Package ‘standR’

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Title Spatial transcriptome analyses of Nanostring’s DSP data in R
Version 1.6.0
Description standR is an user-friendly R package providing functions to assist conducting good-practice analysis of Nanostring’s GeoMX DSP data. All functions in the package are built based on the SpatialExperiment object, allowing integration into various spatial transcriptomics-related packages from Bioconductor. standR allows data inspection, quality control, normalization, batch correction and evaluation with informative visualizations.

biocViews Spatial, Transcriptomics, GeneExpression, DifferentialExpression, QualityControl, Normalization, ExperimentHubSoftware
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standR-package  Tools for analyzing NanoString’s GeoMX spatial transcriptomics data

Description

standR implements a series of functions to facilitate inspection, analysis and visualization of the
NanoString’s GeoMX DSP datasets. standR takes the either the csv files from the Nanostring or
DGEList object as input, allowing for multiple methods to be analyzed together.
Details

standR represents the GeoMX DSP data as SpatialExperiment objects, which can easily be integrated with a wide variety of Bioconductor packages. standR generates various plots, such as QC distribution plots, dimension reduction plots and RLE plots, for quality control of genes and region of interest (ROI) samples. Multiple normalization and batch correction methods are also provided in the package as well, with the ability to compute statistics for assessing the normalization/batch correction results.

Author(s)

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**addPerROIQC**

Add QC statistics to the Spatial Experiment object

**Usage**

```r
addPerROIQC(
  spe_object, 
  sample_fraction = 0.9, 
  rm_genes = TRUE, 
  min_count = 5, 
  design = NULL 
)
```

**Arguments**

- **spe_object**: A SpatialExperiment object
- **sample_fraction**: Double. Genes with low count in more than this threshold of the samples will be removed. Default is 0.9
- **rm_genes**: Logical. Decide whether genes with low count in more than sample_fraction of the samples are removed from the dataset. Default is TRUE.
- **min_count**: Integer. Minimum read count to calculate count threshold. Default is 5.
- **design**: Generate using `model.matrix`, if this is specify, `edgeR::filterByExpr` will be used to filter genes.

**Value**

A SpatialExperiment object
computeClusterEvalStats

*Calculate statistics for evaluating batch correction*

**Description**

Calculate statistics for evaluating batch correction

**Usage**

```r
computeClusterEvalStats(
  spe_object,
  foiColumn,
  precomputed = NULL,
  n_dimension = c(1, 2),
  assay = 2
)
```

**Arguments**

- `spe_object`: A Spatial Experiment object.
- `foiColumn`: A column name indicating the factor of interest to be tested, can be biological factor or batch factor.
- `precomputed`: a dimensional reduction results from `stats::prcomp`. result in `reducedDims(object)` to plot. Default is NULL, we will compute for you.
- `n_dimension`: The top n dimensions to be plotted
- `assay`: a numeric or character, specifying the assay to use (for `SummarizedExperiment` and its derivative classes).

**Value**

A dataframe object containing the clustering evaluating statistics.

**Examples**

```r
library(scater)
data("dkd_spe_subset")
computeClusterEvalStats(dkd_spe_subset, "SlideName")
```
dkd_spe_subset

Description of the standR example datasets

Description
standR-package has 1 datasets:

- dkd_spe_subset Example subset of a GeoMX DSP WTA dataset,

Usage

data("dkd_spe_subset")

Format
A SpatialExperiment object with 3000 rows and 70 samples:

Source


Examples

data(dkd_spe_subset)

drawPCA

Compute and plot the results of a PCA analysis on gene expression data

Description
Compute and plot the results of a PCA analysis on gene expression data

Usage
drawPCA(object, dims = c(1, 2), ...)

## S4 method for signature 'ExpressionSet'
drawPCA(object, dims = c(1, 2), precomputed = NULL, textScale = 1, ...)

## S4 method for signature 'SummarizedExperiment'
drawPCA(
  object,
  dims = c(1, 2),
  assay = 1,
drawPCA

## S4 method for signature 'SingleCellExperiment'
drawPCA(
  object,
  dims = c(1, 2),
  assay = 1,
  precomputed = NULL,
  textScale = 1,
  ...
)

## S4 method for signature 'SpatialExperiment'
drawPCA(
  object,
  dims = c(1, 2),
  assay = 1,
  precomputed = NULL,
  textScale = 1,
  ...
)

Arguments

object       a DGEList, SummarizedExperiment or ExpressionSet object containing gene expression data.
dims         a numeric, containing 2 values specifying the dimensions to plot.
...           aesthetic mappings to pass to ggplot2::aes_string().
precomputed  a dimensional reduction results from stats::prcomp. result in reducedDims(object) to plot.
textScale    a numeric, specifying the relative scale factor to apply to text on the plot.
assay        a numeric or character, specifying the assay to use (for SummarizedExperiment and its derivative classes).

Value

a ggplot2 object

Examples

data("dkd_spe_subset")
drawPCA(dkd_spe_subset)
findBestK

Testing multiple K for RUV4 batch correction to find the best K.

Description

Testing multiple K for RUV4 batch correction to find the best K.

Usage

```r
findBestK(
  spe,
  maxK = 10,
  factor_of_int,
  factor_batch,
  NCGs,
  point_size = 3,
  line_col = "black",
  point_col = "black",
  text_size = 13
)
```

Arguments

- `spe`: A Spatial Experiment object.
- `maxK`: Integer. The max k to test, will test k from 1 to maxK, by default is 10.
- `factor_of_int`: Column name(s) to indicate the factors of interest. This is required for the RUV4 method.
- `factor_batch`: Column name to indicate the batch.
- `NCGs`: Negative control genes. This is required for the RUV4 method.
- `line_col`: Character. Plotting parameter.
- `point_col`: Character. Plotting parameter.

Value

A ggplot object.

Examples

```r
data("dkd_spe_subset")
spe <- findNCGs(dkd_spe_subset, top_n = 100)
findBestK(spe,
  factor_of_int = c("disease_status"),
  factor_batch = "SlideName", NCGs = S4Vectors::metadata(spe)$NCGs
)
```
findNCGs

Get negative control genes from each batch of the data

Description
Get negative control genes from each batch of the data

Usage
findNCGs(spe, n_assay = 2, batch_name = "SlideName", top_n = 200)

Arguments

- **spe** A Spatial Experiment object.
- **n_assay** Integer to indicate the nth count table in the assay(spe) to be used.
- **batch_name** Column name indicating batches.
- **top_n** Integer indicate how many genes to be included as negative control genes.

Value
A Spatial Experiment object, containing negative control genes in the metadata.

Examples

data("dkd_spe_subset")
spe <- findNCGs(dkd_spe_subset, top_n = 100)
S4Vectors::metadata(spe)$NCGs

geomxBatchCorrection
Batch correction for GeoMX data

Description
Batch correction for GeoMX data
**Usage**

```r
gemxBatchCorrection(
  spe,
  k,
  factors,
  NCGs,
  n_assay = 2,
  batch = NULL,
  batch2 = NULL,
  covariates = NULL,
  design = matrix(1, ncol(spe), 1),
  method = c("RUV4", "Limma", "RUVg"),
  isLog = TRUE
)
```

**Arguments**

- `spe` **A Spatial Experiment object.**
- `k` **The number of unwanted factors to use. Can be 0. This is required for the RUV4 method.**
- `factors` **Column name(s) to indicate the factors of interest. This is required for the RUV4 method.**
- `NCGs` **Negative control genes. This is required for the RUV4 method.**
- `n_assay` **Integer to indicate the nth count table in the assay(spe) to be used.**
- `batch` **A vector indicating batches. This is required for the Limma method.**
- `batch2` **A vector indicating the second series of batches. This is specific for the Limma method.**
- `covariates` **A matrix or vector of numeric covariates to be adjusted for.**
- `design` **A design matrix relating to treatment conditions to be preserved, can be generated using `stats::model.matrix` function with all biological factors included.**
- `method` **Can be either RUV4 or Limma or RUVg, by default is RUV4.**
- `isLog` **Logical vector, indicating if the count table is log or not.**

**Value**

A Spatial Experiment object, containing the normalized count and normalization factor. For method RUV4 and RUVg, the W matrices will be saved in the colData of the object.

**Note**

The normalised count is not intended to be used directly for linear modelling. For linear modelling, it is better to include the batch factors/W matrices in the linear model.
References


Examples

data("dkd_spe_subset")
spe <- findNCGs(dkd_spe_subset, top_n = 100)
spe_ruv <- geomxBatchCorrection(spe,
    k = 3,
    factors = c("disease_status", "region"),
    NCGs = S4Vectors::metadata(spe)$NCGs
)

geomxNorm

Perform normalization to GeoMX data

Description

Perform normalization to GeoMX data

Usage

geomxNorm(
    spe_object,
    method = c("TMM", "RPKM", "TPM", "CPM", "upperquartile", "sizefactor"),
    log = TRUE
)

Arguments

spe_object A SpatialExperiment object.
method Normalization method to use. Options: TMM, RPKM, TPM, CPM, upperquartile, sizefactor. RPKM and TPM require gene length information, which should be added into rowData(spe). Note that TMM here is TMM + CPM.
log Log-transformed or not.

Value

A SpatialExperiment object, with the second assay being the normalized count matrix. The normalised count is stored in the assay slot called "logcounts" by default. With method TMM and sizefactor, the norm.factor will be saved in the metadata of the SpatialExperiment object.
Note

The normalised count is not intended to be used directly for linear modelling. For linear modelling, it is better to include the normalized factors in the "norm.factors" column of the DGEList object.

References


Examples

data("dkd_spe_subset")

spe_tmm <- geomxNorm(dkd_spe_subset, method = "TMM")
spe_upq <- geomxNorm(dkd_spe_subset, method = "upperquartile")
spe_deseqnorm <- geomxNorm(dkd_spe_subset, method = "sizefactor")

plotClusterEvalStats

Compare and evaluate different batch corrected data with plotting.

Description

Compare and evaluate different batch corrected data with plotting.

Usage

plotClusterEvalStats(
  spe_list,
  bio_feature_name,
  batch_feature_name,
  data_names,
  colors = NA
)

Arguments

spe_list A list of Spatial Experiment object.
bio_feature_name The common biological variation name.
batch_feature_name The common batch variation name.
data_names Data names.
colors Color values of filing the bars.
plotDR

Value
A ggplot object.

Examples
library(scater)
data("dkd_spe_subset")
spe <- dkd_spe_subset
spe2 <- spe
spe3 <- spe
plotClusterEvalStats(list(spe, spe2, spe3),
  bio_feature_name = "region",
  batch_feature_name = "SlideName", c("test1", "test2", "test3")
)

Description
Compute and plot the results of any dimension reduction methods on gene expression data

Usage
plotDR(object, dims = c(1, 2), ...)

## S4 method for signature 'SingleCellExperiment'
plotDR(object, dims, dimred = "PCA", textScale = 1, ...)

## S4 method for signature 'SpatialExperiment'
plotDR(object, dims, dimred = "PCA", textScale = 1, ...)

Arguments

object        a DGEList, SummarizedExperiment or ExpressionSet object containing gene expression data.
dims          a numeric, containing 2 values specifying the dimensions to plot.
...           aesthetic mappings to pass to ggplot2::aes_string().
dimred        a string or integer scalar indicating the reduced dimension result in reducedDims(object) to plot.
textScale     a numeric, specifying the relative scale factor to apply to text on the plot.

Value
a ggplot2 object
Examples

```r
library(scater)
data("dkd_spe_subset")
spe <- scater::runPCA(dkd_spe_subset)
plotDR(spe, dimred = "PCA")
```

plotGeneQC

Plot gene-wise QC plot

Description

Plot gene-wise QC plot

Usage

```r
plotGeneQC(
  spe,
  top_n = 9,
  ordannots = c(),
  point_size = 1,
  line_type = "dashed",
  line_col = "darkred",
  line_cex = 1,
  hist_col = "black",
  hist_fill = "skyblue",
  bin_num = 30,
  text_size = 13,
  layout_ncol = 1,
  layout_nrow = 2,
  layout_height = c(1, 1),
  ...
)
```

Arguments

- `spe` A SpatialExperiment object.
- `top_n` Integer. Indicating the top n genes will be plotted. Default is 9.
- `ordannots` variables or computations to sort samples by (tidy style).
- `point_size` Numeric. Point size.
- `line_type` Character. Line types for ggplot.
- `line_col` Color for line.
- `line_cex` Cex for line.
- `hist_col` Color for histogram.
plotMDS

hist_fill Fill for histogram.
bin_num Bin numbers for histogram.
text_size Text size.
layout_ncol Integer. Column number for layout. Default is 1.
layout_nrow Integer. Row number for layout. Default is 2.
layout_height Vector of numerics with length of 2. Default is c(1,.4).
... aesthetic mappings to pass to ggplot2::aes() of the dot plots.

Value

A ggplot object

Examples

data("dkd_spe_subset")
spe <- addPerROIQC(dkd_spe_subset)
plotGeneQC(spe)

plotMDS Compute and plot the results of a MDS analysis on gene expression data

Description

Compute and plot the results of a MDS analysis on gene expression data

Usage

plotMDS(
  object,
  dims = c(1, 2),
  precomputed = NULL,
  textScale = 1,
  assay = 1,
  ...
)

# S4 method for signature 'DGEList'
plotMDS(
  object,
  dims = c(1, 2),
  precomputed = NULL,
  textScale = 1,
  assay = 1,
  ...
)
## plotMDS

### S4 method for signature 'ExpressionSet'

```r
plotMDS(
  object,
  dims = c(1, 2),
  precomputed = NULL,
  textScale = 1,
  assay = 1,
  ...
)
```

### S4 method for signature 'SummarizedExperiment'

```r
plotMDS(
  object,
  dims = c(1, 2),
  precomputed = NULL,
  textScale = 1,
  assay = 1,
  ...
)
```

### S4 method for signature 'SingleCellExperiment'

```r
plotMDS(
  object,
  dims = c(1, 2),
  precomputed = NULL,
  textScale = 1,
  assay = 1,
  ...
)
```

### S4 method for signature 'SpatialExperiment'

```r
plotMDS(
  object,
  dims = c(1, 2),
  precomputed = NULL,
  textScale = 1,
  assay = 1,
  ...
)
```

### Arguments

- **object**
  - a DGEList, SummarizedExperiment or ExpressionSet object containing gene expression data.
- **dims**
  - a numeric, containing 2 values specifying the dimensions to plot.
- **precomputed**
  - a dimensional reduction results from either limma::plotMDS.
- **textScale**
  - a numeric, specifying the relative scale factor to apply to text on the plot.
plotPairPCA

assay a numeric or character, specifying the assay to use (for SummarizedExperiment and its derivative classes).

... aesthetic mappings to pass to ggplot2::aes_string().

Value

a ggplot2 object

Examples

data("dkd_spe_subset")
standR::plotMDS(dkd_spe_subset)

Description

Plot pair-wise PCA plots for multiple dimensions

Usage

plotPairPCA(
  spe_object,
  n_dimension = 3,
  precomputed = NULL,
  assay = 2,
  title = NA,
  title.size = 14,
  rmduplabs = FALSE,
  flipcoord = FALSE,
  ...
)

Arguments

  spe_object A SpatialExperiment object.
  n_dimension The top n dimensions to be plotted
  precomputed a dimensional reduction results from stats::prcomp. result in reducedDims(object) to plot. Default is NULL, we will compute for you.
  assay a numeric or character, specifying the assay to use (for SummarizedExperiment and its derivative classes).
  title Character vector, title to put at the top.
  title.size Numeric vector, size of the title.
  rmduplabs Remove duplicated labels from the plot. FALSE by default.
  flipcoord Flip the xy coordinates. FALSE by default.
  ... aesthetic mappings to pass to ggplot2::aes().
Value

A ggplot object.

Examples

data("dkd_spe_subset")
plotPairPCA(dkd_spe_subset)

plotPCAbiplot

Description

Plot PCA bi plot

Usage

plotPCAbiplot(
  spe_object,
  n_loadings = 10,
  dims = c(1, 2),
  precomputed = NULL,
  assay = 1,
  arrow_x = 0,
  arrow_y = 0,
  ...
)

Arguments

  spe_object       A SpatialExperiment object.
  n_loadings      Plot the top n gene loadings
  dims            The top n dimensions to be plotted
  precomputed     a dimensional reduction results from stats::prcomp. result in reducedDims(object) to plot. Default is NULL, we will compute for you.
  assay           a numeric or character, specifying the assay to use (for SummarizedExperiment and its derivative classes).
  arrow_x         a numeric, indicating the x coordinate of the base of the arrow.
  arrow_y         a numeric, indicating the y coordinate of the base of the arrow.
  ...             aesthetic mappings to pass to ggplot2::aes().

Value

A ggplot object.
### Examples

```r
data("dkd_spe_subset")
plotPCAbiplot(dkd_spe_subset)
```

### Description

Compute and plot relative log expression (RLE) values of gene expression data

### Usage

```r
plotRLExpr(object, ordannots = c(), ...)
```

#### S4 method for signature 'DGEList'

```r
plotRLExpr(object, ordannots = c(), ...)
```

#### S4 method for signature 'ExpressionSet'

```r
plotRLExpr(object, ordannots = c(), ...)
```

#### S4 method for signature 'SummarizedExperiment'

```r
plotRLExpr(object, ordannots, assay = 1, ...)
```

### Arguments

- **object**: a DGEList, SummarizedExperiment or ExpressionSet object containing gene expression data.
- **ordannots**: variables or computations to sort samples by (tidy style).
- **...**: aesthetic mappings to pass to `ggplot2::aes_string()`.
- **assay**: a numeric or character, specifying the assay to use (for SummarizedExperiment and its derivative classes).

### Value

A ggplot2 object, containing the RLE plot.

### Examples

```r
data("dkd_spe_subset")
plotRLExpr(dkd_spe_subset)
```
Description

Plot Sample-wise QC plot

Usage

plotROIQC(
  spe_object,
  x_axis = "AOINucleiCount",
  y_axis = "lib_size",
  x_lab = "AOINucleiCount",
  y_lab = "Library size",
  x_threshold = NULL,
  y_threshold = NULL,
  regression_col = "purple",
  hist_col = "black",
  hist_fill = "white",
  bin_num = 50,
  threshold_col = "red",
  threshold_linetype = "dashed",
  layout_ncol = 2,
  layout_nrow = 2,
  layout_height = c(0.8, 2.5),
  layout_width = c(2.5, 0.8),
  ...
)

Arguments

- **spe_object**: A SpatialExperiment object.
- **x_axis**: Numeric feature to plot as x axis.
- **y_axis**: Numeric feature to plot as y axis.
- **x_lab**: Label name for x axis.
- **y_lab**: Label name for y axis.
- **x_threshold**: Threshold to draw.
- **y_threshold**: Threshold to draw.
- **regression_col**: Color for the regression line.
- **hist_col**: Color for the histograms.
- **hist_fill**: Fill for the histograms.
- **bin_num**: Bin numbers for the histograms.
- **threshold_col**: Threshold line color.
threshold_linetype
Threshold line type.

layout_ncol   Column number layout.
layout_nrow   Row number layout.

layout_height Height layout.
layout_width Width layout.

... aesthetic mappings to pass to ggplot2::aes() of the dot plots.

Value
A ggplot object.

Examples
library(ggplot2)
library(patchwork)
data("dkd_spe_subset")
spe <- addPerROIQC(dkd_spe_subset)

plotROIQC(spe)

plotSampleInfo
Plot the user-defined meta data using alluvium plot

Description
Plot the user-defined meta data using alluvium plot

Usage
plotSampleInfo(spe_object, column2plot, textsize = 3)

Arguments

  spe_object   A SpatialExperiment object.
  column2plot  Which columns to plot.
  textsize     text size.

Value
A ggplot object
Examples

```r
library(ggalluvial)

data("dkd_spe_subset")
plotSampleInfo(dkd_spe_subset, column2plot = c("SlideName", "disease_status", "region"))
```

Description

Plot the PCA scree plot.

Usage

```r
plotScreePCA(
  spe_object, 
  dims = ncol(spe_object), 
  precomputed = NULL, 
  assay = 1, 
  bar_color = "black", 
  bar_fill = "royalblue", 
  bar_width = 0.8, 
  point_col = "tomato3", 
  line_col = "tomato3", 
  point_size = 2
)
```

Arguments

- `spe_object`: A SpatialExperiment object.
- `dims`: The top n dimensions to be plotted.
- `precomputed`: A dimensional reduction results from `stats::prcomp`. result in `reducedDims(object)` to plot. Default is NULL, we will compute for you.
- `assay`: A numeric or character, specifying the assay to use (for SummarizedExperiment and its derivative classes).
- `bar_color`: Color for bar.
- `bar_fill`: Fill for bar.
- `bar_width`: Bar width.
- `point_col`: Color for point.
- `line_col`: Color for line.
- `point_size`: Point size.
prepareSpatialDecon

Description

Preparing the inputs for SpatialDecon for doing deconvolution on spatial data

Usage

```r
prepareSpatialDecon(
    spe,
    assay2use = "logcounts",
    negProbeName = "NegProbe-WTX",
    pool = NA
)
```

Arguments

- **spe**: SpatialExperiment object.
- **assay2use**: The name of the assay to use. By default is logcounts.
- **negProbeName**: The name of the negative probe gene. By default is NegProbe-WTX.
- **pool**: A vector indicates the pools of the genes. This is required when there are more than one Negative Probes.

Value

A list of two dataframes. The first data.frame is the normalised count, the second data.frame is the background for the data.

Examples

```r
library(ExperimentHub)
eh <- ExperimentHub()
query(eh, "standR")
countFile <- eh["EH7364"]
sampleAnnoFile <- eh["EH7365"]
```
```r
spe <- readGeoMx(countFile, sampleAnnoFile, rmNegProbe = FALSE)
out <- prepareSpatialDecon(spe)
```

**readGeoMx**

*Import GeoMX DSP data into a spatial experiment object from file paths*

**Description**

Import GeoMX DSP data into a spatial experiment object from file paths.

**Usage**

```r
readGeoMx(
  countFile, sampleAnnoFile, featureAnnoFile = NA, rmNegProbe = TRUE, NegProbeName = "NegProbe-WTX",
  colnames.as.rownames = c("TargetName", "SegmentDisplayName", "TargetName"),
  coord.colnames = c("ROICoordinateX", "ROICoordinateY")
)
```

**Arguments**

- `countFile` tsv file or a dataframe object. Count matrix, with samples in columns and features/genes in rows. The first column is gene names/ids.
- `sampleAnnoFile` tsv file or a dataframe object. Sample annotations.
- `featureAnnoFile` tsv file or a dataframe object. Feature/Gene annotations.
- `rmNegProbe` Logical. Default is TRUE, indicating there are negative probe genes in the data.
- `NegProbeName` Character. Name of negative probe genes, default is NegProbe-WTX.
- `colnames.as.rownames` Vector of characters, length of 3. Column names used to capture gene names, sample names and gene names in countFile, sampleAnnoFile and featureAnnoFile, respectively.
- `coord.colnames` Vector of characters, length of 2. Column names used to capture ROI coordinates.

**Value**

A SpatialExperiment object.
readGeoMxFromDGE

Import GeoMX DSP data into a spatial experiment object from DGEList object

Usage

readGeoMxFromDGE(dge_object, spatialCoord = NULL)

Arguments

dge_object
  a DGEList object (created using edgeR::DGEList).
spatialCoord
  a matrix with coordinates of samples, rowname must be consistent with the col-
names of dge_object.

Value

A SpatialExperiment object.

Examples

# making a simple DGEList object
ng <- 1000
ns <- 10
Counts <- matrix(rbinom(ng * ns, mu = 5, size = 2), ng, ns)
rownames(Counts) <- seq(ng)
y <- edgeR::DGEList(counts = Counts, group = rep(seq(2), each = 5))

# transfer into spatial experiment object
coords <- matrix(rnorm(2 * ns), 10, 2)
spe <- readGeoMxFromDGE(dge_object = y, spatialCoord = coords)
spe
`spe2dge`  

<table>
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<th>spe2dge</th>
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<td>Transfer SpatialExperiment object into DGEList object for DE analysis</td>
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**Description**  
Transfer SpatialExperiment object into DGEList object for DE analysis

**Usage**  
`spe2dge(spe)`

**Arguments**  
- `spe`  
  SpatialExperiment object.

**Value**  
A DGEList.

**Examples**  
```r  
data("dkd_spe_subset")  
  
spe_tmm <- geomxNorm(dkd_spe_subset, method = "TMM")  
dge <- spe2dge(spe_tmm)  ```
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