WCSB, Wageningen University	IBISBA-SOP-WU21
	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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Experimenter 1	Rita Volkers		14/3/2019

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

- BsaI (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

Table 1

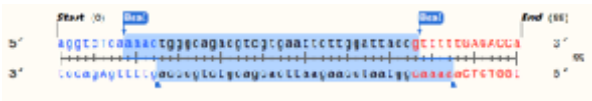
Spacer	Flanks
PAM Spacer	*aggtctcaaaac--gttttgagacca*
Anti-Pam Spacer	*tggtctcaaaaac--gttttgagacct*

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



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

37°C, 5 min → (16°C, 4 min → 37°C, 3 min) x 15 → 37°C, 5 min → 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

IBISBA (grant agreement No 730976) and EmPowerPutida (grant agreement No 635536) projects have received funding from the European Union's Horizon 2020 research and innovation programme.

