Use of METAFECTENE PRO in medium throughput screening of signalling proteins modified by site-directed mutagenesis.

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Introduction:

A drawback of using transfection of individual wells of cells for screening the properties of mutants generated by site-directed mutagenesis is the variation of transfection efficiency across wells. While this is not a major problem when responses are monitored in single cells as for ion channels, or screening of reporter gene activity using the dual glow luciferase assay, in the case of signalling proteins downstream of receptors where quantification of the immediate post-receptor response is desirable, the monitoring of transfection efficiency is a major additional labour-intensive task. Here, a method for transfecting large populations of cells in suspension is presented after which they can be plated in any desired configuration for subsequent analysis. This approach allows detailed pharmacological characterisation and limits the amount of work required for the monitoring of transfection efficiency/ protein expression.

Experimental procedure:

HEK293 cells (1-20 passages) from frozen, are plated in 75cm2 flasks at a density of ~4 x 10^6 cells per flask in 10 ml of DMEM/10% FCS containing 0.2 mg/ml of geneticin and kept in a CO2 incubator at 37°C, in a humidified 95% O2 5% CO2 atmosphere. The maximum amount of METAFECTENE PRO used so far was 10µl per 2 ml of cell suspension added in 100µl of Optimem.

48-72 h later the culture medium is replaced by Hank's balanced salt solution without Mg/Ca (Invitrogen) supplemented with 5 mM EDTA pH8 and trypsin (10x Trypsin-EDTA, Invitrogen) at RT. After 10 min in this solution the cells are detached from the culture-vessel by gentle swirling and collected in a sterile centrifuge tube and pelleted by centrifugation at 300xg for 10 min at RT. The pellet is resuspended in DMEM 10% FCS, cell counts taken and adjusted to 2-2.5 x10^6 cells /ml and 2 ml are added to 100µl of Metafectene-Pro:plasmid DNA mix (2:1) prepared according to the instructions of the manufacturer using Optimem (Invitrogen) as the diluent in a 5 ml sterile poly-propylene screw-cap vial. After brief swirling by hand the vials are placed in a rack which is fixed to a IKA-Vibrax shaker platform operated in a CO2 incubator (Heraeus 6040) and shaken at 400 rpm for 6-8 h at 37°C, in a humidified 95% O2 5% CO2 atmosphere.

On removal of the vials from the incubator no gross cell aggregates or other precipitate should be visible. The cell suspension is triturated by repeatedly (10x) drawing it into a sterile Gilson 1 ml pipette tip in order to reduce the number of small cell aggregates. Subsequently, the suspension is diluted with DMEM FCS to 10^5 cells/ml and plated in a format convenient for subsequent assays.

For screening adenylyl cyclase mutants we add 300µl of cell suspension to 48 well plates (Greiner), 500µl to 24 well plates. Larger aliquots of the suspension at the same dilution are plated to monitor transfection efficiency using luciferase reporter plasmids as well as for the monitoring of protein expression by immunoblots or immunocytochemistry. The cells are used in assays 48-72 h after the initiation of the transfection process.

Results and discussion:

Typical and reproducible transfection efficiency is 60% for HEK cells as estimated by visual monitoring of the expression of EGFP from a CMV promoter-based plasmid through the 40x objective of an Olympus inverted microscope. COS-7 cells were also transfected with similar efficiency with this procedure.