A COMPLETE, MODULAR AND FLEXIBLE PROTEOGENOMIC PIPELINE FOR PEPTIDE NEOANTIGEN DISCOVERY AND VERIFICATION

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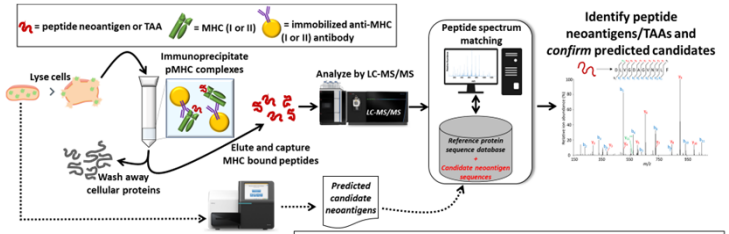
BACKGROUND AND RATIONALE

- Immunotherapy studies in cancer seek to identify non-normal peptide sequences (**neoantigens**) derived from tumor-specific proteoforms, and also aberrantly expressed tumor-associated antigens (**TAAs**) from reference protein sequences, which are bound to the class I or II major histocompatibility complex (**MHC**) and activate the immune system
- Identification of neoantigens relies on next-generation sequencing (**NGS**) to identify genomic or transcriptomic alterations that may encode non-normal, candidate neoantigen peptides, coupled with algorithms that predict their binding to the MHC
- Mass spectrometry (**MS**)-based immunopeptidomics enrich the MHC and utilize LC-tandem mass spectrometry (**LC-MS/MS**) analysis to detect and identify MHC-bound peptides, directly confirming predicted candidates with potential for immunotherapy, including those carrying post-translational modifications (**PTMs**)



Overview of MS-based immunopeptidomics workflow

NEOANTIGEN DISCOVERY AND VERIFICATION: REQUIREMENTS AND SOLUTIONS

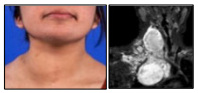
- Multi-omic integration of neoantigen prediction tools from NGS data with MS-based peptidomic analysis tools is a necessity
- Sequence database searching of immunopeptidomic MS/MS data to efficiently identify MHC-bound peptides using "no enzyme" constraints, identifying PTMs, and quantifying peptide abundance using label-free quantification (**LFQ**)
- Ideally these tools would be contained in a single environment, accessible by bench researchers complete with training resources to empower their adoption

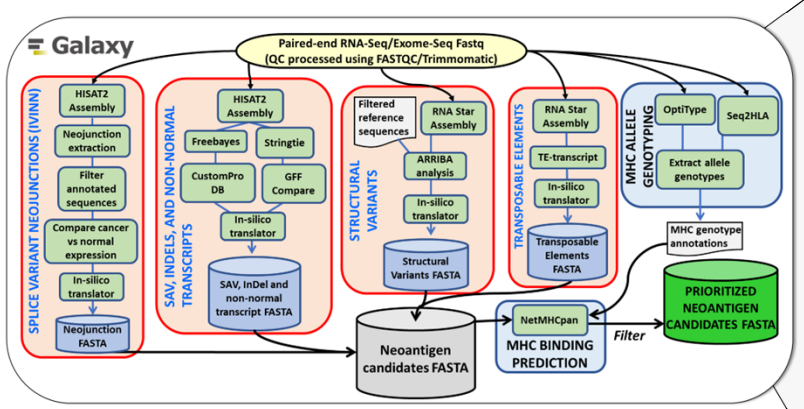
A solution: Integrated multi-omic analysis within the Galaxy ecosystem

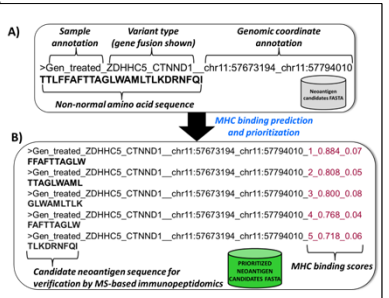
- The Galaxy ecosystem offers a platform to build multi-omic workflows integrating NGS analysis tools, MS/MS analysis tools, complete with access on publicly available instances, and training via the Galaxy Training Network
- Building on the Galaxy for proteomics (**Galaxy-P**) software suite, we are developing workflows for multi-omic neoantigen discovery and verification using state-of-the-art tools, including NGS analysis tools, the FragPipe tool suite (**fragpipe.nesvilab.org**) and PepQuery (**www.pepquery.org**)

RESULTS: NEOANTIGEN PREDICTION FROM NGS DATA

- **Developments guided by ongoing multi-omic study of neoantigens/TAAs from malignant peripheral nerve sheath tumors (MPNST) cells**

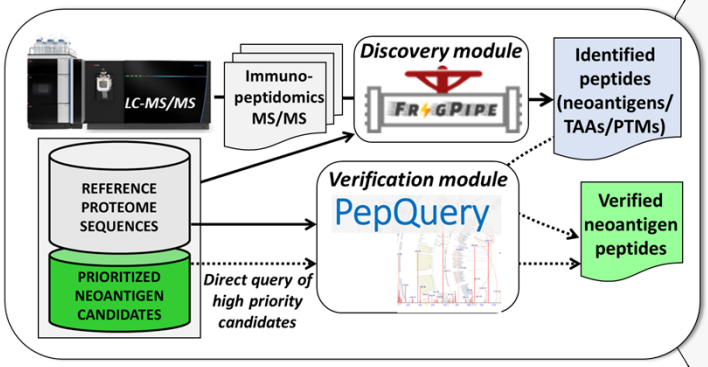




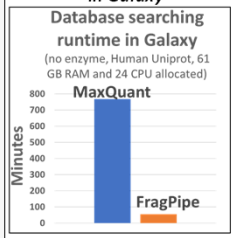


Example FASTA output of candidate neoantigen producing structural variant (A) and prioritization by MHC-binding prediction to generate an annotated FASTA database of prioritized candidates (B).

RESULTS: PEPTIDE NEOANTIGEN DISCOVERY AND VERIFICATION

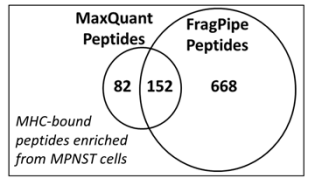


FragPipe performance in Galaxy



Example verified TAA peptides (MPNST cells)

Gene	Peptide	Description
MAGEA4	KVLEHVVRV	Melanoma-associated antigen 4
MAGEC2	GVYAGREHFV	Melanoma-associated antigen C2
MAGEA10	GLYDGMEHL	Melanoma-associated antigen 10
MAGEA2	QVMPKTGL	Melanoma-associated antigen 2
MAGEA3	EVDPIGHLY	Melanoma-associated antigen 3
PRAME	SIQSRYISM	Melanoma antigen expressed in tumors
SOX9	KLADQYPHL	Transcription factor SOX-10



FUTURE PLANS

- Optimize and harden workflows for production-ready deployment
- Publish optimized workflows and tools on accessible Galaxy gateways and in the Galaxy Tool Shed
- Develop Galaxy Training Network tutorials for online, on-demand access

Relevant References

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Acknowledgements

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