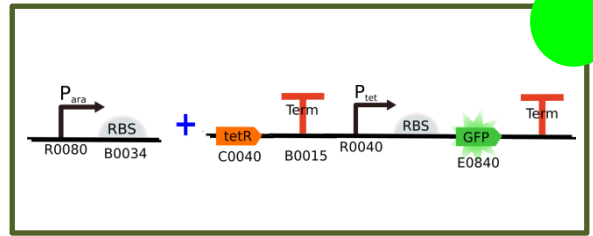


Assembly:

Pa_Term



1st Day:

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

	Part	Size	ng/ μ l
1	Pa_RBS	161 bp	96.9
2	tet_E0840	1745 bp	205.0

	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 20 μ l (μ l)
1	-	2 (M)	-	S	1	P	1	10.3
2	9.2	2 (M)	2	X	1	P	1	4.8

Final Plasmid	Resistance
pSB1A2	Ampicillin

Gel purification

- See PureLink® Quick Plasmide Miniprep Invitrogen™ manual
- Quantify digestion products

Parts	ng/ μ l
Pa_RBS	7.4
tet_E0840	6.7

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	Pa_RBS	6.7
Insert	tet_E0840	18
10x T4 DNA Buffer		3
T4 DNA ligase 1u		0.4
H2O to 30µl		2

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.