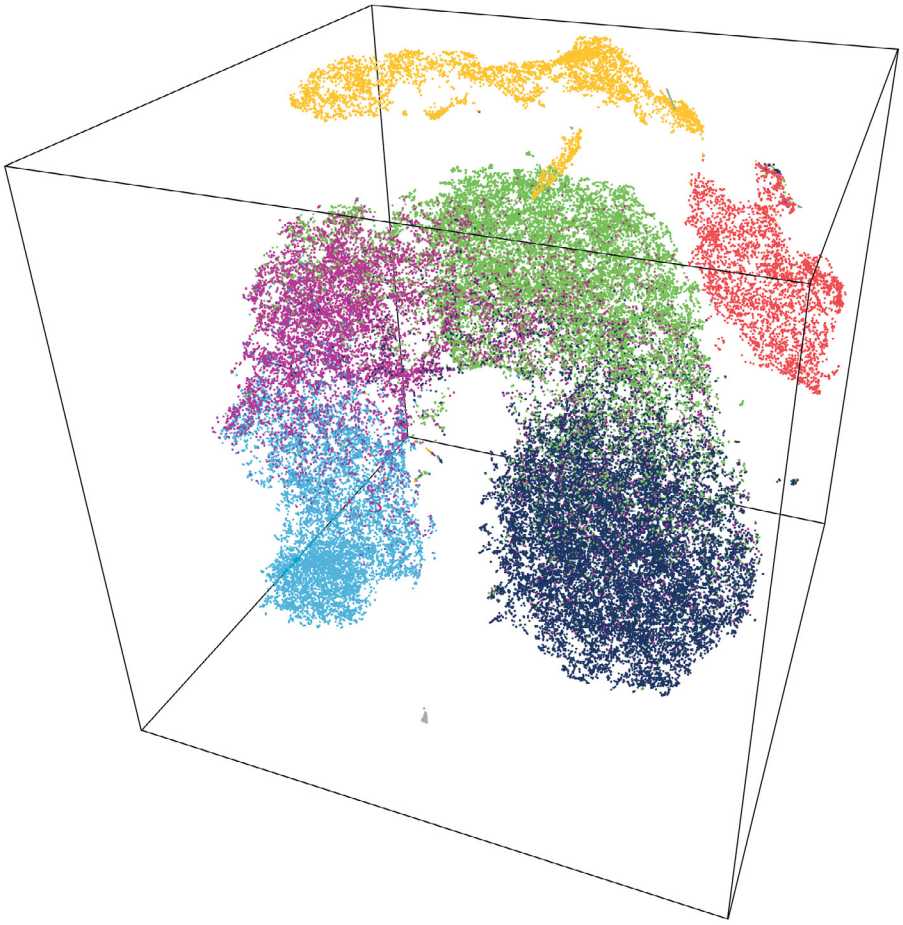
Product Brochure



CHROMIUM™

Single Cell 3' Solution

10xGenomics.com/single-cell

CHROMIUM™

Single Cell 3' Solution

High-throughput single cell transcriptomic measurements enable discovery of gene expression dynamics for profiling individual cell types.

- Complete practical solution for single cell analysis
- Identify rare cell types in heterogeneous populations
- Encapsulate 100-80,000+ cells in 10 minutes
- Wide dynamic range

Introduction

Advances in single cell RNA quantification techniques have enabled comprehensive study of cell subpopulations within a heterogeneous population. We developed the GemCode™ Technology, which combines microfluidics with molecular barcoding and custom bioinformatics software to enable 3' mRNA counting from thousands of single cells.

The Chromium™ Single Cell 3' Solution

Single cells, reagents and a single Gel Bead containing barcoded oligonucleotides are encapsulated into nanoliter-sized GEMs (Gel Bead in emulsion) using the GemCode Technology. Lysis and barcoded reverse transcription of polyadenylated mRNA from single cells are performed inside each GEM. High-quality next generation sequencing libraries are finished in single bulk reaction. Finally, the Chromium™ Software Suite is utilized for processing, analysis and visualization of single cell gene expression data.

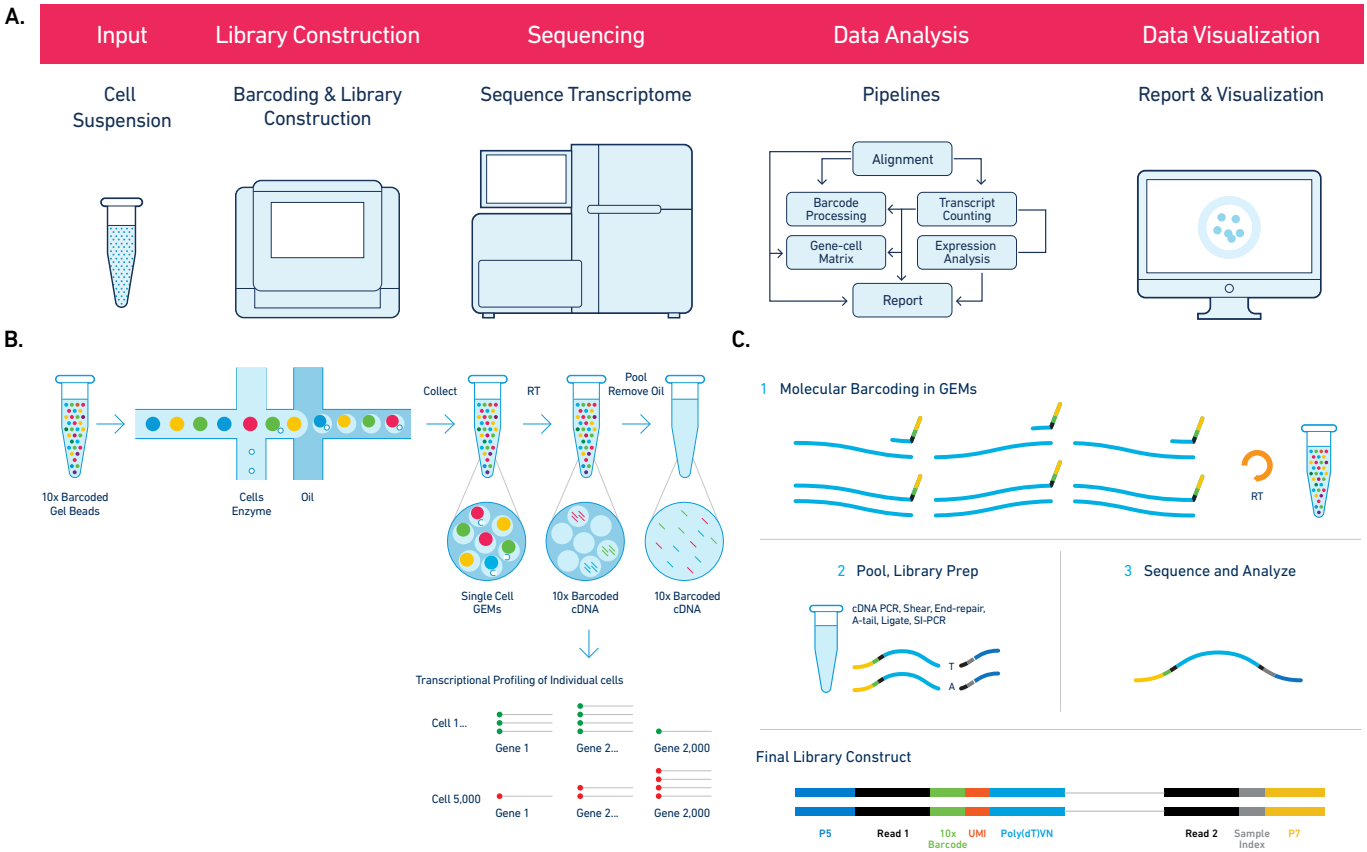


Figure 1. Chromium™ Single Cell 3' Solution. (a) Workflow schematic overview. (b) Formation of GEMs, RT takes place inside each GEM, which is then pooled for cDNA amplification and library construction in bulk. (c) v2 Single Cell Assay schematic overview.

Technical Performance

We analyzed cell lines, peripheral blood mononuclear cell (PBMCs) and bone marrow mononuclear cells (BMMCs) to evaluate the technical performance of the Chromium Single Cell 3' Solution. To verify single cell encapsulation and sensitivity, a mixture of human 293T and mouse NIH/3T3 cells were profiled. 1,015 GEMs contained cells, of which 501 were human only, 514 were mouse only and 3 mixed, indicating an inferred multiplet rate of 0.6%. At ~60,000 reads/cell, a median of ~5,400 genes and ~33,100 transcripts were detected per cell.

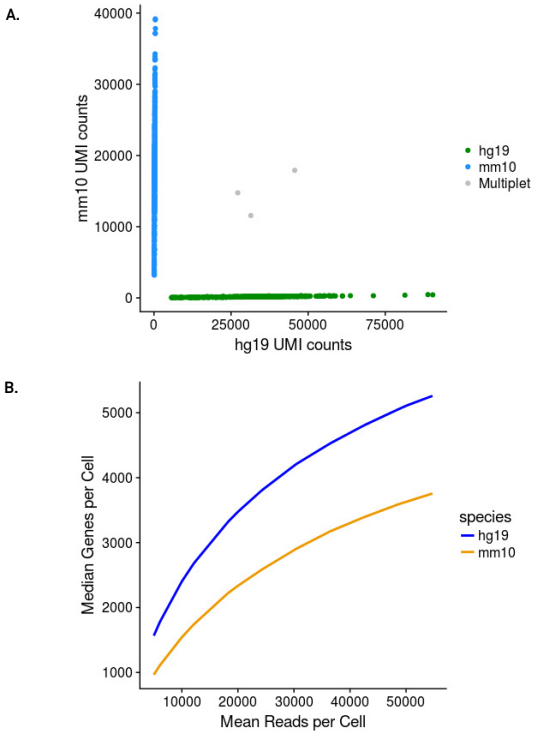


Figure 2. Technical performance. (a) Scatter plot of human and mouse transcript counts detected in GEMs from a mixture of 293T and NIH/3T3 cells. (b) Median genes detected per cell in a mixture of 293T and NIH/3T3 cells as a function of reads per cell.

Chromium™ Single Cell Datasets Available for Download:

- 1.3 Million Brain Cells from E18 Mice
- Mixture of Human (HEK293T) and Mouse (NIH3T3) Cells
- PBMCs from a Healthy Donor

Access these and other single-cell datasets at: support.10xgenomics.com/single-cell/datasets

Immunology Application

Major Subpopulations Observed Within a Heterogeneous PBMC Sample

In Zheng *et al.* (2017) clustering analysis was performed to dissect the heterogeneity of PBMCs using the v1 single cell reagents run on the GemCode™ System. Examination of the most variable genes in each cluster revealed many well-characterized markers for specific subpopulations of PBMCs. We scored ~68,000 PBMCs against the average expression profile of 10 bead-enriched purified PBMC subpopulations, and classified each cell based on its similarity to a purified population. Cell classification was mostly consistent with cell-marker based classification analysis.

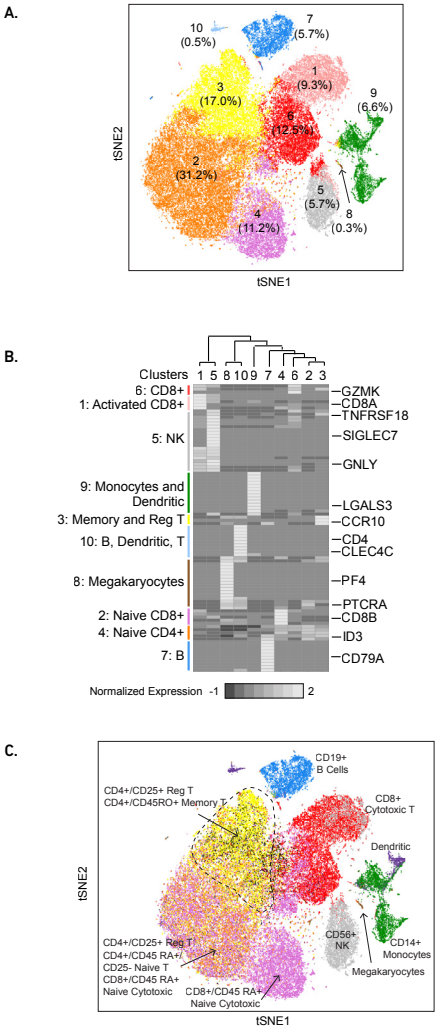


Figure 3. Analysis of 68,000 fresh PBMCs using the v1 single cell reagents run on the GemCode™ System. (a) tSNE plot of 68,000 PBMCs. (b) Top variable genes from each of 10 clusters are normalized and presented in a heat map. Representative markers from each cluster are shown on the right, and the putative cluster ID is shown on the left. (c) tSNE plot of 68,000 PBMCs, with each cell colored by the cell type of purified PBMCs. Approximately 20,000 reads/cell in this experiment (adapted from Zheng *et al.*, 2017).

Cancer Application

Comparison of Specific Subpopulations in AML

Single cell profiling enables comparison of specific subpopulations in frozen PBMC samples from healthy controls and patients with acute myeloid leukemia (AML). This analysis revealed misregulation of the FLT3 pathway that would have been missed by bulk RNA-seq.

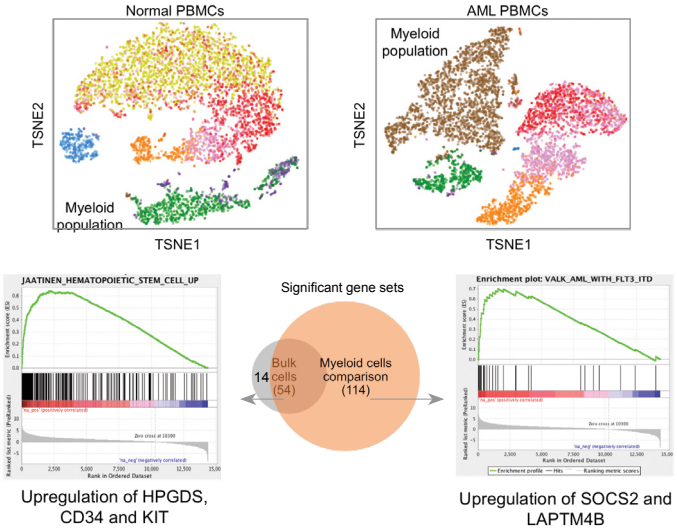


Figure 4. Single cell profiling from healthy and malignant tumor cell samples. Selection of myeloid populations in normal and AML PBMCs. Bottom graphs show an overlap of significant gene sets between bulk RNA-seq and myeloid-cell specific comparisons. Whereas the bulk comparison revealed expected pathways, such as upregulation of stem cell genes, the myeloid-specific comparison revealed upregulation of the FLT3 pathway.

CLL and AML BMMCs Show Expansion of Distinct Populations

We performed single cell analysis of frozen BMMCs from healthy controls, chronic lymphocytic leukemia (CLL) and AML patients. We observed a proliferation of B cells in the CLL sample, and a proliferation of myeloid progenitors in the AML sample, which is consistent with the disease pathology.

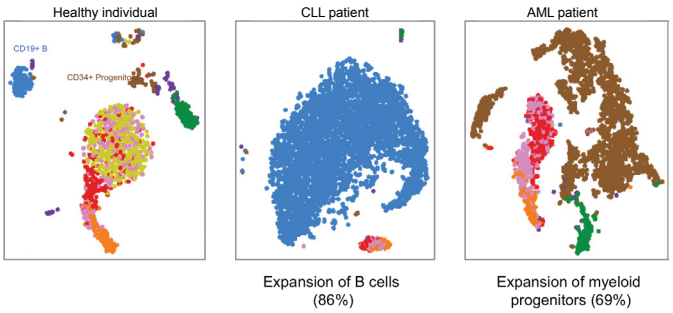


Figure 5. Single cell profiling from healthy and malignant tumor cell samples. Single cell profiling of BMMCs from healthy, CLL and AML patients. ~30,000 reads/cell in this experiment.

Stem Cell Application

Major Subpopulations Among Intestinal Epithelial Cells

To collect intestinal epithelial cells (IECs), a 10cm segment of proximal jejunum was dissected and used to obtain dissociated single epithelial cells as described in Magness *et al.*, 2013. Dead, hematopoietic and endothelial cells were removed, and remaining cells were positively selected with anti-EpCAM. Lgr5+ stem cells were obtained as described above, followed by FACS purification based on expression of Lgr5-green fluorescent protein. Clustering analysis was performed to dissect the heterogeneity of IECs. Examination of the cluster-specific genes revealed many well-characterized markers for subpopulations of IECs. The existence of stem cell population was confirmed by clustering analysis of Lgr5+ stem cells.

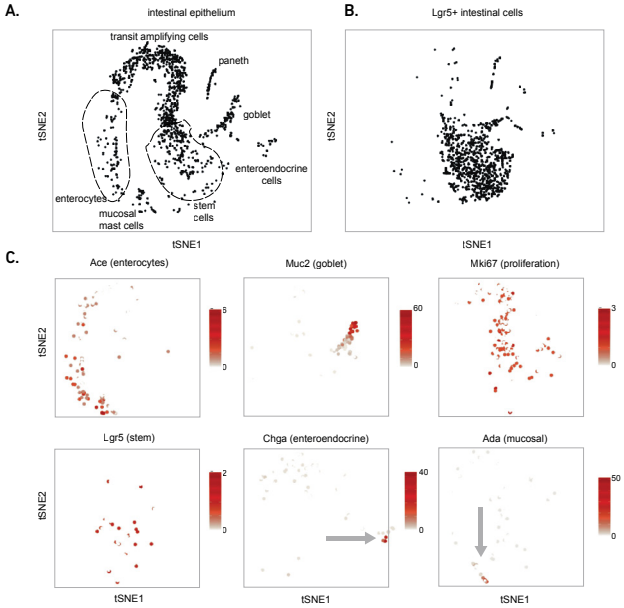


Figure 6. Analysis of ~2,000 intestinal epithelial cells. (a) tSNE plot of ~1k IECs. (b) tSNE plot of ~1k Lgr5+ stem cells. (c) tSNE plot of ~1k IECs, with each cell colored by the normalized expression of markers; Ace: enterocytes (absorptive cells), Muc2: goblet cells, Mki67: proliferation marker (transit amplifying cells), Lgr5: stem cells, Chga: enteroendocrine cells and Ada: mucosal cells. ~10k reads/cell in IECs, and ~70k reads/cell in Lgr5+ stem cells.

Conclusion

We performed high-throughput gene expression profiling of mRNAs in single cells using the Chromium Single Cell 3' Solution. Our scalable approach enables detection of rare cells in a heterogeneous tumor population. Moreover, efficient cell capture enables analysis of clinically relevant sample types with limited cell input.

Literature Cited: *Nature Communications* doi:10.1038/NCOMMS14049.

All Publications are Available at:

10xgenomics.com/resources

Additional Resources:

support.10xgenomics.com/single-cell

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