Automation-friendly, all-in-one SMART-Seq cDNA synthesis and library preparation from cells or total RNA

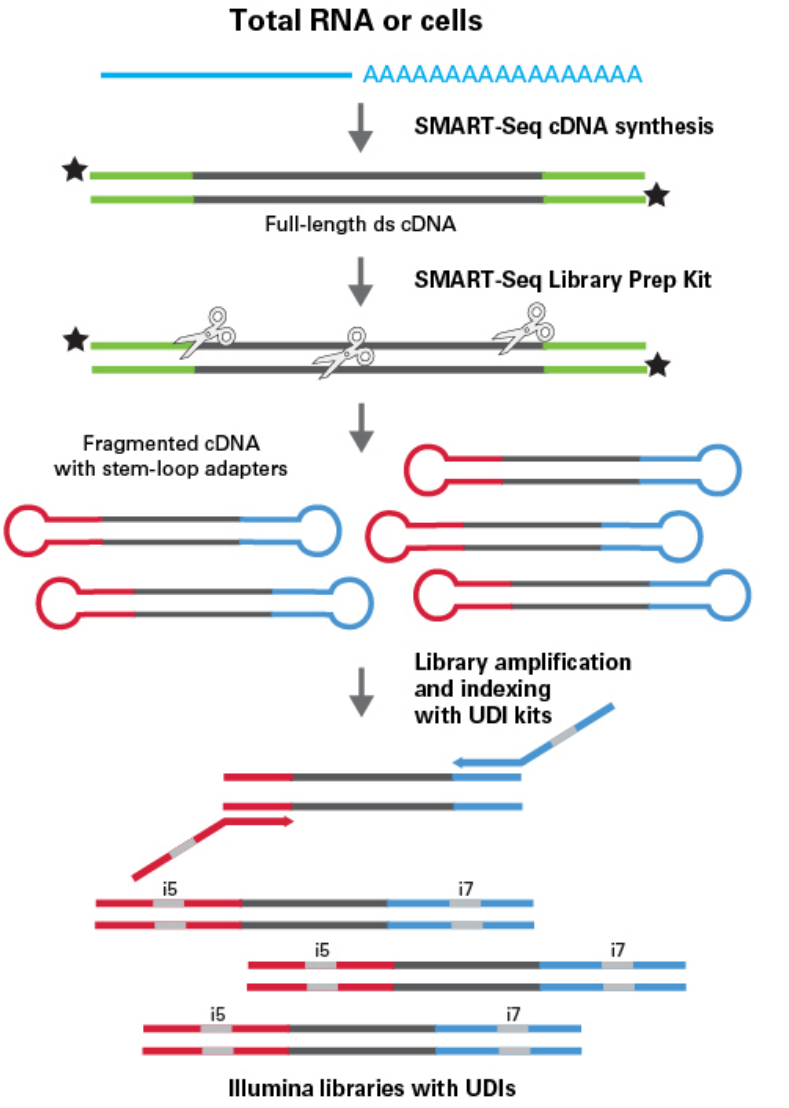
Introduction

The library input quantities (up to 310 µl of 2.5 nM pooled libraries) required to successfully load a high-throughput sequencer, such as the NovaSeq™ system, are much higher than the quantities (5 µl of 4 nM pooled libraries) required for a low-throughput sequencer like the NextSeq® system. For this reason, increased library yield is becoming a very desirable feature for library prep kits. The SMART-Seq HT PLUS Kit (SS-HT PLUS kit) is an automation-friendly version of our SMART-Seq PLUS kits designed to synthesize high-quality, full-length cDNA directly from 1–100 intact cells or ultra-low amounts of total RNA (10 pg–1 ng), and generate a high yield of Illumina-ready sequencing libraries. The kit includes the SMART-Seq HT Kit for generating cDNA, the SMART-Seq Library Prep Kit (not sold separately) for fragmenting the cDNA and amplifying the library, and a unique dual index kit for multiplexing up to 96 samples (Figure 1, Panel A).

For cDNA generation, the SS-HT PLUS kit incorporates our patented SMART (**S**witching **M**echanism **a**t 5′ end of **R**NA **T**emplate) technology. This technology relies on the template-switching activity of reverse transcriptase to enrich for full-length cDNAs and to add defined PCR adapters directly to both ends of the first-strand cDNA (Chenchik et al. 1998). This ensures that the final cDNA libraries contain the entire length of the mRNA, including the 5' end. This strategy maintains a true representation of the original mRNA transcripts, which is a critical factor for gene expression analysis. SMART technology offers unparalleled sensitivity and unbiased amplification of cDNA transcripts, and allows direct cDNA synthesis from intact cells.

For library preparation from the generated full-length cDNA, the SS-HT PLUS kit also incorporates our patented library preparation technology. Unlike other library preparation kits, which are based on ligation of Y-adapters, our approach uses stem-loop adapters to construct high-quality libraries. The easy two-step workflow takes place in a single tube and is completed in about two hours. Sample mix-up, other handling errors, and sample loss are minimized since no intermediate purification steps or sample transfers are necessary (Figure 1, Panel B). The SS-HT PLUS kit also includes unique dual index (UDI) primers for amplification of indexed NGS-ready libraries, allowing the multiplexing of up to 96 samples. Once purified and quantified, the resulting libraries are ready for use on Illumina NGS instruments using standard Illumina sequencing reagents and protocols.

A



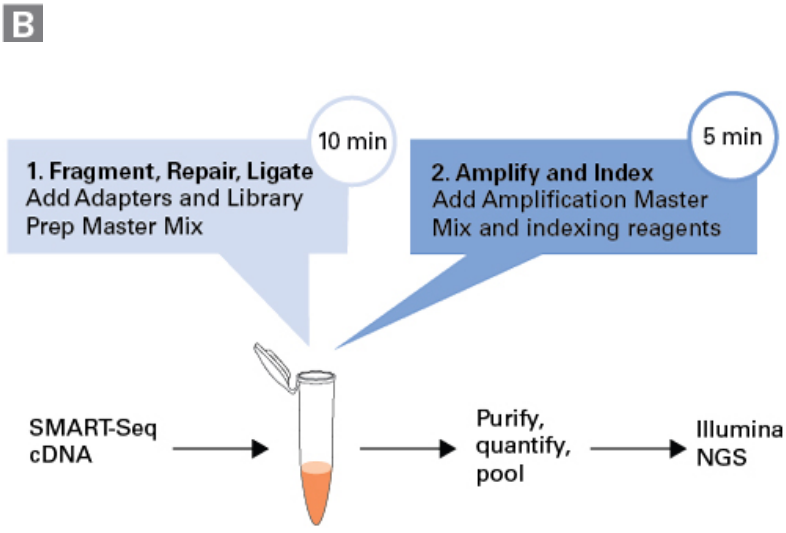


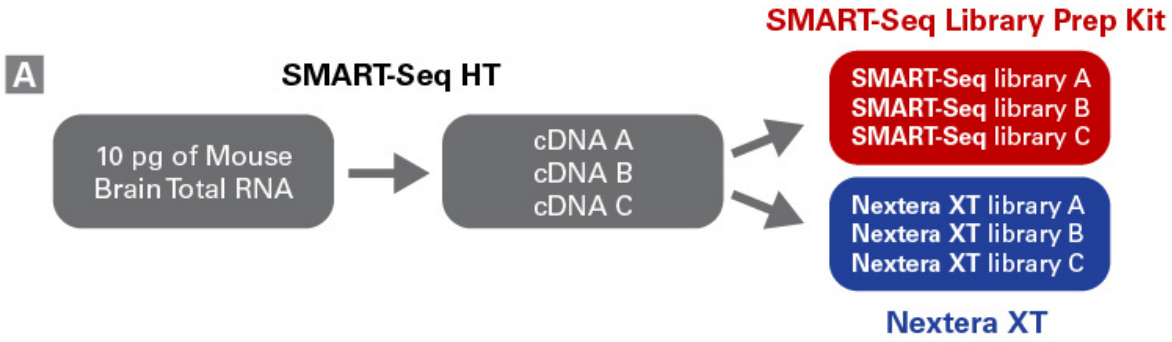
Figure 1. SMART-Seq HT PLUS Kit protocol overview. Panel A. Double-stranded cDNA generated with SMART-Seq HT chemistry is enzymatically fragmented and ligated to stem-loop adapters in a single step. Libraries are then amplified and indexed, creating Illumina-compatible libraries with unique dual indexes. **Panel B.** The library preparation method features a new streamlined two-step protocol that generates sequencing-ready libraries with minimal hands-on

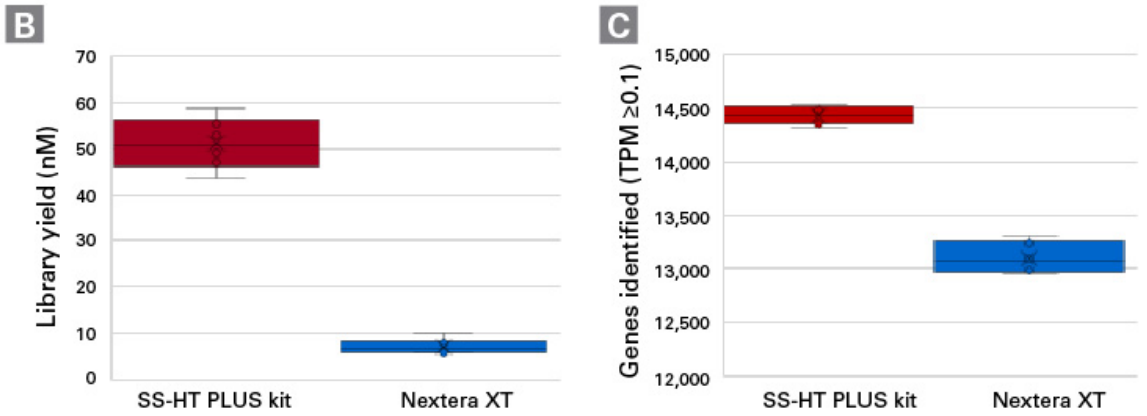
time.

Results

Greater sensitivity and reproducibility for cDNA synthesis

The SMART-Seq HT PLUS kit was designed to provide a streamlined workflow from cells or total RNA to Illumina-ready libraries. To examine kit performance against a gold-standard library preparation kit, 10 pg of Mouse Brain Total RNA was used as input for SMART-Seq HT cDNA synthesis in triplicate. Libraries were then generated from this cDNA using either the SMART-Seq Library Prep Kit portion of the SS-HT PLUS kit or Nextera® XT, and the performance of the two methods was compared (Figure 2, Panel A). The yields obtained from the SS-HT PLUS kit were, on average, 5X higher than yields obtained when using Nextera XT (Figure 2, Panel B), enabling greater flexibility in loading high-throughput sequencers or performing multiple sequencing runs. The libraries were then sequenced on a NextSeq 500, and the number of genes identified over 0.1 transcripts per million (TPM) was reported (Figure 2, Panel C). SS-HT PLUS enabled the detection of 10% more genes compared to Nextera XT, allowing for greater capture of the starting material from the cDNA input. The percentage of reads mapped to exonic regions, intronic regions, intergenic regions, ribosomal RNA (rRNA), and mitochondria were identified and found to be comparable for the two methods (Figure 2, Panel D). Finally, the Pearson and Spearman's correlations calculated from the TPM values were similarly high when using both kits (Figure 2, Panel E), indicating high confidence in the reproducibility of the data.





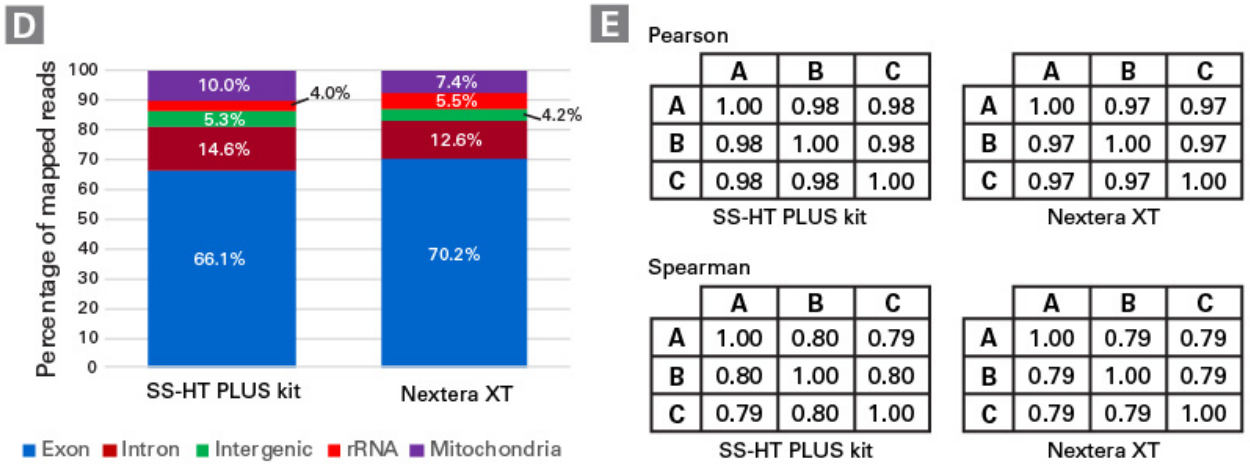


Figure 2. Library preparation with the complete SMART-Seq HT PLUS Kit increases library yield and genes identified compared to Nextera XT. **Panel A.** SMART-Seq HT chemistry was used to produce cDNA from 10 pg of Mouse Brain Total RNA in triplicate. Illumina-compatible libraries were generated from 1 ng or 125 pg using the SMART-Seq Library Prep Kit portion of the SMART-Seq HT PLUS or Nextera XT (Illumina) library prep kits, respectively, and the libraries were sequenced on a NextSeq 500 system. The reads were normalized to 4M paired-end reads and analyzed as described in the methods. **Panel B.** Library yield obtained with the SS-HT PLUS kits are higher than those generated with Nextera XT. **Panel C.** The SS-HT PLUS kit identified 10% more genes than Nextera XT. **Panel D.** As expected, the distribution of mapped reads is similar between the two library preparation methods. **Panel E.** Pearson and Spearman's correlations were calculated from the TPM values obtained from the triplicate cDNA preparations (A to C) processed with the SS-HT PLUS kit or Nextera XT.

Switch with confidence

To evaluate the bias introduced by the library preparation step, the cDNA generated with SMART-Seq HT was processed in duplicate with the SMART-Seq Library Prep Kit portion of the SS-HT PLUS kit or Nextera XT (Figure 3, Panel A). The TPM values were plotted and used to calculate correlations. Looking at the SMART-Seq libraries generated, the correlations obtained when using the same starting cDNA (P: 0.999 & S: 0.90 for cDNA A1 vs. cDNA A2; Figure 3, Panel B left) were higher than those obtained with different input cDNAs (P: 0.98 & S: 0.80 for cDNA A vs. cDNA B; Figure 2, Panel E). We concluded that the main source of variation in the TPM values was coming from the cDNA generation

step.

We also assessed the overlap in the genes identified in SS-HT PLUS versus Nextera XT. The Spearman's correlation (S: 0.90 for SS-HT PLUS cDNA A1 vs. Nextera XT cDNA A1; Figure 3, Panel B, right) was significantly higher than the one obtained when using different cDNA inputs (0.79/0.80 [SS-HT PLUS] & 0.79 [Nextera XT]; Figure 2, Panel E), demonstrating the similarity in gene-identification performance between the two library prep technologies. Finally, as depicted in Figure 3, Panel C, the number of genes identified in the SS-HT PLUS replicates (12,927 genes) was higher compared to those found with the Nextera XT replicates (12,116 genes). Most of the genes identified that did not overlap between replicates showed low TPM values, as depicted by the dots overlying the X- and Y-axis (Figure 3, Panel B). Both methods detected the same 12,311 genes at similar TPM values, as represented by the correlation plot (Panel B) and the Venn diagram (Panel C). Therefore, existing Nextera XT users should see minimal effects on their data when using the new SMART-Seq HT PLUS Kit.

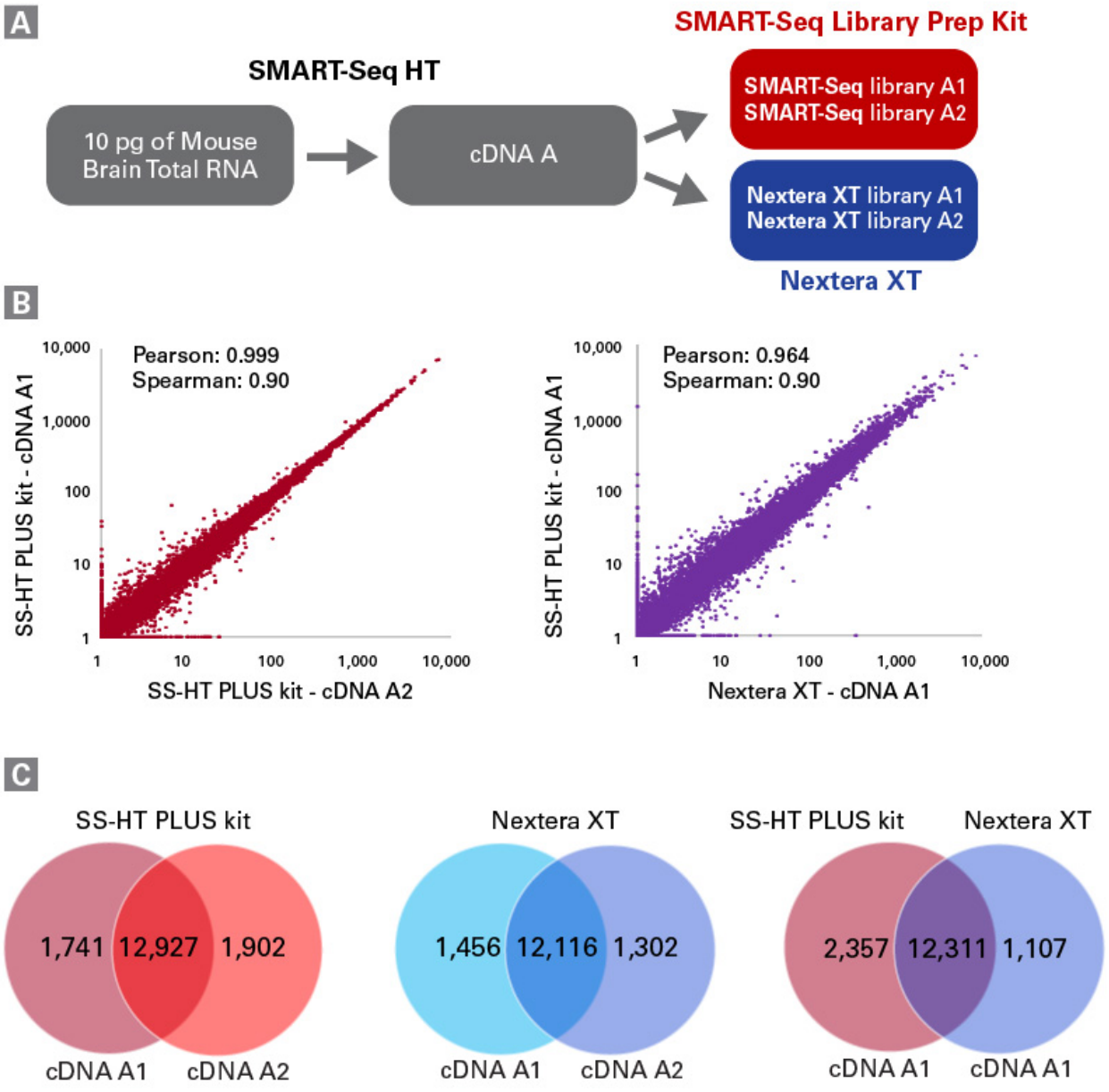


Figure 3. Comparable data between SMART-Seq HT PLUS library prep and Nextera XT kits enable existing users to make the switch. Panel A. Libraries were prepared in duplicate from the same SMART-Seq HT cDNA using either the SMART-Seq Library Prep Kit portion of the SS-HT PLUS kit or Nextera XT. **Panel B.** Correlation plots between libraries made with the SMART-Seq HT PLUS and Nextera XT kits. Pearson and Spearman's correlations were calculated from the TPM values obtained. Scatter plots show the TPM values from all genes with a log10+1 scale. **Panel C.** Venn diagram showing the number of genes identified from the processed cDNA, in duplicate, with the SMART-Seq HT PLUS Kit, Nextera XT, or between both chemistries.

Conclusions

Having a sensitive and reproducible end-to-end approach is crucial to efficiently and easily analyze samples and extract meaningful biological information from the sequencing data. The SMART-Seq HT PLUS Kit combines the proven SMART-Seq cDNA generation approach and user-friendly library preparation chemistry to generate Illumina-compatible libraries ready for sequencing. By using the SMART-Seq HT PLUS Kit, researchers can produce about 5X higher library yields than traditional methods. An increase in the number of genes identified is also observed, which is extremely useful when dealing with difficult samples containing low RNA levels, including clinical research samples.

Methods

cDNA and Illumina libraries were generated from 10 pg of Mouse Brain Total RNA using the SMART-Seq HT PLUS Kit. For comparison between the SMART-Seq Library Prep Kit (not sold separately) and Nextera XT, sequencing libraries were generated using 125 pg of cDNA and the Nextera XT DNA Library Preparation Kit (Illumina) with a quarter of the recommended volumes. The cDNA and sequencing libraries were quantified using Bioanalyzer HS reagents and Qubit, respectively.

Libraries were sequenced on a NextSeq 500 instrument using 2 x 75 bp paired-end reads, and analysis was performed using CLC Genomics Workbench. Reads were downsampled to 4M reads and mapped to the mouse [GRCm38] genome with Ensembl annotations. All percentages shown—including the number of reads that map to introns, exons, or intergenic regions—are percentages of mapped reads in each library. TPMs were reported using CLC.

References

Chenchik, A., Zhu, Y., Diatchenko, L., Li., R., Hill, J. & Siebert, P. Generation and use of high-quality cDNA from small amounts of total RNA by SMART PCR. In *RT-PCR Methods for Gene Cloning and Analysis*. Eds. Siebert, P. & Larrick, J. (BioTechniques Books, MA), pp. 305–319 (1998).

Related Products

Cat. #	Product	Size	License	Quantity	Details
R400748	SMART-Seq® HT PLUS Kit	48 Rxns		*	

The SMART-Seq HT PLUS Kit is a complete kit designed to first generate high-quality cDNA from 1–100 cells or ultra-low amounts of high-integrity total RNA (10 pg–1 ng) and then high-quality Illumina sequencing-ready libraries. Indexes are added using a unique dual index kit (Cat. # R400744 or R400745). This kit supports up to 48 reactions.

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