New CRISPR Protocol

* Digest vector with BbsI (do NOT treat with CIP)
* Design oligo as follows:
  + 5’-CACC(G)-20nt (sense)
  + (C)-20nt-CAAA-5’ (antisense)

*(G): only add (G) if following sequence does not start with G*

*(C): only add (C) if forward primer had (G) added*

* *Annealing of oligos* (10µm stocks):
  + - 2 µL Sense
    - 2 µL Antisense
    - 20 µL 10x Annealing Buffer (e.g. NEB Buffer 3)
    - 176 µL H2O
    - Total: 200 µL in PCR tubes
  + Heat: 95˚C for 2 minutes
  + Allow to cool to room temperature
* Annealed Oligo Phosphorylation
  + 5.8 µL annealed oligo
  + 0.6 µL T4 PNK buffer 10x/T4 ligase buffer
  + 0.6 µL T4 PNK
    - 37˚C 30 minutes incubation
    - 50˚C 20 minutes inactivation
  + Total: 7 µL for ligation
* Ligation
  + 7 µL annealed phosphorylated oligo
  + 1 µL vector
  + 1 µL T4 10x buffer
  + 1 µL T4 DNA ligase
  + Total: 10µL