Tutorial: Processing MaxQuant-Data with Perseus

Perseus 1.5.1.6

What is Perseus?

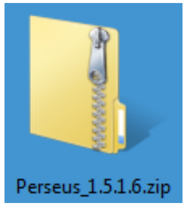
- Powerful statistical software package developed by the Matthias Mann group (Freeware)
- Especially suited to analyze, evaluate and visualize MaxQuant-derived proteomic data

Installation

- Requirements:
  - Operating System: Microsoft Windows Vista or better (but can be run on a Mac via Parallels or Bootcamp etc.)
  - Preinstalled Software: Microsoft .NET Framework 4.5 (http://www.microsoft.com/de-de/download)
  - Perseus Registration Code (free-of-charge online-registration) ➔ click here

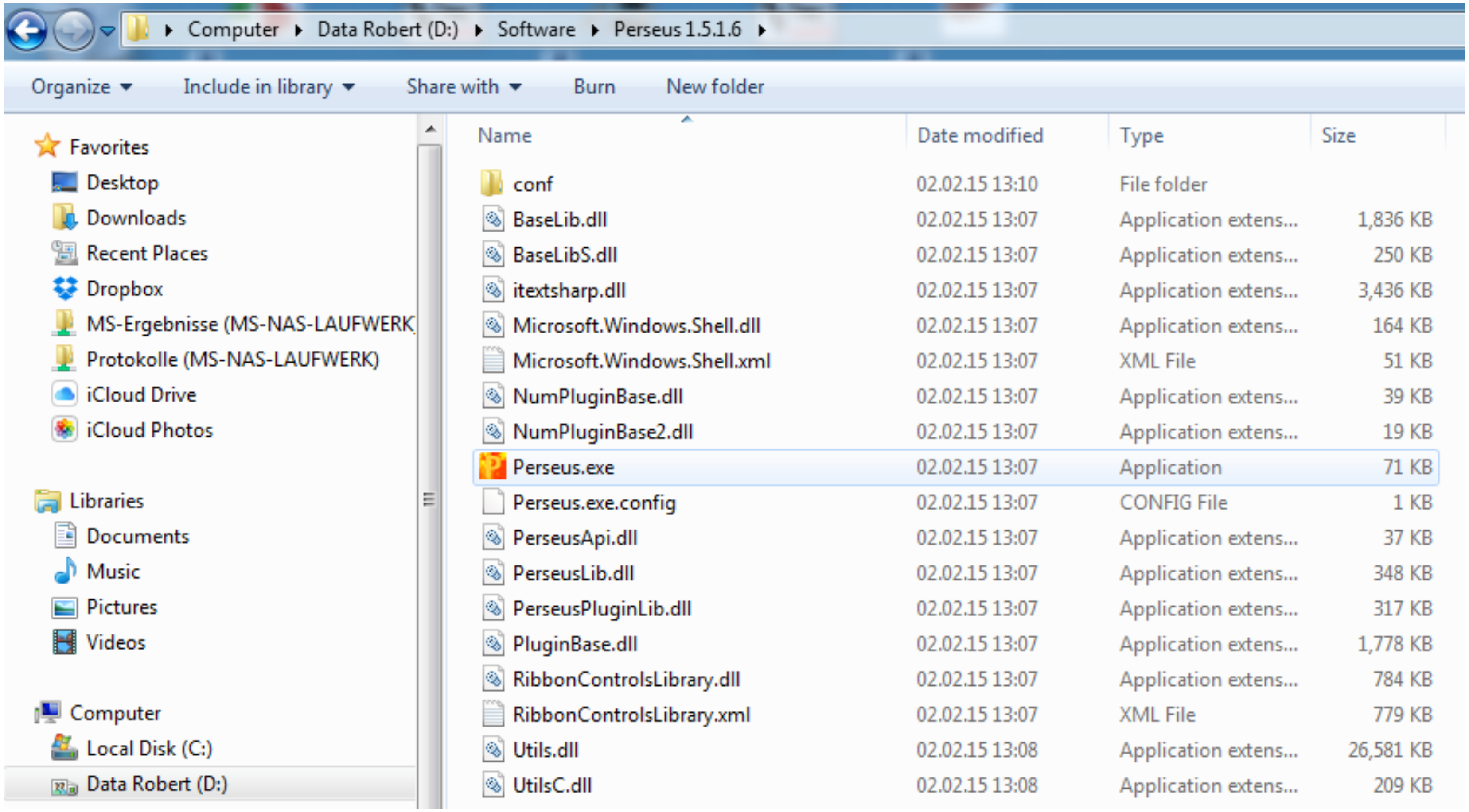
Installation

1. Download Perseus
   - Perseus is provided as a single compressed file (.zip)
   - You can download it by clicking here (Keep your registration code at hand)



2. Uncompress the zip-file and move the containing Perseus folder to a destination of choice

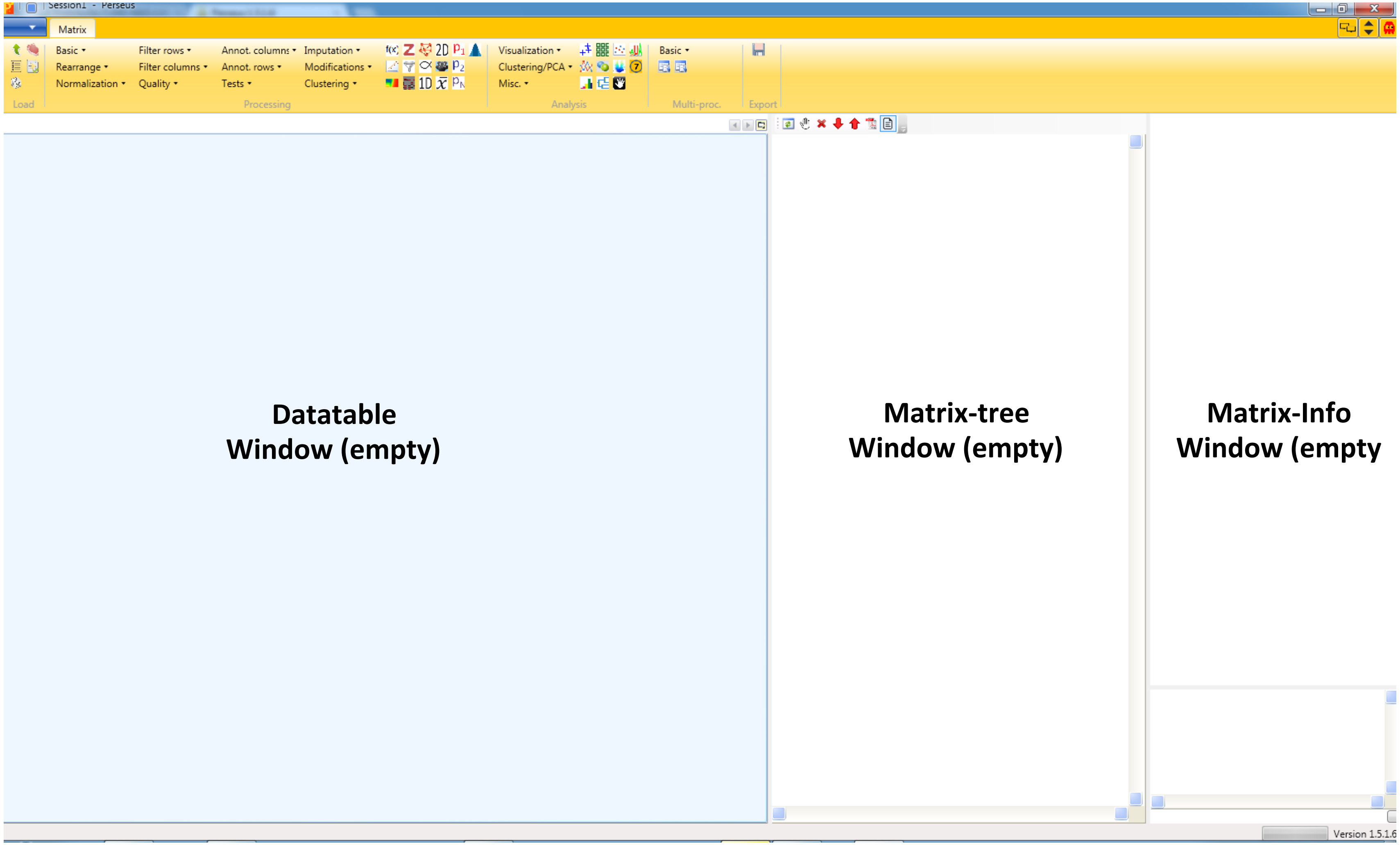
Start Perseus by double clicking Perseus.exe



Name	Date modified	Type	Size
conf	02.02.15 13:10	File folder	
BaseLib.dll	02.02.15 13:07	Application extens...	1,836 KB
BaseLibS.dll	02.02.15 13:07	Application extens...	250 KB
itextsharp.dll	02.02.15 13:07	Application extens...	3,436 KB
Microsoft.Windows.Shell.dll	02.02.15 13:07	Application extens...	164 KB
Microsoft.Windows.Shell.xml	02.02.15 13:07	XML File	51 KB
NumPluginBase.dll	02.02.15 13:07	Application extens...	39 KB
NumPluginBase2.dll	02.02.15 13:07	Application extens...	19 KB
Perseus.exe	02.02.15 13:07	Application	71 KB
Perseus.exe.config	02.02.15 13:07	CONFIG File	1 KB
PerseusApi.dll	02.02.15 13:07	Application extens...	37 KB
PerseusLib.dll	02.02.15 13:07	Application extens...	348 KB
PerseusPluginLib.dll	02.02.15 13:07	Application extens...	317 KB
PluginBase.dll	02.02.15 13:07	Application extens...	1,778 KB
RibbonControlsLibrary.dll	02.02.15 13:07	Application extens...	784 KB
RibbonControlsLibrary.xml	02.02.15 13:07	XML File	779 KB
Utils.dll	02.02.15 13:08	Application extens...	26,581 KB
UtilsC.dll	02.02.15 13:08	Application extens...	209 KB

The software’s main window opens

Main Menu



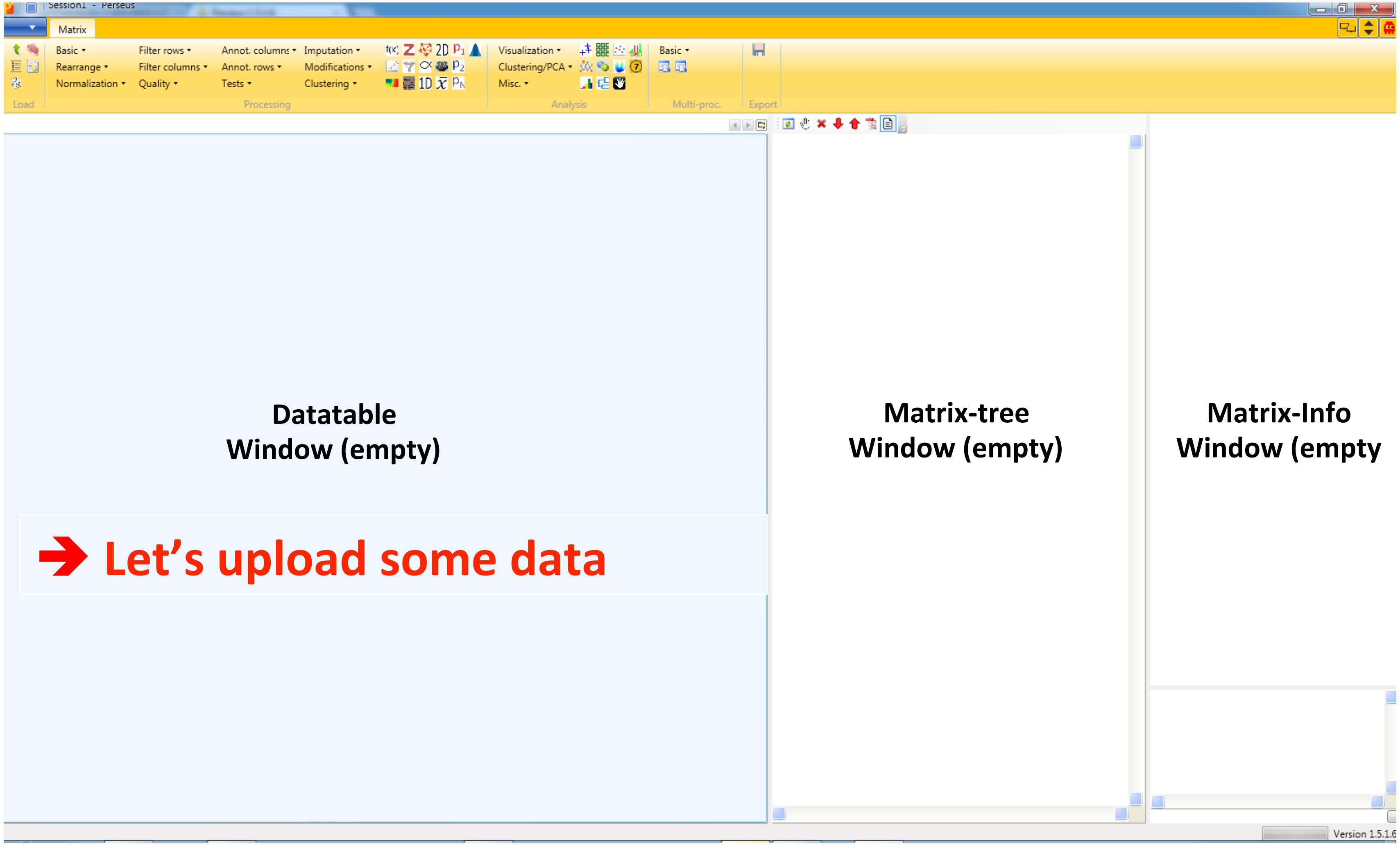
Datatable Window (empty)

Matrix-tree Window (empty)

Matrix-Info Window (empty

The software’s main window opens

Main Menu



Datatable Window (empty)

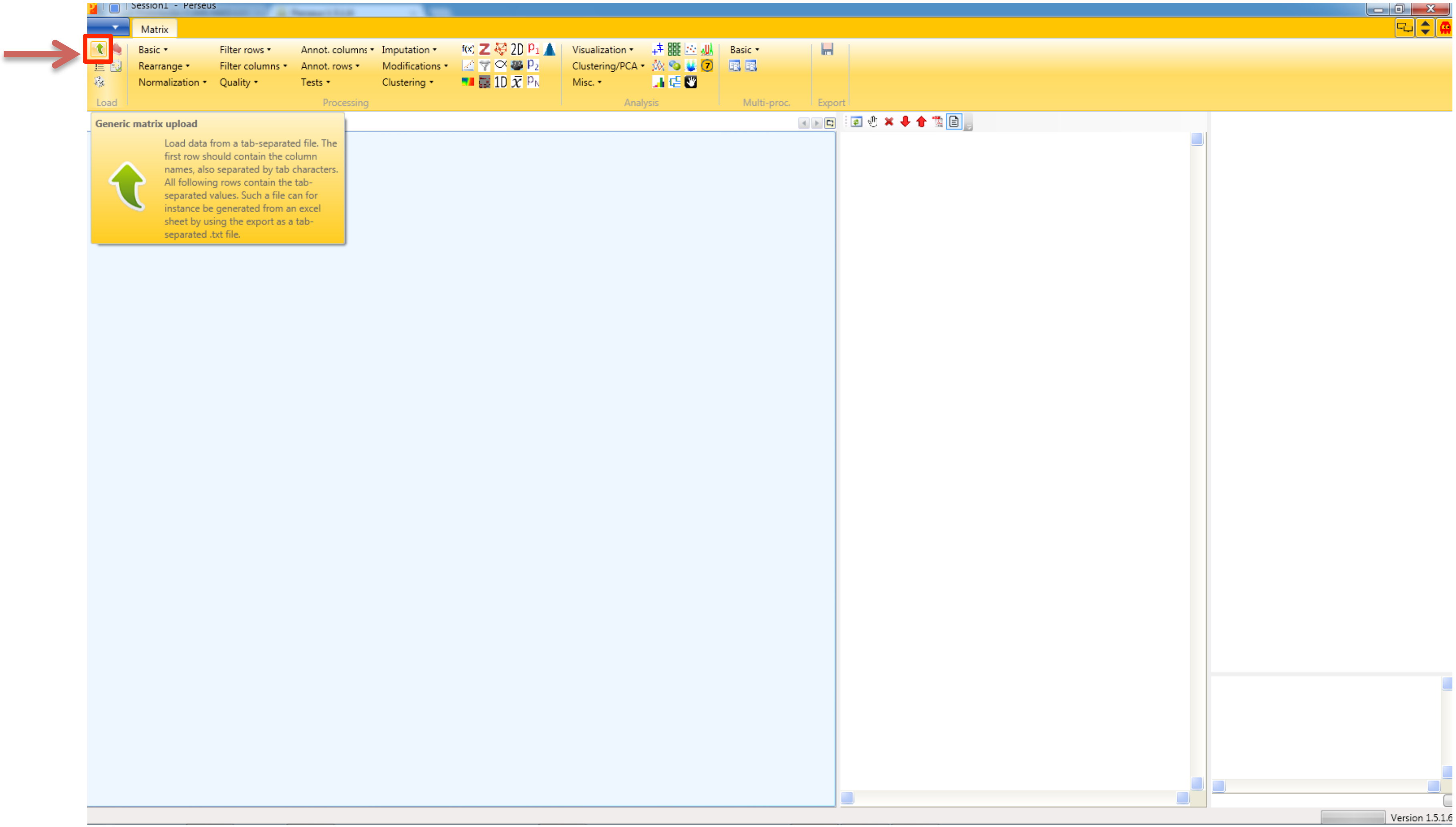
Matrix-tree Window (empty)

Matrix-Info Window (empty

➔ Let’s upload some data

Data upload

Click on the small green arrow in the upper left corner

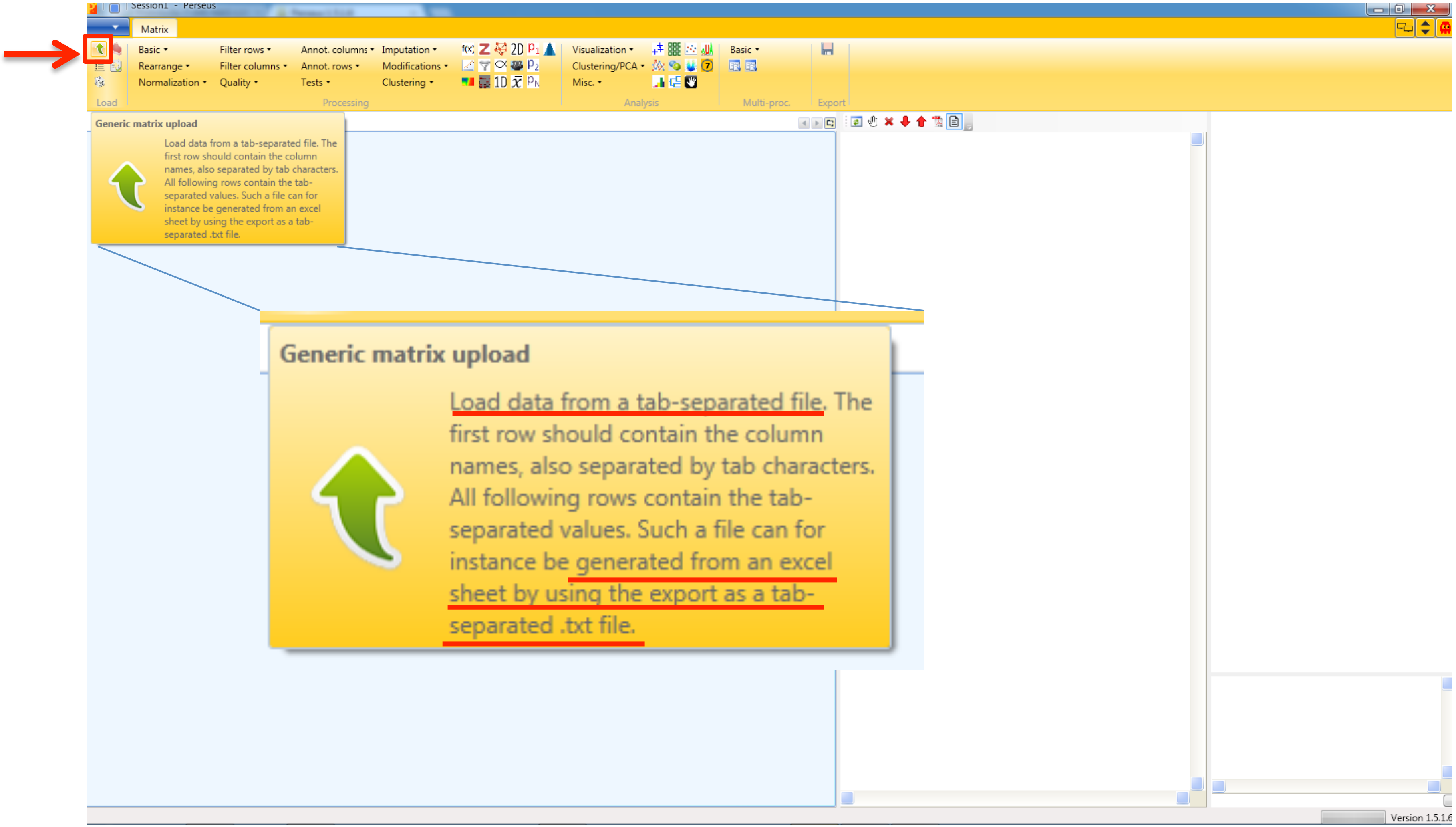


Generic matrix upload

Load data from a tab-separated file. The first row should contain the column names, also separated by tab characters. All following rows contain the tab-separated values. Such a file can for instance be generated from an excel sheet by using the export as a tab-separated .txt file.

Data upload

The data is needed in a tab-separated format ➔ Data export from Excel



Generic matrix upload

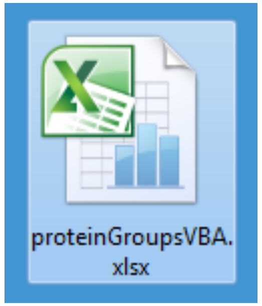
Load data from a tab-separated file. The first row should contain the column names, also separated by tab characters. All following rows contain the tab-separated values. Such a file can for instance be generated from an excel sheet by using the export as a tab-separated .txt file.

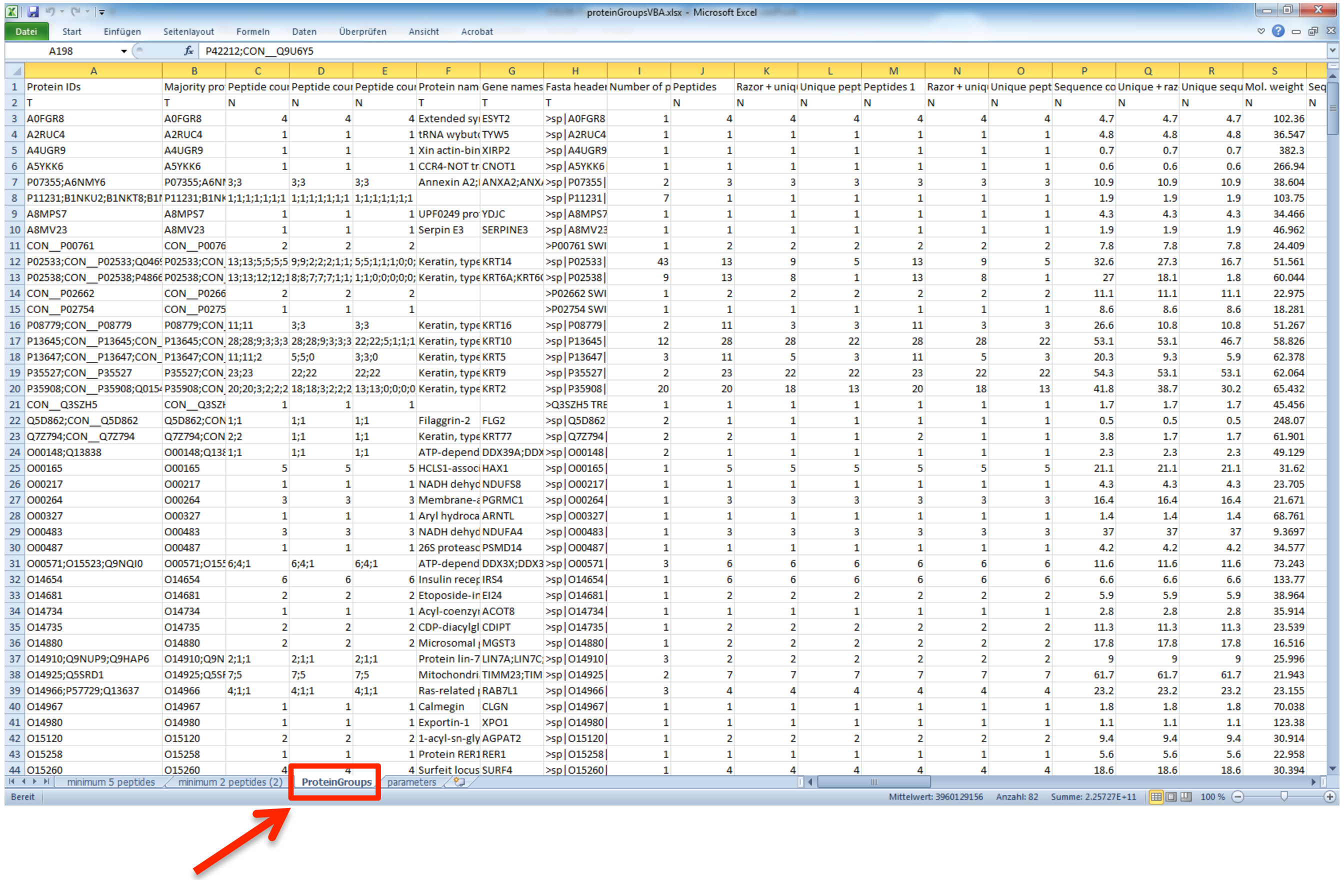
Generic matrix upload

Load data from a tab-separated file. The first row should contain the column names, also separated by tab characters. All following rows contain the tab-separated values. Such a file can for instance be generated from an excel sheet by using the export as a tab-separated .txt file.

Data export from Excel

1.Open Excel-file with protein/peptide results



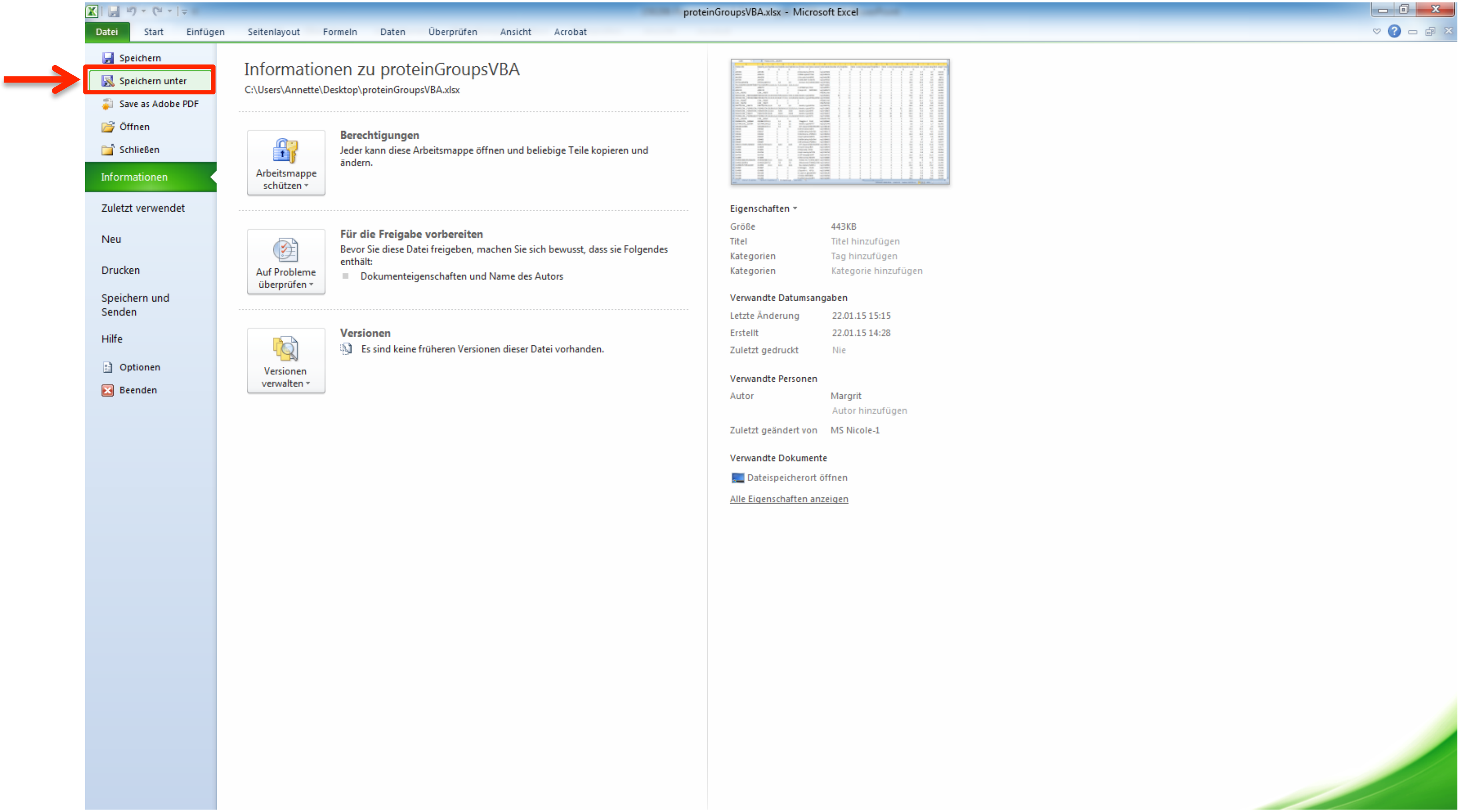


P42212;CON__Q9U6Y5

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	
1	Protein IDs	Majority pro	Peptide cou	Peptide cou	Peptide cou	Protein nam	Gene names	Fasta header	Number of p	Peptides	Razor + uniq	Unique pept	Peptides 1	Razor + uniq	Unique pept	Sequence co	Unique + raz	Unique sequ	Mol. weight	Seq
2	T	T	N	N	N	T	T	T		N	N	N	N	N	N	N	N	N	N	N
3	A0FGR8	A0FGR8	4	4	4	Extended sy	ESYT2	>sp|A0FGR8	1	4	4	4	4	4	4	4.7	4.7	4.7	102.36	
4	A2RUC4	A2RUC4	1	1	1	tRNA wybut	TYW5	>sp|A2RUC4	1	1	1	1	1	1	1	4.8	4.8	4.8	36.547	
5	A4UGR9	A4UGR9	1	1	1	Xin actin-bin	XIRP2	>sp|A4UGR9	1	1	1	1	1	1	1	0.7	0.7	0.7	382.3	
6	A5YKK6	A5YKK6	1	1	1	CCR4-NOT tr	CNOT1	>sp|A5YKK6	1	1	1	1	1	1	1	0.6	0.6	0.6	266.94	
7	P07355;A6NMY6	P07355;A6N	3;3	3;3	3;3	Annexin A2;	ANXA2;ANX	>sp|P07355|	2	3	3	3	3	3	3	10.9	10.9	10.9	38.604	
8	P11231;B1NKU2;B1NKT8;B1	P11231;B1N	1;1;1;1;1;1;1	1;1;1;1;1;1;1	1;1;1;1;1;1;1			>sp|P11231|	7	1	1	1	1	1	1	1.9	1.9	1.9	103.75	
9	A8MPS7	A8MPS7	1	1	1	UPF0249 pro	YDJC	>sp|A8MPS7	1	1	1	1	1	1	1	4.3	4.3	4.3	34.466	
10	A8MV23	A8MV23	1	1	1	Serpin E3	SERPINE3	>sp|A8MV23	1	1	1	1	1	1	1	1.9	1.9	1.9	46.962	
11	CON__P00761	CON__P0076	2	2	2			>P00761 SWI	1	2	2	2	2	2	2	7.8	7.8	7.8	24.409	
12	P02533;CON__P02533;Q046	P02533;CON	13;13;5;5;5;5	9;9;2;2;2;1;1;	5;5;1;1;1;0;0;	Keratin, type	KRT14	>sp|P02533|	43	13	9	5	13	9	5	32.6	27.3	16.7	51.561	
13	P02538;CON__P02538;P486	P02538;CON	13;13;12;12;1	8;8;7;7;7;1;1;	1;1;0;0;0;0;0;	Keratin, type	KRT6A;KRT6	>sp|P02538|	9	13	8	1	13	8	1	27	18.1	1.8	60.044	
14	CON__P02662	CON__P0266	2	2	2			>P02662 SWI	1	2	2	2	2	2	2	11.1	11.1	11.1	22.975	
15	CON__P02754	CON__P0275	1	1	1			>P02754 SWI	1	1	1	1	1	1	1	8.6	8.6	8.6	18.281	
16	P08779;CON__P08779	P08779;CON	11;11	3;3	3;3	Keratin, type	KRT16	>sp|P08779|	2	11	3	3	11	3	3	26.6	10.8	10.8	51.267	
17	P13645;CON__P13645;CON_	P13645;CON	28;28;9;3;3;3	28;28;9;3;3;3	22;22;5;1;1;1	Keratin, type	KRT10	>sp|P13645|	12	28	28	22	28	28	22	53.1	53.1	46.7	58.826	
18	P13647;CON__P13647;CON_	P13647;CON	11;11;2	5;5;0	3;3;0	Keratin, type	KRT5	>sp|P13647|	3	11	5	3	11	5	3	20.3	9.3	5.9	62.378	
19	P35527;CON__P35527	P35527;CON	23;23	22;22	22;22	Keratin, type	KRT9	>sp|P35527|	2	23	22	22	23	22	22	54.3	53.1	53.1	62.064	
20	P35908;CON__P35908;Q015	P35908;CON	20;20;3;2;2;2	18;18;3;2;2;2	13;13;0;0;0;0	Keratin, type	KRT2	>sp|P35908|	20	20	18	13	20	18	13	41.8	38.7	30.2	65.432	
21	CON__Q3SZH5	CON__Q3SZ	1	1	1			>Q3SZH5 TRE	1	1	1	1	1	1	1	1.7	1.7	1.7	45.456	
22	Q5D862;CON__Q5D862	Q5D862;CON	1;1	1;1	1;1	Filaggrin-2	FLG2	>sp|Q5D862	2	1	1	1	1	1	1	0.5	0.5	0.5	248.07	
23	Q7Z794;CON__Q7Z794	Q7Z794;CON	2;2	1;1	1;1	Keratin, type	KRT77	>sp|Q7Z794|	2	2	1	1	2	1	1	3.8	1.7	1.7	61.901	
24	O00148;Q13838	O00148;Q13	1;1	1;1	1;1	ATP-depend	DDX39A;DDX	>sp|O00148|	2	1	1	1	1	1	1	2.3	2.3	2.3	49.129	
25	O00165	O00165	5	5	5	HCLS1-assoc	HAX1	>sp|O00165|	1	5	5	5	5	5	5	21.1	21.1	21.1	31.62	
26	O00217	O00217	1	1	1	NADH dehyd	NDUFS8	>sp|O00217|	1	1	1	1	1	1	1	4.3	4.3	4.3	23.705	
27	O00264	O00264	3	3	3	Membrane-a	PGRMC1	>sp|O00264|	1	3	3	3	3	3	3	16.4	16.4	16.4	21.671	
28	O00327	O00327	1	1	1	Aryl hydroca	ARNTL	>sp|O00327|	1	1	1	1	1	1	1	1.4	1.4	1.4	68.761	
29	O00483	O00483	3	3	3	NADH dehyd	NDUFA4	>sp|O00483|	1	3	3	3	3	3	3	37	37	37	9.3697	
30	O00487	O00487	1	1	1	26S proteasc	PSMD14	>sp|O00487|	1	1	1	1	1	1	1	4.2	4.2	4.2	34.577	
31	O00571;O15523;Q9NQI0	O00571;O15	6;4;1	6;4;1	6;4;1	ATP-depend	DDX3X;DDX3	>sp|O00571|	3	6	6	6	6	6	6	11.6	11.6	11.6	73.243	
32	O14654	O14654	6	6	6	Insulin recep	IRS4	>sp|O14654|	1	6	6	6	6	6	6	6.6	6.6	6.6	133.77	
33	O14681	O14681	2	2	2	Etoposide-in	EI24	>sp|O14681|	1	2	2	2	2	2	2	5.9	5.9	5.9	38.964	
34	O14734	O14734	1	1	1	Acyl-coenzy	ACOT8	>sp|O14734|	1	1	1	1	1	1	1	2.8	2.8	2.8	35.914	
35	O14735	O14735	2	2	2	CDP-diacylgl	CDIPT	>sp|O14735|	1	2	2	2	2	2	2	11.3	11.3	11.3	23.539	
36	O14880	O14880	2	2	2	Microsomal	MGST3	>sp|O14880|	1	2	2	2	2	2	2	17.8	17.8	17.8	16.516	
37	O14910;Q9NUP9;Q9HAP6	O14910;Q9N	2;1;1	2;1;1	2;1;1	Protein lin-7	LIN7A;LIN7C;	>sp|O14910|	3	2	2	2	2	2	2	9	9	9	25.996	
38	O14925;Q5SRD1	O14925;Q5S	7;5	7;5	7;5	Mitochondri	TIMM23;TIM	>sp|O14925|	2	7	7	7	7	7	7	61.7	61.7	61.7	21.943	
39	O14966;P57729;Q13637	O14966	4;1;1	4;1;1	4;1;1	Ras-related	RAB7L1	>sp|O14966|	3	4	4	4	4	4	4	23.2	23.2	23.2	23.155	
40	O14967	O14967	1	1	1	Calmegin	CLGN	>sp|O14967|	1	1	1	1	1	1	1	1.8	1.8	1.8	70.038	
41	O14980	O14980	1	1	1	Exportin-1	XPO1	>sp|O14980|	1	1	1	1	1	1	1	1.1	1.1	1.1	123.38	
42	O15120	O15120	2	2	2	1-acyl-sn-gly	AGPAT2	>sp|O15120|	1	2	2	2	2	2	2	9.4	9.4	9.4	30.914	
43	O15258	O15258	1	1	1	Protein RER1	RER1	>sp|O15258|	1	1	1	1	1	1	1	5.6	5.6	5.6	22.958	
44	O15260	O15260	4	4	4	Surfeit locus	SURF4	>sp|O15260|	1	4	4	4	4	4	4	18.6	18.6	18.6	30.394	

Data export from Excel

2.Click on File (Datei) ➔ Save as (Speichern unter)



Informationen zu proteinGroupsVBA

C:\Users\Annette\Desktop\proteinGroupsVBA.xlsx

Berechtigungen

Jeder kann diese Arbeitsmappe öffnen und beliebige Teile kopieren und ändern.

Für die Freigabe vorbereiten

Bevor Sie diese Datei freigeben, machen Sie sich bewusst, dass sie Folgendes enthält:

- Dokumenteigenschaften und Name des Autors

Versionen

Es sind keine früheren Versionen dieser Datei vorhanden.

Eigenschaften

Größe	443KB
Titel	Titel hinzufügen
Kategorien	Tag hinzufügen
Kategorien	Kategorie hinzufügen

Verwandte Datumsangaben

Letzte Änderung	22.01.15 15:15
Erstellt	22.01.15 14:28
Zuletzt gedruckt	Nie

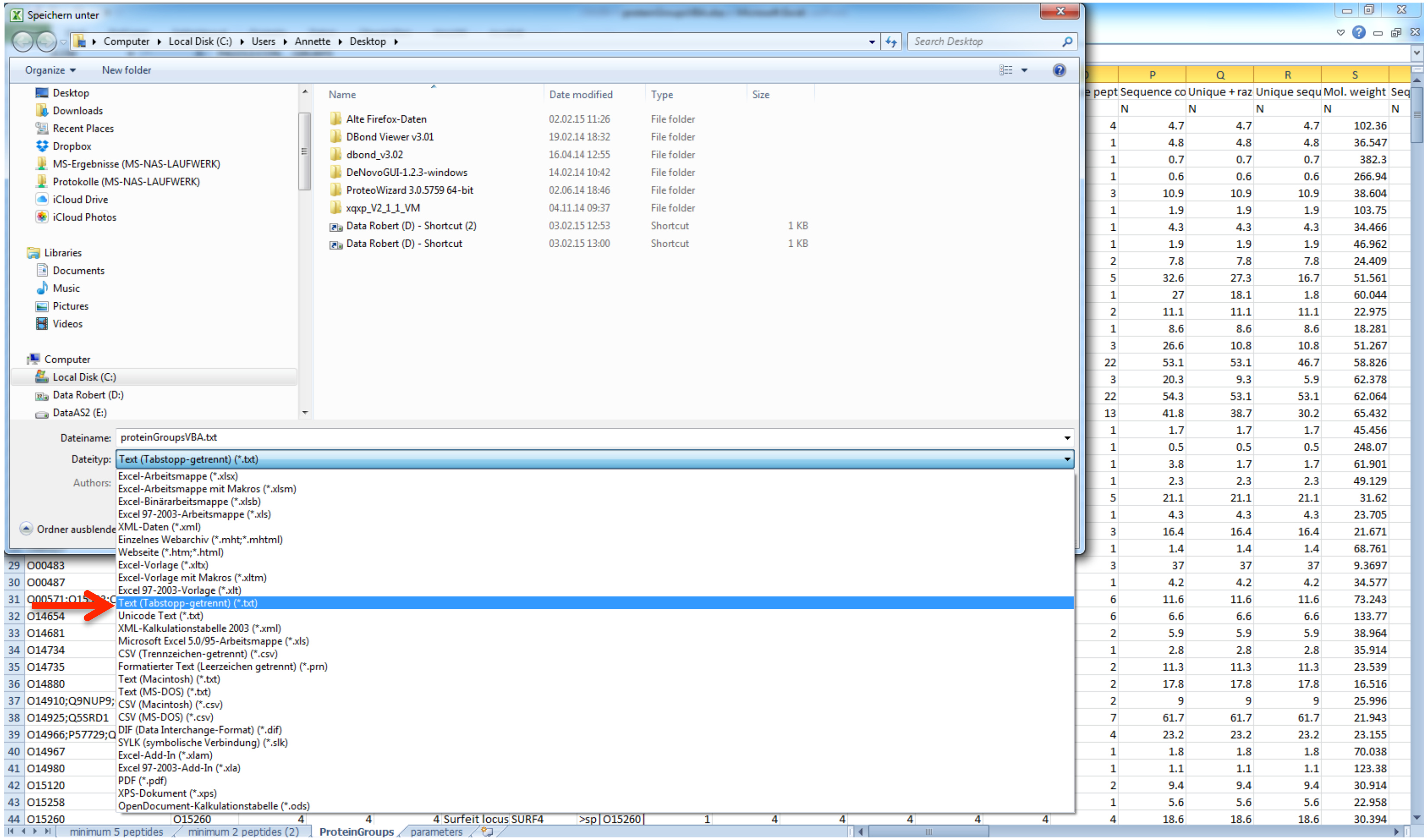
Verwandte Personen

Autor	Margrit Autor hinzufügen
Zuletzt geändert von	MS Nicole-1

Verwandte Dokumente

Data export from Excel

3. As file format (Dateityp) choose “Text (**Tab delimited**) (*.txt)



Name	Date modified	Type	Size
Alte Firefox-Daten	02.02.15 11:26	File folder	
DBond Viewer v3.01	19.02.14 18:32	File folder	
dbond_v3.02	16.04.14 12:55	File folder	
DeNovoGUI-1.2.3-windows	14.02.14 10:42	File folder	
ProteoWizard 3.0.5759 64-bit	02.06.14 18:46	File folder	
xqxp_V2_1_1_VM	04.11.14 09:37	File folder	
Data Robert (D) - Shortcut (2)	03.02.15 12:53	Shortcut	1 KB
Data Robert (D) - Shortcut	03.02.15 13:00	Shortcut	1 KB

Dateiname: proteinGroupsVBA.txt

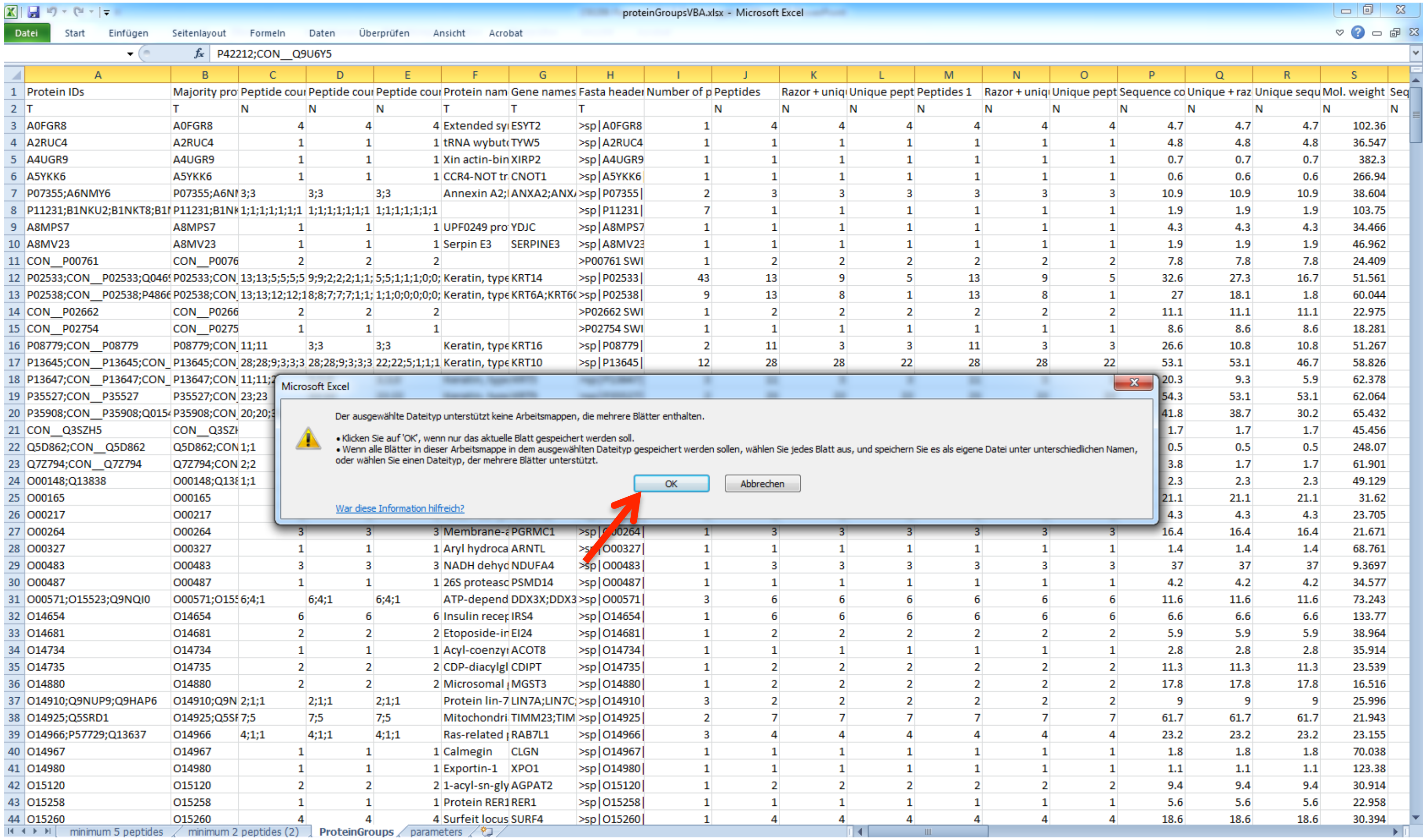
Dateityp: Text (Tabstopp-getrennt) (*.txt)

	A	O	P	Q	R	S
		e pept	Sequence co	Unique + raz	Unique sequ	Mol. weight
			N	N	N	N
		4	4.7	4.7	4.7	102.36
		1	4.8	4.8	4.8	36.547
		1	0.7	0.7	0.7	382.3
		1	0.6	0.6	0.6	266.94
		3	10.9	10.9	10.9	38.604
		1	1.9	1.9	1.9	103.75
		1	4.3	4.3	4.3	34.466
		1	1.9	1.9	1.9	46.962
		2	7.8	7.8	7.8	24.409
		5	32.6	27.3	16.7	51.561
		1	27	18.1	1.8	60.044
		2	11.1	11.1	11.1	22.975
		1	8.6	8.6	8.6	18.281
		3	26.6	10.8	10.8	51.267
		22	53.1	53.1	46.7	58.826
		3	20.3	9.3	5.9	62.378
		22	54.3	53.1	53.1	62.064
		13	41.8	38.7	30.2	65.432
		1	1.7	1.7	1.7	45.456
		1	0.5	0.5	0.5	248.07
		1	3.8	1.7	1.7	61.901
		1	2.3	2.3	2.3	49.129
		5	21.1	21.1	21.1	31.62
		1	4.3	4.3	4.3	23.705
		3	16.4	16.4	16.4	21.671
		1	1.4	1.4	1.4	68.761
29	O00483	3	37	37	37	9.3697
30	O00487	1	4.2	4.2	4.2	34.577
31	O00571;O15	6	11.6	11.6	11.6	73.243
32	O14654	6	6.6	6.6	6.6	133.77
33	O14681	2	5.9	5.9	5.9	38.964
34	O14734	1	2.8	2.8	2.8	35.914
35	O14735	2	11.3	11.3	11.3	23.539
36	O14880	2	17.8	17.8	17.8	16.516
37	O14910;Q9NUP9;	2	9	9	9	25.996
38	O14925;Q5SRD1	7	61.7	61.7	61.7	21.943
39	O14966;P57729;Q	4	23.2	23.2	23.2	23.155
40	O14967	1	1.8	1.8	1.8	70.038
41	O14980	1	1.1	1.1	1.1	123.38
42	O15120	2	9.4	9.4	9.4	30.914
43	O15258	1	5.6	5.6	5.6	22.958
44	O15260	4	18.6	18.6	18.6	30.394

Row 44 (continued): O15260 | 4 | 4 | 4 Surfeit locus SURF4 | >sp|O15260| | 1 | 4 | 4 | 4 | 4 | 4

Data export from Excel

4. Confirm export by clicking OK ➔ Note that only the currently selected datasheet is exported



proteinGroupsVBA.xlsx - Microsoft Excel

fx P42212;CON__Q9U6Y5

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	
1	Protein IDs	Majority pro	Peptide cour	Peptide cour	Peptide cour	Protein nam	Gene names	Fasta header	Number of p	Peptides	Razor + uniq	Unique pept	Peptides 1	Razor + uniq	Unique pept	Sequence co	Unique + raz	Unique sequ	Mol. weight	Seq
2	T	T	N	N	N	T	T	T		N	N	N	N	N	N	N	N	N	N	N
3	A0FGR8	A0FGR8	4	4	4	Extended sy	ESYT2	>sp|A0FGR8	1	4	4	4	4	4	4	4.7	4.7	4.7	102.36	
4	A2RUC4	A2RUC4	1	1	1	tRNA wybut	TYW5	>sp|A2RUC4	1	1	1	1	1	1	1	4.8	4.8	4.8	36.547	
5	A4UGR9	A4UGR9	1	1	1	Xin actin-bin	XIRP2	>sp|A4UGR9	1	1	1	1	1	1	1	0.7	0.7	0.7	382.3	
6	A5YKK6	A5YKK6	1	1	1	CCR4-NOT tr	CNOT1	>sp|A5YKK6	1	1	1	1	1	1	1	0.6	0.6	0.6	266.94	
7	P07355;A6NMY6	P07355;A6N	3;3	3;3	3;3	Annexin A2;	ANXA2;ANX	>sp|P07355|	2	3	3	3	3	3	3	10.9	10.9	10.9	38.604	
8	P11231;B1NKU2;B1NKT8;B1N	P11231;B1NK	1;1;1;1;1;1;1	1;1;1;1;1;1;1	1;1;1;1;1;1;1			>sp|P11231|	7	1	1	1	1	1	1	1.9	1.9	1.9	103.75	
9	A8MPS7	A8MPS7	1	1	1	UPF0249 pro	YDJC	>sp|A8MPS7	1	1	1	1	1	1	1	4.3	4.3	4.3	34.466	
10	A8MV23	A8MV23	1	1	1	Serpin E3	SERPINE3	>sp|A8MV23	1	1	1	1	1	1	1	1.9	1.9	1.9	46.962	
11	CON__P00761	CON__P0076	2	2	2			>P00761 SWI	1	2	2	2	2	2	2	7.8	7.8	7.8	24.409	
12	P02533;CON__P02533;Q046	P02533;CON_	13;13;5;5;5;5	9;9;2;2;2;1;1;	5;5;1;1;1;0;0;	Keratin, type	KRT14	>sp|P02533|	43	13	9	5	13	9	5	32.6	27.3	16.7	51.561	
13	P02538;CON__P02538;P4866	P02538;CON_	13;13;12;12;1	8;8;7;7;7;1;1;	1;1;0;0;0;0;0;	Keratin, type	KRT6A;KRT6	>sp|P02538|	9	13	8	1	13	8	1	27	18.1	1.8	60.044	
14	CON__P02662	CON__P0266	2	2	2			>P02662 SWI	1	2	2	2	2	2	2	11.1	11.1	11.1	22.975	
15	CON__P02754	CON__P0275	1	1	1			>P02754 SWI	1	1	1	1	1	1	1	8.6	8.6	8.6	18.281	
16	P08779;CON__P08779	P08779;CON_	11;11	3;3	3;3	Keratin, type	KRT16	>sp|P08779|	2	11	3	3	11	3	3	26.6	10.8	10.8	51.267	
17	P13645;CON__P13645;CON_	P13645;CON_	28;28;9;3;3;3	28;28;9;3;3;3	22;22;5;1;1;1	Keratin, type	KRT10	>sp|P13645|	12	28	28	22	28	28	22	53.1	53.1	46.7	58.826	
18	P13647;CON__P13647;CON_	P13647;CON_	11;11;2													20.3	9.3	5.9	62.378	
19	P35527;CON__P35527	P35527;CON_	23;23													54.3	53.1	53.1	62.064	
20	P35908;CON__P35908;Q015	P35908;CON_	20;20;													41.8	38.7	30.2	65.432	
21	CON__Q3SZH5	CON__Q3SZ														1.7	1.7	1.7	45.456	
22	Q5D862;CON__Q5D862	Q5D862;CON	1;1													0.5	0.5	0.5	248.07	
23	Q7Z794;CON__Q7Z794	Q7Z794;CON	2;2													3.8	1.7	1.7	61.901	
24	O00148;Q13838	O00148;Q138	1;1													2.3	2.3	2.3	49.129	
25	O00165	O00165														21.1	21.1	21.1	31.62	
26	O00217	O00217														4.3	4.3	4.3	23.705	
27	O00264	O00264	3	3	3	Membrane-a	PGRMC1	>sp|O00264|	1	3	3	3	3	3	3	16.4	16.4	16.4	21.671	
28	O00327	O00327	1	1	1	Aryl hydroca	ARNTL	>sp|O00327|	1	1	1	1	1	1	1	1.4	1.4	1.4	68.761	
29	O00483	O00483	3	3	3	NADH dehyd	NDUFA4	>sp|O00483|	1	3	3	3	3	3	3	37	37	37	9.3697	
30	O00487	O00487	1	1	1	26S proteasc	PSMD14	>sp|O00487|	1	1	1	1	1	1	1	4.2	4.2	4.2	34.577	
31	O00571;O15523;Q9NQI0	O00571;O155	6;4;1	6;4;1	6;4;1	ATP-depend	DDX3X;DDX3	>sp|O00571|	3	6	6	6	6	6	6	11.6	11.6	11.6	73.243	
32	O14654	O14654	6	6	6	Insulin recep	IRS4	>sp|O14654|	1	6	6	6	6	6	6	6.6	6.6	6.6	133.77	
33	O14681	O14681	2	2	2	Etoposide-ir	EI24	>sp|O14681|	1	2	2	2	2	2	2	5.9	5.9	5.9	38.964	
34	O14734	O14734	1	1	1	Acyl-coenzy	ACOT8	>sp|O14734|	1	1	1	1	1	1	1	2.8	2.8	2.8	35.914	
35	O14735	O14735	2	2	2	CDP-diacylgl	CDIPT	>sp|O14735|	1	2	2	2	2	2	2	11.3	11.3	11.3	23.539	
36	O14880	O14880	2	2	2	Microsomal	MGST3	>sp|O14880|	1	2	2	2	2	2	2	17.8	17.8	17.8	16.516	
37	O14910;Q9NUP9;Q9HAP6	O14910;Q9N	2;1;1	2;1;1	2;1;1	Protein lin-7	LIN7A;LIN7C;	>sp|O14910|	3	2	2	2	2	2	2	9	9	9	25.996	
38	O14925;Q5SRD1	O14925;Q5S	7;5	7;5	7;5	Mitochondri	TIMM23;TIM	>sp|O14925|	2	7	7	7	7	7	7	61.7	61.7	61.7	21.943	
39	O14966;P57729;Q13637	O14966	4;1;1	4;1;1	4;1;1	Ras-related	RAB7L1	>sp|O14966|	3	4	4	4	4	4	4	23.2	23.2	23.2	23.155	
40	O14967	O14967	1	1	1	Calmegin	CLGN	>sp|O14967|	1	1	1	1	1	1	1	1.8	1.8	1.8	70.038	
41	O14980	O14980	1	1	1	Exportin-1	XPO1	>sp|O14980|	1	1	1	1	1	1	1	1.1	1.1	1.1	123.38	
42	O15120	O15120	2	2	2	1-acyl-sn-gly	AGPAT2	>sp|O15120|	1	2	2	2	2	2	2	9.4	9.4	9.4	30.914	
43	O15258	O15258	1	1	1	Protein RER1	RER1	>sp|O15258|	1	1	1	1	1	1	1	5.6	5.6	5.6	22.958	
44	O15260	O15260	4	4	4	Surfeit locus	SURF4	>sp|O15260|	1	4	4	4	4	4	4	18.6	18.6	18.6	30.394	

minimum 5 peptides / minimum 2 peptides (2) / **ProteinGroups** / parameters

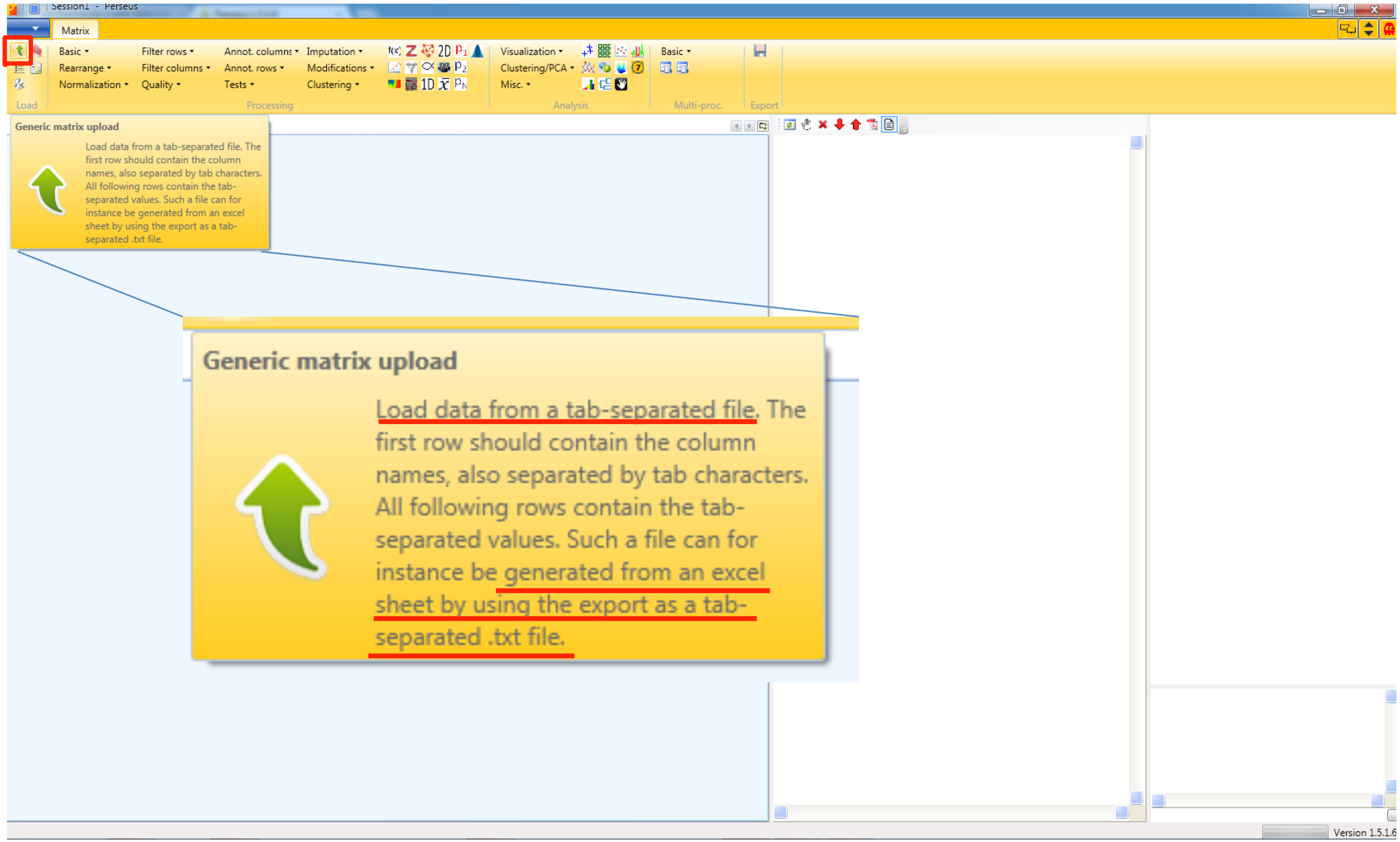
Microsoft Excel

Der ausgewählte Dateityp unterstützt keine Arbeitsmappen, die mehrere Blätter enthalten.

- Klicken Sie auf 'OK', wenn nur das aktuelle Blatt gespeichert werden soll.
- Wenn alle Blätter in dieser Arbeitsmappe in dem ausgewählten Dateityp gespeichert werden sollen, wählen Sie jedes Blatt aus, und speichern Sie es als eigene Datei unter unterschiedlichen Namen, oder wählen Sie einen Dateityp, der mehrere Blätter unterstützt.

Back to Perseus - Data upload

Click here!

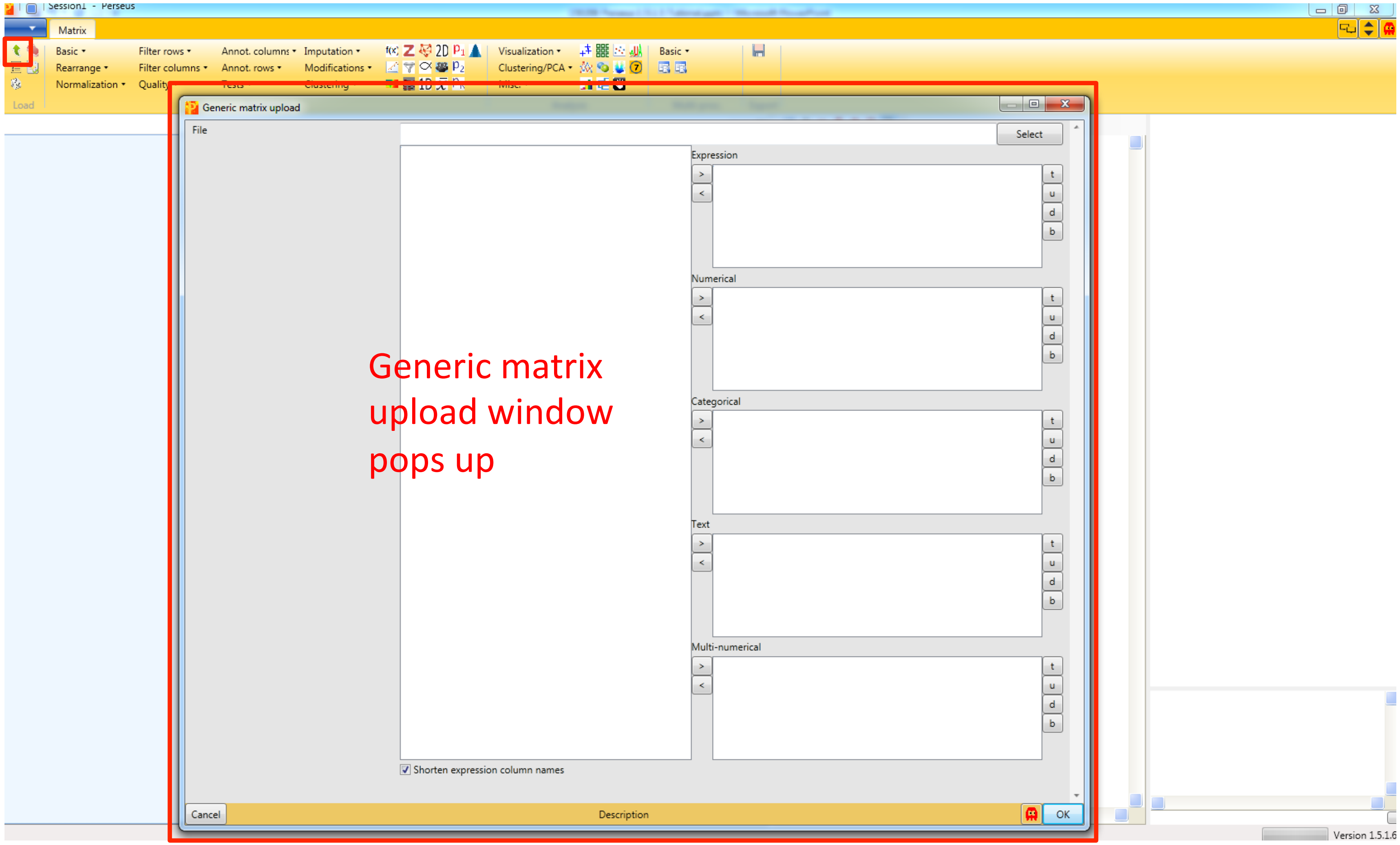


Generic matrix upload

Load data from a tab-separated file. The first row should contain the column names, also separated by tab characters. All following rows contain the tab-separated values. Such a file can for instance be generated from an excel sheet by using the export as a tab-separated .txt file.

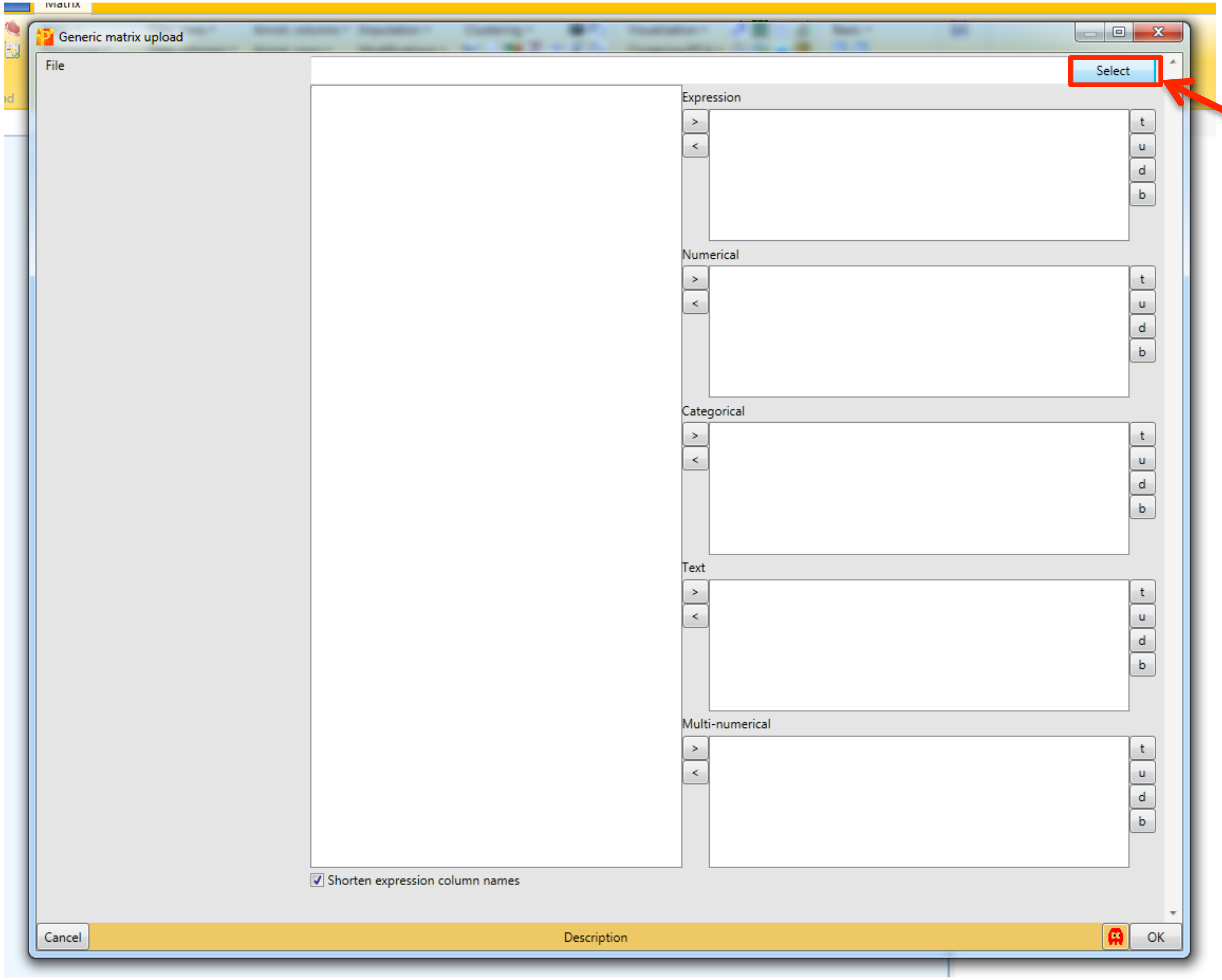
Data upload

Click here!



Generic matrix upload window pops up

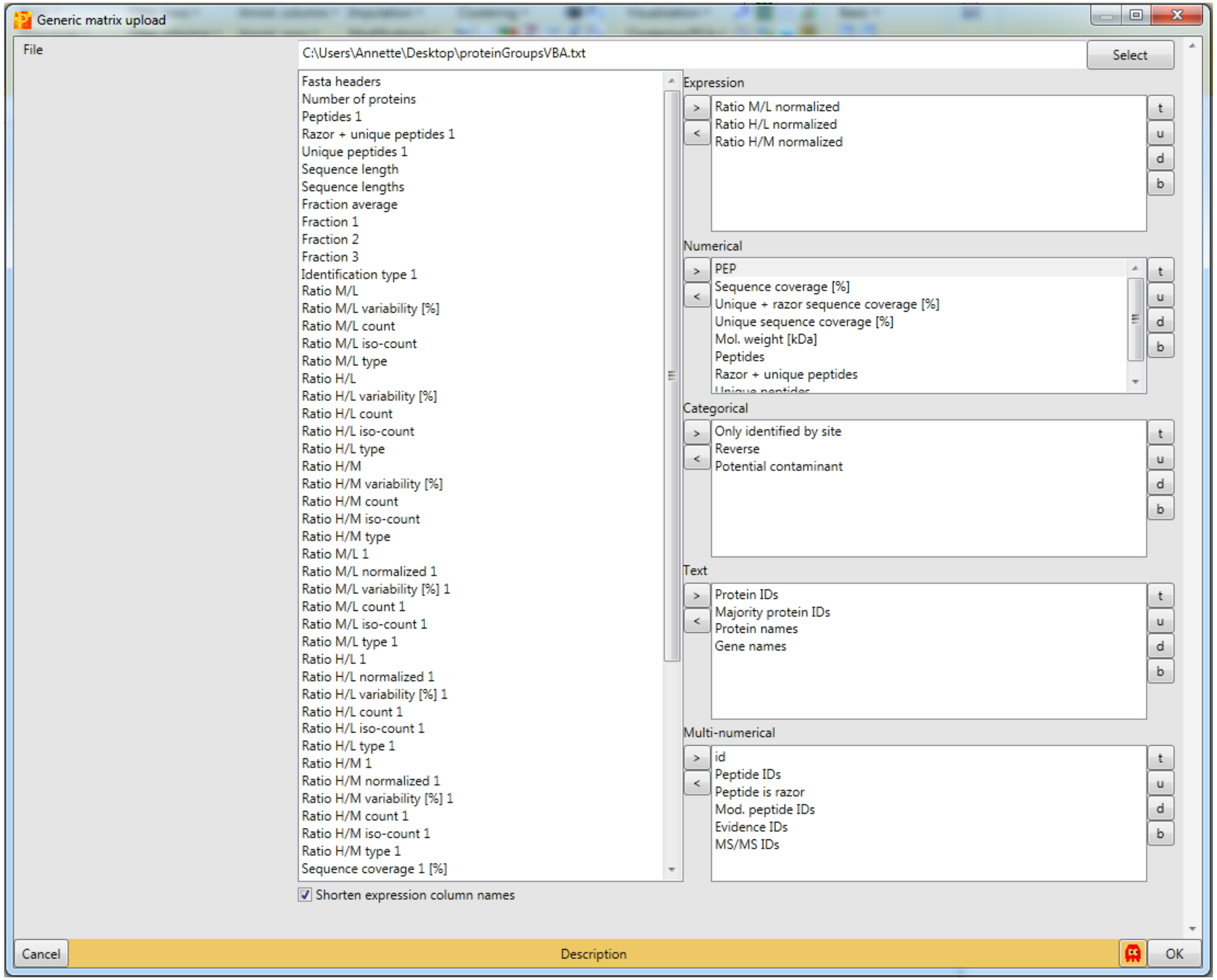
Data upload



☑ Shorten expression column names

Click here and select txt-file!

Data upload



File: C:\Users\Annette\Desktop\proteinGroupsVBA.txt

Fasta headers
Number of proteins
Peptides 1
Razor + unique peptides 1
Unique peptides 1
Sequence length
Sequence lengths
Fraction average
Fraction 1
Fraction 2
Fraction 3
Identification type 1
Ratio M/L
Ratio M/L variability [%]
Ratio M/L count
Ratio M/L iso-count
Ratio M/L type
Ratio H/L
Ratio H/L variability [%]
Ratio H/L count
Ratio H/L iso-count
Ratio H/L type
Ratio H/M
Ratio H/M variability [%]
Ratio H/M count
Ratio H/M iso-count
Ratio H/M type
Ratio M/L 1
Ratio M/L normalized 1
Ratio M/L variability [%] 1
Ratio M/L count 1
Ratio M/L iso-count 1
Ratio M/L type 1
Ratio H/L 1
Ratio H/L normalized 1
Ratio H/L variability [%] 1
Ratio H/L count 1
Ratio H/L iso-count 1
Ratio H/L type 1
Ratio H/M 1
Ratio H/M normalized 1
Ratio H/M variability [%] 1
Ratio H/M count 1
Ratio H/M iso-count 1
Ratio H/M type 1
Sequence coverage 1 [%]

☑ Shorten expression column names

Expression
Ratio M/L normalized
Ratio H/L normalized
Ratio H/M normalized

Numerical
PEP
Sequence coverage [%]
Unique + razor sequence coverage [%]
Unique sequence coverage [%]
Mol. weight [kDa]
Peptides
Razor + unique peptides

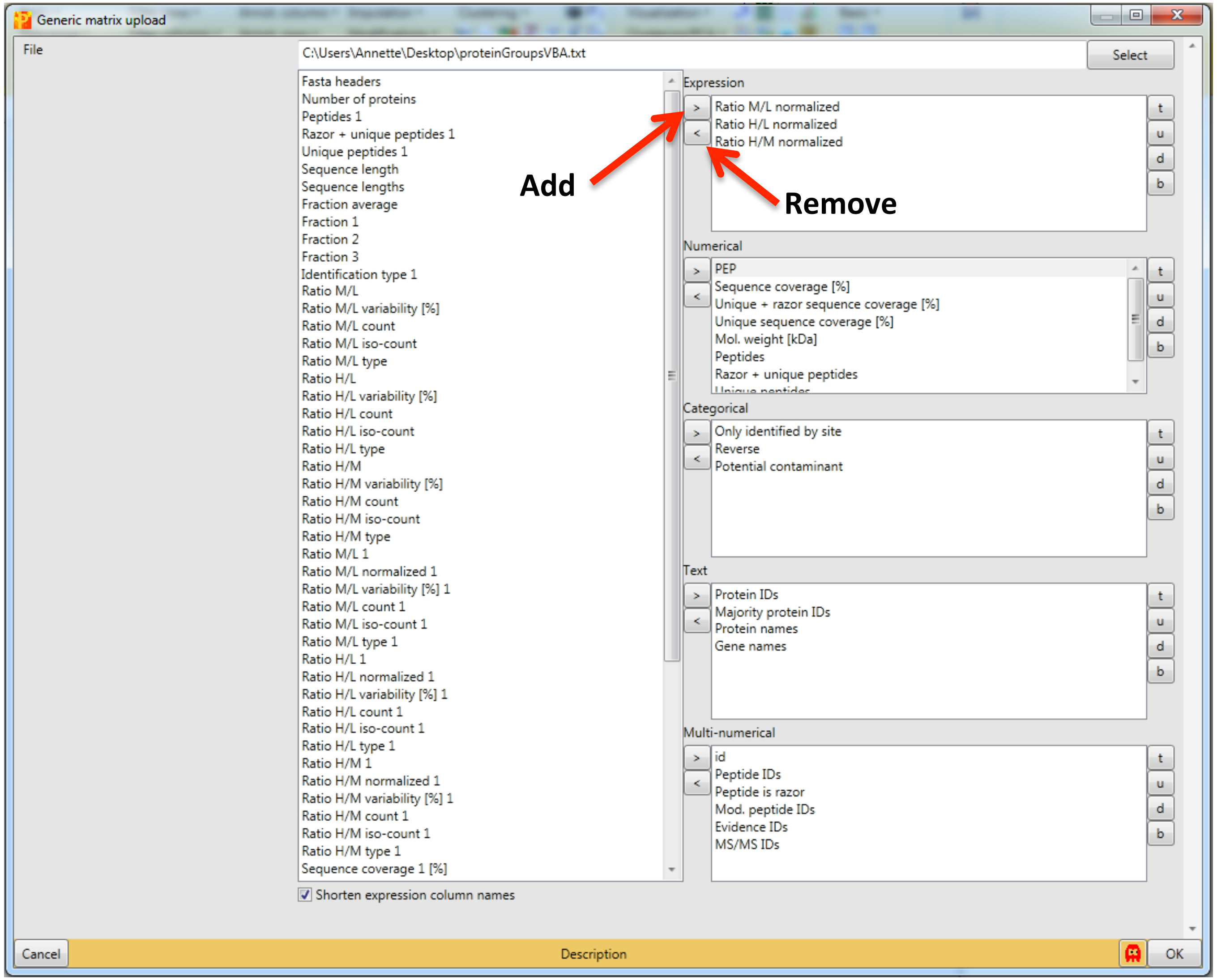
Categorical
Only identified by site
Reverse
Potential contaminant

Text
Protein IDs
Majority protein IDs
Protein names
Gene names

Multi-numerical
id
Peptide IDs
Peptide is razor
Mod. peptide IDs
Evidence IDs
MS/MS IDs

Now you have to define which data columns should be imported into Perseus. You must also define which type of data each column contains.

Data upload



C:\Users\Annette\Desktop\proteinGroupsVBA.txt

Fasta headers
Number of proteins
Peptides 1
Razor + unique peptides 1
Unique peptides 1
Sequence length
Sequence lengths
Fraction average
Fraction 1
Fraction 2
Fraction 3
Identification type 1
Ratio M/L
Ratio M/L variability [%]
Ratio M/L count
Ratio M/L iso-count
Ratio M/L type
Ratio H/L
Ratio H/L variability [%]
Ratio H/L count
Ratio H/L iso-count
Ratio H/L type
Ratio H/M
Ratio H/M variability [%]
Ratio H/M count
Ratio H/M iso-count
Ratio H/M type
Ratio M/L 1
Ratio M/L normalized 1
Ratio M/L variability [%] 1
Ratio M/L count 1
Ratio M/L iso-count 1
Ratio M/L type 1
Ratio H/L 1
Ratio H/L normalized 1
Ratio H/L variability [%] 1
Ratio H/L count 1
Ratio H/L iso-count 1
Ratio H/L type 1
Ratio H/M 1
Ratio H/M normalized 1
Ratio H/M variability [%] 1
Ratio H/M count 1
Ratio H/M iso-count 1
Ratio H/M type 1
Sequence coverage 1 [%]

Expression
Ratio M/L normalized
Ratio H/L normalized
Ratio H/M normalized

Numerical
PEP
Sequence coverage [%]
Unique + razor sequence coverage [%]
Unique sequence coverage [%]
Mol. weight [kDa]
Peptides
Razor + unique peptides
Unique peptides

Categorical
Only identified by site
Reverse
Potential contaminant

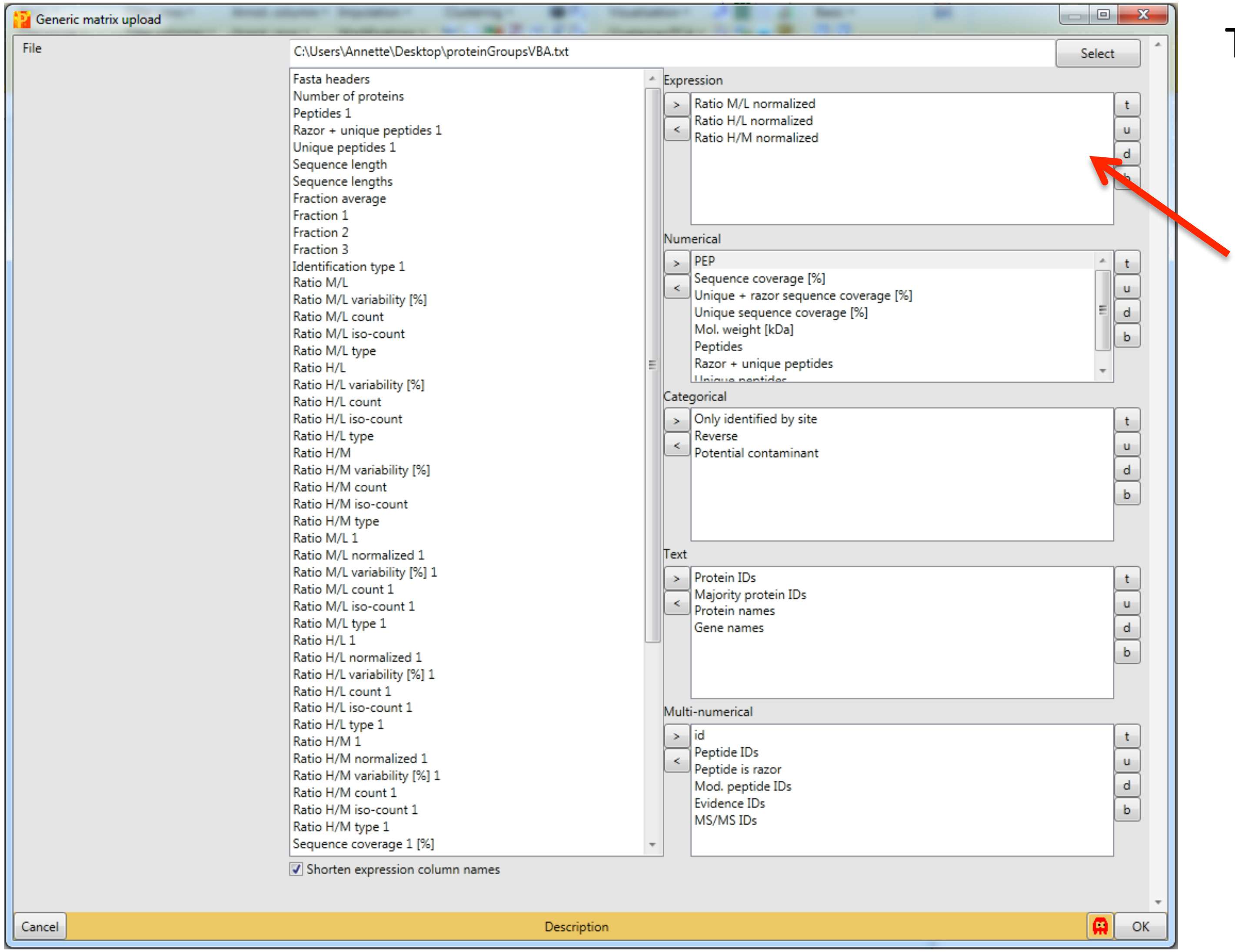
Text
Protein IDs
Majority protein IDs
Protein names
Gene names

Multi-numerical
id
Peptide IDs
Peptide is razor
Mod. peptide IDs
Evidence IDs
MS/MS IDs

☑ Shorten expression column names

Data is added or removed by clicking on the respective buttons.

Data upload



C:\Users\Annette\Desktop\proteinGroupsVBA.txt

Fasta headers
Number of proteins
Peptides 1
Razor + unique peptides 1
Unique peptides 1
Sequence length
Sequence lengths
Fraction average
Fraction 1
Fraction 2
Fraction 3
Identification type 1
Ratio M/L
Ratio M/L variability [%]
Ratio M/L count
Ratio M/L iso-count
Ratio M/L type
Ratio H/L
Ratio H/L variability [%]
Ratio H/L count
Ratio H/L iso-count
Ratio H/L type
Ratio H/M
Ratio H/M variability [%]
Ratio H/M count
Ratio H/M iso-count
Ratio H/M type
Ratio M/L 1
Ratio M/L normalized 1
Ratio M/L variability [%] 1
Ratio M/L count 1
Ratio M/L iso-count 1
Ratio M/L type 1
Ratio H/L 1
Ratio H/L normalized 1
Ratio H/L variability [%] 1
Ratio H/L count 1
Ratio H/L iso-count 1
Ratio H/L type 1
Ratio H/M 1
Ratio H/M normalized 1
Ratio H/M variability [%] 1
Ratio H/M count 1
Ratio H/M iso-count 1
Ratio H/M type 1
Sequence coverage 1 [%]

Expression

Ratio M/L normalized
Ratio H/L normalized
Ratio H/M normalized

Numerical

PEP
Sequence coverage [%]
Unique + razor sequence coverage [%]
Unique sequence coverage [%]
Mol. weight [kDa]
Peptides
Razor + unique peptides

Categorical

Only identified by site
Reverse
Potential contaminant

Text

Protein IDs
Majority protein IDs
Protein names
Gene names

Multi-numerical

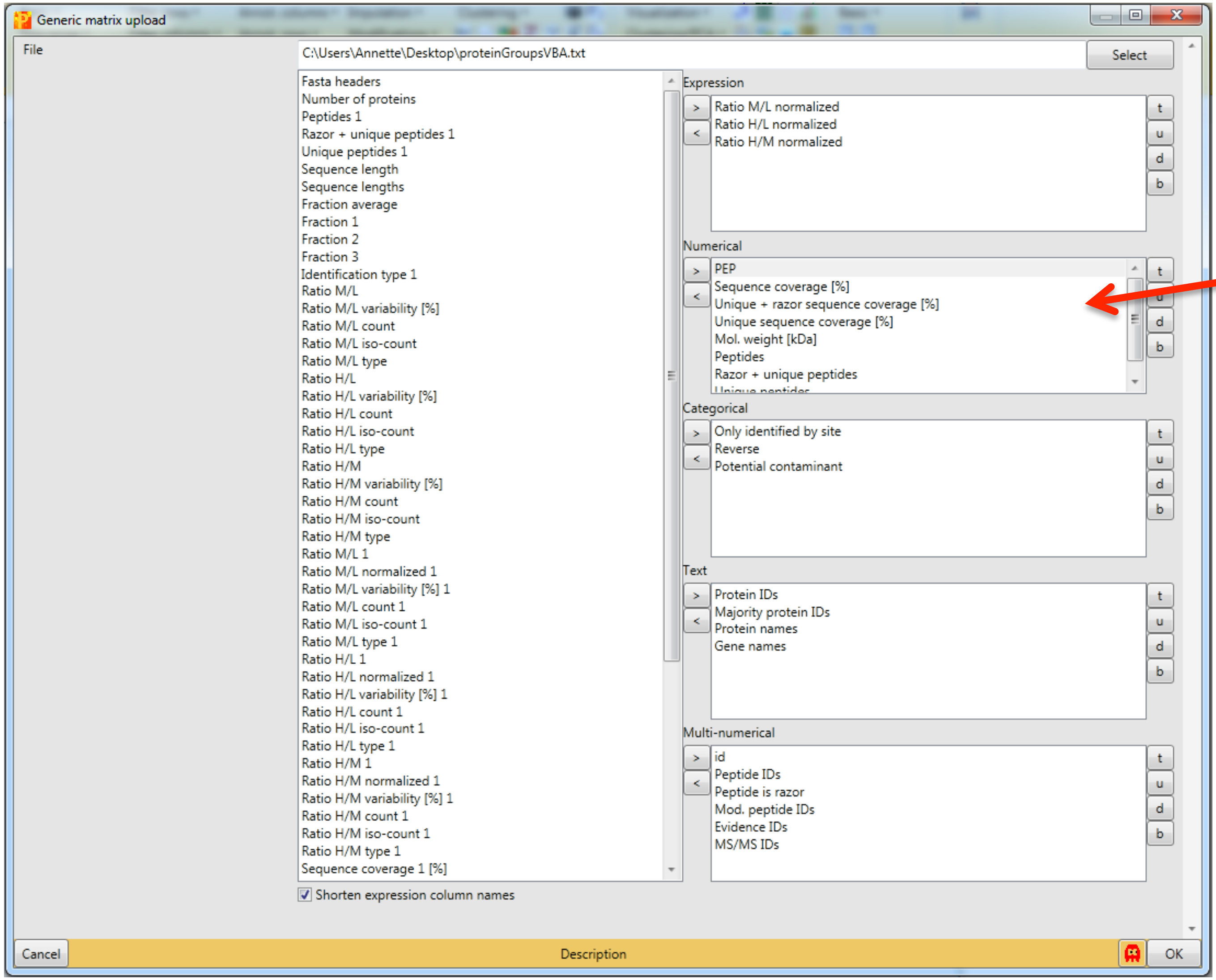
id
Peptide IDs
Peptide is razor
Mod. peptide IDs
Evidence IDs
MS/MS IDs

☑ Shorten expression column names

There are five data types:

1. Expression data = H/L-ratios, Label-free quantification values, Isobaric-label intensities etc.

Data upload



File: C:\Users\Annette\Desktop\proteinGroupsVBA.txt

- Fasta headers
- Number of proteins
- Peptides 1
- Razor + unique peptides 1
- Unique peptides 1
- Sequence length
- Sequence lengths
- Fraction average
- Fraction 1
- Fraction 2
- Fraction 3
- Identification type 1
- Ratio M/L
- Ratio M/L variability [%]
- Ratio M/L count
- Ratio M/L iso-count
- Ratio M/L type
- Ratio H/L
- Ratio H/L variability [%]
- Ratio H/L count
- Ratio H/L iso-count
- Ratio H/L type
- Ratio H/M
- Ratio H/M variability [%]
- Ratio H/M count
- Ratio H/M iso-count
- Ratio H/M type
- Ratio M/L 1
- Ratio M/L normalized 1
- Ratio M/L variability [%] 1
- Ratio M/L count 1
- Ratio M/L iso-count 1
- Ratio M/L type 1
- Ratio H/L 1
- Ratio H/L normalized 1
- Ratio H/L variability [%] 1
- Ratio H/L count 1
- Ratio H/L iso-count 1
- Ratio H/L type 1
- Ratio H/M 1
- Ratio H/M normalized 1
- Ratio H/M variability [%] 1
- Ratio H/M count 1
- Ratio H/M iso-count 1
- Ratio H/M type 1
- Sequence coverage 1 [%]

☑ Shorten expression column names

Expression
- Ratio M/L normalized
- Ratio H/L normalized
- Ratio H/M normalized

Numerical
- PEP
- Sequence coverage [%]
- Unique + razor sequence coverage [%]
- Unique sequence coverage [%]
- Mol. weight [kDa]
- Peptides
- Razor + unique peptides

Categorical
- Only identified by site
- Reverse
- Potential contaminant

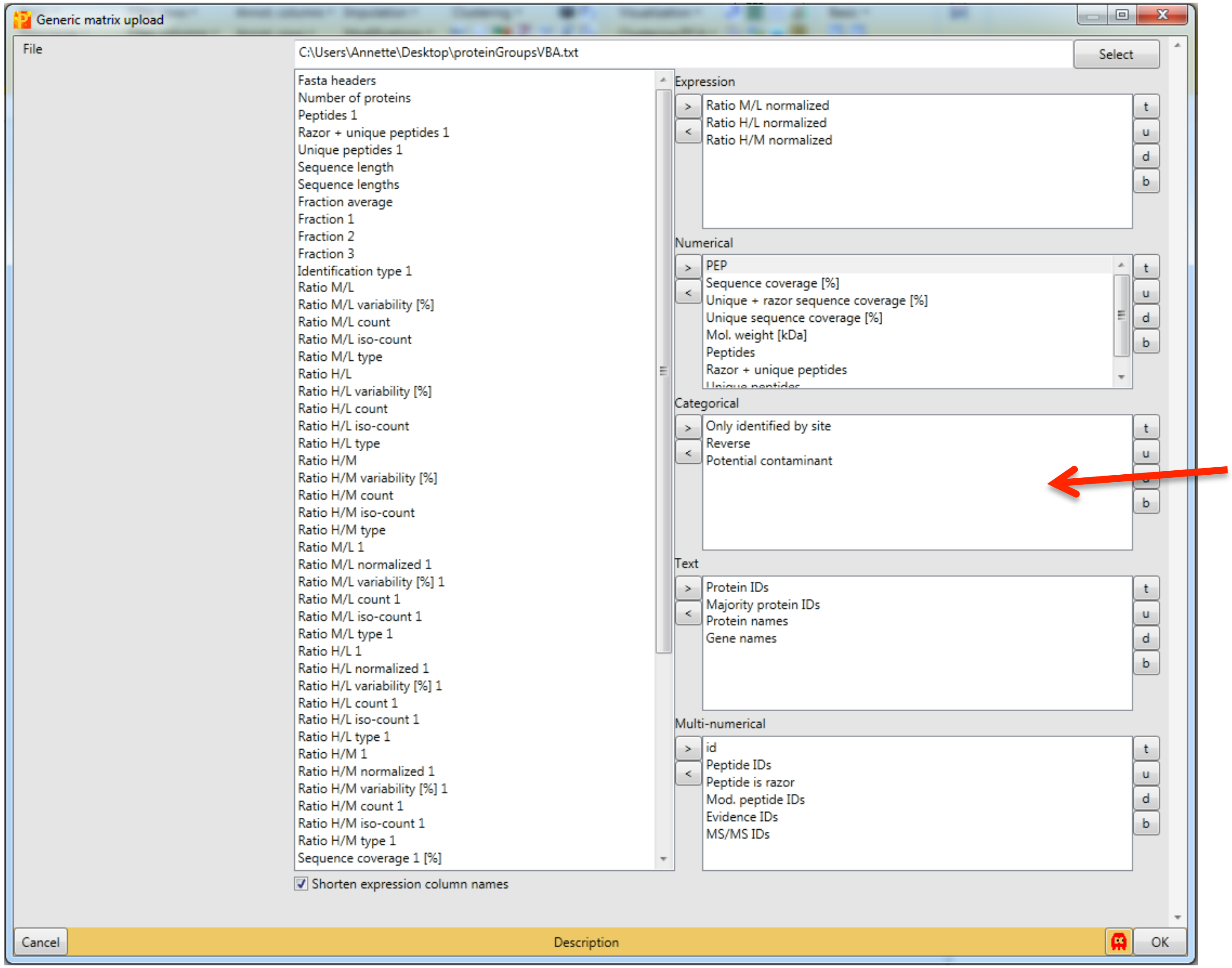
Text
- Protein IDs
- Majority protein IDs
- Protein names
- Gene names

Multi-numerical
- id
- Peptide IDs
- Peptide is razor
- Mod. peptide IDs
- Evidence IDs
- MS/MS IDs

There are five data types:

2. Numerical data = Every column that contains single number values, like: Peptides, Mol. weight, Sequence coverage (%)

Data upload



C:\Users\Annette\Desktop\proteinGroupsVBA.txt

Fasta headers
Number of proteins
Peptides 1
Razor + unique peptides 1
Unique peptides 1
Sequence length
Sequence lengths
Fraction average
Fraction 1
Fraction 2
Fraction 3
Identification type 1
Ratio M/L
Ratio M/L variability [%]
Ratio M/L count
Ratio M/L iso-count
Ratio M/L type
Ratio H/L
Ratio H/L variability [%]
Ratio H/L count
Ratio H/L iso-count
Ratio H/L type
Ratio H/M
Ratio H/M variability [%]
Ratio H/M count
Ratio H/M iso-count
Ratio H/M type
Ratio M/L 1
Ratio M/L normalized 1
Ratio M/L variability [%] 1
Ratio M/L count 1
Ratio M/L iso-count 1
Ratio M/L type 1
Ratio H/L 1
Ratio H/L normalized 1
Ratio H/L variability [%] 1
Ratio H/L count 1
Ratio H/L iso-count 1
Ratio H/L type 1
Ratio H/M 1
Ratio H/M normalized 1
Ratio H/M variability [%] 1
Ratio H/M count 1
Ratio H/M iso-count 1
Ratio H/M type 1
Sequence coverage 1 [%]

Expression
Ratio M/L normalized
Ratio H/L normalized
Ratio H/M normalized

Numerical
PEP
Sequence coverage [%]
Unique + razor sequence coverage [%]
Unique sequence coverage [%]
Mol. weight [kDa]
Peptides
Razor + unique peptides

Categorical
Only identified by site
Reverse
Potential contaminant

Text
Protein IDs
Majority protein IDs
Protein names
Gene names

Multi-numerical
id
Peptide IDs
Peptide is razor
Mod. peptide IDs
Evidence IDs
MS/MS IDs

☑ Shorten expression column names

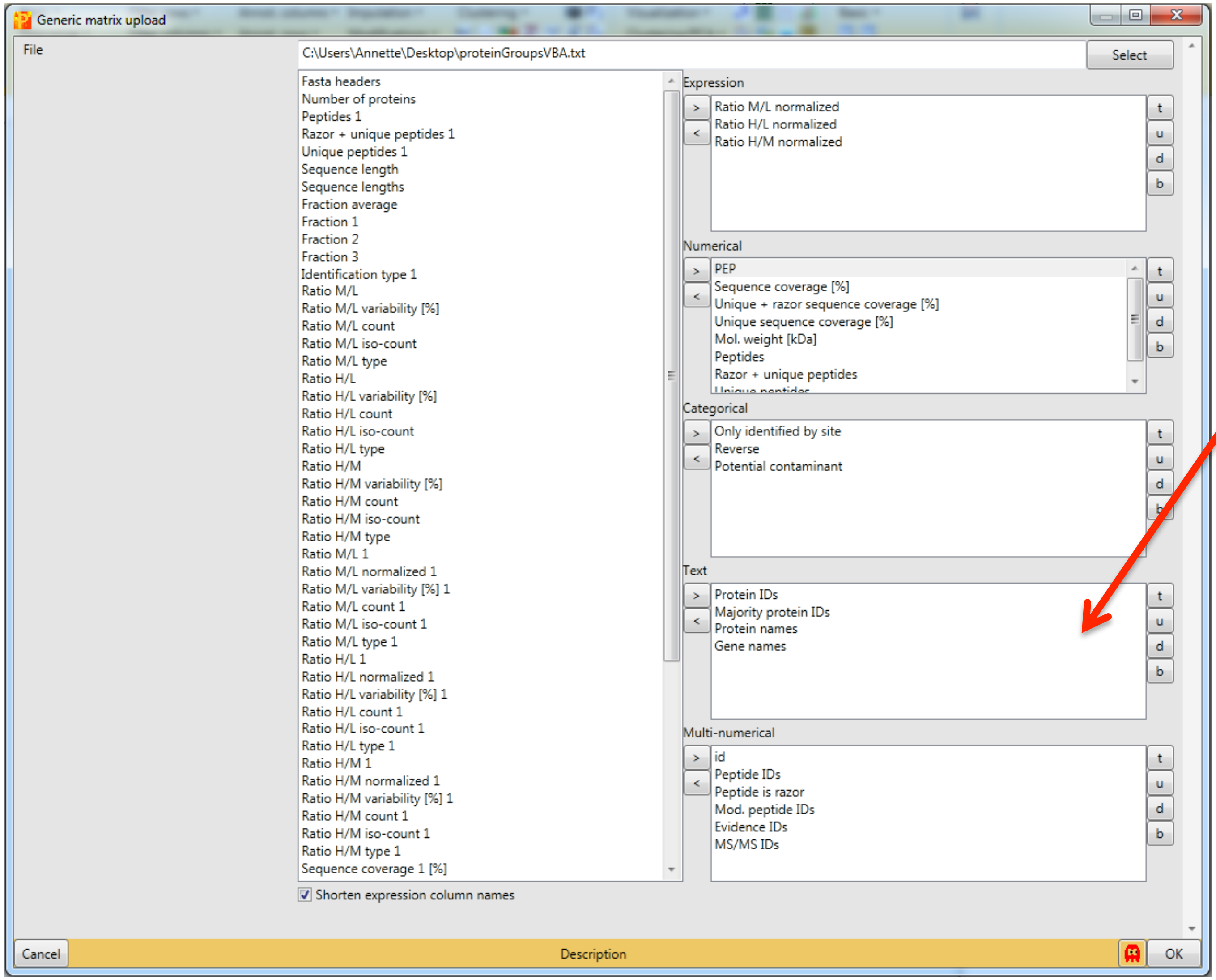
There are five data types:

3. Categorical data

= This is a yes or no data. Every protein group that belongs to a certain category is marked with a plus (+). MaxQuant automatically assigns some of the identified protein groups to three categories:

1. Only identified by site
2. Potential contaminant
3. Reverse

Data upload



C:\Users\Annette\Desktop\proteinGroupsVBA.txt

Fasta headers
Number of proteins
Peptides 1
Razor + unique peptides 1
Unique peptides 1
Sequence length
Sequence lengths
Fraction average
Fraction 1
Fraction 2
Fraction 3
Identification type 1
Ratio M/L
Ratio M/L variability [%]
Ratio M/L count
Ratio M/L iso-count
Ratio M/L type
Ratio H/L
Ratio H/L variability [%]
Ratio H/L count
Ratio H/L iso-count
Ratio H/L type
Ratio H/M
Ratio H/M variability [%]
Ratio H/M count
Ratio H/M iso-count
Ratio H/M type
Ratio M/L 1
Ratio M/L normalized 1
Ratio M/L variability [%] 1
Ratio M/L count 1
Ratio M/L iso-count 1
Ratio M/L type 1
Ratio H/L 1
Ratio H/L normalized 1
Ratio H/L variability [%] 1
Ratio H/L count 1
Ratio H/L iso-count 1
Ratio H/L type 1
Ratio H/M 1
Ratio H/M normalized 1
Ratio H/M variability [%] 1
Ratio H/M count 1
Ratio H/M iso-count 1
Ratio H/M type 1
Sequence coverage 1 [%]

Expression:
Ratio M/L normalized
Ratio H/L normalized
Ratio H/M normalized

Numerical:
PEP
Sequence coverage [%]
Unique + razor sequence coverage [%]
Unique sequence coverage [%]
Mol. weight [kDa]
Peptides
Razor + unique peptides
Unique peptides

Categorical:
Only identified by site
Reverse
Potential contaminant

Text:
Protein IDs
Majority protein IDs
Protein names
Gene names

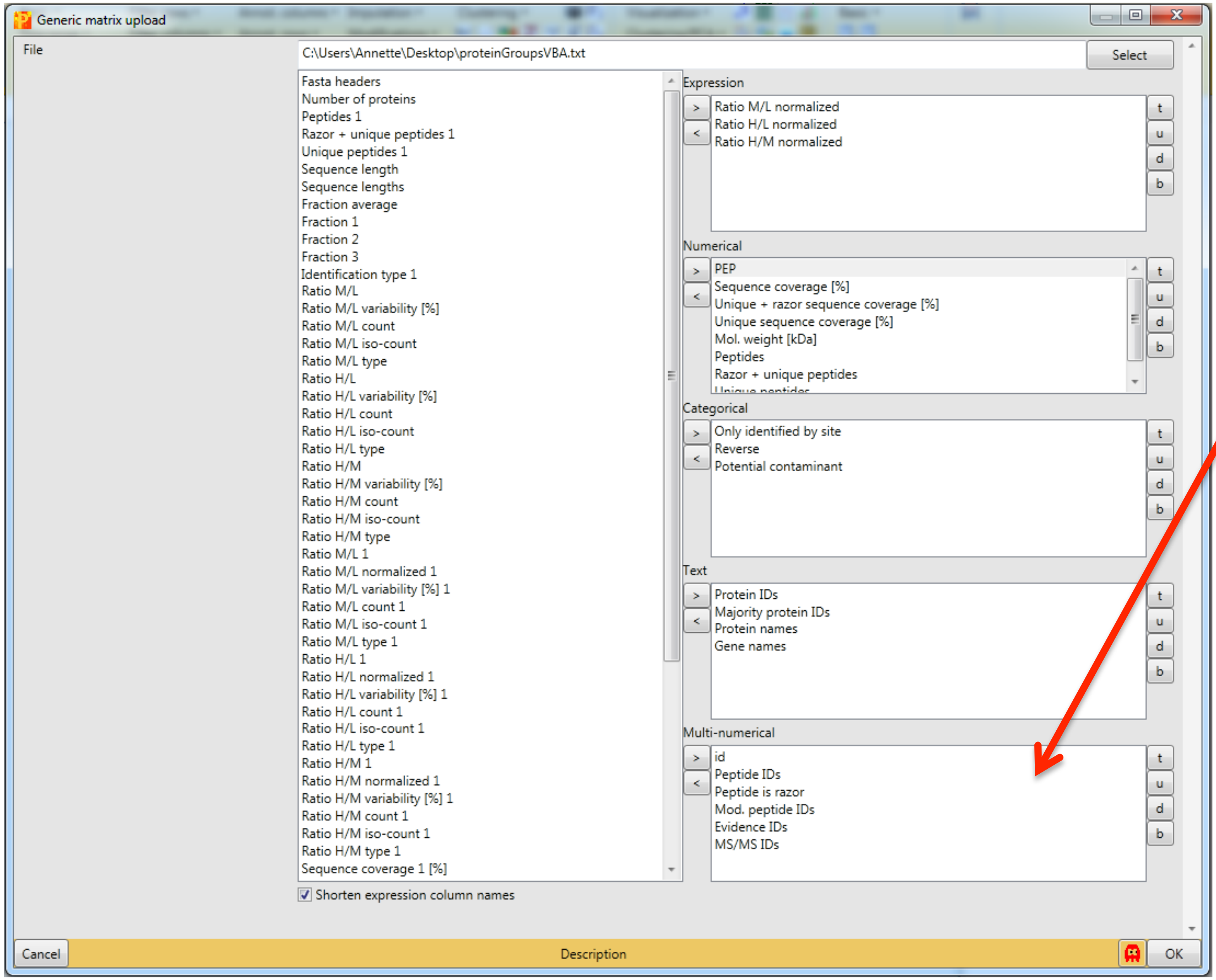
Multi-numerical:
id
Peptide IDs
Peptide is razor
Mod. peptide IDs
Evidence IDs
MS/MS IDs

☑ Shorten expression column names

There are five data types:

4. Text data = Everything that is not a number e.g. Protein IDs (Accession), Protein names, Gene names etc.

Data upload



File: C:\Users\Annette\Desktop\proteinGroupsVBA.txt

Fasta headers
Number of proteins
Peptides 1
Razor + unique peptides 1
Unique peptides 1
Sequence length
Sequence lengths
Fraction average
Fraction 1
Fraction 2
Fraction 3
Identification type 1
Ratio M/L
Ratio M/L variability [%]
Ratio M/L count
Ratio M/L iso-count
Ratio M/L type
Ratio H/L
Ratio H/L variability [%]
Ratio H/L count
Ratio H/L iso-count
Ratio H/L type
Ratio H/M
Ratio H/M variability [%]
Ratio H/M count
Ratio H/M iso-count
Ratio H/M type
Ratio M/L 1
Ratio M/L normalized 1
Ratio M/L variability [%] 1
Ratio M/L count 1
Ratio M/L iso-count 1
Ratio M/L type 1
Ratio H/L 1
Ratio H/L normalized 1
Ratio H/L variability [%] 1
Ratio H/L count 1
Ratio H/L iso-count 1
Ratio H/L type 1
Ratio H/M 1
Ratio H/M normalized 1
Ratio H/M variability [%] 1
Ratio H/M count 1
Ratio H/M iso-count 1
Ratio H/M type 1
Sequence coverage 1 [%]

☑ Shorten expression column names

Expression
Ratio M/L normalized
Ratio H/L normalized
Ratio H/M normalized

Numerical
PEP
Sequence coverage [%]
Unique + razor sequence coverage [%]
Unique sequence coverage [%]
Mol. weight [kDa]
Peptides
Razor + unique peptides
Unique peptides

Categorical
Only identified by site
Reverse
Potential contaminant

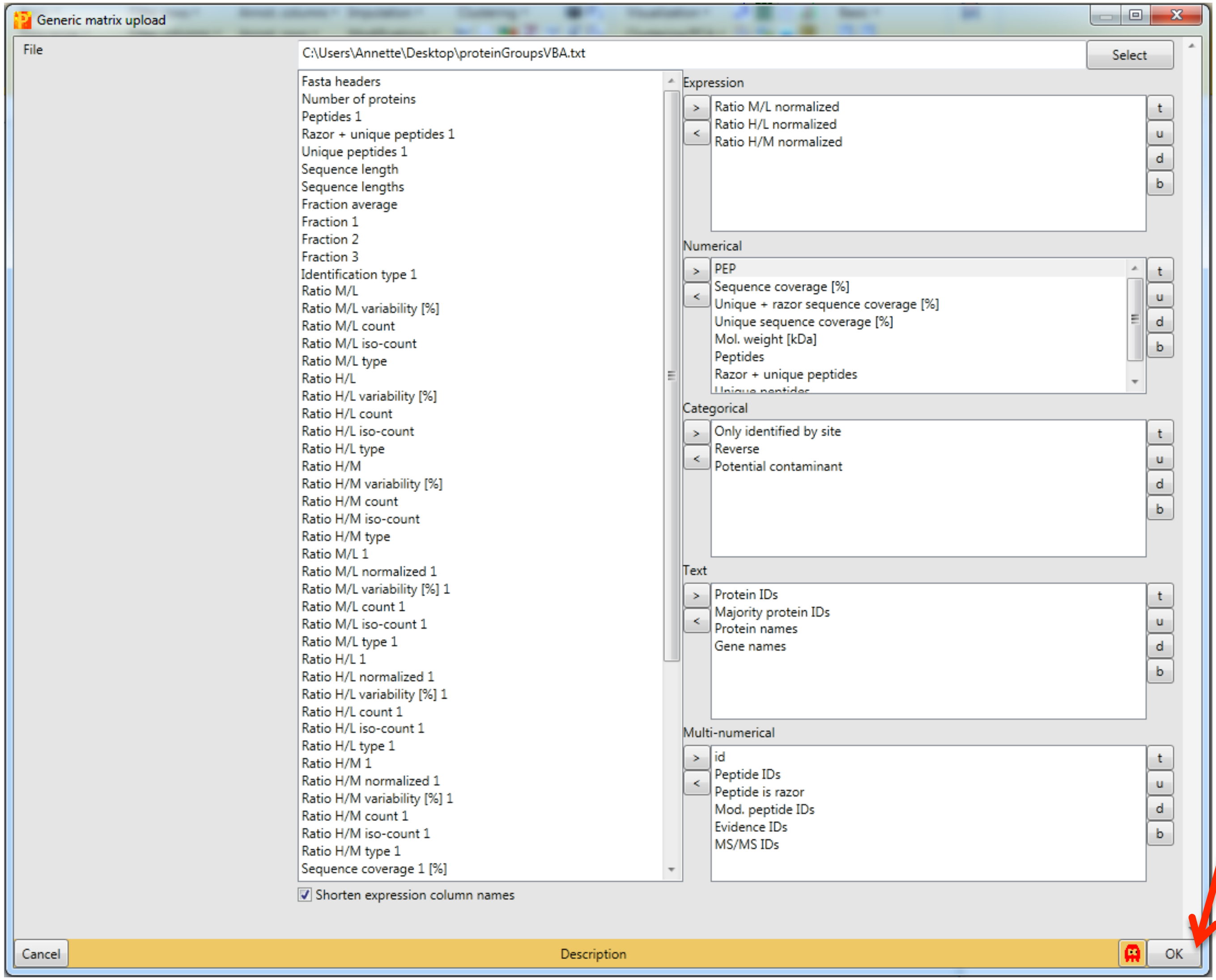
Text
Protein IDs
Majority protein IDs
Protein names
Gene names

Multi-numerical
id
Peptide IDs
Peptide is razor
Mod. peptide IDs
Evidence IDs
MS/MS IDs

There are five data types:

5. Multi-numerical data = Every column that contains more than one single number. Usually these numbers are separated by a semicolon. Examples are the different ID-Columns with link to other files generated by MaxQuant (MS/MS-lists etc.)

Data upload



File C:\Users\Annette\Desktop\proteinGroupsVBA.txt

Fasta headers
Number of proteins
Peptides 1
Razor + unique peptides 1
Unique peptides 1
Sequence length
Sequence lengths
Fraction average
Fraction 1
Fraction 2
Fraction 3
Identification type 1
Ratio M/L
Ratio M/L variability [%]
Ratio M/L count
Ratio M/L iso-count
Ratio M/L type
Ratio H/L
Ratio H/L variability [%]
Ratio H/L count
Ratio H/L iso-count
Ratio H/L type
Ratio H/M
Ratio H/M variability [%]
Ratio H/M count
Ratio H/M iso-count
Ratio H/M type
Ratio M/L 1
Ratio M/L normalized 1
Ratio M/L variability [%] 1
Ratio M/L count 1
Ratio M/L iso-count 1
Ratio M/L type 1
Ratio H/L 1
Ratio H/L normalized 1
Ratio H/L variability [%] 1
Ratio H/L count 1
Ratio H/L iso-count 1
Ratio H/L type 1
Ratio H/M 1
Ratio H/M normalized 1
Ratio H/M variability [%] 1
Ratio H/M count 1
Ratio H/M iso-count 1
Ratio H/M type 1
Sequence coverage 1 [%]

Expression
Ratio M/L normalized
Ratio H/L normalized
Ratio H/M normalized

Numerical
PEP
Sequence coverage [%]
Unique + razor sequence coverage [%]
Unique sequence coverage [%]
Mol. weight [kDa]
Peptides
Razor + unique peptides

Categorical
Only identified by site
Reverse
Potential contaminant

Text
Protein IDs
Majority protein IDs
Protein names
Gene names

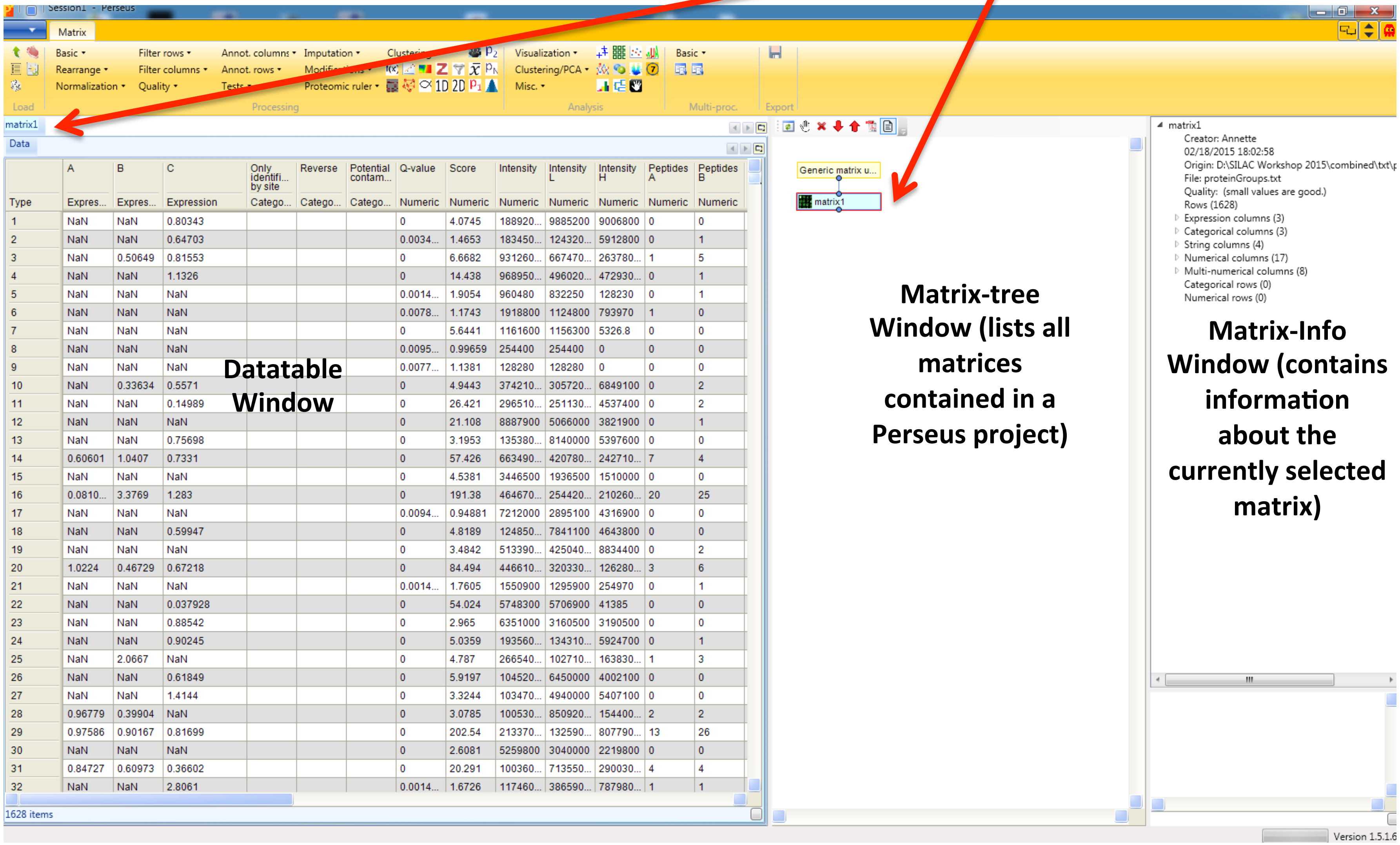
Multi-numerical
id
Peptide IDs
Peptide is razor
Mod. peptide IDs
Evidence IDs
MS/MS IDs

☑ Shorten expression column names

After data selection click OK!

First steps

Data is imported into Perseus in a so-called **matrix**.



	A	B	C	Only identifi... by site	Reverse	Potential contam...	Q-value	Score	Intensity	Intensity L	Intensity H	Peptides A	Peptides B
Type	Expres...	Expres...	Expression	Catego...	Catego...	Catego...	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric
1	NaN	NaN	0.80343				0	4.0745	188920...	9885200	9006800	0	0
2	NaN	NaN	0.64703				0.0034...	1.4653	183450...	124320...	5912800	0	1
3	NaN	0.50649	0.81553				0	6.6682	931260...	667470...	263780...	1	5
4	NaN	NaN	1.1326				0	14.438	968950...	496020...	472930...	0	1
5	NaN	NaN	NaN				0.0014...	1.9054	960480	832250	128230	0	1
6	NaN	NaN	NaN				0.0078...	1.1743	1918800	1124800	793970	1	0
7	NaN	NaN	NaN				0	5.6441	1161600	1156300	5326.8	0	0
8	NaN	NaN	NaN				0.0095...	0.99659	254400	254400	0	0	0
9	NaN	NaN	NaN				0.0077...	1.1381	128280	128280	0	0	0
10	NaN	0.33634	0.5571				0	4.9443	374210...	305720...	6849100	0	2
11	NaN	NaN	0.14989				0	26.421	296510...	251130...	4537400	0	2
12	NaN	NaN	NaN				0	21.108	8887900	5066000	3821900	0	1
13	NaN	NaN	0.75698				0	3.1953	135380...	8140000	5397600	0	0
14	0.60601	1.0407	0.7331				0	57.426	663490...	420780...	242710...	7	4
15	NaN	NaN	NaN				0	4.5381	3446500	1936500	1510000	0	0
16	0.0810...	3.3769	1.283				0	191.38	464670...	254420...	210260...	20	25
17	NaN	NaN	NaN				0.0094...	0.94881	7212000	2895100	4316900	0	0
18	NaN	NaN	0.59947				0	4.8189	124850...	7841100	4643800	0	0
19	NaN	NaN	NaN				0	3.4842	513390...	425040...	8834400	0	2
20	1.0224	0.46729	0.67218				0	84.494	446610...	320330...	126280...	3	6
21	NaN	NaN	NaN				0.0014...	1.7605	1550900	1295900	254970	0	1
22	NaN	NaN	0.037928				0	54.024	5748300	5706900	41385	0	0
23	NaN	NaN	0.88542				0	2.965	6351000	3160500	3190500	0	0
24	NaN	NaN	0.90245				0	5.0359	193560...	134310...	5924700	0	1
25	NaN	2.0667	NaN				0	4.787	266540...	102710...	163830...	1	3
26	NaN	NaN	0.61849				0	5.9197	104520...	6450000	4002100	0	0
27	NaN	NaN	1.4144				0	3.3244	103470...	4940000	5407100	0	0
28	0.96779	0.39904	NaN				0	3.0785	100530...	850920...	154400...	2	2
29	0.97586	0.90167	0.81699				0	202.54	213370...	132590...	807790...	13	26
30	NaN	NaN	NaN				0	2.6081	5259800	3040000	2219800	0	0
31	0.84727	0.60973	0.36602				0	20.291	100360...	713550...	290030...	4	4
32	NaN	NaN	2.8061				0.0014...	1.6726	117460...	386590...	787980...	1	1

1628 items

matrix1
- Creator: Annette
- 02/18/2015 18:02:58
- Origin: D:\SILAC Workshop 2015\combined\txt\p
- File: proteinGroups.txt
- Quality: (small values are good.)
- Rows (1628)
- Expression columns (3)
- Categorical columns (3)
- String columns (4)
- Numerical columns (17)
- Multi-numerical columns (8)
- Categorical rows (0)
- Numerical rows (0)

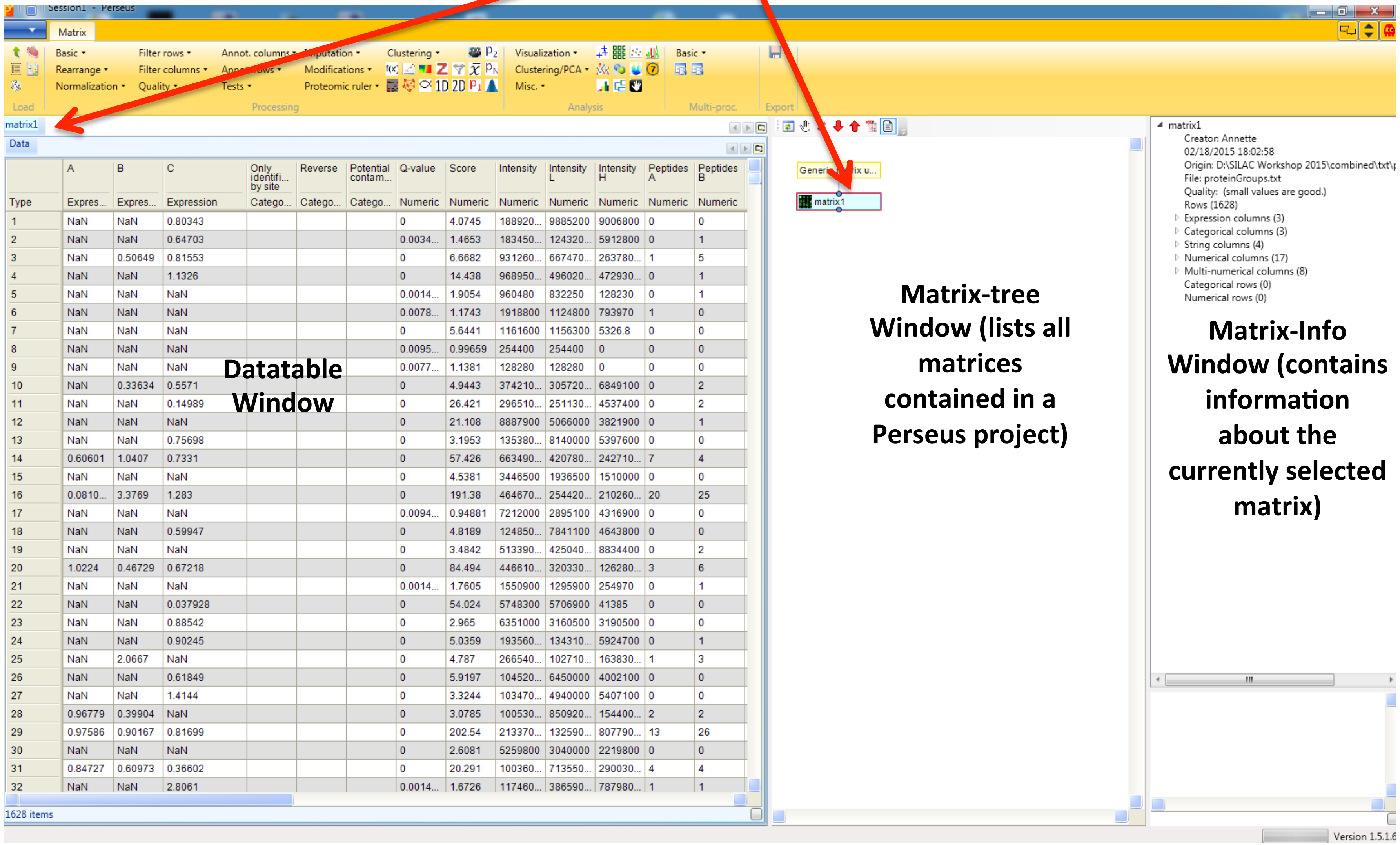
Datatable Window

Matrix-tree Window (lists all matrices contained in a Perseus project)

Matrix-Info Window (contains information about the currently selected matrix)

First steps

Every time you change something a new **matrix** is created. This way you can always go back to a previous processing stage.



	A	B	C	Only identifi... by site	Reverse	Potential contam...	Q-value	Score	Intensity	Intensity L	Intensity H	Peptides A	Peptides B
Type	Expres...	Expres...	Expression	Catego...	Catego...	Catego...	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric
1	NaN	NaN	0.80343				0	4.0745	188920...	9885200	9006800	0	0
2	NaN	NaN	0.64703				0.0034...	1.4653	183450...	124320...	5912800	0	1
3	NaN	0.50649	0.81553				0	6.6682	931260...	667470...	263780...	1	5
4	NaN	NaN	1.1326				0	14.438	968950...	496020...	472930...	0	1
5	NaN	NaN	NaN				0.0014...	1.9054	960480	832250	128230	0	1
6	NaN	NaN	NaN				0.0078...	1.1743	1918800	1124800	793970	1	0
7	NaN	NaN	NaN				0	5.6441	1161600	1156300	5326.8	0	0
8	NaN	NaN	NaN				0.0095...	0.99659	254400	254400	0	0	0
9	NaN	NaN	NaN				0.0077...	1.1381	128280	128280	0	0	0
10	NaN	0.33634	0.5571				0	4.9443	374210...	305720...	6849100	0	2
11	NaN	NaN	0.14989				0	26.421	296510...	251130...	4537400	0	2
12	NaN	NaN	NaN				0	21.108	8887900	5066000	3821900	0	1
13	NaN	NaN	0.75698				0	3.1953	135380...	8140000	5397600	0	0
14	0.60601	1.0407	0.7331				0	57.426	663490...	420780...	242710...	7	4
15	NaN	NaN	NaN				0	4.5381	3446500	1936500	1510000	0	0
16	0.0810...	3.3769	1.283				0	191.38	464670...	254420...	210260...	20	25
17	NaN	NaN	NaN				0.0094...	0.94881	7212000	2895100	4316900	0	0
18	NaN	NaN	0.59947				0	4.8189	124850...	7841100	4643800	0	0
19	NaN	NaN	NaN				0	3.4842	513390...	425040...	8834400	0	2
20	1.0224	0.46729	0.67218				0	84.494	446610...	320330...	126280...	3	6
21	NaN	NaN	NaN				0.0014...	1.7605	1550900	1295900	254970	0	1
22	NaN	NaN	0.037928				0	54.024	5748300	5706900	41385	0	0
23	NaN	NaN	0.88542				0	2.965	6351000	3160500	3190500	0	0
24	NaN	NaN	0.90245				0	5.0359	193560...	134310...	5924700	0	1
25	NaN	2.0667	NaN				0	4.787	266540...	102710...	163830...	1	3
26	NaN	NaN	0.61849				0	5.9197	104520...	6450000	4002100	0	0
27	NaN	NaN	1.4144				0	3.3244	103470...	4940000	5407100	0	0
28	0.96779	0.39904	NaN				0	3.0785	100530...	850920...	154400...	2	2
29	0.97586	0.90167	0.81699				0	202.54	213370...	132590...	807790...	13	26
30	NaN	NaN	NaN				0	2.6081	5259800	3040000	2219800	0	0
31	0.84727	0.60973	0.36602				0	20.291	100360...	713550...	290030...	4	4
32	NaN	NaN	2.8061				0.0014...	1.6726	117460...	386590...	787980...	1	1

1628 items

matrix1
Creator: Annette
02/18/2015 18:02:58
Origin: D:\SILAC Workshop 2015\combined\txt\p
File: proteinGroups.txt
Quality: (small values are good.)
Rows (1628)
Expression columns (3)
Categorical columns (3)
String columns (4)
Numerical columns (17)
Multi-numerical columns (8)
Categorical rows (0)
Numerical rows (0)

Datatable Window

Matrix-tree Window (lists all matrices contained in a Perseus project)

Matrix-Info Window (contains information about the currently selected matrix)

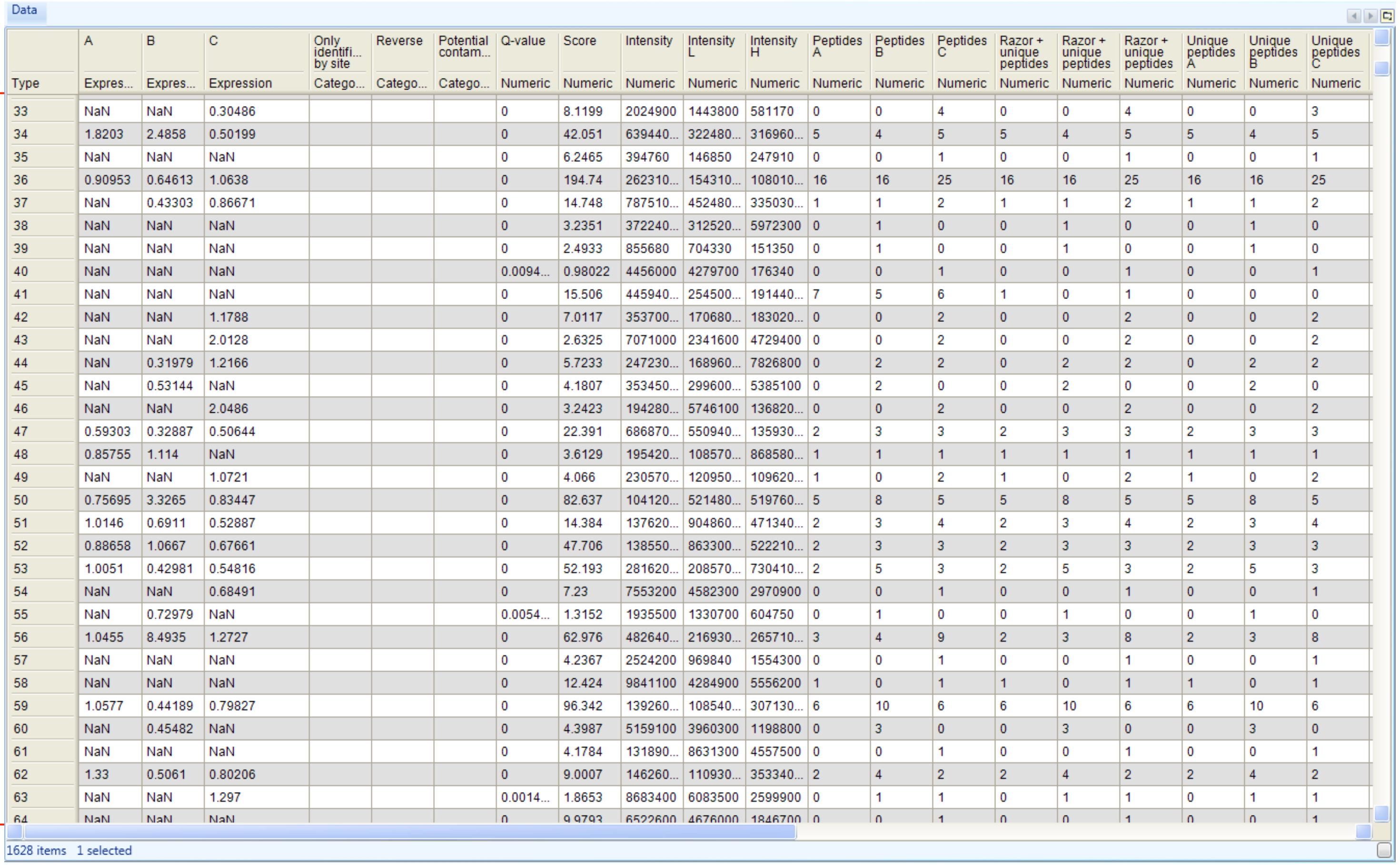
First steps

The Datatable Window

Each column contains one type of data like SILAC ratios, sequence coverage, peptide numbers,…

Data type (as defined during upload)

Each row contains one identified **protein group**



Data

	A	B	C	Only identifi... by site	Reverse	Potential contam...	Q-value	Score	Intensity	Intensity L	Intensity H	Peptides A	Peptides B	Peptides C	Razor + unique peptides	Razor + unique peptides	Razor + unique peptides	Unique peptides A	Unique peptides B	Unique peptides C
Type	Expres...	Expres...	Expression	Catego...	Catego...	Catego...	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric
33	NaN	NaN	0.30486				0	8.1199	2024900	1443800	581170	0	0	4	0	0	4	0	0	3
34	1.8203	2.4858	0.50199				0	42.051	639440...	322480...	316960...	5	4	5	5	4	5	5	4	5
35	NaN	NaN	NaN				0	6.2465	394760	146850	247910	0	0	1	0	0	1	0	0	1
36	0.90953	0.64613	1.0638				0	194.74	262310...	154310...	108010...	16	16	25	16	16	25	16	16	25
37	NaN	0.43303	0.86671				0	14.748	787510...	452480...	335030...	1	1	2	1	1	2	1	1	2
38	NaN	NaN	NaN				0	3.2351	372240...	312520...	5972300	0	1	0	0	1	0	0	1	0
39	NaN	NaN	NaN				0	2.4933	855680	704330	151350	0	1	0	0	1	0	0	1	0
40	NaN	NaN	NaN				0.0094...	0.98022	4456000	4279700	176340	0	0	1	0	0	1	0	0	1
41	NaN	NaN	NaN				0	15.506	445940...	254500...	191440...	7	5	6	1	0	1	0	0	0
42	NaN	NaN	1.1788				0	7.0117	353700...	170680...	183020...	0	0	2	0	0	2	0	0	2
43	NaN	NaN	2.0128				0	2.6325	7071000	2341600	4729400	0	0	2	0	0	2	0	0	2
44	NaN	0.31979	1.2166				0	5.7233	247230...	168960...	7826800	0	2	2	0	2	2	0	2	2
45	NaN	0.53144	NaN				0	4.1807	353450...	299600...	5385100	0	2	0	0	2	0	0	2	0
46	NaN	NaN	2.0486				0	3.2423	194280...	5746100	136820...	0	0	2	0	0	2	0	0	2
47	0.59303	0.32887	0.50644				0	22.391	686870...	550940...	135930...	2	3	3	2	3	3	2	3	3
48	0.85755	1.114	NaN				0	3.6129	195420...	108570...	868580...	1	1	1	1	1	1	1	1	1
49	NaN	NaN	1.0721				0	4.066	230570...	120950...	109620...	1	0	2	1	0	2	1	0	2
50	0.75695	3.3265	0.83447				0	82.637	104120...	521480...	519760...	5	8	5	5	8	5	5	8	5
51	1.0146	0.6911	0.52887				0	14.384	137620...	904860...	471340...	2	3	4	2	3	4	2	3	4
52	0.88658	1.0667	0.67661				0	47.706	138550...	863300...	522210...	2	3	3	2	3	3	2	3	3
53	1.0051	0.42981	0.54816				0	52.193	281620...	208570...	730410...	2	5	3	2	5	3	2	5	3
54	NaN	NaN	0.68491				0	7.23	7553200	4582300	2970900	0	0	1	0	0	1	0	0	1
55	NaN	0.72979	NaN				0.0054...	1.3152	1935500	1330700	604750	0	1	0	0	1	0	0	1	0
56	1.0455	8.4935	1.2727				0	62.976	482640...	216930...	265710...	3	4	9	2	3	8	2	3	8
57	NaN	NaN	NaN				0	4.2367	2524200	969840	1554300	0	0	1	0	0	1	0	0	1
58	NaN	NaN	NaN				0	12.424	9841100	4284900	5556200	1	0	1	1	0	1	1	0	1
59	1.0577	0.44189	0.79827				0	96.342	139260...	108540...	307130...	6	10	6	6	10	6	6	10	6
60	NaN	0.45482	NaN				0	4.3987	5159100	3960300	1198800	0	3	0	0	3	0	0	3	0
61	NaN	NaN	NaN				0	4.1784	131890...	8631300	4557500	0	0	1	0	0	1	0	0	1
62	1.33	0.5061	0.80206				0	9.0007	146260...	110930...	353340...	2	4	2	2	4	2	2	4	2
63	NaN	NaN	1.297				0.0014...	1.8653	8683400	6083500	2599900	0	1	1	0	1	1	0	1	1
64	NaN	NaN	NaN				0	9.9793	6522600	4676000	1846700	0	0	1	0	0	1	0	0	1

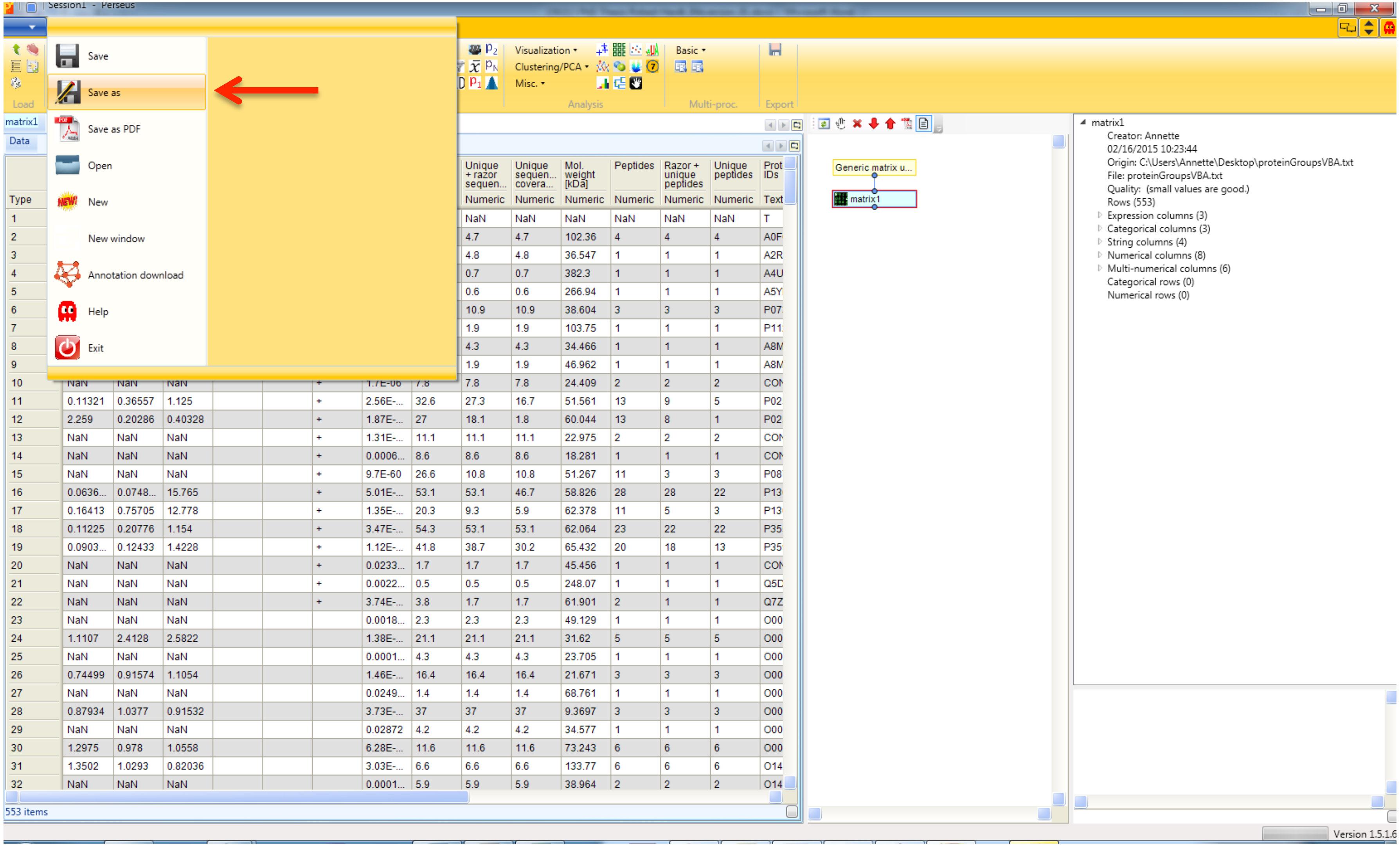
1628 items   1 selected

Protein group???

- MaxQuant does not give you single protein identifications, but so-called protein groups.
- A group contains all the proteins and protein isoforms (present in the searched database), which can be explained by a given set of identified peptides.

First steps

Before processing the data further, first save the Perseus project.

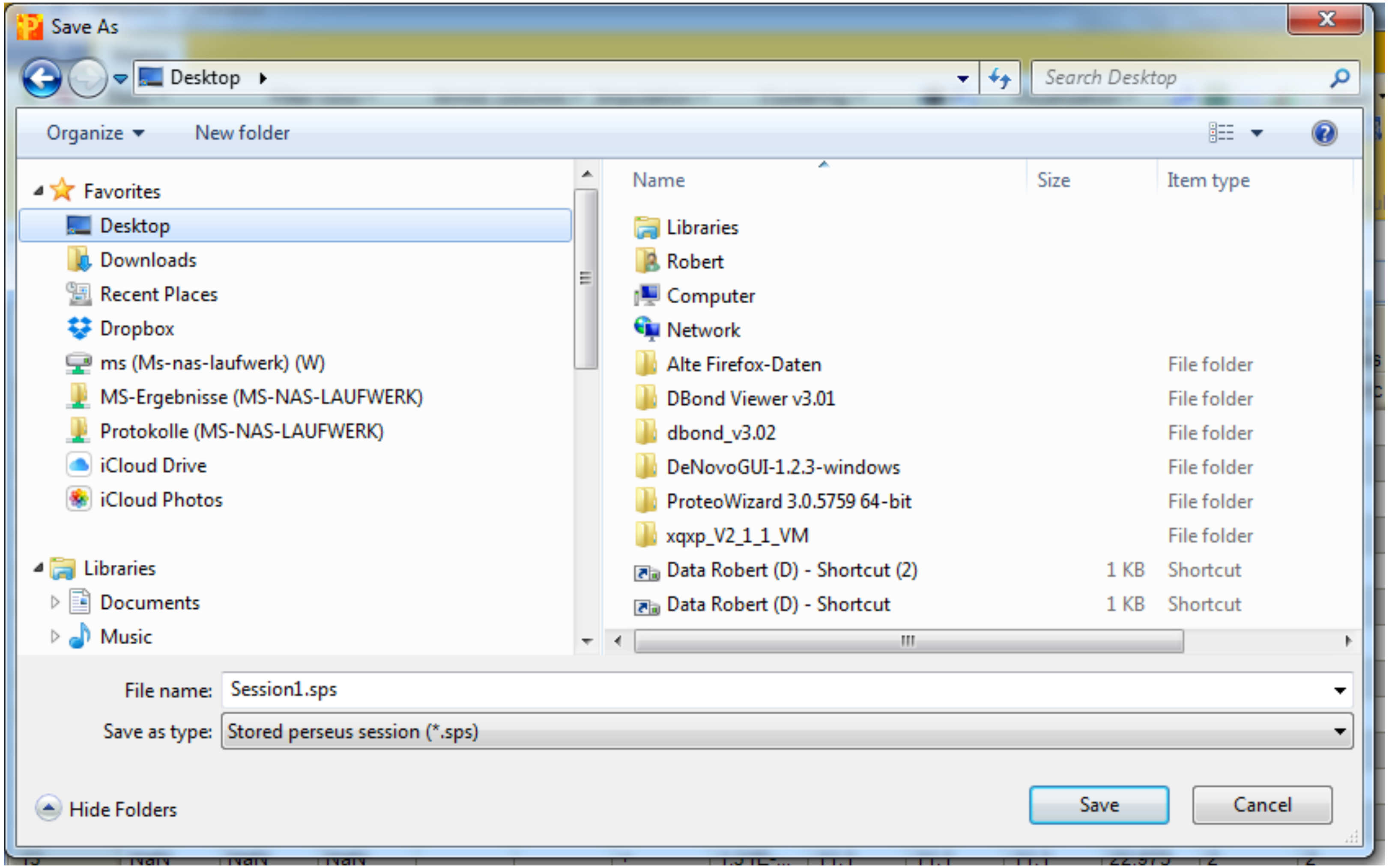


Type									Unique + razor sequen...	Unique sequen... covera...	Mol. weight [kDa]	Peptides	Razor + unique peptides	Unique peptides	Prot IDs
									Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Text
1									NaN	NaN	NaN	NaN	NaN	NaN	T
2									4.7	4.7	102.36	4	4	4	A0F
3									4.8	4.8	36.547	1	1	1	A2R
4									0.7	0.7	382.3	1	1	1	A4U
5									0.6	0.6	266.94	1	1	1	A5Y
6									10.9	10.9	38.604	3	3	3	P07
7									1.9	1.9	103.75	1	1	1	P11
8									4.3	4.3	34.466	1	1	1	A8M
9									1.9	1.9	46.962	1	1	1	A8M
10	NaN	NaN	NaN			+	1.7E-06	7.8	7.8	7.8	24.409	2	2	2	CON
11	0.11321	0.36557	1.125			+	2.56E-...	32.6	27.3	16.7	51.561	13	9	5	P02
12	2.259	0.20286	0.40328			+	1.87E-...	27	18.1	1.8	60.044	13	8	1	P02
13	NaN	NaN	NaN			+	1.31E-...	11.1	11.1	11.1	22.975	2	2	2	CON
14	NaN	NaN	NaN			+	0.0006...	8.6	8.6	8.6	18.281	1	1	1	CON
15	NaN	NaN	NaN			+	9.7E-60	26.6	10.8	10.8	51.267	11	3	3	P08
16	0.0636...	0.0748...	15.765			+	5.01E-...	53.1	53.1	46.7	58.826	28	28	22	P13
17	0.16413	0.75705	12.778			+	1.35E-...	20.3	9.3	5.9	62.378	11	5	3	P13
18	0.11225	0.20776	1.154			+	3.47E-...	54.3	53.1	53.1	62.064	23	22	22	P35
19	0.0903...	0.12433	1.4228			+	1.12E-...	41.8	38.7	30.2	65.432	20	18	13	P35
20	NaN	NaN	NaN			+	0.0233...	1.7	1.7	1.7	45.456	1	1	1	CON
21	NaN	NaN	NaN			+	0.0022...	0.5	0.5	0.5	248.07	1	1	1	Q5D
22	NaN	NaN	NaN			+	3.74E-...	3.8	1.7	1.7	61.901	2	1	1	Q7Z
23	NaN	NaN	NaN				0.0018...	2.3	2.3	2.3	49.129	1	1	1	O00
24	1.1107	2.4128	2.5822				1.38E-...	21.1	21.1	21.1	31.62	5	5	5	O00
25	NaN	NaN	NaN				0.0001...	4.3	4.3	4.3	23.705	1	1	1	O00
26	0.74499	0.91574	1.1054				1.46E-...	16.4	16.4	16.4	21.671	3	3	3	O00
27	NaN	NaN	NaN				0.0249...	1.4	1.4	1.4	68.761	1	1	1	O00
28	0.87934	1.0377	0.91532				3.73E-...	37	37	37	9.3697	3	3	3	O00
29	NaN	NaN	NaN				0.02872	4.2	4.2	4.2	34.577	1	1	1	O00
30	1.2975	0.978	1.0558				6.28E-...	11.6	11.6	11.6	73.243	6	6	6	O00
31	1.3502	1.0293	0.82036				3.03E-...	6.6	6.6	6.6	133.77	6	6	6	O14
32	NaN	NaN	NaN				0.0001...	5.9	5.9	5.9	38.964	2	2	2	O14

matrix1
Creator: Annette
02/16/2015 10:23:44
Origin: C:\Users\Annette\Desktop\proteinGroupsVBA.txt
File: proteinGroupsVBA.txt
Quality: (small values are good.)
Rows (553)
Expression columns (3)
Categorical columns (3)
String columns (4)
Numerical columns (8)
Multi-numerical columns (6)
Categorical rows (0)
Numerical rows (0)

First steps

Perseus projects are saved as sps-files



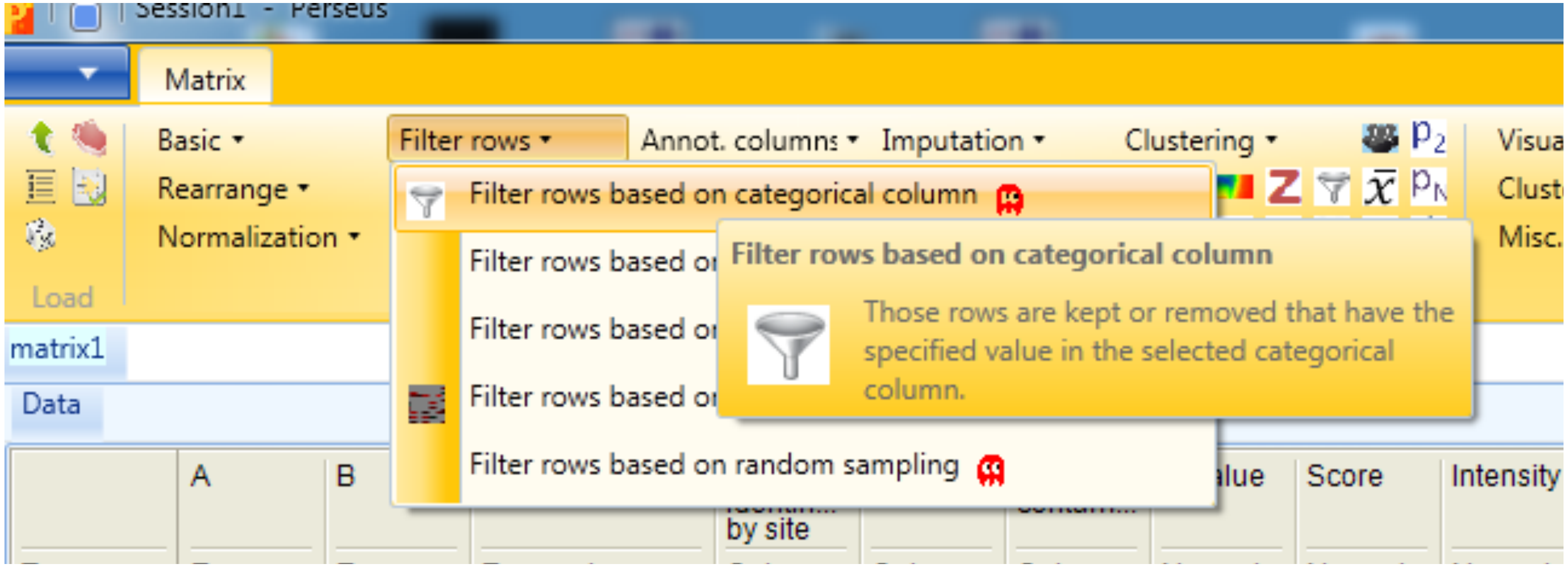
Name	Size	Item type
Libraries		
Robert		
Computer		
Network		
Alte Firefox-Daten		File folder
DBond Viewer v3.01		File folder
dbond_v3.02		File folder
DeNovoGUI-1.2.3-windows		File folder
ProteoWizard 3.0.5759 64-bit		File folder
xqxp_V2_1_1_VM		File folder
Data Robert (D) - Shortcut (2)	1 KB	Shortcut
Data Robert (D) - Shortcut	1 KB	Shortcut

File name: Session1.sps

Save as type: Stored perseus session (*.sps)

First steps

One now starts with the removal of usually irrelevant protein group identifications i.e. “Only identified by site”, “Reverse” and “Potential contaminants” ➔ Click Filter rows - Filter rows based on categorical columns

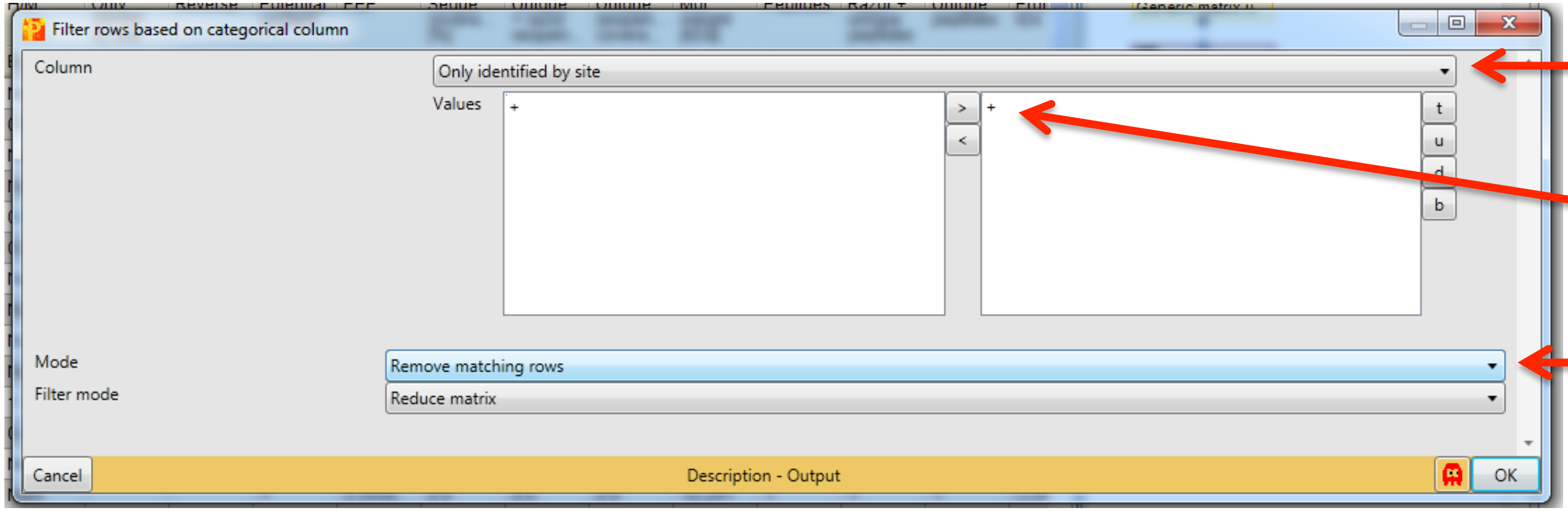


Filter rows based on categorical column

Those rows are kept or removed that have the specified value in the selected categorical column.

First steps

Removal of usually irrelevant protein group identifications



Filter rows based on categorical column

Column: Only identified by site

Values: +

+

Mode: Remove matching rows

Filter mode: Reduce matrix

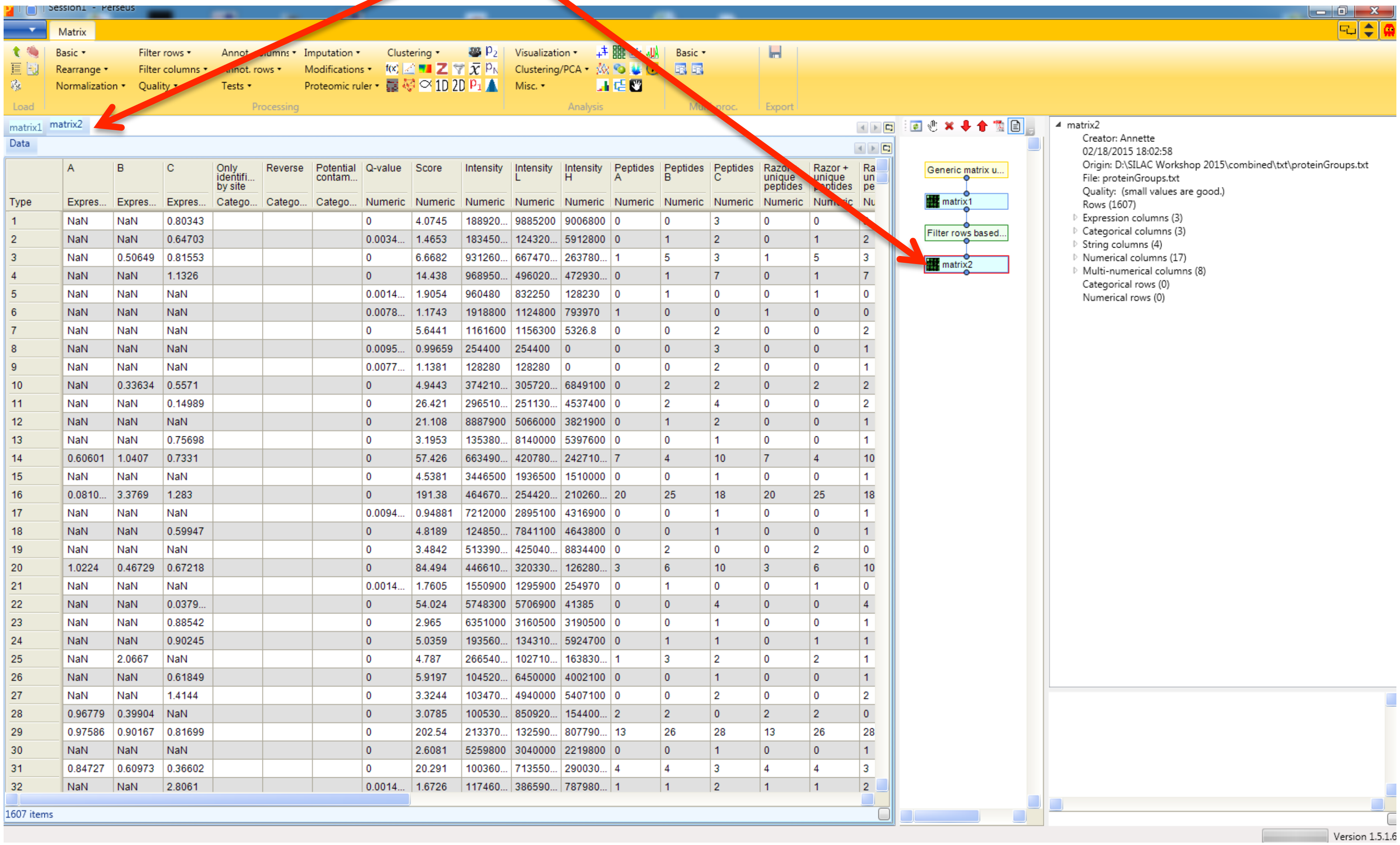
Which column should be searched for?

What value should be looked for?

What should happen to columns matching the criteria?

First steps

Perseus now created a **matrix2** (in a new tab), in which all entries marked with a + in the "Only identified by site"-category have been removed



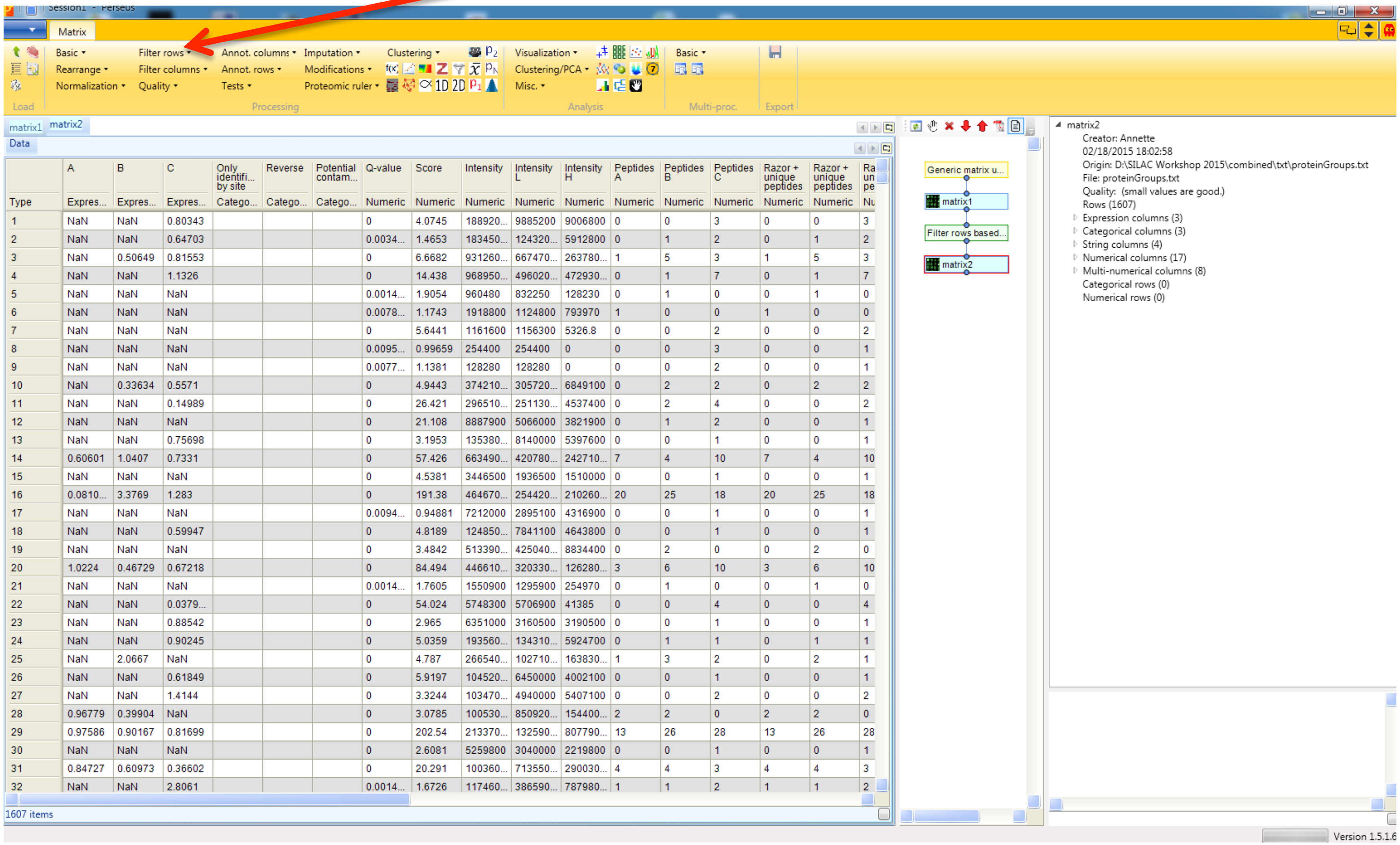
	A	B	C	Only identifi... by site	Reverse	Potential contam...	Q-value	Score	Intensity	Intensity L	Intensity H	Peptides A	Peptides B	Peptides C	Razo unique peptides	Razor + unique peptides	Ra un pe
Type	Expres...	Expres...	Expres...	Catego...	Catego...	Catego...	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Nu
1	NaN	NaN	0.80343				0	4.0745	188920...	9885200	9006800	0	0	3	0	0	
2	NaN	NaN	0.64703				0.0034...	1.4653	183450...	124320...	5912800	0	1	2	0	1	2
3	NaN	0.50649	0.81553				0	6.6682	931260...	667470...	263780...	1	5	3	1	5	3
4	NaN	NaN	1.1326				0	14.438	968950...	496020...	472930...	0	1	7	0	1	7
5	NaN	NaN	NaN				0.0014...	1.9054	960480	832250	128230	0	1	0	0	1	0
6	NaN	NaN	NaN				0.0078...	1.1743	1918800	1124800	793970	1	0	0	1	0	0
7	NaN	NaN	NaN				0	5.6441	1161600	1156300	5326.8	0	0	2	0	0	2
8	NaN	NaN	NaN				0.0095...	0.99659	254400	254400	0	0	0	3	0	0	1
9	NaN	NaN	NaN				0.0077...	1.1381	128280	128280	0	0	0	2	0	0	1
10	NaN	0.33634	0.5571				0	4.9443	374210...	305720...	6849100	0	2	2	0	2	2
11	NaN	NaN	0.14989				0	26.421	296510...	251130...	4537400	0	2	4	0	0	2
12	NaN	NaN	NaN				0	21.108	8887900	5066000	3821900	0	1	2	0	0	1
13	NaN	NaN	0.75698				0	3.1953	135380...	8140000	5397600	0	0	1	0	0	1
14	0.60601	1.0407	0.7331				0	57.426	663490...	420780...	242710...	7	4	10	7	4	10
15	NaN	NaN	NaN				0	4.5381	3446500	1936500	1510000	0	0	1	0	0	1
16	0.0810...	3.3769	1.283				0	191.38	464670...	254420...	210260...	20	25	18	20	25	18
17	NaN	NaN	NaN				0.0094...	0.94881	7212000	2895100	4316900	0	0	1	0	0	1
18	NaN	NaN	0.59947				0	4.8189	124850...	7841100	4643800	0	0	1	0	0	1
19	NaN	NaN	NaN				0	3.4842	513390...	425040...	8834400	0	2	0	0	2	0
20	1.0224	0.46729	0.67218				0	84.494	446610...	320330...	126280...	3	6	10	3	6	10
21	NaN	NaN	NaN				0.0014...	1.7605	1550900	1295900	254970	0	1	0	0	1	0
22	NaN	NaN	0.0379...				0	54.024	5748300	5706900	41385	0	0	4	0	0	4
23	NaN	NaN	0.88542				0	2.965	6351000	3160500	3190500	0	0	1	0	0	1
24	NaN	NaN	0.90245				0	5.0359	193560...	134310...	5924700	0	1	1	0	1	1
25	NaN	2.0667	NaN				0	4.787	266540...	102710...	163830...	1	3	2	0	2	1
26	NaN	NaN	0.61849				0	5.9197	104520...	6450000	4002100	0	0	1	0	0	1
27	NaN	NaN	1.4144				0	3.3244	103470...	4940000	5407100	0	0	2	0	0	2
28	0.96779	0.39904	NaN				0	3.0785	100530...	850920...	154400...	2	2	0	2	2	0
29	0.97586	0.90167	0.81699				0	202.54	213370...	132590...	807790...	13	26	28	13	26	28
30	NaN	NaN	NaN				0	2.6081	5259800	3040000	2219800	0	0	1	0	0	1
31	0.84727	0.60973	0.36602				0	20.291	100360...	713550...	290030...	4	4	3	4	4	3
32	NaN	NaN	2.8061				0.0014...	1.6726	117460...	386590...	787980...	1	1	2	1	1	2

1607 items

matrix2
Creator: Annette
02/18/2015 18:02:58
Origin: D:\SILAC Workshop 2015\combined\txt\proteinGroups.txt
File: proteinGroups.txt
Quality: (small values are good.)
Rows (1607)
Expression columns (3)
Categorical columns (3)
String columns (4)
Numerical columns (17)
Multi-numerical columns (8)
Categorical rows (0)
Numerical rows (0)

First steps

Now repeat the **filtering** for the other two categories!



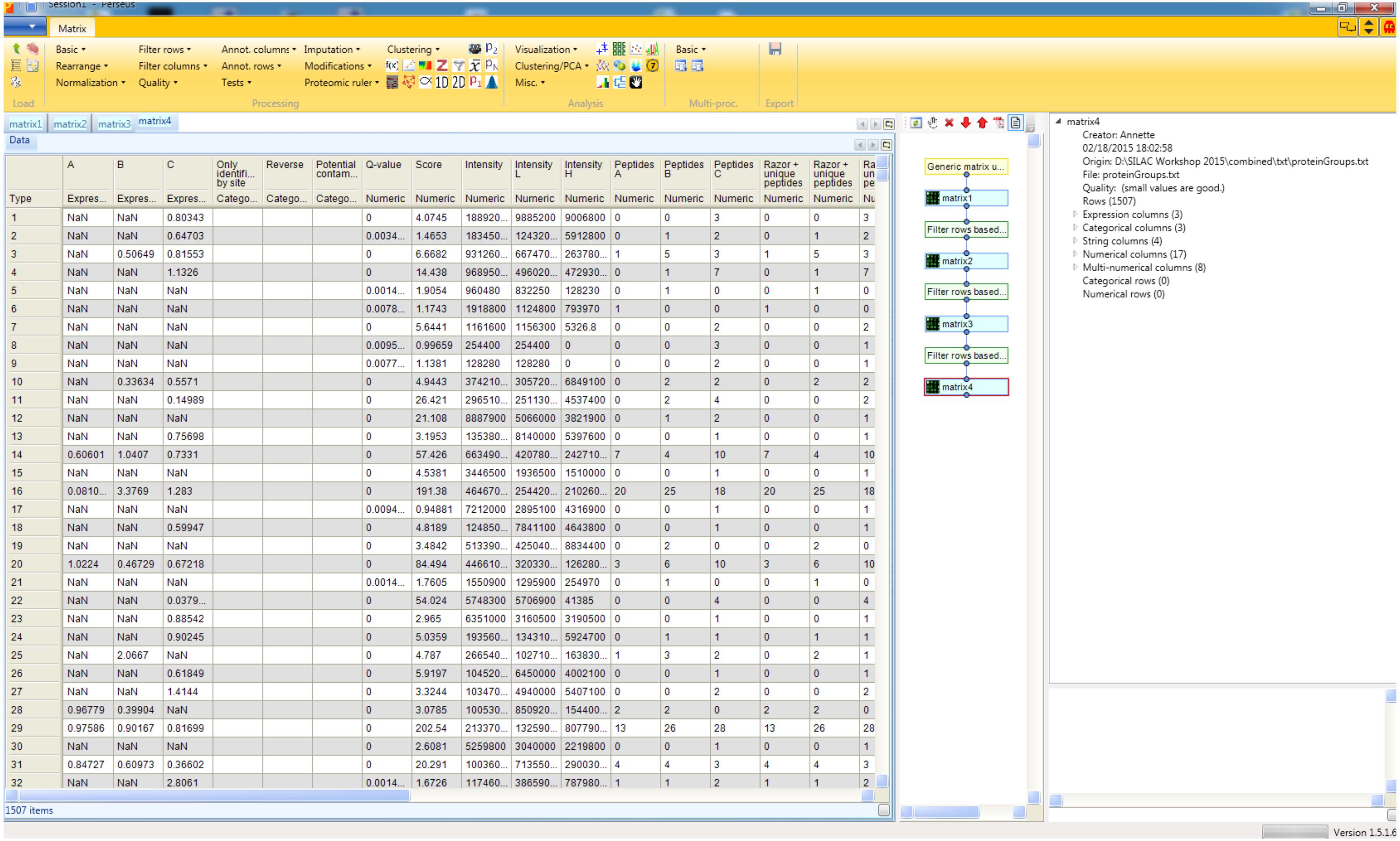
	A	B	C	Only identifi... by site	Reverse	Potential contam...	Q-value	Score	Intensity	Intensity L	Intensity H	Peptides A	Peptides B	Peptides C	Razor + unique peptides	Razor + unique peptides	Ra un pe
Type	Expres...	Expres...	Expres...	Catego...	Catego...	Catego...	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Nu
1	NaN	NaN	0.80343				0	4.0745	188920...	9885200	9006800	0	0	3	0	0	3
2	NaN	NaN	0.64703				0.0034...	1.4653	183450...	124320...	5912800	0	1	2	0	1	2
3	NaN	0.50649	0.81553				0	6.6682	931260...	667470...	263780...	1	5	3	1	5	3
4	NaN	NaN	1.1326				0	14.438	968950...	496020...	472930...	0	1	7	0	1	7
5	NaN	NaN	NaN				0.0014...	1.9054	960480	832250	128230	0	1	0	0	1	0
6	NaN	NaN	NaN				0.0078...	1.1743	1918800	1124800	793970	1	0	0	1	0	0
7	NaN	NaN	NaN				0	5.6441	1161600	1156300	5326.8	0	0	2	0	0	2
8	NaN	NaN	NaN				0.0095...	0.99659	254400	254400	0	0	0	3	0	0	1
9	NaN	NaN	NaN				0.0077...	1.1381	128280	128280	0	0	0	2	0	0	1
10	NaN	0.33634	0.5571				0	4.9443	374210...	305720...	6849100	0	2	2	0	2	2
11	NaN	NaN	0.14989				0	26.421	296510...	251130...	4537400	0	2	4	0	0	2
12	NaN	NaN	NaN				0	21.108	8887900	5066000	3821900	0	1	2	0	0	1
13	NaN	NaN	0.75698				0	3.1953	135380...	8140000	5397600	0	0	1	0	0	1
14	0.60601	1.0407	0.7331				0	57.426	663490...	420780...	242710...	7	4	10	7	4	10
15	NaN	NaN	NaN				0	4.5381	3446500	1936500	1510000	0	0	1	0	0	1
16	0.0810...	3.3769	1.283				0	191.38	464670...	254420...	210260...	20	25	18	20	25	18
17	NaN	NaN	NaN				0.0094...	0.94881	7212000	2895100	4316900	0	0	1	0	0	1
18	NaN	NaN	0.59947				0	4.8189	124850...	7841100	4643800	0	0	1	0	0	1
19	NaN	NaN	NaN				0	3.4842	513390...	425040...	8834400	0	2	0	0	2	0
20	1.0224	0.46729	0.67218				0	84.494	446610...	320330...	126280...	3	6	10	3	6	10
21	NaN	NaN	NaN				0.0014...	1.7605	1550900	1295900	254970	0	1	0	0	1	0
22	NaN	NaN	0.0379...				0	54.024	5748300	5706900	41385	0	0	4	0	0	4
23	NaN	NaN	0.88542				0	2.965	6351000	3160500	3190500	0	0	1	0	0	1
24	NaN	NaN	0.90245				0	5.0359	193560...	134310...	5924700	0	1	1	0	1	1
25	NaN	2.0667	NaN				0	4.787	266540...	102710...	163830...	1	3	2	0	2	1
26	NaN	NaN	0.61849				0	5.9197	104520...	6450000	4002100	0	0	1	0	0	1
27	NaN	NaN	1.4144				0	3.3244	103470...	4940000	5407100	0	0	2	0	0	2
28	0.96779	0.39904	NaN				0	3.0785	100530...	850920...	154400...	2	2	0	2	2	0
29	0.97586	0.90167	0.81699				0	202.54	213370...	132590...	807790...	13	26	28	13	26	28
30	NaN	NaN	NaN				0	2.6081	5259800	3040000	2219800	0	0	1	0	0	1
31	0.84727	0.60973	0.36602				0	20.291	100360...	713550...	290030...	4	4	3	4	4	3
32	NaN	NaN	2.8061				0.0014...	1.6726	117460...	386590...	787980...	1	1	2	1	1	2

1607 items

matrix2
Creator: Annette
02/18/2015 18:02:58
Origin: D:\SILAC Workshop 2015\combined\txt\proteinGroups.txt
File: proteinGroups.txt
Quality: (small values are good.)
Rows (1607)
Expression columns (3)
Categorical columns (3)
String columns (4)
Numerical columns (17)
Multi-numerical columns (8)
Categorical rows (0)
Numerical rows (0)

First steps

1507 entries remain from initially 1628.



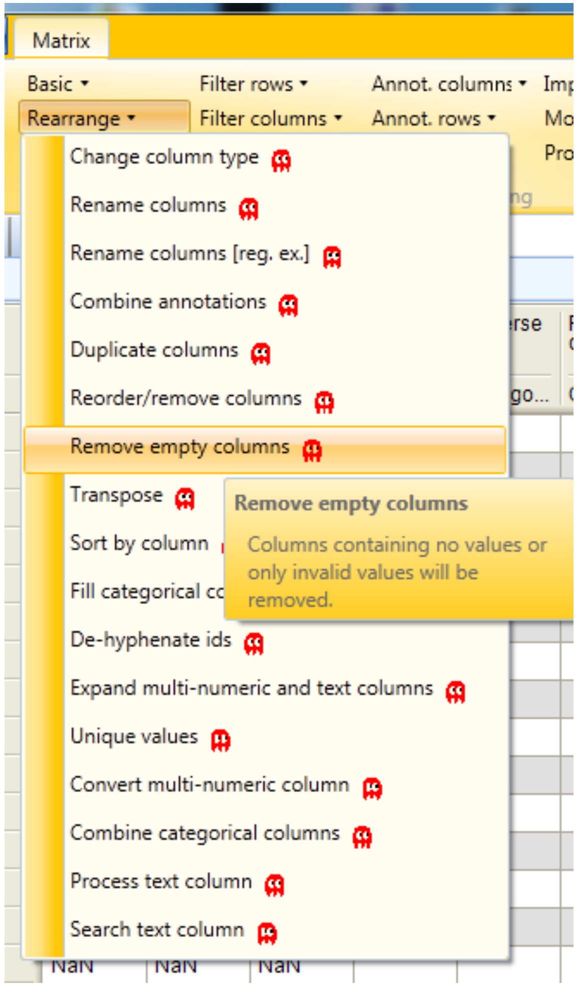
	A	B	C	Only identifi... by site	Reverse	Potential contam...	Q-value	Score	Intensity	Intensity L	Intensity H	Peptides A	Peptides B	Peptides C	Razor + unique peptides	Razor + unique peptides	Ra un pe
Type	Expres...	Expres...	Expres...	Catego...	Catego...	Catego...	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Nu
1	NaN	NaN	0.80343				0	4.0745	188920...	9885200	9006800	0	0	3	0	0	3
2	NaN	NaN	0.64703				0.0034...	1.4653	183450...	124320...	5912800	0	1	2	0	1	2
3	NaN	0.50649	0.81553				0	6.6682	931260...	667470...	263780...	1	5	3	1	5	3
4	NaN	NaN	1.1326				0	14.438	968950...	496020...	472930...	0	1	7	0	1	7
5	NaN	NaN	NaN				0.0014...	1.9054	960480	832250	128230	0	1	0	0	1	0
6	NaN	NaN	NaN				0.0078...	1.1743	1918800	1124800	793970	1	0	0	1	0	0
7	NaN	NaN	NaN				0	5.6441	1161600	1156300	5326.8	0	0	2	0	0	2
8	NaN	NaN	NaN				0.0095...	0.99659	254400	254400	0	0	0	3	0	0	1
9	NaN	NaN	NaN				0.0077...	1.1381	128280	128280	0	0	0	2	0	0	1
10	NaN	0.33634	0.5571				0	4.9443	374210...	305720...	6849100	0	2	2	0	2	2
11	NaN	NaN	0.14989				0	26.421	296510...	251130...	4537400	0	2	4	0	0	2
12	NaN	NaN	NaN				0	21.108	8887900	5066000	3821900	0	1	2	0	0	1
13	NaN	NaN	0.75698				0	3.1953	135380...	8140000	5397600	0	0	1	0	0	1
14	0.60601	1.0407	0.7331				0	57.426	663490...	420780...	242710...	7	4	10	7	4	10
15	NaN	NaN	NaN				0	4.5381	3446500	1936500	1510000	0	0	1	0	0	1
16	0.0810...	3.3769	1.283				0	191.38	464670...	254420...	210260...	20	25	18	20	25	18
17	NaN	NaN	NaN				0.0094...	0.94881	7212000	2895100	4316900	0	0	1	0	0	1
18	NaN	NaN	0.59947				0	4.8189	124850...	7841100	4643800	0	0	1	0	0	1
19	NaN	NaN	NaN				0	3.4842	513390...	425040...	8834400	0	2	0	0	2	0
20	1.0224	0.46729	0.67218				0	84.494	446610...	320330...	126280...	3	6	10	3	6	10
21	NaN	NaN	NaN				0.0014...	1.7605	1550900	1295900	254970	0	1	0	0	1	0
22	NaN	NaN	0.0379...				0	54.024	5748300	5706900	41385	0	0	4	0	0	4
23	NaN	NaN	0.88542				0	2.965	6351000	3160500	3190500	0	0	1	0	0	1
24	NaN	NaN	0.90245				0	5.0359	193560...	134310...	5924700	0	1	1	0	1	1
25	NaN	2.0667	NaN				0	4.787	266540...	102710...	163830...	1	3	2	0	2	1
26	NaN	NaN	0.61849				0	5.9197	104520...	6450000	4002100	0	0	1	0	0	1
27	NaN	NaN	1.4144				0	3.3244	103470...	4940000	5407100	0	0	2	0	0	2
28	0.96779	0.39904	NaN				0	3.0785	100530...	850920...	154400...	2	2	0	2	2	0
29	0.97586	0.90167	0.81699				0	202.54	213370...	132590...	807790...	13	26	28	13	26	28
30	NaN	NaN	NaN				0	2.6081	5259800	3040000	2219800	0	0	1	0	0	1
31	0.84727	0.60973	0.36602				0	20.291	100360...	713550...	290030...	4	4	3	4	4	3
32	NaN	NaN	2.8061				0.0014...	1.6726	117460...	386590...	787980...	1	1	2	1	1	2

1507 items

matrix4
Creator: Annette
02/18/2015 18:02:58
Origin: D:\SILAC Workshop 2015\combined\txt\proteinGroups.txt
File: proteinGroups.txt
Quality: (small values are good.)
Rows (1507)
Expression columns (3)
Categorical columns (3)
String columns (4)
Numerical columns (17)
Multi-numerical columns (8)
Categorical rows (0)
Numerical rows (0)

First steps

Since all three categorical columns are empty now, we can remove them to clean up the table.

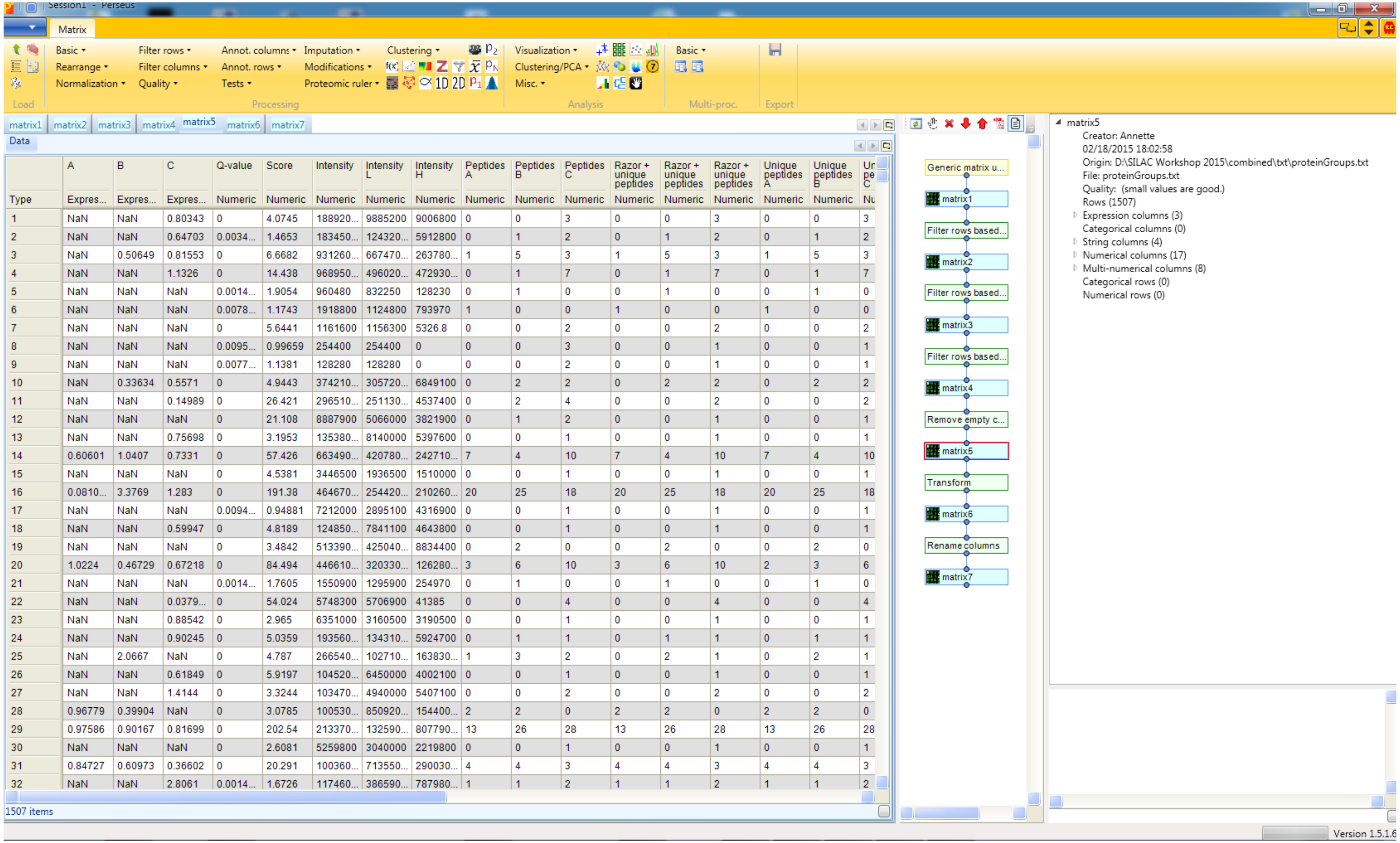


Remove empty columns

Columns containing no values or only invalid values will be removed.

First steps

After removal of empty columns, the data can be processed further…



Data

	A	B	C	Q-value	Score	Intensity	Intensity L	Intensity H	Peptides A	Peptides B	Peptides C	Razor + unique peptides	Razor + unique peptides	Razor + unique peptides	Unique peptides A	Unique peptides B	Ur pe C
Type	Expres...	Expres...	Expres...	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Nu
1	NaN	NaN	0.80343	0	4.0745	188920...	9885200	9006800	0	0	3	0	0	3	0	0	3
2	NaN	NaN	0.64703	0.0034...	1.4653	183450...	124320...	5912800	0	1	2	0	1	2	0	1	2
3	NaN	0.50649	0.81553	0	6.6682	931260...	667470...	263780...	1	5	3	1	5	3	1	5	3
4	NaN	NaN	1.1326	0	14.438	968950...	496020...	472930...	0	1	7	0	1	7	0	1	7
5	NaN	NaN	NaN	0.0014...	1.9054	960480	832250	128230	0	1	0	0	1	0	0	1	0
6	NaN	NaN	NaN	0.0078...	1.1743	1918800	1124800	793970	1	0	0	1	0	0	1	0	0
7	NaN	NaN	NaN	0	5.6441	1161600	1156300	5326.8	0	0	2	0	0	2	0	0	2
8	NaN	NaN	NaN	0.0095...	0.99659	254400	254400	0	0	0	3	0	0	1	0	0	1
9	NaN	NaN	NaN	0.0077...	1.1381	128280	128280	0	0	0	2	0	0	1	0	0	1
10	NaN	0.33634	0.5571	0	4.9443	374210...	305720...	6849100	0	2	2	0	2	2	0	2	2
11	NaN	NaN	0.14989	0	26.421	296510...	251130...	4537400	0	2	4	0	0	2	0	0	2
12	NaN	NaN	NaN	0	21.108	8887900	5066000	3821900	0	1	2	0	0	1	0	0	1
13	NaN	NaN	0.75698	0	3.1953	135380...	8140000	5397600	0	0	1	0	0	1	0	0	1
14	0.60601	1.0407	0.7331	0	57.426	663490...	420780...	242710...	7	4	10	7	4	10	7	4	10
15	NaN	NaN	NaN	0	4.5381	3446500	1936500	1510000	0	0	1	0	0	1	0	0	1
16	0.0810...	3.3769	1.283	0	191.38	464670...	254420...	210260...	20	25	18	20	25	18	20	25	18
17	NaN	NaN	NaN	0.0094...	0.94881	7212000	2895100	4316900	0	0	1	0	0	1	0	0	1
18	NaN	NaN	0.59947	0	4.8189	124850...	7841100	4643800	0	0	1	0	0	1	0	0	1
19	NaN	NaN	NaN	0	3.4842	513390...	425040...	8834400	0	2	0	0	2	0	0	2	0
20	1.0224	0.46729	0.67218	0	84.494	446610...	320330...	126280...	3	6	10	3	6	10	2	3	6
21	NaN	NaN	NaN	0.0014...	1.7605	1550900	1295900	254970	0	1	0	0	1	0	0	1	0
22	NaN	NaN	0.0379...	0	54.024	5748300	5706900	41385	0	0	4	0	0	4	0	0	4
23	NaN	NaN	0.88542	0	2.965	6351000	3160500	3190500	0	0	1	0	0	1	0	0	1
24	NaN	NaN	0.90245	0	5.0359	193560...	134310...	5924700	0	1	1	0	1	1	0	1	1
25	NaN	2.0667	NaN	0	4.787	266540...	102710...	163830...	1	3	2	0	2	1	0	2	1
26	NaN	NaN	0.61849	0	5.9197	104520...	6450000	4002100	0	0	1	0	0	1	0	0	1
27	NaN	NaN	1.4144	0	3.3244	103470...	4940000	5407100	0	0	2	0	0	2	0	0	2
28	0.96779	0.39904	NaN	0	3.0785	100530...	850920...	154400...	2	2	0	2	2	0	2	2	0
29	0.97586	0.90167	0.81699	0	202.54	213370...	132590...	807790...	13	26	28	13	26	28	13	26	28
30	NaN	NaN	NaN	0	2.6081	5259800	3040000	2219800	0	0	1	0	0	1	0	0	1
31	0.84727	0.60973	0.36602	0	20.291	100360...	713550...	290030...	4	4	3	4	4	3	4	4	3
32	NaN	NaN	2.8061	0.0014...	1.6726	117460...	386590...	787980...	1	1	2	1	1	2	1	1	2

1507 items

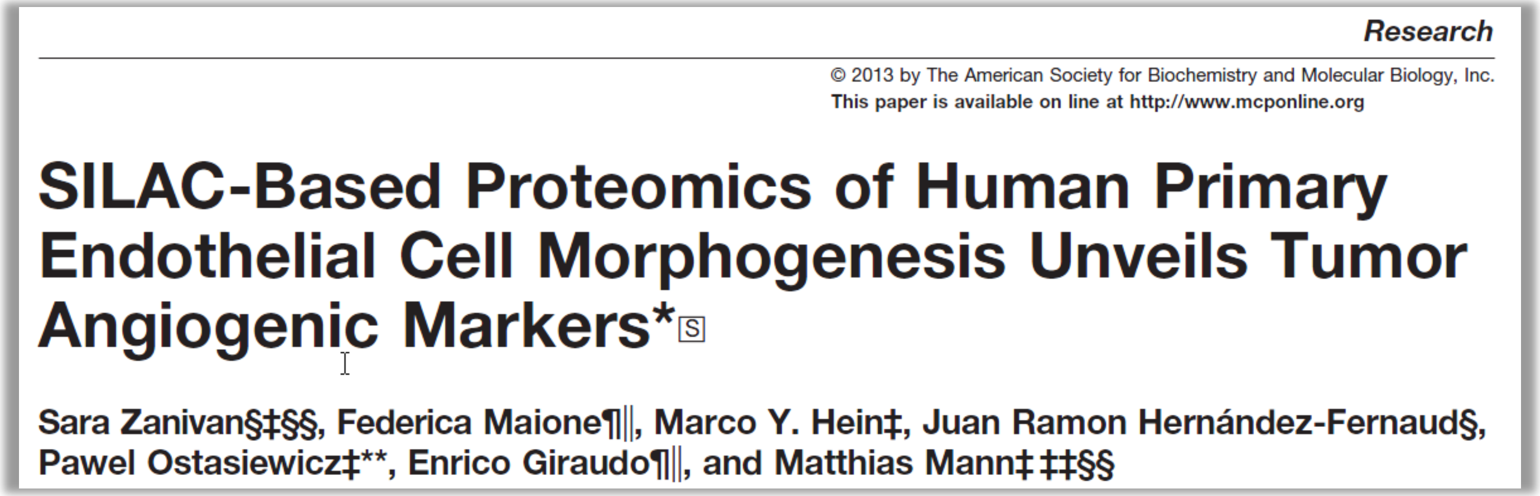
matrix5
- Creator: Annette
- 02/18/2015 18:02:58
- Origin: D:\SILAC Workshop 2015\combined\txt\proteinGroups.txt
- File: proteinGroups.txt
- Quality: (small values are good.)
- Rows (1507)
- Expression columns (3)
- Categorical columns (0)
- String columns (4)
- Numerical columns (17)
- Multi-numerical columns (8)
- Categorical rows (0)
- Numerical rows (0)

More…

- Additional Perseus tutorials at our webpage
  - Analysis of SILAC data
  - Analysis of label-free quantification data (under construction)
  - Analysis of large-scale phosphoproteomics data (under construction)
- Official Perseus documentation
  - http://141.61.102.17/perseus_doku/
- Video tutorials from MaxQuant Summer Schools
  - http://www.youtube.com/channel/UCKYzYTm1cnmc0CFAMhxDO8w

Analysis of SILAC data

- Example dataset from:



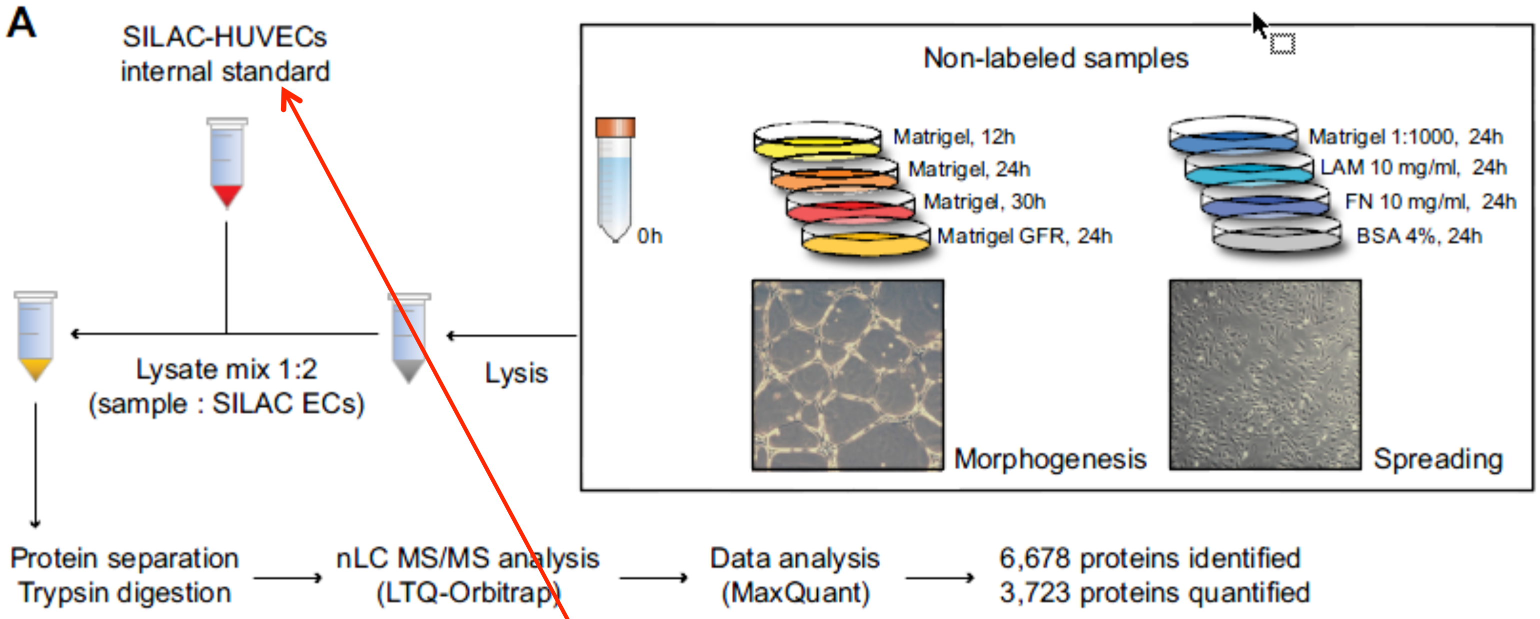
Research

SILAC-Based Proteomics of Human Primary Endothelial Cell Morphogenesis Unveils Tumor Angiogenic Markers[*] S

Sara Zanivan[§‡§§], Federica Maione[¶‖], Marco Y. Hein[‡], Juan Ramon Hernández-Fernaud[§], Pawel Ostasiewicz[‡], Enrico Giraudo[¶‖], and Matthias Mann[‡ ‡‡§§]**

Analysis of SILAC data

- Example dataset:

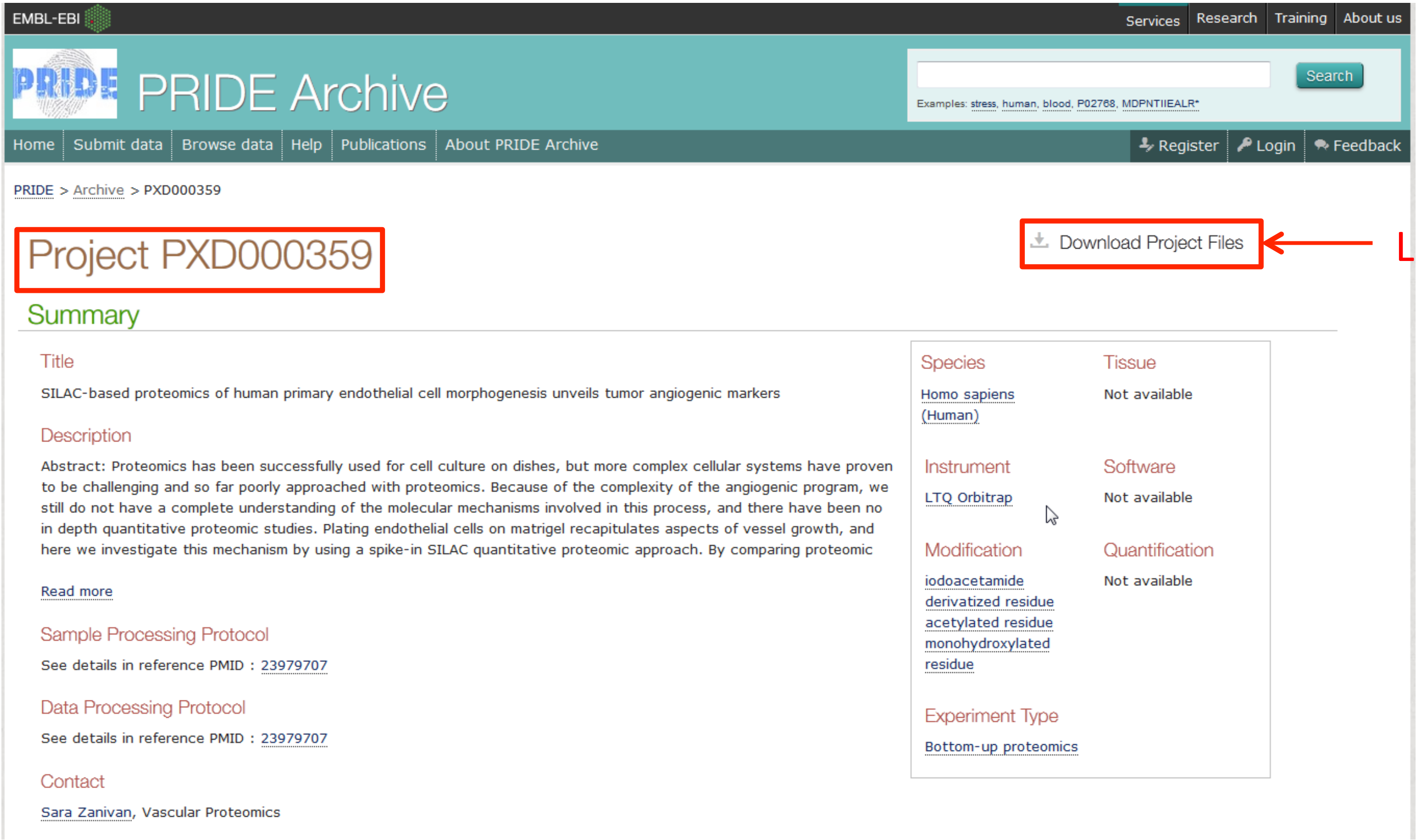


➔ Sample proteins are measured against a heavy labeled reference sample (internal standard)= **Spike-in SILAC**

HUVEC = human umbilical vein endothelial cells

Analysis of SILAC data

- First download the example data from the PRIDE data repository (http://www.ebi.ac.uk/pride/archive/)



Project PXD000359

Summary

Title

SILAC-based proteomics of human primary endothelial cell morphogenesis unveils tumor angiogenic markers

Description

Abstract: Proteomics has been successfully used for cell culture on dishes, but more complex cellular systems have proven to be challenging and so far poorly approached with proteomics. Because of the complexity of the angiogenic program, we still do not have a complete understanding of the molecular mechanisms involved in this process, and there have been no in depth quantitative proteomic studies. Plating endothelial cells on matrigel recapitulates aspects of vessel growth, and here we investigate this mechanism by using a spike-in SILAC quantitative proteomic approach. By comparing proteomic

Read more

Sample Processing Protocol

See details in reference PMID : 23979707

Data Processing Protocol

See details in reference PMID : 23979707

Contact

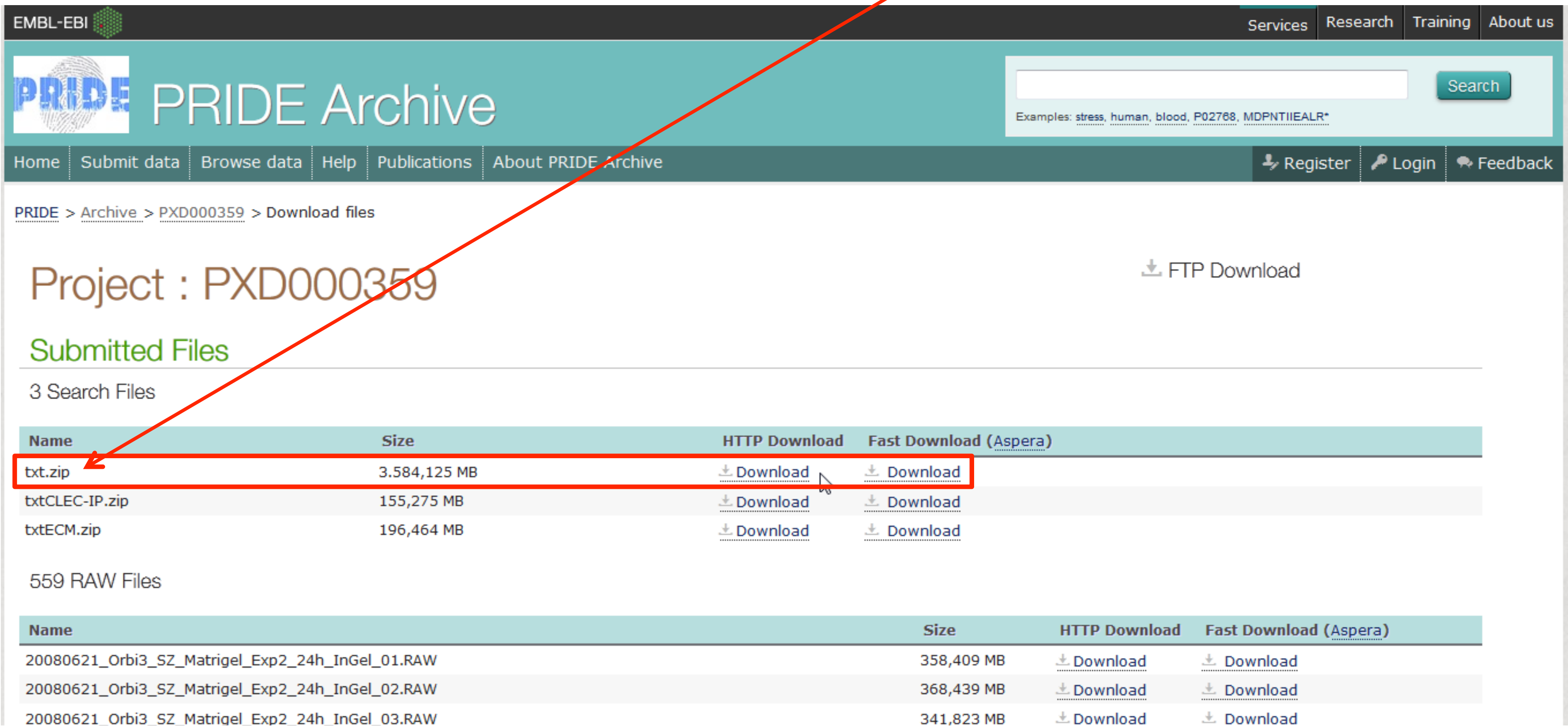
Sara Zanivan, Vascular Proteomics

Species	Tissue
Homo sapiens (Human)	Not available
Instrument	**Software**
LTQ Orbitrap	Not available
Modification	**Quantification**
iodoacetamide derivatized residue acetylated residue monohydroxylated residue	Not available
Experiment Type	
Bottom-up proteomics	

Link to files

Analysis of SILAC data

Just download the **txt.zip** file



Project : PXD000359

Submitted Files

3 Search Files

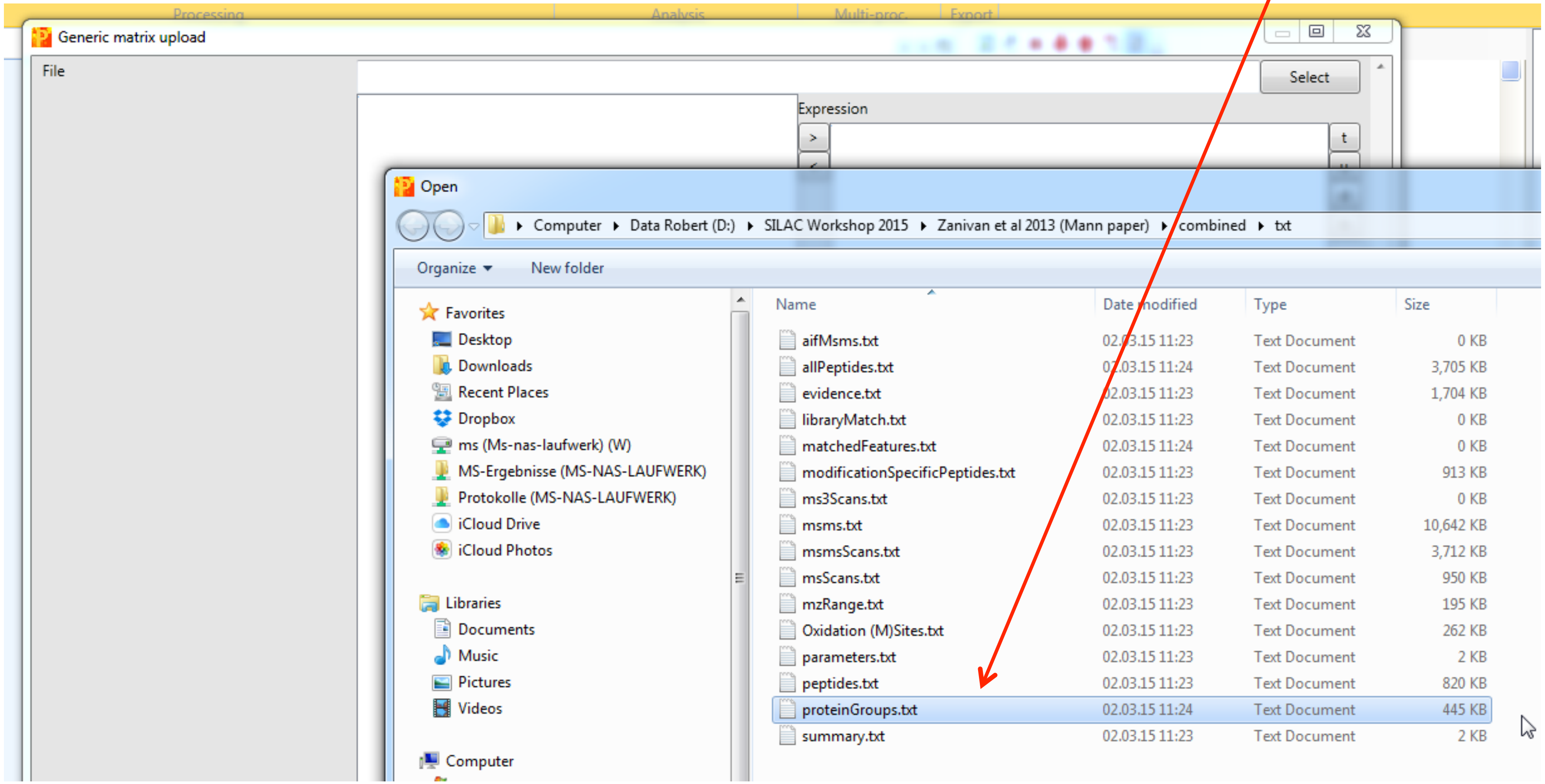
Name	Size	HTTP Download	Fast Download (Aspera)
txt.zip	3.584,125 MB	Download	Download
txtCLEC-IP.zip	155,275 MB	Download	Download
txtECM.zip	196,464 MB	Download	Download

559 RAW Files

Name	Size	HTTP Download	Fast Download (Aspera)
20080621_Orbi3_SZ_Matrigel_Exp2_24h_InGel_01.RAW	358,409 MB	Download	Download
20080621_Orbi3_SZ_Matrigel_Exp2_24h_InGel_02.RAW	368,439 MB	Download	Download
20080621_Orbi3_SZ_Matrigel_Exp2_24h_InGel_03.RAW	341,823 MB	Download	Download

Analysis of SILAC data

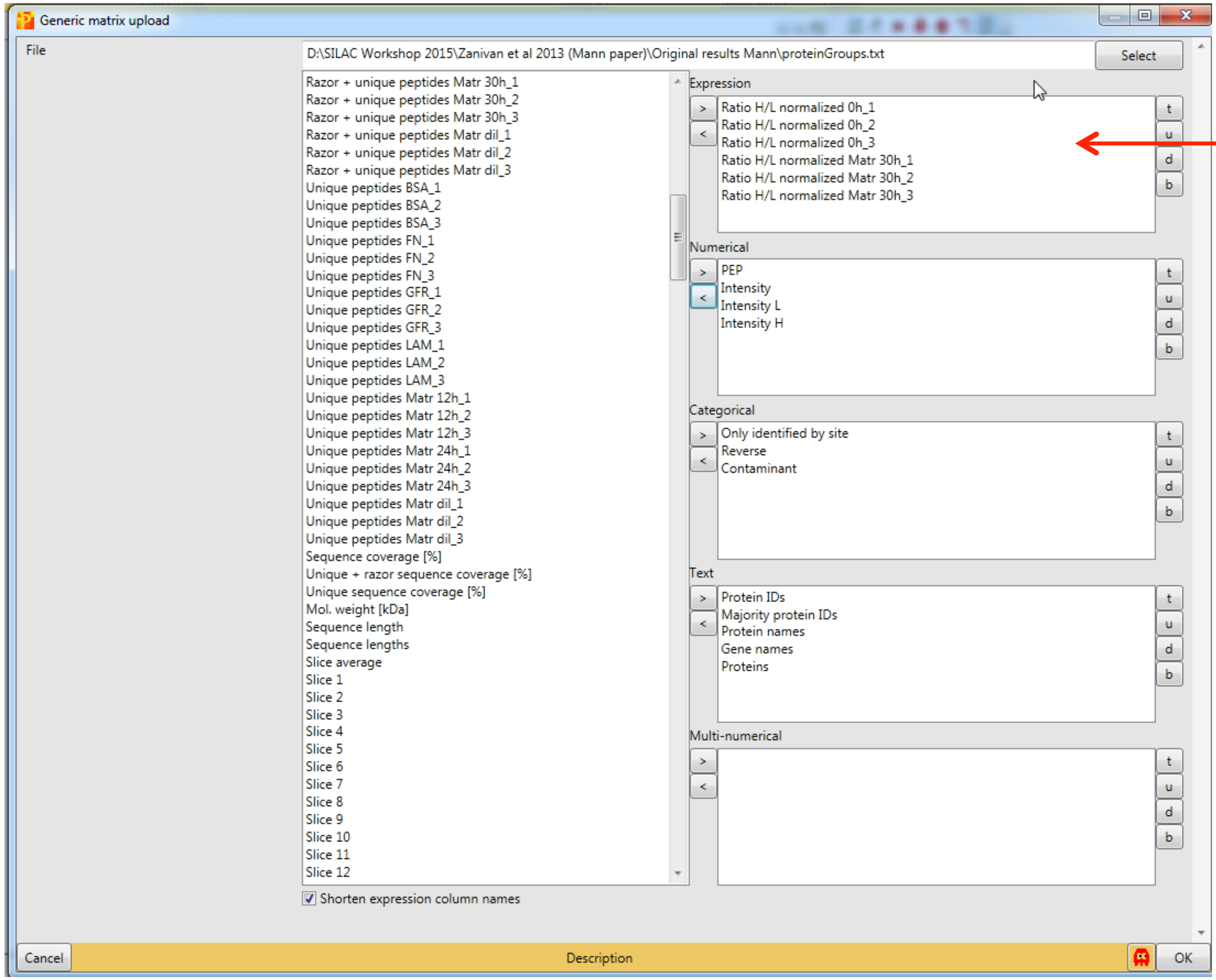
After extracting the zip-file we need the **proteinGroups.txt,** which we import into Perseus



Name	Date modified	Type	Size
aifMsms.txt	02.03.15 11:23	Text Document	0 KB
allPeptides.txt	02.03.15 11:24	Text Document	3,705 KB
evidence.txt	02.03.15 11:23	Text Document	1,704 KB
libraryMatch.txt	02.03.15 11:23	Text Document	0 KB
matchedFeatures.txt	02.03.15 11:24	Text Document	0 KB
modificationSpecificPeptides.txt	02.03.15 11:23	Text Document	913 KB
ms3Scans.txt	02.03.15 11:23	Text Document	0 KB
msms.txt	02.03.15 11:23	Text Document	10,642 KB
msmsScans.txt	02.03.15 11:23	Text Document	3,712 KB
msScans.txt	02.03.15 11:23	Text Document	950 KB
mzRange.txt	02.03.15 11:23	Text Document	195 KB
Oxidation (M)Sites.txt	02.03.15 11:23	Text Document	262 KB
parameters.txt	02.03.15 11:23	Text Document	2 KB
peptides.txt	02.03.15 11:23	Text Document	820 KB
proteinGroups.txt	02.03.15 11:24	Text Document	445 KB
summary.txt	02.03.15 11:23	Text Document	2 KB

Analysis of SILAC data

After extracting the zip-file we need the **proteinGroups.txt,** which we import into Perseus



File: D:\SILAC Workshop 2015\Zanivan et al 2013 (Mann paper)\Original results Mann\proteinGroups.txt

Razor + unique peptides Matr 30h_1
Razor + unique peptides Matr 30h_2
Razor + unique peptides Matr 30h_3
Razor + unique peptides Matr dil_1
Razor + unique peptides Matr dil_2
Razor + unique peptides Matr dil_3
Unique peptides BSA_1
Unique peptides BSA_2
Unique peptides BSA_3
Unique peptides FN_1
Unique peptides FN_2
Unique peptides FN_3
Unique peptides GFR_1
Unique peptides GFR_2
Unique peptides GFR_3
Unique peptides LAM_1
Unique peptides LAM_2
Unique peptides LAM_3
Unique peptides Matr 12h_1
Unique peptides Matr 12h_2
Unique peptides Matr 12h_3
Unique peptides Matr 24h_1
Unique peptides Matr 24h_2
Unique peptides Matr 24h_3
Unique peptides Matr dil_1
Unique peptides Matr dil_2
Unique peptides Matr dil_3
Sequence coverage [%]
Unique + razor sequence coverage [%]
Unique sequence coverage [%]
Mol. weight [kDa]
Sequence length
Sequence lengths
Slice average
Slice 1
Slice 2
Slice 3
Slice 4
Slice 5
Slice 6
Slice 7
Slice 8
Slice 9
Slice 10
Slice 11
Slice 12

Expression
Ratio H/L normalized 0h_1
Ratio H/L normalized 0h_2
Ratio H/L normalized 0h_3
Ratio H/L normalized Matr 30h_1
Ratio H/L normalized Matr 30h_2
Ratio H/L normalized Matr 30h_3

Numerical
PEP
Intensity
Intensity L
Intensity H

Categorical
Only identified by site
Reverse
Contaminant

Text
Protein IDs
Majority protein IDs
Protein names
Gene names
Proteins

Multi-numerical

☑ Shorten expression column names

There is a lot of different samples in the dataset. We only select the 0h (control) and the Matr30h (growth on Matrigel for 30h) samples.

Analysis of SILAC data

Now we make classical SILAC ratios out of the Spike-in SILAC ratios

$$ratio\,0h = heavy\,(Standard)/light\,(0h)$$

&

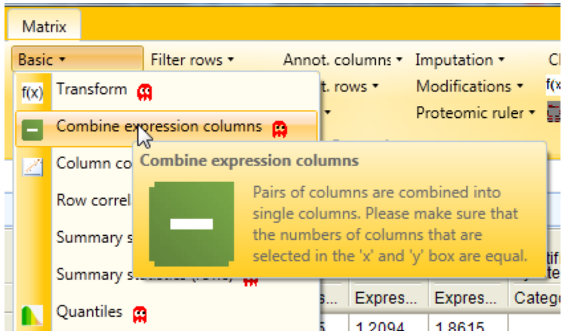
$$ratio\,Matr30h = heavy\,(Standard)/light\,(Matr30h)$$

$$\Downarrow$$

$$ratioMatr30h/0h = heavy\,(standard)/light\,(0h)\ /heavy\,(standard)/light\,(Matr30h)\ = light\,(Matr30h)/light\,(0h)$$

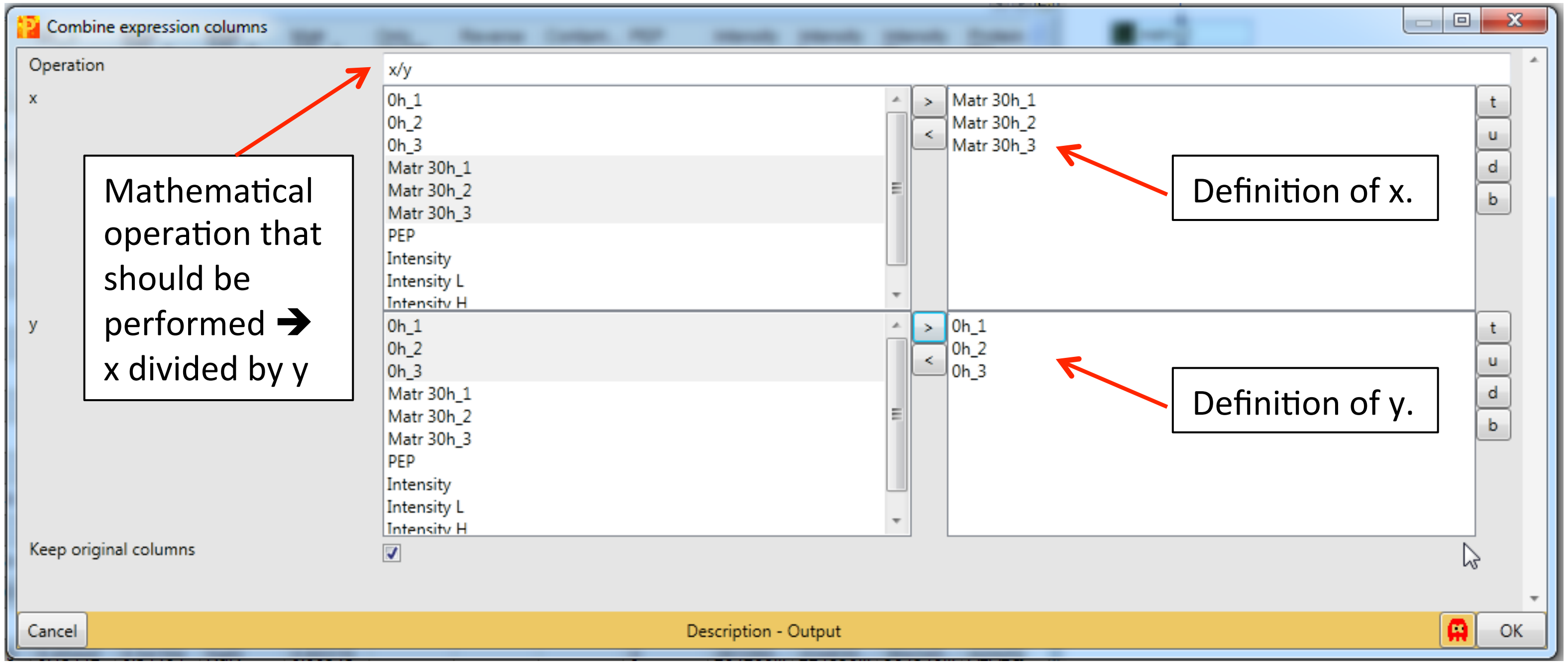
Analysis of SILAC data

Now we make classical SILAC ratios out of the Spike-in SILAC ratios



Combine expression columns

Pairs of columns are combined into single columns. Please make sure that the numbers of columns that are selected in the 'x' and 'y' box are equal.



Operation: x/y

x: Matr 30h_1, Matr 30h_2, Matr 30h_3

y: 0h_1, 0h_2, 0h_3

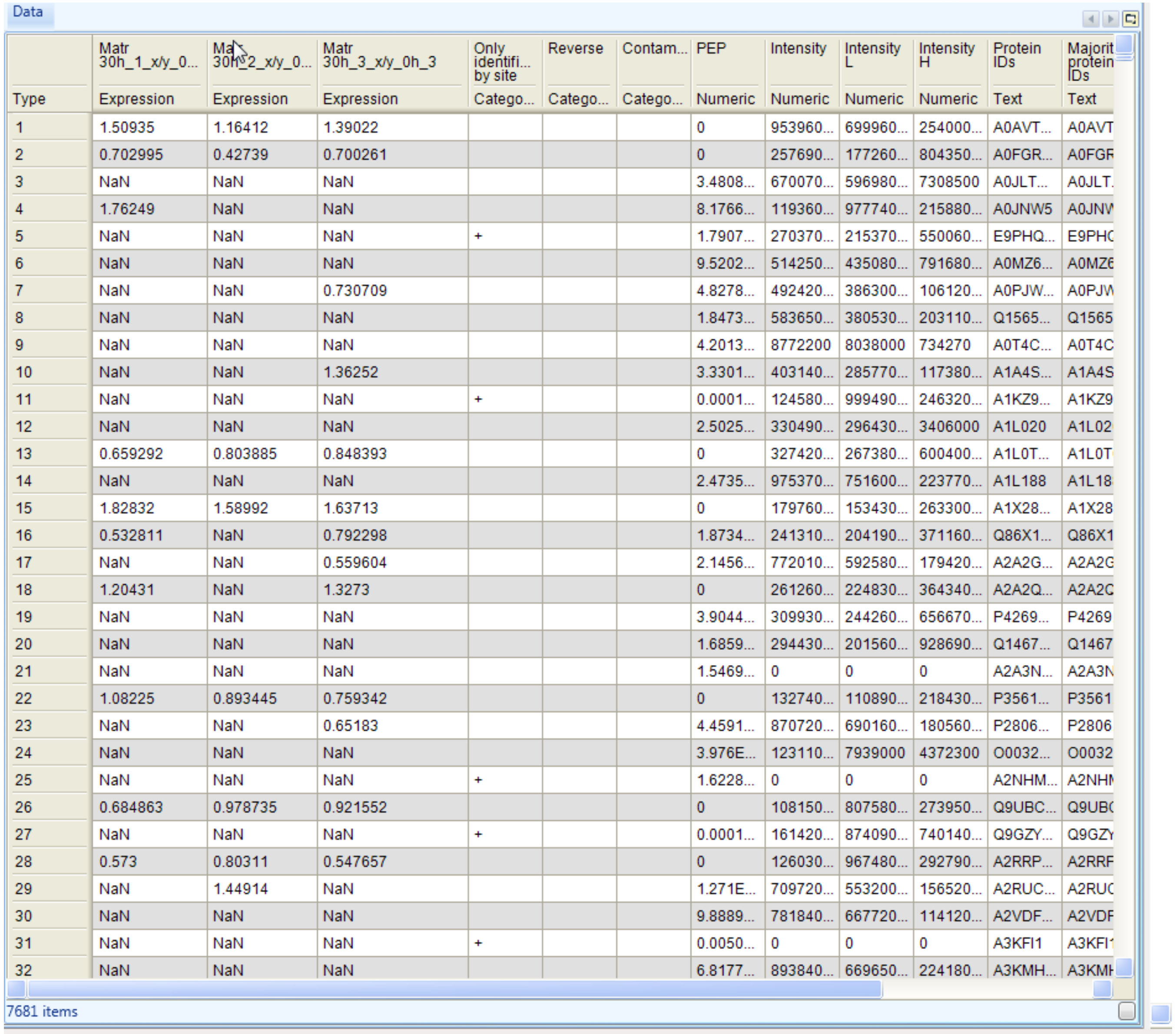
Keep original columns ☑

Mathematical operation that should be performed ➔ x divided by y

Definition of x.

Definition of y.

Analysis of SILAC data



Data

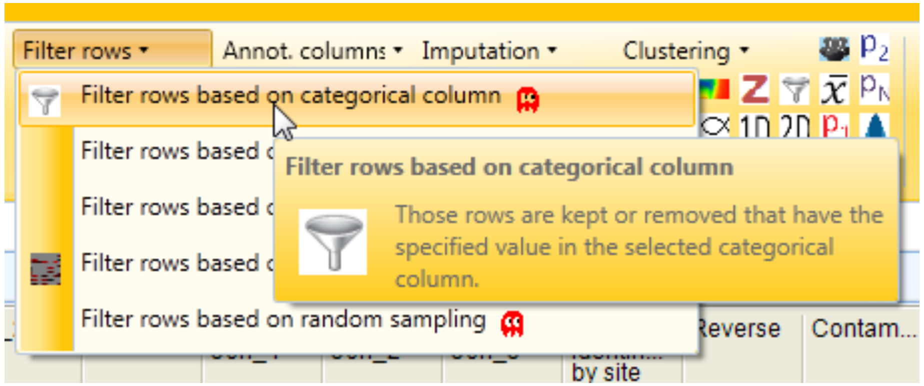
	Matr 30h_1_x/y_0...	Ma 30h_2_x/y_0...	Matr 30h_3_x/y_0h_3	Only identifi... by site	Reverse	Contam...	PEP	Intensity	Intensity L	Intensity H	Protein IDs	Majorit protein IDs
Type	Expression	Expression	Expression	Catego...	Catego...	Catego...	Numeric	Numeric	Numeric	Numeric	Text	Text
1	1.50935	1.16412	1.39022				0	953960...	699960...	254000...	A0AVT...	A0AVT
2	0.702995	0.42739	0.700261				0	257690...	177260...	804350...	A0FGR...	A0FGR
3	NaN	NaN	NaN				3.4808...	670070...	596980...	7308500	A0JLT...	A0JLT.
4	1.76249	NaN	NaN				8.1766...	119360...	977740...	215880...	A0JNW5	A0JNW
5	NaN	NaN	NaN	+			1.7907...	270370...	215370...	550060...	E9PHQ...	E9PHQ
6	NaN	NaN	NaN				9.5202...	514250...	435080...	791680...	A0MZ6...	A0MZ6
7	NaN	NaN	0.730709				4.8278...	492420...	386300...	106120...	A0PJW...	A0PJW
8	NaN	NaN	NaN				1.8473...	583650...	380530...	203110...	Q1565...	Q1565
9	NaN	NaN	NaN				4.2013...	8772200	8038000	734270	A0T4C...	A0T4C
10	NaN	NaN	1.36252				3.3301...	403140...	285770...	117380...	A1A4S...	A1A4S
11	NaN	NaN	NaN	+			0.0001...	124580...	999490...	246320...	A1KZ9...	A1KZ9
12	NaN	NaN	NaN				2.5025...	330490...	296430...	3406000	A1L020	A1L02
13	0.659292	0.803885	0.848393				0	327420...	267380...	600400...	A1L0T...	A1L0T
14	NaN	NaN	NaN				2.4735...	975370...	751600...	223770...	A1L188	A1L18
15	1.82832	1.58992	1.63713				0	179760...	153430...	263300...	A1X28...	A1X28
16	0.532811	NaN	0.792298				1.8734...	241310...	204190...	371160...	Q86X1...	Q86X1
17	NaN	NaN	0.559604				2.1456...	772010...	592580...	179420...	A2A2G...	A2A2G
18	1.20431	NaN	1.3273				0	261260...	224830...	364340...	A2A2Q...	A2A2Q
19	NaN	NaN	NaN				3.9044...	309930...	244260...	656670...	P4269...	P4269
20	NaN	NaN	NaN				1.6859...	294430...	201560...	928690...	Q1467...	Q1467
21	NaN	NaN	NaN				1.5469...	0	0	0	A2A3N...	A2A3N
22	1.08225	0.893445	0.759342				0	132740...	110890...	218430...	P3561...	P3561
23	NaN	NaN	0.65183				4.4591...	870720...	690160...	180560...	P2806...	P2806
24	NaN	NaN	NaN				3.976E...	123110...	7939000	4372300	O0032...	O0032
25	NaN	NaN	NaN	+			1.6228...	0	0	0	A2NHM...	A2NHM
26	0.684863	0.978735	0.921552				0	108150...	807580...	273950...	Q9UBC...	Q9UBC
27	NaN	NaN	NaN	+			0.0001...	161420...	874090...	740140...	Q9GZY...	Q9GZY
28	0.573	0.80311	0.547657				0	126030...	967480...	292790...	A2RRP...	A2RRP
29	NaN	1.44914	NaN				1.271E...	709720...	553200...	156520...	A2RUC...	A2RUC
30	NaN	NaN	NaN				9.8889...	781840...	667720...	114120...	A2VDF...	A2VDF
31	NaN	NaN	NaN	+			0.0050...	0	0	0	A3KFI1	A3KFI1
32	NaN	NaN	NaN				6.8177...	893840...	669650...	224180...	A3KMH...	A3KMH

7681 items

Analysis of SILAC data

Now we again filter out the reverse , potential contaminants & identified by site hits.

7681 protein groups



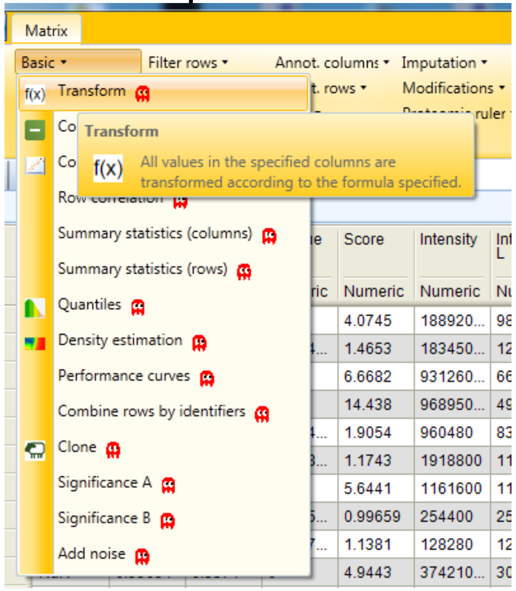
Filter rows based on categorical column

Those rows are kept or removed that have the specified value in the selected categorical column.

6767 protein groups

Analysis of SILAC data

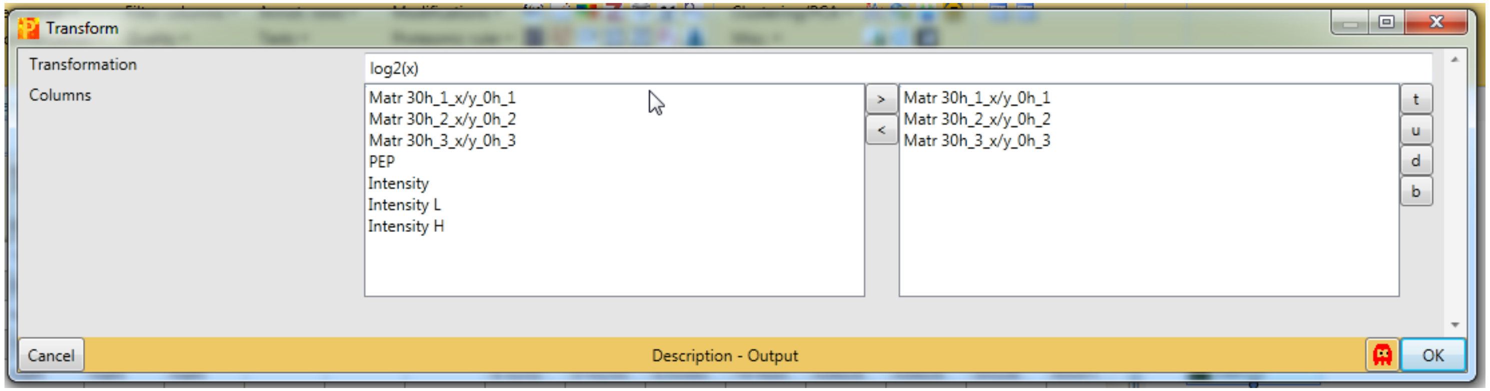
Next we linearize the SILAC ratios by transforming them to their log2-values. This way protein up and downregulations of the same magnitude have equal distances in visual representations.



Transform

f(x) All values in the specified columns are transformed according to the formula specified.

Score	Intensity
Numeric	Numeric
4.0745	188920...
1.4653	183450...
6.6682	931260...
14.438	968950...
1.9054	960480
1.1743	1918800
5.6441	1161600
0.99659	254400
1.1381	128280
4.9443	374210...



Transformation: log2(x)

Columns:

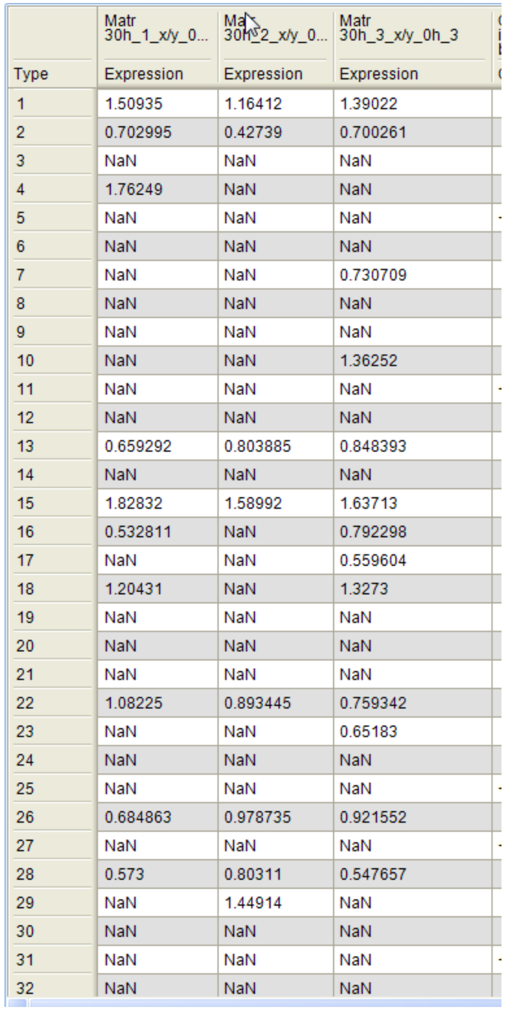
Matr 30h_1_x/y_0h_1
Matr 30h_2_x/y_0h_2
Matr 30h_3_x/y_0h_3
PEP
Intensity
Intensity L
Intensity H

Selected:

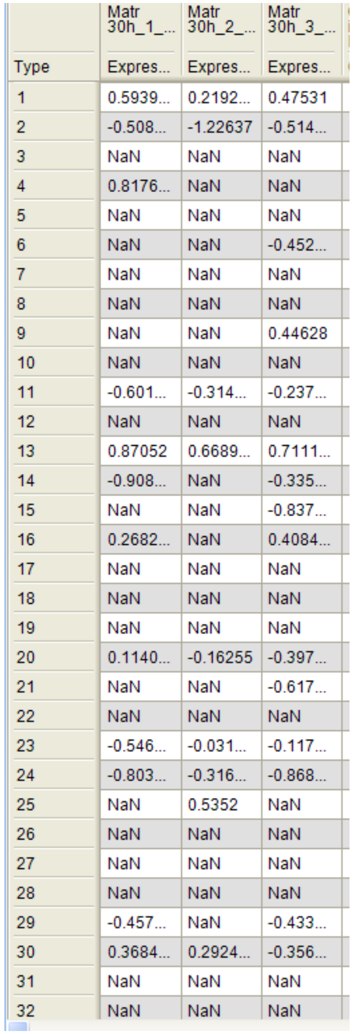
Matr 30h_1_x/y_0h_1
Matr 30h_2_x/y_0h_2
Matr 30h_3_x/y_0h_3

Analysis of SILAC data

The expression ratios are now log2-transformed.



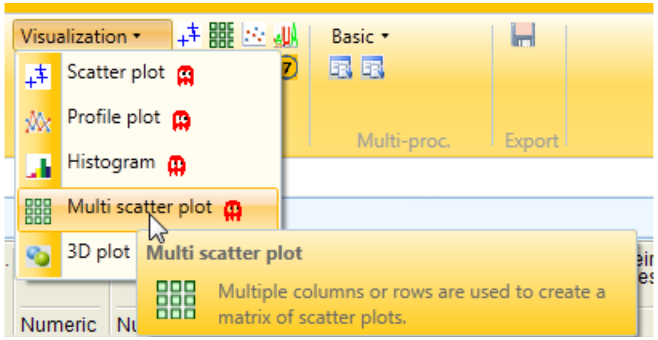
Type	Matr 30h_1_x/y_0... Expression	Ma 30h_2_x/y_0... Expression	Matr 30h_3_x/y_0h_3 Expression
1	1.50935	1.16412	1.39022
2	0.702995	0.42739	0.700261
3	NaN	NaN	NaN
4	1.76249	NaN	NaN
5	NaN	NaN	NaN
6	NaN	NaN	NaN
7	NaN	NaN	0.730709
8	NaN	NaN	NaN
9	NaN	NaN	NaN
10	NaN	NaN	1.36252
11	NaN	NaN	NaN
12	NaN	NaN	NaN
13	0.659292	0.803885	0.848393
14	NaN	NaN	NaN
15	1.82832	1.58992	1.63713
16	0.532811	NaN	0.792298
17	NaN	NaN	0.559604
18	1.20431	NaN	1.3273
19	NaN	NaN	NaN
20	NaN	NaN	NaN
21	NaN	NaN	NaN
22	1.08225	0.893445	0.759342
23	NaN	NaN	0.65183
24	NaN	NaN	NaN
25	NaN	NaN	NaN
26	0.684863	0.978735	0.921552
27	NaN	NaN	NaN
28	0.573	0.80311	0.547657
29	NaN	1.44914	NaN
30	NaN	NaN	NaN
31	NaN	NaN	NaN
32	NaN	NaN	NaN



Type	Matr 30h_1_... Expres...	Matr 30h_2_... Expres...	Matr 30h_3_... Expres...
1	0.5939...	0.2192...	0.47531
2	-0.508...	-1.22637	-0.514...
3	NaN	NaN	NaN
4	0.8176...	NaN	NaN
5	NaN	NaN	NaN
6	NaN	NaN	-0.452...
7	NaN	NaN	NaN
8	NaN	NaN	NaN
9	NaN	NaN	0.44628
10	NaN	NaN	NaN
11	-0.601...	-0.314...	-0.237...
12	NaN	NaN	NaN
13	0.87052	0.6689...	0.7111...
14	-0.908...	NaN	-0.335...
15	NaN	NaN	-0.837...
16	0.2682...	NaN	0.4084...
17	NaN	NaN	NaN
18	NaN	NaN	NaN
19	NaN	NaN	NaN
20	0.1140...	-0.16255	-0.397...
21	NaN	NaN	-0.617...
22	NaN	NaN	NaN
23	-0.546...	-0.031...	-0.117...
24	-0.803...	-0.316...	-0.868...
25	NaN	0.5352	NaN
26	NaN	NaN	NaN
27	NaN	NaN	NaN
28	NaN	NaN	NaN
29	-0.457...	NaN	-0.433...
30	0.3684...	0.2924...	-0.356...
31	NaN	NaN	NaN
32	NaN	NaN	NaN

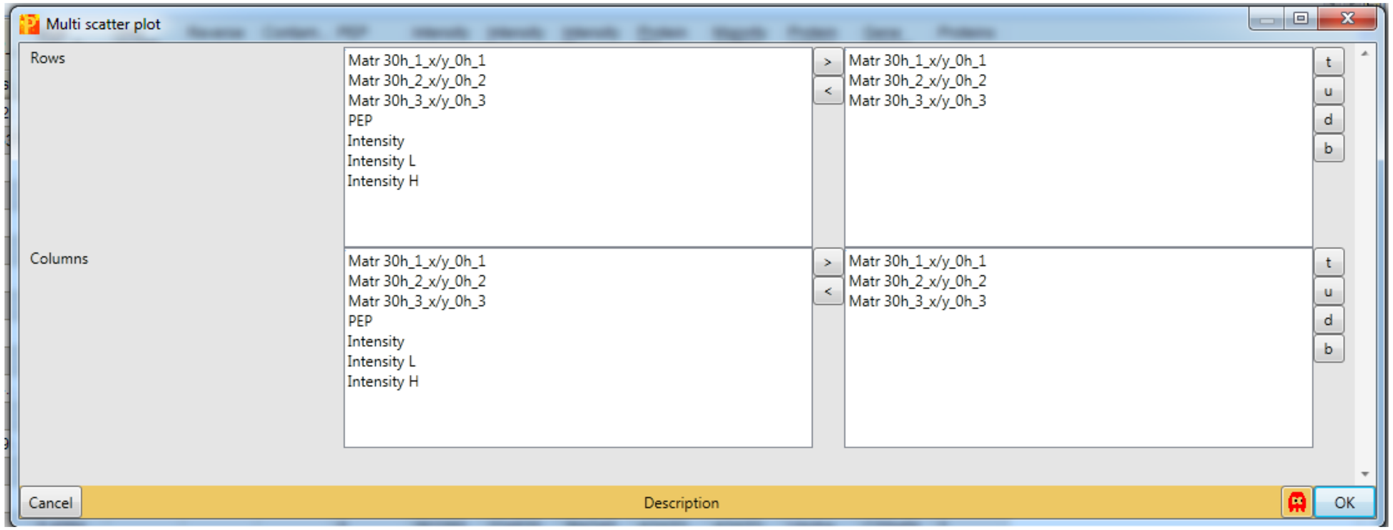
Analysis of SILAC data

To assess the reproducibility of the samples we perform a multi scatter plot. In this the expression values of each sample are compared to all others'.



Multi scatter plot

Multiple columns or rows are used to create a matrix of scatter plots.



Rows

Matr 30h_1_x/y_0h_1
Matr 30h_2_x/y_0h_2
Matr 30h_3_x/y_0h_3
PEP
Intensity
Intensity L
Intensity H

Selected:
Matr 30h_1_x/y_0h_1
Matr 30h_2_x/y_0h_2
Matr 30h_3_x/y_0h_3

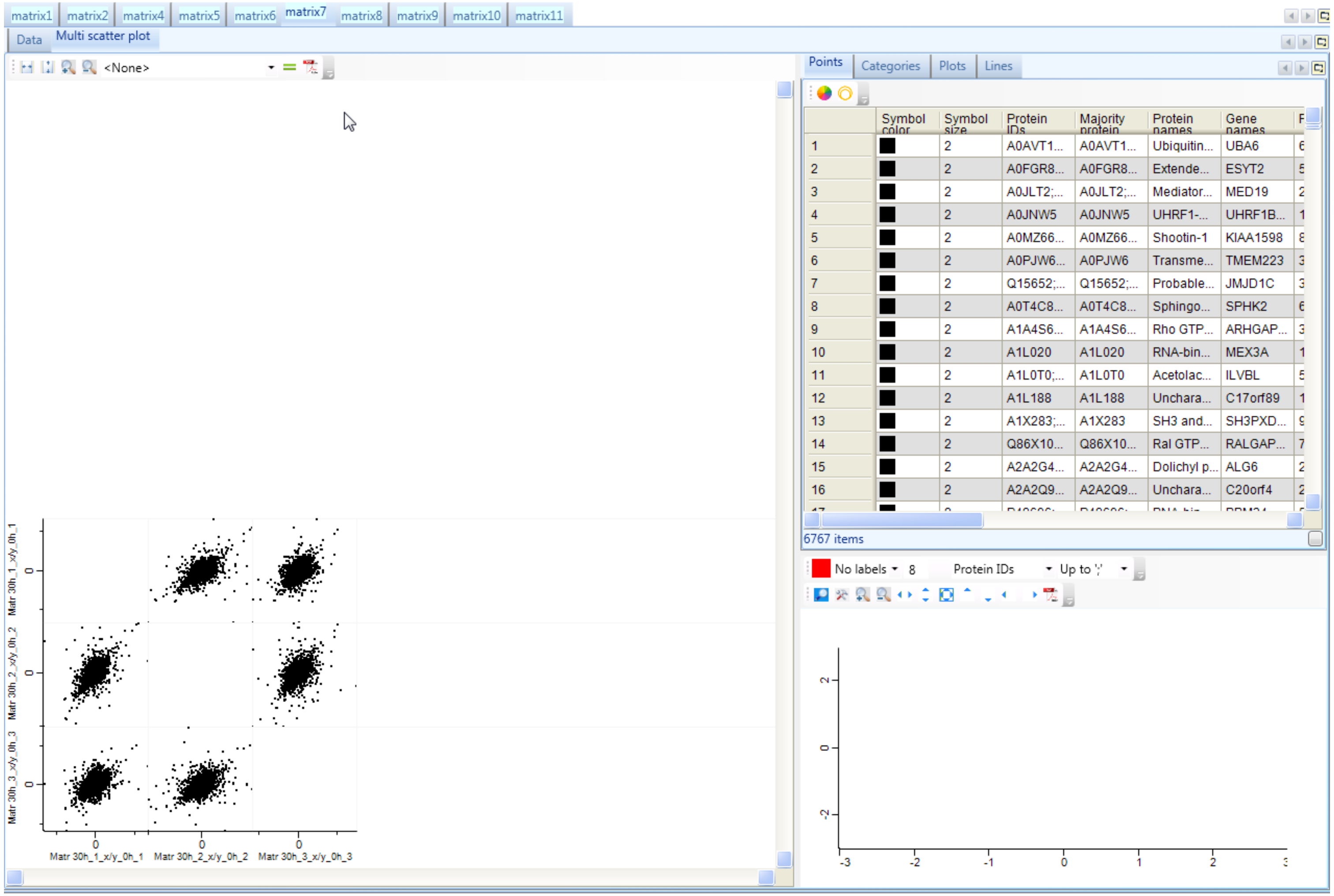
Columns

Matr 30h_1_x/y_0h_1
Matr 30h_2_x/y_0h_2
Matr 30h_3_x/y_0h_3
PEP
Intensity
Intensity L
Intensity H

Selected:
Matr 30h_1_x/y_0h_1
Matr 30h_2_x/y_0h_2
Matr 30h_3_x/y_0h_3

Analysis of SILAC data

To assess the reproducibility of the samples we perform a multi scatter plot. In this the expression values of each sample are compared to all others'.

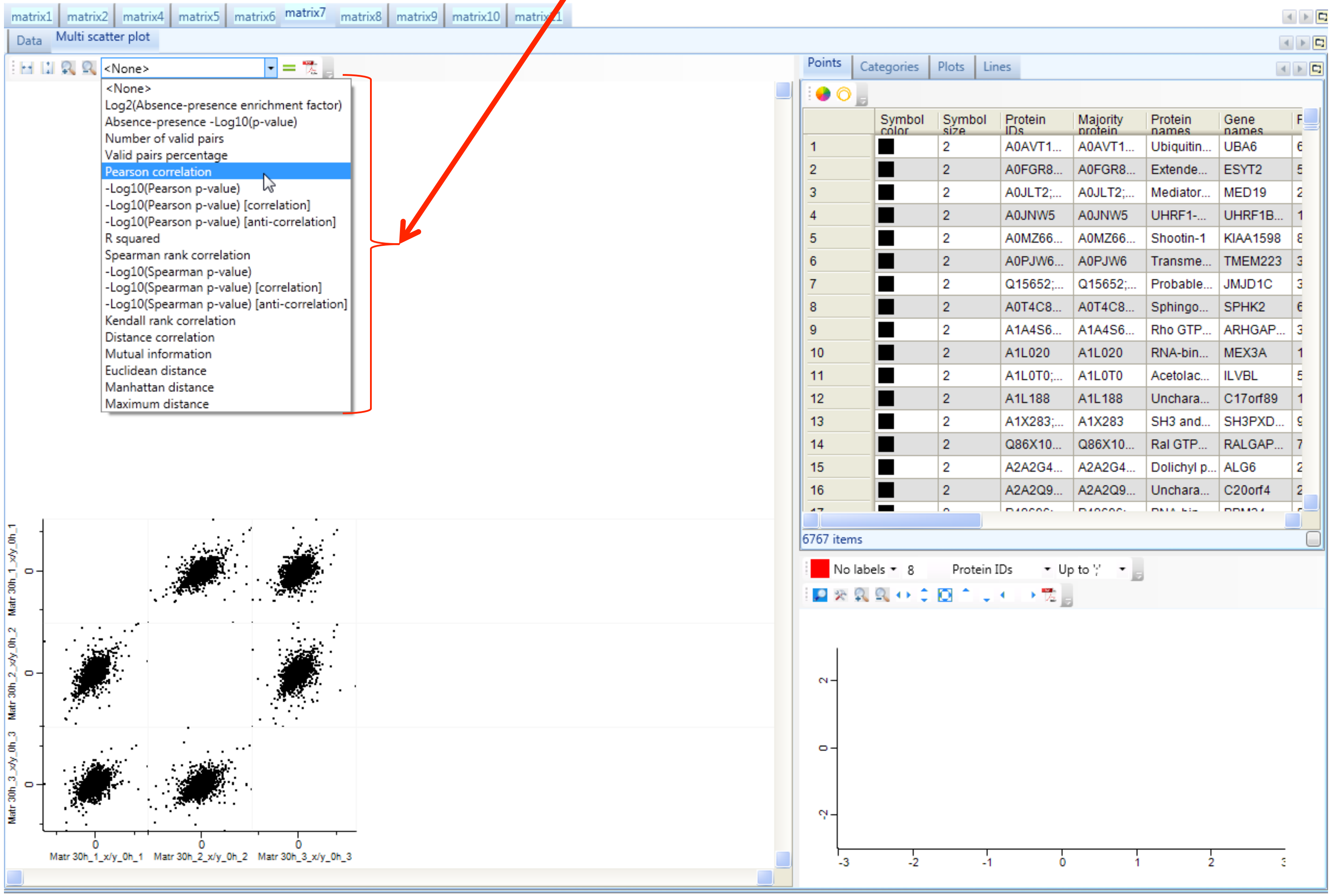


	Symbol color	Symbol size	Protein IDs	Majority protein	Protein names	Gene names
1	■	2	A0AVT1...	A0AVT1...	Ubiquitin...	UBA6
2	■	2	A0FGR8...	A0FGR8...	Extende...	ESYT2
3	■	2	A0JLT2;...	A0JLT2;...	Mediator...	MED19
4	■	2	A0JNW5	A0JNW5	UHRF1-...	UHRF1B...
5	■	2	A0MZ66...	A0MZ66...	Shootin-1	KIAA1598
6	■	2	A0PJW6...	A0PJW6	Transme...	TMEM223
7	■	2	Q15652;...	Q15652;...	Probable...	JMJD1C
8	■	2	A0T4C8...	A0T4C8...	Sphingo...	SPHK2
9	■	2	A1A4S6...	A1A4S6...	Rho GTP...	ARHGAP...
10	■	2	A1L020	A1L020	RNA-bin...	MEX3A
11	■	2	A1L0T0;...	A1L0T0	Acetolac...	ILVBL
12	■	2	A1L188	A1L188	Unchara...	C17orf89
13	■	2	A1X283;...	A1X283	SH3 and...	SH3PXD...
14	■	2	Q86X10...	Q86X10...	Ral GTP...	RALGAP...
15	■	2	A2A2G4...	A2A2G4...	Dolichyl p...	ALG6
16	■	2	A2A2Q9...	A2A2Q9...	Unchara...	C20orf4

6767 items

Analysis of SILAC data

To see how well the three samples correlate we let Perseus do a Pearson correlation. Notice that **other common correlations** can also be calculated.

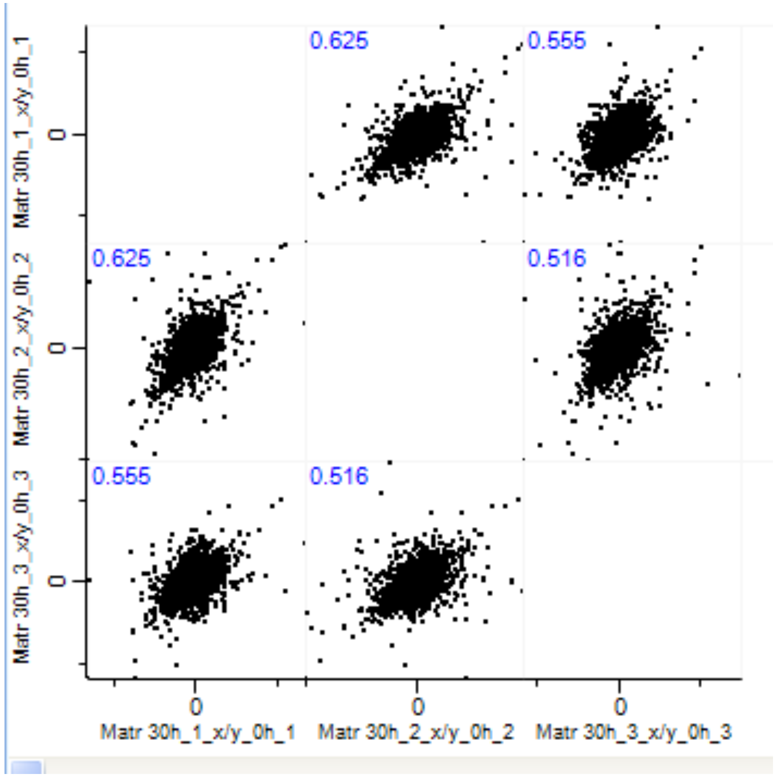


	Symbol color	Symbol size	Protein IDs	Majority protein	Protein names	Gene names
1	■	2	A0AVT1...	A0AVT1...	Ubiquitin...	UBA6
2	■	2	A0FGR8...	A0FGR8...	Extende...	ESYT2
3	■	2	A0JLT2;...	A0JLT2;...	Mediator...	MED19
4	■	2	A0JNW5	A0JNW5	UHRF1-...	UHRF1B...
5	■	2	A0MZ66...	A0MZ66...	Shootin-1	KIAA1598
6	■	2	A0PJW6...	A0PJW6	Transme...	TMEM223
7	■	2	Q15652;...	Q15652;...	Probable...	JMJD1C
8	■	2	A0T4C8...	A0T4C8...	Sphingo...	SPHK2
9	■	2	A1A4S6...	A1A4S6...	Rho GTP...	ARHGAP...
10	■	2	A1L020	A1L020	RNA-bin...	MEX3A
11	■	2	A1L0T0;...	A1L0T0	Acetolac...	ILVBL
12	■	2	A1L188	A1L188	Unchara...	C17orf89
13	■	2	A1X283;...	A1X283	SH3 and...	SH3PXD...
14	■	2	Q86X10...	Q86X10...	Ral GTP...	RALGAP...
15	■	2	A2A2G4...	A2A2G4...	Dolichyl p...	ALG6
16	■	2	A2A2Q9...	A2A2Q9...	Unchara...	C20orf4

6767 items

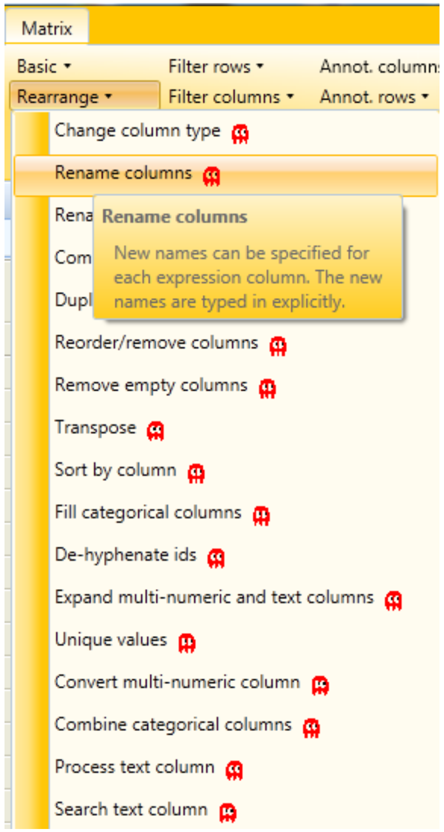
Analysis of SILAC data

The results of the Pearson correlation analysis show a medium degree of correlation between the three samples.



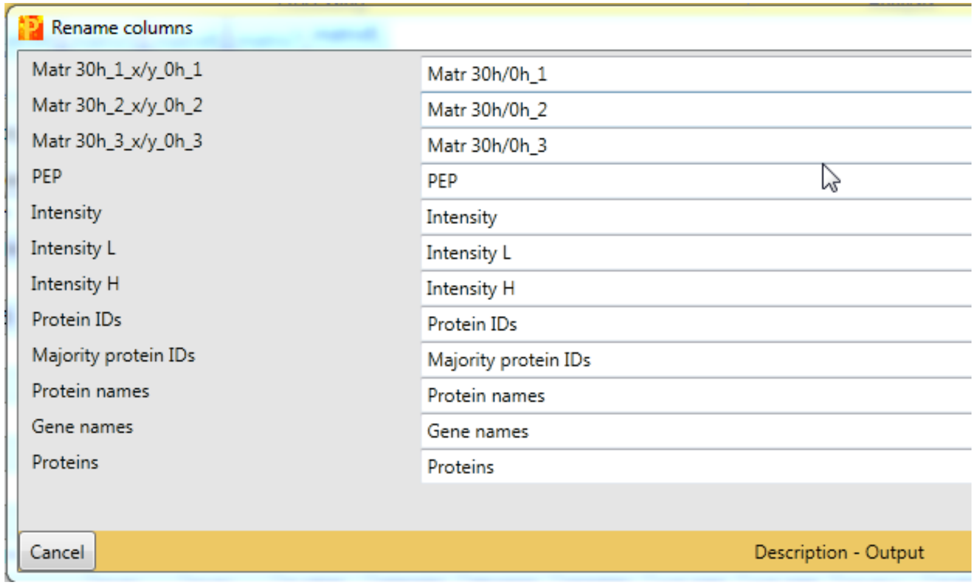
Analysis of SILAC data

At the next step we rename the columns to Matr 30h/0h_....



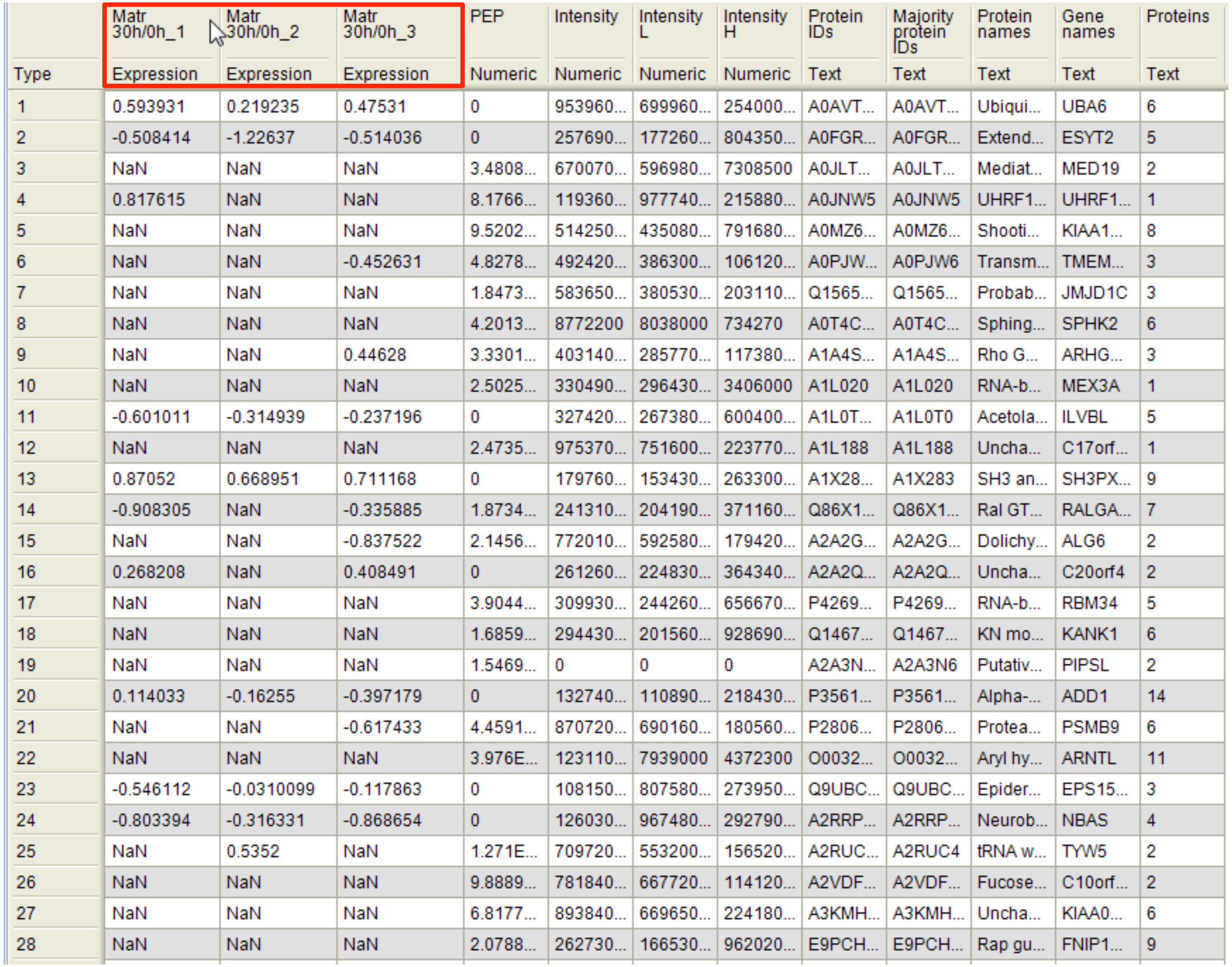
Rename columns

New names can be specified for each expression column. The new names are typed in explicitly.



Matr 30h_1_x/y_0h_1	Matr 30h/0h_1
Matr 30h_2_x/y_0h_2	Matr 30h/0h_2
Matr 30h_3_x/y_0h_3	Matr 30h/0h_3
PEP	PEP
Intensity	Intensity
Intensity L	Intensity L
Intensity H	Intensity H
Protein IDs	Protein IDs
Majority protein IDs	Majority protein IDs
Protein names	Protein names
Gene names	Gene names
Proteins	Proteins

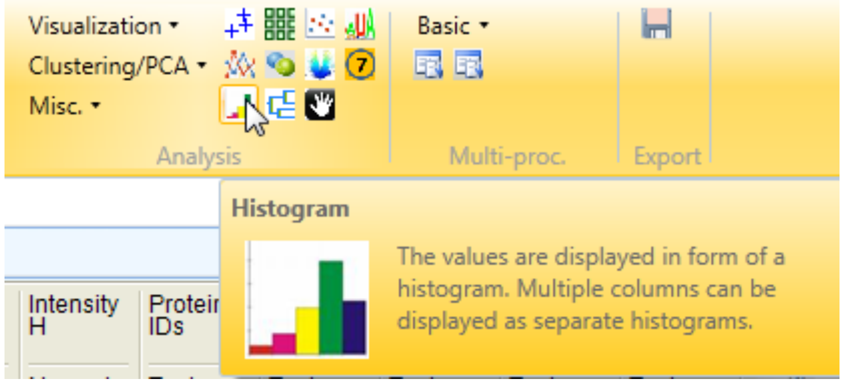
Analysis of SILAC data



	Matr 30h/0h_1	Matr 30h/0h_2	Matr 30h/0h_3	PEP	Intensity	Intensity L	Intensity H	Protein IDs	Majority protein IDs	Protein names	Gene names	Proteins
Type	Expression	Expression	Expression	Numeric	Numeric	Numeric	Numeric	Text	Text	Text	Text	Text
1	0.593931	0.219235	0.47531	0	953960...	699960...	254000...	A0AVT...	A0AVT...	Ubiqui...	UBA6	6
2	-0.508414	-1.22637	-0.514036	0	257690...	177260...	804350...	A0FGR...	A0FGR...	Extend...	ESYT2	5
3	NaN	NaN	NaN	3.4808...	670070...	596980...	7308500	A0JLT...	A0JLT...	Mediat...	MED19	2
4	0.817615	NaN	NaN	8.1766...	119360...	977740...	215880...	A0JNW5	A0JNW5	UHRF1...	UHRF1...	1
5	NaN	NaN	NaN	9.5202...	514250...	435080...	791680...	A0MZ6...	A0MZ6...	Shooti...	KIAA1...	8
6	NaN	NaN	-0.452631	4.8278...	492420...	386300...	106120...	A0PJW...	A0PJW6	Transm...	TMEM...	3
7	NaN	NaN	NaN	1.8473...	583650...	380530...	203110...	Q1565...	Q1565...	Probab...	JMJD1C	3
8	NaN	NaN	NaN	4.2013...	8772200	8038000	734270	A0T4C...	A0T4C...	Sphing...	SPHK2	6
9	NaN	NaN	0.44628	3.3301...	403140...	285770...	117380...	A1A4S...	A1A4S...	Rho G...	ARHG...	3
10	NaN	NaN	NaN	2.5025...	330490...	296430...	3406000	A1L020	A1L020	RNA-b...	MEX3A	1
11	-0.601011	-0.314939	-0.237196	0	327420...	267380...	600400...	A1L0T...	A1L0T0	Acetola...	ILVBL	5
12	NaN	NaN	NaN	2.4735...	975370...	751600...	223770...	A1L188	A1L188	Uncha...	C17orf...	1
13	0.87052	0.668951	0.711168	0	179760...	153430...	263300...	A1X28...	A1X283	SH3 an...	SH3PX...	9
14	-0.908305	NaN	-0.335885	1.8734...	241310...	204190...	371160...	Q86X1...	Q86X1...	Ral GT...	RALGA...	7
15	NaN	NaN	-0.837522	2.1456...	772010...	592580...	179420...	A2A2G...	A2A2G...	Dolichy...	ALG6	2
16	0.268208	NaN	0.408491	0	261260...	224830...	364340...	A2A2Q...	A2A2Q...	Uncha...	C20orf4	2
17	NaN	NaN	NaN	3.9044...	309930...	244260...	656670...	P4269...	P4269...	RNA-b...	RBM34	5
18	NaN	NaN	NaN	1.6859...	294430...	201560...	928690...	Q1467...	Q1467...	KN mo...	KANK1	6
19	NaN	NaN	NaN	1.5469...	0	0	0	A2A3N...	A2A3N6	Putativ...	PIPSL	2
20	0.114033	-0.16255	-0.397179	0	132740...	110890...	218430...	P3561...	P3561...	Alpha-...	ADD1	14
21	NaN	NaN	-0.617433	4.4591...	870720...	690160...	180560...	P2806...	P2806...	Protea...	PSMB9	6
22	NaN	NaN	NaN	3.976E...	123110...	7939000	4372300	O0032...	O0032...	Aryl hy...	ARNTL	11
23	-0.546112	-0.0310099	-0.117863	0	108150...	807580...	273950...	Q9UBC...	Q9UBC...	Epider...	EPS15...	3
24	-0.803394	-0.316331	-0.868654	0	126030...	967480...	292790...	A2RRP...	A2RRP...	Neurob...	NBAS	4
25	NaN	0.5352	NaN	1.271E...	709720...	553200...	156520...	A2RUC...	A2RUC4	tRNA w...	TYW5	2
26	NaN	NaN	NaN	9.8889...	781840...	667720...	114120...	A2VDF...	A2VDF...	Fucose...	C10orf...	2
27	NaN	NaN	NaN	6.8177...	893840...	669650...	224180...	A3KMH...	A3KMH...	Uncha...	KIAA0...	6
28	NaN	NaN	NaN	2.0788...	262730...	166530...	962020...	E9PCH...	E9PCH...	Rap gu...	FNIP1...	9

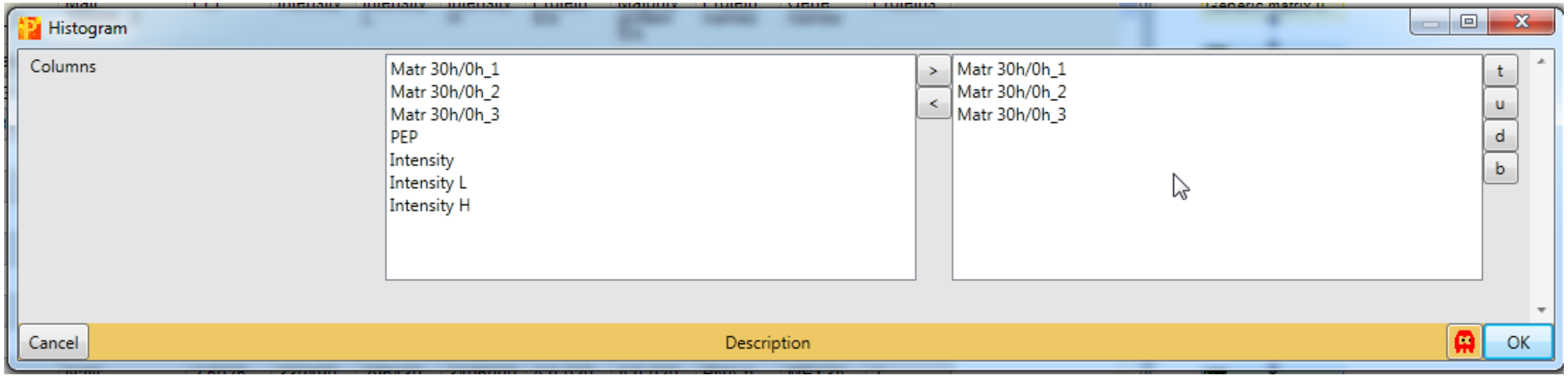
Analysis of SILAC data

To easily get an idea of the SILAC ratio distributions and see if they are normally distributed, we create a histogram.



Histogram

The values are displayed in form of a histogram. Multiple columns can be displayed as separate histograms.



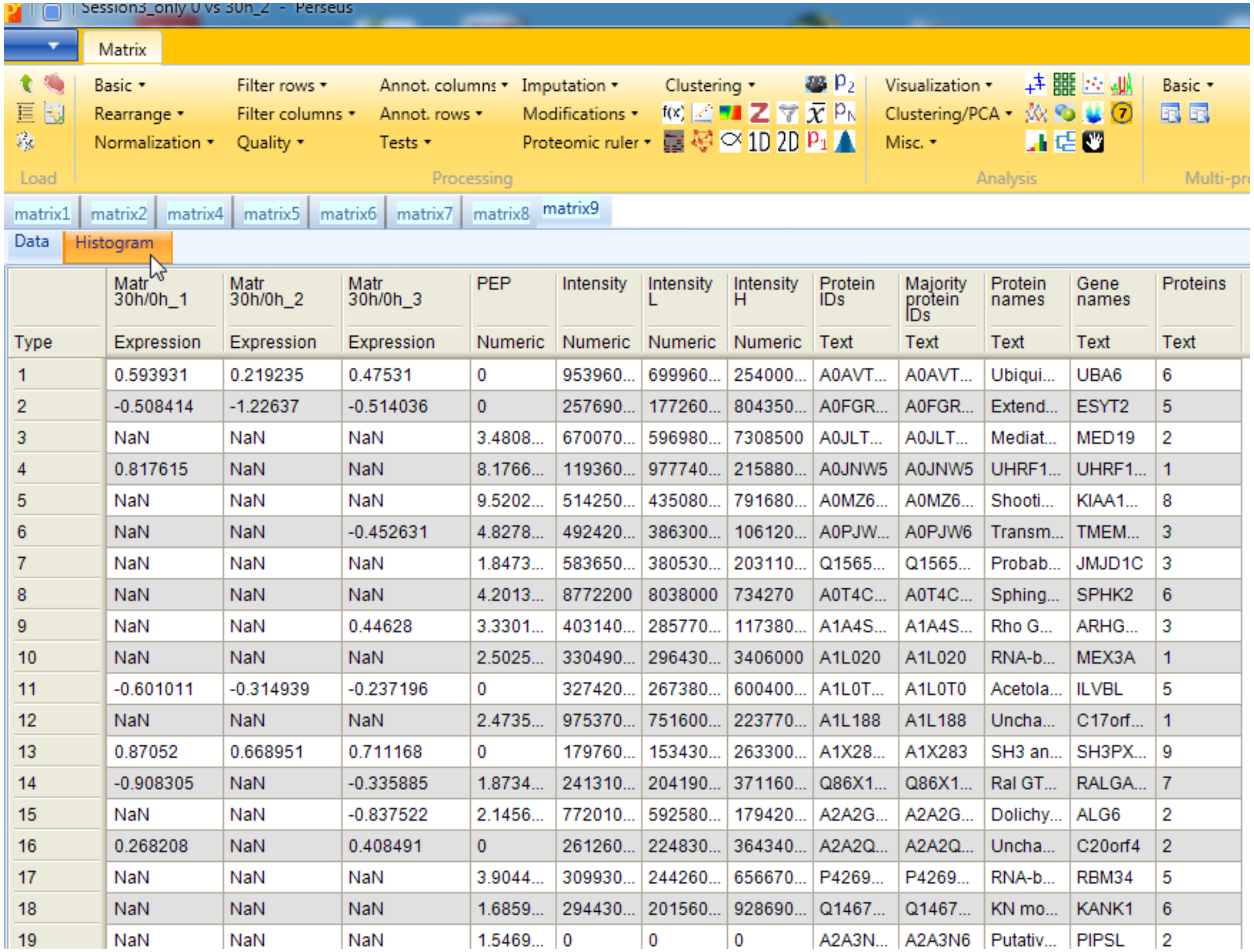
Columns

Matr 30h/0h_1
Matr 30h/0h_2
Matr 30h/0h_3
PEP
Intensity
Intensity L
Intensity H

Matr 30h/0h_1
Matr 30h/0h_2
Matr 30h/0h_3

Analysis of SILAC data

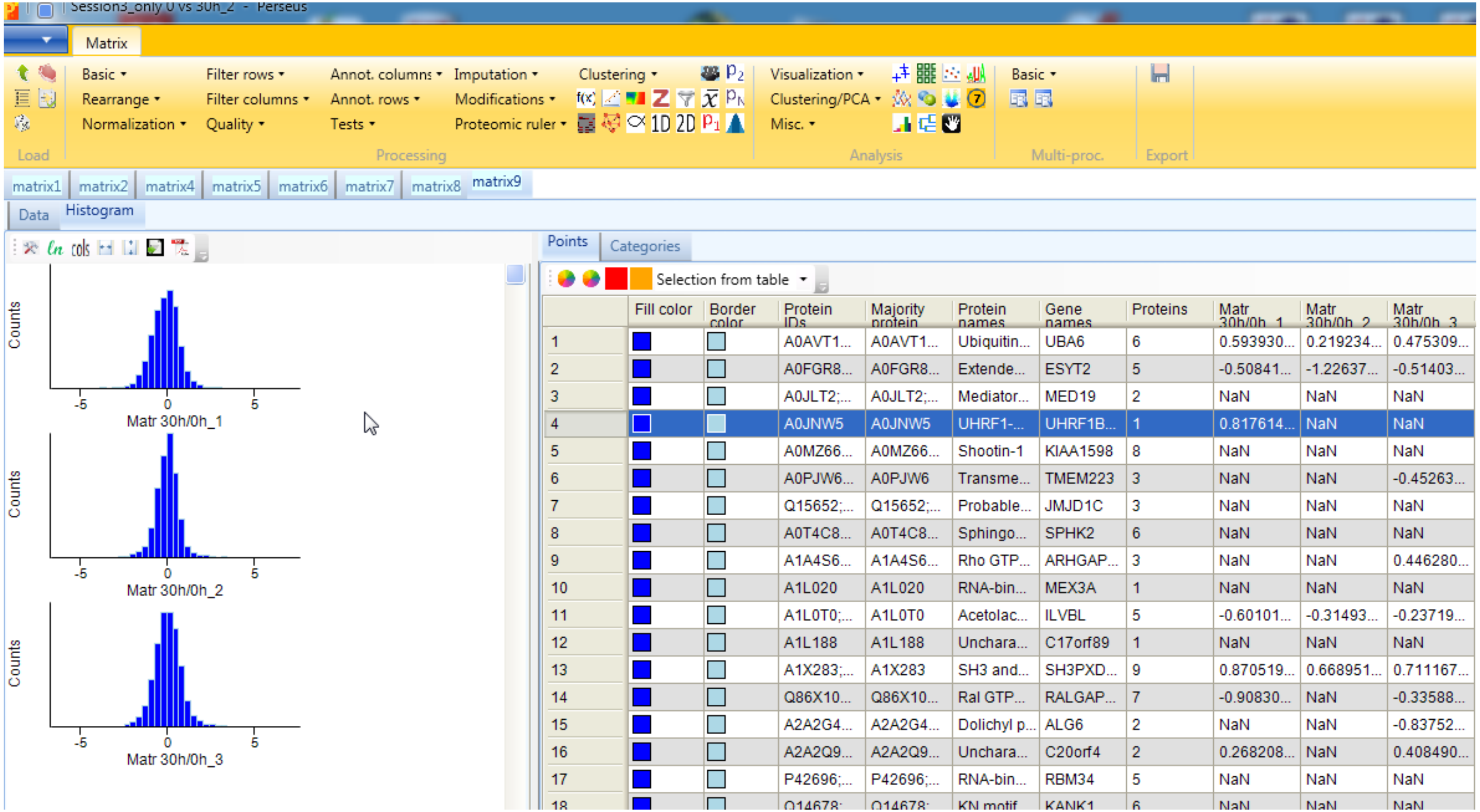
Visualizations are always created within the selected matrix. There they can be found in a separate tab.



	Matr 30h/0h_1	Matr 30h/0h_2	Matr 30h/0h_3	PEP	Intensity	Intensity L	Intensity H	Protein IDs	Majority protein IDs	Protein names	Gene names	Proteins
Type	Expression	Expression	Expression	Numeric	Numeric	Numeric	Numeric	Text	Text	Text	Text	Text
1	0.593931	0.219235	0.47531	0	953960...	699960...	254000...	A0AVT...	A0AVT...	Ubiqui...	UBA6	6
2	-0.508414	-1.22637	-0.514036	0	257690...	177260...	804350...	A0FGR...	A0FGR...	Extend...	ESYT2	5
3	NaN	NaN	NaN	3.4808...	670070...	596980...	7308500	A0JLT...	A0JLT...	Mediat...	MED19	2
4	0.817615	NaN	NaN	8.1766...	119360...	977740...	215880...	A0JNW5	A0JNW5	UHRF1...	UHRF1...	1
5	NaN	NaN	NaN	9.5202...	514250...	435080...	791680...	A0MZ6...	A0MZ6...	Shooti...	KIAA1...	8
6	NaN	NaN	-0.452631	4.8278...	492420...	386300...	106120...	A0PJW...	A0PJW6	Transm...	TMEM...	3
7	NaN	NaN	NaN	1.8473...	583650...	380530...	203110...	Q1565...	Q1565...	Probab...	JMJD1C	3
8	NaN	NaN	NaN	4.2013...	8772200	8038000	734270	A0T4C...	A0T4C...	Sphing...	SPHK2	6
9	NaN	NaN	0.44628	3.3301...	403140...	285770...	117380...	A1A4S...	A1A4S...	Rho G...	ARHG...	3
10	NaN	NaN	NaN	2.5025...	330490...	296430...	3406000	A1L020	A1L020	RNA-b...	MEX3A	1
11	-0.601011	-0.314939	-0.237196	0	327420...	267380...	600400...	A1L0T...	A1L0T0	Acetola...	ILVBL	5
12	NaN	NaN	NaN	2.4735...	975370...	751600...	223770...	A1L188	A1L188	Uncha...	C17orf...	1
13	0.87052	0.668951	0.711168	0	179760...	153430...	263300...	A1X28...	A1X283	SH3 an...	SH3PX...	9
14	-0.908305	NaN	-0.335885	1.8734...	241310...	204190...	371160...	Q86X1...	Q86X1...	Ral GT...	RALGA...	7
15	NaN	NaN	-0.837522	2.1456...	772010...	592580...	179420...	A2A2G...	A2A2G...	Dolichy...	ALG6	2
16	0.268208	NaN	0.408491	0	261260...	224830...	364340...	A2A2Q...	A2A2Q...	Uncha...	C20orf4	2
17	NaN	NaN	NaN	3.9044...	309930...	244260...	656670...	P4269...	P4269...	RNA-b...	RBM34	5
18	NaN	NaN	NaN	1.6859...	294430...	201560...	928690...	Q1467...	Q1467...	KN mo...	KANK1	6
19	NaN	NaN	NaN	1.5469...	0	0	0	A2A3N...	A2A3N6	Putativ...	PIPSL	2

Analysis of SILAC data

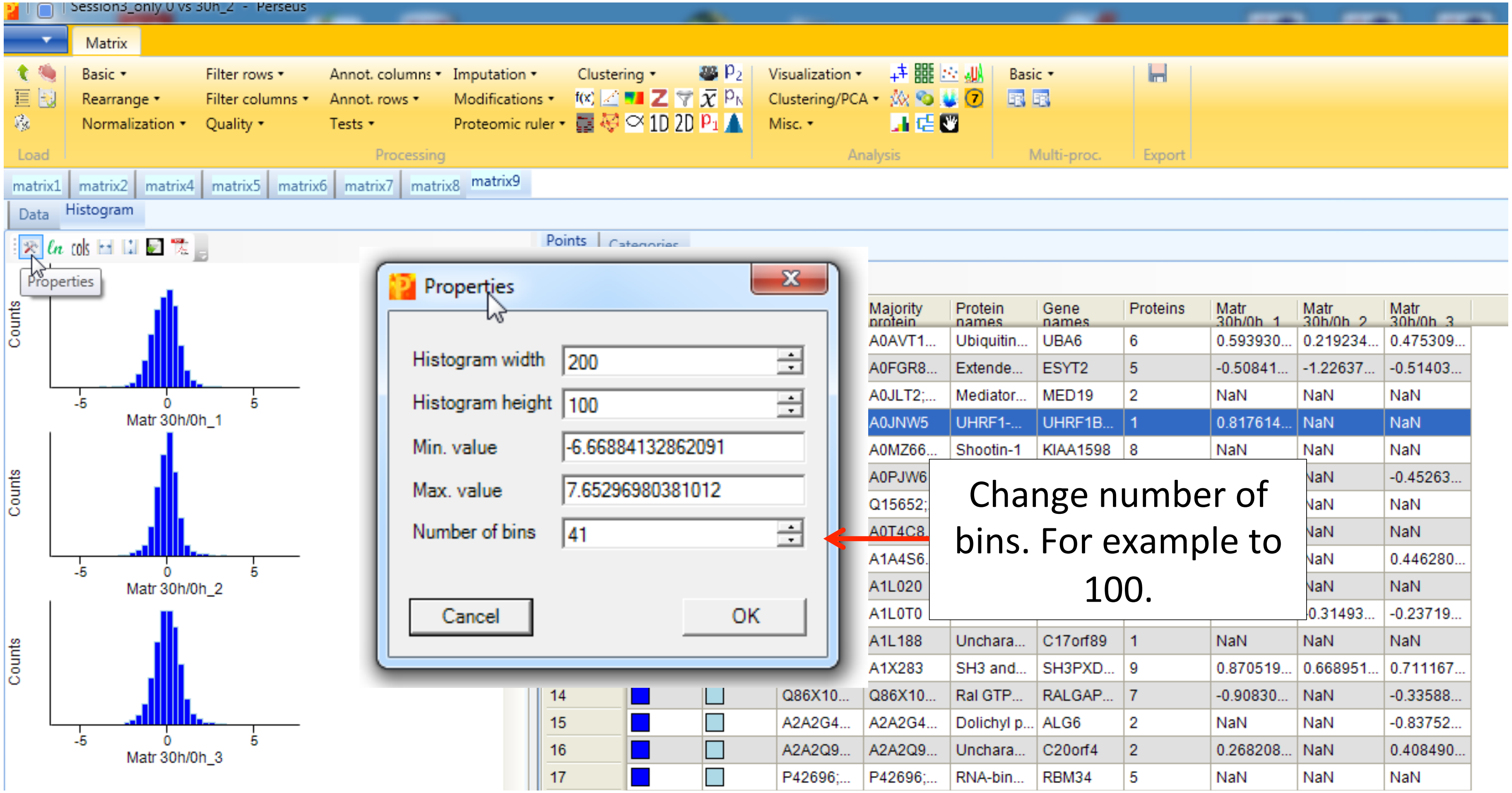
We can observe that the ratios are nicely distributed around 0.



	Fill color	Border color	Protein IDs	Majority protein	Protein names	Gene names	Proteins	Matr 30h/0h_1	Matr 30h/0h_2	Matr 30h/0h_3
1	🟦	☐	A0AVT1...	A0AVT1...	Ubiquitin...	UBA6	6	0.593930...	0.219234...	0.475309...
2	🟦	☐	A0FGR8...	A0FGR8...	Extende...	ESYT2	5	-0.50841...	-1.22637...	-0.51403...
3	🟦	☐	A0JLT2;...	A0JLT2;...	Mediator...	MED19	2	NaN	NaN	NaN
4	🟦	☐	A0JNW5	A0JNW5	UHRF1-...	UHRF1B...	1	0.817614...	NaN	NaN
5	🟦	☐	A0MZ66...	A0MZ66...	Shootin-1	KIAA1598	8	NaN	NaN	NaN
6	🟦	☐	A0PJW6...	A0PJW6	Transme...	TMEM223	3	NaN	NaN	-0.45263...
7	🟦	☐	Q15652;...	Q15652;...	Probable...	JMJD1C	3	NaN	NaN	NaN
8	🟦	☐	A0T4C8...	A0T4C8...	Sphingo...	SPHK2	6	NaN	NaN	NaN
9	🟦	☐	A1A4S6...	A1A4S6...	Rho GTP...	ARHGAP...	3	NaN	NaN	0.446280...
10	🟦	☐	A1L020	A1L020	RNA-bin...	MEX3A	1	NaN	NaN	NaN
11	🟦	☐	A1L0T0;...	A1L0T0	Acetolac...	ILVBL	5	-0.60101...	-0.31493...	-0.23719...
12	🟦	☐	A1L188	A1L188	Unchara...	C17orf89	1	NaN	NaN	NaN
13	🟦	☐	A1X283;...	A1X283	SH3 and...	SH3PXD...	9	0.870519...	0.668951...	0.711167...
14	🟦	☐	Q86X10...	Q86X10...	Ral GTP...	RALGAP...	7	-0.90830...	NaN	-0.33588...
15	🟦	☐	A2A2G4...	A2A2G4...	Dolichyl p...	ALG6	2	NaN	NaN	-0.83752...
16	🟦	☐	A2A2Q9...	A2A2Q9...	Unchara...	C20orf4	2	0.268208...	NaN	0.408490...
17	🟦	☐	P42696;...	P42696;...	RNA-bin...	RBM34	5	NaN	NaN	NaN
18	🟦	☐	Q14678;...	Q14678;...	KN motif...	KANK1	6	NaN	NaN	NaN

Analysis of SILAC data

The histogram can be further formatted and exported as a separate file.



Properties

Histogram width: 200

Histogram height: 100

Min. value: -6.66884132862091

Max. value: 7.65296980381012

Number of bins: 41

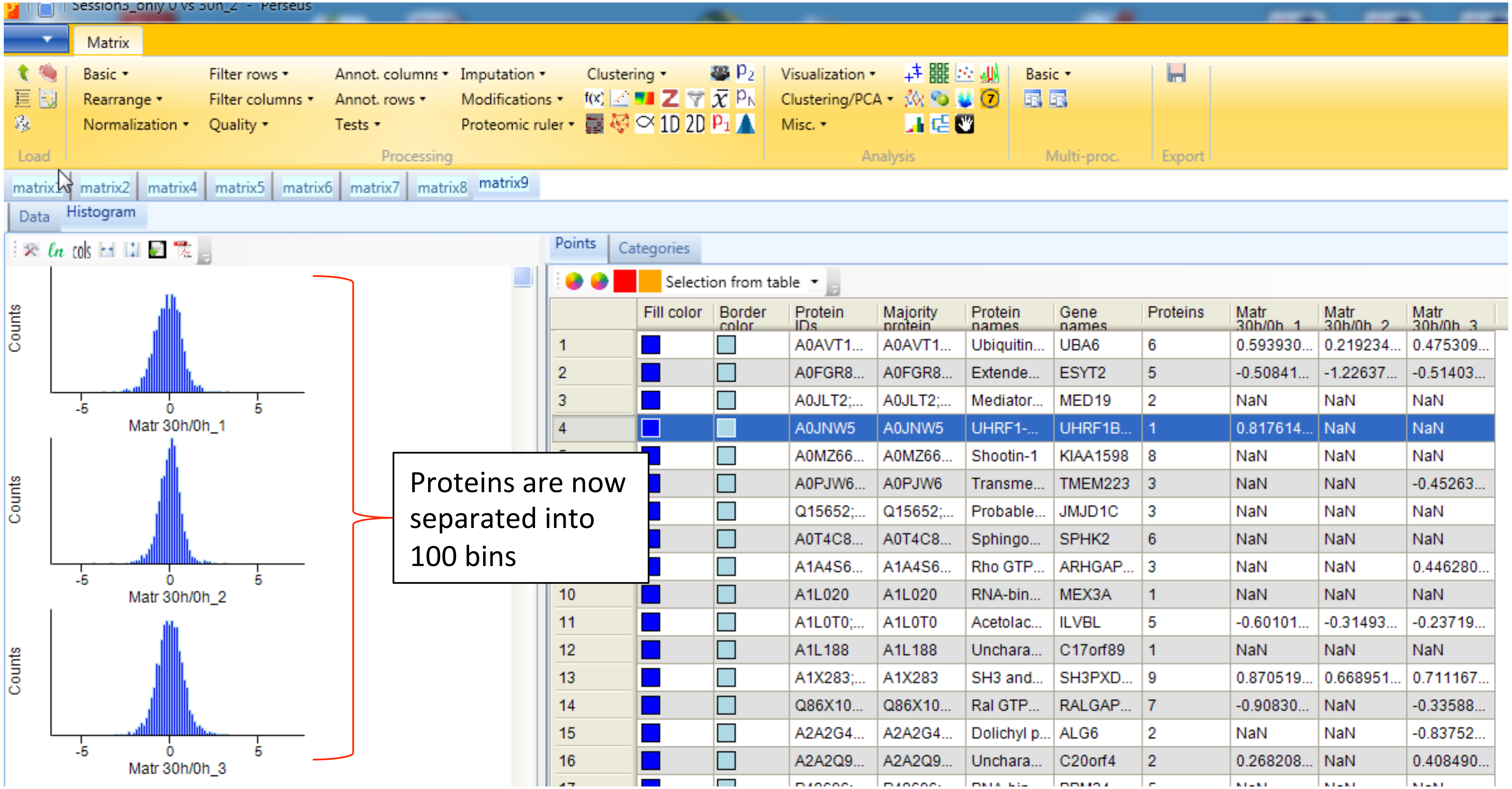
Cancel OK

Change number of bins. For example to 100.

Majority protein	Protein names	Gene names	Proteins	Matr 30h/0h_1	Matr 30h/0h_2	Matr 30h/0h_3
A0AVT1...	Ubiquitin...	UBA6	6	0.593930...	0.219234...	0.475309...
A0FGR8...	Extende...	ESYT2	5	-0.50841...	-1.22637...	-0.51403...
A0JLT2;...	Mediator...	MED19	2	NaN	NaN	NaN
A0JNW5	UHRF1-...	UHRF1B...	1	0.817614...	NaN	NaN
A0MZ66...	Shootin-1	KIAA1598	8	NaN	NaN	NaN
A0PJW6					NaN	-0.45263...
Q15652;					NaN	NaN
A0T4C8					NaN	NaN
A1A4S6					NaN	0.446280...
A1L020					NaN	NaN
A1L0T0					0.31493...	-0.23719...
A1L188	Unchara...	C17orf89	1	NaN	NaN	NaN
A1X283	SH3 and...	SH3PXD...	9	0.870519...	0.668951...	0.711167...
Q86X10...	Ral GTP...	RALGAP...	7	-0.90830...	NaN	-0.33588...
A2A2G4...	Dolichyl p...	ALG6	2	NaN	NaN	-0.83752...
A2A2Q9...	Unchara...	C20orf4	2	0.268208...	NaN	0.408490...
P42696;...	RNA-bin...	RBM34	5	NaN	NaN	NaN

Analysis of SILAC data

The histogram can be further formatted and exported as a separate file.

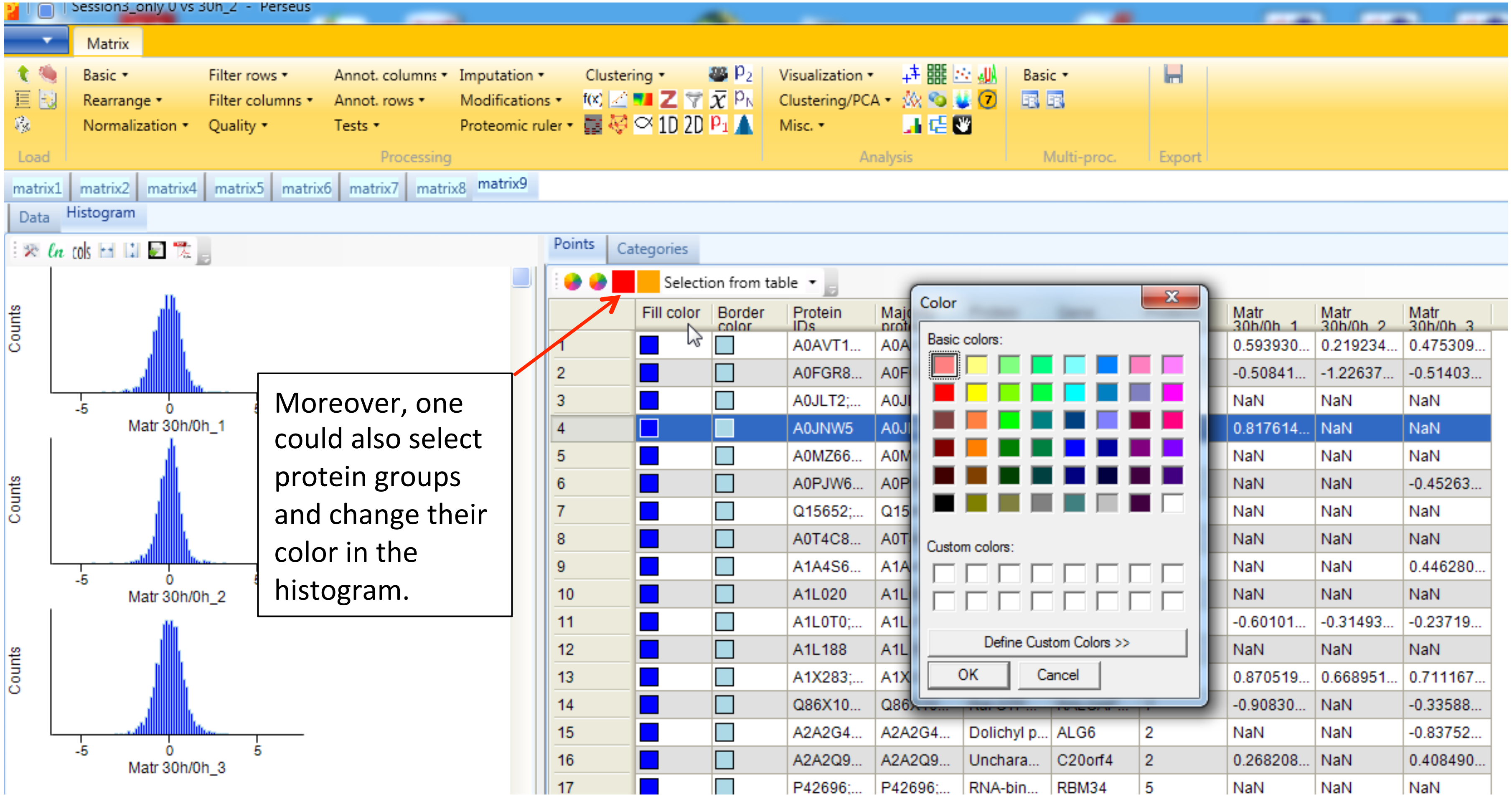


Proteins are now separated into 100 bins

	Fill color	Border color	Protein IDs	Majority protein	Protein names	Gene names	Proteins	Matr 30h/0h_1	Matr 30h/0h_2	Matr 30h/0h_3
1	🟦	☐	A0AVT1...	A0AVT1...	Ubiquitin...	UBA6	6	0.593930...	0.219234...	0.475309...
2	🟦	☐	A0FGR8...	A0FGR8...	Extende...	ESYT2	5	-0.50841...	-1.22637...	-0.51403...
3	🟦	☐	A0JLT2;...	A0JLT2;...	Mediator...	MED19	2	NaN	NaN	NaN
4	🟦	☐	A0JNW5	A0JNW5	UHRF1-...	UHRF1B...	1	0.817614...	NaN	NaN
	🟦	☐	A0MZ66...	A0MZ66...	Shootin-1	KIAA1598	8	NaN	NaN	NaN
	🟦	☐	A0PJW6...	A0PJW6	Transme...	TMEM223	3	NaN	NaN	-0.45263...
	🟦	☐	Q15652;...	Q15652;...	Probable...	JMJD1C	3	NaN	NaN	NaN
	🟦	☐	A0T4C8...	A0T4C8...	Sphingo...	SPHK2	6	NaN	NaN	NaN
	🟦	☐	A1A4S6...	A1A4S6...	Rho GTP...	ARHGAP...	3	NaN	NaN	0.446280...
10	🟦	☐	A1L020	A1L020	RNA-bin...	MEX3A	1	NaN	NaN	NaN
11	🟦	☐	A1L0T0;...	A1L0T0	Acetolac...	ILVBL	5	-0.60101...	-0.31493...	-0.23719...
12	🟦	☐	A1L188	A1L188	Unchara...	C17orf89	1	NaN	NaN	NaN
13	🟦	☐	A1X283;...	A1X283	SH3 and...	SH3PXD...	9	0.870519...	0.668951...	0.711167...
14	🟦	☐	Q86X10...	Q86X10...	Ral GTP...	RALGAP...	7	-0.90830...	NaN	-0.33588...
15	🟦	☐	A2A2G4...	A2A2G4...	Dolichyl p...	ALG6	2	NaN	NaN	-0.83752...
16	🟦	☐	A2A2Q9...	A2A2Q9...	Unchara...	C20orf4	2	0.268208...	NaN	0.408490...

Analysis of SILAC data

The histogram can be further formatted and exported as a separate file.

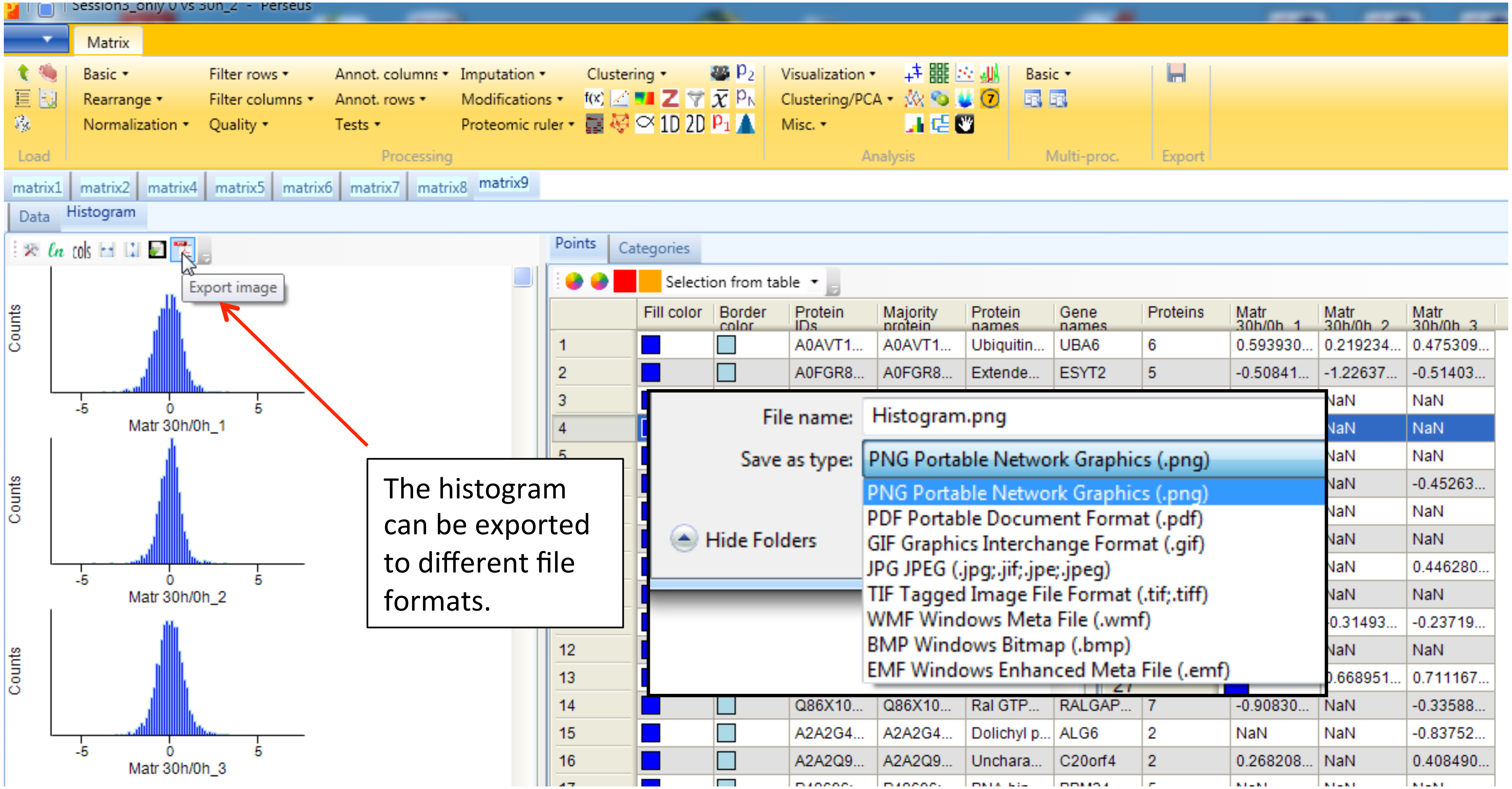


	Fill color	Border color	Protein IDs	Matr 30h/0h_1	Matr 30h/0h_2	Matr 30h/0h_3
1	■	□	A0AVT1...	0.593930...	0.219234...	0.475309...
2	■	□	A0FGR8...	-0.50841...	-1.22637...	-0.51403...
3	■	□	A0JLT2;...	NaN	NaN	NaN
4	■	□	A0JNW5	0.817614...	NaN	NaN
5	■	□	A0MZ66...	NaN	NaN	NaN
6	■	□	A0PJW6...	NaN	NaN	-0.45263...
7	■	□	Q15652;...	NaN	NaN	NaN
8	■	□	A0T4C8...	NaN	NaN	NaN
9	■	□	A1A4S6...	NaN	NaN	0.446280...
10	■	□	A1L020	NaN	NaN	NaN
11	■	□	A1L0T0;...	-0.60101...	-0.31493...	-0.23719...
12	■	□	A1L188	NaN	NaN	NaN
13	■	□	A1X283;...	0.870519...	0.668951...	0.711167...
14	■	□	Q86X10...	-0.90830...	NaN	-0.33588...
15	■	□	A2A2G4...	NaN	NaN	-0.83752...
16	■	□	A2A2Q9...	0.268208...	NaN	0.408490...
17	■	□	P42696;...	NaN	NaN	NaN

Moreover, one could also select protein groups and change their color in the histogram.

Analysis of SILAC data

The histogram can be further formatted and exported as a separate file.



File name: Histogram.png

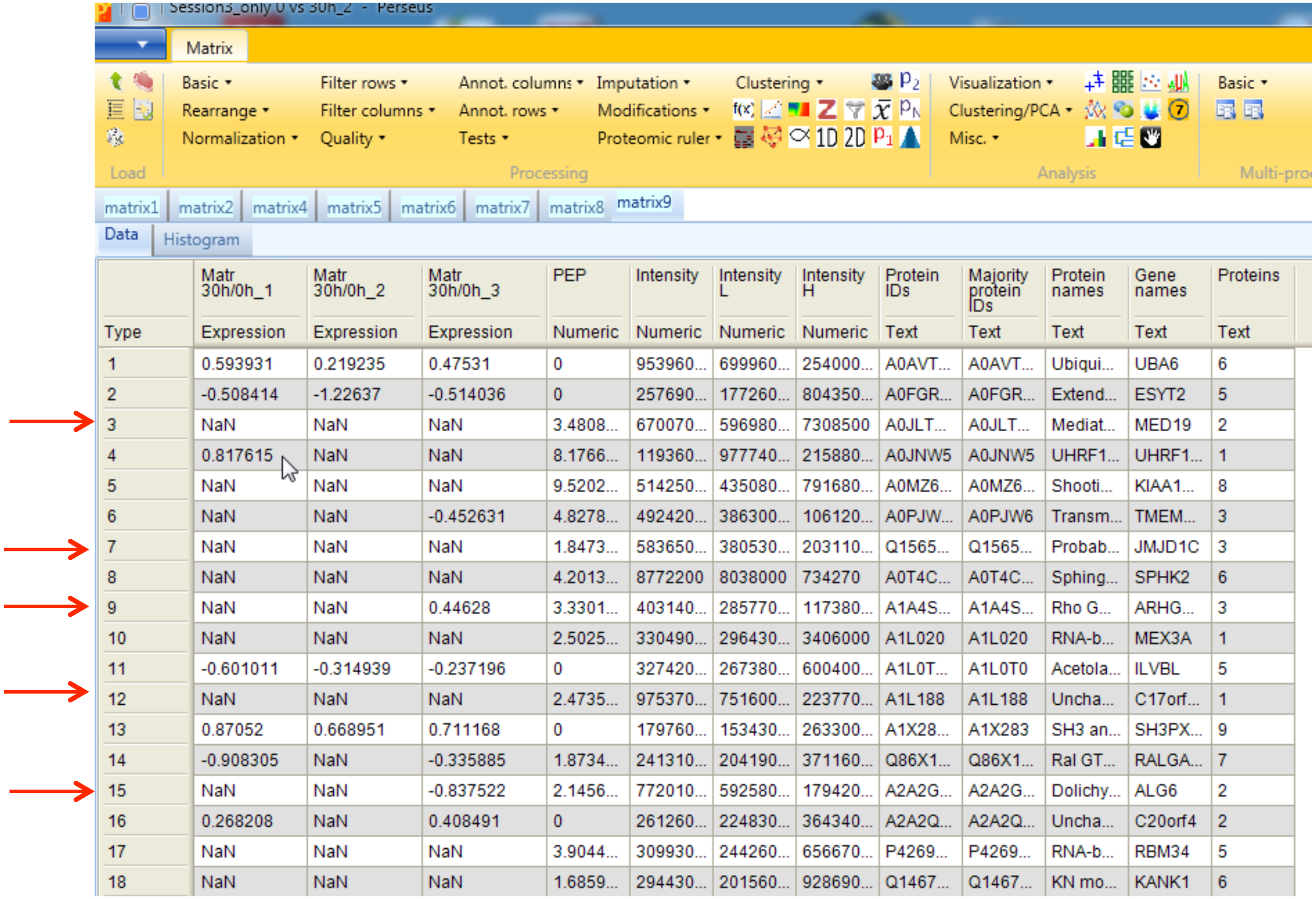
Save as type: PNG Portable Network Graphics (.png)

	Fill color	Border color	Protein IDs	Majority protein	Protein names	Gene names	Proteins	Matr 30h/0h_1	Matr 30h/0h_2	Matr 30h/0h_3
1	🟦	▢	A0AVT1...	A0AVT1...	Ubiquitin...	UBA6	6	0.593930...	0.219234...	0.475309...
2	🟦	▢	A0FGR8...	A0FGR8...	Extende...	ESYT2	5	-0.50841...	-1.22637...	-0.51403...
3										NaN
4										NaN
12										NaN
13										0.711167...
14	🟦	▢	Q86X10...	Q86X10...	Ral GTP...	RALGAP...	7	-0.90830...	NaN	-0.33588...
15	🟦	▢	A2A2G4...	A2A2G4...	Dolichyl p...	ALG6	2	NaN	NaN	-0.83752...
16	🟦	▢	A2A2Q9...	A2A2Q9...	Unchara...	C20orf4	2	0.268208...	NaN	0.408490...

The histogram can be exported to different file formats.

Analysis of SILAC data

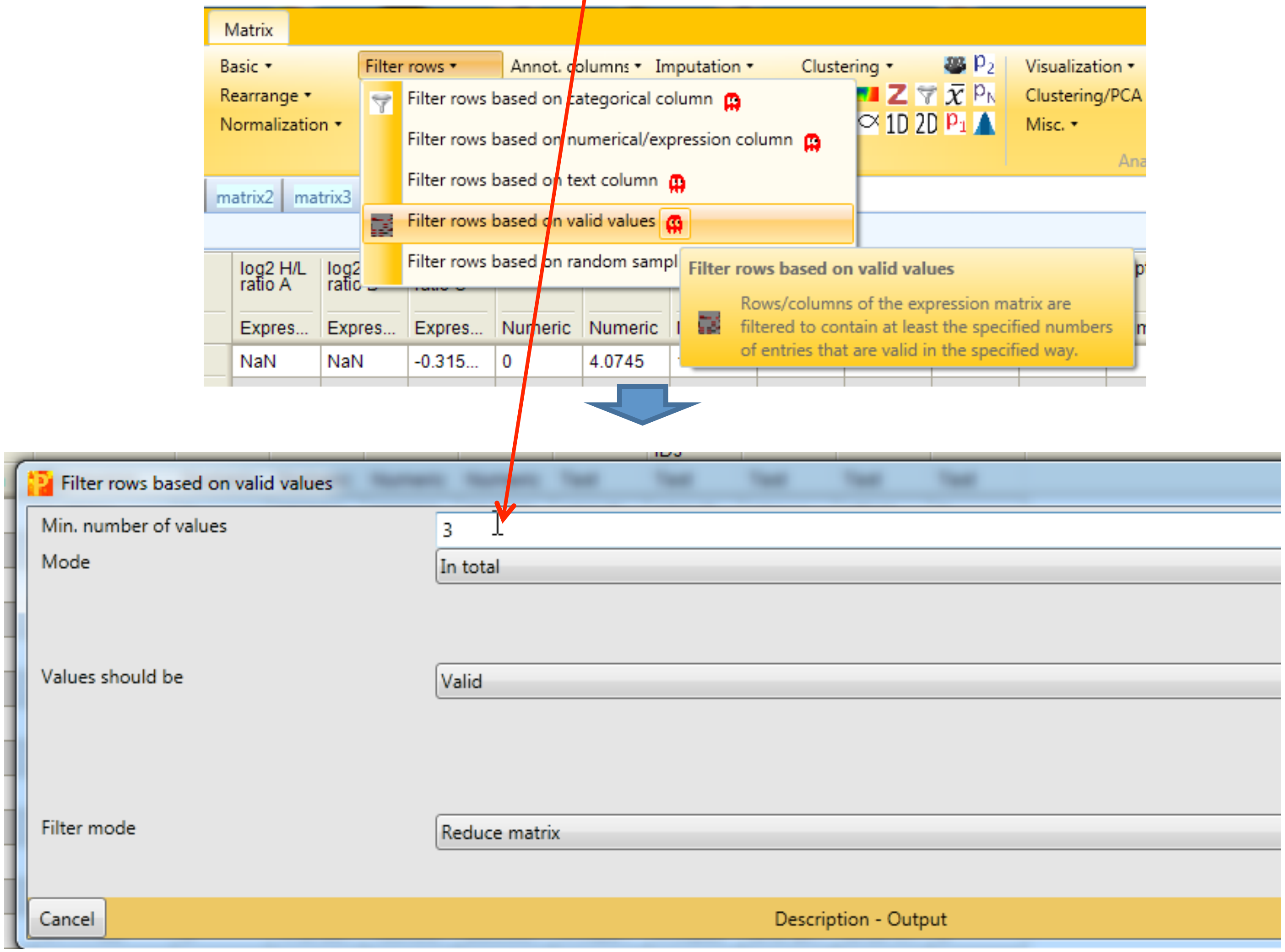
We have a lot of identifications without or only a limited amount of quantitative values (NaN). Since we want to have very reliable quantitative data ,we now remove all entries which have insufficient entries.



	Matr 30h/0h_1	Matr 30h/0h_2	Matr 30h/0h_3	PEP	Intensity	Intensity L	Intensity H	Protein IDs	Majority protein IDs	Protein names	Gene names	Proteins
Type	Expression	Expression	Expression	Numeric	Numeric	Numeric	Numeric	Text	Text	Text	Text	Text
1	0.593931	0.219235	0.47531	0	953960...	699960...	254000...	A0AVT...	A0AVT...	Ubiqui...	UBA6	6
2	-0.508414	-1.22637	-0.514036	0	257690...	177260...	804350...	A0FGR...	A0FGR...	Extend...	ESYT2	5
3	NaN	NaN	NaN	3.4808...	670070...	596980...	7308500	A0JLT...	A0JLT...	Mediat...	MED19	2
4	0.817615	NaN	NaN	8.1766...	119360...	977740...	215880...	A0JNW5	A0JNW5	UHRF1...	UHRF1...	1
5	NaN	NaN	NaN	9.5202...	514250...	435080...	791680...	A0MZ6...	A0MZ6...	Shooti...	KIAA1...	8
6	NaN	NaN	-0.452631	4.8278...	492420...	386300...	106120...	A0PJW...	A0PJW6	Transm...	TMEM...	3
7	NaN	NaN	NaN	1.8473...	583650...	380530...	203110...	Q1565...	Q1565...	Probab...	JMJD1C	3
8	NaN	NaN	NaN	4.2013...	8772200	8038000	734270	A0T4C...	A0T4C...	Sphing...	SPHK2	6
9	NaN	NaN	0.44628	3.3301...	403140...	285770...	117380...	A1A4S...	A1A4S...	Rho G...	ARHG...	3
10	NaN	NaN	NaN	2.5025...	330490...	296430...	3406000	A1L020	A1L020	RNA-b...	MEX3A	1
11	-0.601011	-0.314939	-0.237196	0	327420...	267380...	600400...	A1L0T...	A1L0T0	Acetola...	ILVBL	5
12	NaN	NaN	NaN	2.4735...	975370...	751600...	223770...	A1L188	A1L188	Uncha...	C17orf...	1
13	0.87052	0.668951	0.711168	0	179760...	153430...	263300...	A1X28...	A1X283	SH3 an...	SH3PX...	9
14	-0.908305	NaN	-0.335885	1.8734...	241310...	204190...	371160...	Q86X1...	Q86X1...	Ral GT...	RALGA...	7
15	NaN	NaN	-0.837522	2.1456...	772010...	592580...	179420...	A2A2G...	A2A2G...	Dolichy...	ALG6	2
16	0.268208	NaN	0.408491	0	261260...	224830...	364340...	A2A2Q...	A2A2Q...	Uncha...	C20orf4	2
17	NaN	NaN	NaN	3.9044...	309930...	244260...	656670...	P4269...	P4269...	RNA-b...	RBM34	5
18	NaN	NaN	NaN	1.6859...	294430...	201560...	928690...	Q1467...	Q1467...	KN mo...	KANK1	6

Analysis of SILAC data

We now remove all entries which have insufficient entries. To be very stringent, we remove everything where **only one or two ratios** are present, but this is an individual choice.



Filter rows based on valid values

Rows/columns of the expression matrix are filtered to contain at least the specified numbers of entries that are valid in the specified way.

Filter rows based on valid values

Min. number of values: 3

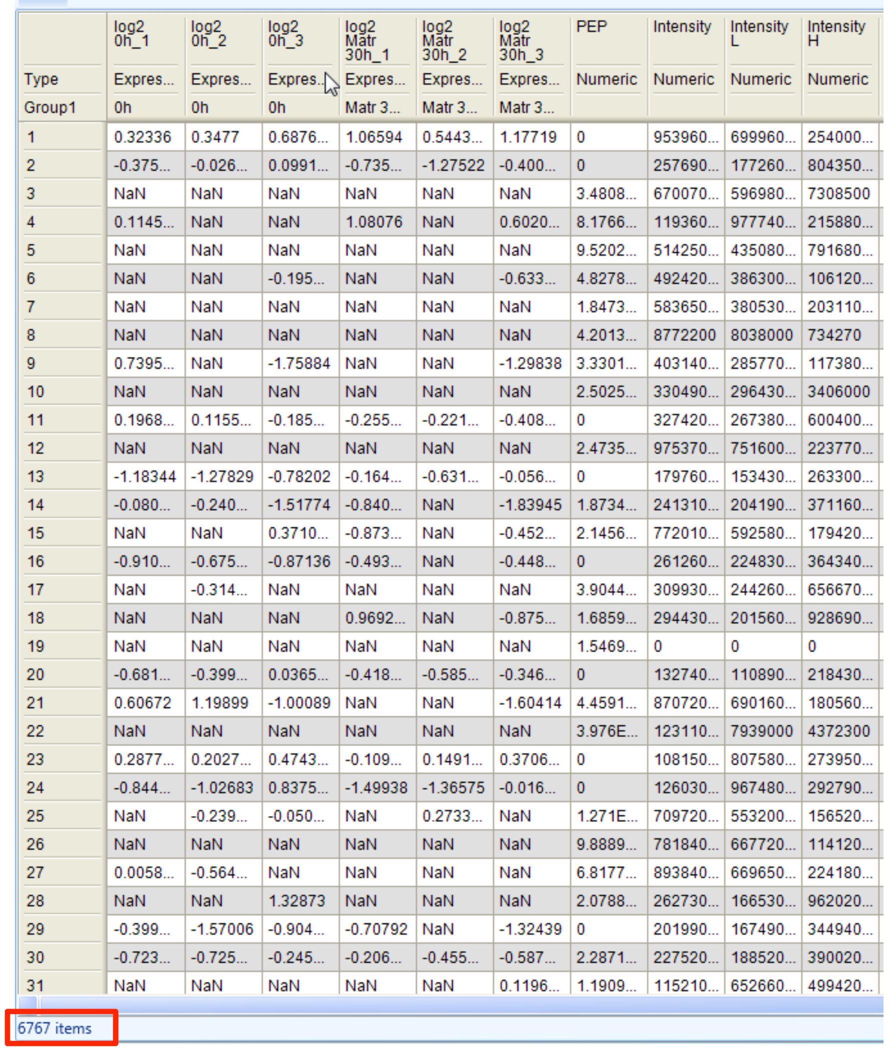
Mode: In total

Values should be: Valid

Filter mode: Reduce matrix

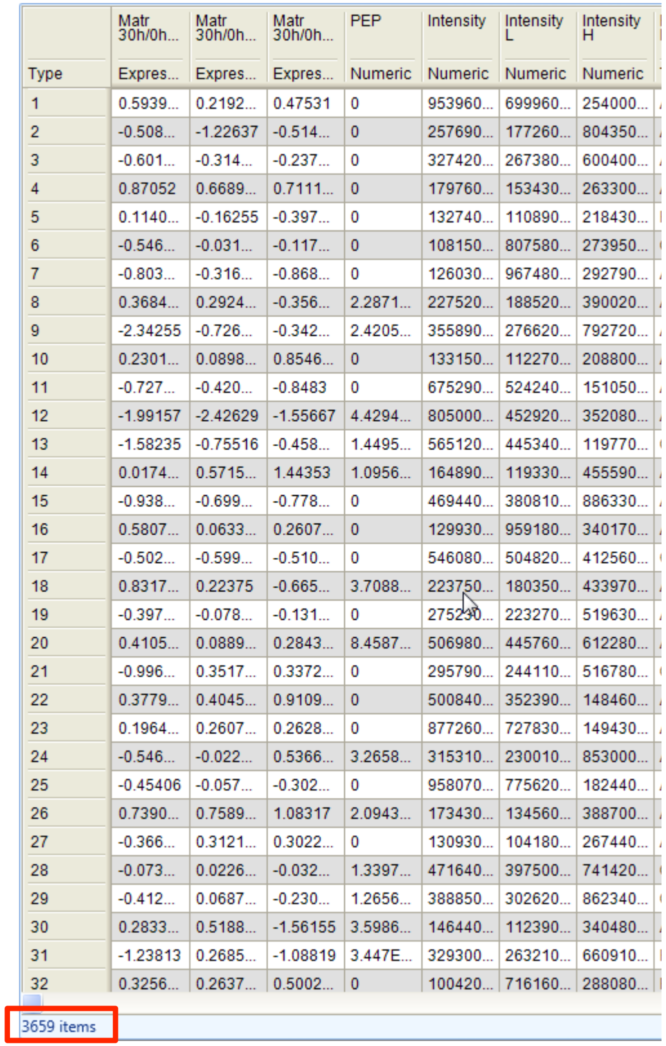
Analysis of SILAC data

The stringent filtering for valid values reduced the number of protein groups from 6767 to 3659



	log2 0h_1	log2 0h_2	log2 0h_3	log2 Matr 30h_1	log2 Matr 30h_2	log2 Matr 30h_3	PEP	Intensity	Intensity L	Intensity H
Type	Expres...	Expres...	Expres...	Expres...	Expres...	Expres...	Numeric	Numeric	Numeric	Numeric
Group1	0h	0h	0h	Matr 3...	Matr 3...	Matr 3...				
1	0.32336	0.3477	0.6876...	1.06594	0.5443...	1.17719	0	953960...	699960...	254000...
2	-0.375...	-0.026...	0.0991...	-0.735...	-1.27522	-0.400...	0	257690...	177260...	804350...
3	NaN	NaN	NaN	NaN	NaN	NaN	3.4808...	670070...	596980...	7308500
4	0.1145...	NaN	NaN	1.08076	NaN	0.6020...	8.1766...	119360...	977740...	215880...
5	NaN	NaN	NaN	NaN	NaN	NaN	9.5202...	514250...	435080...	791680...
6	NaN	NaN	-0.195...	NaN	NaN	-0.633...	4.8278...	492420...	386300...	106120...
7	NaN	NaN	NaN	NaN	NaN	NaN	1.8473...	583650...	380530...	203110...
8	NaN	NaN	NaN	NaN	NaN	NaN	4.2013...	8772200	8038000	734270
9	0.7395...	NaN	-1.75884	NaN	NaN	-1.29838	3.3301...	403140...	285770...	117380...
10	NaN	NaN	NaN	NaN	NaN	NaN	2.5025...	330490...	296430...	3406000
11	0.1968...	0.1155...	-0.185...	-0.255...	-0.221...	-0.408...	0	327420...	267380...	600400...
12	NaN	NaN	NaN	NaN	NaN	NaN	2.4735...	975370...	751600...	223770...
13	-1.18344	-1.27829	-0.78202	-0.164...	-0.631...	-0.056...	0	179760...	153430...	263300...
14	-0.080...	-0.240...	-1.51774	-0.840...	NaN	-1.83945	1.8734...	241310...	204190...	371160...
15	NaN	NaN	0.3710...	-0.873...	NaN	-0.452...	2.1456...	772010...	592580...	179420...
16	-0.910...	-0.675...	-0.87136	-0.493...	NaN	-0.448...	0	261260...	224830...	364340...
17	NaN	-0.314...	NaN	NaN	NaN	NaN	3.9044...	309930...	244260...	656670...
18	NaN	NaN	NaN	0.9692...	NaN	-0.875...	1.6859...	294430...	201560...	928690...
19	NaN	NaN	NaN	NaN	NaN	NaN	1.5469...	0	0	0
20	-0.681...	-0.399...	0.0365...	-0.418...	-0.585...	-0.346...	0	132740...	110890...	218430...
21	0.60672	1.19899	-1.00089	NaN	NaN	-1.60414	4.4591...	870720...	690160...	180560...
22	NaN	NaN	NaN	NaN	NaN	NaN	3.976E...	123110...	7939000	4372300
23	0.2877...	0.2027...	0.4743...	-0.109...	0.1491...	0.3706...	0	108150...	807580...	273950...
24	-0.844...	-1.02683	0.8375...	-1.49938	-1.36575	-0.016...	0	126030...	967480...	292790...
25	NaN	-0.239...	-0.050...	NaN	0.2733...	NaN	1.271E...	709720...	553200...	156520...
26	NaN	NaN	NaN	NaN	NaN	NaN	9.8889...	781840...	667720...	114120...
27	0.0058...	-0.564...	NaN	NaN	NaN	NaN	6.8177...	893840...	669650...	224180...
28	NaN	NaN	1.32873	NaN	NaN	NaN	2.0788...	262730...	166530...	962020...
29	-0.399...	-1.57006	-0.904...	-0.70792	NaN	-1.32439	0	201990...	167490...	344940...
30	-0.723...	-0.725...	-0.245...	-0.206...	-0.455...	-0.587...	2.2871...	227520...	188520...	390020...
31	NaN	NaN	NaN	NaN	NaN	0.1196...	1.1909...	115210...	652660...	499420...

6767 items

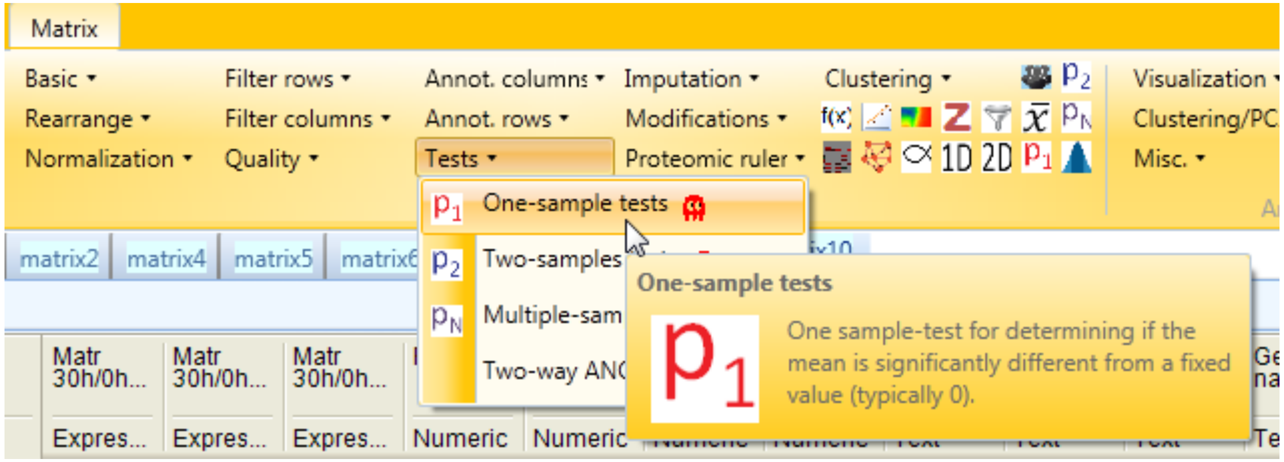


	Matr 30h/0h...	Matr 30h/0h...	Matr 30h/0h...	PEP	Intensity	Intensity L	Intensity H
Type	Expres...	Expres...	Expres...	Numeric	Numeric	Numeric	Numeric
1	0.5939...	0.2192...	0.47531	0	953960...	699960...	254000...
2	-0.508...	-1.22637	-0.514...	0	257690...	177260...	804350...
3	-0.601...	-0.314...	-0.237...	0	327420...	267380...	600400...
4	0.87052	0.6689...	0.7111...	0	179760...	153430...	263300...
5	0.1140...	-0.16255	-0.397...	0	132740...	110890...	218430...
6	-0.546...	-0.031...	-0.117...	0	108150...	807580...	273950...
7	-0.803...	-0.316...	-0.868...	0	126030...	967480...	292790...
8	0.3684...	0.2924...	-0.356...	2.2871...	227520...	188520...	390020...
9	-2.34255	-0.726...	-0.342...	2.4205...	355890...	276620...	792720...
10	0.2301...	0.0898...	0.8546...	0	133150...	112270...	208800...
11	-0.727...	-0.420...	-0.8483	0	675290...	524240...	151050...
12	-1.99157	-2.42629	-1.55667	4.4294...	805000...	452920...	352080...
13	-1.58235	-0.75516	-0.458...	1.4495...	565120...	445340...	119770...
14	0.0174...	0.5715...	1.44353	1.0956...	164890...	119330...	455590...
15	-0.938...	-0.699...	-0.778...	0	469440...	380810...	886330...
16	0.5807...	0.0633...	0.2607...	0	129930...	959180...	340170...
17	-0.502...	-0.599...	-0.510...	0	546080...	504820...	412560...
18	0.8317...	0.22375	-0.665...	3.7088...	223750...	180350...	433970...
19	-0.397...	-0.078...	-0.131...	0	275230...	223270...	519630...
20	0.4105...	0.0889...	0.2843...	8.4587...	506980...	445760...	612280...
21	-0.996...	0.3517...	0.3372...	0	295790...	244110...	516780...
22	0.3779...	0.4045...	0.9109...	0	500840...	352390...	148460...
23	0.1964...	0.2607...	0.2628...	0	877260...	727830...	149430...
24	-0.546...	-0.022...	0.5366...	3.2658...	315310...	230010...	853000...
25	-0.45406	-0.057...	-0.302...	0	958070...	775620...	182440...
26	0.7390...	0.7589...	1.08317	2.0943...	173430...	134560...	388700...
27	-0.366...	0.3121...	0.3022...	0	130930...	104180...	267440...
28	-0.073...	0.0226...	-0.032...	1.3397...	471640...	397500...	741420...
29	-0.412...	0.0687...	-0.230...	1.2656...	388850...	302620...	862340...
30	0.2833...	0.5188...	-1.56155	3.5986...	146440...	112390...	340480...
31	-1.23813	0.2685...	-1.08819	3.447E...	329300...	263210...	660910...
32	0.3256...	0.2637...	0.5002...	0	100420...	716160...	288080...

3659 items

Analysis of SILAC data

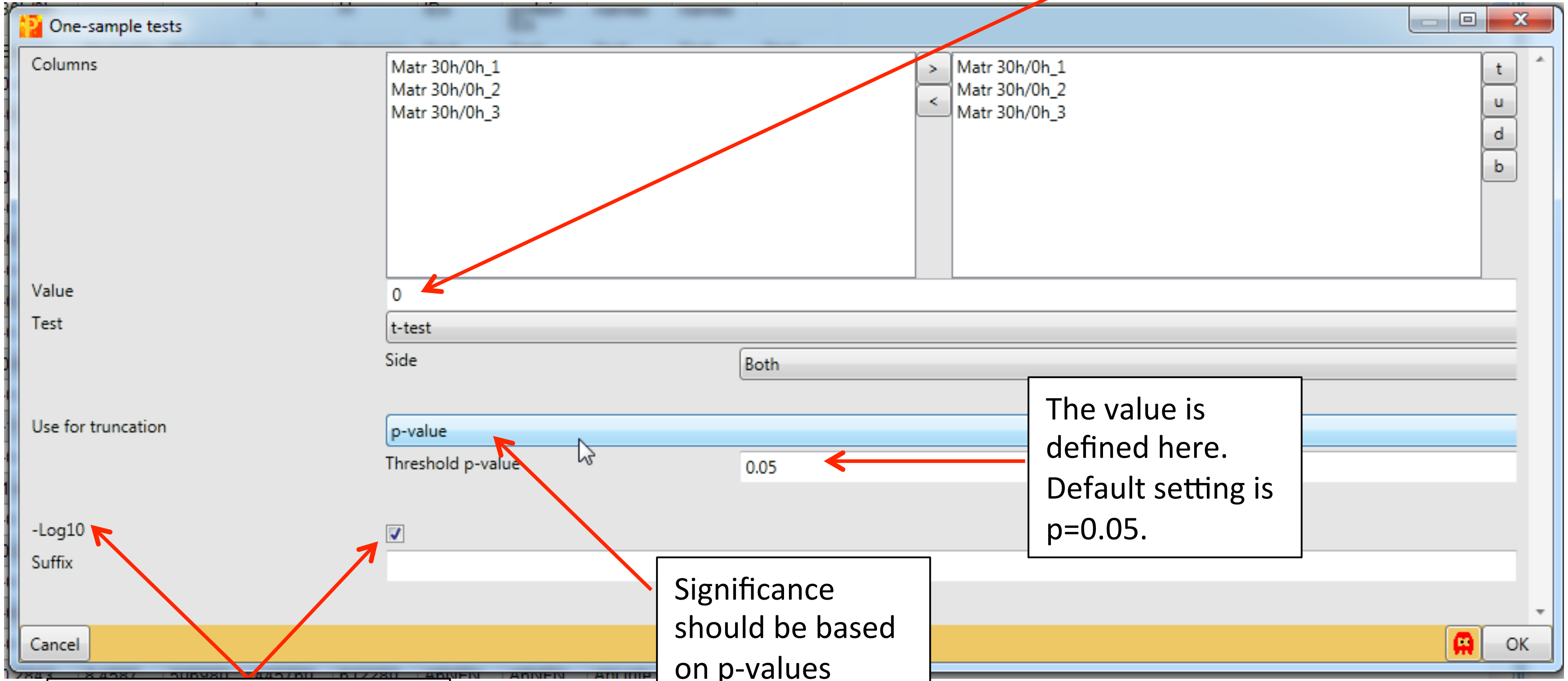
To see which protein groups are significantly changed between both samples (0 vs Matrigel30h) we now perform a one-sample t-test. In this test it is checked which protein groups are significantly differing from a fixed value. This value is set to 0 = ratio 1 = no change, because we work with logarithmic values.



One sample-test for determining if the mean is significantly different from a fixed value (typically 0).

Analysis of SILAC data

To see which protein groups are significantly changed between both samples (0 vs Matrigel30h) we now perform a one-sample t-test. In this test it is checked which protein groups are significantly differing from a fixed value. **This value is set to 0** = ratio 1 = no change, because we work with logarithmic values.



Value 0

Test t-test

Side Both

Use for truncation p-value

Threshold p-value 0.05

-Log10 ☑

Suffix

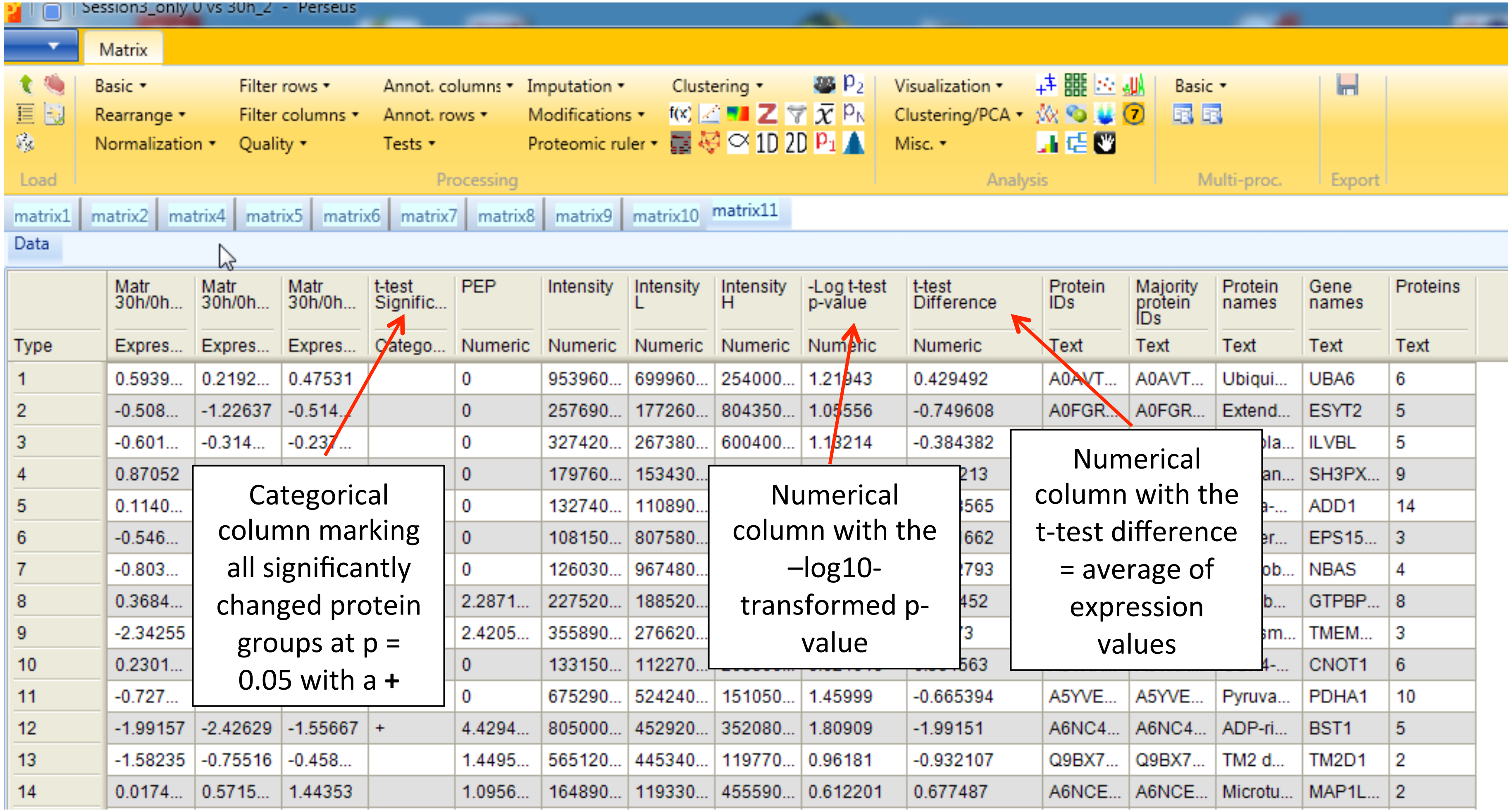
The value is defined here. Default setting is p=0.05.

Significance should be based on p-values

If checked, p-values are given as –log10 (default setting)

Analysis of SILAC data

Three new columns are created in the new matrix. One categorical and two numerical.



	Matr 30h/0h...	Matr 30h/0h...	Matr 30h/0h...	t-test Signific...	PEP	Intensity	Intensity L	Intensity H	-Log t-test p-value	t-test Difference	Protein IDs	Majority protein IDs	Protein names	Gene names	Proteins
Type	Expres...	Expres...	Expres...	Catego...	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Text	Text	Text	Text	Text
1	0.5939...	0.2192...	0.47531		0	953960...	699960...	254000...	1.21943	0.429492	A0AVT...	A0AVT...	Ubiqui...	UBA6	6
2	-0.508...	-1.22637	-0.514...		0	257690...	177260...	804350...	1.05556	-0.749608	A0FGR...	A0FGR...	Extend...	ESYT2	5
3	-0.601...	-0.314...	-0.237...		0	327420...	267380...	600400...	1.13214	-0.384382	[illegible]	[illegible]	la...	ILVBL	5
4	0.87052	[illegible]	[illegible]	[illegible]	0	179760...	153430...	[illegible]	[illegible]	213	[illegible]	[illegible]	an...	SH3PX...	9
5	0.1140...	[illegible]	[illegible]	[illegible]	0	132740...	110890...	[illegible]	[illegible]	565	[illegible]	[illegible]	a-...	ADD1	14
6	-0.546...	[illegible]	[illegible]	[illegible]	0	108150...	807580...	[illegible]	[illegible]	662	[illegible]	[illegible]	er...	EPS15...	3
7	-0.803...	[illegible]	[illegible]	[illegible]	0	126030...	967480...	[illegible]	[illegible]	793	[illegible]	[illegible]	ob...	NBAS	4
8	0.3684...	[illegible]	[illegible]	[illegible]	2.2871...	227520...	188520...	[illegible]	[illegible]	452	[illegible]	[illegible]	b...	GTPBP...	8
9	-2.34255	[illegible]	[illegible]	[illegible]	2.4205...	355890...	276620...	[illegible]	[illegible]	3	[illegible]	[illegible]	sm...	TMEM...	3
10	0.2301...	[illegible]	[illegible]	[illegible]	0	133150...	112270...	[illegible]	[illegible]	563	[illegible]	[illegible]	4-...	CNOT1	6
11	-0.727...	[illegible]	[illegible]	[illegible]	0	675290...	524240...	151050...	1.45999	-0.665394	A5YVE...	A5YVE...	Pyruva...	PDHA1	10
12	-1.99157	-2.42629	-1.55667	+	4.4294...	805000...	452920...	352080...	1.80909	-1.99151	A6NC4...	A6NC4...	ADP-ri...	BST1	5
13	-1.58235	-0.75516	-0.458...		1.4495...	565120...	445340...	119770...	0.96181	-0.932107	Q9BX7...	Q9BX7...	TM2 d...	TM2D1	2
14	0.0174...	0.5715...	1.44353		1.0956...	164890...	119330...	455590...	0.612201	0.677487	A6NCE...	A6NCE...	Microtu...	MAP1L...	2

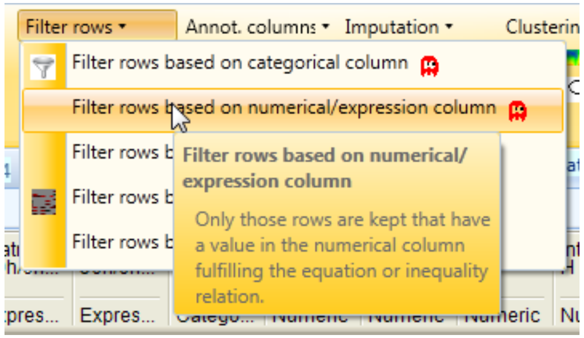
Categorical column marking all significantly changed protein groups at p = 0.05 with a **+**

Numerical column with the –log10-transformed p-value

Numerical column with the t-test difference = average of expression values

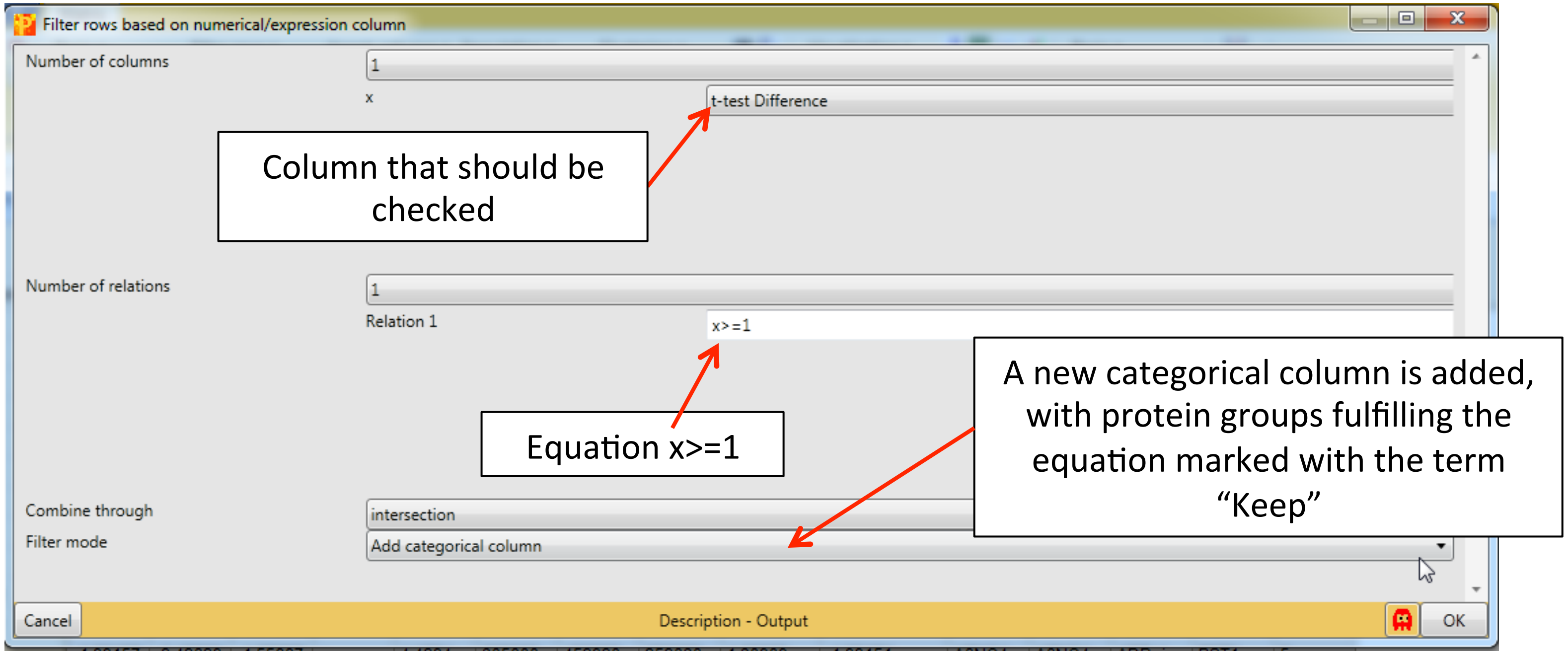
Analysis of SILAC data

Within these significantly changed protein groups we now filter for the ones with t-test differerence ≤ -1 or ≥ 1 (= 2-fold change)



Filter rows based on numerical/ expression column

Only those rows are kept that have a value in the numerical column fulfilling the equation or inequality relation.



Number of columns: 1

x: t-test Difference

Column that should be checked

Number of relations: 1

Relation 1: x>=1

Equation x>=1

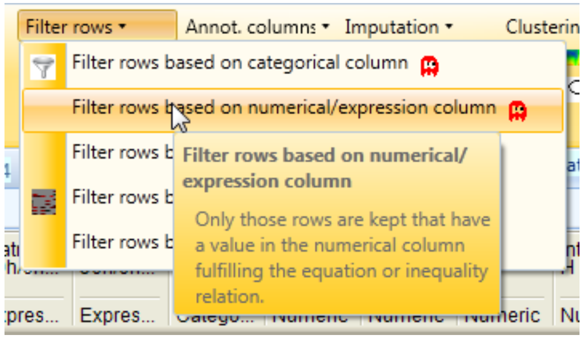
Combine through: intersection

Filter mode: Add categorical column

A new categorical column is added, with protein groups fulfilling the equation marked with the term “Keep”

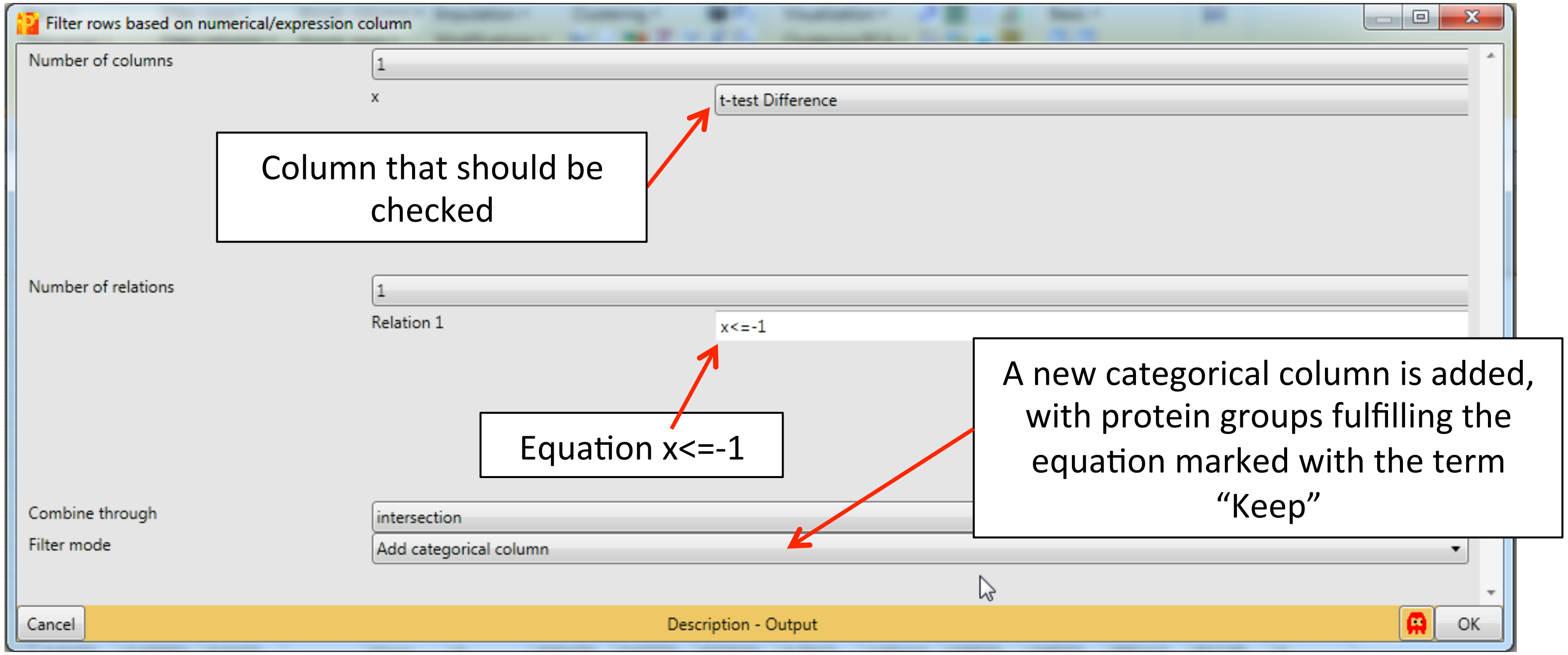
Analysis of SILAC data

Within these significantly changed protein groups we now filter for the ones with a t-test difference ≤ -1 or ≥ 1 (= 2-fold change)



Filter rows based on numerical/ expression column

Only those rows are kept that have a value in the numerical column fulfilling the equation or inequality relation.



Number of columns: 1

x: t-test Difference

Column that should be checked

Number of relations: 1

Relation 1: x<=-1

Equation x<=-1

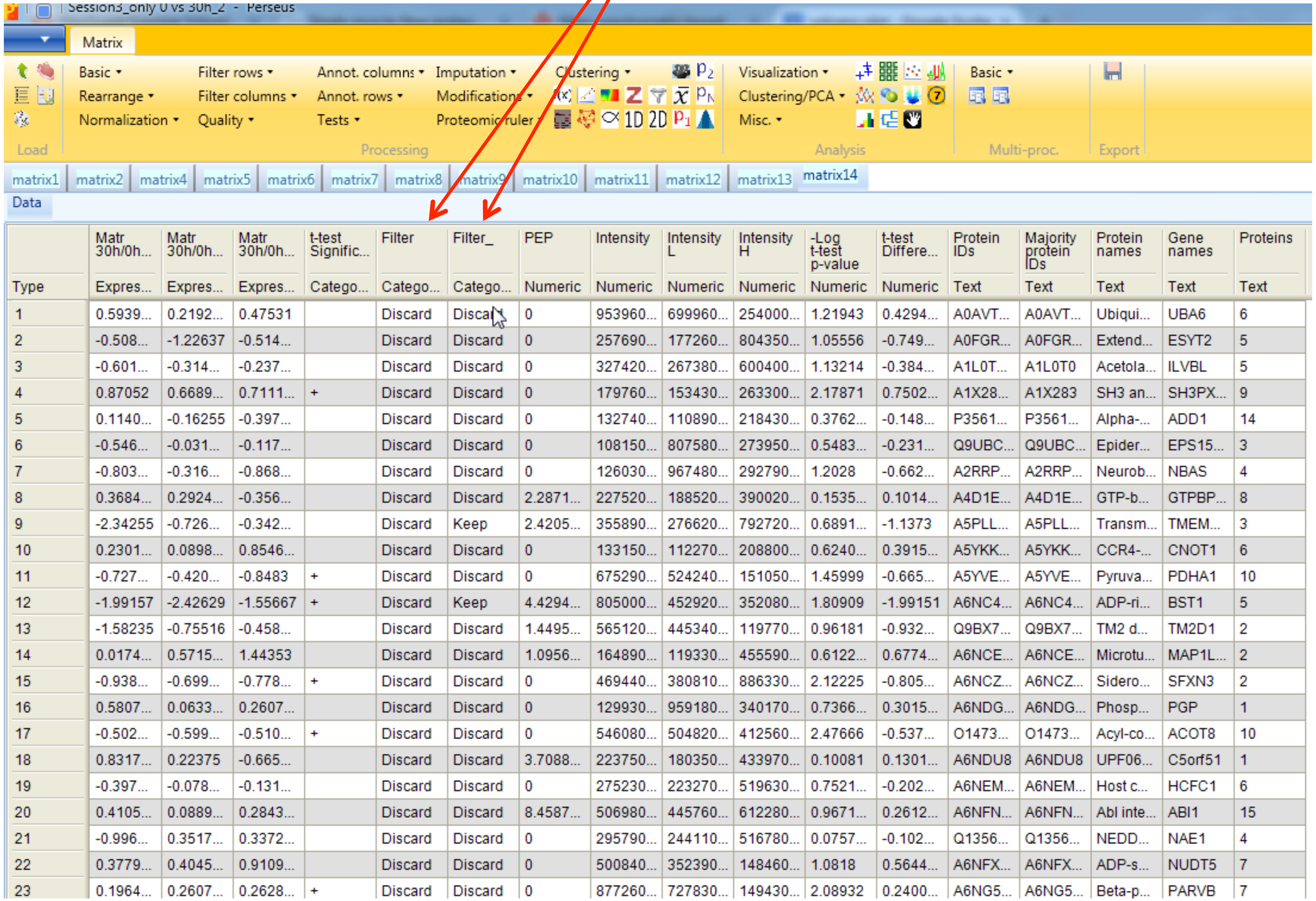
Combine through: intersection

Filter mode: Add categorical column

A new categorical column is added, with protein groups fulfilling the equation marked with the term "Keep"

Analysis of SILAC data

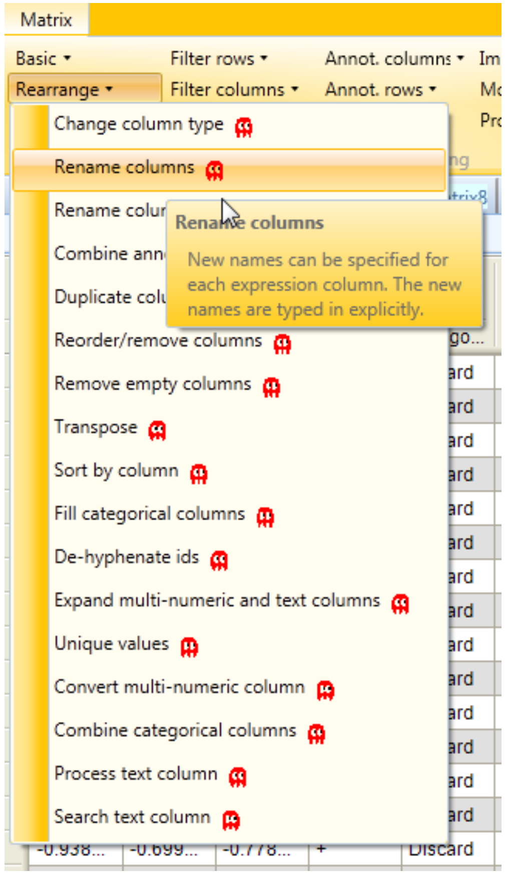
Next we rename the **two added categorical columns** with a meaningful name.



	Matr 30h/0h...	Matr 30h/0h...	Matr 30h/0h...	t-test Signific...	Filter	Filter_	PEP	Intensity	Intensity L	Intensity H	-Log t-test p-value	t-test Differe...	Protein IDs	Majority protein IDs	Protein names	Gene names	Proteins
Type	Expres...	Expres...	Expres...	Catego...	Catego...	Catego...	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Text	Text	Text	Text	Text
1	0.5939...	0.2192...	0.47531		Discard	Discard	0	953960...	699960...	254000...	1.21943	0.4294...	A0AVT...	A0AVT...	Ubiqui...	UBA6	6
2	-0.508...	-1.22637	-0.514...		Discard	Discard	0	257690...	177260...	804350...	1.05556	-0.749...	A0FGR...	A0FGR...	Extend...	ESYT2	5
3	-0.601...	-0.314...	-0.237...		Discard	Discard	0	327420...	267380...	600400...	1.13214	-0.384...	A1L0T...	A1L0T0	Acetola...	ILVBL	5
4	0.87052	0.6689...	0.7111...	+	Discard	Discard	0	179760...	153430...	263300...	2.17871	0.7502...	A1X28...	A1X283	SH3 an...	SH3PX...	9
5	0.1140...	-0.16255	-0.397...		Discard	Discard	0	132740...	110890...	218430...	0.3762...	-0.148...	P3561...	P3561...	Alpha-...	ADD1	14
6	-0.546...	-0.031...	-0.117...		Discard	Discard	0	108150...	807580...	273950...	0.5483...	-0.231...	Q9UBC...	Q9UBC...	Epider...	EPS15...	3
7	-0.803...	-0.316...	-0.868...		Discard	Discard	0	126030...	967480...	292790...	1.2028	-0.662...	A2RRP...	A2RRP...	Neurob...	NBAS	4
8	0.3684...	0.2924...	-0.356...		Discard	Discard	2.2871...	227520...	188520...	390020...	0.1535...	0.1014...	A4D1E...	A4D1E...	GTP-b...	GTPBP...	8
9	-2.34255	-0.726...	-0.342...		Discard	Keep	2.4205...	355890...	276620...	792720...	0.6891...	-1.1373	A5PLL...	A5PLL...	Transm...	TMEM...	3
10	0.2301...	0.0898...	0.8546...		Discard	Discard	0	133150...	112270...	208800...	0.6240...	0.3915...	A5YKK...	A5YKK...	CCR4-...	CNOT1	6
11	-0.727...	-0.420...	-0.8483	+	Discard	Discard	0	675290...	524240...	151050...	1.45999	-0.665...	A5YVE...	A5YVE...	Pyruva...	PDHA1	10
12	-1.99157	-2.42629	-1.55667	+	Discard	Keep	4.4294...	805000...	452920...	352080...	1.80909	-1.99151	A6NC4...	A6NC4...	ADP-ri...	BST1	5
13	-1.58235	-0.75516	-0.458...		Discard	Discard	1.4495...	565120...	445340...	119770...	0.96181	-0.932...	Q9BX7...	Q9BX7...	TM2 d...	TM2D1	2
14	0.0174...	0.5715...	1.44353		Discard	Discard	1.0956...	164890...	119330...	455590...	0.6122...	0.6774...	A6NCE...	A6NCE...	Microtu...	MAP1L...	2
15	-0.938...	-0.699...	-0.778...	+	Discard	Discard	0	469440...	380810...	886330...	2.12225	-0.805...	A6NCZ...	A6NCZ...	Sidero...	SFXN3	2
16	0.5807...	0.0633...	0.2607...		Discard	Discard	0	129930...	959180...	340170...	0.7366...	0.3015...	A6NDG...	A6NDG...	Phosp...	PGP	1
17	-0.502...	-0.599...	-0.510...	+	Discard	Discard	0	546080...	504820...	412560...	2.47666	-0.537...	O1473...	O1473...	Acyl-co...	ACOT8	10
18	0.8317...	0.22375	-0.665...		Discard	Discard	3.7088...	223750...	180350...	433970...	0.10081	0.1301...	A6NDU8	A6NDU8	UPF06...	C5orf51	1
19	-0.397...	-0.078...	-0.131...		Discard	Discard	0	275230...	223270...	519630...	0.7521...	-0.202...	A6NEM...	A6NEM...	Host c...	HCFC1	6
20	0.4105...	0.0889...	0.2843...		Discard	Discard	8.4587...	506980...	445760...	612280...	0.9671...	0.2612...	A6NFN...	A6NFN...	Abl inte...	ABI1	15
21	-0.996...	0.3517...	0.3372...		Discard	Discard	0	295790...	244110...	516780...	0.0757...	-0.102...	Q1356...	Q1356...	NEDD...	NAE1	4
22	0.3779...	0.4045...	0.9109...		Discard	Discard	0	500840...	352390...	148460...	1.0818	0.5644...	A6NFX...	A6NFX...	ADP-s...	NUDT5	7
23	0.1964...	0.2607...	0.2628...	+	Discard	Discard	0	877260...	727830...	149430...	2.08932	0.2400...	A6NG5...	A6NG5...	Beta-p...	PARVB	7

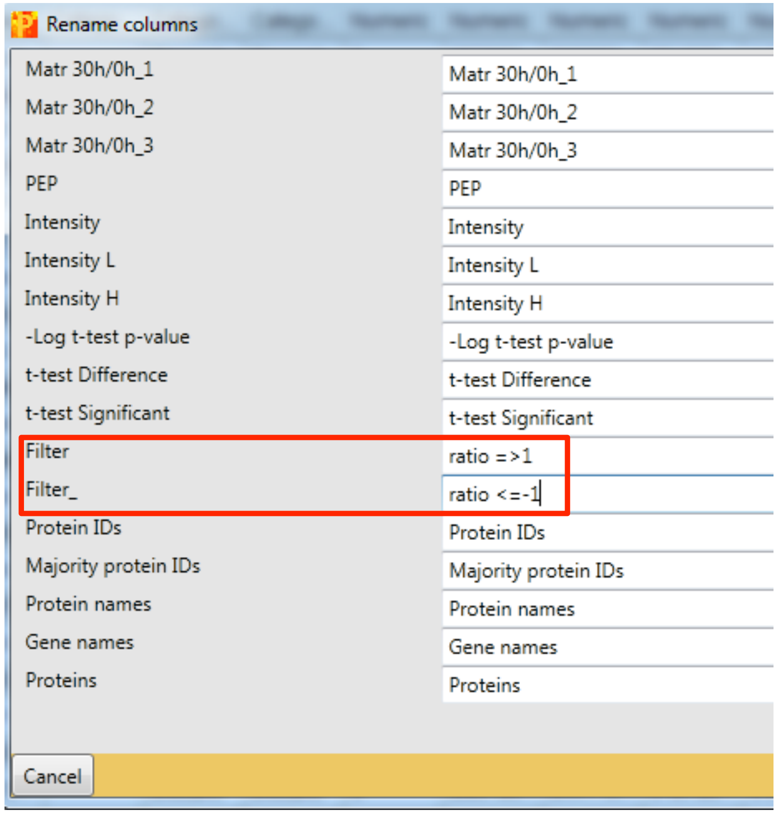
Analysis of SILAC data

Next we rename the **two added categorical columns** with a meaningful name.



Rename columns

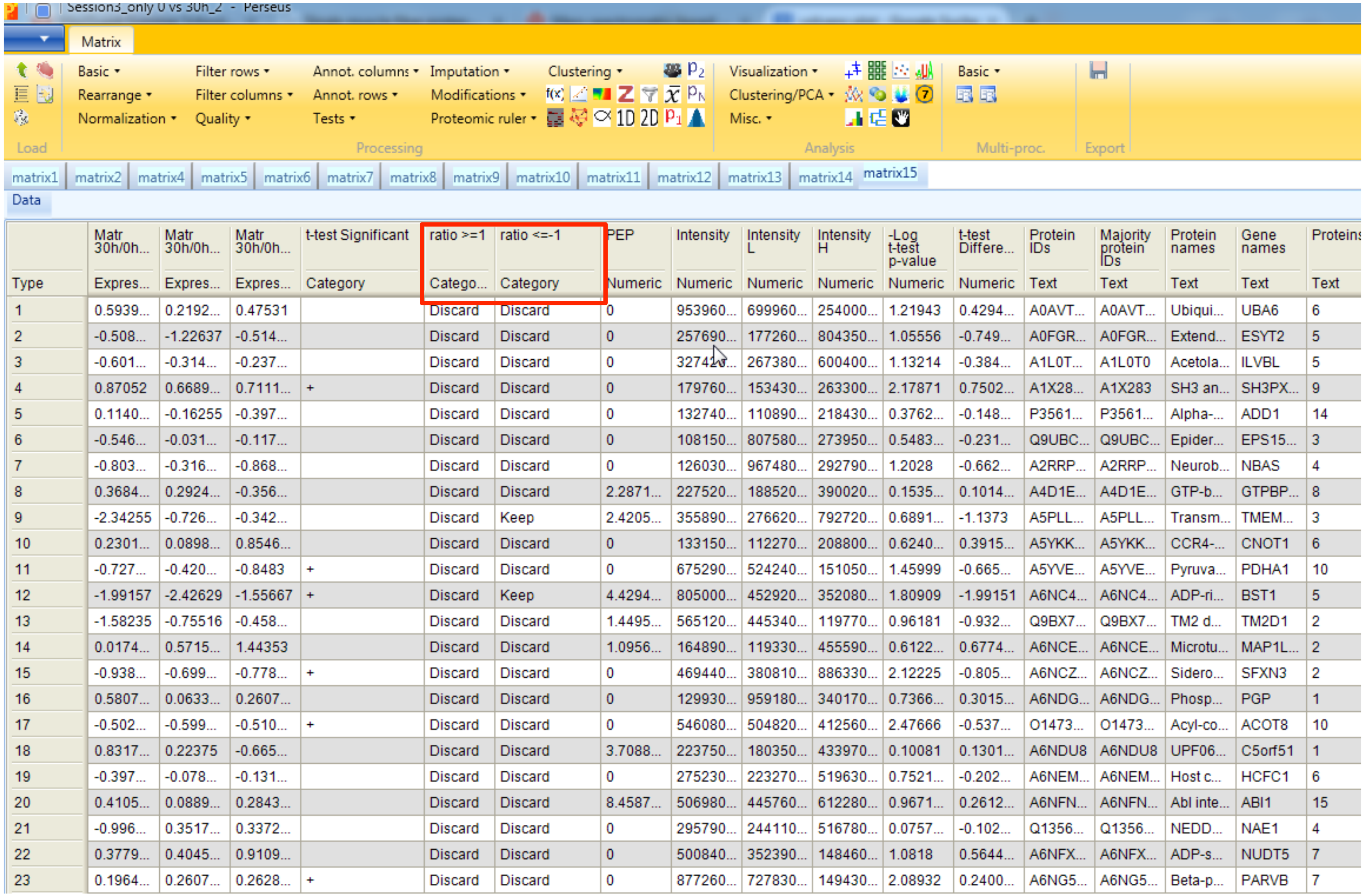
New names can be specified for each expression column. The new names are typed in explicitly.



Matr 30h/0h_1	Matr 30h/0h_1
Matr 30h/0h_2	Matr 30h/0h_2
Matr 30h/0h_3	Matr 30h/0h_3
PEP	PEP
Intensity	Intensity
Intensity L	Intensity L
Intensity H	Intensity H
-Log t-test p-value	-Log t-test p-value
t-test Difference	t-test Difference
t-test Significant	t-test Significant
Filter	ratio =>1
Filter_	ratio <=-1
Protein IDs	Protein IDs
Majority protein IDs	Majority protein IDs
Protein names	Protein names
Gene names	Gene names
Proteins	Proteins

Analysis of SILAC data

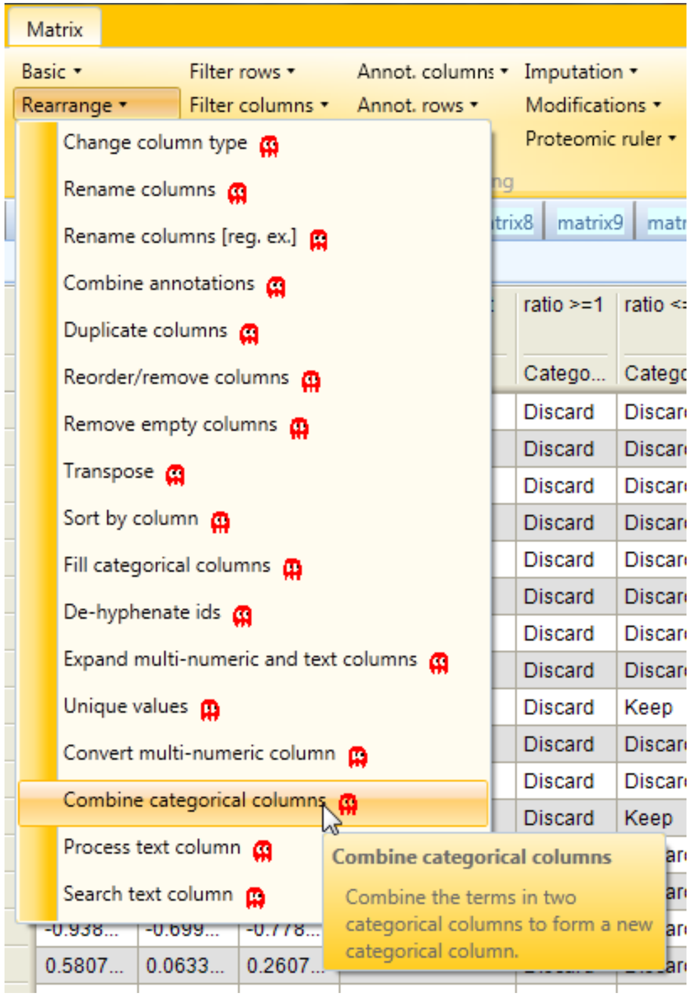
Next we rename the **two added categorical columns** with a meaningful name.



	Matr 30h/0h...	Matr 30h/0h...	Matr 30h/0h...	t-test Significant	ratio >=1	ratio <=-1	PEP	Intensity	Intensity L	Intensity H	-Log t-test p-value	t-test Differe...	Protein IDs	Majority protein IDs	Protein names	Gene names	Proteins
Type	Expres...	Expres...	Expres...	Category	Catego...	Category	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Text	Text	Text	Text	Text
1	0.5939...	0.2192...	0.47531		Discard	Discard	0	953960...	699960...	254000...	1.21943	0.4294...	A0AVT...	A0AVT...	Ubiqui...	UBA6	6
2	-0.508...	-1.22637	-0.514...		Discard	Discard	0	257690...	177260...	804350...	1.05556	-0.749...	A0FGR...	A0FGR...	Extend...	ESYT2	5
3	-0.601...	-0.314...	-0.237...		Discard	Discard	0	32742...	267380...	600400...	1.13214	-0.384...	A1L0T...	A1L0T0	Acetola...	ILVBL	5
4	0.87052	0.6689...	0.7111...	+	Discard	Discard	0	179760...	153430...	263300...	2.17871	0.7502...	A1X28...	A1X283	SH3 an...	SH3PX...	9
5	0.1140...	-0.16255	-0.397...		Discard	Discard	0	132740...	110890...	218430...	0.3762...	-0.148...	P3561...	P3561...	Alpha-...	ADD1	14
6	-0.546...	-0.031...	-0.117...		Discard	Discard	0	108150...	807580...	273950...	0.5483...	-0.231...	Q9UBC...	Q9UBC...	Epider...	EPS15...	3
7	-0.803...	-0.316...	-0.868...		Discard	Discard	0	126030...	967480...	292790...	1.2028	-0.662...	A2RRP...	A2RRP...	Neurob...	NBAS	4
8	0.3684...	0.2924...	-0.356...		Discard	Discard	2.2871...	227520...	188520...	390020...	0.1535...	0.1014...	A4D1E...	A4D1E...	GTP-b...	GTPBP...	8
9	-2.34255	-0.726...	-0.342...		Discard	Keep	2.4205...	355890...	276620...	792720...	0.6891...	-1.1373	A5PLL...	A5PLL...	Transm...	TMEM...	3
10	0.2301...	0.0898...	0.8546...		Discard	Discard	0	133150...	112270...	208800...	0.6240...	0.3915...	A5YKK...	A5YKK...	CCR4-...	CNOT1	6
11	-0.727...	-0.420...	-0.8483	+	Discard	Discard	0	675290...	524240...	151050...	1.45999	-0.665...	A5YVE...	A5YVE...	Pyruva...	PDHA1	10
12	-1.99157	-2.42629	-1.55667	+	Discard	Keep	4.4294...	805000...	452920...	352080...	1.80909	-1.99151	A6NC4...	A6NC4...	ADP-ri...	BST1	5
13	-1.58235	-0.75516	-0.458...		Discard	Discard	1.4495...	565120...	445340...	119770...	0.96181	-0.932...	Q9BX7...	Q9BX7...	TM2 d...	TM2D1	2
14	0.0174...	0.5715...	1.44353		Discard	Discard	1.0956...	164890...	119330...	455590...	0.6122...	0.6774...	A6NCE...	A6NCE...	Microtu...	MAP1L...	2
15	-0.938...	-0.699...	-0.778...	+	Discard	Discard	0	469440...	380810...	886330...	2.12225	-0.805...	A6NCZ...	A6NCZ...	Sidero...	SFXN3	2
16	0.5807...	0.0633...	0.2607...		Discard	Discard	0	129930...	959180...	340170...	0.7366...	0.3015...	A6NDG...	A6NDG...	Phosp...	PGP	1
17	-0.502...	-0.599...	-0.510...	+	Discard	Discard	0	546080...	504820...	412560...	2.47666	-0.537...	O1473...	O1473...	Acyl-co...	ACOT8	10
18	0.8317...	0.22375	-0.665...		Discard	Discard	3.7088...	223750...	180350...	433970...	0.10081	0.1301...	A6NDU8	A6NDU8	UPF06...	C5orf51	1
19	-0.397...	-0.078...	-0.131...		Discard	Discard	0	275230...	223270...	519630...	0.7521...	-0.202...	A6NEM...	A6NEM...	Host c...	HCFC1	6
20	0.4105...	0.0889...	0.2843...		Discard	Discard	8.4587...	506980...	445760...	612280...	0.9671...	0.2612...	A6NFN...	A6NFN...	Abl inte...	ABI1	15
21	-0.996...	0.3517...	0.3372...		Discard	Discard	0	295790...	244110...	516780...	0.0757...	-0.102...	Q1356...	Q1356...	NEDD...	NAE1	4
22	0.3779...	0.4045...	0.9109...		Discard	Discard	0	500840...	352390...	148460...	1.0818	0.5644...	A6NFX...	A6NFX...	ADP-s...	NUDT5	7
23	0.1964...	0.2607...	0.2628...	+	Discard	Discard	0	877260...	727830...	149430...	2.08932	0.2400...	A6NG5...	A6NG5...	Beta-p...	PARVB	7

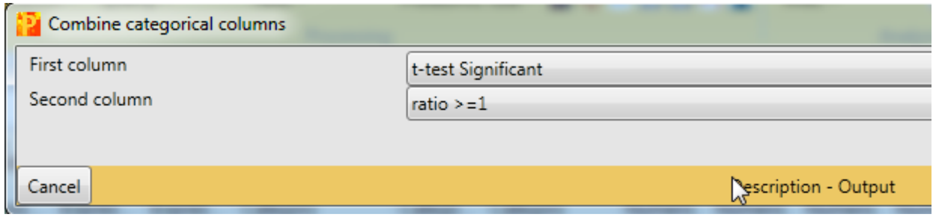
Analysis of SILAC data

Now we combine the new categorical columns with the t-test Significant column.



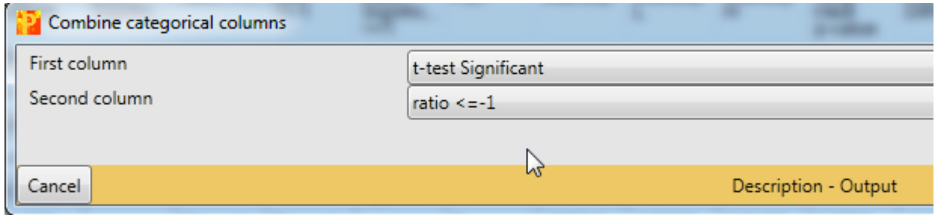
Combine categorical columns

Combine the terms in two categorical columns to form a new categorical column.



First column: t-test Significant

Second column: ratio >=1

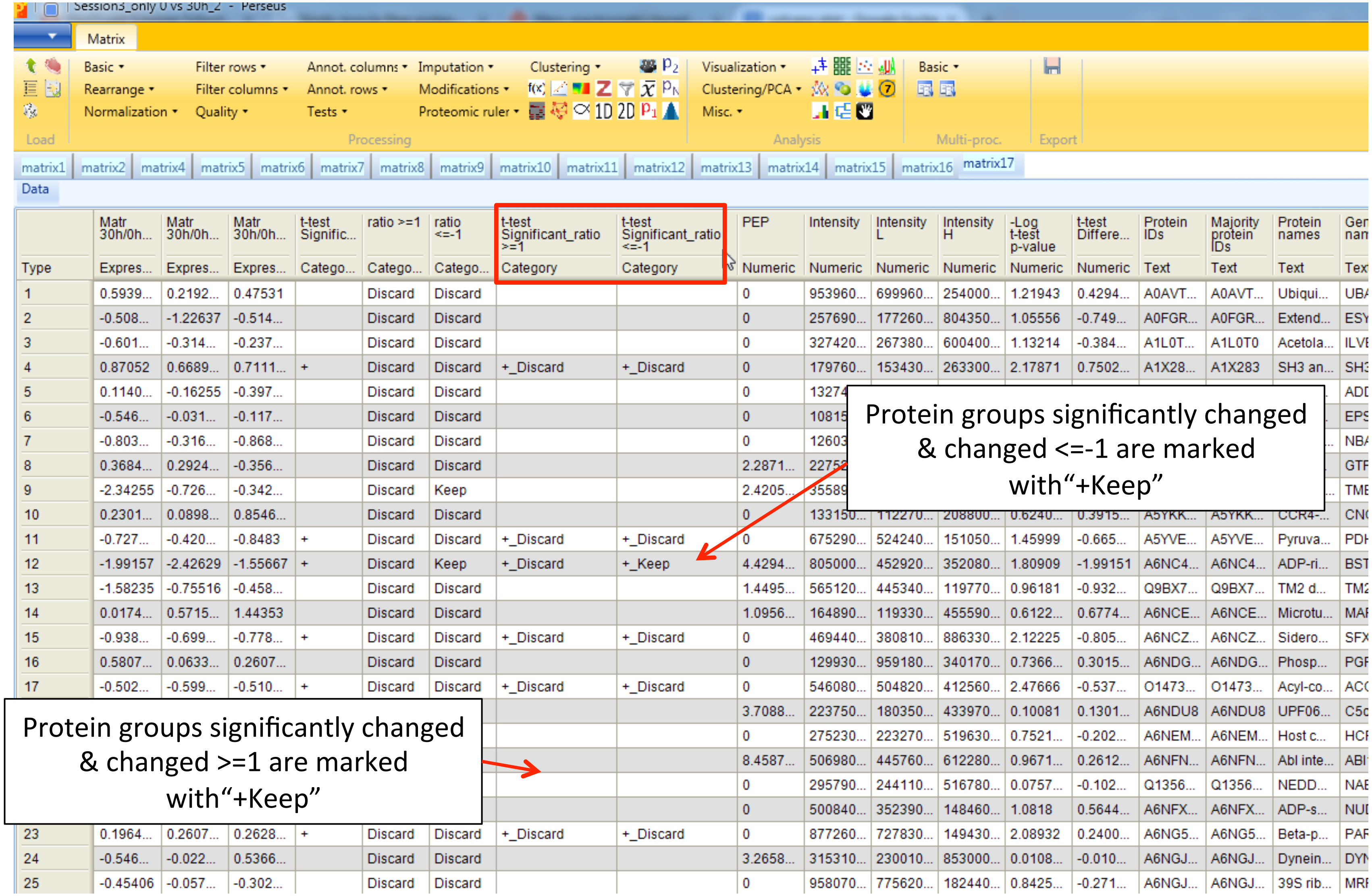


First column: t-test Significant

Second column: ratio <=-1

Analysis of SILAC data

This leads to the generation of two additional categorical columns.



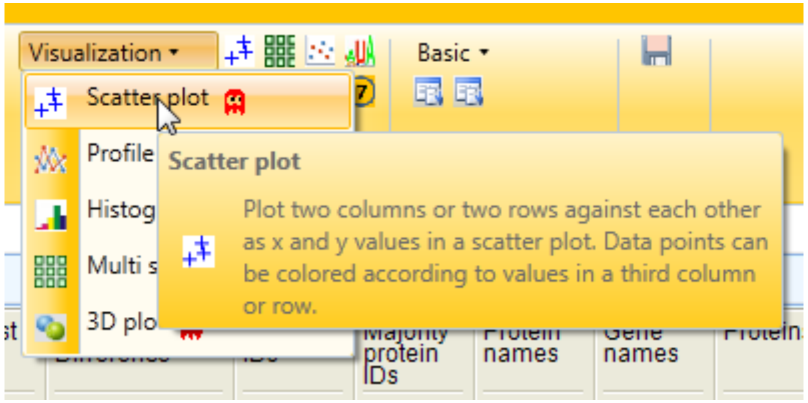
	Matr 30h/0h...	Matr 30h/0h...	Matr 30h/0h...	t-test Signific...	ratio >=1	ratio <=-1	t-test Significant_ratio >=1	t-test Significant_ratio <=-1	PEP	Intensity	Intensity L	Intensity H	-Log t-test p-value	t-test Differe...	Protein IDs	Majority protein IDs	Protein names	Gen nan
Type	Expres...	Expres...	Expres...	Catego...	Catego...	Catego...	Category	Category	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Text	Text	Text	Tex
1	0.5939...	0.2192...	0.47531		Discard	Discard			0	953960...	699960...	254000...	1.21943	0.4294...	A0AVT...	A0AVT...	Ubiqui...	UBA
2	-0.508...	-1.22637	-0.514...		Discard	Discard			0	257690...	177260...	804350...	1.05556	-0.749...	A0FGR...	A0FGR...	Extend...	ESY
3	-0.601...	-0.314...	-0.237...		Discard	Discard			0	327420...	267380...	600400...	1.13214	-0.384...	A1L0T...	A1L0T0	Acetola...	ILVE
4	0.87052	0.6689...	0.7111...	+	Discard	Discard	+_Discard	+_Discard	0	179760...	153430...	263300...	2.17871	0.7502...	A1X28...	A1X283	SH3 an...	SH3
5	0.1140...	-0.16255	-0.397...		Discard	Discard			0	13274								ADD
6	-0.546...	-0.031...	-0.117...		Discard	Discard			0	10815								EPS
7	-0.803...	-0.316...	-0.868...		Discard	Discard			0	12603								NBA
8	0.3684...	0.2924...	-0.356...		Discard	Discard			2.2871...	2275								GTF
9	-2.34255	-0.726...	-0.342...		Discard	Keep			2.4205...	35589								TME
10	0.2301...	0.0898...	0.8546...		Discard	Discard			0	133150...	112270...	208800...	0.6240...	0.3915...	A5YKK...	A5YKK...	CCR4-...	CNC
11	-0.727...	-0.420...	-0.8483	+	Discard	Discard	+_Discard	+_Discard	0	675290...	524240...	151050...	1.45999	-0.665...	A5YVE...	A5YVE...	Pyruva...	PDH
12	-1.99157	-2.42629	-1.55667	+	Discard	Keep	+_Discard	+_Keep	4.4294...	805000...	452920...	352080...	1.80909	-1.99151	A6NC4...	A6NC4...	ADP-ri...	BST
13	-1.58235	-0.75516	-0.458...		Discard	Discard			1.4495...	565120...	445340...	119770...	0.96181	-0.932...	Q9BX7...	Q9BX7...	TM2 d...	TM2
14	0.0174...	0.5715...	1.44353		Discard	Discard			1.0956...	164890...	119330...	455590...	0.6122...	0.6774...	A6NCE...	A6NCE...	Microtu...	MAF
15	-0.938...	-0.699...	-0.778...	+	Discard	Discard	+_Discard	+_Discard	0	469440...	380810...	886330...	2.12225	-0.805...	A6NCZ...	A6NCZ...	Sidero...	SFX
16	0.5807...	0.0633...	0.2607...		Discard	Discard			0	129930...	959180...	340170...	0.7366...	0.3015...	A6NDG...	A6NDG...	Phosp...	PGF
17	-0.502...	-0.599...	-0.510...	+	Discard	Discard	+_Discard	+_Discard	0	546080...	504820...	412560...	2.47666	-0.537...	O1473...	O1473...	Acyl-co...	ACC
									3.7088...	223750...	180350...	433970...	0.10081	0.1301...	A6NDU8	A6NDU8	UPF06...	C5o
									0	275230...	223270...	519630...	0.7521...	-0.202...	A6NEM...	A6NEM...	Host c...	HCF
									8.4587...	506980...	445760...	612280...	0.9671...	0.2612...	A6NFN...	A6NFN...	Abl inte...	ABI
									0	295790...	244110...	516780...	0.0757...	-0.102...	Q1356...	Q1356...	NEDD...	NAE
									0	500840...	352390...	148460...	1.0818	0.5644...	A6NFX...	A6NFX...	ADP-s...	NUD
23	0.1964...	0.2607...	0.2628...	+	Discard	Discard	+_Discard	+_Discard	0	877260...	727830...	149430...	2.08932	0.2400...	A6NG5...	A6NG5...	Beta-p...	PAF
24	-0.546...	-0.022...	0.5366...		Discard	Discard			3.2658...	315310...	230010...	853000...	0.0108...	-0.010...	A6NGJ...	A6NGJ...	Dynein...	DYN
25	-0.45406	-0.057...	-0.302...		Discard	Discard			0	958070...	775620...	182440...	0.8425...	-0.271...	A6NGJ...	A6NGJ...	39S rib...	MRF

Protein groups significantly changed & changed <=-1 are marked with“+Keep”

Protein groups significantly changed & changed >=1 are marked with“+Keep”

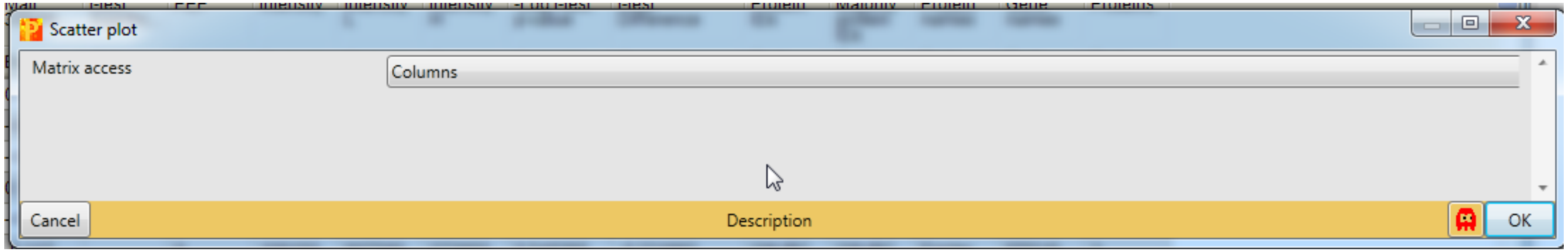
Analysis of SILAC data

An easy way to now visualize these significantly changed protein groups is a volcano plot, which is a type of **scatter plot**.



Scatter plot

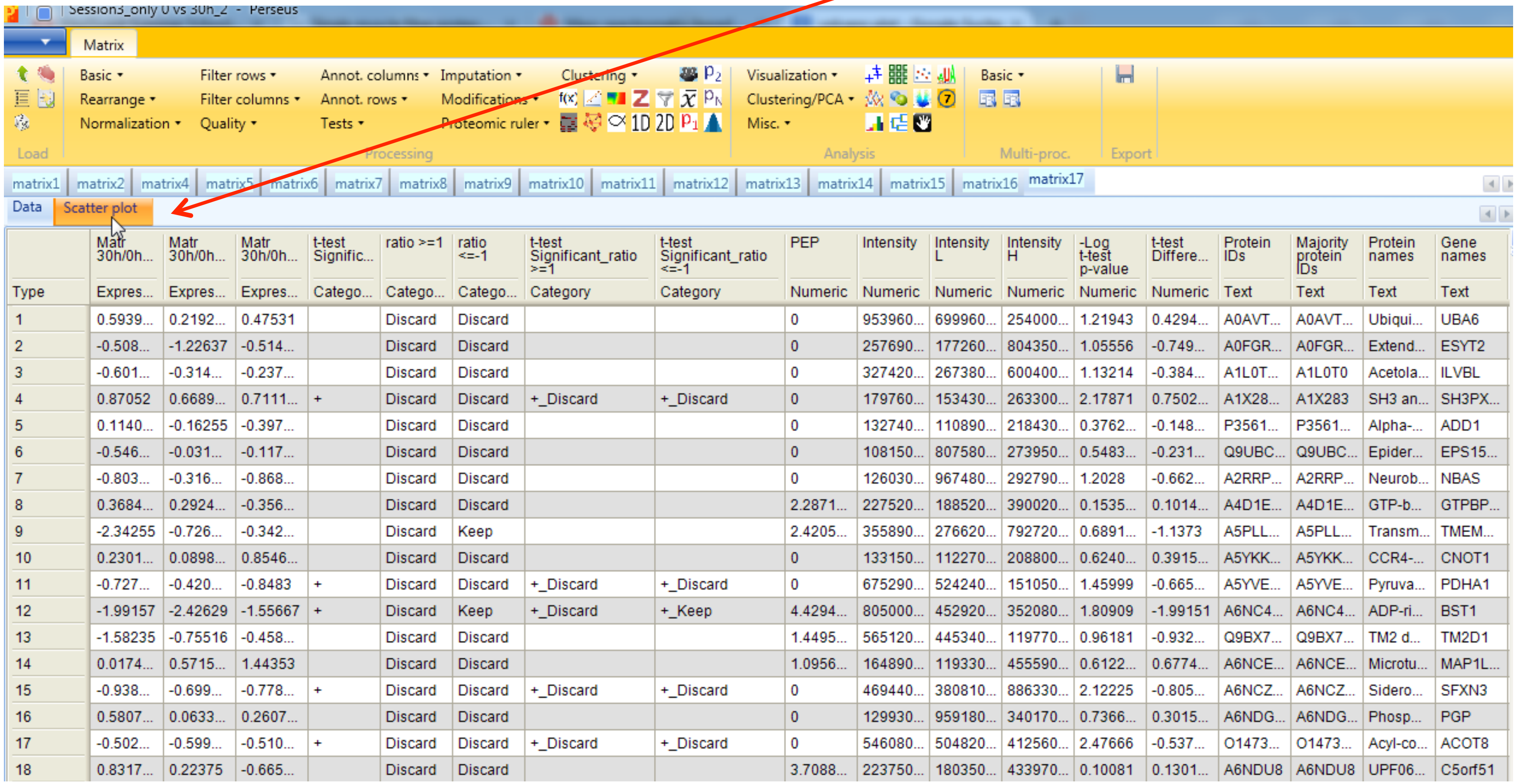
Plot two columns or two rows against each other as x and y values in a scatter plot. Data points can be colored according to values in a third column or row.



Matrix access: Columns

Analysis of SILAC data

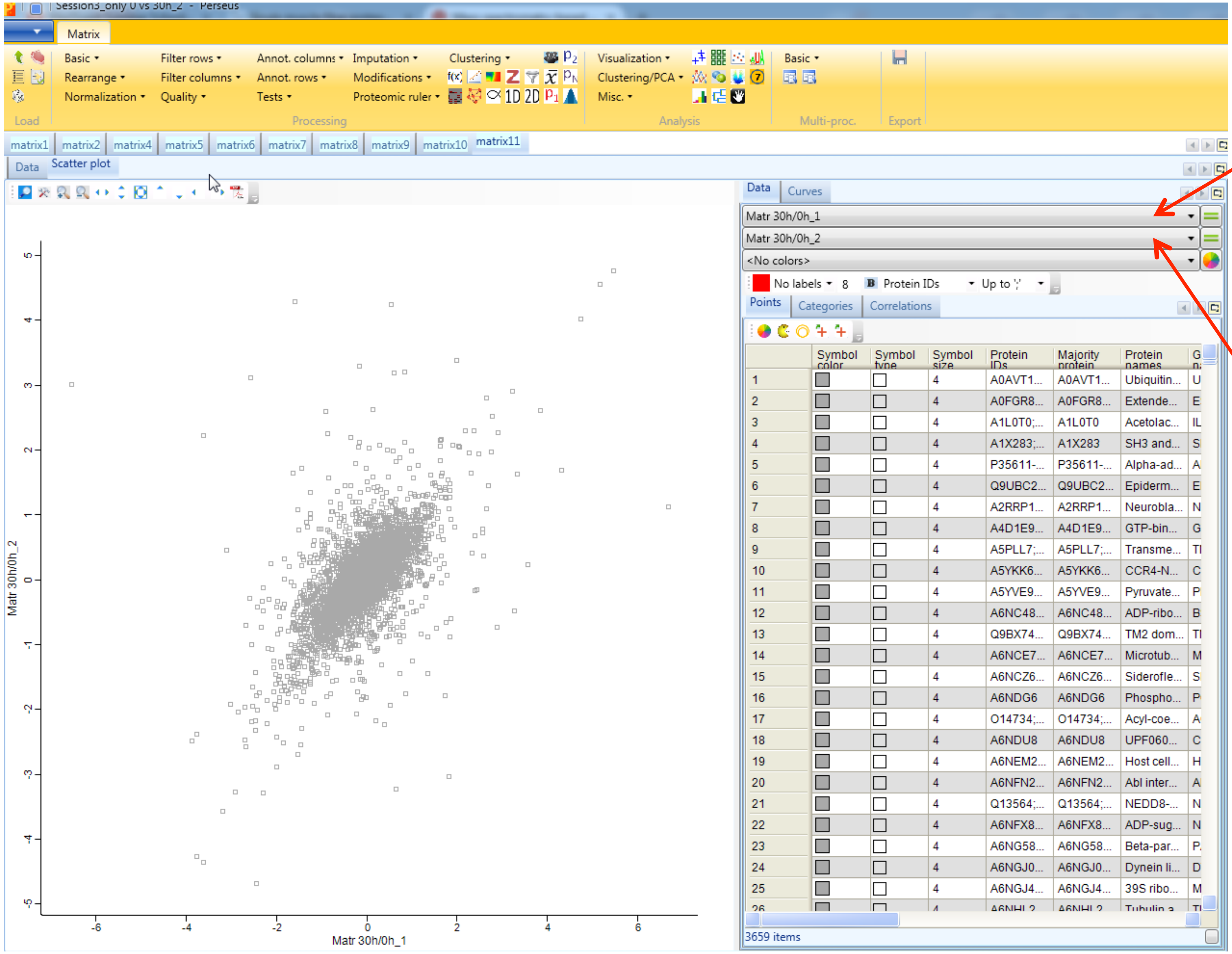
An easy way to visualize these significantly changed protein groups can be achieved by a volcano plot, which is a type of **scatter plot**.



	Matr 30h/0h...	Matr 30h/0h...	Matr 30h/0h...	t-test Signific...	ratio >=1	ratio <=-1	t-test Significant_ratio >=1	t-test Significant_ratio <=-1	PEP	Intensity	Intensity L	Intensity H	-Log t-test p-value	t-test Differe...	Protein IDs	Majority protein IDs	Protein names	Gene names
Type	Expres...	Expres...	Expres...	Catego...	Catego...	Catego...	Category	Category	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Text	Text	Text	Text
1	0.5939...	0.2192...	0.47531		Discard	Discard			0	953960...	699960...	254000...	1.21943	0.4294...	A0AVT...	A0AVT...	Ubiqui...	UBA6
2	-0.508...	-1.22637	-0.514...		Discard	Discard			0	257690...	177260...	804350...	1.05556	-0.749...	A0FGR...	A0FGR...	Extend...	ESYT2
3	-0.601...	-0.314...	-0.237...		Discard	Discard			0	327420...	267380...	600400...	1.13214	-0.384...	A1L0T...	A1L0T0	Acetola...	ILVBL
4	0.87052	0.6689...	0.7111...	+	Discard	Discard	+_Discard	+_Discard	0	179760...	153430...	263300...	2.17871	0.7502...	A1X28...	A1X283	SH3 an...	SH3PX...
5	0.1140...	-0.16255	-0.397...		Discard	Discard			0	132740...	110890...	218430...	0.3762...	-0.148...	P3561...	P3561...	Alpha-...	ADD1
6	-0.546...	-0.031...	-0.117...		Discard	Discard			0	108150...	807580...	273950...	0.5483...	-0.231...	Q9UBC...	Q9UBC...	Epider...	EPS15...
7	-0.803...	-0.316...	-0.868...		Discard	Discard			0	126030...	967480...	292790...	1.2028	-0.662...	A2RRP...	A2RRP...	Neurob...	NBAS
8	0.3684...	0.2924...	-0.356...		Discard	Discard			2.2871...	227520...	188520...	390020...	0.1535...	0.1014...	A4D1E...	A4D1E...	GTP-b...	GTPBP...
9	-2.34255	-0.726...	-0.342...		Discard	Keep			2.4205...	355890...	276620...	792720...	0.6891...	-1.1373	A5PLL...	A5PLL...	Transm...	TMEM...
10	0.2301...	0.0898...	0.8546...		Discard	Discard			0	133150...	112270...	208800...	0.6240...	0.3915...	A5YKK...	A5YKK...	CCR4-...	CNOT1
11	-0.727...	-0.420...	-0.8483	+	Discard	Discard	+_Discard	+_Discard	0	675290...	524240...	151050...	1.45999	-0.665...	A5YVE...	A5YVE...	Pyruva...	PDHA1
12	-1.99157	-2.42629	-1.55667	+	Discard	Keep	+_Discard	+_Keep	4.4294...	805000...	452920...	352080...	1.80909	-1.99151	A6NC4...	A6NC4...	ADP-ri...	BST1
13	-1.58235	-0.75516	-0.458...		Discard	Discard			1.4495...	565120...	445340...	119770...	0.96181	-0.932...	Q9BX7...	Q9BX7...	TM2 d...	TM2D1
14	0.0174...	0.5715...	1.44353		Discard	Discard			1.0956...	164890...	119330...	455590...	0.6122...	0.6774...	A6NCE...	A6NCE...	Microtu...	MAP1L...
15	-0.938...	-0.699...	-0.778...	+	Discard	Discard	+_Discard	+_Discard	0	469440...	380810...	886330...	2.12225	-0.805...	A6NCZ...	A6NCZ...	Sidero...	SFXN3
16	0.5807...	0.0633...	0.2607...		Discard	Discard			0	129930...	959180...	340170...	0.7366...	0.3015...	A6NDG...	A6NDG...	Phosp...	PGP
17	-0.502...	-0.599...	-0.510...	+	Discard	Discard	+_Discard	+_Discard	0	546080...	504820...	412560...	2.47666	-0.537...	O1473...	O1473...	Acyl-co...	ACOT8
18	0.8317...	0.22375	-0.665...		Discard	Discard			3.7088...	223750...	180350...	433970...	0.10081	0.1301...	A6NDU8	A6NDU8	UPF06...	C5orf51

Analysis of SILAC data

In the scatter plot we now have to define, which columns should be plotted against each other. At the moment two **expression values** are selected.



Matr 30h/0h_1

Matr 30h/0h_2

<No colors>

	Symbol color	Symbol type	Symbol size	Protein IDs	Majority protein	Protein names	G n
1	▨	☐	4	A0AVT1...	A0AVT1...	Ubiquitin...	U
2	▨	☐	4	A0FGR8...	A0FGR8...	Extende...	E
3	▨	☐	4	A1L0T0;...	A1L0T0	Acetolac...	IL
4	▨	☐	4	A1X283;...	A1X283	SH3 and...	S
5	▨	☐	4	P35611-...	P35611-...	Alpha-ad...	A
6	▨	☐	4	Q9UBC2...	Q9UBC2...	Epiderm...	E
7	▨	☐	4	A2RRP1...	A2RRP1...	Neurobla...	N
8	▨	☐	4	A4D1E9...	A4D1E9...	GTP-bin...	G
9	▨	☐	4	A5PLL7;...	A5PLL7;...	Transme...	T
10	▨	☐	4	A5YKK6...	A5YKK6...	CCR4-N...	C
11	▨	☐	4	A5YVE9...	A5YVE9...	Pyruvate...	P
12	▨	☐	4	A6NC48...	A6NC48...	ADP-ribo...	B
13	▨	☐	4	Q9BX74...	Q9BX74...	TM2 dom...	T
14	▨	☐	4	A6NCE7...	A6NCE7...	Microtub...	M
15	▨	☐	4	A6NCZ6...	A6NCZ6...	Siderofle...	S
16	▨	☐	4	A6NDG6	A6NDG6	Phospho...	P
17	▨	☐	4	O14734;...	O14734;...	Acyl-coe...	A
18	▨	☐	4	A6NDU8	A6NDU8	UPF060...	C
19	▨	☐	4	A6NEM2...	A6NEM2...	Host cell...	H
20	▨	☐	4	A6NFN2...	A6NFN2...	Abl inter...	A
21	▨	☐	4	Q13564;...	Q13564;...	NEDD8-...	N
22	▨	☐	4	A6NFX8...	A6NFX8...	ADP-sug...	N
23	▨	☐	4	A6NG58...	A6NG58...	Beta-par...	P
24	▨	☐	4	A6NGJ0...	A6NGJ0...	Dynein li...	D
25	▨	☐	4	A6NGJ4...	A6NGJ4...	39S ribo...	M

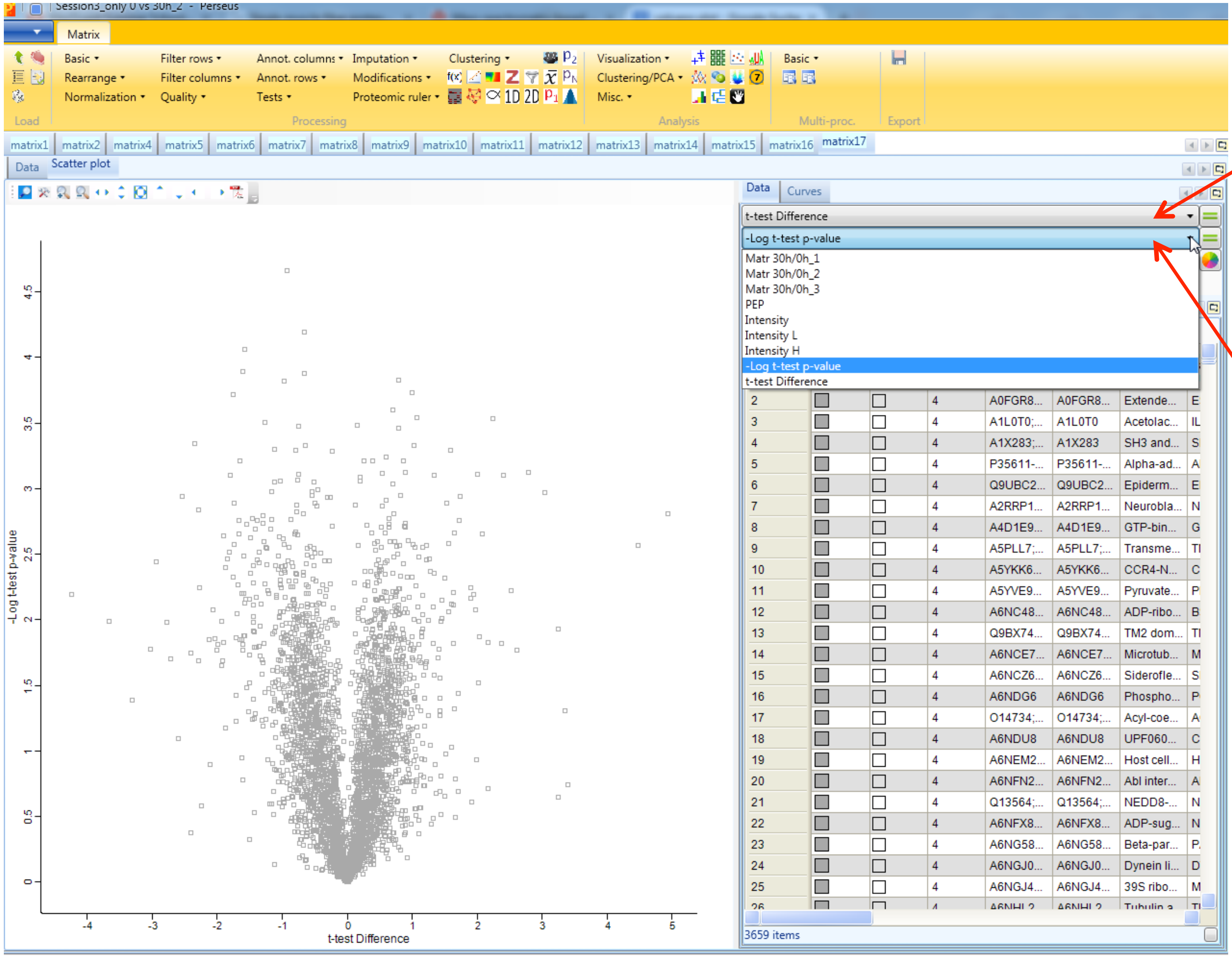
3659 items

Selected x-axis

Selected y-axis

Analysis of SILAC data

For generation of the volcano plot, we now choose the t-test difference for the x- and the –log t-test p-value for the x-axis.



t-test Difference

-Log t-test p-value

2	■	☐	4	A0FGR8...	A0FGR8...	Extende...	E
3	■	☐	4	A1L0T0;...	A1L0T0	Acetolac...	IL
4	■	☐	4	A1X283;...	A1X283	SH3 and...	S
5	■	☐	4	P35611-...	P35611-...	Alpha-ad...	A
6	■	☐	4	Q9UBC2...	Q9UBC2...	Epiderm...	E
7	■	☐	4	A2RRP1...	A2RRP1...	Neurobla...	N
8	■	☐	4	A4D1E9...	A4D1E9...	GTP-bin...	G
9	■	☐	4	A5PLL7;...	A5PLL7;...	Transme...	T
10	■	☐	4	A5YKK6...	A5YKK6...	CCR4-N...	C
11	■	☐	4	A5YVE9...	A5YVE9...	Pyruvate...	P
12	■	☐	4	A6NC48...	A6NC48...	ADP-ribo...	B
13	■	☐	4	Q9BX74...	Q9BX74...	TM2 dom...	T
14	■	☐	4	A6NCE7...	A6NCE7...	Microtub...	M
15	■	☐	4	A6NCZ6...	A6NCZ6...	Siderofle...	S
16	■	☐	4	A6NDG6	A6NDG6	Phospho...	P
17	■	☐	4	O14734;...	O14734;...	Acyl-coe...	A
18	■	☐	4	A6NDU8	A6NDU8	UPF060...	C
19	■	☐	4	A6NEM2...	A6NEM2...	Host cell...	H
20	■	☐	4	A6NFN2...	A6NFN2...	Abl inter...	A
21	■	☐	4	Q13564;...	Q13564;...	NEDD8-...	N
22	■	☐	4	A6NFX8...	A6NFX8...	ADP-sug...	N
23	■	☐	4	A6NG58...	A6NG58...	Beta-par...	P.
24	■	☐	4	A6NGJ0...	A6NGJ0...	Dynein li...	D
25	■	☐	4	A6NGJ4...	A6NGJ4...	39S ribo...	M
26	■	☐	4	A6NHL2	A6NHL2	Tubulin a...	T

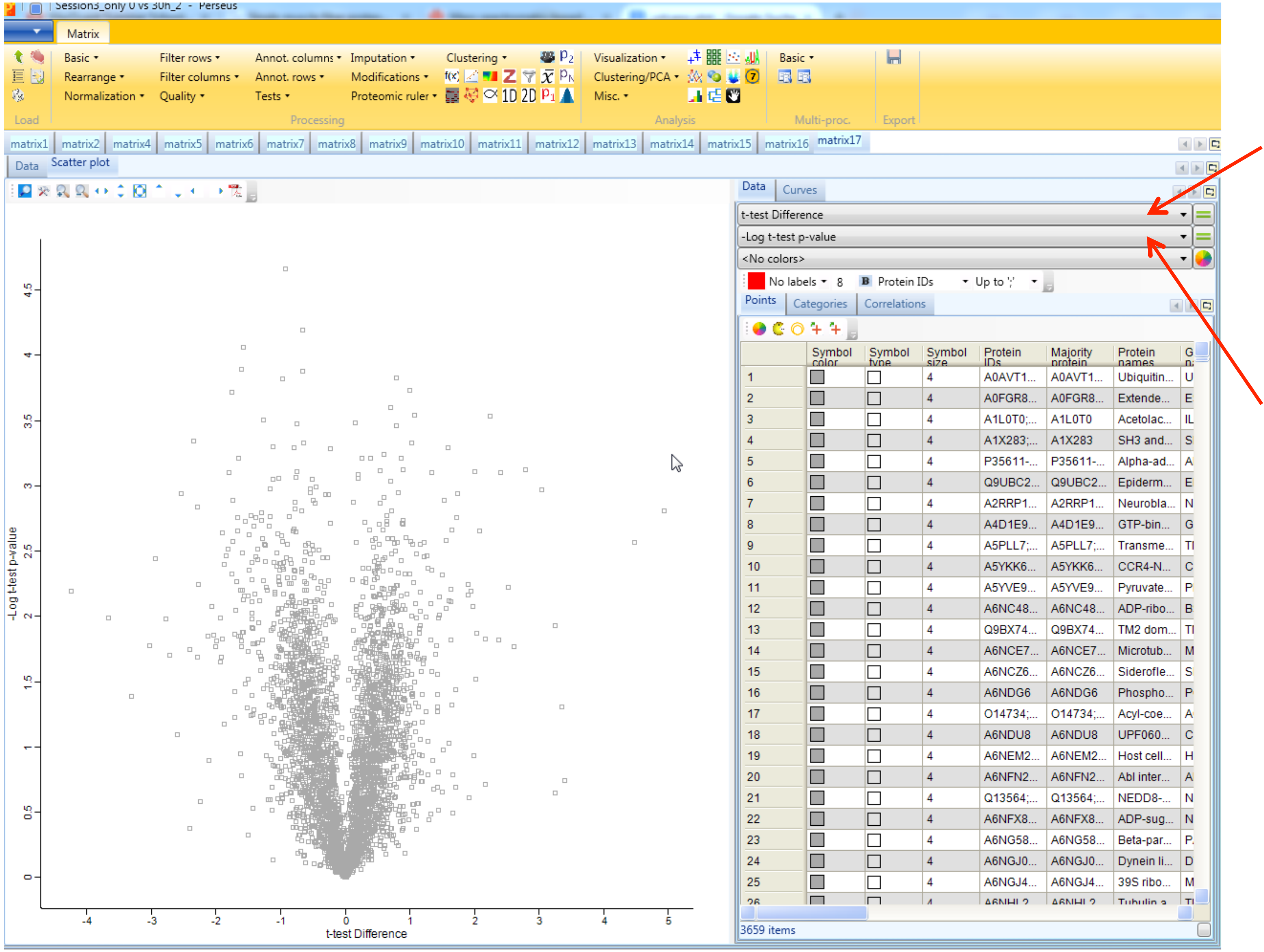
3659 items

Selected x-axis

Selected y-axis

Analysis of SILAC data

For generation of the volcano plot, we now choose the t-test difference for the x- and the –log t-test p-value for the x-axis.



t-test Difference

-Log t-test p-value

<No colors>

No labels 8 Protein IDs Up to ';'

	Symbol color	Symbol type	Symbol size	Protein IDs	Majority protein	Protein names
1	■	☐	4	A0AVT1...	A0AVT1...	Ubiquitin...
2	■	☐	4	A0FGR8...	A0FGR8...	Extende...
3	■	☐	4	A1L0T0;...	A1L0T0	Acetolac...
4	■	☐	4	A1X283;...	A1X283	SH3 and...
5	■	☐	4	P35611-...	P35611-...	Alpha-ad...
6	■	☐	4	Q9UBC2...	Q9UBC2...	Epiderm...
7	■	☐	4	A2RRP1...	A2RRP1...	Neurobla...
8	■	☐	4	A4D1E9...	A4D1E9...	GTP-bin...
9	■	☐	4	A5PLL7;...	A5PLL7;...	Transme...
10	■	☐	4	A5YKK6...	A5YKK6...	CCR4-N...
11	■	☐	4	A5YVE9...	A5YVE9...	Pyruvate...
12	■	☐	4	A6NC48...	A6NC48...	ADP-ribo...
13	■	☐	4	Q9BX74...	Q9BX74...	TM2 dom...
14	■	☐	4	A6NCE7...	A6NCE7...	Microtub...
15	■	☐	4	A6NCZ6...	A6NCZ6...	Siderofle...
16	■	☐	4	A6NDG6	A6NDG6	Phospho...
17	■	☐	4	O14734;...	O14734;...	Acyl-coe...
18	■	☐	4	A6NDU8	A6NDU8	UPF060...
19	■	☐	4	A6NEM2...	A6NEM2...	Host cell...
20	■	☐	4	A6NFN2...	A6NFN2...	Abl inter...
21	■	☐	4	Q13564;...	Q13564;...	NEDD8-...
22	■	☐	4	A6NFX8...	A6NFX8...	ADP-sug...
23	■	☐	4	A6NG58...	A6NG58...	Beta-par...
24	■	☐	4	A6NGJ0...	A6NGJ0...	Dynein li...
25	■	☐	4	A6NGJ4...	A6NGJ4...	39S ribo...

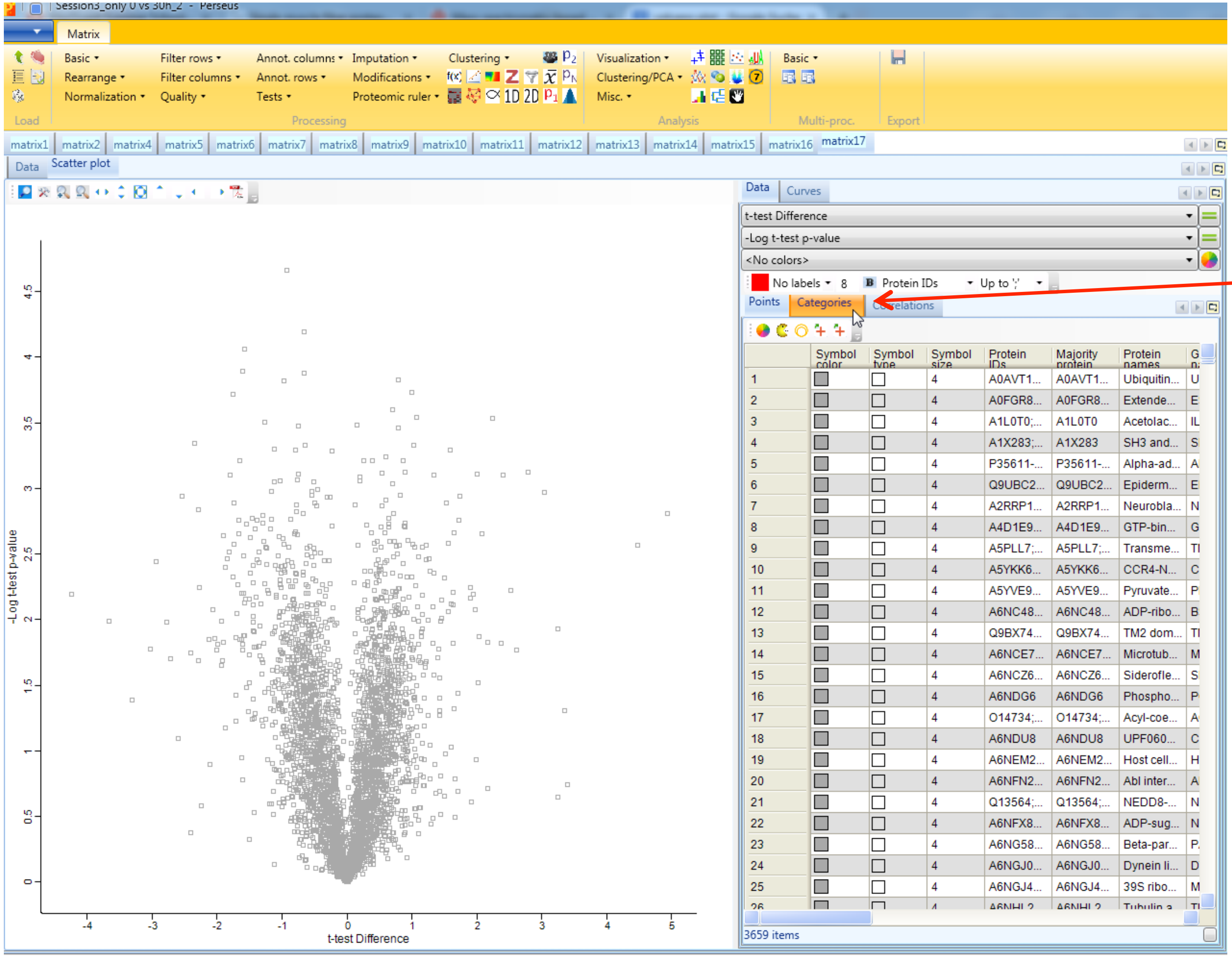
3659 items

Selected x-axis

Selected y-axis

Analysis of SILAC data

Next we highlight the significantly and relevantly changed protein groups.



t-test Difference

-Log t-test p-value

<No colors>

No labels 8 Protein IDs Up to ';'

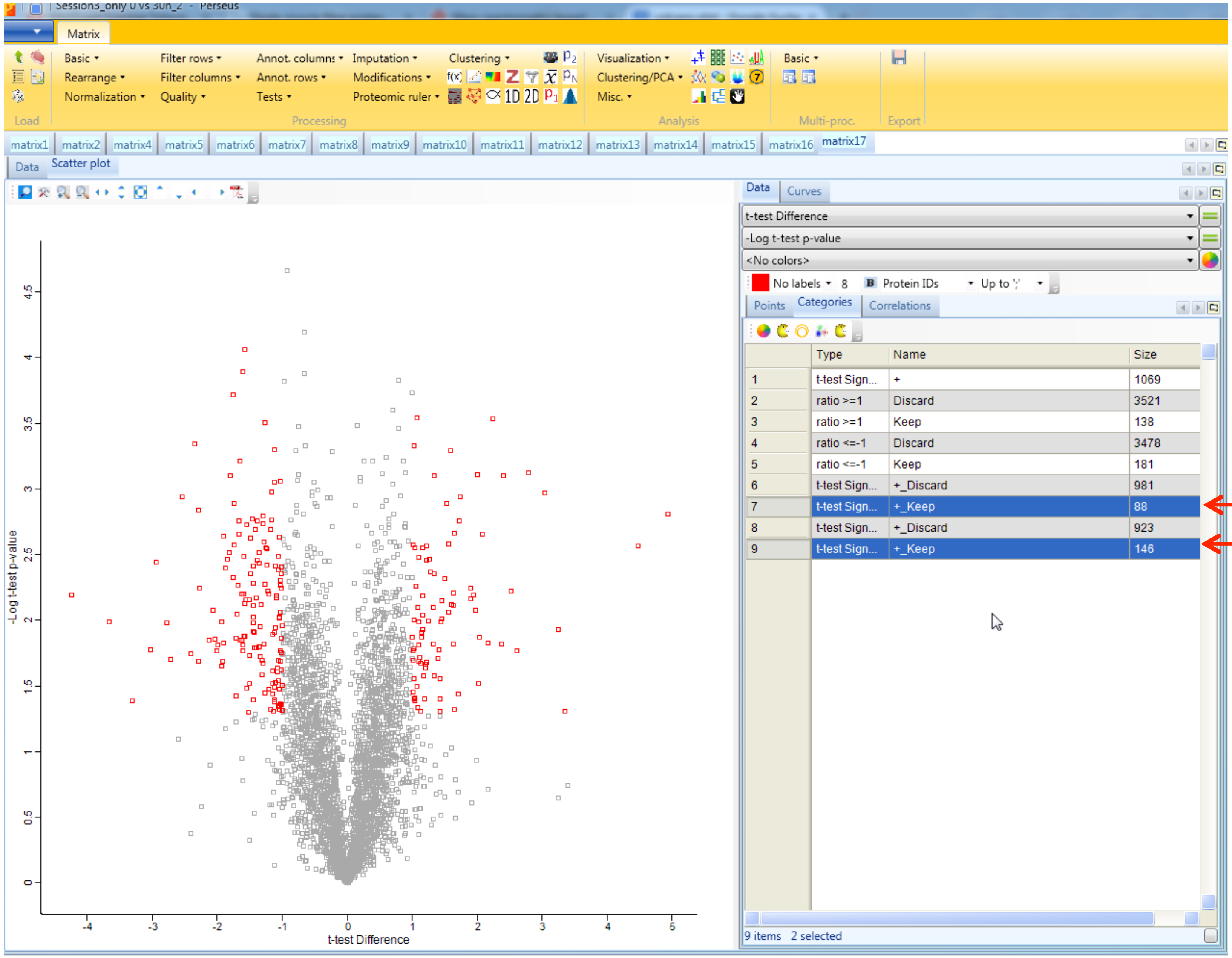
	Symbol color	Symbol type	Symbol size	Protein IDs	Majority protein	Protein names	G
1	■	☐	4	A0AVT1...	A0AVT1...	Ubiquitin...	U
2	■	☐	4	A0FGR8...	A0FGR8...	Extende...	E
3	■	☐	4	A1L0T0;...	A1L0T0	Acetolac...	IL
4	■	☐	4	A1X283;...	A1X283	SH3 and...	S
5	■	☐	4	P35611-...	P35611-...	Alpha-ad...	A
6	■	☐	4	Q9UBC2...	Q9UBC2...	Epiderm...	E
7	■	☐	4	A2RRP1...	A2RRP1...	Neurobla...	N
8	■	☐	4	A4D1E9...	A4D1E9...	GTP-bin...	G
9	■	☐	4	A5PLL7;...	A5PLL7;...	Transme...	T
10	■	☐	4	A5YKK6...	A5YKK6...	CCR4-N...	C
11	■	☐	4	A5YVE9...	A5YVE9...	Pyruvate...	P
12	■	☐	4	A6NC48...	A6NC48...	ADP-ribo...	B
13	■	☐	4	Q9BX74...	Q9BX74...	TM2 dom...	T
14	■	☐	4	A6NCE7...	A6NCE7...	Microtub...	M
15	■	☐	4	A6NCZ6...	A6NCZ6...	Siderofle...	S
16	■	☐	4	A6NDG6	A6NDG6	Phospho...	P
17	■	☐	4	O14734;...	O14734;...	Acyl-coe...	A
18	■	☐	4	A6NDU8	A6NDU8	UPF060...	C
19	■	☐	4	A6NEM2...	A6NEM2...	Host cell...	H
20	■	☐	4	A6NFN2...	A6NFN2...	Abl inter...	A
21	■	☐	4	Q13564;...	Q13564;...	NEDD8-...	N
22	■	☐	4	A6NFX8...	A6NFX8...	ADP-sug...	N
23	■	☐	4	A6NG58...	A6NG58...	Beta-par...	P
24	■	☐	4	A6NGJ0...	A6NGJ0...	Dynein li...	D
25	■	☐	4	A6NGJ4...	A6NGJ4...	39S ribo...	M

3659 items

Select only the protein groups in the previously defined categories-

Analysis of SILAC data

Next we highlight the significantly and relevantly changed protein groups.



t-test Difference

-Log t-test p-value

<No colors>

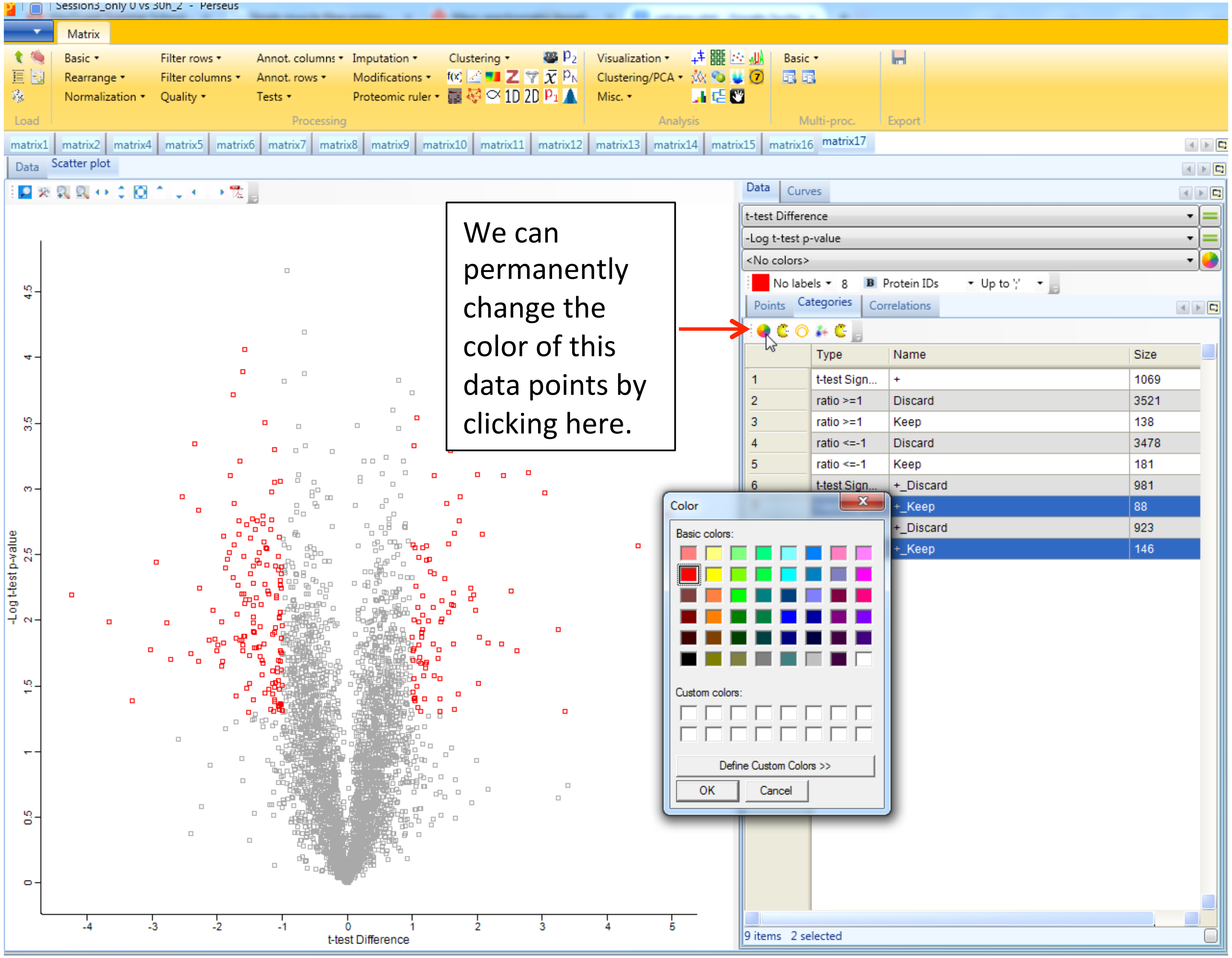
	Type	Name	Size
1	t-test Sign...	+	1069
2	ratio >=1	Discard	3521
3	ratio >=1	Keep	138
4	ratio <=-1	Discard	3478
5	ratio <=-1	Keep	181
6	t-test Sign...	+_Discard	981
7	t-test Sign...	+_Keep	88
8	t-test Sign...	+_Discard	923
9	t-test Sign...	+_Keep	146

9 items 2 selected

By selecting them, their respective data points in the plot become highlighted in red.

Analysis of SILAC data

Next we highlight the significantly and relevantly changed protein groups.



We can permanently change the color of this data points by clicking here.

t-test Difference

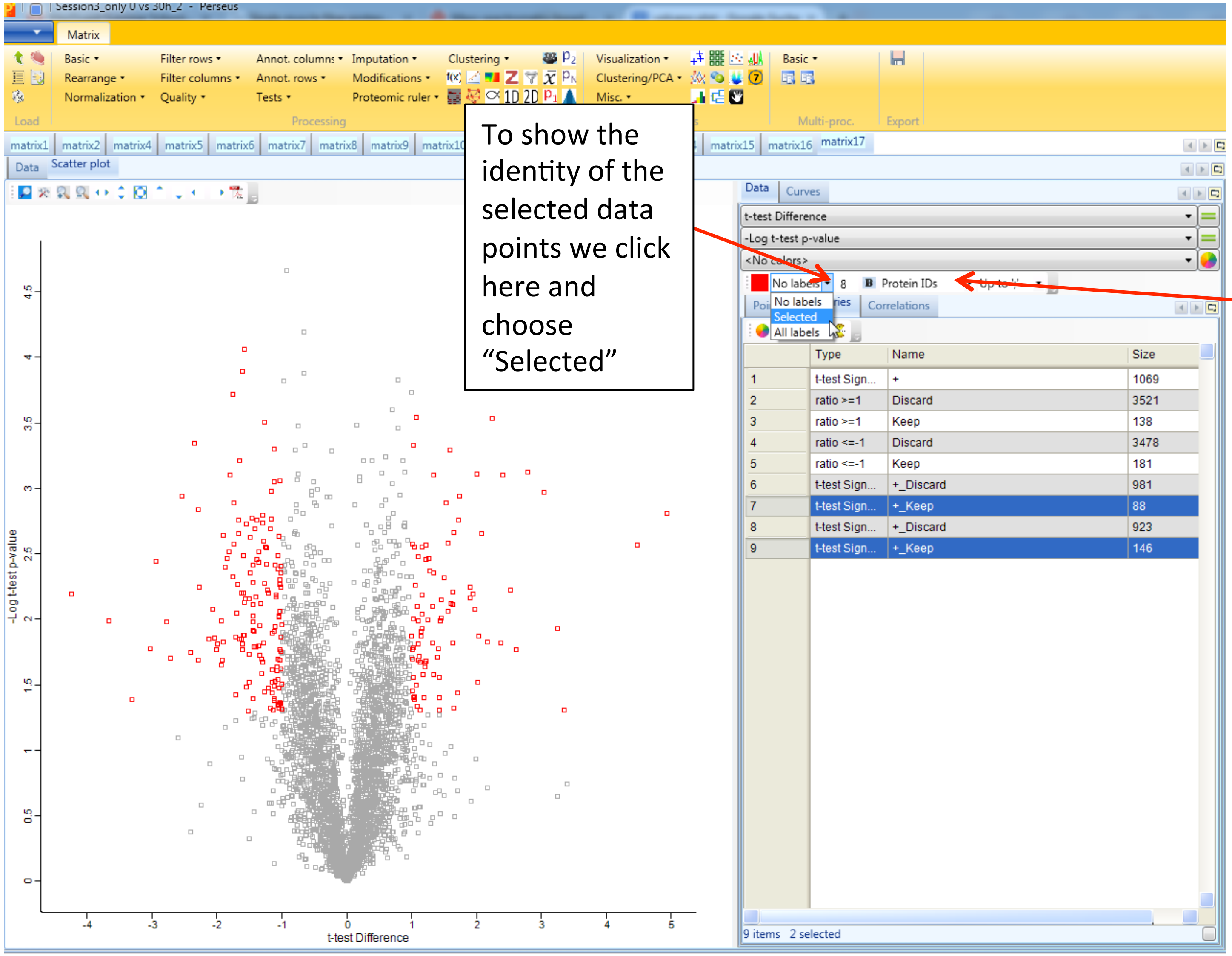
-Log t-test p-value

<No colors>

	Type	Name	Size
1	t-test Sign...	+	1069
2	ratio >=1	Discard	3521
3	ratio >=1	Keep	138
4	ratio <=-1	Discard	3478
5	ratio <=-1	Keep	181
6	t-test Sign...	+_Discard	981
		+_Keep	88
		+_Discard	923
		+_Keep	146

Analysis of SILAC data

Next we highlight the significantly and relevantly changed protein groups.



t-test Difference

-Log t-test p-value

<No colors>

No labels 8 Protein IDs Up to ';'

No labels

Selected

All labels

	Type	Name	Size
1	t-test Sign...	+	1069
2	ratio >=1	Discard	3521
3	ratio >=1	Keep	138
4	ratio <=-1	Discard	3478
5	ratio <=-1	Keep	181
6	t-test Sign...	+_Discard	981
7	t-test Sign...	+_Keep	88
8	t-test Sign...	+_Discard	923
9	t-test Sign...	+_Keep	146

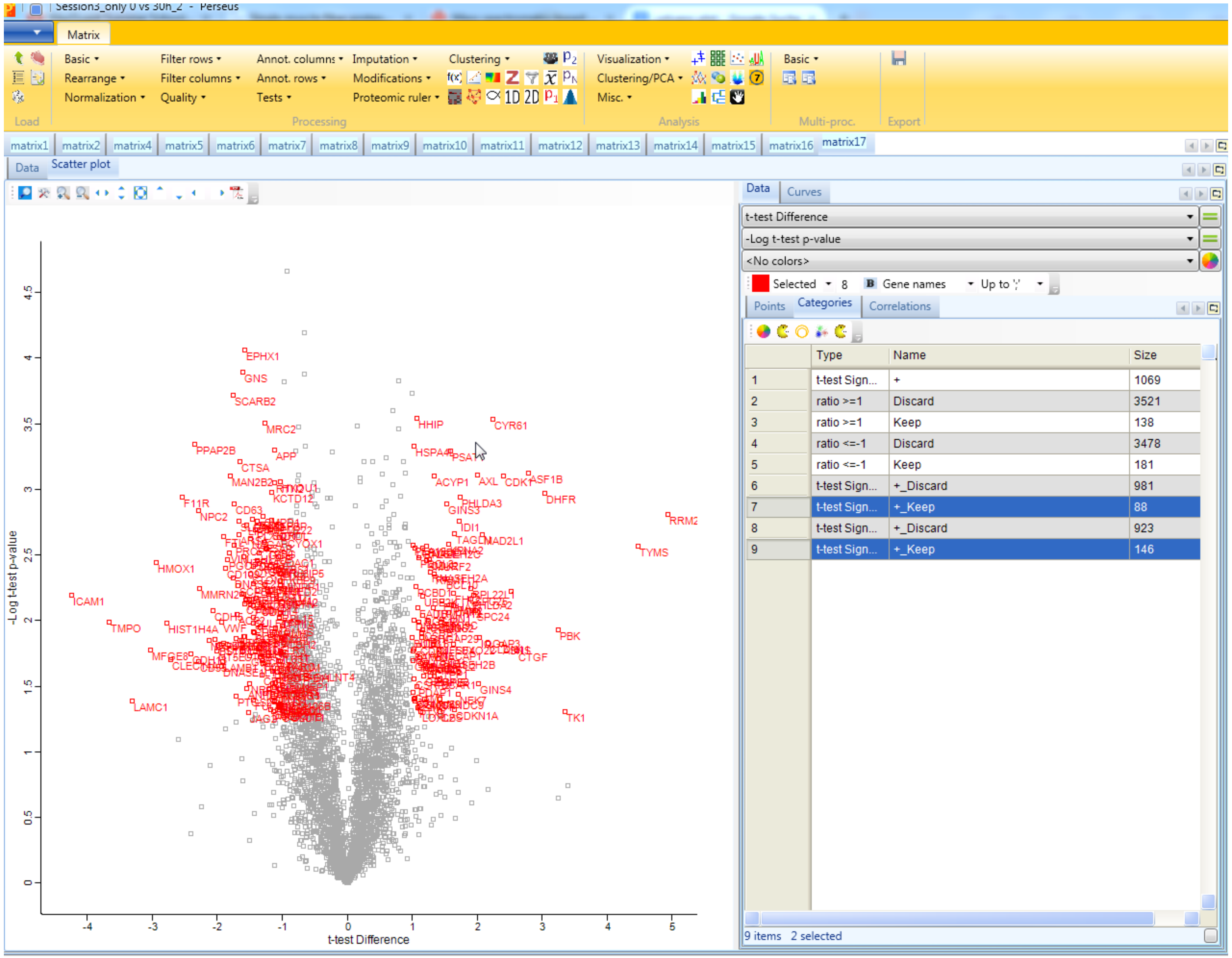
9 items 2 selected

To show the identity of the selected data points we click here and choose "Selected"

As identifier we can choose between: Protein accessions", Gene names etc. We select "Gene names"

Analysis of SILAC data

Next we highlight the significantly and relevantly changed protein groups.



t-test Difference

-Log t-test p-value

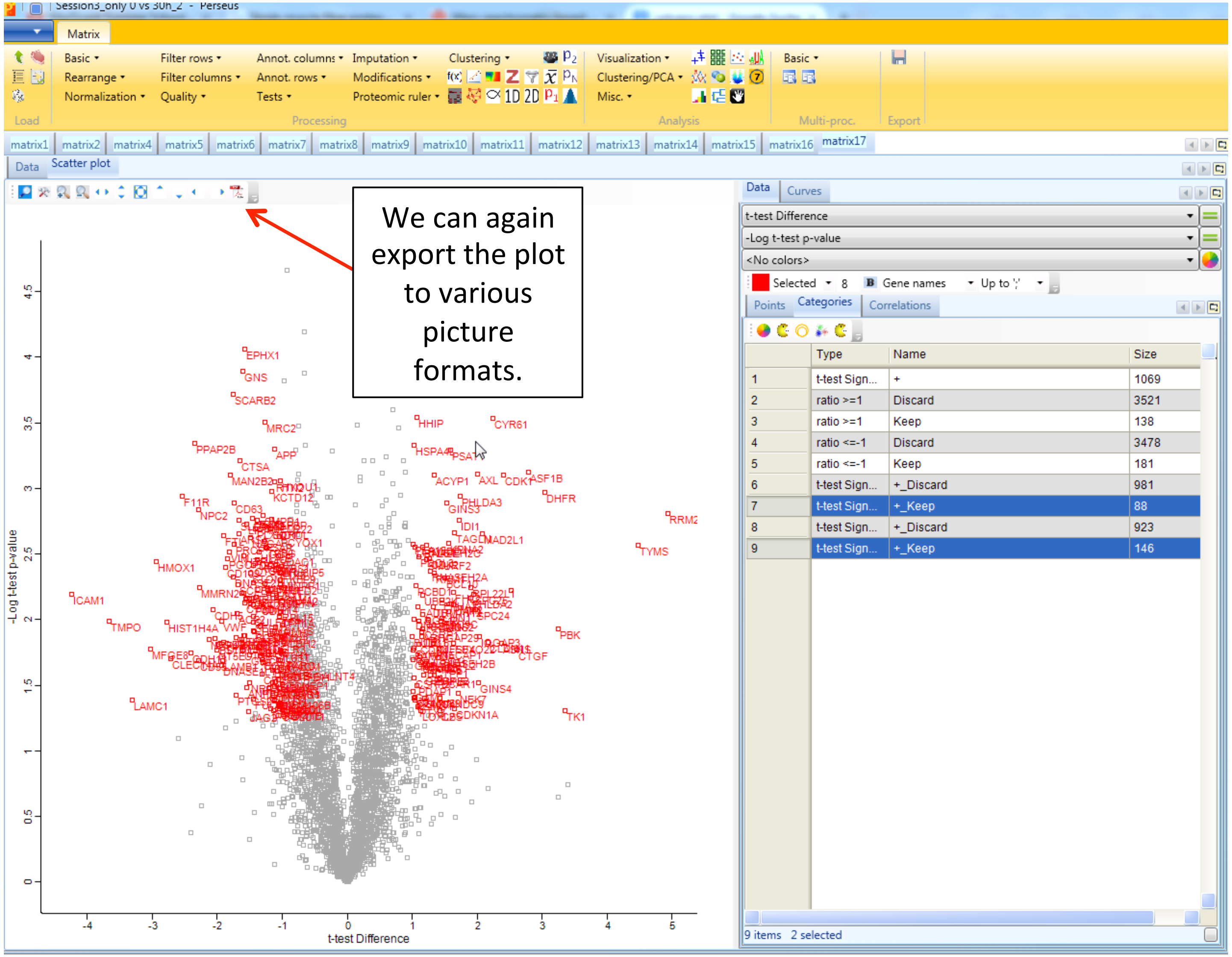
<No colors>

Selected 8 Gene names Up to ';'

	Type	Name	Size
1	t-test Sign...	+	1069
2	ratio >=1	Discard	3521
3	ratio >=1	Keep	138
4	ratio <=-1	Discard	3478
5	ratio <=-1	Keep	181
6	t-test Sign...	+_Discard	981
7	t-test Sign...	+_Keep	88
8	t-test Sign...	+_Discard	923
9	t-test Sign...	+_Keep	146

Analysis of SILAC data

Next we highlight the significantly and relevantly changed protein groups.



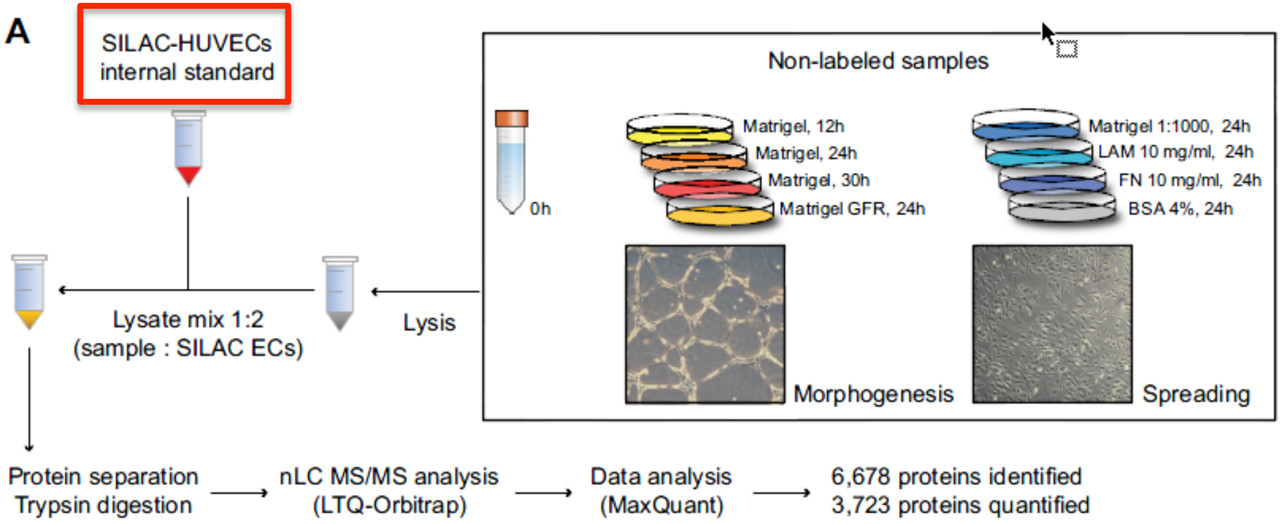
	Type	Name	Size
1	t-test Sign...	+	1069
2	ratio >=1	Discard	3521
3	ratio >=1	Keep	138
4	ratio <=-1	Discard	3478
5	ratio <=-1	Keep	181
6	t-test Sign...	+_Discard	981
7	t-test Sign...	+_Keep	88
8	t-test Sign...	+_Discard	923
9	t-test Sign...	+_Keep	146

9 items 2 selected

We can again export the plot to various picture formats.

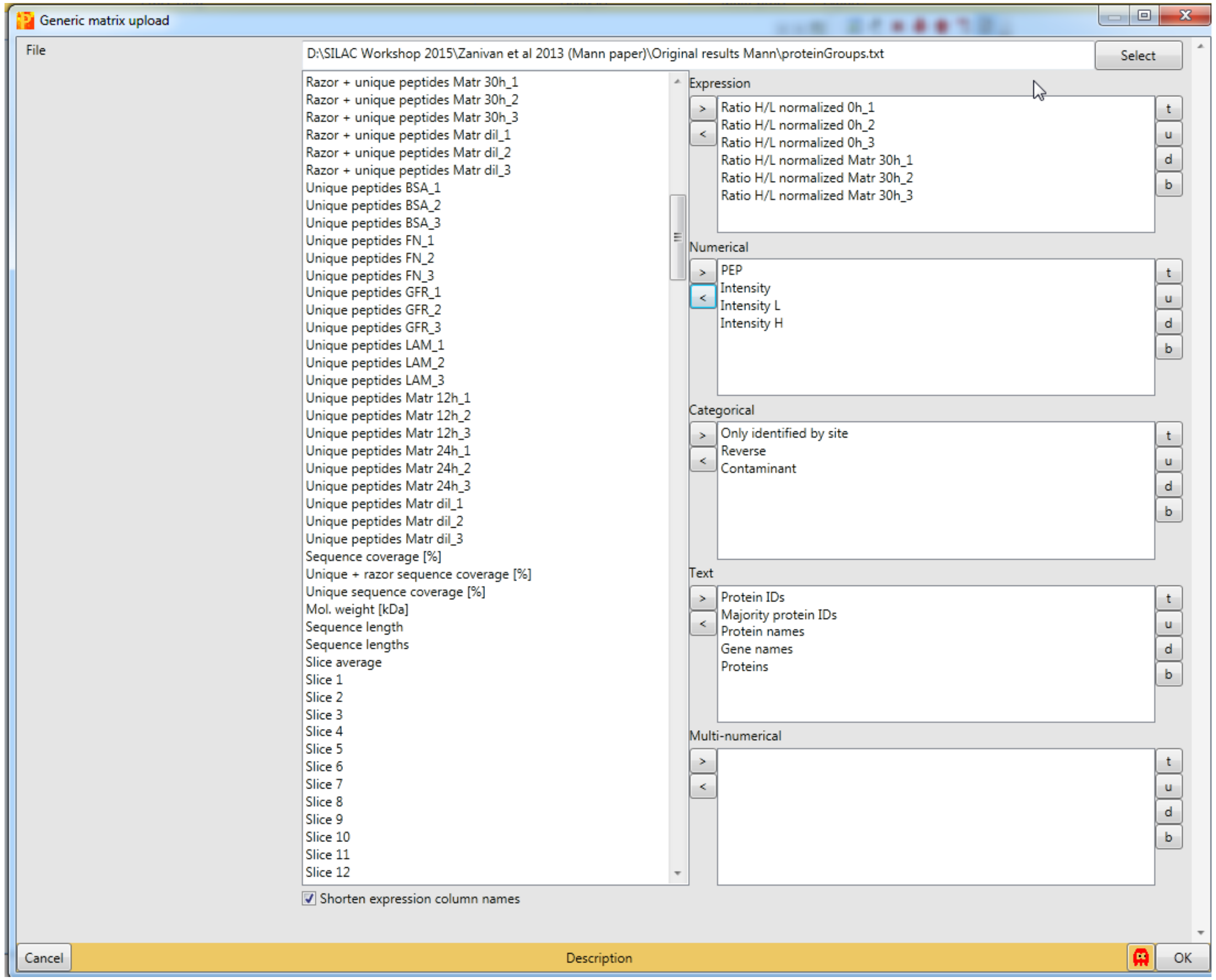
Analysis of SILAC data

- Special case Spike-in SILAC
  - ➢Sample proteins are measured against a heavy labeled reference sample (internal standard)



Analysis of SILAC data

So we again load up the **proteinGroups.txt** into Perseus. And select the 0h and Matr30h H/L ratios



D:\SILAC Workshop 2015\Zanivan et al 2013 (Mann paper)\Original results Mann\proteinGroups.txt

Razor + unique peptides Matr 30h_1
Razor + unique peptides Matr 30h_2
Razor + unique peptides Matr 30h_3
Razor + unique peptides Matr dil_1
Razor + unique peptides Matr dil_2
Razor + unique peptides Matr dil_3
Unique peptides BSA_1
Unique peptides BSA_2
Unique peptides BSA_3
Unique peptides FN_1
Unique peptides FN_2
Unique peptides FN_3
Unique peptides GFR_1
Unique peptides GFR_2
Unique peptides GFR_3
Unique peptides LAM_1
Unique peptides LAM_2
Unique peptides LAM_3
Unique peptides Matr 12h_1
Unique peptides Matr 12h_2
Unique peptides Matr 12h_3
Unique peptides Matr 24h_1
Unique peptides Matr 24h_2
Unique peptides Matr 24h_3
Unique peptides Matr dil_1
Unique peptides Matr dil_2
Unique peptides Matr dil_3
Sequence coverage [%]
Unique + razor sequence coverage [%]
Unique sequence coverage [%]
Mol. weight [kDa]
Sequence length
Sequence lengths
Slice average
Slice 1
Slice 2
Slice 3
Slice 4
Slice 5
Slice 6
Slice 7
Slice 8
Slice 9
Slice 10
Slice 11
Slice 12

☑ Shorten expression column names

Expression

Ratio H/L normalized 0h_1
Ratio H/L normalized 0h_2
Ratio H/L normalized 0h_3
Ratio H/L normalized Matr 30h_1
Ratio H/L normalized Matr 30h_2
Ratio H/L normalized Matr 30h_3

Numerical

PEP
Intensity
Intensity L
Intensity H

Categorical

Only identified by site
Reverse
Contaminant

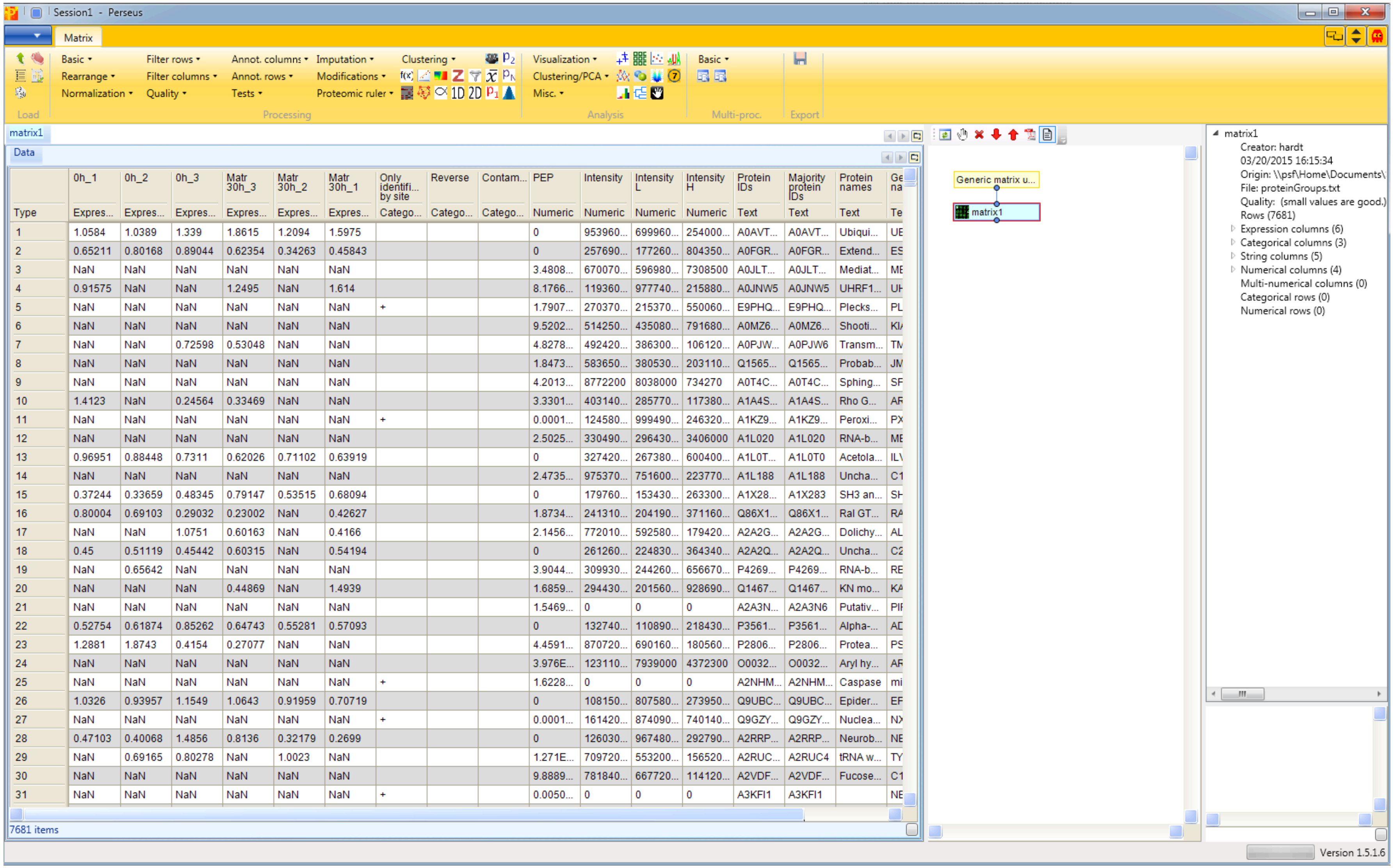
Text

Protein IDs
Majority protein IDs
Protein names
Gene names
Proteins

Multi-numerical

Analysis of SILAC data

So we again load up the **proteinGroups.txt** into Perseus. And select the 0h and Matr30h H/L ratios. Afterwards we filter the contaminats etc.



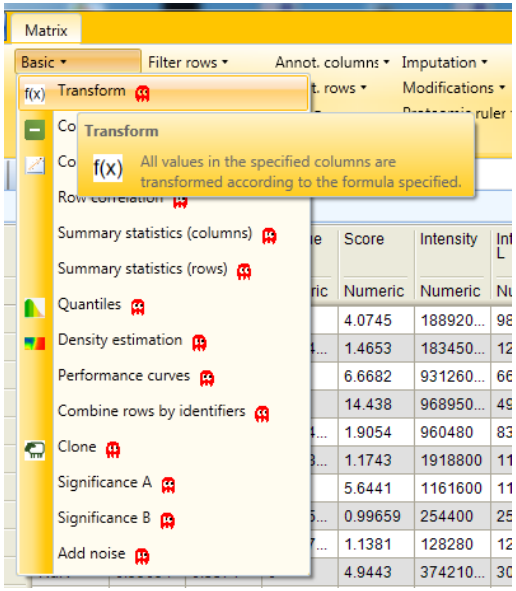
	0h_1	0h_2	0h_3	Matr 30h_3	Matr 30h_2	Matr 30h_1	Only identifi... by site	Reverse	Contam...	PEP	Intensity	Intensity L	Intensity H	Protein IDs	Majority protein IDs	Protein names	Ge na
Type	Expres...	Expres...	Expres...	Expres...	Expres...	Expres...	Catego...	Catego...	Catego...	Numeric	Numeric	Numeric	Numeric	Text	Text	Text	Te
1	1.0584	1.0389	1.339	1.8615	1.2094	1.5975				0	953960...	699960...	254000...	A0AVT...	A0AVT...	Ubiqui...	UE
2	0.65211	0.80168	0.89044	0.62354	0.34263	0.45843				0	257690...	177260...	804350...	A0FGR...	A0FGR...	Extend...	ES
3	NaN	NaN	NaN	NaN	NaN	NaN				3.4808...	670070...	596980...	7308500	A0JLT...	A0JLT...	Mediat...	ME
4	0.91575	NaN	NaN	1.2495	NaN	1.614				8.1766...	119360...	977740...	215880...	A0JNW5	A0JNW5	UHRF1...	UH
5	NaN	NaN	NaN	NaN	NaN	NaN	+			1.7907...	270370...	215370...	550060...	E9PHQ...	E9PHQ...	Plecks...	PL
6	NaN	NaN	NaN	NaN	NaN	NaN				9.5202...	514250...	435080...	791680...	A0MZ6...	A0MZ6...	Shooti...	KI
7	NaN	NaN	0.72598	0.53048	NaN	NaN				4.8278...	492420...	386300...	106120...	A0PJW...	A0PJW6	Transm...	TM
8	NaN	NaN	NaN	NaN	NaN	NaN				1.8473...	583650...	380530...	203110...	Q1565...	Q1565...	Probab...	JM
9	NaN	NaN	NaN	NaN	NaN	NaN				4.2013...	8772200	8038000	734270	A0T4C...	A0T4C...	Sphing...	SF
10	1.4123	NaN	0.24564	0.33469	NaN	NaN				3.3301...	403140...	285770...	117380...	A1A4S...	A1A4S...	Rho G...	AR
11	NaN	NaN	NaN	NaN	NaN	NaN	+			0.0001...	124580...	999490...	246320...	A1KZ9...	A1KZ9...	Peroxi...	PX
12	NaN	NaN	NaN	NaN	NaN	NaN				2.5025...	330490...	296430...	3406000	A1L020	A1L020	RNA-b...	ME
13	0.96951	0.88448	0.7311	0.62026	0.71102	0.63919				0	327420...	267380...	600400...	A1L0T...	A1L0T0	Acetola...	IL
14	NaN	NaN	NaN	NaN	NaN	NaN				2.4735...	975370...	751600...	223770...	A1L188	A1L188	Uncha...	C1
15	0.37244	0.33659	0.48345	0.79147	0.53515	0.68094				0	179760...	153430...	263300...	A1X28...	A1X283	SH3 an...	SH
16	0.80004	0.69103	0.29032	0.23002	NaN	0.42627				1.8734...	241310...	204190...	371160...	Q86X1...	Q86X1...	Ral GT...	RA
17	NaN	NaN	1.0751	0.60163	NaN	0.4166				2.1456...	772010...	592580...	179420...	A2A2G...	A2A2G...	Dolichy...	AL
18	0.45	0.51119	0.45442	0.60315	NaN	0.54194				0	261260...	224830...	364340...	A2A2Q...	A2A2Q...	Uncha...	C2
19	NaN	0.65642	NaN	NaN	NaN	NaN				3.9044...	309930...	244260...	656670...	P4269...	P4269...	RNA-b...	RE
20	NaN	NaN	NaN	0.44869	NaN	1.4939				1.6859...	294430...	201560...	928690...	Q1467...	Q1467...	KN mo...	KA
21	NaN	NaN	NaN	NaN	NaN	NaN				1.5469...	0	0	0	A2A3N...	A2A3N6	Putativ...	PI
22	0.52754	0.61874	0.85262	0.64743	0.55281	0.57093				0	132740...	110890...	218430...	P3561...	P3561...	Alpha-...	AD
23	1.2881	1.8743	0.4154	0.27077	NaN	NaN				4.4591...	870720...	690160...	180560...	P2806...	P2806...	Protea...	PS
24	NaN	NaN	NaN	NaN	NaN	NaN				3.976E...	123110...	7939000	4372300	O0032...	O0032...	Aryl hy...	AR
25	NaN	NaN	NaN	NaN	NaN	NaN	+			1.6228...	0	0	0	A2NHM...	A2NHM...	Caspase	mi
26	1.0326	0.93957	1.1549	1.0643	0.91959	0.70719				0	108150...	807580...	273950...	Q9UBC...	Q9UBC...	Epider...	EF
27	NaN	NaN	NaN	NaN	NaN	NaN	+			0.0001...	161420...	874090...	740140...	Q9GZY...	Q9GZY...	Nuclea...	NX
28	0.47103	0.40068	1.4856	0.8136	0.32179	0.2699				0	126030...	967480...	292790...	A2RRP...	A2RRP...	Neurob...	NE
29	NaN	0.69165	0.80278	NaN	1.0023	NaN				1.271E...	709720...	553200...	156520...	A2RUC...	A2RUC4	tRNA w...	TY
30	NaN	NaN	NaN	NaN	NaN	NaN				9.8889...	781840...	667720...	114120...	A2VDF...	A2VDF...	Fucose...	C1
31	NaN	NaN	NaN	NaN	NaN	NaN	+			0.0050...	0	0	0	A3KFI1	A3KFI1		NE

7681 items

matrix1
Creator: hardt
03/20/2015 16:15:34
Origin: \\psf\Home\Documents\
File: proteinGroups.txt
Quality: (small values are good.)
Rows (7681)
Expression columns (6)
Categorical columns (3)
String columns (5)
Numerical columns (4)
Multi-numerical columns (0)
Categorical rows (0)
Numerical rows (0)

Analysis of SILAC data

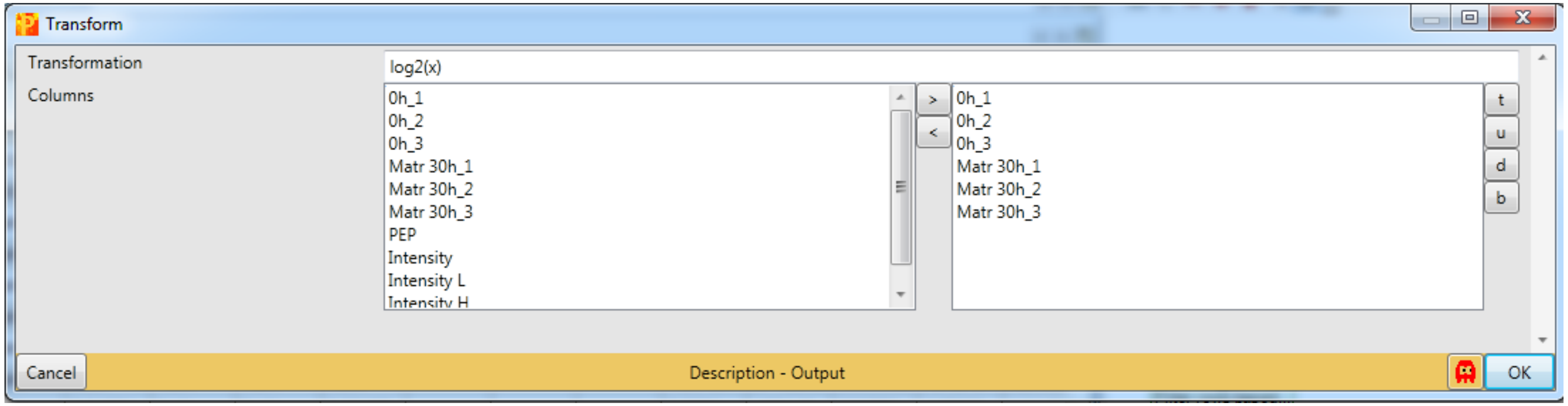
Next we again linearize the SILAC ratios by transforming them to their log2-values.



Transform

All values in the specified columns are transformed according to the formula specified.

Score	Intensity
Numeric	Numeric
4.0745	188920...
1.4653	183450...
6.6682	931260...
14.438	968950...
1.9054	960480
1.1743	1918800
5.6441	1161600
0.99659	254400
1.1381	128280
4.9443	374210...



Transformation: log2(x)

Columns:

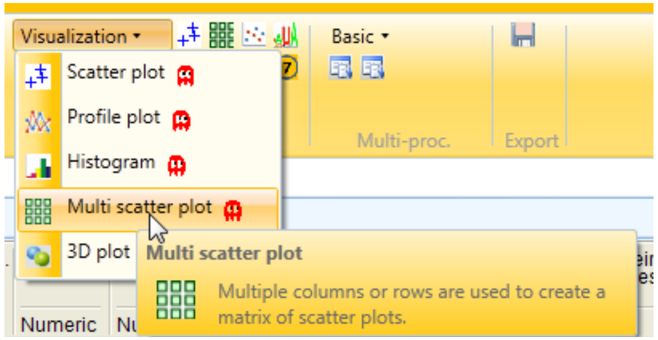
0h_1
0h_2
0h_3
Matr 30h_1
Matr 30h_2
Matr 30h_3
PEP
Intensity
Intensity L
Intensity H

Selected:

0h_1
0h_2
0h_3
Matr 30h_1
Matr 30h_2
Matr 30h_3

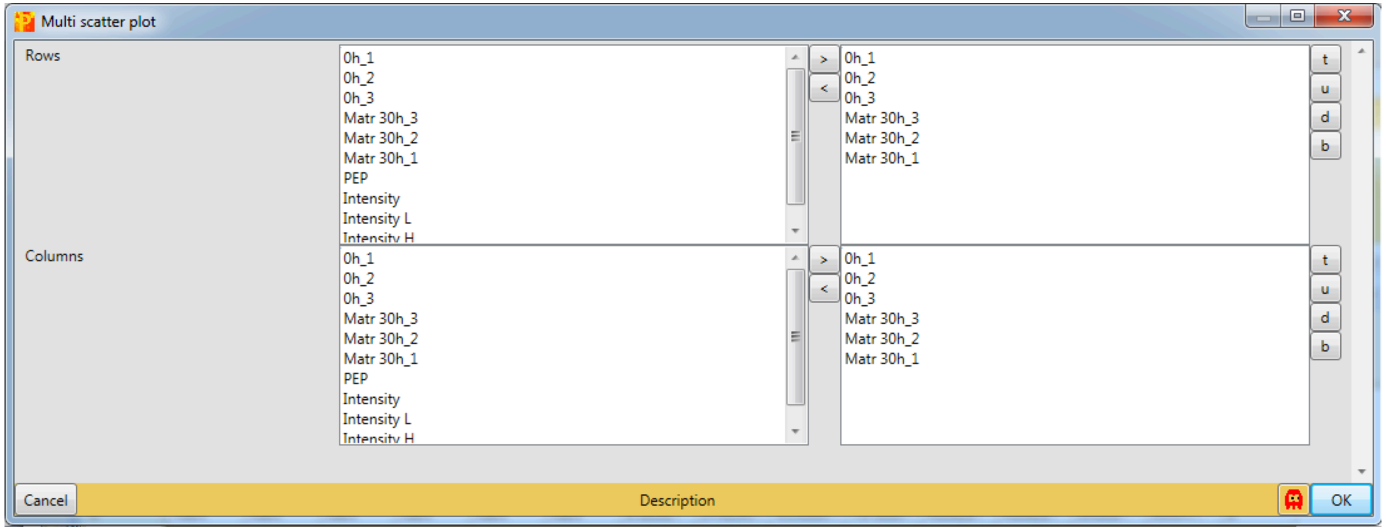
Analysis of SILAC data

As before we do a multi scatter plot to assess the reproducibility of the samples.



Multi scatter plot

Multiple columns or rows are used to create a matrix of scatter plots.



Rows (available): 0h_1, 0h_2, 0h_3, Matr 30h_3, Matr 30h_2, Matr 30h_1, PEP, Intensity, Intensity L, Intensity H

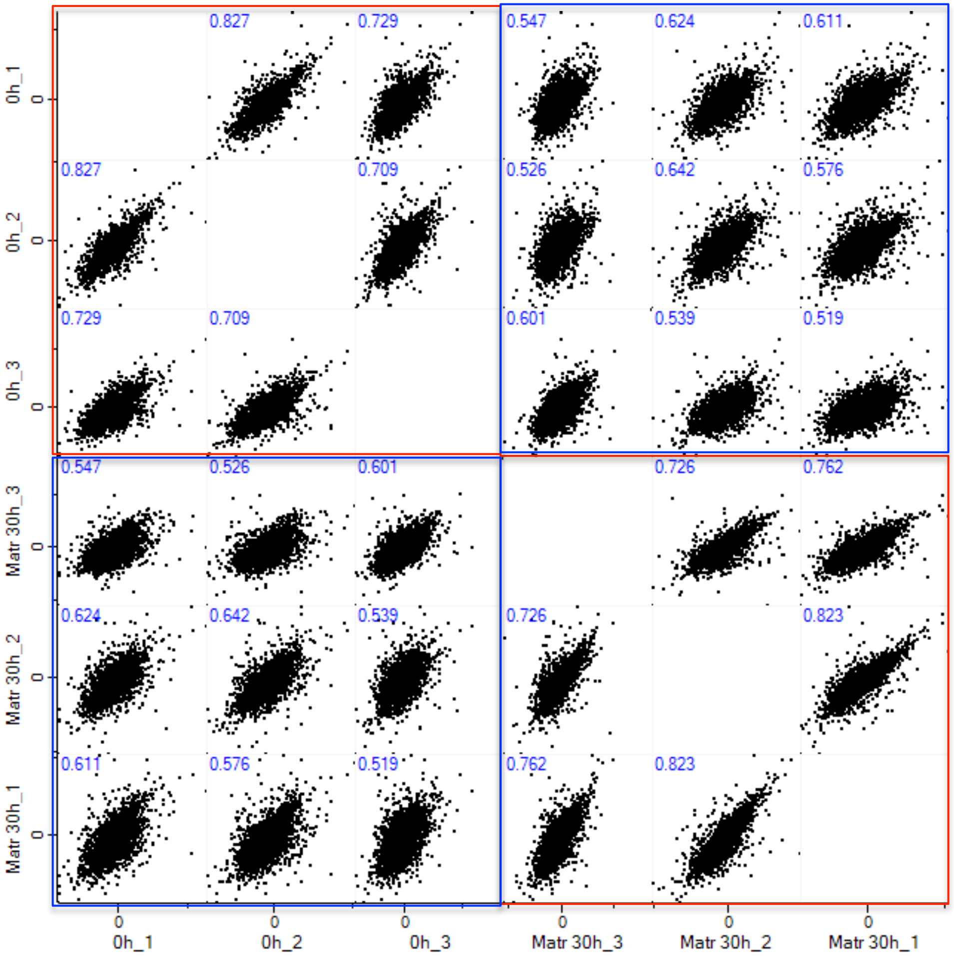
Rows (selected):
0h_1
0h_2
0h_3
Matr 30h_3
Matr 30h_2
Matr 30h_1

Columns (available): 0h_1, 0h_2, 0h_3, Matr 30h_3, Matr 30h_2, Matr 30h_1, PEP, Intensity, Intensity L, Intensity H

Columns (selected):
0h_1
0h_2
0h_3
Matr 30h_3
Matr 30h_2
Matr 30h_1

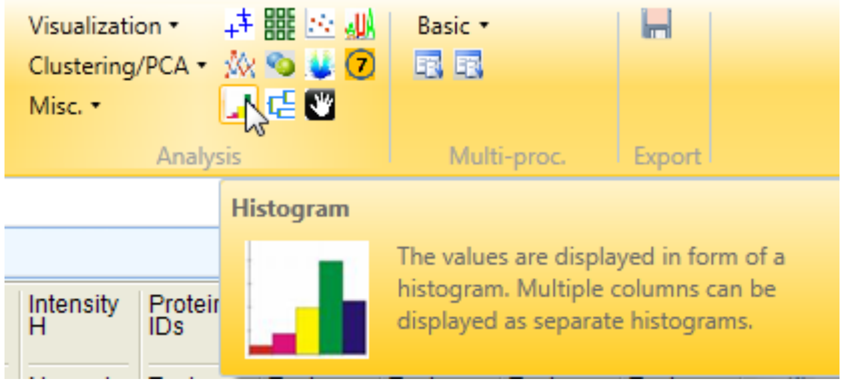
Analysis of SILAC data

As expected the results of the Pearson correlation analysis show a higher correlation between equally-treated then unequally-treated cells.



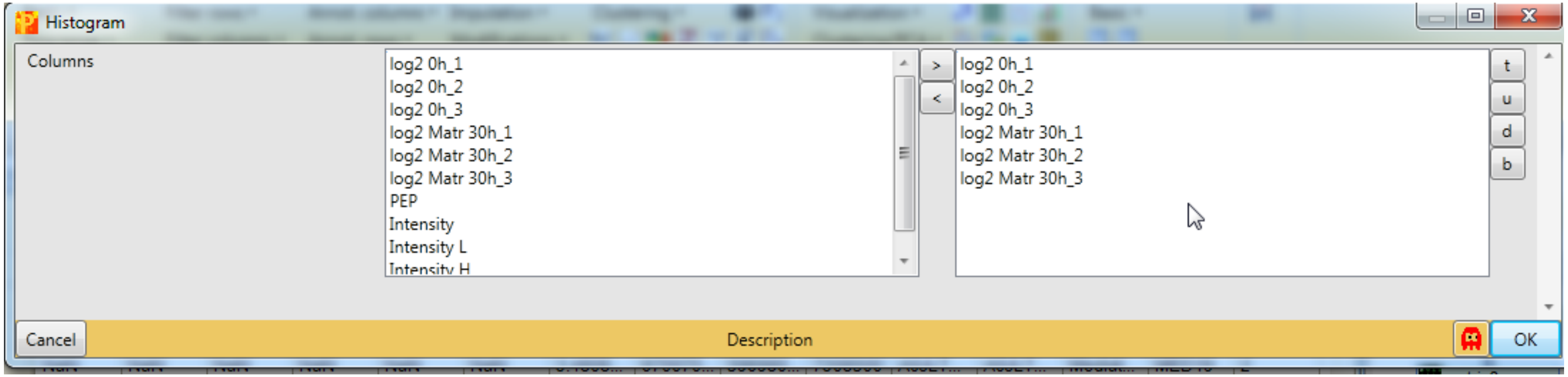
Analysis of SILAC data

In addition to get an idea of the SILAC ratio distributions and see if they are normally distributed, we create a histogram.



Histogram

The values are displayed in form of a histogram. Multiple columns can be displayed as separate histograms.



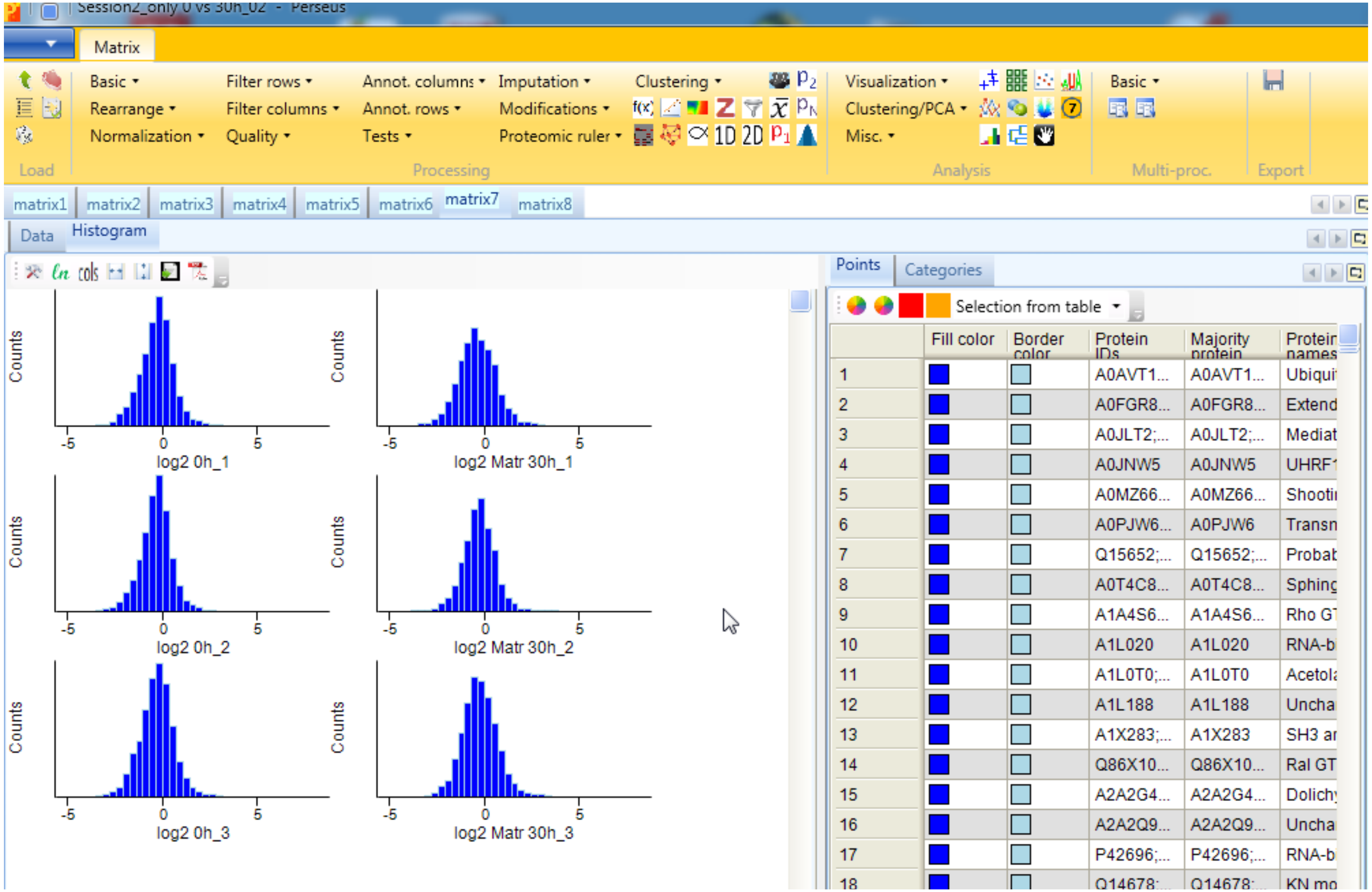
Columns

log2 0h_1
log2 0h_2
log2 0h_3
log2 Matr 30h_1
log2 Matr 30h_2
log2 Matr 30h_3
PEP
Intensity
Intensity L
Intensity H

log2 0h_1
log2 0h_2
log2 0h_3
log2 Matr 30h_1
log2 Matr 30h_2
log2 Matr 30h_3

Analysis of SILAC data

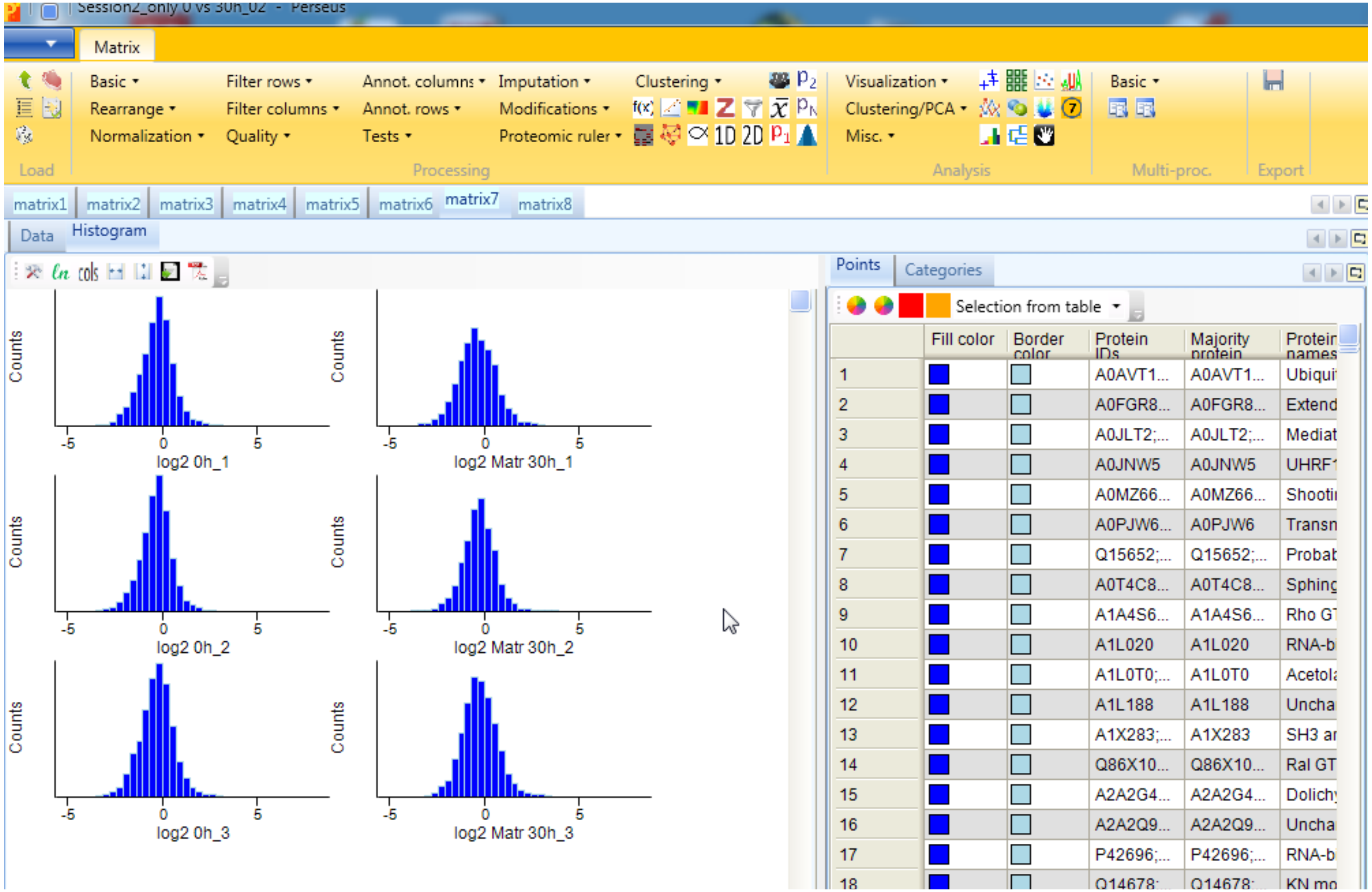
Since we selected the SILAC ratios already normalized by MaxQuant during its search, we can see that they almost completely cluster around 0.



	Fill color	Border color	Protein IDs	Majority protein	Protein names
1	🟦	☐	A0AVT1...	A0AVT1...	Ubiqui
2	🟦	☐	A0FGR8...	A0FGR8...	Extend
3	🟦	☐	A0JLT2;...	A0JLT2;...	Mediat
4	🟦	☐	A0JNW5	A0JNW5	UHRF
5	🟦	☐	A0MZ66...	A0MZ66...	Shooti
6	🟦	☐	A0PJW6...	A0PJW6	Transn
7	🟦	☐	Q15652;...	Q15652;...	Probab
8	🟦	☐	A0T4C8...	A0T4C8...	Sphing
9	🟦	☐	A1A4S6...	A1A4S6...	Rho G
10	🟦	☐	A1L020	A1L020	RNA-b
11	🟦	☐	A1L0T0;...	A1L0T0	Acetol
12	🟦	☐	A1L188	A1L188	Uncha
13	🟦	☐	A1X283;...	A1X283	SH3 ar
14	🟦	☐	Q86X10...	Q86X10...	Ral GT
15	🟦	☐	A2A2G4...	A2A2G4...	Dolich
16	🟦	☐	A2A2Q9...	A2A2Q9...	Uncha
17	🟦	☐	P42696;...	P42696;...	RNA-b
18	🟦	☐	Q14678;...	Q14678;...	KN mo

Analysis of SILAC data

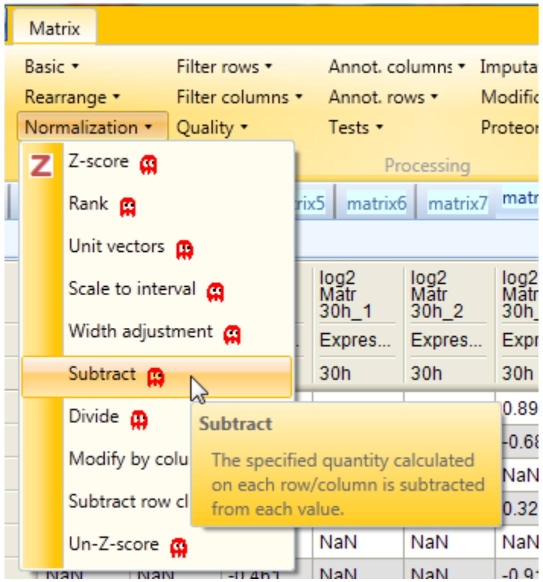
But we can still see a little shift to the left in almost all samples, so we again normalize the data.



	Fill color	Border color	Protein IDs	Majority protein	Protein names
1	🟦	☐	A0AVT1...	A0AVT1...	Ubiqui
2	🟦	☐	A0FGR8...	A0FGR8...	Extend
3	🟦	☐	A0JLT2;...	A0JLT2;...	Mediat
4	🟦	☐	A0JNW5	A0JNW5	UHRF
5	🟦	☐	A0MZ66...	A0MZ66...	Shooti
6	🟦	☐	A0PJW6...	A0PJW6	Transn
7	🟦	☐	Q15652;...	Q15652;...	Probab
8	🟦	☐	A0T4C8...	A0T4C8...	Sphing
9	🟦	☐	A1A4S6...	A1A4S6...	Rho G
10	🟦	☐	A1L020	A1L020	RNA-b
11	🟦	☐	A1L0T0;...	A1L0T0	Acetol
12	🟦	☐	A1L188	A1L188	Uncha
13	🟦	☐	A1X283;...	A1X283	SH3 ar
14	🟦	☐	Q86X10...	Q86X10...	Ral GT
15	🟦	☐	A2A2G4...	A2A2G4...	Dolich
16	🟦	☐	A2A2Q9...	A2A2Q9...	Uncha
17	🟦	☐	P42696;...	P42696;...	RNA-b
18	🟦	☐	Q14678;...	Q14678;...	KN mo

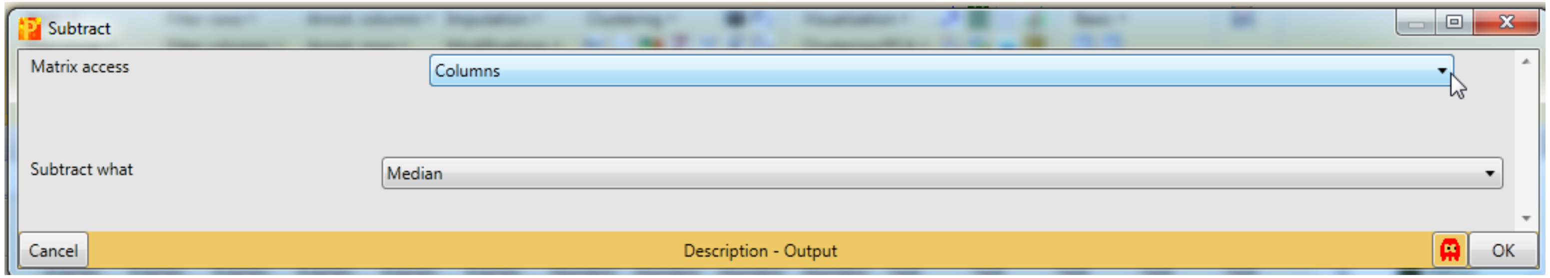
Analysis of SILAC data

Since we have log2-transformed values we normalize each column by subtracting its median.



Subtract

The specified quantity calculated on each row/column is subtracted from each value.



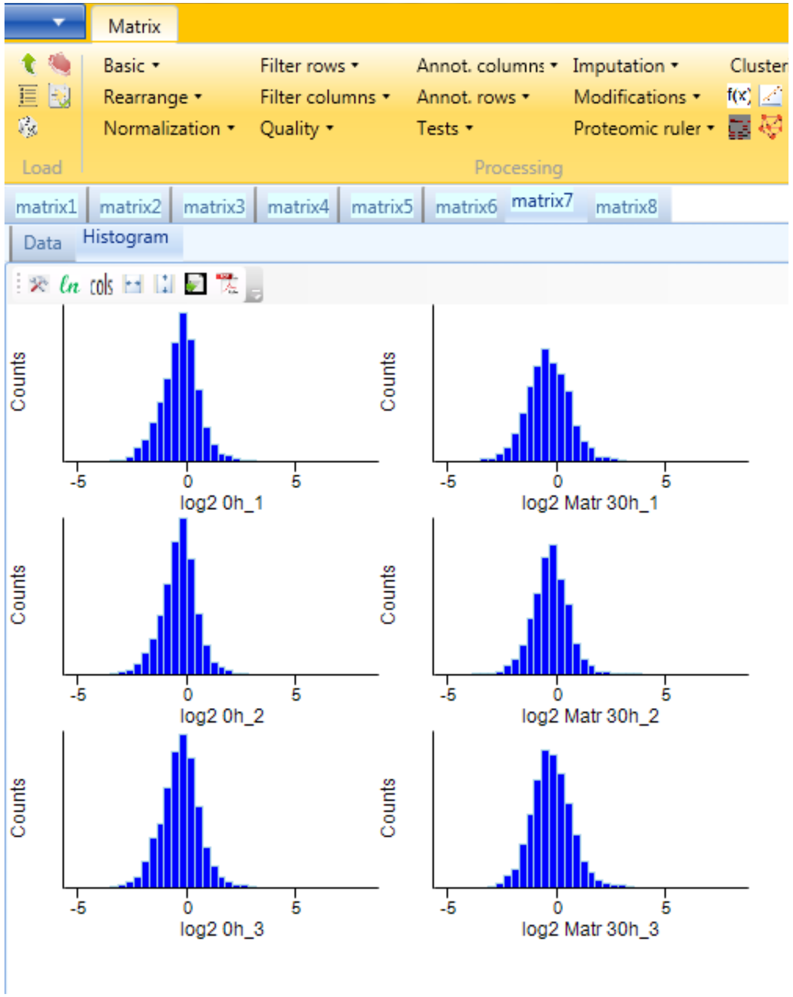
Matrix access: Columns

Subtract what: Median

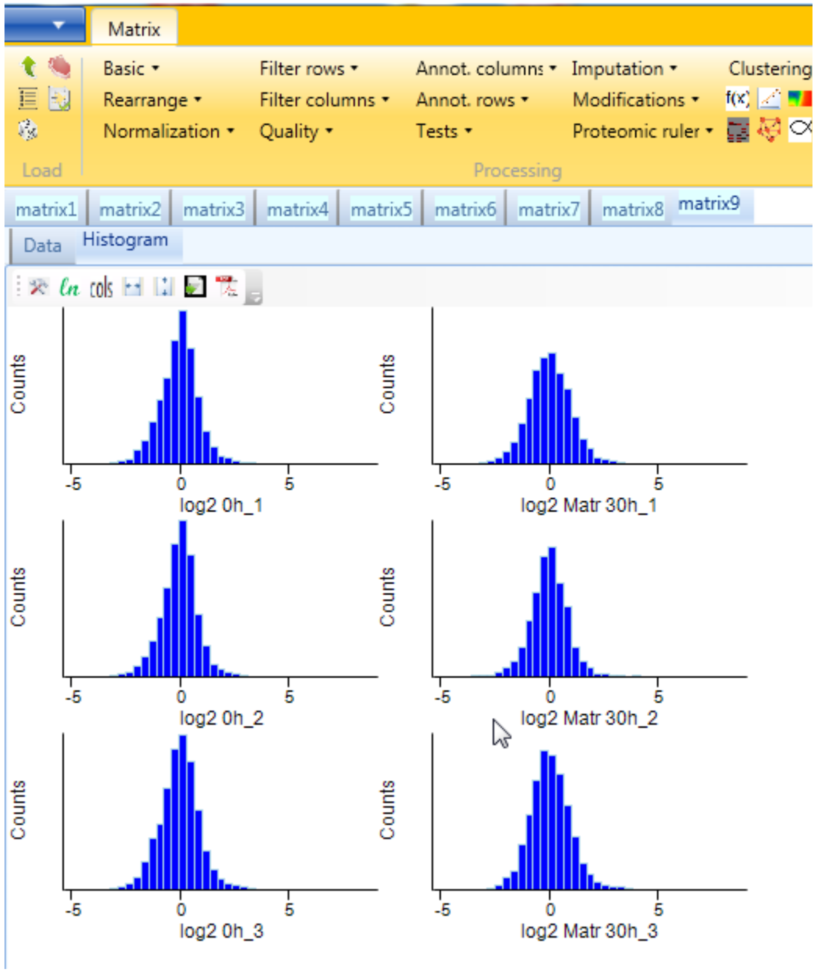
Analysis of SILAC data

After the normalization we again create a histogram.

before



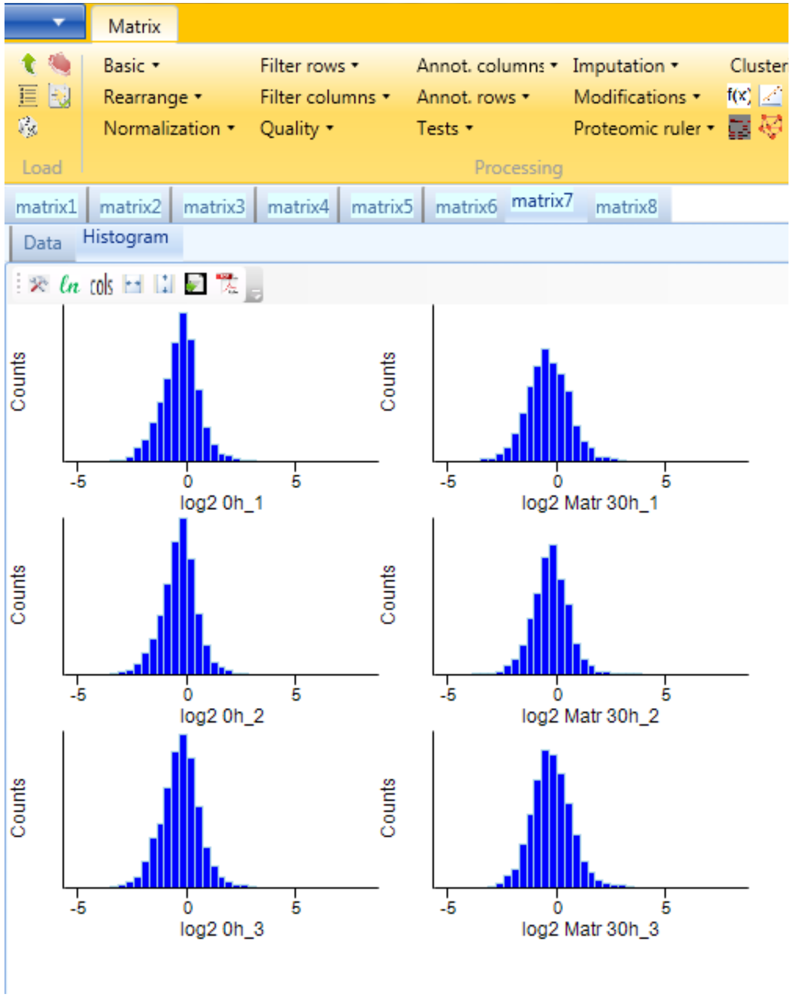
after



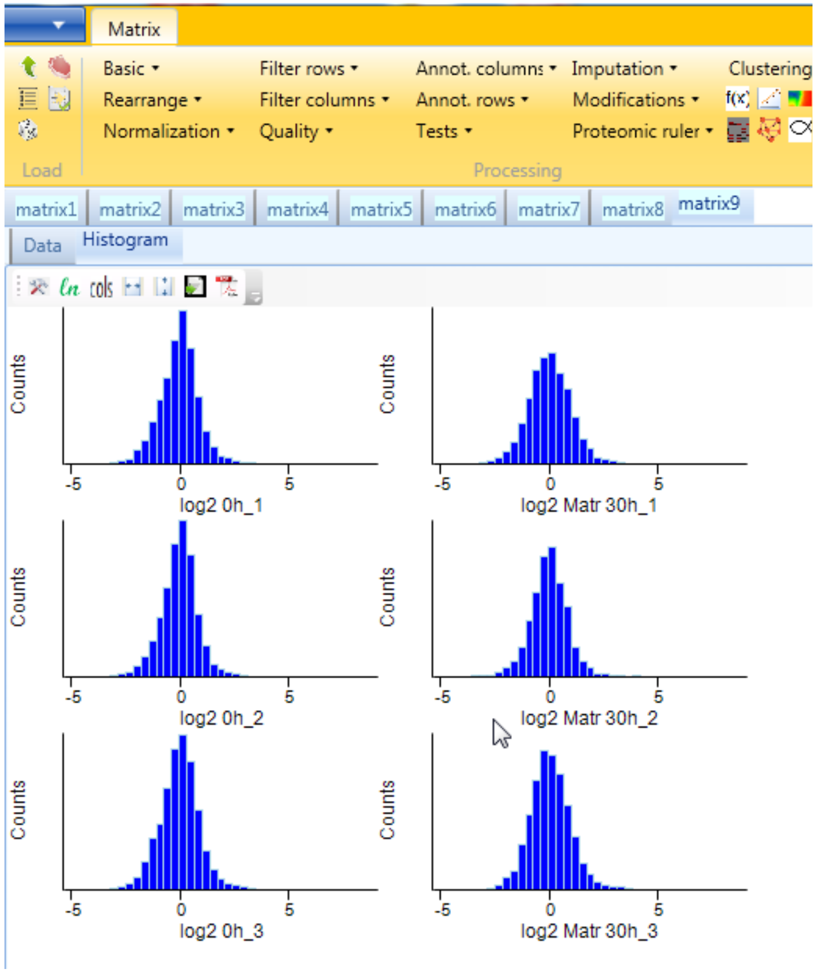
Analysis of SILAC data

Now we can see that the ratios of all experiments are nicely centered around 0.

before

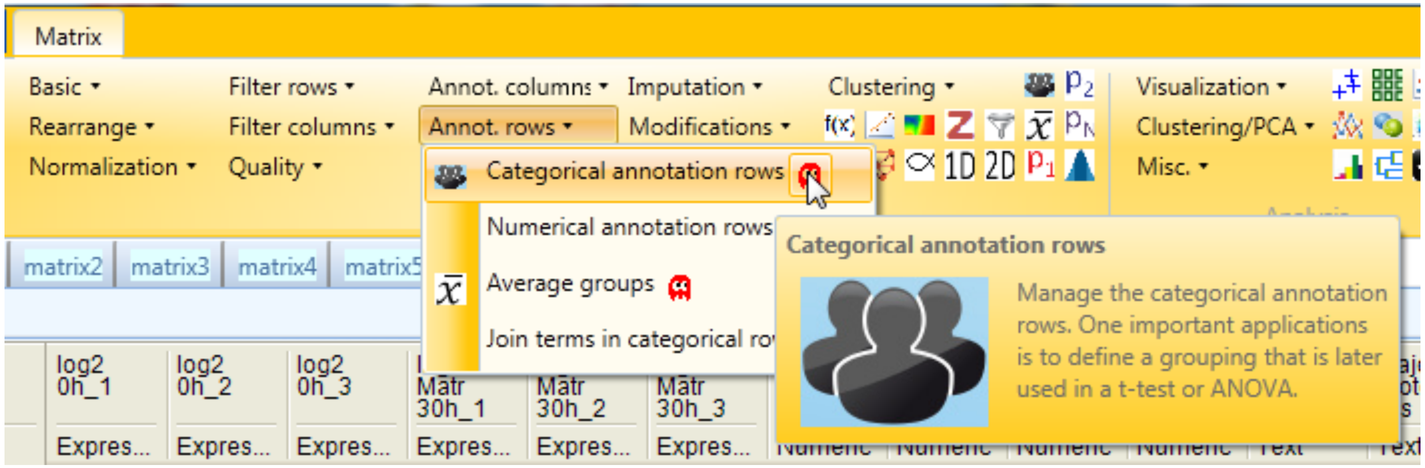


after



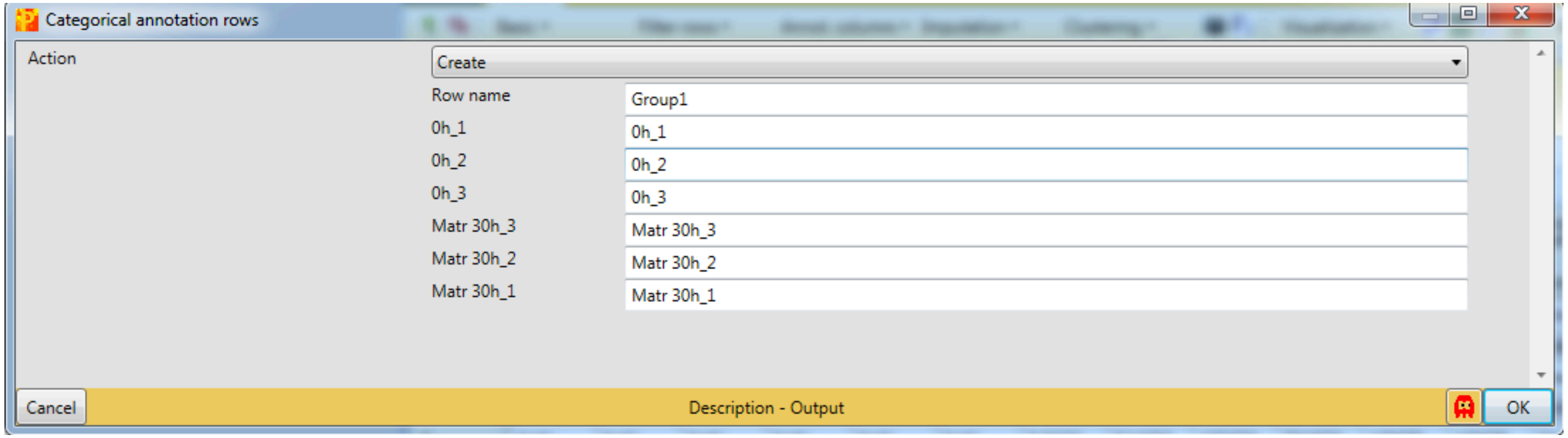
Analysis of SILAC data

Now we define groups for the differentially treated samples.



Categorical annotation rows

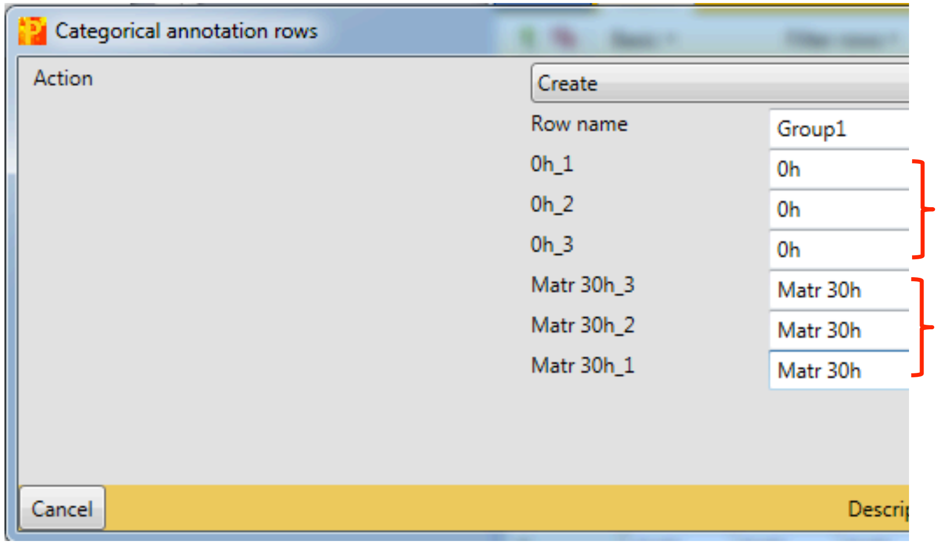
Manage the categorical annotation rows. One important applications is to define a grouping that is later used in a t-test or ANOVA.



Action	Create
Row name	Group1
0h_1	0h_1
0h_2	0h_2
0h_3	0h_3
Matr 30h_3	Matr 30h_3
Matr 30h_2	Matr 30h_2
Matr 30h_1	Matr 30h_1

Analysis of SILAC data

At the next step we define groups for the differentially treated samples.



Action: Create

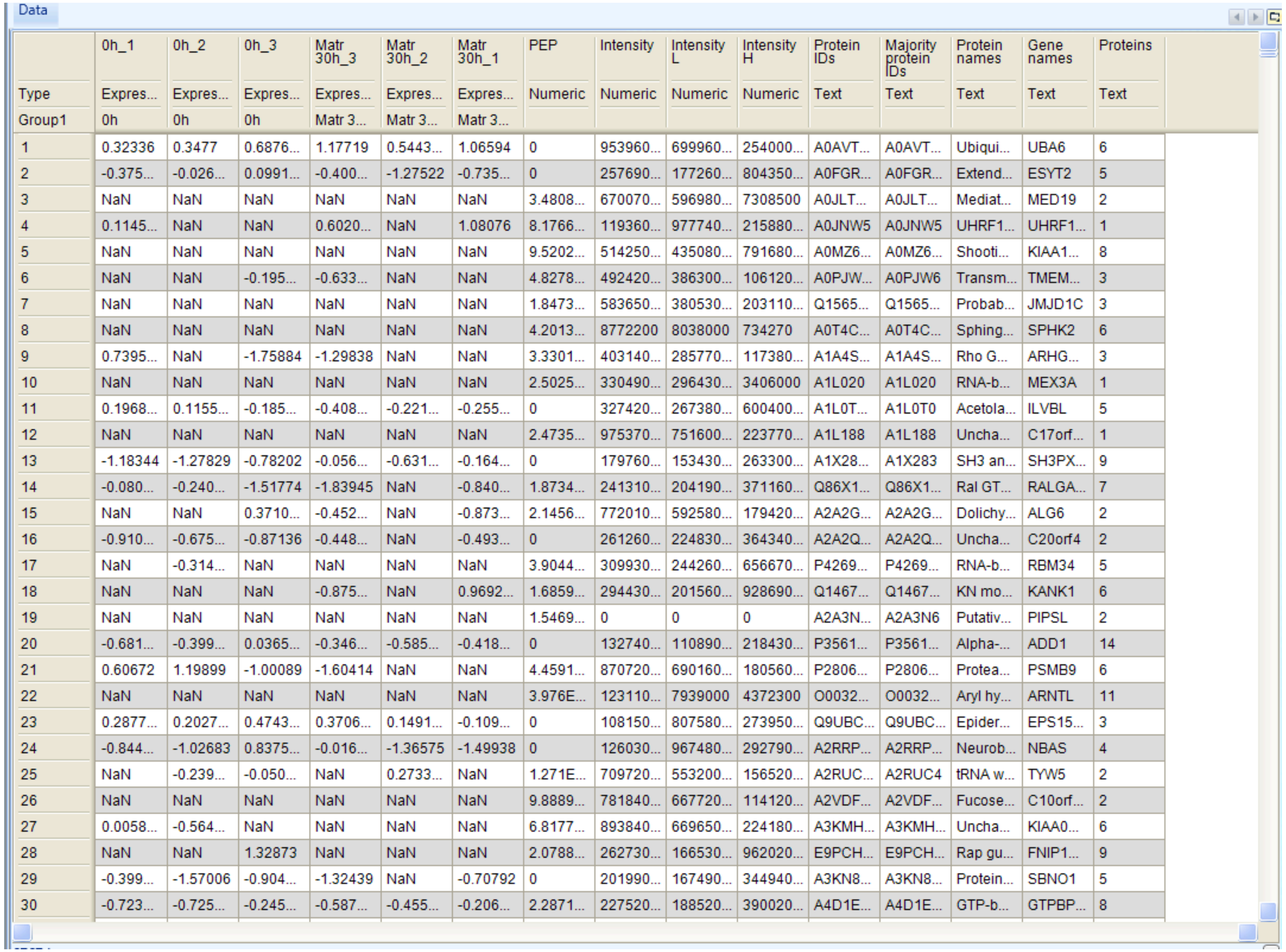
Row name	Group1
0h_1	0h
0h_2	0h
0h_3	0h
Matr 30h_3	Matr 30h
Matr 30h_2	Matr 30h
Matr 30h_1	Matr 30h

This defines the name of the grouping (Here “Group1”). For more complex analyses one could define different groupings within the same Perseus project.

Now you assemble different expression values into groups by giving them the same groupname. In this example the groups are named 0h and Matr30h.

Analysis of SILAC data

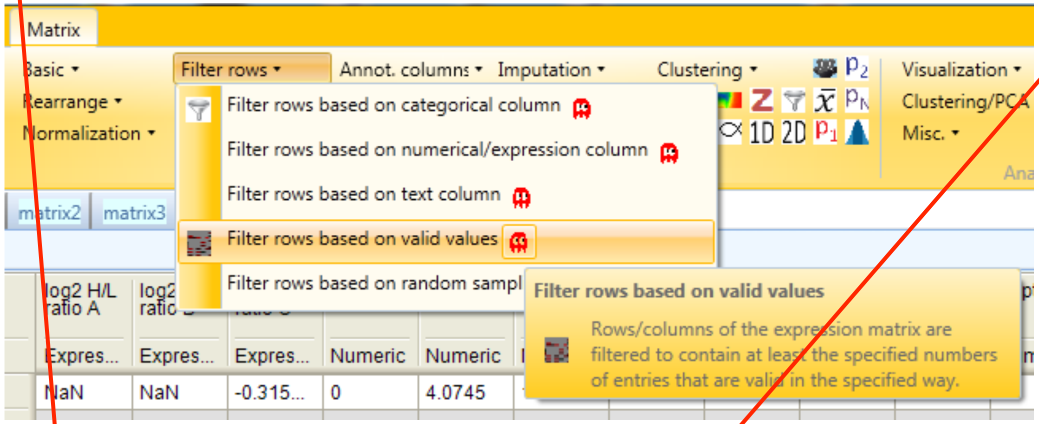
We have a lot of identifications without or only a limited amount of quantitative values (NaN). Since we want to have very reliable quantitative data, we now remove all entries which have insufficient entries.



	0h_1	0h_2	0h_3	Matr 30h_3	Matr 30h_2	Matr 30h_1	PEP	Intensity	Intensity L	Intensity H	Protein IDs	Majority protein IDs	Protein names	Gene names	Proteins
Type	Expres...	Expres...	Expres...	Expres...	Expres...	Expres...	Numeric	Numeric	Numeric	Numeric	Text	Text	Text	Text	Text
Group1	0h	0h	0h	Matr 3...	Matr 3...	Matr 3...									
1	0.32336	0.3477	0.6876...	1.17719	0.5443...	1.06594	0	953960...	699960...	254000...	A0AVT...	A0AVT...	Ubiqui...	UBA6	6
2	-0.375...	-0.026...	0.0991...	-0.400...	-1.27522	-0.735...	0	257690...	177260...	804350...	A0FGR...	A0FGR...	Extend...	ESYT2	5
3	NaN	NaN	NaN	NaN	NaN	NaN	3.4808...	670070...	596980...	7308500	A0JLT...	A0JLT...	Mediat...	MED19	2
4	0.1145...	NaN	NaN	0.6020...	NaN	1.08076	8.1766...	119360...	977740...	215880...	A0JNW5	A0JNW5	UHRF1...	UHRF1...	1
5	NaN	NaN	NaN	NaN	NaN	NaN	9.5202...	514250...	435080...	791680...	A0MZ6...	A0MZ6...	Shooti...	KIAA1...	8
6	NaN	NaN	-0.195...	-0.633...	NaN	NaN	4.8278...	492420...	386300...	106120...	A0PJW...	A0PJW6	Transm...	TMEM...	3
7	NaN	NaN	NaN	NaN	NaN	NaN	1.8473...	583650...	380530...	203110...	Q1565...	Q1565...	Probab...	JMJD1C	3
8	NaN	NaN	NaN	NaN	NaN	NaN	4.2013...	8772200	8038000	734270	A0T4C...	A0T4C...	Sphing...	SPHK2	6
9	0.7395...	NaN	-1.75884	-1.29838	NaN	NaN	3.3301...	403140...	285770...	117380...	A1A4S...	A1A4S...	Rho G...	ARHG...	3
10	NaN	NaN	NaN	NaN	NaN	NaN	2.5025...	330490...	296430...	3406000	A1L020	A1L020	RNA-b...	MEX3A	1
11	0.1968...	0.1155...	-0.185...	-0.408...	-0.221...	-0.255...	0	327420...	267380...	600400...	A1L0T...	A1L0T0	Acetola...	ILVBL	5
12	NaN	NaN	NaN	NaN	NaN	NaN	2.4735...	975370...	751600...	223770...	A1L188	A1L188	Uncha...	C17orf...	1
13	-1.18344	-1.27829	-0.78202	-0.056...	-0.631...	-0.164...	0	179760...	153430...	263300...	A1X28...	A1X283	SH3 an...	SH3PX...	9
14	-0.080...	-0.240...	-1.51774	-1.83945	NaN	-0.840...	1.8734...	241310...	204190...	371160...	Q86X1...	Q86X1...	Ral GT...	RALGA...	7
15	NaN	NaN	0.3710...	-0.452...	NaN	-0.873...	2.1456...	772010...	592580...	179420...	A2A2G...	A2A2G...	Dolichy...	ALG6	2
16	-0.910...	-0.675...	-0.87136	-0.448...	NaN	-0.493...	0	261260...	224830...	364340...	A2A2Q...	A2A2Q...	Uncha...	C20orf4	2
17	NaN	-0.314...	NaN	NaN	NaN	NaN	3.9044...	309930...	244260...	656670...	P4269...	P4269...	RNA-b...	RBM34	5
18	NaN	NaN	NaN	-0.875...	NaN	0.9692...	1.6859...	294430...	201560...	928690...	Q1467...	Q1467...	KN mo...	KANK1	6
19	NaN	NaN	NaN	NaN	NaN	NaN	1.5469...	0	0	0	A2A3N...	A2A3N6	Putativ...	PIPSL	2
20	-0.681...	-0.399...	0.0365...	-0.346...	-0.585...	-0.418...	0	132740...	110890...	218430...	P3561...	P3561...	Alpha-...	ADD1	14
21	0.60672	1.19899	-1.00089	-1.60414	NaN	NaN	4.4591...	870720...	690160...	180560...	P2806...	P2806...	Protea...	PSMB9	6
22	NaN	NaN	NaN	NaN	NaN	NaN	3.976E...	123110...	7939000	4372300	O0032...	O0032...	Aryl hy...	ARNTL	11
23	0.2877...	0.2027...	0.4743...	0.3706...	0.1491...	-0.109...	0	108150...	807580...	273950...	Q9UBC...	Q9UBC...	Epider...	EPS15...	3
24	-0.844...	-1.02683	0.8375...	-0.016...	-1.36575	-1.49938	0	126030...	967480...	292790...	A2RRP...	A2RRP...	Neurob...	NBAS	4
25	NaN	-0.239...	-0.050...	NaN	0.2733...	NaN	1.271E...	709720...	553200...	156520...	A2RUC...	A2RUC4	tRNA w...	TYW5	2
26	NaN	NaN	NaN	NaN	NaN	NaN	9.8889...	781840...	667720...	114120...	A2VDF...	A2VDF...	Fucose...	C10orf...	2
27	0.0058...	-0.564...	NaN	NaN	NaN	NaN	6.8177...	893840...	669650...	224180...	A3KMH...	A3KMH...	Uncha...	KIAA0...	6
28	NaN	NaN	1.32873	NaN	NaN	NaN	2.0788...	262730...	166530...	962020...	E9PCH...	E9PCH...	Rap gu...	FNIP1...	9
29	-0.399...	-1.57006	-0.904...	-1.32439	NaN	-0.70792	0	201990...	167490...	344940...	A3KN8...	A3KN8...	Protein...	SBNO1	5
30	-0.723...	-0.725...	-0.245...	-0.587...	-0.455...	-0.206...	2.2871...	227520...	188520...	390020...	A4D1E...	A4D1E...	GTP-b...	GTPBP...	8

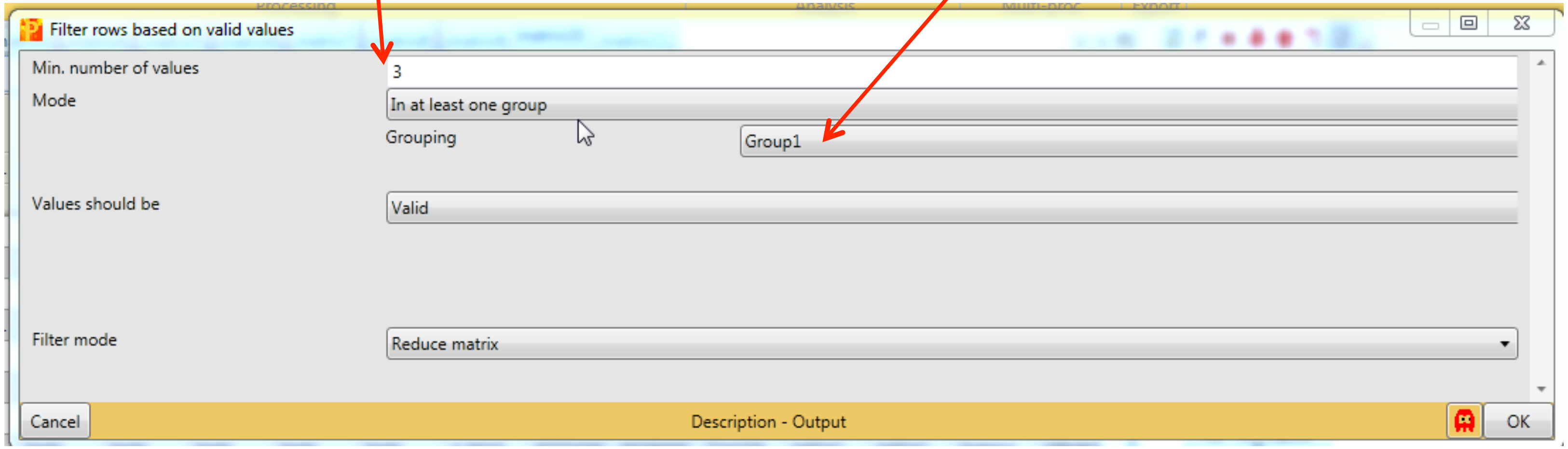
Analysis of SILAC data

We now remove all entries which have insufficient entries. For this we want to have at least **3 valid values** in **one of the previously defined groups** (0 or 30h).



Filter rows based on valid values

Rows/columns of the expression matrix are filtered to contain at least the specified numbers of entries that are valid in the specified way.



Min. number of values: 3

Mode: In at least one group

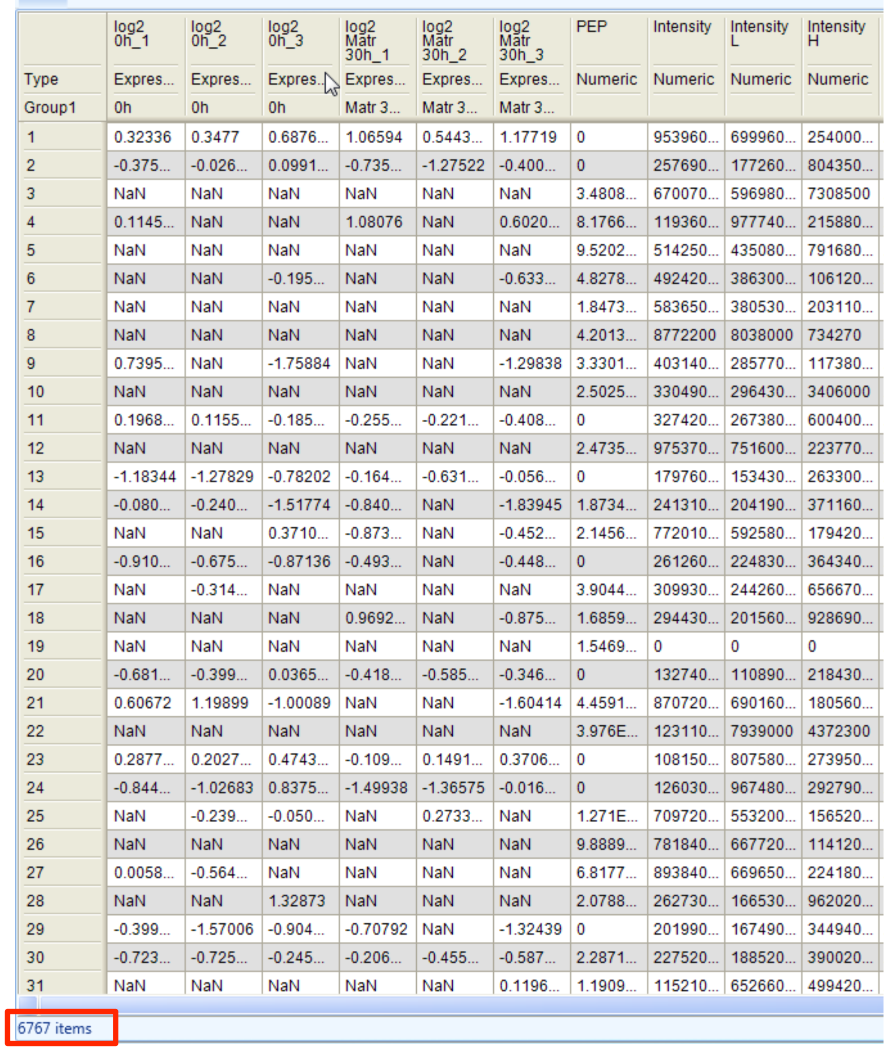
Grouping: Group1

Values should be: Valid

Filter mode: Reduce matrix

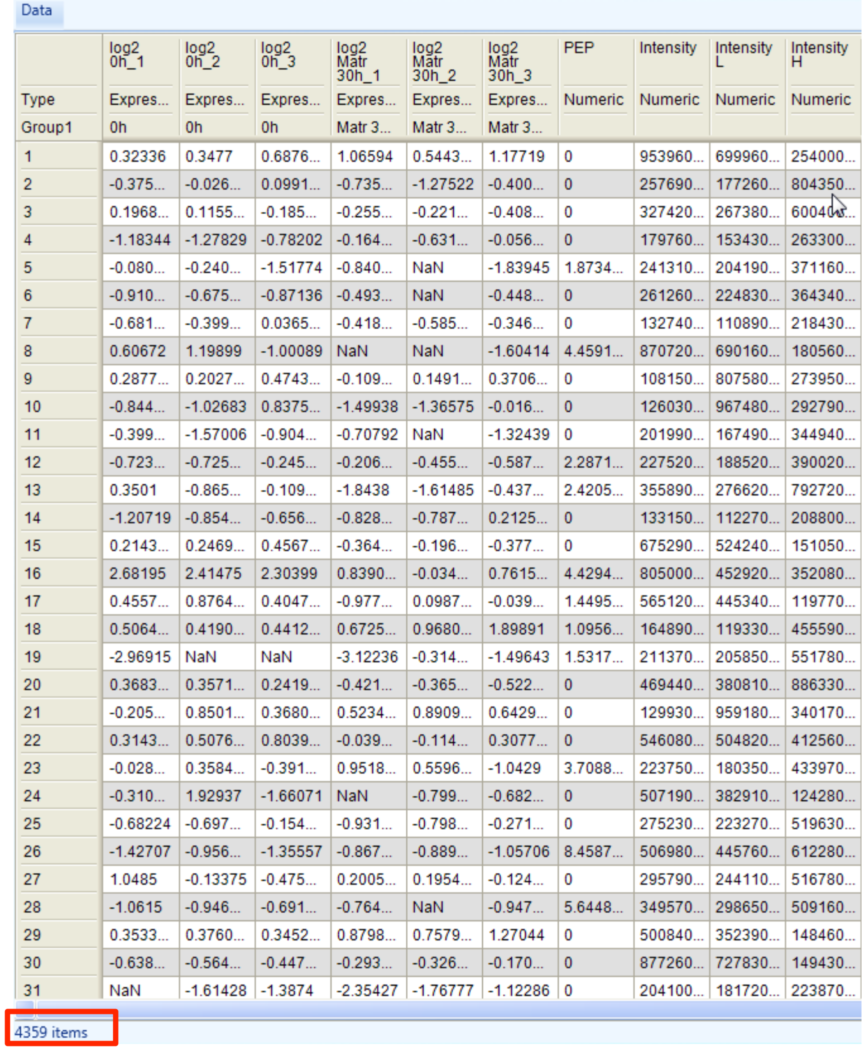
Analysis of SILAC data

The stringent filtering for valid values reduced the number of protein groups from 6767 to 4359



	log2 0h_1	log2 0h_2	log2 0h_3	log2 Matr 30h_1	log2 Matr 30h_2	log2 Matr 30h_3	PEP	Intensity	Intensity L	Intensity H
Type	Expres...	Expres...	Expres...	Expres...	Expres...	Expres...	Numeric	Numeric	Numeric	Numeric
Group1	0h	0h	0h	Matr 3...	Matr 3...	Matr 3...				
1	0.32336	0.3477	0.6876...	1.06594	0.5443...	1.17719	0	953960...	699960...	254000...
2	-0.375...	-0.026...	0.0991...	-0.735...	-1.27522	-0.400...	0	257690...	177260...	804350...
3	NaN	NaN	NaN	NaN	NaN	NaN	3.4808...	670070...	596980...	7308500
4	0.1145...	NaN	NaN	1.08076	NaN	0.6020...	8.1766...	119360...	977740...	215880...
5	NaN	NaN	NaN	NaN	NaN	NaN	9.5202...	514250...	435080...	791680...
6	NaN	NaN	-0.195...	NaN	NaN	-0.633...	4.8278...	492420...	386300...	106120...
7	NaN	NaN	NaN	NaN	NaN	NaN	1.8473...	583650...	380530...	203110...
8	NaN	NaN	NaN	NaN	NaN	NaN	4.2013...	8772200	8038000	734270
9	0.7395...	NaN	-1.75884	NaN	NaN	-1.29838	3.3301...	403140...	285770...	117380...
10	NaN	NaN	NaN	NaN	NaN	NaN	2.5025...	330490...	296430...	3406000
11	0.1968...	0.1155...	-0.185...	-0.255...	-0.221...	-0.408...	0	327420...	267380...	600400...
12	NaN	NaN	NaN	NaN	NaN	NaN	2.4735...	975370...	751600...	223770...
13	-1.18344	-1.27829	-0.78202	-0.164...	-0.631...	-0.056...	0	179760...	153430...	263300...
14	-0.080...	-0.240...	-1.51774	-0.840...	NaN	-1.83945	1.8734...	241310...	204190...	371160...
15	NaN	NaN	0.3710...	-0.873...	NaN	-0.452...	2.1456...	772010...	592580...	179420...
16	-0.910...	-0.675...	-0.87136	-0.493...	NaN	-0.448...	0	261260...	224830...	364340...
17	NaN	-0.314...	NaN	NaN	NaN	NaN	3.9044...	309930...	244260...	656670...
18	NaN	NaN	NaN	0.9692...	NaN	-0.875...	1.6859...	294430...	201560...	928690...
19	NaN	NaN	NaN	NaN	NaN	NaN	1.5469...	0	0	0
20	-0.681...	-0.399...	0.0365...	-0.418...	-0.585...	-0.346...	0	132740...	110890...	218430...
21	0.60672	1.19899	-1.00089	NaN	NaN	-1.60414	4.4591...	870720...	690160...	180560...
22	NaN	NaN	NaN	NaN	NaN	NaN	3.976E...	123110...	7939000	4372300
23	0.2877...	0.2027...	0.4743...	-0.109...	0.1491...	0.3706...	0	108150...	807580...	273950...
24	-0.844...	-1.02683	0.8375...	-1.49938	-1.36575	-0.016...	0	126030...	967480...	292790...
25	NaN	-0.239...	-0.050...	NaN	0.2733...	NaN	1.271E...	709720...	553200...	156520...
26	NaN	NaN	NaN	NaN	NaN	NaN	9.8889...	781840...	667720...	114120...
27	0.0058...	-0.564...	NaN	NaN	NaN	NaN	6.8177...	893840...	669650...	224180...
28	NaN	NaN	1.32873	NaN	NaN	NaN	2.0788...	262730...	166530...	962020...
29	-0.399...	-1.57006	-0.904...	-0.70792	NaN	-1.32439	0	201990...	167490...	344940...
30	-0.723...	-0.725...	-0.245...	-0.206...	-0.455...	-0.587...	2.2871...	227520...	188520...	390020...
31	NaN	NaN	NaN	NaN	NaN	0.1196...	1.1909...	115210...	652660...	499420...

6767 items



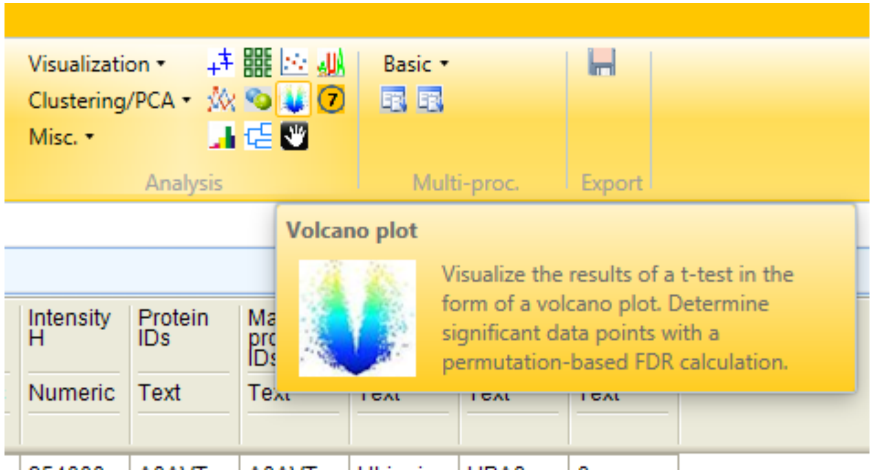
Data

	log2 0h_1	log2 0h_2	log2 0h_3	log2 Matr 30h_1	log2 Matr 30h_2	log2 Matr 30h_3	PEP	Intensity	Intensity L	Intensity H
Type	Expres...	Expres...	Expres...	Expres...	Expres...	Expres...	Numeric	Numeric	Numeric	Numeric
Group1	0h	0h	0h	Matr 3...	Matr 3...	Matr 3...				
1	0.32336	0.3477	0.6876...	1.06594	0.5443...	1.17719	0	953960...	699960...	254000...
2	-0.375...	-0.026...	0.0991...	-0.735...	-1.27522	-0.400...	0	257690...	177260...	804350...
3	0.1968...	0.1155...	-0.185...	-0.255...	-0.221...	-0.408...	0	327420...	267380...	60040[illegible]...
4	-1.18344	-1.27829	-0.78202	-0.164...	-0.631...	-0.056...	0	179760...	153430...	263300...
5	-0.080...	-0.240...	-1.51774	-0.840...	NaN	-1.83945	1.8734...	241310...	204190...	371160...
6	-0.910...	-0.675...	-0.87136	-0.493...	NaN	-0.448...	0	261260...	224830...	364340...
7	-0.681...	-0.399...	0.0365...	-0.418...	-0.585...	-0.346...	0	132740...	110890...	218430...
8	0.60672	1.19899	-1.00089	NaN	NaN	-1.60414	4.4591...	870720...	690160...	180560...
9	0.2877...	0.2027...	0.4743...	-0.109...	0.1491...	0.3706...	0	108150...	807580...	273950...
10	-0.844...	-1.02683	0.8375...	-1.49938	-1.36575	-0.016...	0	126030...	967480...	292790...
11	-0.399...	-1.57006	-0.904...	-0.70792	NaN	-1.32439	0	201990...	167490...	344940...
12	-0.723...	-0.725...	-0.245...	-0.206...	-0.455...	-0.587...	2.2871...	227520...	188520...	390020...
13	0.3501	-0.865...	-0.109...	-1.8438	-1.61485	-0.437...	2.4205...	355890...	276620...	792720...
14	-1.20719	-0.854...	-0.656...	-0.828...	-0.787...	0.2125...	0	133150...	112270...	208800...
15	0.2143...	0.2469...	0.4567...	-0.364...	-0.196...	-0.377...	0	675290...	524240...	151050...
16	2.68195	2.41475	2.30399	0.8390...	-0.034...	0.7615...	4.4294...	805000...	452920...	352080...
17	0.4557...	0.8764...	0.4047...	-0.977...	0.0987...	-0.039...	1.4495...	565120...	445340...	119770...
18	0.5064...	0.4190...	0.4412...	0.6725...	0.9680...	1.89891	1.0956...	164890...	119330...	455590...
19	-2.96915	NaN	NaN	-3.12236	-0.314...	-1.49643	1.5317...	211370...	205850...	551780...
20	0.3683...	0.3571...	0.2419...	-0.421...	-0.365...	-0.522...	0	469440...	380810...	886330...
21	-0.205...	0.8501...	0.3680...	0.5234...	0.8909...	0.6429...	0	129930...	959180...	340170...
22	0.3143...	0.5076...	0.8039...	-0.039...	-0.114...	0.3077...	0	546080...	504820...	412560...
23	-0.028...	0.3584...	-0.391...	0.9518...	0.5596...	-1.0429	3.7088...	223750...	180350...	433970...
24	-0.310...	1.92937	-1.66071	NaN	-0.799...	-0.682...	0	507190...	382910...	124280...
25	-0.68224	-0.697...	-0.154...	-0.931...	-0.798...	-0.271...	0	275230...	223270...	519630...
26	-1.42707	-0.956...	-1.35557	-0.867...	-0.889...	-1.05706	8.4587...	506980...	445760...	612280...
27	1.0485	-0.13375	-0.475...	0.2005...	0.1954...	-0.124...	0	295790...	244110...	516780...
28	-1.0615	-0.946...	-0.691...	-0.764...	NaN	-0.947...	5.6448...	349570...	298650...	509160...
29	0.3533...	0.3760...	0.3452...	0.8798...	0.7579...	1.27044	0	500840...	352390...	148460...
30	-0.638...	-0.564...	-0.447...	-0.293...	-0.326...	-0.170...	0	877260...	727830...	149430...
31	NaN	-1.61428	-1.3874	-2.35427	-1.76777	-1.12286	0	204100...	181720...	223870...

4359 items

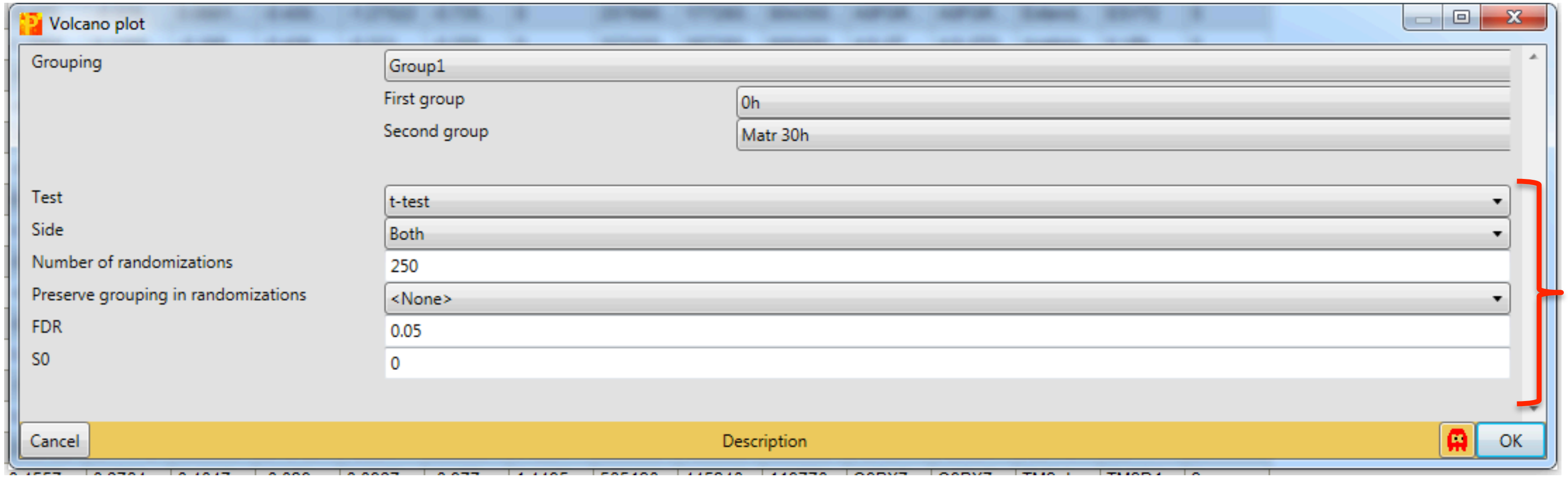
Analysis of SILAC data

To identify protein groups significantly different between experimental conditions we perform a t-test and create a Volcano plot.



Volcano plot

Visualize the results of a t-test in the form of a volcano plot. Determine significant data points with a permutation-based FDR calculation.



Grouping: Group1
First group: 0h
Second group: Matr 30h
Test: t-test
Side: Both
Number of randomizations: 250
Preserve grouping in randomizations: <None>
FDR: 0.05
S0: 0

Two-sided t-test with error corrected p-values (0.05 FDR)