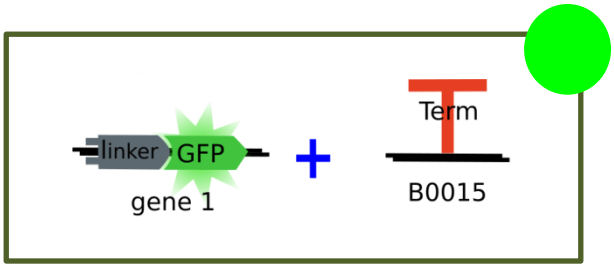
Assembly:

Linker_Term



1st Day:

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

	Part	Size	ηg/µl
1	LinkerGFP	851 bp	111
2	B0015	129 bp	130

	Volume to 1,0 µg (µl)	Buffer 10x (µl)	Enzime 1	Volume (µl)	Enzime 2	Volume (µl)	H_2O to 20µl (µl)
1	9	2 (Tango)	XbaiI	1	PstI	1	7
2	7.5	2 (Tango)	SpeI	1	PstI	1	8.5

Final Plasmid	Resistence
pSB1C3	chloramphenicol

Gel purification

- See PureLink® Quick Gel Extraction Kit Invitrogen™ manual
- Quantify digestion products

Parts	ηg/µl
LinkerGFP	9.8
B0015	12.2

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	B0015	5 µl
Insert	LinkerGFP	4.7 µl
10x T4 DNA Buffer	2 µl	
T4 DNA ligase 1u	0.5 µl	
H2O to 20µl	4.5 µl	

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: Chloramphenicol

4th Day:

Confirmation with NotI