ViTEST

Toehold switch 8/5-8/8

Toehold switch assembly I

EPFL iGEM

Introduction:

There are multiple ways of assembling our toehold switch:

a. Order as gBlock the whole toehold and repressed protein sequence (expensive to order long gBlocks, but the receive product will be ready-to-use)

b. Order toehold as primers and perform PCR on the repressed gene plasmid (cheaper but may not work because the hangover is too long)

c. Divide toehold hangover into multiple primers and preform two-time or three-time PCR (designing multiple sub-primers is time-consuming, and not vastly cheaper than the second method, but has a higher probability of working)

d. Order toehold as gBlock, PCR the plasmid for repressed sequence, perform Gibson assembly (Gibson assembly MasterMix is expensive, but its more flexible to change the repressed sequence)

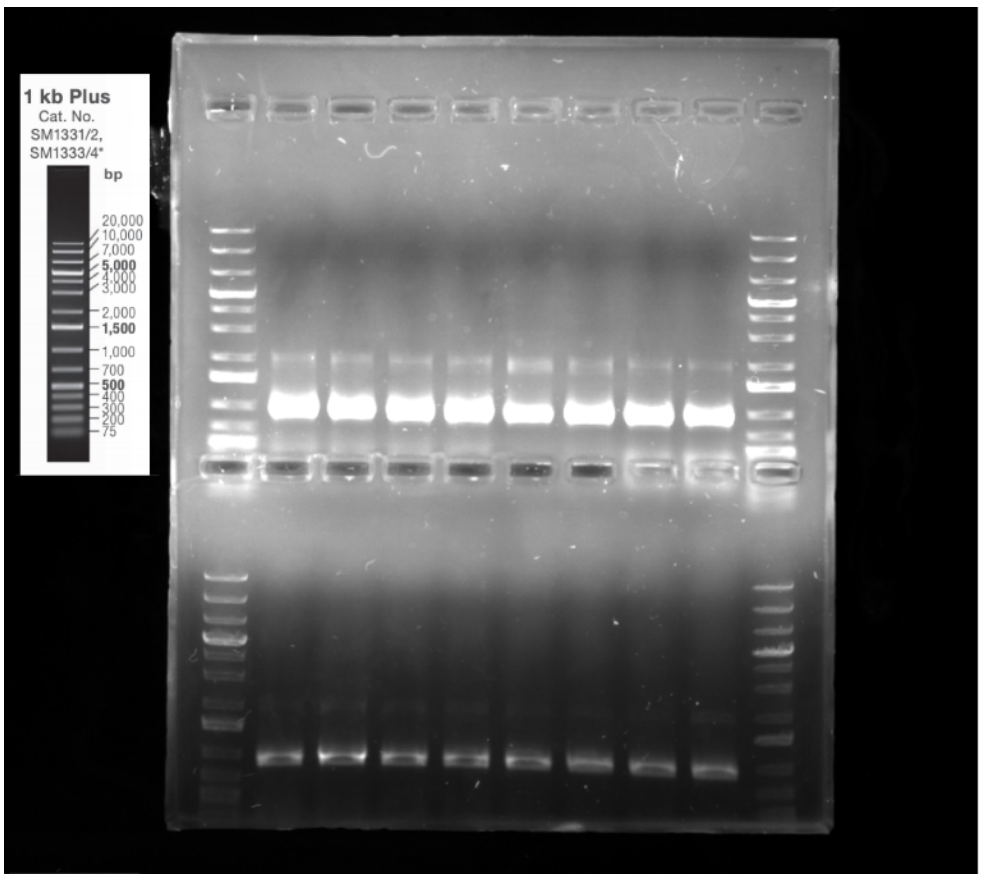
Monday, 5 August 2019

For the first try we chose the second method and used NEB Q5 HIFI as the PCR kits (25uL reaction).

Template	**Fw Primer**	**Rev Primer**	Length	Elongation time	Tm - Q5	Tm used
pJL1-sfGFP	sfGFP_fwd	Toehold_BN_1.1	958	30s	67/68(68)	68
pJL1-sfGFP	sfGFP_fwd	Toehold_BN_1.2	958	30s	67/68(68)	68
pJL1-sfGFP	sfGFP_fwd	Toehold_BN_1.3	958	30s	67/68(68)	68
pJL1-sfGFP	sfGFP_fwd	Toehold_BN_1.4	958	30s	67/68(68)	68

MM	5					
Volume	25					
	Reference (25 uL)	**Per tube**	**Master mix1**	**Master mix2**	**Master mix3**	**Master mix4**
pJL1-sfGFP-2(~60ng/uL)	1	1				
sfGFP_fwd	1.25	1.25	6.25	6.25	6.25	6.25
Toehold_BN_1.1(10uM)	1.25	1.25	6.25			
Toehold_BN_1.2(10uM)				6.25		
Toehold_BN_1.3(10uM)					6.25	
Toehold_BN_1.4(10uM)						6.25
dNTPs (10 mM)	\	\	\	\	\	\
Q5 Reaction Buffer (5X)	\	\	\	\	\	\
Q5 MasterMix	12.5	12.5	62.5	62.5	62.5	62.5
H2O	9	9	45	45	45	45
Total	25	25	120	120	120	120

ladder	BN_1.1_1	BN_1.1_2	BN_1.1_3	BN_1.1_4	BN_1.2_1	BN_1.2_2	BN_1.2_3	BN_1.2_4	ladder
ladder	BN_1.3_1	BN_1.3_2	BN_1.3_3	BN_1.3_4	BN_1.4_1	BN_1.4_2	BN_1.4_3	BN_1.4_4	ladder



Electrophoresis image of first version toehold switch

Tuesday, 6 August 2019

Comments and discussion:

We noticed that there is a band above 1000 bps, it should be the plasmid templet we added in the PCR reaction. As the plasmid is encoded with sfGFP and T7 promotor, it will contaminate the toehold switch and create a false signal even trigger mRNA is not present.

There are two ways to avoid that:

1). Assemble the toehold switch from a template without T7 promotor.

2). Use methylated DNA specific restriction enzyme to digest the residue plasmid.

The first method will be more ideal, but in iGEM distribution we can't find any sfGFP DNA which has the same sequence as ours. Therefore, we went for the second method.

Dpn II restriction enzyme digestion:

Using NEB Dpn II restriction enzyme protocol:

https://www.neb.com/protocols/2012/12/07/optimizing-restriction-endonuclease-reactions

Wednesday, 7 August 2019

In order to control the quality of the PCR products, we sent the samples to sequencing. Each tubes contains 14µg of DNA and a total volume of 10µL.

Thursday, 8 August 2019

The sequence results can be found in appendix 2.1. They indicate that the98% of our products match our desired sequence. Therefore, Toehold switch BN 1.1, 1.2, 1.3, 1.4 are ready to process for the functionality tests.

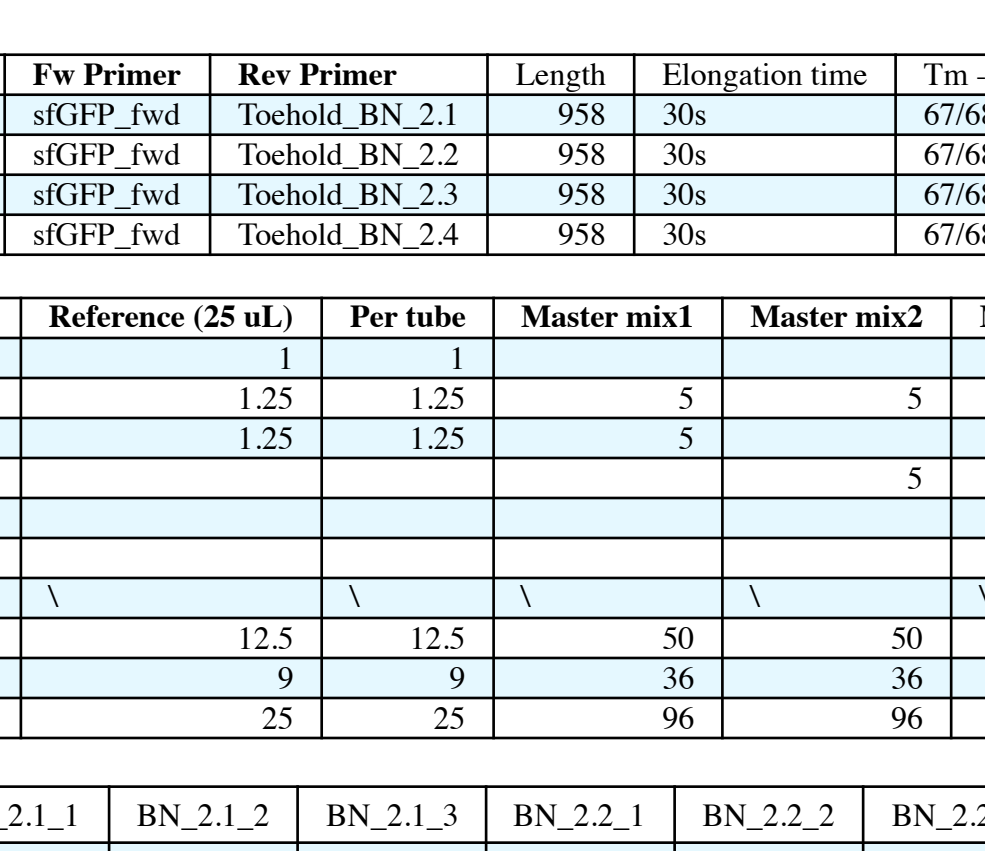
Second version toehold switch assembly:

Using same protocols we assemble the second version of toeholds:

Template	Fw Primer	Rev Primer	Length	Elongation time	Tm - Q5	Tm used
pJL1-sfGFP	sfGFP_fwd	Toehold_BN_2.1	958	30s	67/68(68)	68
pJL1-sfGFP	sfGFP_fwd	Toehold_BN_2.2	958	30s	67/68(68)	68
pJL1-sfGFP	sfGFP_fwd	Toehold_BN_2.3	958	30s	67/68(68)	68
pJL1-sfGFP	sfGFP_fwd	Toehold_BN_2.4	958	30s	67/68(68)	68

	Reference (25 uL)	Per tube	Master mix1	Master mix2	Master mix3	Master mix4
pJL1-sfGFP-2(~70ng/uL)	1	1				
sfGFP_fwd(10uM)	1.25	1.25	5	5	5	5
Toehold_BN_2.1(10uM)	1.25	1.25	5			
Toehold_BN_2.2(10uM)				5		
Toehold_BN_2.3(10uM)					5	
Toehold_BN_2.4(10uM)						5
dNTPs (10 mM)	\	\	\	\	\	\
Q5 MasterMix	12.5	12.5	50	50	50	50
H2O	9	9	36	36	36	36
Total	25	25	96	96	96	96

ladder	BN_2.1_1	BN_2.1_2	BN_2.1_3	BN_2.2_1	BN_2.2_2	BN_2.2_3	ladder
ladder	BN_2.3_1	BN_2.3_2	BN_2.3_3	BN_2.4_1	BN_2.4_2	BN_2.4_3	ladder



Electrophoresis image of second version toehold switch

Discussion/problem shooting :

The PCR products have a huge difference on its yield this time, we thought it is due to the change of the toehold primers which might leads to low affinity between the bases. However later we found out that the new sfGFP plasmid we made from iGEM distribution has different sequence to our Biobits sfGFP plasmid. It means that part of the plasmid inside our template don’t react to our primers at all.

Conclusion/comments:

Toehold switch BN 1.1, 1.2, 1.3, 1.4 are ready to test, BN 2.1, 2.2, 2.3, 2.4 can be used for some test, as there are bands appeared in the desired length. But they might lead to unexpected results.