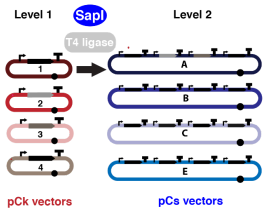
Dec 03, 2019

Loop L2 (even level) SapI type IIS cloning into pCs vectors



Forked from Loop L2 (even level) type IIS cloning - pCs-ye vectors

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Eftychis Frangedakis[1], Susana Sauret-Gueto[2], Anthony West[3], marta tomaselli[2], Nicola Patron[4], Marius Rebmann[2], Jim Haseloff[2]

[1]University of Cambridge; [2]Plant Sciences, University of Cambridge, OpenPlant; [3]previously at Earlham Institute, Norwich; [4]Earlham Institute, Norwich

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Susana Sauret-Gueto
Plant Sciences, University of Cambridge, OpenPlant

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

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Keywords: sapi type iis cloning into pcs vectors protocol, sapi type iis cloning, dna circuit, iis cloning, loop assembly, cloning, loop l2, recursive fabrication, pcs vectors protocol, open system for recursive fabrication, sapi

Abstract

Protocol based on
Pollak B, Cerda A, Delmans M, et al (2019) Loop assembly: a simple and open system for recursive fabrication of DNA circuits. New Phytol 222:628–640

https://doi.org/10.1111/nph.15625

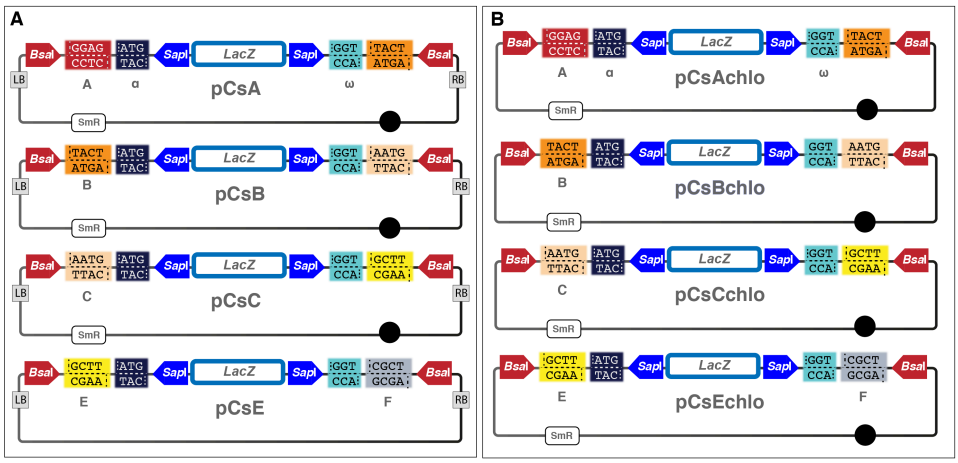
Materials

MATERIALS

- Sterile water
- dATP, 100mM, 25uMoles **Promega Catalog #**U1205
- BSA, molecular biology grade, 20 mg/ml **New England Biolabs Catalog #** B9000S
- Tango Buffer **Thermo Fisher Scientific Catalog #**BY5
- T4 DNA ligase **Thermo Fisher Scientific Catalog #**15224041
- LguI (SapI) **Thermo Fisher Scientific Catalog #**ER1931

Loop pCs vectors

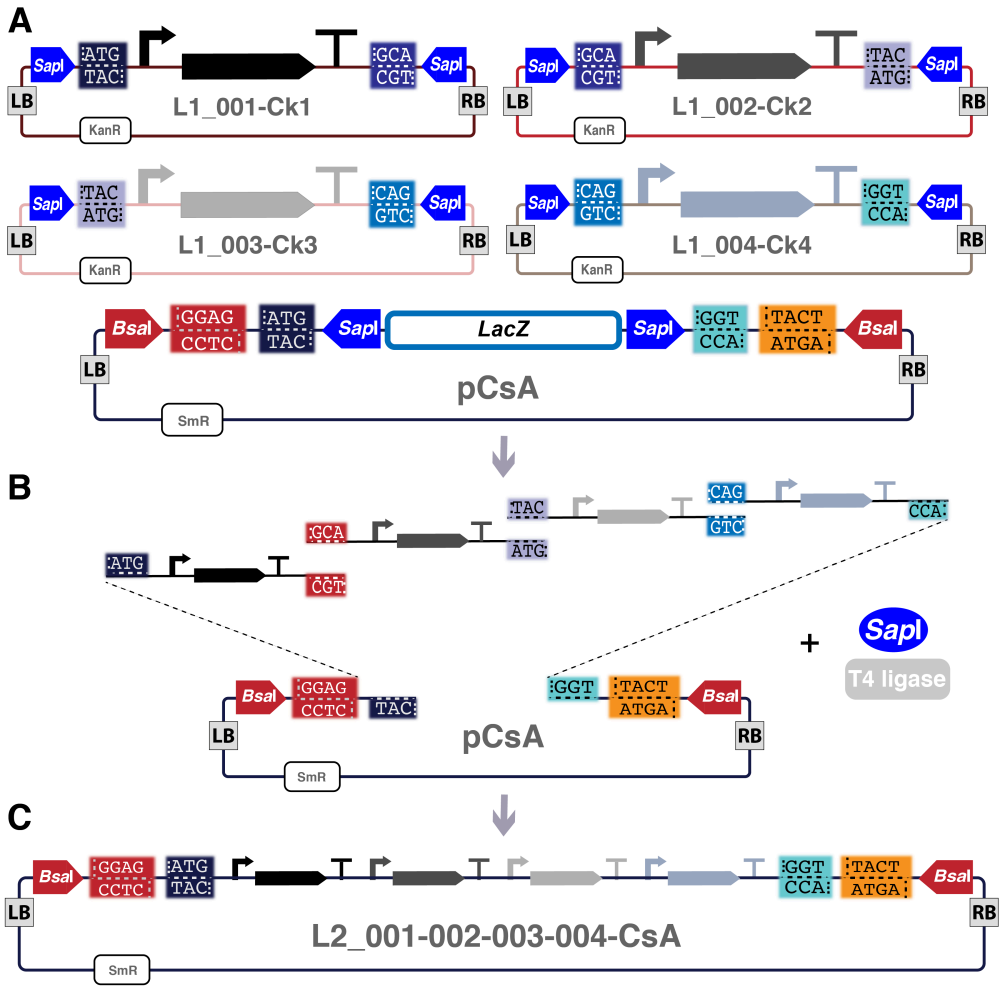
1



Loop vectors for nuclear transformation: pCs (A) and for chloroplast transformation pCschlo (B).
Loop fusion sites in the pCs vectors to assemble different L1 constructs into a L2 construct using a pCs vector and SapI are: a (ATG) and o (GCT).
Loop fusion sites in the pCs vectors to assemble different L2 constructs into a L3 construct using a pCk vector and BsaI are: A (GGAG), B (TACT), C (AATG), E (GCTT) and F (CGCT).
Left (LB) and right border (RB) repeats from nopaline C58 T-DNA for Agrobacterium-mediated nuclear transformation. SmR: spectinomycin bacterial resistance cassette.
LacZ: lacZα cassette for blue-white screening of colonies.

Example of assembly of L1 constructs into a L2 device

2



Loop assembly of four transcription units (L1) into a L2 device using a pCs plasmid and SapI.

Protocol for assembly of L1 constructs into a L2 device

3 Determine the concentration of each DNA plasmid needed (L1 plasmids and pCs acceptor plasmid)with spectrophotometry (Nanodrop).
In the example in step 2, determine concentration of plasmids L1_001-Ck1, L1_002-Ck2, L1_003-Ck3, L1_004-Ck4 and pCsA

4 Prepare aliquots for each plasmid at a concentration of 15 nM for the L1 plasmids and of 7.5 nM for the acceptor pCs vector. With this final concentration, 1 µL of each plasmid is added to the plasmids mix (see step 6).

To calculate the concentration in ng/µL:

- For a final concentration of 15 nM, the concentration in [ng/ul] equals N (the length in bp of the plasmid) divided by 110. This is an approximation of the formula: 15·10^(-9)mol/L x ((607.4 x N) + 157.9)g/mol x 10^(-6)L/µL x 10^9ng/g = concentration (ng/µL)
- For a final concentration of 7.5 nM, the concentration in [ng/ul] equals N divided by 220.

5 Prepare the Loop assembly Even Level reaction master mix (MM) according to according to Table

	Component	Volume (µL)
	Sterile water	2
	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 (LguI) (5 U/µL) (Thermo Fisher)	0.25
	Final volume	5

6 Prepare plasmids mix by adding in a 0.2 mL tube: 1 µL of each of the 4 L1 plasmids , and 1 µL of the pCs (see step 2). Mix well.

7 Add 5 µL of master mix (step 5) to the 5 µL of plasmids mix (step 6), to a final volume of 10 µL. Mix well.

8 Place samples in 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 Loop assembly reaction and then plate on LB agar plates containing 100 µg/mL spectinomycin and 40 µg/mL of X-gal for blue-white screening.

10 Incubate O/N at 37 °C.

11 Colonies with white color are likely to contain a L2 insert cloned into the pCs vector (In the example in step 2: 001-002-003-004)
Blue color colonies will contain undigested pCs vector with LacZ

12 Confirm the presence of the correct insert with Sanger sequencing using the primers pC_F (GCAACGCTCTGTCATCGTTAC) and pC_R (GTAACTTAGGACTTGTGCGACATGTC) for pCs vectors, and pC_F and pC_R2 (CAATCTGCTCTGATGCCGCATAGTTAAG) for pCschlo vectors.