Generation of Edited Induced Pluripotent Stem Cells as Cell Models

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Abstract

The ability to generate patient-specific induced pluripotent stem cells (iPSCs) offers a powerful platform for the generation of physiologically relevant cell models for dissecting basic biology and for use in drug screening. A critical requirement for such models will be generation of homogeneous cell populations that can be characterized and confirmed to be the cell type of interest prior to use in downstream studies.

Reporters driven by lineage-specific promoters are powerful tools to identify and enrich specific cell types in a heterogeneous mixture of cells. In addition, they offer the advantage of enabling live monitoring of cells during complex biological processes. We have generated stable human embryonic stem cell (ESC) lines using non-integrating episomal vectors or site-specific insertion of the reporters into safe-harbor genomic sites. Both methods achieve context-specific expression with measurable GFP in the pluripotent state and absence of GFP after differentiation. While the hOCT4-GFP episomal ESC line is free of genomic effects, reporter expression level is heterogeneous and non-clonal. The hOCT4-GFP ESC line generated using site-specific genomic insertion exhibits a more homogeneous and uniform reporter expression but is not a true indicator of regulated expression by the endogenous promoter. Therefore, the endogenous genomic site was targeted to create lineage-specific reporter knock-in lines. Human iPSC and ESC were edited for the insertion of a GFP-Neoycin fusion expression cassette at the endogenous OCT4 locus using GeneArt™ Precision TALEs and CRISPR/Cas9 based gene editing technologies. Clones with confirmed editing and no detectable off-target insertions, will be expanded and assessed in parallel with existing engineered lines for sensitivity and context-specific expression of the GFP reporter, and will be further used as cell models.

Materials and Methods

• iPSC derivation was carried out with Neonatal Human Dermal Fibroblasts transduced with CytoTune™-iPSC 2.0 Reprogramming Kits.
• Gene expression analysis was carried out with the TaqMan® hPSC Scorecard™ Assay.
• Media containing small molecules were prepared once, on Day 6 of reprogramming and used for the remainder of the experiment.
• Feeder-free Matrices were coated on the day of re-plating. Inactivated MEFs were plated one day before day -7 re-plating.
• CRISPR targets were designed using the MIT CRISPR Design tool (http://crispr.mit.edu/).
• CRISPR sequences were ordered as custom oligos and cloned into Top10 Chemically competent E. coli. Plasmids were confirmed via sequencing (Regenon Inc.)
• G-418 kill curve and Neon optimization assays were generated by treating 100k cells/well with ROCKi for 5hrs pre dissociation and 24hrs, post dissociation. G-418 treatment was initiated after the 24th hour of treatment.

RESULTS

1. Generation of iPSCs: SA-BS2-1-C1

2. Methods to enhance Reprogramming Efficiency

3. Genome Editing

4. CRISPR/Cas9 genome editing is based on the Type-2 CRISPR-Cas system and consists of a CRISPR RNA (crRNA) which can be custom-designed to target homology with specific genomic sequences. The Cas9 endonuclease can be guided to target the section of the genome in order to produce double strand breaks in the DNA.

CONCLUSIONS

CytoTune™ 2.0 is an easy and efficient way to generate iPSC. Resulting iPSC clones are functionally pluripotent as confirmed by marker expression and in vitro differentiation potential, and maintain normal karyotype.

REFERENCES

(1) Da-Woon Jung, et al., (2014) Reprogram or Reboot: Small Molecule Approaches for the Production of Induced Pluripotent Stem Cells and Direct Cell Reprogramming. ACS Chemical Biology 9 (1), 80-95; DOI: 10.1021/cb400754f

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