**Supplementary file 3.** Table of the oligonucleotides/primers used in this study.

| Primer Name | Primer Sequence | Primer Function | Source |
| --- | --- | --- | --- |
| Short\_Biotin\_pHIMAR | (Biot)-CGCCCTGCAGGGATGTCCACGAG | Biotinylated forward primer for amplify Tn-specific sequences during TnSeq | This work |
| NC102 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC | Illumina I7 specific reverse primer for use with Short\_Biotin\_pHIMAR for amplifying Tn-specific sequences during TnSeq | This work |
| Nspacer\_barseq\_pHIMAR | ATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNCGCCCTGCAGGGATGTCCACGAG | Tn specific primer containing Illumina adaptors I5 and P5 as primer extensions. | Wetmore et al., 2015 |
| NEBNext Index 3 Primer for Illumina | CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC- | Illumina I7 specific reverse primer containing an index sequence and an I7 adaptor. | New England Biolabs |
| BarSeq\_R\_P2\_UMI\_Univ | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNGTCGACCTGCAGCGTACG | Barcode specific reverse phasing primer 1, used as a pool with the other phasing primers during BarSeq. Adds Illumina I5 and P5 adaptors. | This work |
| BarSeq\_R\_P2\_UMI\_Univ\_N2 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNGTCGACCTGCAGCGTACG | Barcode specific reverse phasing primer 2, used as a pool with the other phasing primers during BarSeq. Adds Illumina I5 and P5 adaptors. | This work |
| BarSeq\_R\_P2\_UMI\_Univ\_N3 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNGTCGACCTGCAGCGTACG | Barcode specific reverse phasing primer 3, used as a pool with the other phasing primers using BarSeq. Adds Illumina I5 and P5 adaptors. | This work |
| BarSeq\_R\_P2\_UMI\_Univ\_N4 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTCGACCTGCAGCGTACG | Barcode specific reverse phasing primer 4, used as a pool with the other phasing primers during BarSeq. Adds Illumina I5 and P5 adaptors. | This work |
| BarSeq\_R\_P2\_UMI\_Univ\_N5 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNGTCGACCTGCAGCGTACG | Barcode specific reverse phasing primer 5, used as a pool with the other phasing primers during BarSeq. Adds Illumina I5 and P5 adaptors. | This work |
| BarSeq\_F\_i7\_001 | CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_002 | CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTCCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_003 | CAAGCAGAAGACGGCATACGAGATGCCTAAGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_004 | CAAGCAGAAGACGGCATACGAGATTGGTCAGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_005 | CAAGCAGAAGACGGCATACGAGATCACTGTGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_006 | CAAGCAGAAGACGGCATACGAGATATTGGCGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_007 | CAAGCAGAAGACGGCATACGAGATGATCTGGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_008 | CAAGCAGAAGACGGCATACGAGATTCAAGTGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_009 | CAAGCAGAAGACGGCATACGAGATCTGATCGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_010 | CAAGCAGAAGACGGCATACGAGATAAGCTAGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_011 | CAAGCAGAAGACGGCATACGAGATGTAGCCGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_012 | CAAGCAGAAGACGGCATACGAGATTACAAGGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_013 | CAAGCAGAAGACGGCATACGAGATTTGACTGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_014 | CAAGCAGAAGACGGCATACGAGATGGAACTGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_015 | CAAGCAGAAGACGGCATACGAGATTGACATGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_016 | CAAGCAGAAGACGGCATACGAGATGGACGGGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_017 | CAAGCAGAAGACGGCATACGAGATCTCTACGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_018 | CAAGCAGAAGACGGCATACGAGATGCGGACGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_019 | CAAGCAGAAGACGGCATACGAGATTTTCACGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_020 | CAAGCAGAAGACGGCATACGAGATGGCCACGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_021 | CAAGCAGAAGACGGCATACGAGATCGAAACGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_022 | CAAGCAGAAGACGGCATACGAGATCGTACGGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_023 | CAAGCAGAAGACGGCATACGAGATCCACTCGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_024 | CAAGCAGAAGACGGCATACGAGATGCTACCGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_025 | CAAGCAGAAGACGGCATACGAGATATCAGTGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_026 | CAAGCAGAAGACGGCATACGAGATGCTCATGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_027 | CAAGCAGAAGACGGCATACGAGATAGGAATGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_028 | CAAGCAGAAGACGGCATACGAGATCTTTTGGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_029 | CAAGCAGAAGACGGCATACGAGATTAGTTGGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_030 | CAAGCAGAAGACGGCATACGAGATCCGGTGGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_031 | CAAGCAGAAGACGGCATACGAGATATCGTGGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_032 | CAAGCAGAAGACGGCATACGAGATTGAGTGGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_033 | CAAGCAGAAGACGGCATACGAGATCGCCTGGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_034 | CAAGCAGAAGACGGCATACGAGATGCCATGGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_035 | CAAGCAGAAGACGGCATACGAGATAAAATGGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTC CACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |
| BarSeq\_F\_i7\_036 | CAAGCAGAAGACGGCATACGAGATTGTTGGGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGT CCACGAGGTCTCT | Barcode specific forward indexing primer, used during BarSeq. Adds Illumina I7 and P7 adaptors. | This work |