**Protein identification**

Processing Step A: Workflow 1.

(There were the total of 16 protein identification workflow steps A- P which all used the same processing parameters while processing 96 samples in groups of 6 per workflow. The 16 groups were defined).

Result name: 202205013-09-EB3-5F-D

Result file: H:\Pitzer EB3-Rostral Cortex samples\Pitzer EB3-Rostral Cortex samples v2-5\202205013-09-EB3-5F-D.msf

Description: Processing with both full and semi tryptic NIST\_human\_hcd\_tryp libraries and Homo sapiens (sp\_canonical TaxID=9606\_and\_subtaxonomies) (v2022-01-30) fasta, Precursor Detector added. All Sequest HT nodes adjusted to 10ppm and 0.02Da,

Workflow based on template: PWF+PD\_Human\_full-semi\_with\_libraries\_20220615\_EB3\_mods

Creation date: 7/21/2022 9:55:26 AM

Created with Discoverer version: 2.5.0.400

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The workflow tree:

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|-(0) Spectrum Files RC

|-(20) Minora Feature Detector

|-(1) Spectrum Selector

|-(2) Precursor Detector

|-(3) MSPepSearch

|-(4) Percolator

|-(5) Spectrum Confidence Filter

|-(6) MSPepSearch

|-(7) Percolator

|-(8) Spectrum Confidence Filter

|-(9) Sequest HT

|-(10) Percolator

|-(11) Spectrum Confidence Filter

|-(12) Sequest HT

|-(13) Percolator

|-(14) Spectrum Confidence Filter

|-(15) Sequest HT

|-(16) Percolator

|-(17) Spectrum Confidence Filter

|-(18) Sequest HT

|-(19) Percolator

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Processing node 0: Spectrum Files RC

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1. Search Settings:

- File Name(s) (Hidden):

H:\Pitzer EB3-Rostral Cortex samples\Orbitrap raw files\20220506 Pitzer EB3-Rostral cortex\_50cm\202205013-09-EB3-5F-D.raw

H:\Pitzer EB3-Rostral Cortex samples\Orbitrap raw files\20220506 Pitzer EB3-Rostral cortex\_50cm\202205013-15-EB3-32F-D.raw

H:\Pitzer EB3-Rostral Cortex samples\Orbitrap raw files\20220506 Pitzer EB3-Rostral cortex\_50cm\202205013-35-EB3-40F-D.raw

H:\Pitzer EB3-Rostral Cortex samples\Orbitrap raw files\20220506 Pitzer EB3-Rostral cortex\_50cm\20220523-15-EB3-67F-D.raw

H:\Pitzer EB3-Rostral Cortex samples\Orbitrap raw files\20220506 Pitzer EB3-Rostral cortex\_50cm\20220528-27-EB3-7F-D.raw

H:\Pitzer EB3-Rostral Cortex samples\Orbitrap raw files\20220506 Pitzer EB3-Rostral cortex\_50cm\20220528-37-EB3-47F-D.raw

- Protein Database:

contaminants.fasta

Rattus norvegicus (sp\_canonical TaxID=10116\_and\_subtaxonomies) (v2022-06-14)

- Enzyme Name: Trypsin (Full)

- Precursor Mass Tolerance: 10 ppm

- Fragment Mass Tolerance: 0.02 Da

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

2. Regression Settings:

- Regression Model: Non-linear Regression

- Parameter Tuning: Coarse

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Processing node 20: Minora Feature Detector

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1. Peak & Feature Detection:

- Min. Trace Length: 5

- S/N Threshold: 1

- Max. ΔRT of Isotope Pattern Multiplets [min]: 0.2

2. Feature to ID Linking:

- PSM Confidence At Least: High

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Processing node 1: Spectrum Selector

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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: 25

- Upper RT Limit: 135

- 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

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Processing node 2: Precursor Detector

------------------------------------------------------------------

1. General:

- S/N Threshold: 1.5

- S/N Threshold for most abundant peak: 1.5

------------------------------------------------------------------

Processing node 3: MSPepSearch

------------------------------------------------------------------

1. Input Data:

- 1. Spectral Library: human\_hcd\_tryp\_best

- 1. Protein Database: Rattus norvegicus (sp\_canonical TaxID=10116\_and\_subtaxonomies) (v2022-06-14)

- 2. Spectral Library: human\_hcd\_tryp\_good

- 2. Protein Database: Rattus norvegicus (sp\_canonical TaxID=10116\_and\_subtaxonomies) (v2022-06-14)

2. Search Settings:

- Precursor Mass Tolerance: 10 ppm

- Fragment Mass Tolerance: 0.02 Da

- Min. Peak Intensity: 1

------------------------------------------------------------------

Processing node 4: 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

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Processing node 5: Spectrum Confidence Filter

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Filter Settings:

- Spectrum Confidence: Worse Than High

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Processing node 6: MSPepSearch

------------------------------------------------------------------

1. Input Data:

- 1. Spectral Library: human\_hcd\_semitryp

- 1. Protein Database: Rattus norvegicus (sp\_canonical TaxID=10116\_and\_subtaxonomies) (v2022-06-14)

2. Search Settings:

- Precursor Mass Tolerance: 10 ppm

- Fragment Mass Tolerance: 0.02 Da

- Min. Peak Intensity: 1

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Processing node 7: 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

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Processing node 8: Spectrum Confidence Filter

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Filter Settings:

- Spectrum Confidence: Worse Than High

------------------------------------------------------------------

Processing node 9: Sequest HT

------------------------------------------------------------------

1. Input Data:

- Protein Database: Rattus norvegicus (sp\_canonical TaxID=10116\_and\_subtaxonomies) (v2022-06-14)

- Enzyme Name: Trypsin (Full)

- Max. Missed Cleavage Sites: 2

- Min. Peptide Length: 6

- Max. Peptide Length: 150

- Max. Number of Peptides Reported: 10

2. Tolerances:

- Precursor Mass Tolerance: 10 ppm

- Fragment Mass Tolerance: 0.02 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)

------------------------------------------------------------------

Processing node 10: 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

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Processing node 11: Spectrum Confidence Filter

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Filter Settings:

- Spectrum Confidence: Worse Than High

------------------------------------------------------------------

Processing node 12: Sequest HT

------------------------------------------------------------------

1. Input Data:

- Protein Database: Rattus norvegicus (sp\_canonical TaxID=10116\_and\_subtaxonomies) (v2022-06-14)

- Enzyme Name: Trypsin (Full)

- Max. Missed Cleavage Sites: 2

- Min. Peptide Length: 6

- Max. Peptide Length: 150

- Max. Number of Peptides Reported: 10

2. Tolerances:

- Precursor Mass Tolerance: 10 ppm

- Fragment Mass Tolerance: 0.02 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: Deamidated / +0.984 Da (N)

5. Dynamic Modifications (peptide terminus):

- 1. N-Terminal Modification: Carbamidomethyl / +57.021 Da (N-Terminus)

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 13: 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 14: Spectrum Confidence Filter

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Filter Settings:

- Spectrum Confidence: Worse Than High

------------------------------------------------------------------

Processing node 15: Sequest HT

------------------------------------------------------------------

1. Input Data:

- Protein Database: Rattus norvegicus (sp\_canonical TaxID=10116\_and\_subtaxonomies) (v2022-06-14)

- Enzyme Name: Trypsin (Full)

- Max. Missed Cleavage Sites: 2

- Min. Peptide Length: 6

- Max. Peptide Length: 150

- Max. Number of Peptides Reported: 10

2. Tolerances:

- Precursor Mass Tolerance: 10 ppm

- Fragment Mass Tolerance: 0.02 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, K)

- 3. Dynamic Modification: Deamidated / +0.984 Da (N, Q, R)

5. Dynamic Modifications (peptide terminus):

- 1. N-Terminal Modification: Gln->pyro-Glu / -17.027 Da (Q)

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Processing node 16: 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 17: Spectrum Confidence Filter

------------------------------------------------------------------

Filter Settings:

- Spectrum Confidence: Worse Than High

------------------------------------------------------------------

Processing node 18: Sequest HT

------------------------------------------------------------------

1. Input Data:

- Protein Database: Rattus norvegicus (sp\_canonical TaxID=10116\_and\_subtaxonomies) (v2022-06-14)

- Enzyme Name: Trypsin (Full)

- Max. Missed Cleavage Sites: 2

- Min. Peptide Length: 6

- Max. Peptide Length: 150

- Max. Number of Peptides Reported: 10

2. Tolerances:

- Precursor Mass Tolerance: 10 ppm

- Fragment Mass Tolerance: 0.02 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 (C, H, K, M, P, W, Y)

- 2. Dynamic Modification: Carbamidomethyl / +57.021 Da (C, K)

- 3. Dynamic Modification: Acetyl / +42.011 Da (K)

- 4. Dynamic Modification: GG / +114.043 Da (K)

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Processing node 19: 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