Transfection of adult dermis derived human fibroblasts using K2 Transfection System

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Materials:

- DMEM, high glucose, GlutaMAX™ Supplement, HEPES (Gibco)
- Fetal Bovine Serum (Sigma)
- PBS
- Trypsin solution
- Sterile Eppendorf tubes
- 75T cell culture flask (BD bioscience)
- 24-well plate (BD bioscience)
- Plasmid DNA: pE-GFP-C1 (Clontech)

1. Primary human fibroblasts derived from adult human dermis were grown in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin it T75 cell culture flask.
2. Cells from two different patients (40 and 60 years old) were trypsinized after 80% confluence and seeded on 24-well plate in density $10^4/cm^2$ ($2x10^4$ per well in 0,5 ml medium).
3. Next day transfection was performed after treatment with K2 multiplier (10µl) 2h prior transfection and without treatment. Transfection mixtures were prepared 20 minutes prior transfection according to Table I and following protocol:

Table I.

Plate: **24-well plate**
Amount of medium per well: **0,5ml**
K2 multiplier: 10µl, 2h incubation or without K2 multiplier

DNA:K2 ratio					
1:2			1:4		
DNA [µg]	K2 [µl]	DMEM [µl]	DNA [µg]	K2 [µl]	DMEM [µl]
0,3	0,6	30+30	0,3	1,2	30+30
0,5	1	30+30	0,5	2	30+30
0,8	1,6	30+30	0,8	2,4	30+30

The indicated amount of K2 transfection reagent (See Table I) was added to 30 µl of serum-free cell culture medium (DMEM) in a 1.5 ml Eppendorf centrifuge tube. In a separate tube indicated amount of the plasmid DNA (pE-GFP-C1) was mixed with 30 µl of serum-free medium. The media containing K2 and DNA were combined and mixed by pipetting up and down 5 times. The tubes were incubated at room temperature for 20 min for lipid-DNA complexes to form. At the end of the incubation time, the lipid-DNA complex mixtures (60 µl per well) were pipetted onto the cells, mixed by tilting the plates a few times and thereafter incubated at 37°C incubator under 5% CO2. After 24h cells were analyzed under fluorescence microscopy.

Results

Transfection efficiency was similar in cells from both patients. Best efficiency (app. 30%) was obtained after treatment with K2 multiplier, **1:2 K2:DNA ratio**. Efficiency was similar between groups transfected with different DNA amount. **0,3µg** DNA per well seems to be sufficient for transfection of these cells (Figure 1).

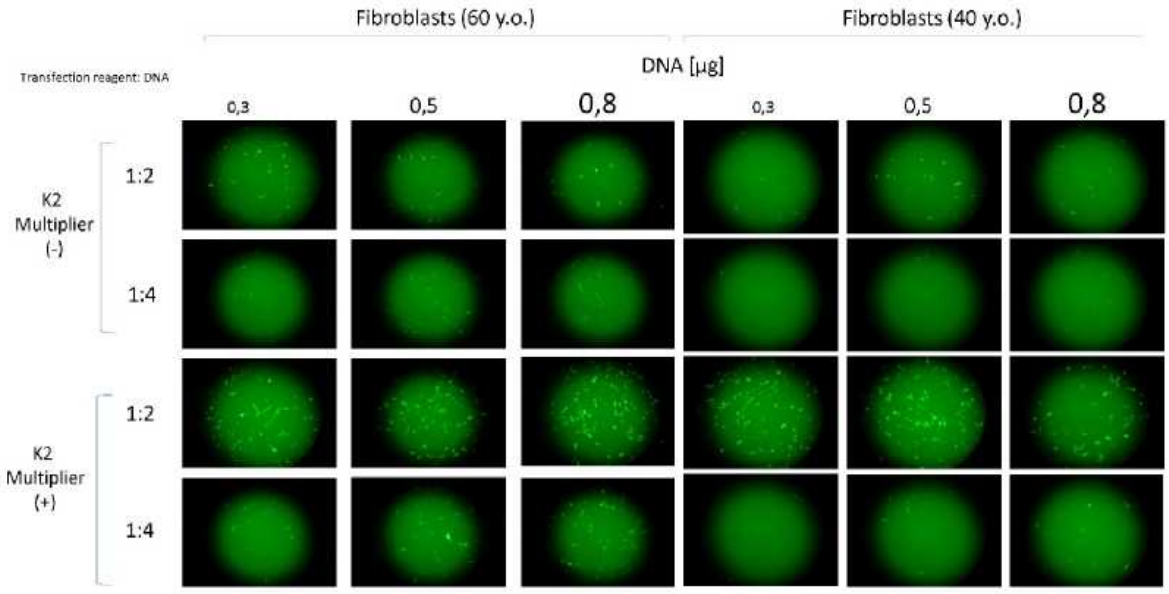

Figure 1. GFP expression in primary dermal human fibroblasts after transfection with different K2:DNA ratio and DNA amount.