

TRANSFECTION OF CELLS WITH GFP (CMUJ Kraków)

Construction of vector containing Green Fluorescence Protein (GFP) gene.

An adenoviral vector containing two reporter genes (enhanced green fluorescent protein (EGFP) and β -galactosidase) under the control of two separate human CMV promoters (Ad-CMV β gal-CMVGFP) was constructed as described (*Konopka et al 2005*). Plasmid pUHG16-3 (*Gossen, Bujard, 1992*) was cleaved with enzymes Bam HI and Xba I to obtain an insert containing the β -galactosidase encoding gene, which was subcloned into the adenoviral shuttle vector pTrack-CMV (*He et al 1998*). The adenoviral vector was prepared by the recombination of pTrackCMV β gal-CMVGFP and Ad-Easy 1 in electrocompetent BJ 5183 bacterial strain. The viral vector Ad-CMV β gal-CMVGFP was produced in 293 packaging cell line according to the procedure described earlier (*He et al 1998*).

- *Transfection*

The transfection of SVF cells by adenoviral vector containing GFP gene was performed by the incubation of 10^6 cells in medium containing viruses (400 MOI). After 24 h incubation in humidified CO₂ incubator (Juan) at 37°C, the Angio medium was changed to the fresh one (without voruses). The fluorescence was observed under the optical Olympus microscope (10 x magnification) containing UV light power supply (lamphouse 50 W Mercury, Olympus). The transfection efficiency was characterized by flow cytometry with the instrument set for fluorescein detection. 10^5 cells after transfection were trypsinized, centrifuged (400 x g, 10 min, room temperature) and fixed in 500 μ l of 2% paraformaldehyde in PBS for flow cytometry.