

Generation of mutation-corrected isogenic LVPEI006-B-1 iPSC line

The LCA12 patient-specific **LVPEI006-B** iPSC line was used as the parental stem cell source to generate mutation-corrected isogenic **LVPEI006-B-1** iPSC lines. CRISPR guide RNA sequence-specific sense and anti-sense DNA oligos were designed to target the mutation region, annealed *in vitro*, and cloned at the BbsI site of the en31FnCas9-ABEmax8.17d vector and sequence confirmed. The RD3 gRNA target region sequence is 5' TCCTCAGATGAGCACTGGGAT 3'. The guide encoding plasmid construct was then transfected into the parental hiPSCs at 50-60% confluency, using the LipofectamineTM Stem Transfection Reagent (Invitrogen Cat. No# STEM00001), according to the manufacturer's instructions. The transfected cells were seeded in low density and then clonally expanded. Single cell clones were manually picked and clonally expanded for genotyping. Genomic DNA was isolated and used as templates for target region-specific PCR using the forward primer: 5' ATGGTGCTGGAGACGCTTAT 3' and the reverse primer: 5' CTCCTGCTTCATCCTCTCCA 3' and the amplicons were subjected to Sanger sequencing to confirm the presence of edits. Clones with confirmed mutation-correction were maintained in Essential 8TM medium (Thermo Fisher Scientific) at 37°C with 5% CO₂ supply and were passaged at regular intervals in 1:6 split ratios using cell dissociation solution (0.5 mM EDTA and 30 mM NaCl in 1X DPBS) and rock inhibitor treatment. Passage 10 cells were characterized for their stemness, pluripotency, and genomic integrity.