August 5 2017 : Efficiency of added Top10 vs Top10-GamS lysate to BL21

1. Aim:

Find out the efficiency of the Top10 lysate compared to the Top10-GamS lysate when added to BL21. This is a repeated experiment as the last one yielded inconclusive results.

2. Materials:

- Lysate from Top10+GamS cells
- Lysate from Top10 cells
- Lysate from BL21 cells
- Platereader
- 384 well plate
- Linear EGFP + T7
- Energy Solution
- Buffer A
- GamS
- Nuclease-free water

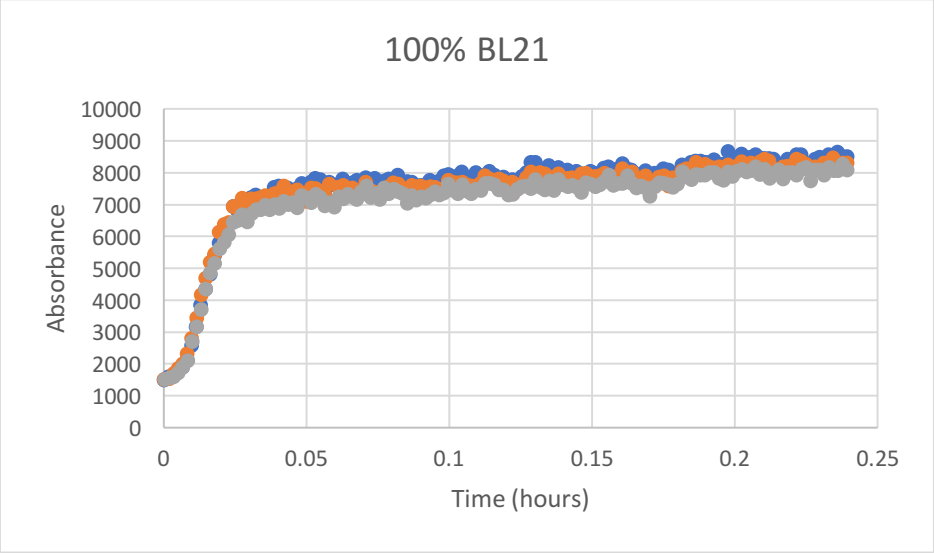
3. Procedure:

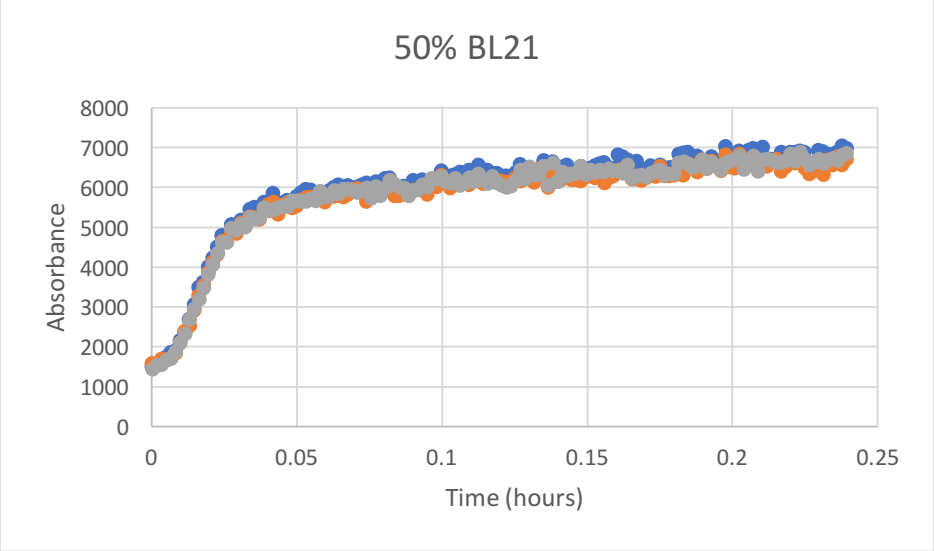
All quantities in microliters. Set up platereader to measure 150 repeats with two minutes between each repeat, at 37C.

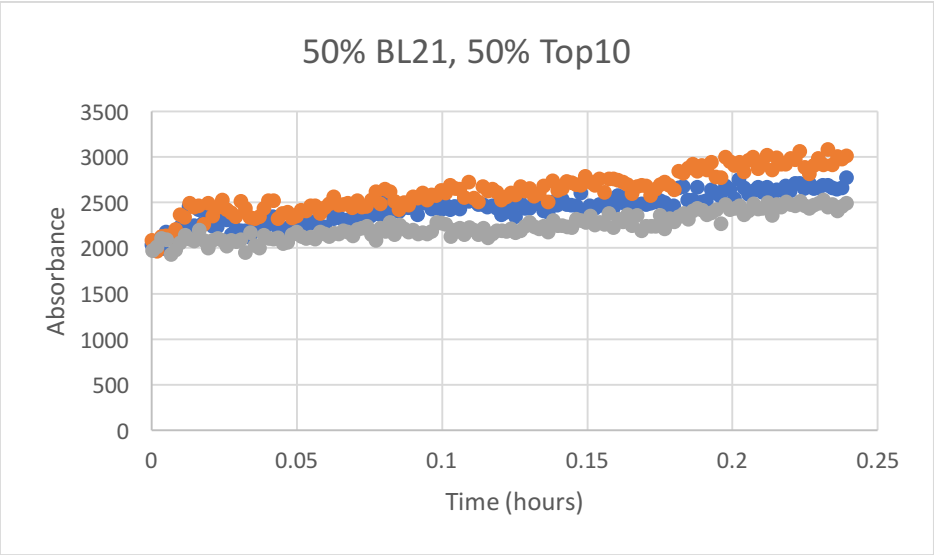
[DNA] initial	DNA quantity	Which Lysate			Energy solution	Buffer A	GamS	H20
		BL21	Top10	Top10-GamS				
Linear DNA T7	0.336	2.5	0	0	2.5	2.5	0.3	1.86
Linear DNA T7	0.336	1.25	0	0	2.5	2.5	0.3	3.11
Linear DNA T7	0.336	1.25	1.25	0	2.5	2.5	0.3	1.86
Linear DNA T7	0.336	1.25	0	1.25	2.5	2.5	0	2.16
none	0.000	2.5	0	0	2.5	2.5	0	2.50
none	0	1.25	1.25	0	2.5	2.5	0	2.50
none	0.000	1.25	0	1.25	2.5	2.5	0	2.5

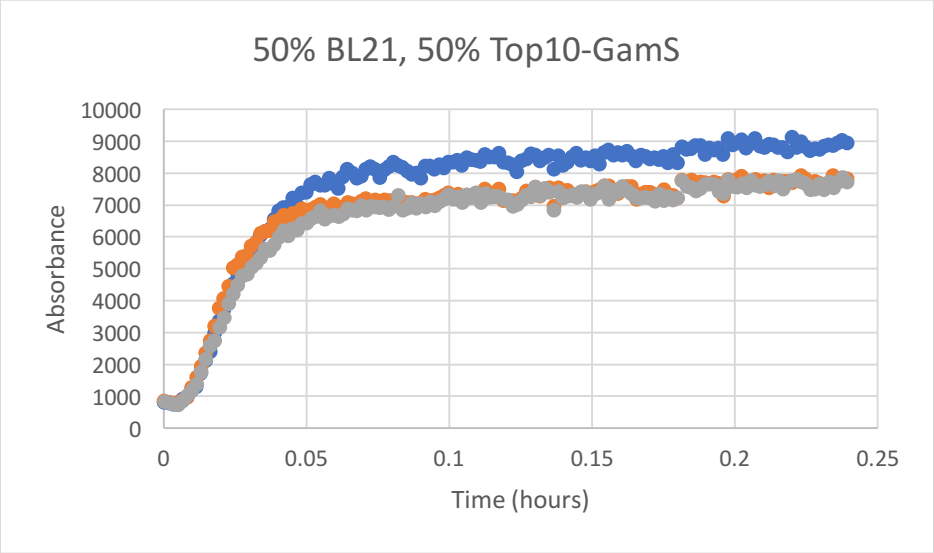
4. Results:

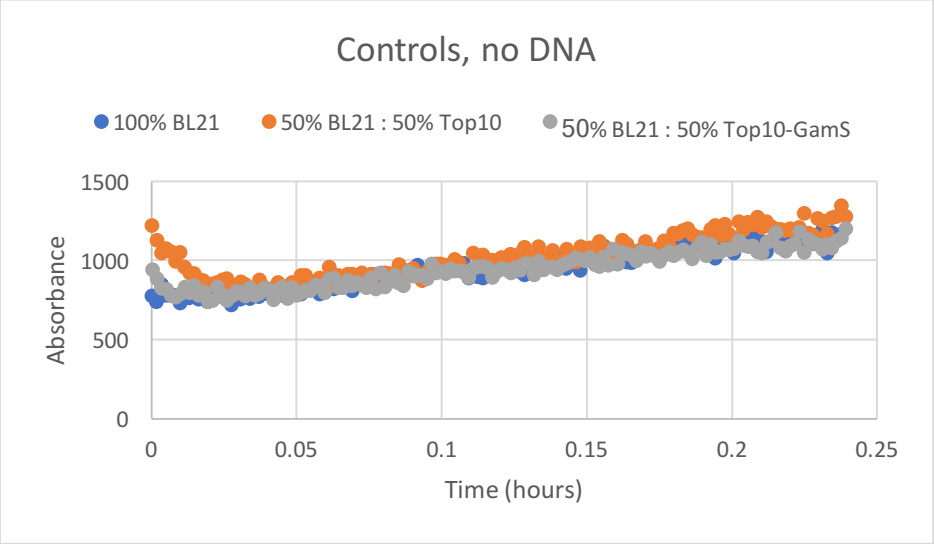
The ratio shown is the BL21 : Top10 ratio, the graph order is the same as on the table.



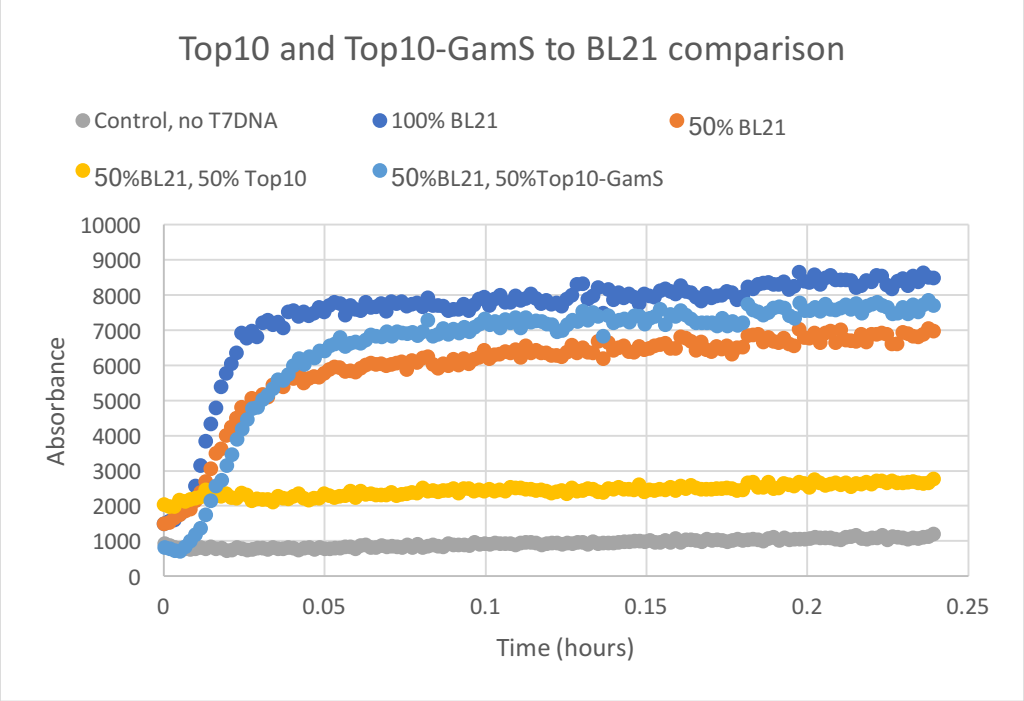



50% BL21, 50% Top10
Absorbance
3500
3000
2500
2000
1500
1000
500
0
0
0.05
0.1
0.15
0.2
0.25
Time (hours)


50% BL21, 50% Top10-GamS
Absorbance
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
0.05
0.1
0.15
0.2
0.25
Time (hours)


Controls, no DNA
100% BL21
50% BL21 : 50% Top10
50% BL21 : 50% Top10-GamS
Absorbance
1500
1000
500
0
0
0.05
0.1
0.15
0.2
0.25
Time (hours)

Integrated chart : the averages of the above graphs were taken



The time scale has the wrong numbers (total reaction time was 6 hours).

5. Conclusion:

The experiment was repeated because the results for 50%BL21, 50%Top10 seemed odd. Yet we get the same result : Very low expression, even though GamS is added and the same amount of BL21 is shown to express quite highly on the graph before.

We need to investigate if / why addition of Top10 lysate to BL21 lysate reaction has this inhibitory effect.

Besides that we can conclude that adding Top10-GamS lysate is a good alternative to adding GamS directly to the reaction.

Graphe:
Le refaire avec le format habituel

- ⇨ **Completer nos résultats de gamS => prouver avec plusieurs graphes**
- ⇨ **Faire des labels plus claires:**
  - **Control**
  - **BL21(DE3) + gamS**

- BL21(DE3) + water
- BL21(DE3) + Top10-gamS
- BL21(DE3) + Top10