Genetic Correction for <u>all</u> inherited retinal disease.

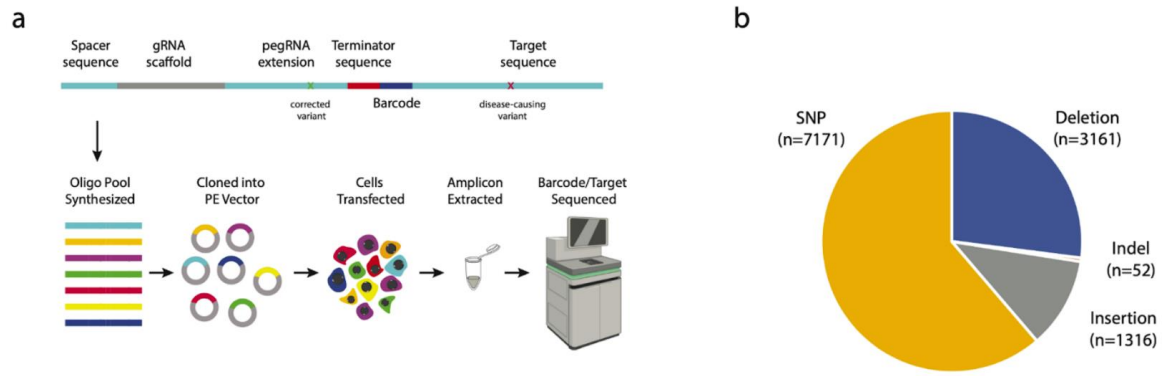
The most recent addition to the gene editing armamentarium "prime editing" has been shown to allow the correction of small genetic changes. However, this new gene editing tool requires specific optimisation for each individual genetic change, and as such needs to be individually tailored. With support from Retina Australia we conducted a large-scale study to identify specific patterns in "prime editing" construct design. Insights from this work could potentially be applied to all inherited retinal diseases when diagnosed early.

Specifically, we investigated the generalizable characteristics of non-engineered prime editing design for efficient proof-in-principle gene correction of disease causing variants in 21 genes implicated in inherited retinal diseases, and associated syndromes (such as Usher, and Bardet Biedl syndrome). We used an "oligopool" approach comprising approximately 12,000 uniquely-barcoded RNA constructs to targeting a synthetically integrated, mutation-specific sequence, which faithfully recapitulates the disease context. To dissect cell-specific variation we characterised the efficiency of genetic correction across eye cells (photoreceptor and RPE-derived cells).

We found that non-engineered extensions should mediate substitution-type edits as compared to indel-type corrections, and that importantly, the desired edit should be placed close to the target site.

This work has helped establish a set of recommendations for the generalizable design of the prime editors for the correction of inherited retinal disease-causing variants. This will facilitate the ongoing clinical translation of gene editing technologies for the potential correction of inherited retinal disease caused by small genetic changes.

Oligo pool amplicon structure comprising 12,000 diverse, non-engineered pegRNAs leveraging the Prime Editor 2 (PE2) system to target a barcoded, lentivirally integrated 50 bp motif recapitulating 21 dysregulated genes implicated in inherited retinal diseases (IRDs). a) Lentiviral particles encoding the oligo pools were transduced into three different cell lines (HEK293A, Y79, and ARPE19), and the cell lines were transfected with a plasmid bearing an antibiotic selectable PE2-system for highthroughput analysis of editing outcomes. b) Breakdown of the targetable edit-types comprised by the oligo pool for the sequence determinants amenable to high editing efficiencies for IRDs. SNPs designate single nucleotide polymorphisms correctable by a single base transition or transversion, whereas deletions are correctable via PE2-mediated insertion. Similarly, insertions are correctable by PE2-mediated deletions, with indels referring to NCBI nomenclature wherein target sequences are correctable with either PE2-mediated insertions or deletions.



Binned features of key sequence determinants reveal cell-line agnostic features conducive towards high editing efficiencies using the PE2 system in HEK293A, ARPE-19 and Y79 cell lines. a) Non-parametric statistical analyses reveal that substitution-type edits constitute the vast majority of efficient PE2-mediated corrections for IRDs (n=1864, n=911, and n=346 for substitutions, insertions and deletions, respectively). b) non-engineered pegRNAs show a preference for a canonical non-cytosine nucleotide as the first nucleotide of the pegRNA extension (n=769, n=448, n=980, and n=924 for adenine, cytosine, guanine, and thymine, respectively). c) High editing efficiency is achieved when the edit is positioned +3 to +5 nucleotides upstream of the putative Cas9-nicking position (n=1117, n=1198, n=516, n=290 for 0 to +2, +3 to +5, +6 to +8, and +9 and more, respectively).