

Supplementary Materials

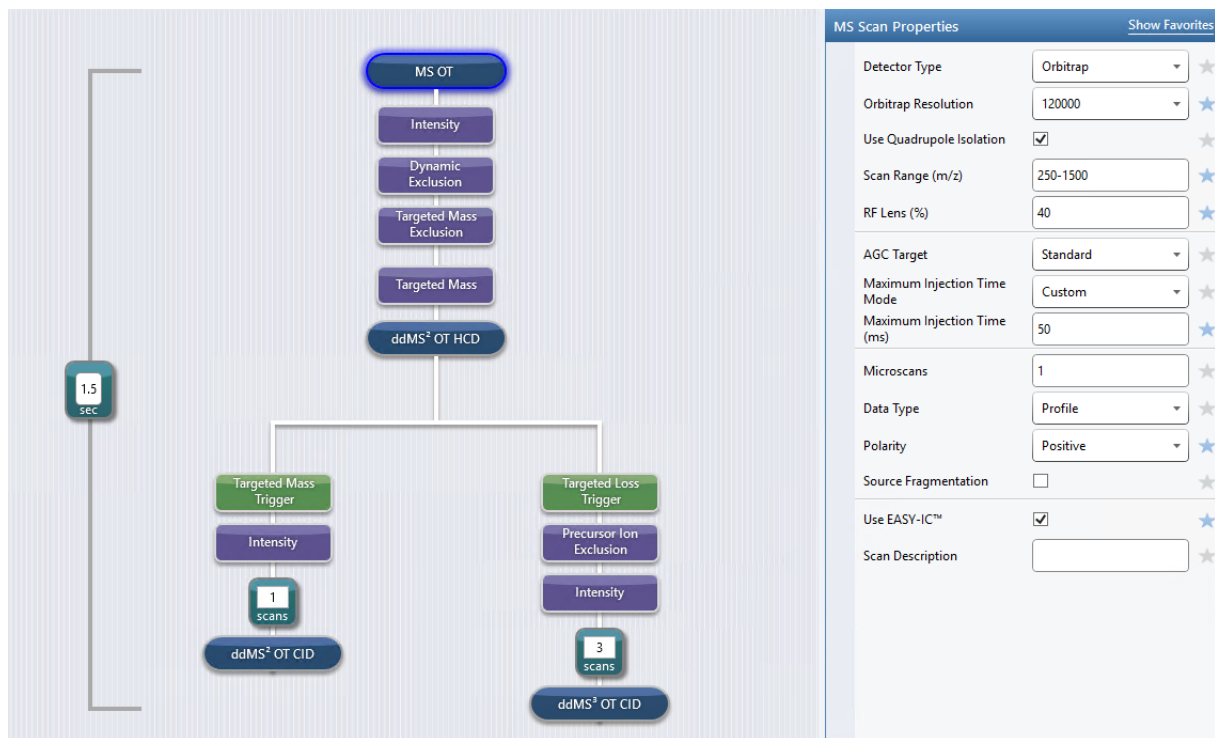


Figure S1: AcquireX DS DDA method – MS orbitrap workflow and masterscan configuration

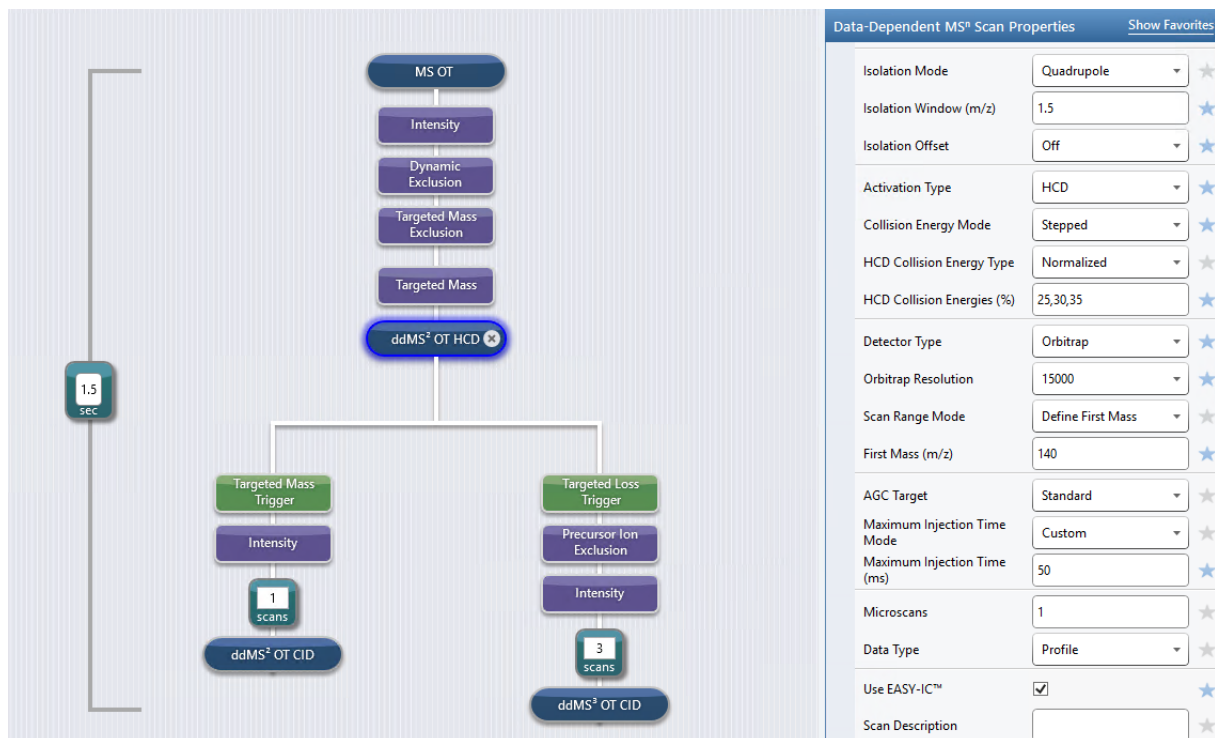


Figure S2: AcquireX DS DDA method – MS orbitrap workflow and ddMS2 HCD configurations

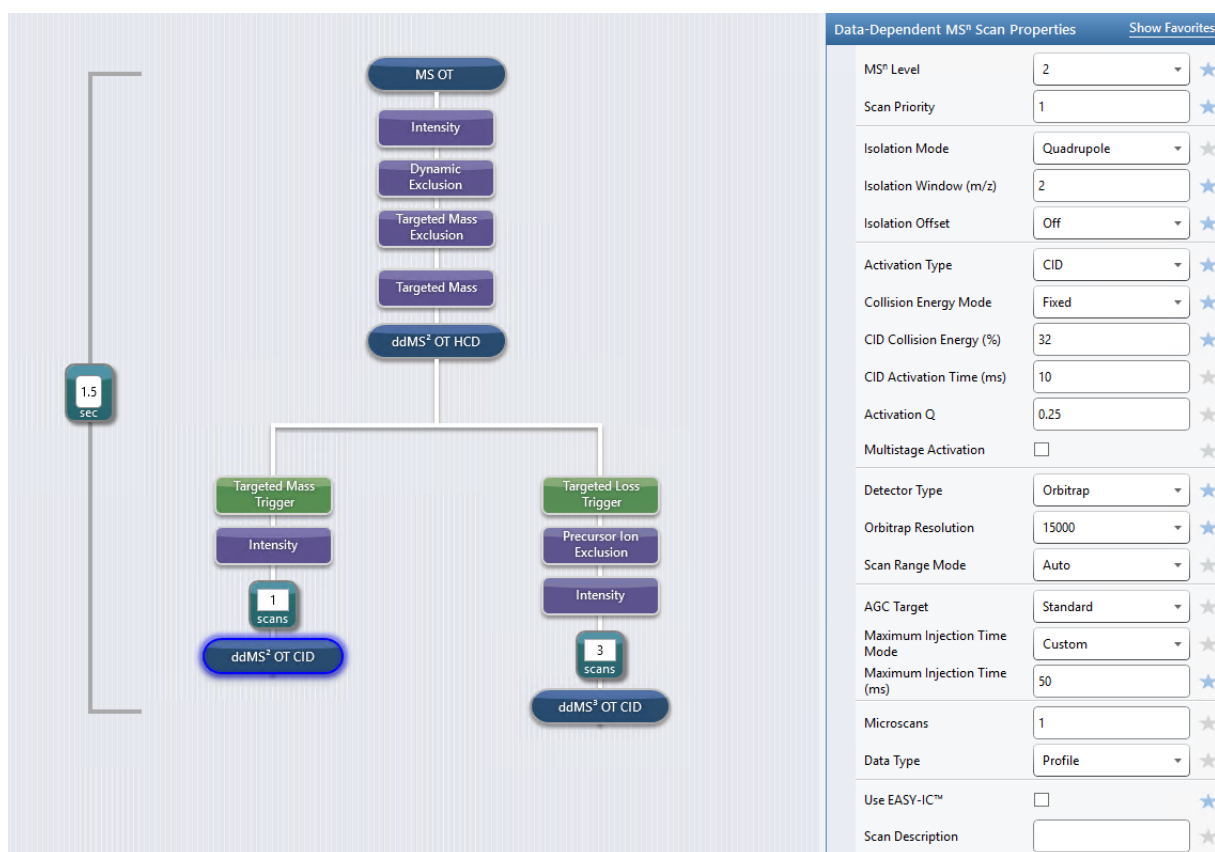


Figure S3: AcquireX DS DDA method – MS orbitrap workflow and ddMS2 CID configurations

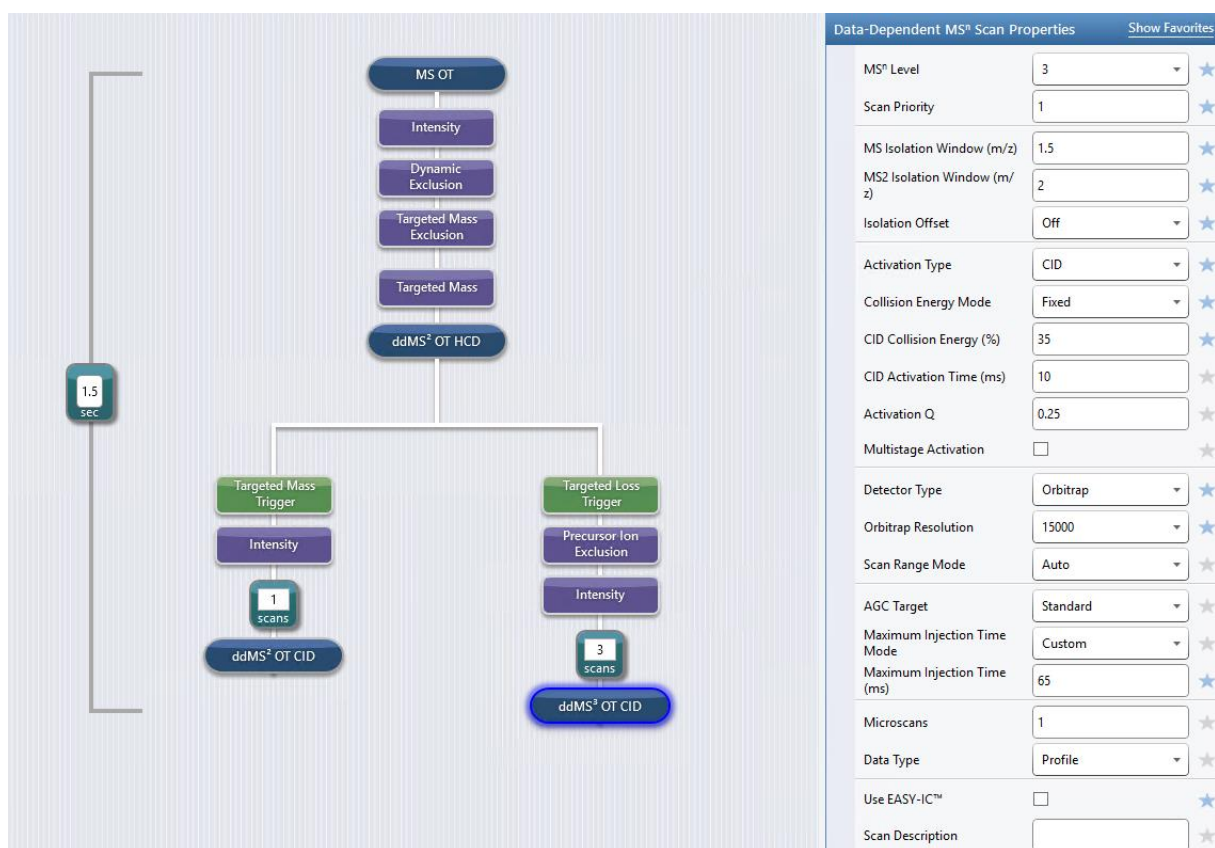


Figure S4: AcquireX DS DDA method – MS orbitrap workflow and ddMS3 HCD configurations

Experiment Details

Experiment Folder: D:\RAW Files Browse

Experiment Name: PorosHA_AGC

Instrument Methods

Full Scan Method: D:\Methods\Test TV Ipidomics AcquireX Fullscan.meth Browse New

MSn Template Method: D:\Methods\Test TV Ipidomics AcquireX MS3_classic.meth Browse New

Experiment Parameters

Exclusion Overlay Factor (default = 0): 5

Exclusion List Peak Window Extension (s) (default = 0 s): 0

Inclusion List Peak Window Extension (s) (default = 0 s): 0

Inclusion List Peak Fragmentation Threshold (%) (default = 50%): 50

Probed ions: [M+H]⁺-1; [M+NH₄]⁺-1; [M+Na]⁺-1

Exclusion Duration (seconds): 4

☐ Enable automatic adding of isotopes

> Component Detection Intensity Parameters

Sequence Design

#	Name	Type	Exclusion Ref	Instrument Method	Val	Inj Vol (µl)
1	Blank_01	Blank		Test TV Ipidomics AcquireX Fullscan	R.D2	2.00 µl
2	Blank_02	Blank		Test TV Ipidomics AcquireX Fullscan	R.D2	2.00 µl
3	Blank_03	Blank		Test TV Ipidomics AcquireX Fullscan	R.D2	2.00 µl
4	Blank_04	Blank		Test TV Ipidomics AcquireX Fullscan	R.D2	2.00 µl
5	Blank_05	Blank		Test TV Ipidomics AcquireX Fullscan	R.D2	2.00 µl
6	Sample_01	Inclusion Reference		Test TV Ipidomics AcquireX Fullscan	R.D1	2.00 µl
7	ID_01	Sample ID		Test TV Ipidomics AcquireX MS3_classic	R.D1	6.00 µl
8	ID_02	Sample ID		Test TV Ipidomics AcquireX MS3_classic	R.D1	6.00 µl
9	ID_03	Sample ID		Test TV Ipidomics AcquireX MS3_classic	R.D1	6.00 µl
10	ID_04	Sample ID		Test TV Ipidomics AcquireX MS3_classic	R.D1	6.00 µl
11	ID_05	Sample ID		Test TV Ipidomics AcquireX MS3_classic	R.D1	6.00 µl

#	Name	Type	Instrument Method	Val	Inj Vol (µl)
12	Blank_1	Unknown	Test TV Ipidomics AcquireX Fullscan	R.D2	2.00 µl
13	Red_1_1	Unknown	Test TV Ipidomics AcquireX Fullscan	R.A1	2.00 µl
14	Blue_1_1	Unknown	Test TV Ipidomics AcquireX Fullscan	R.A2	2.00 µl
15	White_1_1	Unknown	Test TV Ipidomics AcquireX Fullscan	R.A3	2.00 µl
16	QC_1	Unknown	Test TV Ipidomics AcquireX Fullscan	R.D1	2.00 µl
17	Blank_2	Unknown	Test TV Ipidomics AcquireX Fullscan	R.D2	2.00 µl
18	Red_2_1	Unknown	Test TV Ipidomics AcquireX Fullscan	R.B1	2.00 µl
19	Blue_2_1	Unknown	Test TV Ipidomics AcquireX Fullscan	R.B2	2.00 µl
20	White_2_1	Unknown	Test TV Ipidomics AcquireX Fullscan	R.B3	2.00 µl
21	QC_2	Unknown	Test TV Ipidomics AcquireX Fullscan	R.D1	2.00 µl
22	Blank_3	Unknown	Test TV Ipidomics AcquireX Fullscan	R.D2	2.00 µl
23	Red_3_1	Unknown	Test TV Ipidomics AcquireX Fullscan	R.C1	2.00 µl
24	Blue_3_1	Unknown	Test TV Ipidomics AcquireX Fullscan	R.C2	2.00 µl
25	White_3_1	Unknown	Test TV Ipidomics AcquireX Fullscan	R.C3	2.00 µl
26	QC_3	Unknown	Test TV Ipidomics AcquireX Fullscan	R.D1	2.00 µl
27	Blank_4	Unknown	Test TV Ipidomics AcquireX Fullscan	R.D2	2.00 µl
28	Red_1_2	Unknown	Test TV Ipidomics AcquireX Fullscan	R.A1	2.00 µl
29	Blue_1_2	Unknown	Test TV Ipidomics AcquireX Fullscan	R.A2	2.00 µl
30	White_1_2	Unknown	Test TV Ipidomics AcquireX Fullscan	R.A3	2.00 µl

Figure S5: AcquireX DS DDA method – Experiment set up and injection sequence. Line 12-26 in injection sequence repeats 3 times.



Figure S6: Targeted ddMS2 method for spectral library creation – masterscan configuration

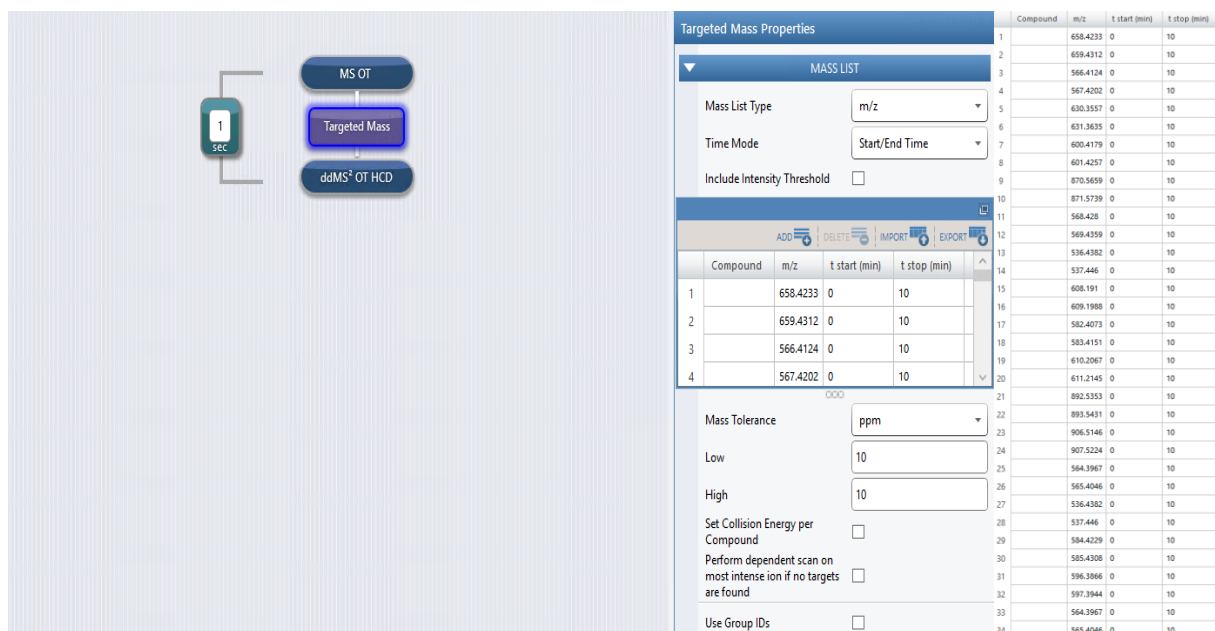


Figure S7: Targeted ddMS2 method for spectral library creation – Target mass list

Table S1: Mass list for pigment library creation

Compound	m/z
Fucoxanthin [M*] ⁺	658.4233
Fucoxanthin [M+H] ⁺	659.4312
Diatoxanthin [M*] ⁺	566.4124
Diatoxanthin [M+H] ⁺	567.4202
Peridinin [M*] ⁺	630.3557

Peridinin [M+H] ⁺	631.3635
Violaxanthin [M*] ⁺	600.4179
Violaxanthin [M+H] ⁺	601.4257
Pheophytin <i>a</i> [M*] ⁺	870.5659
Pheophytin <i>a</i> [M+H] ⁺	871.5739
Zeaxanthin [M*] ⁺	568.428
Zeaxanthin [M+H] ⁺	569.4359
Lycopene [M*] ⁺	536.4382
Lycopene [M+H] ⁺	537.446
Chlorophyll <i>c</i> ₂ [M*] ⁺	608.191
Chlorophyll <i>c</i> ₂ [M+H] ⁺	609.1988
Diadinoxanthin [M*] ⁺	582.4073
Diadinoxanthin [M+H] ⁺	583.4151
Chlorophyll <i>c</i> ₁ [M*] ⁺	610.2067
Chlorophyll <i>c</i> ₁ [M+H] ⁺	611.2145
Chlorophyll <i>a</i> [M*] ⁺	892.5353
Chlorophyll <i>a</i> [M+H] ⁺	893.5431
Chlorophyll <i>b</i> [M*] ⁺	906.5146
Chlorophyll <i>b</i> [M+H] ⁺	907.5224
Canthaxanthin [M*] ⁺	564.3967
Canthaxanthin [M+H] ⁺	565.4046
β-carotene [M*] ⁺	536.4382
β-carotene [M+H] ⁺	537.446

Antheraxanthin [M*] ⁺	584.4229
Antheraxanthin [M+H] ⁺	585.4308
Astaxanthin [M*] ⁺	596.3866
Astaxanthin [M+H] ⁺	597.3944
Alloxanthin [M*] ⁺	564.3967
Alloxanthin [M+H] ⁺	565.4046

The image shows a workflow diagram on the left and a configuration panel on the right. The workflow diagram includes a box labeled '1 sec' connected to a sequence of three boxes: 'MS OT', 'Targeted Mass', and 'ddMS² OT HCD'. The configuration panel, titled 'Data-Dependent MSⁿ Scan Properties', contains the following settings:

- Isolation Mode: Quadrupole
- Isolation Window (m/z): 0.4
- Isolation Offset: Off
- Activation Type: HCD
- Collision Energy Mode: Stepped
- HCD Collision Energy Type: Normalized
- HCD Collision Energies (%): 15,30,45
- Detector Type: Orbitrap
- Orbitrap Resolution: 30000
- Scan Range Mode: Auto
- AGC Target: Standard
- Maximum Injection Time Mode: Auto
- Microscans: 1
- Data Type: Centroid
- Use EASY-IC™: ☐
- Scan Description: (empty field)

Figure S8: Targeted ddMS2 method for spectral library creation – MS orbitrap workflow and ddMS2 CID configurations

The image shows the 'Identification' tab of the LipidSearch software. It is divided into three main sections: 'Batch', 'Database', and 'Peak detection'. The 'Batch' section includes fields for 'Job Name' (set to 'Radiatus') and 'Comment' (set to 'Final'). The 'Database' section shows 'Target Database' set to 'General'. The 'Peak detection' section has 'Recalc. Isotope' set to 'on', 'R.T. interval (Min)' set to '0.01', and 'R.T. Range (Min)' set to '-'. On the right, the 'Search options' panel is visible, showing settings for 'SearchType' (LC), 'ExpType' (LC), 'Precursor tol' (5.0 ppm), 'Product tol' (5.0 ppm), 'Merge Range (Min)' (0.0), 'Min Peak Width (Min)' (0.0), 'Intensity threshold' (0.01, relative), and 'm-Score Threshold' (5.0).

Figure S9: LipidSearch Identification configuration

Identification	Quantitation	Filters	Class	Ion
Quantitation				
Execute Quantitation		<input checked="" type="radio"/> on <input type="radio"/> off		
Mz tol		- 2.5 + 2.5		
tolType		<input type="radio"/> Da <input checked="" type="radio"/> ppm		
Rt range(min.)		- 0.5 + 0.5		

Figure S10: LipidSearch Quantitation configuration

Identification	Quantitation	Filters	Class	Ion
Display Filter				
Toprank filter		<input checked="" type="radio"/> on <input type="radio"/> off		
Main node filter		<input type="radio"/> Off <input type="radio"/> Main isomer peak <input checked="" type="radio"/> All isomer peaks		
m-Score Threshold (Display)		5.0		
c-Score Threshold (Display)		2.0		
FA Priority		<input checked="" type="radio"/> on <input type="radio"/> off		
ID Quality filter		<input checked="" type="checkbox"/> A <input checked="" type="checkbox"/> B <input checked="" type="checkbox"/> C <input checked="" type="checkbox"/> D		

Figure S11: LipidSearch Filters configuration

Identification	Quantitation	Filters	Class	Ion
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Adducts

All

Polarity	Name
negative	<input type="checkbox"/> -H
	<input type="checkbox"/> +HCOO
	<input type="checkbox"/> +CH ₃ COO
	<input type="checkbox"/> +Cl
	<input type="checkbox"/> -2H
	<input type="checkbox"/> -CH ₃
positive	<input checked="" type="checkbox"/> +H
	<input checked="" type="checkbox"/> +NH ₄
	<input checked="" type="checkbox"/> +Na
	<input type="checkbox"/> +Li
	<input type="checkbox"/> +K
	<input type="checkbox"/> +(CH ₃ CH ₂) ₃ NH
	<input type="checkbox"/> +(CH ₃) ₂ NH ₂
	<input type="checkbox"/> +H-H ₂ O
	<input type="checkbox"/> +H-2H ₂ O
	<input type="checkbox"/> +2H

Figure S12: LipidSearch Adduct selection

Parameters

SearchType	Product
ExpType	LC
Normalize Type	None
Alignment Method	Mean
R.T. Tolerance	2.0
Calculate unassigned peak area	on
Filter Type	New Filter
Toprank Filter	on
Main Node Filter	All isomer peaks
m-Score Threshold	5.0
c-Score Threshold	2.0
ID Quality filter	[A, B, C, D]

Sample

c-1	Porosira_NY	TV_TM_201113_Rs_2.raw
c-2	Porosira_NY	TV_TM_201113_Rs_3.raw
c-3	Porosira_NY	TV_TM_201113_Rs_1.raw
s1-1	Porosira_NY	TV_TM_201113_R1_3.raw
s1-2	Porosira_NY	TV_TM_201113_R1_1.raw
s1-3	Porosira_NY	TV_TM_201113_R1_2.raw
s10-1	NY_ID	TV_TM_201213_ID_05.raw
s10-2	NY_ID	TV_TM_201213_ID_04.raw
s10-3	NY_ID	TV_TM_201213_ID_03.raw
s10-4	NY_ID	TV_TM_201213_ID_02.raw
s10-5	NY_ID	TV_TM_201213_ID_01.raw
s2-1	Porosira_NY	TV_TM_201113_R2_2.raw
s2-2	Porosira_NY	TV_TM_201113_R2_3.raw
s2-3	Porosira_NY	TV_TM_201113_R2_1.raw
s3-1	Porosira_NY	TV_TM_201113_R3_3.raw
s3-2	Porosira_NY	TV_TM_201113_R3_2.raw
s3-3	Porosira_NY	TV_TM_201113_R3_1.raw
s4-1	Porosira_NY	TV_TM_201113_B1_3.raw
s4-2	Porosira_NY	TV_TM_201113_B1_1.raw
s4-3	Porosira_NY	TV_TM_201113_B1_2.raw
s5-1	Porosira_NY	TV_TM_201113_B2_3.raw
s5-2	Porosira_NY	TV_TM_201113_B2_2.raw
s5-3	Porosira_NY	TV_TM_201113_B2_1.raw
s6-1	Porosira_NY	TV_TM_201113_B3_2.raw
s6-2	Porosira_NY	TV_TM_201113_B3_3.raw
s6-3	Porosira_NY	TV_TM_201113_B3_1.raw
s7-1	Porosira_NY	TV_TM_201113_W1_3.raw
s7-2	Porosira_NY	TV_TM_201113_W1_1.raw
s7-3	Porosira_NY	TV_TM_201113_W1_2.raw
s8-1	Porosira_NY	TV_TM_201113_W2_3.raw
s8-2	Porosira_NY	TV_TM_201113_W2_2.raw
s8-3	Porosira_NY	TV_TM_201113_W2_1.raw
s9-1	Porosira_NY	TV_TM_201113_W3_3.raw
s9-2	Porosira_NY	TV_TM_201113_W3_2.raw
s9-3	Porosira_NY	TV_TM_201113_W3_1.raw

Figure S13: Alignment settings in LipidSearch

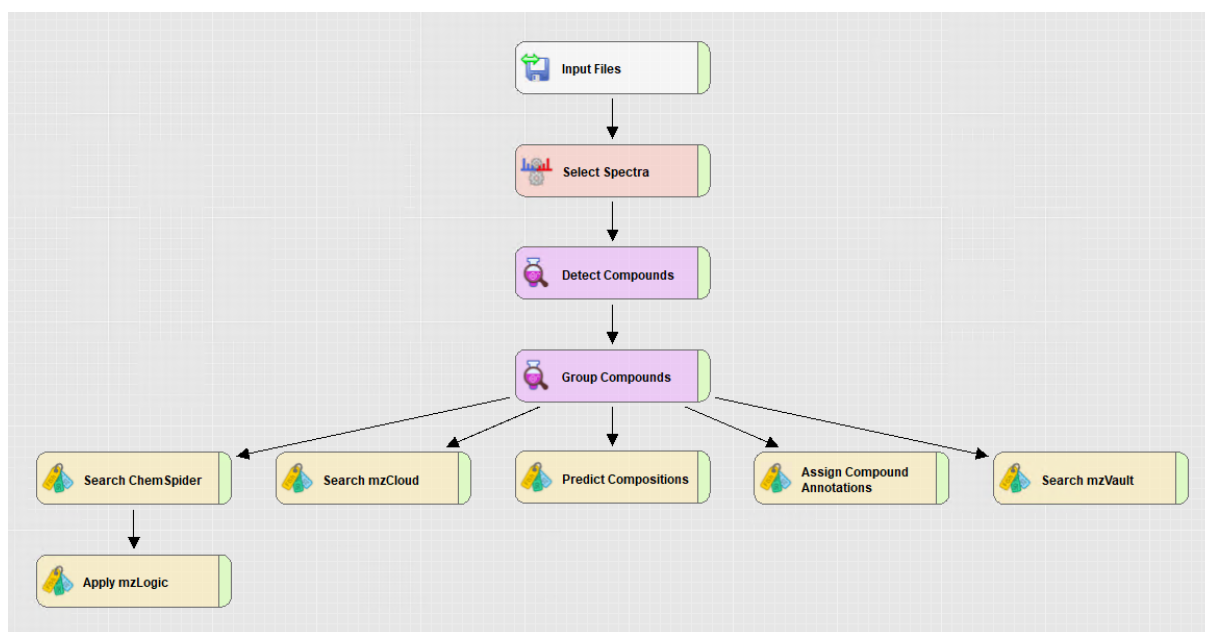


Figure S14: Compound discoverer workflow for data processing

Table S2: Compound Discoverer workflow parameter specifications

	Targeted analysis
Select spectra	
Lower RT limit	0 (lowest available RT is used)
Upper RT limit	0 (highest available RT is used)
Polarity	Any
Detect Compounds	
Mass tolerance	5 ppm
Min. Peak Intensity	250000
Use Most Intense Isotope Only	True
Chromatographic S/N threshold	1.5
Remove Baseline	False
Isotope Pattern Detection	Group Isotopes
Compound detection Ions	[M+2H] ²⁺ , [M+H] ⁺
Group Compounds	
Mass tolerance	5 ppm
RT tolerance	0.2
Align Peaks	False
Preferred Ions	[M+H-H ₂ O] ⁺ , [M+H] ⁺
Areal Integration	Most Common Ion
Area Contribution	3
CV Contribution	10
FWHM to Base Contribution	5
Jaggedness Contribution	5
Modality Contribution	5
Zig-Zag Index Contribution	5
Peak Rating Threshold	4
Number of files	1

Search ChemSpider	
Database(s)	KEGG
Search Mode	By formula or mass
Mass Tolerance	5 ppm
Max. # of results per compound	100
Max. # of Predicted Compounds to be searched for per	3
Apply mzLogic	
Max.# Compounds	0 (all candidates of all compounds are scored)
Max. #mzCloud Similarity Results to consider per	10
Match Factor Threshold	30
Search mzCloud	
Compound Classes	All
Library	Autoprocessed; Reference
Search MSn Tree	False
Identity Search	HighChem High Res
Match Activation Type	True
Match Activation Energy	Match with Tolerance
Activation Energy Threshold	20
Apply Intensity Threshold	True
Similarity Search	None
Match Factor Threshold	60
Use DIA Scans for Search	False
Max. Isolation Width (DA)	500
Match Activation Type	False
Match Activation Energy	Any
Activation Energy Tolerance	100
Apply Intensity threshold	False
Match Factor Threshold	20
Predict Compositions	
Mass Tolerance	10 ppm
Min. Element Counts	C H
Max. Element Counts	C90 H190 Br3 Cl4 N10 O18 P3 S5
Min. RDBE	0
Max. RDBE	40
Min. H/C	0.1
Max. H/C	3.5
Max. # Candidates	10
Intensity Tolerance (%)	30
Intensity Threshold (%)	0.1
S/N Threshold	3
Use Dynamic Recalibration	True
Use Fragments Matching	True
Mass Tolerance	5 ppm
S/N Threshold	3
Assign Compound Annotations	
Mass Tolerance	5 ppm
Data Source #1	MzVault Search
Data Source #2	Predicted Compositions
Data Source #3	mzCloud Search

Data Source #4	ChemSpider Search
Data Source #5	-
Use mzLogic	True
Use Spectral Distance	True
SFit Threshold	20
SFit Range	20
Clear Names	False
Search mzVault	
mzVault Library	pigmentDB2.db
Compound Classes	All
Match Ion Activation Type	True
Match Ion Activation Energy	Match with Tolerance
Ion Activation Energy Tolerance	20
Match Ionization Method	True
Apply Intensity Threshold	True
Precursor Mass Tolerance	10 ppm
Match Analyzer Type	True
Search Algorithm	HighChem HighRes
Match Factor Threshold	50
RT Tolerance (min)	2
Use Retention Time	False