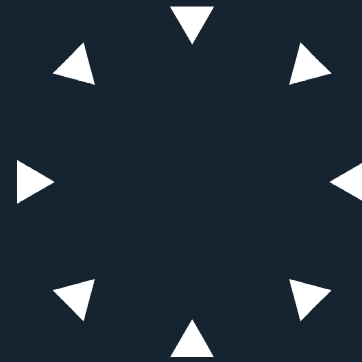


Affinity Selection Mass Spectrometry: From Fundamentals to Confident Hit Identification



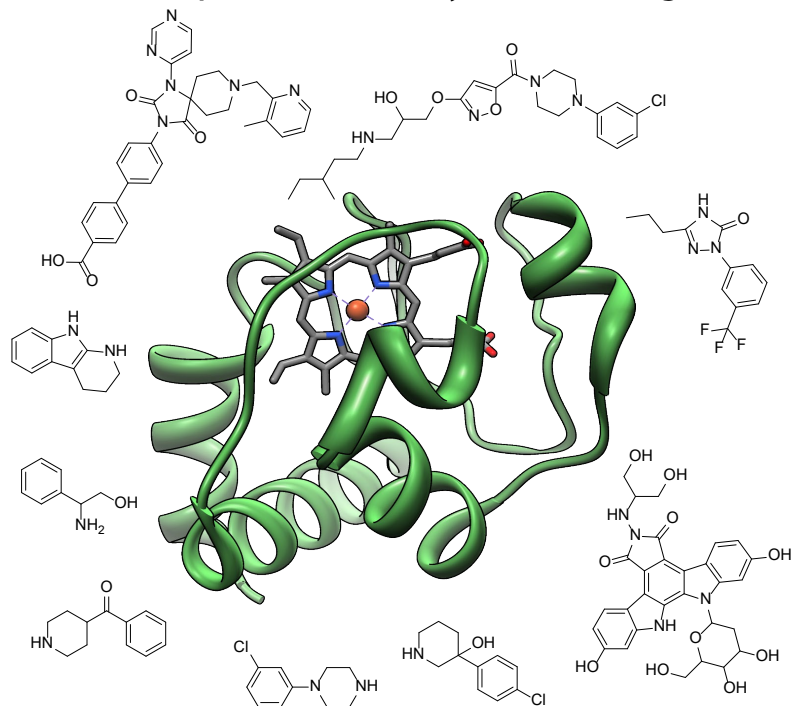
Don D. Nguyen, PhD
2026 -06 -03 Dotmatics Webinar

What is Affinity Selection -Mass Spectrometry (AS-MS) and how does it work?

- High throughput binding assay
- Multiplexed screening for hundreds of cpds/well to single cpds (library dependent)

Affinity Selection-Mass Spectrometry (AS-MS)

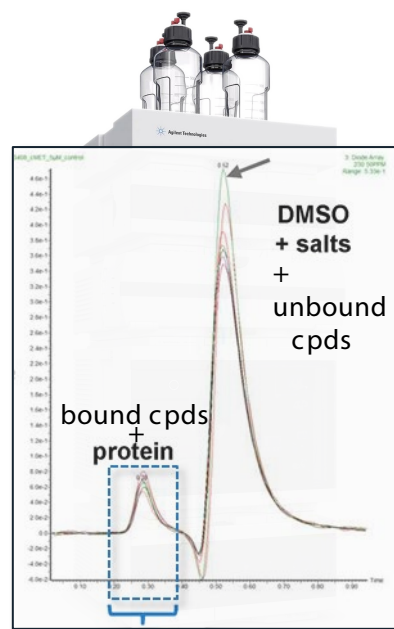
Compound Library Screening



Chromatography

Dimension 1

Size exclusion

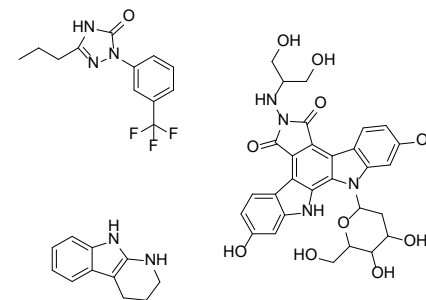


Dimension 2

Reverse phase

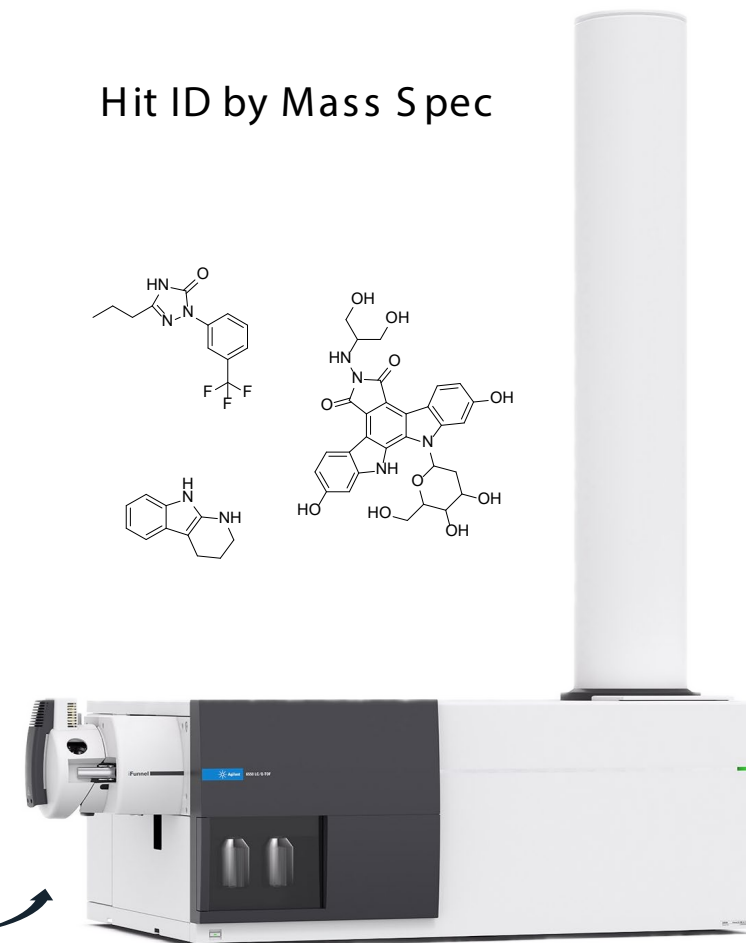


Hit ID by Mass Spec



Protein-compound complex

Protein-compound complex dissociation
(High temp, low pH, aq/org mixture)



What is Affinity Selection -Mass Spectrometry (AS-MS) and how does it work?

- High throughput binding assay
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Benefits and limitations of Affinity Selection -Mass Spectrometry (AS-MS)

Benefits

- High throughput binding assay
- Multiplexed screening for hundreds of cpds/well to single cpds (library dependent)
- In solution, label free, straight forward assay development
- Functional effect independent
- Binding site agnostic. AS-MS is able to:
 - ...query multiple binding sites simultaneously, competition assays
 - ...identify unknown binding sites
- Target tractability, 1^o-, 2^o-screening, orthogonal confirmation, (apparent) K_d

Benefits and limitations of Affinity Selection -Mass Spectrometry (AS-MS)

Benefits

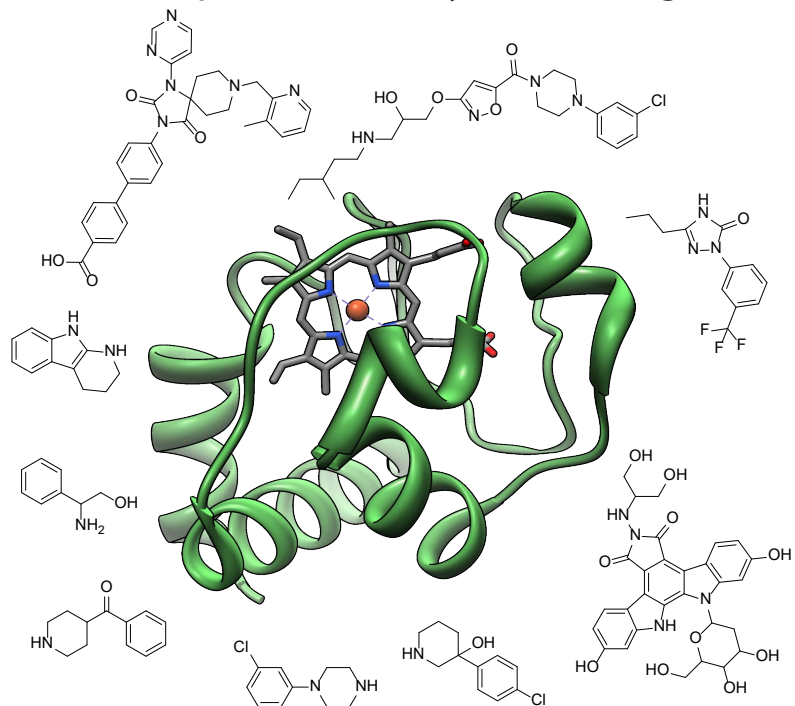
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- Target tractability, 1^o-, 2^o-screening, orthogonal confirmation, (apparent) K_d

Limitations

- Sufficient affinity required ($10e^{-5}$ M minimally)
- Moderate to slow K_{off}
- Moderate protein consumption
- Separation of target+binders (from non-binders)
 - ...performed at 4°C potentially effecting inducible pockets
 - ...is performed in Ammonium acetate/Aqueous/Organic mixtures (not in buffer)

Affinity Selection-Mass Spectrometry (AS-MS)

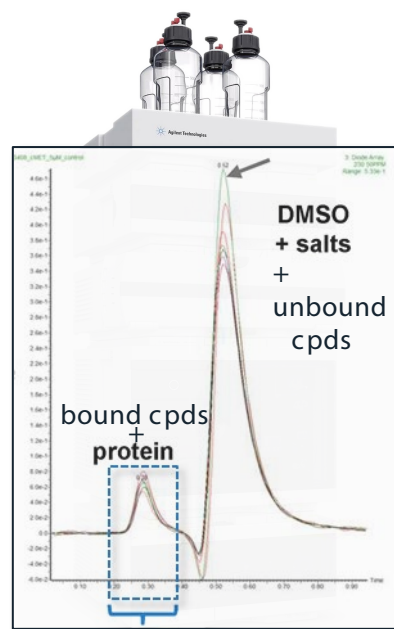
Compound Library Screening



Chromatography

Dimension 1

Size exclusion

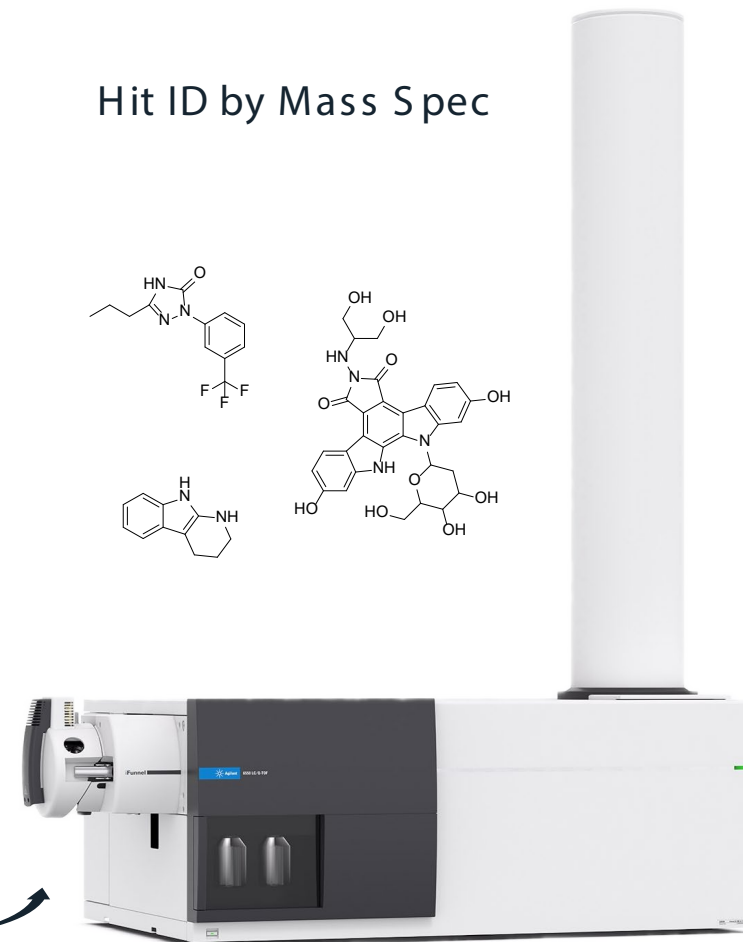
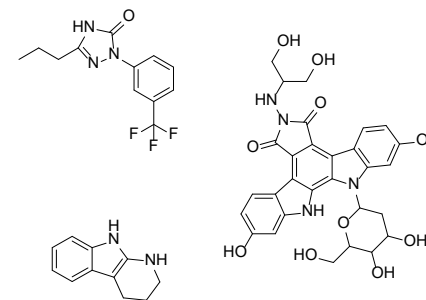


Dimension 2

Reverse phase



Hit ID by Mass Spec



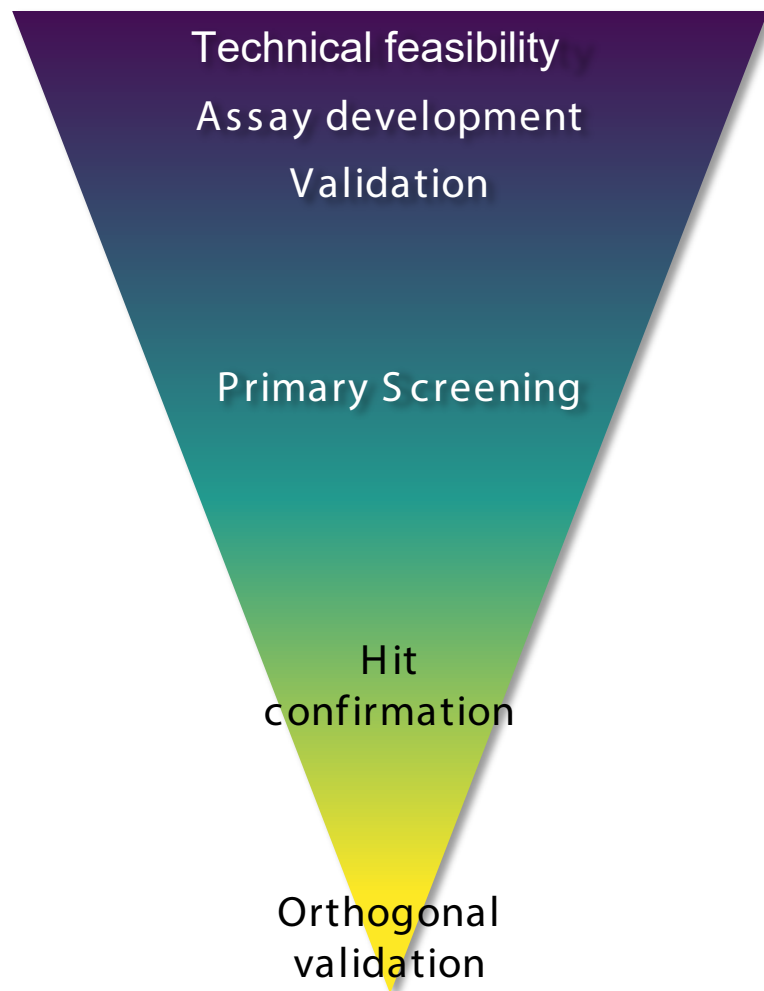
Protein-compound complex

Protein-compound complex dissociation
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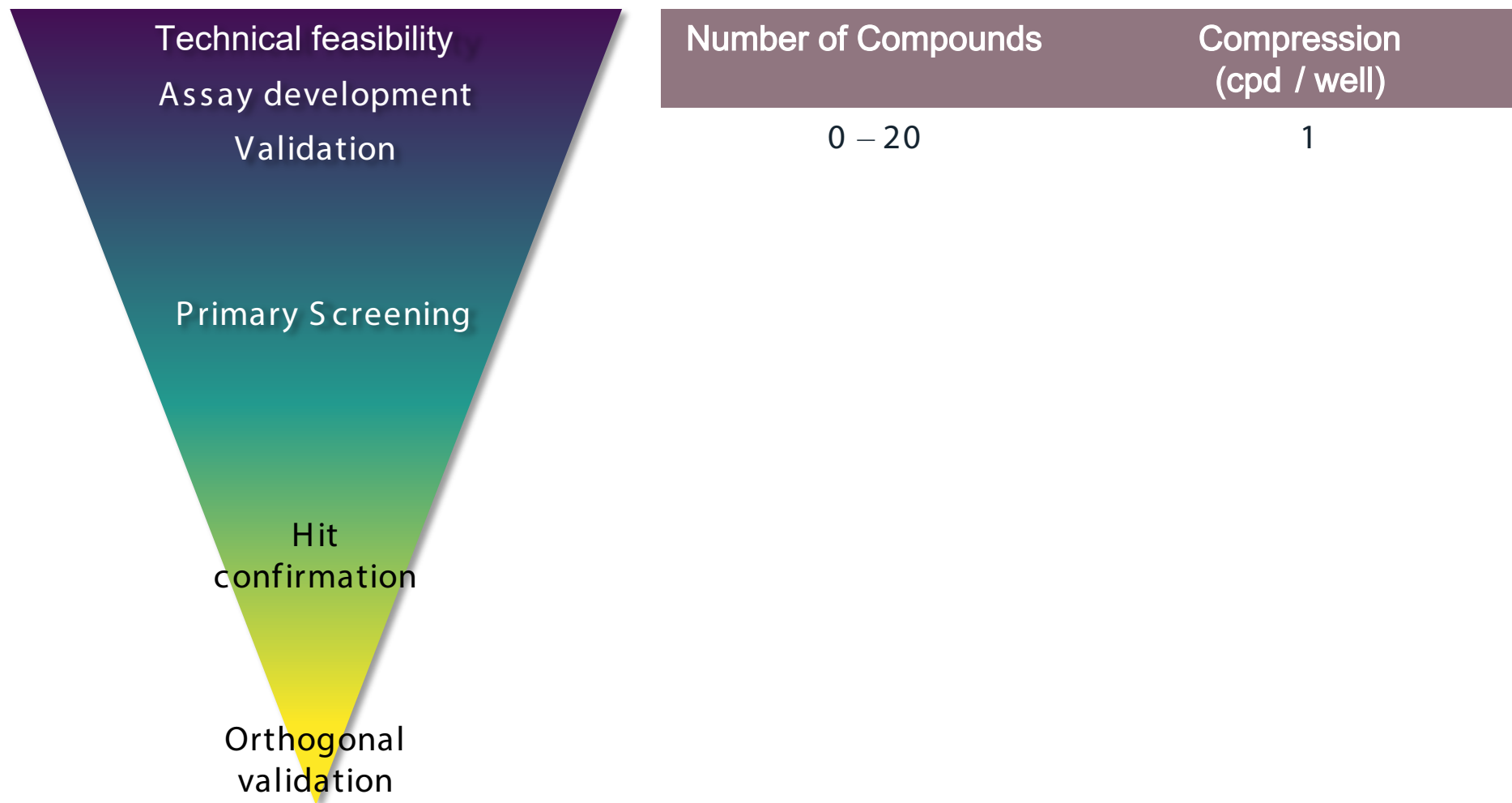
Challenges with AS-MS data analysis



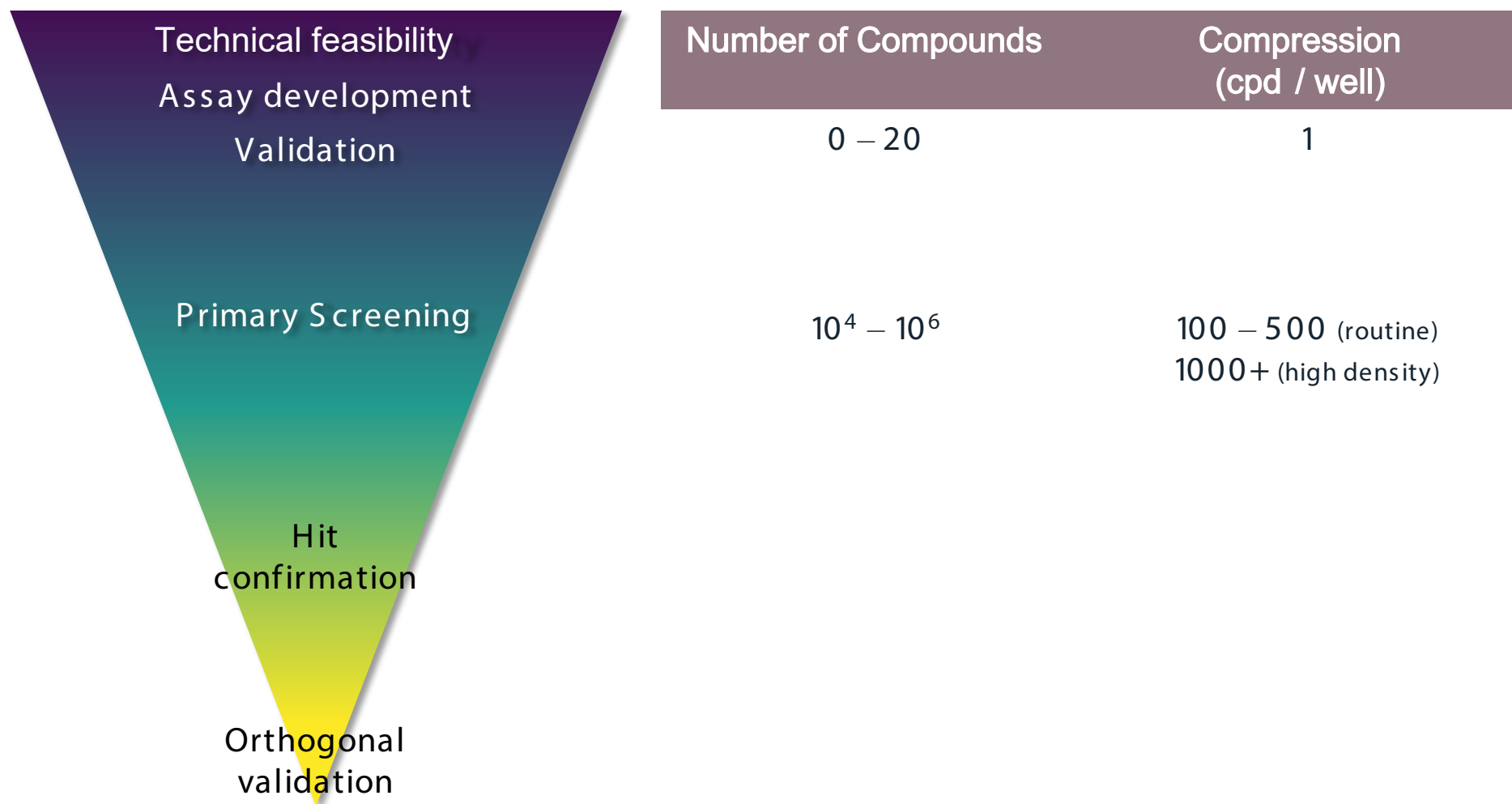
Designing your AS-MS assay cascades



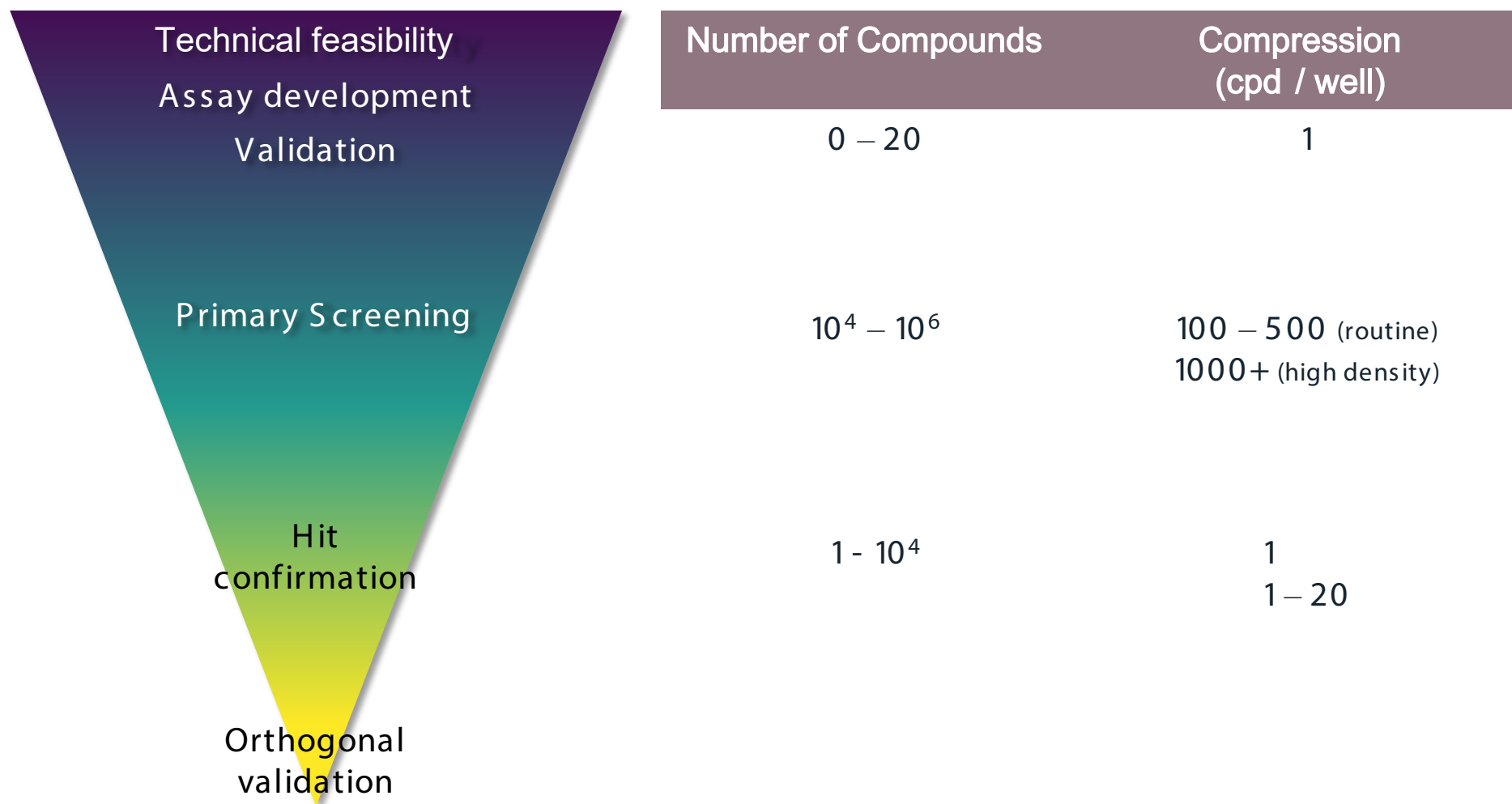
Designing your AS-MS assay cascades



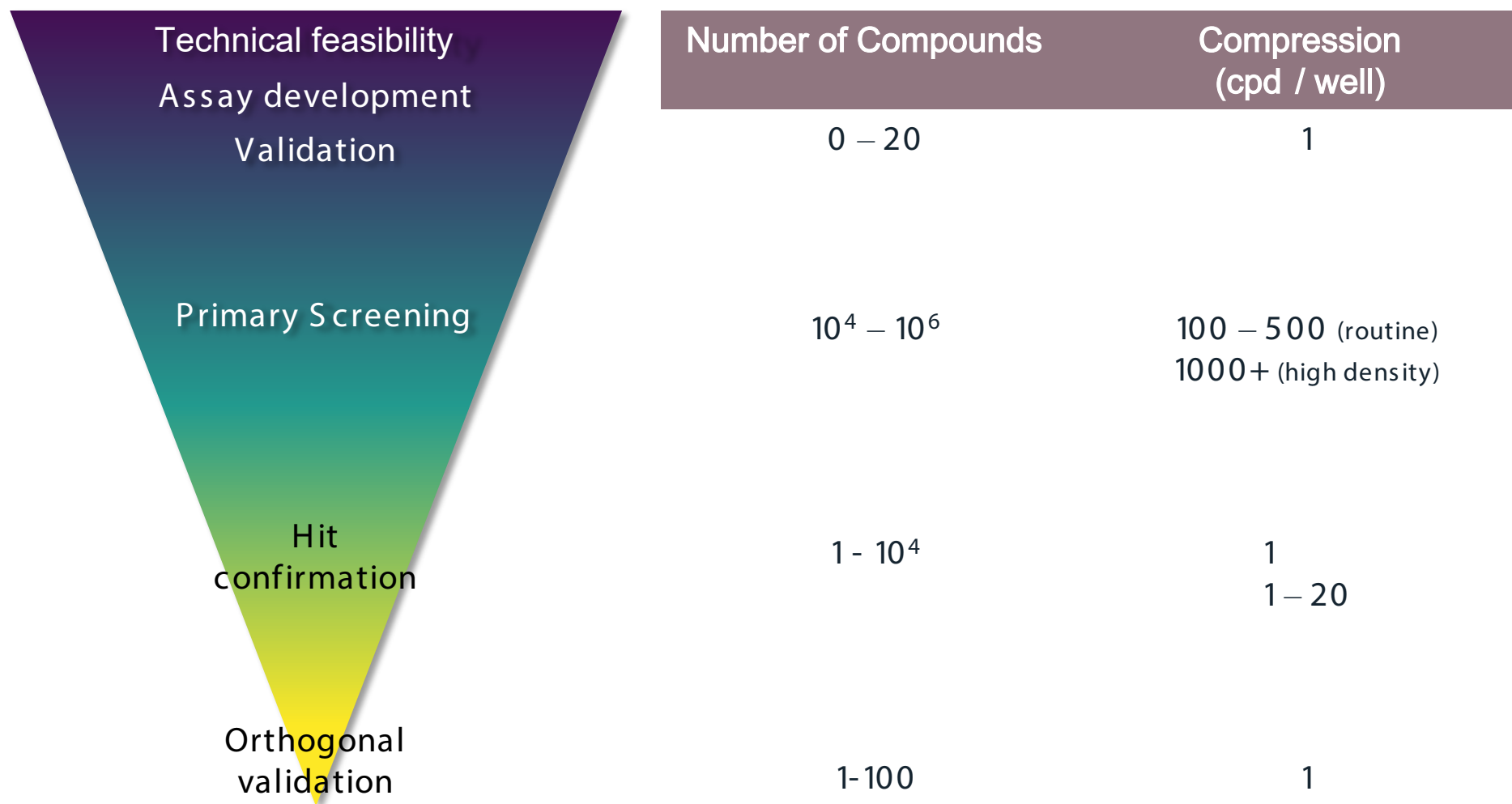
Designing your AS-MS assay cascades



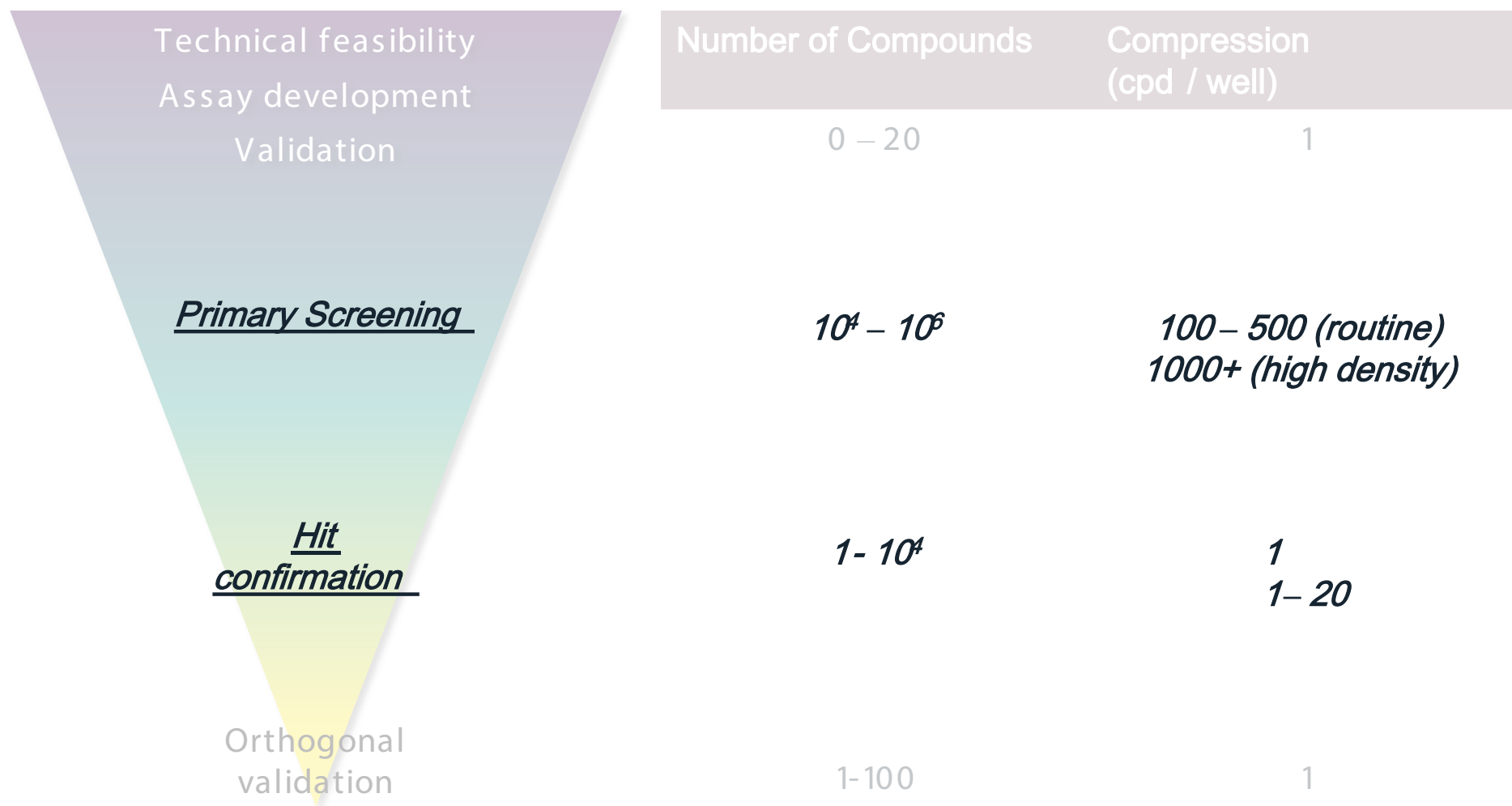
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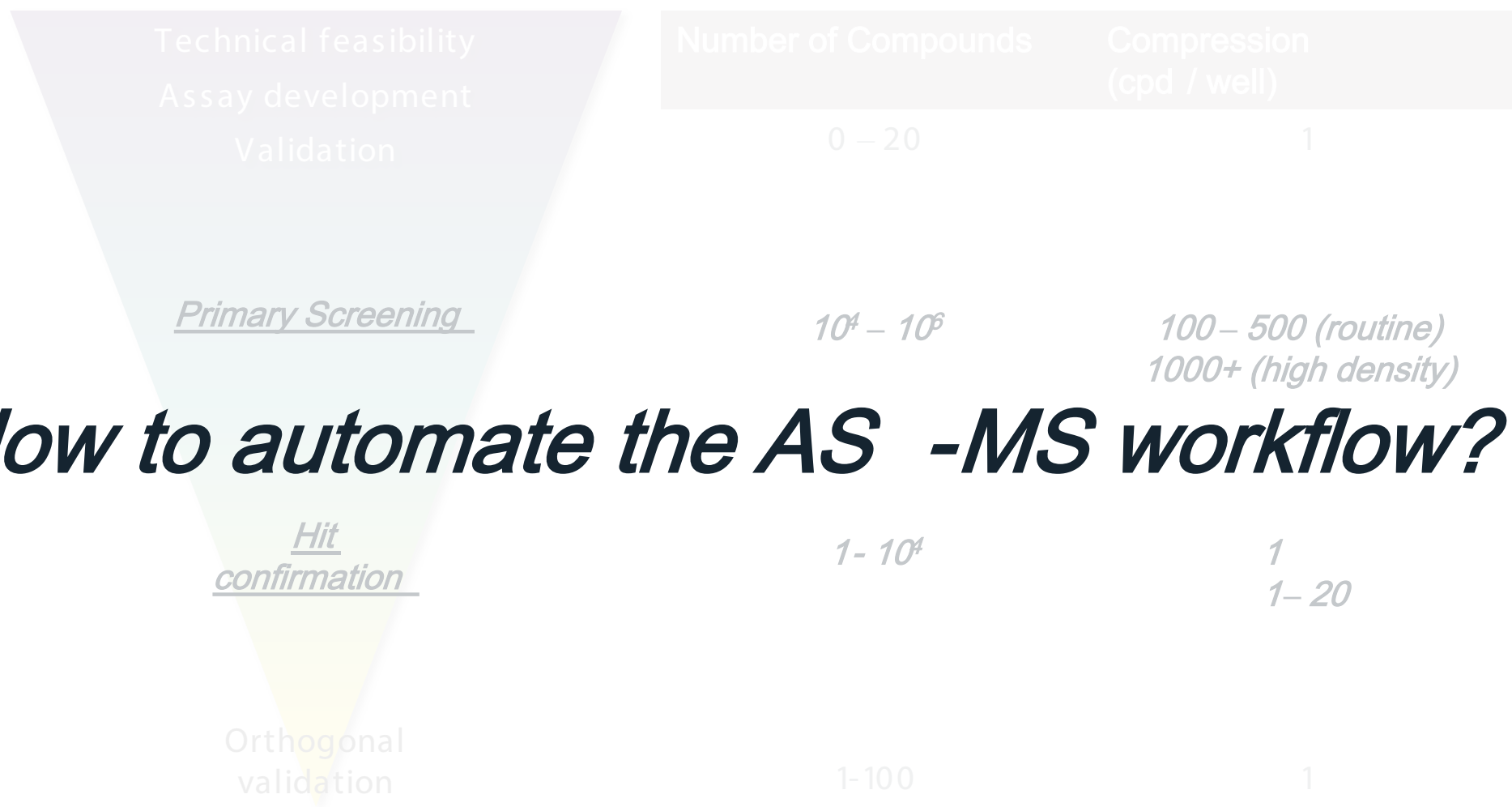
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Challenges with AS -MS data analysis



Challenges with AS -MS data analysis

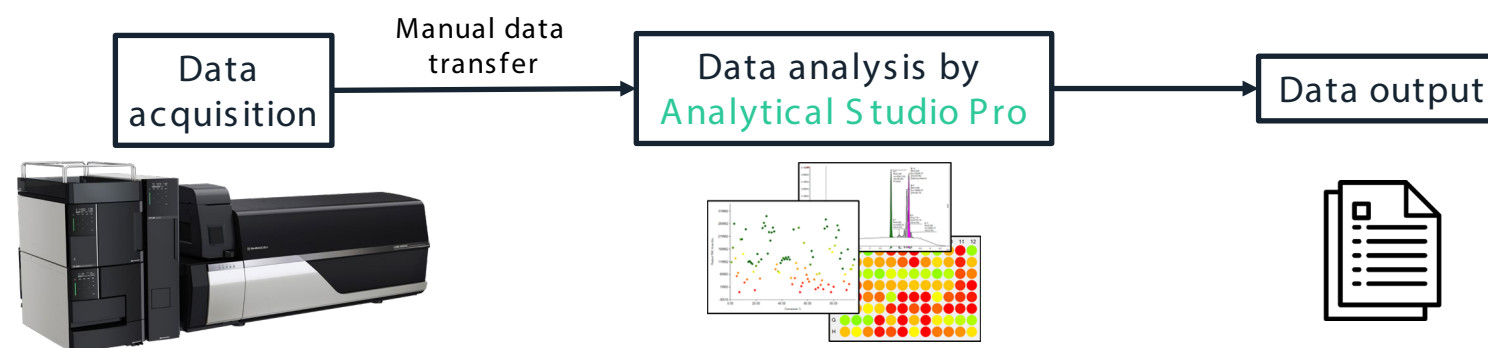


How to automate the AS -MS workflow?

Automating AS-MS:
data flow, processing,
and analysis of
multiplexed and dose
response data



Standalone environment

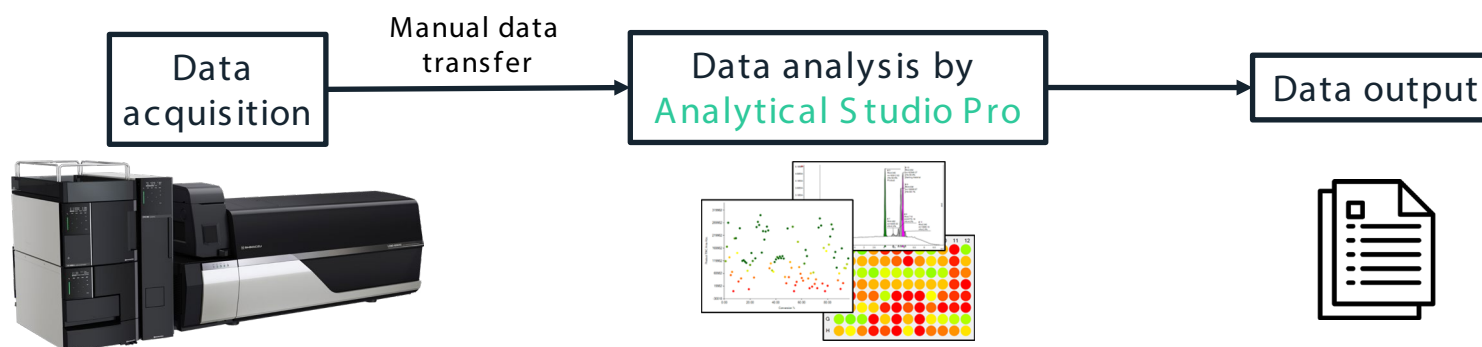


1. Create injection sequence
2. Format SI strings
3. Acquire data

4. Compound list → Create targets file
5. Load targets file
6. Open data
7. Perform filtering+cross-hit filtering

Standalone environment

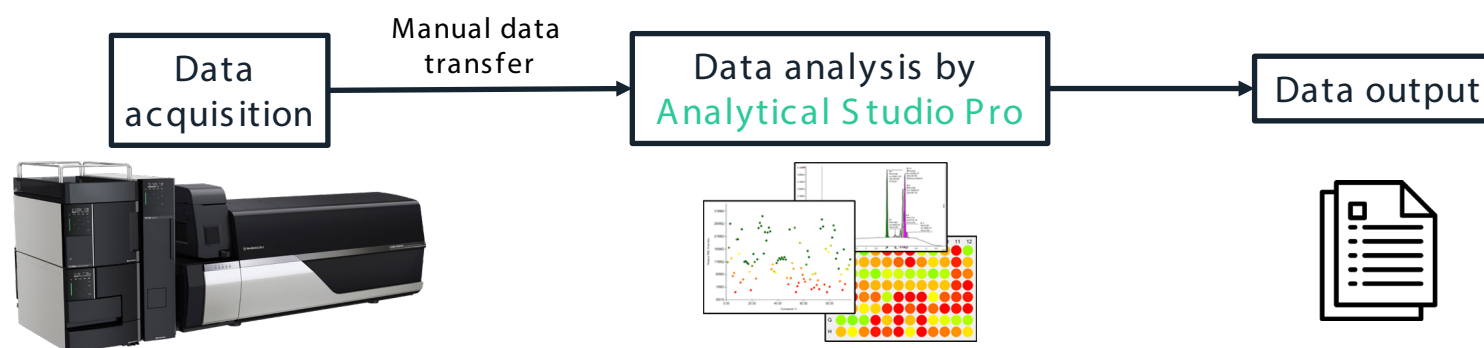
SI=AT!ASMS:CID!Plate123567:PL!01TL;XY;NL;1+8;2+12:F!Formula1_Formula2_Formula..._Formula500:



1. Create injection sequence
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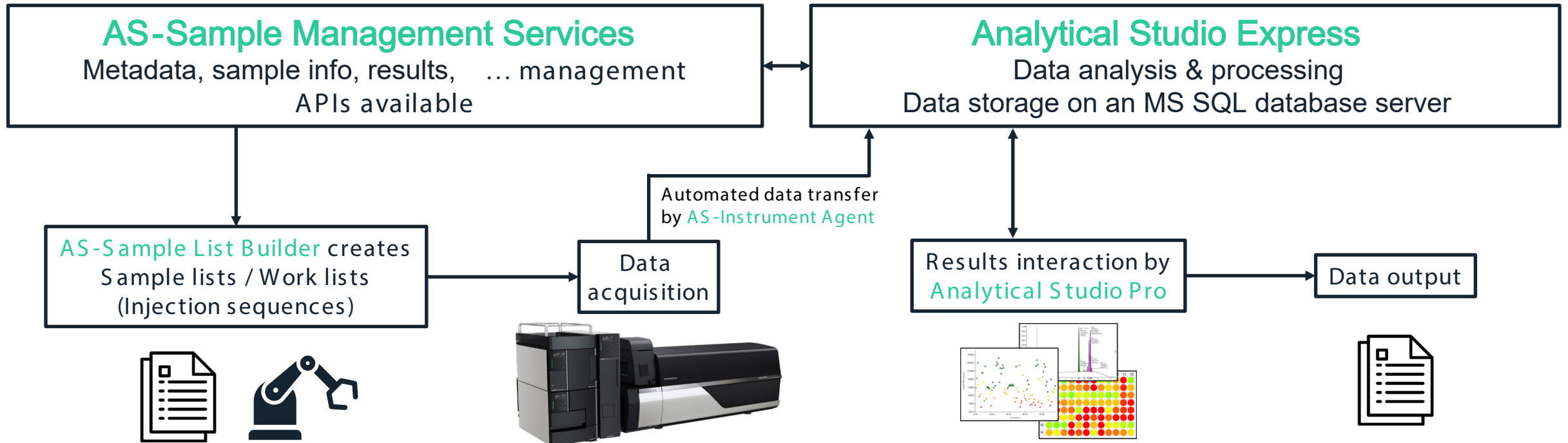
Standalone environment



1. Create injection sequence
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4. Compound list → Create targets file
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AS-MS "Automation"

Analytical Studio Sample Management Environment



Automated AS -MS Workflow

Submission and Sample List Generation

A compound list with ID #'s, formulas, and key details for compound identification and processing is submitted to Analytical Studio Sample Management Services (AS-SMS), which generates a sample list.

Initial Analysis

Data is acquired and is analyzed behind the scenes. Peak integration and compound identification based on mass analysis and isotopic pattern matching.

Cross-Hit Filtering

Users review data and remove false positives by identification of background noise across all samples.

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Submitting a compound list to AS-SMS and generating a sample list

A user:

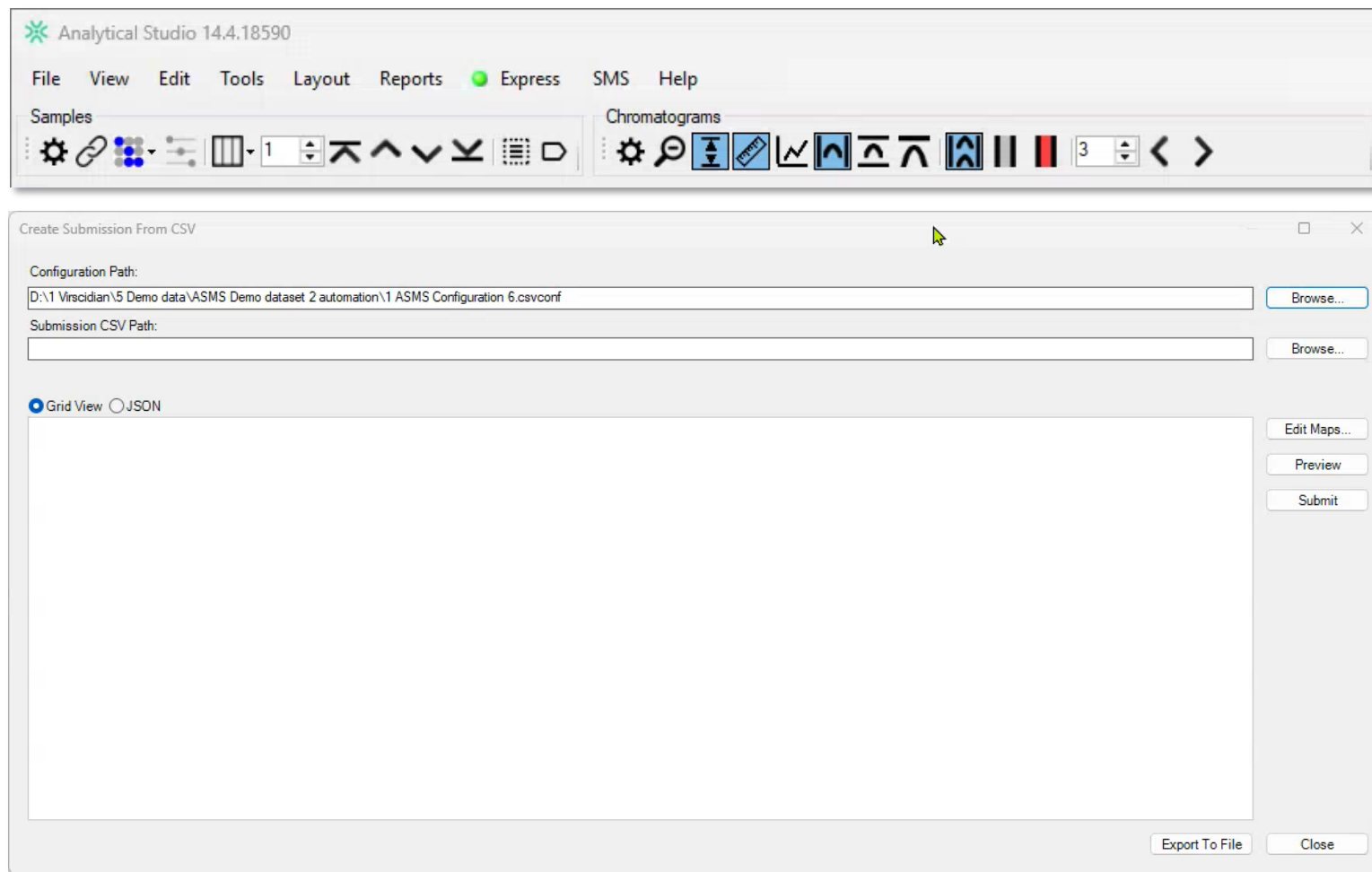
1. Curates a compound list
2. Submits the compound list to AS -SMS
3. Generates a sample list
4. Acquires data

	A	B	C	D	E	F
1	ASMS campaign number	Plate Barcode	PLATE_TYPE	Well	Compound ID	Formula
2	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000001	C17H12N2O4S
3	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000002	C19H29N3O3
4	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000003	C16H11BrN2O3
5	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000004	C17H15NO2
6	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000005	C11H7BrO2
7	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000006	C15H12O6
8	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000007	C21H26FN3O3
9	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000008	C20H24FN3O4
10	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000009	C14H17FN2O6S
11	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000010	C17H15NO5
12	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000011	C15H14N2O4
13	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000012	C16H13NO4S
14	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000013	C20H20N4O5
15	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000014	C18H24N2O2
16	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000015	C17H21N3O3
17	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000016	C10H10F3N3O3
18	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000017	C16H21N3O
19	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000018	C19H17NO4
20	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000019	C15H10F2N2O4S

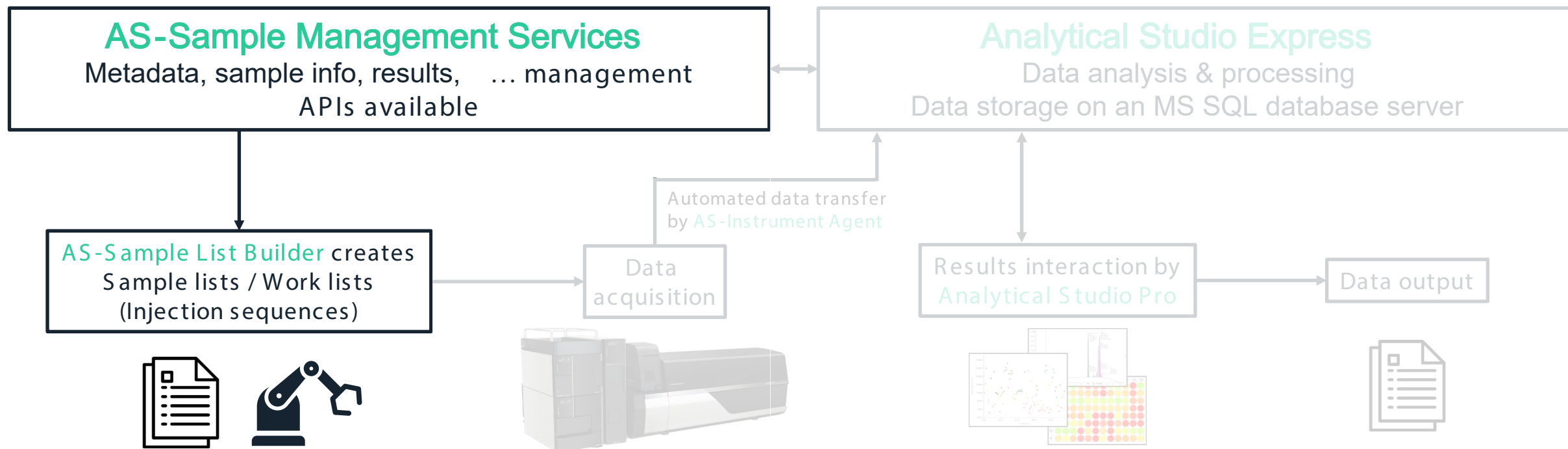
Submitting a compound list to AS-SMS and generating a sample list

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Submitting a compound list to AS-SMS and generating a sample list



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Analytical Studio - Sample List Builder 1.6 RTM

Submissions View Sample Lists View

Submission Browser

Submissions Dashboard

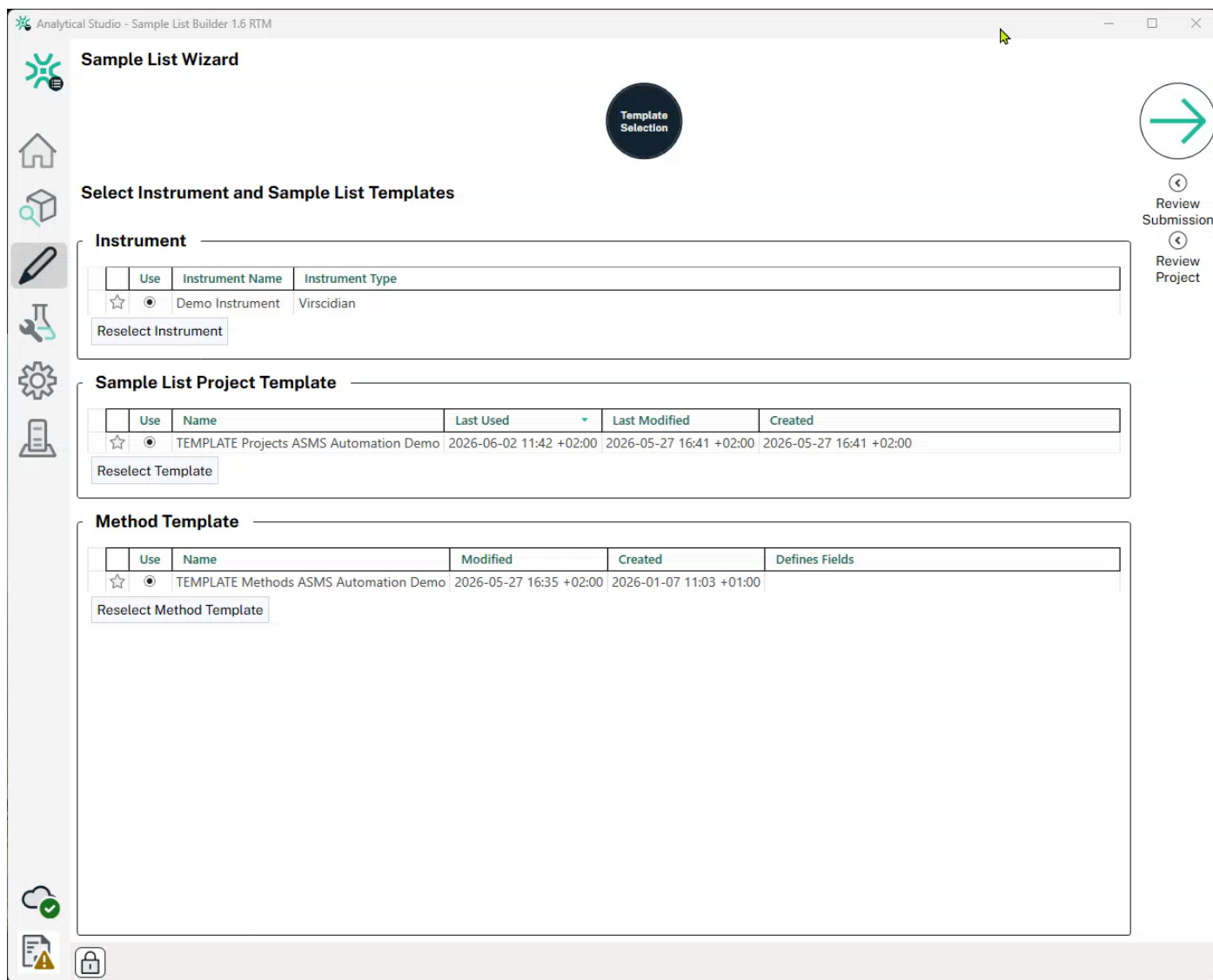
ELN	Submission ID	Parent Submission ID	Submission State	Priority	Project	Workflow Name	Workflow Stage Alias	Needed By
ASMS Automation Demo 1	10	0	Received	0	ASMS	CrudeQC	CrudeQC	

Sample Lists Non-Analytical Sample Lists Submission Lineage Containers External Controls Submission Organization Sample Data

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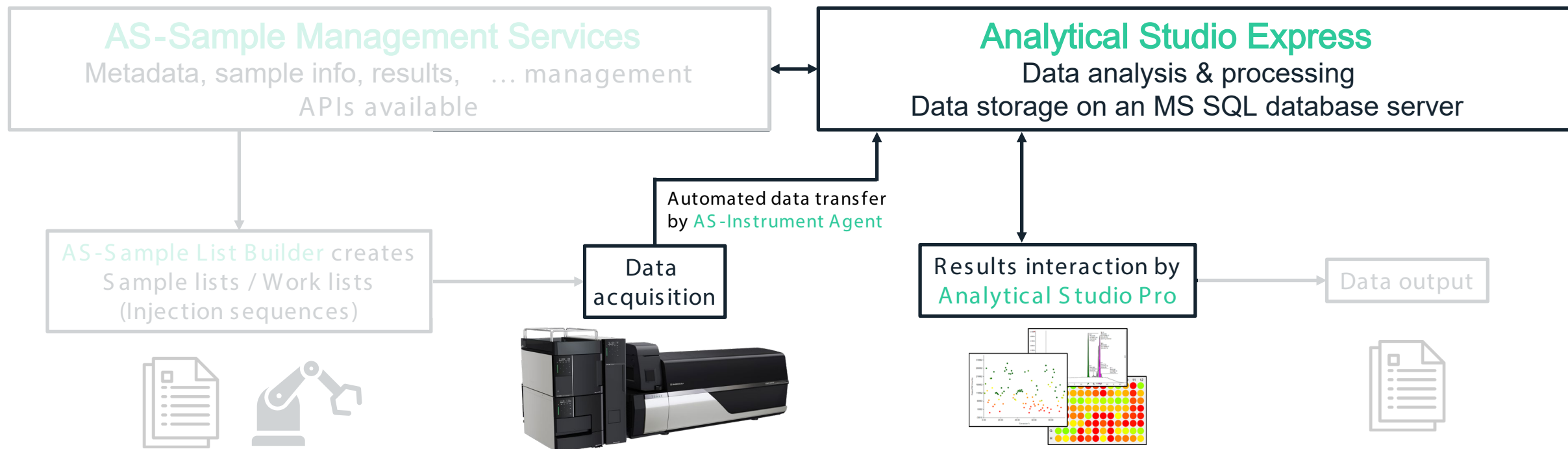
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Users review data and remove false positives by identification of background noise across all samples.

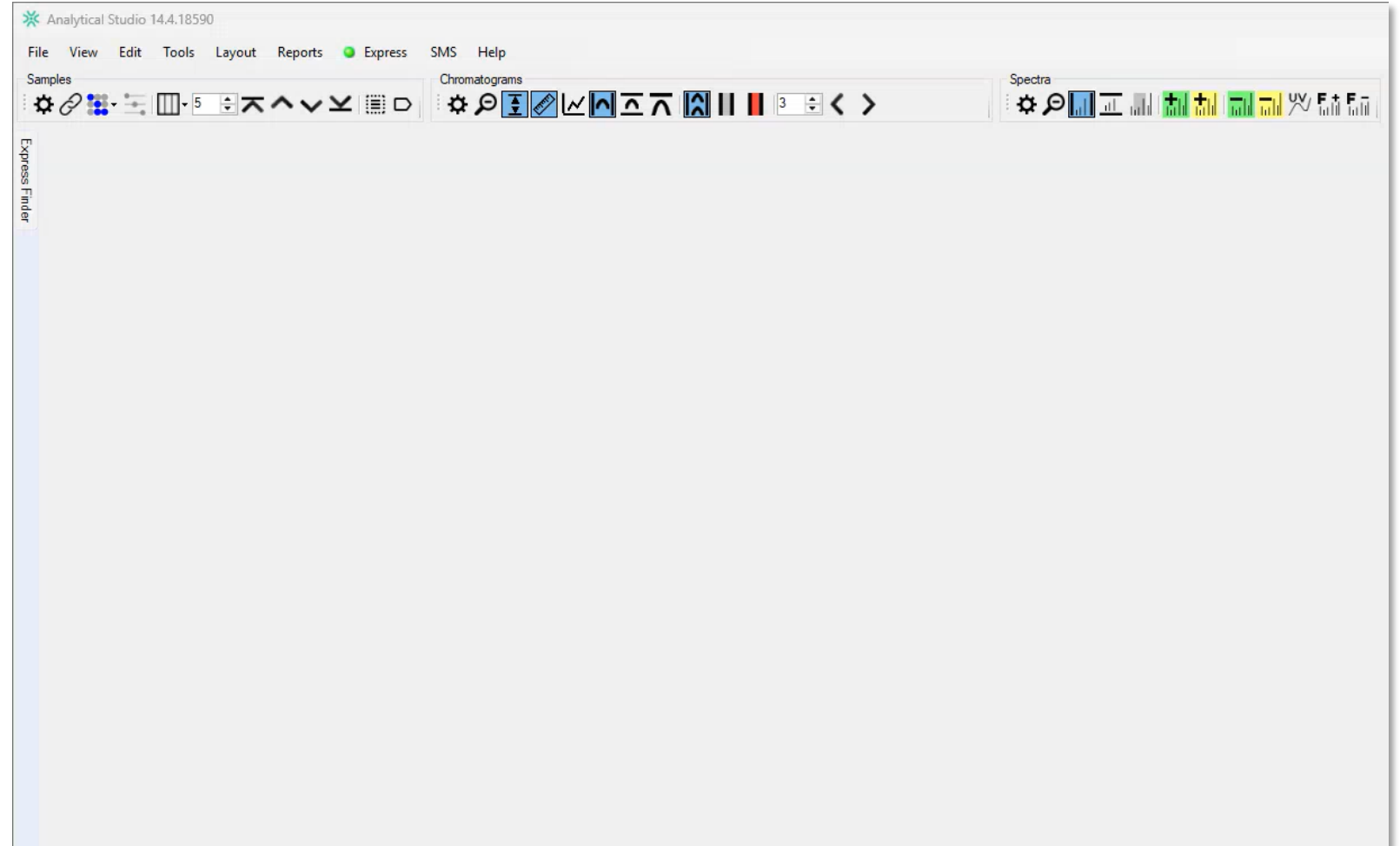
Submitting a compound list to AS-SMS and generating a sample list



Initial analysis: peak integration and compound identification based on mass analysis and isotopic pattern matching

From Analytical Studio -
Express finder:

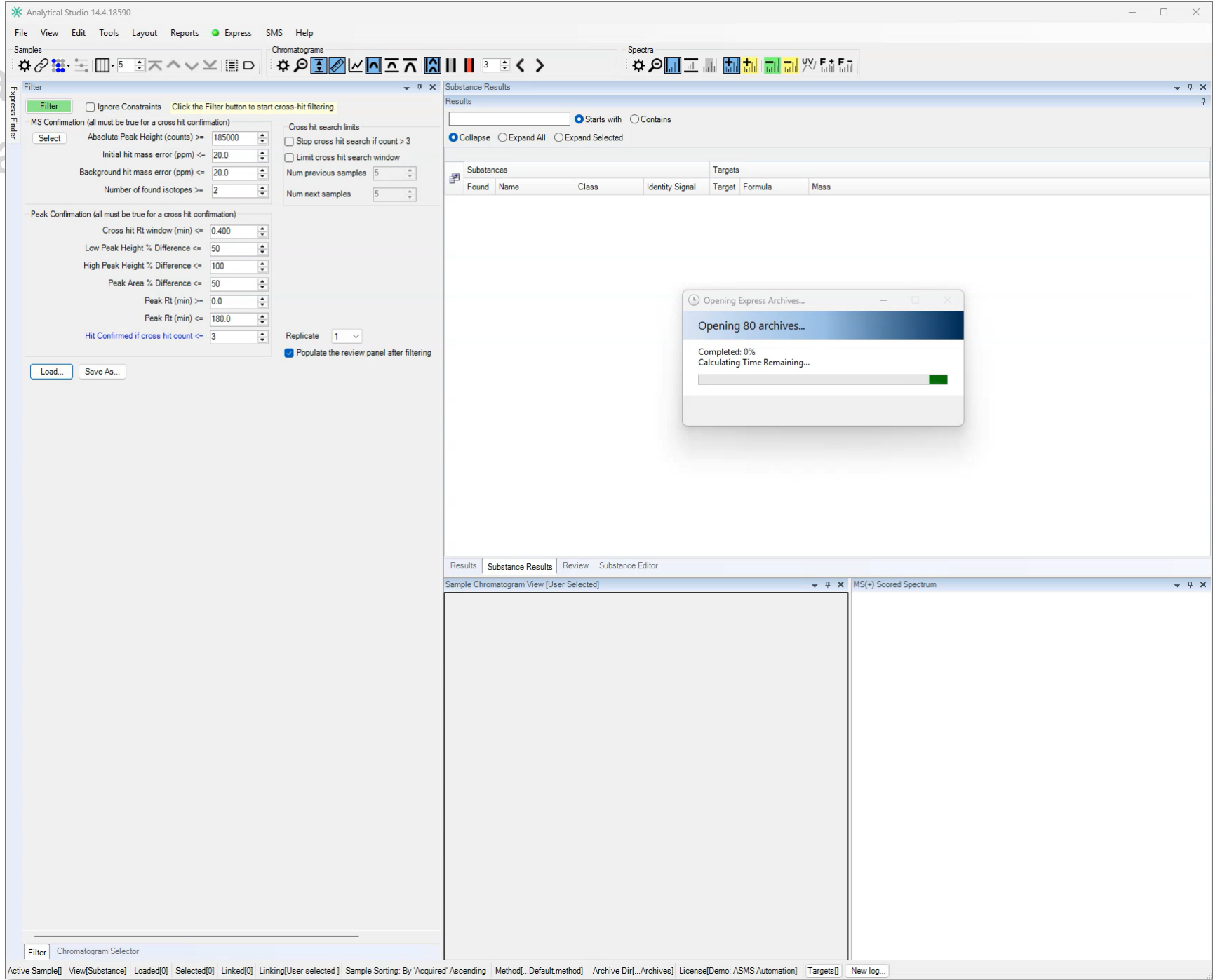
1. Query database
2. Download and (optionally) review results
3. Perform filtering



Initial analysis: peak based on mass ana

From Analytical Studio - Express finder:

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Cross-Hit Filtering background noise

With data downloaded:

1. Perform filtering
2. Interpret results

The screenshot displays the Analytical Studio 14.4.18590 interface. On the left, a grid shows data for Plate123567 across 24 columns and 16 rows (A-P). A 10x10 grid of red squares is visible in the center. Below the grid is a 'Filter' panel with various settings for MS Confirmation, Peak Confirmation, and Cross hit search limits. The main window shows a table of results with columns for Location, Name, C.xHits, F.xHits, # Chrms, Peak Height, Rt(min), Error(ppm), Observed(mz), Expected(mz), and Adducts. The table lists numerous hits, including A03, A04, A05, and A06. Below the table, there are buttons for 'Override selected results using' and 'Sample Chromatogram View'. The bottom right shows two chromatograms: a 'Sample Chromatogram View' and an 'MS(+) Scored Spectrum' for peak 1 at 2.642 min. The spectrum shows a major peak at 2.642 min and several smaller peaks at 394.2411, 395.8849, 396.8849, 397.1504, 398.1504, 398.3470, 399.1524, and 399.3470.

Location	Name	C.xHits	F.xHits	# Chrms	Peak Height	Rt(min)	Error(ppm)	Observed(mz)	Expected(mz)	Adducts
A03	Cpd0000013	0	0	1	3.80E+004	2.642	-9.33	397.1469	397.1506	[M+H]
A03	Cpd0000130	0	0	1	1.32E+006	2.257	9.83	302.1780	302.1751	[M+H]
A04	Cpd0000250	0	0	1	8.30E+004	1.882	9.82	308.1887	308.1856	[M+H]
A04	Cpd0000344	0	0	1	7.26E+004	2.252	1.48	324.1599	324.1594	[M+H]
A04	Cpd0000260	0	0	1	4.19E+004	1.023	9.57	292.1571	292.1543	[M+H]
A05	Cpd0000508	0	0	1	1.79E+005	1.035	8.94_9_16_9	312.1622_312.162	312.1594_312.159	[M+H]_[M+H]_[M+H]_[M+H]
A05	Cpd0000528	0	0	1	1.15E+005	1.002	5.86	264.1259	264.1244	[M+H]
A05	Cpd0000508-2.82	0	0	1	4.27E+004	2.815	8.94_9_16_9	312.1622_312.162	312.1594_312.159	[M+H]_[M+H]_[M+H]_[M+H]
A05	Cpd0000530	0	0	1	1.06E+005	1.880	5.51	308.1887	308.1870	[M+H]
A05	Cpd0000471	0	0	1	6.43E+004	3.537	0.23	349.1118	349.1118	[M+H]
A05	Cpd0000461	0	0	1	6.72E+004	5.857	-0.84	325.1479	325.1481	[M+H]
A05	Cpd0000600	0	0	1	8.58E+005	2.824	-6.70	262.1645	262.1662	[M+H]
A05	Cpd0000564	0	0	1	4.69E+004	4.490	-1.31	386.1970	386.1975	[M+H]
A05	Cpd0000508-1.07	0	0	1	1.61E+005	1.071	8.94_9_16_9	312.1622_312.162	312.1594_312.159	[M+H]_[M+H]_[M+H]_[M+H]
A05	Cpd0000490	0	0	1	7.09E+004	2.384	-4.90	288.1441	288.1455	[M+H]
A05	Cpd0000508-2.13	0	0	1	3.68E+004	2.134	8.94_9_16_9	312.1622_312.162	312.1594_312.159	[M+H]_[M+H]_[M+H]_[M+H]
A05	Cpd0000416	0	0	1	5.53E+004	6.502	6.94	313.1793	313.1771	[M+H]
A05	Cpd0000578	0	0	1	9.05E+004	5.765	-1.12	315.1125	315.1128	[M+H]
A05	Cpd0000423	0	0	1	6.01E+005	2.353	-5.76	252.1441	252.1455	[M+H]
A06	Cpd0000737	0	0	1	8.73E+004	5.753	-0.51	391.1875	391.1877	[M+H]
A06	Cpd0000785	0	0	1	8.91E+004	6.589	-4.89	356.2791	356.2809	[M+H]
A06	Cpd0000788	0	0	1	3.49E+004	3.590	-0.69	288.1929	288.1931	[M+H]
A06	Cpd0000730	0	0	1	5.27E+004	3.720	-0.44	371.1323	371.1325	[M+H]
A06	Cpd0000662	0	0	1	5.19E+004	4.727	-0.50	379.2014	379.2016	[M+H]
A06	Cpd0000732	0	0	1	3.81E+004	1.032	4.65	292.1570	292.1557	[M+H]
A06	Cpd0000694	0	0	1	5.01E+004	2.579	-5.06	261.1333	261.1346	[M+H]
A06	Cpd0000699	0	0	1	5.45E+004	4.060	-0.60	365.1274	365.1276	[M+H]
A06	Cpd0000631	0	0	1	1.52E+006	2.357	-5.97	280.1751	280.1768	[M+H]
A06	Cpd0000775	0	0	1	4.48E+004	5.586	-6.29	369.1247	369.1270	[M+H]
A06	Cpd0000768	0	0	1	8.97E+005	4.120	-5.34	296.1854	296.1870	[M+H]
A06	Cpd0000653	0	0	1	5.24E+004	4.064	7.83	279.0932	279.0910	[M+H]
A06	Cpd0000777	0	0	1	5.41E+004	1.064	4.57	306.1727	306.1713	[M+H]

Cross-Hit Filtering background noise

With data downloaded:

1. Perform filtering
2. Interpret results

The screenshot displays the Analytical Studio 14.4.18590 interface. The top-left panel shows a grid of 24 samples (A-P) across 24 columns, with a 10x10 grid of red squares indicating detected hits. The top-right panel shows the 'Results' table with columns for Location, Name, C x Hits, F x Hits, # Chroms, Peak Height, Rt (min), Error (ppm), Observed (m/z), Expected (m/z), and Adducts. The table lists 61 possible hits, with 6 confirmed hits and a 0.04% hit rate. The bottom-left panel shows the 'Filter' settings, including MS Confirmation (Absolute Peak Height >= 185000, Initial hit mass error <= 20.0, Background hit mass error <= 20.0, Number of found isotopes >= 2) and Peak Confirmation (Cross hit Rt window <= 0.400, Low Peak Height % Difference <= 50, High Peak Height % Difference <= 100, Peak Area % Difference <= 50, Peak Rt (min) >= 0.0, Peak Rt (min) <= 180.0, Hit Confirmed if cross hit count <= 3). The bottom-right panel shows a 'Sample Chromatogram View' for peak #10 at 2.390 minutes, with a mass spectrum plot showing relative intensity (%) versus m/z. The mass spectrum shows a base peak at m/z 386.1443 and other peaks at 382.1870, 387.1476, 388.1443, 389.1476, 390.1476, 391.1476, 392.1476, 393.1476, 394.1476, 395.1476, 396.1476, 397.1476, 398.1476, 399.1476, 400.1476, 401.1476, 402.1476, 403.1476, 404.1476, 405.1476, 406.1476, 407.1476, 408.1476, 409.1476, 410.1476, 411.1476, 412.1476, 413.1476, 414.1476, 415.1476, 416.1476, 417.1476, 418.1476, 419.1476, 420.1476, 421.1476, 422.1476, 423.1476, 424.1476, 425.1476, 426.1476, 427.1476, 428.1476, 429.1476, 430.1476, 431.1476, 432.1476, 433.1476, 434.1476, 435.1476, 436.1476, 437.1476, 438.1476, 439.1476, 440.1476, 441.1476, 442.1476, 443.1476, 444.1476, 445.1476, 446.1476, 447.1476, 448.1476, 449.1476, 450.1476, 451.1476, 452.1476, 453.1476, 454.1476, 455.1476, 456.1476, 457.1476, 458.1476, 459.1476, 460.1476, 461.1476, 462.1476, 463.1476, 464.1476, 465.1476, 466.1476, 467.1476, 468.1476, 469.1476, 470.1476, 471.1476, 472.1476, 473.1476, 474.1476, 475.1476, 476.1476, 477.1476, 478.1476, 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Cross-Hit Filtering removes false positives by identifying background noise across all samples.

Filter Ignore Constraints Click the Filter button to start c

MS Confirmation (all must be true for a cross hit confirmation)

Select Absolute Peak Height (counts) >= 185000

Initial hit mass error (ppm) <= 20.0

Background hit mass error (ppm) <= 20.0

Number of found isotopes >= 2

Peak Confirmation (all must be true for a cross hit confirmation)

Cross hit Rt window (min) <= 0.400

Low Peak Height % Difference <= 50

High Peak Height % Difference <= 85

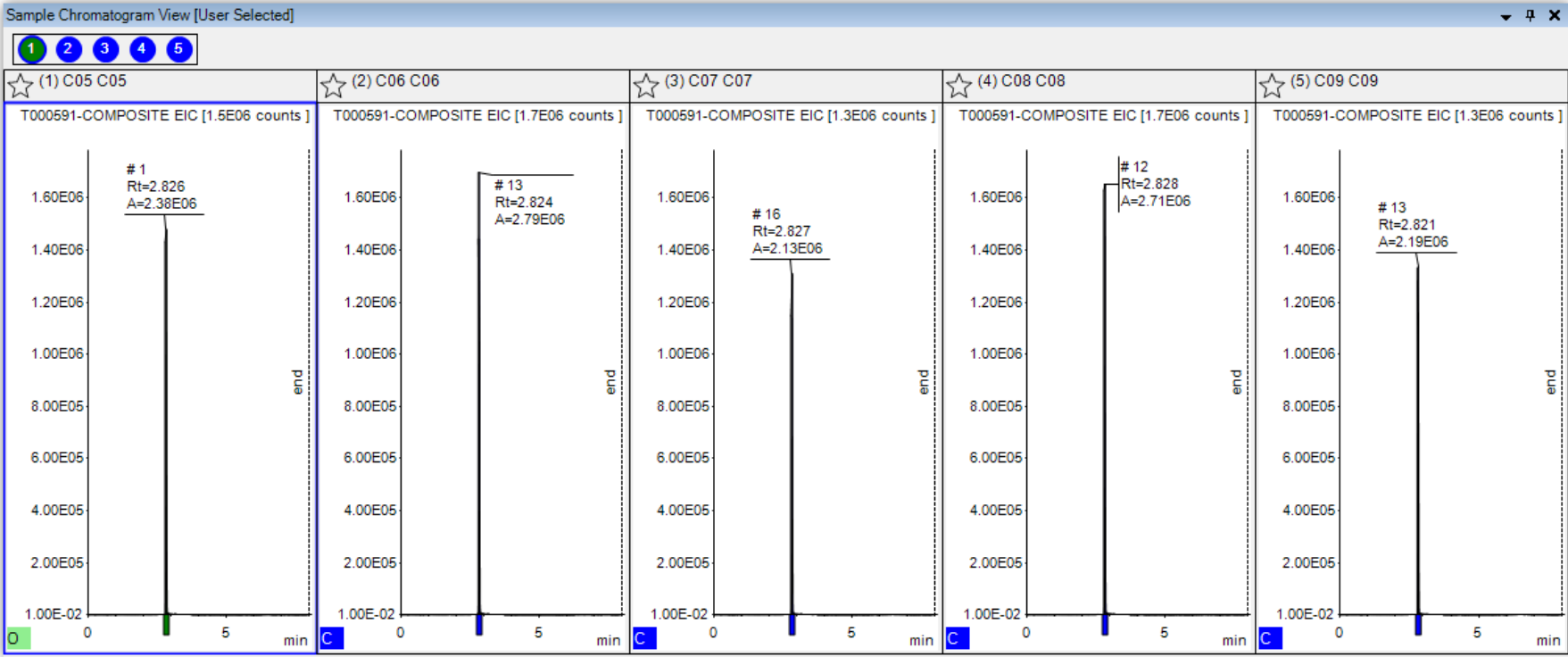
Peak Area % Difference <= 85

Peak Rt (min) >= 0.0

Peak Rt (min) <= 180.0

Hit Confirmed if cross hit count <= 3

Load... Save As...



Cross-Hit Filtering removes false positives by identifying background noise across all samples.

Filter Ignore Constraints **Click the Filter button to start c**

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Peak Confirmation (all must be true for a cross hit confirmation)

Cross hit Rt window (min) <= 0.400

Low Peak Height % Difference <= 50

High Peak Height % Difference <= 85

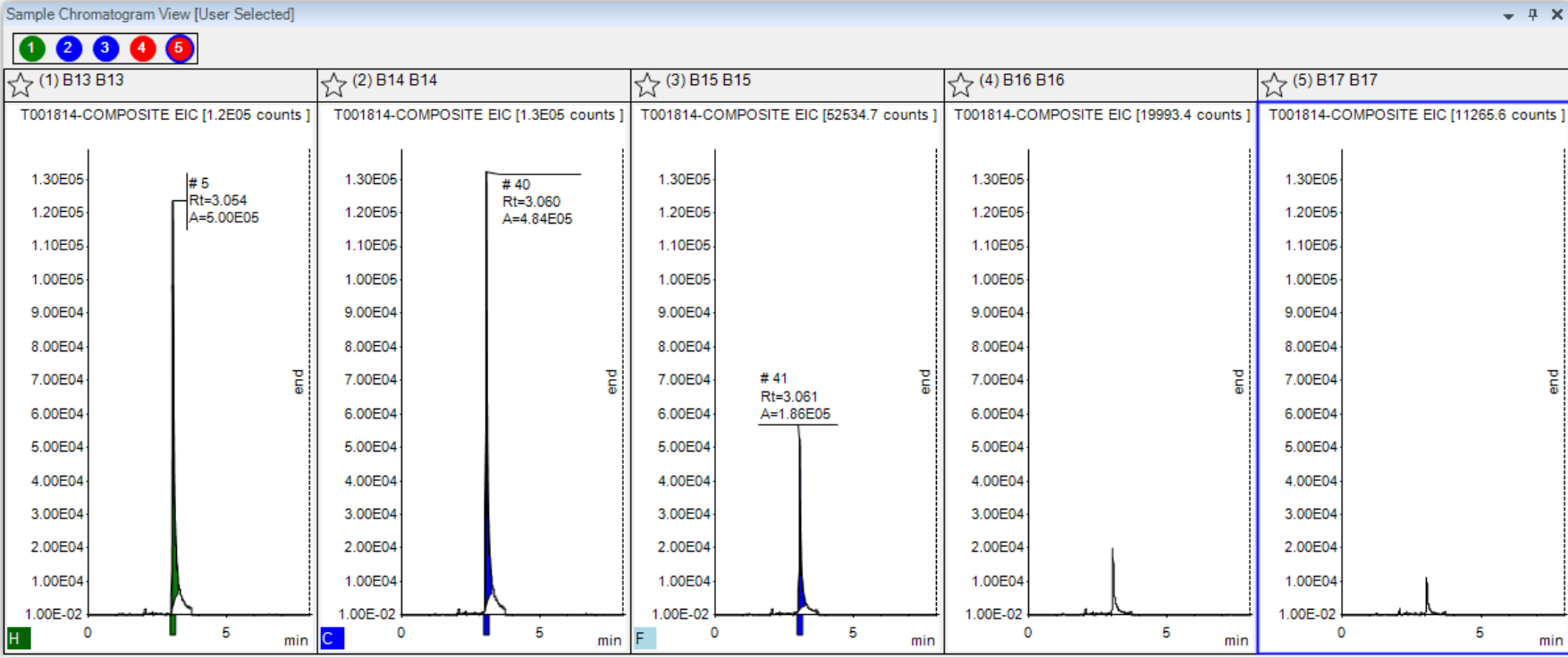
Peak Area % Difference <= 85

Peak Rt (min) >= 0.0

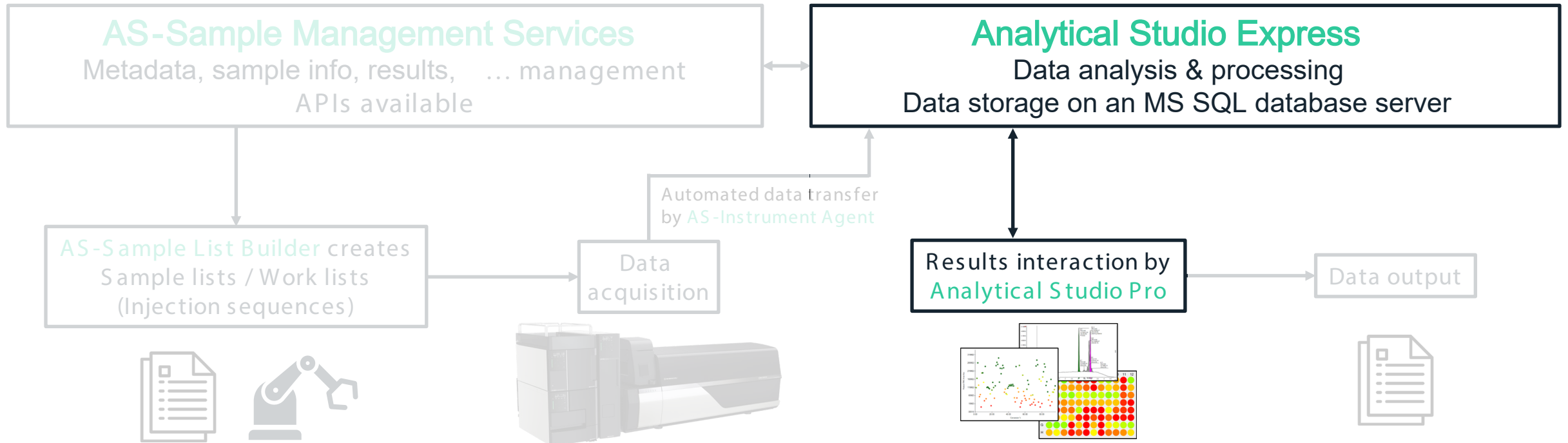
Peak Rt (min) <= 180.0

Hit Confirmed if cross hit count <= 3

Load... Save As...



Cross-Hit Filtering removes false positives by identifying background noise across all samples.



Automated K_d Analysis

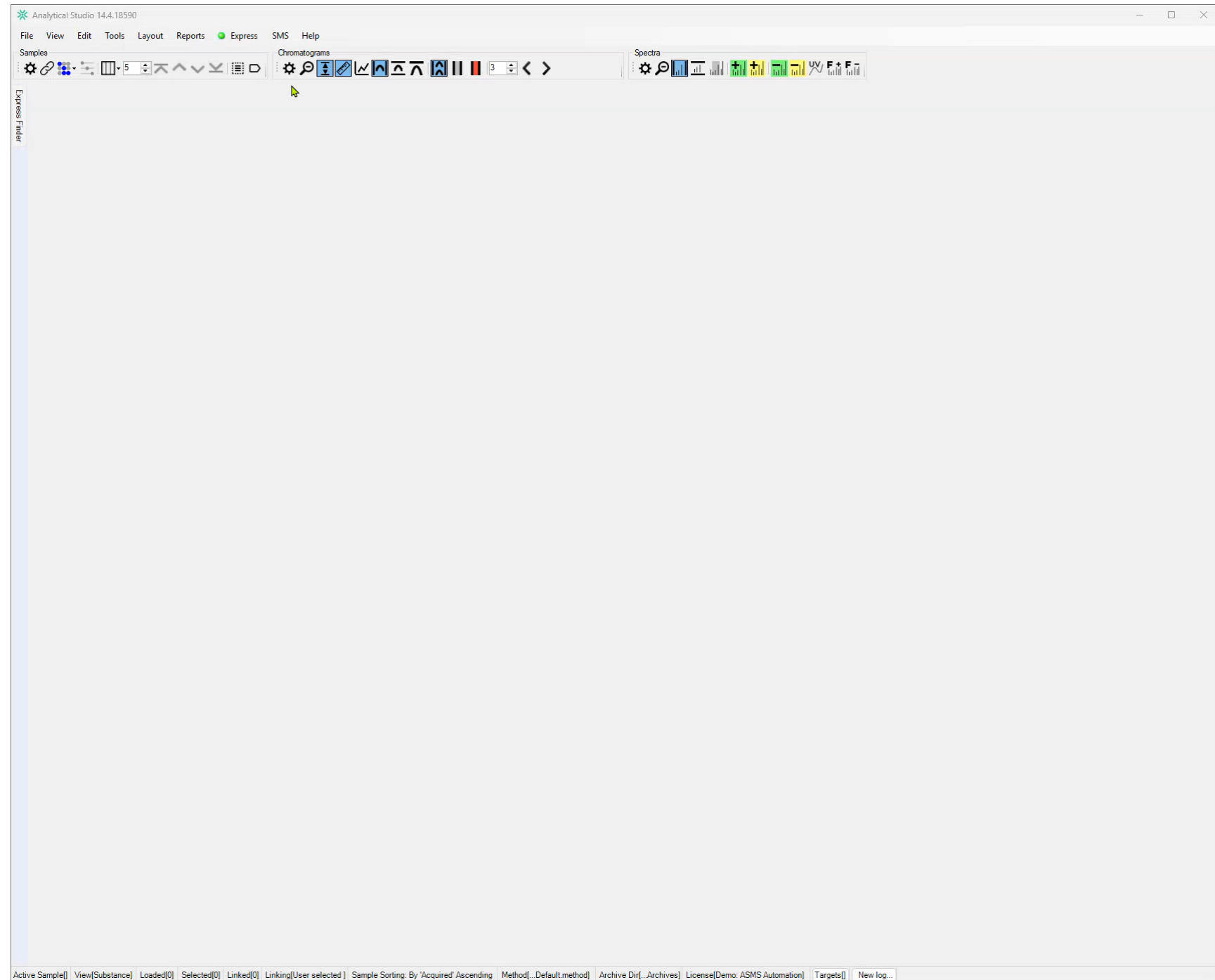
A user:

1. Curates a compound list
2. Submits the compound list to AS -SMS
3. Generates a sample list
4. Acquires data
5. Download data to AS-Pro & interpret results

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AS-MS improvements

	Standalone	AS-SMS+Express
Compound list	Manual	Manual
Sample list	Manually created	Automated via AS -SMS and AS-Sample List Builder
Metadata	Manually curate Sample Info strings	Automated via AS -SMS and AS-Sample List Builder
Initial processing	File > Open > Targets File > Raw data	Download data from AS - Express
Cross-hit filtering	Benchmark	70-80% speed improvement, with the same results
Automation potential	Limited	High potential with API access and integration capabilities

Affinity Selection - Mass Spec

Identifying binders and analyzing dose response data

Automation

Sample list generation, data transfer, and processing

Software improvements

Cross-Hit Filtering and K_d analysis

