BioResearch

Genomic Editing and Nucleofection: ZFN, TALEN, and CRISPR and their Potential for the Generation of Custom Cell Lines and Modified Primary Cells for Use in Cell-Based Assays

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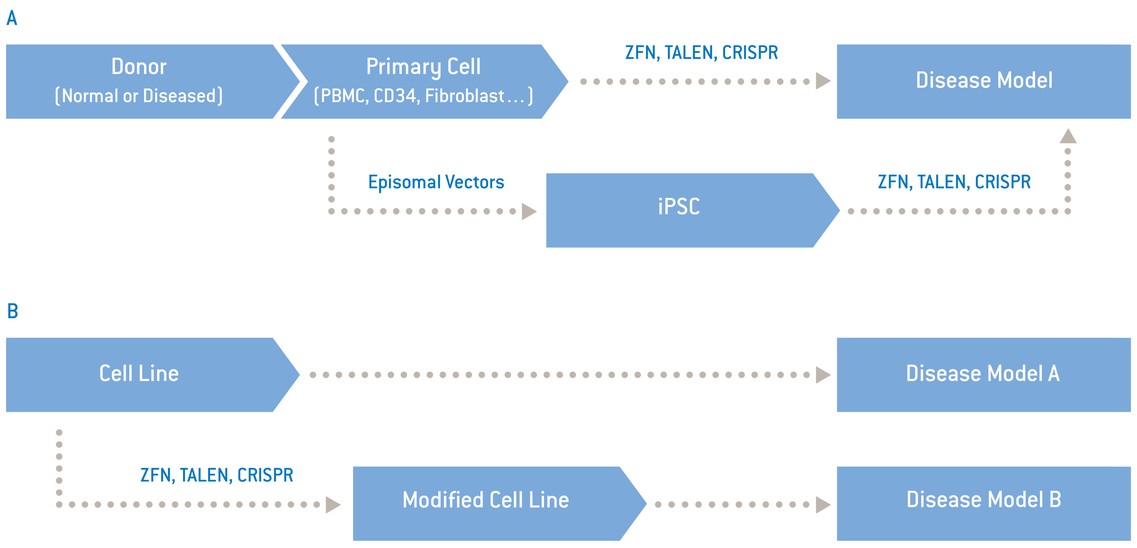
Abstract

Genomic editing technologies like ZFN, TALEN, and CRISPR are leading to a revolution in the research of gene function, and the possible use of clinical transfection to repair and cure the genetic basis of disease. These technologies can be used to insert or delete DNA in the genome, repair defective genes, introduce mutations into specific genes, or create knockouts by the introduction of frame-shift mutations into a gene, and create unique model systems containing specific gene configurations that can be used in cell-based assays.

Regardless of the genome editing technology chosen, however, not only is co-transfection required, but it is critical to get the constructs into the appropriate cell type. Nucleofection excels at the delivery of DNA and other substrates into primary cells and cell lines, and is thus an ideal companion technology for ZFN, TALEN, and CRISPR delivery.

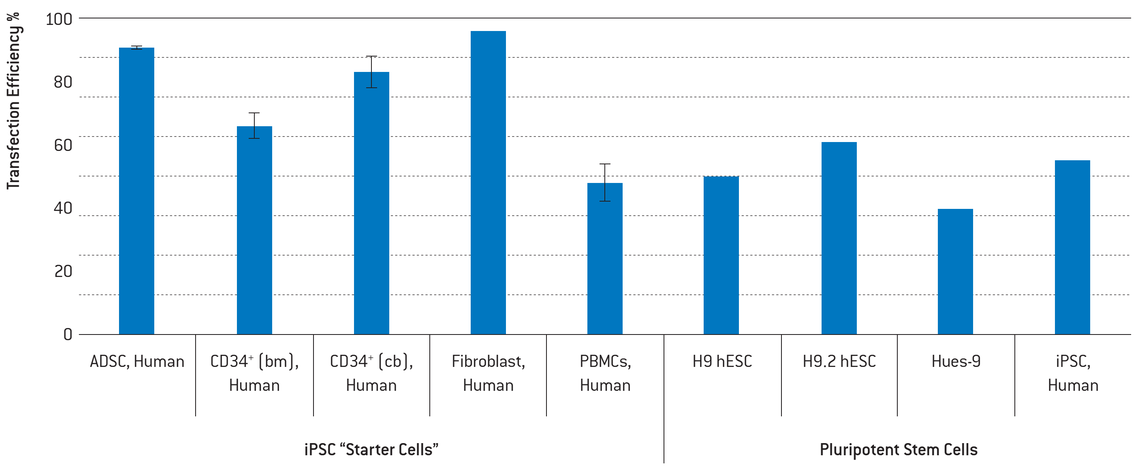
Lonza's Nucleofector™ Technology allows the researcher to transfect primary cells, adult and embryonic stem cells, and generate iPS cells in a non-viral fashion. Nucleofection is an electroporation-based transfection technology that is extremely versatile, with the ability to transfect plasmid DNA, oligonucleotides, siRNAs, mRNA, peptides, or even the necessary genomic editing constructs, with high transfection efficiency and cell viability, allowing researchers to use the most physiologically-relevant primary cells, or cell lines that more closely depict relevant disease states in their research.

Figure 1. Cell-based Assay Strategies



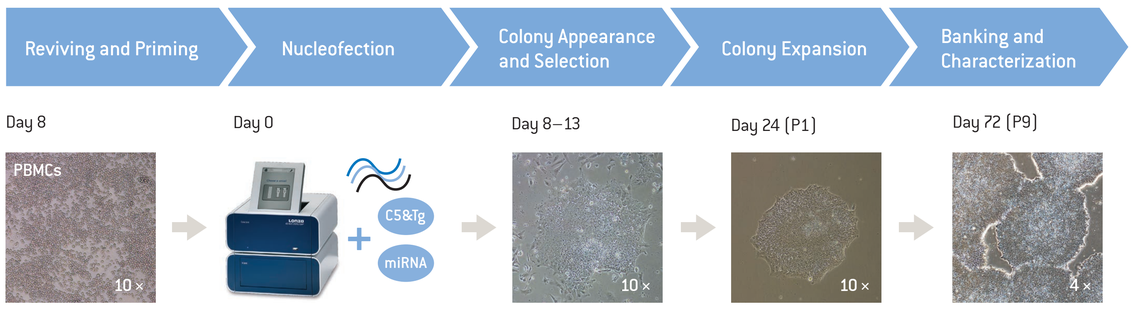
The versatility of Lonza's Nucleofector™ Technology in regard to cells and substrates allows for different strategies to generate cell models for cell-based assays.
A – Direct modification of donor-derived primary cells using genome editing tools or by first generating donor-specific iPSCs and modifying those in a second step.
B – Generation of isogenic cell lines for comparing the effect of a single targeted modification.

Figure 2. High Transfection Efficiencies in Primary Cells and Pluripotent Stem Cells



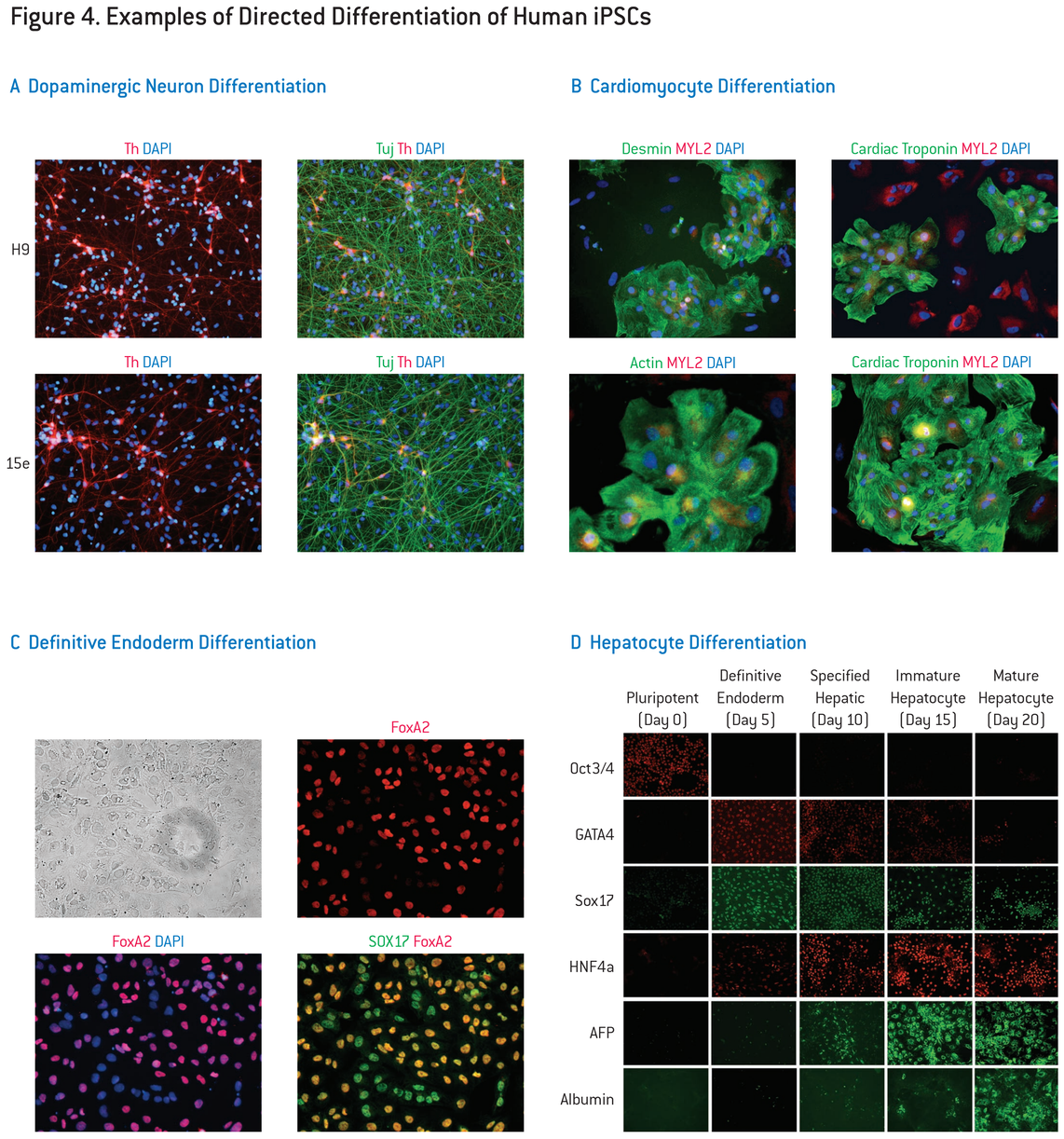
Average transfection efficiencies are shown for primary cell types that can be used as "starter cells" for iPSC generation and for pluripotent stem cell clones.

Figure 3. Overview of Derivation of Human iPSCs



Derivation of hiPSCs using the L7™ hiPSC Reprogramming and hPSC Culture System. Combining Lonza's newly developed hPSC culture system (medium, matrix and passaging solution) with an optimized reprogramming technology results in vector-free hiPSC generation. This reprogramming technology allows robust and efficient derivation of hiPSCs from cord blood (CB)-derived $CD34^+$ cells, and peripheral blood mononuclear cells (PBMNCs).

Figure 4. Examples of Directed Differentiation of Human iPSCs



Human iPSCs derived from human $CD34^+$ or PBMC cells, characterized (data not shown), and then differentiated into dopaminergic neurons (A), cardiomyocytes (B), definitive endoderm (C) or hepatocytes (D). Differentiated cells were stained by cell-type specific markers:
A – Neurons: Th (tyrosine hydroxylase), Tuj (Tubulin β 3)
B – Cardiomyocytes: Desmin, MYL2 (myosin light chain 2), Actin, Cardiac Troponin
C – Endoderm: FoxA2 (forkhead box A2), Sox17 (early endodermal marker)
D – Hepatocytes: Oct3/4 (stem cell marker), GATA4 and Sox17 (early endodermal markers), HNF4a (hepatocyte nuclear factor 4), AFP (α-fetoprotein), Albumin