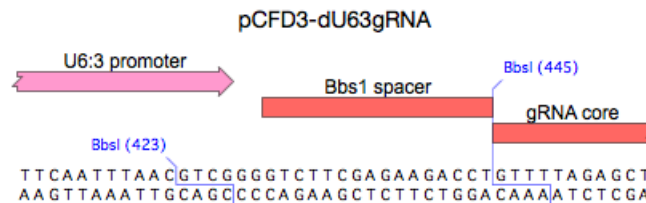


Cloning gRNA expression vectors with pCFD3

Oligo design for pCFD3:



Sense: 5' – GTCG-N19/20

Anti-sense: 5' – AAAC-N19/20 reverse complement

Note that the G at position 4 in the sense oligo is the first base that is transcribed. If your protospacer sequence starts with a G then N will be 19. If it does not start with a G enter all 20 nucleotides behind GTCG.

Resuspend oligos to 100uM.

Set up the following phosphorylation and annealing reaction:

- 1ul sense oligo (100uM)
- 1ul anti-sense oligo (100uM)
- 1ul 10X T4 Ligation Buffer (NEB)
- 6.5ul ddH2O
- 0.5ul T4 PNK (NEB)

Incubate and anneal in a thermocycler:

- 37°C 30min
- 95°C 5min
- ramp down to 25°C at 5°C/min

Set up the following ligation reaction:

- Xul BbsI digested pCFD3 (use 50ng)
- 1ul annealed oligos diluted 1:200
- 1.5ul 10X T4 Ligation Buffer (NEB)
- Xul ddH2O
- 1ul T4 DNA ligase
- total volume 15ul

Ligate 30min at room temperature.

Transform into competent bacteria. Plate on Ampicillin plates.