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Jaydev Upponi, Ph.D.
Science Editor
Journal of Visualized Experiments
1 Alewife Center, Suite 200
Cambridge, MA 02140
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September 18th, 2018

Dear Dr. Upponi,
Enclosed please find our manuscript entitled "An Improved ATAC-seq Protocol with Low Mitochondrial DNA Contamination to Map Genome-Wide Chromatin Architecture in Primary Human CD4+ T Lymphocytes", which we submit for publication to the Journal of Visualized Experiments. This submission follows our pre-submission correspondence. Below we provide a summary of our protocol, highlighting its novelty and implications.
ATAC-seq is a powerful tool in the field of epigenetics, a high throughput and relatively simple method to identify open and accessible chromatin. While ATAC-seq is widely used, current protocols are challenged by contaminating mitochondrial DNA reads that can comprise as much as 50% of sequencing reads. In our manuscript we present an improved ATAC-seq protocol that introduces a modified nuclei lysis buffer that is demonstrated to reduce mitochondrial DNA reads from an average of 50% to 3%. This allows for a large decrease in sequencing costs and higher quality data production from the ATAC-seq pipeline.

The main innovations of our work are:

The efficient isolation, freezing and activation of human primary CD4+ lymphocytes from patient whole blood, allowing for the collection of desired material at different times but simultaneous library construction and sequencing. This provides researchers with the flexibility to collect samples based on availability and research needs, while still avoiding batch bias in Tn5 transposase activity, library preparation, and sequencing.

An improved nuclei lysis buffer that minimizes contaminating mitochondrial DNA reads, allowing for a decrease in sequencing costs. A reduction from an average of 50% mitochondrial DNA reads to 3%, allowing for a 50% reduction in sequencing costs.

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Given the widespread use of ATAC-seq, our improved protocol will benefit a large range of research. This improved ATAC-seq protocol has been validated in various cell types, and has potential promises to improve single cell ATAC-seq library quality as well. The protocol will benefit from visual representation of CD4+ lymphocyte handling and nuclei lysis buffer preparation, making it of interest to the Journal of Visualized Experiments community. Thank you for your consideration.

Sincerely,

Christine S Cheng

Assistant Professor
Department of Biology
Boston University