Metafectene Technical Note

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Testing The New Transfection Agent Metafectene™

We compared the transfection efficiency of the agent Metafectene to Lipid F in osteosarcoma cells (cell line 143B). For this purpose rho0 cells lacking mitochondrial DNA and therefore lacking the mitochondrial respiratory chain, as well as cybrid cells containing wildtyp mitochondria of patient fibroblasts with the MEALS mutation (myoclonic epilepsy, lactic acidosis and strokes) or mitochondria with a mutation on position 3302 of the mitochondrial DNA, were used. The 3302 mutation causes an isolated myopathy of skeletal muscles. The vector used encoded the green fluorescent protein.
Transfection efficiency was qualitatively monitored under a fluorescence microscope.

Cell culture:

The osteosarcoma cybrid cell line 143B rho0 as well as the cybrid lines were propagated in Dulbecco's modified eagles's medium (DMEM), supplemented with 5 % fetal calf serum, 110 µg/ml of sodium pyruvate, 1% penicillin/streptomycin and 50 µg/ml uridine, in a humid atmosphere at 37 °C and 5 % CO_2.
The cells were grown for 18 to 24 hours on coverslips (20mm x 20mm) coated with 1 % gelatine in a 35 mm-well to a confluency level of 80-90 %.

Transfection

Metafectene: We used the protocol proposed by Biontex. The optimized lipid-DNA ratio was 4:1.
4 µl Metafectene and 1µg DNA (pcDNA1 für mtAeq), each in 100 µl medium were mixed in a reaction tube by carefully pipetting up and down. During the incubation time, the cell medium in the wells was changed.
After 20 minutes the solution was again mixed by tapping on the tube before it was added dropwise onto the cells. Routinely 200 µl were used for a 35 mm-well.

Lipid F: We used the protocol proposed by the manufacturer. The optimized lipid-DNA ratio was 3:1.

Fluorescence microscope:

Pictures were taken from the central area of the coverslip at a 50-fold magnification.

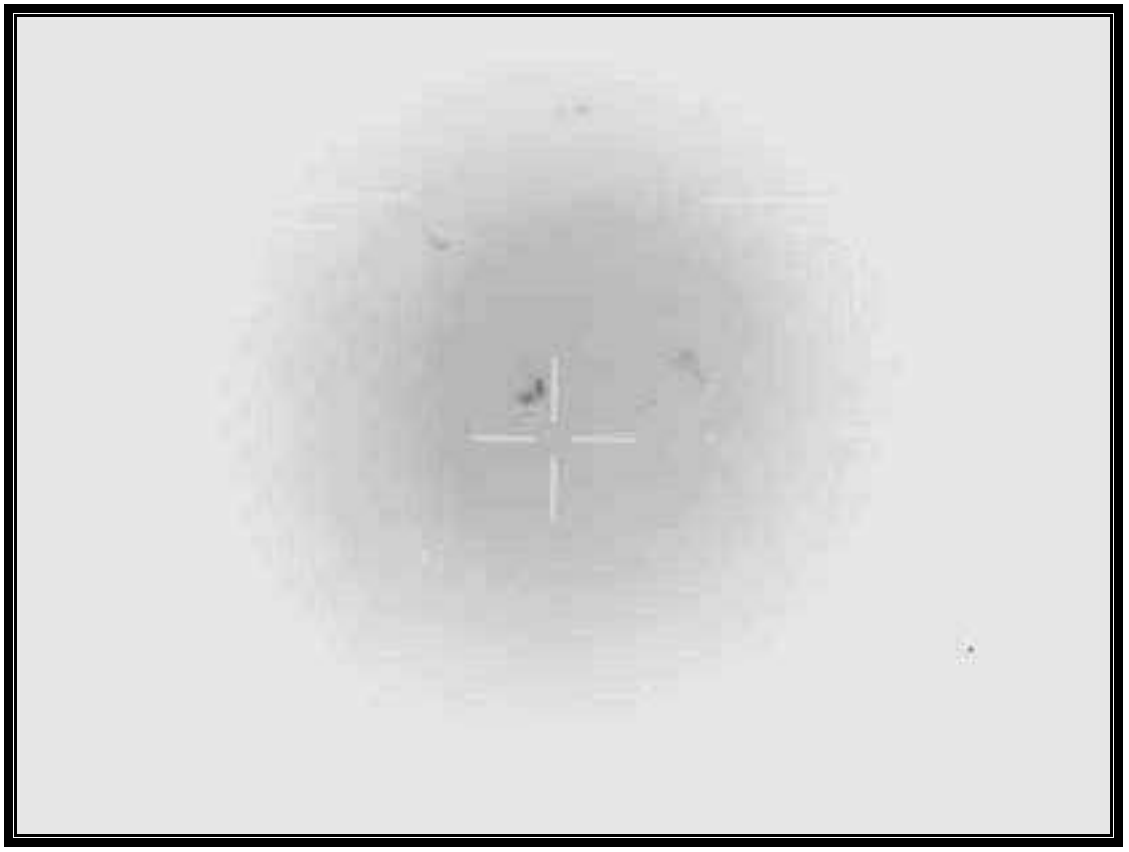
Editing:

The pictures were edited with Adobe Photoshop by resizing, inverting and converting to grayscale.

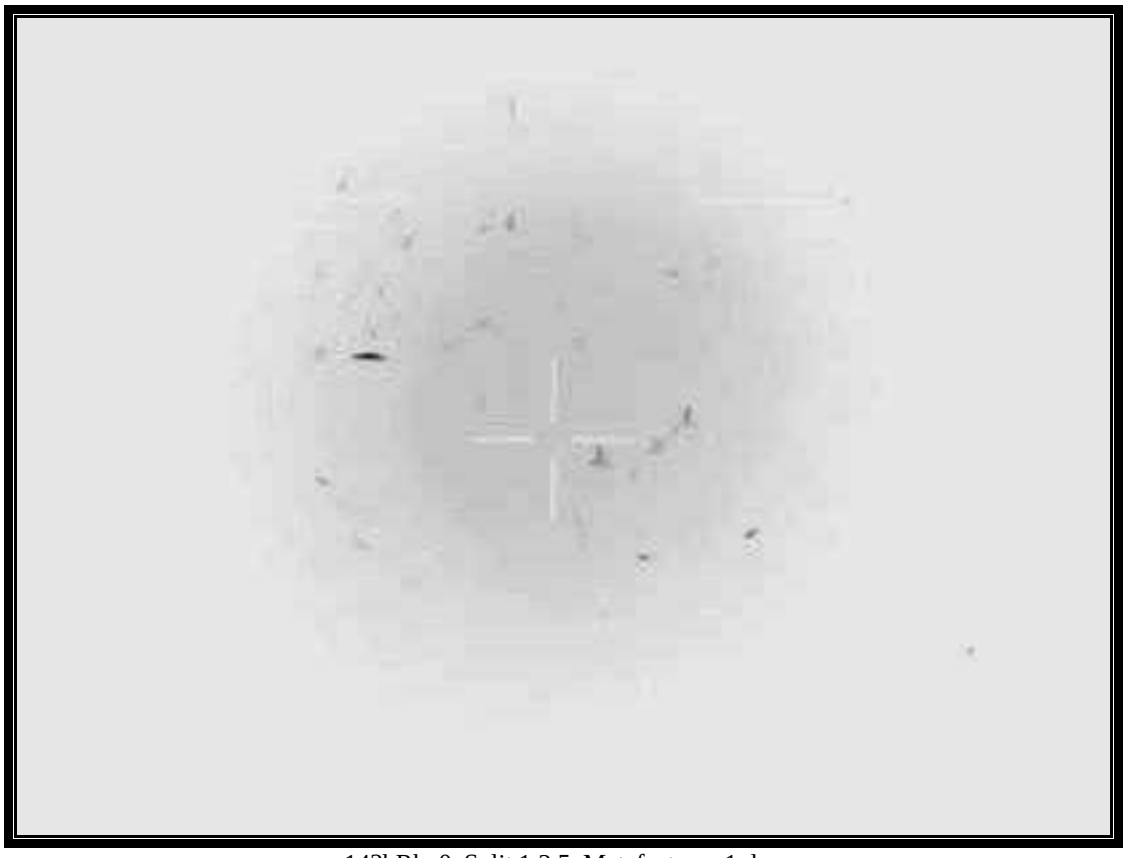
Discussion:

In each of the three cell lines a higher transfection efficiency was observed using Metafectene. While more cells appeared green fluorescent at the same cell density, the cytotoxic effect was less pronounced. Whereas the difference was especially clear for the rho0 cells and wt cybrids, 3302 cells showed only a minor difference.

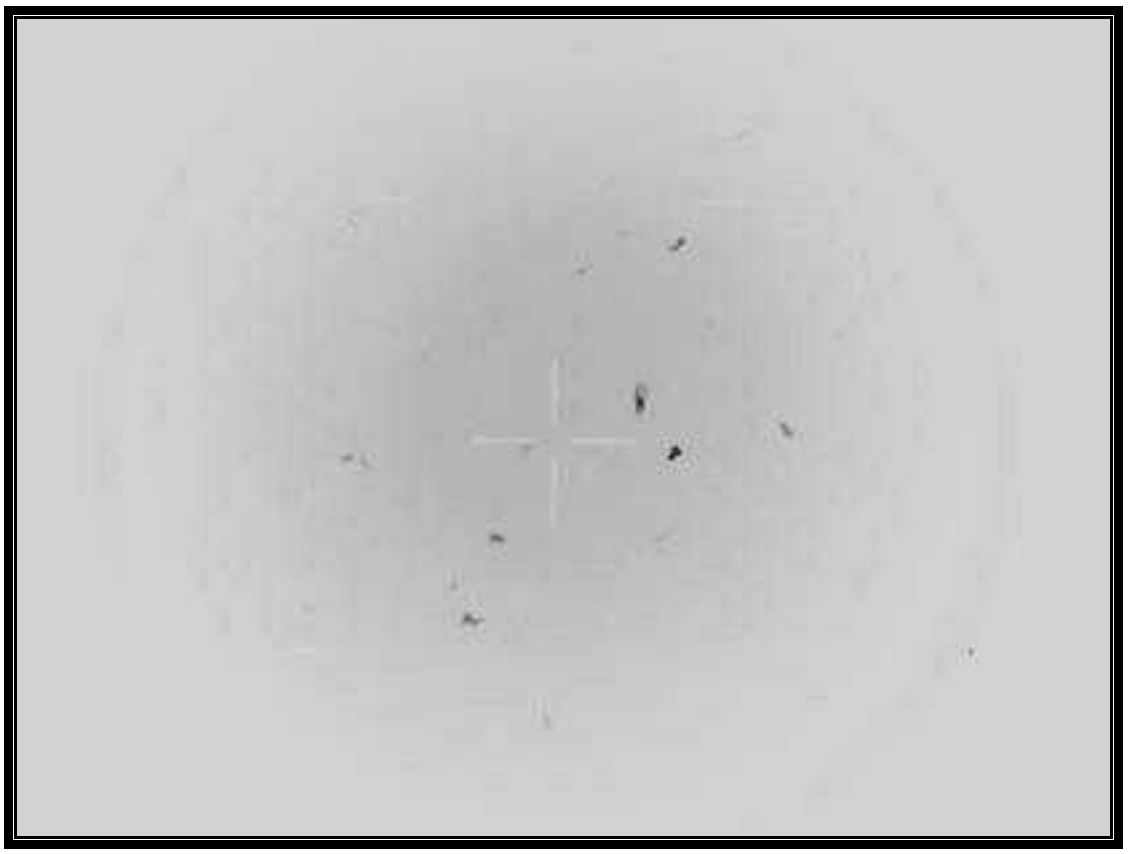
The applied protocol was optimised by variation of the different transfection parameters. DNA amount was kept constant. Incubation longer than 24 hours did not significantly improve transfection efficiency also, changing the cell medium was not necessary. At the Metafectene and DNA concentration used, no toxicity was observed, similar to Lipid F. Higher Metafectene concentrations (up to 10 µl Metafectene for a 35 mm-well) did not show better results (6 µl) or showed a cytotoxic effect (10 µl), respectively.



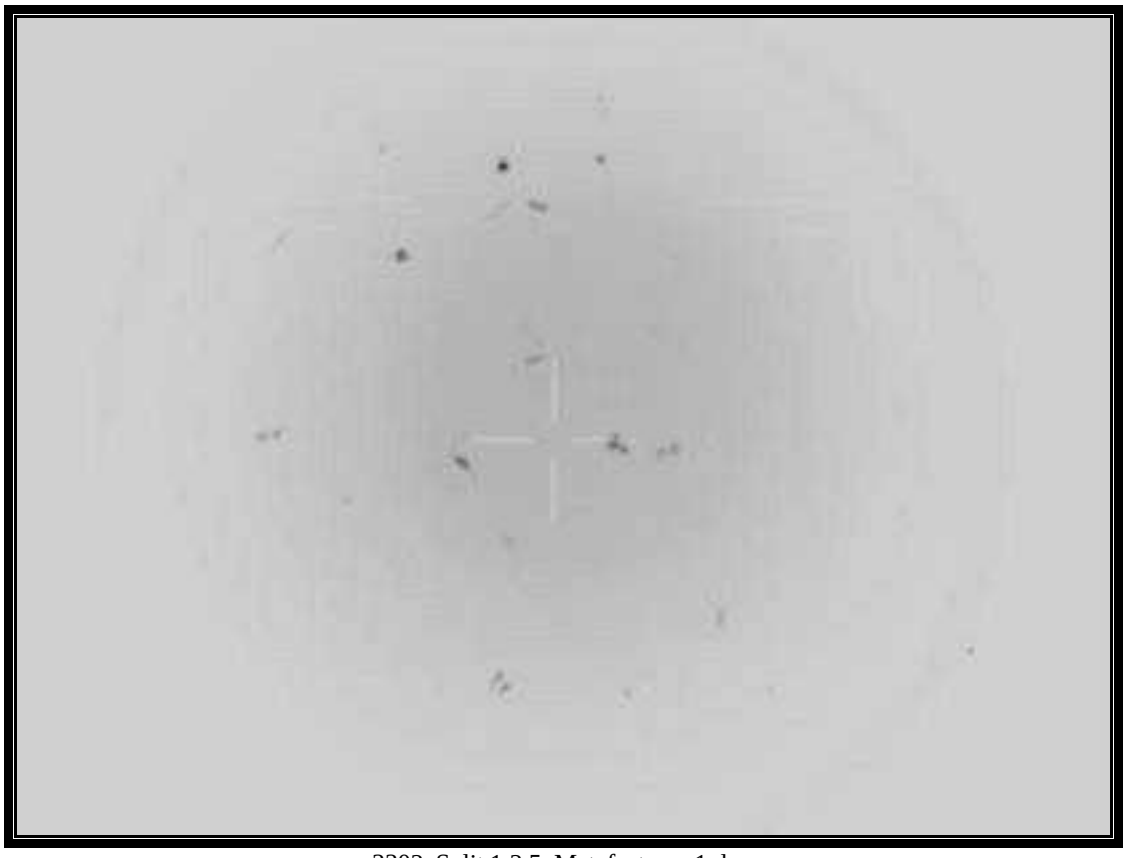
143bRho0, Split 1:2,5, Lipid F-1.day



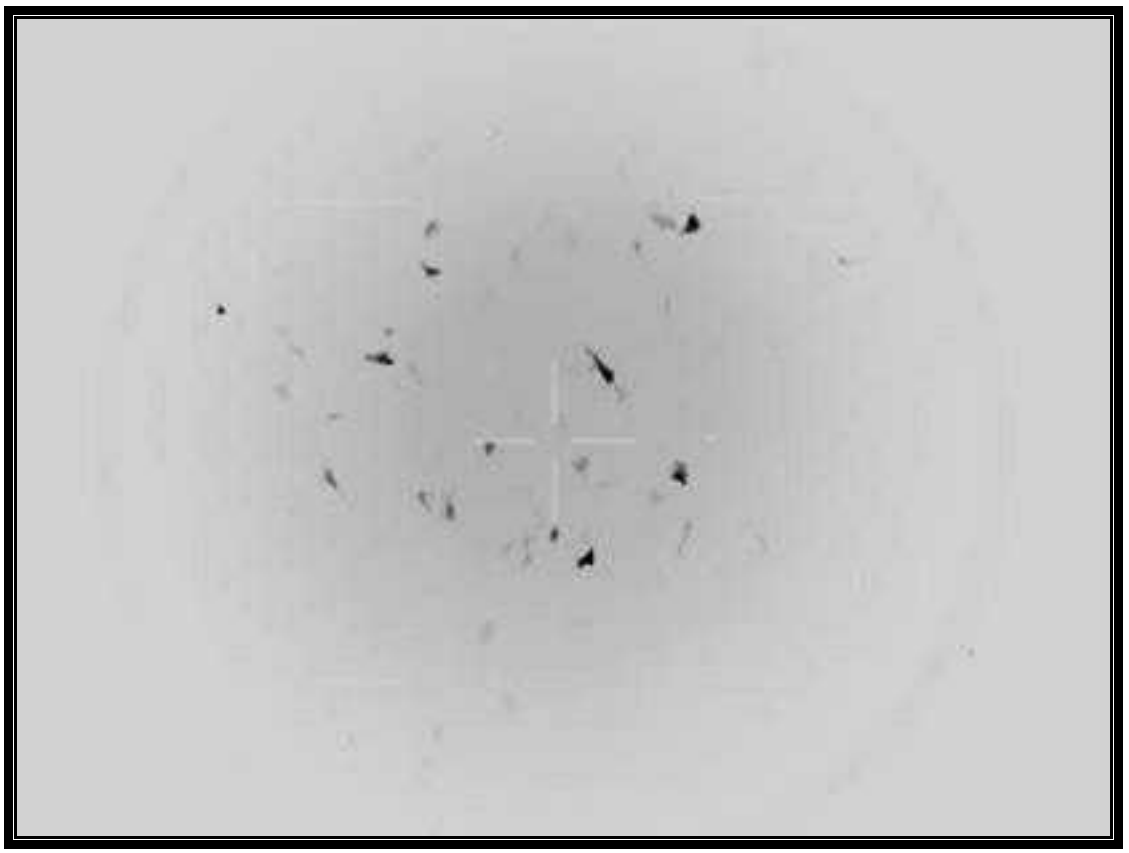
143bRho0, Split 1:2,5, Metafectene -1.day



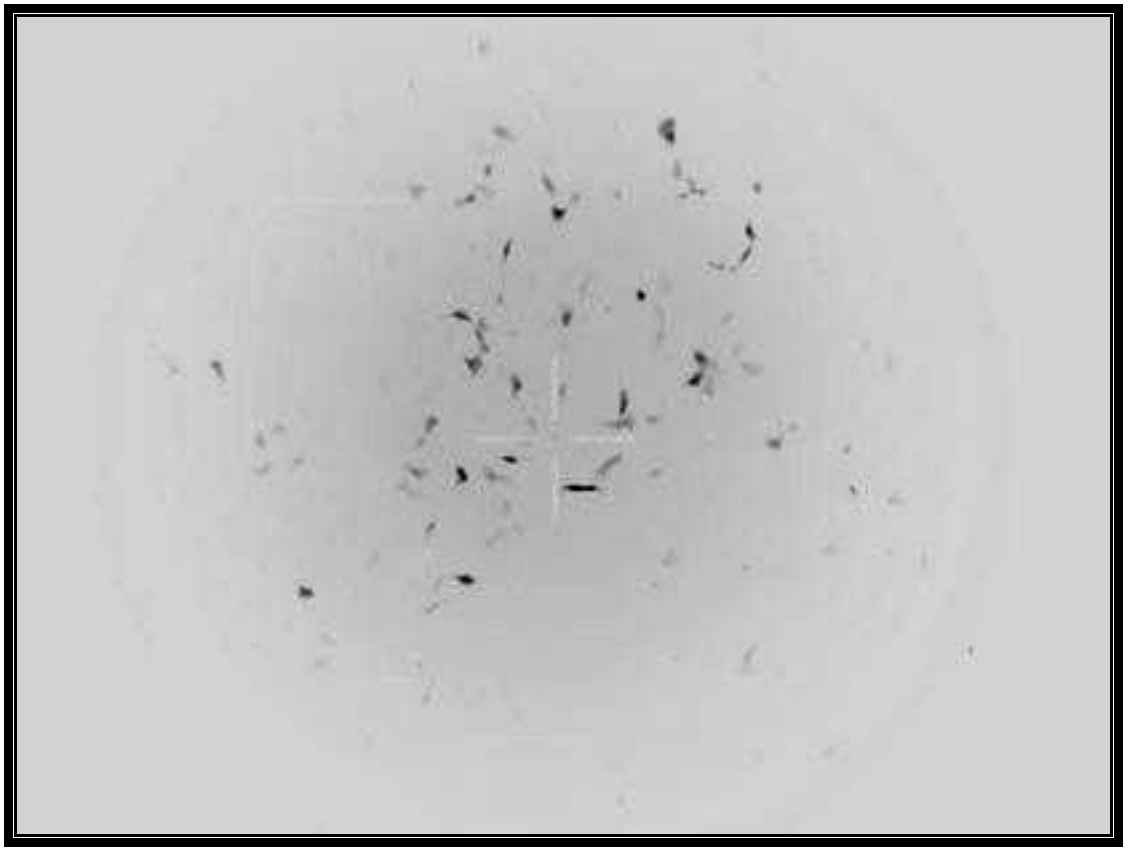
3302, Split :2,5, Lipid F-1.day



3302, Split 1:2,5, Metafectene -1.day



3243wt, Split 1:3, Lipid F -1.day



3243wt, Split 1:3, Metafectene -1.day