Transfection of Jurkat cells with METAFECTENE

Qian Chen and Massimo Gadina

Department of Microbiology and Immunology, Queen's University, Belfast, Northern Ireland

Transfection of eukaryotic cells is widely used to study gene functions and gene regulation. General transfection techniques include introduction of genes into cells with calcium phosphate co-precipitates, or by electroporation, in which a brief electric pulse transiently creates holes in the cell membrane. Suspension cells such as the leukemic T cell line Jurkat are frequently utilized to study the features of interesting genes in immune system function. However, they are notorious for their low efficiency of transfection. Development of an efficient method to transfect these suspension cells has been a major issue for experiments utilizing such cell lines. Cationic liposome-mediated transfection techniques, which incorporate the gene into liposomes that fuse with the cell membrane, are an important addition to other transfection methods. This tecnique allows a high expression of the reporter gene within a short time after transfection. For this purpose, we tested the new transfection reagent MEFAFECTENE, in comparison with another liposome-based reagent: lipid F.

The following experiments, we examined: i) the transfection rates of a suspension cell line Jurkat; ii) the optimal ratio of METAFECTENE to DNA; iii) the transfection efficiency with METAFECTENE compared to that obtained using another commercial lipid based transfection reagent, lipid F.

Materials:
Sterile 6-well tissue culture plates
75 ml cell culture flasks
Sterile Eppendorf tubes

Medium:
RPMI + 10% fetal bovine serum + 1% penicillin
Serum-free RPMI
Sterile water

Cells:
Human derived T-cell line, Jurkat (ECACC 88042803)

Plasmid:
The green fluorescence protein (GFP)-expressing reporter plasmid pEGFP-N1

Transfection reagents:
METAFECTENE (Biontex)
Lipid F

Transfection protocol:

Cells were seeded 24h before transfection (1×10^6cells/well) and incubated overnight in RPMI with 10% serum and antibiotics. The transfection procedure on the next morning was carried out as following: for each transfection, the indicated amount of transfection reagent was added to 50 µl of serum-free RPMI in a 1.5 ml Eppendorf centrifuge tube; in a separate tube, 1 or 2 µg of the plasmic DNA was mixed with 50 µl of serum-free medium; adding media containing lipid into DNA and mixing them gently; the tubes then were allowed to stand at room temperature for 20 min to allow lipid-DNA formation; at the end of this incubation, the lipid-DNA complexes were added onto the cells drop by drop. The cells therefore were incubated at 37˚C degree overnight.

Measurement of the transfection efficiency:

24h after transfection, the cells were washed once with PBS. Each sample (1×10^6cells) was subsequently resuspended in 1 ml PBS and analyzed by flowcytometry- CYAN (Dako cytomation). Results below are shown as histograms of GFP fluorescence versus cell number.

TEST 1: Examining the Transfection Rates of Jurkat Cells by using METAFECTENE and lipid F.

DNA : Lipid = 1 µg : 2 µl

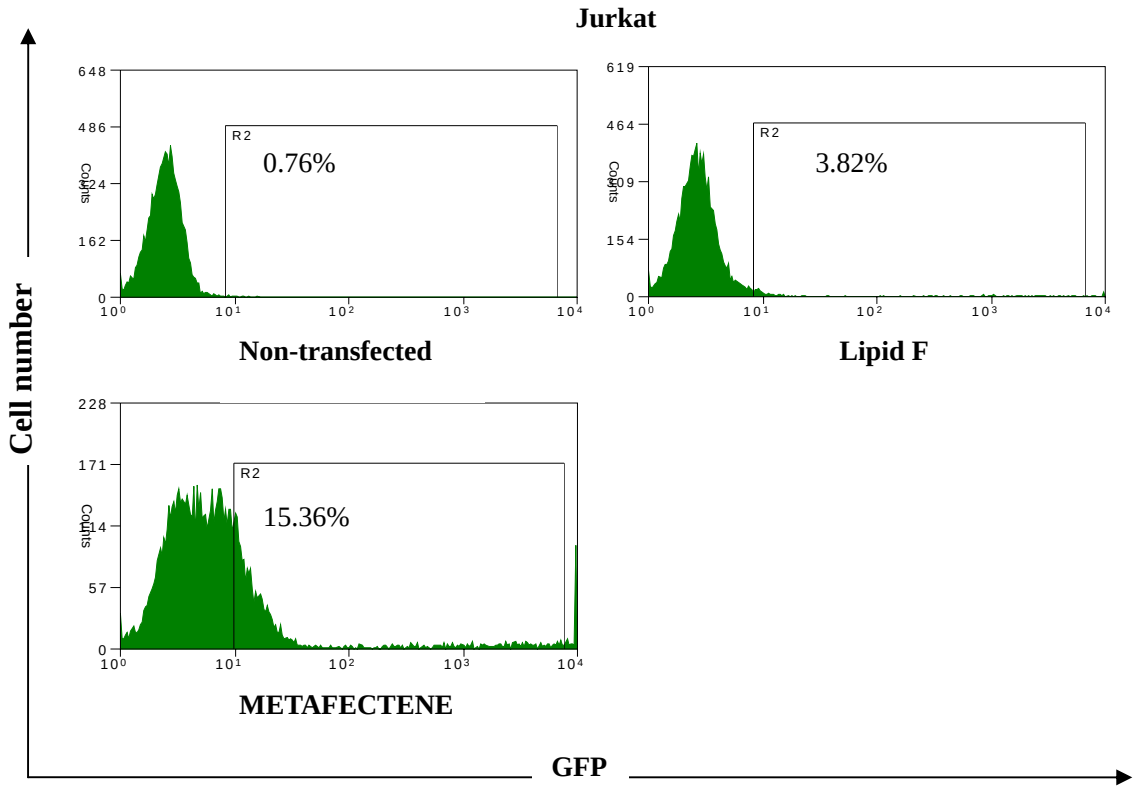

Fig. 1. The transfection rates of Jurkat cells are compared using lipid F (the middle panel) and METAFECTENE (the bottom panel). The results are shown as histograms of GFP fluorescence versus cell number. The percentage of viable cells was determined by gating on the population with GFP-fluorescence.

TEST 2: Optimization of the Transfection for Jurkat cells.

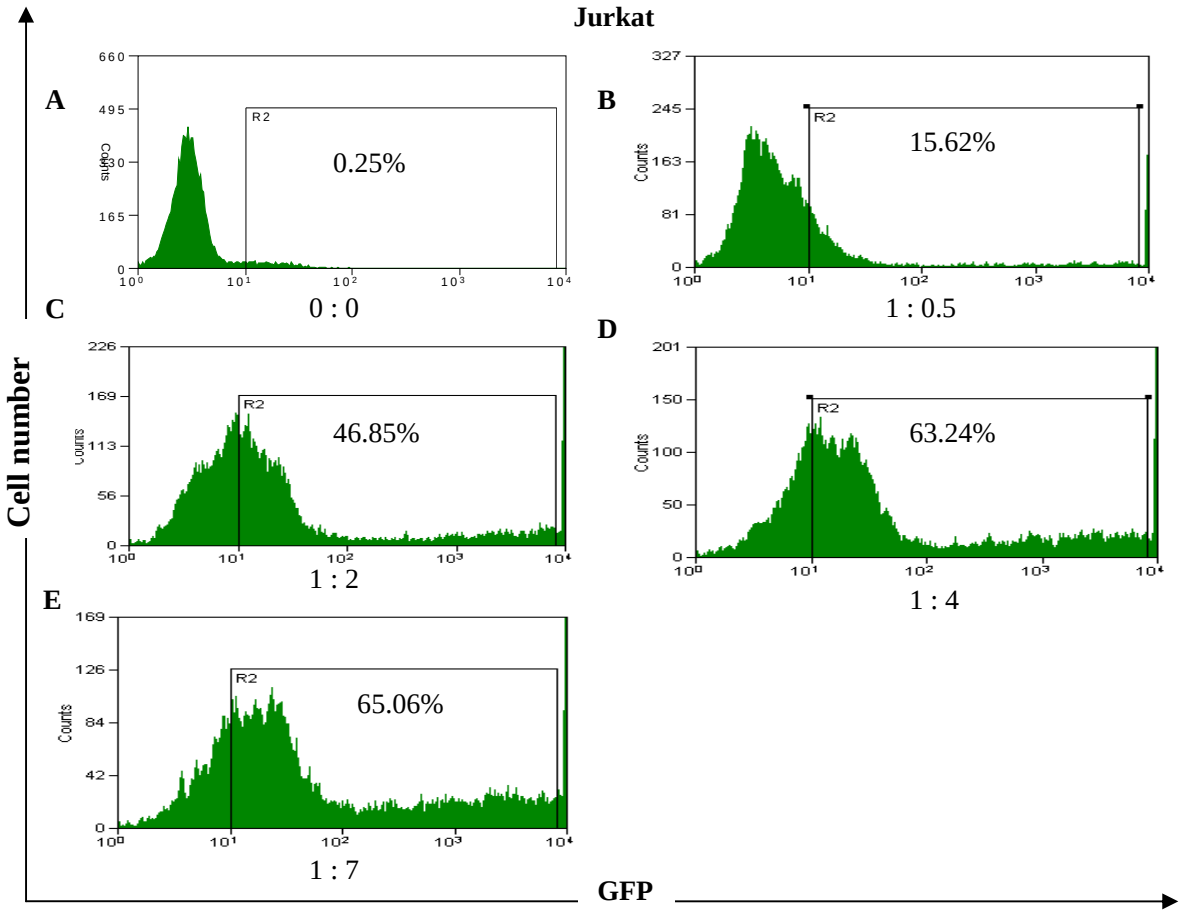

Fig. 2. In order to analyze the influence of different ratios of METAFECTENE to DNA, the experimental conditions are given: (A) Non-transfected Jurkat cells; (B) DNA : METAFECTENE = 2 µg : 1 µl; (C) DNA : METAFECTENE = 2 µg : 4 µl; (D) DNA : METAFECTENE = 2 µg : 8 µl; (E) DNA : METAFECTENE = 2 µg : 14 µl. When 2 µg of DNA was used in the ratio of 1 : 4, we already achieved a clearly high transfection efficiency, 63.24%.

TEST 3: Comparison of METAFECTENE with lipid F by utilizing the optimized Transfection Condition.

DNA : Lipid = 2 µg : 8 µl

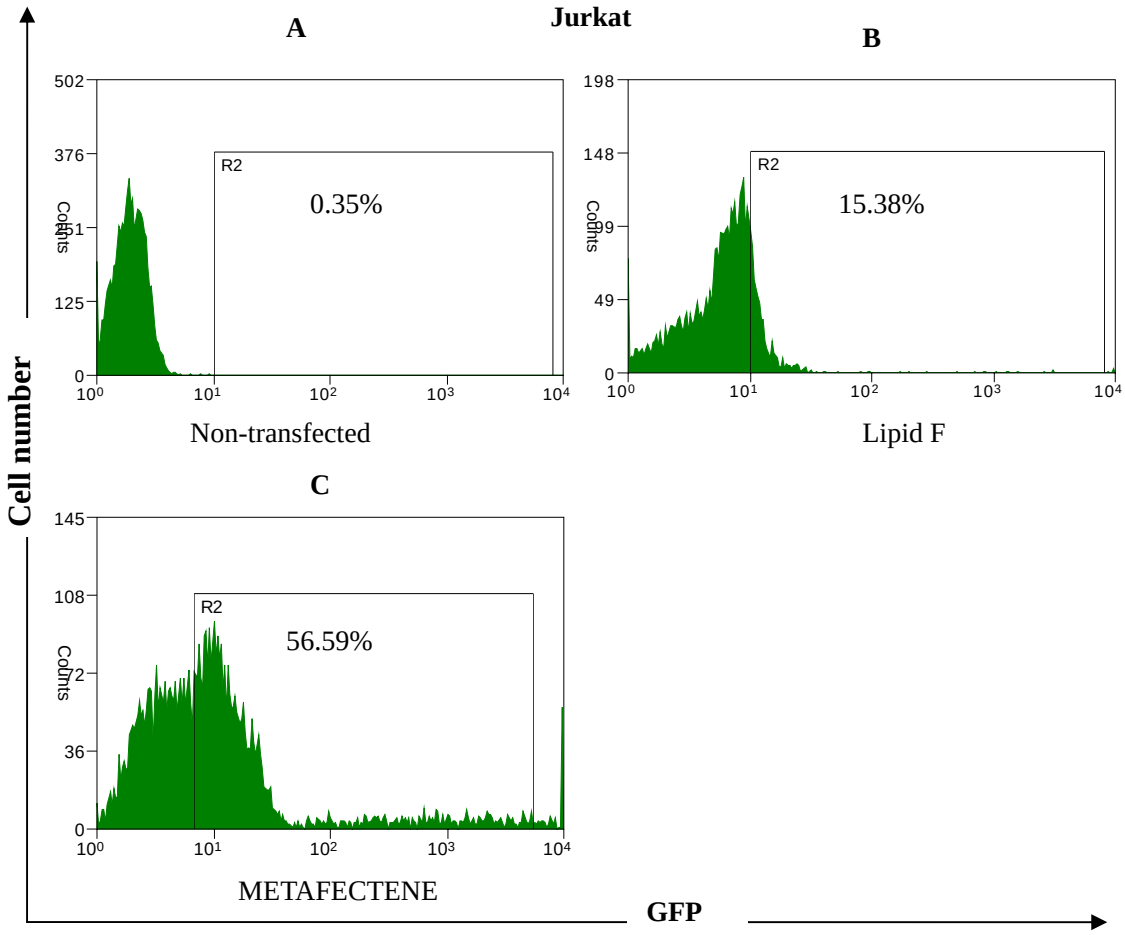

Fig. 3. Using the optimized transfection efficiency for Jurkat cells with (C) the METAFECTENE Reagent, a dramatically high transfection rate of more than 56% is reached. Compare to (B) the lipid F Reagent, which only shows around 15% efficiency.

Conclusion:

Our experiments show that METAFECTENE can be used for the in vitro gene transfection in human suspension cell lines such as the T cell line Jurkat.
Jurkat cells transfected with the lipid F showed considerably lower transfection efficiencies than those transfected with METAFECTENE.
In Jurkat cells, ratios of Reagent:DNA ranging from 1 : 4 to 1 : 7 is optimal for gene incorporation and protein expression.