DNA-Transfection of Mixed Neuron-Glia Cerebellar Cultures using "Biontex K2® Transfection System".

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Cell cultures preparation

Mixed Neuron-Glia Cerebellar Cultures were obtained from CD1 mice at postnatal day 6-7: cerebellum was removed, digested in a trypsin-EDTA solution and triturate in a DNAse solution. Isolated cells were plated at a density of $2x10^5$ per cm^2 onto poly-lysine-coated coverslips ($4cm^2$ surface, incubation with poly-lysine O/N). Each coverslip was inserted in a 35-mm Petri dish containing 1 ml of culture medium (50% Eagle basal medium, 25% Hanks balanced salt solution, 25% horse serum, supplemented with glucose, 200mM L-glutamine and antibiotic/antimycotic solution). Cells were allowed to equilibrate to the *in vitro* conditions at 34°C in 5% CO_2 at least 4 DIV before transfection.

Transfection protocol:

Medium containing serum was changed with serum free medium (Neurobasal Medium-A supplemented with B27 supplement, 100mM L-glutamine and antibiotic/antimycotic solution) and then each Petri dish (containing 1 ml of medium) was added with 20 µl of K2® Multiplier, 2 hours before transfection.

For each Petri dish, 1µg of plasmid DNA (i.e. 1µl of 1 µg/µl solution) was diluted in 15µl of serum-free medium (to obtain solution A) and 2µl of K2® Transfection Reagent in 15µl of serum-free medium (to obtain solution B).

To obtain co-transfections with two different plasmid DNA: add 0.5µg of each plasmid DNA to 15µl of serum-free medium (to obtain solution A) and then follow the standard protocol.

20 min before the ending of incubation with K2® Multiplier, A and B solution were mixed (to obtain the "lipoplex") and incubated RT for 15-20 minutes.

At the ending of incubation with K2® Multiplier lipoplex was added to the cells and incubate O/N. Transfection medium was replaced with fresh serum-free medium and results were analyzed 12-72 hours after. An increment in the rate/intensity of fluorescent cells during this period can be observed.

Results:

Lipoplex transfection method can be successfully applied to mixed Neuron-Glia Cerebellar Cultures. Transfected cells are fluorescent: they can show different level or kind of fluorescence depending on the molecules introduced into the cytoplasm or the nucleus:

Fluorescent cells were observed starting from 24 hours post-transfection. Labelled cells, indicative of successful transfection, express the DNA of interest linked with a fluorescent reporter (fig.1C). Fig. 1 shows a double transfection with a plasmid codifing for a protein of interest linked to a red fluorescent protein (C) and a plasmid codifing for a FRET probe (A-B: the sequence that links acceptor and donor molecules is a protease target: if the enzime is active, it cuts between donor and acceptor, modulating FRET activity. The ratio between donor and acceptor emission intensity was used to measure it - see Alasia et al., 2015).

In this experiment we evaluated if there were any relationship between the expression of the protein of interest linked to the red fluorescent reporter protein and the activity of the protease.

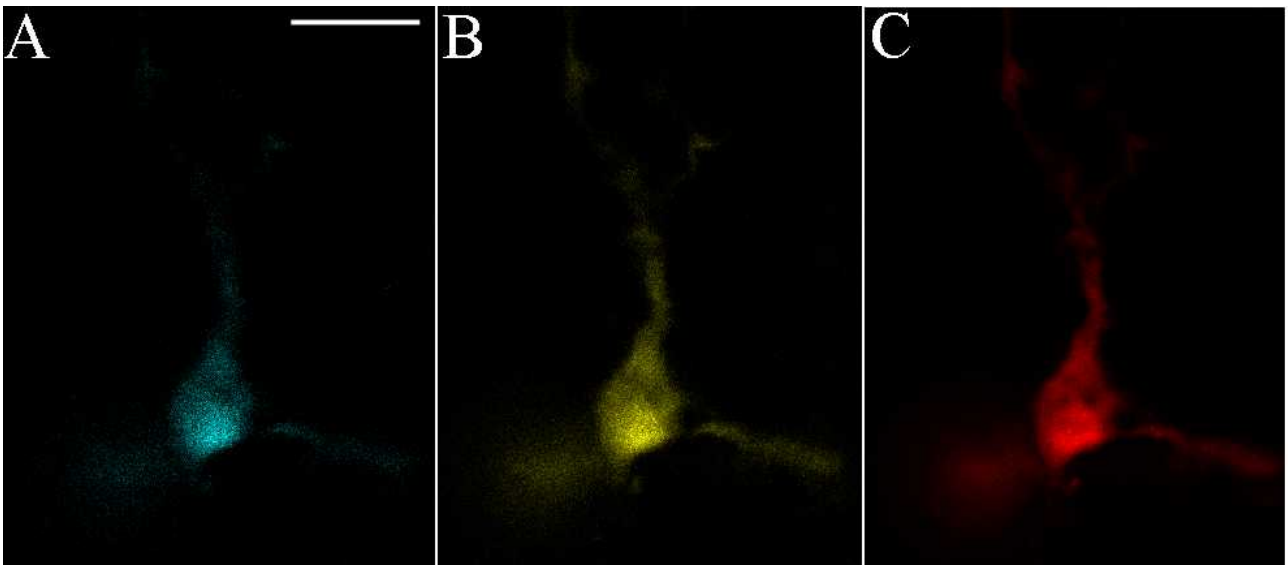

Fig. 1: A-B-C: Co-transfection of a neuron with a plasmid codifing for a red fluorescent protein, emission 618µm (C) and a FRET probe: donor emission 475 nm(A) and acceptor emission 530nm (B). Scale bar = 10µm.

Conclusions:

K2® Transfection System is optimized for cell lines and it promises best results during the exponential growth phase of the cells. Both neurons and glial cell living in cerebellar cultures obtained with this protocol were successfully transfected; they can be discerned through immunofluorescence (by the use of specific antibodies) or, more simply, through morphological features. Isolated neurons from P6-P7 mice are post-mitotic and it minimize the uptake of DNA into the nucleus (on the contrary, glia is actively proliferating). However, with some modifications to the standard protocol, it is possible to obtain successful transfections in neurons too, that are the object of the study. In general, most part of living cells seems to be transfected without toxicity and high level of expression can be observed. This is particularly important to measure FRET activity, that requires high intensity of fluorescence, to allow quantitative measurements.

Bibliography:

Alasia S, Cocito C, Merighi A, Lossi L (2015): Real-Time Visualization of Caspase-3 Activation by Fluorescence Resonance Energy Transfer (FRET); Methods Mol Biol. 2015;1254:99-113

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