Technical Note

Preparation of adenovirus using K2 Transfection System

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In order to obtain the adenovirus containing a gene of interest, the plasmid pAdTrack-CMV was recombined with pAdEasy1 using BJ5183-AD-1 bacteria (BJ5183 containing pAdEasy1 plasmid). The recombined plasmid (tested by PCR and digestion using restriction enzymes) was digested with the PacI restriction enzyme (FastDigest, Thermo). After digestion, PacI was inactived 20 min at 65^{o}C.

PacI linearized plasmid was used for transfection of AD293 cells (Agilent), using K2 Transfection System.

Transfection was performed as following:
A day before transfection, AD293 cells were seeded at a density of 0.5x10^{6}cells in T25 flasks in DMEM 4.5‰ glucose and 10% fetal calf serum. Two hours before transfection, 90 µl K2 Multiplier were added to the cells (at 70% confluency).

Then, solution A and B were prepared: 6 µg PacI linearized plasmid were mixed with 260µl DMEM (Solution A); separately, 21.6 µl K2 transfection reagent was added to 248.4 µl DMEM (Solution B). Then, the solution A was added to the solution B, mixed by pipetting and incubated for 20 min at room temperature. The mixture was added to the cell culture, drop by drop. After 24h, the medium containing the transfection mixture was changed with warmed, fresh medium.

About 50% of the cells showed green fluorescence as a result of GFP expression.

Five days after transfection, AD293 cells were found detached from the culture plate. The cell suspension was collected, centrifuged at 400xg for 10 minutes, and the cells were broken by three freezing / thawing cycles to break the cells and release the adenovirus. The suspension was centrifuged at 7000xg, 10 minutes to sediment cell debris, and the supernatant was further used to infect other AD293 cells, to amplify the adenovirus.

The virus purification was performed by ultracentrifugation of a CsCl discontinuous density.

To test the infectivity of the adenovirus, 5 µl of the viral suspension were added to confluent human endothelial cells (EA Hy926) or murine hepatocytes (HEPA cells), grown in 24-well plates. The cells were infected with a good yield, and the results are presented in the Figure 1.

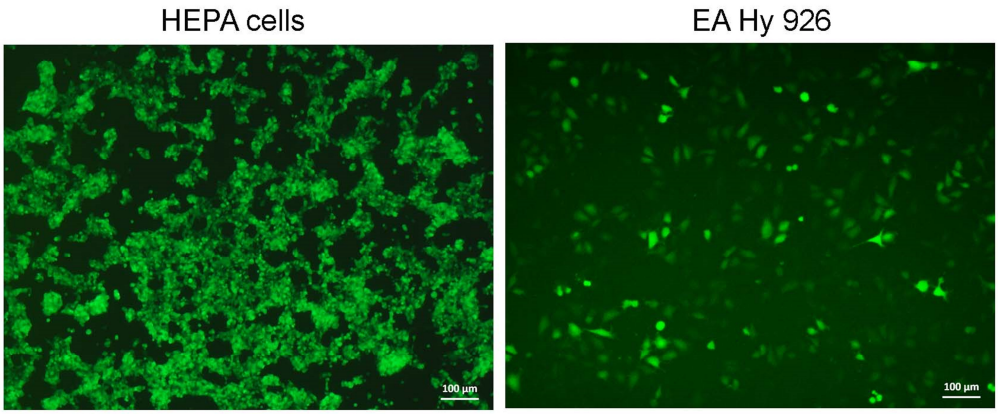

Figure 1. *Adenoviral infection of HEPA and EA Hy926 cells. Green cells represent transduced cells. Murine HEPA cells were 100% transduced, while EA Hy926 cells needed a higher titer to obtain 100% yield of transduction.*