



Title	Scalable, multimodal profiling of chromatin accessibility, gene expression and protein levels in single cells
Author(s)	Chen, Kelvin Yigene
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論文内容の要旨

Synopsis of Thesis

氏 名 Name	CHEN KELVIN YIGENE
論文題名 Title	Scalable, multimodal profiling of chromatin accessibility, gene expression and protein levels in single cells (単一細胞レベルにおけるオープンクロマチン領域解析、遺伝子発現情報ならびにタンパク質定量の同時測定に関する拡張可能な多層的プロファイリング手法の開発)
論文内容の要旨 〔目 的(Purpose)〕 Recent technological advances have enabled massively parallel chromatin profiling with single-cell Assay for Transposase Accessible Chromatin by sequencing (scATAC-seq) in thousands of individual cells. Here, we aimed to extend these approaches with the addition of multiple modalities to complement scATAC-seq and developed ATAC with Select Antigen Profiling by sequencing, ASAP-seq, a tool to simultaneously profile accessible chromatin and protein levels in thousands of single cells. 〔方法ならびに成績(Methods/Results)〕 Our approach pairs sparse scATAC-seq data generated on the 10X Chromium (10X Genomics) with robust detection of hundreds of protein markers and optional capture of mitochondrial DNA (mtDNA) for clonal tracking, thus concomitantly capturing three distinct modalities in single cells. Importantly, ASAP-seq uses a novel bridging approach that repurposes antibody:oligo conjugates designed for existing technologies that pair protein measurements with single cell RNA-seq. This bridging approach introduces a unique 'UBI', which serves as a surrogate for the 'UMI', allowing for quantitative interpretation of protein tag abundances when using commercially available TotalSeq-A (BioLegend) oligonucleotide-antibody conjugates. By benchmarking the assay with the gold-standard 'species mix' experiment, we observed very low cross-species contamination background, demonstrating specificity of protein detection in the assay. Moreover, we found that there was no noticeable decrease in library complexity of the chromatin accessibility data, suggesting that the ATAC quality was comparable to standard scATAC-seq. Extending this to human PBMCs, we demonstrated staining of 9 major immune cell markers (TBNK) with cell-type specific specificity, which correlated well with imputed chromatin accessibility "gene activity scores", suggesting that the staining was specific. We next demonstrated proof-of-principle of the utility of ASAP-seq in CRISPR screening experiments (Perturb-ASAP) by encoding perturbation identities with protein combinatorial barcoding and applied this workflow to dissect perturbation-specific epigenomic changes that occur during primary human CD4 T cell stimulation <i>in vitro</i>. This integrated approach allowed for unbiased discovery of context-dependent coding and non-coding gene regulation modules, exemplified by perturbation-specific chromatin and protein changes that occurred at the IL2RA locus. Lastly, we demonstrate the unique compatibility of ASAPタンイツサイボウレベルニオケルオープンクロマチンリョウイキカイセキ、イデンシハツゲンジョウホウナラビニタンパクシツテイリョウノドウジソクテイニカンスルカクチョウカノウナタソウテキプロファイリングシュホウノカイハツ-seq with intracellular epitope staining of markers such as Granzyme B, Perforin, CTLA-4 and Ki-67 and validated their specificity using CRISPR knockout experiments. Therefore, ASAP-seq offers new possibilities of defining nuanced cell states in single-cell genomics assays with precise quantitation of both surface and intracellular epitopes. 〔総 括(Conclusion)〕 We established a novel method termed ASAP-seq that uniquely enables the simultaneous quantitation of chromatin accessibility and protein epitopes in single-cells. We benchmarked ASAP-seq and demonstrated its utility in enabling unbiased discoveries through profiling of primary tissues and engineered genomic perturbations. In total, our methodological approach and analyses demonstrate the utility of ASAP-seq as modular and powerful tools for charting the complex interplay of gene and protein regulatory layers in single cells.	

論文審査の結果の要旨及び担当者

(申請者氏名) CHEN KELVIN YIGENE			
論文審査担当者	(職)	氏 名	
	主 査	大阪大学特任教授	堀 江 孝 文
	副 査	大阪大学教授	山 崎 隆 二
	副 査	大阪大学教授	長 澤 仁 司

論文審査の結果の要旨

近年、次世代シーケンサーの進歩により単一細胞レベルでの多彩な表現型観測法が開発されているが、同一細胞に関する多層的解析は個別に行われ、統合的解析は*in silico*による推測に依存している。

申請者は、オリゴ標識抗体に結合してscATAC-seqのキャプチャー配列を加えるブリッジオリゴを独自開発し、オープンクロマチンに加え細胞内外のタンパク質を同一細胞から取得可能であるASAP-seq (ATAC with Select Antigen Profiling)を開発し、細胞タグ付けを工夫し多条件を同時に評価可能な系に拡張した。更にヒト細胞に応用してCRISPR-Cas9を用いた特定の23遺伝子のノックアウトによる表現型の摂動を観察し、細胞の分化過程や刺激条件における遺伝学的調節の一端を解明した。

本研究は、単一細胞レベルで細胞間シグナル伝達ネットワークを精緻に解明可能なマルチオミクス手法を提供するものであり、学位の授与に値すると考えられる。