***Editing Efficiency of in vitro Experiments***

***Primary cultures of rhesus airway epithelia.***

Primary rhesus macaque airway epithelial cells were isolated from post mortem tracheal tissues provided by the Tulane National Primate Research Center, Covington, LA. Cultures were prepared in the University of Iowa’s In Vitro Models and Cell Culture Core. Primary rhesus airway epithelia isolated from donor trachea or bronchi were grown at the air-liquid interface on collagen coated Costar Transwell polycarbonate filters (#CLS3413, 0.3 µm2 surface area) as reported previously. Cultures were maintained in media supplemented with Ultro-ser G (USG) and the following antibiotics: penicillin (50 units/ml), streptomycin (50 μg/ml). The cultured cells were maintained at 37°C in 5% CO2. All primary epithelial cells were well-differentiated (>4 weeks old).

***ABE8e-Cas9*** ***RNP formulation and application to cultured epithelia.***

Candidate guide RNAs targeting the rhesus CCR5 gene were screened in rhesus skin fibroblasts. The guide RNA (gRNA) was prepared by combining the crRNA (IDT) and tracrRNA (IDT, Coralville, IA, catalog #1072532) at equimolar concentrations (100 µM), annealing at 95°C for 5 min and renaturation at room temperature as described previously. The RNP was prepared by combining the gRNA and recombinant ABE8e-Cas9 protein in PBS, and incubating at room temperature for 15-20 min. The final concentration of gRNA was 26 µM and ABE8e-Cas9 was 11 µM in a total volume of 10 ml RNP.

***Next Generation Sequencing and analysis.***

Genomic DNA was isolated from the cells using QuickExtract (catalog #QE09050, Lucigen, Middleton, WI) according to the manufacturer’s protocol. The target locus was PCR amplified (KAPA DNA polymerase, Roche, Basel, Switzerland)) using primers designed to the rhesus *CCR5* locus and the appropriate Illumina forward and reverse adaptors as described (table below). Unique Illumina barcoding primer pairs were then added to each sample in a second PCR reaction. Purified PCR products next quantified and sequenced using a single-end read of 200-250 bases on the Illumina MiSeq instrument using the manufacturer’s protocols. Following high throughput sequencing (HTS), the sequencing reads were demultiplexed using MiSeq Reporter (Illumina) and aligned to the appropriate reference genome as previously reporter. Base substitution and indel frequencies were assessed using the software package CRISPResso2, which counts indels of ≥1 base occurring in a 30-base window around the ABE nicking site. Indels were defined as detectable if there is a significant difference (Student’s two-tailed *t*-test, *P* < 0.05) between indel formation in the treated sample and untreated control. Base editing frequencies were further assessed using a previously described MATLAB script. For each *CCR5* modification we determined the target base editing frequencies, bystander edits, and indel frequency. The allelic editing frequency was calculated by the following equation: (% editing-50)/50.

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| Target Gene | gRNA sequence | PAM | Forward Primer\* | Reverse Primer\* | |
| CCR5 | GAG**A**GTTTCTTGTAGGGGAA | CGG | 1: ACTGTCTATATGATTGATTTGCACAACT  2: CGCCTTCAATACTCAATTATTTAACTC | | 1: CCTTTAAAGTCTTTCACTCACAATC  2: GTCAGAGTTTAGAATGAGTTTCAGATT |
| B2M | GAGT**A**GCGCGAGCACAGCTA | AGG | 1: CTGGGCACGCGTTTAATATAAG | 1: CACCAAGGAGAACTTGGAGAA | |

\* Adapters were added to the primer in the following format:

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNN-Forward Primer Sequence

TGGAGTTCAGACGTGTGCTCTTCCGATCT-Reverse Primer Sequence