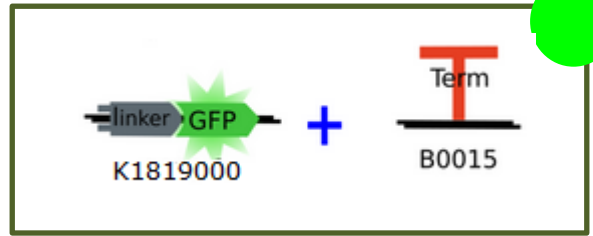


Assembly:

Linker_Term



1st Day:

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

	Part	Size	ng/ μ l
1	K1819000	750 bp	111
2	B0015	129 bp	130

	Volume to 1,0 μ g (μ l)	Buffer 10x (μ l)	Enzyme 1	Volume (μ l)	Enzyme 2	Volume (μ l)	H ₂ O to 20 μ l (μ l)
1	9	2 (Tango)	XbaI	1	PstI	1	7
2	7.5	2 (Tango)	SpeI	1	PstI	1	8.5

Final Plasmid	Resistance
pSB1C3	chloramphenicol

Gel purification

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

Parts	ng/ μ l
K1819000	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 μ l
Insert	LinkerGFP	4.7 μ l
10x T4 DNA Buffer		2 μ l
T4 DNA ligase 1u		0.5 μ l
H2O to 20 μ l		4.5 μ l

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: Chloramphenicol

4th Day:

Confirmation with NotI