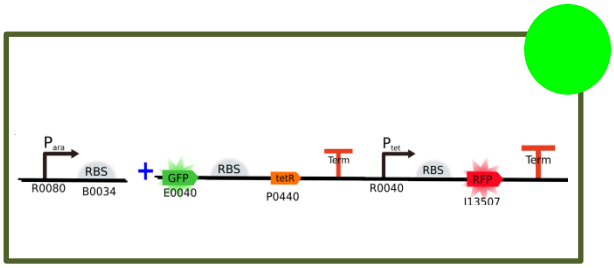
Assembly:

Pa_I13507



1st Day:

EXSP Digestion (see **Enzymatic Digestion Protocol**)

	Part	Size	ηg/µl
1	Pa_RBS	161 bp	94.8
2	GFP_I13507	2585 bp	273.5

	Volume to 1,0 µg (µl)	Buffer 10x (µl)	BSA	Enzime 1	Volume (µl)	Enzime 2	Volume (µl)	H_2O to 20µl (µl)
1	10	2 (M)	-	SpeI	1	PstI	1	6
2	4	2 (M)	-	XbaI	1	PstI	1	12

Final Plasmid	Resistence
pSB1A2	ampicillin

Gel purification

- See PureLink® Quick Plasmide Miniprep Invitrogen™ manual
- Quantify digestion products

Parts	ηg/µl
Pa_RBS	14
GFP_I13507	12.3

Obs: 260/280 in a quality parameter that tells you if your sample is contaminated with proteins. The greater it is compared to 1 the less contaminants you have.

Ligation (see **Ligation Protocol**)

Part containing the plasmid	Pa_RBS	4 µl
Insert	GFP_I13507	10.5 µl
10x T4 DNA Buffer	4 µl	
T4 DNA ligase 1u	0.5 µl	
H2O to 20µl	1	

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

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

- Incubate overnight at 37°C.
- Prepare and sterilize in the autoclave tubes with 6 ml of liquid LB medium.
- Prepare glycerol 40%

2nd Day:

Transformation (see **Transformation Protocol in Escherichia coli DH5-α**)

- Organism: E. coli DH5-α
- Selection: Ampcillin

4th Day:

Confirmation with NotI