

FLEXIBLE, ACCESSIBLE & REPRODUCIBLE WORKFLOWS FOR TANDEM PROTEOGENOMIC AND METAPROTEOMIC ANALYSIS USING THE GALAXYP PLATFORM.

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INTRODUCTION

- Proteogenomics (for identifying unannotated proteoforms) and metaproteomics (for characterizing non-host/multi-organism proteomes) are research areas that extend discoveries beyond the reference proteome.
- For biomedically-relevant proteomics studies, tandem proteogenomic and metaproteomic analysis offers great promise for new discoveries.
- We describe effective and accessible bioinformatic analytical workflows, amenable to creative customization and sharing to foster collaborative research efforts.

GALAXYP

GalaxyP Tools

GalaxyP Workflow

GalaxyP has multiple software tools - some proteomics-specific - and others from the genomics Galaxy framework.

Tools can be used in a sequential manner to generate workflows that can be reused, shared and creatively modified for multiple studies.

Benefits of Galaxy / GalaxyP:

- Software accessibility and usability.
- Share-ability of tools, workflows and histories.
- Reproducibility and ability to test and compare results after using multiple parameters.
- Analytical transparency
- Scalability of data

METHODS & DATASETS

RAW files from multiple datasets (see below) were generated from Orbitrap Velos instrument. The processed peak lists were searched using ProteinPilot™ version 4.5 (AB Sciex) within GalaxyP (usegalaxy.org). the datasets were searched against 3-frame translated cDNA database and the human oral microbial database by using two-step method (Jagtap *et al* 2013). After optimization & testing, multiple workflows were used in a sequential manner to generate inputs for the subsequent workflow. Microbial peptides were identified after using metaproteomic workflows & novel proteoforms were identified after using proteogenomic workflows.

UNLABELED SAMPLE:

- Oral pre-malignant lesion (OPML) dataset was collected as oral exudate using PerioPaper strip method (Kooren *et al* 2011) from an individual with pre-malignant lesion & a matched control sample from adjacent oral cavity.

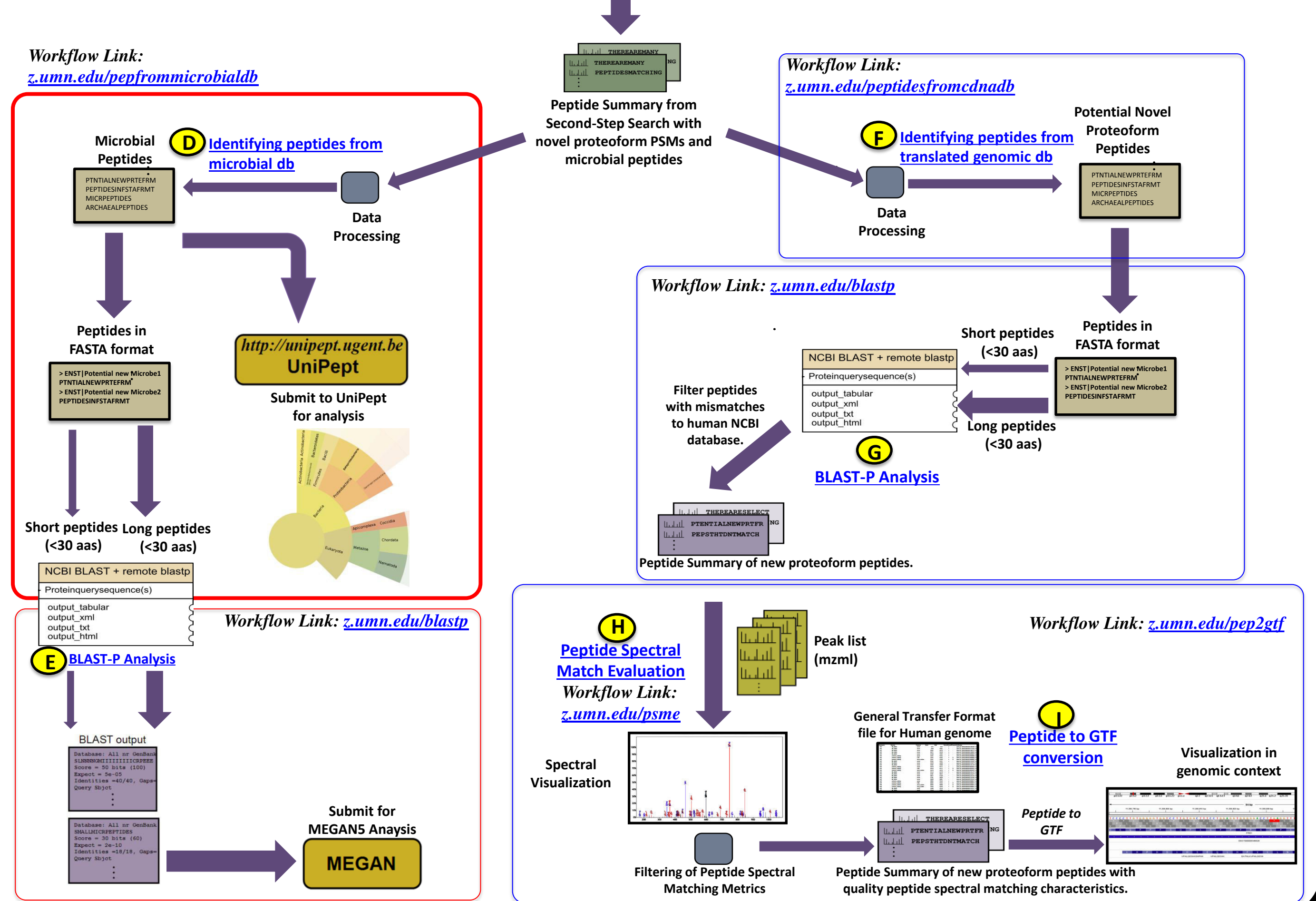
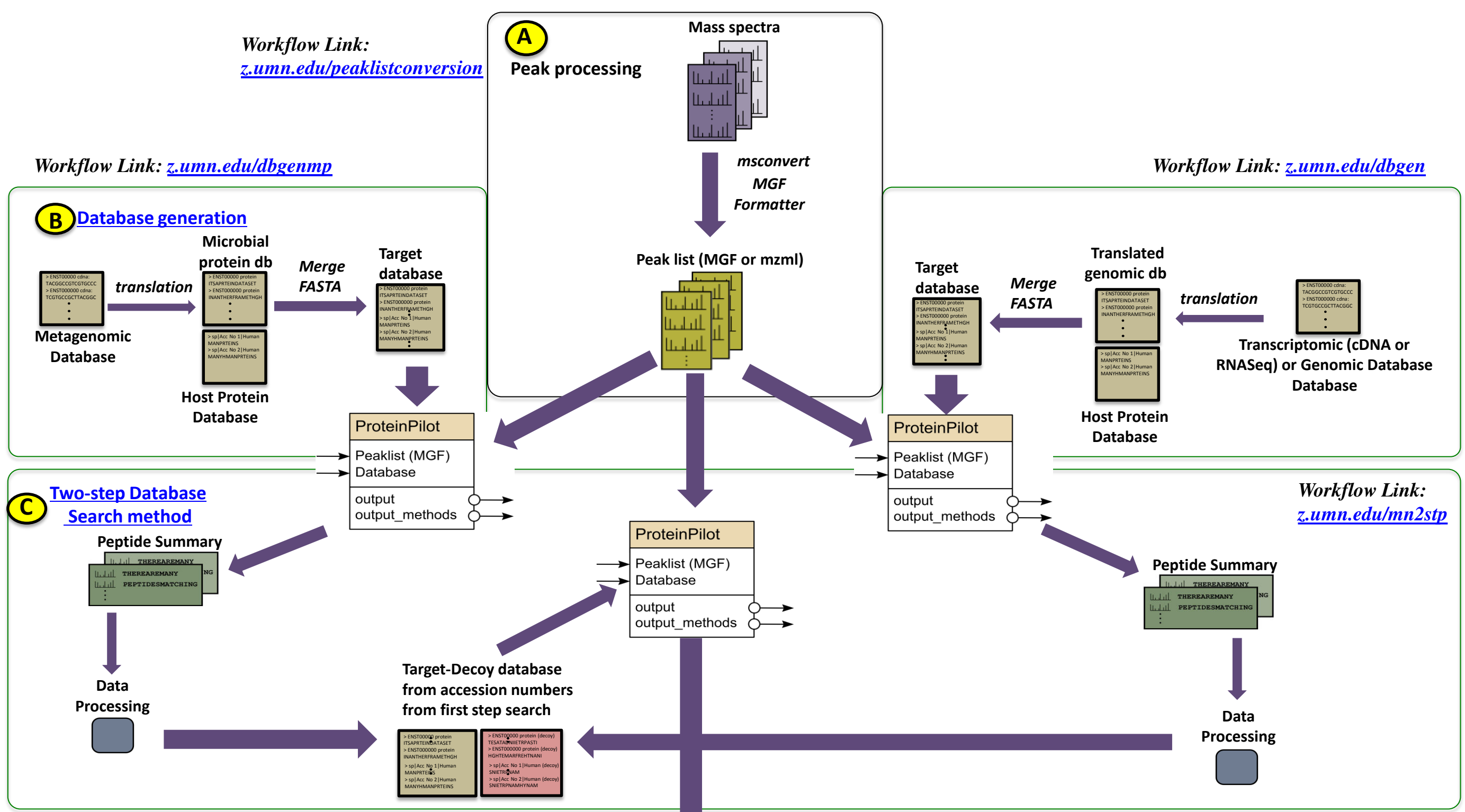
4-plex iTRAQ LABELED SAMPLE:

- Brush biopsies were collected from patients diagnosed with OPML and from patients with Oral Squamous Cell Carcinoma (OSCC). For each patient, brush biopsies from the lesion & the healthy mucosa of corresponding contralateral area were collected (Yang *et al* 2014).

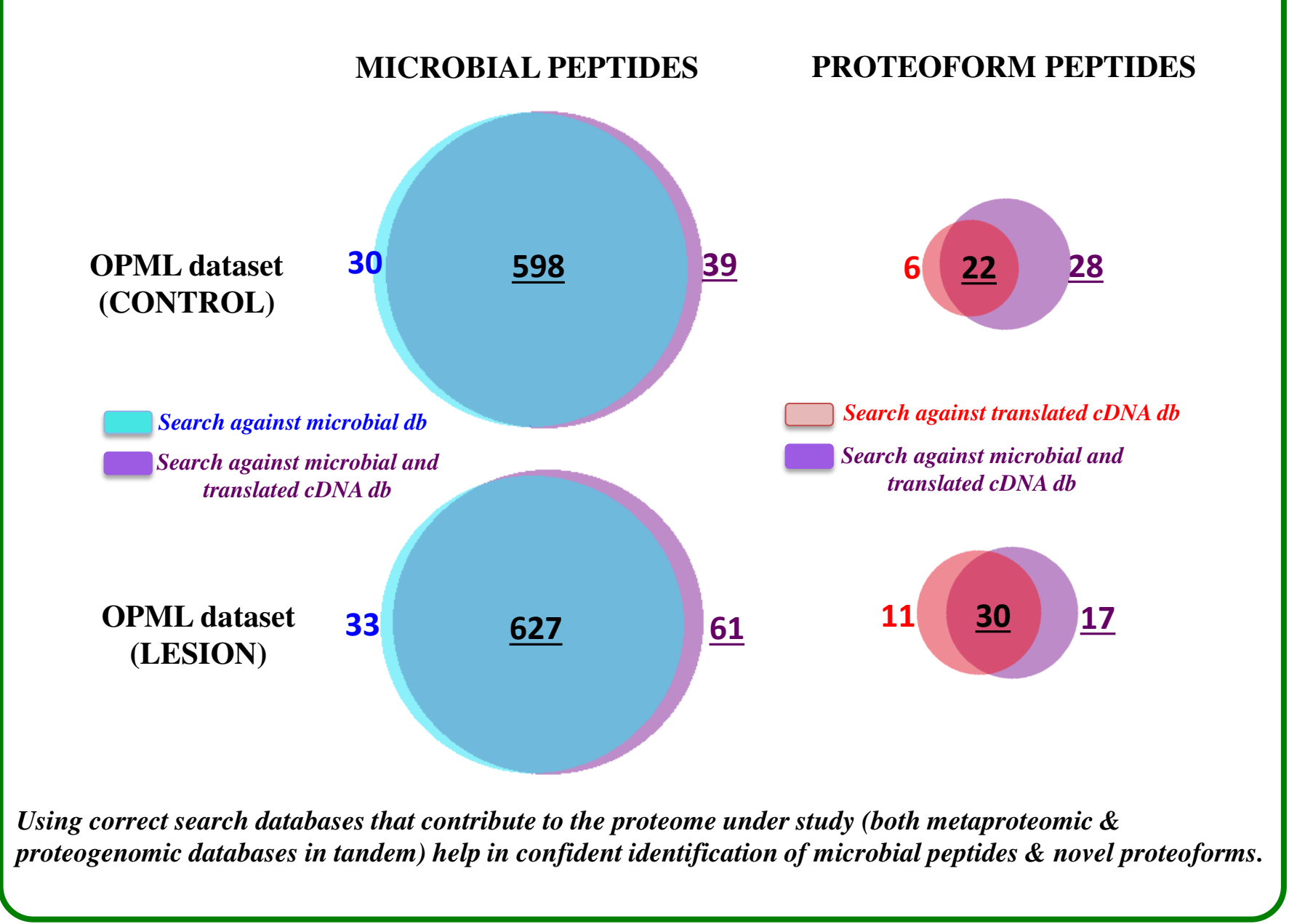
8-plex iTRAQ LABELED SAMPLE:

- Chronic Obstructive Pulmonary Disease (COPD) – linked lung cancer tissue samples were collected & subjected to iTRAQ labeling and 2D LC-MS. Ten replicates of this dataset were searched against the 3-frame translated cDNA database & human oral microbiome database (HOMD) using the two-step method.

WORKFLOWS FOR TANDEM PROTEOGENOMIC AND METAPROTEOMIC ANALYSIS.

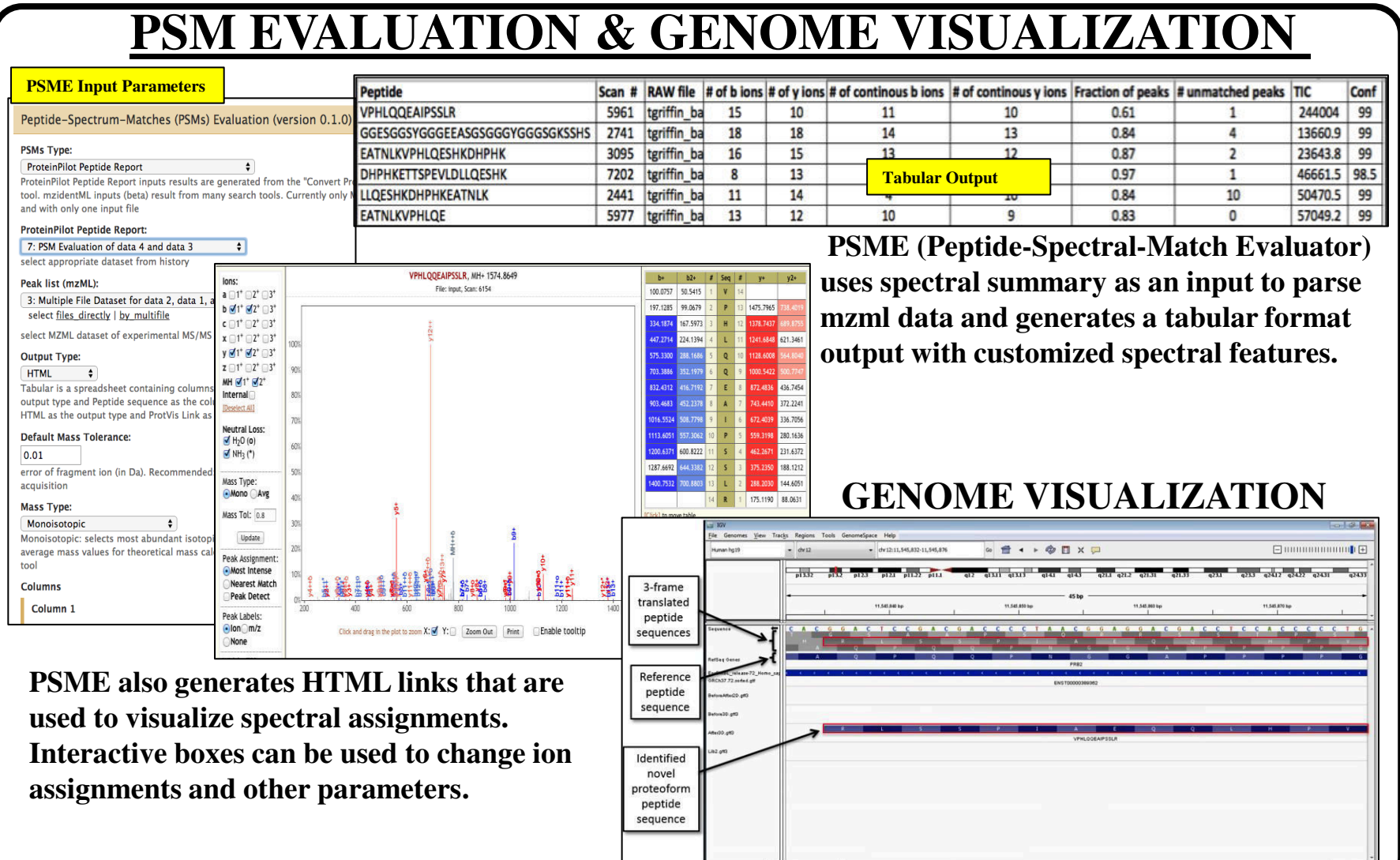


EFFECT OF SEARCH DATABASES ON MICROBIAL & NOVEL PROTEOFORM IDENTIFICATIONS.

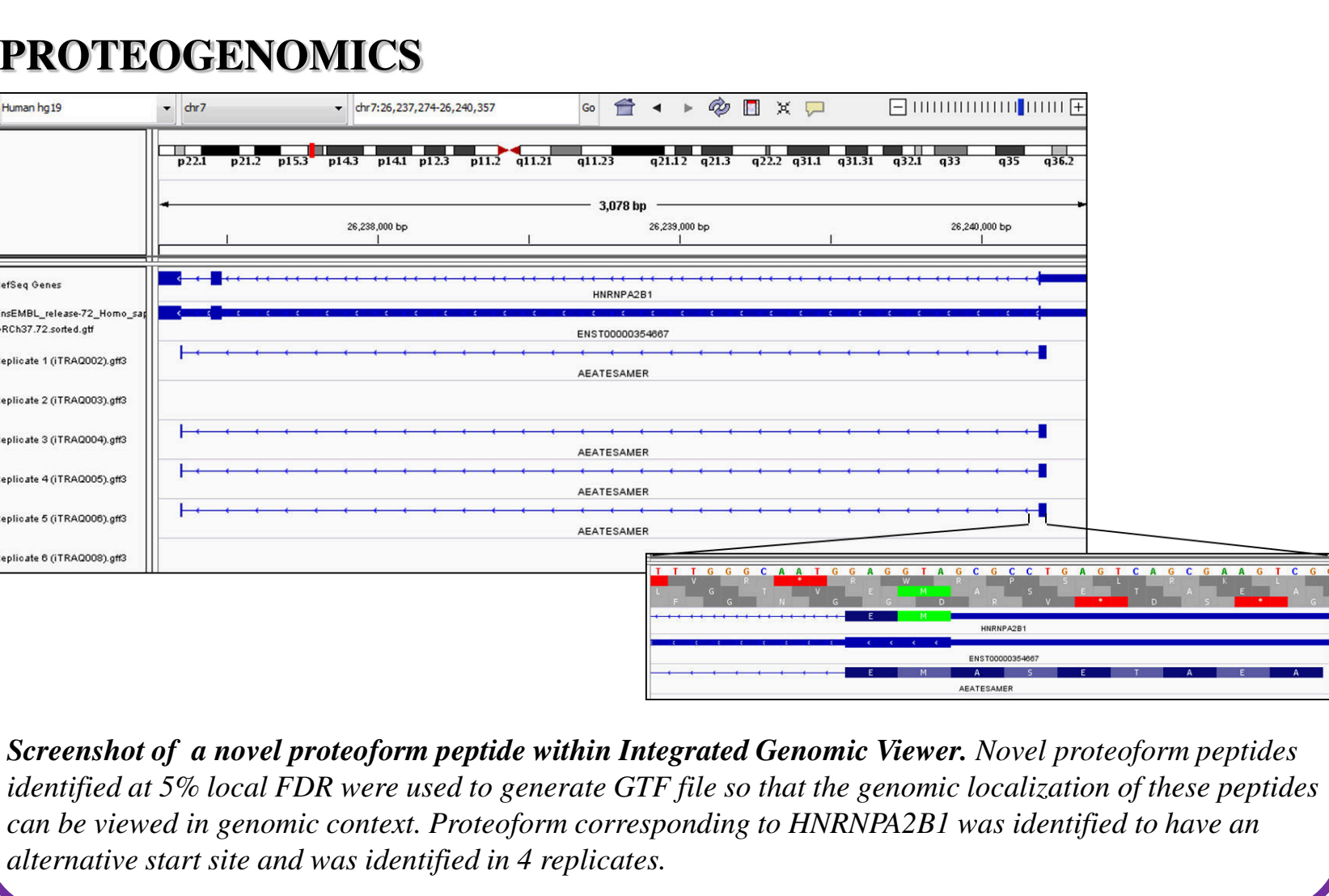
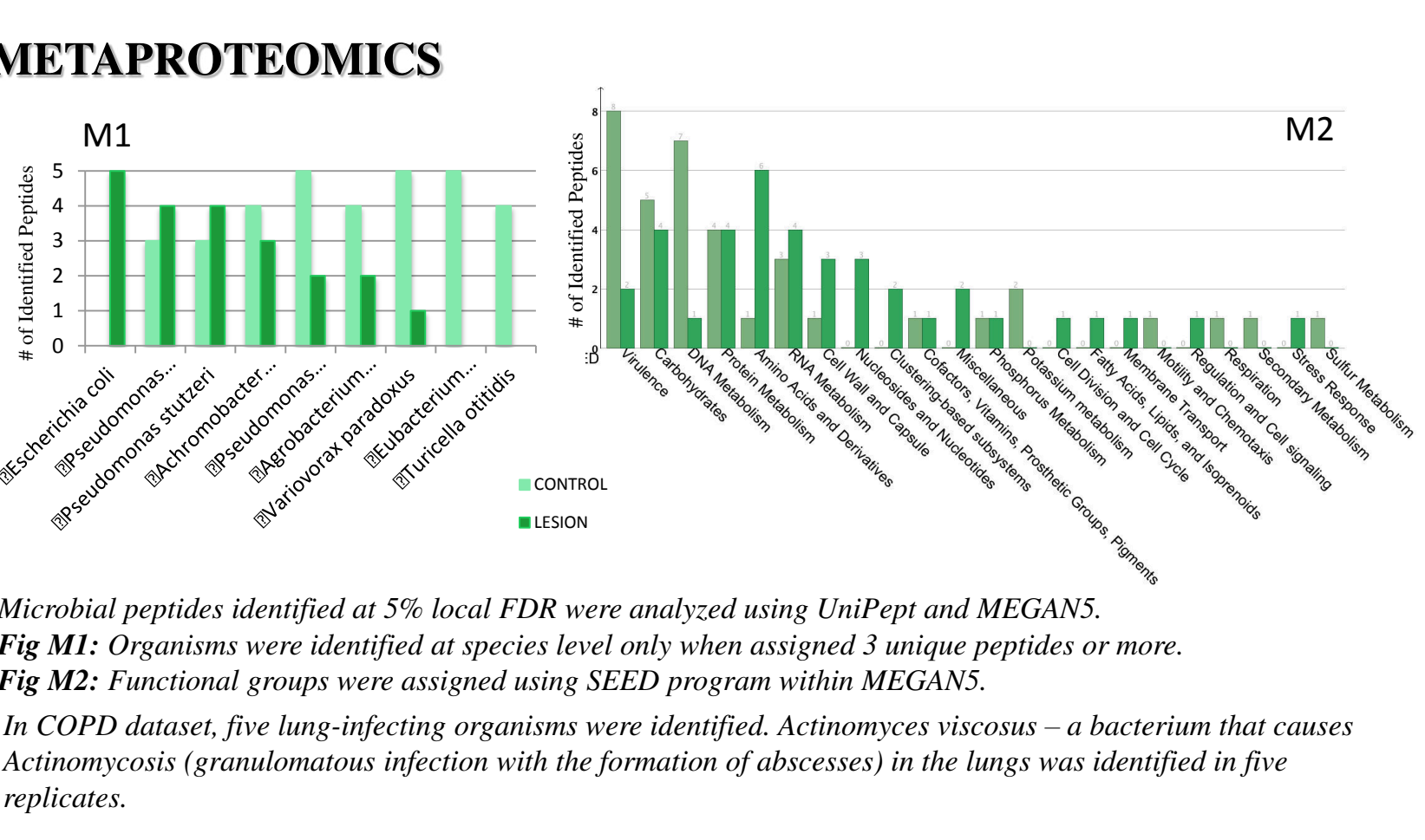


RESULTS SUMMARY

Dataset	RAW Files	Distinct peptides of microbial origin	Number of unique peptides (Species Identified)	Novel proteoform peptides
OPML Control (unlabeled)	7	637	136 (6)	50
OPML Lesion (non-labeled)	7	688	136 (3)	47
Brush Biopsy OSCC (4-plex iTRAQ)	15	1118	166 (6)	6
COPD (10 replicates) (8-plex iTRAQ)	150	87	9 (9)	7



RESULTS



CONCLUSIONS

- We demonstrate the use of a complete platform for tandem metaproteomic / proteogenomic analysis. Workflow for each module/step have been shared for use within Galaxy environment.
- Using both metaproteomic & proteogenomic databases in tandem help in confident identification of microbial peptides & novel proteoforms.
- Using this platform, we have identified microbial peptides & novel proteoforms from both labeled & unlabeled datasets. For example for COPD datasets, the number of identifications from both proteogenomic & metaproteomic databases is limited yet consistent across replicates..
- For metaproteomics studies, identified microbial peptides were used for taxonomic classification (UniPept and MEGAN5) for functional classification (MEGAN5).
- For proteogenomic studies, identified novel proteoforms were validated using PSM evaluation tool and visualizing peptides against the genome.

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