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AAV Production Protocol

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ASAP Collaborative Rese…

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We use this protocol and it's working

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Abstract

Protocol for the production of adeno-associated viruses by the Arenkiel Lab of Team Schlossmacher.

AAV Production Protocol

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2 AAV Production

3 All AAVs were packaged in-house by the Texas Children's Hospital Jan and Dan Duncan Neurological Research Institute's Neuroconnectivity Core, as described below.

4 Cell Culture and Transfection

5 A three-vector system (serotype AAV1, AAV2, AAV5, AAV7, AAV8, AAV9, AAV-DJ/8, AAV-PHP.eB, AAV-PHP.S, AAVrh10, AAV-SCH9 was used for AAV production (Cell Biolabs). HEK-293 cells were plated in 15 cm dishes at a density that yielded ~70% confluency the following day. Cells were then transfected in each plate with 25µg helper plasmid, 25µg serotype specific AAV vector, and 25µg of AAV shuttle vector using polyethylenimine (PEI). In a 1:3 ratio (µg DNA:µg PEI), the solution was added dropwise to cells. After 4-6 hours, the medium was changed to DMEM, 5% FBS, 1x Penicillin/Streptomycin. 48-72 hours later, transfected cells were harvested using a cell scraper. Cells were pelleted by centrifugation at 3500rpm for 10 minutes at 4°C. The supernatant was removed, and the pellet resuspended in TMN (50mM Tris pH8.0, 5mM MgCl2, 0.15M NaCl) at a concentration of 1ml/plate. The resuspended cells were frozen at -80°C overnight.

6 Purification

7 10ul of DNaseI (10mg/ml) and 10ul RNase A (1mg/ml) were added to each plate of defrosted cells in TMN. Plates were incubated at 37°C for 30 minutes, shaking frequently. 100µl of 5% sodium deoxycholate solution in water was added to each plate and mixed gently. Plates were then incubated at 37°C for 10 minutes. The suspended samples were transferred from the plates to tubes and placed on ice for 15 minutes. The tubes were then centrifuged at 3700rpm for 10 minutes and the supernatant was collected.

8 Iodixanol Gradient

9 OptiPrep™ (Millipore Sigma D1556-250ML), or iodixanol, was purchased as a 60% (W/V) stock in water. 15%, 25%, and 40% dilutions of iodixanol were made in PBS-MK (1x PBS, 1 mM MgCl2,

2.5 mM KCl). 2.5µl phenol red solution (0.5% stock in PBS-MK) was added per 1ml of iodixanol solution in the 25% and 60% fractions. The gradient was loaded to the bottom of Beckman OptiSeal 16X67mm tubes (Cat# 362181) starting with 1.5ml 15% iodixanol, 1.3 ml 25%, 1.4ml 40% and finally 1.3ml 60% iodixanol. The supernatant collected from the previous purification step was then placed on top of the gradient. Tubes were centrifuged at 60000rpm for 90 minutes in a Beckman NVT 65 rotor. The clear band below the 60% mark (and below the white cellular debris layer) was collected using a needle and syringe. The collected volume from each tube was approximately 1.5ml.

10 Concentration

11 The goal of this step was to remove the OptiPrep and concentrate the AAV using an Amicon Ultra-15 Centrifugal Filter (Millipore Sigma, UFC9 100 24). An Amicon column was equilibrated with 15ml of DPBS (no Mg, no Ca) by centrifugation at 2500rpm for 5 minutes. The collected band from the OptiPrep gradient was mixed with approximately 40ml of DPBS. The samples were run in batches through the Amicon filter, discarding the filtrate between spins. The virus was then washed three times with 15ml of DPBS with centrifugation after each wash at 2500rpm for 10 minutes. The virus was collected to a sterile microcentrifuge tube, aliquoted, and frozen to -80°C.

12 Viral Titer

13 Titering of virus was performed using Applied Biological Materials qPCR AAV Titer Kit (Cat# G931) and following the manufacturer's recommended protocol. Viral preparations were first diluted to ~108 GC/mL before undergoing viral lysis at room temperature for 3 minutes. A standard curve was generated using five 10-fold serial dilutions of provided Standard Control DNA (dilutions 1/100 to 1/100,000). qPCR components and cycling conditions are found within the manufacturer's accompanying product datasheet. Final titer analysis was performed using the manufacturer's provided calculation file.