K2 Transfection Technical Note

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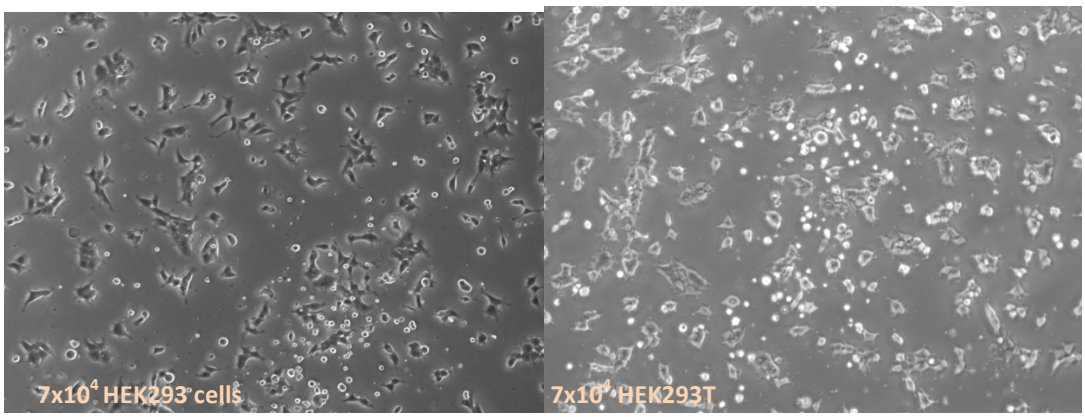
Materials: Plasmid pcDNA-DEST47-TACC3 (expresses a fusion of the TACC3 protein with GFP at C-terminal) (pcDNA-DEST 47 plasmid is from Life Technologies)
175 cm^2 flasks
Sterile Eppendorf tubes
Glass coverslips
Trypsin solution
DMEM + 10% fetal calf serum + Glutamax
HEK293T cells

The following protocol was used to transfect either HEK293 or HEK293T cells with pcDNA-DEST47-TACC3 plasmid (TACC3 is reported to be expressed in cytoplasm and centrosomes - Human Protein Atlas).

$7x10^4$ HEK293T cells were plated in each well of a 24 well plate on a coverslip previously coated with poly-L-lysine in a total volume of 500 µl medium. Cells were incubated in CO_2 incubator at 37°C for 24hrs.

24h later the wells had an approximate coverage of around 30%. One picture per cell line was taken just before transfection:-



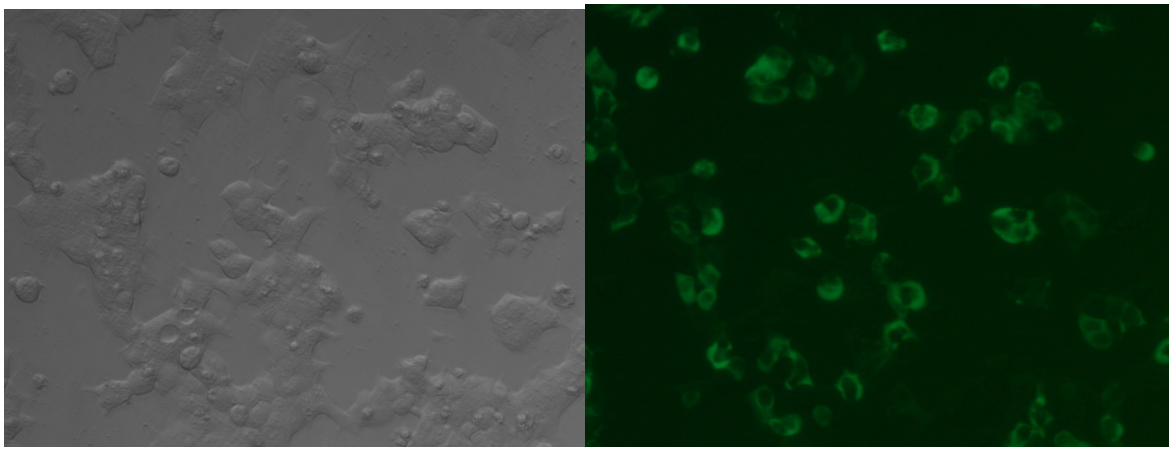
Shortly after, the transfection was performed:-

1) K2 multiplier and K2 transfection reagent were placed at room temperature (RT).
2) Medium in each well was replaced with 330µl (to eventually reach a total of 400µl together with multiplier and DNA:tr complex) complete medium without antibiotics
3) 9.5µl of K2 Multiplier was added to each well and incubated for 2hours
4) Solution A and B were prepared pipetting up and down once. Solution A was added to Solution B pipetting once and incubated at RT for 15min. DNA and tr were not put in contact with the plastic of the tubes.

Solution A: 1 µg DNA in 30µl serum free medium (for TACC3)
Solution B: 2 µl K2 transfection reagent (tr) and 28µl serum free medium

5) 60µl of the mixed solution (A + B) was added to each well and incubated in 37^{o}C CO_2 incubator for 24h

24h post transfection (media was not replaced), expression of TACC3-GFP reflected a high transfection rate (not quantitated).



NOTES

Previous experiments aimed to optimise transfection with K2. Other DNA : TR ratios were tested, 1 µg : 2 µl, 1 µg:3 µl and 1 µg:4 µl (also performed using 0.5ug DNA and 1-4 µl tr). However transfection rate was always high and seemed not to change dramatically.