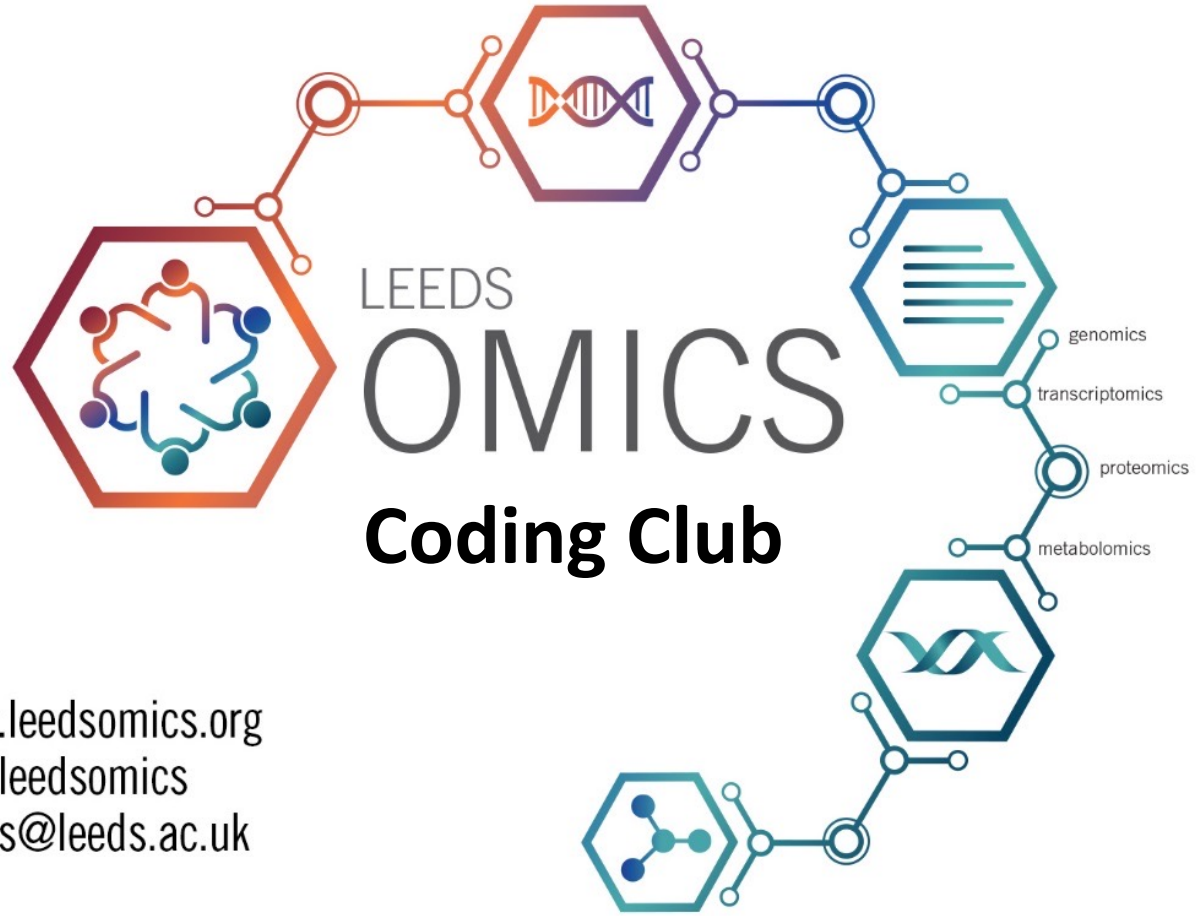

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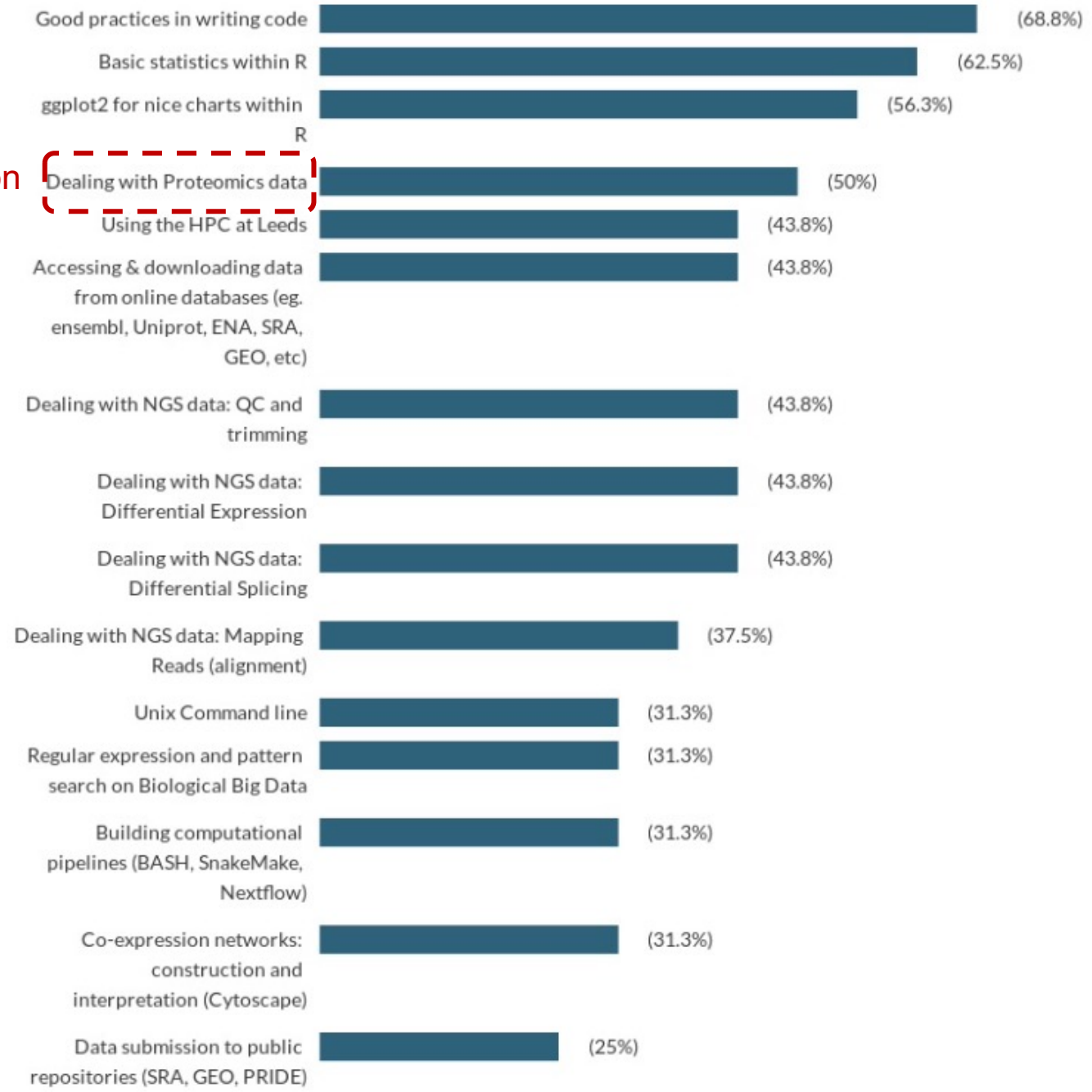
Dealing with Proteomics Data

Club Moderators: Elton Vasconcelos, Euan McDonell

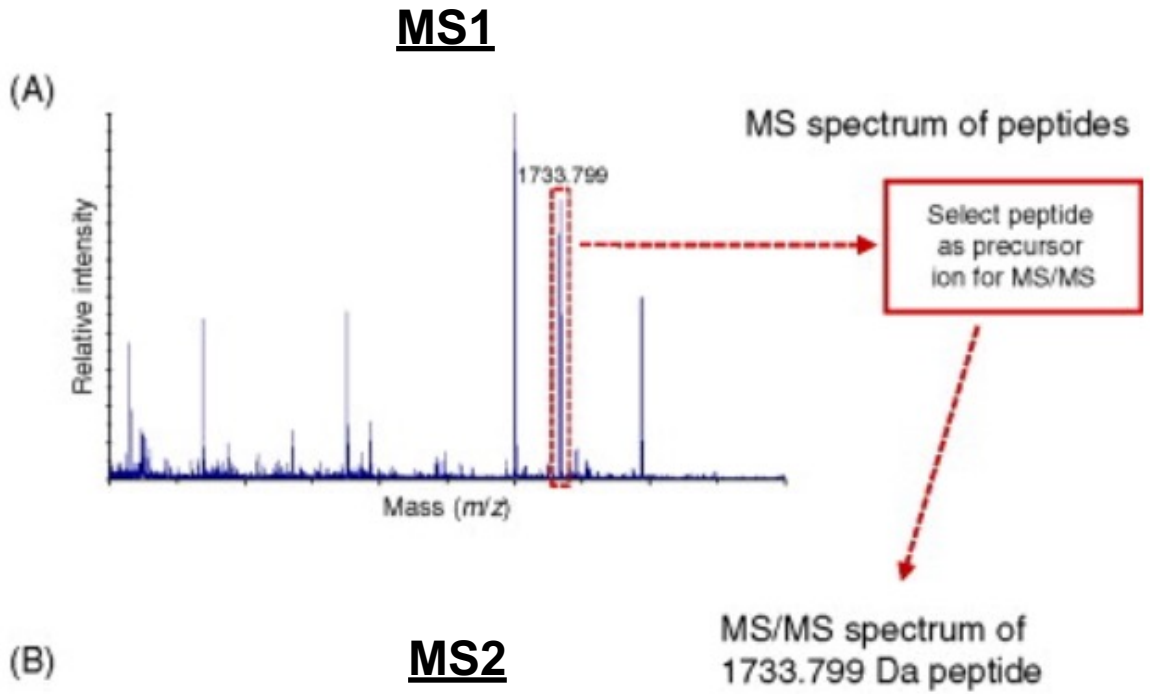
Topics to be addressed - **Survey Result (2021-22)**

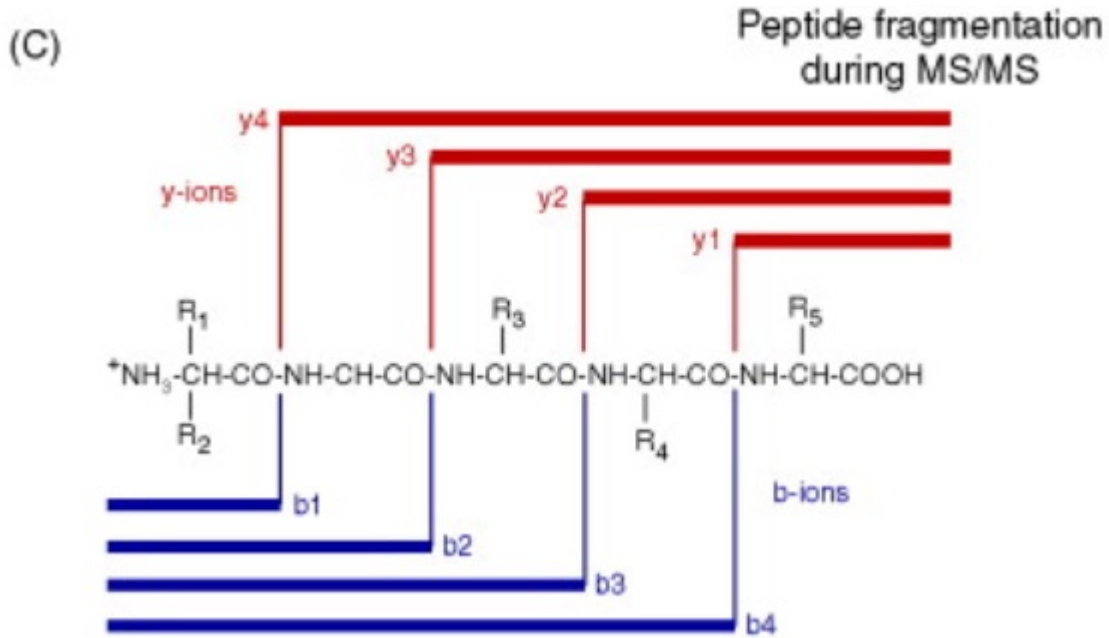
4th session



MS/MS brief summary

Peptides are first detected by MS (panel A). In this example, a 1733.799-Da peptide was subjected to collision-induced dissociation (CID) to achieve peptide fragmentation and the MS/MS spectrum is shown in panel B. Sufficient energy is used to break the peptide bonds which are generally the weakest in the peptide. The trick uses a low enough energy to cause inefficient fragmentation so that peptide fragments are produced with differing numbers of amino acids present. Panel C is a diagrammatic representation of MS/MS fragmentation of peptides due to peptide bond cleavage.





https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/tandem-mass-spectrometry

- Label-free versus label-based proteomics approaches

Feature	Label-free	Label-based
Quantification	Spectral counting and ion intensity-based, relying on precursor ion abundances at the MS^1 level (during the course of protein fragmentation)	Measurement of reporter ions intensities, produced and detected at the MS^2 level (during the course of peptide fragmentation)
Sample preparation	Cost-efficient (no additional step)	Additional labelling steps introduced (expensive commercially available labelling reagents)
Multiplexed analysis	Not possible	3-plex (SILAC); 4- and 8-plex (iTRAQ); 6-, 10-, 11- and 16-plex (TMT)
Instrument time	Time-consuming (one sample per run)	Significantly decreased
Batch effect bias	Increased	Decreased
Proteome coverage	High	Mid

Proteomics Data Analysis Workflow

1. MS raw data conversion to plain text formats

ThermoRawFileParserGUI*

readMgfData ('RforProteomics' R package)

Quality control with plotMzDelta ('MSnbase' R package)

2. Protein Identification, Normalization and Quantification

Comet and CometUI*

Crux for label-free data only

SearchGUI*

Third party software coupled to MS/MS instruments

ProteomeDiscoverer

MaxQuant

IsobarQuant

3. Differential Expression Analysis

Regular t-Test, ANOVA and/or empirical Bayes on label-free data

Limma-adapted (eBayes) functions for iTRAQ/TMT strategies

https://www.biostat.jhsph.edu/~kkammers/software/eupa/R_guide.html

DEP R package https://bioconductor.org/packages/release/bioc/vignettes/DEP/inst/doc/DEP.html

Optional tools

- **PCA and batch effect adjustment**

  data.PC function for PCA (‘gfortify’ R package);

  removeBatchEffect (‘limma’ R package) or ComBat (‘sva’ R package)**;**

- **Expression Correlation**

  Proteomics vs Transcriptomics (*ad hoc* R scripts applying ”Spearman” corr;

  fuzzy c-means-based clustering-> **VSClust**)

Bring your issues on!