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Protocol

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Structure-guided AAV capsid evolution strategies for enhanced CNS gene delivery

In the format provided by the authors and unedited

Supplementary Methods

Determining Viral Titer by qPCR using Roche Light Cycler 480

DNA extraction from Virus

1. Add 10 uL virus to 90uL of DNase I mixture and mix. Incubate for 1 hr at 37° C.
    a. Make sure to include your reference virus in duplicate
2. Add 6 uL of 0.5 M EDTA and mix by vortexing.
3. Add 120 uL of 10% Tween solution, vortex, and incubate for 15 mins

Sample dilution for PCR

4. Add 2 ul of the Tween digested sample to 198 ul of Molecular Biology Grade (MBG) Water to achieve 100× dilution and mix. Set your samples to be quantified aside and continue to serial dilute your reference standard.
5. Make 7 additional standards by performing 2-fold serial dilutions in MBG Water.
    Accurate serial dilutions are critical for achieving correct quantitation. Change pipette tips at each dilution when mixing.

Setting up a plate for qPCR:

6. Prepare your qPCR master mix depending on the number of samples plus 5. Mix the SYBR green, forward and reverse primer and MBG water. Mix by pipetting up and down.

    Per sample

5.0 uL	SyBR Green Master Mix
0.5 uL	Up + Downstream primer (10uM of fwd & rev primer mixture)
2.5 uL	dH_2O

7. Add 8 uL of master mix to the inside wall of each well in row.
8. Add 2 uL dH_2O to the "no template" control reaction.
9. Add 2 uL of standard to each tube.
10. Add 2 uL of sample to each tube.
    Adding the standards in order from lowest to highest minimizes the effect of any cross-contamination on the standard curve.

11. Spin qPCR plate in a Microplate centrifuge
12. Take the plate to the Lightcycler 480 and place the plate in plate reader.

Thermocycler conditions:

Denaturation – 1 cycle
95 ˚C – 10 mins
PCR cycles – 45
95 ˚C for 10 sec
65 ˚C touchdown to 62 ˚C (-0.5 ˚C with 10 cycle delay) for 10 sec
72 ˚C for 10 sec
Melting curve- 1 cycle
95 ˚C for 10 sec
65 ˚C for 1 min
97 ˚C for 1 sec (continuous)

Supplementary Method 1. Determining Viral Titer by qPCR using Roche Light Cycler 480. AAV titer is determined via qPCR with primers specific to the AAV genome and SYBR green DNA polymerase. A viral standard curve is used to quantify vector genome copies. If a viral standard is unavailable, a linearized plasmid consisting of the packaged genome can be used for standard curve generation as described in another *Nature Protocol* [46].