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## Cloning of L0 parts into pUAP-ye for Loop type IIS

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1 Works for me dx.doi.org/10.17504/protocols.io.4wdgxa6

OpenPlant Project



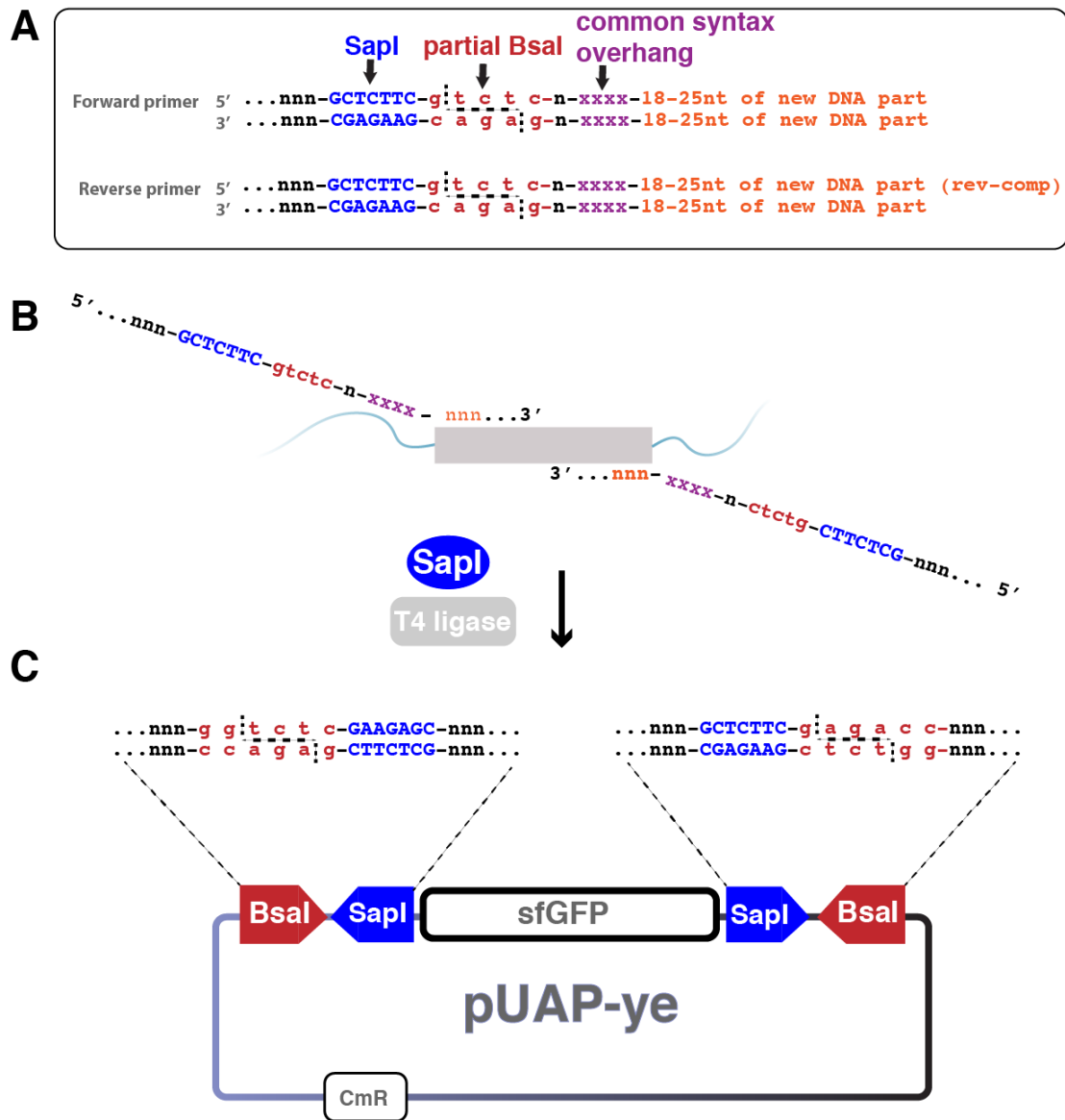
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- 1 Design primers as described in the Figure below to add the necessary overhangs for cloning into pUAP-pe and also the overhangs that correspond to the common syntax.
- 2 DNA parts are PCR amplified from the source DNA part (e.g. plasmid DNA or genomic DNA), using a high fidelity DNA polymerase such as Phusion. Use 10 ng of DNA if the template is plasmid DNA and 100ng if the template is genomic DNA. The cycling conditions are: Denaturation at 98 °C for 30 s. 35 cycles of: denaturation at 98 °C for 10 s, annealing at primer annealing temperature for 30 s, and extension at 72 °C for 15 s/kb. Final extension at 72 °C for 10 min.
- 3 Run the PCR products on a 1.5% (w/v) agarose gel.
- 4 Gel extract the band that corresponds to the size of the amplified DNA part using a kit such as QIAquick Gel Extraction Kit.
- 5 Determine DNA concentration with spectrophotometry (Nanodrop).
- 6 Prepare aliquots of the DNA part at a concentration of 15 nM and of the pUAP-pe acceptor vector at a concentration of 7.5 nM.
- 7 Set up a Type IIS assembly reaction into a 0.2 mL tube according to Table to clone the amplified DNA part into pUAP-pe.

Sterile water	5
pUAP-pe	1
DNA part	1
10x Tango buffer (Thermo Fisher)	1
1 mg/mL bovine serum albumin (NEB)	0.5
T4 DNA ligase (5 U/μL) (Thermo Fisher)	0.25
10mM ATP (SIGMA)	1
SapI (Lgul) (5 U/μL) (Thermo Fisher)	0.25
Final volume	10

- 8 Place samples on a thermocycler and use the following program:  
Assembly: 26 cycles of 37 °C for 3 min and 16 °C for 4 min.  
Termination and enzyme denaturation: 50 °C for 5 min and 80 °C for 10 min.
- 9 Transform 20 µL of chemically competent E. coli cells (transformation efficiency of  $1 \times 10^7$  transformants/µg plasmid DNA) using 2 µL of the assembly reaction and then spread on LB agar plates containing 25 µg/mL chloramphenicol.
- 10 Incubate overnight at 37 °C.
- 11 Select white colonies for sequencing.
- 12 Confirm the presence of the correct insert with Sanger sequencing using the primers UAP\_F and UAP\_R and any additional DNA part specific primers.



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