PCR amplification protocol for deep sequencing verification:

The following PCR amplification scheme was used to obtain library amplicons for next-generation sequencing. Amplicons were then extracted on 1.2% agarose gels using a standard gel extraction kit from Qiagen:

**Mode #1 vector**

|  |
| --- |
| F primer: AATCGGATCTGGAAGTTCTGTTCC |
| R primer: ACAGCCAAGAGCTCTTAATGATG |

PCR protocol:

1uL purified library vector

10uL 5x Q5 buffer

2.5uL 10uM F primer

2.5uL 10uM R primer

1uL 10mM dNTPs

32.5uL water

0.5uL Q5 polymerase

35 cycles, 61C annealing and 45s extension.

**Mode #2 vectors**

|  |
| --- |
| F primer: TCTGGGTCGACTGGTGGTACC |
| R primer: CGTACCATGTAGCTTAATCAGCTGTTAAAGCTT |

1uL purified library vector

10uL 5x Q5 buffer

2.5uL 10uM F primer

2.5uL 10uM R primer

1uL 10mM dNTPs

32.5uL water

0.5uL Q5 polymerase

Did 35 cycles, 65C annealing and 45s extension.

*Note: we have also prepared samples for NGS by performing maxi-prep of plasmid libraries, performing KpnI/HindIII digests, and sequencing the gel-extracted phosphosite DNA insert library. Also, staggered and/or randomized base pairs may be added to primer ends to facilitate Illumina sequencing and multiplexing.*