ILLUMINA PORTFOLIO UPDATE:

Sequencing Power for Every Scale
Systems for every application. For every lab.
Systems for Every Application. For Every Lab.

- **MiniSeq®**: Affordable, simple, powerful
- **MiSeq®**: Speed & simplicity for targeted & small genome sequencing
- **NextSeq®**: Speed & simplicity for every day genomics
- **HiSeq®**: Power & efficiency for large-scale genomics
- **HiSeq X™**: Maximum throughput for whole genome sequencing
MiSeq

0.5–15 Gigabases

12–25 million clusters

Up to 600 cycles (2 x 300 bp)

$99k list price

$545–$1485 run

PE150; 80% > Q30

*List price in USD
MiSeq Instrument

Next-Gen Made Simple: Load & Go

FLEXIBLE DATA OUTPUT

Multiple flow cell options
from 1M reads to 25M
from 300MB to 15GB

READ LENGTH

<table>
<thead>
<tr>
<th>Read Length</th>
<th>Nano</th>
<th>Micro</th>
<th>v2</th>
<th>v3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x50</td>
<td></td>
<td></td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>2x75</td>
<td>4</td>
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<td>15</td>
<td>25</td>
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<tr>
<td>2x150</td>
<td>4</td>
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<tr>
<td>2x250</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2x300</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
MiSeq Reporter (MSR)

- Streamlined on-board analysis workflows
- Most workflows also available on BaseSpace (selectable on Run Setup)
- No user intervention from sample loading to report generation
- Accessible from any computer on the same local network
- All workflows generate FASTQ files that can be analyzed by 3rd party programs
- Stand-alone Windows 64-bit version available for download
NextSeq 500

$250K | list price*

$1000–4000 | run*

PE150 | 75% > Q30

20–120 | gigabases

130–400 million | reads

*List price in USD
One System, Two Output Modes

High

PE150 120 gigabases
400M clusters

Mid

PE150 40 gigabases
130M clusters
HiSeq – Production Power

MORE OUTPUT | FASTER | CHEAPER

PE125 | 4B CLUSTERS

1 TERABASE | 6 DAYS

10 | HUMAN GENOMES | 6 DAYS*

*Assumes 100Gb per 30x human genome

*This information is intended to outline general product direction and it should not be relied on in making a purchasing decision. This material is for information purposes only and may not be incorporated into any contract. This information is not a commitment, promise, or legal obligation to deliver this functionality. The development, release, and timing of any features or functionality described for our products remains at our sole discretion.
From Exomes to Genomes and Everything in Between

*Assumes 100Gb, >30x genome; Nextera Rapid Capture Exome; 50M reads per RNA sample
Targeted Resequencing | Amplicon and Enrichment

- **Amplicon (TSCA)**

  - ULSO
  - DLSO
  - Region of Interest
  - index

- **Enrichment**

  - Biotin probes
  - Streptavidin beads
# Illumina Exome Portfolio Overview

<table>
<thead>
<tr>
<th>Metric</th>
<th>Nextera Rapid Capture Exome*</th>
<th>TruSeq Rapid Exome</th>
<th>TruSeq Exome</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA input</td>
<td>50 ng</td>
<td>50 ng</td>
<td>100 ng</td>
</tr>
<tr>
<td>Sample types</td>
<td>DNA</td>
<td>DNA</td>
<td>DNA and FFPE</td>
</tr>
<tr>
<td>Hands-on time</td>
<td>5 hr</td>
<td>3 hr</td>
<td>6 hr</td>
</tr>
<tr>
<td>Total assay time</td>
<td>1.5 days</td>
<td>1 day</td>
<td>2.5 days</td>
</tr>
<tr>
<td>Hyb time</td>
<td>16 hr</td>
<td>2 hr</td>
<td>16 hr</td>
</tr>
<tr>
<td>On-target %</td>
<td>50%</td>
<td>&gt;75%</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>% coverage at 20X at 8Gb</td>
<td>80%</td>
<td>&gt;85%</td>
<td>&gt;90%</td>
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</tbody>
</table>

*Will EOL in 2016*
# TruSeq Exome

## Exome Content

<table>
<thead>
<tr>
<th>TruSeq Exome</th>
<th>Coverage Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target size</td>
<td>45Mb</td>
</tr>
<tr>
<td>Number of target exons</td>
<td>214,405</td>
</tr>
<tr>
<td>Target content</td>
<td>coding exons</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percent of Exome Covered (by Database)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refseq</td>
</tr>
<tr>
<td>CCDS</td>
</tr>
<tr>
<td>ENSEMBL</td>
</tr>
<tr>
<td>GENCODE v19</td>
</tr>
</tbody>
</table>
TruSeq Exome

Data Summary

- **Proven data quality**
  - Uniform coverage and \( \geq 80\% \) on-target sequencing reads

- **Cost-effective solution**
  - Pool up to 12 pre-enrichment libraries to maximize throughput

- **Accurate, reliable findings**
  - Confidently identify variants with push-button data analysis

Data generated from 50M reads (4Gb) on a HiSeq 2500 in Rapid Run Mode with V2 chemistry using BWA enrichment App on BaseSpace
TruSeq Exome
Beta Data w/ FFPE

- Customer generated data
- FFPE samples were medium quality based on BA traces
- % duplicates <15%
- >70% coverage at 20x

On average: 40x coverage, 70M reads
TruSeq Rapid Exome – Key Metrics

**On Target %**

- 3plex
- 6plex
- 9plex
- 12plex

**Coverage at 10x**

- 3plex
- 6plex
- 9plex
- 12plex

**Precision:** Probability that a called variant is correct

**Recall:** Probability of calling a validated variant

- **100x**
  - Precision: 99.58%
  - Recall: 91.04%

- **NIST (NA12878)**
- **TruSeq Rapid (NA12878)**
Custom applications and concierge service

Custom Applications

- TSCA (TruSeq Custom Amplicon)
- Enrichment
- TREx (TruSeq Targeted RNA expression)

Illumina concierge service for custom designs

- Tier 1 – design only
- Tier 2 – design and functional testing/resynthesis
Small genomes, viruses and amplicons
**Nextera XT DNA Sample Prep**

*The fastest & easiest prep for small genomes, PCR amplicons and plasmids*

- **Rapid Prep**
  - 90 min prep, only 15 min of hands on time

- **Ultra low input**
  - Only 1 ng of input DNA needed

- **Optimized for small genomes, PCR amplicons and plasmids**

- **Innovative sample normalization**
  - No library quantification needed

- **Fastest time to results**
  - DNA to analyzed data in <8 hours with MiSeq
Step 1: Tagmentation of template DNA

Transposomes

Genomic DNA

~ 300 bp

Tagmentation
Step 2: PCR to add adapters and indices

- PCR
- ~300 bp
- Tagmentation
- Reduced-Cycle PCR Amplification
Step 3: Cleanup and Sequence
MiSeq Reporter (MSR)

- Streamlined on-board analysis workflows
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De Novo Assembly in BaseSpace

- Assemble bacteria de novo
  - DNASTAR SeqMan Ngen Performs de novo assembly of bacterial genome sequences

- SPAdes Genome Assembler 3.0
  - Assembly of small genomes from MDA single-cell and standard bacterial data sets

- Velvet de novo Assembly
  - This de-novo assembly pipeline is for bacterial samples using the Velvet assembler and has a focus on samples sequenced with Nextera Mare-pair library kit
Viral Analysis Tools ALL in BaseSpace

- **Kraken Metagenomics**
  - [ccb.jhu.edu/software/kraken](ccb.jhu.edu/software/kraken)
  - Taxonomic analysis of short reads for bacteria, archaea and viral classification

- **PathSeq Virome**
  - Useful for detection of >50,000 human clinically relevant viral genomes

- **DeepChek- HBV v1.3**

- **DeepChek – HCV v1.3**

- **DeepChek HIV**
  - Genotyping/resistance analysis solution for HBV, HCV and HIV with sub-typing, variant calling, and more
16S Metagenomics
16S Metagenomics Library Prep

Illumina-demonstrated Protocol

- A Demonstrated protocol for 16S Metagenomics on MiSeq
  1. 16S protocol for library prep
  2. Sequence 2x250 - 2x300 (V3 recommended for run performance)
  3. Metagenomics workflow (MSR and BaseSpace)

- Method can be used for other amplicons
  - Locus-specific primers with overhang adapters
  - Tm guidelines in UG
16S rRNA Protocol: Summary

- Supported by Illumina TS
- No modifications needed to MiSeq configuration or Sample sheets
- Can utilize MSR 16S Metagenomics analysis
- Uses Illumina developed dual indexing method
- Uses Illumina manufactured index adapters
- Fast protocol; ~4-5 hours
- Library normalization and pooling included in protocol

Image from WiseGeek
Step 1: PCR to amplify regions of interest

*Uses overhang primer pairs*

**Genomic DNA**

- **Locus-specific sequence**: 16S V3-V4 region or custom amplicon
- **Overhang adapter sequence for tailed PCR**
Step 2: 2\textsuperscript{nd} round of PCR adds ILMN indices and adapters

Nextera XT Index primer

Insert to be sequenced

16S V3-V4 or custom amplicon
Step 3: Sequence on the MiSeq System

- **Sequencing order on the MiSeq System**
  - Read 1 – sequence amplicons in forward direction up to 300 nt
  - Index 1 – read first barcode
  - Index 2 – read second barcode (software can now uniquely identify the sample)
  - Read 2 – sequence amplicons in reverse direction up to 300 nt
Both analysis pipelines ① MSR and ② BaseSpace 16S metagenomics use the same classification algorithm and taxonomic database.

- The classification algorithm is a high performance implementation of the published RDP Naïve Bayesian Classifier (http://dx.doi.org/10.1128%2FAEM.00062-07)

- The database is an Illumina-curated version of the GreenGenes Consortium 16S rRNA database. Redundant sequences and entries with missing or partial labels are removed.

- Provides fast, high-accuracy species-level taxonomic classifications.

- Uses full length of Illumina paired-end reads.
MiSeq Reporter (MSR) Analysis

- Gives access to raw classification data.
- Provides per-sample pie chart visualizations by taxonomic level.
BaseSpace 16S Metagenomics Analysis

- New BaseSpace App for easy-to-use, cloud-based analysis
- New interactive visualizations for sample-level classifications
- Aggregate analysis with sample-sample comparisons and visualization
- Easy-to-download, raw-classification data
Metagenomics: 16S rRNA Sequencing

16S Metagenomics
- The 16S Metagenomics app performs taxonomic classification of 16S rRNA targeted amplicon reads using an Illumina-curated version of the GreenGenes taxonomic database

Kraken Metagenomics
- [ccb.jhu.edu/software/kraken](http://ccb.jhu.edu/software/kraken)  
  Taxonomic analysis of short reads for bacteria, archaea and viral classification

QIMME
- QIIME is an open source software package for comparison and analysis of microbial communities, primarily based on high-throughput amplicon sequencing data
Other Amplicons?
Swap out other primers for different 16S Regions

Forward Primer:

5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG- \{Locus-Specific Sequence\}

Overhang Adapter Sequence                      Sequence targeting Amplicon

Reverse Primer:

5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG- \{Locus-Specific Sequence\}

Add the GREEN sequence to any PRIMER PAIR targeting an <~550bp amplicon
Many different RNAs exist - Ribosomal RNA is most abundant RNA species – and least dynamic
# Illumina’s Suite of RNA Library Prep Solutions

<table>
<thead>
<tr>
<th>Total RNA-Seq</th>
<th>mRNA-Seq/ GEx Profiling</th>
<th>Targeted Profiling</th>
<th>miRNA Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruSeq Stranded Total RNA</td>
<td>TruSeq Stranded mRNA</td>
<td>TruSeq RNA Access</td>
<td>TruSeq Targeted RNA Expression</td>
</tr>
</tbody>
</table>

- Coding + ncRNA
- Transcript-level abundance
- Splicing Analysis
- Fusion Discovery
- FFPE compatible

- Coding RNA
- Transcript-level abundance
- Splicing Analysis
- Fusion Discovery

- Coding RNA
- Transcript-level abundance
- Splicing Analysis
- Fusion Discovery
- FFPE Compatible

- 10s-1,000s of targets
- Coding + ncRNA
- Transcript-level abundance
- Fusion Validation
- FFPE Compatible

- miRNA abundance
- isomiR detection
Advantages of TruSeq® RNA Library Prep

- Precise Measurement of Strand Orientation
- Excellent reproducibility
- High dynamic range
- Unparalleled Coverage Quality
Depletion of highly abundant RNA Moieties by RiboZero

- Removes >99.99% of ribosomal RNA by mass
- Reduces “contaminating” rRNA to <10% of all reads (1-6% typical)
- Enables most cost-effective whole-transcriptome analysis
- High efficiency depletion from low quality samples, including FFPE

<table>
<thead>
<tr>
<th>RNA Sample</th>
<th>Kit</th>
<th>Removes EuK. Cytoplasmic rRNA</th>
<th>Removes Mitochondrial rRNA</th>
<th>Removes Bacterial rRNA</th>
<th>Removes Chloroplast rRNA</th>
<th>Removes Globin mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human and other animal species and FFPE RNA</td>
<td>Ribo-Zero™ Magnetic Kit (Human/Mouse/Rat)</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Human and other animal species, FFPE RNA</td>
<td>Ribo-Zero™ Magnetic Gold Kit (Human/Mouse/Rat)</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human microbiome and other bacteria-infected human, mouse, rat samples</td>
<td>Ribo-Zero™ Magnetic Gold Kit (Epidemiology)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
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<tr>
<td>Bacteria (mixed populations of gram-negative and gram-positive bacteria)</td>
<td>Ribo-Zero™ Magnetic Kit (Bacteria)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td>Ribo-Zero™ Magnetic Kit (Gram-Negative Bacteria)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td>Ribo-Zero™ Magnetic Kit (Gram-Positive Bacteria)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
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<tr>
<td>Plant leaf tissue</td>
<td>Ribo-Zero™ Magnetic Kit (Plant Leaf)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
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<tr>
<td>Plant seed or root tissue</td>
<td>Ribo-Zero™ Magnetic Kit (Plant Seed/Root)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
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<tr>
<td>Mammalian blood RNA</td>
<td>Globin-Zero™ Gold Kit</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
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<tr>
<td>Yeast</td>
<td>Ribo-Zero™ Magnetic Kit (Yeast)</td>
<td>✓</td>
<td>✓</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Min Number of Reads/Sample for different Applications

*Translating minimum reads/sample to maximum numbers of samples per Flow Cell*

<table>
<thead>
<tr>
<th>Application</th>
<th>Library Prep Recommendations</th>
<th>Sequencing Recommendations</th>
<th>Max # Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Read Length</td>
<td># Reads / Sample</td>
</tr>
<tr>
<td>Single Cell Sequencing</td>
<td>C1+SMARTer + Nextera XT</td>
<td>2x75 bp</td>
<td>~ 5M</td>
</tr>
<tr>
<td>Small RNA Discovery</td>
<td>TS Small RNA</td>
<td>&lt;1x50 bp</td>
<td>≥ 5M</td>
</tr>
<tr>
<td>Gene expression profiling (GEX)</td>
<td>TS Stranded mRNA</td>
<td>1x75 bp</td>
<td>≥ 10M</td>
</tr>
<tr>
<td>mRNA-Seq</td>
<td>TS Stranded mRNA</td>
<td>2x75 bp</td>
<td>≥ 25M</td>
</tr>
<tr>
<td>RNA Exome Sequencing</td>
<td>TS RNA Access</td>
<td>2x75 bp</td>
<td>≥ 25M</td>
</tr>
<tr>
<td>Total RNA-Seq (High Quality)</td>
<td>TS Total RNA</td>
<td>2x75 bp</td>
<td>≥ 50M</td>
</tr>
<tr>
<td>Total RNA-Seq (Degraded RNA)</td>
<td>TS Total RNA</td>
<td>2x75 bp</td>
<td>≥ 100M</td>
</tr>
<tr>
<td>Total RNA-Seq (FFPE)</td>
<td>TS Total RNA</td>
<td>2x75 bp</td>
<td>≥ 200M</td>
</tr>
</tbody>
</table>
Degraded Samples Present a Challenge for Transcriptome Analysis

- There is an enormous amount of valuable data locked in paraffin blocks
- RNA-Sequencing is the most accurate and powerful technology for transcriptome analysis, however…
- FFPE derived RNA is among the most difficult to analyze because it’s been fragmented and chemically modified by the fixation process

![FFPE vs. Fresh/Frozen RNA](image)
The Challenge: Loss Of Exonic Signal In FFPE Samples

- Poly-A Selected mRNA
- Total RNA-Seq from Fresh
- Total RNA-Seq from FFPE
- PP2CA Gene
TruSeq RNA Library Prep Overview
Focused Sequencing For Higher Throughput

TruSeq Stranded Total RNA - 250M
TruSeq Stranded Total RNA - 30M
TruSeq RNA Access 25M
Access Probes
RefSeq Genes

TruSeq Total RNA
TruSeq RNA Access
Quantitative Capture Across Entire Dynamic Range

- Dynamic range maintained at much lower read depth
- Highly reproducible
TruSeq® RNA Access Can Discover Fusions

- Does not require probes designed specifically for fusions
- Effective for discovering novel fusion events
TruSight® RNA Pan-Cancer Panel

At a glance:

- An RNA enrichment panel targeting 1385 cancer-related genes
- Assess gene fusions, variants and expression profiles
- FFPE compatible
- MiSeq® compatible (8 samples per run)
- BaseSpace® RNA Alignment App for Expression, Variant and Fusion calling

For Research Use Only. Not for use in diagnostic procedures.
New BaseSpace RNA-Seq Alignment App

For Research Use Only. Not for use in diagnostic procedures.
Running the RNA-Seq Alignment App

- Select panel: TruSight RNA Pan-Cancer
Test of RNA Access on Viral Control Samples

- Starting with 10 ng of each (3 replicates per sample):
  - Human Brain RNA (negative for viruses)
  - HeLa (contains rearranged version of HPV-18)
  - UHRR (Universal Human Reference RNA: 10 cell lines; 1/10 is HeLa with HPV-18)
  - HEK293 (contains 5’ portion of Adenovirus 5, ~99% identical to Adenovirus C)
Examples of RNA Access probe placement

These are DNA viruses, so only CDS annotations were probed. RNA viruses are tiled end-to-end

**HPV-18** GenBank X05015.1 circular dsDNA (~7.8 kb)

**Adenovirus C** GenBank NC_001405.1 linear dsDNA (~35 kb)
### TopHat results (alignment to hg19 only)

#### Low alignment % for cell lines and UHR

<table>
<thead>
<tr>
<th>Reads</th>
<th>Number of Reads</th>
<th>% Total Aligned</th>
<th>% Abundant</th>
<th>% Unaligned</th>
<th>Median CV Coverage Uniformity</th>
<th>% Stranded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain-140722a</td>
<td>76/76</td>
<td>1,881,054</td>
<td>97.30%</td>
<td>1.37%</td>
<td>2.70%</td>
<td>1.91</td>
</tr>
<tr>
<td>Brain-140722b</td>
<td>76/76</td>
<td>1,126,882</td>
<td>97.56%</td>
<td>1.26%</td>
<td>2.44%</td>
<td>1.77</td>
</tr>
<tr>
<td>Brain-140722c</td>
<td>76/76</td>
<td>1,030,306</td>
<td>97.37%</td>
<td>1.61%</td>
<td>2.63%</td>
<td>1.74</td>
</tr>
<tr>
<td>HEK293-140722a</td>
<td>76/76</td>
<td>3,269,862</td>
<td>51.60%</td>
<td>0.97%</td>
<td>48.40%</td>
<td>1.92</td>
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<tr>
<td>HEK293-140722b</td>
<td>76/76</td>
<td>3,348,456</td>
<td>51.53%</td>
<td>0.82%</td>
<td>48.47%</td>
<td>1.91</td>
</tr>
<tr>
<td>HEK293-140722c</td>
<td>76/76</td>
<td>4,050,143</td>
<td>51.13%</td>
<td>0.73%</td>
<td>48.87%</td>
<td>1.96</td>
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<tr>
<td>HeLa-140722a</td>
<td>76/76</td>
<td>2,900,950</td>
<td>55.14%</td>
<td>0.90%</td>
<td>44.86%</td>
<td>1.71</td>
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<tr>
<td>HeLa-140722b</td>
<td>76/76</td>
<td>2,336,627</td>
<td>54.88%</td>
<td>1.02%</td>
<td>45.12%</td>
<td>1.62</td>
</tr>
<tr>
<td>HeLa-140722c</td>
<td>76/76</td>
<td>2,180,281</td>
<td>53.86%</td>
<td>1.01%</td>
<td>46.14%</td>
<td>1.72</td>
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<tr>
<td>UHR-140722a</td>
<td>76/76</td>
<td>2,181,189</td>
<td>88.18%</td>
<td>0.92%</td>
<td>11.82%</td>
<td>1.72</td>
</tr>
<tr>
<td>UHR-140722b</td>
<td>76/76</td>
<td>2,685,306</td>
<td>88.12%</td>
<td>0.99%</td>
<td>11.88%</td>
<td>1.78</td>
</tr>
<tr>
<td>UHR-140722c</td>
<td>76/76</td>
<td>2,019,799</td>
<td>87.44%</td>
<td>1.09%</td>
<td>12.56%</td>
<td>1.74</td>
</tr>
</tbody>
</table>

% abundant = rRNA and mitochondrial reads
TruSeq Targeted RNA Expression
Rapid & economical RNA profiling for MiSeq

- Accurate targeting for Human, Mouse & Rat transcriptomes
- Add custom content to Fixed Panels

- Rapid – sample to answer in 1.5 days; <4hrs HOT
- Low RNA input – 50 ng or less
- Low price per sample; 1-384 spls/run

- Over 500,000 predesigned assays
- Custom and pre-validated fixed panels available

<table>
<thead>
<tr>
<th>Pre-Validated Fixed Panels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem Cell</td>
</tr>
<tr>
<td>P53 Pathway</td>
</tr>
<tr>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>NFkB Pathway</td>
</tr>
<tr>
<td>Wnt Pathway</td>
</tr>
</tbody>
</table>
Case Study: SLC25A3

- Two assays that detect transcript A
- One assay that detects transcript B+C
- Two assays that detect all isoforms
Brain: 1,291 counts
Liver: 8,201 counts
UHR: 94,711 counts

Corresponding junction covered by 0, 5, and 109 reads in RNA-Seq

Like Collecting >30 Billion Reads of RNA-Seq Data!!
Short RNAs are key players of gene regulation

- **RNAi**
- microRNA precursor
- viral infection
- 18-26 nt RNA
  - mRNA degradation
  - inhibition of translation
  - gene silencing
TruSeq small RNA

- Total RNA or Purified Small RNA Fragments
  - RNA 3' Adapter
    - 3' Ligation
  - RNA 3' Adapter
    - 5' Ligation
  - RNA 5' Adapter
    - RNA 3' Adapter
      - RT-PCR - 1st Strand Synthesis
      - PCR Amplification
      - Gel Purification (Pooled Index Option)
      - Index Sequence
# TruSeq small RNA

<table>
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<tr>
<th>App</th>
<th>Description</th>
<th>Details</th>
<th>Cons</th>
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</table>
| | Alignment to abundant, mature miRNA, other RNA and genomic. Outputs mature miRNAs, isomiRs and piRNAs. Optional novel precursor discovery and pairwise differential expression analysis. | • miRBase 21 supported for human, mouse & rat  
• 100 samples/200Gb limit  
• 25Gb/sample limit | • Adapter trimming not included  
• No target gene info available  
• Not compatible with Functional Impact or Pathway Analysis apps |
| | Differential miRNA expression analysis between 2 conditions | • Includes adapter trimming  
• miRBase 21 supported for human and mouse and optional download of latest miRBase DB  
• Choice of Outlier replacement from DESeq2 | • Rat genome not supported  
• No miRNA precursor discovery  
• Not compatible with Functional Impact or Pathway Analysis apps |
DNA methylation

Genes that can be expressed

Promoter region

Genes inactivated by DNA methylation

Methylated

Unmethylated
TruSeq DNA Methylation
TruSeq DNA Methylation

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</thead>
<tbody>
<tr>
<td>MethylSeq</td>
<td>TruSeq Methylation Libraries: Whole Genome BiSulfite Sequencing</td>
<td>• Uses Bismark for alignment</td>
<td>• Only Human</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Bismarks maps bisulfite treated sequencing reads to the reference genome and performs methylation calls.</td>
<td>• Single sample analysis</td>
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<tr>
<td></td>
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<td>• Alignment method uses Bowtie2</td>
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</tbody>
</table>
TruSeq Methyl Capture, EPIC

Enrichment bisulfite sequencing library preparation for affordable sequencing of the methylome

Two day workflow, optimized for Illumina instruments, analysis in BaseSpace

Comprehensive fixed EPIC panel overlaps with the CpGs on the MethylationEPIC BeadChip

Custom versions (including human, mouse, rat, and cow) available through DesignStudio within a few months of launch
TruSeq ChIP
Protein-DNA interactions
# ChIP-Seq

**ChIP-Seq for Protein-DNA interaction**

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| | Enriched region identification of Chromosome IP (ChIP-Seq) experiments and motive discovery. | • Alignment & Peak Calling: MACS  
• Peak annotation and Motive Enrichment: HOMER  
• Pairwise analysis recommended  
• ChIP vs Input and ChIP vs ChIP comparisons supported  
• Single ChIP analysis (no control) possible as well | • Identifies "narrow" peaks (i.e transcription factor binding sites), not "broad" peaks (i.e histone modifications).  
• hg19 only reference |
Methods Selector

Methods for transcriptomic analysis:

Ribo-Seq/ART-Seq/GTI-Seq

Active mRNA Translation Sequencing (ART-seq), also called ribosome profiling (Ribo-Seq) or Global Translation Initiation Sequencing (GTI-Seq), isolates RNA that is being processed by the ribosome in order to monitor the translation process. In this method ribosome-bound RNA first undergoes digestion. The RNA is then extracted and the rRNA is depleted. Extracted RNA is reverse-transcribed to cDNA. Deep sequencing of the cDNA provides the sequences of RNAs bound by ribosomes during translation.

References:


Associated kits:
- ARTSeq/TruSeq Ribosome Profiling kit

Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position

Assay for Transposase Accessible Chromatin (ATAC-Seq)

Identifies open chromatin by using Nextera to generate libraries

Uses less starting material than other related methods (DNAse-seq or FAIRE-seq)

Much faster than other related methods
Single-Cell Transcriptomics

- Applications
  - Disease phenotypes with heterogeneous cell types (e.g. cancer)
  - Developmental Biology – cell fate decisions
  - Expression profiling of sub-cellular compartments
  - Single-cell allele-specific expression

The amount of RNA in any given cell strongly depends upon the cell’s size and metabolic state. In general, most cells in the body have 10 to 30 pg of RNA. The SMARTer Ultra Low RNA Kit for Illumina Sequencing allows researchers to generate robust, high-quality RNA sequencing results from the RNA in a single cell.
BaseSpace®

Workflow | Storage | Analysis | Sharing

>4,000 Instruments

>30,000 Users

>240,000 Runs

>60 Apps

Illumina Core Applications

Third Party Applications

BaseSpace Labs Apps
### Illumina Core

- **16S Metagenomics**
- **Amplicon DS**
- **BWA & Isaac Enrichment**
- **BWA & Isaac WGS**
- **Cufflinks Assembly & DE**
- **MethylSeq**
- **RNA Express**
- **Small RNA**
- **TopHat Alignment**
- **TruSeq Amplicon**
- **TruSeq Long-Read Assembly**
- **TruSeq Phasing Analysis**
- **TruSeq Targeted RNA**
- **Tumor Normal**
- **VariantStudio**

### Third-Party

- **DNAStar**
- **DeepChek-HBV, HCV, HIV**
- **EDGC Annotator**
- **Elastic Genome Browser**
- **GeneTalk Variant Analyzer**
- **GENIUS Metagenomics: Know Now**
- **Genomatix Pathway System (GePS)**
- **Genome Profiler**
- **iPathwayGuide**
- **LoFreq Rare Variant Caller**
- **MiRNA Analysis**
- **MyFLq**
- **Novoalign Generic DNA pipeline**
- **OncoMD**
- **PathSEQ Virome**
- **PEDANT Sequence-Analyzer**
- **Phy-Mer**
- **Protein Expression Assembler**
- **Protein Expression Extractor**
- **Protein Expression Workflow**
- **RNA-Seq Translator**
- **SPAdes Genome Assembler**
- **SWATHAtlas Ion Library Generator**
- **The Broad’s IGV**
- **Variant Interpreter**

### BaseSpace Labs

- **FASTQ Toolkit**
- **FASTQC**
- **Kraken Metagenomics**
- **NextBio Annotates RNA-Seq**
- **NextBio Transporter**
- **PicardSpace**
- **Prokka**
- **Prokka Genome Annotation**
- **SRA Import**
- **SRA Submission**
- **SRST2**
- **Variant Calling Assessment Tool**
- **Velvet de novo Assembly**

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For research use only. Not for use in diagnostic procedures.
Thank you!