

Associated Protocol 1: Retro-orbital administration and high-throughput sequencing

ANIMALS. All mice experiments were approved by the Broad Institute IACUC. Wild-type adult C57BL/6J mice (000664) were purchased from the Jackson Laboratory. All mice were housed in a room maintained on a 12 h light and dark cycle with *ad libitum* access to standard rodent diet and water. Animals were randomly assigned to various experimental groups.

RETRO-ORBITAL INJECTIONS. 50 mL of VLPs (containing 4×10^{11} or 7×10^{11} VLPs) were centrifuged for 10 min at 15,000 *g* to remove debris. The clarified supernatant was diluted to 120 mL in 0.9% NaCl (Fresenius Kabi; 918610) right before injection. 1×10^{11} viral genomes (vg) of total AAV was diluted to 120 mL in 0.9% NaCl (Fresenius Kabi; 918610) right before injection. Anesthesia was induced with 4% isoflurane. Following induction, as measured by unresponsiveness to bilateral toe pinch, the right eye was protruded by gentle pressure on the skin, and an insulin syringe was advanced, with the bevel facing away from the eye, into the retrobulbar sinus where VLP or AAV mix was slowly injected. One drop of Proparacaine Hydrochloride Ophthalmic Solution (Patterson Veterinary; 07-885-9765) was then applied to the eye as an analgesic. Genomic DNA was purified from various tissue using Agencourt DNAdvance kits (Beckman Coulter; A48705) following the manufacturer's instructions.

HIGH-THROUGHPUT SEQUENCING OF GENOMIC DNA. Genomic DNA was isolated as described above. Following genomic DNA isolation, 1 mL of the isolated DNA (1–10 ng) was used as input for the first of two PCR reactions. Genomic loci were amplified in PCR1 using PhusionU polymerase (Thermo Fisher Scientific). PCR1 primers for genomic loci are as follows: forward primer 5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNGGCTGCACTTAGAGACCACC-3'; reverse 5'- TGGAGTTCAGACGTGTGCTCTTCCGATCTATGAAGAGCTGATGCTCGCC-3'. PCR1 was performed as follows: 95 C for 3 min; 30–35 cycles of 95 C for 15 s, 61 C for 20 s, and 72 C for 30s; 72 C for 1 min. PCR1 products were confirmed on a 1% agarose gel. 1 mL of PCR1 was used as an input for PCR2 to install Illumina barcodes. PCR2 was conducted for nine cycles of amplification using a Phusion HS II kit (Life Technologies). Following PCR2, samples were pooled and gel purified in a 1% agarose gel using a Qiaquick Gel Extraction Kit (Qiagen). Library concentration was quantified using the Qubit High-Sensitivity Assay Kit (Thermo Fisher Scientific). Samples were sequenced on an Illumina MiSeq instrument (paired-end read, read 1: 200–280 cycles, read 2: 0 cycles) using an Illumina MiSeq 300 v2 Kit (Illumina).

HIGH-THROUGHPUT SEQUENCING DATA ANALYSIS. Sequencing reads were demultiplexed using the MiSeq Reporter software (Illumina) and were analyzed using CRISPResso2 (Clement et al., 2019) as previously described (Doman et al., 2020). Batch analysis mode (one batch for each unique amplicon and sgRNA combination analyzed) was used in all cases. Reads were filtered by minimum average quality score ($Q > 30$) prior to analysis. The following quantification window parameters were used: -w 20 -wc -10. Base editing efficiencies are reported as the percentage of sequencing reads containing a given base conversion at a specific position. Prism 9 (GraphPad) was used to generate dot plots and bar plots.

REFERENCES

Clement, K., Rees, H., Canver, M.C., Gehrke, J.M., Farouni, R., Hsu, J.Y., Cole, M.A., Liu, D.R., Joung, J.K., Bauer, D.E., and Pinello, L. (2019). CRISPResso2 provides accurate and rapid genome editing sequence analysis. *Nat. Biotechnol.* 37, 224–226.

Doman, J.L., Raguram, A., Newby, G.A., and Liu, D.R. (2020). Evaluation and minimization of Cas9-independent off-target DNA editing by cytosine base editors. *Nat. Biotechnol.* 38, 620–628.