

# Package ‘periodicDNA’

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**Type** Package

**Title** Set of tools to identify periodic occurrences of k-mers in DNA sequences

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**Description** This R package helps the user identify k-mers (e.g. di- or tri-nucleotides) present periodically in a set of genomic loci (typically regulatory elements). The functions of this package provide a straightforward approach to find periodic occurrences of k-mers in DNA sequences, such as regulatory elements. It is not aimed at identifying motifs separated by a conserved distance; for this type of analysis, please visit MEME website.

**URL** <https://github.com/js2264/periodicDNA>

**BugReports** <https://github.com/js2264/periodicDNA/issues>

**RoxygenNote** 7.1.0

**Depends** R (>= 4.0), Biostrings, GenomicRanges, IRanges, BSgenome, BiocParallel

**Imports** S4Vectors, rtracklayer, stats, GenomeInfoDb, magrittr, zoo, ggplot2, methods, parallel, cowplot

**Suggests** BSgenome.Scerevisiae.UCSC.sacCer3, BSgenome.Celegans.UCSC.ce11, BSgenome.Dmelanogaster.UCSC.dm6, BSgenome.Drerio.UCSC.danRer10, BSgenome.Hsapiens.UCSC.hg38, BSgenome.Mmusculus.UCSC.mm10, reticulate, testthat, covr, knitr, rmarkdown, pkgdown

**VignetteBuilder** knitr

**biocViews** SequenceMatching, MotifDiscovery, MotifAnnotation, Sequencing, Coverage, Alignment, DataImport

**License** GPL-3 + file LICENSE

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---

|              |                     |
|--------------|---------------------|
| cell_all_REs | <i>cell_all_REs</i> |
|--------------|---------------------|

---

Description

Regulatory elements annotated in C. elegans (cell) according to Serizay et al. 2020, "Tissue-specific profiling reveals distinctive regulatory architectures for ubiquitous, germline and somatic genes", BiorXiv.

Usage

data(cell\_all\_REs)

Format

GRanges

**Source**[BiorXiv](#)**References**

Serizay et al. 2020, "Tissue-specific profiling reveals distinctive regulatory architectures for ubiquitous, germline and somatic genes", BiorXiv. ([DOI](#))

**Examples**

```
data(ce11_all_REs)
table(ce11_all_REs$regulatory_class)
table(ce11_all_REs$which.tissues)
```

---

`ce11_ATACseq`*ce11\_ATACseq*

---

**Description**

Sample of ATAC-seq from mixed tissues in *C. elegans* young adults

**Usage**

```
data(ce11_ATACseq)
```

**Format**

RleList

**Source**[BiorXiv](#)**References**

Serizay et al. 2020, "Tissue-specific profiling reveals distinctive regulatory architectures for ubiquitous, germline and somatic genes", BiorXiv. ([DOI](#))

**Examples**

```
data(ce11_ATACseq)
ce11_ATACseq
```

---

`cell1_proms`*cell1\_proms*

---

**Description**

Promoters annotated in *C. elegans* (cell1) according to Serizay et al. 2020, "Tissue-specific profiling reveals distinctive regulatory architectures for ubiquitous, germline and somatic genes", BiorXiv.

**Usage**

```
data(cell1_proms)
```

**Format**

GRanges

**Source**

[BiorXiv](#)

**References**

Serizay et al. 2020, "Tissue-specific profiling reveals distinctive regulatory architectures for ubiquitous, germline and somatic genes", BiorXiv. ([DOI](#))

**Examples**

```
data(cell1_proms)
table(cell1_proms$which.tissues)
```

---

`cell1_proms_seqs`*cell1\_proms\_seqs*

---

**Description**

Sample of sequences of promoters annotated in *C. elegans* (cell1) according to Serizay et al. 2020, "Tissue-specific profiling reveals distinctive regulatory architectures for ubiquitous, germline and somatic genes", BiorXiv.

**Usage**

```
data(cell1_proms_seqs)
```

**Format**

DNASTringSet

**Source**

[BiorXiv](#)

**References**

Serizay et al. 2020, "Tissue-specific profiling reveals distinctive regulatory architectures for ubiquitous, germline and somatic genes", BiorXiv. ([DOI](#))

**Examples**

```
data(ce11_proms_seqs)
head(ce11_proms_seqs)
```

---

ce11\_TSSs

*ce11\_TSSs*

---

**Description**

Coordinates of promoter TSSs annotated in *C. elegans* (ce11) used in Serizay et al. 2020, "Tissue-specific profiling reveals distinctive regulatory architectures for ubiquitous, germline and somatic genes", BiorXiv.

**Usage**

```
data(ce11_TSSs)
```

**Format**

GRanges

**Source**

[BiorXiv](#)

**References**

Serizay et al. 2020, "Tissue-specific profiling reveals distinctive regulatory architectures for ubiquitous, germline and somatic genes", BiorXiv. ([DOI](#))

**Examples**

```
data(ce11_TSSs)
lengths(ce11_TSSs)
ce11_TSSs[[1]]
```

---

ce11\_WW\_10bp

*ce11\_WW\_10bp*


---

### Description

Sample of WW 10-bp periodicity track generated by getPeriodicityTrack() in ce11 over annotated accessible sites, with default parameters

### Usage

```
data(ce11_WW_10bp)
```

### Format

RleList

### Source

[BiorXiv](#)

### References

Serizay et al. 2020, "Tissue-specific profiling reveals distinctive regulatory architectures for ubiquitous, germline and somatic genes", BiorXiv. ([DOI](#))

### Examples

```
data(ce11_WW_10bp)
ce11_WW_10bp
```

---

getPeriodicity

*A function to compute k-mer periodicity in sequence(s).*


---

### Description

This function takes a set of sequences and a k-mer of interest, map a k-mer of interest in these sequences, computes all the pairwise distances (distogram), normalize it for distance decay, and computes the resulting power spectral density of the normalized distogram.

**Usage**

```

getPeriodicity(x, motif, ...)

## S3 method for class 'DNASTringSet'
getPeriodicity(
  x,
  motif,
  range_spectrum = seq(1, 200),
  BPPARAM = setUpBPPARAM(1),
  roll = 3,
  verbose = TRUE,
  sample = 0,
  n_shuffling = 0,
  cores_shuffling = 1,
  cores_computing = 1,
  order = 1,
  ...
)

## S3 method for class 'GRanges'
getPeriodicity(x, motif, genome = "BSgenome.Celegans.UCSC.ce11", ...)

## S3 method for class 'DNASTring'
getPeriodicity(x, motif, ...)

```

**Arguments**

|                              |                                                                                                                                                                                                                                       |
|------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>x</code>               | a DNASTring, DNASTringSet or GRanges object.                                                                                                                                                                                          |
| <code>motif</code>           | a k-mer of interest                                                                                                                                                                                                                   |
| <code>...</code>             | Arguments passed to S3 methods                                                                                                                                                                                                        |
| <code>range_spectrum</code>  | Numeric vector Range of the distogram to use to run the Fast Fourier Transform on (default: 1:200, i.e. all pairs of k-mers at a maximum of 200 bp from each other).                                                                  |
| <code>BPPARAM</code>         | split the workload over several processors using BiocParallel                                                                                                                                                                         |
| <code>roll</code>            | Integer Window to smooth the distribution of pairwise distances (default: 3, to discard the 3-bp periodicity of dinucleotides which can be very strong in vertebrate genomes)                                                         |
| <code>verbose</code>         | Boolean                                                                                                                                                                                                                               |
| <code>sample</code>          | Integer if > 0, will randomly sample this many integers from the dists vector before normalization. This ensures consistency when looking at periodicity in different genomes, since different genomes will have different GC percent |
| <code>n_shuffling</code>     | Integer, how many times should the sequences be shuffled? (default = 0)                                                                                                                                                               |
| <code>cores_shuffling</code> | integer, Number of cores used for shuffling (used if n_shuffling > 0)                                                                                                                                                                 |

|                 |                                                                                                                                                    |
|-----------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| cores_computing | integer, split the workload over several processors using BiocParallel (used if n_shuffling > 0)                                                   |
| order           | Integer, which order to take into consideration for shuffling (ushuffle python library must be installed for orders > 1) (used if n_shuffling > 0) |
| genome          | genome ID, BSgenome or DNASTringSet object (optional, if x is a GRanges)                                                                           |

### Value

A list containing the results of getPeriodicity function.

- The dists vector is the raw vector of all distances between any possible k-mer.
- The hist data.frame is the distribution of distances over range\_spectrum.
- The normalized\_hist is the raw hist, normalized for decay over increasing distances.
- The spectra object is the output of the FFT applied over normalized\_hist.
- The PSD data frame is the power spectral density scores over given frequencies.
- The motif object is the k-mer being analysed.
- The final periodicity metrics computed by getPeriodicity()

If getPeriodicity() is ran with n\_shuffling > 0, the resulting list also contains PSD values computed when iterating through shuffled sequences.

### Methods (by class)

- DNASTringSet: S3 method for DNASTringSet
- GRanges: S3 method for GRanges
- DNASTring: S3 method for DNASTring

### Examples

```
data(ce11_proms_seqs)
periodicity_result <- getPeriodicity(
  ce11_proms_seqs[1:100],
  motif = 'TT'
)
head(periodicity_result$PSD)
plotPeriodicityResults(periodicity_result)
#
data(ce11_TSSs)
periodicity_result <- getPeriodicity(
  ce11_TSSs[['Ubic. ']][1:10],
  motif = 'TT',
  genome = 'BSgenome.Celegans.UCSC.ce11'
)
head(periodicity_result$PSD)
plotPeriodicityResults(periodicity_result)
#
data(ce11_TSSs)
periodicity_result <- getPeriodicity(
```



```

    ce11_TSSs[['Ubiq.'][1:10],
    motif = 'TT',
    genome = 'BSgenome.Celegans.UCSC.ce11',
    n_shuffling = 10
)
head(periodicity_result$PSD)
plotPeriodicityResults(periodicity_result)

```

---

getPeriodicityTrack      *Function to generate a k-mer periodicity track*

---

## Description

This function takes a set of GRanges in a genome, recover the corresponding sequences and divides them using a sliding window. For each sub-sequence, it then computes the PSD value of a k-mer of interest at a chosen period, and generates a linear .bigWig track from these values.

## Usage

```

getPeriodicityTrack(
  genome = NULL,
  granges,
  motif = "WW",
  period = 10,
  BPPARAM = setUpBPPARAM(1),
  extension = 1000,
  window_size = 100,
  step_size = 2,
  range_spectrum = seq(5, 50),
  smooth_track = 20,
  bw_file = NULL
)

```

## Arguments

|                |                                                                                                                                            |
|----------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| genome         | DNAStringSet, BSgenome or genome ID                                                                                                        |
| granges        | GRanges object                                                                                                                             |
| motif          | character, k-mer of interest.                                                                                                              |
| period         | Integer, the period of the k-mer to study (default=10).                                                                                    |
| BPPARAM        | split the workload over several processors using BiocParallel                                                                              |
| extension      | Integer, the width the GRanges are going to be extended to (default 1000).                                                                 |
| window_size    | Integer, the width of the bins to split the GRanges objects in (default 100).                                                              |
| step_size      | Integer, the increment between bins over GRanges (default 2).                                                                              |
| range_spectrum | Numeric vector, the distances between nucleotides to take into consideration when performing Fast Fourier Transform (default seq_len(50)). |
| smooth_track   | Integer, smooth the resulting track                                                                                                        |
| bw_file        | character, the name of the output bigWig track                                                                                             |

**Value**

Rlelist and a bigWig track in the working directory.

**Examples**

```
data(cell_proms)
track <- getPeriodicityTrack(
  genome = 'BSgenome.Celegans.UCSC.ce11',
  cell_proms[1],
  extension = 200,
  window_size = 100,
  step_size = 10,
  smooth_track = 1,
  motif = 'WW',
  period = 10,
  BPPARAM = setUpBPPARAM(1)
)
track
unlink(
  'BSgenome.Celegans.UCSC.ce11_WW_10-bp-periodicity_g-100^10_smooth-1.bw'
)
```

---

getPeriodicityWithIterations

*A function to compute PSDs with iterations*

---

**Description**

This function computes PSD values of a given k-mer of interest in a set of input sequences. It also iterates the PSD calculation process over shuffled sequences, if `n_shuffling` is used.

**Usage**

```
getPeriodicityWithIterations(x, ...)

## S3 method for class 'DNAStringSet'
getPeriodicityWithIterations(
  x,
  motif,
  n_shuffling = 10,
  cores_shuffling = 1,
  cores_computing = 1,
  order = 1,
  verbose = 1,
  ...
)

## S3 method for class 'GRanges'
getPeriodicityWithIterations(x, genome, ...)
```

**Arguments**

|                 |                                                                                                                          |
|-----------------|--------------------------------------------------------------------------------------------------------------------------|
| x               | DNAStringSet, sequences of interest                                                                                      |
| ...             | Arguments passed to S3 methods                                                                                           |
| motif           | character, k-mer of interest                                                                                             |
| n_shuffling     | integer, Number of shuffling                                                                                             |
| cores_shuffling | integer, Number of cores used for shuffling                                                                              |
| cores_computing | integer, split the workload over several processors using BiocParallel                                                   |
| order           | Integer, which order to take into consideration for shuffling (ushuffle python library must be installed for orders > 1) |
| verbose         | integer, Should the function be verbose?                                                                                 |
| genome          | genome ID, BSgenome or DNAStringSet object (optional, if x is a GRanges)                                                 |

**Value**

Several metrics

**Methods (by class)**

- DNAStringSet: S3 method for DNAString
- GRanges: S3 method for GRanges

**Examples**

```
data(ce11_proms_seqs)
res <- getPeriodicityWithIterations(
  ce11_proms_seqs[1:10],
  genome = 'BSgenome.Celegans.UCSC.ce11',
  motif = 'TT',
  cores_shuffling = 1
)
res$observed_PSD
res$shuffled_PSD
```

---

plotAggregateCoverage *A function to plot aggregated signals over sets of GRanges*

---

**Description**

This function takes one or several RleList genomic tracks (e.g. imported by rtraklayer::import(..., as = 'Rle')) and one or several GRanges objects. It computes coverage of the GRanges by the genomic tracks and returns an aggregate coverage plot.

**Usage**

```
plotAggregateCoverage(x, ...)

## S3 method for class 'CompressedRleList'
plotAggregateCoverage(x, granges, ...)

## S3 method for class 'SimpleRleList'
plotAggregateCoverage(
  x,
  granges,
  colors = NULL,
  xlab = "Center of elements",
  ylab = "Score",
  xlim = NULL,
  ylim = NULL,
  quartiles = c(0.025, 0.975),
  verbose = FALSE,
  bin = 1,
  plot_central = TRUE,
  run_in_parallel = FALSE,
  split_by_granges = FALSE,
  norm = "none",
  ...
)

## S3 method for class 'list'
plotAggregateCoverage(
  x,
  granges,
  colors = NULL,
  xlab = "Center of elements",
  ylab = "Score",
  xlim = NULL,
  ylim = NULL,
  quartiles = c(0.025, 0.975),
  verbose = FALSE,
  bin = 1,
  plot_central = TRUE,
  split_by_granges = TRUE,
  split_by_track = FALSE,
  free_scales = FALSE,
  run_in_parallel = FALSE,
  norm = "none",
  ...
)
```

**Arguments**

|                               |                                                                                                                                                                                                                     |
|-------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>x</code>                | a single signal track ( <code>CompressedRleList</code> or <code>SimpleRleList</code> class), or several signal tracks ( <code>SimpleRleList</code> or <code>CompressedRleList</code> class) grouped in a named list |
| <code>...</code>              | additional parameters                                                                                                                                                                                               |
| <code>granges</code>          | a <code>GRanges</code> object or a named list of <code>GRanges</code>                                                                                                                                               |
| <code>colors</code>           | a vector of colors                                                                                                                                                                                                  |
| <code>xlab</code>             | x axis label                                                                                                                                                                                                        |
| <code>ylab</code>             | y axis label                                                                                                                                                                                                        |
| <code>xlim</code>             | y axis limits                                                                                                                                                                                                       |
| <code>ylim</code>             | y axis limits                                                                                                                                                                                                       |
| <code>quartiles</code>        | Which quantiles to use to determine y scale automatically?                                                                                                                                                          |
| <code>verbose</code>          | Boolean                                                                                                                                                                                                             |
| <code>bin</code>              | Integer Width of the window to use to smooth values by <code>zoo::rollMean</code>                                                                                                                                   |
| <code>plot_central</code>     | Boolean Draw a vertical line at 0                                                                                                                                                                                   |
| <code>run_in_parallel</code>  | Boolean Should the plots be computed in parallel using <code>mclapply</code> ?                                                                                                                                      |
| <code>split_by_granges</code> | Boolean Facet plots over the sets of <code>GRanges</code>                                                                                                                                                           |
| <code>norm</code>             | character Should the signal be normalized ('none', 'zscore' or 'log2')?                                                                                                                                             |
| <code>split_by_track</code>   | Boolean Facet plots by the sets of signal tracks                                                                                                                                                                    |
| <code>free_scales</code>      | Boolean Should each facet have independent y-axis scales?                                                                                                                                                           |

**Value**

An aggregate coverage plot.

**Methods (by class)**

- `CompressedRleList`: S3 method for `CompressedRleList`
- `SimpleRleList`: S3 method for `SimpleRleList`
- `list`: S3 method for list

**Examples**

```
data(ce11_ATACseq)
data(ce11_WW_10bp)
data(ce11_proms)

p1 <- plotAggregateCoverage(
  ce11_ATACseq,
  resize(ce11_proms[1:100], fix = 'center', width = 1000)
)
p1
```

```

proms <- resize(ce11_proms[1:100], fix = 'center', width = 400)
p2 <- plotAggregateCoverage(
  ce11_ATASeq,
  list(
    'Ubiq & Germline promoters' =
      proms[proms$which.tissues %in% c('Ubiq.', 'Germline')],
    'Other promoters' =
      proms[!(proms$which.tissues %in% c('Ubiq.', 'Germline'))]
  )
)
p2

p3 <- plotAggregateCoverage(
  list(
    'atac' = ce11_ATASeq,
    'WW_10bp' = ce11_WW_10bp
  ),
  proms,
  norm = 'zscore'
)
p3

p4 <- plotAggregateCoverage(
  list(
    'ATAC-seq' = ce11_ATASeq,
    'WW 10-bp periodicity' = ce11_WW_10bp
  ),
  list(
    'Ubiq & Germline promoters' =
      proms[proms$which.tissues %in% c('Ubiq.', 'Germline')],
    'Other promoters' =
      proms[!(proms$which.tissues %in% c('Ubiq.', 'Germline'))]
  ),
  norm = 'zscore'
)
p4

p5 <- plotAggregateCoverage(
  list(
    'ATAC-seq' = ce11_ATASeq,
    'WW 10-bp periodicity' = ce11_WW_10bp
  ),
  list(
    'Ubiq & Germline promoters' =
      proms[proms$which.tissues %in% c('Ubiq.', 'Germline')],
    'Other promoters' =
      proms[!(proms$which.tissues %in% c('Ubiq.', 'Germline'))]
  ),
  split_by_granges = FALSE,
  split_by_track = TRUE,
  norm = 'zscore'
)

```

p5

---

plotPeriodicityResults

*Plot the output of getPeriodicity()*


---

## Description

This function plots some results from the result of getPeriodicity(). It plots the raw histogram, the distance-decay normalized histogram and the resulting PSD values. If a shuffled control has been performed by getPeriodicity(), it also displays it.

## Usage

```
plotPeriodicityResults(
  results,
  periods = c(2, 20),
  filter_periods = TRUE,
  facet_control = TRUE,
  xlim = NULL,
  fdr_threshold = 0.05,
  ...
)
```

## Arguments

|                |                                                                     |
|----------------|---------------------------------------------------------------------|
| results        | The output of getPeriodicity function.                              |
| periods        | Vector a numerical vector of length 2, to specify the x-axis limits |
| filter_periods | Boolean Should the x-axis be constrained to the periods?            |
| facet_control  | Boolean should the shuffling plots be faceted?                      |
| xlim           | Integer x axis upper limit in raw and norm. distograms              |
| fdr_threshold  | Float, significance threshold                                       |
| ...            | Additional theme arguments passed to theme_ggplot2()                |

## Value

list A list containing four ggplots

## Examples

```
data(ce11_TSSs)
periodicity_result <- getPeriodicity(
  ce11_TSSs[['Ubiq.']] [1:100],
  genome = 'BSgenome.Celegans.UCSC.ce11',
  motif = 'TT',
  BPPARAM = setUpBPPARAM(1)
```

```
)
head(periodicity_result$PSD)
plotPeriodicityResults(periodicity_result)
plotPeriodicityResults(periodicity_result, xlim = 150)
plotPeriodicityResults(
  periodicity_result, xlim = 150, filter_periods = FALSE
)
plotPeriodicityResults(
  periodicity_result, xlim = 150, facet_control = FALSE
)
```

---

|              |                     |
|--------------|---------------------|
| setUpBPPARAM | <i>setUpBPPARAM</i> |
|--------------|---------------------|

---

**Description**

A function to dynamically select MulticoreParam or SnowParam (if Windows)

**Usage**

```
setUpBPPARAM(nproc = 1)
```

**Arguments**

nproc                      number of processors

**Value**

A BPPARAM object

**Examples**

```
BPPARAM <- setUpBPPARAM(1)
```

---

|               |                                                                                                                                                                          |
|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| theme_ggplot2 | <i>Personal ggplot2 theming function, adapted from roboto-condensed at <a href="https://github.com/hrbrmstr/hrbrthemes/">https://github.com/hrbrmstr/hrbrthemes/</a></i> |
|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

---

**Description**

Personal ggplot2 theming function, adapted from roboto-condensed at <https://github.com/hrbrmstr/hrbrthemes/>



**Usage**

```
theme_ggplot2(
  grid = TRUE,
  border = TRUE,
  base_size = 8,
  plot_title_size = 12,
  plot_title_face = "plain",
  plot_title_margin = 5,
  subtitle_size = 11,
  subtitle_face = "plain",
  subtitle_margin = 5,
  strip_text_size = 10,
  strip_text_face = "bold",
  caption_size = 9,
  caption_face = "plain",
  caption_margin = 3,
  axis_text_size = base_size,
  axis_title_size = 9,
  axis_title_face = "plain",
  axis_title_just = "rt",
  panel_spacing = grid::unit(2, "lines"),
  grid_col = "#cccccc",
  plot_margin = margin(12, 12, 12, 12),
  axis_col = "#cccccc",
  axis = FALSE,
  ticks = FALSE
)
```

**Arguments**

|                                            |                                                                      |
|--------------------------------------------|----------------------------------------------------------------------|
| grid                                       | panel grid ('TRUE', 'FALSE', or a combination of 'X', 'x', 'Y', 'y') |
| border                                     | border if 'TRUE' add border                                          |
| base_size                                  | base font size                                                       |
| plot_title_size, plot_title_margin         | plot title size and margin                                           |
| plot_title_face                            | plot title face                                                      |
| subtitle_face, subtitle_size               | plot subtitle face and size                                          |
| subtitle_margin                            | plot subtitle margin bottom (single numeric value)                   |
| strip_text_face, strip_text_size           | facet label font face and size                                       |
| caption_face, caption_size, caption_margin | plot caption face, size and margin                                   |
| axis_text_size                             | font size of axis text                                               |

|                                                             |                                                 |
|-------------------------------------------------------------|-------------------------------------------------|
| <code>axis_title_face</code> , <code>axis_title_size</code> | axis title font face and size                   |
| <code>axis_title_just</code>                                | axis title font justificationk one of ‘[blmcr]’ |
| <code>panel_spacing</code>                                  | panel spacing (use ‘unit()’)                    |
| <code>grid_col</code>                                       | grid color                                      |
| <code>plot_margin</code>                                    | plot margin (specify with [ggplot2::margin])    |
| <code>axis_col</code>                                       | axis color                                      |
| <code>axis</code>                                           | add x or y axes? ‘TRUE’, ‘FALSE’, “‘xy’”        |
| <code>ticks</code>                                          | ticks if ‘TRUE’ add ticks                       |

**Value**

theme A ggplot theme

**Examples**

```
library(ggplot2)

ggplot(mtcars, aes(mpg, wt)) +
  geom_point() +
  labs(x="Fuel efficiency (mpg)", y="Weight (tons)",
       title="Seminal ggplot2 scatterplot example") +
  theme_ggplot2()
```

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