Paired-Tag: Single-Cell Epigenetic Profiling for Biomarker Discovery

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Epigenetics holds key biomarkers, but has been challenging to study

Epigenome as Biomarker

- Epigenetic dynamics, such as **DNA methylation, histone modifications**, and **transcription factor binding** are essential biomarkers for understanding disease mechanisms and cellular states due to their stable and regulatory roles in gene expression.
- Traditional methods, such as sorting, **suffer from biases** and lack the resolution needed to detect, cell-specific epigenetic variations.
- These limitations hinder the accurate identification and **validation of reliable biomarkers**, impeding advancements in diagnostics and personalized medicine.
- Addressing these challenges is crucial for harnessing the full potential of epigenetic biomarkers in clinical and research settings.

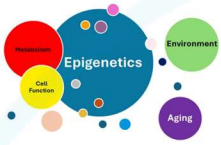


Figure: Epigenetics plays a central role in cell biology, mediating the long-term effects that environment and aging have on cellular metabolism and function – which in turn feed back to impact the epigenome.

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

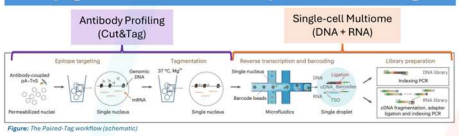


Figure: The Paired-Tag workflow (schematic)

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.

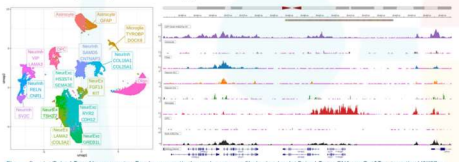


Figure: Droplet Paired-Tag of human cortex. Two human cortical samples were profiled using droplet Paired-Tag scRNA + scCut&Tag, targeting H3K27ac and H3K27me3. Roughly 20,000 cells were recovered after filtering. (Left) The RNA from both experiments were combined for cell type identification and annotation. 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**.

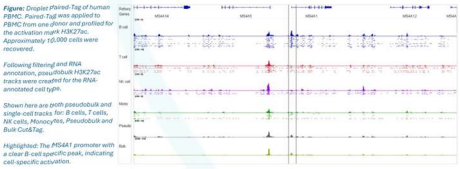


Figure: Droplet Paired-Tag of human PBMC. Paired-Tag was applied to PBMC from one donor and profiled for the activation mark H3K27ac. Approximately 10,000 cells were recovered.

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

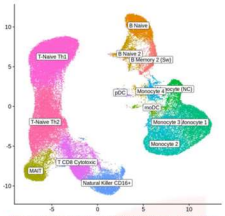


Figure: UMAP embedding of 74,770 PBMCs. Paired-Tag profiling (RNA component)

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**.

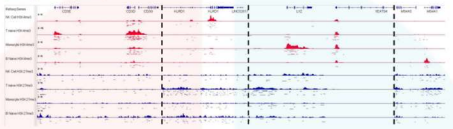


Figure: Pseudobulk and single-cell H3K4me3 and H3K27me3 tracks at major cell type markers

Droplet Paired-Tag integrates with existing single-cell atlases

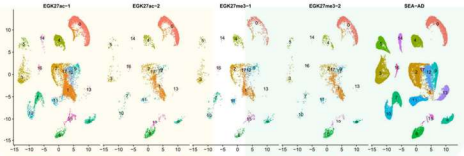


Figure: Integrative embedding of 4 Paired-Tag experiments (RNA only) with the SEA-AD single cell consortium data. Though multiple histone marks are targeted, only the RNA component is needed for seamless data integration.

Paired-tag makes it easy to **annotate** cell types and to **integrate** cell-level epigenetics with existing single-cells studies.

Cell annotation is difficult even for single-cell ATAC data, and presents a significant challenge for single-cell Cut&Tag.

By leveraging transcriptomics, Paired-Tag data can be integrated with existing cell atlases, streamlining analytics and maximizing data value.

Highly-reproducible scCut&Tag signatures even for TFs and PRC

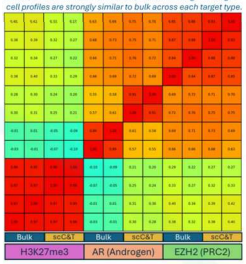


Figure: Genome-wide profile correlations in C4-2 cells between bulk and single-cell Cut&Tag for a histone mark, a transcription factor, and a chromatin remodeler, demonstrating that single-cell profiles are strongly similar to bulk across each target type.

Consistency of Paired-Tag was ensured by comparing genome-wide profile correlations in C4-2 cells between bulk and single-cell Cut&Tag across **H3K27me3** (histone), **AR** [Androgen Receptor] (TF), and **EZH2** (chromatin).

Single-cell profiles closely mirror bulk for each target, highlighting **strong reproducibility** and consistency.

Paired-Tag retains the high accuracy of Cut&Tag down to the single-cell level, providing reliable epigenetic profiling of diverse chromatin targets across cell types.

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.**

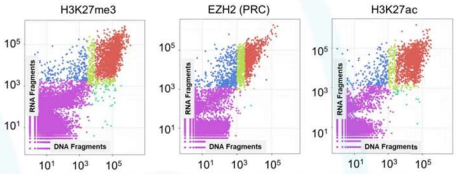


Figure: Recovered DNA and RNA fragments from three Paired-Tag experiments. Colored areas: Ideal (red): >1250 RNA UMI, >2000 DNA UMI; Good (yellow) >1000RNA >1250 DNA; RNA-Low (Green), DNA-Low (Blue), and Empty (purple)

Simple workflow can be multiplexed for up to 4x sample throughput

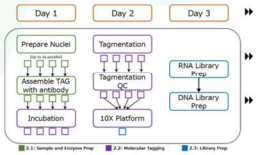


Figure (top): The Paired-Tag Workflow

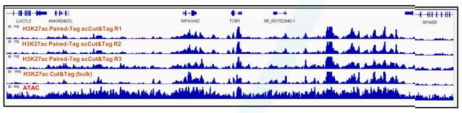


Figure (bottom): The Paired-Tag Workflow

- **Easy-to-use**: Short workflow, existing platform compatibility
- **Flexible**: Off-the-shelf antibodies tailor your experiment to any target
- **High-throughput**: Multiplexing enables up to 4 simultaneous experiment per 10X lane
- **Powerful**: Demonstrated on TFs, chromatin remodelers, and histones

Towards chromatin-based epigenetic biomarker discovery

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

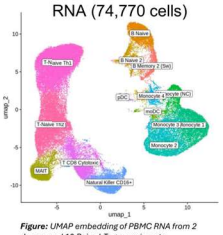


Figure: UMAP embedding of PBMC RNA from 2 donors and 10 Paired-Tag experiments.

- **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)

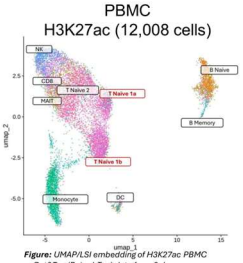
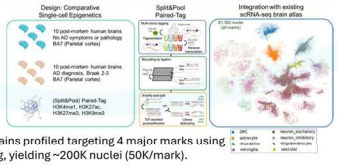


Figure: UMAP/LSI embedding of H3K27ac PBMC scCut&Tag (Paired-Tag) data from 2 donors

- **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

Neuronal Epigenetic De-repression is a key epigenetic marker of Alzheimer's



Epigenetic factors underpin AD and could serve as critical pieces of understanding or biomarkers for the disease.

20 (10 AD, 20 CTL) brains profiled targeting 4 major marks using Split&pool Paired-Tag, yielding ~200K nuclei (50K/mark).

Repressed regions were defined on the basis of H3K9me3 deposition in healthy controls and external ChIP-seq profiling of brain and neuronal cell lines.

In excitatory neurons H3K27ac deposition was observed to a **far greater extent** in AD samples than healthy controls.

Epigenetic de-repression has been posited as a mechanism of AD pathology. This work highlights the power of single-cell epigenetics in uncovering novel biomarkers.

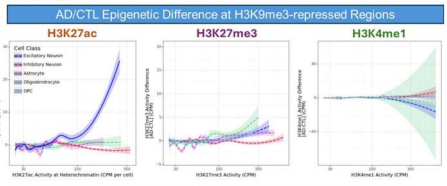


Figure: Average target occupancy (CPM) at repressed regions (x-axis) across various cell lines. (y-axis) The difference in CPM (AD – CTL) for the corresponding element. Notably, the more overall H3K27ac deposition in excitatory neurons, the larger the AD/CTL difference, highlighting the AD-specificity of the epigenetic state.

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 Epigenetic Drivers**: Uncovers key cellular behaviors and processes, spanning contexts from development to disease.
- **Future Potential**: Innovations like Paired-Tag pave the way for deeper biological discoveries and promising therapeutic breakthroughs.

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