

## How to determine the type of ubiquitin formed chains on your favorite substrate

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### INTRODUCTION

In order to determine the type of ubiquitin chain formed on Notch or on Deltex, we generated expression vectors encoding for tagged ubiquitin mutants. These vectors have to be transfected together with the expression vector encoding the protein of interest, then the ubiquitinated proteins are immunoprecipitated and the ubiquitinated forms are detected by anti-VSV western blotting. Note that the extracts and the IPs are done in non-denaturing conditions, therefore several controls are necessary to be sure that the ubiquitinated products derive from the immunoprecipitated proteins, and not from co-immunoprecipitated factors.

### MATERIALS

- The original vectors are available under request to: [christel.brou@pasteur.fr](mailto:christel.brou@pasteur.fr)

### METHODS

- We generate expression vectors encoding vesicular stomatitis virus (VSV)-tagged ubiquitins either wild type (WT), or mutated in all, or mutated in all but one of the critical lysine residues used to polymerize ubiquitin molecules.
- We named these vectors: VSV-Ub WT; VSV-Ub K0 (K6, 11, 27, 29, 33, 48, 63 mutated to R); VSV-Ub K0K29; VSV-Ub K0K48; VSV-Ub K0K63.
- Ubiquitins are under the control of cytomegalovirus (CMV) promotor (pCDNA3-derived vectors).
- The original mutated ubiquitins are generous gifts from R. Baer (Columbia University, New York). They are used as PCR templates and products are inserted into GVSU-pCDNA3 at EcoR1 and Xba1 sites (Brou et al., 2000).
- These expression vectors are used in cotransfecting 293T cells together with an expression plasmid encoding your target.
- A total of 13 µg of DNA for one 10 cm diameter plate (Ca-phosphate precipitation) includes 10 µg of ubiquitin vector.
- 24 or 48 h after transfection cells are harvested, lysed in your favorite IP buffer, and IPed with your specific antibody.
- Ubiquitinated products are revealed by anti-VSV western blotting.

#### We also use the following plasmids

- Flag tagged-ubiquitin, where one critical residue has been mutated to arginine: Flag Ub WT; Flag Ub K29R; Flag Ub K48R; Flag Ub K63R; Flag Ub K0 (K6, 11, 27, 29, 33, 48, 63 mutated to R) and Flag-Ub G76V, where the last glycine is mutated to valine.
- These constructs are made in pCS2-Flag vector and are used in the same way as above, except that IP is done with anti-Flag antibody, and ubiquitinated products are revealed with your specific antibody.

## BIBLIOGRAPHY

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