# Optimisation of C2C12 myoblast transfection

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## Aims:

To optimise the transfection of the C2C12 myoblast cell line with the pEGFP-C1 plasmid (sequence and map available from AddGene). Two transfection agents: Lipofectamine-2000 and polyethylenimine (PEI) will be compared, as PEI may allow reliable transfection with lower amounts of plasmid DNA. This will later enable efficient transfection with multiple pcDNA3.1-ACVR1-Cflag mutant constructs for co-immunoprecipitation or drug screening experiments.

## Experimental details

Plate C2C12 cells at a density of 20,000 cells per well of a 24 well plate in 1mL Growth Medium (DMEM, 10% FCS, 20µg/mL gentamycin).

As per the current protocol in use within the lab, transfect the cells at 40% confluency using Lipofectamine 2000 (Thermo Fisher) as follows:

Dilute Lipofectamine 10x in OptiMEM and then add 5µL, 10µL, 20µL or 25µL of diluted Lipofectamine to a total of 50µL OptiMEM (final concentrations of 0.5µL, 1.0µL, 2.0µ or 2.5µL Lipofectamine per reaction). Incubate at 20°C for 5min. Dilute 0.8µg or 1.6µg of DNA in 50µL OptiMEM, mix with the diluted Lipofectamine and incubate at 20°C for 20min before adding to each well.

At 80% confluency transfect cells with PEI as follows:

Dilute 0.8µg or 1.6µg of DNA in 80µL DMEM. Add PEI at a v/w PEI:DNA ratio of 1:1, 2:1, 3:1, 4:1 or 5:1. Mix well and incubate for 15min at 20°C before adding to each well.

For each transfection include a negative control well in which neither Lipofectamine nor PEI are added.

Bright-field and fluorescent images of each transfection were taken on a Leica DM IL microscope at 24h intervals. (**fig. 1**)

## Conclusions

PEI appears to transfect a greater proportion of C2C12 cells, without killing a subset as Lipofectamine treatment does. In future transfections will be carried out with 4x or 5x PEI treatment rather than with 1.5µL Lipofectamine-2000.

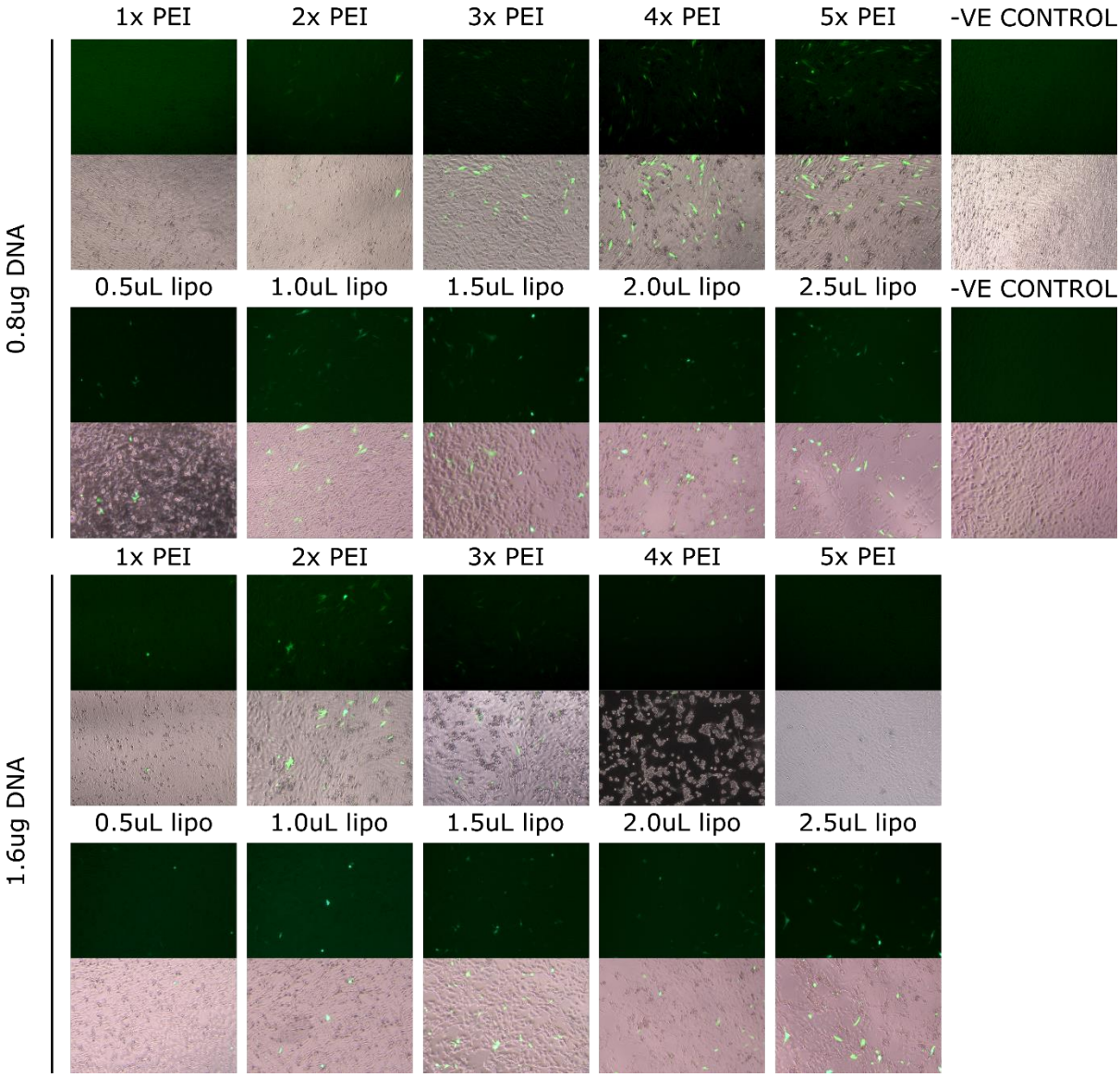


***Figure 1*** *– Fluorescent (top) and merged bright-field and fluorescent images (bottom) of C2C12 cells indicating those cells successfully transfected with pEGFP-C1. Images were taken 24-36h following transfection and their brightness and contrast edited to facilitate scoring of positive or negative cells. Scale bars are 250µM.*