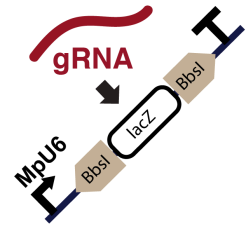




Dec 15, 2025

🌐 gRNA design and cloning with BbsI into Loop plasmid L1_lacZgRNA-Ck2/3

🔗 Forked from [gRNA design and cloning with BbsI into Loop plasmid L1_lacZgRNA-Ck2/3](https://dx.doi.org/10.17504/protocols.io.dm6gp1e8jgzp/v1)



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Protocol status: Working

We use this protocol and it's working

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Keywords: l1-grna transcription unit, cas9 transcription unit via l2 sapi loop assembly, grna target sequence, guide rna target sequence, plasmid l1-laczgrna, cas9 transcription unit, antibiotic resistance transcription unit, dual grna, grna design, grna, rna, l2 sapi loop assembly, bbsi into loop, mpef1 α , cas9

Abstract

This protocol explains how to design and clone the guide RNA target sequence into a L1 plasmid ready to accept the gRNA by cloning with BbsI. L1 plasmids are L1_lacZgRNA-Ck2 and L1_lacZgRNA-Ck3.

If one gRNA target sequence is cloned into the Ck2 plasmid and another one into Ck3 one, the two L1_gRNA transcription units can be combined with an antibiotic resistance transcription unit and a MpEF1 α :Cas9 transcription unit via **L2 SapI Loop assembly**. This allows for dual gRNA editing.



oligo R (100 μ M) 1 μ l

water 8 μ l

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 L1_lacZgRNA-Ck2 or L1_lacZgRNA-Ck3 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	6
10x T4 ligase buffer (NEB)	1
1 mg/mL bovine serum albumin (NEB)	0.5
T4 DNA ligase (5 U/ μ L) (Thermo Fisher)	0.25
BbsI (BpiI) (5 U/ μ L) (Thermo Fisher)	0.25
L1_lacZgRNA-Ck2 or L1_lacZgRNA-Ck3 (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 μ L of reaction and plate on LB agar plates with 50 μ g/mL Kan and X-gal 40 μ g/mL for blue-white screening. Incubate at 37°C for 16 h.
- Confirm with sequencing