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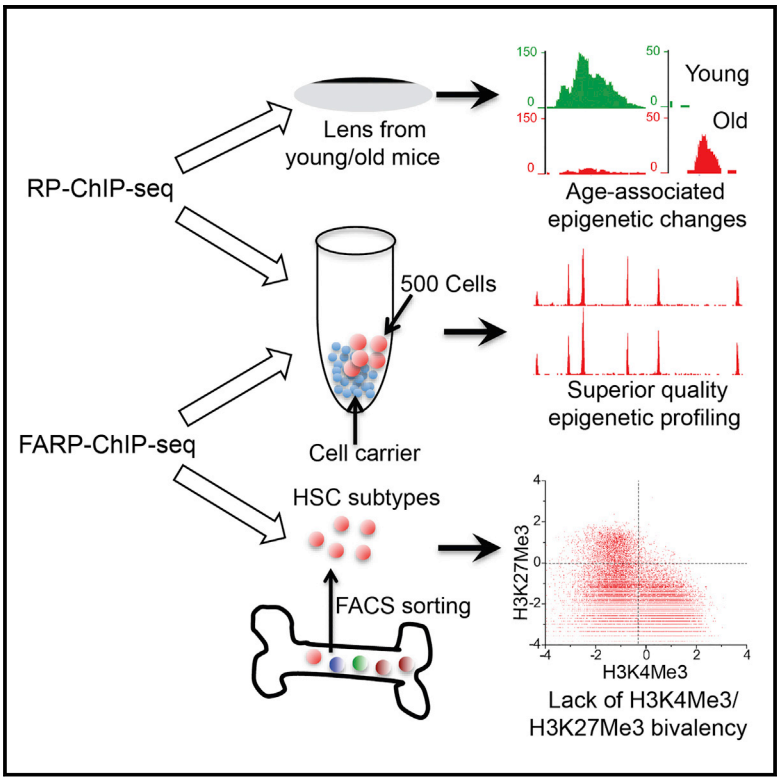
Cell Reports

# Low-Cell-Number Epigenome Profiling Aids the Study of Lens Aging and Hematopoiesis

## Graphical Abstract

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## In Brief

Zheng et al. develop two profiling methods, RP-ChIP-seq and FARP-ChIP-seq, that can be used for as few as 500 cells. Application of the technique reveals age-associated changes in the mouse lens and a lack of H3K4me3/H3K27me3 bivalency on hematopoietic genes in mouse HSCs.

## Highlights

- RP-ChIP-seq enables high-fidelity epigenetic profiling in 500 cells
- FARP-ChIP-seq is generally applicable
- Age-associated epigenome changes in mouse lens are revealed by RP-ChIP-seq
- Lack of H3K4me3/H3K27me3 bivalency on hematopoietic differentiation genes in HSCs

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# Low-Cell-Number Epigenome Profiling Aids the Study of Lens Aging and Hematopoiesis

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## SUMMARY

**Understanding how chromatin modification regulates development and disease can be limited by available material. Despite recent progress, balancing high-quality and reliable mapping using chromatin-immunoprecipitation-based deep sequencing (ChIP-seq) remains a challenge. We report two techniques, recovery via protection (RP)-ChIP-seq and favored amplification RP-ChIP-seq (FARP-ChIP-seq), that provide reproducible mapping in as few as 500 cells. RP-ChIP-seq allows detection of age-associated epigenetic changes in a single mouse lens, whereas FARP-ChIP-seq accurately maps histone H3 lysine 4 trimethylation (H3K4me3) and H3K27me3 in long-term hematopoietic stem cells (LT-HSCs), short-term HSCs (ST-HSCs), and multi-potent progenitors (MPPs) from one mouse. These datasets not only highlight genes that may be involved in lens aging but also indicate a lack of H3K4me3/H3K27me3 bivalency on hematopoietic genes in HSCs.**

## INTRODUCTION

Mapping of epigenome modifications or chromatin regulator/transcription factor binding in a pure cell population is critical for basic and translational research. The ability to map epigenome changes in a cell population during development can shed light on the steps by which different cell lineages establish their transcriptional programs. Mapping the epigenome in a few cells isolated from diseased or healthy tissues may allow the discovery of specific disease-associated changes. Unfortunately, because chromatin immunoprecipitation sequencing (ChIP-seq) requires multi-step manipulations, DNA loss due to irreversible absorption or degradation has made it difficult to reliably obtain high-quality mapping in only a few cells (Park, 2009).

ChIP-seq using standard methods requires nanograms of DNA, and $\geq 10^6$ cells are needed for reliable and high-quality ChIP-seq (Park, 2009). Various strategies have been developed to reduce the cell number needed. One method is to amplify the cells derived from tissues in vitro. Although this is applicable for progenitor/stem cell populations, it is not useful for dissected post-mitotic cells. Culturing and proliferation in vitro may also change progenitor/stem cells, potentially making the genome-wide studies unrepresentative of cells in vivo. Several methods have been developed to facilitate ChIP-seq using thousands or tens of thousands of cells. One of them relies on increasing DNA amplification cycles (Adli et al., 2010; Ng et al., 2013; Shankaranarayanan et al., 2011, 2012), which may introduce mapping bias, as low-abundance ChIP DNA may be underrepresented or lost. Another method utilizes carrier proteins, chemicals, and/or mRNA during ChIP (Zwart et al., 2013), but the absence of carrier during post-ChIP processing still leads to significant DNA loss, thereby compromising ChIP-seq quality.

A third method, called indexing-first ChIP-seq (iChIP-seq) (Lara-Astiaso et al., 2014), uses barcoding and pooling of multiple samples to study the epigenome in multiple hematopoietic lineages. Although the method reduces DNA loss by sample pooling, relying on sorting of fixed cells and sequential ChIP may still lead to DNA loss. Additionally, the on-bead ligation of adapters to chromatin fragments may reduce efficiency. Indeed, 10,000–20,000 sorted hematopoietic cells were used in these iChIP-seq datasets (Lara-Astiaso et al., 2014). Finally, micrococcal nuclease (MNase)-based native ChIP (ultra-low-input micrococcal nuclease-based native ChIP [ULI-NChIP]) was also used for epigenetic mapping (Brind'Amour et al., 2015). While the quality of ULI-NChIP-seq for some epigenetic modifications is reasonable, other modifications were not mapped, indicating that the loss of ChIP DNA during manipulations may result in variable outcomes. Here, we report a new ChIP-seq method for high-fidelity genome-wide profiling using as few as 500 cells and report its applications.

## RESULTS

### Recovery via Protection ChIP-Seq for 500 Cells

An effective way to protect DNA from loss during ChIP-seq is to use agents that behave like DNA and co-purify with chromatin or the DNA of interest during ChIP and library building. This would prevent the loss of DNA due to nonspecific irreversible absorption and degradation by residual contaminating DNases. One straightforward means is to use chromatin as a protection agent. Although this would result in the presence of carrier DNA in the sequencing library, the carrier DNA sequences can be easily