



DDA 定性/定量プロテオーム解析ソフトウェア Scaffold DDA

Scaffold DDA 何ができるか

- DDAデータ : 同定タンパク質の定性/定量データの取り込み
 - * MASCOT Server/Distiller,
Scaffold, etc
- MSFragger と連動したタンパク質の同定・定量
- 定量解析データに基づいた検定とグラフ表示
- GO 情報の表示
- Viewerによる結果ファイルのシェア

解析操作の流れ

- ソフトウェア起動
- 新規作成、Ctrl + N
(menuの「File」-> New やアイコンクリック)
- 検索パラメータ指定、検索開始
- データ取り込み完了
- 属性付与、階層構造化
- データ解析(定性解析、定量解析、検定)
- レポート

対応フォーマット:

定性[同定]

.dat, msr

.mzIdentML

.msf

その他、.mzXML, .d, .tsv …など

定量：
isobalic

定性と同じファイル

定量：
ラベルフリー

.XML(MASCOT Distiller)

mzIdentML [Scaffold Q+S 含む]

.msf

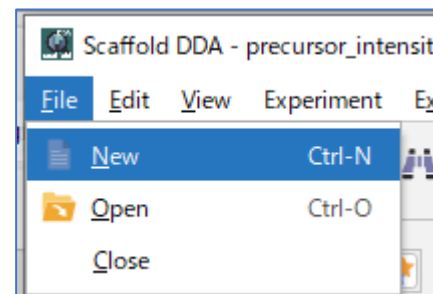
その他のソフトウェアをご利用の場合、英語マニュアルChapter 9 を参照の事

データ取り込み時の画面

データ取り込み開始

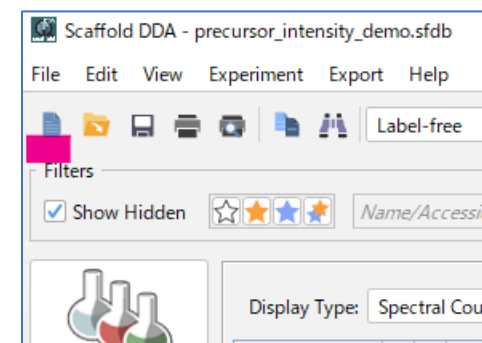
•以下のいずれかの操作を実施

- メニューのFile -> New



- Ctrl + N

- メニューバー下にあるアイコン をクリック



Load Data

[1] Probability Mode

Percolator ... percolator 適用

Prefiltered ... 出力ファイル作成時のfilterのみ適用

No probabilities ... 基準を適用しない

[2] Quantitation Type * Customize可能

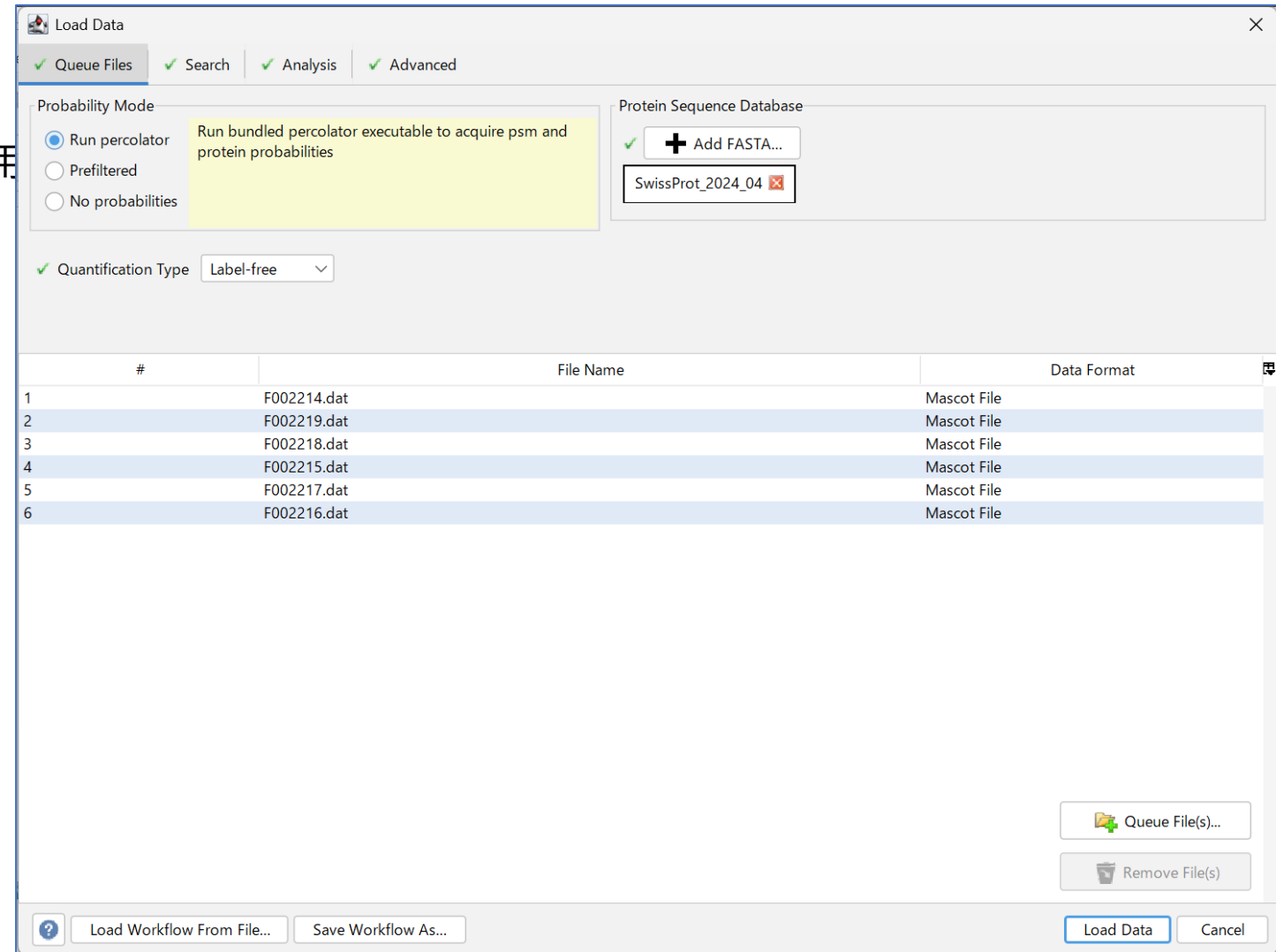
- Label Free [定量計算を行わない時もこちらを選択]
- TMT X-plex (X=2,6,10,11,16)
- iTRAQ Y-plex (Y=4,8)
- SILAC Z-plex (Z=2,3)

[3] Protein Sequence Database

FASTAファイルを選択。

[4] Queue Files

取り込みたいファイルを選択。



Queue files for loading

Look In: 20231109

- F001908.dat
- F001909.dat
- F001910.dat
- F001911.dat
- F001912.dat
- F001913.dat
- F001914.dat
- F001915.dat
- F001916.dat

File Name: F001910.dat "F001911.dat" "F001912.dat" "F001913.dat" "F001914.dat" "F001915.dat" "F001916.dat"

Files of Type: Data Files (*.msf;*.mzid.bz;*.mzml;*.d;*.mzid.gz;*.dat;*.tsv;*.raw;*.dat.gz;*.mzid;*.mgf;*.mzml)

開く 取消

1. Queue File(s)
→ datファイル選択

Please wait

Load Data

Parsing files

Operation 0.9% complete

Load Data

Queue Files Search Thresholds

Quantification Type: Label-free

Protein Sequence Database: SwissProt_2023_01 FASTA Database

Run Percolator

File Name	Data Format
F001914.dat	Mascot DAT File
F001908.dat	Mascot DAT File
F001910.dat	Mascot DAT File
F001912.dat	Mascot DAT File
F001913.dat	Mascot DAT File
F001915.dat	Mascot DAT File
F001911.dat	Mascot DAT File
F001916.dat	Mascot DAT File
F001909.dat	Mascot DAT File

Queue File(s)...

Remove File(s)

Load Workflow From File... Save Workflow As...

Load Data Cancel

2. Load Data ボタン

SciView ODA - ODAAnalysis_06Data.tbl

File Edit View Experiment Export Help

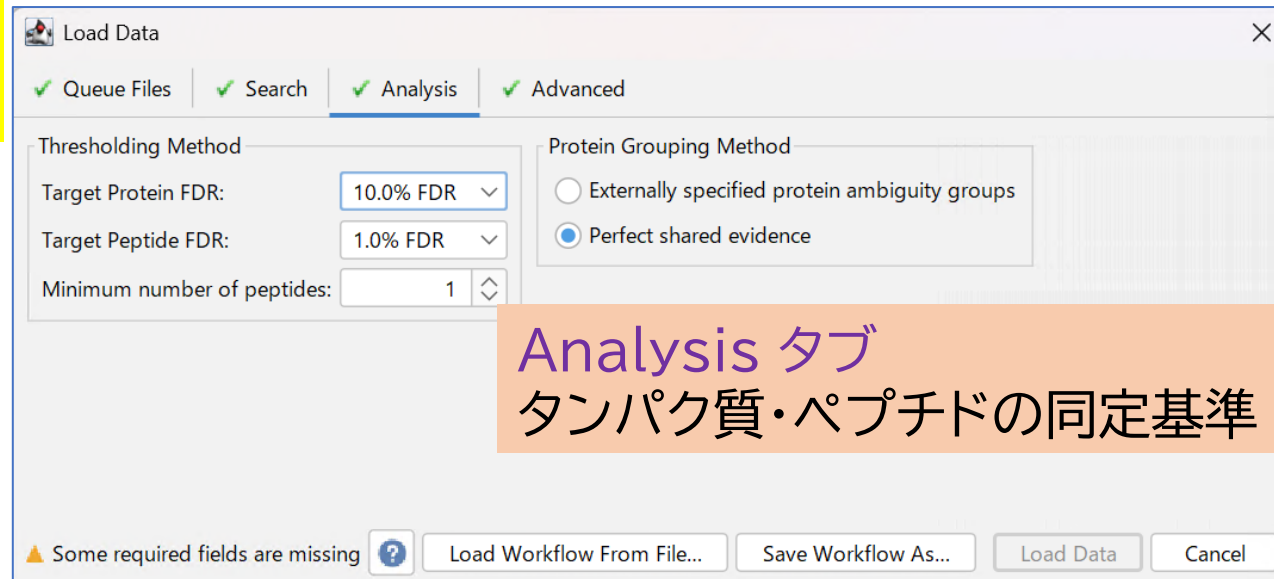
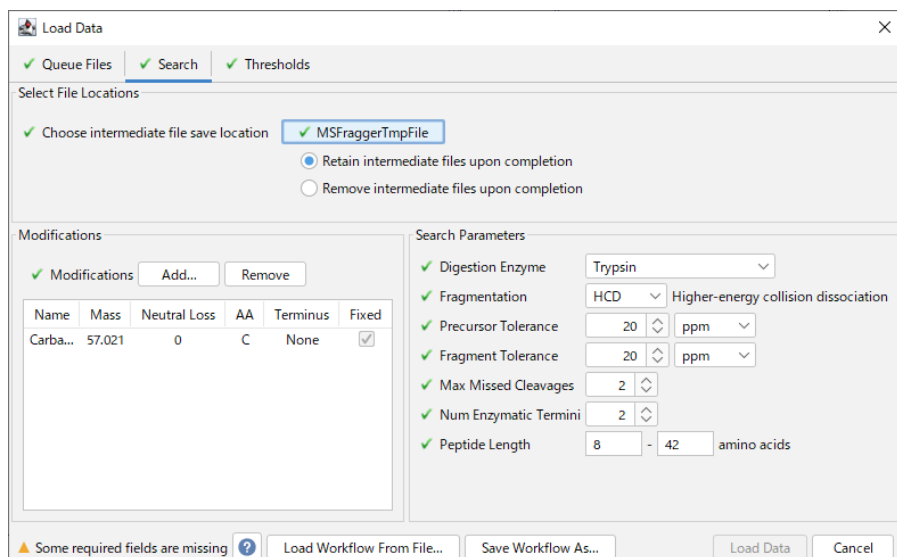
Label-free Summation: MS Sample

Color Legend (Display Value)

Protein Name	Accession Number	Gene Name	Maximum Height	Intensity	00000	00001	00002	00003	00004	00005	00006
Neuroblast differentiation-associated protein ANNAK ...	ANNAK_HUMAN		629 kDa	100%	100	100	100	100	100	100	100
Group of Alpha-emulxin OS-Homo sapiens OX-1906 ...	ENOA_HUMAN (+1)		14 kDa	41%	14	14	14	14	14	14	14
Keratin, type I cytokeletal 6 OS-Homo sapiens OX-1 ...	KC12_HUMAN		54 kDa	41%	54	54	54	54	54	54	54
Filamin-A OS-Homo sapiens OX-9006-ON-FILN1 PE ...	FILN1_HUMAN		281 kDa	25%	281	281	281	281	281	281	281
Group of Heat shock protein HSP 90 beta OS-Equi ...	HSP90_HORSE (+1)		84 kDa	47%	84	84	84	84	84	84	84
Group of Heat shock protein HSP 90 alpha OS-Bos ...	HSP90_BOVIN (+4)		84 kDa	5%	84	84	84	84	84	84	84
DNA dependent protein kinase catalytic subunit OS ...	PRKDC_HUMAN		469 kDa	4%	469	469	469	469	469	469	469
Filamin B OS-Homo sapiens OX-9006-ON-FILB1 PE ...	FILN2_HUMAN		278 kDa	10%	278	278	278	278	278	278	278
Actin, cytoplasmic 1 OS-Camelus dromedarius OX-36 ...	ACTC_CAMEL		42 kDa	4%	42	42	42	42	42	42	42
Heat shock protein HSP 90 alpha OS-Crotalaria gree ...	HSPA90A_CROT		89 kDa	3%	89	89	89	89	89	89	89
Actin, cytoplasmic 18 OS-Streptococcus pyogenes ...	ACTS_STRPY		42 kDa	4%	42	42	42	42	42	42	42
Carbonic dehydratase [cytoplasmic, mitochondrial] ...	CPDH_HUMAN		165 kDa	45%	165	165	165	165	165	165	165
Early cell surface OS-Homo sapiens OX-1048-ON-E ...	EAC1_HUMAN		273 kDa	4%	273	273	273	273	273	273	273
Filamin A OS-Mus musculus OX-10009-ON-FILN1 PE ...	FILN1_MOUSE		281 kDa	7%	281	281	281	281	281	281	281
Heat shock protein HSP 90 alpha OS-Gallus gallus OX ...	HSPA90A_CHICK		84 kDa	4%	84	84	84	84	84	84	84
Group of Heat shock cognate 71 kDa protein OS-L ...	HSP70_BOVIN (+1)		70 kDa	14%	70	70	70	70	70	70	70
Actin, cytoskeletal 1 OS-Lytechinus pictus OX-7803 P ...	ACT1_LYTP1		42 kDa	4%	42	42	42	42	42	42	42
Filamin-A2 OS-Homo sapiens OX-1048-ON-FILN2 ...	FILN2_HUMAN		281 kDa	17%	281	281	281	281	281	281	281
Vimentin OS-Homo sapiens OX-9006-ON-VIM1 PE ...	VIM1_HUMAN		54 kDa	40%	54	54	54	54	54	54	54
Group of Heterogeneous nuclear ribonucleoprotein ...	RNAP2_HUMAN (+1)		34 kDa	1%	34	34	34	34	34	34	34
Keratin, type I cytokeletal 18 OS-Homo sapiens OX ...	KC18_HUMAN		48 kDa	4%	48	48	48	48	48	48	48
Group of Elongation factor 1 alpha 1 OS-Bos taurus O ...	EFA1_BOVIN (+10)		42 kDa	9%	42	42	42	42	42	42	42
Actin, cation 205 OS-Artemia sp. OX-1662 PE-2 SV-1	ACT1_ARTDE		42 kDa	4%	42	42	42	42	42	42	42
Protein OS-Homo sapiens OX-1662 PE-2 SV-1	ACT1_HUMAN		42 kDa	4%	42	42	42	42	42	42	42
Actin, muscle OS-Homo sapiens OX-1662 PE-2 SV-1	ACT2_HUMAN		42 kDa	4%	42	42	42	42	42	42	42
Actin, cytoplasmic 18 OS-Streptococcus pyogenes ...	ACT2_STRPY		42 kDa	4%	42	42	42	42	42	42	42
Actin, muscle OS-Fredericia ostreorum OX-7813 PE-1 S ...	ACT2_FREOS		42 kDa	10%	42	42	42	42	42	42	42
Actin OS-Bugula malay OX-4279 PE-1 SV-1	ACT_BUGMA		42 kDa	3%	42	42	42	42	42	42	42
Actin 11 OS-Limulus polyphemus OX-4980 PE-2 SV-1	ACT1_LIMPO		42 kDa	3%	42	42	42	42	42	42	42
Elongation factor 1 alpha, somatic form OS-Artemia ...	EFA1_ARTDE		50 kDa	9%	50	50	50	50	50	50	50
Actin, muscle OS-Manulaca zenta OX-7130 PE-2 SV-1	ACT2_MANSE		42 kDa	3%	42	42	42	42	42	42	42
Gamma-tubulin OS-Homo sapiens OX-1068-ON-G ...	GTPL_HUMAN		58 kDa	4%	58	58	58	58	58	58	58
Glyoxalase 3-phosphate dehydrogenase OS-H ...	GSP_HUMAN		36 kDa	25%	36	36	36	36	36	36	36
Group of Elongation factor 2 OS-Homo sapiens OX ...	EFA2_HUMAN (+1)		47 kDa	4%	47	47	47	47	47	47	47
Group of Tubulin beta chain OS-Homo sapiens OX ...	TUBB2_BOVIN (+1)		49 kDa	17%	49	49	49	49	49	49	49
Keratin, type I cytokeletal 7 OS-Homo sapiens OX ...	KC7_HUMAN		51 kDa	4%	51	51	51	51	51	51	51
Transferrin OS-Homo sapiens OX-1068-ON-TF_H ...	TF_HUMAN		98 kDa	3%	98	98	98	98	98	98	98
Zenon of Actin, desmosomal medium OS-Thromosoma ...	ACT1_ACAF1 (+1)		42 kDa	4%	42	42	42	42	42	42	42

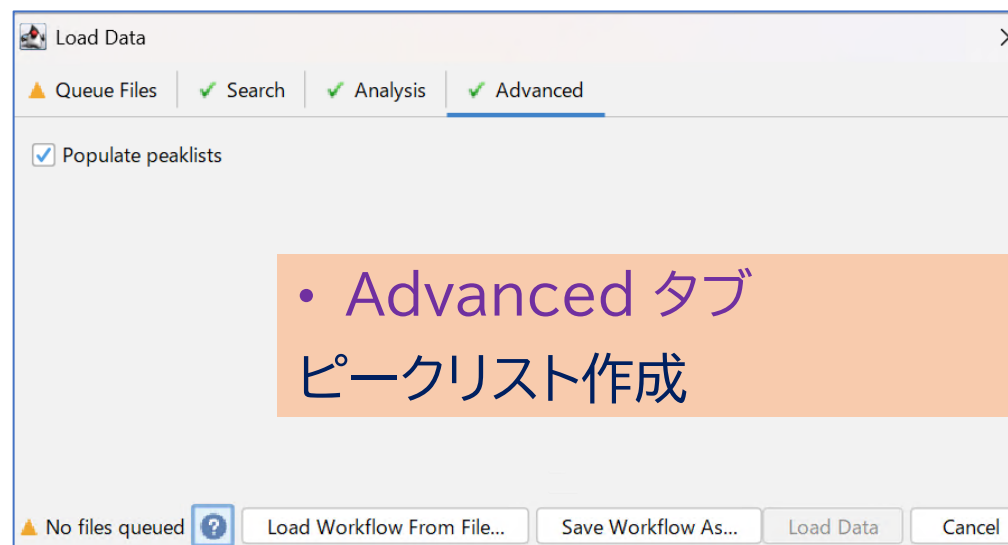
Search タブ、Analysis タブ、Advanced タブ

Load Data ダイアログに含まれる3つのタブ :
Search, Analysis, Advanced



Analysis タブ
タンパク質・ペプチドの同定基準

Search タブ
Rawデータの取り込みから行う場合、
パラメーターを指定



Advanced タブ
ピークリスト作成

ペプチド・タンパク質の定性(同定)

• ペプチド

- 検索結果をもとに、Percolator で再評価
- peptide FDR 1% (デフォルト値、かつ推奨値)

• タンパク質

- 同定ペプチド N以上アサインされている (デフォルト値 N=2)
- protein FDR 設定値を満たす (デフォルト値 FDR 10%)
- clustering ルールに基づき、類似タンパク質をグループ化

Samples 画面

同定されたタンパク質の一覧並びに定量値をはじめとするタンパク質の関連情報が表示

解析結果の概要を確認する上で主体となる画面

Scaffold DDA - precursor_intensity_demo.sfdb

File Edit View Experiment Export Help

Label-free Summarization: MS Sample

Filters: Show Hidden Name/Accession p-value filter GO Term

Display Type: Spectral Counting Value: Total Spectrum Count Normalized Color Options...

Color Legend (Displayed Value) **タンパク質** ≥ 8.00

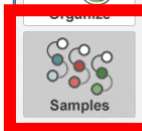
#	Visible	Star	Protein Name	Gene Name	Molecular Weight	Exclusivity	ANOVA CL-Stage BICL Biosample	Taxonomy	GO Information										Quantitative Values									
									developmental process	growth	immune system process	metabolic process	reproduction	reproductive process	Golgi apparatus	cytoplasm	endoplasmic reticulum	membrane	mitochondrion	nucleus	ribosome	binding	Stage 1	Stage 2	Stage 3	Stage 4		
										AF-3913-01A-12	AA-A011-01A-12	AA-A00N-01A-41	AG-A01W-01A-23	AG-A00C-01A-23	AG-A036-01A-22	AG-A007-01A-22	AG-A016-01A-23											
1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=...	ALBU_HUMAN	ALB	69 kDa	100%	0.54	Homo...												63	32	82	30	25	41	21	27
2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of Actin, alpha cardiac muscle 1 OS=Homo sa...	ACTA_HUMAN (+2)	ACTA2 (+2)		33%	0.21	Homo...												29	33	30	19	26	36	20	17
3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of Actin, cytoplasmic 1 OS=Homo sapiens G...	ACTB_HUMAN (+6)	ACTB (+1)		29%	0.0038	Homo...												52	53	35	22	21	20	12	8
4	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Hemoglobin subunit beta OS=Homo sapiens GN=H...	HBB_HUMAN	HBB	16 kDa	100%	0.091	Homo...												10	6	14	19	41	24	26	19
5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Keratin, type I cytoskeletal 18 OS=Homo sapiens GN...	K1C18_HUMAN	KRT18	48 kDa	92%	0.0050	Homo...												44	55	21	11	10	7	13	10
6	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4	VIME_HUMAN	VIM	54 kDa	100%	0.019	Homo...												5	5	12	1	14	24	43	33
7	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Alpha-actinin-4 OS=Homo sapiens GN=ACTN4 PE=...	ACTN4_HUMAN	ACTN4	105 kDa	58%	0.038	Homo...												61	35	24	16	9	7	8	7
8	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Keratin, type II cytoskeletal 8 OS=Homo sapiens GN...	K2C8_HUMAN	KRT8	54 kDa	100%	0.15	Homo...												24	53	8	11	13	5	22	15
9	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of Actinin alpha 1 isoform 3 OS=Homo sapie...	ACTN1_HUMAN (+1)	ACTN1		50%	0.15	Homo...												46	25	18	12	12	23	13	9
10	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4	MYH9_HUMAN	MYH9	227 kDa	96%	0.12	Homo...												34	22	23	20	13	9	19	10
11	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of Filamin A OS=Homo sapiens GN=FLNA PE...	FLNA_HUMAN (+3)	FLNA		96%	0.56	Homo...												19	14	9	11	11	32	19	17
12	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of Hemoglobin alpha 1 OS=Homo sapiens G...	HBA_HUMAN (+1)	HBA1		100%	0.30	Homo...												53	16	40	14	8	5	6	4
13	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of cDNA FLJ32131 fis, clone PEBLM2000267, h...	B3KPS3_HUMAN (+1)			100%	0.068	Homo...												8	4	6	7	32	19	23	14
14	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Pyruvate kinase isozymes M1/M2 OS=Homo sapiens...	KPYM_HUMAN	PKM	58 kDa	100%	0.17	Homo...												12	10	14	6	17	13	19	18
15	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of Histone H2A OS=Homo sapiens PE=2 SV=...	B2R5B3_HUMAN (+10)	HIST2H2AA3 (+9)		100%	0.0033	Homo...												37	31	14	17	10	3	5	3
16	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Collagen alpha-3(VI) chain OS=Homo sapiens GN=C...	CO6A3_HUMAN	COL6A3	344 kDa	100%	0.81	Homo...												9	9	18	7	7	25	20	11
17	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Malate dehydrogenase (Fragment) OS=Homo sapie...	Q7SMT9_HUMAN	MDH2	33 kDa	100%	0.19	Homo...												19	13	7	9	18	12	13	14
18	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Mitochondrial heat shock 60kD protein 1 variant 1 O...	B3GQ57_HUMAN	HSPD1	61 kDa	100%	0.12	Homo...												16	14	14	11	13	12	11	9
19	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Tubulin beta chain OS=Homo sapiens GN=TUBB PE...	T8B5_HUMAN	TUBB	50 kDa	100%	0.0060	Homo...												7	6	8	3	16	16	19	17
20	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of Fibronectin OS=Homo sapiens GN=FN1 PE...	E9PE77_HUMAN (+2)	FN1		100%	0.97	Homo...												19	5	21	6	8	21	11	10
21	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of IQ motif containing GTPase activating prot...	A4QP80_HUMAN (+1)	IQGAP1		100%	0.69	Homo...												14	11	7	10	14	10	15	7
22	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of Protein S100-A9 OS=Homo sapiens GN=S1...	B2RAM6_HUMAN (+1)	S100A9		100%	0.46	Homo...												56	12	31	6	0	1	4	2
23	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Talin-1 OS=Homo sapiens GN=TLN1 PE=1 SV=3	TLN1_HUMAN	TLN1	270 kDa	100%	0.75	Homo...												24	7	11	9	5	14	9	8
24	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Plectin OS=Homo sapiens GN=PLEC PE=1 SV=3	PLEC_HUMAN	PLEC	532 kDa	100%	0.24	Homo...												12	11	11	7	8	9	12	11
25	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Heat shock protein HSP 90-beta OS=Homo sapiens ...	H590B_HUMAN	HSP90AB1	83 kDa	100%	0.024	Homo...												8	4	5	6	14	11	13	13
26	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Immunoglobulin light chain (Fragment) OS=Homo s...	Q0KKI6_HUMAN		24 kDa	100%	0.31	Homo...												15	14	35	7	2	4	4	1
27	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Phosphoglycerate kinase 1 OS=Homo sapiens GN=P...	PGK1_HUMAN	PGK1	45 kDa	100%	0.014	Homo...												14	16	10	9	8	4	6	6
28	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC...	CLH1_HUMAN	CLTC	192 kDa	100%	0.49	Homo...												5	7	3	8	11	9	15	6
29	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2	CO3_HUMAN	C3	187 kDa	100%	0.11	Homo...												17	8	15	11	6	7	3	3
30	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Fibrinogen OS=Homo sapiens GN=FBN1 PE=1 SV=3	FBN1_HUMAN	FBN1	312 kDa	100%	0.37	Homo...												25	10	9	2	4	13	7	5
31	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	DNA-dependent protein kinase catalytic subunit OS...	PRKDC_HUMAN	PRKDC	469 kDa	100%	0.038	Homo...												16	13	4	9	7	7	5	6
32	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 ...	H4_HUMAN	HIST1H4A	11 kDa	100%	0.38	Homo...												6	6	3	7	11	6	8	10
33	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of Spectrin alpha chain, non-erythrocytic 1 O...	A6NG51_HUMAN (+1)	SPTAN1		100%	0.32	Homo...												11	10	4	8	7	6	9	4
34	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Annexin A2 OS=Homo sapiens GN=ANXA2 PE=1 SV...	ANXA2_HUMAN	ANXA2	39 kDa	100%	0.71	Homo...												0	0	2	0	13	6	25	7
35	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of Myosin heavy chain 11 smooth muscle isofo...	B1P543_HUMAN (+1)	MYH11		94%	0.57	Homo...												15	4	4	2	5	20	8	3
36	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Keratin, type I cytoskeletal 19 OS=Homo sapiens GN...	K1C19_HUMAN	KRT19	44 kDa	88%	0.24	Homo...												4	9	4	6	5	4	14	8
37	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Transferrin OS=Homo sapiens GN=TAG1 N PE=1 SV=4	TAG1_HUMAN	TAG1 N	73 kDa	100%	0.11	Homo...												23	12	5	8	1	8	4	1

Protein FDR: 0.57%
1945 Target Proteins
11 Decoy Proteins
(60 total proteins)
Peptide FDR: 0.093%
16160 Target Spectra
15 Decoy Spectra
Thresholds: MS2D

GO情報

定量値

タンパク質



- Organisms
- Samples**
- Proteins
- Visualize
- Publish

表示される「定量値」 [Display Type]

定量データなしの場合

Display Type: Spectral Counting Value: Total Spectrum Count Normalized Color Options...

Color Legend (Displayed Value)

16.00
12.00
8.00
4.00
0.00

Exclusive : ユニークペプチドのみ

定量データありの場合(Precursor Intensity)

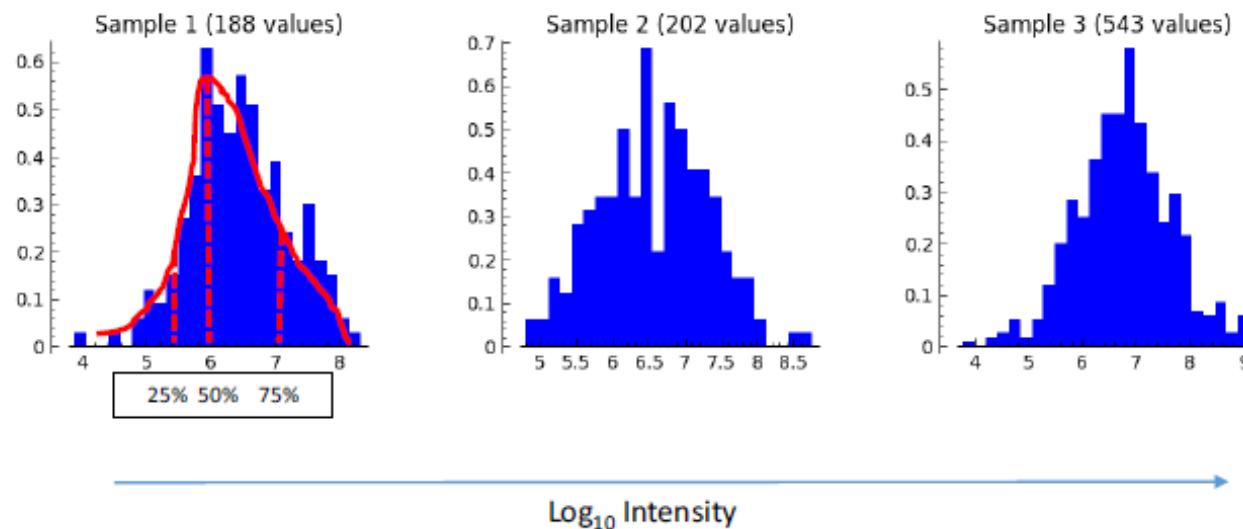
Label-free Summarization: MS Sample

Filters: Show Hidden Name/Accession p-value filter GO Term

Display Type: Precursor Intensity Value: Precursor Intensity Normalized Color Options...

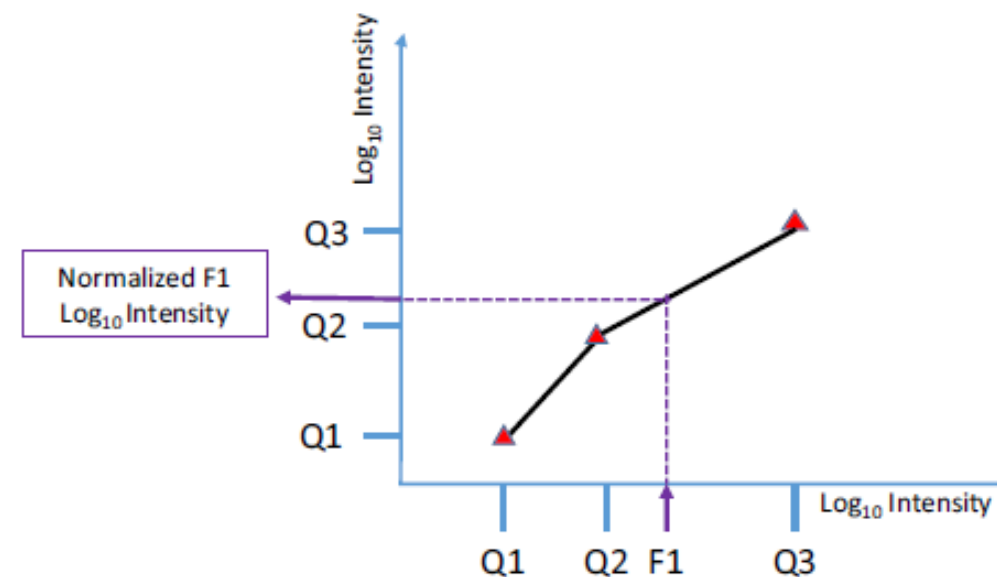
Organize

Normalization



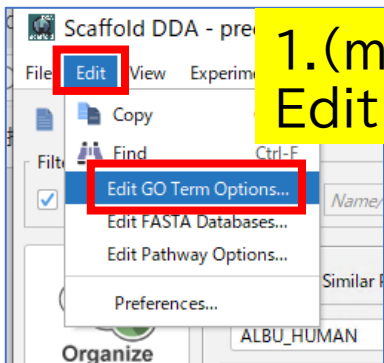
サンプル強度分布によるNormalization

- 全データ vs 各sample、それぞれ強度分布を作成
- 各サンプルにおいて、25%、50%、75%にあたる定量値を、全データ平均にそろえる
- 各四分位数の間は点間を結ぶ直線に従って変換する

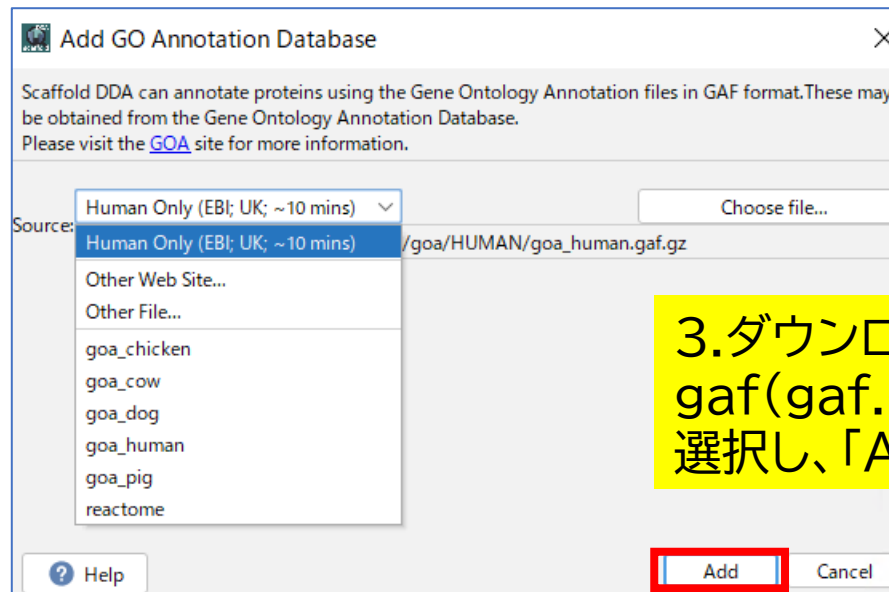
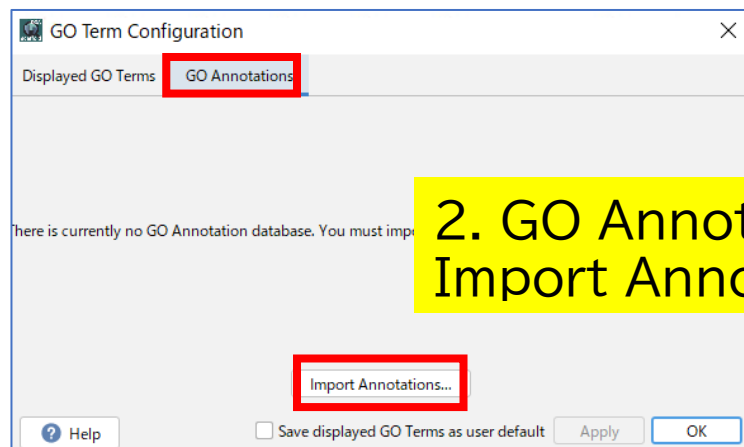


GOファイルのセット方法

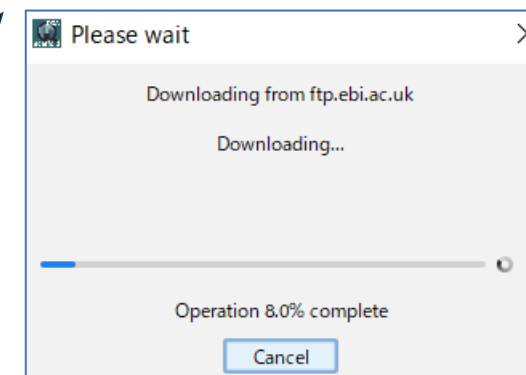
1. (menu) Edit ->
Edit GO Term Options



2. GO Annotations タブ、
Import Annotations

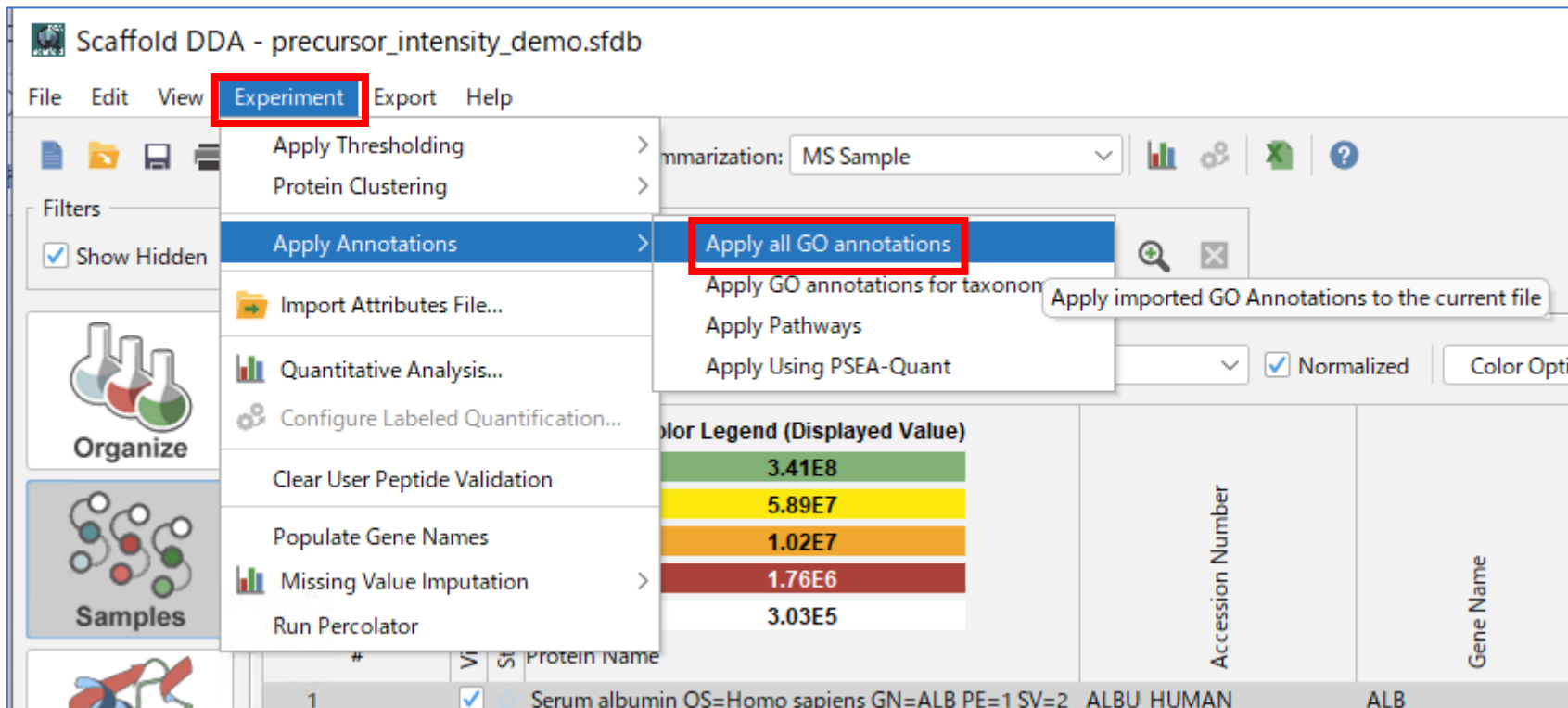


3. ダウンロード先または
gaf(gaf.gz)ファイルを
選択し、「Add」ボタン



GO情報のannotation付与方法

- 1.(menu) Experiment
- > Apply Annotations
- > Apply all GO annotations



The screenshot shows the Scaffold DDA software interface. The 'Experiment' menu is open, and the 'Apply Annotations' option is selected. A sub-menu is displayed, with 'Apply all GO annotations' highlighted. A tooltip for this option reads: 'Apply imported GO Annotations to the current file'. The background shows a data table with columns for '#', 'Protein Name', 'Accession Number', and 'Gene Name'. The first row is highlighted in green and contains the following data:

#	Protein Name	Accession Number	Gene Name
1	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	ALBU HUMAN	ALB

Scaffold DDA - spectrum_counting_demo.sfdb

File Edit View Experiment Export Help

Label-free Summarization: Roast Time

Filters: Show Hidden Name/Accession p-value filter

Display Type: Spectral Counting Value: Weighted Spectrum Count Normalized Color Options...

Color Legend (Displayed Value):

- ≥ 8.00
- 4.00 - 8.00
- 2.00 - 4.00
- 1.00 - 2.00
- < 1.00

#	Visible	Star	Protein Name	Accession Number	Molecular Weight	Exclusivity	Paired t-test CL1: Extraction Method CL2: Temperature x Roast Time	Complete Extraction-Digestion						Soluble Protein Digestion							
								0 C		132C		180C		0 C		132C		180C			
								0 min	5 Min	10 Min	20 Min	5 Min	10 Min	20 Min	0 min	5 Min	10 Min	20 Min	5 Min	10 Min	20 Min
1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Albumin seed storage protein	P93198	16 kDa	38%	0.033	36	50	53	88,500	29	68,500	66,500	131,500	137	101,500	135	121,500	80	33,500
3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Vicilin seed storage protein	Q7Y1C1	70 kDa	21%	0.17	35,083	30,750	30,500	36,667	21,917	37,800	30,750	36,500	45,167	33,083	44,333	39,333	34,083	17,500
4	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	2S albumin seed storage protein	Q7Y1C2																	
5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Seed storage protein	Q2TPW5																	
6	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of 7S vicilin (Fragment) OS=Carya illin...	B3STU4 (+																	
7	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	11S legumin protein OS=Carya illinoensis ...	B5KVH4																	
8	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Rattus norvegicus apolipoprotein A-I precur...	M00001																	
9	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	RecName: Full=Serum albumin; AltName: Fu...	P02769.4																	
10	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	ATPase alpha,F1	gij357982j																	
11	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Oleosin OS=Juglans regia PE=2 SV=1	G8H6H9																	
12	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Non-specific lipid-transfer protein OS=Jugla...	C5H617	12 kDa	100%	0.014	3	4	2	4	0	2	5	12	17	8	15	12	6	4
13	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	RecName: Full=Enolase 2; AltName: Full=2-p...	Q9LE19.1	48 kDa	100%	0.46	3	3	2	4	3	5	1	7	8	5	6	4	0	0
14	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Group of LEA protein [Arachis hypogaea]+2	AA54009.1 (...		100%	0.12	2	3	2	2	2	4	1	3	4	3	7	4	5	0
15	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	GroES-like protein [Arachis hypogaea]	ACF74274.1	21 kDa	100%	0.40	3	3	4	3	4	4	0	2	4	3	4	4	1	0
16	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	60S acidic ribosomal protein P2 OS=Juglans ...	A8QJ72	11 kDa	100%	0.84	1	0	2	3	2	7	2	4	3	3	4	3	2	0
17	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	glutathione peroxidase 1 [Arachis hypogaea]	ACF74299.1	24 kDa	100%	0.48	4	2	3	3	3	4	0	4	4	1	2	0	0	0
18	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	ATP synthase beta subunit	Q9MU05	52 kDa	100%	0.22	4	3	3	4	3	3	2	2	2	0	0	0	0	0
19	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	ubiquitin/ribosomal protein S27a [Arachis hy...	ABI84265.1	18 kDa	100%	0.26	1	2	1	1	0	4	0	5	4	4	4	6	1	0
20	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	alcohol dehydrogenase, partial [Arachis hyp...	AFB69783.1	19 kDa	100%	0.79	2	3	2	1	3	3	0	4	1	3	2	2	0	0
21	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Oleosin OS=Juglans regia PE=2 SV=1	G8H6H8	15 kDa	100%	--	2	4	4	5	1	5	2	0	0	0	0	0	0	0

53 Proteins
4532 Spectra

タンパク質を選択した状態でダブルクリック、またはProteins パネルをクリックすると... →次頁

Proteins 画面

ペプチド/タンパク質の
定性/定量 結果を
より詳細に検証

The screenshot displays the Scaffold DDA software interface. The left sidebar contains navigation options: Organize, Samples, Proteins (highlighted with a red box), Visualize, and Publish. The main window shows a table of identified peptides for protein P93198 (Albumin seed storage protein). Below this, a table of identified spectra is shown. At the bottom, a protein sequence alignment is displayed with various modifications and coverage metrics.

Peptide Sequence	Spectral Match...	Exclusive	Fixed Modifications	Variable Modificati...	Start	Stop	Protein Accession	Peptide Ma...	NTT
DLPNECGISSQR	713	✓	C6 Carbamidomethyl		120	131	P93198	1,374.62	2
DLPNECGISSQRCEIR	1	✓	C6 Carbamidometh...		120	135	P93198	1,932.878	2
GEEMEEMVQSAR	197	✗			108	119	P93198	1,394.581	2
GEEMEEMVQSAR	63	✗		M4 Oxidation	108	119	P93198	1,410.575	2
GEEMEEMVQSAR	56	✗		M7 Oxidation	108	119	P93198	1,410.575	2

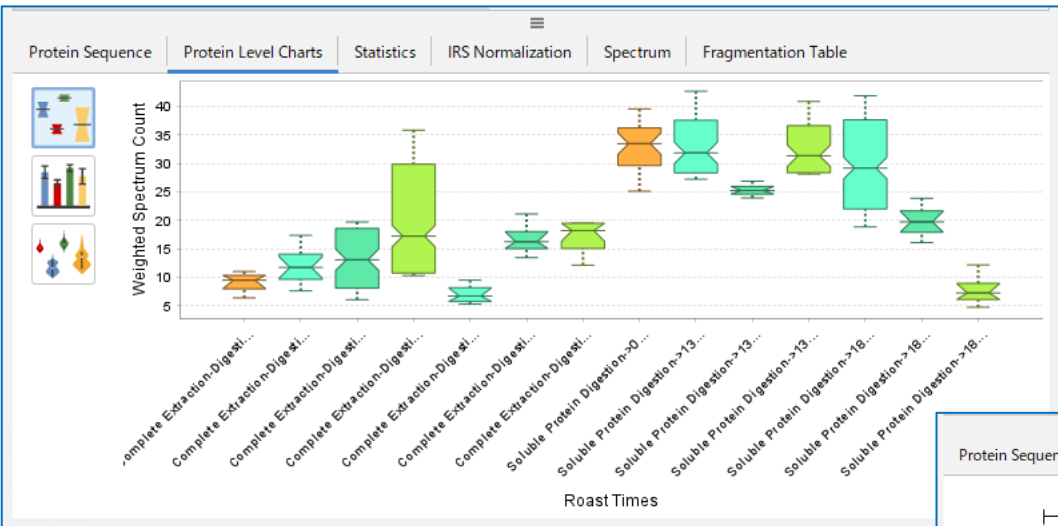
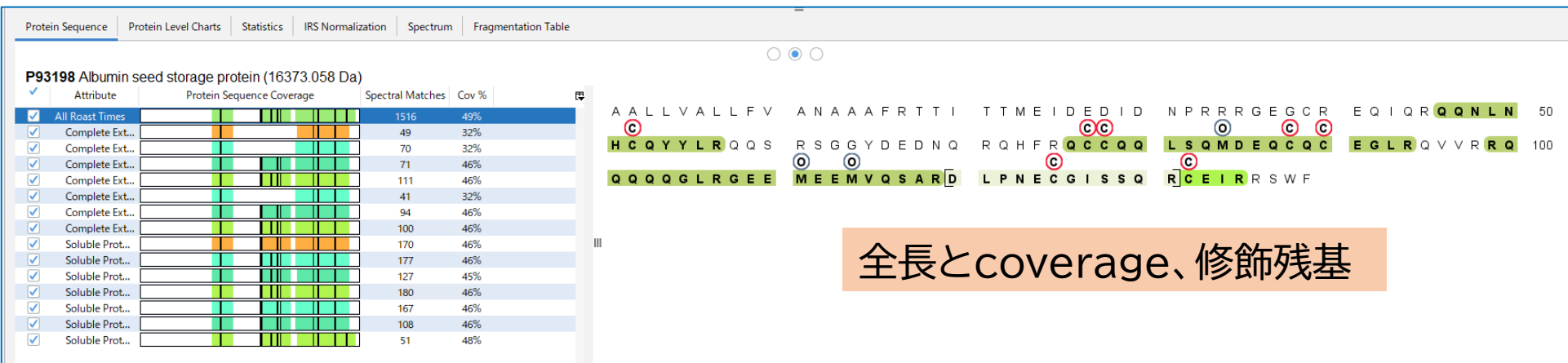
Valid	Sequence	Modifications	Percolator PEP	Mascot:score	Mascot:identity ...	Scaffold:Peptid...	Observed	Charge	Actual Mass	Calculat			
✓	DLPNECGISSQR	Carbamidomet...		55.58	25	100%	688.3	2	1,375	1,375	0.00039	0.28	0.00
✓	DLPNECGISSQR	Carbamidomet...		51.15	25	100%	688.3	2	1,375	1,375	-0.00046	-0.34	0.00
✓	DLPNECGISSQR	Carbamidomet...		23.33	25	97%	688.3	2	1,375	1,375	-0.0012	-0.87	0.00
✓	DLPNECGISSQR	Carbamidomet...		40.31	25	100%	688.3	2	1,375	1,375	0.00088	0.64	0.00
✓	DLPNECGISSQR	Carbamidomet...		50.46	25	100%	688.3	2	1,375	1,375	0.0028	2.1	0.00

P93198 Albumin seed storage protein (16373.058 Da)

Attribute	Protein Sequence Coverage	Spectral Matches	Cov
✓ All Roast Times		1516	49%
✓ Complete Ext...		49	32%
✓ Complete Ext...		70	32%
✓ Complete Ext...		71	46%
✓ Complete Ext...		111	46%
✓ Complete Ext...		41	32%
✓ Complete Ext...		94	46%
✓ Complete Ext...		100	46%
✓ Soluble Prot...		170	46%
✓ Soluble Prot...		177	46%
✓ Soluble Prot...		127	45%
✓ Soluble Prot...		180	46%
✓ Soluble Prot...		167	46%
✓ Soluble Prot...		108	46%

→ スライド 18 関連グラフ

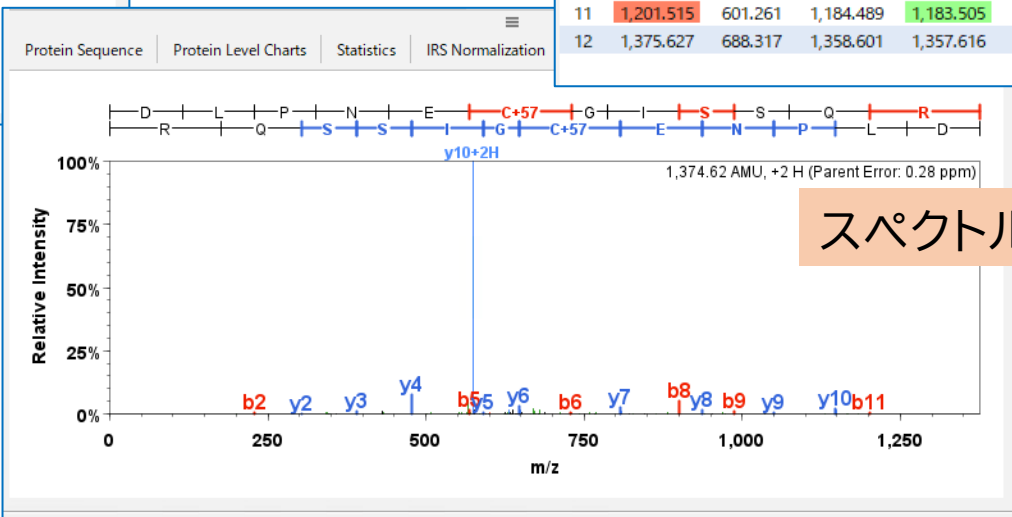
A A L L V A L L F V A N A A A F R T T
T T M E I D E D I D N P R R R G E G C K 40
E Q I Q R **Q Q N L N** **H C Q Y Y L R** Q Q S 60
R S G G Y D E D N Q R Q H F R **Q C C Q Q** 80
L S Q M D E Q C Q C **E G L R** Q V V R R Q 100
Q Q Q Q G L R G E E **M E E M V Q S A R D** 120
L P N E C G I S S Q **R C E I R** R S W F



タンパク質の定量値に基づいたグラフ

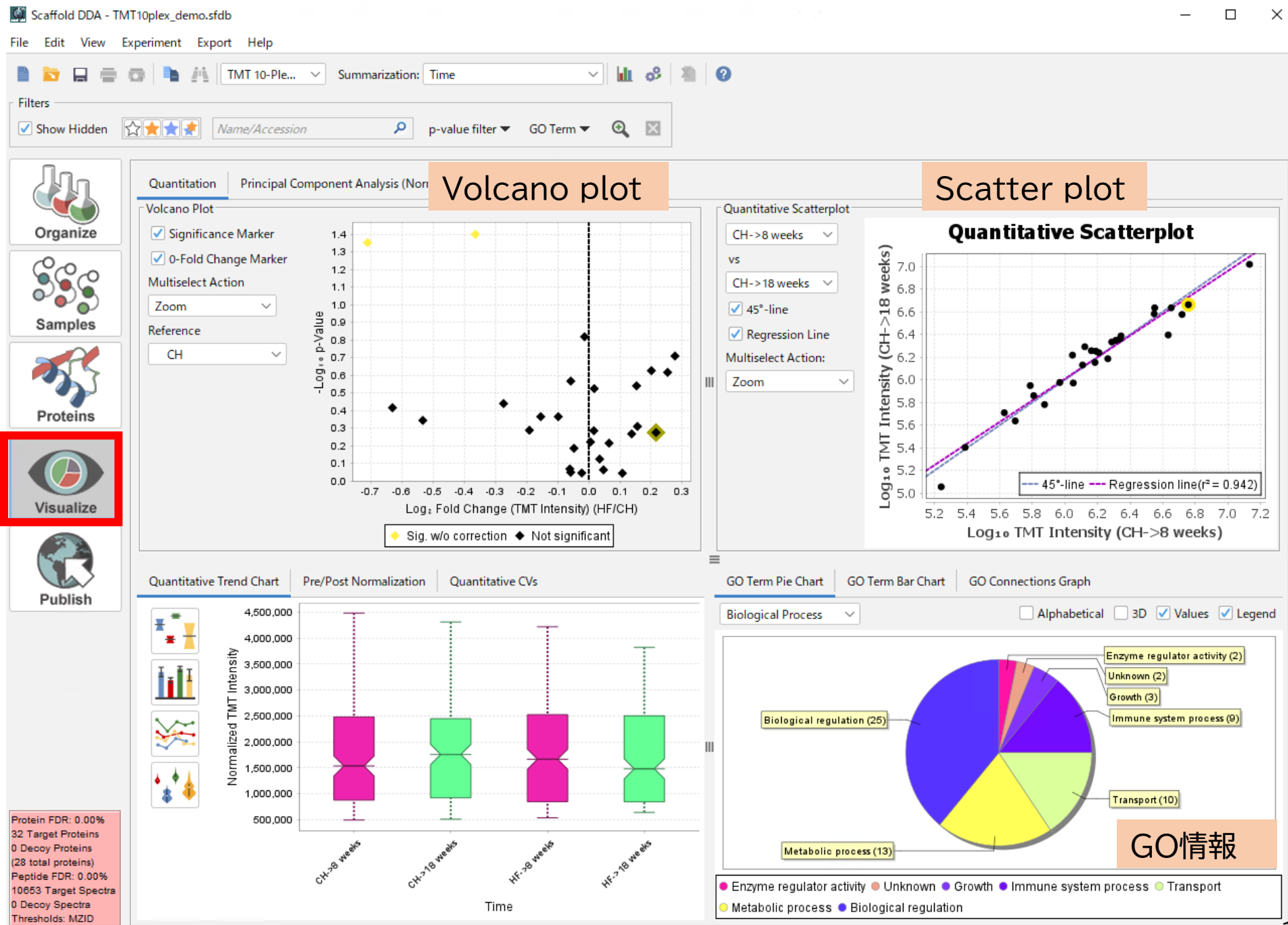
Protein Sequence | Protein Level Charts | Statistics | IRS Normalization | Spectrum | Fragmentation Table

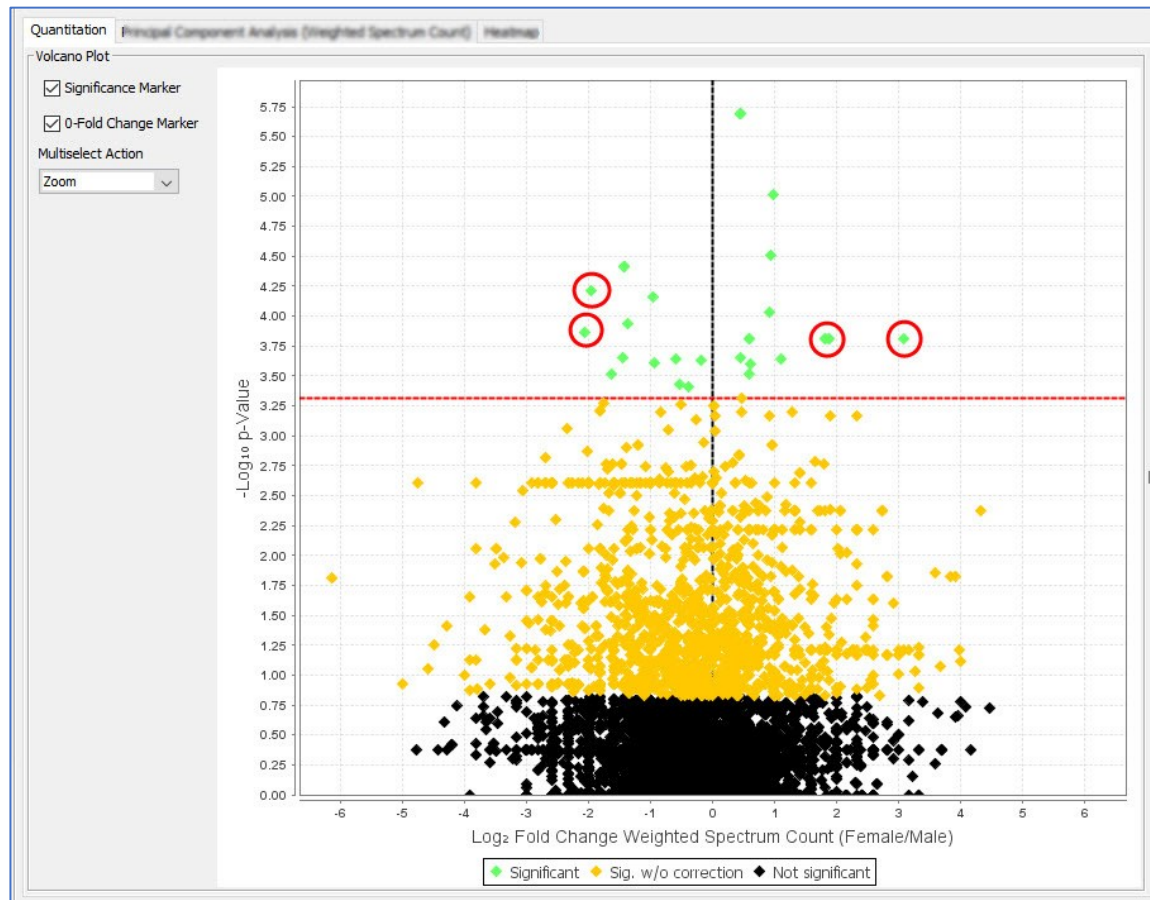
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	116.034			98.024	D	1,375.627	688.317	1,358.601	1,357.617	12
2	229.118			211.108	L	1,260.600	630.804	1,243.574	1,242.590	11
3	326.171			308.160	P	1,147.516	574.262	1,130.490	1,129.505	10
4	440.214		423.187	422.203	N	1,050.463	525.735	1,033.437	1,032.453	9
5	569.257		552.230	551.246	E	936.420	468.714	919.394	918.410	8
6	729.287	365.147	712.261	711.277	C+57	807.378	404.193	790.351	789.367	7
7	786.309	393.658	769.282	768.298	G	647.347	324.177	630.321	629.337	6
8	899.393	450.200	882.366	881.382	I	590.326		573.299	572.315	5
9	986.425	493.716	969.398	968.414	S	477.242		460.215	459.231	4
10	1,073.457	537.232	1,056.430	1,055.446	S	390.210		373.183	372.199	3
11	1,201.515	601.261	1,184.489	1,183.505	Q	303.178		286.151		2
12	1,375.627	688.317	1,358.601	1,357.616	R	175.119		158.092		1



Visualize 画面

- 有意な変動をしているタンパク質の解析
- データの確からしさを検証





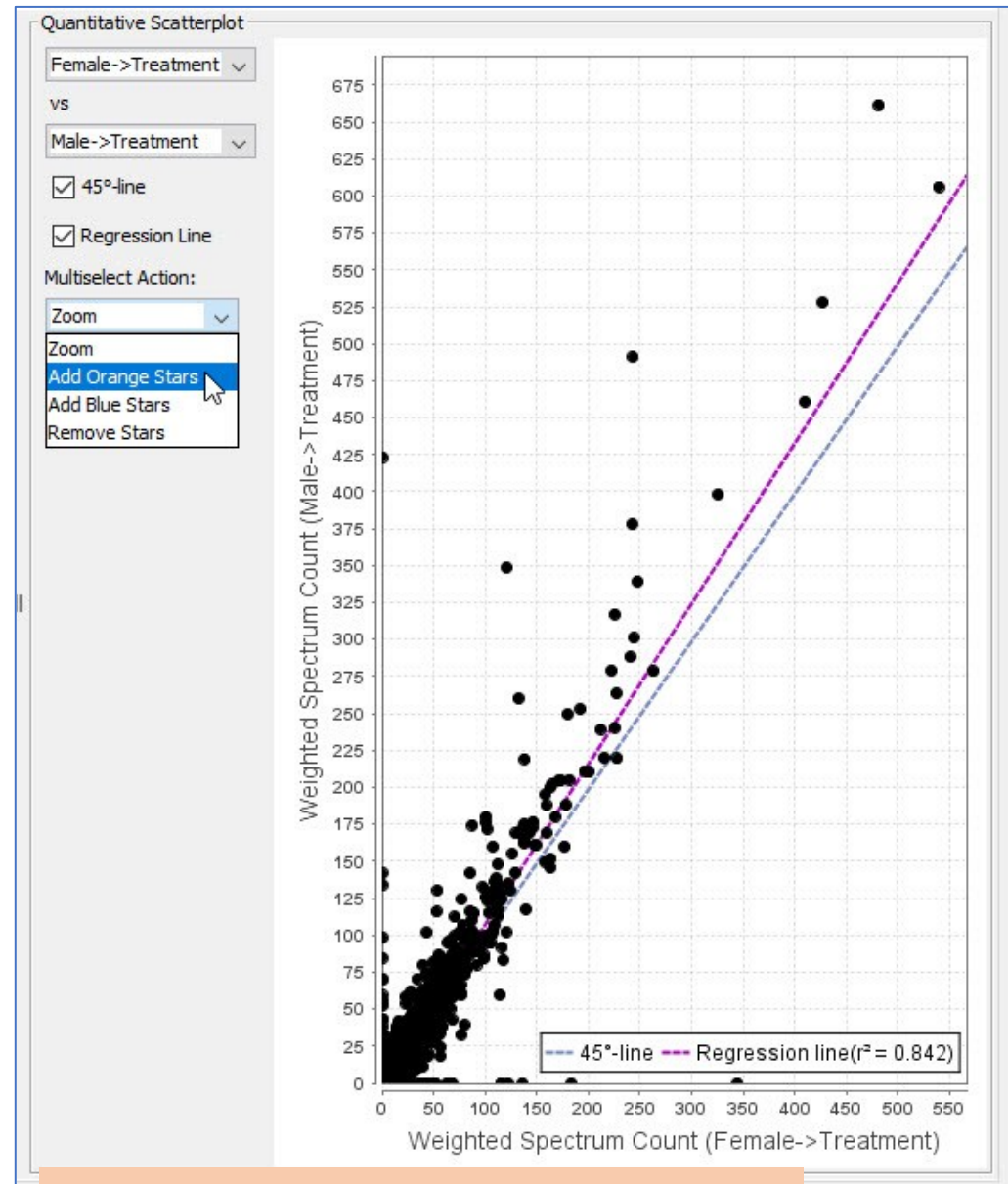
Volcano plot

縦軸 $-\text{Log}_{10}(p)$

横軸 $\text{Log}_2(\text{Fold Change})$

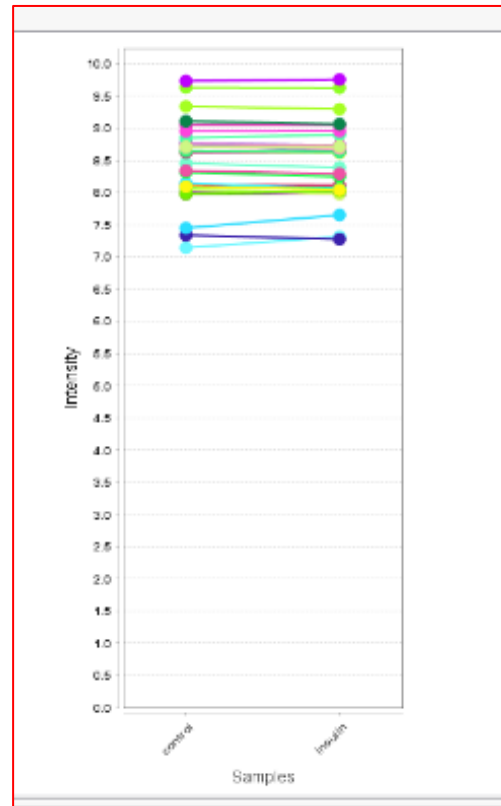
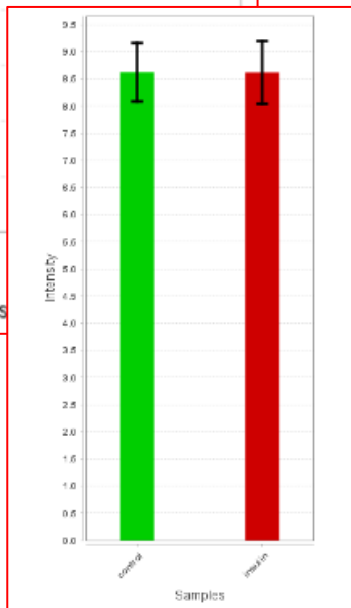
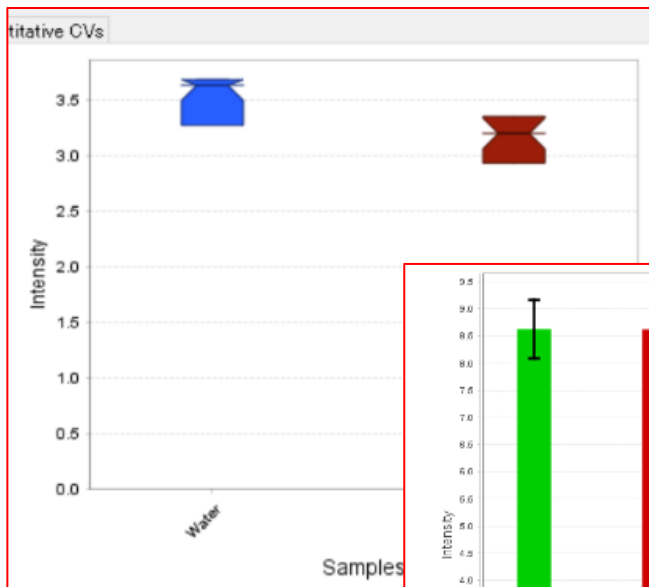
赤点線 多重検定検証の $p(q, \alpha)$

変動タンパク質(例:赤い丸など)を、ratio(横軸)、 p -value両面(縦軸)などを参考に探す

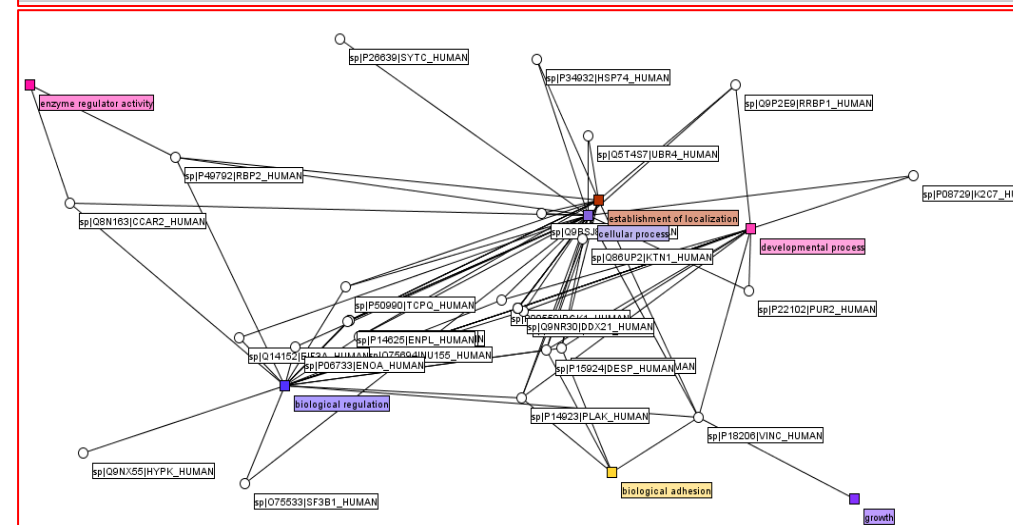
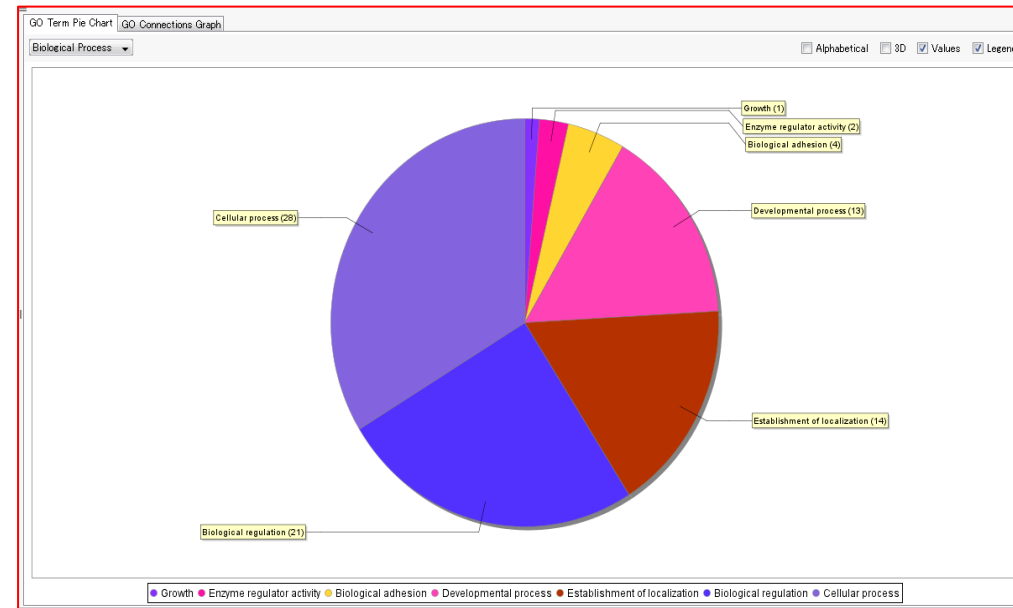


Scatterplot

$y=x$ や回帰直線から大きく外れるタンパク質を探す

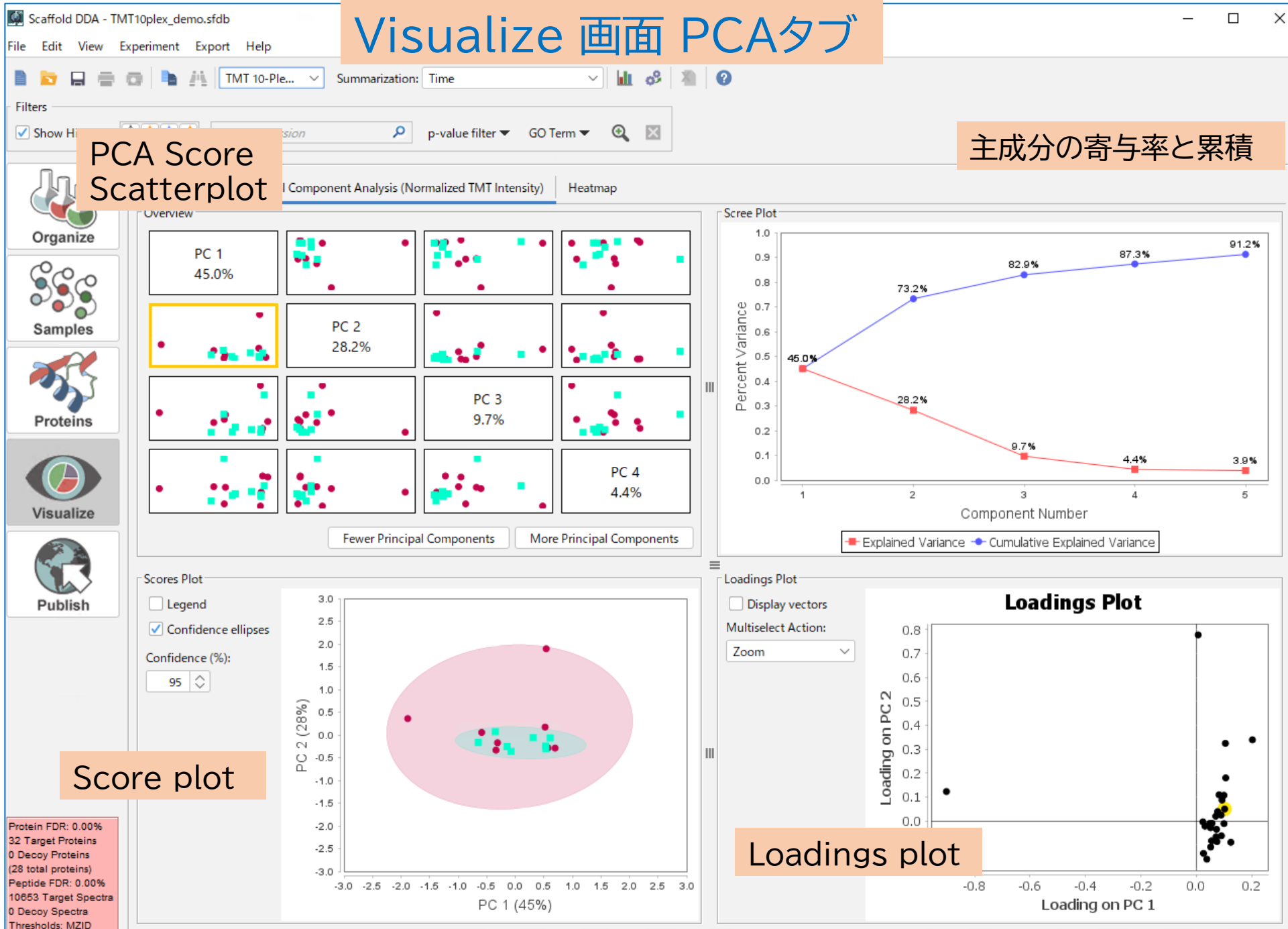


サンプル毎の定量値に関するグラフ
 箱ひげ図(左)、棒グラフ(中)、Trend line(右)
 サンプル毎の定量値ばらつきをチェックする



GO情報
 円グラフ(上) ネットワーク図(下)

Visualize 画面 PCAタブ



主成分の寄与率と累積

Score plot

Loadings plot

Publish 画面

検索パラメータの確認、
論文のMethodに該当する
文章の作成

The screenshot shows the Scaffold DDA software interface. The main window displays a list of parameters for an experiment named 'TMT 10-Plex...'. The parameters are organized into several categories, each with a green dot indicating a status. The 'Publish' button in the left sidebar is highlighted. A summary box at the bottom left provides key statistics. On the right, a 'Method文章' (Method Article) panel is visible, containing sections for 'ANALYSIS OVERVIEW', 'SEARCH', 'CRITERIA FOR PROTEIN IDENTIFICATION', 'QUANTIFICATION', and 'GO ANNOTATION'. Two buttons at the bottom right of the report allow for copying text to the clipboard and exporting the publish report.

Protein FDR: 0.00%
32 Target Proteins
0 Decoy Proteins
(28 total proteins)
Peptide FDR: 0.00%
10653 Target Spectra
0 Decoy Spectra
Thresholds: MZID

Category	Parameter	Value
Protein Grouping	Grouping Method	None
	Protein Clustering Method	None
Threshold Settings	Required Minimum Number of Peptides	55
	Target Peptide FDR	0.1
	Target Protein FDR	0.1
Protein FDR	Achieved Protein FDR	0.0
	Target Protein Count	32
	Decoy Protein Count	0
	Score Used for Protein FDR Thresholding	Scaffold:Protein Probability
	Score Cutoff Used for Protein FDR Thresholding	0
Peptide FDR	Achieved Peptide FDR	0.0
	Target Peptide Count	10653
	Decoy Peptide Count	0
	Score Used for Peptide FDR Thresholding	Scaffold:Peptide Probability
	Score Cutoff Used for Peptide FDR Thresholding	0
Quantitative Settings	Statistical Test	Two-way ANOVA
	Imputation Method	Default
	Quantitative Type	TMT 10-Plex (MS3)
	Purity Correction	TMT 10-Plex Demo Purity Correction
Confidence Settings	Correction Method	Control FDR with standard Benjamini-Hochberg procedure
	Significance Level Type	FDR level q*
	Significance Level	0.05
	Familywise Error Rate	0.0017857143
Heatmap Settings	Linkage Method	Unspecified - Run heatmap
	Distance Metric	Unspecified - Run heatmap
Annotation	FASTA File	No FASTA was specified for annotation

Method文章

ANALYSIS OVERVIEW
Peptide search results were analyzed with Scaffold DDA version 6.2.0. Peptide and protein thresholding, and protein grouping were performed by version <unknown>.

SEARCH
Peptide and protein results were loaded from Scaffold version Scaffold_5.0.2. Peptide identifications were subsequently thresholded to achieve a peptide FDR better than 10.0% on the basis of q -values computed from the score Scaffold:Peptide Probability.

CRITERIA FOR PROTEIN IDENTIFICATION
Proteins were grouped together if they were grouped in every input dataset. Protein groups with a minimum of 55 identified peptides were thresholded to achieve a protein FDR better than 10.0% on the basis of q -values computed from the score Scaffold:Protein Probability.

QUANTIFICATION
Normalization was applied to TMT Intensity and Exclusive TMT Intensity.

GO ANNOTATION
Proteins were annotated with GO terms from: GO_Central, MGI, UniProt, InterPro, GOC, Ensembl, SynGO, ARUK-UCL, RHEA, IntAct, BHF-UCL, Reactome, HGNC-UCL, GOC-OWL, YuBioLab, ParkinsonsUK-UCL, NTNU_SB, CACAO, CAFA, HGNC, DFLAT, Alzheimers_University_of_Toronto, WB, SynGO-UCL, AgBase, PINC, dictyBase, Roslin_Institute and FlyBase.

Copy Text to Clipboard Export Publish Report

Time

p-value filter GO Term

Value: TMT Intensity Normalized Primary factor effect Color Options...

Accession Number	Gene Name	Molecular Weight	Exclusivity	Two-way ANOVA CL1: Diet CL2: Time	Taxonomy	Biological Process										CH		HF								
						biological regulation	enzyme regulator activity	growth	immune system process	metabolic process	transport	cytoplasm	endoplasmic reticulum	membrane	mitochondrion	nucleus	ribosome	binding	catalytic activity	motor activity	structural molecule activity	8 weeks	18 weeks	8 weeks	18 weeks	
Q5SX39	Myh4	223 kDa	100%	0.54	Mus musculus																1.74E5	1.14E5	1.39E5	1.72E5		
A2ASS6	Ttn	3906 kDa	100%	0.24	Mus musculus																	2.44E5	2.53E5	2.82E5	3.67E5	
Q91ZX7	Lrp1	505 kDa	100%	0.15	Mus musculus																	4.95E5	4.33E5	4.05E5	4.51E5	
Q9JHU4	Dync1h1	532 kDa	100%	0.24	Mus musculus																	4.24E5	5.12E5	5.38E5	6.60E5	
P26039	Tln1	270 kDa	100%	0.90	Mus musculus																	7.49E5	6.04E5	6.82E5	6.50E5	
Q9QXS1	Plec	534 kDa	100%	0.49	Mus musculus																	6.43E5	7.25E5	6.47E5	8.96E5	
Q9WTQ5	Akap12	181 kDa	100%	0.87	Mus musculus																	6.12E5	6.88E5	6.15E5	7.71E5	
Q80X90	Finb	278 kDa	95%	0.27	Mus musculus																	9.12E5	9.66E5	9.15E5	7.71E5	
P06909	Cfh	139 kDa	100%	0.040	Mus musculus																	9.29E5	9.46E5	9.01E5	1.16E6	
Q05793	Hspq2	398 kDa	100%	0.86	Mus musculus																					
O70318	Epb41l2	110 kDa	100%	0.60	Mus musculus																					
P97927	Lama4	202 kDa	100%	0.30	Mus musculus																					
Q01853	Vcp	89 kDa	100%	0.45	Mus musculus																					
Q99PL5	Rrbp1	173 kDa	100%	0.52	Mus musculus																					
P02469	Lamb1	197 kDa	100%	0.52	Mus musculus																					

結果表示画面にて、
データをまとめる
単位を変更可能

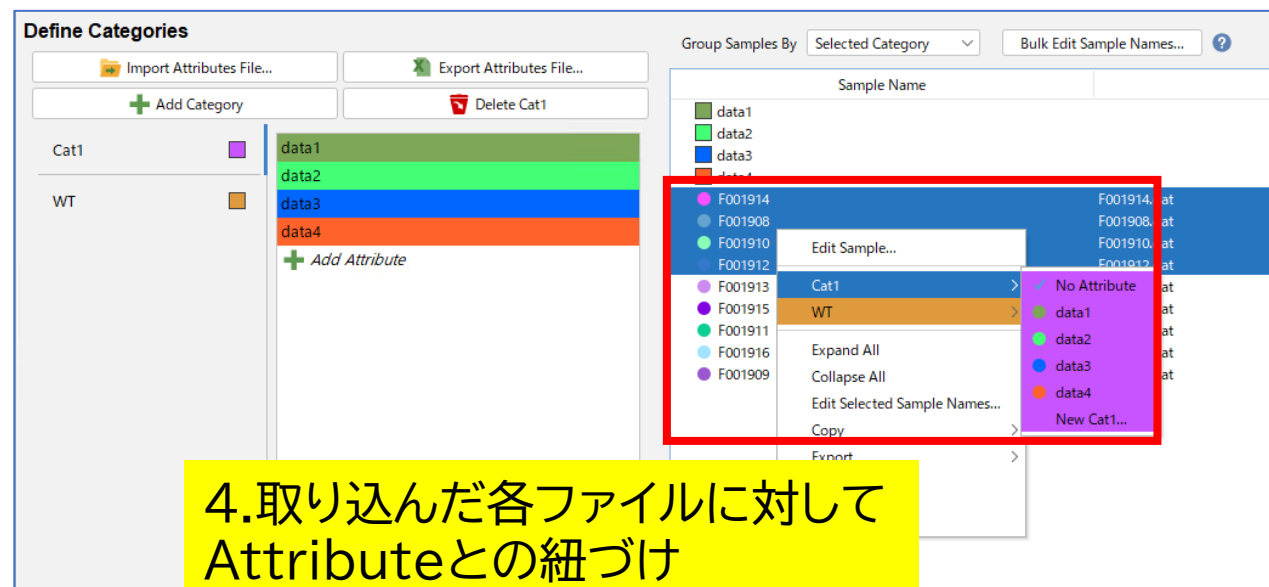
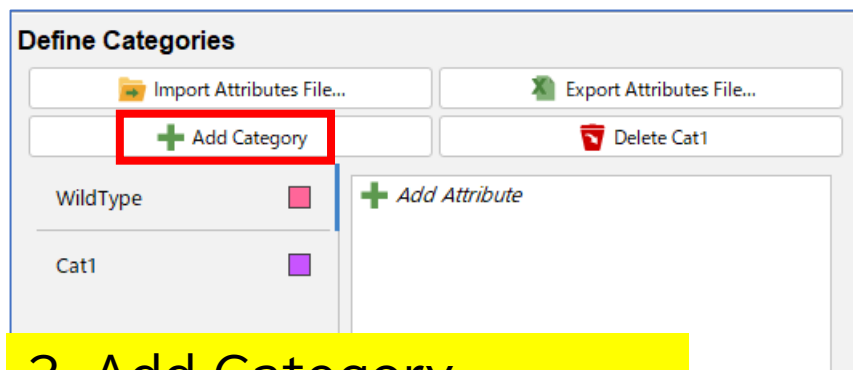
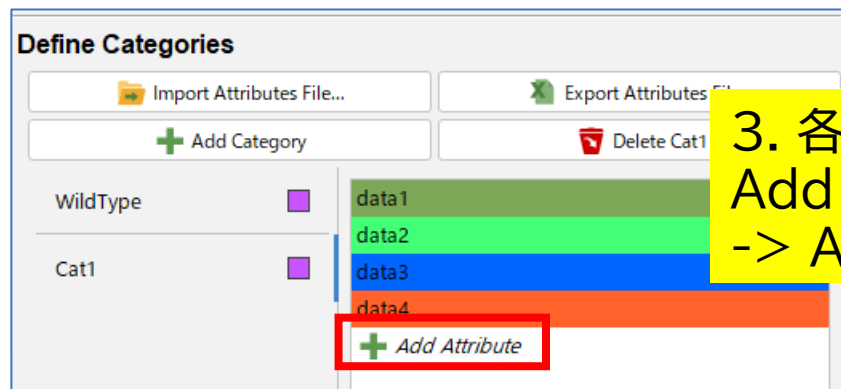
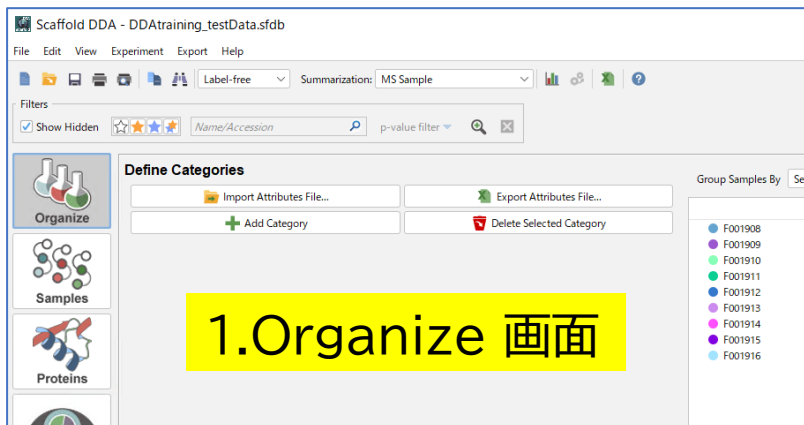
Diet

p-value filter GO Term

Value: TMT Intensity Normalized Primary factor effect Color Options...

Accession Number	Gene Name	Molecular Weight	Exclusivity	Two-way ANOVA CL1: Diet CL2: Time	Taxonomy	Biological Process										CH		HF									
						biological regulation	enzyme regulator activity	growth	immune system process	metabolic process	transport	cytoplasm	endoplasmic reticulum	membrane	mitochondrion	nucleus	ribosome	binding	catalytic activity	motor activity	structural molecule activity	8 weeks	18 weeks	8 weeks	18 weeks		
Q5SX39	Myh4	223 kDa	100%	0.54	Mus musculus																		1.57E5	1.72E5			
A2ASS6	Ttn	3906 kDa	100%	0.24	Mus musculus																			2.44E5	2.91E5		
Q91ZX7	Lrp1	505 kDa	100%	0.15	Mus musculus																			4.44E5	4.40E5		
Q9JHU4	Dync1h1	532 kDa	100%	0.24	Mus musculus																			4.92E5	5.65E5		
P26039	Tln1	270 kDa	100%	0.90	Mus musculus																			6.71E5	6.60E5		
Q9QXS1	Plec	534 kDa	100%	0.49	Mus musculus																			6.86E5	7.64E5		
Q9WTQ5	Akap12	181 kDa	100%	0.87	Mus musculus																			6.88E5	7.11E5		
Q80X90	Finb	278 kDa	95%	0.27	Mus musculus																			9.46E5	9.09E5		
P06909	Cfh	139 kDa	100%	0.040	Mus musculus																			9.89E5	7.68E5		
Q05793	Hspq2	398 kDa	100%	0.86	Mus musculus																			1.31E6	1.26E6		
O70318	Epb41l2	110 kDa	100%	0.60	Mus musculus																			1.37E6	1.37E6		
P97927	Lama4	202 kDa	100%	0.30	Mus musculus																			1.44E6	1.45E6		
Q01853	Vcp	89 kDa	100%	0.45	Mus musculus																			1.48E6	1.02E6		
Q99PL5	Rrbp1	173 kDa	100%	0.52	Mus musculus																			1.55E6	1.57E6		
P02469	Lamb1	197 kDa	100%	0.52	Mus musculus																			1.67E6	1.47E6		

階層設定の操作



検定

Statistical Test

<input type="radio"/> ANOVA / t-test	P	≥ 2 Background \times Concentrations
<input type="radio"/> Permutation Test	NP	≥ 2 Background \times Concentrations
<input type="radio"/> Mann-Whitney U Test	NP	Exactly 2 Background \times Concentrations
<input type="radio"/> Kruskal-Wallis Test	NP	≥ 2 Background \times Concentrations
<input checked="" type="radio"/> None		

Permutation Test

- ノンパラメトリック
- 2群 ○ 3群以上 ○
- ベースはF検定、群間のランダムなデータを入れ替えF値を計算し続ける
- 10000回の入れ替え計算を行い、入れ替え前のF値より有意差以上に差があった回数を10000（データ入れ替えの試行回数）で割った値をp-value とする。

t-test / ANOVA

- パラメトリック
- 2群 ○ 3群以上 ○
- ANOVA両側検定を行う。（2群しかない場合 t検定と同じ。）

Mann Whitney U test / Kruskal-Wallis test

- ノンパラメトリック
- 2群→Mann Whitney U, 3群以上→Kruskal-Wallis
- 同じ分布の形、スケールである事を前提とする

多重比較補正

Multiple Test Correction

Control FWER with Hochberg's step-up and Holm's step-down

Control FDR with standard Benjamini-Hochberg procedure

Control FWER with Hochberg's step-up and Holm's step-down

No correction

多重検定時の第一種過誤に対応する補正

FDR: (False Discovery Rate)

BH法

FWER: Family Wise Error Rate

ホッホベルクのステップアップ手順&ホルムのステップダウン

その他のトピックス(英語マニュアル対応ページ)

・Gene Ontology (→P.29)

Scaffold DDAでGene Ontology情報を付与させるために必要な前処理操作

・タンパク質のグループ化、クラスター化 (→P.156)

シェアペプチドの存在とタンパク質のグループ化ルール

・Report (→P.184)

Scaffold DDAで出力可能なファイル

英語マニュアルのAppendix

- **Appendix A.** Computation of protein and peptide FDR in Scaffold DDA
- **Appendix B.** Rolling Up Intensity Values
- **Appendix C.** Shared Evidence Clustering Algorithm
- **Appendix D.** Distance Based Clustering
- **Appendix E.** Weighted Spectrum counts
- **Appendix F.** Terminology
- **Appendix G.** Heat map clustering
- **Appendix H.** Techniques to Control the Family-wise Error Rate
- **Appendix I.** Using Principal Component Analysis in Scaffold DDA
- **Appendix J.** How PCA is Performed in Scaffold DDA
- **Appendix K.** Description of Mouse Right Click Context Menu Commands

インストール環境、その他

■インストール環境

・対応OS

- Windows 10,11 (64bit)
- Mac (最新版)
- Linux (Ubuntu 16.04 LTS 以降)

・メモリ

最低 4GB以上とあるが、製品版としては32GB以上を強く推奨
(大規模解析なら64GB以上)

・ストレージ

最低 数百 MB 以上。高速SSD の使用を推奨

・CPU

使用可能なコア数の上限が 64コア