Cloning gRNA expression vectors with pC FD2

Oligo design for pC FD2:

Sense:  
5' – CTTCG-N19/20

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

The G at position 5 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 CTTCG. Note that in contrast to cloning with pC FD1 and pC FD3 you need to add a C to the 3’ end of the anti-sense oligo.

Resuspend oligos to 100uM.

Set up the following phosphorylation and annealing reaction:

1 ul sense oligo (100uM)  
1 ul anti-sense oligo (100uM)  
1 ul 10X T4 Ligation Buffer (NEB)  
6.5 ul ddH2O  
0.5 ul 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 pC FD3 (use 50ng)  
1 ul annealed oligos diluted 1:200  
1.5 ul 10X T4 Ligation Buffer (NEB)  
Xul  ddH2O  
1 ul T4 DNA ligase
total volume 15ul

Ligate 30min at room temperature.

Transform into competent bacteria. Plate on Ampicillin plates.