18 September 2017 : ssDNA trigger addition after different times

1. Aim:

To see if adding the trigger at different times has an effect on the lysate reaction.

2. Materials:

- Nuclease-free water
- LacZalpha toehold
- ssDNA short trigger (27B) (500 uM)
- M15 T7 lysate
- Top10-GamS lysate
- Buffer A
- Energy solution
- Substrate (15 mg/mL)
- Plate reader

3. Procedure:

Put into 9 200 uL PCR tubes the following :

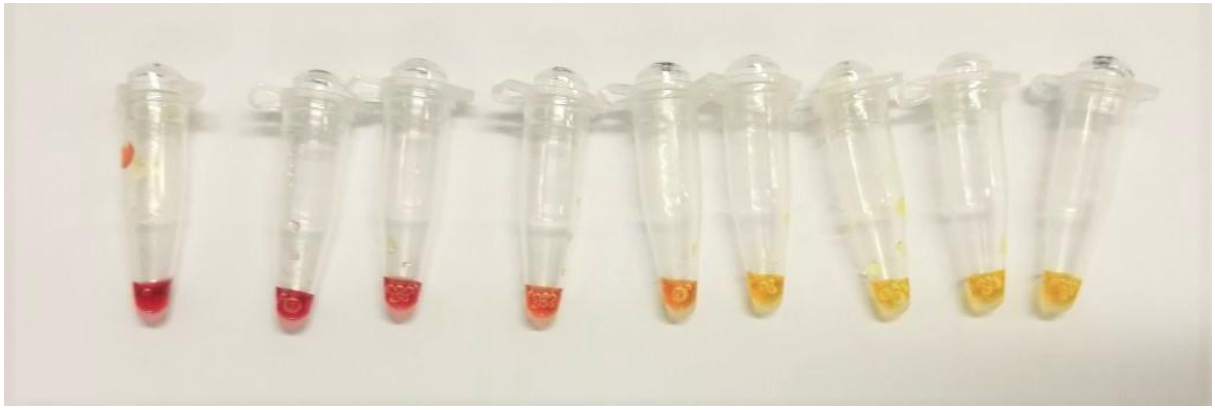
Tube	Which Trigger	Trigger quantity	[DNA] initial	DNA quantity	Which lysate	Lysate quantity	Top10-GamS lysate	Energy solution	Buffer A	Rnase inhib	H20	Substrate	Time of trigger addition
1	ssDNA short	0.2	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.500	0.2	0 min
2	ssDNA short	0.2	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.500	0.2	5 min
3	ssDNA short	0.2	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.500	0.2	10 min
4	ssDNA short	0.2	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.500	0.2	15 min
5	ssDNA short	0.2	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.500	0.2	20 min
6	ssDNA short	0.2	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.500	0.2	30 min
7	ssDNA short	0.2	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.500	0.2	45 min
8	ssDNA short	0.2	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.500	0.2	60 min
9	none	0	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.700	0.2	0 min

4. Results:

Image taken after 30 minutes :

Image taken after 45 minutes :

Image taken after 60 minutes :



5. Conclusion:

Adding the trigger at a later stage to the lysate reaction does not improve the performance of the lysate, as the first tube is by far the one with the best output. In order to keep this diagnostic test simple, we should put trigger to lysate from the beginning, there is no need to incubate the toehold in lysate beforehand (no increased ouput).