



BIO SCIENTIFIC
a PerkinElmer company

NEXTflex[®] Unique Dual Index Barcodes

(for Illumina[®] Platforms)

Catalog #514150

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NEXTflex® Unique Dual Index Barcodes - 514150

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Product Overview

The NEXTflex® Unique Dual Index Barcodes are designed to prepare multiplexed single and paired-end genomic DNA libraries for sequencing using Illumina® platforms. The index and flow cell binding sequences are contained within the NEXTflex® Unique Dual Index Barcodes and attached to the sample insert during adapter ligation. Sample pooling with Unique Dual Index Barcodes allows the user to multiplex up to 96 samples.

Uniquely dual-indexed libraries are libraries prepared with adapters containing two eight base indexes: Index 1 (P7 Index) adjacent to the P7 strand, and Index 2 (P5 Index) adjacent to the P5 strand. None of the indexes found on any given NEXTflex® Unique Dual Index Barcode are used throughout the entire set, which prevents mis-assigned reads from appearing in final data sets.

Each lot of the NEXTflex® Unique Dual Index Barcodes is functionally validated and tested for index purity by sequencing.

Contents, Storage and Shelf Life

The NEXTflex® Unique Dual Index Barcodes Kit contains 96 uniquely dual-indexed barcoded DNA adapters plated in a 96 well plate. See Appendix B for barcode plate configuration. It is recommended that plates are stored at -20°C. The shelf life of each reagent is six months when stored properly.

Kit Contents	Amount
NEXTflex® Unique Dual Index Barcodes 1 - 96* (25 µM)	5 µL
NEXTflex® Primer Mix (12.5 µM)	384 µL

*The Unique Dual Index Barcodes are supplied in duplex form. Do not heat the adapters above room temperature.

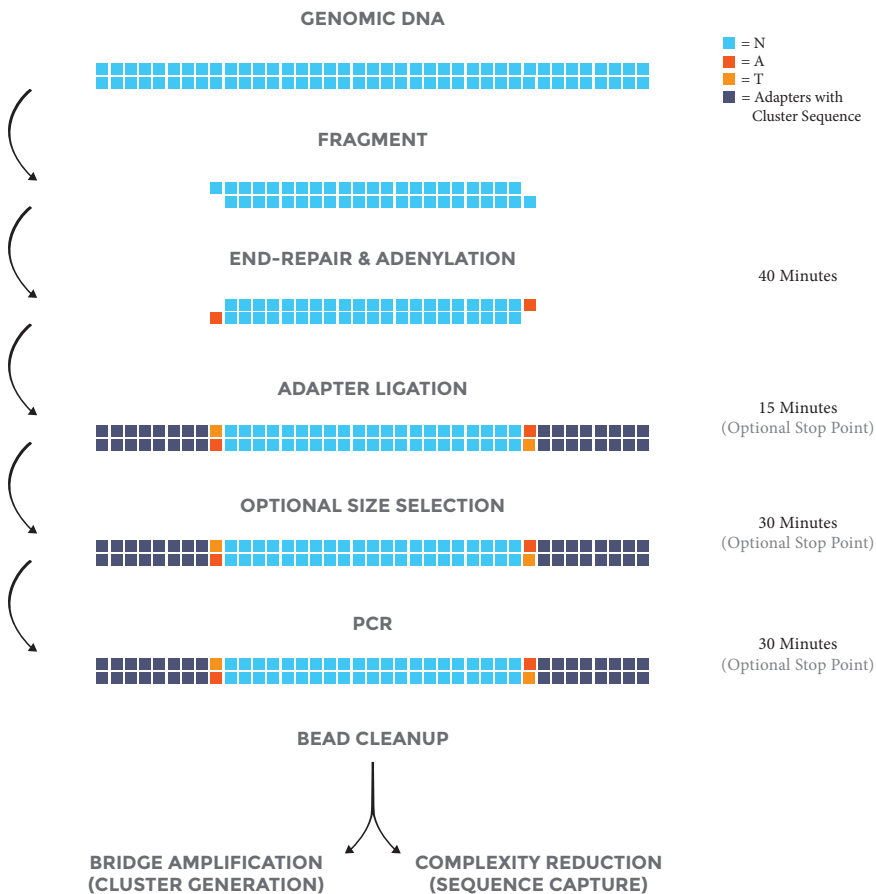
Warnings and Precautions

Bioo Scientific strongly recommends that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor, or contact Bioo Scientific at nextgen@biooscientific.com.

- Do not use the kit past the expiration date.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Do not heat the NEXTflex® Unique Dual Index Barcodes above room temperature.
- Once plate has thawed, spin for one minute before use. This is to ensure all liquid settles to the bottom of the plate.
- The plate seal is intended to be pierced. Do not peel the plate seal from the plate, doing so can easily lead to cross-contamination. Additional thermal heat seals may be applied upon one another to re-seal plate.
- Before use, carefully mix adapters by pipetting up and down several times using a multi-channel pipette with barrier tip. NEVER mix plates by vortexing. Placing a plate on a vortexer to mix samples or barcodes has been proven to result in cross-contamination, even if the plate appears to be securely sealed.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- The NEXTflex™ Primer Mix must be used during PCR amplification. Inadvertent use of an incorrect primer sequence can potentially result in elimination of the index.

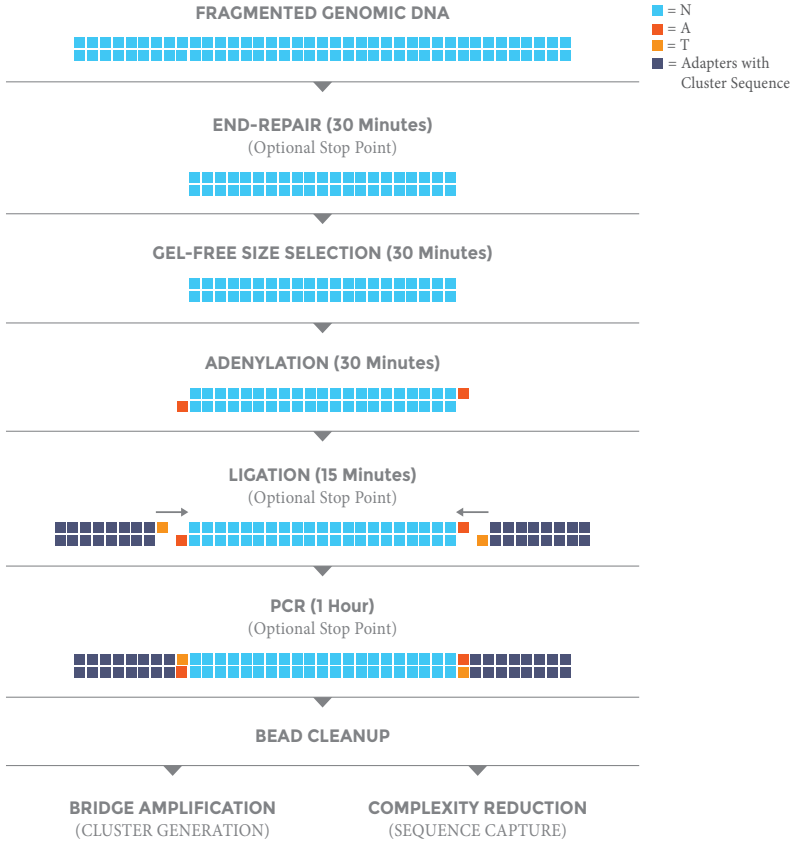
NEXTflex® Rapid DNA-Seq Sample Preparation Flow Chart

Figure 1: Sample flow chart with approximate times necessary for each step.



NEXTflex® DNA-Seq Sample Preparation Flow Chart

Figure 2: Sample flow chart with approximate times necessary for each step.



Oligonucleotide Sequences

NEXTflex®	Sequence (5' → 3')
PCR Primer 1	AATGATACGGCGACCACCGAGATCTACAC
PCR Primer 2	CAAGCAGAAGACGGCATACGAGAT
NEXTflex® Unique Dual Index Barcode	AATGATACGGCGACCACCGAGATCTACAC ¹ XXXXXXXX ¹ ACACTCTTCCCTACACGAGGCTCTCCGATCT GATCGGAAGAGCACACGTCTGAACTCCAGTCAC ² XXXXXXXX ² ATCTCGTATGCCGTCTTCTGCTTG

¹XXXXXXXX denotes the P5 index region of adapter. The index sequences contained in each adapter are listed below.

²XXXXXXXX denotes the P7 index region of the adapter. The index sequences contained in each adapter are listed below.

When entering index sequences for the Illumina® MiniSeq®, NextSeq®, HiSeq® 3000 or HiSeq® 4000 platforms, enter the P5 Index Reverse Complement. For all other Illumina® platforms, enter the P5 Index in the first column.

	P5 Index	P5 Index Reverse Complement	P7 Index
UDI 1	AATAACGT	ACGTTATT	AATCGTTA
UDI 2	TTCTTGAA	TTCAAGAA	GTCTACAT
UDI 3	GGCAGATC	GATCTGCC	CGCTGCTC
UDI 4	CTATGTTA	TAACATAG	GATCAACA
UDI 5	GTTGACGC	GCGTCAAC	CGAAGGAC
UDI 6	ATCTACGA	TCGTAGAT	GATGCCGG
UDI 7	CTCGACAG	CTGTCGAG	CTACGAAG
UDI 8	GAGGCTGC	GCAGCTC	GATGCGTC
UDI 9	CCTCGTAG	CTACGAGG	CTACGGCA
UDI 10	CATAGGCA	TGCCTATG	GATTCTTT
UDI 11	AGATGAAC	GTTTATCT	CTACTCGA
UDI 12	CCGAGTAT	ATACTCGG	GATTCGAG
UDI 13	AATATTGA	TCAATATT	AATCGGCG
UDI 14	GTATACCG	CGGTATAC	TTCGCCGA
UDI 15	GATCCAAC	GTTGGATC	CTGGCCTC
UDI 16	AGATACGC	GCGTATCT	GAACCTAT
UDI 17	GGTATCTT	AAGATACC	CGTATTGG
UDI 18	CCTCTGGC	GCCAGAGG	GAAGCACA
UDI 19	CCATTGTG	CACAATGG	CTTAATAC
UDI 20	ACTACGGT	ACCGTAGT	GAAGTCTT
UDI 21	AAGTGCTA	TAGCACTT	GAAGAGGC

UDI 22	GCCGAACG	CGTTCGGC	CGGATAAC
UDI 23	TGTCCACG	CGTGGACA	GAATCTGG
UDI 24	GACACACT	AGTGTGTC	CTGATTGA
UDI 25	AATATGCT	AGCATATT	AATCCGTT
UDI 26	TTCTCATA	TATGAGAA	TGCGTACA
UDI 27	TCTGTGAT	ATCACAGA	GAATCAAT
UDI 28	CCGAACTT	AAGTTCGG	TGAGTCAG
UDI 29	GTCTAACA	TGTTAGAC	GAATGCTC
UDI 30	GACGCCAT	ATGGCGTC	GAATATCC
UDI 31	GCCAATGT	ACATTGGC	CTTATGAA
UDI 32	CCAACGTC	GACGTTGG	TCGGCACC
UDI 33	GTAGATAA	TTATCTAC	AAGAAGCG
UDI 34	CTTACGGC	GCCGTAAG	CTCACGAT
UDI 35	CCAAGTGC	GCACTTGG	TCGGTCEA
UDI 36	CTAACTCA	TGAGTTAG	TCGGTAAG
UDI 37	AATATCTG	CAGATATT	AAGATACA
UDI 38	TTATATCA	TGATATAA	GTCGCTGT
UDI 39	CTGCGGAT	ATCCGCAG	TCGGATGT
UDI 40	GCGGCTTG	CAAGCCGC	CGAGCCGG
UDI 41	GAGTTGAT	ATCAACTC	CGATTATC
UDI 42	GCACTGAG	CTCAGTGC	TCGAAGCT
UDI 43	GACCACCT	AGGTGGTC	CTATCATT
UDI 44	TGGCTAGG	CCTAGCCA	CGCGCCAA
UDI 45	CCTACCGG	CCGGTAGG	CGAACGGA
UDI 46	GGAGGATG	CATCCTCC	CTACTGAC
UDI 47	CGCTGAAT	ATTCAGCG	TCTTAAGT
UDI 48	TGTGACGA	TCGTCACA	TTAGAGTC
UDI 49	AATAGATT	AATCTATT	AAGACGAA
UDI 50	TTAGCGCA	TGCGCTAA	TTATTATG
UDI 51	GCGGCCGT	ACGGCCGC	CGCTATTA
UDI 52	CAGTAACC	GGTTACTG	TCTATCAG
UDI 53	GCCTAGTA	TACTAGGC	CGGTGGTA
UDI 54	CACGGCGC	GCGCCGTG	TCACCAAT
UDI 55	GGTGCAGA	TCTGCACC	CTGGAAGC
UDI 56	TCGCTGAC	GTCAGCGA	CGTAAGAG
UDI 57	CAGCCAGT	ACTGGCTG	AAGAGAGC
UDI 58	CGTCAACC	GGTTGACG	TCAACGAG
UDI 59	GCCGGCGA	TCGCCGGC	TGCGAGAC

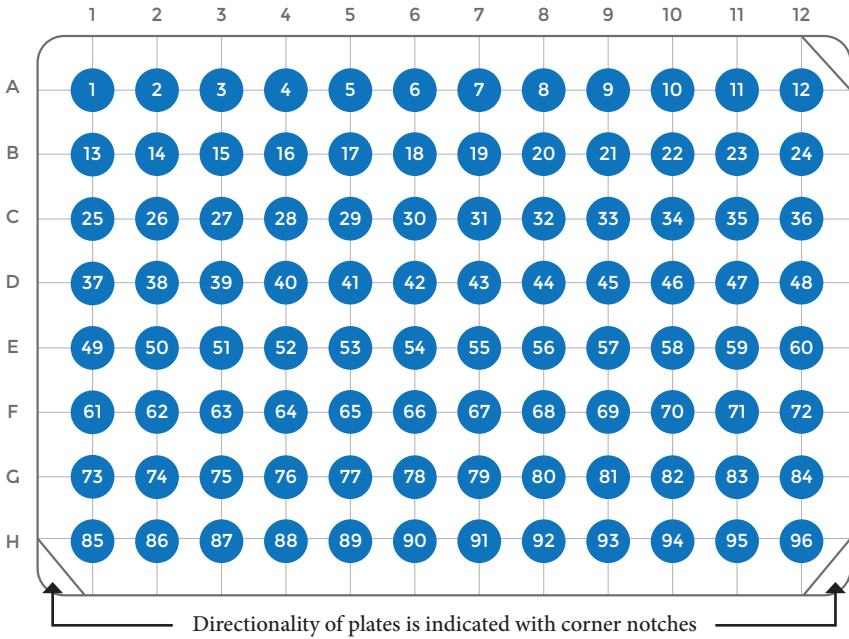
UDI 60	GCCTCCGG	CCGGAGGC	CCTGGTGT
UDI 61	AATAGTCC	GACTATT	AAGTAAGT
UDI 62	TTAGACGT	ACGTCTAA	TGACTGAA
UDI 63	GTGGACTA	TAGTCCAC	AAGACTGT
UDI 64	CACGGACG	CGTCCGTG	CAATGATG
UDI 65	CACTAGAG	CTCTAGTG	CACAGTAA
UDI 66	GCAGATGG	CCATCTGC	TGGTCATT
UDI 67	CTCTCACG	CGTGAGAG	CAACCGTG
UDI 68	GGAATCAC	GTGATTCC	TGGTGCAC
UDI 69	CGTTGACG	CGTCAACG	CCACAATG
UDI 70	CATCAGGT	ACCTGATG	TGTGTGCC
UDI 71	CGTTGTAA	TTACAACG	CACCACGG
UDI 72	GGCACGGT	ACCGTGCC	TGTGTTAA
UDI 73	AATAGCAA	TTGCTATT	AAGTTATC
UDI 74	TGATCGGT	ACCGATCA	GTACAGCT
UDI 75	AGTAGTAT	ATACTACT	CAACTGCT
UDI 76	GTTAGAGG	CCTCTAAC	CATGATGA
UDI 77	CCTTACAG	CTGTAAGG	TGACTACT
UDI 78	GTACATTG	CAATGTAC	CAGAAGAT
UDI 79	GGAGACCA	TGGTCTCC	TGAGGCGC
UDI 80	CGAACACC	GGTGTTCCG	CAGGTTCC
UDI 81	GAGAACAA	TTGTTCTC	TGAACAGG
UDI 82	TGTGAATC	GATTCACA	CAGTGTGG
UDI 83	GGTTAAGG	CCTTAACC	TTCCACCA
UDI 84	AGACCGCA	TGCGGTCT	CCGCTGTT
UDI 85	AATACAGG	CCTGTATT	AAGTTGGA
UDI 86	TGATGGCC	GGCCATCA	GGACAACG
UDI 87	TGTCACCT	AGGTGACA	TTCGAACC
UDI 88	GCTTCGGC	GCCGAAGC	CAGACCAC
UDI 89	CCAGTGGT	ACCACTGG	TTCTGGTG
UDI 90	GCACACGC	GCGTGTGC	CAATCGAA
UDI 91	GTCACGTC	GACGTGAC	AAGTACAG
UDI 92	GCAGCTCC	GGAGCTGC	CCGTGCCA
UDI 93	CATGCAGC	GCTGCATG	CATTGCAC
UDI 94	ACGATTGC	GCAATCGT	TTACCTGG
UDI 95	GACATTCG	CGAATGTC	CTGCAACG
UDI 96	GCGAATAC	GTATTCCG	TACTGTTA

Low Level Multiplexing Guidelines

Each consecutive pair of barcodes found in columns 1 and 2 are fully color balanced and are suitable to be used in a pool of two samples. When designing low-plexity index pools, always include two libraries barcoded with a set of two unique and fully color balanced barcodes to avoid laser color complexity issues during de-multiplexing. Additional libraries may be safely multiplexed with one set of fully color balanced barcodes in a pool.

Plate Format

96 Unique Dual Index Barcoded Adapters / Plate; 5 μ L (2 reaction) / well
Assay Plate: Axygen P-96-450V-C; 500 μ L 96 well "V" Bottom, Clear
Heat Seal: 4titude[®] Pierce Seal 4ti





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We can't wait to hear from you!



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