High Efficiency Transfection of iCell® Cardiomyocytes and Stem Cell Relevant Cell Sources

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Abstract

Stem cell research holds the promise of addressing fundamental questions in biology and revolutionizing therapy for a myriad of diseases. Researchers can utilize stem cells for elucidating disease models through differentiation of both normal and diseased cells into biologically relevant and homogenous cellular lineages. Transfection of nucleic acids such as plasmid DNA, mRNA and siRNA serves as a vital tool in stem cell reprogramming and differentiation processes. *Trans*IT® Transfection Reagents and Ingenio® Electroporation Solution will be highlighted for high efficiency delivery of plasmid DNA and siRNA to iCell Cardiomyocytes and induced pluripotent stem (iPS) cells.

Ideal Entry Points for Transfection in Stem Cell Workflow

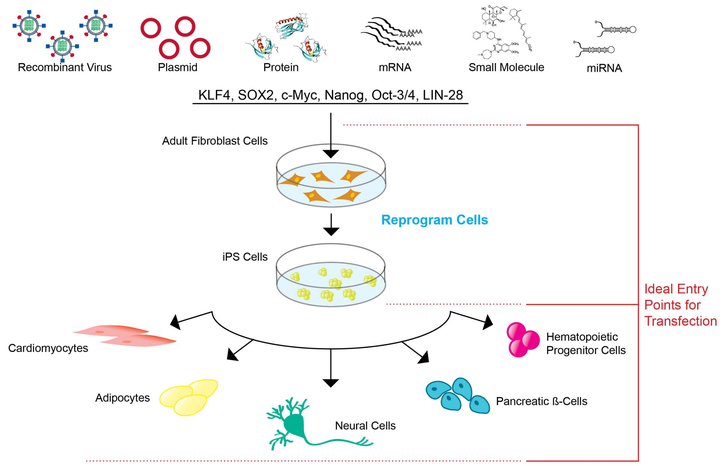

Figure 1. Schematic of Somatic Stem Cell Reprogramming

Somatic cells such as adult fibroblast cells can be transfected or transduced via several methods (e.g. recombinant virus, plasmid, protein, mRNA, small molecule and miRNA) with a combination of transcription factors including KLF4, SOX2, c-Myc, Nanog, Oct-3/4 and LIN-28 to reprogram the cells to a pluripotent state. iPS cells can then be differentiated to a myriad of cell types through growth factor addition and/or transfection of selection markers driven by cell type specific promoters. Stem cell derived cell types such as cardiomyocytes, adipocytes, neural cells, pancreatic β-cells, and hematopoietic progenitor cells can provide researchers with relevant models for their experiments.

mRNA Transfection of Fibroblast Cell Types

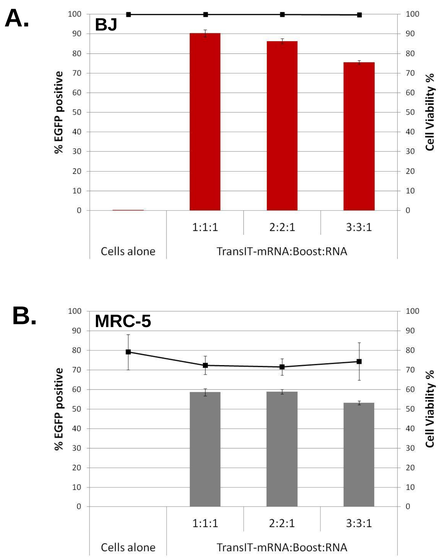

Figure 2. High Efficiency Delivery of mRNA to BJ and MRC-5 Fibroblasts

The *Trans*IT®-mRNA Transfection Kit was used to transfect BJ human neonatal foreskin fibroblasts (A) and MRC-5 human lung fibroblasts with a GFP mRNA incorporating pseudouridine and 5mC modified bases (Trilink Biotechnologies, Inc.). Transfections were performed in 12-well plates using 1-3 µl of *Trans*IT-mRNA Transfection Reagent and mRNA Boost Reagent to deliver 1 µg of RNA (1:1:1, 2:2:1 and 3:3:1, reagent: boost: RNA ratio). Cells were assayed 18 hours post-transfection on a BD LSR II Flow Cytometer. Cell viability was measured using propidium iodide stain (black line).

High Efficiency DNA Transfection of Human iPS Cells

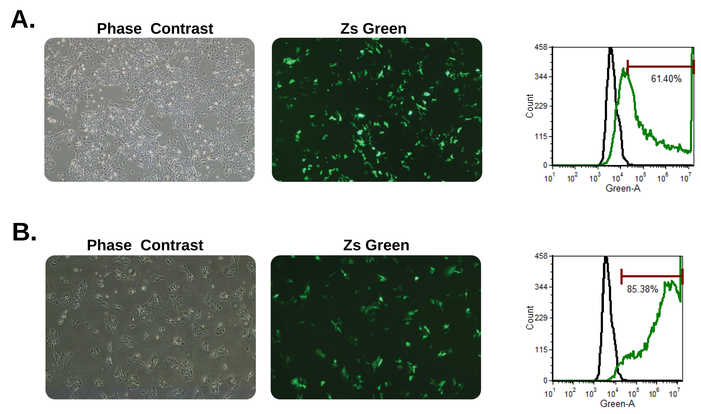

Figure 3. Human iPS Cells Express ZsGreen using *Trans*IT®-2020 Transfection Reagent or Ingenio® Electroporation Solution

The *Trans*IT®-2020 Transfection Reagent was used to transfect 0.5 x 10^6 iPS cells with a ZsGreen™ expression plasmid (Clontech) (A). Transfections were performed in 6-well plates using 7.5 µl of *Trans*IT-2020 Transfection Reagent to deliver 2.5 µg of DNA (3:1, reagent: DNA). The Ingenio Electroporation Kit was used to transfect 2 x 10^6 iPS cells on the Amaxa® Nucleofector® II Device (B). Cells were electroporated with 8 µg ZsGreen expressing plasmid (Clontech) in 100 µl and plated in 6-well plates at 0.33 x 10^6 cells/well. Cells were visualized 24 hours post-transfection and imaged at 4X objective with an Olympus IX71® inverted microscope. Images were acquired using phase contrast and green fluorescence. Cells were assayed at 24 hours post-transfection on an Accuri® Cytometer. The histogram represents the fluorescence intensity of ZsGreen in untransfected cells (black line) compared to cells transfected with plasmid (green line).

Plasmid DNA Delivery to iCell Cardiomyocytes

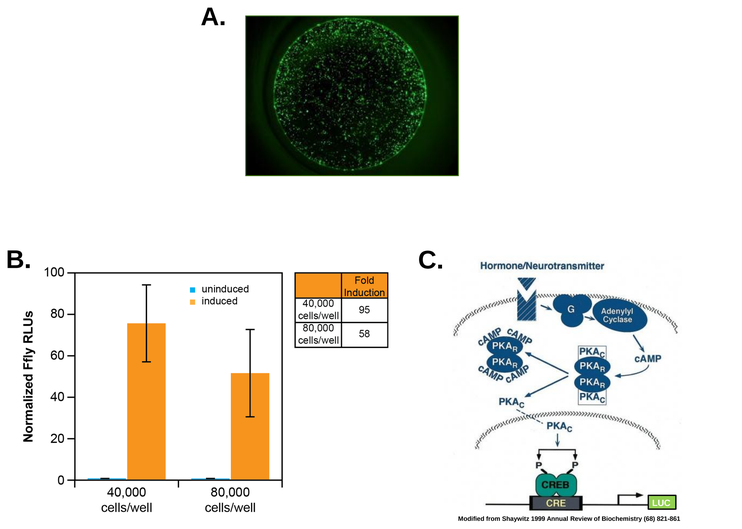

Figure 4. Plasmid DNA Delivery to iCell® Cardiomyocytes using *Trans*IT® -LT1 Transfection Reagent

Panel A illustrate high efficiency transfection of a GFP encoding plasmid. iCell Cardiomyocytes were plated at 20,000 cells/well in a 96 well tissue culture plate coated with 0.1% gelatin. After allowing the cells to recover from thaw, cells were transfected with 100 ng/well of pMAXGFP (Lonza) using *Trans*IT-LT1 Transfection Reagent with a 2:1 reagent-to-DNA ratio according to the manufacturer's instructions. Fluorescent images were taken 3 days post transfection using a Olympus IX71® inverted microscope.

Panel B illustrates cAmp induction measured via a luciferase reporter plasmid. iCell Cardiomyocytes were plated for 5 days and subsequently replated using 40,000 or 80,000 cells/well in a 96 well plate pre-coated with gelatin. Three days post-replating cells were transfected using *Trans*IT-LT1 and a CRE-luciferase reporter plasmid. After 18 hours the cAMP pathway was induced using 10 µM isoproterenol for 6 hours. Luciferase activity was measured using the Promega Dual Glo® Luciferase Assay. Data is normalized to the control reporter.

Panel C is a schematic cAMP mediated luciferase expression. Induction of the cAMP pathway through isoproterenol leads nuclear translocation of cAMP response element-binding protein (CREB) protein and expression of the luciferase reporter protein.

siRNA Knockdown in iCell Cardiomyocytes

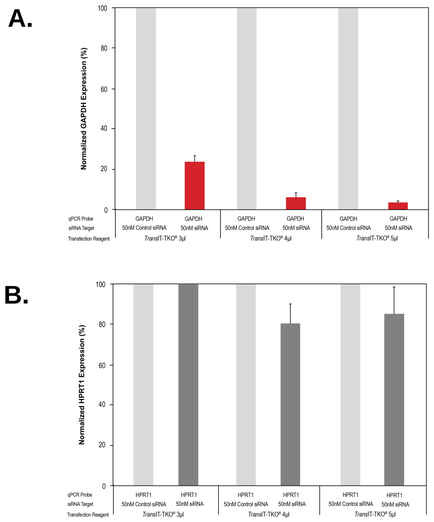

Figure 5. siRNA-mediated Gene Silencing by *Trans*IT-TKO® Transfection of iCell® Cardiomyocytes

Panels A and B show the effect of GAPDH-targeted siRNA on GAPDH (targeted) and HPRT1 (non-targeted) mRNA expression, respectively. iCell Cardiomyocytes were cultured for 7 days in a 12-well cell culture plate before transfection with either control (scrambled) or GAPDH siRNA (sense: GCUCAUUUCCUGGUAUGACUU; antisense: GUCAUACCAGGAAAUGAGCUU) using *Trans*IT-TKO (3 - 5 µl/well). 72 hours post-transfection the GAPDH and HPRT1 (non-targeted) mRNA levels were measured relative to 18s rRNA levels and normalized to the mRNA levels obtained following transfection of the control siRNA in each experiment. The bar graphs show the mean with standard error of the mean (SEM) of three independent transfection complexes.

*Trans*IT® Transfection with Stem Cells and Related Cell Types

Cell Type	Nucleic Acid	Mirus Reagent	Application	Reference
Human embryonic stem cell derived neural progenitors	Plasmid DNA	*Trans*IT-2020	Stem cell transfection	Scientist feedback
Human foreskin fibroblasts	Plasmid DNA	*Trans*IT-2020	DNA transfection	Scientist feedback
Human induced pluripotent stem (iPS) cells	Plasmid DNA	*Trans*IT-2020	Stem cell transfection	Figure 3A.
	Plasmid DNA	Ingenio	Stem cell transfection	Figure 3B.
Human mesenchymal stem cells	Plasmid DNA	*Trans*IT-2020	Stem cell transfection	Scientist feedback
Human skin fibroblasts	Plasmid DNA	*Trans*IT-2020	DNA transfection	Scientist feedback
iCell Cardiomyocytes	Plasmid DNA	*Trans*IT-LT1	Stem cell transfection	Figure 4.
Mouse embryonic fibroblasts	Plasmid DNA	*Trans*IT-2020	DNA transfection	Scientist feedback
Mouse embryonic stem cell derived cardiomyocytes	Plasmid DNA	*Trans*IT-2020	Stem cell transfection	Scientist feedback
Normal human dermal fibroblasts (NHDF)	Plasmid DNA	*Trans*IT-2020	DNA transfection	Scientist feedback
SRF -/- mouse embryonic stem cells	Plasmid DNA	*Trans*IT-LT1	Stem cell transfection	Staus et al. 2007 Arterioscl Throm Vas 27:478-486
BJ human neonatal foreskin fibroblasts	mRNA	*Trans*IT-mRNA	Stem cell reprogramming	Warren et al. 2010 Cell Stem Cell 7(5):618-30.
C3H/10T1/2	mRNA	*Trans*IT-mRNA	Stem cell reprogramming	Angel et al. PLoS ONE 5(7): e11756.
CCD-1109Sk human normal adult skin fibroblasts	mRNA	*Trans*IT-mRNA	Stem cell reprogramming	Angel et al. PLoS ONE 5(7): e11756.
MRC-5 human lung fibroblasts	mRNA	*Trans*IT-mRNA	Stem cell reprogramming	Angel et al. PLoS ONE 5(7): e11756.
Primary human neonatal epidermal keratinocytes	mRNA	*Trans*IT-mRNA	Stem cell reprogramming	Warren et al. 2010 Cell Stem Cell 7(5):618-30.
Primary human lung fibroblasts	mRNA	*Trans*IT-mRNA	Stem cell reprogramming	Warren et al. 2010 Cell Stem Cell 7(5):618-30.
Human mesenchymal stem cells	siRNA	*Trans*IT-TKO	Stem cell differentiation	Andersen et al. 2010 Molecular Therapy 18(11): 2018-2027
iCell Cardiomyocytes	siRNA	*Trans*IT-TKO	Knockdown of gene expression	Figure 5.
Human adipose derived adult stem cells (hADAS)	siRNA	*Trans*IT-siQUEST	Knockdown of gene expression	Wall et al. 2007 American Journal of Physiology 293(5):C1532-8

Conclusions

Mirus Bio nucleic acid delivery reagents enable high efficiency transfection of stem cells and other hard-to-transfect cell types used for stem cell research.

- Perform mRNA transfections in source-specific cell fibroblasts using *Trans*IT-mRNA Transfection Kit
- Transfect plasmid DNA effectively into iPS and differentiated cells with *Trans*IT-LT1 and *Trans*IT-2020 Transfection Reagents
- Introduce siRNA for silencing of differentiated cell types with *Trans*IT-TKO Transfection Reagent
- Electroporate stem cells cost effectively with Ingenio Electroporation Solution