

# Package ‘PhosR’

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**Type** Package

**Title** A set of methods and tools for comprehensive analysis of phosphoproteomics data

**Version** 1.0.0

**Description** PhosR is a package for the comprehensive analysis of phosphoproteomic data. There are two major components to PhosR: processing and downstream analysis. PhosR consists of various processing tools for phosphoproteomics data including filtering, imputation, normalisation, and functional analysis for inferring active kinases and signalling pathways.

**License** GPL-3 + file LICENSE

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**R topics documented:**

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createFrequencyMat	<i>Create frequency matrix</i>
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---

**Description**

Create frequency matrix



```

# extracting flanking sequences
seqWin = mapply(function(x) {
  mid <- (nchar(x)+1)/2
  substr(x, start=(mid-7), stop=(mid+7))
}, seqs)

# The first 10 for demonstration purpose
phospho.L6.ratio = phospho.L6.ratio[1:10,]

# minimum number of sequences used for compiling motif for each kinase.
numMotif=5

motif.mouse.list.filtered <-
  motif.mouse.list[which(motif.mouse.list$NumInputSeq >= numMotif)]

# scoring all phosphosites against all motifs
motifScoreMatrix <-
  matrix(NA, nrow=nrow(phospho.L6.ratio),
        ncol=length(motif.mouse.list.filtered))
rownames(motifScoreMatrix) <- rownames(phospho.L6.ratio)
colnames(motifScoreMatrix) <- names(motif.mouse.list.filtered)

# Scoring phosphosites against kinase motifs
for(i in seq_len(length(motif.mouse.list.filtered))) {
  motifScoreMatrix[,i] <-
    frequencyScoring(seqWin, motif.mouse.list.filtered[[i]])
  cat(paste(i, '.', sep=''))
}

```

---

KinaseFamily

*KinaseFamily*


---

### Description

A summary table of kinase family

### Usage

```
data(KinaseFamily)
```

### Format

An object of class `matrix` (inherits from `array`) with 425 rows and 6 columns.

---

 kinaseSubstrateHeatmap

*Kinase-substrate annotation prioritisation heatmap*


---

## Description

Kinase-substrate annotation prioritisation heatmap

## Usage

```
kinaseSubstrateHeatmap(phosScoringMatrices, top = 3)
```

## Arguments

`phosScoringMatrices`  
a matrix returned from `kinaseSubstrateScore`.

`top`  
the number of top ranked phosphosites for each kinase to be included in the heatmap. Default is 1.

## Value

a pheatmap object.

## Examples

```
data('phospho_L6_ratio')
data('SPSs')
data('PhosphoSitePlus')

grps = gsub('_', '+', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                                function(x){paste(toupper(x[2]), x[3], '',
                                                    sep=';')})))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                             colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSs)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                   ctl = ctl)

phosphoL6 = phospho.L6.ratio.RUV
rownames(phosphoL6) = phospho.site.names

# filter for up-regulated phosphosites
```

```

phosphoL6.mean <- meanAbundance(phosphoL6,
                                grps = gsub('_.++', '', colnames(phosphoL6)))
aov <- matANOVA(mat=phosphoL6, grps=gsub('_.++', '', colnames(phosphoL6)))
phosphoL6.reg <- phosphoL6[(aov < 0.05) &
                          (rowSums(phosphoL6.mean > 0.5) > 0),,drop = FALSE]
L6.phos.std <- standardise(phosphoL6.reg)
rownames(L6.phos.std) <- sapply(strsplit(rownames(L6.phos.std), '~'),
                                function(x){gsub(' ', '', paste(toupper(x[2]), x[3], '', sep=';'))})

L6.phos.seq <- sapply(strsplit(rownames(phosphoL6.reg), '~'),
                      function(x)x[4])

L6.matrices <- kinaseSubstrateScore(PhosphoSite.mouse, L6.phos.std,
                                    L6.phos.seq, numMotif = 5, numSub = 1)

kinaseSubstrateHeatmap(L6.matrices)

```

---

kinaseSubstratePred     *kinaseSubstratePred*

---

## Description

A machine learning approach for predicting specific kinase for a given substrate. This prediction framework utilise adaptive sampling.

## Usage

```

kinaseSubstratePred(
  phosScoringMatrices,
  ensembleSize = 10,
  top = 50,
  cs = 0.8,
  inclusion = 20,
  iter = 5
)

```

## Arguments

phosScoringMatrices	An output of kinaseSubstrateScore.
ensembleSize	An ensemble size.
top	a number to select top kinase substrates.
cs	Score threshold.
inclusion	A minimal number of substrates required for a kinase to be selected.
iter	A number of iterations for adaSampling.

## Value

Kinase prediction matrix

**Examples**

```

data('phospho_L6_ratio')
data('SPSs')

grps = gsub('_', '+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                                function(x){paste(toupper(x[2]), x[3], '',
                                                    sep=';')})))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                             colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSs)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                   ctl = ctl)

phosphoL6 = phospho.L6.ratio.RUV
rownames(phosphoL6) = phospho.site.names

# filter for up-regulated phosphosites
phosphoL6.mean <- meanAbundance(phosphoL6,
                                grps = gsub('_', '+', '', colnames(phosphoL6)))
aov <- matANOVA(mat=phosphoL6, grps=gsub('_', '+', '', colnames(phosphoL6)))
phosphoL6.reg <- phosphoL6[(aov < 0.05) &
                           (rowSums(phosphoL6.mean > 0.5) > 0),,drop = FALSE]
L6.phos.std <- standardise(phosphoL6.reg)
rownames(L6.phos.std) <- sapply(strsplit(rownames(L6.phos.std), '~'),
                                function(x){gsub(' ', '', paste(toupper(x[2]), x[3], '', sep=';')})))

L6.phos.seq <- sapply(strsplit(rownames(phosphoL6.reg), '~'),
                     function(x)x[4])

L6.matrices <- kinaseSubstrateScore(PhosphoSite.mouse, L6.phos.std,
                                   L6.phos.seq, numMotif = 5, numSub = 1)
set.seed(1)
L6.predMat <- kinaseSubstratePred(L6.matrices, top=30)

```

---

 kinaseSubstrateProfile

*Kinase substrate profiling*


---

**Description**

This function generates substrate profiles for kinases that have one or more substrates quantified in the phosphoproteome data.

**Usage**

```
kinaseSubstrateProfile(substrate.list, mat)
```

**Arguments**

`substrate.list` a list of kinases with each element containing an array of substrates.

`mat` a matrix with rows correspond to phosphosites and columns correspond to samples.

**Value**

Kinase profile list.

**Examples**

```
data('phospho_L6_ratio')
data('SPSS')

grps = gsub('_', '+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                                function(x){paste(toupper(x[2]), x[3], '',
                                                    sep=';')}}))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                             colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grp - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSS)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                   ctl = ctl)

phosphoL6 = phospho.L6.ratio.RUV
rownames(phosphoL6) = phospho.site.names

# filter for up-regulated phosphosites
phosphoL6.mean <- meanAbundance(phosphoL6,
                                grp = gsub('_', '+', '', colnames(phosphoL6)))
aov <- matANOVA(mat=phosphoL6, grp=gsub('_', '+', '', colnames(phosphoL6)))
phosphoL6.reg <- phosphoL6[(aov < 0.05) &
                           (rowSums(phosphoL6.mean > 0.5) > 0),,drop = FALSE]
L6.phos.std <- standardise(phosphoL6.reg)
rownames(L6.phos.std) <- sapply(strsplit(rownames(L6.phos.std), '~'),
                                function(x){gsub(' ', '', paste(toupper(x[2]), x[3], '', sep=';')}}))

ks.profile.list <- kinaseSubstrateProfile(PhosphoSite.mouse, L6.phos.std)
```



---

 kinaseSubstrateScore *Kinase substrate scoring*


---

## Description

This function generates substrate scores for kinases that pass filtering based on both motifs and dynamic profiles

## Usage

```
kinaseSubstrateScore(substrate.list, mat, seqs, numMotif = 5, numSub = 1)
```

## Arguments

substrate.list	a list of kinases with each element containing an array of substrates.
mat	a matrix with rows correspond to phosphosites and columns correspond to samples.
seqs	an array containing aa sequences surrounding each of all phosphosites. Each sequence has length of 15 (-7, p, +7).
numMotif	minimum number of sequences used for compiling motif for each kinase. Default is 5.
numSub	minimum number of phosphosites used for compiling phosphorylation profile for each kinase. Default is 1.

## Value

A list of 4 elements. motifScoreMatrix, profileScoreMatrix, combinedScoreMatrix, ksActivityMatrix (kinase activity matrix) and their weights.

## Examples

```
data('phospho_L6_ratio')
data('SPSs')
data('PhosphoSitePlus')

grps = gsub('_', '+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                               function(x){paste(toupper(x[2]), x[3], '',
                                                  sep=';')}}))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                           colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSs)
```

```

phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                ctl = ctl)

phosphoL6 = phospho.L6.ratio.RUV
rownames(phosphoL6) = phospho.site.names

# filter for up-regulated phosphosites
phosphoL6.mean <- meanAbundance(phosphoL6,
                                grps = gsub('_', '+', '', colnames(phosphoL6)))
aov <- matANOVA(mat=phosphoL6, grps=gsub('_', '+', '', colnames(phosphoL6)))
phosphoL6.reg <- phosphoL6[(aov < 0.05) &
                          (rowSums(phosphoL6.mean > 0.5) > 0)],,drop = FALSE]
L6.phos.std <- standardise(phosphoL6.reg)
rownames(L6.phos.std) <- sapply(strsplit(rownames(L6.phos.std), '~'),
                               function(x){gsub(' ', '', paste(toupper(x[2]), x[3], '', sep=';'))})

L6.phos.seq <- sapply(strsplit(rownames(phosphoL6.reg), '~'),
                     function(x)x[4])

L6.matrices <- kinaseSubstrateScore(PhosphoSite.mouse, L6.phos.std,
                                   L6.phos.seq, numMotif = 5, numSub = 1)

```

---

matANOVA

*ANOVA test*


---

## Description

Performs an ANOVA test and returns its adjusted p-value

## Usage

```
matANOVA(mat, grps)
```

## Arguments

mat	An p by n matrix where p is the number of phosphosites and n is the number of samples
grps	A vector of length n, with group or time point information of the samples

## Value

A vector of multiple testing adjusted p-values

## Examples

```

data('phospho_L6_ratio')
data('SPSS')

grps = gsub('_', '+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)

```

```

L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                                function(x){paste(toupper(x[2]), x[3], '',
                                                    sep=';')}}))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                             colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSSs)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                   ctl = ctl)

phosphoL6 = phospho.L6.ratio.RUV
rownames(phosphoL6) = phospho.site.names

# filter for up-regulated phosphosites
phosphoL6.mean <- meanAbundance(phosphoL6, grps = gsub('_+', '',
                                                       colnames(phosphoL6)))
aov <- matANOVA(mat=phosphoL6, grps=gsub('_+', '', colnames(phosphoL6)))

```

---

meanAbundance

*Obtain average expression from replicates*

---

## Description

Obtain average expression from replicates

## Usage

```
meanAbundance(mat, grps)
```

## Arguments

mat	a matrix with rows correspond to phosphosites and columns correspond to samples.
grps	a string specifying the grouping (replciates).

## Value

a matrix with mean expression from replicates

## Examples

```

data('phospho_L6_ratio')
data('SPSS')

grps = gsub('_+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label

```

```

phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                               function(x){paste(toupper(x[2]), x[3], '',
                                                  sep=';')}}))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                           colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSs)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                  ctl = ctl)

phosphoL6 = phospho.L6.ratio.RUV
rownames(phosphoL6) = phospho.site.names

# filter for up-regulated phosphosites
phosphoL6.mean <- meanAbundance(phosphoL6, grps = gsub('_+', '',
                                                       colnames(phosphoL6)))

```

---

medianScaling	<i>Median centering and scaling</i>
---------------	-------------------------------------

---

## Description

Median centering and scaling of an input numeric matrix

## Usage

```
medianScaling(mat, scale = TRUE, grps = NULL, reorder = FALSE)
```

## Arguments

mat	a matrix with rows correspond to phosphosites and columns correspond to samples.
scale	a boolean flag indicating whether to scale the samples.
grps	a string or factor specifying the grouping (replciates).
reorder	whether to reorder by factor.

## Value

A median scaled matrix

**Examples**

```

data('phospho.cells.Ins.sample')
grps = gsub('_[0-9]{1}', '', colnames(phospho.cells.Ins))
phospho.cells.Ins.filtered <- selectGrps(phospho.cells.Ins, grps, 0.5, n=1)

set.seed(123)
phospho.cells.Ins.impute <-
  scImpute(phospho.cells.Ins.filtered,
           0.5,
           grps)[,colnames(phospho.cells.Ins.filtered)]

set.seed(123)
phospho.cells.Ins.impute[,1:5] <- ptImpute(phospho.cells.Ins.impute[,6:10],
                                           phospho.cells.Ins.impute[,1:5], percent1 = 0.6,
                                           percent2 = 0, paired = FALSE)

phospho.cells.Ins.ms <-
  medianScaling(phospho.cells.Ins.impute, scale = FALSE)

```

---

minmax

*Minmax scaling*


---

**Description**

Perform a minmax standardisation to scale data into 0 to 1 range

**Usage**

```
minmax(mat)
```

**Arguments**

**mat** a matrix with rows correspond to phosphosites and columns correspond to condition

**Value**

Minmax standardised matrix

**Examples**

```

data('phospho_L6_ratio')
data('SPSs')

grps = gsub('_.+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                               function(x){paste(toupper(x[2]), x[3], '')}

```

```

                                sep=';'))))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                                colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSs)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                ctl = ctl)

phosphoL6 = phospho.L6.ratio.RUV
rownames(phosphoL6) = phospho.site.names

# filter for up-regulated phosphosites
phosphoL6.mean <- meanAbundance(phosphoL6, grps = gsub('_+', '',
                                colnames(phosphoL6)))
aov <- matANOVA(mat=phosphoL6, grps=gsub('_+', '', colnames(phosphoL6)))
phosphoL6.reg <- phosphoL6[(aov < 0.05) &
                                (rowSums(phosphoL6.mean > 0.5) > 0),,drop = FALSE]
L6.phos.std <- standardise(phosphoL6.reg)
rownames(L6.phos.std) <- sapply(strsplit(rownames(L6.phos.std), '~'),
                                function(x){gsub(' ', '', paste(toupper(x[2]), x[3], '', sep=';'))})

L6.phos.seq <- sapply(strsplit(rownames(phosphoL6.reg), '~'),
                                function(x)x[4])

numMotif = 5
numSub = 1

ks.profile.list <- kinaseSubstrateProfile(PhosphoSite.mouse, L6.phos.std)
motif.mouse.list = PhosR::motif.mouse.list

motif.mouse.list.filtered <-
  motif.mouse.list[which(motif.mouse.list$NumInputSeq >= numMotif)]
ks.profile.list.filtered <-
  ks.profile.list[which(ks.profile.list$NumSub >= numSub)]

# scoring all phosphosites against all motifs
motifScoreMatrix <-
  matrix(NA, nrow=nrow(L6.phos.std),
        ncol=length(motif.mouse.list.filtered))
rownames(motifScoreMatrix) <- rownames(L6.phos.std)
colnames(motifScoreMatrix) <- names(motif.mouse.list.filtered)

# extracting flanking sequences
seqWin = mapply(function(x) {
  mid <- (nchar(x)+1)/2
  substr(x, start=(mid-7), stop=(mid+7))
}, L6.phos.seq)

print('Scoring phosphosites against kinase motifs:')
for(i in seq_len(length(motif.mouse.list.filtered))) {
  motifScoreMatrix[,i] <-

```

```

        frequencyScoring(seqWin, motif.mouse.list.filtered[[i]])
        cat(paste(i, '.', sep=''))
    }
    motifScoreMatrix <- minmax(motifScoreMatrix)

```

---

mIntersect

*Multi-intersection, union*


---

## Description

A recursive loop for intersecting multiple sets.

## Usage

```

mIntersect(x, y, ...)
mUnion(x, y, ...)

```

## Arguments

x, y, ...            objects to find intersection/union.

## Value

An intersection/union of input parameters

## Examples

```

data('phospho_liverInsTC_RUV_sample')
data('phospho_L6_ratio')

site1 <- gsub('~[STY]', '~',
             sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                   function(x){paste(toupper(x[2]), x[3], sep='~')})))
site2 <- rownames(phospho.liver.Ins.TC.ratio.RUV)

# step 2: rank by fold changes
tmp <- do.call(cbind, lapply(split(1:ncol(phospho.L6.ratio), gsub('_exp\\d+',
    '', colnames(phospho.L6.ratio))),
    function(i){rowMeans(phospho.L6.ratio[,i])}))
site1 <- t(sapply(split(data.frame(tmp), site1), colMeans))[, -1])

tmp <- do.call(cbind, lapply(split(1:ncol(phospho.liver.Ins.TC.ratio.RUV),
    gsub(
        '(Intensity\\.)(.*)\\_Bio\\d+',
        '\\2',
        colnames(phospho.liver.Ins.TC.ratio.RUV))),
    function(i){
        rowMeans(phospho.liver.Ins.TC.ratio.RUV[,i])
    })))
site2 <- t(sapply(split(data.frame(tmp), site2), colMeans))

```

```
o <- mIntersect(site1, site2)
```

---

motif.human.list      *List of human kinase motifs*

---

**Description**

A list of human kinase motifs and their sequence probability matrix.

**Usage**

```
data(KinaseMotifs)
```

**Format**

An object of class list of length 380.

---

motif.mouse.list      *List of mouse kinase motifs*

---

**Description**

A list of mouse kinase motifs and their sequence probability matrix.

**Usage**

```
data(KinaseMotifs)
```

**Format**

An object of class list of length 250.

---

motif.rat.list      *List of rat kinase motifs*

---

**Description**

A list of rat kinase motifs and their sequence probability matrix.

**Usage**

```
data(KinaseMotifs)
```

**Format**

An object of class list of length 159.



---

pathwayOverrepresent *Gene set over-representation analysis*

---

### Description

This function performs gene set over-representation analysis using Fisher's exact test.

### Usage

```
pathwayOverrepresent(geneSet, annotation, universe, alter = "greater")
```

### Arguments

geneSet	an array of gene or phosphosite IDs (IDs are gene symbols etc that match to your pathway annotation list).
annotation	a list of pathways with each element containing an array of gene or phosphosite IDs.
universe	the universe/background of all genes or phosphosites in your profiled dataset.
alter	test for enrichment ('greater', default), depletion ('less'), or 'two.sided'.

### Value

A matrix of pathways and their associated substrates and p-values.

### Examples

```
library(limma)

data('phospho_L6_ratio')
data('SPSs')

grps = gsub('_', '+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                               function(x){paste(toupper(x[2]), x[3], '',
                                                  sep=';')}}))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                           colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSs)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                  ctl = ctl)

# divides the phospho.L6.ratio data into groups by phosphosites
```

```

L6.sites <- gsub(' ', '', gsub('~[STY]', '~',
  sapply(strsplit(rownames(phospho.L6.ratio.RUV), '~'),
    function(x){paste(toupper(x[2]), x[3], sep='~')})))
phospho.L6.ratio.sites <- t(sapply(split(data.frame(phospho.L6.ratio.RUV),
  L6.sites), colMeans))

# fit linear model for each phosphosite
f <- gsub('_exp\\d', '', colnames(phospho.L6.ratio.RUV))
X <- model.matrix(~ f - 1)
fit <- lmFit(phospho.L6.ratio.RUV, X)

# extract top-ranked phosphosites for each condition compared to basal
table.AICAR <- topTable(eBayes(fit), number=Inf, coef = 1)
table.Ins <- topTable(eBayes(fit), number=Inf, coef = 3)
table.AICARIns <- topTable(eBayes(fit), number=Inf, coef = 2)

DE1.RUV <- c(sum(table.AICAR[, 'adj.P.Val'] < 0.05),
  sum(table.Ins[, 'adj.P.Val'] < 0.05),
  sum(table.AICARIns[, 'adj.P.Val'] < 0.05))

# extract top-ranked phosphosites for each group comparison
contrast.matrix1 <- makeContrasts(fAICARIns-fIns, levels=X)
contrast.matrix2 <- makeContrasts(fAICARIns-fAICAR, levels=X)
fit1 <- contrasts.fit(fit, contrast.matrix1)
fit2 <- contrasts.fit(fit, contrast.matrix2)
table.AICARInsVSIns <- topTable(eBayes(fit1), number=Inf)
table.AICARInsVSAICAR <- topTable(eBayes(fit2), number=Inf)

DE2.RUV <- c(sum(table.AICARInsVSIns[, 'adj.P.Val'] < 0.05),
  sum(table.AICARInsVSAICAR[, 'adj.P.Val'] < 0.05))

o <- rownames(table.AICARInsVSIns)
Tc <- cbind(table.Ins[o, 'logFC'], table.AICAR[o, 'logFC'],
  table.AICARIns[o, 'logFC'])
rownames(Tc) = gsub('(.*)(&[A-Z])([0-9]+)(;)', '\\1;\\3;', o)
colnames(Tc) <- c('Ins', 'AICAR', 'AICAR+Ins')

# summary phosphosite-level information to proteins for performing downstream
# gene-centric analyses.
Tc.gene <- phosCollapse(Tc, id=gsub(';+', '', rownames(Tc)),
  stat=apply(abs(Tc), 1, max), by = 'max')
geneSet <- names(sort(Tc.gene[,1],
  decreasing = TRUE))[1:round(nrow(Tc.gene) * 0.1)]
#lapply(PhosphoSite.rat, function(x){gsub(';[STY]', ';', x)})

# 1D gene-centric pathway analysis
path1 <- pathwayOverrepresent(geneSet, annotation=Pathways.reactome,
  universe = rownames(Tc.gene), alter = 'greater')

```

**Description**

This function performs gene set enrichment analysis using Wilcoxon Rank Sum test.

**Usage**

```
pathwayRankBasedEnrichment(geneStats, annotation, alter = "greater")
```

**Arguments**

geneStats	an array of statistics (e.g. log2 FC) of all quantified genes or phosphosite with names of the array as gene or phosphosite IDs.
annotation	a list of pathways with each element containing an array of gene IDs.
alter	test for enrichment ('greater', default), depletion ('less'), or 'two.sided'.

**Value**

A matrix of pathways and their associated substrates and p-values.

**Examples**

```
library(limma)

data('phospho_L6_ratio')
data('SPSs')

grps = gsub('_', '+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                               function(x){paste(toupper(x[2]), x[3], '',
                                                  sep=';')})))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                             colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSs)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                  ctl = ctl)

# divides the phospho.L6.ratio data into groups by phosphosites
L6.sites <- gsub(' ', '', gsub('~[STY]', '~',
                              sapply(strsplit(rownames(phospho.L6.ratio.RUV), '~'),
                                      function(x){paste(toupper(x[2]), x[3], sep='~')})))
phospho.L6.ratio.sites <- t(sapply(split(data.frame(phospho.L6.ratio.RUV),
                                                  L6.sites), colMeans))

# fit linear model for each phosphosite
f <- gsub('_exp\\d', '', colnames(phospho.L6.ratio.RUV))
```

```

X <- model.matrix(~ f - 1)
fit <- lmFit(phospho.L6.ratio.RUV, X)

# extract top-ranked phosphosites for each condition compared to basal
table.AICAR <- topTable(eBayes(fit), number=Inf, coef = 1)
table.Ins <- topTable(eBayes(fit), number=Inf, coef = 3)
table.AICARIns <- topTable(eBayes(fit), number=Inf, coef = 2)

DE1.RUV <- c(sum(table.AICAR[, 'adj.P.Val'] < 0.05),
             sum(table.Ins[, 'adj.P.Val'] < 0.05),
             sum(table.AICARIns[, 'adj.P.Val'] < 0.05))

# extract top-ranked phosphosites for each group comparison
contrast.matrix1 <- makeContrasts(fAICARIns-fIns, levels=X)
contrast.matrix2 <- makeContrasts(fAICARIns-fAICAR, levels=X)
fit1 <- contrasts.fit(fit, contrast.matrix1)
fit2 <- contrasts.fit(fit, contrast.matrix2)
table.AICARInsVSIns <- topTable(eBayes(fit1), number=Inf)
table.AICARInsVSAICAR <- topTable(eBayes(fit2), number=Inf)

DE2.RUV <- c(sum(table.AICARInsVSIns[, 'adj.P.Val'] < 0.05),
             sum(table.AICARInsVSAICAR[, 'adj.P.Val'] < 0.05))

o <- rownames(table.AICARInsVSIns)
Tc <- cbind(table.Ins[o, 'logFC'], table.AICAR[o, 'logFC'],
           table.AICARIns[o, 'logFC'])
rownames(Tc) = gsub('(.*)([A-Z])([0-9]+)(;)', '\\1;\\3;', o)
colnames(Tc) <- c('Ins', 'AICAR', 'AICAR+Ins')

# summary phosphosite-level information to proteins for performing downstream
# gene-centric analyses.
Tc.gene <- phosCollapse(Tc, id=gsub(';+', '', rownames(Tc)),
                      stat=apply(abs(Tc), 1, max), by = 'max')

# 1D gene-centric pathway analysis
path2 <- pathwayRankBasedEnrichment(Tc.gene[,1],
                                   annotation=Pathways.reactome,
                                   alter = 'greater')

```

---

Pathways.KEGG

*KEGG pathway annotations*


---

### Description

The data object contains the annotations of KEGG pathways.

### Usage

```
data(Pathways)
```

### Format

An object of class `list` of length 186.

---

Pathways.reactome	<i>Reactome pathway annotations</i>
-------------------	-------------------------------------

---

**Description**

The data object contains the annotations of Reactome pathways.

**Usage**

```
data(Pathways)
```

**Format**

An object of class `list` of length 674.

---

phosCollapse	<i>Summarising phosphosites to proteins</i>
--------------	---

---

**Description**

Summarising phosphosite-level information to proteins for performing downstream gene-centric analyses.

**Usage**

```
phosCollapse(mat, id, stat, by='min')
```

**Arguments**

<code>mat</code>	a matrix with rows correspond to phosphosites and columns correspond to samples.
<code>id</code>	an array indicating the grouping of phosphosites etc.
<code>stat</code>	an array containing statistics of phosphosite such as phosphorylation levels.
<code>by</code>	how to summarise phosphosites using their statistics. Either by 'min' (default), 'max', or 'mid'.

**Value**

A matrix summarised to protein level

**Examples**

```

library(limma)

data('phospho_L6_ratio')
data('SPSs')

grps = gsub('_.+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                               function(x){paste(toupper(x[2]), x[3], '',
                                                  sep=';')}}))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                             colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSs)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                   ctl = ctl)

# divides the phospho.L6.ratio data into groups by phosphosites
L6.sites <- gsub(' ', '', gsub('~[STY]', '~',
                               sapply(strsplit(rownames(phospho.L6.ratio.RUV), '~'),
                                       function(x){paste(toupper(x[2]), x[3], sep='~')})))
phospho.L6.ratio.sites <- t(sapply(split(data.frame(phospho.L6.ratio.RUV),
                                                  L6.sites), colMeans))

# fit linear model for each phosphosite
f <- gsub('_exp\\d', '', colnames(phospho.L6.ratio.RUV))
X <- model.matrix(~ f - 1)
fit <- lmFit(phospho.L6.ratio.RUV, X)

# extract top-ranked phosphosites for each condition compared to basal
table.AICAR <- topTable(eBayes(fit), number=Inf, coef = 1)
table.Ins <- topTable(eBayes(fit), number=Inf, coef = 3)
table.AICARIns <- topTable(eBayes(fit), number=Inf, coef = 2)

DE1.RUV <- c(sum(table.AICAR[, 'adj.P.Val'] < 0.05),
             sum(table.Ins[, 'adj.P.Val'] < 0.05),
             sum(table.AICARIns[, 'adj.P.Val'] < 0.05))

# extract top-ranked phosphosites for each group comparison
contrast.matrix1 <- makeContrasts(fAICARIns-fIns, levels=X)
contrast.matrix2 <- makeContrasts(fAICARIns-fAICAR, levels=X)
fit1 <- contrasts.fit(fit, contrast.matrix1)
fit2 <- contrasts.fit(fit, contrast.matrix2)
table.AICARInsVSIIns <- topTable(eBayes(fit1), number=Inf)
table.AICARInsVSAICAR <- topTable(eBayes(fit2), number=Inf)

DE2.RUV <- c(sum(table.AICARInsVSIIns[, 'adj.P.Val'] < 0.05),

```

```

sum(table.AICARInsVSAICAR[, 'adj.P.Val'] < 0.05))

o <- rownames(table.AICARInsVSIns)
Tc <- cbind(table.Ins[o, 'logFC'], table.AICAR[o, 'logFC'],
            table.AICARIns[o, 'logFC'])
rownames(Tc) = gsub('(.*)([A-Z])([0-9]+)(;)', '\\1;\\3;', o)
colnames(Tc) <- c('Ins', 'AICAR', 'AICAR+Ins')

# summary phosphosite-level information to proteins for performing downstream
# gene-centric analyses.
Tc.gene <- phosCollapse(Tc, id=gsub(';.+', '', rownames(Tc)),
                       stat=apply(abs(Tc), 1, max), by = 'max')

```

---

phospho.cells.Ins      *phospho.cells.Ins*

---

### Description

A subset of phosphoproteomics dataset generated by Humphrey et al., [doi:10.1038/nbt.3327] from two mouse liver cell lines (Hepa1.6 and FL38B) that were treated with either PBS (mock) or insulin.

### Usage

```
data(phospho.cells.Ins.sample)
```

### Format

An object of class `matrix` (inherits from `array`) with 49617 rows and 24 columns.

### Source

doi: 10.1038/nbt.3327 (PXD001792)

### References

Humphrey et al., 2015, doi: 10.1038/nbt.3327

---

phospho.L6.ratio      *phospho.L6.ratio*

---

### Description

An L6 myotube phosphoproteome dataset (accession number: PXD019127).

### Usage

```
data(phospho_L6_ratio)
```

### Format

An object of class `matrix` (inherits from `array`) with 6660 rows and 12 columns.

**Source**

PRIDE accession number: PXD001792

---

phospho.liver.Ins.TC.ratio.RUV  
*phospho\_liverInsTC\_RUV\_sample*

---

**Description**

A subset of phosphoproteomics dataset integrated from two time-course datasets of early and intermediate insulin signalling in mouse liver upon insulin stimulation.

**Usage**

```
data(phospho_liverInsTC_RUV_sample)
```

**Format**

An object of class `matrix` (inherits from `array`) with 5000 rows and 90 columns.

**Source**

PRIDE accession number: PXD001792

**References**

Humphrey et al., 2015

---

PhosphoSite.human      *PhosphoSitePlus annotations for human*

---

**Description**

The data object contains the annotations of kinases and their corresponding substrates as phosphorylation sites in human. It is extracted from the PhosphoSitePlus database. For details of PhosphoSitePlus, please refer to the article: Hornbeck et al. Nucleic Acids Res. 40:D261-70, 2012

**Usage**

```
data(PhosphoSitePlus)
```

**Format**

An object of class `list` of length 379.

**Source**

<https://www.phosphosite.org>



---

PhosphoSite.mouse      *PhosphoSitePlus annotations for mouse*

---

**Description**

The data object contains the annotations of kinases and their corresponding substrates as phosphorylation sites in mouse. It is extracted from the PhosphoSitePlus database. For details of PhosphoSitePlus, please refer to the article: Hornbeck et al. Nucleic Acids Res. 40:D261-70, 2012

**Usage**

```
data(PhosphoSitePlus)
```

**Format**

An object of class list of length 260.

**Source**

<https://www.phosphosite.org>

---

PhosphoSite.rat      *PhosphoSitePlus annotations for rat*

---

**Description**

The data object contains the annotations of kinases and their corresponding substrates as phosphorylation sites in rat. It is extracted from the PhosphoSitePlus database. For details of PhosphoSitePlus, please refer to the article: Hornbeck et al. Nucleic Acids Res. 40:D261-70, 2012

**Usage**

```
data(PhosphoSitePlus)
```

**Format**

An object of class list of length 158.

**Source**

<https://www.phosphosite.org>

plotQC

*A set of function for data QC plot***Description**

The 'panel' parameter allows different type of visualisation for output object from PhosR. 'panel = 0' is used to create a 2\*2 panel of plots including the following. 'panel = 1' is used to visualise percentage of quantification after imputataion. 'panel = 2' is used to visualise dendrogram (hierarchical clustering) of the input matrix. 'panel = 3' is used to visualise abundance level of samples from the input matrix. 'panel = 4' is used to show PCA plot

**Usage**

```
plotQC(mat, cols, labels, panel, ...)
```

**Arguments**

mat	A p by n matrix, where p is the number of phosphosites and n is the number of samples.
cols	A vector of colours to be used in the plot. The length should be equal to the columns of the mat.
labels	A vector of sample names. Used the label points in PCA plot (panel=4)
panel	A numeric value (0-4) to choose the plot type. See description for details.
...	Plotting parameters for base plots

**Value**

A graphical plot

**Examples**

```
# Imputation
data('phospho.cells.Ins.sample')
grps = gsub('_[0-9]{1}', '', colnames(phospho.cells.Ins))
phospho.cells.Ins.filtered <- selectGrps(phospho.cells.Ins, grps, 0.5, n=1)

set.seed(123)
phospho.cells.Ins.impute <-
  scImpute(
    phospho.cells.Ins.filtered,
    0.5,
    grps)[,colnames(phospho.cells.Ins.filtered)]

set.seed(123)
phospho.cells.Ins.impute[,1:5] <- ptImpute(phospho.cells.Ins.impute[,6:10],
phospho.cells.Ins.impute[,1:5], percent1 = 0.6, percent2 = 0, paired = FALSE)

phospho.cells.Ins.ms <- medianScaling(phospho.cells.Ins.impute,
scale = FALSE)

cols <- rep(c('#ED4024', '#7FBF42', '#3F61AD', '#9B822F'), each=6)
```

```

par(mfrow=c(1,2))
plotQC(phospho.cells.Ins.filtered,
       labels=colnames(phospho.cells.Ins.filtered),
       panel = 1, cols = cols)
plotQC(phospho.cells.Ins.ms,
       labels=colnames(phospho.cells.Ins.ms),
       panel = 1, cols = cols)

# Batch correction
data('phospho.L6_ratio')
data('SPSs')

grps = gsub('_', '+', '', colnames(phospho.L6_ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6_ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6_ratio), '~'),
                              function(x){paste(toupper(x[2]), x[3], '',
                                                  sep=';')})))
phospho.L6_ratio = t(sapply(split(data.frame(phospho.L6_ratio), L6.sites),
                           colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6_ratio) %in% SPSs)
phospho.L6_ratio.RUV = RUVphospho(phospho.L6_ratio, M = design, k = 3,
                                  ctl = ctl)

cs = rainbow(length(unique(grps)))
colorCodes = sapply(grps, switch, AICAR=cs[1], Ins=cs[2], AICARIns=cs[3])

# plot after batch correction
par(mfrow=c(1,2))
plotQC(phospho.L6_ratio, panel = 2, cols=colorCodes)
plotQC(phospho.L6_ratio.RUV, cols=colorCodes,
       labels = colnames(phospho.L6_ratio),
       panel=2, ylim=c(-20, 20), xlim=c(-30, 30))

par(mfrow=c(1,2))
plotQC(phospho.L6_ratio, panel = 4, cols=colorCodes,
       labels = colnames(phospho.L6_ratio),
       main='Before Batch correction')
plotQC(phospho.L6_ratio.RUV, cols=colorCodes,
       labels = colnames(phospho.L6_ratio),
       panel=4, ylim=c(-20, 20), xlim=c(-30, 30),
       main='After Batch correction')

```

**Description**

Impute the missing values for mat2 using tail imputation approach if mat1 has more than percent1 (percentage) of quantified values and mat2 has less than percent2 (percentage) quantified values, and vice versa if paired is set to be true. That is if mat2 has percentage of quantified values more than percent1 and mat1 has percentage quantified values less than percent2.

**Usage**

```
ptImpute(mat1, mat2, percent1, percent2, m, s, paired)
```

**Arguments**

mat1	a matrix with rows correspond to phosphosites and columns correspond to replicates within treatment1.
mat2	a matrix with rows correspond to phosphosites and columns correspond to replicates within treatment2.
percent1	a percent indicating minimum quantified percentages required for considering for imputation.
percent2	a percent indicating minimum quantified percentages required for considering for imputation.
m	a numeric number of for controlling mean downshifting.
s	a numeric number of for controlling standard deviation of downshifted sampling values.
paired	a flag indicating whether to impute for both treatment1 and treatment2 (default) or treatment2 only (if paired=FALSE).

**Value**

An imputed matrix

**Examples**

```
data('phospho.cells.Ins.sample')
grps = gsub('_[0-9]{1}', '', colnames(phospho.cells.Ins))
phospho.cells.Ins.filtered <- selectGrps(phospho.cells.Ins, grps, 0.5, n=1)

set.seed(123)
phospho.cells.Ins.impute <-
  scImpute(
    phospho.cells.Ins.filtered,
    0.5,
    grps[,colnames(phospho.cells.Ins.filtered)]

set.seed(123)
phospho.cells.Ins.impute[,1:5] <- ptImpute(phospho.cells.Ins.impute[,6:10],
phospho.cells.Ins.impute[,1:5], percent1 = 0.6, percent2 = 0, paired = FALSE)
```

RUVphospho

*RUV for phosphoproteomics data normalisation***Description**

This is a wrapper implementation of RUVIII for phosphoproteomics data normalisation. This function will call `tailImpute` function to impute all the missing values (if there is any) in the phosphoproteomics data for applying RUVIII. It will then return the normalised values for quantified phosphosites and remove imputed values.

**Usage**

```
RUVphospho(mat, M, ctl, k = NULL, m = 1.6, s = 0.6, keepImpute = FALSE, ...)
```

**Arguments**

<code>mat</code>	a matrix with rows correspond to phosphosites and columns correspond to samples.
<code>M</code>	is the design matrix as defined in RUVIII.
<code>ctl</code>	is the stable phosphosites (or negative controls as defined in RUVIII).
<code>k</code>	is the number of unwanted factors as defined in RUVIII.
<code>m</code>	a numeric number for controlling mean downshifting.
<code>s</code>	a numeric number for controlling standard deviation of downshifted sampling values.
<code>keepImpute</code>	a boolean to keep the missing value in the returned matrix.
<code>...</code>	additional parameters that may be passed to RUVIII.

**Value**

A normalised matrix.

**Examples**

```
data('phospho_L6_ratio')
data('SPSS')

grps = gsub('_', '+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                               function(x){paste(toupper(x[2]), x[3], '',
                                                    sep=';')})))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                           colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)
```

```
# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSs)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                  ctl = ctl)
```

---

scImpute

*Site- and condition-specific (sc) impute*


---

### Description

Impute the missing values for a phosphosite across replicates within a single condition (or treatment) if there are  $n$  or more quantified values of that phosphosite in that condition.

### Usage

```
scImpute(mat, percent, grps)
```

### Arguments

mat	a matrix with rows correspond to phosphosites and columns correspond to replicates within a condition.
percent	a percent from 0 to 1, specifying the percentage of quantified values in any treatment group.
grps	a string specifying the grouping (replicates).

### Value

An imputed matrix

### Examples

```
data('phospho.cells.Ins.sample')
grps = gsub('_[0-9]{1}', '', colnames(phospho.cells.Ins))
phospho.cells.Ins.filtered <- selectGrps(phospho.cells.Ins, grps, 0.5, n=1)

set.seed(123)
phospho.cells.Ins.impute <-
  scImpute(phospho.cells.Ins.filtered,
           0.5,
           grps[,colnames(phospho.cells.Ins.filtered)])
```

---

selectGrps	<i>Select by treatment groups (replicate block)</i>
------------	---

---

**Description**

Select phosphosites that have been quantified in a given percentage of treatment groups (e.g. 0.75 as 3 out of 4 replicates) in n groups.

**Usage**

```
selectGrps(mat, grps, percent, n)
```

**Arguments**

mat	a matrix with rows correspond to phosphosites and columns correspond to samples in replicates for different treatments.
grps	a string specifying the grouping (replicates).
percent	a percent from 0 to 1, specifying the percentage of quantified values in any treatment group.
n	an integer indicating n or more replicates pass the percentage filtering for a phosphosite to be included.

**Value**

a filtered matrix with at least 'percent' quantification in one or more conditions

**Author(s)**

Pengyi Yang, Taiyun Kim

**Examples**

```
data('phospho.cells.Ins.sample')
grps = gsub('_[0-9]{1}', '', colnames(phospho.cells.Ins))

phospho.cells.Ins.filtered <- selectGrps(phospho.cells.Ins, grps, 0.5, n=1)
```

---

selectOverallPercent	<i>Select phosphosite by percentage of quantification</i>
----------------------	---

---

**Description**

Select phosphosites that have been quantified in more than a given percentage of samples

**Usage**

```
selectOverallPercent(mat, percent=NULL, n=NULL)
```

**Arguments**

mat	a matrix with rows correspond to phosphosites and columns correspond to samples in replicates for different treatments.
percent	a percent from 0 to 1, specifying the percentage of quantified values in across all samples for retaining a phosphosite for subsequent analysis.
n	an integer indicating n or more quantified values required for retaining a phosphosite for subsequent analysis.

**Value**

a filtered matrix

**Examples**

```
data('phospho.cells.Ins.sample')

phospho.cells.Ins.filtered <- selectOverallPercent(phospho.cells.Ins, 0.5)

# Before filtering
dim(phospho.cells.Ins)
# After filtering
dim(phospho.cells.Ins.filtered)
```

---

selectTimes	<i>selectTimes</i>
-------------	--------------------

---

**Description**

selectTimes

**Usage**

```
selectTimes(mat, timepoint, order, percent, w)
```

**Arguments**

mat	a matrix with rows correspond to phosphosites and columns correspond to samples in replicates for different treatments.
timepoint	a timepoint as factor with a length equal to the number of columns of mat.
order	a vector specifying the order of timepoints.
percent	a percent (decimal) from 0 to 1, to filter phosphosites with with missing value larger than percent per timepoint.
w	a timepoint window for selection of phosphosites to remove.

**Value**

a filtered matrix



**Examples**

```

data("phospho_liverInsTC_RUV_sample")
timepoint = gsub("(.*)(\\d+[ms])(.*)", "\\2",
                colnames(phospho.liver.Ins.TC.ratio.RUV))
timepoint[which(timepoint == "0m")] = "0s"
timepoint = factor(timepoint)
timepointOrder = c("0s", "5s", "1m", "2m", "3m", "4m", "6m")

# For demonstration purpose, we introduce missing value at 0s
table(timepoint)

phospho.liver.Ins.TC.sim = phospho.liver.Ins.TC.ratio.RUV
rmId = which(timepoint == "0s")

# We replace the values to NA for the first 26 (~60%) of the '0s' samples
# for the first 100 phosphosite as NA
phospho.liver.Ins.TC.sim[1:100,rmId[1:26]] = NA

phospho.liver.Ins.TC.sim = selectTimes(phospho.liver.Ins.TC.sim,
                                       timepoint, timepointOrder, 0.5,
                                       w = length(table(timepoint)))

# Before filtering
dim(phospho.liver.Ins.TC.ratio.RUV)
# After filtering
dim(phospho.liver.Ins.TC.sim)

```

Signalomes

*PhosR Signalomes***Description**

A function to generate signalomes

**Usage**

```
Signalomes(KSR, predMatrix, exprsMat, KOI, threskinaseNetwork=0.9,
           signalomeCutoff=0.5)
```

**Arguments**

KSR	kinase-substrate relationship scoring results
predMatrix	output of kinaseSubstratePred function
exprsMat	a matrix with rows corresponding to phosphosites and columns corresponding to samples
KOI	a character vector that contains kinases of interest for which expanded signalomes will be generated
threskinaseNetwork	threshold used to select interconnected kinases for the expanded signalomes
signalomeCutoff	threshold used to filter kinase-substrate relationships

**Value**

A list of 3 elements. Signalomes, proteinModules and kinaseSubstrates

**Examples**

```

data('phospho_L6_ratio')
data('SPSs')

grps = gsub('_', '+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                                function(x){paste(toupper(x[2]), x[3], '',
                                                    sep=';')}))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                             colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grp - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSs)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                   ctl = ctl)

phosphoL6 = phospho.L6.ratio.RUV
rownames(phosphoL6) = phospho.site.names

# filter for up-regulated phosphosites
phosphoL6.mean <- meanAbundance(phosphoL6, grp = gsub('_', '+', '',
                                                    colnames(phosphoL6)))
aov <- matANOVA(mat=phosphoL6, grp=gsplit('_', '+', '', colnames(phosphoL6)))
phosphoL6.reg <- phosphoL6[(aov < 0.05) &
                           (rowSums(phosphoL6.mean > 0.5) > 0),,drop = FALSE]
L6.phos.std <- standardise(phosphoL6.reg)
rownames(L6.phos.std) <- sapply(strsplit(rownames(L6.phos.std), '~'),
                                function(x){gsub(' ', '', paste(toupper(x[2]), x[3], '', sep=';')}))

L6.phos.seq <- sapply(strsplit(rownames(phosphoL6.reg), '~'),
                      function(x)x[4])

L6.matrices <- kinaseSubstrateScore(PhosphoSite.mouse, L6.phos.std,
                                   L6.phos.seq, numMotif = 5, numSub = 1)
set.seed(1)
L6.predMat <- kinaseSubstratePred(L6.matrices, top=30)

kinaseOI = c('PRKAA1', 'AKT1')

Signalomes_results <- Signalomes(KSR=L6.matrices,
                                 predMatrix=L6.predMat,
                                 exprsMat=L6.phos.std,
                                 KOI=kinaseOI)

```

---

siteAnnotate	<i>Phosphosite annotation</i>
--------------	-------------------------------

---

### Description

This function plots the combined scores of each of all kinases for a given phosphosites

### Usage

```
siteAnnotate(site, phosScoringMatrices, predMatrix)
```

### Arguments

site	site the ID of a phosphosite
phosScoringMatrices	output from function kinaseSubstrateScore()
predMatrix	a prediction matrix from kinaseSubstratePred()

### Value

A graphical plot

### Examples

```
data('phospho_L6_ratio')
data('SPSs')

grps = gsub('_', '+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                                function(x){paste(toupper(x[2]), x[3], '',
                                                    sep=';')})))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                             colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSs)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                   ctl = ctl)

phosphoL6 = phospho.L6.ratio.RUV
rownames(phosphoL6) = phospho.site.names

# filter for up-regulated phosphosites
phosphoL6.mean <- meanAbundance(phosphoL6,
```

```

                                grps = gsub('_', '+', '', colnames(phosphoL6)))
aov <- matANOVA(mat=phosphoL6, grps=gsub('_', '+', '', colnames(phosphoL6)))
phosphoL6.reg <- phosphoL6[(aov < 0.05) &
                          (rowSums(phosphoL6.mean > 0.5) > 0),,drop = FALSE]
L6.phos.std <- standardise(phosphoL6.reg)
rownames(L6.phos.std) <- sapply(strsplit(rownames(L6.phos.std), '~'),
                                function(x){gsub(' ', '', paste(toupper(x[2]), x[3], '', sep=';'))})

L6.phos.seq <- sapply(strsplit(rownames(phosphoL6.reg), '~'),
                      function(x)x[4])

L6.matrices <- kinaseSubstrateScore(PhosphoSite.mouse, L6.phos.std,
                                   L6.phos.seq, numMotif = 5, numSub = 1)
set.seed(1)
L6.predMat <- kinaseSubstratePred(L6.matrices, top=30)

# We will look at the phosphosite AAK1;S677 for demonstration purpose.
site = "AAK1;S677;"
siteAnnotate(site, L6.matrices, L6.predMat)

```

SPSs

*A list of Stably Phosphorylated Sites (SPSs)***Description**

A list of stably phosphorylated sites defined from a panel of phosphoproteomics datasets. For full list of the datasets used, please refer to our preprint for the full list.

**Usage**

```
data(SPSs)
```

**Format**

An object of class character of length 100.

standardise

*Standardisation***Description**

Standardisation by z-score transformation.

**Usage**

```
standardise(mat)
```

**Arguments**

**mat** a matrix with rows correspond to phosphosites and columns correspond to samples.

**Value**

A standardised matrix

**Examples**

```

data('phospho_L6_ratio')
data('SPSs')

grps = gsub('_', '+', '', colnames(phospho.L6.ratio))

# Cleaning phosphosite label
phospho.site.names = rownames(phospho.L6.ratio)
L6.sites = gsub(' ', '', sapply(strsplit(rownames(phospho.L6.ratio), '~'),
                                function(x){paste(toupper(x[2]), x[3], '',
                                                    sep=';')})))
phospho.L6.ratio = t(sapply(split(data.frame(phospho.L6.ratio), L6.sites),
                            colMeans))
phospho.site.names = split(phospho.site.names, L6.sites)

# Construct a design matrix by condition
design = model.matrix(~ grps - 1)

# phosphoproteomics data normalisation using RUV
ctl = which(rownames(phospho.L6.ratio) %in% SPSs)
phospho.L6.ratio.RUV = RUVphospho(phospho.L6.ratio, M = design, k = 3,
                                  ctl = ctl)
phosphoL6 = phospho.L6.ratio.RUV
rownames(phosphoL6) = phospho.site.names

# filter for up-regulated phosphosites
phosphoL6.mean <- meanAbundance(phosphoL6, grps = gsub('_', '+', '',
                                                       colnames(phosphoL6)))
aov <- matANOVA(mat=phosphoL6, grps=gsub('_', '+', '', colnames(phosphoL6)))
phosphoL6.reg <- phosphoL6[(aov < 0.05) &
                           (rowSums(phosphoL6.mean > 0.5) > 0),,drop = FALSE]
L6.phos.std <- standardise(phosphoL6.reg)

```

---

tImpute

*Tail-based impute*


---

**Description**

Tail-based imputation approach as implemented in Perseus.

**Usage**

```
tImpute(mat, m, s)
```

**Arguments**

<code>mat</code>	a matrix with rows correspond to phosphosites and columns correspond to samples.
<code>m</code>	a numeric number for controlling mean downshifting.
<code>s</code>	a numeric number for controlling standard deviation of downshifted sampling values.

**Value**

An imputed matrix

**Examples**

```
data('phospho.cells.Ins.sample')
grps = gsub('_[0-9]{1}', '', colnames(phospho.cells.Ins))
phospho.cells.Ins.filtered <- selectGrps(phospho.cells.Ins, grps, 0.5, n=1)

set.seed(123)
phospho.cells.Ins.impute <- tImpute(phospho.cells.Ins.filtered)
```

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