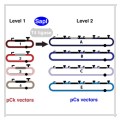

Dec 03, 2019

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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1 Works for me dx.doi.org/10.17504/protocols.io.92fh8bn

OpenPlant Project

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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

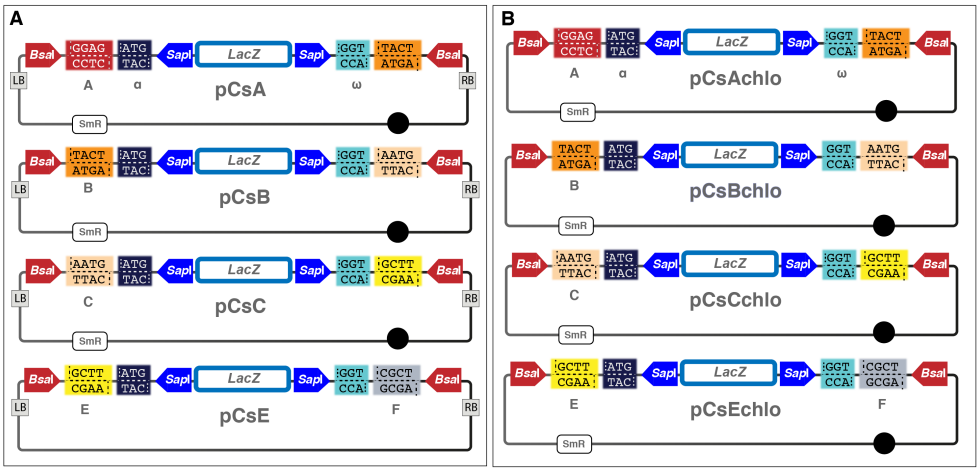
https://doi.org/10.1111/nph.15625

MATERIALS

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

Loop pCs vectors

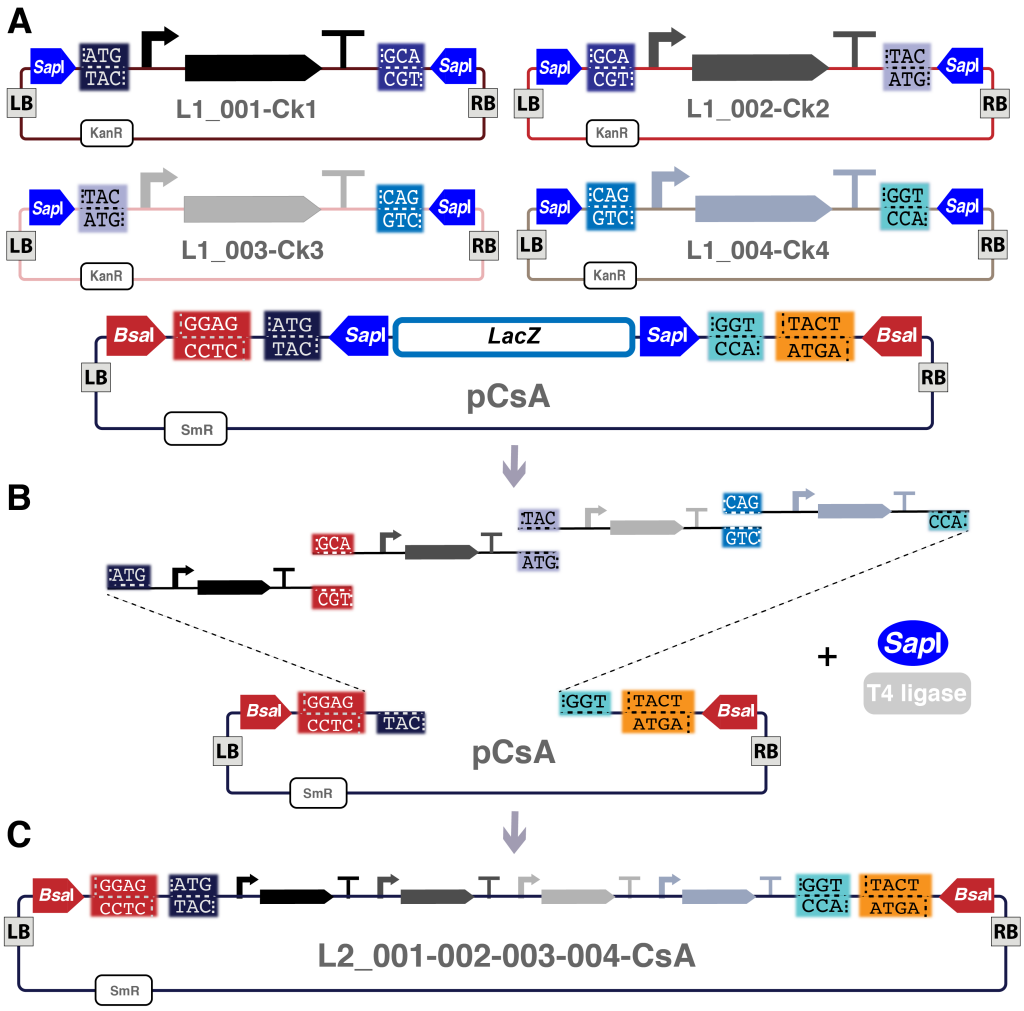
1



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.