DNA Transfection of Mouse pituitary cells GHFT1 using "Biontex K2®Transfection

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Materials and Methods

Cell Culture

Mouse pituitary cells GHFT1 were cultured in a 48-well plate at a density of 1x10^5 cells/well in 250µl HyClone DMEM F12 medium containing 10% newborn calf serum, 2 mM glutamine, and 10 U penicillin /10ng mg/ml streptomycin. The cells were cultured overnight to a confluency of about 90%.

Transfection

The cells were incubated with or without K2® Multiplier for 2hrs before the transfection. For transfection, 20µg DNA (Venus-pcDNA4/TO) was added to 1ml of OptiMEM medium (Gibco). Then 225µl of the DNA solution was transferred to tubes with 225µl of OptiMEM medium containing either 9µl, 13.5µl, 18µl, or 22.5µl of K2 transfection reagent. The solutions were mixed by gently inverting three times, kept at room temperature for 20min, and then transferred to the cell cultures. The following table shows the amount of the K2 multiplier (MP), DNA, and the ratio of DNA and the reagent in each well of 48-well plate.

	MP		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
A	0µl	Ratio	1:2	1:2	1:2	1:2	1:3	1:3	1:3	1:3
		DNA	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5
B	0µl	Ratio	1:4	1:4	1:4	1:4	1:5	1:5	1:5	1:5
		DNA	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5
C	2.5µl	Ratio	1:2	1:2	1:2	1:2	1:3	1:3	1:3	1:3
		DNA	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5
D	2.5µl	Ratio	1:4	1:4	1:4	1:4	1:5	1:5	1:5	1:5
		DNA	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5
E	5.0µl	Ratio	1:2	1:2	1:2	1:2	1:3	1:3	1:3	1:3
		DNA	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5
F	5.0µl	Ratio	1:4	1:4	1:4	1:4	1:5	1:5	1:5	1:5
		DNA	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5

Results

To evaluate and optimize the new transfection system, K2 transfection, mouse pituitary GHFT1 were transfected with Venus/pcDNA4TO plasmid DNA using different ratios of DNA to reagent, amount of DNA or multiplier (Table 1). The fluorescence from the Venus fluorescent protein encoded by Venus-pcDNA4/TO plasmid was observed by epi-fluorescence microscopy at 24hrs and 48hrs after the transfection, and used as a reporter of the transfection efficiency. As shown in Fig.1, fluorescence was detected 24hrs post-transfection (Fig1A) with an increase in fluorescence intensity at 48hrs post transfection (Fig.1B). The analysis of the fluorescence using Image J software showed that 0.3µg DNA (Fig. 2A), DNA to reagent ratio of 1:3 or 4 (Fig. 2B), and 0.25µl multiplier pre-incubation (Fig. 2C) produced the best levels of protein expression.

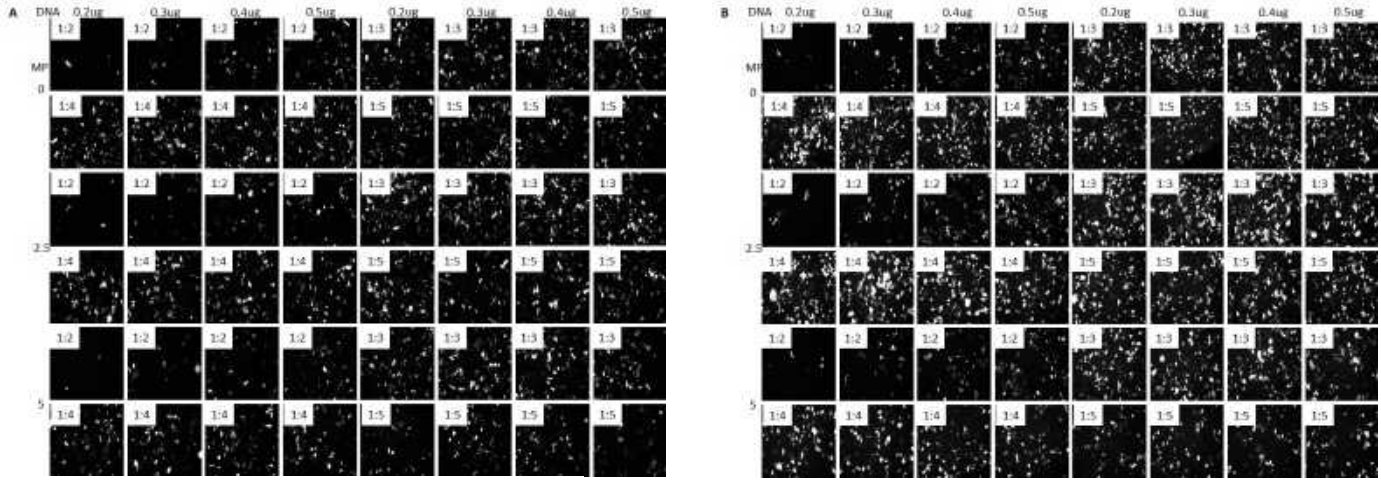

Figure 1. Representative fluorescence images of YFP protein in mouse GHFT1 cells. The cells were transfected with Venus/pcDNA4TO using K2® transfection system. The YFP fluorescence was observed 24hrs (A), and 48hrs (B) after the transfection.

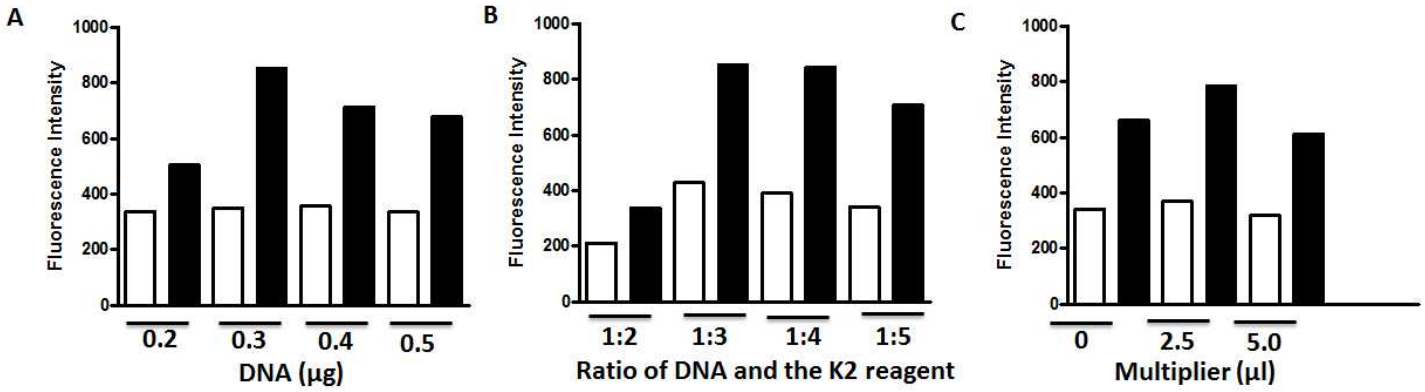

Figure 2. Fluorescence intensity analysis of YFP protein expression under the different conditions. YFP fluorescence intensity was measured for the different amounts of DNA transfected (A), the ratio of DNA to reagent (B), and different amounts of multiplier pre-incubation (C)**.** The black and white bars indicate fluorescence intensity detected 24hrs and 48hrs post-transfection, respectively.

Conclusion

Mouse pituitary cells GHFT1 were transfected at a high efficiency with the K2® transfection system. There was no evidence that the reagents had any adverse affects on cell growth. The direct comparison of the different transfection conditions indicated that 2.5µl K2® Multiplier, and a DNA/K2 transfection reagent ratio of 1:3 had the highest transfection efficiency.