Metafectene Pro Transfection to MDCK

Transfection of pEGFP-wtAQP2 to MDCK-I using Metafectene Pro (Biontex)

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Introduction:

We tested different transfection reagents to find out which one could yield higher transfection efficiency on hard-to-transfect cell type like MDCK.

Materials and methods:

- Cell type: MDCK type I
- Construct: pEGFP-AQP2 (~5.5 kb) coding for GFP-AQP2 fusion protein
- Metafectene Pro (MetaPro) (Biontex)
- Polyethylenimine (PEI, home-made)
- FuGENE 6, FuGENE HD (Roche)
- Serum-free medium: OPTI-MEM I (Invitrogen)
- DMEM medium (Lonza)
- Foetal Calf Serum (FCS)(PAA Laboratories)
- 96-well plate to prepare transfection mix (Cornings)
- 12-well plate (3.8 cm^2/well) , with/without 1.33 cm^2-polycarbonate (semi-permeable) filter (Cornings)

- Transient transfection was performed to cell suspension, with variable amounts of DNA and ratios between (m)DNA and (v)MetaPro to select among the conditions a highest transfection efficiency. The efficiency was also compared between transfections on non-filter well (plastic support) and on filter (polycarbonate support). Transfection efficiency using MetaPro was also compared to those using other reagents such as PEI, FuGENE 6, FuGENE HD.

Experimental procedures / transfection protocol:

- MDCK cells (preferably at 50%-70% confluency) were pre-washed 1x with PBS-EDTA and tryptinized at 37°C for 30 minutes. Cells were resuspended in medium (DMEM+5% FCS) and seeded onto well/filter at density of 100,000 cells per 1 cm^2. For MetaPro transfection, two separate solutions were prepared in 96-well

plate. Solution A contained 50 ul OPTI-MEM and a ug DNA (0.5 ug, 0.75 ug, 1.0 ug DNA), solution B contained 50 ul OPTI-MEM and b ul MetaP. Ratios a:b were 1:1, 1:3, 1:5, 1:7. The transfection mix was prepared by pipetting sol. A into sol. B, and incubated at room temperature for 15-20 minutes. The mix was added to cell suspension, with addition of fresh medium up to 500 ul per well(or filter). Cells were grown at 37°C (5%CO_2), and medium was refreshed after the first 8 hours. After 72 hours, cells were lysed and blotted against rabbit-7 anti-AQP2.

- For other transfection reagents, follow the standard protocol (see references). The amounts of DNA and ratios between DNA:reagent were adjusted for comparison.

Results and discussion:

1. MetaPro was compared to PEI, FuGENE 6, and FuGENE HD. **MetaPro showed the highest transfection efficiency in MDCK type I cells** with up to 60% of transfected cells per plate were observed GFP-positive under fluorescent microscope. For this observation, FuGENE 6 got up to 30% of transfected cells, FuGENE HD got circa 10%, and 0% for PEI (for Western blot results, see appendix 1).
2. **Transfection efficiency using Metafectene Pro was better on plastic support (normal well plate format) than on polycarbonate support (using Cornings' filter)**. Lower transfection efficiency on filter may be caused by a decrease in Metafectene Pro concentration on the apical side either by circulation of medium through the semi-permeable filters, or by possible interaction between the reagent and the filter material. Thus there were less cells transfected on filter (for western blot results, see appendix 2). To minimize the effect of medium circulation and increase the yield of transfected cells on filter, leave the basolateral side empty and refill a few hours (we tested 8 hours) after transfection, or first to transfect the cells on plastic support, and the next day transfer the cells from plastic support to filter. (Data not shown).
3. **Cell morphology was normal with the presence of Metafectene Pro in the medium**.

Conclusion / summary:

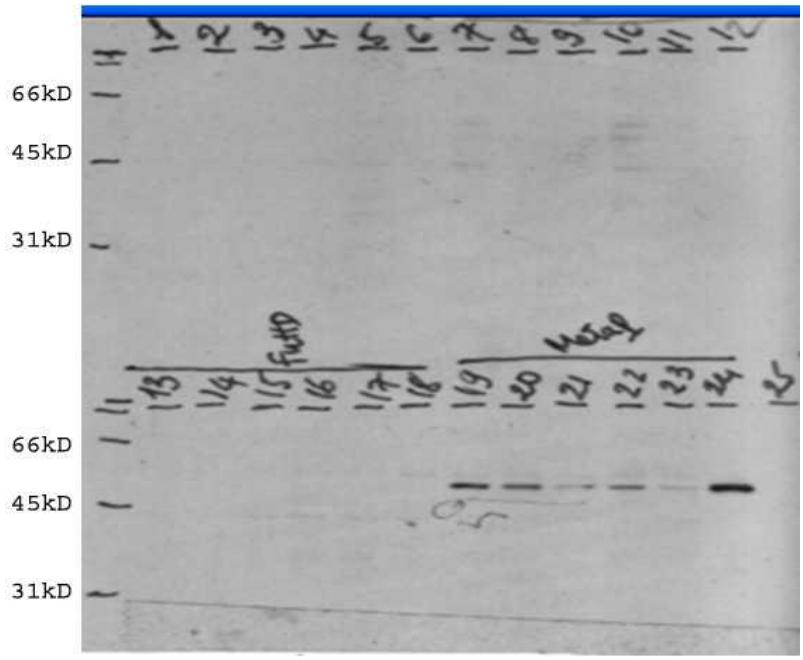
Metafectene Pro works better than other transfection reagents tested on MDCK type I cells. The highest yield of transfected cells using Metafectene Pro was ~60% (with pEGFP-AQP2 vector) under the observation of fluorescent microscope.

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

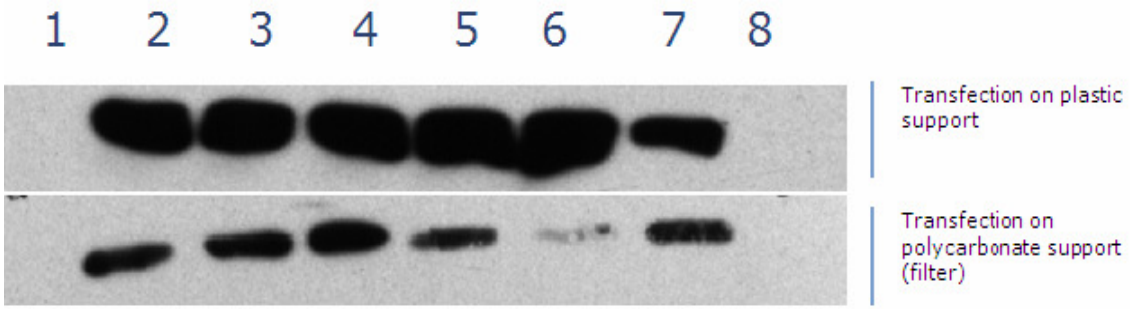
Appendices:

1. Comparison the transfection efficiency between MetaPro and PEI, FuGENE 6, FuGENE HD.



Metafectene Pro showed highest transfection efficiency in MDCK type I when compared to PEI, FuGENE 6, and FuGENE HD. Lanes 1-6: PEI transfection; lanes 8-12: FuGENE 6 transfection; lanes 13-18: FuGENE HD transfection; lanes 19-24: Metafectene Pro transfection; lane 25 negative control. The detection used rabbit7 anti-AQP2, developed by normal ECL (Thermo Scientific) for 5 minutes. The size of the fusion protein is about 58 kD.

2. **Comparison the efficiency between transfection on plastic support and transfection on polycarbonate support (semi-permeable filter).**



Transfection on plastic support (above) showed greater efficiency than on filters (below). From left to right: lanes 1,2,3, 4 used 0.5 ug DNA; lanes 5,6,7,8 used 1.0 ug DNA for transfection. Ratio 1:1 (lanes 1+5); 1:3 (lanes 2+4); 1:5 (lanes 3+7); 1:7 (lanes 4+8). The protein was detected by rabbit7 anti-AQP2. The blots were developed with normal ECL (Thermo Scientific) for 15 minutes.