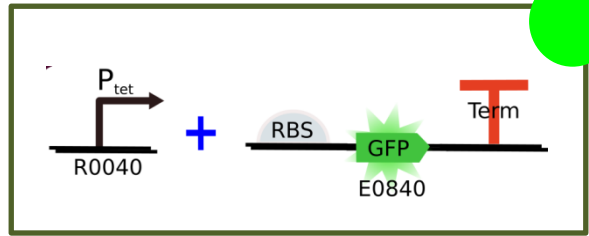


Assembly:

Pt_E0840



1st Day:

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

	Part	Size	ng/ μ l
1	R0040	54 bp	164.3
2	E0840	878 bp	171.0

	Volume to 1,0 μ g (μ l)	Buffer 10x (μ l)	BSA (μ l)	Enzyme 1	Volume (μ l)	Enzyme 2	Volume (μ l)	H ₂ O to 50 μ l (μ l)
1	12.2	2 (M)	-	S	1	P	1	3.80
2	11.7	2 (M)	2	X	1	P	1	2.30

Final Plasmid	Resistance
pSB1C3	chloramphenicol

Gel purification

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

Parts	ng/ μ l
R0040	5.3
E0840	7.3

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	R0040	9.3
Insert	E0840	8.5
10x T4 DNA Buffer		2
T4 DNA ligase 1u		0.4
H2O to 20µl		-

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

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

- 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: Ampicillin

4th Day:

Confirmation with NotI.