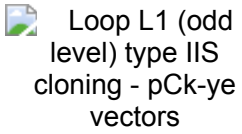
Sep 25, 2019

Loop L1 (odd level) type IIS cloning - pCk-ye vectors



DOI
dx.doi.org/10.17504/protocols.io.4wegxbe

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

[1]University of Cambridge; [2]Plant Sciences, University of Cambridge, OpenPlant;
[3]University of Cambridge, Open Plant

OpenPlant Project

Eftychis Frangedakis
University of Cambridge, Plant Sciences

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.4wegxbe

Protocol Citation: Eftychis Frangedakis, Susana Sauret-Gueto, Anthony West, Nicola Patron, Marta Marta Tomaselli, Marius Rebmann, Jim Haseloff 2019. Loop L1 (odd level) type IIS cloning - pCk-ye vectors. **protocols.io https://dx.doi.org/10.17504/protocols.io.4wegxbe**

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

Created: June 29, 2019

Last Modified: September 25, 2019

Protocol Integer ID: 25254

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

- BsaI - 5,000 units **New England Biolabs Catalog #**R0535L
- T4 DNA Ligase - 20,000 units **New England Biolabs Catalog #**M0202S
- Sterile water
- BSA, molecular biology grade, 20 mg/ml **New England Biolabs Catalog #** B9000S
- 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.

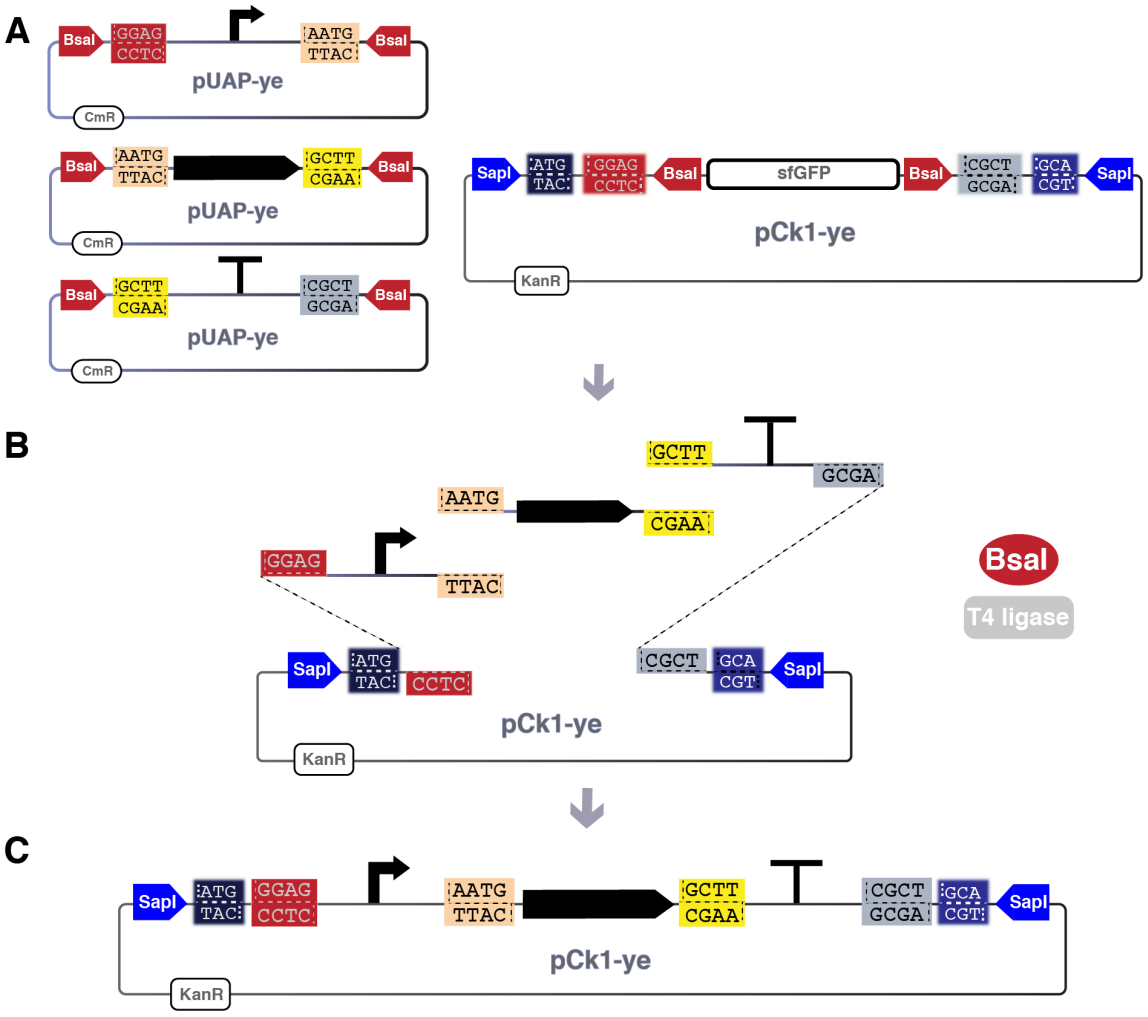
7 Transform 20 µL of chemically competent E. coli cells (transformation efficiency of 1 × 107 transformants/µg plasmid DNA) using 2 µL of the Loop assembly reaction and then plate on LB agar plates containing 50 µg/mL kanamycin.

8 Incubate overnight at 37 °C.

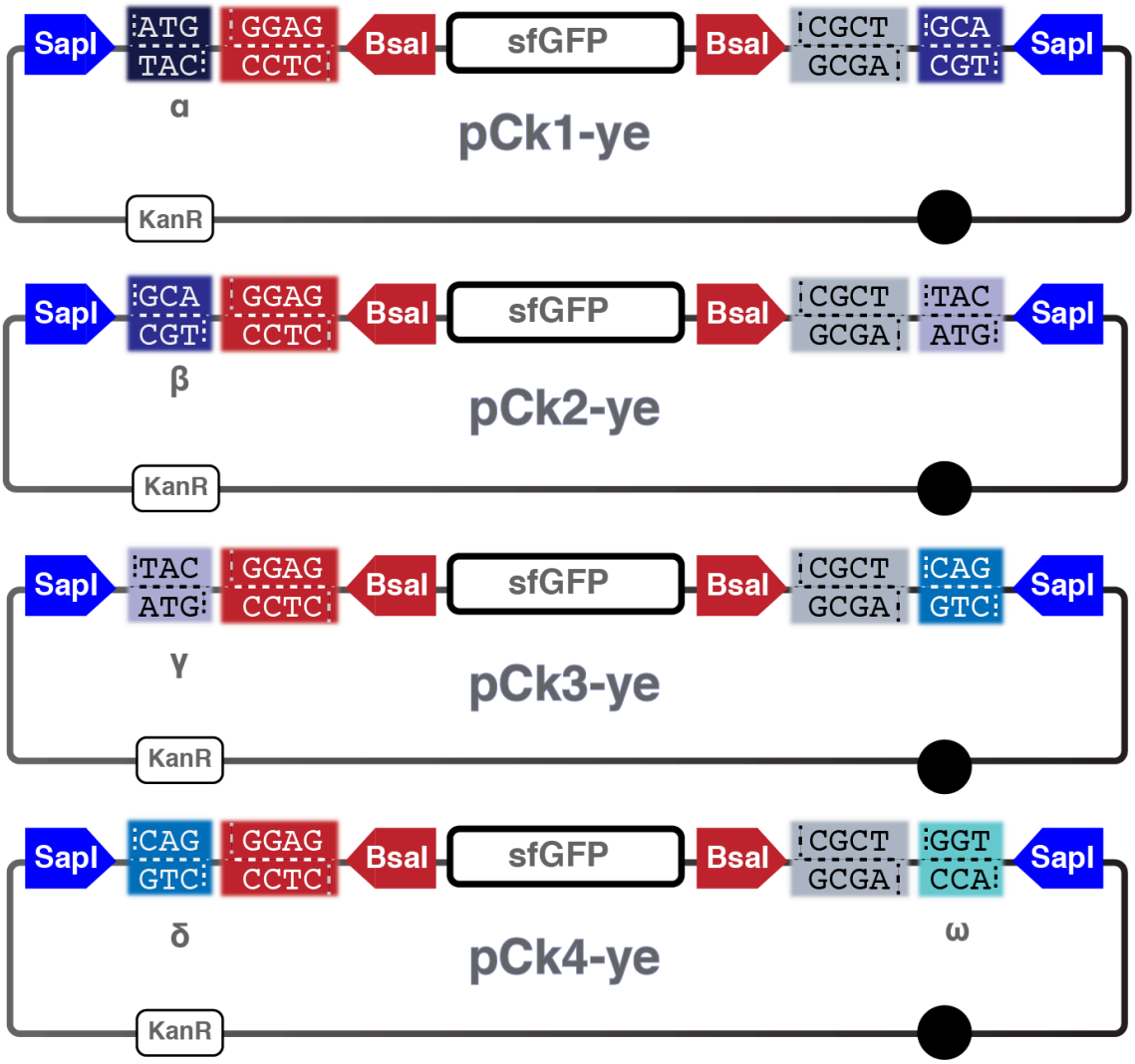
9 Colonies with white color are likely to contain the vector with the insert while yellow color colonies will contain the empty vector.

10 Confirm the presence of the correct insert with Sanger sequencing using the primers pC_F and pC_R

11


A
BsaI
GGAG
CCTC
AATG
TTAC
pUAP-ye
CmR
GCTT
CGAA
CGCT
GCGA
SapI
ATG
TAC
sfGFP
GCA
CGT
pCk1-ye
KanR
B
BsaI
T4 ligase
C

12


SapI
ATG
TAC
GGAG
CCTC
BsaI
sfGFP
BsaI
CGCT
GCGA
GCA
CGT
SapI
α
pCk1-ye
KanR
SapI
GCA
CGT
GGAG
CCTC
BsaI
sfGFP
BsaI
CGCT
GCGA
TAC
ATG
SapI
β
pCk2-ye
KanR
SapI
TAC
ATG
GGAG
CCTC
BsaI
sfGFP
BsaI
CGCT
GCGA
CAG
GTC
SapI
γ
pCk3-ye
KanR
SapI
CAG
GTC
GGAG
CCTC
BsaI
sfGFP
BsaI
CGCT
GCGA
GGT
CCA
SapI
δ
ω
pCk4-ye
KanR