Nextera™ DNA Exome

Simple workflow streamlines library preparation and exome enrichment to accelerate human exome sequencing.

Highlights

- **Flexibility for Different Study Designs**
  Optimized chemistry accommodates low input amounts
- **Efficient Library Prep and Exome Enrichment**
  Rapid, automation-friendly workflow completes in < two days, with only three hours of hands-on time
- **Accurate Variant Calls**
  High coverage and on-target sequencing result in accurate data
- **Integrated Workflow Solution**
  Comprehensive workflow streamlines exome sequencing from library prep through data analysis

Introduction

Exome sequencing has gained recognition in the scientific community as a powerful method for discovering potential causative variants for genetically driven diseases.[1–3] Nextera DNA Exome, previously sold as the TruSeq™ Rapid Exome Kit, delivers a fast exome sequencing workflow, combining library preparation and exome enrichment into a single, streamlined process with no need for DNA shearing equipment.

Flexibility for Different Study Designs

Nextera DNA Exome is optimized for 50 ng of input DNA. Enhanced transposome chemistry results in reduced bias, providing consistent library preparation and exome enrichment across a range of DNA quantities (Figure 1). The transposome enzyme is highly tolerant of varying DNA input amounts, accommodating minor inconsistencies in DNA quantification. The kit supports library pooling before enrichment, enabling labs to run up to 12 samples simultaneously, depending on sample processing volume. Nextera DNA Exome features an automation-friendly workflow to simplify and streamline lab processes even further.

Focused Exonic Content

Nextera DNA Exome is optimized to provide uniform and specific coverage of 45 Mb of exonic content. The probe set is designed to enrich 214,405 exons (Table 2). This focused design, paired with uniform and specific enrichment, enables comprehensive exome sequencing and reliable identification of true, coding variants.

Table 1: Exome Content with Nextera DNA Exome and TruSeq DNA Exome

Coverage Specification	Nextera DNA Exome or TruSeq DNA Exome
Target Size	45 Mb
No. of Target Exons	214,405
Target Content	coding exons
Percent of Exome Covered (by Database)	
RefSeq[a]	99.45%
CCDS[b]	98.83%
ENSEMBL[c]	99.68%
GENCODE v19[d]	99.68%

a. RefSeq - NCBI Reference Sequence Database. www.ncbi.nlm.nih.gov/refseq/. Accessed February 11, 2015.
b. CCDS - Consensus CDS (CCDS) Database. www.ncbi.nlm.nih.gov/projects/CCDS/CcdsBrowse.cgi. Accessed February 11, 2015.
c. ENSEMBL - Ensembl Genome Browser. www.ensembl.org/index.html. Accessed February 11, 2015.
d. GENCODE - GENCODE Project: Encyclopedia of genes and gene variants. www.gencodegenes.org/. Accessed February 11, 2015.

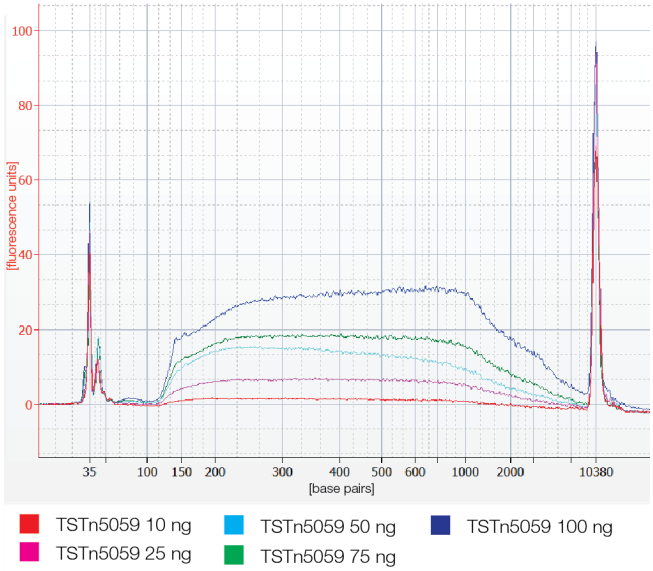

Figure 1: Consistent Tagmentation for Varying Input Amounts—Enhanced transposome chemistry is highly tolerant of varying DNA input amounts, reducing bias and providing consistent results. Libraries were assessed on a Bioanalyzer instrument.

Efficient Library Preparation and Exome Enrichment

Nextera DNA Exome provides library preparation and exome enrichment in less than two days, with just three hours of hands-on time (Figure 3). Library preparation begins with tagmentation, in which genomic DNA is simultaneously fragmented and tagged with adapters (Figure 3A). Tagmented DNA is amplified and sequencing indexes are added by PCR (Figure 3B). Up to 12 libraries are pooled together, and the library pool is concentrated and libraries are denatured into single-stranded DNA (Figure 3C). Biotin-labeled probes specific to the targeted regions are used for two rounds of hybridization (Figure 3D and 3E). The pool is enriched for the desired regions using streptavidin beads that bind to the biotinylated probes. Biotinylated DNA fragments bound to the streptavidin beads are magnetically pulled down from the solution. The enriched DNA fragments are eluted from the beads and further amplified by PCR (Figure 3F). Amplified libraries are cleaned up and ready for sequencing (Figure 3G).

Accurate Variant Calls

Libraries prepared with Nextera DNA Exome deliver high coverage, with 85% of reads covered at 20× depth (Figure 2). Such high coverage results in accurate variant calls. Over 99.58% of variant calls made using Nextera DNA Exome match standard reference data in the National Institute of Standards and Technology (NIST) database (Figure 4).[5,6]

Nextera DNA Exome delivers on average 75% of on-target sequencing reads (Figure 6). This high on-target percentage requires fewer sequencing cycles to reach desired coverage levels, but still achieves uniform coverage for high-confidence results. It also enables sequencing of more exomes per run, allowing labs to maximize their budgets (Table 2).

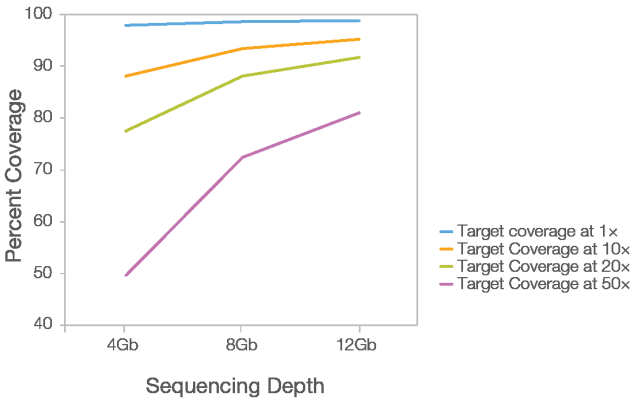

Figure 2: Coverage Efficiency at Varying Depths—Nextera DNA Exome delivers exceptional coverage across varying sequencing depth, with > 80% of targets covered up to 20× depth.

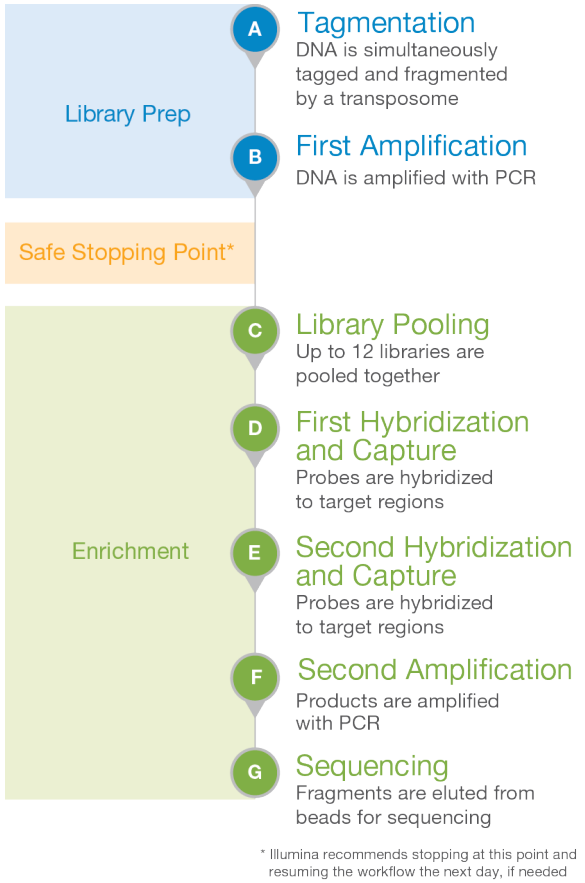

Figure 3: Efficient and Fast Workflow—Nextera DNA Exome completes library preparation and exome enrichment in less than two days and includes a safe stopping point for greater flexibility.

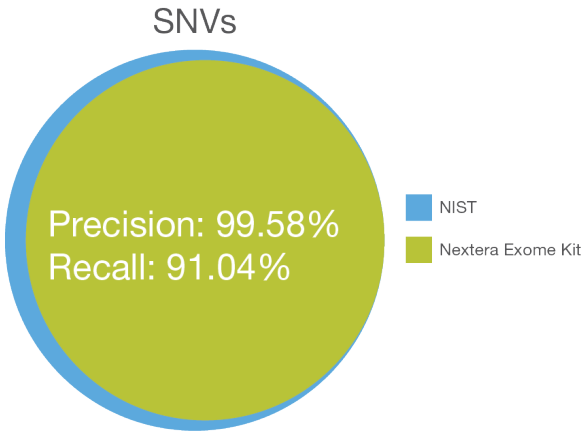

Figure 4: High Correlation with NIST Database—Single nucleotide variant (SNV) calls made with Nextera DNA Exome demonstrate high concordance with standard reference data. The Centre de'Etude du Polymorphism Humain (CEPH) DNA sample NA12878 was sequenced to 100× coverage depth. **Precision** is defined as the probability that a called variant is accurate. **Recall** is defined as the probability of calling a validated variant.

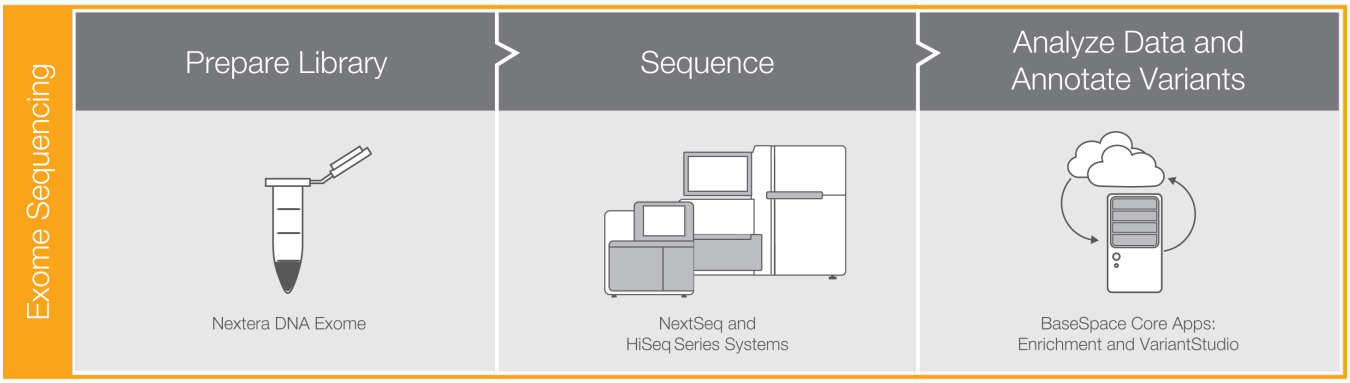

Figure 5: Exome Sequencing Workflow—Nextera DNA Exome is part of an integrated exome sequencing workflow that includes library preparation, sequencing, and data analysis.

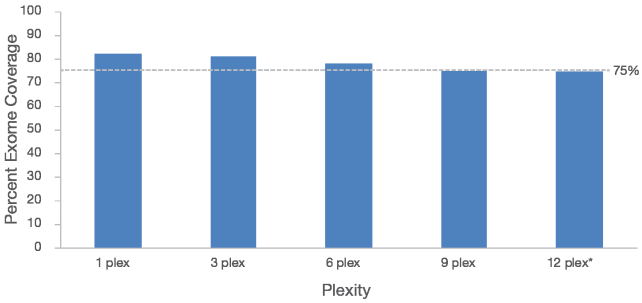

Figure 6: On-Target Enrichment—Nextera DNA Exome delivers on average 75% of on-target sequencing reads at 4 Gb per exome for efficient, cost-effective sequencing.

Table 2: Throughput Comparison with Nextera DNA Exome[a]

Sequencing System	No. of Exomes per Run at 50×	No. of Exomes per Run at 100×
MiSeq Series	1	N/A
NextSeq Series		
Mid-Output Flow Cell	3	2
High-Output Flow Cell	12	6
HiSeq Series		
HiSeq 2500 System Rapid-Run Mode (Dual Flow Cell)	24	12
HiSeq 2500 System High-Output Mode (Dual Flow Cell)	156	78
HiSeq 3000 System	96	48
HiSeq 4000 System (Dual Flow Cell)	192	96

a. Estimated number of exomes sequenced per run is calculated with a mean coverage of 50× and 100×, respectively. Illumina recommends a 2 × 75 bp read length on all sequencers when using Nextera DNA Exome.

Integrated Sequencing Workflow

Nextera DNA Exome is part of a cohesive, supported solution that guides researchers from library preparation through data analysis (Figure 5). The kit combines library preparation and exome enrichment, eliminating the need to purchase indexes, sample purification beads, or other ancillary materials. All components of Nextera DNA Exome are designed, optimized, and analytically validated together, eliminating the need to evaluate multiple, disparate components. Expert Illumina scientists provide a single source of technical and field support for every stage of the workflow. By joining the Illumina community, researchers can harness the expertise of the Illumina support team and collaborate with the large network of scientists using Illumina technology.

Nextera DNA Exome is compatible with the MiSeq™, NextSeq™, HiSeq™, and NovaSeq™ Series of sequencing systems. Illumina sequencing systems use sequencing by synthesis (SBS) chemistry, used to generate more than 90% of the world's sequencing data.* Sequencing data are transferred automatically from Illumina systems to BaseSpace® Sequence Hub, the Illumina genomics computing environment. BaseSpace Sequence Hub removes much of the complexity from the typical analysis workflow, simplifying data analysis and biological interpretation. BaseSpace Sequence Hub offers an established ecosystem of integrated data analysis tools designed for biologists. With BaseSpace Apps, expert-preferred analysis tools are packaged in an intuitive, user-friendly interface, so that any researcher can access trusted analysis pipelines without previous bioinformatics experience (Figure 7). Researchers can choose to analyze exome data using the BWA Enrichment App, which uses the industry-standard BWA/GATK method, or the Isaac™ Enrichment App, which uses the fast and accurate Illumina pipeline.[6]

For biologists investigating the genetic basis of disease, the VariantStudio App enables identification and functional interpretation of disease-associated single nucleotide variants (SNVs) and insertions and deletions (indels). Researchers can rapidly filter and isolate consequential variants to enrich sequencing data with

*Data calculations on file. Illumina, Inc., 2015.

biological context. Significant findings are exported in concise reports. The VariantStudio App enables researchers to explore biological significance in a few simple steps.

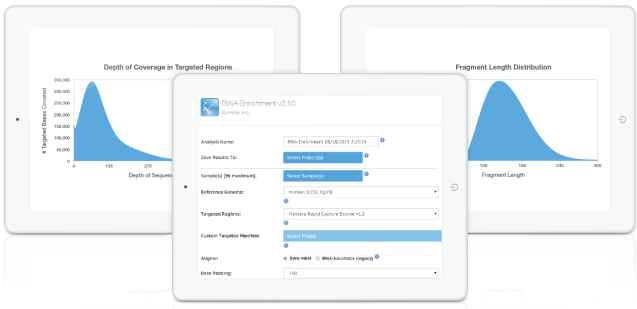

Figure 7: Simplified Data Analysis with BaseSpace Apps—Nextera DNA Exome sequencing data can be easily and securely uploaded to BaseSpace Sequence Hub and analyzed with the BWA Enrichment App. Results are provided in easy-to-read formats.

Exome Sequencing Performance Comparison

Illumina offers two integrated workflow solutions for exome sequencing. Workflows are also available that combine Illumina library prep with TruSeq DNA Exome or Nextera DNA Exome, followed by exome enrichment using xGen® Universal Blockers, xGen Lockdown Reagents, and xGen Exome Research Panel v1.0, available from IDT (Table 3).

Table 3: Exome Workflow Performance Comparison

Metric	TruSeq-xGen[a]	Nextera-xGen[a]	TruSeq Exome	Nextera Exome
DNA Input	100 ng	50 ng	100 ng	50 ng
Sample Types	DNA	DNA	DNA and FFPE	DNA
Hands-On Time	5 hours	2 hours	6 hours	3 hours
Total Assay Time	2.5 days	2 days	2.5 days	2 days
Hybridization Time	4 hours	4 hours	16 hours	2 hours
On-Target %	> 91%	> 92%	> 80%	> 75%
% Coverage at 20×[b]	> 95%	> 85%	> 90%	> 85%

a. Specifications for Illumina-IDT exome enrichment workflows are based on preliminary data posted on BaseSpace Sequence Hub.
b. Percent coverage at 20× was determined for TruSeq-xGen and Nextera-xGen kits with 3.5 Gb of sequencing. Percent coverage at 20× was determined for TruSeq DNA Exome and Nextera DNA Exome with 8 Gb of sequencing.

Summary

Nextera DNA Exome offers a simple, streamlined method for identifying and understanding coding variants with exceptional data accuracy. The fast library preparation and exome enrichment workflow delivers sequencing-ready libraries less than two days and provides flexibility in project planning according to sample volume. As part of a comprehensive workflow consisting of leading sequencing technology and easy-to-use analysis tools, Nextera DNA Exome enables researchers to implement exome sequencing efficiently and cost-effectively.

Learn More

To learn more about exome sequencing, visit www.illumina.com/techniques/sequencing/dna-sequencing/targeted-resequencing/exome-sequencing.html.

Ordering Information

Product	Catalog No.
Nextera Exome Kit (24 samples)	20020616
Nextera Exome Kit (96 samples)	20020617

References

1. Litchfield K, Summersgill B, Yost S, et al. Whole-exome sequencing reveals the mutational spectrum of testicular germ cell tumours. *Nat Commun*. 2015;6:5973.
2. Srivastava S, Cohen JS, Vernon H, et al. Clinical whole exome sequencing in child neurology practice. *Ann Neurol*. 2014;76:473–483.
3. Worthey EA, Mayer AN, Syverson GD, et al. Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. *Genet Med*. 2011;13:255–262.
4. Standard Reference Data (www.nist.gov/srd). Accessed 11 February 2015.
5. Genome in a Bottle Consortium | Advances in Biological and Medical Measurement Science (sites.stanford.edu/abms/giab). Accessed 20 February 2015.
6. Raczy C, Petrovski R, Saunders CT, et al. Isaac: ultrafast whole-genome secondary analysis on Illumina sequencing platforms. *Bioinformatics*. 2013;29:2041–2043.

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