Cloning gRNA into Lentiviral Vectors

Annealing:

23µL ddH$_2$O
1µL 100uM top oligo
1µL 100uM bottom oligo
25µL 2X Annealing Buffer (recipe below)
50µL total reaction volume

Incubate at 95°C for 5min. Let oligos gradually anneal while cooling to RT.
Make a 1:40 dilution of annealed oligos in ddH$_2$O.
Annealed oligos can be stored at -20°C.

Ligation: (can be scaled back for high throughput purposes)

10ng digested vector backbone (digested with BstXI and BlpI)
1µL 1:40 diluted annealed oligo
1µL fresh 10X T4 ligase buffer
1µL T4 ligase (could probably use less especially in master mix)
xx µL ddH$_2$O
10µL total reaction volume

Incubate at RT for 1-4hrs.
Transform into 2.5 µL DH5α bacteria

2X Annealing Buffer:
200mM Potassium acetate
60mM HEPES-KOH pH 7.4
4mM Magnesium acetate

gRNA seq: NNNNNNNNN.....N (N20)

Ligates into 5'BstXI-BlpI3' digested backbone

	5' end	gRNA seq	3' end	Full Sequence
5' forward:	TTG	NNNNNNNNN.....N (N20)	GTTTAAGAGC	TTGNNNNNNNNN.....N (N20)GTTTAAGAGC
3' reverse (not reverse complemented):	CTTGTTG	NNNNNNNNN.....N (N20)	GTTTAAGAGCTAA	CTTGTTGNNNNNNNNN.....N (N20)GTTTAAGAGCTAA

Order 5' forward and reverse compliment of 3' reverse above

Last updated: 4/10/17 by Max Horlbeck, Weissman Lab