2017 Metabolomics Seminars

ThermoFisher
SCIENTIFIC

Pushing the Leading Edge in Protein Quantitation:
Integrated, Precise, and Reproducible Protein Quantitation Workflow Solutions
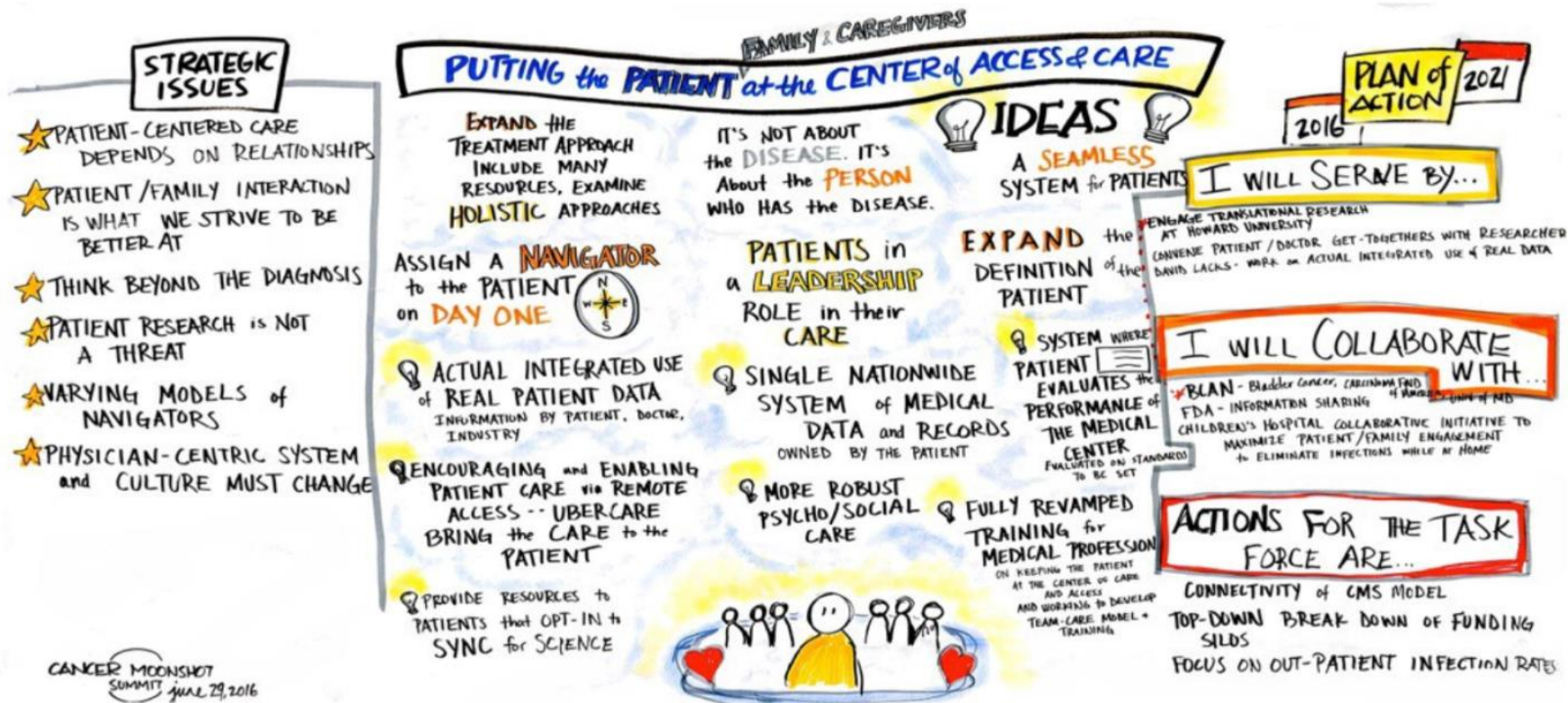
The world leader in serving science

Cancer Moonshot

Goal: To detect cancer at an early stage while providing additional therapies to more patients.

"…It is the proteins that comprise most of the biomarkers that are measured to detect cancers, constitute the antigens that drive immune response and inter and intracellular communications, and it is the proteins that are the drug targets for nearly every targeted therapy that is being evaluated in cancer trials today."
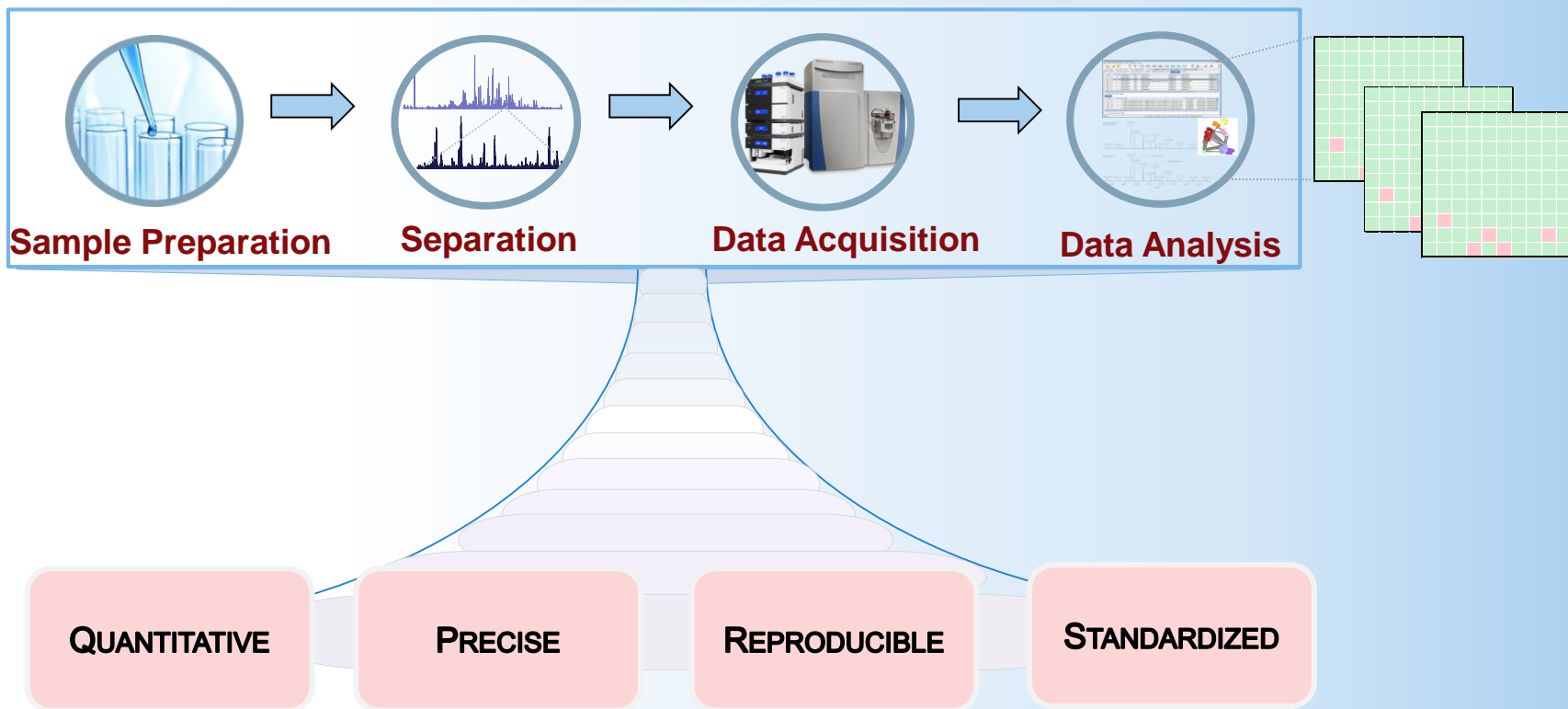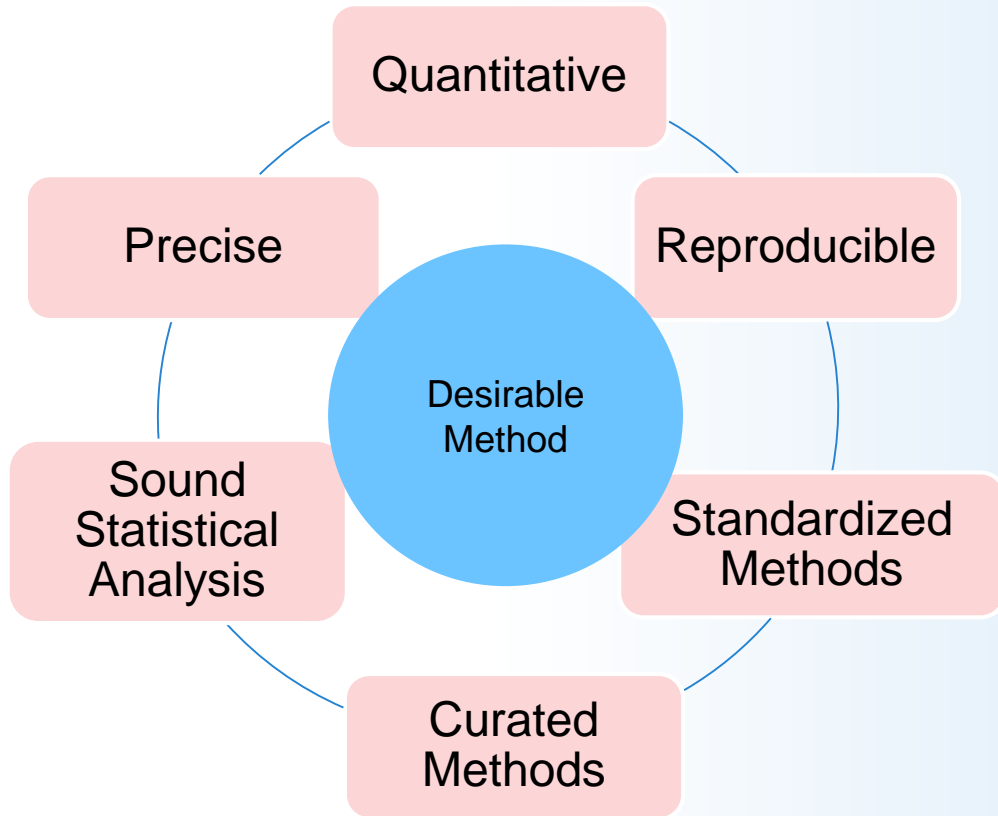
Conrads et al. 2016 Clinical Cancer Research

Quantitative

Reproducible

Precise

Desirable Method

Standardized Methods

Sound Statistical Analysis

Curated Methods

Our Solution

A new standard in quantitative, sensitivity, accuracy and precision

- HR DIA

- DDA+

Workflows

Thermo Fisher
SCIENTIFIC

High-Resolution DIA Workflow

Unparalleled proteome coverage and dynamic range

- Highest depth of proteome coverage and quantitative insight
- Robust quantitative precision

> *Biospecimen profiling*
> *Digital archiving*

DDA+ Workflow

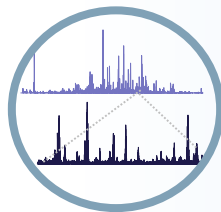*Unsurpassed quantitative precision and reproducibility*

- Unrivaled precision in precursor quantitation
- Maximize complete, reproducible quantitation across samples
- Minimize 'missing values' among samples

> *Cellular signaling studies*
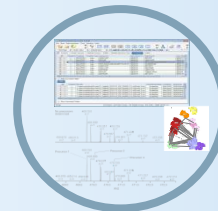> *Mechanism of action studies*
> *PTM profiling*

ThermoFisher
SCIENTIFIC

High-Resolution DIA: Unparalleled Proteome Coverage and Dynamic Range

new
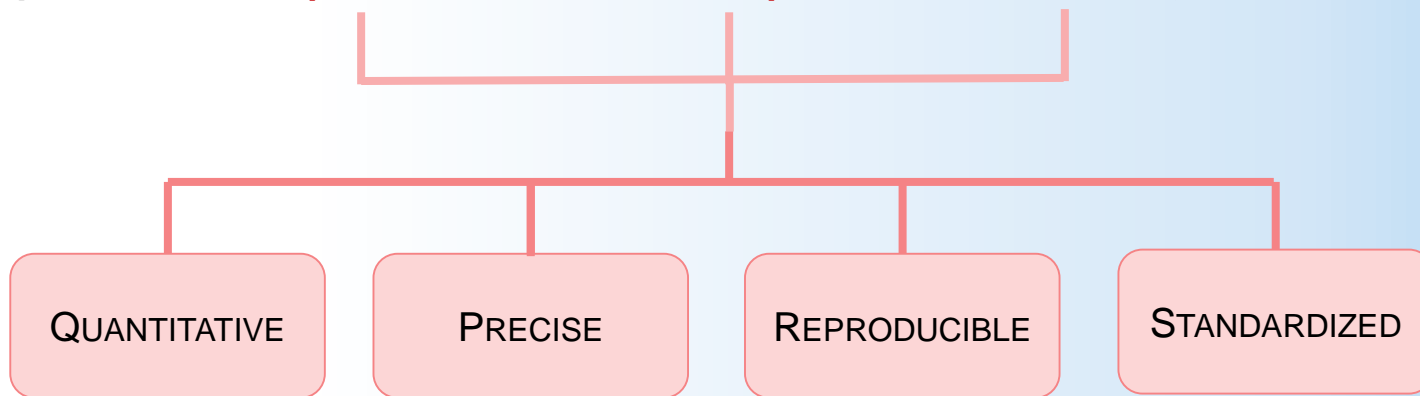
Workflow

Thermo Scientific™ UHPLC Systems

Thermo Scientific™ EASY-Spray™ LC Column

Thermo Scientific™ Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap MS

Spectronaut™ software

- *Thermo Scientific™ UltiMate™ 3000 RSLCnano system*
- *Direct inject or pre-concentration mode*
- *Thermo Scientific™ Viper™ fittings*

- *150 µm ID x 150 mm,*
- *Sensitivity and robustness*
- *RT stability <1% observed for 350 injections*

- *Increased acquisition speed*
- *Advanced precursor determination*
- *Same # of protein IDs half the time*

Designed for Speed and Coverage

ThermoFisher
SCIENTIFIC

Spectronaut Software

Key Benefits

- Spectronaut™ software is **specifically developed for the analysis of DIA & SWATH** data sets
- Data analysis with retention time correction based on spiked reference peptides-HRM calibration kit or iRT Kit
- Spectral library generation from MaxQuant and Thermo Scientific™ Proteome Discoverer™ software search results
- Direct visualization of qualitative and quantitative results on protein level
- Fast data analysis speed in **less than 2 min per run**

Designed for high throughput DIA data analysis

Thermo Fisher
SCIENTIFIC

Balancing Efficiency Without Sacrificing Performance

Nanoflow
- Greater # of proteins
- Greater # of peptides
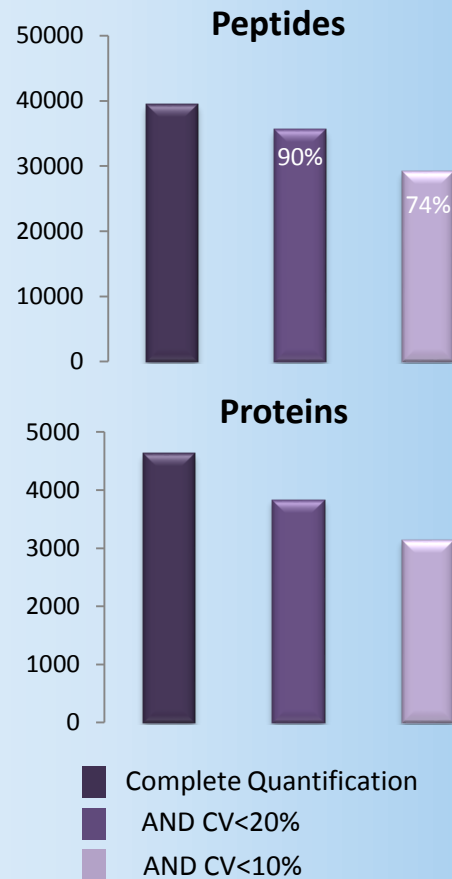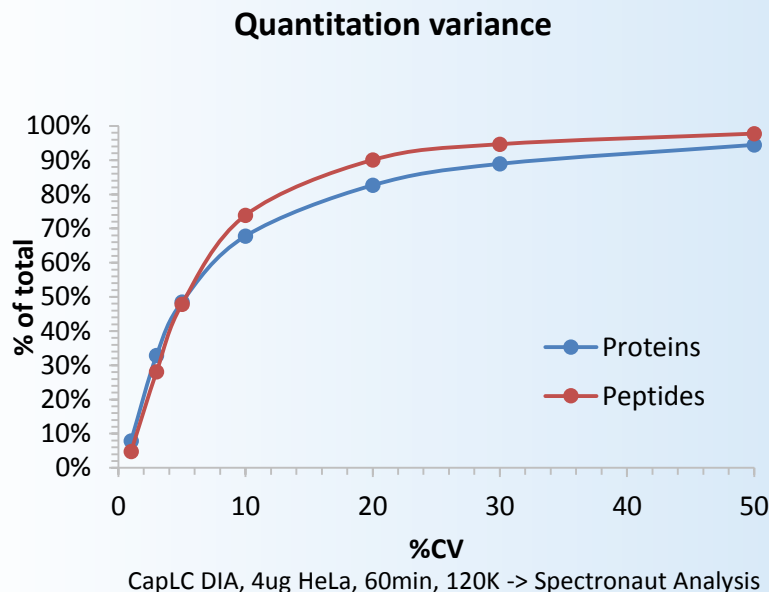- Greater sensitivity
- *Longer total run times*

CapLC
- Greater Efficiency
- Shorter total run time (2X)
- Greater throughput
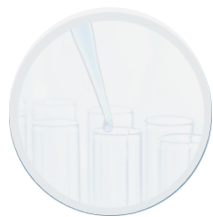- *Comparable protein and peptide id's*
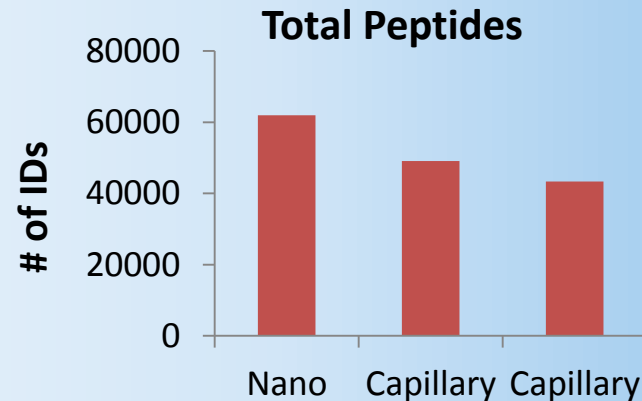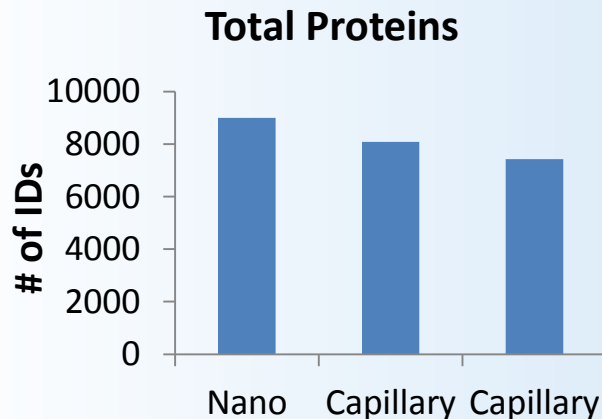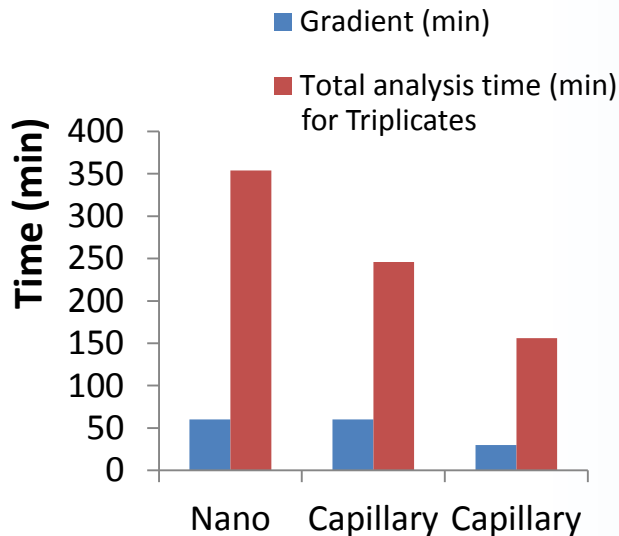
Thermo Fisher
SCIENTIFIC

new

Workflow

UHPLC Systems

EASY-Spray LC Column

Q Exactive HF-X MS

Thermo Scientific™ Proteome Discoverer™ 2.2 software

Proteome Discoverer 2.2
Mass Informatics Platform for Protein Scientists

© Copyright 2008-2017 Thermo Fisher Scientific Inc. All rights reserved. This program is protected by copyright law and international treaties as described in Help About...

thermo scientific
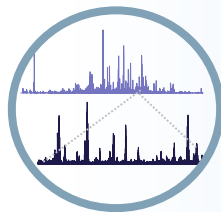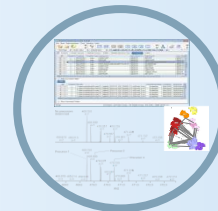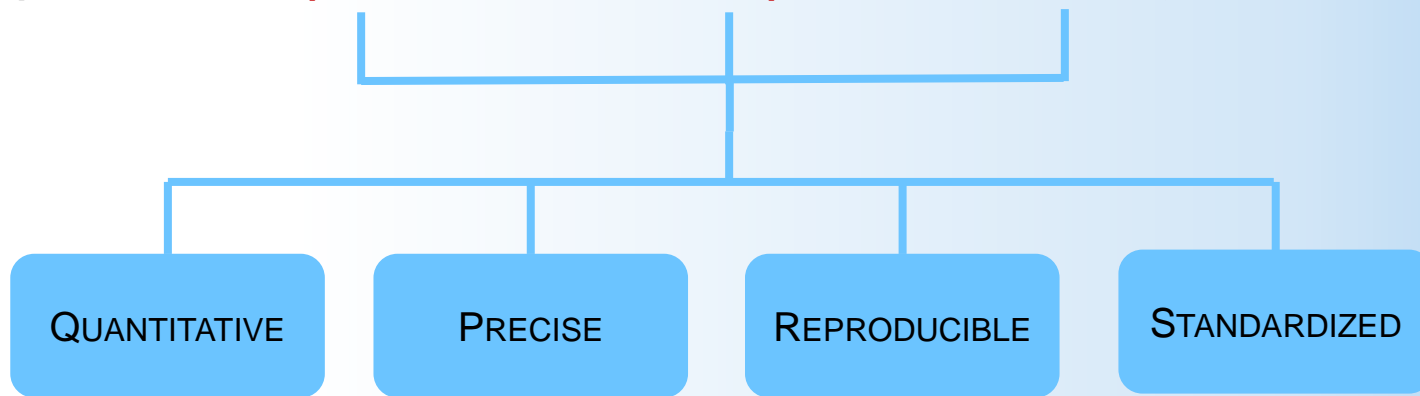
- *UltiMate 3000 RSLCnano system*
- *Direct inject or pre-concentration mode*
- *Viper fittings*

- *150 µm ID x 150 mm,*
- *Sensitivity and robustness*
- *RT stability <1% observed for 350 injections*

- *Increased acquisition speed*
- *Advanced precursor determination*
- *Same # of protein IDs half the time*

## Designed for Precision and Reproducibility

Thermo Fisher
SCIENTIFIC

Proteome Discoverer 2.2 Software

Key Benefits

- Enables large scale, multiplex proteomic studies and captures confident protein results which enables confident reproducibility

- Improved Label-free Quantitation
    - Feature mapping
        - Retention time alignment
        - Feature linking across files

- Minora Feature Detector node
    - Detects chromatographic peaks and features according to the specified quantification approach

- Minimizes 'missing data points' and maximizes quantitative insights
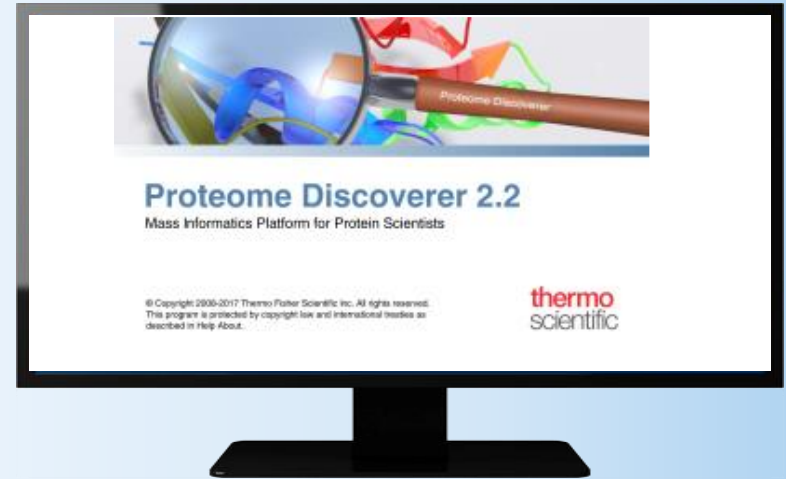
Most comprehensive data analysis platform for qualitative and quantitative proteomics research

Q Exactive HF-X MS

Q Exactive HF MS

Protein ID vs **time**

← 50% →
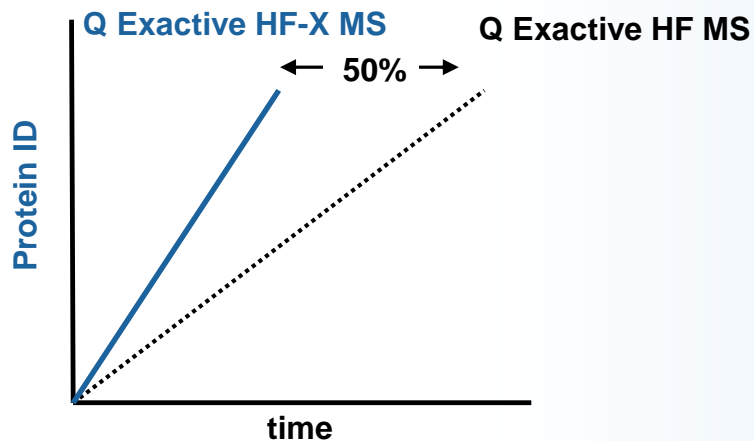
Maximizing protein identifications

- Quick screening of complex samples
- Quality control of complex samples
- Assessment of sample concentration

Saves time and samples in large-scale proteomics efforts

Q Exactive HF-X MS

Peptide ID vs **time**

40%

Q Exactive HF MS
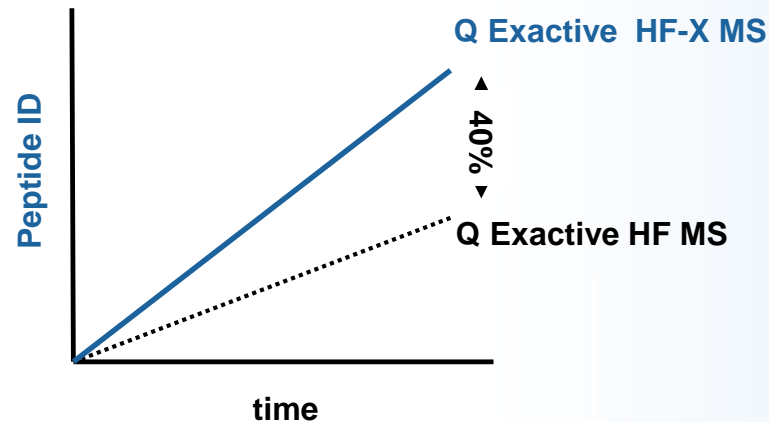
Maximizing peptide identifications

- Highest peptide coverage
- Deep proteome analysis
- Spectral library building

ThermoFisher
SCIENTIFIC

Maintaining Sensitivity at Increased Robustness – capLC with Q Exactive HF-X MS

- Increased sensitivity on QE HF-X
- Increased peptide identifications at higher robustness
- Higher reproducible protein identifications with reduced total run times

Sensitivity

Q- Exactive HF-X MS
Q- Exactive HF MS
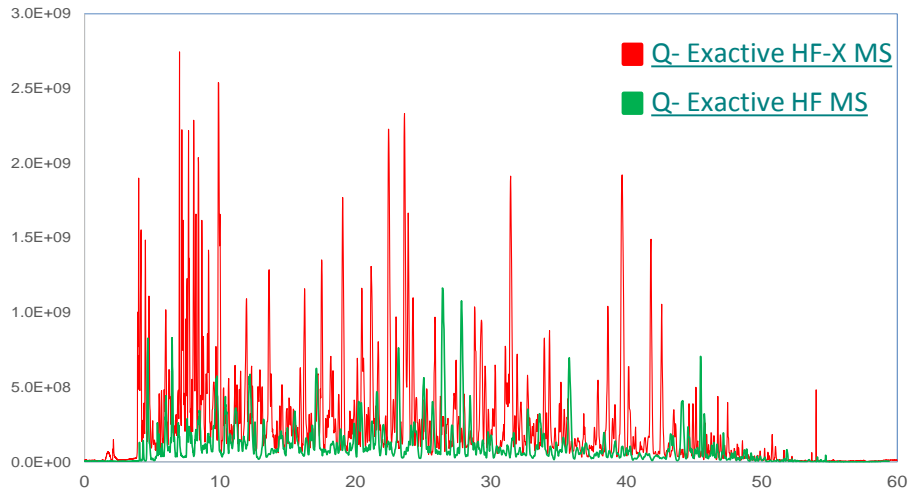
1 ug HeLa + 150 uM x 15 cm Easy Spray

Robustness

Blue= **1 µg (nanoLC)** cell lysate digest
Red = **4 µg** (capLC) cell lysate digest
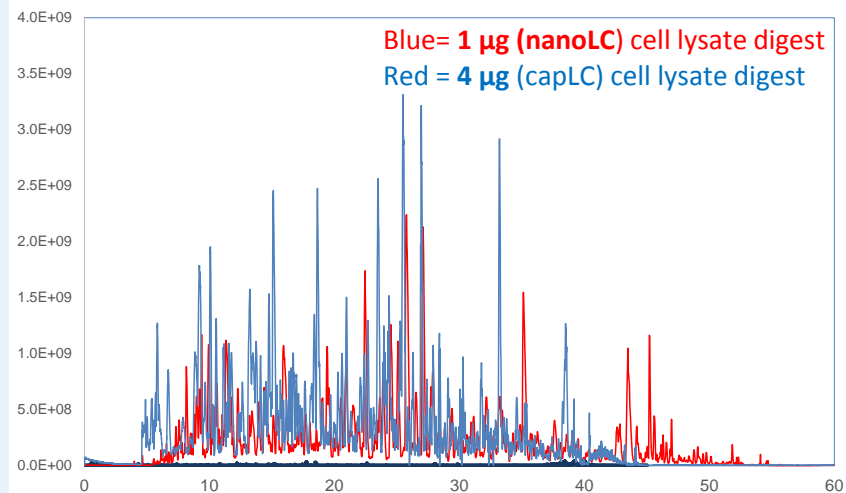
75 µm ID x 15 cm vs 150 µm ID x 15 cm

Thermo Fisher
SCIENTIFIC
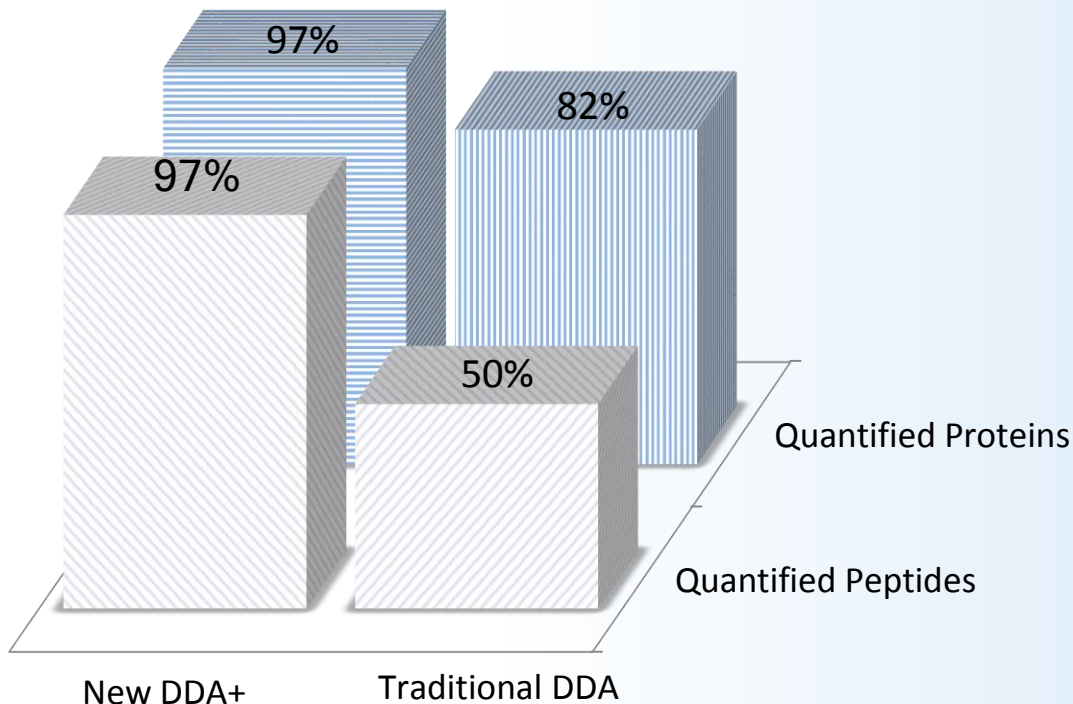
DDA+ workflow compared to DDA

- 15% gain in completely quantified proteins

- 47% gain in completely quantified peptides

- Maximizes quantitation

capLC DDA+, 4ug HeLa, 60min, 120K/7.5K, 19ms, Top 40 -> Proteome Discoverer 2.2 Label-free Quant

Thermo Fisher
S C I E N T I F I C

Completely quantified proteins

Missing data = sparse quantitation
Complete quantitation

Rep 1

Rep 2

Rep 3

Rep 1

Rep 2

Rep 3
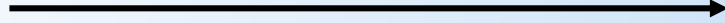
82% quantified during MS

97% quantified from MS and Protein Discoverer 2.2 software

capLC DDA+, 4ug HeLa, 60min, 120K/7.5K, 19ms, Top 40 -> PD 2.2 Label-free Quant

Quantitation　　Precision　　Reproducibility　　Standardization

ThermoFisher
SCIENTIFIC

new

new

Completely quantified peptides
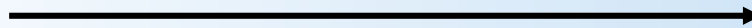
Missing data = sparse quantitation

Complete quantitation

Rep 1

Rep 2
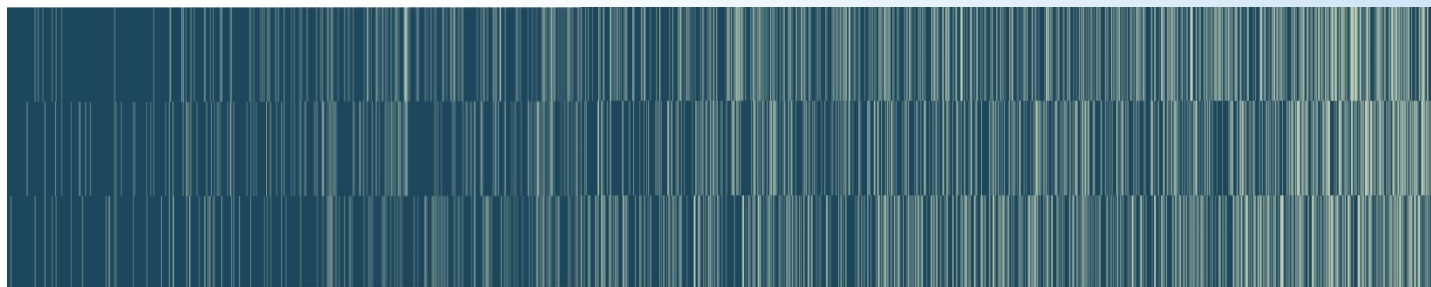
Rep 3

Rep 1

Rep 2

Rep 3

50% quantified during MS

97% quantified from MS and PD 2.2

capLC DDA+, 4ug HeLa, 60min, 120K/7.5K, 19ms, Top 40 -> PD 2.2 Label-free Quant

Quantitation → Precision → Reproducibility → Standardization

ThermoFisher
SCIENTIFIC

DDA+ Enable Unrivaled Quantitative Precision

Protein quantitation variance

% of proteins vs %CV

89%
98%

3329 proteins
completely quantified
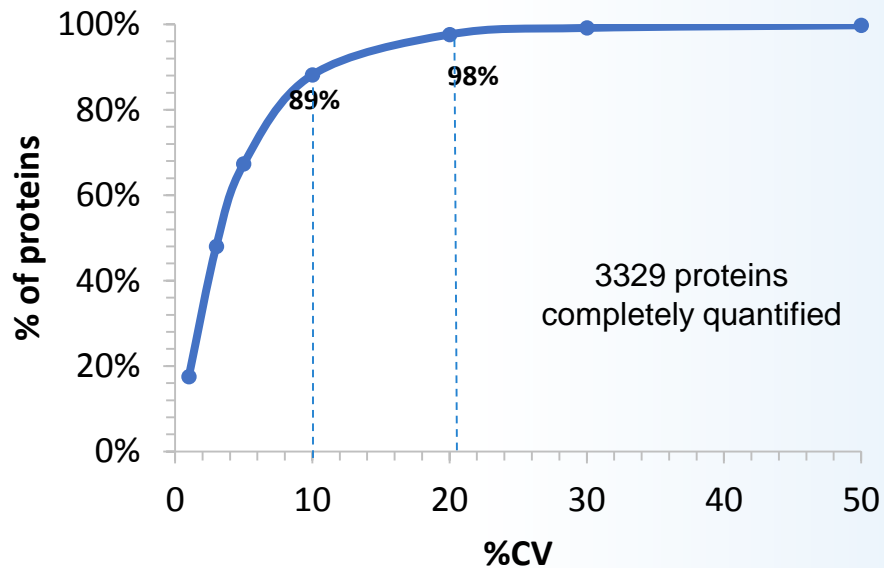
Peptide quantitation variance

% of peptides vs %CV

81%
97%

22525 peptides
completely quantified
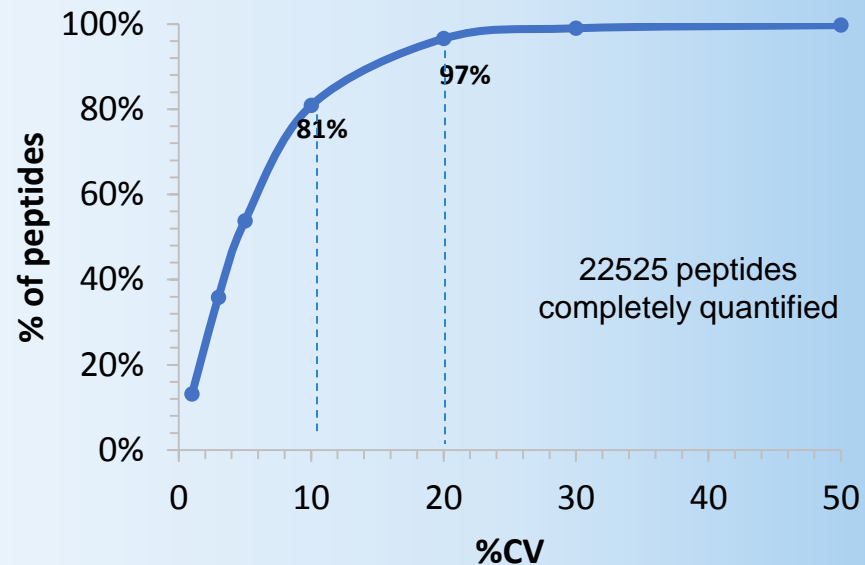
Quantitation | **Precision** | Reproducibility | Standardization

DDA+ Workflow: Greater Reproducibility Between Samples
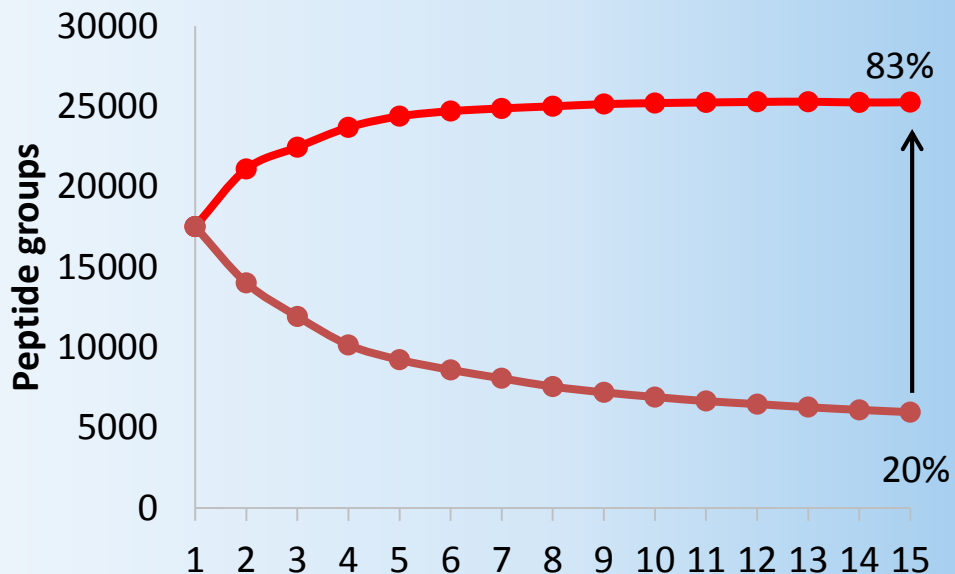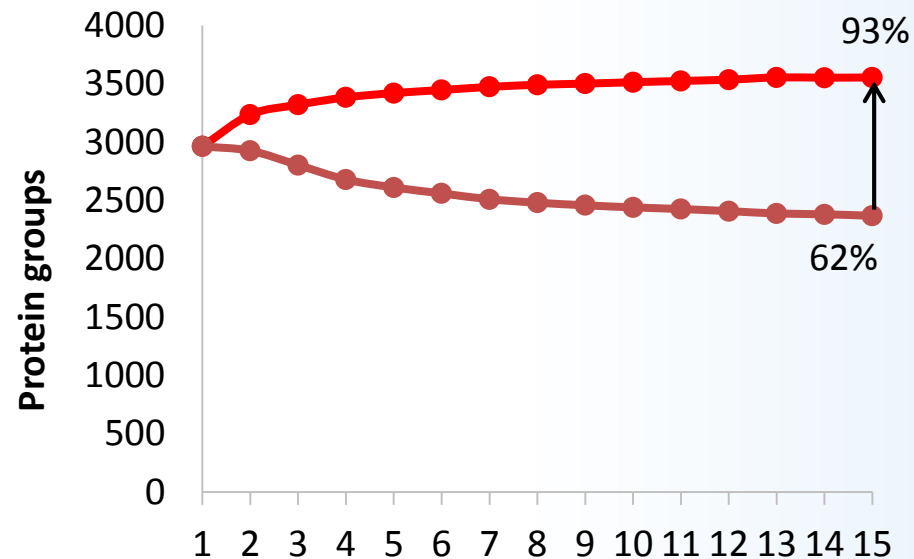
Instrument Standardization Test

- Three Locations

- HeLa digest metrics
  - Protein
  - Peptide
  - PSMs
  - MS/MS

- 60 min gradient

Quantitation → Precision → Reproducibility → **Standardization**

Thermo Fisher
SCIENTIFIC
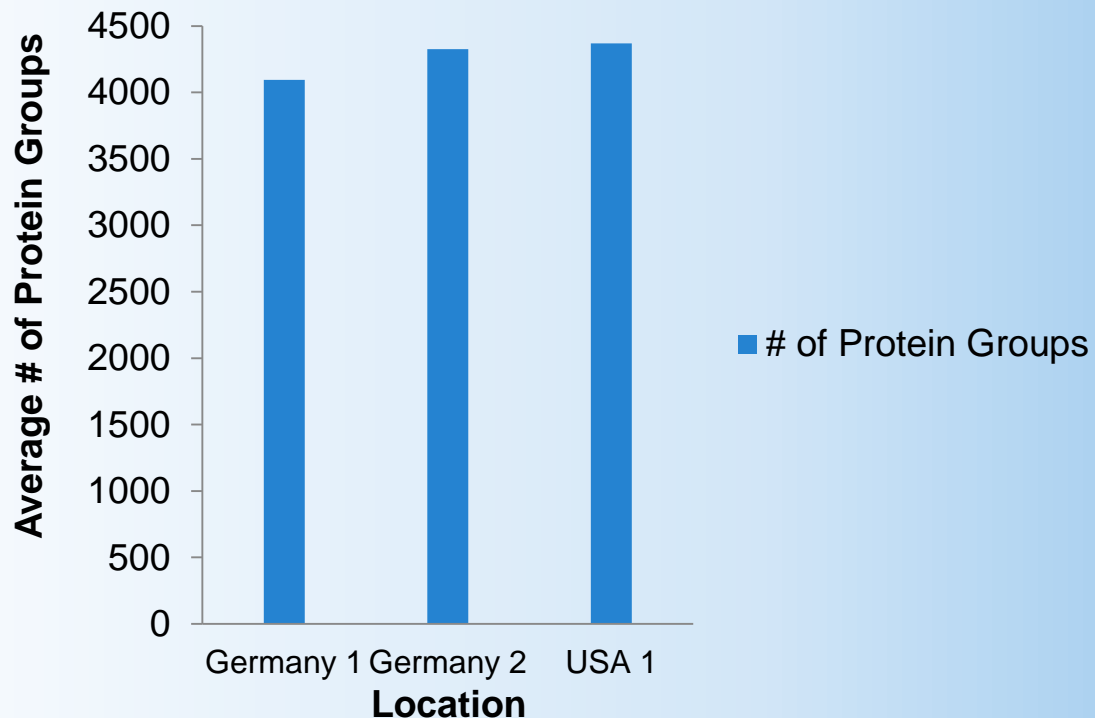
Multi-Center Study to Demonstrate Large-Scale Capabilities

Thermo Fisher BRIMS Center

Objective: Determine analytical robustness and reliability between laboratories

- Comparability of measurements between laboratories and define critical parameters

- Ring trial participants adapt a system suitability test protocol to maintain analytical performance

- Determine the range of accuracy and precision that users can expect to achieve following the standardized product/assay

- The standardization enables the transfer of measurements between laboratories

Phase 1
Labs identified, resources secured, SOP established

Phase 2
Study design, study setup, data collection
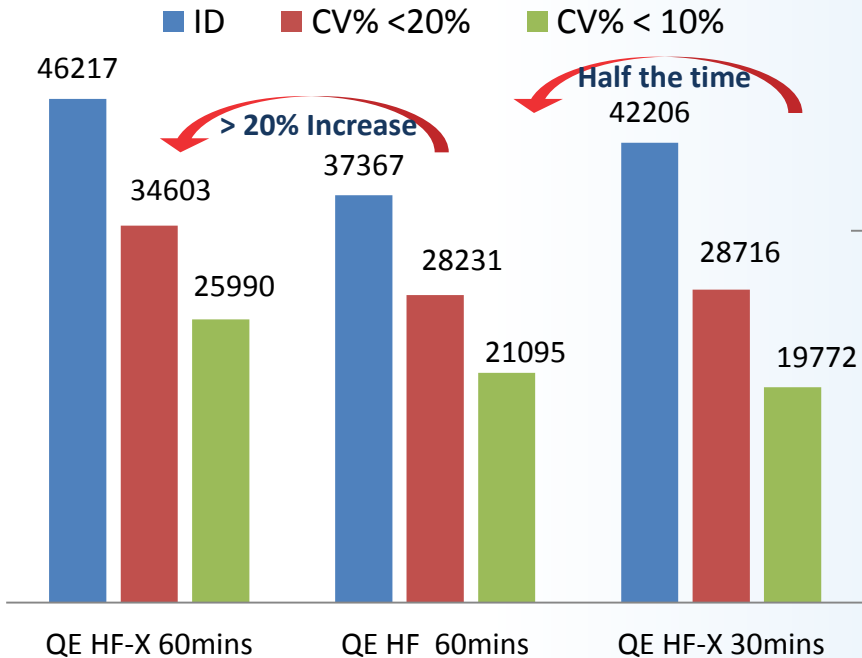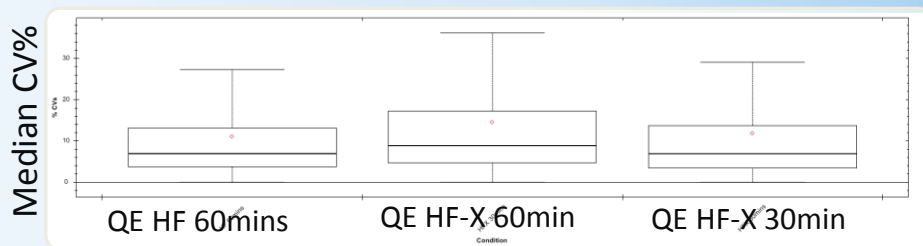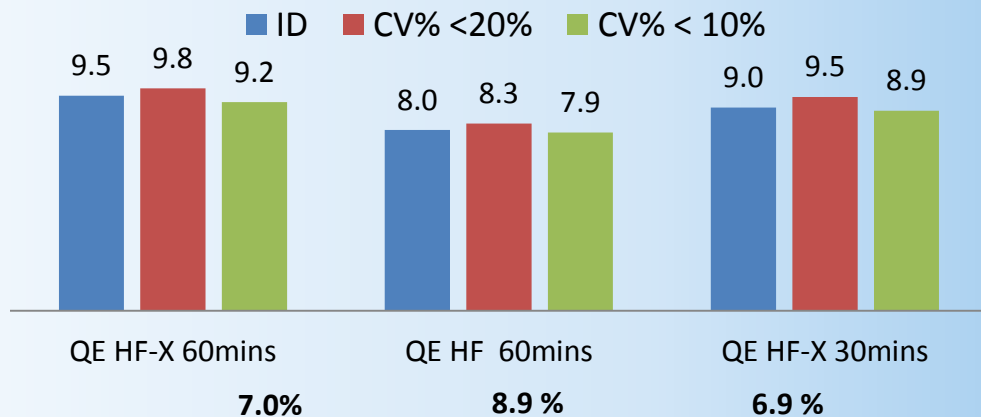
Phase 3
Data review, report-out

Thermo Fisher
SCIENTIFIC

Cancer Moonshot

Questions?

optional slides follow

ThermoFisher
SCIENTIFIC

* 4ug HELA digest, 3 technical replicates, 120k @ MS1
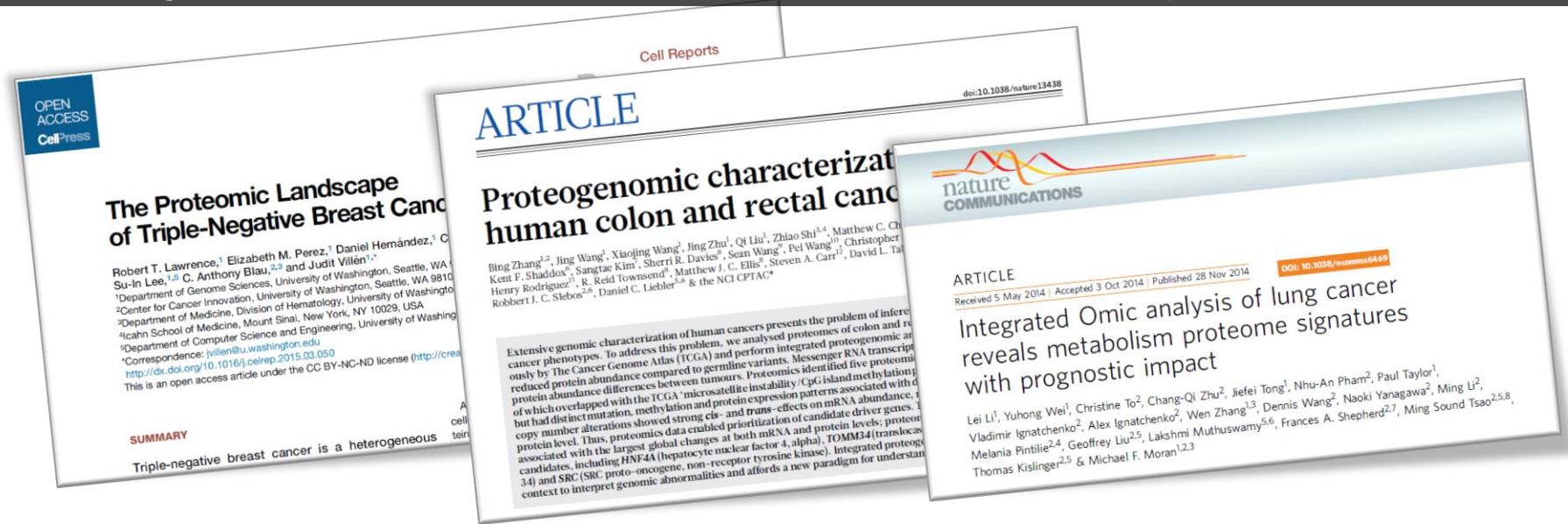
- Proteomics is being used to discover and establish the protein landscape of cancer cells or tissues

- Proteomic measurements complement genomics/transcriptomic measurements by reducing the vast number of potentially actionable somatic mutations and identifying genomic variances that might be actionable

Proteomics Complements Genomics

ThermoFisher
SCIENTIFIC