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High-throughput single-cell transcriptomics on organoids

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Three-dimensional (3D) tissues grown in culture from human stem cells offer the incredible opportunity to analyze and manipulate human development, and to generate patient-specific models of disease. Methods to sequence DNA and RNA in single cells are being used to analyze these so-called 'organoid' systems in high-resolution. Single-cell transcriptomics has been used to quantitate the similarity of organoid cells to primary tissue counterparts in the brain, intestine, liver, and kidney, as well as identify cell-specific responses to environmental variables and disease conditions. The merging of these two technologies, single-cell genomics and organoids, will have profound impact on personalized medicine in the near future.

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Introduction

The human body is composed of an amazing diversity of cell fates, which transition through various states during organ development, disease, and regeneration. Methods to engineer diverse human tissues from stem cells are being developed at a rapid pace in order to study uniquely human organ development and disease in controlled culture environments. Depending on the tissue and disease, these culture models can be initiated from organ-specific adult stem cells or iPSCs generated from skin or blood [1]. These 3D tissues, commonly called 'organoids', offer the promise to more accurately model human development, physiology and disease than conventional 2D culture counterparts by creating complex microenvironments where multiple lineages structurally organize and communicate to maintain a balanced physiological status. However, it has been unclear how precisely these organoid systems recapitulate the cell state-specific gene expression landscapes of the tissues they intend to model. Single-cell genomic methods (reviewed in Ref. [2]) have brought new analytic approaches to characterize these organoid models with more resolution and less bias than previous immunohistological or bulk RNA-seq descriptions of organoid development.

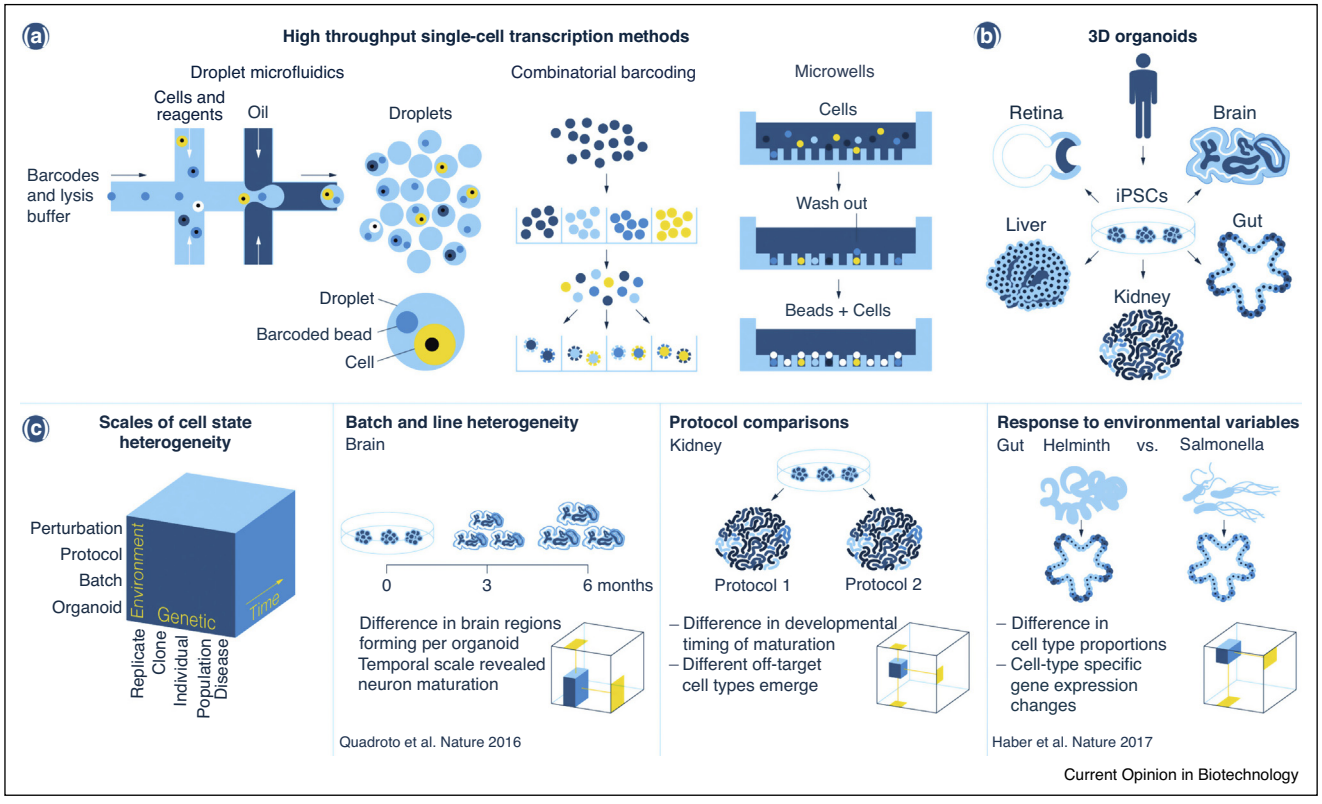
Most single-cell RNA-seq methods require each cell to be physically captured in a small volume where cells can be lysed, and chemistry can be performed on the contents of each individual cell. Capture can be achieved by hand picking or flow cytometric sorting into multi-well plates [3], limited dilutions into wafers containing hundreds of microwells [4], or through valve [5] or droplet-based microfluidic approaches [6•,7•]. In addition, combinatorial barcoding strategies have been developed that enable barcoding of cellular RNAs without physically isolating the cells [8]. Each strategy has particular benefits or drawbacks depending on the application, however the approaches that enable high-throughput (thousands of cells per experiment) are best suited to efficiently sample the complex cellular diversity in organoids and to understand organoid to organoid variability. One limitation of the higher throughput approaches is that only the 3' or 5' end of the transcript is sequenced limiting inquiry into certain features of the transcriptome (e.g. alternative splicing).

These quantitative SCG technologies are being used to study how each cell fate is regulated within complex multilineage human organoids (Figure 1). This data can be used to eventually generate organ-level computational models of human development, which may be used to predict disease mechanisms. Here we review recent advances at the intersection of single-cell genomics (SCG) and human tissue engineering, and highlight existing challenges in the organoid field where high-throughput SCG strategies can have an immediate impact. We concentrate on organoid modeling of the brain, intestine, liver, and kidney, where there has been the most recent progress.

Brain

There have been multiple methods published to generate 3D tissue cultures that resemble the developing human

Figure 1



Single-cell transcriptomics is enabling high-resolution analysis of cell heterogeneity in human organoids.
(a) New high-throughput single-cell transcriptomic methods based on droplet microfluidics, combinatorial barcoding, or microwell technologies have dramatically expanded the number of cells that can be analyzed per experiment, opening up new inroad into quantitative comparisons of cell states. **(b)–(c)** These high-throughput methods are enabling the analysis of human 3D organoids derived from induced pluripotent stem cells (b) across a range of environmental, genetic, and temporal scales (c). High-throughput methods will make it possible to quantitatively assess cell states between organoids, batches, protocols, and various perturbations (environmental scale); between replicates, clones, individuals from the same or different populations, and patients with a genetic disorder (genetic scale); and each of the genetic or environmental impacts on cell state can be assessed over a time course (time scale). The panels highlight that multiple publications have already started to address how various environmental or genetic conditions effect organoid cell states in brain, kidney and gut organoids.

brain. These approaches come in two distinct categories. First, iPSC-derived neuroectoderm can be allowed to self-organize into cerebral organoids that contain multiple interconnected brain regions. Second, iPSC aggregates can be patterned to generate distinct, independent brain regions. In both cases, progenitor cells exhibit very similar morphology and behaviors that have been observed in fetal tissue. Neurons mature, establish synapses, spontaneously fire action potentials, and may even respond to physiological stimuli. Camp, Badsha *et al.* was the first to directly compare cerebral organoids with the early fetal neocortex using scRNA-seq and found that cell composition, lineage relationships, and gene expression programs were largely recapitulated in the organoid cortical regions (Figure 1) [9]. A major limitation of this study was the scRNA-seq technology was low throughput, resulting less than 1000 cells. Quadratto *et al.* substantially advanced the characterization of multi-region cerebral organoids using the Drop-seq method based on droplet microfluidics. It allowed the authors to sequence over 80 000 cells from 31 whole-brain organoids, which clustered into many distinct populations representing different brain developmental identities, including forebrain and retina [10••]. It also revealed, at the transcriptome level, that neurons progressively matured from three to six months in culture along multiple lineages (callosal projection neurons as well as Müller glial and bipolar cells). The authors also provided evidence that organoids from the same bioreactor contained more comparable cell types than between bioreactors, likely due to variation in organoid brain region composition [11].

Birey *et al.* analyzed human iPSC-derived dorsal and ventral forebrain spheroids before and after fusion [12]. The data showed that cells were remarkably well patterned. It was shown for the first time that cells which

migrated from the ventral to the dorsal region could functionally integrate into cortical networks. Migrated cells had increased complexity of dendrites branching, twice higher action potential generation rate than non-migrated or one region cells, expressed the presynaptic and postsynaptic proteins and formed functional synapses. In addition, the results of modeling a neurodevelopmental disorder on forebrain assembled spheroids from different patients' iPSC lines were compatible with the expected disease phenotype. In each of these cases, there was no robust characterization of how iPSC lines from different human individuals, or even different lines from the same individual compare in gene expression space. Such a quantitative characterization will be required in order to understand disease models. Furthermore, it is not clear if cortical organoids can accurately model the expansion of the outer radial glial populations that is observed in later stages of human neocortex development [13]. Indeed, the spatial heterogeneity of organoid cultures naturally requires spatial transcriptomics and there will be major advances in the coming years in this area of research. Finally, recent work has shown that organoids can generate complex network wiring, and it will be interesting to couple single-cell transcriptomics to activity-dependent electrical stimulations [14].

Gut

Gut organoids (stomach, small intestine, colon) can be established either from adult-derived intestinal epithelial stem cells [15,16] or through the differentiation of iPSCs through endoderm derived foregut and hindgut spheroids [17,18]. To date, scRNA-seq on mouse small intestine organoids has provided the most insight into the cellular composition of adult stem cell-derived organoids. Low-throughput methods were used to identify the distinct cardinal populations of the intestinal epithelium (stem cells, paneth cells, goblet cells, enteroendocrine cells, enterocytes) [19]. However, enteroendocrine cells needed to be enriched in order to identify enteroendocrine subpopulations due the use of low-throughput methods. Haber, Biton, Rogel *et al.* generated an atlas of mouse small intestine cell composition from 53 193 number cells. From this survey, they could identify each cell population as well as subtypes of enteroendocrine cells, such as early, middle and late precursors and mature cells [20••]. A new enteroendocrine cell taxonomy was created by comparing the expression of detected genes across two subtypes to canonical classification markers. Results revealed that previously defined markers, for example Sct, Cck, Gcg and Ghrl, were not subtype-specific but expressed across multiple cell types. Interestingly, there was heterogeneity in an enterochromaffin cell population, which split into two distinct subtypes. In addition, the authors compared cell composition and gene expression landscapes in organoids after exposure to different microbe populations. Extended to humans, this strategy will allow controlled experimentation of human intestine to diverse dietary, microbial, or pharmaceutical manipulations. Currently, however, improved organoid culture methods are needed to enable human gut organoids to maintain a balance of progenitors and differentiated cells within a 3D structure, and there is currently no single-cell transcriptomics manuscript published that analyzed human intestinal organoids.

Liver

The dynamic developmental, structural, and cellular heterogeneity of the liver makes it challenging to recapitulate the growth pathways of this organ *in vitro* from pluripotency. The developing fetal liver initially serves as the reservoir for hematopoiesis before structural reorganization and maturation into the major metabolic organ of the body. Multiple different protocols have been developed to differentiate iPSCs in 2D monocultures to hepatic endoderm and then toward 'hepatocyte-like' cells [21]. However, these cells are not functioning as mature, metabolically complete hepatocytes and certain widely used protocols generate cells with only a modest similarity to human hepatocytes, which may even be off target cells with similarity to the intestinal eptithelium [22]. Incorporating additional lineages thought to provide signals that specify hepatic fate acquisition, such as the transverse mesenchyme and nascent endothelium, into a 3D microenvironment has shown great promise in generating hepatic organoids [23]. We have analyzed this system and found that the hepatic cells within these *in vitro* and transplanted human organoids acquire a significant increase in similarity to fetal hepatocytes relative to the 2D counterparts [24]. However, in all cases we have analyzed thus far, there remains a major challenge to generate mature hepatocytes with fully metabolic functionality from human iPSCs.

It has been shown that 2D monocultures of primary adult human hepatocytes can only be maintained short-term due to dedifferentiation and cell death [25]. Recently, protocols were developed to isolate hepatic stem cells (HSCs) from adult tissues and culture HSCs in 3D matrix environments that support proliferation and differentiation of hepatic epithelium [26]. These methods are revealing the potential to maintain differentiated hepatic cells *in vitro*. Single-cell RNA-seq analyses on adult liver could in principle map the transcriptome states of the HSCs and mature hepatocytes *in vivo*, and be used to assess the accuracy and precision of the adult stem-cell derived organoids maintained *in vitro*. In any case, major current protocols lack the cellular diversity (e.g. kupffer cells, stellate cells, bile ducts, portal endothelium, etc.) and the structural organization of the human liver. Spatial maps of mouse liver hepatocyte transcripts confirmed that hepatocytes were ordered into a metabolic hierarchy that correlates with proximity to the portal vein and bile ducts [27]. In the future, full reconstructions of liver development, from fetus to adult, with structural and cell state resolution will enable reverse engineering and bench marking of 3D liver organoid technologies.

Kidney

Mutliple recent studies showed that 3D kidney organoids can be generated from pluripotent stem cells [28–30]. Bulk transcriptome analysis suggested that these organoid systems resemble approximately the first trimester of human kidney development. Recently, single-cell transcriptomics was used to dissect cell composition in human kidney organoids and to identify cell types that could be impacted by disease-related genes predicted from genome-wide association studies [30,31••,32,33]. The mapping of disease-related genes was generally consistent with a scRNA-seq study of the mouse kidney, which highlights that discrete human disease phenotypes are due to mutations in genes that have a cell-type-specific gene expression pattern in the kidney [32]. In Wu *et al*., the authors showed that both organoid systems contain very similar cardinal renal cell types (podocytes, mesenchyme, tubule cells), albeit at different proportions, and that the cells are relatively immature compared to fetal and adult renal cells. The authors also identified multiple populations of non-renal cells, and developed an inhibition strategy based on receptor expression to reduce the prevalence of these off-target cells. This manuscript is a great example of how single-cell transcriptomics can be used not only to assess the quality of the organoids, but also guide the engineering process.

Furthermore, establishment of automated high-throughput human organoid generation platforms that enable testing of culture conditions to enhance cell differentiation, predict chemical toxicity, and phenotype organoids promise rapid innovations in culture methods and assessment of disease phenotypes [34]. Czerniecki *et al*. used scRNA-seq to characterize kidney organoids that had been optimized by robotic manipulation of culture conditions in hundreds of mini-organoids in microwells. Image-based data of marker genes had suggested that the addition of vascular endothelial growth factor (VEGF) increased the abundance of endothelial cells in the organoids. However, single cell RNA-sequencing on 10 535 cells from organoids treated with or without VEGF, revealed that there were very few mature endothelial cells present in the organoids, which had not been resolved by immunohistochemistry. The authors conclude that the VEGF treatment greatly increases the number of endothelial cell progenitors in the organoid cultures, but only a small minority of these cells reaches a mature endothelial cell differentiation state similar to that found *in vivo*. This data further underscores the importance of high-resolution descriptions of cell states that arise in organoid culture systems.

Future prospective

Personalized medicine is on the horizon, where an individual's genome can be integrated with personalized 3D tissue culture models to create avatars of disease particular to the patient. Depending on the tissue and disease, these culture models can be initiated from iPSCs, organ-specific adult stem cells, or cancer tissue. Even though recent protocol enhancements enable longer-term growth and development of 3D organoids, the challenge remains to generate 3D models that recapitulate mature cellular and physiological phenotypes observed in postnatal human tissues. Single-cell sequencing methods are required to assess the accuracy, precision, and efficiency of protocol enhancements. Additionally, because of the cellular complexity and general batch heterogeneity of organoids, it is critical to sample as many cells as economically feasible in multiple organoids per experiment, ideally with spatial [35] and lineage resolution [36]. We expect that over the coming years there will be dedicated efforts to analyze organoids from hundreds of patients with particular diseases from iPSC and adult organoid biobanks. These efforts will require increases in sample throughput either through sample tagging [37], combinatorial barcoding [38•], or random composite measurements [39]. As the field progresses, robust computational strategies will be required to integrate the data and make biological sense of what is sure to be high-information content and extremely complex data.

Conflict of interest statement

Nothing declared.

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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. Clevers H: **Modeling development and disease with organoids**. *Cell* 2016, **165**:1586-1597 http://dx.doi.org/10.1016/j.cell.2016.05.082.

2. Picelli S: **Single-cell RNA-sequencing: the future of genome biology is now**. *RNA Biol* 2016:1-14 http://dx.doi.org/10.1080/15476286.2016.1201618.

3. Picelli S *et al.*: **Smart-seq2 for sensitive full-length transcriptome profiling in single cells**. *Nat Methods* 2013, **10**:1096-1098 http://dx.doi.org/10.1038/nmeth.2639.

4. Han X *et al.*: **Mapping the mouse cell atlas by microwell-seq**. *Cell* 2018, **172**:1091-1107 http://dx.doi.org/10.1016/j.cell.2018.02.001 e1017.

5. Treutlein B *et al.*: **Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq**. *Nature* 2014, **509**:371-375 http://dx.doi.org/10.1038/nature13173.

6. • Macosko EZ *et al.*: **Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets**. *Cell* 2015, **161**:1202-1214 http://dx.doi.org/10.1016/j.cell.2015.05.002.

These two papers presented the first high-throughput single-cell RNA-seq data using droplet microfluidic technology, and the authors made their protocols widely accessible to scientists enabling the establishment of this specialized method in labs around the world. Subsequent commercializations of droplet-based methods have dramatically expanded the single-cell genomics field.

7. • Klein AM *et al.*: **Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells**. *Cell* 2015, **161**:1187-1201 http://dx.doi.org/10.1016/j.cell.2015.04.044.

These two papers presented the first high-throughput single-cell RNA-seq data using droplet microfluidic technology, and the authors made their protocols widely accessible to scientists enabling the establishment of this specialized method in labs around the world. Subsequent commercializations of droplet-based methods have dramatically expanded the single-cell genomics field.

8. Rosenberg AB *et al.*: **Single-cell profiling of the developing mouse brain and spinal cord with split-pool barcoding**. *Science* 2018, **360**:176-182 http://dx.doi.org/10.1126/science.aam8999.

9. Camp JG *et al.*: **Human cerebral organoids recapitulate gene expression programs of fetal neocortex development**. *Proc Natl Acad Sci U S A* 2015, **112**:15672-15677 http://dx.doi.org/10.1073/pnas.1520760112.

10. •• Quadrato G *et al.*: **Cell diversity and network dynamics in photosensitive human brain organoids**. *Nature* 2017, **545**:48-53 http://dx.doi.org/10.1038/nature22047.
This was one of the first explorations of cell diversity in human iPSC-derived organoids using high-throughput single-cell transcriptomics (drop-seq protocol). The higher throughput method enabled comparison of cell composition across organoid batches and time points.

11. Renner M *et al.*: **Self-organized developmental patterning and differentiation in cerebral organoids**. *EMBO J* 2017, **36**:1316-1329 http://dx.doi.org/10.15252/embj.201694700.

12. Birey F *et al.*: **Assembly of functionally integrated human forebrain spheroids**. *Nature* 2017, **545**:54-59 http://dx.doi.org/10.1038/nature22330.

13. Pollen AA *et al.*: **Molecular identity of human outer radial glia during cortical development**. *Cell* 2015, **163**:55-67 http://dx.doi.org/10.1016/j.cell.2015.09.004.

14. Fuzik J *et al.*: **Integration of electrophysiological recordings with single-cell RNA-seq data identifies neuronal subtypes**. *Nat Biotechnol* 2016, **34**:175-183 http://dx.doi.org/10.1038/nbt.3443.

15. Sato T *et al.*: **Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche**. *Nature* 2009, **459**:262-265 http://dx.doi.org/10.1038/nature07935.

16. Stange DE *et al.*: **Differentiated Troy+ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium**. *Cell* 2013, **155**:357-368 http://dx.doi.org/10.1016/j.cell.2013.09.008.

17. Spence JR *et al.*: **Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro**. *Nature* 2011, **470**:105-109 http://dx.doi.org/10.1038/nature09691.

18. McCracken KW *et al.*: **Modelling human development and disease in pluripotent stem-cell-derived gastric organoids**. *Nature* 2014, **516**:400-404 http://dx.doi.org/10.1038/nature13863.

19. Grun D *et al.*: **Single-cell messenger RNA sequencing reveals rare intestinal cell types**. *Nature* 2015, **525**:251-255 http://dx.doi.org/10.1038/nature14966.

20. •• Haber AL *et al.*: **A single-cell survey of the small intestinal epithelium**. *Nature* 2017, **551**:333-339 http://dx.doi.org/10.1038/nature24489.
This manuscript used scRNA-seq to first establish an atlas of cell diversity in the mouse small intestinal epithelium and 3D organoid cultures derived from adult stem cells, and then used scRNA-seq to analyze how each cell type within the intestinal organoid cultures respond to different intestinal microbes. Higher throughput methods enabled comprehensive comparisons of organoids in different environmental conditions.

21. Mallanna SK, Duncan SA: **Differentiation of hepatocytes from pluripotent stem cells**. *Curr Protoc Stem Cell Biol* 2013, **26** http://dx.doi.org/10.1002/9780470151808.sc01gs26 Unit 1G 4.

22. Cahan P *et al.*: **CellNet: network biology applied to stem cell engineering**. *Cell* 2014, **158**:903-915 http://dx.doi.org/10.1016/j.cell.2014.07.020.

23. Takebe T *et al.*: **Vascularized and functional human liver from an iPSC-derived organ bud transplant**. *Nature* 2013, **499**:481-484 http://dx.doi.org/10.1038/nature12271.

24. Camp JG *et al.*: **Multilineage communication regulates human liver bud development from pluripotency**. *Nature* 2017, **546**:533-538 http://dx.doi.org/10.1038/nature22796.

25. Kegel V *et al.*: **Protocol for isolation of primary human hepatocytes and corresponding major populations of non-parenchymal liver cells**. *J Vis Exp* 2016:e53069 http://dx.doi.org/10.3791/53069.

26. Huch M *et al.*: **Long-term culture of genome-stable bipotent stem cells from adult human liver**. *Cell* 2015, **160**:299-312 http://dx.doi.org/10.1016/j.cell.2014.11.050.

27. Halpern KB *et al.*: **Single-cell spatial reconstruction reveals global division of labour in the mammalian liver**. *Nature* 2017, **542**:352-356 http://dx.doi.org/10.1038/nature21065.

28. Takasato M *et al.*: **Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis**. *Nature* 2015, **526**:564-568 http://dx.doi.org/10.1038/nature15695.

29. Morizane R *et al.*: **Nephron organoids derived from human pluripotent stem cells model kidney development and injury**. *Nat Biotechnol* 2015, **33**:1193-1200 http://dx.doi.org/10.1038/nbt.3392.

30. Combes AN *et al.*: **High throughput single cell RNA-seq of developing mouse kidney and human kidney organoids reveals a roadmap for recreating the kidney**. *bioRxiv*. 2017 http://dx.doi.org/10.1101/235499.

31. •• Wu H *et al.*: **Comparative analysis of kidney organoid and adult human kidney single cell and single nucleus transcriptomes**. *bioRxiv*. 2017 http://dx.doi.org/10.1101/232561.
This manuscript quantitatively compared cell composition, similarity to primary tissue, and off-target cell type emergence in two different human kidney organoid protocols derived from iPSCs.

32. Park J *et al.*: **Single-cell transcriptomics of the mouse kidney reveals potential cellular targets of kidney disease**. *Science* 2018, **360**:758-763 http://dx.doi.org/10.1126/science.aar2131.

33. Lindstrom NO *et al.*: **Progressive recruitment of mesenchymal progenitors reveals a time-dependent process of cell fate acquisition in mouse and human nephrogenesis**. *Dev Cell* 2018, **45**:651-660 http://dx.doi.org/10.1016/j.devcel.2018.05.010 e654.

34. Czerniecki SM *et al.*: **High-throughput screening enhances kidney organoid differentiation from human pluripotent stem cells and enables automated multidimensional phenotyping**. *Cell Stem Cell* 2018, **22**:929-940 http://dx.doi.org/10.1016/j.stem.2018.04.022 e924.

35. Lein E, Borm LE, Linnarsson S: **The promise of spatial transcriptomics for neuroscience in the era of molecular cell typing**. *Science* 2017, **358**:64-69 http://dx.doi.org/10.1126/science.aan6827.

36. Spanjaard B *et al.*: **Simultaneous lineage tracing and cell-type identification using CRISPR-Cas9-induced genetic scars**. *Nat Biotechnol* 2018, **36**:469-473 http://dx.doi.org/10.1038/nbt.4124.

37. Stoeckius M *et al.*: **Cell "hashing" with barcoded antibodies enables multiplexing and doublet detection for single cell genomics**. *bioRxiv*. 2017 http://dx.doi.org/10.1101/237693.

38. • Cao J *et al.*: **Comprehensive single-cell transcriptional profiling of a multicellular organism**. *Science* 2017, **357**:661-667 http://dx.doi.org/10.1126/science.aam8940.
This paper described a combinatorial barcoding approach to generate single-cell transcriptomes without first compartmentalizing the cell into a well or droplet. This strategy could in principle be used to scale up the number of conditions (genetic, environmental, or temporal) that can be analyzed in organoids by lowering the cost and also because the method can be performed in 96 well plates, a convenient platform for some organoid culture protocols.

39. Cleary B, Cong L, Cheung A, Lander ES, Regev A: **Efficient generation of transcriptomic profiles by random composite measurements**. *Cell* 2017, **171**:1424-1436 http://dx.doi.org/10.1016/j.cell.2017.10.023 e1418.