

# Package ‘limorhyde2’

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**Type** Package

**Title** Quantify Rhythmicity and Differential Rhythmicity in Genomic Data

**Version** 0.1.1

**Description** Fit linear models based on periodic splines, moderate model coefficients using multivariate adaptive shrinkage, then compute properties of the moderated curves.

**URL** <https://limorhyde2.hugheylib.org>,  
<https://github.com/hugheylib/limorhyde2>

**License** GPL-2

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getDiffRhythmStats	<i>Compute differential rhythm statistics from fitted models</i>
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---

### Description

This function computes differences in rhythmicity between fitted curves for a given pair of conditions.

### Usage

```
getDiffRhythmStats(fit, rhyStats, conds = fit$conds, dopar = TRUE)
```

### Arguments

fit	A <code>limorhyde2</code> object containing data from multiple conditions.
rhyStats	A <code>data.table</code> of rhythmic statistics, as returned by <code>getRhythmStats()</code> , for fitted models in <code>fit</code> .
conds	A character vector indicating the conditions to compare pairwise, by default all conditions in <code>fit</code> .
dopar	Logical indicating whether to run calculations in parallel if a parallel backend is already set up, e.g., using <code>doParallel::registerDoParallel()</code> . Recommended to minimize runtime.

### Value

A `data.table` containing the following differential rhythm statistics:

- `mean_mesor`
- `mean_peak_trough_amp`
- `mean_rms_amp` (only calculated if `rms` to `getRhythmStats()` was `TRUE`)
- `diff_mesor`

- `diff_peak_trough_amp`
- `diff_rms_amp` (only calculated if `rms` to `getRhythmStats()` was TRUE)
- `diff_peak_phase`: circular difference between `-fit$period/2` and `fit$period/2`
- `diff_trough_phase`: circular difference between `-fit$period/2` and `fit$period/2`
- `diff_rhy_dist`: Euclidean distance between polar coordinates (`peak_trough_amp`, `peak_phase`)
- `rms_diff_rhy`: root mean square difference in mean-centered fitted curves (only calculated if `rms` to `getRhythmStats()` was TRUE)

The stats will be based on the value for `cond2` minus the value for `cond1`. The rows of the `data.table` depend on the `'fitType'` attribute of `rhyStats`:

- `'fitType'` is `'posterior_mean'` or `'raw'`: one row per feature per pair of conditions.
- `'fitType'` is `'posterior_samples'`: one row per feature per posterior sample per pair of conditions.

### See Also

[getRhythmStats\(\)](#), [getStatsIntervals\(\)](#)

### Examples

```
library('data.table')

# rhythmicity in one condition
y = GSE54650$y
metadata = GSE54650$metadata

fit = getModelFit(y, metadata)
fit = getPosteriorFit(fit)
rhyStats = getRhythmStats(fit, features = c('13170', '13869'))

# rhythmicity and differential rhythmicity in multiple conditions
y = GSE34018$y
metadata = GSE34018$metadata

fit = getModelFit(y, metadata, nKnots = 3L, condColname = 'cond')
fit = getPosteriorFit(fit)
rhyStats = getRhythmStats(fit, features = c('13170', '12686'))
diffRhyStats = getDiffRhythmStats(fit, rhyStats)
```

---

getExpectedMeas

*Compute expected measurements from fitted models*

---

### Description

This function computes expected measurements (corresponding to the fitted curves) for the specified times and features in all combinations of conditions and covariates (if they exist).

**Usage**

```
getExpectedMeas(
  fit,
  times,
  fitType = c("posterior_mean", "posterior_samples", "raw"),
  features = NULL,
  dopar = TRUE
)
```

**Arguments**

<code>fit</code>	A 'limorhyde2' object.
<code>times</code>	Numeric vector of times, in units of <code>fit\$metadata[[fit\$timeColname]]</code> .
<code>fitType</code>	String indicating which fitted models to use to compute the expected measurements. A typical analysis using <code>limorhyde2</code> will be based on 'posterior_mean', the default.
<code>features</code>	Vector of names, row numbers, or logical values for subsetting the features. NULL indicates all features.
<code>dopar</code>	Logical indicating whether to run calculations in parallel if a parallel backend is already set up, e.g., using <code>doParallel::registerDoParallel()</code> . Recommended to minimize runtime.

**Value**

A data.table.

**See Also**

[getModelFit\(\)](#), [getPosteriorFit\(\)](#), [getPosteriorSamples\(\)](#), [getExpectedMeasIntervals\(\)](#)

**Examples**

```
library('data.table')

y = GSE34018$y
metadata = GSE34018$metadata

fit = getModelFit(y, metadata)
fit = getPosteriorFit(fit)

measObs = mergeMeasMeta(y, metadata, features = c('13170', '12686'))
measFitMean = getExpectedMeas(
  fit, times = seq(0, 24, 0.5), features = c('13170', '12686'))
```

---

`getExpectedMeasIntervals`*Compute credible intervals for expected measurements*

---

### Description

This functions uses posterior samples to quantify uncertainty in the expected measurements from fitted models.

### Usage

```
getExpectedMeasIntervals(expectedMeas, mass = 0.9, method = c("eti", "hdi"))
```

### Arguments

<code>expectedMeas</code>	A <code>data.table</code> of expected measurements for posterior samples, as returned by <a href="#">getExpectedMeas()</a> .
<code>mass</code>	Number between 0 and 1 indicating the probability mass for which to calculate the intervals.
<code>method</code>	String indicating the type of interval: 'eti' for equal-tailed using <a href="#">stats::quantile()</a> , or 'hdi' for highest density using <a href="#">HDInterval::hdi()</a> .

### Value

A `data.table` containing lower and upper bounds of the expected measurement for each combination of feature, time, and possibly condition and covariate.

### See Also

[getExpectedMeas\(\)](#), [getStatsIntervals\(\)](#)

### Examples

```
library('data.table')

y = GSE34018$y
metadata = GSE34018$metadata

fit = getModelFit(y, metadata)
fit = getPosteriorFit(fit)
fit = getPosteriorSamples(fit, nPosteriorSamples = 10L)

measFitSamps = getExpectedMeas(
  fit, times = seq(0, 24, 0.5), fitType = 'posterior_samples',
  features = c('13170', '12686'))
measFitInts = getExpectedMeasIntervals(measFitSamps)
```

---

getModelFit

*Fit linear models for rhythmicity in one or more conditions*


---

### Description

This is the first step in an analysis using `limorhyde2`, the second is to moderate the fits using `getPosteriorFit()`.

### Usage

```
getModelFit(
  y,
  metadata,
  period = 24,
  nKnots = 3L,
  degree = if (nKnots > 2) 3L else 2L,
  sinusoid = FALSE,
  timeColname = "time",
  condColname = NULL,
  covarColnames = NULL,
  sampleColname = "sample",
  nShifts = 3L,
  method = c("trend", "voom", "deseq2"),
  lmFitArgs = list(),
  eBayesArgs = if (method == "trend") list(trend = TRUE) else list(),
  DESeqArgs = list(),
  keepLmFits = FALSE
)
```

### Arguments

<code>y</code>	Matrix-like object of measurements, with rows corresponding to features and columns to samples.
<code>metadata</code>	<code>data.frame</code> containing experimental design information for each sample. Rows of metadata must correspond to columns of <code>y</code> . Row names are ignored.
<code>period</code>	Number specifying the period for the time variable, in the same units as the values in the <code>timeColname</code> column.
<code>nKnots</code>	Number of internal knots for the periodic spline for the time variable.
<code>degree</code>	Integer indicating degree of the piecewise polynomial for the spline.
<code>sinusoid</code>	Logical indicating whether to fit a cosinor-based model instead of a spline-based model.
<code>timeColname</code>	String indicating the column in metadata containing the time at which each sample was acquired.

condColname	String indicating the column in metadata containing the condition in which each sample was acquired. NULL indicates all samples came from the same condition. If not NULL, the model will include main effects and interactions with the terms for time.
covarColnames	Character vector indicating the columns in metadata containing covariates to include in the model. NULL indicates no covariates.
sampleColname	String indicating the column in metadata containing the name of each sample, which must correspond to the column names of <i>y</i> .
nShifts	Number of shifted models to fit. Only used for periodic splines, not for cosinor. Do not change from the default unless you know what you're doing.
method	String indicating method to estimate model coefficients. For microarray data, use 'trend'. For RNA-seq count data, use 'voom' or 'deseq2'.
lmFitArgs	List of arguments passed to <code>limma::lmFit()</code> .
eBayesArgs	List of arguments passed to <code>limma::eBayes()</code> .
DESeqArgs	List of arguments passed to <code>DESeq2::DESeq()</code> .
keepLmFits	Logical indicating whether to keep the complete fit objects from <code>limma</code> or <code>DESeq2</code> . Not needed by any functions in <code>limorhyde2</code> .

## Value

A `limorhyde2` object with elements:

- `metadata`: As supplied above, converted to a `data.table`.
- `timeColname`: As supplied above.
- `condColname`: As supplied above.
- `covarColnames`: As supplied above.
- `coefficients`: Matrix with rows corresponding to features and columns to model terms, including all shifted models.
- `shifts`: Numeric vector indicating amount by which timepoints were shifted for each shifted model.
- `period`: As supplied above.
- `conds`: If `condColname` is not NULL, a vector of unique values of the condition variable.
- `nKnots`: Number of knots.
- `degree`: As supplied above.
- `sinusoid`: As supplied above.
- `nConds`: Number of conditions.
- `nCovs`: Number of covariates.
- `lmFits`: If `keepLmFits` is TRUE, a list of objects from `limma` or `DESeq2`, with length equal to length of the `shifts` element.

## See Also

[getPosteriorFit\(\)](#)

**Examples**

```

library('data.table')

# rhythmicity in one condition
y = GSE54650$y
metadata = GSE54650$metadata

fit = getModelFit(y, metadata)
fit = getPosteriorFit(fit)
rhyStats = getRhythmStats(fit, features = c('13170', '13869'))

# rhythmicity and differential rhythmicity in multiple conditions
y = GSE34018$y
metadata = GSE34018$metadata

fit = getModelFit(y, metadata, nKnots = 3L, condColname = 'cond')
fit = getPosteriorFit(fit)
rhyStats = getRhythmStats(fit, features = c('13170', '12686'))
diffRhyStats = getDiffRhythmStats(fit, rhyStats)

```

---

getPosteriorFit

---

*Compute posterior fit for linear models for rhythmicity*


---

**Description**

This is the second step in an analysis using `limorhyde2`, the first is to fit linear models using `getModelFit()`. This function obtains posterior estimates of coefficients using multivariate adaptive shrinkage (mash), which learns patterns in the data and accounts for noise in the original fits. The defaults for arguments should work well in most cases, so only change them if you know what you're doing.

**Usage**

```

getPosteriorFit(
  fit,
  covMethod = c("data-driven", "canonical", "both"),
  getSigResArgs = list(),
  npc = fit$nKnots,
  covEdArgs = list(),
  overwrite = FALSE,
  ...
)

```

**Arguments**

<code>fit</code>	A <code>limorhyde2</code> object.
<code>covMethod</code>	String indicating the type(s) of covariance matrices to use for the mash fit.

getSigResArgs	List of arguments passed to <code>mashr::get_significant_results()</code> . Only used if <code>covMethod</code> is 'data-driven' or 'both'.
npc	Number of principal components passed to <code>mashr::cov_pca()</code> . Only used if <code>covMethod</code> is 'data-driven' or 'both'.
covEdArgs	List of arguments passed to <code>mashr::cov_ed()</code> . Only used if <code>covMethod</code> is 'data-driven' or 'both'.
overwrite	Logical for whether to recompute the mash fit if it already exists.
...	Additional arguments passed to <code>mashr::mash()</code> .

## Value

A `limorhyde2` object containing everything in `fit` with added or updated elements:

- `mashData`: list of mash data objects
- `mashFits`: list of mash fit objects
- `mashCoefficients`: Matrix of posterior mean coefficients, with rows corresponding to features and columns to model terms.
- `mashIdx`: Vector indicating which model terms were included in the mash fit.

## See Also

[getModelFit\(\)](#), [getRhythmStats\(\)](#), [getExpectedMeas\(\)](#)

## Examples

```
library('data.table')

# rhythmicity in one condition
y = GSE54650$y
metadata = GSE54650$metadata

fit = getModelFit(y, metadata)
fit = getPosteriorFit(fit)
rhyStats = getRhythmStats(fit, features = c('13170', '13869'))

# rhythmicity and differential rhythmicity in multiple conditions
y = GSE34018$y
metadata = GSE34018$metadata

fit = getModelFit(y, metadata, nKnots = 3L, condColname = 'cond')
fit = getPosteriorFit(fit)
rhyStats = getRhythmStats(fit, features = c('13170', '12686'))
diffRhyStats = getDiffRhythmStats(fit, rhyStats)
```

---

getPosteriorSamples *Draw samples from posterior distributions of fitted models*

---

### Description

This is an optional step in an analysis using `limorhyde2`, and is useful for quantifying uncertainty in posterior estimates of fitted curves and rhythmic statistics. The function calls `mashr::mash_compute_posterior_matrices`

### Usage

```
getPosteriorSamples(fit, nPosteriorSamples = 200L, overwrite = FALSE)
```

### Arguments

`fit` A 'limorhyde2' object containing posterior fits.  
`nPosteriorSamples` Number of samples to draw from each posterior distribution.  
`overwrite` Logical indicating whether to recompute posterior samples if they already exist.

### Value

A `limorhyde2` object containing everything in `fit` with added or updated element:

- `mashPosteriorSamples`: a three-dimensional array of coefficients, with dim 1 corresponding to features, dim 2 to model terms, and dim 3 to posterior samples.

### See Also

[getPosteriorFit\(\)](#), [getRhythmStats\(\)](#), [getExpectedMeas\(\)](#), [getStatsIntervals\(\)](#)

### Examples

```
library('data.table')

y = GSE54650$y
metadata = GSE54650$metadata

fit = getModelFit(y, metadata)
fit = getPosteriorFit(fit)
fit = getPosteriorSamples(fit, nPosteriorSamples = 10L)

rhyStatsSamps = getRhythmStats(
  fit, features = c('13170', '13869'), fitType = 'posterior_samples')
rhyStatsInts = getStatsIntervals(rhyStatsSamps)
```

---

getRhythmStats	<i>Compute rhythm statistics from fitted models</i>
----------------	---

---

### Description

This function uses `stats::optim()` to compute various properties of fitted curves with respect to time, potentially in each condition and for each posterior sample, and adjusting for any covariates.

### Usage

```
getRhythmStats(  
  fit,  
  fitType = c("posterior_mean", "posterior_samples", "raw"),  
  features = NULL,  
  dopar = TRUE,  
  rms = FALSE  
)
```

### Arguments

<code>fit</code>	A <code>limorhyde2</code> object.
<code>fitType</code>	String indicating which fitted models to use to compute the rhythmic statistics. A typical analysis using <code>limorhyde2</code> will be based on 'posterior_mean', the default.
<code>features</code>	Vector of names, row numbers, or logical values for subsetting the features. NULL indicates all features.
<code>dopar</code>	Logical indicating whether to run calculations in parallel if a parallel backend is already set up, e.g., using <code>doParallel::registerDoParallel()</code> . Recommended to minimize runtime.
<code>rms</code>	Logical indicating whether to calculate <code>rms_amp</code> .

### Value

A data table containing the following rhythm statistics:

- `peak_phase`: time between 0 and `fit$period` at which the peak or maximum value occurs
- `peak_value`
- `trough_phase`: time between 0 and `fit$period` at which the trough or minimum value occurs
- `trough_value`
- `peak_trough_amp`: `peak_value - trough_value`
- `rms_amp`: root mean square difference between fitted curve and mean value between time 0 and `fit$period` (only calculated if `rms` is TRUE)
- `mesor`: mean value between time 0 and `fit$period`

The rows of the `data.table` depend on the `fit` object and `fitType`:

- `fit` contains data from one condition and `fitType` is `'posterior_mean'` or `'raw'`: one row per feature.
- `fit` contains data from one condition and `fitType` is `'posterior_samples'`: one row per feature per posterior sample.
- `fit` contains data from multiple conditions and `fitType` is `'posterior_mean'` or `'raw'`: one row per feature per condition.
- `fit` contains data from multiple conditions and `fitType` is `'posterior_samples'`: one row per feature per condition per posterior sample.

### See Also

[getModelFit\(\)](#), [getPosteriorFit\(\)](#), [getPosteriorSamples\(\)](#), [getDiffRhythmStats\(\)](#), [getStatsIntervals\(\)](#)

### Examples

```
library('data.table')

# rhythmicity in one condition
y = GSE54650$y
metadata = GSE54650$metadata

fit = getModelFit(y, metadata)
fit = getPosteriorFit(fit)
rhyStats = getRhythmStats(fit, features = c('13170', '13869'))

# rhythmicity and differential rhythmicity in multiple conditions
y = GSE34018$y
metadata = GSE34018$metadata

fit = getModelFit(y, metadata, nKnots = 3L, condColname = 'cond')
fit = getPosteriorFit(fit)
rhyStats = getRhythmStats(fit, features = c('13170', '12686'))
diffRhyStats = getDiffRhythmStats(fit, rhyStats)
```

---

getStatsIntervals      *Compute credible intervals for rhythm or differential rhythm statistics*

---

### Description

This function uses posterior samples to quantify uncertainty in the properties of fitted curves.

### Usage

```
getStatsIntervals(posteriorStats, mass = 0.9, method = c("eti", "hdi"))
```

**Arguments**

- `posteriorStats` A `data.table` of statistics for posterior samples, as returned by `getRhythmStats()` or `getDiffRhythmStats()`.
- `mass` Number between 0 and 1 indicating the probability mass for which to calculate the intervals.
- `method` String indicating the type of interval: 'eti' for equal-tailed using `stats::quantile()`, or 'hdi' for highest density using `HDInterval::hdi()`.

**Value**

A `data.table` containing lower and upper bounds of various statistics for each feature or each feature-condition pair. For `peak_trough_amp` and `rms_amp`, a negative lower bound indicates a rhythm of the opposite phase.

**See Also**

[getRhythmStats\(\)](#), [getDiffRhythmStats\(\)](#), [getExpectedMeasIntervals\(\)](#)

**Examples**

```
library('data.table')

y = GSE54650$y
metadata = GSE54650$metadata

fit = getModelFit(y, metadata)
fit = getPosteriorFit(fit)
fit = getPosteriorSamples(fit, nPosteriorSamples = 10L)

rhyStatsSamps = getRhythmStats(
  fit, features = c('13170', '13869'), fitType = 'posterior_samples')
rhyStatsInts = getStatsIntervals(rhyStatsSamps)
```

---

GSE34018

*Gene expression data for GSE34018*


---

**Description**

Data are based on total RNA, measured by microarray, obtained from livers of wild-type and liver-specific Reverb alpha/beta double knockout mice at various times in a 12h:12h light:dark cycle. To save space and time, the data include only a subset of genes, and so are mainly useful for examples of how to use `limorhyde2`.

**Usage**

GSE34018

**Format**

A list with two elements:

- `y`: Matrix of normalized, log-transformed expression values. Rows correspond to genes (row-names are Entrez Gene IDs) and columns to samples.
- `metadata`: `data.table` with one row per sample. `time` is in hours.

**Source**

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE34018>

**See Also**

[GSE54650](#), [getModelFit\(\)](#)

---

GSE54650

*Gene expression data for GSE54650*

---

**Description**

Data are based on total RNA, measured by microarray, obtained from livers of wild-type mice at various times after transfer to constant darkness. To save space and time, the data include only a subset of genes, and so are mainly useful for examples of how to use `limorhyde2`.

**Usage**

GSE54650

**Format**

A list with two elements:

- `y`: Matrix of normalized, log-transformed expression values. Rows correspond to genes (row-names are Entrez Gene IDs) and columns to samples.
- `metadata`: `data.table` with one row per sample. `time` is in hours.

**Source**

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54650>

**See Also**

[GSE34018](#), [getModelFit\(\)](#)

---

mergeMeasMeta	<i>Merge measurements and metadata</i>
---------------	--

---

### Description

This function is useful for plotting time-courses for individual features.

### Usage

```
mergeMeasMeta(y, metadata, features = NULL, sampleColname = "sample")
```

### Arguments

y	Matrix-like object of measurements, with rows corresponding to features and columns to samples.
metadata	data.frame containing experimental design information for each sample. Rows of metadata must correspond to columns of y. Row names are ignored.
features	Vector of names, row numbers, or logical values for subsetting the features. NULL indicates all features.
sampleColname	String indicating the column in metadata containing the name of each sample, which must correspond to the column names of y.

### Value

A data.table with one row for each sample-feature pair.

### See Also

[getExpectedMeas\(\)](#)

### Examples

```
library('data.table')

y = GSE34018$y
metadata = GSE34018$metadata

fit = getModelFit(y, metadata)
fit = getPosteriorFit(fit)

measObs = mergeMeasMeta(y, metadata, features = c('13170', '12686'))
measFitMean = getExpectedMeas(
  fit, times = seq(0, 24, 0.5), features = c('13170', '12686'))
```

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