

Barcode Submission Instructions

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.

Nextera XT Index Kit v2 - Index 1 (i7) Adapters

Bases in Adapter	i7 Index Name	i7 Bases for Entry on Sample Sheet
TCGCCCTA	N701	TAAGGCGA
CTAGTACG	N702	CGTACTAG
TTCTGCCT	N703	AGGCAGAA

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.

Nextera XT Index Kit v2 - Index 2 (i5) Adapters

Bases in Adapter	i5 Index Name	i5 Bases for Sample Sheet MiSeq, HiSeq 2000/2500	i5 Bases for Sample Sheet MiniSeq, NextSeq, HiSeq 3000/4000
CTCTCTAT	S502	CTCTCTAT	ATAGAGAG
TATCCTCT	S503	TATCCTCT	AGAGGATA
CTAAGGAG	S505	CTAAGGAG	CTCTTAC

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	CTTGTA
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	TCCGAGA		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	GTAAGTAC
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	CAAAAAG
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](#)