An automated, accessible proteogenomic pipeline for high confidence detection and rigorous validation of novel peptide sequence variants in Galaxy-P

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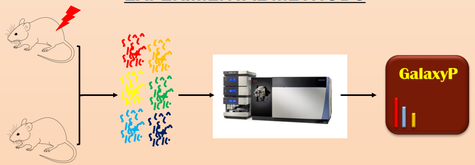
INTRODUCTION

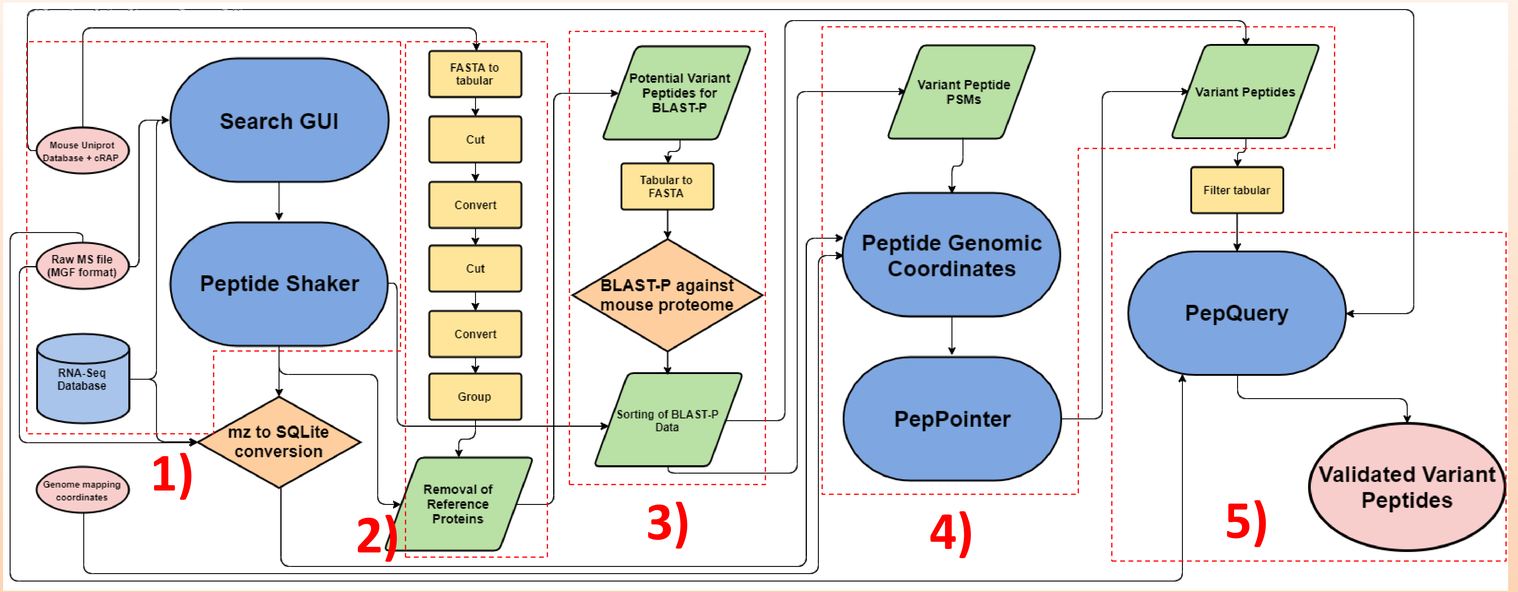
- When bottom-up proteomics experiments utilize FASTA files derived from genomic data, information on proteoforms unique to the samples under analysis is missed (indels, intron retention, alternative splicing, etc.)
- Proteogenomics workflows utilize custom-built FASTA libraries based on transcriptomic data to inform attempts to detect these protein variants
- Many proteogenomics workflows neglect to validate the variants they detect, introducing the potential for false positives
- The PepQuery search engine validates variant peptides by searching MS/MS data against specific variant sequences of interest
- We brought PepQuery into the Galaxy for Proteomics (Galaxy-P) suite and integrated it into an automated workflow to validate newly discovered variant peptides from proteogenomics data
- This workflow was tested using previously created proteogenomics data, and validated variant peptides were verified using targeted mass spectrometry

EXPERIMENTAL METHODS

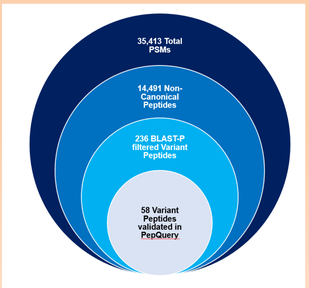


- Samples of control, inflamed (n=3 each) mice were obtained as a part of a study of inflammatory bowel disease
- Proteins were extracted from proximal colon samples and prepped for MS analysis with digestion, TMT-6plex labeling, and concatenation
- Concatenated, labeled peptides were fractionated using high pH reverse-phase fractionation before LC-MS analysis
- Raw MS runs were searched against a FASTA database constructed from RNA-Seq data from comparable samples to determine total peptide spectral matches (PSMs) (Section 1 in workflow)
- Total PSMs are filtered to remove PSMs corresponding to conventional mouse peptides as well as common contaminant peptides (Section 2)
- BLAST-P searching the remaining PSMs against the mouse proteome was used to remove "variant" peptides which have close matches to convention peptides (Section 3)
- Genomic coordinates were assigned to variant peptides (Section 4)
- PepQuery used to validate the identified variant peptides, with the results filtered to remove variants with *any* other potential matches (Section 5)
- Parallel reaction monitoring (PRM) experiments performed to independently validate variant peptides

VARIANT PEPTIDE DETECTION AND VALIDATION WORKFLOW

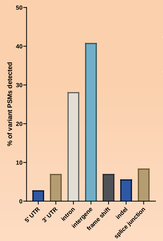


VARIANT PEPTIDES ARE IDENTIFIED AND SUCCESSFULLY VALIDATED VIA OUR WORKFLOW



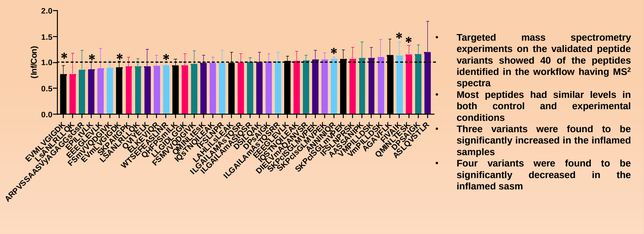
- Initial searches of the eight mouse peptide fractions against an RNA-Seq-supplemented FASTA database revealed 35,413 PSMs
- Comparison against canonical proteins and common MS contaminants leaves half of those PSMs as potential non-canonical peptide variants
- BLAST-P analysis narrows the variant peptides further
- PepQuery analysis shows that 58 variant peptide spectra have no better match in the data, and can be considered valid

Variant Types Detected in MS/MS data



- The variant peptides validated in the workflow correspond to several distinct types of variation
- Most validated variant peptides correspond to peptides from intergenic regions not known to translate into proteins (40% of validated peptides) as well as peptides stemming from retained introns in the proteins (28% of validated peptides)

Ratio of Variant Peptide Abundances in Inflamed and Control Samples



- Targeted mass spectrometry experiments on the validated peptide variants showed 40 of the peptides identified in the workflow having MS² spectra
- Most peptides had similar levels in both control and experimental conditions
- Three variants were found to be significantly increased in the inflamed samples
- Four variants were found to be significantly decreased in the inflamed sasm

SUMMARY

- The PepQuery search engine was brought into the Galaxy-P suite and incorporated into a workflow designed to identify variant peptides in proteogenomic data
- With this new workflow, we were able to identify and validate 58 variant peptides in proximal colon proteogenomics data from an earlier study into inflammatory bowel disease
- Most of the validated 58 variant peptides corresponded to intergenic regions as well as retained introns
- Subsequent validation via targeted mass spectrometry experiments showed direct evidence of 40 of the 58 variant peptides

FUTURE DIRECTIONS

- This workflow will be further tested on open-source proteogenomics datasets to ascertain its ability to detect and validate variant peptides
- An add-on to this workflow is currently in development to automatically generate an inclusion list for the method development of targeted mass spectrometry assays
- A version of this workflow is in development for the label-free quantitation of validated variant peptides in mass spectrometry data

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