isobar for quantification of PTM datasets

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Contents

1 Introduction

isobar [1] version 2 includes modules to facilitate PTM quantification. This vignette describes its parts, and how to use it to generate quantification reports.

```
>   library(isobar) ## load the isobar package
```

Using isobar, automatic report generation is straight-forward given proper input files using the script report/create_reports.R. When called, it parses the globabl properties file report/properties.R and then the properties.R in the current directory. Below is a small example properties.R for creating a PDF Quality Control and XLSX analysis report:

```
type="iTRAQ4plexSpectra"
## peaklist files for quantitation, by default all mgf file in directory
peaklist=list.files(pattern="*\\.mgf$")

## id files, by default all id.csv files in directory
identifications=list.files(pattern="*\\.id.csv$")

modif="PHOS" # modification to track (eg PHOS, ACET, MET)
ptm.info.f <- getPtmInfoFromNextprot
spreadsheet.format="xlsx"
```

Reports will be generated calling path_to_isobar/report/create_reports.R -peptide from the directory containing the peaklists, identifications and properties.R.

2 Modification Site Localization

isobar supports PhosphoRS [5] and Delta Score [4] for modification site localization.

PhosphoRS integration The standalone Java version of PhosphoRS can be downloaded from http://cores.imp.ac.at/uploads/media/PhosphoRS.zip. It features a command line interface to a script which rescores localizations of the modification for each peptide-spectrum match. It uses XML files for input and output, which can be generated and parsed by isobar.

```
> # Generate PhosphoRS XML input file based on MGF and identification file
> #  massTolerance: fragment ion mass tolerance (in Da)
> #  activationType: CID, HCD, or ETD
> writePhosphoRSInput("phosphors.in.xml",
+                     "identifications.id.csv","peaklist.mgf",
+                      massTolerance=0.5,activationType="CID")
```

After calling PhosphoRS (java -jar phosphoRS.jar phosphors.in.xml phosphors.out.xml), the resulting XML file can be read:

```
> # Read PhosphoRS XML output file
> #   simplify: if TRUE, a data.frame is returned, else a list
> #   besthit.only: if TRUE, only the best localization per spectrum is returned
> readPhosphoRSOutput("phosphors.out.xml",simplify=TRUE,besthit.only=TRUE)
```

getPhosphoRSProbabilities is a convenience function calling the writer, the script, and the reader in succession.

```
> getPhosphoRSProbabilities("identifications.id.csv","peaklist.mgf",
+                           massTolerance=0.5,activationType="CID",
+                           phosphors.cmd="java -jar phosphoRS.jar")
```

Delta Score calculation The Mascot Delta Score can be calculated directly by the parser mascotParser2.pl and thresholded (*e. g.* -minDeltaScore=10). For CSV identification files which contain all hits for each spectrum (not just the best one), the function calc.delta.score within the R package is provided.

Using PhosphoRS and Delta Score in Report Generation. When generating an IBSpectra object from peaklist and identifications, via readIBSpectra's argument annotate.spectra.f a function can be plugged in to extend or modify the identification information. This can be used to calculate scores and filter localization scores with filterSpectraDeltaScore) or annotateSpectraPhosphoRS.

```
> # filterSpectraDeltaScore calls calc.delta.score
> #   if no column named delta.score is present in the data frame
> # identifications below a min.delta.score are REMOVED
> ib <- readIBSpectra("identifications.id.csv","peaklist.mgf",
+                        annotate.spectra.f=function(...)
+                          filterSpectraDeltaScore(...,min.delta.score=10))
> # filterSpectraPhosphoRS calls PhosphoRS to caluclate PhosphoRS probabilities
> # identifications below a min.prob (PhosphoRS peptide isoform probability)
> # are marked to be NOT QUANTIFIED (use.for.quant=FALSE), but not removed
> ib <- readIBSpectra("identifications.id.csv","peaklist.mgf",
+                        annotate.spectra.f=
+                          function(...) filterSpectraPhosphoRS(...,min.prob=0.9,
+                             phosphors.cmd="java -jar PhosphoRS.jar"))
```

This can be used in report generation, too, where the `readIBSpectra.args` can be set accordingly in the report properties file `properties.R`:

```
readIBSpectra.args = list(annotate.spectra.f=filterSpectraDeltaScore)
```

or

```
readIBSpectra.args = list(annotate.spectra.f=filterSpectraPhosphoRS)
```

3 Peptide Ratio Calculation

All functions which are available to calculate ratios on protein level can also be used for peptides. The same noise model is appropriate for both.

```
> data(ib_phospho)
> data(noise.model.hcd)
> head(proteinGroup(ib_phospho)@peptideInfo)

      protein              peptide start.pos
2072 A1L390-1      SPLSPTETFSWPDVR      1037
2074 A1L390-2      SPLSPTETFSWPDVR       570
2076 A1L390-3      SPLSPTETFSWPDVR       981
1299   A6NKT7         LLLDLPLQTPHK      1170
783    O00264 GDQPAASGDSDDDEPPPLPR        48
2045 O14497-1            SPFLHSGMK      1604
                                             modif
2072      iTRAQ4plex_Nterm:PHOS:::PHOS::::::::::::
2074      iTRAQ4plex_Nterm:PHOS:::PHOS::::::::::::
2076      iTRAQ4plex_Nterm:PHOS:::PHOS::::::::::::
1299 iTRAQ4plex_Nterm:::::::::PHOS:::iTRAQ4plex_K:
783      iTRAQ4plex_Nterm:::::::PHOS::::::::::::::
2045    iTRAQ4plex_Nterm::::::PHOS:::iTRAQ4plex_K:

> 10^estimateRatio(ib_phospho,noise.model.hcd,peptide="SPLSPTETFSWPDVR")
```

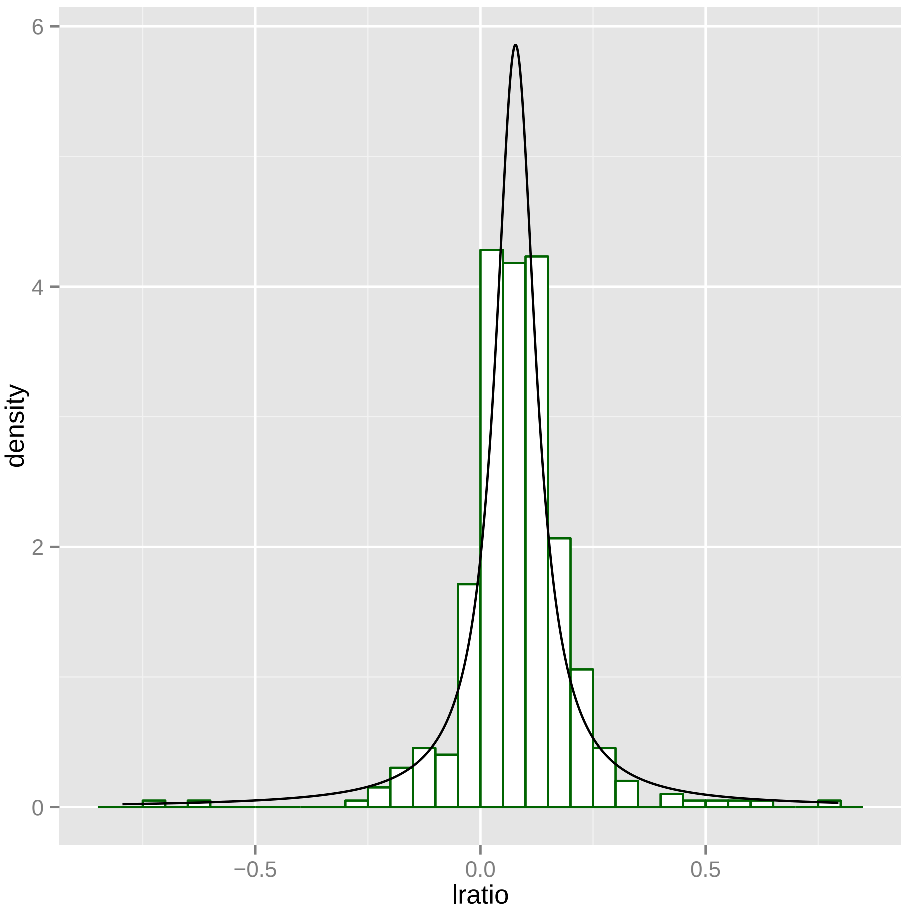
```
          114       115       116      117
114 1.0000000 0.3088721 1.4354943 1.641885
115 3.2375859 1.0000000 4.6497966 5.318776
116 0.6966241 0.2150632 1.0000000 1.143867
117 0.6090561 0.1880132 0.8742276 1.000000
```

By giving a matrix to `estimateRatio`, we can calculate ratios for peptides with specific modifications:

```
> pep.n.modif <- unique(apply(fData(ib_phospho)[,c("peptide","modif")],2,cbind))
> print(head(pep.n.modif))

     peptide
[1,] "AAATPESQEPQAK"
[2,] "AAEAGGAEEQYGFLTTPTK"
[3,] "AAEEQGDDQDSEK"
[4,] "AAPPPGSPAK"
[5,] "AAVGQESPGGLEAGNAK"
[6,] "AAVLSDSEDEEK"
     modif
[1,] "iTRAQ4plex_Nterm:::::::PHOS::::::iTRAQ4plex_K:"
[2,] "iTRAQ4plex_Nterm:::::::::::PHOS::::::::iTRAQ4plex_K:"
[3,] "iTRAQ4plex_Nterm:::::::::::PHOS::iTRAQ4plex_K:"
[4,] "iTRAQ4plex_Nterm:::::::PHOS:::iTRAQ4plex_K:"
[5,] "iTRAQ4plex_Nterm:::::::PHOS::::::::::iTRAQ4plex_K:"
[6,] "iTRAQ4plex_Nterm:::::PHOS::PHOS:::::iTRAQ4plex_K:"

> estimateRatio(ib_phospho,noise.model.hcd,channel1="114",channel2="115",
+                  peptide=head(pep.n.modif),combine=FALSE)[,c("lratio","variance",
+                                                             "n.spectra","p.value.rat")]

      lratio   variance n.spectra  p.value.rat
1 -0.6978020 0.01034090         2 3.394310e-12
2        NaN        Inf         0          NaN
3  0.1388425 0.01052788         2 8.800084e-02
4 -1.0793665 0.04166971         1 6.196545e-08
5 -0.9655771 0.02406589         1 2.419553e-10
6 -0.2164083 0.08305527         7 2.263522e-01

>
```

A ratio distribution can be calculated based on peptide ratios:

```
> suppressPackageStartupMessages(library(distr))
> suppressPackageStartupMessages(library(ggplot2))
> peptide.ratios <- peptideRatios(ib_phospho,noise.model=noise.model.hcd,
+                                    cmbn=matrix(c("114","116"),ncol=1))
> lim <- max(abs(peptide.ratios$lratio),na.rm=TRUE)
> peptide.distr.cauchy <- fitCauchy(peptide.ratios$lratio)
> pseq <- seq(from=-lim,to=lim,length.out=1000)
> ggplot() +
+   geom_histogram(aes(x=lratio,y=..density..),data=peptide.ratios,binwidth=0.05,
+                  color="darkgreen",fill="white") +
+   geom_line(aes(x=x,y=y),color="black",
+             data=data.frame(x=pseq,y=d(peptide.distr.cauchy)(pseq)))
```



Correction with protein ratios. The observed change in concentration of modified peptides in one condition versus another is integrating two separate modes of regulation [6]:

1. Protein expression change
2. Modification state change

In many cases, it thus can be advisable to conduct separate MS quantification runs of the peptides enriched for the modification of interest, AND the global proteome quantification.

In the report generation, data from other experiments can be integrated using the property `compare.to.quant` in `properties.R`:

```
load("../proteome/quant.tbl.rda")         # load proteome quantification table
compare.to.quant=list(proteome=quant.tbl) # set property
rm(quant.tbl)
```

Peptide ratios can also be corrected with proteome ratios of a separate experiment, when giving as `peptide` argument a `matrix` or `data.frame` with columns for 'peptide', 'modif', and 'correct.ratio'. 'correct.ratio' is a log_{10} ratio which will be used to adjust the one calculated for the specific modified peptide.

```
> peptides <- pep.n.modif[1:5,]
> orig.ratio <- estimateRatio(ib_phospho,noise.model.hcd,channel1="114",channel2="115",
+                               peptide=peptides,combine=FALSE)[,c("lratio","variance")]
> peptides.c <- cbind(peptides,correct.ratio=c(0,-1,1,2,-2))
> corr.ratio <- estimateRatio(ib_phospho,noise.model.hcd,channel1="114",channel2="115",
+                               peptide=peptides.c,combine=FALSE)[,c("lratio","variance")]
> data.frame(peptides.c,orig.ratio,corr.ratio)
```

```
              peptide                                                modif
1       AAATPESQEPQAK       iTRAQ4plex_Nterm:::::::PHOS::::::iTRAQ4plex_K:
2 AAEAGGAEEQYGFLTTPTK iTRAQ4plex_Nterm:::::::::::PHOS::::::::iTRAQ4plex_K:
3       AAEEQGDDQDSEK       iTRAQ4plex_Nterm:::::::::::PHOS::iTRAQ4plex_K:
4          AAPPPGSPAK          iTRAQ4plex_Nterm:::::::PHOS:::iTRAQ4plex_K:
5   AAVGQESPGGLEAGNAK   iTRAQ4plex_Nterm:::::::PHOS::::::::::iTRAQ4plex_K:
  correct.ratio     lratio   variance   lratio.1 variance.1
1             0 -0.6978020 0.01034090 -0.3736099  0.1962761
2            -1        NaN        Inf -0.3736099  0.1962761
3             1  0.1388425 0.01052788 -0.3736099  0.1962761
4             2 -1.0793665 0.04166971 -0.3736099  0.1962761
5            -2 -0.9655771 0.02406589 -0.3736099  0.1962761
```

As appearent, the variance stays the same also for corrected ratios. If a fourth column `variance` of the `peptide` argument reports the variance of the correction ratio, it is added to the calculated ratio's variance (assuming independence).

4 Harvesting public PTM databases

neXtProt [3] and PhosphoSitePlus [2] provide information on experimentally determined post-translational modifications. neXtProt focuses on man, and PhosphoSitePlus on man and mouse. Both are manually curated and annotate thousands of residues of post-translationally modified proteins.

`isobar` provides functions to gather their information on identified proteins.

```
> ptm.info <- getPtmInfoFromPhosphoSitePlus(proteinGroup(ib_phospho),modif="PHOS")
> ptm.info <- getPtmInfoFromNextprot(proteinGroup(ib_phospho))
> head(ptm.info)

     .id isoform_ac quality   description evidence first_position last_position
1 A1L390   A1L390-1    GOLD Phosphoserine      EXP             76            76
2 A1L390   A1L390-1  SILVER Phosphoserine      EXP            433           433
3 A1L390   A1L390-1    GOLD Phosphoserine  Curated            533           533
4 A1L390   A1L390-1    GOLD Phosphoserine      EXP            576           576
5 A1L390   A1L390-1    GOLD Phosphoserine      EXP            577           577
6 A1L390   A1L390-1  SILVER Phosphoserine      EXP            614           614
  modification.name modification.accession position
1     Phosphoserine               PTM-0253       76
2     Phosphoserine               PTM-0253      433
3     Phosphoserine               PTM-0253      533
4     Phosphoserine               PTM-0253      576
5     Phosphoserine               PTM-0253      577
6     Phosphoserine               PTM-0253      614
```

For reports, the function can be selected via the property `ptm.info.f` in `properties.R`:

```
protein.info.f = getPtmInfoFromNextprot
```

For PhosphoSitePlus, define the modification to get the correct dataset:

```
ptm.info.f <- function(...) getPtmInfoFromPhosphoSitePlus(...,modification="PHOS")
```

PhosphoSitePlus datasets will be downloaded from their website to 'Phosphorylation_site_dataset.gz' or 'Acetylation_site_dataset.gz', etc (see mapping property of getPtmInfoFromPhosphoSitePlus) unless a file with that name exists.

References

[1] F. P. Breitwieser, A. Müller, L. Dayon, T. Köcher, A. Hainard, P. Pichler, U. Schmidt-Erfurth, G. Superti-Furga, J.-C. Sanchez, K. Mechtler, K. L. Bennett, and J. Colinge. General statistical modeling of data from protein relative expression isobaric tags. *J Proteome Res*, 10(6):2758–2766, Jun 2011.

[2] P. V. Hornbeck, J. M. Kornhauser, S. Tkachev, B. Zhang, E. Skrzypek, B. Murray, V. Latham, and M. Sullivan. Phosphositeplus: a comprehensive resource for investigating the structure and function of experimentally determined post-translational modifications in man and mouse. *Nucleic Acids Res*, 40(Database issue):D261–D270, Jan 2012.

[3] L. Lane, G. Argoud-Puy, A. Britan, I. Cusin, P. D. Duek, O. Evalet, A. Gateau, P. Gaudet, A. Gleizes, A. Masselot, C. Zwahlen, and A. Bairoch. nextprot: a knowledge platform for human proteins. *Nucleic Acids Res*, 40(Database issue):D76–D83, Jan 2012.

[4] M. M. Savitski, S. Lemeer, M. Boesche, M. Lang, T. Mathieson, M. Bantscheff, and B. Kuster. Confident phosphorylation site localization using the mascot delta score. *Mol Cell Proteomics*, 10(2):M110.003830, Feb 2011.

[5] T. Taus, T. Köcher, P. Pichler, C. Paschke, A. Schmidt, C. Henrich, and K. Mechtler. Universal and confident phosphorylation site localization using phosphors. *J Proteome Res*, Nov 2011.

[6] R. Wu, N. Dephoure, W. Haas, E. L. Huttlin, B. Zhai, M. E. Sowa, and S. P. Gygi. Correct interpretation of comprehensive phosphorylation dynamics requires normalization by protein expression changes. *Mol Cell Proteomics*, 10(8):M111.009654, Aug 2011.