Cloning sgRNAs into Lentiviral Vectors

This protocol works for low throughput or medium throughput cloning of sgRNAs 1-96 sgRNAs. If you are cloning many sgRNAs buy your oligos in pre-suspended at 100uM in a plate in an arrayed format that is easy to combine top and bottom oligo by multichannel. For higher throughput we keep everything in PCR plates throughout the protocol until you plate the bacteria.

Annealing:

23µL ddH_2O
1µL 100uM top oligo
1µL 100uM bottom oligo
25µL 2X Annealing Buffer
50µL total reaction volume

Incubate at 95°C for 5min in a PCR machine. Let oligos gradually anneal while cooling to RT (take the plate out and set it on your bench- don't use the default cooling ramp rate on a PCR machine to cool the plate)
Make a 1:20 dilution of annealed oligos in ddH_2O.
Annealed oligos can be stored a -20°C and are stable through at least 2-3 freeze thaws.

Ligation:
100ng digested vector backbone (digested with BstXI and BlpI)
2µL 1:20 diluted annealed oligo
2µL fresh 10X T4 ligase buffer*
1µL T4 ligase
xx µL ddH_2O
20µL total reaction volume

Incubate at RT for 1-4hrs or 16C over night. Or use quick ligase from NEB according to manufacturers protocol with the same amount of input vector and insert.

The amount of vector in this ligation can be scaled back to 5-10ng for high throughput purposes. You can also use 0.2-0.5uL of ligase to save money.

*10X T4 ligase buffer that has been freeze thawed too many times will not work

Transform to DH5α bacteria:

Pre-warm LB agar + amp or carb plate(s) at 37°C.
Combine 20µL cells + 1µL ligation; incubate 30min on ice.

Heat shock cells at 42°C for 45 sec (this can be done in tubes with a water bath or a PCR machine with 96 well plates- prewarm the PCR block to 42C and then set the plate in).
Put immediately back on ice for ~1 min.

For low throughput cloning

add 100µL LB to transformation and recover at 37°C shaking for 1hr.
Plate 50µL of the transformation
Incubate at 37°C overnight.

For higher throughput cloning

Immediately plate all 20uL of transformed bacteria onto a spot on a partitioned plate and streak off the spot to single cell dilution. I use as little as 4uL of bacteria to save money.

Pick 1 colony per sgRNA construct and miniprep (use Zymo Research Zyppy-96 (D4041) for 96 well plates minipreps format). Or use standard mini preps to prep plasmid DNA.

Sanger sequence

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