illumina®

Next-Generation Sequencing

Application Guide

Informatics

Intuitive informatics tools enable critical insights. Essential data can be securely transferred, stored, analyzed, and shared in BaseSpace® Onsite or in the BaseSpace Cloud.

Sequencing

With power for every scale, Illumina provides a complete portfolio of next-generation sequencing (NGS) solutions that are accessible for every study and every lab.

Applications

Illumina offers standard options and features the NeoPrep™ System for reproducible, sequencing-ready libraries with just 30 minutes of hands-on time per run.

APPLICATIONS

INSTRUMENTS

ANALYSIS TOOLS

A global genomics leader, Illumina provides complete sample-to-answer solutions to the research and clinical communities. Illumina technology is responsible for generating 90% of the sequencing data in the world. Through collaborative innovation, Illumina is fueling groundbreaking advancements in the fields of oncology, reproductive health, genetic disease, microbiology, agriculture, and forensic science.

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Applications

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Whole-genome sequencing (large genomes)

Resequence whole genomes and identify variants of interest for disease and population-level studies.

Key strengths

- Combines short inserts and longer reads to allow characterization of any genome
- Provides high-quality, long contig assemblies for *de novo* whole-genome sequencing
- Reveals disease-causing alleles that might not have been identified otherwise
- Captures results for new variants without requiring resequencing

LIBRARY PREP	SEQUENCING	ANALYSIS TOOLS
TruSeq® DNA PCR-Free Sample Preparation Kit N TruSeq Nano DNA Sample Preparation Kit N Nextera® DNA Sample Preparation Kit	NextSeq 500 (1 sample) HiSeq 2500 (1–10 samples) HiSeq X™ Ten (up to 18,000 genomes/year)	If analyzed in BaseSpace **Align/Call variants:** BWA Whole Genome Seq. Isaac Whole Genome Seq. Tumor-Normal Seq. **Annotate/Filter:** VariantStudio **Visualize:** The Broad's IGV

The combination of short inserts and longer reads allows characterization of any genome.

N NeoPrep version available 2015–see page 86

Visit www.illumina.com/largeWG for more details.

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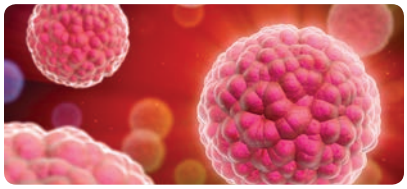
Innovation. Discovery. Application.

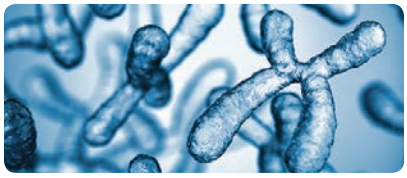


Cancer research

In cancer, whole-genome sequencing can be employed to monitor genomic changes that occur in a tumor, including the presence of markers for good and poor prognosis. Through tumor-normal whole-genome sequencing, researchers can identify the tumor genome and determine differences from non-tumor DNA.

Complex disease

Next-generation sequencing (NGS) is proving to be a powerful tool for genome-wide association studies (GWAS). GWAS allows researchers to identify common genetic variants across populations to establish links between those variants and the traits of individuals, including predisposition to disease.



Genetic disease

Phased sequencing complements human whole-genome sequencing by distinguishing between the genetic contributions from each parent to the chromosomes of the offspring. Inheritance patterns are often important for understanding the basis of genetic disease.

Agrigenomics

Exponential population growth and a changing climate are creating unique challenges for people working to maintain the food supply. To overcome food supply challenges, many are turning to science. NGS is particularly useful in agricultural research, where genomes can be complex and prior knowledge scarce.

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STEP 1
Choose library prep method



Product	TruSeq DNA PCR-Free Sample Preparation Kit	TruSeq Nano DNA Sample Preparation Kit	Nextera DNA Sample Preparation Kit
Most important to me	Best genome coverage	Low input with reduced bias	Fastest assay time
Least important to me	Low DNA input quantity	Assay time	Genome coverage
Input quantity	1–2 µg	100–200 ng	50 ng
Multiplexing	24–96	24–96	96
Assay time	5 hours	6 hours	1.5 hours
Hands-on time	~4 hours	5 hours	~15 minutes
Target insert size	350 bp or 550 bp	350 bp or 550 bp	300 bp–1.5 kb
Fragmentation	Mechanical	Mechanical	Enzymatic

Comparisons within whole-genome sequencing (large genomes) portfolio.

STEP 2
Choose a sequencer



Product	NextSeq 500		HiSeq 2500		HiSeq X Ten
Description	Flexible power Speed and simplicity for everyday genomics		Production power Power and efficiency for large-scale genomics		Population power $1,000 human genome and extreme throughput for population-scale sequencing
On-board clustering	●				
High number of samples per run			●		
Population-scale whole human genome sequencing					●
Large genome samples processed per run	1		1–10		up to 18,000 per year
Run mode	Mid output	High output	Rapid run	High output	
Flow cells processed per run	1	1	1 or 2	1 or 2	1 or 2
Output range	20–39 Gb	30–120 Gb	10–300 Gb	50–1,000 Gb	1.6–1.8 Tb
Run time	15–26 hours	12–30 hours	7–60 hours	< 1–6 days	< 3 days
Reads per flow cell	130 million	400 million	300 million	2 billion	6 billion
Maximum read length	2 x 150 bp	2 x 150 bp	2 x 250 bp	2 x 125 bp	2 x 150 bp

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STEP 3
Obtain results

NextSeq 500 HiSeq 2500

HiSeq X Ten

Align/Call variants

BWA Whole Genome Sequencing B
- Processes whole-genome sequencing data using BWA for alignment
- Uses GATK for variant detection

Isaac Whole Genome Sequencing B
- Performs read mapping using Isaac Genome Alignment Software
- Uses Isaac Variant Detection to identify single nucleotide variants (SNVs), small indels, copy number anomalies (CNAs), and structural variations

Tumor Normal B
- Detects somatic variants from a tumor and matched normal sample pair
- Generates a report for the cross analysis, including quality, variants, and a circos plot

Annotate/Filter

VariantStudio B
- Enables extraction of biological knowledge from variant data by providing a rich annotation database, flexible filtering, and a streamlined variant classification and reporting tool
- Leverages leading annotation databases and a powerful filtering interface to rapidly identify disease-associated variants in data sets ranging from small targeted panels to whole-genome sequencing experiments

Visualize

The Broad's IGV B
- Displays alignments and variants from multiple samples for performing complex variant analysis
- The Broad Institute of MIT and Harvard developed IGV, and Illumina modified it to display BaseSpace data

B BaseSpace

www.illumina.com/largeWG

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Library prep ordering information

TruSeq DNA PCR-Free Sample Preparation Kit

TruSeq DNA PCR-Free Sample Preparation Kits provide simple, all-inclusive library preparation for whole-genome sequencing applications of various organisms, from bacteria to whole human genomes. The kits offer shortened gel-free workflows, the ability to sequence the most challenging regions, and the power to identify the greatest number of variants. Libraries prepared with TruSeq DNA PCR-Free Sample Preparation kits are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 Sequencing System, and HiSeq 2500 sequencer.

Ordering Information

Product	No. of Samples	Catalog No.
TruSeq DNA PCR-Free LT Sample Preparation Kit Set A	24	FC-121-3001
TruSeq DNA PCR-Free LT Sample Preparation Kit Set B	24	FC-121-3002
TruSeq DNA PCR-Free HT Sample Preparation Kit	96	FC-121-3003

TruSeq Nano DNA Sample Preparation Kit

Preserve precious samples with the TruSeq DNA Sample Preparation Kit. Prepare sequencing libraries for low- or high-throughput studies from as little as 100 ng of input DNA in less than a day. Bead-based selection reduces the sample loss associated with gel-based selection. This kit is designed to match the ever-increasing read lengths of Illumina sequencing instruments and is compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, HiSeq 2500 System, and HiSeq X Ten System.

Ordering Information

Product	No. of Samples	Catalog No.
TruSeq Nano DNA LT Sample Preparation Kit Set A	24	FC-121-4001
TruSeq Nano DNA LT Sample Preparation Kit Set B	24	FC-121-4002
TruSeq Nano DNA HT Sample Preparation Kit	96	FC-121-4003

Nextera DNA Sample Preparation Kit

Generate sequencing-ready libraries in under 90 minutes with less than 15 minutes of hands-on time. Nextera technology simultaneously fragments DNA and tags the fragments with sequencing adapters using standard lab equipment. The protocol requires only 50 ng of input DNA. Libraries prepared with Nextera kits are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Samples	Catalog No.
Nextera DNA Sample Preparation Kit	96	FC-121-1031
Nextera DNA Sample Preparation Kit	24	FC-121-1030
Nextera Index Kit (96 indexes)	384	FC-121-1012
Nextera Index Kit (24 indexes)	96	FC-121-1011
TruSeq Dual Index Sequencing Primer Kit, Single Read (single-use kit)		FC-121-1003
TruSeq Dual Index Sequencing Primer Kit, Paired-End Read (single-use kit)		PE-121-1003

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Whole Human Genome Sequencing Services

The only complete research solution.

You've got the samples, but not the dedicated resources or time. You need an experienced service partner to perform whole human genome sequencing to advance your studies.

Illumina offers the only end-to-end research solution, with guaranteed access to industry-leading TruSeq technology through our sequencing platforms, network of partners, and our own FastTrack Services. From discovery to validation, we provide support for every step of your project.

After your whole human genome sequencing project is complete, we'll help you with follow-on studies. Whether it's targeted exome sequencing, SNP discovery, or RNA-Seq, you'll get the in-depth genomic information you need to accelerate your research.

Because your data will be delivered in the format most compatible with popular analysis tools, you can easily continue with any secondary studies of your own.

ign Illumina® Genome Network

Our network of partners with one focus—to perform whole human genome sequencing projects on Illumina systems

FastTrack an Illumina® service

In-house Illumina services offering sequencing and genotyping

illumina CSPro® CERTIFIED SERVICE PROVIDER

Certified service labs producing validated Illumina data

Whole-genome sequencing (small genomes)

Sequence the entire genome of a bacteria, virus, or other microbe, and compare the sequence to that of a known reference.

Key strengths

- Allows investigation of all genes in all organisms present in a given complex sample
- Sequences thousands of organisms in parallel
- Sequences and discovers unculturable organisms
- Detects very low abundance members of the microbial community that might be missed or are too expensive to identify using other methods
- Provides comprehensive analysis of the microbial/viral genome
- Aids discovery of new biomarkers (SNP variants) within a microbial/viral sample by providing distinct gene information from homologous chromosomes, supporting haplotyping, and allele-specific solutions

LIBRARY PREP	SEQUENCING	ANALYSIS TOOLS
Nextera XT DNA Sample Preparation Kit	MiSeq (up to 100 samples) NextSeq 500 (up to 260 samples)	If analyzed in BaseSpace **Align/Call variants:** BWA Whole Genome Seq. Isaac Whole Genome Seq. SPAdes Genome Assembler **Annotate/Filter:** VariantStudio **Visualize:** The Broad's IGV

> We're amazed at what we've been able to accomplish in less than a year.
>
> **Thomas Sutter,** PhD, University of Memphis

Visit www.illumina.com/smallWG for more details.

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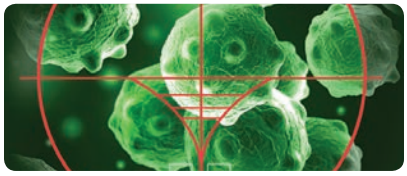
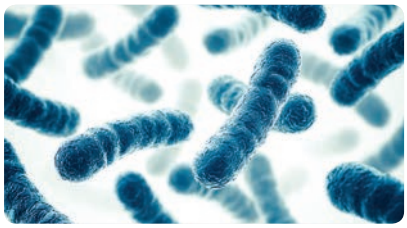
Microbiology

Whole-genome sequencing is an important tool for mapping genomes of novel organisms, finishing genomes of known organisms, comparing genomes across multiple samples, and enables the study of pathogen evolution. Sequencing the entire microbial genome is important for generating accurate reference genomes, for microbial identification, and other comparative genomic studies.



Microbial outbreak identification

The power and speed of NGS was demonstrated during the 2011 enteroaggregative *E. coli* outbreak in Europe, which prompted a rapid scientific response. Using NGS data, researchers quickly generated a high-quality, whole-genome sequence of the bacterial strain, enabling them to increase our understanding of the genetic mutations conferring the increased virulence.



Bacterial and viral screening/identification

Microbiologists and clinicians can use WGS for small genomes towards the identification and confirmation (screening purposes) of a bacterial genome in food testing and human/veterinary (agricultural setting) screening. The ability to effectively sequence a bacterial/viral genome provides the quick and accurate identification of potential pathogens, increasing true positives and decreasing false positive test results.

Bacterial mutagenesis

High-resolution genome data can be instrumental for examining pathogenesis, horizontal gene transfer, pan-genomes, and co-evolution of hosts and symbionts/parasites. The wealth of information enabled by NGS is beneficial for mutational studies of all kinds, including directed evolution strategies, lab adaptation analyses, mutagenesis screens, or temporal and spatial dynamics of epidemics and transmission study.

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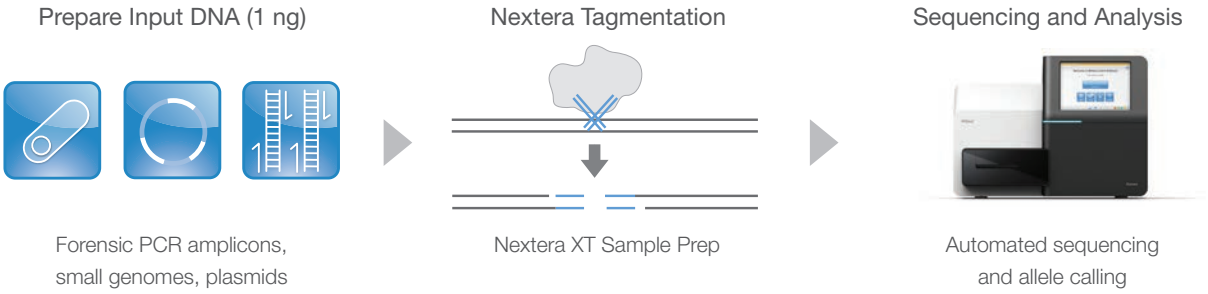
STEP 1
Library prep

Nextera XT Sample Preparation Kit: Fastest and easiest sample prep workflow
Using a single "tagmentation" enzymatic reaction, sample DNA is simultaneously fragmented and tagged with adapters. An optimized, limited-cycle PCR protocol amplifies tagged DNA and adds sequencing indexes (Figure 1). From start to finish, the complete Nextera XT protocol is over 80% faster than other available sample preparation methods and requires the least amount of hands-on time.



STEP 2
Choose a sequencer

Product	MiSeq	NextSeq 500	
Description	Focused power Speed and simplicity for targeted and small genome sequencing	Flexible power Speed and simplicity for everyday genomics	
On-board informatics	●		
Flexibility in number of samples per run		●	
*Small genome samples processed per run	up to 100	up to 260	
Run mode		Mid output	High output
Flow cells processed per run	1	1	1
Output range	0.3–15 Gb	20–39 Gb	30–120 Gb
Run time	5–55 hours	15–26 hours	12–30 hours
Reads per flow cell	25 million	130 million	400 million
Maximum read length	2 x 300 bp	2 x 150 bp	2 x 150 bp

*Assuming a 5 Mb genome at 30x coverage

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STEP 3
Obtain results


MiSeq


NextSeq 500

Align/Call variants

BWA Whole Genome Sequencing B
- Uses BWA for alignment to align NGS reads
- Uses GATK for variant detection

Isaac Whole Genome Sequencing B
- Performs read mapping using Isaac Genome Alignment Software
- Uses Isaac Variant Detection to identify single nucleotide variants (SNVs), small indels, copy number anomalies (CNAs), and structural variations

SPAdes Genome Assembler B
- Analyzes standard isolates and single-cell multiple displacement amplification (MDA) bacterial assemblies
- Offers read error correction tool and iterative short-read genome assembly module

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VariantStudio B
- Enables extraction of biological knowledge from variant data by providing a rich annotation database, flexible filtering, and a streamlined variant classification and reporting tool
- Leverages leading annotation databases and a powerful filtering interface to rapidly identify disease-associated variants in data sets

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The Broad's IGV B
- Displays alignments and variants from multiple samples for performing complex variant analysis
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B BaseSpace

www.illumina.com/smallWG

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Library prep ordering information

Nextera XT DNA Sample Preparation Kit

Generate sequencing-ready libraries from small genomes (bacteria, archaea, viruses), amplicons, and plasmids in less than 90 minutes with only 15 minutes of hands-on time. Nextera XT simultaneously fragments input DNA and tags the fragments with sequencing adapters in a single-tube enzymatic reaction. Nextera XT requires as little as 1 ng of input, supporting a wide array of sample types. Bead-based normalization eliminates the need for library quantification before pooling and sequencing. Libraries prepared with Nextera XT kits are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Indexes	No. of Samples	Catalog No.
Nextera XT DNA Sample Preparation Kit		24	FC-131-1024
Nextera XT DNA Sample Preparation Kit		96	FC-131-1096
Nextera XT Index Kit	24	96	FC-131-1001
Nextera XT Index Kit	96	384	FC-131-1002
TruSeq Dual Index Sequencing Primer Kit, Single Read	single-use kit*		FC-121-1003
TruSeq Dual Index Sequencing Primer Kit, Paired-End Read	single-use kit*		PE-121-1003
Nextera XT Index Kit v2, Set A	96	384	FC-131-2001
Nextera XT Index Kit v2, Set B	96	384	FC-131-2002
Nextera XT Index Kit v2, Set C	96	384	FC-131-2003
Nextera XT Index Kit v2, Set D	96	384	FC-131-2004

*Sequencing primer kits are required for all sequencers except the MiSeq System.

MiSeq Reporter

Streamlined, automated on-board data analysis workflows

MiSeq Reporter is the on-board data analysis tool provided with all MiSeq systems. The simple and intuitive graphical interface makes it easy to get primary and secondary data analysis, as well as quality and coverage information for each sample.

- Load the sample and walk away
- Automatic on-board data analysis and reporting
- Perform data analysis from your desk
- Generate FASTQ files from all workflows
- Access most workflows on BaseSpace

Whole-genome sequencing (long-range)

Sequence novel genomes using long reads that map uniquely to the genome, clarifying highly similar or repetitive regions for accurate *de novo* genome assembly.

Key strengths

- Complements whole-genome sequencing by providing distinct gene information from homologous chromosomes, supporting haplotyping, and allele-specific solutions
- Synthetically assembles long reads for *de novo* and genome-finishing applications with high accuracy
- Offers the industry's lowest DNA input requirement to prepare mate pair libraries as large as 12 kb
- Aids identification of structural variants in complex genomes

LIBRARY PREP	SEQUENCING	ANALYSIS TOOLS
Nextera Mate Pair Sample Preparation Kit TruSeq Synthetic Long-Read DNA Library Prep Kit	NextSeq 500 (1 Nextera kit sample) HiSeq 2500 (10–15+ Nextera kit samples) (4–16 TruSeq kit samples)	If analyzed in BaseSpace **Align/Call variants:** Nextera Mate Pair: • SPAdes Genome Assembler • Velvet *de novo* Assembly TruSeq Synthetic Long-Reads: • TruSeq Long-Read Assembly • TruSeq Phasing Analysis **Visualize:** The Broad's IGV

> "Having larger mate pair libraries has greatly facilitated the assembly of our genomes."
>
> **Thomas Sutter,** PhD, University of Memphis

Visit www.illumina.com/matePair or www.illumina.com/longRange for more details.

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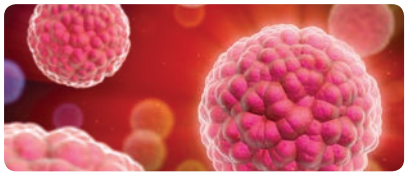
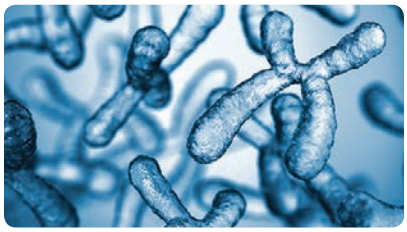


Cancer genetics

Longer sequence reads enable detection of difficult structural variant rearrangements (deletions, inversions, transversions) commonly found in cancer and genetic disease.

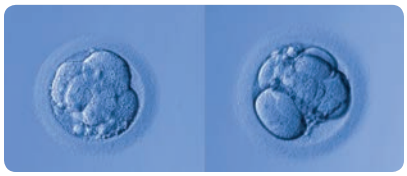


Genetic disease

Longer sequence reads allow for phasing studies that capture unique chromosomal content, including mutations that might differ across chromosome copies. This technique allows researchers to distinguish between maternally and paternally inherited alleles, a distinction that helps establish the association between the number and type of gene mutations and the presence of disease.

Agrigenomics

Longer sequence reads allow researchers to produce more accurate genome assemblies in complex genomes as found in agricultural plants.



Molecular genetics

Long read *de novo* sequencing is an important tool for mapping genomes of novel organisms, finishing genomes of known organisms, or comparing genomes across multiple samples. Sequencing the entire genome is important for generating accurate reference genomes.

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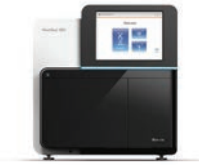
STEP 1
Choose library prep method

Product	Nextera Mate Pair Sample Preparation Kit	TruSeq Synthetic Long-Read DNA Library Prep Kit
Most important to me	Fastest assay time	Highest coverage uniformity, lowest DNA input quantity
Least important to me	Low DNA input quantity	Assay time
Input quantity	1 µg	500 ng
Coverage uniformity	Lower than TruSeq kit	Higher than Nextera kit
Fragment size (bp)	Up to 12 kb	Up to 10 kb
Assay time	1.5 days	3 days
Multiplexing	12	n/a

Comparisons within whole-genome sequencing (long-range) portfolio.

STEP 2
Choose a sequencer



Product	NextSeq 500		HiSeq 2500	
Description	Flexible power Speed and simplicity for everyday genomics		Production power Power and efficiency for large-scale genomics	
Lowest cost to process single sample	●			
Maximize number of samples/run			●	
Long-range samples processed per run	1 Mate Pair sample		4–16 SLR samples 10–15+ Mate Pair samples	
Run mode	Mid output	High output	Rapid run	High output
Flow cells processed per run	1	1	1 or 2	1 or 2
Output range	20–39 Gb	30–120 Gb	10–300 Gb	50–1,000 Gb
Run time	15–26 hours	12–30 hours	7–60 hours	< 1–6 days
Reads per flow cell	130 million	400 million	300 million	2 billion
Maximum read length	2 x 150 bp	2 x 150 bp	2 x 250 bp	2 x 125 bp

www.illumina.com/matePair or www.illumina.com/longRange

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STEP 3
Obtain results

NextSeq 500 HiSeq 2500

Align/Call variants

Nextera Mate Pair:

SPAdes Genome Assembler B

- Analyzes standard isolates and single-cell multiple displacement amplification (MDA) bacterial assemblies
- Offers read error correction tool and iterative short-read genome assembly module

Velvet *de novo* Assembly B

- Assembles *de novo* genomes for bacterial samples
- Offers assembly for libraries prepared with Nextera Mate Pair Sample Preparation Kit

TruSeq Synthetic Long-Read DNA:

TruSeq Long-Read Assembly B

- Assembles synthetic long-reads using data from TruSeq Synthetic Long-Read Library Prep Kit (SLR)
- Assembles reads and corrects for possible errors and misassemblies

TruSeq Phasing Analysis B

- Forms long haplotype contigs using imputation methods
- Reports haplotype blocks across the genome and confidence scores for the phasing

Visualize

The Broad's IGV B

- Displays alignments and variants from multiple samples for performing complex variant analysis
- The Broad Institute of MIT and Harvard developed IGV, and Illumina modified it to display BaseSpace data

B BaseSpace

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Library prep ordering information

Nextera Mate Pair Sample Preparation Kit

The Nextera Mate Pair Sample Preparation Kit is a gel-free method for preparing up to 12 kb mate pair libraries with the industry's lowest DNA input requirement. This kit is designed for applications like *de novo* assembly of small genomes and detection of structural variation. Nextera Mate Pair includes TruSeq DNA Sample Preparation master-mixed reagents to reduce the number of assay steps and hands-on time required. Libraries prepared with Nextera Mate Pair are compatible with the Illumina NextSeq 500 System and HiSeq 2500 System.

Ordering Information

Product	No. of Samples	Catalog No.
Nextera Mate Pair Sample Preparation Kit	48	FC-132-1001

This kit contains Nextera Mate Pair reagents and TruSeq reagents and indexes.

TruSeq Synthetic Long-Read DNA Library Prep Kit (SLR)

The TruSeq Synthetic Long-Read DNA Library Prep Kit is a highly accurate, end-to-end solution that can be used for genome assembly or genome phasing. The library prep kit combines TruSeq and Nextera chemistries with synthetic long-read technology to prepare DNA libraries. The accompanying barcode kit includes 384 indexes for labeling the samples in each well. These indexes are then used after sequencing to construct synthetically long fragments for long-read assembly and phasing analysis. Libraries prepared with the TruSeq Synthetic Long-Read DNA Library Prep Kit are compatible with the Illumina HiSeq 2500 System.

Ordering Information

Product	No. of Samples	Catalog No.
TruSeq Synthetic Long-Read DNA Library Prep Kit	4	FC-126-1001
TruSeq Synthetic Long-Read DNA Barcode Kit	1	FC-126-1002
TruSeq Synthetic Long-Read DNA Barcode Kit	4	FC-126-1003
TruSeq Synthetic Long-Read DNA Accessory Kit		FC-126-1004

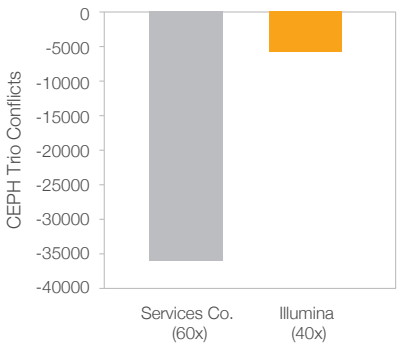
Proven technology.

Most accurate genome at any coverage.

Highest accuracy. More usable data.

Illumina platforms make up the largest installed base of NGS systems worldwide—referenced in over 5,000 peer-reviewed publications, and counting. They're the most trusted and widely adopted for a reason: our proven TruSeq technology, delivering the highest data accuracy in the industry for variant calling.

Whole-genome sequencing involves more than obtaining data with high-quality scores or large coverage depth. It is more important to generate usable data that produces the most callable bases across the entire genome, including in traditionally difficult-to-sequence regions such as high GC.



Services Company data contains over 35,000 consensus conflicts or errors within the genome.

95% GREATER

More usable data: > 95% of the NCBI reference genome. Sequencing performed on an Illumina HiSeq System.

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Epigenetics

Explore the transcriptome with the coverage density and flexibility of NGS to enhance studies of changes in gene expression caused by disease or the environment.

Key strengths

- Enables investigation of epigenetic impact on gene regulation
- Enhances characterization of phenotype of interest
- Identifies potential biomarkers for a range of phenotypes
- Reveals links between epigenetic change and disease development

LIBRARY PREP	SEQUENCING	ANALYSIS TOOLS
Methylation Sequencing Option: EpiGnome Methyl-Seq Kit **Methylation Array Option:** Infinium HumanMethylation450 BeadChip Kit **ChIP-Seq Option:** TruSeq ChIP Sample Preparation Kit	NextSeq 500 (8–24 ChIP-Seq samples) (1 EpiGnome sample) HiSeq 2500 (20–264 ChIP-Seq samples) (1–10 EpiGnome samples)	**BaseSpace Analysis Tools coming soon.** Visit www.illumina.com/informatics for current availability.

> DNA is demethylated at most gene regulatory elements (promoters, enhancers, insulators). This is very exciting because it sort of allows us to peer into the rich landscape of transcriptional regulation in these clinical samples.
>
> **Ben Berman,** PhD, Assistant Professor, Division of Bioinformatics, Department of Preventive Medicine, University of Southern California

Visit www.illumina.com/epigenetics for more details.

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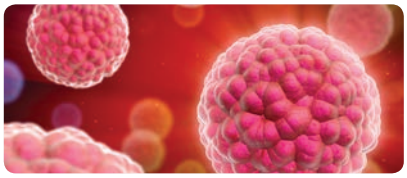


Cancer Research

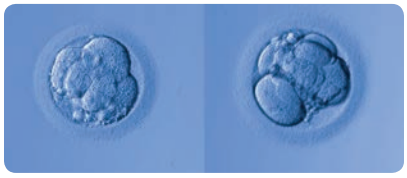
Aberrant methylation is a common epigenetic change in cancer. Sequencing-based methylation analysis enables researchers to identify and track cancer methylation patterns by directly sequencing the methylated genome.

Complex disease

Genetic variants often have functional consequences that contribute to disease. These variants might be inherited or be the result of disease or exposure to environmental mutagens. NGS allows researchers to clarify the relationship between disease and the genetic variants.

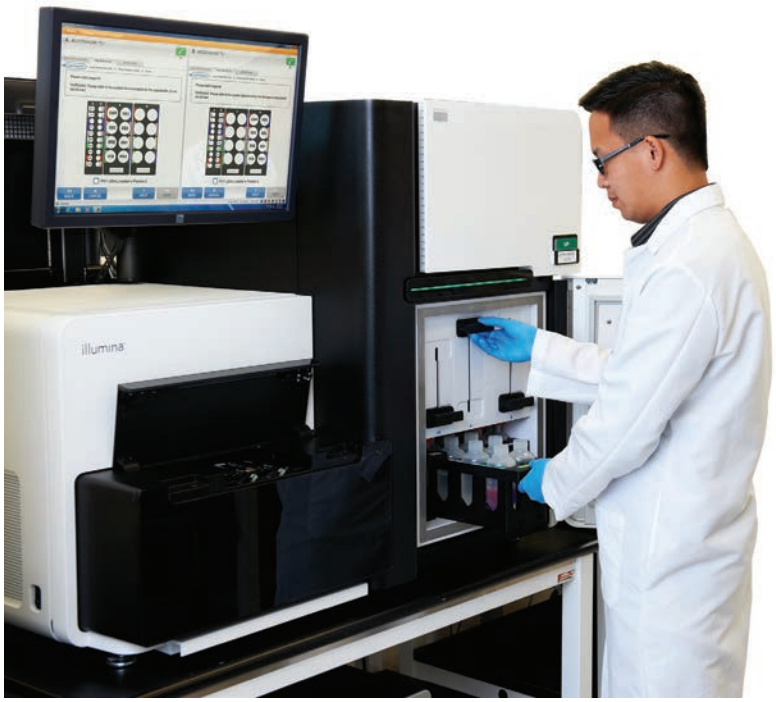
Agrigenomics

Changes to DNA methylation are being investigated for potential down regulation of stress genes during drought, salinity changes, heat, cold, and other environmental changes.



Imprinting

Environmental exposures are able to alter the methylation patterns of the genome along with other epigenetic changes. NGS methylation studies allow researchers a more complete understanding of the etiology of complex genetic diseases, clarifying the contribution of environment and genetic variation.



For Research Use Only

STEP 1
Choose library prep method

Product	EpiGnome™ Methyl-Seq Kit	Infinium® HumanMethylation450 BeadChip Kit	TruSeq ChIP Sample Preparation Kit
Most important to me	Maximum coverage of CpG, CHG, CHH regions	Lowest cost/sample	Understanding location across the genome where a protein of interest is bound
Least important to me	Lowest cost/sample	Coverage density	N/A
Input amount	50 ng	500 ng	5–10 ng
Assay time	1 day	4 days	1.5 days
Multiplexing	12	96	24
FFPE compatible	Yes	Yes	No

Comparisons within epigenetics portfolio.

STEP 2
Choose a sequencer

Product	NextSeq 500		HiSeq 2500	
Description	Flexible power Speed and simplicity for everyday genomics		Production power Power and efficiency for large-scale genomics	
Flexibility in number of samples per run	●			
Maximize number of samples/run			●	
Methylation samples processed per run	8–24 ChIP-Seq; 1 EpiGnome		20–264 ChIP-Seq; 1–10 EpiGnome	
Run mode	Mid output	High output	Rapid run	High output
Flow cells processed per run	1	1	1 or 2	1 or 2
Output range	20–39 Gb	30–120 Gb	10–300 Gb	50–1,000 Gb
Run time	15–26 hours	12–30 hours	7–60 hours	< 1–6 days
Reads per flow cell	130 million	400 million	300 million	2 billion
Maximum read length	2 x 150 bp	2 x 150 bp	2 x 250 bp	2 x 125 bp

www.illumina.com/epigenetics

For Research Use Only

STEP 3
Obtain results

In BaseSpace, you can share data with your collaborators or colleagues across the city or across the globe. For centralized facilities such as Core Labs and service providers, BaseSpace gives you the flexibility to quickly distribute your results to your end-users, or transfer ownership of sequencing runs or analysis projects. Analysis tools for epigenetics will be available soon. Visit www.illumina.com/informatics for current solutions.

Library prep ordering information

EpiGnome Methyl-Seq Kit

EpiGnome produces whole-genome bisulfite sequencing libraries from only 50–100 ng of genomic DNA. The post-bisulfite conversion library construction method yields highly diverse libraries with uniform CpG, CHG, and CHH coverage in less than one day. EpiGnome libraries are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Samples	Catalog No.
EpiGnome Methyl-Seq Kit	12	EGMK81312
EpiGnome Methyl-Seq Kit	24	EGMK91324
EpiGnome Methyl-Seq Kit	96	EGMK91396
EpiGnome Index PCR Primers (12 indexes, 10 reactions each)		EGIDX81312
FailSafe PCR Enzyme Mix (100 units)		FSE51100

Note: Order the EpiGnome Methyl-Seq Kit from www.epicentre.com

Infinium HumanMethylation450 BeadChip Kit

The unique combination of comprehensive, expert-selected coverage, high sample throughput capacity, and affordable price of the HumanMethylation450 BeadChip make it an ideal solution for large sample-size genome-wide DNA methylation studies. Compatible with HiSeq iScan.

Ordering Information

Product	No. of Samples	Catalog No.
Infinium HumanMethylation450 BeadChip Kits	24	WG-314-1003
Infinium HumanMethylation450 BeadChip Kits	48	WG-314-1001
Infinium HumanMethylation450 BeadChip Kits	96	WG-314-1002

TruSeq ChIP Sample Preparation Kit

The TruSeq ChIP Sample Preparation Kit is a simple, cost-effective solution for generating chromatin immunoprecipitation sequencing (ChIP-Seq) libraries from ChIP-derived DNA. ChIP-Seq leverages NGS to quickly and efficiently determine the distribution and abundance of DNA-bound protein targets of interest across the genome. The kits have low DNA input requirements and are compatible with a range of sample sizes. Libraries prepared with TruSeq ChIP Sample Preparation Kits are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Indexes	No. of Samples	Catalog No.
TruSeq ChIP Sample Preparation Kit, Set A	12	48	IP-202-1012
TruSeq ChIP Sample Preparation Kit, Set B	12	48	IP-202-1024

For Research Use Only

Amplicon sequencing

Perform highly targeted resequencing of specific genes of interest, even in difficult-to-sequence areas, with low DNA input.

Key strengths

- Offers unparalleled efficiency for discovering, validating, and screening genetic variants with highly targeted approach
- Multiplexes up to 1,536 amplicons per reaction to achieve coverage of up to 650 kB of cumulative sequence with TruSeq Custom Amplicon Kit
- Delivers improved performance in difficult-to-address GC-rich regions, as well as increased library yield, uniformity, and stability from samples as low as 50 ng with TruSeq Custom Amplicon v1.5

LIBRARY PREP

TruSeq Custom Amplicon Kit (TSCA)

Nextera XT DNA Sample Preparation Kit

Illumina Demonstrated 16S Protocol

SEQUENCING

ATATTTT
GACTTCA
TATAAAA
ATATTTT

MiSeq
(96+ samples)

NextSeq 500
(384 samples)

ANALYSIS TOOLS

If analyzed on MiSeq:
Metagenomics 16S rRNA Workflow
Resequencing Workflow

If analyzed in BaseSpace:
Align/Call variants:
TSCA
- TruSeq Amplicon

Nextera XT DNA Sample Preparation Kit
- Coming soon

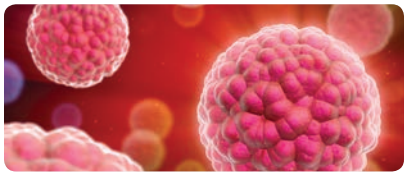
Illumina Demonstrated 16S Protocol
- 16S Metagenomics

Annotate/Filter:
TSCA
- VariantStudio

Visualize:
TSCA
- The Broad's IGV

With targeted resequencing, researchers can focus interrogation on key regions of genomic interest.

Innovation. Discovery. Application.



Cancer research

Custom amplicon sequencing is useful for targeting genes known to be involved in diseases, such as cancer. Efficient, targeted custom amplicon sequencing achieves research results quickly and efficiently.

Microbial genomics

Microbial samples are often complex, requiring researchers to identify and compare the populations and activities of the microbes present within a single sample. High-speed, multiplex 16S amplicon sequencing improves the sensitivity and specificity of taxonomic assignments down to the genus level and species level in some cases.

Public health

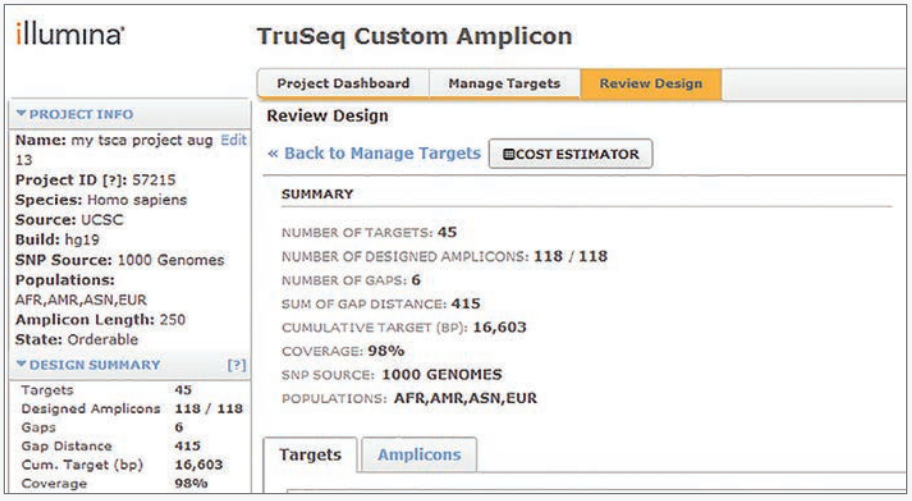
Foodborne illness occurs when people eat food contaminated with pathogenic organisms. NGS allows population health experts to quickly identify the organisms involved in an outbreak, improving health care for the affected people and effectively addressing the source of the outbreak.

DesignStudio

Design primers specific to your desired targeted region with a personalized, web-based sequencing assay design tool

- Receive dynamic feedback to optimize target region coverage
- Reduce the time required to design custom projects
- Access enhanced DNA targeting options, including Coding Sequence Only (CDS)

Personalized, easy-to-use, optimized probe design



Visit www.illumina.com/amplicon for more details.

For Research Use Only

STEP 1
Choose library prep method

Product	TruSeq Custom Amplicon Kit	Nextera XT DNA Sample Preparation Kit
Most important to me	Highly targeted	Fastest assay time, lowest DNA input quantity
Least important to me	Assay time	Amplicons < 300 bp in length
Input quantity	50 ng DNA	1 ng DNA
Assay time	8 hours	90 minutes
Hands-on time	4 hours	65 minutes
FFPE compatible	Yes	No
Design	Customers use Illumina DesignStudio software to design probes targeting their regions of interest.	Independently designed customer probes target amplicons that are at least 300 bp in length.
Fragmentation method	None	Enzymatic

Comparisons within amplicon sequencing portfolio.

STEP 2
Choose a sequencer

Description	MiSeq Focused power Speed and simplicity for targeted and small genome sequencing	NextSeq 500 Flexible power Speed and simplicity for everyday genomics	
On-board informatics	●		
Flexibility in number of samples per run		●	
Amplicons processed per run	96	384	
Run mode		Mid output	High output
Flow cells processed per run	1	1	1
Output range	0.3–15 Gb	20–39 Gb	30–120 Gb
Run time	5–55 hours	15–26 hours	12–30 hours
Reads per flow cell	25 million	130 million	400 million
Maximum read length	2 x 300 bp	2 x 150 bp	2 x 150 bp

www.illumina.com/amplicon

For Research Use Only

STEP 3
Obtain results


MiSeq


NextSeq 500

Align/Call variants

Nextera XT DNA Sample Preparation Kit
Resequencing workflow: M
- Enables WGS for small genomes (< 20 Mb)
- Uses Industry standard informatics tools BWA and GATK to align and call variants

TruSeq Custom Amplicon:
TruSeq Amplicon B
- Aligns targeted regions specified in manifest file for Illumina TruSeq Amplicon panels
- Calls variants and annotates output files

Illumina Demonstrated 16S Protocol
Metagenomics 16S rRNA Workflow M B
- Performs taxonomic classification of 16S rRNA targeted amplicon reads
- Provides interactive visualizations and raw classification output for pre-sample and aggregate analyses

Annotate/Filter

TruSeq Custom Amplicon:
VariantStudio B
- Enables extraction of biological knowledge from variant data by providing a rich annotation database, flexible filtering, and a streamlined variant classification and reporting tool
- Leverages leading annotation databases and a powerful filtering interface to rapidly identify disease-associated variants in data sets

Visualize

TruSeq Custom Amplicon:
The Broad's IGV B
- Displays alignments and variants from multiple samples for performing complex variant analysis
- The Broad Institute of MIT and Harvard developed IGV, and Illumina modified it to display BaseSpace data

B BaseSpace M MiSeq Reporter

For Research Use Only

Library prep ordering information

Nextera XT DNA Sample Preparation Kit

Generate sequencing-ready libraries from small genomes (bacteria, archaea, viruses), amplicons, and plasmids in less than 90 minutes with only 15 minutes of hands-on time. Nextera XT simultaneously fragments input DNA and tags the fragments with sequencing adapters in a single-tube enzymatic reaction. Nextera XT requires as little as 1 ng of input, supporting a wide array of sample types. Bead-based normalization eliminates the need for library quantification before pooling and sequencing. Libraries prepared with Nextera XT kits are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Indexes	No. of Samples	Catalog No.
Nextera XT DNA Sample Preparation Kit		24	FC-131-1024
Nextera XT DNA Sample Preparation Kit		96	FC-131-1096
Nextera XT Index Kit	24	96	FC-131-1001
Nextera XT Index Kit	96	384	FC-131-1002
TruSeq Dual Index Sequencing Primer Kit, Single Read	single-use kit*		FC-121-1003
TruSeq Dual Index Sequencing Primer Kit, Paired-End Read	single-use kit*		PE-121-1003
Nextera XT Index Kit v2, Set A	96	384	FC-131-2001
Nextera XT Index Kit v2, Set B	96	384	FC-131-2002
Nextera XT Index Kit v2, Set C	96	384	FC-131-2003
Nextera XT Index Kit v2, Set D	96	384	FC-131-2004

* Sequencing primer kits are required for all sequencers except the MiSeq System.

TruSeq Custom Amplicon v1.5 Kit

TruSeq Custom Amplicon is a fully customizable, amplicon-based assay for targeted resequencing. Sequence up to 1,536 amplicons in a single reaction using a simple workflow, for coverage of up to 650 kb of cumulative sequence. TruSeq Custom Amplicon requires as little as 50 ng of input gDNA, accommodating many different sample types. Integrated dual indexing supports up to 96 samples per run. TruSeq Custom Amplicon offers a fully integrated DNA-to-data solution, including convenient probe design and ordering using DesignStudio. Libraries prepared with TruSeq Custom Amplicon are compatible with the Illumina MiSeq Desktop Sequencer and NextSeq 500 System.

Ordering Information

Product	No. of Samples	Catalog No.
TruSeq Custom Amplicon v1.5 Kit	96	FC-130-1001
TruSeq Custom Amplicon Index Kit (96 indexes)	384	FC-130-1003
TruSeq Index Plate Fixture Kit		FC-130-1005
TruSeq Index Plate Fixture and Collar Kit (2 each)		FC-130-1007

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Illumina Demonstrated 16S Protocol

Metagenomic studies are commonly performed by analyzing the prokaryotic 16S ribosomal RNA gene (16S rRNA), which is approximately 1,500 bp long and contains 9 variable regions interspersed between conserved regions. Variable regions of 16S rRNA are frequently used in phylogenetic classifications such as genus or species in diverse microbial populations. The Illumina Demonstrated 16S Protocol describes a method for preparing libraries that allows researchers to sequence the variable V3 and V4 regions of the 16S rRNA gene. This protocol can also be used for sequencing other regions with different region-specific primers. The Illumina Demonstrated 16S Protocol is available at www.illumina.com/metagenomics. Sequencing libraries prepared with the Illumina Demonstrated 16S Protocol are compatible with the MiSeq Desktop Sequencer.

Ordering Information

Product	Catalog No.
Nextera XT Index Kit	FC-131-1001
	FC-131-1002
PhiX Control Kit v3	FC-110-3001

Cancer research sequencing panels

Reduce cost per sample and analysis burden with highly focused targeted panels that enable deeper coverage of regions of interest.

Key strengths

- Sequences key genes or regions of interest to high depth using pre-designed, analytically validated panels
- Provides cost-effective, actionable findings for cancer-relevant genes
- Delivers easy-to-interpret results
- Obtains highly accurate results with variants present down to 5% variant allele frequency

LIBRARY PREP

TruSeq Amplicon - Cancer Panel (TSACP)

TruSight Cancer Sequencing Panel

TruSight Tumor Sequencing Panel

TruSight Myeloid Sequencing Panel

SEQUENCING

ATATTTT
GACTTCA
TATAAAA
ATATTTT

MiSeq

NextSeq 500

HiSeq 2500

See page 36 to learn how many samples can be processed on each sequencing platform.

ANALYSIS TOOLS

If analyzed on MiSeq:
TruSight Tumor
- Amplicon–DS Workflow

TruSeq Amplicon - Cancer Panel, TruSight Myeloid:
- TruSeq Amplicon Workflow
  – With Somatic Variant Caller

TruSight Cancer
- Enrichment Workflow

If analyzed in BaseSpace:
Align/Call variants:
- TruSeq Amplicon
  – With Somatic Variant Caller for Tumor Samples
- Amplicon–DS
- BWA Enrichment
- Isaac Enrichment

Annotate/Filter:
VariantStudio

Visualize:
The Broad's IGV

Visit www.illumina.com/cancerPanels for more details.

For Research Use Only

Innovation. Discovery. Application.

Germline testing

The current paradigm is to test individuals believed to be at risk for a few select genes, providing a limited picture and potentially unclear results. NGS enables multiple risk-associated genes to be examined in a single test, providing an expanded view rapidly, accurately, and economically.

- **TruSight Cancer:** The TruSight cancer panel contains 94 genes previously linked to a predisposition to cancer.

Somatic variant detection

Iterative tests on cancer samples delay time to answer and can result in exhaustion of limited tissue. NGS enables multiple genes to be assessed in a single test, providing a single streamlined workflow, decreasing time to answer and producing accurate and highly sensitive results.

- **TruSight Tumor:** Targets 26 genes frequently mutated in solid tumors according to CAP and NCCN guidelines, and clinical trials.
- **TruSight Myeloid:** Targets 54 genes (either targeted exonic regions or full coding sequence) frequently mutated in myeloid malignancies.
- **TruSeq Amplicon - Cancer Panel:** Targets hotspot regions of frequently mutated genes in both solid and hematological cancers.

For Research Use Only

STEP 1
Choose library prep method




Product	TruSight Cancer Sequencing Panel	TruSight Tumor Sequencing Panel	TruSight Myeloid Sequencing Panel	TruSeq Amplicon - Cancer Panel
Key use	Germline mutation detection	Somatic mutation detection in solid tumors	Somatic mutation detection in myeloid malignancies	Somatic mutational hotspots in a broad spectrum of cancers
DNA input	50 ng	30–300 ng	50 ng	150–250 ng
FFPE compatibility	Possible but not supported	Yes	Possible but not supported	Yes
Genomic content	255 kb (~4000 probes, 94 genes)	21 kb (174 amplicons, 26 genes)	~141 kb (568 amplicons, 54 genes)	> 35 kb (212 amplicons, 48 genes)
Assay	Enrichment	Amplicon (dual-stranded)	Amplicon	Amplicon
Read length	2 × 150 bp	2 × 121 bp	2 × 150 bp	2 × 150 bp
Sequencing depth	> 20x per target	> 1,000x per amplicon	95% amplicons at > 500x	~1,000× average coverage
Ideal instrument	MiSeq or NextSeq	MiSeq or HiSeq	MiSeq or NextSeq	MiSeq or NextSeq

STEP 2
Choose a sequencer



Product	MiSeq	NextSeq 500		HiSeq 2500	
Description	Focused power Speed and simplicity for targeted and small genome sequencing.	Flexible power Speed and simplicity for everyday genomics		Production power Power and efficiency for large-scale genomics.	
On-board informatics	●				
Flexibility in number of samples per run		●			
Maximize number of samples/run				●	
Run mode		Mid output	High output	Rapid run	High output
Flow cells processed per run	1	1	1	1 or 2	1 or 2
Output range	0.3–15 Gb	20–39 Gb	30–120 Gb	10–180 Gb	50–1000 Gb
Run time	5–55 hours	15–26 hours	12–30 hours	7–40 hours	< 1 day–6 days
Reads per flow cell	25 million	130 million	400 million	300 million	2 billion
Maximum read length	2 x 300 bp	2 x 150 bp	2 x 150 bp	2 x 150 bp	2 x 125 bp
Number of panels processed per run					
TruSight Cancer	48 (v2 chemistry)	96	96	192	768
TruSight Tumor	4 (v2 chemistry)	34	48	96	384
TruSight Myeloid	8 (v3 chemistry)	40	96	96	480
TSACP	42 (v3 chemistry)	96	96	192	768

For Research Use Only

STEP 3
Obtain results

Align/Call variants

TruSight Tumor:
Amplicon-DS Workflow M B
- Designed for analysis of TruSight Tumor samples
- Optimized for FFPE samples

TruSeq Amplicon - Cancer Panel
TruSight Myeloid Sequencing Panel:
TruSeq Amplicon Workflow M B
- With Somatic Variant Caller

TruSight Cancer:
TruSight Enrichment Workflow M B
- Uses BWA for alignment and GATK for variant calling

BWA Enrichment B
- Aligns using BWA Alignment software and calls variants using GATK
- Industry standard for enrichment

Isaac Enrichment B
- Performs read mapping using Isaac Genome Alignment Software
- Offering 4x faster alignment speeds with the same accuracy as BWA. Designed by Illumina.

Annotate/Filter

VariantStudio B
- Leverages leading annotation databases and a powerful filtering interface to rapidly identify disease-associated variants in data sets
- Enables extraction of biological knowledge from variant data by providing a rich annotation database, flexible filtering, and a streamlined variant classification and reporting tool

Visualize

The Broad's IGV B
- The Broad Institute of MIT and Harvard developed IGV, and Illumina modified it to display BaseSpace data
- Displays alignments and variants from multiple samples for performing complex variant analysis

www.illumina.com/cancerPanels

B BaseSpace

M MiSeq Reporter

For Research Use Only

Library prep ordering information

TruSeq Amplicon - Cancer Panel

The TruSeq Amplicon - Cancer Panel (TSCAP) is a highly multiplexed targeted resequencing assay for detecting somatic hotspot mutations in both solid and hematological cancers. The unique ability of this assay to screen precious FFPE samples for these important variants allows you to unlock a wealth of genomic information for many tumor types. Libraries prepared with the TruSeq Amplicon-Cancer Panel are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Indexes	No. of Samples	Catalog No.
TruSeq Amplicon - Cancer Panel		96	FC-130-1008
TruSeq Custom Amplicon Index Kit	96	384	FC-130-1003
Illumina FFPE QC Kit			WG-321-1001
TruSeq Index Plate Fixture and Collar Kit (2 each)			FC-130-1007
TruSeq Index Plate Fixture Kit			FC-130-1005

TruSight Cancer Sequencing Panel

The TruSight Cancer Sequencing Panel includes genes associated with common and rare cancers, as well as 284 SNPs found to correlate with cancer through genome-wide association studies (GWAS). The TruSight Cancer sequencing panel provides custom oligos targeting identified regions of interest. The panel includes enough product for four enrichment reactions (48 samples) and is compatible with TruSight Rapid Capture kits. Libraries prepared with the TruSight Cancer Sequencing Panel are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	Pooling Plexity	No. of Indexes	No. of Samples	Catalog No.
TruSight Cancer Sequencing Panel (4 enrichments)*			48	FC-121-0202
TruSight Rapid Capture	1	1	8	FC-140-1101
	2	2	8	FC-140-1102
	4	4	16	FC-140-1103
	12	24	48	FC-140-1104
	12	24	96	FC-140-1105
	12	96	288	FC-140-1106

*Requires purchase of a TruSight Rapid Capture kit.

For Research Use Only

TruSight Myeloid Sequencing Panel

The TruSight Myeloid Sequencing Panel covers 15 full genes (exons only) and key exonic regions of 39 additional genes, providing a comprehensive assessment of the key genes involved in myeloid malignancies in a single test. The result is an accurate, cost-effective solution for profiling liquid tumors. Libraries prepared with the TruSight Myeloid Sequencing Panel are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Indexes	No. of Samples	Catalog No.
TruSight Myeloid Sequencing Panel*		96	FC-130-1010
TruSeq Custom Amplicon Index Kit	96	384	FC-130-1003
TruSeq Index Plate Fixture Kit			FC-130-1005
TruSeq Index Plate Fixture and Collar Kit (2 Each)			FC-130-1007

*Requires purchase of a TruSeq Custom Amplicon Index kit.

TruSight Tumor Sequencing Panel

The TruSight Tumor Sequencing Panel takes a deeper view of variation in solid tumors, including lung, colon, melanoma, gastric, and ovarian cancer tumors. This panel allows clinical researchers to identify low-frequency variation across 26 genes for a more comprehensive view of somatic variation. Libraries prepared with the TruSight Tumor Sequencing Panel are compatible with the Illumina MiSeq Desktop Sequencer, the NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Samples	Catalog No.
TruSight Tumor Sequencing Panel Includes library preparation consumables, oligos, DNA QC, and indexes sufficient for 48 samples	48	FC-130-2001
TruSeq Index Plate Fixture Kit		FC-130-1005
TruSeq Index Plate Fixture and Collar Kit (2 each)		FC-130-1007

Select Illumina reagents and consumables are available with product attributes may benefit clinical research laboratories. See page 63 for details.

For Research Use Only

Disease and condition sequencing panels

Target genes and regions thought to be relevant to particular diseases or conditions with focused panels.

Key strengths

- Detects rare variants that might be associated with genetic diseases and enables confident identification of causative *de novo* or inherited rare mutations in a single test
- Provides cost-effective, streamlined, targeted NGS of specific genetic diseases or conditions

LIBRARY PREP

TruSight One Sequencing Panel

TruSight Cardiomyopathy Sequencing Panel

TruSight Inherited Disease Sequencing Panel

TruSight Autism Sequencing Panel

SEQUENCING

ATATTTT
GACTTCA
TATAAAA
ATATTTT

MiSeq

NextSeq 500

HiSeq 2500

See page 42 to learn how many samples can be run on each sequencing platform.

ANALYSIS TOOLS

If analyzed on MiSeq:

- Enrichment Workflow
  - With Somatic Variant Caller for tumor samples
  - With GATK or Isaac for germline samples

If analyzed in BaseSpace:

Align/Call variants:
BWA Enrichment
Isaac Enrichment

Annotate/Filter:
VariantStudio

Visualize:
The Broad's IGV

> We believe that this is going to transform medicine.
>
> **Stephen Kingsmore,** MB, BAO, ChB, DSc, FRCPath, Director of the Center for Pediatric Genomic Medicine, Children's Mercy Hospital

Visit www.illumina.com/diseasePanels for more details.

For Research Use Only

Innovation. Discovery. Application.

One sequencing panel, thousands of inherited conditions

The TruSight One Sequencing Panel is a solution for germline mutation screening of exonic regions in 4,813 genes relevant for clinical research. Designed to cover the most commonly ordered molecular assays, the panel enables labs to perform these assays on-site with a single solution. At the same time, TruSight One facilitates the creation of several smaller sequencing panels with a common workflow, reducing both operational overhead and processing frequency. Genomic targets were identified based on information in the Human Gene Mutation Database (HGMD Professional), the Online Mendelian Inheritance in Man (OMIM) catalog, GeneTests.org, Illumina TruSight panels, and other commercially available sequencing panels.

Inherited cardiac disease research

The increased information obtained from NGS is providing remarkable insight into the genomic and environmental components that underlie inherited cardiac conditions. TruSight Cardio is designed to affordably and accurately screen 174 genes related to 20 inherited cardiac conditions to provide the greatest chance of revealing causative variants.

Inherited disease

TruSight Inherited Disease was initially based on a panel designed for preconception carrier testing for severe, recessive childhood diseases. Dr. Kingsmore and team at Children's Mercy Hospital (CMH) for Pediatric Genomic Medicine in Science Translational Medicine published this panel. Dr. Saunders, FACMG at CMH revised the original content following ACMG guidelines for testing ultra-rare genetic diseases. This revision addresses the needs of medical geneticists with a primary focus on severe recessive diseases with childhood onset. Dr. Ropers added intellectual disability genes, resulting in a panel covering 552 genes.

Autism

Specific genes are emerging as central to the understanding of autism. These genes include genes that have been reported in the Online Mendelian Inheritance in Man (OMIM) database on autism; genes with recurrent mutations resulting in developmental delays; genes with reported mutations as found in case studies involving developmental delay characteristics; and genes from summaries of autism-relevant genes (eg, AutismKB4).

STEP 1
TruSight sequencing panel reference guide

	Maximum Number of Samples at Optimal Kit Configuration							
	Genomic Content Covered	Recommended Read Length	MiSeq v2*	MiSeq v3	NextSeq Mid Output	NextSeq High Output	HiSeq 2500+	DNA Library Preparation
TruSight One	12 Mb	2 x 150		3	12	36	36–228	Included
TruSight Cardiomyopathy	244 kb	2 x 150	12					Included ++
TruSight Inherited Disease	2.25 Mb	2 x 150	1–4	8	48	96	132–660	TruSight Rapid Capture*
TruSight Autism	328 kb	2 x 150	2–36	72	96	96	192–768	TruSight Rapid Capture*

TruSight Rapid Capture kits are available in six different configurations based on desired levels of multiplexing.

* Sample multiplexing accommodates various chemistry and flow cell configurations.

+ Number of samples are flexible to support rapid run and high output modes on the HiSeq 2500.

++ Available in 2015

STEP 2
Choose a sequencer

Product	MiSeq	NextSeq 500		HiSeq 2500	
Description	Focused power Speed and simplicity for targeted and small genome sequencing	Flexible power Speed and simplicity for everyday genomics		Production power Power and efficiency for large-scale genomics	
On-board informatics	●				
Flexibility in number of samples per run		●			
Maximize number of samples/run				●	
Run mode		Mid output	High output	Rapid run	High output
Flow cells processed per run	1	1	1	1 or 2	1 or 2
Output range	0.3–15 Gb	20–39 Gb	30–120 Gb	10–300 Gb	50–1,000 Gb
Run time	5–55 hours	15–26 hours	12–30 hours	7–60 hours	< 1–6 days
Reads per flow cell	25 million	130 million	400 million	300 million	2 billion
Maximum read length	2 x 300 bp	2 x 150 bp	2 x 150 bp	2 x 250 bp	2 x 125 bp

www.illumina.com/diseasePanels

For Research Use Only

STEP 3
Obtain results


MiSeq NextSeq 500


HiSeq 2500

Align/Call variants

Enrichment Workflow: M
- Enables analysis of panels utilizing capture-based methodology, such as the Illumina Exome, TruSight One, and Illumina custom capture panels built in DesignStudio
- Aligns using BWA Alignment software and calls variants using GATK

BWA Enrichment B
- Aligns using BWA Alignment software and calls variants using GATK
- Industry standard for enrichment

Isaac Enrichment B
- Performs read mapping using Isaac Genome Alignment Software
- Uses Isaac Variant Detection to identify single nucleotide variants (SNVs), small indels, copy number anomalies (CNAs), and structural variations
- Offering 4x faster alignment speeds with the same accuracy as BWA; designed by Illumina

Annotate/Filter

VariantStudio B
- Enables extraction of biological knowledge from variant data by providing a rich annotation database, flexible filtering, and a streamlined variant classification and reporting tool
- Leverages leading annotation databases and a powerful filtering interface to rapidly identify disease-associated variants in data sets

Visualize

The Broad's IGV B
- Displays alignments and variants from multiple samples for performing complex variant analysis
- The Broad Institute of MIT and Harvard developed IGV, and Illumina modified it to display BaseSpace data

B BaseSpace M MiSeq Reporter

For Research Use Only

Library prep ordering information

TruSight One Sequencing Panel

The TruSight One Sequencing Panel provides comprehensive coverage of more than 4,800 clinically relevant genes, allowing clinical research labs to analyze all of the genes on the panel or focus on a specific subset. The panel focuses on the exonic regions harboring disease-causing mutations and was designed to cover the most commonly ordered molecular assays, enabling labs to perform these tests with one assay. Libraries prepared with the TruSight One Sequencing Panel are compatible with the Illumina MiSeq Desktop Sequencer, the NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Samples	Catalog No.
TruSight One Sequencing Panel	9	FC-141-1006
TruSight One Sequencing Panel	36	FC-141-1007

TruSight Cardomyopathy Sequencing Panel

TruSight Cardiomyopathy targets genes linked to inherited cardiomyopathies, as well as syndromes like Danon and Fabry disease, Barth syndrome, and Transthyretin Amyloidosis, all of which can present with isolated cardiomyopathy. The TruSight Cardiomyopathy sequencing panel includes custom oligos targeting identified regions of interest. The TruSight Cardiomyopathy Sequencing Panel includes enough product for four enrichment reactions and is compatible with TruSight Rapid Capture. Libraries prepared with the TruSight Cardiomyopathy Sequencing Panel are compatible with the Illumina MiSeq Desktop Sequencer, the NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Indexes	No. of Samples	Catalog No.
TruSight Cardiomyopathy Sequencing Panel	4 enrichments		FC-121-0204
Rapid Capture Kits			
TruSight Rapid Capture Kit	1	8	FC-140-1101
TruSight Rapid Capture Kit	2	8	FC-140-1102
TruSight Rapid Capture Kit	4	16	FC-140-1103
TruSight Rapid Capture Kit	24	48	FC-140-1104 T
TruSight Rapid Capture Kit	24	96	FC-140-1105 T
TruSight Rapid Capture Kit	96	288	FC-140-1106

Select Illumina reagents and consumables are available with product attributes that might be of benefit to clinical research laboratories. See page 63 for details.

For Research Use Only

TruSight Inherited Disease Sequencing Panel

The TruSight Inherited Disease Sequencing Panel focuses on severe, recessive pediatric onset diseases. It targets 552 genes, including coding exons, intron-exon boundaries, and regions known to harbor pathogenic mutations. The TruSight Inherited Disease Sequencing Panel set includes custom oligos targeting identified regions of interest. The panel includes enough product for four enrichment reactions and is compatible with TruSight Rapid Capture. Libraries prepared with the TruSight Inherited Disease Sequencing Panel are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Indexes	No. of Samples	Catalog No.
TruSight Inherited Disease Sequencing Panel	4 enrichments		FC-121-0205
Rapid Capture Kits			
TruSight Rapid Capture Kit	1	8	FC-140-1101
TruSight Rapid Capture Kit	2	8	FC-140-1102
TruSight Rapid Capture Kit	4	16	FC-140-1103
TruSight Rapid Capture Kit	24	48	FC-140-1104
TruSight Rapid Capture Kit	24	96	FC-140-1105
TruSight Rapid Capture Kit	96	288	FC-140-1106

TruSight Autism Sequencing Panel

The TruSight Autism Sequencing Panel targets 101 developmental delay genes linked specifically to autism with custom oligos targeting identified regions of interest. The panel includes enough product for four enrichment reactions and is compatible with TruSight Rapid Capture. Libraries prepared with the TruSight Autism Sequencing Panel are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Indexes	No. of Samples	Catalog No.
TruSight Autism Sequencing Panel	4 enrichments		FC-121-0203
Rapid Capture Kits			
TruSight Rapid Capture Kit	1	8	FC-140-1101
TruSight Rapid Capture Kit	2	8	FC-140-1102
TruSight Rapid Capture Kit	4	16	FC-140-1103
TruSight Rapid Capture Kit	24	48	FC-140-1104
TruSight Rapid Capture Kit	24	96	FC-140-1105
TruSight Rapid Capture Kit	96	288	FC-140-1106

Select Illumina reagents and consumables are available with product attributes that may benefit clinical research laboratories. See page 63 for details.

For Research Use Only

Exome sequencing

Investigate the protein-coding regions of the genome (the exome) with targeted sequencing to uncover genetic influences on disease and population health.

Key strengths

- Identifies variants across a wide range of applications, including population genetics, genetic disease, and cancer studies
- Achieves comprehensive coverage of expertly selected exonic content
- Provides a cost-effective alternative to whole-genome sequencing, as the human genome contains ~85% of known disease-causing variants

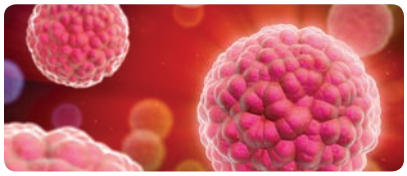
LIBRARY PREP	SEQUENCING	ANALYSIS TOOLS
Nextera Rapid Capture Exome Nextera Rapid Capture Expanded Exome Nextera Rapid Capture Custom Enrichment Kit	NextSeq 500 (up to 9 samples) HiSeq 2500 (up to 150 samples)	If analyzed in BaseSpace: **Align/Call variants:** BWA Enrichment Isaac Enrichment **Annotate/Filter:** VariantStudio **Visualize:** The Broad's IGV

> In a breast cancer study, we profiled hundreds of single cells and discovered that the tumors grew by punctuated clonal expansions, in which hundreds of genomic rearrangements were acquired in short bursts of evolution.
>
> **Nicholas Navin,** PhD, MD Anderson Cancer Center

Visit www.illumina.com/exomeSeq for more details.

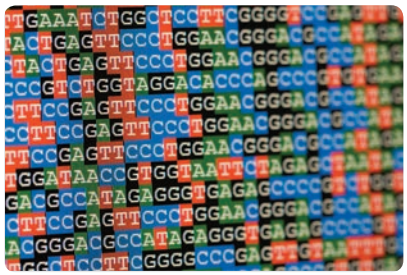
For Research Use Only

Innovation. Discovery. Application.



Cancer research

Exome sequencing allows researchers to focus on only the coding regions. By sequencing only 1–2% of the genome it is possible to sequence to higher depth more economically. By performing tumor-normal exome sequencing, researchers can focus in on the changes most likely to be tumorigenic.

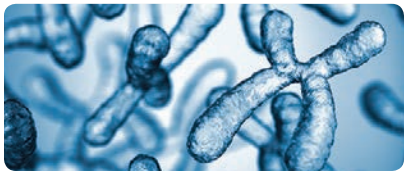


Complex disease

Genetic association and linkage studies with exome sequencing provide researchers with insights into complex diseases through common genetic variants shared between large numbers of individuals. In recent years, researchers have begun to extend complex trait association studies to focus on rare, coding variants that often have impactful functional consequences on affected patients. Researchers taking advantage of the affordability of exome sequencing gain the genomic tools to discover and test for associations between rare coding variants and complex disease directly testing the rare-variant common-disease hypothesis.

Clinical research

Exome sequencing is an increasingly powerful tool for investigation into human disease. It allows evaluation of thousands of protein-coding regions simultaneously with a single test. Exome sequencing accelerates discovery through focused analysis.



Mendelian disorder discovery

Exome sequencing a few unrelated affected individuals is a cost-effective and powerful strategy for identifying the genes related to rare Mendelian disorders. In recent years, experimental and analytical approaches relating to exome sequencing have established a rich framework for discovering the genes underlying unsolved Mendelian disorders.

For Research Use Only

STEP 1
Choose library prep method



Product	Nextera Rapid Capture Exome	Nextera Rapid Capture Expanded Exome	Nextera Rapid Capture Custom Enrichment Kit
Most important to me	Highest coverage of coding exons	Highest coverage of exons, noncoding regions, UTRs, and miRNA	User customizes region of coverage
Least important to me	Coverage of noncoding regions	Narrow targeted regions	Pre-existing probes
Target region size	~37 Mb	~62 Mb	0.5–15 Mb
Target region description	98.3% RefSeq 98.6% CCDS 97.8% Ensembl 98.1% GENCODE v12	95.3% RefSeq 96.0% CCDS 90.6% Ensembl 91.6% GENCODE v12 > 88% RefSeq 5′ and 3′ UTRs > 77% predicted microRNA	Custom probe design
Compatible with add-on	Yes	No	Yes
Recommended read length	2 x 150	2 x 150	2 x 75–2 x 150
Kit configurations (samples/kit)	8, 24, 48, 72, 96	24, 48, 96	48, 96, 288

Comparisons within exome sequencing portfolio.

STEP 2
Choose a sequencer


Product	NextSeq 500		HiSeq 2500	
Description	Flexible power Speed and simplicity for everyday genomics		Production power Power and efficiency for large-scale genomics	
Flexibility in number of samples per run	●			
Maximize number of samples/run			●	
Exomes processed per run	up to 9		up to 150	
Run mode	Mid output	High output	Rapid run	High output
Flow cells processed per run	1	1	1 or 2	1 or 2
Output range	20–39 Gb	30–120 Gb	10–300 Gb	50–1,000 Gb
Run time	15–26 hours	12–30 hours	7–60 hours	< 1–6 days
Reads per flow cell	130 million	400 million	300 million	2 billion
Maximum read length	2 x 150 bp	2 x 150 bp	2 x 250 bp	2 x 125 bp

For Research Use Only

STEP 3
Obtain results

NextSeq 500 HiSeq 2500

Align/Call variants

BWA Enrichment B
- Processes WGS reads using BWA for alignment
- Uses GATK for variant detection

Isaac Enrichment B
- Performs read mapping using Isaac Genome Alignment Software
- Uses Isaac Variant Detection to identify single nucleotide variants (SNVs), small indels, copy number anomalies (CNAs), and structural variations
- Offering 4x faster alignment speeds with the same accuracy as BWA; designed by Illumina

Annotate/Filter

VariantStudio B
- Enables extraction of biological knowledge from variant data by providing a rich annotation database, flexible filtering, and a streamlined variant classification and reporting tool
- Leverages leading annotation databases and a powerful filtering interface to rapidly identify disease-associated variants in data sets

Visualize

The Broad's IGV B
- Displays alignments and variants from multiple samples for performing complex variant analysis
- The Broad Institute of MIT and Harvard developed IGV, and Illumina modified it to display BaseSpace data

www.illumina.com/exomeSeq

B BaseSpace

M MiSeq Reporter

For Research Use Only

Library prep ordering information

Nextera Rapid Capture Exome

The Nextera Rapid Capture Exome Kit provides all-in-one library preparation and exome enrichment. This optimized exome delivers 37 Mb of expertly selected exonic content and requires as little as 4 Gb of sequencing. Custom content can be added using the Nextera Rapid Capture Custom Enrichment kit. Libraries prepared with Nextera Rapid Capture Exome and Expanded Exome are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	Pooling Plexity	No. of Enrichment Reactions	Total No. of Samples	Catalog No.
Nextera Rapid Capture Exome	1	8	8	FC-140-1000
Nextera Rapid Capture Exome	3	8	24	FC-140-1083
Nextera Rapid Capture Exome	6	8	48	FC-140-1086
Nextera Rapid Capture Exome	9	8	72	FC-140-1089
Nextera Rapid Capture Exome	12	2	24	FC-140-1001
Nextera Rapid Capture Exome	12	4	48	FC-140-1002
Nextera Rapid Capture Exome	12	8	96	FC-140-1003

Nextera Rapid Capture Expanded Exome

The Nextera Rapid Capture Expanded Exome delivers all-in-one library preparation and exome enrichment. This optimized exome delivers 62 Mb of genomic content, including expertly selected exonic content, UTRs, and miRNA. Add custom content with the Nextera Rapid Capture Custom Enrichment kit. Libraries prepared with Nextera Rapid Capture Exome are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	Pooling Plexity	No. of Enrichment Reactions	Total No. of Samples	Catalog No.
Nextera Rapid Capture Expanded Exome	12	2	24	FC-140-1004
Nextera Rapid Capture Expanded Exome	12	4	48	FC-140-1005
Nextera Rapid Capture Expanded Exome	12	8	96	FC-140-1006

Nextera Rapid Capture Custom Enrichment Kits

Nextera Rapid Capture Custom Enrichment Kits provide unparalleled access to genomic regions of interest. This custom assay allows researchers to sequence precious samples faster and more efficiently than ever before, using as little as 50 ng of input DNA. The kit offers add-on functionality to refine content over time, or add regions of unique interest to established panels like Nextera Rapid Capture Exome or TruSight content sets. Libraries prepared with the Nextera Rapid Capture Custom Enrichment Kits are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Samples	Catalog No.
Nextera Rapid Capture Custom Compatible with designs of 3,000–10,000 custom enrichment probes	48	FC-140-1007
Nextera Rapid Capture Custom Compatible with designs of 3,000–10,000 custom enrichment probes	96	FC-140-1008
Nextera Rapid Capture Custom Compatible with designs of 3,000–67,000 custom enrichment probes	288	FC-140-1009

For Research Use Only

Concierge service for your custom panel

A dedicated expert from design to delivery.

Custom library panel preparation projects might require special attention. Illumina Concierge supports all your needs–from target region design to functional performance evaluation and optimization to final shipment.

Illumina Concierge

Product Content Service

- Two levels of Concierge Service: design assistance and product optimization
- Thorough consultation of custom design needs from our design experts
- Optimized *in-silico* target coverage and minimized gaps
- Functional product performance improvements
- Coordination of ordering and shipping

	Design Assistance	Product Optimization
Dedicated design expert	●	●
Extended capabilities	●	●
Project management		●
Functional testing		●
Iterative product enhancements		●

For Research Use Only

Metagenomics

Sequence complex microbial and environmental samples to rapidly identify and track emerging diseases, decipher the composition of microbial communities, and contribute to taxonomic classification of microbial species.

Key strengths

- Enables bacteria identification and comparison within a mixed population sample
- Accelerates discovery of new SNP markers that translate to virulence or antibiotic resistance
- Promotes accurate evaluation of microbial biodiversity within a given sample
- Contributes to microbial phylogeny and taxonomy

LIBRARY PREP	SEQUENCING	ANALYSIS TOOLS
Illumina Demonstrated 16S protocol TruSeq DNA PCR-Free Sample Preparation Kit N TruSeq DNA Nano Sample Preparation Kit N Nextera DNA Sample Preparation Kit	MiSeq (150–384 samples) NextSeq 500 (384 samples)	**If analyzed on MiSeq:** Metagenomics 16S rRNA Workflow If analyzed in BaseSpace: Metagenomics 16S

The advent of NGS has enabled researchers to profile entire microbial communities quickly and easily in complex samples.

N NeoPrep version available 2015–see page 86

Visit www.illumina.com/metagenomics for more details.

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Phylogenetic and taxonomic microbiology studies

16S ribosomal RNA (rRNA) sequencing is a common amplicon sequencing method used to identify and compare bacteria present within a given sample. 16S rRNA gene sequencing is a well-established method for studying phylogeny and taxonomy of samples from complex microbiomes or environments that are difficult or impossible to study. Data from 16S studies are used to improve the sensitivity and specificity of taxonomic assignments, down to the species level.

Molecular genetics of microbes

Microbial whole-genome sequencing is an important tool for mapping genomes of novel organisms, finishing genomes of known organisms, or comparing genomes across multiple samples. Sequencing the entire microbial genome is important for generating accurate reference genomes, for microbial identification, and other comparative genomic studies.

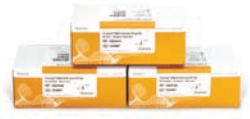
Environmental studies

Shotgun metagenomic sequencing allows researchers to comprehensively sample all genes in all organisms present in a given complex sample. The method enables microbiologists to evaluate bacterial diversity and detect the abundance of microbes in various environments. Shotgun metagenomics also provide a means to study unculturable microorganisms that are otherwise difficult or impossible to analyze.

New and emerging diseases

Metagenomics has become an increasingly important tool in helping to better understand human health, current and new emerging diseases, environmental systems, and the overall taxonomic classification of microbes.

STEP 1
Choose library prep method



Product	TruSeq DNA PCR-Free Sample Preparation Kit	TruSeq Nano DNA Sample Preparation Kit	Nextera DNA Sample Preparation Kit
Most important to me	Lowest bias to achieve highest coverage	Highest coverage from lowest DNA input quantity	Fastest assay time
Least important to me	Low DNA input quantity	Assay time	Genome coverage
Input quantity	1 µg	100 ng	1 ng
Assay time	5 hours	6 hours	90 minutes
Diversity	≥ 1 billion unique fragments	≥ 1 billion unique fragments	< 1 billion unique fragments
Genome coverage	Complete	Complete	Slight bias in coverage
Fragmentation process	Mechanical	Mechanical	Enzymatic

Comparisons within metagenomics sequencing portfolio.

STEP 2
Choose a sequencer


Product	MiSeq	NextSeq 500	
Description	Focused power Speed and simplicity for targeted and small genome sequencing	Flexible power Speed and simplicity for everyday genomics	
On-board informatics	●		
Flexibility in number of samples per run		●	
Metagenomic samples processed per run	384	384	
Run mode		Mid output	High output
Flow cells processed per run	1	1	1
Output range	0.3–15 Gb	20–39 Gb	30–120 Gb
Run time	5–55 hours	15–26 hours	12–30 hours
Reads per flow cell	25 million	130 million	400 million
Maximum read length	2 x 300 bp	2 x 150 bp	2 x 150 bp

www.illumina.com/metagenomics

For Research Use Only

STEP 3
Obtain results

MiSeq NextSeq 500

Align/Call variants

16S Metagenomics M B

- Performs taxonomic classification of 16S rRNA targeted amplicon reads
- Uses an Illumina-curated version of the Greengenes taxonomic database
- Provides interactive visualizations and raw classification output for pre-sample and aggregate analyses

B BaseSpace M MiSeq Reporter

For Research Use Only

Library prep ordering information

TruSeq DNA PCR-Free Sample Preparation Kit

TruSeq DNA PCR-Free Sample Preparation Kits provide simple, all-inclusive library preparation for whole-genome sequencing applications of various organisms, from bacteria to whole human genomes. The kits offer shortened gel-free workflows, the ability to sequence the most challenging regions, and the power to identify the greatest number of variants. Libraries prepared with TruSeq DNA PCR-Free Sample Preparation kits are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Samples	Catalog No.
TruSeq DNA PCR-Free LT Sample Preparation Kit Set A	24	FC-121-3001
TruSeq DNA PCR-Free LT Sample Preparation Kit Set B	24	FC-121-3002
TruSeq DNA PCR-Free HT Sample Preparation Kit	96	FC-121-3003

TruSeq Nano DNA Sample Preparation Kit

Preserve precious samples with the TruSeq DNA Sample Preparation Kit. Prepare sequencing libraries for low- or high-throughput studies from as little as 100 ng of input DNA in less than a day. Bead-based selection reduces the sample loss associated with gel-based selection. This kit is designed to match the ever-increasing read lengths of Illumina sequencing instruments and is compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, HiSeq 2500 System, and HiSeq X Ten System.

Ordering Information

Product	No. of Samples	Catalog No.
TruSeq Nano DNA LT Sample Preparation Kit Set A	24	FC-121-4001
TruSeq Nano DNA LT Sample Preparation Kit Set B	24	FC-121-4002
TruSeq Nano DNA HT Sample Preparation Kit	96	FC-121-4003

Nextera DNA Sample Preparation Kit

Generate sequencing-ready libraries in less than 90 minutes with less than 15 minutes of hands-on time. Nextera technology simultaneously fragments DNA and tags the fragments with sequencing adapters using standard lab equipment. The protocol requires only 50 ng of input DNA. Libraries prepared with Nextera kits are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Samples	Catalog No.
Nextera DNA Sample Preparation Kit	96	FC-121-1031
Nextera DNA Sample Preparation Kit	24	FC-121-1030
Nextera Index Kit (96 indexes)	384	FC-121-1012
Nextera Index Kit (24 indexes)	96	FC-121-1011
TruSeq Dual Index Sequencing Primer Kit, Single Read (single-use kit)		FC-121-1003
TruSeq Dual Index Sequencing Primer Kit, Paired-End Read (single-use kit)		PE-121-1003

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Illumina Demonstrated 16S Protocol

Metagenomic studies are commonly performed by analyzing the prokaryotic 16S ribosomal RNA gene (16S rRNA), which is approximately 1,500 bp long and contains 9 variable regions interspersed between conserved regions. Variable regions of 16S rRNA are frequently used in phylogenetic classifications such as genus or species in diverse microbial populations. The Illumina Demonstrated 16S Protocol describes a method for preparing libraries that allows researchers to sequence the variable V3 and V4 regions of the 16S rRNA gene. This protocol can also be used for sequencing other regions with different region-specific primers. The Illumina Demonstrated 16S Protocol is available at www.illumina.com/metagenomics. Sequencing libraries prepared with the Illumina Demonstrated 16S Protocol are compatible with the Illumina MiSeq Desktop Sequencer.

Ordering Information

Product	Catalog No.
Nextera XT Index Kit	FC-131-1001
	FC-131-1002
PhiX Control Kit v3	FC-110-3001

mRNA sequencing

Obtain a clear and complete view of the coding transcriptome to discover alternative transcripts, gene fusions, and allele-specific expression even from highly degraded samples.

Key strengths

- Enables efficient, comprehensive analysis of the coding transcriptome
- Delivers highly accurate quantification of gene and transcript abundance
- Captures both known and novel features
- Provides an established source of informative biomarkers for a range of phenotypes

LIBRARY PREP

TruSeq Stranded mRNA Library Preparation Kit N

TruSeq RNA Access Library Preparation Kit N

SEQUENCING

ATATTTT
GACTTCA
TATAAAA
ATATTTT

MiSeq
(mRNA-Seq: 1 sample)
(*Gene exp.: 2–3 samples)

NextSeq 500
(mRNA-Seq: 5–16 samples)
(*Gene exp.: 13–40 samples)

HiSeq 2500
(mRNA-Seq: 12–80 samples)
(*Gene Exp.: 30–200 samples)

* Gene Expression Profiling

ANALYSIS TOOLS

If analyzed in BaseSpace:

Align/Call variants:
RNA Express
TopHat Alignment

Count/Compare:
Cufflinks Assembly & DE
RNA Express

Visualize:
The Broad's IGV

Add biological context:
Genomatix Pathway
iPathway Guide

mRNA-Seq has provided new insights into a broad range of studies from complex disease to the ripening process of wine grapes.

N NeoPrep version available 2015–see page 86

Visit www.illumina.com/mRNA for more details.

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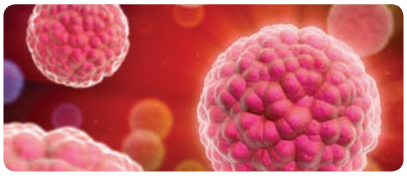


Cancer research

Monitoring cancer gene expression and transcriptome changes with RNA sequencing (RNA-Seq) can help answer research questions about disease classification and progression. Cancers accumulate numerous genetic changes, but typically only a few changes actually drive tumor progression.

Complex disease

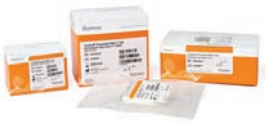
Differences in gene expression are linked to phenotypic variation between individuals. Expression quantitative trait loci (eQTLs) regulate mRNA expression levels, allowing researchers to effectively map expression levels to differences between individuals across the genome.

Agrigenomics

RNA sequencing is revolutionizing the exploration of gene expression in plants and animals, providing novel insights into changing expression levels that occur in development, and during disease and stress conditions. It can be used to elucidate gene and protein function and interactions, identify tissue-specific lists of RNA transcripts produced by an animal or plant genome (mRNAs, noncoding RNAs, and small RNAs), and for SNP discovery.

For Research Use Only

STEP 1
Choose library prep method



Product	TruSeq Stranded mRNA Sample Preparation Kit*	TruSeq RNA Access Library Prep Kit
Most important to me	Complete coding transcriptome with stranded information	Coding transcriptome from low input samples
Least important to me	FFPE compatibility	Lowest cost/sample
FFPE compatible	No	Yes
RNA input	0.1–1 µg high-quality total RNA 10–400 ng previously isolated mRNA	10 ng high-quality total RNA 20 ng degraded total RNA
Assay time	2 days	2.5 days
Stranded	Yes	Yes

* Nonstranded TruSeq RNA v2 still available. See Appendix.
Comparisons within mRNA sequencing portfolio.

STEP 2
Choose a sequencer

Product	MiSeq	NextSeq 500		HiSeq 2500	
Description	Focused power Speed and simplicity for targeted and small genome sequencing	Flexible power Speed and simplicity for everyday genomics		Production power Power and efficiency for large-scale genomics	
Process few samples at a time	●				
Flexibility in number of samples per run		●			
Maximize number of samples/run				●	
mRNA samples processed per run	mRNA-Seq: 1 sample Gene Expression Profiling: 2–3 samples	mRNA-Seq: 5–16 samples Gene Expression Profiling: 13–40 samples		mRNA-Seq:12–80 samples Gene Expression Profiling: 30–200 samples	
Run mode		Mid output	High output	Rapid run	High output
Flow cells processed per run	1	1	1	1 or 2	1 or 2
Output range	0.3–15 Gb	20–39 Gb	30–120 Gb	10–300 Gb	50–1,000 Gb
Run time	5–55 hours	15–26 hours	12–30 hours	7–60 hours	< 1–6 days
Reads per flow cell	25 million	130 million	400 million	300 million	2 billion
Maximum read length	2 x 300 bp	2 x 150 bp	2 x 150 bp	2 x 250 bp	2 x 125 bp

www.illumina.com/mRNA

For Research Use Only

STEP 3
Obtain results

Align/Call variants

RNA Express B
- Aligns RNA-Seq reads with the STAR aligner and assigns aligned reads to genes
- Performs differential gene expression with DESeq2

TopHat Alignment B
- Maps reads
- Performs abundance estimations of reference genes and transcripts
- Offers optional fusion calling
- Calls variants (CNVs and small indels)

Count/Compare

Cufflinks Assembly & Differential Expression (DE) B
- Assembles novel transcripts
- Performs differential expression of novel and reference transcripts

RNA Express B
- Aligns RNA-Seq reads with the STAR aligner and assigns aligned reads to genes
- Performs differential gene expression with DESeq2

Visualize

The Broad's IGV B
- Displays alignments and variants from multiple samples for performing complex variant analysis
- The Broad Institute of MIT and Harvard developed IGV, and Illumina modified it to display BaseSpace data

Add biological context

Genomatix Pathway B
- Associates single gene or list of genes with annotation data
- Offers full range of annotation data including (but not limited to) pathways, diseases, tissues, and small molecules

iPathway Guide B
- Offers differential expression gene analysis, drug interaction analysis, and disease analysis based on enrichment
- Offers coherent cascade analysis on pathways, gene ontology analysis, and more

B BaseSpace

For Research Use Only

Library prep ordering information

TruSeq Stranded mRNA Sample Preparation Kit

The TruSeq Stranded mRNA Sample Preparation Kit provides the clearest and most complete view of the transcriptome with a streamlined, cost-efficient, and scalable solution for mRNA analysis. The kit supports precise measurement of mRNA strand orientation for detection of antisense transcription, enhanced transcript annotation, and increased alignment efficiency. High coverage uniformity enhances the discovery of features such as alternative transcripts, gene fusions, and allele-specific expression. Libraries prepared with TruSeq Stranded mRNA Sample Preparation kits are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	Ribosomal Removal	Configuration	No. of Indexes	No. of Samples	Catalog No.
TruSeq Stranded mRNA Sample Preparation Kit	N/A	Set A	12	48	RS-122-2101
		Set B	12	48	RS-122-2102
		High throughput	96	96	RS-122-2103

TruSeq RNA Access Library Prep Kit

The TruSeq RNA Access Kit uses proven TruSeq Stranded RNA library prep chemistry combined with efficient sequence-specific captures to generate RNA-Seq libraries that focus on the RNA coding regions from degraded samples. TruSeq RNA Access kits require as little as 10 ng of input from intact RNA samples or 20 ng of input RNA from degraded samples. Libraries prepared with TruSeq RNA Access Kits are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Indexes	No. of Samples	Catalog No.
TruSeq RNA Access Library Prep Kit - Set A	12	48	RS-301-2001
TruSeq RNA Access Library Prep Kit - Set B	12	48	RS-301-2002

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Illumina Advantage

Streamline your workflow. Increase your productivity.

Illumina is committed to meeting the unique needs of the clinical research laboratory. Several key products now enhance research productivity and decrease the reverification costs of consumables used in clinical research.

For further information, contact your local account manager.

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Whole transcriptome sequencing

Identify the protein-coding and noncoding areas of the genome to explore the effects of changes in gene expression on phenotype.

Key strengths

- Delivers a complete view of the transcriptome, including both coding and multiple forms of noncoding RNA
- Quantifies gene and transcript abundance
- Captures both known and novel features
- Provides an established source of informative biomarkers for a range of phenotypes

LIBRARY PREP

TruSeq Stranded Total RNA Sample Prep Kit with Ribo-Zero™:
- Human/Mouse/Rat
- Gold
- Globin
- Plant

TruSeq Stranded mRNA N **paired with Ribo-Zero:**
- Epidemiology
- Bacteria
- Yeast

SEQUENCING

ATATTTT
GACTTCA
TATAAAA
ATATTTT

NextSeq 500
(3–10 samples)

HiSeq 2500
(8–96 samples)

ANALYSIS TOOLS

If analyzed in BaseSpace:

Align/Call variants:
RNA Express
TopHat Alignment

Count/Compare:
Cufflinks Assembly & DE
RNA Express

Visualize:
The Broad's IGV

Add biological context:
Genomatix Pathway
iPathway Guide

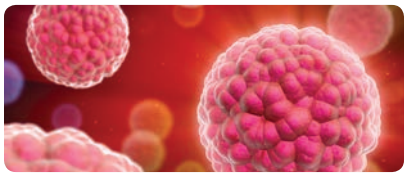
> "Having the deep sequencing coverage with RNA-Seq, we've discovered, and have been validating, tens of thousands of novel genes."
>
> **Christopher E. Mason, PhD,** Assistant Professor, Department of Physiology and Biophysics and the Institute for Computational Biomedicine, Weill Cornell Medical College

N NeoPrep version available 2015–see page 86

Visit www.illumina.com/wholeTranscriptome for more details.

For Research Use Only

Innovation. Discovery. Application.



Cancer research

Monitoring cancer gene expression and transcriptome changes with RNA-Seq can help answer research questions about disease classification and progression. Cancers accumulate numerous genetic changes, but typically only a few changes actually drive tumor progression.

Clinical research on blood samples

Whole-transcriptome analysis of blood-derived RNA requires the removal of two forms of abundant RNA—ribosomal RNA (both cytoplasmic and mitochondrial) as well as globin mRNA, which is present in high levels in whole blood. Traditional removal methods require two independent steps. This two-step process requires additional reagents, a longer workflow, and more input RNA is lost. TruSeq Stranded Total RNA with Ribo-Zero Globin leverages Ribo-Zero chemistry to efficiently remove both forms of abundant RNA in a single, rapid step.

Disease research

Formalin-fixed, paraffin-embedded (FFPE) samples are preserved tissue samples that are important to disease research. Many of these samples represent clinical outcomes, which could provide a potential gold mine of information when linked with underlying expression profiles. Because FFPE samples generally contain partially degraded RNA, transcription analysis can pose challenges and benefit from whole transcriptome solutions.

Agrigenomics

RNA sequencing is revolutionizing the exploration of gene expression in plants and animals, providing novel insights into changing expression levels that occur in development, and during disease and stress conditions. It can be used to elucidate gene and protein function and interactions, identify tissue-specific list of RNA transcripts produced by an animal or plant genome (mRNAs, noncoding RNAs, and small RNAs), and for SNP discovery.

STEP 1
Choose library prep method

Product	TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero	*+TruSeq Stranded mRNA Sample Preparation Kit paired with Ribo-Zero
Most important to me	Stranded information on complete transcriptome	Stranded information from bacteria, yeast, or disease samples
Least important to me	Use of bacteria, yeast, or disease samples	Fastest assay time
FFPE compatible	Yes	Yes
RNA input	0.1–1 µg	0.1–1 µg
Assay time	1.5 days	2 days
Multiplexing	12–96	12–96
Available organisms	Human, mouse, rat; blood; plant; bacteria	Disease samples, bacteria, yeast

* Nonstranded TruSeq RNA v2 still available.

\+ For bacterial, yeast, or disease transcriptome samples, Illumina recommends using the Ribo-Zero Magnetic Kit designed for your sample type in combination with the TruSeq stranded mRNA kits. Visit www.epicentre.com/ribozero for detailed information.

Comparisons within whole transcriptome sequencing portfolio.

STEP 2
Choose a sequencer

Product	NextSeq 500		HiSeq 2500	
Description	Flexible power Speed and simplicity for everyday genomics		Production power Power and efficiency for large-scale genomics	
Process fewer samples at a time	●			
Flexibility in number of samples per run			●	
Maximize number of samples/run	3–10		8–96	
Run mode	Mid output	High output	Rapid run	High output
Flow cells processed per run	1	1	1 or 2	1 or 2
Output range	20–39 Gb	30–120 Gb	10–300 Gb	50–1,000 Gb
Run time	15–26 hours	12–30 hours	7–60 hours	< 1–6 days
Reads per flow cell	130 million	400 million	300 million	2 billion
Maximum read length	2 x 150 bp	2 x 150 bp	2 x 250 bp	2 x 125 bp

www.illumina.com/wholeTranscriptome

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STEP 3
Obtain results

NextSeq 500 HiSeq 2500

Align/Call variants

RNA Express B
- Aligns RNA-Seq reads with the STAR aligner and assigns aligned reads to genes
- Performs differential gene expression with DESeq2

TopHat Alignment B
- Maps reads
- Performs abundance estimations of reference genes and transcripts
- Offers optional fusion calling
- Calls variants (CNVs and small indels)

Count/Compare

Cufflinks Assembly & Differential Expression (DE) B
- Assembles novel transcripts
- Performs differential expression of novel and reference transcripts

RNA Express B
- Aligns RNA-Seq reads with the STAR aligner and assigns aligned reads to genes
- Performs differential gene expression with DESeq2

Visualize

The Broad's IGV B
- Displays alignments and variants from multiple samples for performing complex variant analysis
- The Broad Institute of MIT and Harvard developed IGV, and Illumina modified it to display BaseSpace data

Add biological context

Genomatix Pathway B
- Associates single gene or list of genes with annotation data
- Offers full range of annotation data including (but not limited to) pathways, diseases, tissues, and small molecules

iPathway Guide B
- Offers differential expression gene analysis, drug interaction analysis, and disease analysis based on enrichment
- Offers coherent cascade analysis on pathways, gene ontology analysis, and more

B BaseSpace

For Research Use Only

Library prep ordering information

TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero

TruSeq Stranded Total RNA Sample Preparation kits provide the clearest and most complete view of the transcriptome with a streamlined, cost-efficient, and scalable solution for total RNA analysis. Compatible with a wide range of samples, including low-quality and FFPE, these kits combine the benefits of TruSeq RNA preparation kits with Ribo-Zero ribosomal RNA reduction chemistry. This combination allows analysis of coding and multiple forms of noncoding RNA with precise measurement of strand orientation, uniform coverage, and high-confidence discovery of features such as alternative transcripts, gene fusions, and allele-specific expression. Libraries prepared with the TruSeq Stranded Total RNA Sample Preparation kit are compatible with the Illumina NextSeq 500 System and HiSeq 2500 System.

Ordering Information

Product	Ribosomal Removal	Configuration	No. of Indexes	No. of Samples	Catalog No.
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Human/Mouse/Rat	Cytoplasmic ribosomal RNA	Set A	12	48	RS-122-2201
		Set B	12	48	RS-122-2202
		High throughput	96	96	RS-122-2203
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Gold	Cytoplasmic and mitochondrial ribosomal RNA	Set A	12	48	RS-122-2301
		Set B	12	48	RS-122-2302
		High throughput	96	96	RS-122-2303
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Globin	Cytoplasmic and mitochondrial ribosomal RNA	Set A	12	48	RS-122-2501
		Set B	12	48	RS-122-2502
		High throughput	96	96	RS-122-2503
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Plant	Cytoplasmic, mitochondrial, and chloroplast ribosomal RNA	Set A	12	48	RS-122-2401
		Set B	12	48	RS-122-2402
		High throughput	96	96	RS-122-2503

TruSeq Stranded mRNA Sample Preparation Kit

The TruSeq Stranded mRNA Sample Preparation Kit provides the clearest and most complete view of the transcriptome with a streamlined, cost-efficient, and scalable solution for mRNA analysis. The kit supports precise measurement of mRNA strand orientation for detection of antisense transcription, enhanced transcript annotation, and increased alignment efficiency. High coverage uniformity enhances the discovery of features such as alternative transcripts, gene fusions, and allele-specific expression. Libraries prepared with TruSeq Stranded mRNA Sample Preparation kit are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	Ribosomal Removal	Configuration	No. of Indexes	No. of Samples	Catalog No.
TruSeq Stranded mRNA Sample Preparation Kit	N/A	Set A	12	48	RS-122-2101
		Set B	12	48	RS-122-2102
		High throughput	96	96	RS-122-2103

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Ribo-Zero Magnetic Gold Kit (Epidemiology)

Sequencing is an important tool of discovery for research into human health, disease, development, and more. Add Ribo-Zero to the TruSeq Stranded mRNA library prep workflow to get the most informative sequencing results by removing unwanted ribosomal RNA (rRNA) from the organisms of interest before sequencing.

The Ribo-Zero Magnetic Gold Kit (Epidemiology) effectively removes cytoplasmic and mitochondrial rRNA, including human mitochondrial RNA, from complex samples composed of human, mouse, or rat, and Gram-positive and Gram-negative bacterial RNA. Ribo-Zero Magnetic Gold (Epidemiology) recovers all fragments of human and bacterial non-ribosomal RNA from intact or degraded samples and is applicable to a broad spectrum of bacteria.

Ordering Information

Product	Ribosomal Removal	No. of Reactions	Catalog No.
Ribo-Zero Magnetic Gold Kit (Epidemiology)	Cytoplasmic and mitochondrial	6	MRZE706
		24	MRZE724
		24	MRZY1324

Ribo-Zero Magnetic Kit (Bacteria)

The Ribo-Zero Magnetic Kit (Bacteria) removes ribosomal RNA and cytoplasmic (nuclear-encoded) rRNAs from intact and partially degraded mixtures of Gram-positive and Gram-negative bacteria in a single pass. The sequencing data from samples treated with Ribo-Zero (Bacteria) contain the complete transcriptome of coding and noncoding RNA species, focusing on valuable RNA species leading to the discovery of relevant genes, splice variants, and isoforms.

Ordering Information

Product	Ribosomal Removal	No. of Reactions	Catalog No.
Ribo-Zero Magnetic Kit (Bacteria)	Cytoplasmic	6	MRZMB126
		24	MRZB12424

Ribo-Zero Magnetic Gold Kit (Yeast)

The Ribo-Zero Magnetic Gold Kit (Yeast) is optimized to deplete ribosomal RNA, including cytoplasmic and mitochondrial rRNA, *S. cerevisiae* and *S. pombe*, and might be compatible with other yeast and fungal organisms. Add Ribo-Zero Magnetic Gold (Yeast) to the TruSeq Stranded mRNA library prep workflow to obtain the most informative sequencing results by removing unwanted rRNA before sequencing. Focused sequencing data allow you to characterize novel and low abundance transcripts from the complex yeast transcriptome.

Ordering Information

Product	Ribosomal Removal	No. of Reactions	Catalog No.
Ribo-Zero Magnetic Gold Kit (Yeast)	Cytoplasmic and mitochondrial	6	MRZY1306
		24	MRZY1324

FFPE RNA sequencing

Measure gene expression at the transcript level and discover or validate gene fusions with RNA-Seq of FFPE samples.

Key strengths

- Offers optimized workflows and technologies for FFPE samples
- Derives high-quality genomic data and insight from previously inaccessible samples
- Identifies fusion genes, a critical class of somatic driver mutations, in tumor cells
- Detects and quantifies transcripts, even at low levels of expression, for biomarker development

LIBRARY PREP

TruSeq Stranded Total RNA Sample Prep Kit with Ribo-Zero:
- Human/Mouse/Rat
- Gold
- Globin
- Plant

TruSeq RNA Access N **Library Prep Kit**

TruSeq Targeted RNA Expression Kits

SEQUENCING

ATATTTT
GACTTCA
TATAAAA
ATATTTT

NextSeq 500
(*Whole Trans.: 1–4 samples)
(mRNA: 5–16 samples)
(Targeted: 384 samples)

HiSeq 2500
(*Whole Trans.: 3–20 samples)
(mRNA: 12–80 samples)
(Targeted: 6144 samples)

* Whole transcriptome

ANALYSIS TOOLS

If analyzed in BaseSpace:
Align/Call variants:
RNA Express
TopHat Alignment

Count/Compare:
Cufflinks Assembly & DE
RNA Express

Visualize:
The Broad's IGV

Add biological context:
Genomatix Pathway
iPathway Guide

FFPE samples offer a wealth of information that has been difficult to access historically.

N NeoPrep version available 2015–see page 86

Visit www.illumina.com/FFPErna for more details.

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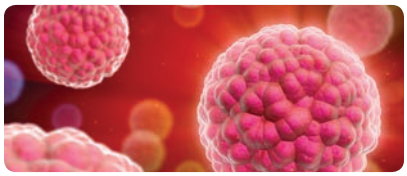
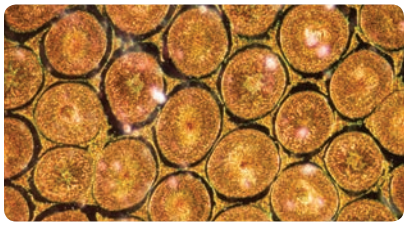


Fixed tumor analysis

Tumor biopsies are commonly FFPE. FFPE RNA sequencing methods allow for complete transcriptome analysis of these samples to enable prognostic or diagnostic assessment, as well as tumor typing from as little as 20 ng of RNA.



Gene expression analysis of archival samples

FFPE tissue samples represent an enormous repository of valuable data that has traditionally been very difficult to access. FFPE RNA sequencing methods can be used to analyze gene expression profiles from these difficult samples, enabling functional annotation of archival samples and biomarker discovery at a whole transcriptome or targeted level.

Fusion gene detection

Gene fusions or translocations are among the most common variants associated with cancer. FFPE RNA sequencing solutions efficiently detect and validate expressed fusion genes in fixed tissue.

Coding SNP analysis

By analyzing only the single nucleotide polymorphisms (SNPs) that are expressed in a particular sample, researchers can focus their study on just the variants that are likely to be causative. FFPE RNA sequencing methods enable comprehensive detection of expressed SNPs.

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STEP 1
Choose library prep method

Product	TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero	TruSeq RNA Access Library Prep Kit	TruSeq Targeted RNA Expression Panel Kits
Most important to me	Transcriptome coverage (coding + noncoding)	Cost/sample, coding coverage	Quantitative information
Least important to me	Lowest input	Noncoding RNA coverage	Discovery power
Input quantity	100 ng	10 ng	50 ng
Assay time	1.5 days	2.5 days	1.5 days
Multiplexing	96	24	384

Comparisons within FFPE RNA sequencing portfolio.

STEP 2
Choose a sequencer

Product	NextSeq 500		HiSeq 2500	
Description	Flexible power Speed and simplicity for everyday genomics		Production power Power and efficiency for large-scale genomics	
Maximize number of samples/run	●			
Flexibility in number of samples per run			●	
Run mode	Mid output	High output	Rapid run	High output
Flow cells processed per run	1	1	1 or 2	1 or 2
Output range	20–39 Gb	30–120 Gb	10–300 Gb	50–1,000 Gb
Run time	15–26 hours	12–30 hours	7–60 hours	< 1–6 days
Reads per flow cell	130 million	400 million	300 million	2 billion
Maximum read length	2 x 150 bp	2 x 150 bp	2 x 250 bp	2 x 125 bp
Number of panels processed per run				
Whole transcriptome	1	4	3	20
mRNA	5	16	12	80
***Targeted RNA**	384	384	6144	6144

* Based on TruSeq indexing and up to 65 targets for MiSeq, 1041 targets for NextSeq, and 651 targets/lane for HiSeq

www.illumina.com/FFPErna

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STEP 3
Obtain results


NextSeq 500


HiSeq 2500

Align/Call variants

RNA Express B
- Aligns RNA-Seq reads with the STAR aligner and assigns aligned reads to genes
- Performs differential gene expression with DESeq2

TopHat Alignment B
- Maps reads
- Performs abundance estimations of reference genes and transcripts
- Offers optional fusion calling
- Calls variants (CNVs and small indels)

Count/Compare

Cufflinks Assembly & Differential Expression (DE) B
- Assembles novel transcripts
- Performs differential expression of novel and reference transcripts

RNA Express B
- Aligns RNA-Seq reads with the STAR aligner and assigns aligned reads to genes
- Performs differential gene expression with DESeq2

Visualize

The Broad's IGV B
- Displays alignments and variants from multiple samples for performing complex variant analysis
- The Broad Institute of MIT and Harvard developed IGV, and Illumina modified it to display BaseSpace data

Add biological context

Genomatix Pathway B
- Associates single gene or list of genes with annotation data
- Offers full range of annotation data including (but not limited to) pathways, diseases, tissues, and small molecules

iPathway Guide B
- Offers differential expression gene analysis, drug interaction analysis, and disease analysis based on enrichment
- Pathway analysis with impact analysis modeled on KEGG pathways and more

B BaseSpace

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Library prep ordering information

TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero

TruSeq Stranded Total RNA Sample Preparation kits provide the clearest and most complete view of the transcriptome with a streamlined, cost-efficient, and scalable solution for total RNA analysis. Compatible with a wide range of samples, including low-quality and FFPE, these kits combine the benefits of TruSeq RNA preparation kits with Ribo-Zero ribosomal RNA reduction chemistry. This combination allows analysis of coding and multiple forms of noncoding RNA with precise measurement of strand orientation, uniform coverage, and high-confidence discovery of features such as alternative transcripts, gene fusions, and allele-specific expression. Libraries prepared with the TruSeq Stranded Total RNA Sample Preparation kit are compatible with the Illumina NextSeq 500 System and HiSeq 2500 System.

Ordering Information

Product	Ribosomal Removal	Configuration	No. of Indexes	No. of Samples	Catalog No.
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Human/Mouse/Rat	Cytoplasmic ribosomal RNA	Set A	12	48	RS-122-2201
		Set B	12	48	RS-122-2202
		High throughput	96	96	RS-122-2203
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Gold	Cytoplasmic and mitochondrial ribosomal RNA	Set A	12	48	RS-122-2301
		Set B	12	48	RS-122-2302
		High throughput	96	96	RS-122-2303
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Globin	Cytoplasmic and mitochondrial ribosomal RNA	Set A	12	48	RS-122-2501
		Set B	12	48	RS-122-2502
		High throughput	96	96	RS-122-2503
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Plant	Cytoplasmic, mitochondrial, and chloroplast ribosomal RNA	Set A	12	48	RS-122-2401
		Set B	12	48	RS-122-2402
		High throughput	96	96	RS-122-2503

TruSeq RNA Access Library Prep Kit

The TruSeq RNA Access Kit uses proven TruSeq Stranded RNA library prep chemistry combined with efficient sequence-specific captures to generate RNA-Seq libraries that focus on the RNA coding regions from degraded samples. TruSeq RNA Access kits require as little as 10 ng of input from intact RNA samples or 20 ng of input RNA from degraded samples. Libraries prepared with TruSeq RNA Access Kit are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Indexes	No. of Samples	Catalog No.
TruSeq RNA Access Library Prep Kit - Set A	12	48	RS-301-2001
TruSeq RNA Access Library Prep Kit - Set B	12	48	RS-301-2002

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TruSeq Targeted RNA Expression Kits

TruSeq Targeted RNA Expression Kits offer highly customizable mid- to high-plex gene expression profiling. TruSeq Targeted RNA overcomes significant challenges in cost and workflow. These assays deliver a fully integrated solution, including convenient online panel design and ordering, a rapid and simple workflow starting with as little as 50 ng of RNA, and automated on-instrument data analysis. User-defined panels of 12–1,000 assays can be created to target genes, isoforms, splice junctions, cSNPs, and fusion genes with the ability to multiplex up to 384 samples. Libraries prepared with TruSeq Targeted RNA Expression Custom Components are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product Name	No. of Samples	Catalog No.
TruSeq Targeted RNA Expression Custom Components		
TruSeq Targeted RNA Custom Kit	48	RT-101-1001
	96	RT-102-1001
TruSeq Targeted RNA Supplemental Content	48	RT-801-1001
	96	RT-802-1001
TruSeq Targeted RNA Expression Fixed Panel Kits		
TruSeq Targeted RNA Apoptosis Panel Kit	48	RT-201-1010
	96	RT-202-1010
TruSeq Targeted RNA Cardiotoxicity Panel Kit	48	RT-201-1009
	96	RT-202-1009
TruSeq Targeted RNA Cell Cycle Panel Kit	48	RT-201-1003
	96	RT-202-1003
TruSeq Targeted RNA Cytochrome p450 Panel Kit	48	RT-201-1006
	96	RT-202-1006
TruSeq Targeted RNA Hedgehog Panel Kit	48	RT-201-1002
	96	RT-202-1002
TruSeq Targeted RNA Neurodegeneration Panel Kit	48	RT-201-1001
	96	RT-202-1001
TruSeq Targeted RNA NFKB Panel Kit	48	RT-201-1008
	96	RT-202-1008
TruSeq Targeted RNA Stem Cell Panel Kit	48	RT-201-1005
	96	RT-202-1005
TruSeq Targeted RNA TP53 Pathway Panel Kit	48	RT-201-1007
	96	RT-202-1007
TruSeq Targeted RNA Wnt Pathway Panel Kit	48	RT-201-1004
	96	RT-202-1004
TruSeq Targeted RNA Expression Index Kits		
TruSeq Targeted RNA Index Kit	48	RT-401-1001
TruSeq Targeted RNA Index Kit A	96	RT-402-1001
TruSeq Targeted RNA Index Kit B	96	RT-402-1002
TruSeq Targeted RNA Index Kit C	96	RT-402-1003
TruSeq Targeted RNA Index Kit D	96	RT-402-1004

Single-cell RNA sequencing

Determine the relationship between single cells and their environments with deep transcriptome sequencing.

Key strengths

- Enables exploration of hereditary information at the most basic level of biological organization
- Identifies intercellular differences previously masked by bulk sampling methods
- Increases understanding of subpopulation responses to environmental cues

LIBRARY PREP

Amplify single cell cDNA for use with:
Nextera XT DNA Sample Preparation Kit

SEQUENCING

ATATTTT
GACTTCA
TATAAAA
ATATTTT

NextSeq 500
*(130–400 mRNA samples)
+(20–100 Whole Transcriptome Samples)

HiSeq 2500
*(300–2000 mRNA samples)
+(60–400 Whole Transcriptome Samples)

ANALYSIS TOOLS

If analyzed in BaseSpace:
Align/Call variants:
RNA Express
TopHat Alignment

Count/Compare:
Cufflinks Assembly & DE
RNA Express

Visualize:
The Broad's IGV

Add biological context:
Genomatix Pathway
iPathway Guide

* Assumes 1M reads per cell
+ Assumes 5M reads per cell

Tumors commonly contain several clonal populations that reflect the ongoing accumulation of mutations. Single-cell genomic methods have the capacity to resolve these complex mixtures of cells.

Visit www.illumina.com/singleCell for more details.

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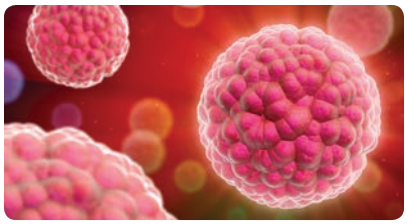
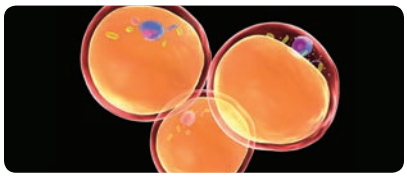


Cancer research

Monitoring cancer gene expression and transcriptome changes with RNA sequencing (RNA-Seq) can help answer research questions about disease classification and progression. Cancers accumulate numerous genetic changes, but typically only a few changes actually drive tumor progression. Evaluating transcriptome profile differences in different areas of a tumor enhances understanding of relapse and metastasis.



Cell biology

Organs consist of myriad phenotypically distinct cells. Single-cell transcriptomics can characterize the function of each of these cell types.

Library prep ordering information

Nextera XT DNA Sample Preparation Kit

Generate sequencing-ready libraries from small genomes (bacteria, archaea, viruses), amplicons, and plasmids in less than 90 minutes with only 15 minutes of hands-on time. Nextera XT simultaneously fragments input DNA and tags the fragments with sequencing adapters in a single-tube enzymatic reaction. Nextera XT requires as little as 1 ng of input, supporting a wide array of sample types. Bead-based normalization eliminates the need for library quantification before pooling and sequencing. Libraries prepared with Nextera XT kits are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Indexes	No. of Samples	Catalog No.
Nextera XT DNA Sample Preparation Kit		24	FC-131-1024
Nextera XT DNA Sample Preparation Kit		96	FC-131-1096
Nextera XT Index Kit	24	96	FC-131-1001
Nextera XT Index Kit	96	384	FC-131-1002
TruSeq Dual Index Sequencing Primer Kit, Single Read	single-use kit*		FC-121-1003
TruSeq Dual Index Sequencing Primer Kit, Paired-End Read	single-use kit*		PE-121-1003
Nextera XT Index Kit v2, Set A	96	384	FC-131-2001
Nextera XT Index Kit v2, Set B	96	384	FC-131-2002
Nextera XT Index Kit v2, Set C	96	384	FC-131-2003
Nextera XT Index Kit v2, Set D	96	384	FC-131-2004

*Sequencing primer kits are required for all sequencers except the MiSeq System.

Targeted RNA sequencing

Design targeted RNA expression panels to focus on RNA sequences of interest or add custom content to fully optimized and experimentally validated panels that focused on specific cell pathways and disease states.

Key strengths

- Offers a highly accurate and specific method for measuring transcripts of interest
- Utilizes qualitative and quantitative information to allow differential expression analysis, allele-specific expression measurement, and fusion gene verification
- Measures dozens to thousands of targets simultaneously
- Is compatible with low-quality or FFPE derived RNA samples

LIBRARY PREP

TruSeq Targeted RNA Expression Kits

See below for complete list.

SEQUENCING

ATATTTT
GACTTCA
TATAAAA
ATATTTT

MiSeq
*(384 samples)

NextSeq 500
*(384 samples)

HiSeq 2500
*(6,144 samples)

ANALYSIS TOOLS

If analyzed on MiSeq:
Targeted RNA Workflow

BaseSpace analysis tools coming soon.

Visit www.illumina.com/informatics for current availability.

* Based on TruSeq indexing and up to 65 targets for MiSeq, 1041 targets for NextSeq 500, and 651 targets/lane for HiSeq

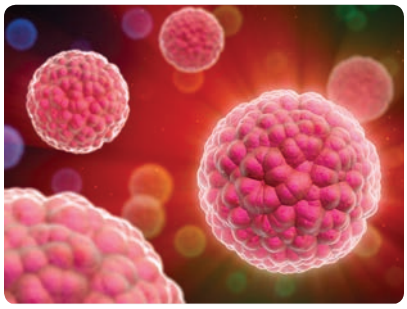
TruSeq Targeted RNA Expression Kits

TruSeq Targeted RNA Custom Kit
TruSeq Targeted RNA Apoptosis Panel Kit
TruSeq Targeted RNA Cardiotoxicity Panel Kit
TruSeq Targeted RNA Cell Cycle Panel Kit
TruSeq Targeted RNA Cytochrome p450 Panel Kit
TruSeq Targeted RNA Hedgehog Panel Kit
TruSeq Targeted RNA Neurodegeneration Panel Kit
TruSeq Targeted RNA NFkB Panel Kit
TruSeq Targeted RNA Stem Cell Panel Kit
TruSeq Targeted RNA TP53 Panel Kit
TruSeq Targeted RNA WNT Pathway Panel Kit

Visit www.illumina.com/targetedExpression for more details.

For Research Use Only

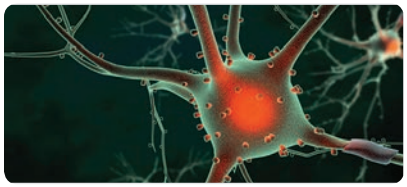
Innovation. Discovery. Application.



Cancer research

To analyze expression of specific cancer genes, TruSeq Targeted RNA Expression provides an ideal solution. Researchers can:

- Choose a panel of assays to study cancer pathways of interest.
- Select gene regions of interest from a comprehensive custom set of validated assays.
- Combine these options, adding content to Illumina-validated panels to create custom panels.
- TruSeq Targeted RNA Expression Kits are designed for the MiSeq system, which has built-in analysis software for the quantitation and visualization of gene expression transcripts.



Neuroscience

The TruSeq Targeted RNA Expression Neurodegeneration Panel is a predesigned gene-expression profiling solution for studying neurodegenerative and neurotoxic pathways such as those implicated in Alzheimer's Disease. The panel targets 77 genes involved in these pathways. All TruSeq Targeted RNA fixed panels are experimentally validated to ensure strong correlation with RNA sequencing.

For Research Use Only

STEP 3
Obtain results

Align/Call variants

Targeted RNA Workflow: M

- Enables analysis of custom and fixed panels utilizing TruSeq Targeted RNA Expression built in the Illumina DesignStudio
- Detects specific transcript isoforms and gene fusions and reports relative expression values

M MiSeq Reporter

For Research Use Only

Library prep ordering information

TruSeq Targeted RNA Expression Custom Components

TruSeq Targeted RNA Expression Kits offer highly customizable mid- to high-plex gene expression profiling and validation. TruSeq Targeted RNA overcomes significant challenges in cost and workflow. These assays deliver a fully integrated solution, including convenient online panel design and ordering, a rapid and simple workflow starting with as little as 50 ng of RNA, and automated on-instrument data analysis. User-defined panels of 12–1,000 assays can be created to target genes, isoforms, splice junctions, cSNPs, and fusion genes with the ability to multiplex up to 384 samples. Libraries prepared with TruSeq Targeted RNA Expression Custom Components are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product Name	No. of Samples	Catalog No.
TruSeq Targeted RNA Expression Custom Components		
TruSeq Targeted RNA Custom Kit	48	RT-101-1001
	96	RT-102-1001
TruSeq Targeted RNA Supplemental Content	48	RT-801-1001
	96	RT-802-1001
TruSeq Targeted RNA Expression Fixed Panel Kits		
TruSeq Targeted RNA Apoptosis Panel Kit	48	RT-201-1010
	96	RT-202-1010
TruSeq Targeted RNA Cardiotoxicity Panel Kit	48	RT-201-1009
	96	RT-202-1009
TruSeq Targeted RNA Cell Cycle Panel Kit	48	RT-201-1003
	96	RT-202-1003
TruSeq Targeted RNA Cytochrome p450 Panel Kit	48	RT-201-1006
	96	RT-202-1006
TruSeq Targeted RNA Hedgehog Panel Kit	48	RT-201-1002
	96	RT-202-1002
TruSeq Targeted RNA Neurodegeneration Panel Kit	48	RT-201-1001
	96	RT-202-1001
TruSeq Targeted RNA NFKB Panel Kit	48	RT-201-1008
	96	RT-202-1008
TruSeq Targeted RNA Stem Cell Panel Kit	48	RT-201-1005
	96	RT-202-1005
TruSeq Targeted RNA TP53 Pathway Panel Kit	48	RT-201-1007
	96	RT-202-1007
TruSeq Targeted RNA Wnt Pathway Panel Kit	48	RT-201-1004
	96	RT-202-1004
TruSeq Targeted RNA Expression Index Kits		
TruSeq Targeted RNA Index Kit	48	RT-401-1001
TruSeq Targeted RNA Index Kit A	96	RT-402-1001
TruSeq Targeted RNA Index Kit B	96	RT-402-1002
TruSeq Targeted RNA Index Kit C	96	RT-402-1003
TruSeq Targeted RNA Index Kit D	96	RT-402-1004

Ribosome profile sequencing

Use deep sequencing of ribosome-protected mRNA fragments to understand ribosomal activity and protein translation at a specific point in time.

Key strengths

- Simplifies translational control investigation
- Measures gene expression
- Identifies translation start sites
- Predicts protein abundance
- Enables translational and co-translational process investigation *in vivo*

LIBRARY PREP

ARTseq Ribosome Profiling Kit-Yeast

ARTseq Ribosome Profiling Kit-Mammalian

SEQUENCING

ATATTTT
GACTTCA
TATAAAA
ATATTTT

MiSeq
(1–2 samples)

NextSeq 500
(7–20 samples)

HiSeq 2500
(15–100 samples)

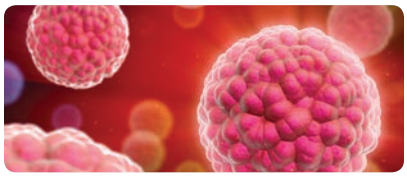
ANALYSIS TOOLS

BaseSpace analysis tools coming soon.

Visit www.illumina.com/informatics for current availability.

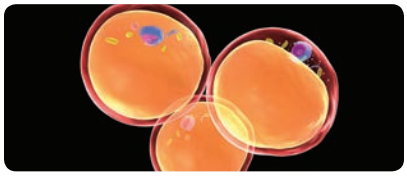
> "Ribosome profiling captures protein abundance information unavailable to mRNA measurements."
>
> **Nicholas Ingolia,** PhD, Carnegie Institution for Science

Innovation. Discovery. Application.



Cancer research

Cell-signaling pathways are commonly deregulated in human cancers. Ribosome profiling has discovered specialized translation of the prostate cancer genome by oncogenic mTOR signaling.*



Cell biology

Ribosome profiling is a powerful technique for investigating translational control (ie, regulation of gene expression). This application bridges the gap from genomics/transcriptomics to proteomics by providing an estimate of protein abundance and translational regulation, thus enhancing mRNA abundance information learned from RNA-Seq.

*Hsieh AC, Liu Y, Edlind MP, *et al*. The translational landscape of mTOR signaling steers cancer initiation and metastasis. Nature. 2012;485(7396):55–61.

Library prep ordering information

ARTseq Ribosome Profiling Kits

The ARTSeq Kit produces RNA-Seq libraries from ribosome–protected mRNA to investigate translational control, measure gene expression, identify translation start sites, predict protein abundance, and investigate translational and co-translational processes *in vivo*. ARTseq Ribosome Profiling Kits are compatible with the Illumina MiSeq Desktop Sequencer, NextSeq 500 System, and HiSeq 2500 System.

Ordering Information

Product	No. of Samples	Catalog No.
ARTseq Ribosome Profiling Kit (Yeast)	12	RPYSC12116
ARTseq Ribosome Profiling Kit (Mammalian)	12	RPHMR12126
Ribo-Zero Magnetic Kit (Human/Mouse/Rat)	24	MRZH11124
Ribo-Zero Gold Kit (Human/Mouse/Rat)	24	MRZG12324
Ribo-Zero Gold Kit (Human/Mouse/Rat)	6	MRZG126
Ribo-Zero Magnetic Gold Kit (Yeast)	24	MRZY1324

Note: Order ARTseq Ribosome Profiling Kits from www.epicentre.com.

Visit www.illumina.com/ribosomeProfiling for more details.

For Research Use Only

MicroRNA sequencing

Generate small RNA libraries directly from total RNA to understand the role of noncoding RNA in RNA silencing and post-transcriptional control of gene expression.

Key strengths

- Examine gene regulation at the transcriptional and post-transcriptional levels
- Query thousands of small RNA sequences with unprecedented sensitivity and dynamic range for both small RNA discovery and profiling applications
- Analyze any small RNA or miRNA without prior sequence or secondary structure information
- Investigate any small RNA between 17 and 35 nucleotides in length

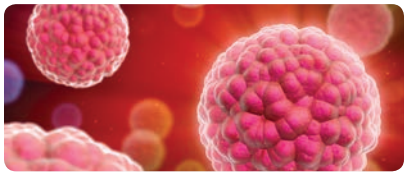
LIBRARY PREP	SEQUENCING	ANALYSIS TOOLS
TruSeq Small RNA Sample Preparation Kit	MiSeq (1–5 samples) NextSeq 500 (25–80 samples) HiSeq 2500 (60–792 samples)	**If analyzed on MiSeq:** Small RNA Workflow **BaseSpace analysis tools coming soon.** Visit www.illumina.com/informatics for current availability.

Query thousands of small RNA sequences with unprecedented sensitivity and dynamic range for both small RNA discovery and profiling applications.

www.illumina.com/miRNA for more details.

For Research Use Only

Innovation. Discovery. Application



Cancer research

Small RNA-Seq enables the discovery and profiling of miRNAs and other small noncoding RNAs present in the cancer transcriptome, at extremely high coverage. Analyze precious tumor samples with high sensitivity to detect mutation levels as low as a single copy per cell.

Complex disease

miRNAs have unique expression profiles in cells of the innate and adaptive immune systems, CNS, and a wide range of diseases, from cancer to psychiatric disorders. Improvements in the depth and sensitivity of high-throughput sequencing technologies allow researchers to profile known and novel miRNAs, and identify their exact sequence and length, which provides insights on RNA editing processes and mutational events.

Library prep ordering information

TruSeq Small RNA Sample Preparation Kit

TruSeq Small RNA Sample Preparation Kits provide a simple, cost-effective solution for generating small RNA libraries directly from total RNA.

Ordering Information

Product	No. of Indexes	Catalog No.
TruSeq Small RNA Sample Preparation Kit	12	RS-200-0012
TruSeq Small RNA Sample Preparation Kit	13–24	RS-200-0024
TruSeq Small RNA Sample Preparation Kit	25–36	RS-200-0036
TruSeq Small RNA Sample Preparation Kit	37–48	RS-200-0048

For Research Use Only

NeoPrep Library Prep System

Library prep reimagined.

Available in 2015, the entirely self-contained NeoPrep System features the precision of digital microfluidics and unparalleled ease of use to reduce your hands-on time and deliver reproducible, high-quality libraries, even with limited starting material.

Sequencing-ready libraries with unrivaled simplicity

With just a few simple steps, the NeoPrep System frees you from the burden of preparing, quantifying, and normalizing DNA and RNA libraries.

- 30 minutes of hands-on time
- 16 sequencing-ready libraries in one run
- High reproducibility
- Compatibility with all Illumina sequencing systems

Kits for the NeoPrep System will include assays from the TruSeq and Nextera portfolios from Illumina, including:

- TruSeq DNA PCR-Free
- TruSeq Nano DNA
- TruSeq Stranded mRNA
- TruSeq RNA Access

NEW! NeoPrep Library Prep System

Stay updated on the latest NeoPrep System developments at www.illumina.com/neoprep.

For Research Use Only

High-throughput library prep automation

Partner-developed and Illumina qualified.

Library prep kits from Illumina can be automated on most general-purpose liquid handling robots. Illumina facilitates kit automation by partnering with leading automation vendors to develop methods for high-throughput library prep.

The resulting Illumina-qualified methods significantly decrease time and expense of method development and optimization, enabling rapid scaling for higher throughput.

	Beckman Coulter	Eppendorf	Hamilton Robotics	PerkinElmer	Tecan
TruSeq Stranded Total RNA Sample Preparation Kit	✔	✔	✔	✔	
TruSeq Stranded mRNA Library Preparation Kit	✔	✔	✔	✔	✔
TruSeq RNA Access Library Preparation Kit			●		
TruSeq Nano DNA Sample Preparation Kit	●				●
Nextera Rapid Capture Exome	●			●	✔
Nextera XT DNA Sample Preparation Kit	●				

✔ Illumina qualified indicates that our analysis has shown that libraries prepared with the method perform comparably to libraries prepared manually.

● Method is available through an automation partner, and is not Illumina qualified.

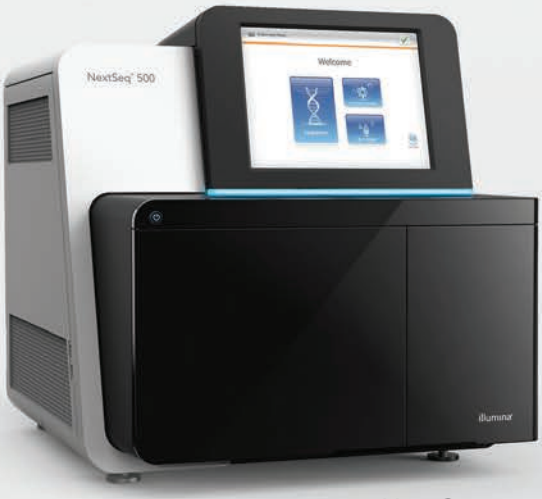
Visit the automation partners web page for the most up-to-date information on available methods at: www.illumina.com/HTautomation.

Contact the automation vendor directly for information on availability, installation, and support, including user guides and application notes.

Visit www.illumina.com/HTautomation for more details.

Instruments

HiSeq X
Population power.



NextSeq 500
Flexible power.

For Research Use Only

HiSeq 2500
Production power.

MiSeqDx
Focused Dx power.

MiSeq
Focused power.

For Research Use Only

Accessible sequencing solutions

Power tailored for every researcher, application, and scale of study.

Product	MiSeq	NextSeq 500	
Description	**Focused power** Speed and simplicity for targeted and small-genome sequencing	**Flexible power** Speed and simplicity for everyday genomics	
Key applications	Small genome, amplicon-targeted gene panel sequencing	Exome, transcriptome, genome sequencing, and more	
Run mode		Mid output	High output
Flow cells processed per run	1	1	1
Output range	0.3–15 Gb	20–39 Gb	30–120 Gb
Run time	5–55 hours	15–26 hours	12–30 hours
Reads per flow cell†	25 million‡	130 million	400 million
Maximum read length	2 x 300 bp‡	2 x 150 bp	2 x 150 bp

* Specifications shown for an individual HiSeq X System. HiSeq X is only available as part of the HiSeq X Ten.

† Clusters passing filter.

‡ For MiSeq V3 Kits only.

HiSeq 2500		HiSeq X Ten*
Production power Power and efficiency for large-scale genomics		**Population power** $1,000 human genome and extreme throughput for population-scale sequencing
Production-scale genome, exome, and transcriptome sequencing		Population-scale whole human genome sequencing
Rapid Run	High output	
1 or 2	1 or 2	1 or 2
10–180 Gb	50–1,000 Gb	1.6–1.8 Tb
7–40 hours	< 1 day–6 days	< 3 days
300 million	2 billion	3 billion
2 x 150 bp	2 x 125 bp	2 x 150 bp

MiSeq/MiSeqDx

Focused power. Speed and simplicity for targeted and small-genome sequencing.

MiSeq System

Focused power

Combining speed, high-quality data, and the longest read lengths, the MiSeq System is ideal for sequencing targeted panels, amplicons, and small genomes. It is a cost-effective alternative to capillary electrophoresis applications, and can perform rapid sequencing and variant detection for time-critical studies.

- Replaces capillary electrophoresis in many applications
- Offers the longest read length of any desktop system
- For Research Use Only

MiSeqDx System

Focused Dx power

Designed specifically for clinical laboratories, the MiSeqDx System is the first FDA-cleared *in vitro* diagnostic NGS system.

- Screening and diagnostic testing
- Data output is tailored to the needs of clinical labs
- Integrated software enables sample tracking, user traceability, and results interpretation

www.illumina.com/MiSeqReagents

Applications for the MiSeq System

Application	Recommended read length	Optimized kits
Whole-genome sequencing		
Small genomes Metagenomics	2 x 150–300	Nextera XT DNA Sample Preparation Kit
		TruSeq DNA Nano Sample Preparation Kit
Targeted DNA sequencing		
Amplicon	2 x 75, 2 x 150 (for Nextera XT amplicons) 2 x 150 (for 150–250 bp TSCA amplicons)	Nextera XT DNA Sample Preparation Kit
		TruSeq Custom Amplicon Kit
Cancer research panels	2 x 150	TruSight Cancer Sequencing Panel
		TruSight Tumor Sequencing Panel
		TruSight Myeloid Sequencing Panel
		TruSeq Amplicon - Cancer Panel
Disease and condition research panels	2 x 150	TruSight One Sequencing Panel
		TruSight Cardiomyopathy Sequencing Panel
		TruSight Inherited Disease Sequencing Panel
		TruSight Autism Sequencing Panel
RNA sequencing		
mRNA	2 x 75	TruSeq Stranded mRNA Sample Preparation Kit
		TruSeq RNA Access Library Prep Kit
Gene expression profiling	1 x 50	TruSeq Stranded mRNA Sample Prep kit
microRNA	1 x 50	TruSeq Small RNA Sample Preparation Kit
Ribosome profiling	1 x 50	ARTseq Ribosome Profiling Kit (Yeast)
		ARTseq Ribosome Profiling Kit (Mammalian)
Targeted RNA sequencing		
Targeted RNA	1 x 50	TruSeq Targeted RNA Custom Kit
		TruSeq Targeted RNA Apoptosis Panel Kit
		TruSeq Targeted RNA Cardiotoxicity Panel
		TruSeq Targeted RNA Cell Cycle Panel Kit
		TruSeq Targeted RNA Cytochrome p450 Panel Kit
		TruSeq Targeted RNA Hedgehog Panel Kit
		TruSeq Targeted RNA Neurodegeneration Panel Kit
		TruSeq Targeted RNA NFkB Panel Kit
		TruSeq Targeted RNA Stem Cell Panel Kit
		TruSeq Targeted RNA TP53 Panel Kit
		TruSeq Targeted RNA WNT Pathway Panel Kit
Regulation applications		
ChIP-Seq	1 x 50	TruSeq ChIP Sample Prep Kit

NextSeq 500

Flexible power. Speed and simplicity for everyday genomics.

NextSeq 500 System

Flexible power

The NextSeq 500 System delivers the power of high-throughput sequencing with the simplicity of a desktop sequencer—transforming exome, transcriptome, and whole-genome sequencing into everyday research tools. High-quality data and accuracy combine with a versatile and flexible platform that lets you switch easily from one application to another. Streamlined sample-to-answer workflows enable you to perform low- and high-throughput studies to support a range of project sizes.

- Only desktop system capable of sequencing a whole human genome in a single run
- Configurable output and fast run time to support a wide range of applications and sample sizes

NextSeq 500 System performance parameters*

Flow cell configuration	Read length (bp)	Output (Gb)	Run time	Data quality
High-output flow cell Up to 400 million single reads Up to 800 million paired-end reads	2 × 150	100–120	29 hours	> 75% > Q30
	2 × 75	50–60	18 hours	> 80% > Q30
	1 × 75	25–30	11 hours	> 80% > Q30
Mid-output flow cell Up to 130 million single reads Up to 260 million paired-end reads	2 × 150	32–39	26 hours	> 75% > Q30
	2 × 75	16–19	15 hours	> 80% > Q30

* Total times include cluster generation, sequencing, and base calling on a NextSeq 500 System. Install specifications are based on Illumina PhiX control library at supported cluster densities (between 129–165K/mm^2 clusters passing filter). Actual performance parameters might vary based on sample type, sample quality, and clusters passing filter. The percentage of bases > Q30 is averaged over the entire run.

High-output kit (120 Gb)	1 Human genome	9 Exomes	10 Transcriptomes	40 Gene expression profiles
Mid-output kit (39 Gb)	3 Exomes	6 Enrichment panels	96 Amplicon panels	12 Gene expression profiles

www.illumina.com/NextSeqReagents

For Research Use Only

Applications for the NextSeq 500 System

Applications	Recommended read length	Optimized kit
Whole-genome sequencing		
Large genomes	2 x 150	TruSeq DNA PCR-Free Sample Prep Kit
		TruSeq Nano DNA Sample Prep Kit
		Nextera DNA Sample Prep Kit
Small genomes	2 x 150	Nextera XT DNA Sample Prep Kit
Exome sequencing		
Amplicon	2 x 75–150 for Nextera XT Amplicons 2 x 150 (for 150–250 bp TSCA amplicons)	Nextera XT DNA Sample Preparation Kit
		TruSeq Custom Amplicon Kit
Exome	2 x 150	Nextera Rapid Capture Exome
		Nextera Rapid Capture Expanded Exome
		Nextera Rapid Capture Custom Enrichment Kit
Cancer research panels	2 x 150	TruSight Cancer Sequencing Panel
		TruSight Tumor Sequencing Panel
		TruSight Myeloid Sequencing Panel
		TruSeq Amplicon - Cancer Panel
Disease and condition research panels	2 x 150	TruSight One Sequencing Panel
		TruSight Cardiomyopathy Sequencing Panel
		TruSight Inherited Disease Sequencing Panel
		TruSight Autism Sequencing Panel
RNA sequencing		
Whole transcriptome	2 x 75	TruSeq Stranded Total RNA Sample Prep Kit
Gene expression profiling	1 x 50	TruSeq Stranded mRNA Sample Prep kit
mRNA	2 x 75	TruSeq Stranded mRNA Sample Prep Kit
		TruSeq RNA Access
microRNA	1 x 50	TruSeq Small RNA Sample Preparation Kit
Ribosome profiling	1 x 50	ARTseq Ribosome Profiling Kit (Yeast)
		ARTseq Ribosome Profiling Kit (Mammalian)
Targeted RNA sequencing		
Targeted RNA	1 x 50	TruSeq Targeted RNA Custom Kit
		TruSeq Targeted RNA Apoptosis Panel Kit
		TruSeq Targeted RNA Cardiotoxicity Panel
		TruSeq Targeted RNA Cell Cycle Panel Kit
		TruSeq Targeted RNA Cytochrome p450 Panel Kit
		TruSeq Targeted RNA Hedgehog Panel Kit
		TruSeq Targeted RNA Neurodegeneration Panel Kit
		TruSeq Targeted RNA NFkB Panel Kit
		TruSeq Targeted RNA Stem Cell Panel Kit
		TruSeq Targeted RNA TP53 Panel Kit
		TruSeq Targeted RNA WNT Pathway Panel Kit

For Research Use Only

HiSeq 2500

Production power. Power and efficiency for large-scale genomics.

HiSeq 2500 System

Production power

Offering a high-powered, cost-effective sequencing solution, the HiSeq 2500 System enables you to quickly and economically perform large-scale high-throughput exome, transcriptome, and whole-genome sequencing projects. The system can process one or two flow cells in parallel and offers flexible run modes. These features let you adjust data output to support a broad range of applications and sample sizes.

- Proven powerhouse configurable for a wide range of applications and sample sizes
- Most economical and efficient solution for large-volume studies

Any application. Any study. Any sample size.

From sample prep through data analysis, we focus on making applications easy, so you can stay focused on your research. Targeted resequencing, gene expression, whole-genome sequencing, epigenetics, and more—we've designed simple, end-to-end solutions for any study. No matter the lab size, research goal, or scale.

www.illumina.com/HiSeqReagents

For Research Use Only

Applications for the HiSeq 2500 System

From whole-genome sequencing to small RNA sequencing, the HiSeq 2500 System offers accurate, fast, and cost-effective solutions for any NGS application.

Application	Recommended read length	Optimized kits
Whole-genome sequencing		
Resequencing	2 x 100–125	TruSeq DNA PCR-Free Sample Prep Kit
		TruSeq Nano DNA Sample Prep Kit
		Nextera DNA Sample Kit
Long range for *de novo*	2 x 100–125	TruSeq Synthetic Long-Read Library Prep Kit
		Nextera Mate Pair Sample Kit
Targeted DNA sequencing		
Exome enrichment	2 x 75–100	Nextera Rapid Capture Exome Kit
		Nextera Rapid Capture Expanded Exome Kit
Custom enrichment	2 x 75	Nextera Rapid Capture Custom Kit
RNA sequencing		
Gene expression profiling	1 x 50	TruSeq Stranded mRNA Sample Prep kit
mRNA sequencing	2 x 75	TruSeq Stranded mRNA Sample Kit
		TruSeq RNA Access
Whole transcriptome	2 x 75	TruSeq Stranded Total RNA Sample Kit
Ribosome profiling	1 x 50	ARTseq Ribosome Profiling Kit (Yeast)
		ARTseq Ribosome Profiling Kit (Mammalian)
Regulation applications		
ChIP-Seq	1 x 50	TruSeq ChIP Sample Prep Kit
Methylation analysis	2 x 75	Epicentre EpiGnome Methyl-Seq Kit

> The HiSeq flexibility allows us to be limited only by our imagination!
>
> **Owen Stephens,** UAMS/MIRT

HiSeq X Ten

Population power. $1,000 human genome and extreme throughput for population-scale sequencing.

HiSeq X Ten

Population power

Composed of 10 HiSeq X Systems, the HiSeq X Ten is the first sequencing platform that breaks the $1,000 barrier for a 30× human genome. The HiSeq X Ten System is ideal for population-scale projects focused on the discovery of genotypic variation to understand and improve human health. It can rapidly sequence tens of thousands of samples at high genome coverage, delivering a comprehensive catalog of human variation within and outside coding regions.

- Tens of thousands of whole human genomes per year
- $1,000 per human genome, including depreciation, sample preparation, and labor

HiSeq X Ten performance parameters*

	Dual flow cell	Single flow cell
Output/run	1.6–1.8 Tb	800–900 Gb
Reads passing filter†	5.3–6 billion	2.6–3 billion
Supported read length	2 × 150	
Run time	< 3 days	
Quality	≥ 75% of bases above Q30 at 2 × 150 bp	

* Specifications based on Illumina PhiX control library at supported cluster densities (between 1,255–1,412 K clusters/mm^2). Supported library preparation kit includes TruSeq Nano DNA HT kit and TruSeq PCR-Free DNA kit with 350 bp target insert size and HiSeq X HD reagents. HiSeq X was designed and optimized for human whole-genome sequencing; other applications and species are not supported.

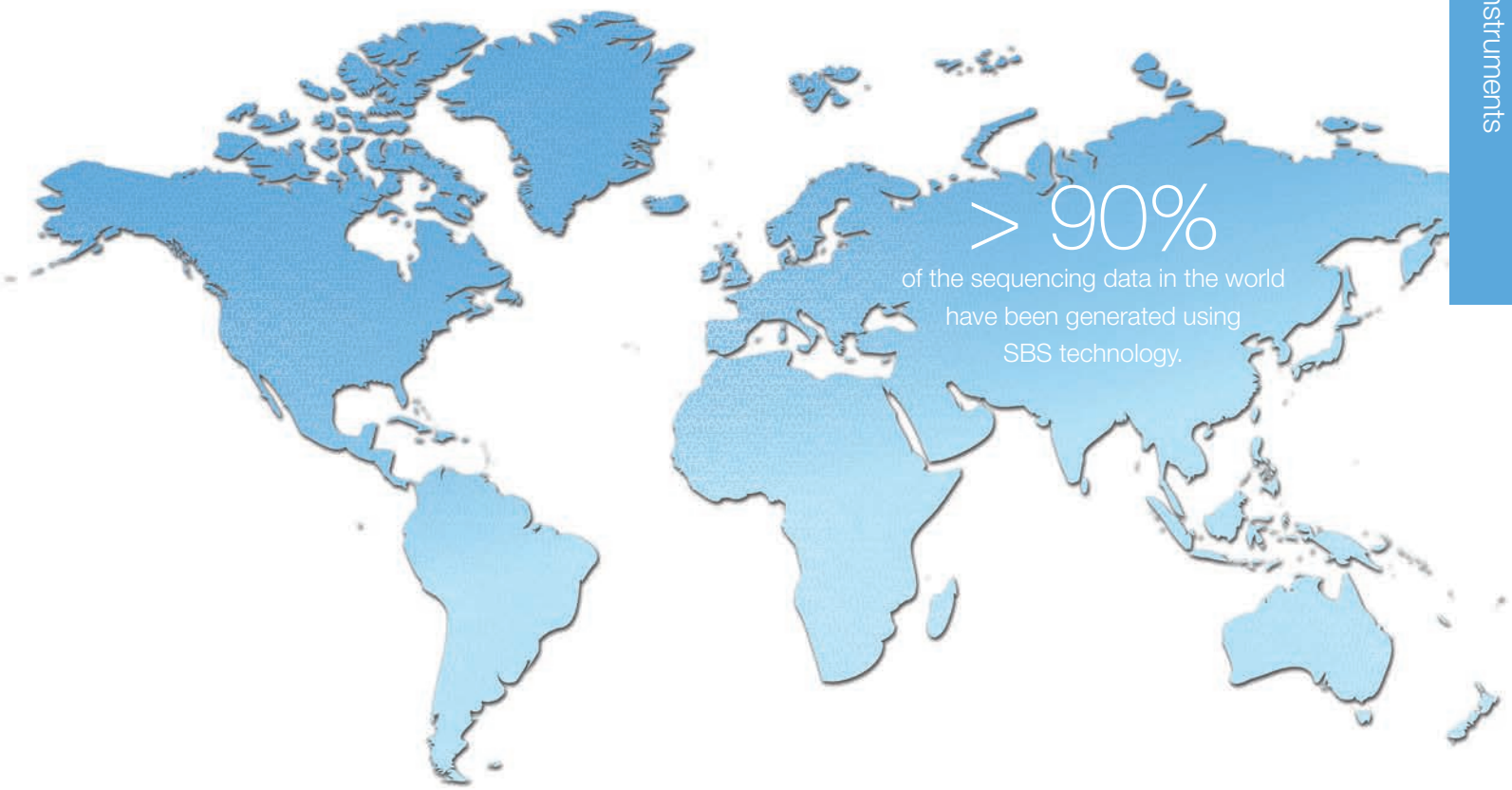
† Single-end reads.

Industry-leading data quality

Proven technology. Highest confidence.

Scientists around the world demand the best data quality. You'll benefit from:

- The greatest accuracy with the highest percentage of sequenced bases over Q30
- The highest yield of error-free reads
- The fewest false positives, false negatives, and miscalls



Analysis Tools

For Research Use Only

Push-button bioinformatics

Data analysis, collaboration, and storage made easy.

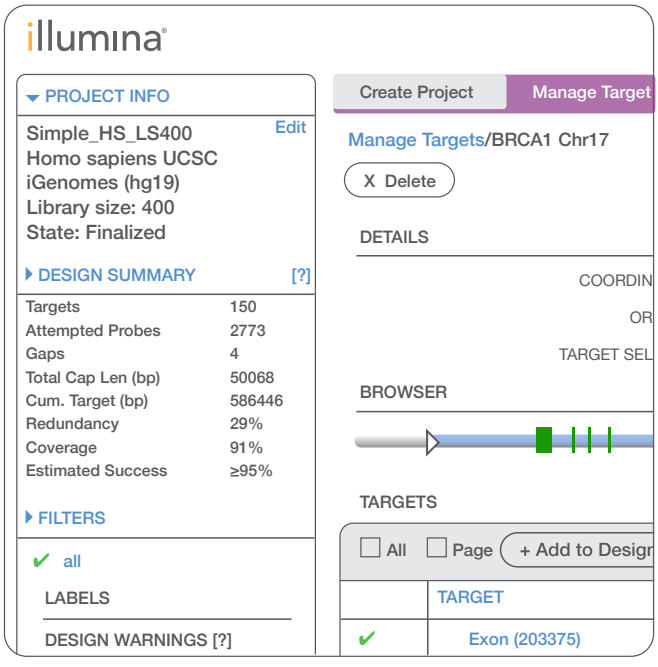
Custom Design	Library Prep	Sequence
Fast and robust design of custom panels for enrichment and Amplicon applications. Seamless ordering of library prep reagents from a unified portal.	Manage samples, libraries, and sequencing runs directly on BaseSpace*. Track thousands of experiments.	Monitor and QC sequencing runs live from anywhere (mobile devies supported).

DesignStudio

Custom applications:

- Amplicon sequencing (TruSeq Custom Amplicon)
- Exome sequencing (Nextera Rapid Capture Custom)
- Targeted RNA sequencing (Custom)

DesignStudio is an interactive, web-based environment that provides dynamic feedback to optimize target region coverage, reducing the time required to design custom projects. Users can personalize and optimize content to fit the needs of their study.



DesignStudio

For Research Use Only

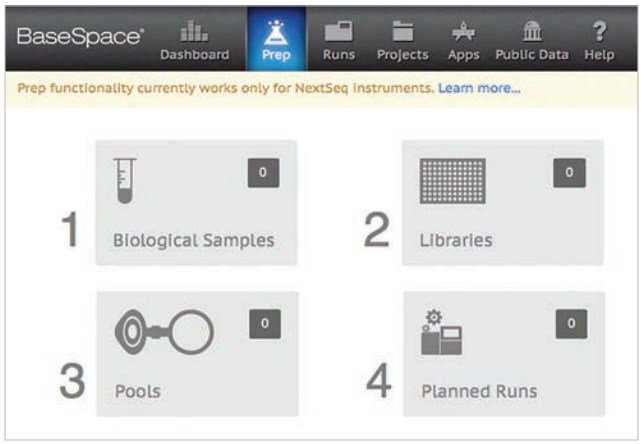
BaseSpace

Analyze			
Align	**Filter/Annotate Count/Compare**	**Visualize**	**Add biological context**
Tailor-made for biologists, based on industry-standard algorithms and a growing ecosystem of third-party apps.	Extract biological knowledge from variant data. Analysis results are presented in intuitive publication-ready graphical reports (advanced users have access to all the files/ results generated by the underlying algorithms). For DNA methods, rich annotation, filtering and report generation is available.	Visualize alignment and variant calling details in a genome browser (IGV).	After running the RNA-Seq Apps on BaseSpace, leverage third-party apps on BaseSpace to perform pathway analysis (Genomatix and iPathwayGuide).

Applications

- Whole-genome sequencing (large genomes)
- Whole-genome sequencing (small genomes)
- Amplicon sequencing (TruSeq Custom Amplicon panels)
- Exome sequencing (Nextera Rapid Capture)
- Exome sequencing (Nextera Rapid Capture Enrichment)
- RNA sequencing (all variations)

NextSeq 500: Prep Tab in BaseSpace



MiSeq, HiSeq 2500 use Illumina Experiment Manager

See the Prep tab in action at www.illumina.com/BaseSpace

Biological Applications (DNA)	Align/Call Variants
Whole-genome sequencing (large genomes)	**BWA Whole Genome Sequencing** • BWA Alignment and GATK Variant Calling • Single nucleotide polymorphisms (SNPs), insertions-deletions (indels), copy number variations (CNV), structural variations (SV) • Works with data from standard whole genome sequencing (WGS) kits
	Isaac Whole Genome Sequencing • Isaac Alignment and Isaac Variant Calling for Nextera exome and TruSight fixed content kits only • Detects SNPs, small indels
	Tumor-Normal Sequencing • Combined analysis of WG tumor-normal data set (tumor sample 80× coverage recommended, matched normal sample 40× coverage recommended) • SNPs, indels, CNVs, SVs • Works with data from WGS kits
Whole-genome sequencing (small genomes)	**BWA Whole Genome Sequencing** • BWA Alignment and GATK Variant Calling • Single nucleotide polymorphisms (SNPs), insertions-deletions (indels), copy number variations (CNVs), structural variations (SVs) • Works with data from standard whole genome sequencing (WGS) kits
	Isaac Whole Genome Sequencing • Isaac Alignment and Isaac Variant Calling for Nextera exome and TruSight fixed content kits only • Detects SNPs, small indels
	SPAdes Genome Assembler • Analyzes standard isolates and single-cell multiple displacement amplicon (MDA) bacterial assemblies • Offers read error correction tool • Offers iterative short-read genome assembly module
Whole-genome sequencing (long-range)	**SPAdes Genome Assembler** • Analyzes standard isolates and single-cell multiple displacement amplicon (MDA) bacterial assemblies • Offers read error correction tool • Offers iterative short-read genome assembly module
	Velvet *de novo* Assembly • Assembles *de novo* genomes for bacterial samples • Offers assembly for libraries prepared with the Nextera Mate Pair Sample Preparation Kit
	TruSeq Long-Read Assembly • Assembly of synthetic long reads from high-quality short reads • Used with the TruSeq Synthetic Long-Read DNA Library Prep Kit • Produces FASTQ file of long reads for use in *de novo* and hybrid assembly, genome finishing, and metagenomic analysis
	TruSeq Phasing • Assigns haplotype information to homologous chromosomes, enabling researchers to phase *de novo* mutations and identify co-inherited alleles • Used with the TruSeq Synthetic Long-Read DNA Library Prep Kit, and requires VCF file of variant calls from 30× WGS. • Produces a phased VCF file of variant phasing calls and confidence scores.

‡VariantStudio

- Enables extraction of biological knowledge from variant data by providing a rich annotation database, flexible filtering, and a streamlined variant classification and reporting tool
- Leverages leading annotation databases and a powerful filtering interface to rapidly identify disease-associated variants in data sets ranging from small targeted panels to whole-genome sequencing experiments

For Research Use Only

Genomes Available			Count/Compare	Visualize
Arabidopsis thaliana *Bos taurus* *Escherichia coli* K-12 DH10B *Escherichia coli* K-12 MG1655	*Drosophila melanogaster* *Homo sapiens* *Mus musculus* PhiX	*Rattus norvegicus* *Rhodobacter sphaeroides* 2.4.1 *Saccharomyces cerevisiae* *Staphylococcus aureus* NCTC 8325	VariantStudio‡	The Broad's IGV‡
Arabidopsis thaliana *Bos taurus* *Escherichia coli* K-12 DH10B *Escherichia coli* K-12 MG1655	*Homo sapiens* *Mus musculus* PhiX *Rattus norvegicus*	*Rhodobacter sphaeroides* 2.4.1 *Saccharomyces cerevisiae* *Staphylococcus aureus* NCTC 8325		
Homo sapiens				
Arabidopsis thaliana *Bos taurus* *Escherichia coli* K-12 DH10B *Escherichia coli* K-12 MG1655	*Drosophila melanogaster* *Homo sapiens* *Mus musculus* PhiX	*Rattus norvegicus* *Rhodobacter sphaeroides* 2.4.1 *Saccharomyces cerevisiae* *Staphylococcus aureus* NCTC 8325	VariantStudio‡	The Broad's IGV‡
Arabidopsis thaliana *Bos taurus* *Escherichia coli* K-12 DH10B *Escherichia coli* K-12 MG1655	*Homo sapiens* *Mus musculus* PhiX *Rattus norvegicus*	*Rhodobacter sphaeroides* 2.4.1 *Saccharomyces cerevisiae* *Staphylococcus aureus* NCTC 8325		
Bacterial				
Bacterial				
Bacterial				The Broad's IGV‡
All genomes (no limitations)			N/A	
Homo sapiens				

‡The Broad's IGV

- Displays alignments and variants from multiple samples for performing complex variant analysis
- The Broad Institute of MIT and Harvard developed IGV, and Illumina modified it to display BaseSpace data

Learn more and sign up at: www.illumina.com/BaseSpace

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Biological Applications (DNA)	Align/Call Variants
Amplicon sequencing	 TruSeq Amplicon • Variant analysis of Illumina TruSeq Amplicon panels Includes somatic variant caller for tumor samples; GATK and Isaac for germline samples • Currently supports Illumina fixed panels: TruSeq Amplicon-Cancer Panel, and TruSight Myeloid Sequencing Panel
	 16S Metagenomics • Taxonomic classification of the 16S rRNA amplicons • Illumina curated version of the GreenGenes taxonomic database • Aggregate analysis among multiple samples • Feature rich and easy to read html and pdf reports
Cancer research sequencing panels	 TruSeq Amplicon • Variant analysis of Illumina TruSeq Amplicon panels • Includes somatic variant caller for tumor samples; GATK and Isaac for germline samples • Currently supports Illumina fixed panels: TruSeq Amplicon-Cancer Panel, and TruSight Myeloid Sequencing Panel
Disease and condition research sequencing panels	 BWA Enrichment • BWA Alignment and GATK Variant Calling for Nextera exome and TruSight fixed content kits only • Detects single nucleotide polymorphisms (SNPs), small insertions-deletions (indels)
	 Isaac Enrichment • Isaac Alignment and Isaac Variant Calling for Nextera exome and TruSight fixed content kits only • Detects SNPs, small indels
Exome sequencing	 BWA Enrichment • BWA Alignment and GATK Variant Calling for Nextera exome and TruSight fixed content kits only • Detects SNPs, small indels
	 Isaac Enrichment • Isaac Alignment and Isaac Variant Calling for Nextera exome and TruSight fixed content kits only • Detects SNPs, small indels
Metagenomics	 16S Metagenomics • Taxonomic classification of the 16S rRNA amplicons • Illumina curated version of the GreenGenes taxonomic database • Aggregate analysis among multiple samples • Feature rich and easy to read html and pdf reports

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- Leverages leading annotation databases and a powerful filtering interface to rapidly identify disease-associated variants in data sets ranging from small targeted panels to whole-genome sequencing experiments

Genomes Available	Count/Compare	Visualize
Cancer panels Myeloid panels Custom amplicon control pools Custom panels	VariantStudio‡	The Broad's IGV‡
Bacterial	N/A	
Cancer panels Myeloid panels Custom amplicon control pools Custom panels	VariantStudio‡	The Broad's IGV‡
Homo sapiens	VariantStudio‡	The Broad's IGV‡
Homo sapiens		
Homo sapiens	VariantStudio‡	The Broad's IGV‡
Homo sapiens		
Bacterial	N/A	

‡The Broad's IGV

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Learn more and sign up at: www.illumina.com/BaseSpace

Biological Applications (RNA)	Align/Call Variants
mRNA sequencing	RNA Express • Rapid gene expression profiling tool incorporating STAR aligner and DESeq • Gene-level gene-expression only
	TopHat Alignment • RNA-Seq alignment using industry-standard tool TopHat2 • Gene fusion calling • Chromosomal single nucleotide polymorphism (cSNP) calling using Isaac Variant Caller Results feed into Cufflinks Assembly & DE App
Whole transcriptome sequencing	RNA Express • Rapid gene expression profiling tool incorporating STAR aligner and DESeq • Gene-level gene-expression only
	TopHat Alignment • RNA-Seq alignment using industry-standard tool TopHat2 • Gene fusion calling • Chromosomal single nucleotide polymorphism (cSNP) calling using Isaac Variant Caller Results feed into Cufflinks Assembly & DE App
FFPE RNA sequencing	RNA Express • Rapid gene expression profiling tool incorporating STAR aligner and DESeq • Gene-level gene-expression only
	TopHat Alignment • RNA-Seq alignment using industry-standard tool TopHat2 • Gene fusion calling • Chromosomal single nucleotide polymorphism (cSNP) calling using Isaac Variant Caller • Results feed into Cufflinks Assembly & DE App
Single-cell RNA sequencing	RNA Express • Rapid gene expression profiling tool incorporating STAR aligner and DESeq • Gene-level gene-expression only
	TopHat Alignment • RNA-Seq alignment using industry-standard tool TopHat2 • Gene fusion calling • Chromosomal single nucleotide polymorphism (cSNP) calling using Isaac Variant Caller • Results feed into Cufflinks Assembly & DE App

BaseSpace is available in the cloud, and as a local appliance installed in your lab.

BaseSpace®

Available on the cloud (Amazon Web Services)

Zero configuration

Zero installation

Zero maintenance

‡The Broad's IGV

- Displays alignments and variants from multiple samples for performing complex variant analysis
- The Broad Institute of MIT and Harvard developed IGV, and Illumina modified it to display BaseSpace data

‡Genomatix Pathway

- Associates single gene or list of genes with annotation data
- Offers full range of annotation data including (but not limited to) pathways, diseases, tissues, and small molecules

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Genomes Available	Count/Compare	Visualize	Add Biological context
Homo sapiens *Mus musculus* *Rattus norvegicus*	Cufflinks Assembly & DE • Differential Expression • Novel/alternate transcript assembly RNA Express • Rapid gene expression profiling tool incorporating STAR aligner and DESeq • Gene-level gene-expression only	The Broad's IGV‡	Genomatix Pathway‡ iPathway Guide‡
Homo sapiens *Mus musculus* *Rattus norvegicus*	Cufflinks Assembly & DE • Differential Expression • Novel/alternate transcript assembly RNA Express • Rapid gene expression profiling tool incorporating STAR aligner and DESeq • Gene-level gene-expression only	The Broad's IGV‡	Genomatix Pathway‡ iPathway Guide‡
Homo sapiens *Mus musculus* *Rattus norvegicus*	Cufflinks Assembly & DE • Differential Expression • Novel/alternate transcript assembly RNA Express • Rapid gene expression profiling tool incorporating STAR aligner and DESeq • Gene-level gene-expression only	The Broad's IGV‡	Genomatix Pathway‡ iPathway Guide‡
Homo sapiens *Mus musculus* *Rattus norvegicus*	Cufflinks Assembly & DE • Differential expression • Novel/alternate transcript assembly RNA Express • Rapid gene expression profiling tool incorporating STAR aligner and DESeq • Gene-level gene-expression only	The Broad's IGV‡	Genomatix Pathway‡ iPathway Guide‡

BaseSpace® ONSITE

Simple local appliance

BaseSpace user interface

Behind customer firewall

Encrypted on-site storage

‡iPathway Guide

- Offers differential expression gene analysis, drug interaction analysis, and disease analysis based on enrichment
- Pathway analysis with impact analysis modeled on KEGG pathways and more

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