

Elucidating epilepsy genetics with advanced sequencing techniques

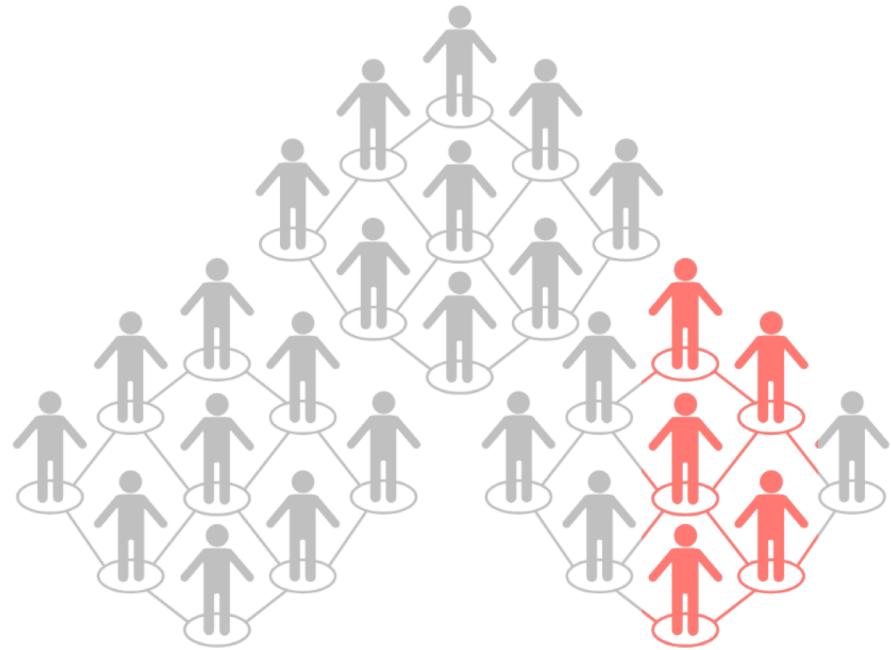
Tracy A. Bedrosian, Ph.D.
Assistant Professor of Pediatrics



Learning Objectives

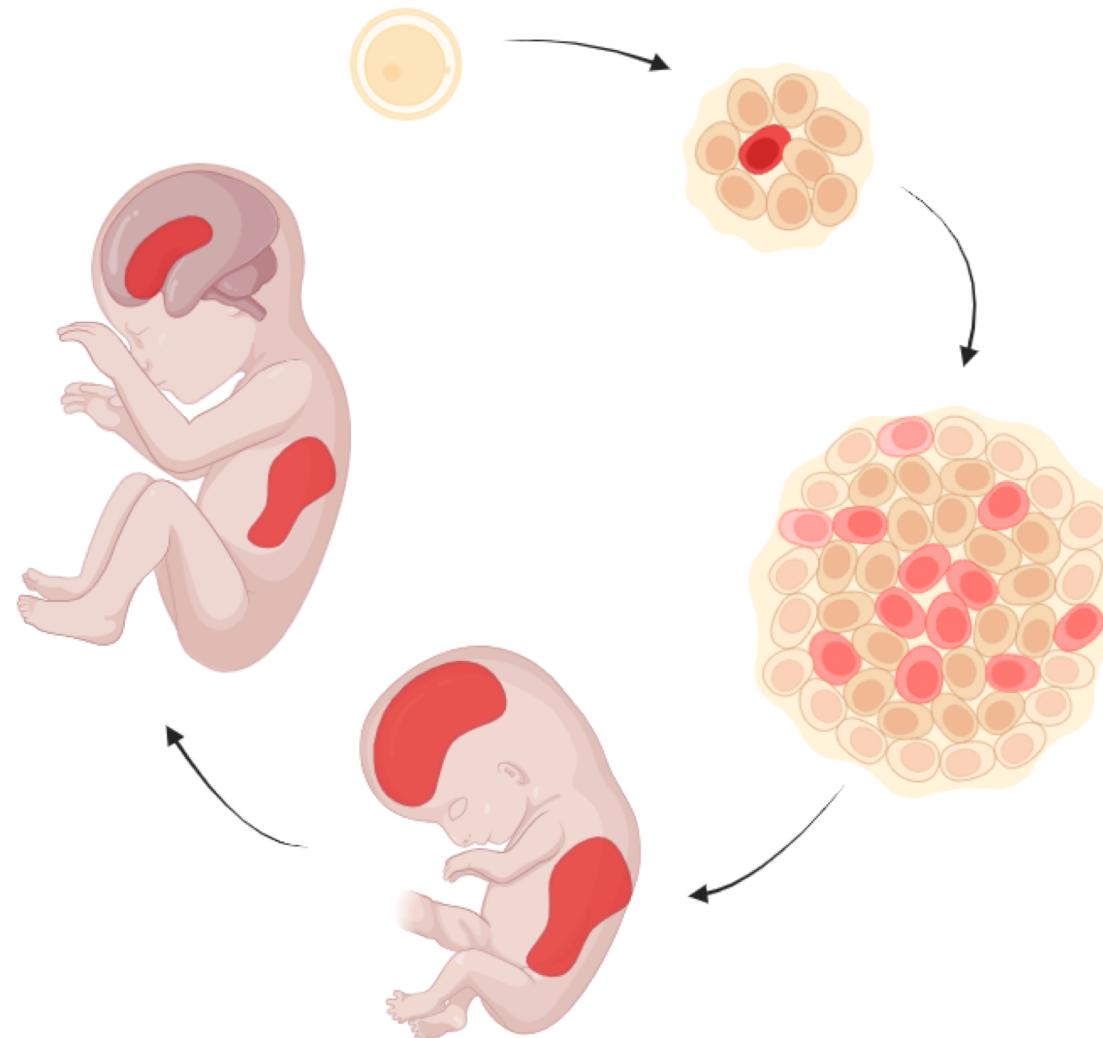
- Understand the role of somatic mosaicism in epilepsy
- Understand the utility of single-cell sequencing for studying mosaicism
- Describe various sequencing / analytical approaches to link somatic genotype and transcriptome at the single-cell level

Drug-resistant epilepsy in children

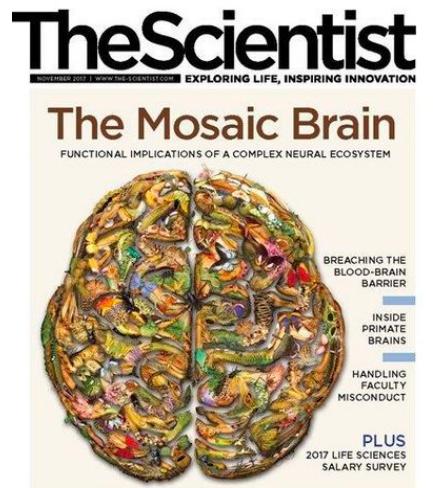
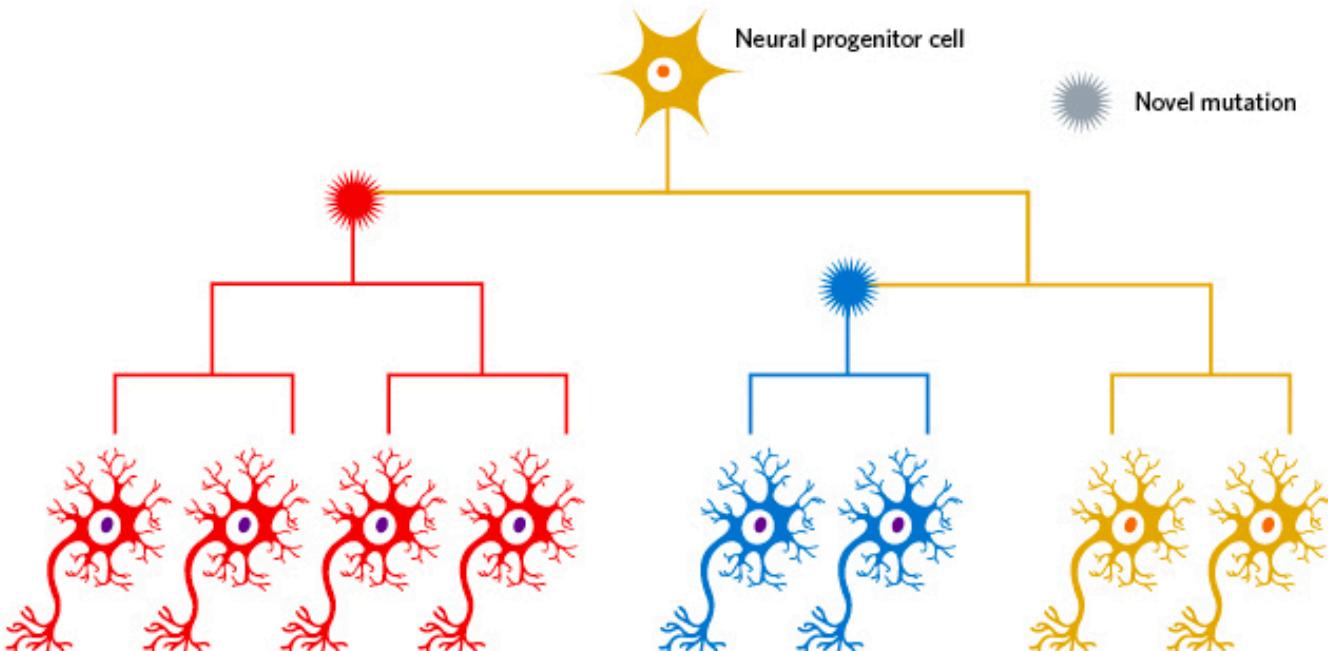


- Surgery is a second-line treatment option
 - Effectiveness is variable
- Uncontrolled seizures are dangerous
- A critical need for effective therapeutic options

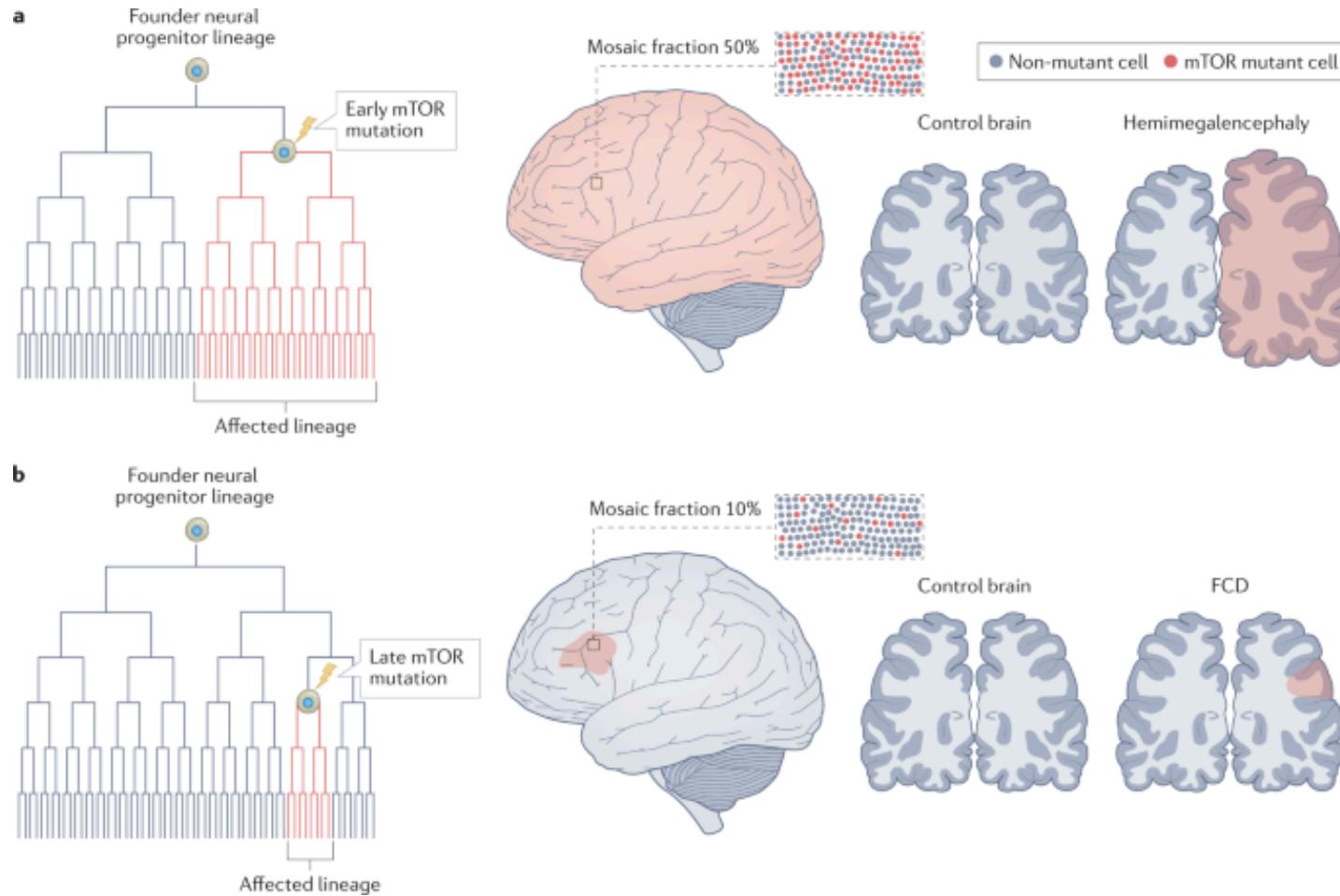
Somatic mosaicism and epilepsy



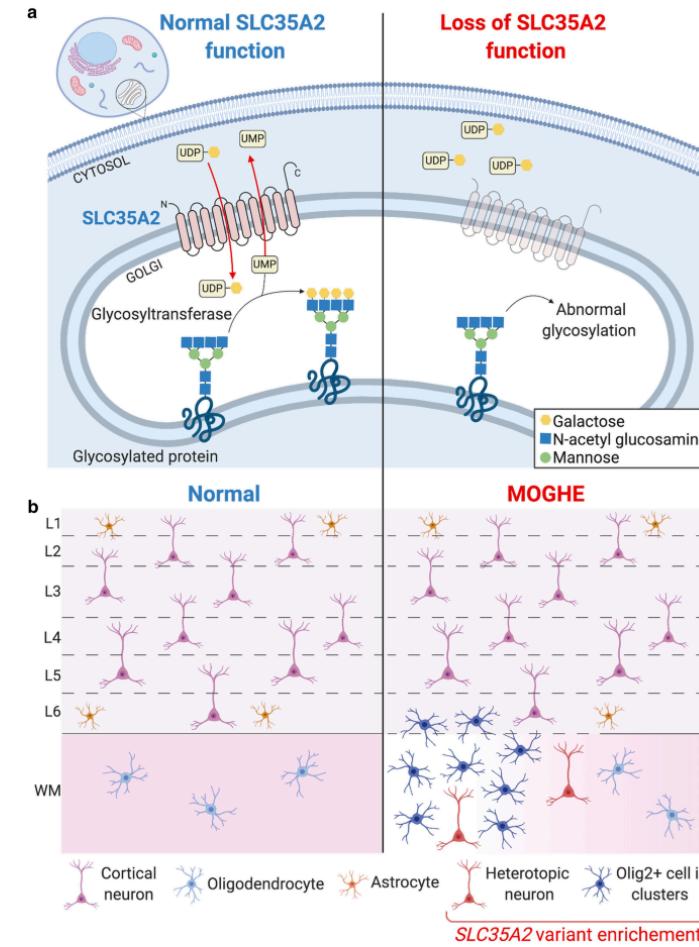
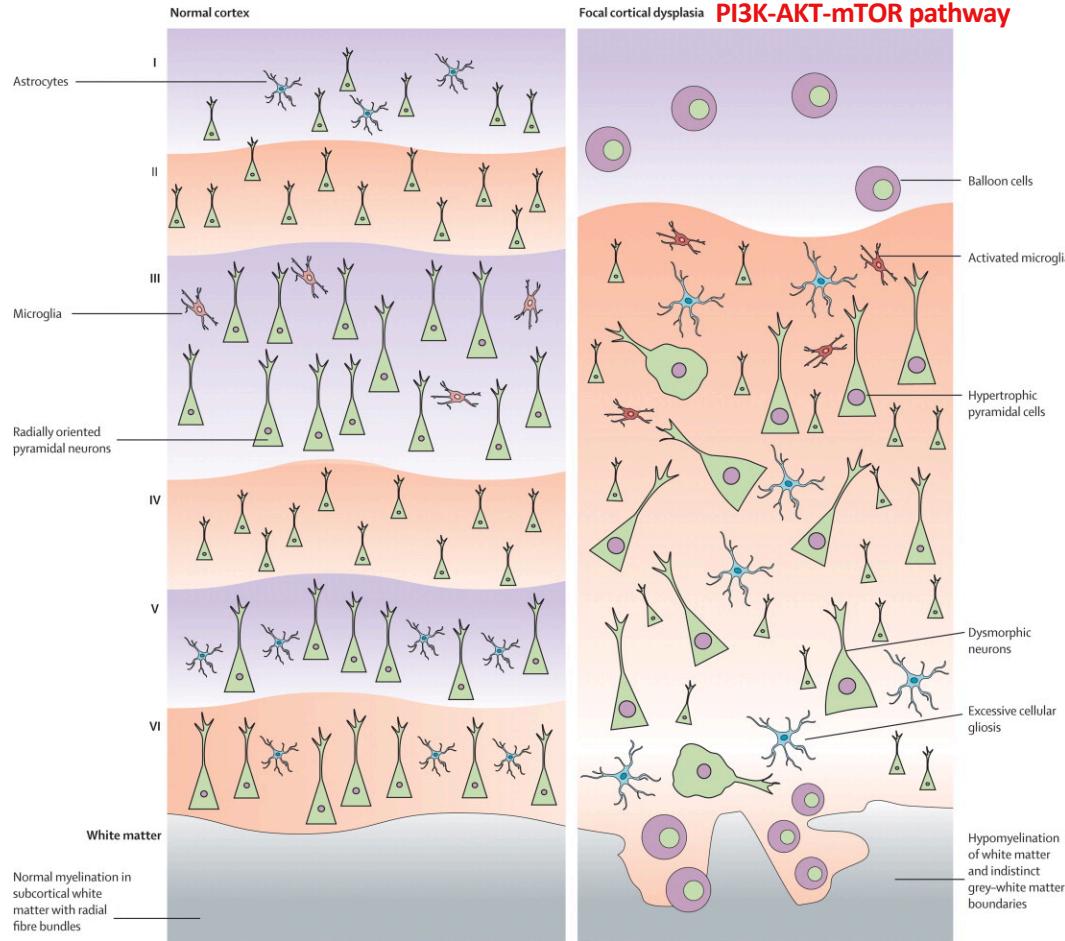
Somatic mosaicism and epilepsy



Somatic variants can affect particular cell lineages



Somatic mosaicism informs pathology (and vice versa)



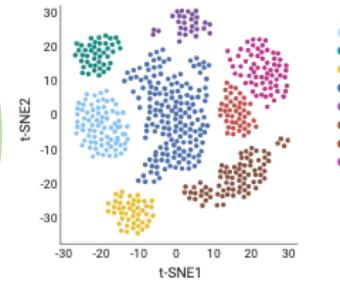
Research objectives



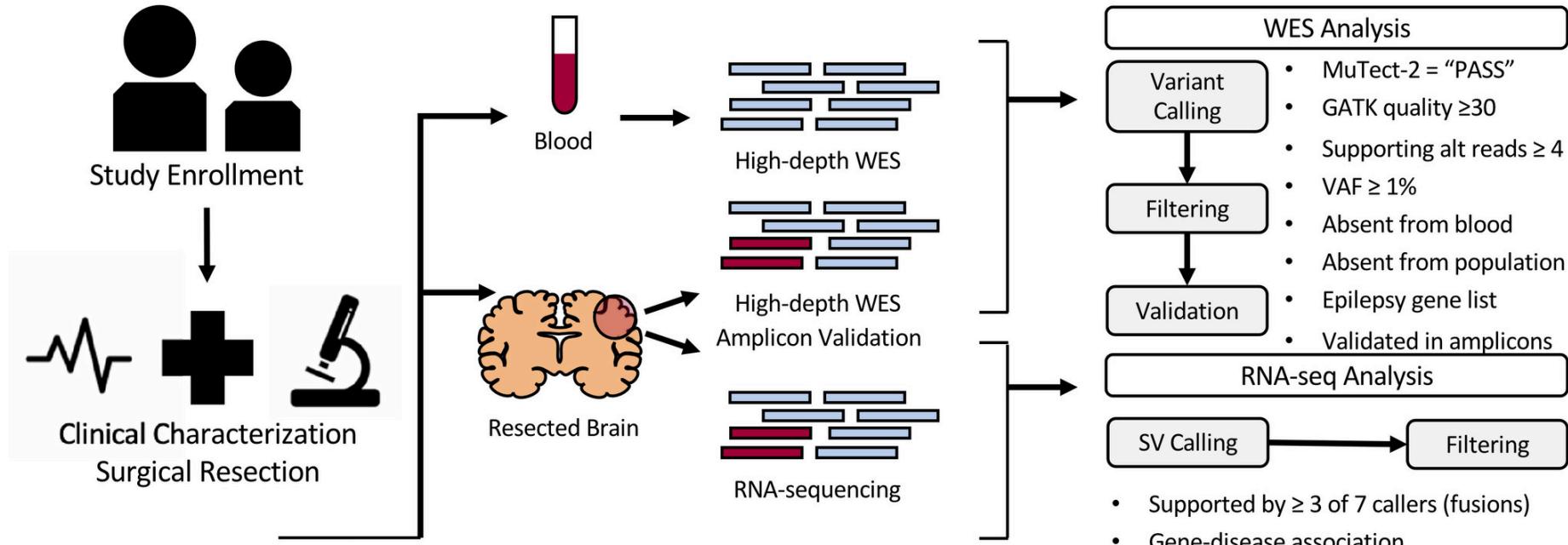
Develop precision
treatment
approaches

Discover genetic
causes of focal
epilepsy

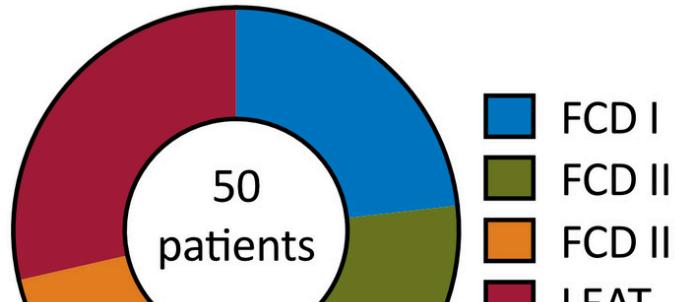
Understand
molecular &
functional effects



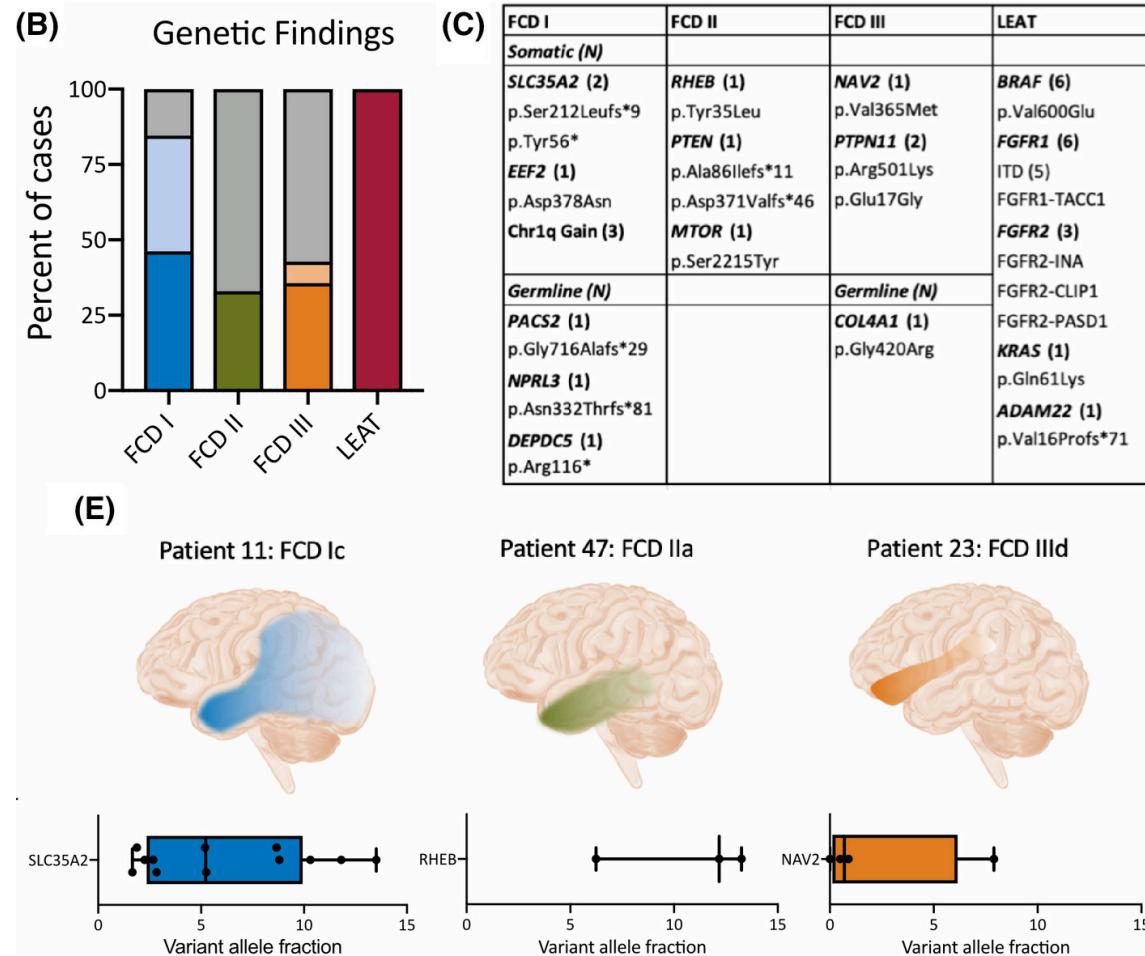
Our approach to gene discovery



Detecting somatic mosaicism in patients



FCD = focal cortical dysplasia
LEAT = long-term epilepsy-associated tumor

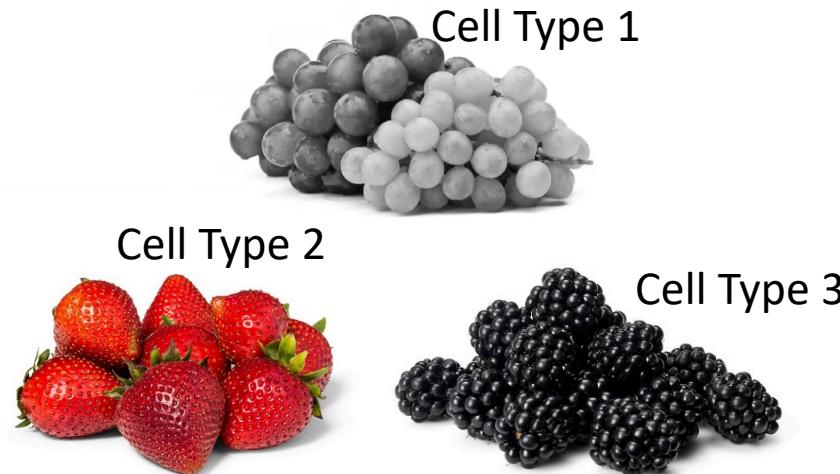


Investigating molecular correlates of mosaicism

Bulk RNA-seq



Single-cell RNA-seq



Single-cell RNA-seq
With Genotype

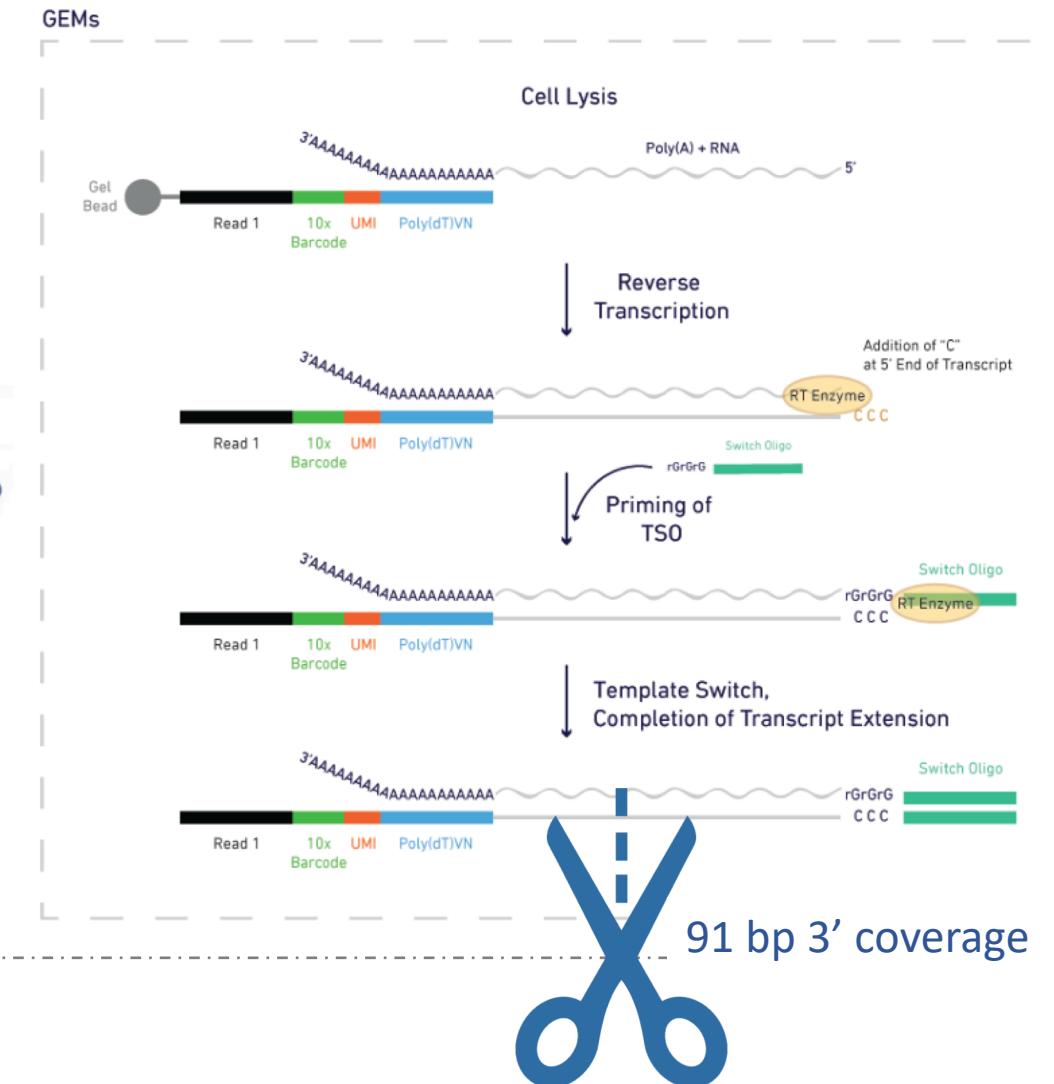
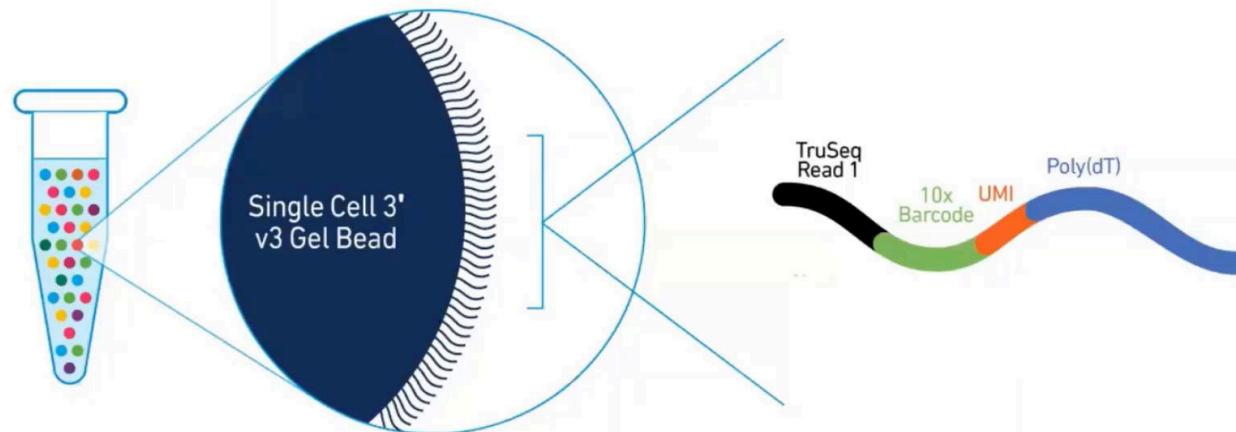
Cell Type 1
Genotype A

Cell Type 1
Genotype B



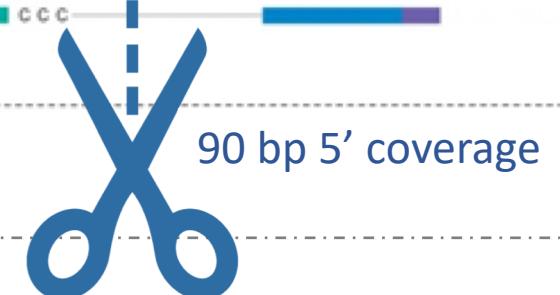
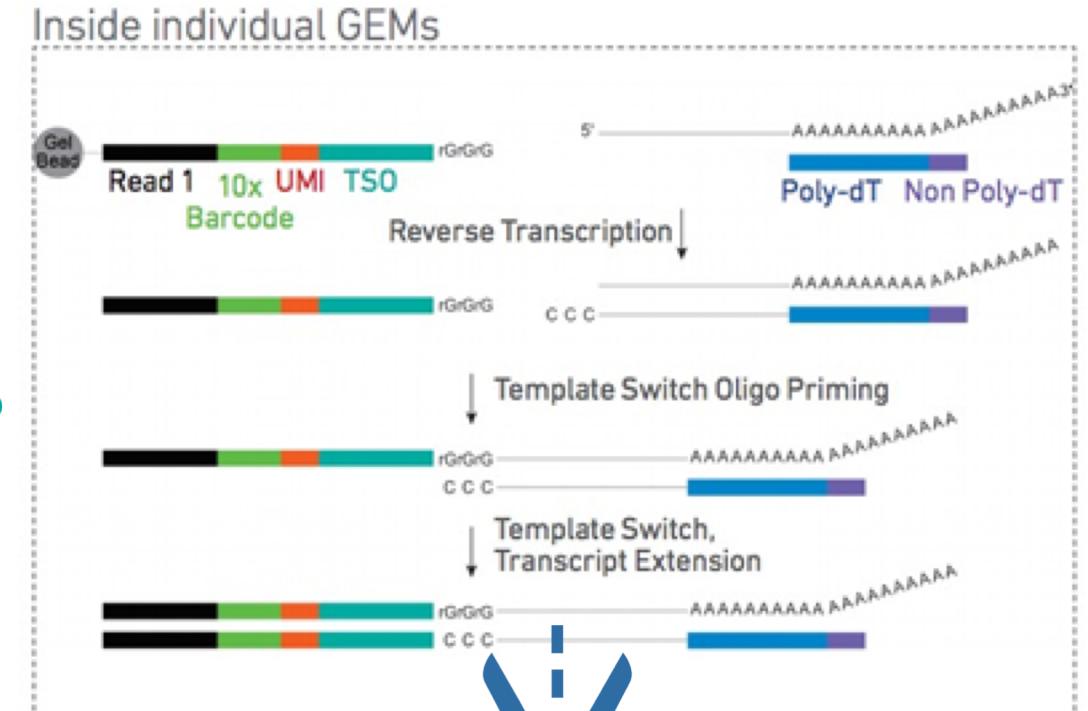
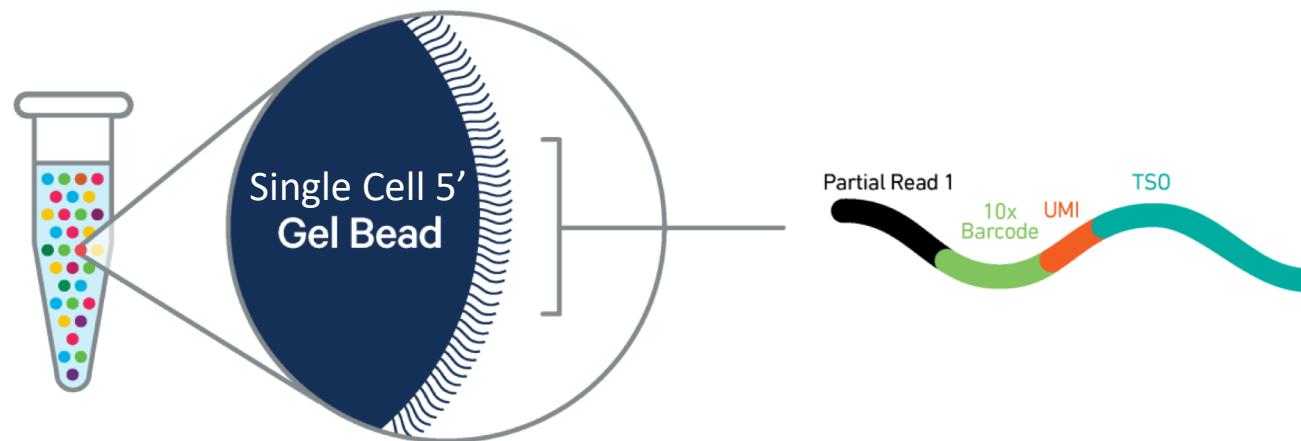
Accessing genotype from transcriptome

10x Genomics 3' Gene Expression



Accessing genotype from transcriptome

10x Genomics 5' Gene Expression



A case study

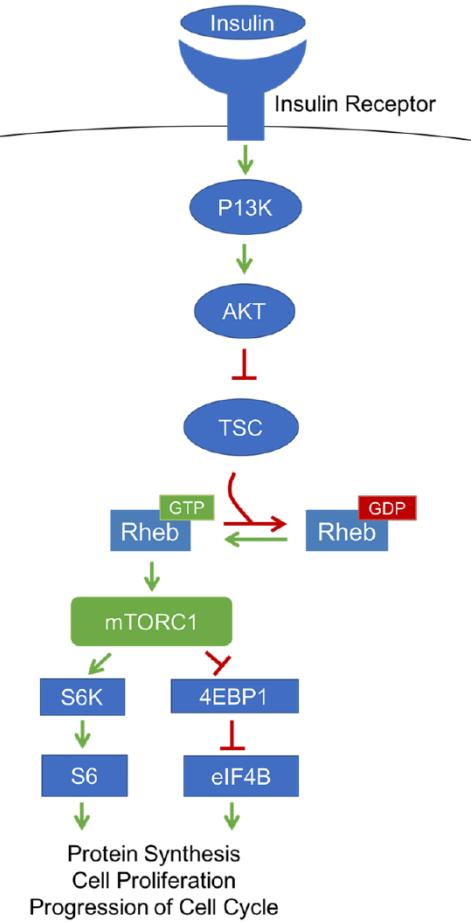
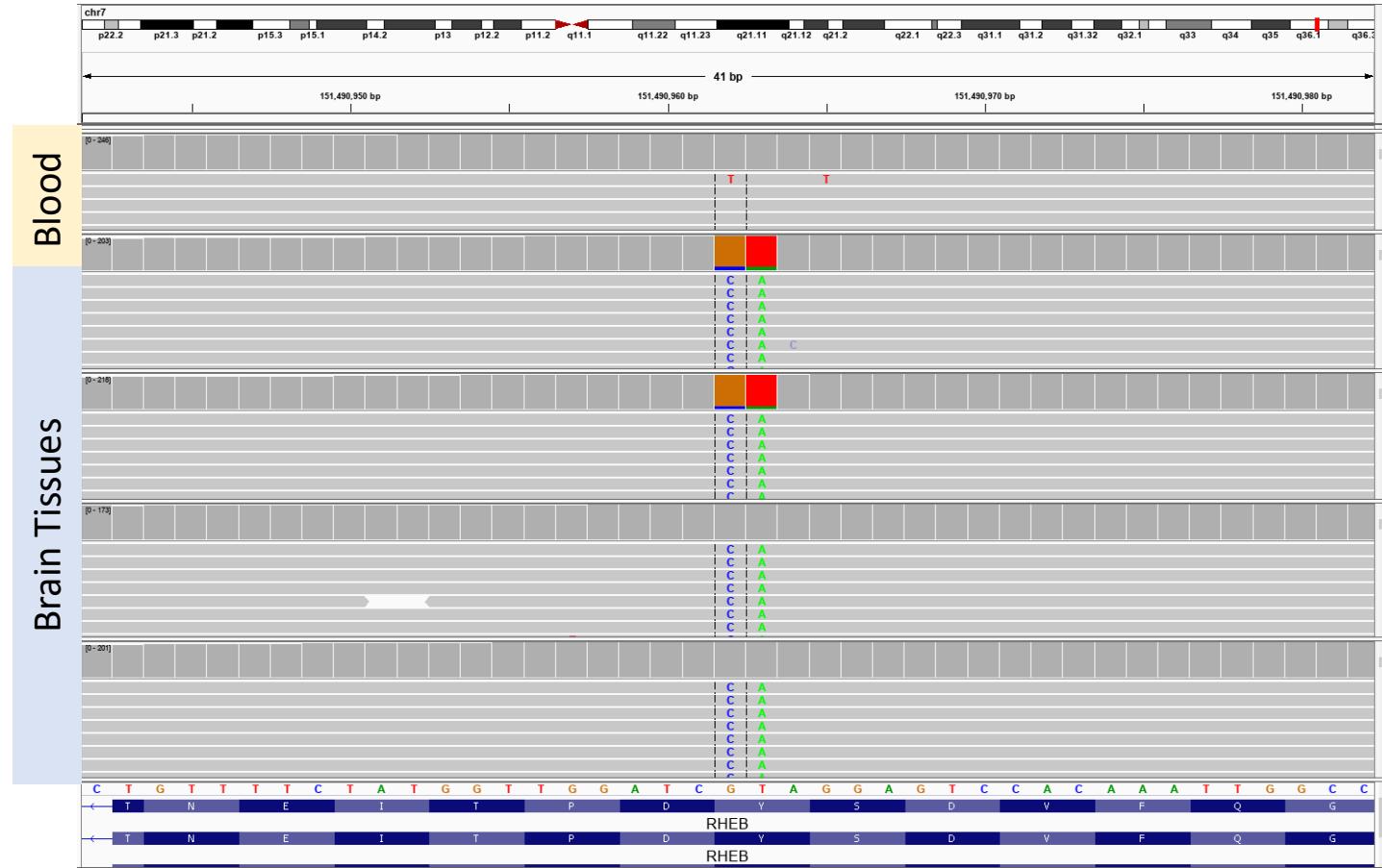


- 6-month old male with a history of infantile spasms, intractable epilepsy
- Left temporal lobe dysplasia
 - Dysmorphic neurons, focal cortical dysplasia type IIa
 - (Suspect activating MTOR pathway variant)

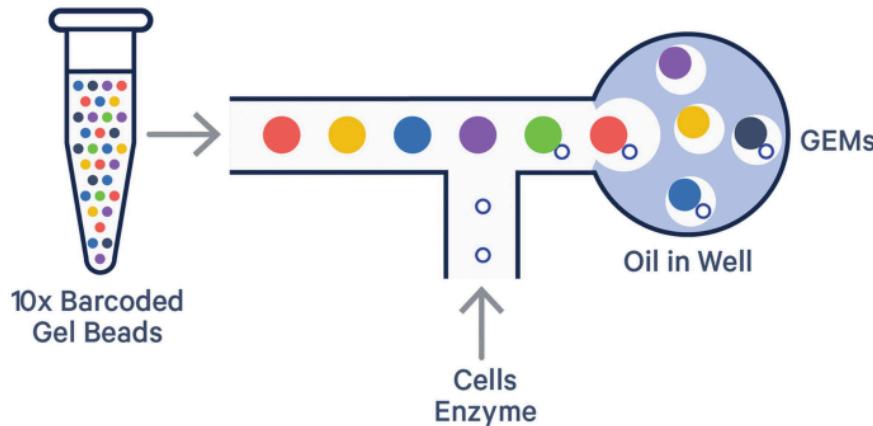
A case study



RHEB p.(Tyr35Leu)



A case study



10XGenomics/
vartrix

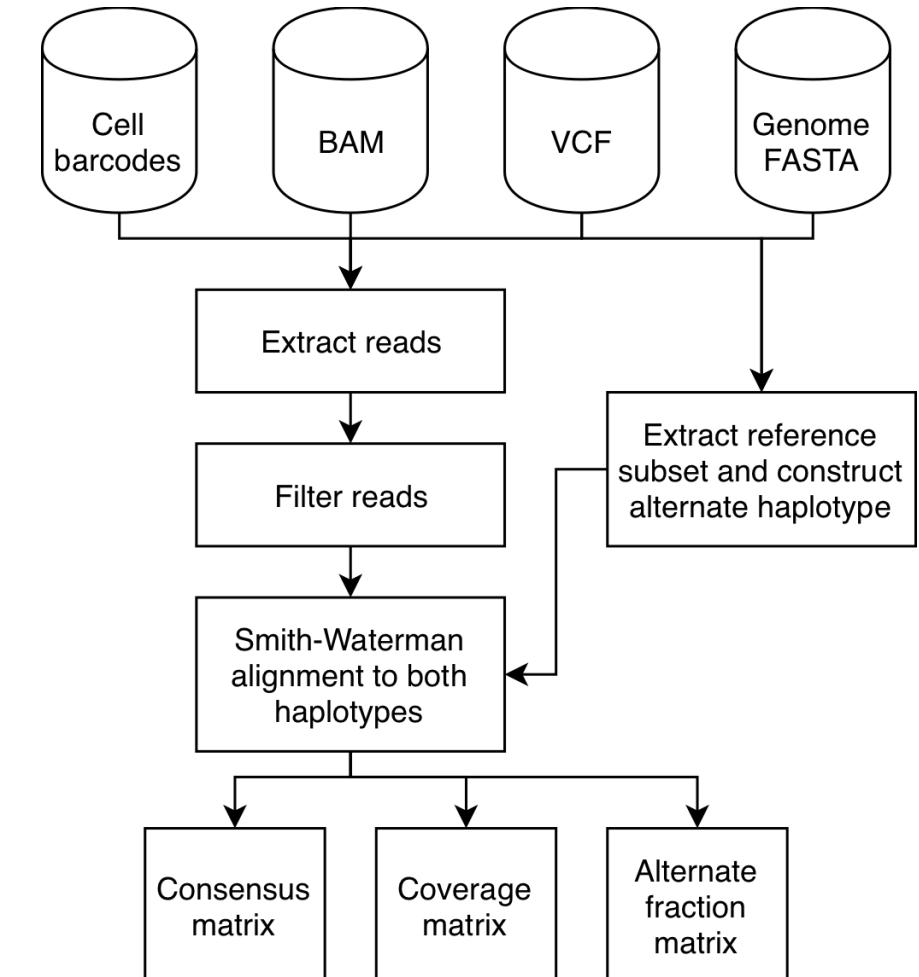
Single-Cell Genotyping Tool

9
Contributors

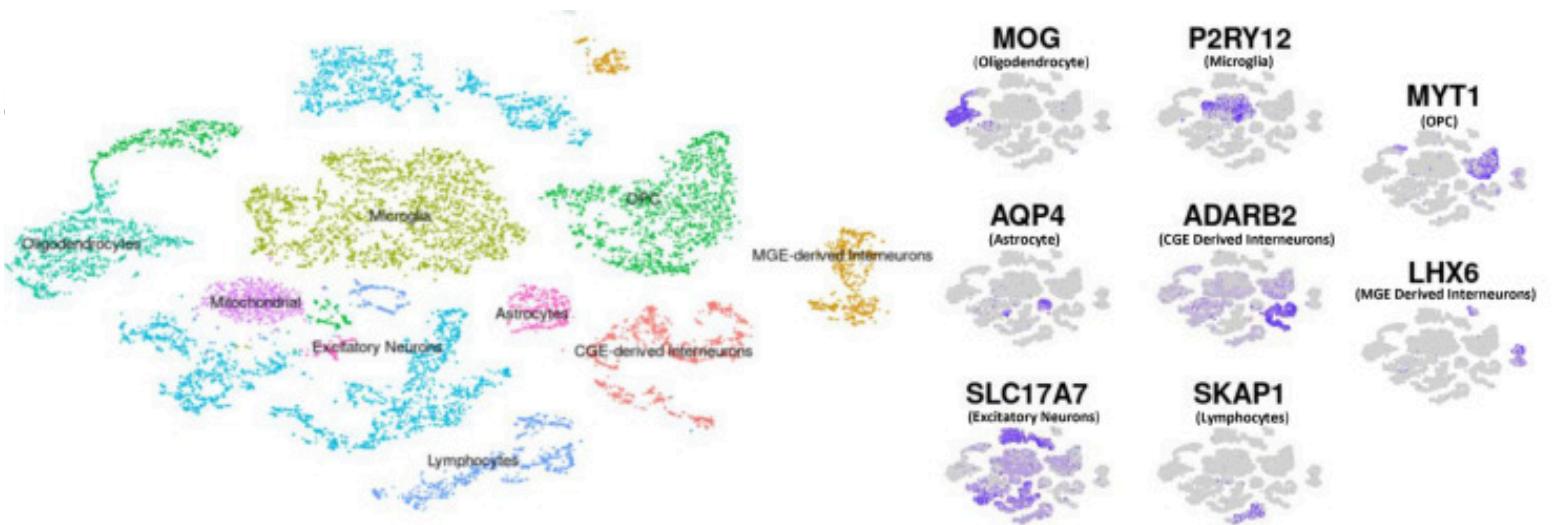
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Issues

163
Stars

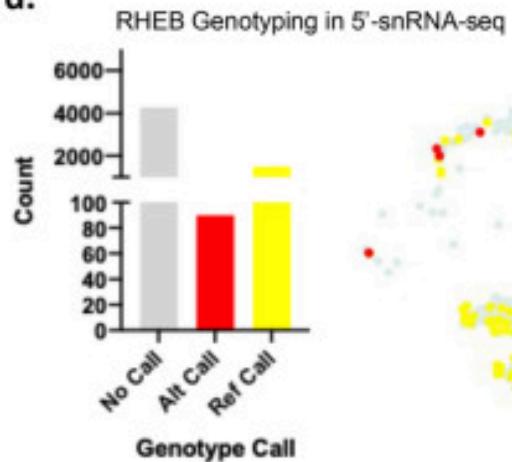
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Forks



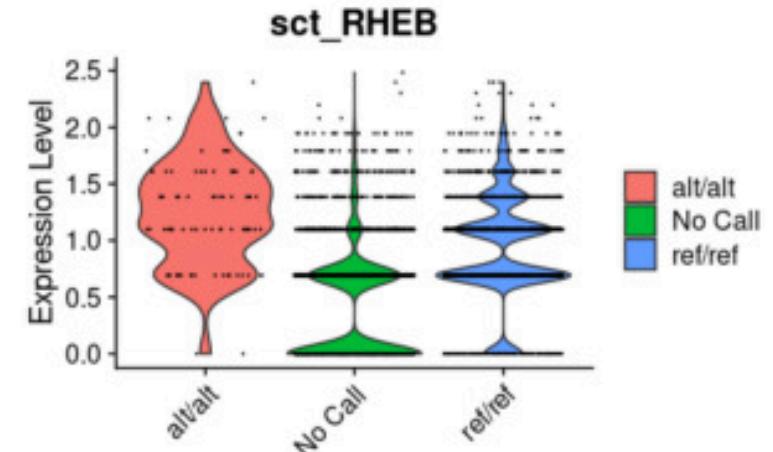
A case study



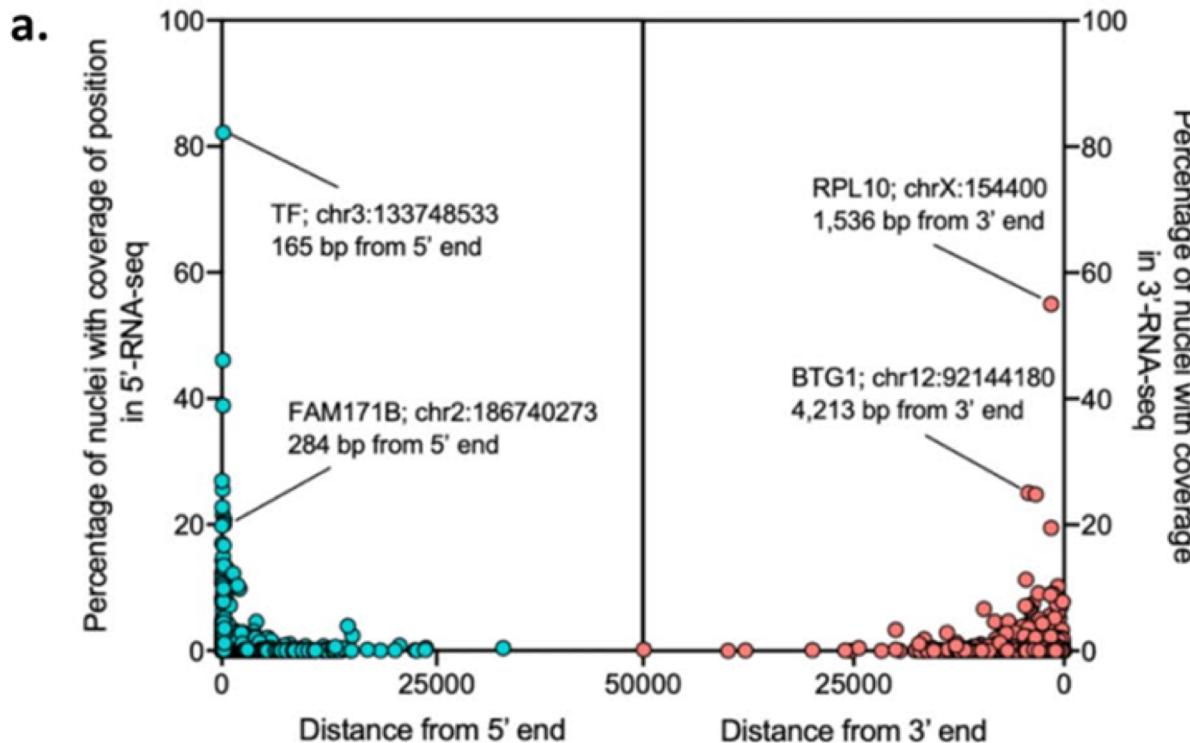
d.



e.



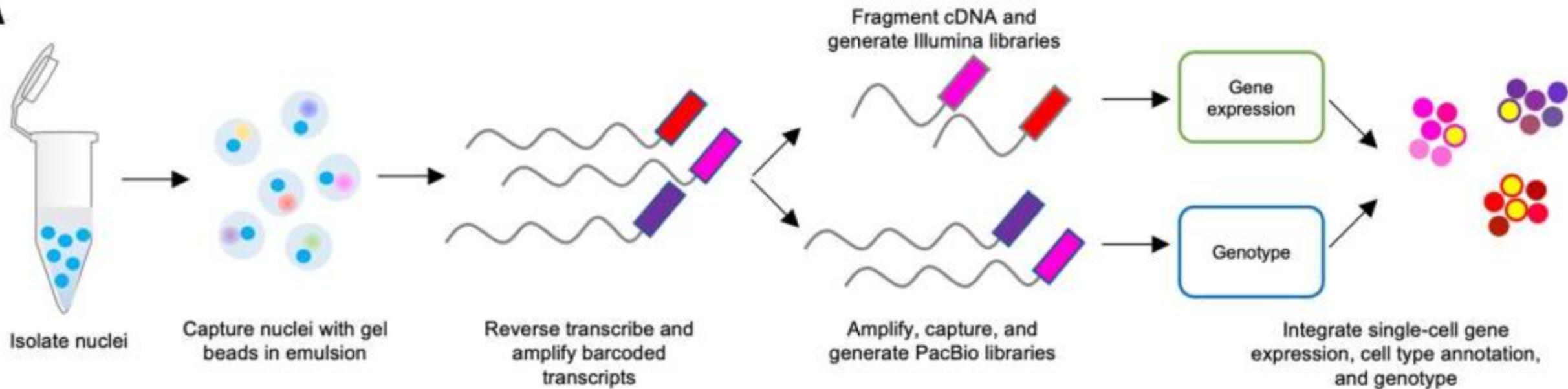
10x data is not *always* perfectly 3' or 5'



Accessing genotype from transcriptome

What about all the other variants far from transcript ends?

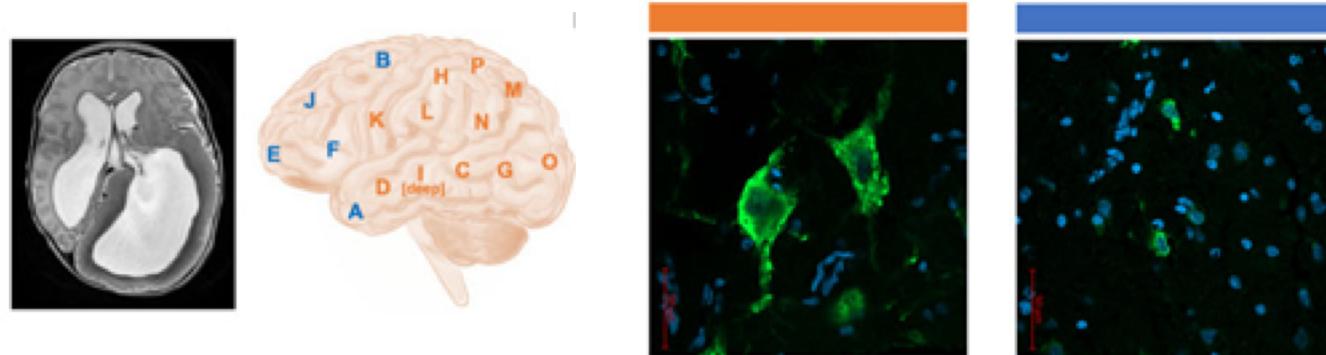
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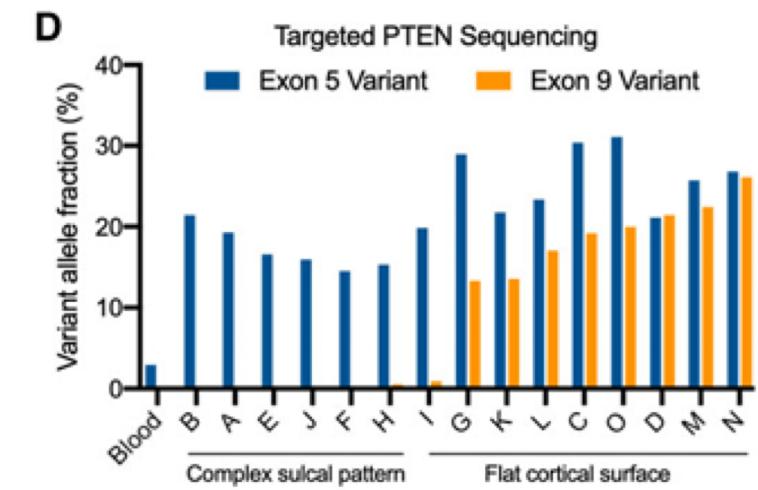
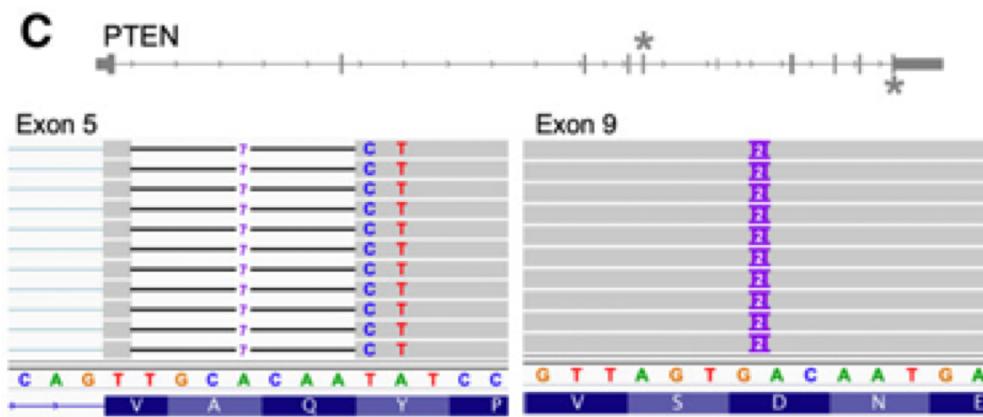
Another case study



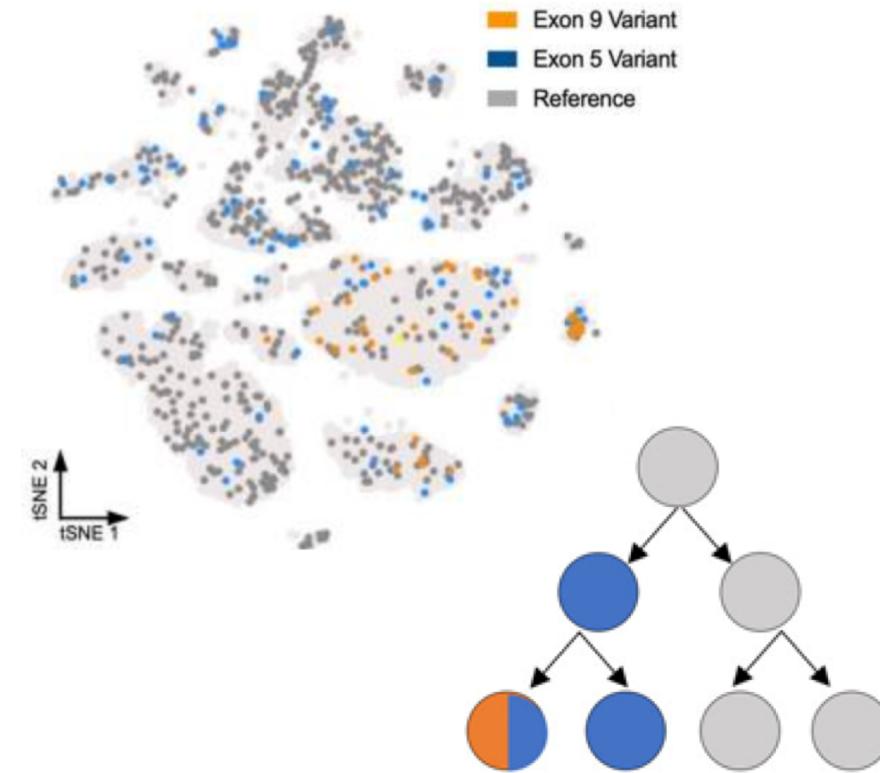
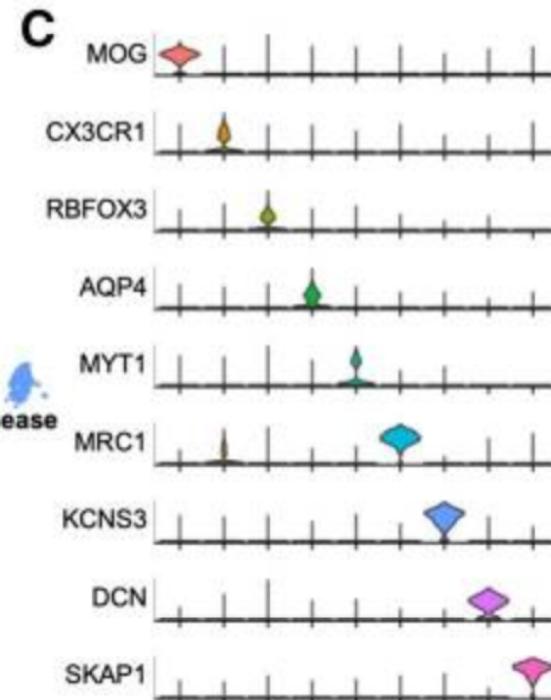
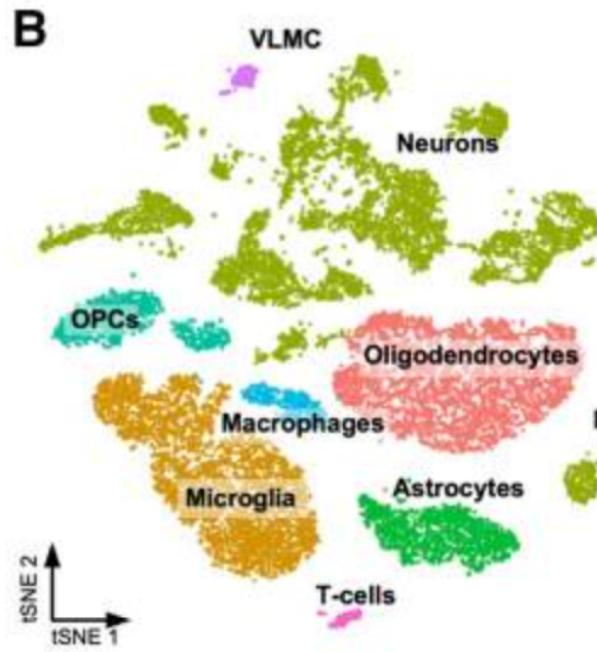
- 1-year old male with a history of intractable epilepsy
- Atypical hemimegalencephaly
 - Anterior → posterior gradient of severity



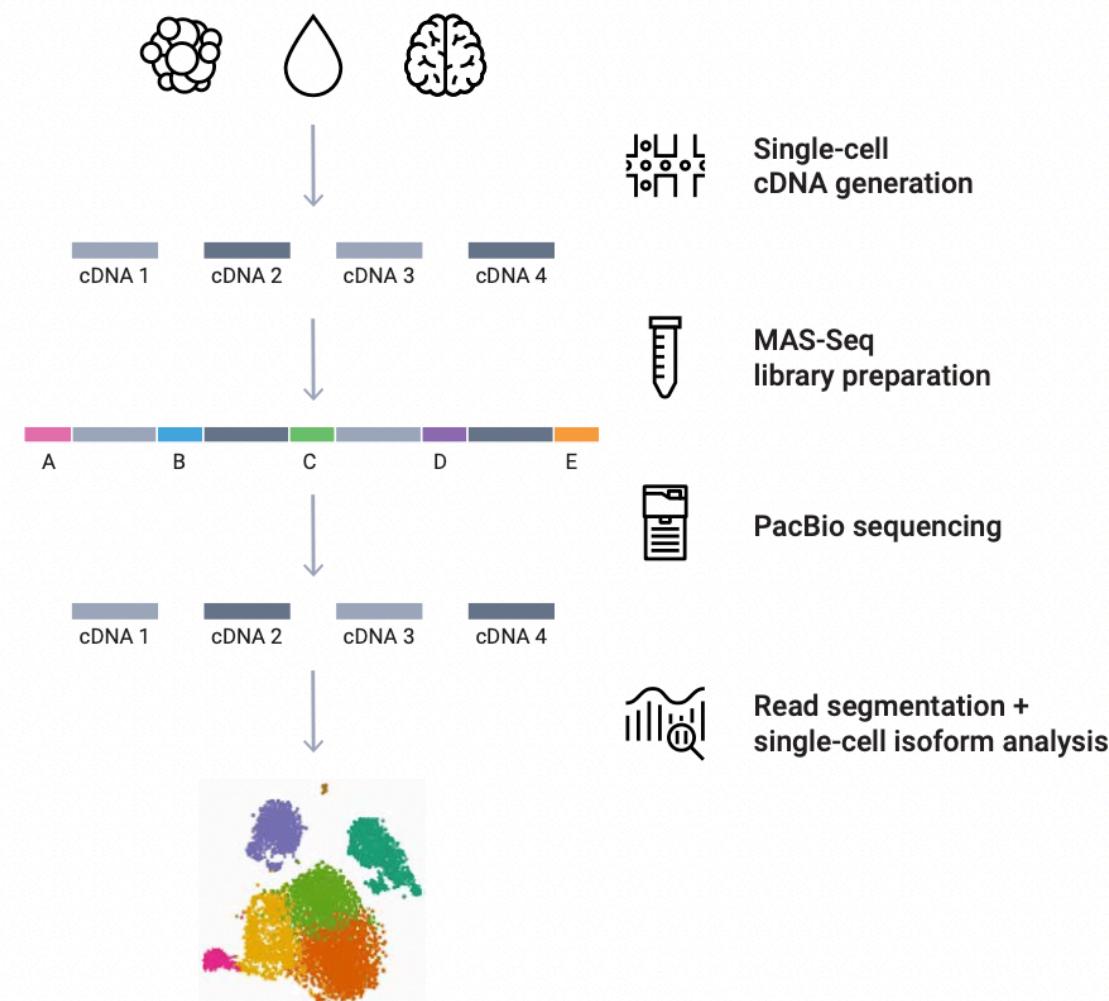
Another case study



Single-cell transcriptomes plus genotyping

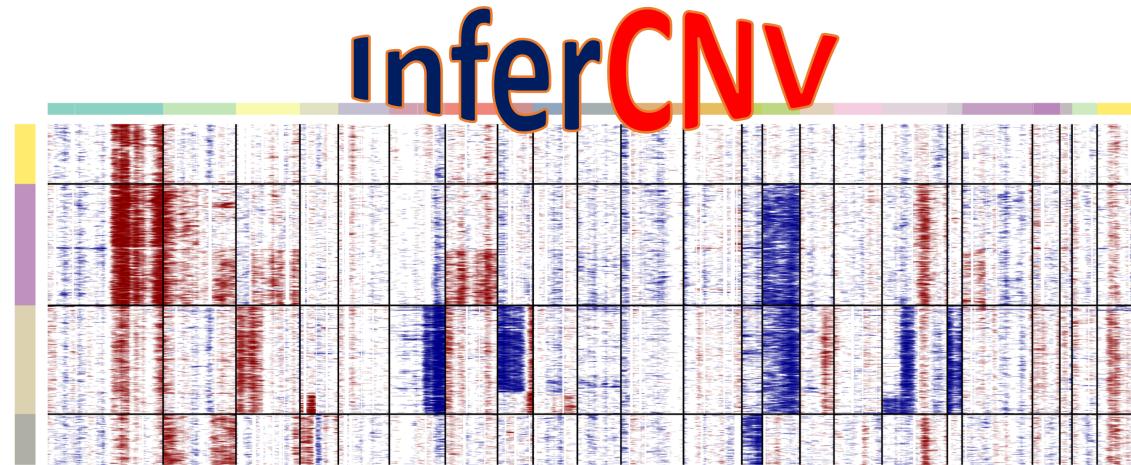


More throughput...MAS-seq!

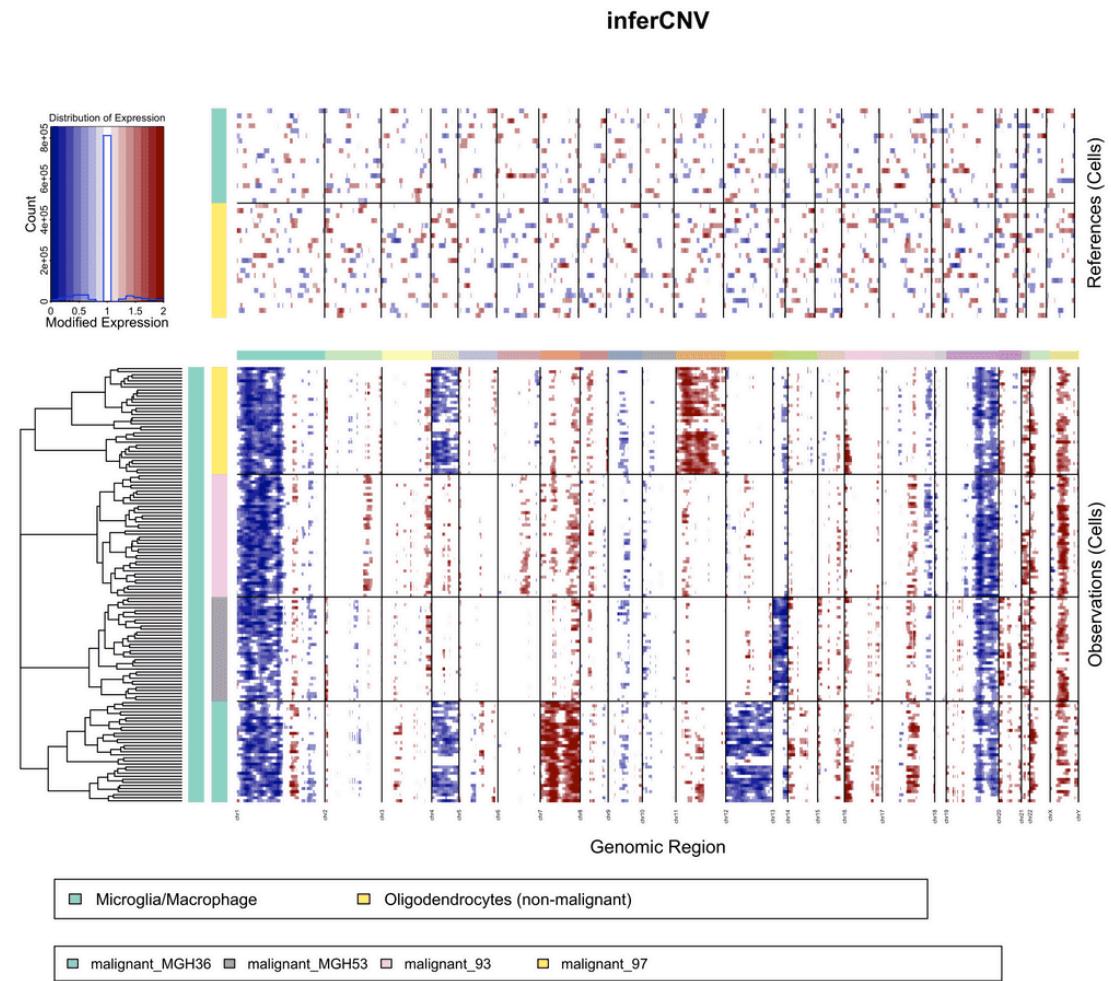


Accessing genotype from transcriptome

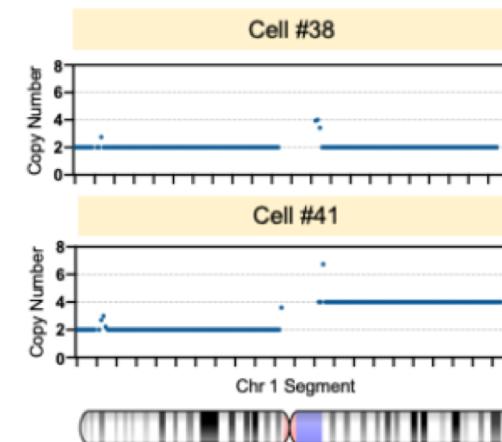
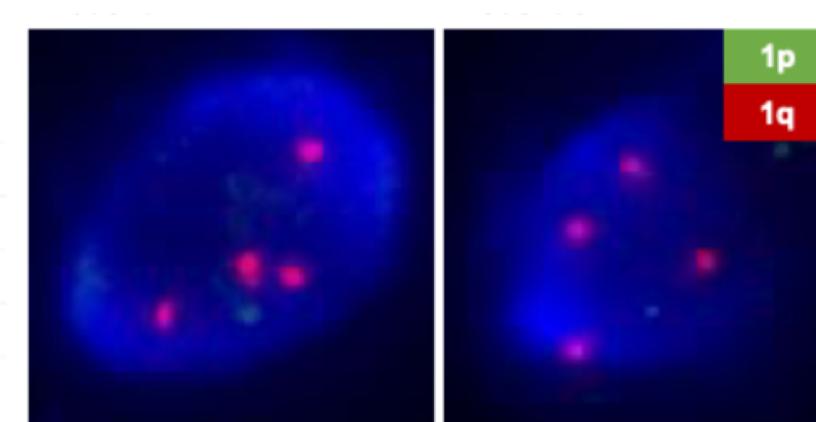
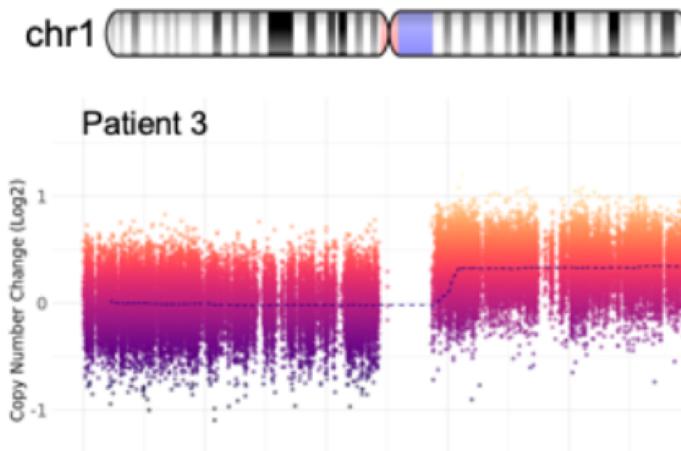
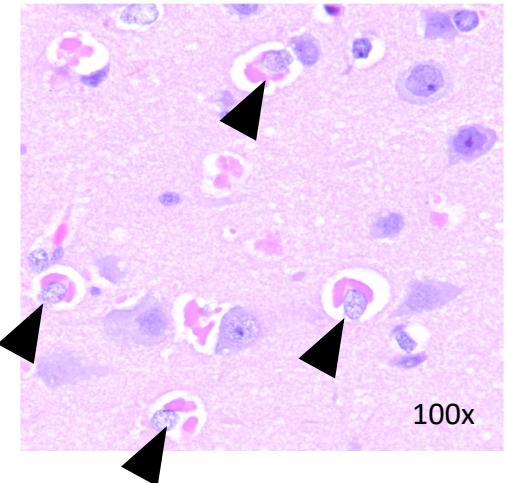
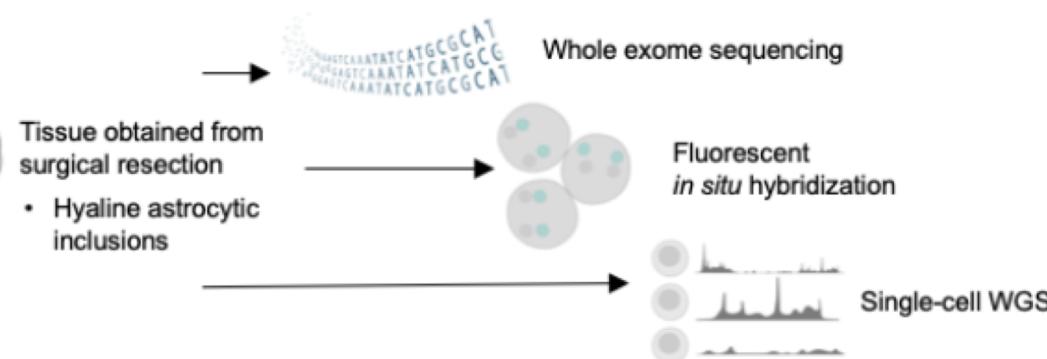
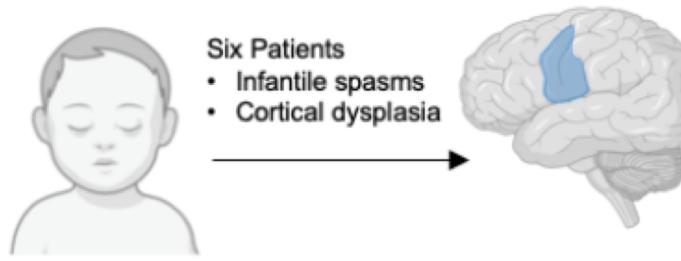
How about copy number variants?



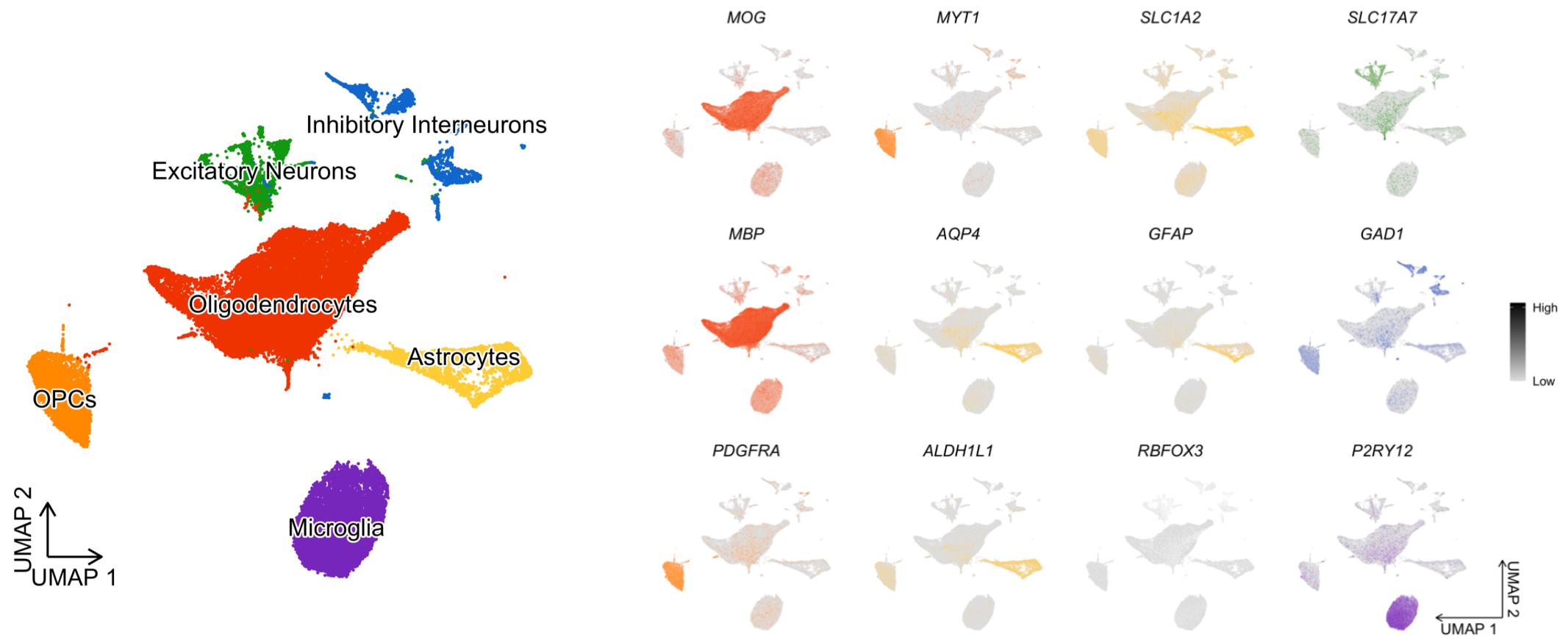
<https://github.com/broadinstitute/infercnv>



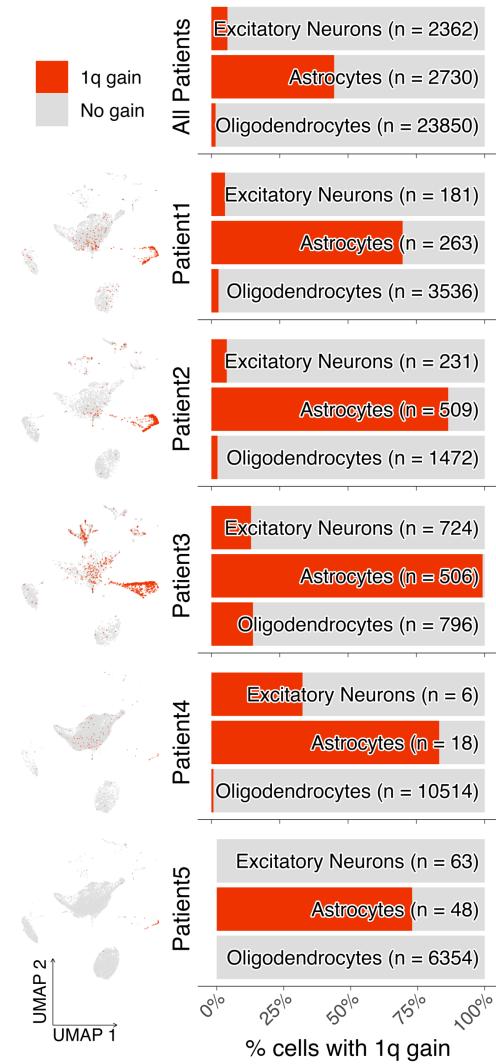
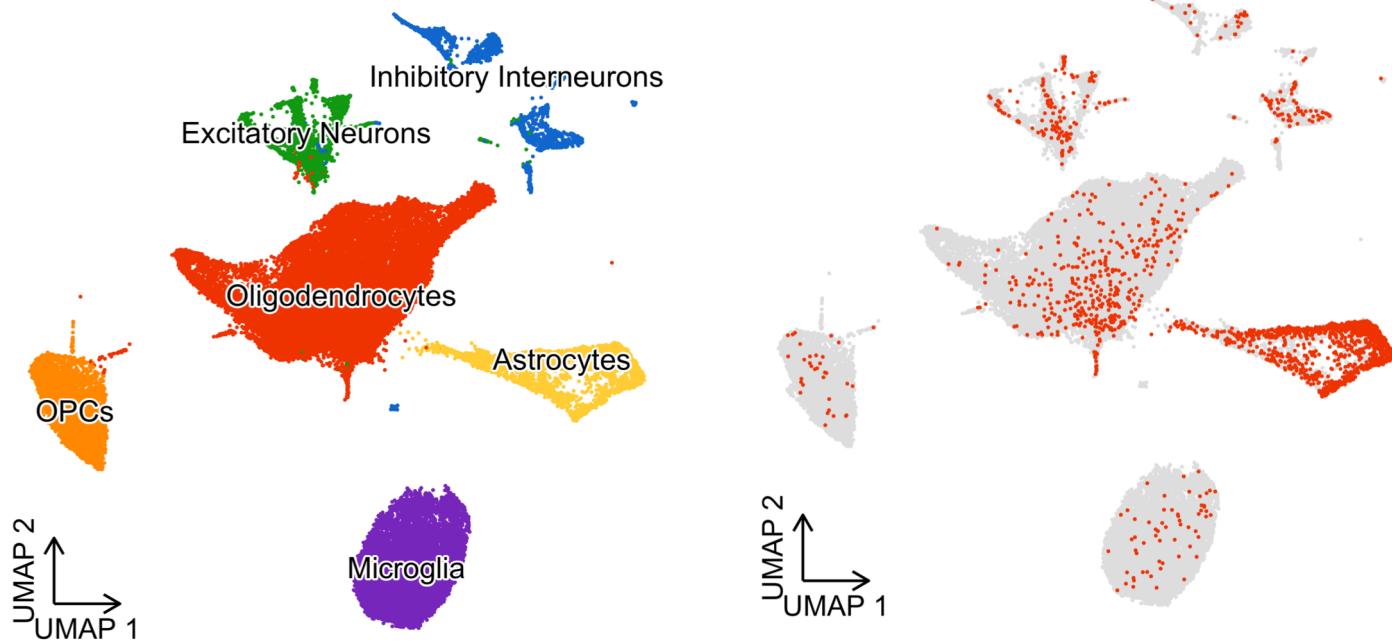
Brain mosaic copy number gain in epilepsy



Single-nuclei RNA-sequencing

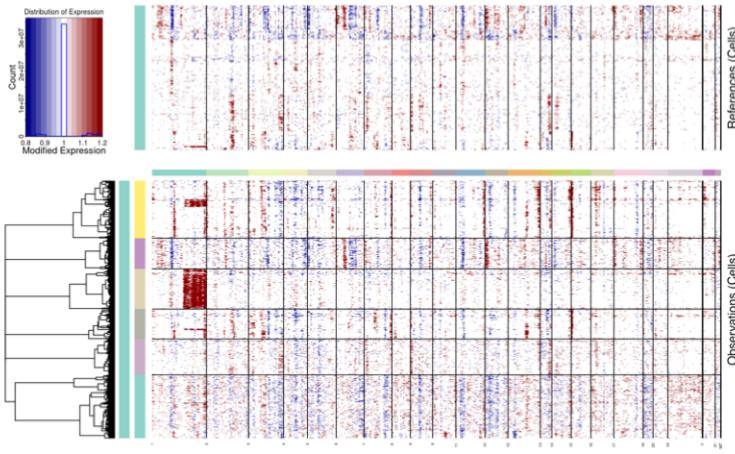


1q gain enriched in astrocytes

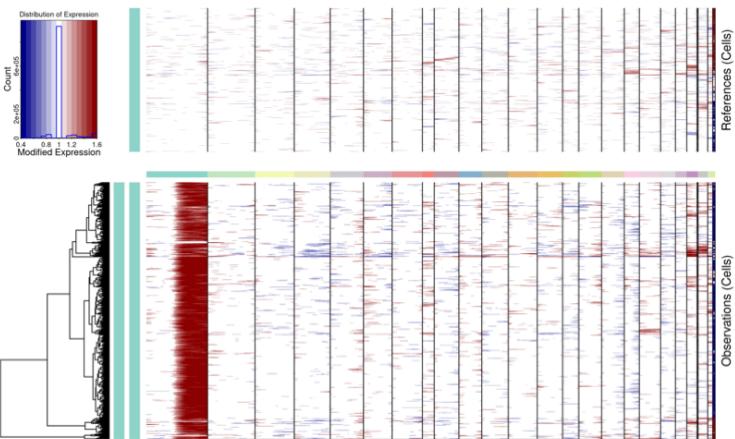


Verifying InferCNV

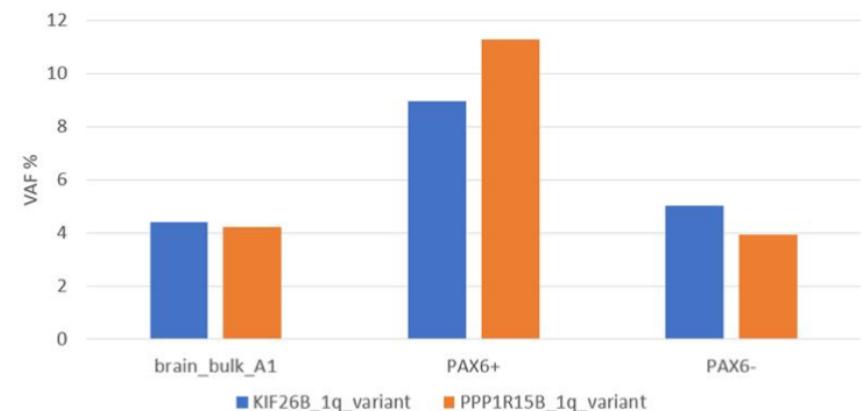
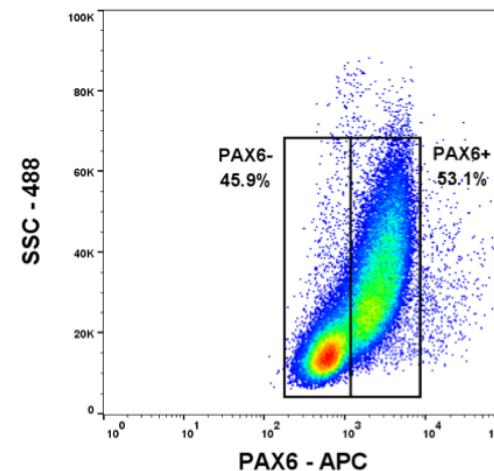
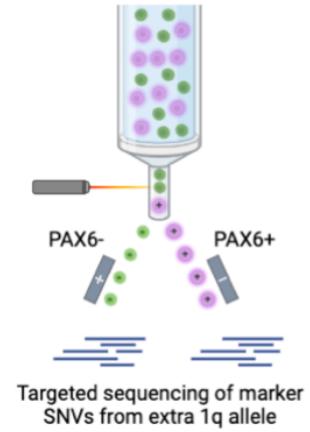
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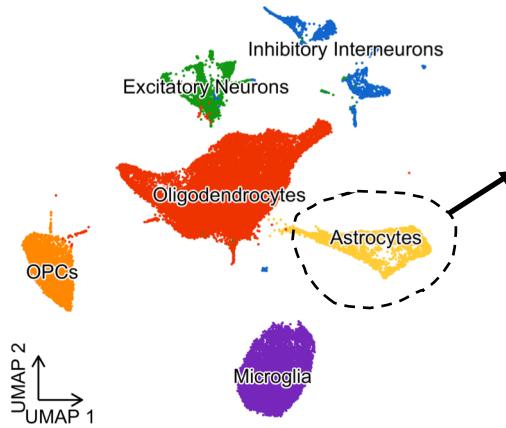
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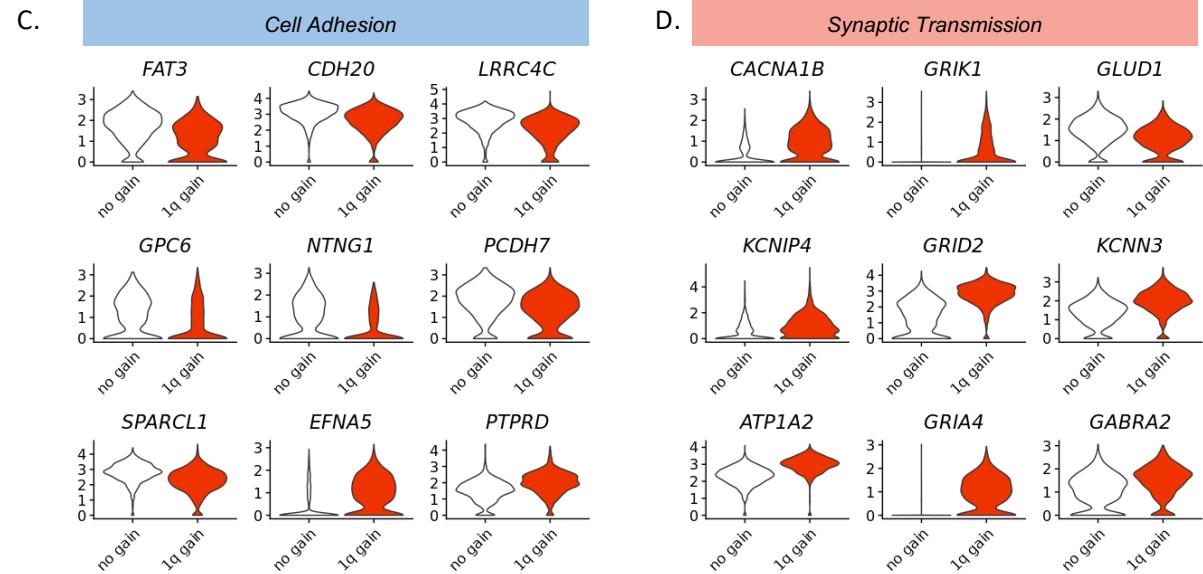
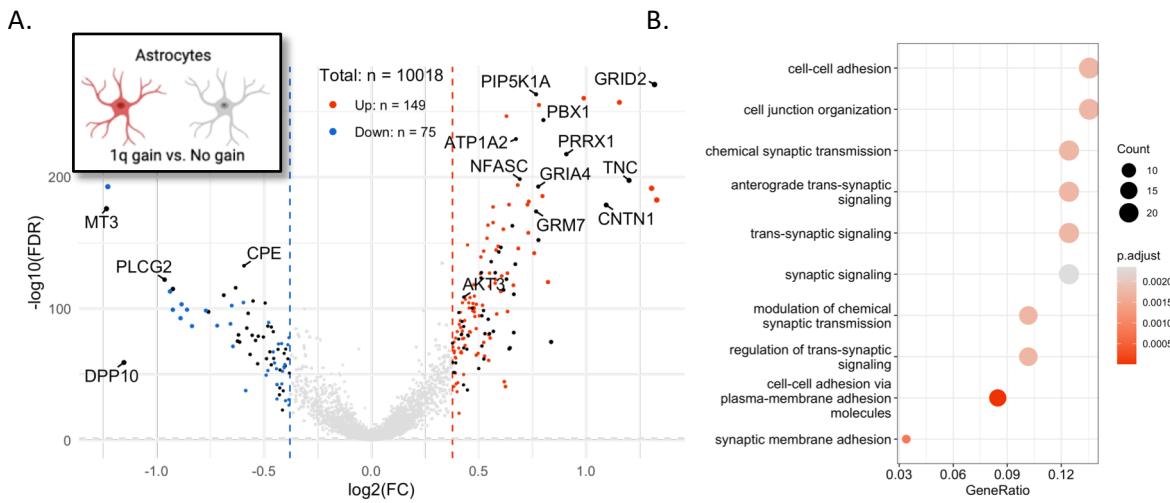
e.



1q gain alters gene expression



Differential expression
MAST



Conclusions

- Somatic mosaicism is an important cause of focal epilepsy
- Single-cell sequencing is a valuable tool for studying signatures of mosaicism that may be lost in bulk RNA-seq
- Various sequencing / analytical approaches can link somatic genotype and transcriptome at the single-cell level
 - Limitations exist; this is an area of active technology development
 - Strong potential to uncover new insights into the molecular consequences of somatic mosaicism

Acknowledgements

Gene Discovery

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NCH Neurology, Neurosurgery, Neuropathology

Single-cell Methods

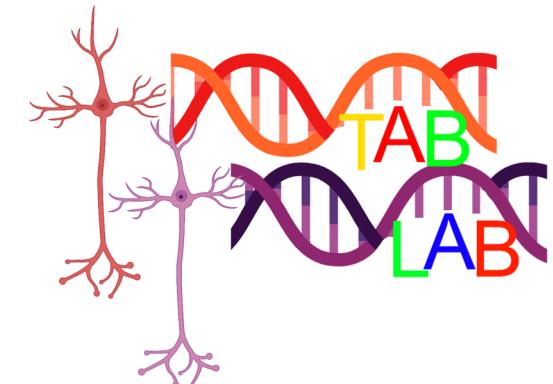
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1q Mosaicism

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Katherine Miller, PhD & Team
Adithe Rivaldi, BS; Noryiyuki Shinagawa, BS
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