SPARC BioCentre
SickKids Proteomics, Analytics, Robotics & Chemical Biology Centre

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?

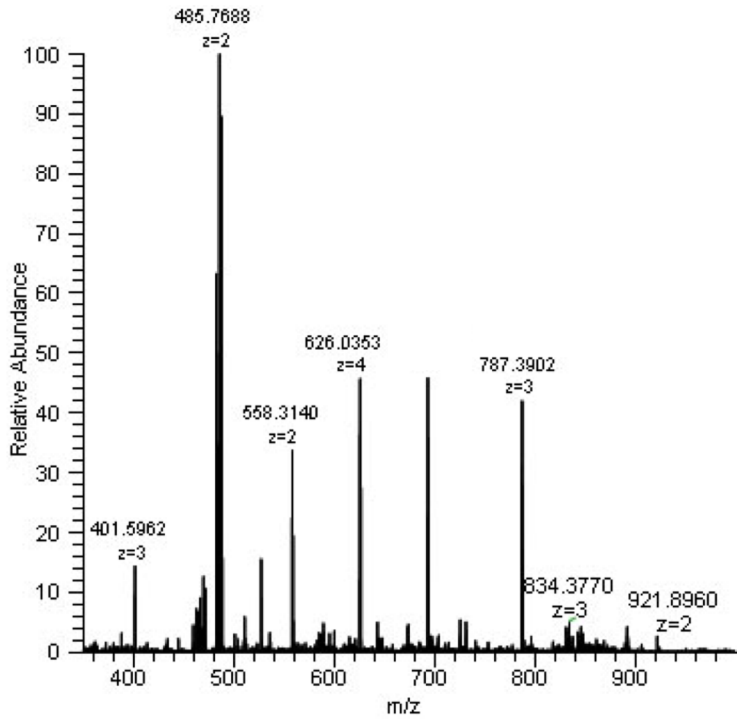
- The entire complement of proteins present in a cell or tissue is known as its proteome.

- Proteomics is the study of proteomes and their functions.

- Proteome is more complex and dynamic than genome
  - 1 gene → ≥ 1 protein
  - Protein modifications
  - △ protein abundance
  - Protein interactions

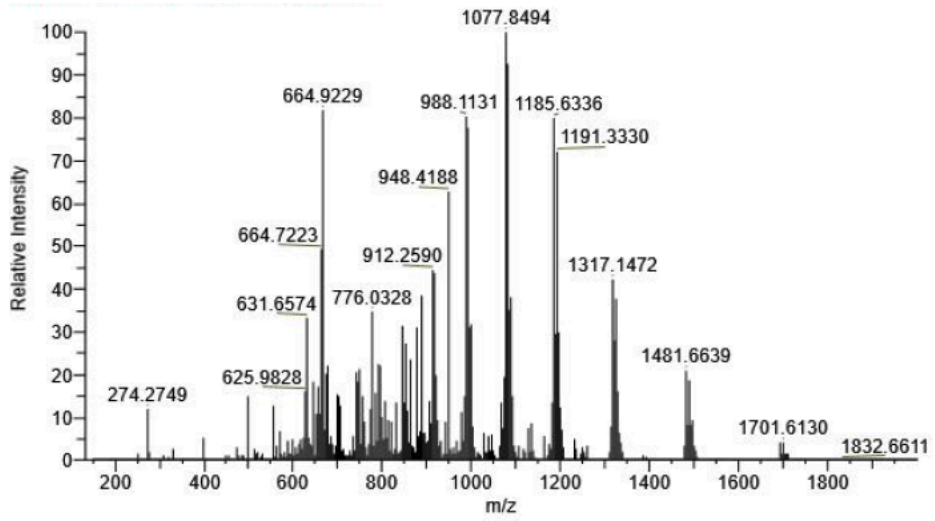
What is Mass Spectrometry?

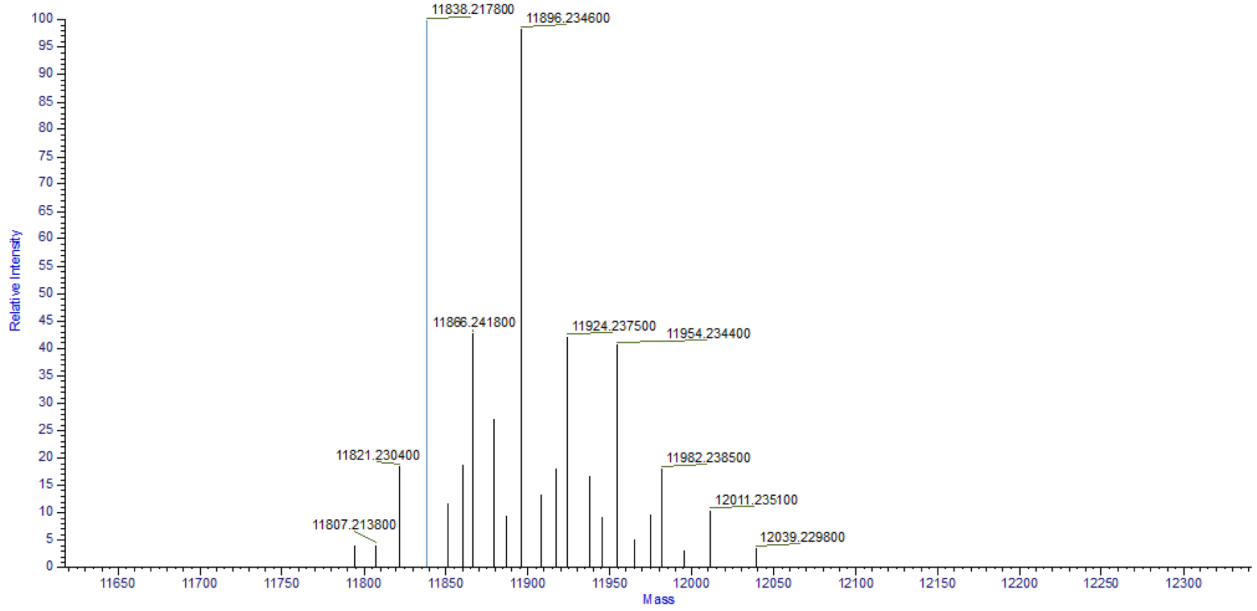
- Analytical technique that measures the mass-to-charge ratio (m/z) of ions
- Results presented as a mass spectrum, a plot of intensity vs. m/z
- At SPARC Molecular Analysis, we perform mass spectrometry on PROTEIN samples



Intact Mass Analysis

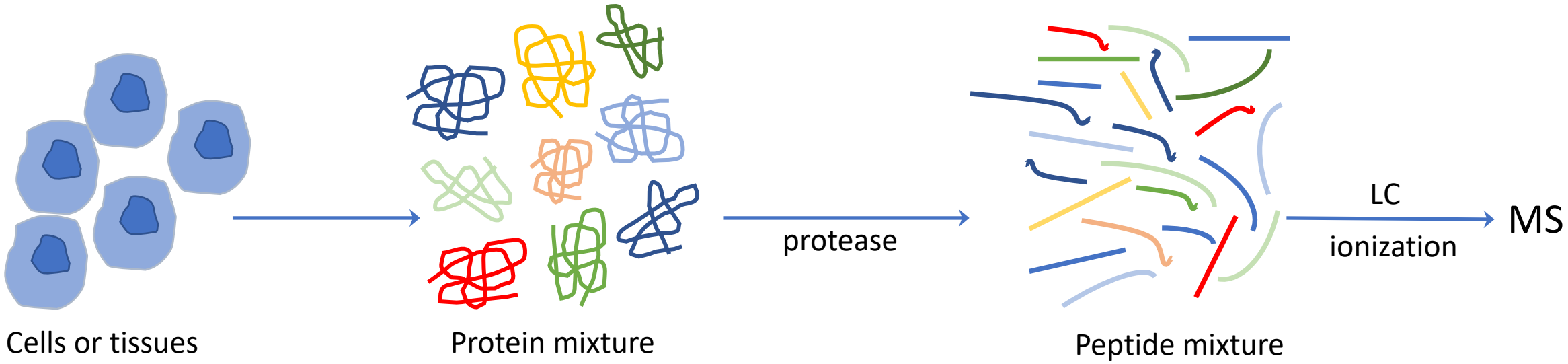
- Purpose: you want to know the precise MASS of your protein
- Suitable for purified protein or peptide
- Sample is NOT digested before analysis but kept intact
- Contact SPARC staff for details of how to get started





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)

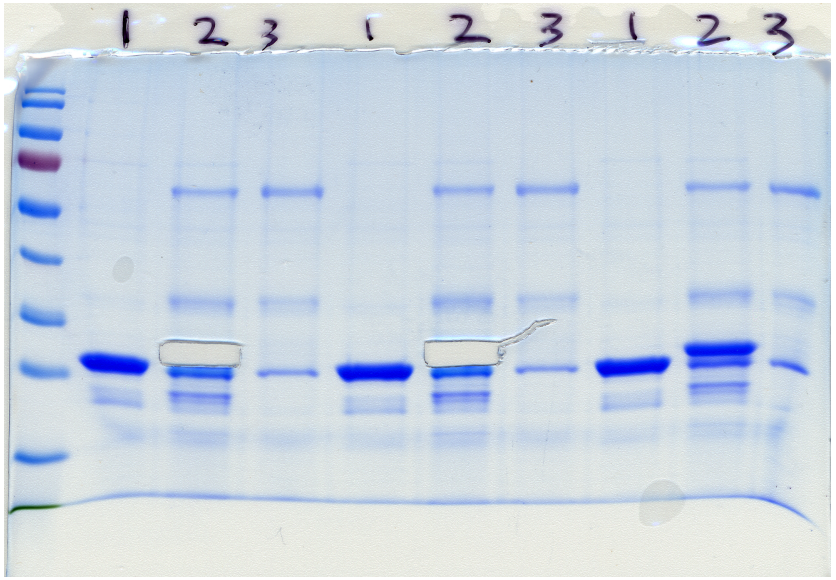


Preparing samples for MS analysis

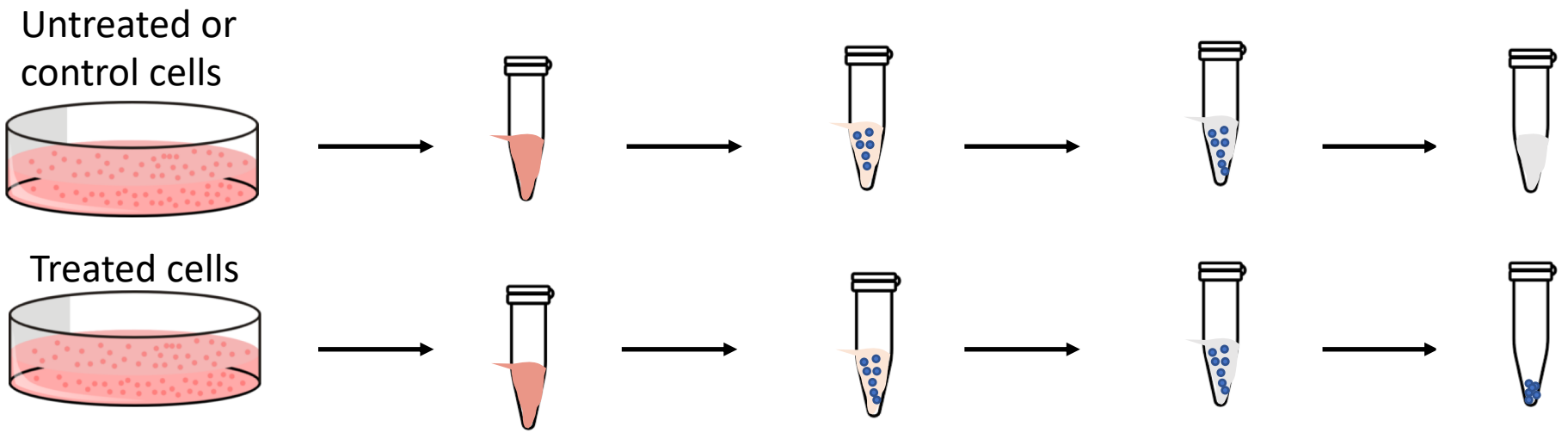
- Minimize keratin contamination!! Wear gloves, lab coat, use clean reagents and glassware.
- We like 10-30 ug of protein sample, but can achieve good results with less depending on sample complexity (1-5 ug).
- We will need to know the biological source or species of your sample (eg. human, mouse, E.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



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

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.

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

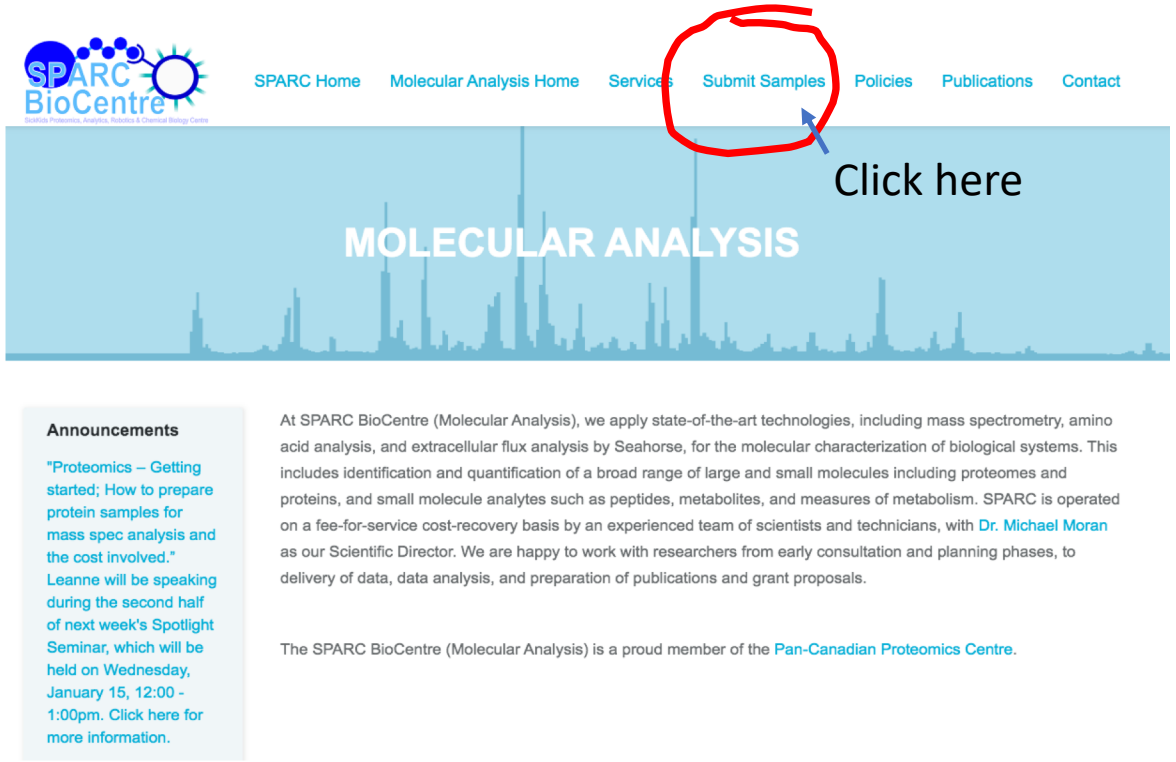
What do you get from us?

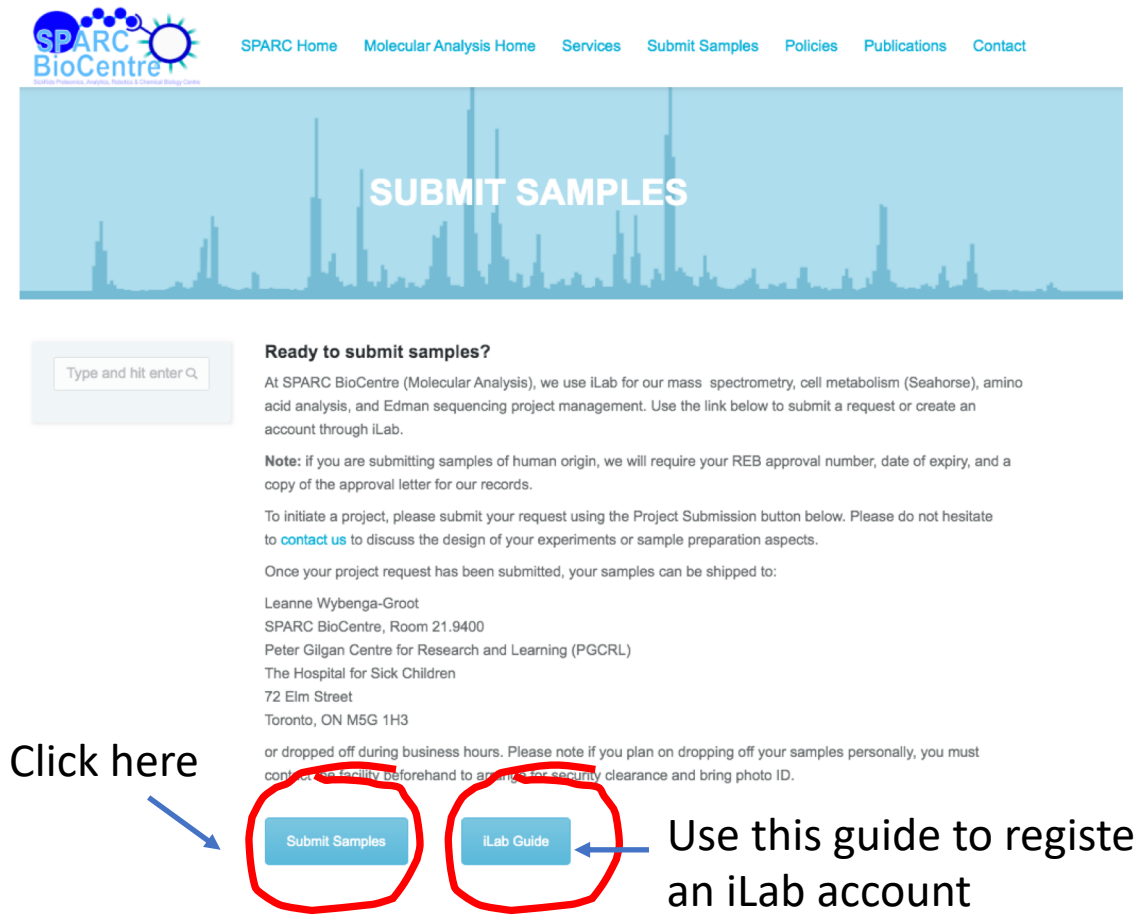
- Consultation – come talk to us in 21.9440 or send an email
- A list of proteins identified in your sample, typically in Scaffold format

#	Visible?	Starred?	Bio View: Identified Proteins (25)	Accessi	Molecul	Protein	Trypsin
1	☑	☆	28S ribosomal protein S35, mitochondrial precursor [Mus musculus], gi|26346603|dbj|BAC369...	gi|148235701 (+3)	36 kDa		1
2	☑	☆	A kinase (PRKA) anchor protein 6 [Mus musculus]	gi|116517311 (+7)	254 kDa		1
3	☑	☆	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	☑	☆	Epha4 protein [Mus musculus]	gi|13435867 (+2)	103 kDa		6
6	☑	☆	Keratin 2 [Mus musculus], gi|187953599|gb|AAI39486.1| Krt2 protein [Mus musculus]	gi|111308159 (+4)	71 kDa		1
7	☑	☆	Krt78 protein [Mus musculus], gi|121933968|gb|AAI27619.1| Krt78 protein [Mus musculus]	gi|111185567 (+4)	55 kDa		1
8	☑	☆	PREDICTED: hypothetical protein [Mus musculus]	gi|149255332	23 kDa		1
9	☑	☆	PREDICTED: myeloid/lymphoid or mixed-lineage leukemia 2 [Mus musculus]	gi|149266757 (+2)	566 kDa		1
10	☑	☆	PREDICTED: similar to 40S ribosomal protein S28 [Mus musculus], gi|148682799|gb|EDL14746...	gi|149272298	8 kDa		1
11	☑	☆	RecName: Full=Ankyrin repeat domain-containing protein 24, gi|112180562|gb|AAH48236.3| ...	gi|123789115 (+2)	106 kDa		1
12	☑	☆	RecName: Full=Nuclear receptor corepressor 1; Short=N-CoR1; Short=N-CoR; AltName: Full=Re...	gi|12643781 (+10)	271 kDa		1
13	☑	☆	actin, gamma, cytoplasmic 1 [Mus musculus]	gi|123298587 (+22)	33 kDa		1
14	☑	☆	envoplakin [Mus musculus], gi|123294499|emb|CAM16385.1| envoplakin [Mus musculus], gi|...	gi|111185907 (+8)	232 kDa		1
15	☑	☆	glutaryl-CoA dehydrogenase, mitochondrial precursor [Mus musculus], gi|113680427|ref|NP_0...	gi|113680425	49 kDa		1
16	☑	☆	glycerophosphodiester phosphodiesterase domain-containing protein 3 [Mus musculus], gi|22...	gi|110431346 (+8)	38 kDa		1
17	☑	☆	keratin, type I cytoskeletal 10 [Mus musculus], gi|26345440|dbj|BAC36371.1| unnamed protei...	gi|112983636 (+20)	57 kDa		1
18	☑	☆	mCG124047 [Mus musculus]	gi|148681587 (+11)	26 kDa		1
19	☑	☆	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	☑	☆	src-like-adapter 2 [Mus musculus], gi|30173326|sp|Q8R4L0.3|SLAP2_MOUSE RecName: Full=S...	gi|124249228	28 kDa		17

What's next?

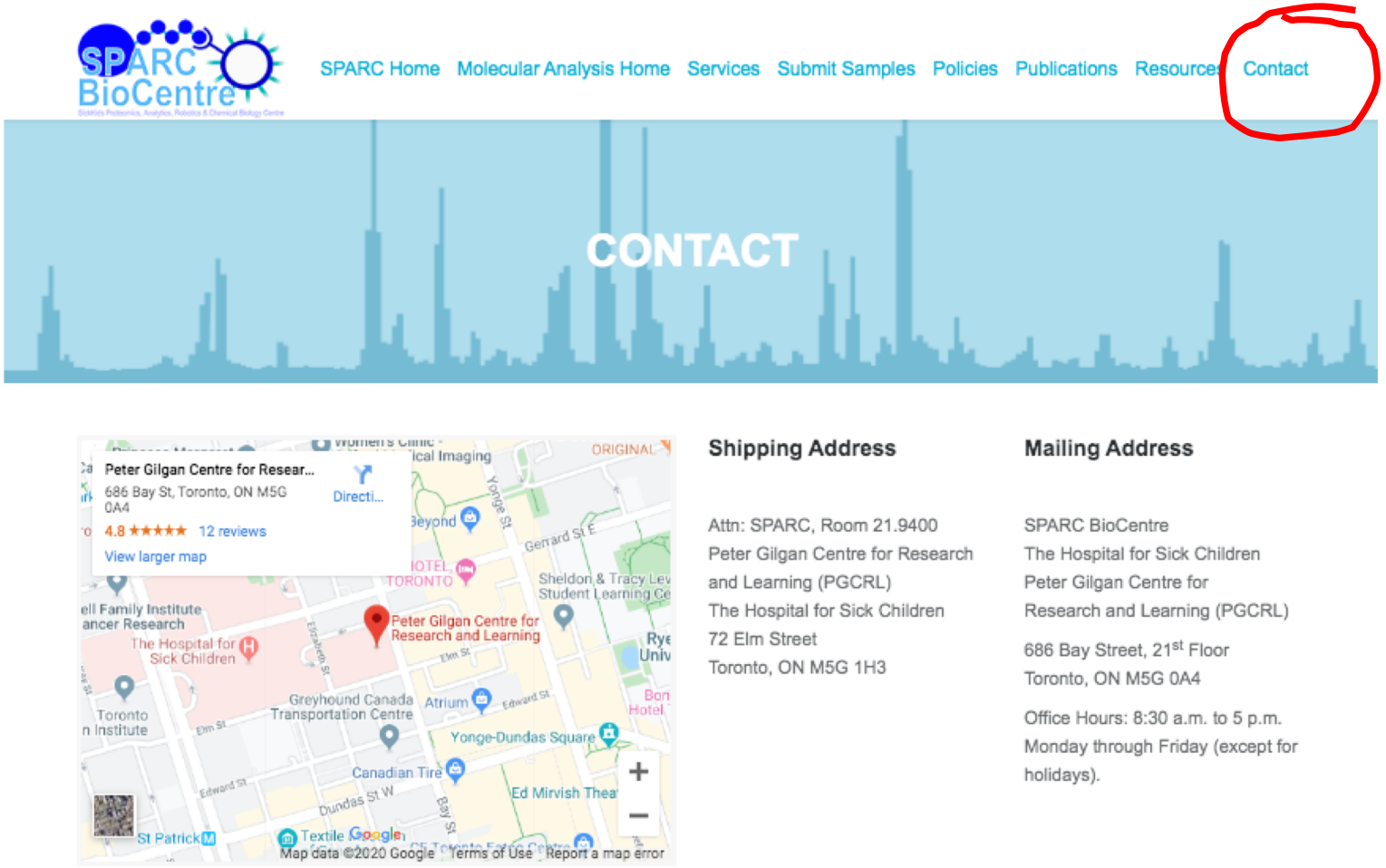
Go to lab.research.sickkids.ca/sparc-molecular-analysis/





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