ViTEST

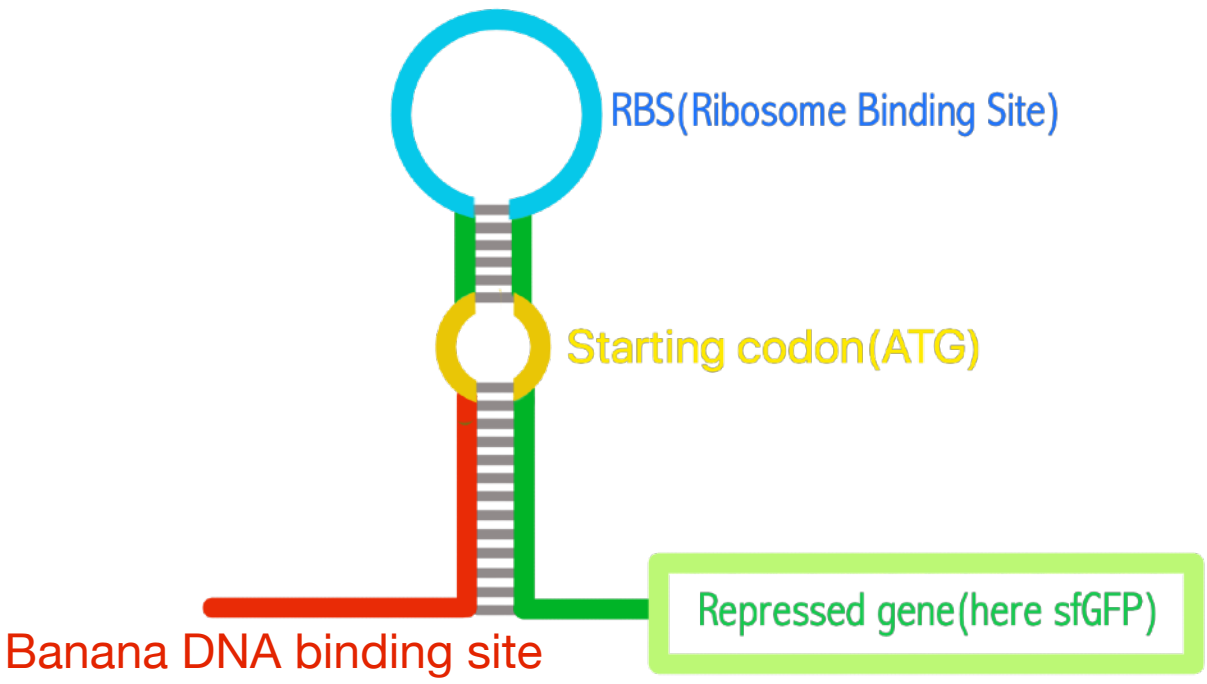
Toehold switch 7/16-7/22

Banana toehold sensor functionality study

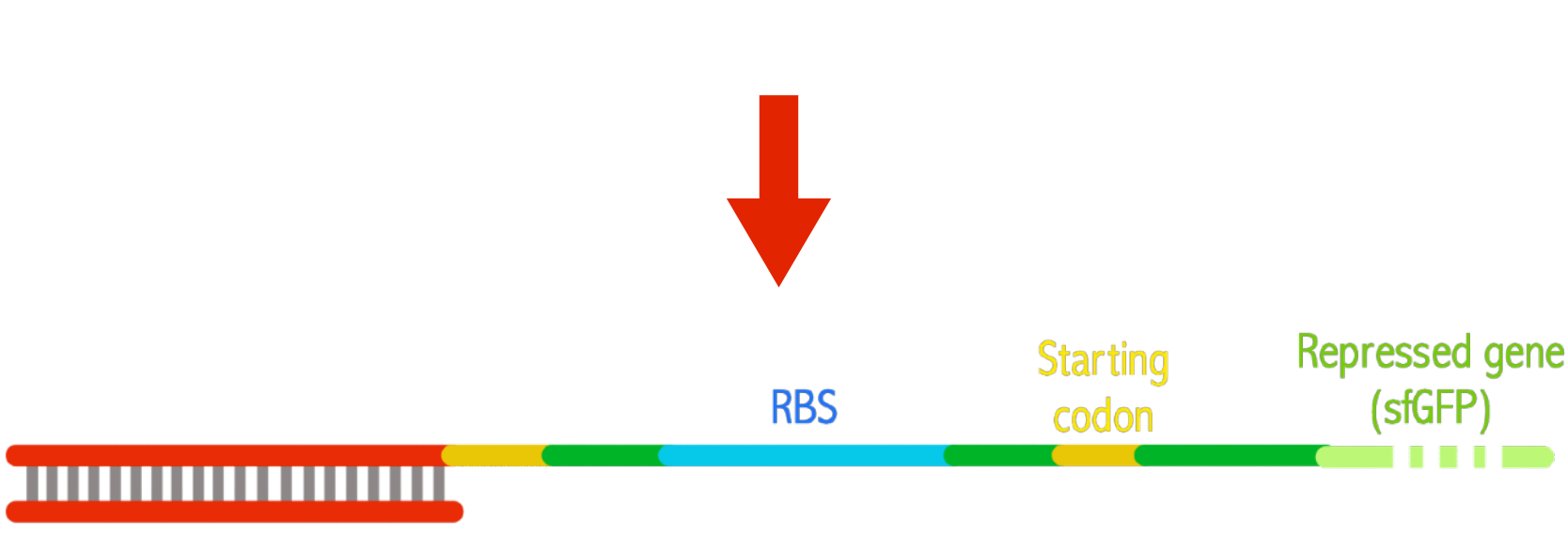
EPFL iGEM

Toehold switch

Target DNA strand



1. Banana DNA template + 2. Toehold switch RNA



3. DNA expression

Introduction:

From our Sumer School program experiment we've ordered a set a banana toehold sensor (Biobits). By preforming tests on the existing toeholds, it helps us to understand the mechanism of toehold. The test is based on the following aspects:

a. Can PCR product activate the toehold switch?

b. Does the activated toehold switch respond to PURExpress (the commercial protein expression system) or OnePot (a homemade protein expression system developed in EPFL lab)?

c. What will happen to the toehold switch if there's no presence of activator?

Tuesday, 16 July 2019

DNA template preparation:

1. Banana Solid crash (BS) 5*PCR tubes:
   1). prepare 5 tubes of 20μL Phire Plant MasterMix.
   2). crush a cut of banana(~5g) use bacteria loop to touch the mush and dip it in the tube.
2. Liquid dilution (BL) 10 tubes:
   1). cut 10mg of banana fruit mix with 1mL of H2O.
   2). make ten times 10 fold dilution of 100μL take 1μL the supernatant as template
3. Pipetting:

Master mix:	**16**		
Volume(μL):	**20**		
	Reference (20 μL)	**Per tube(μL)**	**Master mix(μL)**
H2O	**7**	**7**	**112**
2x Phire Plant MasterMix	**10**	**10**	**160**
Forward Primer (10 uM) *Diluted*	**1**	**1**	**16**
Reverse Primer (10 uM)	**1**	**1**	**16**
Banana solution (different con.)	**1**	**1**	**0**
Total	**20**	**20**	**304**

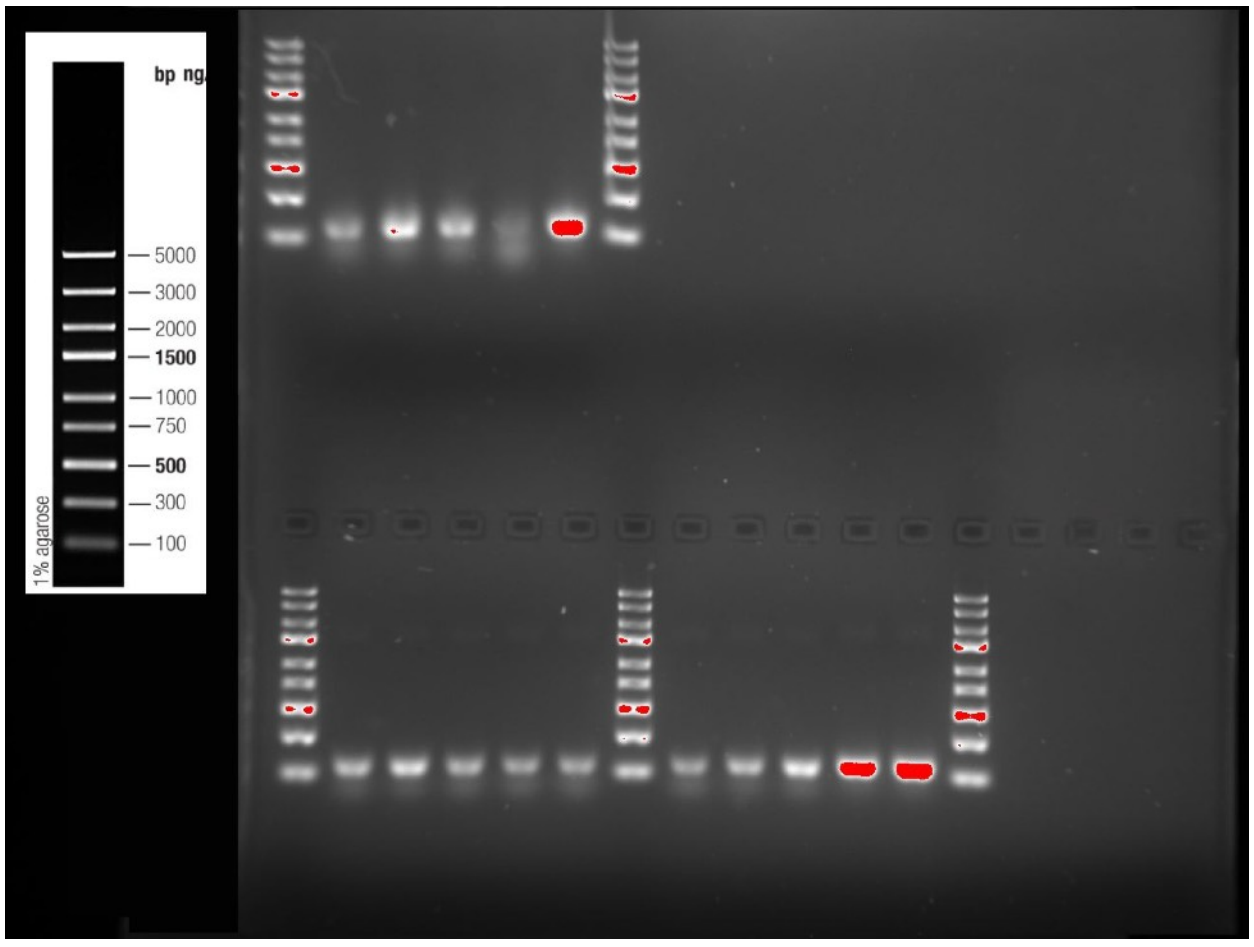
4.PCR setting:

Template	**Fw Primer**	**Rev Primer**	**Length(bp)**	**Elongation time(s)**	**Tm - Phi**	**Tm used (Celsius)**
Banana DNA	**Banana fwd RPA**	**Banana rev RPA**	**168**	**20**	**(63.3/63.6)66.7**	**67**

5. Electrophoresis:

For 168bp PCR products, 2% agarose gel is used.

Gel image:



Gel image banana trigger dsDNA

ladder	BS1	BS2	BS3	BS4	BS5	ladder						
ladder	BL1	BL2	BL3	BL4	BL5	ladder	BL6	BL7	BL8	BL9	BL10	ladder

Comments:

- All green PCR products are good, deeper green ones are better.
- PCR product BS2, BS5, BL8, BL9 and BL10 will be used in the toehold test.

Wednesday, 17 July 2019

6. Purification and DNA concentration measure:

QIAGEN PCR purification toolkits protocol : https://www.qiagen.com/us/products/qiaquick-pcr-purification-kit/#orderinginformation

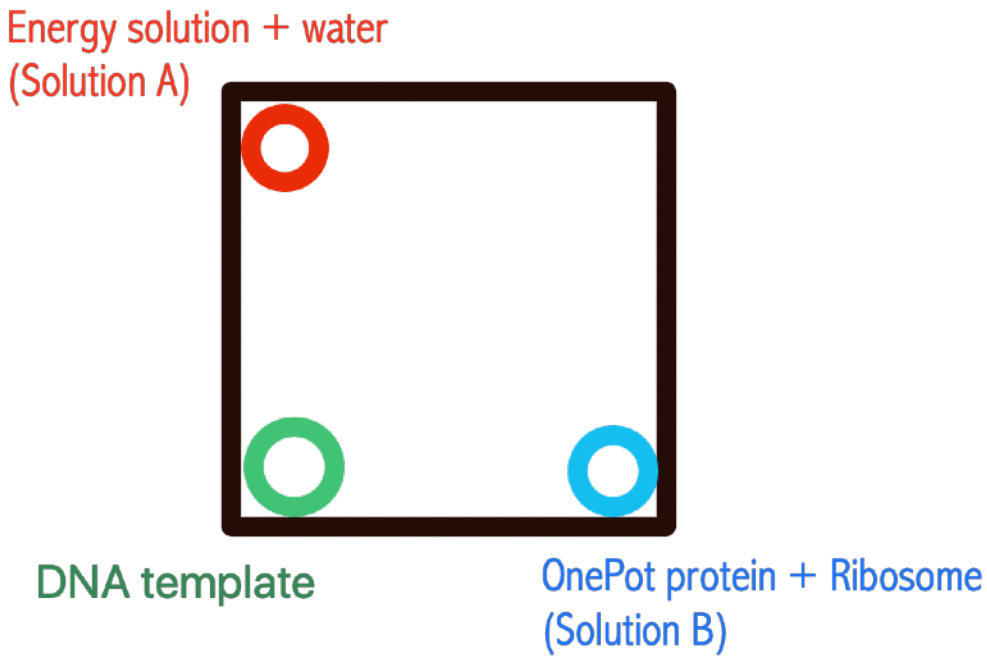
Nano drop measure results:

Banana Concentration	DNA concentration (ng/μL)
BS5	38.4
BL9	27.5
BL10	43.0
BS2	27.3
BL8	24.7

Plate reader florescent measurement:

We use micro-plate reader to measure the fluorescence wave strength in the micro-plate well. In this experiment we took 140 measurements with 30 seconds interval between each measurement.

Pipetting: In order to make sure that the reaction starts right before we start the measurement, the aliquot was pipetted separately in different corners of the well, as shown below:

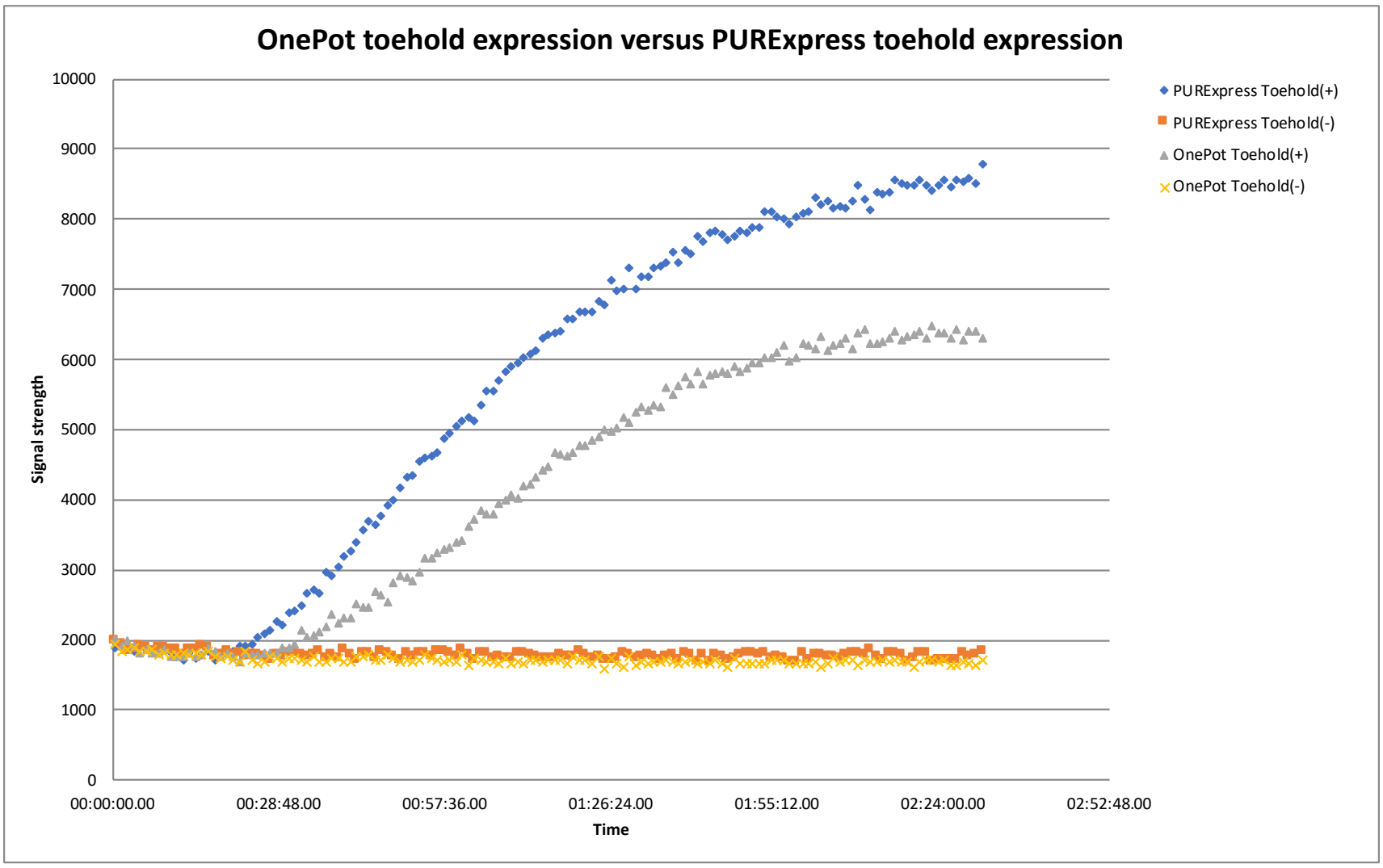


Micro-well pipetting OnePot (PURExpress)

	Reference(10µL)	OnePot toehold	PURE* toehold	PURE* negative	OnePot negative
H2O	**till 10 µL**	**0.9**	**1**	**2**	**1.9**
Energy solution	**4**	**4**	****	****	**4**
OnePot protein	**1.3**	**1.3**	****	****	**1.3**
Ribosome	**1.8**	**1.8**	****	****	**1.8**
T7 promotor	**50(ng)**	****	****	****	****
pCOLA banana sensor sfGFP-1(128ng/µL)		**0.8**	**0.8**	**0.8**	**0.8**
Banana_gBlock(50ng/µL)		**1**	**1**	****	****
A	**4**	****	**4**	**4**	****
B	**3**	****	**3**	**3**	****
RNase inhibitor	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**
Total:	**10**	**10**	**10**	**10**	**10**

After pipetting, the plate was centrifuged at 4000 rpm for 20 seconds, so the aliquots went to the bottom and mixed with each other, 35 uL of chill-out buffer was then added into each well to seal the well. When it's ready, we seal the plate with a plastic membrane dan place it in the plate reader to run the measurement.

The result is in the following diagram:



By principle, in a toehold expression, if trigger DNA fragment is not present, the RBS will stay locked in the hairpin structure, hence no protein expression, which is shown by the ***OnePot Toehold(-)*** and ***PURExpress Toehold(-)***

Conclusions/Comments:

- PCR products can serve as a trigger for toehold regulator.
- Both OnePot and PURExpress allows Toehold regulated reaction.
- A well-designed toehold will have zero leakage when trigger is not present.

From this experiment, we got an idea of how toehold regulation should look like. Base on this structure, we lanced our own toehold design.