Novel methods for RNA and DNA-Seq analysis using SMART® Technology

Andrew Farmer, D. Phil.
Vice President, R&D
Clontech Laboratories, Inc.
Agenda

• Enabling Single Cell RNA-Seq using SMART Technology
  – SMART technology overview / application to RNA-Seq
  – SMARTer Ultra Low RNA Kit for the Fluidigm C1 System
  – SMARTer® Ultra™ Low Input RNA Kit for Sequencing - v3

• Expanding Applications for SMART Technology
  – SMARTer Stranded RNA-Seq Kits
  – RiboGone™: rRNA removal for low input samples
  – SMARTer Universal Low Input RNA Kit for Sequencing
  – DNA SMART™: a novel method for generating ChIP-Seq libraries from low quantities of cells

• Supporting Products for Illumina® Sequencing

Novel methods for RNA and DNA-Seq analysis using SMART® technology
Key aspects of SMART technology

- The SMARTScribe™ Reverse Transcriptase (RT) makes cDNA.
- When the SMARTScribe RT reaches the 5' end of the RNA, its terminal transferase activity adds a few nucleotides.
- The SMARTer Oligonucleotide base-pairs with the non-templated nucleotide stretch, creating an extended template to allow the SMARTScribe RT to continue replicating.
- The SMARTer primer and oligo serve as universal priming sites for cDNA amplification by PCR.
Overview of the SMARTer workflow

- **Ultra Low Input Kit for single cell samples**
  - RIN >8
  - Total RNA or whole cells
  - Oligo dT primed SMART cDNA synthesis
  - Full-length cDNA amplification by LD-PCR

**DAY ONE**

- SMARTer Ultra Low RNA Kit for Illumina Sequencing (UL, UL-HV)
- SMARTer Ultra Low RNA Kit for the Fluidigm C1 System

**DAY TWO**

- Covaris shearing of full-length cDNA
- Low Input Library Prep
- Cluster generation

Novel methods for RNA and DNA-Seq analysis using SMART® technology
Primary sequencing metrics: 
*Mouse brain control RNA*

<table>
<thead>
<tr>
<th>Amount of input total RNA (ng)</th>
<th>Unique Reads</th>
<th>Mapped Reads</th>
<th>rRNA gene</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ng</td>
<td>120</td>
<td>15,000</td>
<td>9,000</td>
<td>6,000</td>
</tr>
<tr>
<td>1 ng</td>
<td>120</td>
<td>15,000</td>
<td>9,000</td>
<td>6,000</td>
</tr>
<tr>
<td>0.1 ng</td>
<td>120</td>
<td>15,000</td>
<td>9,000</td>
<td>6,000</td>
</tr>
<tr>
<td>0.05 ng</td>
<td>120</td>
<td>15,000</td>
<td>9,000</td>
<td>6,000</td>
</tr>
<tr>
<td>0.01 ng</td>
<td>120</td>
<td>15,000</td>
<td>9,000</td>
<td>6,000</td>
</tr>
</tbody>
</table>

### Table:

<table>
<thead>
<tr>
<th>Sequencing Metric</th>
<th>Amount of input total RNA (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 ng</td>
</tr>
<tr>
<td>% mapping to genome</td>
<td>97</td>
</tr>
<tr>
<td>% ribosomal RNA</td>
<td>4.3</td>
</tr>
<tr>
<td>Number of genes detected</td>
<td>16,610</td>
</tr>
</tbody>
</table>
SMART technology: Excellent reproducibility - even at low input

Scatter plots comparing gene counts (i.e., log₂ RPKM values) for replicate samples

- ABRF study of four different low input kits
- ERCCs spiked into three replicas of 50 pg, 500 pg, and 5 ng of total RNA.

High correlation to TaqMan data – even at low input
Why study single cells?

Gene expression profiling in single cells from the pancreatic islets of Langerhans reveals lognormal distribution of mRNA levels

Martin Bengtsson,1,2,4 Anders Ståhlberg,2 Patrik Rorsman,1,3 and Mikael Kubista2

1Department of Experimental Medical Science, Lund University, 221 84 Lund, Sweden; 2Department of Chemistry and Biosciences, Molecular Biotechnology, Chalmers University of Technology and TATAA Biocenter, Lundberg Laboratory, 405 30 Göteborg, Sweden; 3The Oxford Centre for Diabetes, Endocrinology and Metabolism, The Churchill Hospital, Oxford, OX3 7LJ, United Kingdom

Cell-to-cell expression can vary significantly (transcription occurs in bursts)
Average population expression may not necessarily reflect true correlations between genes at the level of the cell
Transcriptome analysis of individual cells

- 12 individual cancer cells were isolated from three different cancer cell lines
  - Four cells each from prostate (PC3 and LNCaP) and bladder (T24)
- Global gene expression profiles were used to analyze each single-cell transcriptome
- Individual cells could be categorized according to their cell line of origin based on their transcriptomes

Revealing single cell expression: Cluster analysis

Down-regulated genes

Up-regulated genes

SMARTer Ultra Low for the Fluidigm C₁

Enrich → Load & Capture → Wash & Stain → Isolate → Lyse, RT & Amplify → Prepare Library → Sequence → Analyze

C₁ Single-Cell Auto Prep System

Any Illumina System

* Slide from Fluidigm

Novel methods for RNA and DNA-Seq analysis using SMART® technology
SMARTer Ultra Low for the Fluidigm C1: Similar mapping performance to the original kit

Transcriptome analysis of individual K562 cells

Analysis of single-cell RNA-seq data
- Average read depth: 3M reads
- >95% cells had >500K total reads
- <1% reads from ribosomal RNA

* Data from Fluidigm
Ensemble single cell data recapitulates the expression profile of the bulk

Cultured HCT116 cells were used as sample input

Agenda

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  – SMARTer Ultra Low Input RNA Kit for Sequencing - v3

• Expanding Applications for SMART Technology
  – SMARTer Stranded RNA-Seq Kits
  – RiboGone: rRNA removal for low input samples
  – SMARTer Universal Low Input RNA Kit for Sequencing
  – DNA SMART: a novel method for generating ChIP-Seq libraries from low quantities of cells

• Supporting Products for Illumina® Sequencing
SMARTer Ultra Low Input RNA-Seq - v3: Benefits

- **Simplified sample prep:** A single-tube protocol works directly on whole cells (fewer steps)

- **Very low input range:** 1–1,000 cells or 10 pg–10 ng of total RNA

- **Improved cDNA amplification:** Better amplification of high GC-content genes

- **Suitable for multiple sequencing platforms:** Compatible with Illumina and Ion Torrent NGS platforms

- **High-quality RNA-seq data:** More genes identified, low rRNA reads, and improved representation of GC-rich genes
SMARTer Ultra Low Input RNA-Seq - v3: Changes

Variations of SMART technology for single cell (or few cells or high quality RNA) transcriptome analysis:

- SMARTer Ultra Low RNA Kit for Illumina Sequencing
- SMARTer Ultra Low RNA Kit for the Fluidigm C₁ System
- SMARTer Ultra Low RNA Kit for Sequencing - v3

Common features:

- Oligo dT priming
- 10 pg sensitivity
- Work directly with cells
- Single tube protocol minimizes handling

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- Work directly with cells
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Common features:

- Oligo dT priming
- 10 pg sensitivity
- Work directly with cells
- Single tube protocol minimizes handling

Improved sensitivity – especially for GC-rich transcripts, simpler protocol, Ion compatible
SMARTer Ultra Low Input RNA-Seq - v3: Workflow

1. Total RNA (RIN > 8) or whole cells
2. SMART cDNA synthesis
3. Full-length cDNA amplification by LD-PCR

**Day One**

- Enzymatic shearing of full-length cDNA
- Covaris shearing of full-length cDNA

**Day Two**

- Ion Xpress Library Prep
- Modified Illumina Nextera® protocol
- Low Input Library Prep Kit

Sequencing
## SMARTer Ultra Low Input RNA-Seq - v3

### Comparison with previous kit

<table>
<thead>
<tr>
<th>Input</th>
<th>100 pg total HBR</th>
<th>10 pg total MBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol</td>
<td>UL - HV</td>
<td>UL - v3</td>
</tr>
<tr>
<td>Number of reads (Millions)</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Mapped to rRNA (%)</td>
<td>4.4%</td>
<td>4.2%</td>
</tr>
<tr>
<td>Mapped to RefSeq (%)</td>
<td>80%</td>
<td>79%</td>
</tr>
<tr>
<td>Mapped uniquely to RefSeq (%)</td>
<td>70%</td>
<td>69%</td>
</tr>
<tr>
<td>Mapped to exons (%)</td>
<td>57%</td>
<td>58%</td>
</tr>
<tr>
<td>Mapped to introns (%)</td>
<td>43%</td>
<td>42%</td>
</tr>
<tr>
<td>Number of genes</td>
<td>11,539</td>
<td>11,631</td>
</tr>
</tbody>
</table>

UL - v3—SMARTer Ultra Low Input RNA Kit for Sequencing - v3  
UL - HV—SMARTer Ultra Low Input RNA for Illumina Sequencing - HV kit  
HBR—Human Brain Total RNA  
MBR—Mouse Brain Total RNA

Sequencing data generated on an Illumina MiSeq® instrument
SMARTer Ultra Low Input RNA-Seq - v3

Better detection of GC-rich genes

A  UL-HV replicates

UL-HV-2 RPKM

UL-HV RPKM

R=0.981

B  UL-v3 replicates

UL-v3-2 RPKM

UL-v3-1 RPKM

R=0.982

C  UL-HV vs. UL-v3

UL-HV RPKM

UL-v3 RPKM

Input: Human Brain Total RNA (100 pg)
Sequencing data generated on an Illumina MiSeq instrument
Reproducibility across input levels
Scatter plots comparing RPKM values for replicate samples

10 pg Human Universal RNA

100 pg Human Universal RNA

R = 0.974

R = 0.997
SMARTer Ultra Low Input RNA-Seq - v3: MAQC comparison:

High accuracy of expression levels

Slope: 0.807
R = 0.816
573 transcripts

100 pg input into SMARTer Ultra Low Input RNA Kit for Sequencing – v3
### SMARTer Ultra Low Input RNA-Seq - v3:
*Primary sequencing metrics from cells*

Sequencing data generated on an Ion Torrent instrument

<table>
<thead>
<tr>
<th>Input</th>
<th>HeLa</th>
<th>Jurkat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 cell</td>
<td>1,000 cells</td>
</tr>
<tr>
<td>Number of reads (Millions)</td>
<td>2.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Mapped to rRNA (%)</td>
<td>0.2%</td>
<td>1.0%</td>
</tr>
<tr>
<td>Mapped to RefSeq (%)</td>
<td>94%</td>
<td>91%</td>
</tr>
<tr>
<td>Mapped uniquely to RefSeq (%)</td>
<td>83%</td>
<td>80%</td>
</tr>
<tr>
<td>Mapped to exons (%)</td>
<td>85%</td>
<td>90%</td>
</tr>
<tr>
<td>Mapped to introns (%)</td>
<td>15%</td>
<td>10%</td>
</tr>
<tr>
<td>Number of genes</td>
<td>9,256</td>
<td>12,586</td>
</tr>
<tr>
<td>Pearson correlation (R)</td>
<td>0.97</td>
<td></td>
</tr>
</tbody>
</table>
Even gene body coverage

![Graph showing even gene body coverage for different cell samples.](image-url)
SMARTer Ultra Low Input RNA-Seq - v3: Summary

- **Simplified sample prep:** A single-tube protocol works directly on whole cells
- **Very low input range:** 1–1,000 cells or 10 pg–10 ng of total RNA
- **Improved cDNA amplification:** The SeqAmp DNA Polymerase better amplifies high GC-content genes
- **Suitable for multiple sequencing platforms:** Compatible with Illumina and Ion Torrent NGS platforms
- **High-quality RNA-seq data:** More genes identified, low rRNA reads, and improved representation of GC-rich genes
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  – DNA SMART: a novel method for generating ChIP-Seq libraries from low quantities of cells

• Supporting Products for Illumina® Sequencing
SMARTer Stranded RNA-Seq Kits: Overview

- Start with full length or degraded rRNA-removed RNA
- Two formats available: 
  **Low-input:** 10–100 ng (total RNA)  
  100 pg–1 ng (polyA-purified RNA)  
  **High-input:** 100 ng–1 μg (total RNA)
- Ligation-free addition of Illumina adapters
- Produce libraries ready for cluster formation in ~5 hours
- >99% of reads map to the correct strand
**SMARTer Stranded RNA-Seq Kits:**

*Mapping data for low input kit*

Mapping statistics from libraries made from poly(A)-purified, Human Brain RNA

<table>
<thead>
<tr>
<th>Sequence Alignment Metrics*</th>
<th>Input RNA</th>
<th>100 ng</th>
<th>10 ng</th>
<th>1 ng</th>
<th>100 pg</th>
</tr>
</thead>
<tbody>
<tr>
<td>% pairs mapped to RefSeq</td>
<td>77%</td>
<td>76%</td>
<td>78%</td>
<td>76%</td>
<td></td>
</tr>
<tr>
<td>% pairs uniquely mapped to RefSeq</td>
<td>74%</td>
<td>74%</td>
<td>75%</td>
<td>74%</td>
<td></td>
</tr>
<tr>
<td>Total exons</td>
<td>65%</td>
<td>65%</td>
<td>64%</td>
<td>66%</td>
<td></td>
</tr>
<tr>
<td>Total introns</td>
<td>35%</td>
<td>35%</td>
<td>36%</td>
<td>34%</td>
<td></td>
</tr>
<tr>
<td>Genes identified (&gt;0.1 FPKM)</td>
<td>15,632</td>
<td>15,642</td>
<td>15,568</td>
<td>14,993</td>
<td></td>
</tr>
<tr>
<td>Number of reads (Millions)</td>
<td>23.8</td>
<td>26.1</td>
<td>27.7</td>
<td>22.3</td>
<td></td>
</tr>
</tbody>
</table>

* All data shown is based on strand-specific alignment.
Comparison of FPKMs at 100pg and 100ng input levels

R = 0.985
SMARTer Stranded RNA-Seq Kits:
Broad linearity of the ERCC standards

(A) ERCC Analysis from 100 ng Libraries

$y = 0.97x + 3.3$
$R = 0.981$
83 transcripts detected

(B) ERCC Analysis from 100 pg Libraries

$y = 0.92x + 3.7$
$R = 0.965$
69 transcripts detected

Novel methods for RNA and DNA-Seq analysis using SMART® technology
Strand specificity can affect counts:

*CDR1 antisense transcript*

Gene Counts By Method

Sequence Coverage*

*CDR1 annotation is on the minus Strand*
Strand specificity can affect counts: *CDR1 antisense transcript*

miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA

Thomas B. Hansen¹, Erik D. Wiklund¹,², Jesper B. Bramsen¹, Sune B. Villadsen¹, Aaron L. Statham², Susan J. Clark², and Jørgen Kjems¹,*

¹Department of Molecular Biology, Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus, Denmark and ²Epigenetics Laboratory, Cancer Program, Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia

sites in the 5’ UTR and ORF of mRNAs (Forman et al., 2008; Orom et al., 2008; Tay et al., 2008). In addition, functional RISC activity has been detected in the nucleus of human cells (Langlois et al., 2005; Robb et al., 2005), and a subset of miRNAs are predominantly nuclear (Hwang et al., 2007; Liao et al., 2010), suggesting that miRNAs may have a variety of biological functions distinct from canonical 3’ UTR target mRNA repression.

In fission yeast and plants, small interfering RNAs (siRNAs)

Strand-specific quantification of CDR1 mRNA and circular AS

To directly assess the effect of miR-671 on both antisense RNA and CDR1 mRNA, we designed a strand-specific qRT-PCR approach (Figure 3A). Interestingly, the antisense levels were several orders of magnitude higher than CDR1 mRNA levels, indicating that the non-coding antisense transcript is the predominant RNA species of the CDR1 locus (Figure 3B).

*CDR1 annotation is on the minus Strand"
rRNA removal from small quantities of RNA: RiboGone

- RiboGone-Mammalian
  - Input compatibility range: 10 ng to 100 ng
  - Sample type: Human, Mouse and Rat
  - Targets nuclear, and some mitochondrial, ribosomal RNA
RiboGone-treated samples maintain accuracy with MAQC data

- Human Universal Reference RNA (HURR) or Human Brain Reference RNA (HBRR)
- Samples treated with the RiboGone - Mammalian kit
- cDNA libraries generated using SMARTer Stranded RNA-Seq Kit

\[
y = 0.8749x - 0.1115 \\
R = 0.860 \\
623 transcripts
\]
Integrated workflow for high-input total RNA samples on Illumina platforms

**A**
- Total RNA
  - rRNA
  - mRNA
  - Add Total RNA
  - Hyb Buffer
  - RiboGone oligos
  - Add RNase H
  - Add DNase
  - Clean up enzymes

**B**
- mRNA
  - 5’ X X X X X
  - 3’
  - SMARTer Stranded Oligo
  - Forward PCR Primer HT
  - First-strand synthesis and tailing by RT

**C**
- RNA-Seq library
  - Read 1
  - Amplify cDNA by PCR with Illumina Indexing Primer Set
  - Reverse PCR primer HT
  - Read 2
  - SMART Stranded N6 Primer

Novel methods for RNA and DNA-Seq analysis using SMART® technology
SMARTer Stranded Total RNA Sample Prep Kit - HI Mammalian

Analysis of sequencing data

Sequence Alignment Metrics (Input 400 ng)

<table>
<thead>
<tr>
<th>RNA source</th>
<th>Human Universal</th>
<th>Human Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of reads (millions)</td>
<td>8.5 (paired end reads)</td>
<td></td>
</tr>
<tr>
<td>Percentage of reads:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rRNA</td>
<td>0.3%</td>
<td>5.3%</td>
</tr>
<tr>
<td>Mapped to genome</td>
<td>94%</td>
<td>88%</td>
</tr>
<tr>
<td>Mapped uniquely to genome</td>
<td>91%</td>
<td>84%</td>
</tr>
<tr>
<td>Exonic</td>
<td>43%</td>
<td>50%</td>
</tr>
<tr>
<td>Intronic</td>
<td>43%</td>
<td>33%</td>
</tr>
<tr>
<td>Intergenic</td>
<td>14%</td>
<td>12%</td>
</tr>
<tr>
<td>Number of genes identified</td>
<td>17,570</td>
<td>17,600</td>
</tr>
<tr>
<td>Percentage of ERCC transcripts with correct strand</td>
<td>99.3%</td>
<td>98.8%</td>
</tr>
</tbody>
</table>
SMARTer Universal Low Input RNA Kit for Sequencing

For total RNA-seq
Illumina or Ion Torrent platforms

Compromised samples
Non-poly(A) RNA
LCM samples

Total RNA
rRNA depletion
SMART cDNA synthesis
cDNA amplification by PCR
Removal of SMART adapters
Sequencing library production
Sequencing

DAY ONE

Sheared PolyA-purified or rRNA-depleted RNA
SMARTer IIA oligonucleotide
First-strand synthesis and tailing by RT
Template switching and extension by RT
Amplify cDNA by PCR with unblocked primer
Double-stranded cDNA
Rsal digestion

DAY TWO

Low Input Library Prep Kit /
Ion Xpress Plus Fragment Library Preparation Kit

Sequencing

Novel methods for RNA and DNA-Seq analysis using SMART® technology
SMARTer Universal Low Input RNA Kit for Sequencing

Reproducibility

Accuracy—Comparison to MAQC

y = 0.959x−0.2491
R = 0.870
585 genes

y = 1.0035x−0.0544
R = 0.881
587 genes

y = 1.073x−0.076
R = 0.884
621 genes

~125 pg

~600 pg

~6 ng
FFPE sequencing (10 ng inputs)

Input Breast Carcinoma FFPE:

Output sequences (RiboGone, Universal Kit):

- rRNA: 1.5%
- Intergenic: 61.9%
- Introns: 20.5%
- Exons: 16.2%

(16,463 genes identified)
DNA SMART ChIP-seq kit:
Technology overview

- Fast, single tube workflow
- Works with both dsDNA and ssDNA
- Generates high complexity libraries from pg amounts of input DNA
- Library complexity (ENCODE standards) preserved with 250–500 pg input

**Fast Track Workflow**

1. **T-tailing**
   - DNA SMART Oligo
   - Add Poly(dA) Primer

2. **Priming and replication**
   - dsDNA or ssDNA

3. **Template switching**
   - Fwd PCR Primer
   - Rev PCR Primer

4. **Addition of adapters and amplification**
   - Read 1
   - Read 2

**Total time: ~4 hours**
Maintenance of complexity and reproducibility even with low input amounts

ChIP assay performed on HEK 293T cells with anti-H3K4me3
Libraries generated with inputs from 4 ng to 50 pg

Complexities in libraries generated by the DNA SMART ChIP-seq Kit

Threshold per ENCODE standards

Overlapping peaks between technical replicates

Novel methods for RNA and DNA-Seq analysis using SMART® technology
Excellent overlap between identified peaks across input levels

Novel methods for RNA and DNA-Seq analysis using SMART® technology
Excellent reproducibility from 1 million to 10,000 cells

1,000,000 cells

200,000 cells

50,000 cells

10,000 cells

1,000,000 cells

ENCODE

RefSeq

GAPDH

Antibody: H3K4me3

No antibody

H3K4me3 / 293 cells (U. Washington)

88% overlap

89% overlap

Novel methods for RNA and DNA-Seq analysis using SMART® technology
Supporting products for Illumina sequencing

Library Quantification Kit

Low Input Library Prep Kit
Library Quantification Kit for Illumina sequencing libraries

RNA-seq Workflow

- Quantitation of fragments containing Illumina adapters
- Accurately detect library concentration prior to sequencing

[Diagram of RNA-seq Workflow]

Structure of a typical fragment from an Illumina sequencing library
Standards provide for accurate quantitation

Minimal lot-to-lot variation for DNA standards

- Plot the C_t values for the 4 standards to generate a standard curve
- Determine the corresponding concentration from the standard curve
**Low Input Library Prep Kit:**
*Library prep for Illumina platforms*

Optimally designed to work downstream of the SMARTer Universal Kit

- **Input:** 50 pg–10 ng of ds cDNA
- **Indexes:** 12
- **Compatibility:** All Illumina platforms

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 rxns</td>
<td>Library construction kit with 12 indexes for Illumina platforms</td>
</tr>
</tbody>
</table>
SMARTer Selection Guide

### RNA-Seq Selection Guide

<table>
<thead>
<tr>
<th>Application</th>
<th>mRNA-Seq (dT priming)</th>
<th>Total RNA-Seq (N₆ priming)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>With strand information</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Without strand information</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ribosomal RNA removal required</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample Type (Input amount)</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells and high-quality total RNA, (1–1,000 cells; 10 pg–10 ng total RNA)</td>
<td>4</td>
</tr>
<tr>
<td>LCM samples, FFPE tissue, prokaryotic samples, or samples with degraded RNA, (2–100 ng total RNA)</td>
<td>2 + 3</td>
</tr>
<tr>
<td>High input total RNA samples, any quality, (100 ng–1 μg total RNA)</td>
<td>3 + 6</td>
</tr>
<tr>
<td>SMARTer Ultra Low Input RNA Kit for Sequencing - v3</td>
<td>5</td>
</tr>
<tr>
<td>SMARTer Stranded RNA-Seq Kit</td>
<td></td>
</tr>
<tr>
<td>SMARTer Universal Low Input RNA Kit for Sequencing</td>
<td></td>
</tr>
<tr>
<td>SMARTer Ultra Low RNA Kit for the Fluidigm C₁ System</td>
<td></td>
</tr>
<tr>
<td>SMARTer Stranded Total RNA Sample Kit - HI Mammalian</td>
<td></td>
</tr>
<tr>
<td>RiboGone - Mammalian (for ribosomal RNA removal)</td>
<td></td>
</tr>
</tbody>
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**INSTRUMENT COMPATIBILITY**

- Illumina® platform
- Fluidigm C₁ cell capture platform
- Compatible with Illumina and Ion Torrent platforms

### NGS Resource Portal: www.clontech.com/ngs
Learning Resources

NGS Resource Portal

**TECH NOTES**
- rRNA removal and Stranded RNA-seq
- The latest in single-cell RNA-seq
- ChIP-seq library preparation
- Our solution for RNA-seq from FFPE samples

**WEBINARS**
- Stranded RNA-seq for low input samples
- New and improved single-cell RNA-seq
- Low-input RNA-seq with Ion Torrent platforms
- See all available webinars

**SCIENTIFIC POSTERS**
- Stranded RNA-seq with SMARTer kits
- SMARTer Ultra Low cDNA synthesis
- Comparison of low-input RNA-seq kits by ABRF
- SMARTer Universal application for FFPE samples
- Low-input ChIP-Seq library preparation

**FAQs**
- Answers to your questions about:
  - RNA-seq
  - ChIP-seq

**TIPS TO GET SMARTer**
- Learn useful tricks for cDNA synthesis, like how to make a magnetic separator or identify the ideal pellet

**CITATION LISTS**
- Publications that have used SMARTer kits for transcriptome analysis

**SELECTION GUIDE**
- Which RNA-seq kit is right for you? View the Selection Guide

Novel methods for RNA and DNA-Seq analysis using SMART® technology
that's
GOOD
science!