PRECISION EDITING OF THE CHO GENOME VIA CRISPR-BASED PRIME EDITING: PROGRESS AND CHALLENGES

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Cell line engineering via CRISPR-based tools has recently enabled precise control to effect gene knockouts and precision insertion of genetic elements. These tools function through a site-specific creation of double stranded DNA break which is resolved through either non-homologous end joining which leaves genetic scars (small insertions or deletions or 'indels') that disrupt gene function (for knockouts), or homology directed repair with a user-supplied template that can insert genetic cargo at a specific location (for insertions). The recent addition of the 'prime editing' method uses the precise control of the Cas9 and couples it with a reverse transcriptase to introduce nucleotide level edits using an extended guide RNA (called a pegRNA) as a user-defined template. When successful, this method enables scarless single nucleotide resolution editing. In this work, we describe efforts toward using this method to study DNA double stranded repair genes in CHO cells that are mutated in conserved regions compared to the same genes in the Chinese Hamster genomes (and other mammals). These single-nucleotide mutations in these genes are potential targets for genomic modification because they may have an impact on CHO cell line stability, a well-established problem in CHO cell lines.

We show progress to alter CHO chromosomal genes to match the protein amino acid sequences with that of the Chinese hamster using the CRISPR-based prime-editing method. This process has been performed with multiple gene targets on both K1 and VRC01 cell lines. While editing efficiencies of this method have been published at rates as high as 50%, our initial attempts showed no successful editing. To increase the desired modification editing efficiency, we describe the development of a two-fluorophore system to be used with fluorescence-activated cell sorting and single cell isolation, a consolidation of genetic parts to reduce the number of plasmids, optimization of PE3 targeting location, and inclusion of RNA structures to prevent degradation of the pegRNA. Preliminary results yielded 1% editing efficiency and subsequent efforts show further progress. We discuss a rationale for the specific challenges CHO cells face using this method. Additionally, we describe a workflow for recursive editing the genome.