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STABLE HUMAN CELL LINE FOR UBIQUITIN RESEARCH

A stable human cell line expressing multiple copies of biotinylated ubiquitin under tight control by tetracycline. Key features:

- Isolates ubiquitinated proteins under denaturing conditions with clean results
- Utilises the strong biotin-streptavidin interaction to enable stringent washing
- Stable and inducible expression of ubiquitin

Potential use as a research tool enabling:

- Clean and easy identification of ubiquitin substrates
- Quantification of ubiquitination
- Studies of the mechanism of ubiquitination
- Ubiquitination inhibitor screens in drug development

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Background

The ubiquitin proteasome system (UPS) is a key pathway regulating cellular protein turnover that is initiated by the covalent attachment of ubiquitin chains to target proteins. Ubiquitination is achieved by a 3 enzyme cascade (E1, E2 and E3), which is highly regulated and large numbers of E2 and E3 enzymes have been identified. The pathway is further regulated by families of deubiquitinases (DUBs), which antagonise the activity of the ubiquitin ligases (figure 1). Multiple ubiquitin molecules can be attached to substrates in various conformations, leading to intricate poly-ubiquitin chains.

Control of this pathway is vital for cell cycle progression and as such, deregulation of ubiquitination is thought to be involved in cancer. Understanding the details of the UPS is therefore crucial, however, current methods available for studying ubiquitination are limited, as they isolate ubiquitin under non-denaturing conditions, leading to contaminated samples with unclear results.

Technology

Dr Catherine Lindon and Dr Ugo Mayor at the University of Cambridge, have generated a human cancer (U2OS) cell line, stably expressing multiple copies of biotinylated ubiquitin (bioUb), under the regulation of tetracycline.

Through using bioUb in these cells, purification of substrates can be carried out under denaturing conditions, resulting in clean preparations without significant loss of material (Figure 2). This allows a more detailed analysis of ubiquitin chain conformations and the ubiquitin mechanism than current methods. Quantification of ubiquitination, understanding of the role of specific ligase and DUB enzymes and analysis of ubiquitin inhibitors, will all be possible using the U2OS bioUb cell line.

Publication

A similar application in Drosophila:
Franco M, et al. A Novel Strategy to Isolate Ubiquitin Conjugates Reveals Wide Role for Ubiquitination during Neural development. Molecular & Cellular Proteomics. 2011 May;10(5) (online publication)

Figure 1: Schematic diagram of the complex ubiquitin pathway

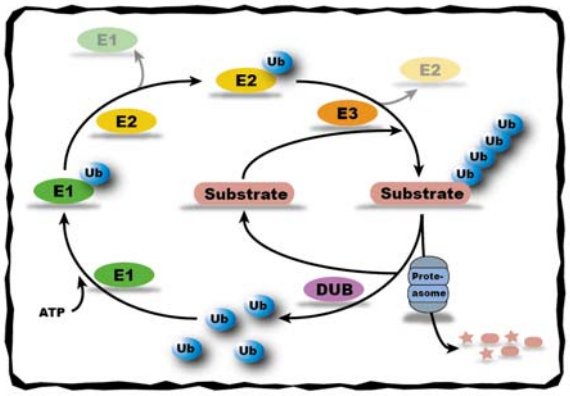
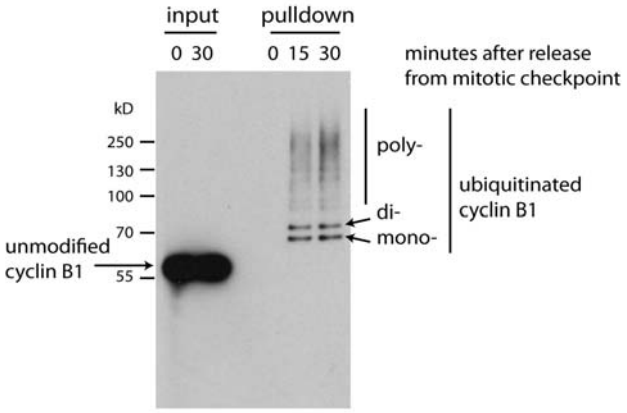


Figure 2: Specific and clean pull-down of ubiquitinated substrate



Cyclin B1 is ubiquitinated and degraded as cells exit mitosis and this is detected cleanly and specifically in streptavidin pulldowns from bioUb cells synchronized at this point in the cell cycle.

Commercialisation

We are seeking to establish non-exclusive licensing relationships for commercialisation of these cells.

TET Systems and Avidity are the owners of the patent portfolios relating to tetracycline regulated gene expression in eukaryotes and the Biotin tag, respectively. Any licensee of the cell lines will require valid licences to the TET System and Biotin tag.