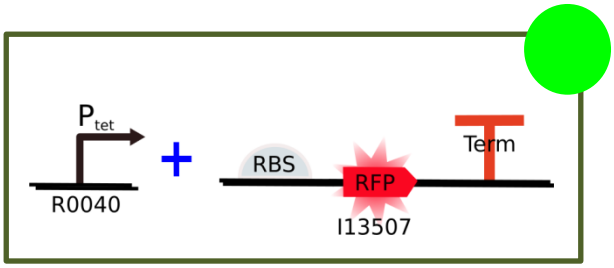
## Assembly:

Pt_I13507




### 1st Day:

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

| | Part | Size | ηg/µl |
|---|---|---|---|
| 1 | R0040 | 164 bp | 78.3 |
| 2 | I13507 | 861 bp | 214 |

| | Volume to 1,0 µg (µl) | Buffer 10x (µl) | BSA | Enzime 1 | Volume (µl) | Enzime 2 | Volume (µl) | $H_2O$ to 20µl (µl) |
|---|---|---|---|---|---|---|---|---|
| 1 | 13.5 | 2 (M) | - | S | 1 | P | 1 | 2.5 |
| 2 | 4.5 | 2 (M) | - | X | 1 | P | 1 | 11.5 |

| Final Plasmid | Resistence |
|---|---|
| pSB1C3 | chloramphenicol |

Gel purification

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

| Parts | ηg/µl |
|---|---|
| R0040 | 8,6 |
| I13507 | 9.8 |

**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.

## 3rd Day:

Ligation (see **Ligation Protocol**)

| | | |
|---|---|---|
| Part containing the plasmid | R0040 | 6 µl |
| Insert | I13507 | 6 µl |
| 10x T4 DNA Buffer | 4 µl | |
| T4 DNA ligase 1u | 0.5 µl | |
| H2O to 20µl | 3.5 | |

**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%

## 4th Day:

Transformation (see **Transformation Protocol in Escherichia coli DH5-α**)

- Organism: E. coli DH5-α
- Selection: Ampcillin

## 5th Day:

Confirmation with NotI