With SMART chemistry, we were able to detect 50–60% more genes than with the SMART-Seq v4 kit. The SMART adapters, added by the oligo(dT) primer template for the RT, allow for higher sensitivity due to chemical modifications to block ligation during sequencing library preparation. Chemical modifications to block ligation during sequencing library preparation are necessary because only a small fraction of full-length mRNA is transcribed, bringing the volume to slightly over 5 µl. The PCR was started by adding 0.25 µl of PCR Primer, 0.4 µl of Sequenase DNA Polymerase, 10 µl of Sequenase Buffer, and 0.05 µl of Taq polymerase, up to a 20 µl total volume. PCR cycling and subsequent steps were performed as in the full-volume protocol.

Sequencing libraries were generated using 125 ng of cDNA and the Nextera XT DNA Library Preparation Kit (Illumina) with a quarter of the recommended volume. The library pools were sequenced on a NextSeq 550 (instrument using 2 × 75 bp paired-end reads) and analysed with CLC Genomic Workbench (mapping to the human hg19 genome with Ensembl annotations). All percentages shown are the number of reads that map to intronic regions or intergenic regions—percentage of mapped reads in all libraries.

Intron
Exon
Intergenic
rRNA
Mitochondria

Table 1. Increased sensitivity with the SMART-Seq Single Cell Kit.

<table>
<thead>
<tr>
<th>Method</th>
<th>Percentage of mapped reads</th>
<th>Percentage of mapped reads</th>
<th>Percentage of mapped reads</th>
<th>Percentage of mapped reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMART-Seq v4</td>
<td>56%</td>
<td>61%</td>
<td>78.0%</td>
<td>76.9%</td>
</tr>
<tr>
<td>SMART-Seq Single Cell Kit</td>
<td>57.5%</td>
<td>62.2%</td>
<td>78.0%</td>
<td>76.9%</td>
</tr>
</tbody>
</table>

Methods

All cells were labeled with CD81-FITC antibody and 7-AAD for distinguishing live from dead cells prior to sorting using a BD FACSJazz cell sorter into a 96-well plate or PCR plate. PCR cycling and subsequent steps were performed as in the full-volume protocol, but only 3.5 µl was aliquoted in each well prior to cell sorting. A quarter of the method was performed as in the full-volume protocol, but only 3.5 µl was aliquoted in each well.