

EpiNext™ NGS Barcode (Index) Set-12

Base Catalog # P-1060

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ NGS Barcode (Index) Set-12 is designed to construct multiplex DNA/RNA libraries used for next-generation sequencing with the Illumina platform including GAllx, HiSeq and MiSeq. This product includes 12 DNA barcodes, each of which contain an index and flow cell binding sequence and can be attached to the sample insert during library preparation. Multiplexing several samples in a single flow cell can be achieved by pooling with the EpiNext™ NGS Barcodes.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the tube/vials. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.



PRODUCT CONTENTS

Component	144 Reactions	Storage Upon Receipt
EpiNext™ Barcodes 1-12 (10 μM)* †	15 μl each	-20°C
EpiNext™ Universal Primer (10 μM)*	155 μl	-20°C
User Guide	1	RT

* Spin the solution down to the bottom prior to use.

† The EpiNext™ NGS Barcode Set-12 contains 12 DNA barcodes with sufficient volume for 12 reactions each, for a total of 144 reactions. The shelf life of each reagent is 6 months when stored at -20°C.

SHIPPING & STORAGE

The product is shipped on frozen ice packs at 4°C.

Upon receipt: Store the following components at -20°C: **EpiNext™ Barcodes 1-12** and **EpiNext™ Universal Primer**. Store all other components at room temperature.

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiNext™ NGS Barcode (Index) Set-12 is tested against predetermined specifications to ensure consistent product quality. Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Thus, only use the User Guide that was supplied with the product when using that product.

Usage Limitation: The EpiNext™ NGS Barcode (Index) Set-12 is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiNext™ NGS Barcode (Index) Set-12 and methods of use contain proprietary technologies by Epigentek.

A BRIEF OVERVIEW

The EpiNext™ NGS Barcode (Index) Set-12 contains indexed sequences that allow efficient multiplexing workflow and flexible setup to be carried out. Pooling barcoded samples into a single flow cell significantly

reduces hands-on time and provides robust data quality in NGS. The EpiNext™ NGS Barcode (Index) Set-12 has the following features:

- The barcodes contained in the EpiNext™ NGS Barcode (Index) Set-12 can be used with single, paired-end and multiplex reads and are compatible with various library preparation workflows used for Illumina NGS, including DNA-seq, RNA-seq, ChIP-seq, MethyIC-seq, MeDIP-seq, hMeDIP-seq, DNA bisulfite-Seq, oxBS-seq and RNA bisulfite-seq.
- The barcodes are also optimized for use with Epigentek's EpiNext™ NGS library preparation kit series including EpiNext™ DNA Library Preparation Kit (Illumina), EpiNext™ High-Sensitivity DNA Library Preparation Kit (Illumina), EpiNext™ Post-Bisulfite DNA Library Preparation Kit (Illumina), EpiNext™ Bisulfite-Seq High-Sensitivity Kit (Illumina), EpiNext™ ChIP-Seq High-Sensitivity Kit (Illumina), EpiNext™ RNA Bisulfite-Seq Kit and EpiNext™ MeDIP-Seq Kit.
- High throughput and cost reduction: Up to 144 multiplexed samples can be processed, which allows multiplexing of 2-12 fragment library samples on a single sequencing flow cell resulting in considerable reduction of per-sample cost.

WORKFLOWS

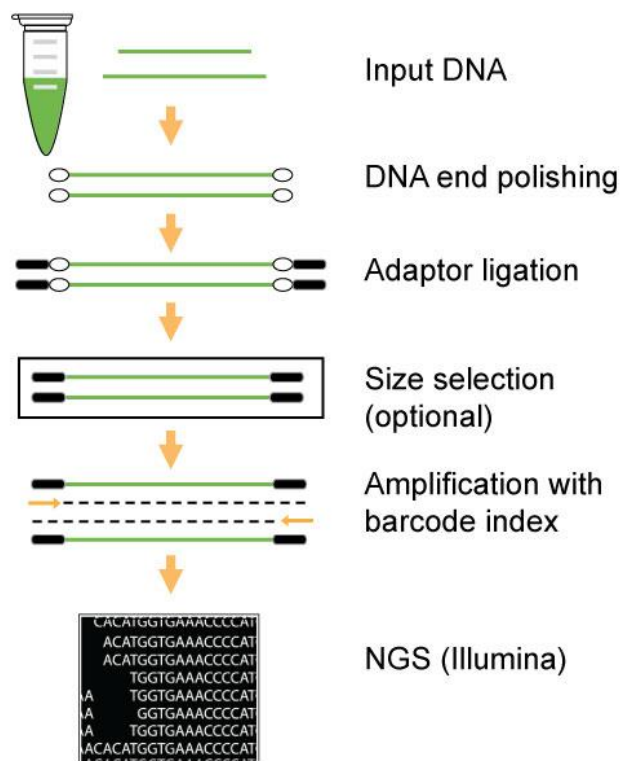


Fig 1. Workflow of the DNA library preparation with EpiNext™ NGS Barcode (Index) Set-12.

EPINEXT™ BARCODED ADAPTOR SEQUENCE

5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNATCTCGTATGCCGTCTTCTGCTTG-3'*

*NNNNNN is 6 nt index sequence contained in the adaptor.

EPINEXT™ UNIVERSAL PRIMER SEQUENCE

5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3'

EPINEXT™ BARCODED ADAPTOR INDEX (5' → 3')

Barcode Adaptor Index 1	ATCACG
Barcode Adaptor Index 2	CGATGT
Barcode Adaptor Index 3	TTAGGC
Barcode Adaptor Index 4	TGACCA
Barcode Adaptor Index 5	ACAGTG
Barcode Adaptor Index 6	GCCAAT
Barcode Adaptor Index 7	CAGATC
Barcode Adaptor Index 8	ACTTGA
Barcode Adaptor Index 9	GATCAG
Barcode Adaptor Index 10	TAGCTT
Barcode Adaptor Index 11	GGCTAC
Barcode Adaptor Index 12	CTTGTA

Note: For low level multiplexing (fewer than 12 indexes are used in a lane for sequencing), it is recommended to use the following indexes:

Pool of 2 samples: Index #6 and 12

Pool of 3 samples: Index #4, 6 and 12

Pool of 4 samples: Index #2, 4, 6 and 12

Pool of 6 samples: Index #2, 4, 5, 6, 7 and 12

APPENDIX: ADDING EPINEXT™ NGS BARCODES TO LIBRARY

The EpiNext™ NGS Barcodes containing different indexed sequences may be used in the library amplification step during the sample library preparation for Illumina NGS. The following is an example protocol:

a. *Prepare the PCR Reactions:*

Thaw all reaction components including master mix, DNA/RNA free water, primer solution and DNA template. Mix well by vortexing briefly. Keep components on ice while in use, and return to -20°C immediately following use. Add components into each PCR tube/well according to the following table:

Component	Size (µl)
HiFi Master Mix (2X)*	12.5 µl
EpiNext™ Universal Primer	1 µl
Barcode Index (any one of 1-12)	1 µl
Adaptor Ligated DNA	10.5 µl
Total Volume	25 µl

* High fidelity PCR amplification enzyme mix from Epigentek. 2X HiFi PCR mix from other supplies could be also used.

b. *Program the PCR Reactions:*

Place the reaction plate in the PCR instrument and set the PCR conditions as follows:

Cycle Step	Temp	Time	Cycle
<i>Activation</i>	98°C	30 sec	1
<i>Cycling</i>	98°C	20 sec	Variable*
	55°C	20 sec	
	72°C	20 sec	
<i>Final Extension</i>	72°C	2 min	1

Note: PCR cycles may vary depending on the input DNA amount. In general, use 12 PCR cycles for 200 ng, 13 cycles for 100 ng, 15 cycles for 50 ng, 17 cycles for 10 ng, and 22 cycles for 1 ng DNA input. Further optimization of PCR cycle number may be required by the end user.

The amplified DNA library can be cleaned up using your own successful method. For the best results and your convenience, we recommend using Epigentek's EpiNext™ DNA Size Selection Kit (P-1059) or The EpiNext™ DNA Purification HT System (P-1061).

RELATED PRODUCTS

DNA Isolation and Cleanup

P-1003	FitAmp™ General Tissue Section DNA Isolation Kit
P-1004	FitAmp™ Plasma/Serum DNA Isolation Kit
P-1006	DNA Concentrator Kit
P-1007	FitAmp™ Gel DNA Isolation Kit
P-1009	FitAmp™ Paraffin Tissue Section DNA Isolation Kit
P-1017	FitAmp™ Urine DNA Isolation Kit
P-1018	FitAmp™ Blood and Cultured Cell DNA Extraction Kit
P-1059	EpiNext™ DNA Size Selection Kit
P-1061	EpiNext™ DNA Purification HT System

Sonication Instruments

EQC-2000	EpiSonic™ 2000 Sonication System
----------	----------------------------------

DNA Enrichment Reaction

P-1015	Methylamp™ Methylated DNA Capture (MeDIP) Kit
P-1038	EpiQuik™ Hydroxymethylated DNA Immunoprecipitation (hMeDIP) Kit

- P-1052 EpiQuik™ MeDIP Ultra Kit
- P-2002 EpiQuik™ Chromatin Immunoprecipitation (ChIP) Kit
- P-2003 EpiQuik™ Tissue Chromatin Immunoprecipitation (ChIP) Kit
- P-2014 EpiQuik™ Plant ChIP Kit
- P-2025 ChromaFlash™ One-Step ChIP Kit
- P-2026 ChromaFlash™ One-Step Magnetic ChIP kit
- P-2027 ChromaFlash™ High-Sensitivity ChIP Kit
- P-2030 EpiNext™ ChIP-Seq High-Sensitivity Kit

PCR Analysis

- P-1029 EpiQuik™ Quantitative PCR Fast Kit

DNA library Prep

- P-1051 EpiNext™ DNA Library Preparation Kit (Illumina)
- P-1053 EpiNext™ High-Sensitivity DNA Library Preparation Kit (Illumina)
- P-1055 EpiNext™ Post-Bisulfite DNA Library Preparation Kit (Illumina)
- P-1056 EpiNext™ Bisulfite-Seq High-Sensitivity Kit (Illumina)

