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## Loop L1 (odd level) BsaI type IIS cloning into pCk vectors V.2

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.92gh8bw](https://doi.org/10.17504/protocols.io.92gh8bw)

OpenPlant Project



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### ABSTRACT

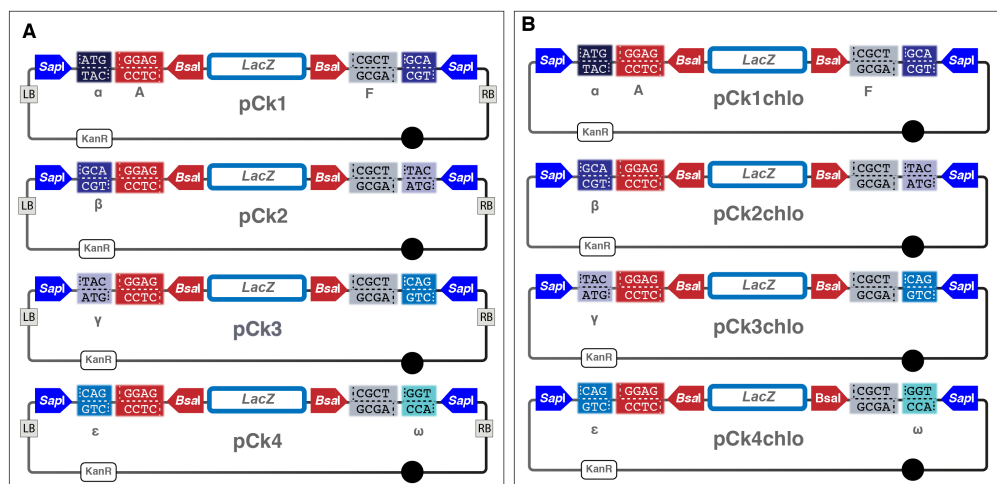
Protocol based on:

[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
BsaI - 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).

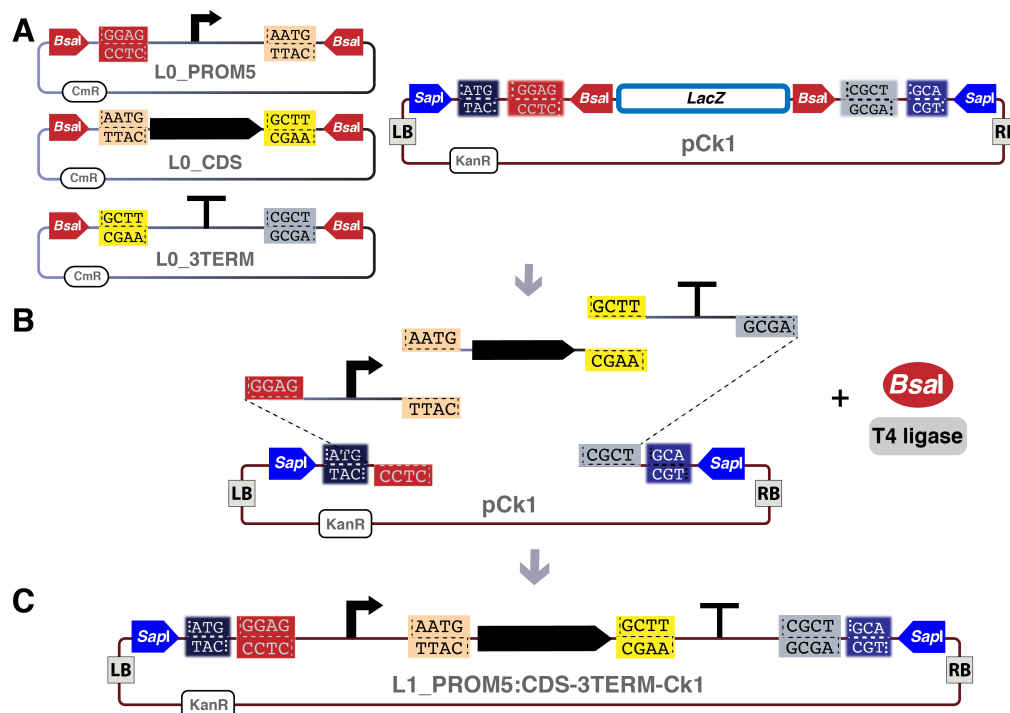
Loop fusion sites in the pCk vectors to assemble different L0 parts into a L1 construct using a pCk vector and BsaI are: A (GGAG) and F (CGCT).

Loop fusion sites in the pCk vectors to assemble different L1 constructs into a L2 construct using a pCs vector and SapI 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 $\alpha$  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 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:

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

- 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 8)

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 , 1 μL of the pCk vector (see step 4), 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 5) to the 5 μL of plasmids mix (step 6), 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 5) for each extra 1 μL of DNA part added in the plasmids mix (step 6).

- 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  $1 \times 10^7$  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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