Transfection of a mouse embryonic olfactory epithelium stem cell line (OP27) using the Biontex K2 transfection system and Lipofectamine 2000.

Dr. rer. biol. hum. Tobias Hohenauer, GCNA, Disease Mechanism Research Core, RIKEN Brain Science Institute, Wako-shi, Saitama, Japan.

Cell culture

OP27 cells were cultured in DMEM 10% FBS at 33°C. On the day prior transfection, cells were plated to reach 80% confluency on the day of transfection.

1. Optimization step K2 system

On the day prior transfection, 50.000 cells/ well of a 24-well cell culture dish were plated in 0.5 ml culture medium. On the day of transfection either 5 or 10 µl of Multiplier solution were added to each well and incubated at 33°C for 2 hours.

Transfections were prepared in serum free medium (Optimem, Life Technologies) as ratios of GFP expression plasmid DNA and K2 transfection reagent of 1:2, 1:4 and 1:6 according to the manufacturer's instructions and complexed DNA was added to the cells dropwise after 20 minutes of incubation at room temperature. Medium was changed after 24 hours and transfection efficiency was determined after 48 hours by FACS analysis (Figure1).

Result

The highest number of GFP positive cells was achieved using 5 µl of Multiplier solution per 0.5 ml culture medium and a DNA: K2 ratio of 1:4 (Figure 1).

2. Comparison of K2 and Lipofectamine 2000

Transfection conditions for Lipofectamine 2000 were used as to the manufacturer's instructions. Transfection conditions for the K2 system were determined as stated above. For the following comparative analysis, transfection was scaled up to 6-well plate format by multiplication of cell number, cell culture medium, DNA amount and transfection reagent volumes by the factor 4. Analysis was carried out in triplicates using FACS (Figure 2).

Result

The K2 system gave rise to slightly higher numbers of transfected cells when used with plasmid DNA then those achieved using Lipofectamine 2000 (Figure 2).

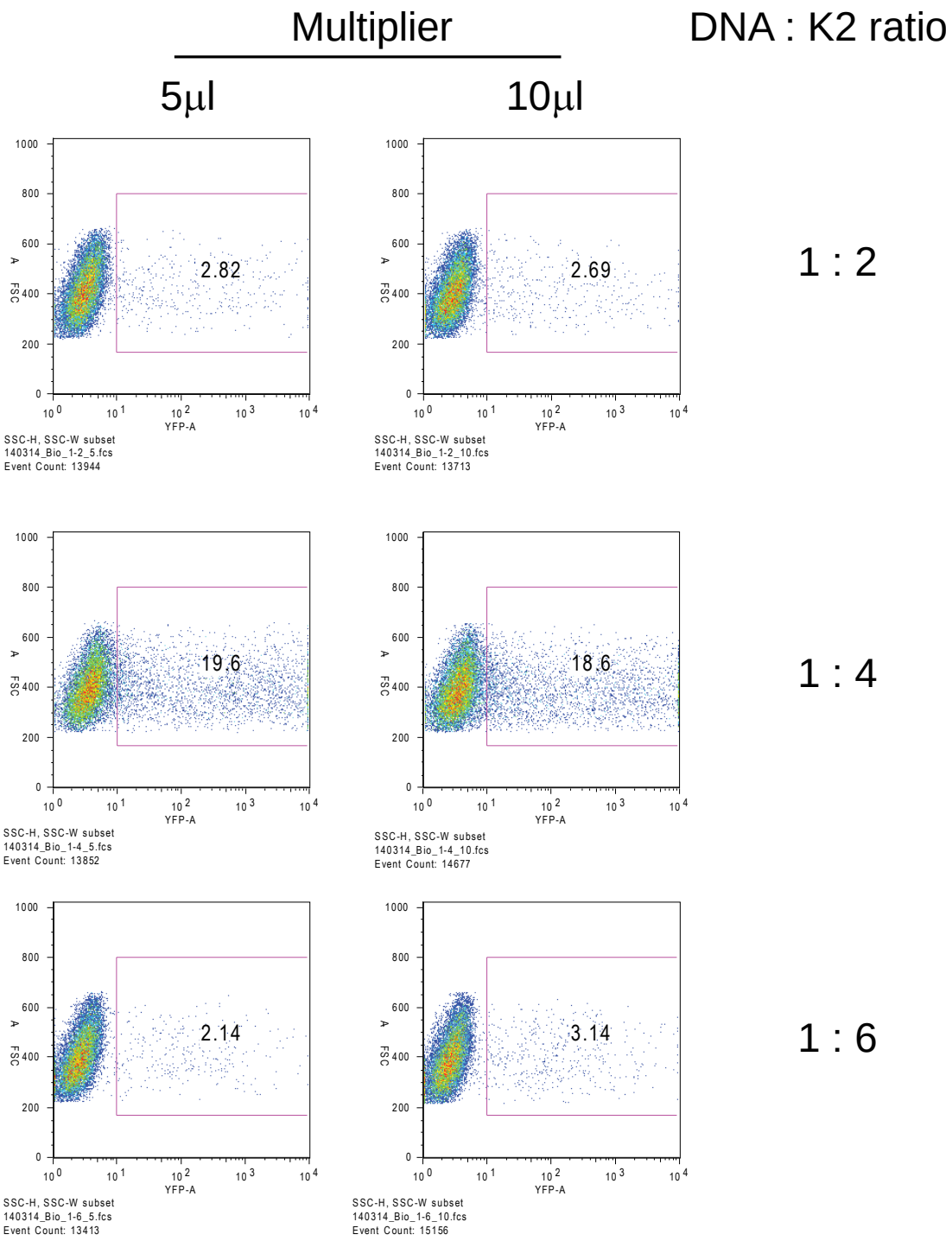
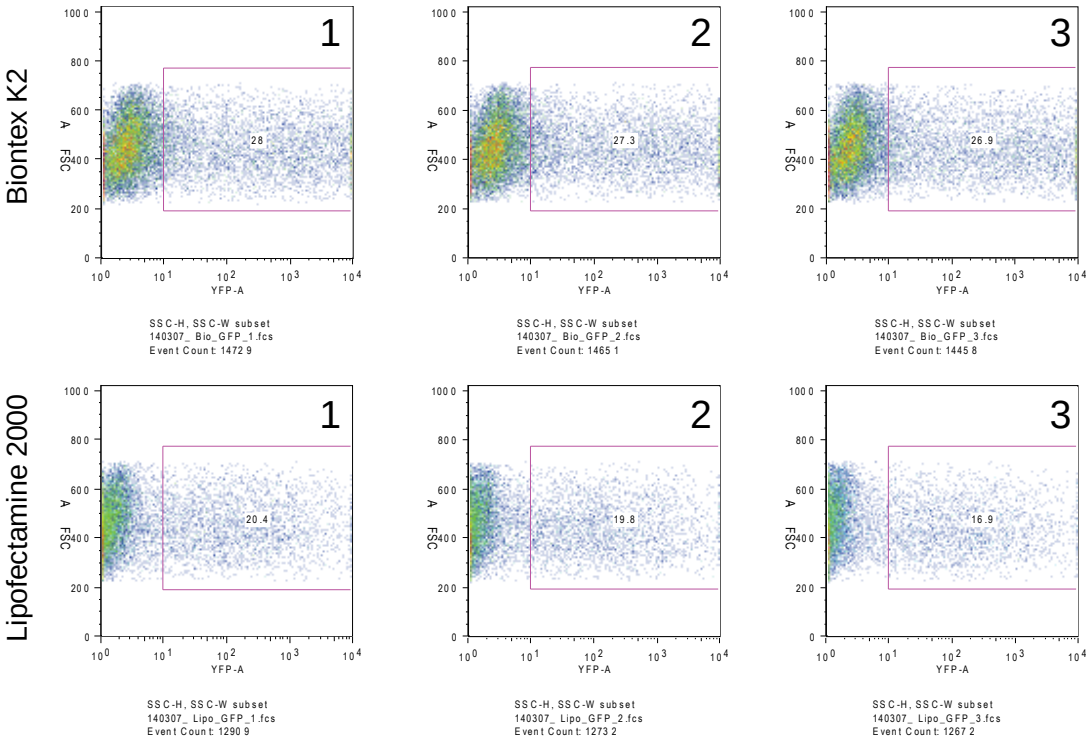


Figure 1: Optimization of K2 transfection conditions. OP27 cells were transfected with GFP expression plasmid using the amounts of Multiplier solution and DNA:K2 ratios indicated and analyzed after 48 hours using FACS.



% GFP positive cells					
Replicate #	1	2	3	avg	STDEV
Biontex K2	28	27.3	26.9	27.4	0.6
Lipofectamine 2000	20.4	19.8	16.9	19.0	1.9

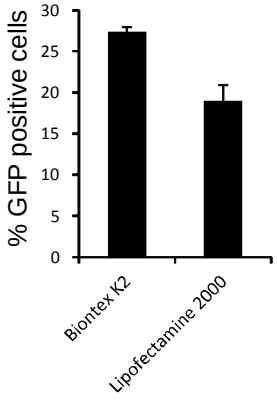


Figure 2: Comparison of the K2 system and Lipofectamine 2000. OP27 cells were transfected in triplicates with GFP expression plasmid and GFP positive cells were identified using FACS 48 hours after transfection.

Appendix

Table 1: Reagent volumes for 24 well plate format, 1:4 ratio

Medium	Multiplier	K2 reagent	Optimem	DNA
0.5 ml	5 µl	2.4 µl	30 µl /30 µl	0.6 µg