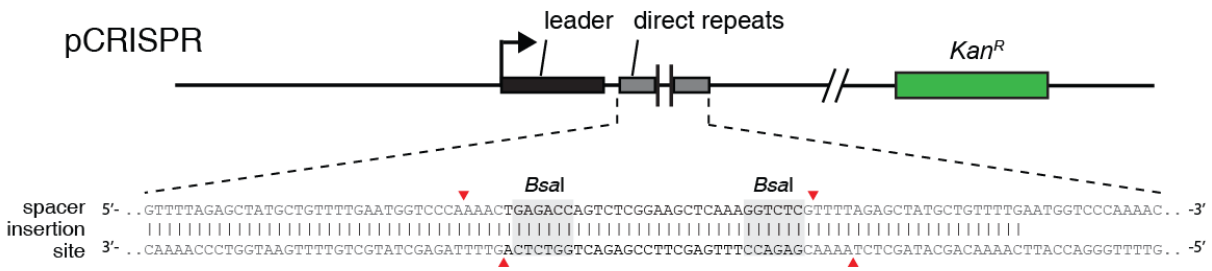
Protocol for new spacer cloning in pCRISPR



Note: the DR-BsaIspc-DR region was accidentally duplicated during construction, which was not shown in the schematic. But this will not interfere with new spacer construction through BsaI digest and oligo cloning.

Vector Digest

-Digest 1-2ug of pCRISPR with BsaI (NEB)

x ul	pCRISPR
1 ul	BsaI (NEB)
5 ul	10X NEB Buffer
0.5 ul	100X BSA
y ul	ddH2O
50 ul	

-Gel purify digested pCRISPR (important for successful cloning)

Oligo Design

-In order to clone a spacer sequence into pCRISPR, synthesize two oligos (IDT) of the following form:

```
5'- AAACNNNNNNNNNNNNNNNNNNNNNNNNNNNNG -3'
        ||||||||||||||||||||||||||||||||
    3'-NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCAAAA -5'
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Phosphorylation

1 ul	oligo I (100 uM)
1 ul	oligo II (100 uM)
5 ul	10X T4 Ligase buffer (NEB)
1 ul	T4 PNK (NEB)
42 ul	ddH2O
50 ul	

Annealing

-Add 2.5 ul of 1M NaCl to the phosphorylated oligo pairs.

-Incubate 5’ @ 95 and slowly cool down to room temperature (use a thermocycler).
(Alternatively, use a heat block and take the block out of the heater and let cool naturally for 2 hours)
-Dilute annealed oligos 10 times

Ligation

1 ul	gel purified, BsaI digested pCRISPR
1 ul	diluted annealed oligos
2 ul	10X T4 Ligase buffer (NEB)
1 ul	T4 ligase
15 ul	ddH2O
20 ul	

-Incubate at RT for 2h or 16C for O/N

Transformation
-*E.coli* DH5α