Integrative Single-Cell Epigenetic and Transcriptomic Profiling of Human PBMCs Using Droplet Paired-Tag

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Single-cell epigenetics advances the understanding of immune function

Epigenetics of Immunity

- Epigenetic mechanisms such as **histone modifica**tions, play a critical role in regulating immune cell differentiation, activation, and function.
- The immune system's complexity arises from its highly **heterogeneous cell populations**, with unique epigenetic landscapes driving their specialized roles in immunity, inflammation, and tolerance.
- Traditional bulk profiling methods **mask the diversity** of epigenetic states by averaging signals across cell types, while cell-sorting techniques disrupt the natural cellular environment and fail to capture subtle regulatory changes.
- **Single-cell** technologies enable simultaneous profiling of chromatin states and transcriptional activity in individual cells, providing **insight into the epigenetic underpinnings** of immune response and autoimmunity.

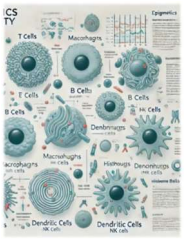

Figure: Epigenetics plays a central role in cell biology, mediating the long-term effects that environment and aging have on immune cell development and function

Paired-Tag Enables Cell Resolution, Holistic Epigenetic and Transcriptomic Profiling

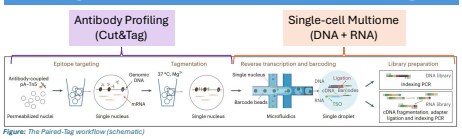

Figure: The Paired-Tag workflow (schematic)

The **Paired-Tag** technology combines single-cell transcriptomics with scCut&Tag (**scRNA + scCut&Tag**) providing the ability to obtain cell-specific epigenetic biomarkers from heterogeneous cell populations. Paired-Tag can map **histone modifications, chromatin remodelers, and transcription factor binding**, for a complete picture of cellular epigenetic state. It is compatible with **several single-cell platforms**, requires no special processing, and **can be multiplexed on-platform** for higher experimental throughput.

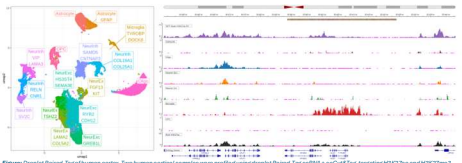

*Figure: Droplet Paired-Tag of human cortex. Two human cortical samples were profiled using droplet Paired-Tag scRNA + scCut&Tag, targeting H3K27ac and H3K27me3. Roughly 20,000 cells were recovered after filtering. (**Left**) The RNA from both experiments were combined for cell type identification and annotation. The resulting UMAP embedding is shown along with cell labels. (**Right**) Following annotation, cell-specific pseudobulk data were computed, and H3K27ac tracks of the TYROBP region are shown, where a clear microglia-specific signal is apparent, despite microglia comprising only 2% of the cells.*

Paired-Tag allows us to capture **cell-specific profiles** from heterogeneous tissues **without sorting**, and to identify cell types, as it easily **integrates with existing single-cell atlases**.

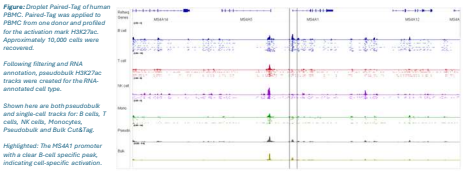

Figure: Droplet Paired-Tag of human PBMC. Paired-Tag was applied to PBMC from one donor and profiled for the activation mark H3K27ac. Approximately 10,000 cells were recovered.

Following filtering and RNA annotation, pseudobulk H3K27ac tracks were created for the RNA-annotated cell type.

Shown here are both pseudobulk and single-cell tracks for: B cells, T cells, NK cells, Monocytes, Pseudobulk and Bulk Cut&Tag.

Highlighted: The MS4A1 promoter with a clear B-cell specific peak, indicating cell-specific activation.

PBMC Profiling: First Chromatin State Atlas of Immune Cells

The immune system must respond to and remember **environmental** exposures and signals. Responses are driven by highly dynamic **epigenetic** mechanisms that have not been examined at single-cell resolution.

We profiled PBMCs from 4 donors for 4 chromatin marks using **Cut&Tag** and **Paired-Tag**, recovering >70,000 **single-cell** profiles.

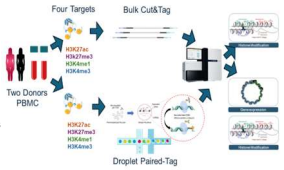

Figure: Experimental design – bulk & single-cell epigenetic profiling of PBMC

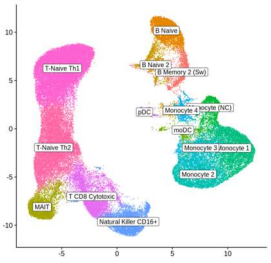

Figure: UMAP embedding of 74,770 PBMCs. Paired-Tag profiling (RNA component)

The RNA component of Paired-Tag enables all experiments to be joined, and **cell types** to be identified **uniformly** and accurately.

By combining single-cell RNA profiling with **single-cell Cut&Tag**, this study presents the first single-cell **chromatin state atlas of human immune cells**.

These data pave the way to **understanding epigenetic mechanisms** of cell states, function, disease response, and aging.

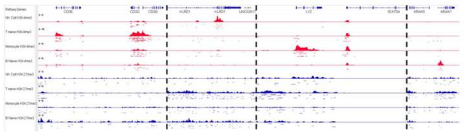

Figure: Pseudobulk and single-cell H3K4me3 and H3K27me3 tracks at major cell type markers

Highly-concordant bulk and single-cell Cut&Tag identifies robust CREs

Comparison of inter-subject **single-cell Paired-Tag** (pseudobulk) **and bulk Cut&Tag** shows that tracks are **highly correlated** within target.

The single-cell data is strongly **concordant** both across Paired-Tag runs, and with corresponding bulk dataset.

Therefore, single-cell processing does **not introduce a bias** or distortion detectable by comparing bulk and pseudobulk.

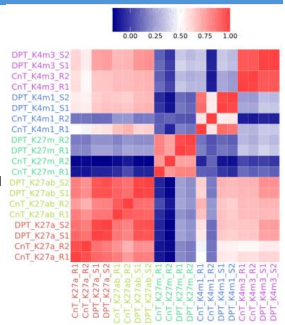

Figure: Correlation of coverage profiles at Regulatory Element DB loci for Paired-Tag and Cut&Tag tracks

Peak calling *using only single-cell Paired-Tag data* recovers **hundreds of thousands of cCREs**. Using MANE-select gene builds, the pattern of these marks is consistent with established biology: H3K27me3 enriching disproportionately for intergenic regions, and active marks (H3Km and H3K27ac) enriching for introns and promoters. Notably H3K4me3 is enriched for promoters and depleted for introns.

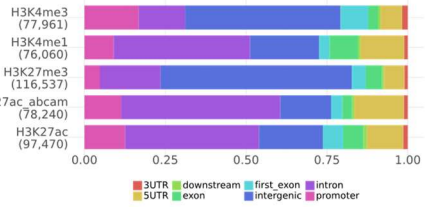

Figure: Called peaks and the proportion of peaks in various genomic regions. MANE-select transcripts.

Broad functional spectrum of PBMC chromatin states in PBMC

Peak calling identified **hundreds of thousands** of candidate cis-regulatory elements (cCREs) in human PBMC, *the majority of which showed a unique histone modification*, typically for repression (H3K27me3). The remaining cCREs were mostly **active** (K27ac + K4me) or **bivalent** (K4me+K27me).

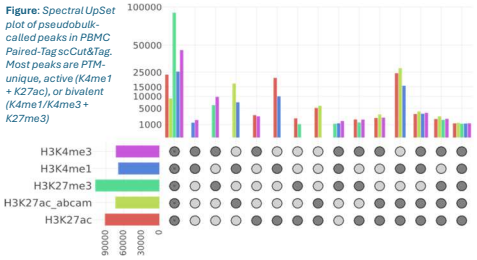

Figure: *Spectral UpSet plot of pseudobulk-called peaks in PBMC Paired-Tag scCut&Tag. Most peaks are PTM-unique, active (K4me1 + K27ac), or bivalent (K4me1/K4me3 + K27me3)*

High-efficiency single-cell profiles

To assess the **profiling efficiency** of Paired-Tag, the number of fragments (UMI) per cell and per modality were compared. **Paired-Tag** was performed on human cortical samples, targeting H3K27ac, H3K27me3 and EZH2.

Across all targets, **1000s of UMI/cell** were recovered, sufficient to perform LSI-based (Signac) cell clustering on Paired-Tag data, and can **produce the coverage equivalent of bulk Cut&Tag with as few as 5,000 cells.**

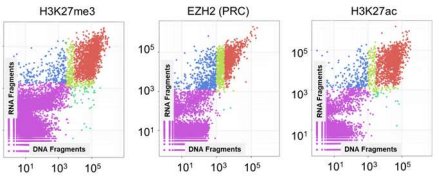

Figure: Recovered DNA and RNA fragments from three Paired-Tag experiments. Colored areas: Ideal (red); >1250 RNA UMI, >2000 DNA UMI; Good (yellow) >1000RNA >1250 DNA; RNA-Low (Green), DNA-Low (Blue), and Empty (purple)

Single-cell profiling of PBMC identifies rare epigenetic T-cell subtype

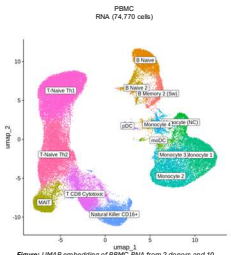

Figure: UMAP embedding of PBMC RNA from 2 donors and 10 Paired-Tag experiments.

- **Profiling Method:** Utilized Paired-Tag to analyze PBMCs from 2 donors, targeting 4 epigenetic marks
- **Data Processing:** CellRanger, Seurat, BigSur; annotated using CellTypist
- **Identified Cell Types**: T cells (2 naïve, CD8, NK, MAIT), B cells (naïve + memory), Monocytes (classical, non-classical), Dendritic (pDC)

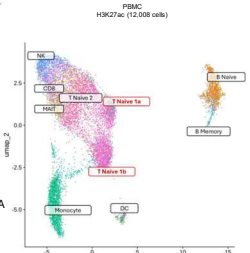

Figure: UMAP/LSI embedding of H3K27ac PBMC scCut&Tag (Paired-Tag) data from 2 donors

- **Epigenetic analysis:** H3K27ac Signac scCut&Tag profiles merged across donors
- **Cluster identification**: LSI identifies 8 clusters, including an additional T-cell cluster not seen in RNA data
- **Discovery of rare subtype**: Rare epigenetically-defined T-cell subtype (~5% overall) was not detectable by RNA alone
- **Significance:** Demonstrates power of single-cell epigenetic profiling to discover novel biomarkers

Identifying type-specific bivalent enhancers underlying immune function

Shared vs. Cell-Type-Specific Regulatory Elements: The majority of CREs are broadly shared, comprising the core epigenomic signature that defines immune cells. Only a few hundred peaks are unique or narrowly shared, highlighting the minimal yet critical fraction of the epigenome that drives immune cell subtype identity.

Single-cell Bivalency: A small subset of CREs are co-marked by H3K27me3 (repressive) and H3K4me3 (active). Paired-Tag single-cell profiling confirms that true bivalency is present at specific loci *within* cell types.

This finding underscores the nuanced role of chromatin state in priming potential transcriptional activity at key regulatory sites.

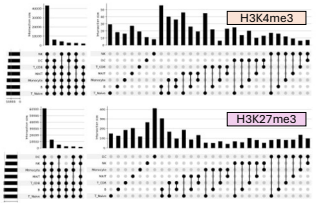

Figure (top): UpSet plot of peak activity across immune cell types

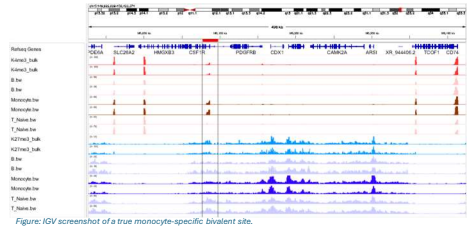

Figure: IGV screenshot of a true monocyte-specific bivalent site.

Conclusions

- **Joint Profiling of Single-Cell Cut&Tag and RNA**: Provides a detailed view of DNA-binding events, similar to ChIP-seq, but with single-cell resolution.
- **Incorporates RNA Profiling**: Identifies cell types and maps interactions between enhancers and their target genes.
- **Valuable Insights into Immune Epigenetics**: Identifies hundreds of thousands of cCREs, including bivalent elements, across major immune cell types
- **Future Potential**: Innovations like Paired-Tag pave the way for deeper biological discoveries and promising therapeutic breakthroughs.

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