# Lab *18.08.19 Test expression of beta-gal in freeze dried reactions of T7M15*

Aim:
Measure the O.D at 595nm of the rehydrated freeze dried reactions to see if they express b-gal at detectable levels

## 1. Materials:

- Freeze-dried reactions tubes
- Nuclease free water
- GamS
- E.coli lacZ
- Substrate (15 mg/ml)
- RNAse inhibitos
- ssDNA short trigger
- Plate reader
- Big and small Centrifuges

## 2. Procedure

Weigh the freeze-dried reaction tubes and see how much water has evaporated ( add that amount when rehydrated as well)
Add the components that are not already in the freeze dried reaction (GamS, RNAse inhibitors, H20 and DNA trigger for the toehold as well as DNA lacZ for the no DNA reactions)
Do three repeats of both reactions.

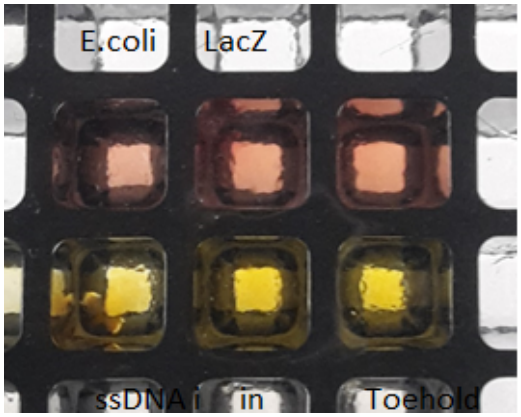
The tables below summarizes what was added to rehydrate the reactions.

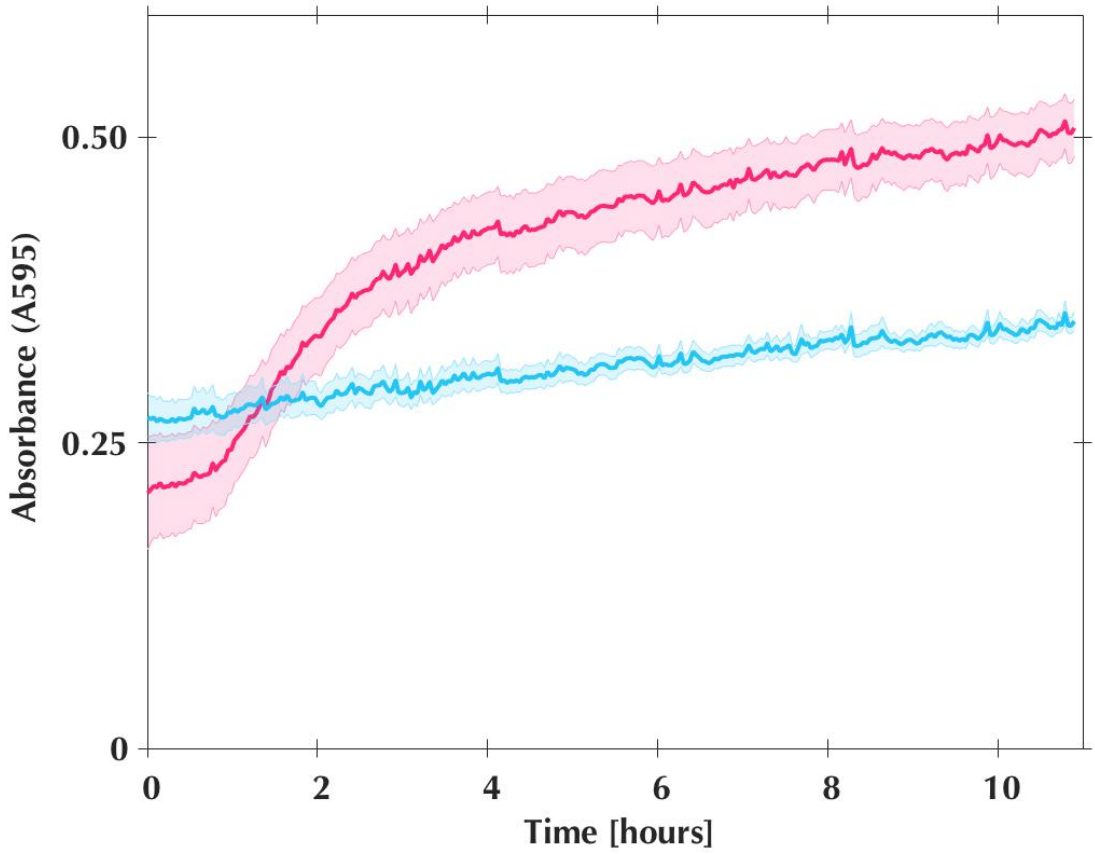
| [DNA] initial | DNA quantity | Which Lysate | | | Energy solution | Buffer A | gamS | Substrate | ssDNA trigger | Rnase inh | H20 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Lysate quantity | | | | | | | | |
| | | | | | | | | | | | |
| **Linear E.Coli LacZ** | 0,15 | M15 | 2,5 | | 2,5 | 2,5 | 0,3 | 0,2 | 0 | 0 | 1,85 |
| **Toehold** | 0,60 | | 2,5 | | 2,5 | 2,5 | 0,3 | 0,2 | 0,4 | 0,1 | 0,90 |

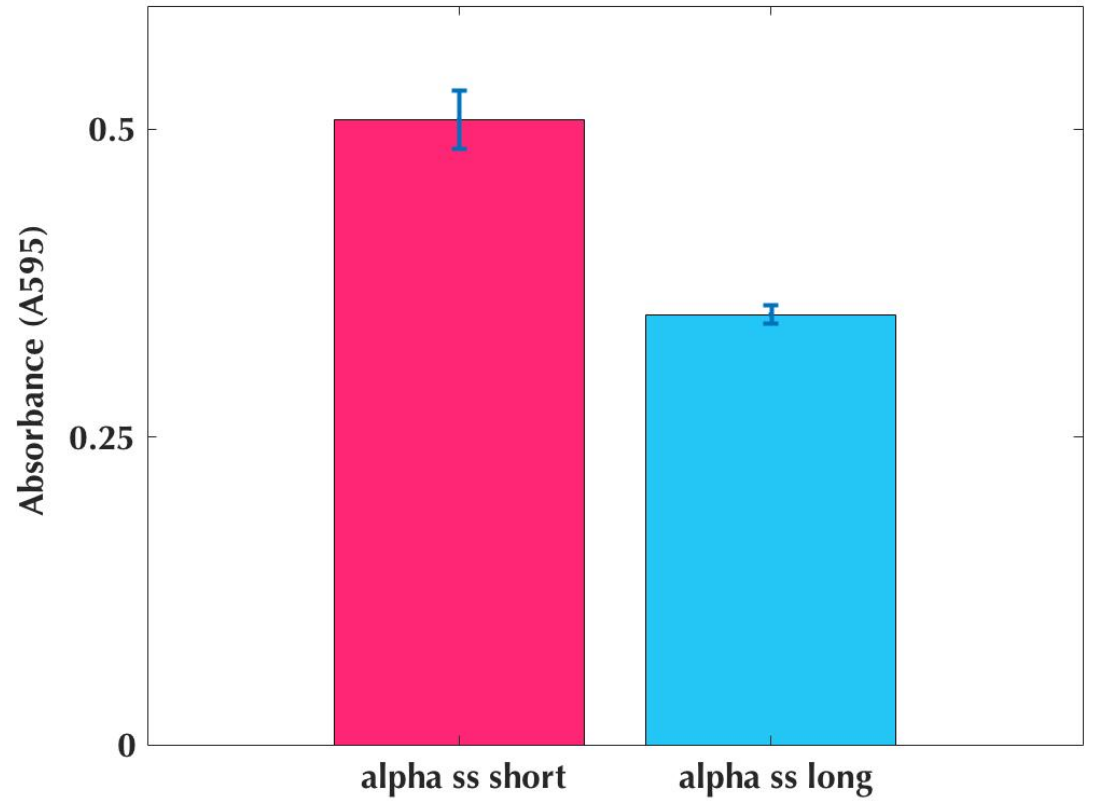
| | | |
|---|---|---|
| **tube 1 (no DNA)** | **E.coli LacZ** | **0,45** |
| | Substrate | 0,60 |
| | GamS | 0,9 |
| | Total water | 33,25 |
| | | |
| **tube 6( toehold)** | ssDNA trigger short | 1,2 |
| | Substrate | 0,6 |
| | RNAse inhib | 0,3 |
| | Total water | 27,9 |
| | GamS | 0,9 |

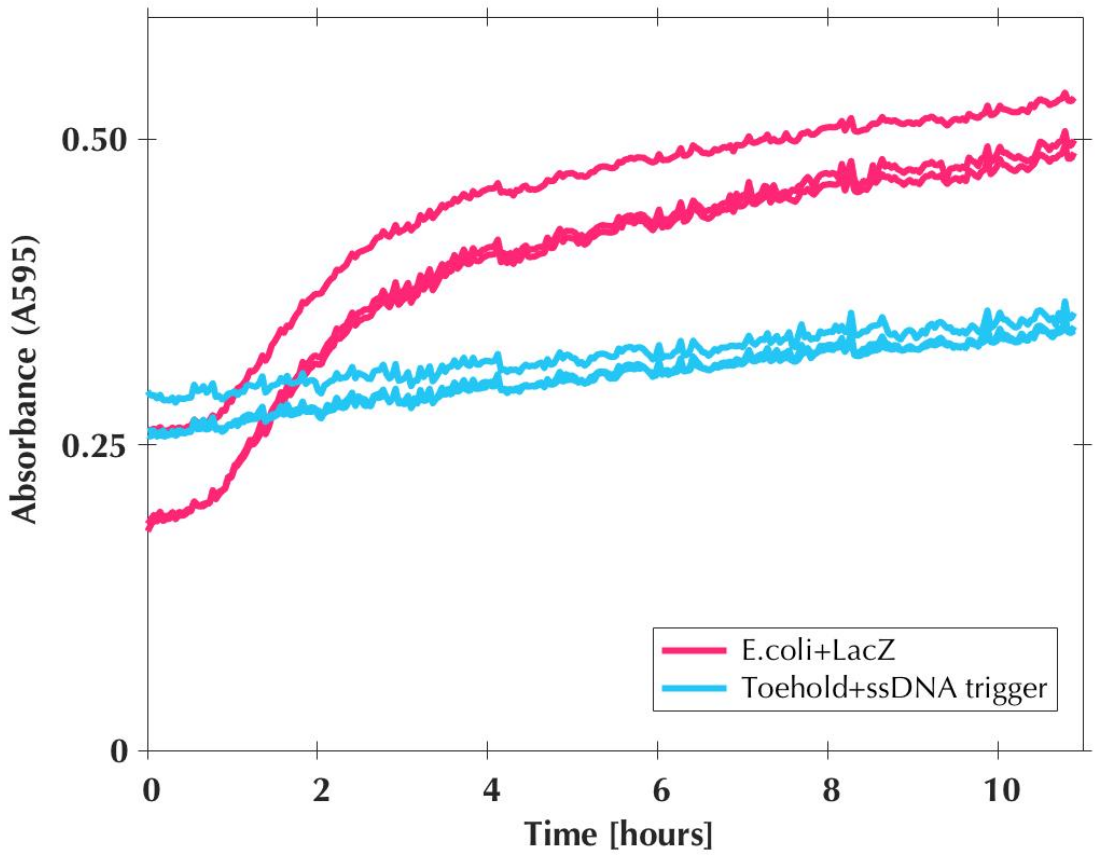
## 3. Labels:

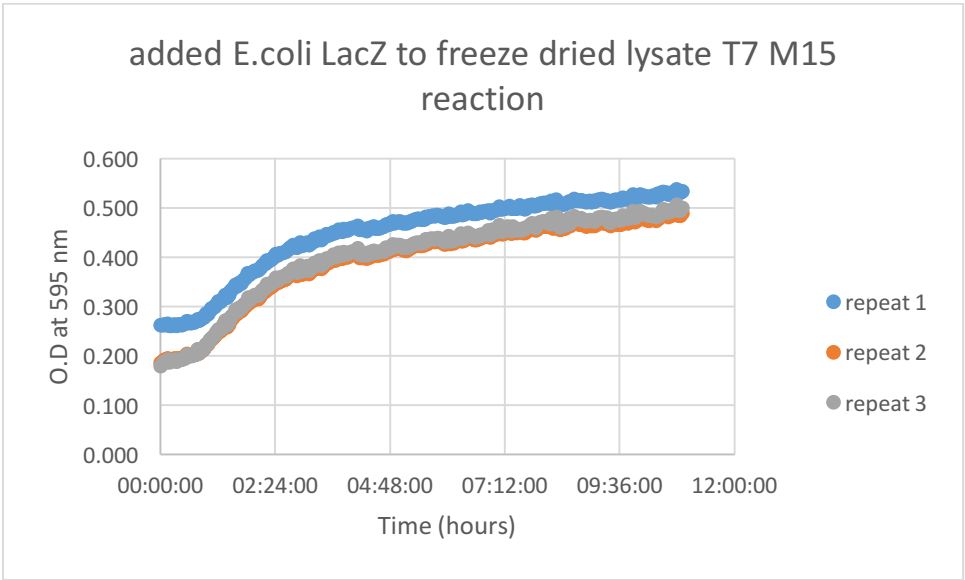
## 4. Results:

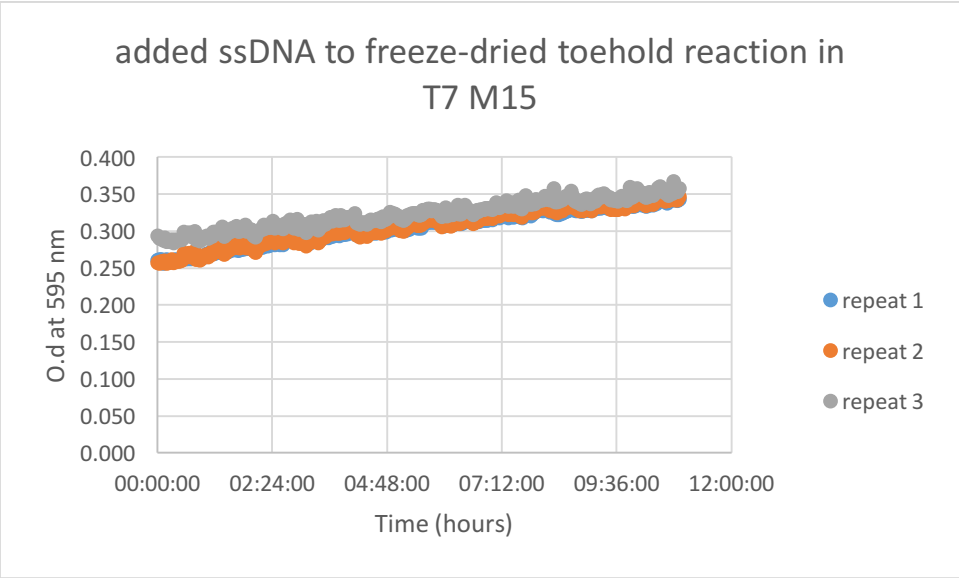



0.50
0.25
0
Absorbance (A595)
0
2
4
6
8
10
Time [hours]


0.5
0.25
0
Absorbance (A595)
alpha ss short
alpha ss long


0.50
0.25
0
Absorbance (A595)
0
2
4
6
8
10
Time [hours]
E.coli+LacZ
Toehold+ssDNA trigger


added E.coli LacZ to freeze dried lysate T7 M15 reaction
0.600
0.500
0.400
0.300
0.200
0.100
0.000
O.D at 595 nm
00:00:00
02:24:00
04:48:00
07:12:00
09:36:00
12:00:00
Time (hours)
repeat 1
repeat 2
repeat 3




## 5. Conclusion:

The levels of expression of beta-gal even though not as high as before recorded where enough to witness a color change in the wells for the freeze dried reactions where we added E.coli lacZ to the M15 T7 lysate.
However, the toehold reactions were unsuccessful as no expression was recorded and the substrate stayed yellow (this experiment needs to be repeated with RNA and not only ssDNA short)