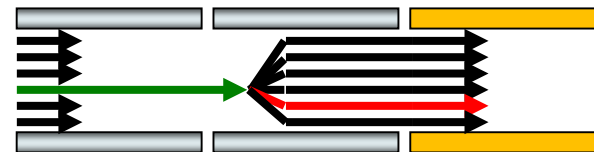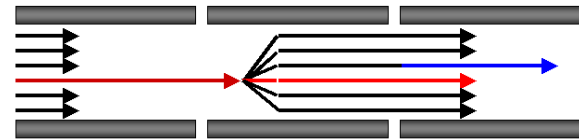# Skyline

Targeted Proteomics Environment

Processing DDA Data with Skyline

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Chromatography-based Quantification

- Hypothesis testing (Verification)

- SRM
- **MS1 chromatogram extraction**
- Targeted MS/MS (PRM)
- Data independent acquisition (DIA/SWATH)

Acquisition	Targeted	Survey
More Selective	PRM	DIA
Less Selective	SRM	DDA

Got HYPOTHESIS??

Discovery versus Validation

‣ Discovery

  ‣ asking your data what changed

‣ Validation

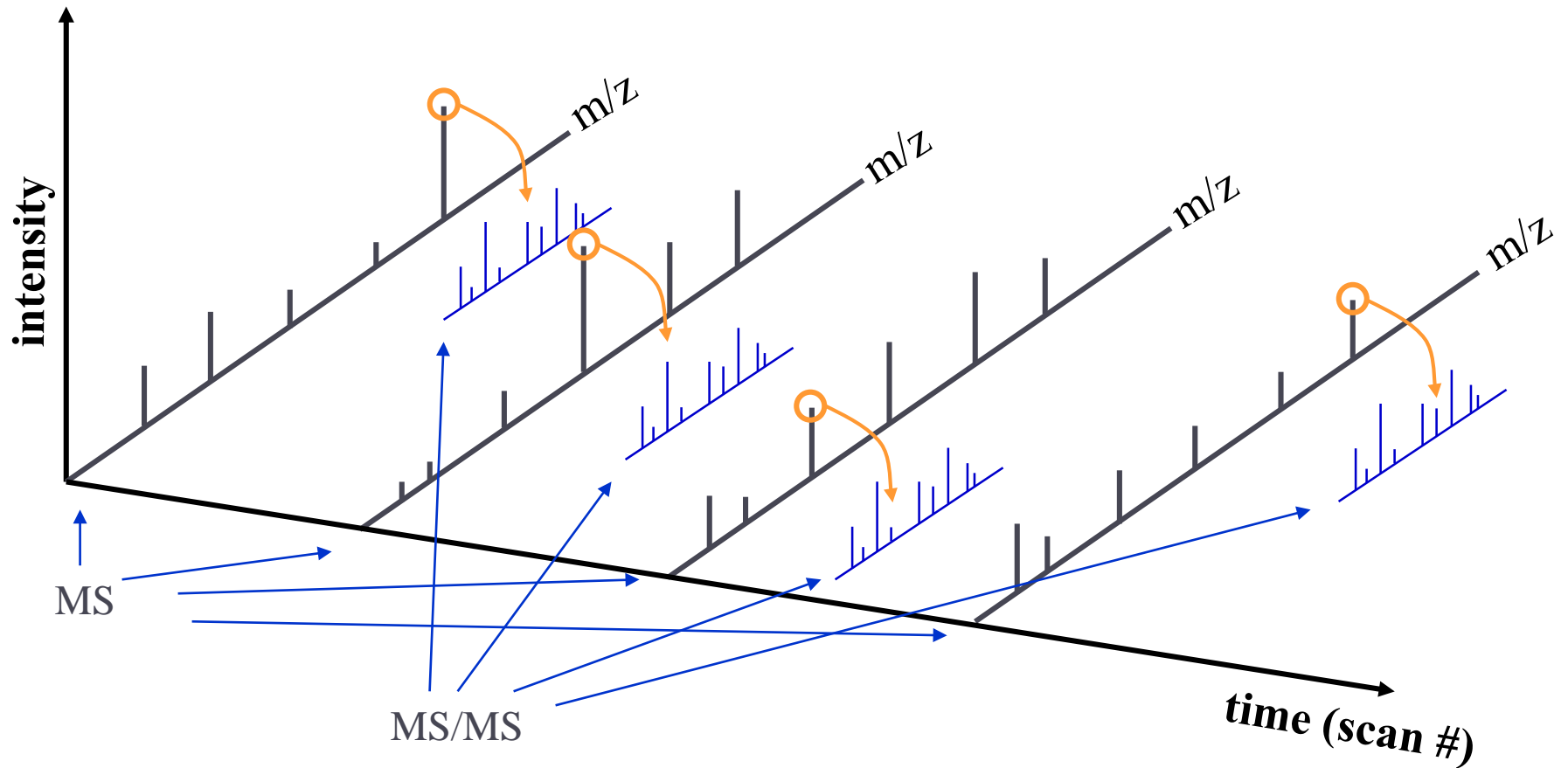 ‣ asking if there is evidence you candidates changed
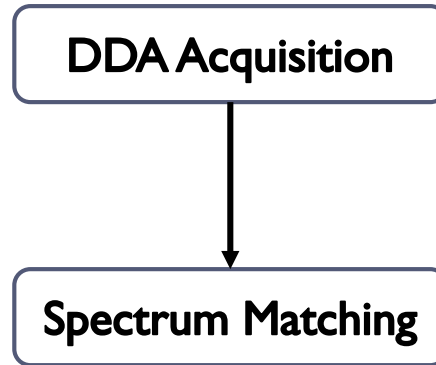
Got HYPOTHESIS??

Overview

DDA Acquisition

DDA Acquisition

Overview
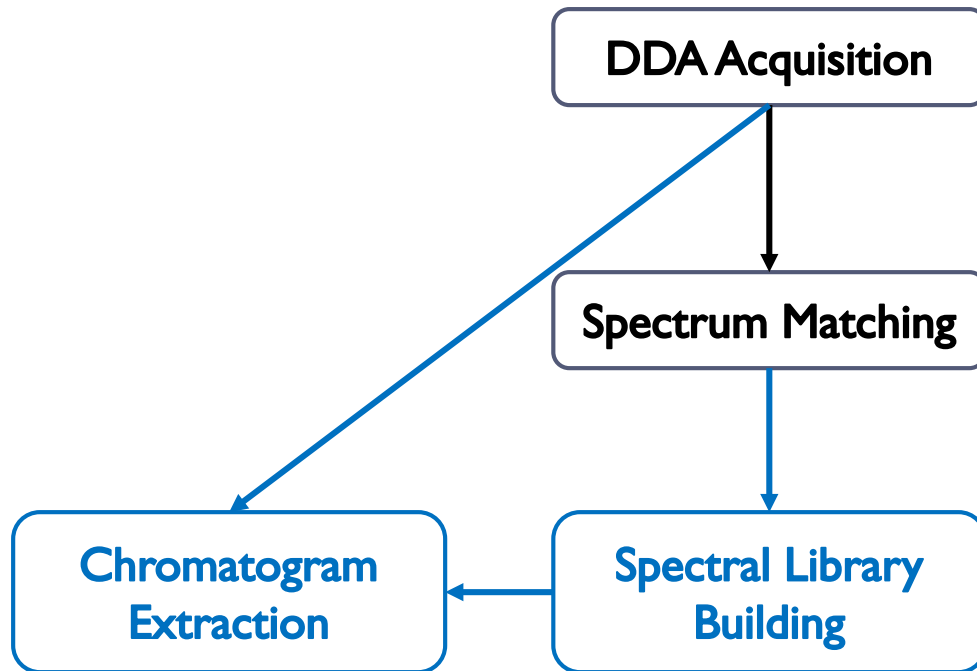
DDA Acquisition

↓

Spectrum Matching

Overview

DDA Acquisition

↓

Spectrum Matching

- *Mascot*
- ByOnic
- Myrimatch / IDPicker
- OMSSA
- MaxQuant Andromeda
- Morpheus
- MSGF+
- PRIDE XML
- Protein Pilot
- Protein Prospector
- Proteome Discoverer (MSF)
- Scaffold – mzIdentML / MGF
- Spectrum Mill
- TPP – pepXML / mzXML files – Peptide Atlas
- X! Tandem
- PLGS - Waters MSe

Overview
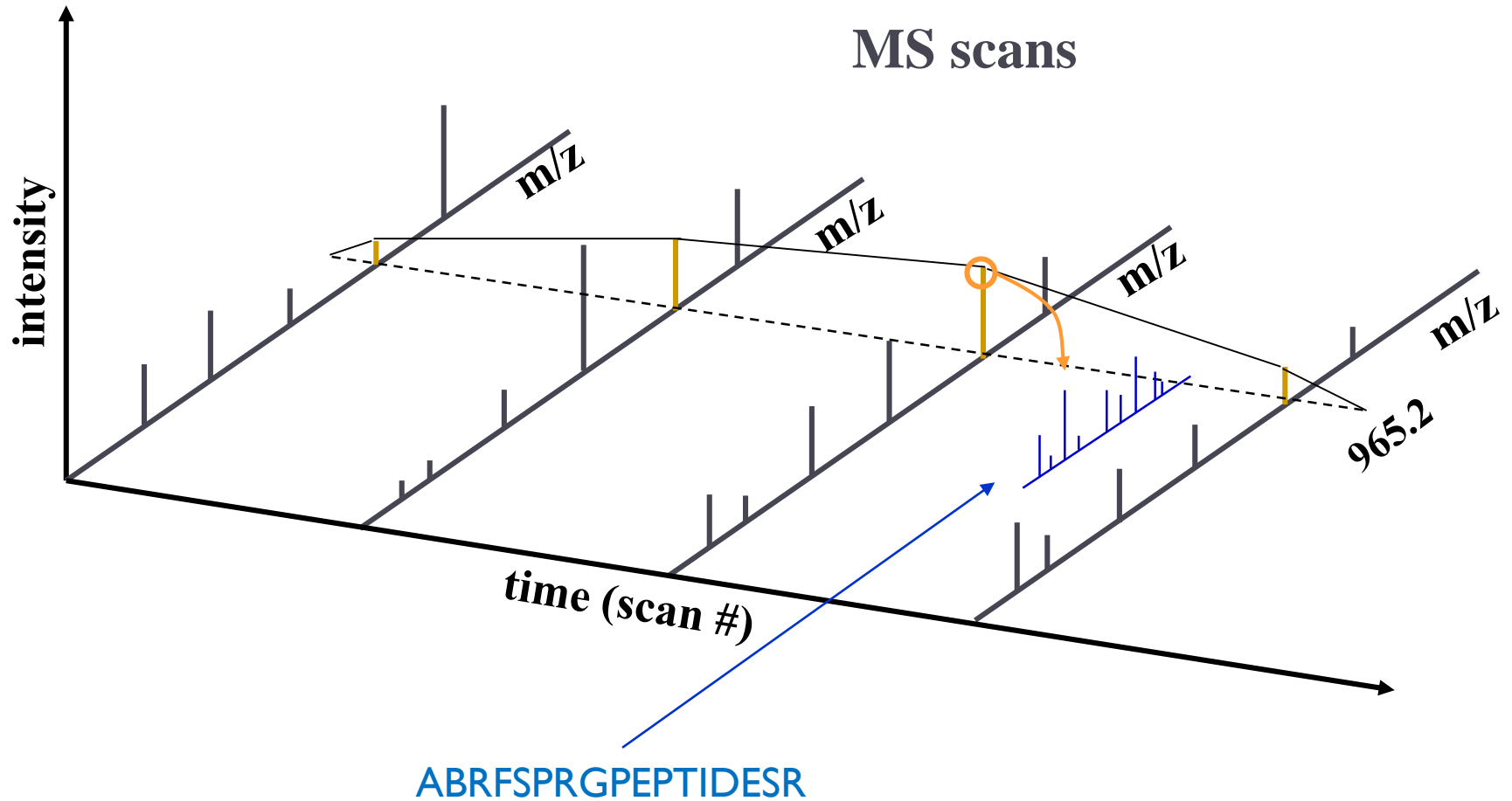
MS1 Chromatogram Extraction

MS1 Chromatogram Extraction in Skyline

Schilling, B. et al. Mol. Cell. Prot. 2012.
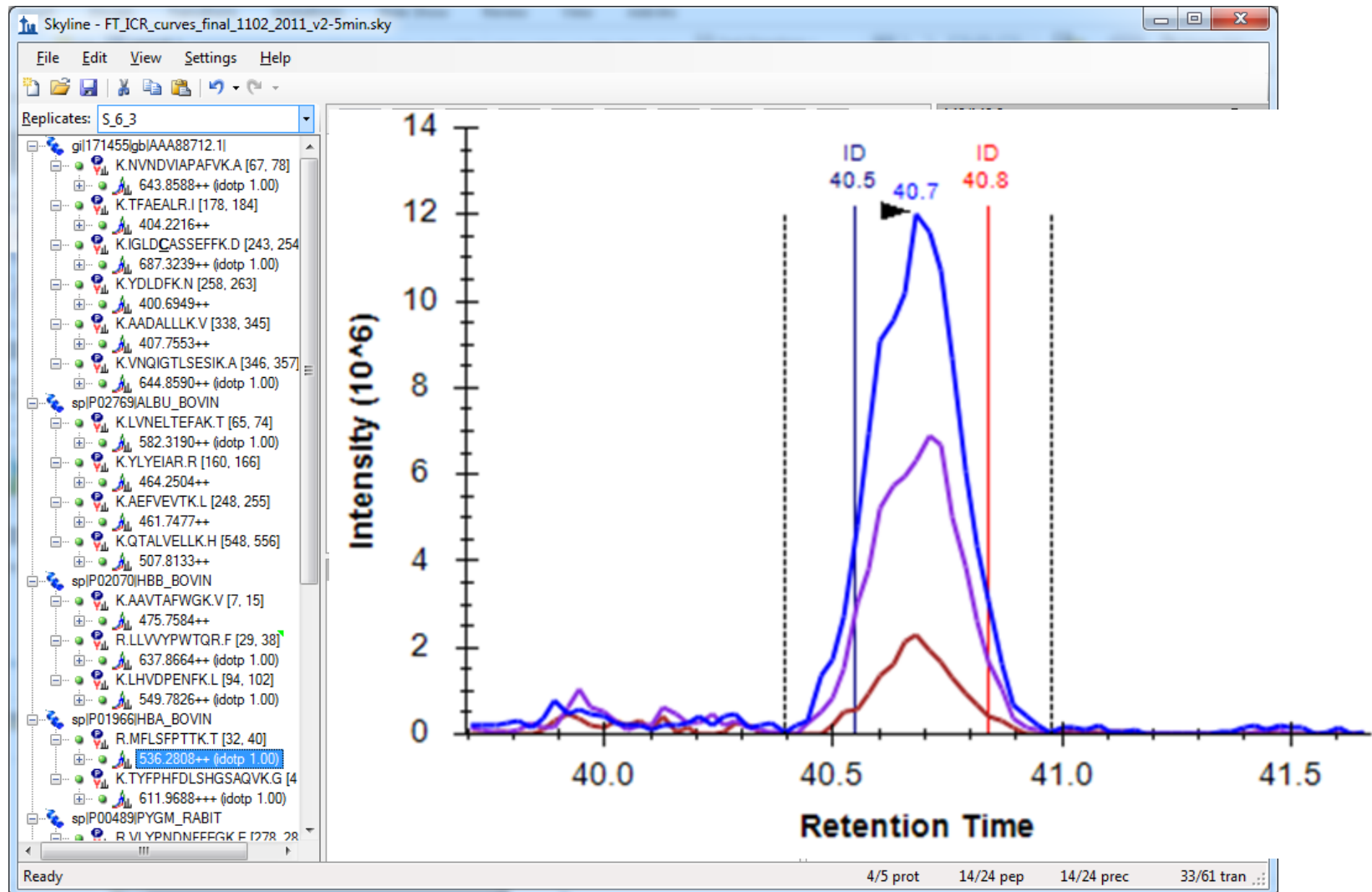
Multiple Instrument Vendors

Overview

Keys to Success with MS1 in Skyline

- Make sure you have ID annotations
    - Diagnose with Spectral Library Explorer
    - http://tinyurl.com/Skyline-missing-ids
    - Use File > Import > Peptide Search
- Review RT alignment in alignment viewer
- Got HYPOTHESIS??
- Review and manually adjust <5% of peaks (targeted)

- Use tutorial
  and webinars

Case Study 2: ABRF iPRG 2014

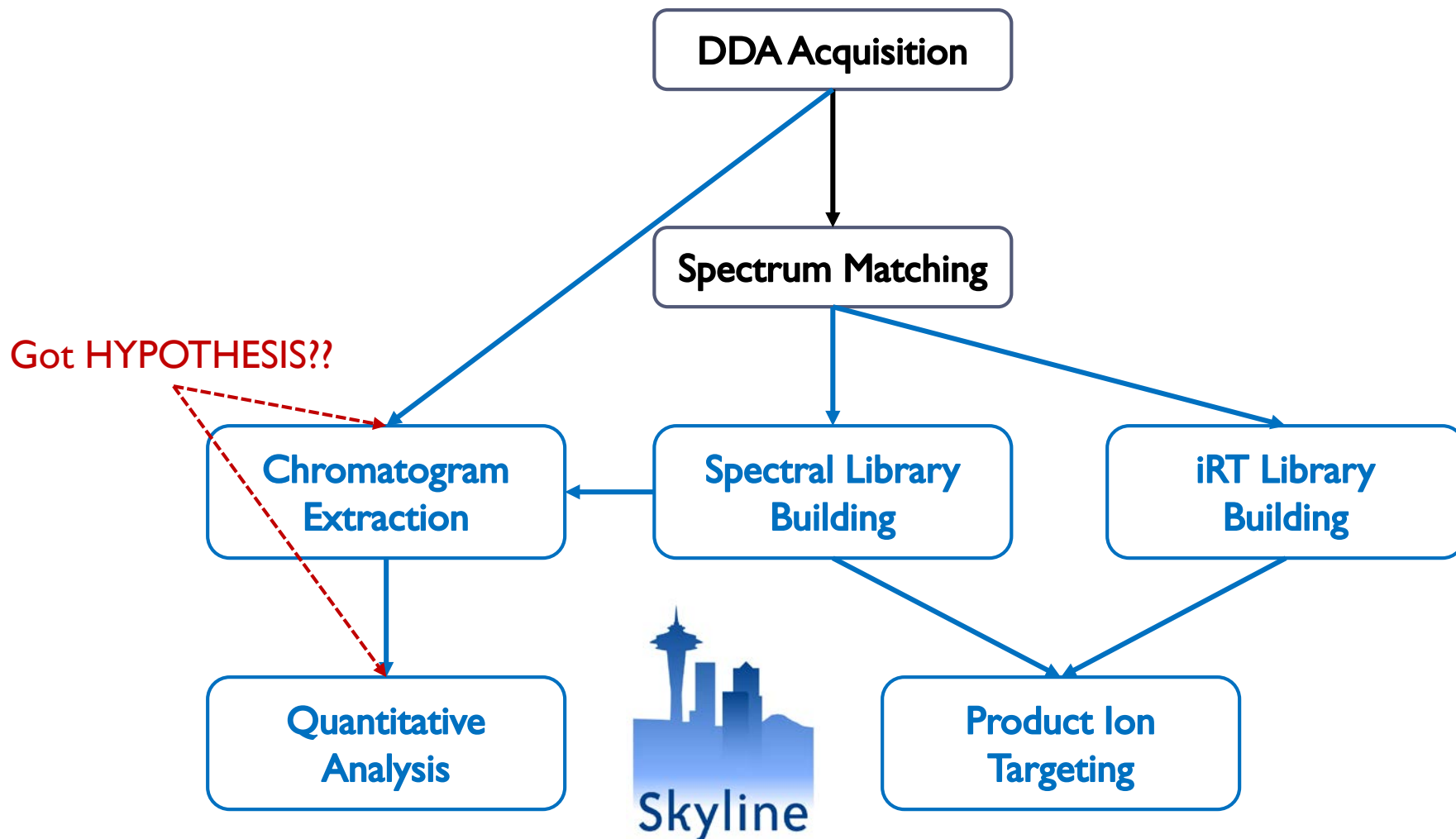|	Fake Accession	Name	Origin	Molecular Weight
A	P44015	Ovalbumin	Chicken Egg White	45KD
B	P55752	Myoglobin	Equine Heart	17KD
C	P44374	Phosphorylase b	Rabbit Muscle	97KD
D	P44983	Beta-Galactosidase	Escherichia Coli	116KD
E	P44683	Bovine Serum Albumin	Bovine Serum	66KD
F	P55249	Carbonic Anhydrase	Bovine Erythrocytes	29KD

Sample Preparation

	A	B	C	D	E	F	(fmol)
Sample 1	65	55	15	2	11	10	+ 200 ng yeast digest
Sample 2	55	15	2	65	0.6	500	+ 200 ng yeast digest
Sample 3	15	2	65	55	10	11	+ 200 ng yeast digest

Group Comparisons

	A	B	C	D		E	F	(fold change)
Sample 1-2	0.85	0.27	0.13	32.5		0.055	50	+ 200 ng yeast digest
Sample 1-3	0.23	0.036	4.33	27.5		0.91	1.1	+ 200 ng yeast digest
Sample 2-3	0.27	0.13	32.5	0.85		16.7	0.022	+ 200 ng yeast digest

Group Comparison Maxima

	A	B	C	D	E	F	(abs log2 fold change)
Sample 1-2	0.2	1.9	2.9	5.0	4.2	5.6	+ 200 ng yeast digest
Sample 1-3	2.1	4.8	2.1	4.8	0.1	0.1	+ 200 ng yeast digest
Sample 2-3	1.9	2.9	5.0	0.2	4.1	5.5	+ 200 ng yeast digest
Maximum	**2.1**	**4.8**	**5.0**	**5.0**	**4.2**	**5.6**	

DDA Runs Searched

	Identified yeast proteins
sample 1-a	3016
sample 1-b	3073
sample 1-c	2905
sample 2-a	2916
sample 2-b	2984
sample 2-c	2907
sample 3-a	2883
sample 3-b	2972
sample 3-c	2913

Comet, OMSA, MSGF+ - iProphet

Discovery to Targeted with Skyline