

**NEXTFLEX® Rapid XP  
DNA-Seq Kit****NEXTFLEX® Rapid XP DNA-Seq  
Pre-Plated Automation Kit**

NOVA-5149-103

**Product Overview**

The NEXTFLEX® Rapid XP DNA-Seq Pre-Plated Automation Kit is designed for DNA library construction from 1 ng – 1 µg of DNA. The kit can be used to prepare single, paired-end, and multiplexed DNA libraries for sequencing using Illumina® platforms. The NEXTFLEX® 1-step Fragmentation, End-Repair, and Adenylation simplifies workflow and shortens hands-on library construction time. An optional bead-based size selection protocol eliminates the need for agarose gel size selection. In addition, the availability of up to 384 unique adapter barcodes facilitates high-throughput applications (available separately).

Automation-friendly, pre-arrayed, single-use reagent plates are now available for use in conjunction with the single-use NEXFLEX® Unique Dual Index barcodes (Set A through Set D) on PerkinElmer® Sciclone® G3 NGSx workstation. Please inquire at [NGS@PerkinElmer.com](mailto:NGS@PerkinElmer.com) for additional details.

## Provided Reagents

### Contents, Storage, and Shelf Life

The NEXTFLEX® Rapid XP DNA-Seq Pre-Plated Automation Kit contains enough material to prepare 96 DNA samples for Illumina® compatible sequencing. The shelf life of all reagents is at least 6 months when stored properly. The Nuclease-free Water, Resuspension Buffer, and the NEXTFLEX® Cleanup Beads XP should be stored at 4°C, and all other components should be stored at -20°C.

All plates are intended for single use only and are not supported for multiple use. Library Prep Construction Plates are built in StorPlate-96V (PerkinElmer, part # 6008290/60088299) while all other plates are built in Hard-Shell® 96-Well PCR Plates (Bio-Rad, part # hsp9631). All plates are sealed using the Adhesive PCR film (LGC Genomics, part # KBS-0606-002). Spin down plates prior to use and carefully unpeel seal to avoid spillage.

Kit Contents	Format
NEXTFLEX® Fragmentation Buffer	Pre-plated in Library Prep Construction Plate
NEXTFLEX® Fragmentation Enzyme Mix	Pre-plated in Library Prep Construction Plate
NEXTFLEX® Ligase Buffer Mix XP	Pre-plated in Library Prep Construction Plate
NEXTFLEX® Ligase Enzyme XP	Pre-plated in Library Prep Construction Plate
NEXTFLEX® PCR Master Mix XP	Pre-plated in Library Prep Construction Plate
NEXTFLEX® Primer Mix XP (50 µM)	Pre-plated in Library Prep Construction Plate
Nuclease-free Water	Pre-plated in Pre-PCR Nuclease-free Water Plate
Resuspension Buffer	Pre-plated in Pre-PCR Resuspension Buffer Plate and Pre-plated in Post-PCR Resuspension Buffer Plate
NEXTFLEX® Cleanup Beads XP	Pre-plated in Pre-PCR Cleanup Beads Plate and Pre-plated in Post-PCR Cleanup Beads Plate

### Library Prep Construction Plate Layout

	Fragmentation Enzyme	Fragmentation Buffer	Ligation Enzyme	Ligation Enzyme	Ligation Buffer	Ligation Buffer	PCR Mix	PCR Primer	Empty	Empty	Empty	Empty
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

### Required Consumables

Part No.	Vendor/ Part Number	Part	Quantity Needed for 96 Samples
6008870	PerkinElmer®	Bio-Rad Hard-Shell®96 Well PCR Plate, Full Skirt	5-7, Dependent on workflow options chosen
111426	PerkinElmer®	Pipette Tip, 150 µL, Art, Box, 10-96 Sterile Racks	12-18 Dependent on workflow options chosen
6008880	PerkinElmer®	PP Storage Plate, 96-Deepwell V-Bottom	1
6008700	PerkinElmer®	12-Column Deepwell Reservoir, 21/25mL, Polypropylene	1
6000030	PerkinElmer®	Universal Lid	1

## Protocol Step Details

The following table lists the recommended incubation times as a guideline for fragmentation. The mode fragment size can be adjusted by changing the duration of incubation at the 35 °C step. These times are recommendations only, and incubation time may need to be optimized for different sample inputs and types to obtain desired mode fragment size.

Input Data	Target Fragment Peak Size			
	200-300	300-400	400-500	500-600
Fragmentation Time (min) at 35 °C				
1 ng	24	15	8	4
10 ng	18	8	5	3
100 ng	15	8	5	3
250 ng	15	8	5	3
500 ng	15	8	5	3
1 µg	14	7	5	3

The following table lists recommended barcoded adapter concentration dilutions for various input amounts for NEXTFLEX® barcoded adapters specified to be at 25 µM, except for the NEXTFLEX® ChIP-Seq barcoded adapters which are provided at 0.6 µM. Pre-plated NEXTFLEX® Unique Dual Index Plates are provided at custom concentrations, so labs should ensure barcode concentration before proceeding. Clarify any questions regarding barcodes with your field application scientist prior to starting the library prep.

Input DNA	Desired Adapter	Adapter Dilution Concentration
1 ng	0.3 µM	1/80
10 ng	0.6 µM	1/40
100 ng	6.25 µM	1/4
250 ng	25 µM	None
500 ng	25 µM	None
1 µg	25 µM	None

For library prep without size selection, the following table lists recommended number of PCR cycles on the thermal cycler.

Input DNA	Number of PCR cycles to produce	
	100 ng libraries	1 µg libraries
1 ng	10-12	13-15
10 ng	6-8	9-11
100 ng	2-3	6-7
250 ng	1-2	4-5
500 ng	0	3-4
1000 ng	0	2-3

For library prep that is intended to include size selection, the following table lists recommendations for the appropriate volumes of NEXTFLEX® Cleanup Beads XP required to size select for library peak sizes (approximated).

Approximate Insert Peak Size (bp)	150-250	250-350	300-500	400-600	500-700
Approximate Library Peak Size (bp)	270-370	370-470	420-620	520-720	620-820
Bead Volume #1	35	32	30	27	24
Bead Volume #2	12	9	8	8	8

For library prep including size selection, the following table lists recommended number of PCR cycles on the thermal cycler.

Input DNA	Number of PCR cycles to produce	
	100 ng libraries	1 µg libraries
10 ng	9-10	11-13
100 ng	4-5	8-9
250 ng	4-5	6-7
500 ng	0-4	4-5
1000 ng	0-4	4-5

Reagents are designed to be used with the automated script.

For any questions, contact your local field application scientist or email [NGS@perkinelmer.com](mailto:NGS@perkinelmer.com).

Research use only. Not for use in diagnostic procedures.