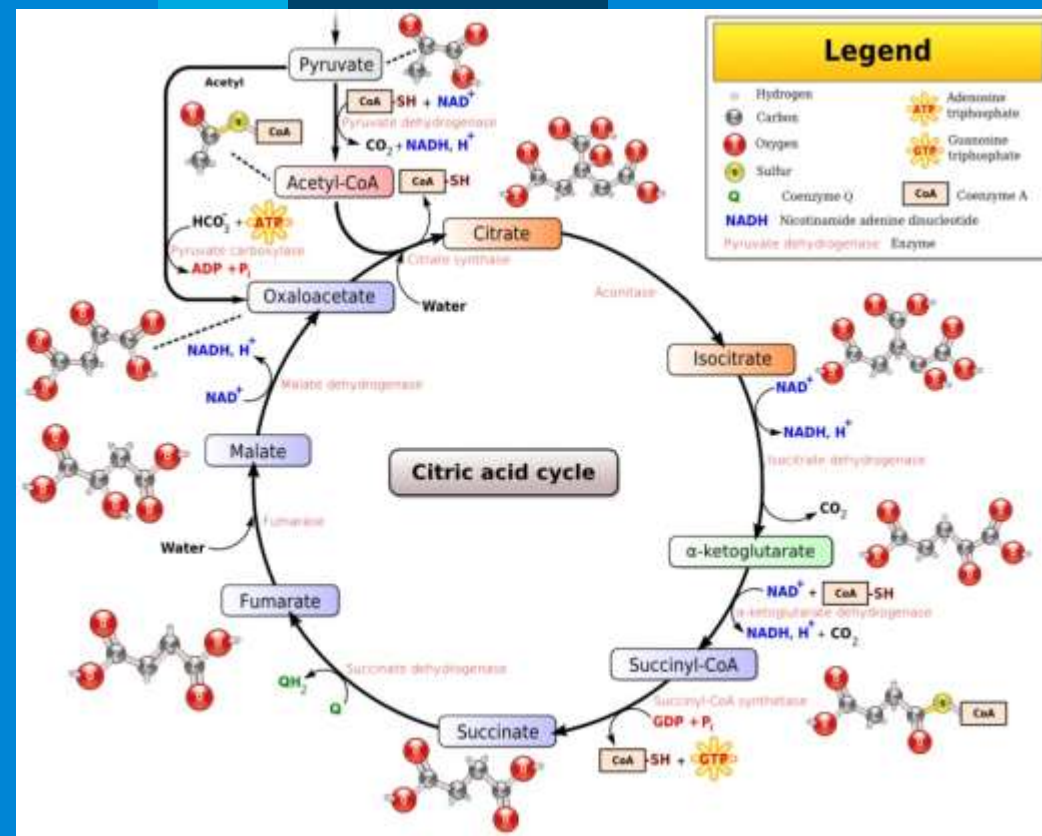


Herramientas y Soluciones en Ciencias -Ómicas y perfilado

AGILENT SEMINAR Universidad de Zaragoza

Jaume C. Morales
Iberia LCMS Product Specialist
Agilent Technologies

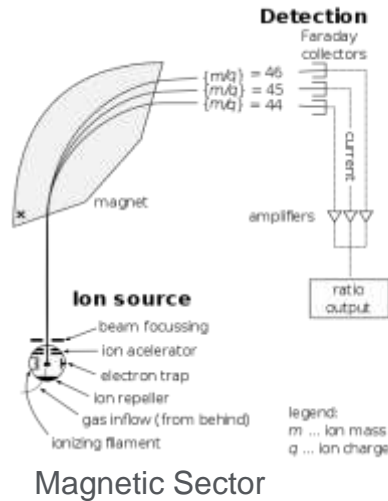


- *Enfoques y estrategias analíticas que nos permiten las últimas tecnologías de LC Mass Spectrometry de alta resolución. **UHPLC QTOF technical details***
- **Agilent proposal Workflows in different scenarios.** *Diferentes estrategias inherentes a investigación en el ámbito de Ciencias de la Vida, así como para el perfilado de Alimentos, Materiales, Procesos...*
- **Herramientas de Agilent y flujos de Trabajo para tomar mejores decisiones en un entorno de Biología integrada.** *Del diseño experimental a las conclusiones, un largo camino para ayudar al investigador.. :*
 - **Datos según modos de Adquisición.** *Complementariedad de las diferentes tecnologías LCMS, GCMS, CEMS, ICPMS*
 - **Deconvolución de datos y herramientas de visualización.** *Como funcionan los algoritmos de Agilent para extraer información de compuestos de un Full Scan.*
 - *Preparación de datos previa al Análisis Estadístico diferencial. **Alineamiento, Normalización, “Baselining” con “Mass Hunter ProFinder”.***
 - *¿Necesito análisis recursivo a través de iteración? Por favor hágamelo fácil.... **Exhaustivo tratamiento de datos para evitar la Pérdida de compuestos.***
 - *Mass Profiler professional. **Análisis Diferencial a través de Interpretaciones, Clustering, PCA, PLRS, modelos de predicción***
 - *Así, ¿Cuales son mis compuestos diferenciales de interés? ¿Como puedo identificarlos? **Librerías empíricas de espectros MS/MS. Agilent METLIN PCDL.***
 - *Análisis de rutas Metabólicas a través de “Pathways Analysis”. **Biología integrada e interpretación biológica de mis datos. Pathways Analysis.***
 - *¿Cuál es mi próximo experimento? **La potencia del enfoque de la Biología integrada.***
- **Movilidad Iónica.** *Una nueva dimensión para extracción de datos más selectiva en muestras complejas. Una nueva herramienta de identificación*
- **Fluxómica. Fácil y rápida** *visualización de la incorporación de sustratos marcados isotópicamente en una ruta metabólica a través de “VistaFlux”.*
- *Método llave en mano para el análisis Metabolómico dirigido en rutina de los **metabolitos del Ciclo Central de Carbono***
- *Sinergias con la medida In-vivo del Metabolismo celular con “Seahorse”.*

Technical features and Analytical Approaches with LC High resolution Mass Spectrometry.



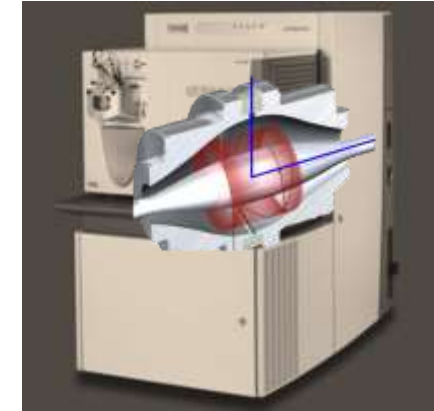
Replica of J. J. Thomson's third mass spectrometer.



Single quadrupole mass spectrometer used for John Fenn's Nobel Prize winning work on electrospray ionization



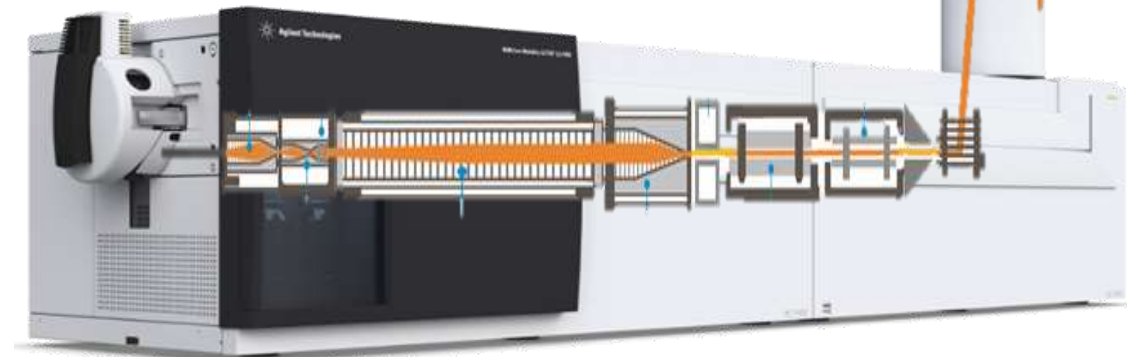
Cyclotron



Orbi

From 54th ASMS Conference on Mass Spectrometry

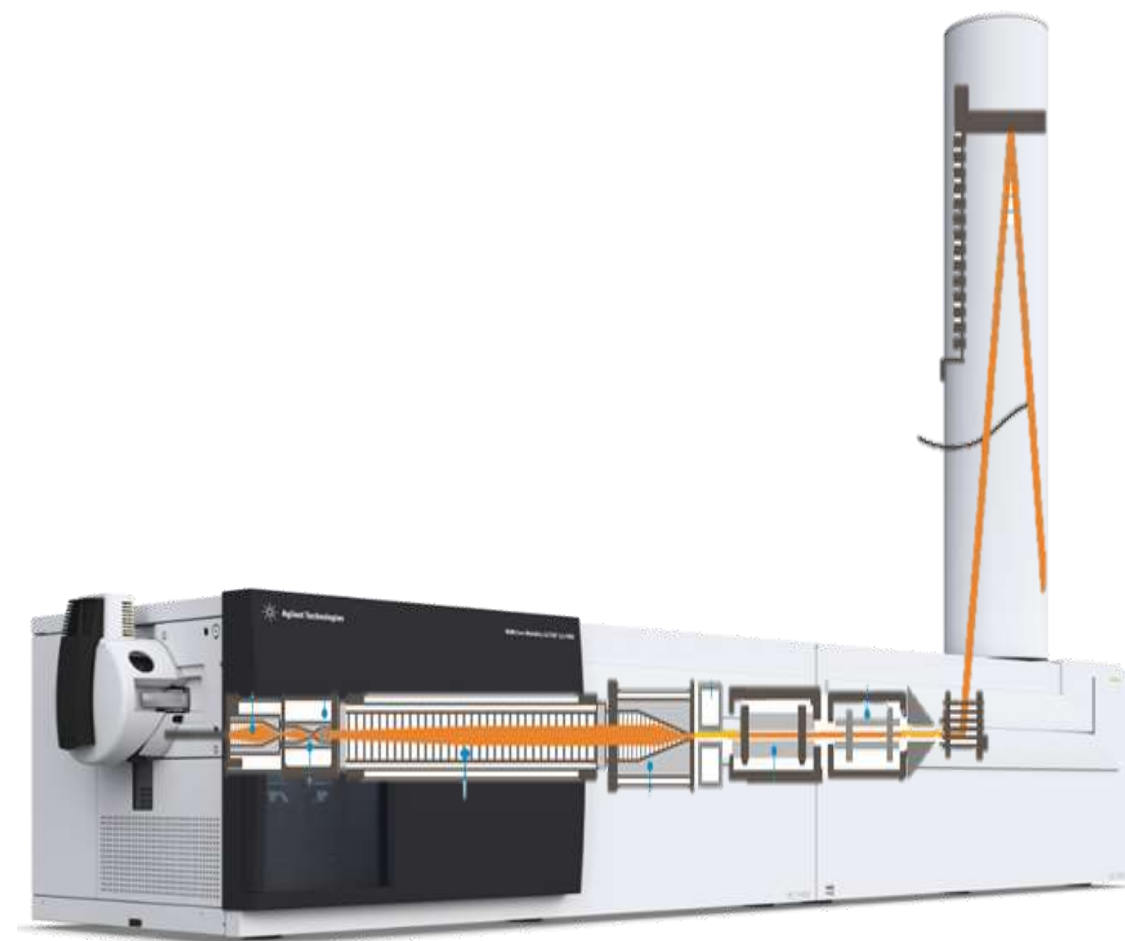
Agilent HR (IMS) QTOF



Technical features and Analytical Approaches with LC High resolution Mass Spectrometry.

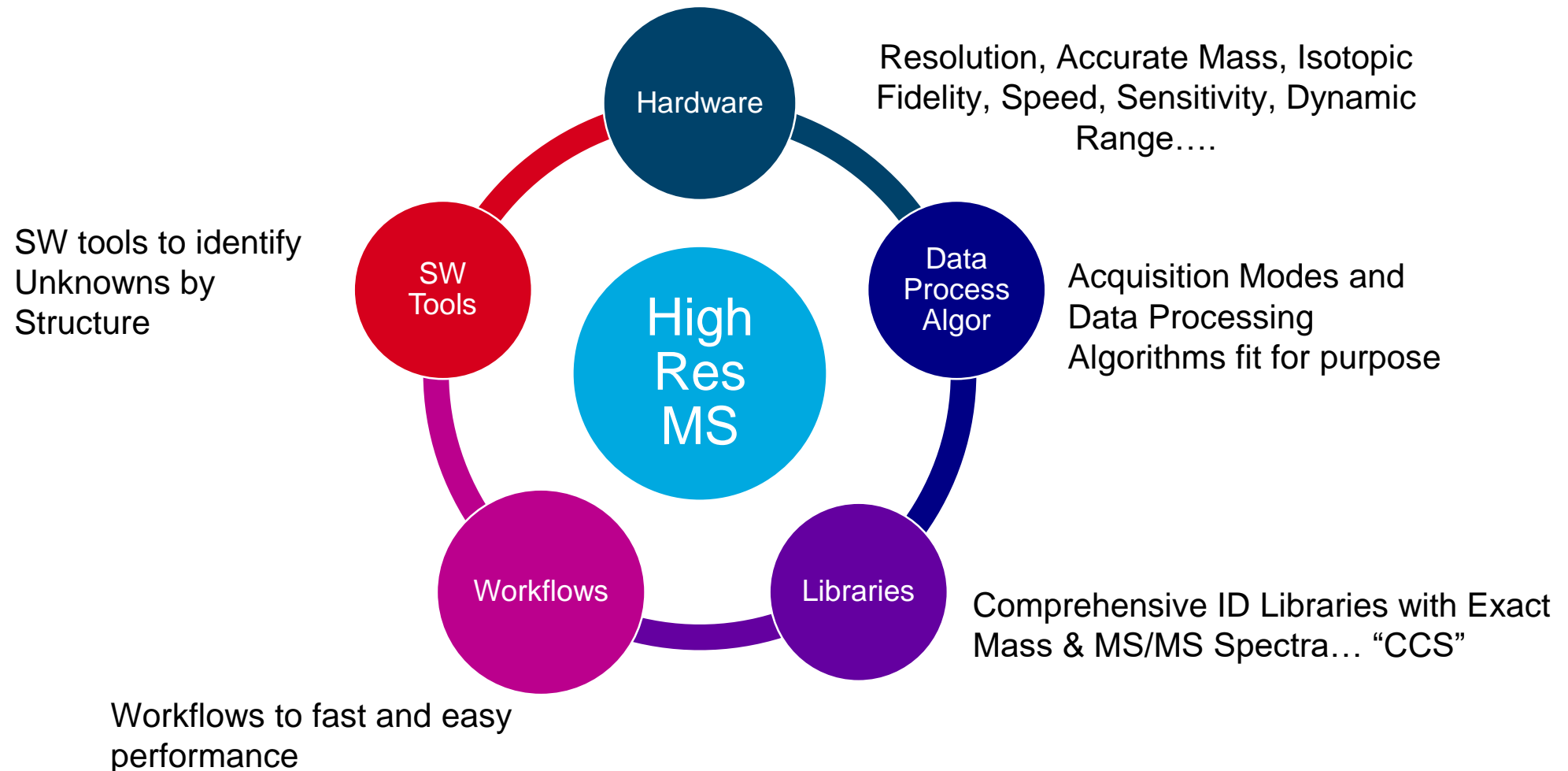
HRMS is absolutely differential MS technology. It allows for :

- Untarget Screening
- Target Screening
- Confirm Suspect compounds
- Sensitive Full Scan analysis
- Structural elucidation
- Identify Unknown Compounds
- -Omics disciplines (Metabolomics, Proteomics,)
- Profiling of samples
- Materials studies



Technical features and Analytical Approaches with LC High resolution Mass Spectrometry.

To work with HRMS is more than an instrument



Technical features and Analytical Approaches with LC High resolution Mass Spectrometry.

Agilent HRMS is based on Time Of Flight technology (TOF)

TOF is just an stop watch measuring time ions take to arrive to detector once they have been shot up at **PULSER**.

Lighter ions arrive before and the heaviest, later.

Reflectron optics provides larger flight path increasing resolution.

Time of flight is calibrated with known compounds (Tuning Mix) so time of ions contrasts with a calibration curve of $t < - > m / z$. This allows to know m/z with High accuracy.

On top of that, Spectral Peak Width is very narrow so we can differentiate very close spectral bands like Isotopic Pattern with High Fidelity.

In general, accurate mass instruments are those who can provide an error mass of $< 5\text{ppm}$.
MS systems based on Quadrupoles have mass error measurements of about $> 150\text{ppm}$.

Agilent systems can provide Mass error $< 1\text{ppm}$ or bellow

Technical features and Analytical Approaches with LC High resolution Mass Spectrometry.

RESOLUTION vs Mass Error

Spectral Resolution allows to get low mass error, on top of other advantages.

Unfortunately Mass error is not proportional to Resolution :

<i>Other Fourier T. systems</i>	Res. ~ 200.000	Mas error <1 ppm
<i>QTOF systems</i>	Res. ~ 60.000	<u>Mas error <0.8 ppm</u>

To identify/confirm a compound through HRMS
we do not only use Mass error.

Technical features and Analytical Approaches with LC High resolution Mass Spectrometry.

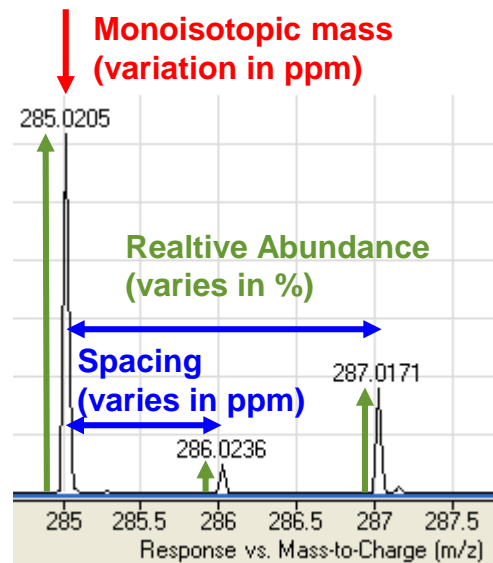
When we identify/confirm a compound through HRMS we do it by :

1. Mass error
2. Isotopic Pattern
3. MS/MS Spectra
4. Rt
5. CCS (*IMS*)

Spectral Resolution allows to get low mass error, on top of other advantages. BUT we don't identify with Resolution itself.

Isotope distribution = isotope ratio accuracy
→ Needs to be <5% even at good mass accuracy to reduce the number of potential database hits or empirical formulae

Scoring based on



Method Editor: Search Database	
Search Database for Compounds	
Search Criteria	Database
Negative Ions	Scoring
Peak Limits	Search Mode
Positive Ions	Search Results
Contribution to overall score	
Mass score	100.00
Isotope abundance score	60.00
Isotope spacing score	50.00
Retention time score	100.00

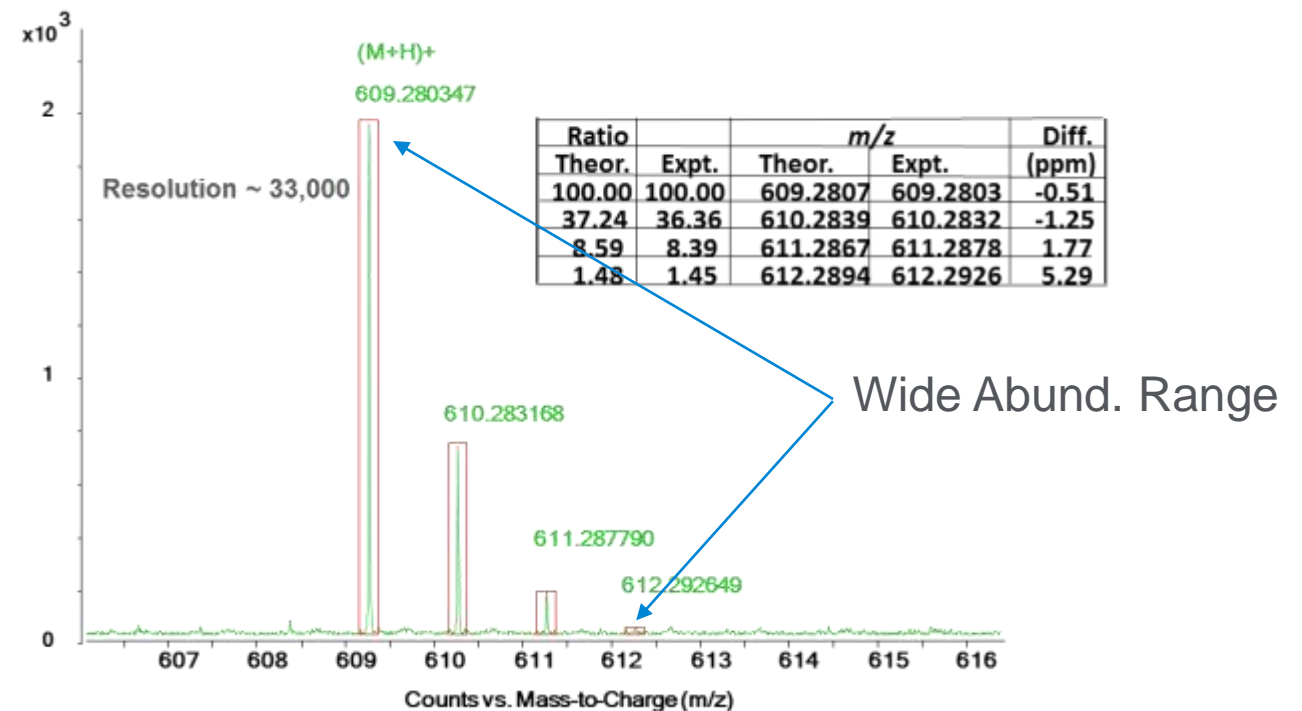
Technical features and Analytical Approaches with LC High resolution Mass Spectrometry.

SO everybody knows HRMS provides very low Mass error measurement BUT...

What other features are important on a HRMS and Why?

- Isotopic Fidelity. Better ID confidence
- Speed. UHPLC & MS/MS Coverage
- Sensitivity.
- MS/MS. Better ID confidence, Diff. Modes
- Dynamic Range. Better ID confidence
- **All at the same time!!!**

Dynamic Range of > 100.000 allows for Better ID



In-spectrum Dynamic Range: >4 Orders

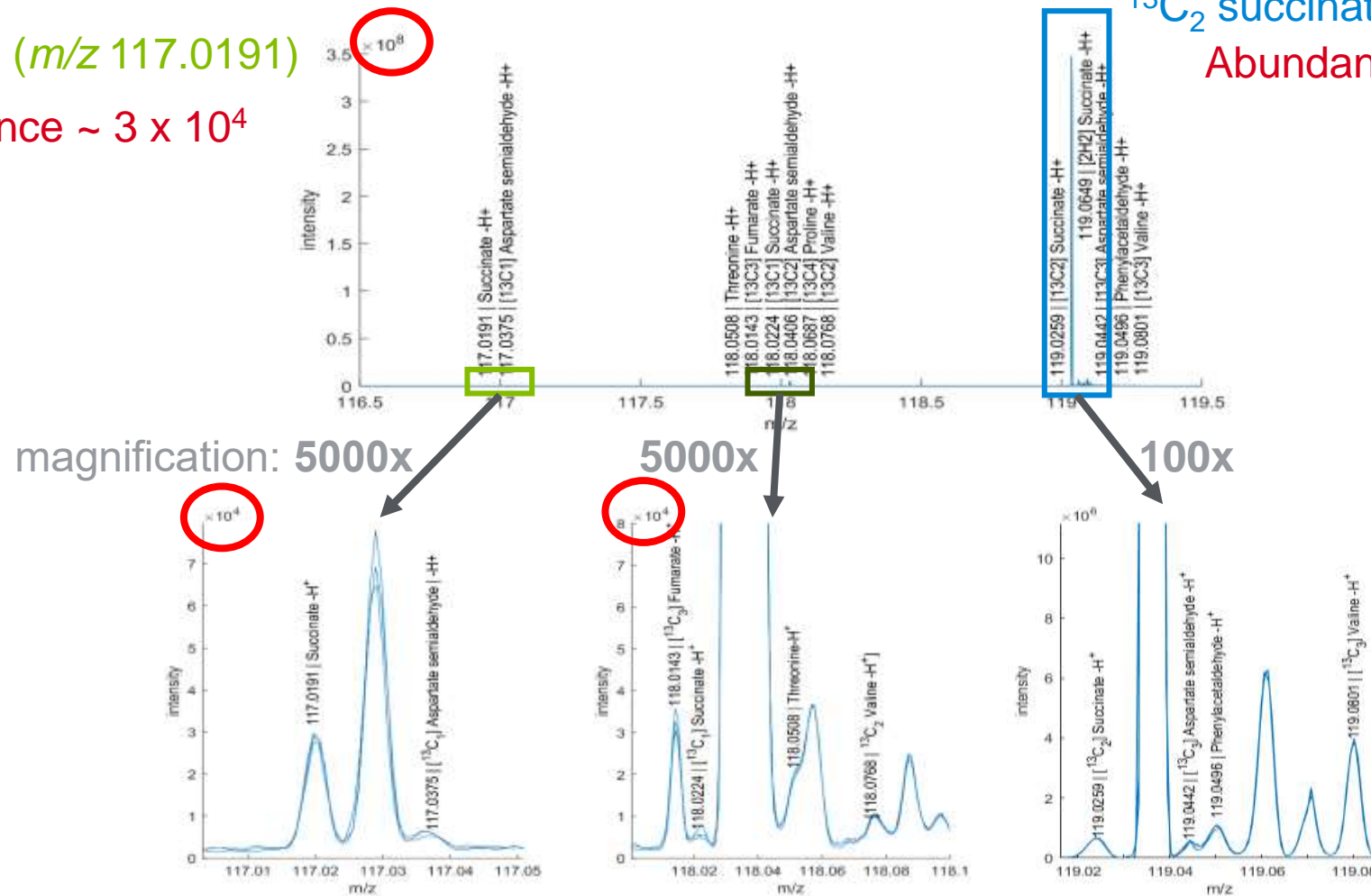
Stable label $^{13}\text{C}_2$ succinate ($1\mu\text{M}$) spiked into E. coli extract

succinate (m/z 117.0191)

Abundance $\sim 3 \times 10^4$

$^{13}\text{C}_2$ succinate (m/z 119.0259).

Abundance $\sim 3 \times 10^8$



HRMS QTOF Approaches

The Rumsfeld Conundrum

... “as we know, there are **known knowns**; there are things we know that we know. There are **known unknowns**; that is to say, there are things that we now know we don't know. But there are also **unknown unknowns** – there are things we do not know we don't know. “

United States Secretary of Defense Donald Rumsfeld

Screening Definitions

Target Screening. - Four Dimensions of Identification (4D-ID) QQQ Emulation



Compounds identified through Accurate Mass, Retention Time, Isotope Pattern and Fragment Confirmation. The quant method is developed via MassHunter Qual and Libraries (PCDLs). Acquisition is in All Ions MS/MS. **Standards Used.**

Suspect Screening - Propose and Identify. WIDEST Screening Approach



A Suspect List PCDL can be created from the encyclopaedic Master PCDLs. Compounds can be found and proposed using *Find-By-Formula*. The proposed list becomes the target or preferred (directed) list for target MS/MS or auto MS/MS. The resulting data can be searched against MS/MS spectra in the Suspect PCDL or Structure Correlated using MSC against the same PCDL if no MS/MS spectra are available.

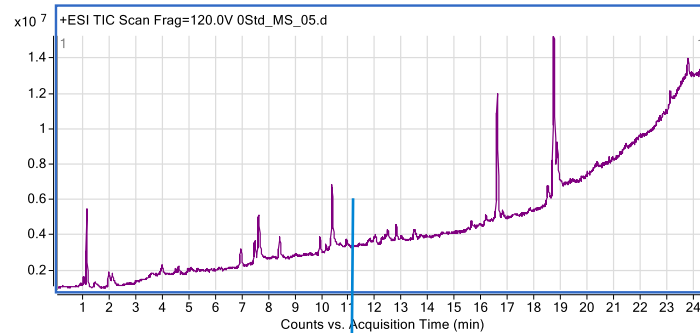
Discovery Profiling - Find, Compare and Identify



Profiling uses Molecular Feature Extraction (MFE) which is a naïve feature finding algorithm, in order to **find & characterize** compounds in a data set. These compounds can then be compared to determine the absence, presence or up- or down-regulation of the compounds from sample group to samples group. Profiling can be performed in single sample comparisons, two sample groups or multiple sample groups and conditions. Significant compounds can be imported into Acquisition in order to generate MS/MS data which is priceless in further compounds identification by formula generation, library search or structural correlation.

MassHunter QUAL : Find By Formula (MS)

Find by Formula (MS) análisis TARGET



¿¿C16H25NO2 ??
Masa= 263.1885Da

Iones posibles :

H⁺ · m/z = 263.1885

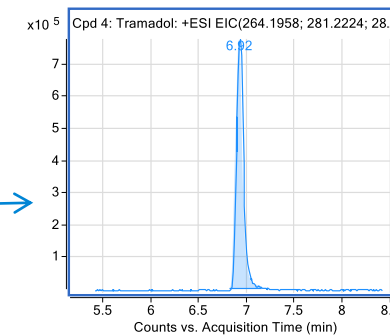
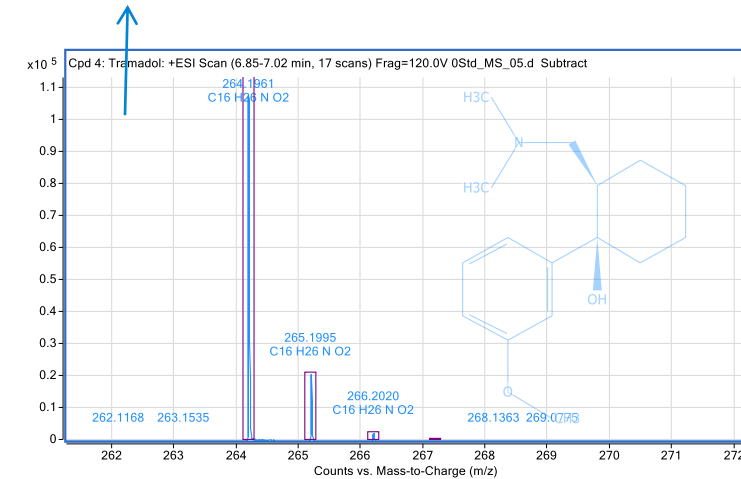
Na⁺ m/z = 264.1958

NH₄⁺ m/z = 286.1778

Extracción de Cromatograma de la Información MS
de todas las especies iónicas posibles :

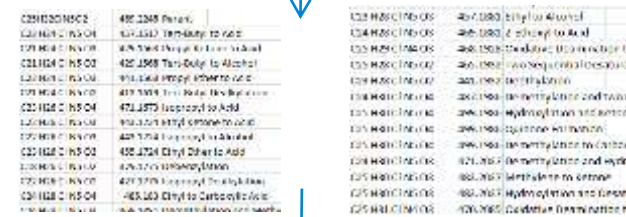
XIC : 263.1885 + 264.1958 + 286.1778

m/z	Ion	Formula	Abundance						
264.1961	(M+H) ⁺	C16 H25 N O2	117959.3						
Best	Formula (M)	Ion Formula	Score	Delta Score	Calc m/z	Diff (ppm)	Mass Match	Abund Match	Spacing Match
✓	C16 H25 N O2	C16 H25 N O2	99.64		264.1958	-1.02	99.41	99.87	99.82
Isotope	Calc Abund Sum%	Abund Sum%	m/z	Calc m/z	Diff (ppm)				
1	83.23	83.49	264.1961	264.1958	-1.01				
2	15.02	14.75	265.1995	265.1991	-1.61				
3	1.62	1.64	266.2020	266.2019	-0.37				
4	0.13	0.12	267.2045	267.2045	6.04				

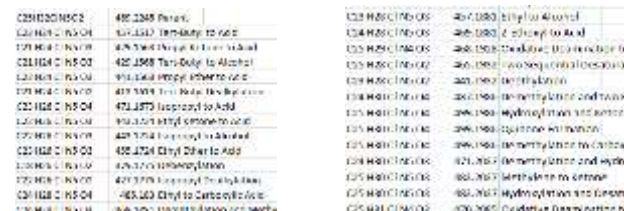


Extracción del espectro de
MS del compuesto

Find by Formula (MS) análisis TARGET



Extracción de Cromatograma de la Información MS
de todas las especies iónicas posibles :



Compound List

Automatically Show Columns

35

FBF

Quadrone (Quadrone)

C12 H14 N12

11.952

205.100

304.1011

68.36

36

FBF

Quadrone (Quadrone)

C12 H14 N12

11.952

205.100

304.1011

68.36

37

FBF

Quadrone (Quadrone)

C12 H14 N12

11.952

205.100

304.1011

68.36

38

FBF

Quadrone (Quadrone)

C12 H14 N12

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39

FBF

Quadrone (Quadrone)

C12 H14 N12

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FBF

Quadrone (Quadrone)

C12 H14 N12

11.952

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41

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Quadrone (Quadrone)

C12 H14 N12

11.952

205.100

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42

FBF

Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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44

FBF

Quadrone (Quadrone)

C12 H14 N12

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45

FBF

Quadrone (Quadrone)

C12 H14 N12

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46

FBF

Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

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Quadrone (Quadrone)

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C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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FBF

Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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FBF

Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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FBF

Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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304.1011

68.36

90

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

C12 H14 N12

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Quadrone (Quadrone)

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FBF

Quadrone (Quadrone)

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304.1011

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Quadrone (Quadrone)

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99

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Quadrone (Quadrone)

C12 H14 N12

11.952

205.100

304.1011

68.36

100

FBF

Quadrone (Quadrone)

C12 H14 N12

11.952

205.100

304.1011

68.36

ID Source

Name

Formula

Score

Score (RT)

RT Diff

Diff (ppm)

Score (Lib)

Score (DB)

Score (MFG)

FBF

Ethephon

C12 H6 Cl O3 P

84.38

-4.15

Species

Ion Formula

Score (MFG)

Score (MFG, MSIMS)

Score (MS)

Score (mass)

Score (iso. abund)

Score (iso. mass)

(M+H)+

84.38

95.57

57.96

m/z (Calc)

Diff (ppm)

Height

Height (Calc)

Height (%)

Height (%) (Calc)

Height Sum (%)

Height Sum (%) (Calc)

144.9616

-2.28

117223.5

130974.4

100

100

73.7

65.9

145.9651

3.73

5945.6

3089.3

5.1

2.4

1.7

3.3

146.9788

-9.06

51933.6

42734.8

44.3

32.6

24

29.2

147.9823

-4.55

2701.8

1006.9

2.3

0.8

0.6

1.5

FBF

Quadrone

C12 H14 N12

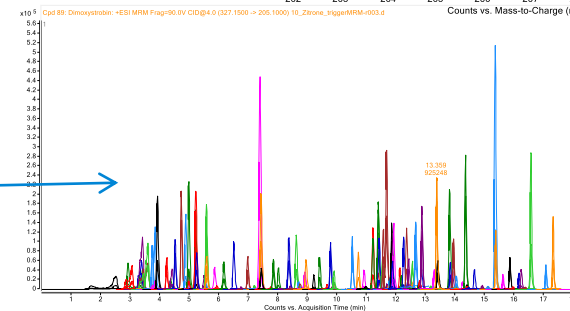
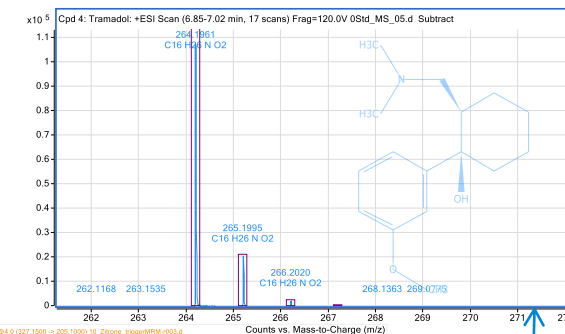
11.952

205.100

304.1011

68.36

MassHunter Automático



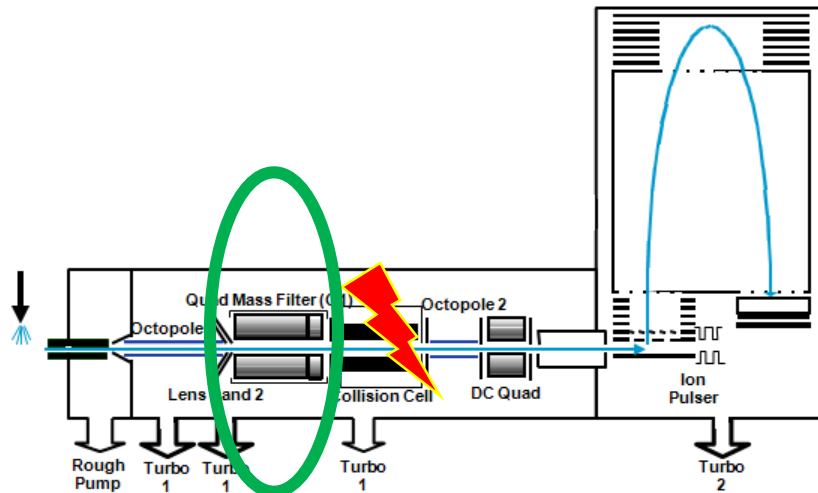
Extracción del espectro de MS de cada compuesto

Target & suspect Screening

All Ions Acquisition

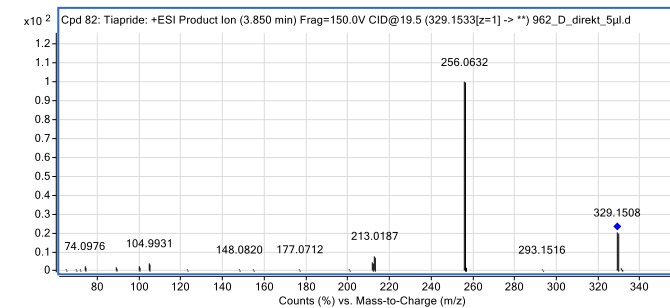
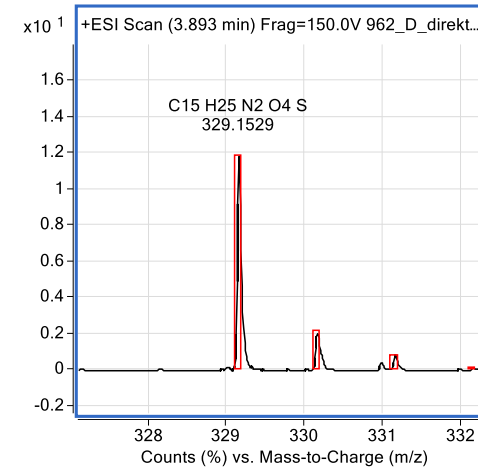
QTOF

ALL-IONS MS & MS/MS Mode



Quadrupole doesn't isolate any ion. NO filtering

Untarget Acquisition Target Process



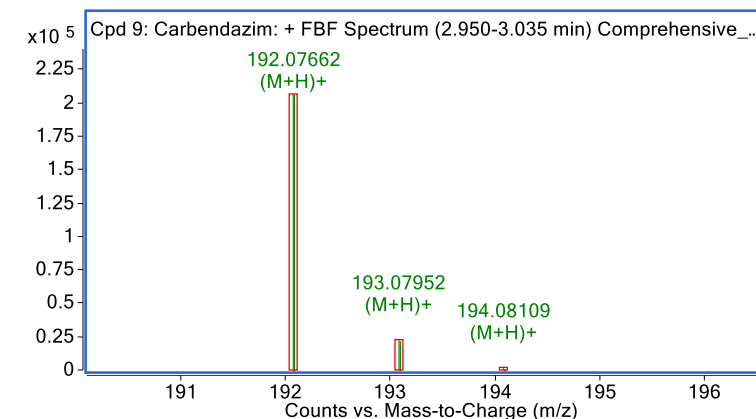
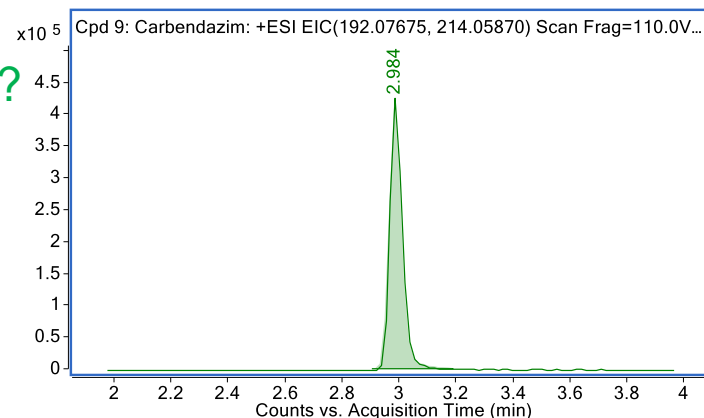
Collision Cell alternates
Energies

All Ions MS/MS – Screening with MS & MS/MS

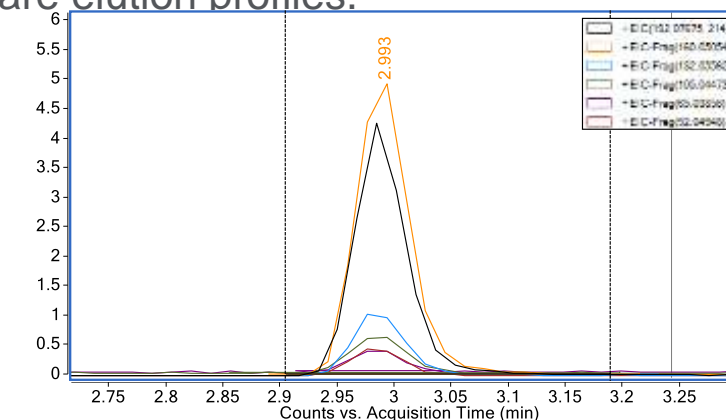
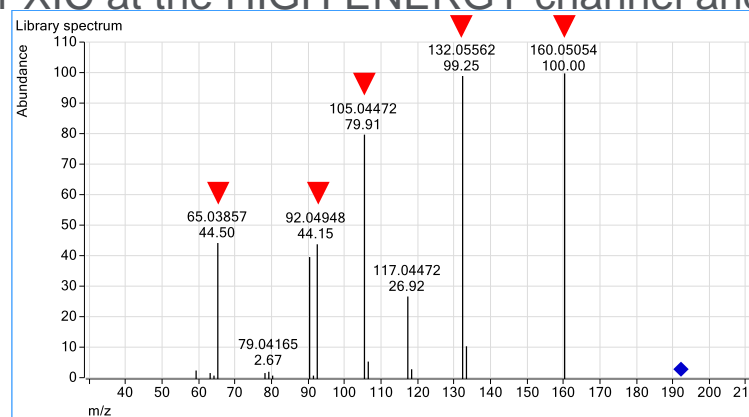
Untarget Acquisition Target Process

- All-Ions Data Process Algorithm is at first stage looking for compounds in a PCDL according Accurate Mass and Isotopic Pattern only at the **LOW ENERGY** Channel.

Score >90 ?

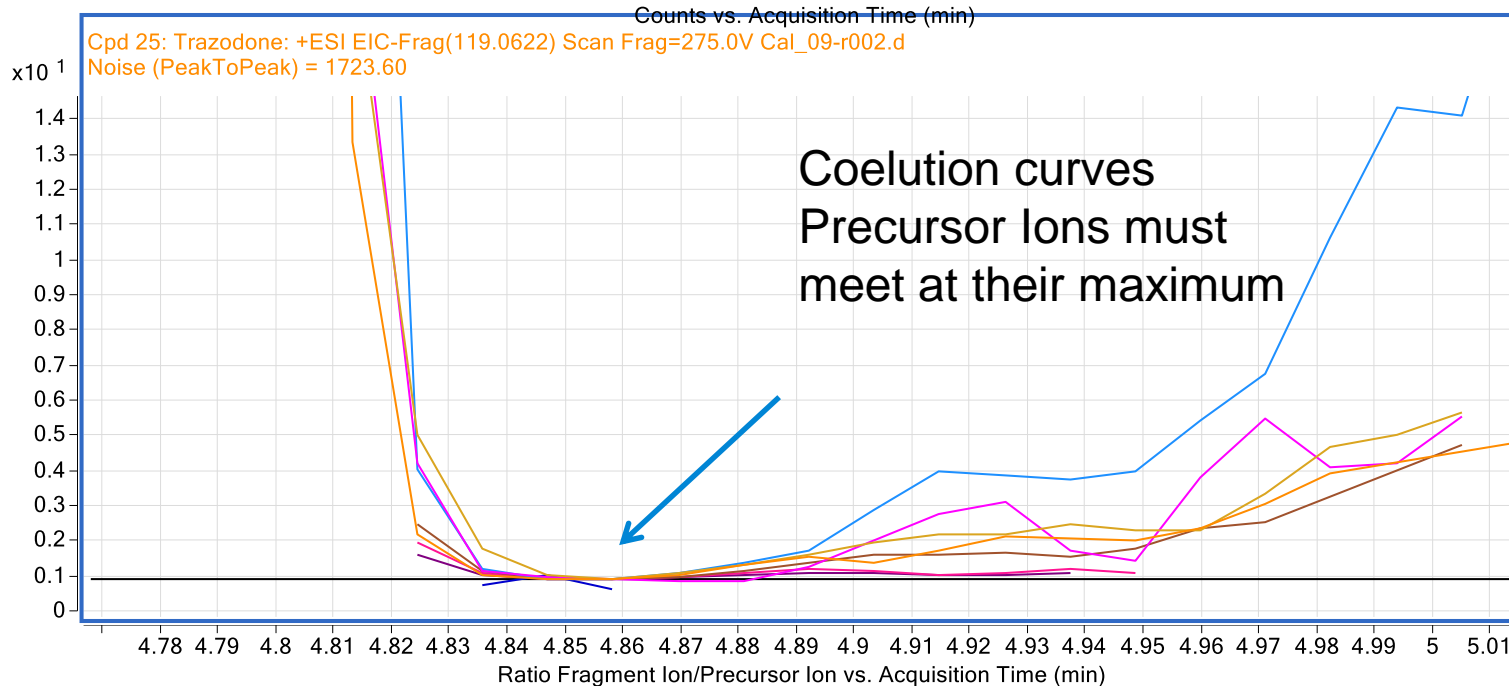
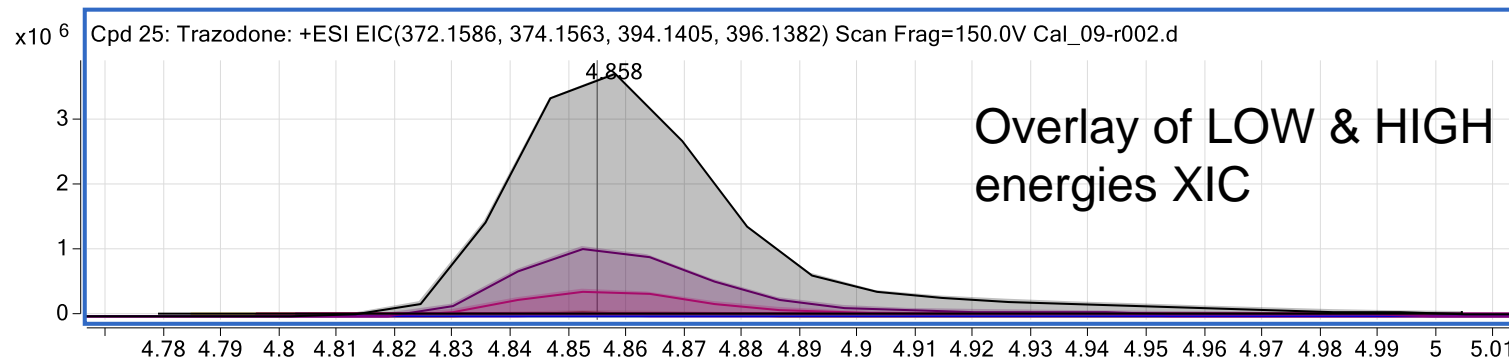


- Only if Score (user setting) is high enough, it queries PCDL Library to list known fragments in MS/MS spectra of compounds to extract their XIC at the **HIGH ENERGY** channel and compare elution profiles.



All Ions MS/MS : Chromatographic Confirmation : Coelution Score

Untarget Acquisition
Target Process



All Ions Screening

Curated or PCDL Libraries

PCDLs by Compounds & Spectra

LC/MS PCDL	Market	PCDL	Compounds with AM MS/MS Spectra	Total number of Spectra	Compounds with RTs
Forensic Toxicology	Forensic Toxicology	>9,200	>3,900	>13,500	0
Pesticides	Food Safety / Environmental	>1,700	>800	>2,700	0
Veterinary Drugs	Food Safety	>2,100	>1,500	>5,200	>120
Mycotoxins	Food Safety	>450	>300	>1,300	0
Water Contaminants	Environmental	>1,400	>1,000	>3,900	>260
METLIN*	Metabolomics / Lipidomics	>79,600**	>9,400	>32,000	>680
NIST 2014 MS/MS	General	>9,300	>9,300	>234,000	0

*METLIN numbers exclude tri- and quatra- peptides in the online METLIN

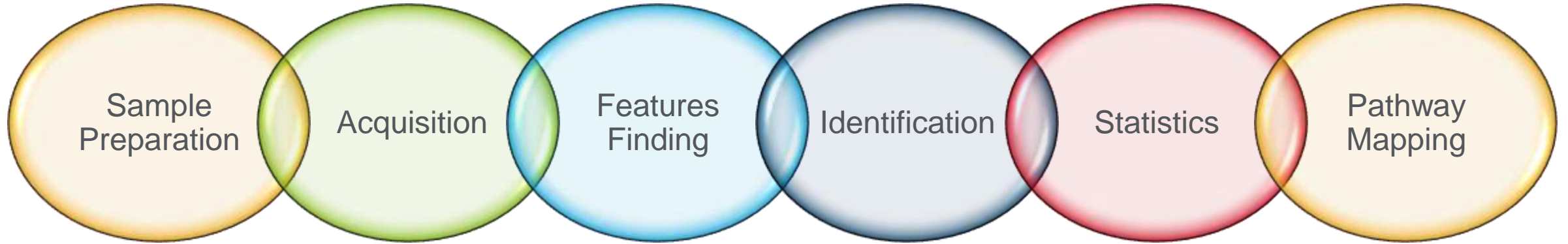
** Plus 168k theoretical



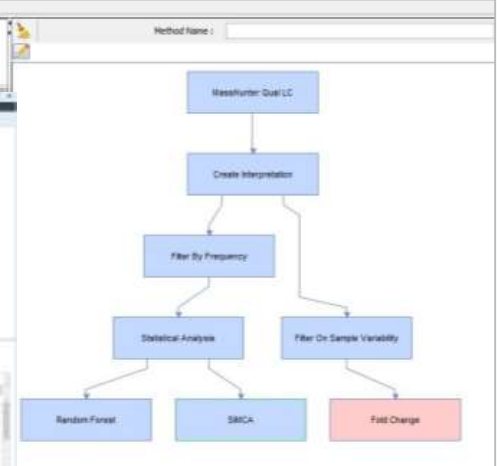
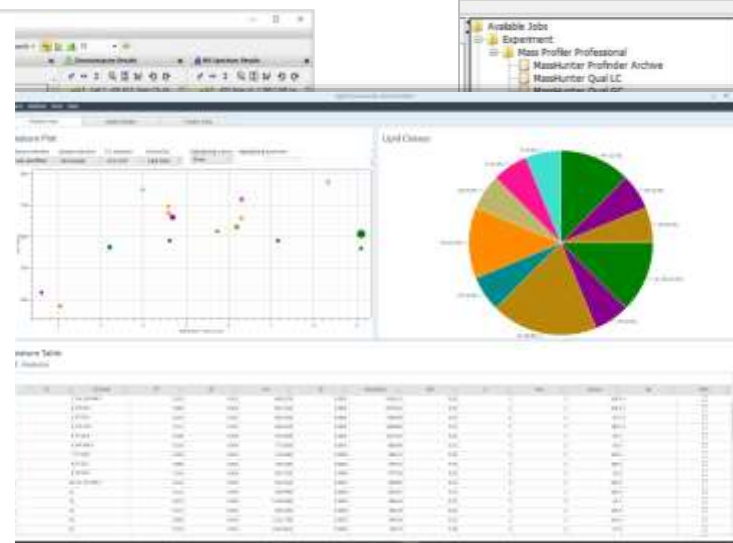
6546 LC/Q-TOF



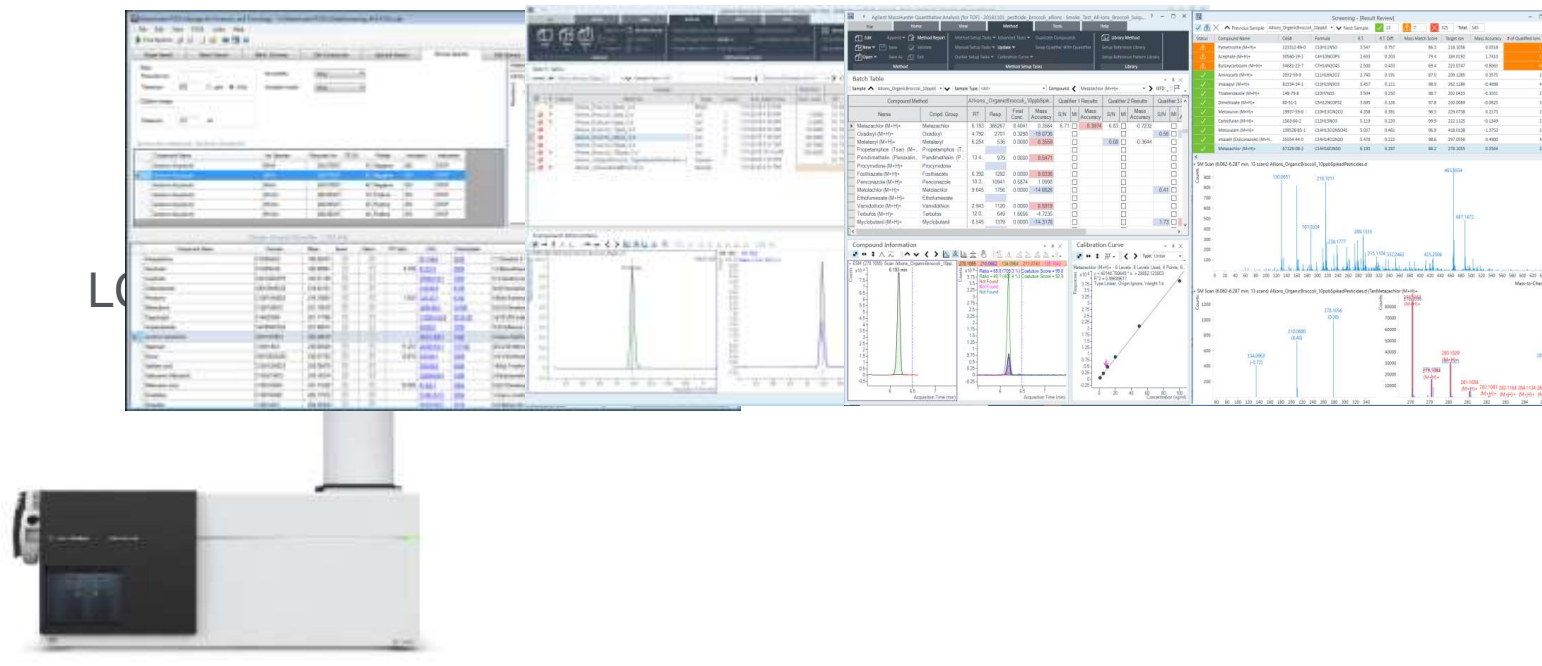
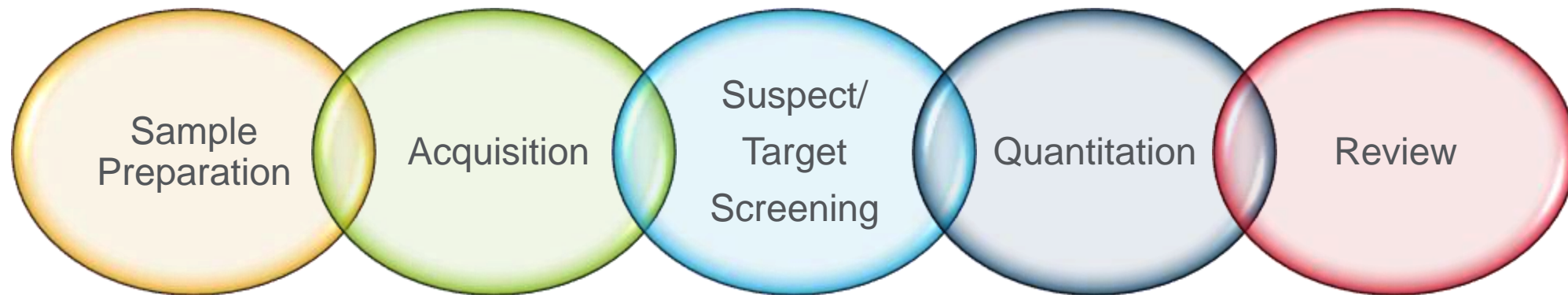
Improving the Agilent Workflows: Metabolomics

725
n

6546
LC/Q-TOF



Improving the Agilent Workflows: Food Safety





6546

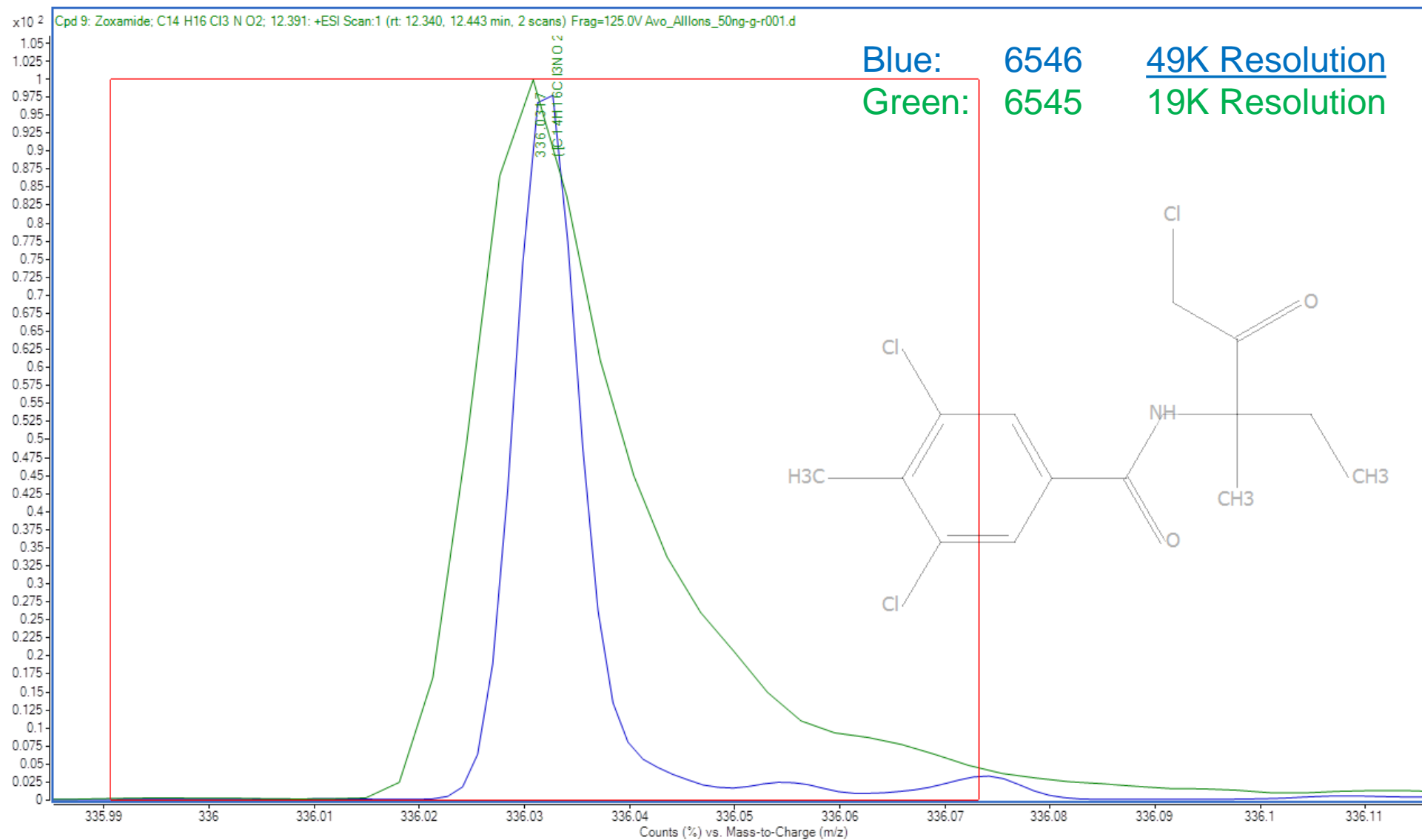
Like the 6545...with a lot more muscle!

- Simultaneous Hi Resolution, Extended Dynamic Range (10Ghz)
- Higher resolution ($>60k$ @ $2722m/z$, $>30k$ @ $118m/z$)
- DIA Quadrupole-resolved All-Ions (Q-RAI)
- Capillary gate valve
- Same sensitivity, isotopic fidelity, robustness from the 6545

Tune Mix – Measured data

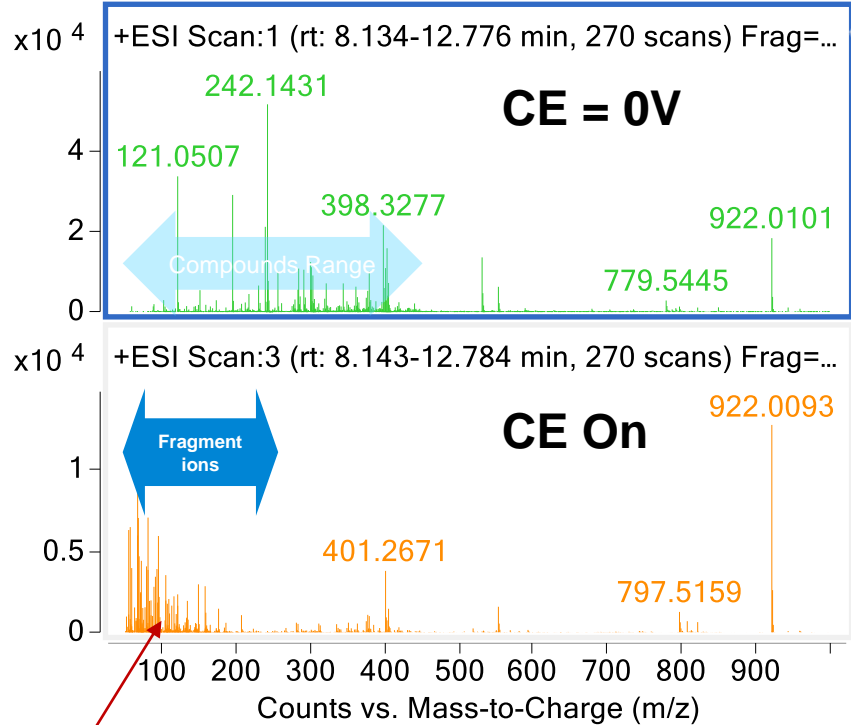


Resolution of 6546A Q-TOF System



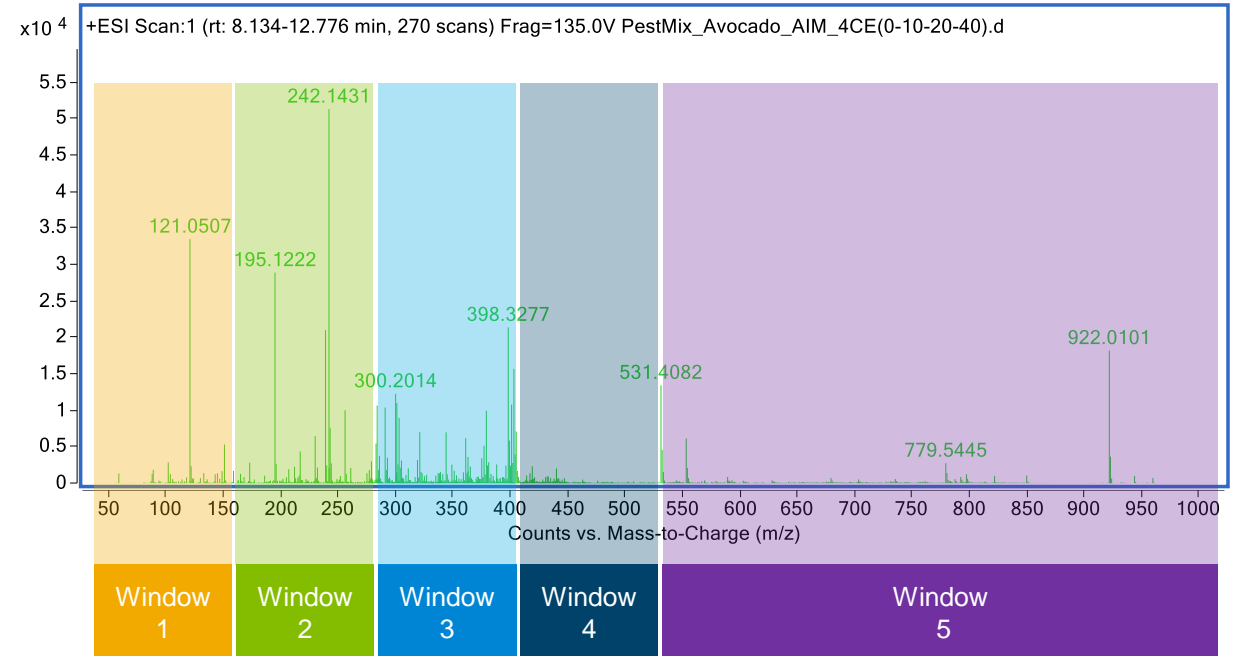
Data Independent Analysis (DIA)

Current: All Ions MS/MS



Too much interference in complex matrix at low m/z when CE applied during All Ions MS/MS

Silhouette: Q-RAI (Quadrupole resolved All-Ions)

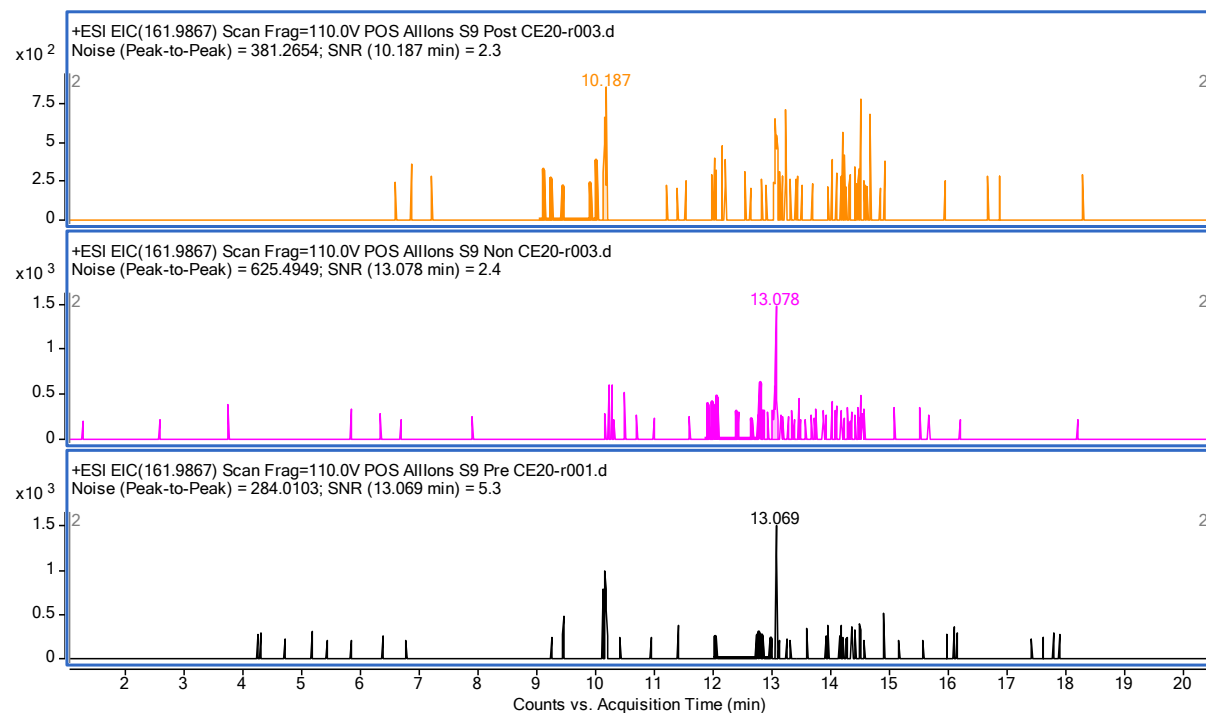


Similar to All Ions, but using the quadrupole to take sequential windows of the mass range to reduce complexity of the MS/MS spectra.

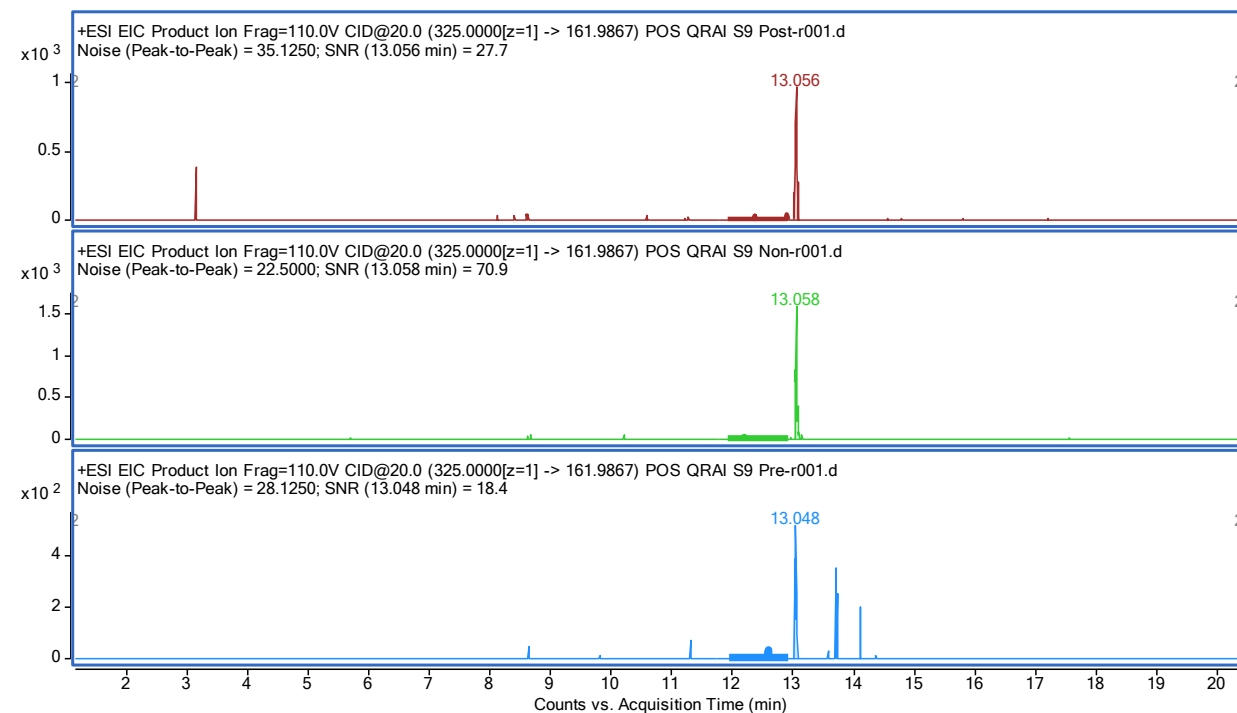
Qualifier Ion

Triclocarban [M+H]⁺ 314.9853 m/z

All Ions

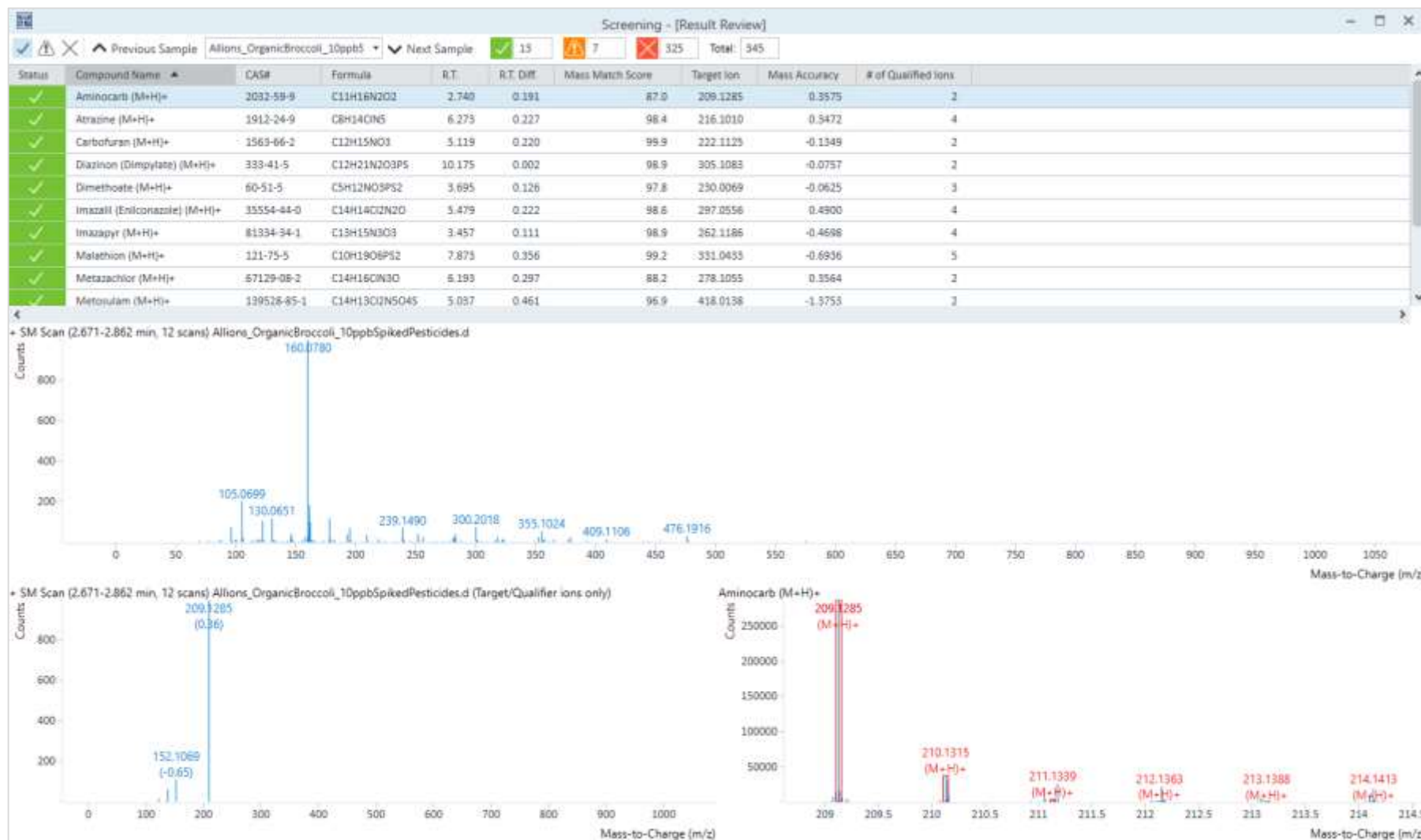


Q-RAI (Quadrupole resolved All Ions)



New: Quant 10 – New viewing tool: LC-QTOF Screener

Ease of use: improving Qual/Quant approaches



Review by “Acceptance”

Screening - [Result Review]									
✓	⚠	✕	Previous Sample	Allions_OrganicBroccoli_10ppb5	Next Sample	✓ 13	⚠ 7	✕ 325	Total: 345
Status	Compound Name	CAS#	Formula	R.T.	R.T. Diff.	Mass Match Score	Target Ion	Mass Accuracy	# of Verified Ions
✓	Dimethoate (M+H)+	60-51-5	C5H12NO3PS2	3.695	0.126	97.8	230.0069	-0.0625	3
✓	Imazalil (Enilconazole) (M...	35554-44-0	C14H14Cl2N2O	5.479	0.222	98.6	297.0556	0.4900	4
✓	Imazapyr (M+H)+	81334-34-1	C13H15N3O3	3.457	0.111	98.9	262.1186	-0.4698	4
✓	Malathion (M+H)+	121-75-5	C10H19O6PS2	7.873	0.356	99.2	331.0433	-0.6936	5



Number of Verified Ions



Target Ion Mass Accuracy



Mass Match Score



RT Difference

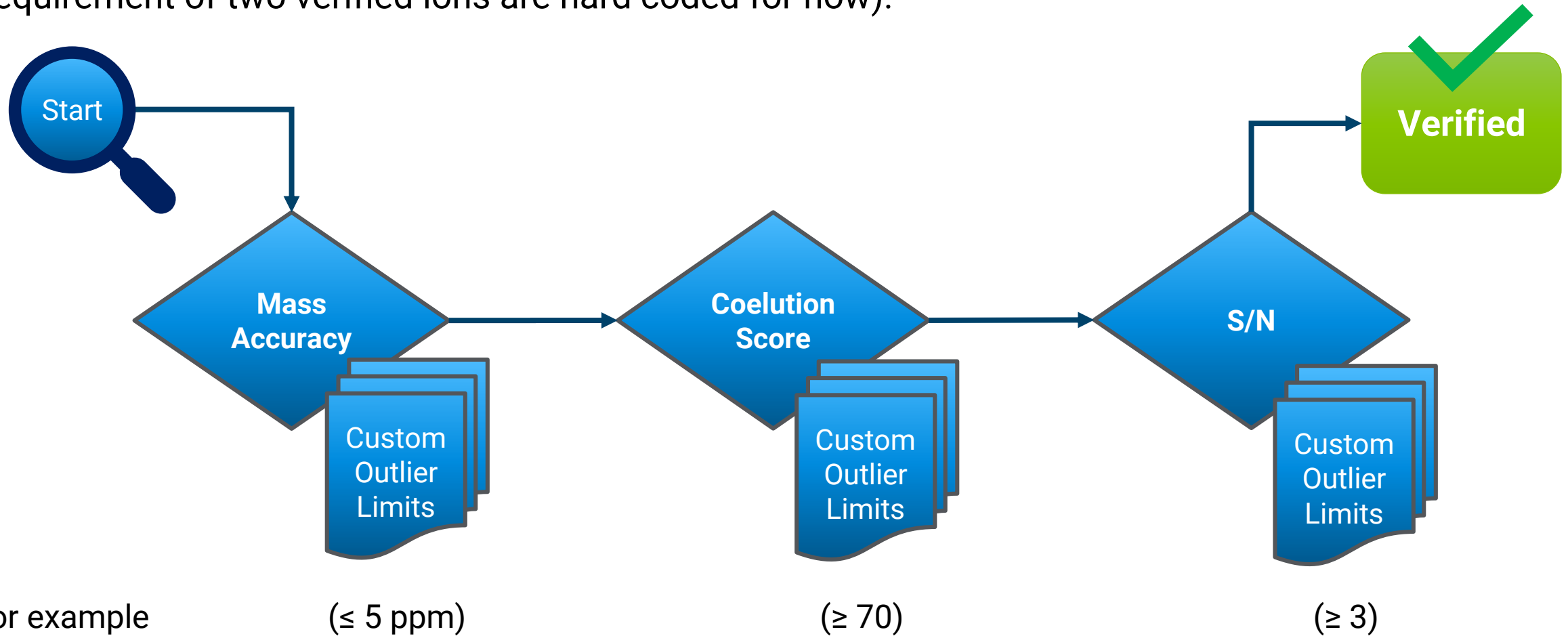
SANTE/11813/2017 Guidelines

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		minimum number of ions	other
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	<u>full scan</u> , limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	<u>2 ions with mass accuracy ≤ 5 ppm^{a, b, c)}</u>	<u>S/N ≥ 3^{d)}</u> Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must <u>fully overlap</u> . Ion ratio: see D12

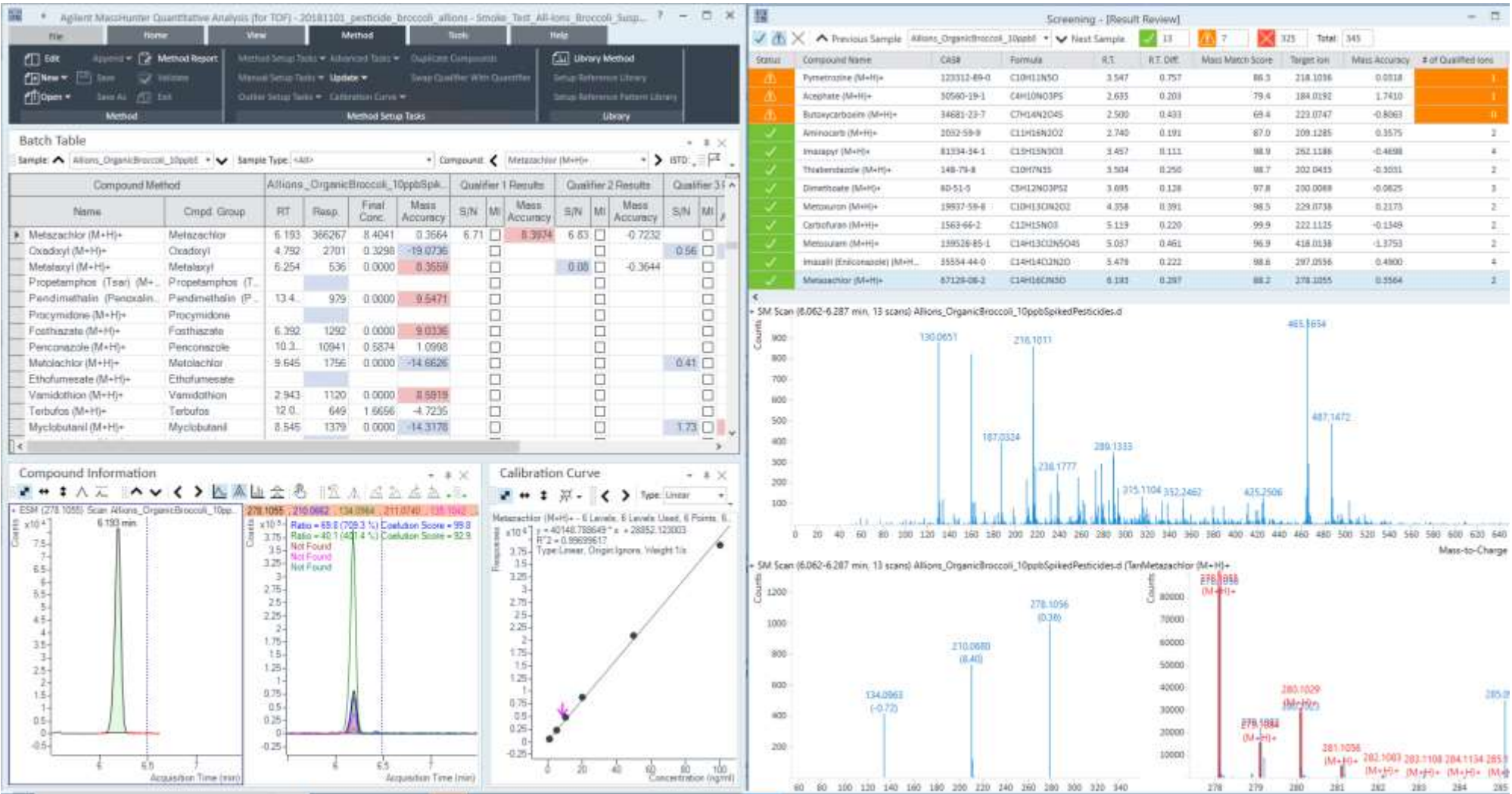
^{a)} preferably including the molecular ion, (de)protonated molecule or adduct ion
^{b)} including at least one fragment ion
^{c)} < 1 mDa for m/z < 200
^{d)} in case noise is absent, a signal should be present in at least 5 subsequent scans

Number of Verified Ions Flowchart

Each extracted ion feature to be evaluated against custom outlier limits
(Requirement of two verified ions are hard coded for now).



Example Results of LC-QTOF Screener



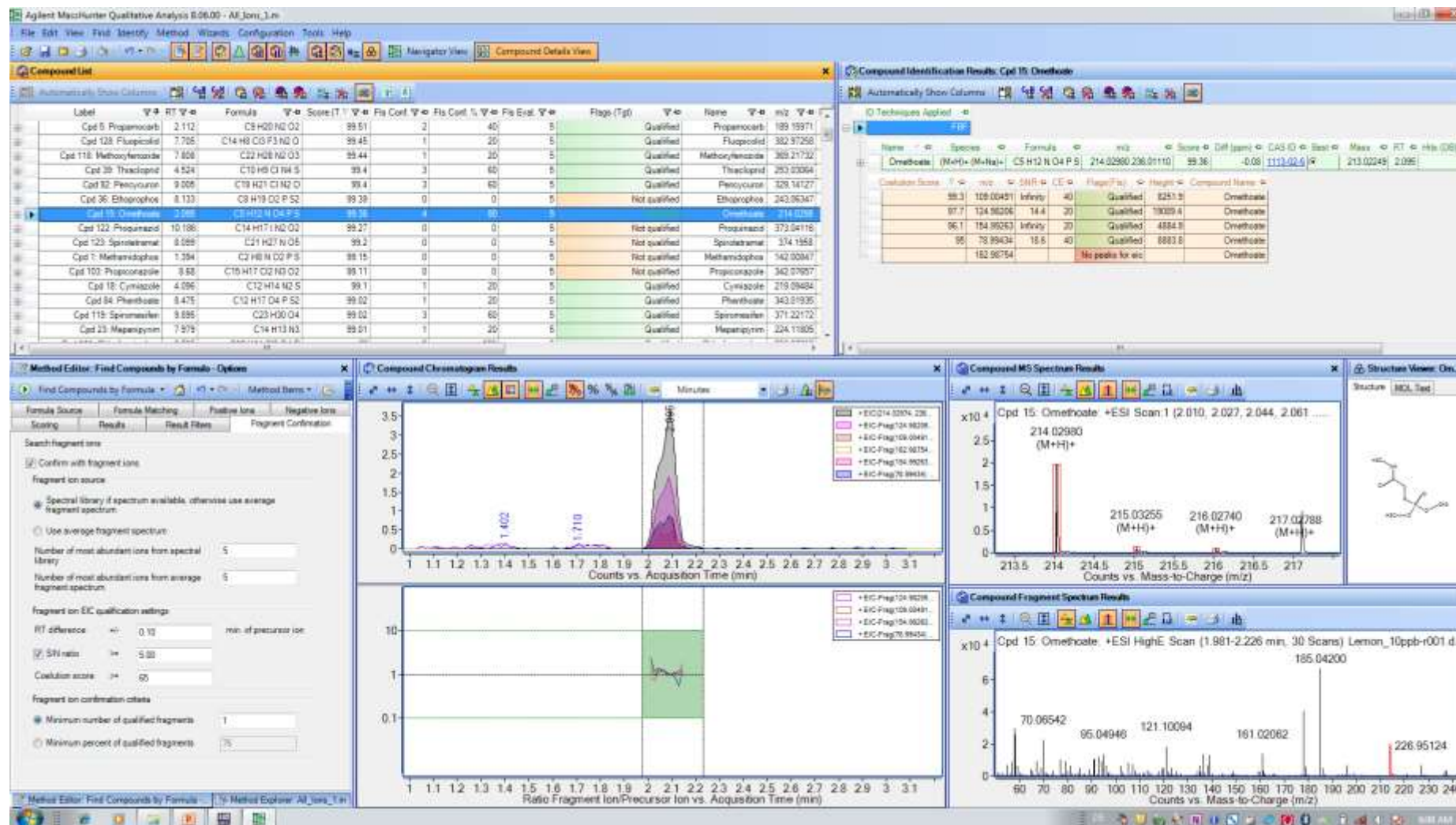
Broccoli sample
(fortified)
14 cpds @ 10 ppb
=> all identified

Target		
Aminocarb		●
Diazinon		●
Dimethoate		●
Imazalil		●
Malathion		●
Metazachlor		●
Molinate		●
Pyraclostrobin		●
Thiabendazole		●
Suspect		
Atrazine		●
Carbofuran		●
Imazapyr		●
Metosulam		●
Metoxuron		●

Qualitative screening using All Ions MS/MS

Results overview of a Suspect Screening

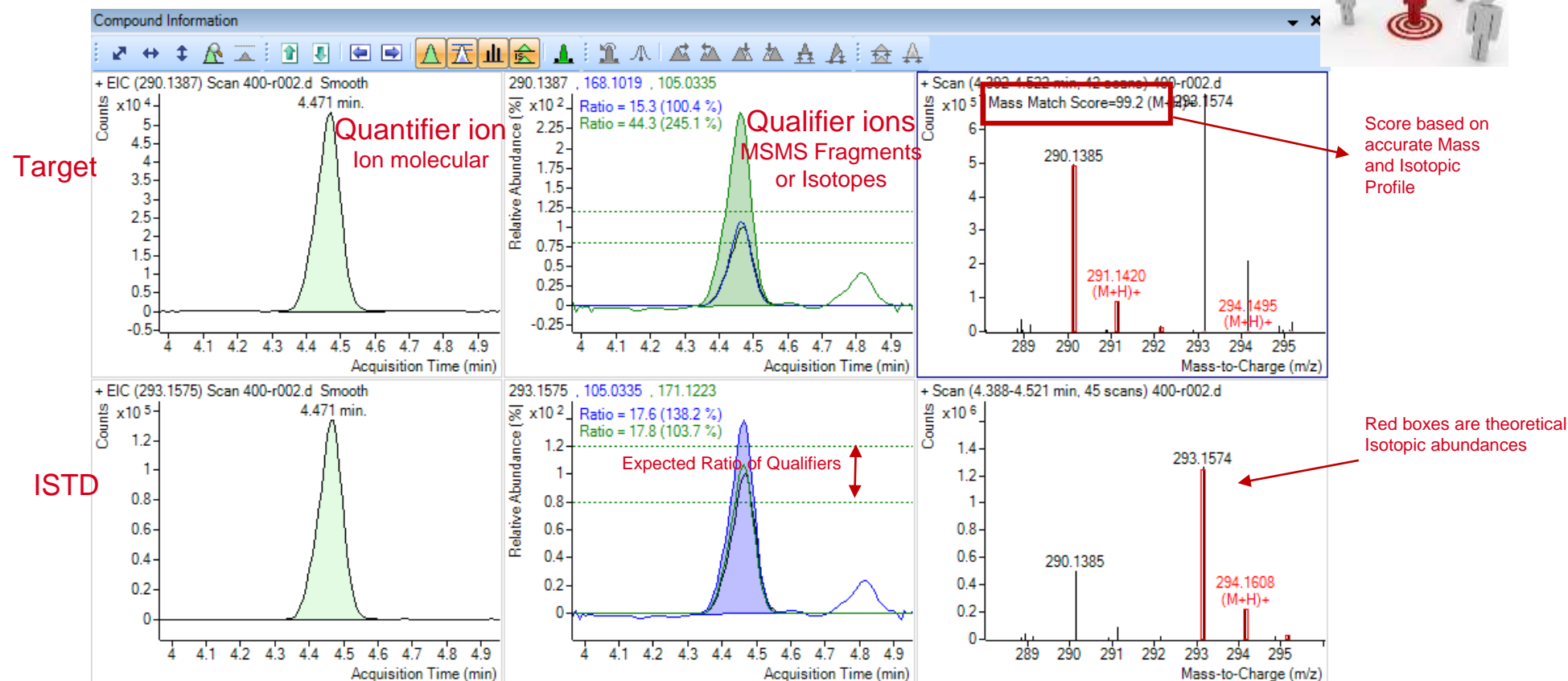
Untargeted Acquisition Target Process



Quantitative screening using All Ions MS/MS

Results overview of a Quant Screening with Standards

Untargeted Acquisition
Target Process



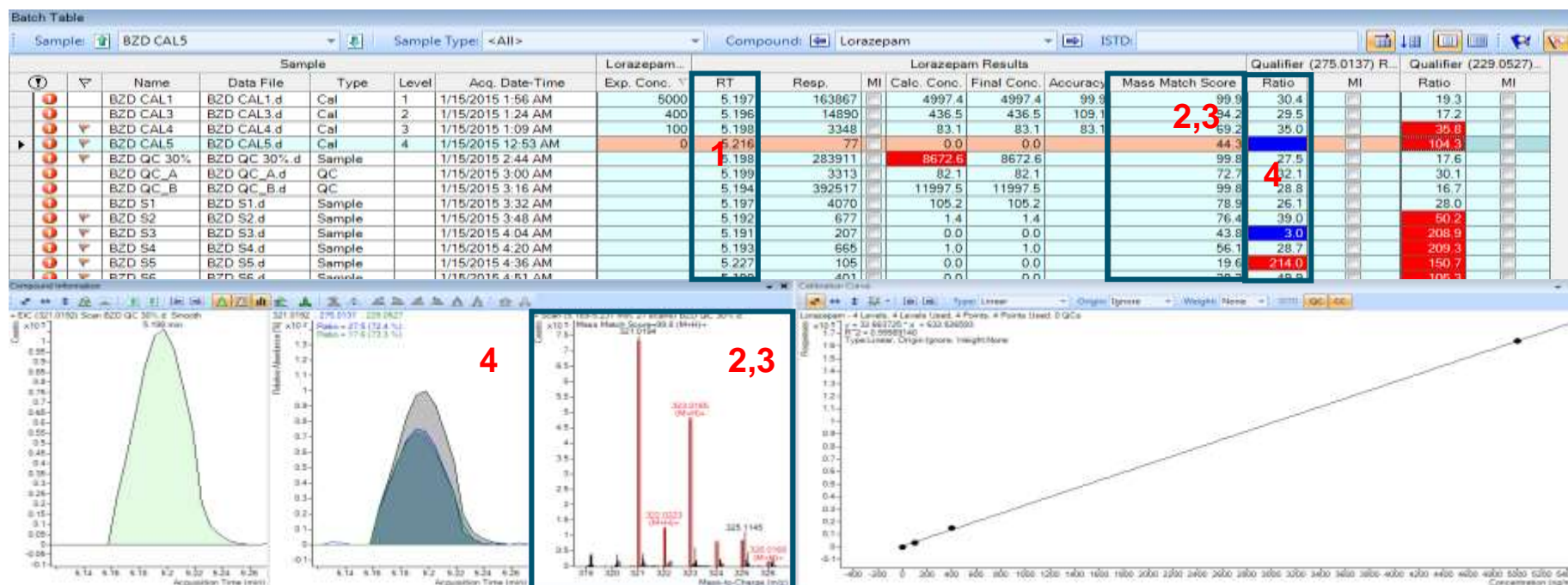
All Ions data in MassHunter Quant

Quantitative screening using All Ions MS/MS

4D-ID Confianza en los resultados

Untargeted Acquisition Target Process

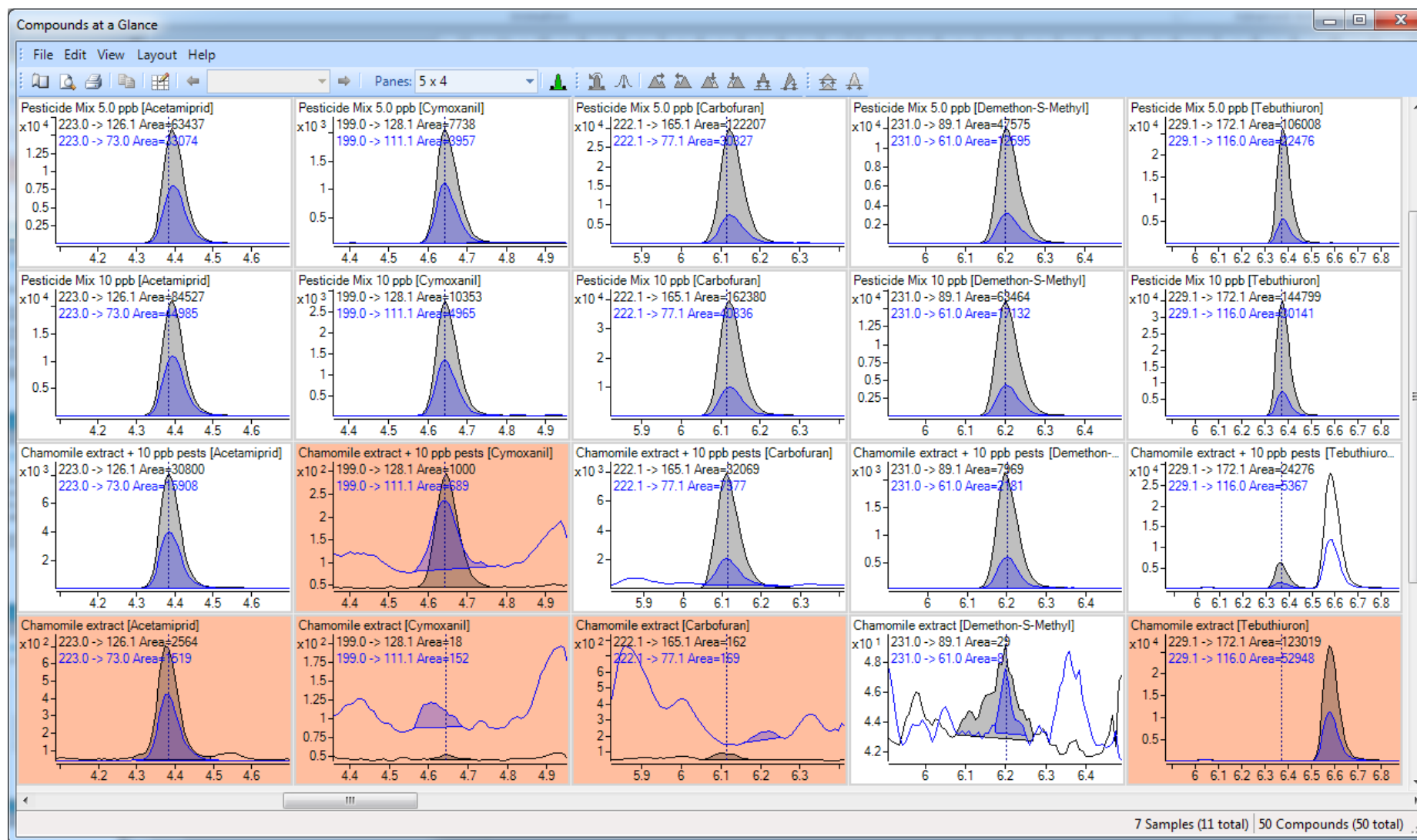
1. Tiempo de retención
2. Masa Exacta
3. Perfil Isotópico del compuesto
4. Fragmentos MSMS como Ion Qualifiers



Quantitative screening using All Ions MS/MS

Compounds at a glance

Untargeted Acquisition
Target Process



Screening Definitions

Target Screening. - Four Dimensions of Identification (4D-ID) QQQ Emulation



Compounds identified through Accurate Mass, Retention Time, Isotope Pattern and Fragment Confirmation. The quant method is developed via MassHunter Qual and Libraries (PCDLs). Acquisition is in All Ions MS/MS. Standards Used.

Suspect Screening - Propose and Identify WIDEST Screening Approach



A Suspect List PCDL can be created from the encyclopaedic Master PCDLs. Compounds can be found and proposed using *Find-By-Formula*. The proposed list becomes the target or preferred (directed) list for target MS/MS or auto MS/MS. The resulting data can be searched against MS/MS spectra in the Suspect PCDL or Structure Correlated using MSC against the same PCDL if no MS/MS spectra are available.

Discovery Profiling - Find, Compare and Identify



Profiling uses Molecular Feature Extraction (MFE) which is a naïve feature finding algorithm, in order to **find & characterize** compounds in a data set. These compounds can then be compared to determine the absence, presence or up- or down-regulation of the compounds from sample group to samples group. Profiling can be performed in single sample comparisons, two sample groups or multiple sample groups and conditions. Significant compounds can be imported into Acquisition in order to generate MS/MS data which is priceless in further compounds identification by formula generation, library search or structural correlation.

- *Enfoques y estrategias analíticas que nos permiten las ultimas tecnologías de LC Mass Spectrometry de alta resolución. **UHPLC QTOF technical details***
- **Agilent proposal Workflows in different scenarios.** *Diferentes estrategias inherentes a investigación en el ámbito de Ciencias de la Vida, así como para el perfilado de Alimentos, Materiales, Procesos...*
- **Herramientas de Agilent y flujos de Trabajo para tomar mejores decisiones en un entorno de Biología integrada.** *Del diseño experimental a las conclusiones, un largo camino para ayudar al investigador.. :*
 - **Datos según modos de Adquisición.** *Complementariedad de las diferentes tecnologías LCMS, GCMS, CEMS, ICPMS*
 - **Deconvolución de datos y herramientas de visualización.** *Como funcionan los algoritmos de Agilent para extraer información de compuestos de un Full Scan.*
 - *Preparación de datos previa al Análisis Estadístico diferencial. **Alineamiento, Normalización, “Baselining” con “Mass Hunter ProFinder”.***
 - *¿Necesito análisis recursivo a través de iteración? Por favor hágamelo fácil.... **Exhaustivo tratamiento de datos para evitar la Perdida de compuestos.***
 - *Mass Profiler professional. **Análisis Diferencial a través de Interpretaciones, Clustering, PCA, PLRS, modelos de predicción***
 - *Así, ¿Cuales son mis compuestos diferenciales de interés? ¿Como puedo identificarlos? **Librerías empíricas de espectros MS/MS. Agilent METLIN PCDL.***
 - *Análisis de rutas Metabólicas a través de “Pathways Analysis”. **Biología integrada e interpretación biológica de mis datos. Pathways Analysis.***
 - *¿Cuál es mi próximo experimento? **La potencia del enfoque de la Biología integrada.***
- **Movilidad Iónica.** *Una nueva dimensión para extracción de datos más selectiva en muestras complejas. Una nueva herramienta de identificación*
- **Fluxómica. Fácil y rápida** *visualización de la incorporación de sustratos marcados isotópicamente en una ruta metabólica a través de “VistaFlux”.*
- *Método llave en mano para el análisis Metabolómico dirigido en rutina de los **metabolitos del Ciclo Central de Carbono***
- *Sinergias con la medida In-vivo del Metabolismo celular con “Seahorse”.*



Chemometric strategy for Life Science -omics and Food Profiling.

Agilent proposal Workflows in different scenarios

Metabolomics, as a discipline to find a **differential metabolite** to correlate with one or multiple independent variables on epidemiological studies has at least two major approaches :

- Holistic or Untarget Metabolomics : Top down approach with massive data to find differences, needs advanced mathematical tools.
- Reductionist or Target Metabolomics : Easiest approach looking for differences in just a limited list of compounds

Genomics/Transcriptomics was first of the –Omics discipline to use advanced Chemometric strategies to resolve large data sets problems.

Untarget Metabolomics and furthermore other omics disciplines like Foodomics, Glycomics, Petrolomics, etc..... leverages from the tools developed in different applications and scenarios.



Chemometric strategy for Life Science -omics and Food Profiling. Agilent proposal Workflows in different scenarios

Metabolomic Studies Introduction

- Classical Metabolomics **objectives on research** area are to find **new Biomarkers** for **early** diseases diagnosis, classify patients,.... There are two important phases :
 - Biomarkers **Discovery** phase will require **massive profiles** of metabolites; hundreds/ **thousands of metabolites** to follow up.
 - Biomarkers **Validation & Diagnosis** phase will require usually to follow up a **reduced number of metabolites**; a few ones/dozens of metabolites to follow up.
- Following such Metabolomics Methodology or approach, other disciplines made progress :
 - Exposomics : as study of the disease-causing effects of environmental factors.
 - Foodomics : Food and Nutrition domains through the application of omics technologies including Nutrigenomics and Nutrigenetics
 - Profiling in general. Food, Materials, etc....



Chemometric strategy for Life Science -omics and Food Profiling. Agilent proposal Workflows in different scenarios

Metabolomic Studies Introduction

- **Metabolomics analysis** to get metabolites profiles are based on:
 - **Chromatography/Mass Spectrometry (LC/MS, GC/MS, CE/MS):** for **all kinds** of metabolites (minoritarian & majoritarian ones). LC/MS is **also used** for Proteomics.
 - **Nuclear Magnetic Resonance (NMR):** **only for majoritarian metabolites.**
- **LC/MS, GC/MS “versus” NMR**
 - **Sensitivity:** LC/MS, GC/MS are able to **detect** metabolites at **much lower concentration** than NMR.
 - **MS sensitivity is $> 10^6$** times better than **NMR.**
 - **MS** requires typically $> 1\text{-}100\text{pg}$ (10^{-12}g) metabolite **NMR** $>200\mu\text{g}$ - 5mg
 - **Sample state:**
 - **LC/MS** requires **liquid samples** (or solid dissolved on aqueous or organic solvent). GC/MS also accepts gaseous samples.
 - **NMR** accepts liquid & solid samples.
 - **Number of Spectras/sample:**
 - **LC-GC/MS:** **thousands** of MS spectra/sample **NMR:** **1 NMR** spectra/sample

A Comprehensive Metabolomics Workflow

Agilent LCMS, CEMS and GCMS

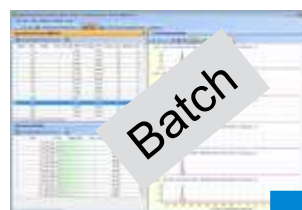
Separate &
Detect



CE-LC-TOF/QTOF
CE-LC-QQQ

Feature
Finding &
Data Prepare

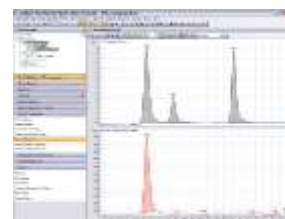
**MassHunter
Profinder**



Batch

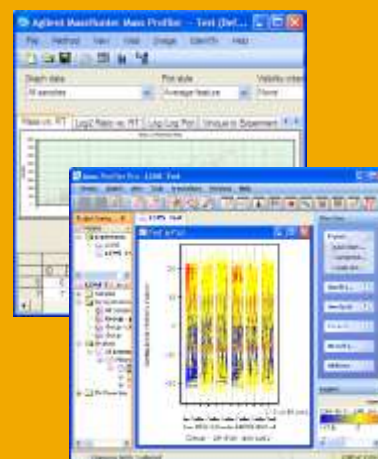
MassHunter Qual

MassHunter Quant



Data Prep. &
Statistics

**Mass Profiler
(Professional)**



**Statistics
Visualization**

ID Browser

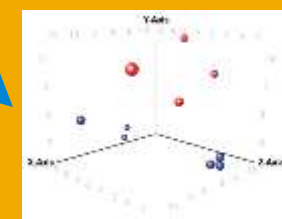


**Annotation &
Identification**

Pathway Analysis //
Profiling



**Pathway
Analysis**



Profiling

*LCMS, CEMS and GCMS Data can be analyzed together in
the same project*

Multi-Omics Open Platform: Mass Profiler Professional

Expression changes represented directly on routes

The screenshot shows the Mass Profiler Professional software interface. On the left is the 'Project Navigator' pane with a tree view containing 'Experiments', 'Samples', 'Interpretations', 'Analysis', and 'My Favorites'. The main workspace displays a biological pathway diagram with nodes and connecting lines. On the right, an 'Interpretation2: Tissue' panel lists genes: LMX1B, NIK2-2, ASCL1, and GATA2, each with a corresponding bar chart. Below this is the 'MS Experiment Creation Wizard (Step 1 of 11)' dialog, which includes a 'Select Data Source' section with radio buttons for 'MassHunter Quant', 'MassHunter Qual', 'MassHunter Qual (GC scan data)', 'MassHunter ICP-MS', 'Chemstation', 'AMDIS', and 'Generic'. A red arrow points from the 'Generic' option to a yellow callout box. Another red arrow points from the 'Interpretation2' panel to a blue callout box. A yellow callout box is also present near the bottom of the pathway diagram.

Projects

**Microarray-based, NGS, q-PCR
Gene Expression/
Transcriptomics Experiments**

**LC/MS, GC/MS, CE/MS, ICP/MS & NMR
based Metabolite / Protein
Abundance Measurements**

**Joint Pathways experiment:
transcriptomics /
metabolomics**

Interpretation2: Tissue

MS Experiment Creation Wizard (Step 1 of 11)

Select Data Source
Choose the data sources that will be used for the experiment

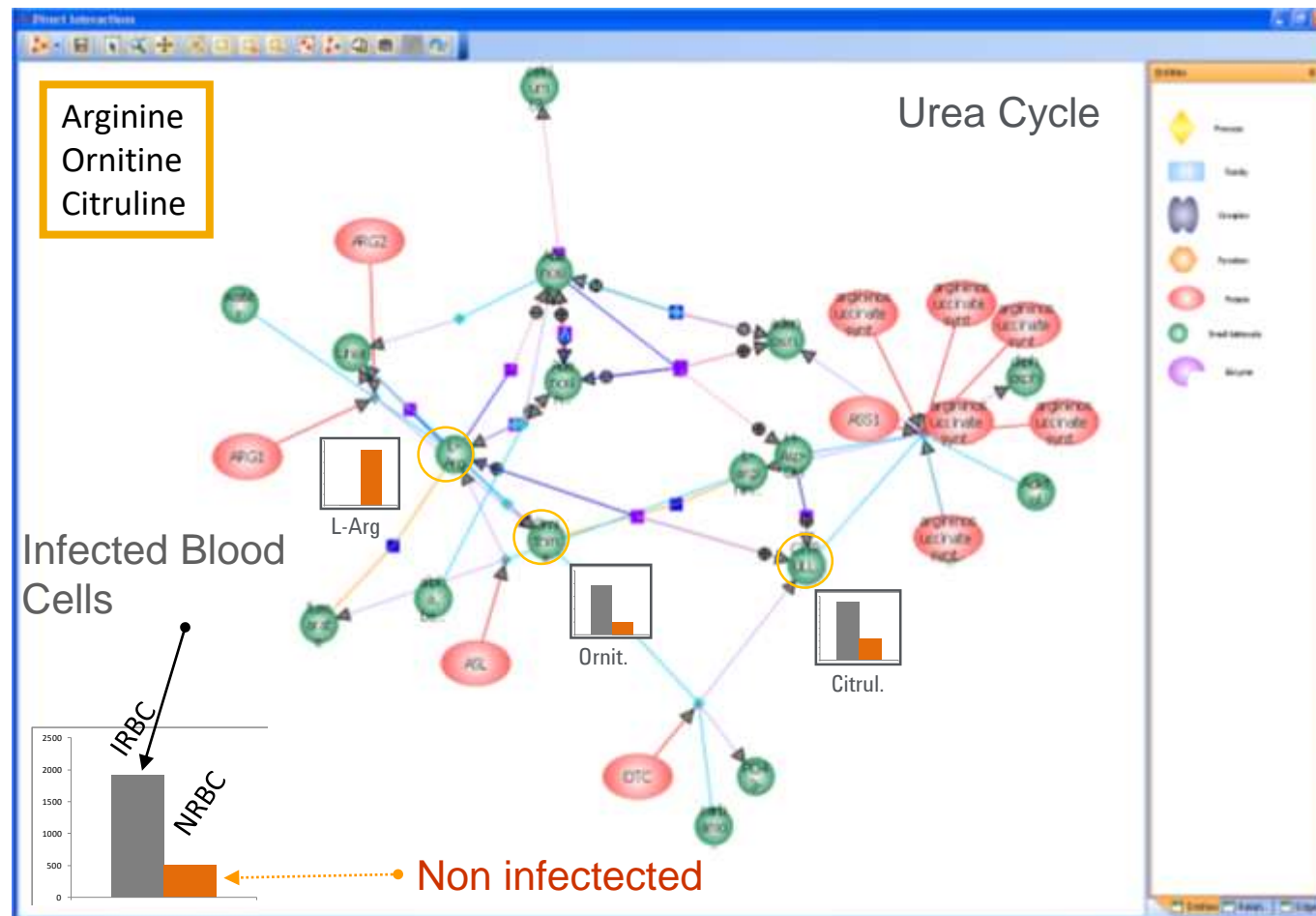
☒ MassHunter Quant
☐ MassHunter Qual
☐ MassHunter Qual (GC scan data)
☐ MassHunter ICP-MS
☐ Chemstation
☐ AMDIS
☐ Generic

**Generic Import for
non Agilent
instruments: *.xls,
*.xlsx, *.TXT or
*.CSV files**

Enrichment Analysis on curated pathways and computationally – derived networks



Differential Abundances of 3 Metabolites of Arginase Route (urea cycle) in Malaria Infected Red Blood Cells (RBS/ erythrocytes).



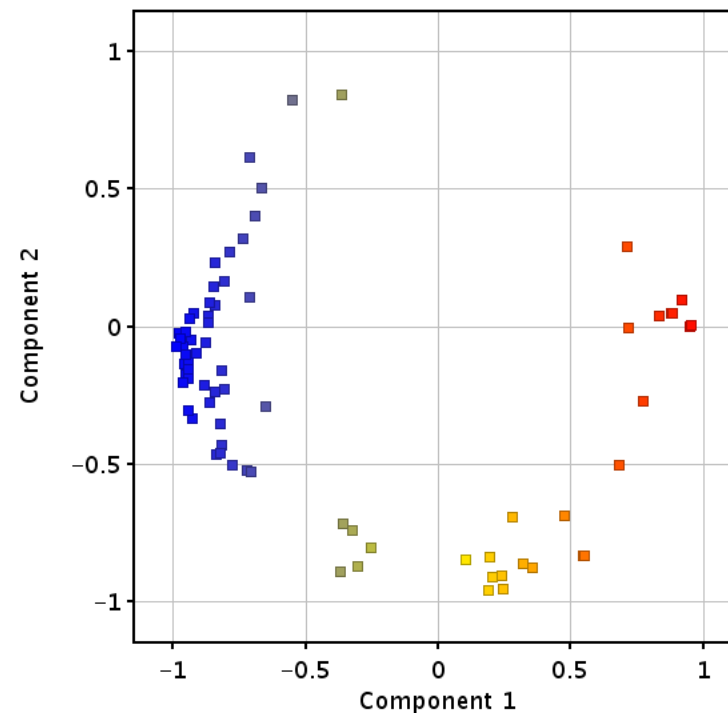
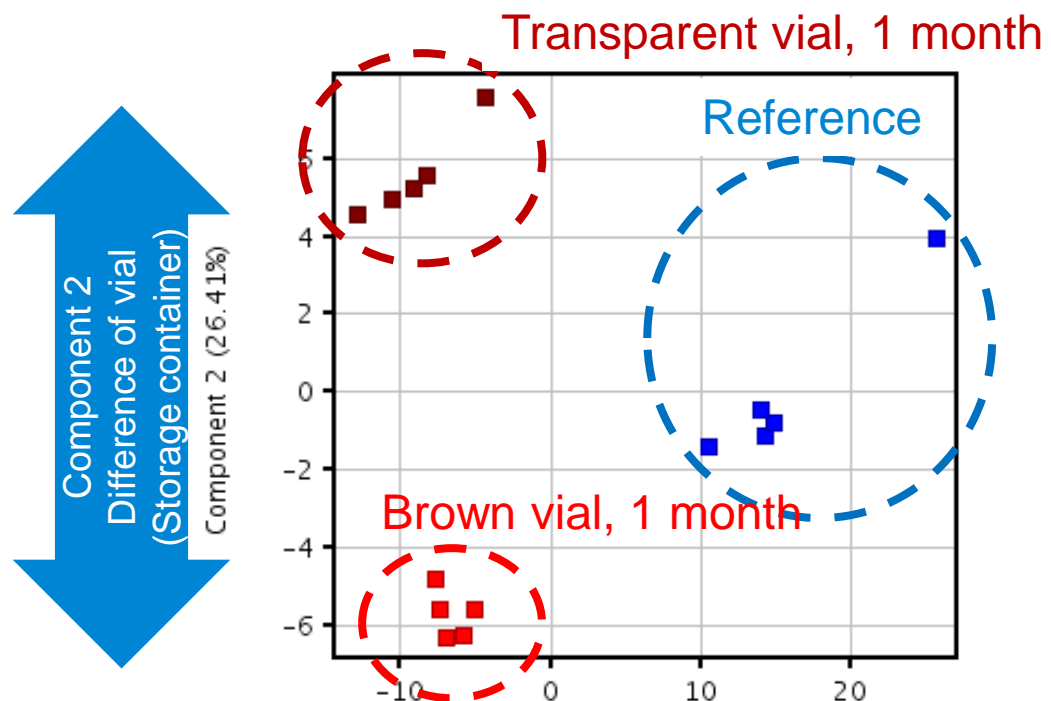
Abstract RBS SAMPLE PREPARATION:

1. - Centrifuge blood at **4°C** 2min (citrate as anticoagulant) and remove **on ice** the supernatant to get the erythrocytes.
2. - Wash with PBS (phosphate buffered saline) to remove the external erythrocytes metabolites.
3. - "Quench" (-25→37°C) and lyse the cell membrane to release the internal erythrocytes metabolites.
4. - Add aqueous phase modifier (methanol) at -20°C.
5. - Add organic phase (chloroform) -25°C.
6. - Do Liquid-liquid extraction at different pH's.
7. - Evaporate in vacuum and do an aqueous extract reconstitution.



Sake (Japanese liquor) deterioration test

PCA 2D Score Plot and Loading Plot



PCA Loading Plot

A point in the loading plot corresponds to a compound

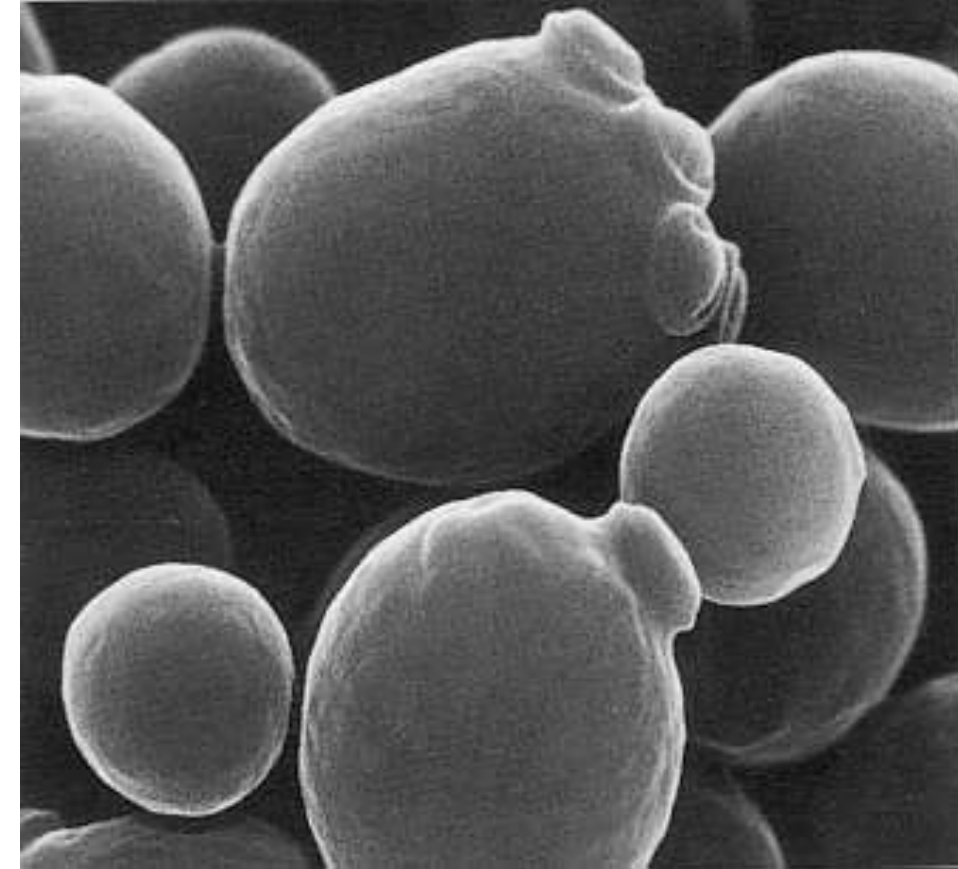
PCA Score Plot

Agilent Omics approaches examples

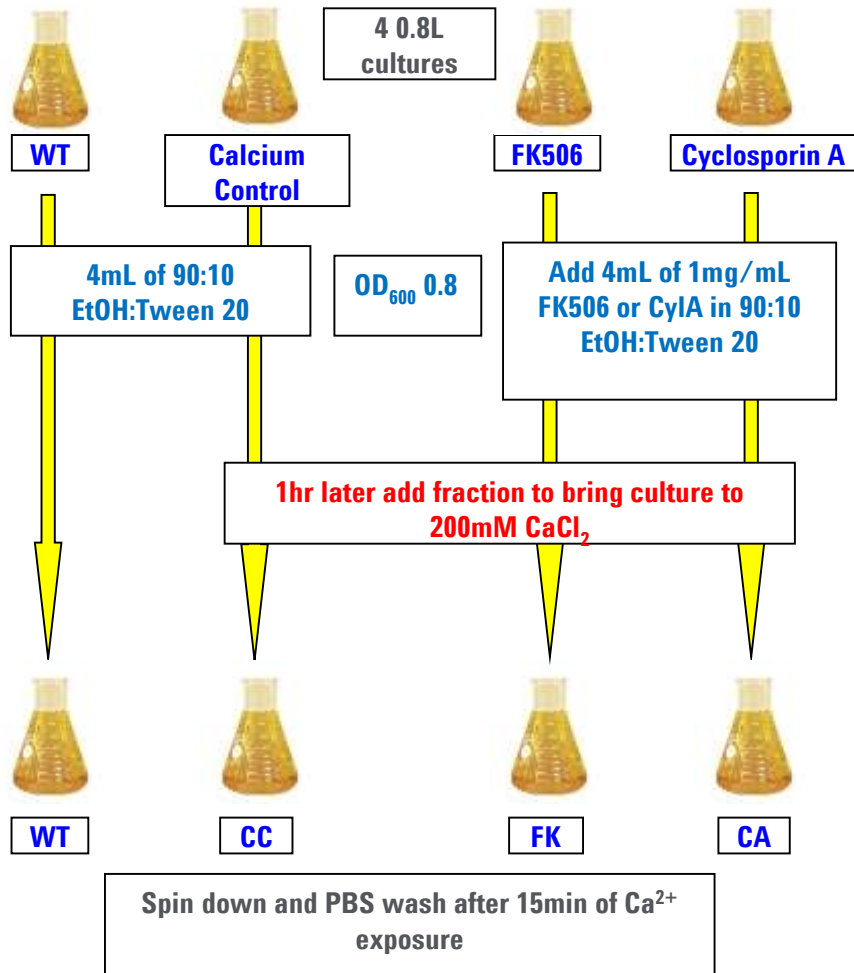
Metabolomics & Food Profiling

Effect of two different drugs on Ca regulation pathway

- *Baker's Yeast is an Ideal Model Organism for Studying Pathways*
- *Saccharomyces cerevisiae* is a widely used model organism
- Biochemistry and pathways are extensively studied
- Fully sequenced genome
- Ideal for “multi-omics” studies with the goal of facilitating research for other organisms.



Yeast Metabolomics :



Experimental Design

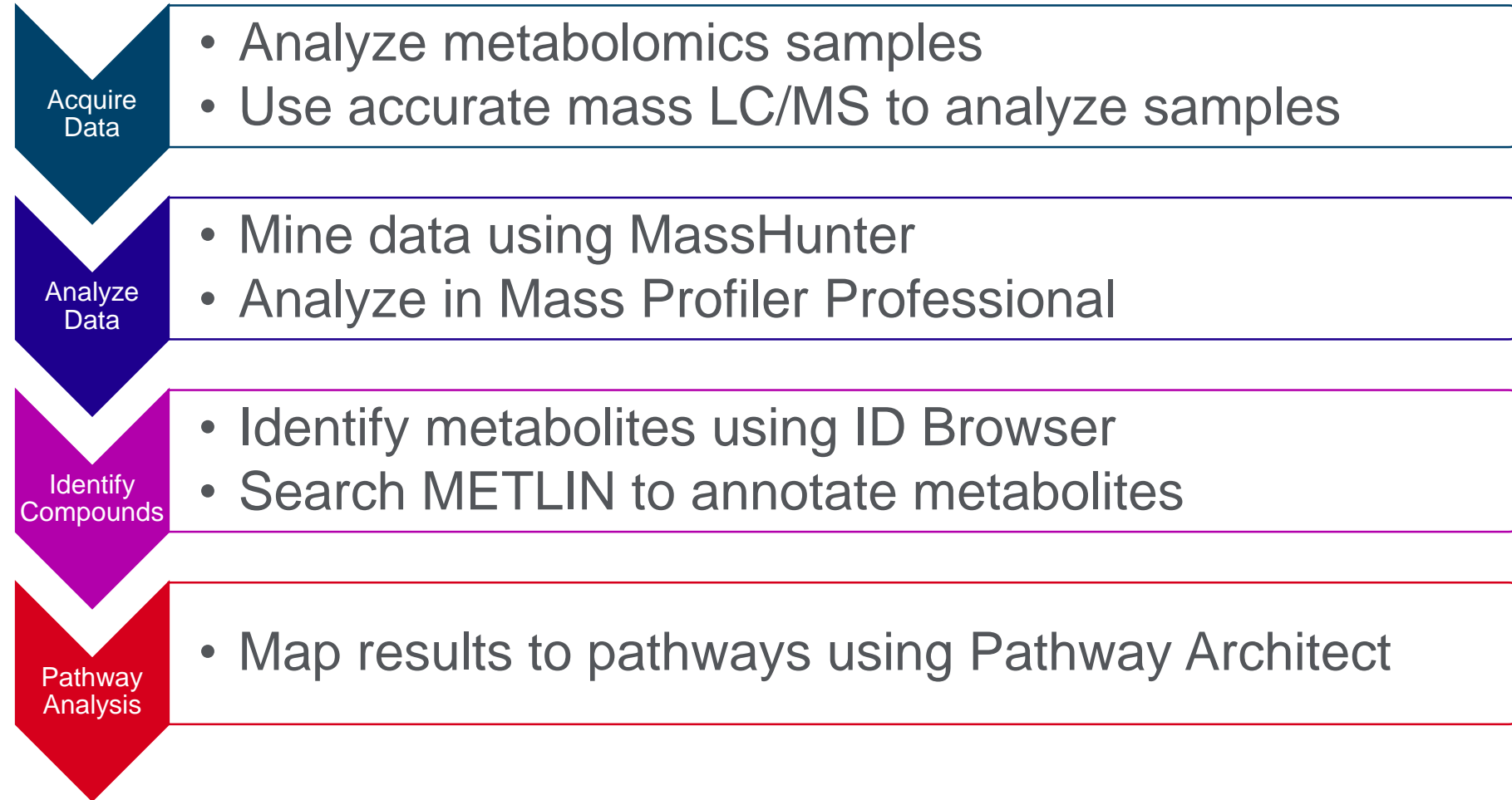
Treatments:

- Wild type (WT) - no treatment
- Calcium control (CC) - CaCl₂
- FK - FK506 and CaCl₂
- CA - Cyclosporin A and CaCl₂

Extraction :

Wet mill with 5:3:3 CHCl₃:CH₃OH:H₂O. Only the aqueous is analyzed

Metabolomics Workflow



TOF/Q-TOF For Discovery Metabolomics

Goal – Detect all metabolites

Data is acquired in Full Scan

Metabolite tracking uses retention time and mass or mass fragments

Statistical analysis is used to find differential metabolites (features)

Feature identification is required for biological interpretation



Ideal for discovery metabolomics

Spectral quality

- Accurate mass
- Good mass resolution
- 5 orders of dynamic range
- High isotope ratio fidelity
- Maintains performance at high acquisition speeds
- Sensitive

Compound identification

- Accurate mass, isotope ratio
- MS/MS with accurate mass, isotope ratio (Q-TOF only)

LC/MS Analysis of Metabolites in Stressed Yeast

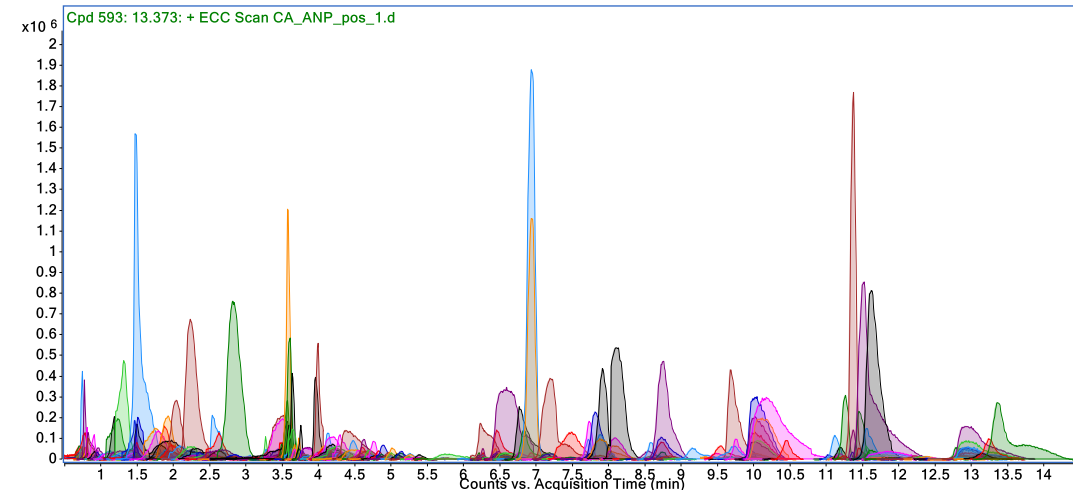
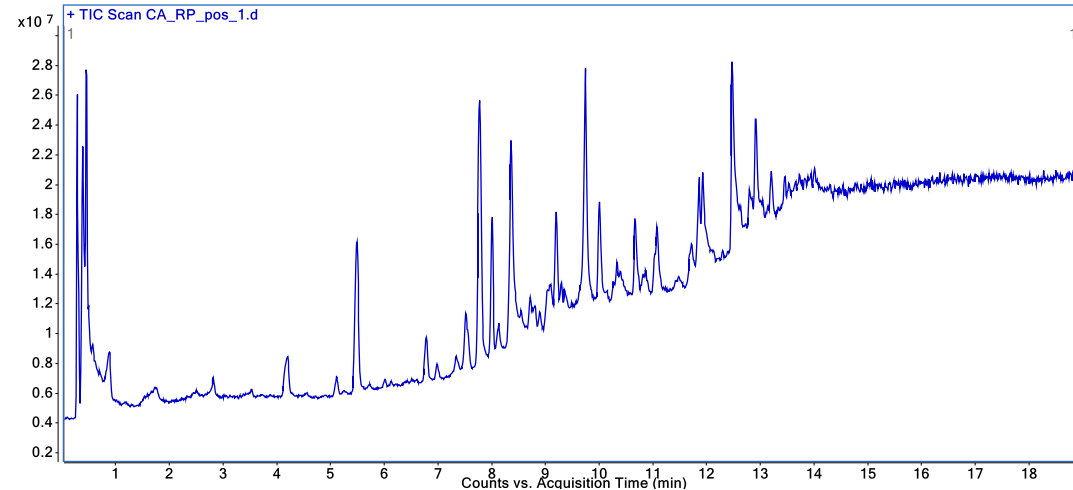
ESI (+) by RP

Polar compounds elute close to the void volume

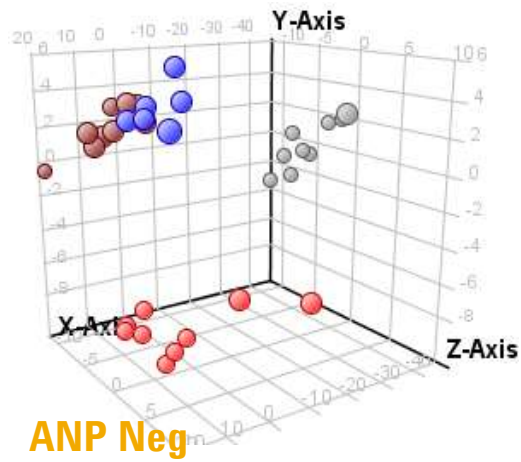
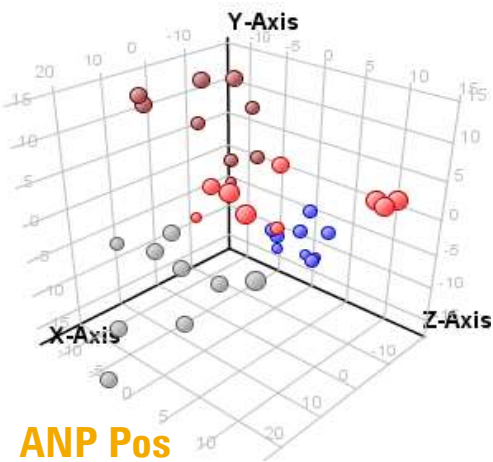
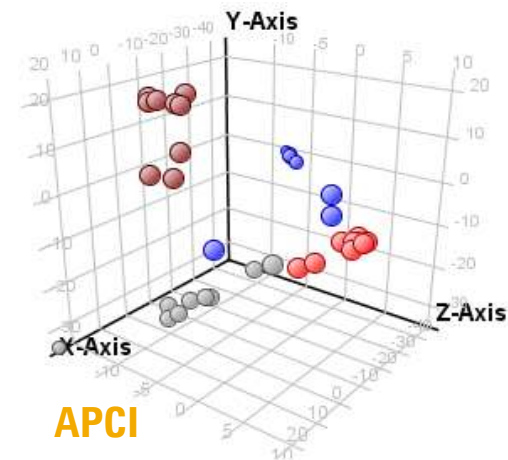
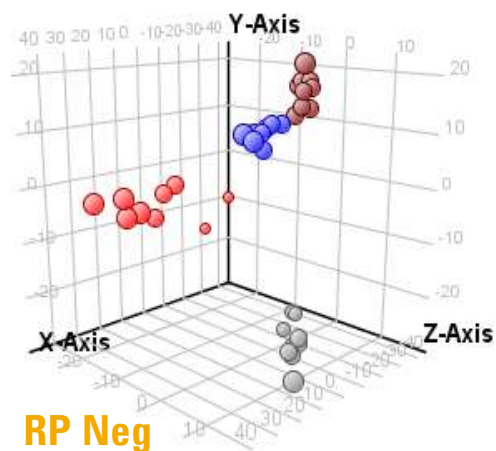
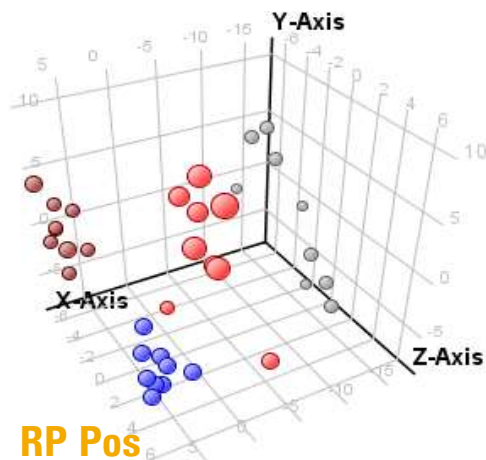
ESI (+) by ANP

ANP separates polar compounds that were in the void volume with RP

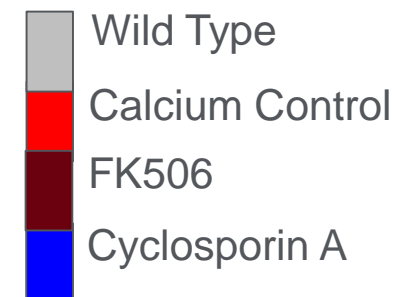
Using both ANP and RP yields more comprehensive coverage



PCA Plots for Yeast Metabolites Using Different Analytical Methodologies

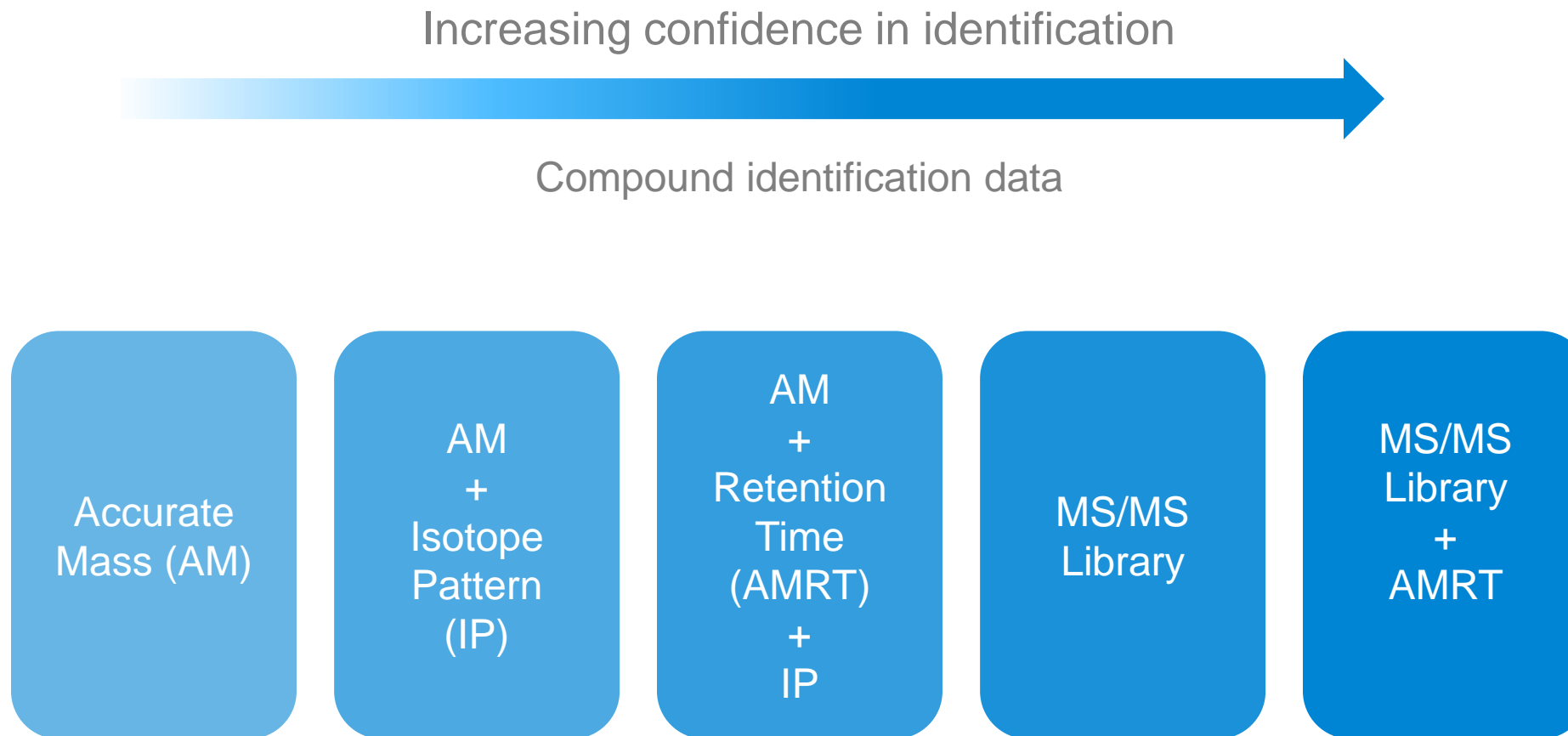


Culture Condition



Increasing Your Confidence in Compound Identification

Confident compound identification is crucial for pathway visualization!



Summary of Yeast Metabolomics Analyses

Differential Features with METLIN Database Annotation

Polar metabolites

- ANP chromatography
- ESI +/-

Non-polar metabolites

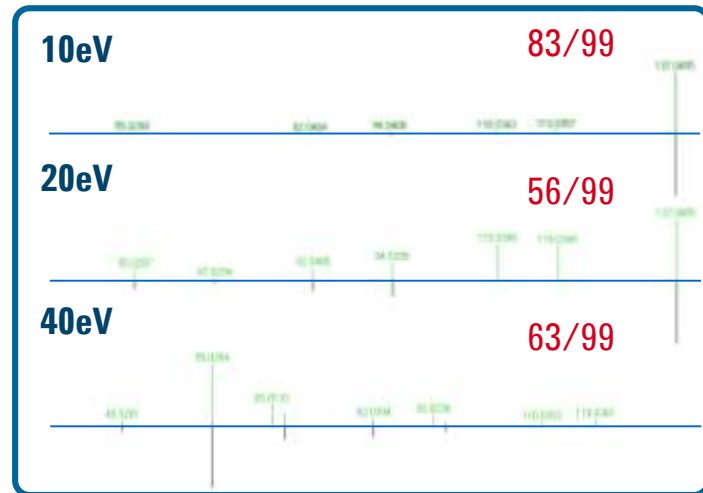
- RP chromatography
- ESI +/-
- APCI

Number of Features

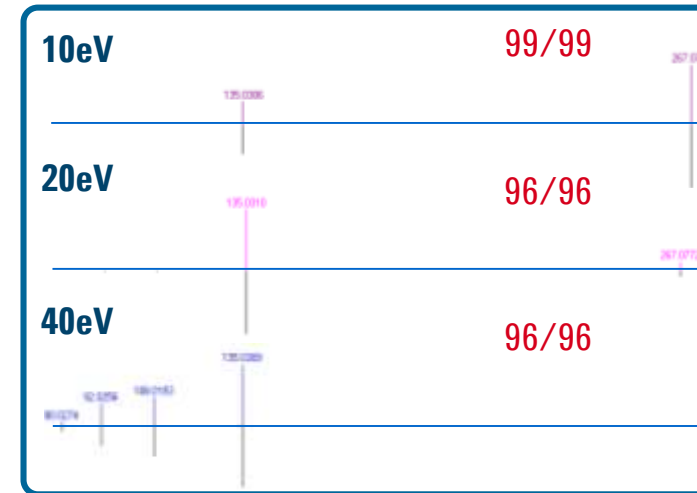
	After QC	METLIN Matches	p<0.05 Cutoff	METLIN Matches
RP-ESI pos	300	112	158	79
RP-ESI neg	523	141	418	115
RP-APCI pos	364	48	333	37
ANP-ESI pos	492	155	145	113
ANP-ESI neg	276	88	213	63

MS/MS Identification Using the Agilent METLIN PCDL Library

Hypoxanthine m/z 137.0458 (+)



Inosine m/z 267.0740 (-)

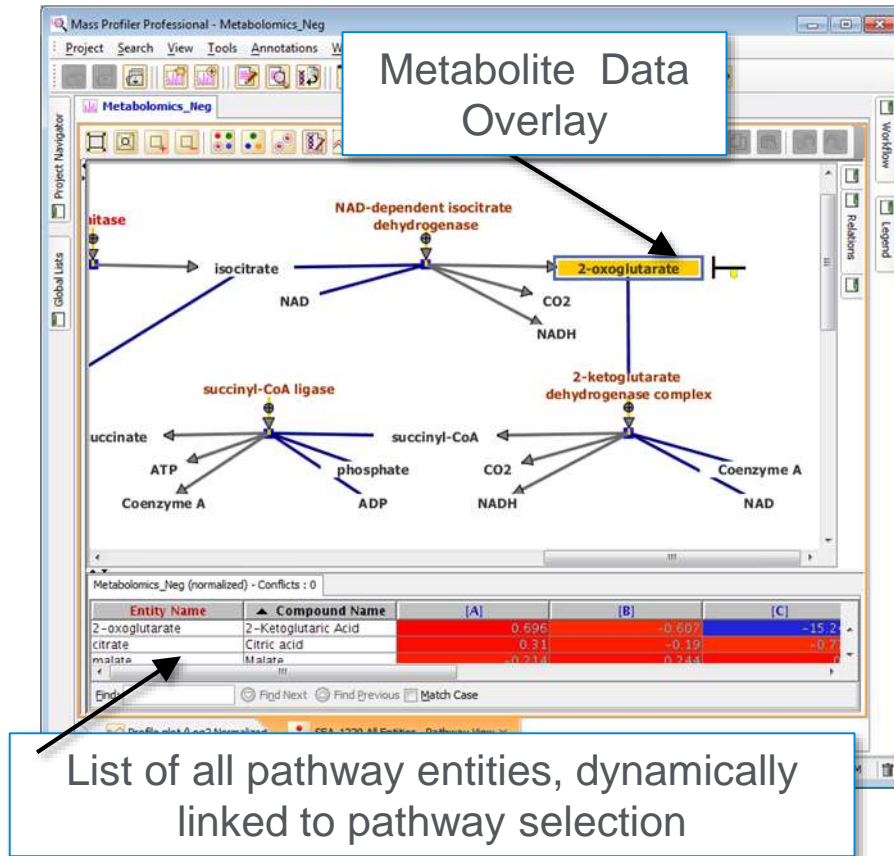


MS/MS spectra library matches:

- MS/MS spectra obtained at 10, 20 and 40eV collision energies
- Matched to METLIN PCDL library spectra
- Displayed as acquired spectra mirrored above library spectra

Pathway Architect

Pathway Architect is an optional module in MassProfiler Professional



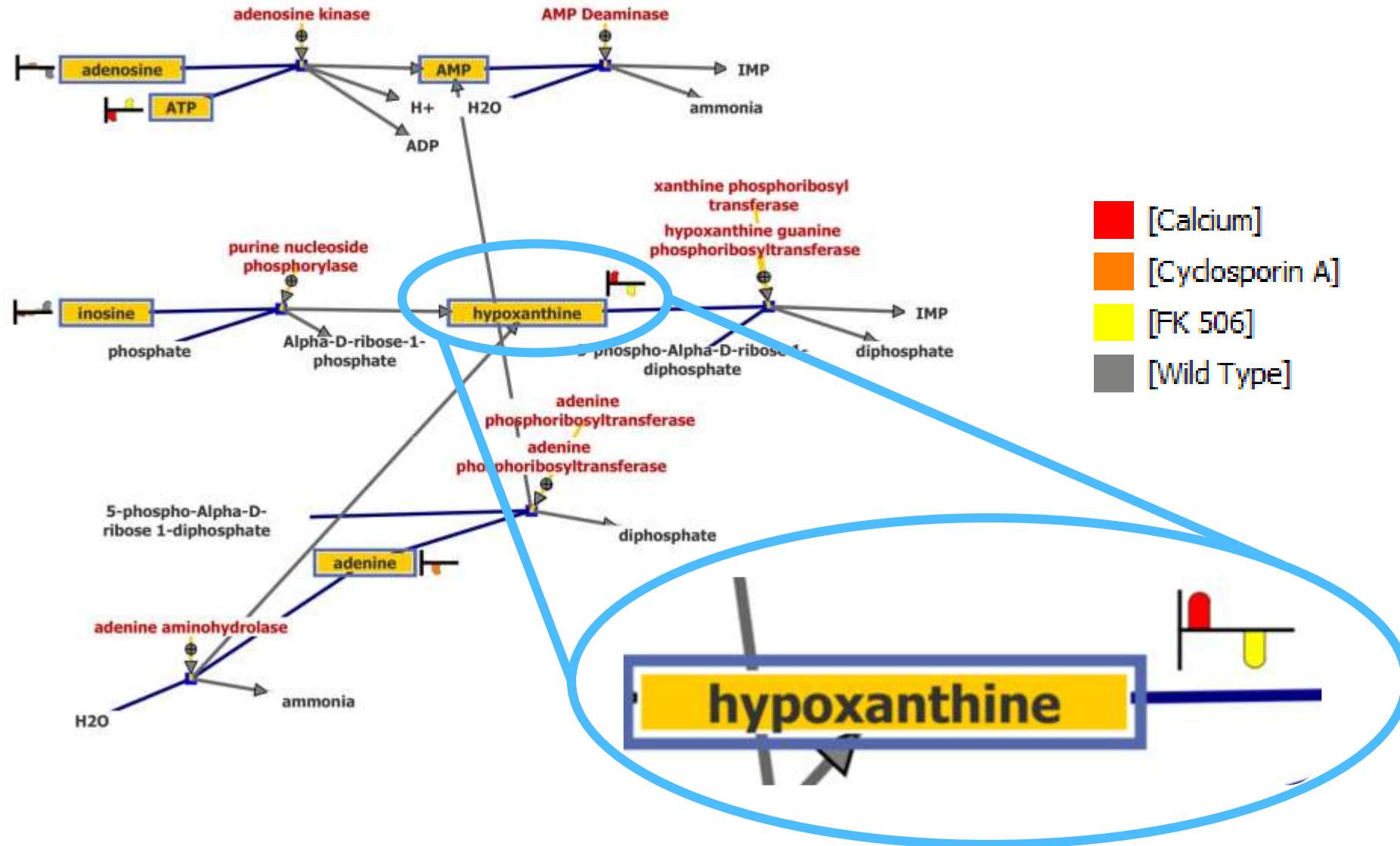
Map and visualize data from one or two types of -omic data on pathways

Search, browse and filter pathways

Supports biological pathways from publicly available databases

- WikiPathways
- BioCyc
- Supported pathway formats
 - BioPAX 3 – Pathway Commons, Reactome, NCI Nature Pathway
 - GPML – PathVisio –custom drawing
- Export compound list from pathways

One of the Stress Activated Pathways in Yeast



Introduction to Food Profiling



The questions contaminant testing won't answer:

- Is this wine cabernet or pinot noir?
- Is this Olive Oil really “Extra Virgin”?
- Is this rice from Japan or from somewhere else?
- How did changing my growing or fermentation process affect my food product?

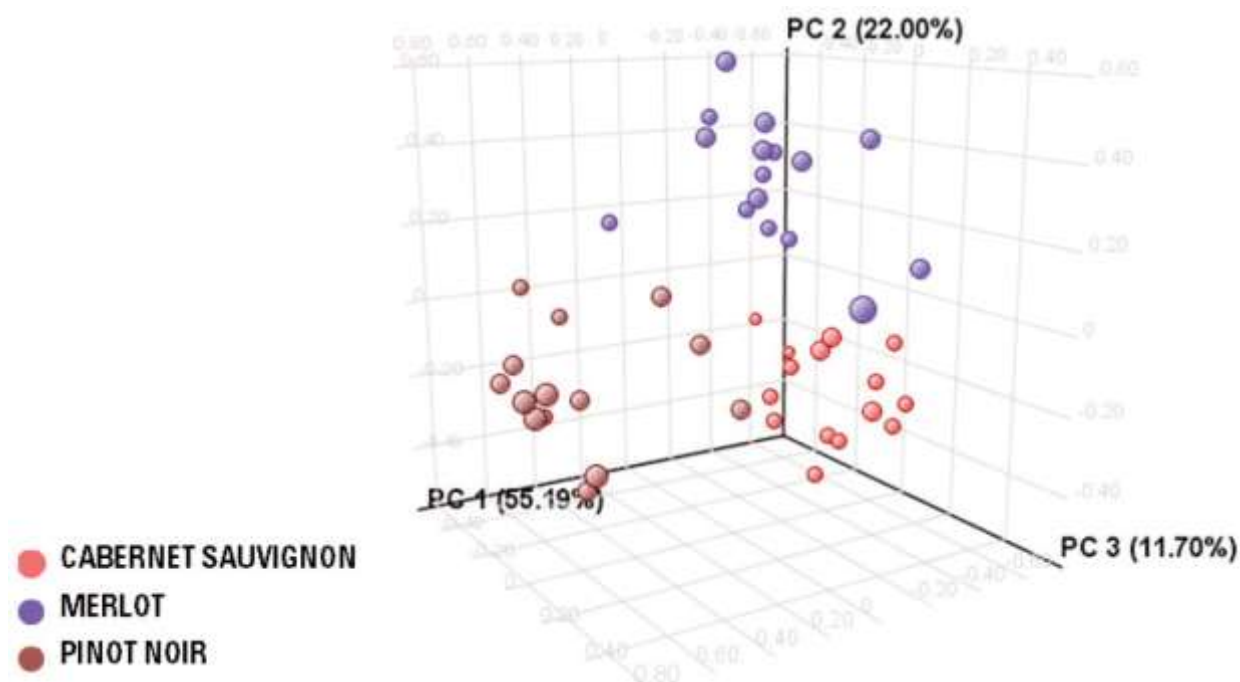
To answer these questions, we need
Food Profiling

Authenticity: Food Type

Determining Wine Varietal by LC/MS



- 45 red wine samples used to create a prediction model
 - 15 Cabernet
 - 16 Merlot
 - 14 Pinot Noir
- Wines sampled varied in geographic origin and vintage
- 5 additional wines which were not part of the original sample set were correctly classified using model



Application note [5990-8451](#)

Determining Wine Varietal by LC/MS

- 45 Red wines
- 3 Varieties: Cabernet Sauvignon (15), Merlot (16), Pinot Noir (14)
- 11 different countries: Czech Republic, Slovakia, France, Italy, Macedonia, Bulgaria, Hungary, Australia, Chile, Germany EE.UU.
- Harvests: 2004 – 2008



SET OF VERY VARIED SAMPLES



Instruments used



Agilent Technologies
1200 RRLC system



Eclipse Plus C18 (2.1×100, 1.8µm)
HILIC Plus C18 (2.1×100, 3.5µm)

Jet Stream ESI source
Multimode ion source



Agilent Technologies
6530 Accurate-Mass Q-TOF LC/MS

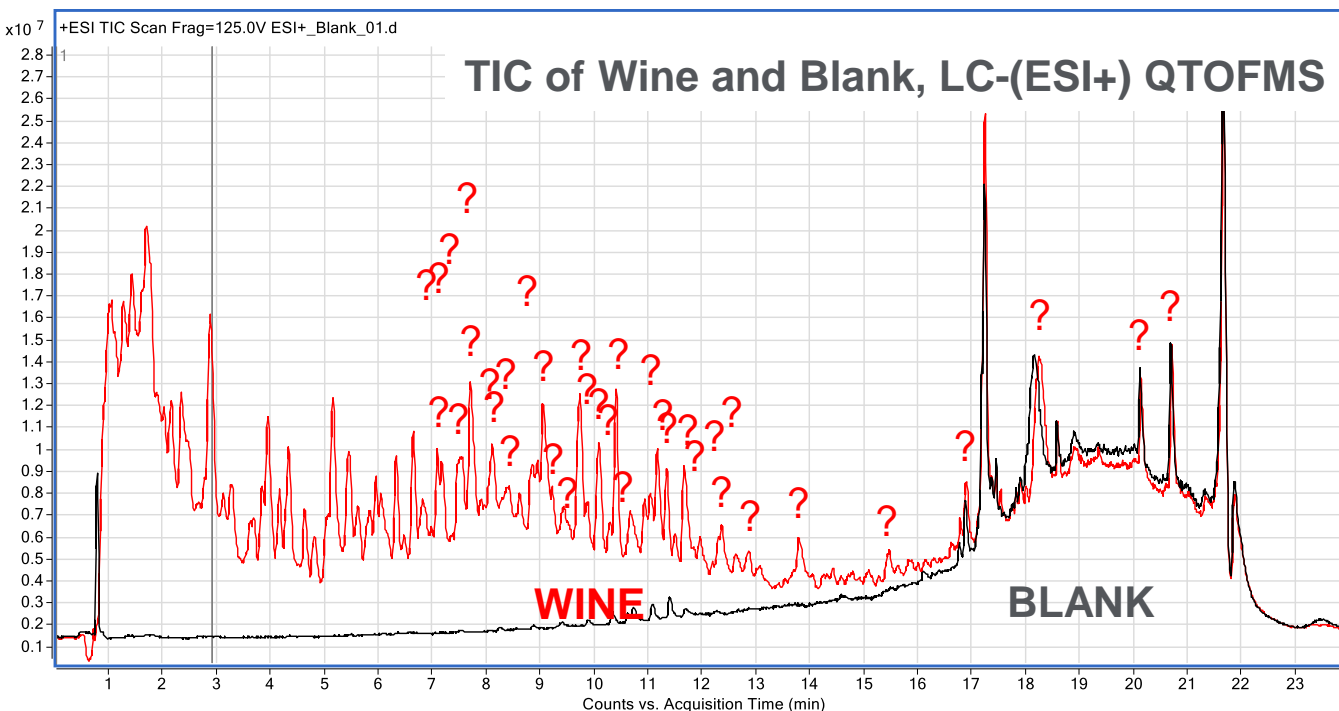


Micro-filtration is the only Sample Prep used

Ondrej Lacina^a, Lukas Vaclavik^a, Jana Hajslova^a, Jerry Zweigenbaum^b

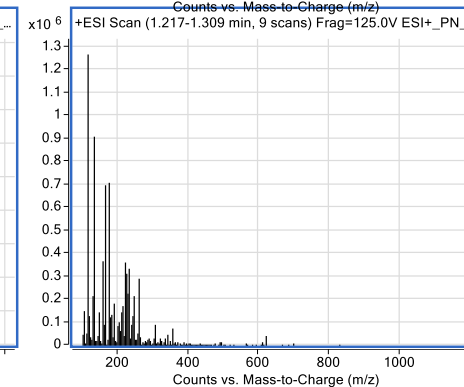
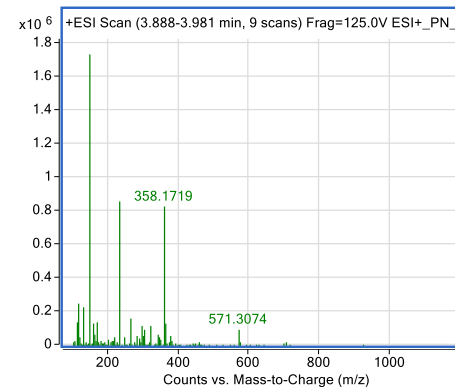
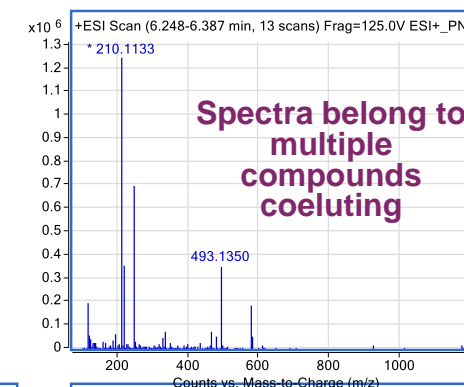
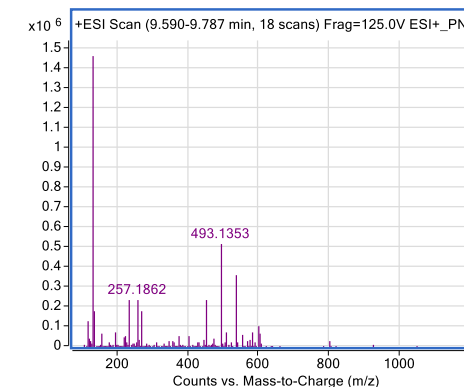
^a Institute of Chemical Technology Prague, Czech Republic ^b Agilent Technologies, Wilmington, DE, USA

Data Processing:

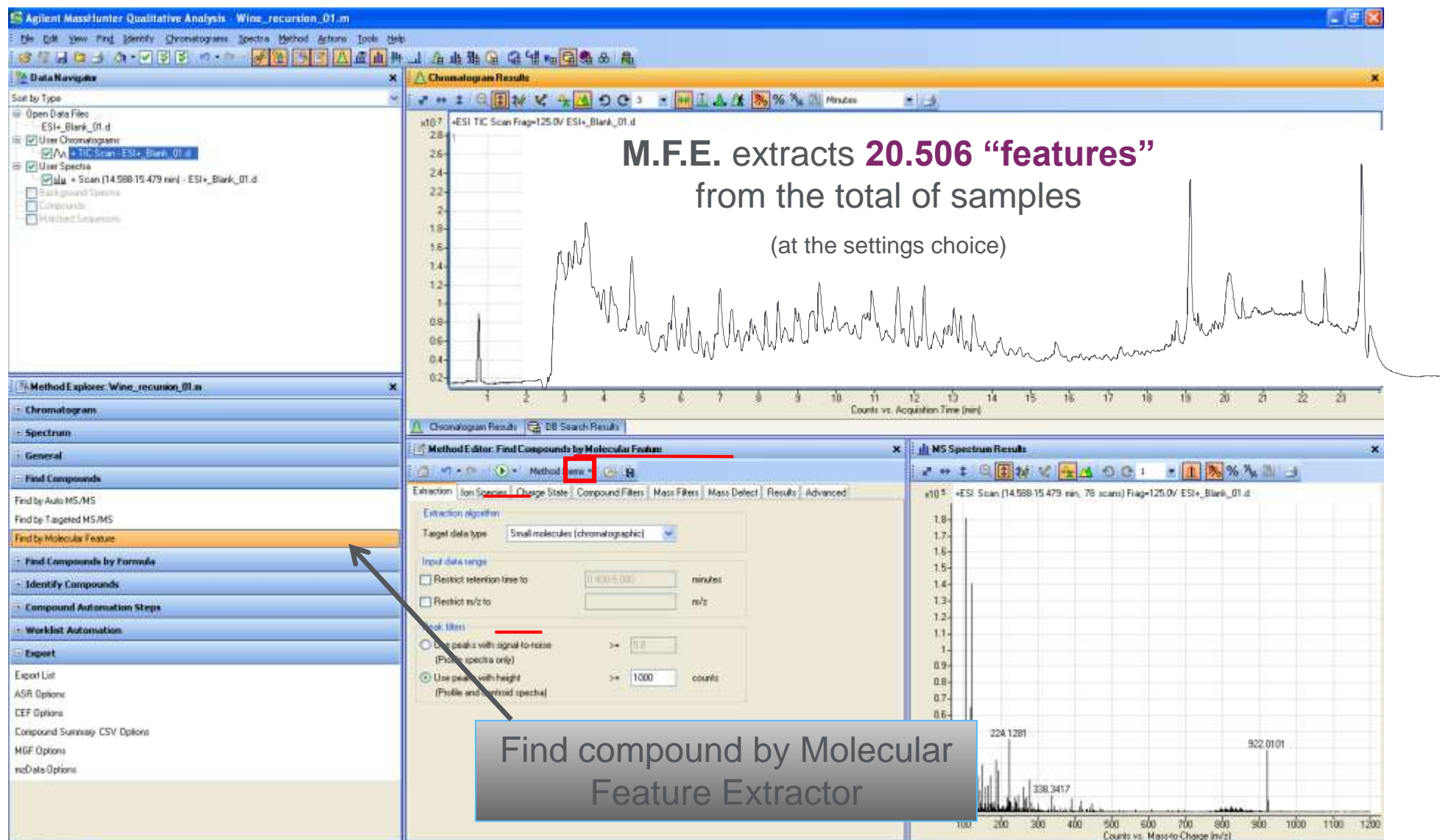


- Very complex data sets.
- Masked minority compounds.

A deconvolution software is needed to characterize all the ionized compounds.

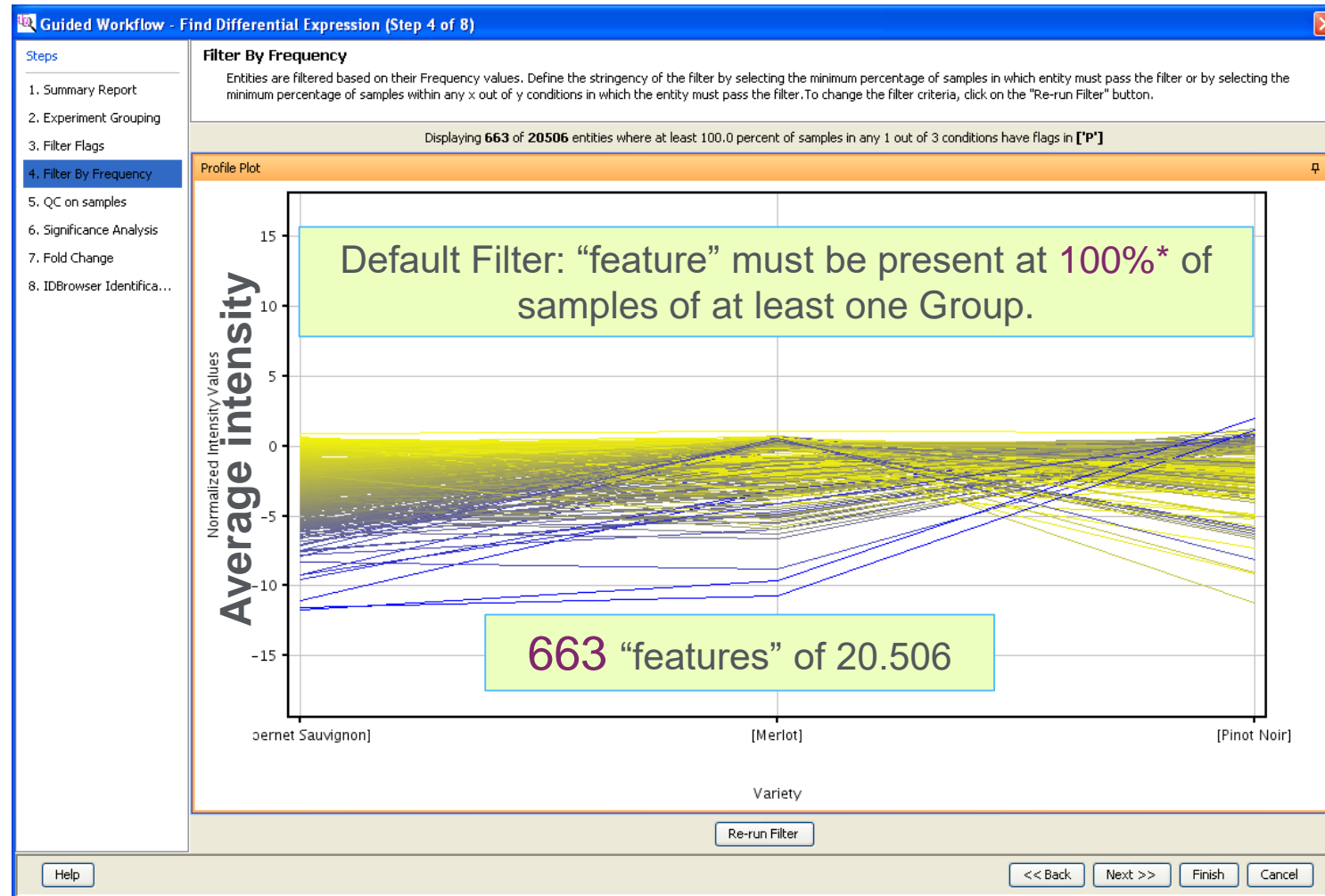


Data Extraction: “Find By Molecular Feature”



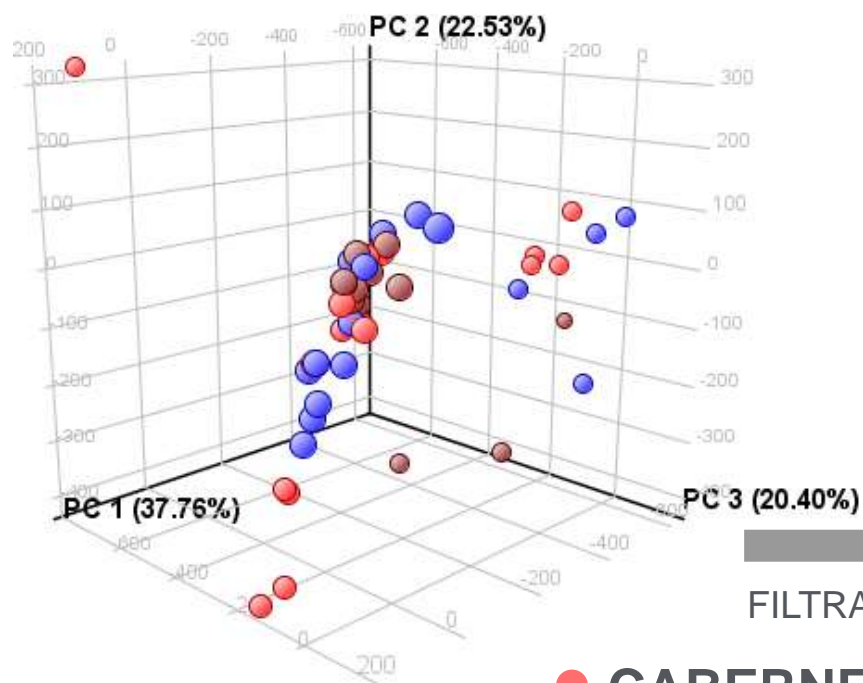
Data Processing with Agilent Mass Profiler Professional: Filtering By Frequencies

* Filtering by 50% “features” would increase from **663** to **3600**

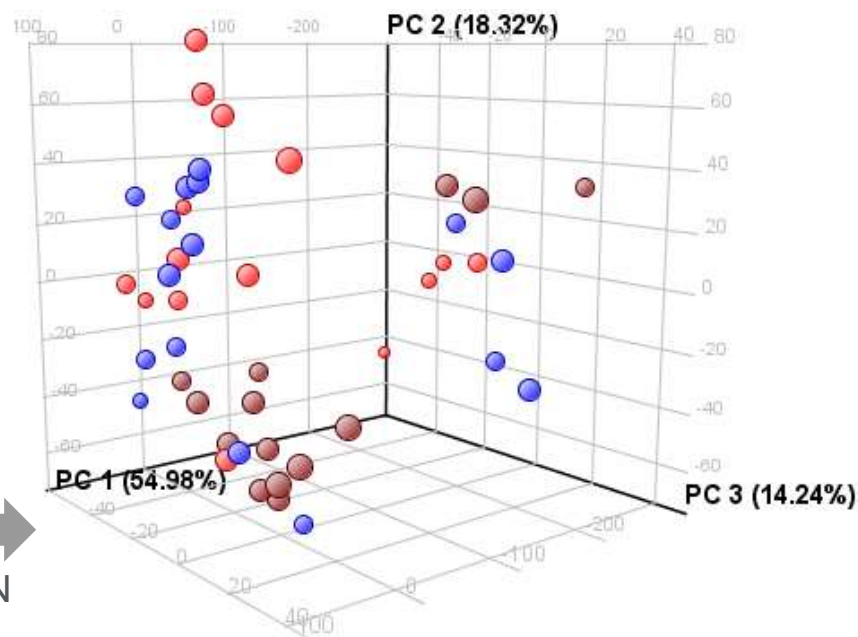


Data Processing with Agilent Mass Profiler Professional: Filtering By Frequencies + PCA

PCA of the data
Initial features (20506)



PCA of the data
Features filtered by Frequencies (3600)



- CABERNET SAUVIGNON
● MERLOT
● PINOT NOIR



Data Processing with Agilent Mass Profiler Professional: Filtering By Frequencies + PCA + ANOVA



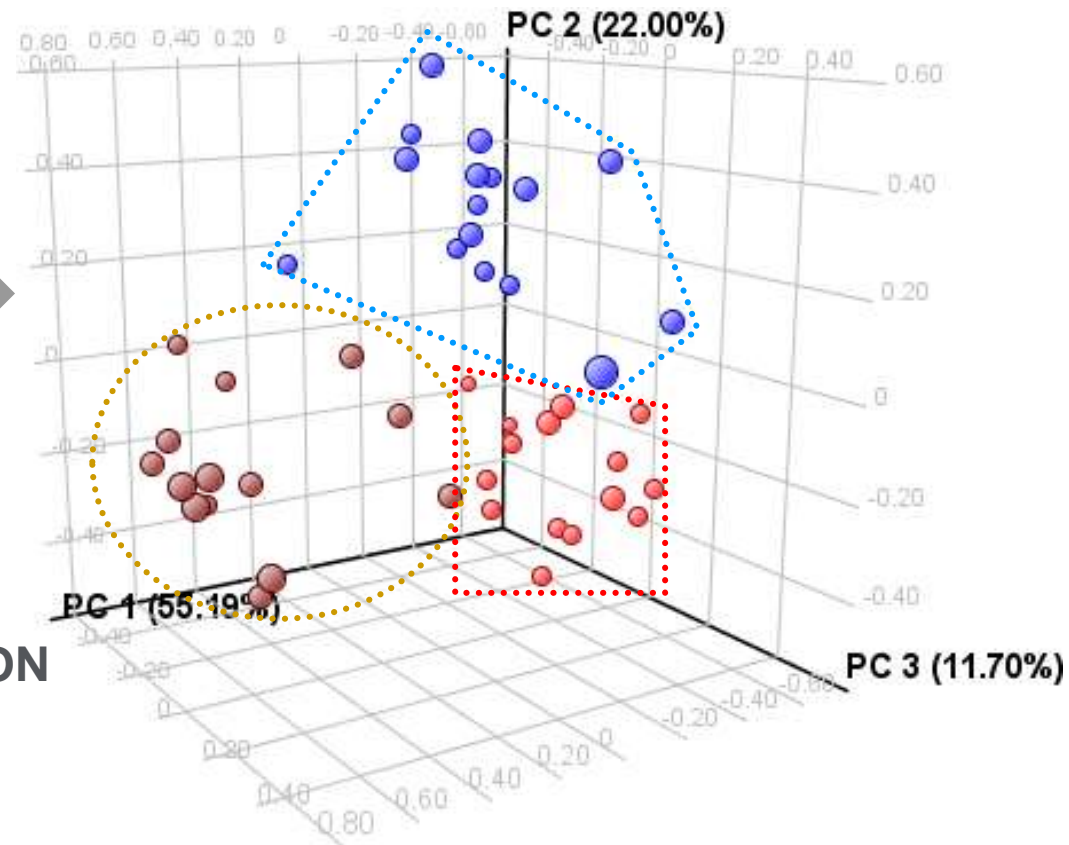
PCA of Data:
Features after ANOVA ($p \leq 0.05$) & Fold Change (≥ 2.0): 26

A good filter of
data is crucial for
a good
Classification fit



FILTRATION

- CABERNET SAUVIGNON
- MERLOT
- PINOT NOIR



Class Prediction Model Validation



Number of samples used for model validation: 45

Class Prediction (Step 3 of 5)

Validation Algorithm Outputs

The validation tables provide the result of the model validation step. The prediction is compared with the true values of the samples. If many mistakes are made in the prediction, press the "Back" button to make changes to the model.

Confusion Matrix

	[Cabernet Sau...	[Merlot] (Predi...	[Pinot Noir] (Pr...	Accuracy
(True) [Cabernet Sauvignon]	15	0	0	100.000
(True) [Merlot]	1	14	1	87.500
(True) [Pinot Noir]	0	0	14	100.000
Overall Accuracy				95.556

- During model validation, 2 MERLOT samples were incorrectly classified.
- All the Cabernet Sauvignon & Pinot Noir were correctly classified.
- **The prediction reliability of the model determined to be of 95.6%***

The model classified correctly 5 of the blind samples (2 CS, 1M, 2 PN).

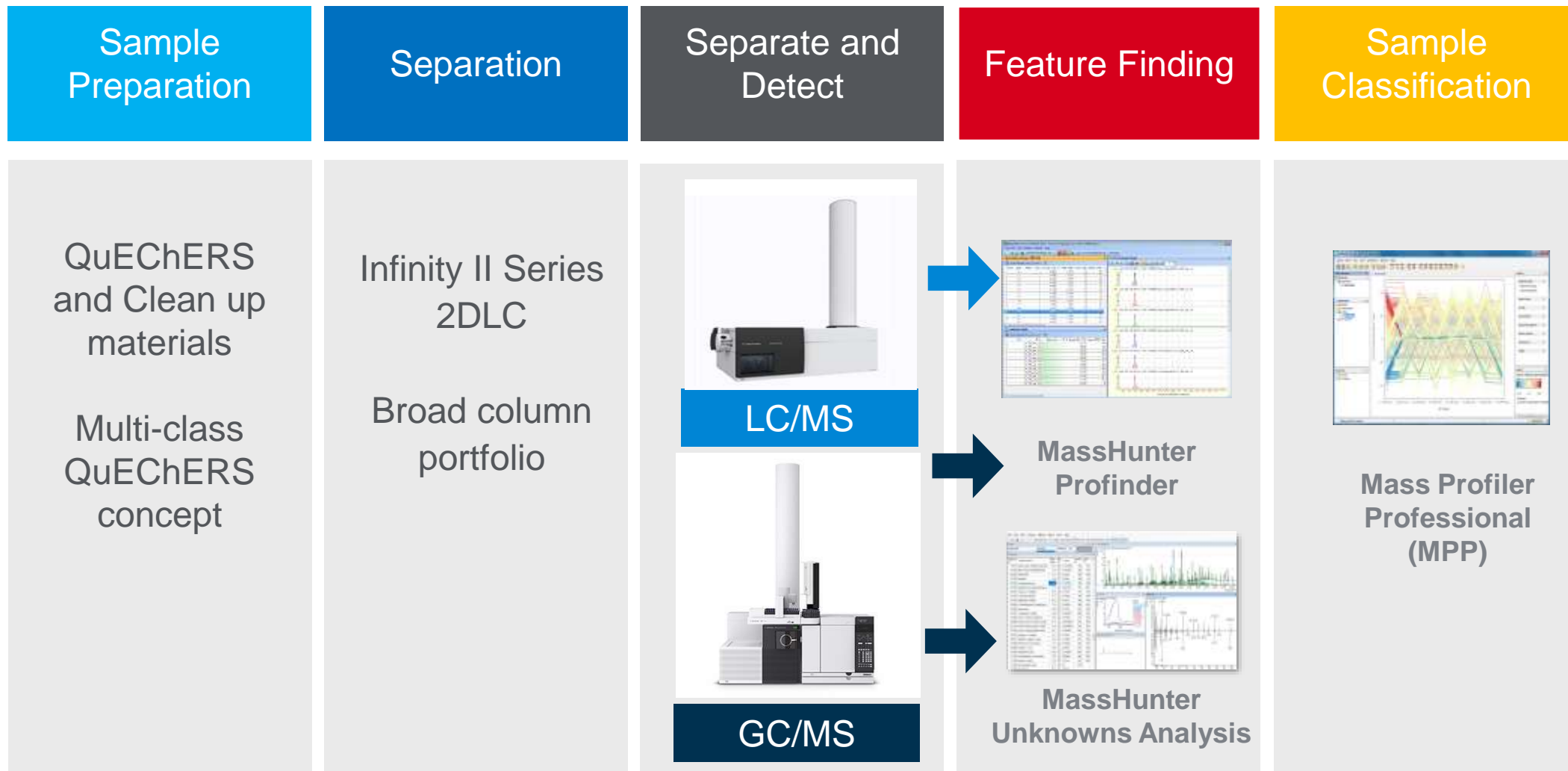
* ANOVA used $p \leq 0.05$.

Food Authenticity Analysis with MPP and MassHunter Classifier

Challenges in Food Authenticity Testing

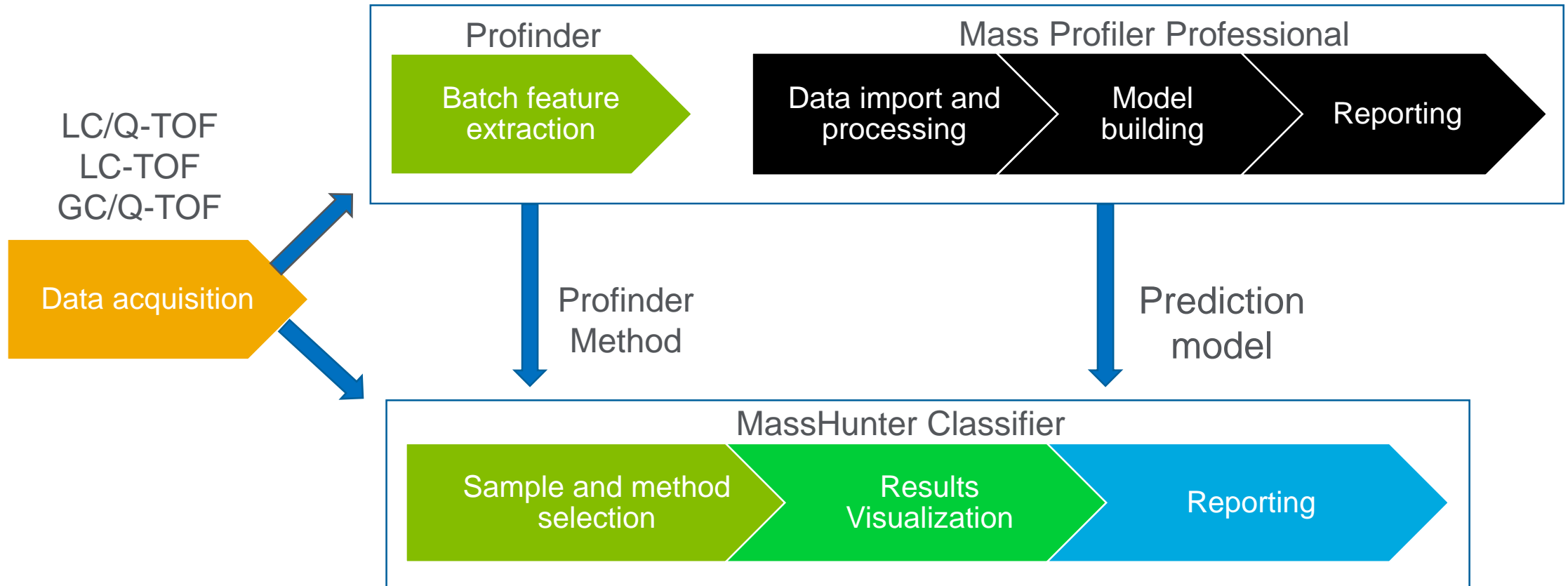
- Meeting regulatory requirements
- Availability of authentic samples
- Speed of analysis
- Extensive method development required
- Extensive validation required

Agilent's Food Authenticity Workflow



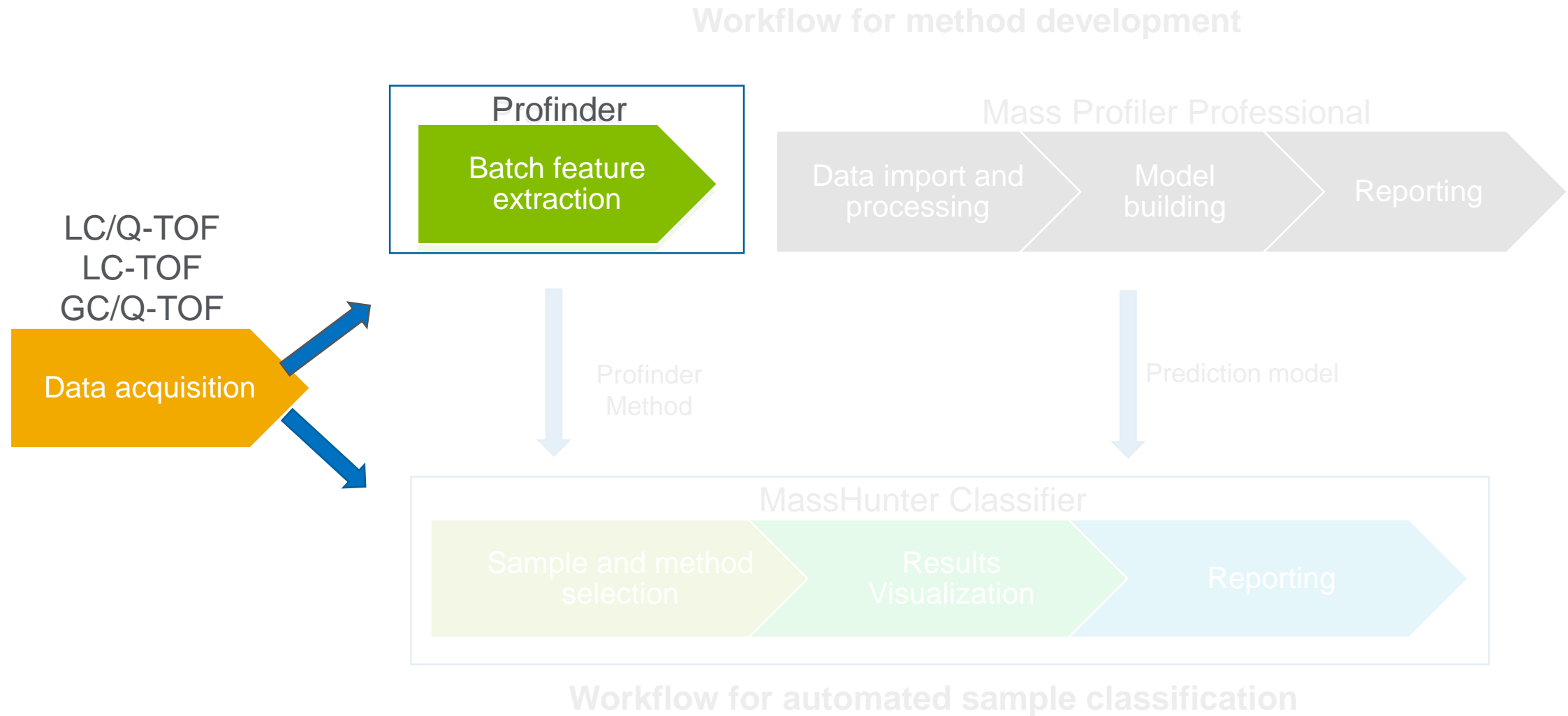
Food Authenticity Workflow

Workflow for Method Development



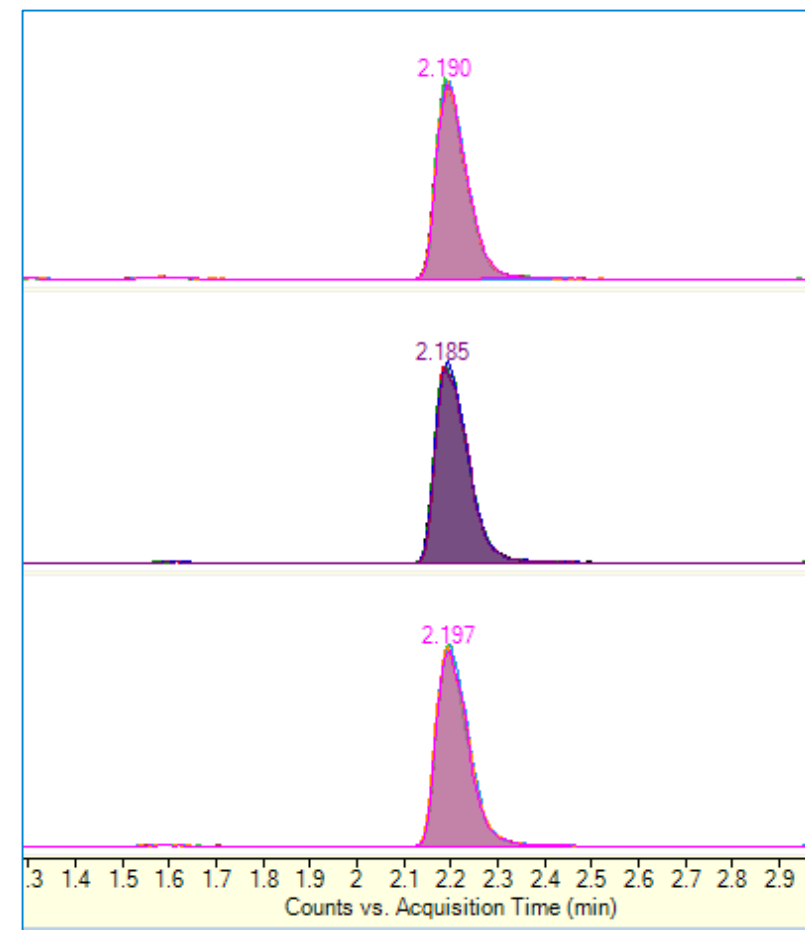
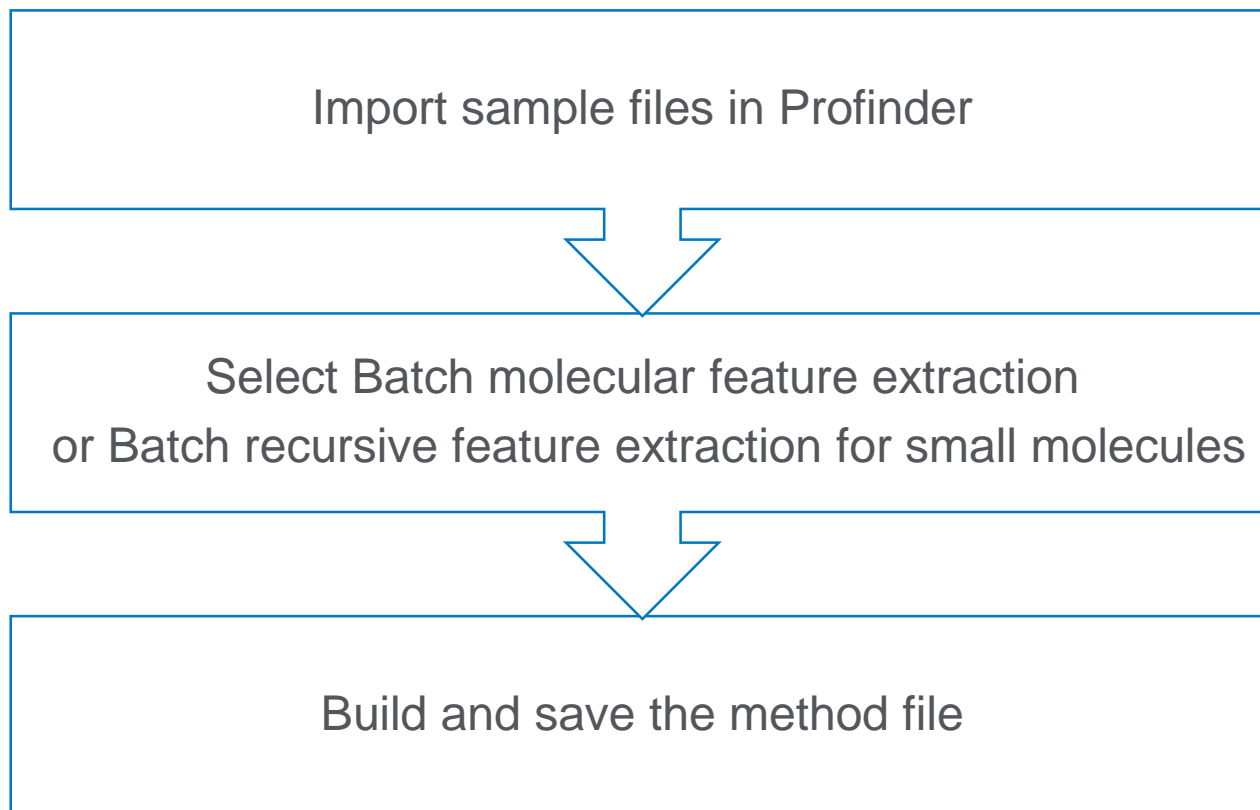
Workflow for Automated Sample Classification

Food Authenticity Workflow

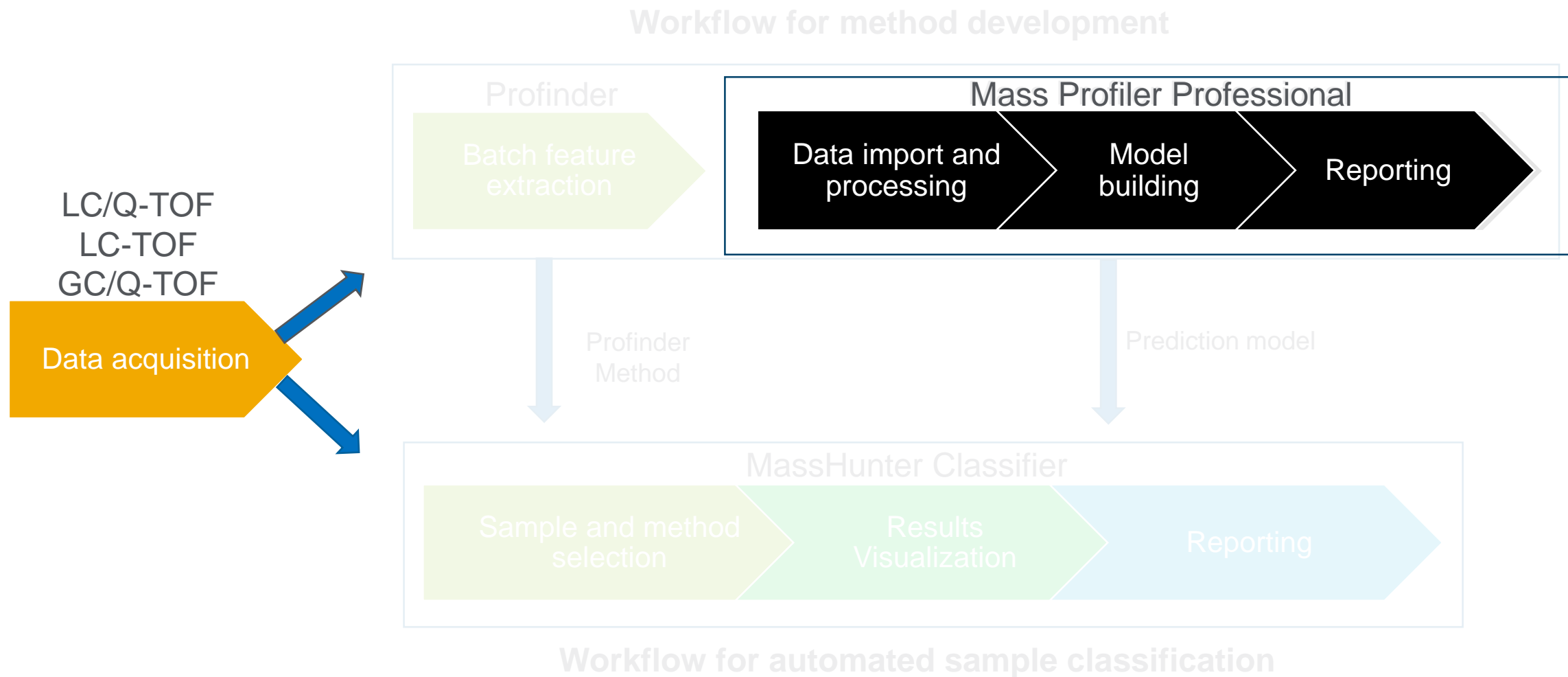


Method Development in MassHunter Profinder

Batch Feature Extraction



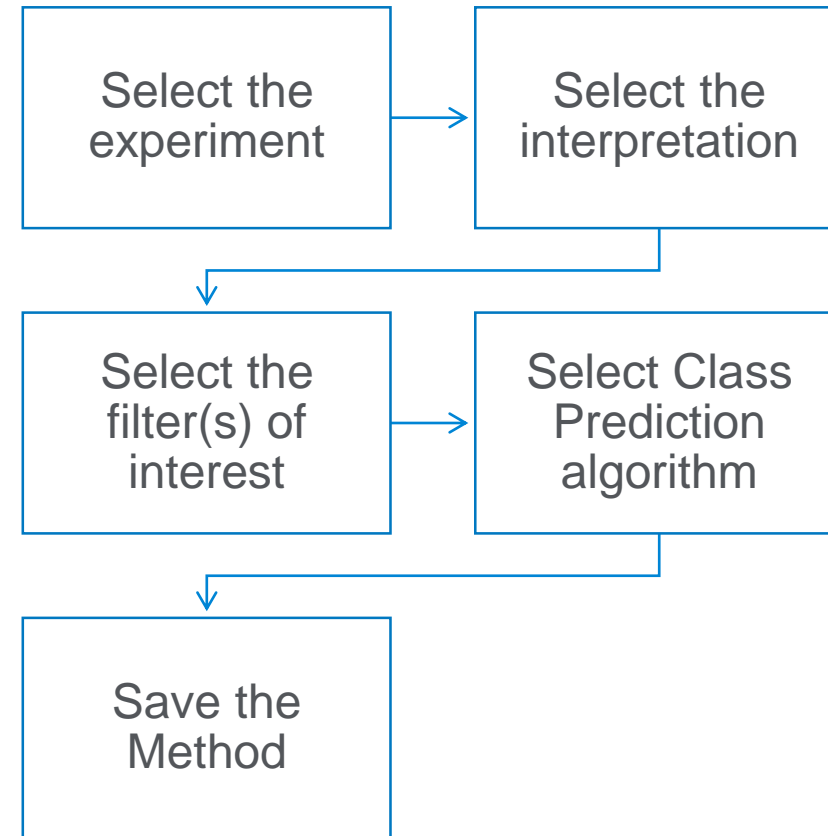
Food Authenticity Workflow



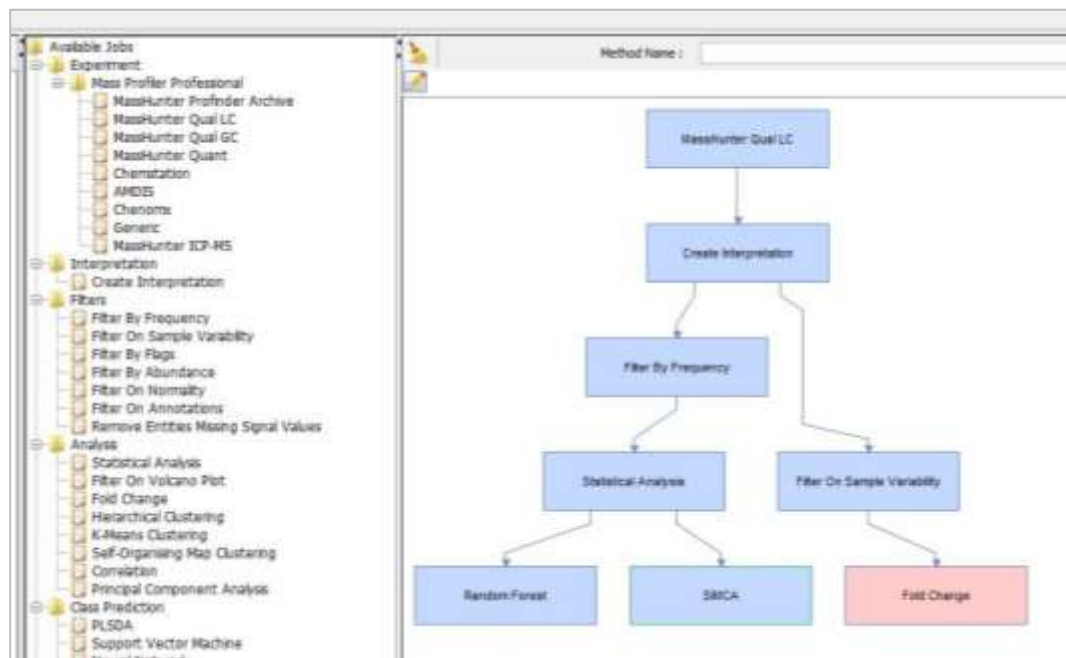
Method Development in MPP

Method manager

- Allows creation, running and monitoring of a method
- Drag and drop the tasks of choice in the drawing area to create a method
- Advanced features like normalization, missing value imputation, etc. can also be configured from the same user interface
- Method can be started as well as exported from the tool
- Exported method gets saved as .m file and can be shared



Creating a Method



Select the tasks of choice and connect them to create a method

The screenshot shows the 'Pipeline -MassHunterQualLCMS.6 (Step 1 of 6)' dialog box. The 'Experiment Type' section has three radio buttons: 'Identified' (selected), 'UnIdentified', and 'Combined (Identified + UnIdentified)'. The 'Organism' dropdown menu is set to 'None'.

Select advanced parameters

Class Prediction Algorithms

Random Forest

New

Linear Discriminant Analysis

SIMCA

Partial least Squares Discrimination

Support Vector Machine

Naive Bayes

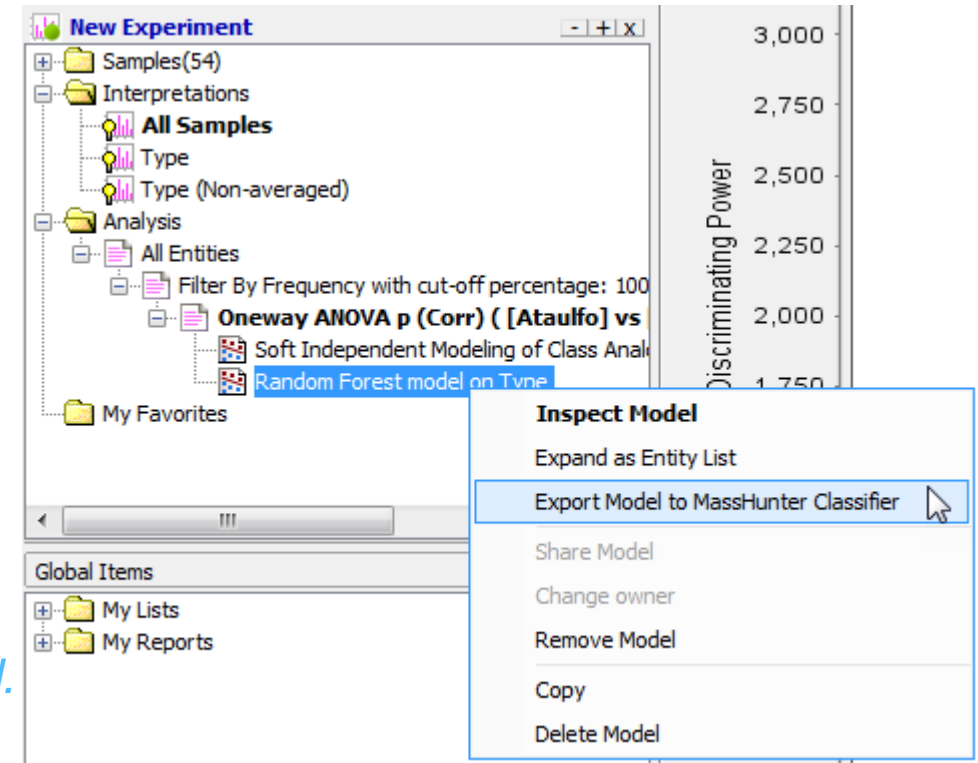
Decision Tree

Neural Network

Export Model to MassHunter Classifier

Exported model is saved in method folder for further use in MassHunter Classifier

For mango data, a prediction model using Random forest was created.



Automated Sample Classification

MassHunter Classifier

Automated Sample Classification Workflow



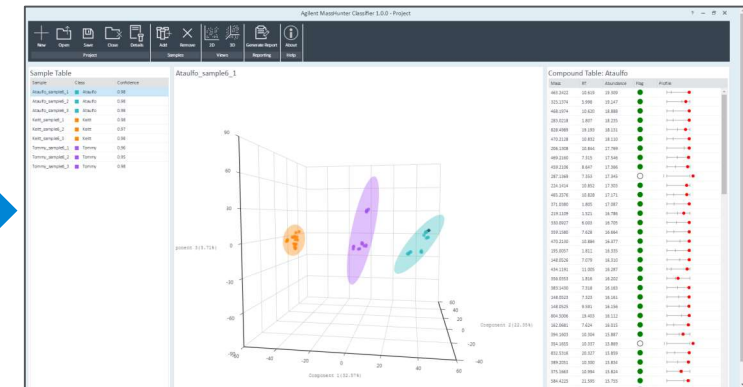
Sample collection



Sample preparation

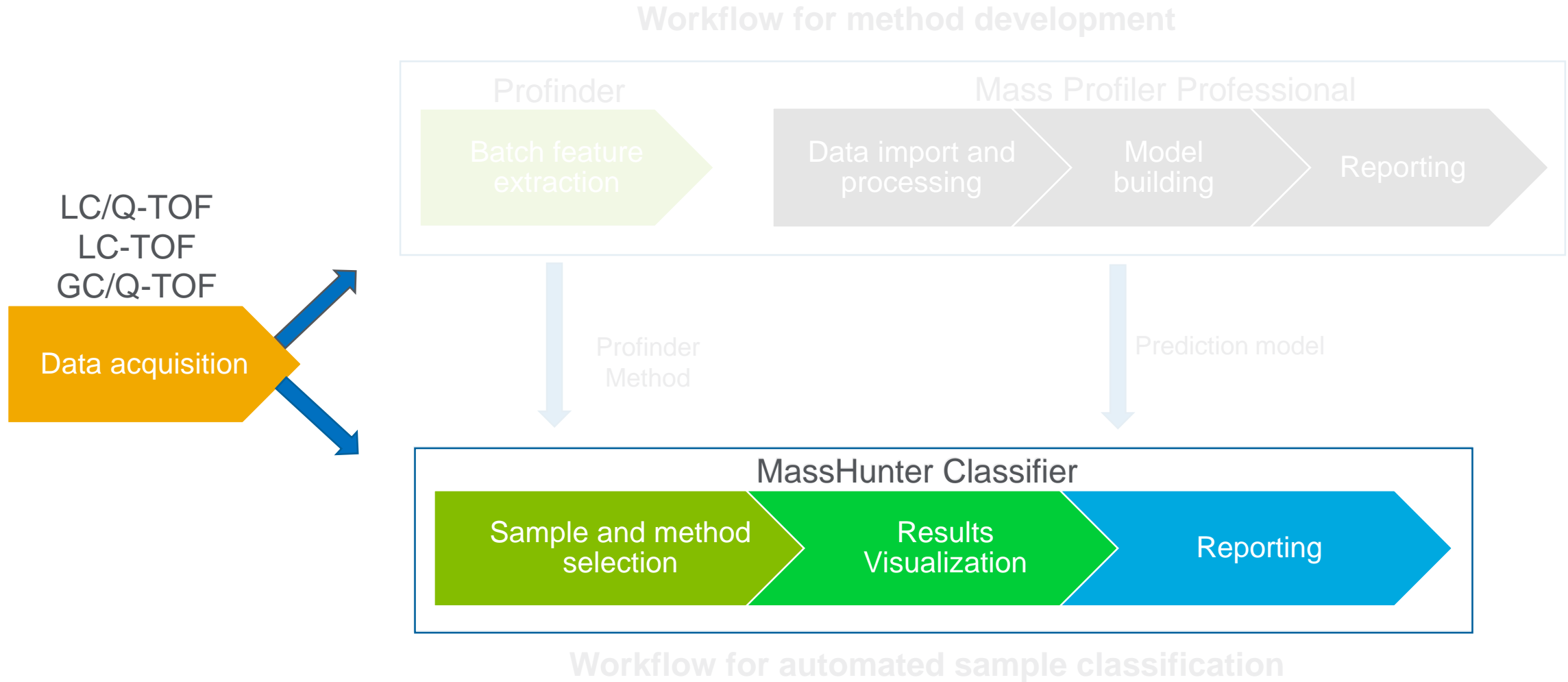


Detection



MassHunter Classifier

Food Authenticity Workflow



Project Creation in MassHunter Classifier

New Project

Project Name: Project

Samples Type: ☒ (.d) ☐ (.cef)

Profinder Method: [Select]

MPP Model: [Select]

Samples: [Select]

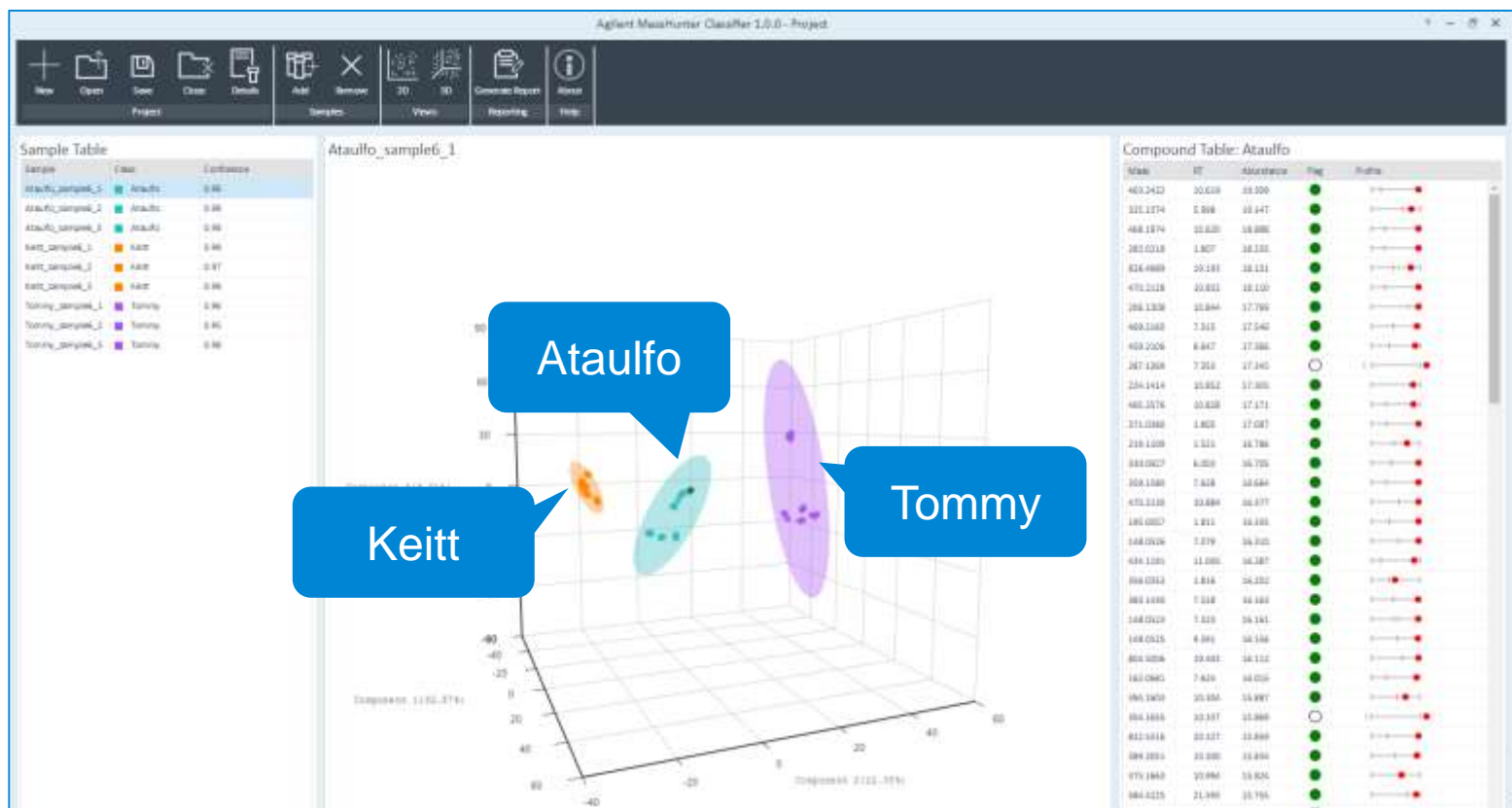
[Submit] [Cancel]

Import Method, Model and Samples

- Profinder method is required when user is working with .d files
- At a time only one MPP model can be imported. To run prediction with another model, a separate project can be created.
- One or more unknown samples can be imported for prediction

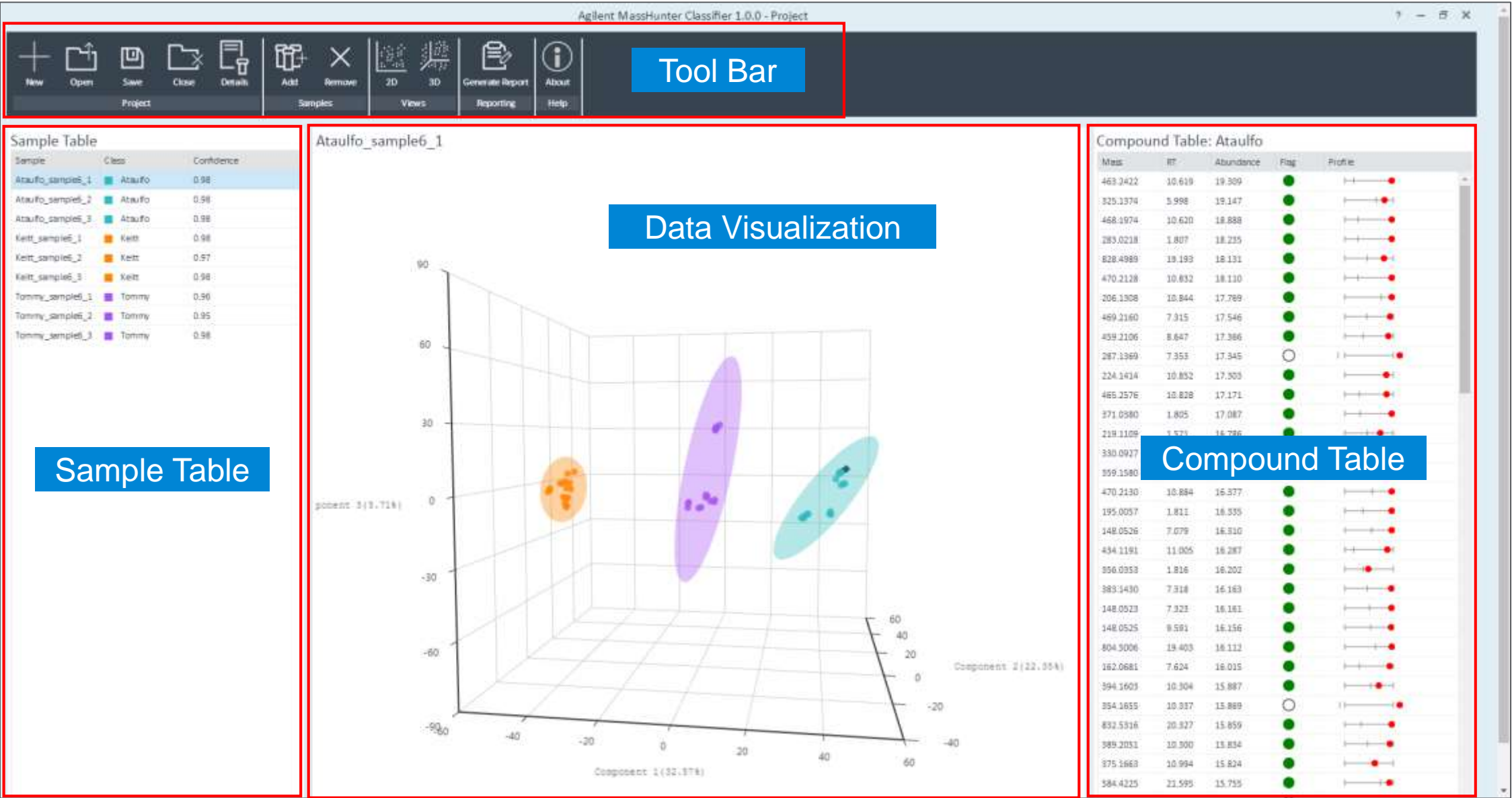
MassHunter Classifier

A simple tool for automated sample classification












- Simple, uncluttered user interface for routine analysis
- Run batch feature extraction and class prediction on previously created classification models
- The tool quickly processes the samples to be classified and user is presented with visualization options such as Principal component Analysis (PCA) to review the results
- User can interactively review the sample classification results and associated compound data before choosing to save a PDF report

MassHunter Classifier User interface



Sample Table

Sample Table

Sample	Class	Confidence
Ataulfo_sample6_1	 Ataulfo	0.98
Ataulfo_sample6_2	 Ataulfo	0.98
Ataulfo_sample6_3	 Ataulfo	0.98
Keitt_sample6_1	 Keitt	0.98
Keitt_sample6_2	 Keitt	0.97
Keitt_sample6_3	 Keitt	0.98
Tommy_sample6_1	 Tommy	0.96
Tommy_sample6_2	 Tommy	0.95
Tommy_sample6_3	 Tommy	0.98

- Sample table shows the predicted class to which unknown sample belongs and the confidence score
- All the samples here are classified with higher than 95% confidence

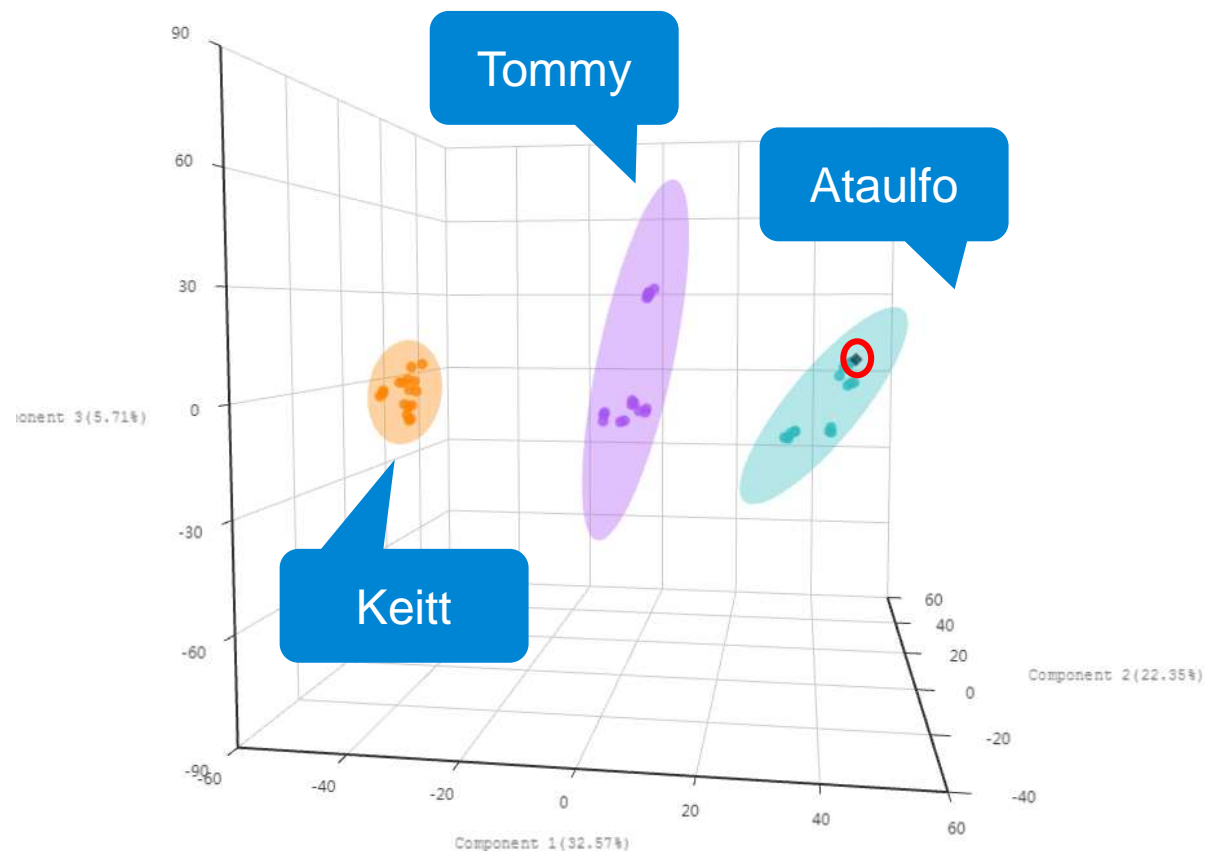
Compound Table

- Compound table shows the list of metabolites used in classification
- Flag column provides information on if a compound contributed to sample classification
- Additional information on compound is seen in profile plot
- Red dot indicates the abundance of the compound in the unknown sample with respect to the abundance distribution of the compound in the training samples for the predicted class

Compound Table: Ataulfo

Mass	RT	Abundance	Flag	Profile
463.2422	10.619	19.309	●	
325.1374	5.998	19.147	●	
468.1974	10.620	18.888	●	
283.0218	1.807	18.235	●	
828.4989	19.193	18.131	●	
470.2128	10.832	18.110	●	
206.1308	10.844	17.769	●	
469.2160	7.315	17.546	●	
459.2106	8.647	17.366	●	
287.1369	7.353	17.345	○	
224.1414	10.852	17.303	●	
465.2576	10.828	17.171	●	
371.0380	1.805	17.087	●	

3D-PCA



- Confidence ellipses in the PCA are drawn for model training data
- Dots on the PCA plot are training samples used for model building
- Diamond structured sample is the unknown Ataulfo sample in 3D PCA plot

Report Generation

Generate Report

Report

Title:

Logo:

Select Samples

<input checked="" type="checkbox"/>	Ataulfo_sample6_1
<input checked="" type="checkbox"/>	Ataulfo_sample6_2
<input checked="" type="checkbox"/>	Ataulfo_sample6_3
<input checked="" type="checkbox"/>	Keitt_sample6_1
<input checked="" type="checkbox"/>	Keitt_sample6_2
<input checked="" type="checkbox"/>	Keitt_sample6_3
<input checked="" type="checkbox"/>	Tommy_sample6_1
<input type="checkbox"/>	Tommy_sample6_2
<input type="checkbox"/>	Tommy_sample6_3

Save:

Agilent | Trusted Answers

MassHunter Classifier Report

Prediction model


Property	Value
Project	MassHunter
Supervised Model	Ada Boost
Model Name	Model Name
Model Type	Model Type
Model File	Model File
Model Path	Model Path
Model Date	Model Date
Model Version	Model Version
Model Author	Model Author

Samples

Sample	Class	Confidence
Ataulfo_sample6_1	Class	Confidence

PCA Plots

Ataulfo_sample6_1



- Information on classified samples can be recorded in a report
- Captured information includes name of model used, samples tested as well as per sample data in a PDF file

Summary

- One stop solution for food authenticity analysis
- Streamlined workflow for batch processing and model building
- Class prediction algorithms to support various applications
- Brand new simplified application for automated sample classification
- Separation in “Scientist” part and “Technician” part
- Standalone software which utilizes our already established platform of Profinder and MPP

- *Enfoques y estrategias analíticas que nos permiten las ultimas tecnologías de LC Mass Spectrometry de alta resolución. **UHPLC QTOF technical details***
- **Agilent proposal Workflows in different scenarios.** *Diferentes estrategias inherentes a investigación en el ámbito de Ciencias de la Vida, así como para el perfilado de Alimentos, Materiales, Procesos...*
- **Herramientas de Agilent y flujos de Trabajo para tomar mejores decisiones en un entorno de Biología integrada.** *Del diseño experimental a las conclusiones, un largo camino para ayudar al investigador.. :*
 - **Datos según modos de Adquisición.** *Complementariedad de las diferentes tecnologías LCMS, GCMS, CEMS, ICPMS*
 - *Deconvolución de datos y herramientas de visualización.* *Como funcionan los algoritmos de Agilent para extraer información de compuestos de un Full Scan.*
 - *Preparación de datos previa al Análisis Estadístico diferencial. Alineamiento, Normalización, “Baselining” con “Mass Hunter ProFinder”.*
 - *¿Necesito análisis recursivo a través de iteración? Por favor hágamelo fácil.... Exhaustivo tratamiento de datos para evitar la Perdida de compuestos.*
 - *Mass Profiler professional. Análisis Diferencial a través de Interpretaciones, Clustering, PCA, PLRS, modelos de predicción*
 - *Así, ¿Cuales son mis compuestos diferenciales de interés? ¿Como puedo identificarlos? Librerías empíricas de espectros MS/MS. Agilent METLIN PCDL.*
 - *Análisis de rutas Metabólicas a través de “Pathways Analysis”. Biología integrada e interpretación biológica de mis datos. Pathways Analysis.*
 - *¿Cuál es mi próximo experimento? La potencia del enfoque de la Biología integrada.*
- **Movilidad Iónica.** *Una nueva dimensión para extracción de datos más selectiva en muestras complejas. Una nueva herramienta de identificación*
- **Fluxómica. Fácil y rápida** *visualización de la incorporación de sustratos marcados isotópicamente en una ruta metabólica a través de “VistaFlux”.*
- *Método llave en mano para el análisis Metabolómico dirigido en rutina de los **metabolitos del Ciclo Central de Carbono***
- *Sinergias con la medida In-vivo del Metabolismo celular con “Seahorse”.*

From experiment design to conclusions, a long way to help scientists. Agilent Tools and workflows to better decisions making at Integrated Biology :

Complementing different analytical technologies LCMS, GCMS, CEMS, ICPMS. Data Acquisition modes.

- For LC HRMS, at first stage, where ID is still not necessary, Full Scan Acquisition or No Data Dependent MS/MS such All Ions is generally the choice of acquisition mode. At second stage, where more ID confidence is needed, a Target acquisition mode could be of interest to get reliable and pure MS/MS spectra of all differential compounds.
- Identification of differential compounds is one of the biggest CHALLENGE, a real bottleneck. Use of analytical technology with strong ID power is crucial to afford such challenge.
- For LCMS & CEMS where Ionization sources are based basically on ESI, **HRMS** is key due to their Qualitative power. (AM, IP, MSMS, CCS).
- For GC techniques where Electron Impact ionization (EI) is such an universal technique with universal libraries, a Single Quadrupole is a very robust and convenient technique. Recent developments of **soft EI** allows to keep some Molecular Ion intact so GCQTOF is also an interesting technique combining EI spectra and HRMS advantages.
- For ICPMS, due to the very low list of possible compounds (periodic table) it is feasible to perform Target Acquisition for all the elements.

From experiment design to conclusions, a long way to help scientists. Agilent Tools and workflows to better decisions making at Integrated Biology :

Complementing different analytical technologies LCMS, GCMS, CEMS, ICPMS. Data Acquisition modes.

- The choice of separation technique is biasing the kind of compounds we will find in terms of polarity.
- Volatile compounds would be difficult to get robust information on a LCMS system as well as other non-polar compounds. GC is in this case the best choice.
- In the other hand, highly polar compounds would be difficult to retain on a regular RP column. An alternative is to use HILIC columns or Capillary Electrophoresis (CE).
- For a comprehensive project considering different techniques it is interesting to have a **Tool** who can handle all these different experiments on the same Data Treatment project pointing out same organism.

Multi-Omics Open Platform: Mass Profiler Professional

Expression changes represented directly on routes

The screenshot displays the Mass Profiler Professional software interface. On the left is the 'Project Navigator' pane, which is divided into sections for 'Experiments', 'MAQC Agilent 8x60 1-color', 'Metabolomics', and 'Significant Pathways for'. The 'Experiments' section shows a tree view with 'Samples', 'Interpretations', and 'Analysis'. The 'MAQC Agilent 8x60 1-color' section shows 'Samples', 'Interpretations', and 'Analysis'. The 'Metabolomics' section shows 'Samples', 'Interpretations', and 'Analysis'. The 'Significant Pathways for' section shows 'Analysis' and 'My Favorites'. The main workspace shows a complex network diagram with various nodes and connecting lines. A red box highlights a specific part of the network, and a red arrow points from a callout box to it. Another red box highlights a section of the interface titled 'Interpretation2: Tissue', which lists genes: LMX1B, NIK2-2, ASCL1, and GATA2. A third red box highlights the 'MS Experiment Creation Wizard (Step 1 of 11)' dialog, which has a 'Select Data Source' section with radio buttons for 'MassHunter Quant', 'MassHunter Qual', 'MassHunter Qual (GC scan data)', 'MassHunter ICP-MS', 'Chemstation', 'AMDIS', and 'Generic'. A red arrow points from a callout box to the 'Generic' option. A fourth red box highlights the 'Joint Pathways' experiment section in the 'Project Navigator'.

Projects

**Microarray-based, NGS, q-PCR
Gene Expression/
Transcriptomics Experiments**

**LC/MS, GC/MS, CE/MS, ICP/MS & NMR
based Metabolite / Protein
Abundance Measurements**

**Joint Pathways experiment:
transcriptomics /
metabolomics**

Interpretation2: Tissue

MS Experiment Creation Wizard (Step 1 of 11)

Select Data Source

Choose the data sources that will be used for the experiment

☒ MassHunter Quant

☐ MassHunter Qual

☐ MassHunter Qual (GC scan data)

☐ MassHunter ICP-MS

☐ Chemstation

☐ AMDIS

☐ Generic

Organism

**Generic Import for
non Agilent
instruments: *.xls,
*.xlsx, *.TXT or
*.CSV files**

Enrichment Analysis on curated pathways and computationally – derived networks

- *Enfoques y estrategias analíticas que nos permiten las ultimas tecnologías de LC Mass Spectrometry de alta resolución. **UHPLC QTOF technical details***
- **Agilent proposal Workflows in different scenarios.** *Diferentes estrategias inherentes a investigación en el ámbito de Ciencias de la Vida, así como para el perfilado de Alimentos, Materiales, Procesos...*
- **Herramientas de Agilent y flujos de Trabajo para tomar mejores decisiones en un entorno de Biología integrada.** *Del diseño experimental a las conclusiones, un largo camino para ayudar al investigador.. :*
 - **Datos según modos de Adquisición.** *Complementariedad de las diferentes tecnologías LCMS, GCMS, CEMS, ICPMS*
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Deconvoluting Data and visualization tools

How does Agilent algorithms get compounds/features lists from a FullScan acquisition.

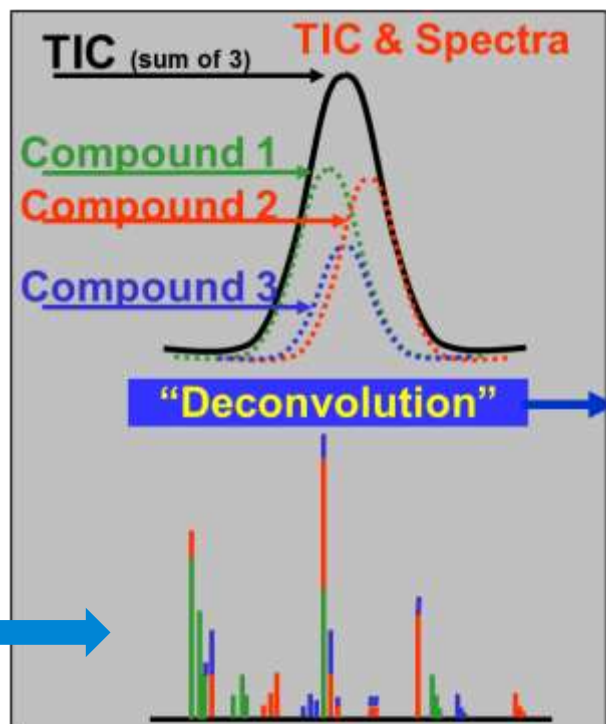
Due Full Scan acquisition, a **deconvolution technique** is needed in order to characterize all possible compounds eluted and ionized on the source.

Different Ionization sources (ESI for LC/CE & EI for GC) need different type of algorithms to extract and characterize compounds (features).

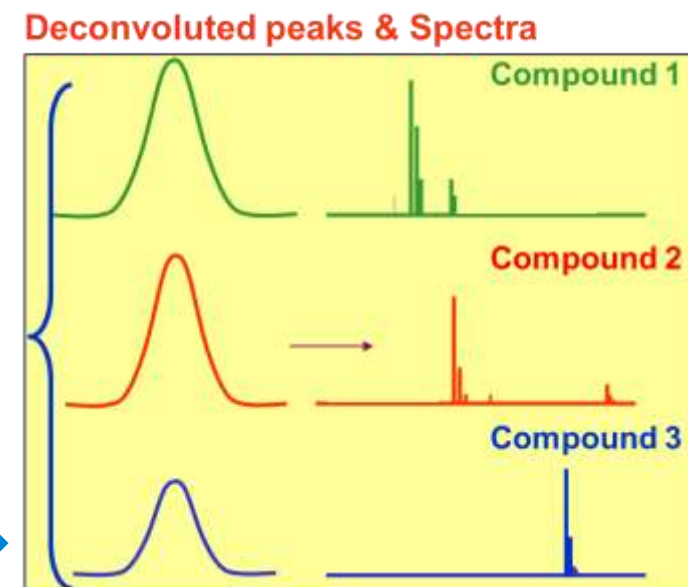
Electron Impact (GCMS) is a destructive ionization technique so **Deconvolution** is based on EI fragments.

Mass Hunter Qual & Quant have a dedicated Deconvolution Algorithm for this type of data, an improved AMDIS version.

The black TIC has three components underneath it. If you take a spectrum at the apex of the black TIC peak, you will see a mixture of three components, as the spectrum shown here on the right with all the green, red, and blue colors.



The MassHunter Deconvolution can pull out these individual components from the total ion chromatogram. So after deconvolution, we can get clean spectra of the matrix, the interference, and the target compound as shown on the right side of the slide.



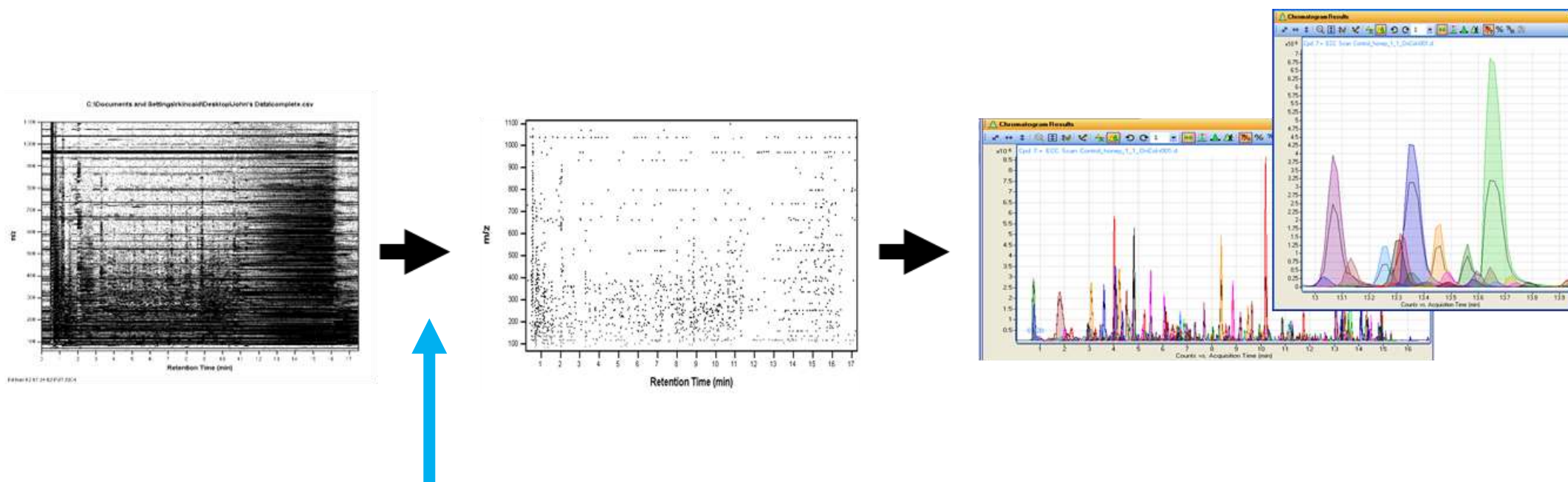
Deconvoluting Data and visualization tools

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Due Full Scan acquisition, a **deconvolution technique** is needed in order to characterize all possible compounds eluted and ionized on the source.

For LC HRMS Agilent developed **Molecular Feature Extraction (MFE)**.

MFE is an advanced deconvolution algorithm working at both spectra and chromatographic level.



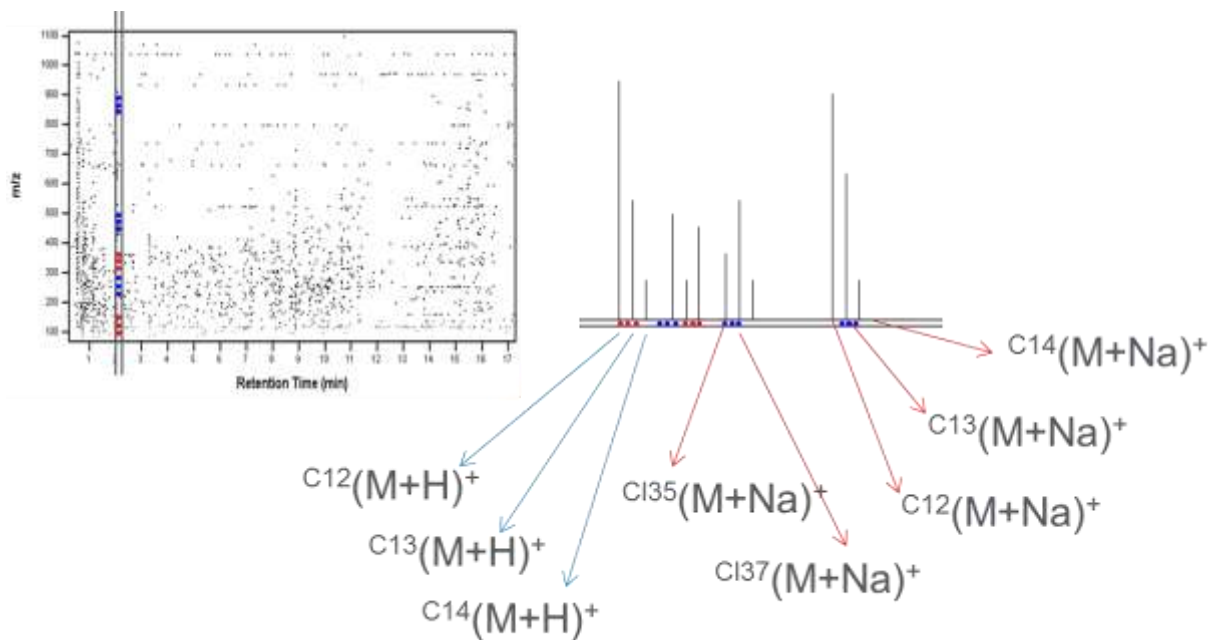
First step is to remove all the m/z which are present along the full chromatogram. This is fix background.

Deconvoluting Data and visualization tools

How does Agilent algorithms get compounds/features lists from a FullScan acquisition.

Molecular Feature Extraction (MFE).

MFE is an advanced deconvolution algorithm working at both spectra and chromatographic level.



Extraction Works in 3 dimensions :

Abundance, RT, m/z

Groups ionic species of same compound based on accurate mass.

Combines signals with chemical relationship (isotopes, adducts, multiple charges, dimers) and “molecular features” (= compounds)

Creates a list of mass intra spectra.

Mass
1071.50627
1050.40797
829.39682
885.40936
1137.49286
1137.48985
1442.63618
1290.59812
1033.4674
664.36901
784.39024
299.37294
299.70937
897.48015
745.33915
973.45314
1672.76497
688.36669
1531.78037
897.38411
788.46911
1501.6093
1398.6487
1248.6202
921.48555
757.41799
911.40588
1120.46384
1748.66086

Different smart settings are used for data mining and noise discrimination.

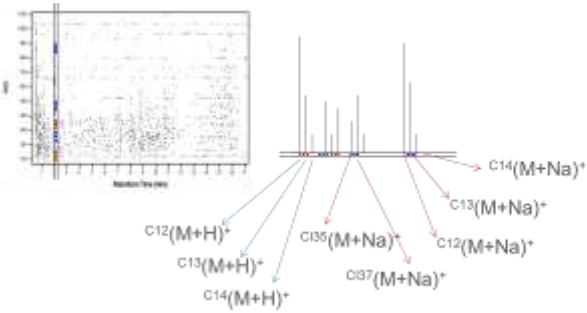
This process is done spectrum by spectrum, only those mass who show a gaussian peak along the time are considered as possible compounds (features)

Deconvoluting Data and visualization tools

How does Agilent algorithms get compounds/features lists from a FullScan acquisition.

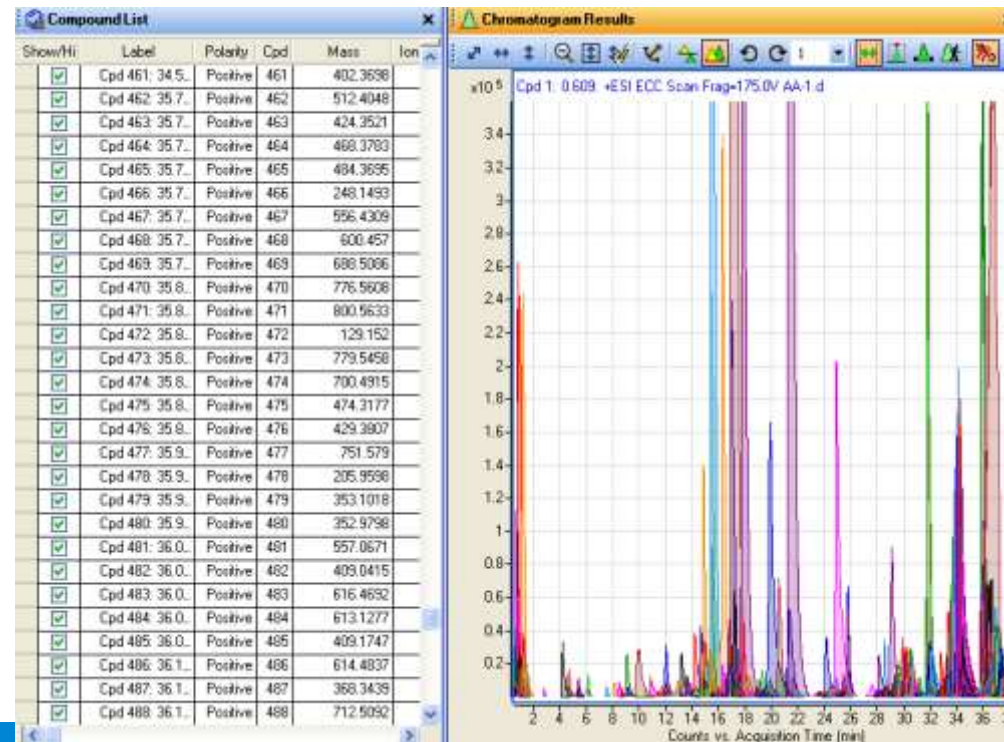
Molecular Feature Extraction (MFE).

MFE is an advanced deconvolution algorithm working at both spectra and chromatographic level.



MFE creates a list of possible compounds (features) characterized by Rt, Abundance, Acc.Mass & Isotopic Pattern.

This data is ready for Chemometric processing among different groups.



- *Enfoques y estrategias analíticas que nos permiten las ultimas tecnologías de LC Mass Spectrometry de alta resolución. **UHPLC QTOF technical details***
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Data prepare for Statistical Analysis.

Alignment, Normalization, Baselining with Mass Hunter ProFinder.

Statistical analysis requires for multiple replicates, both technical and natural

This statistical test requires to prepare data before tests.

Alignment of **Rt** and **Mass** along the different replicates is mandatory to avoid to skip possible compounds due to some instrumental deviation.

Alignment parameters

RT tolerance = \pm (0.0 % + 0.30 min)

Mass tolerance = \pm (10.0 ppm + 2.0 mDa)

But also Normalization and Rt correction can be performed using Standards or Not.

Normalization and RT correction

Abundance normalization

☒ Apply

☐ Without standards

☒ With standards

RT correction

☒ Apply

☐ Without standards

☒ With standards

Display

☐ Raw RT

☒ Corrected RT

Internal standard definitions

No. of internal standards 3

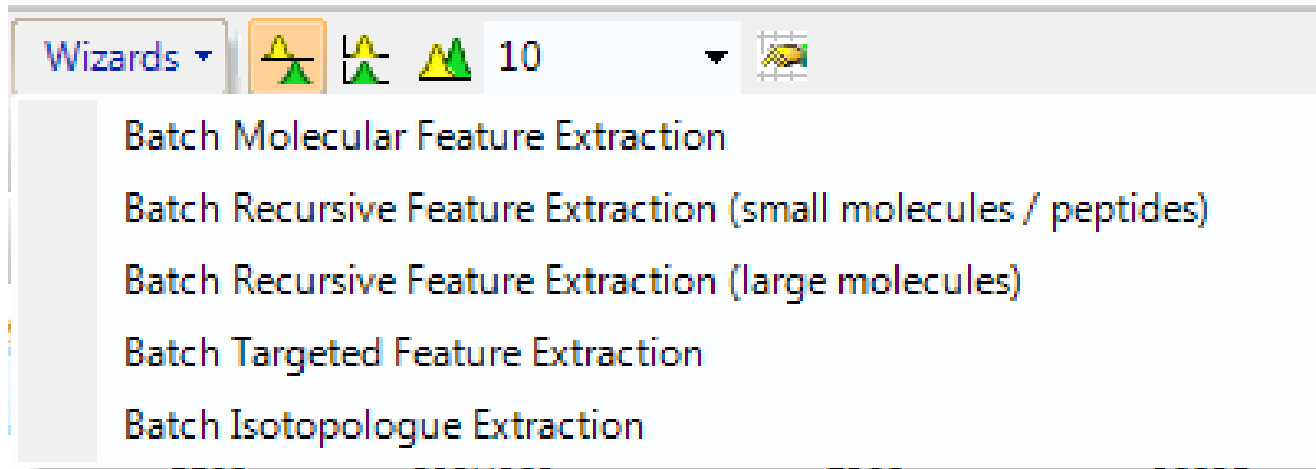
RT(min)	Mass(Da)	Norm.	Corr.
5.1	293.568	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
15.2	502.823	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
27.3	345.851	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Data prepare for Statistical Analysis.

Alignment, Normalization, Baselining with Mass Hunter ProFinder.

Statistical analysis requires for multiple replicates, both technical and natural

Mass Hunter Profinder is a NEW advanced smart tool combining Deconvolution, Data prepare and Recursive Analysis for Small or Large Molecules. It is a Wizard assisted program



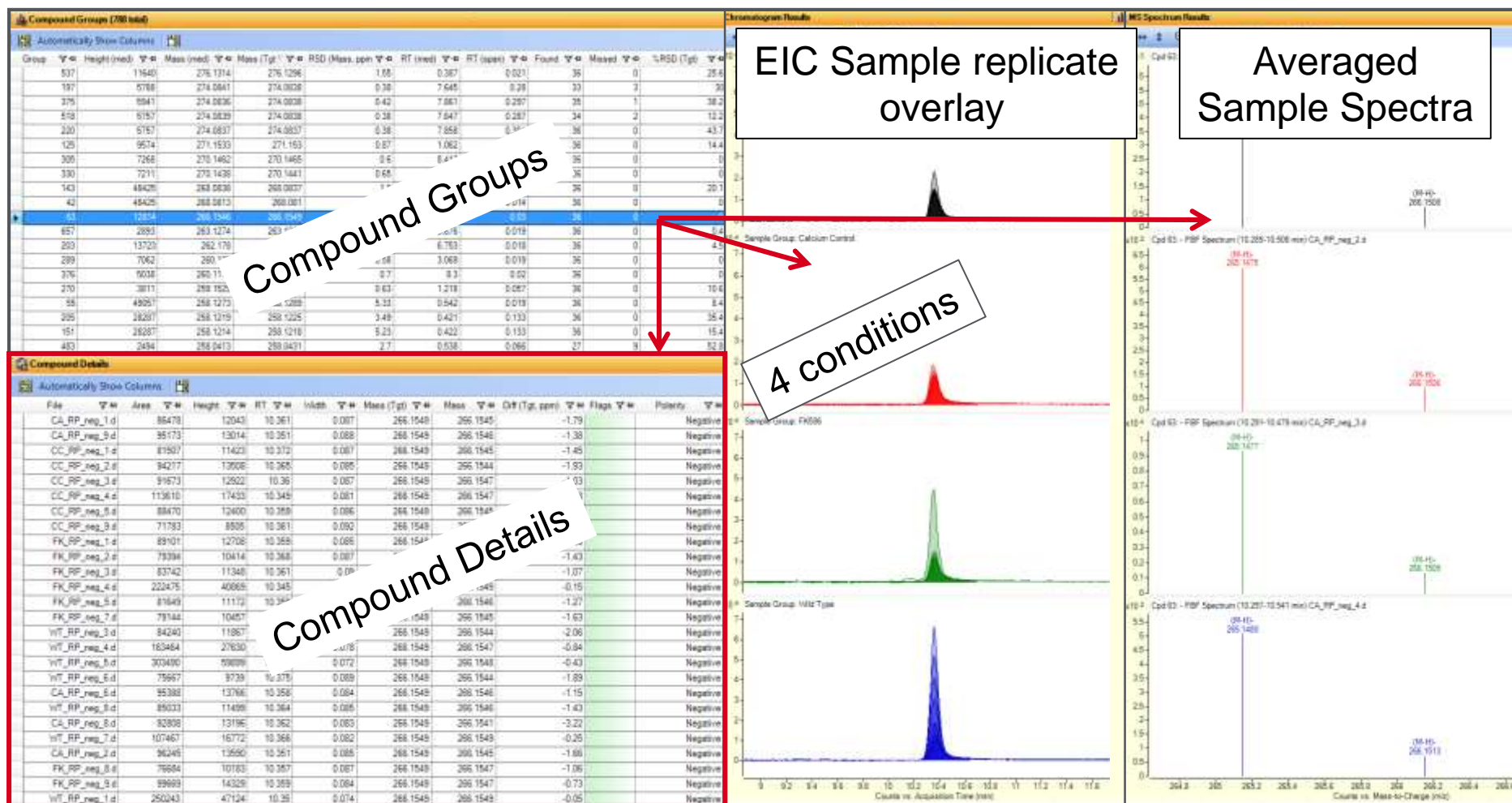
Automatically Deconvolutes with the right technique depending on Data loaded :

- Fragments deconvolution for GCMS
- MFE for LCQTOF

If desired Aligns, Corrects, Normalize and **makes a recursive Analysis.**

Four Profinder Windows:

Compound centric visualization and editing of results



- *Enfoques y estrategias analíticas que nos permiten las últimas tecnologías de LC Mass Spectrometry de alta resolución. **UHPLC QTOF technical details***
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Do I need recursive Analysis?

Please, make it easy ... Comprehensive Data treatment to avoid missing compounds.

Why should I perform Recursive Analysis?

MFE extracts many possible compounds (features) using some filter settings to avoid noise and artifacts.

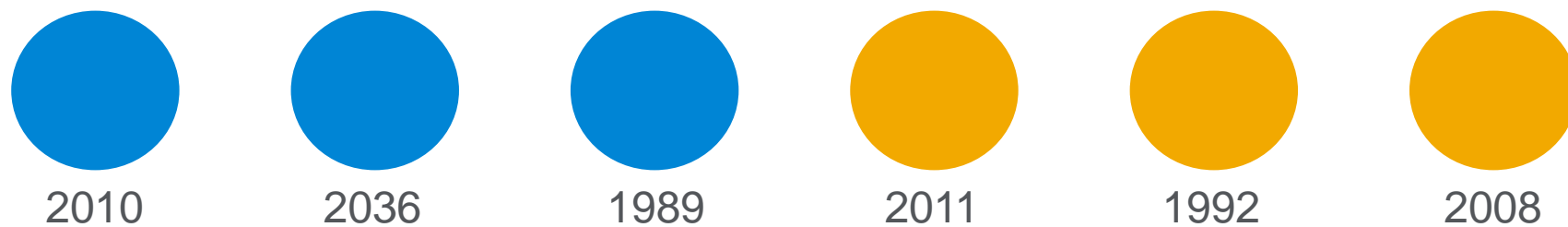
It could be possible some minor compounds would skip MFE on any replicate due to interference or any other reason.

Recursive allows to go deeper in the data and extract without noise filters from data on other replicates.

- Feature extraction using **MFE** (works on 3D data set)
finds targeted AND untargeted OR unknowns
- Alingment & Data prepare
features found across ALL samples, but might be missing in some
- Feature extraction Find by Ions (**works EIC based**)
finds targeted metabolites only (all or significant only), finds lower level missing features

Recursive data extraction in Pro Finder

1. Unbiased feature extraction



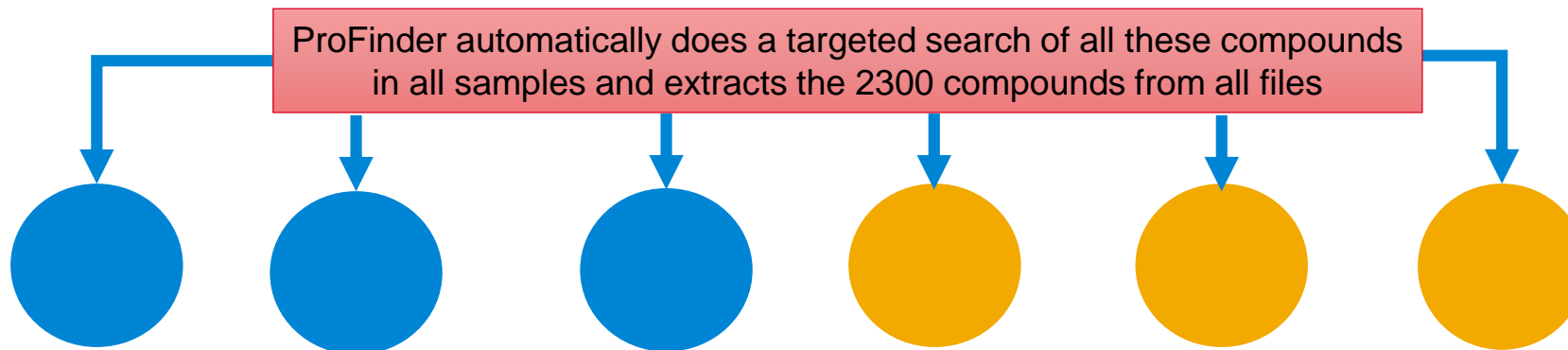
ProFinder makes a 'master list' of all compounds found across all the samples

2300 unique compounds

Unbiased feature detection will always find different numbers of compounds

Peak-picking/feature detection algorithms requires you to set a threshold level

2. Targeted feature extraction

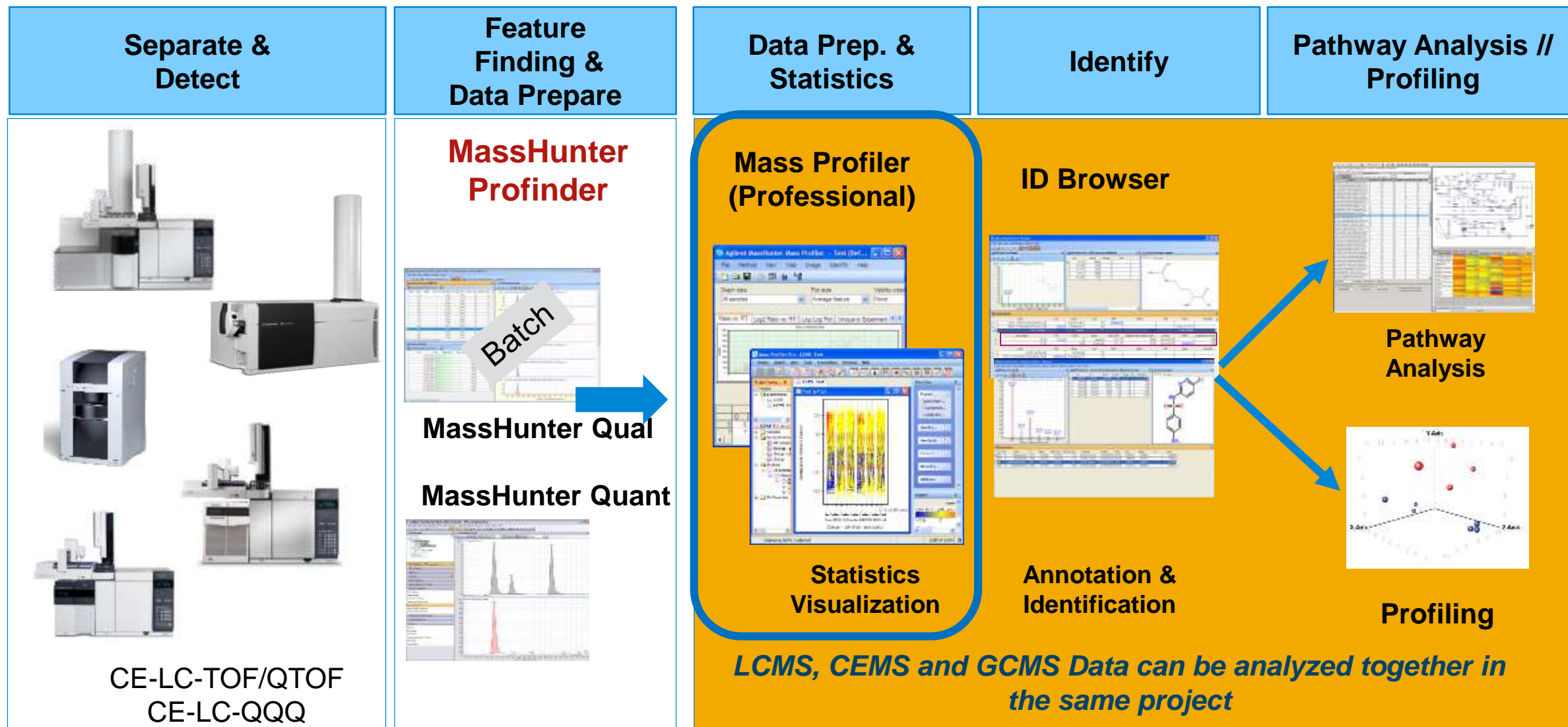


Targeted feature extraction has no threshold, it will extract right down into the noise

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A Comprehensive Metabolomics Workflow

Agilent LCMS, CEMS and GCMS



Mass Profiler professional.

Differential Analysis using Interpretations, clustering, PCA, PLRS, model of prediction

- Agilent Mass Profiler Professional (MPP) software is a powerful chemometrics platform
- Designed to exploit the high information content of mass spectra (MS) data
- Can be used in any MS-based differential analysis to determine relationships among two or more sample groups and variables.
- Provides advanced statistical analysis and visualization tools for GC/MS, LC/MS, CE/MS, and ICP-MS data analysis.
- Also integrates smoothly with Agilent MassHunter Workstation, Profinder, Spectrum Mill, and ChemStation software.
- Is the only platform that provides integrated identification/ annotation of compounds and integrated pathway analysis for metabolomic and proteomic studies.



Project – Workspace, container of Experiments

Experiment – Collection of samples acquired under same instrument method.

Parameter – Variable in the experiment (p.e. Time, Temp, Infected)

Condition – One or more samples representing a common biologic status (p.e. Time 14h)

Interpretation – Samples grouping based on Conditions. (p.e. Time vs Temp)

Entity – Molecular Entity from which we know Rt, Mass and Abundance. It can be “Identified” or “Not Identified”.

Technology – Registry or container of all data acquired under a simple Technology: Metabolomics, Transcriptomics, Proteomics....

Mass Profiler Professional

MPP Terminology



Sample	Grape	Country of Origin
1	Cabernet Sauvignon	USA
2	Cabernet Sauvignon	USA
3	Cabernet Sauvignon	France
4	Cabernet Sauvignon	France
5	Merlot	USA
6	Merlot	USA
7	Merlot	France
8	Merlot	France
9	Pinot Noir	USA
10	Pinot Noir	USA
11	Pinot Noir	France
12	Pinot Noir	France



Interpretation 1: Define samples by parameter **Grape**

Condition1: Cabernet Sauvignon (samples 1-4)

Condition2: Merlot (samples 5-8)

Condition3: Pinot Noir (samples 9-12)

Interpretation 2: Define samples by parameter **Country**

Condition1: USA (samples 1,2,5,6,9,10)

Condition2: France (samples 3,4,7,8,11,12)

Interpretation 3: Define samples by parameter **Grape and Country**

Condition1: Cabernet Sauvignon-USA (samples 1,2)

Condition2: Cabernet Sauvignon-France (samples 3,4)

Condition3: Merlot-USA (samples 5,6)

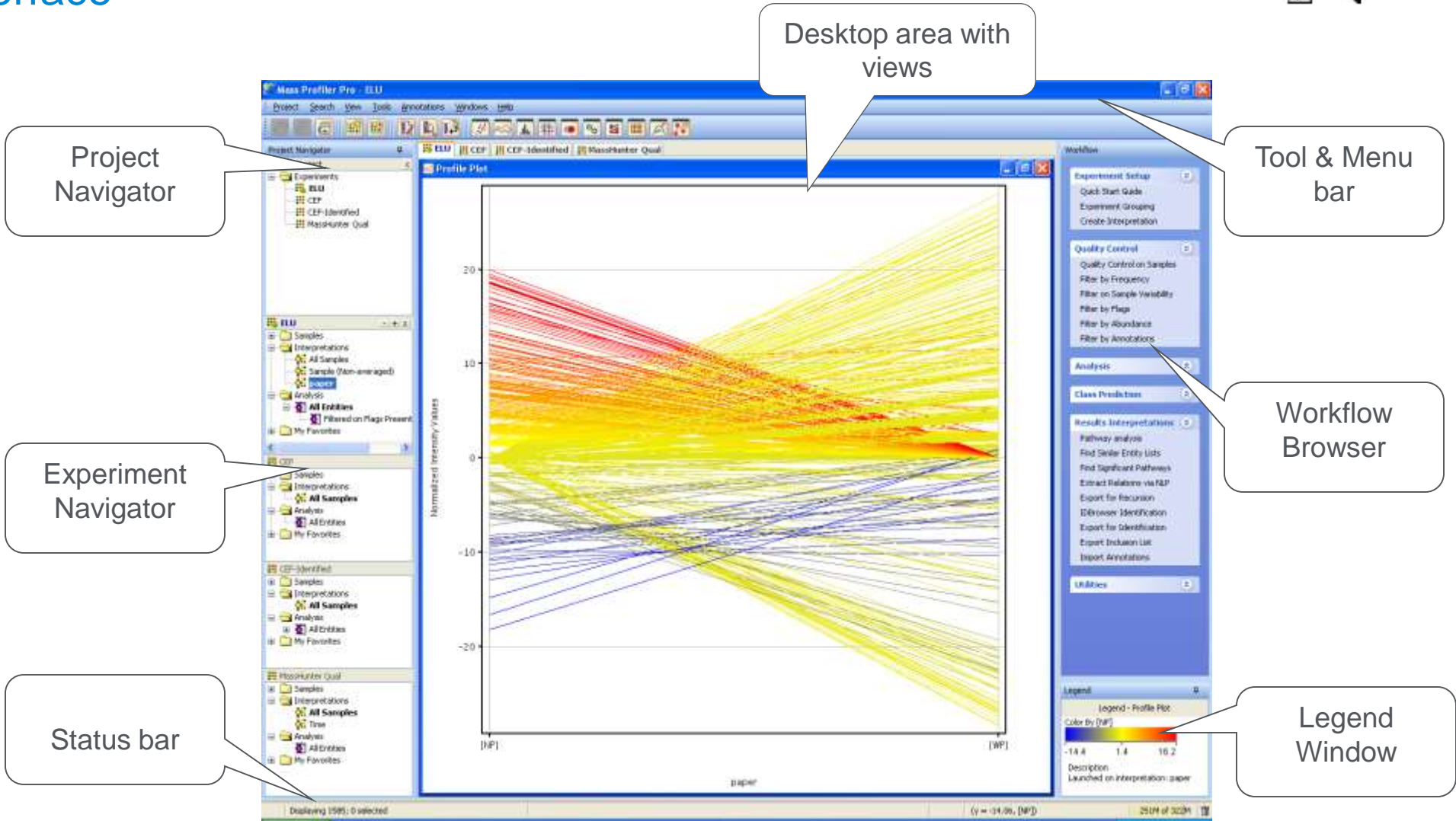
Condition4: Merlot-France (samples 7,8)

Condition5: Pinot Noir-USA (samples 9,10)

Condition6: Pinot Noir-France (samples 11,12)

Mass Profiler Professional

MPP Interface



Mass Profiler Professional

Experiment Creation

There are different choices when creating an experiment including Wizard for novel users or easy tasks.

New Experiment

Experiment description

Enter a name, analysis type, experiment type, a statistical significance test and fold change analysis. "Class Prediction" will guide you through the creation of a new experiment. "Data Import" will guide you through the import of training data.

Experiment name: New
Analysis type: Mass
Experiment type: Core
Workflow type: Analysis
Experiment notes: Analysis
Class Prediction
Data Import

MS Experiment Creation Wizard (Step 1 of 11)

Select Source, Organism and Data to Import

Choose the data source and organism that will be used for the experiment. Data may be imported from files or samples from previous experiments.

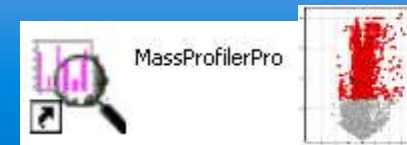
☒ MassHunter Profinder Archive (.PFA)
☐ MassHunter Qual/Profinder/Mass Profiler (.CEF)
☐ MassHunter ICP-MS
☐ AMDIS
☐ Generic

Organism: Homo sapiens
None
Homo sapiens
Mus musculus
Rattus norvegicus
Anopheles gambiae
Arabidopsis thaliana
Bacillus subtilis
Bos taurus

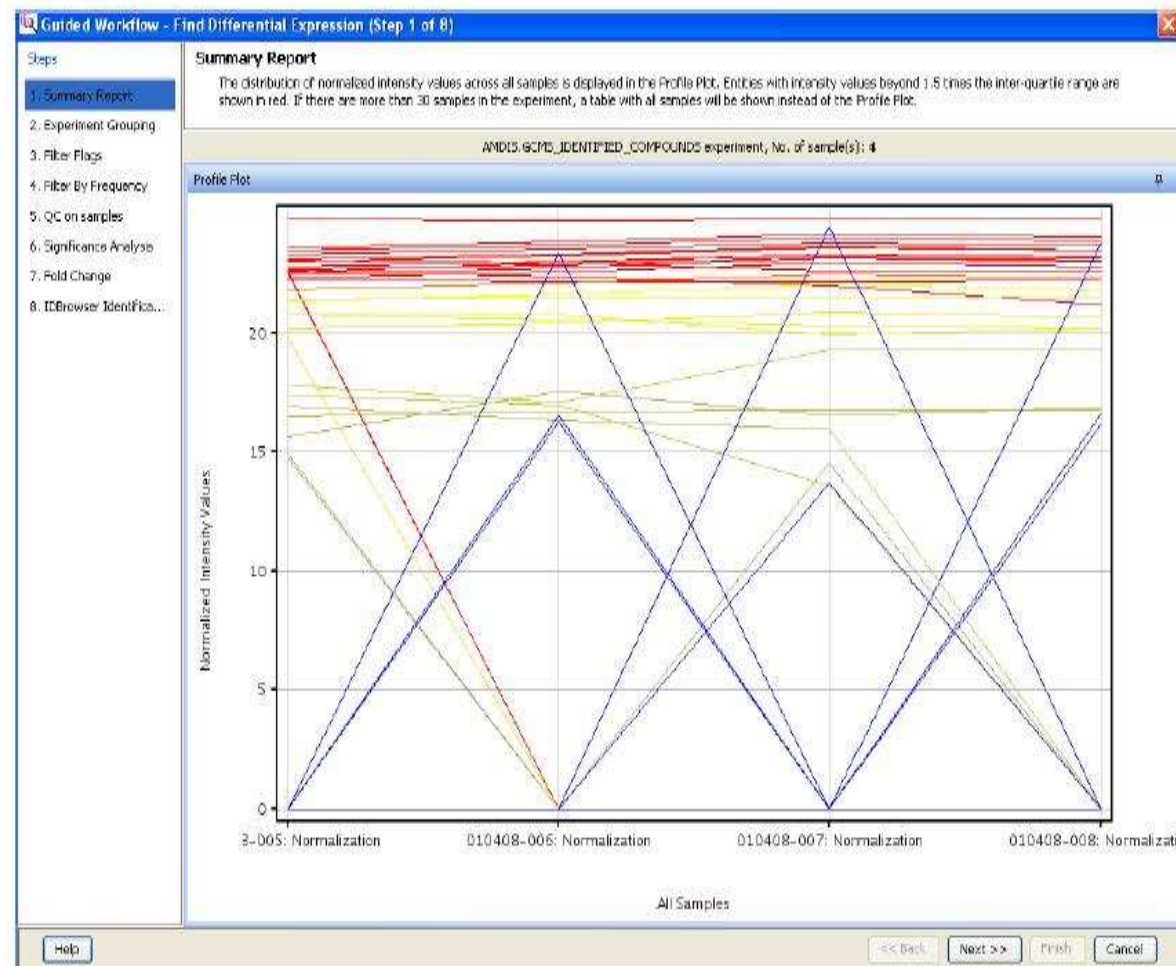
Scientists can use several kinds of data process origin including Generic from other vendors out of AGILENT (with limited software features).

Mass Profiler Professional

Guided Workflow Analysis – Step 1 : Summary Report



1. Summary Report
2. Experiment Grouping
3. Filter on Flags
4. Filter by Frequency
5. QC on Samples
6. Significance Analysis
7. Fold Change
8. ID Browser identification



If < 30 Samples then Profile Plot (shown above) is Generated Baseline

Mass Profiler Professional

Guided Workflow Analysis – Step 2: Grouping



Guided Workflow - Find Differential Expression (Step 2 of 8)

Steps

1. Summary Report
- 2. Experiment Grouping**
3. Filter Flags
4. Filter By Frequency
5. QC on samples
6. Significance Analysis
7. Fold Change
8. IDBrowser Identifica...

Experiment Grouping

Experiment parameters define the grouping or replicate structure of your experiment. Enter experiment parameters by clicking on the "Add Parameter" button. You may enter as many parameters as you like, but only the first two parameters will be used for analysis in the guided workflow. Other parameters can be used in the advanced analysis. You can also edit and re-order parameters and parameter values here.

Displaying **26** sample(s) with **1** experiment parameter(s). To change, use the button controls below.

Experiment Grouping

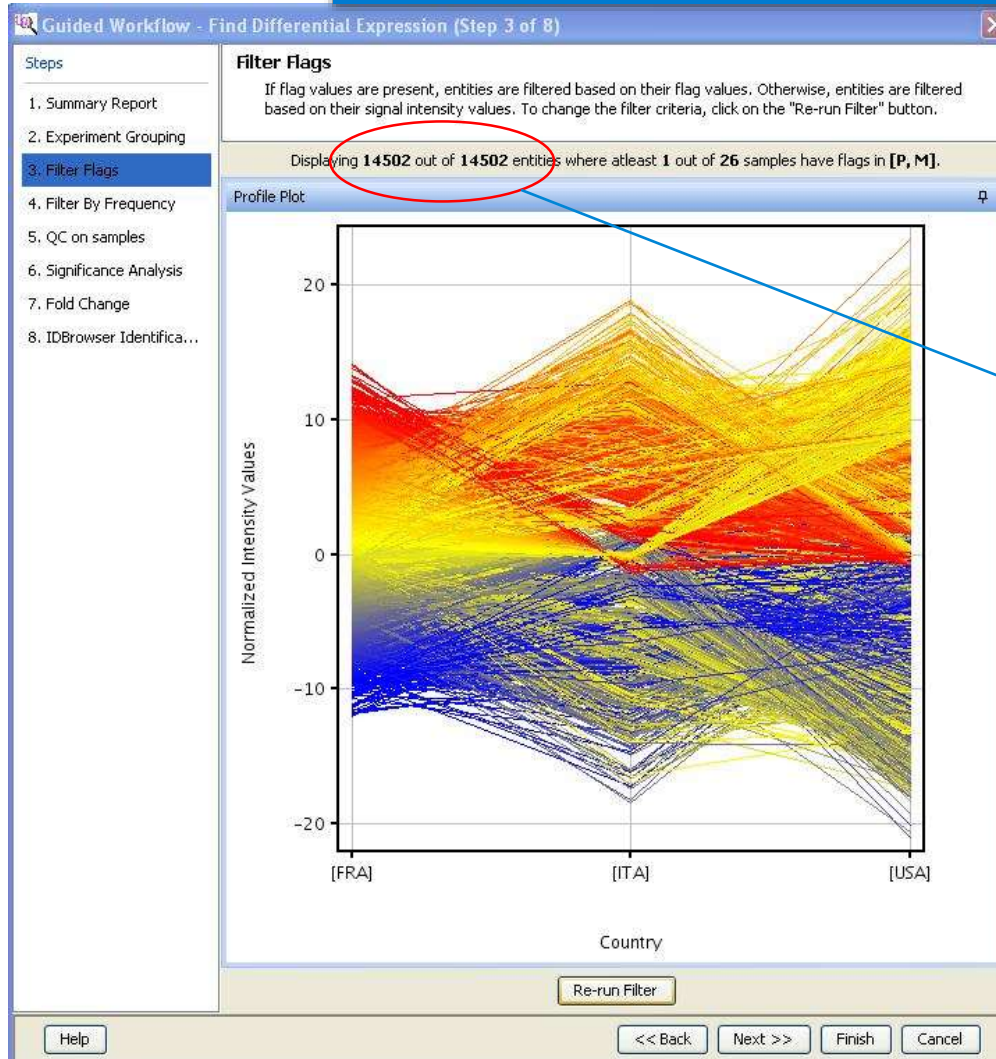
Samples	USA
ESI+ _CS_Calif_1	USA
ESI+ _CS_Calif_1_FK	USA
ESI+ _CS_Calif_2	USA
ESI+ _CS_Calif_3	USA
ESI+ _CS_Fran_1	FRA
ESI+ _CS_Fran_2	FRA
ESI+ _CS_Italy_1	ITA
ESI+ _M_Calif_1	USA
ESI+ _M_Calif_2	USA
ESI+ _M_Fran_1	FRA
ESI+ _M_Fran_2	FRA
ESI+ _M_Fran_3	FRA
ESI+ _M_Fran_4	FRA
ESI+ _M_Italy_1	ITA
ESI+ _PN_Calif_1	USA
ESI+ _PN_Fran_1_01	FRA
ESI+ _PN_Fran_2	FRA
ESI+ _PN_Fran_3	FRA
ESI+ _PN_Fran_4	FRA
ESI+ _PN_Fran_5	FRA
ESI+ _PN_Fran_6	FRA
ESI+ _PN_Fran_7	FRA
ESI+ _PN_Italy_1	ITA
ESI+ _PN_Italy_2	ITA
ESI+ _PN_Italy_3	ITA
ESI+ _PN_Italy_4	ITA

Add Parameter... Edit Parameter... Delete Parameter

<< Back Next >> Finish Cancel

Mass Profiler Professional

Guided Workflow Analysis – Step 3: Filter on Flags



- Can be used to filter out entities which are rarely detected, therefore not very reliable

- Similar to filter by frequency

→ 14502 entities retained out of 14502

Filter Parameters

Acceptable Flags

☒ Present

☒ Marginal

☐ Absent

Retain Entities in which

☒ at least out of 26 samples have acceptable values

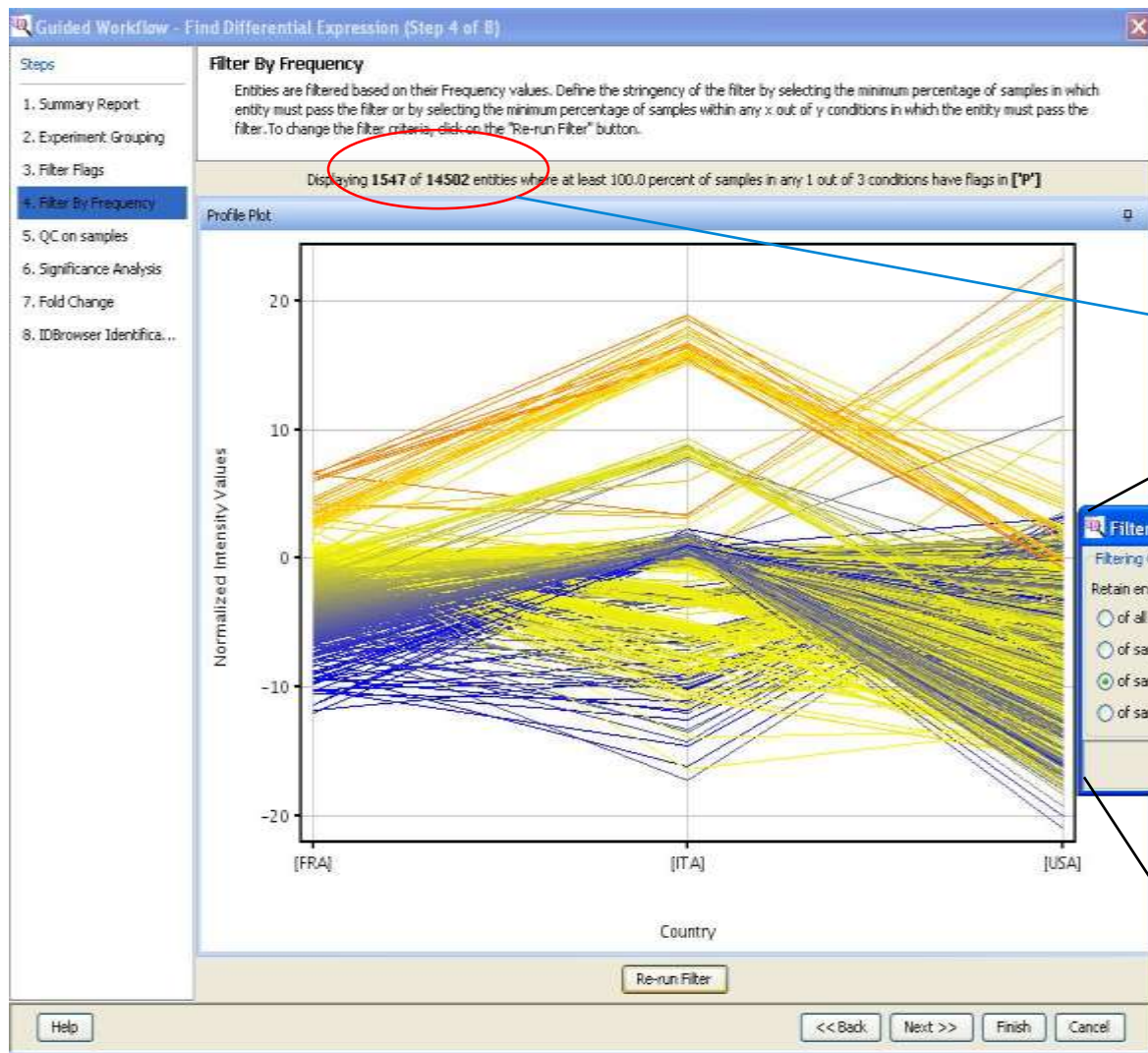
☐ at least % of the values in any out of 3 conditions have acceptable values

OK Cancel

Filter on "Present" and "Marginal" (saturated entity)

Mass Profiler Professional

Guided Workflow Analysis – Step 4: Filter by Frequency



1547 entities retained
out of 14502

Filter Parameters

Filtering Conditions:

Retain entities that appear in at least %

- ☐ of all samples
- ☐ of samples in only one condition
- ☒ of samples in at least one condition
- ☐ of samples within each condition

OK Cancel

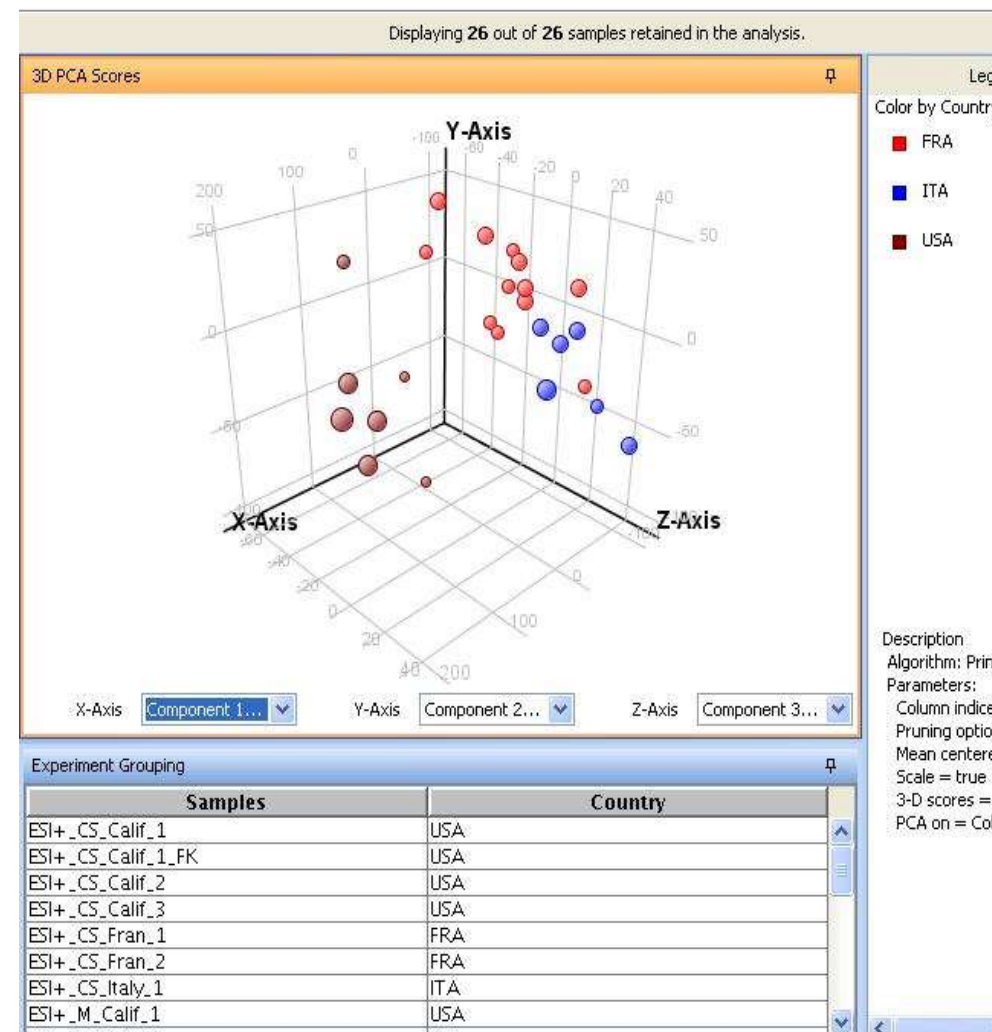
Mass Profiler Professional

Guided Workflow Analysis – Step 5: QC on Samples



QC on samples

Sample quality can be assessed by examining the values in the PCA plot and other experiment specific quality plots.



➤ By default, each sample is plotted according to its values for the first three Principal Components

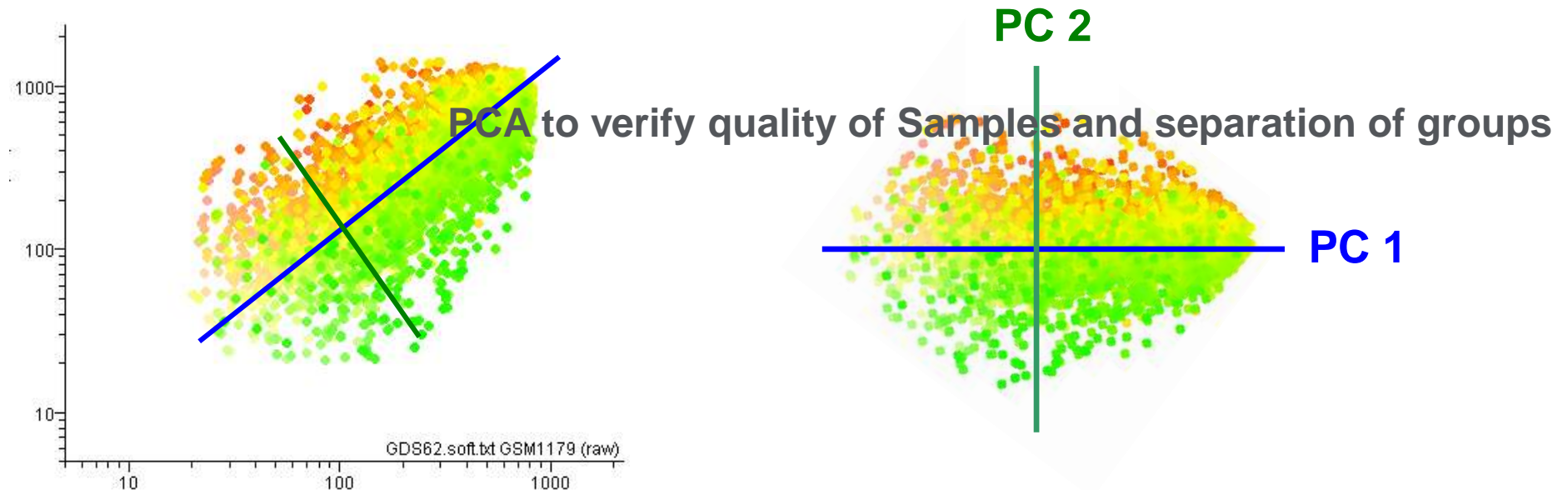
➤ Principal Components are vectors that capture the most variance in the data.

➤ Assumption: samples within an experimental condition should be more similar to each other than to those from different conditions.

➤ Expect to see samples from the same experimental condition to group closer to each other than to samples of a different condition

Mass Profiler Professional

PCA is a variable reduction Method



An eigenvalue-eigenvector decomposition is performed on the covariance matrix of the entity expression values around zero

The eigenvector corresponding to the largest eigenvalue is called the first principal component

Successive principal components are eigenvectors corresponding to each smaller eigenvalue

Mass Profiler Professional

Guided Workflow Analysis – Step 6: Significance Analysis



Depending upon the experimental grouping, **Mass Profiler Professional** performs either T-test or Analysis of Variance (ANOVA) based on the samples.

Guided Workflow - Find Differential Expression (Step 6 of 8)

Steps

1. Summary Report
2. Experiment Grouping
3. Filter Flags
4. Filter By Frequency
5. QC on samples
- 6. Significance Analysis**
7. Fold Change
8. IDBrowser Identifica...

Significance Analysis

Entities are filtered based on their p-values calculated from statistical analysis. To apply a new p-value cut-off, click on "Re-run Analysis" button. You will not be able to proceed to the next step if no entities pass the filter.

Displaying **530** out of **1547** entities satisfying corrected p-value cut-off **0.05**. To change, use the "Re-run Analysis" button below.

Differential Expression Analysis Report

Test Description

Selected Test : Oneway ANOVA
p-value computation: Asymptotic
Multiple Testing Correction: Benjamini-Hochberg

Result Summary

	P all	P < 0.05	P < 0.02	P < 0.01	P < 0.0050	P < 0.0010
Corrected p-value	1547	530	394	343	283	140
Expected by chance		26	7	3	1	0

Entities by Chance False Discovery Rate

p-values

Compound	p-value	Corrected p-value
313.9968@0.7160694	1.3533638E-4	0.0013595155
457.1805@0.89390373	5.532001E-4	0.0037047642
369.1633@0.9154259	0.0011521976	0.0058633215
342.1169@0.93180096	0.0055246614	0.021473998
259.1059@0.96792954	0.0068118055	0.025515407
277.1169@0.9682527	0.014392788	0.044177864
240.1479@0.9680382	0.004107683	0.01663504
189.1007@1.0129546	6.9314125E-4	0.0042267763
129.3197@1.0502836	0.007812585	0.028101565
190.0157@1.058558	0.0040005837	0.016372759
260.0303@1.1603673	0.0032870115	0.014203929
133.0742@1.1728247	0.011550421	0.03746017
122.0405@1.1932591	8.423912E-4	0.004704618
200.0797@1.2358472	0.0012501734	0.0061789723
187.0948@1.2424619	0.014210722	0.042702825

Re-run Analysis

Help << Back Next >> Finish Cancel

Guided Workflow - Find Differential Expression (Step 5 of 8)

Steps

1. Summary Report
2. Experiment Grouping
3. QC on samples
4. Filter Probesets
- 5. Significance Analysis**
6. Fold Change
7. GO Analysis
8. Find Significant Path...

Significance Analysis

Entities are filtered based on their p-values calculated from statistical analysis. To apply a new p-value cut-off, click on "Re-run Analysis" button. You will not be able to proceed to the next step if no entities pass the filter.

Displaying **4762** out of **13555** entities satisfying corrected p-value cut-off **0.05**. To change, use the "Re-run Analysis" button below.

Differential Expression Analysis Report

Test Description

Selected Test : T Test unpaired
p-value computation: Asymptotic
Multiple Testing Correction: Benjamini-Hochberg

Result Summary

	P all	P < 0.05	P < 0.02	P < 0.01	P < 0.005	P < 0.001
FC all	13555	4762	3081	2034	1326	344
FC > 1.1	8661	4528	3023	2012	1321	344
FC > 1.5	2391	1221	980	836	692	288
FC > 2.0	1257	513	398	346	288	172
FC > 3.0	617	219	160	139	122	85
Expected...		238	61	20	6	0

p-values

ProbeNa...	p-value	Correcte...	FCABSOL...	regulation
A_23_P2	0.01133	0.03673	1.48991	down
A_23_P1	0.00164	0.01055	1.24533	down
A_23_P5	0.00237	0.01324	2.62815	up
A_23_P2	5.77787	0.00554	1.11680	down
A_23_P2	0.00222	0.01268	1.29388	up
A_23_P3	2.94803	0.00375	1.62866	down
A_23_P1	8.36640	0.00688	1.47925	down
A_23_P3	2.89185	0.00105	1.56118	up
A_23_P6	0.00762	0.02779	1.112479	down
A_23_P1	0.00967	0.03289	9.713094	down

Volcano Plot

Select pair [Experimental] Vs [Control]

Re-run Analysis

Help << Back Next >> Finish Cancel

Mass Profiler Professional

Guided Workflow Analysis – Statistical Tests



One-way Tests: Compare conditions defined by a single parameter (i.e Grape)

T-test

Merlot ↔ Pinot Noir

ANOVA

Merlot ↔ Pinot Noir ↔ Caber

N-way Tests: Compare conditions defined by 2 or more parameters

2-way ANOVA

Grape
Merlot
Pinot Noir

x

Country
USA
France

3-way ANOVA

Grape
Merlot
Pinot Noir

x

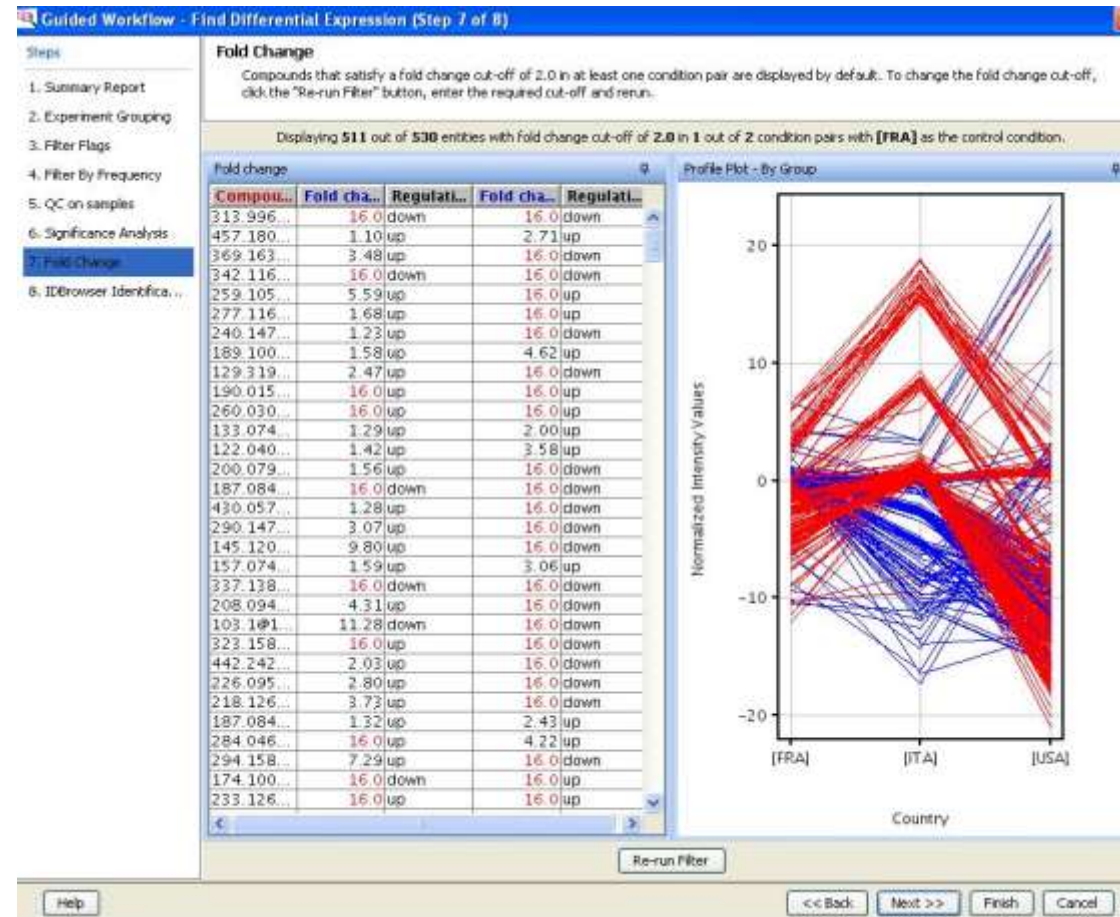
Country
USA
France

x

Vintage
2004
2006

Mass Profiler Professional

Guided Workflow Analysis – Step 7: Fold Change / Volcano



Filter Sets Fold Change > 2.0.. To more

Mass Profiler Professional

Guided Workflow Analysis – Step 8: ID Browser



Once we have a list of differential features, we need to identify them by any library at the first attempt.

GCMS data is more easy due to available universal EI libraries.

For LCHRMS Vendor or user should create their own libraries for a confident and reliable ID.

Label	Name	Formula	Notes	CAS	Overall Score	Mass	Mass
Cpd 106: 2,6-nonadienal 6.2...	2,6-nonadienal	C9 H14 O			85	138.1045	
Cpd 113: 2,6-nonadienal 6.5...	2,6-nonadienal	C9 H14 O			86.12	138.1044	
Cpd 354: Salicylic acid; 7.41...	Salicylic acid	C7 H6 O3	Analgesic, Anti-Inflammatory, A...	69-72-7	76.94	138.0323	
Cpd 253: (+)-Limonene 12.16...	(+)-Limonene	C10 H16			86.88	136.1252	
Cpd 206: p-Cymene 10.079...	p-Cymene	C10 H14			75.29	134.1095	
Cpd 268: p-Cymene 12.758...	p-Cymene	C10 H14			91.31	134.1096	
Cpd 146: Glutaric acid; 7.580...	Glutaric acid	C5 H8 O4	Endogenous Metabolite	110-94-1	86.8	132.0424	
Cpd 32: 2-oxoisocaproic acid...	2-oxoisocaproic acid	C6 H10 O3	Geigy vol.3 p.109	816-66-0	83.12	130.0631	
Cpd 120: 2-oxoisocaproic aci...	2-oxoisocaproic acid	C6 H10 O3	Geigy vol.3 p.109	816-66-0	79.97	130.0631	
Cpd 213: 2-oxoisocaproic aci...	2-oxoisocaproic acid	C6 H10 O3	Geigy vol.3 p.109	816-66-0	85.01	130.0624	
Cpd 34: Mesaconic acid; 2.8...	Mesaconic acid	C5 H6 O4	Citraconic acid, Methylfumaric...	498-24-8	93.83	130.027	
Cpd 60: 6-hydroxy-2-hexyno...	6-hydroxy-2-hexynoic acid	C6 H8 O3			71.35	128.0474	
Cpd 417: 3-octenal 12.657; C...	3-octenal	C8 H14 O			85.36	126.1047	
Cpd 83: Hydroxyhydroquinon...	Hydroxyhydroquinone	C6 H6 O3	Metabolite of quinol	533-73-3	86.77	126.0318	
Cpd 382: Hydroxyhydroquino...	Hydroxyhydroquinone	C6 H6 O3	Metabolite of quinol	533-73-3	65.41	126.0316	

Entities will be annotated upon return to MPP Program

Mass Profiler Professional

Guided Workflow Analysis – Step 8: ID Browser - Return



Guided Workflow - Find Differential Expression (Step 8 of 8)

Steps:

1. Summary Report
2. Experiment Grouping
3. Filter Flags
4. Filter By Frequency
5. QC on samples
6. Significance Analysis
7. Fold Change
8. IDBrowser Identification

IDBrowser Identification

To identify the Entities that passed the fold change cut-off with IDBrowser click on the "IDBrowser Identification" button.

Identify Entities with IDBrowser IDBrowser Identification

Spreadsheet

Compound	Fold change(ITA)	Regulation(ITA) v...	Fold change(USA)	Regulation(USA) v...
313.9968@0.716	16.0	down	16.0	down
457.1805@0.893	1.10	up	2.71	up
269.1631@0.915	3.48	up	16.0	down
342.1169@0.931	16.0	down	16.0	down
259.1059@0.967	5.59	up	16.0	up
277.1169@0.968	1.68	up	16.0	up
240.1479@0.968	1.23	up	16.0	down
189.1007@1.012	1.58	up	4.62	up
129.3197@1.050	2.47	up	16.0	down
190.0157@1.058	16.0	up	16.0	up
260.0303@1.160	16.0	up	16.0	up
133.0742@1.172	1.29	up	2.00	up
122.0405@1.193	1.42	up	3.58	up
200.0797@1.235	1.56	up	16.0	down
187.0848@1.242	16.0	down	16.0	down
430.0574@1.236	1.28	up	16.0	down
290.147@1.2468	3.07	up	16.0	down
145.1208@1.274	9.86	up	16.0	down
157.0742@1.262	1.59	up	3.06	up
337.1383@1.401	16.0	down	16.0	down
208.0949@1.387	4.31	up	16.0	down
103.1@1.3588464	11.28	down	16.0	down
323.1584@1.465	16.0	up	16.0	down
442.2422@1.689	2.03	up	16.0	down
226.0956@1.706	2.80	up	16.0	down
218.1269@1.768	3.73	up	16.0	down
187.0845@1.814	1.32	up	2.43	up
284.0467@1.874	16.0	up	4.22	up
294.1584@1.907	7.29	up	16.0	down
174.1003@2.0179	16.0	down	16.0	down
233.1265@2.156	16.0	up	16.0	up
130.0631@2.690	2.00	up	16.0	down
176.0688@2.769	2.76	up	3.05	up
130.027@2.806418	1.48	up	2.03	up
188.2201@2.84	16.0	down	16.0	down

Help << Back Next >> Finish Cancel

Guided Workflow - Find Differential Expression (Step 8 of 8)

Steps:

1. Summary Report
2. Experiment Grouping
3. Filter Flags
4. Filter By Frequency
5. QC on samples
6. Significance Analysis
7. Fold Change
8. IDBrowser Identification

IDBrowser Identification

To identify the Entities that passed the fold change cut-off with IDBrowser click on the "IDBrowser Identification" button.

Identify Entities with IDBrowser IDBrowser Identification

Spreadsheet

Compound	Fold change(ITA)	Regulation(ITA) v...	Fold change(USA)	Regulation(USA) v...
C18 H6 N2 S2	16.0	down	16.0	down
C17 H31 N O13	1.10	up	2.71	up
His Val Asp	3.48	up	16.0	down
Sucrose	16.0	down	16.0	down
C11 H17 N O6	5.59	up	16.0	up
Quinine	1.68	up	16.0	up
C12 H20 N2 O3	1.23	up	16.0	down
C8 H15 N O4	1.58	up	4.62	up
129.3197@1.050...	2.47	up	16.0	down
C4 H6 N4 O3 S	16.0	up	16.0	up
Inositol phosphate	16.0	up	16.0	up
C5 H11 N O3	1.29	up	2.00	up
C4 H10 O2 S	1.42	up	3.58	up
Barbituric acid, 5-e...	1.56	up	16.0	down
N-(3S-hydroxy-but...	16.0	down	16.0	down
C30 H10 N2 S	1.28	up	16.0	down
C16 H22 N2 O5	3.07	up	16.0	down
C6 H15 N3 O	9.80	up	16.0	down
Ethosuximide M7	1.59	up	3.06	up
C14 H27 N O4 S2	16.0	down	16.0	down
C8 H16 O6	4.31	up	16.0	down
C5 H13 N O	11.28	down	16.0	down
Ala Pro His	16.0	up	16.0	down
C19 H38 O11	2.03	up	16.0	down
Porphobilinogen	2.80	up	16.0	down
Ser Ile	3.73	up	16.0	down
N-(3S-hydroxy-but...	1.32	up	2.43	up
C11 H12 N2 O5 S	16.0	up	4.22	up
Tyr Ile	7.29	up	16.0	down
N2-Acetyl-L-ornith...	16.0	down	16.0	up
C10 H19 N O5	16.0	up	16.0	up
2-oxoisocaproic acid	2.00	up	16.0	down
2-Isopropylmalic acid	2.76	up	3.05	up
Mesaconic acid	1.48	up	2.03	up
C20 H31 N11 S	16.0	up	16.0	down

Help << Back Next >> Finish Cancel

Entities annotated return to MPP Program

- *Enfoques y estrategias analíticas que nos permiten las últimas tecnologías de LC Mass Spectrometry de alta resolución. **UHPLC QTOF technical details***
- **Agilent proposal Workflows in different scenarios.** *Diferentes estrategias inherentes a investigación en el ámbito de Ciencias de la Vida, así como para el perfilado de Alimentos, Materiales, Procesos...*
- **Herramientas de Agilent y flujos de Trabajo para tomar mejores decisiones en un entorno de Biología integrada.** *Del diseño experimental a las conclusiones, un largo camino para ayudar al investigador.. :*
 - **Datos según modos de Adquisición.** *Complementariedad de las diferentes tecnologías LCMS, GCMS, CEMS, ICPMS*
 - **Deconvolución de datos y herramientas de visualización.** *Como funcionan los algoritmos de Agilent para extraer información de compuestos de un Full Scan.*
 - **Preparación de datos previa al Análisis Estadístico diferencial.** *Alineamiento, Normalización, “Baselining” con “Mass Hunter ProFinder”.*
 - **¿Necesito análisis recursivo a través de iteración? Por favor hágamelo fácil....** *Exhaustivo tratamiento de datos para evitar la Pérdida de compuestos.*
 - **Mass Profiler professional.** *Análisis Diferencial a través de Interpretaciones, Clustering, PCA, PLRS, modelos de predicción*
 - **Así, ¿Cuales son mis compuestos diferenciales de interés? ¿Como puedo identificarlos? Librerías empíricas de espectros MS/MS. Agilent METLIN PCDL.**
 - *Análisis de rutas Metabólicas a través de “Pathways Analysis”. Biología integrada e interpretación biológica de mis datos. Pathways Analysis.*
 - *¿Cuál es mi próximo experimento? La potencia del enfoque de la Biología integrada.*
- *Movilidad Iónica. Una nueva dimensión para extracción de datos más selectiva en muestras complejas. Una nueva herramienta de identificación*
- *Fluxómica. Fácil y rápida visualización de la incorporación de sustratos marcados isotópicamente en una ruta metabólica a través de “VistaFlux”.*
- *Método llave en mano para el análisis Metabolómico dirigido en rutina de los metabolitos del Ciclo Central de Carbono*
- *Sinergias con la medida In-vivo del Metabolismo celular con “Seahorse”.*

So, what are my compounds of interest.... Where can I identify them?

Curated or Free MS/MS Libraries. Agilent METLIN PCDL.

PCDLs by Compounds & Spectra

LC/MS PCDL	Market	PCDL	Compounds with AM MS/MS Spectra	Total number of Spectra	Compounds with RTs
Forensic Toxicology	Forensic Toxicology	>9,200	>3,900	>13,500	0
Pesticides	Food Safety / Environmental	>1,700	>800	>2,700	0
Veterinary Drugs	Food Safety	>2,100	>1,500	>5,200	>120
Mycotoxins	Food Safety	>450	>300	>1,300	0
Water Contaminants	Environmental	>1,400	>1,000	>3,900	>260
METLIN*	Metabolomics / Lipidomics	>79,600**	>9,400	>32,000	>680
NIST 2014 MS/MS	General	>9,300	>9,300	>234,000	0

*METLIN numbers exclude tri- and quatra- peptides in the online METLIN

** **Plus 168k theoretical**

Why is Curation Important?

More isn't Always Better

An open-source database may contain over a million spectra

But how many of those spectra are:

- Relevant to your application?
- Duplicates, triplicates...?
- Collected under questionable conditions?
- Contain inaccurate metadata?

Not all Databases and Libraries are Created Equal

Non curated or poorly curated databases and libraries costs you time, money and uncertainty



Questions Laboratories don't want to ask:

- Which of these entries is my molecule?
- Are my identification match scores meaningful?
- Can I trust this source?
- There must be valuable information here..., but where?
- Is this even relevant to my application?

How to Identify the Right Database and Library

Agilent made a huge effort to create Databases and Libraries to ensure results are reliable

Advantages include:

- Developed by experts
- Designed to high standards
- Turn-Key and fully integrated



The PCDL Curation Process

There are four primary steps in the development of an Agilent database or library:

1. Identify the target compound list in collaboration with leading experts
2. Create the compound database with verified information for each target entry
3. Collect mass spectral data using high purity reference standards
4. Create the library with spectra curated according to Agilent's rigorous quality control curation process

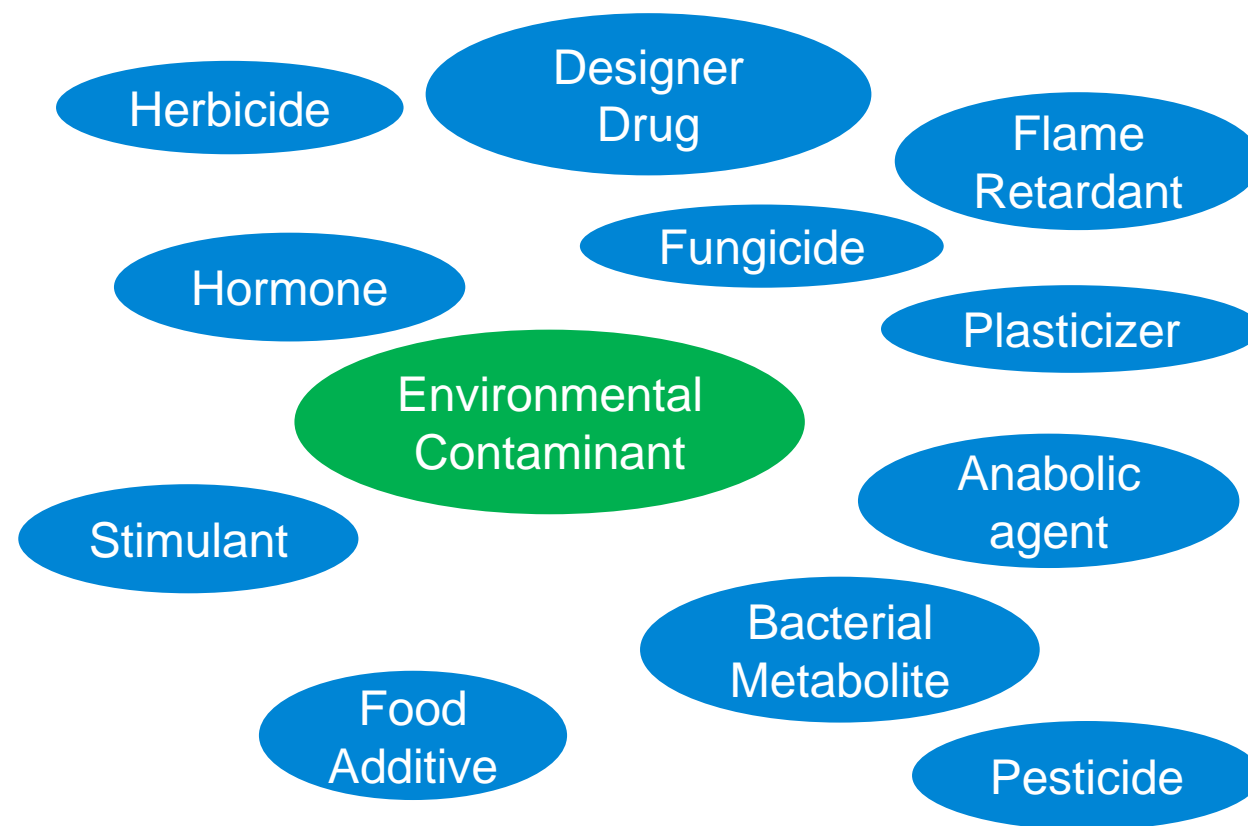
Collaborations with Leading Experts

What's Relevant – the Importance of Targeted Compound Lists

When searching for trace compounds...



...do you really look everywhere?



Curation – Building the Compound Database

Chemical List

MassHunter PCID Manager - LC/MS ESI 11/MassHunter/PCID/E.../AMRT/PCID - isopropyl...

File View ECDL Configuration Links Help

Find Compounds

Compounds Spectra Ion Mobility Import

Compounds search criteria

Must also contain

Must not contain

Search only visible columns Search all columns With spectra With CCS

Ion search mode

Include neutrals

Include anions

Include cations

Tolerances

Mass 10.0

RT 0.1

RI 10.00

Advanced Search

Compound Number: 1006 hits

Name	Formula	Mass	Retention Time	Cation	Anion	CAS	ChemSpider	METLIN	KEGG	HMDB	UNP	IUPAC	NumSpectra
2,4,6-Trimethylbenzylphosphonic acid ethy...	C18H21O3P	316.12283				84434-11-7	10710138					Ethyl (trimethylbenzyl)phosphonate	6
2,4-DCA / 2,4-Dichloroaniline	C6H5Cl2N	160.97991				554-00-7	13860817	70047				2,4-Dichloroaniline	0
2,4-Diaminoanisole	C7H10N2O	138.07931				615-05-4	11481	72941				4-Methoxy-1,3-benzenediamine	0
2,4-Dichlorobenzoic acid	C7H4Cl2O2	189.95883				50-84-0	5583					2,4-Dichlorobenzoic acid	0
2,4-Dichlorobenzylalcohol	C7H6Cl2O	175.97957				1777-82-8	14918					2,4-Dichlorobenzylalcohol	0
2,4-Diethylthioxanthone	C17H16OS	268.09219				82799-44-8	109489					2,4-Diethylthioxanthone	3
2,4-Dimethylphenol (2,4-Xylenol)	C8H10O	122.07316				105-67-9	13839123					2,4-Dimethylphenol	0
2,4-Dinitroaniline	C6H5N3O4	183.02801				97-02-9	7045	70282				2,4-Dinitroaniline	0
2,4-Dinitrophenol	C6H4N2O5	184.01202				51-28-5	1448					2,4-Dinitrophenol	3
2,4-Di-tert-butylphenol	C14H22O	206.16707				96-76-4	7037					2,4-Di-tert-butylphenol	2
2,4-DNT / 2,4-Dinitrotoluene	C7H6N2O4	182.03276				121-14-2	8150					1-Methyl-2,4-dinitrobenzene	0
2,4-Xylidine (2,4-Dimethylaniline)	C8H11N	121.08915				95-68-1	13869462					2,4-Xylidine	0

Common Name
&
Compound Information

1006 hits

	Formula	Mass	Retention Time	Cation	Anion	CAS	ChemSpider	METLIN
phosphonic acid ethy...	C18H21O3P	316.12283				84434-11-7	10710138	
2,4-DCA / 2,4-Dichloroaniline	C6H5Cl2N	160.97991				554-00-7	13860817	70047
2,4-Diaminoanisole	C7H10N2O	138.07931				615-05-4	11481	72941
2,4-Dichlorobenzoic acid	C7H4Cl2O2	189.95883				50-84-0	5583	
2,4-Dichlorobenzylalcohol	C7H6Cl2O	175.97957				1777-82-8	14918	
2,4-Diethylthioxanthone	C17H16OS	268.09219				82799-44-8	109489	
2,4-Dimethylphenol (2,4-Xylenol)	C8H10O	122.07316				105-67-9	13839123	
2,4-Dinitroaniline	C6H5N3O4	183.02801				97-02-9	7045	70282
2,4-Dinitrophenol	C6H4N2O5	184.01202				51-28-5	1448	
2,4-Di-tert-butylphenol	C14H22O	206.16707				96-76-4	7037	
2,4-DNT / 2,4-Dinitrotoluene	C7H6N2O4	182.03276				121-14-2	8150	
2,4-Xylidine (2,4-Dimethylaniline)	C8H11N	121.08915				95-68-1	13869462	

Curation – Building the Compound Database

MassHunter PCID Manager - LC/MS ESI: C:\MassHunter\PCID\ESI\J.LAMRT.PCID - isopentylcR1

File View ECDL Configuration Links Help

Find Compounds

Compounds Spectra Ion Mobility Import

Compounds search criteria

Must also contain

Must not contain

Search only visible columns Search all columns With spectra With CCS

Compound identifier: 10000 hits

Mass	Retention Time	Chelon	Apex	CAS	ChemSpider	RETUN	KEGG	HMPP	UNP	IUPAC	NumSpectra
100P	316.12203			84634-15-7	10711134					Ethyl (benzylcarbamoyl)phenylphosphine oxide	6
DN	162.57801			454-00-7	13882017	7047	14419			2,4-Dichloroaniline	0
N20	138.07931			875-05-4	11481	7241	15010			4-Methoxy-1,3-benzenedione	0

Notes:

E&L compound; Paint additive; Coating additive; Photoinitiator; Printing ink component

Synonyms: 2,4-二乙基噻唑酮, 光引发剂 DETX; Chemcure JETX; DETX; 2,4-Diethylthioxanthen-9-one; Esacure DETX; Genocure DETX; JRCure DETX; Kayacure DETX; Speedcure DETX

SWISS Ordinance (SR 817.023.21)

Deleted CAS: 153859-04-2; 162774-73-4; 676327-59-6

Notes:

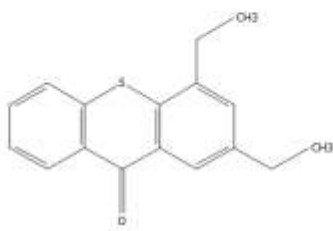
E&L compound; Paint additive; Coating additive; Photoinitiator; Printing ink component

Synonyms: 2,4-二乙基噻唑酮, 光引发剂 DETX; Chemcure JETX; DETX; 2,4-Diethylthioxanthen-9-one; Esacure DETX; Genocure DETX; JRCure DETX; Kayacure DETX; Speedcure DETX

SWISS Ordinance (SR 817.023.21)

Deleted CAS: 153859-04-2; 162774-73-4; 676327-59-6

Structure MOL Text



Collection and Curation of MS/MS Spectra

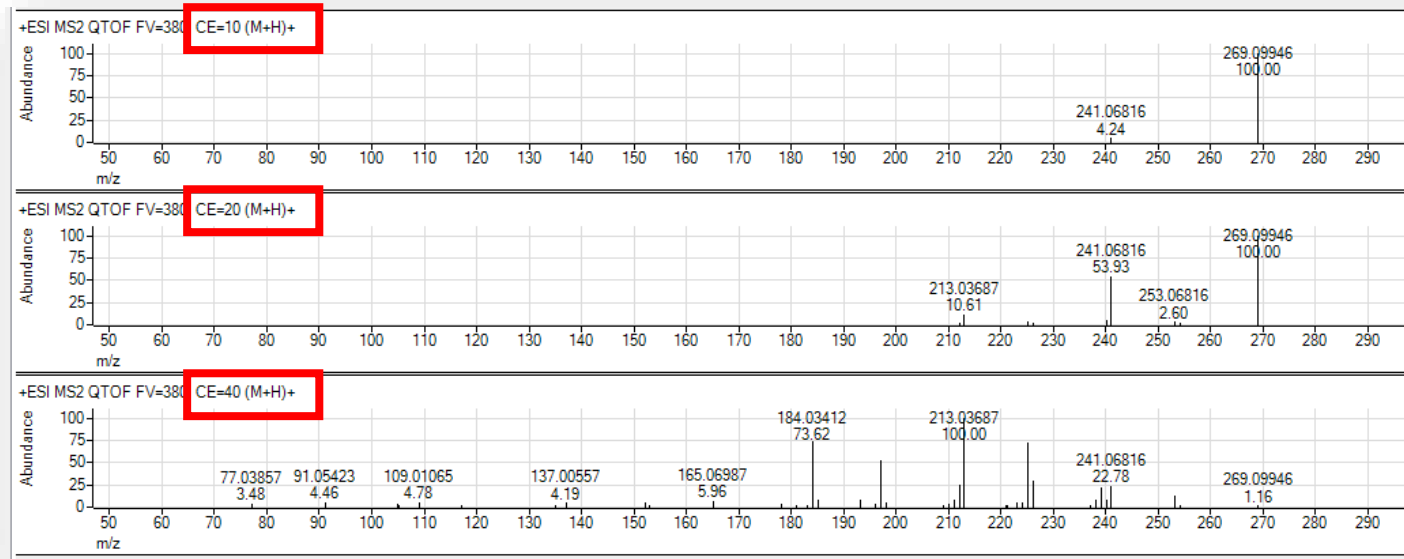
Carefully designed experiments and curation protocols

Data collection

- Flow Injection Analysis (FIA) of pure standards or purified isolates
- Inclusion of commonly analyzed adduct species
- Collected at multiple collisions energies, ion modes, and ion species

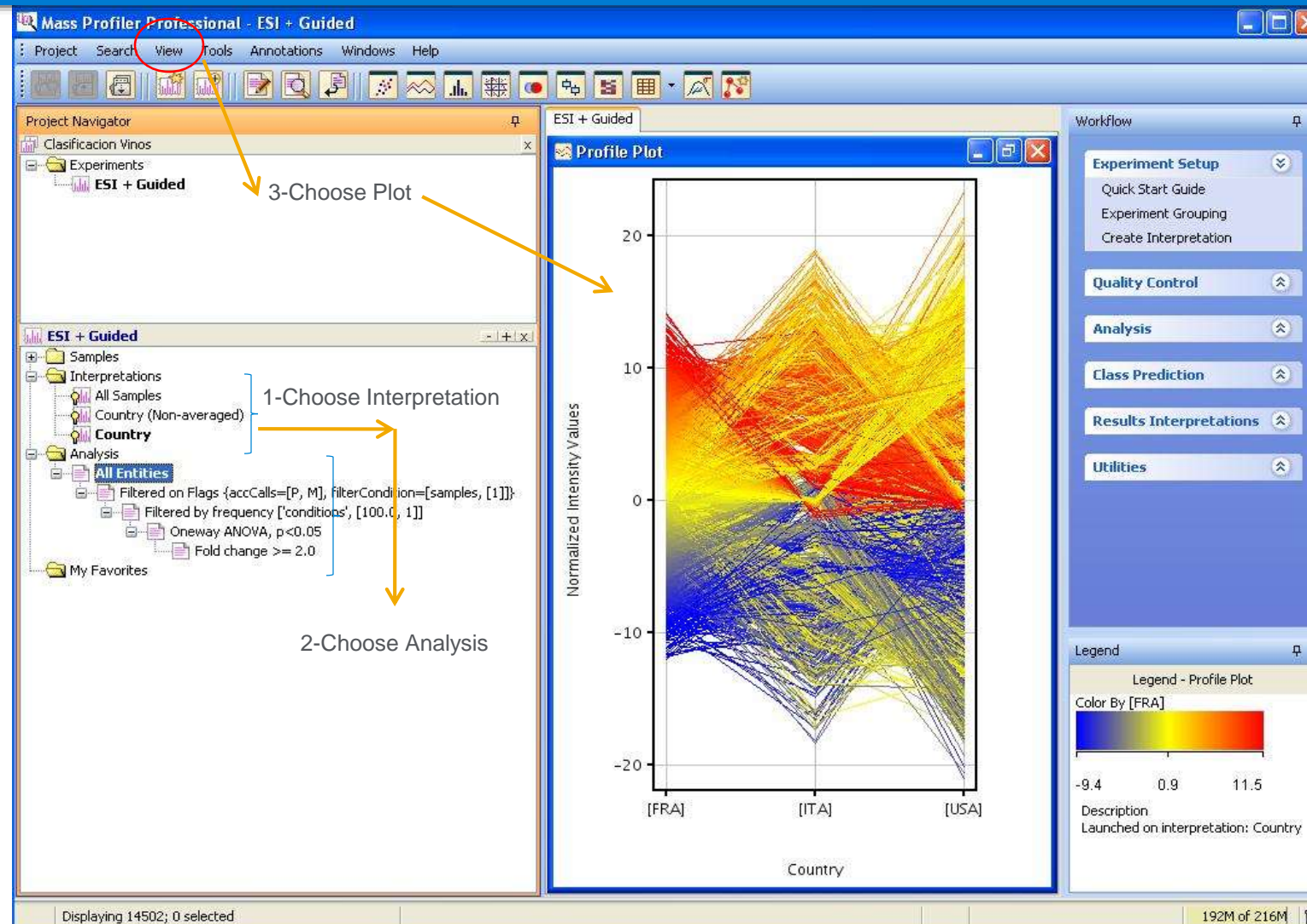
Curation

- Correction to Theoretical accurate mass
- Filtered for signal intensity and curated for spectrum noise and chemical impurities



Mass Profiler Professional

After Guided Workflow

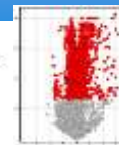


Mass Profiler Professional

Listing Entities



MassProfilerPro



ESI + Guided

- Samples
- Interpretations
 - All Samples
 - Country (Non-averaged)
 - Country**
- Analysis
 - All Entities
 - Filtered on Flags {accCalls=[P, M], filterCondition=[samples, [1]]}
 - Filtered by frequency ['conditions', [100.0, 1]]
 - Oneway ANOVA, p<0.05
 - Fold change >= 2.0**
- My Favorites

Inspect List

Entitylist Inspector

Name: Fold change >= 2.0

Notes: Created from guided workflow step :Fold change analysis
EntityList :Oneway ANOVA, p<0.05
Interpretation :Country
Experiment :ESI + Guided
Fold-Change cut-off :2.0

Creation date: Thu Oct 28 18:15:06 CEST 2010

Last modified date: Thu Oct 28 18:15:06 CEST 2010

Owner: gxuser

Technology: MassHunterQual.LCMS_UNIDENTIFIED_COMPOUNDS.ESI

Number of entities: 511

Experiments: ESI + Guided

Entities | Attributes

Compound	Fold chan...	Regulatio...	Fold chan...	Regulatio...	Annotatio...	Mass
C18 H6 N...	16.0	down	16.0	down	[C18 H6...	313.99680
C17 H31 ...	1.10	up	2.71	up	[C17 H3...	457.18051
His Val Asp	3.48	up	16.0	down	His Val As...	369.16330
Sucrose	16.0	down	16.0	down	Sucrose [...	342.11691
C11 H17 ...	5.59	up	16.0	up	[C11 H1...	259.10590
Queuine	1.68	up	16.0	up	Queuine [...	277.11691
C12 H20 ...	1.23	up	16.0	down	[C12 H2...	240.14790
C8 H15 N...	1.58	up	4.62	up	[C8 H15...	189.10069
129.3197...	2.47	up	16.0	down		129.31970

Find: Find Next Find Previous Match Case

Configure Columns

Help OK Cancel

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Exporting Entities



Export Inclusion List (Step 2 of 2)

Filtering Parameters for Inclusion List
Filtering Parameters for Inclusion List

Inclusion list creation

Retention time window: \pm % + min

☐ Limit number of precursor ions per compound to ion(s)

☐ Minimum ion abundance counts

Exported m/z value

☒ Export monoisotopic m/z
☐ Export highest abundance m/z

Positive ions

☒ +H
☐ +Na
☐ +K
☐ +NH4

Negative ions

☒ -H
☐ +Cl
☐ +Br
☐ +HCOO
☐ +CH3COO
☐ +CF3COO

Charge state preference

☐ Prefer highest abundance charge state(s)
☒ Specify charge state preference order

Inactive

2
3
>3
Unknown

Active

1

Workflow

Quality Control

- Quality Control on Samples
- Filter by Frequency
- Filter on Sample Variability
- Filter by Flags
- Filter by Abundance
- Filter by Annotations

Analysis

- Statistical Analysis
- Filter on Volcano Plot
- Fold Change
- Clustering
- Find Similar Entities
- Principal Component An...
- Find Minimal Masses

Class Prediction

- Build Prediction Model
- Run Prediction

Results Interpretat...

- Pathway analysis
- Find Similar Entity Lists
- Find Significant Pathways
- Extract Relations via NLP
- Export for Recursion
- IDBrowser Identification
- Export for Identification
- Export Inclusion List**
- Import Annotations

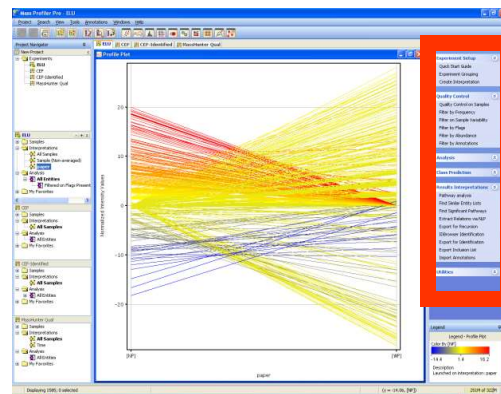
Inclusion list for Target MS/MS on QTOF

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MPP Advanced Workflow



- Use of Experiment Browser
- User defined Interpretations
- User defined Analysis
- Free use of Advanced tools & Utilities



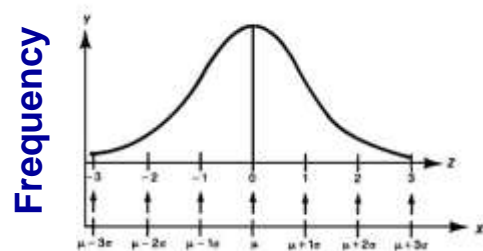
Experiment Browser is designed following usual workflow

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Analysis- Parametric Test



Parametric test



Expression of Entity X

Bell shaped distribution
symmetrical about the
mean

MPP assume that you
have sampled from
populations where
expression of Entity X
follows a normal
distribution

Non- Parametric test

Does not assume normal distribution
Does not assume equal variances
Ranks the order of normalized data across
conditions for analyses

With small number of replicates, non-
parametric tests have less statistical power
than the corresponding parametric tests

P-value Calculation Methods

Asymptotic Method

Permutation Method

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Multiple Testing Correction . P-value Cut-off



What type of error you are more comfortable with

Type I Error (**false positive**):
Calling entities differentially expressed when they really are not

Type II Error (**false negative**):
Not calling entities differentially expressed when they really are

Choice of cut-off trades off between type I and type II errors

5HT1c	0.002364
NFL	0.002649
NMDA2C	0.017181
aFGF	0.027544
GRa3	0.041179
actin	0.045342
nAChRd	0.046372
EGFR	0.0468
bFGF	0.087842
5HT2	0.106591
Brm	0.137903
SOD	0.147089
mGluR2	0.174708
IGF.I	0.223558
SC2	0.274809
trkC	0.288776
mGluR1	0.313801
SC6	0.343059
CNTFR	0.354717
pre-GAD67	0.366955
BDNF.rat	0.417615
GDNF	0.421125
IP3R2	0.421308
L1	0.443525
GAD67	0.462416
H2AZ	0.561907
IP3R1	0.573717
MK2	0.630177
CCO2	0.640797
mGluR3	0.654866
PDGFa	0.659352
IGF.II	0.683554
CNTF	0.690512
nAChRe	0.701041
IGFR2	0.728141
GAP43	0.732078
ODC	0.745628
SC1	0.74575
NT3	0.78811
PTN	0.795557
trk	0.82403
mGluR5	0.8305
cjun	0.839991
Ins2	0.841945
MAP2	0.851833
nenb	0.879299
GRb1	0.888485
TCP	0.892361
GRb2	0.900601
S100beta	0.930265

- p-value=0.05

Truly differentially expressed

Unchanged between populations

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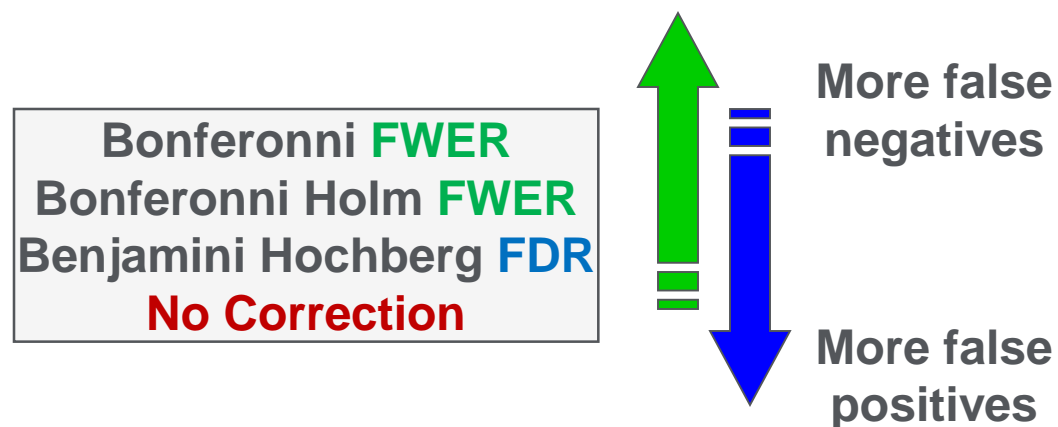
Multiple Testing Correction



1 entity = 1 individual test

- 10000 entities = 10000 tests
- With p -value = 0.05 and analyzing 10000 entities: 500 entities (0.05×10000) likely to appear significant by chance
 - Number of false positives increases proportionally to number of tests being performed

Performing multiple testing correction further decreases the number of false positives



- > **Family-wise Error Rate (FWER)** - Very conservative and does not tolerate any false positives
- > **False Discovery Rate (FDR)** - False positives a percentage of called entities
- > **None** - False positives a percentage of entities being tested

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Multiple Testing Correction . Post Hoc Tests



Decreases rate of false positives, only available in MPP for one-way ANOVA tests

MPP test options:

Tukey's Honestly Significant Difference (HSD) test

Student-Newman-Keuls (SNK) test

Test Description

SNK Post Hoc test. Entities found to be differentially expressed are represented in the blue boxes, while entities found not to be differentially expressed are represented in the orange boxes. To save entities of interest as a new file, select one or multiple boxes and click on the "Union" or "Intersection" button.

Result Summary

Group Name	[Pinot Noir]	[Cab Sav]	[Merlot]
[Pinot Noir]	39	38	12
[Cab Sav]	1	39	25
[Merlot]	27	14	39

Union Intersection

Pink boxes contain entities whose expression does *not* differ significantly between the two conditions

Blue boxes contain entities whose expression differs significantly between the two conditions

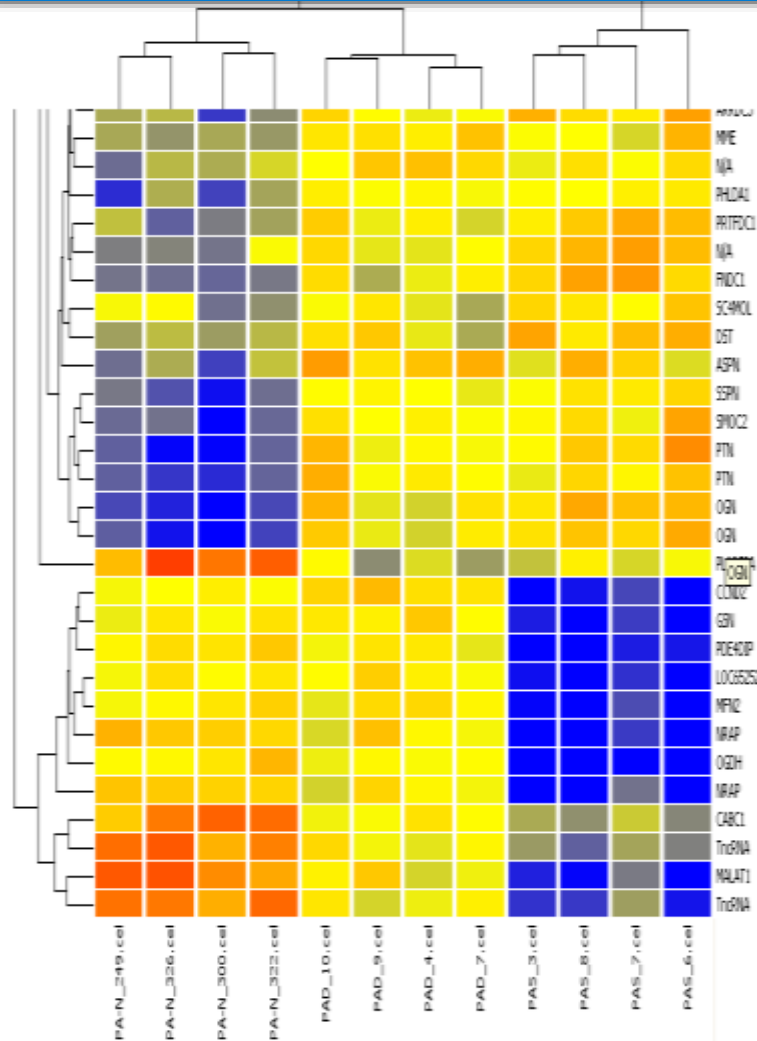


Clustering is an *unsupervised* method for identifying patterns within datasets.

- 1) What do you want to cluster together?
- 2) What similarity metric to select?
- 3) What clustering algorithm will be applied?

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Hierarchical CLUSTERING



Overview:

- Hierarchical clustering algorithm can be used to group entities and conditions based on the **similarity of their expression profiles**
- Performing Hierarchical clustering on both entities and conditions result in a 2-dimensional dendrogram
- **Most similar profiles are joined together** into a group and groups are further joined in a tree structure until all data forms a single group

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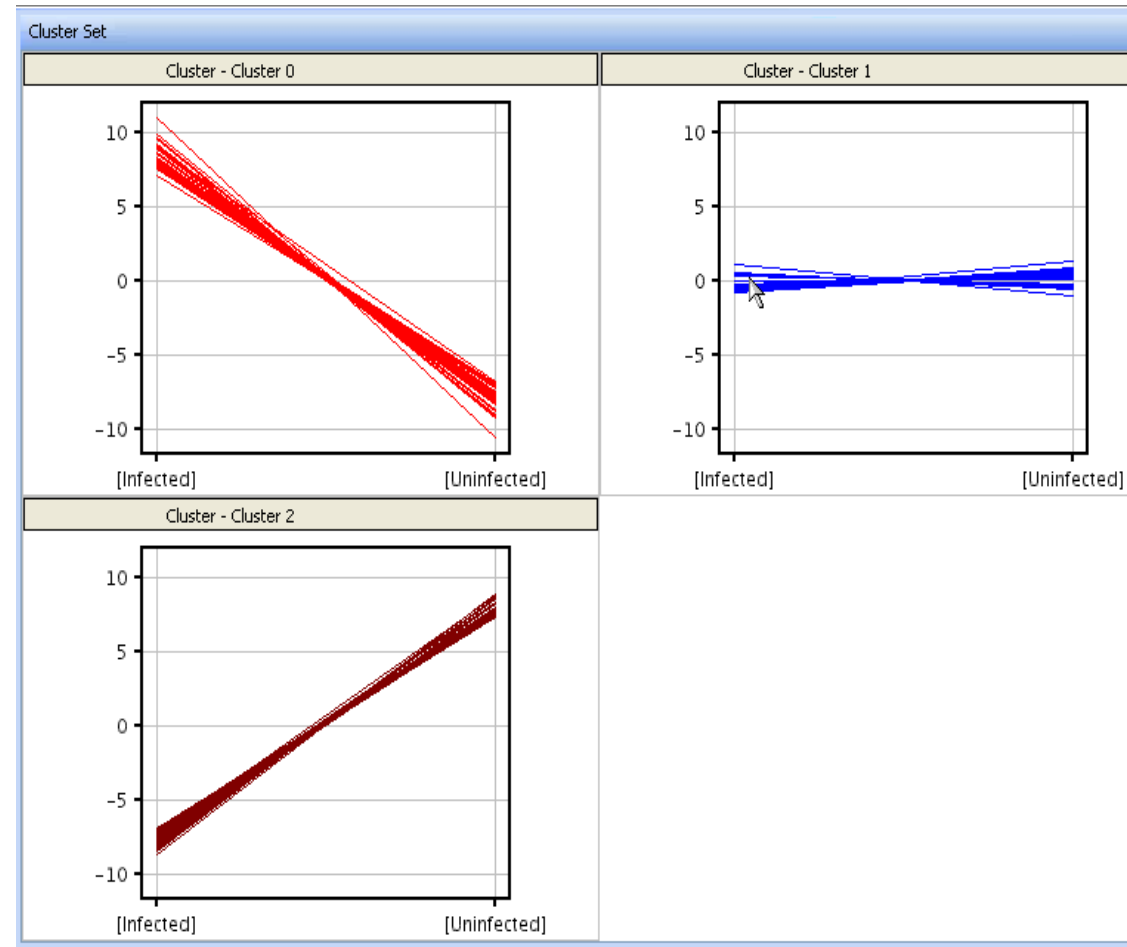
K-means CLUSTERING



Groups of Compounds that behave similarly

Overview:

- User chooses K, the number of clusters to partition selected entities or conditions into
- Algorithm attempts to minimize intra-cluster variability and maximize inter-cluster variability



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Pathways Analysis



Export Inclusion List (Step 2 of 2)

Filtering Parameters for Inclusion List

Filtering Parameters for Inclusion List

Inclusion list creation

Retention time window: \pm % + min

☐ Limit number of precursor ions per compound to ion(s)

☐ Minimum ion abundance counts

Exported m/z value

☒ Export monoisotopic m/z

☐ Export highest abundance m/z

Positive ions

☒ +H

☐ +Na

☐ +K

☐ +NH4

Negative ions

☒ -H

☐ +Cl

☐ +Br

☐ +HCOO

☐ +CH3COO

☐ +CF3COO

Charge state preference

☐ Prefer highest abundance charge state(s)

☒ Specify charge state preference order

Inactive

2

3

>3

Unknown

Active

1

Workflow

Quality Control

Quality Control on Samples

Filter by Frequency

Filter on Sample Variability

Filter by Flags

Filter by Abundance

Filter by Annotations

Analysis

Statistical Analysis

Filter on Volcano Plot

Fold Change

Clustering

Find Similar Entities

Principal Component An...

Find Minimal Masses

Class Prediction

Build Prediction Model

Run Prediction

Results Interpretation

Pathway analysis

Find Similar Entity Lists

Find Significant Pathways

Extract Relations via NLP

Export for Recursion

IDBrowser Identification

Export for Identification

Export Inclusion List

Import Annotations

Inclusion list for Target MS/MS on QTOF

- *Enfoques y estrategias analíticas que nos permiten las últimas tecnologías de LC Mass Spectrometry de alta resolución. **UHPLC QTOF technical details***
- **Agilent proposal Workflows in different scenarios.** *Diferentes estrategias inherentes a investigación en el ámbito de Ciencias de la Vida, así como para el perfilado de Alimentos, Materiales, Procesos...*
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Pathway Analysis

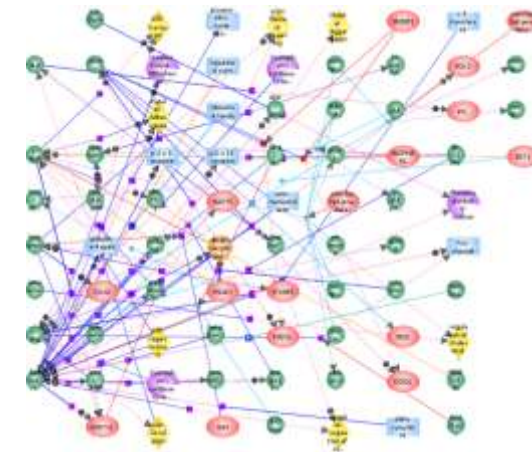


Pathway Analysis allows for Finding Biological Relevance of differential entities

Two types of pathway analysis in MPP:

1. Find Significant Pathways:

Is there a significant enrichment of my entities of interest in a particular pathway?



2. Pathway Analysis:

How do my entities of interest interact in a biochemical network?

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Pathway Analysis



BioPAX (Biological Pathway Exchange) is a standard pathway data exchange format. Pathways in the biopax format will have the extension .owl

MPP users can import pathway data standard pathway sites in BioPAX level 1 or 2 format
www.pathguide.org is a useful website which list repositories of pathways

The screenshot shows the Pathguide website, titled "the pathway resource list". It features a sidebar with navigation links and a main content area with a table of resources.

Navigation Links:

- Proteins/Proteins
- Interactions
- Metabolic Pathways
- Signaling Pathways
- Pathway Diagrams
- Transcription
- Genome / Genes
- Regulatory Networks
- Protein Complexes
- Interactions
- Genetic Interaction
- Protein Maps
- Protein Sequences
- Protein
- Other

Search Filters:

- Organism: [All]
- Availability: [All]
- Standards: [All]
- [Reset] [Search]

Complete Listing of All Pathguide Resources

Pathguide contains information about 200 biological pathway resources. Click on a link to go to the resource home page or Details for a description page. Databases that are new and those supporting BioPAX, CeML, PSI-MI or SBML standards are respectively indicated.

If you know of a pathway resource that is not listed here, or have other questions or comments, please send us an e-mail.

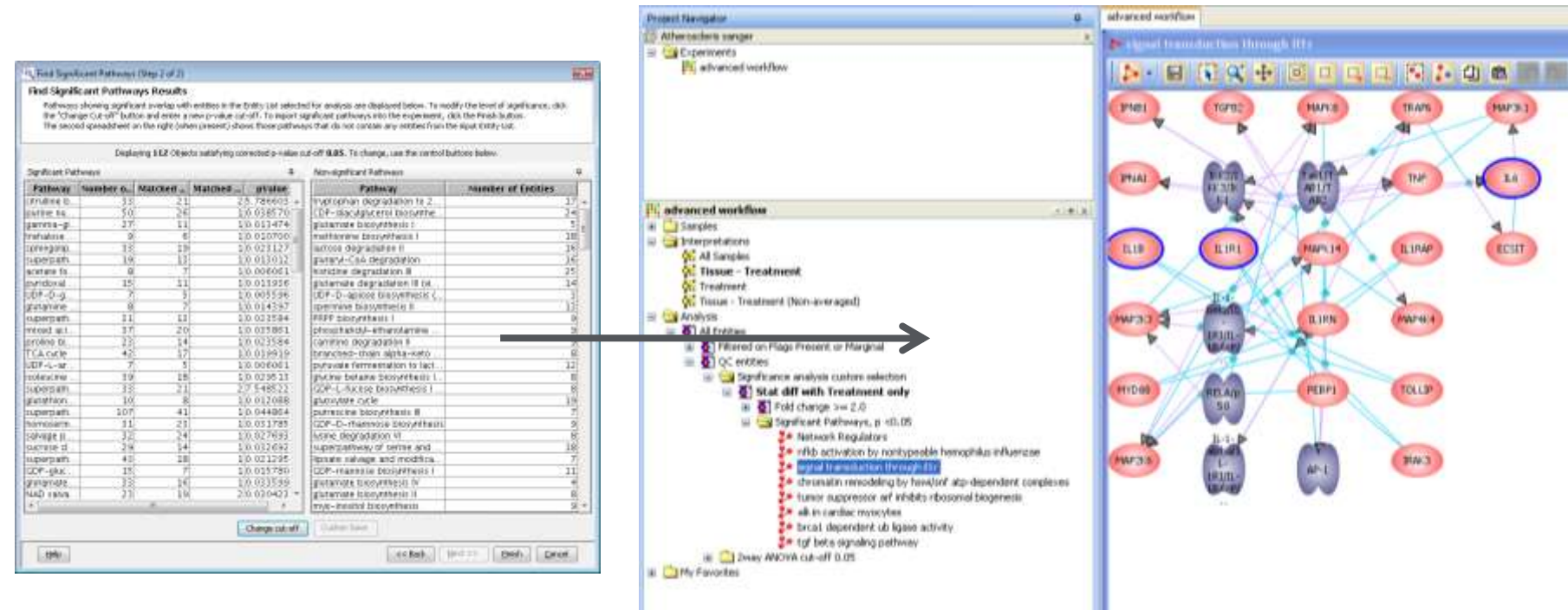
Protein-Protein Interactions

Database Name	Order	Alphabetically	Accession	Standards
3DIP - 3D Interacting Domains	Checkable	1/1/00		
ABCD - Archaeal and Bacterial ABC transporter database	Checkable	1/1/00		
ACPS - Alliance for Cellular Signaling Molecule Pages Database	Checkable	1/1/00		
AFPP - Functional Associations of Proteins in Complete Genomes	Checkable	2/00		
AMAP - Protein Function and Biochemical Pathways Project	Checkable	1/1/00		
ASAP - Amino Acid Signaling Pathway Database	Checkable	1/1/00		
ASPD - Artificial Selected Proteins/Peptides Database	Checkable	1/1/00		
BID - Binding Interface Database	Checkable	2/00		
BIRD - Biomolecular Interaction Network Database	Checkable	1/1/00		
BIGORIN - General Repository for Interaction Databases	Checkable	1/1/00		
BRIT - Biomolecular Relations in Information Transmission and Expression	Checkable	1/1/00		
CAT - Catalog of the Hippocampal CAT neuron	Checkable	1/1/00		
Cancer Cell Map - The Cancer Cell Map	Checkable	1/1/00		
CellSign - CellSign	Checkable	1/1/00		
CSF - Cytokine Signaling Pathway Database	Checkable	2/00		
CTD - Cytokine Target Database	Checkable	1/1/00		
CCID - Database of Covalent Interactions and Bindings	Checkable	1/1/00		
DIM - Domain Interaction Map	Checkable	1/1/00		
DIP - Database of Interacting Proteins	Checkable	1/1/00		
DOMIN - Domain Protein Interactions Database	Checkable	1/1/00		
DoDB - Database of oligomerization domains from lambda experiments	Checkable	1/1/00		
DoP - Database of Protein	Checkable	2/00		
DPC - Database of Protein Crosslinks	Checkable	1/1/00		
DPP - Drosophila Protein Interaction Map Database	Checkable	1/1/00		
DSM - Dynamic Signaling Maps	Checkable	2/00		
FMDB - Functional Molecular Immunology	Checkable	2/00		
FusionDB - Proteins Gene Fusion Events	Checkable	1/1/00		

Databases for any organism of interest can be created using the Biopax files- Rice, zebra fish, chimpanzee, dog

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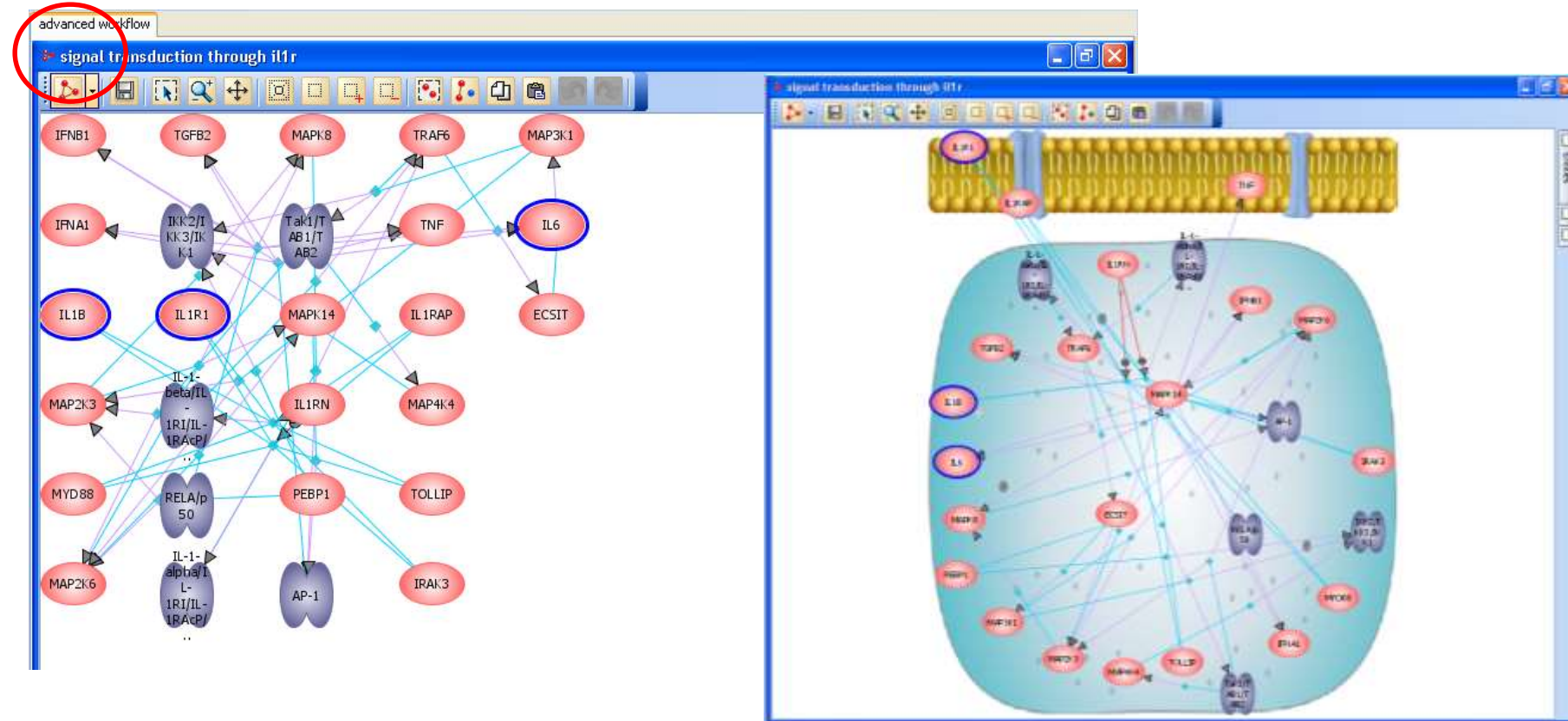
Pathway Analysis – Find Significant Pathway



Is there a significant enrichment of my entities of interest in a particular pathway?

Analysis will be performed on every pathway that has been imported into MPP for the matching organism and every pathway created in MPP

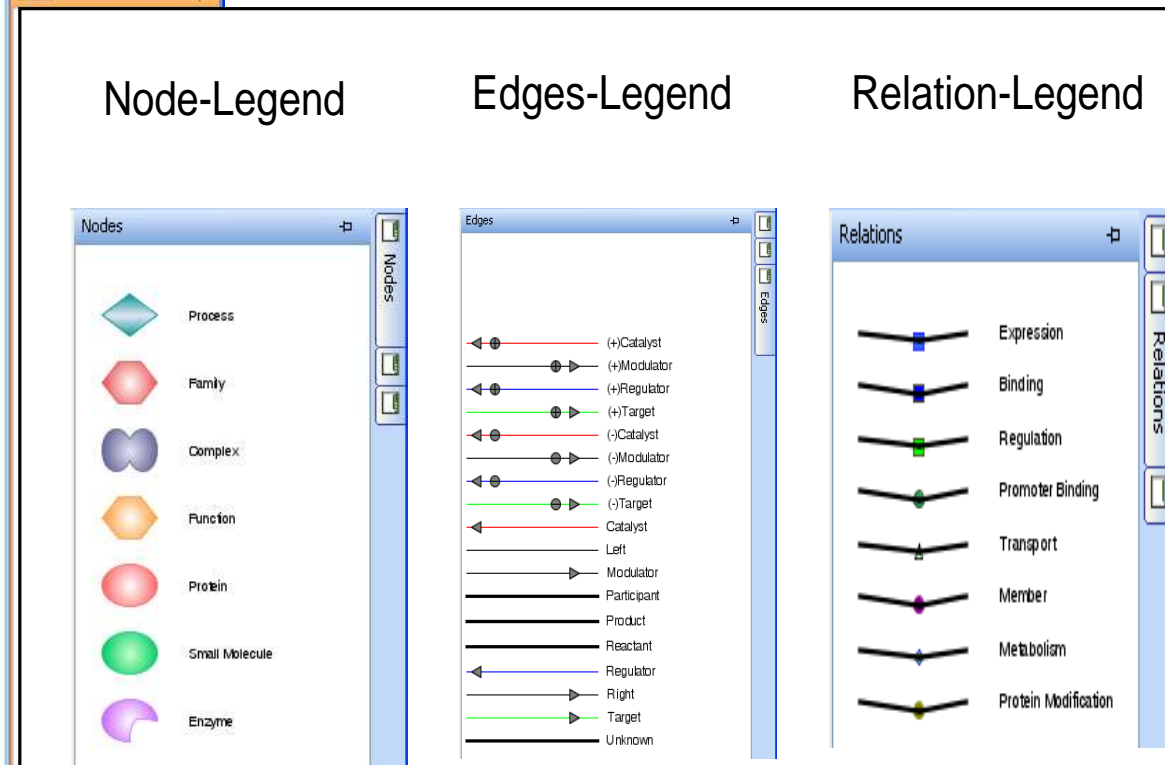
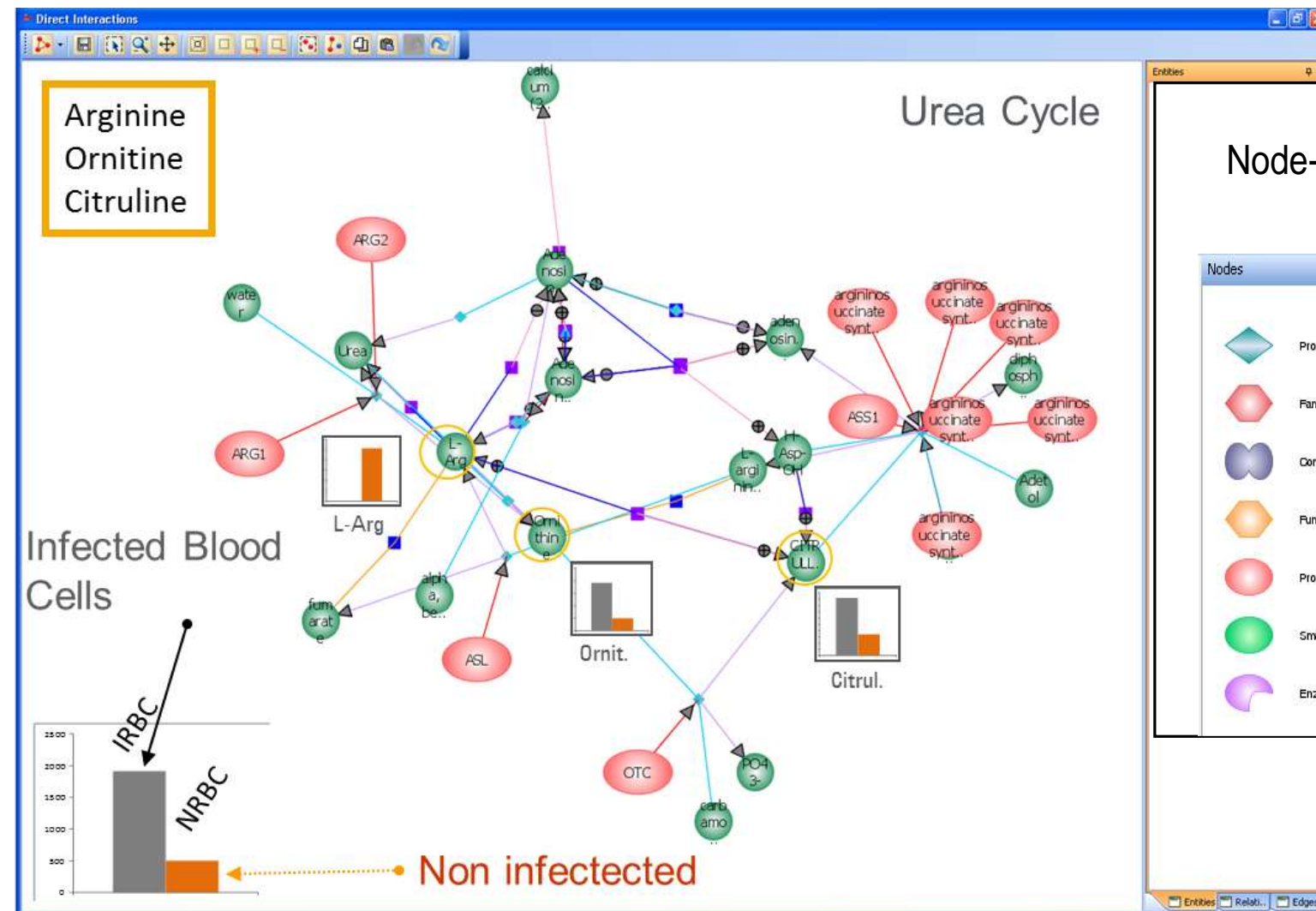
Mass Profiler Professional Pathway Viewer



Layout of entities can be changed – 6 options including cellular view

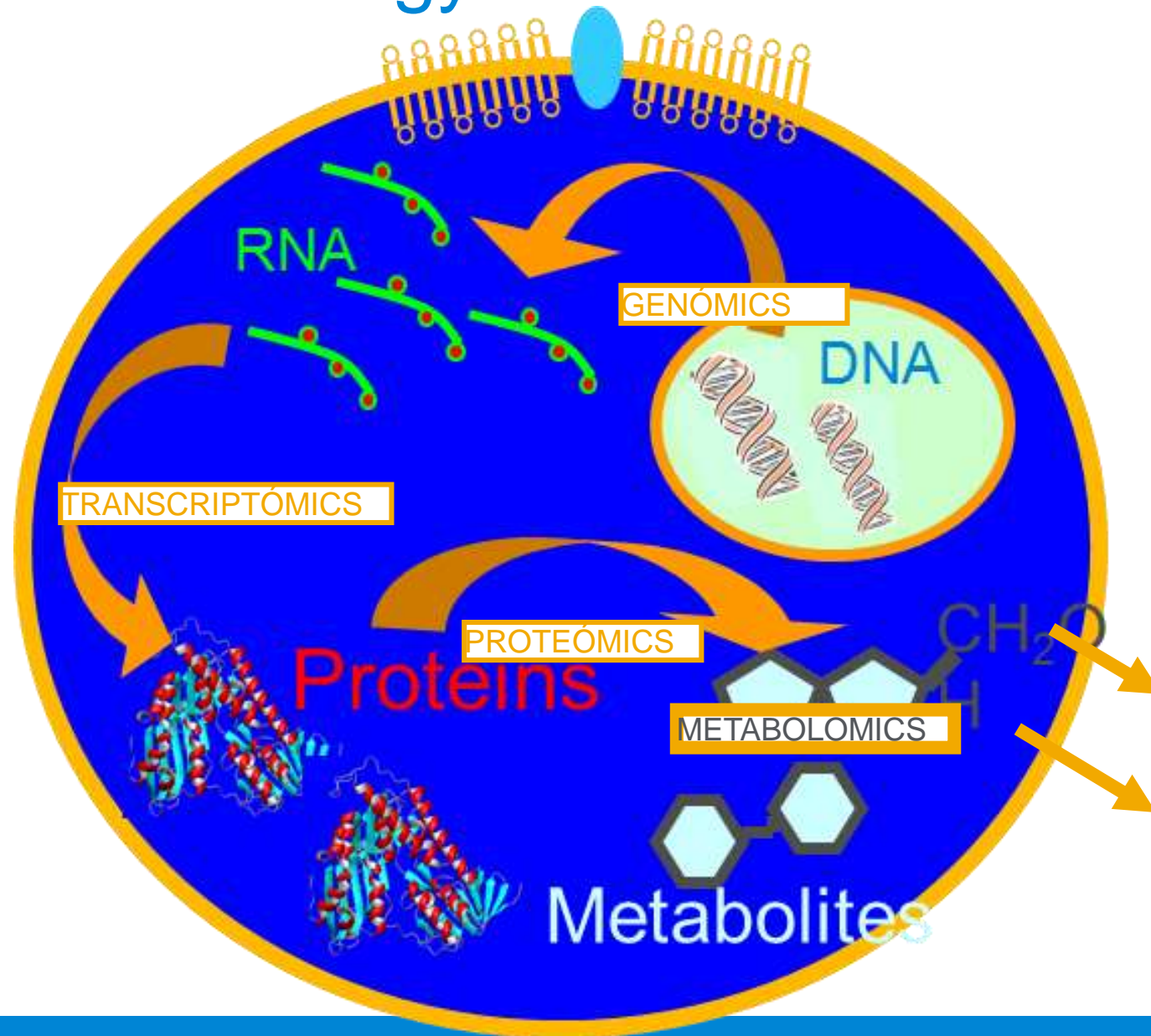
Mass Profiler Professional

Pathway Viewer

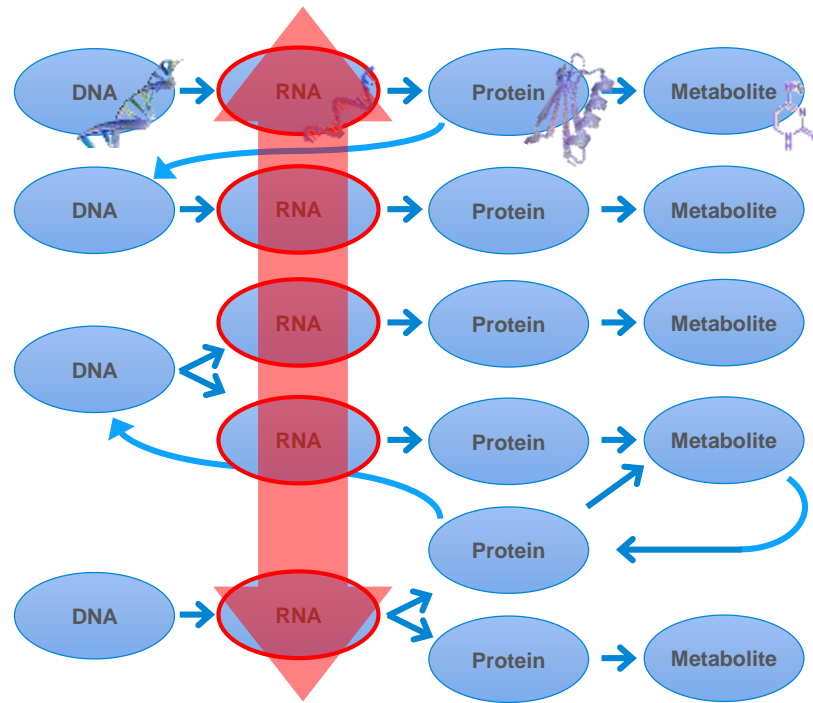


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Classical Biology Process

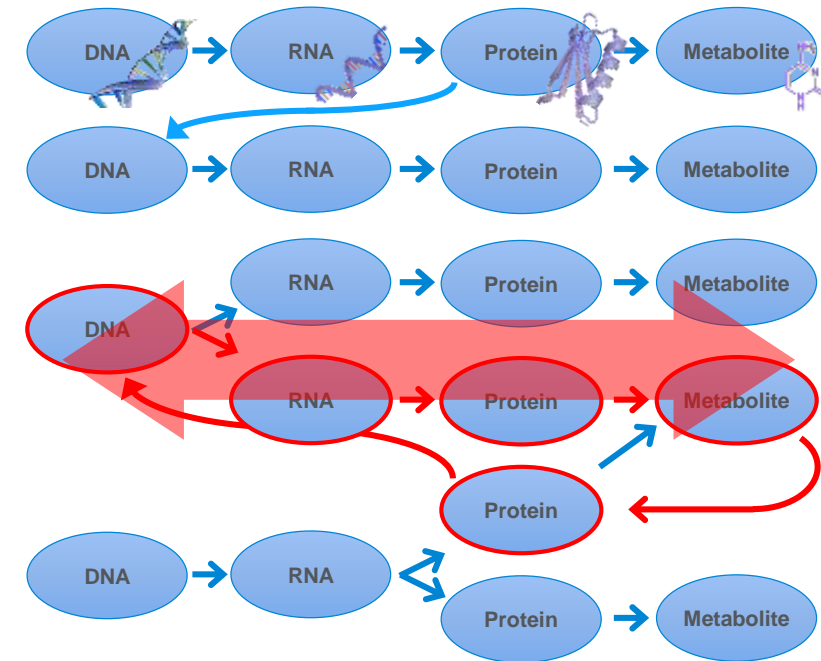


The Biology Challenge



“-Omics”

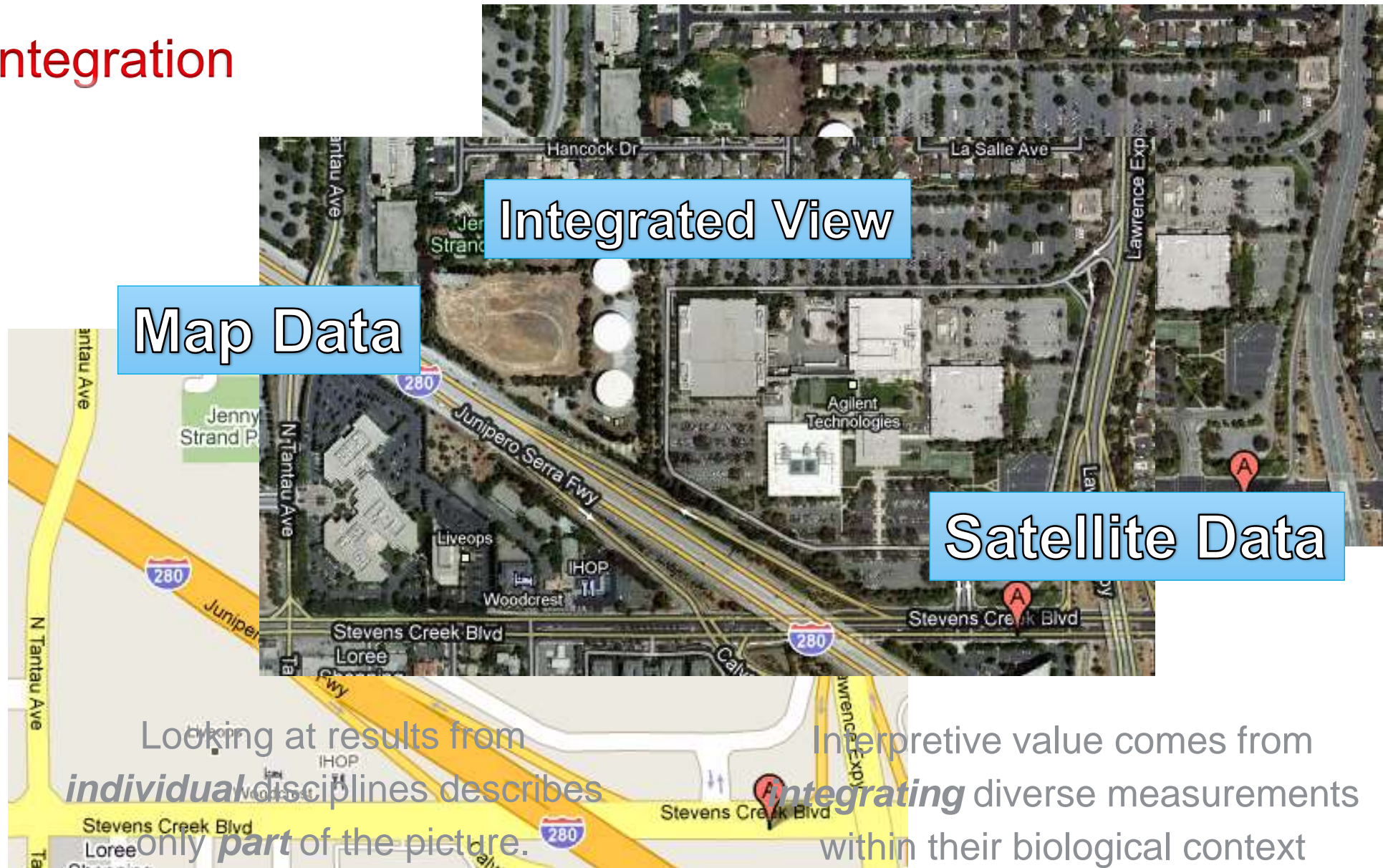
“Classical Biology” approach



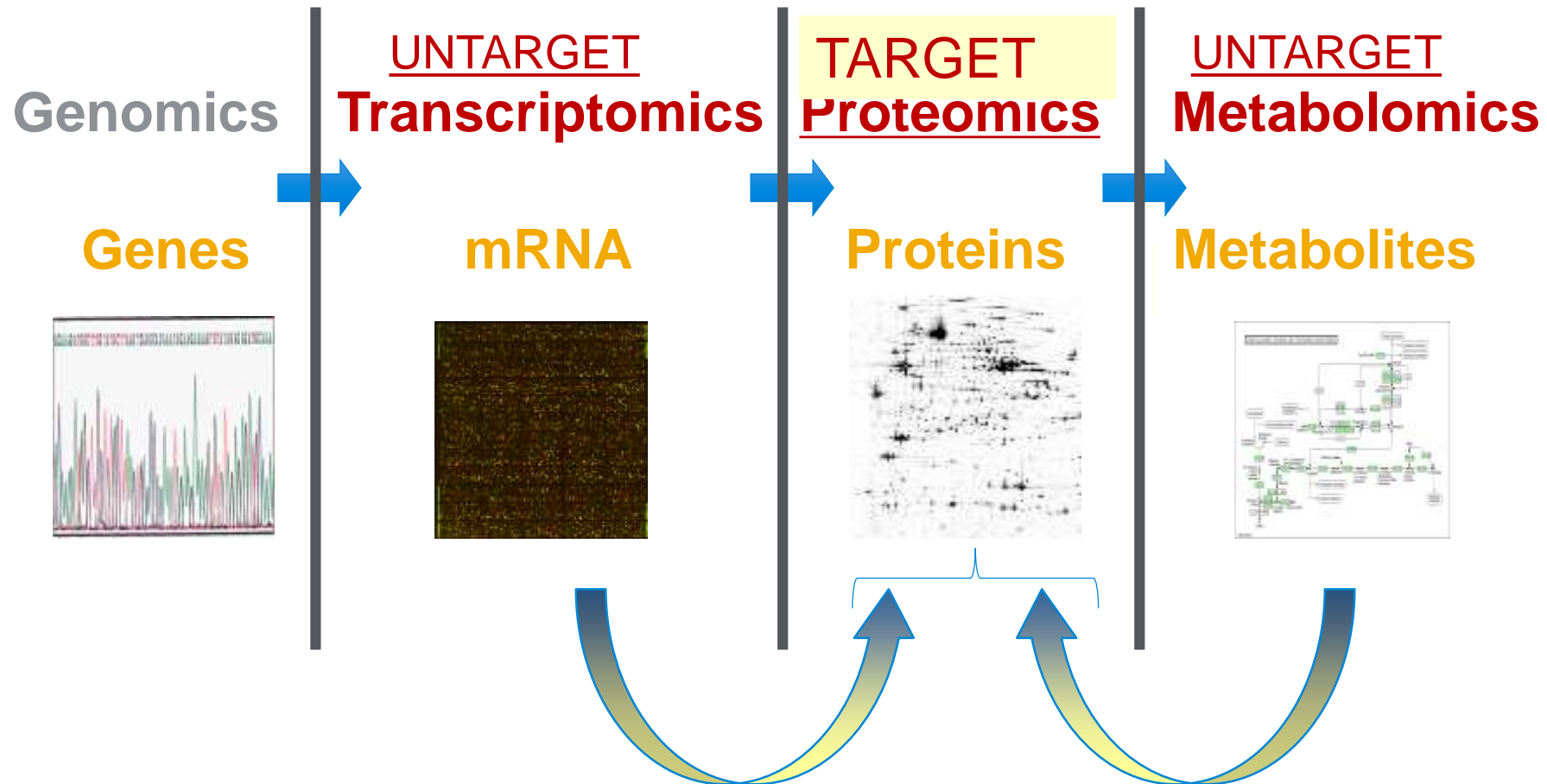
Biological Processes

“Integrated Biology” approach

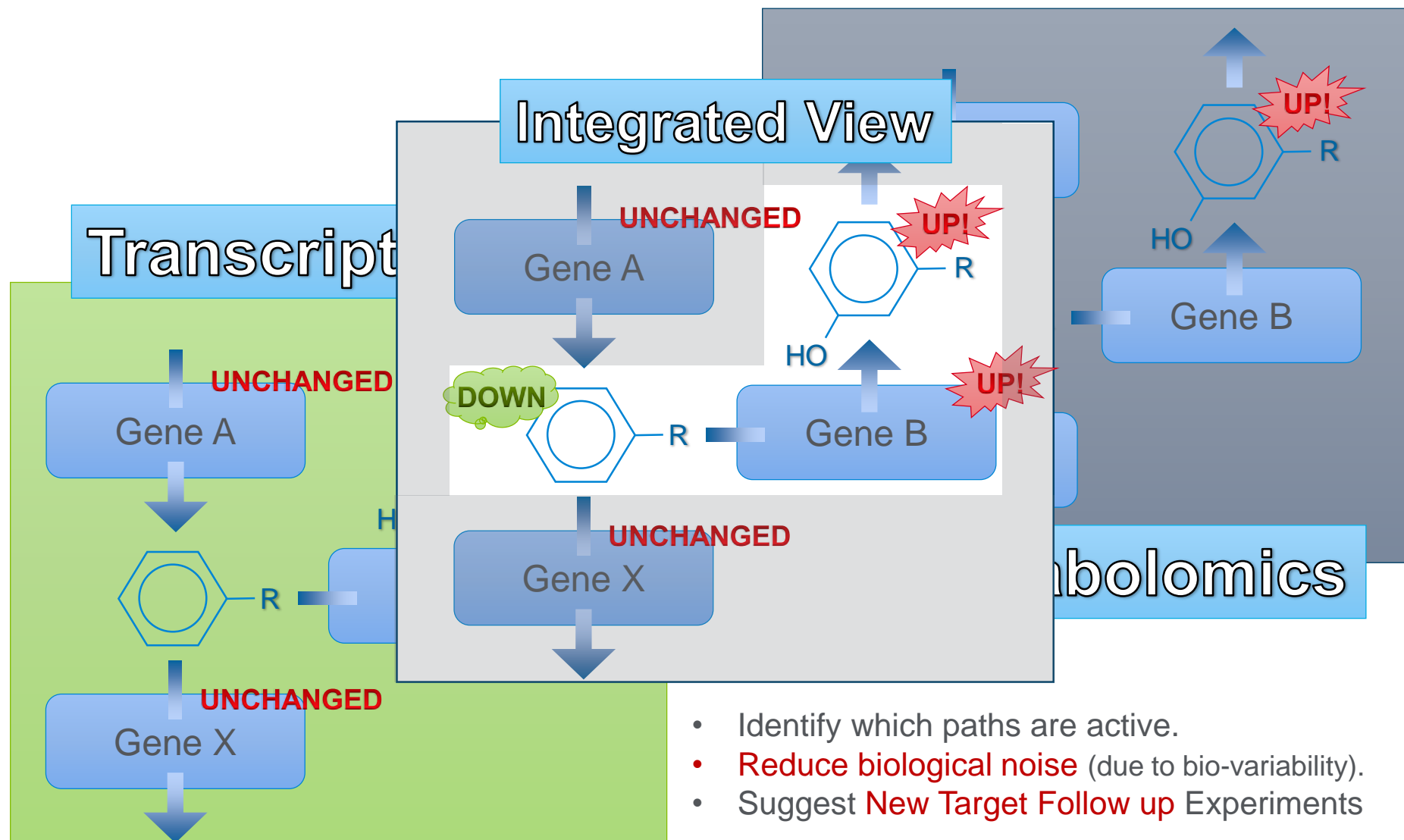
IBS Data Integration



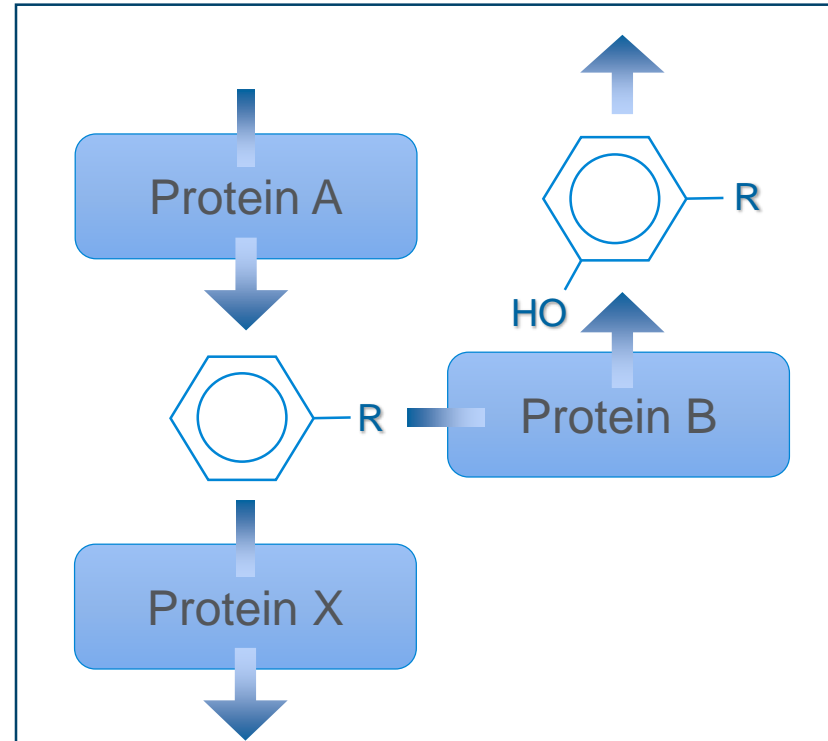
Workflow Strategy to Address Integrated Biology Studies



Multi-omics approaches helps a lot to **reduce** “biological samples noise” and **IMPROVES RELIABILITY OF FINDINGS.**



Integrating Biological Analysis Using Pathways



Sources

- WikiPathways
- BioCyc/MetaCyc
- Generalized BioPax
- KEGG



Platforms

- GeneSpring
- Mass Profiler Professional
- Pathway Architect



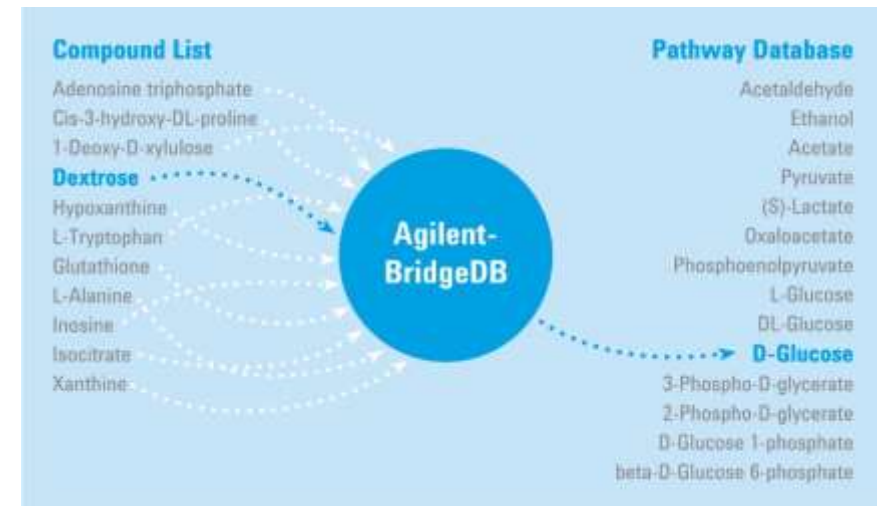
Agilent-BridgeDB

Resolving the Mapping Problem Between Databases

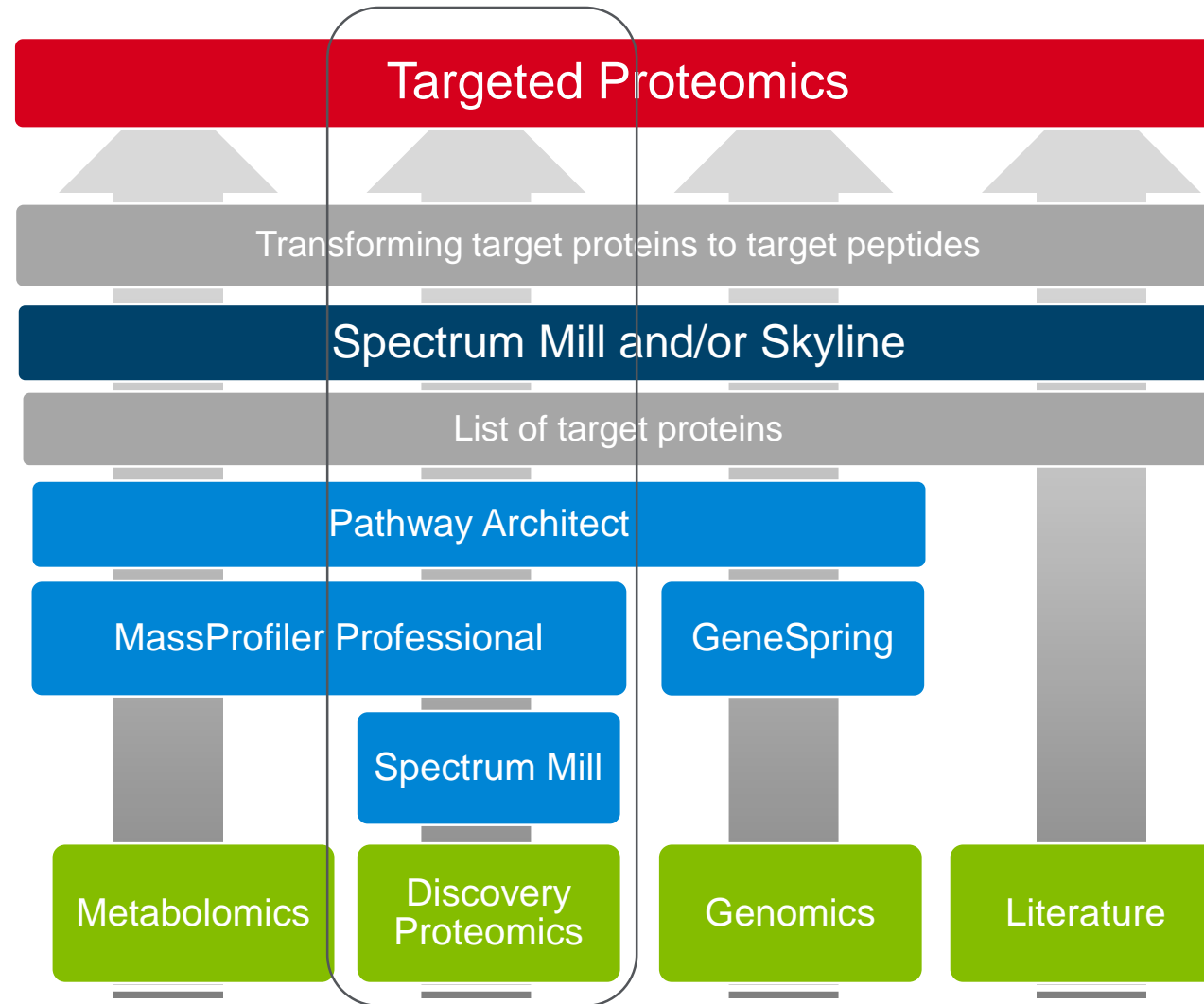
Solves the translation problem of identification names to pathway database names

Automatic – does not require user intervention

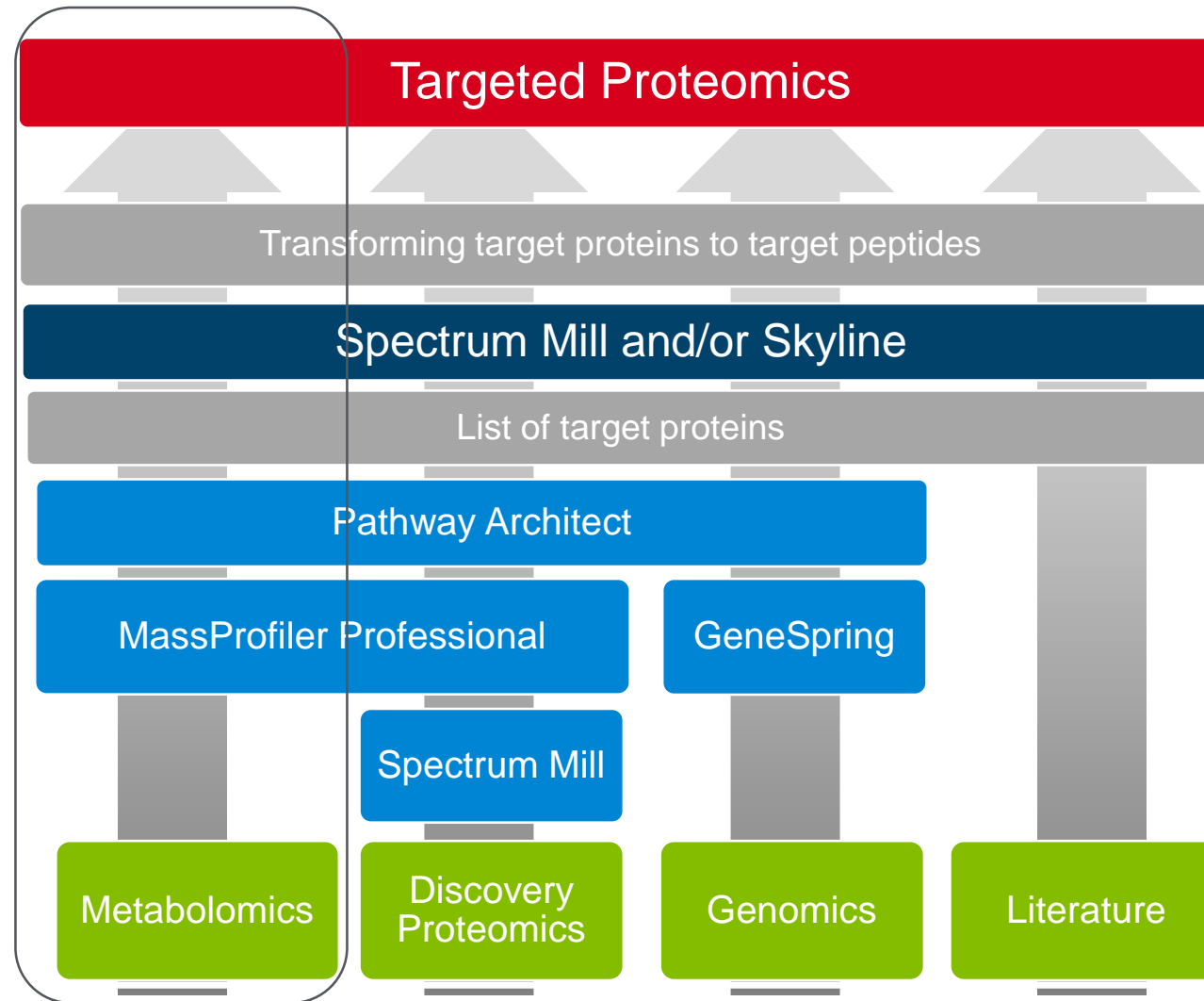
- Metabolites Identifiers
 - KEGG, MetaCyc, PubChem, LMP, HMDB, ChEBI, and CAS
- Proteins Identifiers:
 - Swiss-Prot, UniProt, and UniProt/TrEMBL
- Genes Identifiers:
 - Entrez Gene, GenBank, Ensembl, EC Number, RefSeq, UniGene, HUGO, HGNC, and EMBL



Biology-directed Workflows to Targeted Proteomics



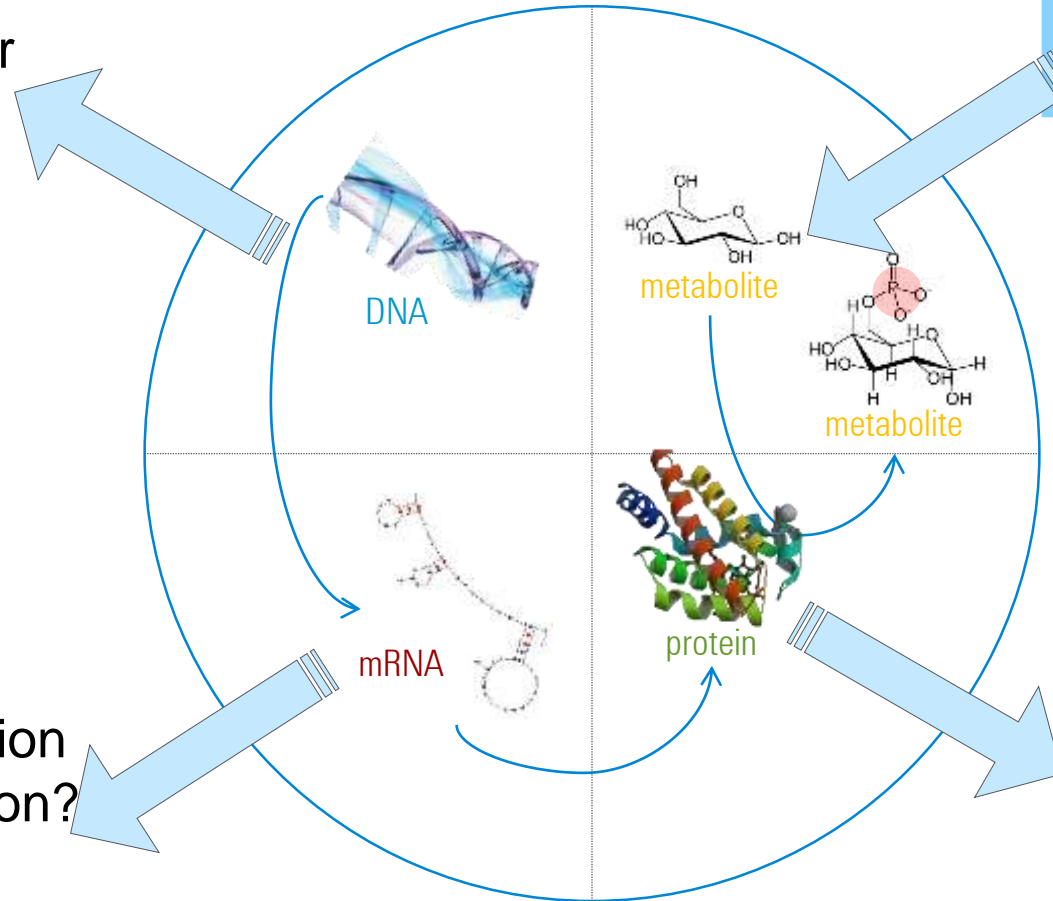
Biology-directed Workflows to Targeted Proteomics



What is the Next Experiment?

- Missense or nonsense mutation?

- Transcriptional regulation?
- Splice variants?



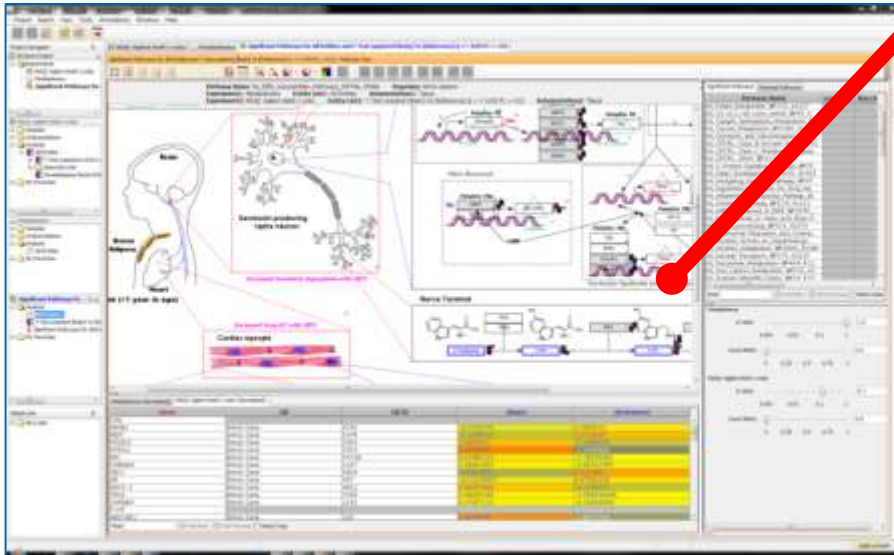
From a metabolic pathway I've identified...

- Differential Protein abundances
- Targeted analyses

Pathway Directed Experiment Creation

Propose new experiments based on pathway analysis

- Re-examine acquired untargeted metabolomics data based on pathway analysis
- Design new experiments (metabolite, protein or genes) based on pathway results interpretation



The screenshot displays the PCDL (Pathway Directed Experiment Design) software interface. It is divided into several sections with yellow headers:

- Build custom metabolite database**: Includes fields for Name, SMILES, CAS, RT, and Formula, along with buttons for Add New, Save to File, Update Database, and Delete Database.
- Custom microarray or NGS design**: Includes fields for Sequencing Platform, Sequencing Technology, and Sequencing Protocol, along with buttons for Design Options, Design Parameters, and Design Results.
- Targeted MS/MS**: Includes fields for Digest Parameters (Digest: Trypsin, Maximum # missed cleavages: 0) and Product Ion Parameters (Show Product Ion Masses). It also has a section for Criteria for Excluding Peptides with checkboxes for Max. # basic residues (RHK), Peptide MH+ Min., Max., AA Composition Filtering, and AAs Required.
- Spectrum Mill**: Includes a section for Protein Position Filtering with checkboxes for Has nearby cleavage site within 3 residues, Contains peptide N-terminal Gln to pyroGlu, Contains protein N-terminus Acetyltable, Contains consensus N-linked glycosylation site, and Contains no variable modification.

Pathways to PCDL: Create custom databases

Select an Organism ➤ Select Pathway(s) ➤ Create new subset KEGG
.cdb

Pathways to PCDL

Settings Tools Help

Pathway Data
Source: KEGG
Organism/Database: All Organisms
Add/Remove

Selection Mode
☒ Pathway Names
☐ Follow Pathway Members
☐ Reaction Partners

Search Text: Clear
Select Highlighted Select All

Prefer Compound Names from
☒ KEGG
☐ METLIN

Create PCDL View Unres

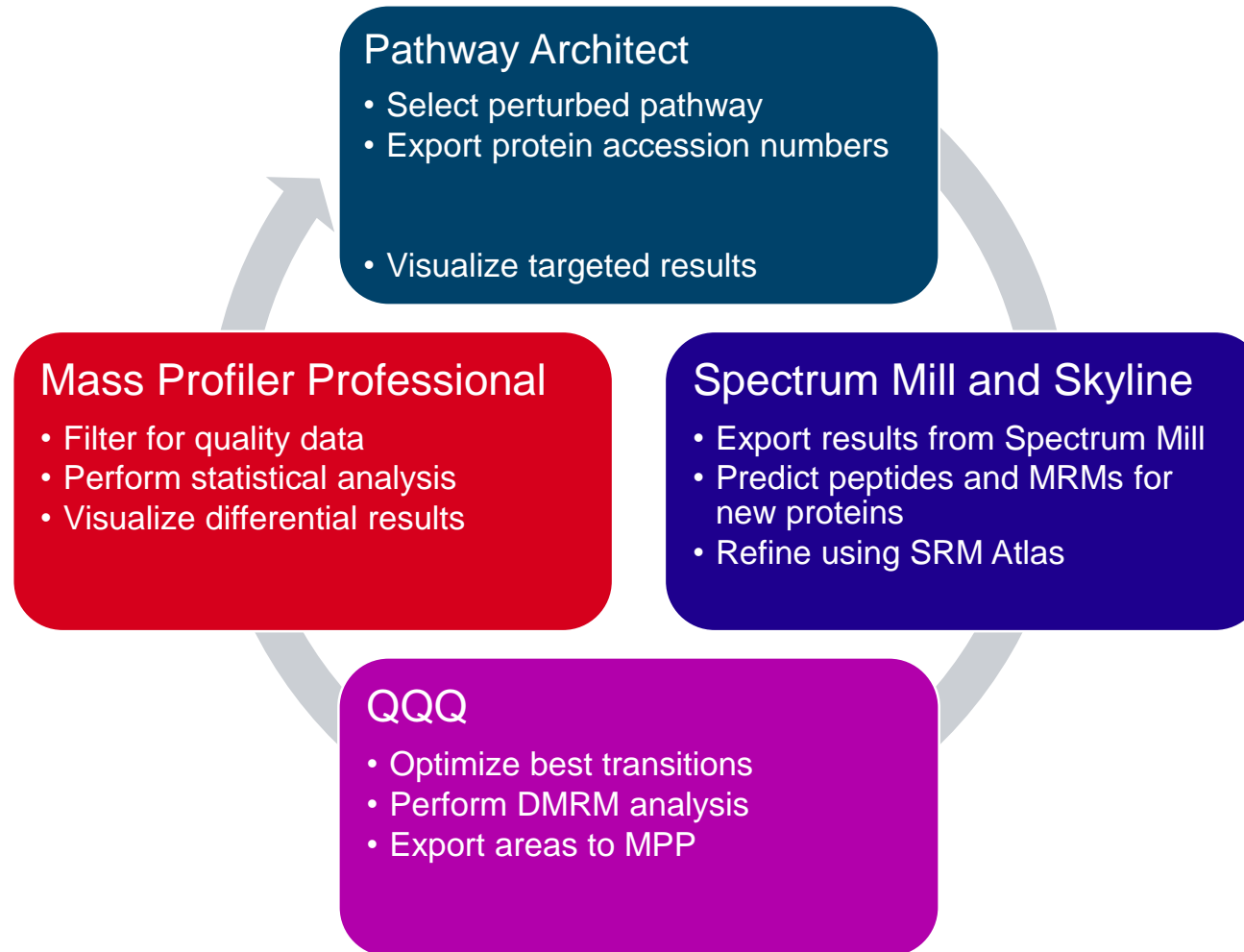
500 Pathways

ID	Name	# of Member Cmpds
ko0010	Glycolysis / Gluconeogenesis	55
ko0020	Citrate cycle (TCA cycle)	38
ko0030	Pentose phosphate pathway	54
ko0040	Pentose and glucuronate interconversions	73
ko0051	Fructose and mannose metabolism	69
ko0052	Galactose metabolism	64
ko0053	Ascorbate and aldarate metabolism	71
ko0061	Fatty acid biosynthesis	61
ko0062	Fatty acid elongation	43
ko0071	Fatty acid metabolism	65
ko0072	Synthesis and degradation of ketone bodies	13
ko0073	Cutin, suberine and wax biosynthesis	38
ko0100	Steroid biosynthesis	60
ko0120	Primary bile acid biosynthesis	58
ko0121	Secondary bile acid biosynthesis	32
ko0130	Ubiquinone and other terpenoid-quinone biosynthesis	106
ko0140	Steroid hormone biosynthesis	114
ko0190	Oxidative phosphorylation	16
ko0195	Photosynthesis	11
ko0196	Photosynthesis - antenna proteins	0
ko0230	Purine metabolism	120
ko0231	Puromycin biosynthesis	21
ko0232	Caffeine metabolism	35
ko0240	Pyrimidine metabolism	102
ko0250	Alanine, aspartate and glutamate metabolism	52
ko0253	Tetracycline biosynthesis	42
ko0260	Glycine, serine and threonine metabolism	85
ko0270	Cysteine and methionine metabolism	101
ko0280	Valine, leucine and isoleucine degradation	60
ko0281	Geraniol degradation	37
ko0290	Valine, leucine and isoleucine biosynthesis	34
ko0300	Valine, leucine and isoleucine biosynthesis	67

668 Unique Resolved Compounds 121 Unresolved Compounds

#	Organism	Selection Mode	Entry ID	Name	# of Cmpd	Del.
1	All Organisms	Pathway	ko0010	Glycolysis / Gluconeogenesis	55	X
2	All Organisms	Pathway	ko0020	Citrate cycle (TCA cycle)	38	X
3	All Organisms	Pathway	ko0030	Pentose phosphate pathway	54	X
4	All Organisms	Pathway	ko0040	Pentose and glucuronate interconversions	73	X
5	All Organisms	Pathway	ko0051	Fructose and mannose metabolism	69	X
6	All Organisms	Pathway	ko0052	Galactose metabolism	64	X
7	All Organisms	Pathway	ko0053	Ascorbate and aldarate metabolism	71	X
8	All Organisms	Pathway	ko0061	Fatty acid biosynthesis	61	X
9	All Organisms	Pathway	ko0062	Fatty acid elongation	43	X
10	All Organisms	Pathway	ko0071	Fatty acid metabolism	65	X
11	All Organisms	Pathway	ko0072	Synthesis and degradation of ketone bodies	13	X
12	All Organisms	Pathway	ko0073	Cutin, suberine and wax biosynthesis	38	X
13	All Organisms	Pathway	ko0100	Steroid biosynthesis	60	X
14	All Organisms	Pathway	ko0120	Primary bile acid biosynthesis	58	X
15	All Organisms	Pathway	ko0121	Secondary bile acid biosynthesis	32	X
16	All Organisms	Pathway	ko0130	Ubiquinone and other terpenoid-quinone biosynthesis	106	X
17	All Organisms	Pathway	ko0140	Steroid hormone biosynthesis	114	X
18	All Organisms	Pathway	ko0190	Oxidative phosphorylation	16	X

Targeted Proteomics: Using Pathway-Directed Information to Inform the Next Experiment



Targeted Proteomics: Agilent 6495 QQQ with iFunnel Technologies

Outstanding sensitivity with iFunnel

Excellent standard flow performance with AJS + 1290

Routine, robust nano LC with HPLC-Chip/QQQ

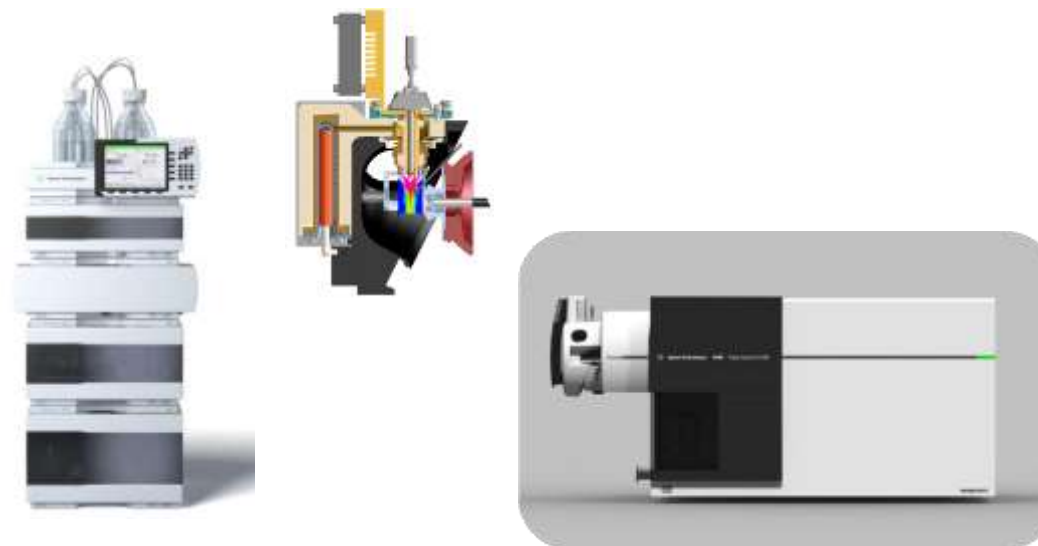
tMRM mode

Skyline workflow manuals

New Skyline Automation tool



Skyline workflow manuals



Skyline – MPP Data Exchange

Importing Targeted Results



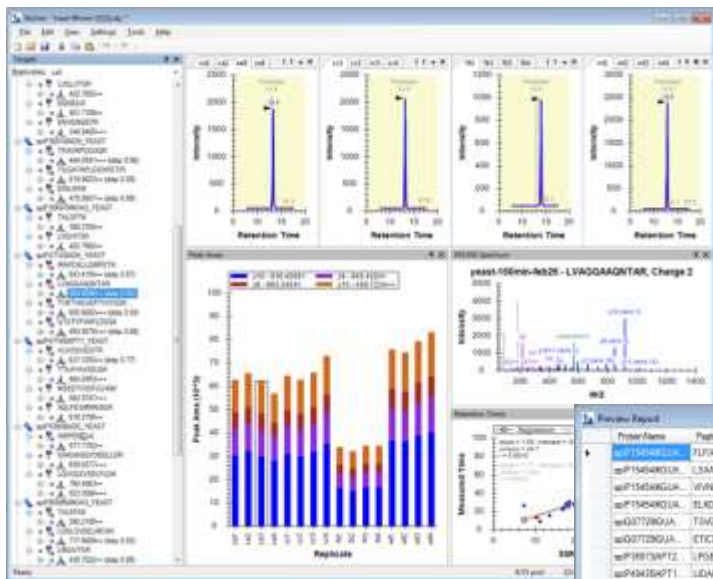
**MassHunter
Mass Profiler
Professional
Software**

MPP 12

Version B.12.00

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Exporting Protein Areas From Skyline to MPP



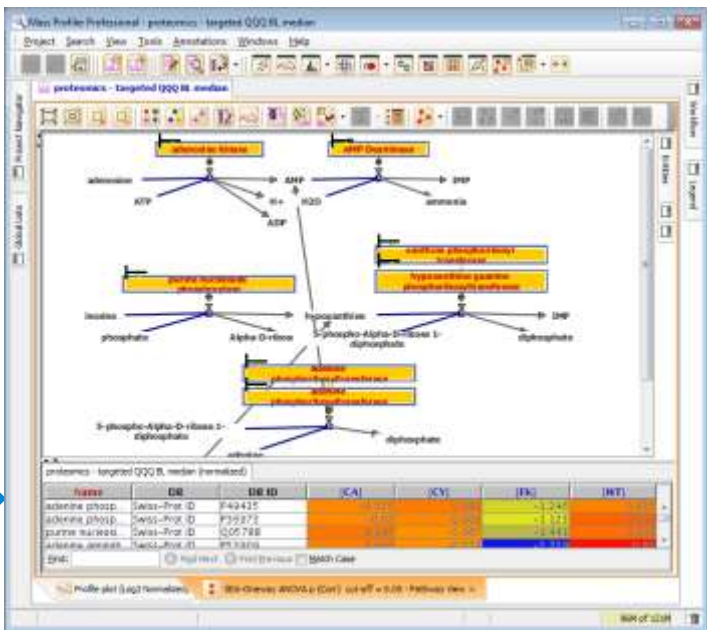
Review and process QQQ results in Skyline

Export results to MPP

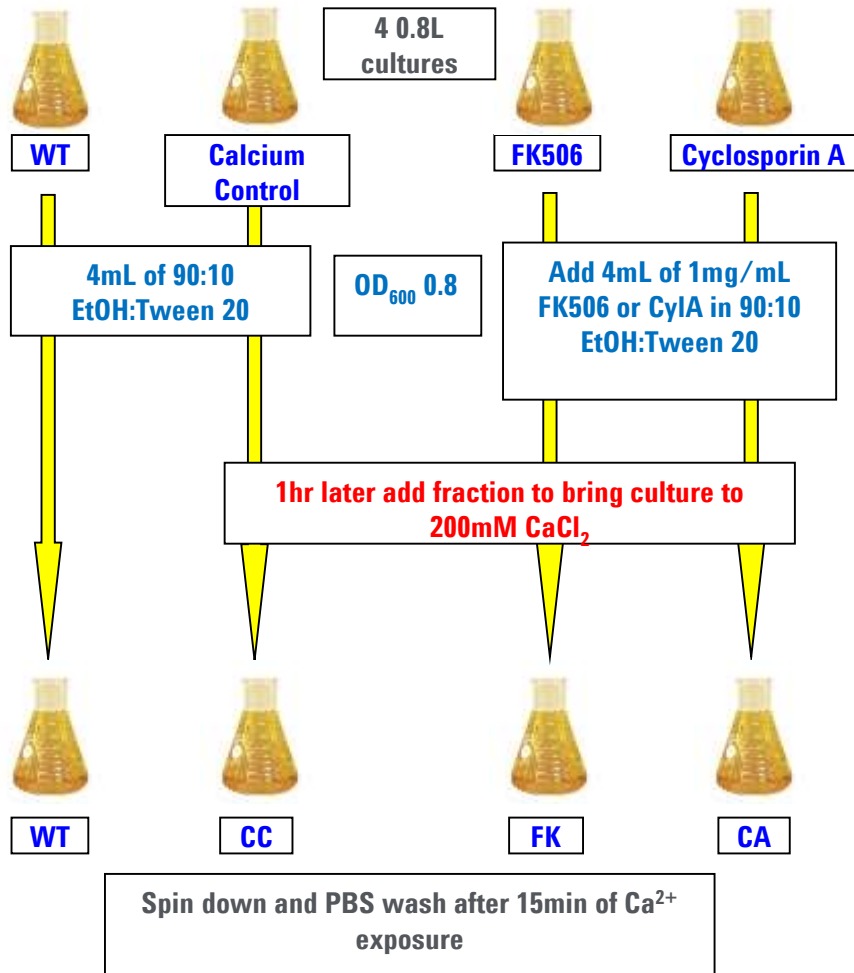
The MPP software interface displays a list of proteins and their associated metabolites. The table lists protein names, peptide sequences, and total ion counts for four different samples (Total Ion 1, Total Ion 2, Total Ion 3, Total Ion 4).

Protein Name	Peptide Sequence	Total Ion 1	Total Ion 2	Total Ion 3	Total Ion 4
Protein 1	Peptide 1	10000	10000	10000	10000
Protein 2	Peptide 2	10000	10000	10000	10000
Protein 3	Peptide 3	10000	10000	10000	10000
Protein 4	Peptide 4	10000	10000	10000	10000

Pathway visualization in Pathway Architect



Yeast Metabolomics :



Experimental Design

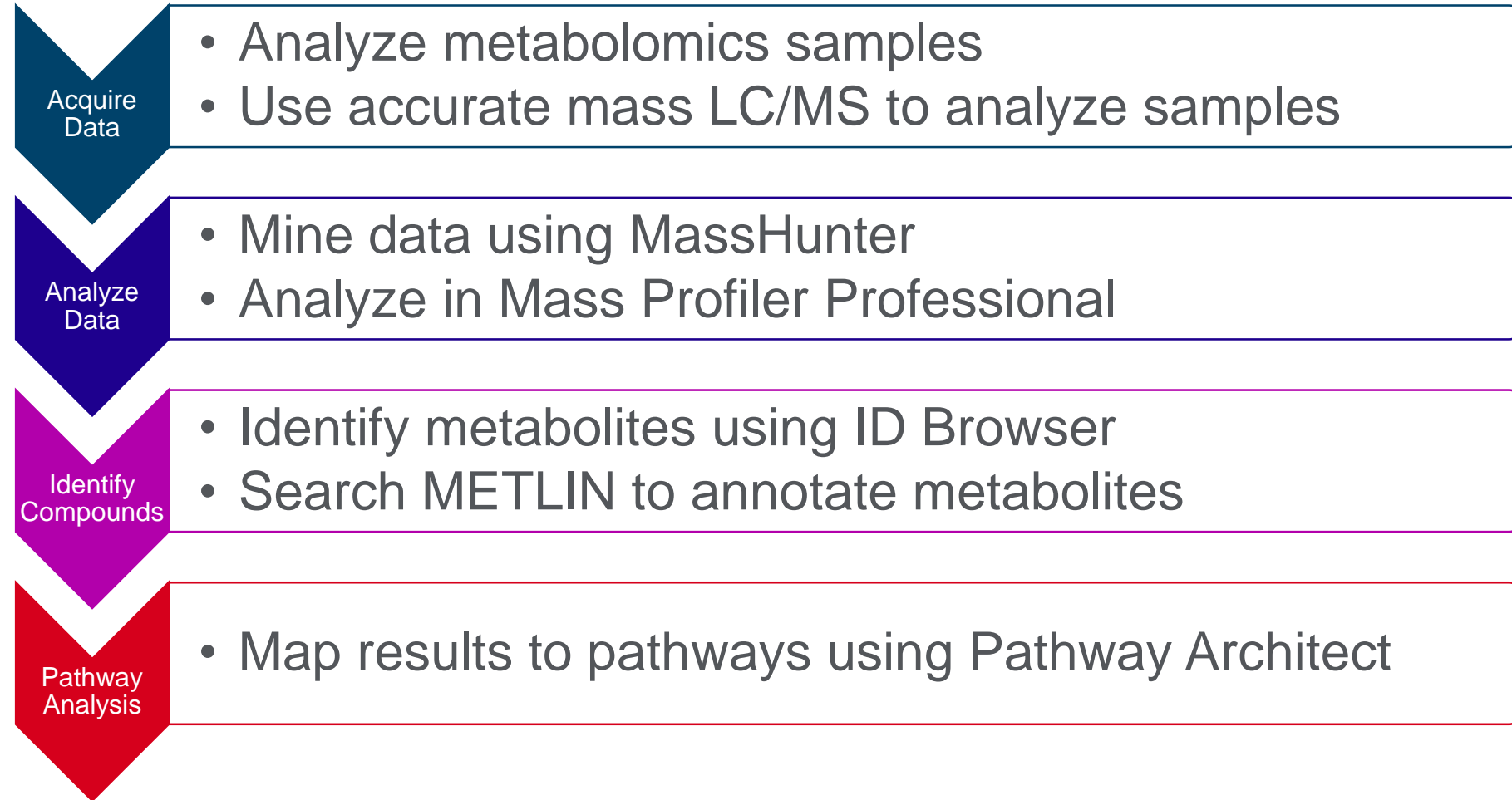
Treatments:

- Wild type (WT) - no treatment
- Calcium control (CC) - CaCl₂
- FK - FK506 and CaCl₂
- CA - Cyclosporin A and CaCl₂

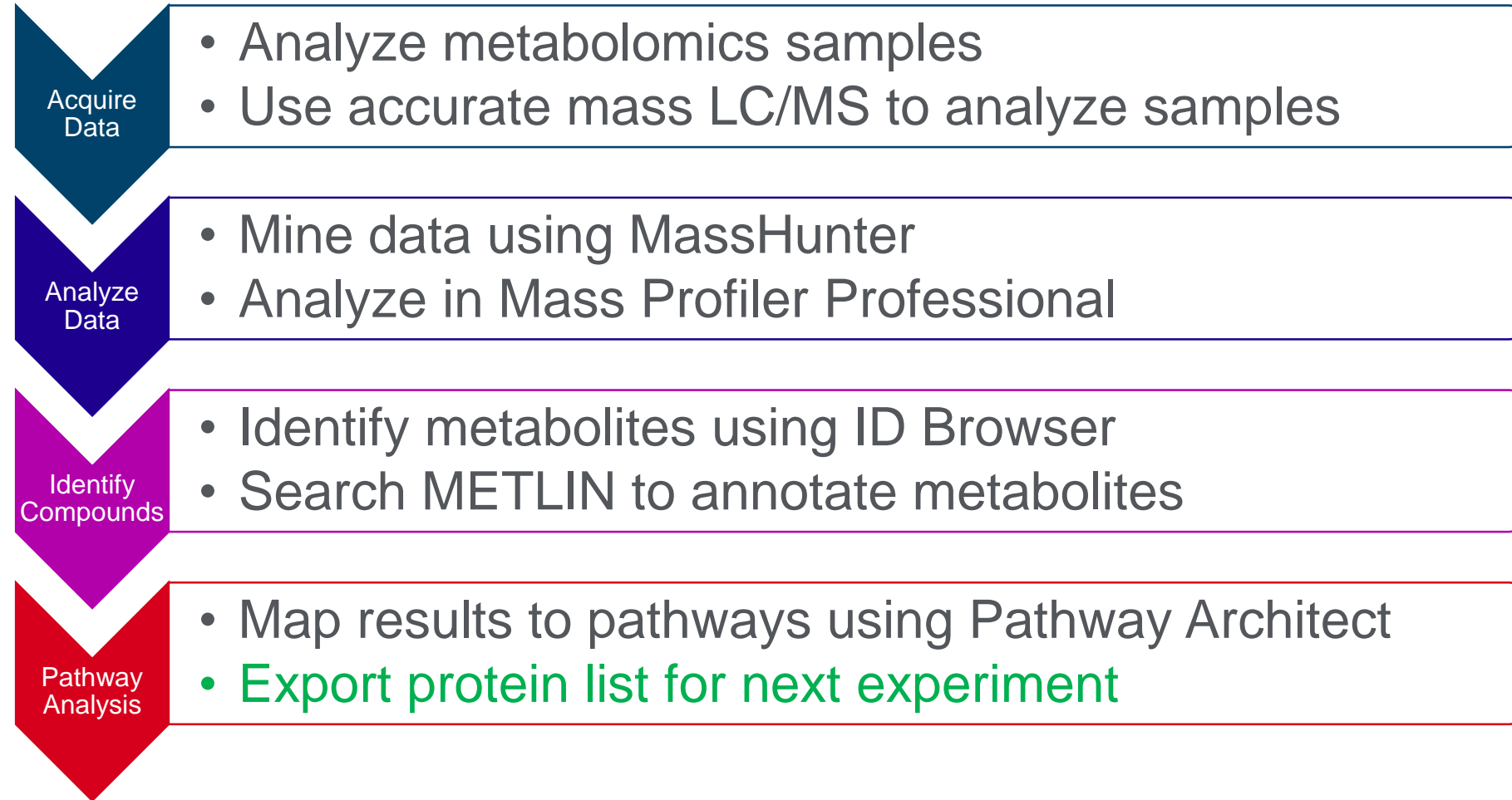
Extraction :

Wet mill with 5:3:3 CHCl₃:CH₃OH:H₂O. Only the aqueous is analyzed

Metabolomics Workflow

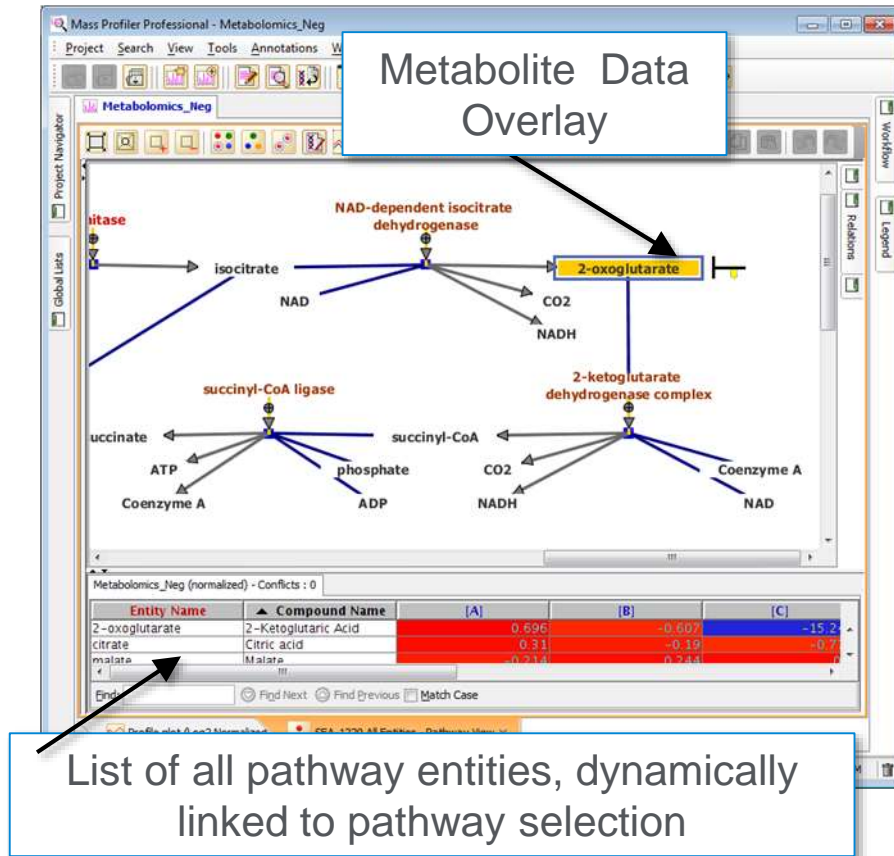


Metabolomics Workflow



Pathway Architect

Pathway Architect is an optional module in MassProfiler Professional



Map and visualize data from one or two types of -omic data on pathways

Search, browse and filter pathways

Supports biological pathways from publicly available databases

- WikiPathways
- BioCyc
- Supported pathway formats
 - BioPAX 3 – Pathway Commons, Reactome, NCI Nature Pathway
 - GPML – PathVisio –custom drawing
- Export compound list from pathways