New Technologies for Sensitive, Low-Input RNA-Seq

Clontech Laboratories, Inc.
Outline

Introduction

Single-Cell-Capable mRNA-Seq Using SMART Technology
- SMARTer® Ultra™ Low RNA Kit for the Fluidigm C_1 System
- SMART-Seq™ v4 Ultra Low Input RNA Kit for Sequencing

Total RNA-Seq Applications
- SMARTer Stranded RNA-Seq Kit
- SMARTer Stranded Total RNA Sample Prep Kit - HI Mammalian
- SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian

Expanding Applications for SMART Technology
- DNA SMART™ ChIP-Seq Kit
Next-Gen Sequencing: RNA-Seq

cDNA synthesis for a complete representation of the transcriptome

- Industry standard for low-input and single-cell RNA-Seq
- Full-length gene body coverage
- Simplified workflow
- Highest sensitivity and reproducibility
- High-quality sequencing libraries

SMART Technology

- Low-input total RNA samples
- Single cells
- Degraded, LCM, or FFPE samples
- Prokaryotic samples
Transcriptome Analysis with NGS

• RNA-seq produces millions of sequences from complex RNA samples. With this powerful approach, we can:
  – Measure gene expression/evaluate differential gene expression between different conditions, cell types, etc.
  – Discover and annotate complete transcripts
  – Discover and characterize alternative splicing (isoforms), polyadenylation & SNPs

• In recent years, more people have been interested in investigating such applications at the single-cell level

The Technology and Biology of Single-Cell RNA Sequencing
SMARTer cDNA Synthesis for NGS

**dT Primed**
- mRNA
- Polyadenylated RNA
- Single cell capacity

**SMART-Seq™ v4 Ultra™ Low Input RNA Kit for Sequencing**
- Use with Ion Torrent or Illumina platforms

**SMARTer Ultra Low RNA Kit for the Fluidigm C1™ System**
- 96 single cells in parallel

**N6 Primed**
- Coding and non-coding RNA
- Non-polyadenylated RNA
- Degraded samples

**SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian**
- Use with Illumina platforms
- Use with highly degraded samples (FFPE)

**SMARTer Stranded Total RNA-Seq Kit**
- Use with Illumina platforms

**SMARTer Stranded Total RNA-Seq Kit - Hi Mammalian**
- Use with typical input RNA samples
Importance of Studying 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

1 Genome Research (2005) 15:1388–1392
Spring Harbor Laboratory Press

- Gene expression can vary significantly between cells (transcription occurs in bursts).
- Average expression of a population may not necessarily correlate with gene expression at the level of an individual cell.

Fig 1. Histograms showing the expression levels of 96 cells expressing ActB in logarithmic and linear scale (inset).
Transcriptome Analysis of Individual Cancer Cells

• Individual cells can be categorized according to their cell line of origin based on their transcriptome

• 12 individual cancer cells were isolated from three different cancer cell lines
  – Four cells each from prostate (PC3 and LNCaP) and bladder (T24) cell lines

• Global gene expression profiles were used to analyze each single-cell transcriptome

Full-length mRNA-seq from single-cell levels of RNA and individual circulating tumor cells.
Transcriptome Analysis of Individual Neurons

Single-cell RNA-seq discovers a wealth of new RNA markers that define discrete groups of neurons

Posted by: RNA-Seq Blog in Commentary 6 days ago 667 Views

from Bio-IT World By Aaron Krof

An adult mouse’s brain, an object not much bigger than the last joint of your pinky finger, contains around 75 million neurons. At the Allen Institute for Brain Science in Seattle, the Mouse Cell Types program, led by Hongkui Zeng, is trying to figure out just how many varieties of neurons make up this vast complex, and what makes each one unique.

Zeng’s research focuses on the primary visual cortex, a tiny sliver of the brain where signals from the eyes are processed and interpreted. Because vision is a relatively well-defined process, it’s thought to be a good model for connecting the behavior of individual neurons to larger brain functions.

“You really can’t understand a system until you understand its parts,” says Bosiljka Tasic, a founding member of the Mouse Cell Types program.

To a shocking extent, those parts are still a mystery. Many supposed cell types are based on little more than what you can see through a microscope: a neuron’s shape, or the pattern of rootlike dendrites extending from its body. These morphological traits, though important, are hard to see in full, and even harder to track methodically across thousands or millions of cells.

This month, Zeng’s team published a study in Nature Neuroscience that takes advantage of new technological developments to get a fine-grained look at the molecular toolkits of single neurons. Using newly refined methods to isolate single cells, Zeng’s lab collected over 1,600 brain cells from the visual cortices of adult mice, intact and in good shape for sequencing. With advances in highly parallel, unbiased RNA sequencing, the group was able to measure each cell’s entire “transcriptome”—the array of RNA molecules that indicate which genes are actively producing proteins—at a depth that reveals even the scarcest RNA traces.

“We think this is probably the most comprehensive survey of a cortical area,” says Tasic, who co-led the study with her colleague Vilas Menon. “Many studies that are coming out now do very shallow sequencing.

http://casestudies.brain-map.org/celltax#section_introa
Our Solutions for mRNA-Seq

**dT Priming**

- mRNA
- Single-cell capacity
- Polyadenylated RNA
- With no strand information

**Ultra-low-input total RNA and 1–1,000 cells**

SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing

Input = 10 pg–10 ng; 1–1,000 cells

- Ultra-low-input total RNA, poly(A⁺) RNA
- Single-cell capacity
- Compatible with Ion Torrent and Illumina® platforms

**SMARTer Ultra Low RNA Kit for the Fluidigm C1 System**

Input = 1–1,000 cells

- 96 single cells in parallel
- Compatible with Illumina platforms

For the Fluidigm C1 cell-capture system
Single-Cell and Ultra-Low Input RNA-Seq Solutions—Timeline

- SMART technology from Clontech
- SMARTer Ultra Low RNA Kit for Illumina Sequencing
- SMARTer Ultra Low RNA Kit for the Illumina C1 System
- SMARTer Ultra Low Input RNA Kit for Illumina Sequencing - v3
- SMARTer Ultra Low Input RNA Kit for Sequencing - v3
- SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing

- 1996
- 2011
- 2013
- 2014
- 2015

RNA sequenced
SMART-Seq2 innovations

1970s
SMARTer Ultra Low RNA Kit for the Fluidigm C₁ System

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

C₁ Single-Cell Auto Prep System

* Slide from Fluidigm
SMART-Seq v4—Advancements

• Input = 10 pg–10 ng; 1–1,000 cells for mRNA-seq

• Optimized template-switching oligo: Incorporates LNA and with our proprietary knowledge of template switching

• Improved sensitivity and reproducibility
  • More genes identified
  • Higher yield

• Simplified protocol

The SMART-Seq v4 kit outperforms all previous generations of SMARTer Ultra Low kits by increasing sensitivity and reproducibility.
SMART-Seq v4—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 SMART-Seq Oligonucleotide base-pairs with the non-templated nucleotide stretch, creating an extended template to allow the SMARTScribe RT to continue replicating.
- The SMART-Seq primer and oligo serve as universal priming sites for cDNA amplification by PCR.
Comparing ULv3, SMART-Seq v4, and SMART-Seq2

<table>
<thead>
<tr>
<th>Input</th>
<th>10 pg Mouse Brain RNA</th>
</tr>
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<tbody>
<tr>
<td>Protocol</td>
<td>ULv3</td>
</tr>
<tr>
<td></td>
<td>SMART-Seq v4</td>
</tr>
<tr>
<td></td>
<td>SMART-Seq2</td>
</tr>
<tr>
<td>Number of PCR cycles</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>18</td>
</tr>
<tr>
<td>Number of reads (Millions)</td>
<td>4.0 (paired-end)</td>
</tr>
<tr>
<td>Replicate</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>No. of transcripts identified</td>
<td>11,647</td>
</tr>
<tr>
<td></td>
<td>10,885</td>
</tr>
<tr>
<td></td>
<td>14,731</td>
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<td></td>
<td>14,813</td>
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<td></td>
<td>12,080</td>
</tr>
<tr>
<td></td>
<td>12,039</td>
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<tr>
<td>Percentage of reads (%)</td>
<td></td>
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<tr>
<td>Mapped to genome</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>96</td>
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<td>72</td>
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<td></td>
<td>93</td>
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<td>Mapped to exons</td>
<td>73</td>
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<td>66</td>
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<td></td>
<td>67</td>
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<td>21</td>
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<td></td>
<td>19</td>
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<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>27</td>
</tr>
<tr>
<td>Mapped to intergenic regions</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>4.7</td>
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<td></td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>5.8</td>
</tr>
</tbody>
</table>

- Reduced background
- Improved sensitivity and reproducibility

Improvements to the template-switching oligo
Comparing ULv3, SMART-Seq v4, and SMART-Seq2

ULv3

SMART-Seq v4

SMART-Seq2

FPKMs <100

R = 0.911
ρ = 0.683

R = 0.972
ρ = 0.820

R = 0.966
ρ = 0.706

FPKMs <100

R = 0.376
ρ = 0.191

R = 0.739
ρ = 0.604

FPKMs <100
### Mapping Statistics from 10 pg–10 ng of MAQC Controls

<table>
<thead>
<tr>
<th>RNA source</th>
<th>HBRR</th>
<th>HURR</th>
<th>HBRR</th>
<th>HURR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input amount</td>
<td>10 pg</td>
<td></td>
<td>10 ng</td>
<td></td>
</tr>
<tr>
<td>Number of paired-end reads (Millions)</td>
<td>2.3</td>
<td></td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Number of PCR cycles</td>
<td>18</td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Transcripts with FPKM &gt;0.1</td>
<td>15,482</td>
<td>15,338</td>
<td>15,421</td>
<td>17,612</td>
</tr>
<tr>
<td>Transcripts with FPKM &gt;1</td>
<td>12,598</td>
<td>12,561</td>
<td>12,707</td>
<td>14,491</td>
</tr>
<tr>
<td>Percentage of reads (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mapped to rRNA</td>
<td>1.2</td>
<td>1.2</td>
<td>1.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Mapped to mitochondria</td>
<td>9.0</td>
<td>9.1</td>
<td>8.8</td>
<td>3.5</td>
</tr>
<tr>
<td>Mapped to genome</td>
<td>92</td>
<td>90</td>
<td>92</td>
<td>94</td>
</tr>
<tr>
<td>Mapped to exons</td>
<td>77</td>
<td>79</td>
<td>78</td>
<td>80</td>
</tr>
<tr>
<td>Mapped to introns</td>
<td>18</td>
<td>17</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Mapped to intergenic regions</td>
<td>5.1</td>
<td>4.9</td>
<td>5.1</td>
<td>5.4</td>
</tr>
<tr>
<td>Duplicates</td>
<td>21</td>
<td>22</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Mapped to ERCC</td>
<td>3.7</td>
<td>3.8</td>
<td>3.8</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Differential Expression Compared to MAQC qPCR Data

10 pg

```
y = 0.7891x + 0.0135
R = 0.783
```

554 transcripts

10 ng

```
y = 1.0535x + 0.0166
R = 0.934
```

841 transcripts
Identification of Long Transcripts

UBR4 (15,882 bp)

Human Brain RNA
10 pg Replicate 1
10 pg Replicate 2
100 pg Replicate 1
100 pg Replicate 2
1 ng Replicate 1
1 ng Replicate 2
10 ng Replicate 1
10 ng Replicate 2
SMARTer cDNA Synthesis for NGS

**dT Primed**  
- mRNA
- Polyadenylated RNA
- Single cell capacity

**N6 Primed**  
- Coding and non-coding RNA
- Non-polyadenylated RNA
- Degraded samples

**SMART-Seq™ v4 Ultra™ Low Input RNA Kit for Sequencing**  
- Use with Ion Torrent or Illumina platforms

**SMARTer Ultra Low RNA Kit for the Fluidigm C1™ System**  
- 96 single cells in parallel

**SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian**  
- Use with Illumina platforms
- Use with highly degraded samples (FFPE)

**SMARTer Stranded Total RNA-Seq Kit**  
- Use with Illumina platforms
- Use with typical input RNA samples

**RiboGone™ - Mammalian**  
- Use with Illumina platforms
Our Solutions for Total RNA-Seq

N6 Priming

- Coding and non-coding RNA
- Non-polyadenylated RNA
- Degraded, FFPE, and LCM samples

With Strand Information

SMARTer Stranded Total RNA Sample Prep Kit - HI Mammalian (RiboGone™ - Mammalian kit built in)
Input = 100 ng–1 µg of total RNA

SMARTer Stranded Total RNA Sample Prep Kit - Low Input Mammalian
(with RiboGone - Mammalian kit)
Input = 10 ng–100 ng of total RNA

SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian
Input = 250 pg–10 ng of total RNA
SMARTer Stranded RNA-Seq with rRNA Depletion

**Total RNA**
- rRNA
- mRNA

Add Total RNA Hyb Buffer

- RiboGone oligos
- Add RNase H
- Add DNase
- Clean up enzymes

**mRNA**

5' XXXXX 5' 3' SMARTer Stranded Oligo

First-strand synthesis and tailing by RT

5' XXXXX 5' SMART Stranded N6 Primer

Forward PCR Primer HT

Reverse PCR primer HT

Amplify cDNA by PCR with Illumina Indexing Primer Set

RNA-Seq library

Read 1 →

Read 2 ←

**Two configurations:**

- Low Input (10 ng–100 ng) = RiboGone - Mammalian + SMARTer Stranded Total RNA Sample Prep

- High Input (100 ng–1 mg) = SMARTer Stranded Total RNA Sample Prep Kit - HI
SMARTer Stranded RNA-Seq for Low-Input Total RNA Samples

Analyses of sequencing data

<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>6.8</td>
<td>7.7</td>
</tr>
<tr>
<td>Number of genes identified</td>
<td>14,563</td>
<td>13,839</td>
</tr>
<tr>
<td>Percentage of reads (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mapped to rRNA</td>
<td>0.9%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Mapped to mtRNA</td>
<td>4.7%</td>
<td>2.9%</td>
</tr>
<tr>
<td>Mapped uniquely to genome</td>
<td>76%</td>
<td>75%</td>
</tr>
<tr>
<td>Mapped to exons</td>
<td>70%</td>
<td>66%</td>
</tr>
<tr>
<td>Mapped to introns</td>
<td>47%</td>
<td>49%</td>
</tr>
<tr>
<td>Mapped to intergenic regions</td>
<td>53%</td>
<td>51%</td>
</tr>
</tbody>
</table>

MAQC Analysis

\[
y = 0.8749x - 0.1115 \\
R = 0.960 \text{ 623 transcripts}
\]
SMARTer Stranded Total RNA Sample Prep Kit - HI Mammalian

Analyses of sequencing data

<table>
<thead>
<tr>
<th>Sequence Alignment Metrics (Input: 400 ng)</th>
<th>Human Universal</th>
<th>Human Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of reads (Millions)</td>
<td>8.5 (paired-end)</td>
<td></td>
</tr>
<tr>
<td>Number of genes identified</td>
<td>17,570</td>
<td>17,600</td>
</tr>
<tr>
<td>Percentage of reads (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mapped to 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>
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<tr>
<td>Mapped to exons</td>
<td>43%</td>
<td>50%</td>
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<tr>
<td>Mapped to introns</td>
<td>43%</td>
<td>33%</td>
</tr>
<tr>
<td>Mapped to intergenic regions</td>
<td>14%</td>
<td>12%</td>
</tr>
<tr>
<td>ERCC transcripts with correct strand</td>
<td>99.3%</td>
<td>98.8%</td>
</tr>
</tbody>
</table>

**MAQC Analysis**

\[ y = 1.0351x + 0.0448 \]
\[ R = 0.927 \]
763 transcripts

**ERCC analysis**

\[ y = 0.9988x + 3.3499 \]
\[ R^2 = 0.9199 \]
SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian

- RNA-seq library prep kit directly resulting in Illumina-compatible libraries
- Input = 250 pg–10 ng of mammalian total RNA
- Incorporates LNA technology, leading to greater sensitivity
- Streamlined workflow, including the depletion of rRNA in the form of ribosomal cDNA using a novel, proprietary technology
- Maintains strand information
- Uses random priming to generate information from coding and non-coding RNA
- Compatible with a range of RNA qualities (e.g., FFPE & LCM samples)
Stranded Total RNA-Seq - Pico Input with ribosomal cDNA depletion

1. **Total RNA**
   - 5′ XXXXX
   - rRNA

2. **First-strand synthesis and tailing by RT**
   - 5′ XXXXX
   - N_x Primer

3. **Template switching and extension by RT**
   - 5′ XXXXX
   - XXXXX

4. **Forward PCR Primer**
   - 5′ XXXXX
   - 5′ XXXXX

5. **Addition of Illumina adapters with barcodes by PCR**
   - from rRNA
   - Cleavage of ribosomal cDNA with ZapR and R-Probes

6. **Enrichment of uncleaved fragments by PCR**

7. **RNA-seq library**
   - Read 1
   - Read 2
## Consistent Sequencing Metrics Across a 100-Fold Input Range

<table>
<thead>
<tr>
<th>Sequencing Alignment Metrics</th>
<th>Mouse Brain Total RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RNA source</strong></td>
<td><strong>Input amount (ng)</strong></td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td><strong>Library yield (ng/μl)</strong></td>
<td>10.5</td>
</tr>
<tr>
<td><strong>Number of reads</strong></td>
<td>2.6 million (paired-end)</td>
</tr>
<tr>
<td><strong>Number of transcripts FPKM &gt;1</strong></td>
<td>12,714</td>
</tr>
<tr>
<td><strong>Pearson/Spearman correlations</strong></td>
<td>0.99/0.93</td>
</tr>
<tr>
<td><strong>Correct strand per biol. annotation (%)</strong></td>
<td>97.7</td>
</tr>
<tr>
<td><strong>Proportion of total reads (%)</strong></td>
<td></td>
</tr>
<tr>
<td><em>Exonic</em></td>
<td>22.6</td>
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<tr>
<td><em>Intronic</em></td>
<td>35.6</td>
</tr>
<tr>
<td><em>Intergenic</em></td>
<td>8.3</td>
</tr>
<tr>
<td><em>rRNA</em></td>
<td>11.2</td>
</tr>
<tr>
<td><em>Mitochondrial</em></td>
<td>8.8</td>
</tr>
<tr>
<td><strong>Overall mapping (%)</strong></td>
<td>86.4</td>
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<tr>
<td><strong>Duplicate rate (%)</strong></td>
<td>12.8</td>
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### Sequencing Alignment Metrics

<table>
<thead>
<tr>
<th>RNA source</th>
<th>Human Liver Total RNA - FFPE</th>
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<tbody>
<tr>
<td>Input amount (ng)</td>
<td>10  1  0.25  0.25</td>
</tr>
<tr>
<td>Ribosomal cDNA removal</td>
<td>Yes  No</td>
</tr>
<tr>
<td>Number of reads</td>
<td>1 million (paired-end)</td>
</tr>
<tr>
<td>Number of transcripts FPKM &gt;1</td>
<td>11,752  11,360  10,368  4,501</td>
</tr>
<tr>
<td>Number of transcripts FPKM &gt;0.1</td>
<td>15,358  14,680  12,793  4,507</td>
</tr>
<tr>
<td>Correct strand per biol. annotation (%)</td>
<td>98.3  98.1  98.3  97.1</td>
</tr>
<tr>
<td>Proportion of total reads (%)</td>
<td></td>
</tr>
<tr>
<td>Exonic</td>
<td>23.9  24.3  21.3  1.7</td>
</tr>
<tr>
<td>Intronic</td>
<td>18.4  19.2  17.1  1.7</td>
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<tr>
<td>Intergenic</td>
<td>2.8   2.7   2.5   1.2</td>
</tr>
<tr>
<td>rRNA</td>
<td>36.7  34.3  34.4  90.1</td>
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<tr>
<td>Mitochondrial</td>
<td>4.0   3.5   3.1   1.4</td>
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<tr>
<td>Overall mapping (%)</td>
<td>85.8  84.0  78.5  96.1</td>
</tr>
<tr>
<td>Duplicate rate (%)</td>
<td>22.6  39.0  52.5  44.0</td>
</tr>
</tbody>
</table>

**250 pg vs. 10 ng Human Liver Total FFPE RNA**

Pearson R=0.99
Spearman ρ=0.69
SMART Technology for DNA Sequencing—Expanding Applications

DNA SMART™ ChIP-Seq Kit

- For low-input ChIP-seq for Illumina platforms
- Single-tube workflow; under 4 hours
- Compatible with dsDNA or ssDNA (100 pg–10 ng)
- Ligation-free addition of Illumina adapters
- Generates high-complexity libraries from picogram amounts of input DNA
Combined ChIP Elute and ChIP-Seq Kits—Workflow

1. Protein-DNA cross-linking
2. Chromatin fragmentation
3. Immunoprecipitation
4. DNA elution/cross-linking reversal
5. DNA purification
6. Your preferred ChIP protocol
7. ChIP Elute Kit
8. dsDNA or ssDNA
9. T-tailing
10. DNA SMART Oligo
11. Replication and tailing by RT
12. DNA SMART Poly(dA) Primer
13. Template switching and extension by RT
14. Forward PCR Primer
15. Addition of adapters and amplification
16. Reverse PCR Primer
17. Read 1 →
18. Read 2
# High-Quality Data from Low-Cell Number ChIP-Seq Experiments

## Sequencing Metrics from Specified Numbers of Cells

<table>
<thead>
<tr>
<th>ChIP antibody</th>
<th>H3K4me3</th>
<th>Input (293T cells)</th>
<th>PCR cycles</th>
<th>Library yield (nM)</th>
<th>Peaks identified</th>
<th>Percentage of reads (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1,000,000</td>
<td>200,000</td>
<td>50,000</td>
<td>10,000</td>
<td></td>
</tr>
<tr>
<td>PCR cycles</td>
<td>15</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Library yield (nM)</td>
<td>86.7</td>
<td>101</td>
<td>44.6</td>
<td>20.5</td>
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<td></td>
</tr>
<tr>
<td>Peaks identified</td>
<td>19,459</td>
<td>19,339</td>
<td>18,549</td>
<td>22,564</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of reads (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reads mapped</td>
<td>92.7</td>
<td>88.6</td>
<td>84.3</td>
<td>75.8</td>
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</tr>
<tr>
<td>Uniquely mapped reads</td>
<td>79.0</td>
<td>74.8</td>
<td>70.4</td>
<td>59.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Useful reads (uniquely mapped, non-duplicates)</td>
<td>66.8</td>
<td>63.5</td>
<td>49.9</td>
<td>34.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-redundant rate</td>
<td>0.85</td>
<td>0.85</td>
<td>0.71</td>
<td>0.57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(ENCODE data from U. Washington—293T cells)
Analysis performed with 15–18 million reads per sample
Robust Libraries from Low-Cell-Number ChIP-Seq Experiments

Reproducibility is maintained for low cell numbers
Summary

Single-Cell-Capable mRNA-Seq Using SMART Technology
- SMARTer Ultra Low RNA Kit for the Fluidigm C₁ System
- SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing

Total RNA-Seq Applications
- SMARTer Stranded RNA-Seq Kit
- SMARTer Stranded Total RNA Sample Prep Kit - HI Mammalian
- SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian
- SMARTer Universal Low Input RNA Kit for Sequencing

Expanding Applications for SMART Technology
- DNA SMART ChIP-Seq Kit
NGS Learning Resources

NGS Resource Portal: www.clontech.com/ngs