sgRNA Cloning Protocol:

1. Anneal the two oligonucleotides together.
   a. Resuspend the oligonucleotides to a final concentration of 100 μM.
   b. Mix the following:

Reagent	Vol.
100 μM Forward Oligo	4.5 uL
100 μM Reverse Oligo	4.5 uL
10X NEB Buffer 2	1 uL
Total	10 uL

   c. Boil 1L of water and then insert the tube containing the above mixture. Boil the mixture for 4 minutes then turn off the heat.
   d. Allow the water to cool very slowly to room temperature (i.e. leave it on your bench at room temperature for several hours).
2. Digest the MLM3636 (Addgene #43860) plasmid with BsmBI. Gel purify the vector backbone for cloning.
3. Dilute the annealed sgRNA 1:100 and use 1 uL of the dilution for each 50 ng of vector. This produces an approximately 3:1 sgRNA:plasmid molar ratio when ligating.
4. Ligate with NEB Quick Ligase.
5. Transform into NEB 10β competent cells.
6. Isolate the plasmid and confirm correct cloning by Sanger sequencing. We used the recommended MLM3636 sequencing primer: 5' – CAGGGTTATTGTCTCATGAGCGG – 3'. For Sanger sequencing, we submit our samples to GeneWiz.