NEXTFLEX® Unique Dual Index Barcodes

(Compatible with Illumina and Element Platforms)

KIT CONTAINS : 8, 24, or 96 BARCODES | 16, 48, or 192 RXNS

USER MANUAL FOR :
#NOVA-514150-eval16
#NOVA-514150-eval48
#NOVA-514150
#NOVA-514151
#NOVA-514152
#NOVA-514153

NEXTFLEX® Unique Dual Index Barcodes

This product is for research use only.

Not for use in diagnostic procedures.

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 384 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 misassigned 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.

Kit Overview

This NEXTFLEX® Unique Dual Index Barcodes Kit contains 8, 24, or 96 uniquely dual-indexed barcoded DNA adapters in plate format for a total of 16, 48, or 192 reactions.

Kit Contents, Storage and Shelf Life

Note: The 16-reaction kit contains UDI barcodes 1-8, the 48-reaction kit contains UDI barcodes 1-24, and the 192-reaction kit contains UDI barcodes 1-96, 97-192, 193-288, or 289-384.

It is recommended that UDI barcodes are stored at -20°C. The shelf life of each reagent is at least 1 year when stored properly.

Kit Contents	Cap Color	Amount (16 rxn / 48 rxn / 192 rxn)	Storage Temp.
NEXTFLEX® Unique Dual Index Barcodes* (25 µM)	PLATE	5 µL each	-20°C
NEXTFLEX® Primer Mix 2.0** (12.5 µM)	GREEN CAP	32 µL / 96 µL / 384 µL	-20°C

**These Unique Dual Index Barcodes are supplied in duplex form. Do not heat the adapters above room temperature. All versions of the kit will be plated column- wise (1-8, 9-16, etc.).*

*** The Primer Mix is only intended for use with the NEXTFLEX® Rapid DNA-seq 2.0 or NEXTFLEX® Rapid XP V2 DNA-seq kits; if the primer mix is to be used with any other DNA-Seq Kits of choice, additional dilutions may be required. For additional guidance, please inquire at https://www.revvity.com/contact-us/technical-support.*

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.revvity.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.
- NEXTFLEX® Unique Dual Index Barcodes have been performance-verified to be used in conjunction with the NEXTFLEX® Rapid DNA-seq 2.0 or NEXTFLEX® Rapid XP V2 DNA-seq kits.
- 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. For the NEXTFLEX® library prep kits that come with their own Primer Mix 2.0, the Primer Mix 2.0 must be used.

Version	Date	Description
V23.10	October 2023	Manual Rebrand
V25.05	May 2025	Quality Statement Addition

SAMPLE PREPARATION PROTOCOL

NEXTFLEX® Rapid XP V2 DNA-seq Flow Chart

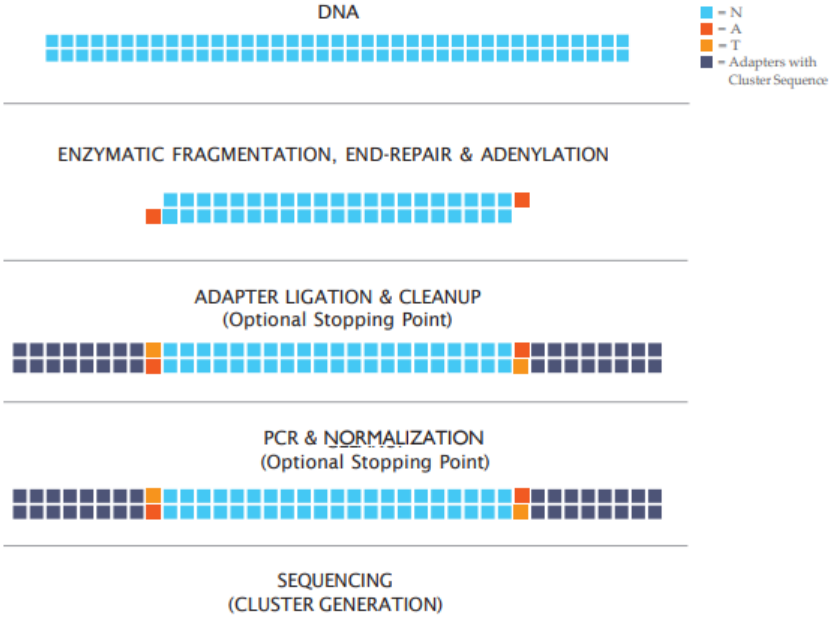


Figure 1: Sample flow chart

NEXTFLEX® Rapid DNA-Seq 2.0 Flow Chart

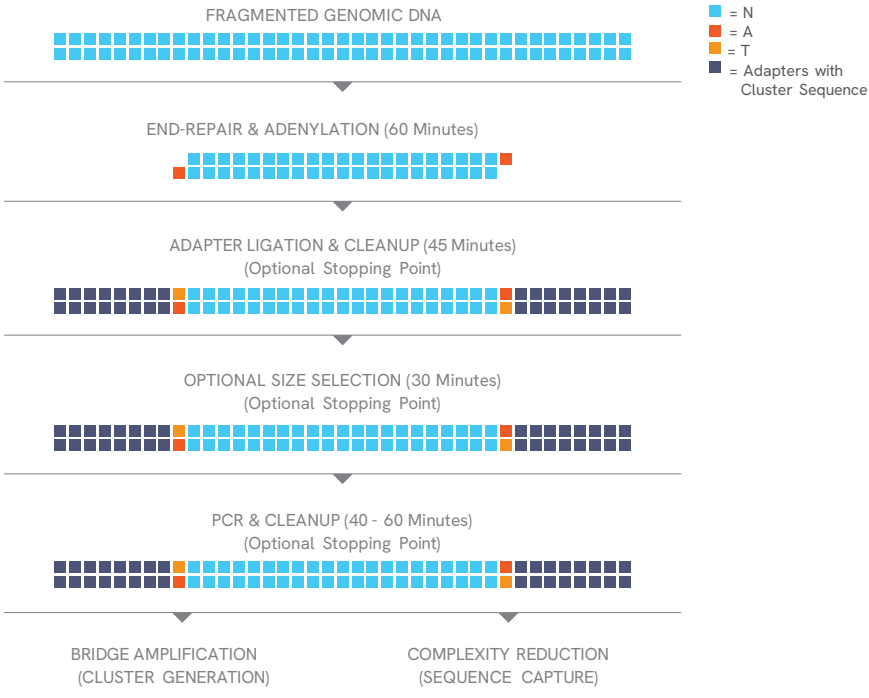


Figure 2: Sample flow chart.

QUALITY STATEMENT

Each lot of NEXFLEX Barcodes undergoes strict QC analysis prior to release. Each component must pass rigorous controls standards, and then afterwards the lot is functionally validated by construction and sequencing of NEXTFLEX Rapid DNA-Seq kit 2.0 libraries on Illumina sequencing platforms. The parameters used to release each lot include, among others:

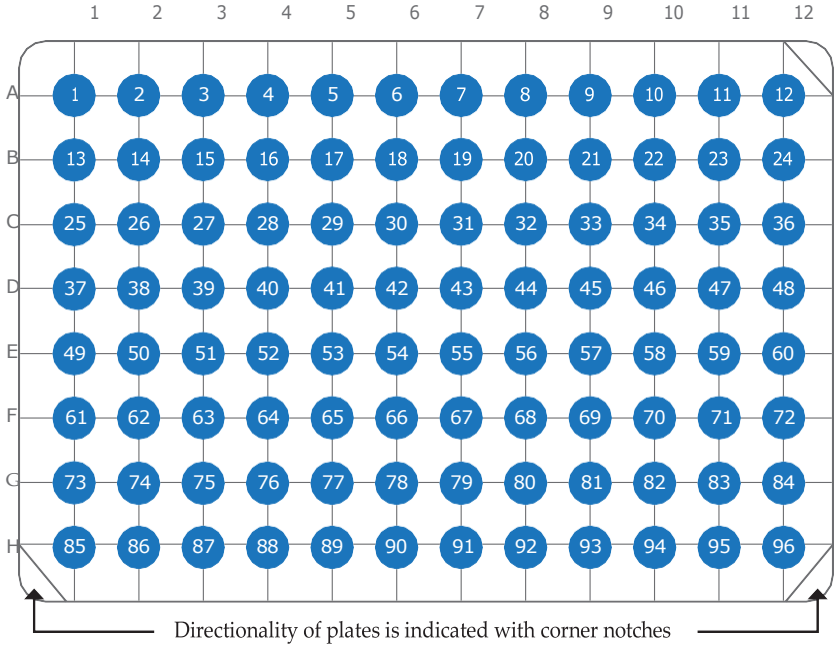
- Reads associated to each index
- % Barcode Purity
- % i5 matching sequence
- % i7 matching sequence

DNA input for quality check are unique sequence-specific amplicons.

APPENDIX A

Plate Format

All part numbers in 96-well plate; 5 µL (2 reaction) / well
Representative Orientation



NOVA-514150-eval16: Contains only Barcodes 1-8 listed in column 1

NOVA-514150-eval48: Contains only Barcodes 1-24 listed in columns 1-3

APPENDIX B

Oligonucleotide Sequences

NEXTFLEX®	Sequence (5′ → 3′)
PCR Primer 1	AATGATACGGCGACCACCGAGATCTACAC
PCR Primer 2	CAAGCAGAAGACGGCATACGAGAT
NEXTFLEX® Unique Dual Index Barcode	AATGATACGGCGACCACCGAGATCTACACXXXXXXXX[1]ACACTC TTTCCCTACACGACGCTCTTCCGATCT GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXX[2]AT CTCGTATGCCGTCTTCTGCTTG

[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.

For a digital copy of indices, please visit our website or this address: https://www.revvity.com/content/nextflex-udi-barcodes-sequences-384-set, or contact us at https://www.revvity.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.

Low Level Multiplexing Guidelines

Barcodes 1 and 2, 13 and 14, 25 and 26, 37 and 38, 49 and 50, 61 and 62, 73 and 74, and 85 and 86 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.

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