

sgRNA Oligo Annealing and Ligation

- Order forward and reverse oligos for your sgRNA whose annealing will result in appropriate sticky ends:



- Forward oligo: CACCGNNNNNNNNNNNNNNNNNNNN
 - Reverse oligo: AACNNNNNNNNNNNNNNNNNNNNNC
- The oligos can then be annealed together:
 - Set up annealing:
 - 1 μL forward oligo (100 μM)
 - 1 μL reverse oligo (100 μM)
 - 1 μL 10x T4 Ligation buffer
 - 7 μL ddH₂O
 - Run annealing program using thermocycler:
 - 37°C for 30 min
 - 95°C for 5 min
 - Ramp down at 0.1°C/s from 95°C to 25°C
- Digest the pLX-sgRNA-BfuAI-2k vector
 - Set up BfuAI digestion:
 - 1 μg pLX-sgRNA-BfuAI-2k
 - 5 μL Buffer 3.1 (NEB)
 - 2 μL BfuAI (NEB)
 - ddH₂O to 50 μL
 - Incubate at 50°C
- Purify the fully digested plasmid
 - Run out digestion product on gel
 - There should be two bands: 7453 bp and 1935 bp
 - **Purify the larger 7453 bp band**, not the 1935 bp stuffer band
- The annealed oligos can then be ligated into the plasmid:
 - Dilute annealed oligos 1:200 in ddH₂O
 - Ligation set up:
 - x μL purified vector (50 ng total)
 - 1 μL diluted oligos
 - 1 μL 10x T4 Ligation buffer
 - 1 μL T4 DNA Ligase
 - ddH₂O to 10 μL
 - Incubate O/N at 16°C
- The ligation product can then be transformed into an appropriate bacterial strain
 - pLX-sgRNA-BfuAI-2k has successfully transformed into the StbI3 strain
 - StbI3 will limit recombination between LTRs in lentiviral vectors