|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Oligo name** | **Sequence (5’ to 3’)** | **Index Identifier** | **Index Sequence** | **Notes** |
| **Barcoding cloning** | |  |  |  |
| Random Barcodes U1 — N20 — U2 | GATGTCCACGAGGTCTCTNNNNNNNNNNNNNNNNNNNNCGTACGCTGCAGGTCGAC |  |  | Random barcodes flanked by universal primer sites |
| FW\_BbsI\_JC | TCACACAAGTTTGTACAAAAAAGCAGGCTGGAGCTCGgaagacATCCCTGATGTCCACGAGGTCTCT |  |  | Forward primer to amplify barcodes for cloning into vector |
| REV\_BbsI\_JC | CTCAACCACTTTGTACAAGAAAGCTGGGTGGATCCgaagacCGCGTTGTCGACCTGCAGCGTACG |  |  | Reverse primer for to amplify barcodes for cloning into vector |
| **Tn-Seq** | |  |  |  |
| JC8 Tn-specific FW Primer | ATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNCCCTGATGTCCACGAGGTCTCT |  |  | Forward primer to sequence transposon insertions; homologous to transposon (Figure 1A, left) |
| P7\_MOD\_TS\_index1 | CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT | Index1 | ATCACG | Indexed reverse primer to sequence transposon insertions; homologous to adapter (Figure 1A, left) |
| P7\_MOD\_TS\_index2 | CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT | Index2 | CGATGT | Indexed reverse primer to sequence transposon insertions; homologous to adapter (Figure 1A, left) |
| P7\_MOD\_TS\_index3 | CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT | Index3 | TTAGGC | Indexed reverse primer to sequence transposon insertions; homologous to adapter (Figure 1A, left) |
| P7\_MOD\_TS\_index4 | CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT | Index4 | TGACCA | Indexed reverse primer to sequence transposon insertions; homologous to adapter (Figure 1A, left) |
| P7\_MOD\_TS\_index5 | CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT | Index5 | ACAGTG | Indexed reverse primer to sequence transposon insertions; homologous to adapter (Figure 1A, left) |
| P7\_MOD\_TS\_index6 | CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT | Index6 | GCCAAT | Indexed reverse primer to sequence transposon insertions; homologous to adapter (Figure 1A, left) |
| P7\_MOD\_TS\_index7 | CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT | Index7 | CAGATC | Indexed reverse primer to sequence transposon insertions; homologous to adapter (Figure 1A, left) |
| P7\_MOD\_TS\_index8 | CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT | Index8 | ACTTGA | Indexed reverse primer to sequence transposon insertions; homologous to adapter (Figure 1A, left) |
| **Bar-seq** | |  |  |  |
| P1\_BS3\_IT001 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNGCACTAGTCGACCTGCAGCGTACG | IT001 | ATCACG | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT002 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNTGTAGCGTCGACCTGCAGCGTACG | IT002 | CGATGT | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT003 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNCGGATTGTCGACCTGCAGCGTACG | IT003 | TTAGGC | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT004 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACCAGTGTCGACCTGCAGCGTACG | IT004 | TGACCA | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT005 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNGTGACAGTCGACCTGCAGCGTACG | IT005 | ACAGTG | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT006 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNTAACCGGTCGACCTGCAGCGTACG | IT006 | GCCAAT | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT007 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNCTAGACGTCGACCTGCAGCGTACG | IT007 | CAGATC | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT008 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGTTCAGTCGACCTGCAGCGTACG | IT008 | ACTTGA | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT009 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNGACTAGGTCGACCTGCAGCGTACG | IT009 | GATCAG | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT010 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNTTCGATGTCGACCTGCAGCGTACG | IT010 | TAGCTT | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT011 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNCATCGGGTCGACCTGCAGCGTACG | IT011 | GGCTAC | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT012 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATGTTCGTCGACCTGCAGCGTACG | IT012 | CTTGTA | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT013 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNAACTGAGTCGACCTGCAGCGTACG | IT013 | AGTCAA | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT014 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNCCTTGAGTCGACCTGCAGCGTACG | IT014 | AGTTCC | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT015 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNACTGTAGTCGACCTGCAGCGTACG | IT015 | ATGTCA | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT016 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCTGCCGTCGACCTGCAGCGTACG | IT016 | CCGTCC | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT017 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNGAGATGGTCGACCTGCAGCGTACG | IT017 | GTAGAG | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT018 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNCGCCTGGTCGACCTGCAGCGTACG | IT018 | GTCCGC | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT019 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNAAAGTGGTCGACCTGCAGCGTACG | IT019 | GTGAAA | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT020 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCGGTGGTCGACCTGCAGCGTACG | IT020 | GTGGCC | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT021 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNGCTTTGGTCGACCTGCAGCGTACG | IT021 | GTTTCG | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT022 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNGCATGCGTCGACCTGCAGCGTACG | IT022 | CGTACG | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT023 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNGGTGAGGTCGACCTGCAGCGTACG | IT023 | GAGTGG | Indexed forward Bar-seq primer (Figure 1A, right) |
| P1\_BS3\_IT024 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCGATGGGTCGACCTGCAGCGTACG | IT024 | GGTAGC | Indexed forward Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT001 | CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT001 | ATCACG | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT002 | CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT002 | CGATGT | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT003 | CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT003 | TTAGGC | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT004 | CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT004 | TGACCA | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT005 | CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT005 | ACAGTG | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT006 | CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT006 | GCCAAT | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT007 | CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT007 | CAGATC | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT008 | CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT008 | ACTTGA | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT009 | CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT009 | GATCAG | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT010 | CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT010 | TAGCTT | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT011 | CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT011 | GGCTAC | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT012 | CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT012 | CTTGTA | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT013 | CAAGCAGAAGACGGCATACGAGATTTGACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT013 | AGTCAA | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT014 | CAAGCAGAAGACGGCATACGAGATGGAACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT014 | AGTTCC | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT015 | CAAGCAGAAGACGGCATACGAGATTGACATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT015 | ATGTCA | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT016 | CAAGCAGAAGACGGCATACGAGATGGACGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT016 | CCGTCC | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT017 | CAAGCAGAAGACGGCATACGAGATCTCTACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT017 | GTAGAG | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT018 | CAAGCAGAAGACGGCATACGAGATGCGGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT018 | GTCCGC | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT019 | CAAGCAGAAGACGGCATACGAGATTTTCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT019 | GTGAAA | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT020 | CAAGCAGAAGACGGCATACGAGATGGCCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT020 | GTGGCC | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT021 | CAAGCAGAAGACGGCATACGAGATCGAAACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT021 | GTTTCG | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT022 | CAAGCAGAAGACGGCATACGAGATCGTACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT022 | CGTACG | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT023 | CAAGCAGAAGACGGCATACGAGATCCACTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT023 | GAGTGG | Indexed reverse Bar-seq primer (Figure 1A, right) |
| P2\_BS3\_IT024 | CAAGCAGAAGACGGCATACGAGATGCTACCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | IT024 | GGTAGC | Indexed reverse Bar-seq primer (Figure 1A, right) |

**Supplementary Table 2. Oligonucleotides used in this study.**