Multi-Q 2: isobaric labeling quantitation analysis with improved accuracy and coverage

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Multi-Q 2 software facilitates isobaric labeling quantitation analysis with improved accuracy and coverage

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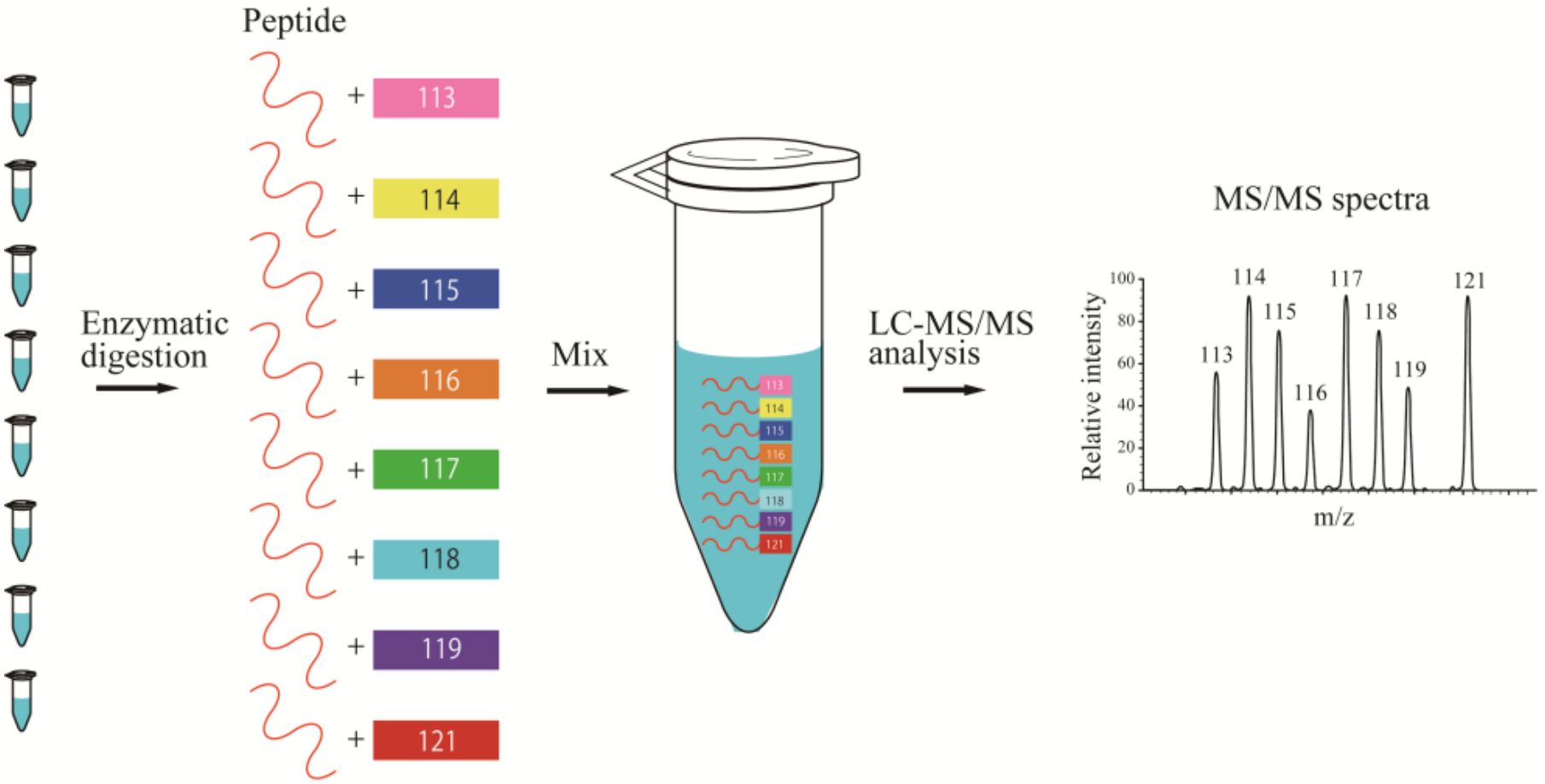
https://app.box.com/s/pvi1nb4ry6s2eqgzcbz21yixwiqvphk9

- Multi-Q 2程式
- SearchResultChecker

https://ms.iis.sinica.edu.tw/comics/software.html

- 所有程式及data set檔案

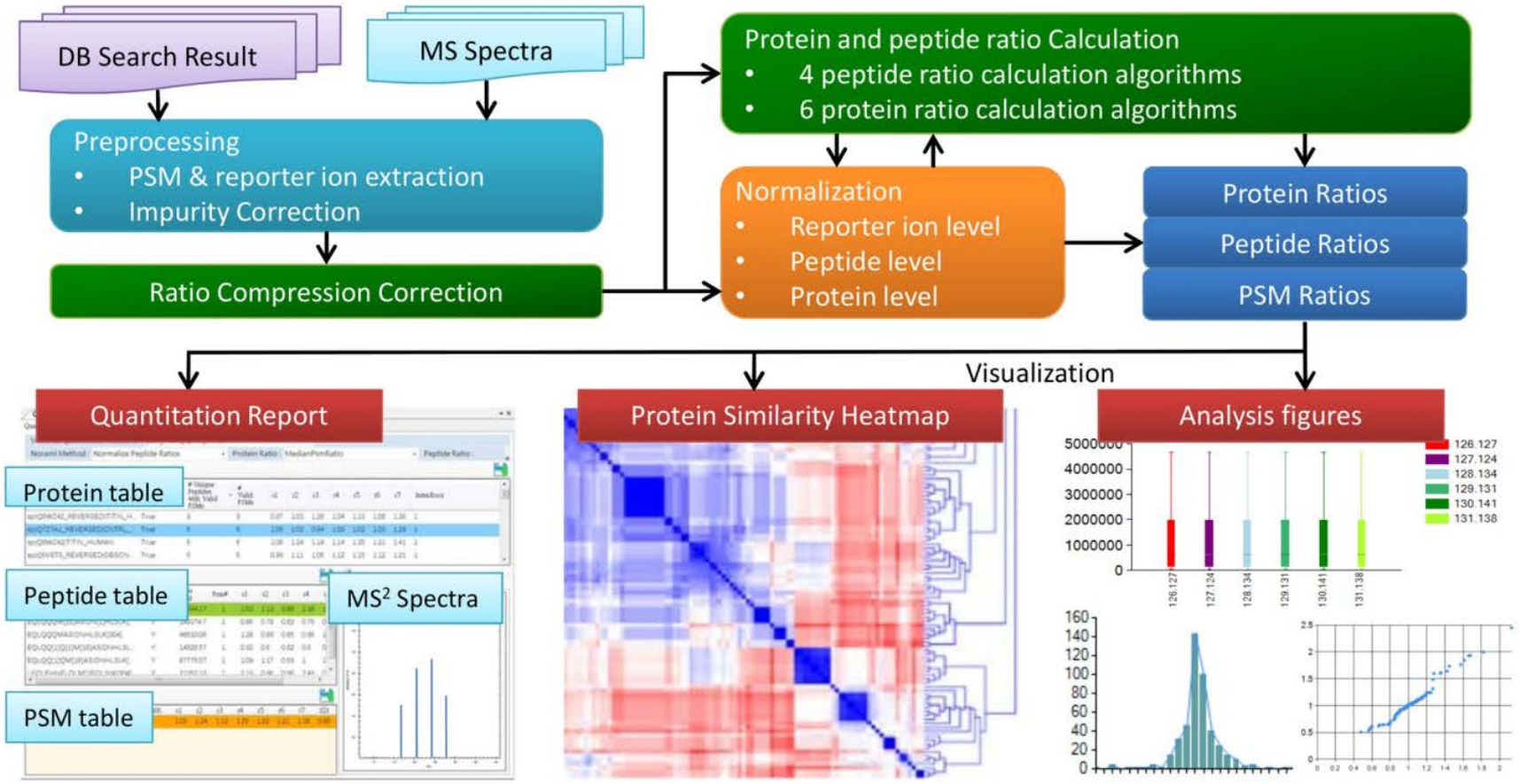
Isobaric Labeling Experiment



Advantage of Multi-Q 2

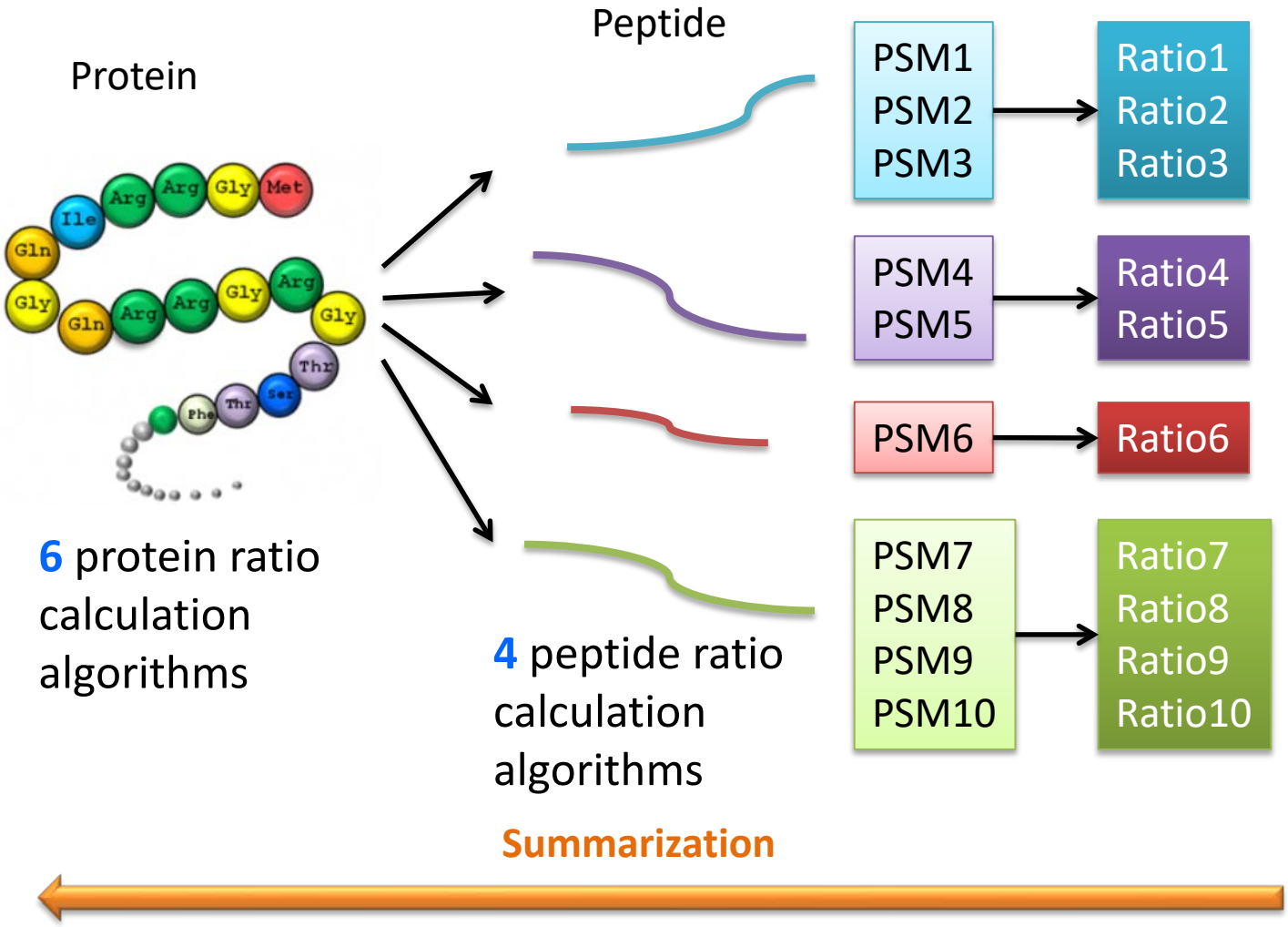
- Improve identification coverage
  - The more peptides and proteins the better
- Improve quantitation accuracy
  - Peptide ratio calculation + protein ratio calculation + normalization
  - The best quantitation algorithmic combinations
- Figure out which algorithmic combination is better under a certain situation
- Support for ratio compression correction
  - Co-isolation ions in MS1
- Graphic user intrefaces and visualization

Overview of Multi-Q 2



Scientific Reports 2021

Quantitation Algorithms



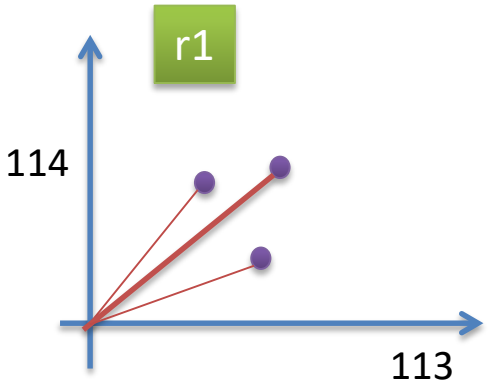
7 different normalization methods

Multi-Q 2 Peptide Ratio Calculation

- Peptide ratio from median of PSM ratios (MedianPsmRatio)

	r1	r2	r3
PSM1			
PSM2			
PSM3			

Median Median Median



- Peptide ratio from weighted PSM ratios (WeightedPsmRatio)

	weight	r1	r2	r3
PSM1	0.7			
PSM2	0.3			

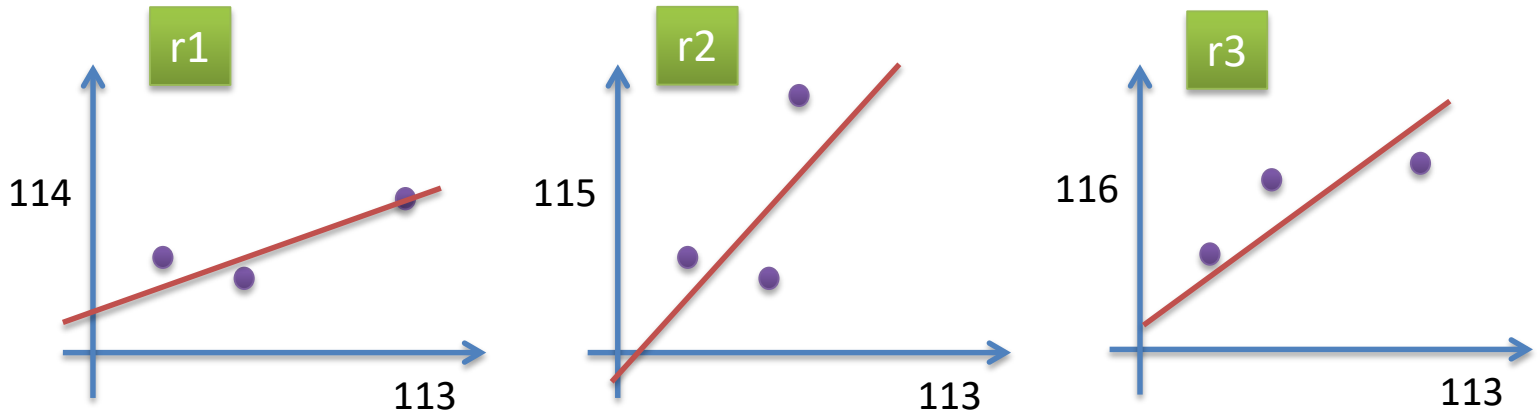
Weighted Avg. Weighted Avg. Weighted Avg.

- Peptide ratio from summation of PSM intensities (SumPsmInten)

	116	117	118	119
PSM1				
PSM2				
PSM3				

Sum Sum Sum Sum → Calculate ratio

- Peptide ratio from slope of linear least square regressions (LinReg)
  - X axis and Y axis represent intensities of two reporter ions, respectively



Multi-Q Protein Ratio Calculation

- Protein ratio from median of PSM ratios (MedianPsmRatio)

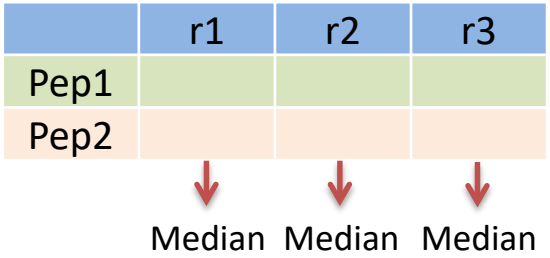
		r1	r2	r3
Pep1	PSM1			
Pep1	PSM2			
Pep1	PSM3	v		v
Pep2	PSM4		v	
Pep2	PSM5			
		↓	↓	↓
		Median	Median	Median

- Protein ratio from trimmed mean of PSM ratios (TrimmedMeanPsmRatio)

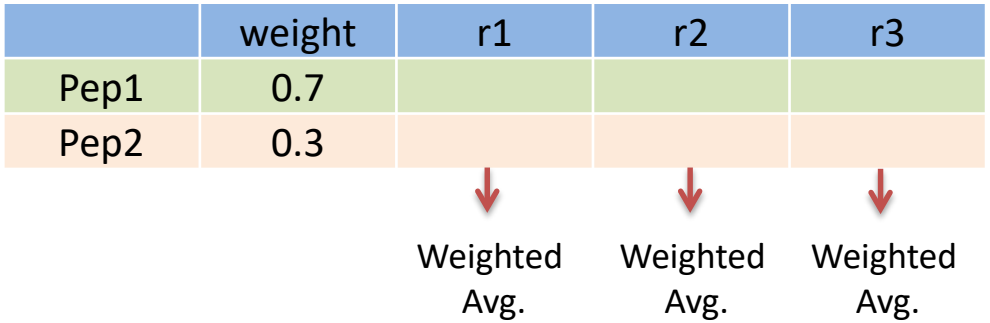
		r1	r2	r3
Pep1	PSM1			
Pep1	PSM2			
Pep1	PSM3			
Pep2	PSM4			
Pep2	PSM5			
		↓	↓	↓
		Trimmed mean	Trimmed mean	Trimmed mean

Trimmed mean factor = 0.2

- Protein ratio from median of peptide ratios (MedianPepRatio)



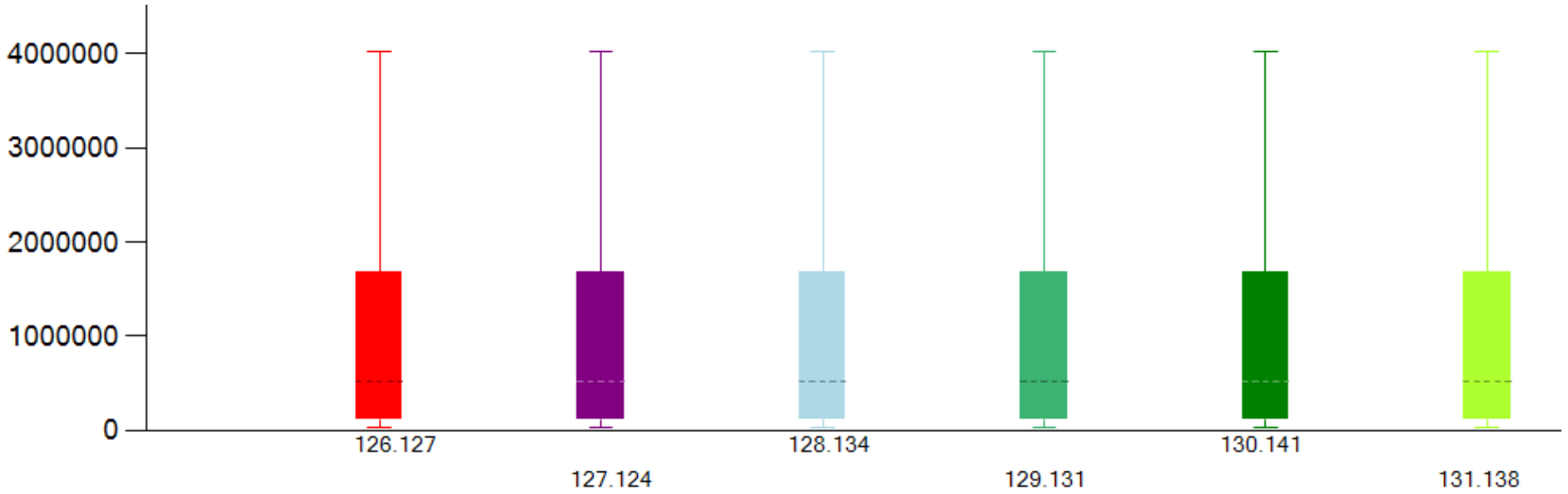
- Protein ratio from weighted peptide ratios (WeightPepRatio)
  - Peptide weight = Median of (113 intensity from all PSMs) + Median of (114 intensity from all PSMs) + …

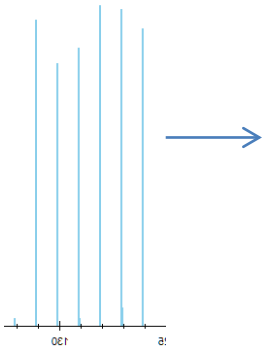


- Protein ratio from weighted PSM ratio (WeightedPsmRatio)
  - The same as peptide ratio calculation
- Protein ratio from summation of PSM intensity (SumPsmInten)

PSM Intensity-based Normalization

- Process
  - Calculate the median intensity for each channel
  - Each reporter ion intensity is normalized by the corresponding median intensity

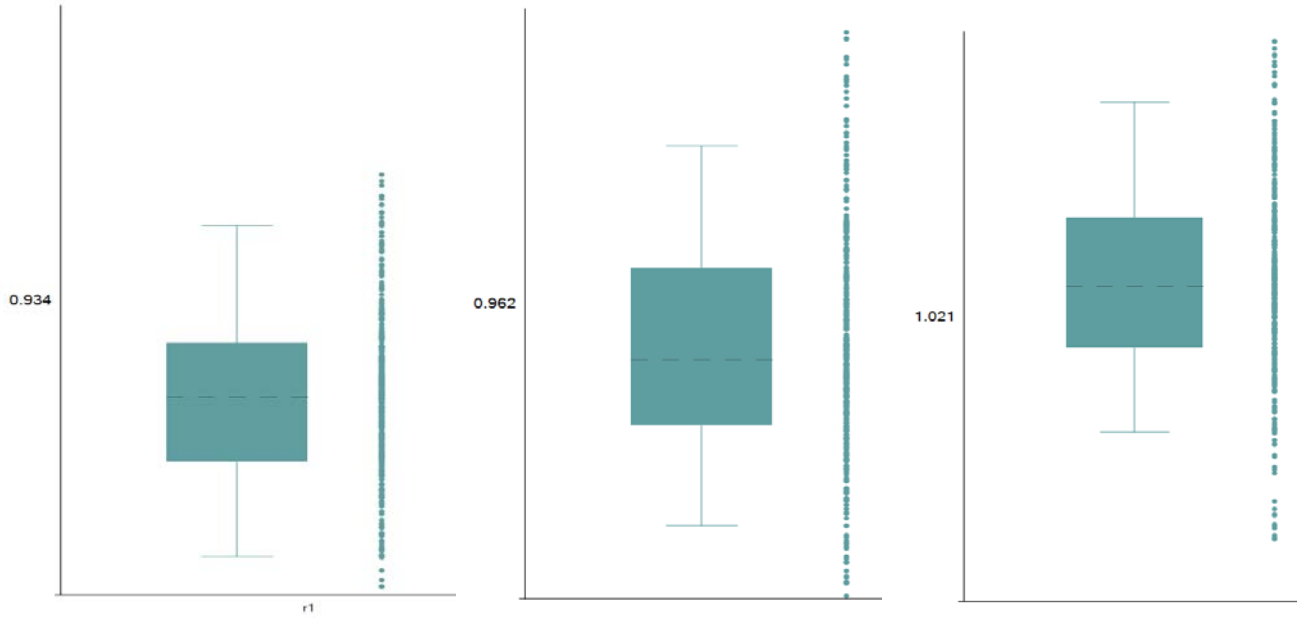




After normalization, relative ratios may be closer to 1

Ratio-based Normalization

- Assumption: abundances of most peptides remain the same in different samples
  - Peptide-level normalization: median of different peptide ratios should be identical
  - Protein-level normalization: median of different protein ratios should be identical
- Process
  - Calculate median values for each ratio (peptide or protein ratio)
  - Normalized ratio = original ratio/median



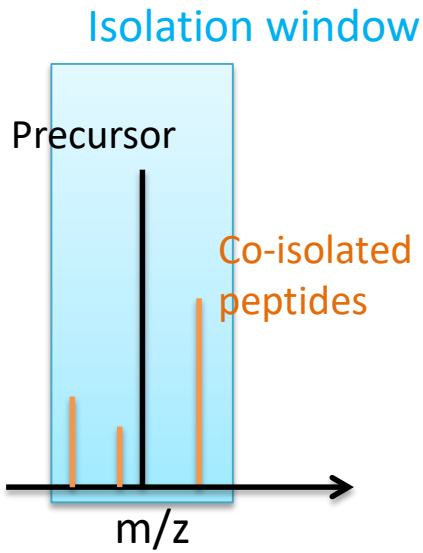
- Caution: If sample ratios are 2:2:1:1:2:2, this method cannot be used

Ratio Compression

- Coeluting peptides within the isolation window → underestimation of peptide/protein abundance differences
  - Ratio compression

- Filtering approach: filter out spectra with high interference
  - Sacrifice quantitation coverage for accuracy

- Algorithmic solution for ratio compression fixing
  - Maximize proteome coverage
  - Maximize quantification accuracy



Ratio Compression Correction

- JPR 2013

Journal of proteome research

Article

pubs.acs.org/jpr

Measuring and Managing Ratio Compression for Accurate iTRAQ/ TMT Quantification

Mikhail M. Savitski,[†,*] Toby Mathieson,[†] Nico Zinn,[†] Gavain Sweetman,[†] Carola Doce,[†] Isabelle Becher,[†] Fiona Pachl,[‡] Bernhard Kuster,[‡,§] and Marcus Bantscheff[†,*]

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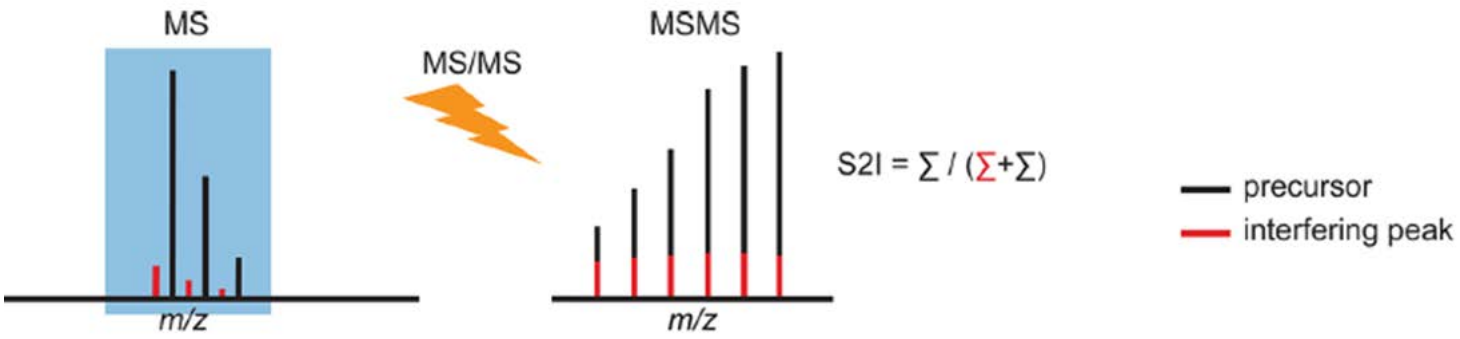


Experiments

- Generate all algorithmic combinations
  - 4 algorithms for peptide ratio calculation
  - 6 protein
  - Ratio compression correction (RCC): on and off
  - 7 normalization approaches
    - None
    - Reporter ion level only
    - Peptide level only
    - Protein level only
    - Reporter ion level + peptide level
    - Reporter ion level + peptide level
    - Reporter ion level + peptide level + protein level

- In total 4 x 6 x 2 x 7 = 336 combinations

Which is the best?

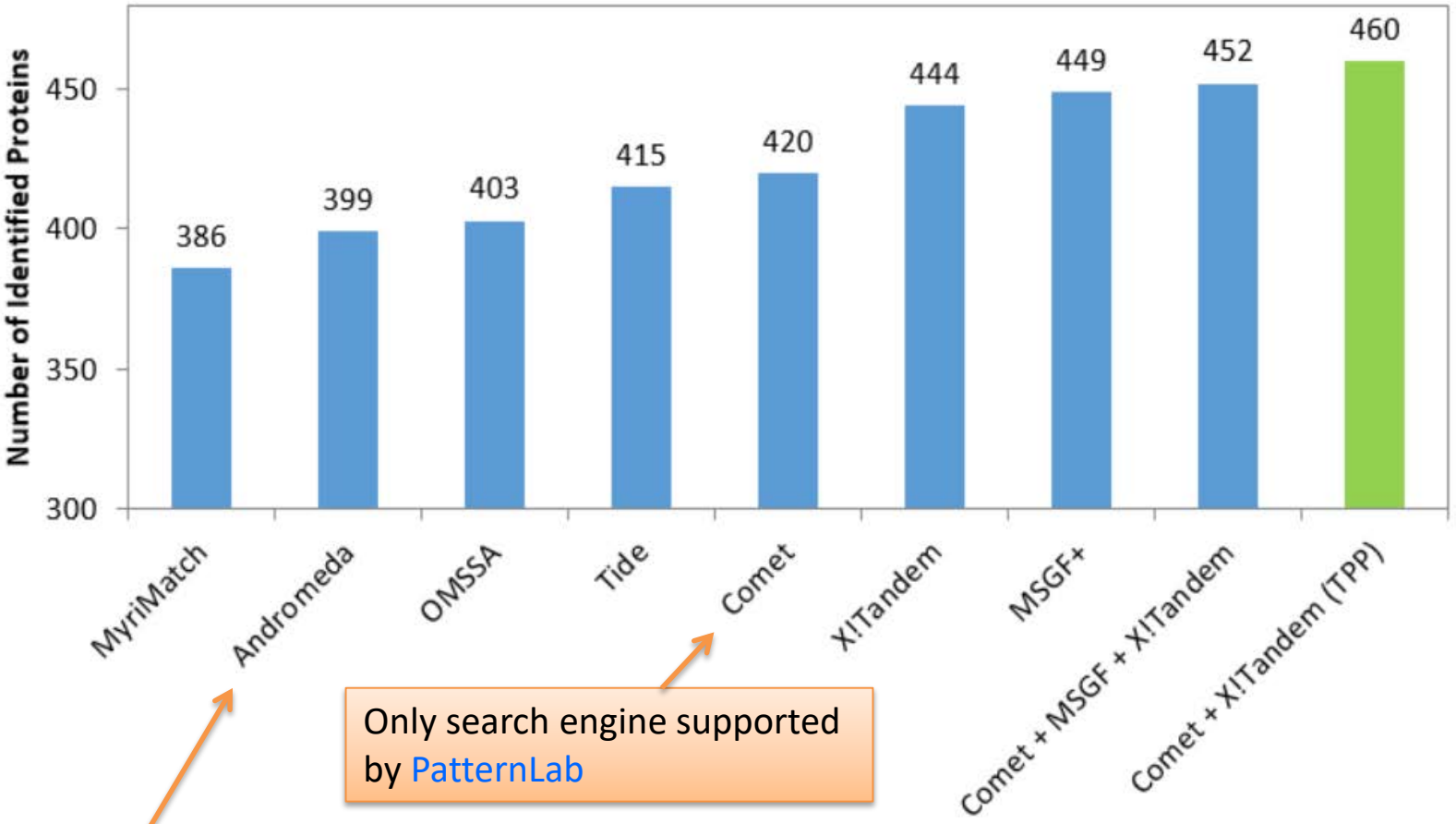
Test Data Set

- Cambridge TMT-6
  - Published in BBA Proteins and Proteomics, 2014
  - Four exogenous proteins were spiked into an equimolar Erwinia carotovora lysate with varying proportions in each channel of quantitation
  - Expected reporter ion ratios:
    - Erwinia peptides: 1:1:1:1:1:1
    - BSA spike (sp|P02769|ALBU_BOVIN): 1:2.5:5:10:5:1 -> Bovine serum albumin (BSA)
    - Enolase spike (sp|P00924|ENO1_YEAST): 10:5:2.5:1:2.5:10 -> Yeast enolase (ENO)
    - PhosB spike (sp|P00489|PYGM_RABIT): 2:2:2:2:1:1 -> Rabbit glycogen phosphorylase (PHO)
    - Cytochrome C spike (sp|P62894|CYC_BOVIN): 1:1:1:1:1:2 -> Bovine cytochrome C (CYT)

	r1	r2	r3	r4	r5
P62894	1	1	1	1	2
P02769	2.5	5	10	5	1
P00924	0.5	0.25	0.1	0.25	1
P00489	1	1	1	0.5	0.5

Identified Protein Number Using Different Search Engines

- Validation software: PeptideShaker



Only search engine supported by MaxQuant

Only search engine supported by PatternLab

PatternLab is proposed by John Yates (Nature Protocol 2016)

Best Algorithmic Combinations

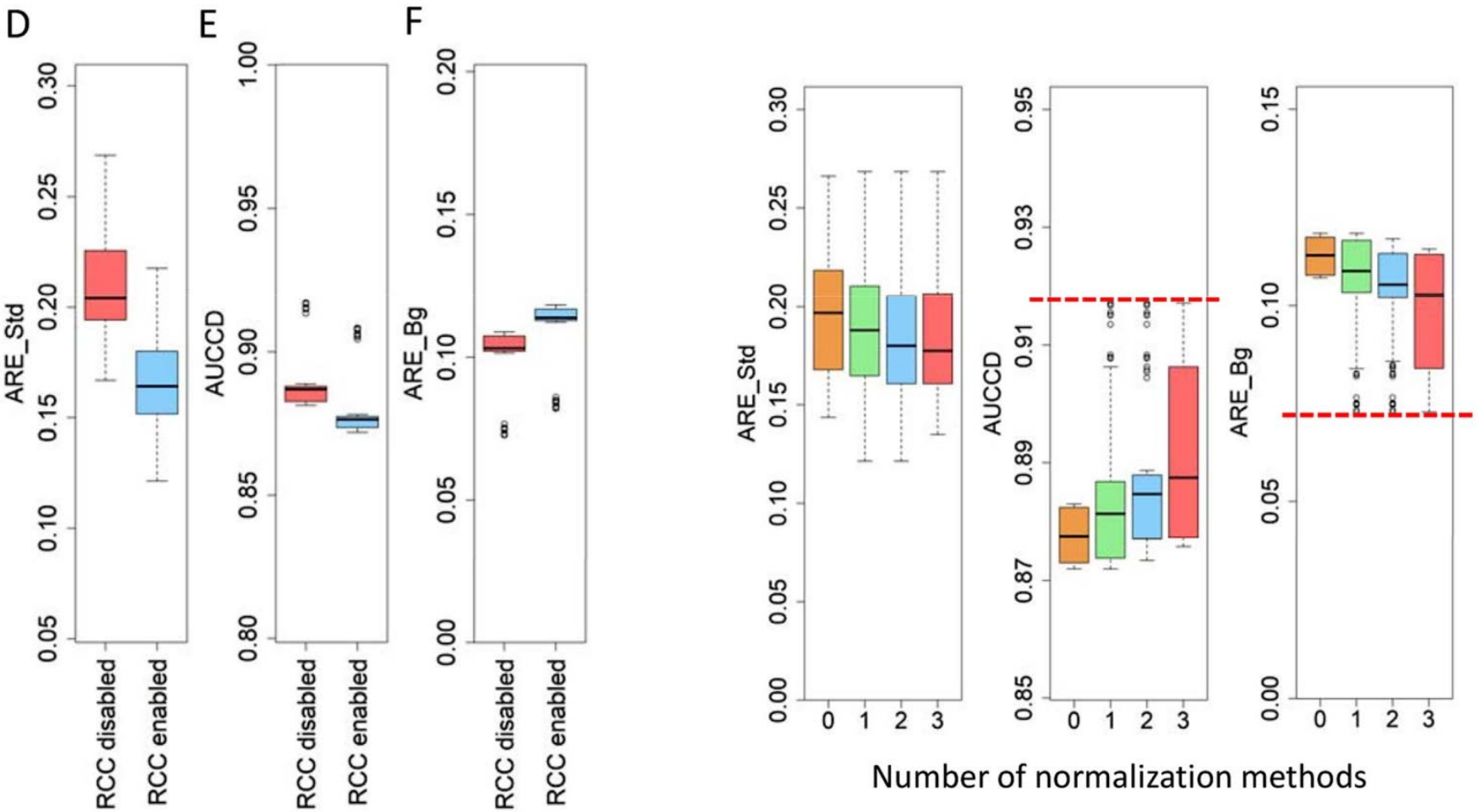
- The best for standard proteins (varying theoretical ratios)
  - MedianPepRatio for protein ratio + LinearRegression for peptide ratio + RCC enabled (MTQ_alg1)

- The best for background proteins (theoretical ratios of 1)
  - WeightedPepRatio for protein ratio + WeightedPsmRatio for peptide ratio + RCC disabled (MTQ_alg2)

- Should use at least one normalization approach
- Several different algorithmic combinations are comparable to these two

ARE: average relative error (the smaller the better)

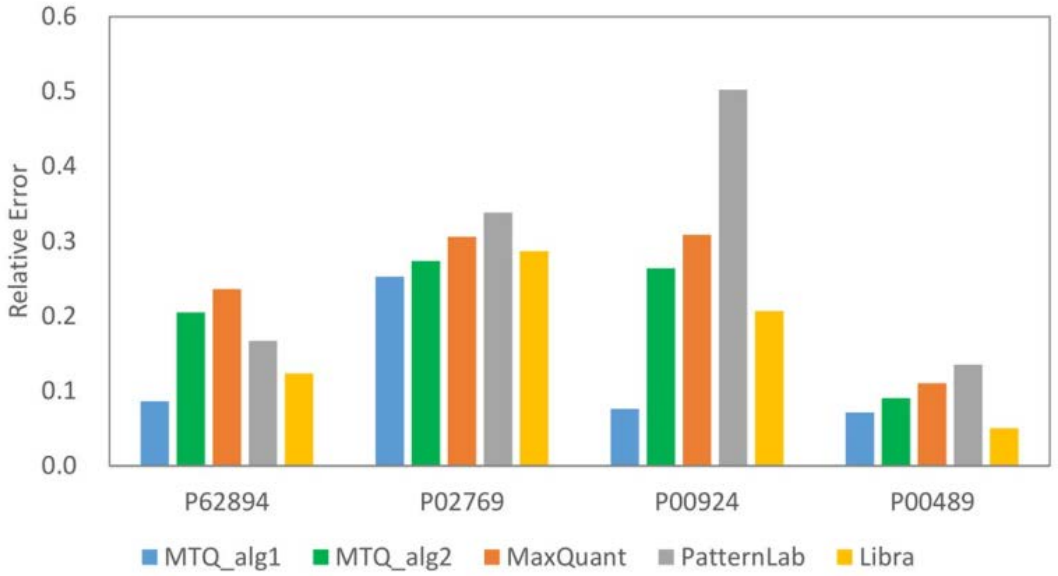
AUCCD: area under the curve of coverage vs deviation (the larger the better)



Turn on ratio compression correction improves quantitation accuracy for standard proteins, but deteriorates accuracy for background proteins

Comparison with Other Tools

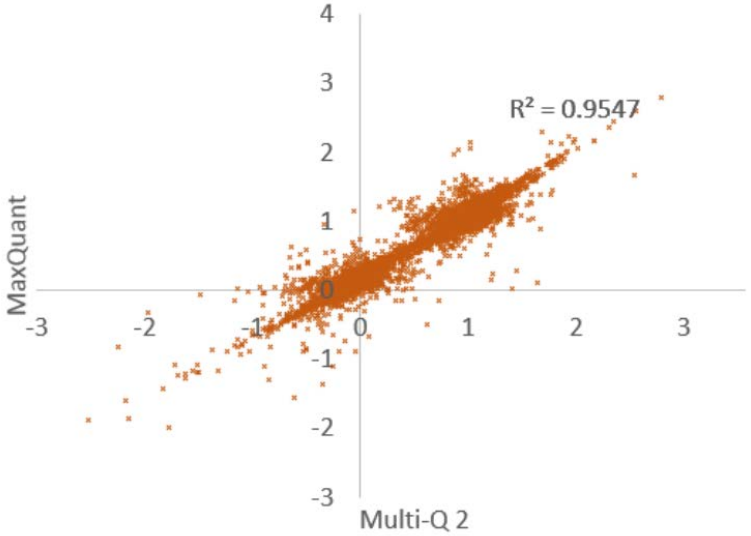
4 standard proteins

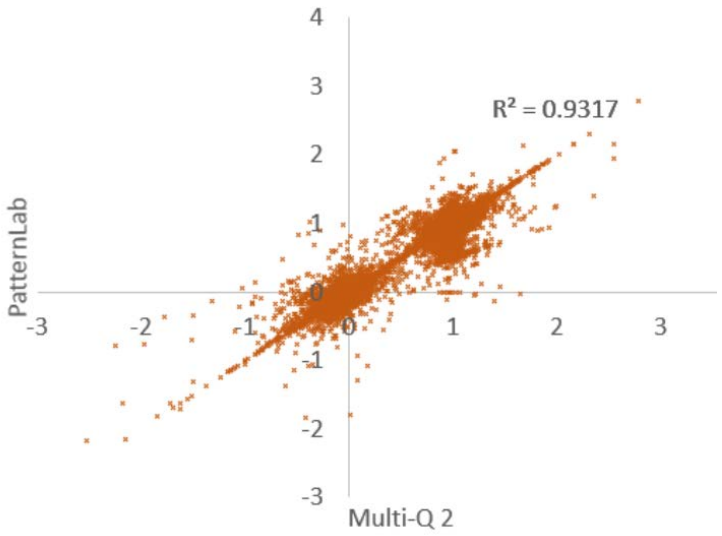


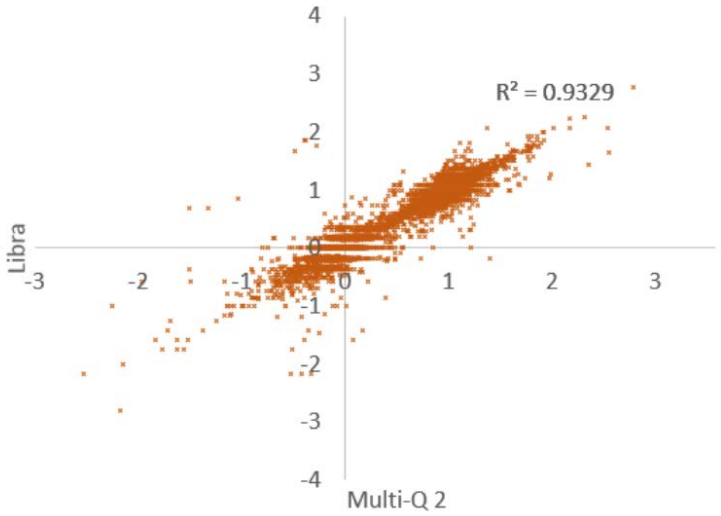
372 common quantifiable proteins

	MTQ_alg1	MTQ_alg2	MaxQuant	PatternLab	Libra
AUCCD	0.886	**0.928***	0.895	0.909	0.902
ARE_Bg	0.105	**0.062**	0.095	0.081	0.088
RMSE	0.139	**0.105**	0.145	0.131	0.125
Mean	0.934	**1.006**	1.055	1.037	0.972
SD	0.122	**0.105**	0.135	0.126	0.122

Correlation of Ratios Calculated with Different Tools







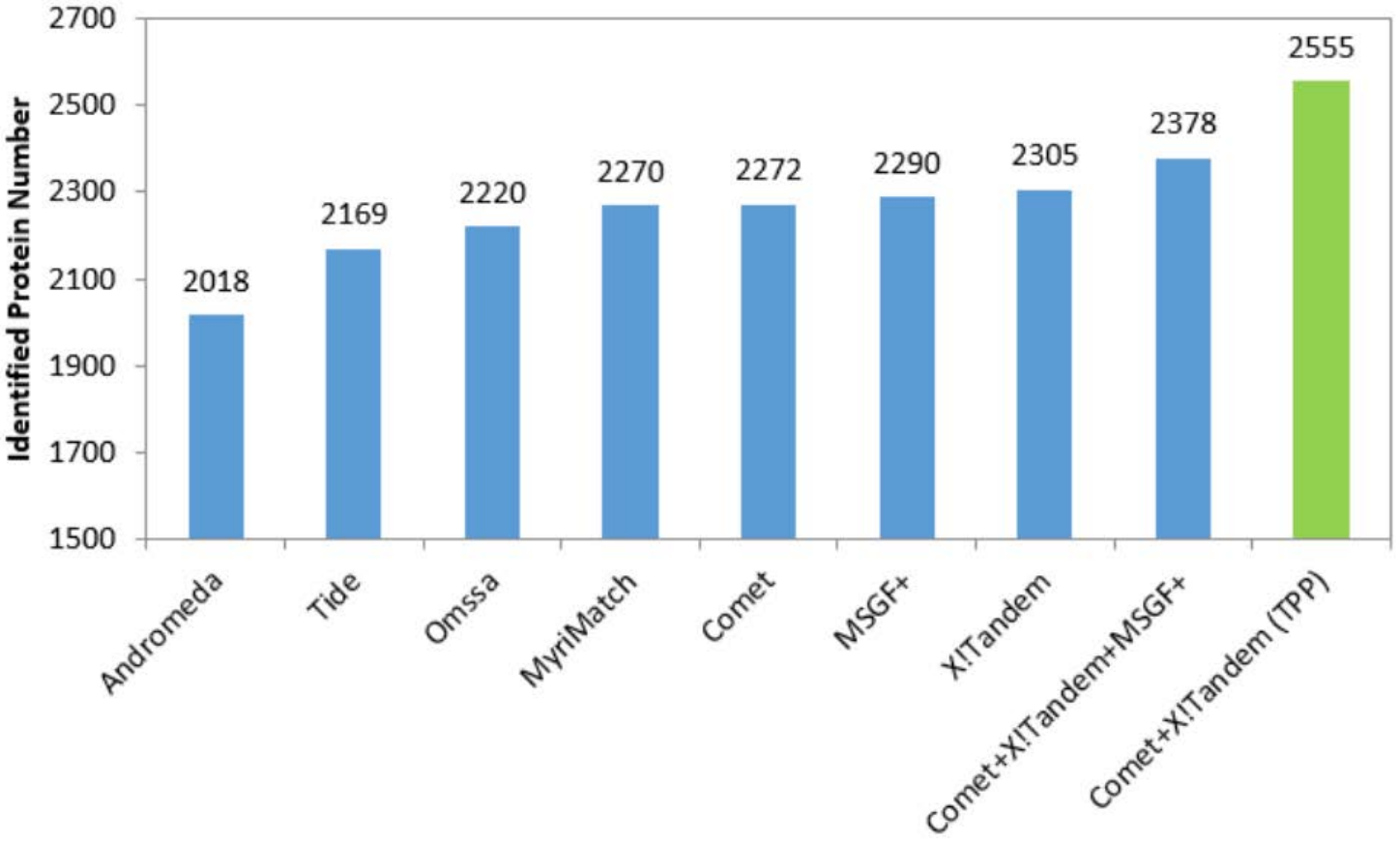
Hultin iTRAQ-8 Data Set

- Data set was published in MCP 2013
  - Lung cancer cell line A549
  - Abundance ratios for 8 channels are 2:2:1:1:2:2:1:1
  - 5 fractions
- Data analysis
  - SearchGUI + PeptideShaker
  - (Comet + Xtandem) + TPP validation (PeptideProphet + iProphet + Mayu)
- The last channel was used as denominator
  - Theretical values of 7 ratios (r1 to r7) are 2, 2, 1, 1, 2, 2, 1

- 24 Combinations
  - 4 algorithms for peptide ratio calculation
  - 6 protein
  - Ratio compression correction (RCC): on and off

Identified and Quantifiable Protein Number

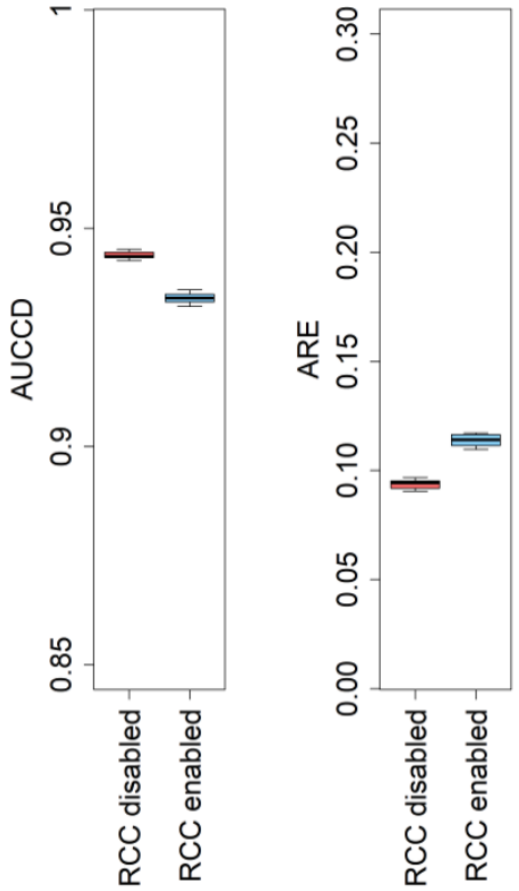
- Quantifiable protein: one whose ratios can be calculated
- Search engine does matter
- Validation pipeline does matter



Quantitation Accuracy

- MTQ_alg2 (best for background proteins in Gatto-TMT6)
  - One of the best in terms of AUCCD and ARE
- MTQ_alg1 (best for std proteins in Gatto-TMT6)
  - Rank 33/48 in terms of AUCCD
  - Rank 32/48 in terms of ARE

	MTQ_alg1	MTQ_alg2	MaxQuant	Libra	PatternLab
AUCCD	0.901	**0.905***	0.889	0.894	0.899
ARE	0.090	**0.086**	0.102	0.094	0.091
RMSE	0.220	**0.218**	0.243	0.228	0.226
Mean1[#]	0.955	0.964	1.102	**0.989**	0.966
Mean2	1.964	1.981	2.099	**1.993**	1.970
SD1	0.155	0.148	0.148	0.156	**0.136**
SD2	**0.253**	0.256	0.263	0.269	0.271



- MedianPepRatio for protein ratio + LinearRegression for peptide ratio + RCC enabled (MTQ_alg1)
- WeightedPepRatio for protein ratio + WeightedPsmRatio for peptide ratio + RCC disabled (MTQ_alg2)

What have we learned

- No single algorithmic combination is the best for all the proteins for all evaluation measures

- Do I need to turn on RCC?
- Which algorithm should I use for quantitation?
- What kind of normalization approach should I use?

Searching for temperature-dependent proteins

- Different channels are samples of different temperatures
  - Channel 114: 55 degree ➔ reference
  - 115: 65
  - 116: 75
  - 117: 80

- R1: 115/114, R2: 116/114, R3: 117/114
- Fold change > 1.5 for any of the ratios
- Using 6 algorithmic combinations to obtain ratios → get candidate protein lists
  - MedianPeptideRatio-LinearRegression
  - WeightedPeptideRatio-WeightedPsmRatio
  - WeightedPsmRatio
  - MedianPsmRatio
  - TreamedMeanPsmRatio
  - SumPsmIntensity
- RCC can be on and off, normalization is applied at reporter ion level

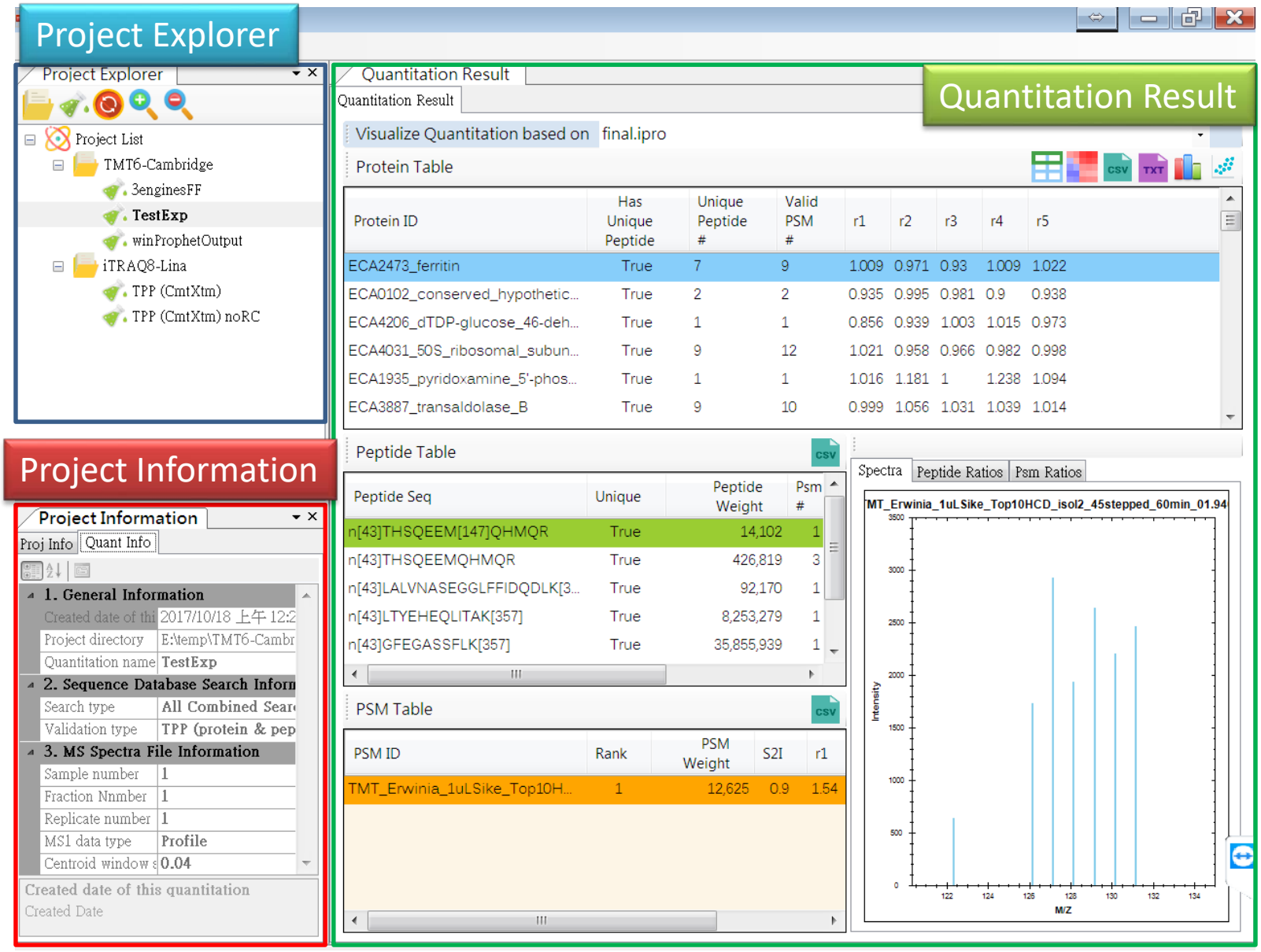
RCC is on

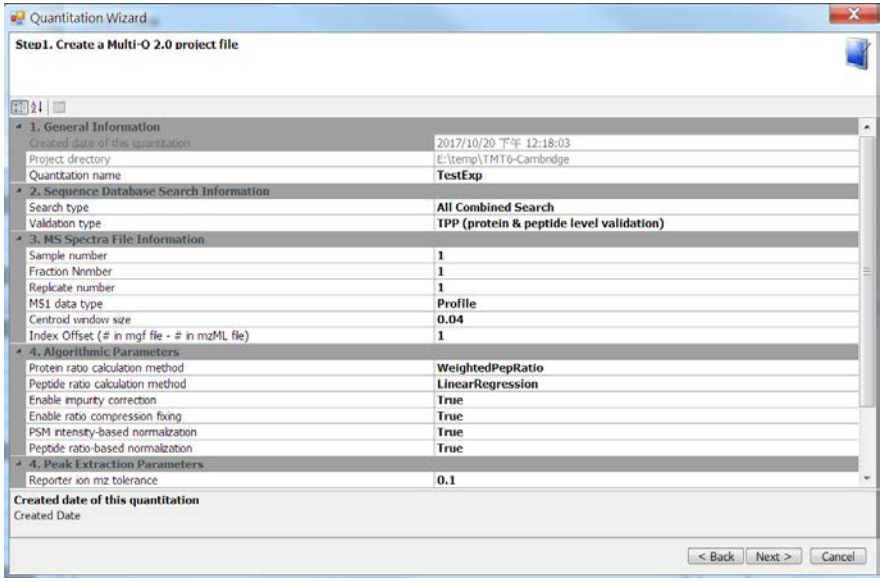
	MedPep-LinReg	WgtPep-WgtPsm	WgtPsm	MedPsm	TrMeanPsm	SumPsmInten
MedPep-LinReg	1	0.78	0.76	0.85	0.84	0.76
WgtPep-WgtPsm	—	1	0.72	0.78	0.78	0.7
WgtPsm	—	—	1	0.72	0.72	0.94
MedPsm	—	—	—	1	0.91	0.71
TrMeanPsm	—	—	—	—	1	0.7
SumPsmInten	—	—	—	—	—	1

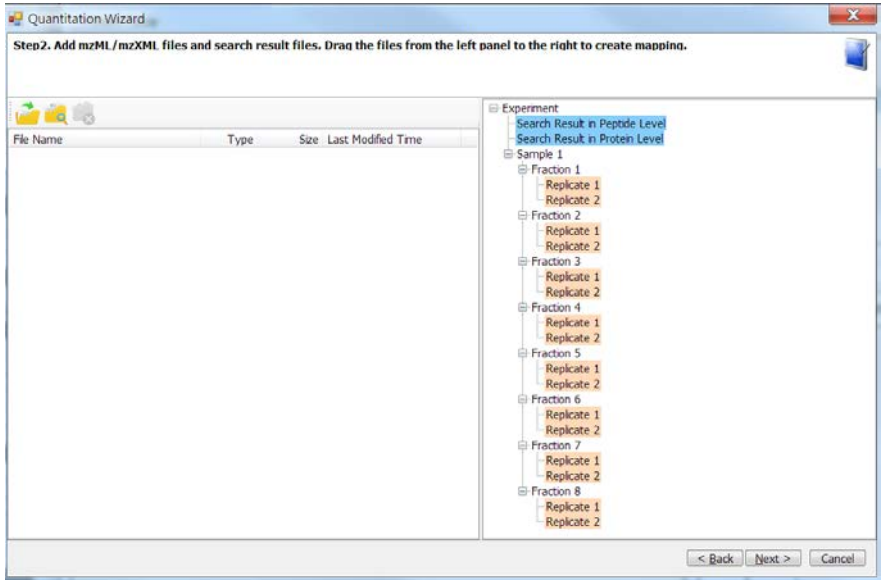
RCC is off

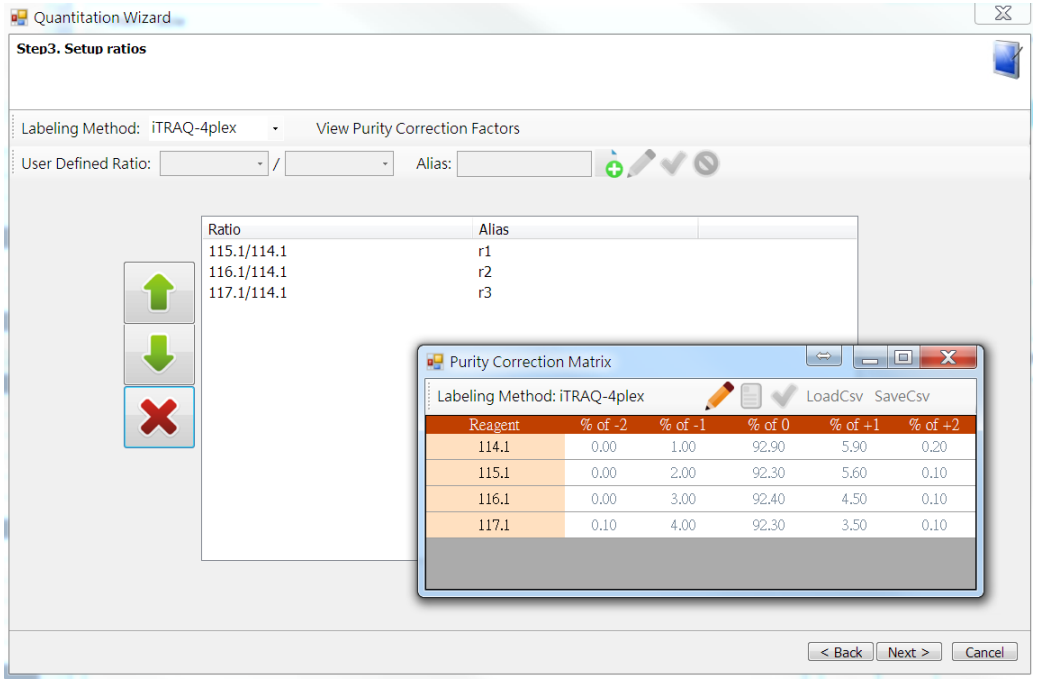
	MedPep-LinReg	WgtPep-WgtPsm	WgtPsm	MedPsm	TrMeanPsm	SumPsmInten
MedPep-LinReg	1	0.77	0.69	0.78	0.78	0.69
WgtPep-WgtPsm	—	1	0.64	0.74	0.74	0.62
WgtPsm	—	—	1	0.61	0.62	0.96
MedPsm	—	—	—	1	0.88	0.61
TrMeanPsm	—	—	—	—	1	0.62
SumPsmInten	—	—	—	—	—	1

Multi-Q 2 User Interface



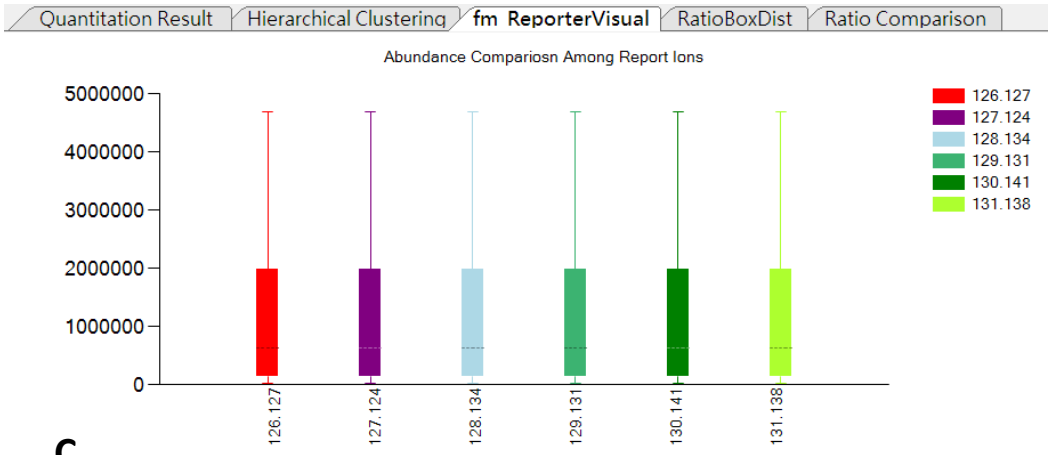

Quantitation Wizard
Step1. Create a Multi-O 2.0 project file
1. General Information
Created date of this quantitation
2017/10/20 下午 12:18:03
Project directory
E:\temp\TMT6-Cambridge
Quantitation name
TestExp
2. Sequence Database Search Information
Search type
All Combined Search
Validation type
TPP (protein & peptide level validation)
3. MS Spectra File Information
Sample number
1
Fraction Number
1
Replicate number
1
MS1 data type
Profile
Centroid window size
0.04
Index Offset (# in mgf file - # in mzML file)
1
4. Algorithmic Parameters
Protein ratio calculation method
WeightedPepRatio
Peptide ratio calculation method
LinearRegression
Enable impurity correction
True
Enable ratio compression fixing
True
PSM intensity-based normalization
True
Peptide ratio-based normalization
True
4. Peak Extraction Parameters
Reporter ion mz tolerance
0.1
Created date of this quantitation
Created Date
< Back
Next >
Cancel


Quantitation Wizard
Step2. Add mzML/mzXML files and search result files. Drag the files from the left panel to the right to create mapping.
File Name
Type
Size
Last Modified Time
Experiment
Search Result in Peptide Level
Search Result in Protein Level
Sample 1
Fraction 1
Replicate 1
Replicate 2
Fraction 2
Replicate 1
Replicate 2
Fraction 3
Replicate 1
Replicate 2
Fraction 4
Replicate 1
Replicate 2
Fraction 5
Replicate 1
Replicate 2
Fraction 6
Replicate 1
Replicate 2
Fraction 7
Replicate 1
Replicate 2
Fraction 8
Replicate 1
Replicate 2
< Back
Next >
Cancel

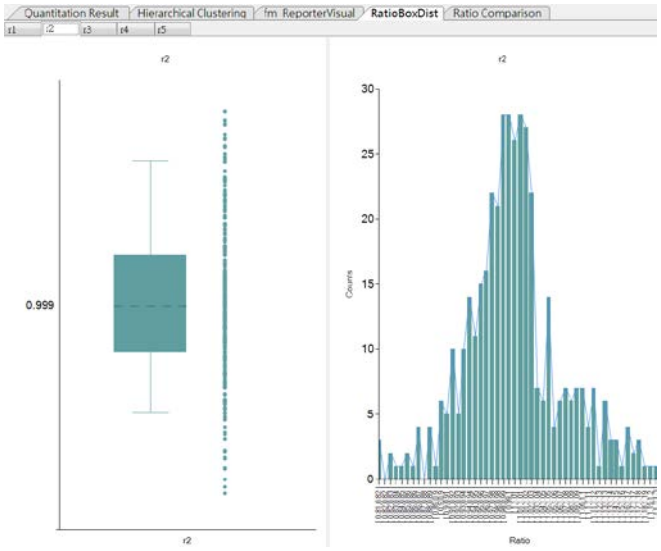

Quantitation Wizard
Step3. Setup ratios
Labeling Method: iTRAQ-4plex
View Purity Correction Factors
User Defined Ratio:
/
Alias:
Ratio
Alias
115.1/114.1
r1
116.1/114.1
r2
117.1/114.1
r3
Purity Correction Matrix
Labeling Method: iTRAQ-4plex
LoadCsv
SaveCsv
Reagent
% of -2
% of -1
% of 0
% of +1
% of +2
114.1
0.00
1.00
92.90
5.90
0.20
115.1
0.00
2.00
92.30
5.60
0.10
116.1
0.00
3.00
92.40
4.50
0.10
117.1
0.10
4.00
92.30
3.50
0.10
< Back
Next >
Cancel

Statistical Analysis Panel

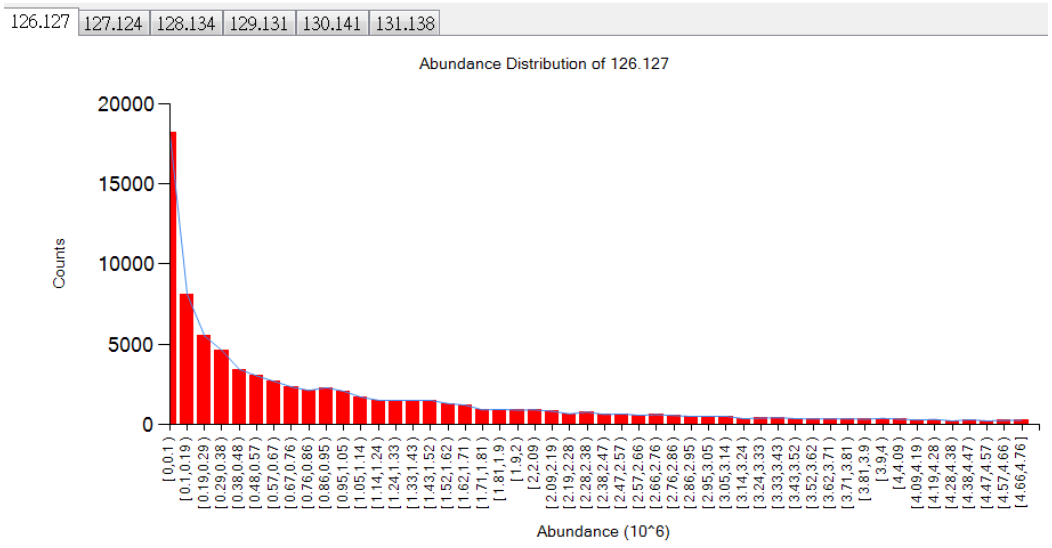
A



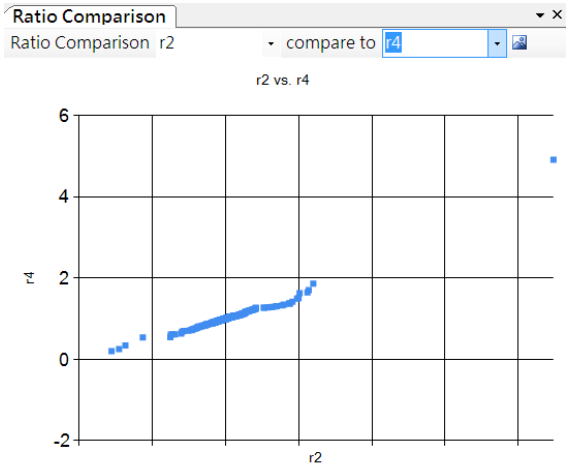
B



C

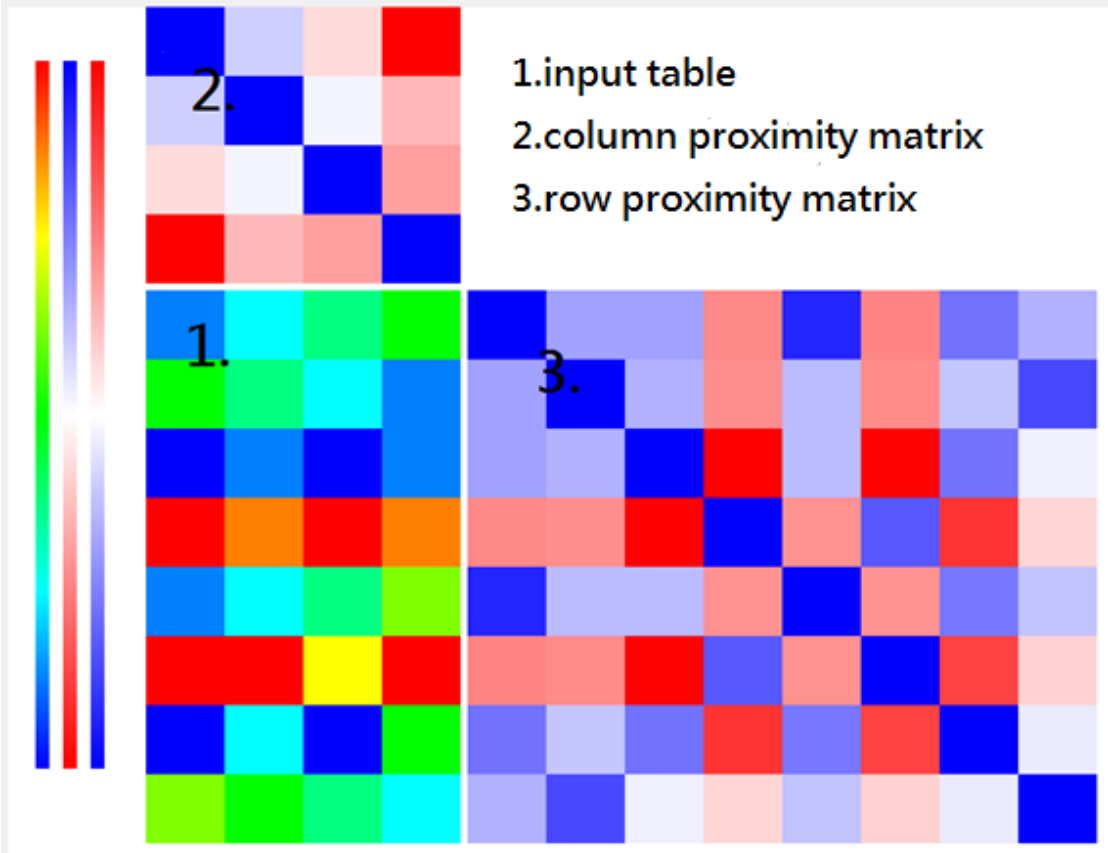


D



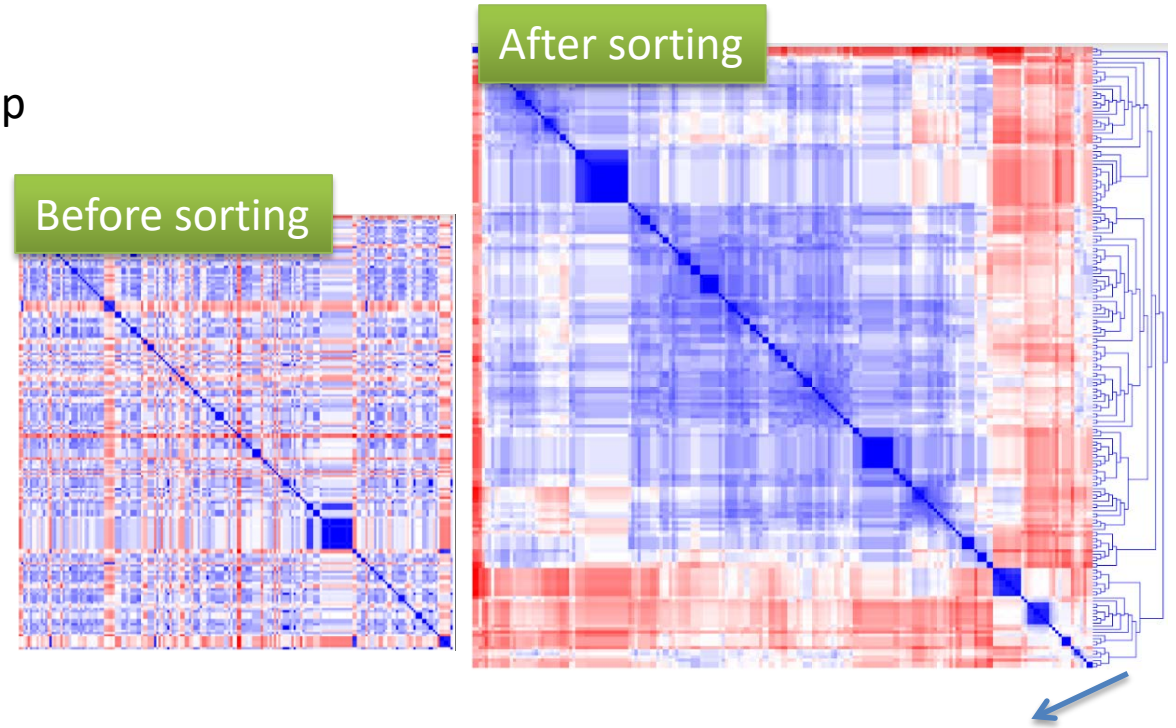
Visualization Module: Protein Similarity Heatmap

1. input table: ratios (x axis) of proteins (y axis)
2. column proximity matrix: correlation between ratios
3. row proximity matrix: correlation between proteins



Protein Similarity Heatmap

- Similarity measures
  - Euclidean distance, Mahattan distance, Pearson's correlation coefficient
- Clustering algorithm
  - Agglomerative hierarchical clustering based on single linkage, complete linkage, and average linkage
- Flip algorithm
  - For data sorting
  - Uncle flip and grandpa flip
- Coloring
  - Red: low correlation
  - Blue: high correlation



Conclusions

- Maximize quantitative protein coverage
  - Supports multiple search engines
  - Supports several popular validation pipelines: TPP, PeptideShaker, PD
- Optimize quantitation accuracy
  - Various ratio calculation algorithms
  - Several evaluation measures
  - Implement a ratio compression correction algorithm
- No single algorithm is the best for all the proteins under all the situations
- Graphical interface for result visualization
- Heat map and hierarchical clustering for further analysis

Acknowledgement

Ting-Yi Sung

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Chu-Ling Ko

Mamie Lih

Yi-Ju Chen

Hands-on Tutorial

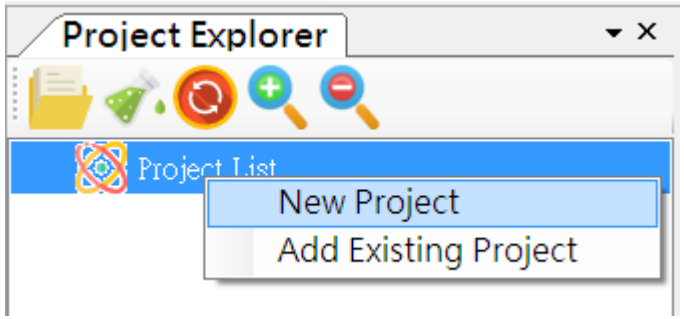
Software Prerequisite

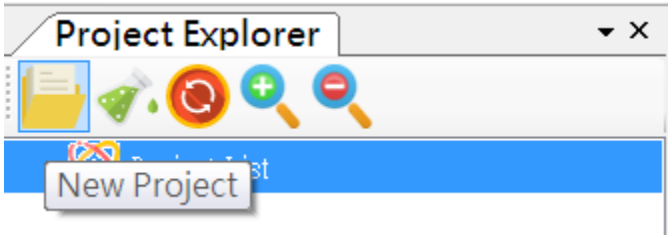
- This slide is available on the web: https://goo.gl/smXFN4
- .net framework 4 or newer version
- Raw data in mzML or mzXML
- Statistically validated identification result
  - "TPP (protein & peptide level validation)"
  - "TPP (peptide level validation)“
  - "PeptideShaker (protein & peptide level validation)"
  - "Proteome Discoverer (protein & peptide level validation)"

Create New Project in Multi-Q 2

- Double click on Multi-Q_v2.exe

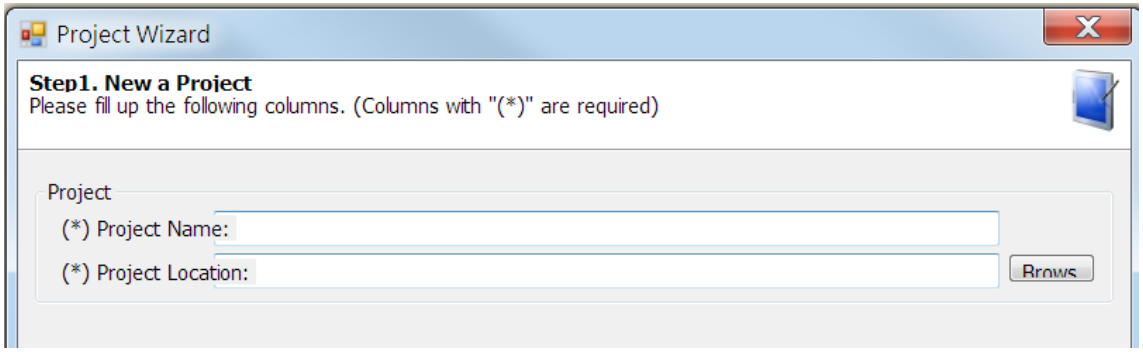
- Create new project
  - Right click on “Project List” in Project Explorer, or
  - Click on “New Project” button on the toolstrip



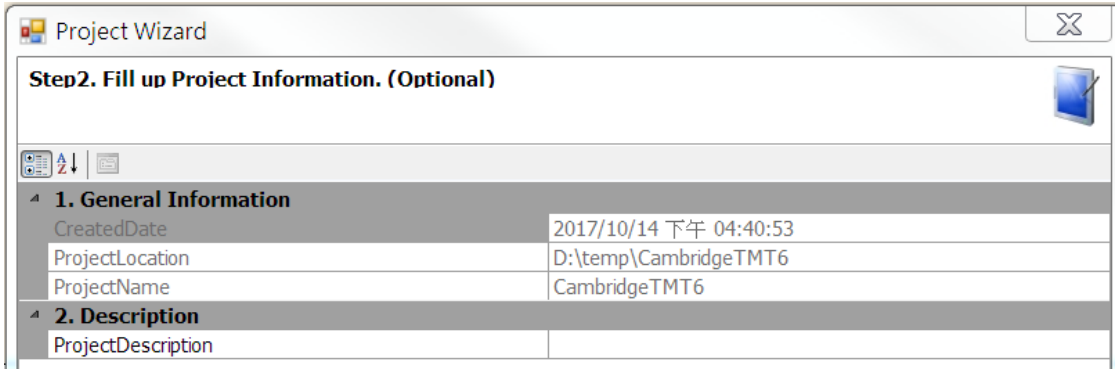


- A project is a folder. In the folder, there can be many quantitation experiments, each of which corresponds to a sub-folder

- In Project Name, type “CambridgeTMT6”. Then, Click on Browse to select a directory for Multi-Q Project.



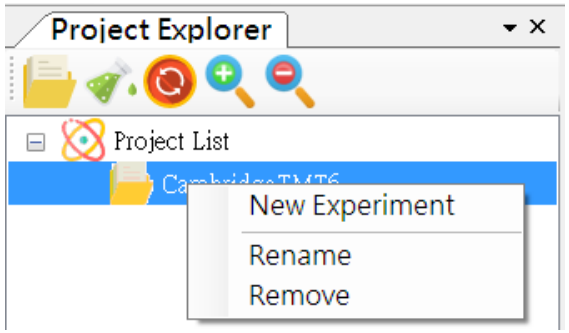
- Suppose you select D:\temp, the next page will look like this

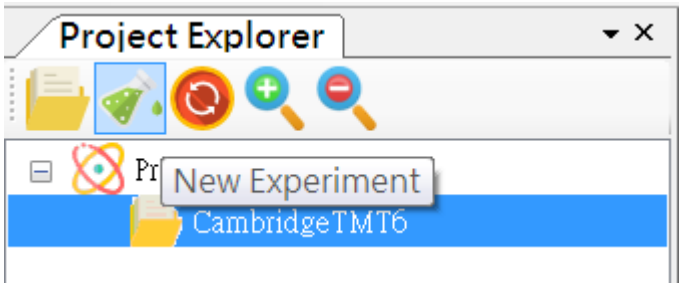


- A new directory will be created in D:\temp

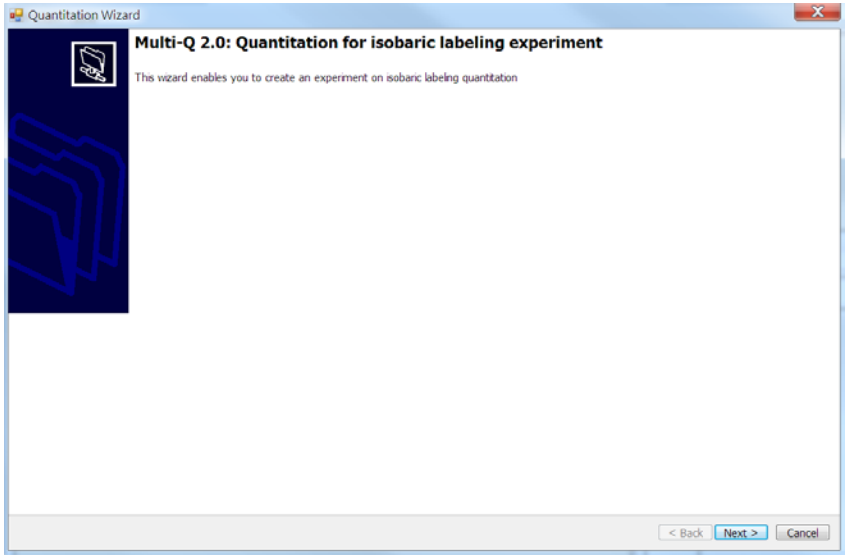
Create New Experiment

- Right click on project node or click on New Experiment button on the toolstrip to create new experiment





- A wizard will pop up, click on “Next”



Quantitation Parameters

1. General Information	
Created date of this quantitation	2017/10/17 下午 07:08:34
Project directory	E:\temp\TMT6-Cambridge
Quantitation name	**TestExp**
2. Sequence Database Search Information	
Search type	**All Combined Search**
Validation type	**PeptideShaker (protein & peptide level validation)**
3. MS Spectra File Information	
Sample number	**1**
Fraction Nnmber	**1**
Replicate number	**1**
MS1 data type	**Profile**
Centroid window size	**0.04**
Index Offset (# in mgf file - # in mzML file)	**1**

Search Type

Individual Search
Run Combined Search
Fraction Combined Search
All Combined Search

Validation Type

TPP (protein & peptide level validation)
TPP (peptide level validation)
PeptideShaker (protein & peptide level validation)
Proteome Discoverer (protein & peptide level validation)

MS1 data type

Centroid

Centroid window size

- Should be large enough to cover multiple profile peaks
- Use ProteoWizard to perform centroiding, then select “Centroid” for MS1 data type

Search Type

3. MS Spectra File Information	
Sample number	**2**
Fraction Nnmber	**3**
Replicate number	**2**

All combined search

- Experiment
  - Search Result in Peptide Level
  - Search Result in Protein Level
  - Sample 1
    - Fraction 1
      - Replicate 1
      - Replicate 2
    - Fraction 2
      - Replicate 1
      - Replicate 2
    - Fraction 3
      - Replicate 1
      - Replicate 2
  - Sample 2
    - Fraction 1
      - Replicate 1
      - Replicate 2
    - Fraction 2
      - Replicate 1
      - Replicate 2
    - Fraction 3
      - Replicate 1
      - Replicate 2

Fraction combined search

- Experiment
  - Sample 1
    - Search Result in Peptide Level
    - Search Result in Protein Level
    - Fraction 1
      - Replicate 1
      - Replicate 2
    - Fraction 2
      - Replicate 1
      - Replicate 2
    - Fraction 3
      - Replicate 1
      - Replicate 2
  - Sample 2
    - Search Result in Peptide Level
    - Search Result in Protein Level
    - Fraction 1
      - Replicate 1
      - Replicate 2
    - Fraction 2
      - Replicate 1
      - Replicate 2
    - Fraction 3
      - Replicate 1
      - Replicate 2

Run combined search

- Experiment
  - Sample 1
    - Fraction 1
      - Search Result in Peptide Level
      - Search Result in Protein Level
      - Replicate 1
      - Replicate 2
    - Fraction 2
      - Search Result in Peptide Level
      - Search Result in Protein Level
      - Replicate 1
      - Replicate 2
    - Fraction 3
      - Search Result in Peptide Level
      - Search Result in Protein Level
      - Replicate 1
      - Replicate 2
  - Sample 2
    - Fraction 1
      - Search Result in Peptide Level
      - Search Result in Protein Level
      - Replicate 1
      - Replicate 2
    - Fraction 2
      - Search Result in Peptide Level
      - Search Result in Protein Level
      - Replicate 1
      - Replicate 2

Individual search

- Experiment
  - Sample 1
    - Fraction 1
      - Replicate 1
        - Search Result in Peptide Level
        - Search Result in Protein Level
      - Replicate 2
        - Search Result in Peptide Level
        - Search Result in Protein Level
    - Fraction 2
      - Replicate 1
        - Search Result in Peptide Level
        - Search Result in Protein Level
      - Replicate 2
        - Search Result in Peptide Level
        - Search Result in Protein Level
    - Fraction 3
      - Replicate 1
        - Search Result in Peptide Level
        - Search Result in Protein Level
      - Replicate 2
        - Search Result in Peptide Level
        - Search Result in Protein Level
  - Sample 2
    - Fraction 1
      - Replicate 1
        - Search Result in Peptide Level
        - Search Result in Protein Level

Validation Type

3. MS Spectra File Information	
Sample number	1
Fraction Nnmber	1
Replicate number	1

TPP (protein and peptide validation)

- Experiment
  - Sample 1
    - Fraction 1
      - Replicate 1
        - Search Result in Peptide Level
        - Search Result in Protein Level

TPP (peptide validation)

- Experiment
  - Sample 1
    - Fraction 1
      - Replicate 1
        - Search Result in Peptide Level

PeptideShaker
(protein and peptide validation)

- Experiment
  - Sample 1
    - Fraction 1
      - Replicate 1
        - Search Result in PSM Level
        - Search Result in Peptide Level
        - Search Result in Protein Level

Proteome Discoverer
(protein and peptide validation)

- Experiment
  - Sample 1
    - Fraction 1
      - Replicate 1
        - Search Result in Peptide Level
        - Search Result in Protein Level

Algorithmic Parameters

4. Algorithmic Parameters	
Protein ratio calculation method	**WeightedPepRatio**
Peptide ratio calculation method	**LinearRegression**
Enable impurity correction	**True**
Enable ratio compression fixing	**True**
PSM intensity-based normalization	**True**
Peptide ratio-based normalization	**True**

Protein ratio calculation method

- MedianPepRatio
- MedianPsmRatio
- WeightedPepRatio
- TrimmedMeanPsmRatio

Peptide ratio calculation method

- WeightedPsmRatio
- LinearRegression
- Median

Peak Extraction Parameters

4. Peak Extraction Parameters	
Reporter ion mz tolerance	**0.1**
Reporter ion mz tolerance (unit)	**Da**
Precursor isolation window size (Da)	**1.2**
Precursor mz tolerance (Da)	**0.02**
Precursor isotopic peak mz tolerance (Da)	**0.02**

Always used by quantitation algorithm

Used only when ratio compression fixing is enabled

Reporter ion extraction for 126.127726 in a TMT-6 data set

→ Search within the range of {126.127726 - 0.1, 126.127726 + 0.1}

126.127726
127.124761
127.131081
128.128116
128.134436
129.131471
129.137790
130.134825
130.141145
131.138180

For TMT-10, the tolerance should be much smaller, say 0.001

Ratio Compression Fixing

- JPR 2013

Journal of proteome research

Article

pubs.acs.org/jpr

Measuring and Managing Ratio Compression for Accurate iTRAQ/ TMT Quantification

Mikhail M. Savitski,[†,*] Toby Mathieson,[†] Nico Zinn,[†] Gavain Sweetman,[†] Carola Doce,[†] Isabelle Becher,[†] Fiona Pachl,[‡] Bernhard Kuster,[‡,§] and Marcus Bantscheff[†,*]

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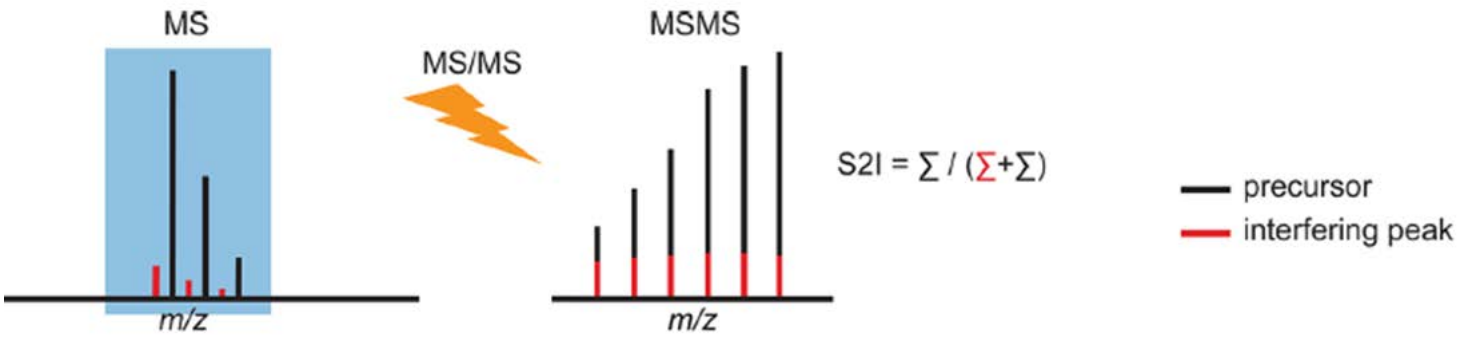
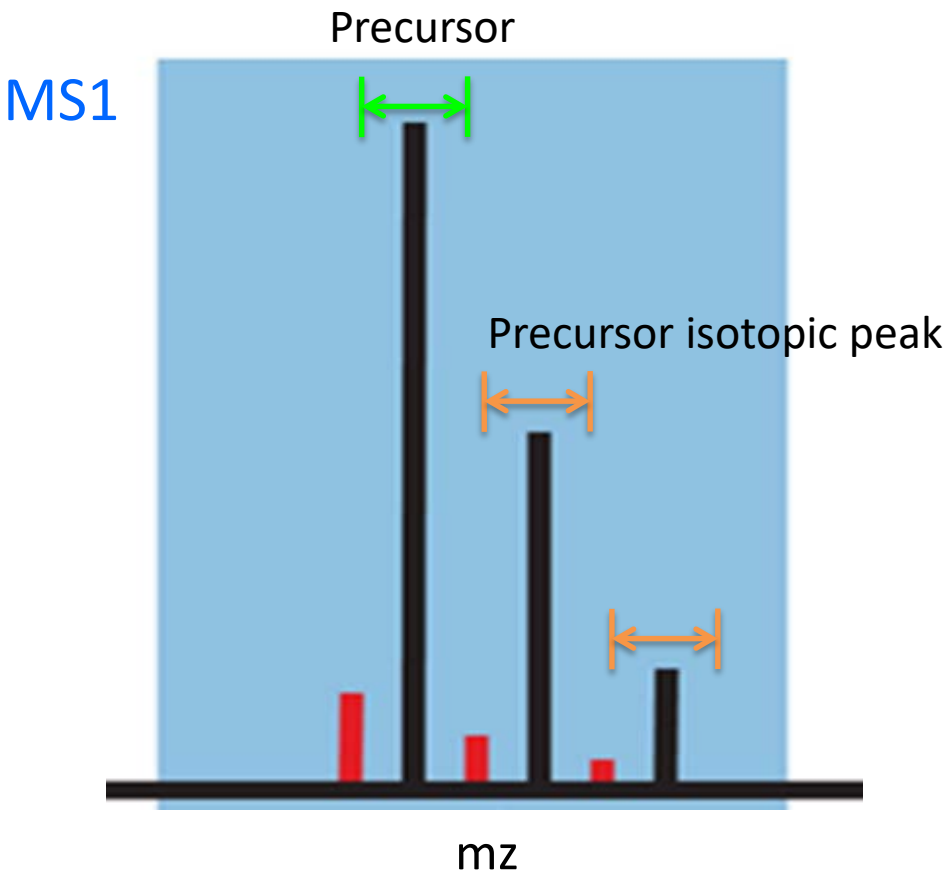
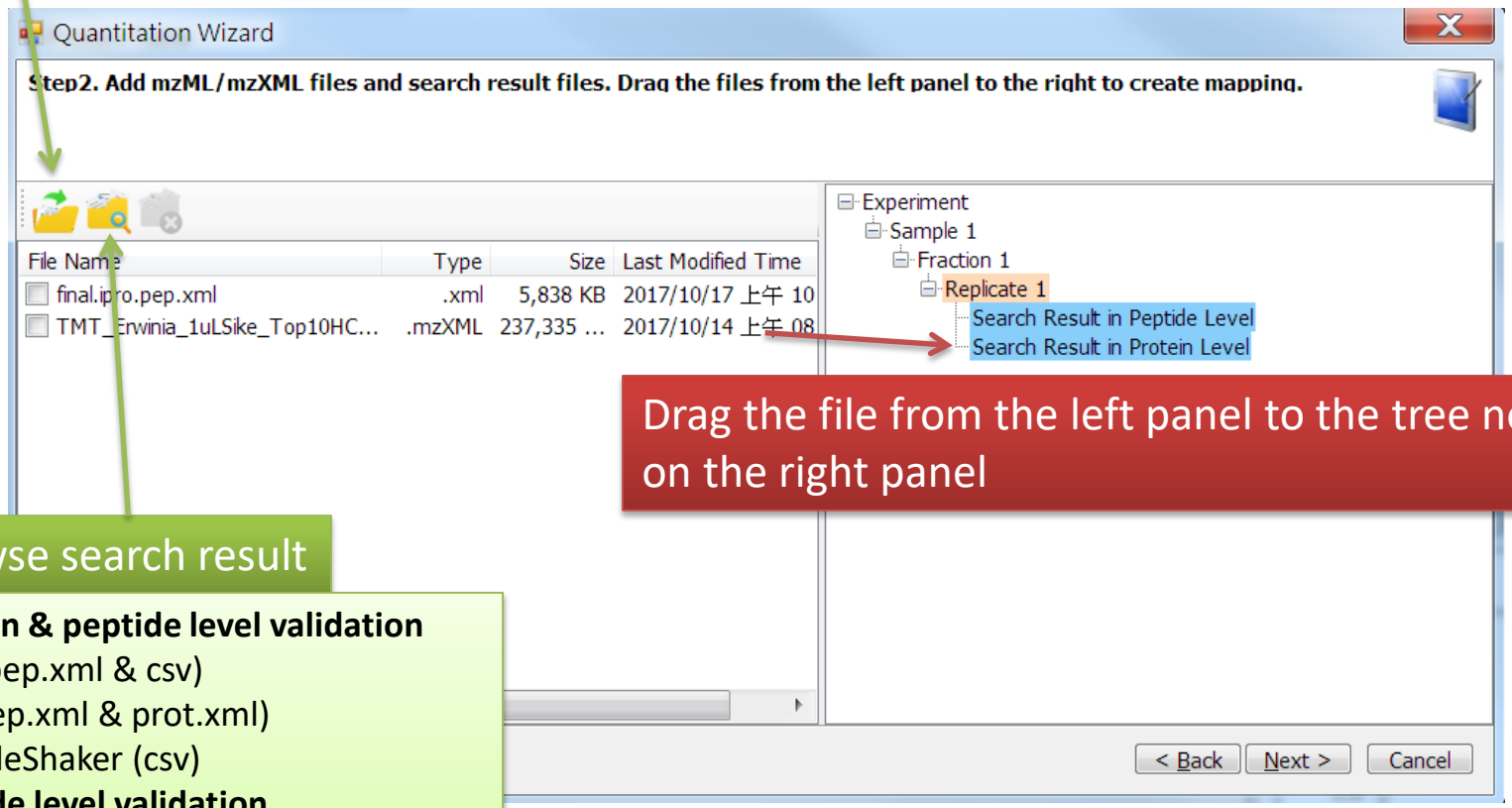


Ratio Compression Fixing Parameters

4. Peak Extraction Parameters	
Reporter ion mz tolerance	**0.1**
Reporter ion mz tolerance (unit)	**Da**
Precursor isolation window size (Da)	**1.2**
Precursor mz tolerance (Da)	**0.02**
Precursor isotopic peak mz tolerance (Da)	**0.02**



Browse mzML or mzXML

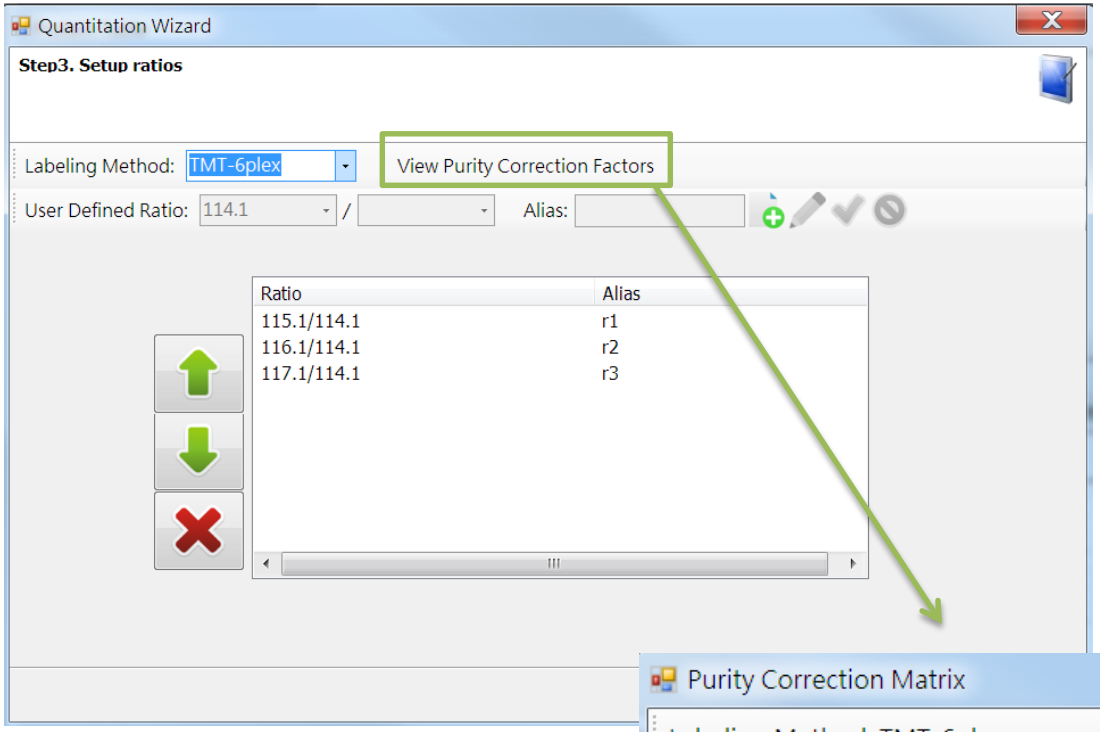


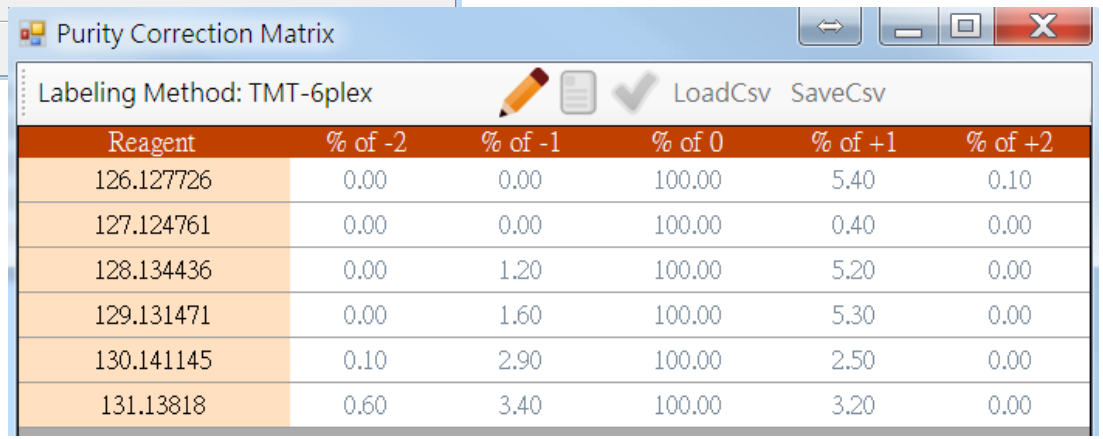
Drag the file from the left panel to the tree node on the right panel

Browse search result

Protein & peptide level validation
TPP (pep.xml & csv)
PD (pep.xml & prot.xml)
PeptideShaker (csv)
Peptide level validation
TPP (pep.xml)

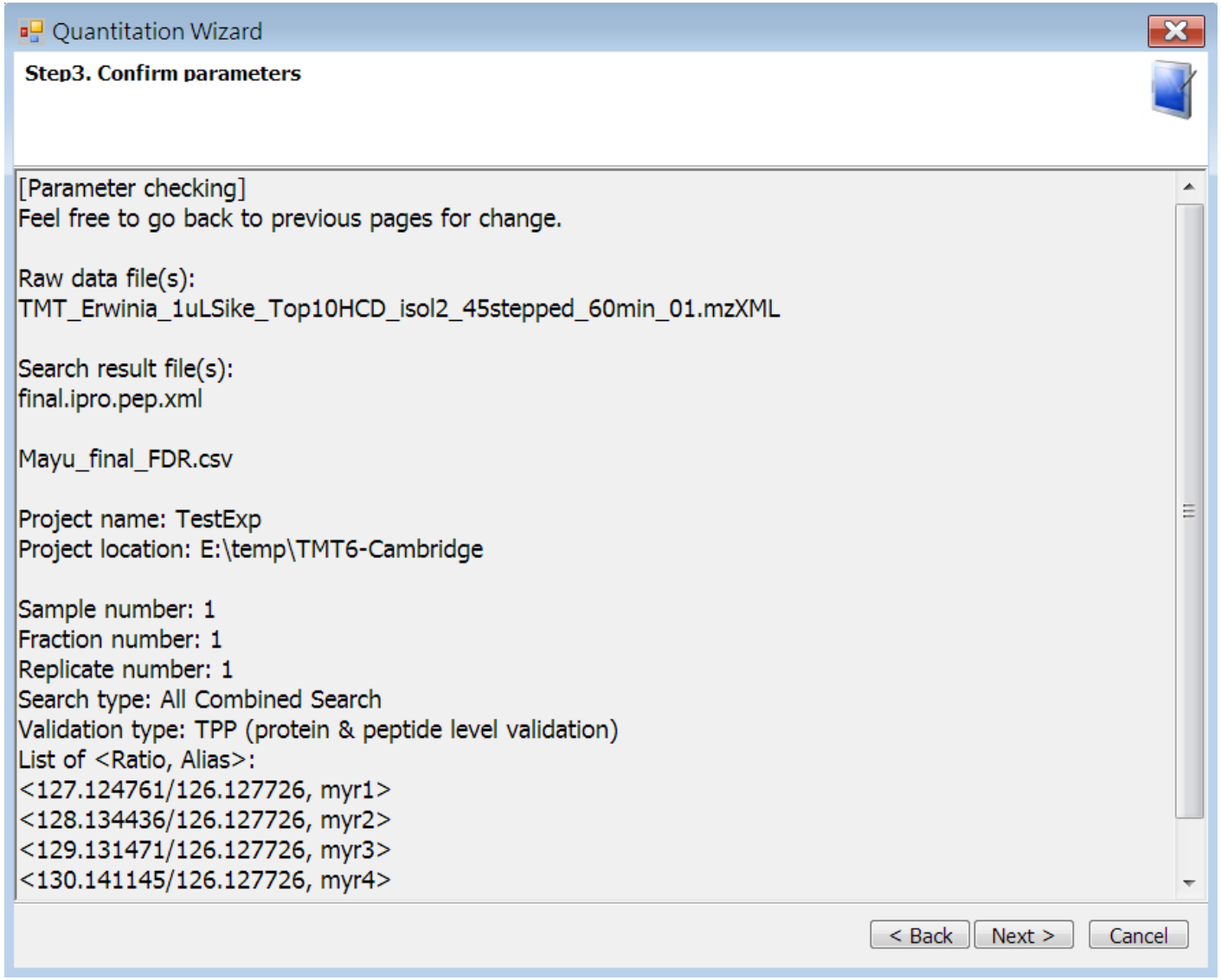
Ratio Editor & Purity Correction Matrix



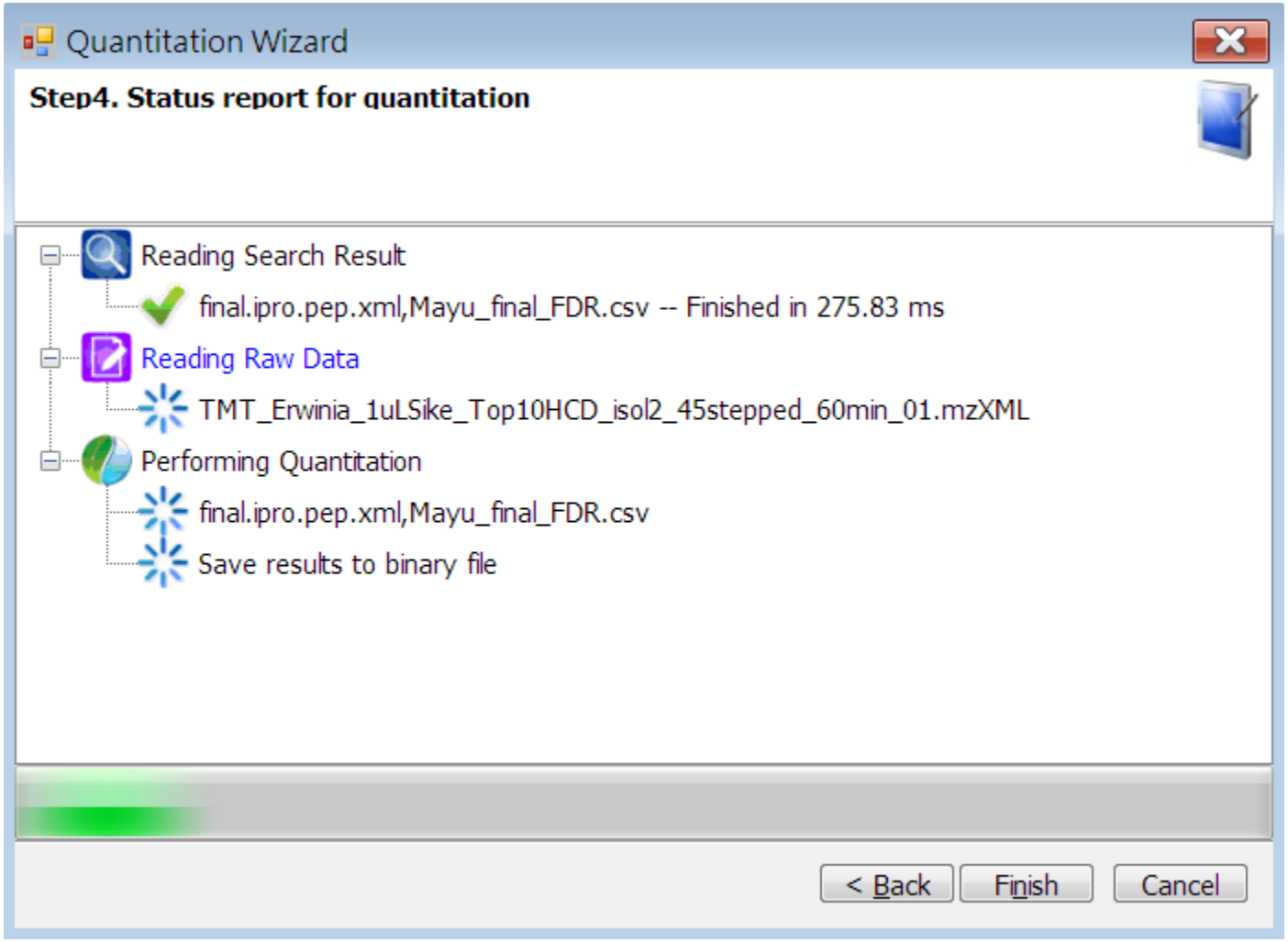


Reagent	% of -2	% of -1	% of 0	% of +1	% of +2
126.127726	0.00	0.00	100.00	5.40	0.10
127.124761	0.00	0.00	100.00	0.40	0.00
128.134436	0.00	1.20	100.00	5.20	0.00
129.131471	0.00	1.60	100.00	5.30	0.00
130.141145	0.10	2.90	100.00	2.50	0.00
131.13818	0.60	3.40	100.00	3.20	0.00

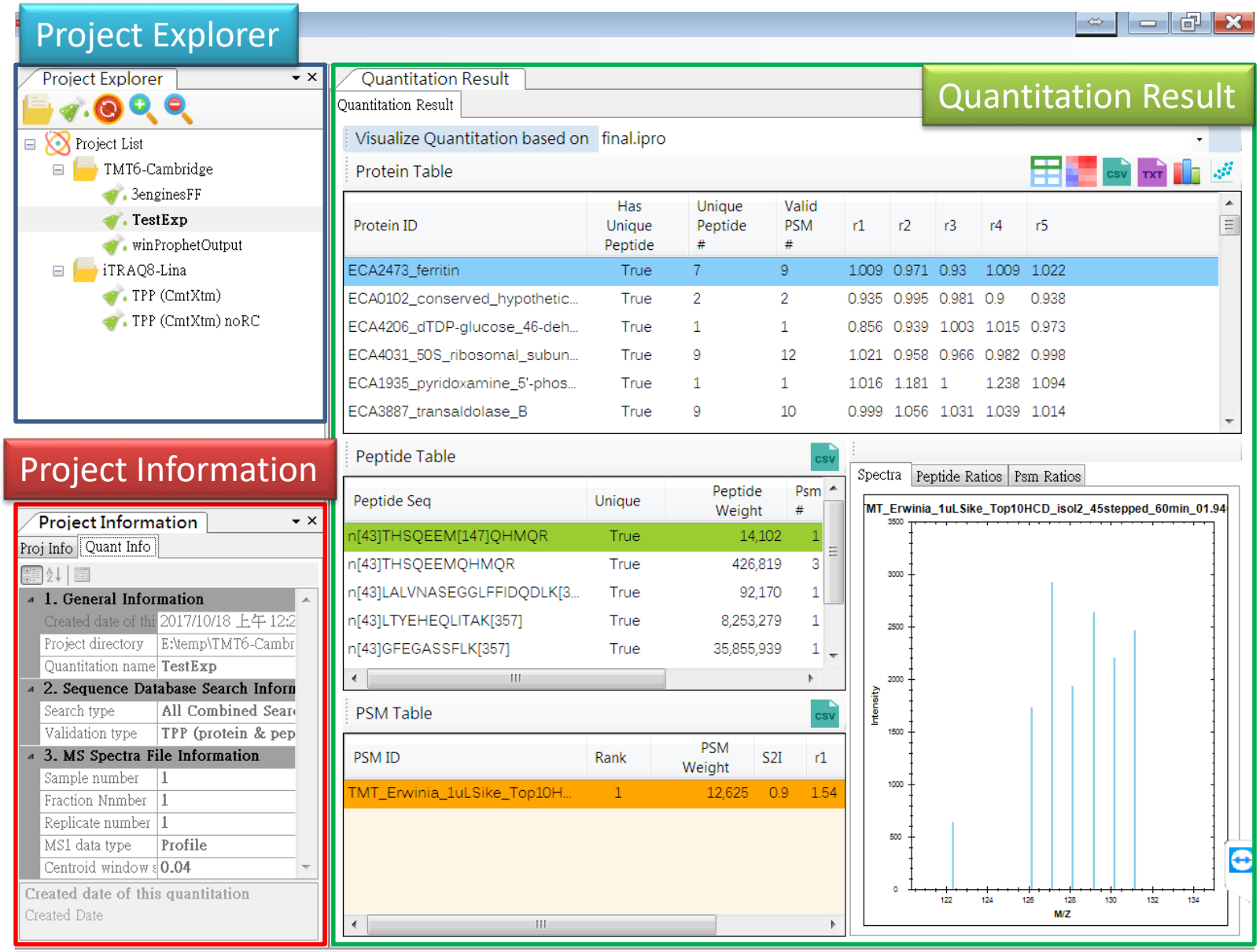
Parameter Confirmation



Process Running



Result Page

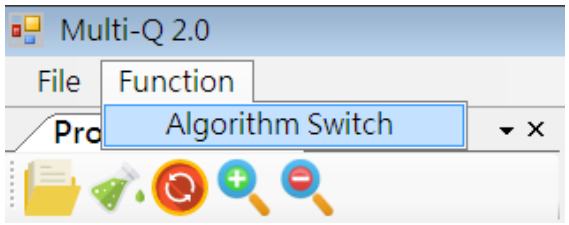

Project Explorer
Project Explorer
Project List
TMT6-Cambridge
3enginesFF
TestExp
winProphetOutput
iTRAQ8-Lina
TPP (CmtXtm)
TPP (CmtXtm) noRC
Quantitation Result
Quantitation Result
Quantitation Result
Visualize Quantitation based on final.ipro
Protein Table
Protein ID | Has Unique Peptide | Unique Peptide # | Valid PSM # | r1 | r2 | r3 | r4 | r5
ECA2473_ferritin | True | 7 | 9 | 1.009 | 0.971 | 0.93 | 1.009 | 1.022
ECA0102_conserved_hypothetic... | True | 2 | 2 | 0.935 | 0.995 | 0.981 | 0.9 | 0.938
ECA4206_dTDP-glucose_46-deh... | True | 1 | 1 | 0.856 | 0.939 | 1.003 | 1.015 | 0.973
ECA4031_50S_ribosomal_subun... | True | 9 | 12 | 1.021 | 0.958 | 0.966 | 0.982 | 0.998
ECA1935_pyridoxamine_5'-phos... | True | 1 | 1 | 1.016 | 1.181 | 1 | 1.238 | 1.094
ECA3887_transaldolase_B | True | 9 | 10 | 0.999 | 1.056 | 1.031 | 1.039 | 1.014
Peptide Table
Peptide Seq | Unique | Peptide Weight | Psm #
n[43]THSQEEM[147]QHMQR | True | 14,102 | 1
n[43]THSQEEMQHMQR | True | 426,819 | 3
n[43]LALVNASEGGLFFIDQDLK[3... | True | 92,170 | 1
n[43]LTYEHEQLITAK[357] | True | 8,253,279 | 1
n[43]GFEGASSFLK[357] | True | 35,855,939 | 1
PSM Table
PSM ID | Rank | PSM Weight | S2I | r1
TMT_Erwinia_1uLSike_Top10H... | 1 | 12,625 | 0.9 | 1.54
Spectra
Peptide Ratios
Psm Ratios
MT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.94
Intensity
M/Z
Project Information
Project Information
Proj Info
Quant Info
1. General Information
Created date of thi 2017/10/18 上午 12:2
Project directory E:\temp\TMT6-Cambr
Quantitation name TestExp
2. Sequence Database Search Inform
Search type All Combined Sear
Validation type TPP (protein & pep
3. MS Spectra File Information
Sample number 1
Fraction Nnmber 1
Replicate number 1
MS1 data type Profile
Centroid window s 0.04
Created date of this quantitation
Created Date

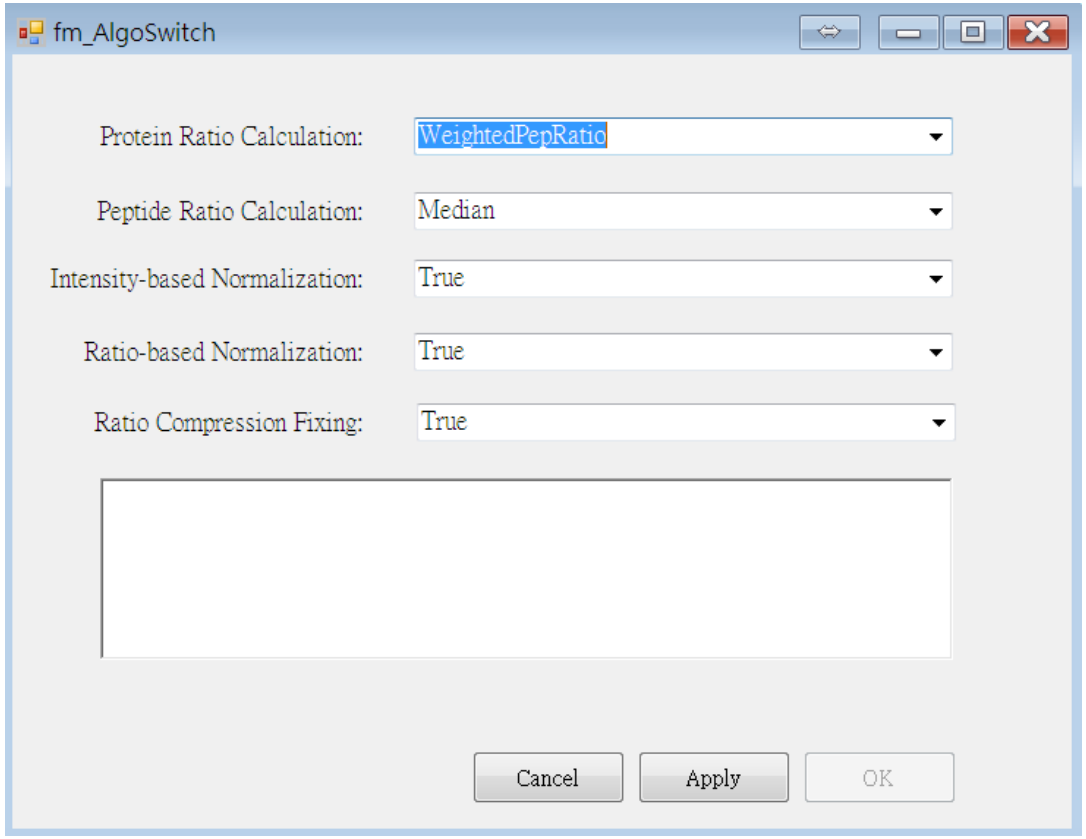
Files in Experiment Directory

Gatto-TMT6.conf	2021/4/6 下午 04:52	CONF 檔案	9 KB
Gatto-TMT6.procData	2021/4/6 下午 04:52	PROCDATA 檔案	118,789 KB
Gatto-TMT6.qResultDi	2021/4/6 下午 04:52	QRESULTDI 檔案	2,355 KB
Gatto-TMT6.sResultDi	2021/4/6 下午 04:52	SRESULTDI 檔案	879 KB

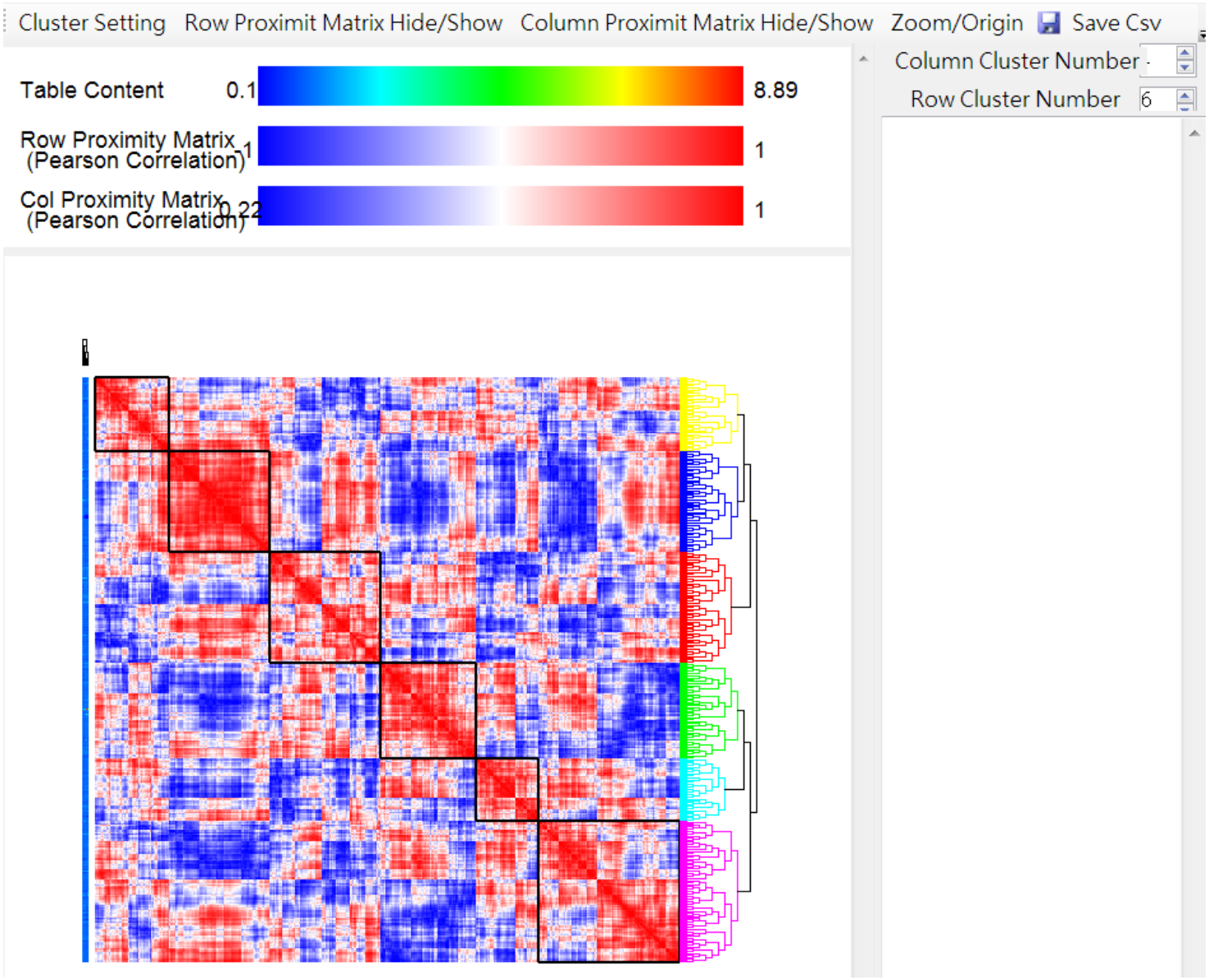
Gatto-TMT6.conf: all the parameter settings
Gatto-TMT6.procData: binary file for the raw data (mzML/mzXML)
Gatto-TMT6.qResultDi: quantitation result
Gatto-TMT6.sResultDi: database search result

Switch to Different Quantitation Algorithms

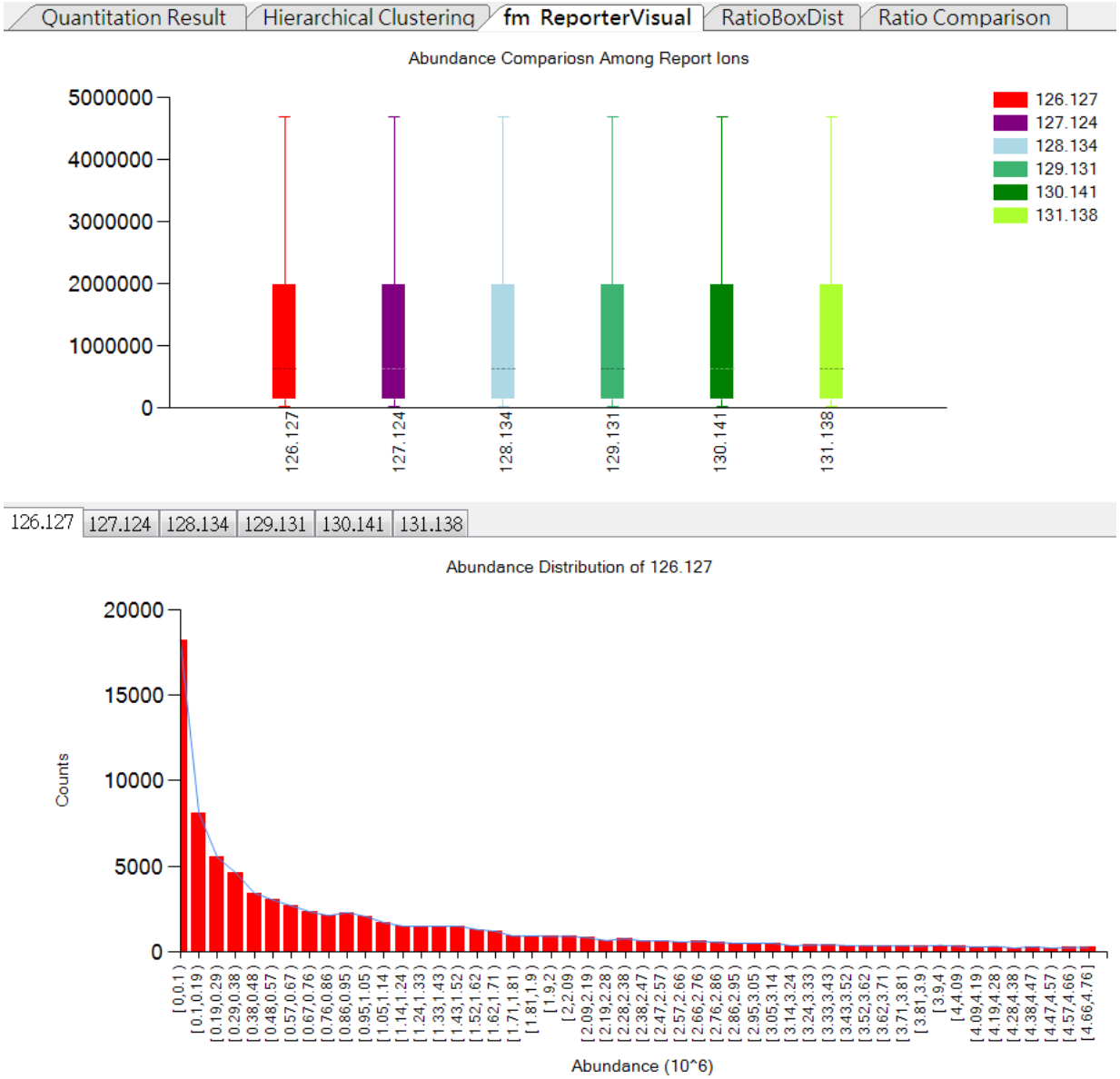




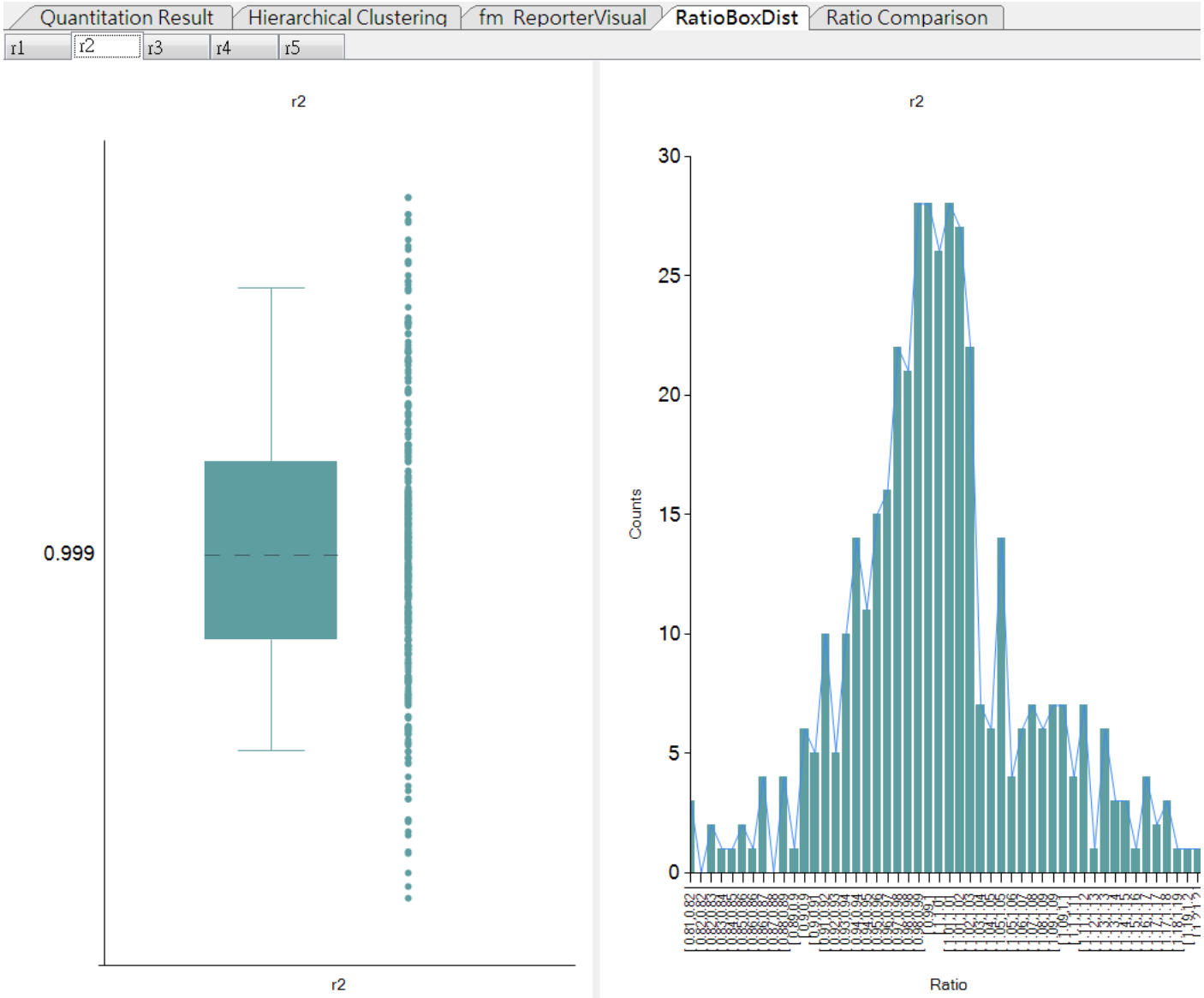
Heat Map & Protein Clustering



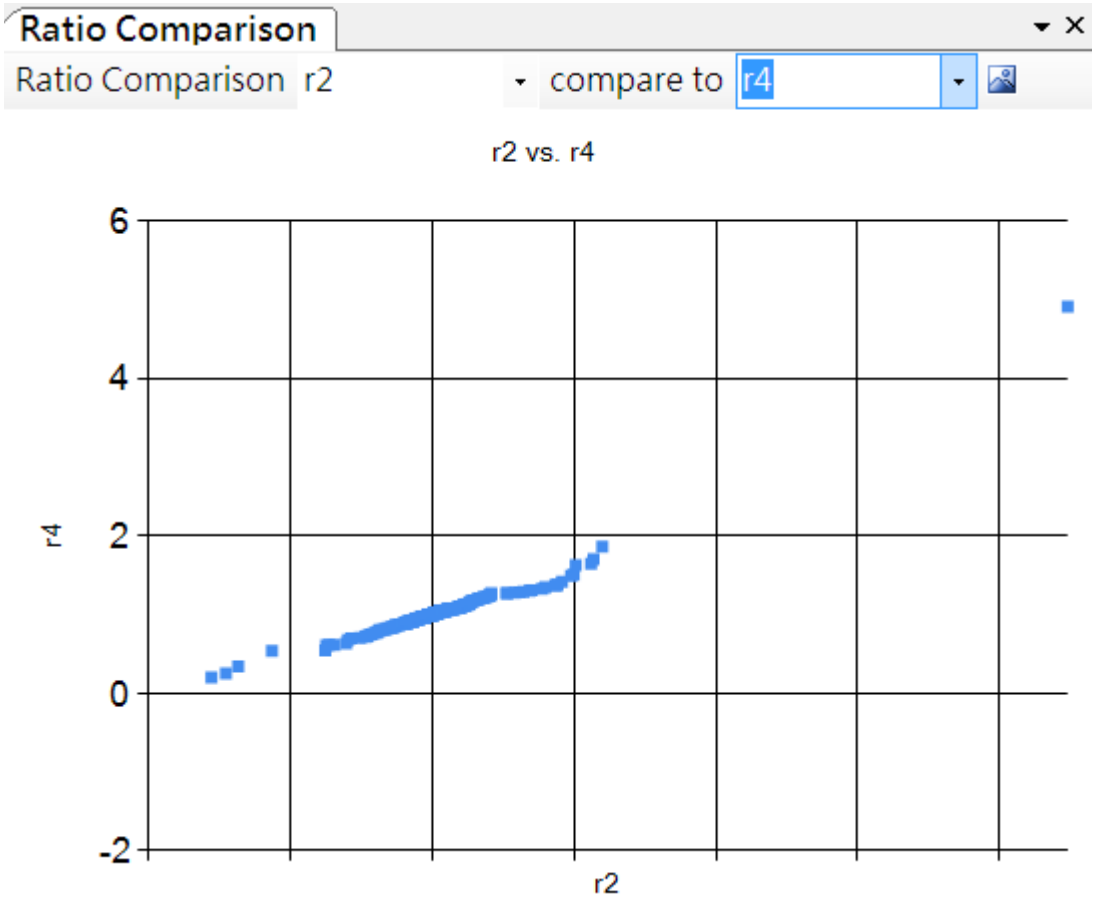
Reporter Ion Abundance Analysis



Distribution of a Single Ratio



Ratio vs Ratio Scatter Plot



Search Result Checker

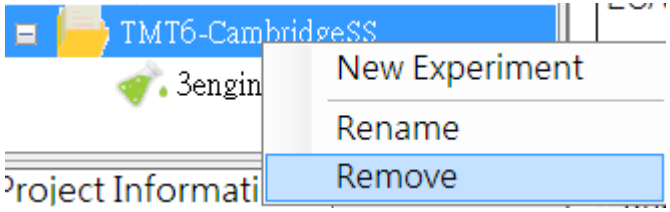
- Check whether the search result files are valid for Multi-Q 2 quantitation

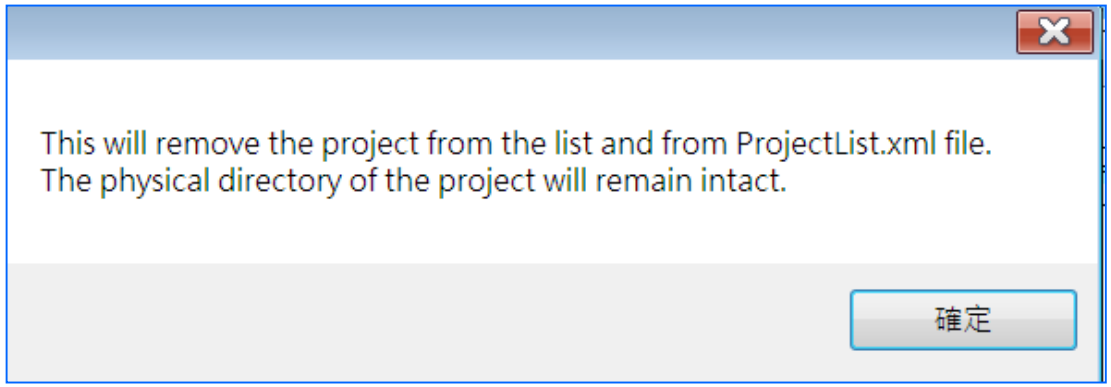
```
$ SearchResultChecker.exe
< command usage >
-t [num]              | the search result validation type
                        1 : TPP (peptide level validation)
                        2 : TPP (protein & peptide level validation)
                        3 : PeptideShaker (protein & peptide level validation)
                        4 : Proteome Discoverer (protein & peptide level validation)
-i [file1,file2,...] | validation file names connected with comma in the order : psm,peptide,protein
                        names should be in the order : psmfile,peptidefile,proteinfile
-d [directory_path]   | the directory of the validation files;
                        no need if the input file names are the full path
                        If the path consists of space(s), please use quotation mark to enclose it.
                           For example, -d "D:\Work\My folder"

End
```

- TPP (protein & peptide level validation)
  - SearchResultChecker -t 2 -i peptidefile.pep.xml,proteinfile.csv -d D:\work\dataset

Project Explorer Operations

- Project Level
  - Remove project – project will be removed from Project Explorer and from ProjectList.xml, but the folder will not be deleted





  - Add existing project

Project Explorer Operations

- Experiment level
  - Rename experiment
  - Remove experiment – have to manually delete the Experiment folder