Central Techniques in Omics Exam 2022

Short answer questions:

1. Ion mobility mass spectrometry (IMS-MS) is implemented in several mass spectrometers to improve biomolecule characterization by 4D type identification.

   • *Sketch an IMS based mass spectrometer in details and explain how Ion mobility separation is aided by a combination of gas flow and electrical fields.*

   • *Collisional cross section (CCS) can improve identification and quantification of proteins. Explain why and the values of 4D?*

2. Functional protein array technologies enable detection of key biomarkers in biofluids such as autoantibodies or microvesicles.

   • *Discuss and explain shortly difference in setup and usage of protein microarrays?*

3. Protein presence *in situ* can be accomplished by MS analysis and validated by several complementary techniques.

   • *Discuss and explain shortly the use of ImagingMS for tissue profiling and in-situ drug quantification?*

   • *Explain shortly the similarities and differences of Western blotting for protein quantification in comparison to immunohistochemistry (IHC)?*

Computational questions:

Amyloid plaques (Aβ plaques) are one of the hallmarks of Alzheimer's disease (AD). The main constituent of Aβ plaques is beta-amyloid peptides but a complex interplay of other infiltrating proteins also co-localizes. Researchers have investigated the content of amyloid plaques by LC-MS in a mouse model treated with a drug.

Your aim is to perform protein identification using four selected raw files using the search engine MaxQuant and perform label-free quantification Perseus by software available from CTO course homepage and compare treated versus control (ctl).

• Explain shortly the selected search parameters in the box (right) and type of raw data from the used mass spectrometer (Orbitrap QExactive)

• Illustrate the data processing outcome with screen dumps.
• Import the archive search result in Peresus.
    Import files with LFQ values and selected output of own choice
    In the grouping step group according to control or
• Illustrate the data processing outcome with screen dumps.
• You can filter data, process and use the PCA and Volcano plot to illustrate the separation of patients.
• What impact will missing LFQ values have and how can you increase the proteome coverage experimentally? Suggest alternative technologies and methods.

Assay question:

The article by Aggrawal et al., 2021, Role of Multiomics Data to Understand Host−Pathogen Interactions in COVID-19 Pathogenesis (https://dx.doi.org/10.1021/acs.jproteome.0c00771?ref=pdf) reviews and discusses a range of complementary Omics technologies including integration of transcriptomic and proteomic data for increased understanding of the pathobiology of SARS-CoV-2 in humans.

Read the manuscript critically and structure you reply by answering the below guiding questions. The level of details and accuracy may affect the grading of this part.

1. (10p) The 30-kb SARS-CoV-2 viral genome codes for two polyproteins (figure 1) and the S protein found on the surface of the viral particle enables host infection into the human lung tissue by the ACE2 receptor.
    Describe and sketch the light microscope for imaging of post-mortem lung biopsy tissue. What central steps are necessary for detection of the ACE2 or S-protein by fluorescence or immunohistochemistry?
    Analysis of COVID-19 disease groups with varying severity has shown altered blood profiles as biomarkers, including lower lymphocyte counts and up-regulation of biomarkers interleukin-6 (IL-6; very low abundant protein) and C-reactive protein (CRP; may be high abundant). Suggest with argumentation for appropriate technologies to quantitively measure each class of molecule.
2. (10p) The authors highlight proteomic workflows using protein array and mass spectrometry-based technologies to profile the biological samples (urine, plasma immune cells) to group and detect potential biomarkers (Figure 2).
    Explain in our own words in details how each high-throughput platform can be used for targeted and untargeted proteomics.
    Explain differences in experimental processing of patient samples for detection and data analysis that allows specific detection of regulated proteins across the three groups.
3. (10p) Next-generation sequencing or NGS is a pillar of high-throughput omics that has aided the understanding of the dynamics of the viral genome and how it interacts and coevolves with the host.
    How can the mRNA-protein data be used in a proteogenomics strategy to investigate variants of e.g. Spike protein mutations?
    Explain the difference in long and short-read technologies for mapping the viral

genomes. If possible then shortly outline a wetlab experiment that would allow purification and sequencing of SARS-CoV-2 (note: you have not had the CTO wet-lab and any answer will respect that).

Is any specific type of NGS hardware optimal for each type of sequencing above and why?

4. (10p) The researchers highlight many biological findings measuring plasma and sera proteomic based changes approach to profile biofluid or tissue samples.

   Explain how the new instrument technologies like PASEF and DIA-PASEF technological approaches can increase the proteome coverage?

   Suggest approaches to increase proteome coverage of the COVID-19 samples?

5. (10p) Complementary techniques central to Omics studies may be used to validate host response to SARS-CoV-2 infection the viral-host related inflammation related biomarkers e.g RdRp-IP1, RdRp, CRP, IL10 highlighted in this review.

   Explain in your own words and drawings the technology and differences between

   1. RT-qPCR,
   2. Somalogic based proteomics
   3. Nanostring based biomarker analysis

   for detection of the proposed biomarkers in tissue or biofluids obtained from the patients.