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Data-Independent Acquisition (DIA) Data Processing using Spectronaut/directDIA (Biognosys): Whole Proteome Analysis of Human Whole Lung Tissue

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ABSTRACT

Proteolytic peptide measurement of Human whole lung tissue using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Acquisition by Data-Independent Acquisition (DIA) on an Orbitrap Eclipse Tribrid Mass Spectrometer for peptide/protein identification and quantification by processing DIA data using Spectronaut software.

MATERIALS

- Spectronaut software (version 17.6.230428.55965; Biognosys)

Protocol status: Working
We use this protocol and it's working

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Keywords: LC-MS/MS, DIA, Orbitrap Eclipse, Proteomics, Mass Spectrometry, Spectronaut, Lung , Human

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1 Search the DIA data with Spectronaut using the spectral library-free directDIA algorithm against a Human UniProt-SwissProt proteome database (https://www.uniprot.org/proteomes), applying the settings described in **Table 1**.

Pulsar Search		
Peptides	Enzymes/Cleavage Rules	Trypsin/P
	Digest Type	Specific
	Max Peptide Length	52
	Min Peptide Length	7
	Missed Cleavages	2
	Toggle N-terminal N	True
Labeling	Channel 1	False
	Channel 2	False
	Channel 3	False
Modifications	Max Variable Modifications	5
	Fixed Modifications	Carbamidomethyl (C)
	Variable Modifications	Acetyl (Protein N-term) Oxidation (M)
Speed-Up	Use Dynamic IM Peak Filter	True
	Target TIC Fraction	0.9
	MS2 Index	Automatic
Identification	PSM FDR	0.01
	Peptide FDR	0.01
	Protein Group FDR	0.01
	directDIA Workflow	directDIA+ (Deep)
	PTM Localization Filter	False
Tolerances	Thermo Orbitrap	
	Calibration Search	Dynamic
	MS1 Correction Factor	1
	MS2 Correction Factor	1
	Main Search	Dynamic
	MS1 Correction Factor	1
	MS2 Correction Factor	1
Workflow	Fragment Ion Selection Strategy	Intensity Based
	In-Silico Generate Missing Channels	False
	Use DNN Predicted Ion Mobility	Auto
Result Filters	Fragment Ions	
	Ion AA Length (min)	N = 3
	Ion Charge	False
	Ion Loss Type	False
	Ion Type	False
	m/z	Min = 300; Max = 1,800
	Relative Intensity	Min = 5
	Precursors	
	Amino Acids	False
	Best N Fragments per Peptide	Min = 3; Max = 6
	Best N Peptides per Protein Group	False
	Channel Count	False
	FASTA Matched	False
	Missed Cleavage	False
	Modifications	None
	Peptide Charge	False
	Proteotypicity	False

DIA Analysis		
XIC Extraction	XIC IM Extraction Window	Dynamic
	Correction Factor	1
	XIC RT Extraction Window	Dynamic
	Correction Factor	1
	MS1 Mass Tolerance Strategy	Dynamic
	Correction Factor	1
	MS2 Mass Tolerance Strategy	Dynamic
	Correction Factor	1
Calibration	MZ Extraction Strategy	Maximum Intensity
	Allow source-specific iRT Calibration	True
	Precision iRT	True
	Exclude De-amidated Peptides	True
	iRT <-> RT Regression Type	Local (Non-Linear) Regression
	MS1 Mass Tolerance Strategy	System Default
	MS2 Mass Tolerance Strategy	System Default
Identification	Precursor Qvalue Cutoff	0.01
	Precursor PEP Cutoff	0.2
	Protein Qvalue Cutoff (Experiment)	0.01
	Protein Qvalue Cutoff (Run)	0.05
	Protein PEP Cutoff	0.75
	Single Hit Definition	By Stripped Sequence
	Exclude Single Hit Proteins	False
	Exclude Duplicate Assays	True
	Exclude Predicted Fragment Scores	False
	Generate Decoys	True
	Decoy Generation Method	Mutated
	Preferred Fragment Source	NN Predicted Fragments
	Decoy Limit Strategy	Dynamic
	Library Size Fraction	0.1
	Pvalue Estimator	Kernel Density Estimator
Quantification	Precursor Filtering	Identified (QValue)
	Imputation Strategy	Use Background Signal
	Proteotypicity Filter	None
	Protein LFQ Method	Automatic
	Quantify MS Level	MS2
	Quantify Type	Area
	Cross-Run Normalization	True
	Normalization Filter Type	None
	Normalization Strategy	Local Normalization
	Row Selection	Identified in at least 1 Run (Sparse)
	Interference Correction	True
	Only Identified Peptides	True
	Exclude All Multi-Channel Interferences	True
	MS1 Min	2
	MS2 Min	3
	Major (Protein) Grouping	by Protein Group Id
	Minor (Peptide) Grouping	by Stripped Sequence
	Major Group Quantity	Sum peptide quantity
	Major Group Top N	Min = 1; Max = 7
	Minor Group Quantity	Sum precursor quantity
	Minor Group Top N	Min = 1; Max = 10
PTM Workflow	PTM Localization	False

Workflow	Method Evaluation	False
	MS2 DeMultiplexing	Automatic
	Profiling Strategy	iRT Profiling
	Carry-over exact Peak Boundaries	None
	Profiling Row Selection	Minimum QValue Row Selection
	Qvalue Threshold	0.01
	Profiling Target Selection	Automatic Selection
	Run Limit for directDIA Library	-1
	Unify Peptide Peaks Strategy	None
Protein Inference	Protein Interference Workflow	Automatic
	Inference Algorithm	IDPicker
Post Analysis	Differential Abundance Testing	Unpaired t-test
	Assume Equal Variance	False
	Group-Wise Testing Correction	True
	Difference Abundance Grouping	Major Group (Quantification Settings)
	Smallest Quantitative Unit	Precursor Ion (Quantification Settings)
	Use All MS-Level Quantities	False
	Calculate Explained TIC	None
	Calculate Sample Correlation Matrix	False
	Hierarchical Clustering	True
	Distance Metric	Manhattan Distance
	Linkage Strategy	Ward's Method
	Order Runs by Clustering	True
	Z-score Transformation	False

Table 1. Settings for the data-independent acquisition data processing with Spectronaut/directDIA