Ubiquigent

WHITE PAPER

PROTEOME*profiler*

A multiplexed, mass spectrometry assay to report changes in the cellular proteome

Overview

The PROTEOME*profiler*™ assay offers customers ultimate flexibility for characterising changes in the cellular proteome in a range of biological settings, for example, comparing the proteomes of diseased and healthy cells or tissues or analysing the response to a perturbation, whether that be from a small molecule, genetic modification, or some other treatment.

The introduction of separation and analytical strategies such as multidimensional liquid chromatography (LC) of peptides coupled to high-performance tandem mass spectrometry (MS) has led to a dramatic increase in the depth and breadth of sampling of a given proteome. In turn, the introduction of various protocols and reagents now enables the identification and quantitation of proteins in multiplex format to provide relative quantification across different samples in a single run, significantly improving precision and accuracy as well as cost per sample.

Applications of PROTEOME*profiler* in protein degradation drug discovery

Understanding the modulation of the entire cellular proteome is an essential element of any targeted protein degradation drug discovery programme. In this context, PROTEOME*profiler* can be employed to:

Identify and validate drug targets

- Identify targets of interest by highlighting disease-related changes in protein homeostasis in an entirely unbiased manner (by comparing proteomic changes between diseased and healthy tissues/cell lines)
- Produce robust target validation, e.g., analysis of the proteomic changes between isogenic cell lines may offer further confirmation of the relevance of these targets (and associated pathway modulation) to disease
- Provide proof of concept and determine the consequences of deubiquitylase (DUB) inhibitor or proteolysis targeting chimeras (PROTAC)/molecular glue-mediated target protein downregulation in a disease relevant cell line, e.g., target genes may be genetically

Version 001

silenced or deleted by siRNA and CRISPR-type approaches to mimic small molecule mechanism of action (MOA).

Determine compound mechanism of action

- Characterise the activity of your compound; report the relative efficacy (EC_{50} values), selectivity, concentration, and time dependencies of deubiquitinase (DUB) inhibitors, PROTACs, molecular glues, DUBTACs and similar agents
- Confirm the primary target in cells; comprehensively reveal relevant pathway and proteomic alterations
- Demonstrate MOA of your compounds by treating disease-relevant cells with candidate compounds and analyse the impact on the proteome in an entirely unbiased manner
- Support programme SAR and lead series decisions by comparing active and inactive analogues or tool compounds, and across different pharmacophores
- Generate independent data to assist with due diligence activities on in-licensing opportunities
- Identify potential biomarkers to assist and accelerate clinical development

Key features

- **Widely applicable** - the understanding of global proteomic changes is highly important to the development of compounds in the protein degradation/stabilisation space across multiple modalities, including PROTACs, molecular glues, DUB inhibitors and DUBTACs
- **Informative** - unbiased, proteome-wide information to confirm degradation of protein target AND any other proteomic changes to dissect compound MOA, generate new hypotheses, and identify candidate pharmacodynamic markers for use in the clinical setting
- **Efficient** - benefiting from the large dynamic range of the MS platform, generate information about changes in the abundance of multiple proteins present at different orders of magnitude in the cell
- **Cost-effective** - multiplex experiments of up to 16 samples allow for robust statistical analyses and comparison of conditions in a single experiment
- **Flexible** - investigate different cell lines, tissues, compounds, or examine dose responses or time-courses in a single experiment
- **Physiologically relevant** - use unmodified, disease-relevant cells or tissues of your choice

Options

The PROTEOME*profiler* assay is highly flexible, allowing any composition of samples/conditions up to 16-plex per experiment. Customers are encouraged to discuss the goals and design of their experiment with Ubiquigent™. Customers may supply cell pellets/lysates for MS sample preparation by Ubiquigent or may elect to delegate the entire project from design to completion to Ubiquigent, incorporating all upstream elements, such as cell propagation, compound/siRNA treatments, generation of lysates and preparation of samples for MS.

PROTEOME*profiler* assay workflow summary

A schematic representation of the PROTEOME*profiler* experimental workflow is presented in Figure 1.

- The design of the experiment is finalised, taking note of the availability of up to a total of 16 samples per experiment, and incorporating the need for appropriate controls and replicates.

- Following cell treatment with compounds/siRNA transfection as appropriate, cell pellets are prepared and will be lysed by Ubiquigent according to the requirements set out in the document '*Requirements for Customer-supplied samples for PROTEOMEprofilerv1.pdf*'.
- Samples are enzymatically digested for MS analysis and labelled with individual mass tags.
- Offline fractionation of samples (to yield 15-20 fractions) is performed prior to LC-MS/MS analysis to reduce sample complexity and improve the detection of low abundance targets.
- The abundance of each protein identified by MS is reported as a fold change relative to the control sample(s).

Compound submission and study design

Accessing PROTEOME*profiler* is as easy as accessing any assay within our Drug Discovery Platform. Study designs are discussed and agreed with each customer upfront to ensure that any specific requirements are met.

Once agreed, customers are then asked to complete a *'PROTEOMEprofiler sample submission form'* confirming the following:

- Description of the samples being submitted for analysis in PROTEOME*profiler* - i.e., providing an indication of the species, and highlighting which, if any, are replicate samples.
- Allocation of samples to groups and choice of which sample groups are to be compared in order to calculate ratio.
- Full disclosure of the components of any lysis buffer and protocol to be used to prepare the lysates*, along with the expected protein yields from the submitted cell pellets.

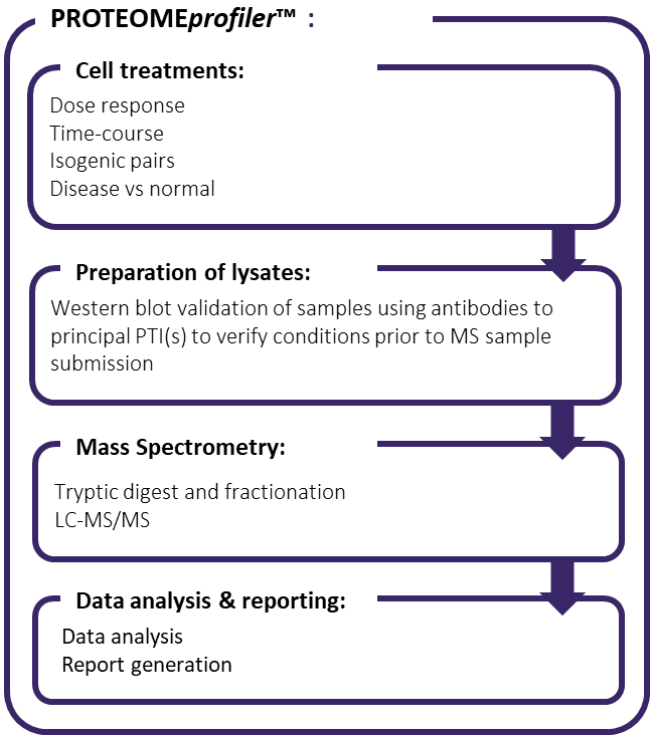

Figure 1: PROTEOMEprofiler workflow.

Customers may supply cell pellets directly to Ubiquigent; Ubiquigent will then process the samples for analysis by MS. The customer is responsible for pre-validating samples prior to submission to Ubiquigent, for example by ensuring that parallel cell pellets from the same experiment yield the expected change(s) in the target protein or phenotype in an appropriate analytical and/or functional assay. Alternatively, Ubiquigent can treat cells as briefed by the customer and similarly confirm key responses prior to the submission of aliquots from the same experiment to MS. PTI: Protein Target(s) of Interest.

*This is only applicable if you have optimised your protein extraction protocol for your particular cell type or tissue and wish Ubiquigent to use this protocol for lysate preparation from your cell pellets. Otherwise, Ubiquigent will use RIPA buffer.

Customers are encouraged to validate samples prior to submission to PROTEOME*profiler*, e.g., Western blots demonstrating the expected change in abundance of one or more PTIs obtained from aliquots from the same

experiment, expected phenotypic response or proof of gene knockout status.

Customers may discuss with Ubiquigent if there is a desire for cell treatments and sample verification to be undertaken by us. In such cases customers will need to supply cell line(s), antibodies (where appropriate) and provide appropriate experimental method guidance. The customer is responsible for ensuring compliance with all licences and permissions in relation to supply of any cell lines, cell pellets, cell lysates or antibodies or any and all other experimental materials provided to Ubiquigent required to carry out their project.

The Process

Ubiquigent will issue a Master Services Agreement (MSA), generate Work Orders (WO) for each individual project and supply a PROTEOME*profiler* submission form. Alternatively, customer may request quotes through Ubiquigent's marketplace at Science Exchange or Scientist.com.

Together with Ubiquigent, customer should finalise the study design and send the submission form and samples, test compound(s), antibodies, or cell lines (as applicable) to Ubiquigent.

Upon receipt of a fully executed MSA, a signed WO, a completed submission form and all materials, Ubiquigent will then schedule the study and provide progress updates.

Once the study has been completed, a fully annotated Customer Report will be sent to the customer with an offer to arrange a post project meeting to discuss the results.

Data Analysis and Reporting

Ubiquigent will provide the customer with an annotated Customer Report typically comprising:

- A complete list of identified proteins with fold-changes between averages of the sample groups to be compared as defined in the submission form and p-values obtained via Welch's t-test.
- A graphical illustration of the fold-change and p-value data in the form of a Volcano plot.
- A heatmap of hierarchical clustering of protein abundance in each sample.
- Gene ontology and protein interaction analysis of proteins with significant fold-changes between sample groups to be compared as defined in the submission form.

Customers should discuss with Ubiquigent whether any additional analyses are required for their project.

We look forward to discussing how PROTEOME*profiler* can accelerate and enhance your drug discovery programme

Contact us: services@ubiquigent.com