Improve **shotgun proteomics** identifications with **b10prot** R package.

Create **tidy**verse-style **workflows**.

Proteomics Identification Workflow in R

Gorka Prieto [1,]

@GorkaEhu

gorka.prieto@ehu.eus

Nerea Osinalde [2] **Pedro Navarro** [3] **Jesús Vázquez** [4]

[1] *Department of Communications Engineering, University of the Basque Country (UPV/EHU)*

[2] *Department of Biochemistry and Molecular Biology, University of the Basque Country (UPV/EHU)*

[3] *Chromatography & Mass Division Software, Thermo Fisher Scientific (Bremen) GmbH*

[4] *Cardiovascular Proteomics Laboratory, Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC) and CIBER de Enfermedades Cardiovasculares*

Motivation

- **Shotgun proteomics**: Preferred method for large-scale protein identification.
- **Challenge**: Need for robust statistical methods to automate identification and reduce false positives.
- **Our contribution**:
  - Adapted our existing protein inference and scoring tools (PAnalyzer, LPGF, refined FDR).
  - Integrated seamlessly with shotgun proteomics workflows using R.
- **Goal**: Make these tools more accessible to a wider scientific audience via R.

Approach

PSMs

- A `data.frame` with the following columns:
  - `psmScore`, `rank`, `isDecoy`, `peptideRef`, `proteinRef`, `geneRef` (optional)
- Select `rank=1` PSMs

Peptides

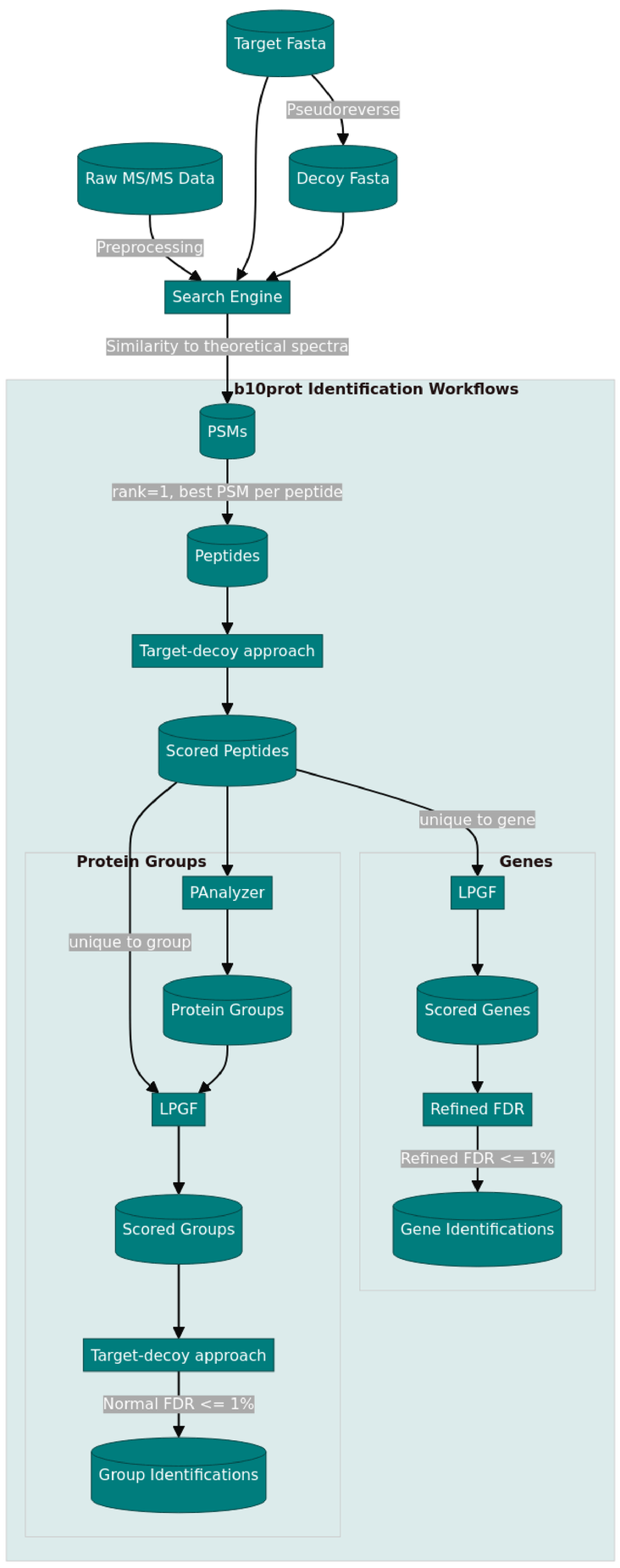
- Best PSM per peptide as `pepScore`
- Target-decoy approach **(Elias and Gygi 2007)** to assign statistical significance **(Käll et al. 2008)**:
  - p-value, FDR, q-value
- Identified peptides: q-value <= 1%
- For upper level (eg. protein) scores we will use:
  - Peptide-level `LP` = cologaritm of p-value

Proteins (or Genes)

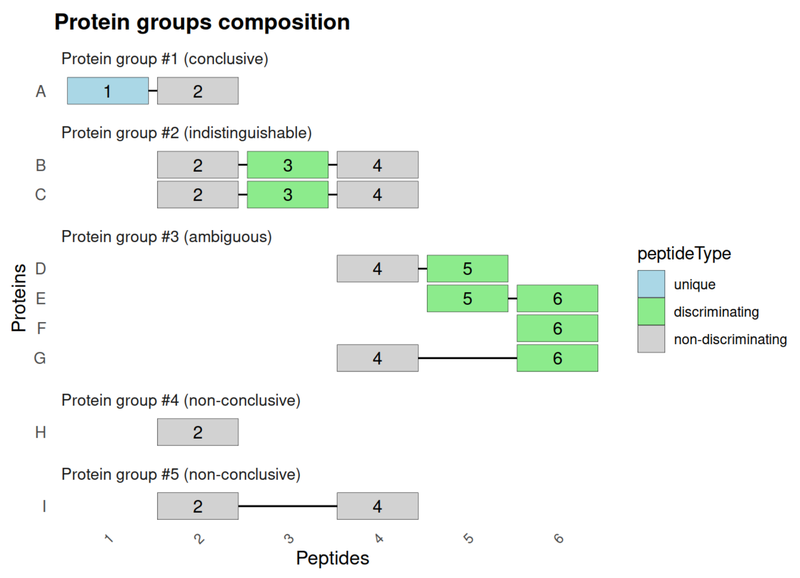
- Consider only peptides **unique** to one protein
- Calculate **LPGF** score **(Prieto and Vázquez 2020)** from:
  - `LP` peptide scores
  - `n` number of matched peptides
  - `m` number of identified peptides
- Protein-level **refined FDR** **(Prieto and Vázquez 2020)** using LPGF score

Protein Groups

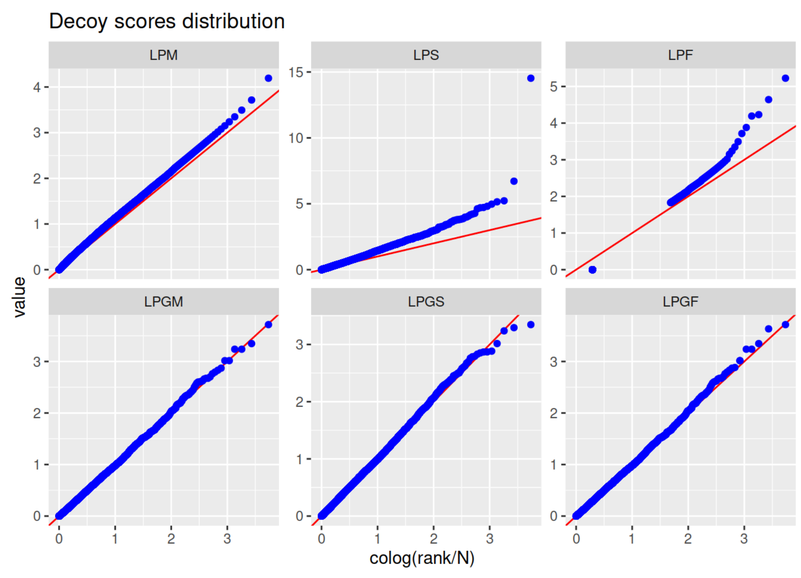
- Construct protein groups using **PAnalyzer** **(Prieto et al. 2012)**
- Consider only peptides **unique** to one group
- Calculate group-level **LPGF** score
- Group-level **refined FDR** using LPGF score



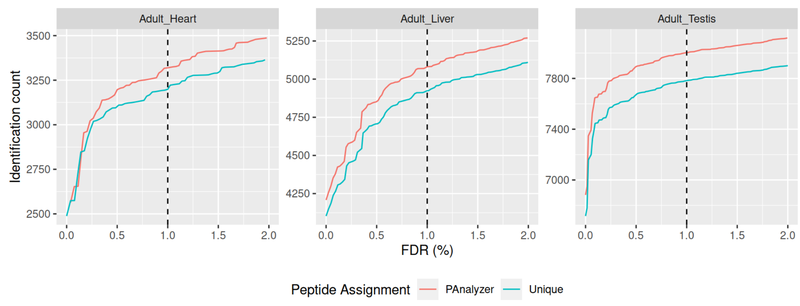
- **PAnalyzer** can now be used from R:



- **LPGF** and **FDRr** implemented in R:



- Approx. 3% improvement using protein groups:



- Approx. 5% improvement compared to other scores:
  - **Navarro et al. (2024)**

Results

- New **b10prot** R package: https://akrogp.github.io/b10prot/

References

Elias, Joshua E., and Steven P. Gygi. 2007. "Target-Decoy Search Strategy for Increased Confidence in Large-Scale Protein Identifications by Mass Spectrometry." *Nature Methods* 4 (3): 207–14.

Käll, Lukas, John D. Storey, Michael J. MacCoss, and William Stafford Noble. 2008. "Assigning Significance to Peptides Identified by Tandem Mass Spectrometry Using Decoy Databases." *Journal of Proteome Research* 7 (1): 29–34.

Navarro, Pedro, Waqas Nasir, Kai Fritzemeier, Gorka Prieto, Víctor M. Guerrero-Sánchez, Jesús Vázquez, and Christoph Henrich. 2024. "Performance of LPGF Protein Validation in diverse sample types and mass spectrometry workflows." *72nd ASMS Conference on Mass Spectrometry and Allied Topics*. Anaheim, California.

Prieto, Gorka, Kerman Aloria, Nerea Osinalde, Asier Fullaondo, Jesus M. Arizmendi, and Rune Matthiesen. 2012. "PAnalyzer: A software tool for protein inference in shotgun proteomics." *BMC Bioinformatics* 13 (1): 288.

Prieto, Gorka, and Jesús Vázquez. 2020. "Protein Probability Model for High-Throughput Protein Identification by Mass Spectrometry-Based Proteomics." *Journal of Proteome Research* 19 (3): 1285–97.

-=]b10prot[=-

eman ta zabal zazu