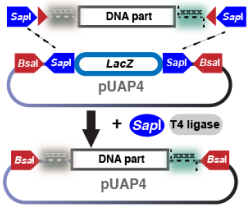
Feb 18, 2020 Version 2

Cloning of standardized L0 parts into pUAP4 for Loop type IIS assembly V.2



Version 1 is forked from 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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Abstract

Protocol for cloning of DNA L0 parts into pUAP4 using SapI type IIS assembly.

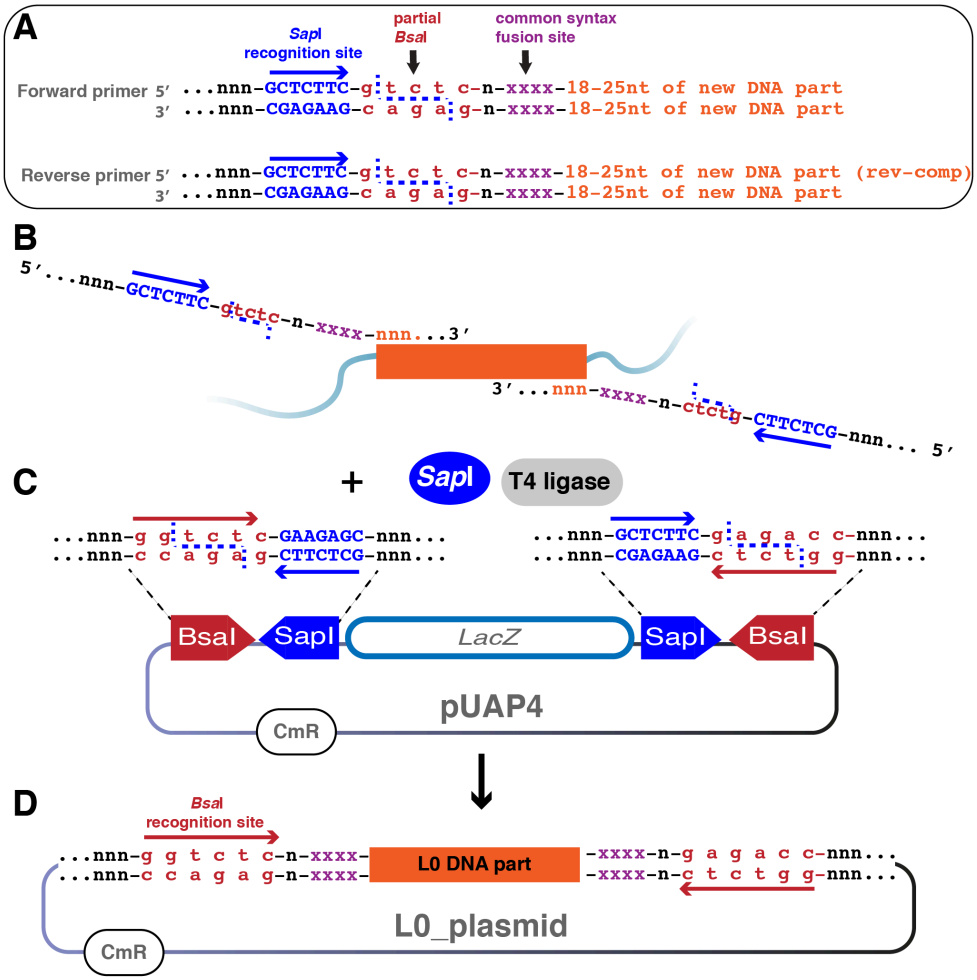
A standarised L0 part has at its end 5' and 3' fusion sites that follow the **common syntax**.

For Marchantia L0 parts, we commonly use eight positions and nine fusion sites, by combining positions A2-A3 for proximal promoter (PROMP), and B1-B2 for 5′ untranslated region (5UTR). The other types of parts are: A1 for distal promoter (PROMD), B3 for coding sequence with start codon and no stop codon (CDS1), B4 for coding sequence without start or stop codon (CDS2), B5 coding sequence without start codon and with stop codon (CTAG), B6 for 3′ untranslated region (3UTR) and C1 for transcription terminator (TERM). Parts can span multiple fusion sites, like A1-A3 for promoter (PROM), A1-B2 for promoter with 5′ UTR (PROM5), B3-B4 for coding sequence with start codon and no stop codon for N-terminal fusion with CTAG (CDS12), B3-B6 for coding sequence with start and stop codons (CDS), or B6-C1 for 3′ UTR with terminator (3TERM).

Troubleshooting

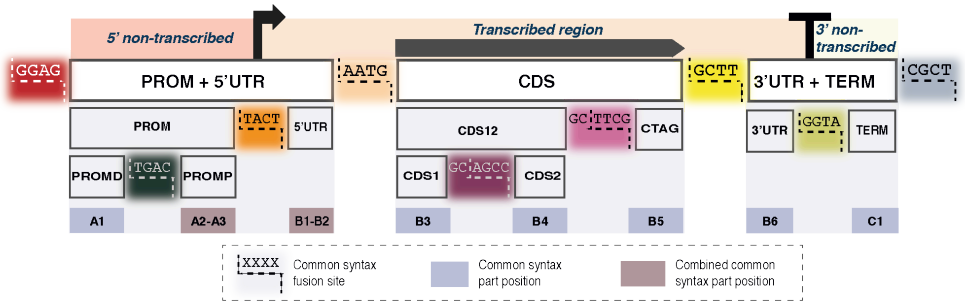
Summary of primers and cloning into pUAP4

1



Design of primers for cloning of L0 parts into pUAP4 vectors. (A) To clone a standardizsed L0 part flanked by the 5' and 3' common syntax fusion sites specific to a type of part, the fusion sites are added to the DNA part by PCR, using specially designed primers. Primers include, in order from the 5' end: 3 random bp (in black), the 7 bp SapI recognition site (in blue), 5 bp that correspond to a partial BsaI recognition site (in red), one random bp for spacing (in black), and then the common syntax fusion site (in purple) followed by 18-25 bp complementary to the DNA part to be amplified (in orange). (B) The DNA part should be amplified with a high fidelity DNA polymerase and (C) it is cloned into pUAP4 using SapI type IIS assembly to produce (D) the desired L0 plasmid. Once cloned into pUAP4, the full BsaI recognition site is reconstituted. BsaI type IIS cloning will be used to assemble multiple L0 parts into a transcription unit (L1). Blue arrows: SapI recognition site. Blue dashed lines: SapI cleavage site. Red arrows: BsaI recognition site. CmR: chloramphenicol bacterial resistance cassette. LacZ: lacZα cassette for blue-white screening of colonies (negative blue colonies contain undigested pUAP4, positive white colonies contain a L0 part inserted into pUAP4).

2 Common syntax fusion sites.



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PLEASE NOTE THAT:
- The **AATG** fusion site includes the start ATG codon for the CDS.

- A CDS part has both a start codon and a STOP codon
- CDS12 and CDS1 parts have a start codon but NO stop codon
- A CDS2 part has NO start codon and NO Stop Codon
- A CTAG part has NO start codon and it does have a Stop Codon

PCR amplification of DNA part

3 Design primers as described in step 1 to add the SapI recognition site, a partial BsaI recognition site, and the common syntax fusion site.

4 L0 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.

5 Run the PCR products on a 1.5% (w/v) agarose gel.

6 Gel extract the band that corresponds to the size of the amplified L0 part using a kit such as QIAquick Gel Extraction Kit.

SapI assembly

7 Determine L0 part and pUAP4 concentration with spectrophotometry (Nanodrop).

8 Prepare aliquots at a concentration of 15 nM for the L0 part and of 7.5 nM for the pUAP4 acceptor vector.

9 Set up a SapI Type IIS assembly reaction into a 0.2 mL tube according to Table:

	Sterile water	5 µL
	pUAP4	1 µL
	Amplified L0 part	1 µL
	10x Tango buffer (ThermoFisher)	1 µL
	1 mg/mL bovine serum albu	0.5 µL

	min (NEB)	
	T4 DNA ligase (5 U/µL) (ThermoFisher)	0.25 µL
	10mM ATP (SIGMA)	1 µL
	SapI (LguI) (5 U/µL) (ThermoFisher)	0.25 µL
	Final volume	10 µL

10 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.

11 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 and 40 µg/mL of X-gal for blue-white screening.

12 Incubate overnight at 37 °C.

13 Select white colonies for sequencing.

14 Confirm the presence of the correct insert with Sanger sequencing using the primers UAP_F (CTCGAGTGCCACCTGACGTCTAAGAAAC) and UAP_R (CGAGGAAGCCTGCATAACGCGAAGTAATC) and any additional DNA part specific primers.