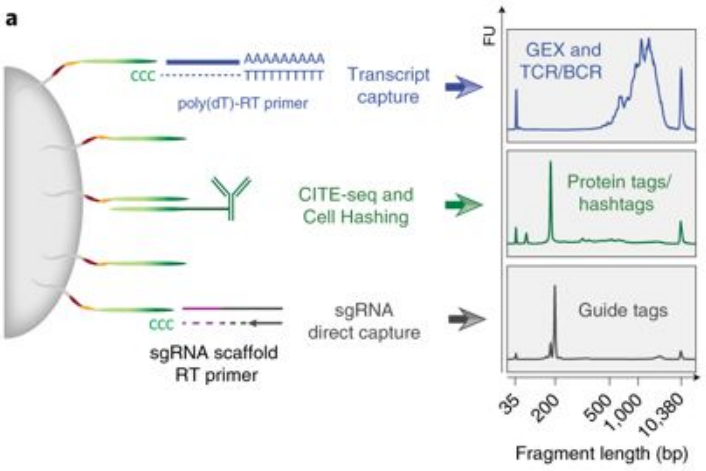
Single Cell Transcriptomics

Newsletter May 2019

Paper 1

Mimitou et al. [Multiplexed detection of proteins, transcriptomes, clonotypes and CRISPR perturbations in single cells](). *Nature methods*, 2019.

During the last couple of years, the Technology Innovation group has been developing tools for combining high-throughput single cell RNA-seq assays with other approaches, for example the detection of protein markers using DNA oligo-labeled antibodies against cell surface markers (CITE-seq). Then, they followed up this work by using the same concept to barcode individual samples allowing multiplexing of single cell RNA sequencing experiments in a method named Cell Hashing.

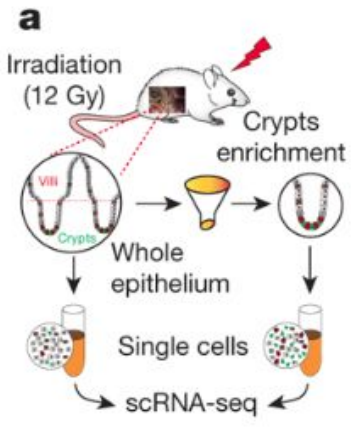


Here, they extend the use of CITE-seq and the related Cell Hashing method for multiplexing and doublet detection, to 5′ capture-based scRNA-seq methods (exemplified by the 10x Genomics system) allowing the detection of surface proteins together with the scRNA-seq and clonotype features. They combined CITE-seq and Cell Hashing with the ability to directly detect single guide RNAs used for CRISPR screens with a new method named ECCITE-seq (expanded CRISPR-compatible cellular indexing of transcriptomes and epitopes by sequencing). As proof of principle for the method, they used it to characterize malignant populations in a sample from a patient with cutaneous T cell lymphoma. Combining modalities enabled fine dissection of specific cell subtypes and helped reveal a transcriptomic signature of malignant cells in this type of cancer, but it can be applied to many other fields.

Paper 2

Ayyaz et al. [Single-cell transcriptomes of the regenerating intestine reveal a revival stem cell.]() *Nature*, 2019.

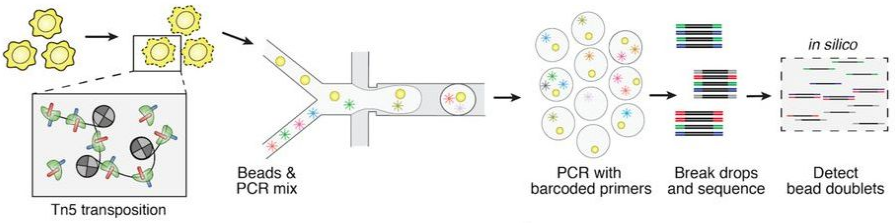
Damaged intestinal epithelium is able to regenerate although the multipotent LGR5+ crypt-base cells forming it are lost after injury. Other different populations have been proposed to repair the damaged intestine, such as reserve stem cells, progenitors of absorptive enterocytes or slow cycling LGR5+ cells. However, it is unclear whether cellular plasticity or distinct cell populations are driving the regeneration.



In order to define the mechanisms underlying intestine repair, Ayyaz et al. profiled the transcriptome of single-cells from healthy and irradiated intestine using 10x genomics platform. They identified a new distinct, damage-induced quiescent cell type that they named the revival stem cell (revSC) that were rare in non-irradiated intestines. They are present along irradiated crypts and were distinguished by expression of several genes involved in inflammatory responses, DNA damage responses and cell survival, such as clusterin (Clu), annexin A1 (Anxa1), Mif23, Npm124 and cyclin D1 (Ccnd1). Furthermore, Yap1 - a transcriptional regulator important for intestine regenerations - was expressed exclusively in revSC, and deleting Yap1 in the intestinal epithelium suppressed emergence of endogenous CLU+ cells. In conclusion, they claim that revSC are rare, unique and quiescent crypt cells induced by intestinal damage in a YAP1-dependent manner mediating the homeostatic turnover.

Paper 3

Lareau et al. [Droplet-based combinatorial indexing for massive scale single-cell epigenomics.](#) *bioRxiv*, 2019.



Current methods for single-cell Assay for Transposase Accessible chromatin (scATAC-seq) remain either relatively low-throughput (100s to 1,000s of cells/experiment) or provide low-complexity data (1,000s of fragments per cell). The authors describe a new method for for single-cell chromatin accessibility profiling using droplet microfluidics and ATAC-seq. They load Tn5 transposase with barcoded DNA adapters to add well-specific DNA barcodes to open chromatin. Following barcoded transposition, transposed cells are pooled and loaded at high density to co-encapsulate multiple barcoded cells with multiple beads in each droplet. Then, droplet co-encapsulation of transposed cells with barcoded beads and PCR reagents, we perform library preparation. They applied this new approach to obtain an epigenomic atlas from 502.207 resting and stimulated human bone marrow cells.

Paper 4

Razmara et al., [Recount-brain: a curated repository of human brain RNA-seq datasets metadata](#)**.** *bioRxiv, 2019.*

Sex	Female	Male		
Age/Development	Fetus	Child	Adolescent	Adult
Race/Ethnicity	Asian	Black	Hispanic	White
Tissue Site 1	Cerebral cortex	Hippocampus	Brainstem	Cerebellum
Tissue Site 2	Frontal lobe	Temporal lobe	Midbrain	Basal ganglia
Tissue Site 3	Dorsolateral prefrontal cortex	Superior temporal gyrus	Substantia nigra	Caudate
Hemisphere	Left	Right		
Brodmann Area	1-52			
Disease Status	Disease	Neurological control		
Disease	Brain tumor	Alzheimer's disease	Parkinson's disease	Bipolar disorder
Tumor Type	Glioblastoma	Astrocytoma	Oligodendroglioma	Ependymoma
Clinical Stage 1	Grade I	Grade II	Grade III	Grade IV
Clinical Stage 2	Primary	Secondary	Recurrent	
Viability	Postmortem	Biopsy		
Preparation	Frozen	Thawed		

In 2017, John Hopkins researchers created recount2 package, which aggregates expression and phenotypic information over 2041 different RNA-seq studies. Now they released next version: recount-brain. It gathers scRNA-seq brain data from SRA, GTEx and TCGA repositories, filling and unifying missing phenotypic metadata. The repository has 4,431 human brain tissue samples from 62 projects and provides web-interface to search over these samples. As a demonstration, authors took a study on effects of post-mortem interval on transcription and replicated it taking data from 9 different studies, which increased power and gave new findings.

Workshop:

Computational workshop in single-cell data analysis

Friday, May 10, 2019 from 8:30 AM to 3:30 PM

Faculty Club, Panum Building

Next Single Cell Seminar - 28th June, Mærsk Tower, Top Floor

9:00 – 9:40 **Andrea Asenjo Martinez, Khodosevich group, BRIC**
Maturation of mouse inhibitory interneurons during postnatal neurogenesis

10:00 – 10:40 **Gaurav Singh Rathore, Kirkeby group, Danstem**
Mapping human neurogenesis in an in vitro model of human brain development using scRNAseq

10:00 – 10:40 Coffee and Discussion

Other:

The Brain Mosaic: Cellular heterogeneity in the CNS (2nd edition)

10 - 11 October 2019, Leuven, Belgium

[Registration](#) open until 26 September

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