



## FOR REFERENCE PURPOSES

**This manual is for Reference Purposes Only. DO NOT use this protocol to run your assays. Periodically, optimizations and revisions are made to the kit and protocol, so it is important to always use the protocol included with the kit.**

### NEXTflex™ DNA Barcodes - 6

(Illumina Compatible)

Catalog #514101 (48 reactions)



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**NEXTflex™ DNA Barcodes 6 - 514101**

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## Product Overview

The NEXTflex™ DNA Barcodes are designed to prepare multiplexed single and paired-end genomic DNA libraries for sequencing using Illumina® platforms. The index and flow cell binding sequence are designed within the NEXTflex™ DNA Barcodes and are attached onto the sample insert during adapter ligation. Pooling with NEXTflex DNA Barcodes allows the user to multiplex several samples in a single flow cell.

## Contents, Storage and Shelf Life

The NEXTflex™ DNA Barcodes contain 6 DNA-Seq Barcoded Adapters with enough material for 8 reactions each, for a total of 48 reactions. The shelf life of each reagent is 12 months when stored at -20°C.

Kit Contents	Amount
NEXTflex™ DNA Barcode Adapter 1 - 6* (25 µM)	20 µL
NEXTflex™ Primer Mix (12.5 µM)	96 µL

\*The Barcode Adapters are supplied in duplex form. Do not heat the adapter above room temperature.

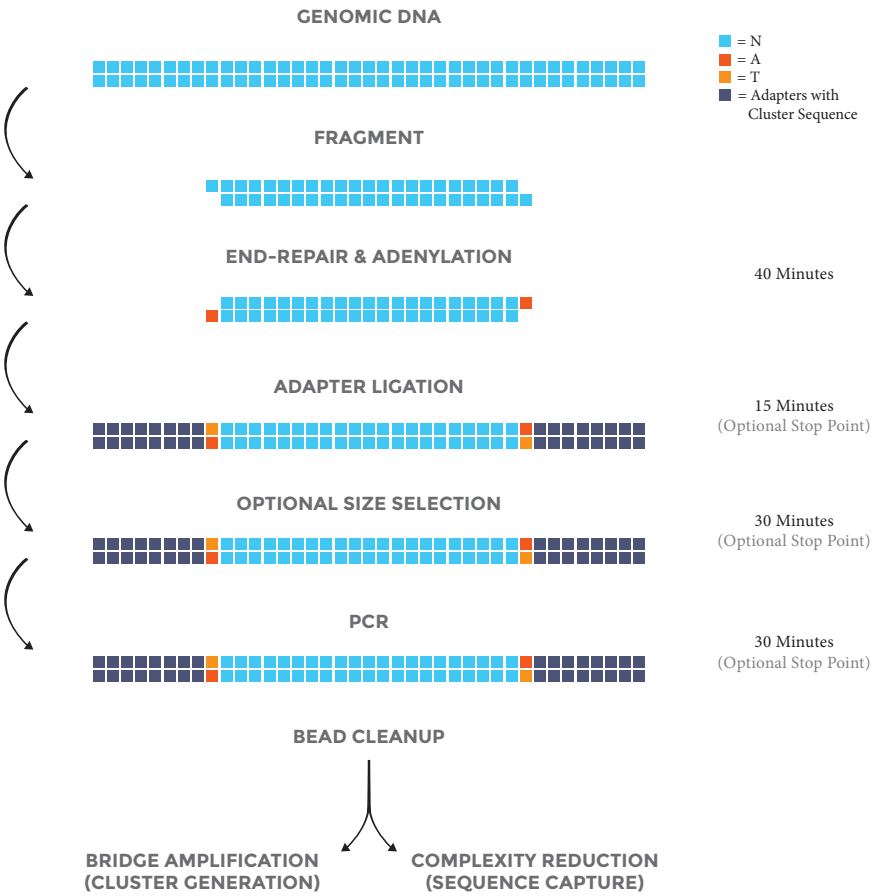
## Warnings and Precautions

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](mailto: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 DNA-Seq Barcoded Adapters above room temperature.
- 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.

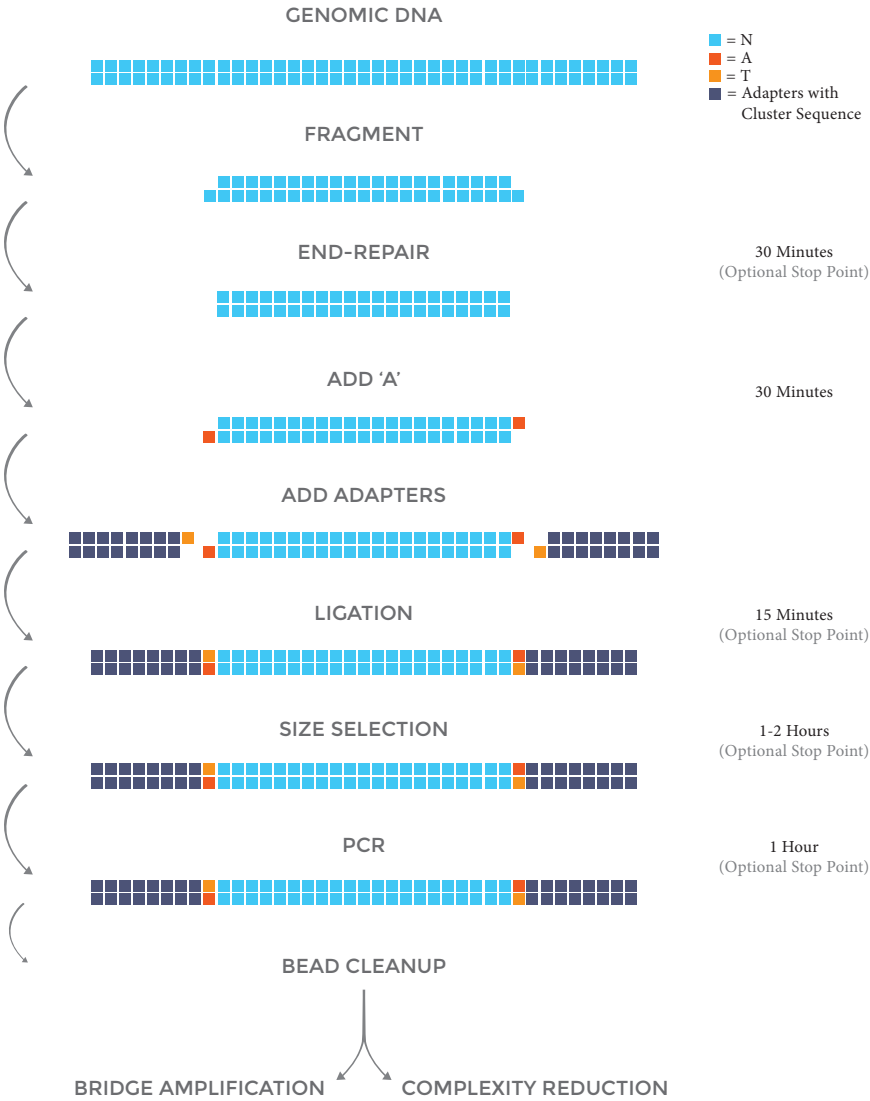
NEXTflex™ Rapid DNA-Seq Sample Preparation Flow Chart

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™ DNA-Seq Barcode Adapter	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT GATCGGAAGAGCACAGCTCTGAACCTCCAGTCACXXXXXXATCTCGTATGCGGCTCTTCTGCTTG

<sup>1</sup>XXXXXX denotes the index region of adapter. The index sequences contained in each adapter are listed below.

NEXTflex™ DNA-Seq Index		NEXTflex™ DNA-Seq Index	
Barcode Adapter 1	CGATGT	Barcode Adapter 25	ACTGAT
Barcode Adapter 2	TGACCA	Barcode Adapter 26	ATGAGC
Barcode Adapter 3	ACAGTG	Barcode Adapter 27	ATTCCT
Barcode Adapter 4	GCCAAT	Barcode Adapter 28	CAAAAG
Barcode Adapter 5	CAGATC	Barcode Adapter 29	CAACTA
Barcode Adapter 6	CTTGTA	Barcode Adapter 30	CACCGG
Barcode Adapter 7	ATCACG	Barcode Adapter 31	CACGAT
Barcode Adapter 8	TTAGGC	Barcode Adapter 32	CACTCA
Barcode Adapter 9	ACTTGA	Barcode Adapter 33	CAGGCG
Barcode Adapter 10	GATCAG	Barcode Adapter 34	CATGGC
Barcode Adapter 11	TAGCTT	Barcode Adapter 35	CATTTT
Barcode Adapter 12	GGCTAC	Barcode Adapter 36	CCAACA
Barcode Adapter 13	AGTCAA	Barcode Adapter 37	CGGAAT
Barcode Adapter 14	AGTTCC	Barcode Adapter 38	CTAGCT
Barcode Adapter 15	ATGTCA	Barcode Adapter 39	CTATAC
Barcode Adapter 16	CCGTCC	Barcode Adapter 40	CTCAGA
Barcode Adapter 17	GTAGAG	Barcode Adapter 41	GCGCTA
Barcode Adapter 18	GTCCGC	Barcode Adapter 42	TAATCG
Barcode Adapter 19	GTGAAA	Barcode Adapter 43	TACAGC
Barcode Adapter 20	GTGGCC	Barcode Adapter 44	TATAAT
Barcode Adapter 21	GTTCG	Barcode Adapter 45	TCATTC
Barcode Adapter 22	CGTACG	Barcode Adapter 46	TCCC GA
Barcode Adapter 23	GAGTGG	Barcode Adapter 47	TCGAAG
Barcode Adapter 24	GGTAGC	Barcode Adapter 48	TCGGCA

## Low Level Multiplexing

2 barcodes: (4, 6) or (3, 19)

3 barcodes: (4, 6, and any other barcode) / (3, 19, and any other barcode) / (1, 5, 19) or (3, 12, 15) or other 3 barcode combinations (many combinations are possible)

4 barcodes: (4, 6, and two other barcodes) / (3, 19, and two barcode) / (3, 4, 6, 19) / (1, 2, 5, 16) or other 4 barcode combinations (many combinations are possible)

## Illumina Compatible RNA NGS Kits and Adapters

Catalog #	Product
5138-01	NEXTflex™ Rapid RNA-Seq Kit (8 reactions)
5138-02	NEXTflex™ Rapid RNA-Seq Kit (48 reactions)
5138-07	NEXTflex™ Rapid Directional RNA-Seq Kit (8 reactions)
5138-08	NEXTflex™ Rapid Directional RNA-Seq Kit (48 reactions)
512911	NEXTflex™ RNA-Seq Barcodes –6
512912	NEXTflex™ RNA-Seq Barcodes – 12
512913	NEXTflex™ RNA-Seq Barcodes – 24
512914	NEXTflex™ RNA-Seq Barcodes – 48
512916	NEXTflex-96™ RNA-Seq Barcodes

5130-01	NEXTflex™ qRNA-Seq™ Kit 4 barcodes (8 reactions)
5130-02	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set A (48 reactions)
5130-03	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set B (48 reactions)
5130-04	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set C (48 reactions)
5130-05	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set D (48 reactions)

5130-01D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 4 barcodes (8 reactions)
5130-02D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set A (48 reactions)
5130-03D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set B (48 reactions)
5130-04D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set C (48 reactions)
5130-05D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set D (48 reactions)

5132-01	NEXTflex™ Small RNA Sequencing Kit (24 reactions)
5132-02	NEXTflex™ Small RNA Sequencing Kit (48 reactions)
5132-03	NEXTflex™ Small RNA Sequencing Kit v2 (24 reactions)
5132-04	NEXTflex™ Small RNA Sequencing Kit v2 (48 reactions)
513305	NEXTflex™ Small RNA Barcode Primers -12 (Set A)
513306	NEXTflex™ Small RNA Barcode Primers -12 (Set B)
513307	NEXTflex™ Small RNA Barcode Primers -12 (Set C)
513308	NEXTflex™ Small RNA Barcode Primers -12 (Set D)

512979	NEXTflex™ Poly(A) Beads (8 reactions)
512980	NEXTflex™ Poly(A) Beads (48 reactions)
512981	NEXTflex™ Poly(A) Beads (100 reactions)



## Illumina Compatible DNA NGS Kits and Adapters

Catalog #	Product
4201-01	NEXTflex™ 16S V4 Amplicon-Seq Kit – 4
4201-02	NEXTflex™ 16S V4 Amplicon-Seq kit – 12
4201-03	NEXTflex™ 16S V4 Amplicon-Seq kit – 24
4201-04	NEXTflex™ 16S V4 Amplicon-Seq kit – 48
4201-05	NEXTflex™ 16S V4 Amplicon-Seq kit – 96
4201-06	NEXTflex™ 16S V4 Amplicon-Seq kit – 192
4201-07	NEXTflex™ 16S V4 Amplicon-Seq kit – 288
4202-01	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit - 4
4202-02	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit - 12
4202-03	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit - 48
4202-04	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit - 1-96
4202-05	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit - 97-192
4202-06	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit - 193-288
4202-07	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit - 289-384

5140-01	NEXTflex™ DNA Sequencing Kit (8 reactions)
5140-02	NEXTflex™ DNA Sequencing Kit (48 reactions)
5144-01	NEXTflex™ Rapid DNA-Seq Kit (8 reactions)
5144-02	NEXTflex™ Rapid DNA-Seq Kit (48 reactions)
5150-01	NEXTflex™ Cell Free DNA-Seq Kit (8 reactions)
5150-02	NEXTflex™ Cell Free DNA-Seq Kit (48 reactions)
514101	NEXTflex™ DNA Barcodes – 6
514102	NEXTflex™ DNA Barcodes – 12
514103	NEXTflex™ DNA Barcodes – 24
514104	NEXTflex™ DNA Barcodes – 48
514105	NEXTflex-96™ DNA Barcodes (Plate Format)
514106	NEXTflex-96™ DNA Barcodes (Tube Format)
514160	NEXTflex™ Dual-Indexed DNA Barcodes (1-96)
514161	NEXTflex™ Dual-Indexed DNA Barcodes (97-192)

5119-01	NEXTflex™ Bisulfite-Seq kit (8 reactions)
5119-02	NEXTflex™ Bisulfite-Seq kit (48 reactions)
511911	NEXTflex™ Bisulfite-Seq Barcodes – 6
511912	NEXTflex™ Bisulfite-Seq Barcodes – 12
511913	NEXTflex™ Bisulfite-Seq Barcodes - 24
5118-01	NEXTflex™ Methyl-Seq 1 Kit (8 reactions)
5118-02	NEXTflex™ Methyl-Seq 1 Kit (48 reactions)

511921	NEXTflex™ Msp 1 (8 reactions)
511922	NEXTflex™ Msp 1 (48 reactions)

5143-01	NEXTflex™ ChIP-Seq Kit (8 reactions)
5143-02	NEXTflex™ ChIP-Seq Kit (48 reactions)
514120	NEXTflex™ ChIP-Seq Barcodes – 6
514121	NEXTflex™ ChIP-Seq Barcodes – 12
514122	NEXTflex™ ChIP-Seq Barcodes – 24
514123	NEXTflex™ ChIP-Seq Barcodes – 48
514124	NEXTflex-96™ ChIP-Seq Barcodes

5140-51	NEXTflex™ Pre-Capture Combo Kit (6 barcodes)
5140-52	NEXTflex™ Pre-Capture Combo Kit (12 barcodes)
5140-53	NEXTflex™ Pre-Capture Combo Kit (24 barcodes)
5140-56	NEXTflex™ Pre-Capture Combo Kit (48 barcodes)
5140-54	NEXTflex™ Pre-Capture Combo Kit (96 barcodes)
514131	NEXTflex™ DNA Barcode Blockers - 6 for SeqCap
514132	NEXTflex™ DNA Barcode Blockers - 12 for SeqCap
514133	NEXTflex™ DNA Barcode Blockers - 24 for SeqCap
514136	NEXTflex™ DNA Barcode Blockers - 48 for SeqCap
514134	NEXTflex™ DNA Barcode Blockers - 96 for SeqCap

5142-01	NEXTflex™ PCR-Free DNA Sequencing Kit (8 reactions)
5142-02	NEXTflex™ PCR-Free DNA Sequencing Kit (48 reactions)
514110	NEXTflex™ PCR-Free Barcodes – 6
514111	NEXTflex™ PCR-Free Barcodes – 12
514112	NEXTflex™ PCR-Free Barcodes – 24
514113	NEXTflex™ PCR-Free Barcodes – 48

## DNA Fragmentation

Catalog #	Product
5135-01	AIR™ DNA Fragmentation Kit (10 reactions)
5135-02	AIR™ DNA Fragmentation Kit (40 reactions)



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