Comparison of Different NGS Library Construction Methods for Single-Cell Sequencing

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Abstract
Recent advances in whole genome amplification (WGA), whole transcriptome amplification (WTA) technologies and next-generation sequencing (NGS) have enabled whole genome or transcriptome sequencing at the single cell level. Single-cell sequencing studies have yielded new insights into the heterogeneity of the genome and transcriptome in individual cells. Such heterogeneity at the single-cell level has been shown to be closely related to cellular function, differentiation, development, and heterogeneity. A critical element of the single-cell sequencing workflow is sequencing library construction following WGA or WTA. An efficient library construction method is required to convert a high percentage of the DNA fragments to an adapter-ligated sequencing library and to ensure high sequence complexity of the library. Furthermore, uniform representation of all genomic regions in a sequencing library is essential for retaining all important sequence information.

GeneRead and Nextera Library Prep Workflow for Single-Cell Sequencing
Following MDA-based WGA or WTA from 1–1000 cells, the amplified genomic DNA or cDNA, respectively, can be subjected to library construction using either a PCR-free, adapter-ligation-based library prep method (GeneRead) or tagmentation-based library prep method (Nextera). The workflows show the steps involved in sequencing library construction. Both GeneRead and Nextera protocols can be completed in under 2 hours. The GeneRead library construction protocol combines all enzymatic reactions and tagmentation in one reaction tube and does not require PCR-based amplification. The Nextera method requires an intermediate cleanup step between tagmentation and PCR-amplification, but does not require DNA fragmentation.

Sequencing Data Quality
All 4 sequencing libraries were sequenced on an Illumina MiSeq instrument with a MiSeq Rapid Reagent Kit v2 (300-cycle, paired-end sequencing, 2X150). FastQC software (Babraham Bioinformatics) was used to analyze the sequencing data quality. As shown in the per-base-GC-content (1A–1D) and per-base-sequence-content (2A–2D), the Nextera libraries had characteristics bias in the first 20 nucleotides (C and G). This is likely caused by the bias of the Nextera transposase in recognizing genome sequences for tagmentation. GeneRead libraries from A REPI-g-amplified gDNA and B REPI-g amplified cDNA. Nextera libraries from C REPI-g amplified gDNA and D REPI-g amplified cDNA. The reverse reads of the same libraries showed some patterns in per-base-GC content as the forward reads.

Comparison of Library Quality Metrics
To further evaluate the quality of the GeneRead and Nextera sequencing libraries, we analyzed the following metrics: percentages of the WGA-gDNA reads mapped to the B-subunit reference genome, and WGA-gDNA reads with Phred score of 30; GC bias metric, plotted as normalized sequence content vs genomic regions with different GC contents, and distribution of reads for different RNA species for the in the WGA-gDNA libraries.

Summary
Both GeneRead and Nextera library prep methods:
• Are compatible with REPI-g WGA and WTA for single cell sequencing
• Provide streamlined protocols to construct libraries in under 2 hours
• Deliver high library quality for sequencing MDA-amplified gDNA and cDNA
GeneRead library prep:
• PCR free library prep for minimal bias
• High coverage uniformly
• Requires additional DNA fragmentation
Nextera library prep:
• Conditional fragmentation and adapter ligation suitable for automation
• Requires PCR amplification
• GC bias with low coverage of AT-rich region
• Bias at the ends of sequencing reads

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