Comparative study of Metafecten Pro and Lipofectamine 2000 by Reverse Transfection Cell Microarray (RTCM)

Introduction

Reverse Transfection cell microarray (RTCM) is a good method for parallel high throughput analyzes of gene function in mammalian cells. By allowing the simultaneous transfection of an immense number of different recombinant DNA or RNA constructs into cells this method opens up the possibility to conduct several hundreds of cell culture experiments at the same time on a single glass slide(Sturzl *et al.*, 2008). Standartisation of RTCM is very important for getting optimal results. Therefore, the new transfection reagenz Metafecten Pro was tested with 20 different ratio of DNA : Tranfectionreagenz to optimize it for RTCM.

Materials and Methods

Materials

Metafectene PRO, a polycationic liposomal transfection reagent, was obtained from Biontex Laboratories GmbH (Munich, Germany). Optimem was purchased from Invitrogen (Karlsruhe, Germany). Succhrose was obtained from Merck (Darmstadt, Germany). DMEM, penicillin, streptomycin and L-glutamine solutions were purchased from PAA (Cölbe, Germany). The plasmid encoding eGFP was used for evaluating transfection efficiency.

Cells

Human cervical epithelial cancer cell line HeLa, Human embryonic kidney cell line HEK293 and Green monkey kidney cell line Vero were cultivated in Dulbecco´s modified Eagle´s Medium (DMEM) supplemented with 10% fetal calf serum (Biochrome, Berlin, Germany), penicillin(50 U/ml), streptomycin (50 µg/ml) and L-glutamine (2mM).

Preparation of spotting solution, spotting and cell seeding

1-4µg eGFP plasmid was mixed with 2-4µl Metafecten Pro or Lipofectamine 2000. 3µl Optimem containing 0,4M sucrose were added. The mixture was filed with water to a final volume of 11,5µl. To form complexes the solution was mixed by pipetting up and down and incubated for 20 min at room temperature. Plasmid-/ transfection solution was mixed with 7,25µl 0.2% gelatine (Sigma) and transferred to a 384- Well plate. Subsequently the plate was cooled to 12°C and spotting was carried out with a contact printer VersArray ChipWriterPro (Biorad) using solid pins (PTS 600) with a tip diameter of 600µm and a center to center distance of 1100µm. Different cell lines were seeded onto the printed chips at a density to reach full-confluencey after 48h (table 1). Signal detection is carried out with a laser scanner (FLA 5000) with a resolution of 25µm. Evaluation of data was done with a quantification software (AIDA).

Results

Reverse Transfection of different cell lines with Metafecten Pro

Transfectionreagenz was complexed with the eGFP plamid at differents DNA to Transfectionreagenz ratios and tested with different cell lines (Table 1).

Cell line	Seeded cells per cm^2	Transfection efficiency
HEK 293	$1x10^5$ cells	80%
HeLa	$2,5x10^4$ cells	30-50%
Vero	$2,15x10^4$ cells	10%

Table 1 Technical information about cell seeding and transfection efficiency for RTCM

In all three cells reverse transfected with Metafecten Pro showed high variations depending on DNA to transfection reagenz ratio. But the optima were only slightly reproducible in a second screen. Transfection efficiency was the best in HEK293 cells. In HeLa and Vero cells only few cells were transfected.

Comparison of reverse transfection efficiency of Lipofectamin 2000 with Metafecten Pro

Next we compared the transfection efficiency of Metafecten Pro with a well standardised transfection reagenz Lipofectamine 2000 which was already published to work good in reverse transfetcion (Kato et al., 2004; Uchimura et al., 2005). In HEK293- and HeLa cells Metafecten Pro induces higher expression than Lipofectamine 2000 but showed much more dependent on DNA : Lipofectamine ratio (Figure 2A+B) which were not possible to establish. In Vero cells for the most part of the experimente Lipofectamine 2000 induces higher expression. The expression level after Metafecten transfection was in the best case the same as with Lipofectamine 2000, but as in HEK 293 and HeLa cells also much more dependent on the DNA : Transfectionreagenz ratio (Figure 2 C) which were also not possible to establish.

References

Kato K, Umezawa K, Miyake M, Miyake J, Nagamune T (2004). Transfection microarray of nonadherent cells on an oleyl poly(ethylene glycol) ether-modified glass slide. *Biotechniques* **37:** 444-8, 450, 452.

Sturzl M, Konrad A, Sander G, Wies E, Neipel F, Naschberger E *et al* (2008). High throughput screening of gene functions in Mammalian cells using reversely transfected cell arrays: review and protocol. *Comb Chem High Throughput Screen* **11:** 159-72.

Uchimura E, Yamada S, Uebersax L, Yoshikawa T, Matsumoto K, Kishi M *et al* (2005). On-chip transfection of PC12 cells based on the rational understanding of the role of ECM molecules: efficient, non-viral transfection of PC12 cells using collagen IV. *Neurosci Lett* **378:** 40-3.

Figure legends

Figure 1

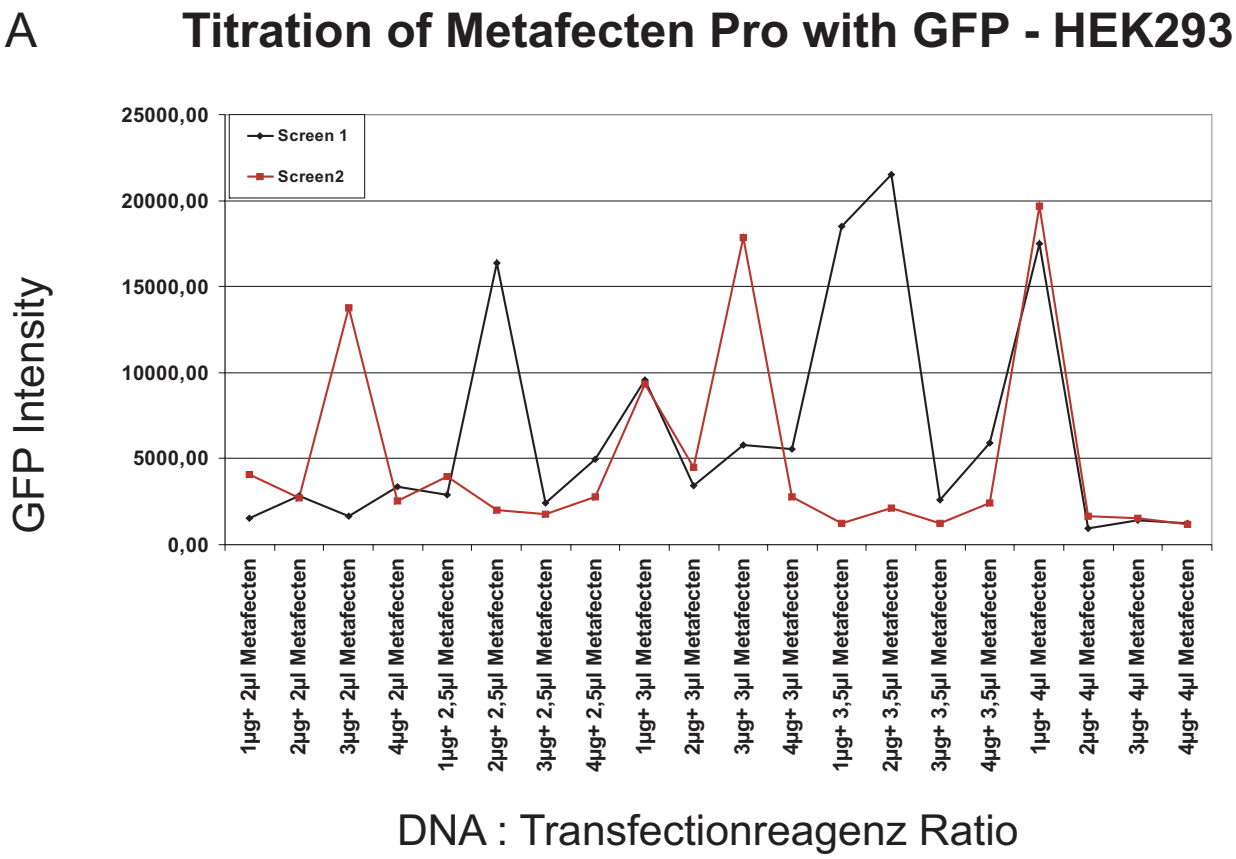
HEK293- (A), HeLa- (B) and Vero- (C) cells were reverse transfected with GFP using different ratio of DNA to transfectionreagenz. GFP intensität was measured by FLA5000 and quantified by AIDA software. The results are showing 2 independent screens.

Figure 2

HEK293- (A), HeLa- (B) and Vero- (C) cells were reverse transfected with GFP using two differenent transfectionreagenzes (Lipofectamine 2000, red; Metafecten Pro, black). Different ratio of DNA to transfectionreagenz were analysed and GFP intensität was measured by FLA5000. Quantification was done with AIDA software.

Figure 1

A


Titration of Metafecten Pro with GFP - HEK293
GFP Intensity
25000,00
20000,00
15000,00
10000,00
5000,00
0,00
Screen 1
Screen2
1µg+ 2µl Metafecten
2µg+ 2µl Metafecten
3µg+ 2µl Metafecten
4µg+ 2µl Metafecten
1µg+ 2,5µl Metafecten
2µg+ 2,5µl Metafecten
3µg+ 2,5µl Metafecten
4µg+ 2,5µl Metafecten
1µg+ 3µl Metafecten
2µg+ 3µl Metafecten
3µg+ 3µl Metafecten
4µg+ 3µl Metafecten
1µg+ 3,5µl Metafecten
2µg+ 3,5µl Metafecten
3µg+ 3,5µl Metafecten
4µg+ 3,5µl Metafecten
1µg+ 4µl Metafecten
2µg+ 4µl Metafecten
3µg+ 4µl Metafecten
4µg+ 4µl Metafecten
DNA : Transfectionreagenz Ratio

B

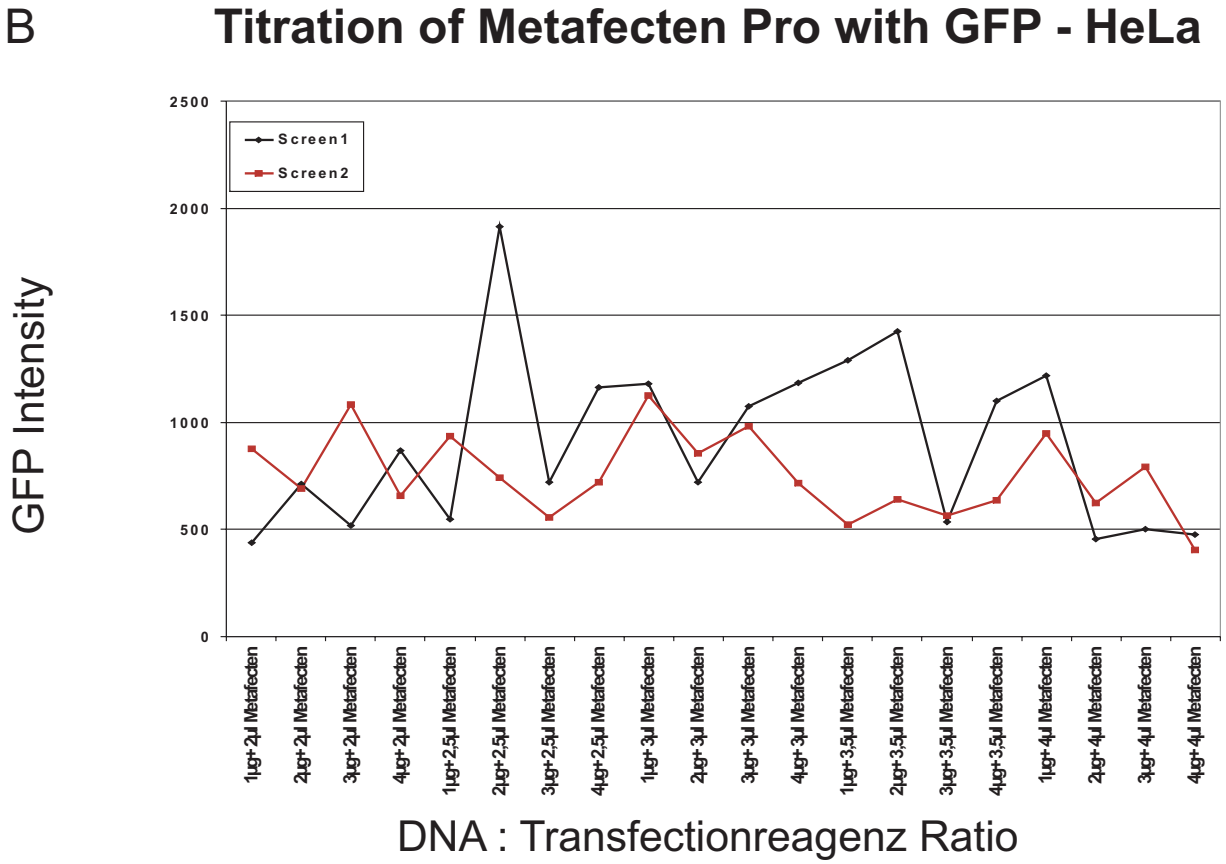

Titration of Metafecten Pro with GFP - HeLa
GFP Intensity
2500
2000
1500
1000
500
0
Screen1
Screen2
1µg+2µl Metafecten
2µg+2µl Metafecten
3µg+2µl Metafecten
4µg+2µl Metafecten
1µg+2,5µl Metafecten
2µg+2,5µl Metafecten
3µg+2,5µl Metafecten
4µg+2,5µl Metafecten
1µg+3µl Metafecten
2µg+3µl Metafecten
3µg+3µl Metafecten
4µg+3µl Metafecten
1µg+3,5µl Metafecten
2µg+3,5µl Metafecten
3µg+3,5µl Metafecten
4µg+3,5µl Metafecten
1µg+4µl Metafecten
2µg+4µl Metafecten
3µg+4µl Metafecten
4µg+4µl Metafecten
DNA : Transfectionreagenz Ratio

Figure 1

C

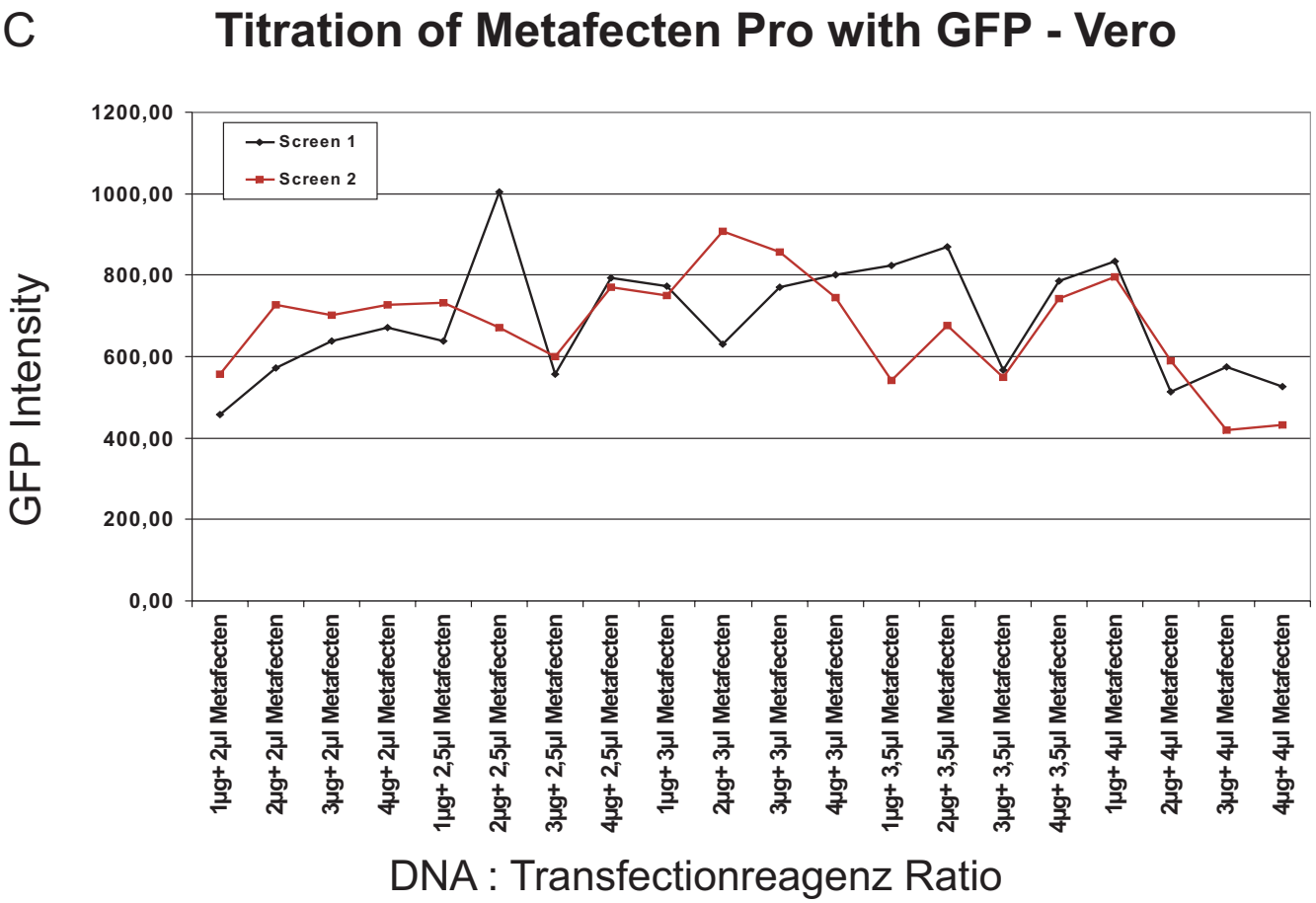
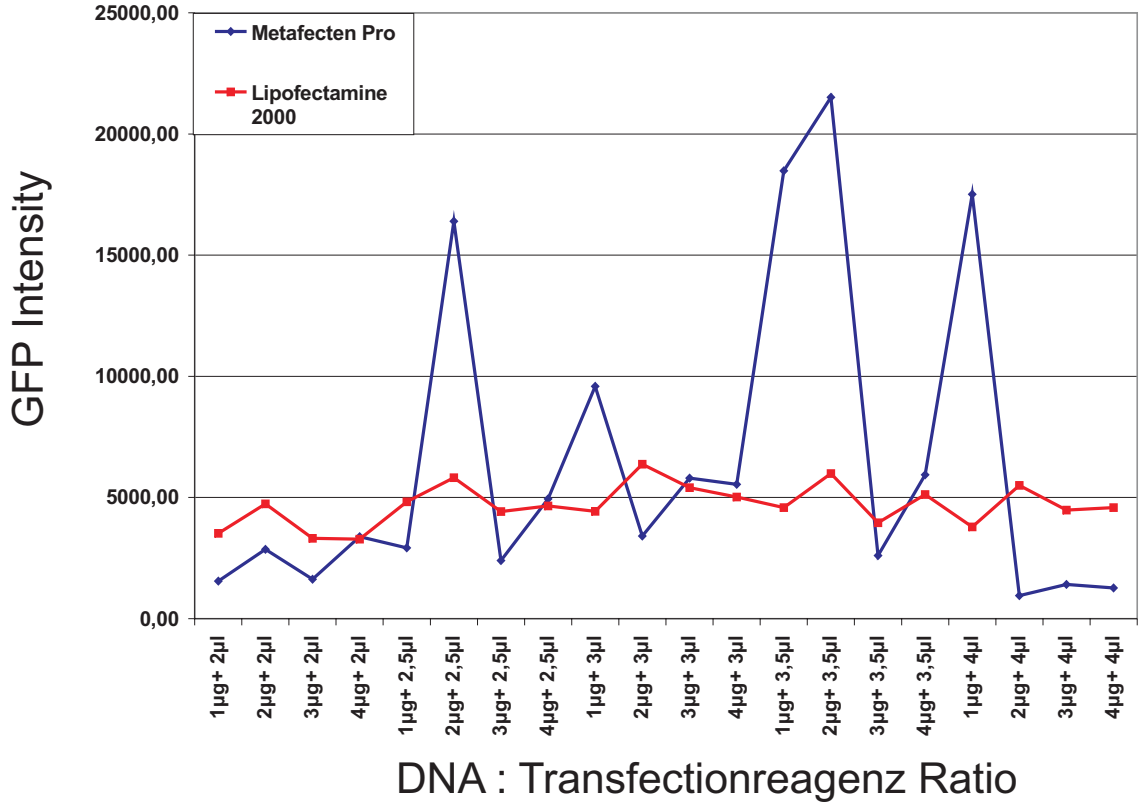

Titration of Metafecten Pro with GFP - Vero
GFP Intensity
1200,00
1000,00
800,00
600,00
400,00
200,00
0,00
Screen 1
Screen 2
1µg+ 2µl Metafecten
2µg+ 2µl Metafecten
3µg+ 2µl Metafecten
4µg+ 2µl Metafecten
1µg+ 2,5µl Metafecten
2µg+ 2,5µl Metafecten
3µg+ 2,5µl Metafecten
4µg+ 2,5µl Metafecten
1µg+ 3µl Metafecten
2µg+ 3µl Metafecten
3µg+ 3µl Metafecten
4µg+ 3µl Metafecten
1µg+ 3,5µl Metafecten
2µg+ 3,5µl Metafecten
3µg+ 3,5µl Metafecten
4µg+ 3,5µl Metafecten
1µg+ 4µl Metafecten
2µg+ 4µl Metafecten
3µg+ 4µl Metafecten
4µg+ 4µl Metafecten
DNA : Transfectionreagenz Ratio

Figure 2

A **Comparison of Metafecten Pro and Lipofectamine 2000 - HEK293 -**



B **Comparison of Metafecten Pro and Lipofectamine 2000 - HeLa -**

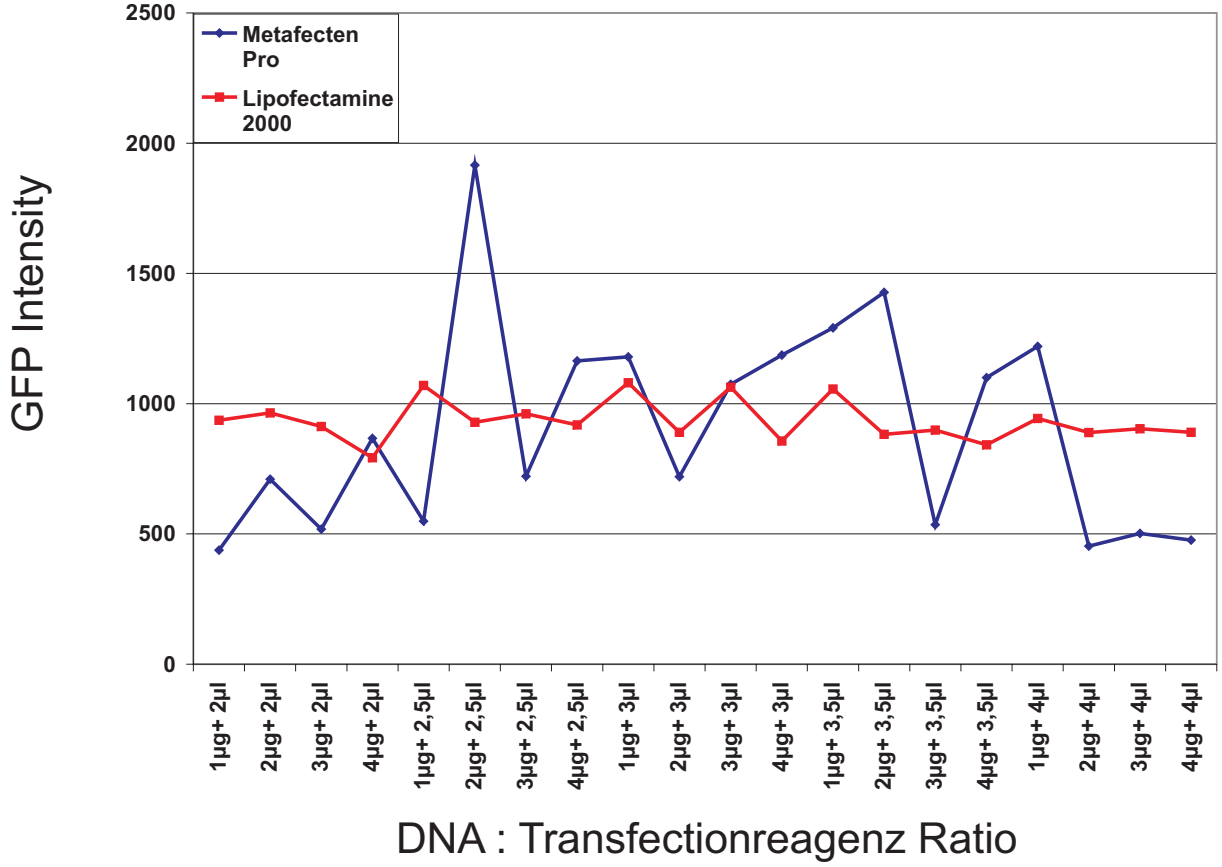


Figure 2

C **Comparison of Metafecten Pro and Lipofectamine 2000 - Vero -**