EPA-1 Optimization of precise genome editing in human induced pluripotent stem cells

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Patient-specific induced pluripotent stem cells (iPSCs) that carry disease-relevant genetic mutations provide promising source for in vitro disease modeling. However, variable genetic background of each iPSC, which hampers disease-relevant phenotypical changes, presents a particularly significant impediment to this modeling approaches. Here, we present a generally applicable solution to this key problem by demonstrating the generation of a panel of isogenic disease and control cell lines from hiPSCs that differ exclusively at a specific gene of interest by combining TALENs (or CRISPR/Cas9)-mediated genome editing and iPSC culture technology.

First, to make a knockout mutation in a gene, we electroporate a donor plasmid containing drug resistant gene with TALEN (or Cas9/sgRNA) vector to introduce homology directed repair (HDR). Electroporated hiPSCs are plated onto dishes with drug resistant mouse embryonic fibroblast (MEF) and subjected with drug selection. The resulting colonies are selected and analyzed by PCR.

Next, to make a frameshift or missense mutation in a gene, we introduce non-homologous end joining (NHEJ). However, analyzed colonies are often mixtures of targeted and non-targeted cells and required sib-selection. In our method, we prepare iPSC line containing drug resistance gene in a safe harbor locus. Cells are electroporated with TALEN (or Cas9/sgRNA) vector, mixed with a normal iPSC line without drug resistance, and then plated onto drug resistant MEF. Mixed normal iPSCs are expected to sustain the viability of single cell-derived, gene-targeted iPSCs and be easily removed by drug selection. Eventually we are able to obtain homogenous cell population with a targeted mutation from the resultant colonies.

Taken together, our efficient strategies for precise gene editing in hiPSCs will enable sophisticated genome engineering, which is suitable for in vitro modeling of various diseases.

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