Application Note

Transfection of mIC_{c12} cells with Metafectene

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Cells
mICc12, mouse intestinal cells, adherent

Cell Culture
The cells are splitted one time per week at a ratio of 1:5 to 1:10. Medium exchanged each second day.
Description of the medium according to Dr. A. Vandewalle (1) (INSERUM U478, Faculte X. Bichat, 16 rue Henri Huchard, B.P. 416, 75870 Paris Cedex 18 France)
for 500 ml (final concentrations):
HAMF`12 250 ml
DMEM 250 ml
Insulin 5 mikrog/ml
Dexamethasone 50 nM
Selenium 60nM
Transferrin 5 mikrog/ml
Triiodothyronine 1nM
EGF 10ng/ml
HEPES 20mM
Glutamine 2 mM
FCS (fetal calv serum; heat inactivated) 2%

This medium is also used during Transfection.

Day 1: Seeding

Trypsinize the cells; add medium and count them.
Add 2 ml medium per well in **three** 6-well plates.
Add 500 000 cells per well to the first plate;
Add 450 000 cells per well to the second plate;
Add 300 000, 400 000, 450 000 (2x), 500 000 and 600 000 to the wells of the third plate;
incubate for 16-18 h.

Day 2: Transfection

Prepare 2 sterile tubes for each transfection (1 for DNA and one for Metafectene (MF)).
Add 100 µl PBS(-Mg –Ca; Invitrogen) (room temperature) to each tube. Add either DNA (pcCMV FUSII-zeo) or MF to the tubes (see table)

Plate 1

well	1	2	3	4	5	6
DNA (µg)	2	2	2	2	1	4
MF (µl)	5	10	15	20	10	10

Plate 2

well	1	2	3	4	5	6
DNA (µg)	1	2	3	4	1	3
MF (µl)	2.5	5	7.5	10	5	5

Plate 3

well	1	2	3	4	5	6
DNA (µg)	2	2	2	2	2	0
MF (µl)	5	5	5	5	5	0

Transfer the MF solutions into the DNA containing tubes; mix gently; incubate at RT 20 min.
Add the transfection solutions to the 6-well plate.
Incubate in the incubator for 30 h.

Day 3: Assay and Evaluation

Assess transfection efficiency 1. transfected cells are fluorescent under blue light (EGFP excitation at 530 nm).
2. luciferase activity per well;

Cell lysis: add 150 µl reporter lysis buffer (RLB)(Promega).
Freeze the plate
Dilute the lysate 20 x in RLB and assay 5 µl in a Turner luminometer for 15 s.

Results:

Plate 1:
In wells 1 and 6 app. 10 and 12% of the cells were transfected; in the other wells less (3-5%). Luciferase assay gives similar results. Optimal concentration 5 µl MF and 2 µg plasmid DNA.

Plate 2:
Between 8-10% of the cells were transfected. In the well no.:6; there was not a higher percentage of cells transfected rather did transfected cells fluorescence stronger:

Plate 3:
Little impact of cell density on transfection efficiency.

Recommendations: 5-6 µl MF and 2 µg plasmid DNA; seed 400 000-500 000 cells /well in a 6-well plate.

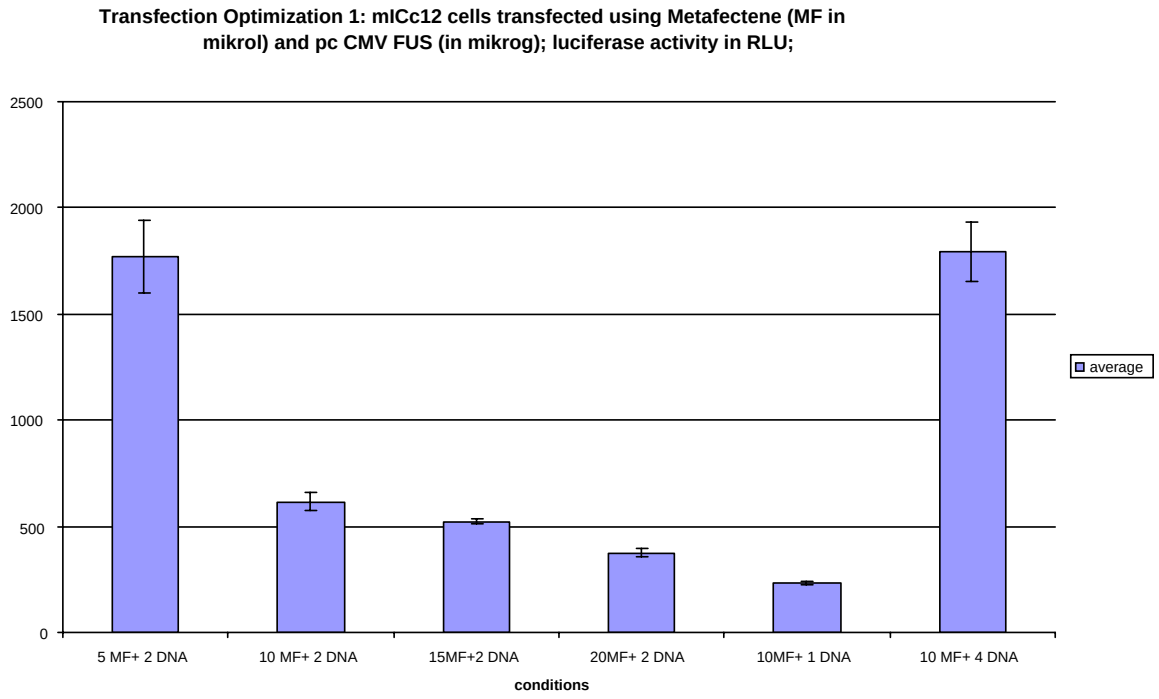

Figure 1

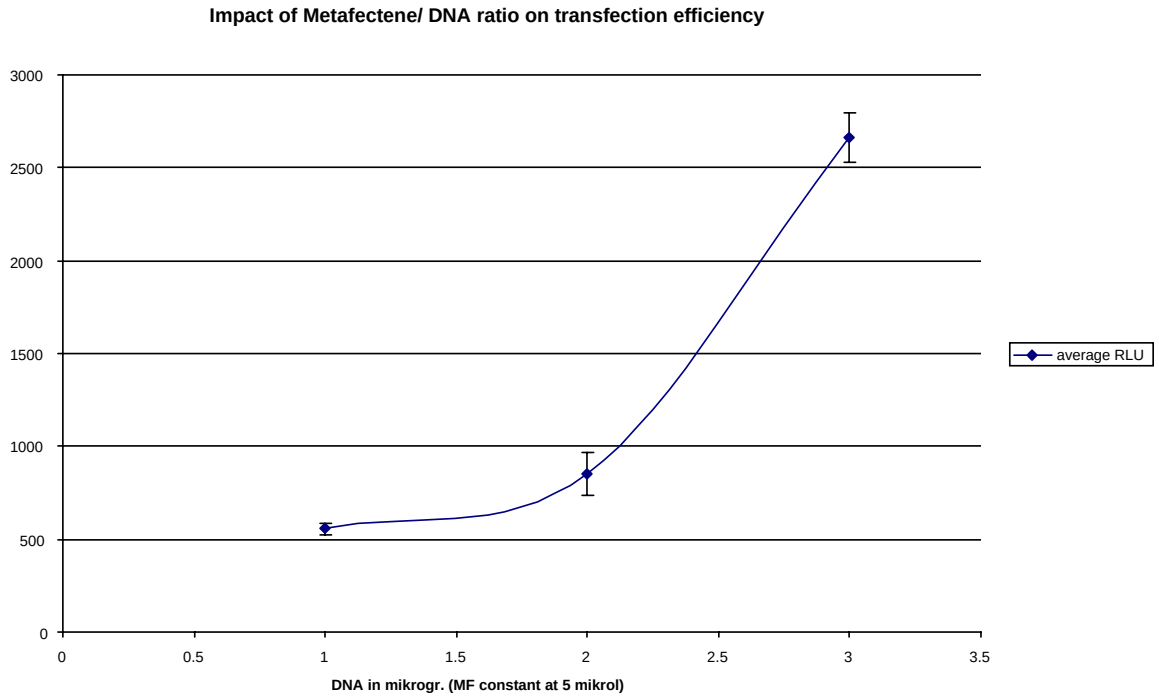

Figure 2

References:

(1) Bens, A., et al. 1996. Trans-immortalized intestinal cells (m-ICc12) that maintain a crypt phenotype. Am.J. Physiol. 270:C1666-C1674