K4 technical note

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Materials: plasmid pEGFP-C1 (GenBank Accession #: U55763)
ARPE-19 cells (ATCC CRL-2302)
DMEM:F12 medium supplemented with 10% FBS
Sterile 24well tissue culture plates
Sterile 1.5ml snap cap tubes
OptiMEM (serum-free medium)
K4 transfection system

Optimization of ARPE-19 transfection:

ARPE-19 were grown in DMEM:12 supplemented with 10% FBS, glutamine, penicillin and streptomycin to near confluency. One day prior transfection the cells were trypsinized and seeded onto sterile 24well cell culture plates at a density of 200,000 cells per well in 0.5ml of medium. At the day of transfection, the cells were covering about 80% of the growth area and mitosis was ongoing.

Optimization of the transfection with K4 transfection reagent was carried out as follows:

All reagents were prewarmed to room temperature.
A total of 6 DNA concentrations (from 0.3 µg to 0.8 µg per well) and 4 different different DNA to transfection reagent ratios were tested.
To prepare the cells for transfection, 5µl of K4 Multiplier were added to each well, the plate was swirled once and the cells were incubated for 30min at 37°C.
During this incubation time the lipoplexes were prepared:
Purified pEGFP-C1 plasmid DNA was diluted in OptiMEM to a final concentration of 10ng/µl.
The K4 transfection reagent was diluted as follows:

For 1:2 ratio: 175µl OptiMEM + 7µl of K4 transfection reagent
For 1:3 ratio: 175µl OptiMEM + 10.5µl of K4 transfection reagent
For 1:4 ratio: 175µl OptiMEM + 14µl of K4 transfection reagent
For 1:5 ratio: 175µl OptiMEM + 17.5µl of K4 transfection reagent

Then 175µl of the DNA solution was added to each of the transfection reagent dilutions. The solutions were mixed by pipetting once up and down.
Incubation for 20min at room temperature.
At the end of both incubation times the lipoplex solutions were added dropwise to the wells. 30, 40, 50, 60, 70, 80µl of the each mixture per well (A1-A6, B1-B6, C1-C6, D1-D6).
The plate was swirled once before further incubation at 37°C.

24 hours post transfection the DNA containing medium was removed and replaced by fresh growth medium.

2 days post transfection GFP expression was assessed by fluorescence microscopy (Figure 1).

Results and Discussion:

Cell survival appeared above 90%. Transfection efficiency reached up to about 25% and was found to be highly dependent on the ratio of DNA to the transfection reagent. A ratio of 1:4 resulted in the highest efficiencies. Furthermore, lower amounts of DNA were favorable. DNA amounts of 0.3 µg to 0.5µg per well gave the best results.

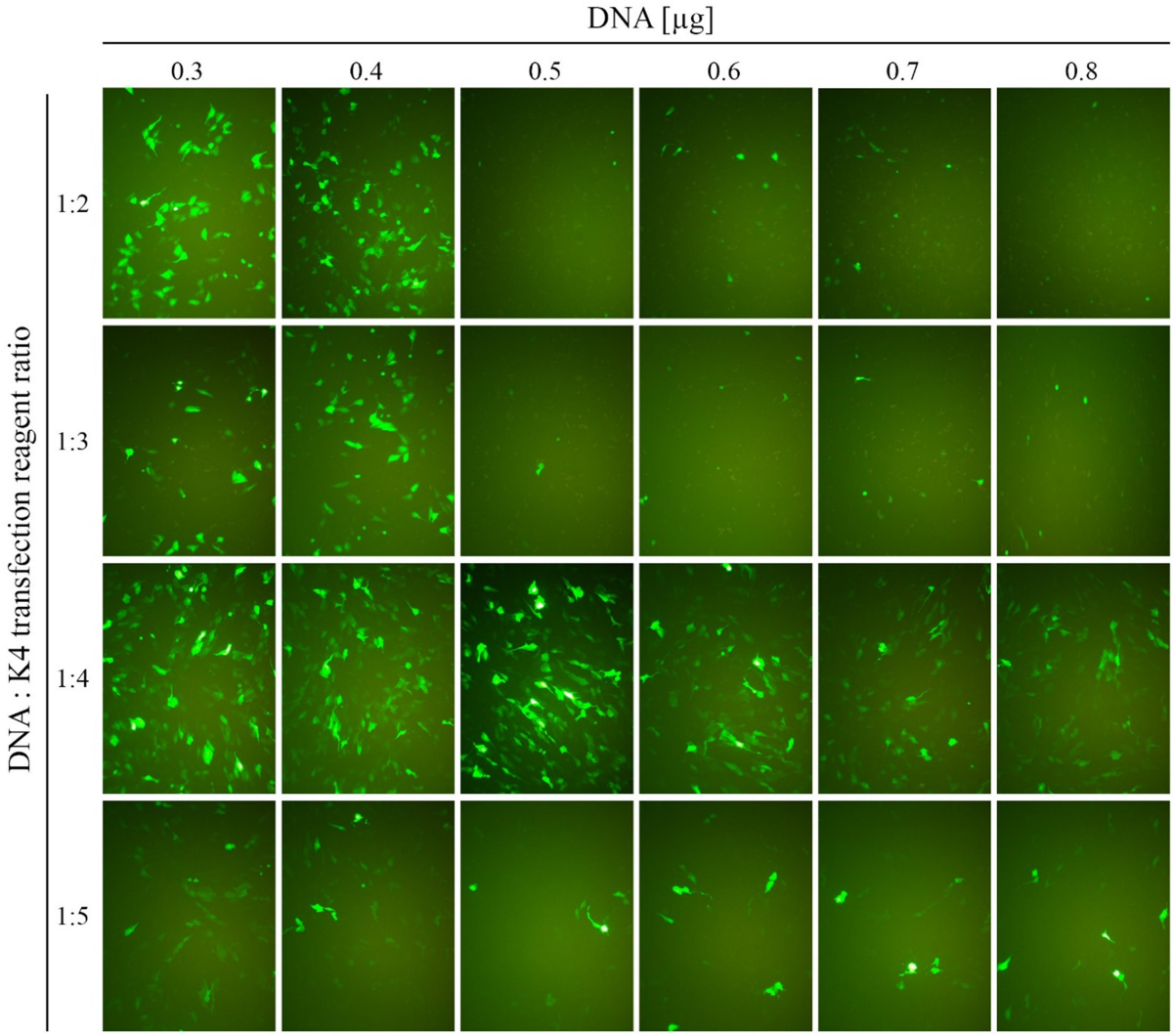

Figure 1: ARPE cells were transfected with a pEGFP plasmid using the K4 transfection reagent. Images of GFP expressing cells were taken at 2 days post transfection.