High-throughput single cell DNA methylation coupled with true positive displacement liquid handling technology enables epigenetic profiling of thousands of single cells

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SCALE biosciences

[1]Archana Bettadapur, [1]Adam Barner, [2]Melissa Netwal, [1]Sanika Khare, [1]Cam Jansen, [1]Dominic Skinner

[1]Scale Biosciences, San Diego, CA, USA [2]SPT Labtech, Melbourn, Cambridgeshire, UK

Abstract

Single cell DNA methylation sequencing is a powerful technique in epigenetics that provides unprecedented insights into cellular heterogeneity and gene regulation. Scale Bio provides an easy-to-use and fully kitted solution, leveraging highly parallelized barcoding technology to make single cell methylation analysis accessible to more researchers with their single cell DNA methylation (scMET) kit. In this study, we explore the benefits of automating the Scale Bio single cell methylation protocol with SPT Labtech's firefly® to facilitate the analysis of single cell methylomes isolated from human peripheral blood mononuclear cells (PBMCs) compared to a library prepared in parallel using manual methods.

Introduction

Scale Bio utilizes the cell itself as a compartment to perform rounds of sequential barcoding in a plate-based workflow, eliminating the need for complex instrumentation. This technology has been successfully adapted to assess whole genome DNA methylation at the single cell level, offering a robust, affordable, high-throughput protocol that enhances yield, diversity, and coverage.

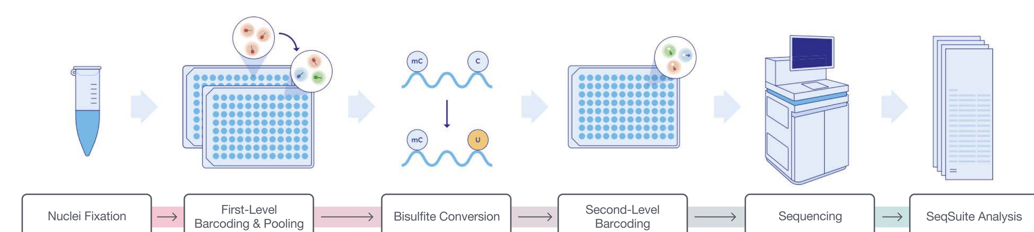

Figure 1. **Scale Bio Single Cell Methylation Kit v1.1 Workflow. Bisulfite conversion and downstream library construction steps were automated on the SPT Labtech firefly.**

While this has increased accessibility to single cell analysis significantly without requiring specialized instruments, automation of these methods can significantly reduce consumable consumption and streamline processing time while reducing user to user variability and strain.

firefly utilizes true positive displacement technology, which is well-suited to working with cell and nuclei suspension due to its gentle approach. It enables precise and dynamic dispensing with a significant reduction in dead volumes and reduces the requirement for plastic waste inherent to traditional pipetting methods.

Methods

Isolated nuclei from PBMCs were fixed, barcoded, sorted, and lysed per the Scale Bio Single Cell Methylation Kit protocol. Lysed samples were stored at -20°C for two months and thawed on ice prior to downstream processing. All reagents and master-mixes were prepared off-deck according to the Scale Bio Single Cell Methylation Kit Protocol and divided equally between the manual and automated libraries. firefly was used for all chilled incubation, dispense, and vortex steps; plate and reservoir thermal modules on the firefly were actively cooled to 0°C for all chilled processing steps. Cell wash steps were done manually using the ScaleBio spin funnel, as well as purification of the final pooled indexed PCR library from a subset of wells. Fragment size was determined using the Agilent Tapestation DS5000 High Sensitivity DNA Kit before sequencing on a NextSeq2000. Sequencing data from automated and manually generated libraries were analyzed with ScaleBio Seq Suite: MET v1.1.0.

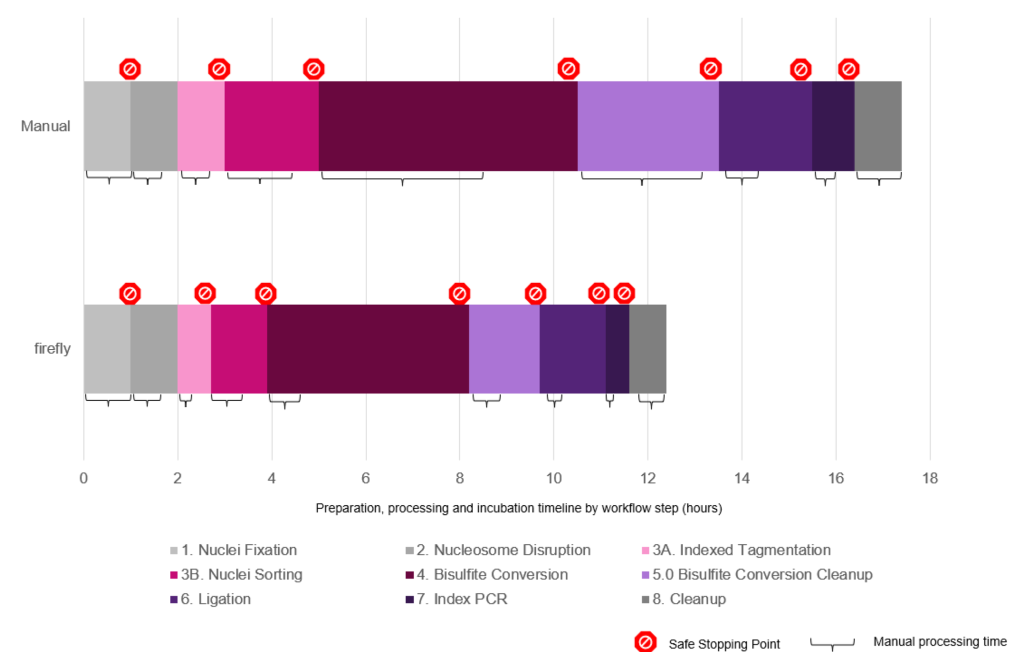

Figure 2. **Processing time by workflow step. The method developed on firefly reduced overall processing time by 35% and manual processing by over 50%.**

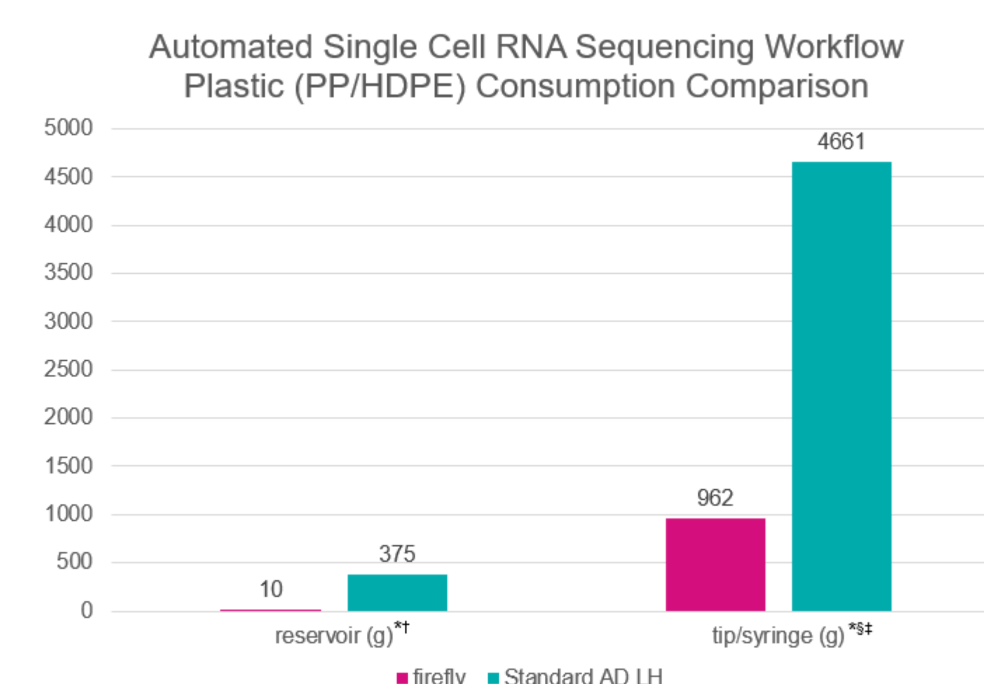

Figure 3. **Reservoir and tip type comparison.**

*SPT Labtech firefly/dragonfly reservoir (1 g), SPT Labtech firefly/dragonfly syringe (4 g). †LP SBS 96-well diamond bottom reservoir weight (23.3 g) based on Axygen (RES-SW96-LP), other SBS reservoir types may differ. §Single tip weight (1.6 g) based non-filter 125 µL Apricot tips, other tip weights may differ. ‡Tip box not included in weight calculation.

Results

Manually prepared libraries and those generated with firefly showed comparable metrics, maintaining high cell recovery and robust cytosine coverage.

Cluster projection of the single nuclei were performed using genome non-overlapping 5Kb bins quantified by ALLCools (ALL methyl-Cytosine tools). Marker genes were identified using Differentially Methylated Regions (DMRs) and annotated based on known methylation signatures, demonstrating high resolution of PBMC population based on known marker genes per Figure 3.

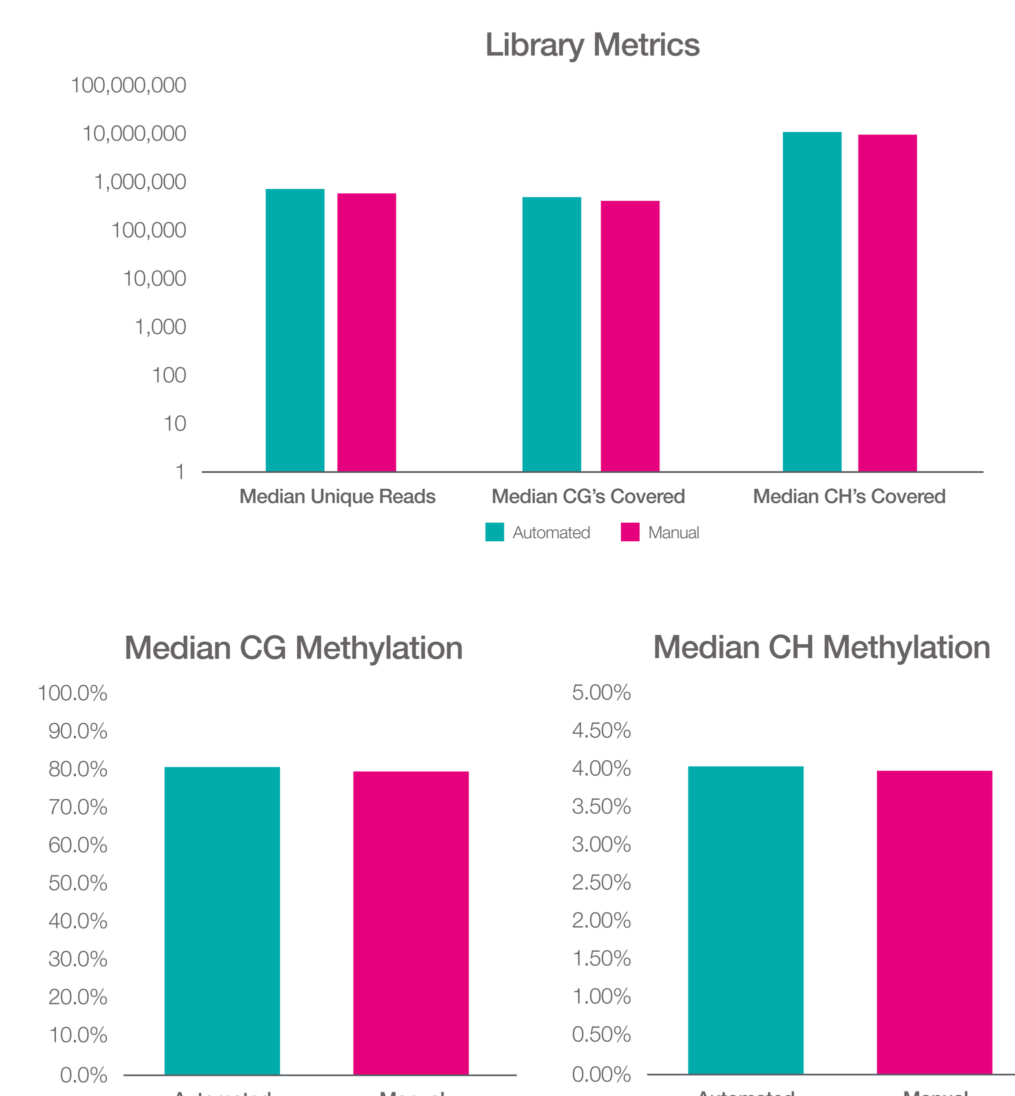

Figure 3. a) **Library metrics of median unique reads, CG, and CH coverage were comparable across automated and manual libraries;** b) **and** c) **High median CG methylation and low median CH methylation demonstrates efficient bisulfite conversion in both library preparation methods.**

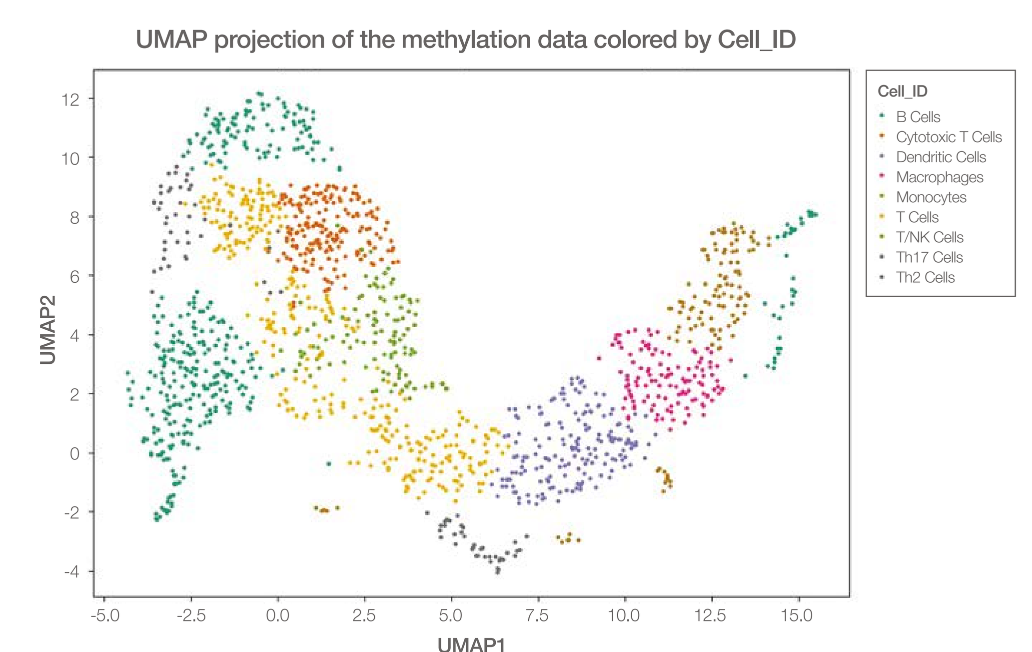

Figure 4. **UMAP projection of the methylation data colored by cluster. Cell type annotation was performed using analysis of Differentially Methylated Regions (DMRs).**

The UMAP cluster projection was colored by library type, indicating that the samples from the automated and manual libraries were evenly distributed across clusters without bias from library preparation method.

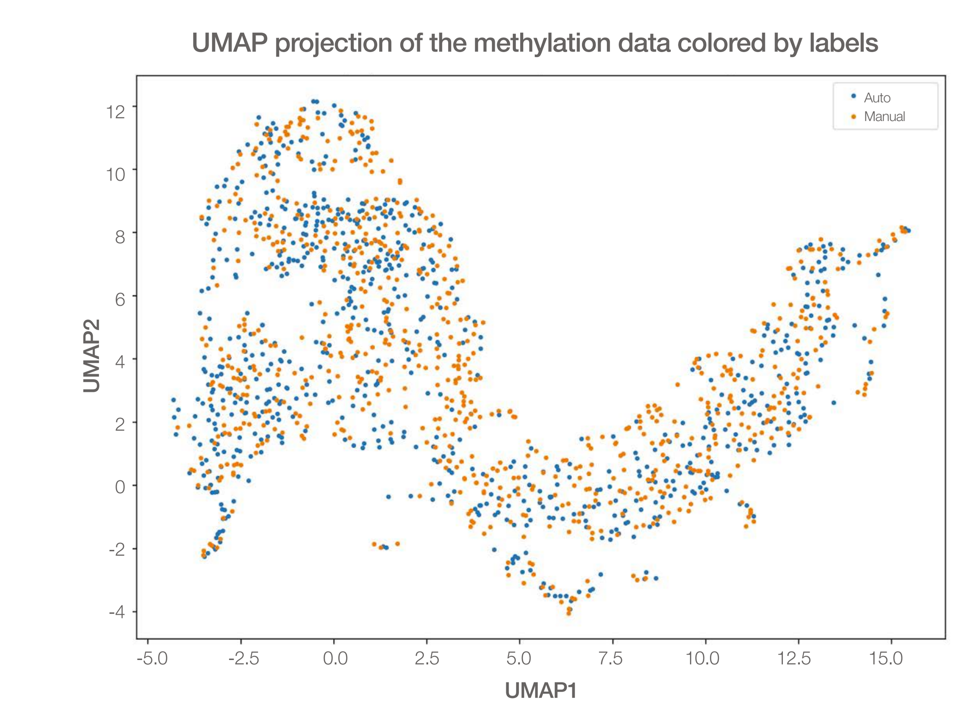

Figure 5. **UMAP projection of the methylation data clustered colored by library preparation method.**

Further bioinformatic analysis showed an even representation of all cell types across both library types, further confirming comparable data quality and biological relevance regardless of preparation method (Figure 6).

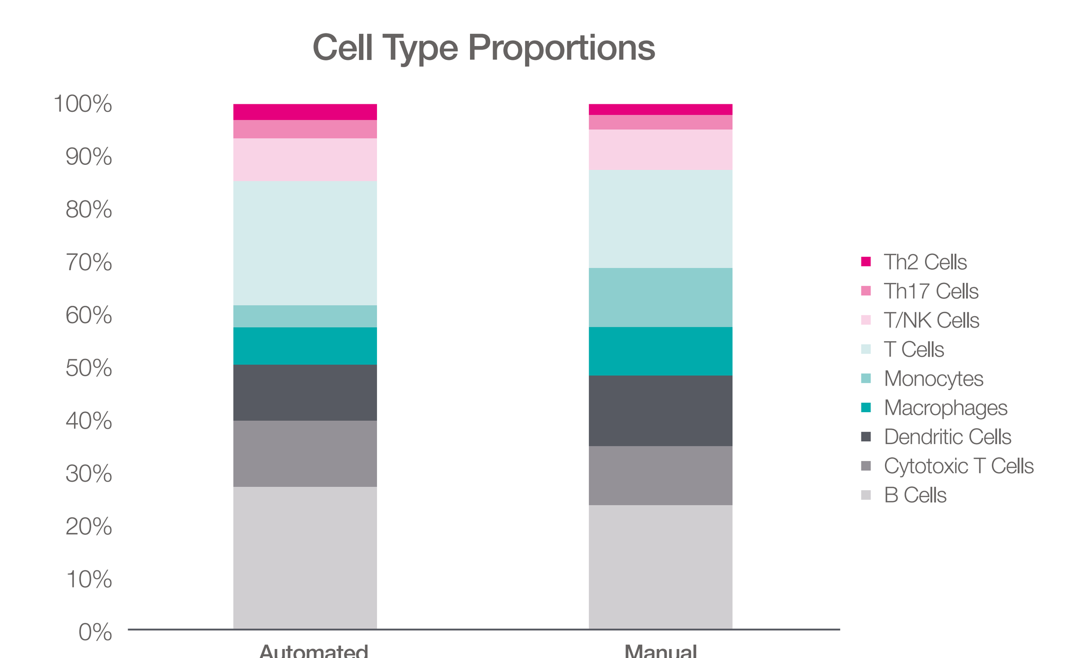

Figure 6. **Cell type proportions compared across automated and manually prepared libraries.**

Conclusion

The easy-to-use, plate-based technology of the Scale Bio Single Cell Methylation Sequencing Kit coupled with firefly's efficient and sustainable platform provide an automated workflow that reduced workflow time by over 35% and hands-on time by over 50%. Automation on the firefly facilitates profiling of complex samples, maintaining sensitivity, specificity, and accuracy in identifying DNA methylation sites and providing insights into cellular heterogeneity and trajectories, facilitating the research required to study disease by allowing scientists to scale and standardize their workflows for reproducible and consistent data sets.

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