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Nextera™ Flex for Enrichment

A fast, integrated workflow for a wide range of target enrichment applications encompassing custom panels, fixed panels, and whole-exome sequencing.

Highlights

- **Fast library prep and enrichment workflow**

Provides a time-saving solution that is 85% faster than standard Illumina library prep and enrichment

- **Integrated sample input**

Enhances library preparation efficiency with integrated protocols for blood and saliva

- **Wide range of applications**

Enables advanced study designs in cancer research, genetic disease research, and whole-exome sequencing

Introduction

The Nextera Flex for Enrichment solution combines versatile, simple, and fast library prep and enrichment functionality for targeted enrichment and exome sequencing applications. It offers extraordinary flexibility for input type, input amount, and a wide range of supported enrichment sequencing applications encompassing custom panels, fixed panels, and whole-exome sequencing from Illumina or third-party vendors (Table 1).

Nextera Flex for Enrichment uses innovative bead-based chemistry with a simplified, single hybridization step (Figure 1). With the Nextera Flex for Enrichment workflow, DNA extraction can be processed directly from fresh blood and saliva samples with the Flex Lysis Reagent Kit and Saliva Lysis Protocol, respectively, for additional time savings.

Fast and flexible library preparation and enrichment workflow

A key component of the Nextera Flex for Enrichment solution is On-Bead Tagmentation, which uses bead-bound transposomes to mediate a uniform tagmentation reaction. This strategy provides several significant advantages.

- For gDNA inputs ≥ 50 ng, accurate quantitation of the initial DNA sample is not required, as insert fragment size is not affected, saving time and costs associated with kits and reagents.
- On-Bead Tagmentation eliminates the need for separate DNA fragmentation steps, saving time and costs associated with related consumables.
- For gDNA inputs between 50–1000 ng, saturation-based DNA normalization eliminates the need for individual library quantitation and normalization steps before enrichment.
- Novel 90-minute single hybridization protocol enables enrichment in less than four hours.

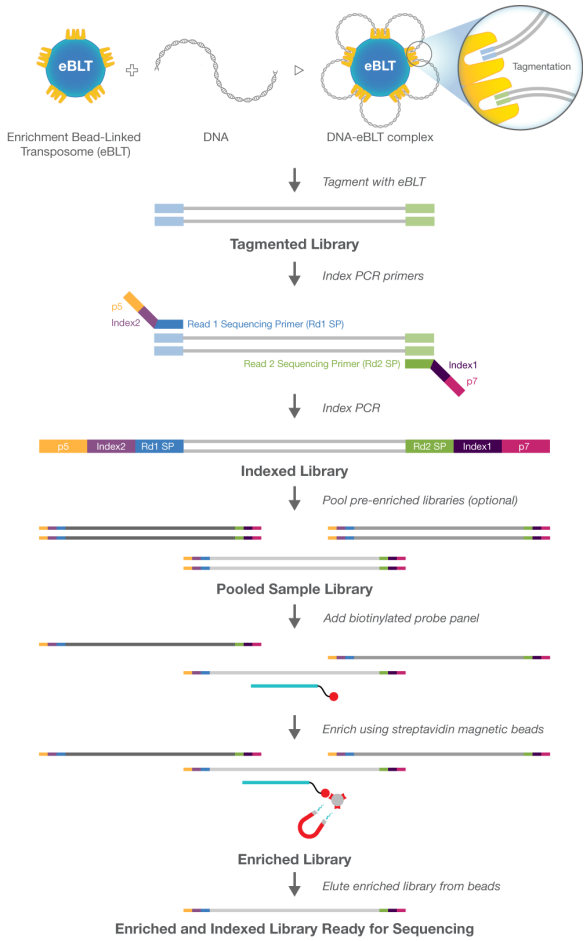

Figure 1: Nextera Flex for Enrichment assay chemistry—A uniform tagmentation reaction mediated by eBLTs followed by a single hybridization reaction enables a fast and flexible workflow.

Table 1: Nextera Flex for Enrichment specifications

Parameter	Specification
DNA input type	gDNA, whole blood, saliva, DNA extracted formalin-fixed, paraffin-embedded (FFPE) tissue
DNA input verified[a]	10–1000 ng
Sample multiplexing	96 unique dual indexes (UDIs)
Pre-enrichment pooling[b]	1-plex or 12-plex verified and supported
Supported sequencing systems	All Illumina systems
Total workflow time[c]	~ 6.5 hours

a. DNA inputs as low as 10 ng are possible, but will not provide saturation-based DNA normalization

b. Other enrichment plexities are possible, but have not been verified. Additional optimization may be required and optimal results are not guaranteed

c. Includes library preparation, enrichment, and library normalization/pooling steps

Figure 2: Nextera Flex for Enrichment delivers the fastest Illumina enrichment workflow—Workflow times are based on processing 12 samples at 12-plex enrichment. Times may vary depending on equipment used, number of samples processed, automation procedures, or user experience.

Table 2: Comparison of Illumina enrichment workflows

	Nextera Flex for Enrichment	TruSeq DNA Exome	Nextera DNA Exome	Nextera Rapid Capture Custom Enrichment
Integrated DNA option[a]	✓	—	—	—
Flexible, broad DNA input range	✓	—	—	—
Library normalization included[b]	✓	—	—	—
FFPE compatible	✓	✓	—	—
DNA input required	10–1000 ng	100 ng	50 ng	50 ng
Total library prep and enrichment time[c]	~6.5 hours	2.5 days	2 days	1.5 days
Insert size[d]	150–220 bp	150 bp	150–220 bp	230 bp
Sample index sets	96 unique dual indexes	24 single indexes, 96 dual indexes	24 single indexes, 96 dual indexes	24 single indexes, 96 dual indexes

a. Integrated lysis protocols available for blood and saliva

b. Library normalization occurs with ≥ 50 ng gDNA input

c. Total library prep and enrichment time includes library preparation, library normalization/pooling, and enrichment

d. Degraded FFPE DNA may result in smaller insert sizes

Fastest Illumina enrichment workflow

The Nextera Flex for Enrichment solution supports liquid-handling systems for library prep automation and produces a workflow with the lowest number of steps and the fastest total workflow time in the Illumina enrichment portfolio (Figure 2)(Table 2).

Integrated DNA Input

DNA extraction can be processed directly from whole blood or saliva samples. The optional Flex Lysis Reagent Kit has been optimized and validated for Nextera Flex for Enrichment library preparation from whole blood and is integrated into the workflow for maximum efficiency. The lysis protocols feature bead-based reagents and require less than 30 minutes of hands-on time.

Illumina offers a diverse portfolio of services to support a broad range of applications, including service partnerships and programs, training, and consulting throughout the NGS workflow. Learn more >

Optimized performance across Illumina sequencing systems

The robust and straightforward Nextera Flex for Enrichment solution yields reliable results across all Illumina sequencing systems by providing > 90% on-target reads, > 95% uniformity, and low PCR duplicate rate (Table 3). Nextera Flex for Enrichment is optimized for high-throughput systems (Figure 3)(Table 4).

Figure 3: Nextera Flex for Enrichment provides optimized performance across Illumina sequencing systems—The Nextera Flex for Enrichment solution is compatible with all Illumina sequencing systems, including high-throughput, production-scale systems.

Table 3: Comparison of performance[a]

Parameter[b]	Nextera Flex for Enrichment	Nextera Flex for Enrichment	Nextera Flex for Enrichment	TruSeq DNA Exome	Nextera DNA Exome
Panel	Illumina Exome Panel	Exome Panel X	Exome Panel Y	Illumina Exome Panel	Illumina Exome Panel
Panel size	45 Mb	39 Mb	33 Mb	45 Mb	45 Mb
Probe size	80 bp	120 bp	120 bp	80 bp	80 bp
Padded read enrichment (on-target)[c]	85%	91%	91%	85%	75%
Fragment length median	~ 200 bp	~ 200 bp	~ 200 bp	~ 150 bp	~ 200 bp
Coverage at 20×	93%	96%	97%	90%	85%
Uniformity of coverage[c]	95%	97%	98%	85%	85%
Read depth per sample	30M PF clusters	25M PF clusters	20M PF clusters	40M PF clusters	40M PF clusters
SNV precision	99%	99%	99%	99%	99%
SNV recall	94%	94%	95%	89%	91%

a. Data represents example comparison data. Actual performance specifications may vary.

b. The analysis was run on 48 samples (all NA12878 Coriell samples) per condition. Data analysis was performed using the Enrichment BaseSpace App.

c. See the BaseSpace App User Guide[3] for additional details.

Table 4: Sample throughput per flow cell with Nextera Flex for Enrichment

Panel	iSeq 100 System	MiniSeq System		MiSeq System			NextSeq Series	
		Mid	High	v2	v2 Nano/ Micro	v3	Mid	High
Fixed panels								
TruSight One	NR	NR	2	1	0/0	3	12	36
TruSight One Expanded	NR	NR	1	0	0/0	1	7	24
TruSight Cancer	4	8	24	12	1/4	24	96	384
TruSight Cardio	4	8	24	12	1/4	24	96	384
Custom panels								
2000 probes	8	16	50	30	2/8	50	260	384
5000 probes	2	4	12	8	1/2	12	65	200
10,000 probes	1	2	6	4	0/1	6	33	100

Abbreviations: Mid, mid output; High, high output; NR, not recommended

Panel	NextSeq Series		HiSeq Series		NovaSeq 6000 System			
	Mid	High	2500 RR/HO	3000/4000	SP	S1	S2	S4
Illumina Exome	5	16	12/80	100	64	128	164	384
Exome Panel X	5	16	12/80	100	64	128	164	384
Exome Panel Y	6	20	15/100	125	80	160	205	384

Abbreviations: Mid, mid output; High, high output; RR, rapid-run mode; HO, high-output mode (v4)

DNA enrichment for a broad range of applications

By combining exceptional enrichment performance and the proven accuracy of Illumina sequencing by synthesis (SBS) chemistry, the Nextera Flex for Enrichment solution supports both fixed and custom panels of varying sizes, including whole-exome sequencing, for advanced study designs in a variety of areas (Figure 5). Furthermore, Nextera Flex for Enrichment is compatible with Illumina and third-party enrichment probes/panels, which enables content portability for increased flexibility.

Accurate Data

Nextera Flex for Enrichment produces highly uniform and consistent insert sizes, across a wide DNA input range, delivering uniform and consistent library yields.[1] Also, it provides high coverage uniformity and padded read enrichment for custom, fixed, and exome panels (Figure 4). Nextera Flex for Enrichment enables accurate single nucleotide variant (SNV) (Figure 6 and Table 3) and insertion/deletion (indel) recall and precision, as compared to other Illumina enrichment solutions.

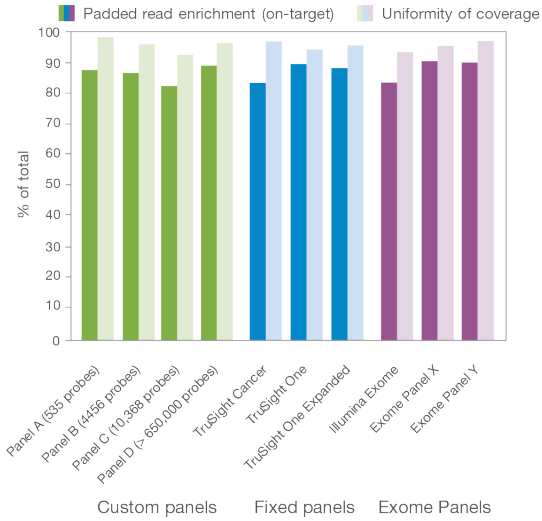

Figure 4: High coverage uniformity and padded read enrichment—Nextera Flex for Enrichment provides high coverage uniformity and on-target padded read enrichment for custom, fixed, and exome panels.

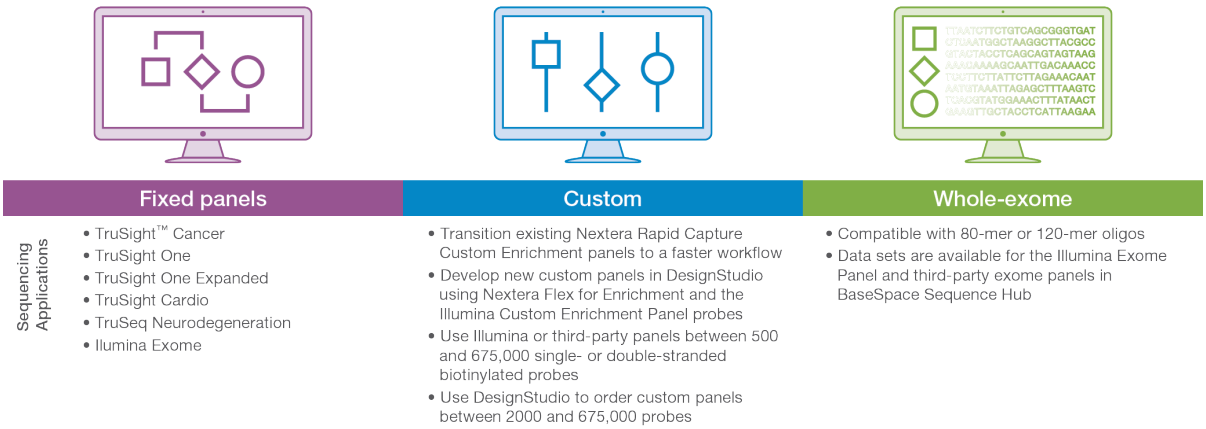

Figure 5: Broad range of applications with Nextera Flex for Enrichment—Nextera Flex for Enrichment can be used to support a broad range of applications including fixed panels, custom panels, and whole-exome sequencing.

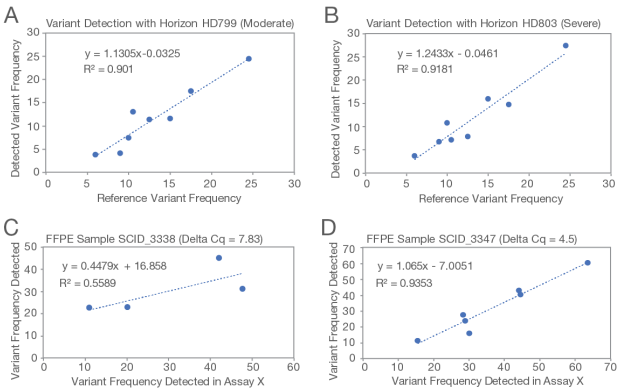

Figure 6: Accurate variant calling—Nextera Flex for Enrichment provides low abundance somatic variant calling for (A, B) cell line FFPE control human reference DNA samples and (C, D) real world FFPE samples with observed variant frequency showing significant correlation with frequencies from an orthologous sequencing assay.

Summary

Nextera Flex for Enrichment features the fastest workflow in the Illumina enrichment portfolio. The user-friendly, automation-compatible solution supports users of all experience levels and provides a common workflow for a variety of experimental designs. On-Bead Tagmentation chemistry enables support for a wide range of DNA input amounts, various sample types, and a broad range of applications, including fixed panels, custom panels, and whole-exome sequencing. Furthermore, Nextera Flex for Enrichment is compatible with Illumina and third-party enrichment probes/panels, which enables content portability. The innovative Nextera Flex for Enrichment solution combined with the power of Illumina SBS chemistry provides an optimal targeted enrichment and exome sequencing experience.

References

1. Illumina (2017). Nextera DNA Flex Library Preparation Kit Data Sheet. Accessed September 10, 2018.
2. Illumina (2017). BWA Enrichment v2.1 BaseSpace App Guide. Accessed September 28, 2018.

Ordering Information

Order Nextera Flex products online at www.illumina.com

Product	Catalog No.
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents 96 samples (8, 12-plex enrichment reactions)	20025524
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents 16 samples (16, 1-plex enrichment reactions)	20025523
Nextera DNA Flex Pre-Enrichment Library Prep Reagents (96 samples)	20025520
Nextera DNA Flex Pre-Enrichment Library Prep Reagents (16 samples)	20025519
Flex Lysis Reagent Kit (for blood lysis)	20018706
IDT for Illumina - Nextera DNA Unique Dual Indexes - Set A (96 indexes, 96 samples)	20027213
IDT for Illumina - Nextera DNA Unique Dual Indexes - Set B (96 indexes, 96 samples)	20027214
IDT for Illumina - Nextera DNA Unique Dual Indexes - Set C (96 indexes, 96 samples)	20027215
IDT for Illumina - Nextera DNA Unique Dual Indexes - Set D (96 indexes, 96 samples)	20027216
IDT for Illumina - Nextera DNA Unique Dual Indexes - Set A–D (384 indexes, 384 samples)	20027217
Illumina Exome Panel (8 enrichment reactions)	20020183
TruSight Cancer (8 enrichment reactions)	FC-121-0202
TruSight One (6 enrichment reactions)	20029227
TruSight One Expanded (6 enrichment reactions)	20029226
TruSight Cardio (8 enrichment reactions)	20029229
TruSeq Neurodegeneration (8 enrichment reactions)	20029550
Illumina Custom Enrichment Panel	20025371

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