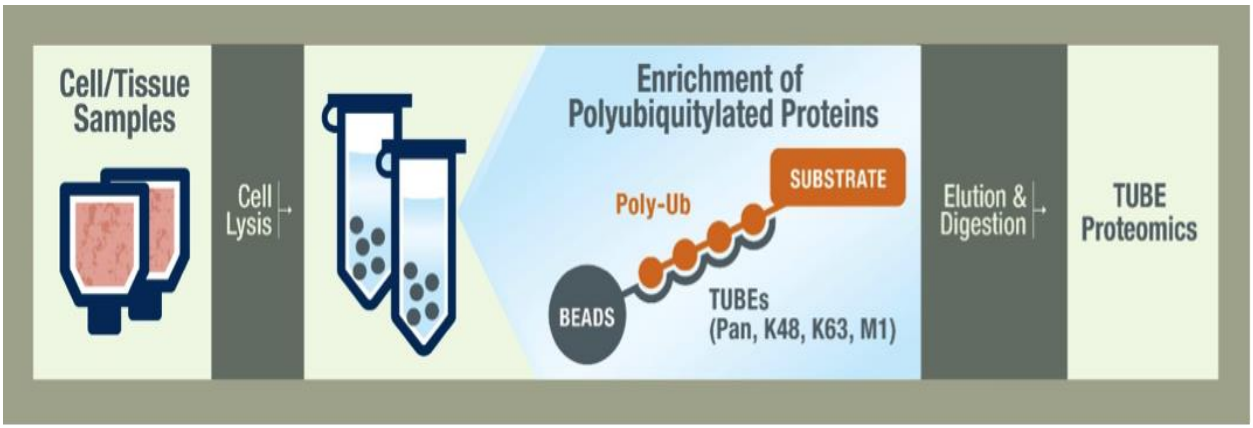
Ubiquitin Proteomics

TUBE-based Mass Spec Analysis

Simple, Efficient and Reproducible



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Analysis Report

Project Name	PROTAC Drug Effect on Ubiquitin Signature of Dopaminergic Neurons
Sample Description	Frozen cell pellets of SH-SY5Y
Number of Samples	4
Samples List	1- Negative Control 2- PROTAC, E3 Ligase or DUB Drug 3- E3 Ligase or DUB Inhibitor + PROTAC E3 Ligase or DUB Drug
Project Client	LifeSensors

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Analytical Service Report

Summary

In this project, 2,000 proteins were identified for the 4 samples provided. 200 proteins were detected to be ubiquitinated. 15,000 peptides were identified, 250 peptides are ubiquitinated. For detailed information, please read the following report and the attached supplementary data.

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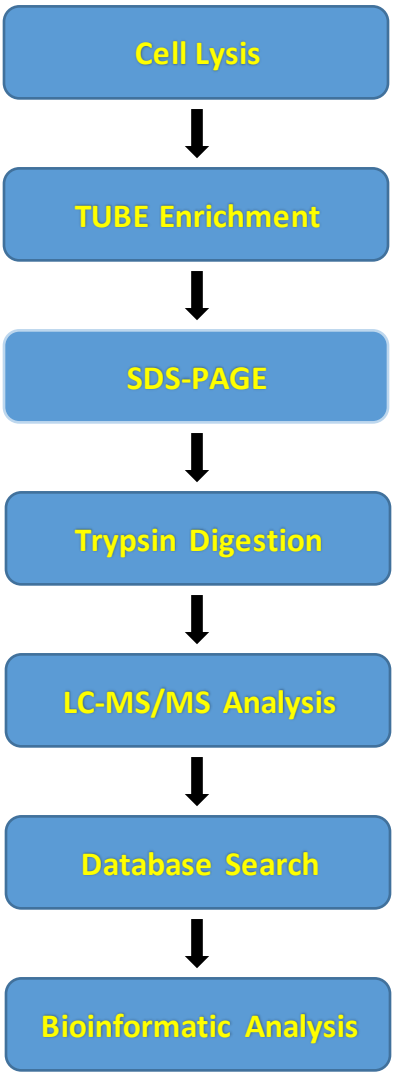
Experimental Flowchart



1. Methods

1.1 Sample Preparation

1.1.1. Whole Cell Protein Extracts

A. Lysis Buffer, containing protease inhibitor cocktail was added to the cells.
B. Cells were lysed on ice for 15 min.
C. Lysates were clarified by high-speed centrifugation
D. The supernatants were collected and were used for further TUBE enrichment and analysis.
E. Protein concentration of each supernatant was determined using the BCA Protein Assay Kit.

1.1.2. TUBE Enrichment

A. Ubiquitinated samples are enriched using TUBE pull down
B. Specific TUBEs used is dependent on the research questions being addressed
C. LifeSensors' MS compatible buffers are used to elute captured protein

1.2. SDS-PAGE and LC-MS/MS analysis

A. Samples are separated on gels and stained before analysis
B. Samples undergo trypsin digest
C. The duration of the analysis is dependent on the level of detail needed by our client, we will help you determine the best way to run your samples

1.3. Data Analysis

A. Specific bioinformatic searches will be performed based on your sample type
B. Our team has experience with a number of cell types, from many model species
C. Analysis includes
    a. Protein identification
    b. Ubiquitin sites and distribution
    c. Protein mass distribution
    d. Protein sequence coverage
    e. Peptide number distribution
    f. Bioinformatic analysis
    g. Summary of findings
D. We are here to help you get the most out of your data. We offer customer support after the analysis and can provide more in-depth analysis of specific targets.

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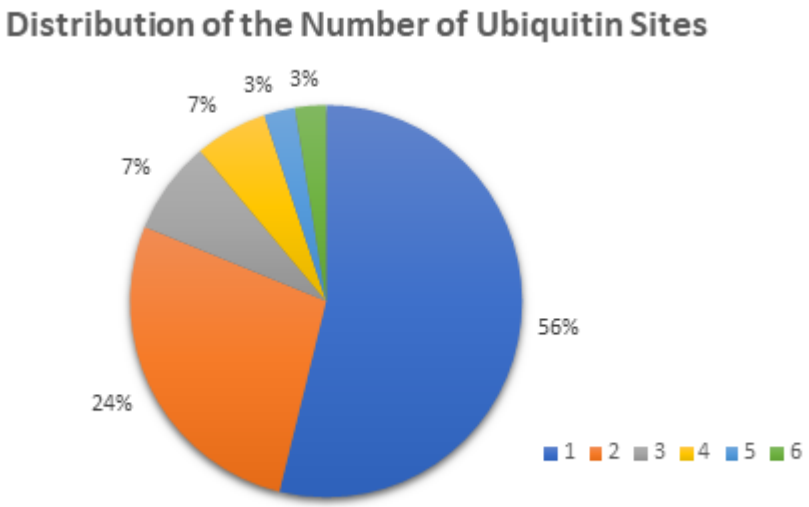
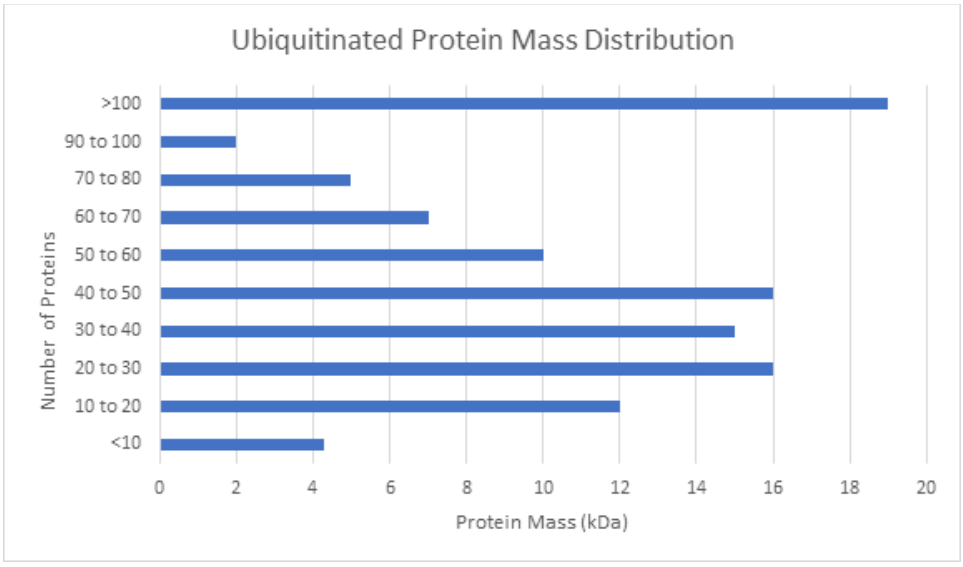
Example Data

Below are examples of data generated using TUBE- based mass spec technology with pan-selective TUBEs. Here the ubiquitinated proteins have been differentiated from whole cell lysate. This methodology is sensitive enough to detect the number of ubiquitin sites on a given protein. The number of ubiquitinated proteins detected was assigned to the number of ubiquitin sites that they carry. In this case over half of the ubiquitnated proteins detected were monoubiquitinated. When studying the effects of a drug or genetic knockout global shifts in the ubiquitome is a good place to start your analysis.



Other global analysis provided by LifeSensors includes protein mass distribution of all proteins as well as ubiquitinated proteins. The number of ubiquitinated proteins detected was assigned to different intervals of protein mass. Here there is an interesting cluster of proteins around 30 kDa, and over 100 kDa. The ubiquitin pathway is clearly playing a role in clearing and otherwise influencing larger proteins in this experiment.



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