Metafectene Technical Note

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Materials:
Plasmid pEGFP-N1 (Clontech)
Sterile 12-well tissue culture plates
10cm culture plates
Trypsin 0.25% (GIBCO)
DMEM + 10% FCS + Penicillin/Streptomycin
MDCK (Madin Darby Canine Kidney) cells
EMCs (Epicardial Mesothelial Cells - Eid et al. 2000)

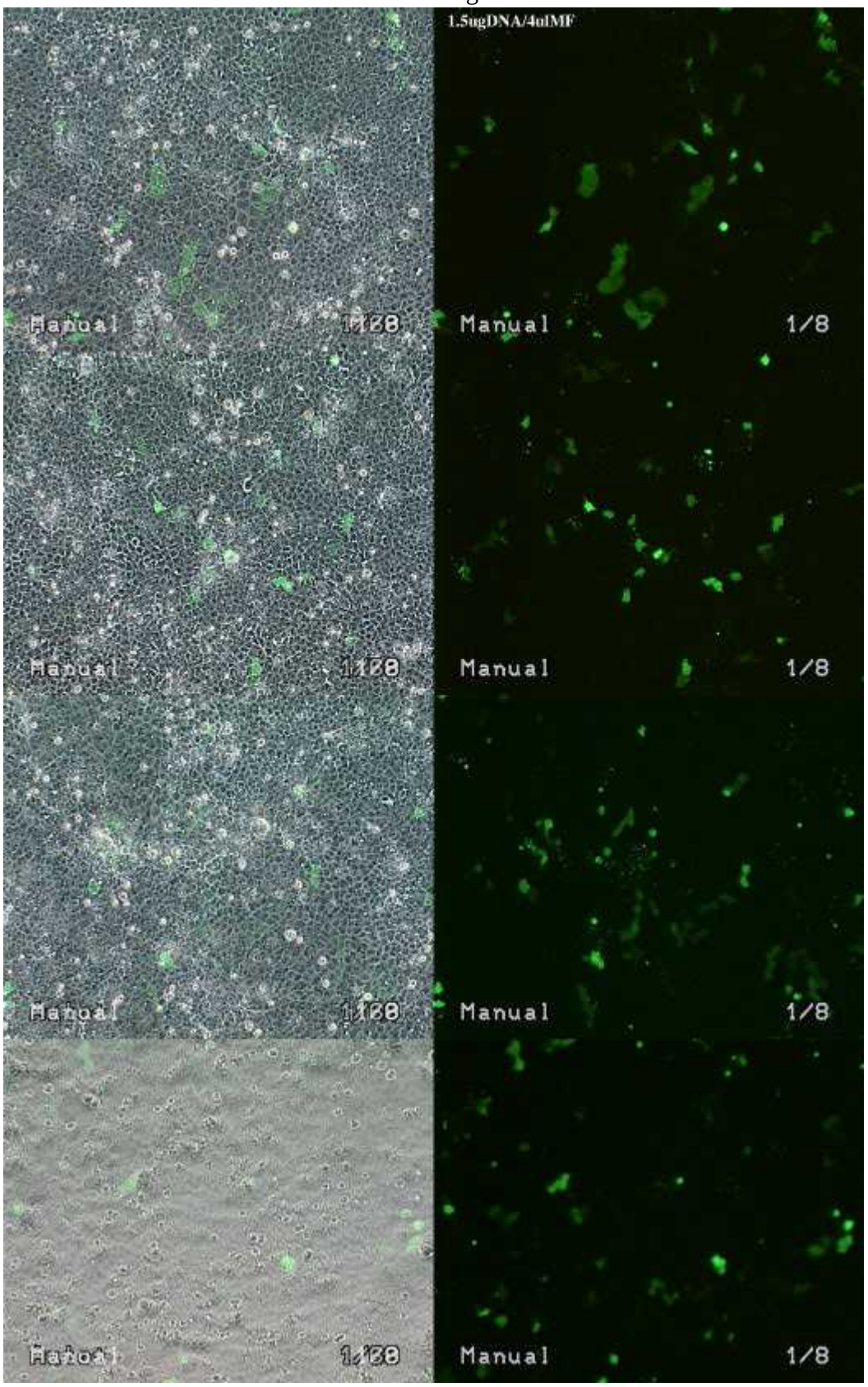
Optimization for transfection of epithelial cells:
Cells were grown on 10cm plates in DMEM (10%FCS+P/S) to confluency, trypsinized, and seeded onto 12-well plates. Cells were transfected with pEGFP at 50-70% optical confluency. Transfection efficiency was recorded ~24 hours later when cells are truly confluent.

Optimization for transfection using METAFECTENE:

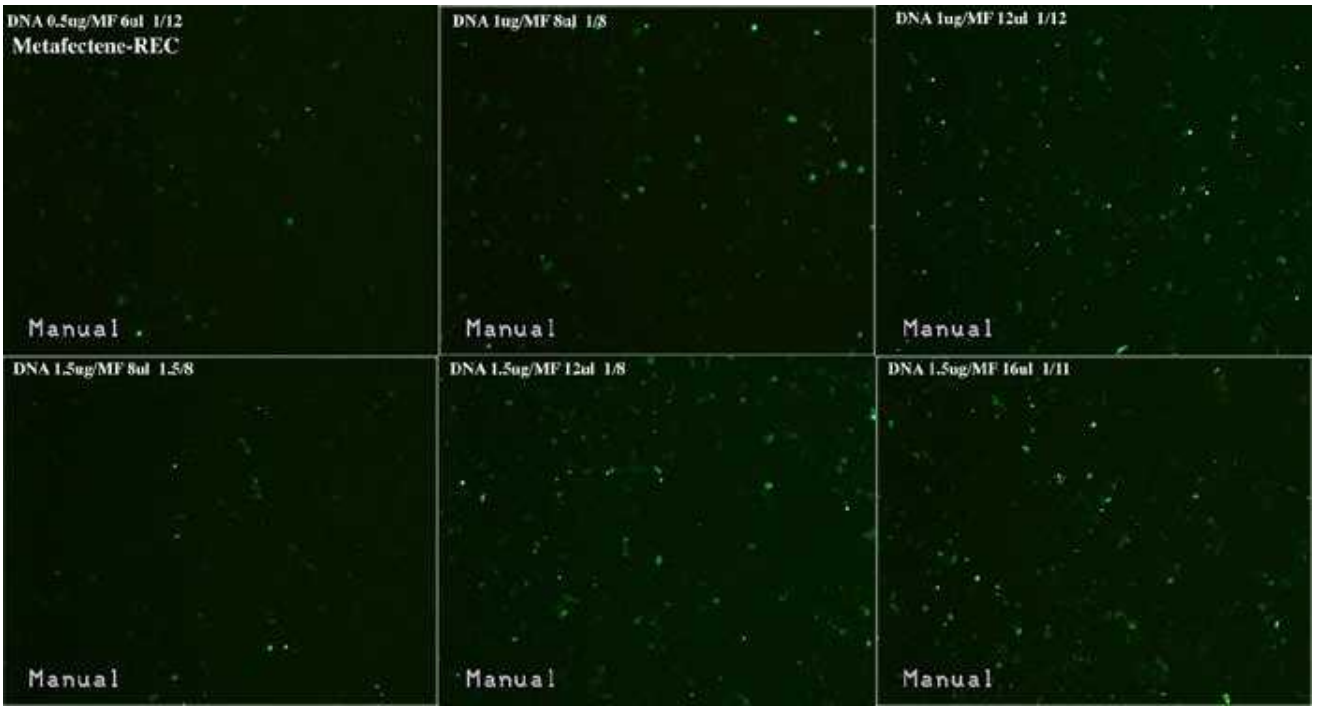
				MDCK	EMC
	DNA (ug)	METAFECTENE	Ratio	% transfected cells	% transfected cells
A	0.5	1	1/2	4	0
B	0.5	2	1/4	1	<1
C	0.5	4	1/8	1	1
D	0.5	6	1/12	< 1	1
E	1	2	1/2	6	0
F	1	4	1/4	5	<1
G	1	8	1/8	< 1	5
H	1	12	1/12	< 1	5
I	1.5	4	1/2.6	7	2
J	1.5	8	1/5.3	4	2
K	1.5	12	1/8	< 1	5
L	1.5	16	1/11	< 1	5

METAFECTENE was added to 50ul of serum-free DMEM or Optimem in a 1.5ml tube. In another tube, plasmid DNA was added to 50ul of serum-free DMEM or Optimem. The tubes were combined and incubated for 20 minutes at RT so lipid-DNA complexes could form. Fresh media containing serum but free of antibiotics was added to cells growing in 12 well plates. The transfection mixture was added to cells and placed in a 37? incubator (5% CO_2) for either 4 hours or overnight. Twenty-four hours after transfection, live cells were assessed for transfection efficiency using an inverted microscope.

MDCK cells transfected with 1.5ug DNA with 4ul of Metafectene



EMCs transfected with differing ratios of DNA/MF



Conclusions:
For both MDCK and EMCs, transfection efficiencies were low, as is common for epithelial lines. However, as compared with a comparable transfection reagent, MDCK cells transfected with METAFECTENE resulted in a markedly higher transfection efficiency. However, transfection of EMCs was approximately the same when comparing METAFECTENE and a comparable transfection reagent.

Duplicate experiments were performed where METAFECTENE was combined with DNA in either serum-free DMEM or Optimem and no difference in efficiency was observed. Likewise, the cells were incubated in the transfection mixture for either 4 hours or overnight (no difference in efficiency was observed).

The transfection of MDCK cells appeared to be better when the ratio of DNA to METAFECTENE was higher (between 1:2 and 1:4), and when a higher concentration of DNA was used (1-1.5ug).