

Towards a safe and effective gene therapy for *SLC6A1*

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12/4/25

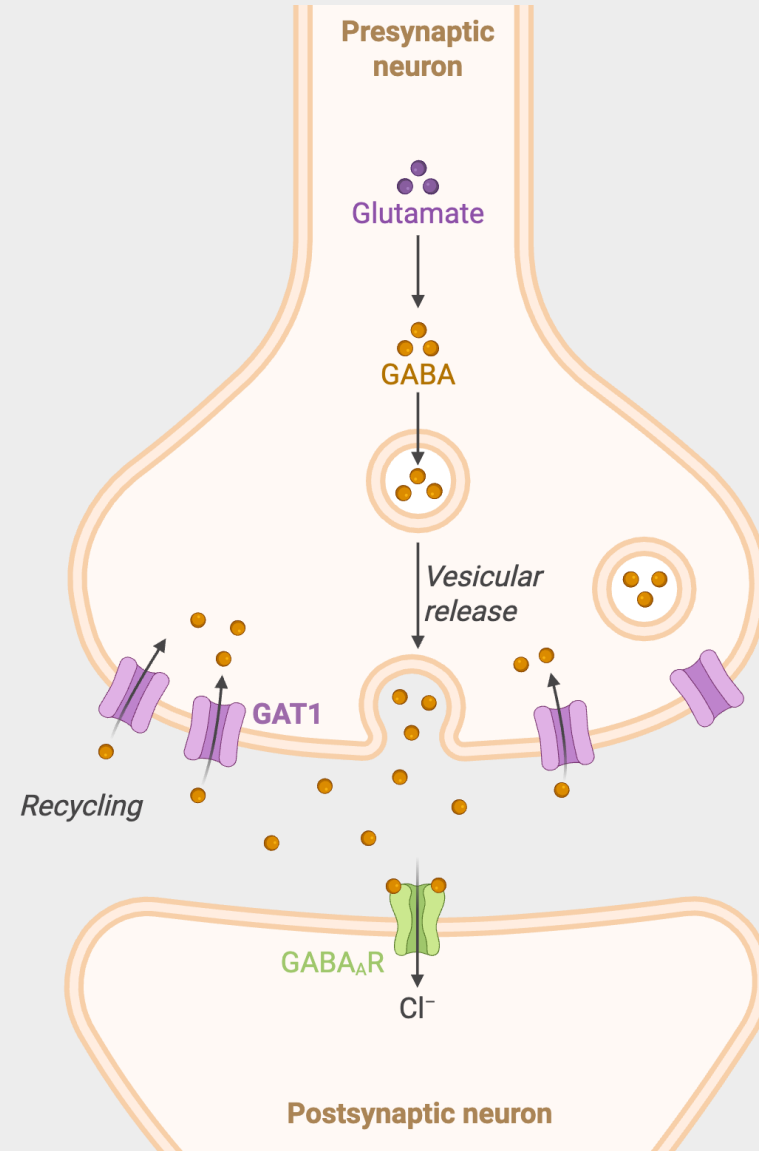


SLC6A1 expression is restricted to a neuronal subtype

GAT1 removes GABA from the synaptic space to allow its recycling

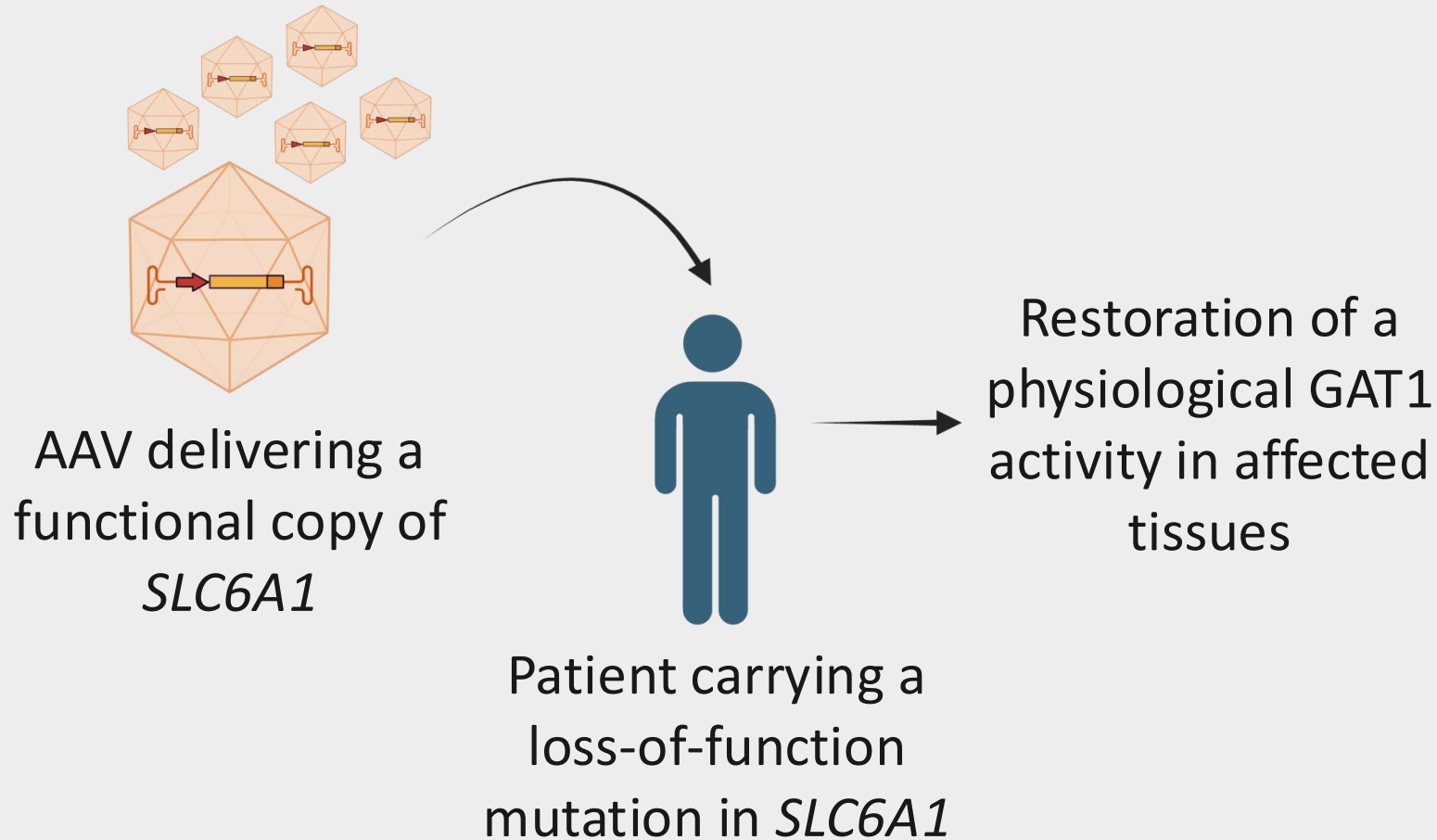
It is expressed in GABAergic neurons, a fraction of all cortical neurons

The regulation of *SLC6A1* expression might be essential for a safe and effective AAV-based gene therapy

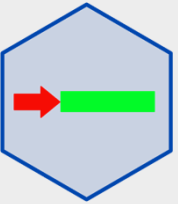


Guastella et al., 1990; Minelli et al., 1995; Scimemi et al., 2014

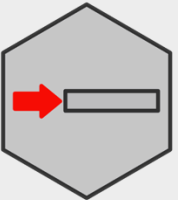
AAV-based gene addition therapy



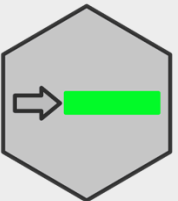
Optimize the delivery of the transgene



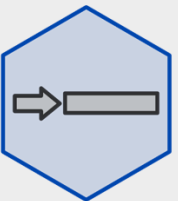
SLC6A1 promoter



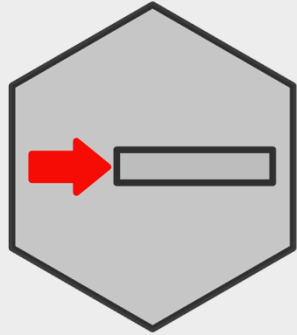
SLC6A1 cassette



AAV capsid



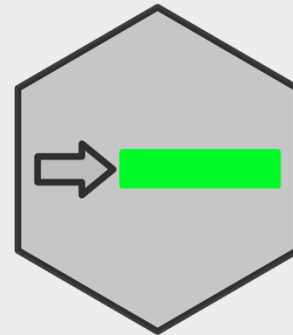
Increasing the safety and efficacy of gene addition



SLC6A1 promoter

Regulates where and when
SLC6A1 is expressed

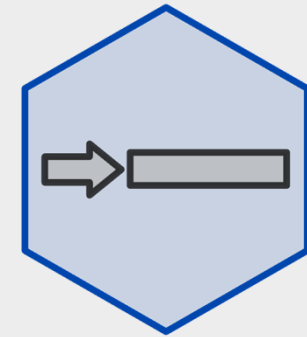
Controls the strength of
expression



SLC6A1 cassette

Contains the *SLC6A1* coding
sequence

Can contain additional introns
and regulatory sequences

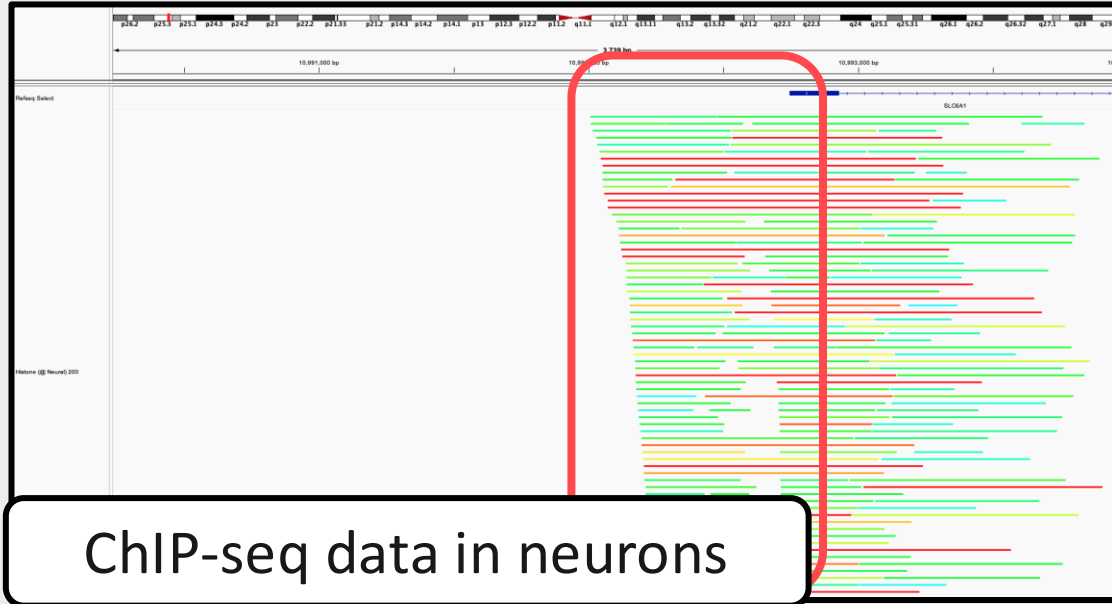
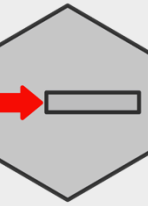


AAV capsid

Packages and delivers the
transgene

Controls which cells will be
transduced

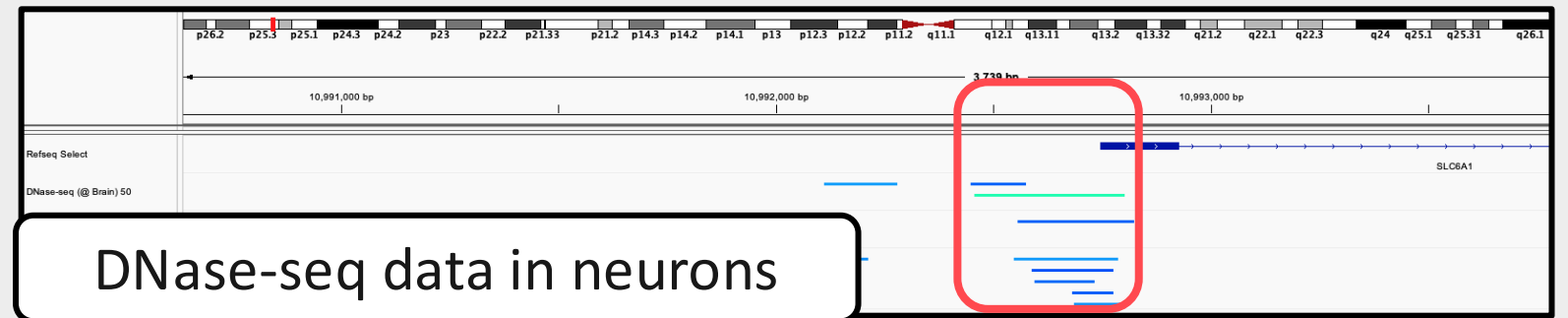
SLC6A1 promoter design



ChIP-seq data in neurons



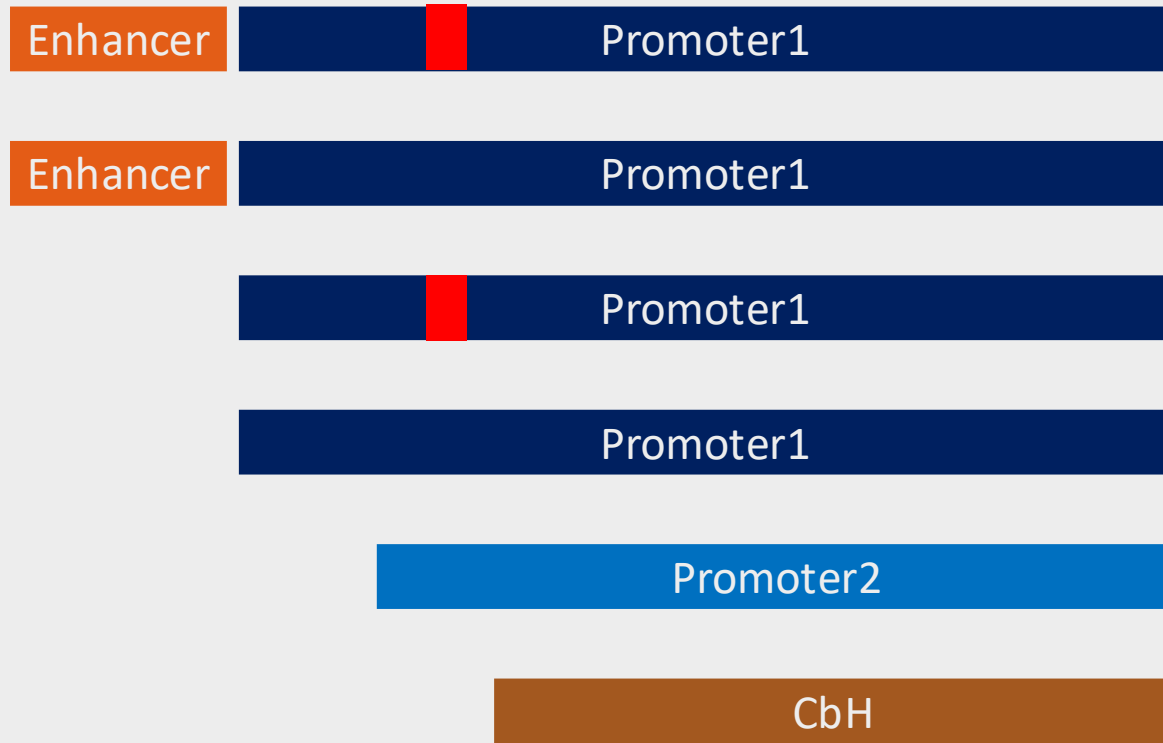
ATAC-seq data in neurons



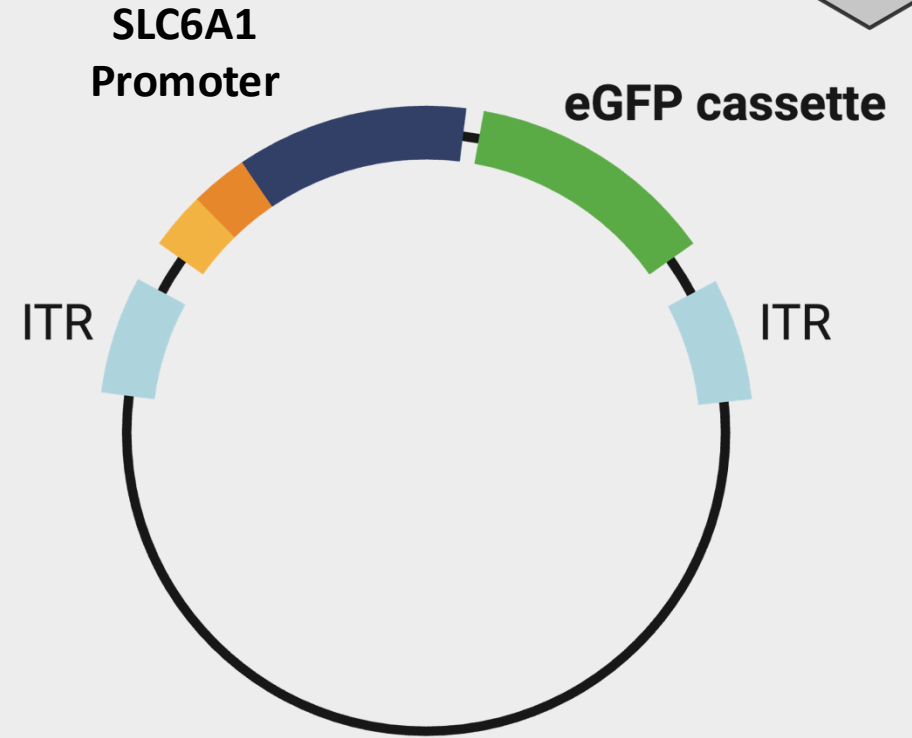
DNase-seq data in neurons

<https://chip-atlas.org>

SLC6A1 promoter validation



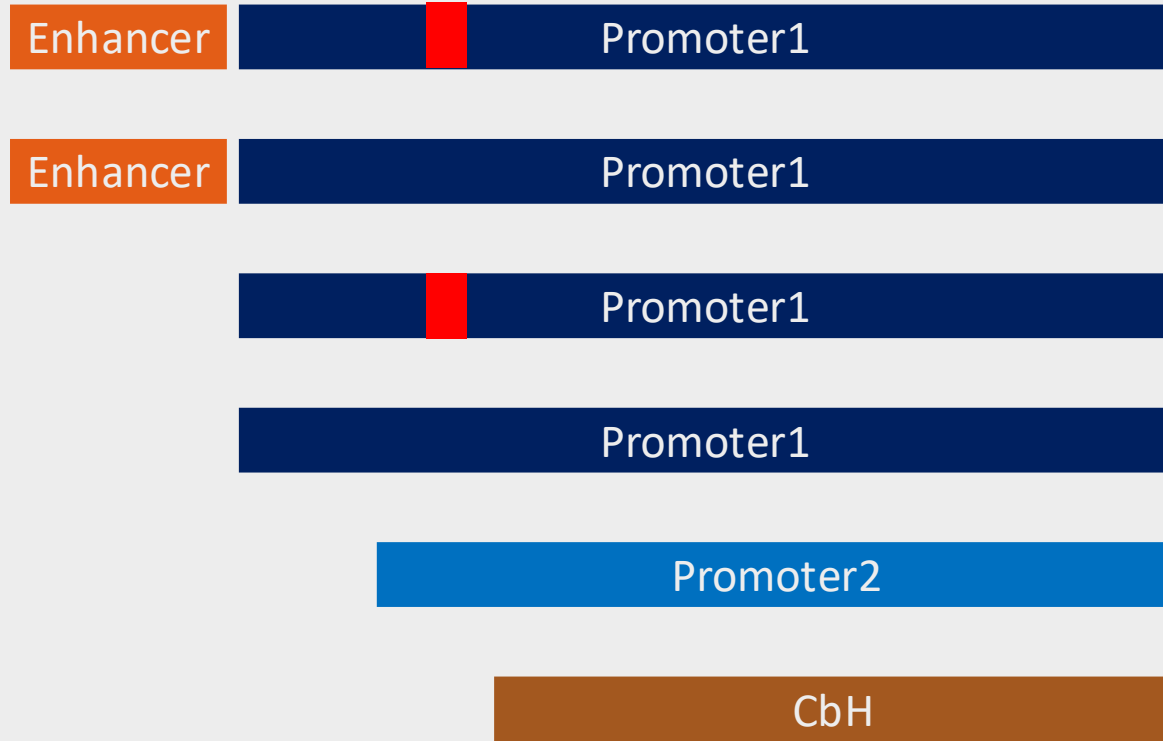
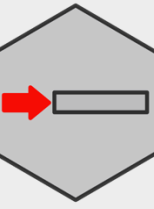
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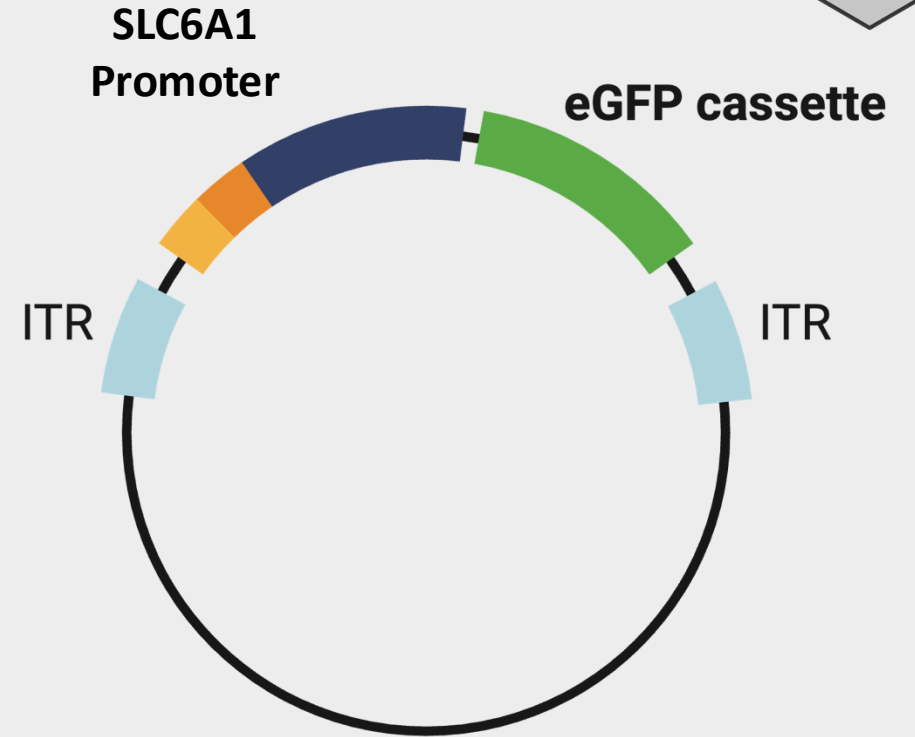
Polymorphism: 21 bp insertion that creates an enhancer element and potentiates the activity of the *SLC6A1* promoter¹

(1) Hirunsatit et al., 2009

SLC6A1 promoter validation



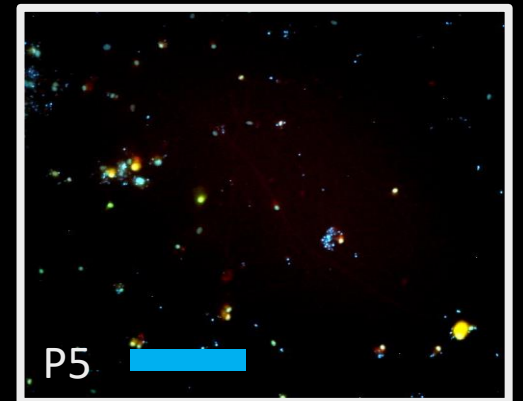
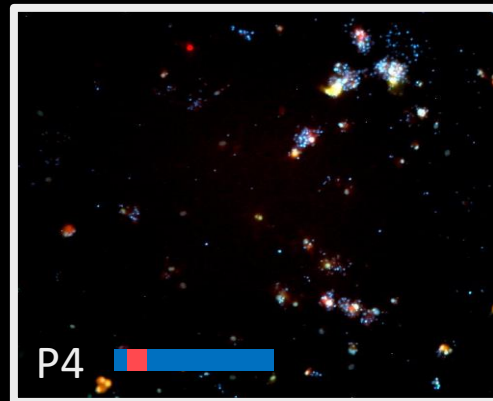
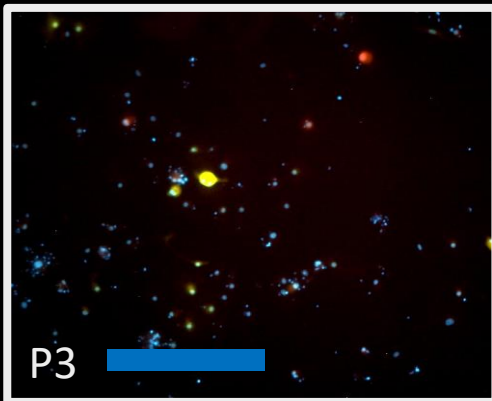
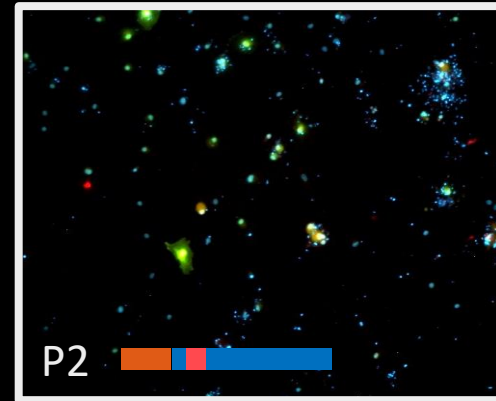
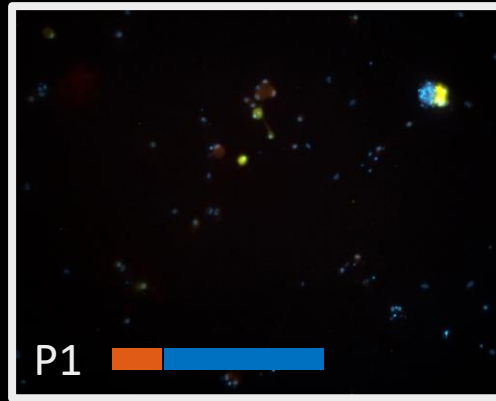
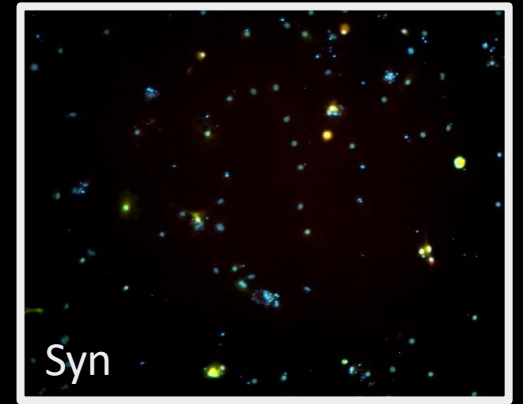
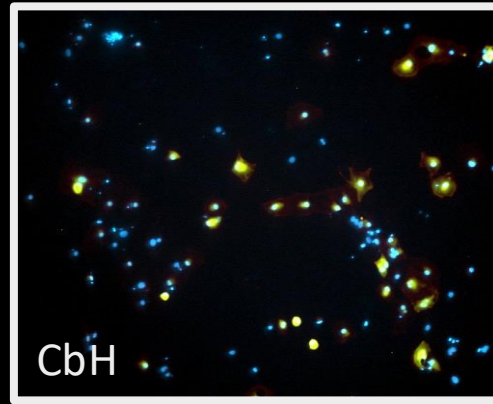
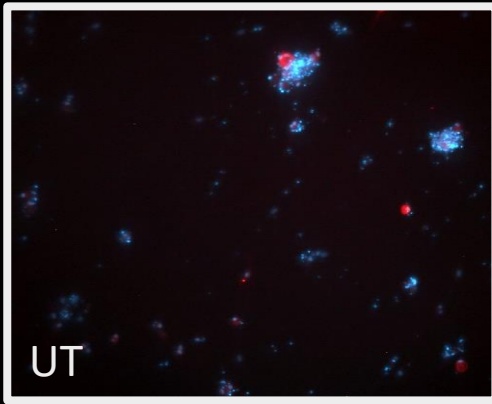
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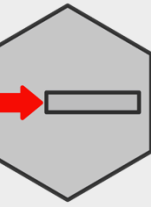


Select *in vitro* the most **specific** and **effective** *SLC6A1* promoter

Promoter screening in human neurons

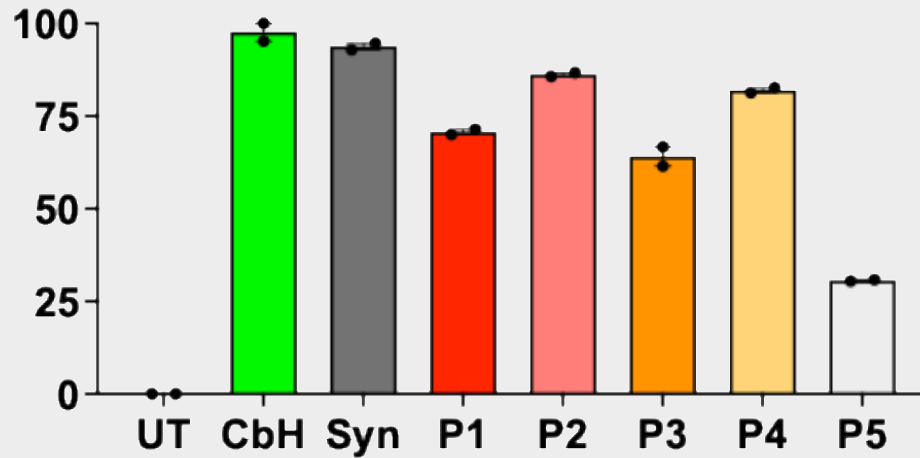
DAPI GAT1 GFP





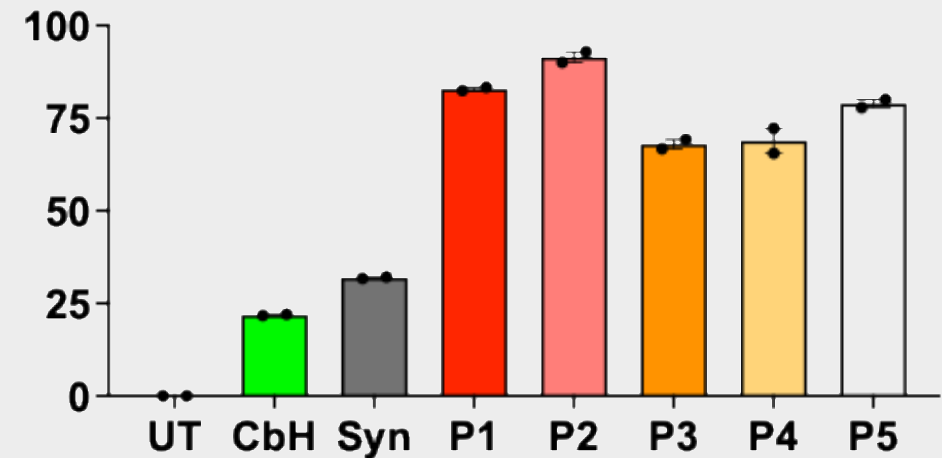
P2 is highly specific towards GABAergic neurons

Targeting efficiency



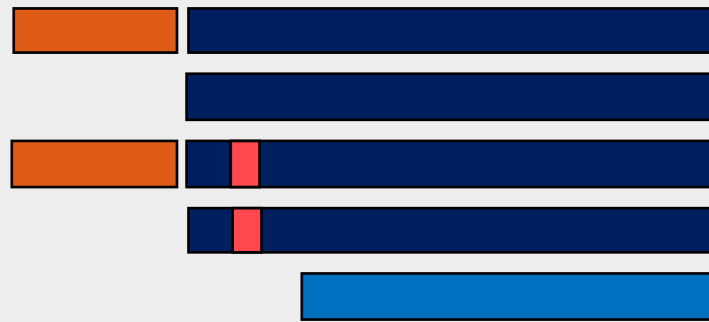
- CbH and Syn target nearly 100% of all GAT1+ neurons
- P2 (86%) is the most effective *SLC6A1* promoter

Targeting specificity



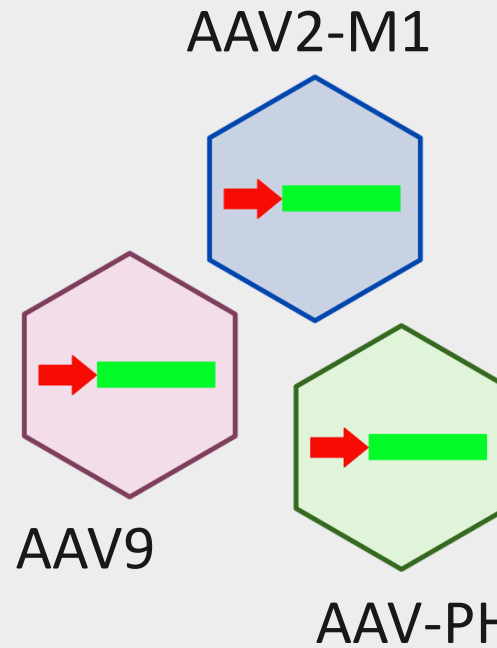
- Syn and CbH target other cell types
- P1 and P2 are highly specific (>85%)

In vivo promoter and capsid selection

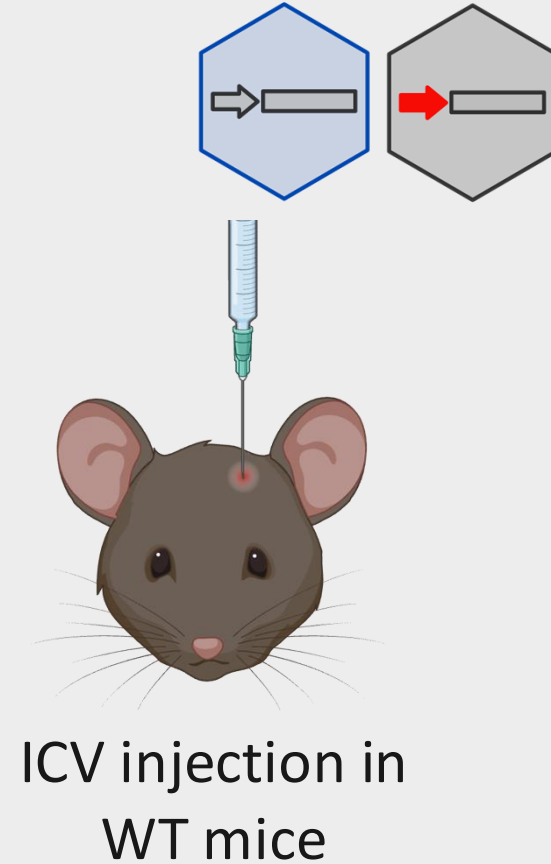


5 versions of the *SLC6A1* promoter

X



N=15
→

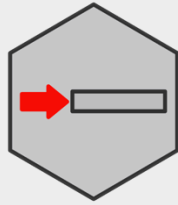


ICV injection in WT mice

Select *in vivo* the most **specific** and **effective** *SLC6A1* promoter and AAV capsid

In collaboration with Paz Lab, Gladstone Institute

P2 is highly specific towards GABAergic neurons

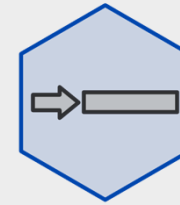


SLC6A1 promoter

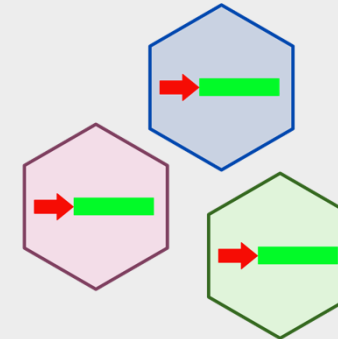


P2 is effective and specific in targeting human GABAergic neurons

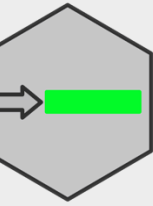
The *in vivo* screening will allow to further validate the *SLC6A1* promoter



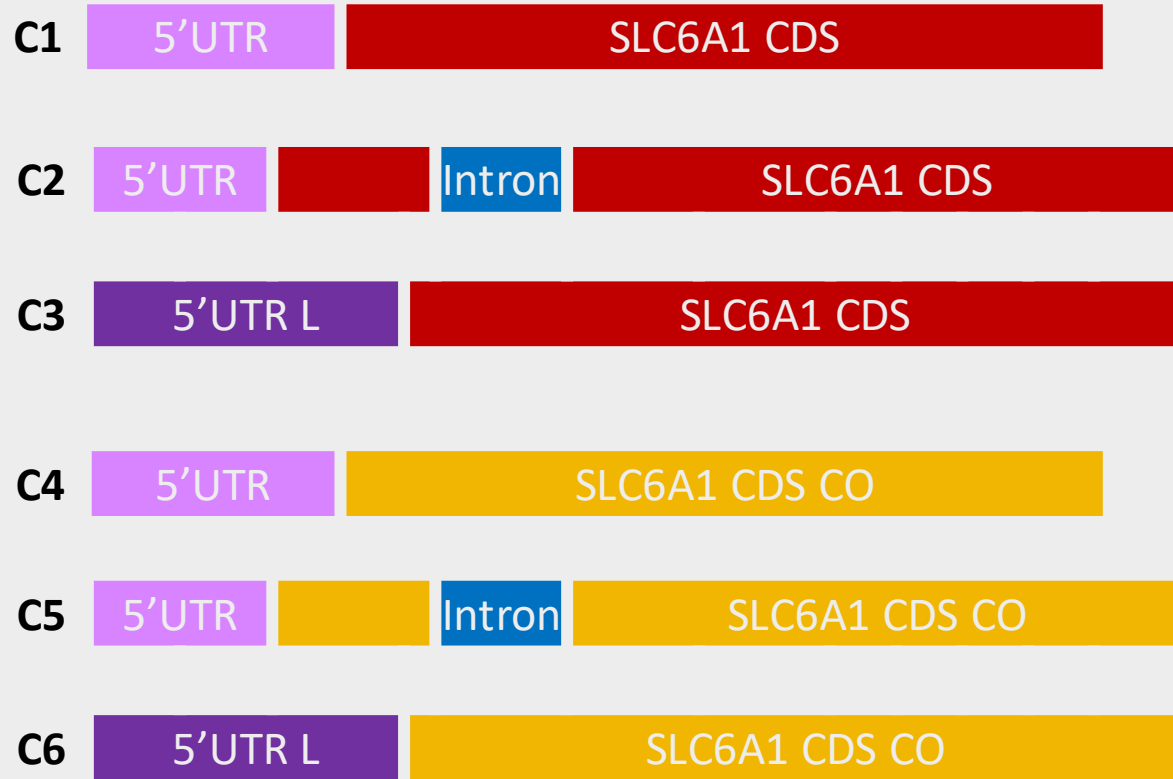
AAV capsid



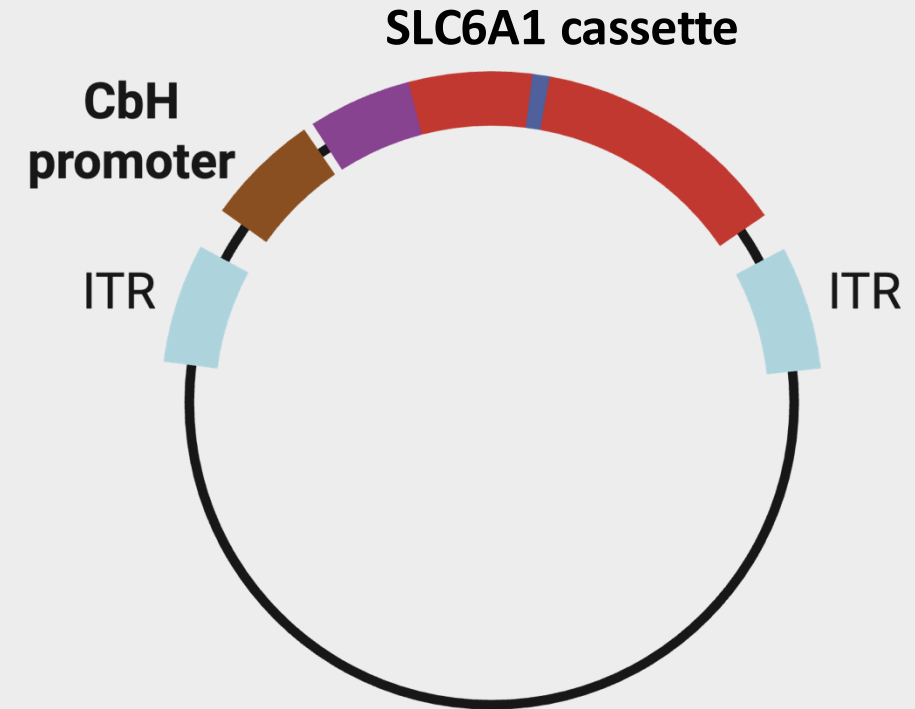
The *in vivo* screening will allow to select the best performing capsid



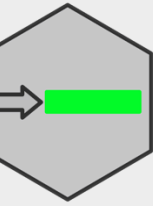
Cassette design: enhancers and introns



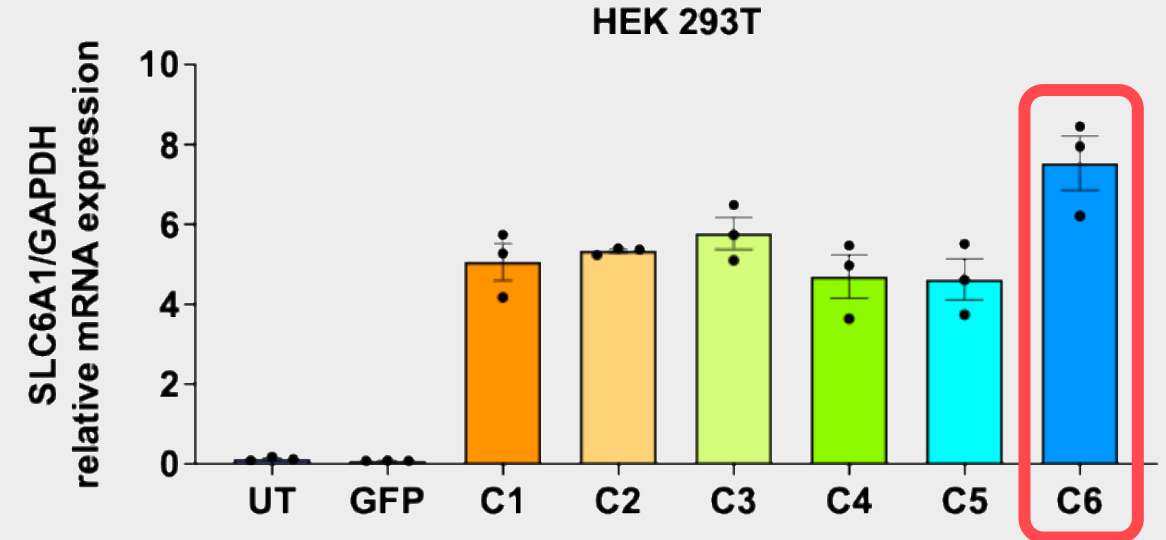
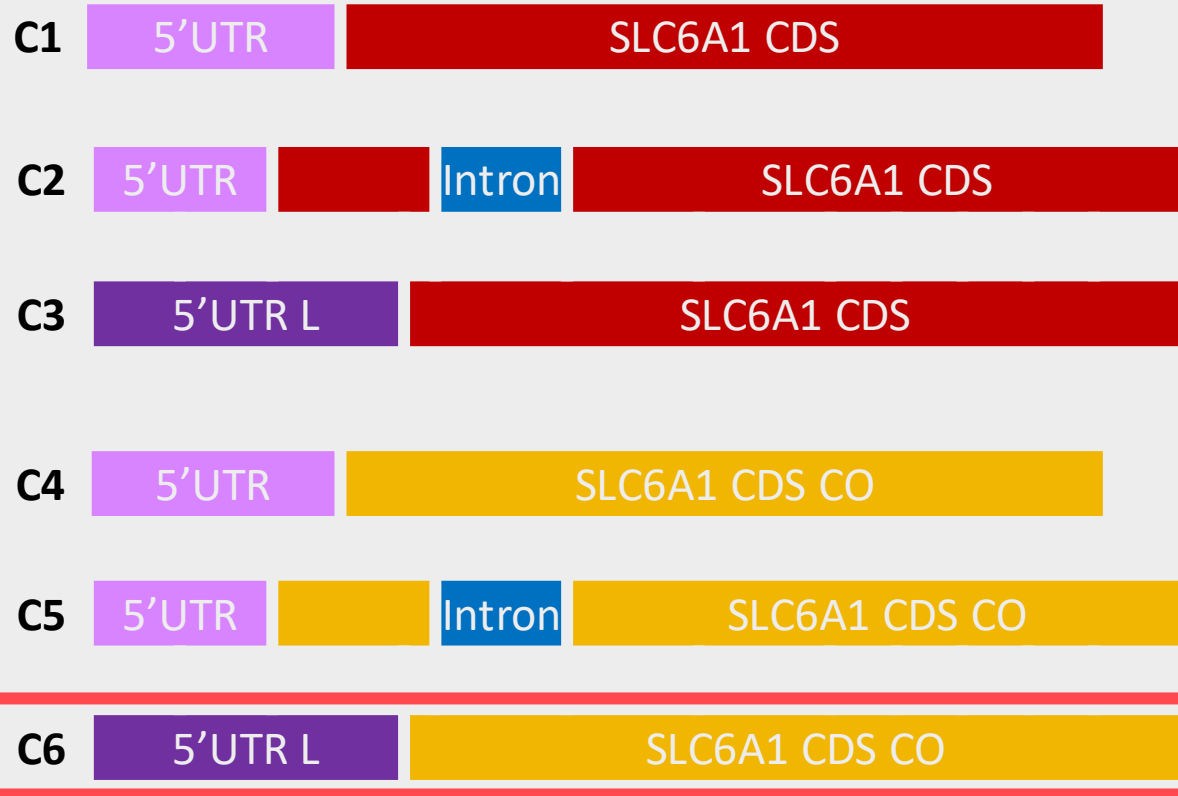
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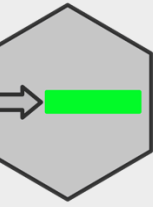
Select the most **effective** *SLC6A1* cassette



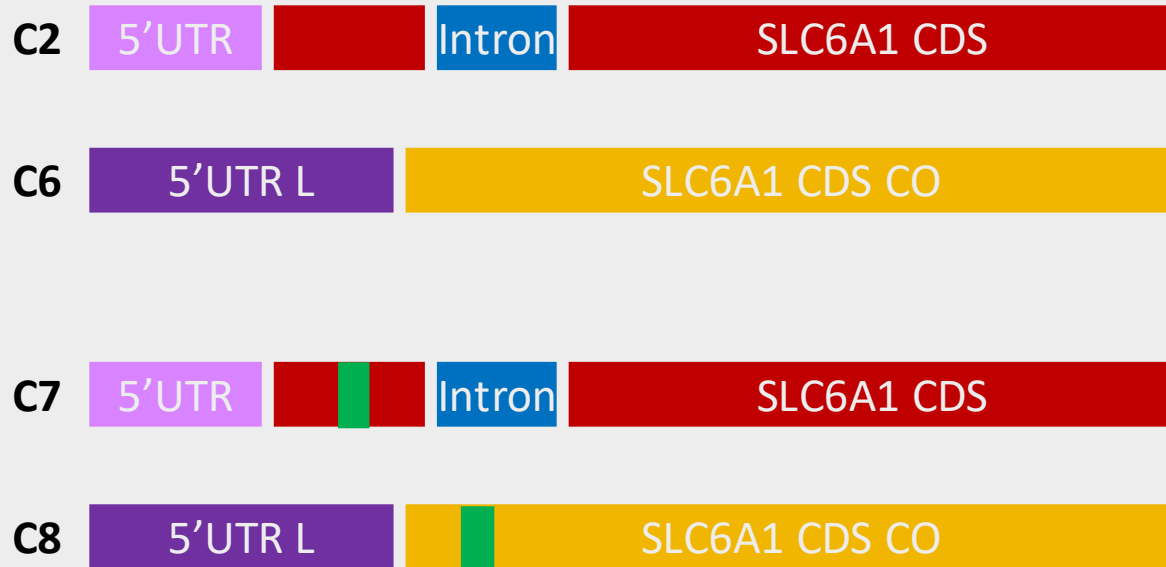
Cassette design: enhancers and introns



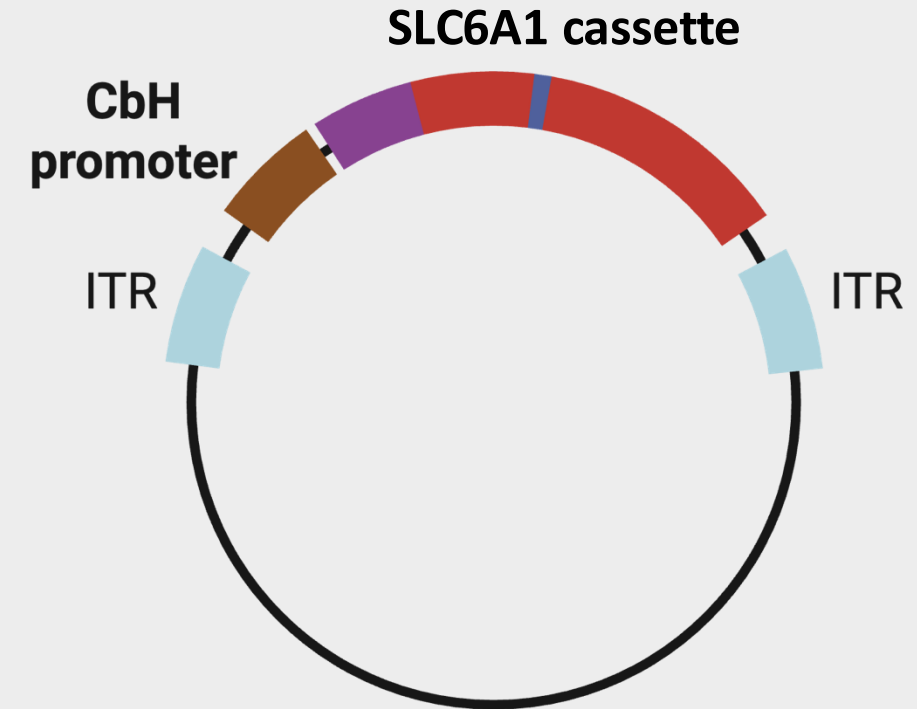
Select the most **effective** *SLC6A1* cassette




Cassette design: polymorphism



To be
cloned in

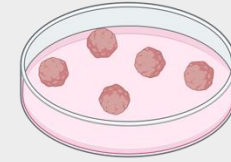
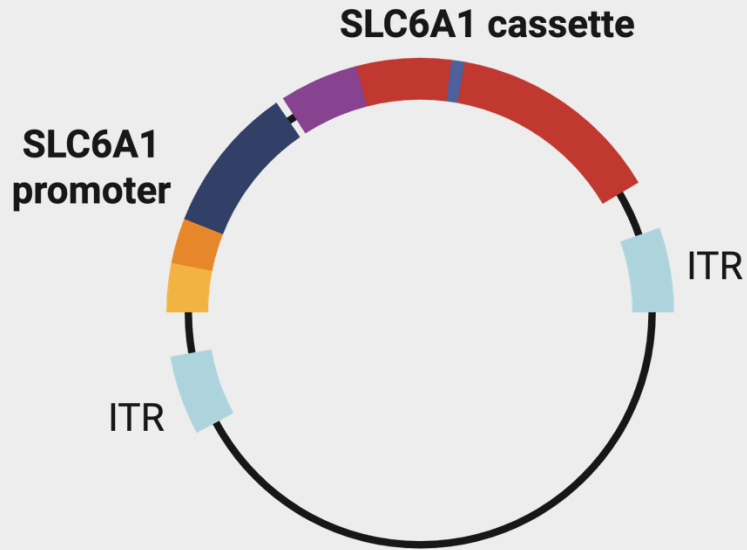
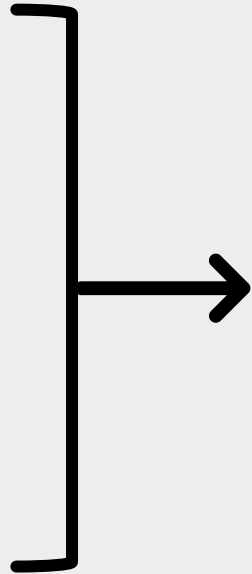
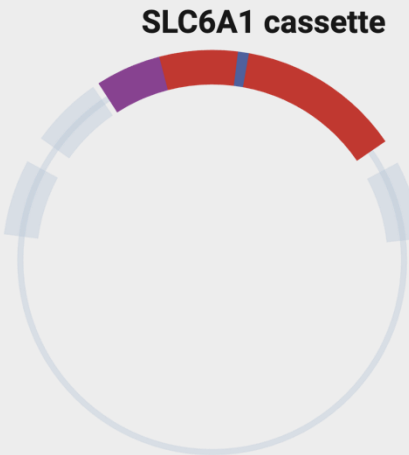
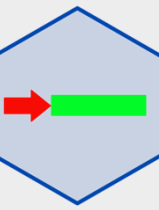


 **Polymorphism c.259T>C (p.Phe87Leu)**

- Predicted to be non pathogenic
- Allows for higher GAT1 activity¹

(1) Silva et al., 2024

Future plans



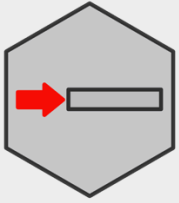
Transduction of patient-derived brain organoids



ICV injection in *Slc6a1*^{+/-} mice

In collaboration with Paz Lab, Gladstone Institute

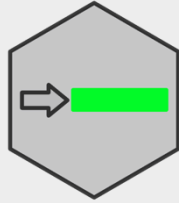
Future plans



SLC6A1 promoter

- ✓ Selected the best promoter *in vitro*

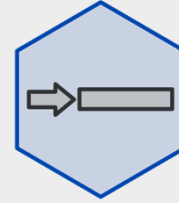
Finish analyzing samples to select promoter in vivo



SLC6A1 cassette

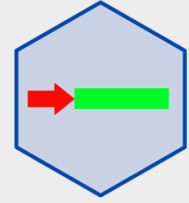
- ✓ Selected the best cassettes *in vitro*

Test non-pathogenic variant in vitro



AAV capsid

Finish analyzing samples to select capsid in vivo

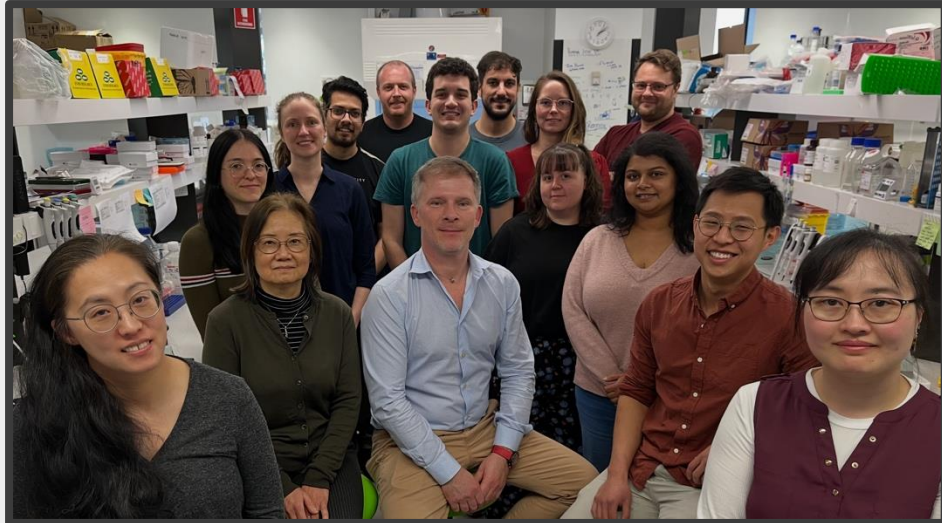


Therapeutic product

- ✓ Generated iPSC from 2 patients

Waiting for selection of the best promoter and cassette

Acknowledgments



CMRI

Leszek Lisowski

Andrea Perez Iturralde

Hardik Parate

Florencia Haase

Suzanne Scott

Maddison Knight

Gladstone Institute

Jeanne Paz

Yuliya Voskobiyny

Bret Holt



Reaching to Cure Genetic Epilepsy

SLC6A1KIDS 



THANKS!

Do you have any questions?

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