Single-nucleus RNA sequencing to find transcriptomic diversity in human brain neurons

Blue B. Lake, PhD
Kun Zhang Laboratory
Department of Bioengineering
University of California, San Diego

The Human Brain

100 Billion neurons; 1 Trillion cells; arguably >1,000 cell types

NIH UCSD SCAP-T center:
Generate 10,000 single-cell transcriptomes from the cortex and map to the 3D brain structure.

http://www.scap-t.org/

Why Single Cell?

Number of genes with fold change >3

Cell extraction from adult brain

LCM of cortex sections

Limitations:
1. Post-mortem
2. Primary tissue
3. Difficulty to isolate intact cells
4. Potential for RNA degradation
5. Limited options (e.g. low RNA from LCM)

SNS: Human Brain Neurons

Single Nucleus Sequencing (SNS)

- Enables cellular level analyses on challenging tissue sources
  - e.g. Human postmortem tissue repositories
  - Any plant/animal tissues that are difficult to isolate intact cells

- No cellular cross contamination
  - e.g. Highly adherent oligodendrocytes in neuronal isolates

- Stabilized RNA
  - Nuclear RNA that is not reliant on live/dead state

- Sorting capacity
  - e.g. Neuronal nuclear antigen (NeuN)

- Scalable
  - Cryopreservation of sorted nuclei for deeper sampling

BA8 – Eye Fields
BA10 – Prefrontal cortex
BA17 – Visual cortex
BA21 – Auditory Cortex, Speech
BA22 – Auditory Cortex, Wernicke’s Area
BA41/42 – Auditory Cortex
SmartSeq Modification:
Improved Transcript Coverage using Randomer

Poly-T + Randomer

- Improved transcript coverage
- More consistent cDNA amplification
- More protein coding genes

SmartSeqPlus:
- Improved transcript coverage
- More consistent cDNA amplification
- More protein coding genes

SmartSeq Modification:
Improved Transcript Coverage using Randomer

Poly-UT + Randomer

- RNA Antisense
- lincRNA
- Protein-coding

SmartSeqPlus:
- No increase in Ribosomal reads
- Unaffected expression values

SmartSeq Modification:
Improved Sampling using Small Chips and Poly-dddC

- Nuclei freezing for increased sampling depth
- Small C1 Chips to capture smaller nuclei
- Recovery of RNA from smaller nuclei
- Consistent gene expression values


SNS: Human Brain Neurons

- Fluidigm C1 Chip
- BA8 – Eye Fields
- BA10 – Prefrontal cortex
- BA17 – Visual cortex
- BA21 – Auditory Cortex, Speech
- BA22 – Auditory Cortex, Wernicke’s Area
- BA41/42 – Auditory Cortex

Smaller C1 Capture Sites to Minimize Doublets

SNS: Human Brain Neurons

- Fluidigm C1 Chip
- BA8 – Eye Fields
- BA10 – Prefrontal cortex
- BA17 – Visual cortex
- BA21 – Auditory Cortex, Speech
- BA22 – Auditory Cortex, Wernicke’s Area
- BA41/42 – Auditory Cortex
Nuclei vs Whole Cell

**ERCC Spike-In R Values**

**Mapping Statistics**

**Nuclei Show More Intronic Reads**

Mouse Cortical Nuclei

Cortical Cells

Hippocampal Cells

Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq

- Nuclei expression values correlate highly with intact cells
- Intronic sequences accurately predict gene expression differences

Nuclei vs Whole Cell

**Mouse Nuclei**

- Neurons
- Glia

SNS: Human Brain Neurons

- Neurons
- Neurons + Glia

*Zhang et al. J Neurosci 34, 11929-11947 (2014)

**Human Nuclei**

- Neurons
- Neurons + Glia

Nuclei can accurately predict cell types

**Clustering & Classification**

\[ n = 905 \]

\[ n = 2178 \]

In = Inhibitory Neurons

Ex = Excitatory Neurons

**Clustering & Classification**

\[ n = 905 \]
Two Broad Neuronal Types

*Fraction of Positive:

Inhibitory Neurons (e.g., Interneurons)  Excitatory Neurons (e.g., Pyramidal Neurons)


Interneuron Sub-Types

Excitatory Neuron Sub-Types

BA Specific Expression

BA Specific Subtypes

Layer 4 granular neuron subtypes
Summary

Single-nucleus transcriptome sequencing (SNS) is a scalable approach for difficult-to-dissociate tissues

SNS on the adult human brain reveals expected cortical subtypes

SNS reveals heterogeneity between cortical regions:
- Subtype proportions
- Intra-Subtype transcriptome variations

Acknowledgements

TSRI
- Jerold Chun
- Swami Kaeser
- Yuen Yung
- Julian Wong
- Diane Bushman

UCSD CBC/CMM
- Wei Wang
- Rui Ji
- Andre Wildberg

Illumina
- Jian-Bing Fan
- Neeraj Salathia
- Raahees Vijayaraghavan
- Richard Shen
- Mostafa Ronaghi
- Fiona Kaper

CJ Support:
- Fluidigm
- Manisha Ray
- Bob Jones

Funding support
NIMH SCAP U01 (Roadmap)