Since the emergence of next-generation sequencing (NGS), the importance of and demand for single-cell analysis have risen rapidly. Extracting meaningful biological information from the small amount of RNA present in each cell requires a high-throughput, low-input, and high-sensitivity RNA-seq preparation method. The SMART-Seq Single Cell Kit has been developed for this need, as described in the SMART-Seq Single Cell Kit User Manual. Libraries were distinguished from dead cells prior to sorting using a BD FACSJazz cell sorter into a quarter volume of the reverse transcription mastermix was added to start the reverse transcription reaction. Chemical modifications to block ligation during sequencing library preparation are nontemplated nucleotides (indicated by Xs) that hybridize to the SMART-Seq scTSO, providing a new template for the RT. The amplified cDNA is then purified, quantified, and used for sequencing library preparation.

**Methods**

**Figure 2** and **Figure 3**: All cells were labeled with CD45-PE antibody and 14AG12C1 to distinguish live from dead cells prior to sorting using a BD FACSAria cell sorter into a 96-well plate or PCR strips. After sorting, cells were flash-frozen on dry ice and then stored at -80°C until ready to use. Unless otherwise noted, all libraries created with the SMART-Seq Single Cell Kit were generated from 2 µg of UHR total RNA for the quarter-volume workflow (except for the intron analysis). All libraries were processed using the NEBNext protocol. As described in the SMART-Seq Single Cell Kit User Manual, libraries were processed with either the NEBNext or SMART-Seq protocols.

**Figure 4**: The SMART-Seq Single Cell Kit outperforms all current commercial and noncommercial full-length methods, particularly with as little as 2 pg of total RNA.

**Figure 5**: The SMART-Seq Single Cell Kit shatters sensitivity seen with the Smart-seq2 protocol. Libraries were prepared from fresh B cells and fixed with formaldehyde. SMART-Seq Single Cell Kit libraries were then sequenced on a HiSeq 2500 using quarter-volume on the Illumina platform. Panel A: SSe2 has a similar percentage of reads mapped regardless of the PCR cycling, unlike Smart-seq2 (SS2). This indicates that small variations in the workload have a greater impact on sensitivity of Smart-seq2 (SS2) than the SMART-Seq single cell technology.

**Figure 6**: The SMART-Seq Single Cell Kit shatters sensitivity seen with the Smart-seq2 protocol. Libraries were prepared from fresh B cells and fixed with formaldehyde. SMART-Seq Single Cell Kit libraries were then sequenced on a HiSeq 2500 using quarter-volume on the Illumina platform. Panel A: SSe2 has a similar percentage of reads mapped regardless of the PCR cycling, unlike Smart-seq2 (SS2). This indicates that small variations in the workload have a greater impact on sensitivity of Smart-seq2 (SS2) than the SMART-Seq single cell technology.