

4/5 September 2017 : Test new M15 T7 and M15 T7 YPTG lysates

1. Aim:

Make sure all lysates work as expected to use them in later experiments.

2. Materials:

- Nuclease-free water
- LacZ toehold purified (4 September)
- LacZ alpha toehold, not purified (5 September)
- ssDNA short trigger
- M15 T7 batch 1 lysate
- M15 T7 batch 2 lysate
- M15 T7 grown on YPTG batch 1 lysate
- M15 T7 grown on YPTG batch 2 lysate
- Top10-GamS lysate (tested)
- Top10-GamS lysate (new, not tested yet)
- Buffer A
- Energy solution
- Substrate
- Plate reader

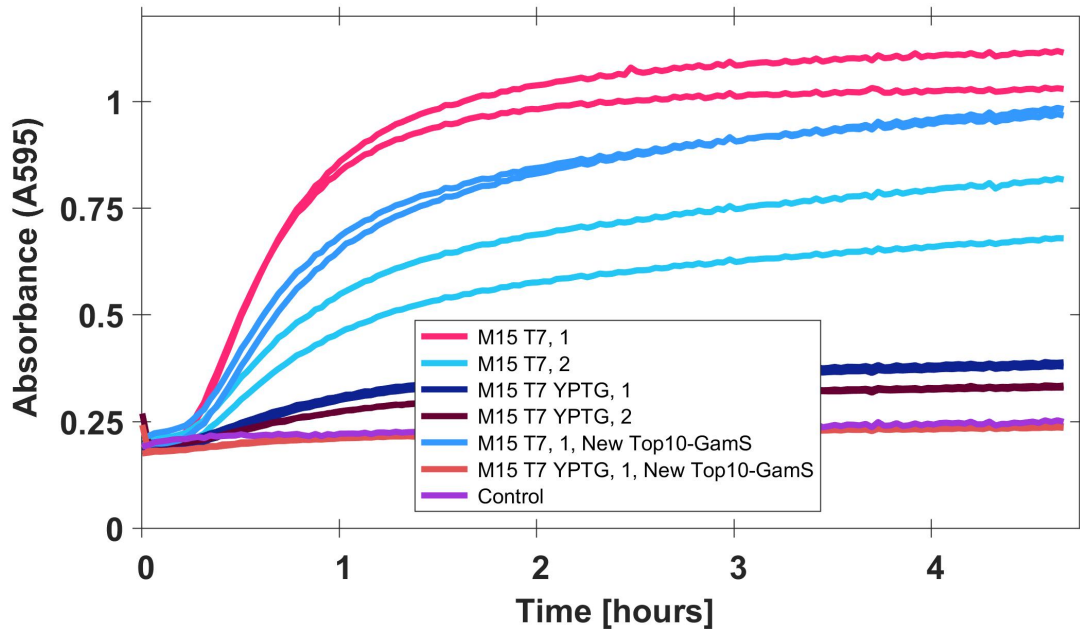
3. Procedure:

Which trigger	Trigger quantity	Which DNA	quantity	Which lysate	Lysate quantity	Top10-GamS lysate	Energy solution	Buffer A	H2O	Substrate
short trigger	0.2	LacZalpha Toehold	0.35	M15-T7 1	1.25	1.25	2.5	2.5	1.8	0.2
short trigger	0.2	LacZalpha Toehold	0.35	M15-T7 2	1.25	1.25	2.5	2.5	1.8	0.2
short trigger	0.2	LacZalpha Toehold	0.35	M15-T7 Y1	1.25	1.25	2.5	2.5	1.8	0.2
short trigger	0.2	LacZalpha Toehold	0.35	M15-T7 Y2	1.25	1.25	2.5	2.5	1.8	0.2
short trigger	0.2	LacZalpha Toehold	0.35	M15-T7 1 + Topnew	1.25	1.25	2.5	2.5	1.8	0.2
short trigger	0.2	LacZalpha Toehold	0.35	M15-T7 Y1 + Topnew	1.25	1.25	2.5	2.5	1.8	0.2
none	0	LacZalpha Toehold	0.35	M15 T7 1	1.25	1.25	2.5	2.5	2.0	0.2
none	0	LacZalpha Toehold	0.35	M15 T7 2	1.25	1.25	2.5	2.5	2.0	0.2
none	0	LacZalpha Toehold	0.35	M15 T7 Y1	1.25	1.25	2.5	2.5	2.0	0.2
none	0	LacZalpha Toehold	0.35	M15 T7 Y2 + Topnew	1.25	1.25	2.5	2.5	2.0	0.2

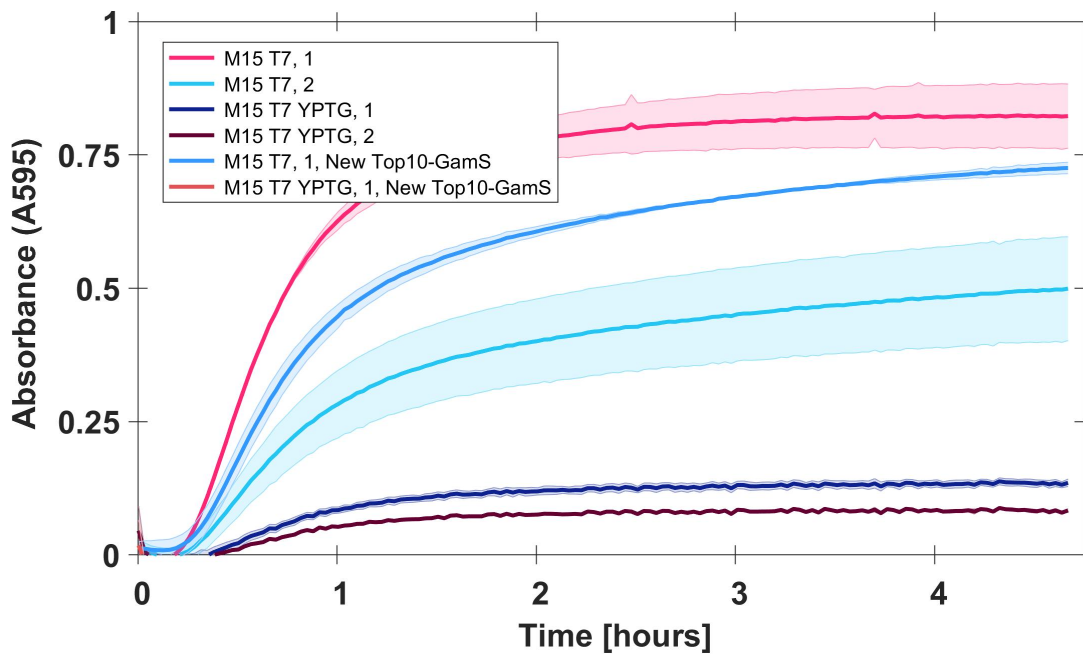
4. Results :

Experiment from 4 September gave no conclusive results (controls exploded). The experiment was redone on 5 September.

Results from 5 September repeated experiment : (showing blank)



Shaded error graphs (blank was subtracted). The last label of the legend can't be seen as it was below the control.



Error bars of end point measurements (control was subtracted)

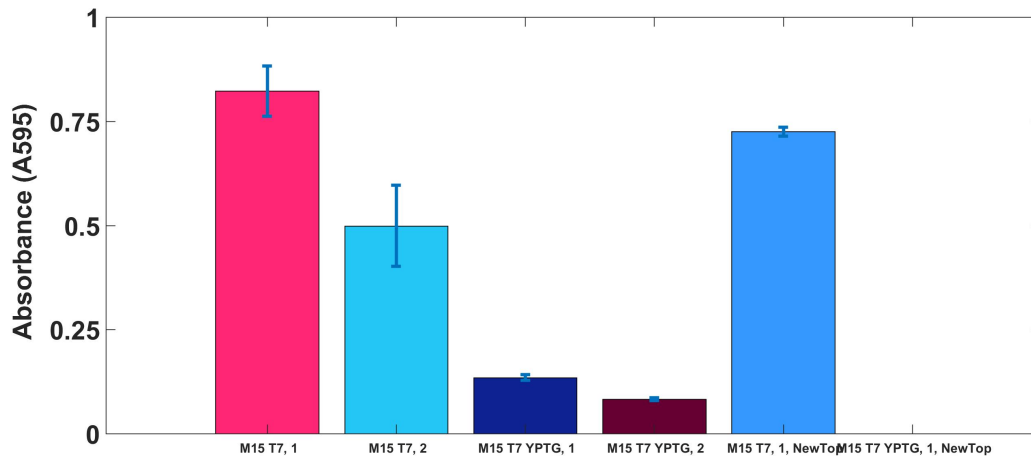
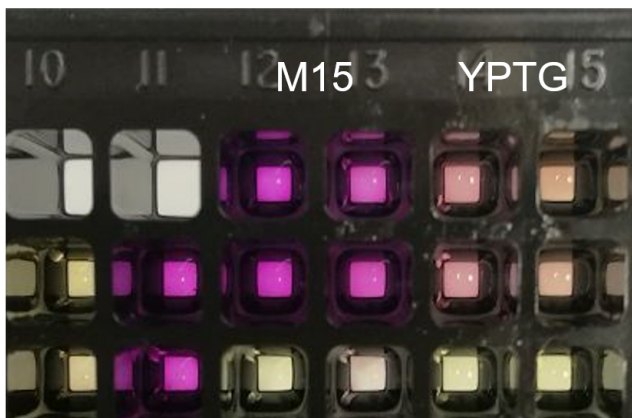


Image of the wells after the plate reader measurements (12 hours later):



Columns 12-15, third row : negative controls of each lysate

Column 12 : M15 T7 batch 1
 Column 13 : M15 T7 batch 2
 Column 14 : M15 T7 YPTG batch 1
 Column 15 : M15 T7 YPTG batch 2
 Column 11 : M15 T7 batch 1 + untested Top10-GamS
 Column 10 : M15 T7 YPTG batch 1 + untested Top10-GamS

5. Conclusion:

The new M15 T7 lysates work well, where batch 1 had better expression than batch 2 (but both will give an output visible by eye).

The M15 T7 lysates grown on YPTG show very little expression (and also weak colour change), so it will probably not be used for future experiments.

The so far untested Top10-GamS lysate is usable, although overall expression with it was weaker than with the normal Top10-GamS lysate.