**Submitting Barcodes to HTSF**

*If you are submitting prepared libraries or pools of prepared libraries, please read these instructions for listing your index (barcode) sequence.*

The HTSF sequences many different types of samples with various bioinformatics pipelines. In order to simplify processing, we ask that you always submit barcodes as if they are running on the HiSeq 2500, even if you are requesting a different sequencing platform. We will internally modify them, if needed, for your specific workflow. Please also submit the full length index sequence.

For example, follow Illumina’s guidelines for entering the i7 index. If sequencing a dual-indexed library, list the i7 index as index 1.

 

If sequencing a dual-indexed library, list the i5 index as index 2. Do NOT use Illumina’s guidelines for HiSeq 3000/4000 runs. Submit barcodes as if you are running on HiSeq 2500 or MiSeq.



Please use the following reference tables to help you determine how to submit your barcode sequence. If you have used custom barcodes, or a library prep kit that is not listed here, please contact HTSF about how to submit your barcodes.

For single-indexed Illumina (TruSeq LT or ChIP kits) or Kapa Biosystems libraries:

|  |  |
| --- | --- |
| i7 Index Name | i7 Bases for Sample Sheet |
| Index 1 | ATCACGA |
| Index 2 | CGATGTA |
| Index 3 | TTAGGCA |
| Index 4 | TGACCAA |
| Index 5 | ACAGTGA |
| Index 6 | GCCAATA |
| Index 7 | CAGATCA |
| Index 8 | ACTTGAA |
| Index 9 | GATCAGA |
| Index 10 | TAGCTTA |
| Index 11 | GGCTACA |
| Index 12 | CTTGTAA |
| Index 13 | AGTCAAC |
| Index 14 | AGTTCCG |
| Index 15 | ATGTCAG |
| Index 16 | CCGTCCC |
| Index 18 | GTCCGCA |
| Index 19 | GTGAAAC |
| Index 20 | GTGGCCT |
| Index 21 | GTTTCGG |
| Index 22 | CGTACGT |
| Index 23 | GAGTGGA |
| Index 25 | ACTGATA |
| Index 27 | ATTCCTT |

For dual-indexed Illumina (TruSeq HT kits) or Kapa Biosystems libraries:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| i7 Index Name | i7 Bases for Sample Sheet |  | i5 Index Name | i5 Bases for Sample Sheet |
| D701 | ATTACTCG |  | D501 | TATAGCCT |
| D702 | TCCGGAGA |  | D502 | ATAGAGGC |
| D703 | CGCTCATT |  | D503 | CCTATCCT |
| D704 | GAGATTCC |  | D504 | GGCTCTGA |
| D705 | ATTCAGAA |  | D505 | AGGCGAAG |
| D706 | GAATTCGT |  | D506 | TAATCTTA |
| D707 | CTGAAGCT |  | D507 | CAGGACGT |
| D708 | TAATGCGC |  | D508 | GTACTGAC |
| D709 | CGGCTATG |  |  |  |
| D710 | TCCGCGAA |  |  |  |
| D711 | TCTCGCGC |  |  |  |
| D712 | AGCGATAG |  |  |  |

For Illumina TruSeq Small RNA or BIOO Scientific NEXTflex™ Small RNA libraries:

|  |  |
| --- | --- |
| i7 Index Name | i7 Bases for Sample Sheet |
| RPI1  | ATCACG |
| RPI2 | CGATGT |
| RPI3 | TTAGGC |
| RPI4 | TGACCA |
| RPI5 | ACAGTG |
| RPI6 | GCCAAT |
| RPI7 | CAGATC |
| RPI8 | ACTTGA |
| RPI9 | GATCAG |
| RPI10 | TAGCTT |
| RPI11 | GGCTAC |
| RPI12 | CTTGTA |
| RPI13 | AGTCAA |
| RPI14 | AGTTCC |
| RPI15 | ATGTCA |
| RPI16 | CCGTCC |
| RPI17 | GTAGAG |
| RPI18 | GTCCGC |
| RPI19 | GTGAAA |
| RPI20 | GTGGCC |
| RPI21 | GTTTCG |
| RPI22 | CGTACG |
| RPI23 | GAGTGG |
| RPI24 | GGTAGC |
| RPI25 | ACTGAT |
| RPI26 | ATGAGC |
| RPI27 | ATTCCT |
| RPI28 | CAAAAG |
| RPI29 | CAACTA |
| RPI30 | CACCGG |
| RPI31 | CACGAT |
| RPI32 | CACTCA |
| RPI33 | CAGGCG |
| RPI34 | CATGGC |
| RPI35 | CATTTT |
| RPI36 | CCAACA |
| RPI37 | CGGAAT |
| RPI38 | CTAGCT |
| RPI39 | CTATAC |
| RPI40 | CTCAGA |
| RPI41 | GACGAC |
| RPI42 | TAATCG |
| RPI43 | TACAGC |
| RPI44 | TATAAT |
| RPI45 | TCATTC |
| RPI46 | TCCCGA |
| RPI47 | TCGAAG |
| RPI48 | TCGGCA |

For Illumina Nextera libraries (Nextera DNA, Nextera XT, and Nextera Rapid Capture kits)\*:

The i5 index names vary for different Nextera products as follows:

• N50x—Nextera DNA

• S50x—Nextera XT

• E50x—Nextera Rapid Capture

\*Please note: The i5 index adapter [N/S/E]501 has been discontinued and replaced with [N/S/E]517. Be careful to note which index you have in your kit.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| i7 Index Name | i7 Bases for Sample Sheet |  | i5 Index Name | i5 Bases for Sample Sheet |
| N701 | TAAGGCGA |  | [N/S/E]501 | TAGATCGC |
| N702 | CGTACTAG |  | [N/S/E]502 | CTCTCTAT |
| N703 | AGGCAGAA |  | [N/S/E]503 | TATCCTCT |
| N704 | TCCTGAGC |  | [N/S/E]504 | AGAGTAGA |
| N705 | GGACTCCT |  | [N/S/E]505 | GTAAGGAG |
| N706 | TAGGCATG |  | [N/S/E]506 | ACTGCATA |
| N707 | CTCTCTAC |  | [N/S/E]507 | AAGGAGTA |
| N708 | CAGAGAGG |  | [N/S/E]508 | CTAAGCCT |
| N709 | GCTACGCT |  | [N/S/E]517 | GCGTAAGA |
| N710 | CGAGGCTG |  |  |  |
| N711 | AAGAGGCA |  |  |  |
| N712 | GTAGAGGA |  |  |  |

An Excel file with common Illumina barcodes is available for download here.

*Link to Illumina Barcode list 2017.xlsx*