Library Verification

Primer Sequences
NI-956: 5′-GATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
NI-1032: 5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTATGCGGCCGGTACCCAG
NI-798: 5′-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTC
NI-826: 5′-CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTG

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