

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 (<u>http://www.microsoft.com/de-de/download</u>)
 - Perseus Registration Code (free-of-charge onlineregistration) → <u>click here</u>

Installation

- 1. Download Perseus
 - Perseus is provided as a single compressed file (.zip)



- You can download it by clicking <u>here</u> (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

| 😋 🔾 🗸 🕨 ocomputer 🔸 Data Robert (D | D:) ▶ Software ▶ Perseus 1.5.1.6 ▶ | | | |
|--------------------------------------|------------------------------------|----------------|--------------------|-----------|
| Organize 👻 Include in library 👻 Shar | re with 🔻 🛛 Burn 🛛 New folder | | | |
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| 🐌 Downloads | 🚳 BaseLib.dll | 02.02.15 13:07 | Application extens | 1,836 KB |
| 📳 Recent Places | 🚳 BaseLibS.dll | 02.02.15 13:07 | Application extens | 250 KB |
| 😌 Dropbox | 🚳 itextsharp.dll | 02.02.15 13:07 | Application extens | 3,436 KB |
| 🕌 MS-Ergebnisse (MS-NAS-LAUFWERK | Microsoft.Windows.Shell.dll | 02.02.15 13:07 | Application extens | 164 KB |
| Protokolle (MS-NAS-LAUFWERK) | Microsoft.Windows.Shell.xml | 02.02.15 13:07 | XML File | 51 KB |
| iCloud Drive | 🚳 NumPluginBase.dll | 02.02.15 13:07 | Application extens | 39 KB |
| 🛞 iCloud Photos | NumPluginBase2.dll | 02.02.15 13:07 | Application extens | 19 KB |
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| 🥱 Libraries 🗧 | Perseus.exe.config | 02.02.15 13:07 | CONFIG File | 1 KB |
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| 🔢 Data Robert (D:) | 🚳 UtilsC.dll | 02.02.15 13:08 | Application extens | 209 KB |

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The software's main window opens



Click on the small green arrow in the upper left corner



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



1.Open Excel-file with protein/peptide results

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| A198 - | <i>f</i> _x P42 | 212;CON_Q | 19U6Y5 | | | | | | | | | | | | | | |
| A | В | С | D | E | F | G | н | L J | K | L | M | N | 0 | Р | Q | R | S |
| Protein IDs | Majority pro | Peptide cou | ur Peptide co | our Peptide o | our Protein nam | Gene names | Fasta header | Number of p Peptides | Razor + un | iq Unique pep | t Peptides 1 | Razor + uniq | Unique per | ot Sequence co | Unique + raz Un | ique sequ Ma | ol. weigh |
| 2 T | т | N | N | N | т | т | т | N | N | N | N | N | N | N | N N | N | |
| A0FGR8 | A0FGR8 | | 4 | 4 | 4 Extended sy | ESYT2 | >sp A0FGR8 | 1 | 4 | 4 1 | 4 4 | 4 4 | | 4 4.7 | 4.7 | 4.7 | 102.3 |
| A2RUC4 | A2RUC4 | | 1 | 1 | 1 tRNA wybut | TYW5 | >sp A2RUC4 | 1 | 1 | 1 7 | 1 7 | 1 1 | | 1 4.8 | 4.8 | 4.8 | 36.54 |
| 5 A4UGR9 | A4UGR9 | | 1 | 1 | 1 Xin actin-bir | XIRP2 | >sp A4UGR9 | 1 | 1 | 1 7 | 1 7 | 1 1 | | 1 0.7 | 0.7 | 0.7 | 382 |
| 5 A5YKK6 | A5YKK6 | | 1 | 1 | 1 CCR4-NOT tr | CNOT1 | >sp A5YKK6 | 1 | 1 | 1 / | 1 1 | 1 1 | | 1 0.6 | 0.6 | 0.6 | 266. |
| 7 P07355;A6NMY6 | P07355;A6N | 3;3 | 3;3 | 3;3 | Annexin A2; | ANXA2;ANX | >sp P07355 | 2 | 3 | 3 / | 3 7 | 3 3 | | 3 10.9 | 10.9 | 10.9 | 38.6 |
| P11231;B1NKU2;B1NKT8;B1 | LT P11231;B1NK | 1;1;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 1.9 | 1.9 | 1.9 | 103. |
| 9 A8MPS7 | A8MPS7 | | 1 | 1 | 1 UPF0249 pro | DIC | >splA8MPS7 | 1 | 1 | 1 : | 1 7 | 1 1 | | 1 4.3 | 4.3 | 4.3 | 34.4 |
| 0 A8MV23 | A8MV23 | | 1 | 1 | 1 Serpin E3 | SERPINE3 | >splA8MV23 | 1 | 1 | 1 | 1 : | 1 1 | | 1 1.9 | 1.9 | 1.9 | 46.9 |
| 1 CON P00761 | CON P0076 | | 2 | 2 | 2 | | >P00761 SWI | 1 | 2 | 2 | 2 : | 2 2 | | 2 7.8 | 7.8 | 7.8 | 24.4 |
| 2 P02533:CON P02533:0046 | 5 P02533:CON | 13:13:5:5:5: | - 5 9:9:2:2:?·1· | :1: 5:5:1:1:1: | 0:0: Keratin, typ | KRT14 | >sp P025331 | 43 | 13 | 9 | 5 17 | 3 9 | | 5 32.6 | 27.3 | 16.7 | 51.5 |
| 3 P02538:CON P02538:P486 | 6 P02538:CON | 13:13:12:12 | 18:8:7:7:7:1 | :1: 1:1:0:0:0: | 0:0: Keratin, typ | KRT64:KRT6 | >sn P02538 | 9 | 13 | 8 | 1 12 | 3 8 | | 1 27 | 18.1 | 1.8 | 60.0 |
| 4 CON P02662 | CON P0266 | | 2 | 2 | 2 | | >P02662 SWI | 1 | 2 | 2 | , | 2 2 | | 2 11.1 | 11.1 | 11.1 | 22.9 |
| 5 CON P02754 | CON P0275 | | 1 | 1 | 1 | | >P02754 SWI | 1 | 1 | 1 | 1 | 1 1 | | 1 86 | 8.6 | 8.6 | 18.2 |
| 5 P09779:CON P09779 | D09779-CON | 11-11 | 2.2 | 2.2 | Koratin typ | KPT16 | >col 0097791 | 2 | 11 | 2 | 2 1 | 1 2 | | 2 26.6 | 10.9 | 10.9 | 51.2 |
| 7 P12645(CONP08775 | PIB//J,CON | 20,20,0,2,2, | 3,5 | 3,5 | 111 Koratin, typ | KRT10 | >sp[P00775] | 12 | 20 | | 2 20 | 2 39 | | 5 20.0 | E2 1 | 46.7 | 50.0 |
| P13643,CON_P13643,CON | P13043,CON | 20,20,3,5,5, | 5 20,20,3,5,5 | 2,22,22,3,1 | Koratin, typ | VDTE | >sp[P13043] | 12 | 11 | co 24 | 20 | 3 20 | 4 | 2 30.2 | 33.1 | 40.7 | 50.0 |
| P15047,CON_P15047,CON | _ P15047,CON | 22,22 | 3,3,0 | 3,3,0 | Keratin, typ | KNTO | >sp[P15047] | 3 | 22 | 3 3 | , <u>1</u> | L J | | 20.3 | 5.5 | 5.5 | 62.5 |
| 9 P35527;CON_P35527 | P35527;CON | 23;23 | 22;22 | 22;22 | Keratin, typ | KRT9 | >sp[P35527] | 2 | 23 . | 12 24 | 2 Z: | 3 22 | 2 | 2 54.3 | 53.1 | 53.1 | 02.0 |
| 0 P35908;CON_P35908;Q015 | 52 P35908;CON | 20;20;3;2;2; | 2 18;18;3;2;2 | 2;2 13;13;0;0; | U;U Keratin, typ | EKRIZ | >sp1P359081 | 20 | 20 . | 18 1: | 3 20 | J 18 | 1 | .3 41.8 | 38.7 | 30.2 | 65.4 |
| I CON_Q3SZH5 | CON_Q3SZ | | 1 | 1 | 1 | | >Q35ZH5 TRE | 1 | 1 | 1 1 | 1 1 | 1 1 | | 1 1./ | 1.7 | 1.7 | 45.4 |
| 2 Q5D862;CON_Q5D862 | Q5D862;CON | 1;1 | 1;1 | 1;1 | Filaggrin-2 | FLG2 | >sp Q5D862 | 2 | 1 | 1 1 | 1 1 | 1 1 | | 1 0.5 | 0.5 | 0.5 | 248 |
| 3 Q7Z794;CON_Q7Z794 | Q72794;CON | 2;2 | 1;1 | 1;1 | Keratin, typ | EKRT77 | >sp Q7Z794 | 2 | 2 | 1 1 | 1 3 | 2 1 | | 1 3.8 | 1.7 | 1.7 | 61.9 |
| 4 000148;Q13838 | 000148;Q138 | 1;1 | 1;1 | 1;1 | ATP-depend | DDX39A;DD | >sp 000148 | 2 | 1 | 1 1 | 1 1 | 1 1 | | 1 2.3 | 2.3 | 2.3 | 49.1 |
| 5 000165 | 000165 | | 5 | 5 | 5 HCLS1-assoc | HAX1 | >sp 000165 | 1 | 5 | 5 5 | 5 5 | 5 5 | | 5 21.1 | 21.1 | 21.1 | 31 |
| 6 000217 | 000217 | | 1 | 1 | 1 NADH dehyd | NDUFS8 | >sp 000217 | 1 | 1 | 1 1 | 1 1 | 1 1 | | 1 4.3 | 4.3 | 4.3 | 23.7 |
| 7 000264 | O00264 | | 3 | 3 | 3 Membrane- | PGRMC1 | >sp 000264 | 1 | 3 | 3 3 | 3 3 | 3 3 | | 3 16.4 | 16.4 | 16.4 | 21.6 |
| 8 000327 | O00327 | | 1 | 1 | 1 Aryl hydroca | ARNTL | >sp 000327 | 1 | 1 | 1 2 | 1 2 | 1 1 | | 1 1.4 | 1.4 | 1.4 | 68.7 |
| 9 000483 | O00483 | | 3 | 3 | 3 NADH dehyd | NDUFA4 | >sp 000483 | 1 | 3 | 3 | 3 2 | 3 3 | | 3 37 | 37 | 37 | 9.36 |
| 0 000487 | O00487 | | 1 | 1 | 1 26S protease | PSMD14 | >sp 000487 | 1 | 1 | 1 : | 1 7 | 1 1 | | 1 4.2 | 4.2 | 4.2 | 34.5 |
| 1 000571;015523;Q9NQI0 | 000571;0155 | 6;4;1 | 6;4;1 | 6;4;1 | ATP-depend | DDX3X;DDX3 | >sp 000571 | 3 | 6 | 6 (| 5 (| 5 6 | | 6 11.6 | 11.6 | 11.6 | 73.2 |
| 2 014654 | O14654 | | 6 | 6 | 6 Insulin rece | IRS4 | >sp 014654 | 1 | 6 | 6 (| 5 (| 5 6 | | 6 6.6 | 6.6 | 6.6 | 133. |
| 3 014681 | O14681 | | 2 | 2 | 2 Etoposide-in | EI24 | >sp 014681 | 1 | 2 | 2 : | 2 : | 2 2 | | 2 5.9 | 5.9 | 5.9 | 38.9 |
| 4 014734 | 014734 | | 1 | 1 | 1 Acyl-coenzy | ACOT8 | >sp 014734 | 1 | 1 | 1 7 | 1 7 | 1 1 | | 1 2.8 | 2.8 | 2.8 | 35.9 |
| 5 014735 | 014735 | | 2 | 2 | 2 CDP-diacylg | CDIPT | >sp 014735 | 1 | 2 | 2 / | 2 7 | 2 2 | | 2 11.3 | 11.3 | 11.3 | 23.5 |
| 6 014880 | 014880 | | 2 | 2 | 2 Microsomal | MGST3 | >sp 014880 | 1 | 2 | 2 | 2 2 | 2 2 | | 2 17.8 | 17.8 | 17.8 | 16.5 |
| 7 014910;Q9NUP9;Q9HAP6 | 014910;Q9N | 2;1;1 | 2;1;1 | 2;1;1 | Protein lin-7 | LIN7A;LIN7C | >sp 014910 | 3 | 2 | 2 | 2 : | 2 2 | | 2 9 | 9 | 9 | 25.9 |
| 8 014925:05SRD1 | 014925:055 | 7:5 | 7:5 | 7:5 | Mitochondr | TIMM23:TIM | >sp 014925 | 2 | 7 | 7 | , . | 7 7 | | 7 61.7 | 61.7 | 61.7 | 21.9 |
| 014966:P57729:013637 | 014966 | 4:1:1 | 4:1:1 | 4:1:1 | Ras-related | RAB7L1 | >sp 014966 | 3 | 4 | 4 / | 4 / | 4 4 | | 4 23.2 | 23.2 | 23.2 | 23.1 |
| 0 014967 | 014967 | | 1 | 1 | 1 Calmegin | CLGN | >sp 014967 | 1 | 1 | 1 | 1 | 1 1 | | 1 18 | 1.8 | 1.8 | 70 0 |
| 1 014980 | 014980 | | 1 | 1 | 1 Exportin-1 | XPO1 | >sn 014980 | 1 | 1 | 1 | | 1 1 | | 1 11 | 1.1 | 1.1 | 122 |
| 2 015120 | 015120 | | 2 | 2 | 2 1-acyl-sp. db | AGRAT2 | >sp[015120] | 1 | 2 | 2 | | 2 2 | | 2 04 | 9.4 | 9.4 | 30.0 |
| 2 015359 | 015259 | | 1 | 1 | 1 Protoin PCP | 10001 | >cp[015120] | 1 | 1 | 1 | | 1 1 | | 1 5.6 | 5.4 | 5.6 | 20.9 |
| 4 015250 | 015256 | | | | 4 Susfait I | CUDEA | ~sp[015258 | 1 | 4 | 4 | | . 1 | | 1 5.0 | 3.0 | 3.0 | 22.9 |
| 0000 1 1 1 m / DU | 1112/001 | | | 4 | 4 SUITEIT IOCUS | COURT A | <>010152601 | | (4) | - AL | . / | | | 4 18.6 | 0.61 | LX D | 30.3 |



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



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

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| 🕮 Recent Places | s | Alte Firefox-Daten | 02.02.15 11:26 | Filefolder | | | | | 4 | 4.7 | 4.7 | 4.7 | 102.36 |
| Dropbox | - | DBond Viewer v3.01 | 19.02.14 18:32 | File folder | | | | | 1 | 4.8 | 4.8 | 4.8 | 36.547 |
| MS-Ergebniss | se (MS-NAS-LAUFWERK) | bond_v3.02 | 16.04.14 12:55 | File folder | | | | | 1 | 0.7 | 0.7 | 0.7 | 382.3 |
| Protokolle (M | AS-NAS-LAUEWERK) | DeNovoGUI-1.2.3-windows | 14.02.14 10:42 | File folder | | | | | 1 | 0.6 | 0.6 | 0.6 | 266.94 |
| iCloud Drive | | ProteoWizard 3.0.5759 64-bit | 02.06.14 18:46 | File folder | | | | | 3 | 10.9 | 10.9 | 10.9 | 38.604 |
| iCloud Photo | | xqxp_V2_1_1_VM | 04.11.14 09:37 | File folder | | | | | 1 | 1.9 | 1.9 | 1.9 | 103.75 |
| | | 🔊 Data Robert (D) - Shortcut (2) | 03.02.15 12:53 | Shortcut | 1 KB | | | | 1 | 4.3 | 4.3 | 4.3 | 34.466 |
| 🚞 Librarian | | 🔊 Data Robert (D) - Shortcut | 03.02.15 13:00 | Shortcut | 1 KB | | | | 1 | 1.9 | 1.9 | 1.9 | 46.962 |
| De sum ente | | | | | | | | | 2 | 7.8 | 7.8 | 7.8 | 24.409 |
| Documents | | | | | | | | | 5 | 32.6 | 27.3 | 16.7 | 51.561 |
| a) Music | | | | | | | | | 1 | 27 | 18.1 | 1.8 | 60.044 |
| Pictures | | | | | | | | | 2 | 11.1 | 11.1 | 11.1 | 22.975 |
| Videos | | | | | | | | | 1 | 8.6 | 8.6 | 8.6 | 18.281 |
| _ | | | | | | | | | 3 | 26.6 | 10.8 | 10.8 | 51.267 |
| Computer | | | | | | | | | 22 | 53.1 | 53.1 | 46.7 | 58.826 |
| Local Disk (C: | :) | | | | | | | | 3 | 20.3 | 9.3 | 5.9 | 62.378 |
| 🔞 Data Robert (| (D:) | | | | | | | | 22 | 54.3 | 53.1 | 53.1 | 62.064 |
| 👝 DataAS2 (E:) | Ŧ | | | | | | | | 13 | 41.8 | 38.7 | 30.2 | 65.432 |
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| Detaitor | Test (Telestern estered) (***) | | | | | | | | 1 | 0.5 | 0.5 | 0.5 | 248.07 |
| Dateityp: | First (Tabstopp-getrennt) (".bt) | | | | | | | | 1 | 3.8 | 1.7 | 1.7 | 61.901 |
| Authors: | Excel-Arbeitsmappe (".xisx) Excel-Arbeitsmappe mit Makros (*.xism) | | | | | | | | 1 | 2.3 | 2.3 | 2.3 | 49.129 |
| | Excel-Binärarbeitsmappe (*.xlsb) | | | | | | | | 5 | 21.1 | 21.1 | 21.1 | 31.62 |
| A . | Excel 97-2003-Arbeitsmappe (*.xls) | | | | | | | | 1 | 4.3 | 4.3 | 4.3 | 23.705 |
| Ordner ausblend | Einzelnes Webarchiv (*.mht:*.mhtml) | | | | | | | | 3 | 16.4 | 16.4 | 16.4 | 21.671 |
| | Webseite (*.htm;*.html) | | | | | | | 2 | 1 | 1.4 | 1.4 | 1.4 | 68.761 |
| 29 000483 | Excel-Vorlage (*.xltx) Excel-Vorlage mit Makros (*.vltm) | | | | | | | - 8 | 3 | 37 | 37 | 37 | 9.3697 |
| 30 000487 | Excel 97-2003-Vorlage (*.xit) | | | | | | | - | 1 | 4.2 | 4.2 | 4.2 | 34.577 |
| 31 000571:015. 30 | Text (Tabstopp-getrennt) (*.txt) | | | | | | | | 6 | 11.6 | 11.6 | 11.6 | 73.243 |
| 32 014654 | Unicode Text (*.txt) XML-Kalkulationstabelle 2003 (*.vml) | | | | | | | - | 6 | 6.6 | 6.6 | 6.6 | 133.77 |
| 33 014681 | Microsoft Excel 5.0/95-Arbeitsmappe (*.xls) | | | | | | | - | 2 | 5.9 | 5.9 | 5.9 | 38.964 |
| 34 014734 | CSV (Trennzeichen-getrennt) (*.csv) | | | | | | | - | 1 | 2.8 | 2.8 | 2.8 | 35.914 |
| 35 014735 | Formatierter Lext (Leerzeichen getrennt) (*.p | orn) | | | | | | - | 2 | 11.3 | 11.3 | 11.3 | 23.539 |
| 36 O14880 | Text (MS-DOS) (*.txt) | | | | | | | - | 2 | 17.8 | 17.8 | 17.8 | 16.516 |
| 37 O14910;Q9NUP9 | CSV (Macintosh) (*.csv) | | | | | | | - | 2 | 9 | 9 | 9 | 25.996 |
| 38 O14925;Q5SRD1 | CSV (MS-DOS) (*.csv) DIE (Data Interchange-Format) (* dif) | | | | | | | - | 7 | 61.7 | 61.7 | 61.7 | 21.943 |
| 39 O14966;P57729;C | SYLK (symbolische Verbindung) (*.slk) | | | | | | | | 4 | 23.2 | 23.2 | 23.2 | 23.155 |
| 40 014967 | Excel-Add-In (* xlam) | | | | | | | | 1 | 1.8 | 1.8 | 1.8 | 70.038 |
| 41 014980 | Excel 97-2003-Add-In (*.xla) | | | | | | | | 1 | 1.1 | 1.1 | 1.1 | 123.38 |
| 42 015120 | XPS-Dokument (*.xps) | | | | | | | - | 2 | 9.4 | 9.4 | 9.4 | 30.914 |
| 43 015258 | OpenDocument-Kalkulationstabelle (*.ods) | | | | | | | | 1 | 5.6 | 5.6 | 5.6 | 22.958 |
| 44 015260 | 015260 4 | 4 4 Surfeit locus | SURF4 >sp 0152 | 60 1 | 4 4 | 4 4 | | 4 | 4 | 18.6 | 18.6 | 18.6 | 30.394 |

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

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| | A | P | | D | E C | c | G | u II | 1 | 1.1 | K | I M | N | | 0 | D | 0 | D | c | Ξ |
| 1 | Protein IDs | Majority pro | Pentide cou | u Pentide cou | u Pentide cou | u Protein nam | Gene names | Fasta header | Number of n Per | ntides Ra | zor + uniquilini | que nent Pentide | s 1 Razor - | + unia Unia | ue nent Segu | ence co l In | ique + raz Uni | | J weight Seg | |
| 2 | T | т | N | N | N | T | T | T | N | N N | N | N | N | N | N N | N | N | N | N N N | |
| 3 | A0EGR8 | A0FGR8 | | 1 . | 4 . | 4 Extended sy | / ESYT2 | >splA0EGR8 | 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-bi | n 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 t | r 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;1; | l l | | >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 | CONP0076 | | 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 | L; 5;5;1;1;1;0;0 |); Keratin, typ | e KRT14 | >sp P02533 | 43 | 13 | 9 | 5 | 13 | 9 | 5 | 32.6 | 27.3 | 16.7 | 51.561 | |
| 13 | P02538;CON_P02538;P486 | 6 P02538;CON | 13;13;12;12; | 18;8;7;7;7;1;1 | L; 1;1;0;0;0;0;0; |); Keratin, typ | e KRT6A;KRT60 | >sp P02538 | 9 | 13 | 8 | 1 | 13 | 8 | 1 | 27 | 18.1 | 1.8 | 60.044 | |
| 14 | CONP02662 | CONP0266 | i : | 2 | 2 | 2 | | >P02662 SWI | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 11.1 | 11.1 | 11.1 | 22.975 | |
| 15 | CONP02754 | CONP0275 | 5 | 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, typ | e 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, typ | e 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;7 Mic | rosoft Excel | 1.00 | Renative, Spr. | | 1000 | | | | | | | × | 20.3 | 9.3 | 5.9 | 62.378 | |
| 19 | P35527;CON_P35527 | P35527;CON | 23;23 | USUIT EXCEI | 100.00 | Sanation, Sanat | | | 1 | | - | - | | 100 | | 54.3 | 53.1 | 53.1 | 62.064 | |
| 20 | P35908;CON_P35908;Q015 | 2 P35908;CON | 20;20;3 | Der aus | gewählte Dateity | p unterstützt kei | ne Arbeitsmappen | , die mehrere Blät | ter enthalten. | | | | | | | 41.8 | 38.7 | 30.2 | 65.432 | |
| 21 | CON_Q3SZH5 | CON_Q3SZ | | A Nicker | Sie auf 'OK' we | nn nur das aktue | lle Blatt gespeiche | rt werden soll | | | | | | | | 1.7 | 1.7 | 1.7 | 45.456 | |
| 22 | Q5D862;CON_Q5D862 | Q5D862;CON | 1;1 | •Wenn | alle Blätter in die | ser Arbeitsmappe | e in dem ausgewäh | lten Dateityp ges | peichert werden solle | en, wählen Sie j | edes Blatt aus, un | d speichern Sie es als | eigene Datei un | nter unterschied | dlichen Namen, | 0.5 | 0.5 | 0.5 | 248.07 | |
| 23 | Q7Z794;CON_Q7Z794 | Q7Z794;CON | 2;2 | oder wä | ihlen Sie einen Di | ateityp, der mehr | ere Blätter unters | tützt. | | | | | | | | 3.8 | 17 | 1.7 | | |
| 24 | O00148;Q13838 | O00148;Q13 | | | | | | | | | | | | | | | 1.7 | | 61.901 | |
| 25 | O00165 | 000165 | 1;1 | | | | | | OK | Abbrechen | 1 | | | | | 2.3 | 2.3 | 2.3 | 61.901 49.129 | |
| 26 | 000217 | 000105 | 1;1 | Mar die | en Tofernalisa k | lfueisle? | | | ОК | Abbrechen | | | | | | 2.3 21.1 | 2.3 21.1 | 2.3 21.1 | 61.901 49.129 31.62 | |
| 27 | 000217 | 000165 | .1;1 | <u>War die</u> | se Information h | lfreich? | | 1 | OK | Abbrechen | | | | | | 2.3 21.1 4.3 | 2.3 21.1 4.3 | 2.3 21.1 4.3 | 61.901 49.129 31.62 23.705 | |
| | 000264 | 000165 000217 000264 | | <u>War die</u> | se Information h | lfreich? 3 Membrane- | e PGRMC1 | >sp[c_J0264] | ок | Abbrechen 3 | 3 | 3 | 3 | 3 | 3 | 2.3 21.1 4.3 16.4 | 2.3 21.1 4.3 16.4 | 2.3 21.1 4.3 16.4 | 61.901 49.129 31.62 23.705 21.671 | |
| 28 | 7 000264 000327 | 000165 000217 000264 000327 | | <u>War die</u> 3 | se Information h 3 1 | lfreich? 3 Membrane- 1 Aryl hydroci | €PGRMC1 a ARNTL | >sp 2,00264 >sr_(000327 | ок | Abbrechen 3 1 | 3 1 | 3 1 | 3 1 | 3 1 | 3 | 2.3 21.1 4.3 16.4 1.4 | 2.3 21.1 4.3 16.4 1.4 | 2.3 21.1 4.3 16.4 1.4 | 61.901 49.129 31.62 23.705 21.671 68.761 | |
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| 28 29 30 | 000217 000264 000327 000483 000487 | 000165 000217 000264 000327 000483 000487 | | <u>War die</u> 3 1 3 1 | se Information h 3 1 3 1 | lfreich? 3 Membrane- 1 Aryl hydroci 3 NADH dehy 1 26S proteas | e PGRMC1 a ARNTL d NDUFA4 c PSMD14 | >sp 0.00264 >sp 0.00327 >sp 0.00483 >sp 0.00487 | ок [| Abbrechen 3 1 3 1 | 3 1 3 1 | 3 1 3 1 | 3 1 3 1 | 3 1 3 1 | 3 1 3 1 | 2.3 21.1 4.3 16.4 1.4 37 4.2 | 2.3 21.1 4.3 16.4 1.4 37 4.2 | 2.3 21.1 4.3 16.4 1.4 37 4.2 | 61.901 49.129 31.62 23.705 21.671 68.761 9.3697 34.577 | |
| 28 29 30 31 | 000254 000327 000483 000487 000487 | 000165 000217 000264 000327 000483 000487 000571;015 | 6;4;1 | War die 3 1 3 3 4 6;4;1 | se Information h | lfreich? 3 Membrane- 1 Aryl hydroca 3 NADH dehy 1 26S proteas ATP-depend | e PGRMC1 a ARNTL d NDUFA4 c PSMD14 d DDX3X;DDX3 | >sp 0.00264 >sp 0.00327 >sp 0.00483 >sp 0.00487 >sp 0.00571 | ОК 1 1 1 3 | Abbrechen 3 1 3 1 6 | 3 1 3 1 6 | 3 1 3 1 6 | 3 1 3 1 6 | 3 1 3 1 6 | 3 1 3 1 6 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 | 1.7 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 | 61.901 49.129 31.62 23.705 21.671 68.761 9.3697 34.577 73.243 | - |
| 28 29 30 31 32 | 000264 000264 000327 000483 000487 000571;015523;Q9NQI0 000574;015523;Q9NQI0 | 000165 000217 000264 000327 000483 000487 000571;0155 014654 | 56;4;1 | War die 3 1 3 5 5 | se Information h | Ifreich2 3 Membrane- 1 Aryl hydroca 3 NADH dehy 1 26S proteas ATP-depeno 5 Insulin rece | e PGRMC1 a ARNTL d NDUFA4 c PSMD14 d DDX3X;DDX3 g IRS4 | >sp Cu0264 >sp O00327 >sp O00483 >sp O00487 >sp O00571 >sp O14654 | ОК 1 1 1 1 3 1 | Abbrechen 3 1 3 1 6 6 6 | 3 1 3 1 6 6 6 | 3 1 3 1 6 6 | 3 1 3 1 6 6 | 3 1 3 1 6 6 | 3 1 3 1 6 6 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 | 61.901 49.129 31.62 23.705 21.671 68.761 9.3697 34.577 73.243 133.77 | |
| 28 29 30 31 32 33 | 000264 000327 000483 000483 000877 000571;015523;Q9NQI0 014654 014654 | 000165 000217 000264 000327 000483 000487 000571;015 014654 014681 | 6;4;1 | War die 3 1 3 4 5 5 2 | se Information h | Ifreich? 3 Membrane- 1 Aryl hydroca 3 NADH dehy 1 26S proteas ATP-depend 5 Insulin rece 2 Etoposide-i | ¢ PGRMC1 a ARNTL d NDUFA4 c PSMD14 d DDX3X;DDX3 ç IRS4 r EI24 | >sp 0.00264] >sr 0.00327 >sp 0.00483 >sp 0.00487 >sp 0.00571 >sp 0.14654 >sp 0.14681 | ок 1 1 1 1 3 1 1 1 | Abbrechen 3 1 3 1 6 6 6 2 | 3 1 3 1 6 6 6 2 | 3 1 3 1 6 6 2 | 3 1 3 1 6 6 6 2 | 3 1 3 1 6 6 2 | 3 1 3 1 6 6 2 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 5.9 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 5.9 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 5.9 | 61.901 49.129 31.62 23.705 21.671 68.761 9.3697 34.577 73.243 133.77 38.964 | |
| 28 29 30 31 32 33 34 | 000264 000237 000483 000487 000487 000687 014654 014654 014681 014734 | 000183 000217 000264 000327 000483 000487 000571;015 014654 014681 014734 | 6;4;1 | War die 3 1 3 1 6;4;1 5 2 | se Information h | Ifreich2 3 Membrane- 1 Aryl hydroca 3 NADH dehy 1 26S proteas ATP-depend 5 Insulin rece 2 Etoposide-i 1 Acyl-coenzy | e PGRMC1 a ARNTL d NDUFA4 c PSMD14 d DDX3X;DDX3 ç IRS4 n EI24 n ACOT8 | >sp 00264 >sp 000327 >sp 000487 >sp 000487 >sp 000571 >sp 014654 >sp 014734 >sp 014734 | ок 1 1 1 1 1 3 1 1 1 1 1 | Abbrechen 3 1 3 1 6 6 6 2 1 | 3 1 3 1 6 6 6 2 1 | 3 1 3 1 6 6 2 1 | 3 1 3 1 6 6 2 1 | 3 1 3 1 6 6 2 1 | 3 1 3 1 6 6 2 1 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 5.9 2.8 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 5.9 2.8 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 5.9 2.8 | 61.901 49.129 31.62 23.705 21.671 68.761 9.3697 34.577 73.243 133.77 38.964 35.914 | |
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| 28 29 30 31 32 33 34 35 36 37 | 000264 000327 000483 000487 000571;015523;Q9NQI0 004654 014681 014734 014734 014735 014735 014880 014735 | 000217 000264 000327 000483 000487 000571;015 014654 014681 014735 014880 014910;09N | 6;4;1 2;1;1 | War die 8 1 3 5 6;4;1 5 2 1 2 2;1;1 7 7 7 7 | se Information h | Ifreich2 3 Membrane- 1 Aryl hydroci 3 NADH dehyi 1 265 proteasi ATP-depend 5 Insulin rece 2 Etoposide-ii 1 Acyl-coenzy 2 CDP-diacylg 2 Microsomal Protein lin- | ₹ PGRMC1 a ARNTL c PSMD14 c PSMD14 d DDX3x;DDX3 ç IRS4 r EI24 r ACOT8 i (CDIPT i (MGST3 7 LIN7A;LIN7C; | >sp 0.00264 >su 000327 >sp 000483 >sp 000487 >sp 000487 >sp 014654 >sp 014681 >sp 014734 >sp 014734 >sp 014734 >sp 014735 >sp 014880 >sp 014920 | ок 1 1 1 1 3 1 1 1 1 1 1 1 3 3 | Abbrechen 3 1 3 1 6 6 6 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 | 3 1 3 1 6 6 2 1 1 2 2 2 2 2 | 3 1 3 1 6 6 2 1 2 2 2 2 2 2 | 3 1 3 1 6 6 2 1 1 2 2 2 2 2 2 | 3 1 3 1 6 6 2 1 2 2 2 2 2 2 | 3 1 3 1 6 6 2 1 2 2 2 2 2 2 2 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 5.9 2.8 11.3 17.8 9 (1.7) | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 5.9 2.8 11.3 17.8 9 (17) | 2.3 21.1 4.3 16.4 37 4.2 11.6 6.6 5.9 2.8 11.3 17.8 9 (1)2 | 61.901 49.129 31.62 23.705 21.671 68.761 9.3697 34.577 73.243 133.77 73.243 133.77 38.964 35.914 23.539 16.516 25.996 | |
| 28 29 30 31 32 33 34 35 36 37 38 | 000264 00027 000483 000487 000571;015523;Q9NQI0 014654 014734 014734 014735 014880 014910;Q9NUP9;Q9HAP6 014910;Q9NUP9;Q9HAP6 | 000217 000264 000327 000483 000487 000571;015 014654 014654 014735 014735 014880 014910;09N 014925;05S | 2;1;1 2;1;1 7;5 | War die 3 1 3 6;4;1 5 2 2 2 2 2;1;1 7;5 1 1 2 2 1 2 2 1 1 2 1 1 5 1 1 1 1 1 1 | se Information h | Ifrech2 3 Membrane- 1 Aryl hydroca 3 NADH dehy 1 265 proteas: ATP-depend 5 Insulin rece 2 Etoposide-i 1 Acyl-coenzy 2 CDP-diacylg 2 CDP-diacylg 2 Microsomal Protein lin- Mitochondr | PGRMC1 a ARNTL c NDUFA4 c PSMD14 d DDX3x;DDX3 ; IRS4 r EI24 r ACOT8 d CDIPT ; MGST3 7 LIN7A;LIN7C; i TIMM23;TIM | >sp 0.00264 >sp 0.00327 >sp 0.00483 >sp 0.00483 >sp 0.00487 >sp 0.04654 >sp 0.14654 >sp 0.14734 >sp 0.14735 >sp 0.14735 >sp 0.14735 >sp 0.14800 >sp 0.14910 >sp 0.14925 | ок 1 1 1 1 1 1 1 1 1 1 1 1 1 | Abbrechen 3 1 3 1 6 6 6 6 2 1 1 2 2 2 2 2 2 7 7 | 3 1 3 1 6 6 6 2 1 2 2 2 2 2 2 2 2 7 7 | 3 3 1 6 6 2 1 2 2 2 2 7 7 | 3 1 3 1 6 6 6 2 1 2 2 2 2 7 7 | 3 1 3 1 6 6 2 1 2 2 2 2 2 7 | 3 1 3 1 6 6 6 2 1 2 2 2 2 2 2 7 7 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 5.9 2.8 11.3 17.8 9 61.7 9.2 2.2 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 5.9 2.8 11.3 17.8 9 61.7 9 | 2.3 21.1 4.3 16.4 1.4 37 4.2 11.6 6.6 5.9 2.8 2.8 11.3 17.8 9 6.17 9 | 61.901 49.129 31.62 23.705 21.671 68.761 9.3697 34.577 73.243 133.77 38.964 35.914 23.539 16.516 25.996 21.943 | |
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Back to Perseus - Data upload







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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 is added or removed by clicking on the respective buttons.



There are five data types:

1. Expression data

= H/L-ratios, Labelfree quantification values, Isobariclabel intensities etc.





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



There are five data types:

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



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

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| 1 | NaN | NaN | 0.80343 | | | | 0 | 4.0745 | 188920 | 9885200 | 9006800 | 0 | 0 | | Categorical columns (3) |
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Every time you change something a new **matrix** is created. This way you can always go back to a previous processing stage.

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| 3 | NaN | 0.50649 | 0.81553 | | | | 0 | 6.6682 | 931260 | 667470 | 263780 | 1 | 5 | | | Numerical columns (17) |
| 4 | NaN | NaN | 1.1326 | | | | 0 | 14.438 | 968950 | 496020 | 472930 | 0 | 1 | | •••• | Categorical rows (0) |
| 5 | NaN | NaN | NaN | | | | 0.0014 | 1.9054 | 960480 | 832250 | 128230 | 0 | 1 | | Matrix-tree | Numerical rows (0) |
| 6 | NaN | NaN | NaN | | | | 0.0078 | 1.1743 | 1918800 | 1124800 | 793970 | 1 | 0 | | Window (lists all | |
| 7 | NaN | NaN | NaN | | | | 0 | 5.6441 | 1161600 | 1156300 | 5326.8 | 0 | 0 | | window (lists all | iviatrix-info |
| 8 | NaN | NaN | NaN | | - 1- 1 - | | 0.0095 | 0.99659 | 254400 | 254400 | 0 | 0 | 0 | | matrices | Window (contains |
| 9 | NaN | NaN | NaN D | ατατά | able | | 0.0077 | 1.1381 | 128280 | 128280 | 0 | 0 | 0 | | matrices | |
| 10 | NaN | 0.33034 NoN | 0.5571 | Vind | 0.44 | | 0 | 4.9443 | 206510 | 305720 | 4527400 | 0 | 2 | | contained in a | information |
| 12 | NaN | NaN | 0.14969 V | VIIIG | UW | | 0 | 20.421 | 290510 | 5066000 | 3921000 | 0 | 1 | | contained in a | mormation |
| 13 | NaN | NaN | 0.75698 | | | | 0 | 3 1953 | 135380 | 8140000 | 5397600 | 0 | 0 | | Perseus project) | about the |
| 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 | | | currently selected |
| 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 | | | matrix) |
| 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 | 0.01849 | | | | 0 | 5.9197 | 104520 | 6450000 | 4002100 | 0 | 0 | | | × > |
| 21 | 0.06770 | 0.20004 | 1.4144 NoN | | | | 0 | 2.0795 | 103470 | 4940000 | 154400 | 2 | 2 | | | |
| 20 | 0.90779 | 0.990167 | 0.81699 | | | | 0 | 202 54 | 213370 | 132590 | 807790 | 4 | 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 item | s | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | Version 1.5.1.6 |

The Datatable Window

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

| | | | 1 | | | | | | | | | | | | | | | | | | | 1 |
|-------------------|----|------------|------------|----------------|------------|-----------------------------|---------|------------------|---------|---------|-----------|----------------|----------------|---------------|---------------|---------------|-------------------------------|-------------------------------|-------------------------------|-------------------------|-------------------------|-------------------------|
| | | Data | | | | | | | | | | | | | | | | | | | | |
| Data type (as | | | A | В | С | 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 |
| defined during — | | Туре | Expres | Expres | Expression | Catego | Catego | Catego | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric |
| unload) | | 33 | NaN | NaN | 0.30486 | | | | 0 | 8.1199 | 2024900 | 1443800 | 581170 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 3 |
| upioau) | | 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 | 1.1/88 | | | | 0 | 7.0117 | 353700 | 1/0680 | 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 | 0.51979 | 1.2100 | | | | 0 | 0.7233 | 247230 | 108900 | 7820800 | 0 | 2 | 2 | 0 | 2 | 2 | 0 | 2 | 2 |
| | | 40 | NaN | 0.53144 NoN | 2.0496 | | | | 0 | 4.1007 | 104290 | 299000 | 126020 | 0 | 2 | 2 | 0 | 2 | 2 | 0 | 2 | 2 |
| | | 40 | 0.59303 | 0.32887 | 0.50644 | | | | 0 | 22 391 | 686870 | 550940 | 135930 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 3 | 3 |
| Each row contains | | 48 | 0.85755 | 1.114 | NaN | | | | 0 | 3.6129 | 195420 | 108570 | 868580 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| one identified | ור | 49 | NaN | NaN | 1.0721 | | | | 0 | 4.066 | 230570 | 120950 | 109620 | 1 | 0 | 2 | 1 | 0 | 2 | 1 | 0 | 2 |
| one identified | | 50 | 0.75695 | 3.3265 | 0.83447 | | | | 0 | 82.637 | 104120 | 521480 | 519760 | 5 | 8 | 5 | 5 | 8 | 5 | 5 | 8 | 5 |
| protein group | | 51 | 1.0146 | 0.6911 | 0.52887 | | | | 0 | 14.384 | 137620 | 904860 | 471340 | 2 | 3 | 4 | 2 | 3 | 4 | 2 | 3 | 4 |
| proteingroup | | 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 |
| | | h4 | NaN | MaN | NaN | | | | | 99793 | 6522600 | 4676000 | 1846700 | | | | | | 1 | 0 | 0 | |
| | | 1628 items | 1 selected | | | | | | | | | | | | | | | | | | | ſ |

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.

Before processing the data further, first <u>save</u> the Perseus project.

| | bession1 - I | Perseus | | | | | | | - 14 h | | Table Inc. | | | and had it. | |
|-----------------|--------------|---------------|---------|----------|-----------|------|-----------------------------|-------------------------------------|-------------------------|--------------------------|-------------------------------|--------------------|------------|---|--|
| - | | | | | | | | | | | | | | | R. 🗘 🗭 |
| | Sav | e | | | | | 2 p2 7 x p1 | Visualizati Clustering Misc 🔹 | on • 44 //PCA • 🎊 | i 課 ⊵ "∥ So ¥ (7 C | Basic • | | • | | |
| Load | Sav | e as | | — | | | | inise. | Analysis | | Mult | ti-proc. | Export | | |
| matrix1 Data | Sav | e as PDF | | | | | | | Anaysis | | | a proc. | |) · • • • • • • • • • • • • • • • • • • | matrix1 Creator: Annette 02/16/2015 10:23:44 |
| | Op | en | | | | | Unique + razor sequen | Unique sequen covera | Mol. weight [kDa] | Peptides | Razor + unique peptides | Unique peptides | Prot | Generic matrix u | Origin: C:\Users\Annette\Desktop\proteinGroupsVBA.txt File: proteinGroupsVBA.txt Quality: (small values are good.) |
| Туре | NEW! Nev | N | | | | | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Text | matrix1 | Rows (553) |
| 1 | | | | | | | NaN | NaN | NaN | NaN | NaN | NaN | Т | | Expression columns (3) Categorical columns (3) |
| 2 | Nev | w window | | | | | 4.7 | 4.7 | 102.36 | 4 | 4 | 4 | A0F | | String columns (4) |
| 3 | ** | | | | | | 4.8 | 4.8 | 36.547 | 1 | 1 | 1 | A2R | | Numerical columns (8) Multi-numerical columns (6) |
| 4 | Anr 😽 | notation down | nload | | | | 0.7 | 0.7 | 382.3 | 1 | 1 | 1 | A4U | | Categorical rows (0) |
| D | . | | | | | | 0.0 | 0.0 | 200.94 | 1 | 1 | 1 | ADY D07 | | Numerical rows (0) |
| 7 | Hel | р | | | | | 10.9 | 10.9 | 102.75 | 1 | 1 | 1 | P11 | | |
| 8 | | | | | | | 4.3 | 4.3 | 34.466 | 1 | 1 | 1 | ASM | | |
| 9 | | | | | | | 1.9 | 1.9 | 46.962 | 1 | 1 | 1 | A8M | | |
| 10 | INAIN | INAIN | Nan | | - 1./E-U0 | 1.8 | 7.8 | 7.8 | 24.409 | 2 | 2 | 2 | 100 | | |
| 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 | 100 | | |
| 14 | NaN | NaN | NaN | + | ► 0.0006 | 8.6 | 8.6 | 8.6 | 18.281 | 1 | 1 | 1 | 100 | | |
| 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 | 8 0.75705 | 12.778 | + | + 1.35E | 20.3 | 9.3 | 5.9 | 62.378 | 11 | 5 | 3 | P13 | | |
| 18 | 0.11225 | 6 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 | 100 | | |
| 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./ | 01.901 | 2 | 1 | 1 | 0/2 | | |
| 23 | 1 1107 | 2 4 1 2 9 | 2 5922 | | 1.205 | 2.3 | 2.3 | 2.3 | 49.129 | 5 | 5 | 5 | 000 | | |
| 24 | NaN | NaN | NaN | | 0.0001 | 4.3 | 43 | 4.3 | 23 705 | 1 | 1 | 1 | 000 | | |
| 26 | 0 74490 | 0.91574 | 1 1054 | | 1.46F- | 16.4 | 16.4 | 16.4 | 21.671 | 3 | 3 | 3 | 000 | | |
| 27 | NaN | NaN | NaN | | 0.0249 | 1.4 | 14 | 1.4 | 68 761 | 1 | 1 | 1 | 000 | | |
| 28 | 0.87934 | 1.0377 | 0.91532 | | 3.73E | 37 | 37 | 37 | 9.3697 | 3 | 3 | 3 | 000 | | |
| 29 | NaN | NaN | NaN | | 0.02872 | 4.2 | 4.2 | 4.2 | 34.577 | 1 | 1 | 1 | 000 | | |
| 30 | 1.2975 | 0.978 | 1.0558 | | 6.28E | 11.6 | 11.6 | 11.6 | 73.243 | 6 | 6 | 6 | 000 | | |
| 31 | 1.3502 | 1.0293 | 0.82036 | | 3.03E | 6.6 | 6.6 | 6.6 | 133.77 | 6 | 6 | 6 | 014 | | |
| 32 | NaN | NaN | NaN | | 0.0001 | 5.9 | 5.9 | 5.9 | 38.964 | 2 | 2 | 2 | 014 | | |
| 552 iterre | | | | | | | | _ | | | _ | | | | |
| 553 items | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | Version 1.5.1.6 |

Perseus projects are saved as sps-files



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



Removal of usually irrelevant protein group identifications



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

| | Session1 - Pe | erseus | - | - | | | | | - | | | | | - | | | | | | |
|------------|---------------|---------------|-------------|----------|------------|--------------|------------|---------------|------------------|-------------|-----------|----------|----------|----------|----------|---------|-------|---------------------------------------|-----|---|
| | Matrix | | | | | | | | | | | | | | | | | | | |
| 1 🧐 | Basic 🔹 | Filter | r rows • | Annot | Jumns • | Imputation • | Clust | ering • | 🐸 P ₂ | Visualizati | ion 🔹 📫 | 111111 | Basic • | | | | | | | |
| E 🖸 | Rearrange • | Filter | r columns 🔹 | unnot. r | rows • | Modification | s • f(x) 📈 | î 💶 Z 🦻 | \bar{x}^{p_N} | Clustering | g/PCA 🔹 🕺 | (🔨 🔐 🖸 | | | | | | | | |
| 1 <u>1</u> | Normalizatio | on • Qual | lity • | Tests • | | Proteomic ru | iler 🔹 📰 🕀 | \simeq 1D 2 | D P1 🔺 | Misc. • | | i 🖆 💟 | | | | | | | | |
| Load | | | | F | Processing | | | | | | Analysis | | Mu | proc. | Export | | | | _ | |
| matrix1 | matrix2 | 5 | | | | | | | | | | | | | | | 4 🕨 🗖 | j 🔝 🖑 🗶 🖊 👚 🛅 📑 | . 4 | Creator Appette |
| Data | | | | | | | | | | | | | | | | | 4 ▶ 🖪 | | | 02/18/2015 18:02:58 |
| | А | В | С | Only | Reverse | Potential | Q-value | Score | Intensity | Intensity | Intensity | Peptides | Peptides | Peptides | Razor | Razor + | Ra | Generic matrix u | | Origin: D:\SILAC Workshop 2015\combined\txt\proteinGroups.txt |
| | | | | by site | | contant | | | | L | | <u>^</u> | D | Č | peptides | potides | pe | | | Quality: (small values are good.) |
| Туре | Expres | Expres | Expres | Catego | . Catego | . Catego | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Nu | matrix1 | | Rows (1607) |
| 1 | NaN | NaN | 0.80343 | | | | 0 | 4.0745 | 188920 | 9885200 | 9006800 | 0 | 0 | 3 | 0 | 0 | | Filter rows based | | Expression columns (3) Categorical columns (3) |
| 2 | NaN | NaN | 0.64703 | | | | 0.0034 | 1.4653 | 183450 | 124320 | 5912800 | 0 | 1 | 2 | 0 | 1 | 2 | | | String columns (4) |
| 3 | NaN | 0.50649 | 0.81553 | | | | 0 | 6.6682 | 931260 | 667470 | 263780 | . 1 | 5 | 3 | 1 | 5 | 3 | matrix2 | | Numerical columns (17) Multi-numerical columns (8) |
| 4 | NaN | NaN | 1.1320 | | | | 0.0014 | 14.438 | 968950 | 496020 | 472930 | . 0 | 1 | / | 0 | 1 | 1 | , , , , , , , , , , , , , , , , , , , | | Categorical rows (0) |
| 0 | NaN | NaN | NaN | | | | 0.0014 | 1.9054 | 960480 | 832250 | 128230 | 0 | 1 | 0 | 1 | 1 | 0 | | | Numerical rows (0) |
| 7 | NoN | NaN | NoN | | | | 0.0078 | 5.6441 | 1161600 | 1124000 | 5226.9 | 0 | 0 | 2 | 0 | 0 | 2 | | | |
| 8 | NaN | NaN | NaN | | | | 0.0095 | 0.00650 | 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 2.0667 | 0.90245 | | | | 0 | 5.0359 | 193560 | 134310 | 5924700 | 0 | 1 | 1 | 0 | 1 | 1 | | | |
| 20 | NaN | 2.0007 | 0.61940 | | | | 0 | 4./8/ | 200040 | 6450000 | 4002400 | 0 | 0 | 2 | 0 | 2 | 1 | | | |
| 20 | NoN | NaN | 0.01849 | | | | 0 | 2.2244 | 104520 | 4040000 | 4002100 | 0 | 0 | 2 | 0 | 0 | 2 | | | |
| 28 | 0.96770 | 0.39904 | NaN | | | | 0 | 3.0785 | 100530 | 850920 | 154400 | 2 | 2 | 0 | 2 | 2 | 2 | | | - |
| 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 item | s | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | Version 1.5.1.6 |

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

| | essioni - Pe | erseus | - | - | | | - | | - | | | | | | | | | | |
|------------|--|----------------|-------------|------------------|-----------|--|---------------------------|-------------------|------------------|----------------|----------------|---------------|---------------|---------------|-------------------|-------------------|-------|-------------------|---|
| | Matrix | | | | | | | | | | | DDF | 1. J. | | | | | | |
| | Basic • | Filte | r rows * | Annot. o | columns • | Imputation | Clust | ering • | ₩ P ₂ | Visualizati | ion • 🕂 | F iii: 🖄 🔐 | Basic • | | | | | | |
| 三日日 | Rearrange • | Filte | r columns • | Annot. r | rows • | Modification | ns 🔹 🔣 🖉 | | | Clustering | /PCA • 🕵 | × 🐿 😻 🔽 | 100,000 | | | | | | |
| 15 | Normalizatio | on • Qua | anty • | rests * | | Proteomic r | uler • 🔛 🦄 | r ∽ id z | | WISC. * | | | | | | | | | |
| Load | and the second | | | ŀ | rocessing | | | | 1 | | Analysis | | Mul | ti-proc. | Export | | | | 4 112 |
| matrix1 | naurixz | | | | | | | | | | | | | | | | < ▶ □ | : E 🖑 🛪 🕈 🖀 🔳 🕫 | Creator: Annette |
| Data | _ | | | | | | | | | | | | | | | | < ▶ □ | | 02/18/2015 18:02:58 |
| | A | В | С | Only identifi | Reverse | Potential contam | Q-value | Score | Intensity | Intensity L | Intensity H | Peptides A | Peptides B | Peptides C | Razor + unique | Razor + unique | Ra | Generic matrix u | File: proteinGroups.txt |
| Tune | Everes | Everee | Everee | by site | Catago | Catago | Numerie | Numerie | Numerie | Numerie | Numerie | Numerie | Numorio | Numerie | peptides | peptides | pe | All matrix 1 | Quality: (small values are good.) |
| туре | Expres | Expres | Expres | Catego | . Catego. | Catego | Numeric | Numeric 4.0745 | 100000 | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | | indurix i | Rows (1607) Expression columns (3) |
| 2 | NaN | NaN | 0.60343 | | | | 0.0024 | 4.0745 | 100920 | 104000 | 5010000 | 0 | 1 | 3 | 0 | 1 | 2 | Filter rows based | Categorical columns (3) |
| 2 | NaN | 0.50640 | 0.04703 | | | | 0.0034 | 1.4005 | 024260 | 667470 | 262790 | 1 | 5 | 2 | 1 | 5 | 2 | | String columns (4) |
| 3 | NaN | 0.50049 NaN | 1 1226 | | | | 0 | 14 439 | 931200 | 496020 | 472020 | . 1 | 1 | 7 | 0 | 1 | 7 | matrix2 | Multi-numerical columns (17) Multi-numerical columns (8) |
| 5 | NaN | NaN | NaN | | | | 0.0014 | 1 9054 | 960490 | 832250 | 129230 | 0 | 1 | 0 | 0 | 1 | 0 | | Categorical rows (0) |
| 6 | NaN | NaN | NaN | | | | 0.0078 | 1.3034 | 1918800 | 1124800 | 793970 | 1 | 0 | 0 | 1 | 0 | 0 | | Numerical rows (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 item: | ; | | | | | | | 2 | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | Version 1.5.1. |

First steps entries remain from initially 1628.

| <mark>. </mark> | essioni - Pe | erseus | - | - | | | | | - | | | | | - | | | | | |
|------------------|--------------|-------------|-------------|---------------------|------------|--------------|-----------|----------|----------------------|-------------|-----------|----------|----------|----------|--------------------|--------------------|-------|-------------------|---|
| | Matrix | | | | | | | | | | | | | | | | | | 도 💭 🗢 🚇 |
| 1 🧐 | Basic • | Filter | r rows • | Annot. c | olumns • I | Imputation • | Clust | ering • | 3 P2 | Visualizati | on • 📫 | F 🎫 🗠 🚚 | Basic • | | | | | | |
| 王曰 | Rearrange • | Filter | r columns • | Annot. re | ows • I | Modification | s • 🛛 🕅 🗵 | 2 🖬 Z 🛐 | $\overline{x} p_{N}$ | Clustering | /PCA 🔹 🐝 | x 💊 😃 🔽 | | | | | | | |
| \$ | Normalizatio | on • Qual | lity • | Tests • | l. | Proteomic ru | ler 🔹 🕎 ∛ | 🕴 🗠 1D 2 | D 🖭 👗 | Misc. • | <u>,</u> | l 🧲 💟 | | | | | | | |
| Load | | | | P | rocessing | | | | | | Analysis | | Mult | ti-proc. | Export | | | | |
| matrix1 | matrix2 ma | atrix3 matr | rix4 | | | | | | | | | | | | | | < > C | 🗆 🕑 🗙 🐥 🎓 % 🖹 🖕 | ▲ matrix4 |
| Data | | | | | | | | | | | | | | | | | < > C | | Creator: Annette 02/18/2015 18:02:58 |
| | A | в | С | Only | Reverse | Potential | Q-value | Score | Intensity | Intensity | Intensity | Peptides | Peptides | Peptides | Razor + | Razor + | Ra | Generic matrix u | Origin: D:\SILAC Workshop 2015\combined\txt\proteinGroups.txt |
| | | | | identifi by site | | contam | | | | L | н | A | В | C | unique peptides | unique peptides | pe | | File: proteinGroups.txt |
| Туре | Expres | Expres | Expres | Catego | Catego | Catego | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numerio | NU | matrix1 | Quality: (small values are good.) Rows (1507) |
| 1 | NaN | NaN | 0.80343 | | | | 0 | 4.0745 | 188920 | 9885200 | 9006800 | 0 | 0 | 3 | 0 | 0 | 3 | | Expression columns (3) |
| 2 | NaN | NaN | 0.64703 | | | | 0.0034 | 1.4653 | 183450 | 124320 | 5912800 | 0 | 1 | 2 | 0 | 1 | 2 | Filter rows based | Categorical columns (3) String columns (4) |
| 3 | NaN | 0.50649 | 0.81553 | | | | 0 | 6.6682 | 931260 | 667470 | 263780 | . 1 | 5 | 3 | 1 | 5 | 3 | matrix 2 | Numerical columns (17) |
| 4 | NaN | NaN | 1.1326 | | | | 0 | 14.438 | 968950 | 496020 | 472930 | . 0 | 1 | 7 | 0 | 1 | 7 | inauticz | Multi-numerical columns (8) Coloradia (20) |
| 5 | NaN | NaN | NaN | | | | 0.0014 | 1.9054 | 960480 | 832250 | 128230 | 0 | 1 | 0 | 0 | 1 | 0 | Filter rows based | Numerical rows (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 | matrix3 | |
| 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 | Filter rows based | |
| 10 | NaN | 0.33634 | 0.5571 | | | | 0 | 4.9443 | 374210 | 305720 | 6849100 | 0 | 2 | 2 | 0 | 2 | 2 | matrix4 | |
| 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 item: | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | Version 1.5.1.6 |

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


First steps

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

| 2 I 🗋 I 🎽 | ession1 - Pe | rseus | | - | | | 8 | | - | | | | | | | | | | | | |
|------------|--------------|-----------|----------------|-----------|-----------|--------------|------------|-----------|-------------------|-------------|------------|----------|----------|----------|---------|---------|-------|-------------------|------|--|--------------------------------|
| - | Matrix | | | | | | | | | | | | | | | | | | | | Fu 🔷 🛱 |
| 1 1 | Basic • | Filter | rows • | Annot. c | olumns • | Imputation • | Clust | ering • | 🐸 P ₂ | Visualizati | on• + | BBE 🖂 🚚 | Basic • | | | | | | | | |
| 三日 | Rearrange 🔹 | Filter | columns • | Annot. re | ows 🔹 | Modification | s • f(x) 🛛 | 🕯 🎫 Z 🔊 | $\bar{x}^{p_{N}}$ | Clustering | /PCA 🔹 🎎 | : 🔊 😃 🔽 | | | | | | | | | |
| \$ | Normalizatio | n • Quali | ity • | Tests • | | Proteomic ru | iler 🔹 🧱 🗄 | 🖓 🗠 1D 2 | D 🖭 🛦 💧 | Misc. • | _ _ | de 🖉 | | | | | | | | | |
| Load | | | | Pi | rocessing | | | | | | Analysis | | Mult | ti-proc. | Export | | | | | | |
| matrix1 | matrix2 ma | trix3 mat | rix4 matrix | 5 matrix6 | 5 matrix | 7 | | | | | | | | | | | 4 🕨 🗖 | 🛛 🕑 🗶 🐥 🎓 🖫 🗈 🛓 | ⊿ ma | trix5 | |
| Data | | | | | | | | | | | | | | | | | 4 🕨 🖸 | | | 02/18/2015 18:02:58 | |
| | A | в | С | Q-value | Score | Intensity | Intensity | Intensity | Peptides | Peptides | Peptides | Razor + | Razor + | Razor + | Unique | Unique | Ur | Generic matrix u | | Origin: D:\SILAC Workshop 2015\c | .ombined\txt\proteinGroups.txt |
| | | | | | | | L | н | A | в | C | peptides | peptides | peptides | A | B | C Pe | | | File: proteinGroups.txt Quality: (small values are good.) | |
| Туре | Expres | Expres | Expres | Numeric | Numeri | c Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Numeric | Nu | matrix1 | | Rows (1507) | |
| 1 | NaN | NaN | 0.80343 | 0 | 4.0745 | 188920 | 9885200 | 9006800 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | Cittan and based | Þ | Expression columns (3) Categorical columns (0) | |
| 2 | NaN | NaN | 0.64703 | 0.0034 | 1.4653 | 183450 | 124320 | 5912800 | 0 | 1 | 2 | 0 | 1 | 2 | 0 | 1 | 2 | Pliter rows based | Þ | String columns (4) | |
| 3 | NaN | 0.50649 | 0.81553 | 0 | 6.6682 | 931260 | 667470 | 263780 | 1 | 5 | 3 | 1 | 5 | 3 | 1 | 5 | 3 | matrix2 | Þ | Numerical columns (17) | |
| 4 | NaN | NaN | 1.1326 | 0 | 14.438 | 968950 | 496020 | 472930 | 0 | 1 | 7 | 0 | 1 | 7 | 0 | 1 | 7 | | V | Categorical rows (0) | |
| 5 | NaN | NaN | NaN | 0.0014 | 1.9054 | 960480 | 832250 | 128230 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | Filter rows based | | Numerical rows (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 | matrix3 | | | |
| 8 | NaN | NaN | NaN | 0.0095 | 0.99659 | 254400 | 254400 | 0 | 0 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 1 | Filter rows based | | | |
| 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 | matrix4 | | | |
| 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 | Remove empty c | | | |
| 13 | NaN | NaN | 0.75698 | 0 | 3.1953 | 135380 | 8140000 | 5397600 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | matrix5 | | | |
| 14 | 0.60601 | 1.0407 | 0.7331 | 0 | 57.426 | 663490 | 420780 | 242710 | / | 4 | 10 | 1 | 4 | 10 | / | 4 | 10 | | | | |
| 15 | NaN | NaN | NaN | 0 | 4.5381 | 3446500 | 1936500 | 1510000 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | Transform | | | |
| 10 | 0.0810 | 3.3709 | 1.283 NoN | 0 0004 | 191.38 | 404070 | 204420 | 210200 | 20 | 25 | 18 | 20 | 25 | 18 | 20 | 25 | 18 | | | | |
| 10 | NoN | NaN | 0.50047 | 0.0094 | 4.0400 | 104050 | 2095100 | 4510900 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 1 | matrix6 | | | |
| 10 | NaN | NaN | 0.59947 NoN | 0 | 4.0109 | 512200 | 125040 | 4043600 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | Rename columns | | | |
| 20 | 1 0224 | 0.46720 | 0.67219 | 0 | 94 4042 | 446610 | 220220 | 126290 | 2 | 6 | 10 | 2 | 6 | 10 | 2 | 2 | 6 | | | | |
| 20 | NaN | NaN | NaN | 0.0014 | 1 7605 | 1550900 | 1205000 | 254970 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | matrix7 | | | |
| 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 | | | | | | | | | | | | | | | | | | | | | |
| 1507 items | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | Version 1.5.1.6 |

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
 - <u>http://141.61.102.17/perseus_doku/</u>
- Video tutorials from MaxQuant Summer Schools
 - <u>http://www.youtube.com/channel/</u> <u>UCKYzYTm1cnmc0CFAMhxD08w</u>

• Example dataset from:

Research

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

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

• Example dataset:



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

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| Project PXD000359 | | wnload Project Files | ← ι | ink to |
| Summary | | | | |
| Title | Species | Tissue | | |
| SILAC-based proteomics of human primary endothelial cell morphogenesis unveils tumor angiogenic markers | Homo sapiens | Not available | | |
| Description | (Human) | | | |
| 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 approached more | Instrument | Software | | |
| still do not have a complete understanding of the molecular mechanisms involved in this process, and there have been no | LTQ Orbitrap | Not available | | |
| 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 | Modification | Quantification | | |
| Read more | iodoacetamide derivatized residue | Not available | | |
| Sample Processing Protocol | acetylated residue | | | |
| See details in reference PMID : 23979707 | residue | | | |
| Data Processing Protocol | Experiment Type | | | |
| See details in reference PMID : 23979707 | Bottom-up proteomics | | | |
| Contact | | | | |
| Sara Zanivan, Vascular Proteomics | | | | |

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| txt.zip | 3.584,125 MB | 🗄 Download 📐 | 🗄 Download | | | |
| txtCLEC-IP.zip | 155,275 MB | 🖄 Download | 🗄 Download | | | |
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| 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 Matricel | Exp2 24h InGel 03.RAW | | 341,823 MB | | 🗄 Download | |

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

| Processing | Analysis | | Multi-proc. Export | | | _ | |
|-------------------------|---------------------------------|-------|----------------------------------|----------------------|---------------|-----------|---|
| 🔁 Generic matrix upload | | | | | | | |
| File | | | | | Select | - | |
| | | | Expression | | | | |
| | | | > | / | t | | |
| | | | | | | | |
| | Dpen 📴 Open | | | | | | |
| | Computer 🕨 Data Robert (D | D:) 🕨 | SILAC Workshop 2015 | (Mann paper) 🕨 combi | ned ▶ txt | | |
| | Organize 👻 New folder | | | | | | |
| | ★ Favorites | - | Name | Date modified | Туре | Size | |
| | 📃 Desktop | | 📋 aifMsms.txt | 02.03.15 11:23 | Text Document | 0 KB | |
| | 🐌 Downloads | | allPeptides.txt | 07.03.15 11:24 | Text Document | 3,705 KB | |
| | Recent Places | | evidence.txt | 02.03.15 11:23 | Text Document | 1,704 KB | |
| | 😌 Dropbox | | 📋 libraryMatch.txt | 02.03.15 11:23 | Text Document | 0 KB | |
| | 🖵 ms (Ms-nas-laufwerk) (W) | | matchedFeatures.txt | 02.03.15 11:24 | Text Document | 0 KB | |
| | MS-Ergebnisse (MS-NAS-LAUFWERK) | | modificationSpecificPeptides.txt | 02.03.15 11:23 | Text Document | 913 KB | |
| | Protokolle (MS-NAS-LAUFWERK) | | ms3Scans.txt | 02.03.15 11:23 | Text Document | 0 KB | |
| | iCloud Drive | | msms.txt | 02.03.15 11:23 | Text Document | 10,642 KB | |
| | 🛞 iCloud Photos | | msmsScans.txt | 02.03.15 11:23 | Text Document | 3,712 KB | |
| | | Ξ | msScans.txt | 02.03.15 11:23 | Text Document | 950 KB | |
| | 🥽 Libraries | | mzRange.txt | 02.03.15 11:23 | Text Document | 195 KB | |
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| | J Music | | parameters.txt | 02.03.15 11:23 | Text Document | 2 KB | |
| | E Pictures | | 📋 peptides.txt | 02.03.15 11:23 | Text Document | 820 KB | |
| | Videos | | proteinGroups.txt | 02.03.15 11:24 | Text Document | 445 KB | N |
| | | | 📄 summary.txt | 02.03.15 11:23 | Text Document | 2 KB | 5 |
| | Computer | | | | | | |

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

| Generic matrix upload | | | |
|-----------------------|---|---|------------------|
| ile | D:\SILAC Workshop 2015\Zanivan et al 2013 (Mar | nn paper)\Original results Mann\proteinGroups.txt | Select |
| | 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 | 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_11 Ratio H/L normalized Matr 30h_2 Ratio H/L normalized Matr 30h_3 | t u d b |
| | 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_2 | Numerical PEP Intensity L Intensity H | t u d b |
| | 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_3 Unique peptides Matr 24h_3 Unique peptides Matr dil_1 Unique peptides Matr dil_2 Unique peptides Matr dil_3 | Categorical > Only identified by site < | t u d b |
| | Sequence coverage [%] Unique + razor sequence coverage [%] Unique sequence coverage [%] Mol. weight [kDa] Sequence length Sequence lengths Slice average Slice 1 Slice 2 Slice 3 | Text > Protein IDs Protein names Gene names Gene names Proteins Proteins | t u d b |
| | Slice 4 Slice 5 Slice 6 Slice 7 Slice 8 Slice 9 Slice 10 Slice 11 Slice 12 | Multi-numerical | t u d b |
| | Shorten expression column names | | |
| ncel | Desc | ription | С |

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.

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)



ratioMatr30h/0h=heavy(standard)/light(0h) /heavy
(standard)/light(Matr30h) = light(Matr30h)/light
(0h)

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



| Data | | | | | | | | | | | | < > C |
|----------|---------------------|--------------------------------|------------------------|-----------------------------|---------|--------|---------|-----------|----------------|----------------|----------------|---------------------------|
| | Matr 30h_1_x/y_0 | Ma 30H ^o 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 |
| Туре | 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 | A0FGF |
| 3 | NaN | NaN | NaN | | | | 3.4808 | 670070 | 596980 | 7308500 | A0JLT | A0JLT. |
| 4 | 1.76249 | NaN | NaN | | | | 8.1766 | 119360 | 977740 | 215880 | A0JNW5 | A0JNV |
| 5 | NaN | NaN | NaN | + | | | 1.7907 | 270370 | 215370 | 550060 | E9PHQ | E9PHC |
| 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 | A2A2C |
| 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 | Q9UB(|
| 27 | NaN | NaN | NaN | + | | | 0.0001 | 161420 | 874090 | 740140 | Q9GZY | Q9GZY |
| 28 | 0.573 | 0.80311 | 0.547657 | | | | 0 | 126030 | 967480 | 292790 | A2RRP | A2RRF |
| 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 | АЗКМН |
| | | | | | | | | | | | | |
| 081 Item | 5 | | | | | | | | | | | |

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

7681 protein groups



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.



The expression ratios are now log2-transformed.

| | Matr 30h_1_x/y_0 | Ma 30M_2_x/y_0 | Matr 30h_3_x/y_0h_3 | i |
|------|---------------------|-------------------|------------------------|---|
| Туре | Expression | Expression | Expression | C |
| 1 | 1.50935 | 1.16412 | 1.39022 | Т |
| 2 | 0.702995 | 0.42739 | 0.700261 | |
| 3 | NaN | NaN | NaN | Т |
| 4 | 1.76249 | NaN | NaN | |
| 5 | NaN | NaN | NaN | F |
| 6 | NaN | NaN | NaN | |
| 7 | NaN | NaN | 0.730709 | Т |
| 8 | NaN | NaN | NaN | |
| 9 | NaN | NaN | NaN | Т |
| 10 | NaN | NaN | 1.36252 | |
| 11 | NaN | NaN | NaN | F |
| 12 | NaN | NaN | NaN | |
| 13 | 0.659292 | 0.803885 | 0.848393 | Т |
| 14 | NaN | NaN | NaN | |
| 15 | 1.82832 | 1.58992 | 1.63713 | Т |
| 16 | 0.532811 | NaN | 0.792298 | |
| 17 | NaN | NaN | 0.559604 | Τ |
| 18 | 1.20431 | NaN | 1.3273 | |
| 19 | NaN | NaN | NaN | Γ |
| 20 | NaN | NaN | NaN | Τ |
| 21 | NaN | NaN | NaN | Γ |
| 22 | 1.08225 | 0.893445 | 0.759342 | |
| 23 | NaN | NaN | 0.65183 | Γ |
| 24 | NaN | NaN | NaN | Γ |
| 25 | NaN | NaN | NaN | 4 |
| 26 | 0.684863 | 0.978735 | 0.921552 | Γ |
| 27 | NaN | NaN | NaN | 4 |
| 28 | 0.573 | 0.80311 | 0.547657 | |
| 29 | NaN | 1.44914 | NaN | [|
| 30 | NaN | NaN | NaN | |
| 31 | NaN | NaN | NaN | - |
| 32 | NaN | NaN | NaN | |

| | Matr 30h_1 | Matr 30h_2 | Matr 30h_3 |
|------|---------------|---------------|---------------|
| Туре | Expres | Expres | 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 |

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 | a Lotar, MF reach parts parts | a Mappin Protect Series, Protects | |
|--------------------|---|---|------------------|
| 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 | Matr 30h_1_x/y_0h_1 | t u d b |
| 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 | Matr 30h_1_x/y_0h_1 Matr 30h_2_x/y_0h_2 Matr 30h_3_x/y_0h_3 | t u d b |
| Caraal | D | | |
| Cancer | Desc | npuon | W OK |

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'.

| matrix1 matrix2 matrix4 matrix5 matrix6 matrix7 matrix8 matrix9 matrix10 matrix11 | | | | | | | 4 | |
|---|-----------|-----------------------|-----------|----------------|---------------------|------------------|---------------|---|
| | Points | | Dista 12 | | | | 4 | |
| | | Categories | Plots Lir | nes | | | 4 | |
| | | | | | | | | |
| 10 million and | _ | Symbol | Symbol | Protein IDs | Majority protein | Protein names | Gene names | |
| | 1 | | 2 | A0AVT1 | A0AVT1 | Ubiquitin | UBA6 | 6 |
| | 2 | | 2 | A0FGR8 | A0FGR8 | Extende | ESYT2 | 5 |
| | 3 | | 2 | A0JLT2; | A0JLT2; | Mediator | MED19 | 2 |
| | 4 | | 2 | A0JNW5 | A0JNW5 | UHRF1 | UHRF1B | 1 |
| | 5 | | 2 | A0MZ66 | A0MZ66 | Shootin-1 | KIAA1598 | 8 |
| | 6 | | 2 | A0PJW6 | A0PJW6 | Transme | TMEM223 | 3 |
| | 7 | | 2 | Q15652; | Q15652; | Probable | JMJD1C | 3 |
| | 8 | | 2 | A0T4C8 | A0T4C8 | Sphingo | SPHK2 | 6 |
| | 9 | | 2 | A1A4S6 | A1A4S6 | Rho GTP | ARHGAP | 3 |
| | 10 | | 2 | A1L020 | A1L020 | RNA-bin | MEX3A | 1 |
| | 11 | | 2 | A1L0T0; | A1L0T0 | Acetolac | ILVBL | 5 |
| | 12 | | 2 | A1L188 | A1L188 | Unchara | C17orf89 | 1 |
| | 13 | | 2 | A1X283; | A1X283 | SH3 and | SH3PXD | S |
| | 14 | | 2 | Q86X10 | Q86X10 | Ral GTP | RALGAP | 7 |
| | 15 | | 2 | A2A2G4 | A2A2G4 | Dolichyl p | ALG6 | 2 |
| | 16 | | 2 | A2A2Q9 | A2A2Q9 | Unchara | C20orf4 | 2 |
| | 47 | - | - | D40000 | Diacoc. | DALA SI- | DD1/24 | |
| | 6767 iter | ns | | | | | | |
| Mutt 300.1 Juy | No | labels ▼ 8 🕵 🔍 ↔ ‡ | Protein | IDs ▼U | oto Y 🔹 | | | |
| Matt 301, 2, 24, 201, 2 | 6- | | | | | | | |
| | 0 | | | | | | | |
| Matr 30h_1_x/y_0h_1 Matr 30h_2_x/y_0h_2 Matr 30h_3_x/y_0h_3 | -3 | -2 | 1 -1 | 0 | 1 | 2 | 3 | |

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

| matrix1 matrix2 matrix4 matrix5 matrix6 matrix7 matrix8 matrix9 matrix10 matrix1 | | | | | | | | • • • |
|--|--------------------|------------|--------------|--------------------|-------------------|--------------------|---------------|-------|
| Data Multi scatter plot | | | | | | | 4 |) Þ 🖸 |
| · · · · · · · · · · · · · · · · · · · | Points | Categories | Plots Li | nes | | | 4 | ▶ □ |
| <none></none> | | | | | | | | |
| Log2(Absence-presence enrichment factor) Absence-presence -Log10(p-value) | | Symbol | Symbol | Protein | Majority | Protein | Gene | F |
| Number of valid pairs | 1 | cólor | size 2 | IDs A0AVT1 | nrotein A0AVT1 | names Ubiquitin | names UBA6 | 6 |
| Valid pairs percentage | 2 | | 2 | A0FGR8 | A0FGR8 | Extende | ESYT2 | 5 |
| -Log10(Pearson p-value) | 3 | | 2 | A0JLT2: | A0JLT2: | Mediator | MED19 | 2 |
| -Log10(Pearson p-value) [correlation] | 4 | | 2 | A0JNW5 | A0JNW5 | UHRF1 | UHRF1B | 1 |
| -Logitu(Pearson p-value) [anti-correlation] R squared | 5 | | 2 | A0MZ66 | A0MZ66 | Shootin-1 | KIAA1598 | 8 |
| Spearman rank correlation | 6 | | 2 | A0PJW6 | A0PJW6 | Transme | TMEM223 | 3 |
| -Log10(Spearman p-value) -Log10(Spearman p-value) [correlation] | 7 | | 2 | Q15652; | Q15652; | Probable | JMJD1C | 3 |
| -Log10(Spearman p-value) [anti-correlation] | 8 | | 2 | A0T4C8 | A0T4C8 | Sphingo | SPHK2 | 6 |
| Kendall rank correlation | 9 | | 2 | A1A4S6 | A1A4S6 | Rho GTP | ARHGAP | 3 |
| Mutual information | 10 | | 2 | A1L020 | A1L020 | RNA-bin | MEX3A | 1 |
| Euclidean distance | 11 | | 2 | A1L0T0; | A1L0T0 | Acetolac | ILVBL | 5 |
| Manhattan distance Maximum distance | 12 | | 2 | A1L188 | A1L188 | Unchara | C17orf89 | 1 |
| | 13 | | 2 | A1X283; | A1X283 | SH3 and | SH3PXD | S |
| | 14 | | 2 | Q86X10 | Q86X10 | Ral GTP | RALGAP | 7 |
| | 15 | | 2 | A2A2G4 | A2A2G4 | Dolichyl p | ALG6 | 2 |
| | 16 | | 2 | A2A2Q9 | A2A2Q9 | Unchara | C20orf4 | 2 |
| · · · · · · · · · · · · · · · · · · · | 47 | - | - | D40000 | D 40000 | DATA NO. | DD1124 | 1 |
| | 6767 iter | ns | | | | | | |
| | No | labels 🔻 8 | Protein | IDs ▼ U | oto 🖞 🔹 | | | |
| | 1 22 | 0.0* | D 1 * | < → ¹ 2 | | 2 | | |
| Wate | | | - Leo - | 1 1 1 1 1 1 | 2 | | | |
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| | 0 - | | | | | | | |
| | | | | | | | | |
| | ~ | | | | | | | |
| | | | | | | | | |
| 0 0 Matr 30h_1_x/y_0h_1 Matr 30h_2_x/y_0h_2 Matr 30h_3_x/y_0h_3 | Ļ | 2 | | Ļ | | | | |
| | -3 | -2 | -1 | 0 | | 2 | | |

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



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



| Rename columns | |
|----------------------|----------------------|
| 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 |
| | |
| Cancel | Description - Output |

| | 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 |
|------|------------------|------------------|------------------|---------|-----------|----------------|----------------|----------------|----------------------------|------------------|---------------|----------|
| Туре | 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 |

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







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

| | Session3_only 0 vs | 30h_2 - Perse | us | _ | | | | | | | | |
|-----------|--------------------|------------------|------------------|-----------|---------------|----------------|----------------|---------------------------------|----------------------------|------------------|---------------|-----------|
| - | Matrix | | | | | | | | | | | |
| 🕇 🧐 | Basic 🔻 | Filter rows • | Annot. colu | mns • Imp | utation • | Clusteri | ng 🕶 🕴 | 😃 🍳 🔋 🗸 | isualization | · 井 昭 | E 🖂 🛺 | Basic 🔹 |
| 亘 🛃 | Rearrange • | Filter column | s • Annot. row | s∙ Moo | difications • | f(x) 🗹 ! | 💶 Z 🍸 : | \overline{x} P _N c | lustering/P | са 📲 🎊 🙎 | 0 😺 🔽 | E E |
| \$ | Normalization • | Quality 🔹 | Tests 🔹 | Prot | teomic ruler | • 📑 🖓 (| \simeq 1D 2D | P1 🛕 🛛 N | Aisc. 🔹 | - 🛃 G | | |
| Load | | | Proc | essing | | | | | | Analysis | | Multi-pro |
| matrix1 | matrix2 matrix4 | 4 matrix5 m | atrix6 matrix7 | matrix8 n | natrix9 | | | | | | | |
| Data H | listogram | | | | | | | | | | | |
| | 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 |
| Туре | 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 |

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

| <mark>8</mark> I 🗆 I | Session3_only 0 vs 30h_2 - Perse | us | | | | | | | | | - | | |
|----------------------|----------------------------------|--------------------------------|---------------------------|------------|------------------|-----------------|-----------|------------------|-------------|----------|------------------|------------------|------------------|
| - | Matrix | | | | | | | | | | | | |
| 1 🦦 | Basic • Filter rows • | Annot. columns • Imputation | Clust | tering • | 🐸 P ₂ | Visualization • | -井 闘: | 🔄 🚚 🛛 Basi | c• | | | | |
| EN | Rearrange • Filter columns | s • Annot. rows • Modificatio | ns 🔹 🕅 | 🛯 🖬 Z 🏹 | \bar{x} PN | Clustering/PC/ | A = 🎊 💁 🕯 | 0 | 8 | | | | |
| 廢 | Normalization • Quality • | Tests • Proteomic r | uler 🔹 🧱 将 | 🖗 🗠 1D 2D | P1 🛕 | Misc. • | - 📕 🤮 🕻 | | | | | | |
| Load | | Processing | | | | Ar | nalysis | N | /ulti-proc. | Export | | | |
| matrix1 | matrix2 matrix4 matrix5 ma | atrix6 matrix7 matrix8 matrix9 | | | | | | | | | | | |
| Data | Histogram | | | | | | | | | | | | |
| : 33 fr | rale 🖂 🗈 🖼 🕾 | | Points | Categories | | | | | | | | | |
| : ~ cn | | | | Calaati | | the s | | | | | | | |
| <i>"</i> | | | | Selecti | on from ta | able 🔹 📑 | | D | | | | | |
| nut | | | | Fill color | Border | Protein IDs | notein | Protein names | Gene | Proteins | Matr 30h/0h 1 | Matr 30h/0h_2 | Matr 30h/0h_3 |
| ŏ | | | 1 | | | A0AVT1 | A0AVT1 | Ubiquitin | UBA6 | 6 | 0.593930 | 0.219234 | 0.475309 |
| | | | 2 | | | A0FGR8 | A0FGR8 | Extende | ESYT2 | 5 | -0.50841 | -1.22637 | -0.51403 |
| | -5 0 5 | - | 3 | | | A0JLT2; | A0JLT2; | Mediator | MED19 | 2 | NaN | NaN | NaN |
| | Matr 30h/0h_1 | 2 | 4 | | | A0JNW5 | A0JNW5 | UHRF1 | UHRF1B | 1 | 0.817614 | NaN | NaN |
| | | | 5 | | | A0MZ66 | A0MZ66 | Shootin-1 | KIAA1598 | 8 | NaN | NaN | NaN |
| nts | | | 6 | | | A0PJW6 | A0PJW6 | Transme | TMEM223 | 3 | NaN | NaN | -0.45263 |
| Cou | | | 7 | | | Q15652; | Q15652; | Probable | JMJD1C | 3 | NaN | NaN | NaN |
| | | | 8 | | | A0T4C8 | A0T4C8 | Sphingo | SPHK2 | 6 | NaN | NaN | NaN |
| | - <u></u> | - | 9 | | | A1A4S6 | A1A4S6 | Rho GTP | ARHGAP | 3 | NaN | NaN | 0.446280 |
| | -5 0 5 Matr 30h/0h_2 | | 10 | | | A1L020 | A1L020 | RNA-bin | MEX3A | 1 | NaN | NaN | NaN |
| | | | 11 | | | A1L0T0; | A1L0T0 | Acetolac | ILVBL | 5 | -0.60101 | -0.31493 | -0.23719 |
| s | | | 12 | | | A1L188 | A1L188 | Unchara | C17orf89 | 1 | NaN | NaN | NaN |
| Ino | | | 13 | | | A1X283; | A1X283 | SH3 and | SH3PXD | 9 | 0.870519 | 0.668951 | 0.711167 |
| 0 | | | 14 | | | Q86X10 | Q86X10 | Ral GTP | RALGAP | 7 | -0.90830 | NaN | -0.33588 |
| L | | _ | 15 | | | A2A2G4 | A2A2G4 | Dolichyl p | ALG6 | 2 | NaN | NaN | -0.83752 |
| | -5 0 5 Matr 30b/0b 3 | | 16 | | | A2A2Q9 | A2A2Q9 | Unchara | C20orf4 | 2 | 0.268208 | NaN | 0.408490 |
| | wat son/on_s | | 17 | | | P42696; | P42696; | RNA-bin | RBM34 | 5 | NaN | NaN | NaN |
| | | | 18 | | | 014678 | 014678 | KN motif | KANK1 | 6 | NaN | NaN | NaN |

| P | | Session3_only U vs . | 30h_2 - Perseus | <u> </u> | - | | | - | | | | _ | | - | | |
|-----|--------|----------------------|----------------------|-------------|-------------------|------------|------------|------------------|---------------|-----------------------|--------------------|---------------|----------|----------------------|----------------------|----------------------|
| | • | Matrix | | | | | | | | | | | | | | |
| 1 | ۰ | Basic 🔹 | Filter rows * | Annot. colu | Imns • Imputation | • Clust | ering • | 🐸 P ₂ | Visualization | • <mark>+</mark> ‡ == | 🗄 🛄 🛛 Basi | ic • | | | | |
| 1 | | Rearrange • | Filter columns 🔹 | Annot. row | s • Modification | ns 🔹 🕅 🔀 | 🖞 🎫 Z 🏹 | \bar{x}^{p_N} | Clustering/PC | A + 🖄 🧐 I | 20 🖪 | -R | | | | |
| 1 | | Normalization • | Quality 🕶 | Tests 🔹 | Proteomic r | uler 🔹 🧱 🕅 | a 🗠 1D 2D | P ₁ | Misc. • | - 1 C . | * | | | | | |
| L | bad | | | Proc | essing | | | | Д | nalysis | 1 | Multi-proc. | Export | | | |
| m | atrix1 | matrix2 matrix4 | matrix5 matrix | 6 matrix7 | matrix8 matrix9 | | | | | | | | | | | |
| D | ata H | listogram | | | | | | | | | | | | | | |
| | r ln | cols 🖂 💷 🛃 🛣 | _ | | | Points | Categories | | | | | | | | | |
| ſ | Proper | ties | | | Duranting | | | | x | | | | | | | |
| 12 | Toper | | | | Properties | | | | | Majority | Protein | Gene | Proteins | Matr | Matr | Matr |
| 100 | | | | | | | | | | A0AVT1 | names Ubiquitin | Dames UBA6 | 6 | 30h/0h 1 0 593930 | 30h/0h 2 0 219234 | 30h/0h_3 0.475309 |
| Ŭ | | | | | Histogram wid | th 200 | | | ÷ | A0FGR8 | Extende | ESYT2 | 5 | -0.50841 | -1 22637 | -0.51403 |
| | | <u> </u> | <u> </u> | | I Barran Kata | -ht land | | | _ | A0JLT2: | Mediator | MED19 | 2 | NaN | NaN | NaN |
| | | -5 0 Matr 30h/0 | 5)h_1 | | Histogram neig | gnt 100 | | | | A0JNW5 | UHRF1 | UHRF1B | 1 | 0.817614 | NaN | NaN |
| | | | | | Min. value | -6.668 | 884132862 | 091 | | A0MZ66 | Shootin-1 | KIAA1598 | 8 | NaN | NaN | NaN |
| uts | | h | | | Manager | 7.050 | 00000001/ | 110 | | A0PJW6 | | | | r | NaN | -0.45263 |
| Cou | | | | | Max. value | 17.602 | 363803810 | 012 | | Q15652; | Cha | nge n | umbe | r of | NaN | NaN |
| | | | | | Number of bins | s 41 | | | ㅋ 🖊 | A0T4C8. | hinc | Eoro | vamn | lo to | NaN | NaN |
| | | | <u> </u> | | | , | | | | A1A4S6. | DI115. | 1016 | латтр | | NaN | 0.446280 |
| | | -5 0 Matr 30h/0 | b_2 | | | | | | | A1L020 | | 10 | 0. | | NaN | NaN |
| | | | | | Cancel | | | OK | | A1L0T0 | 1 | | ••• | | 0.31493 | -0.23719 |
| lts | | | | | | 1 | - | | | A1L188 | Unchara | C17orf89 | 1 | NaN | NaN | NaN |
| 100 | | | | 6 | | | | | | A1X283 | SH3 and | SH3PXD | 9 | 0.870519 | 0.668951 | 0.711167 |
| Ĩ | | | | | | 14 | | | Q86X10 | Q86X10 | Ral GTP | RALGAP | 7 | -0.90830 | NaN | -0.33588 |
| | | | • <u>_</u> | | | 15 | | | A2A2G4 | A2A2G4 | Dolichyl p | ALG6 | 2 | NaN | NaN | -0.83752 |
| | | -5 0 Matr 30h/0 | b h_3 | | | 16 | | | A2A2Q9 | A2A2Q9 | Unchara | C20orf4 | 2 | 0.268208 | NaN | 0.408490 |
| | | | | | | 17 | | | P42696; | P42696; | RNA-bin | RBM34 | 5 | NaN | NaN | NaN |

| <mark>2</mark> [_ |) Sessions_only U | /s 30n_2 - Perseus | | - | | | | | | | _ | | - | | |
|---------------------|---------------------|-------------------------------|-----------|---------------------|-----------------------------|---------------|-------------------|-----------------|-----------|------------|-------------|-------------|----------|----------|----------|
| - | Matrix | | | | | | | | | | | | | | |
| 1 | Basic • | Filter rows • | Annot. co | olumns • Imputation | Cluster | ing • | 🐸 P ₂ | Visualization • | - 井 嘂 : | 😳 🚚 📔 Basi | c • | | | | |
| E | Rearrange • | Filter columns 🔹 | Annot. ro | ws • Modificatio | ns 🔹 🕅 🗹 | 🖬 Z 🕅 | \overline{x} PN | Clustering/PC | A • 🎊 💁 🕻 | 0 | 8 | | | | |
| 暾 | Normalization | Quality - | Tests • | Proteomic | ruler 🔹 🧱 😽 | \cong 1D 2D | P1 🛕 | Misc. • | - 📕 🗲 🕻 | * | | | | | |
| Load | | | Pr | ocessing | | | | A | nalysis | | Aulti-proc. | Export | | | |
| matrix | matrix2 matrix | x4 matrix5 matrix | x6 matrix | 7 matrix8 matrix9 | | | | | | | | | | | |
| Data | Histogram | | | | | | | | | | | | | | |
| : 🙊 : | En rok 🖂 🖾 🛃 🚏 | | | | Points Ca | tegories | | | | | | | | | |
| | | - | _ | | : | Select | ion from t | able 🔹 | | | | | | | |
| ş | | | | | | Fill color | Border | Protein | Majority | Protein | Gene | Proteins | Matr | Matr | Matr |
| uno | n n n | | | | | | color | IDs T | nrotein | names | names | 1 Totelling | 30h/0h 1 | 30h/0h 2 | 30h/0h 3 |
| 0 | , i i i i | 1 | | | 1 | _ | | AUAV11 | AUAV11 | Ubiquitin | UBA6 | 0 | 0.593930 | 0.219234 | 0.475309 |
| | | lun | | | 2 | _ | | A0FGR8 | A0FGR8 | Extende | ESYT2 | 5 | -0.50841 | -1.22637 | -0.51403 |
| | -5 0 | 5 | | | 3 | | | A0JLT2; | A0JLT2; | Mediator | MED19 | 2 | NaN | NaN | NaN |
| | Matr 30ł | n/0h_1 | | | 4 | | | A0JNW5 | A0JNW5 | UHRF1 | UHRF1B | 1 | 0.817614 | NaN | NaN |
| | | | | | - | ۳ ۲ | | A0MZ66 | A0MZ66 | Shootin-1 | KIAA1598 | 8 | NaN | NaN | NaN |
| ints | L | | | Proteins a | re now | | | A0PJW6 | A0PJW6 | Transme | TMEM223 | 3 | NaN | NaN | -0.45263 |
| Col | , i i i i | | | separated | into | | | Q15652; | Q15652; | Probable | JMJD1C | 3 | NaN | NaN | NaN |
| | | 1 | | 100 him | inte | | | A0T4C8 | A0T4C8 | Sphingo | SPHK2 | 6 | NaN | NaN | NaN |
| | | 100 mm | | 100 bins | | | | A1A4S6 | A1A4S6 | Rho GTP | ARHGAP | 3 | NaN | NaN | 0.446280 |
| | -5 Matr 30ł | n/0h_2 | | | 10 | | | A1L020 | A1L020 | RNA-bin | МЕХЗА | 1 | NaN | NaN | NaN |
| | dda | | | | 11 | | | A1L0T0; | A1L0T0 | Acetolac | ILVBL | 5 | -0.60101 | -0.31493 | -0.23719 |
| Its | | | | | 12 | | | A1L188 | A1L188 | Unchara | C17orf89 | 1 | NaN | NaN | NaN |
| our | | L | | | 13 | | | A1X283; | A1X283 | SH3 and | SH3PXD | 9 | 0.870519 | 0.668951 | 0.711167 |
| Ŭ | , | | | | 14 | | | Q86X10 | Q86X10 | Ral GTP | RALGAP | 7 | -0.90830 | NaN | -0.33588 |
| | | lillion | | | 15 | | | A2A2G4 | A2A2G4 | Dolichyl p | ALG6 | 2 | NaN | NaN | -0.83752 |
| | -5 0 Matr 304 | 1/0h 3 | | | 16 | | | A2A2Q9 | A2A2Q9 | Unchara | C20orf4 | 2 | 0.268208 | NaN | 0.408490 |
| | 11120 001 | | | | 47 | | | D40000 | D40000 | DALA NO. | DDM24 | - | NI-NI | NI-NI | N1-N1 |

| <mark>2</mark> C | Sess | ion3_only 0 vs 30h_2 | - Perseus | (| | | _ | | | | | | - | | | | |
|--------------------|----------|--|--------------|-------------------------|---------|----------------------------|------------|----------------------------------|-----------------|-------------|---------------|-------------|-------------|--------|----------------------|----------------------|----------------------|
| | м | atrix | | | | | | | | | | | | | | | |
| - 🐮 🕸 | Ba | sic • Filter | rows * | Annot. columns • Imputa | ation | Cluste | ring • 🚦 | 🗴 p ₂ | Visualization • | ÷ | ₩ ≥ 嘂 # | Basic | • | | | | |
| E | Re | arrange 🔹 🛛 Filter | columns • | Annot. rows • Modifi | icatior | ns 🔹 🕅 🗹 | 💶 Z 💎 : | $\overline{\chi}$ p _N | Clustering/PC/ | ۵ - ۵ | x 💊 😻 7 | | | | | | |
| ® | No | ormalization 🔹 Quali | ity • | Tests • Protec | omic r | uler 🔹 📰 🥳 | 🗠 1D 2D I | P ₁ | Misc. • | 1 | l 🧲 🖉 👘 | | | | | | |
| Load | | | | Processing | | | | | Ar | nalysis | | Mu | ılti-proc. | Export | | | |
| matri | x1 ma | trix2 matrix4 mat | rix5 matrix6 | 5 matrix7 matrix8 mat | trix9 | | | | | | | | | | | | |
| Data | Histo | gram | | | | | | | | | | | | | | | |
| 1 20 | ln rok I | - 11 🔽 📆 📜 | | | | Points C | ategories | | | | | | | | | | |
| | | | | | | | Selectio | on from ta | able 🔹 | | | | | | | | |
| ts | | | | | | 7 | Fill color | Border | Protein | Maio | Color | | | × | Matr | Matr | Matr |
| Cour | | 1 | | | | | 5 | color | IDs A0AVT1 | ford A0A | Basic colors: | : | | | 30h/0h 1 0.593930 | 30h/0h 2 0 219234 | 30h/0h_3 0.475309 |
| Ŭ | | ALC: N | | | | 2 | | | A0FGR8 | AOF | | | | | -0.50841 | -1.22637 | -0.51403 |
| | Ļ | | Mor | eover one | | 3 | | | A0JLT2: | A0JI | | | | | NaN | NaN | NaN |
| | -5 | Matr 30h/0h_1 | | d alaa aalaat | | 4 | | | A0JNW5 | A0J | | | | 1 | 0.817614 | NaN | NaN |
| | | .lt | cour | d also select | | 5 | | | A0MZ66 | A0M | | | | | NaN | NaN | NaN |
| nts | | | prot | ein groups | | 6 | | | A0PJW6 | A0P | | | | | NaN | NaN | -0.45263 |
| Cou | | , 1 | and | change their | | 7 | | | Q15652; | Q15 | | | | | NaN | NaN | NaN |
| | | a de la companya de la | colo | r in the | | 8 | | | A0T4C8 | AOT | Custom color | rs: | | | NaN | NaN | NaN |
| | -5 | | | i ili tile | | 9 | | | A1A4S6 | A1A | | | | | NaN | NaN | 0.446280 |
| | | Matr 30h/0h_2 | histo | ogram. | | 10 | | | A1L020 | A1L | | | | | NaN | NaN | NaN |
| | | .titu | | | - | 11 | | | A1L0T0; | A1L | | | | | -0.60101 | -0.31493 | -0.23719 |
| uts | | | | | | 12 | | | A1L188 | A1L | Det | fine Custor | m Colors >> | | NaN | NaN | NaN |
| Cou | | | | | | 13 | | | A1X283; | A1X | ОК | Can | cel | | 0.870519 | 0.668951 | 0.711167 |
| | | | | | | 14 | | | Q86X10 | Q86 | | | | | -0.90830 | NaN | -0.33588 |
| | -5 | 0 | 5 | | | 15 | | | A2A2G4 | A2A | 2G4 Dolic | chyl p A | ALG6 | 2 | NaN | NaN | -0.83752 |
| | | Matr 30h/0h_3 | ~ | | | 16 | | | A2A2Q9 | A2A | 2Q9 Unch | nara C | C20orf4 | 2 | 0.268208 | NaN | 0.408490 |
| | | | | | | 17 | | | P42696; | P426 | 596; RNA- | -bín F | RBM34 | 5 | NaN | NaN | NaN |

| 210 | Sessions_only UVs | 30n_2 - Perseus | | | | | | | | | _ | | - | | |
|-----------|---|---|------------------|-----------------|----------|----------------|------------|---------------|-----------------------|---------------|----------------------|--------------|----------|----------|----------|
| • | Matrix | | | | | | | | | | | | | | |
| 1 | Basic 🔹 | Filter rows • | Annot. columns • | Imputation • | Clusteri | ng • | 🐸 P2 | Visualization | - <mark>+</mark> == : | 🔄 🚚 📔 Basi | ic • | | | | |
| 三国 | Rearrange • | Filter columns 🔹 | Annot. rows • | Modifications • | f(x) 🗹 🛛 | 💶 Z 🏹 | $ar{x}$ PN | Clustering/PC | A • 🔣 💁 🛔 | 20 🖪 | -R | | | | |
| 1 | Normalization • | Quality 🕶 | Tests 🔹 | Proteomic ruler | • 📑 🤤 (| \simeq 1D 2D | P1 🛕 | Misc. • | - 📕 🤤 🕻 | * | | | | | |
| Load | | | Processing | 3 | | | | A | nalysis | 1 | Multi-proc. | Export | | | |
| matrix | 1 matrix2 matrix4 | 4 matrix5 matrix | 6 matrix7 matr | ix8 matrix9 | | | | | | | | | | | |
| Data | Histogram | | | | | | | | | | | | | | |
| : 🗴 (| n rok 🖂 🖾 🛐 🛣 | | | Po | ints Cat | tegories | | | | | | | | | |
| | 4 | | | | | Selecti | on from t | able 🔻 | | | | | | | |
| <u>so</u> | , lit, ^{Lit} | Export Image | | | | Fill color | Border | Protein | Majority | Protein | Gene | Proteins | Matr | Matr | Matr |
| onu | dia. | ·\\ | | | | | color | IDs | nrotein | names | names | r roteinis | 30h/0h 1 | 30h/0h 2 | 30h/0h 3 |
| 0 | 1 Martin | | | $\frac{1}{2}$ | | | | AUAVT1 | AUAVT1 | Obiquitin | UBA6 | 0 | 0.593930 | 0.219234 | 0.475309 |
| | | m | | 2 | | | | AUFGR8 | AUFGR8 | Extende | ESTIZ | 5 | -0.50841 | -1.22037 | -0.51403 |
| | -5 0 Matr 30b/ | 5 0h 1 | \mathbf{i} | 3 | | | F | ile name: | Histogram | n.png | | | | NaN | NaN |
| | Maa oon | 011_1 | | 4 | _ | 5 | C | I | | LL Net | di Combi | | | NaN | NaN |
| ts. | , , (| | The | isto grano | | 1 | Sav | e as type: | PING Porta | ble Netwo | rk Graphi | cs (.png) | | NaN | -0.45263 |
| no | | | Iner | nstogram | | | | | PNG Porta | ble Netwo | rk Graphi | cs (.png) | | NaN | NaN |
| 0 | , | | can b | e exporte | d 🛉 | | Hide Fo | Iders | CIE Granhi | ne Docum | ent Form | at (.pdf) | | NaN | NaN |
| [| | lin m | to dif | ferent file | | | | indicity. | IPG IPFG (| ing: iif: ing | niger om er inea) | nac (.gir) | | NaN | 0.446280 |
| | -5 0 Matr 30h/ | 5 0h 2 | form | atc | - | <u> </u> | | | TIF Tagged | I Image Fil | e Format | (.tif:.tiff) | | NaN | NaN |
| | dd. | | TOTT | ats. | | | | | WMF Wind | lows Meta | File (.wm | nf) | | 0.31493 | -0.23719 |
| ts. | | | | 12 | 2 | | | | BMP Wind | ows Bitma | p (.bmp) | | | NaN | NaN |
| uno | | | | 1: | 3 | | | | EMF Wind | ows Enhar | iced Meta | a File (.emf |) | 0.668951 | 0.711167 |
| 0 | , Januari, J | | | 14 | 4 | | | Q86X10 | Q86X10 | Ral GTP | RALGAP | 7 | -0.90830 | NaN | -0.33588 |
| L | | http://www.com/com/com/com/com/com/com/com/com/com/ | | 1: | 5 | | | A2A2G4 | A2A2G4 | Dolichyl p | ALG6 | 2 | NaN | NaN | -0.83752 |
| | -5 0 Matr 30h/ | 5 0h 3 | | 10 | 5 | | | A2A2Q9 | A2A2Q9 | Unchara | C20orf4 | 2 | 0.268208 | NaN | 0.408490 |
| | | | | 4 | , | | | D40000 | D40000 | DATA NO. | DDM04 | - | NI-81 | NI-NI | NI-NI |

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.

| 1 0 1 36 | essions_only 0 vs | 30n_2 - Perse | eus | - | | | _ | | | | | |
|------------|---|--------------------------------|--|-------------------------------|---|--------------------|----------------------------|---|--|------------------------|---------------|------|
| • | Matrix | | | | | | | | | | | |
| ★ 🧐 亘 🔝 | Basic • Rearrange • Normalization • | Filter rows • Filter column | Annot. colu is • Annot. row: Tests • | ımns ▼ Imp s ▼ Moc Prot | utation • difications • eomic ruler | Clusteri f(x) 🗹 | ng • • Z 🐨 : ∝ 10 20 | $\overline{\mathbf{x}}$ \mathbf{p}_2 V $\overline{\mathbf{x}}$ $\mathbf{p}_{\mathbf{N}}$ C | 'isualization lustering/P ∕lisc. ▼ | • + # 日 CA • 公 • CA | | Basi |
| Load | Normalization | Quanty | Proc | essing | conne ruier | 894 °V | 10 20 | | | Analysis | - | |
| LOad | | | PIOC | essing m | natrix9 | | | | | Analysis | | |
| Data US | matrix2 matrix4 | matrixo m | latrixo matrix/ | matrixe | | | | | | | | |
| HIS | stogram | 0 | 0 | | (| 1 | L | 1 | | 1 | | |
| | 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 | Pro |
| Туре | Expression | Expression | Expression | Numeric | Numeric | Numeric | Numeric | Text | Text | Text | Text | Tex |
| 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 |

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



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 |
|------------|--------------|--------------|--------------|-----------------------|-----------------------|-----------------------|---------|-----------|----------------|----------------|
| Туре | 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 |
| 6767.1 | | | | | | | | | | |
| oror items | | | | | | | | | | |

| Type Expres Expres Expres Numeric Numeric <t< th=""><th></th><th>Matr 30h/0h</th><th>Matr 30h/0h</th><th>Matr 30h/0h</th><th>PEP</th><th>Intensity</th><th>Intensity L</th><th>Intensity H</th></t<> | | Matr 30h/0h | Matr 30h/0h | Matr 30h/0h | PEP | Intensity | Intensity L | Intensity H |
|--|-------------|----------------|----------------|----------------|---------|-----------|----------------|----------------|
| 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 -0.342 2.4205 355890 276620 792720 10 0.2301 0.4883 0 675290 524240 151050 12 -1.99157 -2.42629 -1.55667 | Туре | Expres | Expres | Expres | Numeric | Numeric | Numeric | Numeric |
| 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 -0.342 2.4205 355890 276620 792720 10 0.2301 0.4208 0.8483 0 675290 524240 1515050 12 -1.99157 -2.42629 -1.55667 4.4294 805000 452920 352080 13 -1.58235 <td>1</td> <td>0.5939</td> <td>0.2192</td> <td>0.47531</td> <td>0</td> <td>953960</td> <td>699960</td> <td>254000</td> | 1 | 0.5939 | 0.2192 | 0.47531 | 0 | 953960 | 699960 | 254000 |
| 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 0.727 -0.420 -0.8483 0 675290 524240 151050 12 -1.99157 -2.42629 -1.55667 4.4294 805000 452920 352080 13 -1.58235 | 2 | -0.508 | -1.22637 | -0.514 | 0 | 257690 | 177260 | 804350 |
| 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 < | 3 | -0.601 | -0.314 | -0.237 | 0 | 327420 | 267380 | 600400 |
| 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 12 -1.99157 -2.42629 -1.55667 4.4294 805000 452920 352080 13 -1.58235 -0.75516 -0.458 1.44955 565120 445340 119770 14 0.0174 0.5715 1.44353 1.0956 164890 380810 886330 15 <th< td=""><td>4</td><td>0.87052</td><td>0.6689</td><td>0.7111</td><td>0</td><td>179760</td><td>153430</td><td>263300</td></th<> | 4 | 0.87052 | 0.6689 | 0.7111 | 0 | 179760 | 153430 | 263300 |
| 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 151500 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 16489440 380810 886330 15 - | 5 | 0.1140 | -0.16255 | -0.397 | 0 | 132740 | 110890 | 218430 |
| 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 1.44953 1.0956 164890 119330 455590 14 0.0174 0.5715 1.44353 1.0956 164890 380810 886330 15 -0.938 -0.699 -0.778 0 546080 504820 412560 | 6 | -0.546 | -0.031 | -0.117 | 0 | 108150 | 807580 | 273950 |
| 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 15150 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 86330 16 0.5807 0.0633 0.2207 0 546080 442560 17 -0.502 -0 | 7 | -0.803 | -0.316 | -0.868 | 0 | 126030 | 967480 | 292790 |
| 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 546080 412560 17 -0.502 -0.599 -0.510 0 546080 445760 612280. 20 0.4105 0.0788 | 8 | 0.3684 | 0.2924 | -0.356 | 2.2871 | 227520 | 188520 | 390020 |
| 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 20 0.4105 | 9 | -2.34255 | -0.726 | -0.342 | 2.4205 | 355890 | 276620 | 792720 |
| 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 | 10 | 0.2301 | 0.0898 | 0.8546 | 0 | 133150 | 112270 | 208800 |
| 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 | 11 | -0.727 | -0.420 | -0.8483 | 0 | 675290 | 524240 | 151050 |
| 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 </td <td>12</td> <td>-1.99157</td> <td>-2.42629</td> <td>-1.55667</td> <td>4.4294</td> <td>805000</td> <td>452920</td> <td>352080</td> | 12 | -1.99157 | -2.42629 | -1.55667 | 4.4294 | 805000 | 452920 | 352080 |
| 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 | 13 | -1.58235 | -0.75516 | -0.458 | 1.4495 | 565120 | 445340 | 119770 |
| 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 | 14 | 0.0174 | 0.5715 | 1.44353 | 1.0956 | 164890 | 119330 | 455590 |
| 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 275290 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 -0.454 | 15 | -0.938 | -0.699 | -0.778 | 0 | 469440 | 380810 | 886330 |
| 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 275240 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 -0.57 -0.302 0 958070 775620 182440 26 0.7390 <td>16</td> <td>0.5807</td> <td>0.0633</td> <td>0.2607</td> <td>0</td> <td>129930</td> <td>959180</td> <td>340170</td> | 16 | 0.5807 | 0.0633 | 0.2607 | 0 | 129930 | 959180 | 340170 |
| 18 0.8317 0.22375 -0.665 3.7088 223750 180350 433970. 19 -0.397 -0.078 -0.131 0 275240 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 -0.577 -0.302 0 958070 775620 182440 26 0.7390 0.7589 1.08317 2.0943 173430 134560 388700 27 -0.366 | 17 | -0.502 | -0.599 | -0.510 | 0 | 546080 | 504820 | 412560 |
| 19 -0.397 -0.078 -0.131 0 27529b 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 1.3397 471640 397500 741420. 28 -0.073< | 18 | 0.8317 | 0.22375 | -0.665 | 3.7088 | 223750 | 180350 | 433970 |
| 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 0.0286 -0.230 1.2656 38850 302620 862340 30 | 19 | -0.397 | -0.078 | -0.131 | 0 | 275230 | 223270 | 519630 |
| 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 0.28 | 20 | 0.4105 | 0.0889 | 0.2843 | 8.4587 | 506980 | 445760 | 612280 |
| 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 | 21 | -0.996 | 0.3517 | 0.3372 | 0 | 295790 | 244110 | 516780 |
| 23 0.1964 0.2607 0.2628 0 877260 727830 149430. 24 -0.546 -0.022 0.5366 3.2658 315310 23010 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 4060910 31 -1.23813 0.2685 0.5002 0 100420 716160 <td>22</td> <td>0.3779</td> <td>0.4045</td> <td>0.9109</td> <td>0</td> <td>500840</td> <td>352390</td> <td>148460</td> | 22 | 0.3779 | 0.4045 | 0.9109 | 0 | 500840 | 352390 | 148460 |
| 24 -0.546 -0.022 0.5366 3.2658 315310 23010 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. | 23 | 0.1964 | 0.2607 | 0.2628 | 0 | 877260 | 727830 | 149430 |
| 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 | 24 | -0.546 | -0.022 | 0.5366 | 3.2658 | 315310 | 230010 | 853000 |
| 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 | 25 | -0.45406 | -0.057 | -0.302 | 0 | 958070 | 775620 | 182440 |
| 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 | 26 | 0.7390 | 0.7589 | 1.08317 | 2.0943 | 173430 | 134560 | 388700 |
| 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 | 27 | -0.366 | 0.3121 | 0.3022 | 0 | 130930 | 104180 | 267440 |
| 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 | 28 | -0.073 | 0.0226 | -0.032 | 1.3397 | 471640 | 397500 | 741420 |
| 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. | 29 | -0.412 | 0.0687 | -0.230 | 1.2656 | 388850 | 302620 | 862340 |
| 31 -1.23813 0.2685 -1.08819 3.447E 329300 263210 660910. 32 0.3256 0.2637 0.5002 0 100420 716160 288080. | 30 | 0.2833 | 0.5188 | -1.56155 | 3.5986 | 146440 | 112390 | 340480 |
| 32 0.3256 0.2637 0.5002 0 100420 716160 288080. | 31 | -1.23813 | 0.2685 | -1.08819 | 3.447E | 329300 | 263210 | 660910 |
| | 32 | 0.3256 | 0.2637 | 0.5002 | 0 | 100420 | 716160 | 288080 |
| Jibbil stores | 2650 itorra | | _ | _ | | _ | | |

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.

| Matrix | | | | | |
|-------------------------|----------------------|-----------------------------|---------------------|---|-----------------|
| Basic 🔹 | Filter rows 🕶 | Annot. columns • | Imputation • | Clustering • 🛛 🖉 🗛 | Visualization |
| Rearrange 🔹 | Filter columns 🔹 | Annot. rows • | Modifications • | tee 🗹 💶 Z 🐨 🗴 PN | Clustering/PC |
| Normalization \bullet | Quality • | Tests • | Proteomic ruler • | 📲 😽 🗠 1D 2D 🎦 🛕 | Misc. 🔹 |
| | | p ₁ One-sample | tests 🛱 | | A |
| matrix2 matrix4 | matrix5 matrix | p ₂ Two-sample | s One-sample tes | sts | |
| | | p _N Multiple-sar | " D 0 | ne sample-test for determin | ing if the |
| Matr Mat 30h/0h 30h | r Matr /0h 30h/0h | Two-way AN | " P ₁ " | ean is significantly different alue (typically 0). | from a fixed Ge |
| Expres Exp | res Expres | Numeric Numer | ic mannene ma | | Te |

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.



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

| <mark>8</mark> 🗋 . | bession3_only | 0 vs 30h_2 | - Perseus | <i>.</i> | - | | | | - | | | | | - | | - |
|------------------------|---------------|------------|-------------|-----------|-------------|--------------|------------|-----------|----------------------|-----------|----------|----------|----------------|------------------|--------|----------|
| • | Matrix | | | | | | | | | | | | | | | |
| 1 🧐 | Basic 🔹 | Filter | rows • | Annot. co | olumns 🔹 In | nputation • | Clust | ering • | 2 P2 | Visualiza | ation • | 사고 꽤 타 | 📙 🛛 Basic | • | | |
| 三日 | Rearrange 🔹 | Filter | columns • | Annot. ro | ws∙ N | lodification | s 🔹 🕅 🖂 | 🕯 🎫 Z 🔊 | \bar{x}_{P_N} | Clusteri | ng/PCA 🔹 | A 💁 👪 🛛 | 7 🖪 🖬 | 8 | | |
| 1 | Normalizatio | n 🔹 Quali | ty • | Tests • | P | roteomic ru | iler 🔹 🧱 将 | 🕴 🗠 1D 21 | D 🖭 🛕 👘 | Misc. 🔹 | | 📕 🖆 🕙 | | | | |
| Load | | | | Pr | ocessing | | | | | | Analys | sis | м | ulti-proc. | Export | |
| matrix1 | matrix2 ma | trix4 mat | rix5 matrix | x6 matrix | 7 matrix8 | matrix9 | matrix10 | matrix11 | | | | | | | | |
| Data | | N | | | | | | | | | | | | | | |
| | Matr | Matr | Matr | t-test | PEP | Intensity | Intensity | Intensity | -Log t-test | t-test | | Protein | Majority | Protein | Gene | Proteins |
| | 30h/0h | 30h/0h | 30h/0h | Signific | | | LÍ | ΗÍ | p-value | Diffe | rence | IDs | protein IDs | names | names | |
| Туре | Expres | Expres | Expres | Catego | Numeric | Numeric | Numeric | Numeric | Numeric | Num | eric | Text | Text | Text | Text | Text |
| 1 | 0.5939 | 0.2192 | 0.47531 | | 0 | 953960 | 699960 | 254000 | 1.21943 | 0.429 | 9492 | A0AVT | A0AVT | Ubiqui | UBA6 | 6 |
| 2 | -0.508 | -1.22637 | -0.514. | | 0 | 257690 | 177260 | 804350 | 1.05556 | -0.74 | 9608 | A0FGR | A0FGR | Extend | ESYT2 | 5 |
| 3 | -0.601 | -0.314 | -0.237 | | 0 | 327420 | 267380 | 600400 | 1.13214 | -0.38 | 4382 | Nlune | | bla | ILVBL | 5 |
| 4 | 0.87052 | | | | 0 | 179760 | 153430 | | | | 213 | NUIT | ierical | an | SH3PX | 9 |
| 5 | 0.1140 | Ca | tegoric | al | 0 | 132740 | 110890 | Nι | imerical | | 565 | column | with th | ne 🚛 | ADD1 | 14 |
| 6 | -0.546 | colur | nn mar | king | 0 | 108150 | 807580 | colun | nn with ⁻ | the | 662 | t-test d | ifferen | ce _{er} | EPS15 | 3 |
| 7 | -0.803 | all si | ignifica | ntly 🛛 | 0 | 126030 | 967480 | _ | -log10- | | 793 | = ave | rage of | ob | NBAS | 4 |
| 8 | 0.3684 | chan | ged pro | otein | 2.2871 | 227520 | 188520 | trans | formed | p- | 452 | expr | ession | b | GTPBP | 8 |
| 9 | -2.34255 | gro | une at i | n – | 2.4205 | 355890 | 276620 | | | I. | 3 | 2/P | | sm | TMEM | 3 |
| 10 | 0.2301 | | | р – | 0 | 133150 | 112270 | | value | | .563 | va | lues | 4 | CNOT1 | 6 |
| 11 | -0.727 | 0.0 | 5 With a | a + | 0 | 675290 | 524240 | 151050 | 1.45999 | -0.66 | 5394 | A5YVE | A5YVE | Pyruva | PDHA1 | 10 |
| 12 | -1.99157 | -2.42629 | -1.55667 | + | 4.4294 | 805000 | 452920 | 352080 | 1.80909 | -1.99 | 151 | A6NC4 | A6NC4 | ADP-ri | BST1 | 5 |
| 13 | -1.58235 | -0.75516 | -0.458 | | 1.4495 | 565120 | 445340 | 119770 | 0.96181 | -0.93 | 2107 | Q9BX7 | Q9BX7 | TM2 d | TM2D1 | 2 |
| 14 | 0.0174 | 0.5715 | 1.44353 | | 1.0956 | 164890 | 119330 | 455590 | 0.612201 | 0.677 | 7487 | A6NCE | A6NCE | Microtu | MAP1L | 2 |

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



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



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

| <mark>5</mark> 🗍 : | Session3_only | 0 vs 30h_2 | - Perseus | _ | _ | _ | | | _ | | _ | | - | | | | |
|-----------------------|----------------|----------------|----------------|--------------------|-------------|--------------|------------|-----------|-------------------|----------------|---------------------------|-------------------|----------------|----------------------------|------------------|---------------|----------|
| Matrix | | | | | | | | | | | | | | | | | |
| 1 | Basic 🔹 | Filter | rows * | Annot. co | olumns • Ii | mputation • | Clust | ering • | 避 p ₂ | Visualizati | ion • + | 器 🖂 💵 | Basic 🕶 | | | | |
| EN | Rearrange 🔹 | Filter | columns • | Annot. ro | ows ▼ _ N | Iodification | - 🗶 🗵 | 1 🖬 Z 🔊 | \overline{x} PN | Clustering | /PCA 🔹 🎊 | S 😺 7 | | | | | |
| 廢 | Normalizatio | n • Quali | ity • | Tests 🔹 | P | roteomic ru | iler / 🕎 将 | 🕴 🏹 1D 2 | D 🖭 🛕 💧 | Misc. 🔹 | - Ja | 년 🕙 | | | | | |
| Load | | | | Pr | ocessing | | / | | | | Analysis | | Mult | ti-proc. | Export | | |
| matrix1 | matrix2 ma | trix4 mat | rix5 matri | x6 matrix | 7 matrix8 | matrix9 | matrix10 | matrix11 | matrix12 | matrix13 | matrix14 | | | | | | |
| Data | | | | | | | | | | | | | | | | | |
| | 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 |
| Туре | 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 | Disca | 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 | Кеер | 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 | Кеер | 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 | 01473 | 01473 | 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 |
Next we rename the **two added categorical columns** with a meaningful name.

| Ma | atrix | | | |
|-----|----------------|----------------------------|-------------------|---------|
| Bas | ic • | Filter rows • | Annot. colum | ns 🕶 Im |
| Rea | arrange 🔹 | Filter columns 🔹 | Annot. rows • | Mo |
| | Change colu | mn type 🧰 | | Pro |
| | Rename colu | imns 🙀 | | ng |
| | Rename colu | Renarive column | 15 | itriv8 |
| | Combine an | ⁿ New names car | n be specified fo | or 📄 |
| | Duplicate co | each expression | n column. The n | ew |
| | Reorder/rem | iove columns | a in explicitly. | go |
| | Remove em | oty columns 🙃 | | ard |
| | Т | · · · · · · · · · · · · | | ard |
| | Transpose | 8 | | ard |
| | Sort by colu | mn 🗛 | | ard |
| | Fill categoric | al columns 👖 | | ard |
| | De-hyphena | te ids 👩 | | ard |
| | Expand mult | i-numeric and text | columns 👩 | ard |
| | | _ | | ard |
| | Unique value | es 🔛 | | ard |
| | Convert mul | ti-numeric column | A | ard |
| | Combine cat | egorical columns | 9 | ard |
| | Process text | column 🙀 | | ard |
| | Search text of | olumn 👝 | | ard |
| T | 0.9380.0 | 099U.//8 | + UIS | card |

| 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 |
| | |

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

| | Session3_only U vs 3Uh_2 - Perseus | | | | | | | | | | | | | | | | |
|---------|---|------------|------------|------------------|------------|-----------------|-----------|----------------------------|--------------|------------|----------|----------|--------|--------|----------|---------|------|
| • | Matrix | | | | | | | | | | | | | | | | |
| 1 🐐 | Basic 🔹 | Filter | rows * | Annot. columns • | Imputatio | n • Clusterir | ng • 🛛 | 😃 📴 🔰 V | isualization | - 井 | E 🗠 🚛 👔 | Basic 🔹 | | - | | | |
| 亘 🛃 | Rearrange • | Filter | columns • | Annot. rows 🔹 | Modificat | ions 🔹 🚺 🗹 🗖 | 🛛 Z 🝸 : | \bar{x} P _N ⊂ | lustering/P(| CA 🔹 🎊 🔦 | 0 😃 🔽 | FR FR | | | | | |
| 1 | Normalizatio | on 🔹 Qual | ity • | Tests 🔹 | Proteomi | : ruler 🔹 🕎 🤯 S | × 1D 2D | Р1 🛕 🛛 М | lisc. 🕶 | 🚽 🛃 🕻 | W | | | | | | |
| Load | | | | Processing | , | | | | A | Analysis | | Multi-p | roc. E | xport | | | |
| matrix1 | matrix2 ma | atrix4 mat | rix5 matri | x6 matrix7 matr | ix8 matrix | 9 matrix10 m | atrix11 m | atrix12 m | atrix13 m | atrix14 ma | trix15 | | | | | | |
| Data | | | | | | | | | | | | | | | | | |
| | Matr Matr Matr Significant ratio >=1 ratio <=-1 | | | | | | | | | | | | | | | | |
| Туре | 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 | 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 | Кеер | 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 | Кеер | 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 | 01473 | 01473 | 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 |

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

| | Matrix | | | | | | | |
|---|-------------|---|--|-------------------------------|-----------|--|--|--|
| E | Basic 🕶 | Filter rows • | Annot, columns | Imputatio | n • | | | |
| F | Rearrange • | Filter columns | Annot. rows • | Modificat | ions • | | | |
| ľ | Change co | lumn type 👩 | | Proteomic | : ruler 🕶 | | | |
| | Rename co | lumns a | | ng | | | | |
| | | | | trix8 matrix | 9 matr | | | |
| 1 | Rename co | olumns [reg. ex.] 🙀 | | | | | | |
| - | Combine a | nnotations 😭 | | ratio >=1 | ratio <: | | | |
| | Duplicate (| olumns 🙀 | | 1000 - 1 | Tauto 44 | | | |
| | Reorder/re | move columns 👩 | | Catego | Catego | | | |
| | Pomovoor | matu columna 🗖 | | Discard | Discar | | | |
| | Remove er | npty columns 🙀 | | Discard | Discar | | | |
| | Transpose | ផ | | Discard | Discar | | | |
| | Sort by col | umn 🔐 | | Discard | Discar | | | |
| | Fill catego | rical columns 🙃 | | Discard | Discar | | | |
| | De-hypher | ate ids 👩 | | Discard | Discar | | | |
| | e injplier | | | Discard | Discar | | | |
| | Expand mu | ilti-numeric and te | xt columns 🙀 | Discard | Discar | | | |
| | Unique val | ues 🛱 | | Discard | Кеер | | | |
| | Convert m | ulti-numeric colum | in 😭 | Discard | Discar | | | |
| ſ | Combine o | ategorical columns | . 0 | Discard | Discar | | | |
| | Deserves | | 2 | Discard | Keep | | | |
| | Process te | a column 😭 | Combine categor | ical columns | ar | | | |
| | Search tex | Search text column 😭 Combine the terms in two | | | | | | |
| | -0.9381 | 0.0990.//8 | categorical colun categorical colun | ins to form a in. | new ar | | | |

This leads to the generation of two additional categorical columns.

| 21012 | Session3_only U vs 3Uh_2 - Perseus | | | | | | | | | | | | | | | | | |
|---------|------------------------------------|----------------|----------------|--------------------|-------------|---------------|-----------------------------|-----------------------------|-------------|-----------|----------------|-----------------------|--------------------|-------------------|----------------|---------------------|------------------|-------|
| | Matrix | | | | | | | | | | | | | | | | | |
| 1 1 | Basic • | Filter | rows * | Annot. co | olumns 🕶 Ir | mputation • | Clustering • | 🦉 P2 Visua | lization • | . 308 부 | Ba: | sic 🔹 | | | | | | |
| EN | Rearrange • | Filter | columns • | Annot. ro | ows • N | Aodification | s 🔹 f(x) 🗹 🗾 🖊 | $\forall \bar{x} p_N$ Clust | ering/PCA • | 🔆 🕥 🚺 | 7 5 | ER. | | | | | | |
| 1 | Normalizatio | n • Quali | ity • | Tests 🔹 | P | roteomic ru | ıler • 📑 🏹 🗠 1D | 2D P1 🛕 Misc. | • | _1 Ci 🕻 | 7 | | | | | | | |
| Load | | | | Pr | rocessing | | | | Anal | /sis | | Multi-proc. | Ехро | rt | | | | |
| matrix1 | matrix2 ma | trix4 mat | rix5 matri | x6 matrix | 7 matrix8 | matrix9 | matrix10 matrix1 | 1 matrix12 matri | x13 matrix | d4 matri | x15 matrix | _{x16} matrix | 17 | | | | | |
| Data | | _ | _ | | - | | | | _ | _ | _ | _ | | | | | | |
| | Matr 30h/0h | Matr 30h/0h | Matr 30h/0h | t-test Signific | ratio ≻=1 | ratio <=-1 | t-test Significant_ratio | t-test Significant_ratio | PEP | Intensity | Intensity L | Intensity H | -Log t-test | t-test Differe | Protein IDs | Majority protein | Protein names | Ger |
| Type | Expres | Expres | Expres | Catego | Catego | Catego | Category | Category | Numeric | Numeric | Numeric | Numeric | p-value 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 | UB/ |
| 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 | | | | | | | | ADE |
| 6 | -0.546 | -0.031 | -0.117 | | Discard | Discard | | | 0 | 10815 | Protei | in gro | ups si | gnific | antly | chang | ged | EPS |
| 7 | -0.803 | -0.316 | -0.868 | | Discard | Discard | | | 0 | 12603 | 8, | chan | σ_d < | 1 ar | , ma | rkod | | . NB/ |
| 8 | 0.3684 | 0.2924 | -0.356 | | Discard | Discard | | | 2.2871 | 22752 | G | chan | gcu | – ± ai | | INCU | | GTF |
| 9 | -2.34255 | -0.726 | -0.342 | | Discard | Кеер | | | 2.4205 | 35589 | | | with | '+Kee | р″ | | | . TME |
| 10 | 0.2301 | 0.0898 | 0.8546 | | Discard | Discard | | | 0 | 133150 | 112270 | 208800 | 0.6240 | 0.3915 | A5YKK | A5YKK | CCR4 | CNO |
| 11 | -0.727 | -0.420 | -0.8483 | + | Discard | Discard | +_Discard | +_Discard | 0 | 675290 | 524240 | 151050 | 1.45999 | -0.665 | A5YVE | A5YVE | Pyruva | PDF |
| 12 | -1.99157 | -2.42629 | -1.55667 | + | Discard | Кеер | +_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 | 01473 | Acyl-co | ACC |
| Drote | nin aro | une ci | ignific | anthy | chang | bor | | | 3.7088 | 223750 | 180350 | 433970 | 0.10081 | 0.1301 | A6NDU8 | A6NDU8 | UPF06 | C5c |
| PIOLE | | ups si | igninc | antiy | Chang | geu | | | 0 | 275230 | 223270 | 519630 | 0.7521 | -0.202 | A6NEM | A6NEM | Host c | HCF |
| | & char | iged > | >=1 ar | e mai | rked | - | \rightarrow | | 8.4587 | 506980 | 445760 | 612280 | 0.9671 | 0.2612 | A6NFN | A6NFN | Abl inte | ABI |
| | with"+Keen" | | | | | | | | | | | | | | | | | |
| | _ | vvici1 | | ۲ | | | | | 0 | 500840 | 352390 | 148460 | 1.0818 | 0.5644 | A6NFX | A6NFX | ADP-s | NU |
| 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 5066 | | Discound | Discound | | | | | | 050000 | 0.0400 | 0.040 | A CNIO I | | | DVN |
| | -0.540 | -0.022 | 0.5300 | | Discard | Discard | | | 3.2658 | 315310 | 230010 | 853000 | 0.0108 | -0.010 | ADINGJ | A6NGJ | Dynein | UII |

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



| Scatter plot | | |
|---------------|-------------|------|
| Matrix access | Columns | • |
| | | |
| | | |
| č | | * |
| Cancel | Description | 🛱 ок |

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

| 2101 | I Dessions_only U vs 3Uh_2 - Perseus | | | | | | | | | | | | | | | | | |
|---------|--|--------------------------------|------------------------------|-----------------------------------|------------------------|--|---|---|-------------|--------------------------------|-------------------|----------------|--------------------|-------------------|----------------|---------------------|------------------|---------------|
| • | Matrix | | | | | | | | | | | | | | | | | |
| | Basic • Rearrange • Normalizatio | Filter Filter on • Quali | rows * columns * ity * | Annot. co Annot. ro Tests • | olumns • In ows • N | mputation • Aodification roteomic ru | Clustering • f(&) 🗹 💶 Z uler • 🧱 🍀 🌱 10 | $\begin{array}{c c} & \mathbf{p}_2 \\ \hline & \mathbf{x} \\ \hline & \mathbf{x} \\ \mathbf{z} \\ \mathbf{z} \\ \mathbf{p}_1 \\ \mathbf{x} \\ \mathbf{x} \\ \mathbf{z} \\ $ | ering/PCA • | ≥ 罪 4 ● ≪ ‰ ● 5 Ⅰ | - 🛄 🛛 Ba (7) 💽 | sic • | н | | | | | |
| Load | | 4 | | PI | rocessing | | | 1 1 | Anal | ysis | _ | Multi-proc. | Expo | rt | | | | |
| matrix1 | matrix2 ma | itrix4 mat | rix5 matri | x6 matrix | 7 matrix8 | matrix9 | matrix10 matrix1 | 1 matrix12 matri | x13 matrix | <14 matrix | x15 matri | c16 matrix. | 17 | | | | | 4 1 |
| | | < | | | | | | | | | | | | | | | | 4 1 |
| | Máťr 30h/0h | Matr 30h/0h | Matr 30h/0h | t-test Signific | ratio >=1 | ratio <=-1 | t-test Significant_ratio | t-test Significant_ratio | PEP | Intensity | Intensity L | Intensity H | -Log t-test | t-test Differe | Protein IDs | Majority protein | Protein names | Gene names |
| Туре | Expres | Expres | Expres | Catego | Catego | Catego | Category | Category | Numeric | Numeric | Numeric | Numeric | p-value 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 | Кеер | | | 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 | Кеер | +_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 | 01473 | 01473 | 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 |

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.

| | Matrix | | | | | | | | | |
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| 1 | Basic • Filter rows • Annot. columns • Imputation • Clustering • 🦉 P ₂ | Visualization • 井 嘂 这 📣 | Basic 🔹 | | | | | | | |
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| 1 | Normalization • Quality • Tests • Proteomic ruler • 🧱 🖓 🗠 1D 2D 🖭 🛕 | Misc. • 🎿 🔁 🖤 | | | | | | | | |
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| | | 1 | color | | size 4 | A0AVT1 | A0AVT1 | Diquitin U | | |
| | | 2 | | | 4 | A0FGR8 | A0FGR8 | Extende E | | |
| | | 3 | | | 4 | A1L0T0; | A1L0T0 | Acetolac IL | | Selected y-axis |
| 01- | | 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 | | |
| 2 | | 8 | | | 4 | A4D1E9 | A4D1E9 | GTP-bin G | | |
| 40 | | 9 | | | 4 | ASPLL7; | ASPLL7; | Transme 11 | | |
| ³ | | 10 | | | 4 | A51KK0 | A5Y1/E0 | Pyruvate P | | |
| Matr | | 12 | | | 4 | A6NC48 | A6NC48 | ADP-ribo B | | |
| | | 13 | | | 4 | Q9BX74 | Q9BX74 | TM2 dom TI | | |
| 7- | | 14 | | | 4 | A6NCE7 | A6NCE7 | Microtub M | | |
| | | 15 | | | 4 | A6NCZ6 | A6NCZ6 | Siderofle S | | |
| | | 16 | | | 4 | A6NDG6 | A6NDG6 | Phospho P | | |
| 177 | | 17 | | | 4 | 014734; | 014734; | Acyl-coe A | | |
| | | 18 | | | 4 | A6NDU8 | A6NDU8 | UPF060 C | | |
| 9- | | 19 | | | 4 | A6NEM2 | A6NEM2 | Host cell H | | |
| | | 20 | | | 4 | A6NFN2 | A6NFN2 | Abl inter A | | |
| | | 21 | | | 4 | Q13564; | Q13564; | NEDD8 N | | |
| 4- | | 22 | | | 4 | A6NEX8 | A6NEX8 | ADP-sug N | | |
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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.

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| 13 | Normalization • | Quality • | Tests • | Proteomic ruler | • 📰 😽 🗠 1D 2D 🎦 💧 | Misc. • | L 🖆 💟 | | | | | | | | | |
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| | | | • • • • | | | | 4 | | | 4 | A1X283; | A1X283 | SH3 and | S | | |
| | | | | | | | 5 | | | 4 | P35611 | P35611 | Alpha-ad | A | | |
| с – | | | | | | | 6 | | | 4 | Q9UBC2 | Q9UBC2 | Epiderm | E | | |
| | | | | | | | 7 | | | 4 | A2RRP1 | A2RRP1 | Neurobla | N | | |
| e | | | | | | | 8 | | | 4 | A4D1E9 | A4D1E9 | GTP-bin | G | | |
| -Val | | | | | | | 9 | | | 4 | A5PLL7; | A5PLL7; | Transme | TI | | |
| estp | | | | | | | 10 | | | 4 | A5YKK6 | A5YKK6 | CCR4-N | С | | |
| Ę. | | | | | | | 11 | | | 4 | A5YVE9 | A5YVE9 | Pyruvate | P | | |
| -~ F | | | | | 8 | | 12 | | | 4 | A6NC48 | A6NC48 | ADP-ribo | В | | |
| | - | | | | | | 13 | | | 4 | Q9BX74 | Q9BX74 | TM2 dom | TI | | |
| | c | | · · · · · · · · · · · · · · · · · · · | | | | 14 | | | 4 | A6NCE7 | A6NCE7 | Microtub | M | | |
| 9 | | | | | | | 15 | | | 4 | A6NCZ6 | A6NCZ6 | Siderofle | S | | |
| | | | | | | | 16 | | | 4 | A6NDG6 | A6NDG6 | Phospho | P | | |
| | | | 88.0 | | 8 | | 17 | | | 4 | 014734; | 014734; | Acyl-coe | A | | |
| | | | | | _0 | | 18 | | | 4 | A6NDU8 | A6NDU8 | UPF060 | С | | |
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| 9 | | | | | | | 21 | | | 4 | Q13564; | Q13564; | NEDD8 | Ν | | |
| ē | | | | | | | 22 | | | 4 | A6NFX8 | A6NFX8 | ADP-sug | N | | |
| | | | | | | | 23 | | | 4 | A6NG58 | A6NG58 | Beta-par | P. | | |
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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.

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| 1.5 | | | | | | | | 15 | | | 4 | A6NDG6 | A6NDG6 | Phospho | P | | | |
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| 210 | Session3_only U vs 3Uh_2 - Perseus | and the second |
|------------|---|--|
| - | Matrix | |
| 1 1 | Basic T Filter rows Annot, columns Imputation Clustering Wisualization | · 建翻 / Basic · _ |
| TE EX | Rearrange • Filter columns • Annot. rows • Modifications • 🕼 🗹 💶 Z 🐨 🕱 Ph Clustering/PCA | A • 30 🗞 🕊 7 🖪 🖪 |
| 1 | Normalization • Quality • Tests • Proteomic ruler • 🗮 🤯 🖾 10 20 Pr 🔺 Misc. • | |
| Load | Drocersing Ass | nalurir Multi-proc Export |
| Load | And | narysis multi-proc. Export |
| matrix1 | I matrix2 matrix4 matrix5 matrix6 matrix7 matrix8 matrix9 matrix10 matrix11 matrix12 matrix13 matrix | atrix14 matrix15 matrix16 matrix17 |
| Data | Scatter plot | |
| i 🗾 🕺 | $\mathbb{R} \otimes \mathbb{Q} \leftrightarrow \mathbb{Q} \wedge \mathbb{Q} \wedge \mathbb{Q} \otimes \mathbb{R}$ | Data Curves |
| | | t-test Difference 🔻 |
| | | -Log t-test p-value |
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| | | Selected * 8 B Gene names * IIn to '' * |
| 4.5 | | Dointe Categories Correlations |
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| 4- | PEPHX1 | Type Name Size |
| | GNS a | 1 t-test Sign + 1069 |
| | SCARB2 | 2 ratio >=1 Discard 3521 |
| 85- | | 3 ratio >=1 Keep 138 |
| | | 4 ratio <=-1 Discard 3478 |
| | CTSA | 5 ratio <=-1 Keep 181 |
| <u>е</u> - | | 6 t-test Sign +_Discard 981 |
| | F11R CD63 CTD12 GINS3 DHFR | 7 t-test Sign +_Keep 88 |
| æ | | RRM2 8 t-test Sign +_Discard 923 |
| alu 5 | | 9 t-test Sign +_Keep 146 |
| 5 DF | | |
| t-tes | | |
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| | -4 -3 -2 -1 0 1 2 3 4 5 | 5 9 items 2 selected |
| | riesi Dinerence | |

| 210 | Session3_only U vs 3Uh_2 - Perseus | | | | | | | |
|-----------|------------------------------------|--|-----------------------------|-----------------------|---------------------|-------------------------|------------------------|-------|
| • | Matrix | | | | | | | |
| 1 | Basic Filter rows | Annot. columns • Imputation • | Clustering 🔹 🦉 📴 | Visualization • 📫 🏭 | 🔆 🚛 🛛 Basic | • | | |
| E 🖸 | Rearrange • Filter columns • | Annot. rows • Modifications • | 100 🗹 💶 Z 🍸 🗴 Pr | Clustering/PCA + 🐰 👒 | 💐 🔽 🖪 E | 3 | | |
| R. | Normalization • Quality • | Tests • Proteomic ruler | 🔸 🧱 🏹 🗠 1D 2D 🎦 🛕 | Misc. • 💦 🔒 🔂 | * | | | |
| Load | | Processing | | Analysis | м | ulti-proc. | Export | |
| matrix1 | L matrix2 matrix4 matrix5 matrix6 | matrix7 matrix8 matrix9 m | atrix10 matrix11 matrix12 | matrix13 matrix14 mat | rix15 matrix1 | 5 matrix17 | | < > C |
| Data | Scatter plot | | | | | | | |
| | | | | | Data Curv | res | | |
| | | | can again | | t-test Differe | nce | | •= |
| | | vve | can again | | -log t-test p | -value | | |
| | | expo | rt the plot | | <no colors=""></no> | volue | | |
| | | | | | Selecte | d - 0 B (| Sono namor 🐨 Unito V 🐨 | |
| 4.5 | | to | various | | Points Ca | tegories Co | relations | |
| | | | | | | 2 0 | | |
| | 0 | ۹ ° | nciure | | | 💓 💊 📑 | Marra | Cine |
| 4- | EP | | rmats | | | туре | Name | Size |
| | GN | | initiatis. | | 1 | t-test Sign | + | 1069 |
| | SCA | RB2 | | | 2 | ratio >=1 | Discard | 3521 |
| е Э | | MRC2 ^D D | IP CYR61 | | 3 | ratio <= 1 | Reep | 138 |
| | PPAP2B | | PA4PSAT | | 4 | ratio <=-1 | Discard | 3478 |
| | | SA 28209 | ACYP1 AXL CDKASF1B | | 6 | t toet Sign | + Discard | 001 |
| <u></u> - | F11R Popper | KCTD12 | BHLDA3 | | 7 | t-test Sign | + Keen | 901 |
| | NPC2 SP | | | RRM2 | 8 | t-test Sign | + Discard | 923 |
| alue | Falan | | TAGEMAD2L1 | TYME | 9 | t-test Sign | + Keep | 146 |
| 4 P-V | HMOX1 | | NURF2 | 1100 | - | | | |
| t-tes | | | RNBSEHZA BD19-, JBPL2219 | | | | | |
| 5 | | | | | | | | |
| · · · | | | PBK | | | | | |
| | | | CTGF | | | | | |
| <u>بو</u> | CLCCODS ANAGE | C HORSCHUNT4 | | | | | | |
| - | LAMC1 PT | | | | | | | |
| | | | TK1 | | | | | |
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| | | 900 - 10 - 10 - 10 - 10 - 10 - 10 - 10 - | | | | | | |
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| 9 | | | | | | | | |
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| 0- | | | | | | | | |
| | | | | | | | | |
| | -4 -3 -2 | -1 0 1 | 2 3 | 4 5 | | | | |
| | | t-test Difference | | | 9 items 2 se | elected | | |

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



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

| Generic matrix upload | | | | | |
|-----------------------|--|--------|-----|---|------------------|
| File | D:\SILAC Workshop 2015\Zanivan et al 2013 (Mann paper) | \Origi | nal | results Mann\proteinGroups.txt | Select |
| | 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 BSA_3 Unique peptides FN_1 Unique peptides FN_2 | A. | Ex | pression 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 Jumerical | t u d b |
| | Unique peptides FN_3 Unique peptides GFR_1 Unique peptides GFR_2 Unique peptides GFR_3 Unique peptides GFR_3 Unique peptides LAM_1 Unique peptides LAM_2 Unique peptides Matr 12h_1 Unique peptides Matr 12h_2 Unique peptides Matr 12h_3 | | | PEP Intensity Intensity L Intensity H Int | t u d b |
| | 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 + razor sequence coverage [%] | | Te | Reverse Contaminant | d |
| | Mol. weight [kDa] Sequence length Slice average Slice 1 Slice 2 Slice 3 Slice 4 | | M | Majority protein IDs Protein names Gene names Proteins ulti-numerical | u d b |
| | Dife 5 Silee 5 Silee 7 Silee 8 Silee 9 Silee 10 Silee 11 Silee 12 Shorten expression column names | Ŧ | | > | t u d b |
| | | | | | - |
| Cancel | Description | | | | 🛱 ок |

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.

| 21019 | Session1 - Pe | rseus | | | | | | | | | | | | | | | | | |
|------------|---------------|-----------|-----------|---------------|----------------|----------------|------------|------------|-------------------------------|-------------|-----------|-------------|---------|-----------|-------------|---------|-----|-------------------|---|
| | Matrix | | | | | | | | | | | | | | | | | | |
| 1 1 | Basic • | Filter | rows • | Annot, c | olumns • I | mputation • | Cluste | erina • | 😅 þ ₂ | Visualizati | on• 📫 | 885 1:0- 20 | Basic • | | | | | | |
| EN | Rearrange • | Filter | columns • | Annot. n | ows • 1 | Modification | s + f(x) 📈 | Z 7 | \overline{x} P _N | Clustering | /PCA • 🎊 | S 😺 🔽 | | | | | | | |
| - | Normalizatio | on • Qual | ity • | Tests • | F | Proteomic ru | ler • 📷 🍇 | 1D 2 |) P1 🛦 | Misc. • | | c= 🖤 | | | | | | | |
| Load | | | | P | rocessing | | | | | | Analysis | | Mult | i-proc. | Export | | | | |
| matrix1 | | | | | | | | | | | | | | | | | | n : 🖬 🕪 🗶 🛔 🛧 🐄 📄 | 4 matrix1 |
| Data | | | | | | | | | | | | | | | | | | | Creator: hardt |
| | | | 1.01.0 | | | | | - | | | | | | | | | | | 03/20/2015 16:15:34 |
| | Un_1 | Un_2 | Un_3 | Matr 30h_3 | Matr 30h_2 | Matr 30h_1 | identifi | Reverse | Contam | PEP | intensity | L | H | IDs | protein | names | na | Generic matrix u | File: proteinGroups.txt |
| Type | Expros | Expres | Expres | Expres | Expres | Expres | Catego | Catego | Catego | Numeric | Numeric | Numeric | Numeric | Text | IDS Text | Text | То | matrix 1 | Quality: (small values are good.) |
| 1 | 1.0594 | 1 0290 | 1 220 | 1.9615 | 1 2004 | 1 5075 | Catego | Catego | Catego | 0 | 052060 | 600060 | 254000 | | | Libiqui | 110 | | Expression columns (6) |
| 2 | 0.65211 | 0.00160 | 0.90044 | 0.62254 | 0.24262 | 0.45942 | | | | 0 | 257600 | 177260 | 204000 | ADEGP | ADEGP | Extend | ES | | Categorical columns (3) |
| 2 | NaN | NaN | NoN | NoN | 0.34203 NaN | 0.43043 NoN | | | | 2 1000 | 670070 | 506090 | 7209500 | | | Modiat | ME | | String columns (5) Numerical columns (4) |
| | 0.91575 | NaN | NaN | 1 2/05 | NaN | 1.614 | | | | 8 1766 | 119360 | 977740 | 215880 | A0.INI//5 | A0.INI//5 | LIHRE1 | LIF | | Multi-numerical columns (0) |
| 5 | NaN | NaN | NaN | NaN | NaN | NaN | + | | | 1 7907 | 270370 | 215370 | 550060 | FORHO | FORHO | Placks | PI | | Categorical rows (0) |
| 6 | NaN | NaN | NaN | NaN | NaN | NaN | | | | 9.5202 | 51/250 | 435080 | 791680 | A0M76 | A0M76 | Shooti | KU | | Numerical rows (0) |
| 7 | NaN | NaN | 0.72598 | 0.53048 | NaN | NaN | | | | 4.8278 | 492420 | 386300 | 106120 | AOP IW | AOP IW6 | Transm | TN | | |
| 8 | NaN | NaN | NaN | NaN | NaN | NaN | | | | 1.8473 | 583650 | 380530 | 203110 | 01565 | 01565 | Probab | .IN | | |
| 9 | NaN | NaN | NaN | NaN | NaN | NaN | | | | 4 2013 | 8772200 | 8038000 | 734270 | A0T4C | A0T4C | Sobing | SE | | |
| 10 | 1 4123 | NaN | 0.24564 | 0.33469 | NaN | NaN | | | | 3 3301 | 403140 | 285770 | 117380 | A1A4S | A1A4S | Rho G | AF | | |
| 11 | NaN | NaN | NaN | NaN | NaN | NaN | + | | | 0.0001 | 124580 | 999490 | 246320 | A1K79 | A1K79 | Peroxi | PX | | |
| 12 | NaN | NaN | NaN | NaN | NaN | NaN | | | | 2 5025 | 330490 | 296430 | 3406000 | A11 020 | A1L 020 | RNA-b | ME | | |
| 13 | 0.96951 | 0 88448 | 0.7311 | 0.62026 | 0 71102 | 0.63919 | | | | 0 | 327420 | 267380 | 600400 | A1L 0T | A1L 0T0 | Acetola | | | |
| 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 | SF | | |
| 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 | PIF | | |
| 22 | 0.52754 | 0.61874 | 0.85262 | 0.64743 | 0.55281 | 0.57093 | | | | 0 | 132740 | 110890 | 218430 | P3561 | P3561 | Alpha | AE | | |
| 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 | AF | | |
| 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 | N | | |
| 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 | ; | | | | | | | | | | | | | | | | | <u> </u> | |
| | | | | | | | | | | | | | | | | | | | Version 1.5.1.6 |

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

| | Mat | rix | | | | |
|---|-------------|---|----------------|-----------------------|--------------|-------|
| | Basic | Filter rows Anno | ot. co | olumns • I | mputation • | |
| | f(x) | Transform 🙀 | t. ro | ows ▼ _ ! | Modification | s • |
| | ۵ | Co Transform | - | |)ti-ru | ler ' |
| | Z | Co f(x) All values in the specified transformed according to | l col o the | umns are formula s | pecified. | |
| _ | | Summany statistics (columns) | | | 1 | |
| | | Summary statistics (courtins) | e | Score | Intensity | L |
| | | | ric | Numeric | Numeric | N |
| - | | Quantiles 😭 | Г | 4.0745 | 188920 | 98 |
| | - | Density estimation 🙀 | ł | 1.4653 | 183450 | 12 |
| | | Performance curves 🤮 | | 6.6682 | 931260 | 66 |
| | | Combine rows by identifiers | | 14.438 | 968950 | 49 |
| | | Clone 🛱 | ł | 1.9054 | 960480 | 83 |
| | ~ ~/ | | 3 | 1.1743 | 1918800 | 11 |
| | | Significance A 🥵 | L | 5.6441 | 1161600 | 11 |
| | | Significance B 🥵 | 5 | 0.99659 | 254400 | 25 |
| | | Add noise 😗 | | 1.1381 | 128280 | 12 |
| | - | | 1 | 4.9443 | 374210 | 30 |



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





| Multi scatter plot | | | |
|--------------------|--|-------------|--------------------------------------|
| Rows Columns | 0h_1 0h_2 0h_3 Matr 30h_3 Matr 30h_2 Matr 30h_1 PEP Intensity Intensity L Intensity L Intensity H 0h_1 0h_2 0h_3 Matr 30h_3 Matr 30h_3 Matr 30h_1 PEP Intensity Intensity L Intensity L Intensity L | | t u d b t u d b |
| Cancel | | Description | 🙀 ок |

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



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







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

| 2 ' 🗆 | Session2_only 0 vs Son_02 - Perseus | - | - | | - | |
|------------|-------------------------------------|-------------------------------|---------------------------|--------------------------|--------------------|-----------|
| • | Matrix | | | | | |
| 1 🐐 | Basic • Filter rows • | Annot. columns • Imputation • | Clustering • 🦉 🗛 | Visualization • 🕂 🏭 🔛 | 🔐 Basic 🔹 | |
| 三 🕄 | Rearrange • Filter columns • | Annot. rows Modifications | 📧 🗹 🎫 Z 🝸 $\bar{\chi}$ PN | Clustering/PCA 🔹 🎊 🚳 😃 | | |
| 18 18 | Normalization • Quality • | Tests • Proteomic ruler • | 📑 🍕 🗠 1D 2D 🎦 🛕 | Misc. • 🍡 🛃 🖼 🖤 | | |
| Load | | Processing | | Analysis | Multi-proc. | Export |
| matrix1 | matrix2 matrix3 matrix4 matrix5 | matrix6 matrix7 matrix8 | | | | < ▶ □ |
| Data | Histogram | | | | | < > C |
| 🗄 🙊 ln | cols 🖂 🔝 🛃 🏗 📕 | | | Points Categories | | < > 🖬 |
| | | | | 🗄 🤣 🥑 📕 🔤 Selection fron | n table 🔹 | |
| Ints | 완 | . | | Fill color Borde | er Protein Majorit | / Protein |
| C | | | | 1 | A0AVT1 A0AVT | 1 Ubiqui |
| | iiiiii. | | | 2 | A0FGR8 A0FGR | 8 Extend |
| | 5 0 5 | -5 0 5 | - | 3 | A0JLT2; A0JLT2 | ; Mediat |
| | log2 0h_1 | log2 Matr 30h_1 | | 4 | A0JNW5 A0JNW | 5 UHRF1 |
| | | | | 5 | A0MZ66 A0MZ6 | 6 Shootii |
| uts. | e e | I I. | | 6 | A0PJW6 A0PJW | 6 Transn |
| Cou | 5 | | | 7 | Q15652; Q15652 | 2; Probat |
| | | | | 8 | A0T4C8 A0T4C | B Sphing |
| 4 | | L <u>IIIII</u> | | 9 | A1A4S6 A1A4S | 6 Rho G1 |
| - | log2 0h_2 | -5 0 5 log2 Matr 30h_2 | ~ | 10 | A1L020 A1L020 | RNA-b |
| | | | | 11 | A1L0T0; A1L0T0 |) Acetola |
| <u>t</u> s | | - <u></u> | | 12 | A1L188 A1L188 | 3 Uncha |
| Sour | jun jung | | | 13 | A1X283; A1X283 | 3 SH3 ar |
| | | | | 14 | Q86X10 Q86X1 | D Ral GT |
| 4 | | L | _ | 15 | A2A2G4 A2A2G | 4 Dolich |
| - | 5 0 5 log2.0h.3 | -5 0 5 log2 Matr 30h 3 | | 16 | A2A2Q9 A2A2Q | 9 Uncha |
| | loge on_o | log2 maa oon_o | | 17 | P42696; P42696 | ; RNA-b |
| | | | | 18 | Q14678: Q1467 | 3: KN mo |

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

| <mark>2</mark> ' 🗆 | Session2_only 0 vs 30n_02 - Perseus | | - | | | |
|--------------------|---|------------------------------|-----------------------------|--------------------------|------------------|---------|
| • | Matrix | | | | | |
| 1 🧐 | Basic • Filter rows • A | nnot. columns • Imputation • | Clustering • 🦉 🗛 🖓 | Visualization 🔹 🕂 🏭 🖂 🚛 | Basic 🔹 | |
| 三 🕄 | Rearrange • Filter columns • A | nnot. rows • Modifications • | 100 🗹 🎫 Z 🝸 $\bar{\chi}$ PN | Clustering/PCA 🔹 🎊 😒 😃 7 | | |
| 18 18 | Normalization • Quality • T | ests • Proteomic ruler • | 📑 苓 🗠 1D 2D 🎦 🛕 | Misc. 🔹 🛛 📑 🖼 👹 | | |
| Load | | Processing | | Analysis | Multi-proc. | Export |
| matrix1 | matrix2 matrix3 matrix4 matrix5 | matrix6 matrix7 matrix8 | | | | < ▶ □ |
| Data | Histogram | | | | | < > C |
| 🗄 🙊 ln | cols 🖂 🔝 💽 🏗 🛒 | | | Points Categories | | < Þ 🖬 |
| | | | | Selection from tak | ole 🔹 | |
| Ints | ti t | <u>d.</u> | | Fill color Border | Protein Majority | Protein |
| õ | . Š | | | 1 | A0AVT1 A0AVT1. | Ubiqui |
| | | | | 2 | A0FGR8 A0FGR8. | Extend |
| | | -5 0 5 | - 1 | 3 | A0JLT2; A0JLT2;. | Mediat |
| | log2 0h_1 | log2 Matr 30h_1 | | 4 | A0JNW5 A0JNW5 | UHRF1 |
| | | | | 5 | A0MZ66 A0MZ66. | Shootii |
| st | ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ | L . | | 6 | A0PJW6 A0PJW6 | Transn |
| Cou | | | | 7 | Q15652; Q15652; | Probat |
| _ | | | | 8 | A0T4C8 A0T4C8. | Sphing |
| 4 | | L | | 9 | A1A4S6 A1A4S6. | Rho G1 |
| - | log2 0h_2 | -5 0 5 log2 Matr 30h_2 | ~ | 10 | A1L020 A1L020 | RNA-b |
| | d. | | | 11 | A1L0T0; A1L0T0 | Acetola |
| 알 | | L | | 12 | A1L188 A1L188 | Uncha |
| Sour | , in the second s | | | 13 | A1X283; A1X283 | SH3 ar |
| о | | | | 14 | Q86X10 Q86X10. | Ral GT |
| L | | L | _ | 15 | A2A2G4 A2A2G4. | Dolich |
| - | 5 0 5 log2.0h.3 | -5 0 5 log2 Matr 30h 3 | | 16 | A2A2Q9 A2A2Q9. | Uncha |
| | 1092 01_0 | logz maa oon_o | | 17 | P42696; P42696; | RNA-b |
| | | | | 18 | Q14678: Q14678: | KN mo |

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





| Subtract | And others, Support of States, | |
|---------------|--|------|
| Matrix access | Columns | |
| Subtract what | Median | |
| Cancel | Description - Output | 🛱 ок |

After the normalization we again create a histogram.

before



after



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



before

Matrix Basic • Filter rows • Annot, columns * Imputation • Clustering f(x) Z Rearrange • Filter columns 🔹 Annot, rows • Modifications ¹ $\square \bigcirc \bigcirc \bigcirc$ 10 Normalization • Quality • Tests • Proteomic ruler • matrix4 matrix5 matrix6 matrix7 matrix8 matrix9 matrix2 matrix3 matrix1 Histogram Data 🛪 la cols 🖂 🖬 📰 📆 🚬 Counts Counts -5 -5 5 ò log2 0h 1 log2 Matr 30h 1 Counts Counts -5 5 -5 b log2 Matr 30h_2 Ó log2 0h_2 Counts Counts -5 -5 ò 5 ò log2 0h_3 log2 Matr 30h_3

after

Now we define groups for the differentially treated samples.





| Categorical annotation rows | 1. Scillar | The second | | family 1 | ····· | |
|-----------------------------|------------|------------|-------------|----------|-------|----|
| 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 | | | | |
| | | | | | | |
| | | | | | | |
| Cancel | | Descriptio | on - Output | | | ОК |

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

| ction | Create | |
|-------|------------|----------|
| | Row name | Group1 |
| | 0h_1 | 0h |
| | 0h_2 | 0h |
| | 0h_3 | Oh |
| | 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.

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 |
|--------|----------|----------|----------|---------------|---------------|---------------|---------|-----------|----------------|----------------|----------------|----------------------------|------------------|---------------|----------|
| Туре | 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 |

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).

| M | latrix | | |
|-----------------------------------|---------------------|--|----------|
| la | sic • Filte | Filter rows 🔹 Annot. columns 🔹 Imputation 🔹 Clustering 🔹 🦉 P2 Visualization 🔹 | |
| R.e | earrange 🔹 😽 😽 | Filter rows based on categorical column 🚌 💶 Z 🏹 $ar{\chi}$ P _N Clustering/PCA | |
| Ne | ormalization • | Filter rows based on numerical/expression column | |
| | | Filter rows based on text column | |
| m | atrix2 matrix3 | Eiter rows based on valid values | |
| | | | |
| | log2 H/L log2 | Filter rows based on random sampl Filter rows based on valid values p | |
| | | Rows/columns of the expression matrix are | |
| | Expres Expres | es Expres Numeric Numeric intered to contain a reactine specified numbers in of entries that are value in the specified way. | |
| | | | |
| | | | |
| | | | |
| Processing | | | |
| Filter rows based on valid values | | | |
| Min. number of values | 3 | | A |
| Mode | In at least one gro | e group | |
| | Grouping | Group1 💆 | |
| | | | |
| Values should be | Valid | | |
| | | | |
| | | | |
| Filter mode | Reduce matrix | v | _ |
| | Incource matrix | , | |
| Concel | | Description Output | |
| Cancer | | Description - Output | |

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

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 |
|--------|--------------|--------------|--------------|-----------------------|-----------------------|-----------------------|---------|-----------|----------------|----------------|
| Туре | 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 |
| | - | | | | | | | | | |

| | 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 |
|--------|--------------|--------------|--------------|-----------------------|-----------------------|-----------------------|---------|-----------|----------------|----------------|
| Туре | 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 | 600400 |
| 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. |

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

| Visualization • + Clustering/PCA • & Misc. • | ŧ III i⊵ " ≪ ∾ ¥ 0 ∎ c= ¥ | Basi | ic • | | | |
|--|---------------------------------|----------|--|---|--|--|
| Analysis | ; | 1 | Multi-proc. | Export | | |
| | Volc | ano plot | | | | |
| Intensity Protein H IDs | Ma prc ID: | | Visualize th form of a ve significant of permutatio | e results of olcano plot. data points n-based FD | a t-test in the Determine with a R calculation. | |
| Numeric Text | Тел | TEAL | TEAL | TEAL | - | |
| | 1000 | 1 | | - | | |

| Volcano plot | | | x | 1 |
|-------------------------------------|---------------|---------------|----|--------------|
| Grouping | Group1 | | • | |
| | First group | Oh | | |
| | Second group | Matr 30h | | |
| | | | | |
| Test | t-test | | • | Two-sided |
| Side | Both | | - | t-test with |
| Number of randomizations | 250 | | | error |
| Preserve grouping in randomizations | <none></none> | | - | |
| FDR | 0.05 | | | corrected p- |
| SO | 0 | | | values (0.05 |
| | | | Ļ | FDR) |
| Cancel | [| Description 🙀 | ОК | |