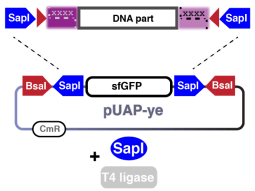
Sep 25, 2019

Cloning of L0 parts into pUAP-ye for Loop typeIIS



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Protocol status: Working
We use this protocol and it's working

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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 (ThermoFisher)	1
	1 mg/mL bovine serum albumin (NEB)	0.5
	T4 DNA ligase (5 U/µL) (ThermoFisher)	0.25
	10mM ATP (SIGMA)	1
	SapI (LguI) (5 U/µL) (ThermoFisher)	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 × 10^7 transformants/µg plasmid DNA) using 2 µL of the assembly reaction and then spread

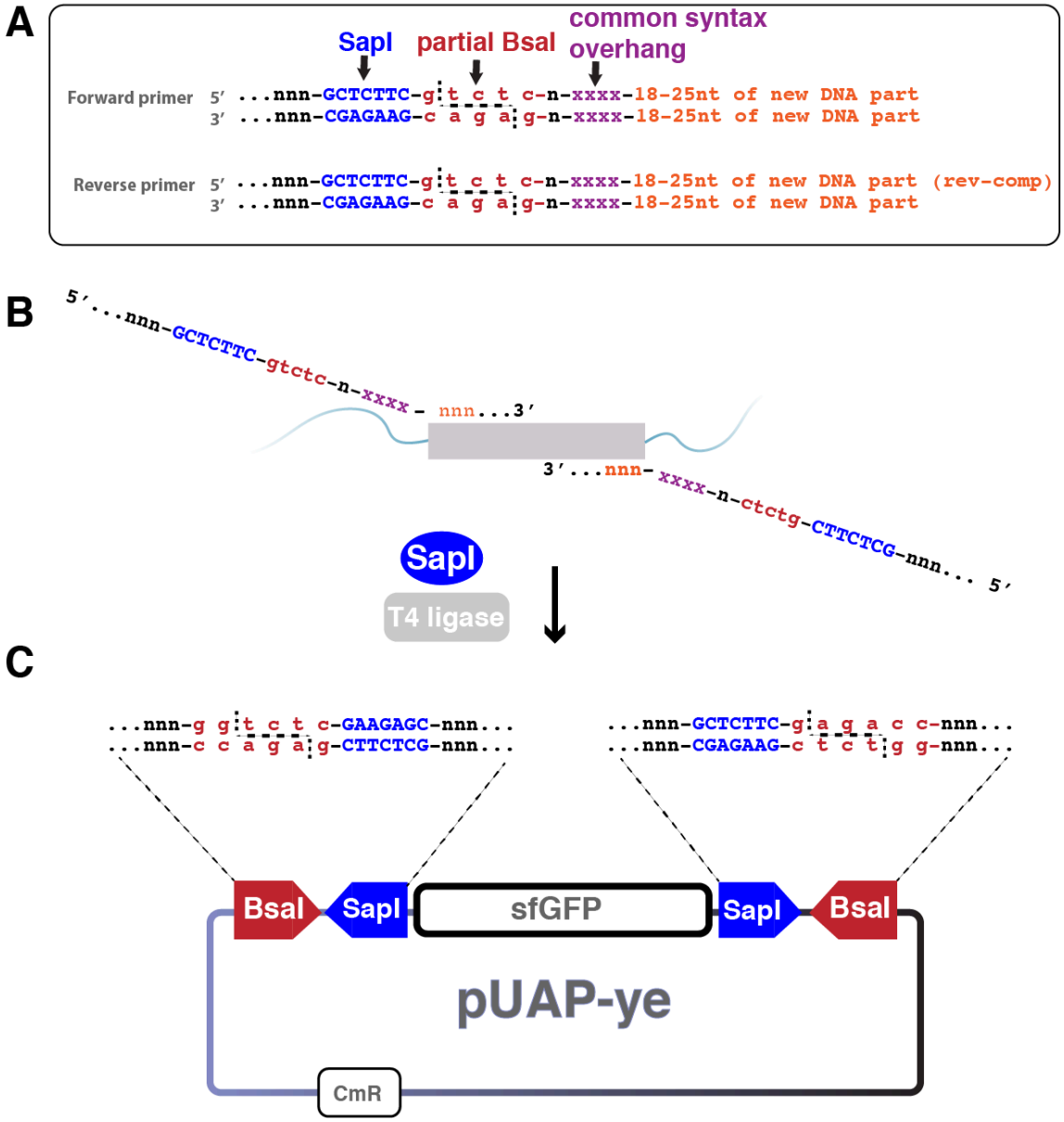
on LB agar plates containing 25 µg/mL chloramphenicol.

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.

13


A
SapI
partial BsaI
common syntax overhang
Forward primer
5′ ...nnn-GCTCTTC-g t c t c-n-xxxx-18-25nt of new DNA part
3′ ...nnn-CGAGAAG-c a g a g-n-xxxx-18-25nt of new DNA part
Reverse primer
5′ ...nnn-GCTCTTC-g t c t c-n-xxxx-18-25nt of new DNA part (rev-comp)
3′ ...nnn-CGAGAAG-c a g a g-n-xxxx-18-25nt of new DNA part
B
5′...nnn-GCTCTTC-gtctc-n-xxxx - nnn...3′
3′...nnn- xxxx-n-ctctg-CTTCTCG-nnn... 5′
SapI
T4 ligase
C
...nnn-g g t c t c-GAAGAGC-nnn...
...nnn-c c a g a g-CTTCTCG-nnn...
...nnn-GCTCTTC-g a g a c c-nnn...
...nnn-CGAGAAG-c t c t g g-nnn...
BsaI
SapI
sfGFP
SapI
BsaI
pUAP-ye
CmR