

Sep 24, 2019

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.4wegxbe

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

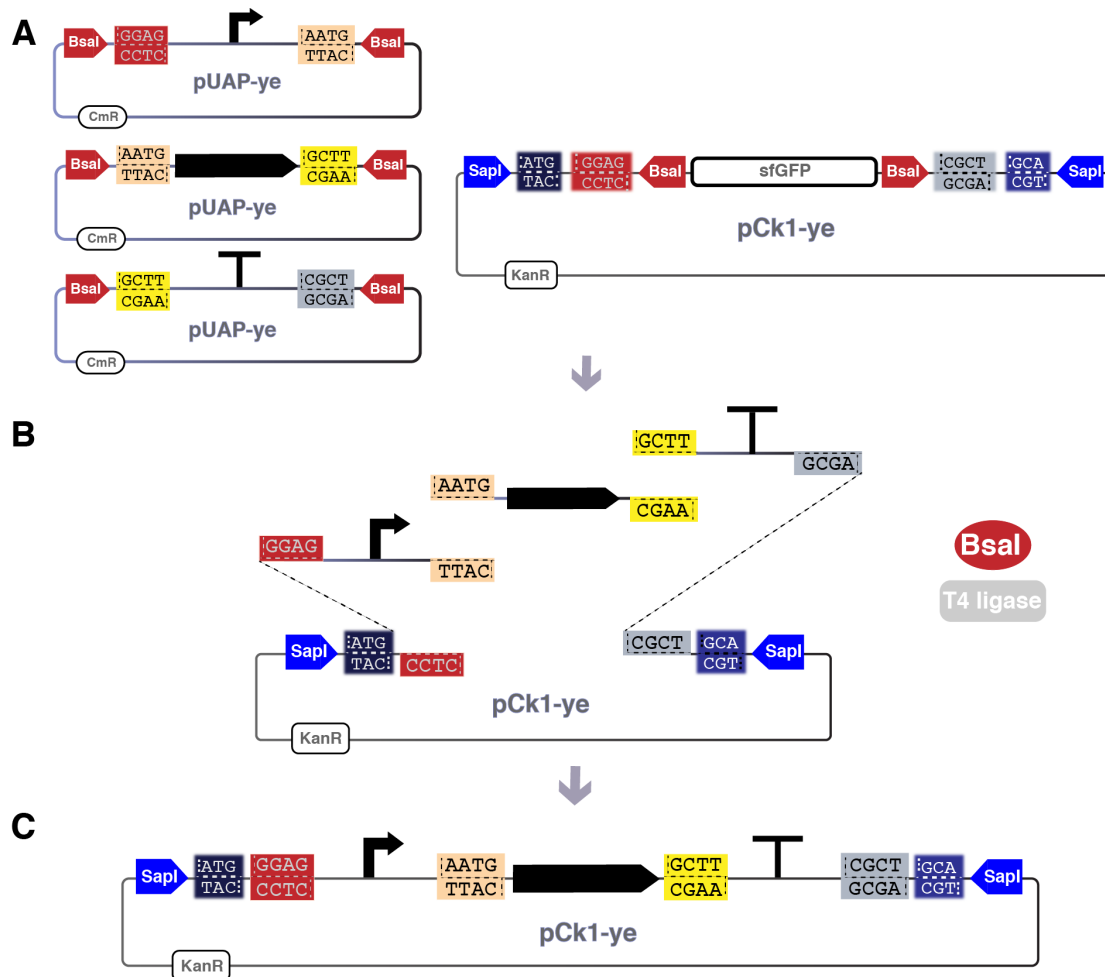
- 1 Determine the concentrations of the DNA parts by spectrophotometry (Nanodrop).
- 2 Prepare aliquots for DNA parts to be assembled at a concentration of 15 nM and of the pCk vector at a concentration of 7.5 nM.

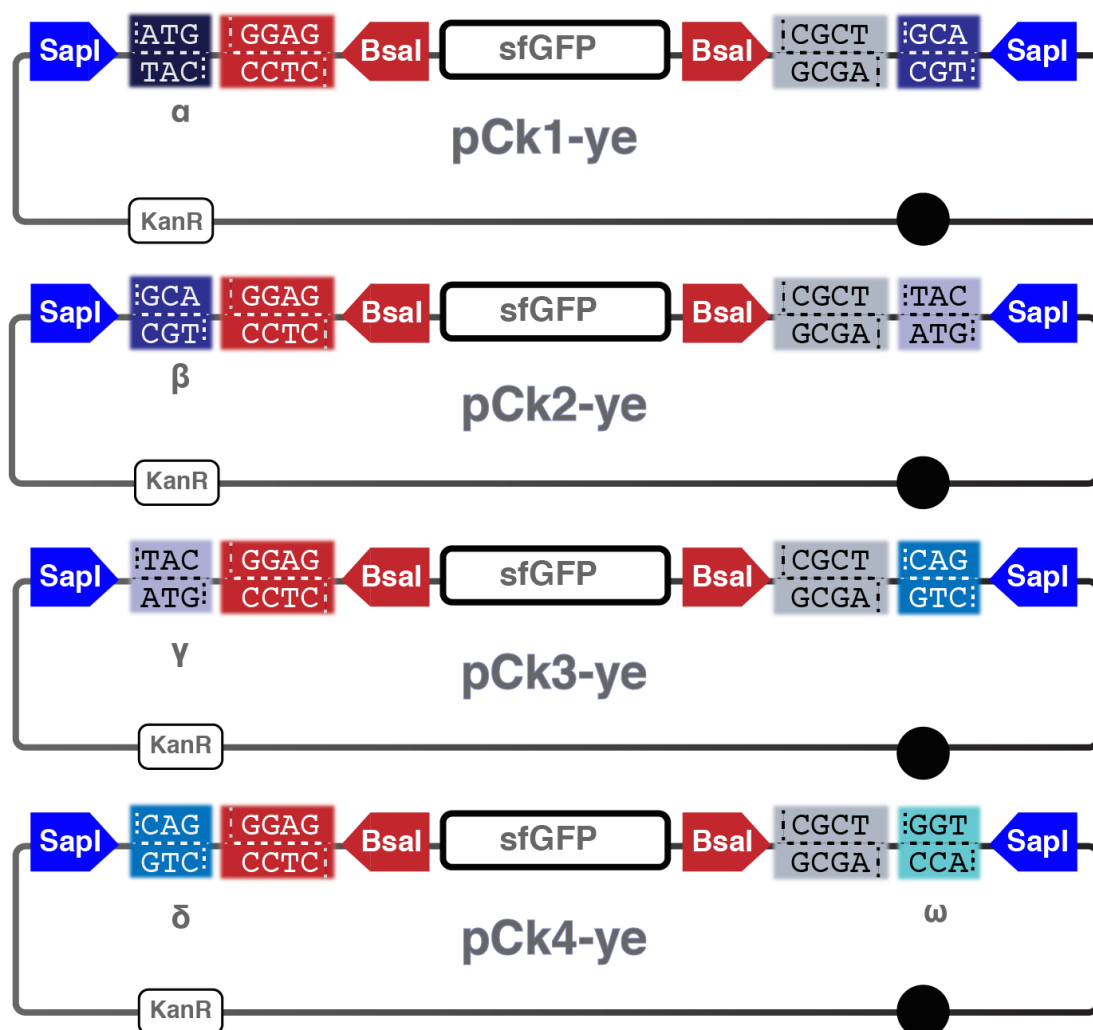
To calculate the concentration needed for each part to assemble (not the backbone) in ng/μL, divide the length of the plasmid where they are cloned by 100. For the backbone, divide the plasmid length by 200. In this way you can prepare aliquots of your parts and backbones and add 1 μL of each one to the plasmid mix.


- 3 . Prepare Loop assembly Level 1 reaction master mix (MM) according to Table, if four or less number of parts are assembled into a pCk-pe vector.

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

- 4 Prepare plasmids mix for each reaction, by adding in a 0.2 mL tube, 1 μL of each DNA part aliquot (see step 2), 1 μL of the pCk-pe vector and sterile water up to 5 μL. Mix well.
- 5 Add 5 μL of master mix to the 5 μL of plasmids mix, to a final volume of 10 μL. Mix well. If more than 4 DNA parts are to be assembled into a pCk-pe vector, reduce the water volume in the MM by 1 μL for each extra 1 μL of DNA part added in the plasmids mix.
- 6 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.
- 7 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.
- 8 Incubate overnight at 37 °C.
- 9 Colonies with white color are likely to contain the vector with the insert while yellow color colonies will contain the empty vector.
- 10 Confirm the presence of the correct insert with Sanger sequencing using the primers pC_F and pC_R





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