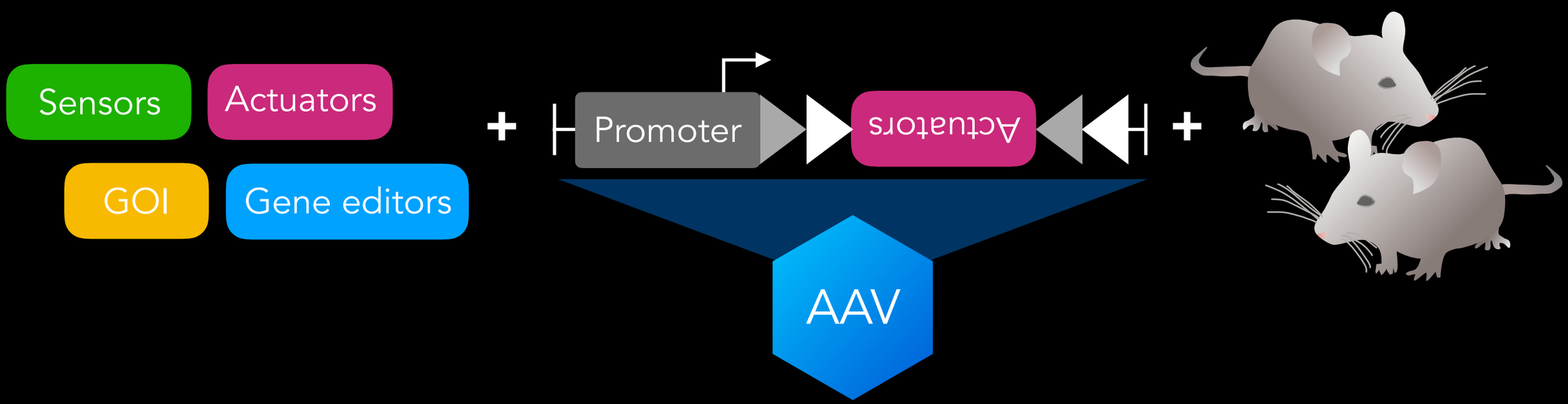
Genetically encoded tools, AAV vectors and transgenic mice are a powerful and flexible combination for studying how specific cell types and circuits impact behavior and learning

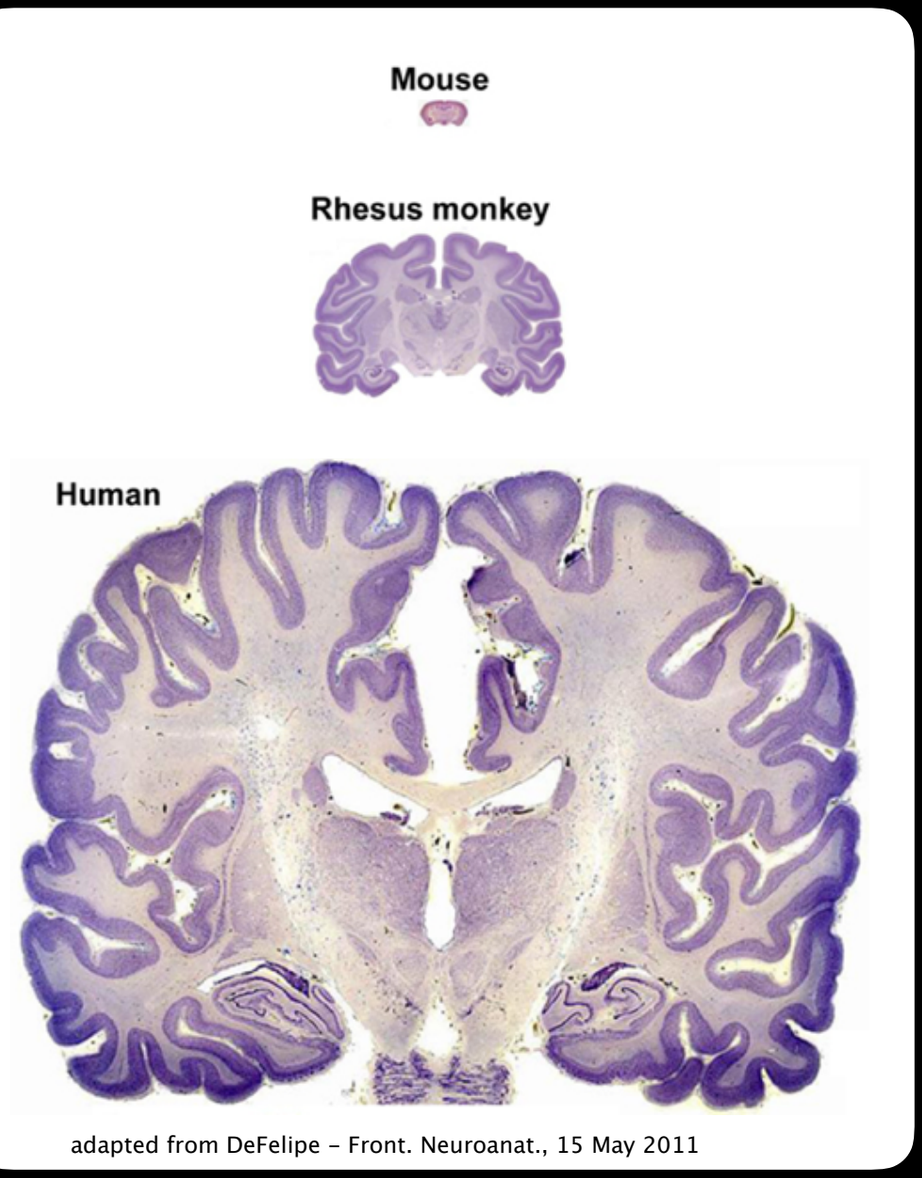


Genetically encoded tools

Cre/Flp-dependent AAVs

Transgenic Animals (Cre/Flp)

Enabling cell type specific neuromodulation in the absence of transgenics

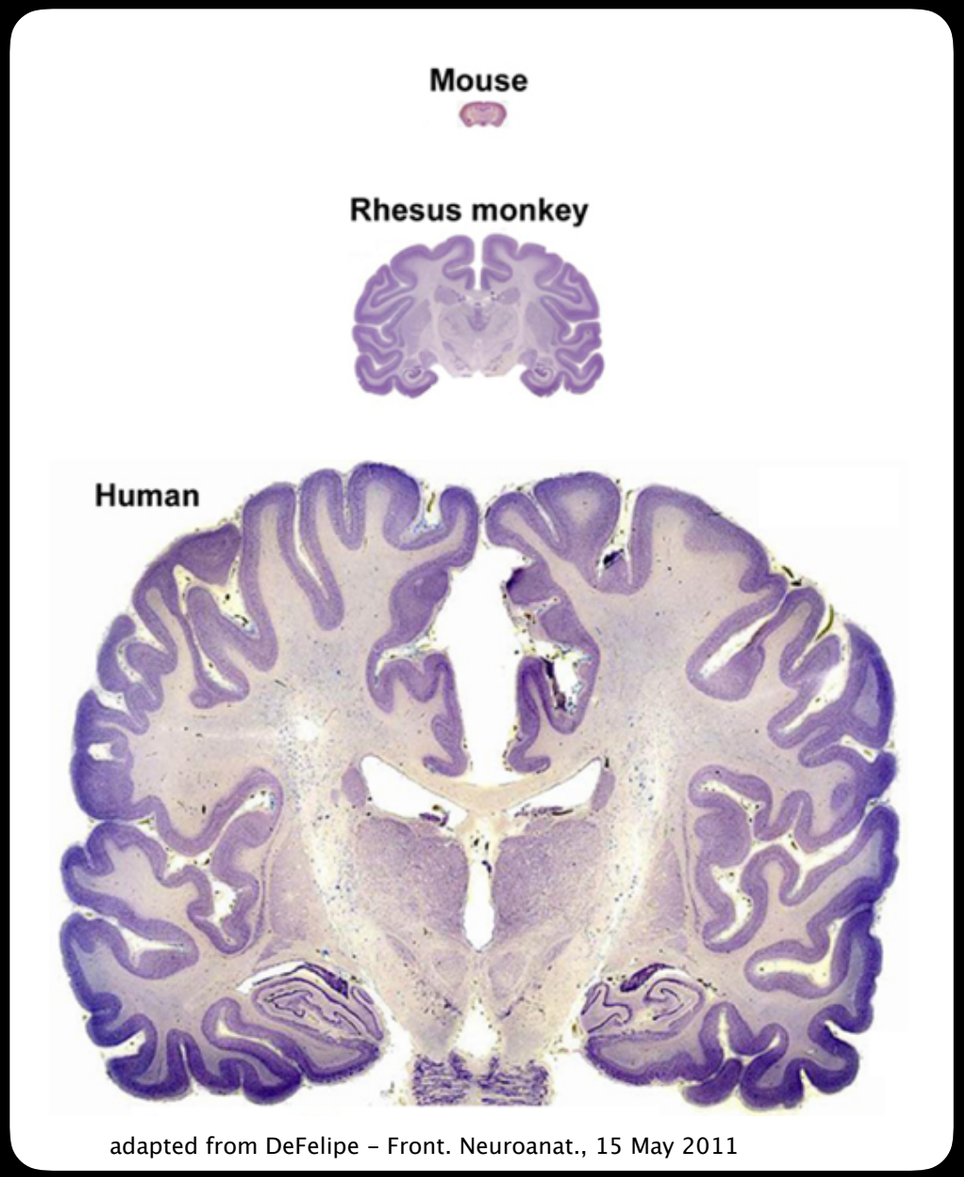


adapted from DeFelipe – Front. Neuroanat., 15 May 2011

Objectives

- Develop vehicles for safe and efficient noninvasive CNS-wide gene transfer
- Enable targeted gene expression in specific neuronal and glial populations
- Develop improved intersectional strategies for specific circuits
- Avoid immune responses to foreign transgenes and viral components

Enabling cell type specific neuromodulation in the absence of transgenics



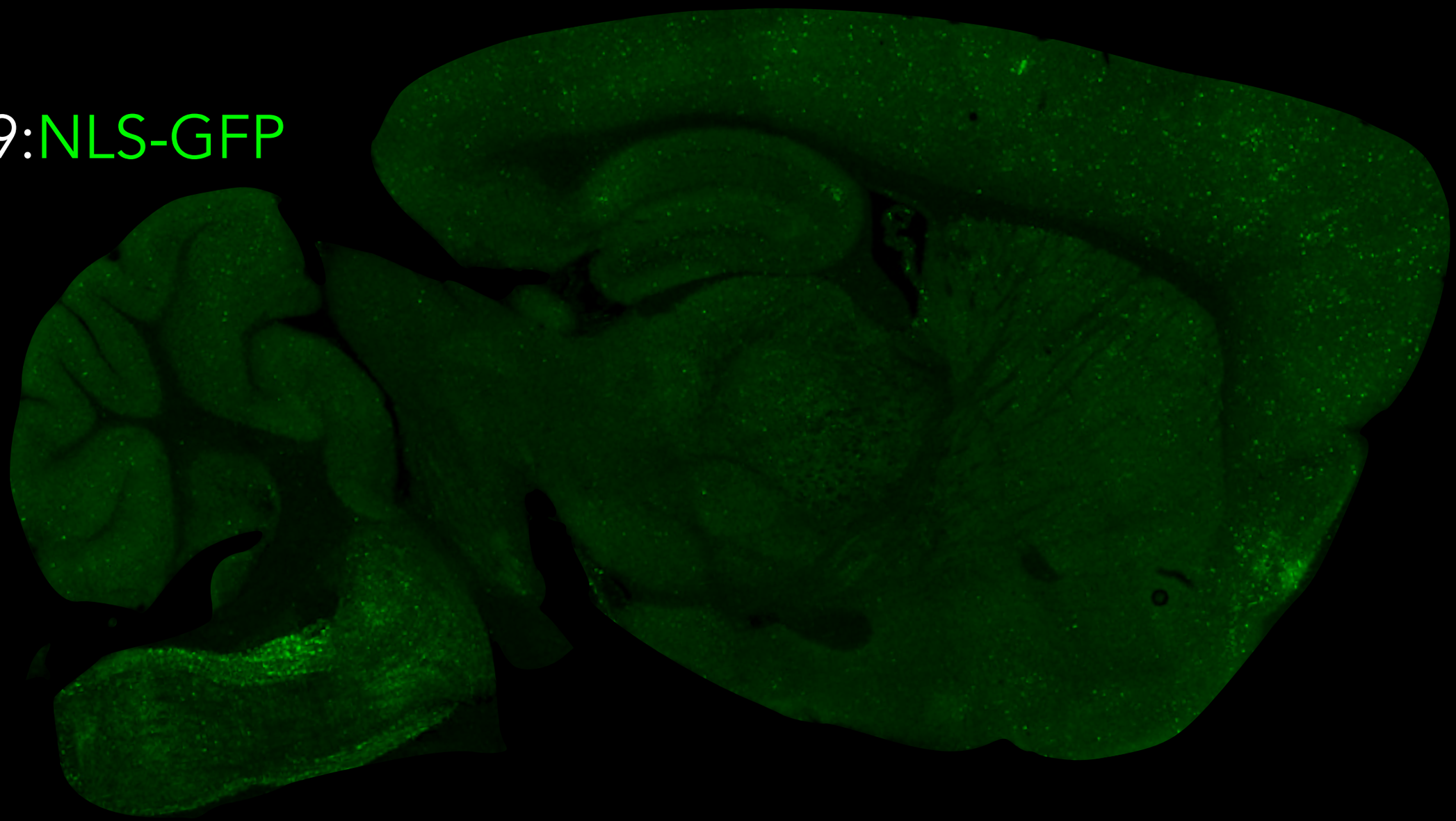
adapted from DeFelipe – Front. Neuroanat., 15 May 2011

Objectives

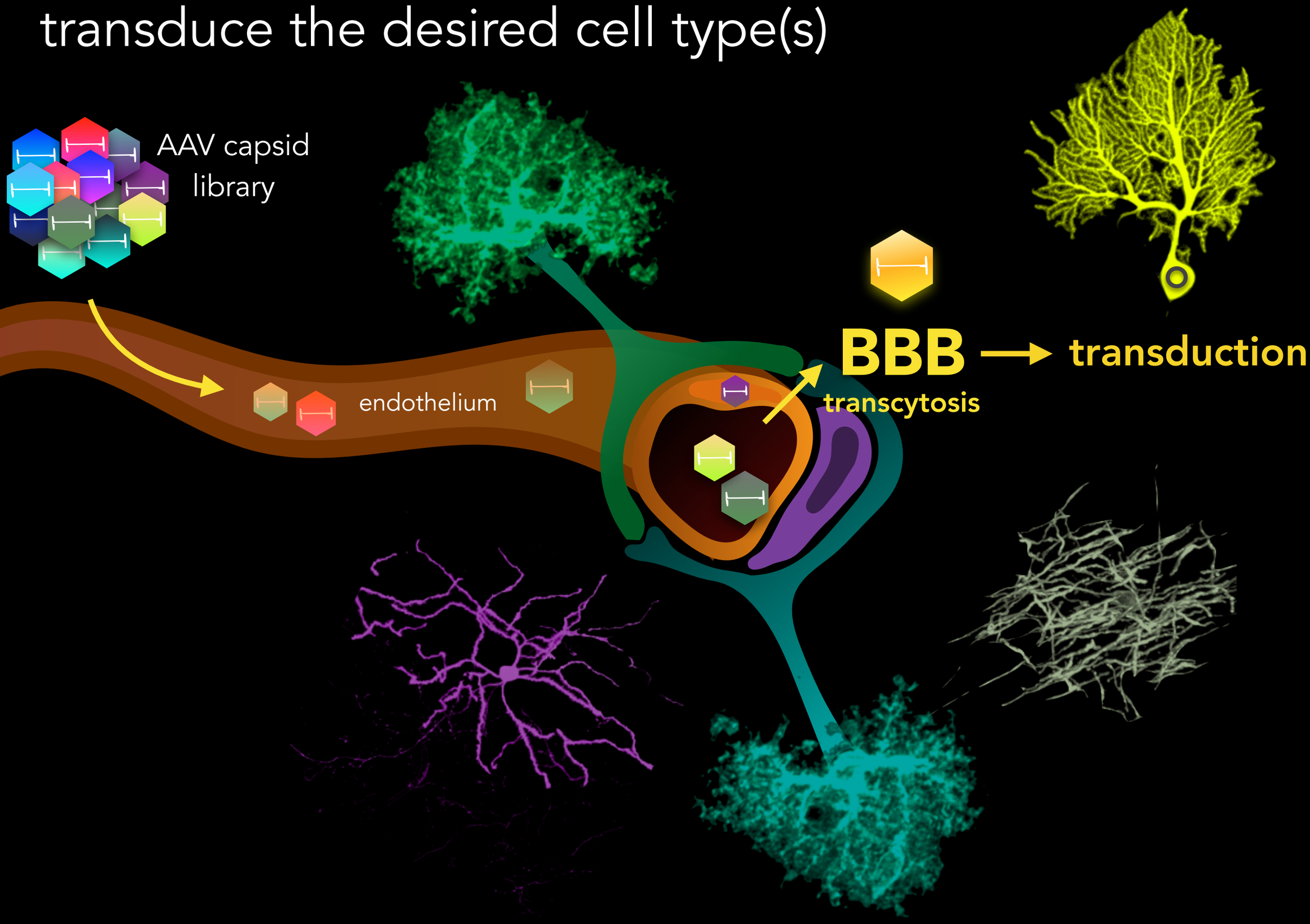
- Develop vehicles for safe and efficient noninvasive CNS-wide gene transfer
- Enable targeted gene expression in specific neuronal and glial populations
- Develop improved intersectional strategies for specific circuits
- Avoid immune responses to foreign transgenes and viral components

Intravenous AAV9 provides widespread but sparse transduction

AAV9:NLS-GFP



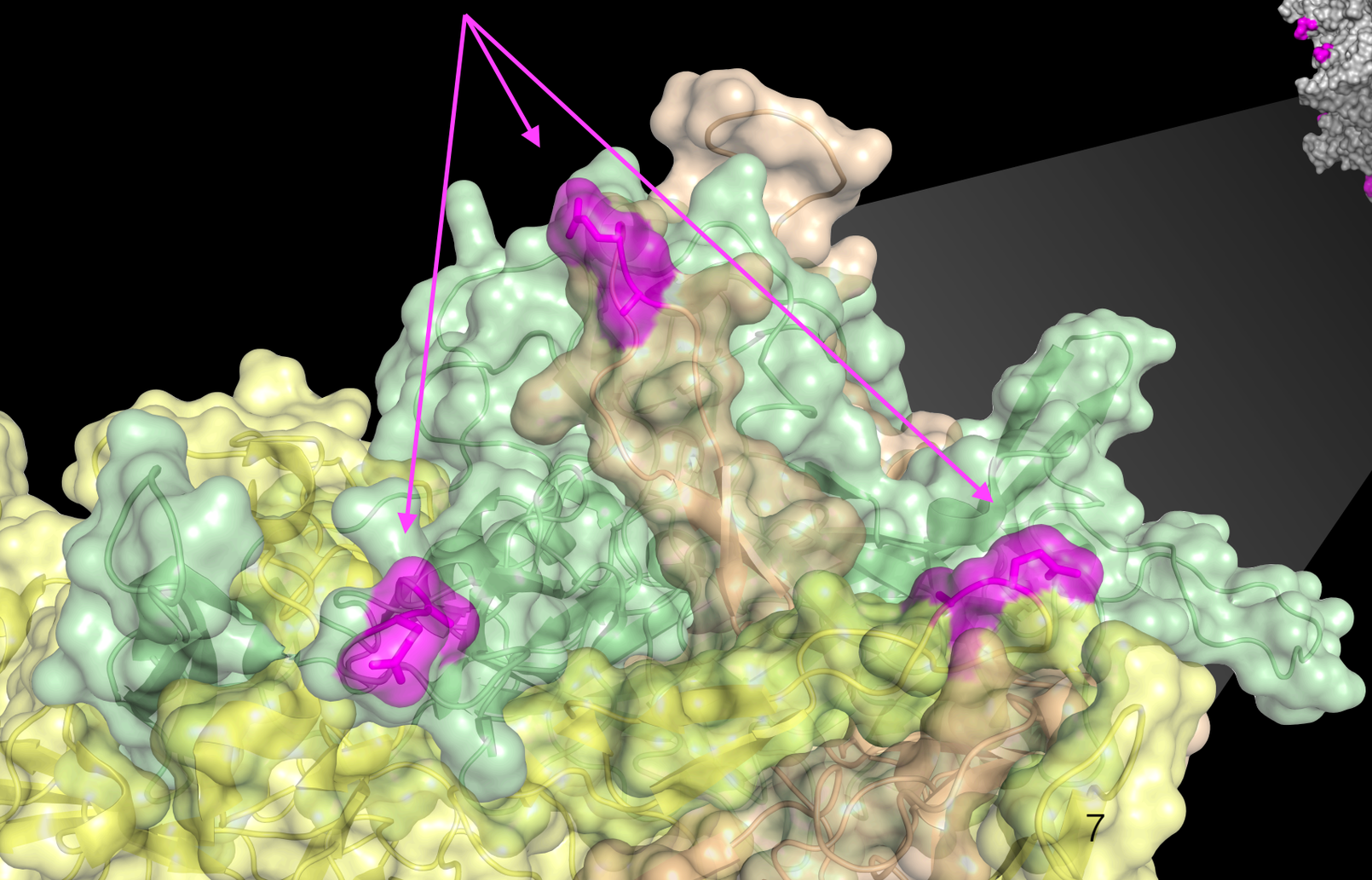
In vivo selection for AAVs that cross the BBB and transduce the desired cell type(s)



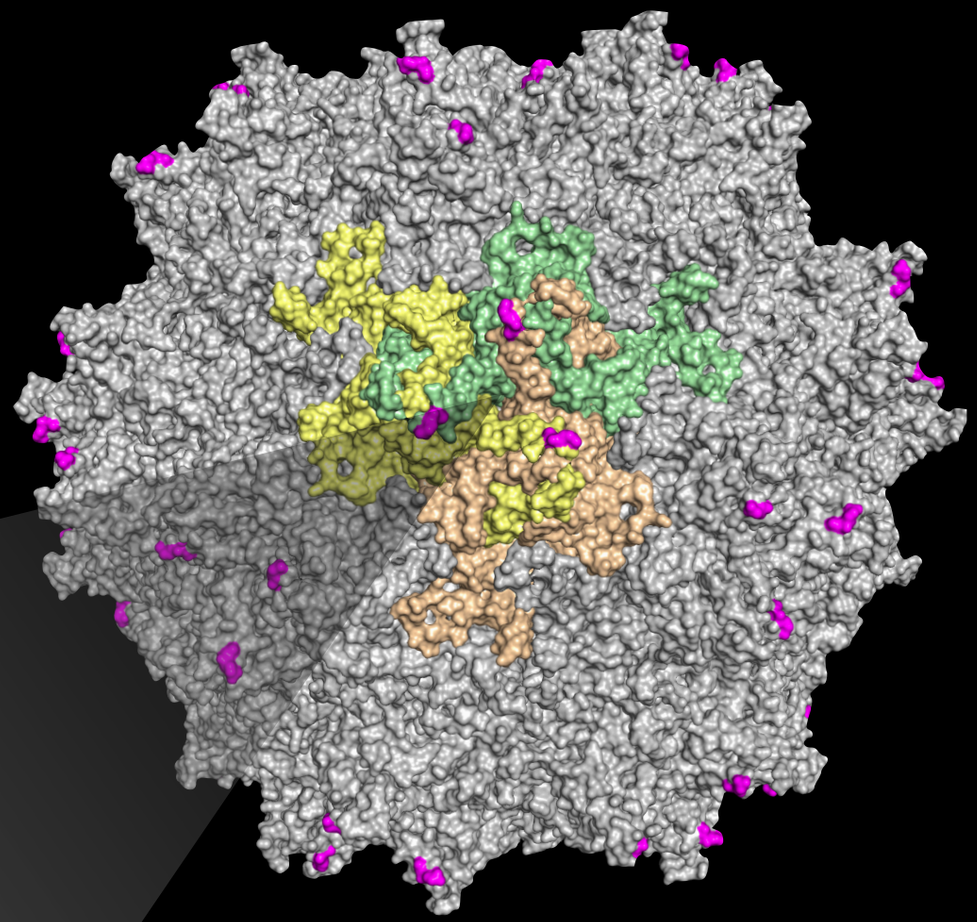
In vivo selection for AAVs that cross the BBB and transduce the desired cell type(s)

Library design

7-mer NNK insertion
between AA588-589

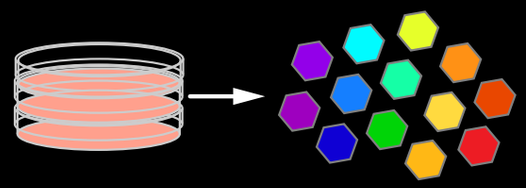


AAV9 K449R



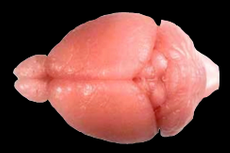
Using CREATE to select for AAVs that cross the blood brain barrier

generate DNA library



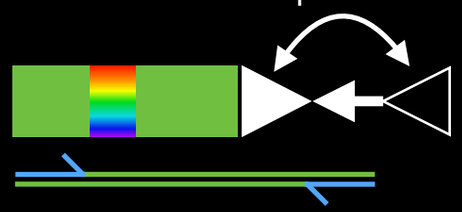
generate and purify capsid virus library

administer to Cre transgenic animal



collect tissues

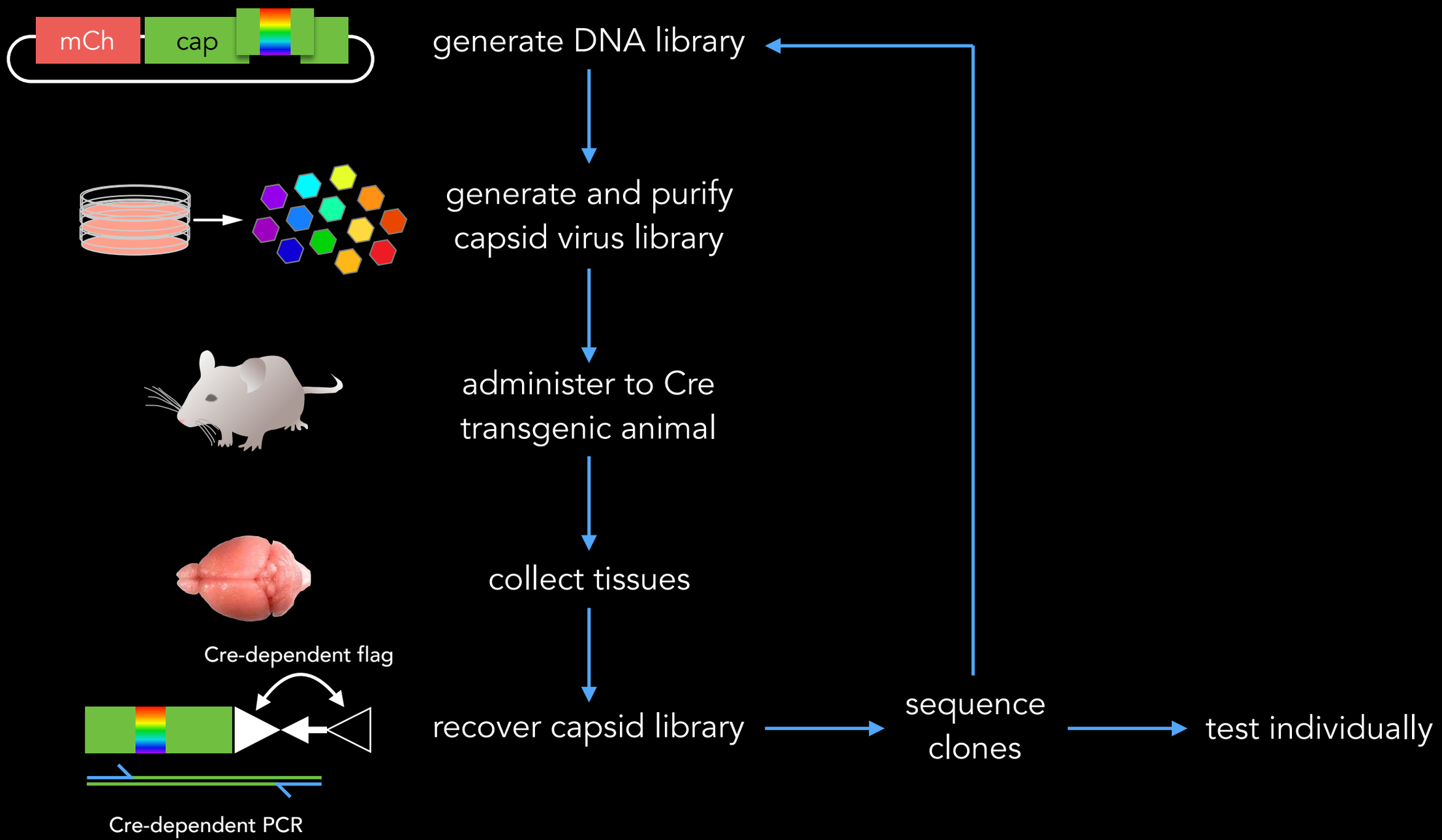
Cre-dependent flag



Cre-dependent PCR

recover capsid library

Using CREATE to select for AAVs that cross the blood brain barrier

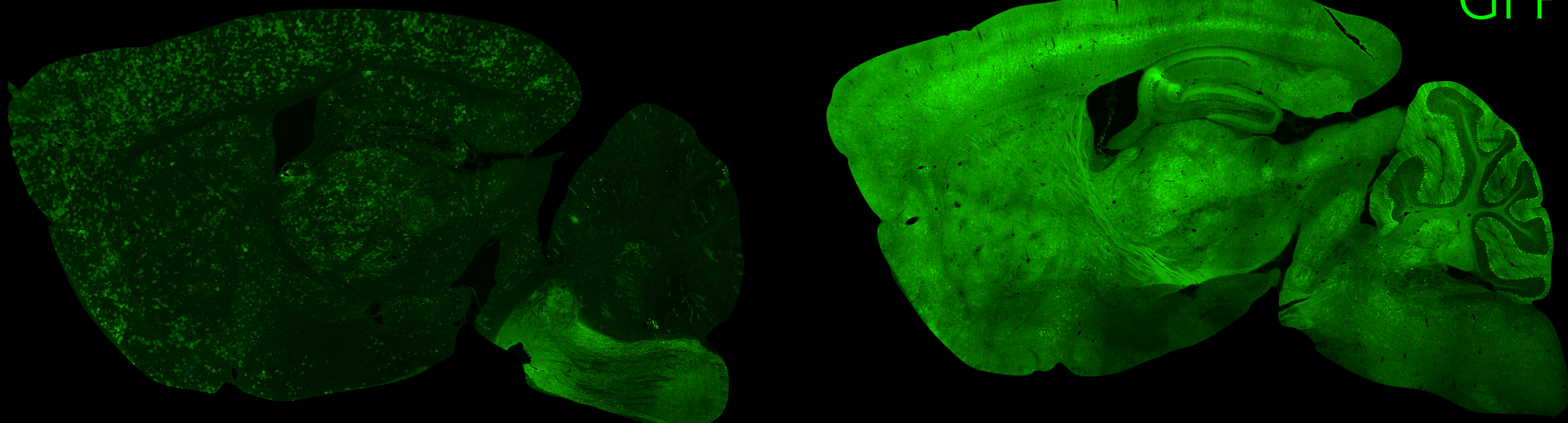


The AAV-PHP.B variant transduces the adult mouse brain with high efficiency

AAV9

AAV-PHP.B

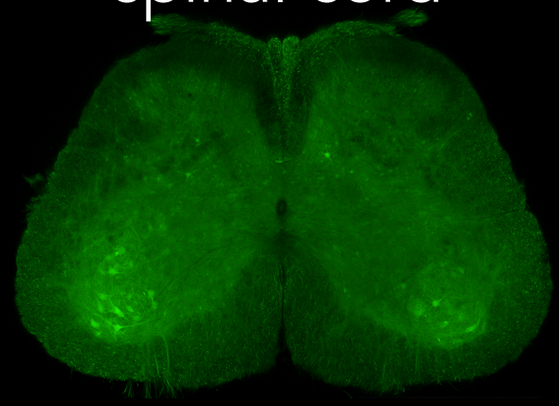
GFP

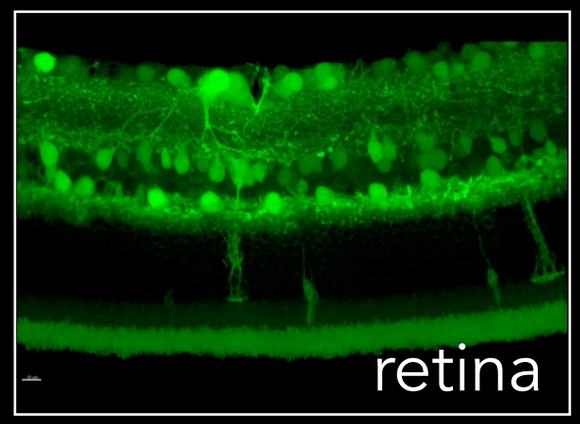


$1x10^{12}$ vg/mouse

Typical dose for direct inj $1x10^{9}$-$1x10^{10}$

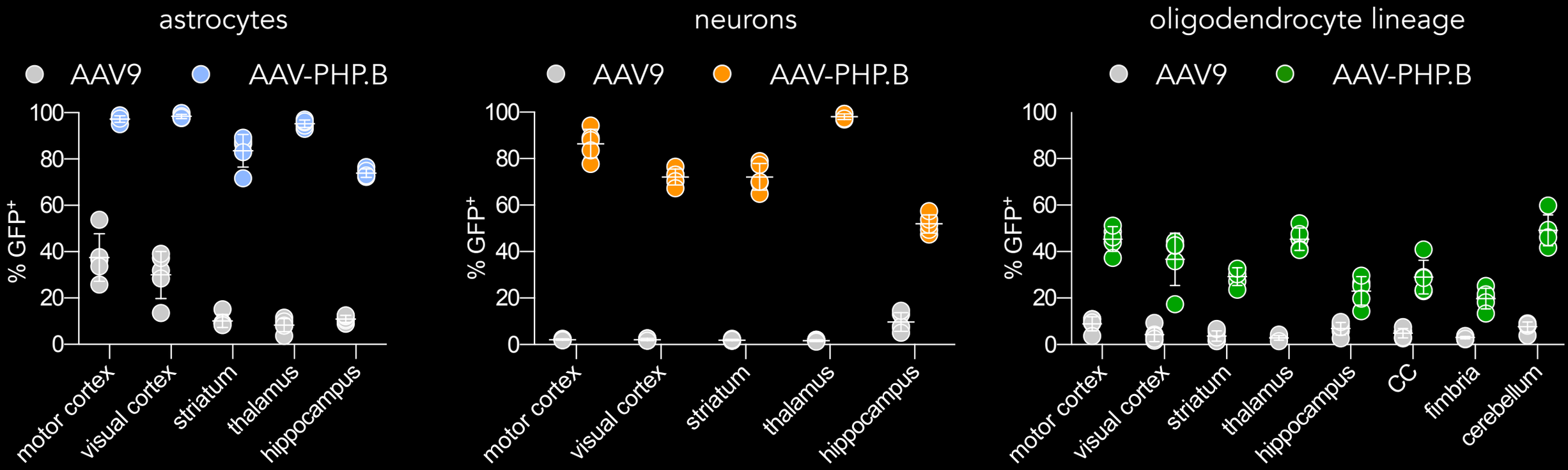
spinal cord





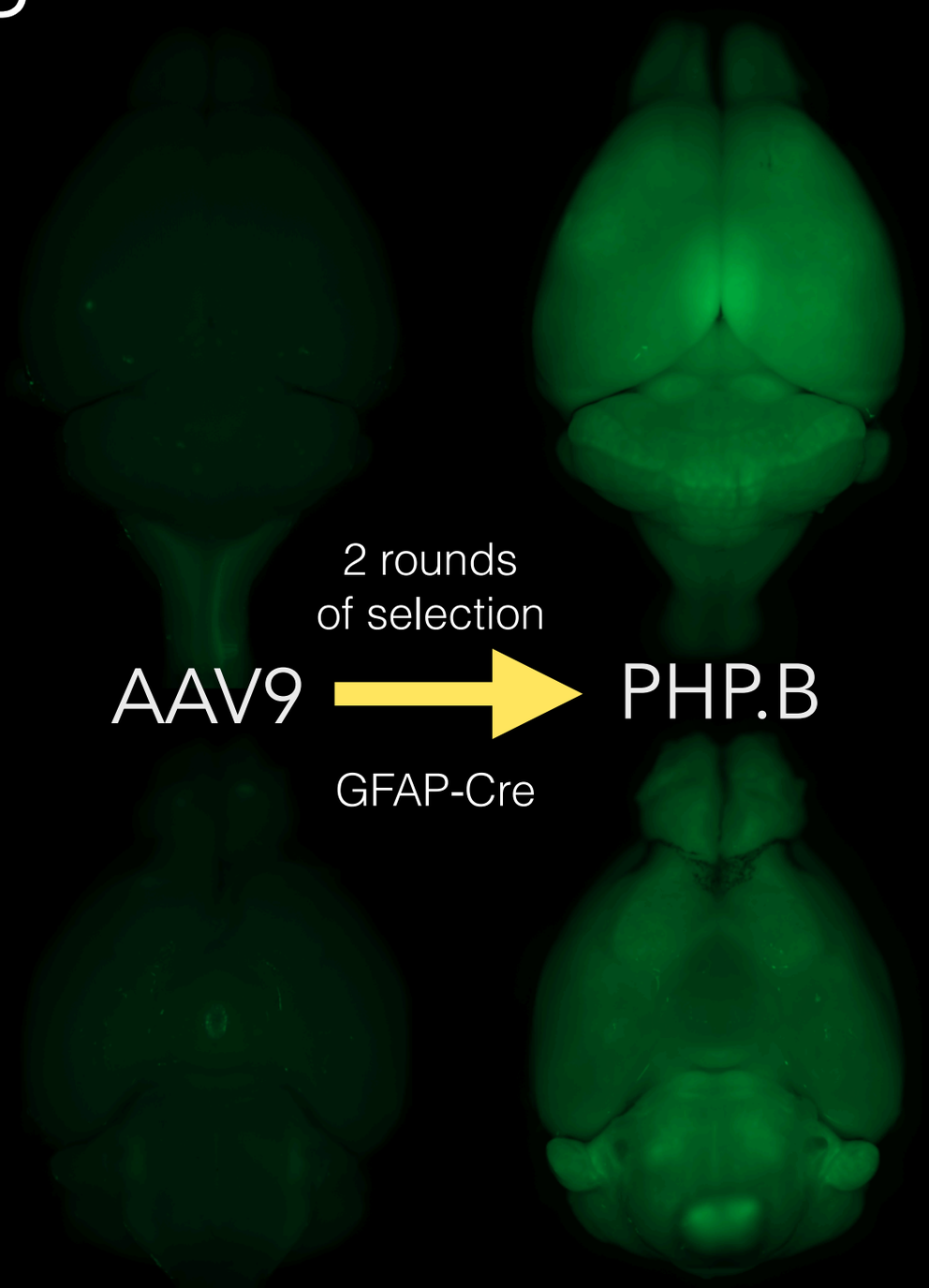
Deverman *et al. Nature Biotechnology* 2016

AAV-PHP.B efficiently transduces astrocytes, neurons and oligodendrocyte lineage cells



Deverman *et al. Nature Biotechnology* 2016

Evolution of a further enhanced CNS-targeted AAV



2 rounds
of selection

AAV9 → PHP.B

GFAP-Cre

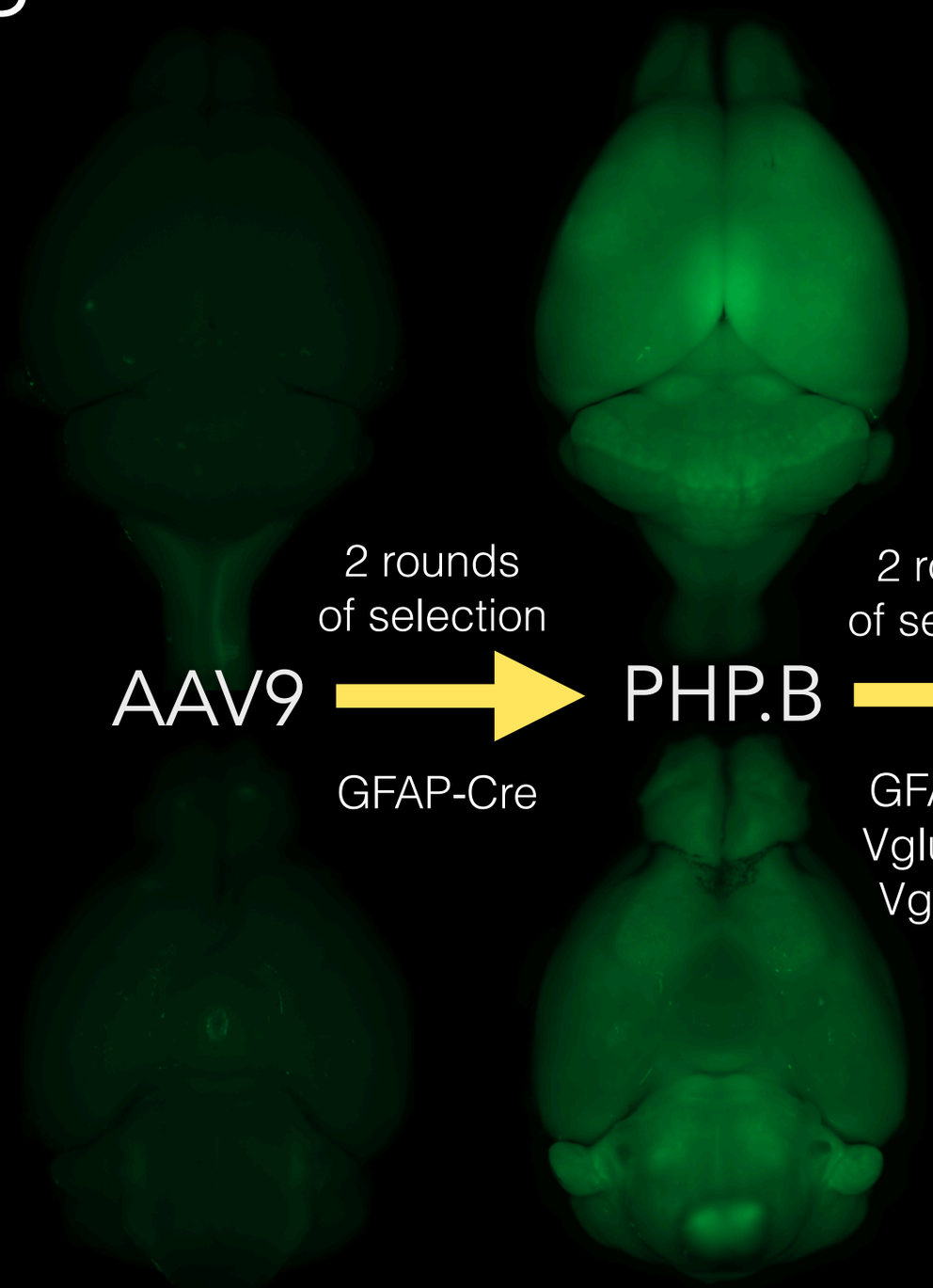
$1x10^{12}$ vg

$1x10^{11}$ vg

Ken Chan

Chan *et al. Nature Neurosci* 2017

Evolution of a further enhanced CNS-targeted AAV



AAV9 → (GFAP-Cre) → PHP.B → (GFAP-Cre, Vglut2-Cre, Vgat-Cre)

2 rounds of selection

2 rounds of selection

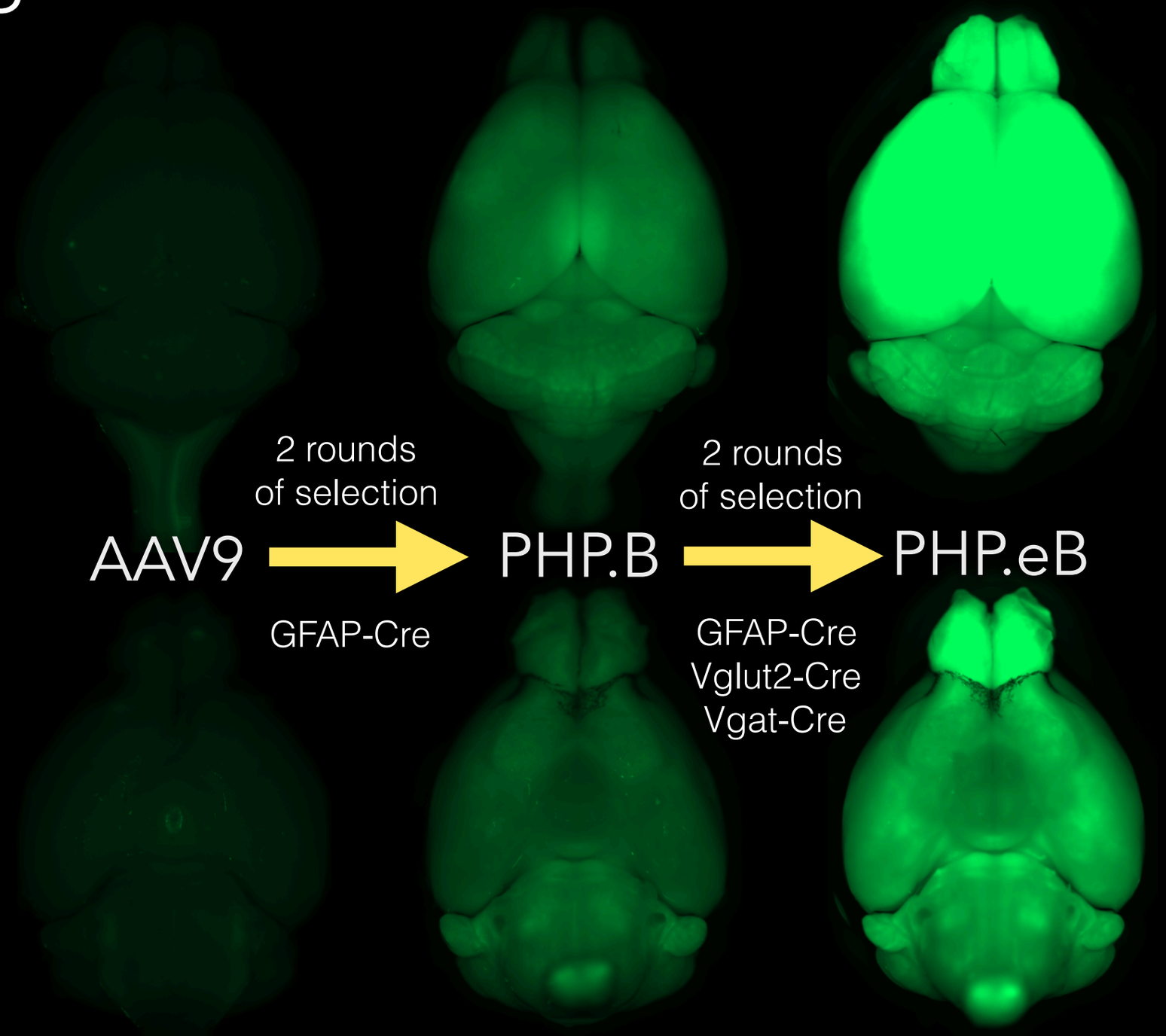
$1x10^{12}$ vg

$1x10^{11}$ vg

Ken Chan

Chan *et al. Nature Neurosci* 2017

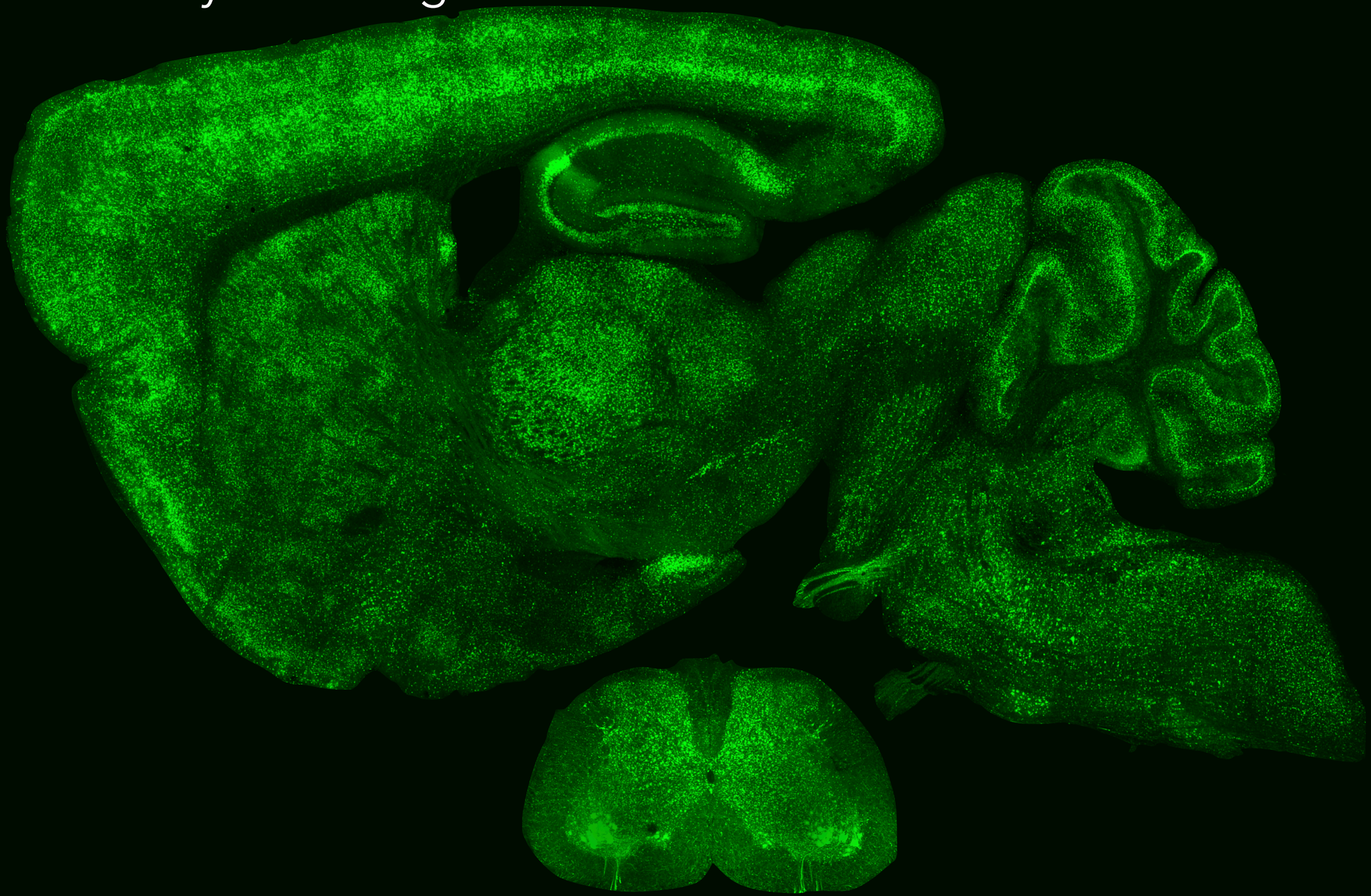
Evolution of a further enhanced CNS-targeted AAV



Ken Chan

Chan *et al. Nature Neurosci* 2017

AAV-PHP.eB enables efficient transduction of neurons and astrocytes throughout the CNS at lower virus doses



Chan *et al*. Nature Neurosci 2017