



NGS Library Prep Kits for Illumina® & Ion Torrent™ Sequencing



#### Introduction

PerkinElmer offers a robust portfolio of next-generation sequencing (NGS) solutions developed with expertise spanning from extraction to analysis, which enables a reliable, robust, and automated start-to-finish bundle for all your NGS library prep. The complete portfolio includes nucleic acid extraction solutions with corresponding instruments, consumables, and complete reagent bundles to simplify nucleic acid and NGS library analysis, workstations that automate and streamline the library preparation, robust and comprehensive library prep kits, and user-friendly software for the analysis of complex sequencing data.

PerkinElmer's NGS workflow solutions are designed to solve the researcher's problems. From improving nucleic acid yields, to increasing throughput, to reducing bias, we have the solution you need.

This product catalogue highlights the PerkinElmer NGS library preparation barcodes and kit solutions available. The NGS Workflow Solution is also provided as a comprehensive overview and additional details can be found in the sections focusing on automation.

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#### PerkinElmer's NGS Workflow Solutions

## **EXTRACTION TO ANALYSIS**



NEXT GENERATION SEQUENCING WORKFLOW

SAMPLE DISAGGREGATION		OMNI Technology
NUCLEIC ACID ISOLATION	1 7	chemagen <sup>™</sup> Technology
QUANTITATION OF NUCLEIC ACIDS		Plate Reader
VISUAL QC & SIZING OF NUCLEIC ACIDS		Microfluidic Devices
NANO DISPENSING		FlexDrop™ iQ™ Dispenser
LIBRARY PREPARATION REAGENTS	Piters	NEXTFLEX® Library Prep Kits
AUTOMATED LIBRARY PREPARATION		Automated Liquid Handlers
LIBRARY QUALITY CONTROL		Microfluidic Devices
ANALYZE		

Third version of this document: January 2022

The latest updates available are on the website:

https://perkinelmer-appliedgenomics.com/home/applications/ngs-workflows

### Kit selection by application

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Application	Wh	ole 3	diffe Chi	R.sed Lipper	isulfite Tar	gette RIVA	anscr.	Me'	int.
NEXTFLEX® Rapid 2.0 DNA-seq Kit 2.0	•	•	•		•			•	
NEXTFLEX® Rapid XP DNA-seq Kit	•				•			•	
NEXTFLEX® Cell Free DNA-seq Kit 2.0	•	•			•				
NEXTFLEX® ChIP-seq Kit			•						
NEXTFLEX® Bisulfite-seq Kit				•					
NEXTFLEX® Methyl-1 seq Kit				•					
NEXTFLEX® Rapid RNA Kit						•			
NEXTFLEX® Rapid Directional RNA Kit 2.0						•			
NEXTFLEX® small RNA Kit v3							•		
NEXTFLEX® Combo-Seq™ mRNA/ miRNA Kit						•	•		
NEXTFLEX® 16S V1-V3 Amplicon- Seq Kits 2.0								•	
NEXTFLEX® 16S V4 Amplicon-Seq Kits 2.0								•	
NEXTFLEX® 18S ITS Amplicon-Seq Kits								•	
NEXTFLEX® 16S V4 & 18S ITS Amplicon-Seq Kits								*	
NEXTFLEX® Amplicon Panels					•				•
NEXTFLEX® DNA-seq Kit for Ion Torrent	•	•	•			*			•
NEXTFLEX® Cell Free DNA-seq Kit for Ion Torrent	•	•							•

For any more information about the kits, please contact NGS@perkinelmer.com

<sup>\*</sup> Untested but should be compatible.

\*\* Single-index, dual-index, and unique dual-index barcodes available separately.

\*\*\* PerkinElmer also provides nucleic acid isolation kits, detailed at https://perkinelmer-appliedgenomics.com/home/cfdna-cfrna-isolation/

# DNA LIBRARY PREPARATION SOLUTIONS

# NEXTFLEX® RAPID DNA-SEQ KIT 2.0

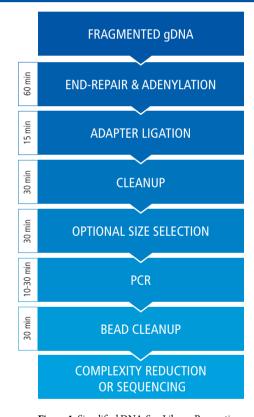
#### A Robust, User-friendly Protocol that Produces Quality Sequencing-ready Libraries

For labs that routinely run DNA sequencing, PerkinElmer® has developed the NEXTFLEX® rapid DNA-seq kit 2.0 that boasts high yields of quality sequencing libraries from fragmented genomic DNA in the form of a convenient, all-inclusive kit that includes the library prep reagents, barcoded adapters, and cleanup beads. Labs have the option of multiplexing up to 384 samples using the NEXTFLEX-HT™ barcodes or up to 1536 samples using the NEXTFLEX® unique dual index barcodes.

Be sure to explore our NextPrep-Mag<sup>™</sup> cfDNA automated isolation kits on the chemagic<sup>™</sup> instruments and our NextPrep-Mag<sup>™</sup> urine cfDNA isolation kits. https://perkinelmer-appliedgenomics.com/home/products/cfdna-cfrna-isolation/

#### **KEY FEATURES**

- Robust yields
- Quality sequencing data
- Genome coverage
- GC bias
- Mapping rate
- Mitigation of duplication rate
- PCR-free compatible workflow
- Low input, down to 1 ng, compatible workflow
- Automated on the Sciclone® G3 NGS and Zephyr® G3 NGS workstation



**Figure 1.** Simplified DNA-Seq Library Preparation Workflow.

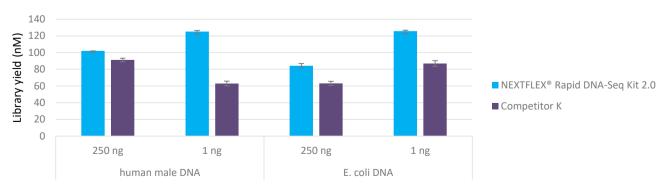
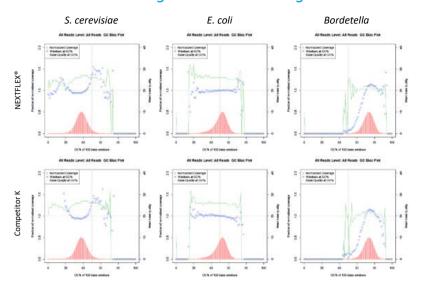


Figure 2. Libraries prepared with NEXTFLEX® rapid DNA-seq kit 2.0 show higher yield than libraries prepared with Competitor K's kit.

#### Reduced GC Bias & Higher Genome Coverage



**Figure 3.** Small genome libraries prepared with the NEXTFLEX° rapid DNA-seq kit 2.0 show less GC bias and higher genome coverage than libraries prepared with Competitor K's kit.



#### **ORDERING INFORMATION**

Catalog #	Kit Name	Quantity
NOVA-5188-01	NEXTFLEX® Rapid DNA-Seq Kit 2.0	8 rxn
NOVA-5188-02	NEXTFLEX® Rapid DNA-Seq Kit 2.0	48 rxn
NOVA-5188-03	NEXTFLEX® Rapid DNA-Seq Kit 2.0	96 rxn
NOVA-5188-11	NEXTFLEX <sup>®</sup> Rapid DNA-Seq Kit 2.0 (with NEXTFLEX-HT <sup>™</sup> barcodes)	8 rxn
NOVA-5188-12	NEXTFLEX <sup>®</sup> Rapid DNA-Seq Kit 2.0 (with NEXTFLEX-HT <sup>™</sup> barcodes)	48 rxn
NOVA-5188-13	NEXTFLEX® Rapid DNA-Seq Kit 2.0 (with NEXTFLEX-HT™ barcodes)	96 rxn

#### **Application note:**

**NEXTFLEX® Rapid DNA-Seq Kit 2.0: DNA Library Preparation that Improves Sequencing Performance** https://perkinelmer-appliedgenomics.com/wp-content/uploads/marketing/NEXTFLEX/rapid\_dna-seq\_2-0/ Rapid\_DNA-Seq\_2-0\_App\_Note\_AG011806\_06\_AP.pdf

To discuss which NEXTFLEX® barcodes are best suited for your Illumina® sequencing platform application, please contact ngs@perkinelmer.com or discover more at https://perkinelmer-appliedgenomics.com/home/products/library-preparation-kits/dna-library-prep-kits/nextflex-rapid-dna-seq-2-0/

# NEXTFLEX® RAPID XP DNA-SEQ KIT

## Get Xtra Performance out of Your Library Preparation

The NEXTFLEX® Rapid XP DNA-Seq kit produces libraries for Illumina® sequencing with high yield, high coverage, low duplication rates, and low GC bias. The kit incorporates an enzymatic fragmentation step into a reproducible, streamlined, and single-tube protocol. This all-inclusive kit contains the fragmentation enzyme, library preparation reagents, and cleanup/size selection beads that have been optimized to

ensure reliable performance. The NEXTFLEX® Rapid XP DNA-Seq library prep kit is compatible with your NEXTFLEX® barcodes of choice, providing a flexible solution. All NEXTFLEX® barcodes are color-balanced and have undergone proprietary purity QC metrics to give you reliable sequencing results.

#### **KEY FEATURES**

- Reliable: Consistent yields and high-quality sequencing metrics
- Convenient: Kit includes fragmentation enzyme and cleanup/size selection beads
- Streamlined: Single step for fragmentation, endrepair, and adenylation
- Flexible: Chemistry optimized with NEXTFLEX® color-balanced barcodes that allow a wide range of multiplexing (2 up to 1536 samples in one run)
- Pre-arrayed library prep reagents available to simplify user experience
- Efficient: Automated on the PerkinElmer® Sciclone®
   G3, the Sciclone® G3 NGSx iQ™ and Zephyr® G3 NGS workstations



 $NEXTFLEX^{\circ}\ Rapid\ XP\ DNA-Seq\ kit\ workflow.$ 

#### **Application notes:**

**NEXTFLEX® Rapid XP DNA-Seq Kit: High-Performance DNA Library Preparation with Enzymatic Fragmentation** https://perkinelmer-appliedgenomics.com/wp-content/uploads/marketing/NEXTFLEX/rapid\_xp/
Rapid XP DNA-Seq App Note AG011810 05 AP.pdf

NEXTFLEX® Rapid XP DNA-Seq it Automated Library Preparation on Sciclone® G3 NGS(x) Workstation https://perkinelmer-appliedgenomics.com/wp-content/uploads/marketing/Sciclone/NEXTFLEX\_Rapid\_XP\_DNA-Seq\_Kit\_Automated\_Library\_Prep\_Sciclone\_G3\_NGSx\_App\_Note.pdf

The Sciclone® G3 NGSx IQ™ Workstation & NEXTFLEX® Rapid XP DNA-seq Pre-Plated Automation Kit https://perkinelmer-appliedgenomics.com/wp-content/uploads/marketing/NEXTFLEX/rapid\_xp/Sciclone\_NGSx\_iQ\_NEX\_Rapid\_XP\_DNA-Seq\_Pre-Plated\_Auto\_Kit\_App\_Note.pdf

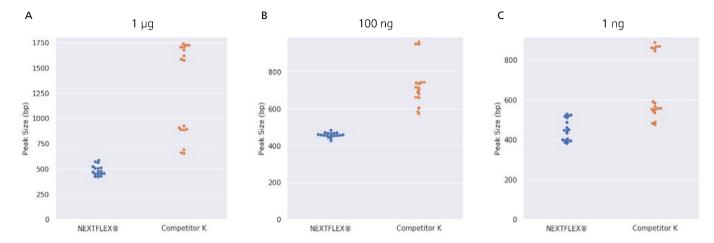


Figure 1. Reliable performance of NEXTFLEX® Rapid XP DNA-seq fragmentation enzyme. DNA is fragmented more evenly and into smaller sizes using the NEXTFLEX® Rapid XP DNA-seq kit than Competitor K's kit. Each dot indicates each library peak size. (A) Libraries were prepared from 1 μg of DNA with 7 minutes of fragmentation with 2 PCR cycles: NEXTFLEX® Rapid XP DNA-seq kit (484 ± 52 bp, 11 %, n=17), Competitor K's kit (1266 ± 451 bp, 36 %, n=17). (B) Libraries were prepared from 100 ng of DNA with 8 minutes of fragmentation with 5 PCR cycles: NEXTFLEX® Rapid XP DNA-seq kit (457 ± 12 bp, 3 %, n=18), Competitor K's kit (730 ± 119 bp, 16 %, n=17). (C) Libraries were prepared from 1 ng of DNA with 15 minutes of fragmentation with 12 PCR cycles: NEXTFLEX® Rapid XP DNA-seq kit (448 ± 54 bp, 12 %, n=17), Competitor K's kit (628 ± 160 bp, 25 %, n=17). All different input libraries were generated from 6 different commercially available DNA samples. (mean ± standard deviation, coefficient of variation).

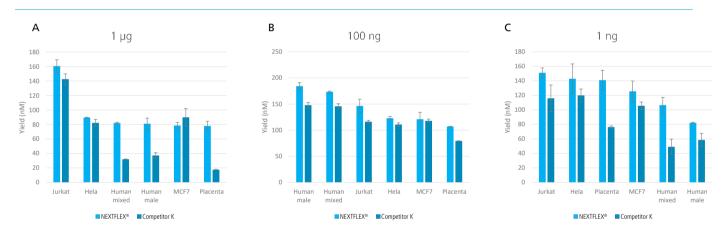


Figure 2. High yield obtained for libraries prepared using the NEXTFLEX® Rapid XP DNA-seq kit. Libraries were prepared using both NEXTFLEX® Rapid XP DNA-seq kit and Competitor K's kit. (A) Libraries were prepared from 1 μg of DNA with 7 minutes of fragmentation with 2 PCR cycles. (B) Libraries were prepared from 100 ng of DNA with 8 minutes of fragmentation with 5 PCR cycles. (C) Libraries were prepared from 1 ng of DNA with 15 minutes of fragmentation with 12 PCR cycles.

Be sure to explore the NEXTFLEX® Rapid XP DNA-seq Pre-Plated Automation Kit and the NEXTFLEX® Unique Dual Index Barcodes for Automation.

#### ORDERING INFORMATION

Catalog #	Kit Name	Quantity
NOVA-5149-01	NEXTFLEX® Rapid XP DNA-Seq Kit	8 rxn
NOVA-5149-02	NEXTFLEX® Rapid XP DNA-Seq Kit	48 rxn
NOVA-5149-03	NEXTFLEX® Rapid XP DNA-Seq Kit	96 rxn
NOVA-5149-103	NEXTFLEX® Rapid XP DNA-seq Pre-Plated Automation Kit	96 rxn

To discuss which NEXTFLEX® barcodes are best suited for your Illumina® sequencing platform application, please contact ngs@perkinelmer.com or discover more at https://perkinelmer-appliedgenomics.com/home/products/library-preparation-kits/dna-library-prep-kits/nextflex-rapid-xp-dna-seq-kit/

# NEXTFLEX® CELL FREE DNA-SEQ KIT 2.0

- Designed for low sample input Only 1 ng of input DNA required
- Accelerated workflow requiring ~ 2 hours, with minimal hands-on time
- Enhanced Adapter Ligation Technology offers a larger number of unique sequencing reads
- Flexible adapter barcode options Bundle kits with up to 1536 unique dual index barcodes
- Compatible with Illumina® sequencing platforms
- Protocols available for automation

## Optimized ctDNA and cfDNA Library Prep

The NEXTFLEX® Cell Free DNA-Seq Kit 2.0 for Illumina® Library Preparation is optimized for library construction from low input amounts of circulating tumor DNA (ctDNA) or cell free fetal DNA (cffDNA) isolated from cell free fluids. Common research applications include sequencing circulating tumor cells using liquid biopsies (ctDNA) and sequencing cell free fetal DNA (cffDNA).

## Optimized for Low-Input DNA from Cell-Free Fluids

The NEXTFLEX® Cell Free DNA-Seq Kit 2.0 can be used to construct libraries compatible with Illumina® platforms from 1 ng of DNA in two hours or less (Figure 1). This kit delivers high coverage and reduced bias, along with flexible multiplexing options. The NEXTFLEX® Cell Free DNA-Seq Kit 2.0 also features Enhanced Adapter Ligation Technology, offering improved ligation efficiency, and resulting in library preps with a larger number of unique sequencing reads (Figure 2).



Figure 1. The NEXTFLEX® Cell Free DNA-Seq 2.0 Flow Chart

#### Library validation

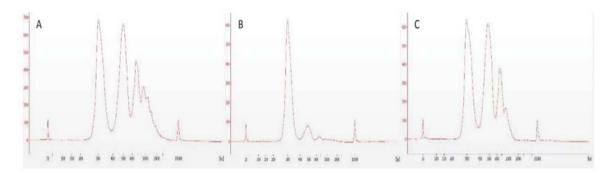


Figure 2. Nucleosome Enriched Library

10ng input of cfDNA was used for the enrichment (or no enrichment) and prepared using the rapid cell-free DNA-seq kit standard protocol A) No enrichment

- B) Mono-nucleosome enrichment
- C) Mono-, di-, tri-nucleosome enrichment

#### Flexible Multiplexing Options

We offer flexible multiplexing options compatible with the NEXTFLEX® Cell Free DNA-Seq Kit 2.0. The kit is designed to be used with the NEXTFLEX® NGS barcodes.

Users interested in different degrees of multiplexing should contact NGS@perkinelmer.com

Be sure to explore our NextPrep-Mag<sup>™</sup> cfDNA automated isolation kits on the chemagic<sup>™</sup> instruments and our NextPrep-Mag<sup>™</sup> urine cfDNA isolation kits. https://perkinelmer-appliedgenomics.com/home/products/cfdna-cfrna-isolation/

Cat #	Product	Quantity
NOVA-5150-01	NEXTflex® Cell Free DNA-Seq Kit 2.0	8 rxn
NOVA-5150-02	NEXTflex® Cell Free DNA-Seq Kit 2.0	48 rxn

<sup>\*</sup> compatible with a wide range of NEXTFLEX® barcodes, please inquire.

#### **Application notes:**

https://perkinelmer-appliedgenomics.com/wp-content/uploads/marketing/High-Throughput-End-to-End-Cell-Free-DNA-Analysis-Workflow-from-Plasma.pdf

 $https://perkinelmer-applied genomics.com/wp-content/uploads/2019/12/cfDNA-Automated-Kit-App-Note-AG021902\_22\_AP\_print.pdf$ 

# NEXTFLEX® BISULFITE-SEQ KIT

- Compatible with total bisulfite-seq and reduced representation
- Single nucleotide resolution of methylation sites
- Uracil insensitive polymerase designed for bisulfite-converted DNA
- Methylome-level assessment with broad genome coverage
- Enhanced adapter ligation technology with NEXTFLEX® Ligation
- Bead-based cleanup
- Automation-friendly workflow is compatible with liquid handlers
- Methylated barcoded adapters for multiplexing
- Functionally validated with Illumina® sequencing platforms

#### Designed for RRBS and WGBS

The NEXTFLEX® Bisulfite-Seq Kit is designed work with reduced representation or total bisulfite-converted DNA libraries and is compatible with single, paired-end and multiplexed DNA libraries on Illumina® sequencing platforms. NEXTFLEX® Bisulfite-Seq Kit can be used with reduced representation by utilizing the NEXTFLEX® Msp1 Restriction Enzyme, which is available separately, that leads to selective amplification of CpG regions, resulting in focused sequence depth. The NEXTFLEX® Bisulfite-Seq Kit can also be used without the Msp1 Restriction Enzyme for genomewide methyl-seq analysis, including under-represented CpG regions.

#### **Complete Methylome Analysis**

The NEXTFLEX® Bisulfite-Seq Kit is a versatile kit designed to facilitate assessment of the methylation state of the genome and simplify workflow by using master mixed reagents and magnetic bead based cleanup to reduce pipetting and eliminate time-consuming steps in library preparation. In addition, the availability of up to 24 methylated NEXTFLEX® Bisulfite-Seq barcoded adapters makes multiplexing simple. This kit features Enhanced Adapter Ligation Technology, resulting in library preps with a larger number of unique sequencing reads. This specially designed NEXTFLEX® ligation enzymatic mix allows users to perform ligations with longer adapters and superior ligation efficiencies.

Cat #	Product	Quantity
NOVA-5119-01	NEXTFLEX® Bisulfite-Seq Kit	8 rxns
NOVA-5119-02	NEXTFLEX® Bisulfite-Seq Kit	48 rxns
NOVA-511911	NEXTFLEX® Bisulfite-Seq Barcodes - 6	48 rxns
NOVA-511912	NEXTFLEX® Bisulfite-Seq Barcodes - 12	96 rxns
NOVA-511913	NEXTFLEX® Bisulfite-Seq Barcodes - 24	192 rxns
NOVA-511921	NEXTFLEX® MSP1 Restriction Enzyme	8 rxns
NOVA-511922	NEXTFLEX® MSP1 Restriction Enzyme	48 rxns

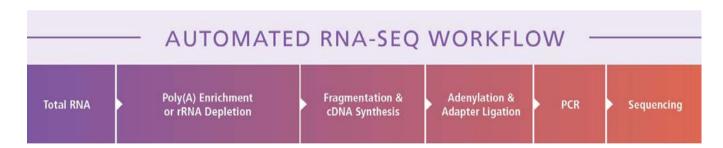
# RNA LIBRARY PREPARATION SOLUTIONS

# NEXTFLEX® RAPID DIRECTIONAL RNA-SEQ KIT 2.0

- Reliable: High coverage uniformity with low duplication rate
- Convenient: Optimized with reverse transcriptase and cleanup/size selection beads
- Streamlined: Simple protocol validated with NEXTFLEX® poly(A) beads 2.0 and NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat)
- Flexible: Designed to work with NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes that allow a wide range of multiplexing (2 up to 384 samples in one run)\*
- Efficient: Automated on the Sciclone® G3 NGSx, Sciclone® G3 NGSx iQ™, and Zephyr® G3 NGS workstations

#### New & Improved Library Preparation Kit for your RNA Sequencing Needs

The NEXTFLEX® rapid directional RNA-seg kit 2.0 produces libraries for Illumina® sequencing instruments with high coverage uniformity, low duplication rates, strand specificity and minimal rRNA contamination when used with the NEXTFLEX® Poly(A) Beads 2.0 (10 ng - 5 μg) or NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) (5 ng - 1 µg). This kit includes reverse transcriptase, necessary library preparation reagents, and cleanup/size selection beads optimized to ensure reliable performance. The kit involves a simple library preparation protocol hat has been validated with the updated NEXTFLEX® poly(A) beads 2.0 and NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) to accommodate total RNA as input. The NEXTFLEX® rapid directional RNA-seq kit 2.0 is designed to be used with NEXTFLEX® RNASeg 2.0 Unique Dual Index Barcodes (6.25µM), which are color-balanced and have undergone proprietary purity QC metrics to generate reliable sequencing results for every sample.

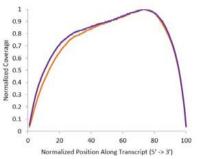


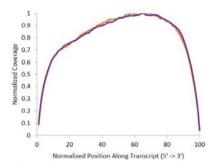
Can be automated on three PerkinElmer platforms. See "Automation" part of this booklet.

Cat #	Product	Quantity
NOVA-5198-0X	NEXTFLEX® RAPID DIRECTIONAL RNA-SEQ KIT 2.0	8, 48 or 96 rxns
NOVA-51296X	NEXTFLEX® RIBONAUT rRNA DEPLETION (HUMAN, MOUSE, RAT)	8, 48 or 96 rxns
NOVA-51299X	NEXTFLEX® POLY(A) BEADS 2.0	8, 48 or 96 rxns
NOVA-51292X	NEXTFLEX® RNA-SEQ 2.0 UNIQUE DUAL INDEX BARCODES (BARCODE 1-384)	192 rxns
NOVA-524100	NEXTFLEX® RNA-SEQ 2.0 UNIQUE DUAL INDEX BARCODES (BARCODE 1-1,536)	3,072 rxns

<sup>\*</sup> set of 1536 UDIs available upon request, please inquire

## NEXTFLEX® RAPID DIRECTIONAL RNA-SEQ KIT 2.0 with NEXTFLEX® POLY(A) BEADS 2.0





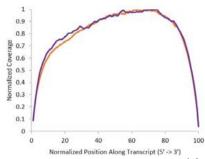


Figure 1. The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrates even coverage along transcripts compared to the Competitor N kit. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® Poly(A) Beads 2.0 and the Competitor N Poly(A) enrichment kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Competitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using paired-end mode (2×76 bp). Reads were trimmed using cutadapt and mapped to the Gencode v30 reference using bowtie2. The coverage along transcripts was calculated using the BBMap pileup tool.

#### Lower Duplication Rate

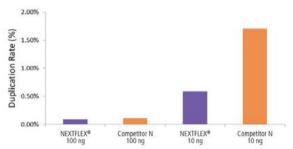


Figure 2. The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrate lower duplication rate compared to the Competitor N kit. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent #740000) using the NEXTFLEX® Poly(A) Beads 2.0 and the Competitor N Poly(A) enrichment kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Competitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using paired-end mode (2x76 bp). Reads were trimmed using cutadapt, mapped to the Gencode v30 reference using bowtie2, and randomly downsampled to 100k reads. Duplication rate was calculated using the fastp all-in-one FASTQ preprocessor.

#### **Superior Strandedness**

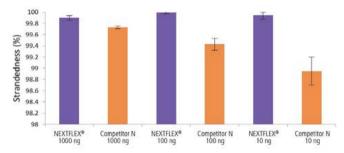


Figure 3. The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrate superior strandedness than the Competitor N kit. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent #740000) containing ERCC RNA Spike-In mix (Thermo Fisher® Scientific #4456740) using the NEXTFLEX® Poly(A) Beads 2.0 and the Competitor N Poly(A) enrichment kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Competitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using paired-end mode (2x76 bp). Reads were trimmed using cutadapt and mapped to the ERCC92 reference using bowtie2. Strandedness was calculated using SAMtools.

#### Lower Levels of rRNA Contamination

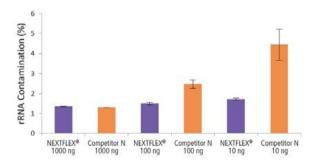
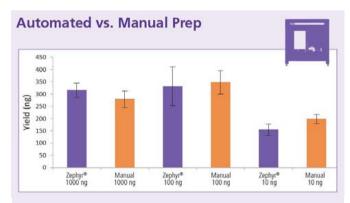


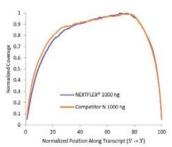
Figure 4. The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 delivers libraries containing lower levels of rRNA contamination than the Competitor N kit. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent #740000) using the NEXTFLEX® Poly(A) Beads 2.0 and the Competitor N Poly(A) enrichment kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Competitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using paired-end mode (2x76 bp). The reads were trimmed using cutadapt and the percent of rRNA was determined by using bowtie2 to map reads to human rRNA. The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrated superior removal of \$5\$, \$5.8\$, 12\$, 16\$, 18\$, and 28\$ rRNA species compared to the Competitor N kit.

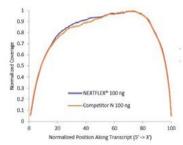


**Figure 5.** Libraries prepared using the Zephyr $^\circ$  G3 NGS workstation and manually deliver comparable yields using the NEXTFLEX $^\circ$  Rapid Directional RNA-Seq kit 2.0. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent $^\circ$  #740000) using the NEXTFLEX $^\circ$  Poly(A) Beads 2.0. Libraries were generated using the NEXTFLEX $^\circ$  Rapid Directional RNA-Seq kit 2.0. Final library concentrations were quantified using the Qubit $^\circ$  2.0 fluorometer (Thermo Fisher $^\circ$  Scientific #Q32866).

## NEXTFLEX® RAPID DIRECTIONAL RNA-SEQ KIT 2.0 with NEXTFLEX® RIBONAUT™ rRNA DEPLETION KIT

#### Robust Gene Coverage





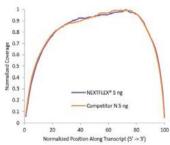


Figure 1. The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrates even coverage along transcripts compared to the Competitor N kit. rRNA-depleted total RNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) and the Competitor N rRNA-depletion kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Competitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using single-end mode (1x151 bp). Reads were trimmed using cutadapt, mapped to the Gencode v30 reference transcriptome using bowtie2, and randomly downsampled to 720k reads. The coverage along transcripts was calculated using the BBMap pileup tool.

#### Low Duplication Rate

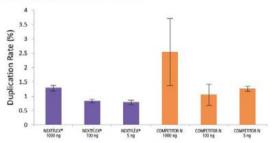


Figure 2. The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrate low duplication rates compared to the Competitor N kit. rRNA-depleted total RNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) and the Competitor N rRNA-depletion kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Competitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using single-end mode (1x151 bp). Reads were trimmed using cutadapt, mapped to the Gencode v30 reference transcriptome using bowtie2, and randomly downsampled to 28k reads. Duplication rate was calculated using the fastp all-in-one FASTQ preprocessor.

#### Reliable Directionality

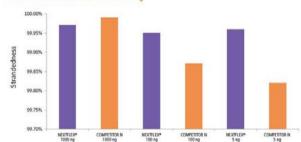


Figure 3. The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrates comparable directionality relative to the Competitor N kit. rRNA-depleted total RNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) and the Competitor N rRNA-depletion kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Competitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using single-end mode (1x151 bp). Reads were trimmed using cutadapt and mapped to the Gencode v30 reference transcriptome using bowtie2. Reads from respective samples were combined and downsampled for a total of 800k reads each. Strandedness was calculated using the fastp all-in-one FASTQ preprocessor.

#### Low Levels of rRNA Contamination

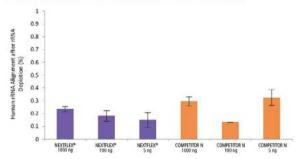


Figure 4. The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 delivers libraries containing low levels of rRNA contamination compared to the Competitor N kit. rRNA-depleted total RNA was isolated from Universal Human Reference RNA (Agilent #740000) using the NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) and the Competitor N rRNA-depletion kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Competitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using single-end mode (1x151 bp). The reads were trimmed using cutadapt and the percent of rRNA was determined by using bowtie2 to map reads to human rRNA. The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrated superior removal of 5S, 5.8S, 12S, 16S, 18S, and 28S rRNA species compared to the Competitor N kit.

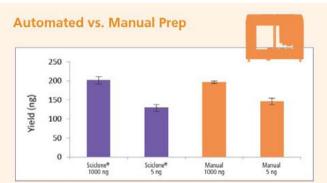


Figure 5. Libraries prepared using the Sciclone® G3 NGSx workstation and manually deliver comparable yields using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0. rRNA-depleted total RNA was isolated from Universal Human Reference RNA (Agilent #740000) using the NEXTFLEX® RiboNaut™ rRNA depletion kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0. Final library concentrations were uantified using the Qubit® 2.0 fluorometer (Thermo Fisher Scientific #Q32866).

## NEXTFLEX® RIBONAUT™ rRNA DEPLETION KIT (HUMAN / MOUSE / RAT)

## New rRNA depletion for total RNA-seq applications

- Hybridization and bead-based protocol for rRNA depletion
- • Optimized for use with 5 ng – 1  $\mu g$  of total RNA as starting material
- Validated using the NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0 and automated on the Sciclone® G3 NGSx and Zephyr® G3 NGS workstations

### The NEXTFLEX® RiboNaut™ Kit Ensures Effective rRNA Depletion using Hybridization Technology

The NEXTFLEX® RiboNaut™ rRNA depletion kit (human / mouse / rat) is an effective method to remove rRNA contamination while enabling labs to interrogate additional RNA species in a sample, not only limited to intact mRNAs. The kit utilizes subtractive hybridization technology consisting of a mixture of biotinylated oligos complementary to rRNAs to pull them out of the sample using streptavidin-coated magnetic beads. The kit has been optimized for use with 5 ng − 1 μg of total RNA as starting material to deplete cytoplasmic and mitochondrial rRNAs. The kit has been verified to be compatible with human, mouse and rat, and it may be compatible with other mammalian species.

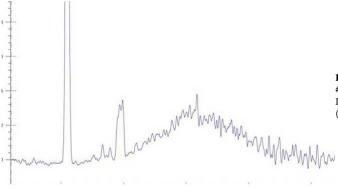


Figure 1. Example of 1 µg of Universal Human Reference RNA (Agilent\* #740000) after rRNA depletion. 1 µL of rRNA-depleted RNA was run on the LabChip\* GXII Touch\* HT instrument using the RNA Pico Assay Reagent Kit (# CLS960012) and a DNA/RNA/Charge Variant Assay (# 760435).

#### **Optimized to Produce Robust Performance Data from Total RNA Samples**

The NEXTFLEX® RiboNaut™ rRNA Depletion kit (human / mouse / rat) shows robust performance data using total RNA samples for gene coverage along a transcript, duplication rate, directionality, and rRNA contamination. Prior to rRNA depletion and subsequent RNA-seq, qualification of total RNA integrity using the LabChip® GXII Touch™ HT instrument.

As an alternative mRNA-Seq solution for intact poly(A)-tailed mRNA species, PerkinElmer offers the NEXTFLEX® Poly(A) Beads 2.0 for use NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 or other applications.

Cat #	Product	Quantity
NOVA-512961	NEXTFLEX® RIBONAUT RRNA DEPLETION KIT (HUMAN, MOUSE, RAT)	8 rxns
NOVA-512962	NEXTFLEX® RIBONAUT RRNA DEPLETION KIT (HUMAN, MOUSE, RAT)	48 rxns
NOVA-512963	NEXTFLEX® RIBONAUT RRNA DEPLETION KIT (HUMAN, MOUSE, RAT)	96 rxns

## NEXTFLEX® POLY(A) BEADS 2.0

#### Updated magnetic bead-based nRNA purification for RNA sequencing applications

- 10 ng 5 µg total starting RNA
- Magnetic bead-based protocol
- No organic solvents or precipitation step required
- Validated using the NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0
- Magnetic bead-based protocol for purification of pure, intact mRNA upstream of RNA-Seq library prep (no organic solvents or precipitation step required)
- $\bullet$  Optimized for use with 10 ng – 5  $\mu g$  of total RNA as starting material
- Validated using the NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0 and automated on the Sciclone® G3 NGSx and Zephyr® G3 NGS workstations

### NEXTFLEX® Poly(A) Beads 2.0 for Reducing Ribosomal rRNA Reads in RNA-seq Libraries

Several approaches have been used to remove ribosomal RNA (rRNA) from total RNA samples for RNA-Seq library preparation. Removal of rRNA avoids the waste of reagents and bioinformatics resources to sequence and align ~85% of total RNA comprising rRNA (which is generally not a target of interest in NGS sequencing experiments). PerkinElmer's NEX-TFLEX® Poly(A) beads 2.0 kit offers an easy and cost-effective method for removing rRNA in RNA-Seq libraries. This product takes advantage of the tract of adenosine residues present at the 3' ends of a vast majority of protein-coding mRNAs. Hybridization of these poly(A) tails to tracts of complementary thymidines ("oligo dT") immobilized on solid supports allows the retrieval of poly(A)+ RNAs by recovering the solid support along with the hybridized RNA complexes.



#### The NEXTFLEX® Poly(A) Beads 2.0 Kit Offer a Streamlined Solution for High mRNA Recovery

The NEXTFLEX® Poly(A) Beads 2.0 kit provides improved mRNA yields, lower rRNA contamination, and a more simplified protocol. The kit offers a convenient method for the purification of pure, intact mRNA upstream of RNA-Seq library prep. Poly(A)-tailed mRNA is isolated via magnetic beads conjugated to oligo(dT), and separation using a magnetic stand allows for high mRNA recovery. The intact mRNA is eluted in small volumes, thereby eliminating the need for precipitation. These beads have been validated with the new and improved NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0.

Figure 1. Example of 1 µg of Universal Human Reference RNA (Agilent\* #740000) after Poly(A) enrichment using the NEXTFLEX\* Poly(A) Beads 2.0. 3 µL Poly(A) enriched RNA was run on the LabChip\* GXII Touch™ HT instrument using the RNA Pico Assay Reagent Kit (# CLS960012) and a DNA/RNA/Charge Variant Assay LabChip (# 760435)

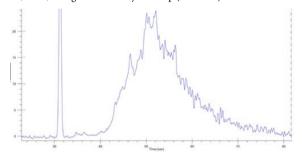
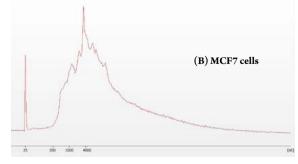


Figure 2. NEXTFLEX® Poly(A) Beads 2.0 yield clean mRNA with minimal rRNA carry-over from 5 μg of total RNA input from (A) mouse brain and (B) MCF7 cells. ~1.5% rRNA carry-over was observed based on sequencing data.



Cat #	Product	Quantity
NOVA-512991	NEXTFLEX® POLY(A) BEADS 2.0	8 rxns
NOVA-512992	NEXTFLEX® POLY(A) BEADS 2.0	48 rxns
NOVA-512993	NEXTFLEX® POLY(A) BEADS 2.0	96 rxns

# NEXTFLEX® SMALL RNA-SEQ KIT V3

#### **KEY FEATURES**

- Completely automated protocol with inputs ≥200 ng total RNA
- Greater discovery/detection rates decrease sequencing cost
- Substantially reduces ligation-associated bias
- Automated on the PerkinElmer® Sciclone® G3 NGS workstation and Zephyr® G3 NGS workstation
- Now available with up to 384 UDIs on certain Illumina® platforms.

Be sure to explore our cell free RNA isolation kit NextPrep™ Magnazol™ cfRNA Isolation Kit https://perkinelmer-appliedgenomics.com/home/cfdna-cfrna-isolation/

#### Automated Gel-Free or Low Input Small RNA Library Prep

The NEXTFLEX® small RNA-seq kit v3 uses patented and patent-pending technology to provide a reduced-bias small RNA library preparation solution for Illumina® sequencing platforms with gel-free or low-input options. Our approach to reducing ligation-associated bias involves the use of adapters with randomized bases at the ligation junctions, resulting in greatly decreased bias in comparison to standard protocols. This reduction in bias results in data that more accurately represent abundances of small RNAs in the starting material. In addition, reduction of bias allows more miRNAs to be detected with fewer total reads, increasing efficiency and reducing cost for small RNA sequencing.

The NEXTFLEX small RNA-Seq kit v3 also allows for gel-free small RNA library preparation. This is possible thanks to the dual approach used for adapter-dimer reduction. Unprecedented reduction of adapter-dimer formation allows completely gel-free small RNA library prep when starting with ≥200 ng of total RNA, which in turn allows for a fully automated solution on the PerkinElmer® Sciclone® G3 NGS Workstation, the Sciclone® G3 NGSx iQTM workstation and Zephyr® G3 NGS Workstation.

The NEXTFLEX® small RNA-seq v3 automation kit with UDIs uses the same popular chemistry and build in a new dimension of data quality, integrity, and accuracy with the integration of Unique Dual Indexes (UDIs) for Illumina® sequencing on the MiSeq®, HiSeq® 2000/2500, and Nova-Seq® platforms. The addition of UDI barcodes for small RNA sequencing allows confident multiplexing of up to 192 samples, all while mitigating the risk of index hopping and spread of signal that can occur on a patterned flow cell. The purity of each index sequence is validated by sequencing for high confidence in the resulting sequencing data quality.

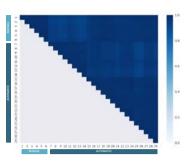
The NEXTFLEX® small RNA-seq v3 automation kit with UDIs is compatible with cell-free RNA, such as RNA isolated from plasma. Users who wish to deplete the abundant tRNA fragments and Y RNA fragments found in different sample types should also adopt the use the NEXTFLEX® tRNA/YRNA blocker (not included in kit).

	NEXTFLEX® Small RNA-Seq Kit v3	TruSeq® Small RNA Library Prep Kit	NEBNext® Small RNA Library Prep Kit	QIAseq® miRNA Library Prep Kit
Randomized Adapters for Reduced Bias	Yes	No	No	No
Gel-free Protocol	Yes	No	Yes	Yes
Automation Script Available	Yes	No	No	No
All Enzymes Included	Yes	No	Yes	Yes
Unique Dual Indexes Included for Automation	Yes*	No	No	No
Up to 192 Barcodes Available	Yes	No	No	No

 $<sup>^{*}</sup>$  on the Illumina MiSeq, HiSeq 2000/2500 instruments. These primers behave as single index primers on other Illumina sequencers.

#### **Sequencing Metrics**

	Reads Processed	Insert <15 bp (%)	Reads Passing Filter	miRBase Alignment Rate of Filtered Reads (%)	miRBase Alignment Rate of All Reads (%)	miRNA Groups Detected
Manual Average	143287.0	23.9	108982.5	61.9	47.1	376.2
Manual SD	0.0	4.0	5736.2	2.7	3.5	14.4
Automated Average	143287.0	21.8	112116.8	60.1	47.0	392.6
Automated SD	0.0	3.4	4838.9	4.9	3.3	10.7



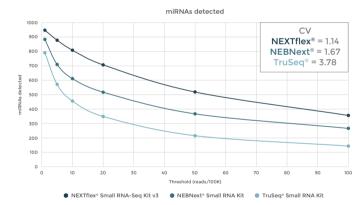
Pair-wise correlation of miRNA expression in replicate libraries created manually and on the PerkinElmer\* Sciclone\* NGS Workstation.

"We recommend using the BiooScientific NEXTFLEX kit, as it detects the largest number of miRNAs, owing to its 4 N random adaptor sequence that ameliorates ligation bias."

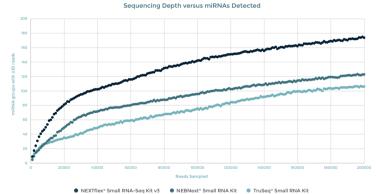
Yeri et al. Evaluation of commercially available small RNASeq library preparation kits using low input RNA. BMCGenomics. 2018. 19:331

"For the detection of human (unmodified) miRNAs the standard NEXTFLEX protocol performed best..."

Dard-Dascot et al. Systematic comparison of small RNA library preparation protocols for next-generation sequencing. BMCGenomics. 2018. 19:118



miRNAs detected at various thresholds - Sequencing results from small RNA libraries created in triplicate from 1 ng of Miltenyi® miRXplore® Universal Reference, an equimolar mixture of 963 miRNAs. The number of miRNAs detected at various thresholds is shown. The inset shows the Coefficient of Variation of the 963 miRNAs in each sample.



**Sequencing Depth vs. miRNAs Detected** - Small RNA libraries were created in triplicate from the indicated amount of human brain total RNA. The indicated number of reads was sampled from each library and the average number of miRNA groups with ≥20 reads determined. The inset shows the number of reads required to detect 100 miRNA groups at a threshold of ≥20 reads.

#### ORDERING INFORMATION

Catalog #	Kit Name	Quantity
NOVA-5132-05	NEXTFLEX® Small RNA-Seq Kit v3 (8 barcodes)	8 rxns
NOVA-5132-06	NEXTFLEX® Small RNA-Seq Kit v3 (48 barcodes)	48 rxns
NOVA-5132-18	NEXTFLEX® Small RNA-Seq Kit v3 with Unique Dual Index Barcodes (1 – 96) for Automation	96 rxn
NOVA-5132-19	NEXTFLEX® Small RNA-Seq Kit v3 with Unique Dual Index Barcodes (97-192) for Automation	96 rxn
NOVA-5132-20	NEXTFLEX® Small RNA-Seq Kit v3 with Unique Dual Index Barcodes (193-288) for Automation	96 rxn
NOVA-5132-21	NEXTFLEX® Small RNA-Seq Kit v3 with Unique Dual Index Barcodes (289-384) for Automation	96 rxn
NOVA-5132-22	NEXTFLEX® Small RNA-Seq Kit v3 with Unique Dual Index Barcodes (1-48)	48 rxns
NOVA-5132-23	NEXTFLEX® Small RNA-Seq Kit v3 with Unique Dual Index Barcodes (49-96)	48 rxns
NOVA-5132-24	NEXTFLEX® Small RNA-Seq Kit v3 with Unique Dual Index Barcodes (97-144)	48 rxns
NOVA-5132-25	NEXTFLEX® Small RNA-Seq Kit v3 with Unique Dual Index Barcodes (145-192)	48 rxns
NOVA-5132-26	NEXTFLEX® Small RNA-Seq Kit v3 with Unique Dual Index Barcodes (193-240)	48 rxns
NOVA-5132-27	NEXTFLEX® Small RNA-Seq Kit v3 with Unique Dual Index Barcodes (241-288)	48 rxns
NOVA-5132-28	NEXTFLEX® Small RNA-Seq Kit v3 with Unique Dual Index Barcodes (289-336)	48 rxns
NOVA-5132-29	NEXTFLEX® Small RNA-Seq Kit v3 with Unique Dual Index Barcodes (337-384)	48 rxns

#### White papers:

#### Eliminating Bias in the Characterization of Small RNAs Derived from Extracellular Vesicles

 $\underline{https://perkinelmer-appliedgenomics.com/wp-content/uploads/marketing/NEXTFLEX/small\_rna/Eliminating-Bias-in-the-Characterization-of-Small-RNAs-Derived-from-Extracellular-Vesicles.pdf$ 

#### Applications for Small RNA-Seq in the Development of Biomarkers for Cognitive Diseases

https://perkinelmer-appliedgenomics.com/wp-content/uploads/marketing/NEXTFLEX/small\_rna/Small-RNA-Seq-in-the-Development-of-Biomarkers-for-Cognitive-Diseases.pdf

#### Increasing Ligation Efficiency and Discovery of miRNAs for Small RNA NGS Sequencing Library Prep with Plant Samples

 $\underline{https://perkinelmer-appliedgenomics.com/wp-content/uploads/marketing/NEXTFLEX/miRNA/Increasing-Ligation-Efficiency-and-Discovery-of-miRNAs-for-Small-RNA-NGS-Sequencing-Library-Prep-with-Plant-Samples.pdf$ 

#### Reduced-Bias Small RNA Library Preparation with Gel-Free or Low-Input Options

https://perkinelmer-appliedgenomics.com/wp-content/uploads/marketing/NEXTFLEX/miRNA/Reduced-Bias-Small-RNA-Library-Preparation-Final.pdf

#### **Small RNA Library Preparation from Human Biofluids**

https://perkinelmer-appliedgenomics.com/wp-content/uploads/2020/03/Small-RNA-Library-Prep-Human-Biofluids-App-Note-AG031911\_01\_AP\_Print.pdf

## NEXTFLEX® tRNA/YRNA BLOCKERS FOR SMALL RNA-SEQ AND RNA-SAQ APPLICATIONS

Frustrated that your small RNA sequencing reads are being depleted by abundant tRNA and YRNA fragments that don't contribute to your research? Annoyed that you are losing reads that are going to waste? Just block it, then prep!

To maximize the efficiency of the patented and patent-pending reduced ligation bias technology of the NEXT-FLEX® Small RNA-Seq Kit v3, which also excels at preparing libraries using cfRNA – such as RNA isolated from plasma – labs can now couple this popular chemistry with the NEXTFLEX® tRNA/YRNA blockers. These blockers can be seamlessly integrated into the library prep setup to allow for more miRNA discovery for the same sequencing depth.

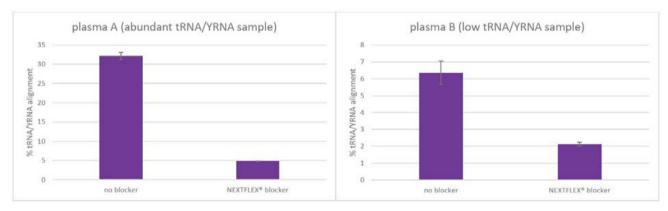


Figure 1. Blocking efficiency of the NEXTFLEX $^{\circ}$  tRNA/YRNA blockers. Libraries were prepared using the NEXTFLEX $^{\circ}$  small RNA-seq kit v3 from cf RNA isolated from 600  $\mu$ L of commercially available plasma that had either high tRNA/YRNA abundance or low tRNA/YRNA abundance using a modified protocol on the PerkinElmer $^{\circ}$  Zephry $^{\circ}$  G3 NGS workstation. Libraries were sequenced on the Illumina $^{\circ}$  MiSeq $^{\circ}$  instrument, and results were analyzed for  $^{\circ}$  alignment to tRNA/YRNA using a proprietary script.

Please inquire at NGS@perkinelmer.com for blockers against abundant miRNAs in blood.

Cat #	Product	Quantity
NOVA-51312X	NEXTflex® tRNA/YRNA Blockers	8, 48, 96 rxn
NOVA-5132-0X	NEXTFLEX® Small RNA-Seq Kit v3	8 & 48 rxn (Manual)
NOVA-5132-18 to -21	NEXTFLEX® Automated Small RNA-Seq Kit v3 with UDIs* (UDI 1 384)	96 rxns (Automation)
NOVA-5132-22 to -28	NEXTFLEX® Small RNA-Seq Kit v3 with UDIs* (UDI 1-384)	48 rxns (Manual)

<sup>\*</sup> These barcodes behave as single index barcodes, not as UDIs, when run on an Illumina\* MiniSeq\*, HiSeq\* 3000/4000, HiSeq\*X, NextSeq\* or NovaSeq\* platform. Up to 384 samples can be multiplexed at a time using barcodes 1 – 384.

## NEXTFLEX® COMBO-SEQ™ mRNA/miRNA KIT

#### **KEY FEATURES**

- Combined library prep workflow for both small RNA and mRNA sequencing
- Completely gel-free protocol from inputs as low as 5 ng
- Compatible with total RNA inputs, no poly(A) selection or rRNA depletion required
- Complete kit solution, including cleanup/size selection beads and barcodes
- Automated on the PerkinElmer® Sciclone® G3 NGS/ NGSx workstation

### Facilitating Both High- and Low-throughput Needs for Illumina® Multiplexing

Eight UDI barcoded primers are included in the small reaction size NEXTFLEX® Combo-Seq™ kit and forty-eight UDI barcoded primers are included in the large reaction size kit. UDIs read as single index reads on certain instruments, including the Illumina® MiniSeq®, NextSeq®, NovaSeq®, HiSeq® 3000 or HiSeq® 4000 platforms. If interested in reading both indices when using these sequencers, contact NGS@perkinelmer.com for possible solutions.

## Small RNA & mRNA NGS library preparation in a single workflow

Gel-free, Combined Small RNA and mRNA Library Prep with Randomized Adapters to Reduce Ligation Bias and UDIs to Prevent Sample Mis-assignment on Illumina® Platforms

The NEXTFLEX® Combo-Seg™ Kit enables the user to generate combined mRNA and small RNA libraries in a single workflow using 5 ng - 100 ng of total RNA inputs, without requiring upfront costly and tedious rRNA depletion or poly(A) selection. The kit utilizes patented and patent-pending technology that allows processing of mRNA fragments and small RNA for library construction. Like the best-in-class, NEXTFLEX® small RNA-seg kit v3, the NEXTFLEX® Combo-Seg™ mRNA/miRNA library preparation kit utilizes adapters with randomized ends to greatly reduce bias compared to standard protocols. This allows a more accurate representation of small RNA in the starting material and cost-saving due to efficient sequencing. Adapter-dimer reducing techniques built into the protocol enables gel-free workflow from inputs as low as 5 ng of total RNA. As a result, the kit is streamlined and Automation-friendly from start-to-finish. Libraries with robust yield can be prepared within one day using a manual or automated protocol.

The NEXTFLEX® Combo-Seq™ kit has already yielded interesting results and taken my research in new directions. The ability to simultaneously measure miRNAs and their mRNA targets from small amounts of RNA has been truly invaluable.

Kristen Thomas, PhD, Children's Research Hospital, Tennessee, USA

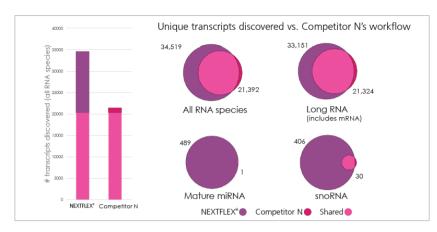


Figure 1. NEXTFLEX® Combo-Seq™ mRNA/miRNA kit discovers ~60% more transcripts compared to Competitor N's mRNA-seq workflow. Libraries were generated from 20 ng of Biochain Human Universal RNA (R4234565) using the NEXTFLEX® Combo-Seq mRNA/ miRNA Kit and Competitor N's directional mRNA-seq workflow. Sequencing libraries were downsampled to 10M reads and processed using the exceRpt pipeline (https://hub. docker.com/r/rkitchen/excerpt). Pipeline output files were used to enumerate "transcripts discovered." Transcripts were only marked "discovered" if >= 3 reads mapped to a given transcript and if each read aligned in the appropriate direction (sense for the NEXTFLEX® kit and antisense for Competitor N's kit). For "long RNA," transcripts were counted using the "geneLevel" read counts files. In the figure, pink indicates transcripts which were detected by both workflows, whereas purple and red refer to NEXTFLEX® and Competitor N, respectively.

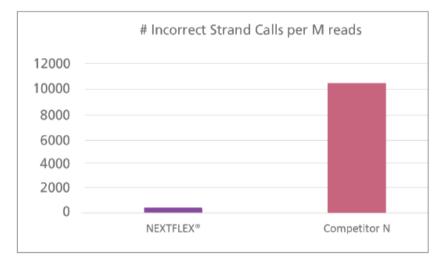


Figure 2. The NEXTFLEX® Combo-Seq™ mRNA/miRNA kit gives higher confidence in strand calls compared to Competitor N's directional mRNA-seq workflow. Reads were mapped to synthetic ERCC's to determine orientation. NEXTFLEX® chemistry has >99.9% directionality whereas Competitor N is only ~99% directional (data not shown), which allows for higher directionality/ strandedness and lower rate of false-positives regarding directionality.

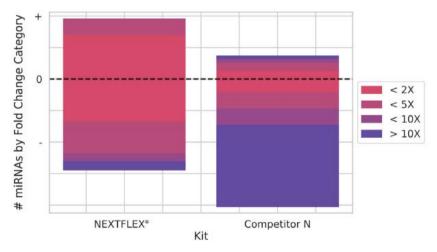


Figure 3. The NEXTFLEX® Combo-Seq™ mRNA/miRNA kit gives more accurate representation of miRNAs than Competitor N's small RNA-seq kit. In order to assess bias, libraries were generated from a pool of 963 equimolarly pooled miRNAs using the NEXTFLEX® Combo- Seq™ mRNA/miRNA kit and a commercially available small RNA-seq kit from Competitor N. Individual miRNAs were binned into categories based on their representation in the sequencing library in terms of fold-change from expected. With the NEXTFLEX® Combo-Seq™ mRNA/miRNA kit, only 5.8% of miRNAs deviate more than 10-fold from the expected value, compared with 30.1% of miRNAs with Competitor N.

Cat#	Product	Quantity
NOVA-5139-01	NEXTFLEX® Combo-Seq mRNA/miRNA Kit	8 rxns
NOVA-5139-02	NEXTFLEX® Combo-Seq mRNA/miRNA Kit	48 rxns
NOVA-5139-53	NEXTFLEX® Combo-Seq™ mRNA/miRNA Kit (Barcodes 1-96) Automation	96 rxns
NOVA-5139-54	NEXTFLEX® Combo-Seq <sup>™</sup> mRNA/miRNA Kit (Barcodes 97-192) Automation	96 rxns



## NEXTFLEX® NGS HYBRIDIZATION PANELS

- Optimized workflow, from one provider
- All-inclusive reagent set containing: DNA fragmentation reagents, barcodes, library preparation reagents, sequence specific blockers and hybrid capture reagents
- Meticulously designed for effective capture of single nucleotide variants (SNV), small insertions and deletions (indels) and copy number variations (CNV)
- Effective library preparation from small amount of DNA – complete solution with high quality DNA extraction from dried blood spots (DBS)
- Single DBS can be used for both enzymatic and genetic testing without the need of resampling
- Dedicated easy to use software

The NEXTFLEX® NGS Hybridization Panels are part of a comprehensive workflow for genetic testing offered by PerkinElmer, a single-source solution that includes nucleic acid isolation, NGS library preparation, QC, data analysis and data interpretation. Meticulously designed hybrid capture covers targeted gene areas including the exons with at least five base pair flanking. PerkinElmer also provides end-to-end support for all steps of the workflow - all this conveniently from one provider. All the reagents are compatible with Illumina® sequencing platforms.

For more information contact NGS.info@perkinelmer.com

Step	Catalog #	Product	Target	Reagent size
	NOVA-6001-16	NEXTFLEX® Duchenne Muscular Dystrophy NGS Hybridization Panel (RUO)	DMD	16 rxns
	NOVA-6002-16	NEXTFLEX® Lysosomal Storage Disorders NGS Hybridization Panel (RUO)	ARSB, GAA, GALC, GALNS, GLA, GLB1, GUSB, IDS, IDUA, SMPD1, SUMF1	16 rxns
Library Preparation & Hybrid Capture	NOVA-6003-16	NEXTFLEX® Targeted NGS Panel 1 (RUO) genes related to endocrine and metabolic disorders, hemoglobinopathies, and primary immunodeficiencies	79 genes	16 rxns
NOV	NOVA-6004-16	NEXTFLEX® Targeted NGS Panel 2 (RUO) genes related to amino acid disorders, fatty acid disorders and organic acid disorders	68 genes	16 rxns
	NOVA-6005-16	NEXTFLEX® Core Exome NGS Panel (RUO)	~6000 genes	16 rxns
Result Analysis	NOVA-6100-1	NEXTFLEX® NGS Data Analysis Platform		Annual license

# NEXTFLEX® UNIVERSAL BLOCKERS

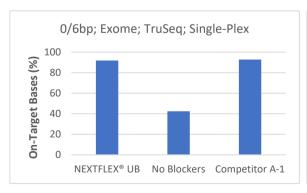
- Effective in blocking single-index or dual-index libraries
- Compatible with all NEXTFLEX® barcodes and all other ligation-based and tagmentation-based workflows for Illumina® sequencing
- Simplifies and reduces the cost of target capture without sacrificing performance
- 96 barcode blockers available

## Improve the Number of On-Target Reads

During target enrichment, adapters can interact with the complementary adapter sequence strand of another library molecule. The NEXTFLEX® Universal Blockers are designed to prevent concatemerization of library molecules. By blocking this complementary adapter sequence interaction, the number of on-target reads improves dramatically and the depth of enrichment increases.

#### One Set of Blockers for All Applications

The NEXTFLEX® Universal Blockers are designed for use with indexed adapters for Illumina® sequencing platforms. They are effective in blocking single-indexed or dual-indexed libraries, without being affected by index length or the presence of a unique molecular index (UMI).



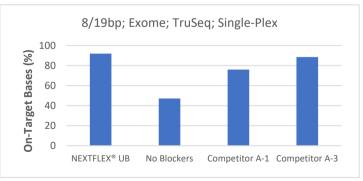
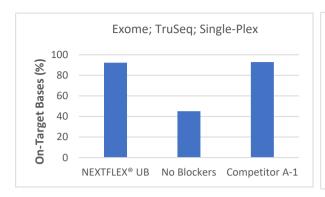


Figure 1. NEXTFLEX\* Universal Blockers (UB) work from 0-19 bp unblocked regions (barcodes and/or UMIs). Competitor A1- and A-3 are two separate products from Competitor A.



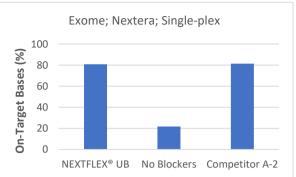


Figure 2. NEXTFLEX $^{\circ}$  Universal Blockers can block Illumina $^{\circ}$  TruSeq $^{\circ}$  or Nextera $^{\circ}$ -style libraries. Competitor A1- and A-2 are two separate products from Competitor A.

#### Compatible with Multiple Workflows

The NEXTFLEX® Universal Blockers are compatible with ligation-based workflows, such as NEXTFLEX® or Illumina® TruSeq™ library prep kits, and with any libraries prepared with NEXTFLEX® DNA Barcodes, NEXTFLEX-96™ DNA Barcodes, NEXTFLEX HT™ Barcodes, NEXTFLEX® Dual-Index Barcodes and NEXTFLEX® Unique Dual Index Barcodes.

They are also compatible with tagmentation-based workflows, such as Illumina® Nextera<sup>™</sup> library prep kits.

#### ORDERING INFORMATION

Catalog #	Kit Name	Quantity
NOVA-5143231	NEXTFLEX® Universal Blockers	8 rxns
NOVA-5143232	NEXTFLEX® Universal Blockers	48 rxns
NOVA-5143233	NEXTFLEX® Universal Blockers	96 rxns

## NEXTFLEX® AMPLICON PANELS

- 100% coverage of exons, flanking intron-exon boundaries, and other regions
- High uniformity and on-target reads
- Up to 384 unique barcodes allowing for high multiplexing capabilities
- 20 ng DNA isolated from fresh or frozen samples required for detection of germline mutations
- 40 ng of DNA isolated from FFPE samples for detection of somatic mutations
- Simple and fast protocols reduce time required for library prep
- Fluid workflow and modular design allows quick adoption of multiple panels that can be run on a single cartridge

The NEXTFLEX® Amplicon Panels are clinical research tools that are easily scalable, simple to use, fast, and cost-effective solutions for targeted sequencing. The kits include primers flanking the regions of interest, library prep reagents, clean-up beads, and barcodes needed for the construction of libraries compatible with Illumina® sequencing platforms and Ion Torrent™ sequencing platforms.

Inquire about custom amplicon panels for your specific needs to NGS@perkinelmer.com.

CANCER PREDISPOSITION & CANCER			
Panel	Catalog #		
BRCA1/2	NOVA-4221		
BRCA1/2 Plus-1	NOVA-4224		
CEBPA	NOVA-4249		
Colorectal Cancer-1	NOVA-4234		
Colorectal Cancer-2	NOVA-4248		
HBOC-1	NOVA-4239		
HBOC-2	NOVA-4240		
Myeloid	NOVA-4260		
TP53	NOVA-4251		
BRCA FFPE	NOVA-4222		
TP53 FFPE	NOVA-4252		

NEWBORN SYNDROMES & INFERTILITY			
Panel	Catalog #		
PDE8B, EN2, NLGN4X, CDKL5, NLGN3, MECP2, RPL10	NOVA-4264		
CYP21A2	NOVA-4244		
CFTR	NOVA-4231		
DMD	NOVA-4256		
SUMF1, GLB1, IDUA, ARSB, GUSB, SMPD1, GALC, GALNS, GAA, GLA, IDS	NOVA-4259		
FBN1	NOVA-4257		
NPSH1, NPSH2, WT1	NOVA-4246		
ARHGDIA, DGKE, LAMB2, PLCE1	NOVA-4254		
NF1, NF2	NOVA-4258		
PAH	NOVA-4250		
FSHB, FSHR, LHB, LHCGR	NOVA-4261		
AR, CATSPER1, CFTR, FSHR, LHCGR	NOVA-4262		

INHERITED SYNDROMES & CONDITIONS			
Panel	Catalog #		
Congenital Hyperinsulism	NOVA-4245		
Cardiovascular Disease	NOVA-4255		
Epilepsy	NOVA-4236		
Mediterranean Fever	NOVA-4237		
MODY-1	NOVA-4232		
MODY-2	NOVA-4233		
MODY-3	NOVA-4243		
MODY-4	NOVA-4238		
MODY-5	NOVA-4267		
Obesity-1	NOVA-4241		
Obesity-2	NOVA-4242		
Periodic Fever-1	NOVA-4247		
Periodic Fever-2	NOVA-4253		

# METAGENOMICS SOLUTIONS

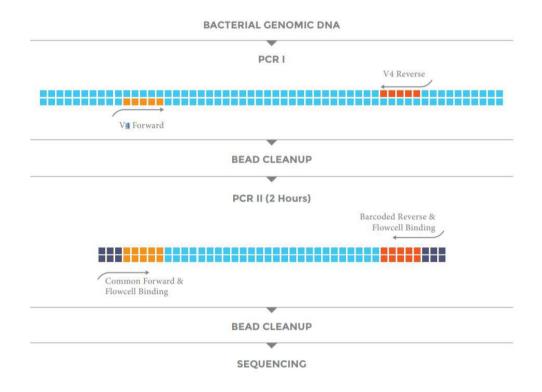
#### **KEY FEATURES**

- Up to 384 barcodes for high-level multiplexing
- Lot-validated kits for confidence in performance
- Practical kit sizes for both low- and highthroughput projects
- Streamlined workflow

#### NEXTFLEX® Metagenomics Amplicon-Seq Kit Series

PerkinElmer offers a robust NEXTFLEX® portfolio for targeted next-generation sequencing (NGS) metagenomics solutions for both 16S and 18S rRNA applications. Catering to your lab's specific needs, we offer three distinct 16S rRNA panels that have been designed to target the 16S rRNA V1-V3, V4, and V5-V6 hypervariable regions, respectively. We also offer targeted sequencing for 18S rRNA ITS1 and ITS2 to allow taxonomic assignment of fungal and micro-eukaryotic species. Now you can also analyze bacteria, fungus and micro-eukaryotes in a single run with the NEXTFLEX® 16S V4 & 18S ITS Amplicon-Seq Kit.

By utilizing NGS, entire communities within a sample can be efficiently analyzed from minimal quantities of DNA, without biases that could potentially be introduced by culturing in other non-NGS methods. The NEXTFLEX® metagenomics amplicon-seq kit series for 16S and/or 18S rRNA maximize the economy and speed of targeted sequencing by offering exceptional values for labs needing a metagenomics solution.



 $\textbf{Figure 1.} \ Representative \ workflow \ for \ 16S\ V4\ ampicon-seq\ kit.$ 

#### **NEXTFLEX® 16S Amplicon-Seq Kits**



Figure 1. S chematic representation of targeted regions covered by the NEXTFLEX°~16S series of amplicon-seq kits

#### **NEXTFLEX® 18S ITS Amplicon-Seq Kit**



 $\textbf{Figure 2.} \ Schematic \ representation \ of the \ ITS1 \ and \ ITS2 \ regions \ covered \ by \ the \ NEXTFLEX ^*18S \ ITS \ amplicon-seq \ kit$ 

#### ORDERING INFORMATION

Catalog #	Kit Name	Quantity
	16S METAGENOMIC KITS	
NOVA-4202-0X series	NEXTFLEX® 16S V1-V3 Amplicon-Seq Kit	24, 96 or 192 rxns
NOVA-4203-0X series	NEXTFLEX® 16S V4 Amplicon-Seq Kit 2.0	24, 96 or 192 rxns
	18S METAGENOMIC KITS	
NOVA-4210-0X series	NEXTFLEX® 18S ITS Amplicon-Seq Kit	24 rxns
	NEW TO THE METAGENOMIC FAMILY!	
NOVA-4213-0X series	NEXTELEX® 165 V4 & 185 ITS Amplicon-Sea Kit	8 or 48 rxns

	NEW TO THE METAGENOMIC FAMILY!	
NOVA-4213-0X series	NEXTFLEX® 16S V4 & 18S ITS Amplicon-Seq Kit	8 or 48 rxns
NOVA-4213-13	NEXTFLEX® 16S V4 & 18S ITS Amplicon-Seq Automation Kit	96 rxn

#### **Technical note available:**

#### **Metagenomics Studies of Plant-associated Microbial Communities**

https://perkinelmer-appliedgenomics.com/wp-content/uploads/marketing/ NEXTFLEX/16S/16S\_V5-V6\_Amplicon-Seq\_Kit\_Tech\_Note\_AG011812\_12\_TN.pdf

## MOLECULAR INVERSION PROBES

#### **KEY ADVANTAGES**

- Workflow much simpler than hybridization enrichment – only 4 touch-points, sample prepared in a single tube/well so no need for sample transfer tracking
- Fast to design most new designs ship within 6 weeks
- No upper limit on panel size
- Consistent uniform and deep coverage, and high
- accuracy from overlapping probe design
- Easy to use color-coded reagents
- UMIs and UDIs standard
- Easy to automate and readily scalable
- Suited for working with low quantities of DNA

#### Simple, Flexible Targeted NGS

Molecular Inversion Probes (MIPs) allow custom targeting of specific regions of the genome with a simple workflow. The MIP assay design includes tiling single stranded DNA probes across the target of interest. The probes contain sequences on their 5' and 3' ends that are complementary to the target, and are joined by a backbone keeping them in close proximity therefore reducing off target binding. The loops are then filled-in to capture the target region between the 5' and 3' probes.

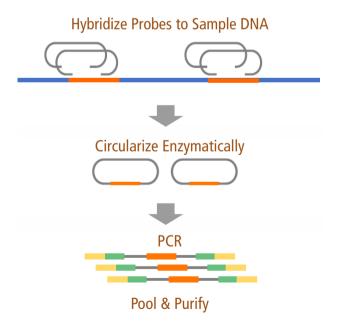


Figure 1. Summary of the Molecular Inversion Probe workflow

#### **MIPs**

# Hybridize Circular Conversion PCR Pool + Cleanup

No quantitation needed within workflow No cleanup needed until samples are pooled One tubewell per sample

Figure 2. Comparison of workflows between molecular inversion probes and hybridization capture.

# NEXTFLEX® Extended Carrier Panel Target size 291,211bp Hands on time 1 hour Sample prep time 3.5 hours + overnight hyb

#### Competitor T "Fast" Protocol



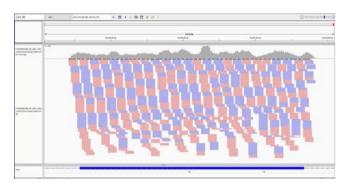


Figure 3. IGV view of NGS reads from overlapping molecular inversion probe design

#### **Custom NEXTFLEX® Panels**

Custom panels can be designed to genes, breakpoints and CNVs. The design can be augmented with amplicons for pseudogenes, fuzzy breakpoints and short repeat expansions.

#### **Example Custom NEXTFLEX® Extended Carrier Panel**

Contains redundantly tiled Molecular Inversion Probes (MIPs) for 1722 targets. Targets include:

- 1. Complete coding exonic sequences of 89 genes, at least 15 bases of exon-adjacent intronic sequences, and Clinvar database-specified pathogenic/likely pathogenic mutations located in introns or promoters of these genes,
- 2. Mutation hotspots in additional 16 genes,
- 3. Breakpoint probes for nine large deletions and one duplication,
- 4. Selected mutations in 11 genes relevant to infertility conditions and microdeletions in three Azoospermia Factor regions of chromosome Y.

#### Molecular Inversion Probe Performance on Genomic DNA (Average 0.9x106 Reads per Sample)

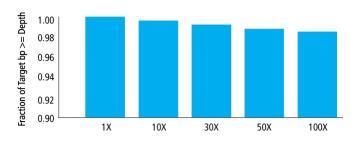
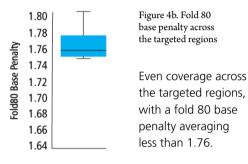


Figure 4a. Fraction of targets with depth of coverage ranging from 1x up to 100x

Consistently high depth of coverage across the target regions, with >99% of targeted bases having >100x depth



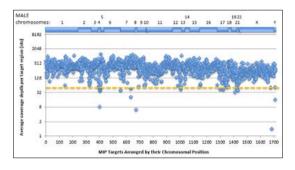
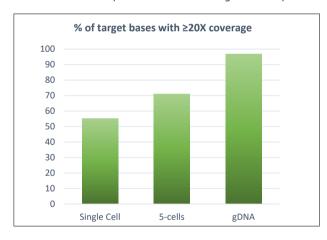


Figure 4c. Average depth of coverage across all 1,722 targets

Greater than 97.5% of targets generated greater than 30x coverage. Further customization of the probe pool is possible.

#### Molecular inversion probes performance on limited template (2x106 reads)

Standard MIP kit performance on whole genome amplified cells.



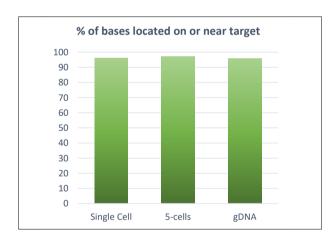


Figure 5. Target specificity and depth of coverage from single cell and 5 cell whole genome amplified templates, compared to unamplified genomic DNA using a standard molecular inversion probe panel and protocol

With no prior optimisation and using a standard genomic DNA kit and workflow, greater than 95% of the sequencing data was from bases on target or within 250bp up or downstream. Greater than 55% of targets from single cell templates and 70% of targets from 5 cell templates obtained at least 20x depth of coverage. This equates to 950 target regions from single cells and 1,224 target regions from 5 cells. A high sensitivity kit is also available.

## **BARCODES**

## NEXTFLEX® NGS BARCODES FOR DNA-SEQ

#### **NEXTFLEX® NGS BARCODES FOR DNA-SEQ:**

- NEXTFLEX-HT™ Barcodes
   Up to 384 single-index barcodes specifically designed for the highest flexibility for DNA-seq applications.
- NEXTFLEX® DNA Barcodes
   Traditional single-indexed barcoded adapters with up to 48 multiplexing options.
- NEXTFLEX-96™ Barcodes
   A set of 96 barcoded adapters for routine high-throughput DNA-seq applications.
- NEXTFLEX® ChIP-Seq Barcodes
   Lower concentration barcoded adapters for use with low input DNA-seq applications. Up to 48 multiplexing options are available.
- NEXTFLEX-96<sup>™</sup> ChIP-Seq Barcodes
   A set of 96 lower concentration barcoded adapters for use with low input, high throughput DNA-seq applications.
- NEXTFLEX® PCR-Free Barcodes
   High concentration barcoded adapters for PCR-free applications. Up to 48 multiplexing options are available.
- NEXTFLEX® Bisulfite-Seq Barcodes
- NEXTFLEX® Unique Dual Index Barcodes
   Up to 1,536 barcodes designed to specifically
   mitigate the index hopping or spread of signal
   phenomenon associated with Illumina® platforms
   that utilize a patterned flow cell.

## DNA-SEQ, CHIP-SEQ, BISULFITE-SEQ

The NEXTFLEX® Barcodes are barcoded adapters that provide flexibility and high-throughput capabilities for Illumina® sequencing applications. Multiplexing with NEXTFLEX® Barcodes drastically increases scale while reducing costs by allowing the user to pool multiple libraries in a single flow cell lane.

These barcodes can be used with single read or pairedend read sequencing and are compatible with NEXTFLEX® library prep kits designed for Illumina® sequencing, and other Illumina® library preparation workflows that involve the ligation of adapters to adenylated fragments.

- Single- and unique dual-indexed adapters for multiplexing Illumina® libraries
- Compatible with both paired-end and single-read Illumina® sequencing
- Maximum flexibility of Illumina® pooling combinations
- Sequence verified for index purity

#### NEXTFLEX-HT™ Barcodes

#### 384 Single-Index Barcodes Enabling Deep Multiplexing for Sequencing on Illumina® Platforms

The NEXTFLEX-HT<sup>TM</sup> Barcodes for Illumina® sequencing platforms are single-index adapters that provide unprecedented flexibility and high-throughput capabilities in sequencing applications. Multiplexing with NEXTFLEX-HT<sup>TM</sup> Barcodes drastically increases scale while reducing costs by allowing the user to pool multiple libraries in a single flow cell lane.

These barcodes can be used with single read or paired-end read sequencing and are compatible with NEXTFLEX® library prep kits designed for Illumina® sequencing, and other Illumina® library preparation workflows that involve the ligation of adapters to adenylated fragments. Currently 384 barcodes are available; however, greater than 2,000 are ready to be developed.

#### **Dual Error Correction Prevents Ambiguity**

The NEXTFLEX-HT™ Barcodes utilize a single-index adapter containing a 12 nt unique sequence. The 12 nt indices are designed with a Hamming Distance of at least five throughout the entire series of 2380, permitting dual error correction. Dual error correction enables proper differentiation between samples by preventing ambiguity from PCR errors or sequencing instrument miscalling. The NEXTFLEX-HT™ index is contained within the adapter sequence, making PCR enrichment optional.

#### **Reduce Run-to-Run Sample Carryover and Sample Crosstalk**

Even users who don't routinely perform high-throughput multiplexing benefit from the superior design of the NEXT-FLEX-HT™ Barcodes. The ability to avoid using the same set of indices consecutively reduces concerns about run-to-run sample carryover and sample crosstalk. Plus, each pair of consecutive barcodes are fully color balanced and can be used for low level multiplexing. The sets of 96 NEXTFLEX-HT™ Barcodes are supplied in 96-well plates with each well containing 2 reactions of each barcode.

- Single-index adapters for multiplexing up to 384 Illumina libraries
- Compatible with both paired-end and single-read Illumina® sequencing platforms
- Maximum flexibility of Illumina-suitable pooling combinations

#### ORDERING INFORMATION

Catalog #	Kit Name	Quantity
NOVA-514174	NEXTFLEX-HT™ Barcodes 1-96 (In 96-well Plate)	192 rxns
NOVA-514175	NEXTFLEX-HT™ Barcodes 97-192 (In 96-well Plate)	192 rxns
NOVA-514176	NEXTFLEX-HT™ Barcodes 193-288 (In 96-well Plate)	192 rxns
NOVA-514177	NEXTFLEX-HT™ Barcodes 289-384 (In 96-well Plate)	192 rxns

## NEXTFLEX® UNIQUE DUAL INDEX BARCODES

## Mitigate Index Hopping & Spread of Signal on Patterned Flow Cells with the NEXTFLEX® Unique Dual Index Barcodes

The NEXTFLEX® unique dual index barcodes are barcoded adapters for sequencing on Illumina® platforms that provide unprecedented data security in sequencing applications. Increased mis-assignment of indexes has been shown to occur on Illumina® sequencing instruments that feature a patterned flow cell and exclusion amplification technology. The NEXTFLEX® unique dual index barcodes are designed to specifically mitigate the index hopping or spread of signal phenomenon associated with Illumina® platforms that utilize a patterned flow cell. Index mis-assignment can lead to increased false positive rates, which are especially detrimental to sensitive applications.

Multiplexing with NEXTFLEX® unique dual index barcodes drastically increases processing capacity while reducing costs by allowing the user to pool multiple libraries in a single flow cell lane.

Illumina® sequencing instruments that utilize a patterned flow cell and exclusion amplification technology is known to suffer from increased levels of sample mis-assignment. The use of NEXTFLEX® unique dual index barcodes prevents such mis-assigned reads from appearing in final data sets allowing for the highest assurance of data integrity.

#### **KEY FEATURES**

- Unique dual index adapters for multiplexing up to 384 Illumina® sequencing libraries
- Mitigate index hopping or spread of signal that can occur during sequencing
- Decreases level of mis-assigned reads in sequencing data
- Compatible with both paired-end and single-read Illumina® sequencing

#### Amplicon

			2	3	4	5	6	7	8
	1	99.84	0.02	0.02	0.04	0.02	0.02	0.07	0.08
	2	0.03	99.82	0.02	0.06	0.03	0.02	0.21	0.09
	3	0.03	0.03	99.86	0.07	0.02	0.03	0.11	0.1
Index	4	0.02	0.03	0.02	99.58	0.02	0.02	0.09	0.09
Ĕ	5	0.03	0.03	0.03	0.09	99.85	0.04	0.13	0.09
		0.02	0.02	0.01	0.05	0.02	99.83	0.08	0.06
		0.01	0.02	0.01	0.02	0.01	0.01	99.15	0.04
	8	0.03	0.04	0.02	0.09	0.03	0.03	0.16	99 46

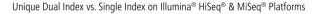
Samples Demultiplexed viaUnique 8nt i7 Index

#### Amplicon

			2		4	5			8
	1	100	0	0	0	0	0	0	0
	2	0	99.9	0	0	0	0	0	0
	3	0	0.01	100	0	0	0.01	0	0
Index	4	0	0	0	100	0	0	0	0
ĭ	5	0	0	0	0	100	0	0	0
	6	0	0	0	0	0	99.9	0	0
		0	0	0	0	0	0	100	0
	8	0	0	0	0	0	0	0	100

Samples Demultiplexed via Unique 8nt i7 Index & Unique 8nt i5 Index

## Figure 1. NEXTFLEX® unique dual index Barcodes decrease index mis-assignment on the Illumina® HiSeq® 4000 platform. A set of libraries prepared using NEXTFLEX® unique dual index barcodes was sequenced on the HiSeq® 4000 sequencing platform. The numbers indicate percentage of correct insert reads assigned to index sequences. The resultant data was demultiplexed twice: first by taking only the unique I7 index into account (left panel), and second by taking both unique I7 and I5 indexes into account (right panel). By assessing both the unique I7 and I5 indexes, sequence mis-assignment was drastically decreased.



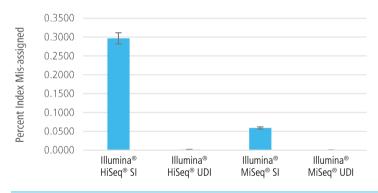


Figure 2. NEXTFLEX® Unique Dual Index Barcodes increase confidence in sequencing data. The data generated on the HiSeq® 4000 platform showed a dramatic reduction in the percentage of misassigned reads, and the data generated on the MiSeq® platform also showed a reduction. The results indicate that the NEXTFLEX® unique dual index barcodes greatly reduce the amount of mis-assigned reads in data sets, and that this occurs at a higher rate on the instruments that use exclusion amplification chemistry and patterned flow cells.

#### **ORDERING INFORMATION**

Catalog #	Kit Name	Quantity
NOVA-514150-EVAL16	NEXTFLEX® Unique Dual Index Barcodes 1-8 (in tubes)	16 rxns
NOVA-514150-EVAL48	NEXTFLEX® Unique Dual Index Barcodes 1-24 (in tubes)	48 rxns
NOVA-514150	NEXTFLEX® Unique Dual Index Barcodes 1-96 (in 96-well plate)*	192 rxns
NOVA-514151	NEXTFLEX® Unique Dual Index Barcodes 97-192 (in 96-well plate)*	192 rxns
NOVA-514152	NEXTFLEX® Unique Dual Index Barcodes 193-288 (in 96-well plate)*	192 rxns
NOVA-514153	NEXTFLEX® Unique Dual Index Barcodes 289-384 (in 96-well plate)*	192 rxns

## NEXTFLEX® UNIQUE DUAL INDEX BARCODES (SET OF 1,536)

#### You Asked, We Listened!!

#### **KEY FEATURES**

- Save time and sequencing costs by multiplexing up to 1,536 samples in a single run on the highthroughput Illumina® NovaSeq® sequencer or any other Illumina® NGS platform
- Avoid costly mistakes due to sample swapping by having ready access to 1,536 unique dual index barcodes off-the-shelf
- Boost your confidence in sample identification using functionally validated unique dual indices

The new set of 1,536 NEXTFLEX® Unique Dual Index barcodes were designed to maximize flow cell capacity and minimize risk. The cost per sample is greatly reduced while mitigating chances of sample mix-up by means of unprecedented multiplexing options and enhanced barcode rotation capability between runs. The 1,536 adapters have unique 10 nucleotide indexes on both ends and each lot is functionally validated to increase data security by mitigating sample mis-assignment caused through index hopping and signal spreading, especially on sequencers with patterned flow cells such as the Illumina NovaSeq® sequencer or other Illumina® NGS platforms.

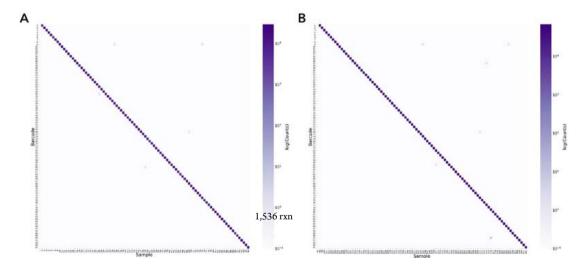


Figure 1. Heatmap of barcode purity. Samples barcoded with a representative set of 192 unique barcodes from NEXTFLEX $^{\circ}$  Unique Dual Index Barcodes (set of 1,536) plotted against counts barcodes found within the reads. The color bar is logarithmically scaled to depict low levels of counts more easily, and zero values are converted to one magnitude lower than the dataset's minimum non-zero value. A diagonal line is expected for 100% purity. Barcode purity for two sets of 96 barcodes was observed at  $\geq$  99.9% for all UDIs.

Catalog #	Kit Name	Quantity
NOVA-534100	NEXTFLEX® Unique Dual Index Barcodes (1,536 barcodes, 3,072 rxn)	3,072 rxn
NOVA-534101	NEXTFLEX® Unique Dual Index Barcodes (1-384)	768 rxns
NOVA-534102	NEXTFLEX® Unique Dual Index Barcodes (385-768)	768 rxns
NOVA-534103	NEXTFLEX® Unique Dual Index Barcodes (769-1152)	768 rxns
NOVA-534104	NEXTFLEX® Unique Dual Index Barcodes (1153-1536)	768 rxns

#### NEXTFLEX® NGS BARCODES FOR RNA-SEQ

#### **NEXTFLEX® NGS BARCODES FOR RNA-SEQ:**

- NEXTFLEX® RNA-Seq Barcodes
   Traditional single-indexed barcoded adapters with up to 48 multiplexing options.
- NEXTFLEX-96<sup>TM</sup> RNA-Seq Barcodes
   A set of 96 barcoded adapters for routine high-throughput RNA-seq applications.
- NEXTFLEX® RNA-Seq Unique Dual Index Barcodes
   Up to 1,536 barcodes designed to specifically
   mitigate the index hopping or spread of signal
   phenomenon associated with Illumina® platforms
   that utilize a patterned flow cell
- NEXTFLEX RNA Barcodes https://perkinelmer-appliedgenomics.com/home/products/library-preparation-kits/nextflex-ngs-barcodes/nextflex-rna-barcodes/)
- **NEXTFLEX-96 RNA Barcodes** https://perkinelmer-appliedgenomics.com/home/products/library-preparation-kits/nextflex-ngs-barcodes/nextflex-96-rna-barcodes/#:~:text=%20NEXTFLEX-96%E2%84%A2%20RNA-Seq%20Barcodes%20%201%20Index%20contained,5%20 Compatible%20with%20Illumina%20%C2%AE%20next-generation...%20More%20)
- **NEXTFLEX RNA-Seq UDI Adapters** https://perkinelmer-appliedgenomics.com/home/products/library-preparation-kits/nextflex-ngs-barcodes/nextflex-unique-dual-index-barcodes/nextflex-rna-seq-udi-adapters/



#### **AUTOMATION**

With our optimized nucleic acid extraction, DNA/RNA quantitation, automated library preparation, NGS library QC prep kits and technical expertise, you have an ally who understands your underlying science and can provide the solutions that address the full breadth of your scientific requirements

- all from a single-source who delivers the application support you need to be productive right from the start.

#### Sample disaggregation

OMNI International, the newest member of the PerkinElmer family, provides a uniquely diverse line of laboratory homogenizers. OMNI streamlines your sample preparation process with easy-to-use solutions designed for grinding, lysing, and homogenizing samples prior to downstream analysis. Fast, accurate, repeatable results every time.

Check products at www.omni-inc.com

#### Cell counting

Nexcelom Bioscience is the expert in automatic cell counting and analysis. With sophisticated proprietary technologies, Nexcelom is a clear leader and innovator in the field of image-based cytometry for cell analysis.

Check products from at www.nexcelom.com

#### PKeye<sup>™</sup> Mobile Operations Monitor

The PKeye Workflow Monitor provides researchers 24/7 access to their PerkinElmer instruments. From an office, a conference room, or even from the comfort of home, the PKeye workflow monitor provides valuable information such as:

- Data snapshots
- Instrument status
- Workflow step notifications
- Errors
- Video recordings

All accessible via the Cloud internet interface. With the PKeye workflow monitor you can receive push notifications via SMS or email giving you instant information to ensure your samples are moving through extraction, quality control and liquid handling processes. Currently supported PerkinElmer instruments are:

- JANUS® G3 Liquid Handlers
- Sciclone® G3 Liquid Handlers
- Zephyr® G3 Liquid Handlers
- LabChip® GX Touch™ Instrument
- chemagic<sup>™</sup> 360 Platform

#### **Nucleic** acid isolation

Based on the patented chemagen Magnetic Bead Technology, PerkinElmer offers different solutions for nucleic acid isolation. Discover which solution meets your sample volume, your throughput, or your automation needs. More information about the technology, the kits, and the chemagic<sup>TM</sup> instruments at: https://chemagen.com/

#### The chemagic<sup>™</sup> Prepito<sup>®</sup> instrument:

Bench top instrument for automated RNA and DNA isolation



- Up to 12 samples per run
- Sample volume capacity up to 1 ml
- Kits available for a large variety of sample materials (e.g., blood, serum and plasma, tissues, etc.)
- Cost-efficient integrated buffer dispensing and use of standard plastic devices
- Barcode reader for optimal sample management
- Touch panel software for ease of use
- USB slot for simple data transfer

Size: 57 x 50 x 57 cm (W/D/H)

#### The chemagic<sup>™</sup> 360 instrument:

Flexible automated high-throughput DNA and RNA isolation



- Vast flexibility for reliable automated DNA and RNA purification workflowsSample volumes from 10 µl - 10 mlHuge kit portfolio for various sample materials
- Throughput from 96 to 4000 samples per day
- No cross-contamination
- Ultra-fast protocols available for nucleic acid purification
- Flexible reagent dispensing to keep running expenses low
- Convenient and improved sample management
- Intuitive software
- Bar code reading for sample tracking
- LIMS compatible log files
- Compact designEasy to integrate with liquid handling platforms

Size: 80 cm x 80 cm x 90 cm (W/D/H)

#### The chemagic<sup>™</sup> Prime<sup>™</sup> Junior instrument:

Medium-throughput automated DNA and RNA isolation and assay setup

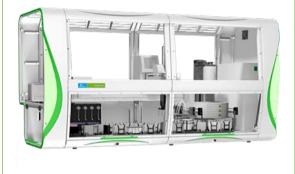


- Complete workflow from primary sample to assay setup
- Walk-away automation for up to 48 samples
- Suitable for diverse samples like blood, plasma, saliva, urine, swabs, feces, etc.
- Customizable to your needs: Pipetting, eluate transfer, normalization, assay, PCR, NGS setup
- JANUS® G3 technology for reliable pipetting and setup capabilities
- Suitable for even challenging applications e.g., long-read sequencing, MLPA, etc.
- No waste of reagents due to buffer dispensing according to the number of samples
- Intuitive WinPREP® software for easy operation
- Full traceability at any time during the process: Barcode tracking and LIMS compatible
- Optional add-ons: UV light and 2D barcode reader

Size 1.9 m x 0.87 x 1.70 m (L/W/H) incl. table

#### The chemagic™ Prime™ instrument:

Very high-throughput automated DNA and RNA isolation and assay setup



- Streamlined walk-away automation for nucleic acid isolation and assay setup with minimized hands-on time
- Flexible sample volumes from 10 µl to 10 ml
- High sample capacity: process up to 192 samples in a single run
- Suitable for various sample materials
- Large selection of kits available
- Designed to prevent cross-contamination
- Proven nucleic acid purification technology and protocols
- Optimized reagent dispensing to lower running expenses by decreasing the cost per sample
- Integrated liquid handling unit for pipetting, eluate transfer, normalization, assay, PCR, NGS setup
- Automated barcode reading for full sample traceability and tracking compatible with your LIMS system
- Intuitive software for easy handling

Size:  $2.3 \text{ m} \times 0.9 \text{ m} \times 1.9 \text{ m}$  (L/W/H) - incl. table

#### Quantification & quality control of DNA, RNA & NGS libraries

DNA and RNA quantitation and sizing can be done in seconds using automated capillary electrophoresis separation.

#### LabChip® GX Touch™ Nucleic Acid Analyzer:

Microfluidic capillary electrophoresis analysis of nucleic acids & NGS libraries



- LabChip® microfluidic technology to modernize gel electrophoresis
- Fully automated genomic samples analysis in real time, sample process in as fast as 30 seconds
- Digitized data format for convenient analysis, review, share and archive
- Quantitative metric of RNA and DNA sample integrity to ensure only the best samples go downstream
- Native high throughput capability to support up to 384 samples in a single run

Size: 69cm x 51cm x 49 cm

#### **VICTOR Nivo® Plate Reader:**

High-throughput quantification of nucleic acids



- Rapid, high-throughput solution for accurately quantifying nucleic acids and NGS libraries
- Analysis of 96 samples taking less than 2 minutes

Size: 20cm x 50cm x 27 (W/D/H)

#### Nanoliter, non-contact dispensing

#### FlexDrop™ iQ™ Non-contact Dispenser



Delivers rapid, precise, and flexible dispensing from up to 96-source wells into 96, 384, or 1536 well plates with a dead volume of 1  $\mu$ L. This can drastically reduce cost by eliminating reagent waste and facilitating reaction miniaturization. This instrument is ideal for:

- Dispensing mastermix, probe and primers for highthroughput PCR
- Dispensing beads, buffers and enzymes into medium to high-throughput NGS kits, such as NEXTFLEX® NGS kits

Size: 471 x 575 x 378 mm (L/W/H)

#### Automated library preparation

With a portfolio of dedicated NGS automated workstations to match your throughput needs, PerkinElmer offers over 100 vendor-qualified library kit methods that are pre-developed, tested and can enable users to be running library prep assays in a week. Additionally, PerkinElmer offers solutions with varying throughput, capacity, and dynamic volume range to meet all of your NGS sample preparation needs. Choose from the Sciclone® G3 NGS, or Zephyr® G3 NGS platforms.

#### Zephyr® G3 NGS workstation



This workstation is an affordable, semi-automated benchtop solution designed for medium-throughput NGS needs and can construct up to 48 NGS libraries per day. This compact workstation is equipped with:

- A highly precise, 96-channel pipetting head
- An integrated gripper
- On-deck temperature and shaking options
- A waste tip chute
- Many pre-written NGS methods such as: DNA, RNA, amplicon and exome library construction
- Applications development support for automating any new kits

Size for the Zephyr® G3 NGSx Workstation: 660mm x762mm x 585mm (H/W/D)

#### Sciclone® G3 NGSx workstations



This workstation is designed for batch processing of up to 96 samples at a time. It includes:

- A highly precise, 96-channel pipetting head
- An integrated gripper to facilitate moving labware around the deck
- On-deck temperature and shaking options for precise heating and shaking of your NGS library preparation
- A waste tip chute to seamlessly remove consumables from the deck
- A fully enclosed workspace
- Below deck storage to accommodate large NGS protocols

Size: 1219mm x 711mm (W/D)

#### Sciclone® G3 NGSx iQ™ workstation



Designed with more automation and for longer walk-away times, this workstation includes all the features of the Sciclone G3 NGSx Workstation (above) and also includes:

- An integrated on-deck thermal cycler (ODTC) to enable full automation of PCR preparation
- Twister® III robotic arm to load up to 27 tip boxes of any consumable type.
- This provides increased walk-away time for users and lowers variability and risk, such as incubation and PCR timing.

Size: 1708mm x 807mm (W/D)

#### Sciclone ® G3 NGSx HT Workstation



Fully Automated, Walk-Away Miniaturized Workflows

For very high throughput NGS protocols, the Sciclone G3 NGSx HT Workstation is includes all the features of the Sciclone G3 NGSx Workstation (above) and also is equipped with:

- A highly precise, 384-channel, low volume pipetting head, to precisely pipette liquids down to 500 nL
- An integrated ODTC to enable full automation of PCR preparation
- Twister III robotic arm to load up to 27 tip boxes of any consumable type.

The Sciclone G3 NGSx HT Workstation can process 384 samples at once and specializes in reaction miniaturization to drastically reduce reagent volumes and per-sample costs while simultaenously increasing the throughput of your NGS assays.

Size: 1708mm x 807mm (W/D)

#### Integrated laboratory automation solutions for genomic workflows

With experience designing integrated automated laboratory solutions for a number of genomic applications including nucleic acid isolation, RNAi screening, CRISPR fragment analysis, PCR, and gene expression analysis, PerkinElmer's robotics experts can design and provide custom workstations to meet your automation needs, big or small.

More information at https://www.perkinelmer.com/fr/category/integrated-laboratory-automation

#### explorerTM G3 workstation

Example Configuration (low throughput)



explorerTM G3 workstation iX15 to integrated e.g.

- ZEPHYR® G3 NGS liquid handling workstation
- JANUS® G3 Mini 8-tip liquid handling workstation supporting normalization and pooling
- Victor Nivo Multimode Reader for quantification
- Analytik Jena TRobot II or INHECO ODTC thermal cycler
- Random access labware racks
- plate::handlerTM Flex robotic arm, barcode reader and plate:worksTM scheduling software

#### Example Configuration (low/medium throughput)



explorerTM G3 workstation iX20 to integrate e.g.

- JANUS® G3 Mini 8-tip liquid handling workstation with PlateStak labware stacker
- Labcyte ECHO low volume liquid handler
- Victor Nivo Multimode Reader for quantification
- Multiple Analytik Jena TRobot II thermo cycler
- Random access labware racks
- plate::handlerTM Flex robotic arm, barcode reader and plate:worksTM scheduling software

#### Example Configuration (high throughput)



explorerTM G3 workstation iX40 to integrated e.g.

- multiple Sciclone G3 NGS, Zephyr G3 NGS or JANUS® G3 liquid handling workstation
- Victor Nivo for DNA quantification
- multiple Analytik Jena TRobot II thermo cycler
- LabChip® GX Touch™ analyzer for Library QC
- Automated plate Hotels and incubators
- Automated plate sealer and peeler
- Reagent dispenser
- plate::handlerTM Flex robotic arm, barcode reader and plate:worksTM scheduling software

#### **Automated protocols**

PerkinElmer's team of application scientists is continuously releasing new automated methods to address emerging applications. By being responsive to latest trends in the NGS field, you can be assured we can meet your future automation needs.

The tables below list the kits and protocols that are currently automated on the Sciclone®, JANUS®, and Zephyr® G3 liquid handlers.

To see the most up to date list of protocols, please check on the webpage https://perkinelmer-appliedgenomics. com/home/products/automated-liquid-handling/ automated-ngs-protocols/

ast and		p Methods for Your NGS Workflow			PLATFORMS		
	COMPANY	PRODUCT	JANUS® G3	Sciclone® G3	Sciclone® G3	Sciclone® G3	Zephyr®
				NGSx	NGSx iQ™	NGSx HT	
	PerkinElmer	NEXTFLEX® Rapid XP DNA-Seq Kit		Х	Х		Х
	PerkinElmer	NEXTFLEX® Rapid DNA-Seq Kit 2.0		Х	Х		Х
	PerkinElmer	NEXTFLEX® Rapid DNA-Seq Kit		X	X	Х	
	PerkinElmer PerkinElmer	NEXTFLEX® Cell Free DNA-Seq Kit 2.0 PG-Seg® Kit		X X	Х		X
	10x Genomics®	Genomic Preparation		X			
	ArcherDx®	Universal DNA Reagent Kit v2		X			
	Illumina®	Nextera® XT DNA Kit	Х	X	Х		Х
	Illumina®	TruSeq® DNA PCR-Free Kit	Х	Х	Х		
	Illumina®	TruSeq® Nano DNA Kit	Х	Х			
	Illumina®	Illumina® DNA Prep	Х	Х	Х		
	Illumina®	Illumina® DNA Prep with Enrichment		Х	Х		Х
	Illumina®	DNA PCR-Free Library Prep Tagmentation		Х	Х		
	NEB®	NEBNext <sup>®</sup> Ultra <sup>™</sup> II DNA Library Prep for Illumina <sup>®</sup> Instruments		Х			Х
	NEB®	NEBNext® Ultra™ DNA Kit	Х	Х			Х
NIA Library	NEB®	NEBNext® dsDNA Fragmentase™ Kit		Х			
NA Library reparation	NuGen®	Celero DNA-Seq			Х		
its	NuGen®	Ovation® Ultralow DR Multiplex System (1-96)		Χ			Х
	PacBio <sup>®</sup>	2kb Template Preparation & Sequencing	X	Х			Х
	PacBio®	10kb Template Preparation & Sequencing	Х	Х			Χ
	PacBio®	20kb Template Preparation Using BluePippin™ Size-Selection System		Х			Х
	PacBio®	Greater than 10kb Template Preparation Using AMPure® PB Beads		Х			Х
	PacBio®	HiFi SMRTbell® Libraries with SMRTbell Express Template Prep Kit 2.0		Х	Х		
	PacBio®	Multiplexed Microbial Library Prep with SMRTbell Express Template Prep Kit 2.0		Х			
	Roche®	DNA HTP Library Preparation Kit	Х	Х			Х
	Roche®	DNA HyperPrep™ Kit	Х	Х	Х		Х
	Roche®	DNA HyperPlus™ Kit	Х	Х	Х		Х
	Roche®	KAPA HyperCap v3		Х			
	Thermo Fisher®	lon Torrent™ lon Express® Fragment Library Prep Kit		Х			Х
	Thermo Fisher®	Ion Express® Plus gDNA Fragment Library Prep Kit		Х		Х	
	Twist <sup>®</sup>	Library Preparation Kit		Х	Х		
	QIAGEN®	QIAseq® FX DNA Library Kit		Х			
	Swift®	Accel-NGS® 2S Plus DNA Library Kit		Х			

Fast and	Lasy Library Prep I	Methods for Your NGS Workflow			PLATFORMS		
	COMPANY	PRODUCT	JANUS® G3	Sciclone® G3	Sciclone® G3	Sciclone® G3	Zephyr®
				NGSx	NGSx iQ™	NGSx HT	
	PerkinElmer	NEXTFLEX® Rapid RNA-Seq Kit		Х			Х
	PerkinElmer	NEXTFLEX® Rapid Directional RNA-Seq Kit with Poly(A) Beads 2.0		Х	Χ		Х
	PerkinElmer	NEXTFLEX® Rapid Directional qRNA-Seq Kit		Χ			
	PerkinElmer	NEXTFLEX® Combo-Seq™ mRNA/miRNA Kit		Х	Χ		
	10X Genomics®	Chromium™ Single Cell 3' Library Kit v3					Х
	Agilent®	SureSelect® Strand-Specific RNA Library Prep Kit	Х		Χ		
	Illumina®	RNA Prep with Enrichment (L) Tagmentation		Χ	Χ		
	Illumina®	TruSeq® RNA Access Kit		Χ			
	Illumina®	TruSeq® Stranded mRNA (poly-A selection) Kit		Х	Х		Х
	Illumina®	TruSeq® Stranded Total RNA™ Kit	Х	Х	Х		Х
	Illumina®	TruSeq® RNA Library Prep Kit v2		Х	Х		Х
	Illumina®/Epicentre	Ribozero™/Scriptseq™ Stranded RNA Kit		Х			
	Lexogen®	autoSENSE™ mRNA-Seq Library Prep Kit for Illumina® Sequencing		Х			
	Lexogen®	autoQuantSeq™ 3'mRNA-Seq Library Prep Kit for Illumina® Sequencing		Х			
RNA Library	NEB®	NEBNext® Ultra™ RNA Library Prep Kit for Illumina® Sequencing		Х			Х
reparation	NEB®	NEBNext® Ultra™ RNA II Library Prep Kit for Illumina® Sequencing		Х			
Cits	NuGen®	Ovation® Human Blood RNA-Seq Library Systems		Х			
	NuGen®	Ovation® Human FFPE RNA-Seq Multiplex System		Х			
	NuGen®	Ovation® RNA-Seq System v2		Х			
	NuGen®	Ovation® Universal RNA-Seq System (strand-specific)		Х			
	Roche®	mRNA HyperPrep™ Kit		Х			Х
	Roche®	RNA HyperPrep™ Kit		Х	Х		Х
	Roche®	RNA HyperPrep™Kit with RiboErase™		Χ			Х
	Roche®	Stranded RNA-Seq Kit		Х			Х
	Roche®	Stranded RNA-Seq Kit with RiboErase™	X	Х			Х
	Roche®	Stranded mRNA-Seq Kit		Х			Х
	Takara formerly Clontech	SMARTer® Stranded Total RNA-Seq Kit v2 Pico Input Mammalian		Х			
	Takara formerly Clontech	SMART-Seq® v4 Ultra® Low Input RNA Kit for Seqeuncing		Х			
	Takara formerly Clontech	SMART-Seq® HT Kit				Х	
	Thermo Fisher®	(Ion Torrent™) Ion Total RNA Kit v2	X				
	Twist®	cDNA Library Preparation Kit for ssRNA Virus Detection	Х	Х	Х		

	COMPANY	PRODUCT	JANUS® G3	Sciclone® G3	Sciclone® G3	Sciclone® G3	Zephy
				NGSx	NGSx iQ™	NGSx HT	
	PerkinElmer	NEXTFLEX® Small RNA-Seq Kit v3		Х	X	NOSKIII	Х
nall RNA Library eparation Kits	PerkinElmer	NEXTFLEX® Small RNA-Seq Kv3 Automation Kit with UDIs		X	X		X
	reixiliLillei						^
	PerkinElmer	NEXTFLEX® Variant Seq™ SARS-CoV-2 Kit	Х	Х	Х		Х
	10x Genomics®	Exome Preparation		Х			
	Agilent®	HaloPlex® Kit	X	X			
	Agilent®	SureSelect® XT2 Kit	X	X	X		Х
	Agilent®	SureSelect® XT Kit  SureSelect® XT HS	Х	Х	X		
	Agilent® Agilent®	SureSelect® QXT Kit		Х	^		Х
	Cergentis®	TLA Targeted Locus Amplification Kit	Х	X			^
	IDT®	xGen Hybridization and Wash Kit		Λ	Х		
	IDT®/Epicentre	XGen® Exome Capture Kit		Х	X		
	Illumina®	COVIDSeq™ Test		X	X		Х
	Illumina®	Nextera® Rapid Capture Kit		Х			Х
	Illumina®	TruSeq® Rapid Exome Kit	Х	Х			
	Illumina®	TruSeq <sup>®</sup> Custom Amplicon v1.5 Kit	Х				Х
	Illumina®	TruSeq® Custom Amplicon Low Input Kit		Х			Х
	Illumina®	TruSeq® Exome Kit		Х			
ome/Target pture Library	Illumina®	TruSeq® Focus Panel		Χ			
eparation Kits	Illumina®	TruSeq® Cancer HotSpot Panel v2		Х			
	Illumina®	TruSeq® Comprehensive Panel v3		Х			
	Illumina®	TruSight® Cancer Kit		Х			
	Illumina®	TruSight® Enrichment Kit	Х				
	Illumina®	TruSight® Rapid Capture Kit		Х			
	Illumina®	TruSight® Tumor Kit	X				
	Illumina®	TruSight® Tumor 15 Kit	Х	V			
	NEB®	NEBNext® Direct® Cancer HotSpot Panel		X			
	NuGen®	Ovation® Target Enrichment System		X			
	Roche®	SeqCap® EZ Kit		Х			
	Roche®	SeqCap® EZ HyperPrep® Kit		Х			
	Thermo Fisher®	lon TargetSeq™ Exome Kit	X				
	Thermo Fisher®	Ion Ampliseq® Panels	X				
	Twist®	Human Core Exome Kit		Х	Χ		
	Twist®	Twist® Custom Panels		Х	Х		
	Twist®	Fast Hybridization Target Enrichment		Χ	Х		
	PerkinElmer	NEXTFLEX® 16S V1-V3 Amplicon-Seq Kit		Х			
	PerkinElmer	NEXTFLEX® 16S V4 Amplicon-Seq Kit 2.0		X			
	PerkinElmer	NEXTFLEX® 18S ITS Amplicon-Seq Kit		Х			
tagenomics	PerkinElmer	NEXTFLEX® 16S V4 & 18S ITS Amplicon-Seq Kit		Х			
rary paration Kits	PerkinElmer	NEXTFLEX® Rapid XP DNA-Seq Kit		Х	Х		>
,	PerkinElmer	NEXTFLEX® Rapid DNA-Seq Kit 2.0		Х			>
	PerkinElmer	NEXTFLEX® Rapid DNA-Seq Kit		Х			
	NuGen®	Ovation® Ultralow Methyl-Seq Library System		Х			
	PerkinElmer	NEXTFLEX® Poly(A) Bead Selection	Х	Х			>
	PerkinElmer	Normalization & Pooling Protocol	X	Х			>
	Beckman Coulter®	SPRI® Purification Protocol	Х	Х			Х
upplementary	Beckman Coulter®	SPRI® Size Selection Protocol	Х	Х			Х
	NuGen®	Encore® Biotin Module		Х			
	NuGen®	Ovation® FFPE WTA System		Х			
olications &	NuGen®	Ovation® Pico WTA System v2		Х			
tocols	Thermo Fisher®	PicoGreen® Quantitation		Х			>
	Thermo Fisher®	Qubit <sup>®</sup> Quantitation					>
	Thermo Fisher®	Quant-iT® Quantitation					>
	Roche®	SYBR® FAST qPCR Kit		Х			
	Roche®	Library Quant Kit	Х	Х			>

#### NEXTFLEX® VARIANT-SEQ<sup>TM</sup> SARS-COV-2 KIT V2

#### Now available with normalization beads

- Complete solution –Reagents, barcodes, beads, and analysis software included
- Pressure Tested Developed and optimized using real samples
- Robust Same input volume regardless of Ct value
- Minimal Sample Prep No new extraction or normalization prior to library prep
- High-throughput Up to 1,536 indexes available
- Automated Automation available in Sciclone® G3 NGS/NGSx, Sciclone® G3 NGSx iQ™ and JANUS® G3 liquid handlers
- Fast sequencing 1 x 36 nt sequencing possible, for guick turnaround time
- No minimum purchase requirements

The new NEXTFLEX® Variant-Seq<sup>TM</sup> SARS-CoV-2 Kit v2 introduces proprietary NEXTFLEX® normalization beads, which provide a consistent mass and sequencing cluster density for all samples in a library pool, regardless of their viral load. This shortens the time needed for quantification and pooling preparation for sequencing in a high throughput lab by 3 hours.

The NEXTFLEX® Variant-Seq™ SARS-CoV-2 workflow is optimized to quickly, easily, and affordably identify all mutations in a SARS-CoV-2 PCR-positive sample. It utilizes an amplicon-based target enrichment workflow which offers many advantages over hybridization capture-based protocols, most notably speed, scalability, and cost.

#### Whole Genome Sequencing of the SARS-CoV-2 Genome

As of March 2021, SARS-CoV-2 has infected 115 million people and caused over 2.56 million deaths.1 The discovery of particularly aggressive variants such as B.1.1.7 and B.1.351 has shown that monitoring for mutations associated with changes to infection outcome and transmission of SARS-CoV-2 is critical to ensure the success of vaccination programs and establish robust public health responses.2 Once a sample is detected as positive for COVID-19 using an RT-PCR assay, the next question that needs to be answered is, does the sample contain a SARS-CoV-2 Variant of Concern (VOC). NGS is ideal for this as its high resolution enables it to identify all mutations, known and unknown, providing insights to SARS-CoV-2 infection and transmission.

#### Track & Identify SARS-CoV-2 Variants

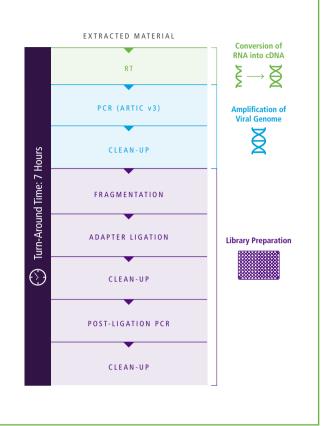
The NEXTFLEX® Variant-Seq™ SARS-CoV-2 Kit has optimized the identification and tracking of SARS-CoV-2 variants. Sample preparation has been simplified, throughput has been increased, and multiple automation solutions offer flexibility based on your lab's needs. Along with a complete, sample-to-answer workflow, PerkinElmer is here to help labs meet their SARS-CoV-2 variant detection challenges.

The NEXTFLEX® Variant-Seq™ SARS-CoV-2 Kit is powered by COSMOSID® software, converting sequencing data into a concise sample report within minutes. These reports are compliant with the CDC requirements for tracking variants. The Pangolin lineage, used by the CDC to track variants, is included in the reports. Additionally, the SARS-CoV-2 consensus created using the report can be easily submitted to NCBI and GISAID.

#### SARS-CoV-2 WGS Simplified

The NEXTFLEX® Variant-Seq™ SARS-CoV-2 kit simplifies the identification of existing and new variants of the SARS-CoV-2 virus by greatly reducing the sample preparation needed prior to sequencing. The NEXTFLEX® Variant-Seq™ SARS-CoV-2 workflow begins with synthesizing cDNA from previously extracted nucleic acids from a sample already identified as containing SARS-CoV-2 using RT-PCR. A single universal volume of the extracted nucleic acids is used as template, with no prior dilution step. The viral genome is then amplified using the ARTIC v3 primer pools, provided in the kit. The PCR product is purified and without quantification or normalization goes directly through a fast library preparation protocol to generate libraries that are compatible with Illumina® sequencing.

By eliminating the need for a repeat nucleic acid extraction and complicated sample preparation required by most other NGS-based protocols, the entire procedure from sample to finalized library ready for sequencing only takes 7 hours.



#### Maximize Your Variant Detection Throughput While Reducing Your Sequencing Costs

The NEXTFLEX® Variant-Seq<sup>™</sup> SARS-CoV-2 kit includes up to 1,536 unique dual index barcodes, supplied in four sets of 384 barcodes each, for ultra high-throughput multiplexing on the Illumina® NovaSeq® sequencer or other Illumina® platform. The 1,536 NEXTFLEX® UDIs maximize your sequencing throughput and increase your workflow efficiencies. By multiplexing 1,536 samples per run, you can:

- Sequence 4x as many samples per run
- Get answers faster by reducing the numbers of runs needed
- Reduce sequencing reagent costs by up to 4x

#### Maximize Your Variant Detection Throughput While Reducing Your Sequencing Costs

Plug-and-play protocols have been developed for automating the NEXTFLEX® Variant-Seq™ SARS-CoV-2 kit on the Sciclone® G3 NGS/NGSx, Sciclone® G3 NGSx iQ™ and JANUS® G3 liquid handlers. These multiple automation options offer labs the flexibility to meet their specific throughput and hands-on time needs.

Let us help you track and identify SARS-CoV-2 variants.

#### HIVE<sup>TM</sup> scRNAseq SOLUTION

Capture, storage, & processing: all in one workflow



#### **Enabling Multi-site & Multitimepoint Sample Collection**

The HIVETM scRNAseq solution is a combined sample storage and single-cell profiling system. The HIVETM device delivers versatility by enabling sample collection at different sites or at different timepoints and sample processing at the same location where they are collected or at a centralized processing laboratory.

Biological resolution at the level of individual cells is powering the next phase of precision health. The HIVETM scRNAseq Solution integrates sample storage and single cell profiling into a complete workflow, solving the issues that limit single cell RNA analysis by:

- Enabling multi-site and multi-timepoint sample collection
- Maintaining sample integrity through storage, shipping, and processing
- Increasing the recovery rates of fragile cells
- Facilitating loading of larger sample volumes
- Delivering flexible and scalable workflows by enabling batch processing
- Removing the need for specialized instrumentation

The HIVE™ scRNAseq Solution is a portable, handheld, single-use device that enables gentle capture, robust storage, and easy processing for the analysis of single-cell samples. The HIVE™ scRNAseq Solution will expand single-cell analysis in basic, translational, pre-clinical, and clinical research applications.

#### Easy to Use

The HIVE<sup>TM</sup> scRNAseq solution incorporates reagents and a single-use, handheld, shippable HIVE<sup>TM</sup> device that can be used in almost any lab environment. No specialized equipment is required to process the samples from single cells through library preparation and the workflow can be run in most labs.

#### THE HIVE™ scRNAseq SOLUTION









Sample Capture & Storage

RNA Library Prep

Data Analysis

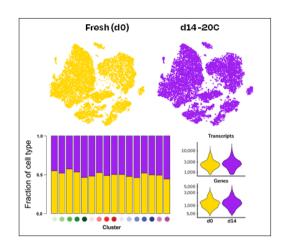
The HIVE™ scRNAseq solution delivers comprehensive biological representation, including fragile and sparse cells.

With a sample storage solution built into the capture and processing workflows, the HIVE<sup>TM</sup> devices provide a stable environment to arrest cells in their current states and protect the sample over time.

#### **Maintaining Sample Integrity**

Cell quality is not compromised when using the HIVE<sup>TM</sup> device during storage, shipping or processing. As can be seen in Figure 1, single cell libraries prepared from fresh cells and cells stored at -20°C for 14 days prior to processing have similar profiles, showing no loss of cell population diversity, number of transcripts or genes detected.

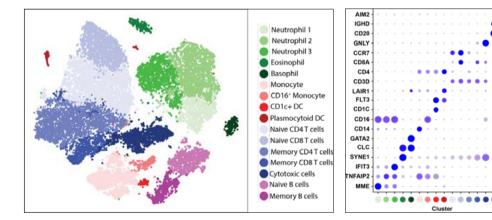
Figure 1. Comparison between samples processed while fresh or stored at -20°C for 14 days prior to processing.



#### Increasing the Recovery Rates of Fragile Cells

Cells isolated using the HIVE<sup>TM</sup> device settle gently into microwells within a matter of minutes. This change enables the analysis of cells that are typically difficult to analyze either because of their fragile membrane structures or because they have been stimulated or transfected which can induce fragility. Figure 2 shows the HIVE<sup>TM</sup> scRNAseq workflow is able to recover granulocytes which are difficult to detect with other platforms because of their fragility.

Figure 2. Illustrates 24,720 cells were captured in six HIVE<sup>ss</sup> devices. The cell types processed include granulocytes such as neutrophils, eosinophils, and basophils.



#### Facilitating Loading of Larger Sample Volumes

Other systems for single cell analysis only allow small sample loading volumes, typically around 50  $\mu$ L. To ensure you are not missing critical information available in the primary sample, the HIVE<sup>TM</sup> scRNAseq solution allows you to load much larger volumes of up to 4 mL.

#### Delivering Flexible & Scalable Workflows

The HIVETM scRNAseq workflow reduces experimental and inter-site variability seen in other single cell analysis platforms. The workflow also delivers flexibility of processing location and throughput. The samples can be easily processed where they are collected or stored and shipped to a central facility for processing. By allowing safe sample storage, samples can be batched to improve lab efficiency.

#### AUTOMATED cfDNA & cfRNA WORKFLOW

#### Solutions:

- Plasma Fractionation
- cfDNA isolation from plasma
- cfDNA isolation from urine
- QC & sizing of cfDNA
- Library preparation reagents
- Library preparation automation
- Library quality control

### Volume Verification or Volume-driven Pipetting: Two Ways to Automate cfDNA & cfRNA Workflows

Volume verification allows the user to set a desired level of plasma required to provide an adequate yield for downstream analysis. The JANUS G3 Blood iQ workstation verifies the volume in each tube and will only process those with adequate available plasma.

In volume-driven pipetting the workstation transfers all available plasma volume based on the calculated plasma height. This is particularly useful in some cfDNA workflows where obtaining the highest volume of plasma is necessary to increase the likelihood of uncovering a rare variant or mutation.

Both methods create an efficient and reliable first step in the genetic analysis of fractionated blood. This powerful tool combined with other PerkinElmer products, such as the chemagic™ 360 nucleic acid extractor, LabChip GX-Touch nucleic acid analyzer, and the Sciclone® G3 NGSx iQ™ workstation, enables a fully automated genetic analysis workflow from blood, a common sample matrix for complex workflows.

#### Improving the Efficiency of cfDNA and cfRNA Workflows

Research is being focused on developing clinical research reagents in the fields of prenatal disorders and malignant disease monitoring using DNA recovered from cell-free fluids. Cell-free DNA (cfDNA) is a sample type of choice due to its quick, minimally invasive method of collection that is amenable to analyzing multiple time points and multiple samples at a time.

Circulating DNA can be highly fragmented and present at low concentrations, which may pose a number of challenges that need to be overcome to achieve reliable cfDNA extraction. This has led to a need for more efficient methods for extracting, processing, and analyzing cfDNA. To meet this need, PerkinElmer has developed a complete, automated workflow for extracting cfDNA and performing subsequent next generation sequencing applications.

#### cfRNA Biomarkers

Cell-free circulating tumor RNA, or cfRNA shed from tumor cells, may mediate intercellular communication and may be used as biomarkers for diseases and monitoring of therapies.

#### Improve the Efficiency of Your cfDNA and cfRNA Workflows

Our automated and optimized end-to-end solutions allow labs to minimize errors, reduce hands-on time, and increase throughput and reproducibility. Flexible solutions based on your testing and throughput requirements are available to meet your lab's needs.

#### **AUTOMATED cfDNA & cfRNA WORKFLOW**







PRECISE FRAGMENT
SIZE DISTRIBUTION
& QUANTITATION OF
LIBRARIES

Library Quality Control:
LabChip® GX-Touch™ Microfluidic Technology

PerkinElmer understands centralized biobanking facilities face demanding challenges to setup an efficient and scalable workflow for storage and processing of biological specimens. These challenges include: sample traceability, blood fractionation, quality control, and sample preparation for genetic analysis. The new JANUS G3 Blood iQ workstation is the first step in a seamless, efficient, and traceable workflow for the genetic analysis of cfDNA, cfRNA, and genomic DNA from fractionated blood.

The JANUS G3 Blood iQ workstation is the premium biobanking solution for unattended automation of any blood fractionation workflow. The workstation features:

- Maximize plasma and buffy coat recovery with the proprietary image analysis software
- Reformat to and record individual tube & plate IDs with the integrated gripper & 2D barcode options
- All storage and tips are on deck with the large deck capacity
- Process samples quickly with the pre-developed protocols or easily develop custom protocols with WinPrep software
- Precision blood transfers with the highly reproducible liquid transfer technology
- The JANUS G3 Blood iQ workstation provides traceable, efficient, and reproducible blood fractionation necessary for the isolation of gDNA, cfDNA, and cfRNA from buffy coat and plasma layers of fractionated blood.

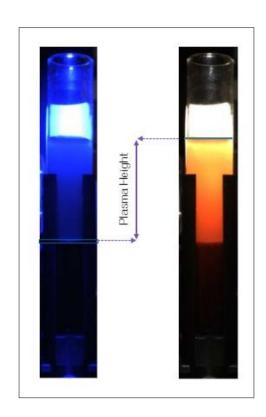


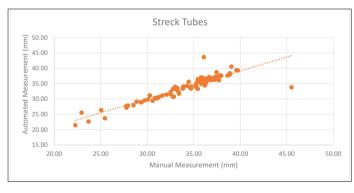
#### **Blood Layer Detection**

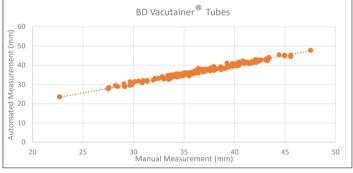
Proprietary imaging technology allows the JANUS G3 Blood iQ workstation to precisely identify plasma and buffy coat layers in centrifuged blood tubes. A blue image is taken and identifies the buffy layer height based on a bounded square around the pixels identified to contain white blood cells. A white image is taken and identifies the plasma layer height through points on the plasma layer meniscus and their relative position to the buffy layer. This imaging technology is driven by a deeplearning based image analysis model which precisely identifies the distinct blood layers. Subsequently, the WinPrep™ software calculates volumes based on the layer height and tube geometries. Height calculations are unique to each tube. This important technology outputs the below parameters which are also stored in a CSV file which can be seamlessly integrated with laboratory information management systems.



- Input Tube Barcodes
- Available Plasma Volume
- Actual Plasma Volume Transferred
- Available Buffy Coat Volume
- Actual Buffy Coat Volume Transferred







The above graphs show the difference between manually measured plasma heights and measurements from the JANUS G3 Blood iQ imaging system.

#### **Blood Tube Compatibility**

The JANUS G3 Blood iQ Workstation can accurately identify the plasma and buffy

coat layers in a variety of centrifuged input blood tubes. Compatible tube types include:

- 10 mL Streck Cell-Free DNA BCT® Tubes
- 10 mL BD Vacutainer® Plus Plastic Tube with lavender BD Hemogard™ Closure
- 10 mL PreAnalytiX PAXgene® Blood Circulating Cell-Free DNA Tubes
- 9 mL Greiner Bio-one VACUETTE® Tubes

#### Sample Traceability

The JANUS G3 Blood iQ Workstation utilizes a blood tube scanning camera and a whole rack barcode scanner

to accurately track each individual sample's journey through the blood fractionation process. These integrated 2D barcode scanners captures barcodes from destination tubes to destination well plates providing full traceability of samples from input test tube to output microfuge tubes in a convenient export file.



KIT SELECTION TARGET SEQUENCING **END TO END WORKFLOWS** 

#### JANUS G3 VariSpan Technology: Precision Pipetting for cfDNA, cfRNA & gDNA Analysis

The JANUS G3 Blood iQ workstation comes with an 8-channel VariSpan pipetting module which enables industry leading accuracy and precision variable pipetting. The VariSpan pipetting arm enables aspirating from the blood tubes and reformatting to any well plate configuration. The four main pipetting tips used in this workstation, shown below, allow for complex plasma and buffy coat extraction in fractionated blood.

Matrix	Volume	Accuracy (Standard Error)	Precision (Random Error)
Plasma	5.0 mL	+/-5%	5.0% CV
Plasma	500 μL	+/-10%	5.0% CV
Buffy Coat	900 μL	+/-5%	5.0% CV
Buffy Coat	100 μL	+/-10%	5.0% CV

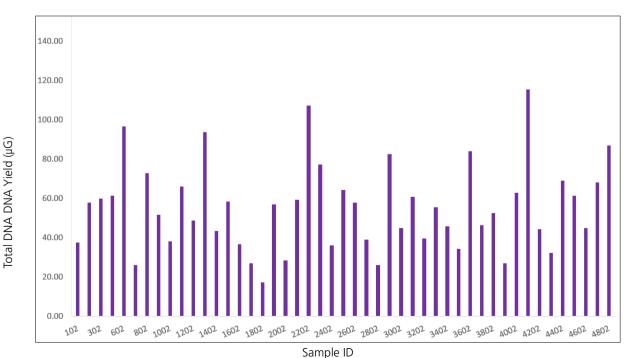
#### **PLASMA TIP** 5 mL Conductive Filter Tip **BUFFY COAT TIP** 900 µL Wide Bore Conductive Filter Tip

#### Downstream Extraction: Integrated Gripper and Deck Capacity

The JANUS G3 Blood iQ workstation is equipped with generous deck capacity and an integrated gripper to fully automate downstream workflows such as magnetic-bead based nucleic acid extraction. This instrument can further prepare wash and elution plates for nucleic acid extraction platforms such as the chemagic<sup>™</sup> 360 instrument.

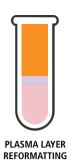
#### High DNA Yield from Buffy Coat

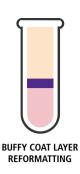
Samples were processed in an automated workflow using the JANUS G3 Blood iQ workstation. On average, 58.7 µg of total genomic DNA was extracted from the buffy coat layer.



#### Out of the Box Protocols

The Janus Blood iQ workstation comes with pre-developed protocols for blood fractionation. Users have the ability to reformat the plasma layer, the buffy coat layer or both to various destination labware. All you need to do is input your desired transfer volumes (or automated image-determined volumes) and click Go!







#### **PKeye<sup>™</sup> Mobile Operations Monitor**

PerkinElmer has created the PKeye mobile operations monitor which allows you to remotely monitor your workstation from ar office, conference room or even your own home. Integrating multiple on-deck cameras to the JANUS G3 Blood iQ workstation, the PKeye mobile operations monitor enables:

- Remote error notifications
- Video clips of each individual error
- Full run videos, locally stored, for future troubleshooting

You will now truly be able to walk-away from your workstation and focus on your intellectual contributions to the laboratory.

# THE PKEYE" WORKFLOW MONITOR: REVEAL THE PULSE OF YOUR LAB Pata On-Demand Just-in-Time Manual Interventions Cloud-based Instrument Logs PReal-time Progress Monitoring Video Recording Status Notifications Status Notifications Status Notifications LabChip® GX Touch® Analyzer Chemagic® 360 Estractor

Part Number	Description
CJM80B5	JANUS G3 Blood iQ Workstation, Standard, IVD
CJM9GB5	JANUS G3 Blood iQ Workstation, Standard, Gripper, IVD
CJL80B5	JANUS G3 Blood iQ Workstation, Expanded, IVD
CJL8GB5	JANUS G3 Blood iQ Workstation, Expanded, Gripper, IVD
YJM80B5	JANUS G3 Blood iQ Workstation, Standard, RUO
YJM9GB5	JANUS G3 Blood iQ Workstation, Standard, Gripper, RUO
YJL80B5	JANUS G3 Blood iQ Workstation, Expanded, RUO
YJL8GB5	JANUS G3 Blood iQ Workstation, Expanded, Gripper, RUO
CLS154586	Kit, Well Plate Tube ID, Flatbed Barcode Scanner
CLS151491	JANUS G3 Instrument Table
CLS153622	PKeye Hardware
CLS153940	PKeye Cloud Set-up & Integration

# TARGETED SEQUENCING OF DNA FROM DRIED BLOOD SPOT SAMPLES

#### Introduction

Dried blood spots (DBS) are frequently used for newborn screening, large population-based surveys, and biobanking. DBS sampling delivers many benefits not realized by other blood sampling protocols including:

- Minimal blood volume required (approximately 30 100 µL per spot)
- Simple collection which does not require a phlebotomist
- Easy transportation and space saving storage
- Sample integrity protected during transportation and storage

Because of these benefits, DBS are used in many research, pathogen detection, and biobanking applications. PerkinElmer's optimized DBS workflow solution provides a tested and validated method for targeted sequencing of DNA extracted from DBS.

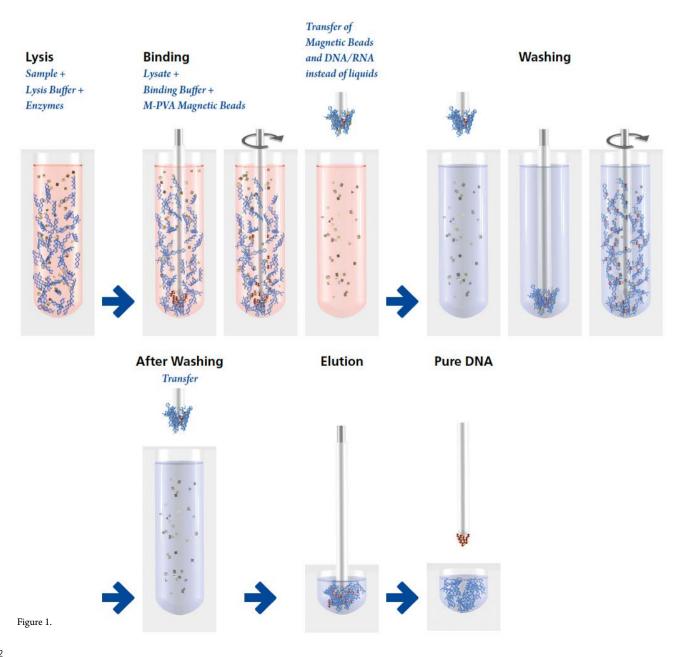


#### Improving Quality and Yield of DNA Isolation from DBS

The DBS Puncher™ instrument was used to punch two 4.75 mm dried blood spot samples per extraction into 96-well microtitration plates from 96 blood cards. DNA was isolated from the 96 DBS samples using the chemagic™ DNA CS200 DNA Kit automated on the chemagic™ 360 instrument. Both instruments are capable of high throughput operations, working in a 96 well format.

The chemagic<sup>™</sup> 360 instrument is one of several PerkinElmer chemagen<sup>™</sup> instruments for nucleic acid extraction using the proprietary chemagen<sup>™</sup> separation technology.

chemagen™ Technology addresses challenges associated with the isolation of high molecular weight (HMW) gDNA from DBS samples by eliminating the alkaline lysis and centrifugation steps traditionally used during isolation which denature and fragment nucleic acids. The chemagen™ Technology's automated magnetic separation procedure (Figure 1) features magnetizable rotating rods combining the transfer and suspension of chemagen™ M-PVA magnetic beads to isolate and purify ultra-pure, HMW gDNA from DBS. The M-PVA magnetic beads have a high affinity for nucleic acids and low protein binding, resulting in very pure DNA and RNA elution.



#### Nucleic Acid Automation Designed for Your Lab's Needs

With flexibility in throughput, capacity, and sample type, high quality manufacturing standards, and outstanding customer support, PerkinElmer's chemagic<sup>TM</sup> nucleic isolation automation delivers solutions designed to meet your specific need.



#### chemagic™ 360 Instrument

With the ability to isolate DNA from 96 to 1,000 DBS samples per day and the flexibility to isolate DNA or RNA from any other human sample type, the chemagic™ 360 Instrument is designed to meet labs' high-throughput nucleic acid isolation needs.



#### chemagic™ Prepito® Instrument

The chemagic™ Prepito® instrument is ideal for lower-through-put labs, isolating DNA from less than 100 samples per day.

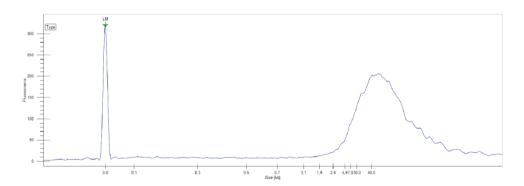


Figure 2: Representative electropherogram made with the LabChip® GX Touch™ nucleic acid analyzer using the Genomic DNA chip of DNA extracted from DBS with the chemagic™ 360 instrument.

The DNA extracted from each sample was eluted into 60  $\mu$ l of elution buffer. With an average yield of ~600 ng of genomic DNA at an average concentration of 10 ng/ $\mu$ l, enough DNA was extracted from every sample for downstream NGS analysis.

To ensure the quality the extracted DNA was analyzed using the LabChip® GX Touch™ nucleic acid analyzer with the Genomic DNA chip and reagents kit (Figure 2).

DNA extracted using the chemagen<sup>TM</sup> Technology from DBS can be taken for a variety of downstream applications. Data below illustrates its application with targeted sequencing which relies on pure, high quality DNA as input material.

#### Targeted Sequencing from DNA Extracted from DBS Samples

Five of the afore mentioned DBS gDNA samples served as input for targeted sequencing. The DNA was sheared to a target peak size of approximately 150 - 200 bp using the Covaris® E220 focused ultrasonicator. Libraries were constructed from 200 ng of DNA with the Agilent® SureSelect® XT low input reagent kits for library preparation and the Agilent® SureSelect® XT DMD kit for hybrid-capture-based target enrichment. Both the library preparation and target capture protocols were automated on the Sciclone® G3 NGSx workstation. The samples were sequenced on an Illumina® NovaSeq® sequencer.

Table 1: Sequencing data generated from libraries constructed using the Agilent\* SureSelect\* XT low input reagent kit for library preparation and the Agilent\* SureSelect\* XT DMD kit for hybrid-capture-based target enrichment from DNA isolated from 5 DBS samples using the chemagic $^{m}$  360 instrument.

DBS Sample	Total reads	% Mapped Reads	pped Reads			% Ta	arget	
·				age (BED)	10–20x		30–40x	>40x
1	10470234	99%	10%	162.49	0.88	1.1	1.37	96.24
2	21593676	99%	10%	183.68	0.63	0.8	0.96	97.41
3	19451036	99%	17%	138.55	0.76	0.94	1.1	96.8
4	18497402	99%	18%	134.26	0.51	0.74	0.87	88.12
5	10512000	100%	14%	180.96	0.58	0.79	0.85	97.6

#### Conclusion

Genomic DNA extracted from DBS is an attractive sample type because of the many handling and storage benefits it offers. Using the chemagen<sup>TM</sup> Technology, DBS can now be used for NGS analysis, which has been challenging in the past, because other technologies could not deliver such pure and high quality DNA required for NGS analysis. The described DBS workflow consisting of sampling, transportation, gDNA extraction, DNA and library QC, and library preparation automation facilitates targeted NGS sequencing of DNA extracted from DBS.

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Reference	Name	Quantity		
	LIBRARY PREP // DNA SEQ			
NEXTFLEX® RAPID DNA-SEQ KIT 2.0 (PCR-free compatible)				
NOVA-5188-01	NEXTFLEX® Rapid DNA-Seq Kit 2.0	8 rxn		
NOVA-5188-02	NEXTFLEX® Rapid DNA-Seq Kit 2.0	48 rxn		
NOVA-5188-03	NEXTFLEX® Rapid DNA-Seq Kit 2.0	96 rxn		
NOVA-5188-11	NEXTFLEX® Rapid DNA-Seq Kit 2.0 (with NEXTFLEX-HT™ barcodes)	8 rxn		
NOVA-5188-12	NEXTFLEX® Rapid DNA-Seq Kit 2.0 (with NEXTFLEX-HT™ barcodes)	48 rxn		
NOVA-5188-13	NEXTFLEX® Rapid DNA-Seq Kit 2.0 (with NEXTFLEX-HT™ barcodes)	96 rxn		
NEXTFLEX® RAPIC	XP DNA-SEQ KIT (PCR-free compatible)			
NOVA-5149-01	NEXTFLEX® Rapid XP DNA-Seq Kit	8 rxns		
NOVA-5149-02	NEXTFLEX® Rapid XP DNA-Seq Kit	48 rxns		
NOVA-5149-03	NEXTFLEX® Rapid XP DNA-Seq Kit	96 rxns		
NOVA-5149-103	NEXTFLEX® Rapid XP DNA-Seq Pre-Plated Automation Kit	96 rxns		
NEXTFLEX® CELL	FREE DNA-SEQ KIT 2.0*			
NOVA-5150-01	NEXTFLEX® Cell Free DNA-Seq Kit 2.0	8 rxns		
NOVA-5150-02	NEXTFLEX® Cell Free DNA-Seq Kit 2.0	48 rxns		
* For use with the N	lextPrep-Mag™ cfDNA automated isolation kit (NOVA-3825 series) or NextPrep-Mag™ urine cfDNA isolation l	kit (NOVA-3826 series)		
NEXTFLEX® BISUL	FITE-SEQ KIT			
NOVA-5119-01	NEXTFLEX® Bisulfite-Seq Kit	8 rxns		
NOVA-5119-02	NEXTFLEX® Bisulfite-Seq Kit	48 rxns		
NOVA-511911	NEXTFLEX® Bisulfite-Seq Barcodes - 6	48 rxns		
NOVA-511912	NEXTFLEX® Bisulfite-Seq Barcodes - 12	96 rxns		
NOVA-511913	NEXTFLEX® Bisulfite-Seq Barcodes - 24	192 rxns		
NOVA-511921	NEXTFLEX® MSP1 Restriction Enzyme	8 rxns		
NOVA-511922	NEXTFLEX® MSP1 Restriction Enzyme	48 rxns		
NEXTFLEX® DNA-	SEQ KIT FOR ION PGM™ and ION PROTON™ (PCR-free compatible)			
NOVA-4001-01	NEXTFLEX® DNA-Seq Kit for Thermo Scientific™ Ion PGM™ and Ion Proton™ sequencers	8 rxns		
NOVA-4001-02	NEXTFLEX® DNA-Seq Kit for Thermo Scientific™ Ion PGM™ and Ion Proton™ sequencers	48 rxns		
NEXTFLEX® CELL	FREE DNA-SEQ KIT for Thermo Scientific™ Ion PGM™ and Ion Proton™ sequencers			
NOVA-4002-01	NEXTFLEX® Cell Free DNA-Seq Kit for Thermo Scientific™ Ion PGM™ and Ion Proton™ sequencers	s 8 rxns		
NOVA-4002-02	NEXTFLEX® Cell Free DNA-Seq Kit for Thermo Scientific™ Ion PGM™ and Ion Proton™ sequencers	s 48 rxns		

#### References

Reference	Name	Quantity		
LIBRARY PREP // RNA SEQ				
NEXTFLEX® SMALL RNA-SEQ KIT v3*				
NOVA-5132-05	NEXTFLEX® Small RNA-Seq Kit v3 (8 barcodes)	8 rxns		
NOVA-5132-06	NEXTFLEX® Small RNA-Seq Kit v3 (48 barcodes)	48 rxns		
NOVA-51312X	NEXTFLEX® TRNA/YRNA Blockers	8, 48, 96 rxn		
NOVA-5132-08	NEXTFLEX® Small RNA Sequencing Kit v3 Automation Kit	96 rxns		
NOVA-5132-18	NEXTFLEX® Automated Small RNA-Seq kKit v3 with UDIs (UDI 1-96 or 97-192)	96 rxns		
NOVA-5132-19	NEXTFLEX® Automated Small RNA-Seq Kit v3 with UDIs (UDI 97-192)	96 rxns		
NOVA-5132-20	NEXTFLEX® Automated Small RNA-Seq Kit v3 with UDIs (UDI 193-288)	96 rxns		
NOVA-5132-21	NEXTFLEX® Automated Small RNA-Seq Kit v3 with UDIs (UDI 289-384)	96 rxns		
NOVA-5132-22	NEXTFLEX® Small RNA-Seq Kit v3 with UDIs (1-48)	48 rxns		
NOVA-5132-23	NEXTFLEX® Small RNA-Seq Kit v3 with UDIs (49-96)	48 rxns		
NOVA-5132-24	NEXTFLEX® Small RNA-Seq Kit v3 with UDIs (97-144)	48 rxns		
NOVA-5132-25	NEXTFLEX® Small RNA-Seq Kit v3 with UDIs (145-192)	48 rxns		
NOVA-5132-26	NEXTFLEX® Small RNA-Seq Kit v3 with UDIs (193-240)	48 rxns		
NOVA-5132-27	NEXTFLEX® Small RNA-Seq Kit v3 with UDIs (241-288)	48 rxns		
NOVA-5132-28	NEXTFLEX® Small RNA-Seq Kit v3 with UDIs (289-336)	48 rxns		
* Compatible with	the NextPrep™ Magnazol cfRNA isolation kit (NOVA-3830-01)			
NEXTFLEX® RAPID	DIRECTIONAL RNA-SEQ KIT 2.0			
NOVA-5198-01	NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0	8 rxns		
NOVA-5198-02	NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0	48 rxns		
NOVA-5198-03	NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0	96 rxns		
NOVA-512991	NEXTFLEX® Poly(A) Beads 2.0	8 rxns		
NOVA-512992	NEXTFLEX® Poly(A) Beads 2.0	48 rxns		
NOVA-512993	NEXTFLEX® Poly(A) Beads 2.0	96 rxns		
NOVA-512961	NEXTFLEX® RiboNaut rRNA Depletion Kit (Human, Mouse, Rat)	8 rxns		
NOVA-512962	NEXTFLEX® RiboNaut rRNA Depletion Kit (Human, Mouse, Rat)	48 rxns		
NOVA-512963	NEXTFLEX® RiboNaut rRNA Depletion Kit (Human, Mouse, Rat)	96rxns		

References					
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Reference	Name	Quantity			
	LIBRARY PREP // AMPLICON PANELS				
· ·	on Panels for Cancer Predisposition & Cancer*				
· · · · · · · · · · · · · · · · · · ·	on Panels for Inherited Syndromes and Conditions*				
<u> </u>	on Panels for Newborn Syndromes and Infertility*				
* Pre-designed par	* Pre-designed panels, custom panels, and up to 384 barcodes available. Please inquire.				
	LIBRARY PREPARATION & HYBRID CAPTURE	ı			
NOVA-6001-16	NEXTFLEX® Duchenne Muscular Dystrophy NGS Hybridization Panel (RUO)	16 rxns			
NOVA-6002-16	NEXTFLEX® Lysosomal Storage Disorders NGS Hybridization Panel (RUO)	16 rxns			
NOVA-6003-16	NEXTFLEX® Targeted NGS Panel 1 (RUO) genes related to endocrine and metabolic disorders, hemoglobinopathies, and primary immunodeficiencies	16 rxns			
NOVA-6004-16	NEXTFLEX® Targeted NGS Panel 2 (RUO) enes related to amino acid disorders, fatty acid disorders and organic acid disorders	16 rxns			
NOVA-6005-16	NEXTFLEX® Core Exome NGS Panel (RUO)	16 rxns			
NOVA-6100-1	NEXTFLEX® NGS Data Analysis Platform	Annual license			
NEXTFLEX® DNA B	ARCODE BLOCKERS				
NOVA-514134	NEXTFLEX-96™ DNA Barcode Blockers - 96	128-192 rxns			
	UNIVERSAL BLOCKERS				
NOVA-5143231	NEXTFLEX® Universal Blockers	8 rxns			
NOVA-5143232	NEXTFLEX® Universal Blockers	48 rxns			
NOVA-5143233	NEXTFLEX® Universal Blockers	96 rxns			
110VA-3143233		90 IXIIS			
NEVTELEV® 4.66 VA	LIBRARY PREP // METAGENOMIC AMPLICON-SEQ				
	-V3 AMPLICON-SEQ KIT	24			
NOVA-4202-02	NEXTFLEX® 16S V1-V3 Amplicon-Seq Kit (12 Barcodes)	24 rxns			
NOVA-4202-03	NEXTFLEX® 16S V1-V3 Amplicon-Seq Kit (48 Barcodes)	96 rxns			
NOVA-4202-04	NEXTFLEX® 16S V1-V3 Amplicon-Seq Kit (Barcodes 1-96)	192 rxns			
NOVA-4202-05	NEXTFLEX® 16S V1-V3 Amplicon-Seq Kit (Barcodes 97-192)	192 rxns			
NOVA-4202-06	NEXTFLEX® 16S V1-V3 Amplicon-Seq Kit (Barcodes 193-288)	192 rxns			
NOVA-4202-07	NEXTFLEX® 16S V1-V3 Amplicon-Seq Kit (Barcodes 289-384)	192 rxns			
	AMPLICON-SEQ KIT 2.0	l <sub>-</sub> .			
NOVA-4203-02	NEXTFLEX® 16S V4 Amplicon-Seq Kit 2.0 (12 Barcodes)	24 rxns			
NOVA-4203-03	NEXTFLEX® 16S V4 Amplicon-Seq Kit 2.0 (48 Barcodes)	96 rxns			
NOVA-4203-04	NEXTFLEX® 16S V4 Amplicon-Seq Kit 2.0 (Barcodes 1-96)	192 rxns			
NOVA-4203-05	NEXTFLEX® 16S V4 Amplicon-Seq Kit 2.0 (Barcodes 97-192)	192 rxns			
NOVA-4203-06	NEXTFLEX® 16S V4 Amplicon-Seq Kit 2.0 (Barcodes 193-288)	192 rxns			
NOVA-4203-07	NEXTFLEX® 16S V4 Amplicon-Seq Kit 2.0 (Barcodes 289-384)	192 rxns			
	S AMPLICON-SEQ KITS	I			
NOVA-4210-02	NEXTFLEX® 18S ITS Amplicon-Seq Kit (12 Barcodes)	24 rxns			
	\$ 18S ITS AMPLICON-SEQ KITS	I			
NOVA-4213-01	NEXTFLEX® 16S V4 & 18S ITS Amplicon-Seq Kit (Barcodes 1-8)	8 rxns			
NOVA-4213-02	NEXTFLEX® 16S V4 & 18S ITS Amplicon-Seq Kit (Barcodes 1-48)	48 rxns			
NOVA-4213-13	NEXTFLEX® 16S V4 & 18S ITS Amplicon-Seq Automation Kit (Barcodes 1-96)	96 rxns			
LIBRARY PREP // MOLECULAR INVERSION PROBES PANELS					
NEXTFLEX® Custom MIP Panel*					
* custom panels w	ith UDI and UMI available. Please inquire.				

References				
Reference	Name	Quantity		
Reference		Quantity		
NEVTELEY LITTM DADGO	BARCODES FOR ILLUMINA® DNA-SEQ			
NEXTFLEX-HT™ BARCO		102		
NOVA-514174	NEXTFLEX_HTTM Barcodes - 1-96 (plate)	192 rxns		
NOVA-514175	NEXTFLEX-HTTM Barcodes - 97-192 (plate)	192 rxns		
NOVA-514176	NEXTFLEX LITTM Barcodes - 192-288 (plate)	192 rxns 192 rxns		
NOVA-514177	NEXTFLEX-HTTM Barcodes - 289-384 (plate)			
NOVA-514153	NEXTFLEX® Unique Dual Index Barcodes - Set D (Barcodes 289 - 384)	192 rxns		
NEXTFLEX® DNA BARC		40		
NOVA-514101	NEXTFLEX® DNA Barcodes - 6	48 rxns		
NOVA-514102	NEXTFLEX® DNA Barcodes - 12	96 rxns		
NOVA-514103	NEXTFLEX® DNA Barcodes - 24	192 rxns		
NOVA-514104	NEXTFLEX® DNA Barcodes - 48	384 rxns		
NEXTFLEX-96™ DNA B				
NOVA-514105	NEXTFLEX-96™ DNA Barcodes - (plate)	768 rxns		
NOVA-514106	NEXTFLEX-96™ DNA Barcodes - (tubes)	768 rxns		
NEXTFLEX® CHIP-SEQ B				
NOVA-514120	NEXTFLEX® ChIP-seq Barcodes - 6	48 rxns		
NOVA-514121	NEXTFLEX® ChIP-seq Barcodes - 12	96 rxns		
NOVA-514122	NEXTFLEX® ChIP-seq Barcodes - 24	192 rxns		
NOVA-514123	NEXTFLEX® ChIP-seq Barcodes - 48	384 rxns		
NEXTFLEX® PCR-FREE E	BARCODES			
NOVA-514110	NOVA-514110	NOVA-514110		
NOVA-514112	NOVA-514112	NOVA-514112		
NEXTFLEX® BISULFITE-S	SEQ BARCODES BARCODES			
NOVA-511911	NEXTFLEX® Bisulfite-Seq Barcodes - 6	48 rxns		
NOVA-511912	NEXTFLEX® Bisulfite-Seq Barcodes - 6	96 rxns		
NOVA-511913	NEXTFLEX® Bisulfite-Seq Barcodes - 6	192 rxns		
NEXTFLEX® UNIQUE DU	JAL INDEX BARCODES (1-384)			
NOVA-514150-EVAL16	NEXTFLEX® Unique Dual Index Barcodes 1-8 (in tubes)	16 rxns		
NOVA-514150-EVAL48	NEXTFLEX® Unique Dual Index Barcodes 1-24 (in tubes)	48 rxns		
NOVA-514150	NEXTFLEX® Unique Dual Index Barcodes - Set A (Barcodes 1 - 96)	192 rxns		
NOVA-514151	NEXTFLEX® Unique Dual Index Barcodes - Set B (Barcodes 97 - 192)	192 rxns		
NOVA-514152	NEXTFLEX® Unique Dual Index Barcodes - Set C (Barcodes 193 - 288)	192 rxns		
NOVA-514153	NEXTFLEX® Unique Dual Index Barcodes - Set D (Barcodes 289 - 384)	192 rxns		
NEXTFLEX® UNIQUE DU	JAL INDEX BARCODES FOR ULTRA HIGH-THROUGHPUT MULTIPLEXING (1-1,536)			
NOVA-534100	NEXTFLEX® Unique Dual Index Barcodes (1-1,536 )	3,072 rxns		
NOVA-534101	NEXTFLEX® Unique Dual Index Barcodes (1-384)	768 rxns		
NOVA-534102	NEXTFLEX® Unique Dual Index Barcodes (385-768)	768 rxns		
NOVA-534103	NEXTFLEX® Unique Dual Index Barcodes (769-1152)	768 rxns		
NOVA-534104	NEXTFLEX® Unique Dual Index Barcodes (1153-1536)	768 rxns		

BARCODES FOR ILLUMINA® RNA-SEQ				
NEXTFLEX® RNA-SEQ BARCODES				
NOVA-512911	NEXTFLEX® RNA-Seq Barcodes - 6	48 rxns		
NOVA-512912	NEXTFLEX® RNA-Seq Barcodes - 12	96 rxns		
NOVA-512913	NEXTFLEX® RNA-Seq Barcodes - 24	192 rxns		
NOVA-512914	NEXTFLEX® RNA-Seq Barcodes - 48	384 rxns		
NEXTFLEX-96™ RNA-SEQ BARCODES				
NOVA-512915	NEXTFLEX-96™ RNA-Seq Barcodes - (plate)	768 rxns		
NEXTFLEX® RNA-SEQ UNIQUE DUAL INDEX BARCODES (1-384)				
NOVA-512920-EVAL16	NEXTFLEX® RNA-Seq Unique Dual Index Barcodes 1-8 (in tubes)	16 rxns		
NOVA-512920-EVAL48	NEXTFLEX® RNA-Seq Unique Dual Index Barcodes (in tubes)	48 rxns		
NOVA-512920	NEXTFLEX® RNA-Seq Unique Dual Index Barcodes (Barcodes 1 - 96)	192 rxns		
NOVA-512921	NEXTFLEX® RNA-Seq Unique Dual Index Barcodes (Barcodes 97 - 192)	192 rxns		
NOVA-512922	NEXTFLEX® RNA-Seq Unique Dual Index Barcodes (Barcodes 193 - 288)	192 rxns		
NOVA-512923	NEXTFLEX® RNA-Seq Unique Dual Index Barcodes (Barcodes 289 - 384)	192 rxns		
	BARCODES FOR ION TORRENT™ SEQ			
NEXTFLEX® DNA BARCO	ODES FOR ION TORRENT™ SEQUENCERS			
NOVA-401001	NEXTFLEX® DNA Barcodes for Ion PGM™ and Ion Proton™ - 8	48 rxns		
NOVA-401002	NEXTFLEX® DNA Barcodes for Ion PGM™ and Ion Proton™ - 16	96 rxns		
NOVA-401003	NEXTFLEX® DNA Barcodes for Ion PGM™ and Ion Proton™ - 32	192 rxns		
NOVA-401004	NEXTFLEX® DNA Barcodes for Ion PGM™ and Ion Proton™ - 64	384 rxns		
NOVA-401005	NEXTFLEX® DNA Barcodes for Ion PGM™ and Ion Proton™ - 96	768 rxns		

For more information about the kits, please contact NGS@perkinelmer.com

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