DNA-transfection of mouse MC7-L1 cells using "Biontex K2® Transfection System".

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Materials and Methods

Cell culture

Murine MC7-L1 cells, a mammary epithelial cell line derived from a murine mammary ductal carcinoma (Lanari C et al., Cancer Res. 2001; 61:293-302), were cultured in 6 cm tissue culture plates, with antibiotic-free DMEM-F12 medium containing supplemented to 5% fetal calf serum. The amount of medium for each dish was 4 mL Cells were plated at 1 x 10^6 cells (green fluorescent protein (GFP)) or 7 x 10^5 (progesterone receptor A (PRA)) per plate and incubated approximately 16 hours prior to transfection. Transfection was performed when cells reached 50% confluence.

Cell transfection

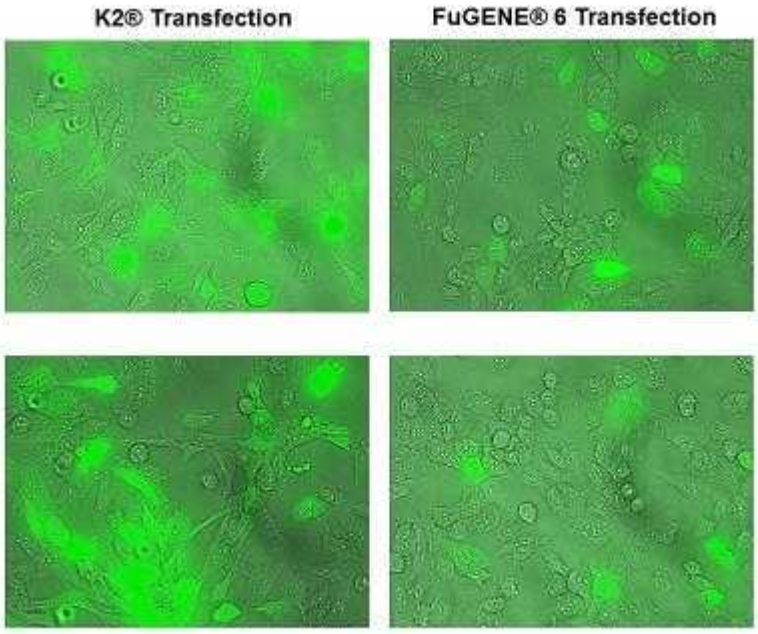
Cells were treated with K2 ® Multiplier (25 µl/plate) for 2 hours before DNA transfection. K2® Multiplier was dripped slowly onto the medium with gentle mixing. K2® Transfection Reagent (4 µl/µg DNA) was mixed with DMEM-F12 medium and incubated at room temperature during preparation of the DNA. Plasmid DNA encoding either GFP (1 µg/plate) or PRA (2 µg/plate) was mixed with DMEM-F12 medium and this DNA solution was added to the solution containing the K2® Transfection Reagent, and then mixed by tapping and followed by 20 minutes incubation at room temperature. This transfection solution was applied to cells by slowly dripping into the medium with gentle mixing. Transfections were then incubated at 37 ^{0}C and 5% CO_2 for 24 hours. For transfections with FuGENE® 6 reagent, conditions were identical to those for the K2® transfection system, except that the ratio of FuGENE® 6 reagent to DNA was 2 µl/µg DNA. Transfection efficiency was estimated by fluorescence microscopy. For PRA detection, cells were stained with a fluorescence antibody directed against PRA prior to the microscopy.

Results

The K2 ® transfection system is 3-4 times more efficient than the FuGENE ® 6 transfection system.

Transfection of MC7-L1 cells was performed for GFP expression comparing the K2® to FuGENE® reagents. Fluorescent microscopy showed a dramatic increase in the number of fluorescent cells in transfections that utilized K2® in comparison to FuGENE® 6 reagent (Figure 1A). Quantitation showed a 4-fold increase in transfection efficiency (Figure 1B).

A



B

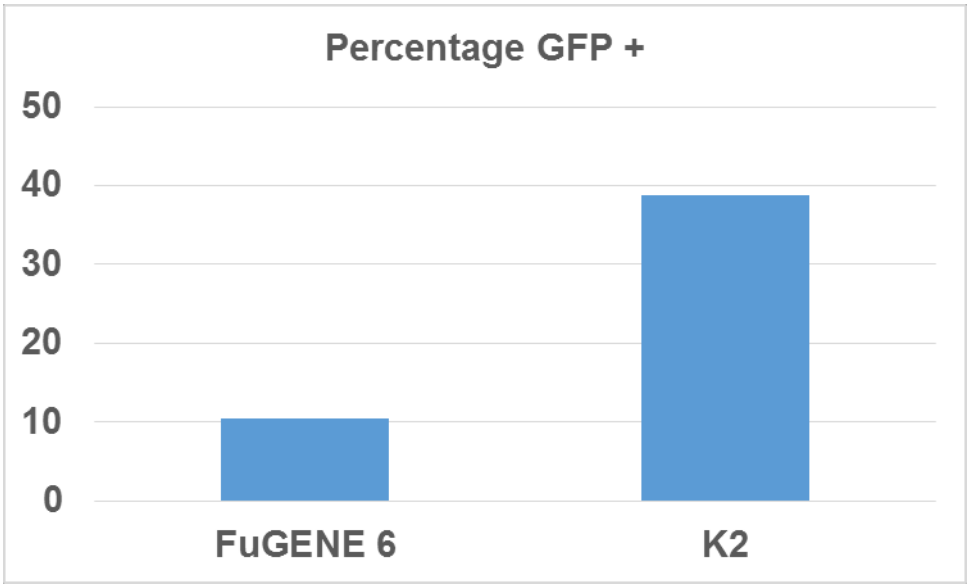
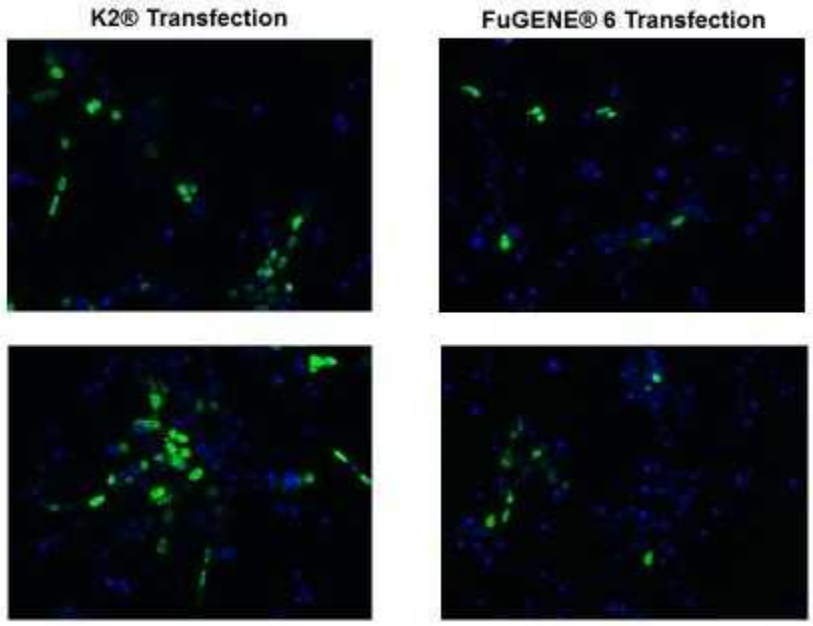


Figure 1. A. Fluorescent microscopy of GFP-transfected MC7-L1 cells using K2® vs. FuGENE® 6. Detection of fluorescence was gated to eliminate background observed in untransfected cells. Representative images are shown. B. The mean percentage of GFP fluorescent cells from three randomly selected visual fields is presented. K2® was about 4-fold more efficient in transfection than FuGENE® 6; *p* = 0.001.

Transfection of MC7-L1 cells was also performed for PRA expression comparing the K2® to FuGENE® 6 reagents. Fluorescent microscopy showed a similar dramatic increase in the number of fluorescent cells in transfections that utilized K2® in comparison to FuGENE® 6 reagent (Figure 2A). Quantitation showed a 4-fold increase in transfection efficiency (Figure 2B).

A



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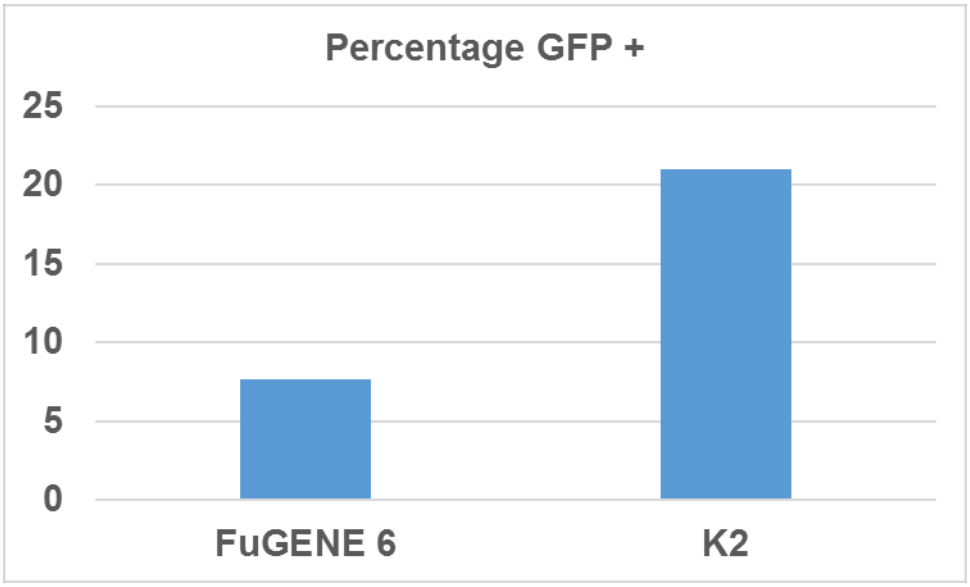

Figure 1. A. Fluorescent microscopy of PRA-transfected MC7-L1 cells using K2® vs. FuGENE® 6. Detection of fluorescence was gated to eliminate

background observed in cells transfected with an "empty" vector. Representative images are shown. B. The mean percentage of PRA fluorescent cells from four randomly selected visual fields is presented. K2® was about 3-fold more efficient in transfection than FuGENE® 6; *p* = 0.002.

Conclusions

Transfection of two different plasmids into murine MC7-L1 cells, a mammary epithelial cell line derived from a murine mammary ductal carcinoma, with the K2® Transfection system proved to be 3 to 4-fold more efficient than the commonly used FuGENE® 6 transfection reagent. Transfection with either system yielded cells that appeared healthy, grew to similar densities, and possessed normal morphology by microscopic examination, suggesting that exposure to the K2® Transfection system had no negative effect on cell growth or metabolism. The K2® Transfection system is an effective system for introducing ectopic gene expression into a significant proportion of an experimental cell culture.