

# Package “"proteoQ"”

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## Introduction to proteoQ

Chemical labeling using tandem mass tag (TMT) has been commonly applied in mass spectrometry (MS)-based quantification of proteins and peptides. The `proteoQ` tool is designed to aid automated and reproducible analysis of proteomics data. It interacts with an `Excel` spread sheet for dynamic sample selections, aesthetics controls and statistical modelings. The arrangement allows users to put data manipulation behind the scene and apply metadata to openly address biological questions using various informatic tools. In addition, the entire workflow is documented and can be conveniently reproduced upon revisiting.

The tool currently processes the peptide spectrum matches (PSM) tables from Mascot searches for 6-, 10- or 11-plex TMT experiments. Peptide and protein results are then produced with users' selection of parameters in data filtration, alignment and normalization. The package further offers a suite of tools and functionalities in statistics, informatics and data visualization by creating ‘wrappers’ around published R routines.

## Installation

To install this package, start R (version “3.6”) and enter:

```
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
BiocManager::install(c("Biobase", "GSVA", "Mfuzz", "limma"))

if (!requireNamespace("devtools", quietly = TRUE))
  install.packages("devtools")
devtools::install_github("qzhang503/proteoQ")
```

## Application – Part I

In this section I illustrate the following applications of `proteoQ`:

- Summarization of PSM data to peptide and protein reports.
- Visualization of quality metrics in peptide and protein data.
- Partial or complete re-normalization of data when needed

The data set I use in this section corresponds to the proteomics data from Mertins et al.(2018). In the study, two different breast cancer subtypes, triple negative (WHIM2) and luminal (WHIM16), from patient-derived

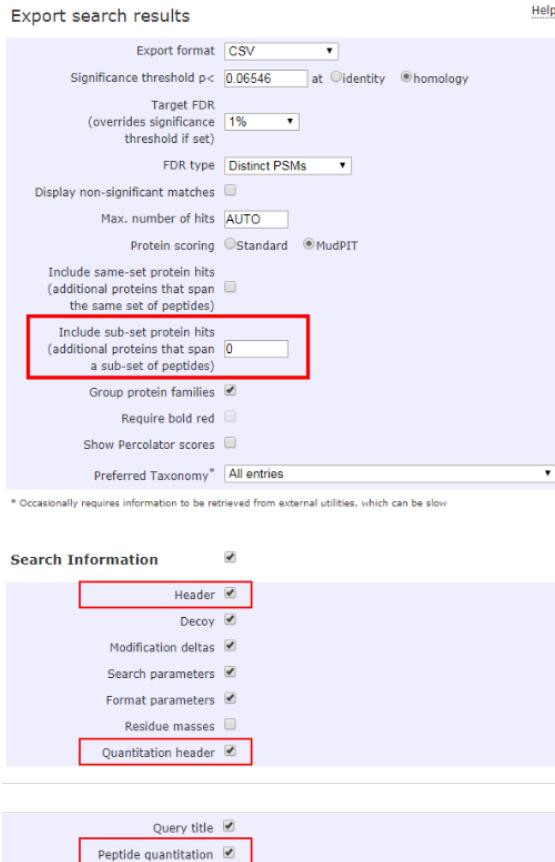


Figure 1: Mascot\_export

xenograft (PDX) models were assessed by three independent laboratories. At each site, lysates from WHIM2 and WHIM16 were each split and labeled with 10-plex TMT at equal sample sizes and repeated on a different day. This results in a total of 60 samples labeled under six 10-plex TMT experiments. The samples under each 10-plex TMT were fractionated by off-line Hp-RP chromatography(2011), followed by LC/MS analysis. The raw PSM results from Mascot searches are stored in a companion R package, `proteoQDA` and are accessible through the following installation:

```
devtools::install_github("qzhang503/proteoQDA")
```

## Set up the experiments

We first set up a working directory:

```
dat_dir <- "c:\\The\\First\\Example"
```

The workflow begins with PSM table(s) in a `csv` format from the Mascot search engine. When exporting PSM results, I typically set the option of `Include sub-set protein hits` to 0 with my opinionated choice in satisfying the principle of parsimony. The options of `Header` and `Peptide quantitation` should be checked to include the search parameters and quantitative values. The `filename(s)` of the export(s) will be taken as is.<sup>1</sup>

---

<sup>1</sup>The default file names begin with letter F, followed by six digits and ends with .csv in file name extension.

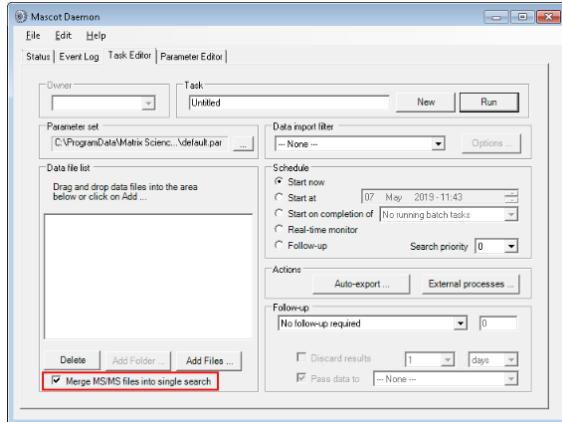


Figure 2: Mascot\_daemon

The same peptide sequence under different PSM files can be assigned to different protein IDs when inferring proteins from peptides using algorithms such as greedy set cover. To avoid such ambiguity in protein inference, I typically enable the option of **Merge MS/MS files into single search** in Mascot Daemon. If the option is disabled, peptide sequences that have been assigned to multiple protein IDs will be removed for now when constructing peptide reports.

The merged search may become increasingly demanding in computing powers with growing data sets. In the example, I combined the MS peak lists from the Hp-RP fractions within the same 10-plex TMT experiment, but not the lists across experiments. This results in a total of six pieces of PSM results in **Mascot** exports. To get us started, we go ahead and copy the PSM files that we have prepared in **proteoQDA** over to the working directory:

```
library(proteoQDA)
cptac_csv_1(dat_dir)
```

The workflow involves an **Excel** template containing the metadata of multiplex experiment numbers, including TMT channels, LC/MS injection indices, sample IDs, reference channels, RAW MS data file names and addditional fields from the users. The default file name for the experimental summary is **expt\_smry.xlsx**. If samples were fractionated off-line prior to LC/MS, a second **Excel** template will also be filled out to link multiple RAW MS file names that are associated to the same sample IDs. The default file name for the fractionation summary is **frac\_smry.xlsx**.<sup>2</sup> The description of the column keys in the **Excel** files can be found from the help document by entering `?proteoQ::load_expts` from a R console. We next copy over a pre-compiled **expt\_smry.xlsx** and a **frac\_smry.xlsx** to the working directory:

```
cptac_expt_1(dat_dir)
cptac_frac_1(dat_dir)
```

We now have all the pieces that are required by **proteoQ** in place. Let's have a quick glance at the **expt\_smry.xlsx** file. We note that no reference channels were indicated under the column **Reference**. With **proteoQ**, the **log2FC** of each species in a given sample is calculated either (a) in relative to the reference(s) within each multiplex TMT experiment or (b) to the mean of all samples in the same experiment if reference(s) are absent. Hence, the later approach will be employed to the exemplary data set that we are working with. In this special case, the mean of a given species in each TMT experiment is the average of five WHIM2 and five WHIM16 samples, which is biologically equivalent across TMT experiments.

As a final step of the setup, we will load the experimental summary and some precomputed results:

---

<sup>2</sup>To extract the names of RAW files under a `raw_dir` folder: `extract_raws(raw_dir)`

```
library(proteoQ)
load_expts()
```

## Summarize PSMs to peptides and proteins

*Process PSMs* — In this section, I demonstrate the summarisation of PSM data to peptides and proteins. We start by processing PSM data from `Mascot` outputs:

```
# Generate PSM reports
normPSM(
  rptr_intco = 1000,
  rm_craps = FALSE,
  rm_krts = FALSE,
  rm_outliers = FALSE,
  plot_violins = TRUE
)

# or accept the default parameters
normPSM()
```

PSM outliers will be assessed at a basis of per peptide and per sample at `rm_outliers = TRUE`, which can be a slow process for large data sets. To circumvent repeated efforts in the assessment of PSM outliers, we may set `rm_outliers = FALSE` and `plot_violins = TRUE` when first executing `normPSM()`. We then visually inspect the violin plots of reporter-ion intensity. Empirically, PSMs with reporter-ion intensity less than 1,000 are trimmed and samples with median intensity that is 2/3 or less to the average of majority samples are removed from further analysis.<sup>3</sup>

*Summarize PSMs to peptides* — We next summarise PSM to peptides.

```
# Generate peptide reports
normPep(
  id = pep_seq,
  method_psm_pep = median,
  method_align = MGKernel,
  range_log2r = c(5, 95),
  range_int = c(5, 95),
  n_comp = 3,
  seed = 749662,
  maxit = 200,
  epsilon = 1e-05
)
```

At `id = pep_seq_mod`, peptide sequences that are different in variable modifications will be treated as different species. The log2FC of peptide data will be aligned by median centering across samples by default. If `method_align = MGKernel` is chosen, log2FC will be aligned under the assumption of multiple Gaussian kernels.<sup>4</sup> The parameter `n_comp` defines the number of Gaussian kernels and `seed` set a seed for reproducible fittings. The parameters `range_log2r` and `range_int` define the range of log2FC and the range of reporter-ion intensity, respectively, for use in the scaling of standard deviation across samples.

<sup>3</sup>The sample removal and PSM re-processing can be achieved by deleting the corresponding entries under the column `Sample_ID` in `expt_smry.xlsx`, followed by the re-load of the experiment, `load_expts()`, and the re-execution of `normPSM()` with desired parameters.

<sup>4</sup>Density kernel estimates can occasionally capture spikes in the profiles of log2FC for data alignment. Users will need to inspect the alignment of ratio histograms and may optimize the data normalization with different combinations of tuning parameters before proceeding to the next steps.

Let's compare the log2FC profiles with and without scaling normalization:<sup>5</sup>

```
# without the scaling of log2FC
pepHist(
  scale_log2r = FALSE,
  ncol = 10
)

# with the scaling of log2FC
pepHist(
  scale_log2r = TRUE,
  ncol = 10
)
```

There are 60 panels of histograms in each plot, which may not be easy to explore as a whole. Instead, we will break the plots down by their data origins. We begin with modifying the `expt_smry.xlsx` file by adding the columns BI, JHU and PNNL. Each of the new columns includes sample entries that are tied to their laboratory origins.

<https://www.youtube.com/embed/3B5et8VY3hE>

We now are ready to plot histograms for each subset of data.<sup>6</sup> In this document, we only display the plots using the BI subset:

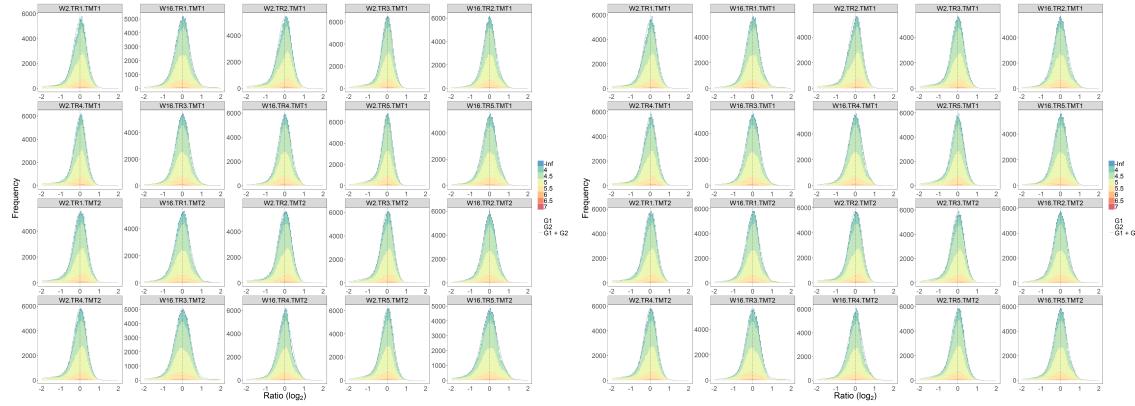
```
# without the scaling of log2FC
pepHist(
  scale_log2r = FALSE,
  col_select = BI,
  filename = Hist_BI_N.png,
  ncol = 5
)

# with the scaling of log2FC
pepHist(
  scale_log2r = TRUE,
  col_select = BI,
  filename = Hist_BI_Z.png,
  ncol = 5
)
```

\*NB\*: We interactively told `pepHist()` that we are interested in sample entries under the newly created

<sup>5</sup>`normPep()` will report log2FC results both before and after the scaling of standard deviations.

<sup>6</sup>system files will be automatically updated from the modified `expt_smry.xlsx`



As expected, the widths of log2FC profiles become more consistent after the scaling normalization. However, such adjustment may cause artifacts when the standard deviation across samples are genuinely different. I typically test `scale_log2r` at both TRUE and FALSE, then make a choice in data scaling together with my a priori knowledge of the characteristics of both samples and references.<sup>7</sup> I will use the same data set to illustrate the impacts of references in scaling normalization in Lab 1. Alignment of log2FC against housekeeping or normalizer protein(s) is also available. This seems suitable when the quantities of proteins of interest are different across samples where the assumption of constitutive expression for the vast majority of proteins may not hold.

*Summarize peptides to proteins* — We then summarise peptides to proteins using a two-component Gaussian kernel.

```
# Generate protein reports
normPrn(
  id = gene,
  method_pep_prn = median,
  method_align = MGKernel,
  range_log2r = c(5, 95),
  range_int = c(5, 95),
  n_comp = 2,
  seed = 749662,
  fasta = "C:\\\\Results\\\\DB\\\\Refseq\\\\RefSeq_HM_Frozen_20130727.fasta",
  maxit = 200,
  epsilon = 1e-05
)
```

Similar to the peptide summary, we inspect the alignment and the scaling of ratio profiles, and re-normalize the data if needed.<sup>8</sup>

```
# without the scaling of log2FC
prnHist(
  scale_log2r = FALSE,
  ncol = 10
)

# with the scaling of log2FC
prnHist(
```

<sup>7</sup>The default is `scale_log2r` = TRUE throughout the package. When calling functions involved parameter `scale_log2r`, users can specify explicitly `scale_log2r` = FALSE or define its value under the global environment.

<sup>8</sup>Parameter `fasta` is solely used for the calculation of protein percent coverage. Precomputed data will be used if no `fasta` database is provided.

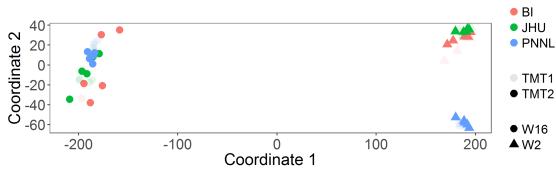


Figure 3: Peptide\_MDS

```
scale_log2r = TRUE,
ncol = 10
)
```

## Application – Part II

In this section I illustrate the following applications of `proteoQ`:

- Basic informatic analysis and linear modeling against the peptide and protein data.

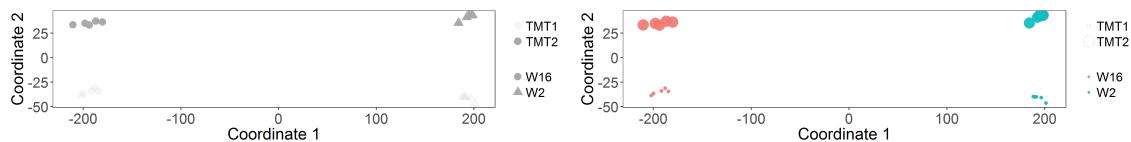
### MDS and PCA plots

In this section, we visualize MDS, PCA and Euclidean distance against the peptide data at `scale_log2r = TRUE`. We start with metric MDS for peptide data:

```
# data from all three laboratories
pepMDS(
  show_ids = FALSE
)
```

It is clear that the WHIM2 and WHIM16 samples are well separated by the Euclidean distance of log2FC (**Figure 2A**). We next take the JHU data subset as an example to explore batch effects in the proteomic sample handling:

```
# `JHU` subset
pepMDS(
  col_select = JHU,
  filename = MDS_JHU.png,
  show_ids = FALSE
)
```



We immediately spot that all samples are coded with the same color (**Figure 2B**). This is not a surprise as the values under column `expt_smry.xlsx::Color` are exclusively JHU for the JHU subset. For similar reasons, the two different batches of TMT1 and TMT2 are distinguished by transparency, which is governed by column `expt_smry.xlsx::Alpha`. We may wish to modify the aesthetics using different keys: e.g., color coding by WHIMs and size coding by batches, without the recourse of writing new R scripts. One solution is to link the attributes and sample IDs by creating additional columns in `expt_smry.xlsx`. In this example, we have had coincidentally prepared the column `Shape` and `Alpha` to code WHIMs and batches, respectively. Therefore, we can recycle them directly to make a new plot (**Figure 2C**):

```
# `JHU` subset
pepMDS(
  col_select = JHU,
  col_fill = Shape, # WHIMs
  col_size = Alpha, # batches
  filename = MDS_JHU_new_aes.png,
  show_ids = FALSE
)
```

The `prnMDS` performs MDS for protein data. For PCA analysis, the corresponding functions are `pepPCA` and `prnPCA` for peptide and protein data, respectively.

While MDS approximates Euclidean distances at a low dimensional space. Sometime it may be useful to have an accurate view of the distance matrix. Functions `pepEucDist` and `prnEucDist` plot the heat maps of Euclidean distance matrix for peptides and proteins, respectively. They are wrappers of (`pheatmap`) and inherit many parameters therein. Supposed that we are interested in visualizing the distance matrix for the JHU subset:

```
# `JHU` subset
pepEucDist(
  col_select = JHU,
  annot_cols = c("Shape", "Alpha"),
  annot_colnames = c("WHIM", "Batch"),

  # parameters from `pheatmap`
  display_numbers = TRUE,
  number_color = "grey30",
  number_format = "%.1f",

  clustering_distance_rows = "euclidean",
  clustering_distance_cols = "euclidean",

  fontsize = 16,
  fontsize_row = 20,
  fontsize_col = 20,
  fontsize_number = 8,

  cluster_rows = TRUE,
  show_rownames = TRUE,
  show_colnames = TRUE,
  border_color = "grey60",
  cellwidth = 24,
  cellheight = 24,
  width = 14,
  height = 12,
```

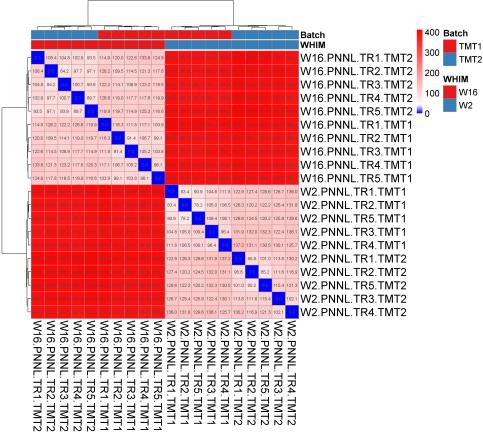


Figure 4: Peptide\_EucDist

```

    filename = EucDist_JHU.png
)

```

Parameter `annot_cols` defines the tracks to be displayed on the top of distance-matrix plots. In this example, we have chosen `expt_smry.xlsx::Shape` and `expt_smry.xlsx::Alpha`, which encodes the WHIM subtypes and the batch numbers, respectively. Parameter `annot_colnames` allows us to rename the tracks from `Shape` and `Alpha` to `WHIM` and `Batch`, respectively, for better intuition. We can alternatively add columns `WHIM` and `Batch` if we choose not to recycle columns `Shape` and `Alpha`.

## Correlation plots

In this section, we visualize the batch effects through correlation plots. The `proteoQ` tool currently limits itself to a maximum of 44 samples for a correlation plot. In the demo, we will perform correlation analysis against the PNNL data subset. By default, samples will be arranged diagonally from upper left to bottom right by the row order of `expt_smry.xlsx::Sample_ID` within a subset. We have learned from the earlier MDS analysis that the batch effects are smaller than the differences between W2 and W16. We may wish to put the TMT1 and TMT2 groups adjacent to each other for visualization of more nuance batch effects, followed by the correlational comparison of WHIM subtypes. We can achieve this by supervising sample IDs at a customized order. In the `expt_smry.xlsx`, I have prepared an `Order` column where samples within the JHU subset were arranged in the descending order of W2.TMT1, W2.TMT2, W16.TMT1 and W16.TMT2. Now we tell the program to look for the `Order` column for sample arrangement:

```

# Correlation plots of peptide data
pepCorr(
  col_select = PNNL,
  col_order = Order,
  filename = PNNL.png,

  use_log10 = TRUE,
  scale_log2r = TRUE,
  min_int = 3.5,
  max_int = 6.5,
  min_log2r = -2,
)

```

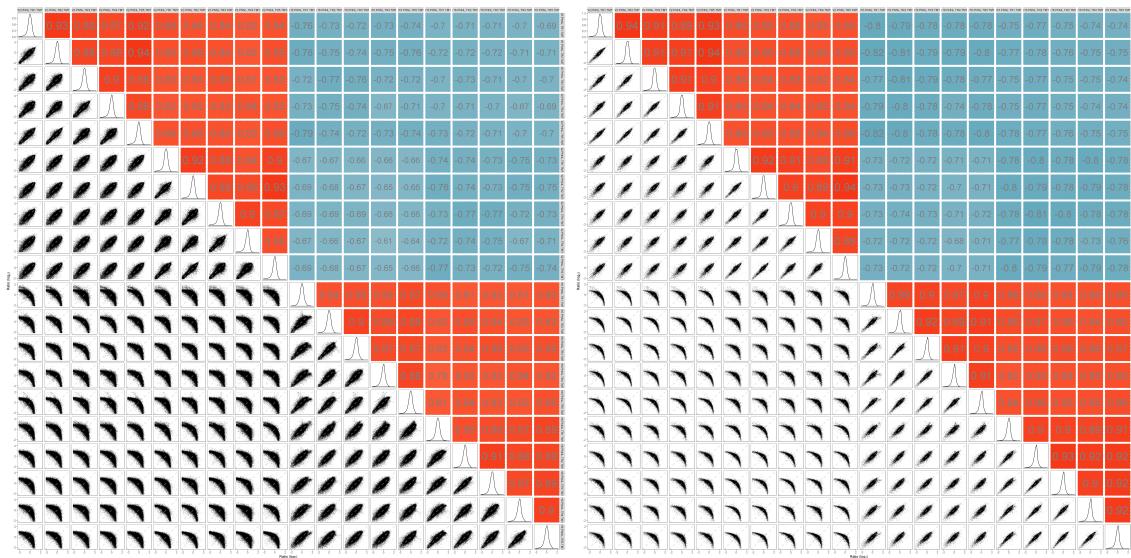
```

max_log2r = 2,
width = 24,
height = 24
)

# Correlation plots of protein data
prnCorr(
  col_select = PNNL,
  col_order = Order,
  filename = PNNL.png,

  use_log10 = TRUE,
  scale_log2r = TRUE,
  min_int = 3.5,
  max_int = 6.5,
  min_log2r = -2,
  max_log2r = 2,
  width = 24,
  height = 24
)

```



More items under construction...

The following performs of heat map visualization against protein data:

```

# Protein heat maps
prnHM(
  xmin = -1,
  xmax = 1,
  x_margin = 0.1,
  annot_cols = c("Group", "Color", "Alpha", "Shape"),
  annot_colnames = c("Group", "Lab", "Batch", "WHIM"),
  cluster_rows = TRUE,
  cutree_rows = 10,
  show_rownames = FALSE,
  show_colnames = TRUE,

```

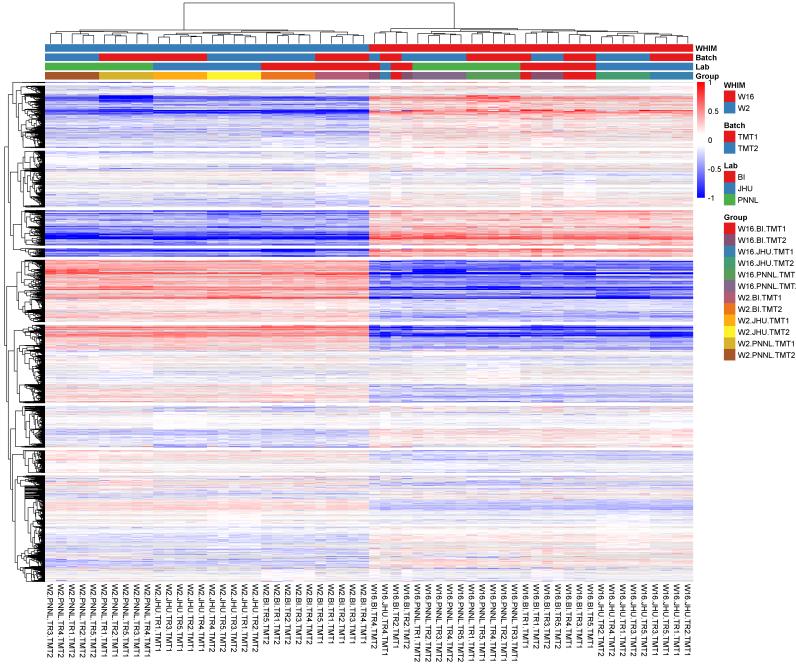


Figure 5: Protein\_heatmap

```
fontsize_row = 3,
cellwidth = 14,
width = 18,
height = 12
)
```

### Significance tests and volcano plot visualization

In this section, we perform the significance analysis of peptide and protein data. The approach of contrast fit is used in proteoQ (Chambers, J. M. (1992) Linear models; limma, Gordon Smith). We first define the contrast groups for significance tests. For this purpose, I have devided the samples by their WHIM subtypes, laboratory locations and batch numbers. This ends up with entries of W2.BI.TMT1, W2.BI.TMT2 etc. under the `expt_smry.xlsx::Term` column. The interactive environment between the Excel file and the proteoQ tool allows us to enter more columns of contrasts when needed. For instance, we might also be interested in a more course comparison of inter-laboratory differences without batch effects. The corresponding contrasts of W2.BI, W2.BI etc. can be found under a pre-made column, `Term_2`. Having these columns in hand, we are now ready to perform significance tests for peptides and protein species. In the demo, we will analyze protein data and perform volcano plot visualization:

```
# Protein significance tests
prnSig(
impute_na = FALSE,
W2_bat = ~ Term["(W2.BI.TMT2-W2.BI.TMT1)", "(W2.JHU.TMT2-W2.JHU.TMT1)", "(W2.PNNL.TMT2-W2.PNNL.TMT1)", # W2_loc_bat = ~ Term["((W2.BI.TMT1-W2.JHU.TMT1)-(W2.BI.TMT2-W2.JHU.TMT2))", "((W2.BI.TMT1-W2.PNNL.TMT1-W2_loc = ~ Term_2["W2.BI-W2.JHU", "W2.BI-W2.PNNL", "W2.JHU-W2.PNNL"] # location effects
)
```

## TMT2 – TMT1 (n = 16, 917)

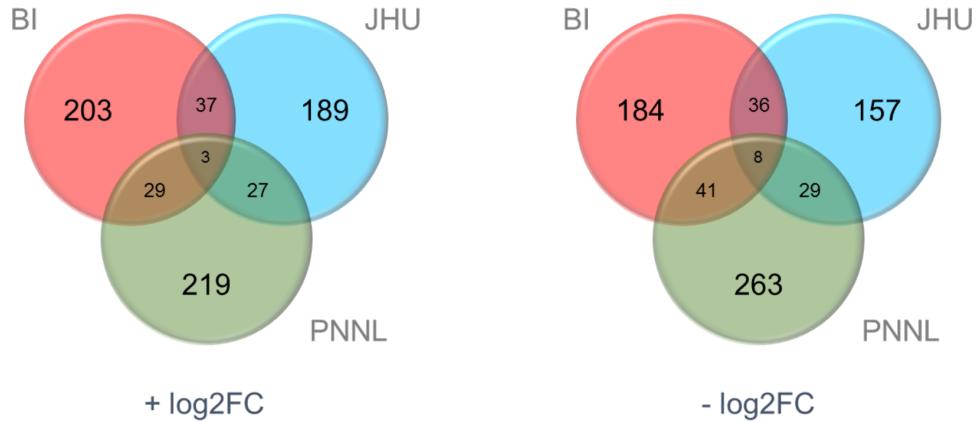
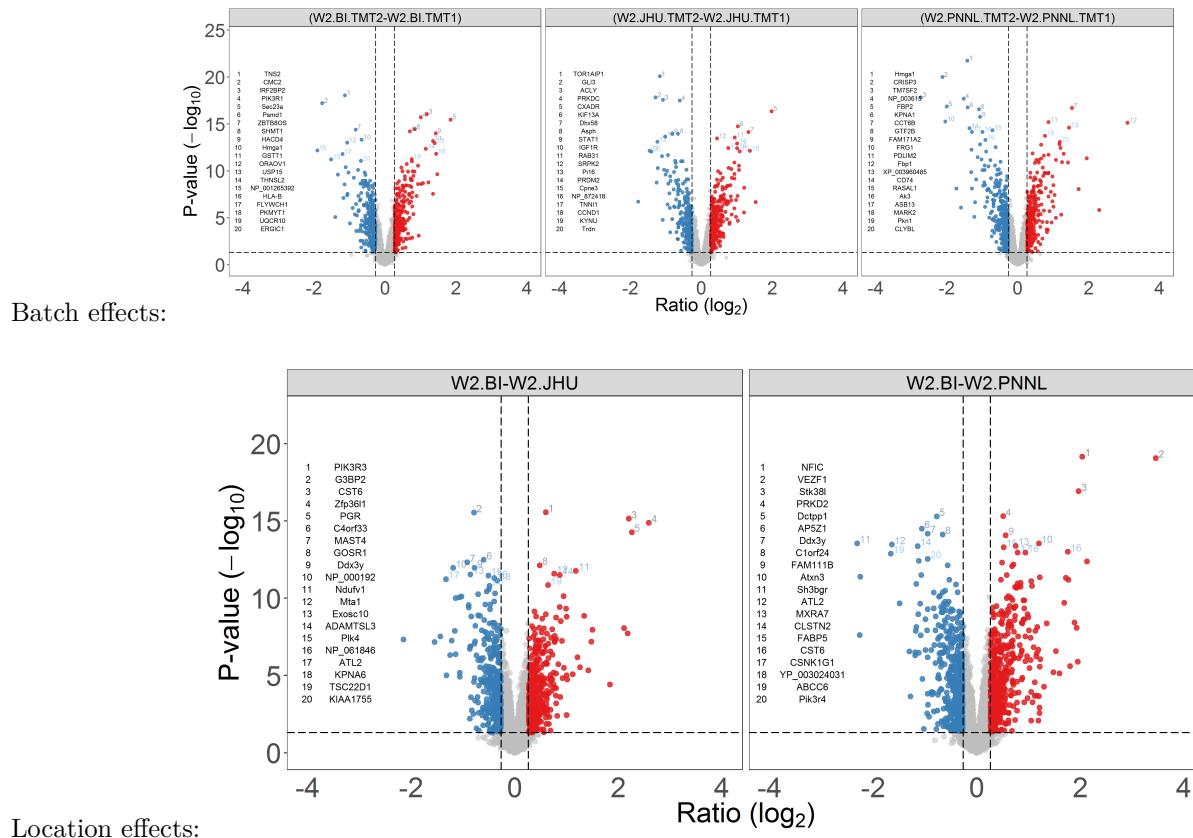


Figure 6: img\_venn\_batches

```
# Volcano plots
prnVol()
```

Note that we have informed the prnSig function to look for contrasts under columns Term and Term\_2, followed by the contrast pairs in square brackets. Pairs of contrasts are separated by comma.



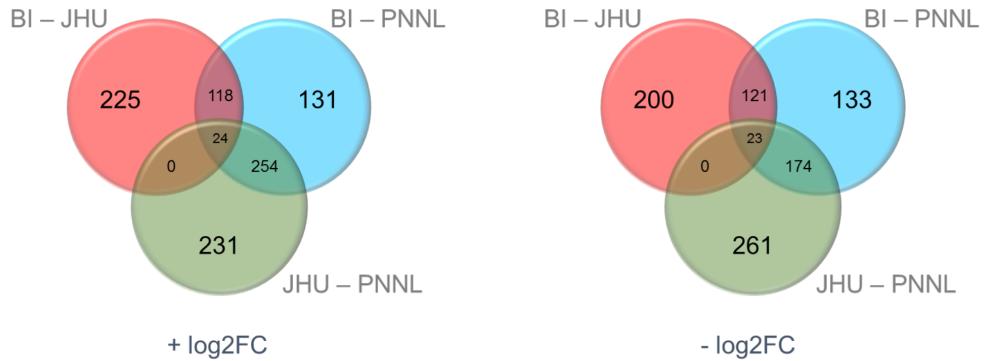


Figure 7: img\_venn\_location

The following performs the imputation of peptide and protein data:

```
# Impute missing values
pepImp(m = 2, maxit = 2)
prnImp(m = 5, maxit = 5)
```

The following performs the trend analysis against protein expressions:

```
# Soft clustering in protein expressions by trends
anal_prnTrend(
  scale_log2r = TRUE,
  n_clust = 6
)

# Visualization of trends
plot_prnTrend()
```

The following performs the NMF analysis against protein data:

```
# Protein NMF
library(NMF)

# NMF analysis
anal_prnNMF(
  # col_group = Group, # optional a priori knowledge of sample groups
  scale_log2r = TRUE,
  r = 6,
  nrun = 200
)

# Consensus heat map
plot_prnNMFCon(
  r = 6,
  annot_cols = c("Color", "Alpha", "Shape"),
  annot_colnames = c("Lab", "Batch", "WHIM"),
  width = 10,
  height = 10
)
```

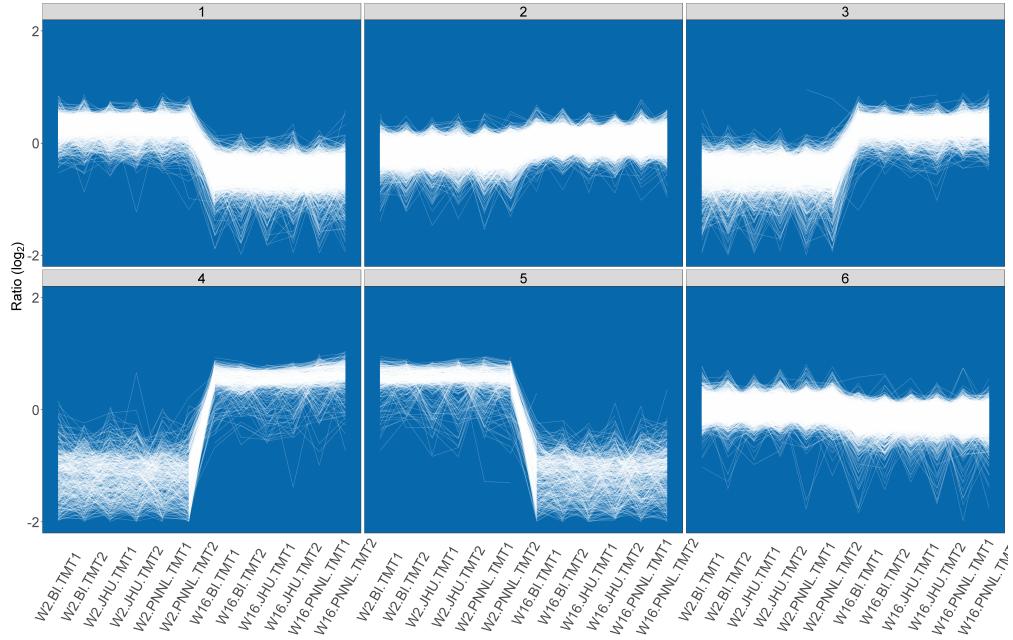


Figure 8: Protein\_trends

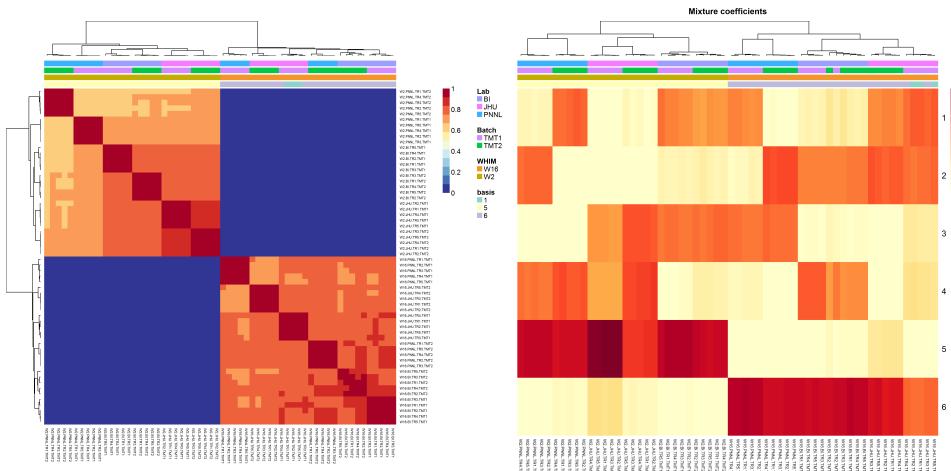
```

# Coefficient heat map
plot_prnNMFCoef(
r = 6,
annot_cols = c("Color", "Alpha", "Shape"),
annot_colnames = c("Lab", "Batch", "WHIM"),
width = 10,
height = 10
)

# Metagene heat map(s)
plot_metaNMF(
r = 6,
annot_cols = c("Color", "Alpha", "Shape"),
annot_colnames = c("Lab", "Batch", "WHIM"),

fontsize = 8,
fontsize_col = 5
)

```



The following performs GSVA:

```
prnGSVA(
  scale_log2r = TRUE,
  gset_nm = c("go_sets",
    # "kegg_sets",
    "c2_msig")
)
```

The following maps gene sets under the environment of volcano plot visualization:

```
gsvaMap(
  scale_log2r = TRUE,
  pval_cutoff = 1E-5,
  show_sig = "pVal"
)
```

### Lab: Choices of references

In this lab, we explore the effects of reference choices on data normalization. We first copy data over to the file directory specified by `temp_dir`, followed by PSM, peptide normalization and histogram visualization of peptide `log2FC`.

```
# directory setup
temp_dir <- "c:\\\\The\\\\W2_ref\\\\Example"
library(proteoQDA)
cptac_csv_1(temp_dir)
cptac_expt_ref_w2(temp_dir)
cptac_frac_1(temp_dir)

# analysis
library(proteoQ)
load_expts(temp_dir, expt_smry_ref_w2.xlsx)

normPSM()
```

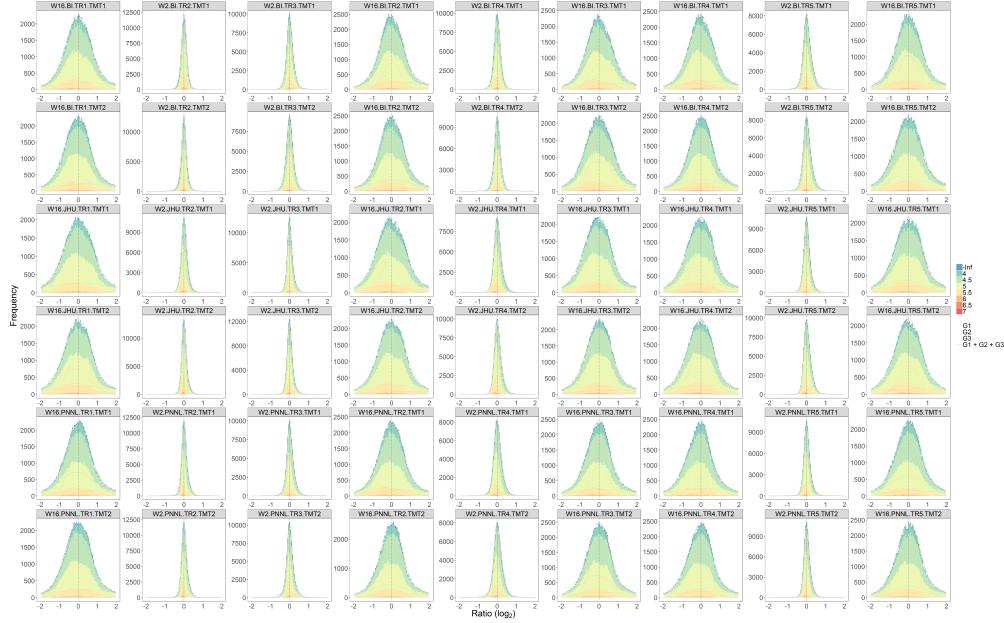


Figure 9: Peptide\_reference\_effect\_1

```

normPep(
  id = pep_seq,
  method_psm_pep = median,
  method_align = MGKernel,
  range_log2r = c(5, 95),
  range_int = c(5, 95),
  n_comp = 3,
  seed = 911,
  maxit = 200,
  epsilon = 1e-05
)

# visualization
pepHist(
  scale_log2r = FALSE,
  ncol = 9
)

```

Notice that in the above histogram the log2FC profiles of WHIM2 samples are much narrower than those of WHIM16 (**Figure S1A**). This will occur when a reference is more similar to one group of sample(s) than the other. In our case, the reference is one of WHIM2. The difference in the breadth of log2FC profiles between the WHIM16 and the WHIM2 groups is likely due to the genuine difference in their proteomes. If the above argument is valid, a scaling normalize would moderate, and thus bias, the quantitative difference in proteomes between WHIM2 and WHIM16.

We alternatively seek a “center-of-mass” representation for uses as references. We select one WHIM2 and one WHIM16 from each 10-plex TMT. The proteoQ tool will average the signals from designated references. Therefore, the derived reference can be viewed as a mid point of the WHIM2 and the WHIM16 proteomes. We next perform analogously the data summary and histogram visualization. With the new reference, we have achieved log2FC profiles that are more comparable in breadth between WHIM2 and WHIM16 samples. With the new reference, a scaling normalization may be suitable at later steps.

```

# directory setup
temp_dir_2 <- "c:\\The\\W2_W16_ref\\Example"
library(proteoQDA)
cptac_csv_1(temp_dir_2)
expt_smry_ref_w2_w16(temp_dir_2)
cptac_frac_1(temp_dir_2)

# analysis
library(proteoQ)
load_expts(temp_dir_2, expt_smry_ref_w2_w16.xlsx)

normPSM()

normPep(
  id = pep_seq,
  method_psm_pep = median,
  method_align = MGKernel,
  range_log2r = c(5, 95),
  range_int = c(5, 95),
  n_comp = 3,
  seed = 911,
  maxit = 200,
  epsilon = 1e-05
)

# visualization
pepHist(
  scale_log2r = FALSE,
  ncol = 8
)

```

## Lab: Peptide subsets

In addition to the global proteomes, the CPTAC publication contains phosphopeptide data from the same samples.(2018) In this lab, we will explore the stoichiometry of phosphopeptide subsets in relative to the combined data sets of `global + phospho` peptides. We first performed a search aganist the combined `global and phospho` data. The search results are available in `proteoQDA`. We next copy the result files over, followed by the analysis and visualization of the BI subset:

```

# directory setup
temp_phospho_dir <- "c:\\The\\Phosphopeptide\\Example"
library(proteoQDA)
cptac_csv_2(temp_phospho_dir)
cptac_expt_2(temp_phospho_dir)
cptac_frac_2(temp_phospho_dir)

# analysis
library(proteoQ)
load_expts(temp_phospho_dir, expt_smry.xlsx)

normPSM()

```

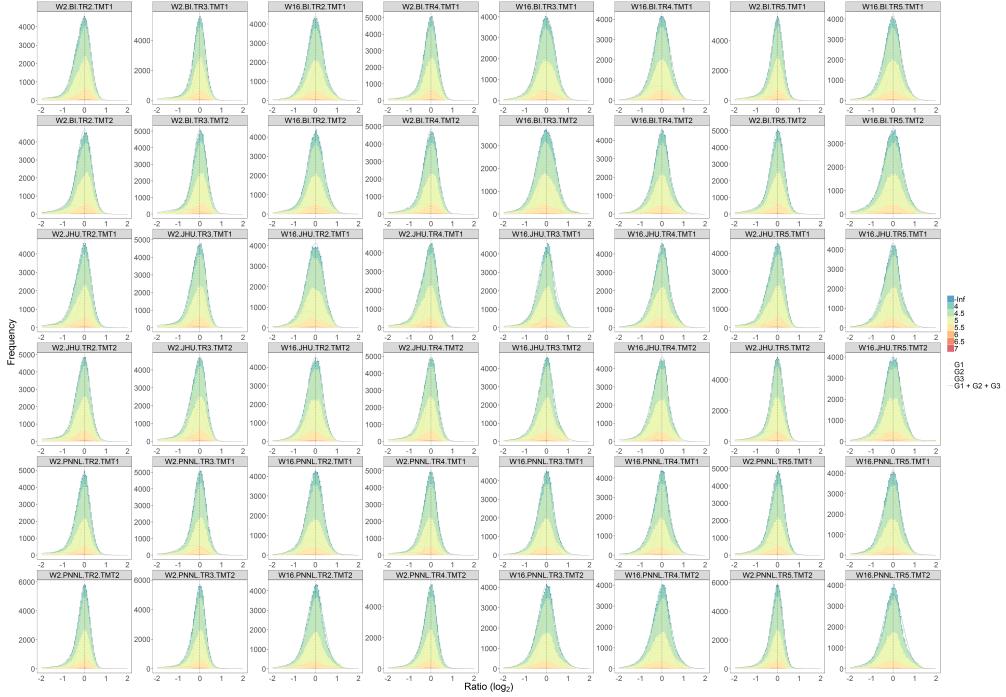


Figure 10: Peptide\_reference\_effect\_2

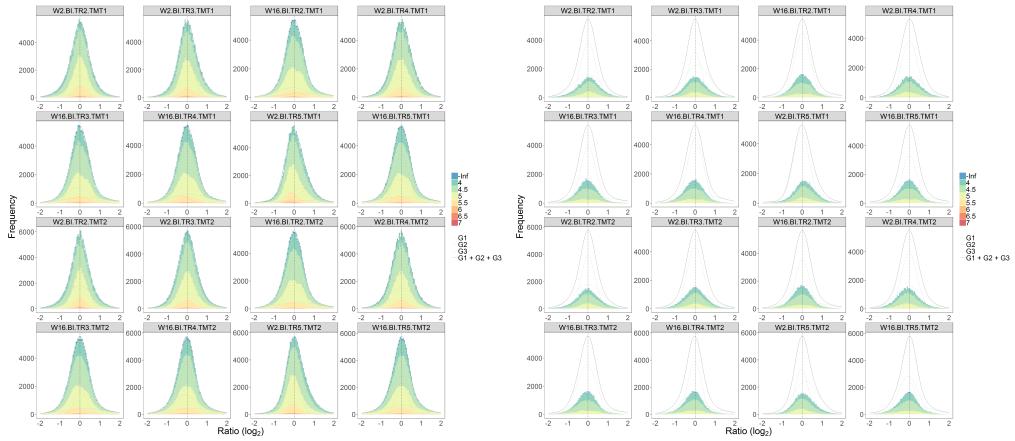
```

normPep(
  id = pep_seq_mod, # peptides with different variable modifications
  method_psm_pep = median,
  method_align = MGKernel,
  range_log2r = c(5, 95),
  range_int = c(5, 95),
  n_comp = 3,
  seed = 749662,
  maxit = 200,
  epsilon = 1e-05
)

# all peptides
pepHist(
  col_select = BI,
  scale_log2r = TRUE,
  ncol = 4,
  filename = "BI_all_peptides.png"
)

# phospho subsets
pepHist(
  col_select = BI,
  scale_log2r = TRUE,
  pep_pattern = "sty",
  ncol = 4,
  filename = "BI_pSTY.png"
)

```



## References

- Philipp, Martins. 2018. "Reproducible Workflow for Multiplexed Deep-Scale Proteome and Phosphoproteome Analysis of Tumor Tissues by Liquid Chromatography-Mass Spectrometry." *Nature Protocols* 13 (7): 1632–61. <https://doi.org/10.1038/s41596-018-0006-9>.
- Wang, Y. 2011. "Reversed-Phase Chromatography with Multiple Fraction Concatenation Strategy for Proteome Profiling of Human MCF10A Cells." *Proteomics*. 11 (10): 2019–26. <https://doi.org/10.1002/pmic.201000722>.