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Cloning individual AAV capsid variants

Forked from a private protocol

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

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Abstract

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

Protocol materials

- MscI - 1,250 units **New England Biolabs Catalog #**R0534L
- AgeI - 1,500 units **New England Biolabs Catalog #**R0552L
- pUCmini-iCAP-PHP.B plasmid **addgene Catalog #**103002
- Q5 Hot Start High-Fidelity 2X Master Mix - 500 rxns **New England Biolabs Catalog #**M0494L
- NEB Stable Competent E.coli (High Efficiency) - 20×0.05 ml **New England Biolabs Catalog #**C3040H

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 00:00:10 , 60 °C for 00:00:30 and 72 °C for 00:00:10 using 50s
Q5 Hot Start High-Fidelity 2X Master Mix - 500 rxns **New England Biolabs Catalog #**M0494L

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) - 20×0.05 ml **New England Biolabs Catalog #**C3040H

6 Select colonies on carbenicillin/ampicillin-LB-agar plates.