

# Package: crmn (via r-universe)

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**Title** CCMN and Other Normalization Methods for Metabolomics Data

**Depends** pcaMethods (>= 1.56.0), Biobase, methods

**Description** Implements the Cross-contribution Compensating Multiple standard Normalization (CCMN) method described in Redestig et al. (2009) Analytical Chemistry <https://doi.org/10.1021/ac901143w> and other normalization algorithms.

**URL** <https://github.com/hredestig/crmn>

**License** GPL (>= 3)

**Collate** 'classes.R' 'crmn-package.R' 'misc.R' 'norm.R' 'generic.R'

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**Repository** <https://hredestig.r-universe.dev>

**RemoteUrl** <https://github.com/hredestig/crmn>

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analytes	<i>Accessor for the analytes</i>
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---

**Description**

Subset an data set to only contain the analytes.

**Usage**

analytes(object, standards=NULL, ...)

**Arguments**

object	an ExpressionSet, matrix or data.frame
standards	a logical vector indicating which rows are internal analytes
...	not used

**Value**

subsetting dataset

**Author(s)**

Henning Redestig

**Examples**

```
data(mix)
analytes(mix)
analytes(exprs(mix), fData(mix)$tag == 'IS')
```

---

analytes_eset	<i>Accessor for the analytes</i>
---------------	----------------------------------

---

**Description**

Subset an expression set to remove the internal standards

**Usage**

```
analytes_eset(object, where = "tag", what = "IS", ...)
```

**Arguments**

object	an ExpressionSet
where	Column index or name of fData which equals what for the ISs (and something else for the analytes)
what	What the column where does not equal for analytes. Can be vector values too.
...	not used

**Value**

ExpressionSet

**Author(s)**

Henning Redestig

**Examples**

```
data(mix)
analytes(mix)
fData(mix)$test <- fData(mix)$tag
analytes(mix, where="test")
```

---

analytes_other	<i>Accessor for the analytes</i>
----------------	----------------------------------

---

**Description**

Subset an expression set to remove the internal standards

**Usage**

```
analytes_other(object, standards, ...)
```

**Arguments**

object	an ExpressionSet
standards	a logical vector indicating which rows are internal standards
...	not used

**Value**

ExpressionSet

**Author(s)**

Henning Redestig

**Examples**

```
data(mix)
analytes(exprs(mix), fData(mix)$tag == 'IS')
```

---

crmn	<i>CRMN</i>
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---

**Description**

Normalize metabolomics data using CCMN and other methods

**Details**

Package: crmn  
Type: Package  
Developed since: 2009-05-14  
Depends: Biobase, pcaMethods (>= 1.20.2), pls, methods  
License: GPL (>=3)  
LazyLoad: yes

A package implementing the 'Cross-contribution compensating multiple standard normalization' described in Redestig et al. (2009) Analytical Chemistry, <https://doi.org/10.1021/ac901143w>. Can be used to normalize metabolomics data. Do `openVignette("crmn")` to see the manual.

**Author(s)**

Henning Redestig

---

dropunusedlevels      *Drop unused levels*

---

**Description**

Drop unused factor levels in a data frame.

**Usage**

```
dropunusedlevels(x)
```

**Arguments**

x                    the data frame

**Author(s)**

Henning Redestig

**Examples**

```
iris[1:10,]$Species  
dropunusedlevels(iris[1:10,])$Species
```

---

`makeX-methods`*Make X*

---

**Description**

Construct a design matrix

**Usage**

```
makeX(object, factors, ...)
```

```
## S4 method for signature 'ANY,matrix'
```

```
makeX(object, factors, ...)
```

```
## S4 method for signature 'ExpressionSet,character'
```

```
makeX(object, factors, ...)
```

**Arguments**

`object` an ExpressionSet

`factors` column names from the pheno data of object or a design matrix

`...` not used

**Details**

Make a design matrix from the pheno data slot of an expression set, taking care that factors and numerical are handled properly. No interactions are included and formula is the most simple possible, i.e.  $y \sim -1 + \text{term1} + \text{term2} + \dots$ . Can also be given anything as object in which case factor must be a design matrix. In that case the same design matrix is returned.

**Value**

a design matrix

**Author(s)**

Henning Redestig

**Examples**

```
data(mix)
makeX(mix, "runorder")
runorder <- mix$runorder
makeX(mix, model.matrix(~-1+runorder))
```

---

method-methods	<i>Accessor for the method</i>
----------------	--------------------------------

---

**Description**

Get the method

**Usage**

```
method(object, ...)
```

```
method(object, ...)
```

**Arguments**

object	an nFit object
--------	----------------

...	not used
-----	----------

**Value**

the method (content differs between normlization methods)

**Author(s)**

Henning Redestig

---

mexprs-methods	<i>Matrix safe accessor of expression slot</i>
----------------	--

---

**Description**

Get the expression data from an ExpressionSet or just return the given matrix

**Usage**

```
mexprs(object)
```

```
mexprs(object)
```

```
## S4 method for signature 'ExpressionSet'
```

```
mexprs(object)
```

**Arguments**

object	an ExpressionSet or matrix
--------	----------------------------

**Value**

the expression data

**Author(s)**

Henning Redestig

**Examples**

```
data(mix)
head(mexprs(mix))
head(mexprs(exprs(mix)))
```

---

mexprs-rep-methods      *Accessor*

---

**Description**

Matrix safe setter of expression slot

**Usage**

```
mexprs(object) <- value

## S4 replacement method for signature 'ExpressionSet,matrix'
mexprs(object) <- value

mexprs(object) <- value
```

**Arguments**

object	an ExpressionSet or matrix
value	the value to assign

**Details**

Set the expression data in an ExpressionSet or just return the given matrix

**Value**

the expression data

**Author(s)**

Henning Redestig



**Examples**

```
data(mix)
test <- mix
mexprs(test) <- exprs(mix) * 0
head(mexprs(test))
test <- exprs(mix)
mexprs(test) <- test * 0
head(mexprs(test))
```

---

mix

*Dilution mixture dataset.*

---

**Description**

Mixture dilution series

**Usage**

```
data(mix)
```

**Details**

Multi-component dilution series. GC-TOF/MS measurements by Miyako Kusano. Input concentrations are known and given in the original publication.

**Author(s)**

Henning Redestig

**Examples**

```
data(mix)
fData(mix)
exprs(mix)
pData(mix)
```

---

model-methods

*Accessor for the model*

---

**Description**

Get the model

**Usage**

```
model(object, ...)
```

```
model(object, ...)
```

**Arguments**

object	an nFit object
...	not used

**Value**

the model (content differs between normalization models)

**Author(s)**

Henning Redestig

---

nFit	<i>Normalization model</i>
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---

**Description**

Common class representation for normalization models.

**Author(s)**

Henning Redestig

---

normalize	<i>Normalize a metabolomics dataset</i>
-----------	---

---

**Description**

Normalization methods for metabolomics data

**Usage**

```
normalize(object, method, segments = NULL, ...)
```

**Arguments**

object	an ExpressionSet
method	the desired method
segments	normalization in a cross-validation setup, only to use for validation/QC purposes.
...	passed on to normFit and normPred

**Details**

Wrapper function for normFit and normPred

**Value**

the normalized dataset

**Author(s)**

Henning Redestig

**See Also**

normFit, normPred

**Examples**

```
data(mix)
normalize(mix, "crmn", factor="type", ncomp=3)
#other methods
normalize(mix, "one")
normalize(mix, "avg")
normalize(mix, "nomis")
normalize(mix, "t1")
normalize(mix, "ri")
normalize(mix, "median")
normalize(mix, "totL2")
## can also do normalization with matrices
Y <- exprs(mix)
G <- with(pData(mix), model.matrix(~-1+type))
isIS <- with(fData(mix), tag == "IS")
normalize(Y, "crmn", factor=G, ncomp=3, standards=isIS)
```

---

normFit

*Fit a normalization model*

---

**Description**

Fit the parameters for normalization of a metabolomics data set.

**Usage**

```
normFit(
  object,
  method,
  one = "Succinate_d4",
  factors = NULL,
  lg = TRUE,
  fitfunc = lm,
  formula = TRUE,
  ...
)
```

**Arguments**

object	an ExpressionSet or a matrix (with samples as columns) in which case the standards must be passed on via . . .
method	chosen normalization method
one	single internal standard to use for normalization
factors	column names in the pheno data slot describing the biological factors. Or a design matrix directly.
lg	logical indicating that the data should be log transformed
fitfunc	the function that creates the model fit for normalization, must use the same interfaces as lm.
formula	if fitfunc has formula interface or not
. . .	passed on to standardsFit, standards, analytes

**Details**

Normalization is first done by fitting a model and then applying that model either to new data or the same data using normPred. Five different methods are implemented.

**t1** divide by row-means of the  $L_2$  scaled internal standards

**one** divide by value of a single, user defined, internal standard

**totL2** divide by the square of sums of the full dataset

**nomis** See Sysi-Aho et al.

**crmn** See Redestig et al.

**Value**

a normalization model

**Author(s)**

Henning Redestig

**References**

Sysi-Aho, M.; Katajamaa, M.; Yetukuri, L. & Oresic, M. Normalization method for metabolomics data using optimal selection of multiple internal standards. *BMC Bioinformatics*, 2007, 8, 93

Redestig, H.; Fukushima, A.; Stenlund, H.; Moritz, T.; Arita, M.; Saito, K. & Kusano, M. Compensation for systematic cross-contribution improves normalization of mass spectrometry based metabolomics data *Anal Chem*, 2009, 81, 7974-7980

**See Also**

normPred, standards, model.matrix

**Examples**

```

data(mix)
nfit <- normFit(mix, "crmn", factors="type", ncomp=3)
slplot(sFit(nfit)$fit$pc, scol=as.integer(mix$runorder))
## same thing
Y <- exprs(mix)
G <- model.matrix(~-1+mix$type)
isIS <- fData(mix)$tag == 'IS'
nfit <- normFit(Y, "crmn", factors=G, ncomp=3, standards=isIS)
slplot(sFit(nfit)$fit$pc, scol=as.integer(mix$runorder))

```

normPred

*Predict for normalization***Description**

Predict the normalized data using a previously fitted normalization model.

**Usage**

```
normPred(normObj, newdata, factors = NULL, lg = TRUE, predfunc = predict, ...)
```

**Arguments**

normObj	the result from normFit
newdata	an ExpressionSet or a matrix (in which case the standards must be passed on via ...), possibly the same as used to fit the normalization model in order to get the fitted data.
factors	column names in the pheno data slot describing the biological factors. Or a design matrix.
lg	logical indicating that the data should be log transformed
predfunc	the function to use to get predicted values from the fitted object (only for crmn)
...	passed on to standardsPred, standardsFit, odestandards, analytes

**Details**

Apply fitted normalization parameters to new data to get normalized data. Current can not only handle matrices as input for methods 'RI' and 'one'.

**Value**

the normalized data

**Author(s)**

Henning Redestig

**See Also**

normFit

**Examples**

```
data(mix)
nfit <- normFit(mix, "crmn", factor="type", ncomp=3)
normedData <- normPred(nfit, mix, "type")
splot(pca(t(log2(exprs(normedData))))), scol=as.integer(mix$type))
## same thing
Y <- exprs(mix)
G <- with(pData(mix), model.matrix(~-1+type))
isIS <- fData(mix)$tag == 'IS'
nfit <- normFit(Y, "crmn", factors=G, ncomp=3, standards=isIS)
normedData <- normPred(nfit, Y, G, standards=isIS)
splot(pca(t(log2(normedData))), scol=as.integer(mix$type))
```

---

pcaMuffle

*Muffle the pca function*

---

**Description**

PCA and Q2 issues warnings about biasedness and poorly estimated PCs. The first is non-informative and the poorly estimated PCs will show up as poor overfitting which leads to a choice of fewer PCs i.e. not a problem. This function is mean to muffle those warnings. Only used for version of pcaMethods before 1.26.0.

**Usage**

```
pcaMuffle(w)
```

**Arguments**

w                    a warning

**Value**

nothing

**Author(s)**

Henning Redestig

---

`plot.nFit`*Plot a statistics for CRMN normalization model*

---

**Description**

Simple plot function for a CRMN normalization model.

**Usage**

```
## S3 method for class 'nFit'  
plot(x, y = NULL, ...)
```

**Arguments**

<code>x</code>	an <code>nFit</code> object
<code>y</code>	not used
<code>...</code>	passed on to the scatter plot calls

**Details**

Shows Tz and the optimization (if computed) of the PCA model. The number of components used for normalization should not exceed the maximum indicated by Q2. The structure shown in the Tz plot indicate the analytical variance which is exactly independent of the experimental design. The corresponding loading plot shows how this structure is capture by the used ISs.

**Value**

nothing

**Author(s)**

Henning Redestig

**See Also**

`splot`

**Examples**

```
data(mix)  
nfit <- normFit(mix, "crmn", factors="type", ncomp=2)  
plot(nfit)
```

---

sFit-method	<i>Accessor for the standards model</i>
-------------	---

---

**Description**

Get the sFit

**Usage**

```
sFit(object, ...)
```

```
sFit(object, ...)
```

**Arguments**

object	an nFit object
...	not used

**Value**

the sFit is only defined for CRMN

**Author(s)**

Henning Redestig

---

show	<i>Show method for nFit</i>
------	-----------------------------

---

**Description**

Show some basic information for an nFit model

**Usage**

```
## S4 method for signature 'nFit'  
show(object)
```

**Arguments**

object	the nFit object
--------	-----------------

**Value**

prints some basic information



**Author(s)**

Henning Redestig

**Examples**

```
data(mix)
normFit(mix, "avg")
```

---

show\_nfit
*Show nfit***Description**

Show method for nFit

**Usage**

```
show_nfit(object)
```

**Arguments**

object            the nFit object

**Value**

prints some basic information

**Author(s)**

Henning Redestig

---

standards
*Accessor for the Internal Standards***Description**

Subset an data set to only contain the labeled internal standards.

**Usage**

```
standards(object, standards=NULL, ...)
```

**Arguments**

object            an ExpressionSet, matrix or data.frame  
standards        a logical vector indicating which rows are internal standards  
...                not used

**Value**

subsampled dataset

**Author(s)**

Henning Redestig

**Examples**

```
data(mix)
standards(mix)
standards(exprs(mix), fData(mix)$tag == 'IS')
```

---

standardsFit

*Standards model*


---

**Description**

Fit a model which describes the variation of the labeled internal standards from the biological factors.

**Usage**

```
standardsFit(object, factors, ncomp = NULL, lg = TRUE, fitfunc = lm, ...)
```

**Arguments**

object	an ExpressionSet or a matrix. Note that if you pass a matrix have to specify the identity of the standards by passing the appropriate argument to standards.
factors	the biological factors described in the pheno data slot if object is an ExpressionSet or a design matrix if object is a matrix.
ncomp	number of PCA components to use. Determined by cross-validation if left NULL
lg	logical indicating that the data should be log transformed
fitfunc	the function that creates the model fit for normalization, must use the same interfaces as lm.
...	passed on to Q2, pca (if pcaMethods > 1.26.0), standards and analytes

**Details**

There is often unwanted variation in among the labeled internal standards which is related to the experimental factors due to overlapping peaks etc. This function fits a model that describes that overlapping variation using a scaled and centered PCA / multiple linear regression model. Scaling is done outside the PCA model.

**Value**

a list containing the PCA/MLR model, the recommended number of components for that model, the standard deviations and mean values and Q2/R2 for the fit.

**Author(s)**

Henning Redestig

**See Also**

makeX, standardsPred

**Examples**

```
data(mix)
sfit <- standardsFit(mix, "type", ncomp=3)
splot(sfit$fit$pc)
## same thing
Y <- exprs(mix)
G <- model.matrix(~-1+mix$type)
isIS <- fData(mix)$tag == 'IS'
sfit <- standardsFit(Y, G, standards=isIS, ncomp=3)
```

---

standardsPred

*Predict effect for new data (or get fitted data)*


---

**Description**

Predicted values for the standards

**Usage**

```
standardsPred(model, newdata, factors, lg = TRUE, ...)
```

**Arguments**

model	result from standardsFit
newdata	an ExpressionSet or matrix with new data (or the data used to fit the model to get the fitted data)
factors	the biological factors described in the pheno data slot if object is an ExpressionSet or a design matrix if object is a matrix.
lg	logical indicating that the data should be log transformed
...	passed on to standards and analytes

**Details**

There is often unwanted variation in among the labeled internal standards which is related to the experimental factors due to overlapping peaks etc. This predicts this effect given a model of the overlapping variance. The prediction is given by  $\hat{X}_{IS} = X_{IS} - X_{IS}B$

**Value**

the corrected data

**Author(s)**

Henning Redestig

**See Also**

makeX, standardsFit

**Examples**

```
data(mix)
fullFit <- standardsFit(mix, "type", ncomp=3)
sfit <- standardsFit(mix[,-1], "type", ncomp=3)
pred <- standardsPred(sfit, mix[,1], "type")
cor(scores(sfit$fit$pc)[1,], scores(fullFit$fit$pc)[1,])
## could just as well have been done as
Y <- exprs(mix)
G <- model.matrix(~-1+mix$type)
isIS <- fData(mix)$tag == 'IS'
fullFit <- standardsFit(Y, G, ncomp=3, standards=isIS)
sfit <- standardsFit(Y[,-1], G[-1,], ncomp=3,
                    standards=isIS)
pred <- standardsPred(sfit, Y[,1,drop=FALSE], G[1,,drop=FALSE], standards=isIS)
cor(scores(sfit$fit$pc)[1,], scores(fullFit$fit$pc)[1,])
```

---

standards\_eset

*Accessor for the Internal Standards*


---

**Description**

Subset an data set to only contain the labeled internal standards.

**Usage**

```
standards_eset(object, where = "tag", what = "IS", ...)
```

**Arguments**

object	an ExpressionSet
where	Column index or name in fData which equals what for the ISs
what	What the column where equals for ISs
...	not used

**Value**

subsetting dataset

**Author(s)**

Henning Redestig

**Examples**

```
data(mix)
standards(mix)
fData(mix)$test <- fData(mix)$tag
standards(mix, where="test")
```

---

standards\_other

*Accessor for the Internal Standards*

---

**Description**

Subset an data set to only contain the labeled internal standards.

**Usage**

```
standards_other(object, standards, ...)
```

**Arguments**

object	an matrix or data.frame
standards	a logical vector indicating which rows are internal standards
...	not used

**Value**

subsetting dataset

**Author(s)**

Henning Redestig

**Examples**

```
data(mix)
standards(exprs(mix), fData(mix)$tag == 'IS')
```

---

weightnorm	<i>Normalize by sample weight</i>
------------	-----------------------------------

---

**Description**

Normalize samples by their weight (as in grams fresh weight)

**Usage**

```
weightnorm(object, weight = "weight", lg = FALSE)
```

**Arguments**

object	an ExpressionSet
weight	a string naming the pheno data column with the weight or a numeric vector with one weight value per sample.
lg	is the assay data already on the log-scale or not. If lg, the weight value is also log-transformed and subtraction is used instead of division.

**Details**

Normalize each sample by dividing by the loaded sample weight. The weight argument is taken from the pheno data (or given as numerical vector with one value per sample). Missing values are not tolerated.

**Value**

the normalized expression set

**Author(s)**

Henning Redestig

**Examples**

```
data(mix)
w <- runif(ncol(mix),1, 1.3)
weightnorm(mix, w)
```

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