

Supplementary Materials:

Supplemental File 1. Proteome Discoverer processing and consensus of workflow parameters.

Supplemental File 1: Proteome Discoverer Analysis Settings

Analysis Settings

Processing Step A: Workflow

Result name: 20120315_New_Decellularized_Analysis

Description: Processing workflow for precursor area quantification. Ion trap-detected HCD spectra using SequestHTwith Percolator validation. Specify the FASTA database and any additional modifications.

Workflow based on template: PWF_Tribrid_Precursor_Area_Quan_OT HCD_SequestHTnew_Percolator_mus

Creation date: 4/13/2021 4:31:51 PM

Created with Discoverer version: 2.4.1.15

The workflow tree:

- |-(0) Spectrum Files RC
- |-(1) Spectrum Selector
- |-(2) Sequest HT
- |-(3) Percolator
- |-(4) Minora Feature Detector

Processing node 0: Spectrum Files RC

1. Search Settings:

- File Name(s) (Hidden):

C:\PDStudies\202103_Lincoln_OIMvalve_Project\20120315_C57_BR1_Decellularized.raw
C:\PDStudies\202103_Lincoln_OIMvalve_Project\20120315_C57_BR1_Decellularized_rep.raw
C:\PDStudies\202103_Lincoln_OIMvalve_Project\20120315_C57_BR2_Decellularized.raw
C:\PDStudies\202103_Lincoln_OIMvalve_Project\20120315_C57_BR2_Decellularized_rep.raw
C:\PDStudies\202103_Lincoln_OIMvalve_Project\20120315_C57_BR3_Decellularized.raw
C:\PDStudies\202103_Lincoln_OIMvalve_Project\20120315_C57_BR3_Decellularized_rep.raw
C:\PDStudies\202103_Lincoln_OIMvalve_Project\20120315_OIM_BR1_Decellularized.raw
C:\PDStudies\202103_Lincoln_OIMvalve_Project\20120315_OIM_BR1_Decellularized_rep.raw
C:\PDStudies\202103_Lincoln_OIMvalve_Project\20120315_OIM_BR2_Decellularized.raw
C:\PDStudies\202103_Lincoln_OIMvalve_Project\20120315_OIM_BR2_Decellularized_rep.raw
C:\PDStudies\202103_Lincoln_OIMvalve_Project\20120315_OIM_BR3_Decellularized.raw
C:\PDStudies\202103_Lincoln_OIMvalve_Project\20120315_OIM_BR3_Decellularized_rep.raw

- Protein Database: Mus musculus (SwissProt TaxID=10090) (v2017-10-25)

- Enzyme Name: Trypsin (Full)

- Precursor Mass Tolerance: 20 ppm

- Fragment Mass Tolerance: 0.5 Da

- 1. Dynamic Modification: Carbamidomethyl / +57.021 Da (C)

2. Regression Settings:

- Regression Model: Non-linear Regression

- Parameter Tuning: Coarse

Processing node 1: Spectrum Selector

1. General Settings:

- Precursor Selection: Use MS1 Precursor
- Use Isotope Pattern in Precursor Reevaluation: True
- Provide Profile Spectra: Automatic

2. Spectrum Properties Filter:

- Lower RT Limit: 0
- Upper RT Limit: 0
- First Scan: 0
- Last Scan: 0
- Lowest Charge State: 0
- Highest Charge State: 0
- Min. Precursor Mass: 350 Da
- Max. Precursor Mass: 5000 Da
- Total Intensity Threshold: 0
- Minimum Peak Count: 1

3. Scan Event Filters:

- MS Order: Is Not MS1
- Min. Collision Energy: 0
- Max. Collision Energy: 1000
- Scan Type: Is Full

4. Peak Filters:

- S/N Threshold (FT-only): 1.5

5. Replacements for Unrecognized Properties:

- Unrecognized Charge Replacements: Automatic
- Unrecognized Mass Analyzer Replacements: ITMS
- Unrecognized MS Order Replacements: MS2
- Unrecognized Activation Type Replacements: CID
- Unrecognized Polarity Replacements: +
- Unrecognized MS Resolution@200 Replacements: 60000
- Unrecognized MSn Resolution@200 Replacements: 30000

6. Precursor Pattern Extraction:

- Precursor Clipping Range Before: 2.5 Da
- Precursor Clipping Range After: 5.5 Da

Processing node 2: Sequest HT

1. Input Data:

- Protein Database: Mus musculus (SwissProt TaxID=10090) (v2017-10-25)
- Enzyme Name: Trypsin (Full)
- Max. Missed Cleavage Sites: 4
- Min. Peptide Length: 4
- Max. Peptide Length: 144
- Max. Number of Peptides Reported: 10

2. Tolerances:

- Precursor Mass Tolerance: 20 ppm
- Fragment Mass Tolerance: 0.05 Da
- Use Average Precursor Mass: False
- Use Average Fragment Mass: False

3. Spectrum Matching:

- Use Neutral Loss a Ions: True
- Use Neutral Loss b Ions: True
- Use Neutral Loss y Ions: True

- Use Flanking Ions: True
- Weight of a Ions: 0
- Weight of b Ions: 1
- Weight of c Ions: 0
- Weight of x Ions: 0
- Weight of y Ions: 1
- Weight of z Ions: 0

4. Dynamic Modifications:

- Max. Equal Modifications Per Peptide: 3
- Max. Dynamic Modifications Per Peptide: 4
- 1. Dynamic Modification: Oxidation / +15.995 Da (M)
- 2. Dynamic Modification: Carbamidomethyl / +57.021 Da (C)
- 3. Dynamic Modification: Acetyl / +42.011 Da (K, R)

6. Dynamic Modifications (protein terminus):

- 1. N-Terminal Modification: Acetyl / +42.011 Da (N-Terminus)
- 2. N-Terminal Modification: Met-loss / -131.040 Da (M)
- 3. N-Terminal Modification: Met-loss+Acetyl / -89.030 Da (M)

Processing node 3: Percolator

1. Target/Decoy Strategy:

- Target/Decoy Selection: Concatenated
- Validation based on: q-Value

2. Input Data:

- Maximum Delta Cn: 0.05
- Maximum Rank: 0

3. FDR Targets:

- Target FDR (Strict): 0.01
- Target FDR (Relaxed): 0.05

Processing node 4: Minora Feature Detector

1. Peak & Feature Detection:

- Min. Trace Length: 5
- Max. Δ RT of Isotope Pattern Multiplets [min]: 0.2

2. Feature to ID Linking:

- PSM Confidence At Least: High

Consensus Step : Workflow

Result name: 20120315_New_Decellularized_Analysis

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Workflow based on template: CWF_Comprehensive_Enhanced

Annotation_LFQ_and_Precursor_Quan_relaxed-SN3

Creation date: 4/13/2021 4:31:56 PM

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The workflow tree:

- |- (0) MSF Files
- |- (1) PSM Grouper
- |- (2) Peptide Validator
- |- (3) Peptide and Protein Filter
- |- (4) Protein Scorer
- |- (5) Protein Grouping
- |- (6) Peptide in Protein Annotation
- |- (7) Protein FDR Validator
- |- (8) Protein Annotation
- |- (9) Protein Marker
- |- (10) Feature Mapper
- |- (11) Precursor Ions Quantifier

Post-processing nodes:

- |- (12) Result Statistics
- |- (13) Display Settings
- |- (14) Data Distributions

Processing node 0: MSF Files

1. Storage Settings:

- Spectra to Store: Identified or Quantified
- Feature Traces to Store: All

2. Merging of Identified Peptide and Proteins:

- Merge Mode: Globally by Search Engine Type

3. FASTA Title Line Display:

- Reported FASTA Title Lines: Best match
- Title Line Rule: standard

4. PSM Filters:

- Maximum Delta Cn: 0.05
- Maximum Rank: 0
- Maximum Delta Mass: 0 ppm

Hidden Parameters:

- MSF File(s):
C:\PDStudies\202103_Lincoln_OIMvalve_Project\202103_Lincoln_OIMvalveProject\20120315_New_Decellul
arized_Analysis.msf

Processing node 1: PSM Grouper

1. Peptide Group Modifications:

- Site Probability Threshold: 75

Processing node 2: Peptide Validator

1. General Validation Settings:

- Validation Mode: Automatic (Control peptide level error rate if possible)
- Target FDR (Strict) for PSMs: 0.01
- Target FDR (Relaxed) for PSMs: 0.05
- Target FDR (Strict) for Peptides: 0.01

- Target FDR (Relaxed) for Peptides: 0.05

2. Specific Validation Settings:

- Validation Based on: q-Value
- Target/Decoy Selection for PSM Level FDR Calculation Based on Score: Automatic
- Reset Confidences for Nodes without Decoy Search (Fixed score thresholds): False

Processing node 3: Peptide and Protein Filter

1. Peptide Filters:

- Peptide Confidence At Least: Medium
- Keep Lower Confident PSMs: True
- Minimum Peptide Length: 4
- Remove Peptides Without Protein Reference: False

2. Protein Filters:

- Minimum Number of Peptide Sequences: 1
- Count Only Rank 1 Peptides: False
- Count Peptides Only for Top Scored Protein: False

Processing node 4: Protein Scorer

No parameters

Processing node 5: Protein Grouping

1. Protein Grouping:

- Apply strict parsimony principle: True

Processing node 6: Peptide in Protein Annotation

1. Flanking Residues:

- Annotate Flanking Residues of the Peptide: True
- Number Flanking Residues in Connection Tables: 1

2. Modifications in Peptide:

- Protein Modifications Reported: Only for Master Proteins

3. Modifications in Protein:

- Modification Sites Reported: All And Specific
- Minimum PSM Confidence: High
- Report Only PTMs: True

4. Positions in Protein:

- Protein Positions for Peptides: Only for Master Proteins

Processing node 7: Protein FDR Validator

1. Confidence Thresholds:

- Target FDR (Strict): 0.01
- Target FDR (Relaxed): 0.05

Processing node 8: Protein Annotation

1. Annotation Aspects:

- 1. Aspect: Biological Process
- 2. Aspect: Cellular Component
- 3. Aspect: Molecular Function
- 4. Aspect: None
- 5. Aspect: None
- 6. Aspect: None

Processing node 9: Protein Marker

5. Annotate Species:

- As Species Map: False
- As Species Names: False

6. Mark Additional Entities:

- Annotation Groups: False
- Pathway Groups: False
- Modification Sites: True
- Peptide Isoform Groups: True

Processing node 10: Feature Mapper

1. Chromatographic Alignment:

- Perform RT Alignment: True
- Maximum RT Shift [min]: 10
- Mass Tolerance: 10 ppm
- Parameter Tuning: Coarse

2. Feature Linking and Mapping:

- RT Tolerance [min]: 0
- Mass Tolerance: 0 ppm
- Min. S/N Threshold: 0

Processing node 11: Precursor Ions Quantifier

1. General Quantification Settings:

- Peptides to Use: Unique + Razor
- Consider Protein Groups for Peptide Uniqueness: True
- Use Shared Quan Results: True
- Reject Quan Results with Missing Channels: False

2. Precursor Quantification:

- Precursor Abundance Based On: Intensity
- Min. # Replicate Features [%]: 0

3. Normalization and Scaling:

- Normalization Mode: Total Peptide Amount
- Scaling Mode: On All Average

4. Exclude Peptides from Protein Quantification:

- For Normalization: Use All Peptides
- For Protein Roll-Up: Use All Peptides
- For Pairwise Ratios: Exclude Modified

5. Quan Rollup and Hypothesis Testing:

- Protein Abundance Calculation: Summed Abundances
- N for Top N: 3
- Protein Ratio Calculation: Pairwise Ratio Based
- Maximum Allowed Fold Change: 100

- Imputation Mode: None
- Hypothesis Test: t-test (Background Based)

6. Quan Ratio Distributions:

- 1st Fold Change Threshold: 2
- 2nd Fold Change Threshold: 4
- 3rd Fold Change Threshold: 6
- 4th Fold Change Threshold: 8
- 5th Fold Change Threshold: 10

Processing node 12: Result Statistics

No parameters

Processing node 13: Display Settings

1. General:

- Filter Set:

```
### Filter Set MasterProteinFilter.filterset contains the following filters:
### Row Filter for TargetProtein:
### Master is equal to Master
###
```

```
'magellan filter set' 1 'MasterProteinFilter.filterset' FiltersetProperties 1 'LastFileName'
'C:\Users\frank.berg\Desktop\MasterProteinFilter.filterset' Filter 'TargetProtein' 1 NARY_AND 1 =
FilterConditionProperties 1 'NamedComparableFilterCondition/DisplayPropertyHint' 'Master' property
'Thermo.PD.EntityDataFramework.MasterProteinAssessment, Thermo.Magellan.EntityDataFramework'
'IsMasterProtein' constant 'Thermo.PD.EntityDataFramework.MasterProteinAssessment,
Thermo.Magellan.EntityDataFramework' 'IsMasterProtein'
```

Consensus Step Validation

Result name: 20120315_New_Decellularized_Analysis

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Peptide Validator nodes:

Processing node 2: Peptide Validator

1. General Validation Settings:

- Validation Mode: Automatic (Control peptide level error rate if possible)
- Target FDR (Strict) for PSMs: 0.01
- Target FDR (Relaxed) for PSMs: 0.05
- Target FDR (Strict) for Peptides: 0.01

- Target FDR (Relaxed) for Peptides: 0.05

2. Specific Validation Settings:

- Validation Based on: q-Value
- Target/Decoy Selection for PSM Level FDR Calculation Based on Score: Automatic
- Reset Confidences for Nodes without Decoy Search (Fixed score thresholds): False

Additional information:

Used validation mode: 'Automatic (Control peptide level error rate if possible)'.

All PSMs have PEPs. Qquality will be used for peptides.

Updated peptide confidences using qquality.

Protein Validator nodes:

Processing node 7: Protein FDR Validator

1. Confidence Thresholds:

- Target FDR (Strict): 0.01
- Target FDR (Relaxed): 0.05

Processing Step A: Validation

Result name: 20120315_New_Decellularized_Analysis

Result

file:

C:\PDStudies\202103_Lincoln_OIMvalve_Project\202103_Lincoln_OIMvalveProject\20120315_New_Decellularized_Analysis.msf

Description: Processing workflow for precursor area quantification. Ion trap-detected HCD spectra using SequestHTwith Percolator validation. Specify the FASTA database and any additional modifications.

Workflow based on template: PWF_Tribrid_Precursor_Area_Quan_OT HCD_SequestHTnew_Percolator_mus

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Psm Validator nodes:

Processing node 3: Percolator

1. Target/Decoy Strategy:

- Target/Decoy Selection: Concatenated
- Validation based on: q-Value

2. Input Data:

- Maximum Delta Cn: 0.05
- Maximum Rank: 0

3. FDR Targets:

- Target FDR (Strict): 0.01
- Target FDR (Relaxed): 0.05

Validation for Processing Node: Sequest HT (2)

Percolator Output:

Results for Sequest HT (2):

Iteration 1: Estimated 17989 PSMs with q<0.01
Iteration 2: Estimated 28312 PSMs with q<0.01
Iteration 3: Estimated 32642 PSMs with q<0.01
Iteration 4: Estimated 34325 PSMs with q<0.01
Iteration 5: Estimated 35492 PSMs with q<0.01
Iteration 6: Estimated 35817 PSMs with q<0.01
Iteration 7: Estimated 36014 PSMs with q<0.01
Iteration 8: Estimated 36000 PSMs with q<0.01
Iteration 9: Estimated 36076 PSMs with q<0.01
Iteration 10: Estimated 36066 PSMs with q<0.01

Learned normalized SVM weights for the 3 cross-validation splits:

Split1	Split2	Split3	FeatureName
0.0798	-0.0085	0.0491	XCorr
0.2026	0.2049	0.2273	Delta Cn From Second PSM
1.2600	1.5484	1.3428	Binomial Score
-0.3721	-0.4406	-0.4221	Isolation Interference [%]
-0.2304	-0.2181	-0.4164	MH+ [Da]
0.6353	2.3288	0.9372	Delta Mass [Da]
0.3888	-0.6898	0.4365	Delta Mass [ppm]
-1.1355	0.0818	1.1024	Absolute Delta Mass [Da]
-5.0238	-8.6499	-7.9411	Absolute Delta Mass [ppm]
0.2569	0.2469	0.3857	Peptide Length
0.0000	0.0000	0.0000	Is z=1
-0.2940	-0.3761	-0.2998	Is z=2
0.1964	0.2429	0.2040	Is z=3
0.1682	0.2364	0.1850	Is z=4
0.1151	0.1607	0.0818	Is z=5
0.1299	0.1350	0.1181	Is z>5
-1.3697	-1.6907	-1.5056	# Missed Cleavages
0.0263	0.0609	0.0075	Log Peptides Matched
0.5587	0.7057	0.5429	Log Total Intensity
0.2410	0.2507	0.2310	Fraction Matched Intensity [%]
-0.7115	-0.8580	-0.8230	Fragment Coverage Series A, B, C [%]
-0.1624	-0.2024	-0.1853	Fragment Coverage Series X, Y, Z [%]
-0.3604	-0.4170	-0.3223	Log Matched Fragment Series Intensities A, B, C
-0.4408	-0.5106	-0.3823	Log Matched Fragment Series Intensities X, Y, Z
0.0705	0.0521	0.1017	Longest Sequence Series A, B, C
0.0323	0.0129	0.0352	Longest Sequence Series X, Y, Z
0.1638	0.0668	0.1481	IQR Fragment Delta Mass [Da]
-0.1203	-0.1763	-0.1065	IQR Fragment Delta Mass [ppm]
-0.2419	-0.1056	-0.2392	Mean Fragment Delta Mass [Da]
0.0805	0.0072	0.0535	Mean Fragment Delta Mass [ppm]
0.3097	0.4030	0.3582	Mean Absolute Fragment Delta Mass [Da]
-0.3768	-0.2851	-0.3753	Mean Absolute Fragment Delta Mass [ppm]
-13.9003	-18.5101	-15.8022	m0

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Consensus Step Quantification
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- Precursor Abundance Based On: Intensity
- Min. # Replicate Features [%]: 0

3. Normalization and Scaling:

- Normalization Mode: Total Peptide Amount
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