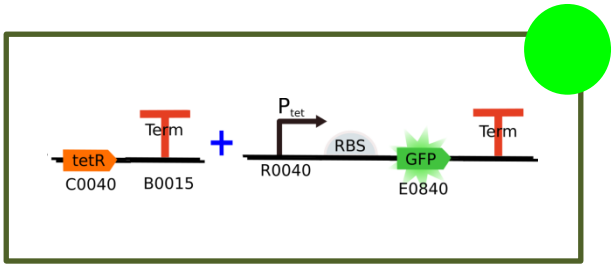
Assembly:

Tet_E0840



1st Day:

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

	Part	Size	ηg/µl
1	Tet_Term	813 bp	104.3
2	Pt_E0840	932 bp	265.0

	Volume to 1,0 µg (µl)	Buffer 10x (µl)	BSA (µl)	Enzime 1	Volume (µl)	Enzime 2	Volume (µl)	H2O to 20µl (µl)
1	6.4	2 (M)	-	S	1	P	1	9.6
2	12.2	2 (M)	2	X	1	P	1	3.8

Final Plasmid	Resistence
pSB1C3	chloramphenicol

Gel purification

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

Parts	ηg/µl
Tet_Term	13.6
Pt_E0840	9.9

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	**Tet_Term**	3.7
Insert	Pt_E0840	5
10x T4 DNA Buffer	2	
T4 DNA ligase 1u	0.4	
H2O to 20µl	8.9	

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.