Single Cell Transcriptomics

Newsletter Oktober 2022

Paper 1

Borm, Lars E. ***et al.*** [Scalable in situ single-cell profiling by electrophoretic capture of mRNA using EEL FISH](), *Nature Biotechnology*, 2022

Enhanced ELectric Fluorescence in situ Hybridization (EEL FISH) from the Linnarsson lab is now published. Current methods in spatial transcriptomic are dominated by a trade off in resolution versus throughput. In regular smFISH many z-stack pictures have to be captured, making the imaging process very slow. In EEL FISH the imaging time is elegantly sped up by electrophoresing the mRNA out of the tissue and onto a slide. The tissue is then removed and several square centimeters can be imaged in days time. They implemented a barcoded prove design to detect 448 genes in 16 cycles in one color channel. They applied EEL to a full sagittal section of the mouse brain where 440 genes were measured in a little over two days of imaging, which enabled the study of spatial regions, gradients and borders defined by gene expression. They also applied EEL to the human visual cortex and found that EEL greatly reduced the highly autofluorescent lipofuscin deposits that normally restrict RNA detection by smFISH in human tissue.

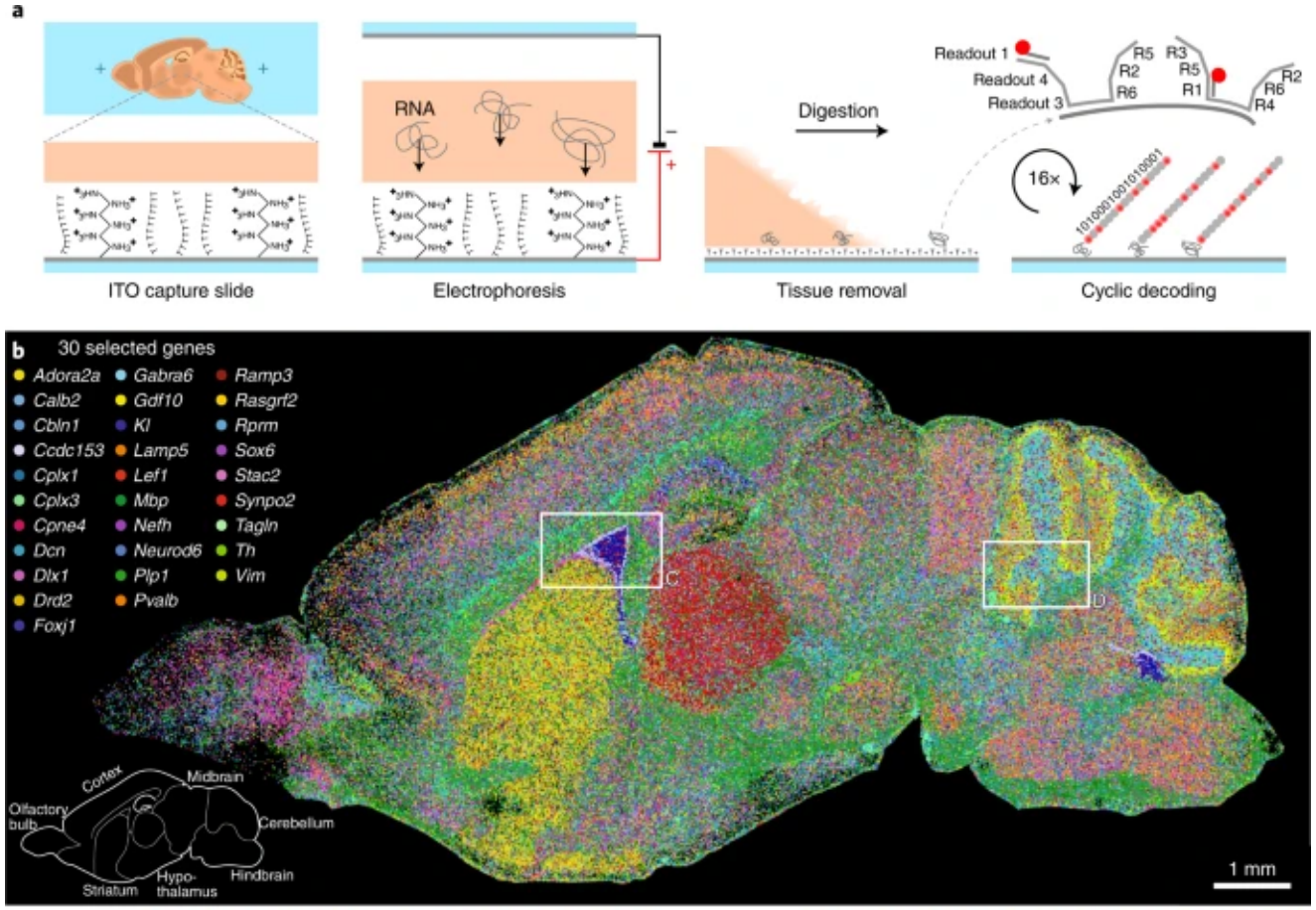
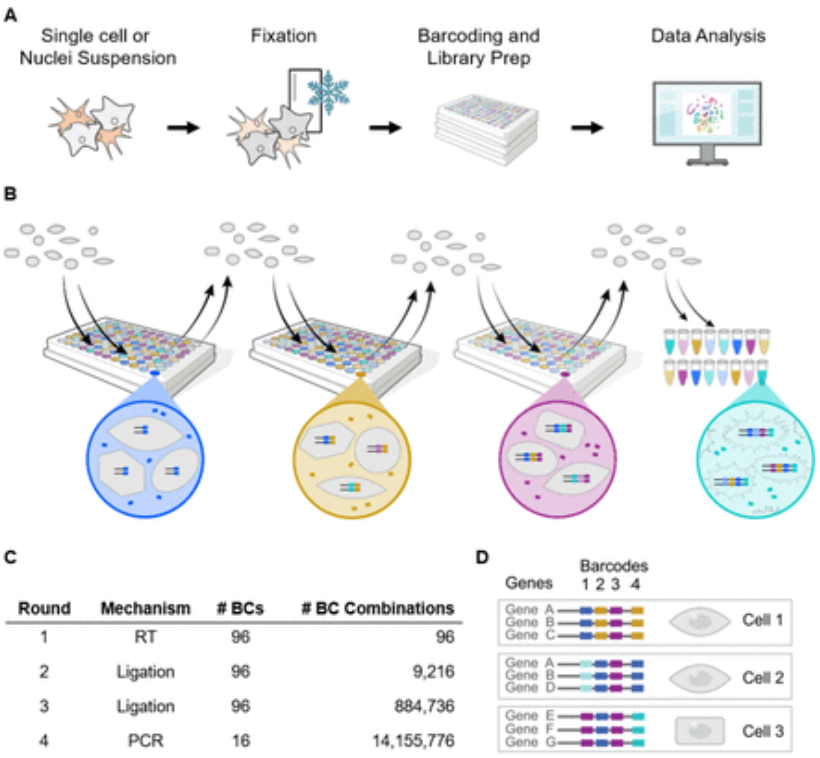


Fig 1.: ***a*** *Schematic illustration of the EEL workflow: RNA transfer by electrophoresis onto the indium tin oxide (ITO) coated slide, tissue removal and cyclic fluorescent decoding.* ***b*** *Results of EEL on a sagittal mouse brain section, where every dot is a single molecule of RNA belonging to one of the 30 selected genes to highlight large anatomical structures. The total experiment contained 440 genes.*

Paper 2

Tran, V *et al.* High sensitivity single cell RNA sequencing with split pool barcoding *bioRxiv, 2022*

Parse Biosciences presents here the version 2 chemistry of their combinatorial barcoding scRNA-seq method. This updated version provides greater sensitivity (2.5x increase transcript & 1.8x increase gene detection) across cells/nuclei from various sample types. The workflow of split pool combinatorial barcoding scRNA-seq is depicted in figure 2. This method makes it possible to scale projects to hundreds of samples and millions of cells, overcoming limitations of previous droplet based technologies. Moreover, no specialized lab equipment is needed.



Round	Mechanism	# BCs	# BC Combinations
1	RT	96	96
2	Ligation	96	9,216
3	Ligation	96	884,736
4	PCR	16	14,155,776

*Fig 2.: **A** Single cell or nuclei suspension needs to be fixed and can then be stored until proceeding to barcoding and further steps of the workflow. **B** For barcoding, the fixed cells or nuclei are distributed in a 96-well plate containing the barcodes. During the split-pool barcoding process the cells are several times pooled and redistributed to generate uniquely labeled transcripts according to cell of origin. Then, cells are lysed and barcoded transcripts undergo library preparation. **C** With each round of split-pool barcoding, the total number of barcode combinations grows exponentially. The Evercode WT Mega configuration generates over fourteen million barcode combinations, enough to uniquely label one million cells or nuclei with very low doublet rates. **D** Single cell transcriptomes are constructed by grouping transcripts containing the same four barcode combinations.*

Paper 3

Ma, S *et al.* Molecular and cellular evolution of the primate dorsolateral prefrontal cortex, *Science*, 2022

In this study they assessed over 600,000 single-nucleus transcriptomes from adult human, chimpanzee, macaque, and marmoset dorsolateral prefrontal cortex (dlPFC). On the human samples they performed sn-multiomics (snATACseq + snRNAseq). The granular dlPFC is an evolutionary specialization of primates that is centrally involved in cognition. Its dysfunction has been implicated in the etiology of many neuropsychiatric disorders. While most of the identified cell subtypes were conserved across species, some differences were observed: for example a layer (L) 2-3 intratelencephalic subtype absent in marmosets, an inhibitory neuron subtype exclusive to marmosets, and a microglial subtype detected only in humans.

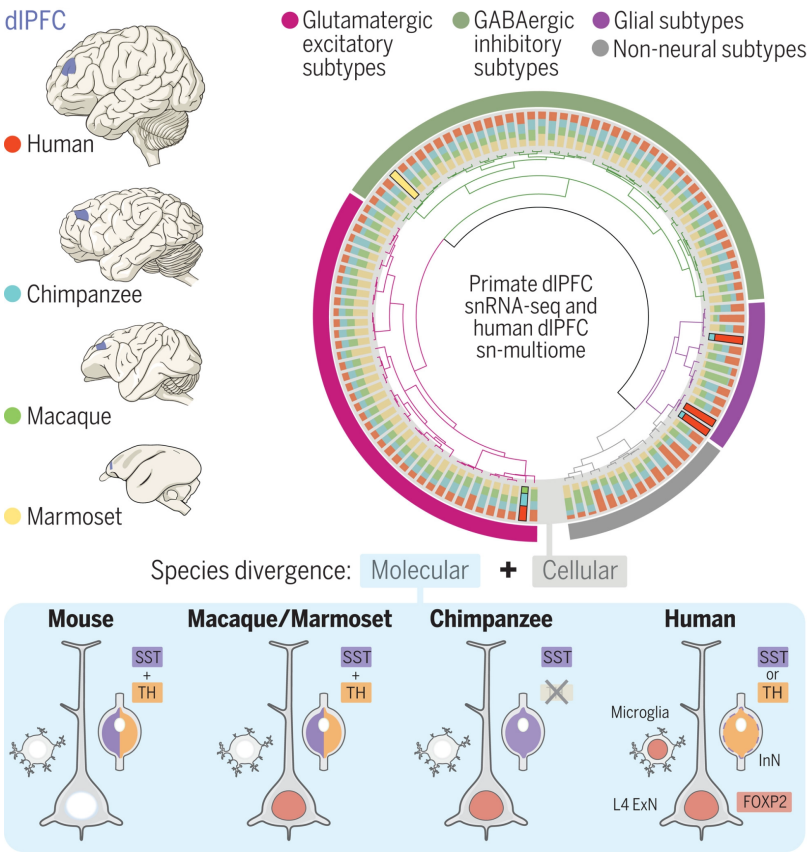


*Fig 3.: **Top left** Homologous regions of the dlPFC dissected for snRNA-seq and sn-multiome analyses. **Top right** 114 hierarchically organized (dendrogram) transcriptomically defined cell subtypes distributed across the four species (bar plots; same color code as in the top left panel), with species-specific variations highlighted. **Bottom** Notable molecular changes across species featured by species-specific FOXP2 expression and the human-specific posttranscriptional switching between SST and TH.*

Comparisons of cell type proportions showed that L2-3 intratelencephalic neurons underwent substantial expansion in humans compared with other species as well as in Catarrhini as compared with marmosets. These results confirm and extend theories of primate cortical expansion. Furthermore, they found that the neuropsychiatric risk gene *FOXP2* exhibited human-specific expression in microglia and primate-specific expression in L4 excitatory neurons. By integrating chromatin accessibility and gene coexpression, they identified cis-regulatory elements regulating *FOXP2* expression and constructed *FOXP2* regulatory networks including downstream targets mirroring the cell type- and species-specific *FOXP2* expression patterns.

Next Single Cell Seminar

Date: 28th October 2022, location and speaker to be announced

If you would like to announce anything single cell related, being it job announcement, event, your published paper, technology development etc., please contact us.

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