

A New Lipid Software Workflow for Processing Orbitrap-based Global Lipidomics Data in Translational and Systems Biology Research

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Overview

Purpose: We present a new workflow for high-resolution Thermo Scientific™ Orbitrap™-based mass spectrometers for lipidomics using a model system consisting of a wild-type strain vs. knockout for Co-Q production in yeast¹.

Methods: Lipids in yeast mitochondria were analyzed by high resolution LC-MS and MS/MS. Lipid Search® software, an MS² based search using a comprehensive lipid database, was used to identify the lipid species and determine significant differences.

Results: The yeast lipidomics results obtained from the LC/MS data using Lipid Search are comparable to results obtained using infusion lipidomics. We also compared the lipids identified using metabolomics analysis of the same data set – component finding and molecular weight (MW) search for assignment of metabolites and lipids. Due to the complexity of lipid extracts we found that the comprehensive lipid database MS² search method is superior to the accurate mass based MW search for lipidomics.

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Methods

Phenotypes of WT (wild-type) and Knockout (KO) Yeast Strains (*S. Cerevisiae*)

WT yeast continue to grow after glucose is exhausted from the media (Diauxic shift point) whereas KO yeast have a defect in Coenzyme Q production and do not grow after the shift. Duplicate biological replicates of WT and KO yeast were collected post shift for metabolomic/lipidomic analyses and analyzed by LC-MS.

Sample Preparation

Yeast were treated with zymolase, homogenized and mitochondria were enriched by differential centrifugation. Mitochondrial protein levels were determined by BCA assay. Mitochondria (~0.25 mg) were extracted 3 times with 400 µL of IPA for 10 min at 4 °C. After centrifugation, supernatants were combined and vacuum dried. Samples were dissolved in 250 µL of 65:35:5 Acetonitrile, Isopropanol, Water with 5 µg/mL17:0 PG.

Liquid Chromatography–Mass Spectrometry (LC-MS)

Thermo Scientific™ Accela™ 1250 chromatograph and Accela Open autosampler, 10 µL Injection. Column: 2.1 x 100 mm C18, 2.7µm operated at 260 µL/min and 55 °C. The RP HPLC method¹ is described in S. Bird, et al., *Anal. Chem.* **2011**, 83, 940–949, 6648–6657. A Thermo Scientific™ Q Exactive™ high-resolution Orbitrap mass spectrometer was operated at 70K resolution for electrospray ionization pos. ion LC-MS and 35K for Top5 MS/MS (CE 35).

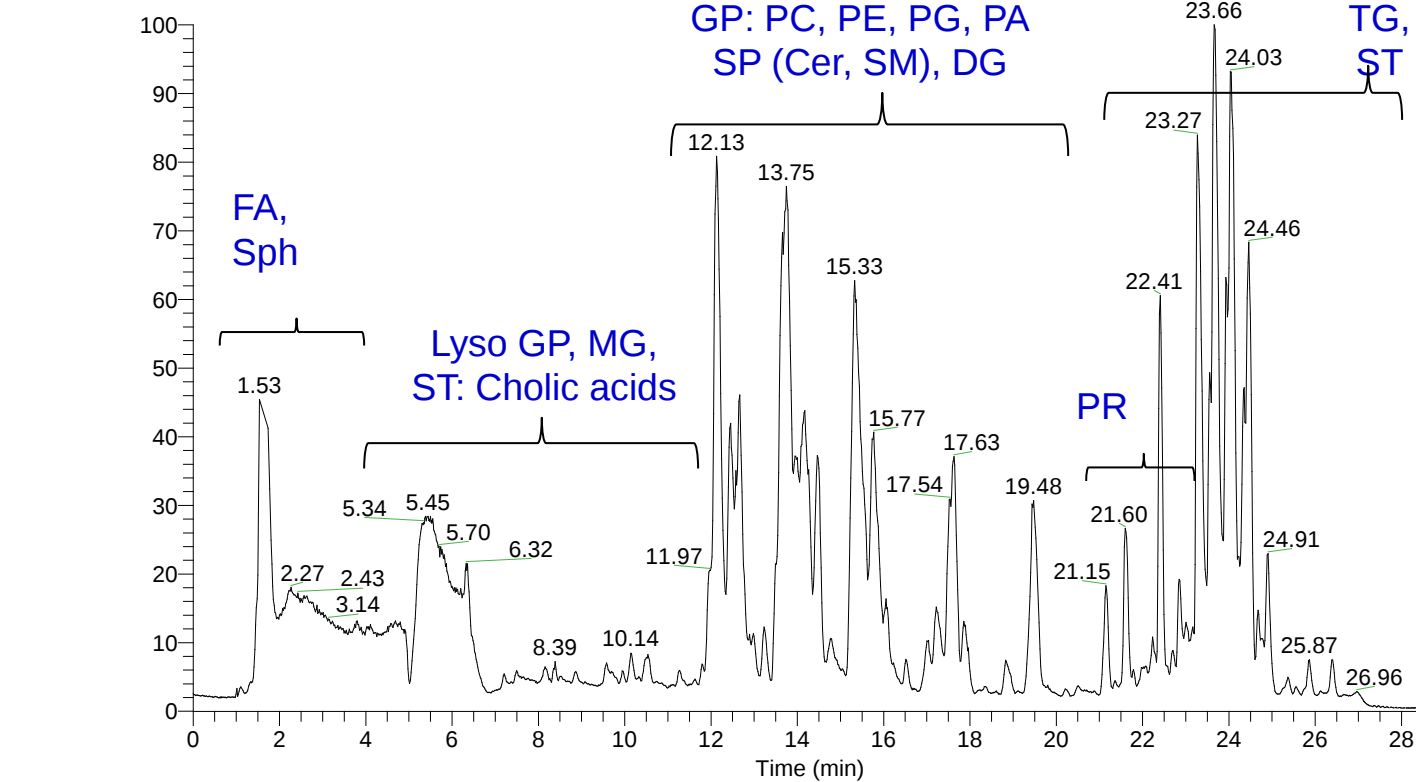
Data Analysis Software

Metabolomics –Thermo Scientific™ SIEVE™ and Lipidomics – Thermo Scientific™ Lipid Search™.

Table 1. Lipid complexity from the LIPID MAPS Structure Database (LMSD)²

Lipid Category		# Class	# Sub-Class	# Lipids
FA	Fatty acyis	14	36	5,787
GL	Glycerolipids	6	19	7,568
GP	Glycerophospholipids	21	120	8,001
SP	Sphingolipids	10	31	4,317
ST	Sterol lipids	6	38	2,678
PR	Prenol lipids	5	21	1,200
SL	Saccharolipids	6	7	1,293
PK	Polyketides	15	28	6,741
Total		83	300	37,585

FIGURE 1. LC-MS chromatograms of lipids from WT and KO yeast.

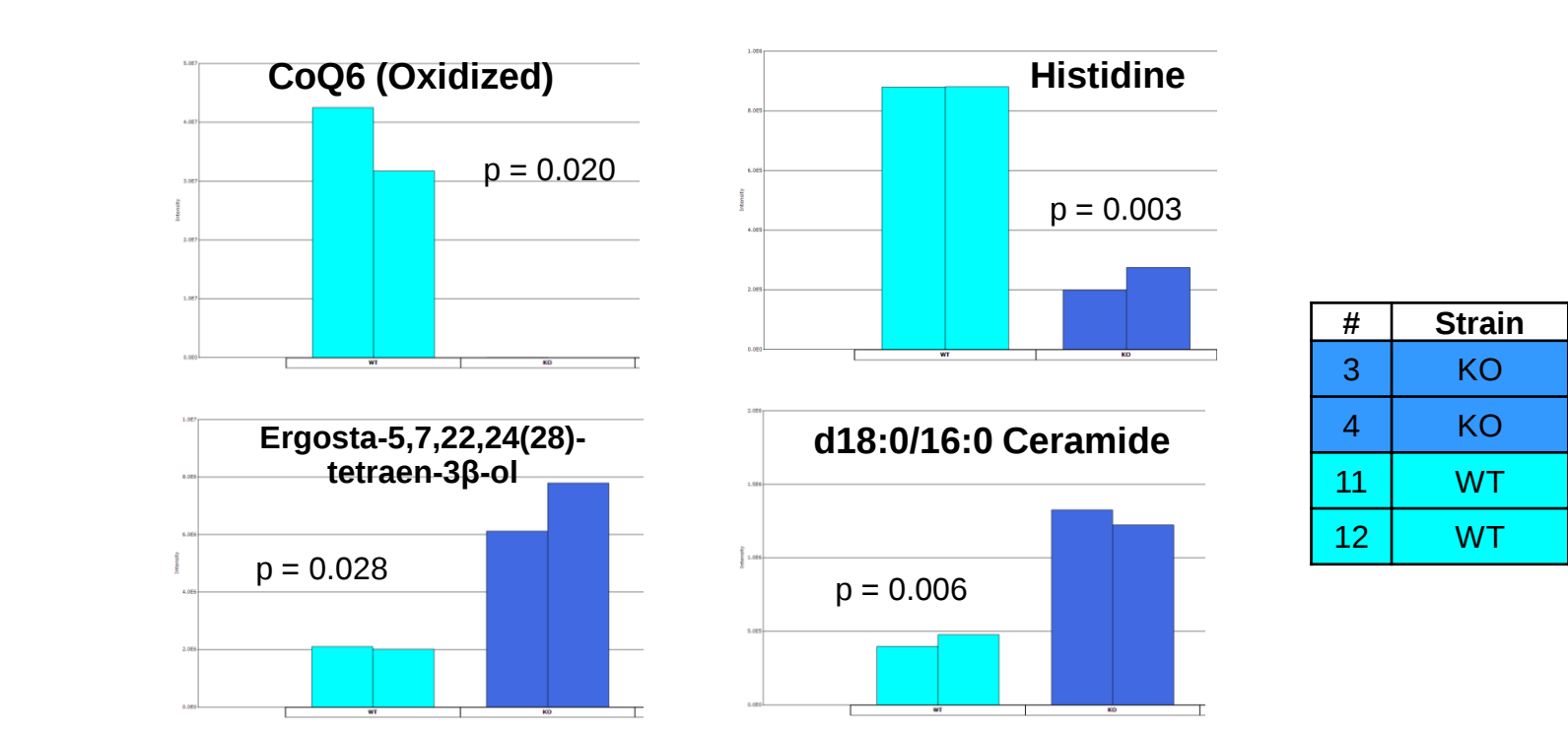


Results

High-Resolution LC-MS Data – Metabolomics Analysis

To characterize the yeast phenotypes we analyzed the sample extracts using an LC-MS method suited for analysis of both metabolites and lipids. The LC-MS chromatogram from WT yeast (Figure 1) shows the regions where lipid classes elute during the LC gradient. Metabolomics analysis using an accurate-mass search tentatively identified 160 metabolites and lipids were present. t-Test statistics (Figure 2) show key metabolite differences.

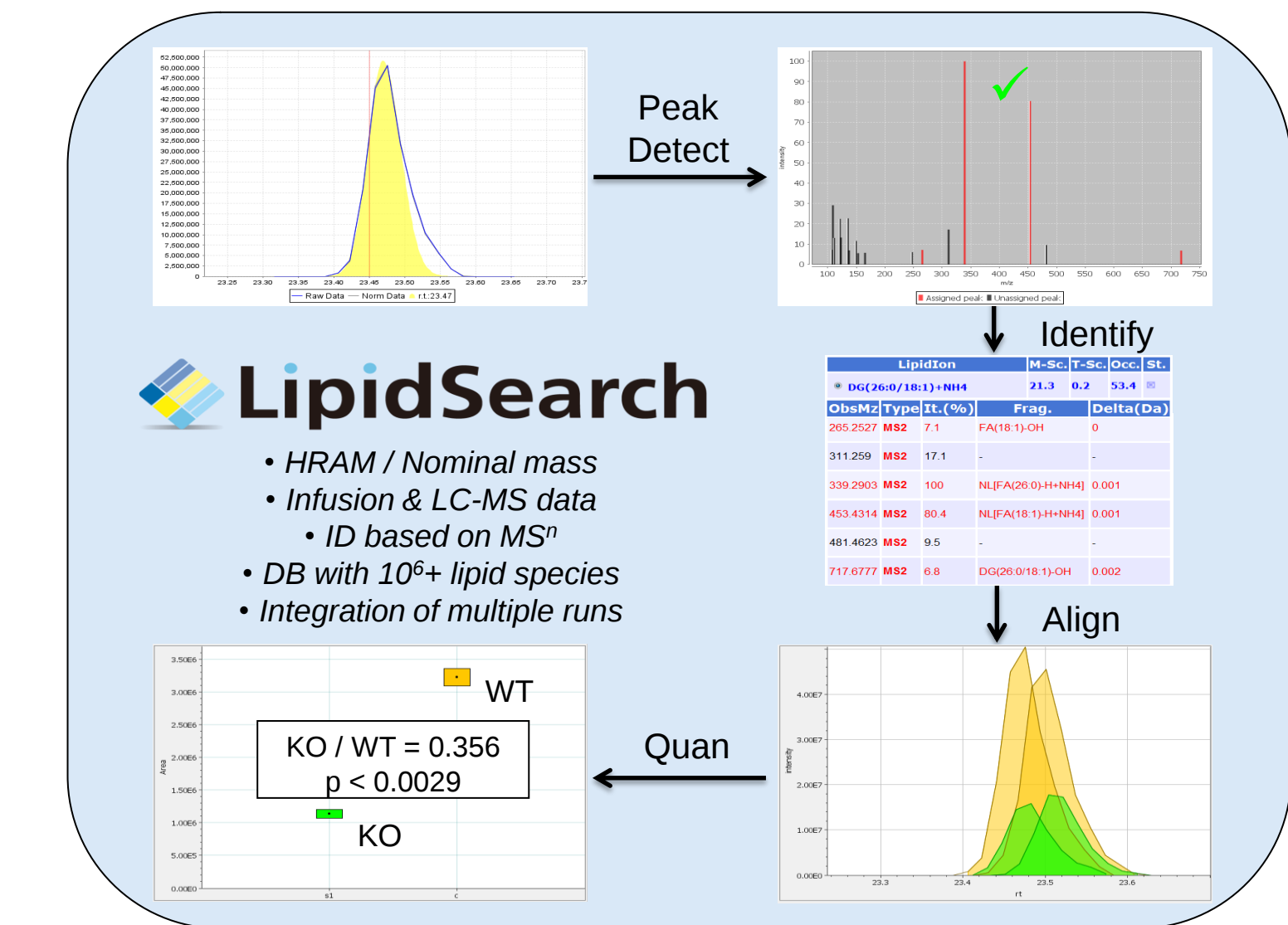
FIGURE 2. Significant metabolite differences observed WT vs. KO yeast.



LC-MS/MS Data Processing Workflow using Lipid Search Software (Figure 3)

- 1) Peak Detection.** Read raw files, MSⁿ and precursor ion accurate masses.
- 2) Identification.** Candidate molecular species are identified by searching a large database > 1,000,000 entries of accurate masses (lipid precursor and fragment ions) predicted from each potential lipid structure and positive / negative ion adducts.
- 3) Alignment.** The search results for each individual sample are aligned within a time window and the results are combined into a single report.
- 4) Quantification.** The accurate-mass extracted ion chromatograms are integrated for each identified lipid precursor and the peak areas are obtained.
- 5) Statistical Analysis.** t-Tests determine which lipid species are significantly different between sample vs. control groups, and results are displayed in a whisker plot.

FIGURE 3. Lipid Search software LC-MS workflow.



Lipid Search Identification and Alignment

LC-MS raw data files containing full scan and data dependent-MS/MS were searched for PL, GL, SP and Co-enzyme lipid classes using a mass tolerance of 5 ppm for precursor ions and 10 ppm for product ions (Figure 4a). The search results from the 4 samples were aligned using a 0.25 min tolerance window and a combined report was generated (Figure 4b).

FIGURE 4a. Search results for yeast lipids

	Name	RawData	Type	ExpType	Process	Result	Regist	Update
2013-05-25 21:54:24	300Post_1aw		Producer	LC	F I Q	0.238 0.2521	20130505 21:54:24	20130505 22:01:01
2013-05-25 21:54:24	300Post_1aw		Producer	LC	F I Q	0.202 0.2621	20130505 21:54:24	20130505 22:01:01
2013-05-25 21:54:24	120VPost_1aw		Producer	LC	F I Q	0.202 0.2720	20130505 21:54:24	20130505 21:58:01
2013-05-25 21:54:24	170VPost_1aw		Producer	LC	F I Q	0.245 0.2609	20130505 21:54:24	20130505 21:58:01