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Loop L1 (odd level) Bsal type IIS cloning into pCk vectors V.1

Forked from [Loop L1 \(odd level\) type IIS cloning - pCk-ye vectors](#)

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1 Works for me [dx.doi.org/10.17504/protocols.io.92bh8an](https://doi.org/10.17504/protocols.io.92bh8an)

OpenPlant Project



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ABSTRACT

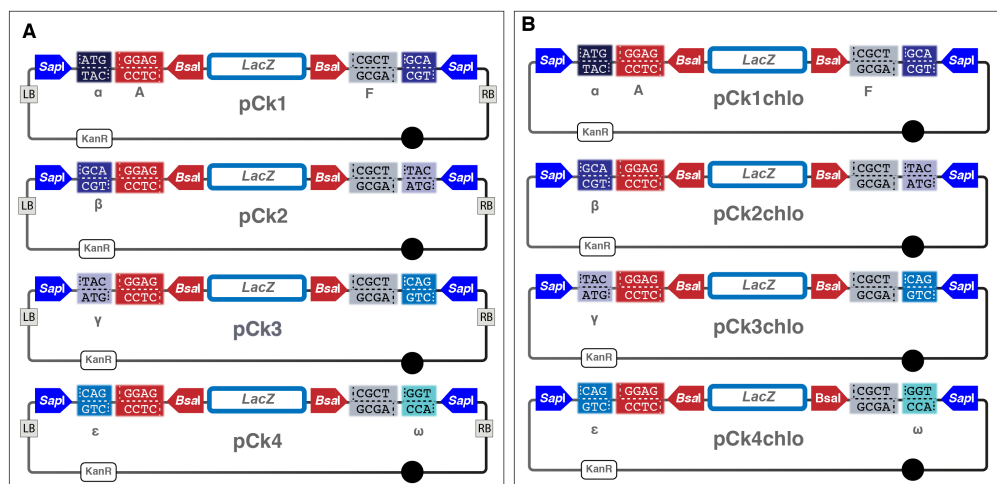
Protocol based on:

[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
Bsal - 5,000 units	R0535L	New England Biolabs
T4 DNA Ligase - 20,000 units	M0202S	New England Biolabs
Sterile water		
BSA, molecular biology grade, 20 mg/ml	B9000S	New England Biolabs
10X NEB T4 DNA ligase buffer		New England Biolabs



Loop vectors for nuclear transformation: pCks (A) and for chloroplast transformation pCkchlo (B).

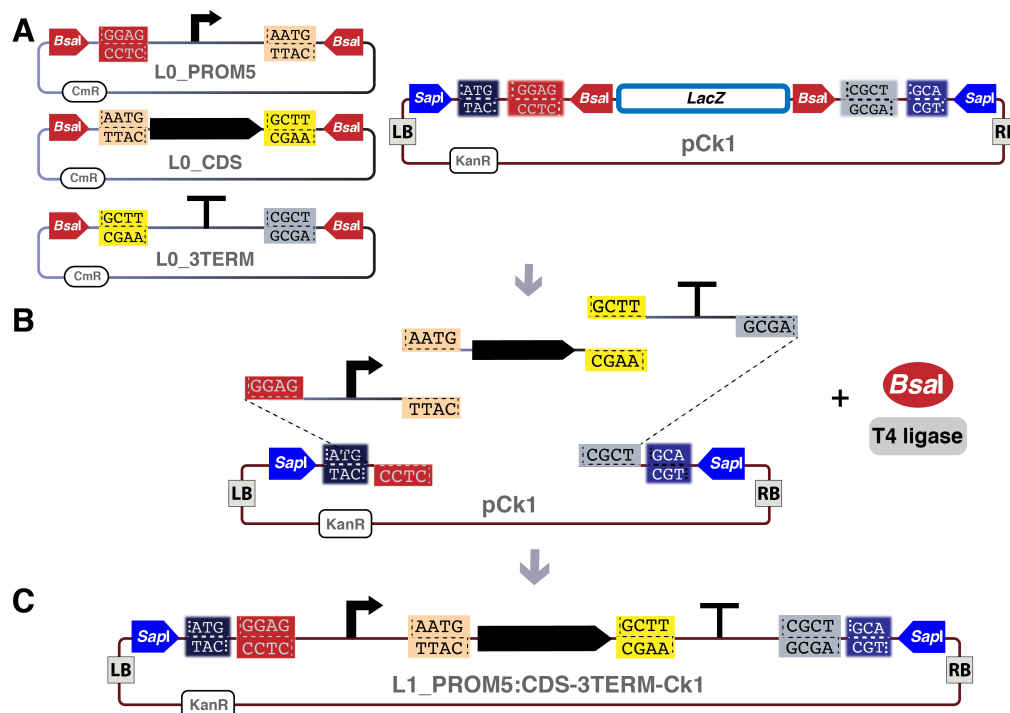
Loop fusion sites in the pCk vectors to assemble different L0 parts into a L1 construct using a pCk vector and BsaI are: A (GGAG) and F (CGCT).

Loop fusion sites in the pCk vectors to assemble different L1 constructs into a L2 construct using a pCs vector and SmaI are: a (ATC), b (GCA), d (TAC), e (CAG) and o (GCT).

Left (LB) and right border (RB) repeats from nopaline C58 T-DNA for Agrobacterium-mediated nuclear transformation. KanR: kanamycin bacterial resistance cassette. LacZ: lacZ α cassette for blue-white screening of colonies.

Example of assembly of L0 parts into a transcription unit (L1)

2



Loop assembly of multiple L0 parts into a transcription unit (L1) using a pCk plasmid and BsaI.

Protocol for assembly of L0 parts into a transcription unit (L1)

- Determine the concentrations of each DNA plasmid needed (L0 plasmids and pCk acceptor plasmid) by spectrophotometry (Nanodrop).
In the example in step 2, determine concentration of plasmids L0_PROM5, L0_CDS, L0_3TERM and pCk1.

- Prepare aliquots for each plasmid at a concentration of 15 nM for the L0 plasmids and of 7.5 nM for the acceptor pCk vector. With this final concentration, 1 µL of each plasmid is added to the plasmid mix (see step 4).

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:

$$15 \cdot 10^{-9} \text{ mol/L} \times ((607.4 \times N) + 157.9) \text{ g/mol} \times 10^{-6} \text{ L/}\mu\text{L} \times 10^9 \text{ ng/g} = \text{concentration (ng/}\mu\text{L)}$$

- For a final concentration of 7.5 nM, the concentration in [ng/ul] equals N divided by 220.

- 5 Prepare Loop assembly Level 1 reaction master mix (MM) according to Table , if four or less number of L0 parts are assembled into a pCk vector (otherwise see step 6)

Components	Volume (μL)
Sterile water	3
10x T4 ligase buffer (NEB)	1
1 mg/mL bovine serum albumin (NEB)	0.5
T4 DNA ligase at 400 U/μL (NEB)	0.25
10 U/μL BsaI (NEB)	0.25
Final volume	5

- 6 Prepare plasmids mix by adding in a 0.2 mL tube: 1 μL of each L0 plasmid (see step 2), 1 μL of the pCk vector and sterile water up to 5 μL. Mix well.

When 4 L0 parts are assembled into a pCk plasmid, the volume of the plasmid mix is 5 μL, and thus no volume of water is added.

- 7 Add 5 μL of MM (step 3) to the 5 μL of plasmids mix (step 4), to a final volume of 10 μL. Mix well.
- 8 If more than 4 L0 parts are to be assembled into a pCk vector, reduce the water volume in the MM by 1 μL (step 3) for each extra 1 μL of DNA part added in the plasmids mix .
- 9 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.
- 10 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 50 μg/mL kanamycin and 40 μg/mL of X-gal for blue-white screening.
- 11 Incubate overnight at 37 °C.
- 12 Colonies with white color are likely to contain an L1 insert cloned into the pCk vector (In the example in step 2: PROM5:CDS-3TERM)
Blue color colonies will contain undigested pCk vector with LacZ
- 13 Confirm the presence of the correct insert with Sanger sequencing using the primers pC_F (GCAACGCTCTGTCATCGTTAC) and pC_R (GTAACCTAGGACTTGTGCGACATGTC) for pCk vectors, and pC_F and pC_R2 (CAATCTGCTCTGATGCCGCATAGTTAAG) for pCkchlo vectors.



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