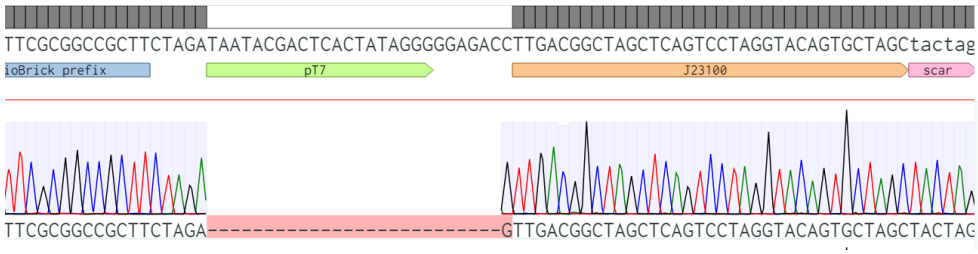
August –October 2016

Construction of *in silico* designed Switches and Triggers

Construction of switch standard backbone:

1. KS1- RFP is first transferred from pSB1A2 to pSB4C5 using Xhoi and Psti.
2. "Xbai-pT7-Eco31I-J23100-RBS- Eco31I-Linker-Xbai" is constructed by overlapping 2 oligoes.
3. We intended to insert "Xbai-pT7-Eco31I-J23100-RBS- Eco31I-Linker-Xbai" into KS1-RFP- pSB4C5 to construct standard backbone. Experiment repeated 5 times but failed.
4. In the second experiment, one colony was found to be red. Sequencing result shown that deletion of "pT7-Eco31I" occurred.



5. A pair of Q5 mutagenesis primers were designed to insert pT7-Eco31I into that construct to construct standard backbone.
6. Mutagenesis was performed once and proceed to sequencing.
7. Repeat the procedures until correct sequencing result is obtained.

Construction of trigger standard backbone:

1. "Xbai-pT7- Eco31I-I20260-Eco31I-SpeI-PstI" is first made by PCR using I20260 (a GFP generating device) as template.
2. "Xbai-pT7- Eco31I-I20260-Eco31I-SpeI-PstI" is inserted to pSB1K3 backbone to construct trigger standard backbone. Construct is confirmed by GFP colony and plasmid-PCR.

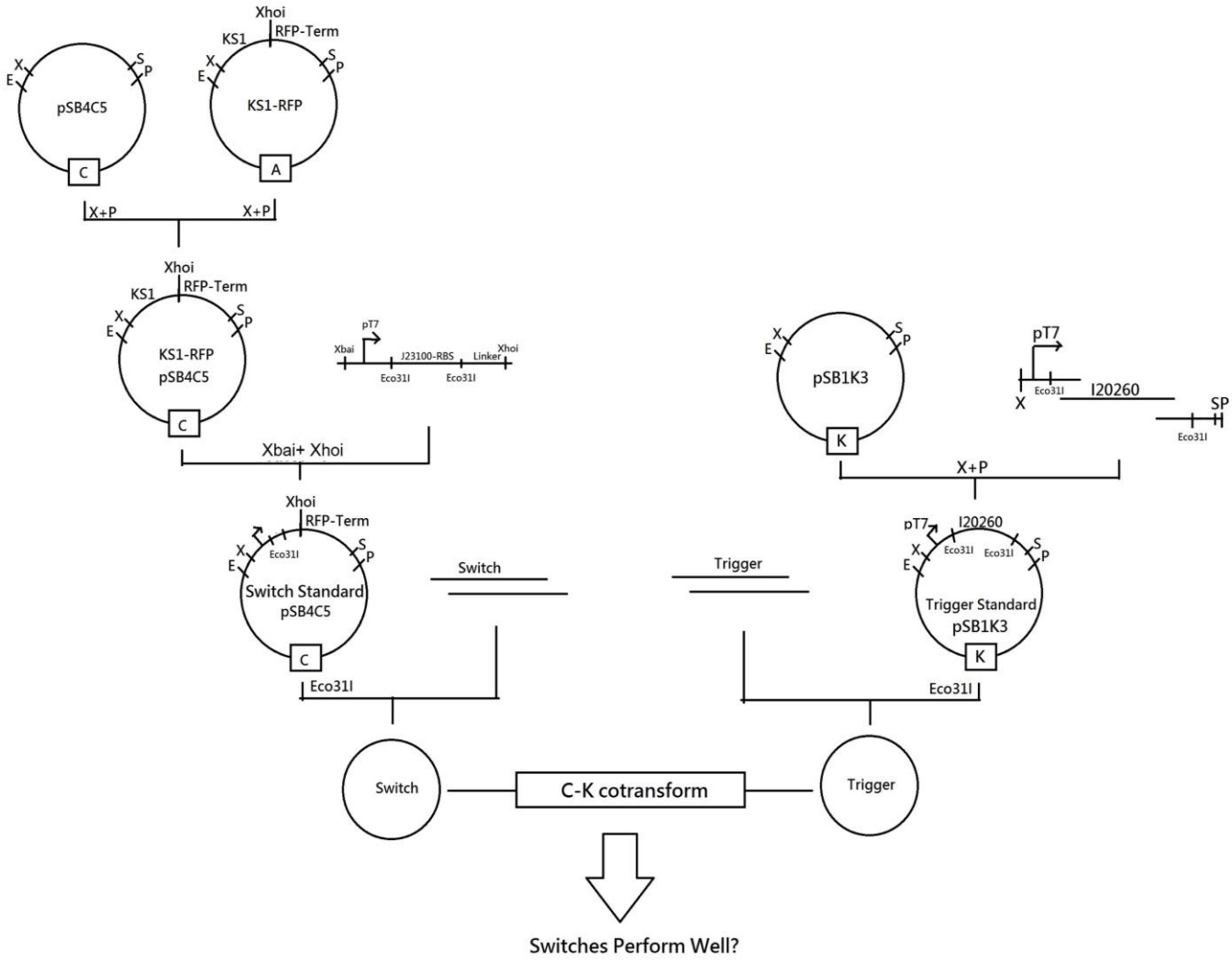
Construction of trigger construct:

1. Trigger is inserted into the standard backbone.
2. Sequencing result showed that trigger were correctly inserted. Construction of other 3 trigger will still carry on.

November 2016-February 2017

Co-transformation of switch and trigger

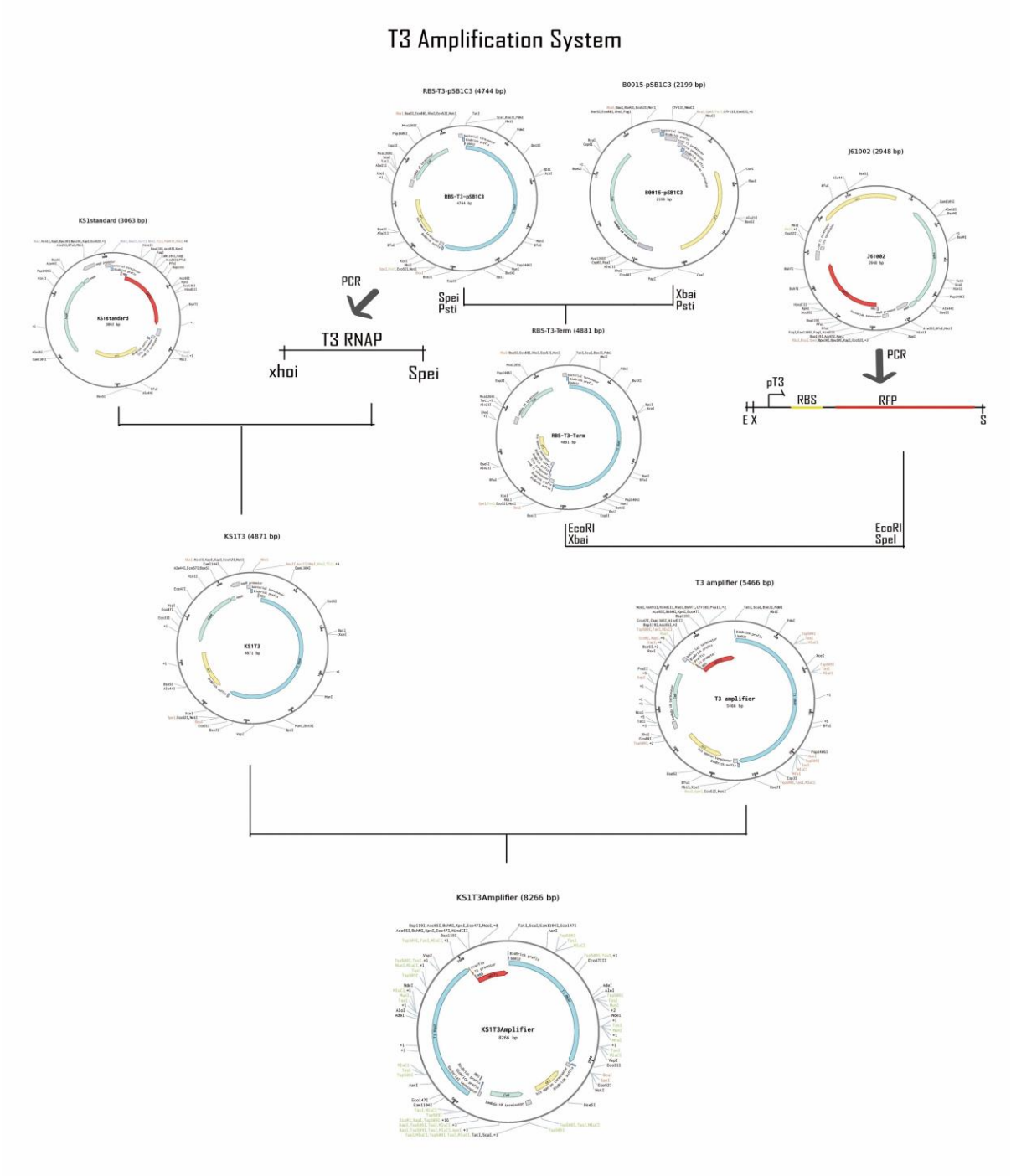
After successful construction of switch and trigger, we test their specificity by co-transformation whereas the selection is made by C/K antibiotics. The ratio of [switch] and [trigger] for co-transformation is 1:2.



March2017-April2017

T3 amplification system

The T3 amplification system includes integration T3 RNAP into KS-1 standard plasmid and co-transformation with t3 amplifier, which is constructed by using iGEM distribution kit. Unfortunately, we cannot obtain the positive result. The workflow is summarised below:



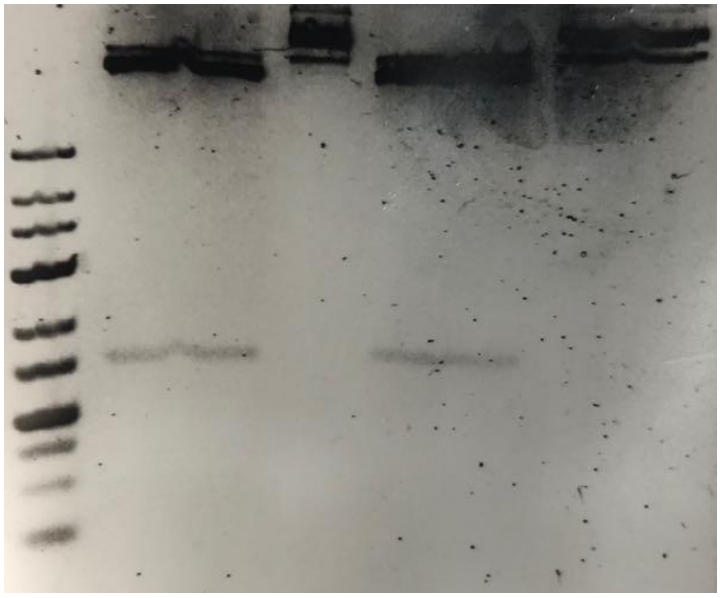


Figure 1: Restriction digestion mapping of B0015 on 3% DNA agarose gel. The terminator (129 bp) is isolated by gel purification.

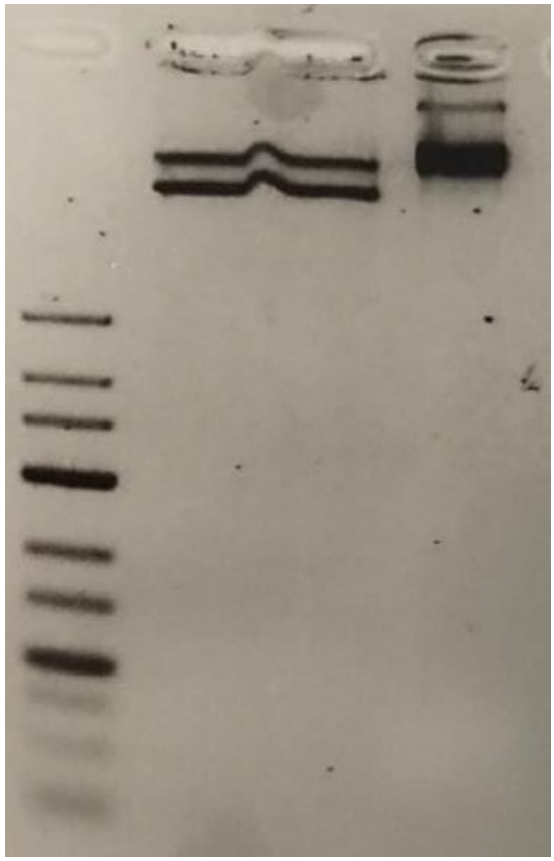


Figure 2: The ligation product is run in 3% DNA agarose gel. No conclusion could be drawn since wrong ladder is used.

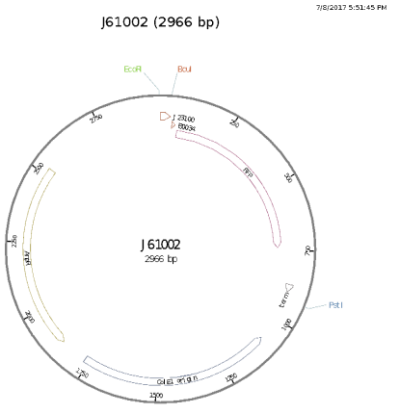
May 2017-June 2017

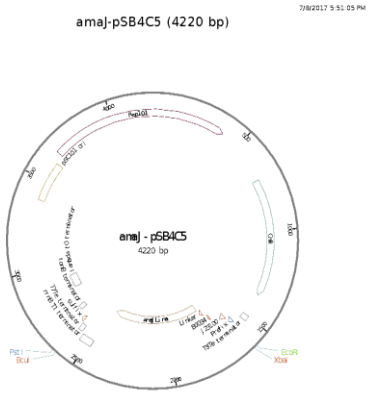
Characterization of chromoprotein

Cloning of plasmid

Transformation of plasmids using electroporation

By digestion and ligation, plasmids were constructed as follow where constitutive promoter (J23100) is used. Plasmids are transformed into E.coli by Ca2+ heat shock. The cells are incubated at 37 º C overnight (~16 hrs).





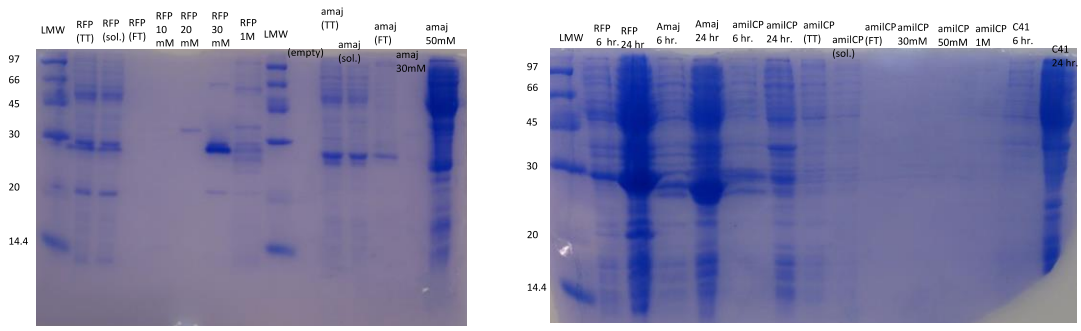
Culturing

Day 0: Prepare the medium according to protocol
Day 1: Pick single colony of C41 cells to 5ml LB solution with 1x antibiotics to grow starter.
Day 2: 1% Inoculation in two 1L conical flask, each with 250 ml 2XYT solution 1x antibiotics overnight.

Protein extraction

1. Sin down 100ml cells in 50 ml falcon.
2. Wash cell pellet with 40 ml cool TE buffer.
3. Spin down cells, discard supernatant carefully.
4. Re-suspend cells with cold 15 ml Protein Lysis Buffer (PLB).
5. Sonicate on ice for 30 s 12.
6. Spin at 4°C at 13000 speed for 5 min
7. Transfer supernatants to new set of tubes.
8. Dialysis.

The protein purification was carried out using *HiTrap Q HP Ion exchange column, Ge Health Care* followed by Hydrophobic interaction chromatography, Biored.



Remarks:
LMW: protein ladder
TT: Total protien (1.5㎕l)
Sol.: Soluble protein (1.5㎕l)
FT: flow throw: 1.5 (㎕l)
Fluorescent protein/chromorprotein: 5㎕l
Cell extracted after 6 hours inoculation: 1.752 ㎕g
Cell extracted after 24 hours inoculation:2㎕l
RFP:~30kDa; amaj:~25kDa;amilCP:~25ka (Calculated acording to iGEM registry DNA sequence)

Figure 3. SDS–PAGE analysis of purification of chromo/fluorescent proteins by ion-exchange chromatography. It is shown that the protein purity is not up to standard, the amajLime and mRFP is proceed to HIC.

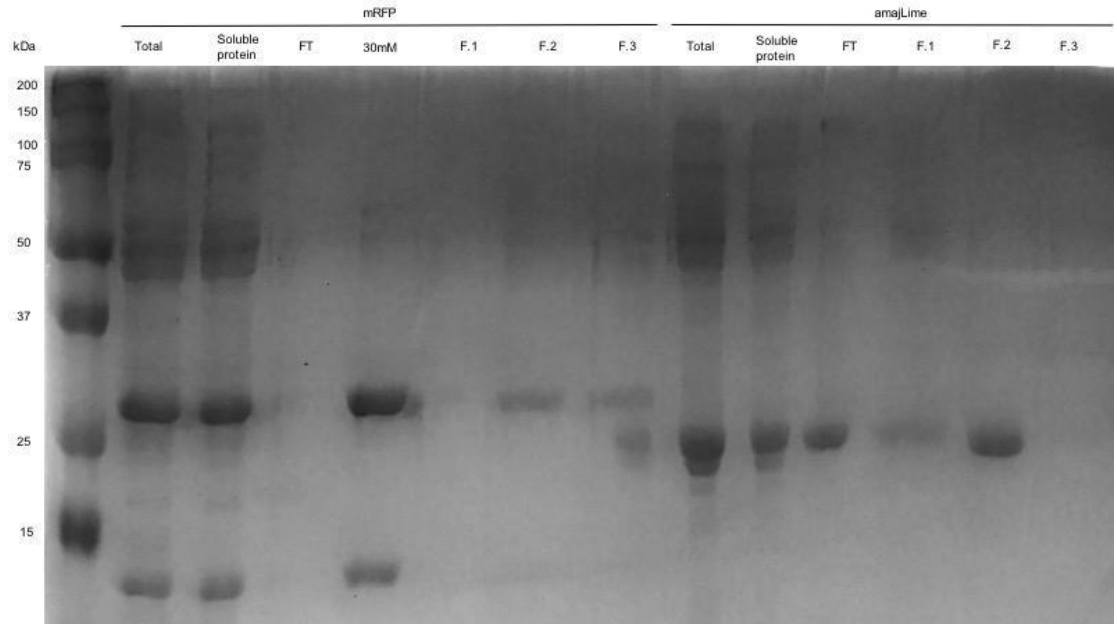


Figure 4. SDS–PAGE analysis of purification of amajLime (left) and mRFP (right) proteins by ion-exchange chromatography and followed by hydrophobic-interaction chromatography HIC.

pH test

1. Diluted protein into buffers to 50 µg ranging in pH from 2-12 in 96-well plates.
2. Determine absorbance/ fluorescence by Plate reader

The final value was correlated with fluorescein concentration. The raw data of fluorescence is summarized as follow:

	pH2	pH4	pH6	pH8	pH10	pH12
Replica1	23	20	14858	17862	13223	12810
Replica2	21	22	15429	17251	12978	11981
Replica3	20	14	16127	16508	15933	11968

July 2017-August 2017

Fluorescent signal by switch and trigger co-transformation

Fluorescent signal is measured by plate reader at 584 nm (excitation) and 607nm (emission). The raw data (trial1) is recorded as follow:

	Fluoresencent signal result					
Switch	PB2-1		PB2-2		PB2-3	
Trigger	-	+	-	+	-	+
	8	12	10	15	11	148
	10	15	9	12	14	29
	12	12	13	18	11	232
	H5-1		H5-2		H5-3	
	-	+	-	+	-	+
	11	15	10	23	13	16
	17	15	15	15	13	14
	12	12	11	18	17	11
	N1-1		N1-2		N1-3	
	-	+	-	+	-	+
	11	66	10	61	12	12
	14	122	14	20	12	12
	12	40	14	44	13	10
	H7-1		H7-2			
	-	+	-	+		
	12	50	13	293		
	15	29	8	34		
	16	32	13	294		

September 2017

Collaboration with HKUST

Total of nine plasmids were transformed by Ca2+ heat shock while three colonies were picked for inoculation until it reached 0.6 OD600. Cell culture were transferred to ach well and make triplicates at 200minutes. The raw data summarized below.

21_9 analysis.xlsx

October 2017

Construction of standard backbone

Since iGEM requires standard backbone pSB1C3 for shipping, we later sub-cloned our biobricks into pSB1C3. Agarose electrophoresis and sequencing revealed that all biobricks were successfully inserted. Below showed our 1% agarose gel photo (our biobricks ~ 1000bp, pSB1C3 ~ 2000bp):