

Proteomics – Getting started

How to prepare protein samples for mass spectrometry analysis at SPARC

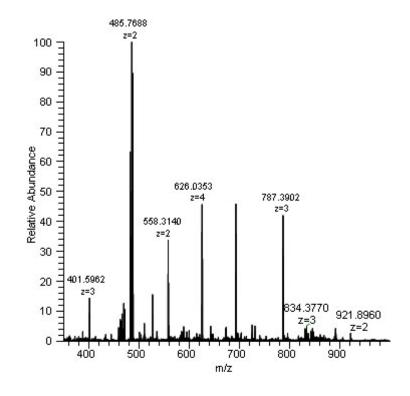
Presented by Leanne Wybenga-Groot at SickKids Spotlight Series, Jan. 15, 2020

What is Proteomics?

- The entire complement of proteins present in a cell or tissue is known as its proteome.
- Proteomics is the study of proteomes and their functions.
- Proteome is more complex and dynamic than genome
 - 1 gene \rightarrow \geq 1 protein
 - Protein modifications
 - △ protein abundance
 - Protein interactions

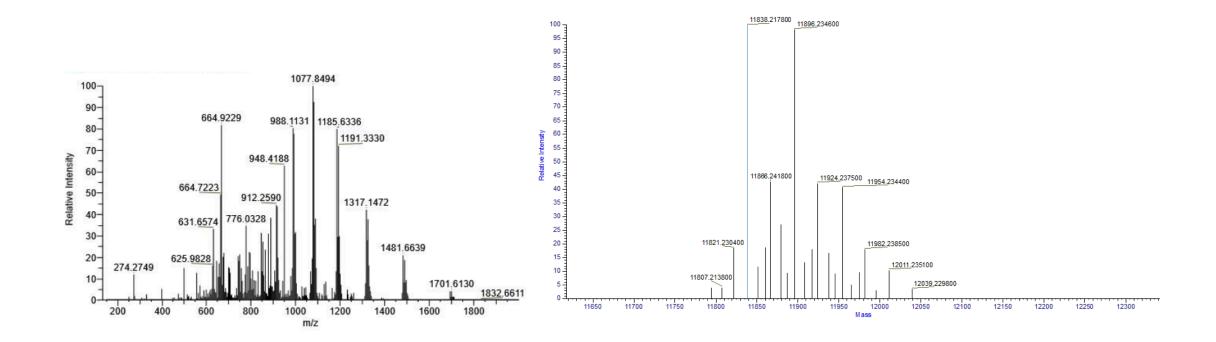
What is Mass Spectrometry?

- Analytical technique that measures the mass-to-charge ratio (m/z) of ions
- Results presented as a mass spectrum, a plot of intensity vs. m/z
- At SPARC Molecular Analysis, we perform mass spectrometry on PROTEIN samples



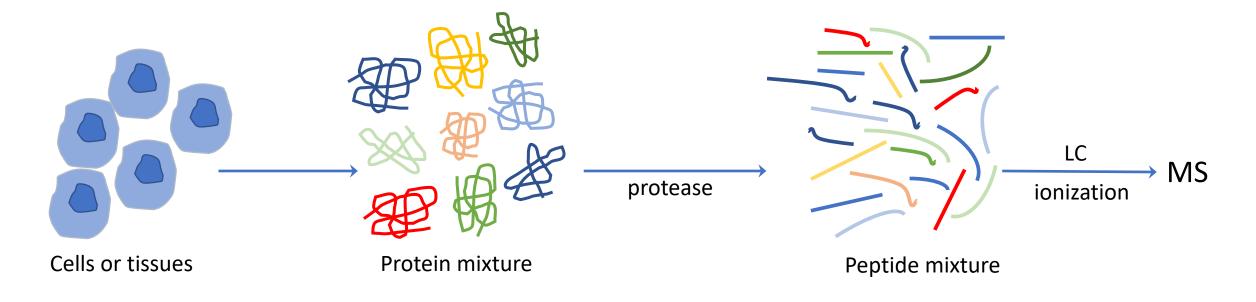
Intact Mass Analysis

- Purpose: you want to know the precise MASS of your protein
- Suitable for purified protein or peptide
- Sample is NOT digested before analysis but kept intact
- Contact SPARC staff for details of how to get started



Bottom-up MS

- Purpose: you want to identify proteins in a complex sample
- Suitable for cell & tissue lysates, BioID or IP experiments, gel bands, etc
- Proteins are digested into peptides with a protease, introduced into MS and identified by tandem MS (MS/MS)
- Liquid chromatography is used to separate the complex sample into simpler samples and introduce peptides to MS (LC MS/MS)

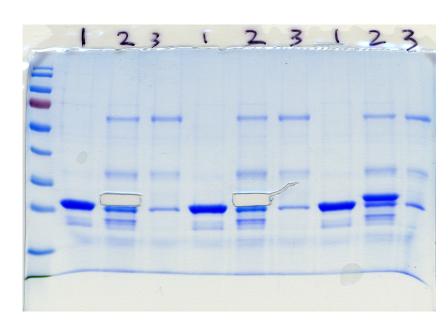


Preparing samples for MS analysis

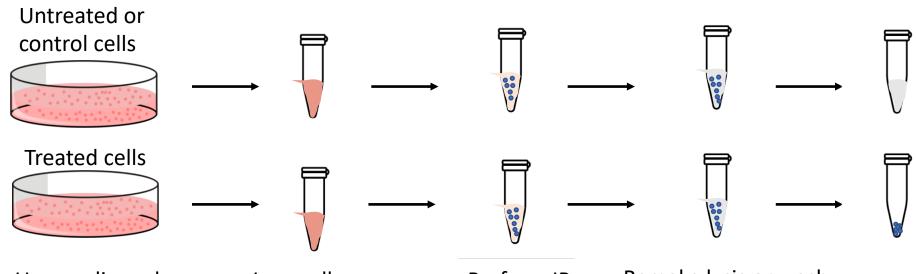
- Minimize keratin contamination!! Wear gloves, lab coat, use clean reagents and glassware.
- We like 10-30 ug of protein sample, but can achieve good results with less depending on sample complexity (1-5 ug).
- We will need to know the biological source or species of your sample (eg. human, mouse, E. coli)
- We prefer to know the protein concentration of your sample
- We need to know what solvent or buffer your sample is in

Preparing a gel band for MS analysis

- Use new glass plates for SDS-PAGE and clean, new boxes for staining.
- Cut out band in laminar flow hood using clean materials.
- Cut gel slice 2-3 mm wide only; avoid excess gel!
- Do not combine multiple gel bands in same tube.
- Store gel slices in 1% acetic acid at 4°C.



Preparing IP or BioID samples for MS



Elute proteins with ammonium hydroxide (see website protocol) or something specific to beads. Bring eluate in new tube, removed from beads.

OR

Remove liquid, freeze beads, and send for MS analysis.

Use media and harvest as normal; aim for 1-10 mg of starting material

Lyse cells as normal, measure concentration

Perform IP as normal, ~10-30 μL beads

Remake lysis or wash buffer with NO glycerol or detergent; wash beads 2-3X with this (or PBS). Protease, phosphatase inhibitors OK

Preparing solutions for MS analysis

Cell or tissue lysates

- Can use detergents in lysis buffer; we will perform acetone precipitation or S-Trap to remove it, but expect to lose sample
- Sample is more complex, thus need 3-4 hr LC MS/MS run
- Can be labelled with TMT tags and multiplexed (run together). For this, need 50-100 µg of protein per sample.

Secretomes

- Collect 25-50 mL media from cell plate
- Serum free for at least 24 hr
- We will concentrate media and process as normal

What do you get from us?

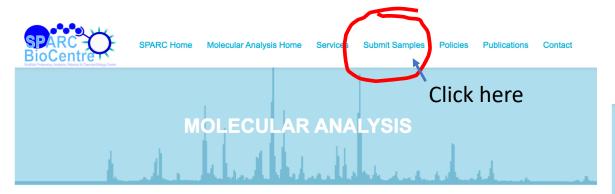


- Consultation come talk to us in 21.9440 or send an email
- A list of proteins identified in your sample, typically in Scaffold format

		No. 1		_	
	sible?	99	cessi	Molecul	Protein Trypsin
	1.5	토Bio View: Bloentified Proteins (25)	A 00	≝	₹ Ğ
#				≥ 36 kDa	<u> </u>
	_	28S ribosomal protein S35, mitochondrial precursor [Mus musculus], gi 26346603 dbj BAC369.	•		1
2	~	A kinase (PRKA) anchor protein 6 [Mus musculus]	gi 116517311 (+7)	254 kDa	1
3	\checkmark	ATPase, H+ transporting, lysosomal accessory protein 1, isoform CRA_a [Mus musculus]	gi 148697878 (+7)	48 kDa	1
4	✓	🕯 Bbx protein [Mus musculus]	gi 116283990 (+17)	60 kDa	1
5	\checkmark	🕯 Epha4 protein [Mus musculus]	gi 13435867 (+2)	103 kDa	6
6	\checkmark	keratin 2 [Mus musculus], gi 187953599 gb AAI39486.1 Krt2 protein [Mus musculus]	gi 111308159 (+4)	71 kDa	1
7	\checkmark	🕯 Krt78 protein [Mus musculus], gi 121933968 gb AAI27619.1 Krt78 protein [Mus musculus]	gi 111185567 (+4)	55 kDa	1
8	\checkmark	PREDICTED: hypothetical protein [Mus musculus]	gi 149255332	23 kDa	1
9	\checkmark	PREDICTED: myeloid/lymphoid or mixed-lineage leukemia 2 [Mus musculus]	gi 149266757 (+2)	566 kDa	1
10	\checkmark	PREDICTED: similar to 40S ribosomal protein S28 [Mus musculus], gi 148682799 gb EDL14746.	gi 149272298	8 kDa	1
11	\checkmark	🌣 RecName: Full=Ankyrin repeat domain-containing protein 24, gi 112180562 gb AAH48236.3	. gi 123789115 (+2)	106 kDa	1
12	\checkmark	RecName: Full=Nuclear receptor corepressor 1; Short=N-CoR1; Short=N-CoR; AltName: Full=Re.	gi 12643781 (+10)	271 kDa	1
13	\checkmark	🕯 actin, gamma, cytoplasmic 1 [Mus musculus]	gi 123298587 (+22)	33 kDa	1
14	\checkmark	🌣 envoplakin [Mus musculus], gi 123294499 emb CAM16385.1 envoplakin [Mus musculus], gi	gi 111185907 (+8)	232 kDa	1
15	\checkmark	glutaryl-CoA dehydrogenase, mitochondrial precursor [Mus musculus], gi 113680427 ref NP_0.	gi 113680425	49 kDa	1
16	\checkmark	glycerophosphodiester phosphodiesterase domain-containing protein 3 (Mus musculus), gi 22.	gi 110431346 (+8)	38 kDa	1
17	\checkmark	keratin, type I cytoskeletal 10 [Mus musculus], gi 26345440 dbj BAC36371.1 unnamed protei	gi 112983636 (+20)	57 kDa	1
18	\checkmark	mCG124047 [Mus musculus]	gi 148681587 (+11)	26 kDa	1
19	\checkmark	mCG142699, isoform CRA_a [Mus musculus]	gi 148664748 (+3)	121 kDa	1
20	✓	mCG145060 [Mus musculus]	gi 148707543	12 kDa	1
21	~	neuron navigator 1, isoform CRA_a [Mus musculus]	gi 148707629 (+3)	201 kDa	1
22	$\overline{\checkmark}$	src-like-adapter 2 [Mus musculus], gi 30173326 sp Q8R4L0.3 SLAP2_MOUSE RecName: Full=S	3.1	28 kDa	17
	_	see and analysis a firms marriage 2 and 2	9.,		

What's next?

Go to lab.research.sickkids.ca/sparc-molecular-analysis/



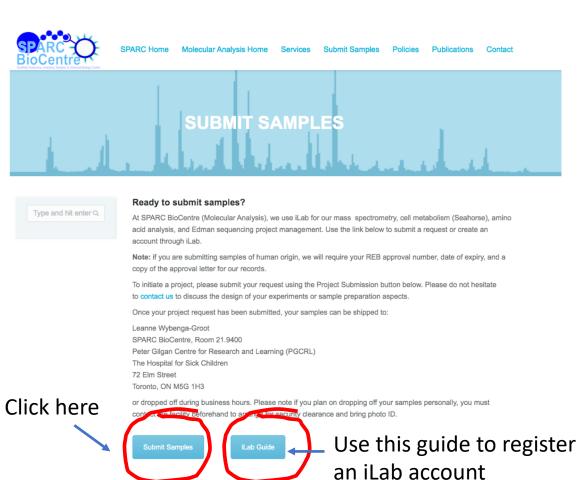
Announcements

"Proteomics – Getting started; How to prepare protein samples for mass spec analysis and the cost involved."

Leanne will be speaking during the second half of next week's Spotlight Seminar, which will be held on Wednesday, January 15, 12:00 - 1:00pm. Click here for more information.

At SPARC BioCentre (Molecular Analysis), we apply state-of-the-art technologies, including mass spectrometry, amino acid analysis, and extracellular flux analysis by Seahorse, for the molecular characterization of biological systems. This includes identification and quantification of a broad range of large and small molecules including proteomes and proteins, and small molecule analytes such as peptides, metabolites, and measures of metabolism. SPARC is operated on a fee-for-service cost-recovery basis by an experienced team of scientists and technicians, with Dr. Michael Moran as our Scientific Director. We are happy to work with researchers from early consultation and planning phases, to delivery of data, data analysis, and preparation of publications and grant proposals.

The SPARC BioCentre (Molecular Analysis) is a proud member of the Pan-Canadian Proteomics Centre

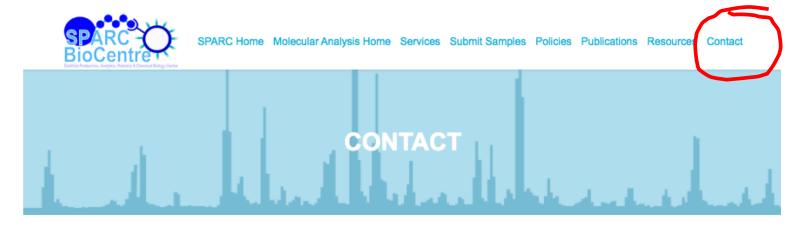


Once you have an iLab account...

- Request a service and fill in form as best you can; provide Cost Centre # if you are internal to SickKids. Otherwise, provide billing info.
- We will price the service and contact you if we need more info
- PI (or lab designate) approves the cost of service
- Bring samples to us
- We process your sample and send data via email/Dropbox within 1-2 weeks of your samples arriving for standard projects
- Your PI will be invoiced through SickKids myFinance at the end of each month

Questions??

 Contact one of us or pop by PGCRL 21.9440





Shipping Address

Attn: SPARC, Room 21.9400
Peter Gilgan Centre for Research
and Learning (PGCRL)
The Hospital for Sick Children
72 Elm Street
Toronto, ON M5G 1H3

Mailing Address

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Peter Gilgan Centre for
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686 Bay Street, 21st Floor
Toronto, ON M5G 0A4

Office Hours: 8:30 a.m. to 5 p.m. Monday through Friday (except for holidays).

^{**}Please note when dropping off samples to the SPARC BioCentre, you must make arrangements with someone from SPARC to add your name to the PGCRL security list. If your name is not on the security list, you will not be allowed access to the SPARC labs.



Leanne Wybenga-Groot, PhD Facility Manager Rm. 21.9703



Craig Simpson, PhD Project Manager Rm. 21.9440