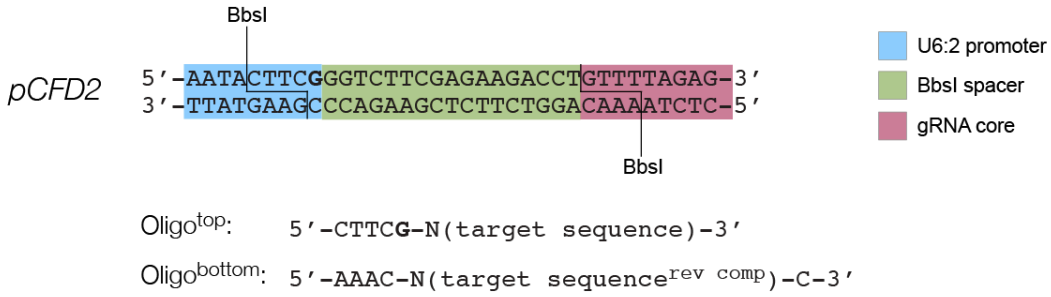
Cloning gRNA expression vectors with pCFD2

Oligo design for pCFD2:



Sense: 5’ – CTTC**G**-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 pCFD1 and pCFD3 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:

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:

37ºC	30min
95ºC	5min
ramp down to 25ºC at 5ºC/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.