AAVehicle: Novel Assembly Activating Protein Domain Shuffling for Enhanced Adeno-Associated Virus Capsid Formation and Gene Therapy as Treatment for Genetic Disorders

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Every one in five adults carry a genetic mutation related disease (Cross 2017). Current neoadjuvant and nanoparticle therapies are ineffective in treatment due to rapid tumor drug resistance, short-term drug delivery, immune system rejection, toxicity, and lack of precise receptor targeting. Adeno-associated virus (AAV) has been identified as a promising candidate for gene therapy, but faces setbacks in capsid assembly due to the coevolution of its capsid proteins, the assembly activating protein (AAP) and VP proteins. This research develops an AAP domain shuffling technique to generate a library of artificially evolved and optimized AAP variants promoting enhanced AAV capsid assembly. The process involves generating new random domain-shuffled AAP inserts, ligating them into plasmid backbones, cloning the plasmids, and transfecting them into AAV-293 cells to produce diverse AAV capsids. Initially, domain DNA overhang sequences were identified using ApE to enable complete shuffling. Ligation confirmed the presence of domain shuffled AAP inserts in 28 out of 30 plasmids. The random shuffling method was further assessed through DNA sequencing which characterized the generated inserts. Every clone with a shuffled insert contained the hydrophobic region domain (HR), with the highest producing AAP having HR-CC and the others with HR-PR. This highlights HR's critical role in optimal capsid assembly. After shuffling, 93% of plasmids yielded AAV production, with a high concentration of 3*10^9 vg/mL titres. The produced AAV particles display clinical potential in developing accessible, AAV gene therapy treatments more efficiently at an industry-scale through enhanced capsid assembly with less resources.

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