



MAY 23, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n2bvj87ebgk5/v1

External link:
<https://rdcu.be/c9Vzw>

Protocol Citation: Sripriya Ravindra Kumar, Timothy F. Shay, Xinhong Chen, David Brown, Tatyana Dobрева, Qin Huang, Xiaozhe Ding, Yicheng Luo, Pétur H. Einarsson, Alon Greenbaum, Min J. Jang, Benjamin E. Deverman, Viviana Gradinaru 2023. Cloning individual AAV capsid variants. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.n2bvj87ebgk5/v1>

MANUSCRIPT CITATION: Ravindra Kumar, S., Miles, T.F., Chen, X. *et al.* Multiplexed Cre-dependent selection yields systemic AAVs for targeting distinct brain cell types. *Nat Methods* **17**, 541–550 (2020). <https://doi.org/10.1038/s41592-020-0799-7>

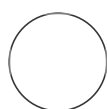
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🌐 Cloning individual AAV capsid variants

Forked from a private protocol

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ABSTRACT












This protocol describes the procedure to clone individual AAV capsid variants for single variant characterization.

Protocol status: Working
We use this protocol and it's working

Created: May 08, 2023

Last Modified: May 23, 2023

PROTOCOL integer ID:
81615

- 1 Digest  pUCmini-iCAP-PHP.B plasmid addgene Catalog #103002 with
 MscI - 1,250 units New England Biolabs Catalog #R0534L and
 AgeI - 1,500 units New England Biolabs Catalog #R0552L
- 2 Design 100 bp primers for the desired plasmid variant that cover the insertion region with ~20 bp overlap of the backbone
- 3 To synthesize the double-stranded DNA fragment, anneal the primers by PCR with 20 cycles of  98 °C for ,  60 °C for  and  72 °C for  using  Q5 Hot Start High-Fidelity 2X Master Mix - 500 rxns New England Biolabs Catalog #M0494L 50s
- 4 Assemble the variant-specific fragment into the digested backbone by Gibson assembly
- 5 Transform the assembled plasmid into  NEB Stable Competent E.coli (High Efficiency) - 20x0.05 ml New England Biolabs Catalog #C3040H
- 6 Select colonies on carbenicillin/ampicillin-LB-agar plates.

