



XiaLab Analytics

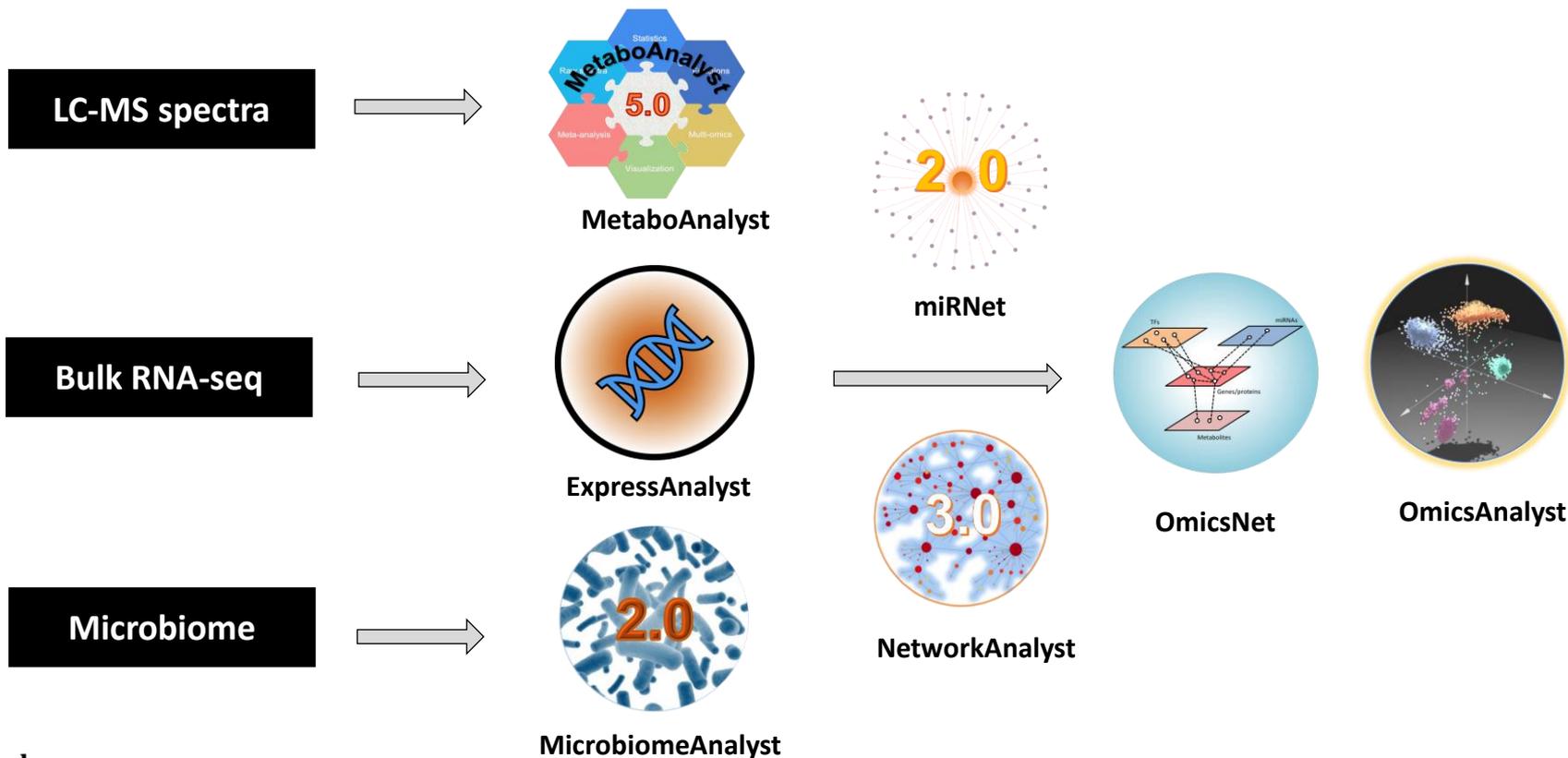
Empowering researchers through trainings, tools and AI

<https://www.xialab.ca> • contact@xialab.ca

Omic Data Science Training Course



Raw data → statistics → networks → functions



Our Resources

Recordings & slides

- <https://www.xialab.ca> under the “Training” tab within 3 days after lecture

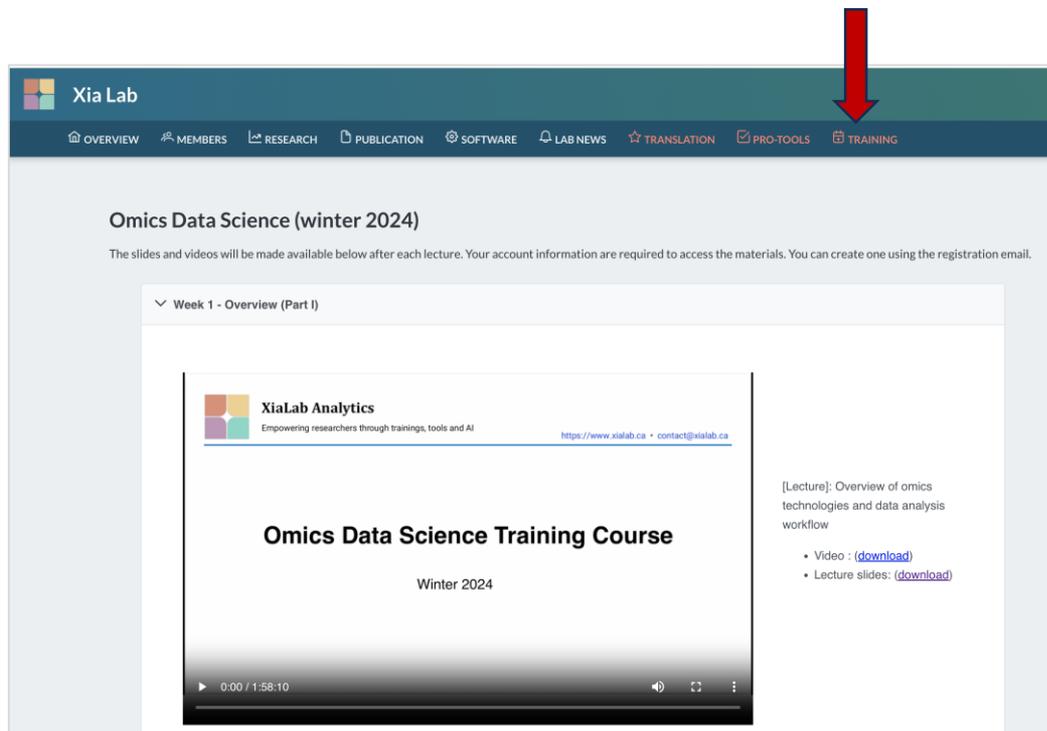
- **Faster with Firefox**

Community tool:

- <https://www.#####.ca>

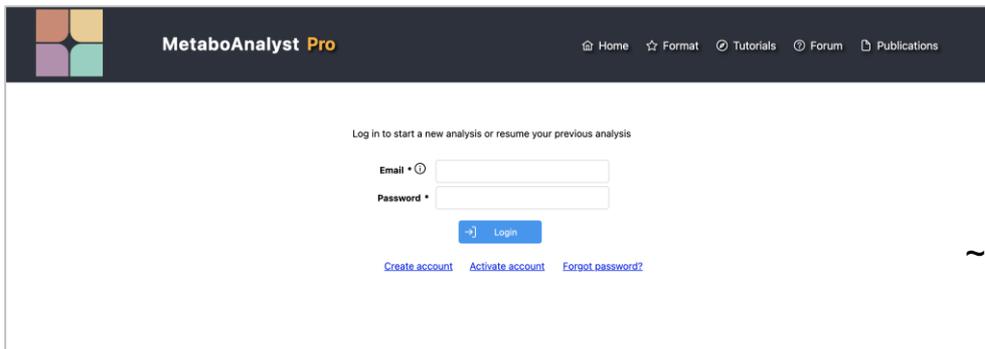
Pro Tools:

- <https://pro.#####.ca>
 - ❖ You will be assigned to one of the cloud nodes



The screenshot shows the Xia Lab website interface. At the top, a dark blue navigation bar contains the Xia Lab logo and several menu items: OVERVIEW, MEMBERS, RESEARCH, PUBLICATION, SOFTWARE, LAB NEWS, TRANSLATION, PRO-TOOLS, and TRAINING. A large red arrow points to the TRAINING tab. Below the navigation bar, the main content area is titled 'Omics Data Science (winter 2024)'. A sub-section is labeled 'Week 1 - Overview (Part I)'. The central focus is a video player showing a slide titled 'Omics Data Science Training Course' for 'Winter 2024'. The slide header includes the XiaLab Analytics logo and the tagline 'Empowering researchers through trainings, tools and AI'. To the right of the video player, there is a list of resources: '[Lecture]: Overview of omics technologies and data analysis workflow' with links for 'Video : (download)' and 'Lecture slides: (download)'. The video player controls at the bottom show a progress bar at 0:00 / 1:58:10.

About the “pro” tools



MetaboAnalyst Pro

Home Format Tutorials Forum Publications

Log in to start a new analysis or resume your previous analysis

Email

Password

Login

[Create account](#) [Activate account](#) [Forgot password?](#)

Community version
~ 5000 users / day
~ 100s concurrent users

A total of six tools have their “pro” versions

- <https://pro.metaboanalyst.ca>
- <https://pro.microbiomeanalyst.ca>
- <https://pro.expressanalyst.ca>
- <https://pro.omicsnet.ca>
- <https://pro.omicsanalyst.ca>
- <https://pro.mirnet.ca>

1. Dedicated computing
2. Live report & project management
3. More stable
4. Prioritized support



Our Syllabus

Topic	Date	Lecture	Lab
Omics Data Science Foundations	Jan. 6	Omics data processing, statistics and visualization	--
	Jan. 13	From raw data to functional insights	--
Transcriptomics	Jan. 20	Gene expression data analysis (part I)	ExpressAnalyst & NetworkAnalyst
	Jan. 27	Gene expression data analysis (part II)	ExpressAnalyst & Seq2Fun
Proteomics, Networks, & Biomarkers 	Feb. 3	Biological network analysis & gene regulatory networks	NetworkAnalyst & miRNet
	Feb. 10	Proteomics & biomarker analysis	ExpressAnalyst & MetaboAnalyst
Metabolomics	Feb. 17	Targeted metabolomics data analysis	MetaboAnalyst
	Feb. 24	LC-MS untargeted metabolomics data analysis	MetaboAnalyst
Microbiomics	Mar. 2	Marker gene data analysis	MicrobiomeAnalyst
	Mar. 9	Shotgun metagenomics data analysis	MicrobiomeAnalyst
Multi-omics	Mar. 16	Knowledge-driven multi-omics integration	OmicsNet
	Mar. 23	Data-driven multi-omics integration	OmicsAnalyst



Schedule for today



Time	Topics
9:00 – 9:10	General introduction & recap
9:10 – 9:40	Proteomics data analysis workflow
9:45 – 10.15	Live demo & hands on
10:20 – 10:50	Biomarker analysis
10:55 – 11:25	Live demo & hands on
Summary and discussion	

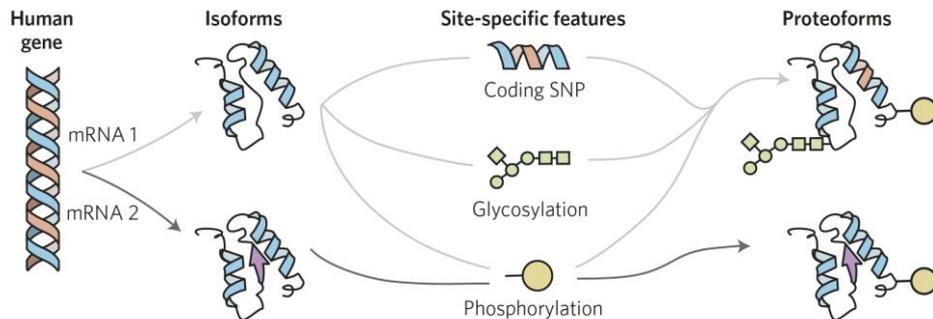


Proteomics & proteoform

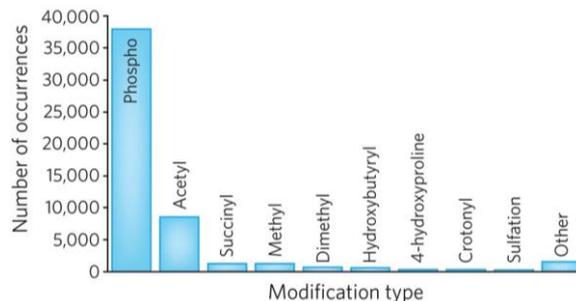
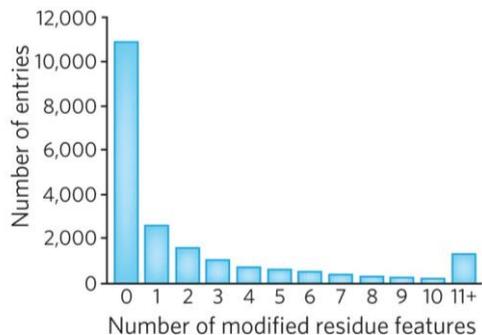
- Proteomics: study the entire set of proteins produced by a cell
 - Include functional, structural and quantitative proteomics
- A **proteoform** is defined by its exact amino acid sequence combined with any post translational modifications.
 - Each “protein” arising from a gene may exist in numerous different proteoforms.
- Technological platform is similar to metabolomics
 - LC-MS based shotgun proteomics
- Statistical & functional analysis are similar to transcriptomics (proteins ↔ genes)



Protein & their proteoforms



It is estimated to have at least ~6 million proteoforms (2016)



<https://www.nature.com/articles/nchembio.2576>

Three types of proteomics research

1. Structural proteomics (protein 3D structures)
2. Functional proteomics (large-scale PPI studies)
 - Yeast two-hybrid screening (Y2H)
 - Affinity purification coupled to mass spectrometry.
3. Quantitative proteomics (abundance & biomarkers)
 - Gel-based proteomics
 - Protein microarrays
 - **MS-based proteomics (shotgun proteomics)**



Structural Proteomics

nature methods

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[nature](#) > [nature methods](#) > [editorials](#) > article

Editorial | [Published: 11 January 2022](#)

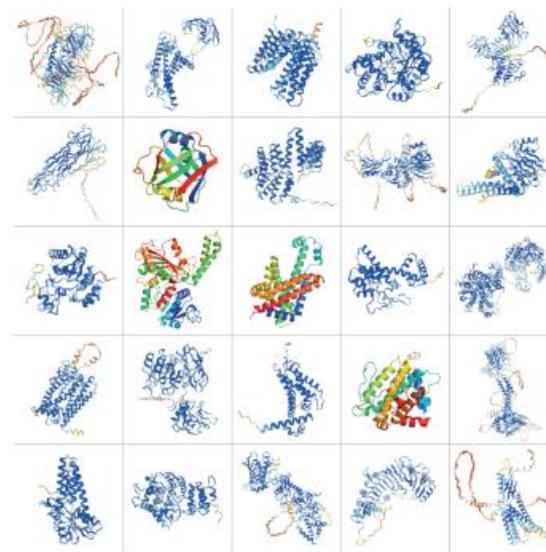
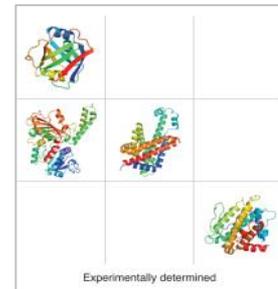
Method of the Year 2021: Protein structure prediction

[Nature Methods](#) 19, 1 (2022) | [Cite this article](#)

27k Accesses | 17 Citations | 428 Altmetric | [Metrics](#)

Deep Learning based approaches for protein structure prediction have sent shock waves through the structural biology community. We anticipate far-reaching and long-lasting impact.

AlphaFold



Expanded coverage with structure prediction

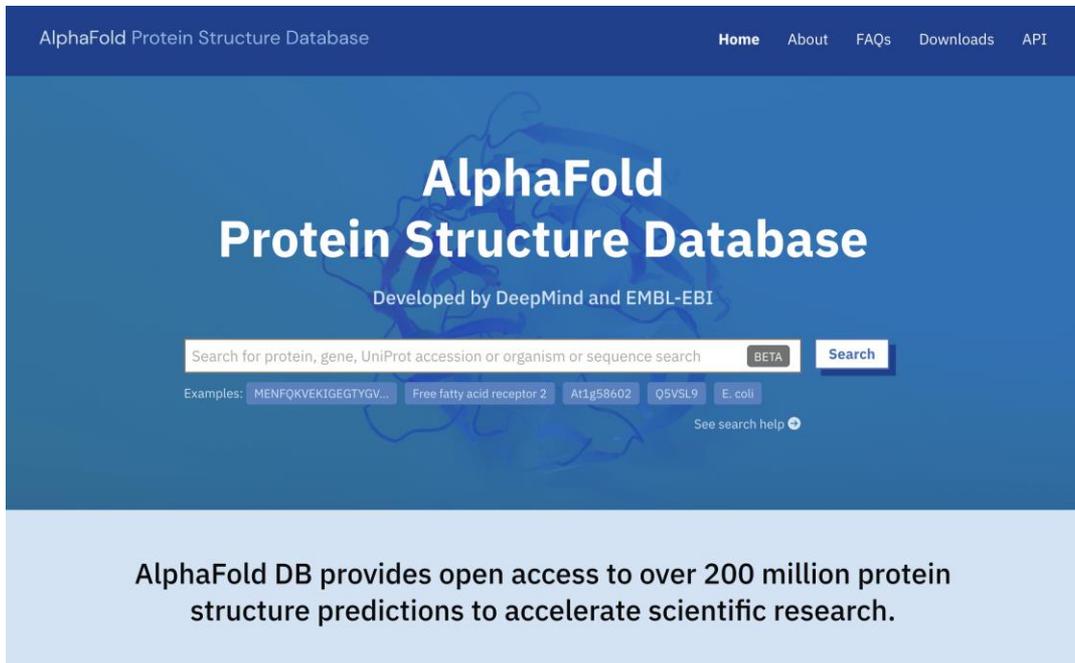


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Structural Proteomics (II) - a “solved” problem?

- AlphaFold was trained on protein chains in the PDB.
- Can make a strong prediction based on a multiple sequence alignment alone.
 - ✓ Templates are not a critical input to make an accurate prediction
 - ✓ Can ignore templates if they appear unhelpful



AlphaFold Protein Structure Database

Home About FAQs Downloads API

AlphaFold Protein Structure Database

Developed by DeepMind and EMBL-EBI

Search for protein, gene, UniProt accession or organism or sequence search BETA Search

Examples: MENFOKVEKIGEGTYGV... Free fatty acid receptor 2 A1g58602 Q5VSL9 E. coli

[See search help](#)

AlphaFold DB provides open access to over 200 million protein structure predictions to accelerate scientific research.

➔ predict the impact of **single mutations** on protein stability



Functional proteomics – PPI

nature

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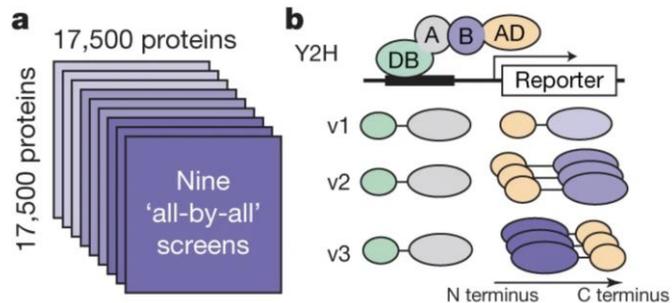
Article | [Published: 08 April 2020](#)

A reference map of the human binary protein interactome

[Katja Luck](#), [Dae-Kyum Kim](#), [Luke Lambourne](#), [Kerstin Spirohn](#), [Bridget E. Begg](#), [Wenting Bian](#), [Ruth Brignall](#), [Tiziana Cafarelli](#), [Francisco J. Campos-Laborie](#), [Benoit Charlotheaux](#), [Dongsic Choi](#), [Atina G. Coté](#), [Meaghan Daley](#), [Steven Deimling](#), [Alice Desbuleux](#), [Amélie Dricot](#), [Marinella Gebbia](#), [Madeleine F. Hardy](#), [Nishka Kishore](#), [Jennifer J. Knapp](#), [István A. Kovács](#), [Irma Lemmens](#), [Miles W. Mee](#), [Joseph C. Mellor](#), ... [Michael A. Calderwood](#) ✉ [+ Show authors](#)

[Nature](#) **580**, 402–408 (2020) | [Cite this article](#)

68k Accesses | **534** Citations | **453** Altmetric | [Metrics](#)



HuRI

The Human Reference Interactome

PROTEINS INTERACTIONS
9094 64006

Literature Benchmark

PROTEINS INTERACTIONS
6047 13441

<http://www.interactome-atlas.org/>

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Imaging-based analysis

1. Load the gel image
2. Lay the grid
3. Annotate the spots
4. Compare with database/archived gel images
5.

The screenshot displays the GelScape web application interface. The main window shows a gel image titled "KIDNEY_HUMAN EXPERIMENT" with a grid overlaid. The y-axis is labeled "MW(KD)" and ranges from 267.78 to 42.22. The x-axis is labeled "pI" and ranges from 4 to 10. A spot is highlighted with a red circle and labeled "P53".

The interface includes a navigation menu with buttons: "Load Gel", "Grid&Axes", "Annotate&View", "Manipulate Gel", "Morph&Compare", "GelBank", and "Log Off".

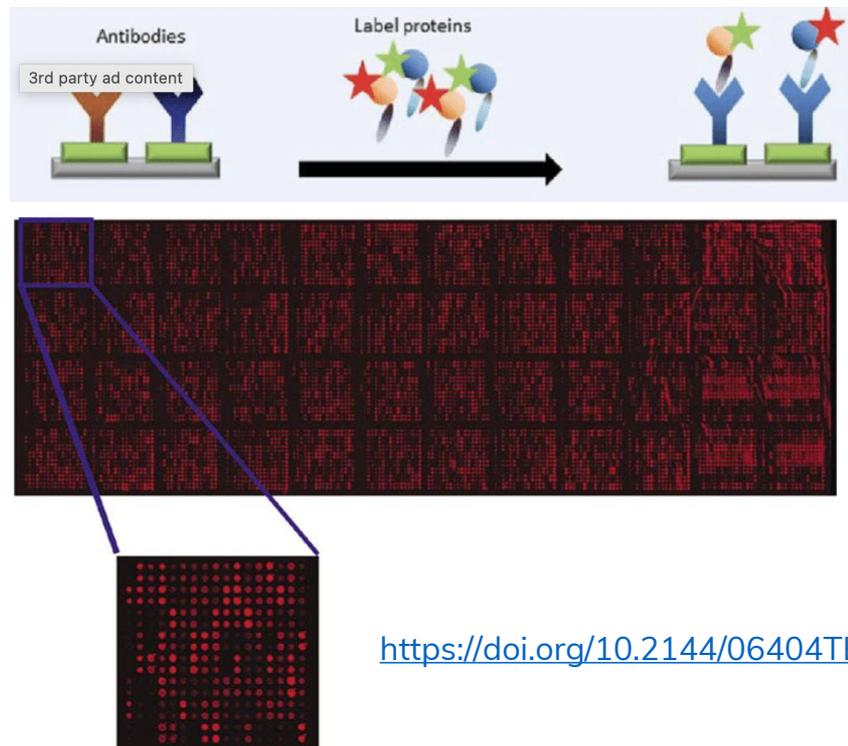
On the right side, there is a "Date of Entry" field set to "18-Oct-2003" and a "Back to Annotate/View" button. Below this is a "Protein Spot Card" window showing the following information:

- Annotation Method: Manual
- Protein Name: Manual
- Spot Label: P53
- Mass Spec. File: [Add]
- SProt/GenBank ID: P04404
- Organism: Sus scrofa (Pig)
- Cellular Location: Neuroendocrine and endocrine se granules
- Protein Sequence: SAALALLLC AGQVIALPVN SPNKGDTLV MKCIVEVISD TLKPSPPMPV SOECFETLRG DERILSILRH QNLLKLODL ALQGAKEKSH QQKQSSYED ELSEVLEKQN DQAELEKQTE EASSKAAEK RGDSEVEKKN DEDADGAKPQ

At the bottom right, there is a "Delete Mark" button and a "Java Applet Window" label.

Protein microarrays (I)

- Immobilize probes on protein chip
 - **Antibodies**
 - Aptamers
 - Affibodies
- Targeted proteins can be detected either by direct labeling or using a reporter antibody in sandwich assay format



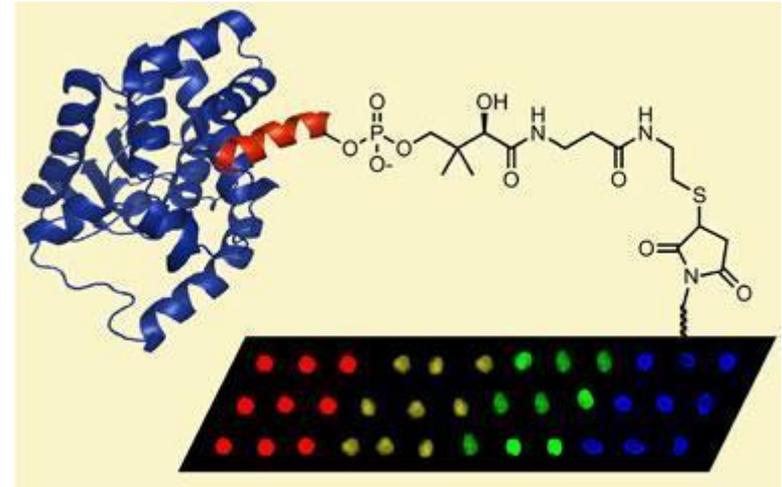
<https://doi.org/10.2144/06404TE01>



Protein microarrays (II) – many challenges

Production of reliable, consistent, high-throughput proteins that are **correctly folded & functional** are very challenging

- Require a lot more steps in its creation than does a DNA chip
- Different surface chemistries
- Requiring long time in storage
- Reducing non-specific binding by the capture agents
- Complete representation of the proteome

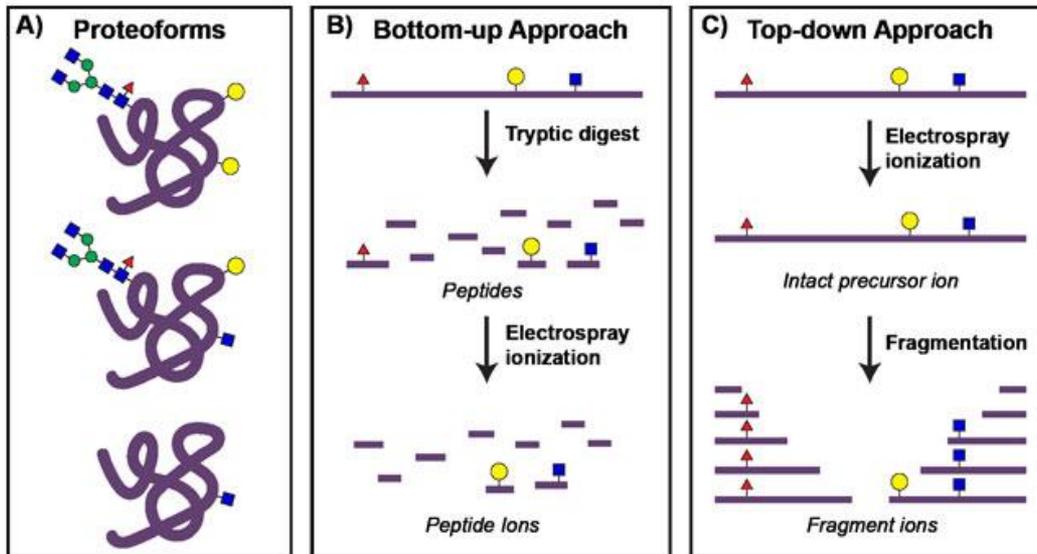


DOI: 10.1021/ja8030278



MS-based quantitative proteomics

- Bottom-up:
 - **Shotgun proteomics**
 - The protein samples are first proteolytically digested into peptides before analyzing in MS
- Top-down:
 - Intact proteins are directly analyzed by MS



<https://www.technologynetworks.com/proteomics/articles/a-fusion-of-proteomic-practices-the-indisputable-complementarity-of-bottom-up-and-top-down-337094>

Latest breakthrough in MS-based proteomics



nature biotechnology



Article

<https://doi.org/10.1038/s41587-023-02099-7>

Ultra-fast label-free quantification and comprehensive proteome coverage with narrow-window data-independent acquisition

Received: 14 June 2023

Accepted: 13 December 2023

Published online: 01 February 2024

Check for updates

Ulises H. Guzman^{1,7}, Ana Martinez-Val^{1,7}, Zilu Ye^{1,2,7}, Eugen Dar
Tabiwang N. Arrey³, Anna Pashkova³, Santosh Renuse⁴, Eduard Den
Johannes Petzoldt³, Amelia C. Peterson³, Florian Harking¹, Ole Øste
Rasmus Rydbirk⁵, Susana Aznar⁶, Hamish Stewart³, Yue Xuan⁶
Daniel Hermanson⁴, Stevan Horning³, Christian Hock³, Alexander M
Vlad Zabrouskov⁴ & Jesper V. Olsen¹✉

Mass spectrometry (MS)-based proteomics aims to characterize comprehensive proteomes in a fast and reproducible manner. Here we present the narrow-window data-independent acquisition (nDIA) strategy consisting of high-resolution MS1 scans with parallel tandem MS (MS/MS) scans of ~200 Hz using 2-Th isolation windows, dissolving the differences between data-dependent and -independent methods. This is achieved by pairing a quadrupole Orbitrap mass spectrometer with the asymmetric track lossless (Astral) analyzer which provides >200-Hz MS/MS scanning speed, high resolving power and sensitivity, and low-ppm mass accuracy. The nDIA strategy enables profiling of >100 full yeast proteomes per day, or 48 human proteomes per day at the depth of ~10,000 human protein groups in half-an-hour or ~7,000 proteins in 5 min, representing 3× higher coverage compared with current state-of-the-art MS. Multi-shot acquisition of offline fractionated samples provides comprehensive coverage of human proteomes in ~3 h. High quantitative precision and accuracy are demonstrated in a three-species proteome mixture, quantifying 14,000+ protein groups in a single half-an-hour run.



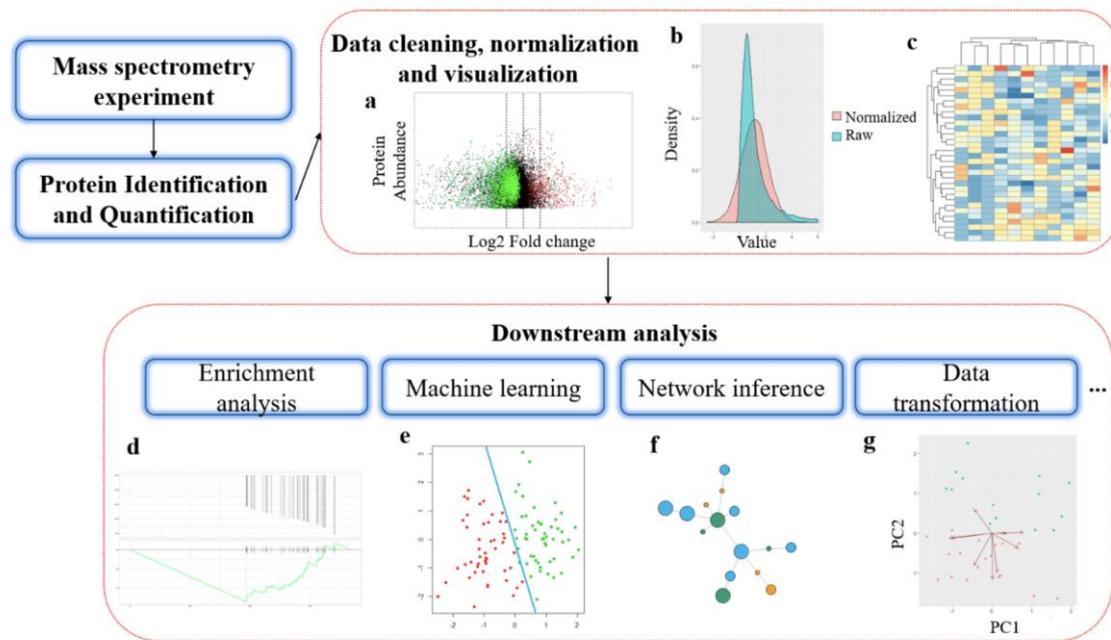
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General bioinformatics workflow

1. Data preprocessing
 - Protein ID & quantification
2. Data processing & normalization
3. Statistical analysis
4. Functional analysis

#1 and #2 are unique to omics technology. #3 and #4 are common



<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7216093/>

Data Pre-processing



MS-based protein identification

- Searching against the fragmentation spectra databases
 - A target database is established from in silico digestion of all expressed or hypothetical protein sequences. Then a peptide spectrum match score is calculated for each fragmentation spectra and all theoretical fragmentation spectra information from the target database.
 - ❖ Mascot, SEQUEST, etc
- de novo peptide sequencing
 - The peptide sequence is determined from fragmentation spectra information and fragmentation method
 - ❖ DeepNovo-DIA

Mascot is the benchmark for identification, characterisation and quantitation of proteins using mass spectrometry data. Learn more about the tools developed by Matrix Science to get the best out of your data, whatever your chosen instrument. Mascot is a great choice for **researchers, core labs, industry** and **educators**.



Mascot Server

Mascot Server is a powerful search engine for identifying proteins and peptides from primary sequence databases using mass spectrometry data. Fast, parallel execution is combined with probabilistic scoring, chemical and post-translational modifications, iTRAQ/TMT quantitation, robust FDR estimation, top-down protein identification, Percolator rescoring, spectral libraries, intact crosslinking, and more.

- > **Overview**
- > **FREE search**
- > **Online documentation**
- > **Training course**
- > **Technical support**
- > **Licensing**
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<https://www.matrixscience.com/>



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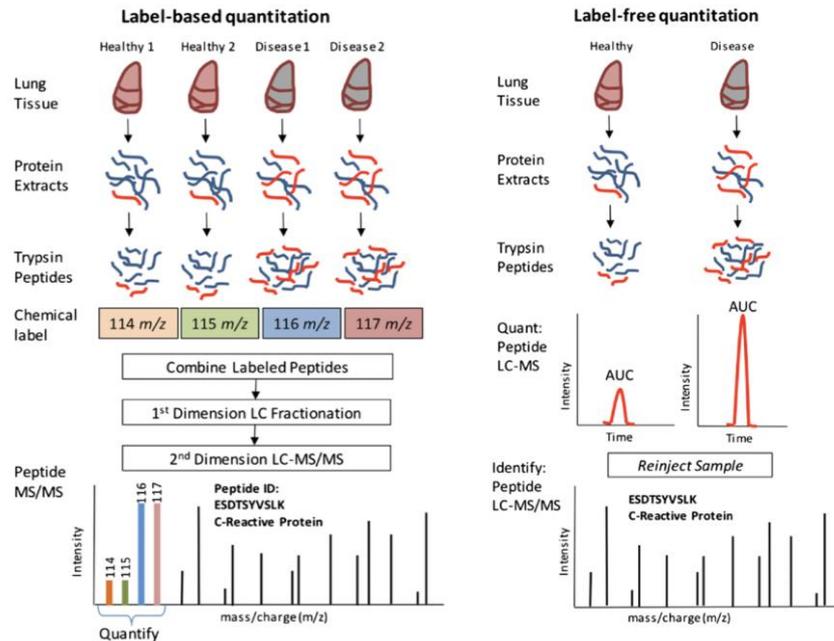
MS-based protein quantification

1. Labeling methods

- Using isobaric stable isotope labeled chemical tags
 - Have identical mass, vary in terms of distribution of heavy isotopes in their structure
 - Distribution of the Isotope patterns allowing the relative quantifications
- Mix samples (multiplexing)

2. Label-free methods

- Different samples are acquired from separate LC-MS/MS experiments
- Quantification based on the peak area (or ion intensity in LC-MS) or spectral mass counting (MS2)



<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4883989/>



MaxQuant for protein quantification

- Supporting both label-based and label-free methods

PROTOCOL UPDATE

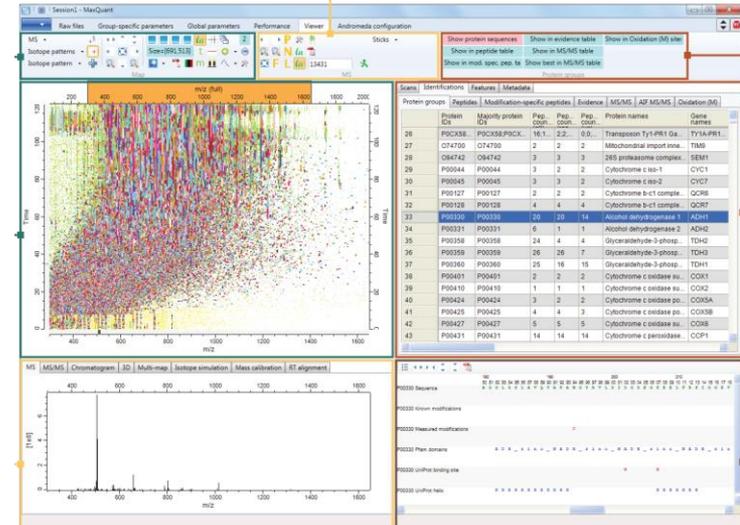
The MaxQuant computational platform for mass spectrometry-based shotgun proteomics

Stefka Tyanova^{1,2}, Tikira Temu^{1,2} & Juergen Cox¹

¹Computational Systems Biochemistry, Max-Planck Institute for Biochemistry, Martinsried, Germany. ²These authors contributed equally to this work. Correspondence should be addressed to J.C. (cox@biochem.mpg.de).

Published online 27 October 2016; doi:10.1038/nprot.2016.136

MaxQuant is one of the most frequently used platforms for mass-spectrometry (MS)-based proteomics data analysis. Since its first release in 2008, it has grown substantially in functionality and can be used in conjunction with more MS platforms. Here we present an updated protocol covering the most important basic computational workflows, including those designed for quantitative label-free proteomics, MS1-level labeling and isobaric labeling techniques. This protocol presents a complete description of the parameters used in MaxQuant, as well as of the configuration options of its integrated search engine, Andromeda. This protocol update describes an adaptation of an existing protocol that substantially modifies the technique. Important concepts of shotgun proteomics and their implementation in MaxQuant are briefly reviewed, including different quantification strategies and the control of false-discovery rates (FDRs), as well as the analysis of post-translational modifications (PTMs). The MaxQuant output tables, which contain information about quantification of proteins and PTMs, are explained in detail. Furthermore, we provide a short version of the workflow that is applicable to data sets with simple and standard experimental designs. The MaxQuant algorithms are efficiently parallelized on multiple processors and scale well from desktop computers to servers with many cores. The software is written in C# and is freely available at <http://www.maxquant.org>.

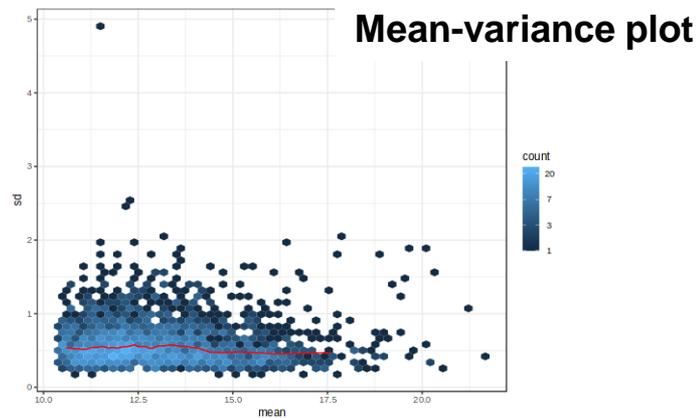
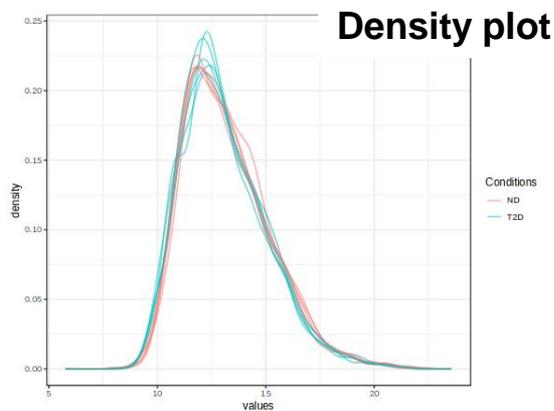
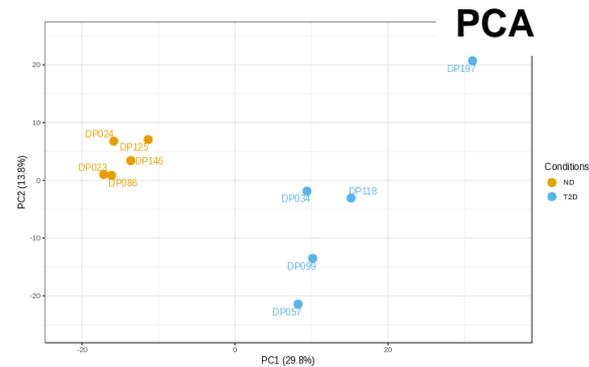
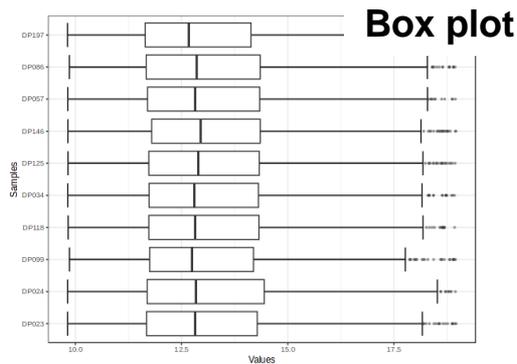


Data Processing & Normalization

**QC => Missing Values => Data Filtering
=> Data Normalization**

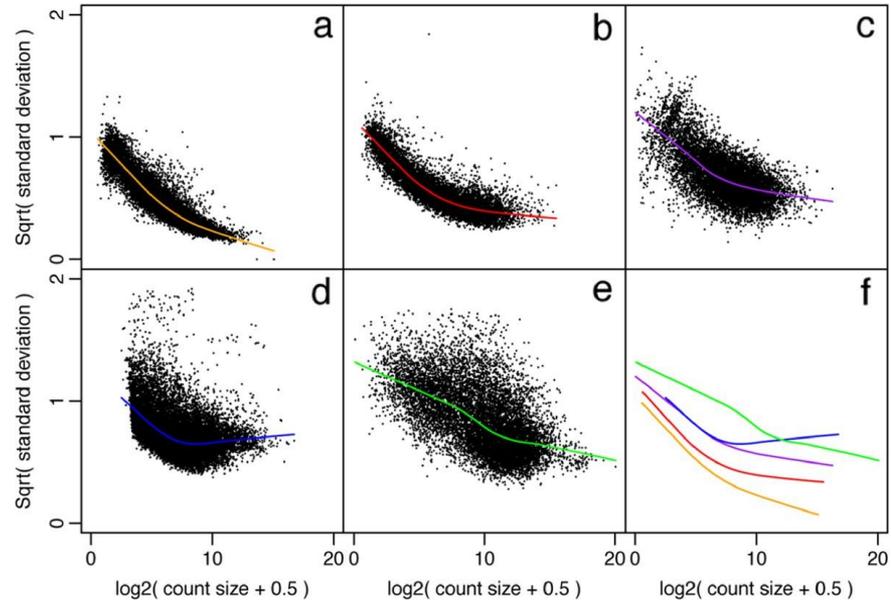


Understanding QC plots in ExpressAnalyst



QC based on mean-variance plot

- Showing a decreasing trend between the means and variances resulting from a combination of technical variation in the sequencing experiment and biological variation amongst replicates.
 - Experiments with high biological variation usually result in flatter trends, where variance values plateau at high expression values.
 - Experiments with low biological variation tend to result in sharp decreasing trends.



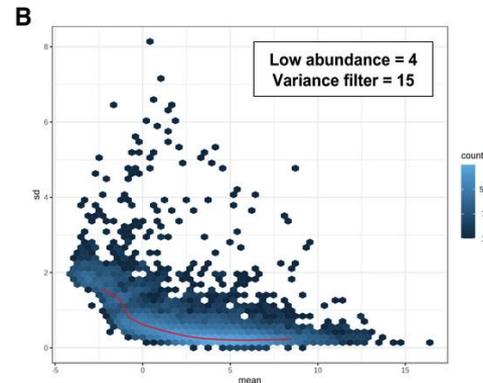
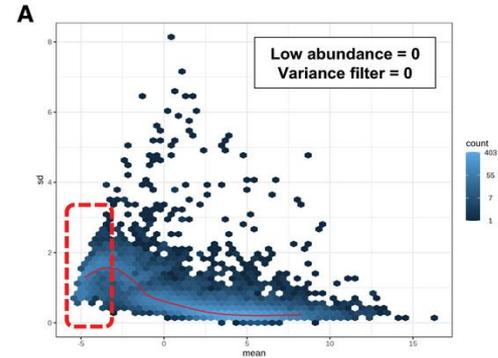
Ordered by increasing levels of biological variation in datasets

<https://pubmed.ncbi.nlm.nih.gov/24485249/>



Mean-variance (MA) plot for filtering

- Visual check on the level of filtering performed upstream.
- If filtering of lowly-expressed genes is insufficient, a drop in variance levels can be observed at the low end of the expression scale due to very small counts.
- If this is observed, return to the earlier filtering step and increase the expression threshold applied to the dataset.



Dealing with missing data

- Missing at random: missingness can be fully accounted for by variables where there is complete information
 - Predictable using other features of the data
- Missing not at random: where there is a systematic explanation
 - Below detection limit in MS-based proteomics or metabolomics.

Step 1. Remove features with too many missing values

Remove features with > % missing values

Step 2. Estimate the remaining missing values

Replace by LoDs (1/5 of the minimum positive value of each variable)

Exclude variables with missing values

Replace by column (feature)

Estimate missing values using

- KNN (feature-wise)
- KNN (sample-wise)
- PPCA
- BPCA
- SVD Impute



Variance stabilization normalization (VSN)

- Originally designed for microarray to overcome the limitations of log transformations by accommodating negative values and minimizing the inflated variance around low signal intensities
- Scale data from different samples into the same level through parametric transformations and maximum likelihood estimation
- Eliminate the dependency between variances and mean abundances

The screenshot shows a configuration panel for VSN normalization. It is divided into two main sections: 'Filtering' and 'Normalization'. Under 'Filtering', there are three settings: 'Filter unannotated features' with a checked checkbox, 'Low abundance' with a slider set to 4, and 'Variance filter' with a slider set to 15. Under 'Normalization', there are seven radio button options: 'None', 'Variance Stabilizing Normalization (VSN)' (which is selected), 'Upper Quantile Normalization', 'Normalization by median', 'Linear regression normalization', 'Local regression normalization', and 'Log2 transform data' (which has an unchecked checkbox).

Filtering:

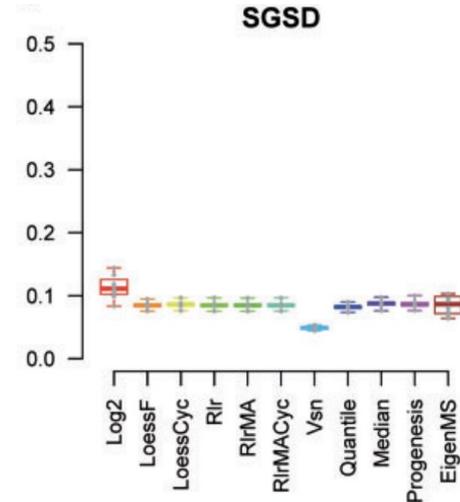
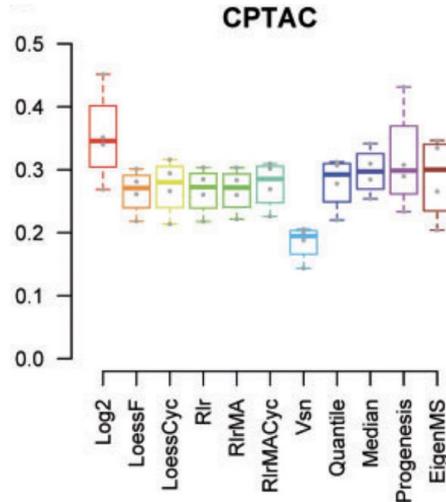
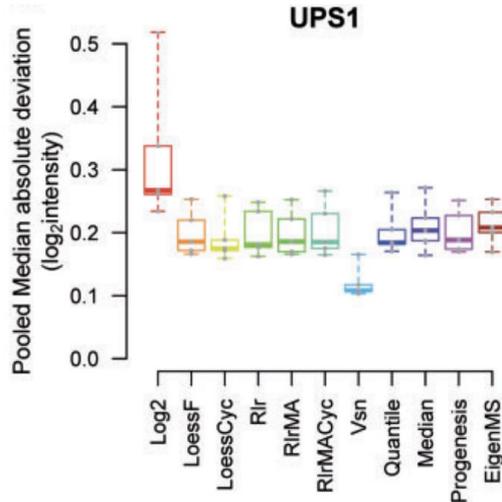
- Filter unannotated features:
- Low abundance:
- Variance filter:

Normalization:

- None
- Variance Stabilizing Normalization (VSN)
- Upper Quantile Normalization
- Normalization by median
- Linear regression normalization
- Local regression normalization
- Log2 transform data

MS-based proteomics data normalization

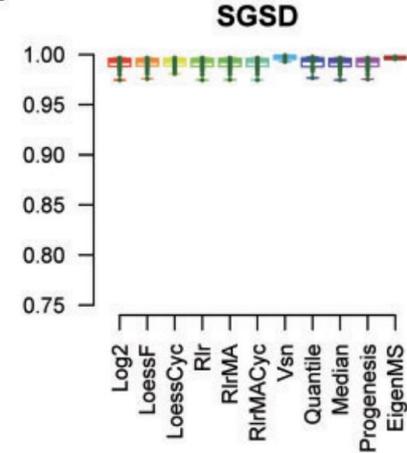
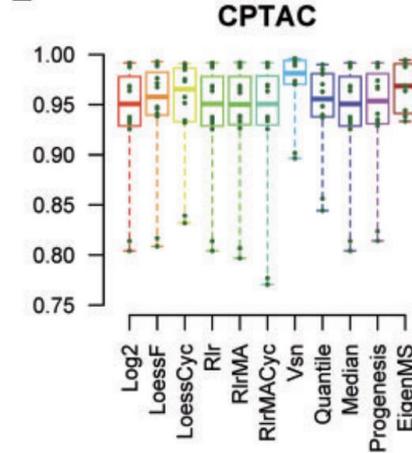
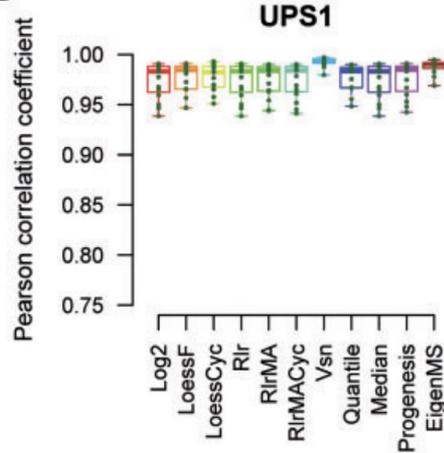
Variance among house keeping genes
(smaller => better)



<https://pubmed.ncbi.nlm.nih.gov/27694351/>

MS-based proteomics data normalization

Pearson correlation coefficients among house keeping genes (higher => better)



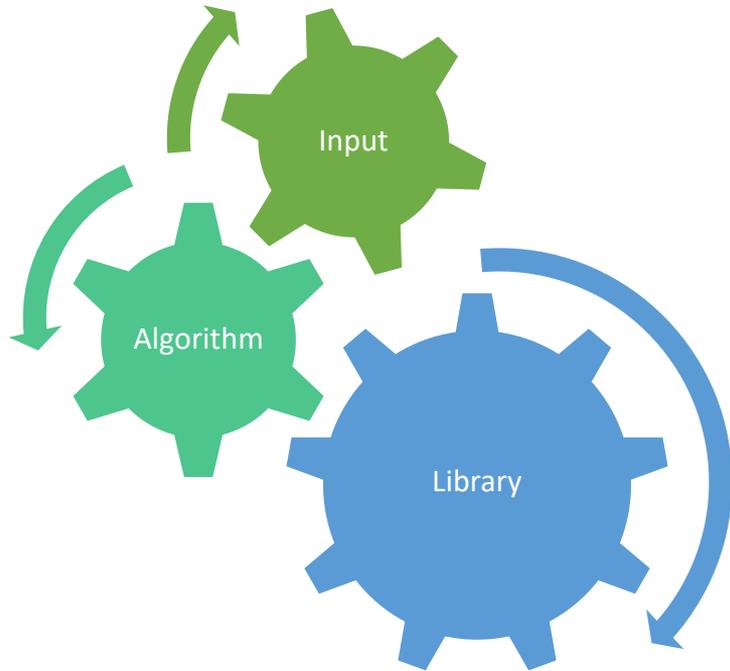
<https://pubmed.ncbi.nlm.nih.gov/27694351/>



Enrichment analysis



Three components in enrichment analysis



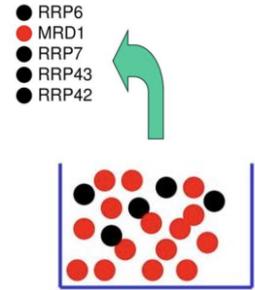
1. Input: define the signals of interest
 - **Significant features**
 - Complete ranked list
 - A data table
2. Define functions libraries:
 - Pathways, gene sets
3. Perform enrichment tests evaluate the coordinated changes of a group.
 - Over-representation analysis (ORA)
 - Permutation based approaches

Enrichment Tests

We need to calculate the null distribution - how often we can see this by random chance?

- Model driven
 - Hypergeometric distribution
- Data driven
 - Don't know - we can use permutation to get this

$$f_X(k|N, K, n) = \frac{\binom{K}{k} \binom{N-K}{n-k}}{\binom{N}{n}}$$



	Sampled	Not Sampled	Total
success	k	K-k	K
non-success	n-k	(N-K)-(n-k)	N-K
Total	n	N-n	N

Over-representation analysis (ORA)

Potential sources of bias with ORA

ORA assumption: all genes/proteins can be measured with equal reliability or opportunity. However, the realities are:

1. Technological bias
 - Platform (MS or microarray) capture a limited subset of “gene/protein universe”
2. Biological bias
 - A given cell type or tissue have specialized proteome / transcriptome
3. Sampling bias
 - Could be introduced during sample prep (selectively enrich some groups)



Addressing bias (I) – providing background

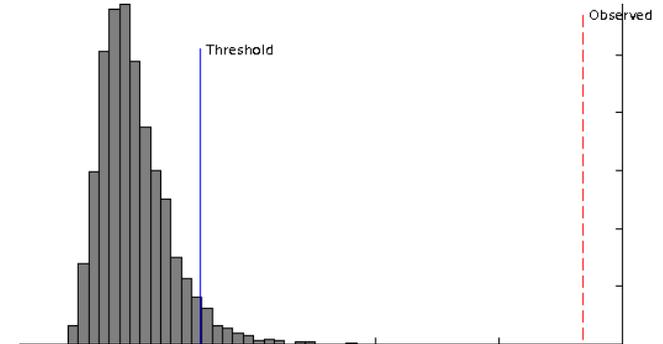
- Better define the “background universe” reflecting the technical and biological context of the current study
 - This information is provided in data table upload
 - i.e. all genes / proteins measured are already present in the raw abundance table)
 - For gene/protein list upload, you can optionally upload the complete list



Addressing bias (II) – using permutations

To estimate null distribution from the current data

- Transcriptomics / proteomics
 - GSEA
- miRNA-omics
 - Unbiased sampling (introduced in the previous lecture)
- Metabolomics
 - Mummichog
 - will be introduced in the next session



Learning more on computational proteomics

❖ Computational Mass Spectrometry @ Pacific Northwest National Laboratory

Welcome!

- 1 Isobaric Quantification: Proteomics
 - 1.1 Prepare MS/MS Identifications
 - 1.2 Prepare Reporter Ion Intensities
 - 1.3 Create Study Design Tables
 - 1.4 Create Quantitative Cross-tab
 - 1.5 Create MSnSet
- 2 Isobaric Quantification: Phosphoprot...
 - 2.1 Prepare MS/MS Identifications
 - 2.2 Prepare Reporter Ion Intensities
 - 2.3 Create Study Design Tables
 - 2.4 Create Quantitative Cross-tab
 - 2.5 Create MSnSet
- 3 Spectral Counting
- 4 Feature ID Conversion
 - 4.1 Conversion with biomaRt
 - 4.2 Conversion with AnnotationHub
 - 4.3 Conversion Using FASTA Headers
- 5 Exploratory Data Analysis

Proteomics Data Analysis in R/Bioconductor

Tyler Sagendorf
May 27, 2022

Welcome!

This tutorial is very much a work-in progress. Even sections that appear finished are likely to be changed. I will update this when significant progress is made. Thank you for your patience.

It is highly recommended to review the resources below before continuing with the rest of the tutorial.

- Proteomics Overview
 - Protein Analysis by Shotgun/Bottom-up Proteomics
 - Modern Proteomics – Sample Preparation, Analysis and Practical Applications
 - Liquid Chromatography Mass Spectrometry-Based Proteomics: Biological and Technological Aspects
- Mass Spectrometry
 - Warwick School of Life Sciences Teaching Animations
 - Tandem Mass Spectrometry for Peptide and Protein Sequence Analysis

<https://pnnl-comp-mass-spec.github.io/proteomics-data-analysis-tutorial/index.html>



Live Demo & Hands On



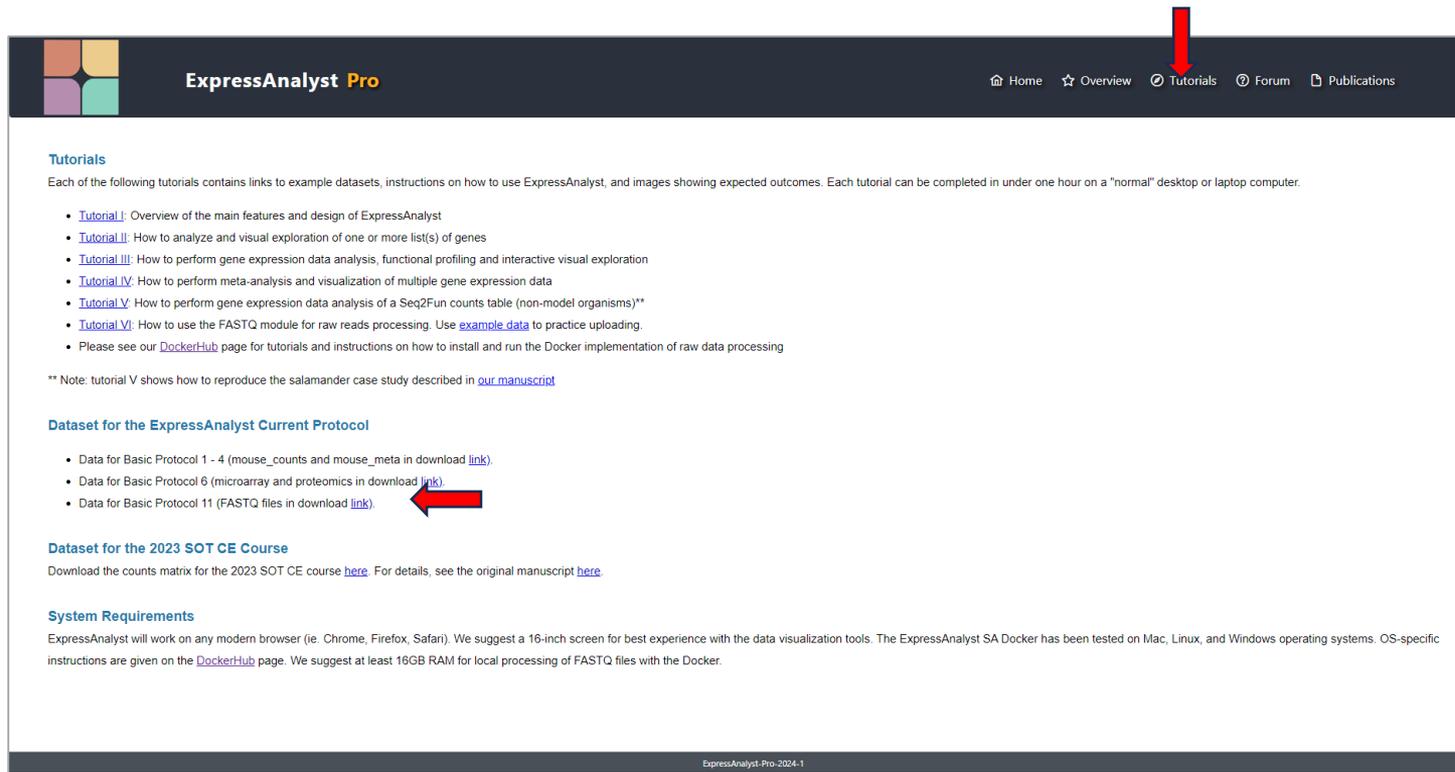
Proteomics vs RNA-seq data

- Proteomics data:
 - LC-MS
 - Intensity based, has decimal values.
 - Lower coverage (a subset of whole proteome)
 - Often have missing values

- RNA-seq data
 - High-throughput sequencing
 - Count based, integer
 - In some cases, can be decimal in the case of fractional counts (depending of the algorithm used)
 - Capture most genes
 - Do not have missing values



Example dataset



ExpressAnalyst Pro

Home Overview **Tutorials** Forum Publications

Tutorials

Each of the following tutorials contains links to example datasets, instructions on how to use ExpressAnalyst, and images showing expected outcomes. Each tutorial can be completed in under one hour on a "normal" desktop or laptop computer.

- [Tutorial I](#): Overview of the main features and design of ExpressAnalyst
- [Tutorial II](#): How to analyze and visual exploration of one or more list(s) of genes
- [Tutorial III](#): How to perform gene expression data analysis, functional profiling and interactive visual exploration
- [Tutorial IV](#): How to perform meta-analysis and visualization of multiple gene expression data
- [Tutorial V](#): How to perform gene expression data analysis of a Seq2Fun counts table (non-model organisms)**
- [Tutorial VI](#): How to use the FASTQ module for raw reads processing. Use [example data](#) to practice uploading.
- Please see our [DockerHub](#) page for tutorials and instructions on how to install and run the Docker implementation of raw data processing

** Note: tutorial V shows how to reproduce the salamander case study described in [our manuscript](#)

Dataset for the ExpressAnalyst Current Protocol

- Data for Basic Protocol 1 - 4 (mouse_counts and mouse_meta in [download link](#)).
- Data for Basic Protocol 6 (microarray and proteomics in [download link](#)).
- Data for Basic Protocol 11 (FASTQ files in [download link](#)).

Dataset for the 2023 SOT CE Course

Download the counts matrix for the 2023 SOT CE course [here](#). For details, see the original manuscript [here](#).

System Requirements

ExpressAnalyst will work on any modern browser (ie. Chrome, Firefox, Safari). We suggest a 16-inch screen for best experience with the data visualization tools. The ExpressAnalyst SA Docker has been tested on Mac, Linux, and Windows operating systems. OS-specific instructions are given on the [DockerHub](#) page. We suggest at least 16GB RAM for local processing of FASTQ files with the Docker.

ExpressAnalyst.Pro-2024-1

Example dataset

#NAME	D034	D057	D086	D099	D118	D125	D146	D197	D023	D024
#CLASS: Diagnosis	T2D	T2D	ND	T2D	T2D	ND	ND	T2D	ND	ND
CRYAB	12.08658823	12.64878448	12.28710506	NA	13.29318423	12.50848808	13.41511448	13.54087129	NA	11.80891996
HDLBP	16.00951903	15.85587266	16.31787505	15.38619931	15.61725193	15.78135854	15.8326181	15.08939838	16.26661951	15.76832481
EHD1	11.82545124	13.4000789	12.83612759	13.29662656	12.53035756	12.64786312	13.16280041	12.98266615	13.14742926	12.90561264
SERPINA1	NA	NA	14.35252462	NA	13.36863623	13.27338767	NA	NA	14.01216748	12.25590661

Proteomics data often have missing data (LC-MS) .
In RNA-seq, they would be simply 0 count.



Missing value imputation

Home > Upload > Integrity Check

Data integrity check

Omics data overview Metadata overview **Missing value estimation**

Missing values in proteomics data will cause difficulties for downstream analysis. There are several different methods for this purpose. The default method replaces all the missing values with a small values (the half of the minimum positive values in the original data) assuming to be the detection limit. The assumption of this approach is that most missing values are caused by low abundance metabolites (i.e. below the detection limit).

ExpressAnalyst also offers other methods, such as replace by mean/median, k-nearest neighbours based on similar features - KNN (feature-wise), k-nearest neighbours based on similar samples - KNN (sample-wise), probabilistic PCA (PPCA), Bayesian PCA (BPCA) method, singular value decomposition (SVD) method to impute the missing values (ref). Note for KNN, k is set to 10 (the default value). Please choose the one that is the most appropriate for your data.

Total missing value detected: 0

Step 1. Remove features with too many missing values

Remove features with > % missing values

Step 2. Estimate the remaining missing values

Replace by LoDs (1/5 of the minimum positive value of each variable)

Exclude variables with missing values

Replace by column (feature)

Estimate missing values using



Missing value imputation: assumptions

- Missing due to the abundance level is lower than detection limit:
 - Replace by 1/5 of the lowest value for that variable --> default option in ExpressAnalyst
- Missing due to technical or human error:
 - Replace by mean/median



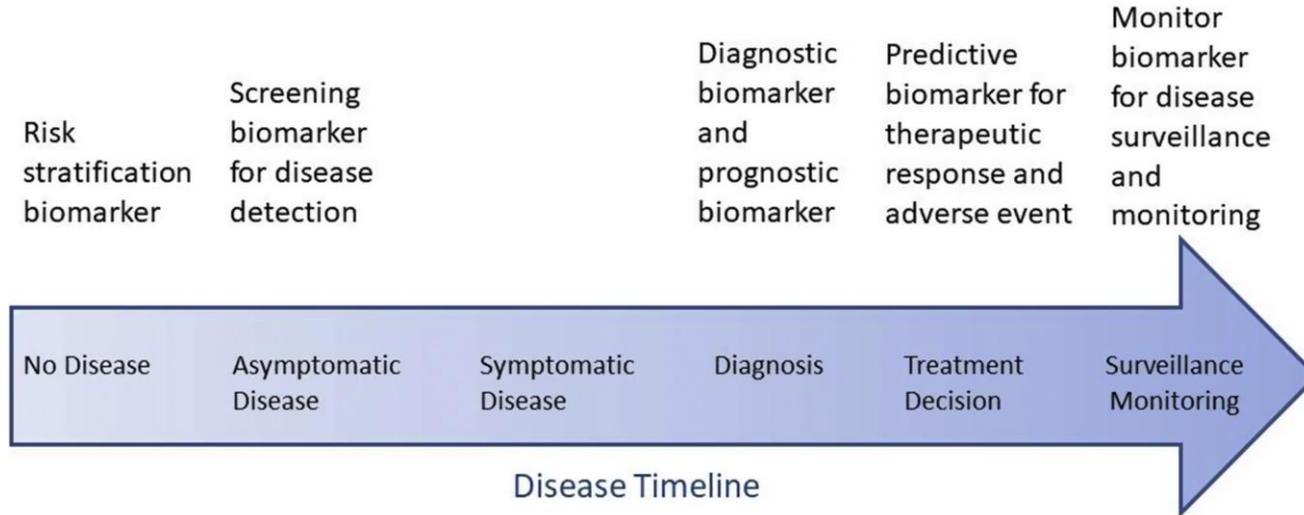
Schedule for today

Time	Topics
9:00 – 9:10	General introduction & recap
9:10 – 9:40	Proteomics data analysis workflow
9:45 – 10.15	ExpressAnalyst live demo & hands on
10:20 – 10:50	Biomarker analysis
10:55 – 11:25	MetaboAnalyst live demo & hands on
Summary and discussion	



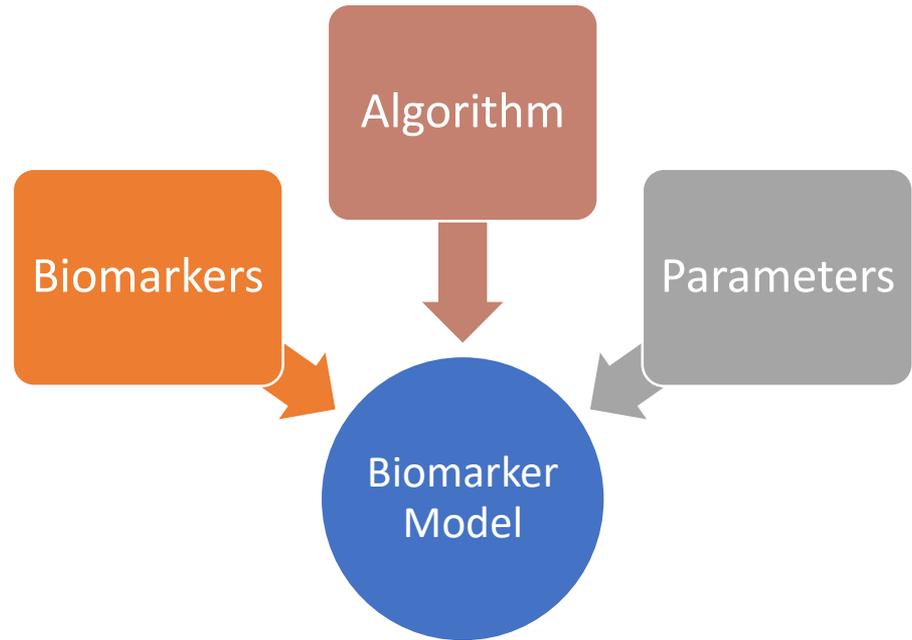
What are Biomarkers

A characteristic that is **objectively measured** and **evaluated** as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic indication.



Key components in biomarker analysis

- The main activities in biomarker analysis involve selecting biomarkers and tuning parameters based on a few well-established classification algorithms.
- Evaluating performance of biomarker models is based on their capacities to classify new samples using cross validation (CV).
- Permutation tests are often used to evaluate whether a classifier has learned anything better than random guessing (null model).



Overview of biomarker analysis

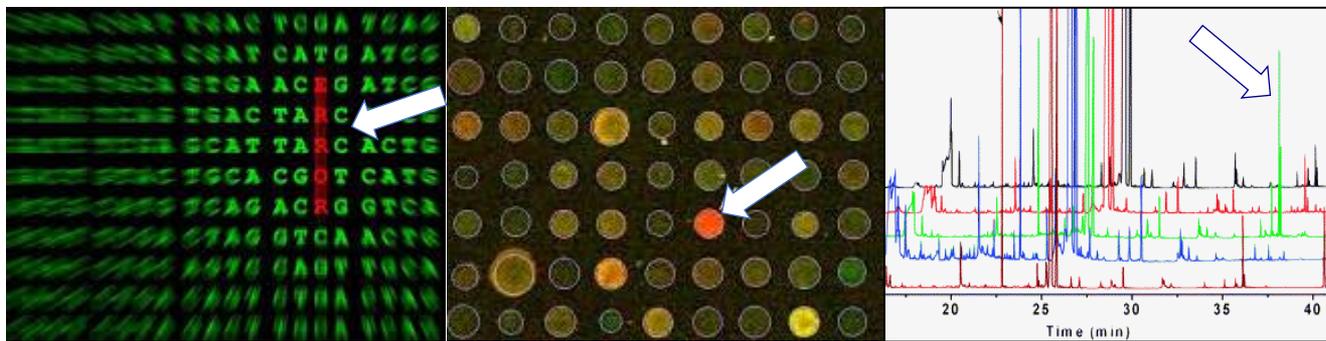
1. Biomarker selection
 - ✓ Identification of an optimal subset of features that will provide the maximal discriminating power between the diseased and healthy samples
2. Performance evaluation
 - ✓ Assessment & validation of the panel of biomarkers
3. Model creation
 - ✓ Developing a fixed mathematical equation or computer algorithm, which combines the panel of selected biomarkers into a single test score with the aim of accurately predicting a particular clinical outcome,



Measuring biomarkers

We look for biomarkers that

- Can be measured earlier
- Can be measured more easily or frequently
- Can be measured with higher precision, or less subjective



Genetic
Variation

Gene
Expression

Metabolite
Concentration



Desirable properties of biomarkers

- Objectively measurable
 - Can be reliably measured
 - By different platforms or technologies
 - Clear identifications
 - Absolute quantification
 - Interpretable?
- High sensitivity, high specificity

Finger-prick blood test for Alzheimer disease



Convenient & accurate

Evaluating Performances

Balanced data (control group and disease group ~same size)

➤ **Accuracy**

- 9/13 correct => 69% accuracy

➤ **Error rate**

- $1 - \text{accuracy} = 31\%$

Not suitable for imbalanced data

- In a population, cancer incidence is low: ~5 cases in 1000 people. If a classifier predict all people to be healthy, then it is 99.5% accurate (majority vote)
 - Need to develop other measurements



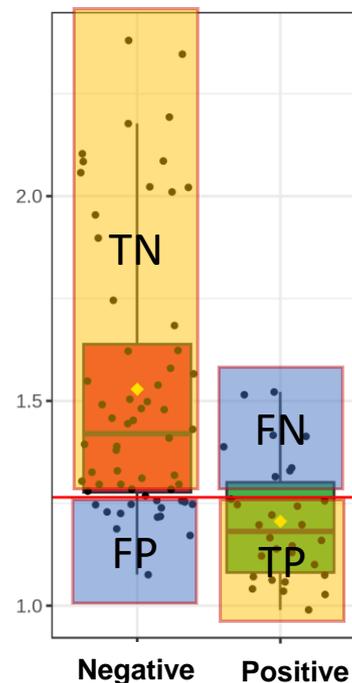
Evaluating Biomarker Performance

- Performance evaluation in clinical diagnosis (usually very unbalanced i.e., few case, most normal)
- True positives (TP)
 - True negatives (TN)
 - False positives (FP)
 - False negatives (FN)

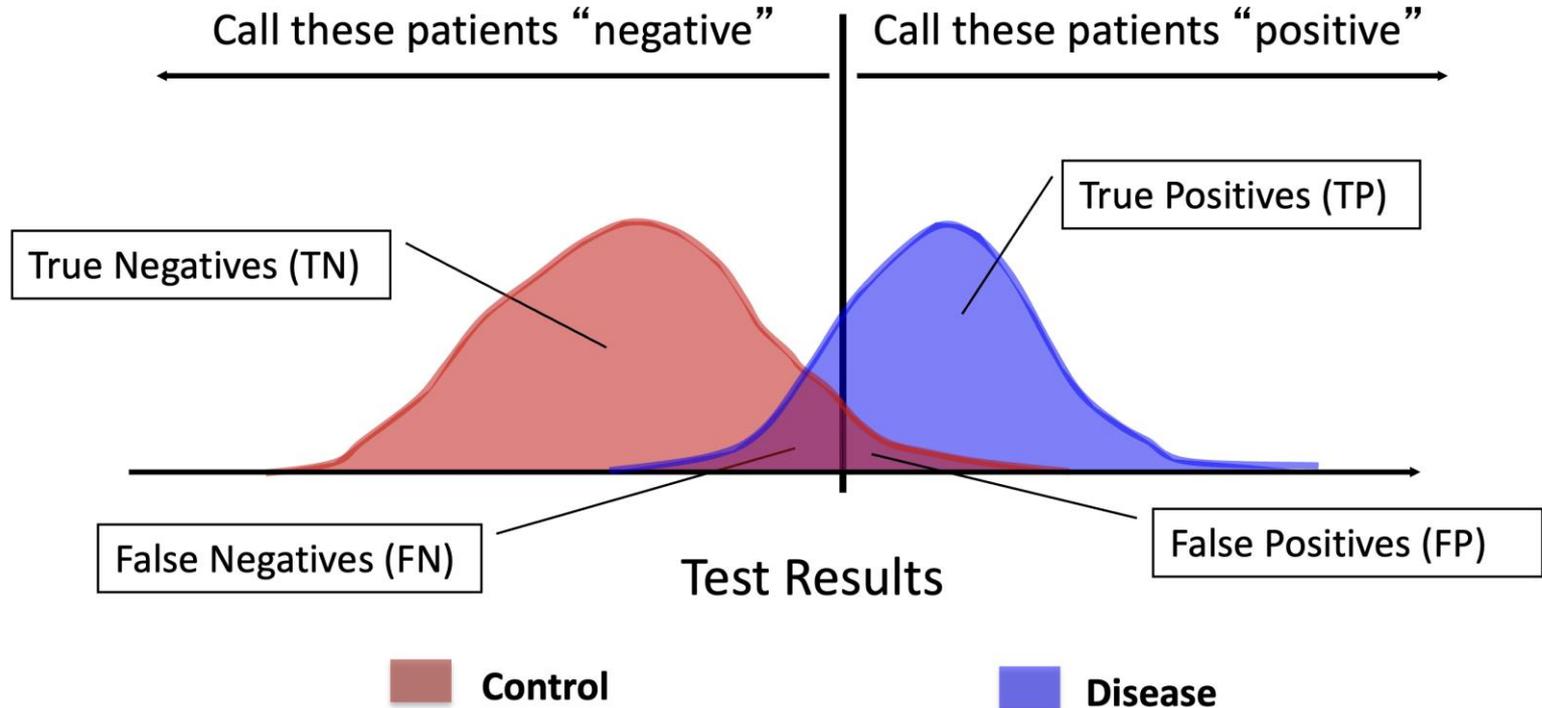
	p' (Predicted)	n' (Predicted)
p (Actual)	True Positive	False Negative
n (Actual)	False Positive	True Negative

Evaluating Biomarkers - Sensitivity & Specificity

- Sensitivity: true positive rate
 - $Sn = TP / (TP + FN)$
 - The probability of a positive test result given that a subject has an actual positive outcome
- Specificity: true negative rate
 - $Sp = TN / (TN + FP)$
 - The probability of a negative test result given that a subject has an actual negative outcome

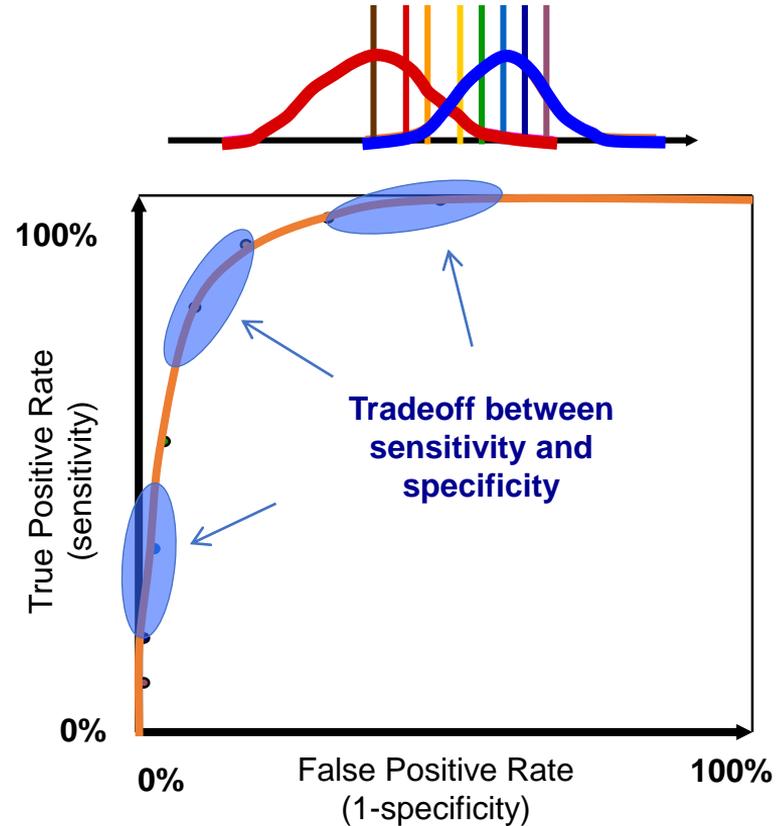


Another illustration



Receiver Operating Characteristic (ROC) curves

- A historic name from radar studies
- Very popular in biomedical applications
 - To assess biomarker performance.
 - To compare different biomarker models
- A graphical plot of the true positive rate (TPR) vs. false positive rate (FPR), for a binary classifier (i.e. positive/negative) as its cutoff point is varied



Sensitivity, Specificity & ROC curve

Two important performance measures in a diagnostic tests

- Sensitivity (true positive rate)
- Specificity (true negative rate)

Cutoff dependent

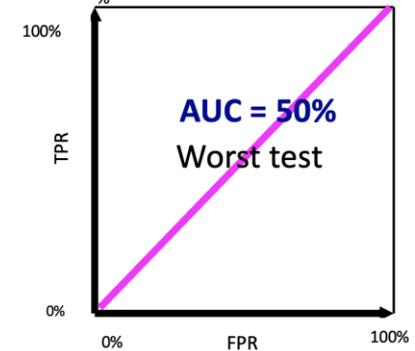
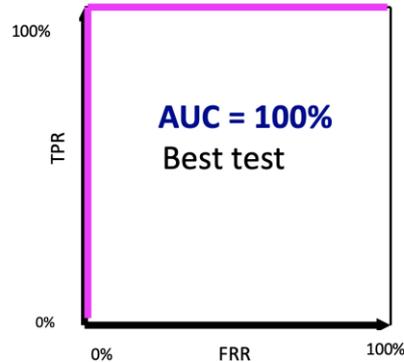
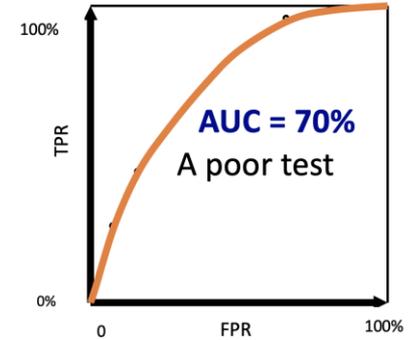
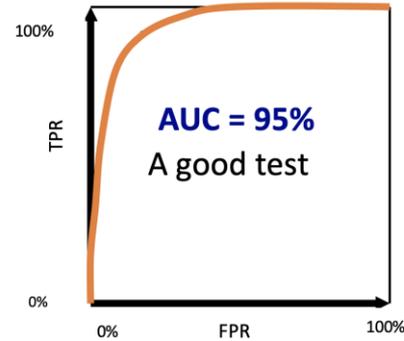
- Increase cutoff, will improve specificity, decrease sensitivity

ROC curves integrate these two measures



Area under an ROC curve (AUC)

- Overall measure of a test performance
- AUC is the probability that a classifier will rank a randomly chosen positive case higher than a randomly chosen negative one
- Comparisons between two tests based on differences between (estimated) AUCs



From Biomarkers to ROC curves

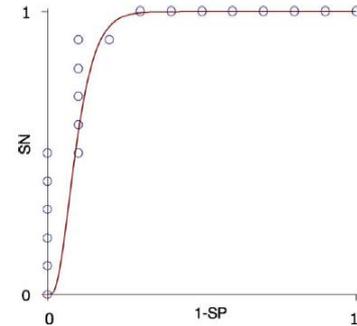


How to construct ROC curves

Input: a score on a univariate scale

- A test gives continuous value (i.e. blood *Glucose* level)

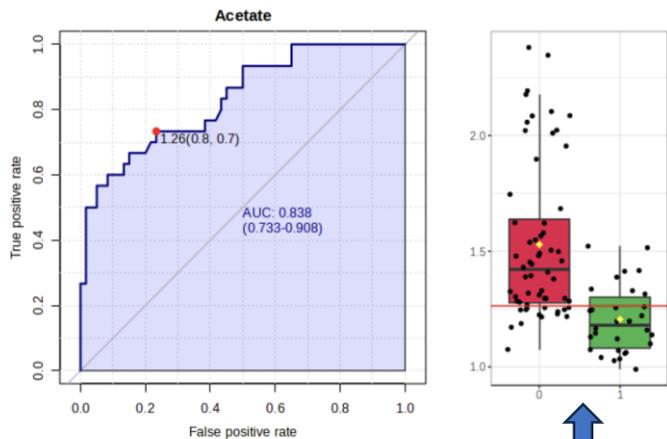
2-h plasma glucose (mmol/L)		Threshold-dependent values*					
Healthy	Diseased	TP	TN	FP	FN	1 - SP	SN
		10	0	10	0	1.00	1.00
4.86		10	1	9	0	0.90	1.00
5.69		10	2	8	0	0.80	1.00
6.01		10	3	7	0	0.70	1.00
6.06		10	4	6	0	0.60	1.00
6.27		10	5	5	0	0.50	1.00
6.37		10	6	4	0	0.40	1.00
6.55		10	7	3	0	0.30	1.00
7.29	7.29	9	8	2	1	0.20	0.90
7.82		9	9	1	1	0.10	0.90
	9.22	8	9	1	2	0.10	0.80
	9.79	7	9	1	3	0.10	0.70
	11.28	6	9	1	4	0.10	0.60
	11.83	5	9	1	5	0.10	0.50
12.06		5	10	0	5	0.00	0.50
	18.48	4	10	0	6	0.00	0.40
	18.50	3	10	0	7	0.00	0.30
	20.49	2	10	0	8	0.00	0.20
	22.66	1	10	0	9	0.00	0.10
	26.01	0	10	0	10	0.00	0.00



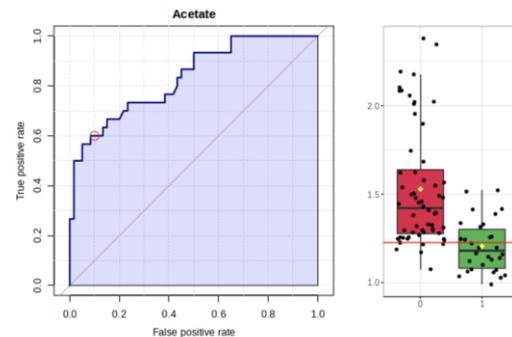
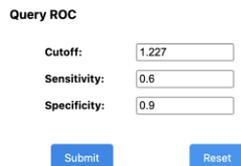
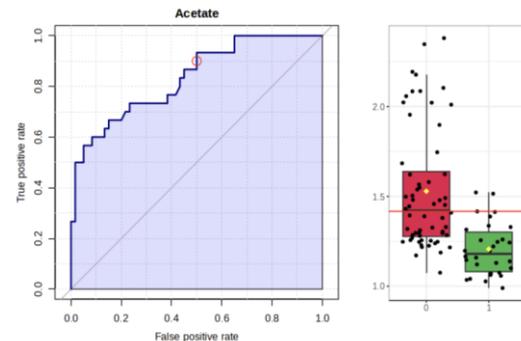
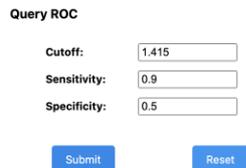
TA Lasko, et al (2005)

Straightforward for a single biomarker

Abundance values can be used directly to predict Positive/Negative

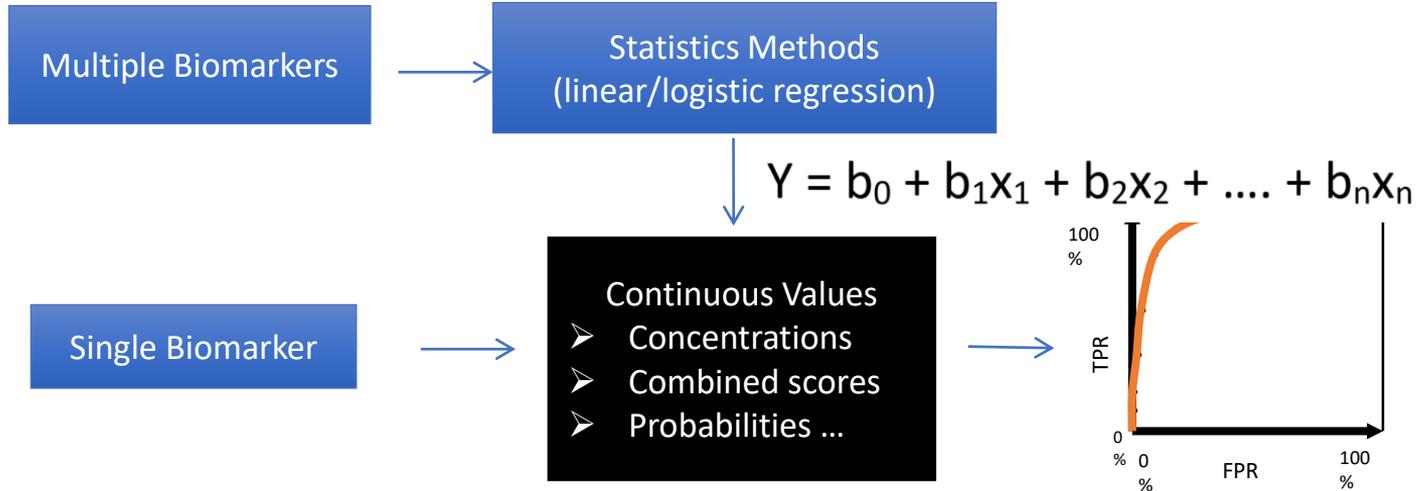


Move the cut-off
(red line)



How to deal with multiple biomarkers?

- Input: a score on a univariate scale
 - A classifier that produces a continuous score (i.e. likelihood, probabilities)



The challenges of Omics Data

Omics data is usually of small sample size with large number of variables
($n \ll p$)

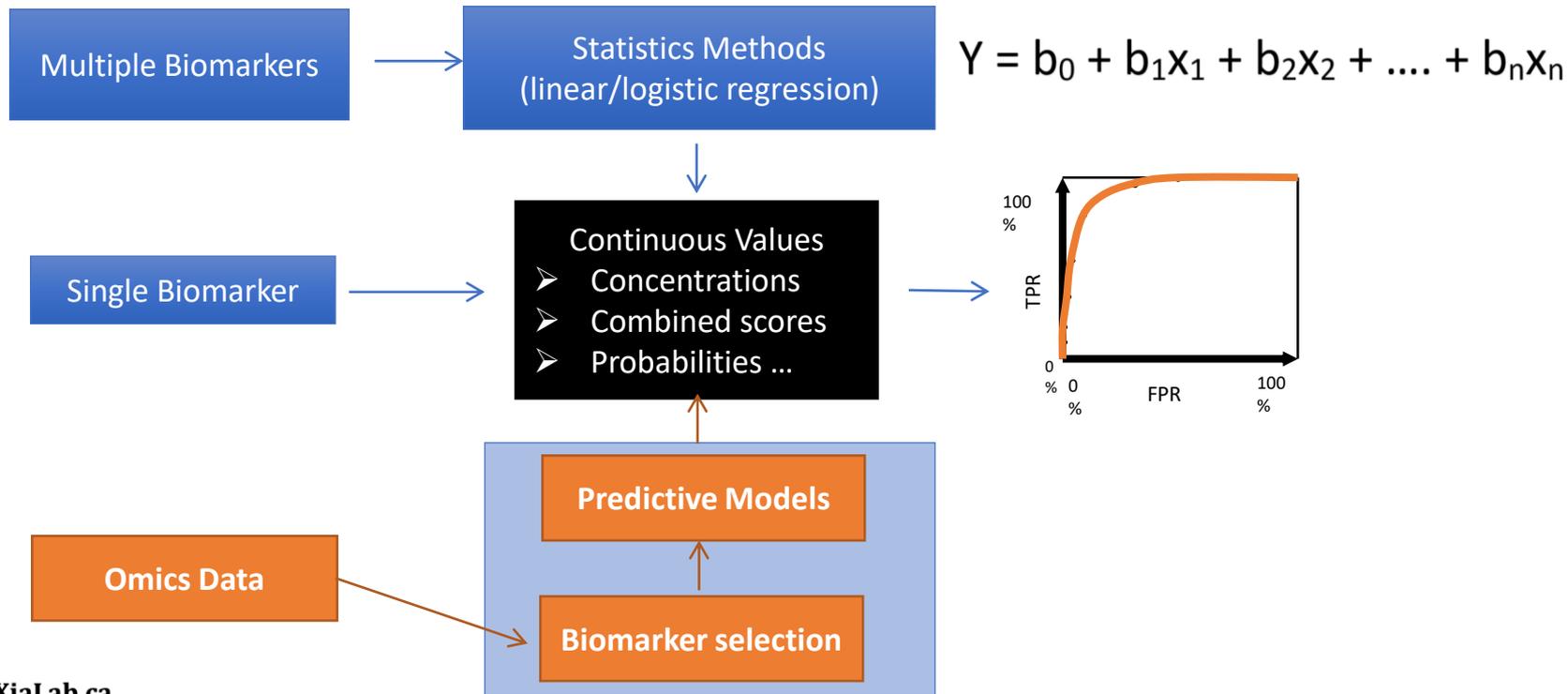
- SNPs (~ 1,000,000s)
- Gene expressions (~10,000s)
- Compound concentrations (~ 1000s)
- ➔ Linear/logistic regression will not work ($n > p$)

Need new strategies

- Computationally efficient;
- Not susceptible to over-fitting;
- ➔ Advanced machine learning approaches

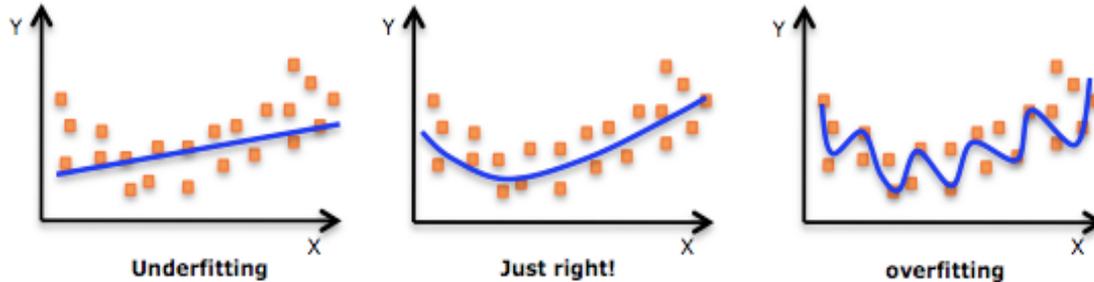


Dealing with omics data



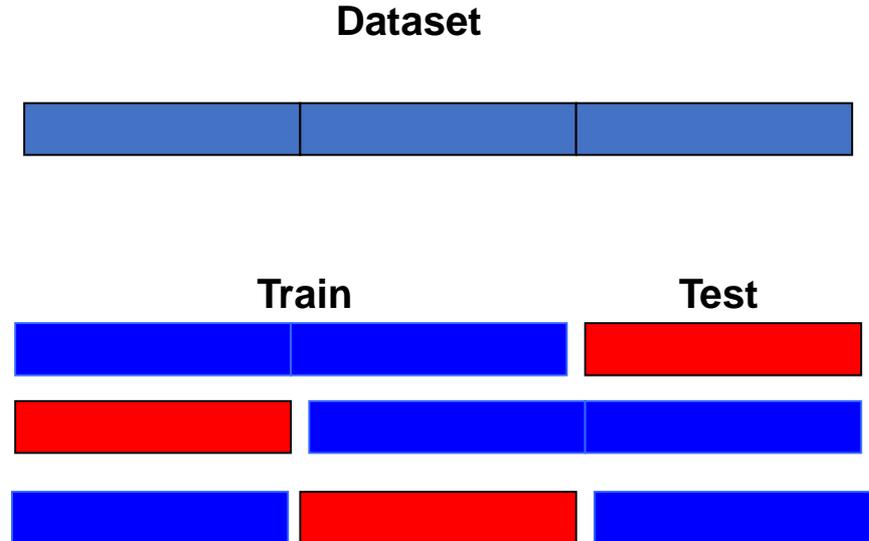
Overfitting issue

- Fitted model performs well for the current data
- Fitted model is not good for prediction of new data – prediction error is underestimated
- Model is too elaborate, models “noise” that will not be the same for new data



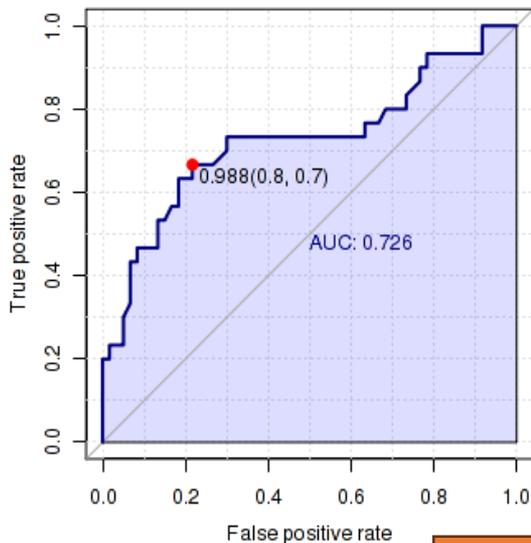
Addressing overfitting issue

- **Cross validation** – whether the model can predict on new events
 - Prediction accuracy
 - Sum of squares captured by the model (R^2)
 - Cross-validated R^2 (also known as Q^2)
- **Permutation tests** – whether the model captures real signals compared to null

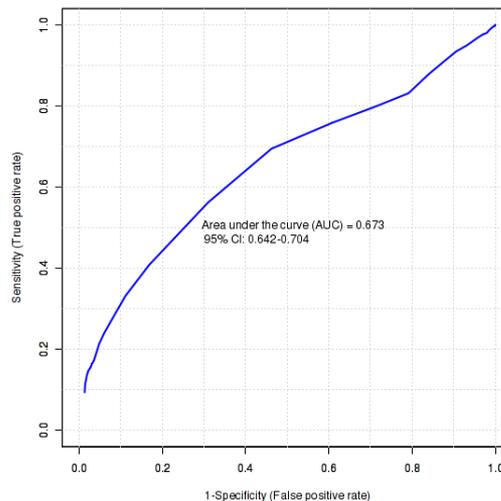


Over-fitting in classical univariate ROC curve

AUC Classical approach: 0.726



AUC CV-based: 0.673



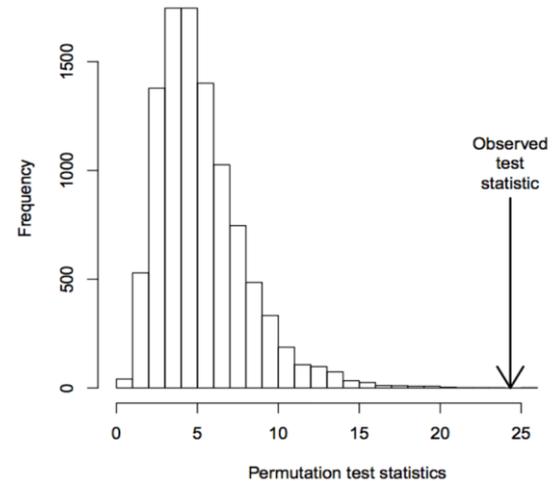
Classical univariate based on **all** data points
(no separation of training & testing)



Permutation Tests

To test whether your model is significantly different from the null models

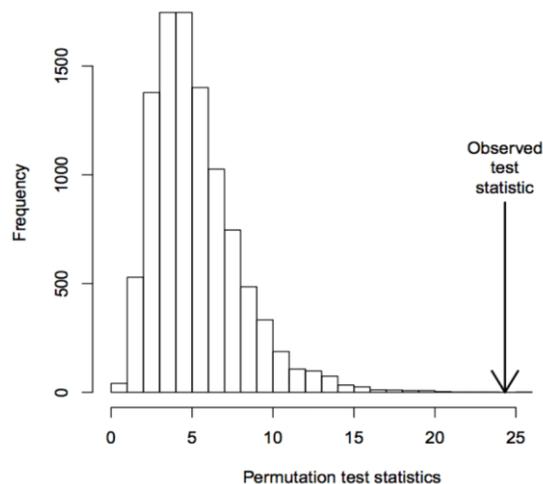
1. Randomly shuffle the class labels (y) and build the (null) model between new y and x ;
2. Test whether there is still the similar performance;
3. We can compute empirical p values
 - If the result is similar as the permuted results (i.e. null model), then we can **not** say y and x is significantly correlated



Permutations

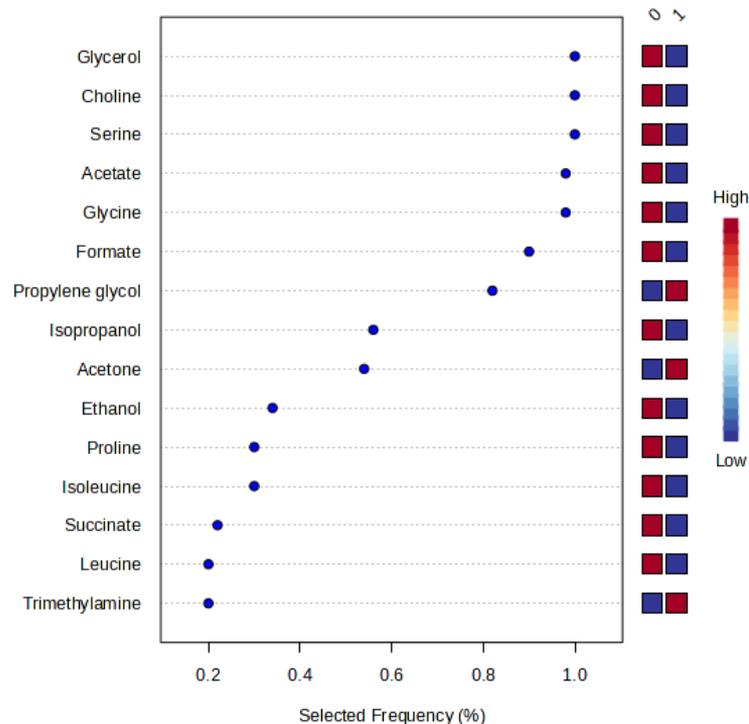
To test whether your model is significantly different from the null models

1. Randomly shuffle the class labels (y) and build the (null) model between new y and x ;
2. Test whether there is still the similar performance;
3. We can compute empirical p values
 - If the result is similar as the permuted results (i.e. null model), then we can **not** say y and x is significantly correlated



Model instability (variance)

- Multiple biomarker models will be created during cross validation-based subset of datasets
- Each model will use the same algorithm, but “slightly” different ingredients
 - Biomarkers
 - Parameters
- More severe when sample size are small



Biomarker Module in MetaboAnalyst

Biomarker selection & model creation

- Support vector machine (SVM);
- Random forests;
- Partial least squares;

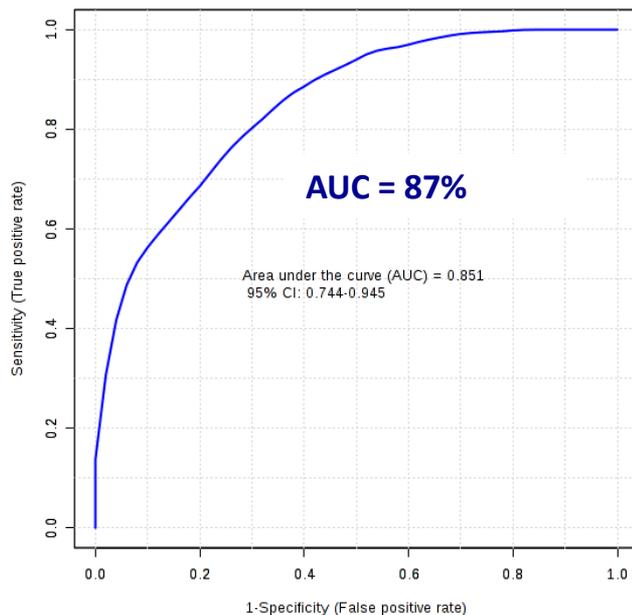
Balanced repeated random sampling & cross validation

ROC curves are generated by Monte-Carlo cross validation (MCCV) using balanced sub-sampling. In each MCCV, two thirds (2/3) of the samples are used to evaluate the feature importance. The top 2, 3, 5, 10 ...100 (max) important features are then used to build classification models which is validated on the 1/3 the samples that were left out. The procedure were repeated multiple times to calculate the performance and confidence interval of each model.

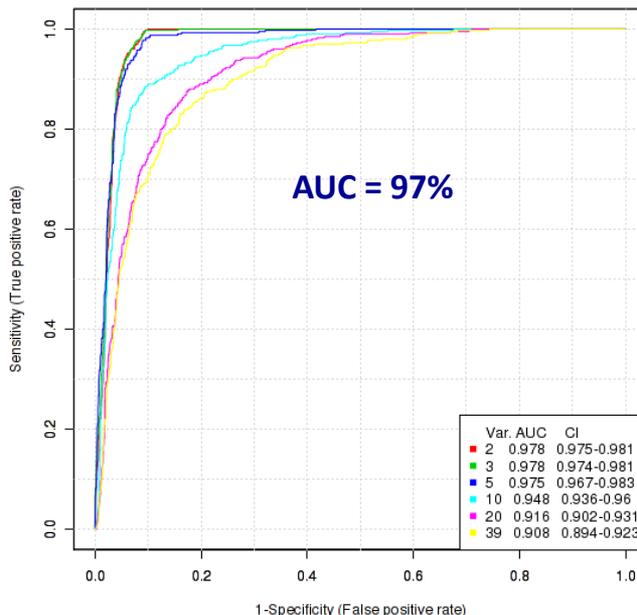
- Smooth ROC curve
- Confidence intervals
- Imbalanced samples



Manual or automatic biomarker selection



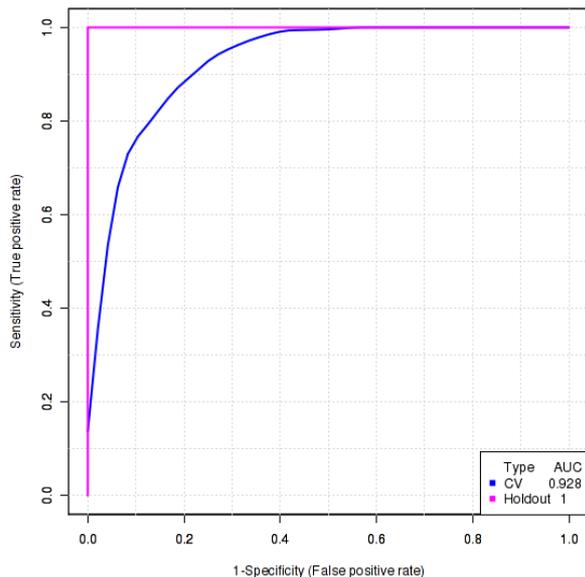
Manual selected biomarkers



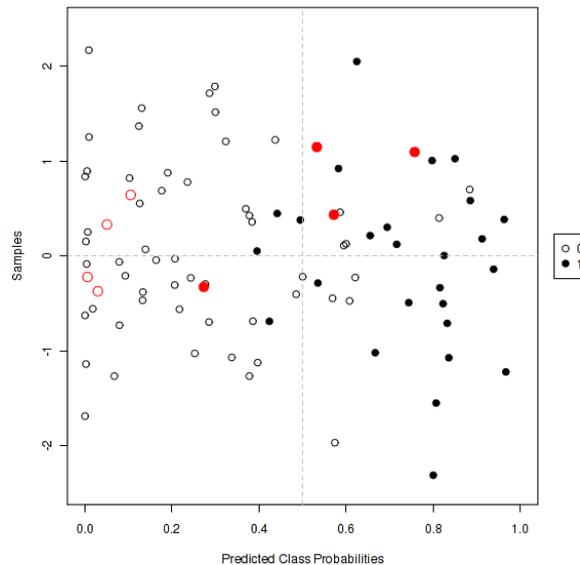
Biomarkers selected by SVM

Predicting new samples with current model

AUC = 1



Accuracy = 7/8



Final Remark

Omics data is usually used for the first-round screening for potential biomarkers

- Need independent validation

Biomarkers are not necessarily mechanistically related to the underlying disease

- Association \neq causality



Live Demo & Hands On



Biomarker Module

The screenshot displays the MetaboAnalyst Pro web interface. At the top, the logo and navigation menu (Home, Format, Tutorials, Forum, Publications) are visible. The main content area is a grid of available modules categorized by input data type. A red arrow points to the 'Biomarker Analysis' module in the 'Generic Format' row. Below the grid, three detailed boxes provide descriptions for 'Statistical Analysis [one factor]', 'Statistical Analysis [metadata table]', and 'Biomarker Analysis'.

Input Data Type	Available Modules (click on a module to proceed)						Start from your Saved Projects
Raw Spectra (mzML, mzXML or mzData)				LC-MS Spectra Processing			
MS Peaks (peak list or intensity table)				Functional Analysis	Functional Meta-analysis		
Annotated Features (compound list or table)			Enrichment Analysis	Pathway Analysis	Joint-Pathway Analysis	Network Analysis	
Generic Format (.csv or .txt table files)	Statistical Analysis [one factor]	Statistical Analysis [metadata table]	Biomarker Analysis	Statistical Meta-analysis	Power Analysis	Other Utilities	

>> Statistical Analysis [one factor]

This module offers various commonly used statistical and machine learning methods including t-tests, ANOVA, PCA, PLS-DA and Orthogonal PLS-DA. It also provides clustering and visualization tools to create dendrograms and heatmaps as well as to classify data based on random forests and SVM.

>> Statistical Analysis [metadata table]

This module aims to detect associations between phenotypes and metabolomics features with considerations of other experimental factors / covariates based on general linear models coupled with PCA and heatmaps for visualization. More options are available for two-factors / time-series data.

>> Biomarker Analysis

This module performs various biomarker analyses based on receiver operating characteristic (ROC) curves for a single or multiple biomarkers using well-established methods. It also allows users to manually specify biomarker models and perform new sample prediction.

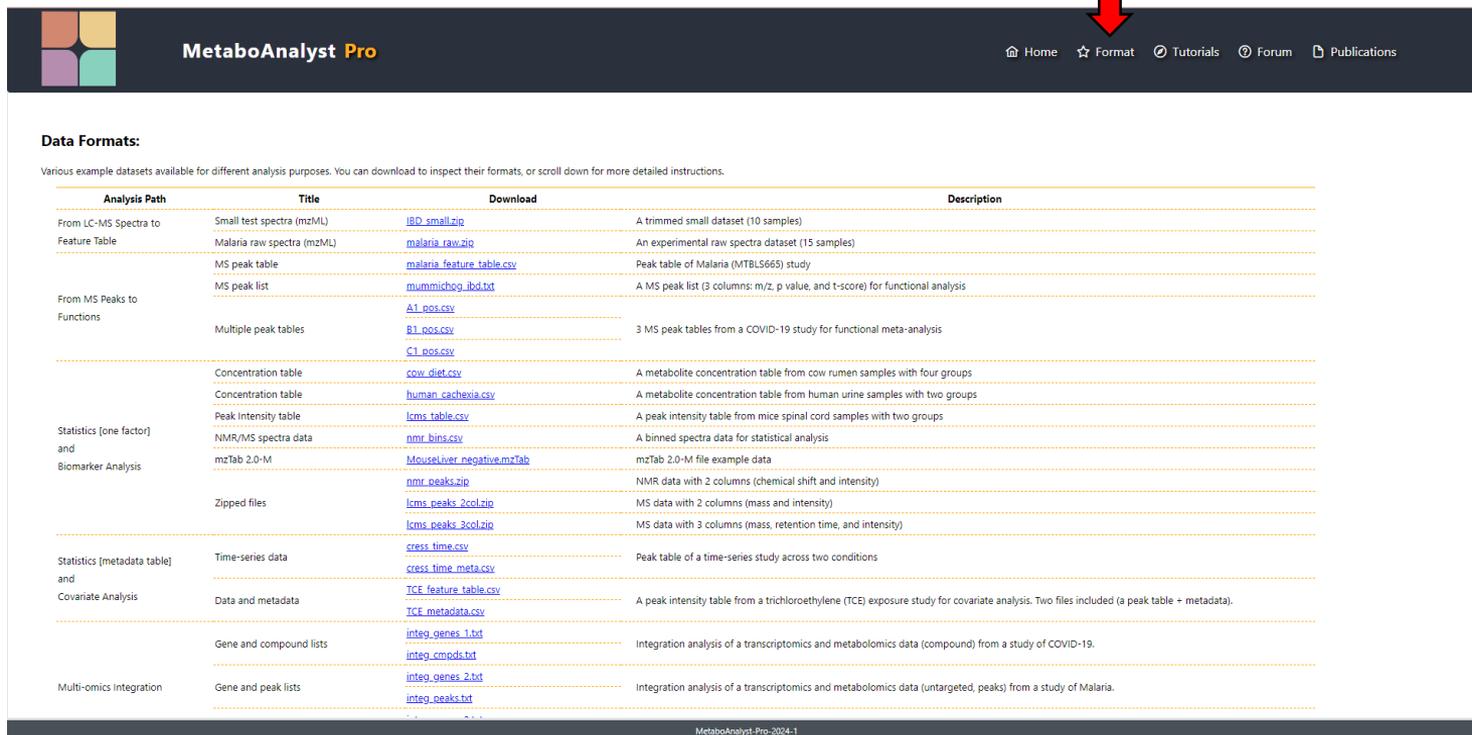
>> Enrichment Analysis

>> Pathway Analysis (targeted)

>> Network Explorer

MetaboAnalyst-Pro-2024-1

Dataset Format



MetaboAnalyst Pro

Home Format Tutorials Forum Publications

Data Formats:

Various example datasets available for different analysis purposes. You can download to inspect their formats, or scroll down for more detailed instructions.

Analysis Path	Title	Download	Description	
From LC-MS Spectra to Feature Table	Small test spectra (mzML)	l8D_small.zip	A trimmed small dataset (10 samples)	
	Malaria raw spectra (mzML)	malaria_raw.zip	An experimental raw spectra dataset (15 samples)	
From MS Peaks to Functions	MS peak table	malaria_feature_table.csv	Peak table of Malaria (MTBLS665) study	
	MS peak list	mummichog_lbd.txt	A MS peak list (3 columns: m/z, p value, and t-score) for functional analysis	
	Multiple peak tables	A1_pos.csv		
		B1_pos.csv		3 MS peak tables from a COVID-19 study for functional meta-analysis
Statistics (one factor) and Biomarker Analysis	Concentration table	cow_diet.csv	A metabolite concentration table from cow rumen samples with four groups	
	Concentration table	human_cachexia.csv	A metabolite concentration table from human urine samples with two groups	
	Peak intensity table	lcms_table.csv	A peak intensity table from mice spinal cord samples with two groups	
	NMR/MS spectra data	nmr_bins.csv	A binned spectra data for statistical analysis	
	mzTab 2.0-M	MouseLiver_negative.mzTab	mzTab 2.0-M file example data	
	Zipped files		nmr_peaks.zip	NMR data with 2 columns (chemical shift and intensity)
		lcms_peaks_2col.zip	MS data with 2 columns (mass and intensity)	
		lcms_peaks_3col.zip	MS data with 3 columns (mass, retention time, and intensity)	
Statistics (metadata table) and Covariate Analysis	Time-series data	cress_time.csv	Peak table of a time-series study across two conditions	
		cress_time_meta.csv		
Data and metadata		TCE_feature_table.csv	A peak intensity table from a trichloroethylene (TCE) exposure study for covariate analysis. Two files included (a peak table + metadata).	
		TCE_metadata.csv		
Gene and compound lists		integ_genes_1.txt	Integration analysis of a transcriptomics and metabolomics data (compound) from a study of COVID-19.	
		integ_cpds.txt		
		integ_genes_2.txt		
Multi-omics integration	Gene and peak lists	integ_genes.txt	Integration analysis of a transcriptomics and metabolomics data (untargeted, peaks) from a study of Malaria.	
		integ_peaks.txt		

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Dataset format

MetaboAnalyst Pro Home Format Tutorials Forum Publications

Gene and compound lists [integ_cmds.txt](#) Integration analysis of a transcriptomics and metabolomics data (compound) from a study of COVID-19.

Multi-omics integration
Gene and peak lists [integ_genes_2.txt](#) Integration analysis of a transcriptomics and metabolomics data (untargeted, peaks) from a study of Malaria.
[integ_peaks.txt](#)
Protein and compound lists [integ_genes_3.txt](#) Integration analysis of a proteomics and metabolomics data (compounds, HMDB) from a study of COVID-19.
[integ_cmds_3.txt](#)

General One-factor / Paired Metadata / Time-series Peak lists MS spectra **Biomarker** Meta-analysis Peaks to path Network

Exploratory biomarker analysis

The data format is same as the one-factor data with samples in rows or columns, followed immediately by class labels. Please note, ROC curve-based biomarker analysis is only defined for two-group analysis. If your data contains multiple groups, you need to specify which two groups you want to investigate.

Creating biomarker models to predict new samples

You can create biomarker models to predict new samples (with unknown class) using the **ROC Tester**. To do this, you need to upload a data that contains both the samples with class labels and the samples whose class label need to be predicted (**leave their sample labels empty**). A screenshot is shown below.

Patient ID	Label	Acetate	Acetone	Adipate	Alanine	Asparagine	Betaine	Carnitine	Citrate	Creatine
Control_01	0	126.47	9.49	38.09	314.19	159.17	109.95	265.07	3714.5	196.37
Control_02	0	212.72	11.82	327.01	871.31	157.59	244.69	120.3	2617.57	212.72
Control_03	0	314.19	4.44	131.63	464.05	89.12	116.75	25.03	862.64	221.41
Control_04	0	37.34	206.44	144.03	589.93	273.14	278.66	200.34	13629.61	85.63
Control_05	0	407.48	44.26	15.03	1118.79	42.52	391.51	84.77	854.06	105.64
Control_06	0	81.45	14.44	25.28	237.46	157.59	66.69	40.04	1958.63	200.34
Control_07	0	51.42	3.25	8.41	336.97	71.52	149.9	127.74	3944.19	383.75
Control_08	0	7.46	2.8	3.53	69.41	13.87	15.33	9.87	788.4	5.81
Disease_01	1	9.97	8.67	8.25	102.51	32.79	31.19	7.32	1669.03	35.16
Disease_02	1	100.48	9.12	14.59	962.95	221.41	149.9	487.85	4675.07	126.47
Disease_03	1	27.94	9.49	18.54	164.02	32.14	219.2	230.44	3533.34	1450.99
Disease_04	1	30.88	7.92	259.82	507.7	64.72	137	35.87	854.06	1863.11
Disease_05	1	55.15	9.21	11.02	217.02	32.14	167.34	14.88	1772.24	125.21
Disease_06	1	95.58	8.67	9.03	167.34	47.94	56.83	16.95	323.76	102.51
Disease_07	1	69.41	6.23	3.16	34.47	13.33	41.68	24.53	265.07	11.7
Disease_08	1	79.84	3.16	4.81	26.84	14.3	4.06	18.36	80.64	18.54
Unknown_01		91.84	17.64	22.87	441.42	79.04	157.59	62.8	897.85	419.89
Unknown_02		70.81	4.22	15.8	188.67	54.05	78.26	24.05	2489.91	170.72
Unknown_03		42.52	9.39	12.43	237.46	35.87	60.34	12.06	4447.07	97.51
Unknown_04		82.27	3.82	20.49	333.62	61.56	68.72	15.18	2643.87	55.7

If you would like to predict new samples, leave their class label empty

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Dataset Format

Samples can be either in rows or columns, first row/column is samples, second row/column should be metadata group. Only one metadata group is supported.

Samples		Metadata									
Patient ID	Label	Acetate	Acetone	Adipate	Alanine	Asparagine	Betaine	Carnitine	Citrate	Creatine	
Control_01	0	126.47	9.49	38.09	314.19	159.17	109.95	265.07	3714.5	196.37	
Control_02	0	212.72	11.82	327.01	871.31	157.59	244.69	120.3	2617.57	212.72	
Control_03	0	314.19	4.44	131.63	464.05	89.12	116.75	25.03	862.64	221.41	
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Control_05	0	407.48	44.26	15.03	1118.79	42.52	391.51	84.77	854.06	105.64	
Control_06	0	81.45	14.44	25.28	237.46	157.59	66.69	40.04	1958.63	200.34	
Control_07	0	51.42	3.25	8.41	336.97	71.52	149.9	127.74	3944.19	383.75	
Control_08	0	7.46	2.8	3.53	69.41	13.87	15.33	9.87	788.4	5.81	
Disease_01	1	9.97	8.67	8.25	102.51	32.79	31.19	7.32	1669.03	35.16	
Disease_02	1	100.48	9.12	14.59	962.95	221.41	149.9	487.85	4675.07	126.47	
Disease_03	1	27.94	6.49	18.54	164.02	32.14	219.2	230.44	3533.34	1450.99	
Disease_04	1	30.88	7.92	259.82	502.7	64.72	137	35.87	854.06	1863.11	
Disease_05	1	55.15	9.21	11.02	217.02	32.14	167.34	14.88	1772.24	125.21	
Disease_06	1	95.58	8.67	9.03	167.34	47.94	56.83	16.95	323.76	102.51	
Disease_07	1	69.41	6.23	3.16	34.47	13.33	41.68	24.53	265.07	11.7	
Disease_08	1	79.84	3.16	4.81	26.84	14.3	4.06	18.36	80.64	18.54	
Unknown_01		91.84	17.64	22.87	441.42	79.04	157.59	62.8	897.85	419.89	
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Unknown_04		82.27	3.82	20.49	333.62	61.56	68.72	15.18	2643.87	55.7	

Samples without metadata labels for prediction

Our Syllabus

Topic	Date	Lecture	Lab
Omics Data Science Foundations	Jan. 6	Omics data processing, statistics and visualization	--
	Jan. 13	From raw data to functional insights	--
Transcriptomics	Jan. 20	Gene expression data analysis (part I)	ExpressAnalyst & NetworkAnalyst
	Jan. 27	Gene expression data analysis (part II)	ExpressAnalyst & Seq2Fun
Proteomics & Biological Networks	Feb. 3	Biological network analysis & gene regulatory networks	NetworkAnalyst & miRNet
	Feb. 10	Proteomics & biomarker analysis	ExpressAnalyst & MetaboAnalyst
Metabolomics	Feb. 17	Targeted metabolomics data analysis	MetaboAnalyst
	Feb. 24	LC-MS untargeted metabolomics data analysis	MetaboAnalyst
Microbiomics	Mar. 2	Marker gene data analysis	MicrobiomeAnalyst
	Mar. 9	Shotgun metagenomics data analysis	MicrobiomeAnalyst
Multi-omics	Mar. 16	Knowledge-driven multi-omics integration	OmicsNet
	Mar. 23	Data-driven multi-omics integration	OmicsAnalyst



Tutorials

Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis

Jasmine Chong,¹ David S. Wishart,^{4,5,6} and Jianguo Xia^{1,2,3,6}

¹Institute of Parasitology,

²Department of Animal Science
Canada

³Department of Microbiology
Canada

⁴Department of Computing

⁵Department of Biological

Basic Protocol 1: Data uploading, processing, and normalization

Basic Protocol 2: Identification of significant variables

Basic Protocol 3: Multivariate exploratory data analysis

Basic Protocol 4: Functional interpretation of metabolomic data

Basic Protocol 5: Biomarker analysis based on receiver operating characteristic (ROC) curves

Basic Protocol 6: Time-series and two-factor data analysis

Basic Protocol 7: Sample size estimation and power analysis

Basic Protocol 8: Joint pathway analysis

https://www.dropbox.com/s/pm6t6w2qo8q1z95/CPIB_MetaboAnalyst4.pdf?dl=0



**We would like to hear your
comment & feedback**

contact@xialab.ca

See (most of) you next week!

