Primers for 1st Step:

Forward primers

P104 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNCGATGTTTAATATGGACTAAAGGAGGCTTTT

P105 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNACAGTGTTAATATGGACTAAAGGAGGCTTTT

P111 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNTGACCATTAATATGGACTAAAGGAGGCTTTT

P112 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGCCAATTTAATATGGACTAAAGGAGGCTTTT

P122 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNATCACGTTAATATGGACTAAAGGAGGCTTTT

P124 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGGCTACTTAATATGGACTAAAGGAGGCTTTT

P125 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNTAGCTTTTAATATGGACTAAAGGAGGCTTTT

P130 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNTCTCCCTTAATATGGACTAAAGGAGGCTTTT

P131 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGACGTCTTAATATGGACTAAAGGAGGCTTTT

P132 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGAGGTTTTAATATGGACTAAAGGAGGCTTTT

P135 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGTTAACTTAATATGGACTAAAGGAGGCTTTT

P137 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNAACCACTTAATATGGACTAAAGGAGGCTTTT

Two mismatches between P132 and P125.

Two mismatches between P132 and P131.

P123 (CAGATC) is removed, because it seems a bad primer from PCR result.

Second Generation Forward primers

 P201 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGCTTGATTAATATGGACTAAAGGAGGCTTTT

 P202 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNCAAGAATTAATATGGACTAAAGGAGGCTTTT

 P203 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNATGCGATTAATATGGACTAAAGGAGGCTTTT

 P204 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNCATAGGTTAATATGGACTAAAGGAGGCTTTT

 P205 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNTTTTTGTTAATATGGACTAAAGGAGGCTTTT

 P206 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNTGGAAGTTAATATGGACTAAAGGAGGCTTTT

 P207 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNCCGGGCTTAATATGGACTAAAGGAGGCTTTT

 P208 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNCTACTCTTAATATGGACTAAAGGAGGCTTTT

 P209 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNAGTGATTTAATATGGACTAAAGGAGGCTTTT

 P210 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNTTCGGTTTAATATGGACTAAAGGAGGCTTTT

 P211 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGAATCGTTAATATGGACTAAAGGAGGCTTTT

 P212 ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNCGCATATTAATATGGACTAAAGGAGGCTTTT

Reverse Primers

P101 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNTATATACGCTCGAATTCAAGCTTAGATCTGATA

P108 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCGCTCTATCTCGAATTCAAGCTTAGATCTGATA

P109 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGAGACGTCTTCGAATTCAAGCTTAGATCTGATA

P110 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNATACTGCGTTCGAATTCAAGCTTAGATCTGATA

P126 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNACTAGCAGATCGAATTCAAGCTTAGATCTGATA

P127 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNTGAGCTAGCTCGAATTCAAGCTTAGATCTGATA

P128 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCTGCTACTCTCGAATTCAAGCTTAGATCTGATA

P129 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGCGTACGCATCGAATTCAAGCTTAGATCTGATA

P133 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCCTCTCCGCTCGAATTCAAGCTTAGATCTGATA

P134 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNTTAATCCTCTCGAATTCAAGCTTAGATCTGATA

P136 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNATCCGAGTATCGAATTCAAGCTTAGATCTGATA

P138 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCCGTACACATCGAATTCAAGCTTAGATCTGATA

Two mismatches between P129 and P138.

Second Generation Reverse Primers

P213 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCTTCTCAAATCGAATTCAAGCTTAGATCTGATA

P214 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNAGGGTTTATTCGAATTCAAGCTTAGATCTGATA

P215 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGGCGTCCGTTCGAATTCAAGCTTAGATCTGATA

P216 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGTATTGTAATCGAATTCAAGCTTAGATCTGATA

P217 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGCGCGCTCTTCGAATTCAAGCTTAGATCTGATA

P218 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGCACTCTTCTCGAATTCAAGCTTAGATCTGATA

P219 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNTGTTTGAATTCGAATTCAAGCTTAGATCTGATA

P220 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCCTGCACACTCGAATTCAAGCTTAGATCTGATA

P221 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGATCTGCAATCGAATTCAAGCTTAGATCTGATA

P222 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGCTTAAGGCTCGAATTCAAGCTTAGATCTGATA

P223 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCAATGTTGATCGAATTCAAGCTTAGATCTGATA P224 CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCACCGACAGTCGAATTCAAGCTTAGATCTGATA

Primers for 2nd step PCR:

PE2 AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCxT

PE1 CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCxT

Expected Sequencing Result:

NNNNNNNNAACCACTTAATATGGACTAAAGGAGGCTTTTGTCGACGGATCCGATATCGGTACCNNNNNAANNNNNTTNNNNNTTNNNNNATAACTTCGTATAATGTATGCTATACGAAGTTATTGCGCGGTGATCACTTATGGtaccgTTCGTATAATGTgTaCTATACGAAGTTATTCCTAAAACTATTTCTTCTTTCACCTGGTACCGATATCAGATCTAAGCTTGAATTCGATGTGTACGGNNNNNNNN

ForwardPrimer-Kpnl-BC1s-LoxP-Bcll-Lox5171/71-BC-Kpnl-ForwardPrimer

NNNNNNNNAACCACTTAATATGGACTAAAGGAGGCTTTTGTCGACGGATCCGATATCGGTACCNNNNNAANNNNNTTNNNNNTTNNNNNATAACTTCGTATAATGTATGCTATACGAAGTTATTGCGCGGTGATCACTTATGGtaccgTTCGTATAATGTgTaCTATACGAAcggtaNNNNNAANNNNNTTNNNNNTTNNNNNGGTACCGATATCAGATCTAAGCTTGAATTCGATGTGTACGGNNNNNNNN

ForwardPrimer-Kpnl-BC1s-LoxP-Bcll-Lox5171/66/71-BC2s-Kpnl-ForwardPrimer