Proposal Title

Enabling Single-cell and Locus-specific Chromatin Proteomics at the University of Michigan

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Executive Summary

Problem: Single cell transcriptional profiling has revealed considerable heterogeneity of gene expression even within genetically identical populations of cells. These differences are postulated to contribute to cell fate decisions both during normal development and in diseases. However, the source of this transcriptional heterogeneity is unclear. Changes in the chromatin state of defined loci are prime candidates to underlie transcriptional heterogeneity between cells with an apparently identical developmental potential. **The ability to profile chromatin at the single cell and single locus resolution, therefore, offers the possibility to deconvolve cellular heterogeneity.** However, single-cell chromatin profiling and single-locus chromatin proteomics remain a significant hurdle in the field of chromatin biology. Indeed, many groups across the University of Michigan desire the capability to profile chromatin at increasingly low-input and higher resolution levels. **In this Biosciences Initiative Exploratory Funding (BIEF) proposal, we propose to develop, import, and distribute to the University of Michigan research community reagents and technologies that will enable single-cell and locus-specific chromatin proteomics.** This capability will permit the elucidation of fundamental mechanisms underlying cell- and locus-specific transcriptional heterogeneities both during normal development and in disease.

Strategies to Address Problem:

1. Develop/import technologies for the *in vitro* development and production of recombinant intellectual property-free 'nanobodies' to detect chromatin modifiers and modifications with high specificity and reproducibility.

2. Develop/implement the capability to examine distributions of chromatin marks and chromatin accessibility at or approaching single cell resolution.

3. Develop/implement technologies to identify chromatin modifying factors and their accessory proteins at individual loci.

Why the Biosciences Initiative: This proposal seeks to standardize new enabling technologies, rather than to address a specific biological question. Such proposals are difficult to be funded by traditional federal funding mechanisms from the NIH, NSF, NOAA, etc.

Resources Required/Requested:

- Three post-docs each funded at 30% effort to travel and train with experts at host institutions and standardize the protocols at UM for each of the first three bulleted Aims under "Deliverables" below.
- Funding to organize a Chromatin Proteomics Workshop to disseminate the technologies.

Deliverables:

- Optimize and standardize a protocol to generate nanobodies against histone post-translational modifiers and modifications (PTMs). As proof-of-principle, we will develop two to three nanobodies against the common chromatin PTMs.
- Import and standardize protocols to profile chromatin modification/state and chromosome organization in low-input samples approaching single cells. As proof-of-principle, profile H3K27me3 and H3K4me3 in such samples by ChIP-Seq; chromatin accessibility by ATAC-Seq; and, chromosome organization by Hi-C.
- Import and standardize a protocol to purify proteins bound to chromatin modifiers at specific loci. As proof-of-principle, we will purify proteins bound to a sequence at HOXA loci that demarcates posterior and anterior HOXA gene clusters that are important for normal development and diseases.
- Distribute the protocols via a week-long intensive Chromatin Workshop with a capacity for 30 trainees as well as via the Epigenomics Core.

We believe that the successful completion of the proposal would position the UM at the forefront of chromatin epigenomics and proteomics technologies. The expertise gained would help labs across the UM campus as well as facilitate recruitment of new faculty (please see letters of support by Chairs of Biomedical Engineering in the College of Engineering, Environmental Health Sciences at the School of Public Health, MCDB in LSA, and Human Genetics, Pathology and Biological Chemistry in the Medical School).