Docket #: S20-449

QUANTIFICATION OF CELLULAR PROTEINS USING BARCODED BINDING MOIETIES

Technology Reference: Chan Zuckerberg CZB-190S; Stanford S20-449

Summary

Researchers at Stanford have developed a method enabling quantification of intracellular protein levels using oligonucleotide-barcoded antibodies.

Multiplexing single cell technologies affords researchers the ability to characterize cell states and identify gene regulatory programs across various cell types. The application of sequencing-based surface protein quantification using barcoded antibodies to intracellular or nuclear protein targets has been challenging. Nuclear targets are particularly challenging due to high levels of background oligo-antibody staining, potentially driven by the conjugated single stranded DNA (ssDNA) oligo. However, quantification of nuclear transcription factors, the drivers of gene regulation, would enable critical understanding of gene regulatory processes. For this reason, several approaches to reduce non-specific staining include saturating cells with single stranded nucleic acids or other charged polymers to block non-specific binding to ssDNA oligos.

Stage of Research

The inventors have developed methods quantify intracellular and nuclear targets within a single cell using antibodies conjugated to barcoded oligonucleotides. The inventors reduced non-specific oligo-antibody staining by coating the conjugated ssDNA oligo with single-stranded DNA binding proteins (SSBs). The inventors demonstrate the utility of this technology in profiling CD4 memory T cells by combining SSB-coated oligo-antibodies in nuclear protein quantification with ATAC-seq and RNA-seq (NEAT-seq).

Stage of Development
Research – *in vitro*

Applications

- Directly measure single cell protein levels of cytoplasmic or nuclear targets, like transcription factors, with improved signal over background.
- Oligo-antibody staining in combination with other single cell genomic measurements (e.g., ATAC-seq, RNA-seq, Hi-C, etc.) or perturbating screening for comprehensive cell profiling.

Advantages

- Modular and flexible design can be applied to any target binding moiety (e.g., antibody fragment, ligand, aptamer, etc.) and incorporation to existing single cell pipelines (i.e., split-pool protocols and microfluidics)
- Single-stranded DNA binding proteins (SSBs) bind with high affinity to ssDNA in a sequence non-specific manner, with low affinity for nuclear dsDNA.
- SSBs are compatible with oligo PCR amplification steps during library generation

Publications

- Chen AF, Parks B, Kathiria AS, Ober-Reynolds B, Goronzy JJ, Greenleaf WJ. NEAT-seq: simultaneous profiling of intra-nuclear proteins, chromatin assessibility and gene expression in single cells. *Nature Methods.* 2022.

Patents

- Published Application: WO2022164893
- Published Application: 20240125797

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