



Metabolomics and Integrative omics: from data production to analysis

28-29 Aprile 2022 | BARI



Metabolomics Workflow and Data Analysis: a Yeast case

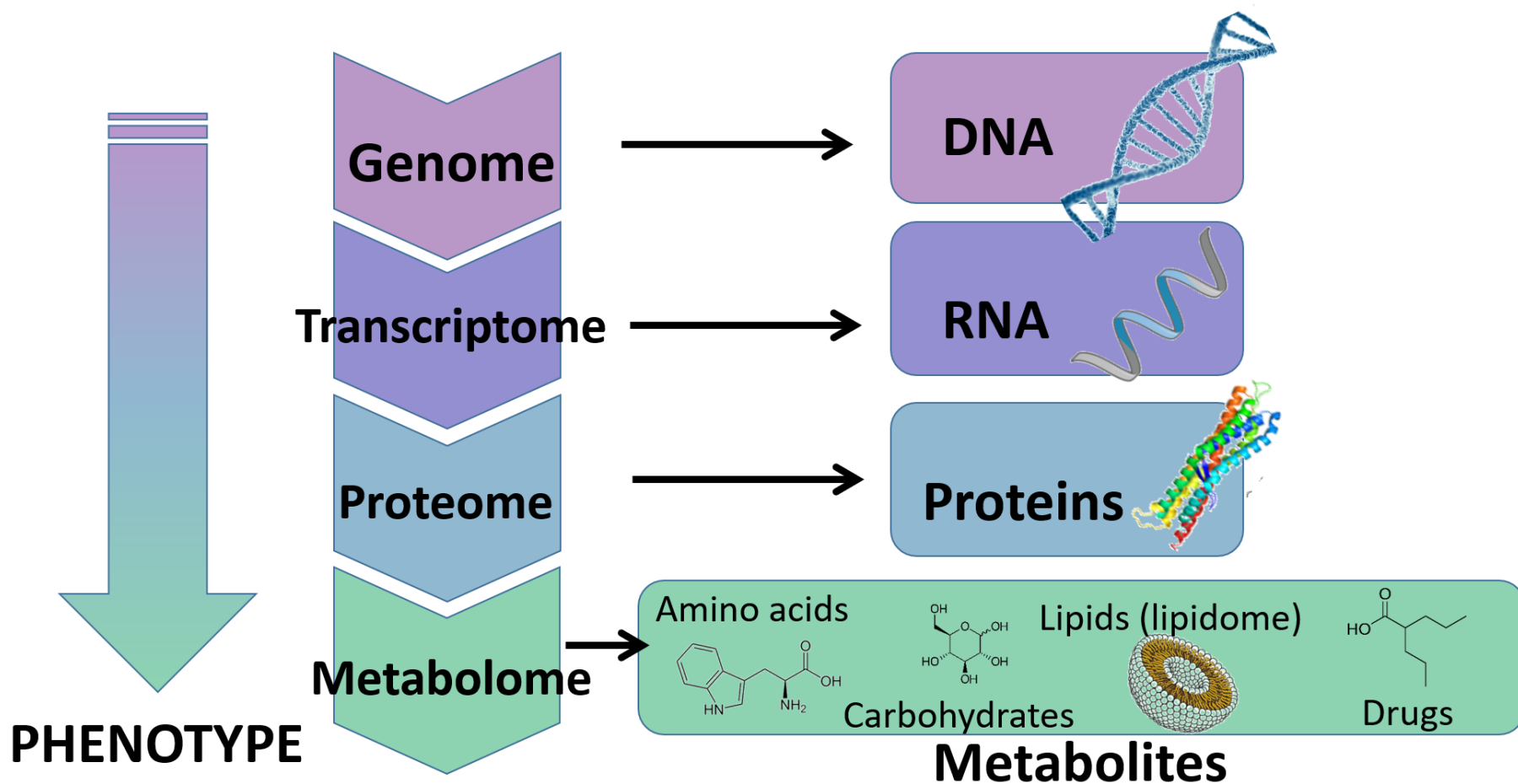
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Institute of Biomembranes, Bioenergetics
and Molecular Biotechnologies

Bari, Italy

«Integrative-Omics»



Experimental Design

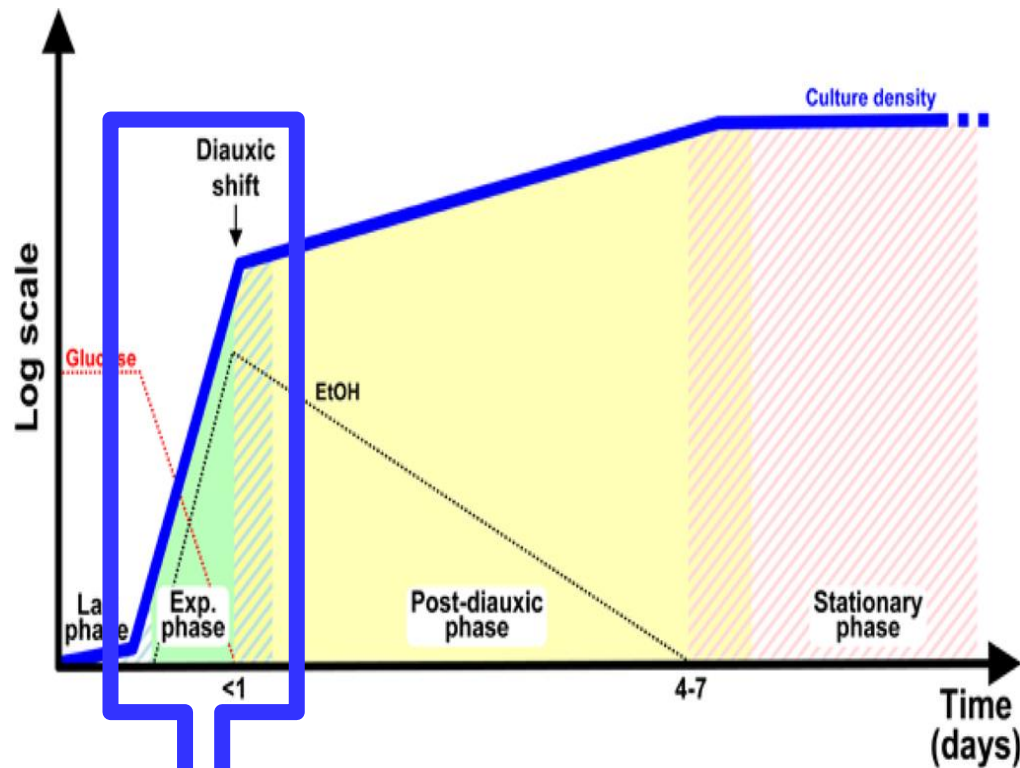
- Aim(s) of Metabolomics analysis
- Which matrix?
- Which target compounds?
- Suitable Metabolomics analysis System



Saccharomyces cerevisiae as a model organism

Yeast can be easily **grown in** strictly defined liquid or solid cultures allowing complete **control** of environmental parameters

Growth phases of *Saccharomyces cerevisiae*

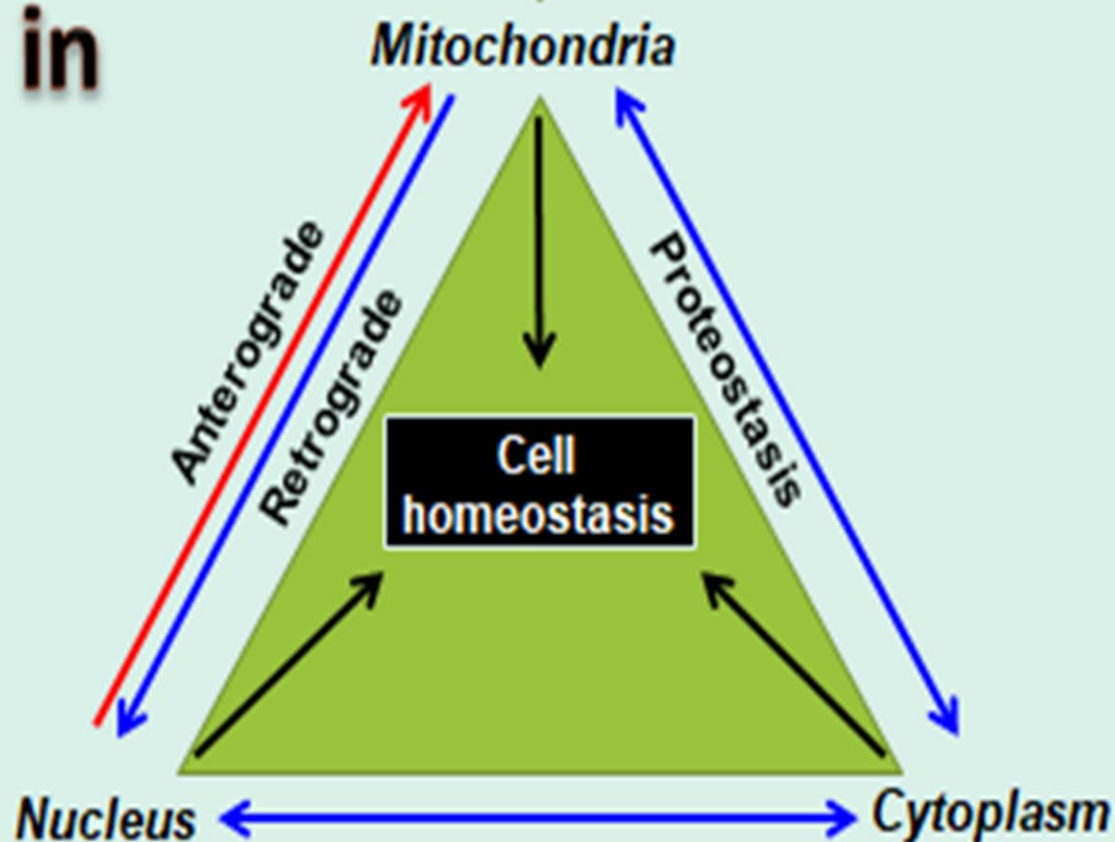


**Proliferating cell
model**

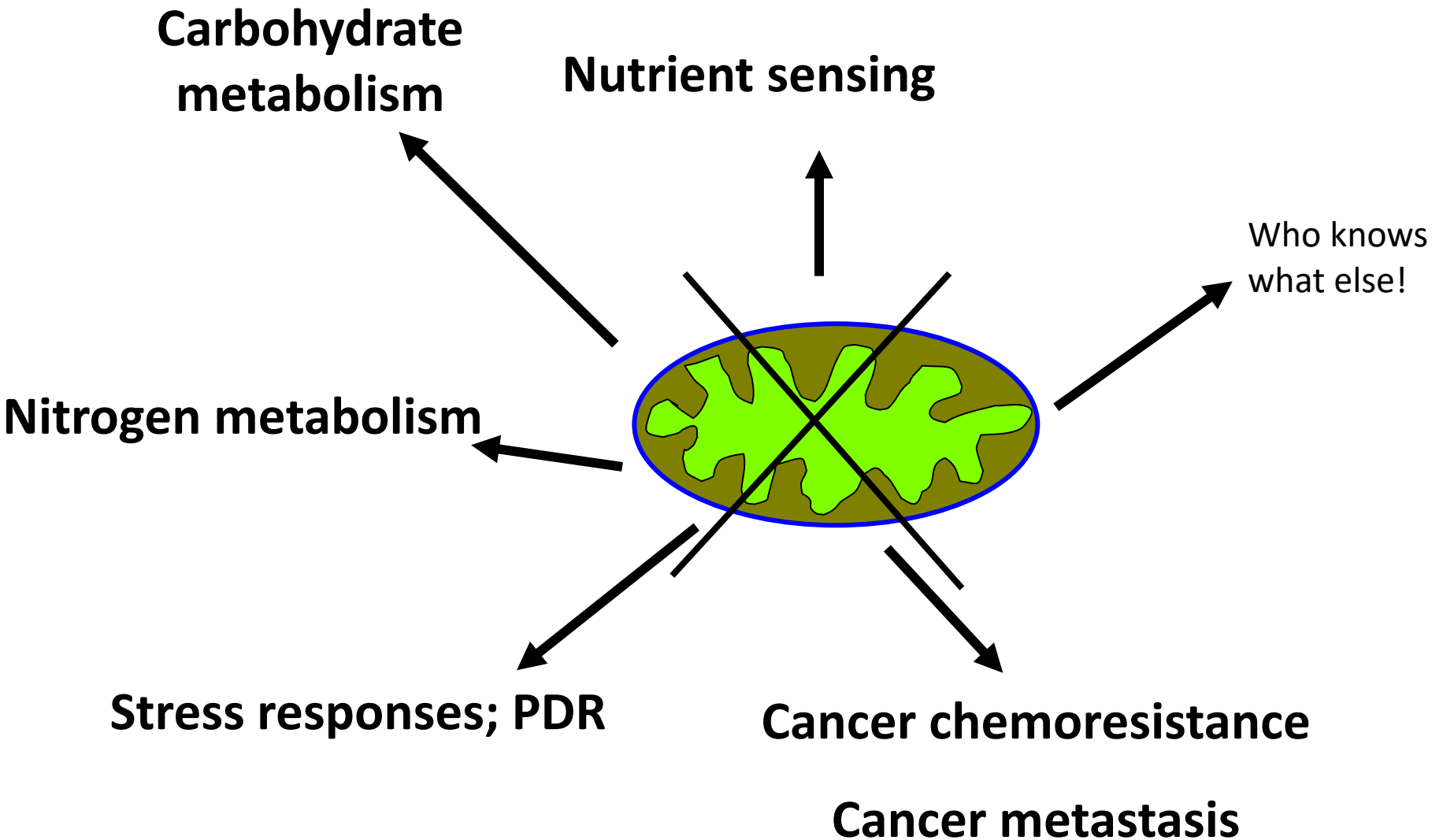
Environmental cues

out

in



Mitochondrial Dysfunction

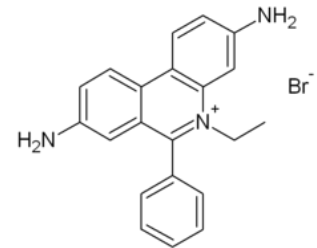


Case study

*In order to study mitochondrial dysfunction we used cells **depleted of mtDNA**.*

Sample Preparation :

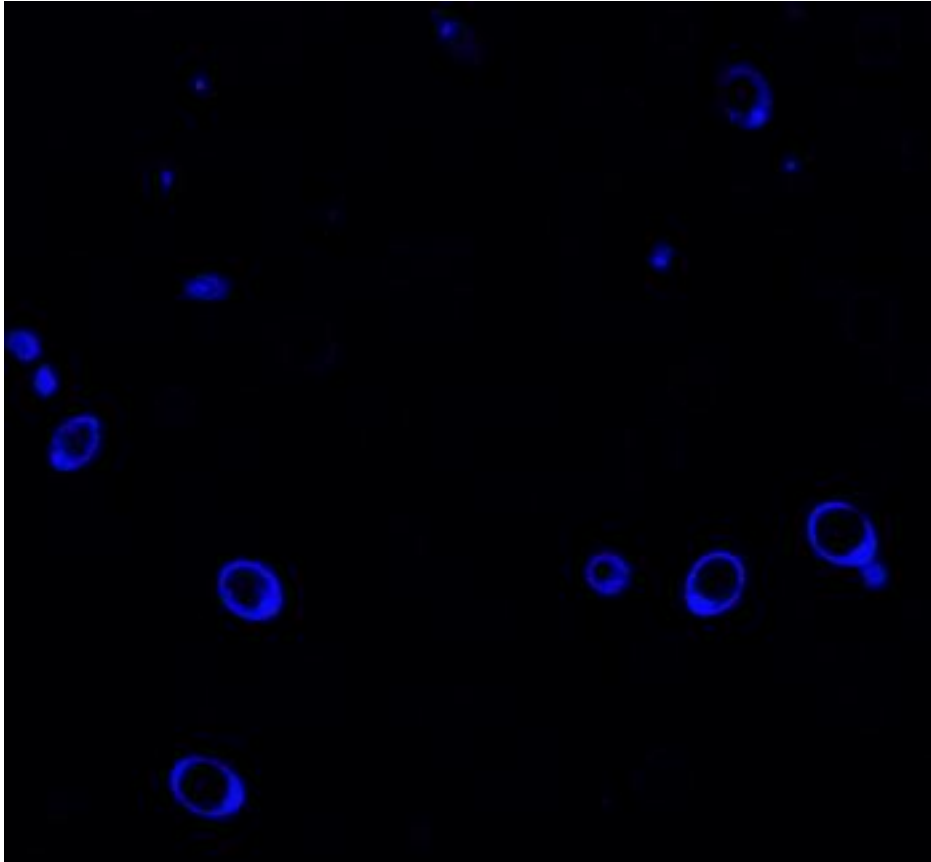
- **YSBN1** prototrophic yeast strain
- **EtBr** to remove mtDNA
- Selective plating on glycerol medium for ρ^0 isolation



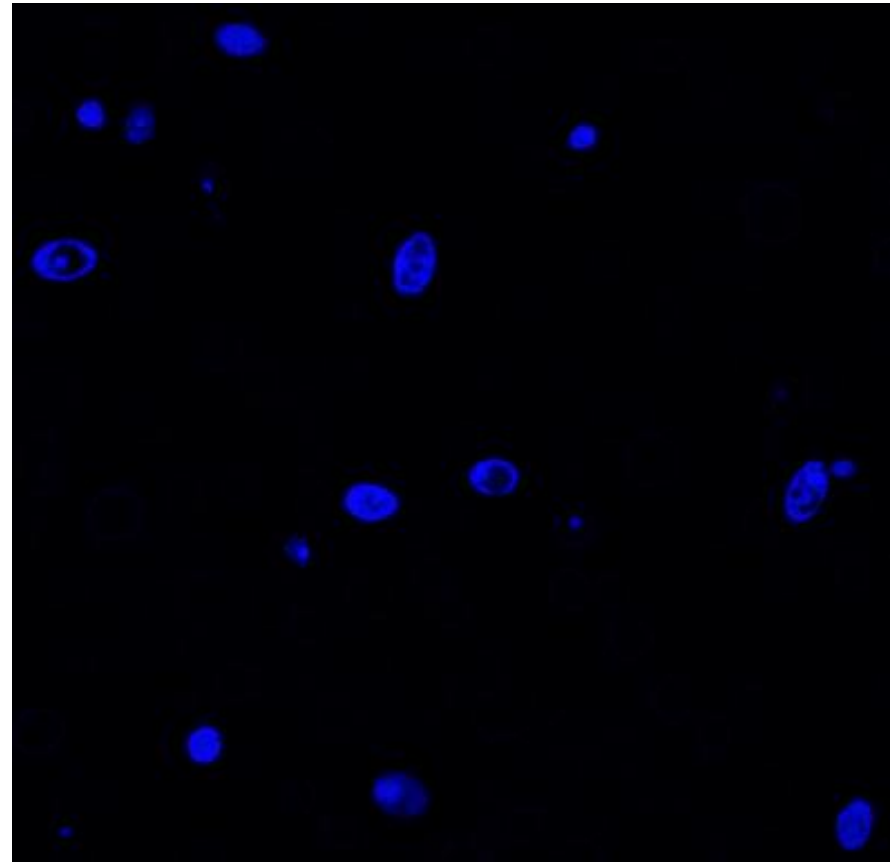
Ethidium bromide

Confocal Microscopy

ρ^0



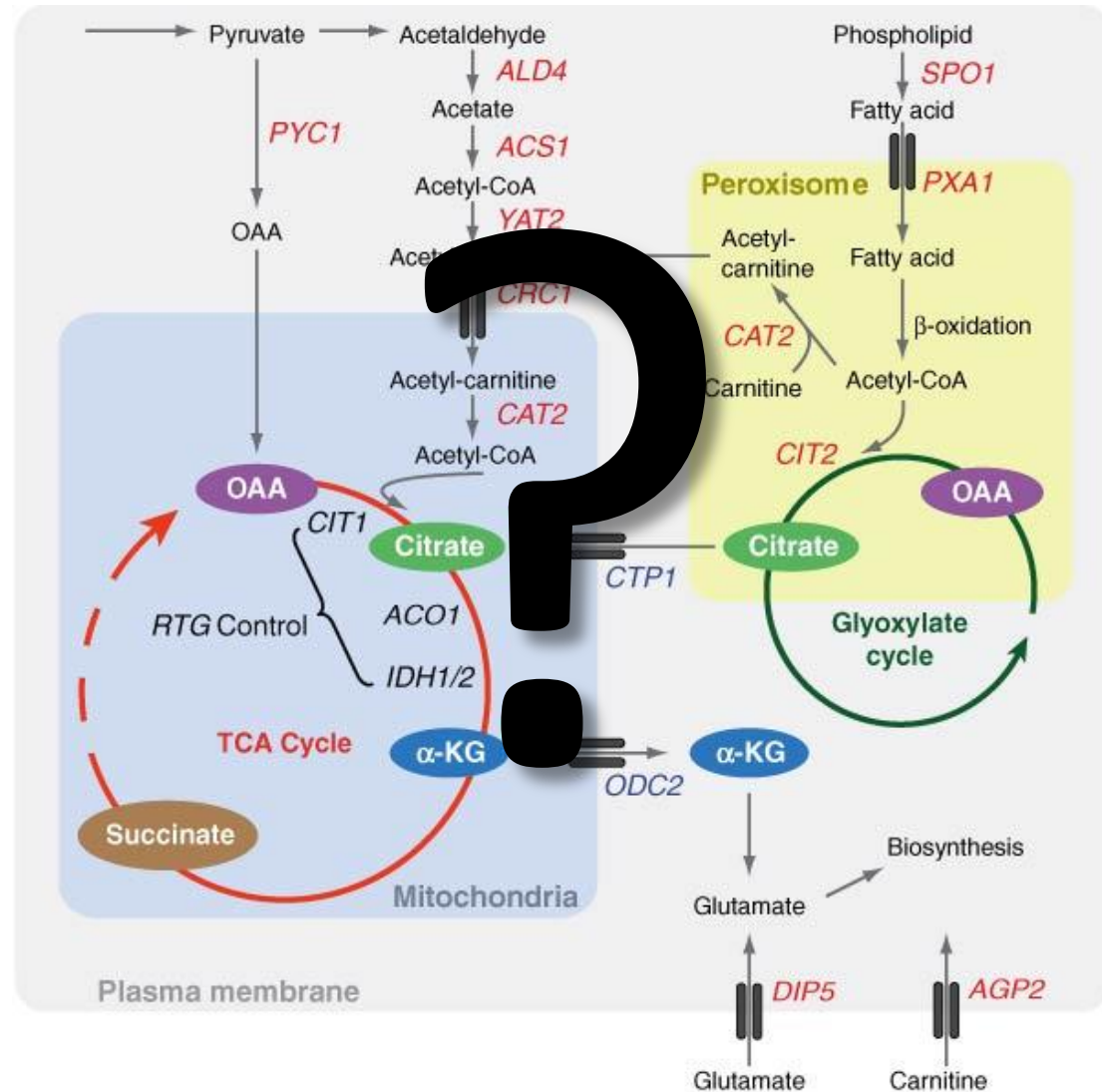
ρ^+



DAPI stained cells

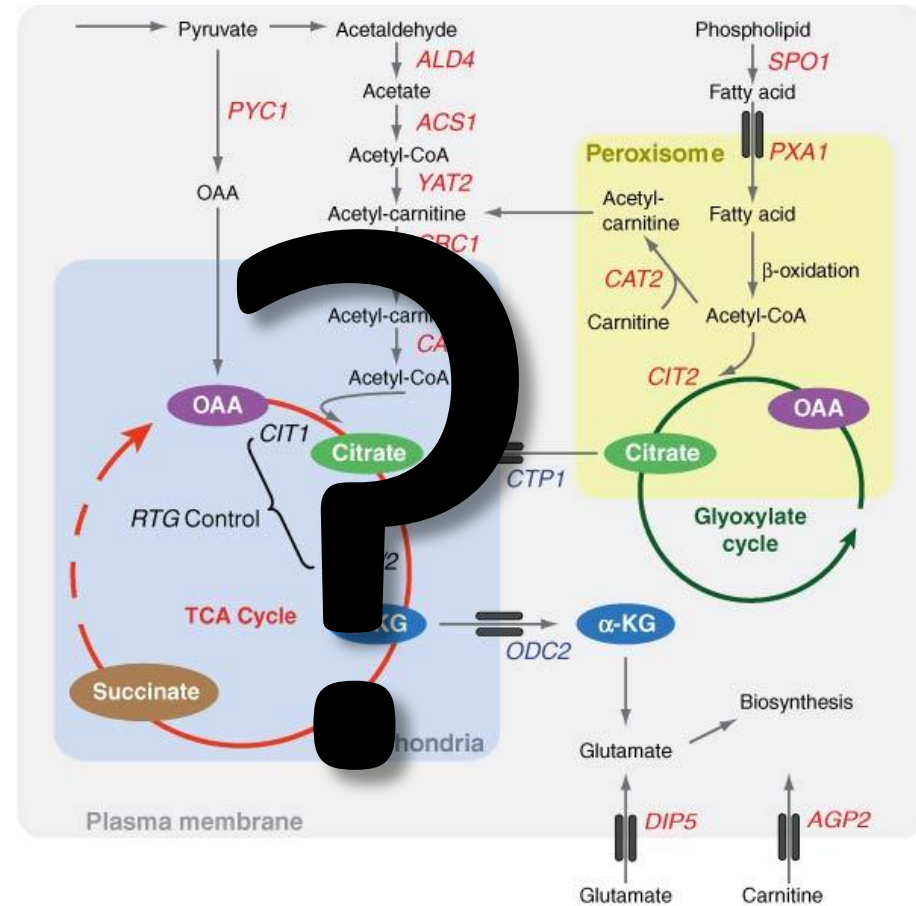
Retrograde target genes activated in ρ^0 yeast cells


Metabolic reconfiguration of respiratory-deficient yeast cells through activation of mitochondrial RTG signaling as inferred from transcript profiling



Retrograde target genes activated in ρ^0 yeast cells

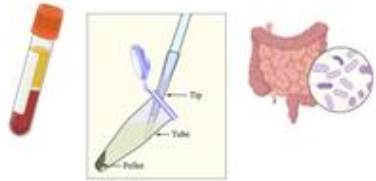
The scope of our work was to analyze the differences in metabolomic profile between ρ^0/ρ^+ cells in order to determine the **actual metabolic reconfiguration** caused by mitochondrial retrograde pathway activation.



 Liu Z, Butow RA. 2006. Annu. Rev. Genet. 40:159–85

Metabolomics Workflow

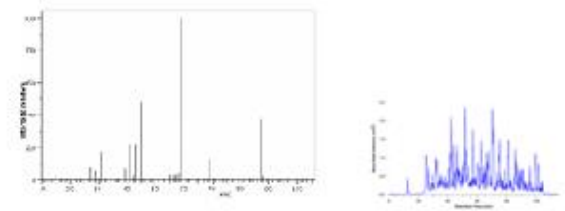
Sample Preparation



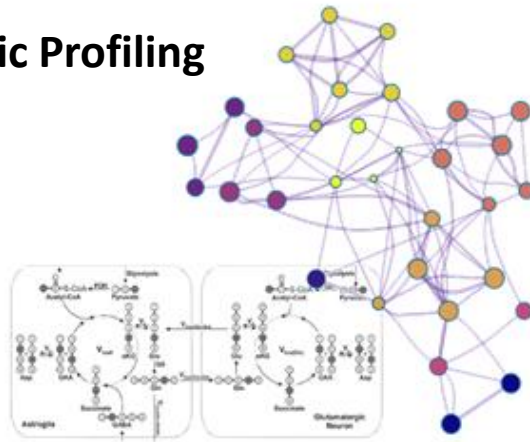
Data Acquisition



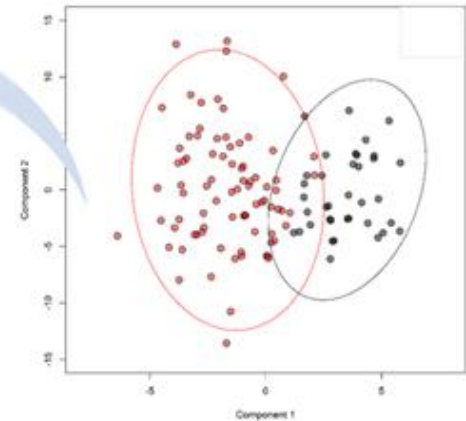
Data Processing



Metabolomic Profiling



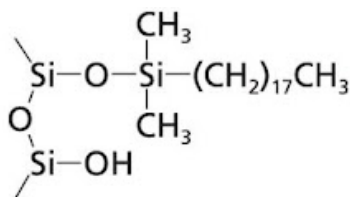
Statistical Analysis



Chromatographic column choice

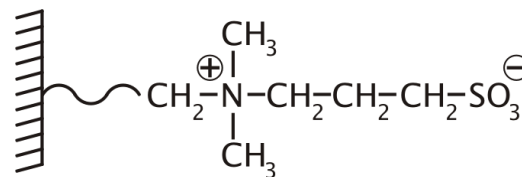
- UHPLC Column:

Column **C18** RPC



✓ Hydrophobic molecule

HILIC (hydrophilic interaction liquid chromatography)

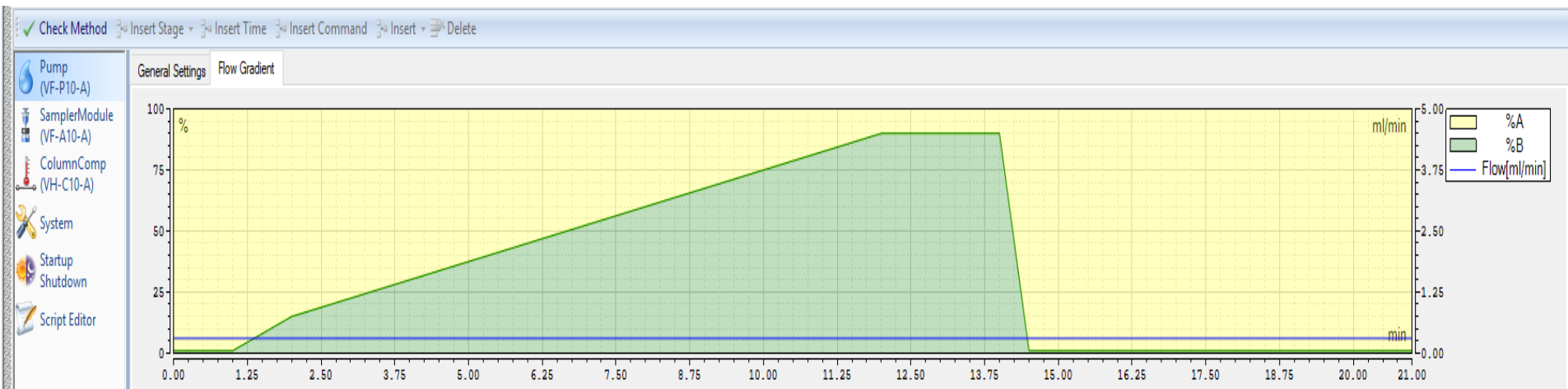


✓ Hydrophilic molecule



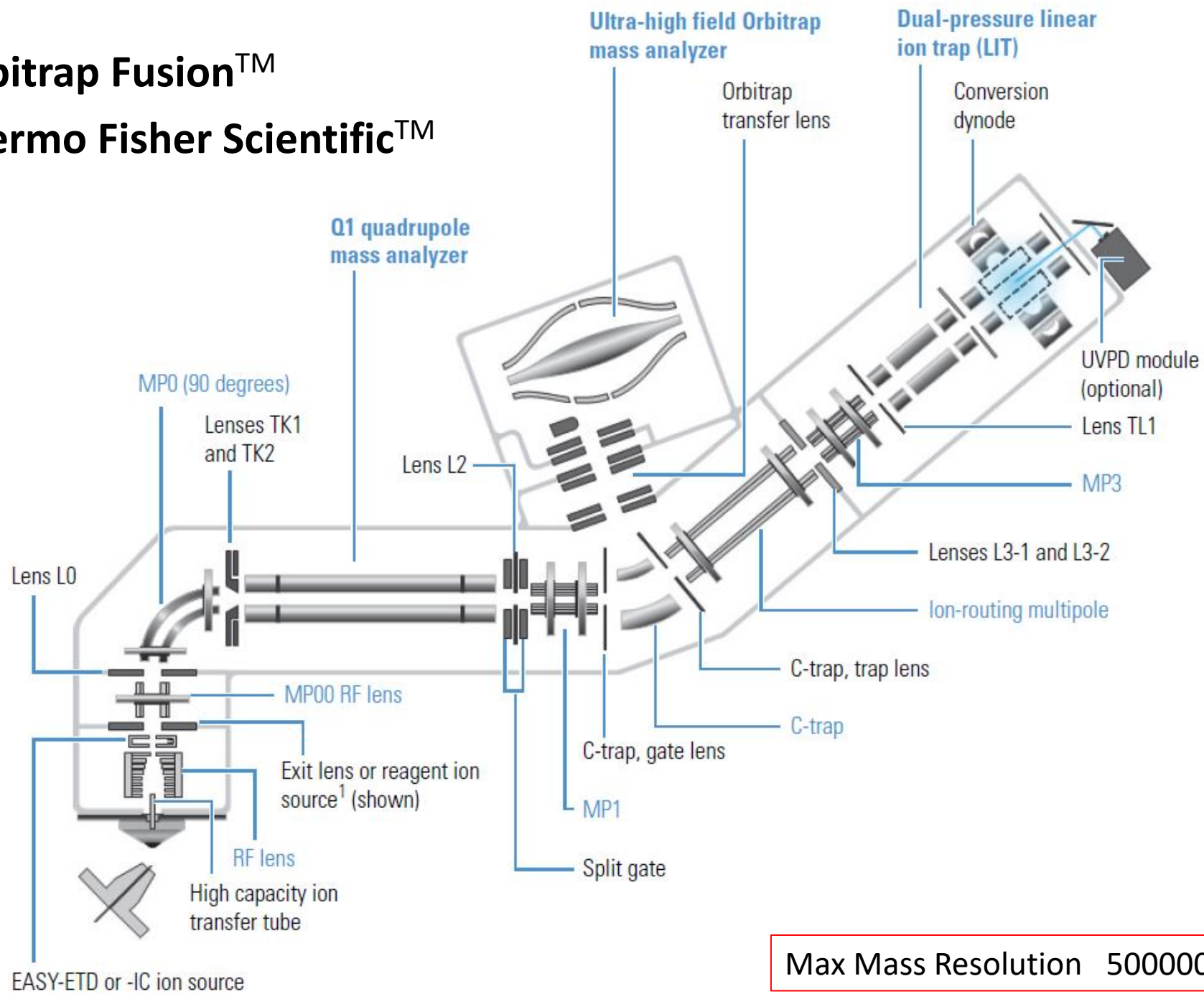
LC - MSⁿ

LC – Vanquish™ UHPLC System



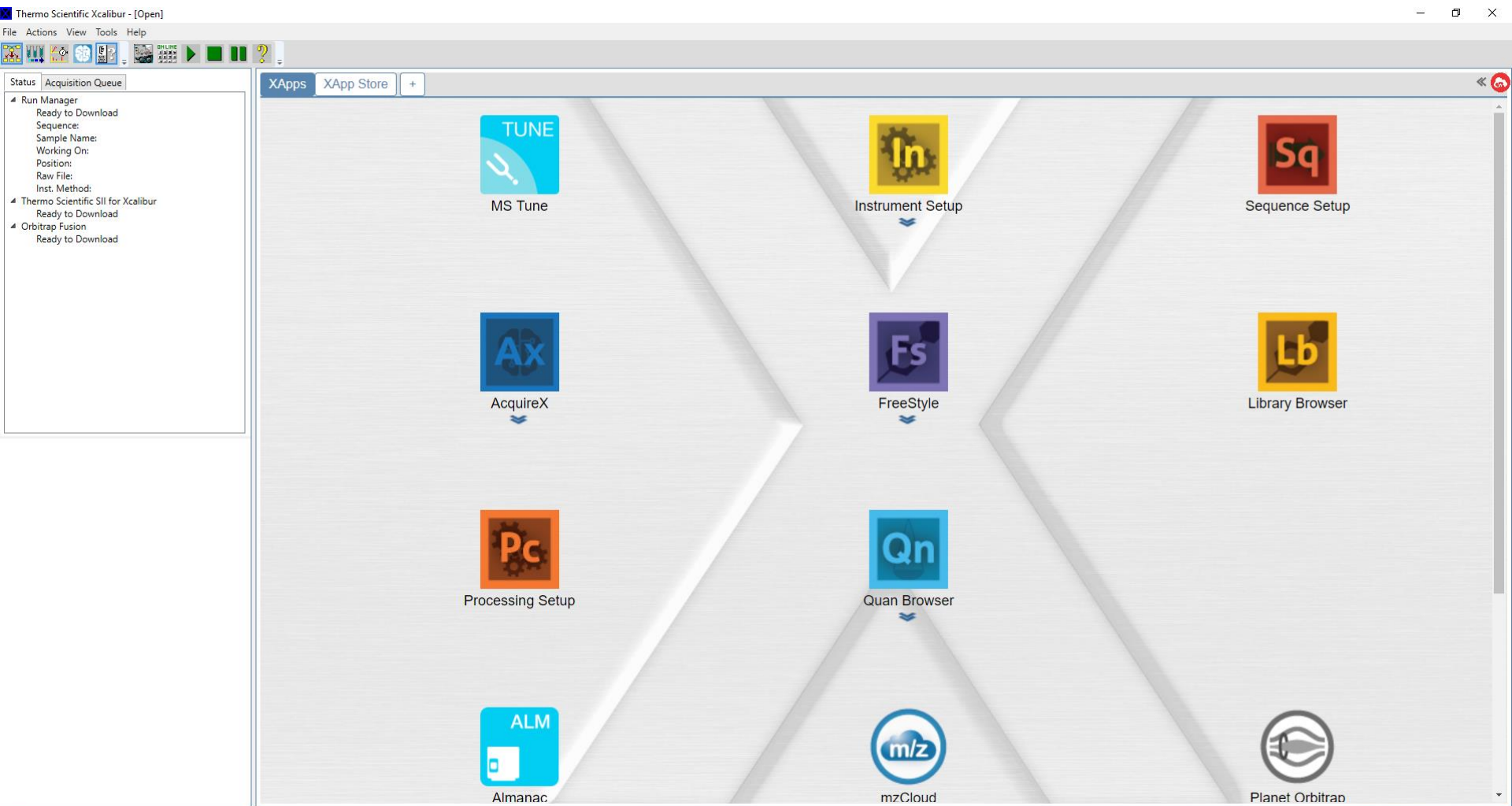
Orbitrap Fusion™

Thermo Fisher Scientific™



LC - MSⁿ

- MS¹ Resolution **120000**
- MS² **HCD** Fragmentation (**20, 40, 90**)
- MS³ **CID** Fragmentation (**30%**)
- Instrumental **Internal Calibration**



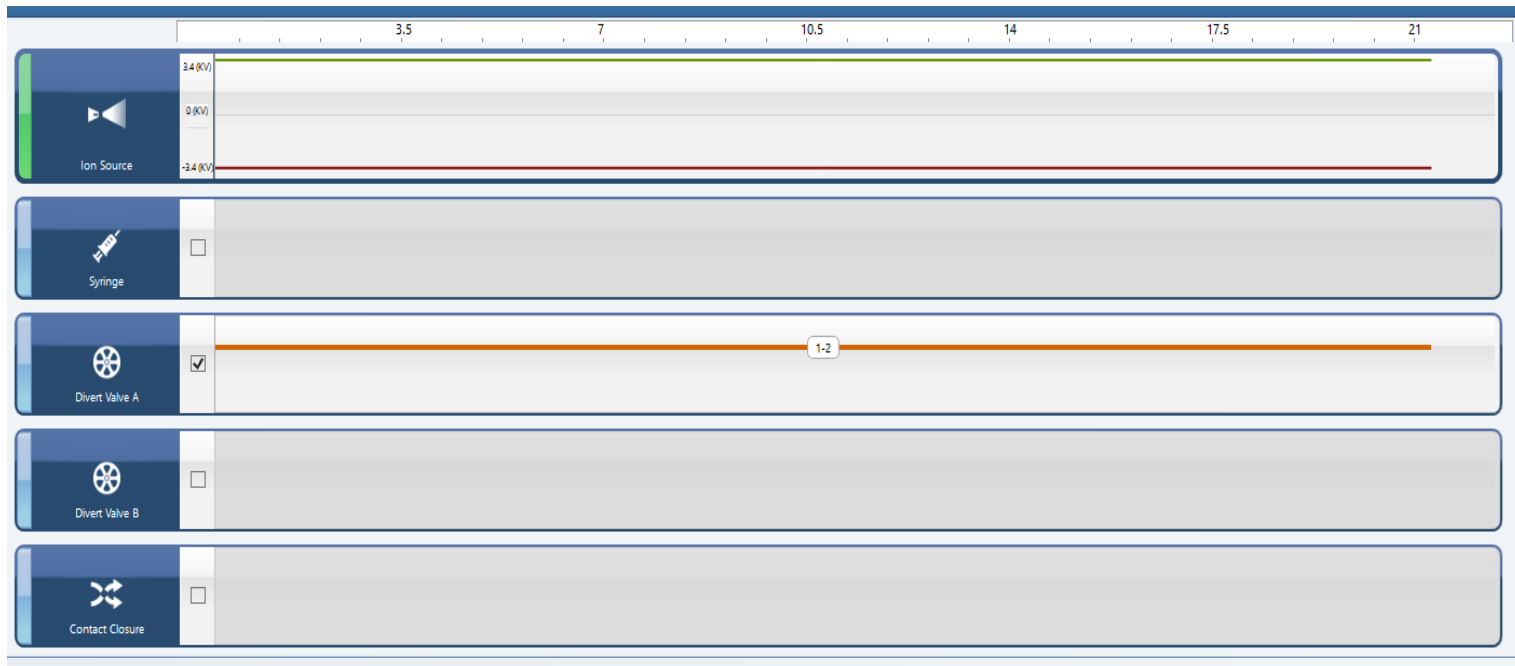
LC - MSⁿ

C18 column → Positive mode
→ Negative mode

HILIC column → Positive mode
→ Negative mode

- Ion Source

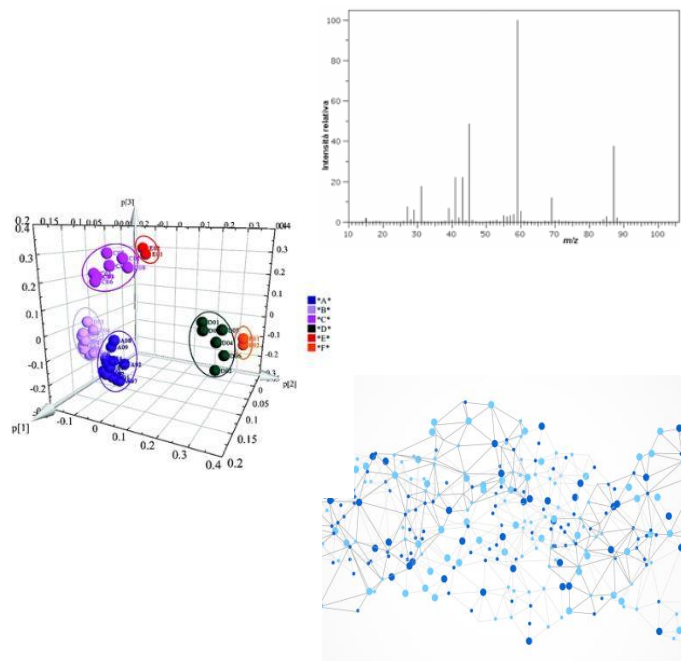
H-ESI



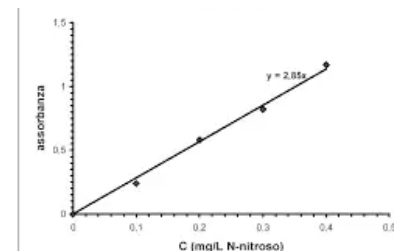
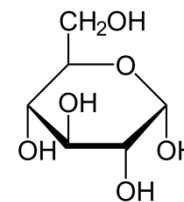
Analysis Mode



Untargeted



Targeted

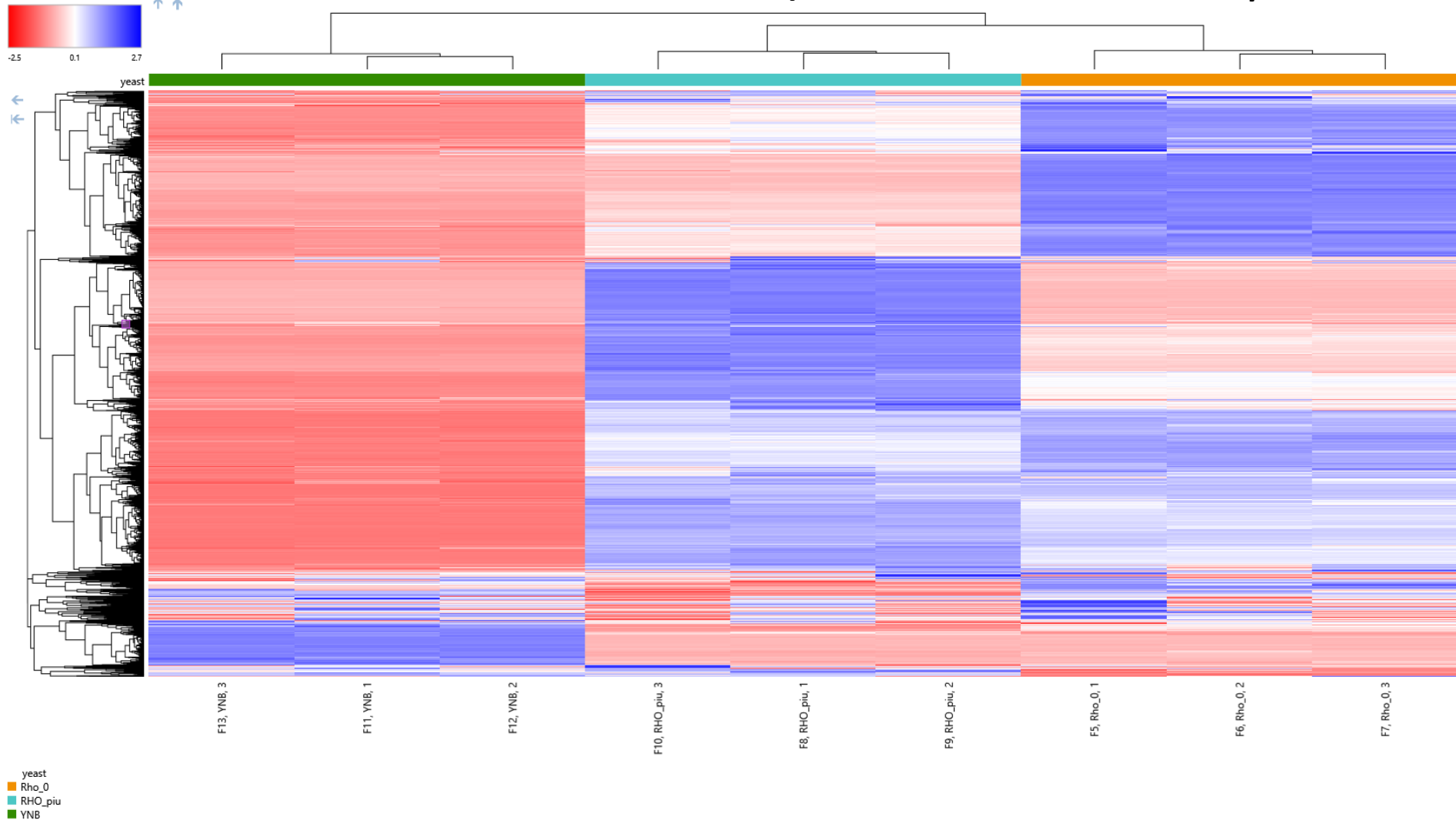


Untargeted Analysis

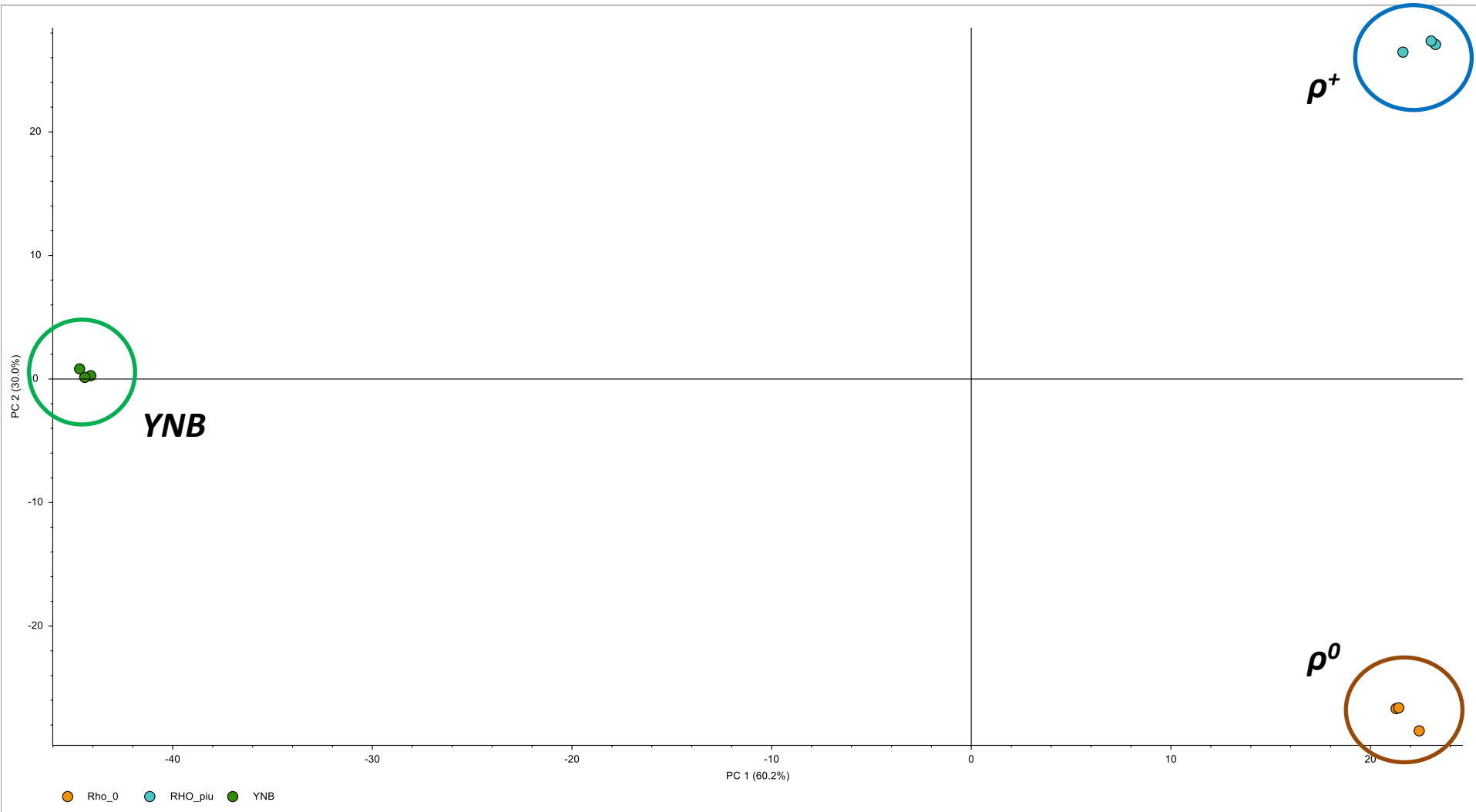
Metabolomic fingerprint



Data Source: Compounds
Distance Function: Euclidean
Linkage Method: Complete
Scaling: Scale Before Clustering
Normalized data: no

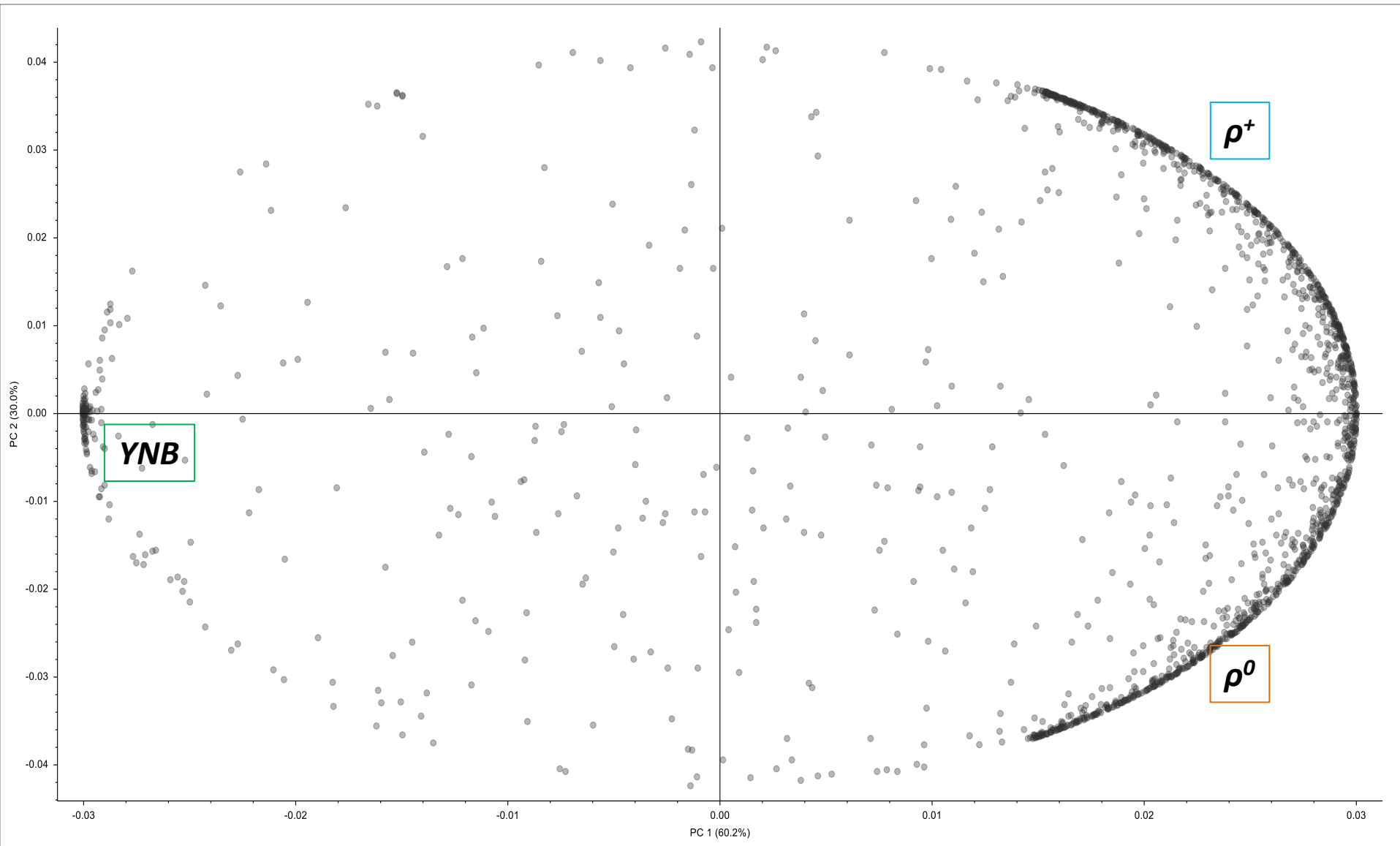


Principal Component Analysis (PCA)

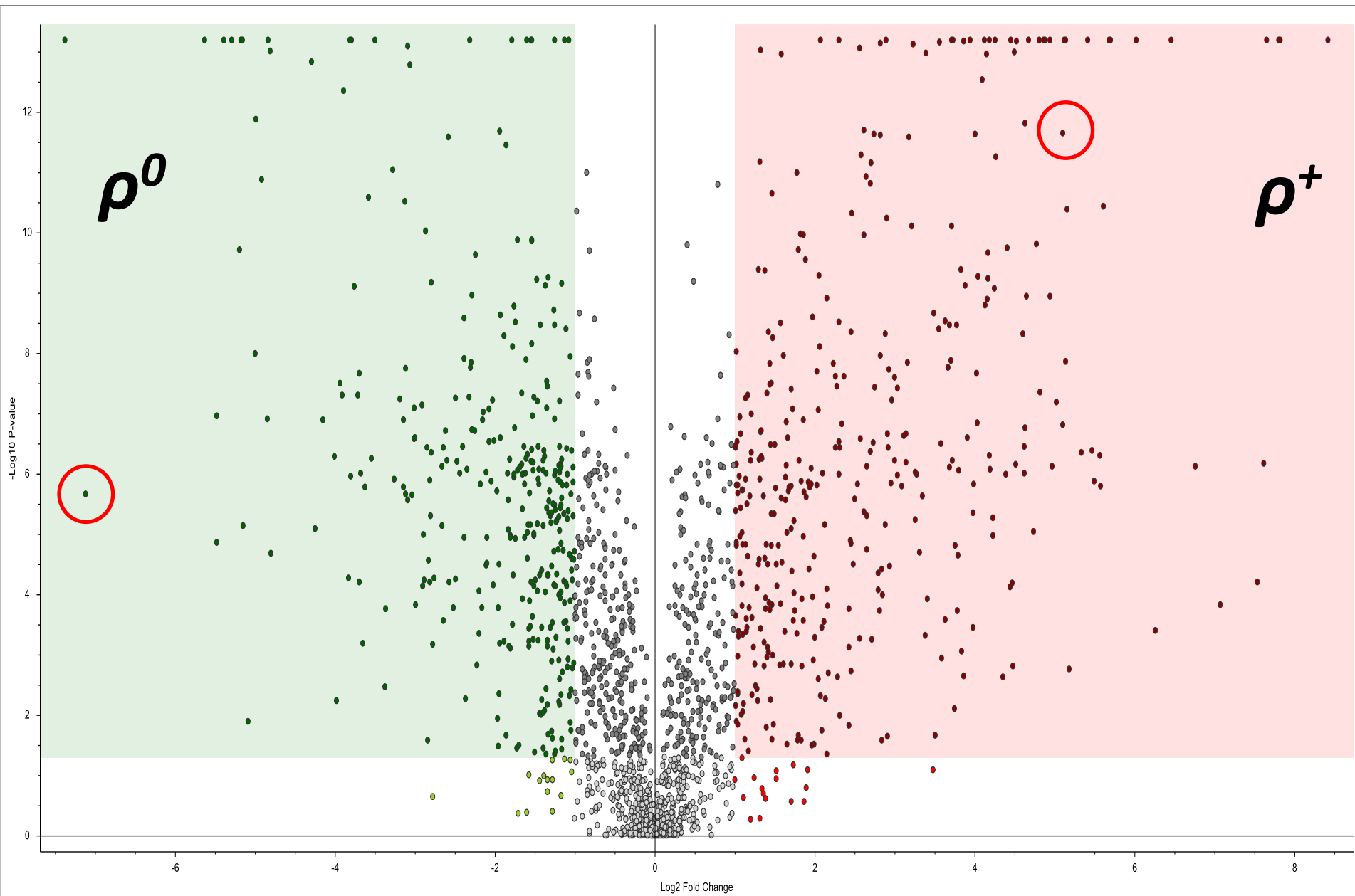


90.2% Variance

Loading Plot



Volcano Plot



Draw new molecules

Compound Discoverer 3.3.0.550

File Reporting Lists & Libraries View Window Help

Start Page Job Queue yeast ysbn1 rho0 rho+ hilic ysbn1 rho- rho0 neg hilic YSBN1 R+ e R0 pos YSBN1 R+ e R0 neg

Chromatograms

Group By: ☐ Replicate (3/4) ☒ Yeast (2/4) ☐ Sample Type (1/2) ☐ File (5/10)

Filter By: ☐ Replicate ☒ Yeast ☐ Sample Type ☐ File

Intensity [counts] (10⁶)

RT [min]

7.082

Mass Spectrum

F5 #1031, RT=7.076 min, MS1, FTMS (-)
F2 #4646, RT=7.030 min, MS1, FTMS (-)
F2 #4656, RT=7.037 min, MS2, FTMS (-), (HCD, DDA, 488.1621@[20.40.90], -1)
F2 #4658, RT=7.039 min, MS3, ITMS (-), (CIC)
F2 #4659, RT=7.039 min, MS3, ITMS (-), (CIC)
F2 #4660, RT=7.040 min, MS3, ITMS (-), (CIC)
F2 #4697, RT=7.056 min, MS1, FTMS (-)
F2 #4711, RT=7.071 min, MS2, FTMS (-), (HCD, DDA, 488.1621@[20.40.90], -1)

ID_01 (F2) #4656, RT=7.037 min, MS2, FTMS (-), (HCD, DDA, 488.1621@[20.40.90], -1)
simil polipeptide- N-Acetyl-1-aspartylglutamic acid, C18 H27 N5 O11
Fish Coverage: 9 Matched, 2 Unmatched, 10 Skipped

Intensity [counts] (10⁶)

m/z

102.0558 146.04570 C5 H8 N O4 [M+e]-1 128.03519 C5 H6 N O3 [M+e]-1 145.56711 300.27081 488.16342

Compound Annotation Editor

ChemSpider...

Apply Fish scoring

Save Cancel

ChemSpider...

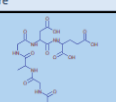
Apply Fish scoring

Save Cancel

Compounds

Tags	Checked	Name	Formula	Annot. Sol
1	<input checked="" type="checkbox"/>	simil polipeptide- N-Acetyl-1-aspartylglutamic acid	C18 H27 N5 O11	
2	<input checked="" type="checkbox"/>	α , α -Trehalose	C12 H22 O11	
3	<input type="checkbox"/>		C12 H20 N6 O13	
4	<input type="checkbox"/>	N- α -Acetyl-L-asparagine	C6 H10 N2 O4	
5	<input type="checkbox"/>			
6	<input type="checkbox"/>	3-(5-tert-Butyl-3-hydroxy-1,2-oxazol-4-yl)-L-alanine	C10 H16 N2 O4	

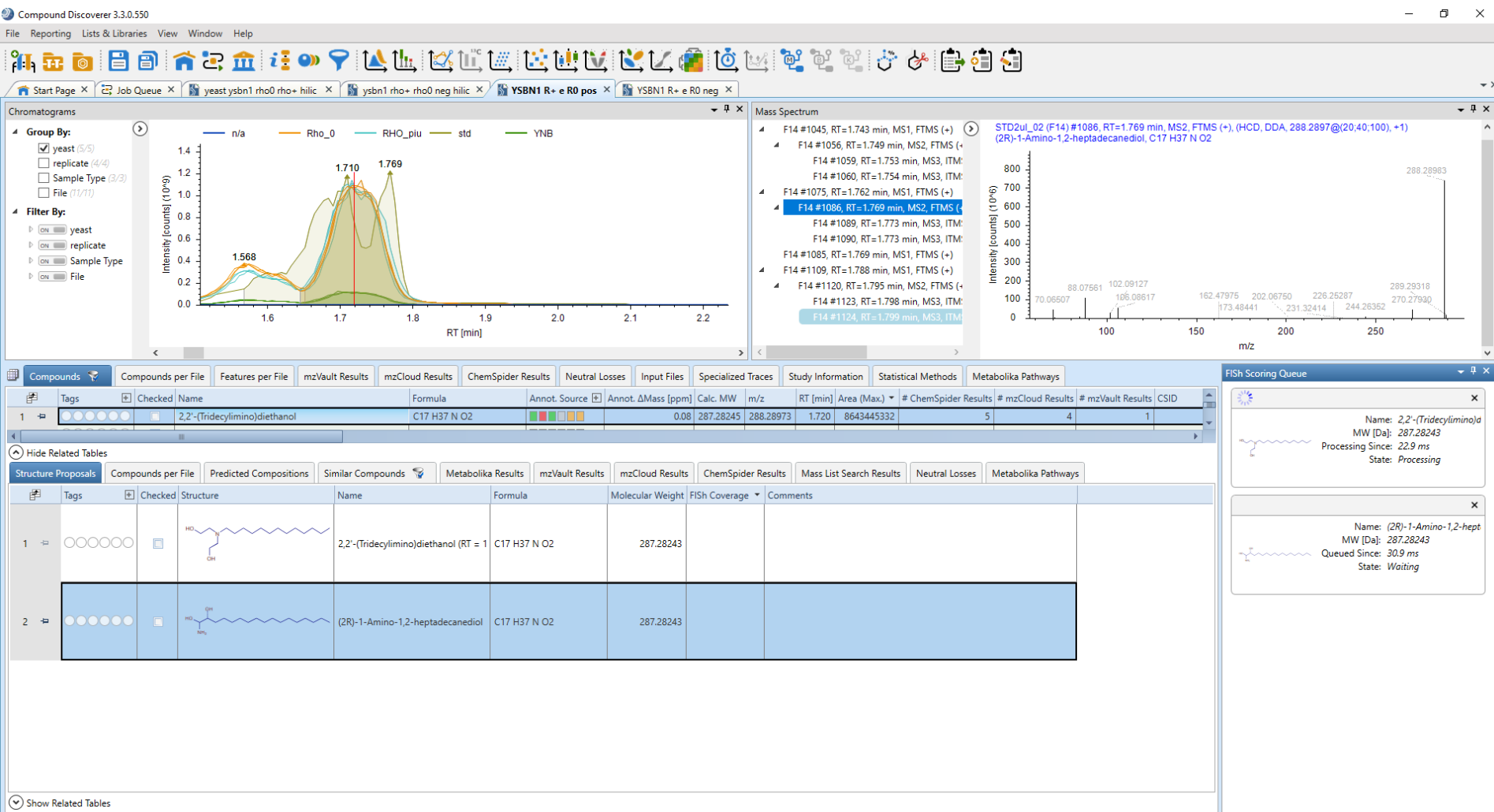
Structure Proposals

Tags	Checked	Structure	Name	Formula
2	<input checked="" type="checkbox"/>		simil polipeptide- N-Acetyl-1-aspart	C18 H27 N5 O11

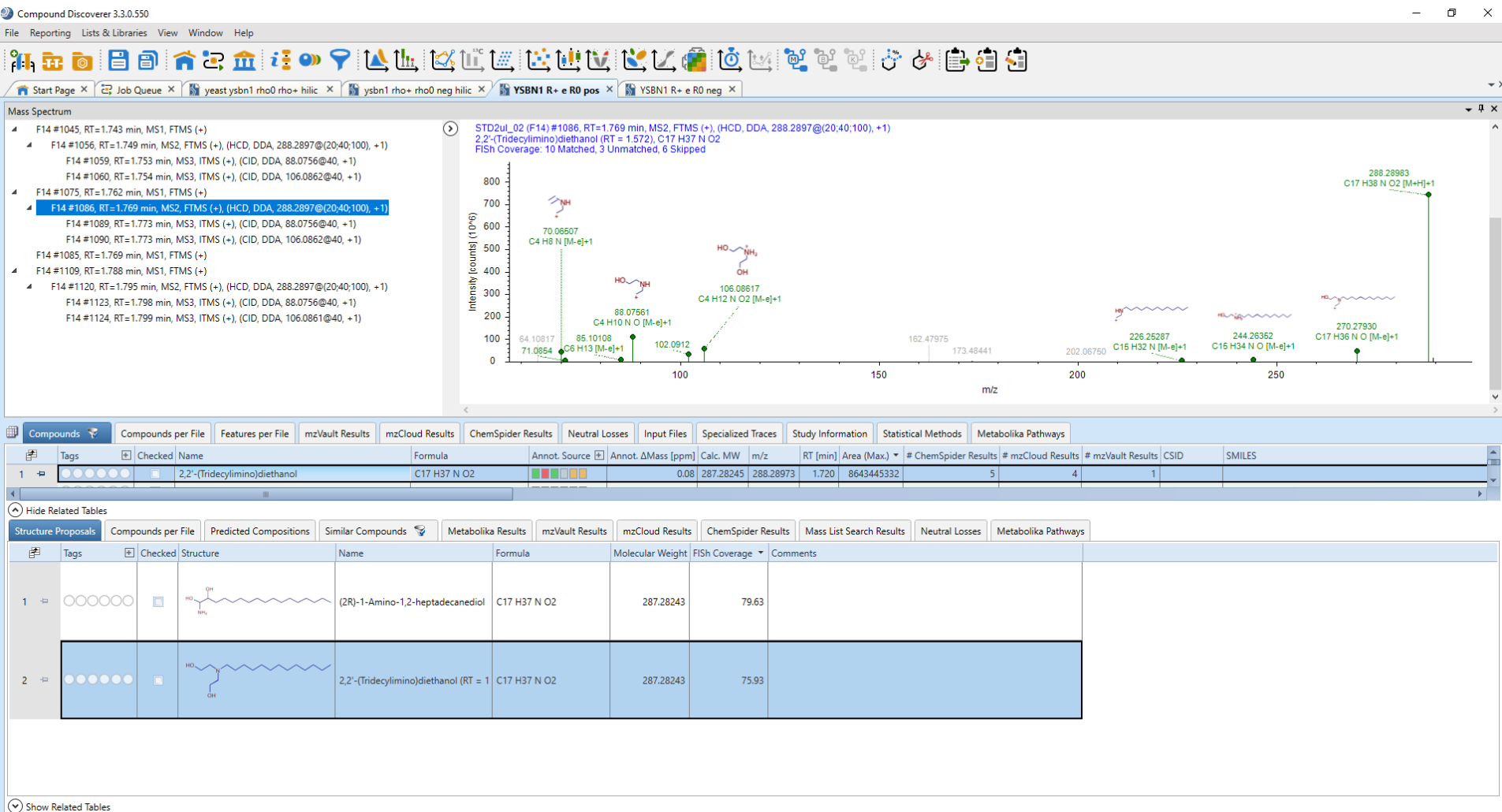
Metabolika Results

Area (Max.)	# ChemSpider Results	# mzCloud Results	CSID	SMILES
840384630	0	4		
3601275754	66	29	9046871	C([C@@H]1[C@H]([C@@H]1[C@H]([C@H]1O1)O1)O1)O1
1233643	0	0		
683556	0	4		
446212	0	0		
393792	1	0	394522	CC(C)C1c1c(no1)O[C@H]1C(=O)O)N

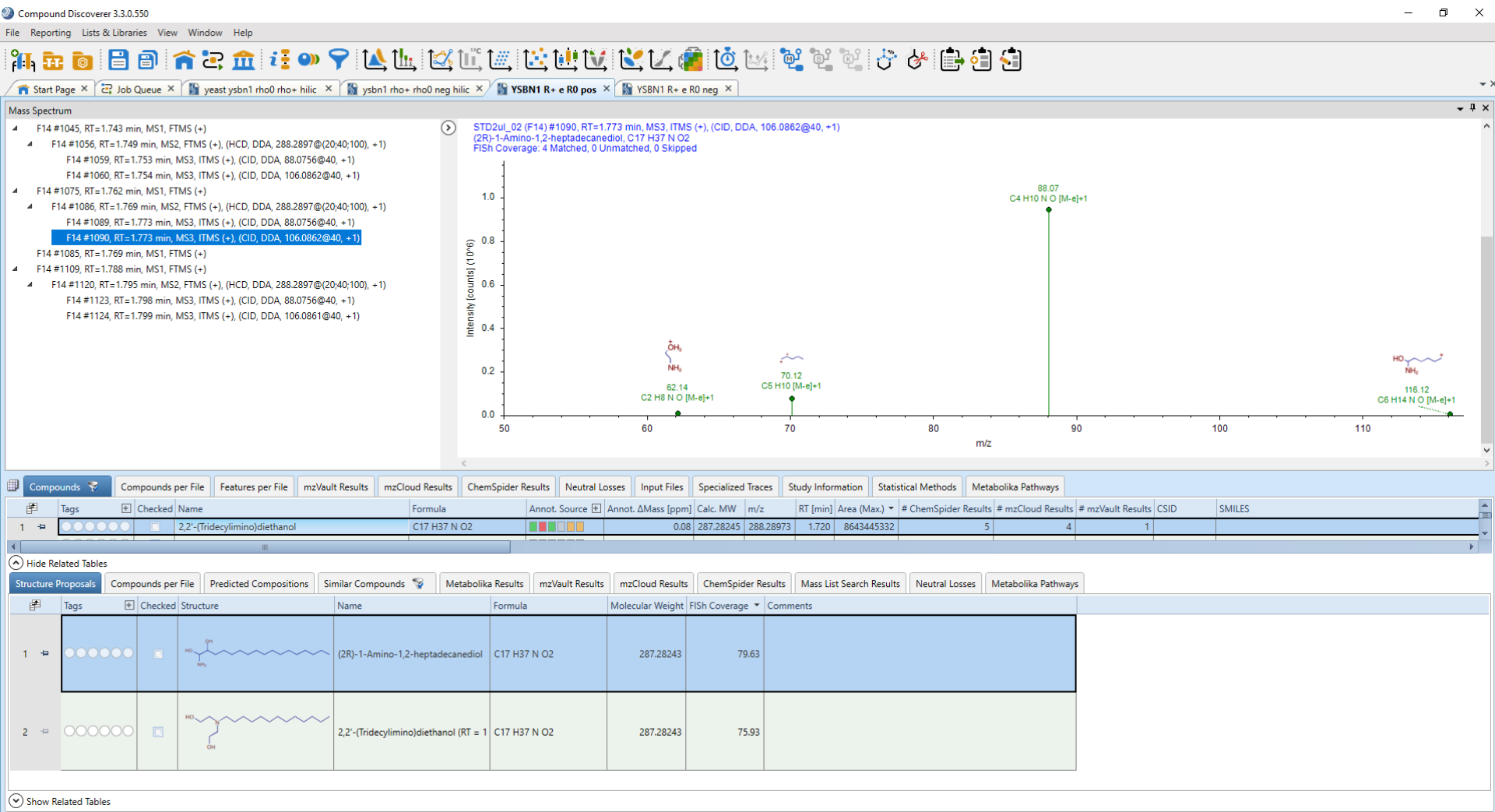
Fish scoring



MS²

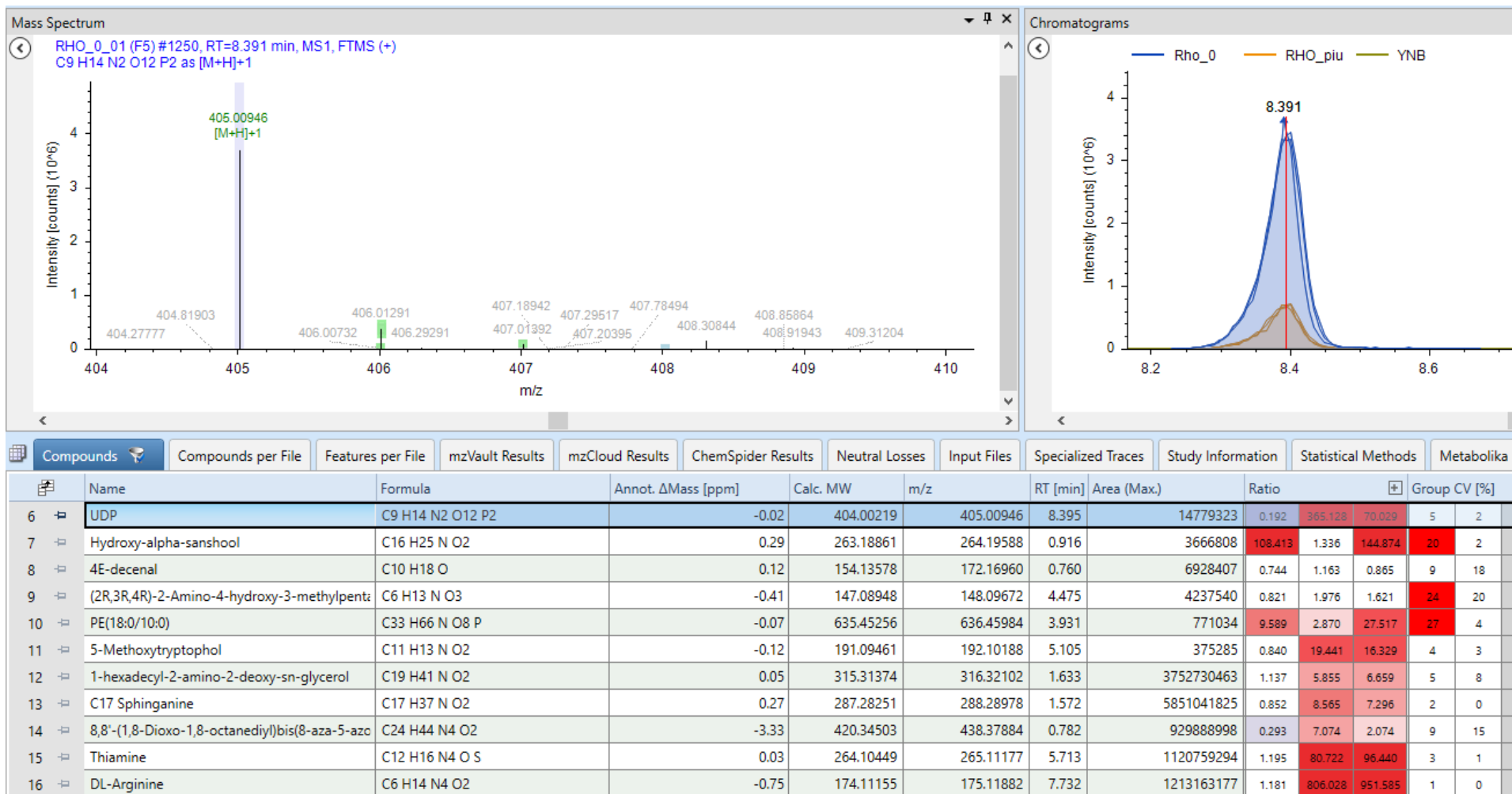


MS³



Differential Metabolomic Profile

- > 1800 compounds
- Phospholipids, purine pyrimidine compounds, A.A.



The **interlaboratory** comparison shows that **absolute concentration** can vary by up to threefold, even for identical sample processing. However, when comparing only the relative concentration differences between growth conditions or strains, all laboratories deliver a consistent picture⁶



Ratiometric measurements still have much higher reliability than do absolute estimates.

Conclusions

Experimental Design: choose the correct analysis strategy

Sample preparation: decide the most appropriate extraction for our goal

Targeted and Untargeted Analysis: Pros and cons

MSⁿ : fragmentation is useful for the determination of unknown or isobaric compounds

Multionics integration → Metabolic **Pathway**

Acknowledgement



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S C I E N T I F I C

Igor Fochi

CNR.BiOmicS

