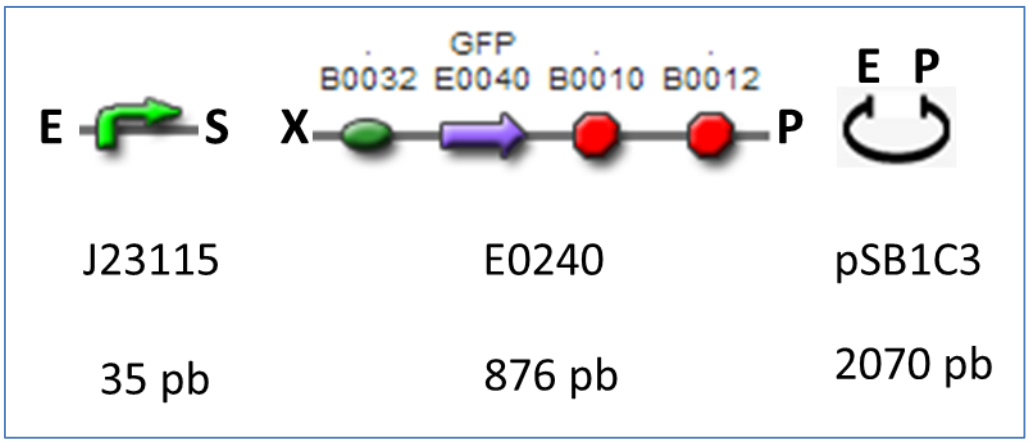
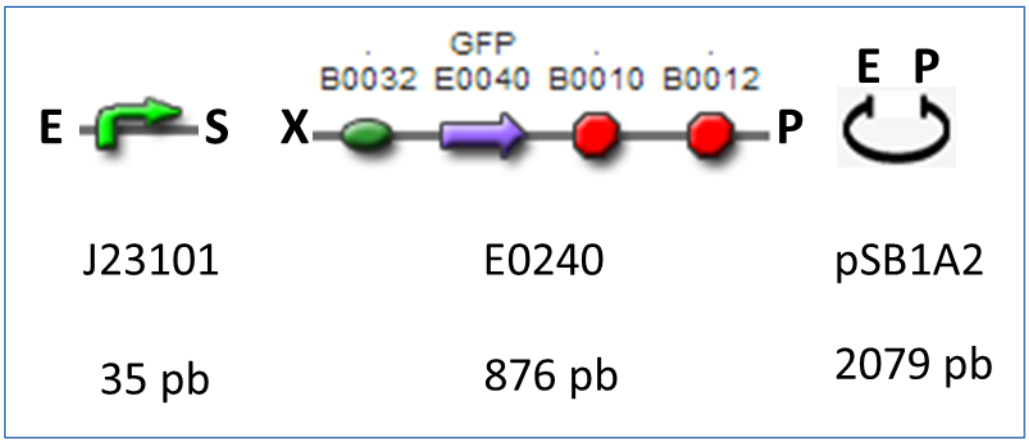
Biobrick Device 2



Biobrick Device 3



1st Day – August 29th, 2014

EXSP Digestion

Assembly 3

Parts	**Concentration (ng/uL)**	**Volume to 2,5 ug (uL)**	**Buffer X10 (uL)**	**FastDigest® EcoRI NEB (uL)**	**FastDigest® XbaI NEB (uL)**	**FastDigest® SpeI NEB (uL)**	**FastDigest® PstINEB (uL)**	**H_2O**
J23115	225	12	5	1	-	1	-	31
E0240	81	31	5	-	1	-	1	12
pSB1C3								

Assembly 2

Parts	**Concentration (ng/uL)**	**Volume to 2,5 ug (uL)**	**Buffer X10 (uL)**	**FastDigest® EcoRI NEB (uL)**	**FastDigest® XbaI NEB (uL)**	**FastDigest® SpeI NEB (uL)**	**FastDigest® PstINEB (uL)**	**H_2O**

J23101	307	8.2	5	1	-	1	-	34.8
E0240	81	31	5	-	1	-	1	12.0
pSB1A2	181	13.8	5	1	-	-	1	29.2

2nd Day – August 30th, 2014

Agarose (1%) gel electrophoresis

- Total digestion product volume of each part DNA was applied to the agarose gel 1% stained with ethidium bromide (0.5 ug/mL) and bands showing the corresponding size were excised from the gel and purified according the Wizard SV gel and PCR clean up kit protocol (Promega)**.**

- The purified products were quantified using NanodropTM 1000 (Thermoscientific)

Ligation

Assembly 3

Linear plasmid (50 ng)	**2 uL**	
Insert: Plasmid 6:1	J23115	E0240
	2 uL	10 uL
T4 DNA Ligase buffer (10X)	2 uL	
T4 DNA ligase (1U)	1 uL	
H_2O to 20 uL	5 uL	

Assembly 2

Linear plasmid (50 ng)	**2 uL**	
Insert: Plasmid 6:1	J23101	E0240
	2 uL	10 uL
T4 DNA Ligase buffer (10X)	2 uL	
T4 DNA ligase (1U)	1 uL	
H_2O to 20 uL	5 uL	

To determinate the amount of DNA necessary we used the following equation

$$Insert\ ng = plasmid\ ng \times \frac{insert\ bp}{plasmid\ bp} \times insert{:}\ plasmid\ ratio$$

The Ligation reactions were Incubated overnight at 37 ºC.

Meanwhile, we prepared and sterilized in the autoclave tubes of liquid LB medium and glycerol 40%.

3rd Day - August 31th, 2014

Transformation in competent *Escherichia coli* TOP10 (Invitrogen) using cloranphenicol (70 ug/mL) for selection of positive clones with Assembly 1 and ampicillin (100 ug/mL)) for selection of positive clones with Assembly 2. The Transformation protocol is presented below:

Transformation in Escherichia coli (TOP10 - Invitrogen)

Materials
- 1,5ml tube
- Styrofoam box with ice
- Agar plate with antibiotic
- Competent cells
- Plasmidial DNA
- Centrifuge
- Water bath at 42°C
- Liquid LB medium
- Shaker

Method
- Briefly spin the competent cells and put then on ice
- Add 50ng of plasmidial DNA in a 1,5ml tube
- Add the 50ul of competent cell in the same tube
- Keep the tube on ice for 25min
- Put the tube in a 42° water bath for 2 min
- Put the tube on ice for 5 min
- Add 200ul of liquid LB
- Incubate at 250rpm/37°C/1 hour
- Plate the the solution in a Agar plate with the appropriate antibiotic
- Incubate the plate at 37°C overnight

4th Day - September 1st, 2014

Inoculate 2 colonies in 5 mL LB mediumcontaining the correspondent antibiotic.

5th Day - September 2nd, 2014

We prepared glycerol stocks of the clones, using 250 uL of clone culture + 250 uL of sterile glycerol 40%. The samples were stocked at -80ºC.

4,75 mL of each clone culture was submmited to Plasmidial Extraction using PureLink Invitrogen Protocol.

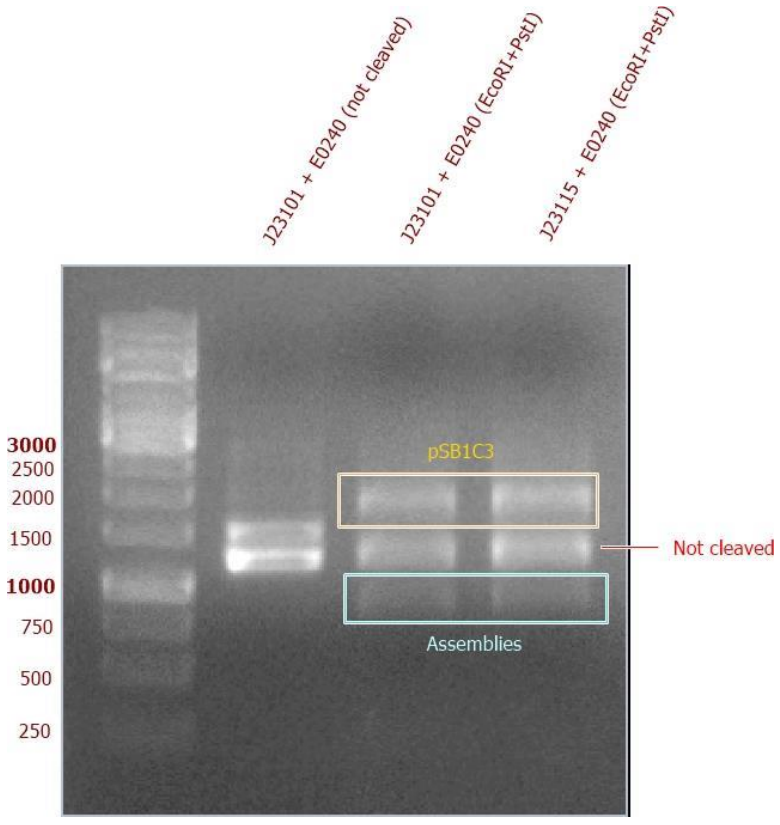
Assemble confirmation - September 3rd, 2014

We performed an EP Digestion as follows to confirm the assemble formation:

	Volume to 300 ng (uL)	**Buffer x10 (uL)**	**FastDigest® EcoRI NEB (uL)**	**FastDigest® PstI NEB (uL)**	**H_2O to 10 uL (uL)**
Assembly 3	3	1	0.5	0.5	5
Assembly 2	3	1	0.5	0.5	5

Agarose (1%) gel electrophoresis

- Confirmation reaction product for one clone was applied to the agarose gel 1% stained with ethidium bromide (0.5 ug/mL). The result is shown in the figure bellow:



The results confirm Assembly 3 and Assembly 2 formation.