August 1 2017 : Characterization of M15 T7 lysate and M15 T7 lysate from cells grown on YPTG

1. Aim:

To investigate protein synthesis of M15 T7 cell lysates with and without Top10-GamS as well as compare the usual M15 T7 lysate with lysate made from M15 T7 cells grown on YPTG.

2. Materials:

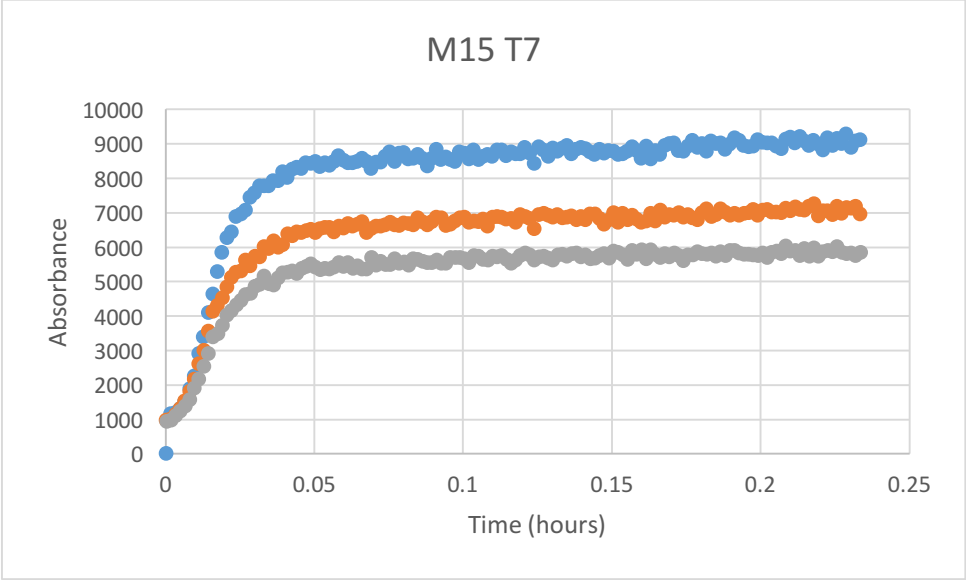
- Lysate from Top10+GamS cells
- Lysate from M15 T7 cells
- Lysate from M15 T7 cells grown on YPTG
- Platereader
- 384 well plate
- Linear GFP + T7
- Energy Solution
- Buffer A
- GamS
- Nuclease-free water

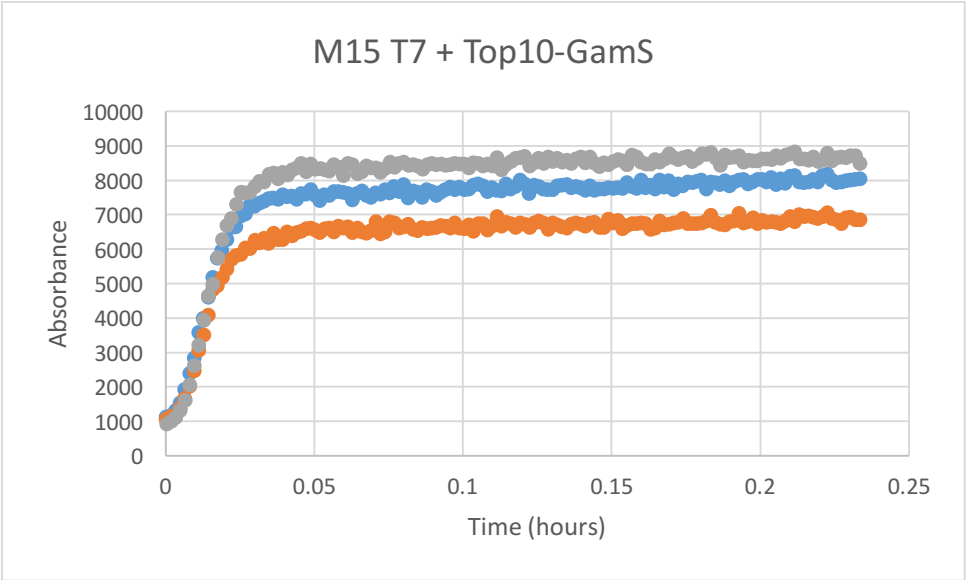
3. Procedure:

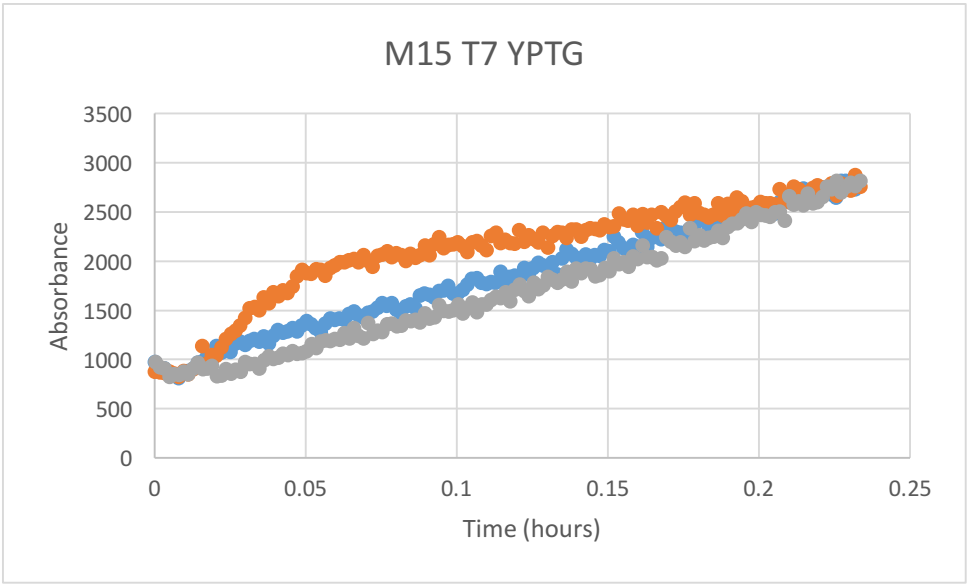
[DNA] initial	DNA quantity	Which lysate	Lysate quantity	Energy solution	Buffer A	GamS	H20
Linear DNA T7	0.336	M15-T7	2.5	2.5	2.5	0.3	1.86
Linear DNA T7	0.336	M15-T7 + Top10	2.5	2.5	2.5	0	2.16
Linear DNA T7	0.336	M15-T7 YPTG	2.5	2.5	2.5	0.3	1.86
Linear DNA T7	0.336	M15-T7 YPTG + Top10	2.5	2.5	2.5	0	2.16
none	0	M15-T7	2.5	2.5	2.5	0	2.5
none	0	M15-T7 YPTG	2.5	2.5	2.5	0	2.5
none	0	Top10-GamS	2.5	2.5	2.5	0	2.5

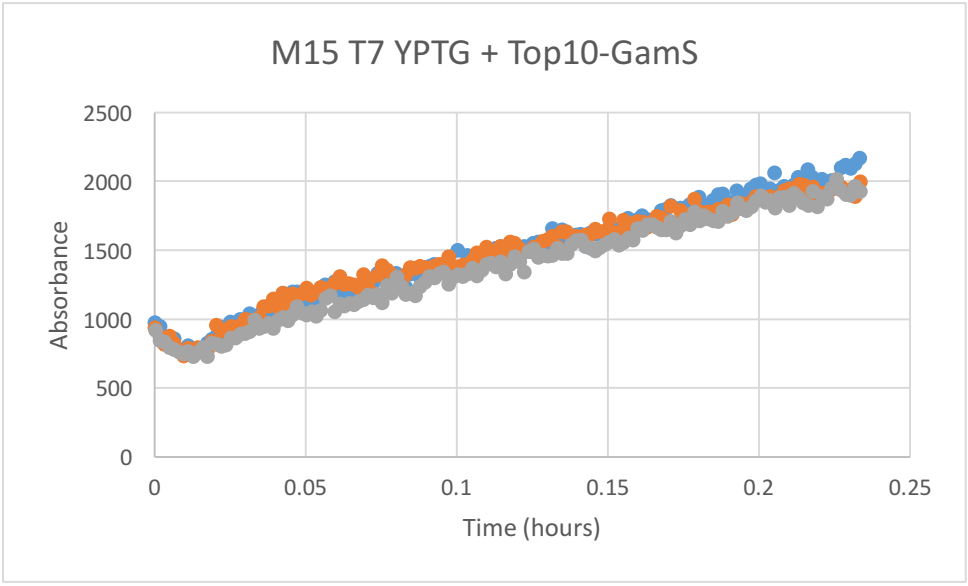
All quantities in microliters. Set up platereader to measure 150 repeats with two minutes between each repeat, at 37C.

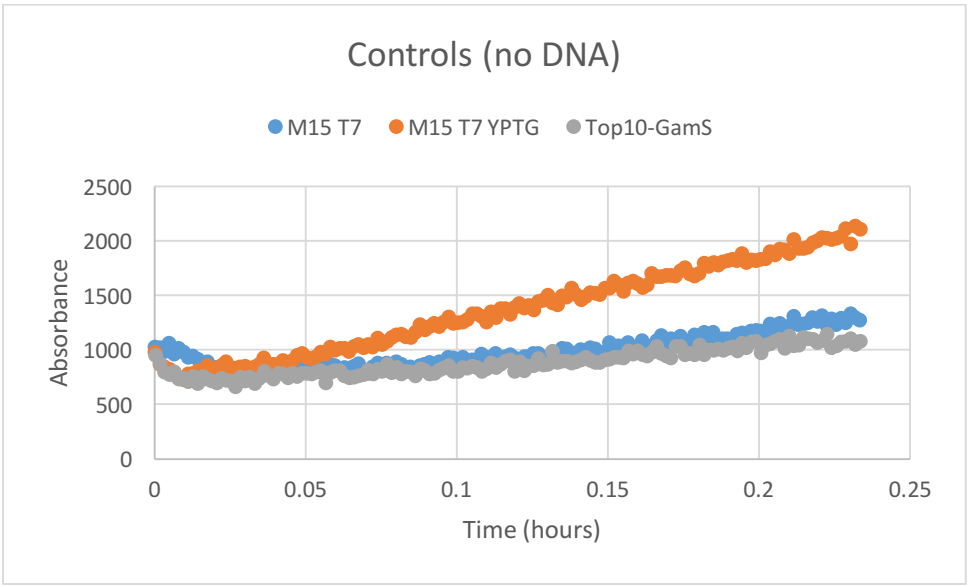
4. Results:


M15 T7
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)

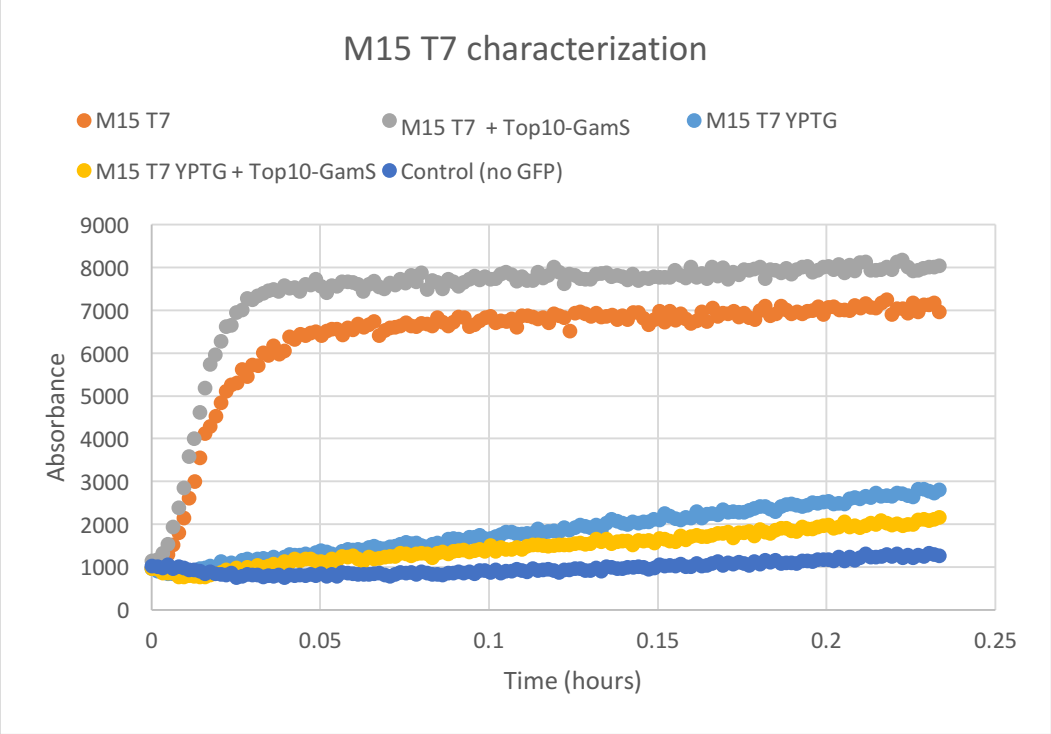

M15 T7 + Top10-GamS
Absorbance
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
0.05
0.1
0.15
0.2
0.25
Time (hours)


M15 T7 YPTG
Absorbance
3500
3000
2500
2000
1500
1000
500
0
0
0.05
0.1
0.15
0.2
0.25
Time (hours)


M15 T7 YPTG + Top10-GamS
Absorbance
2500
2000
1500
1000
500
0
0
0.05
0.1
0.15
0.2
0.25
Time (hours)


Controls (no DNA)
M15 T7
M15 T7 YPTG
Top10-GamS
Absorbance
2500
2000
1500
1000
500
0
0
0.05
0.1
0.15
0.2
0.25
Time (hours)

Integrated chart with the average taken of the graphs before :



5. Conclusion:

The repeats are similar and enable us to draw the following conclusions:

The M15 T7 lysate works better with Top10-GamS than with GamS added in protein form (compare: it takes half the M15 T7 lysate, yet the average protein synthesis is higher. Top10-GamS does not have the T7 polymerase, so all the GFP synthesis is due the M15 lysate, whose productivity is enhanced).

There was an error with the M17 T7 YPTG lysate, maybe the wrong lysate was taken, as we see expression comparable to the negative control (nonexistent).

The M15 T7 YPTG part of this experiment should be repeated for conclusion.

Graphe: enlever les YPTG
Label:
M15-T7 + gamS
M15-T7 + Top10-gamS

Both control