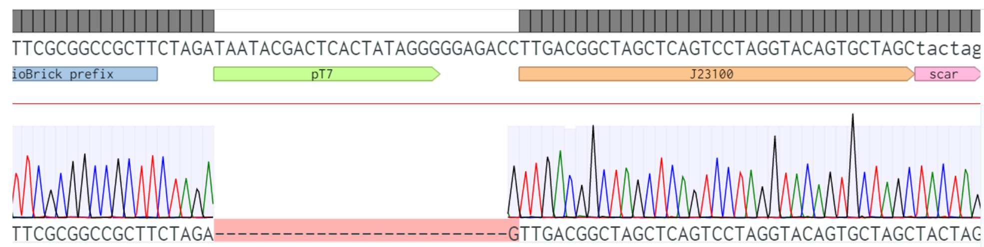
August –October 2016

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.

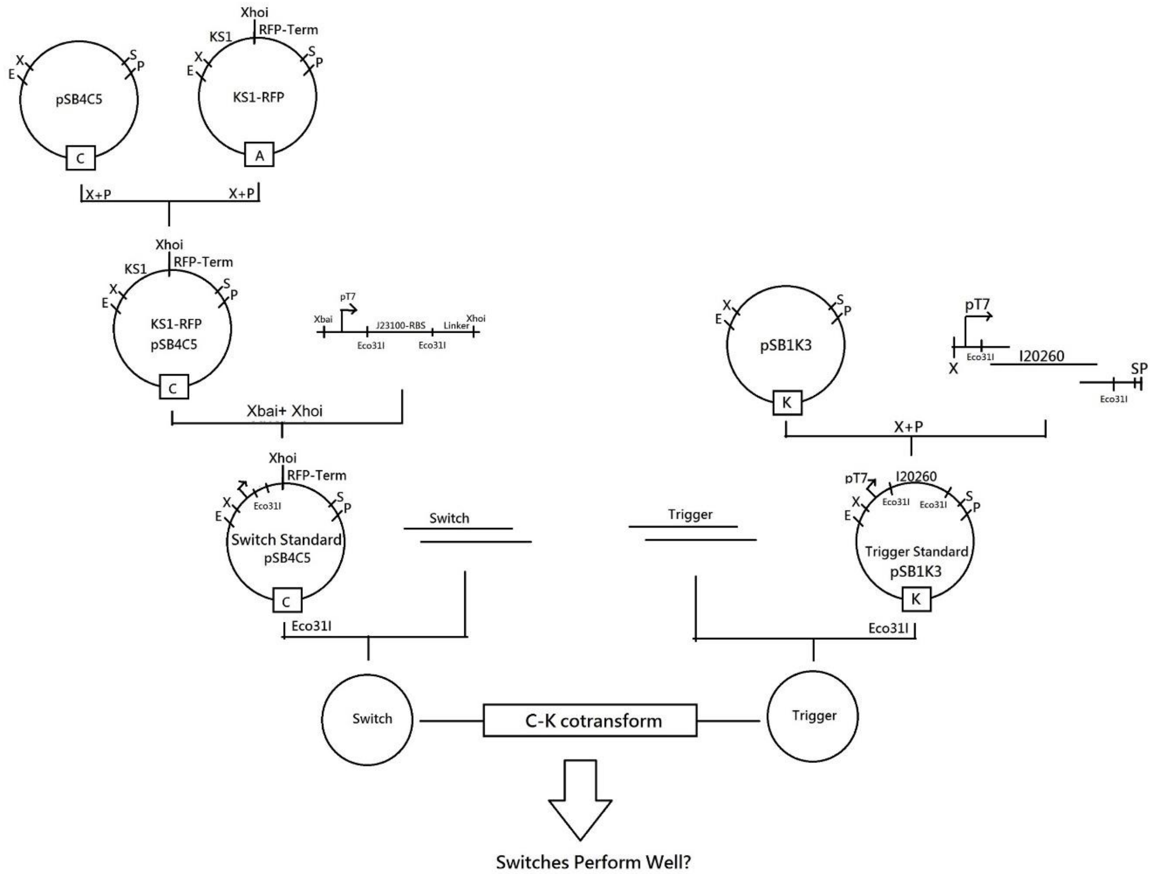
Construction of trigger construct:

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

Workflow of Co-transformation of switch and trigger

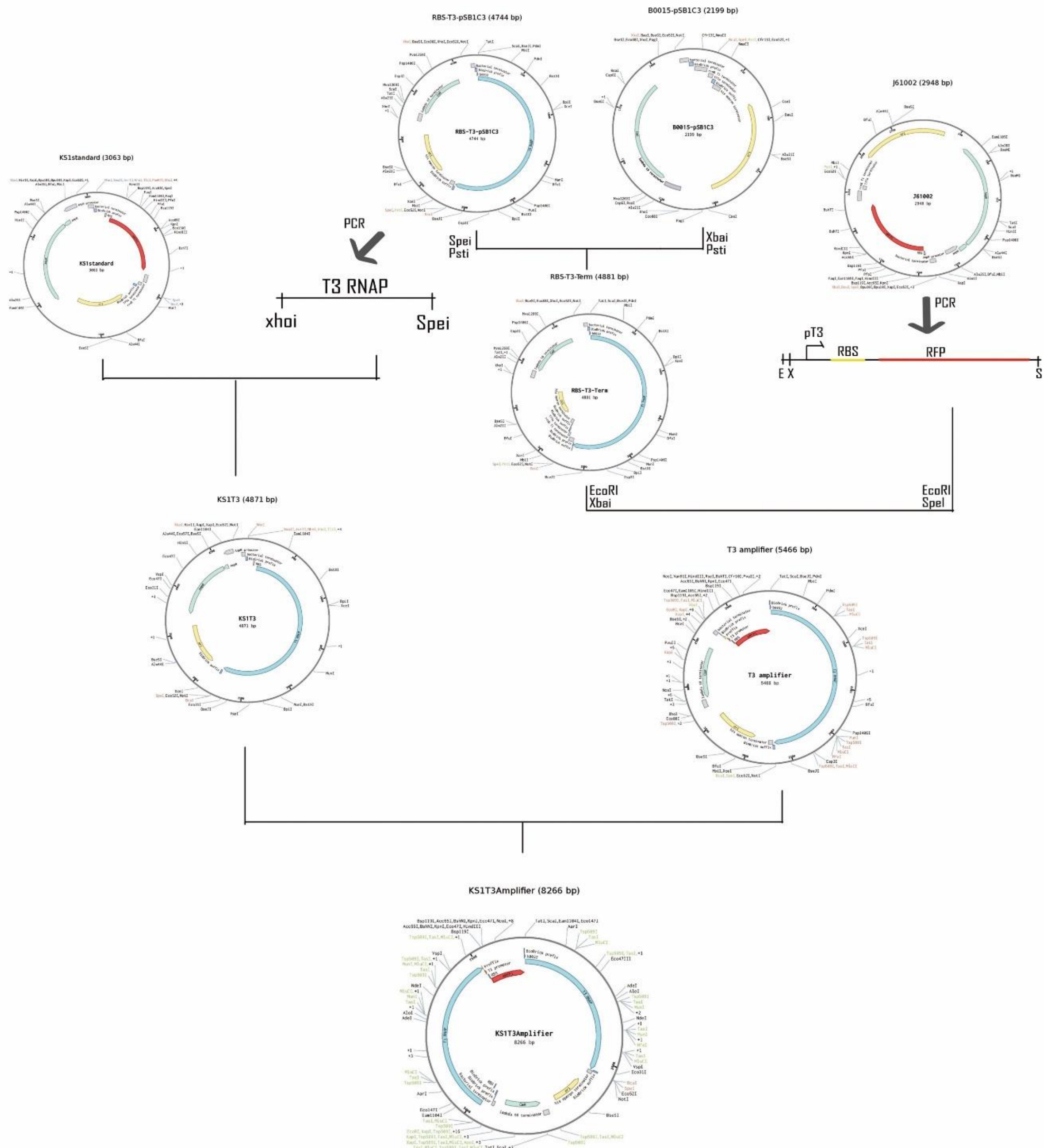
After successful construction of switch and trigger, we test their specificity by co-transformation whereas the selection is made by C/K antibiotics. The ratio of [switch] and [trigger] for co-transformation is 1:2.



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:



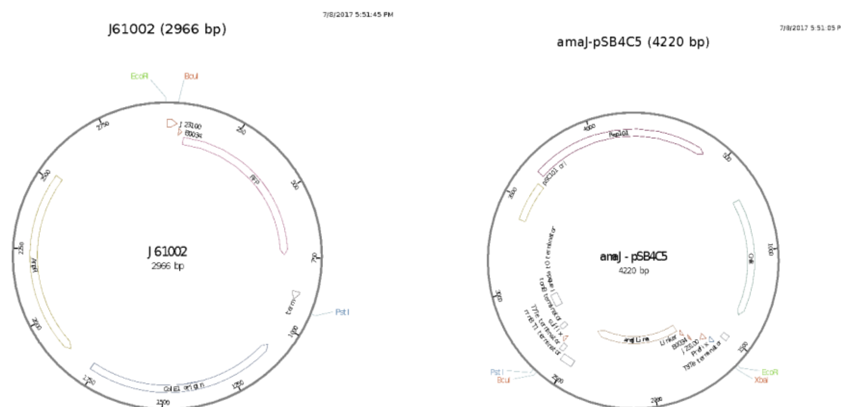
May 2017-June 2017

Characterization of chromoprotein

Cloning of plasmid

Transformation of plasmids using electroporation

1. Thaw a tube of competent cells (usually 100 µL) on ice, and use as soon as possible.
2. Pipette 50 ng DNA to the solution surface of competent cells.
3. Put the tube on ice for 5 min.
4. Heat shock: Put the tube at 42 oC for 45 s to 2 min.
5. Put the tube in ice for 5 min.
6. Transfer the cells to a 1.5 mL or 2 mL microfuge tube
7. Add 1 ml LB broth or SOC medium.
8. Incubate the tube at 37 oC shaker for 45 – 90 min with shaking (~ 250 rpm).
9. Spread 3 dilution of cells (10-fold serial dilution) onto the pre-warmed agar plate (with suitable antibiotics), each in 50 – 100 µl.
10. Incubate the agar plates upside down at 37 oC incubator overnight (~16 hrs).
11. Wrap the plates with parafilm and store at 4 oC for further use.



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.](#)

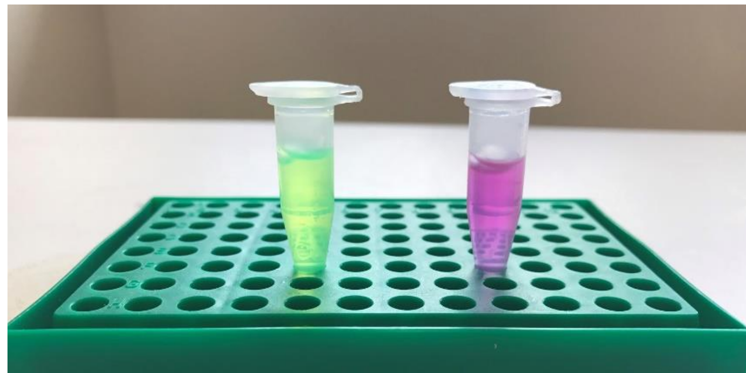
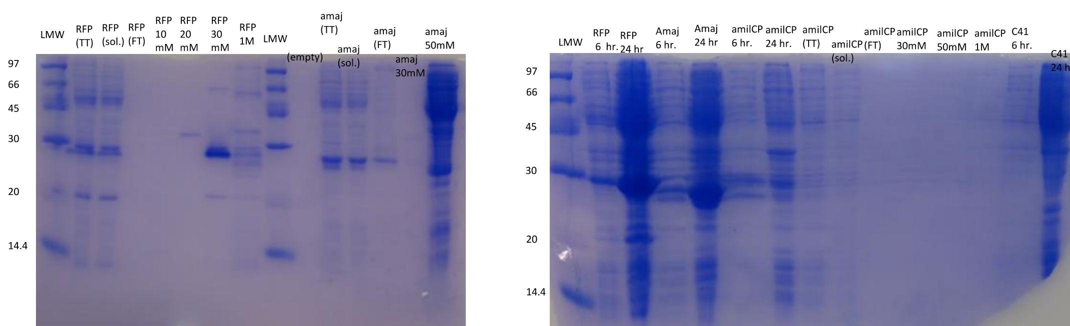


Figure 1. Fluorescent proteins extracted from C41 after overnight expression



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

Figure 2. SDS–PAGE analysis of purification of chromo/fluorescent proteins by ion-exchange chromatography

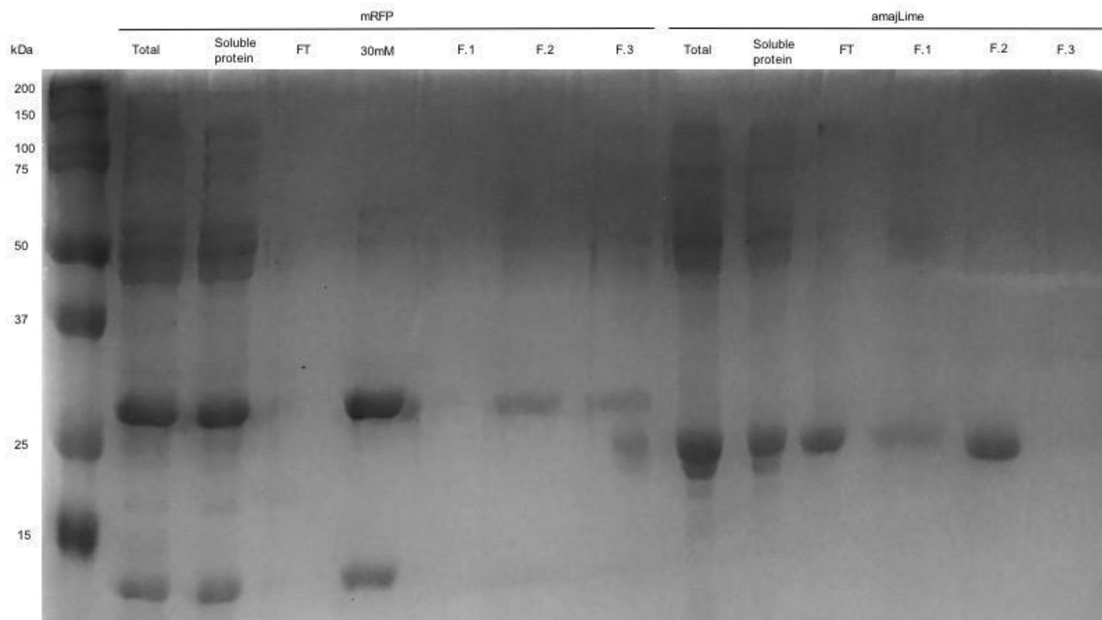


Figure 3. SDS–PAGE analysis of purification of amajLime (left) and mRFP (right)

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

July 2017-August 2017

Fluorescent signal by switch and trigger co-transformation

Andrew

Switch	PB2-1		PB2-2		PB2-3		H5-1		H5-2		H5-3		N1-1		N1-2	
Trigger	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
	8.487793	11.80899	10.48317	15.15511	11.19639	147.5115	10.5322	14.77591	10.35953	22.52336	13.02981	15.901	11.13907	65.75966	9.562767	60.66929
	9.758588	15.23867	9.040479	11.84042	13.60397	28.91654	17.28134	14.91274	15.31101	15.39154	13.25266	13.75958	13.84295	121.5119	14.03878	19.79925
	11.51109	11.86813	12.56105	17.87435	10.88443	232.1375	11.72502	11.5096	11.49816	18.08703	17.27975	11.28559	12.41435	40.15571	13.7075	43.87178
	N1-3		H7-1		H7-2		H7-3		N9-1		N9-2		N9-3			
	-	+	-	+	-	+	-	+	-	+	-	+	-	+		
	12.03609	12.20238	11.78379	50.33138	12.8413	292.7109	8.330226	11.49629	22.02352	38.26817	11.6433	259.7058	12.3001	11.34303		
	11.95493	11.69427	15.34088	28.65752	7.569286	34.04126	12.547	13.30838	11.94197	18.47741	10.2879	218.9986	8.108341	12.64081		
	13.30396	9.635743	16.27781	32.40564	12.82415	294.3948	11.13022	12.34796	8.910478	33.72504	10.03264	148.4237	11.65629	8.713262		

September 2017
Collaboration with HKUST and UCCKE