Library Verification

Primer Sequences
NI-956: 5’-GATCGGAAGAGCGTAGCTCGGTGAGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATTT
NI-1032: 5’-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTATGCGGCCTCCAG
NI-798: 5’-AATGATACGGCGACCGAGATCTACACTCTTTCCCTACACGACGCTC
NI-826: 5’-CAAGCAGAAGACGGCATACGATATTGGCGTGACTGGAGTTCAGACGACGCTG

1. Prepare first-round PCR reaction with 25. µl 2x Q5 High-Fidelity Master Mix (NEB M0492S)
   a. Primers are NI-956 and NI-1032, 2.5 µl each at 10 µM
   b. Template is 100 ng plasmid library
   c. Add ultra-pure water to a final volume of 50 µl
   d. PCR conditions are
      i. 30 s initial denaturation at 98 ºC
      ii. 12 cycles of
         10 s denaturation at 98 ºC
         15 s annealing at 65 ºC
         10 s extension at 72 ºC
      iii. 2 min final extension at 72 ºC
2. Purify PCR amplicon using 100 µl (2.0 volumes) AMpure XP beads (A63880)
3. Prepare second-round PCR reaction with 25. µl 2x Q5 High-Fidelity Master Mix
   a. Primers are NI-798 and NI-826, 2.5 µl each at 10 µM
      Note: These are Illumina TruSeq universal forward and indexed reverse primers
   b. Template is 1.0 µl purified first-round PCR amplicon
   c. Add ultra-pure water to a final volume of 50 µl
   d. PCR conditions are
      i. 30 s initial denaturation at 98 ºC
      ii. 12 cycles of
         5 s denaturation at 98 ºC
         10 s annealing at 65 ºC
         10 s extension at 72 ºC
      iii. 2 min final extension at 72 ºC
4. Purify PCR amplicon using 100 µl (2.0 volumes) AMpure XP beads