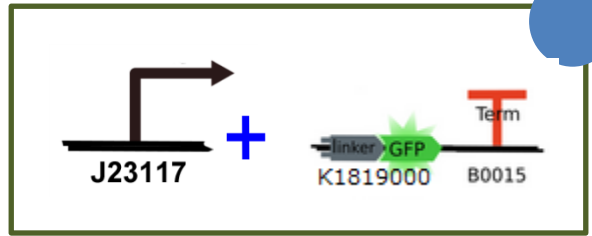


Assembly:

linkerGFP_TermC



1st Day:

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

| | Part | Size | ng/ μ l |
|---|-------------|--------|-------------|
| 1 | linker_Term | 879 bp | 125 |
| 2 | J23117 | 35 bp | 147.6 |

| | Volume to 1,0 μ g (μ l) | Buffer 10x (μ l) | Enzyme 1 | Volume (μ l) | Enzyme 2 | Volume (μ l) | H ₂ O to 20 μ l (μ l) |
|---|----------------------------------|-----------------------|----------|-------------------|----------|-------------------|---|
| 1 | 8 | 2 (Tango) | XbaI | 1 | PstI | 1 | 8 |
| 2 | 6.8 | 2 (Tango) | SpeI | 1 | PstI | 1 | 9.2 |

| Final Plasmid | Resistance |
|---------------|-----------------|
| pSB1C3 | chloramphenicol |

Gel purification

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

| Parts | ng/ μ l |
|-------------|-------------|
| linker_Term | 6.2 |
| J23117 | 22.7 |

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 | J23117 | 2.2 μ l |
| Insert | LinkerGFP | 11.5 μ l |
| 10x T4 DNA Buffer | | 2 μ l |
| T4 DNA ligase 1u | | 0.4 μ l |
| H2O to 20 μ l | | 3.9 μ 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%

2nd Day:

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

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

4th Day:

Confirmation with NotI