



Automated Quantification and Analysis of SILAC-iTRAQ Dual-labeled Data

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Overview

- We have developed software that enables development of a pipeline for automated quantification of dual-labeled data
- We tied together existing, proven tools, in a novel manner to develop this software pipeline
- Implementation was done on the Galaxy-P framework, an extension of Galaxy. Galaxy is an open, web-based platform for data intensive biomedical research¹.

Methods

- MaxQuant² is used for SILAC quantification
- Extension to iQuant³ is used for iTRAQ quantification
- Mconvert (part of ProteoWizard platform) is used for file conversion and input file splitting
- Galaxy-P is used to tie together the different tools and generate a combined quantification report.

Results

- Software for automated quantification of dual-labeled SILAC-iTRAQ data in an open source, web-based workflow application (Galaxy-P)

Introduction

- Development of software for analysis of proteomics data has traditionally depended heavily on whether labeling was done metabolically (e.g., SILAC) or chemically (e.g., iTRAQ)
- Both approaches are generally mutually exclusive precluding dual (metabolic and chemical) labeling
- SILAC-iTRAQ dual-labeling offers capabilities for studying protein dynamics not accessible using either SILAC or iTRAQ technologies
- Protein abundance as well as degree of modification can be measured simultaneously on the same HPLC-MS run
- SILAC-iTRAQ dual-labeling makes it possible to determine dynamic proteomic profiles at different stages of a cellular process
- Currently there is no software available for quantification of SILAC-iTRAQ dual-labeled data
- We extend previously developed software for accurate protein quantification of iTRAQ data to enable quantification of SILAC-iTRAQ dual-labeled data

Dual-labeling

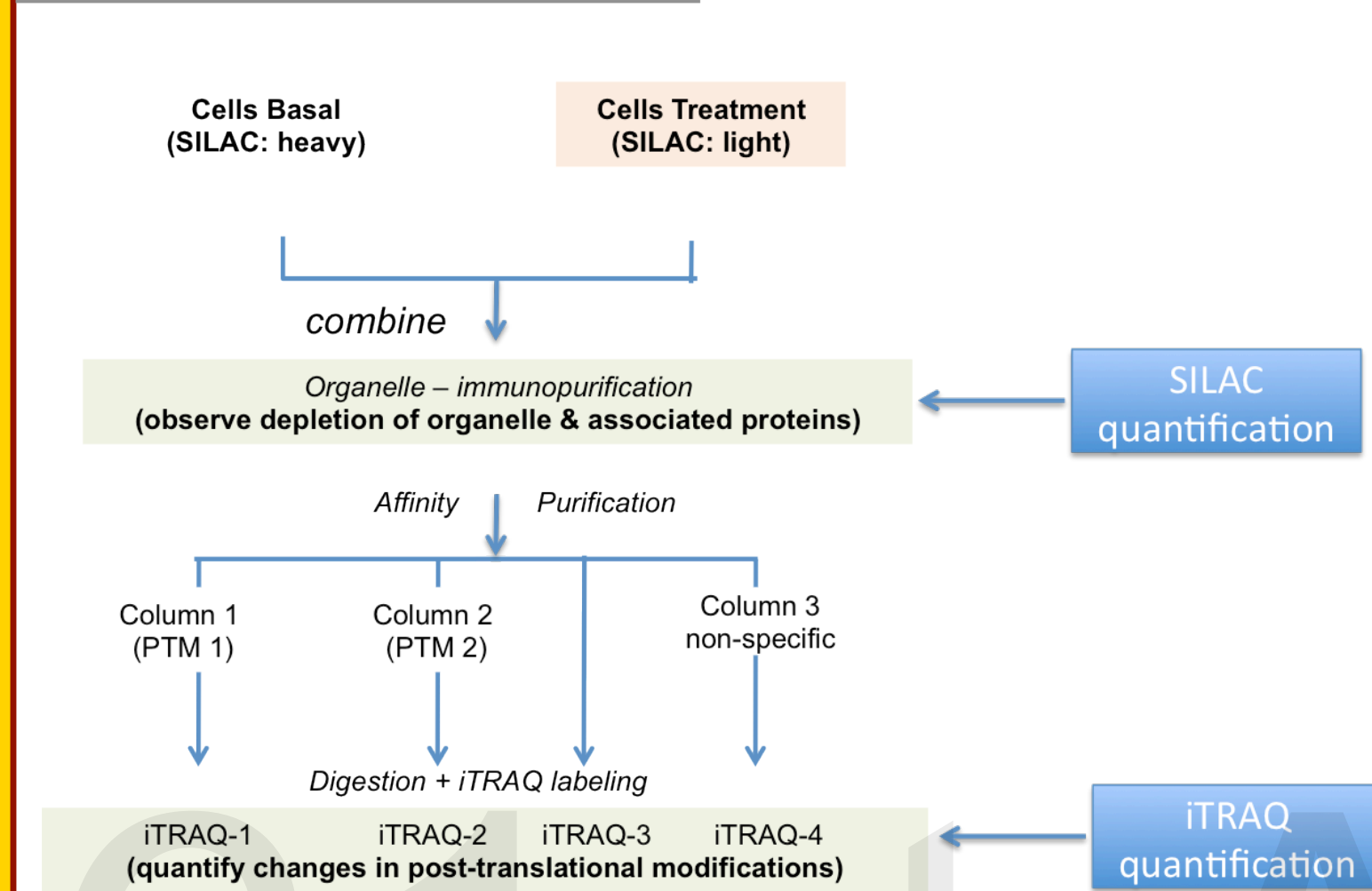


Figure 1: schematic diagram of SILAC-iTRAQ dual-labeling

SILAC: Stable Isotope Labeling by Amino acids in Cell culture
iTRAQ: isobaric Tag for Relative and Absolute Quantification
PTM: post-translational modification

Quantification Pipeline

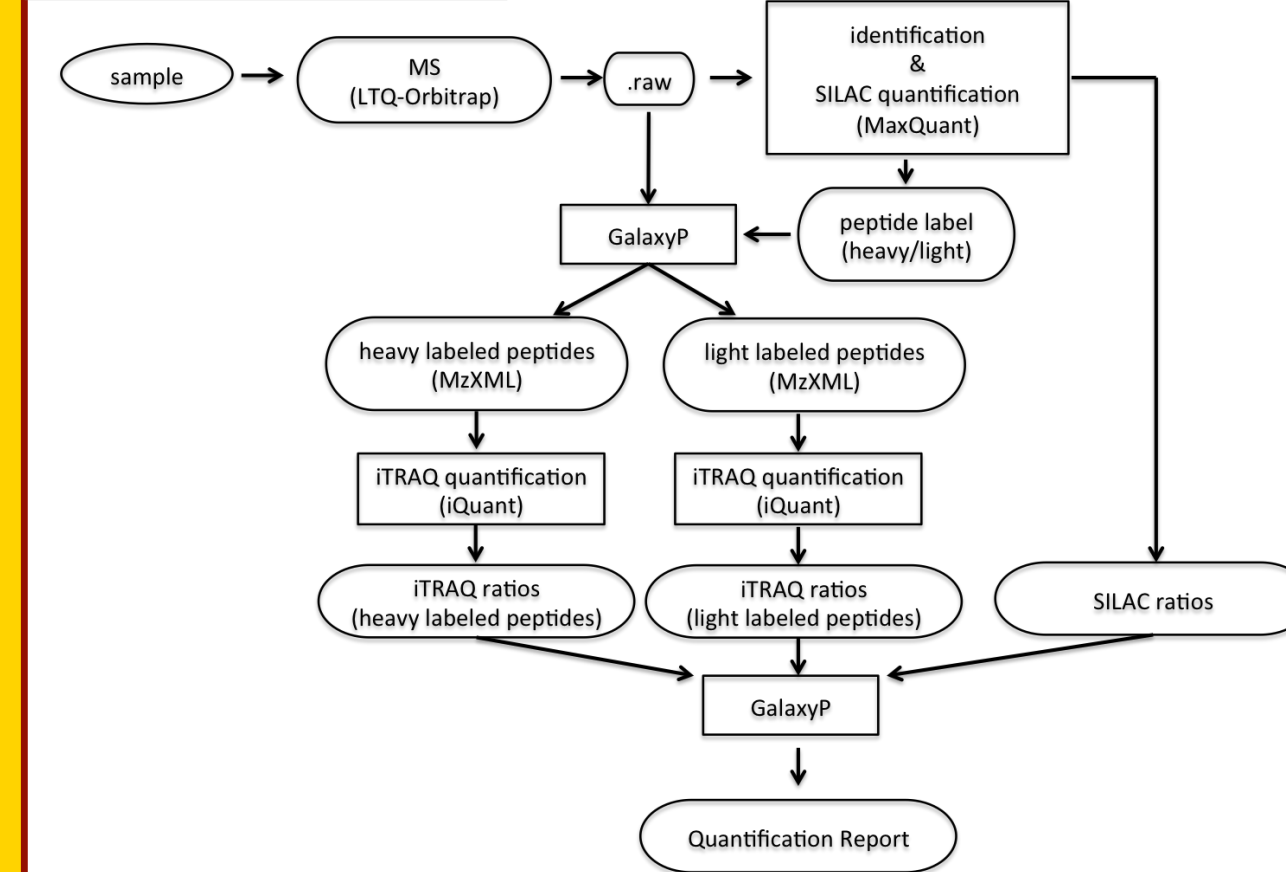


Figure 2: schematic diagram of the quantification pipeline

- Consists of five main steps:
 - peptide grouping and protein identification
 - SILAC quantification
 - separation of heavy labeled peptides and light labeled peptides
 - iTRAQ quantification
 - generation of a combined quantification

Results

- We have successfully implemented and tested this workflow using test cell samples that were dual SILAC-iTRAQ labeled on our local web-based server
- All tools used in implementing this workflow are open source and available to any user

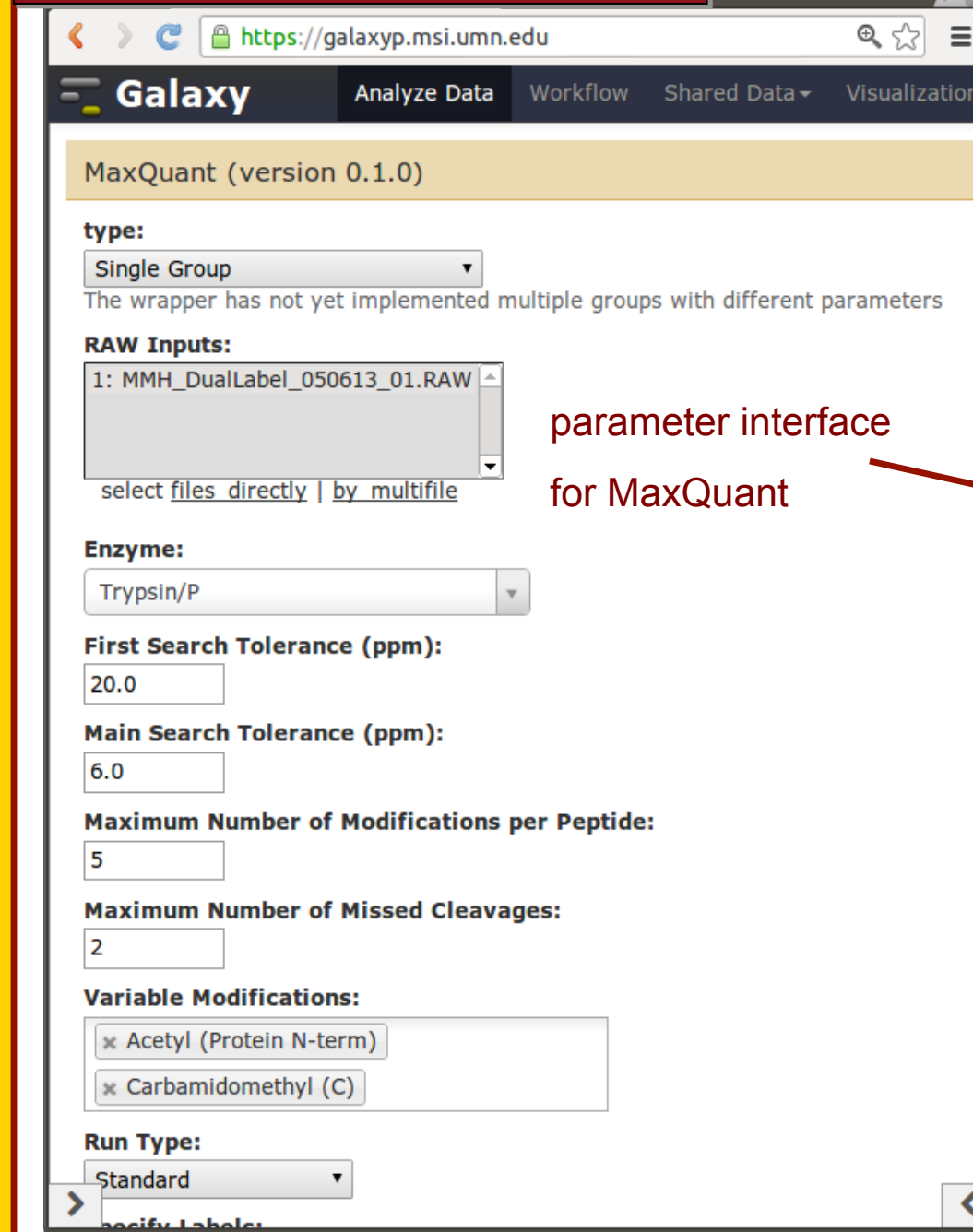
Future Work

- Package the software for easy local installation by users who wish to have their own local copy of this web-based application
- Make Amazon cloud images available as well as an easy interface for launching them for users wishing to use this tool without any additional customization
- Add a cross-platform, open-source alternative to MaxQuant (e.g., SILACAnalyzer which is part of OpenMS toolkit) for SILAC quantification

References / Acknowledgements

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Implementations of pipeline in Galaxy-P



parameter interface for MaxQuant

RAW file from instrument

protein database (fasta file)

