

August 11 2017 : Test all lysates with E.Coli LacZ

1. Aim:

To have a comparative experiment where all lysates are tested in the same conditions (same plate) and with the same DNA. This can give us good evidence of relative expression levels, which is an integral part of lysate characterization.

2. Materials:

- Lysate from Top10-GamS cells
- Lysate from Top10 cells
- Lysate from M15 cells
- Lysate from M15+T7 cells
- Lysate from M15-T7 cells grown on YPTG
- Lysate from DH5alpha cells
- Lysate from BL21 cells
- Lysate from BL21 cells grown on YPTG
- Platerreader
- 384 well plate
- Linear E.Coli LacZ
- Energy Solution
- Buffer A
- GamS
- Nuclease-free water
- Substrate

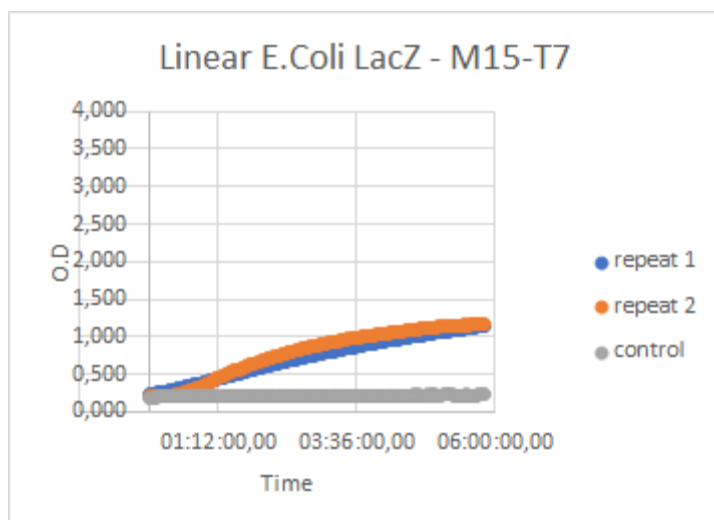
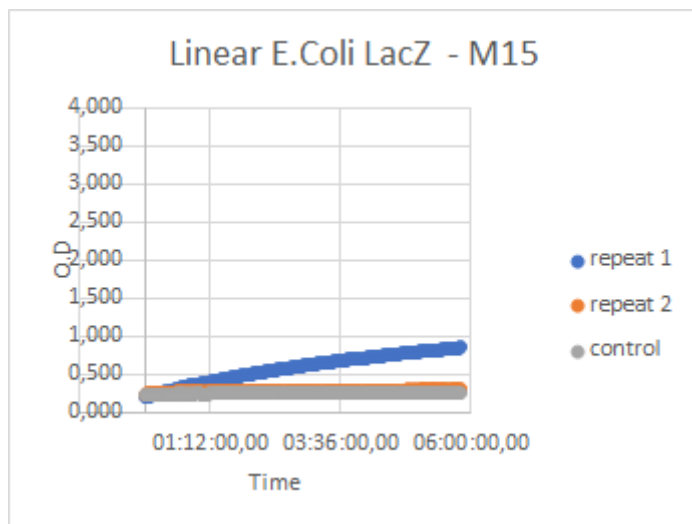
3. Procedure:

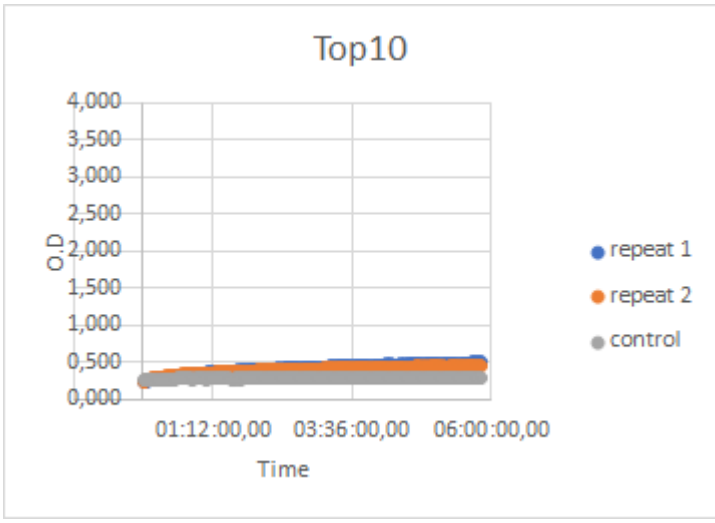
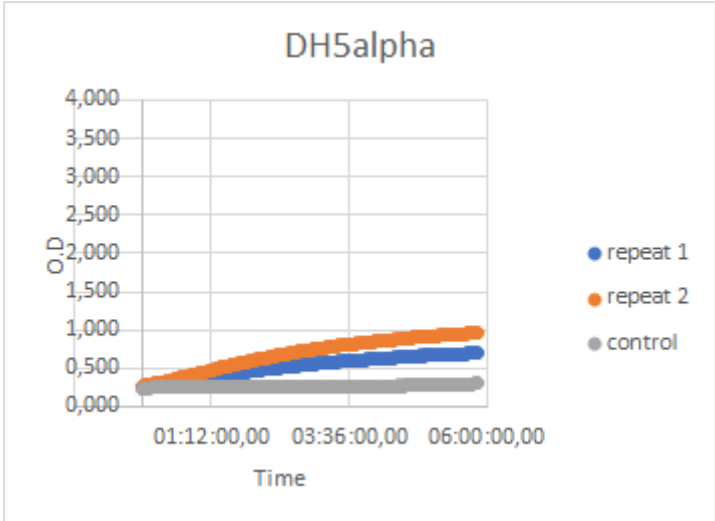
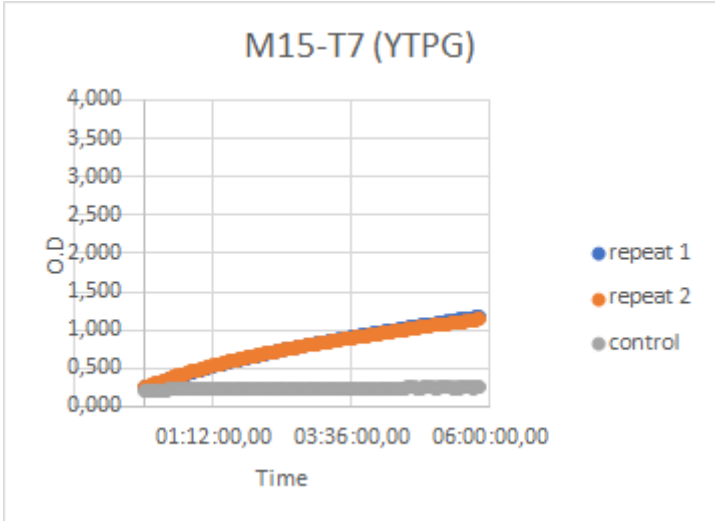
All quantities in microliters. Set up platerreader to measure 150 repeats with two minutes between each repeat, at 37C.

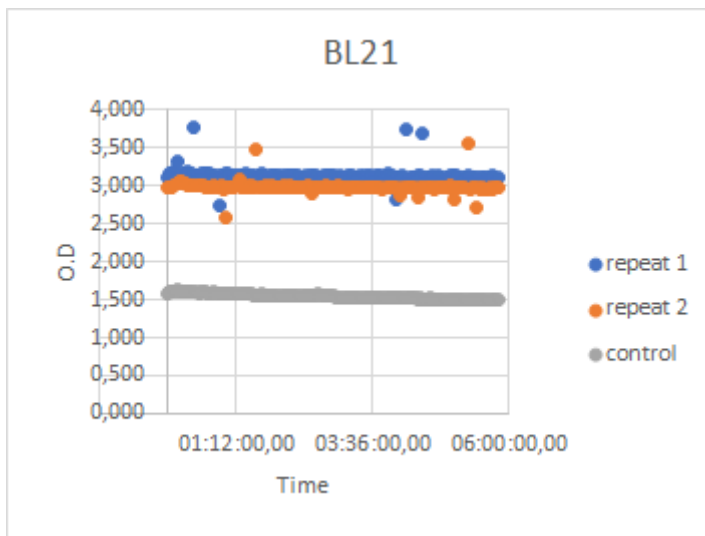
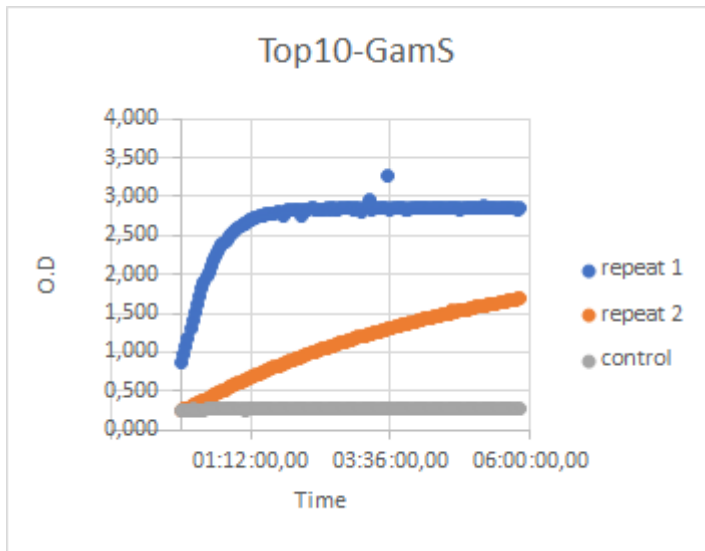
[DNA] initial	DNA quantity	Which Lysate	Lysate quantity	Energy solution	Buffer A	gamS	Substrate	H2O
Linear E.Coli LacZ	0,15	M15	2,5	2,5	2,5	0,3	0,2	1,85
Linear E.Coli LacZ	0,15	M15 T7	2,5	2,5	2,5	0,3	0,2	1,85
Linear E.Coli LacZ	0,15	M15 T7 YPTG	2,5	2,5	2,5	0,3	0,2	1,85
Linear E.Coli LacZ	0,15	DH5alpha	2,5	2,5	2,5	0,3	0,2	1,85
Linear E.Coli LacZ	0,15	Top10	2,5	2,5	2,5	0,3	0,2	1,85
Linear E.Coli LacZ	0,15	Top10-Gams	2,5	2,5	2,5	0,3	0,2	1,85
Linear E.Coli LacZ	0,15	BL21	2,5	2,5	2,5	0,3	0,2	1,85
Linear E.Coli LacZ	0,15	BL21 YPTG	2,5	2,5	2,5	0,3	0,2	1,85
none	0	M15	2,5	2,5	2,5	0	0,2	2,30

none	0	M15 T7	2,5	2,5	2,5	0	0,2	2,30
none	0	M15 YPTG T7	2,5	2,5	2,5	0	0,2	2,30
none	0	DH5alpha	2,5	2,5	2,5	0	0,2	2,30
none	0	Top10	2,5	2,5	2,5	0	0,2	2,30
none	0	Top10-GamS	2,5	2,5	2,5	0	0,2	2,30
none	0	BL21	2,5	2,5	2,5	0	0,2	2,30
none	0	BL21 YPTG	2,5	2,5	2,5	0	0,2	2,30

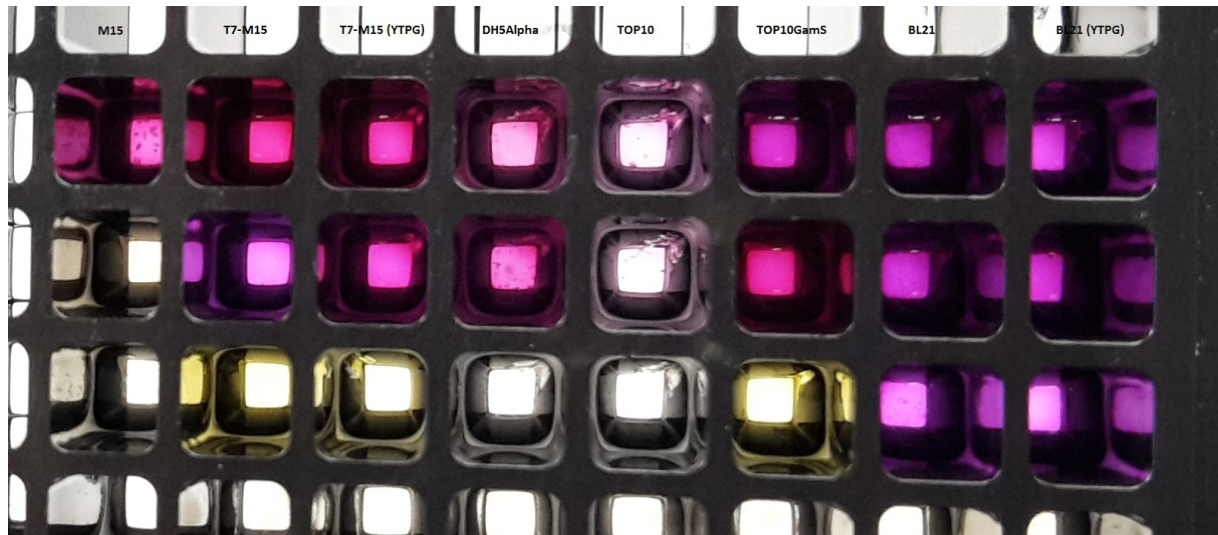
4. Results:







Picture of the 386 well plate:

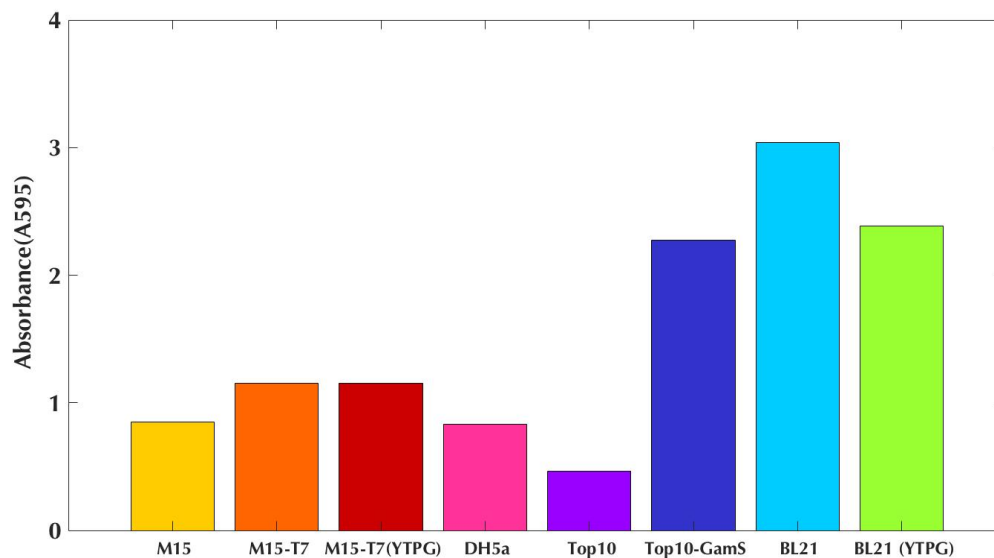


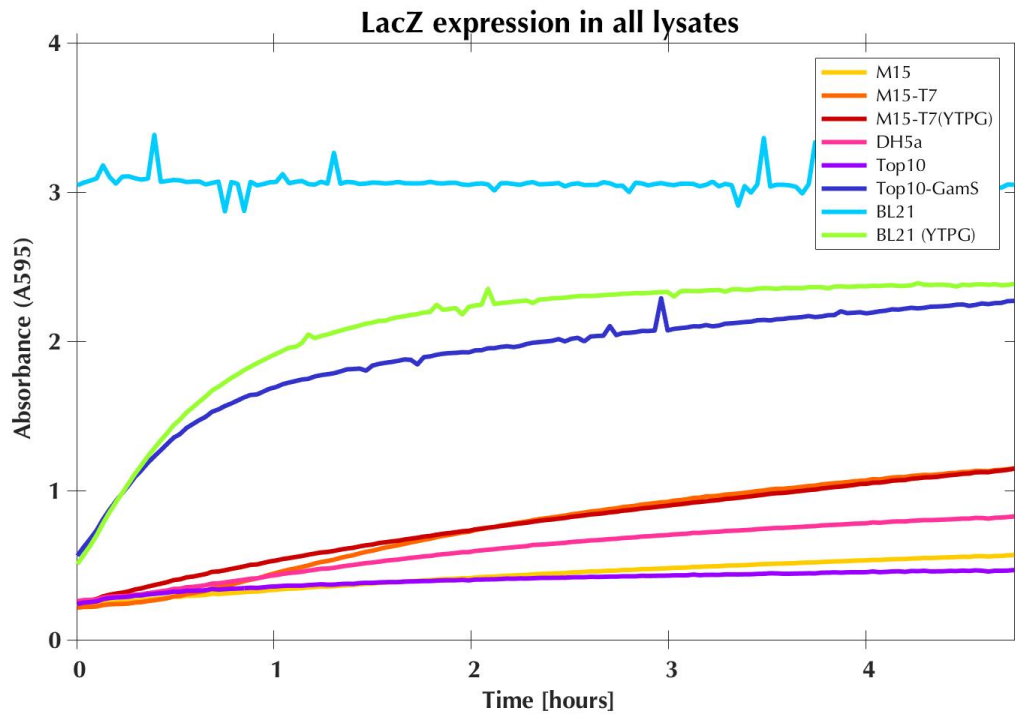
5. Conclusion:

All lysates except for Top10 have a noticeable colour change, which is confirmation of the plate reader data, where all lysates except Top10 show expression levels in the range of 0,5 – 2,5.

We also conclude that even if the expression levels are not very high (as for instance with DH5alpha), the colour change is non-ambiguous and conclusive.

The BL21 YPTG lysate had lower and slower expression compared to BL21 lysate. M15 T7 YPTG and M15 T7 expressed beta-gal on similar levels.





Graphe + bars:

Séparer les graphes car on a l'impression qu'ils ne marchent pas

⇒ **Graphe 1 =**

- BL21
- BL21 YPTG
- Top10-gamS

⇒ **Graphe 2 =**

- M15-T7
- M15-T7 YPTG
- DH5a
- Top10

⇒ **Garder les control visibles**

