

The role of single-cell sequencing in studying tumour evolution

Maximilian Mossner  ^{1*} Ann-Marie C. Baker  ¹ Trevor A. Graham  ¹

¹ Evolution and Cancer Laboratory, Centre for Genomics and Computational Biology, Barts Cancer Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London, EC1M 6BQ, UK

Abstract

Tumour evolution is a complex interplay between the acquisition of somatic (epi)genomic changes in tumour cells and the phenotypic consequences they cause, all in the context of a changing microenvironment. Single-cell sequencing offers a window into this dynamic process at the ultimate resolution of individual cells. In this review, we discuss the transformative insight offered by single-cell sequencing technologies for understanding tumour evolution.

Keywords

single cell sequencing, tumour evolution, scRNA-Seq, scDNA-Seq

Peer Review

The peer reviewers who approve this article are:

- James Hicks**, Michelson Center for Convergent Biosciences, Dornsife College, University of Southern California, Los Angeles, California
Competing interests: No competing interests were disclosed.
- Floris Foijer**, European Research Institute for the Biology of Ageing, University of Groningen, University Medical Center Groningen, A. Deusinglaan 1, Groningen, 9713 AV, The Netherlands
Diana Spierings, European Research Institute for the Biology of Ageing, University of Groningen, University Medical Center Groningen, A. Deusinglaan 1, Groningen, 9713 AV, The Netherlands
Competing interests: No competing interests were disclosed.

***Corresponding author:** Maximilian Mossner (m.mossner@qmul.ac.uk)

Competing interests: The authors declare that they have no competing interests.

Grant information: TG acknowledges funding from Cancer Research UK (A19771), the Wellcome Trust (202778/Z/16/Z) and the National Institutes of Health via the Cancer Systems Biology Consortium U54 scheme (CA217376). MM has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under Research Executive Agency grant agreement 608765.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2021 Mossner M et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Mossner M, Baker AMC and Graham TA. **The role of single-cell sequencing in studying tumour evolution.** Faculty Reviews 2021 10:(49) <https://doi.org/10.12703/r/10-49>

Published: 26 May 2021, Faculty Reviews 10:(49) <https://doi.org/10.12703/r/10-49>

Introduction

Intra-tumour heterogeneity (ITH) and evolution are deeply intertwined. Heterogeneity is “fuel to the fire” of evolution – the population of cells in a tumour cannot adapt to a new selection pressure if there is no variation in the population for natural selection to operate upon. And because new (epi)genetic mutations are acquired each time a tumour cell divides, the process of tumour growth (and so tumour evolution) inevitably generates heterogeneity within a tumour¹.

For many decades, the prevalent opinion was that tumours generally follow a strictly “linear” evolutionary trajectory whereby the sequential addition of “driver” genomic aberrations was associated with a clonal expansion leading to replacement of the complete tumour population. In this paradigm, tumour progression followed with the accumulation of driver alterations². However, multi-region sequencing of bulk tumour tissues has now shown that ITH, in particular the frequent coexistence of subclones with different driver alterations, is common in all tumour types, prominently including lung^{3,4}, hematologic^{5,6}, colon^{7,8}, breast^{9,10} and brain^{11,12} neoplasia.

A mechanistic understanding of the process of carcinogenesis requires understanding the drivers and dynamics of tumour evolution¹³. Consequently, predicting and indeed modulating the response of a tumour to treatment¹⁴, or whether a pre-malignant lesion will become tumourous in the future^{15,16}, both require predicting tumour evolution¹⁶. Indeed, recent work suggests that evolutionary trajectories are recurrent across tumours, and biomarkers that identify these trajectories could enable personalised tumour medicine^{17–19}.

ITH is a read-out of tumour evolutionary dynamics²⁰ and consequently measurement of ITH has prognostic relevance: pan-tumour analyses of whole exome sequencing data from The Cancer Genome Atlas revealed that tumours harbouring between two and four detectable clones have poorer prognoses than those containing fewer than two or more than four clones²¹, and clonal diversity of copy number alterations predicts survival in lung tumours²². Treatment resistance, tumour relapse and development of metastases can coincide with the expansion of “new” clones that harbour specific driver genomic alterations, conferring phenotypic advantages in the changing microenvironment^{5,6,23}. In this context, one should be careful not to mistake passenger mutations arising through neutral evolution as contributors to evolutionary adaptation since somatic mutations accumulate randomly throughout cell divisions and many have no apparent effect on the fitness of a (sub)clone⁸. One considerable caveat in the assessment of ITH is the fact that generally we can sample only a small fraction of the entire tumour, thus limiting our ability to detect minor subclones. Extrapolating conclusions to the bulk tumour therefore should be done with caution. Translating measurements of tumour evolution, such as quantification of ITH, will require tumour sampling to be standardised (for example, number, position and size of biopsies) and tumour cellularity to be considered as these factors can confound interpretation of molecular composition²⁴.

However, recent data indicates that even in tumour populations with largely identical genomes, a high degree of cell *plasticity* – extensive phenotypic cell-to-cell variability – is observed^{25–28}, which can be caused by, among other factors, heterogeneity at the transcriptomic or epigenomic level or both. From an evolutionary perspective, this is a highly important notion as it is tumour cell phenotypes and not genotypes that are undergoing selection.

Next-generation sequencing (NGS) techniques have contributed tremendously to our knowledge of the molecular architectures of tumours. However, the majority of data has been generated using DNA or RNA derived from “bulk” tumour tissue (Figure 1A), almost inevitably consisting of complex mixtures of tumour cells and stromal cells, such as fibroblasts,

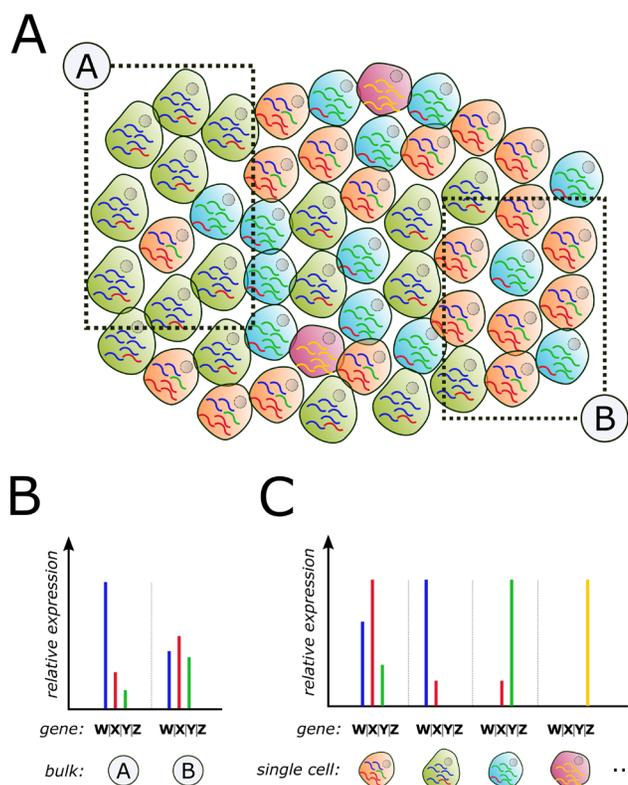


Figure 1. Dissecting molecular profiles of heterogeneous tumour cell populations by using bulk and single-cell methodology.

(A) Intra-tumour heterogeneity usually manifests in the form of mixed cell populations with diverse molecular traits. As illustrated in this example, many tumours frequently show the coexistence of cell clones with different transcriptomic makeups and strongly varying population sizes. Although analysis of multicellular bulk pieces “A” and “B” reveals differences in the RNA expression profiles, these could be (inaccurately) attributed as intrinsic, tumour cell-specific changes (B). Only by using single-cell next-generation sequencing analysis, transcriptome profiles can be directly associated with specific cell types (such as tumour and various types of stromal cells) and allow the cell type complexity within heterogeneous tumour lesions to be enumerated (C). Importantly, only single-cell screening allows sensitive *de novo* identification of rare cell subpopulations that otherwise would be missed entirely or lie below the level of detection when bulk tissue analysis is used.

macrophages and lymphocytes, which confound the analysis. Bioinformatics tools have been created to deconvolute the individual cell types from bulk RNA sequencing (RNA-Seq)²⁹⁻³² but are complicated by the necessity for prior knowledge of transcriptome patterns that uniquely determine each cell type so that the signal from these cells can be isolated in the downstream analysis. However, since there is a dynamic interplay between tumour cells and their microenvironment, it is reasonable to expect that RNA expression of the same cell “type” can show inter-tumour and intra-tumour variability in gene expression³³ (Figure 1A, B) and furthermore that this variation is an important determinant of tumour biology. Thus, bulk analysis risks missing important tumour biology.

The recent evolution of powerful single-cell sequencing techniques provides a unique opportunity to directly overcome these limitations, allowing highly unbiased assessments of

tumour heterogeneity – both subclonal heterogeneity of tumour cells and the cell types present in the stroma – with an unprecedented level of sensitivity (Figure 1A, C). In the following sections, we provide an overview of common methods and recent innovations achieved through the application of single-cell analysis.

Single-cell sequencing in tumours

Uni-modal techniques

One of the first single-cell sequencing applications was a single-cell RNA-Seq (scRNA-Seq) protocol established in 2009 by Tang *et al.*³⁴. This paved the way for a vast range of highly sophisticated single-cell next generation sequencing (scNGS) methods that characterise genomic, transcriptomic, epigenomic and proteomic patterns that will be explained in more detail below. To provide a quick overview, a summary of common methods can be viewed in Table 1.

Table 1. Overview of commonly used single-cell next-generation sequencing techniques.

Type of analysis	Method	Application/Features
RNA sequencing	SMART-Seq2 ³⁵	- Full-length mRNA sequencing
	SMART-Seq3 ³⁶	- Full-length mRNA sequencing - UMI support
	Quartz-Seq ³⁷	- Full-length mRNA sequencing
	MATQ-Seq ³⁸	- Full-length mRNA + lncRNA sequencing
	SUPeR-Seq ³⁹	- Full-length mRNA + lncRNA sequencing
	Holo-Seq ⁴⁰	- Full-length mRNA + lncRNA + small RNA sequencing
	MARS-Seq2 ⁴¹	- 3-prime end mRNA sequencing - UMI support
	CEL-Seq2 ⁴²	- 3-prime end mRNA sequencing - UMI support
	mcSCRIB-Seq ⁴³	- 3-prime end mRNA sequencing - UMI support
DNA sequencing	STRT-Seq ⁴⁴	- 5-prime end mRNA sequencing - UMI support
	Degenerate oligonucleotide primed polymerase chain reaction ⁴⁵	++CNV / –SNV detection
	Multiple displacement amplification ⁴⁶⁻⁴⁹	–CNV / +++SNV detection
	Ampli1 ⁵⁰	++CNV / +SNV detection
	STRAND-Seq ⁵¹⁻⁵³	+++CNV detection (SNV unknown)
Epigenomic sequencing	“Direct library preparation” ^{54,55}	+++CNV / ++SNV detection
	Bisulfite sequencing ^{56,57}	- CpG DNA methylation analysis
	Drop-ChIP-Seq ⁵⁸	- Chromatin immune precipitation analysis for histone modifications
	scATAC-Seq ^{59,60}	- Chromatin accessibility analysis
	CUT&Tag ⁶¹	- Targeted chromatin-protein association profiling

Abbreviations: CNV, copy number variant; lncRNA, long non-coding RNA; mRNA, messenger RNA; SNV, single-nucleotide variant; UMI, unique molecular index. Suitability of DNA sequencing applications is represented on a scale from (–) to (+++). Although a range of individual single-cell RNA sequencing (scRNA-Seq) protocols show overlapping characteristic features, the final choice of method depends primarily on the requirement for quantitative analysis (for example, number of cells) versus qualitative analysis (for example, full-length assessment for splicing/fusion transcripts). As a rule of thumb, the cost of library preparation and sequencing is related to the breadth of data per cell obtained.

By far the most extensive variety of scNGS protocols in use today are scRNA-Seq approaches, which differ in aspects such as cell isolation methods, type of transcript coverage, cDNA transcription, and library amplification. Another important aspect represents the utilisation of unique molecular identifiers (UMIs), which are random DNA sequences embedded in library adapters that uniquely “tag” a cellular RNA/DNA fragment before polymerase chain reaction (PCR) amplification. By reading UMI tags after library preparation and sequencing, PCR

duplicates can be easily distinguished from biological replicates, thereby generating absolute counts of the initial fragments present.

In general, scRNA-Seq protocols can be divided into full-length transcript approaches, such as SMART-Seq²³⁵, Quartz-Seq³⁷, MATQ-Seq³⁸ or SUPeR-Seq³⁹, and protocols sequencing short parts of the 3’ or 5’ end of RNA transcripts, such as MARS-Seq²⁴¹, CEL-Seq²⁴², mcSCRb-Seq⁴³ or STRT-Seq⁴⁴ (Figure 2A).

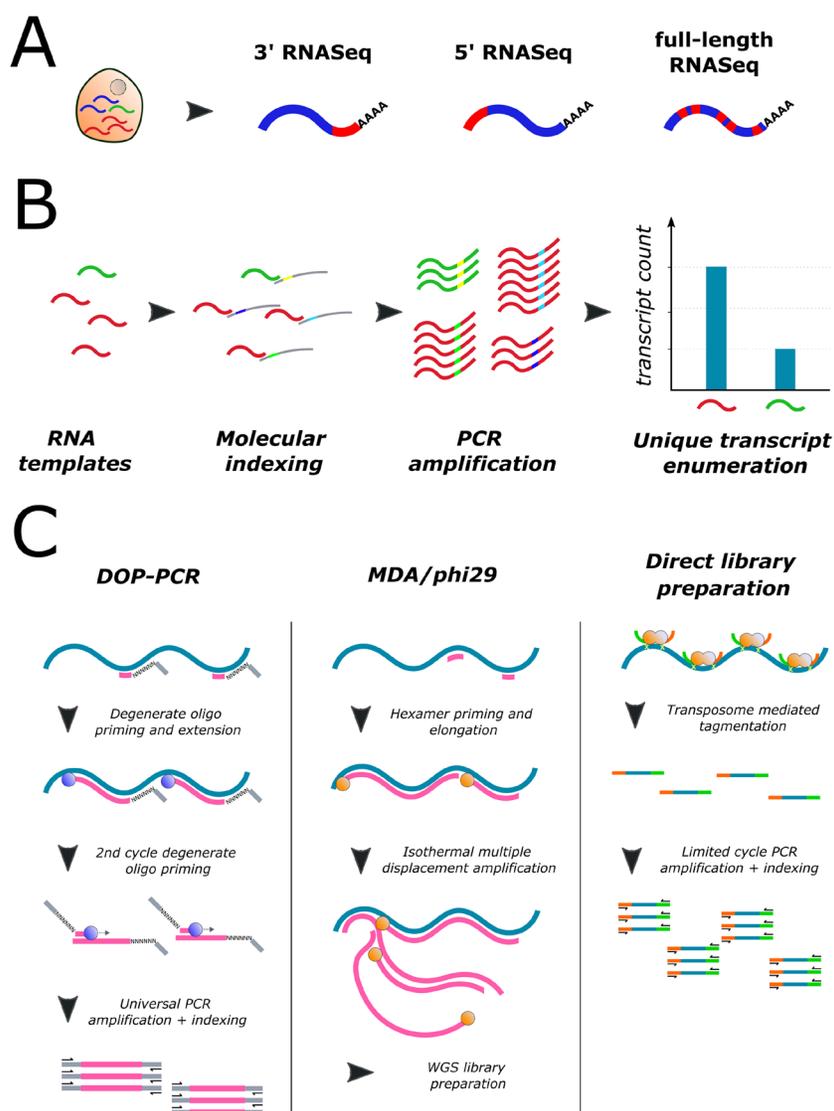


Figure 2. Single-cell sequencing strategies. (A) Single-cell RNA sequencing approaches can be split broadly into techniques that sequence either the 3’ or 5’ end of individual RNA transcripts or random parts of the full transcript molecule. (B) The scarcity of starting RNA templates in a single cell is a major confounding factor leading to polymerase chain reaction (PCR) amplification bias and inaccurate quantification of transcript levels. Using adapters with “unique molecular indexes” (UMIs) (random nucleotide sequences) for reverse transcription allows tagging of individual transcripts before the PCR amplification step. After sequencing and mapping of amplified libraries, the abundance of initial RNA transcripts can be estimated by counting the number of UMIs for any detected RNA transcript, thereby mitigating amplification bias. (C) Overview of the most common amplification techniques for assessment of genomic DNA profiles in single cells. DOP-PCR, degenerate oligonucleotide primed polymerase chain reaction; MDA, multiple displacement amplification; WGS, whole genome sequencing.

Full-length scRNA-Seq has the advantage of identifying alternative transcript isoforms, fusion transcript events and (to a limited extent) single-nucleotide variants (SNVs) and insertion/deletion variants (INDELs) throughout entire RNA transcripts. The most commonly used full-length protocol, SMART-Seq2, has been shown to detect the largest number of transcripts per cell but exhibits higher amplification noise due to lack of UMI utilisation⁶² although this was addressed recently in the updated SMART-Seq3 protocol³⁶. It also has significantly higher costs per cell unless the transposase enzyme for the final library tagmentation step is produced in-house⁶³. Whereas the majority of scRNA-Seq protocols use oligo-dT priming of poly-A mRNA species, MATQ-Seq³⁸, SUPeR-Seq³⁹ and Holo-Seq⁴⁰ provide total RNA expression profiles, including long non-coding RNA and circular RNA species, that are increasingly recognised as playing a key role in tumour pathogenesis. To date, Holo-Seq⁴⁰ is the only method capable of capturing expression of small RNAs (for example, microRNAs or enhancer RNAs) in scRNA-Seq. Although these full-length methods provide exceptional insight into the complexity of single-cell transcriptomes, they inevitably require several-fold-higher sequencing depth compared with their 3'/5' biased counterparts.

In comparison with full-length protocols, 3' and 5' assays benefit from the (optional but straightforward) inclusion of UMIs enabling more accurate transcript quantification (Figure 2B). In addition, the possibility to barcode RNA of individual cells means that these methods allow early multiplexing of cell samples, thereby increasing the library preparation throughput by magnitudes and reducing costs. While these protocols are predominantly carried out in various plate formats, emulsion droplet-based techniques that are able to encapsulate single cells into oil droplets for separate processing (for example, the 10x Chromium platform⁶⁴, inDrop⁶⁵ and Drop-Seq⁶⁶) have recently emerged. These technologies have an even higher throughput with combined capturing and library preparation of tens of thousands of cells per sample and highly reduced library processing costs per cell. However, the drawbacks of such approaches are the high upfront infrastructure costs and the inability to map generated cell profiles to preanalytical assessments, such as fluorescence-activated cell sorting (FACS)-based epitope profiling or cellular morphology.

Single-cell approaches for genomic DNA profiling (scDNA-Seq) have experienced similar advances in the development of increasingly sensitive methods (Figure 2C). A single cell contains a minute amount of DNA (typically two copies of each allele as opposed to multiple mRNA copies of each gene) and therefore traditional scDNA-Seq protocols have required either the whole genome or loci of interest to be preamplified prior to library preparation. Generally, for whole genome amplification (WGA), isothermal multiple displacement amplification (MDA) and variations thereof have been the methods of choice because of their wide breadth of genome coverage^{46,47}. However, MDA is prone to allelic imbalance/dropouts and GC amplification bias when only a single diploid genome is used

as the starting template, leading to relatively high error rates in SNV/INDEL calling and less precise detection of copy number variants (CNVs)⁴⁸. Subsequent methods used FACS to isolate cells in the G₂/M phase of the cell cycle to increase the starting amount of DNA for WGA, which significantly reduced error rates⁶⁷. Further optimisations of MDA and library chemistry increased SNV/INDEL detection rates to about 90%⁴⁹ but did not improve the poor detection of CNVs. An alternative method of single-cell WGA is degenerate oligonucleotide primed PCR (DOP-PCR) and this results in high coverage uniformity and therefore very accurate CNV detection but highly limited SNV detection because of low breadth of genome coverage, usually around 10%⁴⁵. The recent development of the *Amplif* technology (Menarini Silicon Biosystems, Huntingdon Valley, PA, USA) improves on CNV detection and allelic dropout rates by implementing an optimised strategy using enzymatic digestion, adapter ligation followed by single-primer amplification⁵⁰. A further alternative method called STRAND-Seq⁵¹, and variations thereof developed by van den Bos *et al.*⁵² and Bakker *et al.*⁵³, includes a preamplification-free approach, which leads to more precise CNV karyotyping and even allows delineation of the parental origin of alleles in single cells. Generally, the choice of WGA method has to be directed by the type of genomic data that is intended to be interrogated.

An elegant approach to circumvent the problems associated with WGA has been developed by Zahn *et al.* and uses transposome tagmentation directly on single-cell genomic DNA⁵⁴. This technique, named “direct library preparation”, uses a one-pot reaction for DNA fragmentation and adapter ligation, omits preamplification before library construction and thereby provides a wide breadth of genome coverage that allows highly accurate CNV detection even with low coverage sequencing (<0.1×). In this approach, much like in the above-mentioned STRAND-Seq method^{52,53}, the lack of preamplification allows researchers to easily identify and remove PCR duplicates because of the unique fragmentation pattern of a single-cell genome, resulting in vastly improved noise reduction. Intriguingly, genome allelic balance remains largely preserved and enables sensitive SNV/INDEL calling by combining single-cell genomes *in silico* to create a “synthetic bulk”; for example, single cells are assigned to a subclonal population by using their CNV profiles derived from the same scDNA-Seq dataset. More recently, the method has been scaled to allow processing of tens of thousands of cells with compatibility to commercially available single-cell processing solutions⁵⁵.

Although the above-mentioned techniques allow deep insight into ITH at single-cell resolution, spatial information is lost because in the generation of single-cell suspensions for library preparation the tissue architecture is destroyed. To address this problem, several groups developed *in situ* analysis technologies^{68–70} that have single-cell – or near cellular – resolution. For example, our group recently validated BaseScope for robust detection of mutation-specific RNA as a means to measure ITH in a spatial context⁷¹. The higher-throughput

“slide-seq” method interrogates global RNA expression profiles with single-cell resolution in virtually any type of sectioned tissue⁷⁰. To achieve this, the authors engineered arrays of 10- μ m, uniquely barcoded beads with known positions that capture mRNA species upon transfer from tissue sections via oligo-dT priming, enabling high-resolution spatial transcriptome mapping of tens of thousands of single cells per section to be achieved. In theory, genetic information (CNVs and potentially SNVs) extracted from the RNA data can be used to spatially map genotypes concurrently with the RNA-based phenotypic characterisation. Of note, by implementing an innovative *in situ* RNA-Seq approach, STARmap expands on the possibilities of slide-seq and enables even three-dimensional spatial single-cell transcriptomic profile resolution⁶⁹.

Recent advances in single-cell processing have also allowed tumour epigenetics to be investigated on a larger scale and at cellular resolution. Widely used methods include single-cell bisulfite sequencing for measuring DNA CpG methylation^{56,57}, droplet-based single-cell chromatin immunoprecipitation (drop-ChIP) for assessment of histone modifications⁵⁸, single-cell transposase-accessible chromatin sequencing (scATAC-Seq) for analysing chromatin accessibility^{59,60} and cleavage under targets and tagmentation (CUT&Tag) for measuring custom chromatin protein distribution⁶¹. Although they still provide, on a per-cell level, only a small fraction of the epigenetic information obtained from bulk sample measurements, these techniques represent essential tools for connecting phenotype and genotype features of tumour evolution on a cellular level.

Analysis and interpretation of scNGS datasets must be performed with appreciation of certain caveats. For example, it is important to note that tissue dissociation protocols for the generation of single-cell suspensions can be quite harsh, causing fragile states of single cells and RNA species in particular and this may bias detection to “hardy” cells. Moreover, owing to the scarcity of template material in a single cell and hence the necessity for strong signal amplification, stochastic dropout of biological features is inevitable. As such, quality control of the data and selection of adequate computational analysis strategies are of utmost importance to ensure that the results reflect true biological heterogeneity between and within samples and not simply variable quality of input material or batch processing artefacts. Methods to robustly handle these intrinsic sources of noise are essential: Excellent reviews of scNGS computational analysis pipelines can be found elsewhere^{72–76}.

Multi-modal techniques

Mapping multiple biological features within the same cell is an important step towards understanding the molecular interdependencies that lead to phenotypic diversity within tumours. As such, there is currently much interest in combining multiple uni-modal single-cell techniques to develop multi-modal scNGS approaches. For example, TARGET-Seq combines an improved SMART-Seq2 scRNA-Seq protocol with targeted SNV/INDEL genotyping by using custom primers amplifying

loci from both RNA and DNA in the same reaction, thereby dramatically improving the high allelic dropout rate usually observed with mutation calling using (full-length) scRNA-Seq data only⁷⁷. Similar approaches for successful mapping of transcriptome and targeted genotype profiles of single cells have been reported by two other groups^{78,79} using modifications of 3' scRNA-Seq protocols. The use of 3' RNA-Seq, in comparison with TARGET-Seq, as a genotyping source might be a limiting factor for the genotyping success rate as mutations in the 5' region as well as in marginally expressed genes may not be detected. Though significantly expanding on our abilities to unify genotypic and transcriptomic cell profiles, the limitation of the above methodologies is that prior knowledge of the mutations to be interrogated is required. Combined genome and transcriptome sequencing (“G&T-Seq”) has been the first method to provide a more unbiased screening solution and allows the assessment of complex genomic profiles in parallel with transcriptomic profiles^{80,81}. With biotinylated oligo-dT capture probes, RNA moieties of a single cell can be physically separated from the DNA and both can be subjected independently to the aforementioned scNGS protocols.

The REAP-Seq and CITE-Seq protocols combine scRNA-Seq and cell-surface proteomics by labelling single cells with antibody–oligonucleotide conjugates before extracting RNA^{82,83}. The oligonucleotides of these conjugates carry epitope-specific barcodes and poly-A tails and therefore can be conveniently quantified by integrating them in common scRNA-Seq library protocols. In a similar approach, the RAID-Seq method employs scRNA-Seq of fixed single cells and simultaneously allows intracellular (phospho)proteins to be quantified⁸⁴. These approaches are compatible with high-throughput microfluidics platforms, such as that of 10x Genomics, and can be highly multiplexed to simultaneously assess dozens of protein epitopes and the corresponding transcriptomic profile of a single cell.

Insights from single-cell next-generation sequencing into tumour evolution

With a highly effective arsenal of single-cell methods at hand today, recent studies have revealed exciting novel insights into tumour heterogeneity and evolutionary principles. For example, Gao *et al.* investigated patterns of aneuploidy in triple-negative breast tumour (TNBC) by generating genome-wide CNV profiles of 1,000 single cells from 12 patients⁸⁵. Interestingly, the investigators' analysis revealed highly stable karyotypes across individual tumour clones and only rare events of “metastable” tumour cells showed additional single aberrations, presumably denoting evolutionary dead ends. The data was consistent with a mathematical model whereby CNVs were acquired in a “punctuated burst” early in TNBC tumourigenesis rather than through gradual acquisition. Notably, though, the model did not describe the effects of selection – which could conceivably lead to a clonal expansion and the clonality of CNVs observed in the data.

Another study characterised oestrogen receptor–positive breast tumour via scRNA-Seq in combination with cellular

barcoding analysis of bulk tumour cells (the ClonTracer system)⁸⁶, revealing that resistance to anti-oestrogen therapy occurs mainly through selection of pre-existing clones⁸⁷. Notably, resistance development appears to be further promoted by increased activity of the KDM5 histone demethylase family, which is frequently observed in patients, mainly by epigenetically induced enhanced transcriptomic heterogeneity, and can be reversed by KDM5 inhibition. Intriguingly, cellular barcoding in conjunction with scRNA-Seq data also indicated that, in contrast to anti-oestrogen treatment, eventual acquisition of resistance to KDM5 inhibition is attributed to epigenetic and not genetic changes, highlighting different paths of evolutionary adaptation to therapy.

Subjecting TNBC single-cell to scDNA-Seq, followed by longitudinal analysis in TNBC tumours before, during, and after chemotherapy, also revealed the outgrowth of rare pre-existing clones upon chemoresistance development⁸⁸, underlining the clinical relevance of measuring ITH. Intriguingly, the parallel scRNA-Seq analysis on a separate set of single cells from the same tissues uncovered that resistance-associated transcriptional profiles partly pre-existed and were further reshaped during therapy to fully establish the transcriptional signature of phenotypic resistance⁸⁸ via upregulation of receptor tyrosine kinase, epithelial-mesenchymal transition and immune-associated expression pathways²⁵.

H3K27M-mutant glioma represents a highly fatal tumour subtype that occurs during early childhood and is assumed to be linked to the activation of proliferative brain development programs. By using scRNA-Seq, Filbin *et al.* were able, for the first time, to delineate the phenotypic composition of these tumours at cellular resolution, revealing that they are composed mainly of a stem-like oligodendrocyte precursor subset with highly proliferative and tumour-propagating characteristics but that differentiated cells represent only a minority of the tumour population⁸⁹. Notably, complementing their primary tumour analysis with patient-derived xenografts indicated that platelet-derived growth factor receptor alpha (PDGFRA) and BMI1 are involved in maintaining the stem-like phenotype and therefore represent potential therapeutic targets in this inoperable and aggressive disease.

Although scRNA-Seq profiling provides detailed insight into cell-specific transcriptional states, some tumour types such as acute myeloid leukaemia (AML) can to a certain extent show a variety of differentiation phenotypes similar to that observed in healthy tissue homeostasis, which makes it necessary to accurately determine cell genotypes in parallel. As described earlier, van Galen *et al.* performed simultaneous scRNA-Seq and targeted sequencing of patient-specific mutations (present in RNA transcripts) from the very same cell⁷⁹. By using a machine learning classifier, they revealed that AML single-cell transcriptomes indeed closely resembled up to six normal

myeloid differentiation states and their compositions were highly variable between patients and associated with genetic background and clinical outcome. Furthermore, their approach allowed unbiased assessment of AML stem-like cells, which revealed a strong upregulation of stress response, redox signaling and self-renewal programs compared with normal hematopoietic stem cells and identified CD36 and CD74 as novel putative leukaemia stem cell markers for potential therapeutic exploitation.

In an effort to unravel the complex relationship between tumour and stromal cells, recent studies from the Teichman lab using scRNA-Seq analyses in human- and mouse-derived melanomas revealed the requirement of a delicate balance of the immune complement system for maintaining tumour growth⁹⁰. The group's "CellPhoneDB" tool uses multicellular scRNA-Seq data to identify cell-cell communication networks by identifying receptor-ligand pairings that underlie biological multicellular interactions⁹¹.

Collectively, although the findings described here are only a small representation of recent achievements made possible by single-cell investigations, they highlight the immediate clinical relevance of scNGS and the potential to transform our understanding of tumour ITH and mechanisms of clonal evolution.

Conclusions

Single-cell sequencing techniques have proven to be powerful tools to dissect the heterogeneity of tumour cells. Investigating the genomic, transcriptomic and epigenomic features of individual tumour and stromal cells within a tumour, and their interactions, already revealed important biological mechanisms underlying the propagation and maintenance of tumour development. The ongoing refinement of these methods and vast increase in throughput will soon allow even deeper and more precise characterisation of spatial and temporal tumour evolution, microenvironmental interactions and immune crosstalk. The exciting new possibilities offered by single-cell technologies are likely to translate to new prognostic tools and personalised treatment strategies in the near future, extending the survival of tumour patients and improving clinical management.

Abbreviations

AML, acute myeloid leukaemia; CNV, copy number variant; FACS, fluorescence-activated cell sorting; INDEL, insertion/deletion variant; ITH, intra-tumour heterogeneity; MDA, multiple displacement amplification; NGS, next generation sequencing; PCR, polymerase chain reaction; RNA-Seq, RNA sequencing; scDNA-Seq, single-cell DNA sequencing; scNGS, single-cell next-generation sequencing; scRNA-Seq, single-cell RNA sequencing; SNV, single-nucleotide variant; TNBC, triple-negative breast cancer; UMI, unique molecular identifier; WGA, whole genome amplification

References

Faculty Opinions Recommended

1. Greaves M, Maley CC: **Clonal evolution in cancer.** *Nature.* 2012; **481**(7381): 306–13.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
2. Jones S, Chen WD, Parmigiani G, et al.: **Comparative lesion sequencing provides insights into tumor evolution.** *Proc Natl Acad Sci U S A.* 2008; **105**(11): 4283–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
3. de Bruin EC, McGranahan N, Mitter R, et al.: **Spatial and temporal diversity in genomic instability processes defines lung cancer evolution.** *Science.* 2014; **346**(6206): 251–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
4. Zhang J, Fujimoto J, Zhang J, et al.: **Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing.** *Science.* 2014; **346**(6206): 256–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
5. Mossner M, Jann JC, Wittig J, et al.: **Mutational hierarchies in myelodysplastic syndromes dynamically adapt and evolve upon therapy response and failure.** *Blood.* 2016; **128**(9): 1246–59.
[PubMed Abstract](#) | [Publisher Full Text](#)
6. Walter MJ, Shen D, Ding L, et al.: **Clonal architecture of secondary acute myeloid leukemia.** *N Engl J Med.* 2012; **366**(12): 1090–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
7. Cross W, Kovac M, Mustonen V, et al.: **The evolutionary landscape of colorectal tumorigenesis.** *Nat Ecol Evol.* 2018; **2**(10): 1661–72.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
8. Sottoriva A, Kang H, Ma Z, et al.: **A Big Bang model of human colorectal tumor growth.** *Nat Genet.* 2015; **47**(3): 209–16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
9. Barry P, Vatsiou A, Spiteri I, et al.: **The Spatiotemporal Evolution of Lymph Node Spread in Early Breast Cancer.** *Clin Cancer Res.* 2018; **24**(19): 4763–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
10. Yates LR, Gerstung M, Knappskog S, et al.: **Subclonal diversification of primary breast cancer revealed by multiregion sequencing.** *Nat Med.* 2015; **21**(7): 751–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
11. Sottoriva A, Spiteri I, Piccirillo SGM, et al.: **Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics.** *Proc Natl Acad Sci U S A.* 2013; **110**(10): 4009–14.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
12. Spiteri I, Caravagna G, Cresswell GD, et al.: **Evolutionary dynamics of residual disease in human glioblastoma.** *Ann Oncol.* 2019; **30**(3): 456–63.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
13. Zahir N, Sun R, Gallahan D, et al.: **Characterizing the ecological and evolutionary dynamics of cancer.** *Nat Genet.* 2020; **52**(8): 759–67.
[PubMed Abstract](#) | [Publisher Full Text](#)
14. Gatenby RA, Brown JS: **Integrating evolutionary dynamics into cancer therapy.** *Nat Rev Clin Oncol.* 2020; **17**(11): 675–86.
[PubMed Abstract](#) | [Publisher Full Text](#)
15. Curtius K, Wright NA, Graham TA: **An evolutionary perspective on field cancerization.** *Nat Rev Cancer.* 2018; **18**(1): 19–32.
[PubMed Abstract](#) | [Publisher Full Text](#)
16. Maley CC, Aktipis A, Graham TA, et al.: **Classifying the evolutionary and ecological features of neoplasms.** *Nat Rev Cancer.* 2017; **17**(10): 605–19.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Caravagna G, Giarratano Y, Ramazzotti D, et al.: **Detecting repeated cancer evolution from multi-region tumor sequencing data.** *Nat Methods.* 2018; **15**(9): 707–14.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
18. Turajlic S, Xu H, Litchfield K, et al.: **Tracking Cancer Evolution Reveals Constrained Routes to Metastases: TRACERx Renal.** *Cell.* 2018; **173**(3): 581–594.e12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
19. Turajlic S, Xu H, Litchfield K, et al.: **Deterministic Evolutionary Trajectories Influence Primary Tumor Growth: TRACERx Renal.** *Cell.* 2018; **173**(3): 595–610.e11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. Hu Z, Sun R, Curtis C: **A population genetics perspective on the determinants of intra-tumor heterogeneity.** *Biochim Biophys Acta Rev Cancer.* 2017; **1867**(2): 109–26.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
21. Andor N, Graham TA, Jansen M, et al.: **Pan-cancer analysis of the extent and consequences of intratumor heterogeneity.** *Nat Med.* 2016; **22**(1): 105–13.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
22. Jamal-Hanjani M, Wilson GA, McGranahan N, et al.: **Tracking the Evolution of Non-Small-Cell Lung Cancer.** *N Engl J Med.* 2017; **376**(22): 2109–21.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Faculty Opinions Recommendation](#)
23. Misale S, Yaeger R, Hobor S, et al.: **Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer.** *Nature.* 2012; **486**(7404): 532–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
24. Litchfield K, Stanislaw S, Spain L, et al.: **Representative Sequencing: Unbiased Sampling of Solid Tumor Tissue.** *Cell Rep.* 2020; **31**(5): 107550.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Faculty Opinions Recommendation](#)
25. Brady SW, McQuerry JA, Qiao Y, et al.: **Combating subclonal evolution of resistant cancer phenotypes.** *Nat Commun.* 2017; **8**(1): 1231.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
26. Hata AN, Niederst MJ, Archibald HL, et al.: **Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition.** *Nat Med.* 2016; **22**(3): 262–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. Sharma SV, Lee DY, Li B, et al.: **A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations.** *Cell.* 2010; **141**(1): 69–80.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
28. Woolston A, Khan K, Spain G, et al.: **Genomic and Transcriptomic Determinants of Therapy Resistance and Immune Landscape Evolution during Anti-EGFR Treatment in Colorectal Cancer.** *Cancer Cell.* 2019; **36**(1): 35–50.e9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
29. Cao S, Wang Z, Gao F, et al.: **An R Implementation of Tumor-Stroma-Immune Transcriptome Deconvolution Pipeline using DeMixT.** *bioRxiv.* 2019; 566075.
[Publisher Full Text](#)
30. Li Y, Xie X: **A mixture model for expression deconvolution from RNA-seq in heterogeneous tissues.** *BMC Bioinformatics.* 2013; **14** Suppl 5(Suppl 5): S11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
31. Wang X, Park J, Susztak K, et al.: **Bulk tissue cell type deconvolution with multi-subject single-cell expression reference.** *Nat Commun.* 2019; **10**(1): 380.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
32. Wang Z, Cao S, Morris JS, et al.: **Transcriptome Deconvolution of Heterogeneous Tumor Samples with Immune Infiltration.** *iScience.* 2018; **9**: 451–60.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
33. Tirosh I, Izar B, Prakadan SM, et al.: **Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq.** *Science.* 2016; **352**(6282): 189–96.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
34. Tang F, Barbacioru C, Wang Y, et al.: **mRNA-Seq whole-transcriptome analysis of a single cell.** *Nat Methods.* 2009; **6**(5): 377–82.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Faculty Opinions Recommendation](#)
35. Picelli S, Björklund ÅK, Faridani OR, et al.: **Smart-seq2 for sensitive full-length transcriptome profiling in single cells.** *Nat Methods.* 2013; **10**(11): 1096–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
36. Hagemann-Jensen M, Ziegenhain C, Chen P, et al.: **Single-cell RNA counting at allele and isoform resolution using Smart-seq3.** *Nat Biotechnol.* 2020; **38**(6): 708–14.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Faculty Opinions Recommendation](#)
37. Sasagawa Y, Nikaïdo I, Hayashi T, et al.: **Quartz-Seq: A highly reproducible and sensitive single-cell RNA sequencing method, reveals non-genetic gene-expression heterogeneity.** *Genome Biol.* 2013; **14**(4): R31.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
38. Sheng K, Cao W, Niu Y, et al.: **Effective detection of variation in single-cell transcriptomes using MATQ-seq.** *Nat Methods.* 2017; **14**(3): 267–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Faculty Opinions Recommendation](#)
39. Fan X, Zhang X, Wu X, et al.: **Single-cell RNA-seq transcriptome analysis of linear and circular RNAs in mouse preimplantation embryos.** *Genome Biol.* 2015; **16**(1): 148.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
40. Xiao Z, Cheng G, Jiao Y, et al.: **Holo-Seq: Single-cell sequencing of holo-transcriptome.** *Genome Biol.* 2018; **19**(1): 163.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

41. Keren-Shaul H, Kenigsberg E, Jaitin DA, *et al.*: **MARS-seq2.0: An experimental and analytical pipeline for indexed sorting combined with single-cell RNA sequencing.** *Nat Protoc.* 2019; 14(6): 1841–62.
[PubMed Abstract](#) | [Publisher Full Text](#)
42. Hashimshony T, Senderovich N, Avital G, *et al.*: **CEL-Seq2: Sensitive highly-multiplexed single-cell RNA-Seq.** *Genome Biol.* 2016; 17: 77.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
43. Bagnoli JW, Ziegenhain C, Janjic A, *et al.*: **Sensitive and powerful single-cell RNA sequencing using mcSCR-seq.** *Nat Commun.* 2018; 9(1): 2937.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
44. Natarajan KN: **Single-Cell Tagged Reverse Transcription (STRT-Seq).** *Methods Mol Biol.* 2019; 1979: 133–53.
[PubMed Abstract](#) | [Publisher Full Text](#)
45. Baslan T, Kendall J, Rodgers L, *et al.*: **Genome-wide copy number analysis of single cells.** *Nat Protoc.* 2012; 7(6): 1024–41.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
46. Hou Y, Song L, Zhu P, *et al.*: **Single-cell exome sequencing and monoclonal evolution of a JAK2-negative myeloproliferative neoplasm.** *Cell.* 2012; 148(5): 873–85.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Faculty Opinions Recommendation](#)
47. Zong C, Lu S, Chapman AR, *et al.*: **Genome-wide detection of single-nucleotide and copy-number variations of a single human cell.** *Science.* 2012; 338(6114): 1622–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
48. Hou Y, Wu K, Shi X, *et al.*: **Comparison of variations detection between whole-genome amplification methods used in single-cell resequencing.** *Gigascience.* 2015; 4: 37.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
49. Leung ML, Wang Y, Waters J, *et al.*: **SNES: Single nucleus exome sequencing.** *Genome Biol.* 2015; 16(1): 55.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
50. Borgström E, Paterlini M, Mold JE, *et al.*: **Comparison of whole genome amplification techniques for human single cell exome sequencing.** *PLoS One.* 2017; 12(2): e0171566.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
51. Falconer E, Hills M, Naumann U, *et al.*: **DNA template strand sequencing of single-cells maps genomic rearrangements at high resolution.** *Nat Methods.* 2012; 9(11): 1107–12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
52. van den Bos H, Spierings DCJ, Taudt AS, *et al.*: **Single-cell whole genome sequencing reveals no evidence for common aneuploidy in normal and Alzheimer's disease neurons.** *Genome Biol.* 2016; 17(1): 116.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
53. Bakker B, Taudt A, Belderbos ME, *et al.*: **Single-cell sequencing reveals karyotype heterogeneity in murine and human malignancies.** *Genome Biol.* 2016; 17(1): 115.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
54. Zahn H, Steif A, Laks E, *et al.*: **Scalable whole-genome single-cell library preparation without preamplification.** *Nat Methods.* 2017; 14(2): 167–73.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Faculty Opinions Recommendation](#)
55. Laks E, McPherson A, Zahn H, *et al.*: **Clonal Decomposition and DNA Replication States Defined by Scaled Single-Cell Genome Sequencing.** *Cell.* 2019; 179(5): 1207–1221.e22.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
56. Farlik M, Sheffield NC, Nuzzo A, *et al.*: **Single-cell DNA methylome sequencing and bioinformatic inference of epigenomic cell-state dynamics.** *Cell Rep.* 2015; 10(8): 1386–97.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
57. Smallwood SA, Lee HJ, Angermueller C, *et al.*: **Single-cell genome-wide bisulfite sequencing for assessing epigenetic heterogeneity.** *Nat Methods.* 2014; 11(8): 817–20.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
58. Rotem A, Ram O, Shores N, *et al.*: **Single-cell ChIP-seq reveals cell subpopulations defined by chromatin state.** *Nat Biotechnol.* 2015; 33(11): 1165–72.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
59. Cusanovich DA, Daza R, Adey A, *et al.*: **Multiplex single cell profiling of chromatin accessibility by combinatorial cellular indexing.** *Science.* 2015; 348(6237): 910–4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
60. Lareau CA, Duarte FM, Chew JG, *et al.*: **Droplet-based combinatorial indexing for massive-scale single-cell chromatin accessibility.** *Nat Biotechnol.* 2019; 37(8): 916–24.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Faculty Opinions Recommendation](#)
61. Kaya-Okur HS, Wu SJ, Codomo CA, *et al.*: **CUT&Tag for efficient epigenomic profiling of small samples and single cells.** *Nat Commun.* 2019; 10(1): 1930.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
62. Ziegenhain C, Vieth B, Parekh S, *et al.*: **Comparative Analysis of Single-Cell RNA Sequencing Methods.** *Mol Cell.* 2017; 65(4): 631–643.e4.
[PubMed Abstract](#) | [Publisher Full Text](#)
63. Picelli S, Björklund AK, Reinius B, *et al.*: **Tn5 transposase and tagmentation procedures for massively scaled sequencing projects.** *Genome Res.* 2014; 24(12): 2033–40.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
64. Zheng GXY, Terry JM, Belgrader P, *et al.*: **Massively parallel digital transcriptional profiling of single cells.** *Nat Commun.* 2017; 8: 14049.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
65. Zilionis R, Nainys J, Veres A, *et al.*: **Single-cell barcoding and sequencing using droplet microfluidics.** *Nat Protoc.* 2017; 12(1): 44–73.
[PubMed Abstract](#) | [Publisher Full Text](#)
66. Bageritz J, Raddi G: **Single-Cell RNA Sequencing with Drop-Seq.** *Methods Mol Biol.* 2019; 1979: 73–85.
[PubMed Abstract](#) | [Publisher Full Text](#)
67. Wang Y, Waters J, Leung ML, *et al.*: **Clonal evolution in breast cancer revealed by single nucleus genome sequencing.** *Nature.* 2014; 512(7513): 155–60.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
68. Ke R, Mignardi M, Pacureanu A, *et al.*: **In situ sequencing for RNA analysis in preserved tissue and cells.** *Nat Methods.* 2013; 10(9): 857–60.
[PubMed Abstract](#) | [Publisher Full Text](#)
69. Wang X, Allen WE, Wright MA, *et al.*: **Three-dimensional intact-tissue sequencing of single-cell transcriptional states.** *Science.* 2018; 361(6400): eaat5691.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
70. Rodrigues SG, Stickels RR, Goeva A, *et al.*: **Slide-seq: A scalable technology for measuring genome-wide expression at high spatial resolution.** *Science.* 2019; 363(6434): 1463–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
71. Baker AM, Huang W, Wang XMM, *et al.*: **Robust RNA-based in situ mutation detection delineates colorectal cancer subclonal evolution.** *Nat Commun.* 2017; 8(1): 1998.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
72. Chen G, Ning B, Shi T: **Single-Cell RNA-Seq Technologies and Related Computational Data Analysis.** *Front Genet.* 2019; 10: 317.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
73. Kiselev VY, Andrews TS, Hemberg M: **Challenges in unsupervised clustering of single-cell RNA-seq data.** *Nat Rev Genet.* 2019; 20(5): 273–82.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Faculty Opinions Recommendation](#)
74. Vieth B, Parekh S, Ziegenhain C, *et al.*: **A Systematic Evaluation of Single Cell RNA-Seq Analysis Pipelines: Library preparation and normalisation methods have the biggest impact on the performance of scRNA-seq studies.** *bioRxiv.* 2019; 583013.
[Publisher Full Text](#)
75. Zhang L, Zhang S: **Comparison of Computational Methods for Imputing Single-Cell RNA-Sequencing Data.** *IEEE/ACM Trans Comput Biol Bioinform.* 2020; 17(2): 376–89.
[PubMed Abstract](#) | [Publisher Full Text](#)
76. Efremova M, Teichmann SA: **Computational methods for single-cell omics across modalities.** *Nat Methods.* 2020; 17(1): 14–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
77. Rodriguez-Meira A, Buck G, Clark SA, *et al.*: **Unravelling Intratumoral Heterogeneity through High-Sensitivity Single-Cell Mutational Analysis and Parallel RNA Sequencing.** *Mol Cell.* 2019; 73(6): 1292–1305.e8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
78. Nam AS, Kim KT, Chaligne R, *et al.*: **Somatic mutations and cell identity linked by Genotyping of Transcriptomes.** *Nature.* 2019; 571(7765): 355–60.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
79. van Galen P, Hovestadt V, li MHW, *et al.*: **Single-Cell RNA-Seq Reveals AML Hierarchies Relevant to Disease Progression and Immunity.** *Cell.* 2019; 176(6): 1265–1281.e24.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
80. Bronner IF, Lorenz S: **Combined Genome and Transcriptome (G&T) Sequencing of Single Cells.** *Methods Mol Biol.* 2019; 1979: 319–62.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Faculty Opinions Recommendation](#)

81. Macaulay IC, Teng MJ, Haerty W, *et al.*: **Separation and parallel sequencing of the genomes and transcriptomes of single cells using G&T-seq.** *Nat Protoc.* 2016; **11**(11): 2081–103.
[PubMed Abstract](#) | [Publisher Full Text](#)
82.  Peterson VM, Zhang KX, Kumar N, *et al.*: **Multiplexed quantification of proteins and transcripts in single cells.** *Nat Biotechnol.* 2017; **35**(10): 936–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Faculty Opinions Recommendation](#)
83.  Stoeckius M, Hafemeister C, Stephenson W, *et al.*: **Simultaneous epitope and transcriptome measurement in single cells.** *Nat Methods.* 2017; **14**(9): 865–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
84. Gerlach JP, van Buggenum JAG, Tanis SEJ, *et al.*: **Combined quantification of intracellular (phospho-)proteins and transcriptomics from fixed single cells.** *Sci Rep.* 2019; **9**(1): 1469.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
85. Gao R, Davis A, McDonald TO, *et al.*: **Punctuated copy number evolution and clonal stasis in triple-negative breast cancer.** *Nat Genet.* 2016; **48**(10): 1119–30.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
86. Bhang HE, Ruddy DA, Radhakrishna VK, *et al.*: **Studying clonal dynamics in response to cancer therapy using high-complexity barcoding.** *Nat Med.* 2015; **21**(5): 440–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
87. Hinohara K, Wu HJ, Vigneau S, *et al.*: **KDM5 Histone Demethylase Activity Links Cellular Transcriptomic Heterogeneity to Therapeutic Resistance.** *Cancer Cell.* 2018; **34**(6): 939–953.e9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
88.  Kim C, Gao R, Sei E, *et al.*: **Chemoresistance Evolution in Triple-Negative Breast Cancer Delineated by Single-Cell Sequencing.** *Cell.* 2018; **173**(4): 879–893.e13.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
89.  Filbin MG, Tirosh I, Hovestadt V, *et al.*: **Developmental and oncogenic programs in H3K27M gliomas dissected by single-cell RNA-seq.** *Science.* 2018; **360**(6386): 331–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
90.  Davidson S, Efremova M, Riedel A, *et al.*: **Single-Cell RNA Sequencing Reveals a Dynamic Stromal Niche That Supports Tumor Growth.** *Cell Rep.* 2020; **31**(7): 107628.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [Faculty Opinions Recommendation](#)
91.  Efremova M, Vento-Tormo M, Teichmann SA, *et al.*: **CellPhoneDB: Inferring cell-cell communication from combined expression of multi-subunit ligand-receptor complexes.** *Nat Protoc.* 2020; **15**(4): 1484–506.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Faculty Opinions Recommendation](#)