# NEXTFLEX® Unique Dual Index Barcodes (Set A)

(For Illumina® Platforms)

Catalog #NOVA-514150

 V18.01

**This product is for research use only.**
**Not for use in diagnostic procedures.**

# NEXTflex® Unique Dual Index Barcodes - NOVA-514150

## 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 NEXTflex® Unique Dual Index Barcodes allows the user to multiplex up to 96 samples using this set on its own, or up to 192 samples when used in conjunction with other available sets of NEXTflex® Unique Dual Index Barcodes.

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 purty by sequencing.

## Contents, Storage and Shelf Life

The NEXTflex® Unique Dual Index Barcodes Kit contains 96 uniquely dual-indexed barcoded DNA adapters plated along the rows 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.

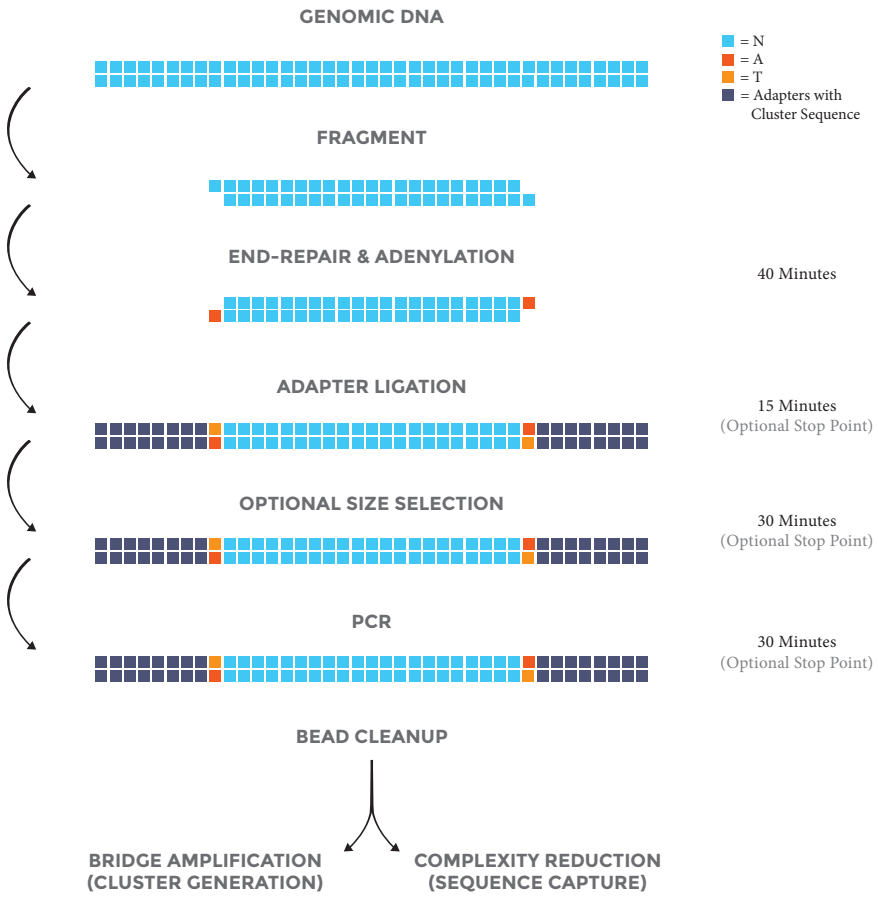
## Warnings and Precautions

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 multichannel 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 | AATGATACGGCGACCACCGAGATCTACACXXXXXXXX[1]ACACTCTTTCCCTACACGACGCTCTTCCGATCT GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXX[2]ATCTCGTATGCCGTCTTCTGCTTG |

[1]XXXXXXXX denotes the P5 index region of adapter. The index sequences contained in each adapter are listed below.
[2]XXXXXXXX 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 | GCAGCCTC | GATGCGTC |
| UDI 9 | CCTCGTAG | CTACGAGG | CTACGGCA |
| UDI 10 | CATAGGCA | TGCCTATG | GATTCCTT |
| UDI 11 | AGATGAAC | GTTCATCT | 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 | GAACTTAT |
| 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 | TCGGTCGA |
| 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 |

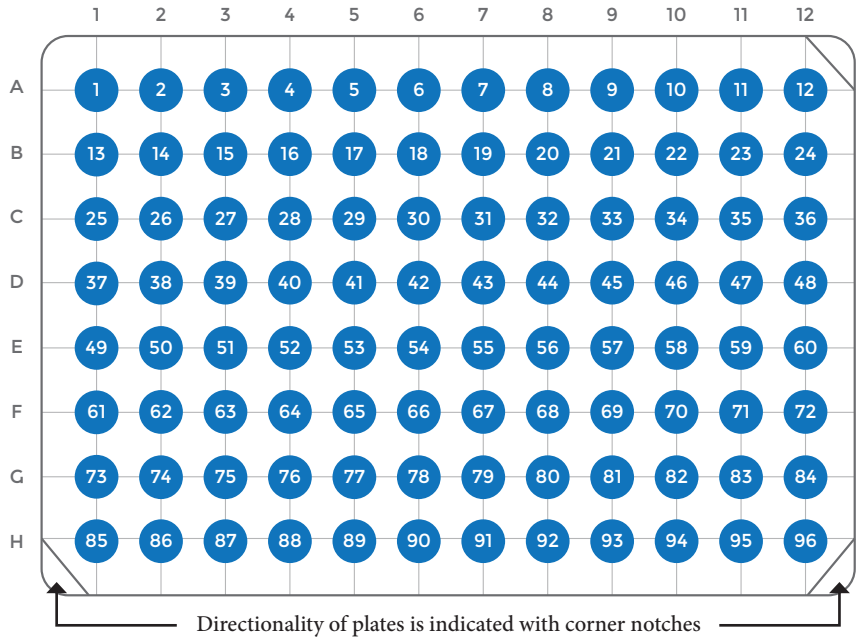
| | | | |
|---|---|---|---|
| UDI 60 | GCCTCCGG | CCGGAGGC | CCTGGTGT |
| UDI 61 | AATAGTCC | GGACTATT | AAGTAAGT |
| UDI 62 | TTAGACGT | ACGTCTAA | TGACTGAA |
| UDI 63 | GTGGACTA | TAGTCCAC | AAGACTGT |
| UDI 64 | CACGGACG | CGTCCGTG | CAATGATG |
| UDI 65 | CACTAGAG | CTCTAGTG | CACAGTAA |
| UDI 66 | 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 | GGTGTTCG | 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 | GTATTCGC | 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 µL (2 reaction) / well
Assay Plate: Axygen P-96-450V-C; 500 µL 96 well "V" Bottom, Clear
Heat Seal: 4titude® Pierce Seal 4ti



## WE WANT TO HEAR FROM YOU!

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# Bioo Scientific

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