A high-throughput method for single-cell ATAC-seq on the ICELL8 Single-Cell System

Introduction

Epigenomic information provides great insight into gene regulation, but is complicated by the fact that single cells (even those of the same type) may have distinct epigenomic profiles. While recent years have seen great strides being made in single-cell next-generation sequencing methods, single-cell approaches to examine chromatin state have historically been complex, low-throughput, and/or cost-prohibitive. Assay for transposase-accessible chromatin using sequencing (ATAC-seq) is a widely used approach to detect chromatin accessibility, which is a crucial component of genome regulation. Recently, the Greenleaf lab at Stanford University, in conjunction with Takara Bio USA, Inc. (TBUSA), developed a simple, rapid ATAC-seq workflow to generate sequencing-ready libraries from single cells using the highly flexible ICELL8 Single-Cell System. This methodology allows for the generation of libraries from 1,000–2,000 single cells in as little as 4–5 hours with an on-chip workflow.

Results

ATAC-seq workflow on the ICELL8 platform

ICELL8 single-cell systems allow for the automated isolation and dispensing of single cells into 5,184-nanowell blank ICELL8 chips (Figure 1). Cell viability is assayed using Hoechst and propidium iodide staining and wells bearing live single cells are automatically identified using CellSelect Software, eliminating time-consuming manual identification steps and saving reagents by dispensing reagents only in targeted wells. Tagmentation, addition of indexes, and PCR amplification are then performed on-chip (Figure 1), minimizing technical variability and providing a simple 4–5-hour workflow. The PCR amplicons are then pooled off-chip and purified using size-selection beads to generate sequencing-ready libraries. (For a detailed protocol, please refer to the protocol page).

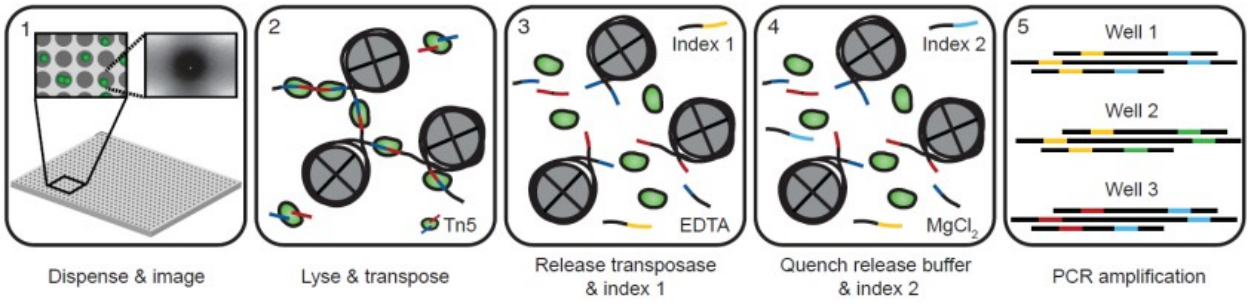

Figure 1. Schematic of the ATAC-seq workflow on the ICELL8 system.

ATAC-seq reveals distinct cell populations based upon chromatin accessibility at transcription factor start sites

Peripheral blood mononuclear cells (PBMCs), B, CD4+ T, CD8+ T, monocyte, and T cells were isolated from whole blood. Following isolation, they were dispensed using the ICELL8 platform. A total of 2,333 single cells passing QC metrics were used to generate and sequence high-complexity single-cell ATAC-seq libraries (Figure 2) with an average of ~14,000 fragments per cell.

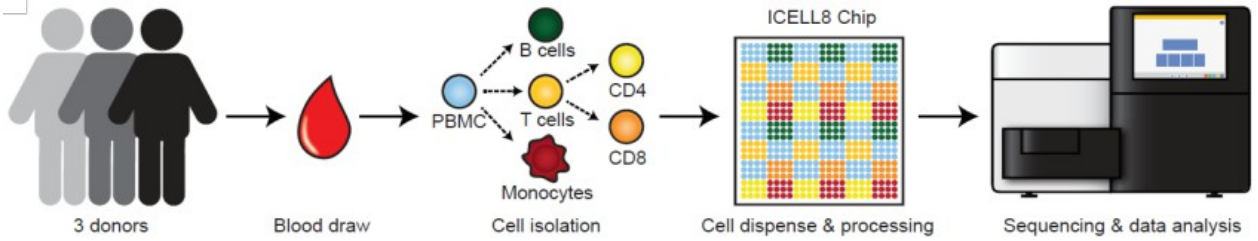

Figure 2. Experimental design for using ATAC-seq to identify epigenomic states of multiple cell types from human donors.

Analysis of chromatin accessibility within transcriptional start sites revealed the robust grouping of cells into three major clusters: B cells, T cells, and monocytes (Figure 3). PBMC subpopulations demonstrate cell-type-specific chromatin accessibility patterns concordant with the various isolated cell types (Figure 3, Panel A). Consistent with previously published literature, examination of the PU.1 DNA binding motif revealed increased accessibility specifically within monocytes and B cells (Figure 3, Panel B), while C/EBPα and RUNX1 motifs were more accessible only in monocytes and T cells, respectively (Figure 3, Panels C–D).

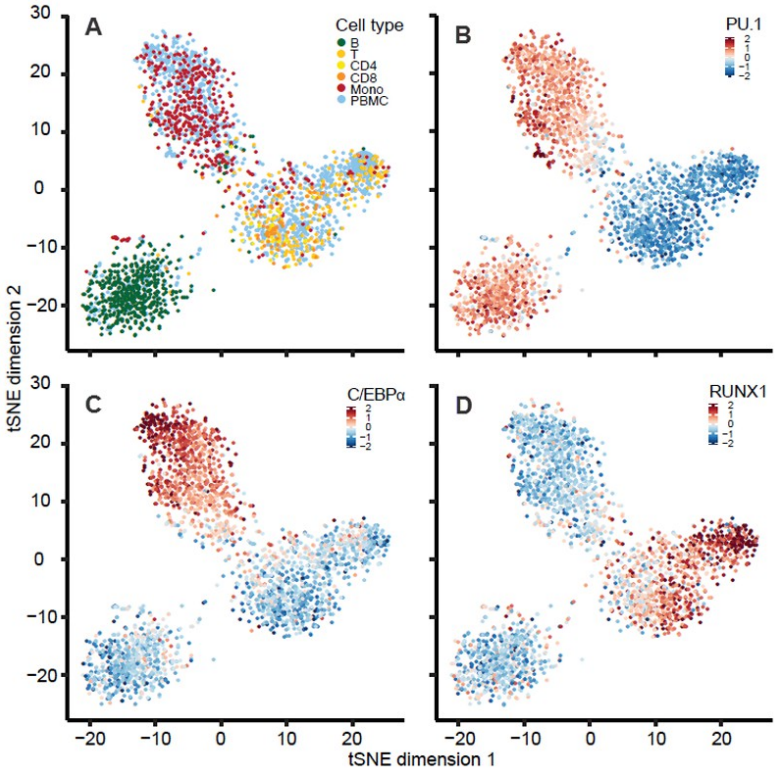

Figure 3. t-SNE plots of chromatin accessibility reveal robust clustering of chromatin accessibility patterns within specific hematopoietic cell types. Chromatin accessibility profiles for all cells (**Panel A**), PU.1 (**Panel B**), C/EBPα (**Panel C**), and RUNX1 (**Panel D**) reveal discrete clustering of major cell types derived from human blood.

Conclusions

This protocol, developed by the Greenleaf lab in conjunction with TBUSA, allows for the rapid generation of libraries for single-cell ATAC-seq. The ICELL8 Single-Cell System streamlines this workflow, provides automated cell isolation/imaging, and enables a simple, rapid 4–5-hour on-chip workflow. These methods enable the rapid, high-throughput processing of 1,000–2,000 cells/chip with an average library complexity of ~14,000 fragments/cell, sufficient to recapitulate previously described chromatin accessibility profiles and identify specific cell types within a highly complex starting sample (e.g., human blood).

Methods

Complete methods used in the generation of the above data may be obtained from Mezger *et al.*, 2018, while a streamlined protocol for single-cell ATAC-seq on the ICELL8 Single-Cell System may be obtained directly from TBUSA.

References

Mezger, A. *et al.* High-throughput chromatin accessibility profiling at single-cell resolution. *Nat. Commun.* **9**, 3647 (2018).

Takara Bio USA, Inc.
United States/Canada: +1.800.662.2566 • Asia Pacific: +1.650.919.7300 • Europe: +33.(0)1.3904.6880 • Japan: +81.(0)77.565.6999