DNA-transfection of murine embryonic fibroblasts (MEFs) using "Biontex K2® Transfection System"

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Background

Murine embryonic fibroblasts (MEFs) derived from genetically modified animals vary in phenotype and propensity for DNA-transfection. Here the efficiencies of two different transfection reagents are compared using two cell lines, one genetically wild type (*wt*), the other a mutant line with a flat cell phenotype (*mut*).

Materials and Methods

Murine embryonic fibroblasts (MEFs) were cultured in high glucose DMEM (Sigma #D5796) containing 10% foetal calf serum, 100U ml^{-1} penicillin, 0.1mg ml^{-1} streptomycin in 24-well plates (Corning/Costar).
Polyethylenimine (PEI, Polysciences) was solubilised at pH 7.0 in PBS, filter sterilised and diluted to a 20mM working concentration in sterile PBS.
Plasmids pEGFP (green fluorescent protein) and pUbA-Rluc (renilla luciferase) (70ng of each) were introduced in a cocktail with pUC8 to give a total of 600ng DNA per well.

One day prior to transfection, cells were seeded at a density of $0.5x10^5$ per well. Two hours prior to transfection, cells were treated with 5µl K2® Multiplier per well. Transfection reagent and DNA samples were prepared separately in 1.5 ml microfuge tubes by mixing with Opti-MEM (see table for volumes per well). DNA/Opti-MEM solutions were added to K2 transfection reagent/Opti-MEM solutions and mixed by gentle pipetting. The mixtures were allowed to stand at RT for 15 minutes and then added to the wells with gentle mixing.

Table. Volumes and amounts of components for 1 well of 24-well plate

Well size (ø in mm)	Volume of medium (ml)	K2® multiplier (µl)	K2® transfection reagent/Opti-MEM (µl)	DNA/Opti-MEM (µl)	DNA cocktail (ng total)
15.6	0.5	5	2.4/25	<2.5/25	600

For transfections with PEI an optimised version of the standard protocol and comparable amounts of expression plasmids were used. GFP expression was monitored after 24h by fluorescence microscopy; luciferase expression was determined after 48h using the Stop and Glow system (Promega).

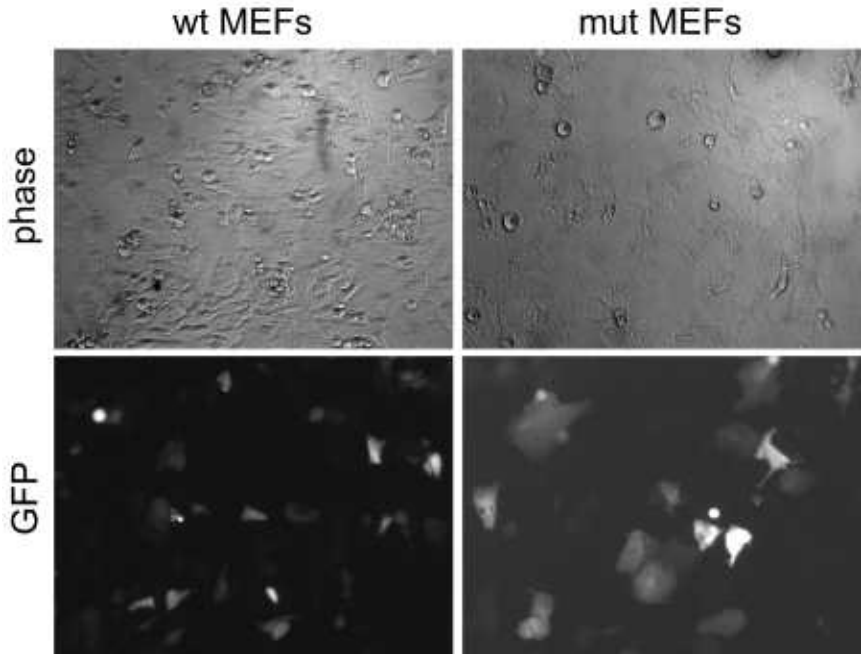
Results

Little or no GFP expression was apparent in *mut* MEFs transfected with pEGFP using PEI (not shown). Significant GFP fluorescence was seen in *wt* and *mut* cells transfected with the Biontex K2® Transfection System (see figure 1), whereby the *mut* MEFs were transfected less effectively, as anticipated.

To quantify relative transfection efficiencies levels of renilla luciferase activity in the cells were quantified (figure 2). The Biontex K2® System was ten-fold more efficient than PEI in both *wt* and *mut* MEFs. The mutant MEFs were transfected 8-fold less efficiently with either method.

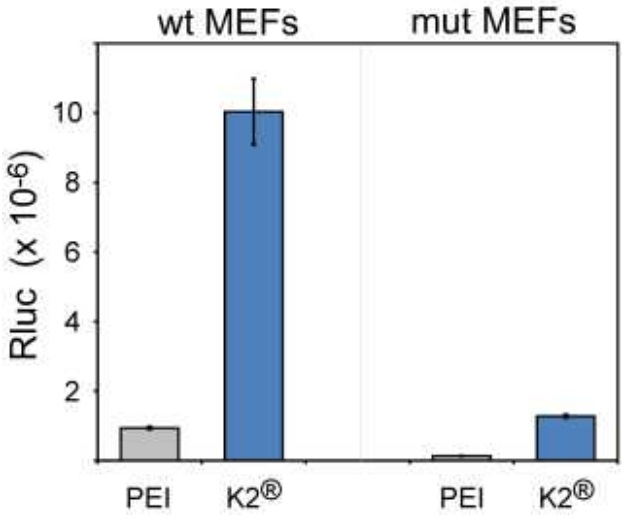
Figures

Figure 1



Cells transfected with pEGFP using the Biontex K2® Transfection System were imaged after 24h on a Zeiss Axiovert microscope at 20x magnification.

Figure 2



Cells transfected with a Rluc expression vector using the Biontex K2® Transfection System were harvested after 48h and processed for luciferase measurement using the Stop and Glow system (Promega). Values represent averages of 6 wells; error bar shows SEM.

Conclusions

The Biontex K2® Transfection System transfects cells at >50% confluency with efficiencies approx 10-fold higher than linear PEI, including cells refractory to transfection by conventional methods. No overt toxicity was observed and cells continued to proliferate for up to 48h post transfection.