

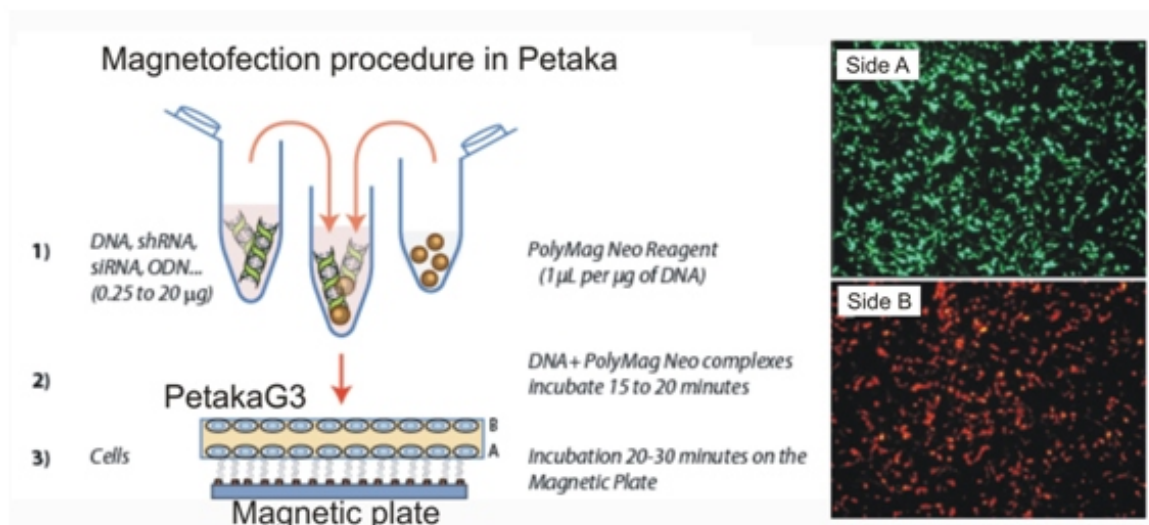
Celartia and OZBioscience start collaboration for High efficiency magneto assisted cell transfection optimization

The Biotech Company OZ Bioscience (**Parc scientifique et Technologique de Luminy 163 Avenue de Luminy, case 922-13288 Marseille, Cedex 9**) developed very successful transfections with Petaka to demonstrate the ability to transfect cells seeded on both sides of PetakaG3™ using Magnetofection. Celartia will enter in a developing program to put together joint products in the market with the aim of providing high efficient tools for scientist.

Highly efficient transfection was achieved on both primary cultured cells and usual established cell lines, such as SV40 cells, both cultured up to limit of confluence, close to the verge of dormancy (see protocol in next pages).

Transfections were performed using PolyMag-Neo as the Magnetofection transfection reagent. 24 hours later, cells were tested for Green Fluorescence expression & observed by direct epifluorescence microscopy. Results showed that most of cells express high level of green fluorescence protein indicating that PolyMag Neo was able to transfect cells on PetakaG3 device with very high efficiency. Results are comparable to what is usually observed in "classic" culture cell vessels.

The same group showed that the two sides of the same PetakaG3 device can be transfected with two different plasmids, one on each side respectively (figure 4)



Research Report

Transfection of cells on PetakaG3™ cell culture device (Celartia Europe) With Magnetofection™ technology (OZ Biosciences)



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Transfection of cells on PetakaG3™ cell culture device (Celartia Europe) With Magnetofection™ technology (OZ Biosciences)

A – One Side transfection

The very first experiment was conducted in order to establish the ability to transfect cells seeded on one side of PetakaG3™ using Magnetofection.

Due to its high efficiency on both primary and usual cell lines, PolyMag Neo reagent was chosen as the Magnetofection transfection reagent.

Briefly, 2.10^6 COS-7 cells (African Green Monkey SV40-transformed kidney fibroblast) were seeded on one side of PetakaG3 according to manufacturer instruction. 24 hours later, cells were transfected using 12µg pVectOZ-GFP plasmid (cat # PL00120) with 12 µL PolyMag Neo (PG cat # PG60100) for a 1:1 ratio according to OZ Biosciences instruction (see detailed protocol below). The day after Green Fluorescence expression was observed by epifluorescence microscopy:

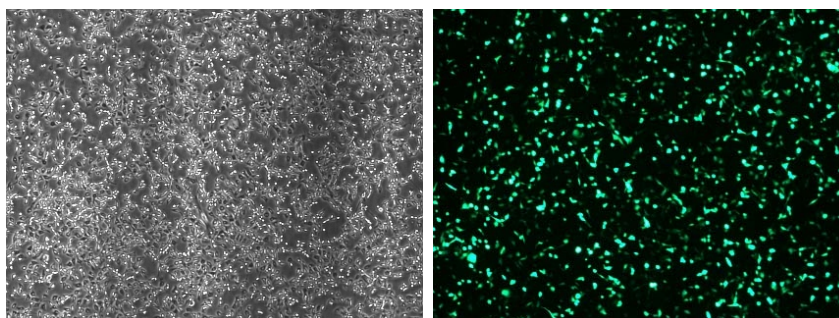


Figure 1 : COS7 were transfected on PetakaG3 device using 12 µg of DNA and 12 µL of PolyMag Neo.

Results showed that most of cells express high level of green fluorescence protein indicating that PolyMag Neo was able to transfect cells on PetakaG3 device with very high efficiency. Results are comparable to what is usually observed in [classical](#) culture cell vessels.

B [Two-sided](#) transfection

The goal of the experiment was to transfect **each side** of **one** PetakaG3 cell culture device with **two** different reporter genes encoding plasmids.

- Cells on one side should express Red Fluorescent Protein and only RFP ;
- Cells on the other side should express Green Fluorescent protein and only GFP

First HEK-293T (human embryonic kidney) cells were seeded on one side 1, and 6 hours later, on the other side 2 of the same Petaka device according to manufacturer instruction manual. Then, the day after cells on side 1 were transfected by Magnetofection with PolyMag Neo and 6 hours later, the same transfection protocol was applied to cells on the other side, 2. Finally, fluorescence on the both sides was assessed by epifluorescence microscopy.

Results showed that the two sides of the same PetakaG3 device can be transfected with two different plasmids, one on each side respectively (figure 2):

- Cells one side expressed Red Fluorescent Protein and only RFP
- Cells on the other side expressed Green Fluorescent protein and only GFP

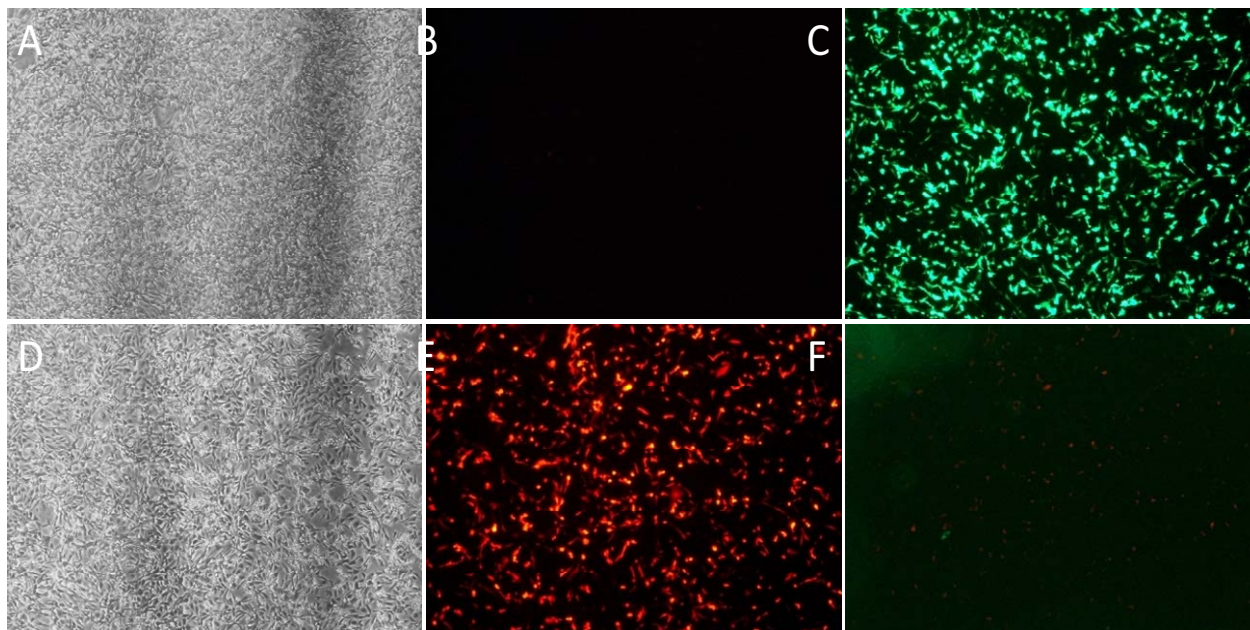


Figure 2 : HEK-293T cells seeded on the two sides of one PetakaG3 device were transfected using 12 µg DNA and 12 µL of PolyMag Neo: Side 1 (A,B, and C) was transfected with GFP encoding plasmid, Side 2 (D,E and F) was transfected using RFP plasmid. Fluorescence was observed 24 h after transfection.

Images of cells on side one (figure 2, A, B and C) showed that fluorescence is only observed in the GFP channel (C) and not in the RFP channel (B).

Images of cells on side two (figure 2, D, E and F) showed that fluorescence is only observed in the RFP channel (E) and not in the GFP channel (F) (only 2 cells seem to express GFP). The red cells observed in GFP channel come from the green filter bandwidth (Red is though visible in green).

Some important points:

- Cells must be washed after the first transfection and the plate must be kept on the transfected side.
- Time between two transfection experiments may vary depending cell type but it is important to wait at least 6 hours between the two transfection experiments.

Avoiding washing procedure or shortening the gap between the two transfection experiments can lead to gene expression on the wrong side (cells will be transfected both in Green and Red on one or the two sides).

In annex: protocol for two-sided transfection.

Annex: Two-sided PetakaG3™ Transfection protocol with PolyMag Neo

Day 0: Cell culturing adapted from Celartia PetakaG3™ protocol

T0 : Cell seeding on one side

01. Prepare cell suspension: 1.5×2.10^6 cells are suspended in 20 mL complete DMEM
02. Inject cells in PetakaG3 according to the manufacturer protocol
03. After pressure balance is done by squishing, place PetakaG3 in horizontal position for 6 hours on one side

T6 : Cell seeding on the other side

04. Replace medium from PetakaG3 by 20 mL complete DMEM containing 1.5×2.10^6 cells
 05. Place PetakaG3 in horizontal position on the other side
- two sides of the device are seeded

D1 : transfecting Both sides of PetakaG3

Allow reagents to reach Room temperature (RT) before use. It is recommended to add medium first in tube and then, dilute the DNA.

T0 : Transfection on one side

A. Prepare DNA/PolyMag Neo complexes

06. Dilute 12 µg of DNA in 500 µL DMEM without any complement
07. Add 12 µL PolyMag Neo in a polypropylene tube
08. Add DNA mixture to PolyMag Neo
09. Allow complexes formation by incubation 20 min at RT

B. Transfection

10. Withdraw 1 mL of culture medium from PetakaG3
11. Add complexes
12. Add 500 µL complete medium in order to "push" whole complexes preparation in PetakaG3 device
13. Mix well by rocking PetakaG3 to correctly spread complexes within the whole plate
14. Place PetakaG3 in horizontal position on the magnetic plate: one side is transfected
15. Incubate 30 min □ 1 hour in standard condition in horizontal position.




C. Washing Procedure

16. Place PetakaG3 in vertical position and keep magnetic plate in contact
17. Replace culture medium with fresh culture medium while keeping magnetic plate in contact
18. Incubate 30 min more on magnetic plate in horizontal position
19. Remove magnetic plate and keep Petaka G3 in horizontal position for 6 hours

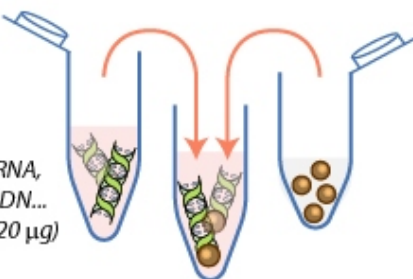
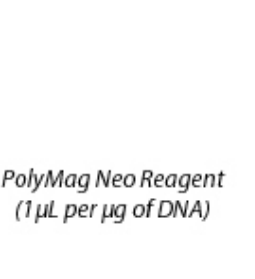

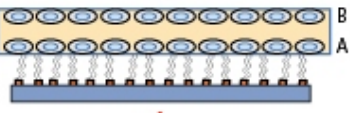
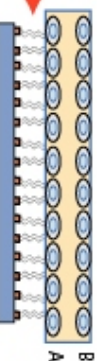
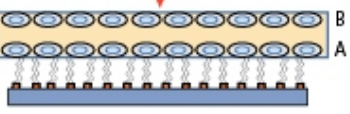

T6 : Transfection on the other side

20. Go through steps 06 to 18 for the other side of PetakaG3
21. Place PetakaG3 in vertical position until assaying.

Preparation of cells in PetakaG3 cell culture device

- 1)  A
Seed the cells on one side of the PetakaG3 cell culture device
6 hours incubation
- Flip the PetakaG3 
- 2)  A
B
Seed the cells on the other side of the PetakaG3 cell culture device

Magnetofection Procedure

- 1)  DNA, shRNA, siRNA, ODN... (0.25 to 20 µg)  PolyMag Neo Reagent (1 µL per µg of DNA)
- 2)  DNA + PolyMag Neo complexes incubate 15 to 20 minutes
- 3)  B
A
Cells Incubation 20-30 minutes on the Magnetic Plate
- 4)  B
A
Wash and replace culture medium
- 5)  B
A
Incubation 20-30 minutes on the Magnetic Plate and then 6 hours at 37°C
- 6) **6 hours later repeat steps 1 to 5 for face B**
-  B
A
Assay (24h / 72h)