Dreamfect™ Stem– Results

Stem cells are widely investigated due to their regenerative potential. In many cases, it would prove useful to enhance or silence some of their features through gene delivery strategies to harness their full potential cell/gene-based therapy. Because stem cells behaviour, physiology and properties are quite distinctive to other cells, OZ Biosciences has developed a specific transfection reagent for stem cells. DreamFect™ Stem enables transfection of multipotent stem cells, embryonic and iPS with high efficiency and very low toxicity. Its specific biodegradable composition allows to transfect stem cells in presence of serum and to maintain their undifferentiated stage and capacities to differentiate.

DreamFect™ Stem main Benefits:

1. High transfection efficiency for multipotent stem cells, embryonic and iPS
2. Minimized toxicity due to reagent biodegradability and low DNA amount required
3. Cell phenotype and differentiation potential are not affected
4. Serum Compatible
5. Simple, Ready-to-use and rapid (no specific buffer)

DreamFect™ Stem transfection efficiency on different multipotent stem cells

DreamFect™ Stem exhibits high transfection efficiency on human adult multipotent stem cells such as MSC (Mesenchymal Stem Cells), AdSC (Adipose derived Stem Cells), AFSC (Amniotic Fluid Stem Cells), and KG1a (leukemic stem-like cells; Fig. 1, Fig. 2). A dose-response study demonstrates that the best results are achieved for a ratio of 3 µL of DreamFect™ Stem per µg DNA (Fig. 3).

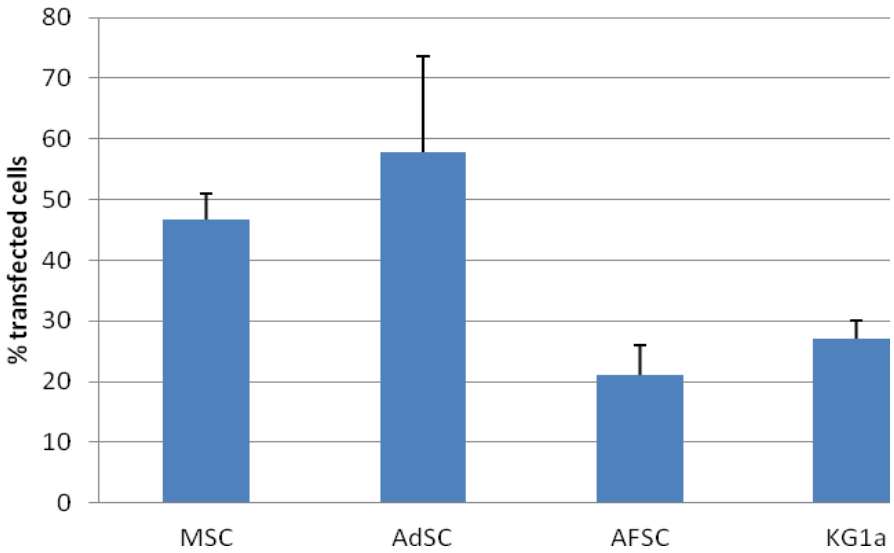

Figure 1: Several human stem cells (MSC, AdSC, AFSC, KG1a) were transfected with 0.5 g of pVectOZ-GFP plasmid DNA and 1.5 L per well of DreamFect™ Stem reagent per well in a 24-well plate. Percentage of GFP positive cells were measured 48h post transfection by flow cytometry.

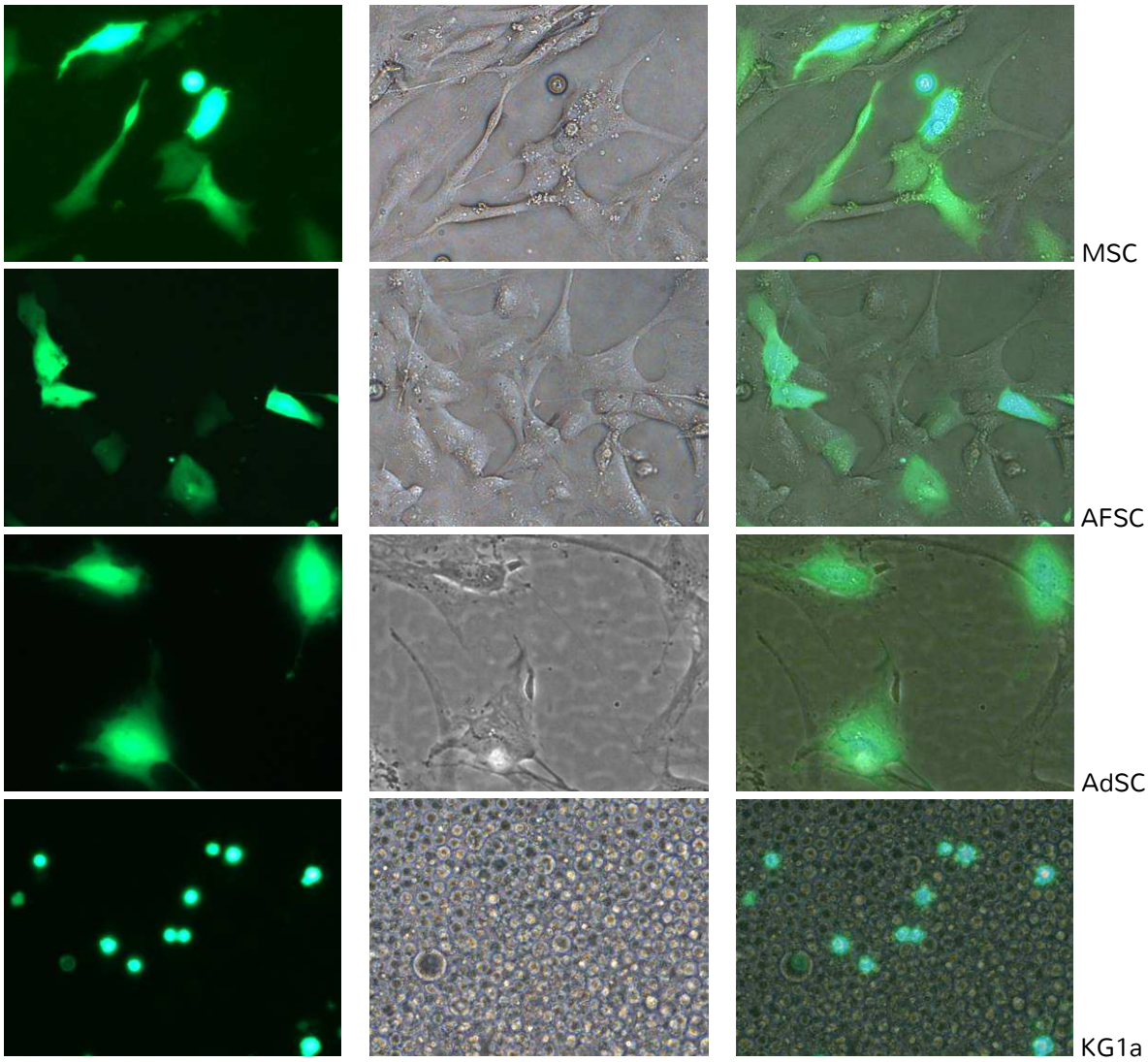

Figure 2: Adherent stem cells (MSC, AdSC, AFSC) and suspension stem cells-like (KG1a) were transfected with 0.5 g of pVectOZ-GFP plasmid DNA and 1,5 L of DreamFect™ Stem per well in a 24-well plate. Transfection efficiency was assessed by fluorescence microscopy 48h post transfection (Magnification x 200).

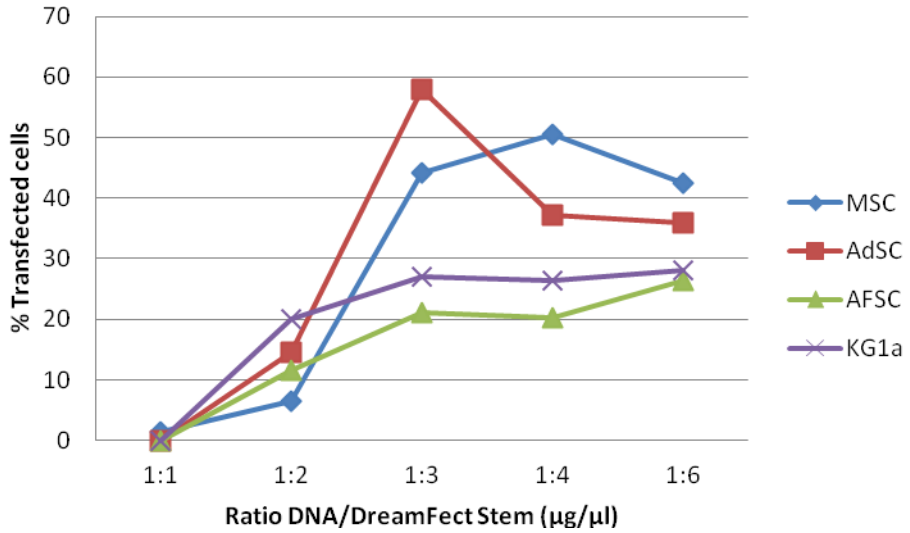

Figure 3: Several human stem cells (MSC, AdSC, AFSC, KG1a) were transfected with 0.5 g of pVectOZ-GFP plasmid DNA and different amounts of DreamFect™ Stem (from 0.5 to 3 µL) per well in a 24-well plate. Percentage of transfected cells were measured 48h post transfection by flow cytometry.

DreamFect™ Stem outperforms other transfection reagents

Transfection efficiency

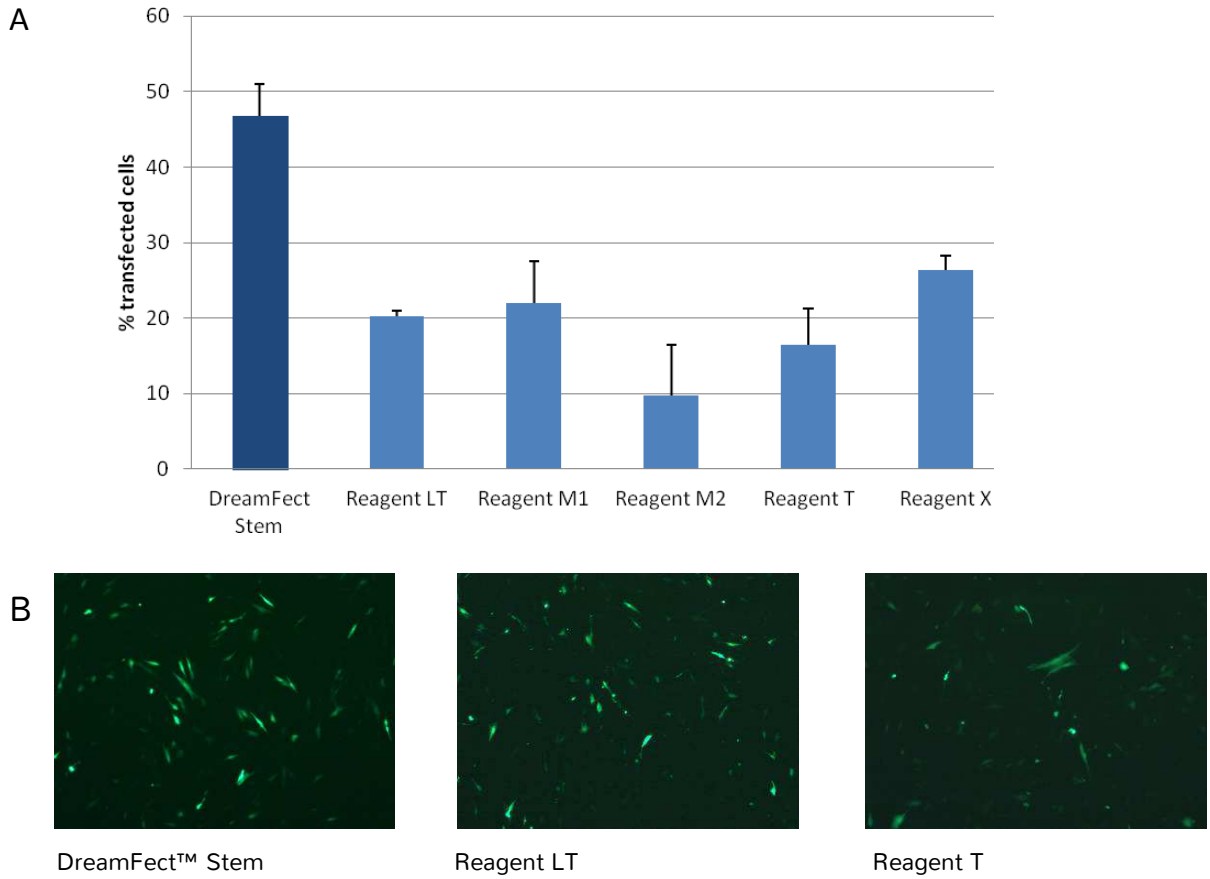

Figure 4: Human mesenchymal stem cells were transfected with 0.5 g of pVectOZ-GFP plasmid DNA and 1.5 L of DreamFect™ Stem or with competitors' reagents (according to manufacturers' manuals). Transfection efficiency was monitored by flow cytometry (A) and fluorescent microscopy (B) 48h after transfection (Magnification x 40).

Toxicity

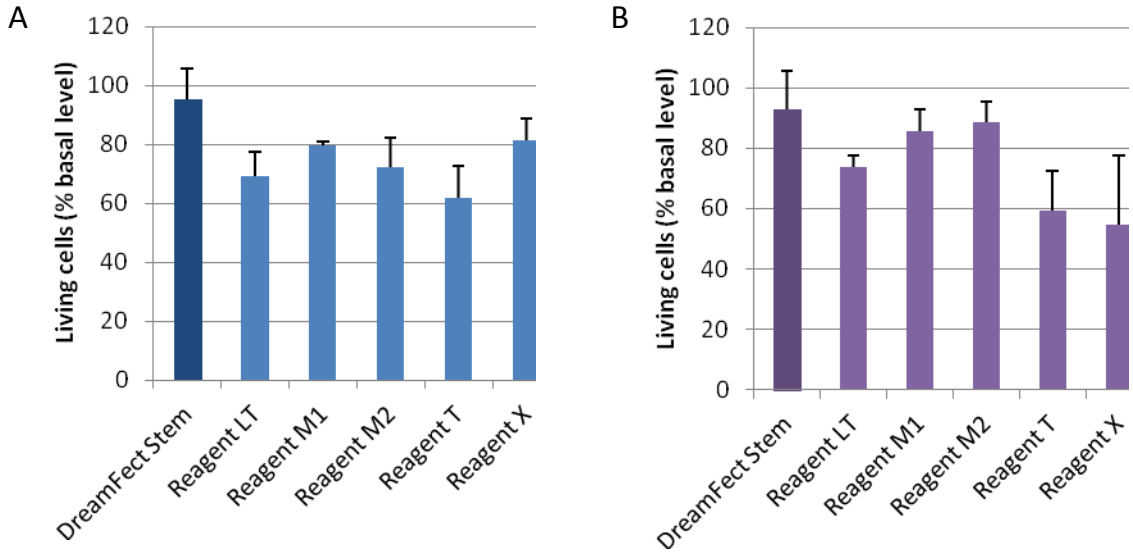

Figure 5: Human mesenchymal stem cells (A) and KG1a cells (B) were transfected with 0.5 g of pVectOZ-GFP plasmid DNA and 1.5 L of DreamFect™ Stem or with competitors' reagents (according to manufacturers' manuals). 24 hours post-transfection, % of living cells was analyzed by MTT assay.

DreamFect™ Stem does not affect the differenciation potential

Several publications reported that transfection via cationic lipids do not cause any changes in their phenotypic profiles and differentiation capacity of stem cells (Yap et al, Malaysian J Pathol, 2009; Madeira *et al*. J Biomed Biotech, 2010). As shown in Figure 6, human mesenchymal stem cells transfected with DreamFect™ Stem retain their potential to differentiate into osteoblasts when cultured in osteogenic differentiation medium.

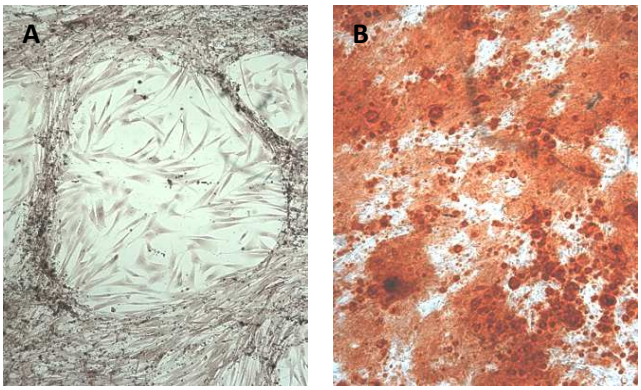

Figure 6: Two days after transfection with DreamFect™ Stem, MSC were stimulated using an osteogenic medium; and 18 days after stimulation, the cells were analyzed using alizarin red staining. Bright-field microscopy images of the transfected nonstimulated (A) and stimulated (B) MSCs 20 days after transfection (Magnification x 40).

DreamFect™ Stem efficiency does not depend on passage numbers

As some stem cells are quite expensive and/or hard to obtain, they have to be cultivated for several passages in order to reach clinically relevant numbers of cells. Thus, the influence of stem cells passage number on DreamFect™ Stem transfection efficiency was studied. Early and late passages of MSC, AdSC, AFSC and KG1A showed comparable transfection profiles, suggesting that the capability of DreamFect™ Stem to deliver genes to adult stem cells is relatively constant (Fig. 7, Fig. 8).

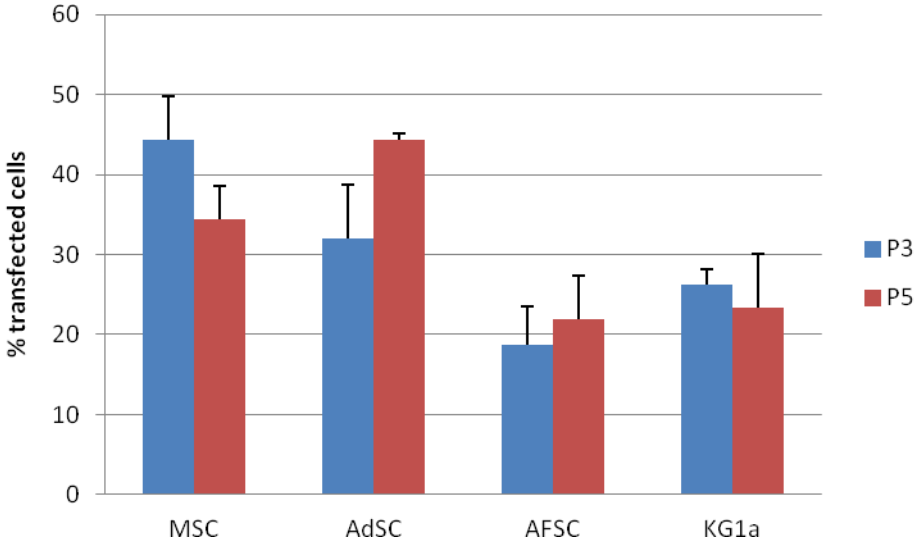

Figure 7: Different types of human multipotent stem cells at passage 3 (P3) and 5 (P5) were transfected with 0.5 g of pVectOZ-GFP plasmid DNA and 1.5 L of DreamFect™ Stem per well in a 24-well plate. Percentage of transfected cells was measured 48h post transfection by flow cytometry.

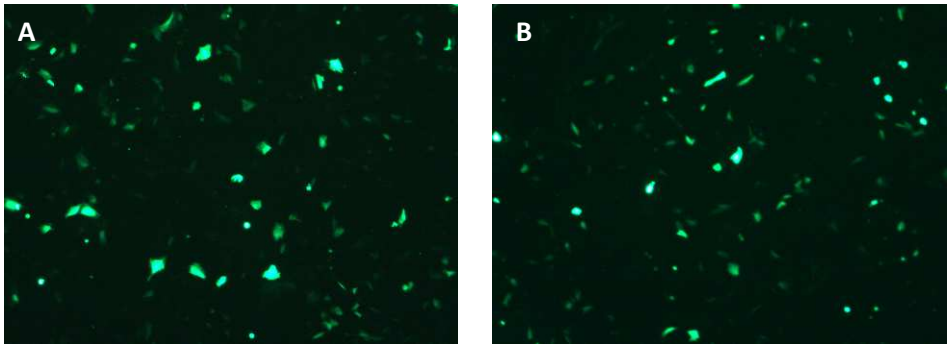

Figure 8: Amniotic fluid stem cells at passage 3 (A) and passage 5 (B) were transfected with 0.5 g of pVectOZ-GFP plasmid DNA and 1.5 L of DreamFect Stem per well in a 24-well plate. Transfection efficiency assessed by fluorescence microscopy 48h post transfection (Magnification x 4)

DreamFect™ Stem efficiency does not depend on plating density

It has been shown that plating density may induce changes in mesenchymal stem cells proliferation, cell morphology and differentiation potential (Neuhuber *et al.,* Exp Hematol., 2008). To determine if the transfection of stem cells with DreamFect™ Stem is altered by plating density, we conducted transfection experiments on MSC and AdSC exhibiting 50% or 70% confluency. Our results show that with DreamFect™ Stem, transfection profiles of MSC and AdSC are comparable. The effect of Stem cell density on transfection efficiency with DreamFect™ Stem is not significant (Fig. 9).

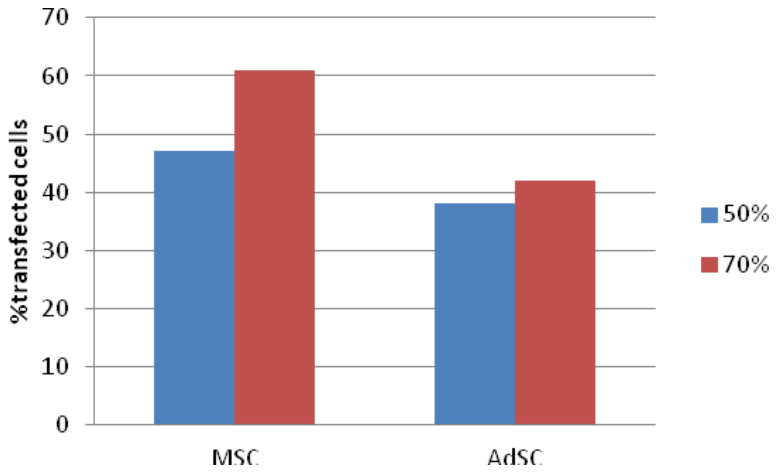

Figure 9: Human mesenchymal stem cells at 50% or 70% confluency were transfected with 0.5 g of pVectOZ-GFP plasmid DNA and 1.5 L of DreamFect™ Stem per well in a 24-well plate. Percentage of transfected cells were measured 48h post transfection by flow cytometry.

Medium change improves DreamFect™ Stem efficiency

In our transfection protocol, we highly suggest to change the culture medium of your stem cells 4 to 6h after transfection experiments. This step improves the transfection efficiency of DreamFect™ Stem (Fig 10, Fig. 11).

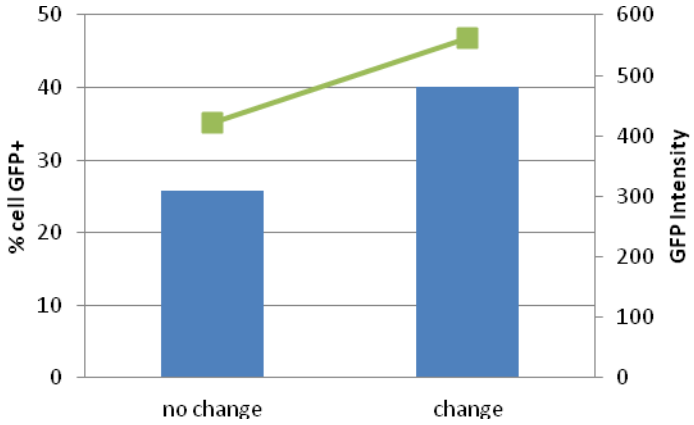

Figure 10: Human mesenchymal stem cells were transfected with 0.5 g of pVectOZ-GFP plasmid DNA and 1.5 L of DreamFect Stem. The culture medium was changed or not 4 hours post-transfection. Transfection efficiency was monitored by flow cytometry 48h after transfection.

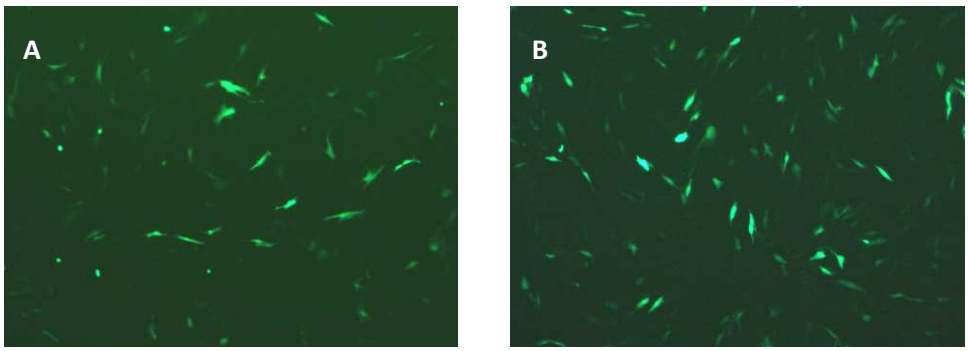

Figure 11: Human Mesenchymal Stem Cells were transfected with 0.5 g of pVectOZ-GFP plasmid DNA and 1.5 L of DreamFect Stem. The culture medium was changed (A) or not (B) 4 hours post-transfection. Transfection efficiency assessed by fluorescence microscopy 48h post transfection (Magnification x 40)

DreamFect™ Stem enables to work in complete cell culture medium

It has been shown that working in serum-free medium may lead to spontaneous cell differentiation of various stem cells and to heterogeneity within the treated cell population. DreamFect™ Stem allows you to work in complete cell culture medium and to avoid this kind of disagreement.

DreamFect™ Stem allows high transfection rate of embryonic stem cells

DreamFect™ Stem exhibits high transfection efficiency on embryonic stem cells (Fig. 12)

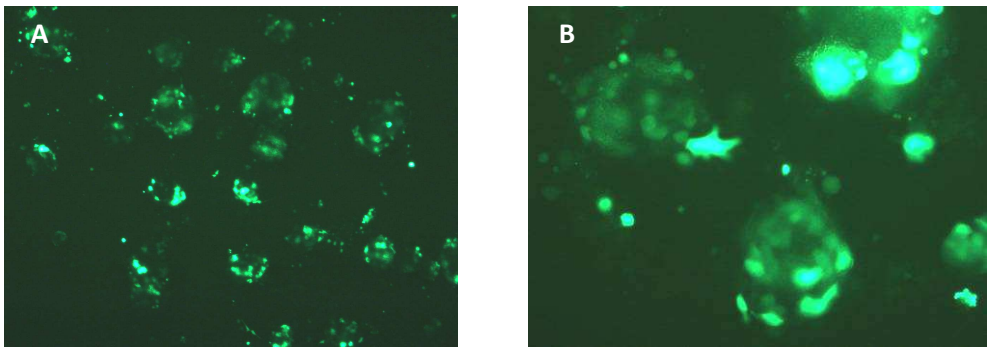

Figure 12: mouse Embryonic Stem Cells growing on mitotically inactivated feeder cells were transfected with 1 g of pVectOZ-GFP plasmid DNA and 3 L of DreamFect™ Stem per well in a 24-well plate. Transfection efficiency was assessed by fluorescence microscopy 48h post transfection (Magnification x 40 (A) and x 200 (B)).

Bibliography

- Madeira C, Mendes RD, Ribeiro SC, Boura J S, Aires-Barros MR, da Silva CL and Cabral JMS. Nonviral Gene Delivery to Mesenchymal Stem Cells Using Cationic Liposomes for Gene and Cell Therapy. J Biomed Biotechnol. 2010; 2010: 735349
- Neuhuber B, Swanger SA, Howard L, Mackay A, Fischer I. Effects of plating density and culture time on bone marrow stromal cell characteristics. Exp. Hematol. 2008; 36:1176–1185.
- Yap FL, Cheong SK, Ammu R and Leong CF. Transfected human mesenchymal stem cells do not lose their surface markers and differenciation properties. Malaysian J Pathol, 2009; 113-120.