

Quadruple-Indexed 3RAD Libraries

ACGACGCTCTCCGATCTCCGAATGCTAGXXXXXXXXXXXXAATTACGTTAGAGATCGGAAGAGCACACGTAAATCC
 TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGAGGCTTACGATCXXXXXXXXXXXXTTAATGCAATCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
 P

Ligated product

First cycle

ACGACGCTCTCCGATCTCCGAATGCTAGXXXXXXXXXXXXAATTACGTTAGAGATCGGAAGAGCACACGTAAATCC
 AATGATACGGCGACCACCGAGATCTACACACCGACAAACACTCTTTCCCTAC →
 TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGAGGCTTACGATCXXXXXXXXXXXXTTAATGCAATCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
 P

AATGATACGGCGACCACCGAGATCTACACACCGACAAACACTCTTTCCCTACACGACGCTCTCCGATCTCCGAATGCTAGXXXXXXXXXXXXAATTACGTTAGAGATCGGAAGAGCACACGTCTGAATCCAGTCAC
 TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGAGGCTTACGATCXXXXXXXXXXXXTTAATGCAATCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
 1st PCR product

Second cycle

AATGATACGGCGACCACCGAGATCTACACACCGACAAACACTCTTTCCCTACACGACGCTCTCCGATCTCCGAATGCTAGXXXXXXXXXXXXAATTACGTTAGAGATCGGAAGAGCACACGTCTGAATCCAGTCAC
 GACTTGAGGTCAGTG ← TCCAGTGA TAGAGCATA CGGCAGAAGACGAAC
 iTru5 primer → AATGATACGGCGACCACCGAGATCTACACACCGACAAACACTCTTTCCCTAC
 TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGAGGCTTACGATCXXXXXXXXXXXXTTAATGCAATCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
 iTru7 primer

AATGATACGGCGACCACCGAGATCTACACACCGACAAACACTCTTTCCCTACACGACGCTCTCCGATCTCCGAATGCTAGXXXXXXXXXXXXAATTACGTTAGAGATCGGAAGAGCACACGTCTGAATCCAGTCAC
 TTACTATGCCGCTGGTGGCTTAGATGTGGCTGTTGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGAGGCTTACGATCXXXXXXXXXXXXTTAATGCAATCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGTCCAGTGA TAGAGCATA CGGCAGAAGACGAAC
 2nd PCR product

Third cycle

AATGATACGGCGACCACCGAGATCTACACACCGACAAACACTCTTTCCCTACACGACGCTCTCCGATCTCCGAATGCTAGXXXXXXXXXXXXAATTACGTTAGAGATCGGAAGAGCACACGTCTGAATCCAGTCAC
 GACTTGAGGTCAGTG ← TCCAGTGA TAGAGCATA CGGCAGAAGACGAAC
 iTru5 primer → AATGATACGGCGACCACCGAGATCTACACACCGACAAACACTCTTTCCCTAC
 TTACTATGCCGCTGGTGGCTTAGATGTGGCTGTTGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGAGGCTTACGATCXXXXXXXXXXXXTTAATGCAATCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGTCCAGTGA TAGAGCATA CGGCAGAAGACGAAC
 iTru7 primer

AATGATACGGCGACCACCGAGATCTACACACCGACAAACACTCTTTCCCTACACGACGCTCTCCGATCTCCGAATGCTAGXXXXXXXXXXXXAATTACGTTAGAGATCGGAAGAGCACACGTCTGAATCCAGTCACAGTCACTTCTCGTATGCCGCTTCTGCTTG
 TTACTATGCCGCTGGTGGCTTAGATGTGGCTGTTGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGAGGCTTACGATCXXXXXXXXXXXXTTAATGCAATCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGTCCAGTGA TAGAGCATA CGGCAGAAGACGAAC

Full length 3RAD PCR product