Application Note:

Metafectene Pro Transfection to mpkCCD cells

1 Title of Experiment:

Transfection of pEGFP-C1 and pGL3-Control to mpkCCD cells.

2 Author, Institute and address:

M.L.A. Kortenoeven and P.M. Deen
Department of Physiology
Nijmegen Centre for Molecular Life Sciences
Radboud University Nijmegen Medical Centre
P.O. Box 9101, 6500 HB Nijmegen

3 Introduction:

Transfection of mpkCCD has been very inefficient using conventional methods like PEI or lipofectamine, resulting in about 5% positive cells. Therefore, we tested different transfection reagents which could yield higher transfection efficiency on mpkCCD cells.

4 Materials and methods:

- 24-well plate for transfection
- 96-well plate for preparing transfection mix
- $mpkCCD_{c14}$ cells, preferably 50-70% confluency before trypsinization
- Metafectene PRO (Biontex)
- Fugene 6 and Fugene HD (Roche)
- PBS
- Growth medium (Dulbecco's modified Eagle's medium/Ham's F-12, 1:1 v/v; 60 nM sodium selenate; 5 µg/ml transferrin; 2 mM glutamine; 50 nM dexamethasone; 1 nM triiodothyronine; 10 ng/ml epidermal growth factor; 5 µg/ml insulin; 20 mM D-glucose; 2% fetal calf serum; and 20 mM HEPES, pH 7.4)
- DNA constructs: pEGFP-c1and pGL3-Control

mpkCCD cells were seeded in a 24 wells plate and after one day transfection was performed using either Metafectene Pro, Fugene 6 or Fugene HD. Variable amounts of DNA and ratios of DNA to reagent were used to select the condition yielding the highest transfection efficiency. Transfection was also performed to cells in suspension, immediately after seeding.

5 Experimental procedures / transfection protocol:

mpkCCD cells (preferably 50-70% confluency) were pre-washed with PBS-EDTA and trypsinized at 37°C for 30 minutes. Cells were resuspended in growth medium and seeded onto the well at a density of 50000-80000 cells per cm^2, resulting in 40-60% confluency after 1 day.
Transfection was also performed to cells in suspension, immediately after seeding. In this case, cells were seeded at a density of 150.000 cells per cm^2.

For MetaPro transfection, two separate solutions were prepared in a 96-well plate. Solution A contained the DNA in PBS (total 30 µl for a 24-wells plate), solution B contained Metafectene Pro in PBS (total 30 µl). Solution A was added to solution B and incubated at room temperature for 15-20 minutes. The mix was added to 800 µl of normal growth medium on the cells in the well and mixed by making "eights". Cells were grown at 37°C (5%CO2), and medium was refreshed the next day.
For Fugene 6 and Fugene HD, transfection was performed according to manufacturer's instructions (see references).
Three days after transfection, the amount of green (GFP-positive) cells was estimated under a fluorescent microscope or luciferase activity was measured using the Luciferase Assay System (Promega) and a luminometer.

6 Results and discussion:

- MetaPro was compared to Fugene 6 and Fugene HD. MetaPro showed the highest transfection efficiency in mkpCCD cells, with up to 70% of GFP-positive cells observed under a fluorescent microscope. Fugene 6 resulted in about 10% of transfected cells, Fugene HD got circa 5%. Transfection with a luciferase vector PGL3-control also gave the highest luciferase activity in cells transfected by MetaPro.

- Transfection turned out to be most efficient using the following conditions:
  - 0,5 µg DNA / 100.000 cells
  - Ratio metafectene to DNA = 1 : 4

  This resulted in transfection efficiencies of 50-70%, see appendix.
- Transfection to cells in suspension, immediately after seeding gave similar results to transfection of cells seeded one day before. In this case, less DNA gave better results (0,25 µg / 100.000 cells)

7 Conclusion / summary:

Metafectene Pro works better than other transfection reagents tested on mpkCCD cells. The highest yield of transfected cells using Metafectene Pro was about 70%.

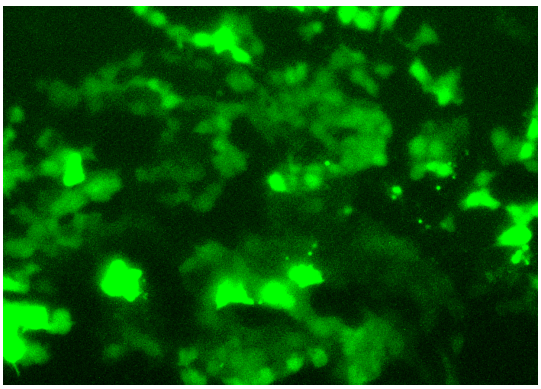
8 References:

1. Transfection protocol Metafectene Pro referred to Biontex Laboratories
   URL:http://www.biontex.com/con_4_6_4/cms/front_content.php?idcat=77

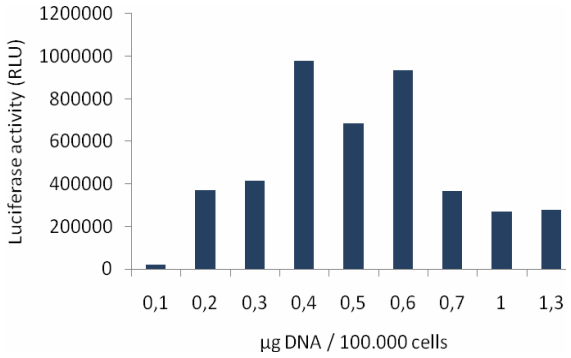
2. Transfection protocols FuGENE 6 / FuGENE HD referred to Roche Applied Science
   URL:https://www.roche-applied-science.com/sis/transfection/applications.jsp

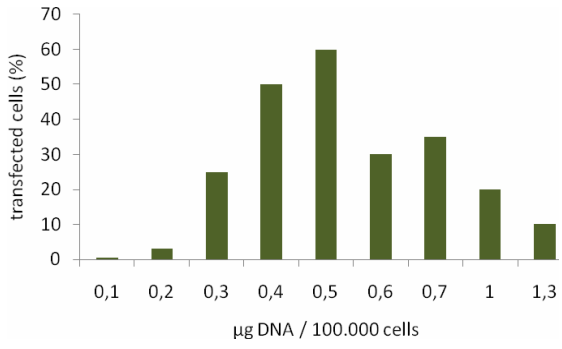
9 Appendix: Tables and/or figures:

1. GFP-positive cells 3 days after transfection using Metafectene Pro



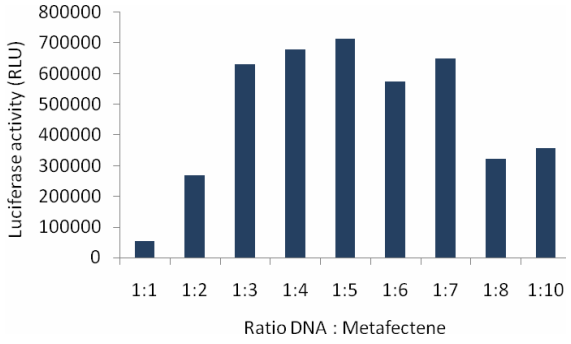
2. Different amounts of transfected DNA

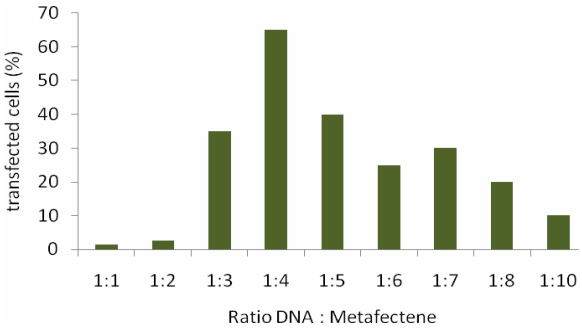




mpkCCD cells were transfected with different amounts of pGL3-control and luciferase activity was measured, as shown on the left, or mpkCCD cells were transfected with different amounts of pEGFP-c1 and the amount of transfected cells was estimated, as shown on the right

3. Different ratio's of DNA : Metafectene Pro





mpkCCD cells were transfected with different ratio's of pGL3-control to Metafectene Pro and luciferase activity was measured, as shown on the left, or mpkCCD cells were transfected with different ratio's of pEGFP-c1 to Metafectene Pro and the amount of transfected cells was estimated, as shown on the right