Lab 04.08.18 Test Toehold with long and short ssDNA trigger

1. Aim:

Test whether the trigger binds to the toehold and results in the unwinding of the RBS, transcription of the lacZ gene and production of the beta-gal

2. Materials:

- Lysate T7-M15
- Buffer A
- Nuclease free water
- Energy Solution
- ssDNA trigger short
- ssDNA trigger long
- Toehold iG1 PCR
- Substrate (15 mg/ml)
- RNAse inhibitors
- GamS
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3. Procedure

Add the following components to a 200ul pcr tube:

- 1 Reaction trigger short+plasmid
- 1 Reaction trigger short+iG1
- 1 Reaction triggerlong+plasmid
- 1 Reaction trigger long+iG1
- 1 Control plasmid (No trigger)
- 1 Control linear template (No trigger)

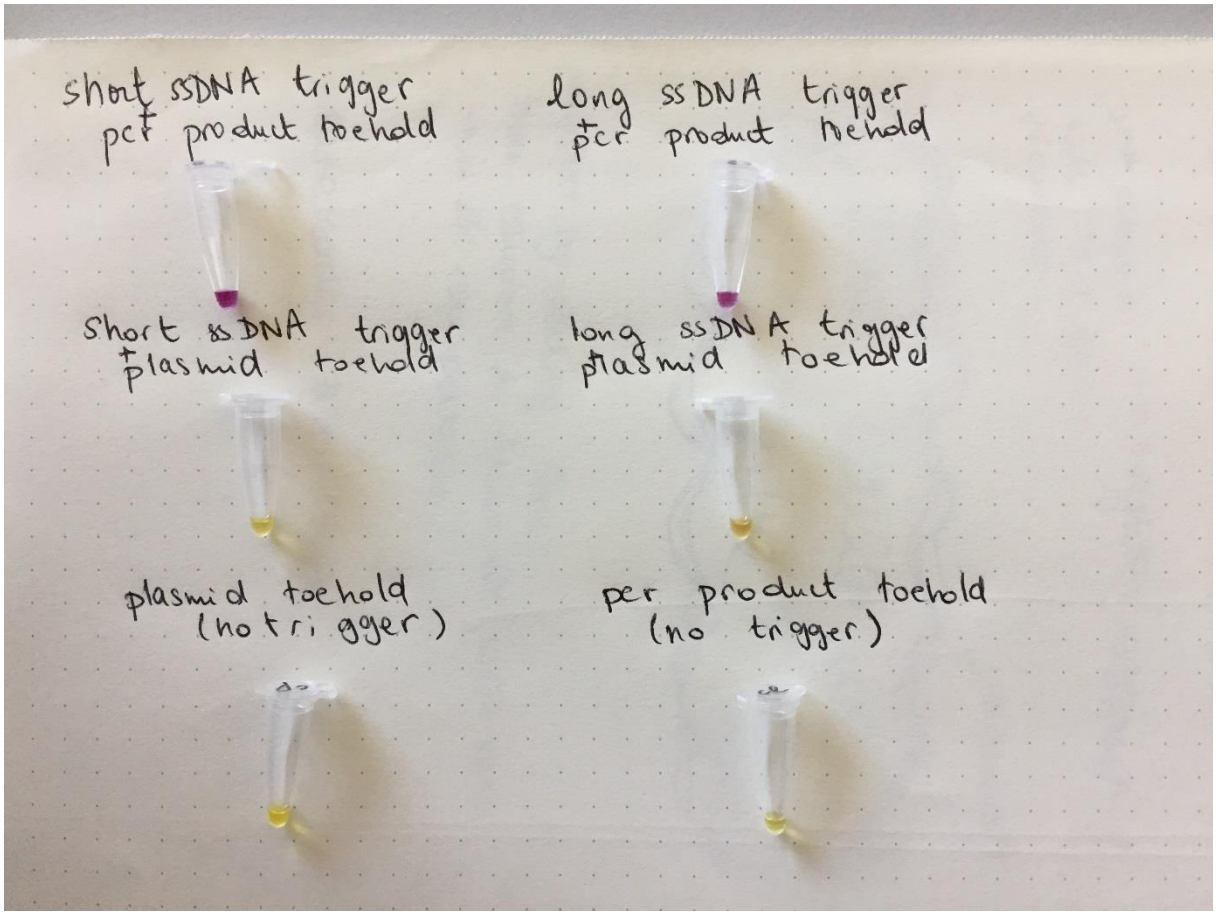
Plasmid	volume to add
toehold	1,38
trigger	0,2
RNASE	0,2
Substrate	0,2
lysate	2,5
ES	2,5
Buffer A	2,5

Nuclease free water	0,52
Total volume	10

Linear DNA template	
toehold	1,38
trigger	0,2
RNASE	0,2
Substrate	0,2
lysate	2,5
ES	2,5
Buffer A	2,5
Nuclease free water	0,22
GamS	0,3
Total Volume	10

And trigger controls.

4. Results:



5. Conclusion:

The toehold works in its linear DNA template form and does not in its plasmid form (problem with a stop codon?)

The trigger unwind the RBS and b-gal is produced.