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Application Note

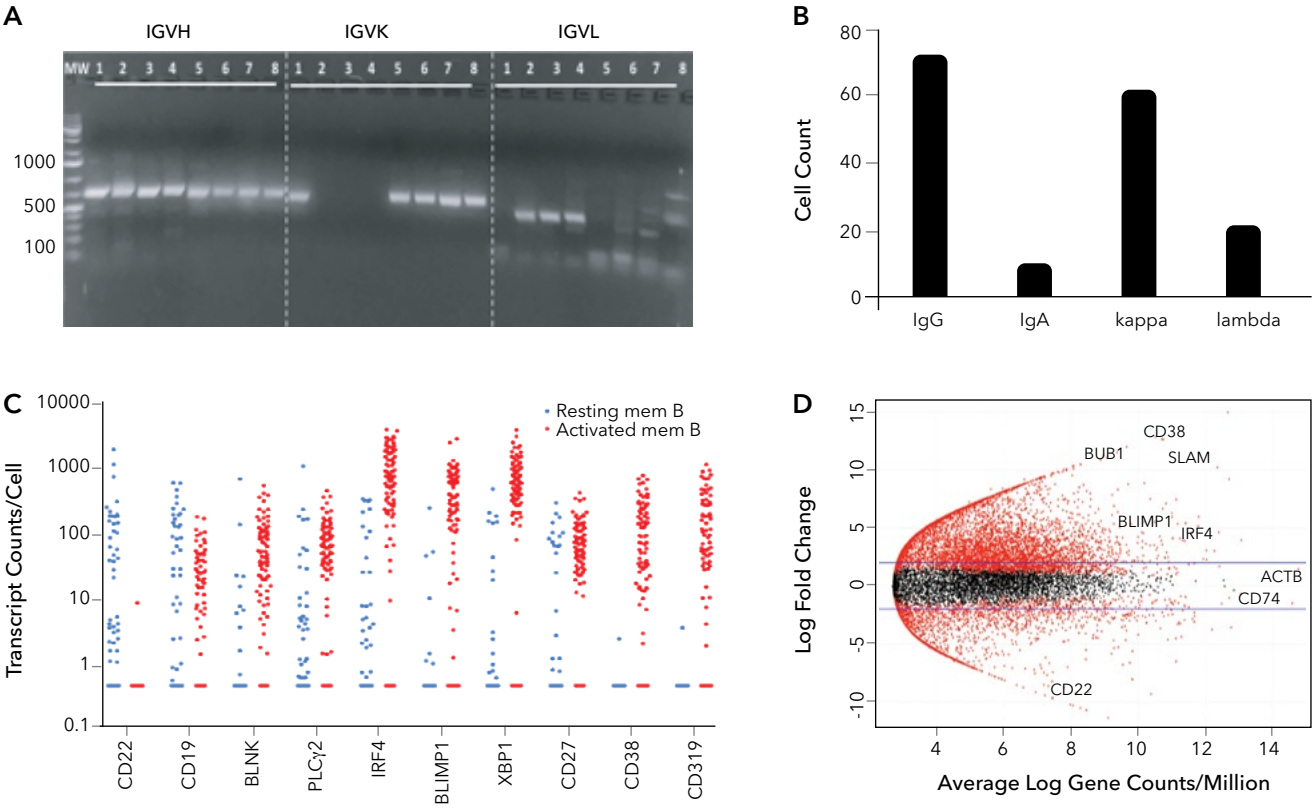
B Cell Isolation for Single Cell Genomics

Introduction

Single-cell RNA-sequencing (scRNA-seq) allows for dissection of gene expression profiles from individual cells, offering insights into sample heterogeneity and the genomic variability underlying molecular phenotypes. scRNA-seq begins with single cell isolation, which has traditionally been limited to expensive and complicated FACS instruments, often inconveniently housed in core facilities. Namocell's single cell dispensers offer an alternative to FACS isolation, enabling rapid and reliable deposition of single cells for multiomics approaches. In this application note, the Namo Single Cell dispenser was used to isolate single activated B cells labeled with both a cell viability dye and a fluorescent, cell type-specific marker. Gene expression was quantified for both resting and activated B cells to identify transcriptional changes associated with the activated cell state.

Methods

Primary human memory B cells were stimulated in bulk on mCD40L-3T3 feeder cells for 6 days. Cells were harvested and stained with anti-human CD27-PE and the cell viability dye CellTracker Green. The Namo Single Cell Dispenser was used to sort and dispense PE-positive and FITC-positive cells into a 96-well PCR plate pre-loaded with 4 µL of lysis buffer per well. Following cell lysis, cDNA was synthesized from each well and libraries were prepared for RNA-sequencing. Gene-specific amplification (qPCR) was also performed for the VH and VL antibody variable domains of each single cell. Libraries were sequenced on an Illumina NextSeq and gene expression was compared between activated and resting B cells.



Data courtesy of Krista McCutcheon and Eric Waltari (Antibody Discovery Scientists)

Results

In the 96-well plate, 93 wells (97%) contained cDNA following amplification. Sanger sequencing was used to determine that 100% of these cells were single cells, and not doublets. Expression of immunoglobulin genes via qPCR confirmed the uniform presence of heavy chain IGVH and the presence of either the kappa or lambda light chains (IGVK, IGVL; Figure 1A depicts 8 representative cells). Figure 1B shows quantification of the number of cells found with each heavy and light chain. Gene expression changes were observed across several genes implicated in B cell activation (Figure 1C) and differential expression analysis further identified a set of genes up- or down-regulated as a result of cell activation (Figure 1D). As expected, resting cells expressed much higher levels of CD22 and activated cells showed induction of IRF4 expression. Overall, the Namo Single Cell Dispenser provides a fast benchtop sorting and dispensing platform for seamless downstream single-cell genomic analyses.