

# Package ‘Pirat’

February 21, 2025

**Type** Package

**Title** Precursor or Peptide Imputation under Random Truncation

**Description** Pirat enables the imputation of missing values (either MNARs or MCARs) in bottom-up LC-MS/MS proteomics data using a penalized maximum likelihood strategy. It does not require any parameter tuning, it models the instrument censorship from the data available. It accounts for sibling peptides correlations and it can leverage complementary transcriptomics measurements.

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**BiocViews** Proteomics, MassSpectrometry

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|          |  |
|----------|--|
| envPirat | <i>Creates a BasiliskEnvironment class</i> |
|----------|--|

---

### Description

Please refer to the package ‘basilisk’.

### Usage

```
envPirat
```

### Format

An object of class BasiliskEnvironment of length 1.

**Value**

An instance of the class 'BasiliskEnvironment'

---

|                |  |
|----------------|--|
| estimate_gamma | <i>Estimate missingness parameters Gamma</i> |
|----------------|--|

---

**Description**

Estimate missingness parameters Gamma

**Usage**

```
estimate_gamma(pep.ab.table, mcar = FALSE)
```

**Arguments**

|              |  |
|--------------|--|
| pep.ab.table | The peptide or precursor abundance matrix, with molecules in columns and samples in row. |
| mcar         | If TRUE, forces gamma_1 = 0.   |

**Value**

A list of the containing missingness parameters gamma\_0 and gamma\_1.

**Examples**

```
data(subbouyssie)
estimate_gamma(subbouyssie$peptides_ab)
```

---

|                 |  |
|-----------------|--|
| estimate_psi_df | <i>Estimate psi and degrees of freedom</i> |
|-----------------|--|

---

**Description**

Estimate the inverse-gamma parameters from the distribution of observed peptide variances in an abundance table.

**Usage**

```
estimate_psi_df(pep.ab.table)
```

**Arguments**

|              |   |
|--------------|---|
| pep.ab.table | The peptide or precursor abundance matrix, with molecules in columns and samples in row (can contain missing values). |
|--------------|---|

**Value**

List containing estimated fitted hyperparameters df (degrees of freedom) and psi (inverse scale).

**Examples**

```
data(subbouyssie)
obj <- subbouyssie
# Keep only fully observed peptides
obs2NApep <- obj$peptides_ab[ ,colSums(is.na(obj$peptides_ab)) <= 0]
estimate_psi_df(obs2NApep)
```

---

get\_indexes\_embedded\_prot

*Indexes of PGs embedded in each others*

---

**Description**

Returns indexes of PGs that are embedded in others

**Usage**

```
get_indexes_embedded_prot(adj)
```

**Arguments**

adj                    An adjacency matrix between precursors/peptides and PGs

**Value**

A vector of indices

**Examples**

```
data(subbouyssie)
get_indexes_embedded_prot(subbouyssie$adj)
```

---

```
impute_block_llk_reset
```

*Impute each PG.*

---

### Description

Imputes each PG separately and return the results for each PG.

### Usage

```
impute_block_llk_reset(
  data.pep.rna.crop,
  psi,
  pep_ab_or = NULL,
  df = 1,
  nu_factor = 2,
  max_pg_size = NULL,
  min.pg.size2imp = 1,
  verbose = FALSE,
  ...
)
```

### Arguments

|                                |   |
|--------------------------------|---|
| <code>data.pep.rna.crop</code> | A list representing dataset   |
| <code>psi</code>               | Inverse scale parameter for IW prior of peptides abundances   |
| <code>pep_ab_or</code>         | In case we impute a dataset with pseudo-MVS, we can provide the ground truth abundance table, such that imputation will be done only for pseudo-MVs. This will accelerate imputation algorithm. |
| <code>df</code>                | Estimate degree of freedom of the IG distribution fitted on observed variance.  |
| <code>nu_factor</code>         | Multiplication factor on degree of freedom. 2 by default.   |
| <code>max_pg_size</code>       | Maximum PGs size authorized for imputation. PG size is plitted if its size is above this threshold.   |
| <code>min.pg.size2imp</code>   | Minimum PG size to impute after splitting. PGs for which size is greater are not imputed. Should be lower than <code>max_pg_size</code> to have effect.   |
| <code>verbose</code>           | A boolean (FALSE as default) which indicates whether to display more details on the process   |
| <code>...</code>               | Additional arguments  |

### Value

A list containing imputation results for each PG, the execution time, and adjacency matrix between peptides and PGs corresponding to the imputed PGs.

**Examples**

```

Py_impute_block_llk_reset <- function(data.pep.rna.mis, psi) {
  proc <- basilisk::basiliskStart(envPirat)

  func <- basilisk::basiliskRun(proc,
    fun = function(arg1, arg2) {

      imputed_pgs <- Pirat::impute_block_llk_reset(arg1, arg2)
      imputed_pgs
    }, arg1 = data.pep.rna.mis, arg2 = psi)

  basilisk::basiliskStop(proc)
  func
}

data(subbouyssie)
obs2NApep <- subbouyssie$peptides_ab[, colSums(is.na(subbouyssie$peptides_ab)) <= 0]
res_hyperparam <- estimate_psi_df(obs2NApep)
psi <- res_hyperparam$psi
Py_impute_block_llk_reset(subbouyssie, psi)

```

---

```
impute_block_llk_reset_PG
```

*Impute each PG.*

---

**Description**

Imputes each PG separately accounting for transcriptomic dataset and returns the results for each PG.

**Usage**

```

impute_block_llk_reset_PG(
  data.pep.rna.crop,
  psi,
  psi_rna,
  rna.cond.mask,
  pep.cond.mask,
  pep_ab_or = NULL,
  df = 2,
  nu_factor = 1,
  max_pg_size = NULL,
  max.pg.size2imp = 1,
  verbose = FALSE,
  ...
)

```

**Arguments**

|                                |   |
|--------------------------------|---|
| <code>data.pep.rna.crop</code> | A list representing dataset, with mRNA normalized counts and mRNA/PGs adjacency table.  |
| <code>psi</code>               | Inverse scale parameter for IW prior of peptides abundances   |
| <code>psi_rna</code>           | Inverse scale parameter for IW prior of mRNA abundances   |
| <code>rna.cond.mask</code>     | Vector of size equal to the number of samples in mRNA abundance table, containing indices of conditions of each sample.   |
| <code>pep.cond.mask</code>     | Vector of size equal to the number of samples in peptide abundance table, containing indices of conditions of each sample.  |
| <code>pep_ab_or</code>         | In case we impute a dataset with pseudo-MVS, we can provide the ground truth abundance table, such that imputation will be done only for pseudo-MVs. This will accelerate imputation algorithm. |
| <code>df</code>                | Estimate degree of freedom of the IG distribution fitted on observed variance.  |
| <code>nu_factor</code>         | Multiplication factor on degree of freedom. 2 by default.   |
| <code>max_pg_size</code>       | Maximum PGs size authorized for imputation. PG size is splitted if its size is above this threshold.  |
| <code>max.pg.size2imp</code>   | Maximum PG size to impute after splitting. PGs for which size is greater are not imputed. Should be lower than <code>max_pg_size</code> to have effect.   |
| <code>verbose</code>           | A boolean (FALSE as default) which indicates whether to display more details on the process   |
| <code>...</code>               | Additional parameters   |

**Value**

A list containing imputation results for each PG, the execution time, and adjacency matrix between peptides and PGs corresponding to the imputed PGs.

**Examples**

```
Py_impute_block_llk_reset_PG <- function(data.pep.rna.crop, ...) {
  proc <- basilisk::basiliskStart(envPirat)

  func <- basilisk::basiliskRun(proc,
    fun = function(arg1, ...) {
      Pirat::impute_block_llk_reset_PG(arg1, ...)
    }, arg1 = data.pep.rna.crop, ...)
  basilisk::basiliskStop(proc)
  func
}

data(subprobers)
obj <- subprobers
# Keep only fully observed peptides
obs2NApep <- obj$peptides_ab[, colSums(is.na(obj$peptides_ab)) <= 0]
res_hyperparam_pep = estimate_psi_df(obs2NApep)
```

```

psi_pep <- res_hyperparam_pep$psi
obs2NArna <- obj$rnas_ab[,colSums(obj$rnas_ab == 0) <= 0]
res_hyperparam_rna = estimate_psi_df(obs2NArna)
psi_rna <- res_hyperparam_rna$psi
# paired proteomic transcriptomic setting
cond_mask <- seq(nrow(obj$peptides_ab))
imputed_pgs <- Py_impute_block_llk_reset_PG(
  data.pep.rna.crop = obj,
  psi = psi_pep,
  psi_rna = psi_rna,
  rna.cond.mask = cond_mask,
  pep.cond.mask = cond_mask)

```

---

|                    |  |
|--------------------|--|
| impute_from_blocks | <i>Impute abundance table from PGs results</i> |
|--------------------|--|

---

### Description

From imputation results in each PG and the associate adjacency peptide/PG matrix,imputes the original abundance table. .

### Usage

```
impute_from_blocks(logs.blocks, data.pep.rna, idx_blocks = NULL)
```

### Arguments

|              |  |
|--------------|--|
| logs.blocks  | List of PGs imputation results, that also contains related peptide/PGs adjacency matrix. |
| data.pep.rna | List representing the dataset not yet imputed  |
| idx_blocks   | Indices of PGs for which imputation results should be integrated                         |

### Value

The original peptide abundance table with imputed values.

### Examples

```

Py_impute_block_llk_reset <- function(data.pep.rna.mis, psi) {
  proc <- basilisk::basiliskStart(envPirat)

  func <- basilisk::basiliskRun(proc,
    fun = function(arg1, arg2) {

      imputed_pgs <- Pirat::impute_block_llk_reset(arg1, arg2)
      imputed_pgs
    }
  )
}

```



```
    }, arg1 = data.pep.rna.mis, arg2 = psi)

  basilisk::basiliskStop(proc)
  func
}

data(subbouyssie)
obj <- subbouyssie
# Keep only fully observed peptides
obs2NApep <- obj$peptides_ab[, colSums(is.na(obj$peptides_ab)) <= 0]
res_hyperparam <- estimate_psi_df(obs2NApep)
psi <- res_hyperparam$psi
imputed_pgs <- Py_impute_block_llk_reset(obj, psi)
impute_from_blocks(imputed_pgs, obj)
```

---

pipeline\_llkimpute      *Pirat imputation function*

---

## Description

Imputation pipeline of Pirat. First, it creates PGs. Then, it estimates parameters of the penalty term (that amounts to an inverse-Wishart prior). Second, it estimates the missingness mechanism parameters. Finally, it imputes the peptide/precursor-level dataset with desired extension.

## Usage

```
my_pipeline_llkimpute(data.pep.rna.mis, ...)

pipeline_llkimpute(
  data.pep.rna.mis,
  pep.ab.comp = NULL,
  alpha.factor = 2,
  rna.cond.mask = NULL,
  pep.cond.mask = NULL,
  extension = c("base", "2", "T", "S"),
  mcar = FALSE,
  degenerated = FALSE,
  max.pg.size.pirat.t = 1,
  verbose = FALSE
)
```

## Arguments

data.pep.rna.mis  
Parameter 'data.pep.rna.mis' of the function 'pipeline\_llkimpute()'

|                     |   |
|---------------------|---|
| ...                 | Additional parameters for the function 'pipeline_llkimpute()'   |
| pep.ab.comp         | The pseudo-complete peptide or precursor abundance matrix, with samples in row and peptides or precursors in column. Useful only in mask-and-impute experiments, if one wants to impute solely peptides containing pseudo-MVs.  |
| alpha.factor        | Factor that multiplies the parameter alpha of the penalty described in the original paper.  |
| rna.cond.mask       | Vector of indexes representing conditions of samples of mRNA table, only mandatory if extension == "T". For paired proteomic and transcriptomic tables, should be c(1:n_samples).   |
| pep.cond.mask       | Vector of indexes representing conditions of samples of mRNA table, only mandatory if extension == "T". For paired proteomic and transcriptomic tables, should be c(1:n_samples).   |
| extension           | If NULL (default), classical Pirat is applied. If "2", only imputes PGs containing at least 2 peptides or precursors, and remaining peptides are left unchanged. If "S", Pirat-S is applied, considering sample-wise correlations only for singleton PGs. If "T", Pirat-T is applied, thus requiring <code>**rnas_ab**</code> and <code>**adj_rna_pg**</code> in list <code>**data.pep.rna.mis**</code> , as well as non-NULL <code>**rna.cond.mask**</code> and <code>**pep.cond.mask**</code> . Also, the maximum size of PGs for which transcriptomic data can be used is controlled with <code>**max.pg.size.pirat.t**</code> . |
| mcar                | If TRUE, forces $\gamma_1 = 0$ , thus no MNAR mechanism is considered.  |
| degenerated         | If TRUE, applies Pirat-Degenerated (i.e. its univariate alternative) as described in original paper. Should not be TRUE unless for experimental purposes.   |
| max.pg.size.pirat.t | When extension == "T", the maximum PG size for which transcriptomic information is used for imputation.   |
| verbose             | A boolean (FALSE as default) which indicates whether to display more details on the process   |

**Value**

The imputed `**data.pep.rna.mis$peptides_ab**` table.

The imputed `**data.pep.rna.mis$peptides_ab**` table.

NA

**See Also**

[pipeline\_llkimpute()]

**Examples**

```
# Pirat classical mode
data(subbouyssie)
myResult <- my_pipeline_llkimpute(subbouyssie)

# Pirat with transcriptomic integration for singleton PGs
data(subprobers)
```

```

nsamples = nrow(subroppers$peptides_ab)
myResult <- my_pipeline_llkimpute(subroppers,
  extension = "T",
  rna.cond.mask = seq(nsamples),
  pep.cond.mask = seq(nsamples),
  max.pg.size.pirat.t = 1)

## Not run:
myResult <- pipeline_llkimpute(subbouyssie)

## End(Not run)

```

---

pirat2SE

*Convert Pirat dataset to SummarizedExperiment*


---

### Description

This function converts the original dataset structure into a `SummarizedExperiment`.

### Usage

```
pirat2SE(peptides_ab, adj, mask_prot_diff = NULL, mask_pep_diff = NULL)
```

### Arguments

|                             |  |
|-----------------------------|--|
| <code>peptides_ab</code>    | the peptide or precursor abundance matrix to impute, with samples in row and peptides or precursors in column;   |
| <code>adj</code>            | a <code>n_peptide x n_protein</code> adjacency matrix between peptides and proteins containing 0 and 1, or TRUE and FALSE. Can contain: <code>**rnas_ab**</code> , the mRNA normalized count matrix, with samples in row and mRNAs in column; <code>**adj_rna_pg**</code> , a <code>n_mrna x n_protein</code> adjacency matrix <code>n_mrna</code> and proteins containing 0 and 1, or TRUE and FALSE; |
| <code>mask_prot_diff</code> | (Optional) boolean vector of size equal to the number of proteins, indicating whether proteins are ground truth differentially abundant (typically in spike-in benchmark datasets).  |
| <code>mask_pep_diff</code>  | (Optional) boolean vector of size equal to the number of peptides, indicating whether peptides are ground truth differentially abundant (typically in spike-in benchmark datasets).  |

### Value

An instance of the class `'SummarizedExperiment'`

## Examples

```
data(subbouyssie)
peptides_ab <- subbouyssie$peptides_ab
adj <- subbouyssie$adj
mask_prot_diff <- subbouyssie$mask_prot_diff
mask_pep_diff <- subbouyssie$mask_pep_diff
obj <- pirat2SE(peptides_ab, adj, mask_prot_diff, mask_pep_diff )
obj
```

---

plot2hists

*Plot 2 histograms*

---

## Description

Plot 2 histograms on the same graph.

## Usage

```
plot2hists(
  d1,
  d2,
  name1 = "name1",
  name2 = "name2",
  titlename = "myTitle",
  xlab = "",
  freq = TRUE
)
```

## Arguments

|           |  |
|-----------|--|
| d1        | vector of values for the first histogram                                       |
| d2        | vector of values for the first histogram                                       |
| name1     | Label for first histogram  |
| name2     | Label for 2nd histogram  |
| titlename | Title of figure  |
| xlab      | X-axis label   |
| freq      | If True, bins heights correspond to raw counts, otherwise bins are normalized. |

## Value

A plot

**Examples**

```
v1 <- 1:10
v2 <- 5:25
plot2hists(v1, v2)
```

---

plot\_pep\_correlations *Empirical density of peptide correlations*

---

**Description**

Plot empirical densities of correlations between peptides within PG and at random, estimated by gaussian kernel. Note that only correlations between fully observed peptides are considered here.

**Usage**

```
plot_pep_correlations(pep.data, titlename = NULL, xlabel = "Correlations")
```

**Arguments**

|           |                              |
|-----------|------------------------------|
| pep.data  | List representing dataset    |
| titlename | Title of the graph displayed |
| xlabel    | Label of x-axis              |

**Value**

The ggplot2 graph

**Examples**

```
data(subbouyssie)
plot_pep_correlations(subbouyssie, 'test')
```

---

rm\_pg\_from\_idx\_merge\_pg

*Remove PGs by index and merge*

---

**Description**

Remove PG by index and merge transcripts (if transcriptomic information is available) of PG included in one another (under condition that they have peptide). Then it removes transcripts without PG. Do not remove peptides that are left without PG.

**Usage**

```
rm_pg_from_idx_merge_pg(l_pep_rna, pg_idx)
```

**Arguments**

`l_pep_rna`      A list representing dataset, formatted as in `pipeline_llkimpute` function  
`pg_idx`          Vector of indices

**Value**

A list representing dataset.

**Examples**

```
data(ropers)  
idxs_emb_prot = get_indexes_embedded_prot(ropers$adj)  
ropers_wo_emb_prot = rm_pg_from_idx_merge_pg(ropers, idxs_emb_prot)
```

---

ropers

*Ropers dataset*

---

**Description**

This dataset corresponds to ‘Ropers2021’ dataset, described in Pirat article.

**Format**

A list containing: - `peptides_ab`: numeric matrix of precursors log<sub>2</sub> abundances. - `adj`: adjacency matrix between peptides and PGs - `rnas_ab`: numeric matrix of gene expression log<sub>2</sub> counts from mRNA analysis. - `adj_rna_pg`: adjacency matrix between genes and PGs

**Value**

A dataset

**References**

Ropers, D., Couté, Y., Faure, L., Ferré, S., Labourdette, D., Shabani, A., Trouilh, L., Vasseur, P., Corre, G., Ferro, M., Teste, M. A., Geiselmann, J., & de Jong, H. (2021). Multiomics Study of Bacterial Growth Arrest in a Synthetic Biology Application. *ACS Synthetic Biology*, 10(11), 2910–2926. [https://doi.org/10.1021/ACSSYNBIO.1C00115/SUPPL\\_FILE/SB1C00115\\_SI\\_010.ZIP](https://doi.org/10.1021/ACSSYNBIO.1C00115/SUPPL_FILE/SB1C00115_SI_010.ZIP)

---

|                |                            |
|----------------|----------------------------|
| split_large_pg | <i>Split too large PGs</i> |
|----------------|----------------------------|

---

**Description**

Randomly splits PGs with too many peptides/precursors, while keeping other PGs untouched. The new PGs created all have size equal to size\_max. Hence, some peptides can be duplicated in the new PGs created.

**Usage**

```
split_large_pg(adj, size_max)
```

**Arguments**

|          |  |
|----------|--|
| adj      | Adjacency matrix between peptides and PGs. |
| size_max | Maximum PG size desired.                   |

**Value**

New adjacency matrix between peptides and PGs.

**Examples**

```
data(subbouyssie)
split.obj <- split_large_pg(subbouyssie$adj, 5)
```

---

|                   |   |
|-------------------|---|
| split_large_pg_PG | <i>Splits too large PGs in proteogenomics context</i> |
|-------------------|---|

---

**Description**

Randomly splits PGs with too many peptides/precursors, while keeping other PGs untouched, and adapts adjacency matrix between mRNA and PGs accordingly. The new PGs created all have size equal to size\_max (including peptides and mRNAs). Hence, some peptides and mRNA can be duplicated in the new PGs.

**Usage**

```
split_large_pg_PG(adj, size_max, adj_rna_pg)
```

**Arguments**

|            |  |
|------------|--|
| adj        | Adjacency matrix between peptides and PGs. |
| size_max   | Maximum PG size desired.                   |
| adj_rna_pg | Adjacency matrix between mRNA and PGs.     |

**Value**

List containing new adjacency matrix between peptides and PGs, and new adjacency matrix between mRNA and PGs.

**Examples**

```
data(subropers)
split.obj <- split_large_pg_PG(subropers$adj, 5, subropers$adj_rna_pg)
```

---

|             |                             |
|-------------|-----------------------------|
| subbouyssie | <i>Sub-Bouyssie dataset</i> |
|-------------|-----------------------------|

---

**Description**

This dataset is extracted from the original ‘Bouyssie2020’ dataset mentioned in Pirat article, where only 5 PGs were randomly selected.

**Format**

A list containing: - peptides\_ab: numeric matrix of peptide (or precursors) log2 abundances. - adj: adjacency matrix between peptides and PGs.

**Value**

A dataset

**References**

Bouyssié, D., Hesse, A. M., Mouton-Barbosa, E., Rompais, M., MacRon, C., Carapito, C., Gonzalez De Peredo, A., Couté, Y., Dupierris, V., Burel, A., Menetrey, J. P., Kalaitzakis, A., Poisat, J., Romdhani, A., Burlet-Schiltz, O., Cianférani, S., Garin, J., & Bruley, C. (2020). Proline: an efficient and user-friendly software suite for large-scale proteomics. *Bioinformatics*, 36(10), 3148–3155. <https://doi.org/10.1093/BIOINFORMATICS/BTAA118>

---

|           |                           |
|-----------|---------------------------|
| subropers | <i>Sub-Ropers dataset</i> |
|-----------|---------------------------|

---

**Description**

This dataset is extracted from the original ‘Ropers2021’ dataset described in Pirat article, where only 10 PGs were randomly selected.



**Format**

A list containing: - peptides\_ab: numeric matrix of peptide (or precursors) log2 abundances. - adj: adjacency matrix between peptides and PGs - rnas\_ab: numeric matrix of gene expression log2 counts from mRNA analysis. - adj\_rna\_pg: adjacency matrix between genes and PGs

**Value**

A dataset

**References**

Ropers, D., Couté, Y., Faure, L., Ferré, S., Labourdette, D., Shabani, A., Trouilh, L., Vasseur, P., Corre, G., Ferro, M., Teste, M. A., Geiselmann, J., & de Jong, H. (2021). Multiomics Study of Bacterial Growth Arrest in a Synthetic Biology Application. *ACS Synthetic Biology*, 10(11), 2910–2926. [https://doi.org/10.1021/ACSSYNBIO.1C00115/SUPPL\\_FILE/SB1C00115\\_SI\\_010.ZIP](https://doi.org/10.1021/ACSSYNBIO.1C00115/SUPPL_FILE/SB1C00115_SI_010.ZIP)

---

wrapper\_pipeline\_llkimpute

*Imputation method using SummarizedExperiment dataset*

---

**Description**

This function imputes data from an instance of the SummarizedExperiment structure data. After a conversion step, it calls the function ‘my\_pipeline\_llkimpute’.

**Usage**

```
wrapper_pipeline_llkimpute(se, ...)
```

**Arguments**

se                    An instance of the class SummarizedExperiment  
...                    Additional arguments to pass to ‘my\_pipeline\_llkimpute()’

**Value**

See my\_pipeline\_llkimpute() function

**Examples**

```
data(subbouyssie)  
obj <- pirat2SE(subbouyssie$peptides_ab, subbouyssie$adj,  
subbouyssie$mask_prot_diff, subbouyssie$mask_pep_diff )  
res <- wrapper_pipeline_llkimpute(obj)
```

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