

NEXTFLEX[®] Unique Dual Index Barcodes

(Compatible with Illumina[®] and Element
platforms[®])

KIT CONTAINS: 16, 24, 48, 96 OR 1,536 BARCODES | 16, 24, 48,
96 OR 1,536 RXNS

USER MANUAL FOR:

#NOVA-634100-EVAL16 (Barcodes 1-16)

#NOVA-634100-EVAL24 (Barcodes 1-24)

#NOVA-634100-EVAL48 (Barcodes 1-48)

#NOVA-634100 (Full set 16 barcode plates, barcodes 1-1,536)

#NOVA-634101 to -16 (Individual plates with 96 barcodes each)

NEXTFLEX® Unique Dual Index Barcodes (Set of 1536)

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This product is for research use only.

Not for use in diagnostic procedures.

This manual is proprietary to Revvity and intended only for customer use in connection with the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose without the prior written consent of Revvity. Follow the protocol included with the kit.

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GENERAL INFORMATION

Product Overview

The NEXTFLEX® Unique Dual Index Barcodes are designed to prepare multiplexed single and paired-end genomic DNA libraries for sequencing using Illumina® and Element® 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 1536 samples.

Uniquely dual-indexed libraries are libraries prepared with adapters containing two ten 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 of 1536, which prevents mis-assigned reads from appearing in final data sets. The set of 1536 UDI barcodes are not compatible for use with other NEXTFLEX® Unique Dual Index Barcodes (Set of up to 384).

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

Kit Contents, Storage & Shelf Life

The NEXTFLEX® Unique Dual Index Barcodes Kits contains 16, 24, 48 or 96 uniquely dual-indexed barcoded DNA adapters plated column-wise in a 96-well plate, for a total of 1,536 different barcodes. See Appendix B for barcode plate configuration. It is recommended that plates and NEXTFLEX® Primer Mix (50 µM) are stored at -20°C. The shelf life of each reagent is at least 1 year when stored properly.

Kit Contents	Cap Color	Amount	Storage Temp.
NEXTFLEX® Unique Dual Index Barcodes 1 - 96* (25 µM)	PLATE	2.5 µL per well	-20°C
NEXTFLEX® Primer Mix 2.0** (50 µM)	GREEN CAP	32/48/96/192 µL	-20°C

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

** The Primer Mix 2.0 is only intended for use with the NEXTFLEX® Rapid DNA-Seq Kit 2.0 or NEXTFLEX® Rapid XP DNA-Seq Kit; if the primer mix 2.0 is to be used with any other DNA-Seq kits of choice, additional dilutions may be required. For additional guidance, please inquire at NGS@revvity.com. Each box contains a Primer Mix 2.0 vial at the amount indicated above.

Warnings and Precautions

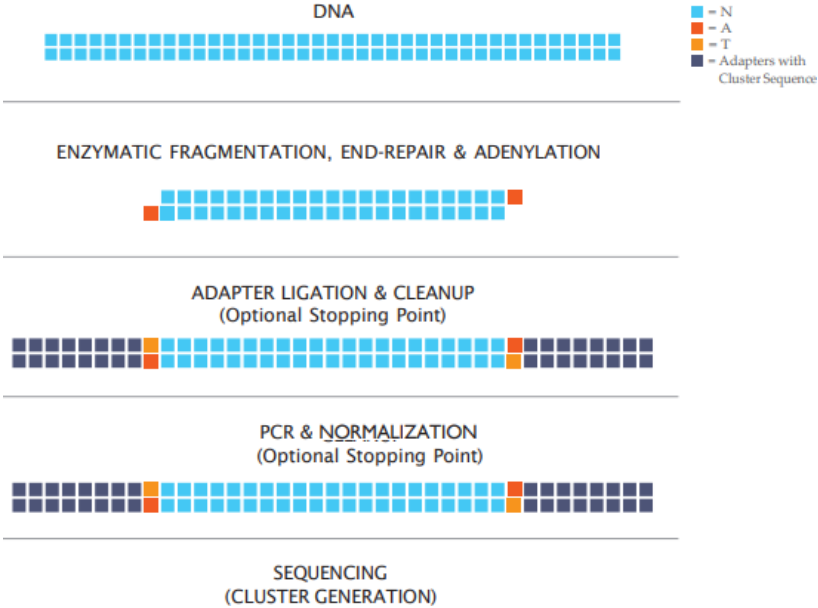
- We strongly recommend that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor, or contact us at <https://www.revivity.com/contact-us/technical-support> and choose the “Next Gen Sequencing” category.
- 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 2.0 is only compatible with the NEXTFLEX® Rapid DNA-Seq Kit 2.0 and NEXTFLEX® Rapid XP v2 DNA-Seq Kit at the concentration it is supplied in. If the NEXTFLEX® Unique Dual Index Barcodes are used with other NEXTFLEX® kits, the Primer Mix 2.0 must be diluted prior to use. Make sure to verify that the Primer Mix concentration is compatible at its stock concentration or dilute with nuclease-free water prior to use. The NEXTFLEX® Primer Mix 2.0 should always be used with the NEXTFLEX® Unique Dual Index Barcodes for PCR amplification even if alternative primers are supplied with your kit of choice. Inadvertent use of an incorrect primer sequence can potentially result in elimination of the index. Primer Mix included with the NEXTFLEX® library prep kits should always be used instead of the Primer Mixes that come with the NEXTFLEX barcodes. For additional information, please contact <https://www.revivity.com/contact-us/technical-support>.

Revision History

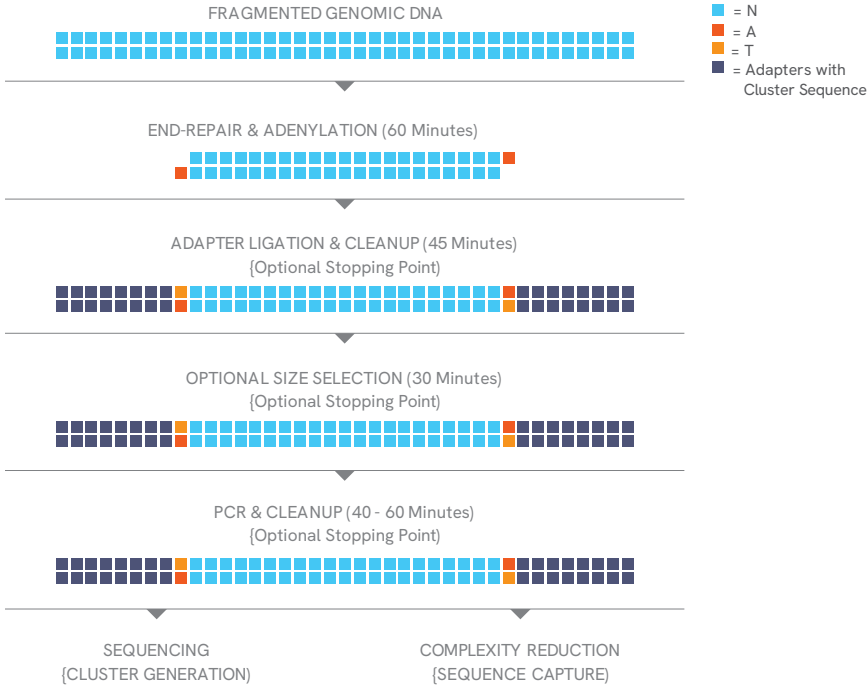
Version	Date	Description
V24.11	November 2024	Product Launch

SAMPLE PREP PROTOCOL

NEXTFLEX® Rapid XP v2 DNA-Seq Sample Preparation Flow Chart



NEXTFLEX® Rapid DNA-Seq 2.0 Sample Preparation Flow Chart



APPENDIX A

Oligonucleotide Sequences

NEXTFLEX®	Sequence (5' → 3')
PCR Primer 1	AATGATACGGCGACCACCGAGATCTACAC
PCR Primer 2	CAAGCAGAAGACGGCATACGAGAT
NEXTFLEX® UDI Barcode	AATGATACGGCGACCACCGAGATCTACACXXXXXXXXXX ¹ ACACTCTTTCCTACACGACGCTCTCCGA TCT GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXXX ² ATCTCGTATGCCGTCTTCTGCTTG

XXXXXXXXXX¹ denotes the P5 index region of adapter. The index sequences contained in each adapter are listed below.

XXXXXXXXXX² denotes the P7 index region of the adapter. The index sequences contained in each adapter are listed below.

For a digital copy of indices, you may contact your local distributor, or contact us at <https://www.revvy.com/contact-us/technical-support> and choose the "Next Gen Sequencing" category.

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. For additional information, please contact <https://www.revvy.com/contact-us/technical-support>.

APPENDIX B

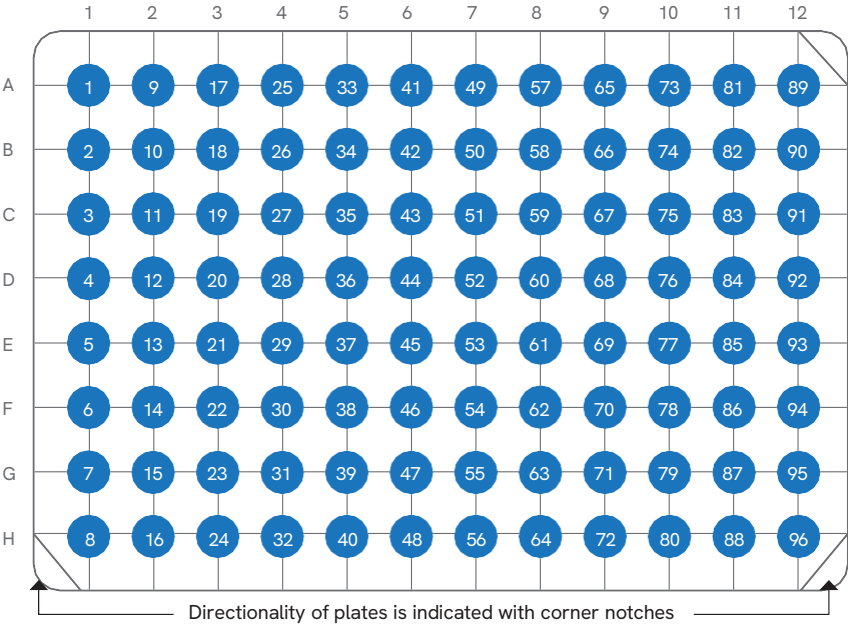
Part number Description

Part Number	Description	Number of Rxn
NOVA-634100-EVAL16	Barcodes 1-16	16
NOVA-634100-EVAL24	Barcodes 1-24	24
NOVA-634100-EVAL48	Barcodes 1-48	48
NOVA-634100	Barcodes 1-1,536	1,536
NOVA-634101	Barcodes 1-96	96
NOVA-634102	Barcodes 97-192	96
NOVA-634103	Barcodes 193-288	96
NOVA-634104	Barcodes 289-384	96
NOVA-634105	Barcodes 385-480	96
NOVA-634106	Barcodes 481-576	96
NOVA-634107	Barcodes 577-672	96
NOVA-634108	Barcodes 673-768	96
NOVA-634109	Barcodes 769-864	96
NOVA-634110	Barcodes 865-960	96
NOVA-634111	Barcodes 961-1,056	96
NOVA-634112	Barcodes 1,057-1,152	96
NOVA-634113	Barcodes 1,153-1,248	96
NOVA-634114	Barcodes 1,249-1,344	96
NOVA-634115	Barcodes 1,345-1,440	96
NOVA-634116	Barcodes 1,441-1,536	96

APPENDIX C

Plate Format

All part numbers in 96-well plate; 2.5 μ L (1 reaction) / well
Representative Orientation



- NOVA-634100-EVAL16: Contains only Barcodes 1-16 listed in column 1-2
- NOVA-634100-EVAL24: Contains only Barcodes 1-24 listed in column 1-3
- NOVA-634100-EVAL48: Contains only Barcodes 1-48 listed in column 1-6



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