

GeneJuice[®] mediated transfection of plasmid vectors into C2C12, Sol8, and 293T cell lines

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Abstract

The use of non-viral gene delivery systems to deliver transgenes into muscle cell lines for *in vitro* expression studies is hampered by the reduced efficiency of transfection. Here we compare proprietary transfection reagents and demonstrate that GeneJuice[®] Transfection Reagent, a proprietary formulation of a non-toxic cellular protein and a small amount of a novel polyamine, achieves above 95% transfection efficiency in cell lines such as 293T, which express the Large T antigen. GeneJuice mediated transfection into C2C12 cells shows a third higher expression levels of the reporter gene, pCMV β , than achieved by transfection mediated using Lipofectamine[™] 2000.

Introduction

Cationic lipid formulations to promote DNA transfer into cultured eukaryotic cells were introduced in the late 1980s (Felgner 1987). Early pioneering work by the Wolff group (Wolff 1990) showed that naked plasmid DNA could be used for gene delivery into muscle cells and a large number of studies have followed, demonstrating various ways of increasing the efficiency of non-viral naked DNA transfection into muscle cells. The aim of the present study is to compare the efficiencies of various proprietary transfection reagents and to optimize transfection in muscle cell lines, using naked plasmid DNA as the vector and measuring the expression level of a reporter gene.

Materials and Methods

Cell lines:

- C2C12 (Yaffe 1977, ATCC No. CRL-1772); adherent mouse muscle myoblast cell line cultured in DMEM + 10% fetal calf serum (FCS).
- 293T; transformed human kidney cell line cultured in DMEM/10% FCS.
- Sol8 (Daubas 1988); mouse soleus muscle cell line cultured in DMEM/20% FCS.

Expression vector system:

pCMV β , plasmid vector expressing bacterial β -galactosidase driven by the CMV immediate early promoter.

Transfection reagents:

- Cationic lipid agents: Lipofectamine 2000 (Invitrogen), Effectene[®] Transfection Reagent (QIAGEN).
- Activated dendrimer agents: SuperFect[®] and PolyFect[®] Transfection Reagents, (QIAGEN).
- GeneJuice[®] Transfection Reagent (Novagen).
- Calcium phosphate

Cell culture:

6-well plates seeded at 2×10^5 cells per well.

The β -galactosidase histochemistry assay was carried out as previously described (Dodds 1998). Transfection efficiency was determined 48 h post transfection by counting the number of positive blue cells and the total number of cells using a Leica[®] light microscope (10X magnification). Reported cell counts are averaged from three fields of observation. Quantitation was verified using a β -Gal ELISA kit (Roche) according to the manufacturer's procedure.

Results

The initial comparison of transfection efficiencies did not include GeneJuice Reagent. Transfection efficiency of the *lacZ* reporter gene into C2C12 cells was compared for four different commercially available *in vitro* transfection reagents (Effectene, Lipofectamine 2000, SuperFect, PolyFect) and DNA/calcium phosphate precipitates. For this optimization study the amount of DNA was varied and amount of reagent kept constant, according to manufacturers' protocols (Table 1). Three DNA:transfection agent ratios were tested. a) DNA at half the manufacturer's recommended amount, b) DNA at the recommended

Table 1. Transfection reagent volumes and DNA concentrations

Transfection Reagent	Reagent volume (μ l)	1/2 Recommended DNA conc. (μ g)	Recommended DNA conc. (μ g)	2X Recommended DNA Conc. (μ g)
GeneJuice	4	N/A	1	N/A
Lipofectamine 2000	10	2	5	10
Effectene	10	0.2	0.4	1
PolyFect	10	1	2	5
SuperFect	10	1	2	5
CaPO ₄	24	2	5	10

Transfection reagent comparison

manufacturer's amount and c) DNA at twice the manufacturer's recommended amount. Transfection efficiency was observed to be highest across all three DNA ratios in this comparison using Lipofectamine 2000 (Figure 1).

Optimization and comparison of GeneJuice® reagent with Lipofectamine™ 2000

This study was carried out to determine the optimal efficiency of GeneJuice in mediating transfection into C2C12 muscle cells and to compare this with the transfection efficiency of the previously optimized Lipofectamine 2000. Following transfection and β -galactosidase histochemistry staining it was observed that a ratio of 1 μ g DNA:4 μ l GeneJuice reagent obtained significantly higher transfection efficiency of the *lacZ* reporter gene, as determined by the number of positive blue cells following assay for β -galactosidase enzyme, than the optimized Lipofectamine 2000 (Figures 2 and 3).

GeneJuice mediated transfection into C2C12 and Sol8 cell lines

Murine muscle cell lines are known to transfect poorly *in vitro*. This study was carried out to determine the transfection efficiency of the GeneJuice reagent in a different murine cell line using the reporter gene as described previously. Expressed β -galactosidase levels were determined by the β -gal ELISA and it was observed that expressed protein levels were over 50% greater in Sol8 compared to C2C12 cells. Transfection was carried out using the optimized GeneJuice parameters as determined previously (Figure 4).

Conclusion

Sol8 cells are characterized as slow twitch soleus muscle cells and differ from fast twitch muscle cells in the amount of structural proteins, including cell surface proteins, pattern of transcript

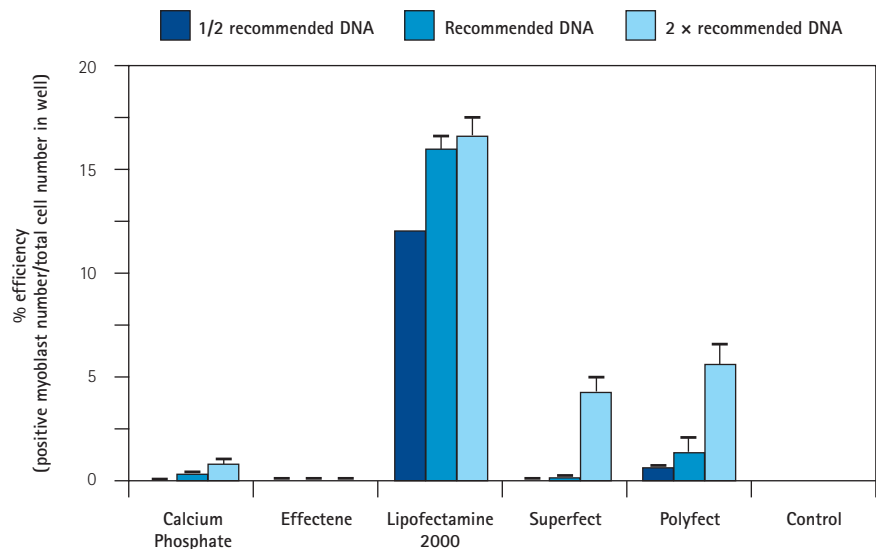


Figure 1. Comparing β -galactosidase expression levels in C2C12 cells following transfection with pCMV β mediated by various transfection reagents

Transfection was carried out using purified endotoxin- and lipopolysaccharide-free pCMV β . The different transfection reagent protocols were carried out using half, standard, and twice the amount of pDNA, according to the manufacturer's recommended protocol. The experiment was carried out in triplicate and, following β -galactosidase histochemistry staining, positive blue cell count averages were determined. The bars represent the percent efficiency of transfection determined as the ratio of positive blue myoblasts in the total number of cells in a 1 mm² field of observation. Lipofectamine 2000 at 2 μ g, 5 μ g, and 10 μ g DNA was observed to obtain relatively the greatest efficiency of transfected cells. For controls, no DNA complexes were added.

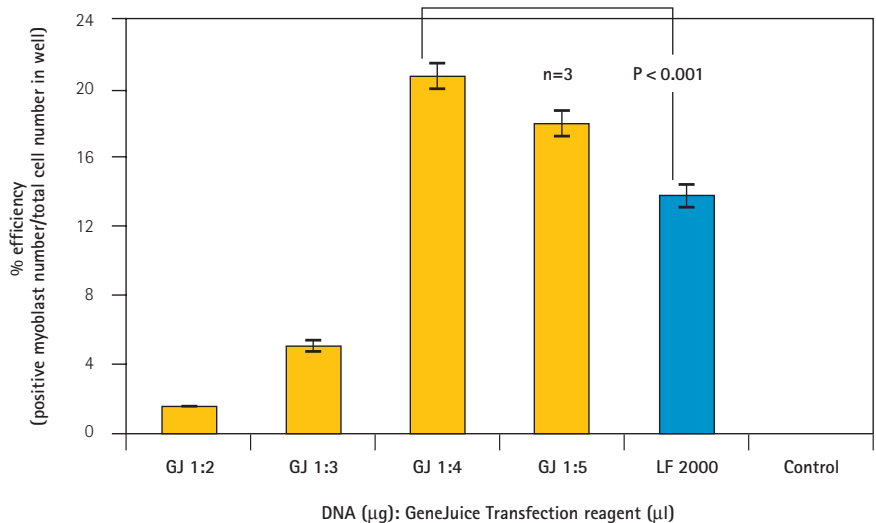


Figure 2. Optimization of GeneJuice Transfection Reagent

Varying ratios of reporter gene pDNA:GeneJuice were transfected into C2C12 muscle cells (according to manufacturer's protocol) and compared against optimal transfection conditions using Lipofectamine 2000. Cells were seeded at a density of 2×10^5 cells/well in standard 6-well plates. The cells were then viewed at 10X magnification and the number of positive blue cells in three different fields of observation was recorded. The assay was carried out in triplicate. The percent efficiency of transfection was determined as previously stated. It was observed that GeneJuice produced a greater degree of transfection at a ratio of 1 μ g pDNA:4 μ l GeneJuice Transfection Reagent. For controls, no DNA complexes were added.

expression, and metabolic enzymes (Pette 1997). The increased number of slow twitch muscle cell surface receptor proteins, which can act as anchors for cationic DNA complexes, may explain

the difference in internalization of reporter vector and thereby the level of expressed protein observed. Cultured muscle cells were generally observed (continued bottom of page 13)