	IBISBA-SOP-WU21
WCSB, Wageningen University	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Design of spacers and cloning of them in pSEVA231-CRISPR

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Instruction

Design of spacers and cloning of them in pSEVA231-CRISPR

1. Introduction / Purpose

The purpose of the current protocol is the design of CRISPR-spacers ready to be ligated in the plasmid pSEVA231-CRISPR.

Keywords: CRISPR-cas - spacer - pCRISPR - design

2. Equipment and chemicals

2.1. Equipment

- PCR machine
- Heating block (thermomixer)

2.2. Chemicals

- Bsal (New England Biolabs)
- T4 ligase
- T4 ligase buffer
- 100x Bovine Serum Albumin (BSA)
- Kanamycin

2.3. Strains

• *E. coli* DH5α

2.4. Other materials

• pSEVA231-CRISPR

3. Media and Buffers

- SOC medium
- LB medium

4. Procedures

Spacer design

Use the online tool BPROM (Softberry) to define the gene promoter and sequence between the promoter and the ATG

Find the available PAM (-NGGs) and choose the most suitable (usually the closer to the promoter the higher down-regulation)

Select 30 nucleotides (5'-3') before the PAM of the template or non-template strand (Fig.1)

Copy paste the appropriate flanks and order the single strand DNAs as primers (Table 1)

Figure 1

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Table 1

Spacer	Flanks
PAM Spacer	a ggtctc aaaacgttttt gagacc a
Anti-Pam Spacer	t ggtctc aaaaaogtttt gagacc t

Oligo annealing and Ligation

Annealing Mix together: 1 ul oligo 1 (100 uM) 1 ul oligo 2 (100 uM) 2.5 ul NaCl (1 M) 45.5 ul ddH2O

- Incubate for 5 minutes at 95°C and slowly cool down to room temperature (use a thermocycler) (Alternatively, use the heating block and take the block out of the heater and let it cool for 2 hours)
- Dilute the annealed oligos Fig. 2 10 times
- The annealed oligos should have the structure of Fig. 2.

Figure 2

	Start (0)	an in	find (55)
5'	aggtorea	asactgggcagscotcgtcaattettggattaccgtttttbAsACCa	31
31	Cocagnys	LttyacceptotycagcacttaspacetaatyccaaseaCTC1001	51

Ligation Mix together: 2 ul diluted annealed oligo 2 ul Plasmid (10-15ng/ul) 2 ul MetaAssembly Buffer

37°C, 5 min \rightarrow (16°C, 4 min \rightarrow 37°C, 3 min) x 15 \rightarrow 37°C, 5 min \rightarrow 85°C, 15 min

- Transform to *E.coli* DH5a
- Screen colonies by colony PCR with primers 86/87
- Select the colonies with the small DNA product and send for sequencing with the same primers

Primer sequences: 86: CTGGATTCTCACCAATAAAAAACG 87: TCTAGGGCGGCGGAT

5. Remarks / troubleshooting

6. Biosafety

No biosafety issues were associated with this protocol.

7. Acknowledgements



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