Performance advances and workflow simplifications for single cell RNA-Seq and DNA-Seq
Next-generation sequencing

Breaking performance barriers
A complete portfolio for next-gen sequencing

• Sensitivity
• Reproducibility
• Reliability
• Ease of use
• Scalability
SMARTer Whole transcriptome analysis

**Single-cell or ultra-low-input RNA-seq**
- 1–1,000 cells; 10 pg–10 ng RNA
  - Oligo(dT) priming
    - intact cells or high-quality RNA (RIN 8–10)
  - SMART-Seq HT Kit
    - cDNA synthesis from 1–100 cells or 10 pg–1 ng total RNA
  - SMART-Seq v4 3' DE Kit
    - cDNA synthesis from 1–100 cells or 10 pg–1 ng total RNA
  - SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing
    - cDNA synthesis from 1–1,000 cells or 10 pg–10 ng total RNA

**Total RNA-seq**
- 100 pg–1 µg RNA; random priming
  - Random priming
    - intact cells or low-quality RNA
  - SMART-Seq Stranded Kit
    - Library construction from 1–1,000 mammalian cells or 10 pg–10 ng mammalian total RNA
  - SMART-Seq v4 Ultra Low Input RNA Kit for the Fluidigm C1® System
    - cDNA synthesis from single cells
  - mRNA depletion
    - for human, mouse, or rat
  - rRNA(−) or poly A(+) RNA depleted or enriched RNA from any species
  - SMART-Seq Universal Low Input RNA Kit for sequencing
    - cDNA synthesis from 250 pg–10 ng (RIN 2–10 or DV200 >25)
  - SMART-Seq Total RNA Sample Prep Kit - Low Input Mammalian
    - Library construction from 10–100 ng (RIN 3–10)
  - SMART-Seq Total RNA Sample Prep Kit - Hi Mammalian
    - Library construction from 100 pg–10 ng
  - SMART-Seq Stranded Total RNA Sample Prep Kit - Hi Mammalian
    - Library construction from 100 ng–1 µg (RIN 3–10)
SMART-Seq Stranded RNA-Seq

- Simple workflow from 1–1,000 intact cells or isolated total RNA
- High sensitivity, as determined by number of transcripts identified
- Reproducible chemistry enables confidence in your data
- Accurate detection of coding and noncoding transcripts
Experimental overview

- Tumor Cell Dissociation
- Labeling and FACS
- SMART-Seq Library Prep
- Sequencing & Analysis
Excellent mapping statistics from cells with extremely low RNA content

Distribution of reads (% of total)

- Exonic
- Intronic
- Intergenic
- rRNA
- Mitochondria
- Unmapped/other

Genes detected (TPM >1)

CD45 (N=18)  EpCAM (N=47)
Uniform gene-body coverage with the SMART-Seq Stranded Kit

Normalized read coverage

- CD45+ cell C
- CD45+ cell D
- EpCAM+ cell C
- EpCAM+ cell B

Graphs showing gene expression levels for ACTB and CD69 genes.
SMARTer DNA Sequencing

DNA sequencing

Low-input DNA-seq
- General application
  - SMARTer ThruPLEX DNA-Seq Kit
    - Library construction from 50 pg-50 ng DNA
- Specific application
  - SMARTer ThruPLEX Tag-seq Kit
    - Library construction with incorporation of unique molecular tags from 1 ng-50 ng DNA
  - SMARTer ThruPLEX Plasma-Seq Kit
    - Library construction from <1 ng-30 ng cfDNA isolated from plasma

Single-cell whole genome amplification and DNA-seq
- SMARTer PicoPLEX Gold DNA-Seq kit
  - Library construction from 1-5 mammalian cells or <15-30 pg gDNA

Epigenomic profiling
- ChIP-seq
  - SMARTer ThruPLEX DNA-Seq Kit
    - Library construction from 50 pg-80 ng ChIP DNA (double-stranded)
- Methylated DNA-seq
  - EpiXplore Meth-Seq DNA Enrichment Kit
    - Library construction from 25 ng-1 μg gDNA
  - DNA SMART ChIP-Seq kit
    - Library construction from 100 pg-10 ng ChIP DNA (single-stranded or double-stranded)
PicoPLEX Gold Single Cell DNA-Seq

- Fast, simple workflow: from cells to libraries in 3 hours, with minimal hands on time
- Best in class performance: superior reproducibility and sensitivity
- Flexible kit configuration: UDIs available for use on the NovaSeq
PicoPLEX Gold: fast, simple library prep

ONE
Add Enzyme Extraction Master Mix

TWO
Add Pre-Amplification Master Mix

THREE
Pre-amplification cleanup

FOUR
Add Amplification Master Mix

Single cell → AMPure → Amplified library → Analysis

5 min
5 min
15 min
5 min

30 min Hands-on time
## High-fidelity detection of single nucleotide variants

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<th>PicoPLEX WGA v2</th>
<th>Kit D</th>
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<td><strong>Depth of SNV position</strong></td>
<td></td>
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<tr>
<td>≥10</td>
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<td><strong>Allele frequency ≥20%</strong></td>
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<td><strong>SNVs called (of 74 validated)</strong></td>
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January, 2019

CONFIDENTIAL | Oncology Research Program
Accurate detection of segmental aneuploidies with low-pass sequencing

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