

**Scope of Services**

The Invitrogen business of Life Technologies Corporation (“Invitrogen”) will profile a specified number of ubiquitin ligase(s) of interest in the absence and presence of inhibitor(s) in an *in vitro* Ubiquitin Ligase Substrate Identification with Inhibitor assay using ProtoArray® microarrays that contain more than 9,000 human proteins as outlined below on behalf of the “Client”.

This Statement of Work specifies Invitrogen’s ProtoArray® Ubiquitin Ligase Substrate Identification with Inhibitor assay for this project.

**Service includes:**

1. Characterization of ubiquitin ligase substrates on ProtoArray® Human Protein Microarrays v5.0 (containing more than 9,000 unique proteins spotted in duplicate) and inhibition of ubiquitination in an *in vitro* ubiquitin ligase assay.
2. Controls and samples are as listed below. This service includes ten (10) ProtoArray® microarrays in total.
3. Probing of ProtoArrays® v5.0 at one (1) concentration of E1 (100 nM, unless otherwise specified by Client), one (1) concentration of E2 (500 nM, unless otherwise specified by Client), and two (2) ligase concentrations (50 nM and 250 nM, unless otherwise specified by Client), in duplicate, in the presence of one (1) concentration of biotin-ubiquitin (100 µg/ml) and ATP regenerating enzymes, using a total of four (4) microarrays, and detection of interactions using Alexa Fluor® 647-streptavidin.
4. Probing of ProtoArrays® v5.0 at one (1) concentration of E1, one (1) concentration of E2, and two (2) ligase concentrations in the presence of inhibitor (25 µM, unless otherwise specified by Client), in duplicate, in the presence of one (1) concentration of biotin-ubiquitin and ATP regenerating enzymes, using a total of four (4) microarrays, and detection of interactions using Alexa Fluor® 647-streptavidin.
5. Probing of one (1) ProtoArray® v5.0 with detection reagent only as a negative control.
6. Probing of one (1) ProtoArray® v5.0 with E1, E2, biotin-ubiquitin, ATP regenerating enzymes, and Alexa Fluor® 647-streptavidin as a negative control.

**Notes:**

- a. *When multiple ligases and/or inhibitors are tested simultaneously, only one of each negative control will be performed.*
- b. *Invitrogen does not guarantee that any interactions of the ligase of interest will be observed on the array, that observed interactions are specific to the ligase of interest, or that the inhibitor of interest will inhibit ubiquitination.*

**Timeline:** 4 weeks upon initiation of service project

**Service does not include:**

1. Shipment of a ProtoArray® microarray.
2. E1, E2, or E3 enzymes.
3. Inhibitor compounds.
4. Assay performed at the same ligase activity for multiple ligases.

**Client provides:**

1. Ligase (at least two aliquots of 20 µl at 100 µM).
2. E1 (at least two aliquots of 20 µl at 100 µM).

## ProtoArray® Ubiquitin Ligase Substrate Identification with Inhibitor Assay

3. E2 (at least two aliquots of 20 µl at 100 µM).
4. Inhibitor compound in solvent (50 µl of 100 µM).
5. Solvent only (no compound, at least 50 µl of solvent)
6. Signed Quotation and related Purchase Order.
7. Project Initiation Sheet

### Invitrogen delivers:

1. Comprehensive project report that includes all data of candidate ligase substrates, and results of the negative controls for the ProtoArray® Ubiquitin Ligase Substrate Identification Assay.
2. Image files and raw data.

### Ordering information:

Service Description	Catalog Number
ProtoArray® Ubiquitin Ligase Substrate Identification Service	Custom Quote

### Timeline:

Upon receipt of all of the following: (i) this Quotation signed by Client; (ii) the Purchase Order related to this Quotation; and (iii) the Client Materials, Invitrogen will place the project in the current queue for execution. The Client will be informed of the predicted start date. Once the project is initiated, Invitrogen will use commercially reasonable efforts to supply the Deliverables to Client within the specified timelines. The timeline may be extended for orders of >3 ligases.

### Project management:

Our project management team will be overseeing the work on this project and will keep you apprised of the status.

### Terms and conditions:

Invitrogen's Terms and Conditions for Provision of Services govern the service work provided hereunder and are available upon request. Furthermore, this service may be covered by one or more Limited Use Label Licenses (See Technical Support section of [Invitrogen.com](http://Invitrogen.com) for specific Limited Use Label Licenses). By use of this service, you accept the terms and conditions of all applicable Limited Use Label Licenses. This service is performed for research use only.

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