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Finding needles in a haystack: dissecting tumor heterogeneity with single-cell transcriptomic and chromatin accessibility profiling

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Tumor evolution often results in a wealth of heterogeneous cancer cell types within a single tumor — heterogeneity that can include epigenetic and gene expression changes that are impossible to identify from histological features alone. The invasion of cancer cells into nearby healthy tissue, accompanied by the infiltration of responding immune cells, results in an even more complex architecture of tumor and non-tumor cells. However, bulk genomics-based methods can only assay the aggregate transcriptomic and epigenetic profiles across all of this rich cellular diversity. Such bulk averaging hides small subpopulations of tumor cells with unique phenotypes that might result in therapeutic resistance or metastatic progression. The advent of single-cell-based genomics assays for measuring transcription and chromatin accessibility – particularly scRNA-seq and scATAC-seq – has enabled the dissection of cell-types within tumors at a scale and resolution capable of unraveling the epigenetic and gene expression programs of rare and unique cellular subpopulations. This Review focuses on recent advances in scRNA-seq and scATAC-seq technologies and their application to cancer biology in the context of furthering our understanding of tumor heterogeneity.

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Introduction

Tumors generally comprise a complex mélange of cell types, including malignantly transformed cells infiltrating normal somatic tissue with variable levels of immune response, all existing within a complex microenvironment [1]. Genomics-based assays such as whole-exome sequencing, RNA sequencing (RNA-seq), and ATAC sequencing (ATAC-seq) are starting to resolve the diversity of cellular phenotypes within human cancers. The overall goal of these assays has been to define a trajectory of molecular patterns that underlie malignant transformation, to map the cell autonomous signals between tumor cells and surrounding stroma, and/or to determine the magnitude of phenotypic diversity of cells within the tumor itself and thereby expose cells that may have specific therapeutic weaknesses. Here we review current methods for performing scRNA-seq and/or scATAC-seq, the advantages and limitations of performing single-cell compared to bulk assays on tumor samples, and how we hope such technologies will help to unpack both the common and unique biology of individual tumors.

Single cell transcriptomic profiling identifies rare cancer subpopulations within tumors

In the last decade, advances in scRNA-seq technologies have rapidly changed our understanding of tumor heterogeneity throughout cancer progression. Computational methods aimed at deconvoluting subpopulations from bulk RNA-seq data have provided some understanding of the breadth of this cellular diversity [2]; however, cell clustering based on single-cell transcriptomic profiles provides substantially more resolving power capable of identifying new cellular states. In particular, RNA isolation and barcoding techniques have evolved from initial well-based applications with modest throughput (Smart-seq, CEL-seq2) [3•,4], to split-and-pool approaches that enable the sequential barcoding of mRNA transcripts originating from separate cells (sci-RNA-seq) [5], to the droplet-based encapsulation of single cells using microfluidics platforms (Drop-seq, inDrops, Seq-Well, and 10x Chromium) [6–8] (previously reviewed [9]). While traditional microwell-based methods consistently detect higher numbers of genes per cell, split-and-pool and droplet-based methods generally allow the processing of significantly more cells (up to 10 000 or more cells in a single sample). The parallel development of computational packages to analyze these increasingly large scRNA-seq datasets has been reviewed in depth previously [9].

For detecting rare subpopulations of cancer cells with the capacity for therapeutic resistance and/or metastatic progression, the higher throughput methods are perhaps most appropriately matched for finding these potential

'needles' in the cellular 'haystack.' For example, these methods have been used to identify pre-metastatic cancer cells from non-small-cell lung cancer primary tumors [10,11], revealing that cancer cells can begin to adopt a metastatic-like transcriptomes before leaving the primary tumor and not just after colonization at a secondary site. This type of observation has widespread implications for how patients are stratified into risk categories in the clinic and has the potential to identify biomarkers to help predict whether a tumor has already seeded micrometastases before diagnosis. In addition, scRNA-seq has been used to characterize the responses of infiltrating T-lymphocytes in models of basal and squamous cell carcinoma and non-small cell lung cancer [12,13]. In the case of basal and squamous cell carcinoma, scRNA-seq was used to identify the specific subpopulation of T-lymphocytes responsible for initiating an immune response following checkpoint blockade, providing further insight into why non-inflamed, 'cold' tumors might be resistant to immunotherapy.

Engineered RNA transcripts can also be introduced into the cell and read out alongside single-cell transcriptomes to provide orthogonal information, including the presence of CRISPR/Cas9 sgRNAs that perturb cellular function [14–16] or lineage tracing cassettes that track the relationships between cells following cell division [17,18]. Along these lines, scRNA-seq methods that combine sgRNA read-out can substantially enrich information derived from traditional proliferation-based CRISPR screens. In particular, cellular responses to perturbations may be categorized into patterns depending on how the entire transcriptome changes. For example, Hill *et al.* revealed how the transcriptome of the breast cancer cell line MCF7 responds differently to the chemotherapeutic drug doxorubicin depending on which tumor suppressor genes have been mutated by CRISPR/Cas9 sgRNAs [19]. Similar experiments have the potential to inform why specific genotypes of tumors might be more or less susceptible to a range of possible chemotherapy regimens available. These types of studies are especially important in an age where clinical trials are increasingly enrolling patients based on the molecular profiles of their tumors rather than on the organ in which their tumor originated [20]. Lineage tracing methods (reviewed in depth here [21]), either using engineered evolvable cassettes or naturally existing variation in mitochondrial genomes [22], are also proving to be indispensable tools for understanding the clonal expansions that bring about tumor heterogeneity. Although their use in the cancer field is just beginning, lineage tracing cassettes have already been used to understand the metastatic trajectory of solid tumors by mapping the most recent ancestry of pre-metastatic cells within primary tumors and comparing their transcriptomes to cells within metastases [23•]. Such techniques will likely become increasingly essential for understanding the molecular drivers of metastatic progression.

Along these lines of 'RNA-seq plus' assays that add data enhancing biological interpretation to 'vanilla' scRNA-seq are methods that combine transcriptome analysis with DNA-indexed affinity reagents (generally antibodies), thereby enabling simultaneous characterization of a diversity of surface proteins along with the transcriptome [24]. These methods have immense promise to provide appropriate FACS markers linked to interesting expression phenotypes, enabling the isolation of these cells and potential downstream phenotypic analysis or mechanistic study. Similarly, unpacking the relationships between immunophenotypic surface markers and genomics-based profiles could allow for the easy stratification of patients based on novel combinations of surface markers.

Single cell chromatin accessibility reveals regulatory mechanisms of cancers

Profiling chromatin accessibility at single cell resolution, most commonly using scATAC-seq, can help to identify regions in the genome that are 'open' or accessible to the binding of trans-factors that may drive gene expression programs. Thus, in addition to stratifying subsets of transcriptionally distinct cells within a heterogeneous population, scATAC-seq provides a window into the underlying epigenetic regulatory mechanisms that drive cell type differences.

The technological evolution of scATAC-seq has substantial similarities to the dynamics observed for scRNA-seq; namely, initial implementations were of relatively modest throughput, while subsequent implementations using split-and-pool or droplet-based protocols have radically increased the number of single cells that can be studied at once. Similar to scRNA-seq, computational methods to analyze such large datasets are rapidly growing and have been reviewed previously [25,26]. Recent scATAC-seq applications have included defining an array of epigenetic regulators that drive T-cell exhaustion in basal cell carcinoma [27] and identifying the transcription factor RUNX2 as a master regulator of extracellular matrix remodeling in a mouse model of non-small cell lung cancer [28•]. Importantly, scATAC-seq has the power to identify patterns of changes that are not readily apparent at the transcriptome-level; for example, widespread changes in the accessibility of transcription factor binding motifs might offer a more mechanistic explanation for the differences between non-metastatic and metastatic cells compared to seemingly disparate transcriptomic changes. However, while scRNA-seq technologies can readily be combined with orthogonal read-outs such as CRISPR/Cas9 sgRNAs, lineage tracing methods, or the sequencing-based characterization of surface markers, applications that add 'bells and whistles' to standard scATAC-seq datasets are currently limited. For example, a lentiviral sgRNA sequence only has a single copy integrated into genomic DNA per cell but might have hundreds of copies being expressed per cell at the RNA

level. While this limitation does not entirely preclude doing CRISPR screens with a scATAC-seq read-out, it does restrict our ability to associate sgRNAs to chromatin accessibility profiles to ~50% of cells assayed in a given experiment [29]. Combining scATAC-seq with an RNA read-out will likely be the most efficient way to relate orthogonal readouts back to chromatin accessibility, and this strategy has already been applied to read-out CRISPR/Cas9 sgRNA transcripts from differentiating keratinocyte cells and to read-out T-cell receptor transcripts from leukemia samples combined with scATAC-seq [30,31].

The development of multi-omic approaches to dissect tumor heterogeneity

scRNA-seq and scATAC-seq provide highly complementary information regarding both the transcriptional phenotype and underlying regulatory logic of the cell state; thus, generating independent single-cell transcriptomic and chromatin accessibility profiling from portions of the same sample can provide synergistic data sets for deeper mechanistic insights into cancer. For example, integrative analysis using these two single-cell methods helped to identify genes with both differential gene expression and local accessibility differences in a rare form of leukemia called mixed-phenotype acute lymphoblastic leukemia (MPAL) [32••]. These analyses then enabled the identification of putative oncogenic transcription factors (e.g. RUNX1) that directly regulate these genes, opening up new avenues for personalized therapeutic targets. Furthermore, with multiple sophisticated informatics packages such as Seurat and ArchR to streamline, standardize, and visualize these analyses, multi-omic data integration has become far more broadly accessible [33,34•].

While joint chromatin accessibility and transcriptomic profiling appears promising, several limitations make these types of studies challenging. First, these methods rely on correctly linking cells to their 'nearest neighbor' across two highly distinct data types to provide an integrated manifold combining RNA and chromatin accessibility landscapes. Such a linking across independent single-cell data sets becomes especially difficult when analyzing tumor subpopulations with relatively subtle distinctions. In addition to these analytical hurdles, the technical difficulties of splitting an initial sample into multiple portions to perform separate analyses might not be feasible for rare and limited samples, including many biopsy and metastatic samples.

Have your cake and eat it too: the promise of simultaneous transcriptomic and chromatin accessibility profiling of individual cancer cells

Simultaneous generation of scATAC-seq and scRNA-seq data from the same cell solves the challenges of independent application of these techniques, and appears to be a major methodology for the future of multi-omic exploration of cancer biology. Four high throughput joint-assay strategies have been reported: sci-CAR-seq [35], Paired-seq [36], SNARE-seq [37], and SHARE-seq [38•]. While highly scalable, the first two methods for single-cell ATAC-seq and RNA-seq in the same cell, sci-CAR-seq and Paired-seq, are operationally complex and require multiple barcoded Tn5 preparations, which likely has hindered their widespread adoption. In contrast, SNARE-seq and SHARE-seq rely on the ligation of barcoded adapters to cDNA and ATAC fragments in droplets or in a split-and-pool format, respectively, resulting in a substantial simplification that may accelerate broader adoption of these approaches. Additionally, SHARE-seq increases the number of ATAC-seq fragments detected per cell by at least ~7-fold and the number of distinct transcripts by RNA-seq detected per cell by at least ~3-fold compared to previous methods, allowing similar data quality to standard independent assays [38•]. Furthermore, 10x Genomics has recently launched the first commercial droplet-based kit for joint scATAC-seq and scRNA-seq on nuclei, reporting data quality from the joint assay similar to that observed when these assays are deployed separately. Altogether, these multi-omic techniques directly link the transcriptome of a cell to its accessibility profile to provide more information per cell for more thorough cell type and functional annotations, especially for rare cell types in a tumor or in precious primary samples.

In addition to overcoming the limitations of performing scATAC-seq and scRNA-seq separately, multi-omics assays allow techniques that require an RNA read-out to be directly associated with ATAC fragments, such as lineage tracing of RNA barcodes, detecting sgRNA IDs in large-scale CRISPR/Cas9 perturbations, and future addition of affinity-based DNA readouts. Further, we expect that modeling the effects of commonly mutated genes in cell culture, spheroid, and/or mouse tumor models followed by a simultaneous transcriptomic and chromatin accessibility read-out will soon become a common method for dissecting the transcriptional and epigenetic phenotype from the mutational heterogeneity of cancer.

Conclusion

The single-cell and multi-omics methods described in this Review are just the beginning of the genomics-based techniques that will be employed in cancer research in the years to come. While initial applications focused on cell type annotations in the tumor and its microenvironment, improvements in independent scRNA-seq and scATAC-seq methodologies as well as wider adoption of multi-omic approaches will quickly expand and deepen our functional insight into rare cancer subpopulations, limited primary biopsies and metastases, and the mechanics of immune evasion. Adapting these assays to include

orthogonal information, including reading out CRISPR/Cas9 perturbation genotypes, surface marker expression, and/or transcription factor levels, will further enhance our understanding of tumor heterogeneity and ideally will help us predict if and how cells will respond to therapeutics.

Conflict of interest statement

W.J.G. is a consultants for 10x Genomics who has licensed IP associated with ATAC-seq. W.J.G. has additional affiliations with Guardant Health (consultant) and Protillion Biosciences (co-founder and consultant).

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as

- • of special interest
- •• of outstanding interest

1. Lawson DA, Kessenbrock K, Davis RT, Pervolarakis N, Werb Z: **Tumour heterogeneity and metastasis at single-cell resolution**. *Nat Cell Biol* 2018, **20**:1349-1360.

2. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA: **Robust enumeration of cell subsets from tissue expression profiles**. *Nat Methods* 2015, **12**:453-457.

3. • Hagemann-Jensen M, Ziegenhain C, Chen P, Ramsköld D, Hendriks G-J, Larsson AJM, Faridani OR, Sandberg R: **Single-cell RNA counting at allele and isoform resolution using Smart-seq3**. *Nat Biotechnol* 2020, **38**:708-714

The authors describe latest updates to Smart-seq for the most sensitive single cell RNA sequencing method, detecting up to 150 000 unique molecules per cell. While low throughput, Smart-seq3's sensitivity enables isoform and allele counting for more detailed cell annotations.

4. Hashimshony T, Senderovich N, Avital G, Klochendler A, de Leeuw Y, Anavy L, Gennert D, Li S, Livak KJ, Rozenblatt-Rosen O *et al.*: **CEL-Seq2: sensitive highly-multiplexed single-cell RNA-Seq**. *Genome Biol* 2016, **17**:77.

5. Cao J, Packer JS, Ramani V, Cusanovich DA, Huynh C, Daza R, Qiu X, Lee C, Furlan SN, Steemers FJ *et al.*: **Comprehensive single-cell transcriptional profiling of a multicellular organism**. *Science* 2017, **357**:661-667.

6. Gierahn TM, Wadsworth MH, Hughes TK, Bryson BD, Butler A, Satija R, Fortune S, Love JC, Shalek AK: **Seq-Well: portable, low-cost RNA sequencing of single cells at high throughput**. *Nat Methods* 2017, **14**:395-398.

7. Klein AM, Mazutis L, Akartuna I, Tallapragada N, Veres A, Li V, Peshkin L, Weitz DA, Kirschner MW: **Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells**. *Cell* 2015, **161**:1187-1201.

8. Macosko EZ, Basu A, Satija R, Nemesh J, Shekhar K, Goldman M, Tirosh I, Bialas AR, Kamitaki N, Martersteck EM *et al.*: **Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets**. *Cell* 2015, **161**:1202-1214.

9. Hwang B, Lee JH, Bang D: **Single-cell RNA sequencing technologies and bioinformatics pipelines**. *Exp Mol Med* 2018, **50**:96.

10. Marjanovic ND, Hofree M, Chan JE, Canner D, Wu K, Trakala M, Hartmann GG, Smith OC, Kim JY, Evans KV *et al.*: **Emergence of a high-plasticity cell state during lung cancer evolution**. *Cancer Cell* 2020, **38**:229-246.e13.

11. Laughney AM, Hu J, Campbell NR, Bakhoum SF, Setty M, Lavallée V-P, Xie Y, Masilionis I, Carr AJ, Kottapalli S *et al.*: **Regenerative lineages and immune-mediated pruning in lung cancer metastasis**. *Nat Med* 2020, **26**:259-269.

12. Li A, Herbst RH, Canner D, Schenkel JM, Smith OC, Kim JY, Hillman M, Bhutkar A, Cuoco MS, Rappazzo CG *et al.*: **Longitudinal single cell profiling of regulatory T cells identifies IL-33 as a driver of tumor immunosuppression**. *bioRxiv* 2019 http://dx.doi.org/10.1101/512905.

13. Yost KE, Satpathy AT, Wells DK, Qi Y, Wang C, Kageyama R, McNamara KL, Granja JM, Sarin KY, Brown RA *et al.*: **Clonal replacement of tumor-specific T cells following PD-1 blockade**. *Nat Med* 2019, **25**:1251-1259.

14. Jaitin DA, Weiner A, Yofe I, Lara-Astiaso D, Keren-Shaul H, David E, Salame TM, Tanay A, van Oudenaarden A, Amit I: **Dissecting immune circuits by linking CRISPR-pooled screens with single-cell RNA-seq**. *Cell* 2016, **167**:1883-1896.e15.

15. Datlinger P, Rendeiro AF, Schmidl C, Krausgruber T, Traxler P, Klughammer J, Schuster LC, Kuchler A, Alpar D, Bock C: **Pooled CRISPR screening with single-cell transcriptome readout**. *Nat Methods* 2017, **14**:297-301.

16. Dixit A, Parnas O, Li B, Chen J, Fulco CP, Jerby-Arnon L, Marjanovic ND, Dionne D, Burks T, Raychowdhury R *et al.*: **Perturb-Seq: dissecting molecular circuits with scalable single-cell RNA profiling of pooled genetic screens**. *Cell* 2016, **167**:1853-1866.e17.

17. Raj B, Wagner DE, McKenna A, Pandey S, Klein AM, Shendure J, Gagnon JA, Schier AF: **Simultaneous single-cell profiling of lineages and cell types in the vertebrate brain**. *Nat Biotechnol* 2018, **36**:442-450.

18. Chan MM, Smith ZD, Grosswendt S, Kretzmer H, Norman TM, Adamson B, Jost M, Quinn JJ, Yang D, Jones MG *et al.*: **Molecular recording of mammalian embryogenesis**. *Nature* 2019, **570**:77-82.

19. Hill AJ, McFaline-Figueroa JL, Starita LM, Gasperini MJ, Matreyek KA, Packer J, Jackson D, Shendure J, Trapnell C: **On the design of CRISPR-based single-cell molecular screens**. *Nat Methods* 2018, **15**:271-274.

20. Malone ER, Oliva M, Sabatini PJB, Stockley TL, Siu LL: **Molecular profiling for precision cancer therapies**. *Genome Med* 2020, **12**:8.

21. Wagner DE, Klein AM: **Lineage tracing meets single-cell omics: opportunities and challenges**. *Nat Rev Genet* 2020, **21**:410-427.

22. Ludwig LS, Lareau CA, Ulirsch JC, Christian E, Muus C, Li LH, Pelka K, Ge W, Oren Y, Brack A *et al.*: **Lineage tracing in humans enabled by mitochondrial mutations and single-cell genomics**. *Cell* 2019, **176**:1325-1339.e22.

23. • Quinn JJ, Jones MG, Okimoto RA, Yosef N, Bivona TG, Weissman JS: **Continuous lineage recording reveals rapid, multidirectional metastasis in a lung cancer xenograft model in mouse**. *bioRxiv* 2020 http://dx.doi.org/10.1101/2020.04.16.045245

The authors apply their previous work in Ref. [17] to bridge the metastatic phenotype of lung cancer xenografts in mouse to the underlying transcriptomes, revealing potential metastatic driver candidates. Furthermore, this approach explored the sequence and topology of tumor seeding from the primary xenografts at a single cell resolution.

24. Stoeckius M, Hafemeister C, Stephenson W, Houck-Loomis B, Chattopadhyay PK, Swerdlow H, Satija R, Smibert P: **Simultaneous epitope and transcriptome measurement in single cells**. *Nat Methods* 2017, **14**:865-868.

25. Baek S, Lee I: **Single-cell ATAC sequencing analysis: from data preprocessing to hypothesis generation**. *Comput Struct Biotechnol J* 2020, **18**:1429-1439.

26. Chen H, Lareau C, Andreani T, Vinyard ME, Garcia SP, Clement K, Andrade-Navarro MA, Buenrostro JD, Pinello L: **Assessment of**

computational methods for the analysis of single-cell ATAC-seq data. *Genome Biol* 2019, **20**:241.

27. Satpathy AT, Granja JM, Yost KE, Qi Y, Meschi F, McDermott GP, Olsen BN, Mumbach MR, Pierce SE, Corces MR *et al.*: **Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion**. *Nat Biotechnol* 2019, **37**:925-936.

28. • LaFave LM, Kartha VK, Ma S, Meli K, Del Priore I, Lareau C, Naranjo S, Westcott PMK, Duarte FM, Sankar V *et al.*: **Epigenomic state transitions characterize tumor progression in mouse lung adenocarcinoma**. *Cancer Cell* 2020, **38**:212-228.e13

The authors used single cell ATAC-seq to define the RUNX family transcription factors as the major epigenetic regulators of metastatic progression in a mouse model of lung adenocarcinoma. This study highlights the strength of scATAC-seq to simultaneously annotate cell types and directly explore the underlying *trans* factors driving the cancer progression.

29. Pierce SE, Granja JM, Greenleaf WJ: **High-throughput single-cell chromatin accessibility CRISPR screens enable unbiased identification of regulatory networks in cancer**. *bioRxiv* 2020 http://dx.doi.org/10.1101/2020.11.02.364265.

30. Satpathy AT, Saligrama N, Buenrostro JD, Wei Y, Wu B, Rubin AJ, Granja JM, Lareau CA, Li R, Qi Y *et al.*: **Transcript-indexed ATAC-seq for precision immune profiling**. *Nat Med* 2018, **24**:580-590.

31. Rubin AJ, Parker KR, Satpathy AT, Qi Y, Wu B, Ong AJ, Mumbach MR, Ji AL, Kim DS, Cho SW *et al.*: **Coupled single-cell CRISPR screening and epigenomic profiling reveals causal gene regulatory networks**. *Cell* 2019, **176**:361-376.e17.

32. •• Granja JM, Klemm S, McGinnis LM, Kathiria AS, Mezger A, Corces MR, Parks B, Gars E, Liedtke M, Zheng GXY *et al.*: **Single-cell multiomic analysis identifies regulatory programs in mixed-phenotype acute leukemia**. *Nat Biotechnol* 2019, **37**:1458-1465

The authors performed multiomic integration of single cell DNA accessibility, transcriptomics, and protein abundance of normal hematopoiesis and mixed-phenotype acute leukemia to define leukemia-specific key regulators, such as *RUNX1*.

33. Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM, Hao Y, Stoeckius M, Smibert P, Satija R: **Comprehensive integration of single-cell data**. *Cell* 2019, **177**:1888-1902.e21.

34. • Granja JM, Corces MR, Pierce SE, Bagdatli ST, Choudhry H, Chang H, Greenleaf W: **ArchR: an integrative and scalable software package for single-cell chromatin accessibility analysis**. *bioRxiv* 2020 http://dx.doi.org/10.1101/2020.04.28.066498

ArchR enables standardized and streamlined processing, analysis, and visualization of single cell ATAC data along with multiomic integrations in a highly memory efficient manner, opening up these complex analytical approaches to a wider audience.

35. Cao J, Cusanovich DA, Ramani V, Aghamirzaie D, Pliner HA, Hill AJ, Daza RM, McFaline-Figueroa JL, Packer JS, Christiansen L *et al.*: **Joint profiling of chromatin accessibility and gene expression in thousands of single cells**. *Science* 2018, **361**:1380-1385.

36. Zhu C, Yu M, Huang H, Juric I, Abnousi A, Hu R, Lucero J, Behrens MM, Hu M, Ren B: **An ultra high-throughput method for single-cell joint analysis of open chromatin and transcriptome**. *Nat Struct Mol Biol* 2019, **26**:1063-1070.

37. Chen S, Lake BB, Zhang K: **High-throughput sequencing of the transcriptome and chromatin accessibility in the same cell**. *Nat Biotechnol* 2019, **37**:1452-1457.

38. • Ma S, Zhang B, LaFave L, Chiang Z, Hu Y, Ding J, Brack A, Kartha VK, Law T, Lareau C *et al.*: **Chromatin potential identified by shared single cell profiling of RNA and chromatin**. *bioRxiv* 2020 http://dx.doi.org/10.1101/2020.06.17.156943

SHARE-seq provides a high throughput joint single cell transcriptomic and chromatin accessibility profiling with comparable sensitivity to performing the assays independently.