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.