Dami cell transfection
Comparison of Lipofectamin 3000 and K4 Transfection System

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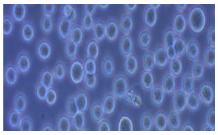
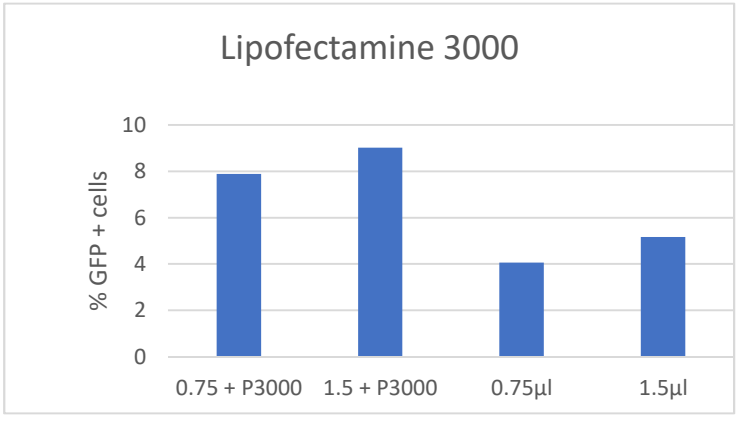
Dami cells are a human megakaryocytic cells. This cell line was established from the blood of a patient with megakaryoblastic leukaemia. Dami cells are a good model of megakaryocytes. They were grown in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum and 1% antibiotics and maintained in a tissue culture incubator at 37°C with 5% CO_2. Cells were seeded in a 24 well plate (70,000 cells/well) and stimulated with 100nM Phorbol 12-myristate 13-acetate (PMA) for 24 hours, to allow cells to adhere to glass slides. On the day of transfection, cell confluency was 80-90%. Several different transfection reagents were used during the optimisation process, as Dami cell transfection rate was generally very low, even at optimal conditions.

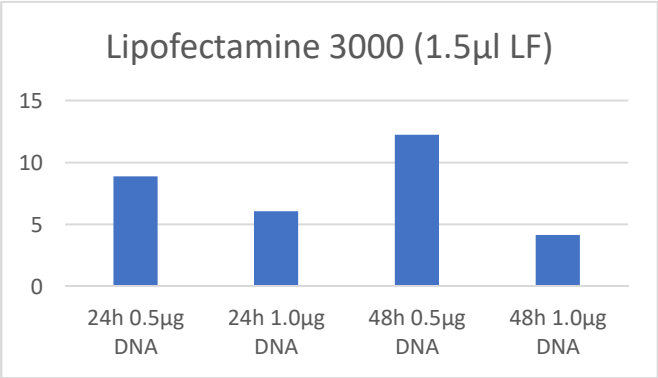


Lipofectamine 3000

Cells were transfected according to manufacturer's instructions. For optimisation purposes, cells were transfected with GFP-MSCV plasmids. To find optimal conditions, Dami cells were transfected with either 0.75µl or 1.5µl of Lipofectamine and in the presence or absence of P3000; P3000 appears to produce DNA precipitates. In all conditions, 0.5µg GFP-MSCV plasmid was added, and transfection left for 24 hours. GFP positive cells were counted in 10 fields of view. 1.5µl + P3000 were optimal conditions, however transfection rate was still low.



Further optimisation was carried out using the optimal conditions. Two concentrations of DNA were tried, 0.5µg or 1µg, and transfection left for 24 or 48 hours. Highest transfection rate was 48h with 0.5µg DNA. However, transfection with this reagent at these conditions is not favourable due to high levels of DNA precipitates that are visible as DAPI dots.



K4 transfection reagent

K4 transfection was carried out at manufacturers suggested conditions for 24 well plate. 5µl of K4 multiplier was added to each well 30 mins before transfection and 0.5µg DNA was used, and transfection was left for 48h. Moreover, transfection with K4 does not produce DNA precipitates as is seen with other transfection reagents, therefore making it the preferable and most efficient transfection reagent for Dami cells.