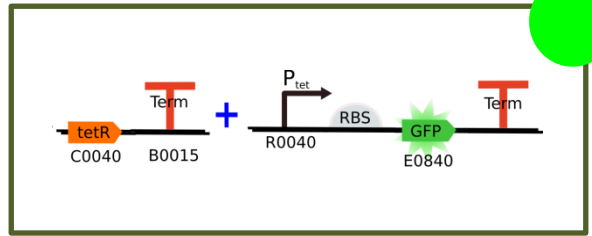


Assembly:

Term_E0840



1st Day:

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

	Part	Size	ng/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)	Enzyme 1	Volume (μl)	Enzyme 2	Volume (μl)	H ₂ O 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	Resistance
pSB1C3	chloramphenicol

Gel purification

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

Parts	ng/μl
Tet_Term	13.6
Pt_E0840	9.9

Obs: 260/280 is 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

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

- 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.