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Spotlight

Multiomic chromatin and transcription profiling with EpiDamID

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DamID maps protein-genome interactions using DNA adenine methyltransferase tethered to individual chromatin proteins. In a recent issue of *Molecluar Cell*, Rang et al. introduce EpiDamID, a powerful extension of DamID suitable for mapping histone marks while simultaneously measuring mRNA levels in single cells.

Genome-wide mapping of histone modifications and chromatin regulatory proteins is essential for understanding how chromatin architecture impacts global gene expression in any eukaryotic system. Chromatin immunoprecipitation followed by sequencing (or ChIP-seq) has been the preferred technique for analyzing genome-wide protein-DNA interactions since its inception 15 years ago. However, ChIP-seq has traditionally required large numbers of cells, complicating studies in which the starting material is limiting. In addition, ChIP-seq studies on heterogeneous populations of cells, such as many human tissue samples, provide average profiles of all chromatin states in each population. There has long been a need for new single-cell chromatin mapping techniques suitable for characterization of heterogeneous samples. Several techniques adapted to single cells have recently been described, including single-cell ChIP-based approaches and adaptations of CUT&RUN (cleavage under targets and release using nuclease) and CUT&Tag (cleavage under targets and tagmentation), among other methods (Klein and Hainer, 2020). More recently, multi-omics approaches have been developed that allow researchers to measure multiple features (typically mRNA levels and one other feature) simultaneously in single cells. Techniques like Paired-Tag, CoTECH (combined assay of transcriptome and enriched chromatin binding), and SET-seq (same-cell epigenome and transcriptome sequencing) enable mapping of histone modifications and measurement of gene expression in the same cells (Sun et al., 2021; Xiong et al., 2021; Zhu et al., 2021). These techniques all employ modifications of CUT&Tag, where protein A fused to the transposase from the bacterial transposon Tn5 (pA-Tn5), which is targeted to specific chromosomal loci via binding of the pA module to antibodies specific for chromatin proteins. DNA surrounding the chromatin mark is then marked by the insertion of adapters from pA-Tn5, allowing specific amplification and sequencing of genomic regions near the chromatin protein of interest. While these methods are of broad utility and have been implemented in many systems, they all depend on the availability and specificity of antibodies for each chromatin protein or histone mark.

DNA adenine methyltransferase identification (or DamID) is an alternative method for mapping chromatin protein binding sites that does not depend on antibodies (van Steensel and Henikoff, 2000). In this method, the protein of interest is expressed as a fusion protein with the bacterial Dam DNA methyltransferase. Consequently, Dam activity is largely restricted to regions near the binding sites of the protein of interest. Dam methylates adenines at GATC motifs within DNA. Consequently, the sensitivity of genomic GATC motifs to the methylation-dependent DpnI restriction enzyme indicates the presence of the protein of interest. Unlike ChIP, DamID reveals protein-DNA contact histories as methylation occurs in living cells and m6A is maintained after the Dam fusion protein releases from chromatin, at least until new DNA synthesis takes place. Newer variants of DamID have been developed to study different features of chromatin biology like Split DamID, which examines co-occupancy of chromatin proteins (Hass et al., 2015), and RNA-DamID, which measures interactions between nuclear RNAs and genomic loci (Cheetham and Brand, 2018). In addition, many of these techniques have been adapted to profile single cells, enabling studies of heterogeneous cell populations. While DamID and its offshoots have numerous advantages, one major weakness is their inability to map histone post-translational modifications that mark active, silent, or other regulatory states.

To integrate single-cell DamID with measurements of gene expression, Jop Kind and colleagues previously developed scDam&T-seq, a method in which single-cell DamID profiles are coupled with quantification of mRNA levels in the same cell (Rooijers et al., 2019). Like other DamID derivatives, scDam&T-seq does not require antibodies or Tn5 transposase fusions and is suitable for measuring cell-type-specific transcriptional programs in heterogeneous tissues or during dynamic transitions, such as embryonic development. In their new study, published in *Molecular Cell*, Rang et al. (2022) have taken the existing scDam&T-seq technique a step further with the development of a new method they call EpiDamID, which is capable of mapping histone modifications in single cells. In EpiDamID, the authors fuse Dam to proteins known to specifically interact with individual histone modifications, (i.e., chromatin "reader" domains), as well as endogenous chromatin-binding proteins and single-chain variable fragment antibodies that target specific chromatin epitopes (Figure 1). In control experiments, unfused Dam was shown to methylate accessible

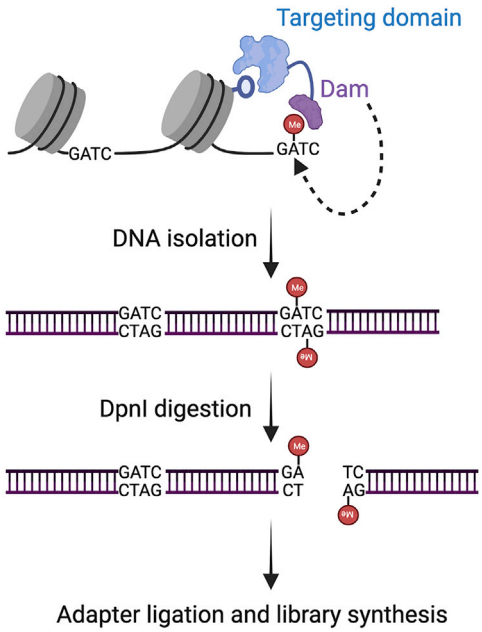

Figure 1. Simplified workflow for chromatin profiling by EpiDamID
Epigenetic "reader" domains target Dam methylase to specific chromatin loci, where adenine residues within GATC motifs are methylated. Methylated GATC sites are cleaved and identified by deep sequencing after adapter ligation, as in DamID protocols.

chromatin, whereas fusion of Dam to various chromatin binding modules resulted in significant enrichment of GATC methylation at regions specific for each fusion. EpiDamID successfully identified several different chromatin domains, including transcriptionally active regions (using Dam fused to readers of active marks), constitutive heterochromatin (with Dam fused to readers of H3K9me3), and facultative heterochromatin (Polycomb) domains (with Dam fused to readers of H3K27me3 or Polycomb proteins).

After validation of EpiDamID by comparison with other mapping approaches in cell lines, the authors go on to profile chromatin structure in more complex systems. For experiments using heterogeneous populations of cells, clustering of cells by chromatin profiles revealed that EpiDamID can successfully distinguish different cell types and captures cell type-specific features of chromatin structure. To better understand the targets and functions of Polycomb group proteins, the authors jointly profiled transcriptome and Polycomb domains in mouse embryoid bodies by targeting the two main types of Polycomb repressive complexes with EpiDamID: PRC1, using full length RING1B protein fused to Dam, and PRC2, using a H3K27me3-mintbody—a modification-specific intracellular antibody—fused to Dam. They show that genes encoding transcription factors are more likely to be Polycomb targets, accumulating higher levels of H3K27me3 and RING1B, suggesting the presence of hierarchical gene regulatory networks. The authors went on to demonstrate the utility of EpiDamID *in vivo*, using zebrafish embryo development as a model system. In this context, they identified broad heterochromatic domains specifically found within notochord. Overall, the authors demonstrated that EpiDamID is well suited to study cell-type-specific epigenetic regulation and can uncover new biological insights by integrating chromatin profiles with transcriptional outputs in the same cells.

In summary, EpiDamID shows considerable promise for epigenomic profiling, adding to a growing toolkit of single-cell chromatin profiling approaches. In some ways, EpiDamID may be considered complementary to approaches based on CUT&Tag since these methods all have distinct sets of advantages and weaknesses. Since adenine methylation is more stable than binding of most chromatin proteins, EpiDamID can be used to track the spatial and temporal distribution of epigenetic marks. On the other hand, EpiDamID comes with its own limitations as the authors have noted. Expression of Dam fusion proteins must be tested and potentially optimized for each new experimental system, as the activity of expression constructs vary by cell type and experimental model. It is possible that higher or lower levels of Dam fusion protein expression may lead to higher-than-optimal background or low signal, respectively, in some models. Since background GATC methylation will always be present due to intrinsic DNA binding by Dam, untethered Dam must therefore be utilized as a control. Like other DamID techniques, the resolution of EpiDamID is a function of GATC sequence density and accessibility in the genome, limiting the precision with which binding sites of proteins of interest can be determined. Consequently, EpiDamID may be best suited for chromatin profiling studies of large chromosomal domains, such as lamin associated domains, nucleolar associated domains, subtelomeric regions, and others.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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