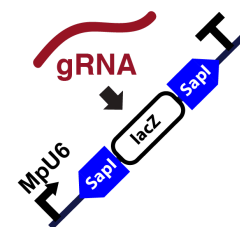


Dec 04, 2019

gRNA design and cloning with Sapl into Loop plasmid L2_lacZgRNA-Cas9-CsA

Forked from [gRNA design and cloning into Loop L2 plasmids \(L2_gRNA-Cas9-CsA and L2_gRNA-CsA plasmids\)](#).



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Protocol status: Working

We use this protocol and it's working

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Protocol Integer ID: 30550

Keywords: guide rna target sequence, l2 plasmid, cas9, cloning, grna design, rna, sapi into loop, grna, csa this protocol, clone

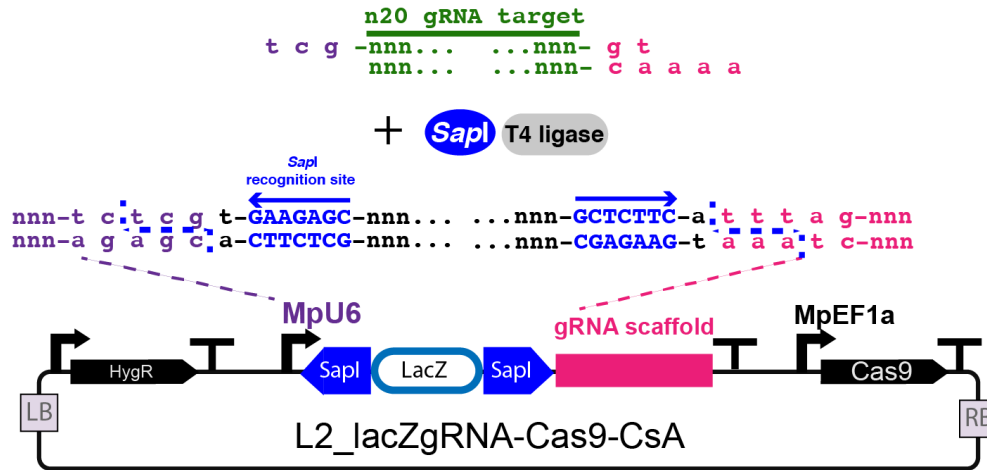
Abstract

This protocol explains how to design and clone the guide RNA target sequence into a L2 plasmid ready to accept the gRNA by cloning with SapI (L2 plasmid also contains a cassette to express Cas9)

Troubleshooting

Summary of design of gRNA and cloning into L2 with SapI

1



Design of oligos for gRNA SapI mediated cloning into L2_lacZgRNA-Cas9-CsA vector. The L2_lacZgRNA-Cas9-CsA SapI digested vector has AGC and TTT overhangs. Therefore, oligos for gRNA should be designed such that the forward strand has a 5' overhang of TCG and the reverse strand has a 5' overhang of gt-AAA (addition of "gt" nucleotides is necessary to reconstitute the full sequence of the gRNA scaffold in pink). Blue arrows: SapI recognition site. Blue dashed lines: SapI cleavage site. LacZ: lacZα cassette for blue-white screening of colonies.

Protocol for design of gRNA and cloning into L2 with SapI

2 **gRNA oligo design**

Order two oligos that contain the forward and reverse guide sequence plus the overhangs necessary for ligation (highlighted with bold) into L2_lacZgRNA-Cas9-CsA:

oligo F: 5'- **TCG**-NNNNNNNNNNNNNNNNNNNNNNNNNNNNNN-**gt** 3'

oligo R: 5'-**AAAac**-NNNNNNNNNNNNNNNNNNNNNNNNNNNNNN-3'

Note: Standard de-salted oligos are ok

3 **Oligo annealing**

Mix oligos with water as follow:

oligo F (100μM) 1μl

oligo R (100μM) 1μl

water 8μl



Total volume 10 μ l

Anneal in a thermocycler using the following parameters: 37°C for 30 min, 95°C for 5 min and then ramp down to 25°C at 5°C per min. After annealing the gRNA can be directly cloned into L2_lacZgRNACas9-CsA plasmid without the need of any further processing (step 4).

4 **Cloning into backbone vector**

In a 0.2 mL tube set up the following reaction:

Component	Volume (μ L)
Sterile water	5
10x Tango buffer (Thermo Fisher)	1
1 mg/mL bovine serum albumin (NEB)	0.5
T4 DNA ligase (5 U/ μ L) (Thermo Fisher)	0.25
10mM ATP (SIGMA)	1
SapI (LguI) (5 U/ μ L) (Thermo Fisher)	0.25
L2_lacZgRNACas9-Csa (25-50 ng)	1
annealed oligo	1
Final volume	10

- Place samples on the thermocycler and incubated using the following program:



Assembly: 15 cycles: 3 minutes at 37°C and 4 minutes at 16°C

Termination: 5 minutes at 50°C and 10 minutes at 80°C

- Transform chemically competent using 1 µL of reaction and plate on LB agar plates with 100 µg/mL spec and X-gal 40. Incubate at 37 oC for 16 h.
- Confirm with sequencing