OHRI Proteomics Core Facility

Spring 2024 Updates

The Ottawa Hospital RESEARCH INSTITUTE | L'Hôpital d'Ottawa INSTITUT DE RECHERCHE

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Hello clients of the Proteomics Core,

I have thoroughly enjoyed continuing the delivery of high-quality LC-MS data since last May's managerial transition. The Proteomics Core has served dozens of projects in the Ottawa research community over the past year. Having continued with Lawrence's existing services through 2023, I am looking forward to expanding the offered services to better meet the needs of your projects.

What's New for Spring 2024:

- **Expanded Options for Protein Digestion**. This means clients can submit samples to the Core in a wider array of formats beyond traditional gel bands.
    - In-solution digestion of cell lysates and purified proteins using trifluoroethanol
    - SP3 Protein purification from cell lysates of known protein concentration
    - On-bead digestion of proteins that have already been bound to magnetic beads by the client (such as BioID or Co-IP)
    - Premium varieties of trypsin are available depending on project aims (such as PTM or quantitative analysis)

- **Re-Optimized Default LC-MS methods.** The existing default LC-MS methods have been built upon by re-optimizing all parameters; boosting protein detections by more than 10%. Now, the default 40-minute CID method has been replaced with a 45-minute method using HCD fragmentation. It is important to note that all previously used LC-MS methods are still fully available for use to allow direct comparisons to past projects, but they must be specifically requested in the service request form. It is recommended for new projects to switch to these new methods.

- **Targeted Protein Analysis**. Does your lab have challenges detecting a protein of interest using antibody-dependent methods, such as Western blotting? Targeted LC-MS involves tailoring the MS method to detect peptides specific to your protein of interest. This strategy comes with greater sensitivity than traditional exploratory LC-MS analysis and superior quantitative accuracy than Western blotting.

- **New Service Request and Service Report forms**. Service request and service report forms are being re-configured and will be distributed later this spring.

- **Pricing Changes.** The pricing structure remains unchanged; a cost-effective subscription service is still available for high-volume users, and internal labs (OHRI/uOttawa) will continue to receive a discounted rate compared to external labs. In order to follow the Core's cost-recovery model and meet the rising cost of consumables, the fees of some services have been modestly adjusted.

- **Proteomics Seminar.** Keep your eyes peeled for an announcement in the Fall, as a "Proteomics Basics" presentation series is in the works. This will help with the design of your experiments, raise awareness of alternative strategies to reach your research goals, and establish a foundation of proteomics knowledge for trainees.

If any of the services described above catch your interest, please reach out as I am happy to discuss. Furthermore, I invite you to review the new menus of pricing and services offered by the Proteomics Core in the pages to follow.

Whether you need global proteome quantitation of cell lysates or the identification of interacting proteins from pulldowns, let's make this year "proteomically productive"!

Andrew Macklin
OHRI Proteomics Core Facility Manager

Annual Subscription

The Annual Subscription service will continue to be offered as a cost-effective option for high-volume clients. As a subscriber, your lab will enjoy steep discounts on all services, and a higher priority in times of long sample queues. To demonstrate the large discounts, the prices for a batch of five samples with and without a subscription are listed below when they are processed by commonly bundled services.

For the upcoming fiscal year, the annual subscription fee has been raised from 6500$ to 6750$ to keep up with rising consumable costs. Recurring subscription revenue is critical for financial planning and maintaining the Core's operation.

The one-year subscription period begins April 1st. If interested, please ask Andrew for the enrolment form and detailed terms and conditions.

		Example Price for a Batch of Five Samples ($)	
Bundled Services	**What's Included?**	**Internal, Non-Subscriber (OHRI/uOttawa)**	**Subscribing Lab**
Standard Gel Band Service	• In-gel protein digestion • LC-MS using the default 45min gradient • Protein ID by Mascot and Scaffold report file	1875	250
"Peptide 45" Extract Service (For peptide extracts ready for LC-MS)	• Ziptip desalting of pre-digested peptides • LC-MS using the default 45min gradient • Protein ID by Mascot and Scaffold report file	1050	150
In-depth Gel Band Service (For High Sequence Coverage / PTM Analysis)	• In-gel digestion using Trypsin Platinum • LC-MS using the default 45min gradient • Interpretation of PTM sites and detected sequences of interest by Mascot and/or Maxquant • Scaffold and Scaffold PTM report files	2275 + 1080 $ to add CID and ETD fragmentation methods (optional)	315 + 205 $ to add CID and ETD fragmentation methods (optional)
Quantitative Proteomic Analysis for Cell Lysates	• In-solution or SP3 protein digestion using Trypsin-LysC mix • Ziptip desalting • LC-MS using 90min method • Maxquant analysis and Scaffold report file	2645	430

Sample Prep Services (see footnotes)

Digestion Service	Sample Format	Price per Sample ($)	
		Internal (OHRI/uOttawa)	External
In-Gel	* SDS-PAGE Gel Band	175	200
SP3 Protein Purification and On-bead Digestion	† Lysates or purified protein (of known concentration)	175	200
In-Solution (Trifluoroethanol-based)		150	175
Pre-Bound On-bead Digestion	‡ BioID or IP using magnetic beads	150	175
Ziptip	§ Peptide desalting	10	15
Additional Sample Preparation	E.g. sample drying and resuspension in an LC-MS compatible buffer	100 $ / hour	150 $ / hour

General Notes: Digestion services default to using reliable, sequencing-grade trypsin. Premium varieties of trypsin can be used at an added cost.

- Samples intended for quantitative global proteomics (in-solution or SP3 on-bead digestion) may benefit from using the Trypsin-LysC protease mix. The addition of LysC enhances peptide recovery and improves quantitative reproducibility. Upgrading to this protease will cost an additional 10$ per sample and 5$ for subscribers.
- Gel bands requiring high sequence coverage for PTM and isoform analysis are suggested to be digested by Trypsin Platinum. It has greater cleavage site specificity, meaning fewer artifacts and improved spectral database matching. Upgrading to this trypsin will cost an additional 10$ per sample for nonsubscribers, and 5$ for subscribers.
- Alternative proteases AspN and GluC are available at a premium cost in specific circumstances. This is usually after trypsin proves to be unfruitful in detecting the peptide of interest. Please ask Andrew if this would be suitable for your needs.

***** As in the past, proteins can be provided to the Core as SDS-PAGE gel bands submersed in 1% Acetic Acid Solution. When handling the gel and excising your bands, make a diligent effort to limit keratin contamination (perform in a cell culture or laminar flow hood, always wear gloves). When excising the bands, please limit the amount of "blank gel" around the band. Do not include dye front or stacking gel. If running a cell lysate, BioID, or IP on a gel and you wish to the entire lane in a gel band format, please divide each gel lane into 3-5 gel bands. The Core cannot accept gel bands that exceed 1cm x 1cm in size. Gels must be stained by MS-compatible Coomassie or Silver stain.

† Lysates with known protein concentration (such as determined by Bradford or BCA) are to be provided frozen or lyophilized. Cell pellets must have been washed and re-pelleted with PBS a minimum of three times before lysis to remove FBS contaminant proteins. The protein concentrations of each sample and lysis buffer components are to be detailed on the service request form. Lysates are to be stored frozen at -80^{o}C before providing them to the Core.

‡ If the client wishes the Core perform on-bead digestion (such as BioID, Co-IP, etc.), the client can bind proteins to magnetic beads before sample submission. Since it is not advised to freeze these samples (unlike the other three sample formats), the Core will need to digest these samples shortly after receiving them. As such, please ensure the following submission conditions have been met:

- The client must provide 2 full business days' notice of when they will be submitting samples so Proteomics Core staff can arrange their schedules accordingly
- This type of sample cannot be submitted to the Proteomics Core on Fridays, since the digestion protocol would carry over into the weekend.
- If samples are received later than 2 PM on the agreed submission date, the samples will be stored at 4^{o}C overnight until they can be digested the following morning.
- The beads (once proteins are bound) must have been washed by the client at minimum three times with 50mM Ammonium Bicarbonate (pH = 8) to remove detergents in lysis and binding buffers. This will be the last step performed by the client before submission to the Core.

§ Zip-tipping will be performed by the Proteomics Core for all samples except those originating from gel bands. This is to remove salts, particulates, and detergents in buffers that would clog and contaminate the LC-MS instrumentation

LC-MS Analysis Services (see footnotes)

Pricing changes:

- The rate for 60 minutes of LC-MS time is unchanged from 2023-24, but the prices for shorter and longer gradients have been adjusted to meet the needs of a shifting proteomic landscape. While the price for short gradients (45 minutes) has been slightly increased, the price for long gradients (90 and 180 minutes) has been scaled down. The goal for this revised pricing structure is to appeal to a wider spectrum of projects requiring longer gradients which were prohibitively expensive, and incentivize projects using short-gradient projects to use longer methods and reap the rewards of boosted peptide detections.
- Due to the stochastic nature of LC-MS analysis, duplicate injection of the same sample will yield ~ 20% more protein hits and instill further confidence in the proteins detected by the original LC-MS run. To incentivize this boost, the cost of LC-MS time for a second replicate has been discounted by 50% from the price of the first injection. Similarly, additional injections for alternative fragmentation methods (such as in PTM studies) are discounted at the same rate.

		Price Per LC-MS Run ($)	
New, Default LC-MS Method	**Application**	**Internal (OHRI/uOttawa)**	**External**
45min HCD	Low Proteome Complexity Protein ID and Quant	200	250
60min HCD	Low-Medium Proteome Complexity Protein ID and Quant	250	300
90min HCD	Medium-High Proteome Complexity Protein ID and Quant	325	400
180min HCD	High Proteome Complexity Protein ID and Quant	400	500
* Targeted Method (45, 60 and 90min)	Robust and sensitive detection of select proteins	Same rate per hour of LC-MS time as above	
Add-ons			
** Runs with CID fragmentation, ETD fragmentation, or higher HCD Collision Energy	Enhanced PTM and High Sequence Coverage Analysis	50% of the original HCD run's cost	
*** Technical Duplicate Injection	Maximizing proteins detected in a sample	50% of the original HCD run's cost	

Legacy methods, but they are still available for use (same hourly rates as the new default methods)			
40min CID	Pre-2024 default method	200	250
45min HCD	Pre-2024 custom method	200	225
55min CID	Pre-2024 custom method	230	275
85min CID	Pre-2024 custom method	375	450

***** Targeted method development and optimization not included. This is the price for a run once the method targeting your proteins of interest has been developed

****** Generally, when detecting PTMs, HCD is a good fragmentation method to use on the first attempt. Other methods such as CID and ETD provide alternative fragmentation patterns and may yield different database matches. In PTM studies, the sample will initially be analyzed using an HCD method that uses higher collision energies than the typical sequencing method. If the client wishes to also analyze by CID and ETD, this must be specified on the request form. The cost of this LC-MS running is 50% discounted from the original HCD price since the sample will already have been prepared and loaded.

******* If you want technical duplicate analysis, please request it on the service request form. The price of the second injection is discounted 50% from the price of the original run since the sample will already have been prepared and loaded.

Proteomics Data Processing Services

Analysis Type	Application	Price Per Analysis ($)	
		Internal (OHRI/uOttawa)	External
Standard Protein Identification	Standard Protein Identification with Semi-Quantitation by Mascot's Spectral Counts	* The First Scaffold file is included	
Protein Quantitation by Maxquant LFQ	Protein Quantitation by LFQ + Scaffold file	15 $ per hour of raw data	20 $ per hour of raw data
Interpretation of High-sequence coverage and PTM analysis	Comparing two conditions to identify the source of mass shift (PTM, isoform, etc.)	300 $ per project	450 $ per project
Targeted Method Development	Robust and sensitive detection of a few select proteins of interest	50 $ per protein	75 $ per protein
Additional Custom Analysis, Consultation or Interpretation	* Upon request	100 $ per hour	150 $ per hour
LC-MS .raw files Sent to Client	Client wishes to do their proteome database searching	Free (upon request)	

* Additional Mascot analyses using different search parameters than those originally requested are 50$ per Scaffold file for subscribers, 100$ for internal, 150$ for external (the 1-hour rate for additional custom analysis)

Mass Cytometry

Mass cytometry is a hybrid of flow cytometry and mass spectrometry which allows for the antibody-dependent measurement of up to 50 proteins across millions of cells. No digestion. Single-cell resolution. By switching out fluorophores for rare earth metal tags, we're able to leverage the power and sensitivity of mass spectrometry to analyze which antibodies bind to their targets and highlight novel cell populations.

The Standard Biotools Helios instrument at OHRI is fully staffed and we can help you from the initial experiment design to high-dimensional analysis.

If this is of interest to you, please reach out to our CyTOF specialist Damian Carragher at **dcarragher@ohri.ca**