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