

Title: An AAV toolbox for enhanced transduction efficiency with regional and/or cell-type specificity in the CNS and PNS after systemic delivery

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Abstract: Recombinant adeno-associated viruses (rAAVs) are extensively used for in vivo gene transfer. Capsids and rAAV genomes that enable widespread and efficient gene expression following noninvasive administration with potential for expression that is restricted to target elements (cell types and/or defined nuclei) are needed. We therefore applied the capsid selection method CREATE (Cre-recombination-based AAV targeted evolution; Deverman et al., Nat. Biotechnol., 2016) to evolve and select for novel capsids that more efficiently or more selectively transduce specific cell populations. Here we report several novel capsids with neural tropisms. First, AAV-PHP.eB, an enhanced version of the previously reported AAV-PHP.B vector, is able to transduce 69% of DAPI+ cortical cells after intravenous delivery of  $1 \times 10^{11}$  vg per adult mouse. Second, AAV-PHP.S, transduces neurons throughout the PNS. At a dose of  $1 \times 10^{12}$  vg per adult mouse, intravenous administration of AAV-PHP.S transduces 82% of PGP95+ dorsal root ganglion neurons. In addition, because of the efficiency of AAV-PHP.S following systemic delivery, it is able to efficiently transduce difficult-to-access sensory neurons, such as those in the cardiac ganglia, or peripheral sensory networks that are widespread, such as the enteric nervous system. Third, we have identified an AAV-PHP.B variant, AAV-PHP.N, which shows strong selectivity for CNS neurons, and reduced liver transduction. To achieve targeted gene expression in nontransgenic animals, we are also presenting on the use of rAAV vectors with both cell type-specific promoters and with miRNA binding sites in the 3' UTR to reduce expression in specific neural cell types. By pairing previously described cell type specific promoters such as serotonergic (FEV), human synapsin 1 (hSyn1), interneuron (Dlx5/6), tyrosine hydroxylase (TH) and Purkinje Cell (PCP), with the AAV-PHP vectors, we can achieve both high efficiency and specificity of gene expression in the CNS and PNS. Finally, we have developed a two-component viral vector system to control the density of labeling when systemically delivering genes with AAV-PHP viruses. We demonstrate the utility of such a system by separately encoding spectrally distinct fluorescent proteins under various gene regulatory elements for single-cell morphology studies. Collectively, the versatility and efficiency of these viral capsids and viral vectors provides an expansion to the current AAV toolbox with potential for efficient and versatile gene manipulation studies.