable in AJs sample due to large STD in CN's T7-9, and T7-10 samples.

deGFP - Easily discernible difference in promoter sequences. Again especially in AJ's samples. T7-8 significantly lower level of expression than T7-9 and T7-10. Significant difference in overall fluorescence units between both reporters again highlighting increased sensitive of deGFP for reporting under these conditions

conclusion

T7-8, -9, -10 move from low to high expression strength within the high expression level promoter category. deGFP again is highlighted as the more sensitive reporter.

Overall conclusions

The collection spans ~ 1000 fold range of expression across promoters. This is observed when using amajLime and deGFP as reporters. PlamGFP exhibits a slightly reduced dynamic range. This highlights that reporter choice can affect the overall outcome of PURE quantification. Furthermore, based on spectral and photophysical characteristics alone it would be difficult to choose an optimum reporter from the three used; however, it is clear empirically that deGFP is the most sensitive. Interestingly, the strength of all promoter sequences seems to be directly correlated with the amount of mutations the sequence has in comparison to the consensus T7 promoter sequence. Perhaps this notion could be used to increase

the overall breadth of the collection by engineering more promoters within each expression category. Despite these experiments enabling the characterisation of the promoter collection and providing an insight for protein reporter choice in PURE. I suspect more accurate data on the performance of this promoter collection would be generated by using light-up aptamers rather than proteins in future.