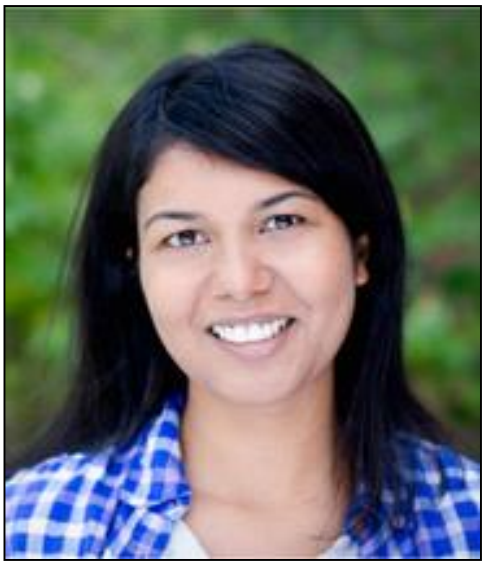
Donnelly Centre
PDF and RA Seminar Series

Global investigation of cell lineage and specification in the vertebrate brain with single-cell transcriptomics



Dr. Bushra Raj
Post Doctoral Fellow
Alex Schier Lab
Harvard University

DATE: June 8th, 2018
TIME: 11:00 – 12:00 pm
LOCATION: Red Room

A central goal in the field of developmental biology is to understand how the brain is specified and organized regionally, cellularly and molecularly. A fundamental component of realizing this vision is characterizing the origins and fates of cells during development. Critical insights into these processes have been gained using genetic markers, perturbations and fate mapping. However, analyses have been largely restricted to small gene sets or particular cell types resulting in 'local' views of neural development. To advance the field further, efforts need to be directed towards obtaining a global view of the genealogy of the brain.

We developed a technology, scGESTALT, which combines cell type identification by single-cell RNA sequencing with lineage recording by CRISPR-Cas9 cumulative barcode editing to simultaneously determine cell identity and lineage relationships. We sequenced ~60,000 transcriptomes from the juvenile zebrafish brain and identified more than 100 cell types and marker genes, generating a global catalogue of cell types in a vertebrate brain. In addition, we implemented a strategy for in vivo barcode editing across two time windows that encompass early and late embryogenesis, and obtained >10,000 distinct barcodes. We isolated thousands of single-cell transcriptomes and their associated lineage barcodes from the brain with droplet microfluidics. Using the patterns of shared edits between barcodes, we generated lineage trees with hundreds of branches and tips representing the associated cell types. Inspection of the trees identified restrictions at the level of cell types and brain regions.

Using a time series of single-cell transcriptomics data of >140,000 cells spanning 10 developmental stages from embryo to larva, and a trajectory reconstruction algorithm (URD), we are now now generating large-scale cell specification trees during zebrafish brain development to interrogate molecular regulation of neuronal cell diversity at a global scale. Combining this with scGESTALT will enable one to query the relationship between lineage and cell identity for thousands of cells during development.

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