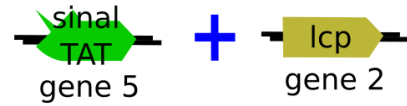


## Assembly:

TAT\_Lcp



## 1<sup>st</sup> Day:

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

	Part	Size	ng/ $\mu$ l
1	Lcp	1194 bp	125
2	signalTAT	246 bp	112

	Volume to 1,0 $\mu$ g ( $\mu$ l)	Buffer 10x ( $\mu$ l)	Enzyme 1	Volume ( $\mu$ l)	Enzyme 2	Volume ( $\mu$ l)	H <sub>2</sub> O to 20 $\mu$ l ( $\mu$ l)
1	8	2 (Tango)	X	1	SacI	1	8
2	7	2 (Tango)	X	1	SacI	1	9

Final Plasmid	Resistance
pSB1C3	chloramphenicol

### Gel purification

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

Parts	ng/ $\mu$ l
signal TAT	7.6
Lcp	2.9

**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	Lcp	6.5 $\mu$ l
Insert	signalTA T	4.2 $\mu$ l
10x T4 DNA Buffer		2 $\mu$ l
T4 DNA ligase 1u		0.5 $\mu$ l
H2O to 20 $\mu$ l		6.8 $\mu$ l

**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%

### **2<sup>nd</sup> Day:**

---

Transformation (see **Transformation Protocol in Escherichia coli DH5- $\alpha$** )

- Organism: E. coli DH5- $\alpha$
- Selection: Chloramphenicol

### **4<sup>th</sup> Day:**

---

Confirmation with NotI