

AAV6 Vector Design, Production, and Purification Adeno-associated virus serotype 6 (AAV6) vectors were constructed using the pAAV-MCS plasmid, which contains inverted terminal repeats (ITRs) from AAV2. Additionally, all AAV6 vectors underwent ddPCR for precise quantification of vector genomes. Additionally, a tracking vector for targeted hematopoietic stem/progenitor cells (HSPCs) post-transplantation was developed, featuring a bi-cistronic Nluc cDNA-T2A-TurboGFP cassette driven by the SFFV promoter, with 400 bp homology arms to HBB. The gcHBB-SCD vector was assembled with Gibson Assembly Mastermix and features 2.4 kb of homologous sequence to HBB, including specific SNP variations. In-process testing included silver stain SDS-PAGE analysis, bioburden assessment, and viral genomic titer determination via qPCR. The cells were expanded to a final volume of 3L in spinner flasks and co-transfected with the plasmids using a polyethylenimine (PEI) transfection method.