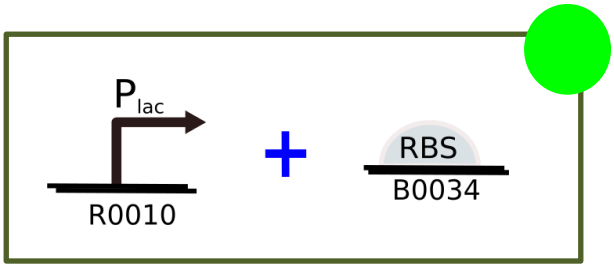
Assembly:

Pl_RBS



1st Day:

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

	Part	Size	ηg/µl
1	R0010	200 bp	75.7
2	B0034	12bp	103.2

	Volume to 1,0 µg (µl)	Buffer 10x (µl)	BSA	Enzime 1	Volume (µl)	Enzime 2	Volume (µl)	H_2O to 50µl (µl)
1	3	2 (M)	-	E	1	S	1	13
2	6.3	2 (M)	-	E	1	X	1	9.7

Final Plasmid	Resistence
pSB1A2	ampicillin

Gel purification

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

Parts	ηg/µl
R0010	9.1
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	R0010	2
10x T4 DNA Buffer	2	
T4 DNA ligase 1u	0.4	
H2O to 20µl	12	

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

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

- 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: Ampcillin

4th Day:

Confirmation with NotI