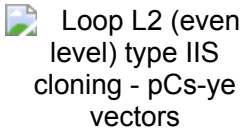
Sep 24, 2019

Loop L2 (even level) type IIS cloning - pCs-ye vectors



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

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

Protocol Citation: Eftychis Frangedakis, Susana Sauret-Gueto, Anthony West, Marta Marta Tomaselli, Nicola Patron, Marius Rebmann, Jim Haseloff 2019. Loop L2 (even level) type IIS cloning - pCs-ye vectors. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4wcgxaw

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

Created: June 28, 2019

Last Modified: September 24, 2019

Protocol Integer ID: 25252

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

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

1 Determine DNA parts concentration with spectrophotometry (Nanodrop).

2 Prepare aliquots for DNA parts to be assembled at a concentration of 15 nM and of the pCs-pe 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 the Loop assembly Even Level reaction 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

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 pCs-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.

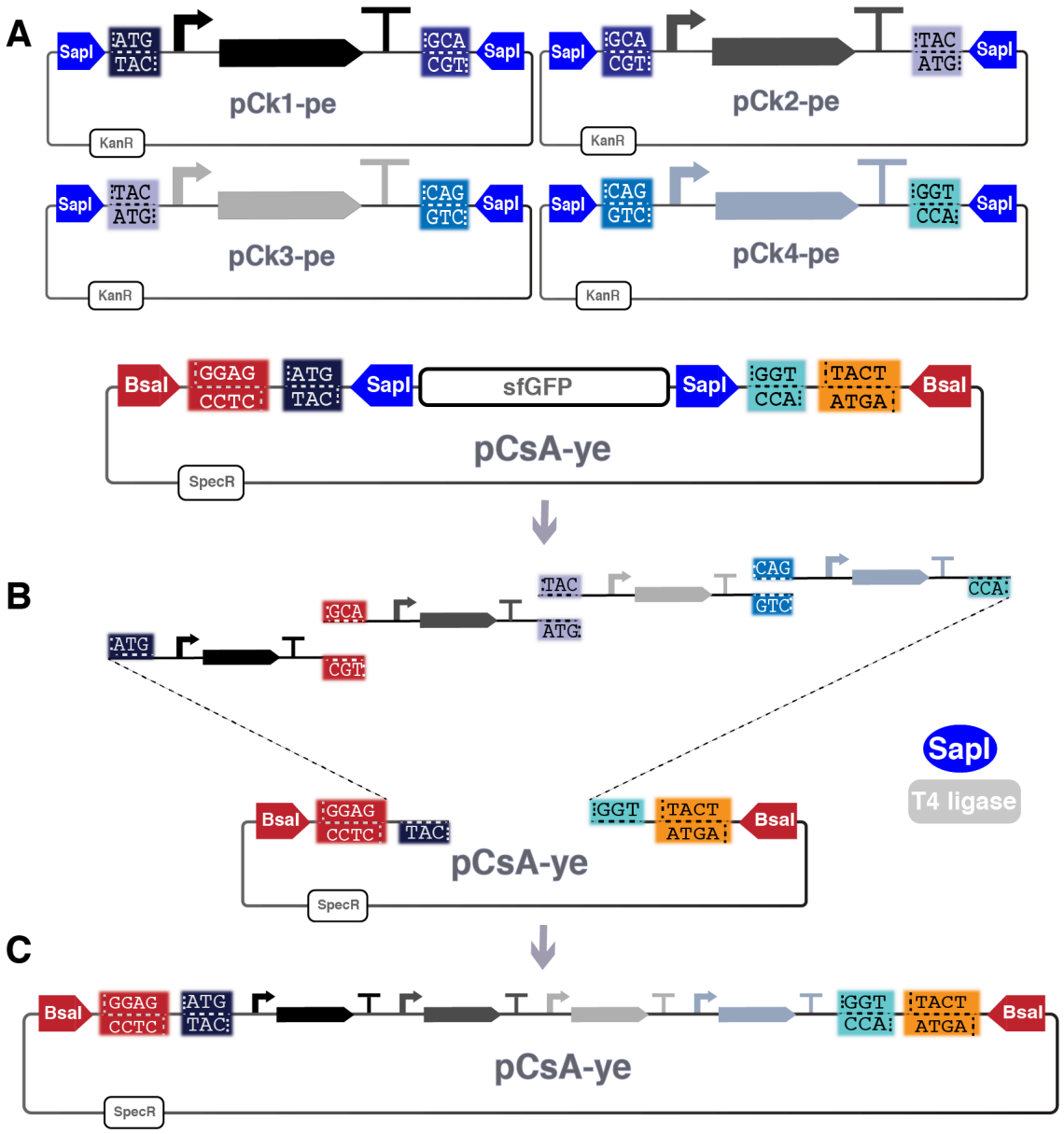
7 Transform 20 µL of chemically competent E. coli cells (transformation efficiency of 1 × 107 transformants/µg plasmid DNA) using 2 µL of the Loop assembly reaction and then plate on LB agar plates containing 100 µg/mL spectinomycin.

8 Incubate O/N 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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CGT
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ATG
CAG
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CAG
GTC
GGT
CCA
pCk4-pe
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GGT
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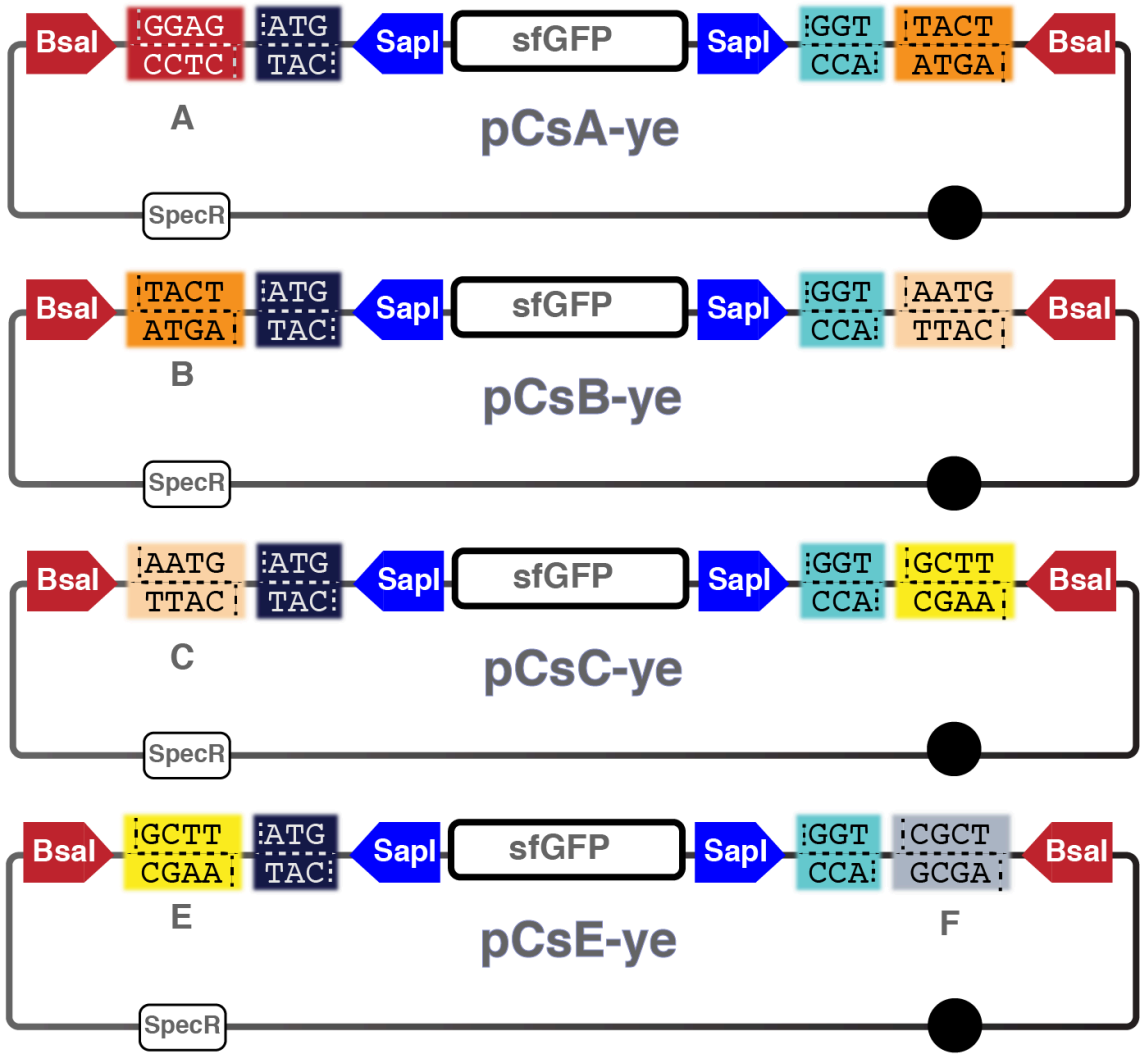
ATGA
pCsA-ye
SpecR
B
T4 ligase
C

12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