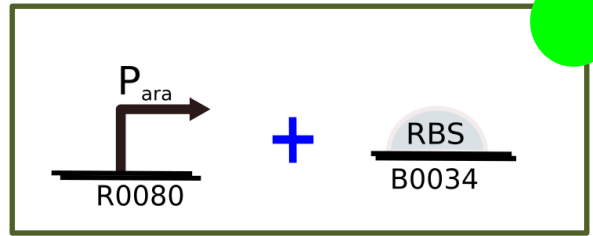


Assembly:

Pa_RBS



1st Day:

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

	Part	Size	$\eta\text{g}/\mu\text{l}$
1	R0080	149 bp	108.2
2	B0034	12bp	103.2

	Volume to 1,0 μg (μl)	Buffer 10x (μl)	BSA	Enzyme 1	Volume (μl)	Enzyme 2	Volume (μl)	H ₂ O to 20 μl (μl)
1	6.8	2 (M)	-	E	1	S	1	9.2
2	6.3	2 (M)	-	E	1	X	1	9.7

Final Plasmid	Resistance
pSB1A2	ampicillin

Gel purification

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

Parts	$\eta\text{g}/\mu\text{l}$
R0080	4.2
B0034	14.3

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	B0034	3.5
Insert	R0080	2.5
10x T4 DNA Buffer		2
T4 DNA ligase 1u		0.4
H2O to 20µl		11.5

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