

METAFECTENE application note

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Transfection of CRFK cells

The feline epithelial cell line „Crandell Feline Kidney“ (CRFK; ATCC: CCL-94) supports *in vitro* propagation of feline viruses. Especially, CRFK cells have proven as a useful tool for replication of cloned viruses after transfection of replication competent viral DNA. As in any other cell culture system, a critical aspect for the successful expression of viral DNA clones is of course efficacy of transfection. Therefore we looked for a simple, inexpensive and reliable technique which allows transfection of DNA into CRFK cells at a high rate and results in rapid and strong expression of transfected genes.

Materials and Methods

Adherent CRFK cells were grown in RPMI medium supplemented with antibiotics and 10% FCS. For application of the CaCl₂/BBS method, cells were seeded in 6-well plates, for other methods in 12-well plates. Cell density was adjusted to 1x10⁵ cells/ml and 3ml/well were seeded in 6well, 1ml/well in 12-well plates. Transfection was done according to published procedures and protocols provided by the manufacturer the following day. DNA used for transfection consisted of a CMV promoter-driven β-galactosidase construct. For the CaCl₂/BBS method 2.5 to 10μg DNA were taken. Assays with reagent 1, a lipid-based reagent, and Metafectene were done with 1-3μg and 0.5-2μg DNA using 2 to 32 μl and 2 to 16μl of transfection reagent, respectively. With the CaCl₂/BBS method, cells were washed the day following transfection. The other plates were washed after 48 hours. At day 3 past transfection, cells were fixed and incubated with β-galactosidase substrate. Cells were screened for appearance of blue β-galactosidase product using a light microscope after 1 hour and again the following day. Efficacy of transfection was estimated from the relation of fixed cells to blue-colored cells. Photos of representative wells were taken. Assays were done independently for at least two times.

Table1:

Method	DNA [ug]	Reagent [ul]	Efficiency [%]
CaCl ₂ /BBS	2.5-10		5-15
Reagent 1	1	2-16	0-5
	2	8-24	15-40
	3	20-32	30-50
Metafectene	0.5	1-4	0-15
	0.5	6	70
	1	2-12	40-60
	2	4-16	50-70

Results and Discussion:

It was clearly demonstrated that compared with the other applied techniques the CaCl₂/BBS method was of low efficacy. Changes in medium or variation of incubation time did not result in improved transfer rates.

Compared to CaCl₂/BBS, reagent 1 allowed more efficient but still not satisfactory delivery of construct DNA into cell nuclei. A major disadvantage of this technique was a significant loss of cell viability with increasing reagent volume used for transfection. Because maturation of virus particles takes at least 3 days following transfection, stable viability of the cells is necessary for effective virus release into tissue culture supernatant. Using Metafectene, optimal transfection results are obtained with minimal toxicity for the cells. Surprisingly, quite low amounts of DNA (0.5μg) as well as of reagent (6μl) were needed for successful transfection. Moreover, the level of gene expression was higher compared to the other reagent. Following transfection with Metafectene, cells showed an intense color reaction already 20 minutes past fixation. In contrast using reagent 1 for transfection it needed overnight incubation to detect single cells displaying strong blue staining. Using Metafectene, efficacy of transfection was highly reproducible. Presence of D-MEM tissue culture medium did not interfere with the reaction.

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Figure1:

CRFK cells were transfected with a CMV promoter/ β -galactosidase construct, fixed and activity analyzed *in situ*.

A) CaCl_2 /BBS with 10 μg DNA B) Reagent 1 with 2 μg DNA/8 μl reagent C) Metafectene with 0.5 μg DNA/6 μl reagent

