

Luciferase-reporter based DUB screen

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INTRODUCTION

Components of a signalling pathway may be either activated or inactivated by covalent attachment of one or more ubiquitin moieties. The activity of such signal transduction pathways can often be measured using reporter genes e.g. the Firefly/Renilla dual-luciferase reporter system. In order to specifically study the involvement of de-ubiquitinating enzymes (DUBs) in signalling pathways, we have constructed a short hairpin RNA (shRNA) library targeting the gene family of human DUBs. Co-transfection of the DUB library can be used in combination with various reporters to identify DUBs that affect the activity of any chosen pathway under various conditions.

METHODS

Protocol for a Firefly/Renilla luciferase reporter screen

First an appropriate cell line must be chosen to study the signalling pathway of interest. In this cell line, experiments must be performed to titrate the amount of reporter and possible stimulant so that inhibition of a DUB involved in regulating the pathway will be able to repress, as well as super-activate reporter activity. For screens performed so far the amounts of Firefly and Renilla reporters are 0.125-0.25 micrograms, and 0.25-2.5 nanograms /24-well, respectively, while we have used 0.625-0.75 micrograms of DUB pool/24-well. These amounts are when using the calcium-phosphate protocol for transfection.

Day 1:

Cells are seeded in 24-well dishes, with 2 wells/DUB pool.

Day 2:

A master mix containing Firefly and Renilla reporter is made, and aliquoted in 55 tubes, followed by addition of the 55 individual DUB pools. Thereafter cells are transfected with Calcium-phosphate or other chosen methods.

Day 3:

Cells are washed with PBS and given fresh medium

Day 4:

Appropriate stimulus is added to cells

Day 5:

Cell lysis in and luciferase measurement.

NB! It is important that lysis and luciferase measurement occurs three days after transfection, since it is only at this time that phenotypes conferred by DUB inhibition become evident in our experience.

Follow-up:

When the screen data have been analyzed and potential hits identified, these must be tested again comparing them to pools that did not alter signalling.

This must be done to ensure that hits are not the result of noise in the screen.

BIBLIOGRAPHY

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