Package 'DegNorm'

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Type Package

Title DegNorm: degradation normalization for RNA-seq data

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Description This package performs degradation normalization in bulk RNA-seq data to improve differential expression analysis accuracy. It provides estimates for each gene within each sample.

License LGPL (>= 3)

Depends R (>= 4.0.0), methods

Imports Rcpp (>= 1.0.2),GenomicFeatures, txdbmaker, parallel, foreach, S4Vectors, doParallel, Rsamtools (>= 1.31.2), GenomicAlignments, heatmaply, data.table, stats, ggplot2, GenomicRanges, IRanges, plyr, plotly, utils,viridis

LinkingTo Rcpp, RcppArmadillo,S4Vectors,IRanges

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Contents

`DegNorm-package` *DegNorm: degradation normalization for RNA-seq data*

Description

`DegNorm` is an R package for degradation normalication for bulk RNA-seq data.DegNorm, short for degradation normalization, is a bioinformatics pipeline designed to correct for bias due to the heterogeneous patterns of transcript degradation in RNA-seq data.

Details

DegNorm is a data-driven approach for RNA-Seq normalization resulting in the adjusted read count matrix. This adjustment applies to each gene within each sample, accounting for sample- and gene-specific degradation bias while simultaneously controlling for the sequencing depth. The algorithm at the center of DegNorm is the rank-one over-approximation of a gene's coverage score matrix, which is comprised of the different samples' coverage score curves along the transcript for each gene. For each gene, DegNorm estimates (1) an envelope function representing the ideal shape of the gene's coverage curve when no degradation is present, and (2) scale factors for each sample (for said gene) that indicates the relative abundance of the gene within the sample.

functions: `read_coverage_batch,degnorm,plot_coverage,plot_heatmap,plot_corr,plot_boxplot`

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References

DegNorm reference:

Xiong, B., Yang, Y., Fineis, F. Wang, J.-P., DegNorm: normalization of generalized transcript degradation improves accuracy in RNA-seq analysis, Genome Biology, 2019,20:75

coverage_res_chr21 *Example CoverageClass data*

Description

Example of CoverageClass data from DegNorm package. It is the output from read_coverage_batch function for human chromosome 21.

Usage

```
data(coverage_res_chr21)
```

Format

A coverageClass list of the following

coverage a list of converage matrices for all genes within each sample

counts a data.frame of read counts for all genes within each sample.

Examples

```
data(coverage_res_chr21)
summary_CoverageClass(coverage_res_chr21)
```

degnorm *Main function to perform degradation normalization.*

Description

degnorm calcualtes the degradation index score for each gene within each sample and return the degradation-normalized read counts.

Usage

```
degnorm(read_coverage,counts,iteration,loop,down_sampling=1,grid_size=10,
cores=1)
```

Arguments

read_coverage	a list of converage matrices, one per gene
counts	dataframe of read counts, each row for one gene, and column for sample. The order and number of genes must match the order in read_coverage matrices.
iteration	iteration number for degnorm algorithm. 5 is sufficient.
loop	iteration number inside of nonnegative matrix factorization-over approximation. Default is 100.
down_sampling	1 for yes (default) and 0 for no. If yes, average coverage score is calcualted on a grid of size specified by grid_size argument. The new coverage matrix formed by the grid average score will be used for baseline selection. This increases the efficiency of algorithm while maintaining comparable accuracy.

grid_size	default size is 10 bp.
cores	number of cores. Default number if 1. Users should input the maximum possible number of cores for efficiency.

Value

degnorm outputs a list of following objects:

counts	a data.drame of read counts for each gene within each sample.
counts_normed	a data.drame of degradation-normalized read counts for each gene within each sample.
DI	a matrix of degradation index scores for each gene within each sample.
K	normalizing scale factor for each gene within each sample after accounting for degradation normalization.
convergence	convergence tag; 0 = degnorm was not done on this gene because smaller counts or too short length.1 = degnorm was done with baseline selection. 2 = degnorm done without baseline selection because gene length (after filtering out low count regions)<200 bp. 3= baseline was found, but DI score is too large. 4 = baseline selection didn't coverge.
envelop	list of the envelop curves for all genes.

Examples

```
##coverage_res_chr21 is a \code{CoverageClass} object from DegNorm Package.
data(coverage_res_chr21)
res_DegNorm = degnorm(read_coverage = coverage_res_chr21[[1]],
                      counts = coverage_res_chr21[[2]],
                      iteration = 2,
                      down_sampling = 1,
                      grid_size=10,
                      loop = 20,
                      cores=2)
```

DegNorm-plot-functions

Degradation index (DI) score plot functions

Description

DegNorm provides three functions for visualization gene-/sample-wise degradation.

Usage

```
plot_corr(DI)
plot_heatmap(DI)
plot_boxplot(DI)
```

Arguments

DI	a matrix or data.frame of degradation index (DI) scores with each row corresponding to one gene and each column for a sample.

Details

plot_corr plots the correlation matrix of DI scores between samples. plot_heatmap plots the heatmap of DI scores. Left is ploted in descending order of average DI scores of genes where each row corresponds to one gene. In the right plot, DI scores were sorted within each sample and plotted in descending order. plot_boxplotplots the boxplot of DI scores by samples.

Value

These functions return a boxplot of DI scores by sample, a heatmap of DIS scores of all genes in all samples and a correlation plot of DI scores between samples respectively.

Examples

```
## res_DegNorm_chr21 is degnorm otuput stored in sysdata.Rda
data(res_DegNorm_chr21)
plot_boxplot(res_DegNorm_chr21$DI)
plot_heatmap(res_DegNorm_chr21$DI)
plot_corr(res_DegNorm_chr21$DI)
```

---

plot_coverage *Coverage plot functions for DegNorm*

Description

plot_coverage plots the before- and after-degradation coverage curves

Usage

```
plot_coverage(gene_name, coverage_output, degnorm_output, group=NULL, samples=NULL)
```

Arguments

gene_name	the name of the gene whose coverage coverage to be plotted.
coverage_output	CoverageClass object, the output from function coverage_cal_batch.
degnorm_output	DegNormClass object, the output from function DegNorm.
group	a vector of integers or character strings indicating the biological conditions of the samples. Coverage curves will be plotted in the same color for the same group. Default is NULL. By default all curves will plotted in different colors.
samples	a string vector for the subset of samples to be plotted. NULL means all samples to be plotted. The length of samples must be of the same length of group if both specified.

Details

plot_coverageoutputs the coverage curves before- and after-degradation normalization.

Value

The coverage curve before and after degradation normalization.

Examples

```
## gene named "SOD1",plot coverage curves
data(coverage_res_chr21)
data(res_DegNorm_chr21)
plot_coverage(gene_name="SOD1", coverage_output=coverage_res_chr21,
degnorm_output=res_DegNorm_chr21, group=c(0,1,1))
```

`read_coverage` *Function to calculate read coverage score for one bam file*

Description

This function judges whether bam file is single-end and paired-end, and generate bam file index if needed. It calls function `paired_end_cov_by_ch` or `single_end_by_ch`. It takes multiple-core structure for parallel computing for efficiency.

Usage

```
read_coverage(bam_file,all_genes,cores)
```

Arguments

`bam_file`	The name of the bam file.
`all_genes`	An GRangesList object. It's the parsed genes annotation file from GTF file.
`cores`	number of cores to use.

Details

This function judges whether bam file is single-end and paired-end, and generate bam file index if needed. It takes multiple-core structure for parallel computing for efficiency.

Value

This function returns a coverageClass object. It contains a list of: (1) a list of coverage score for each gene in RLE format and (2) a dataframe for read counts

See Also

`read_coverage_batch`

`read_coverage_batch` *Compute the read coverage score and read counts for all genes in batch mode*

Description

This function calls `read_coverage` to compute read coverage score and read counts for all genes and samples.

Notes:

1. Coverage score is calculated per gene, i.e., concatenation of all exons from the same gene.
2. We follow the HTseq protocol for counting valid reads or read pairs for each gene.
3. When reading the alignment file, `isSecondaryAlignment` is set to `FALSE` to avoid redundant counting.
4. For paired-end data, `isPaired` is set to `TRUE`. We do not recommend setting `isProperPair` to `TRUE` as some fragment lengths may exceed 200 bp.
5. Users can modify `scanBamParam` in the R code as needed.

Usage

```
read_coverage_batch(bam_file_list, gtf_file, cores = 1)
```

Arguments

`bam_file_list`	A character vector of BAM file paths.
`gtf_file`	The GTF file that RNA-seq reads were aligned to.
`cores`	Number of processor cores to use. Default = 1.

Value

A list with the following components:

`coverage`	A list of coverage matrices for all genes within each sample.
`counts`	A `data.frame` of read counts for all genes within each sample.

See Also

`read_coverage`

Examples

```
## Read BAM file and GTF file from the package
bam_file_list <- list.files(
    path = system.file("extdata", package = "DegNorm"),
    pattern = ".bam$", full.names = TRUE
)
gtf_file <- list.files(
    path = system.file("extdata", package = "DegNorm"),
    pattern = ".gtf$", full.names = TRUE
)

## Run read_coverage_batch to calculate read coverage curves and read counts
coverage_res <- read_coverage_batch(bam_file_list, gtf_file, cores = 2)
```

res_DegNorm_chr21 *Example DegNormClass data*

Description

Example of DegNormClass data from DegNorm package. It is the output from degnorm function for human chromosome 21.

Usage

```
data("res_DegNorm_chr21")
```

Format

A DegNormClass list of the following items:

counts a data.drame of read counts for each gene within each sample.

counts_normed a data.drame of degradation-normalized read counts for each gene within each sample.

DI a matrix of degradation index scores for each gene within each sample.

K normalizing scale factor for each gene within each sample after accounting for degradation normalization.

convergence convergence tag; 0 = degnorm was not done on this gene because smaller counts or too short length.1 = degnorm was done with baseline selection. 2 = degnorm done without baseline selection because gene length (after filtering out low count regions)<200 bp. 3= baseline was found, but DI score is too large. 4 = baseline selection didn't coverge.

envelop a list of the envelop curves for all genes.

Examples

```
data(res_DegNorm_chr21)
summary_DegNormClass(res_DegNorm_chr21)
```

summary_CoverageClass *Summary method for CoverageClass.*

Description

It prints a summary of the data objects contained in the list from read_coverage_batch.

Usage

```
summary_CoverageClass(object)
```

Arguments

object	CoverageClass from coderead_coverage_batch.

Value

On-screen plot of summary of `CoverageClass` object.

Examples

```
## Summary of coverage_cal_batch output (CoverageClass)
data(coverage_res_chr21)
summary_CoverageClass(coverage_res_chr21)
```

`summary_DegNormClass` *Summary method for DegNormClass.*

Description

It prints a summary of the data objects contained in the list from degnorm function.

Usage

```
summary_DegNormClass(object)
```

Arguments

`object`	DegNormClass from `degnorm` function.

Value

On-screen summary of `DegNormClass` object.

Examples

```
## Summary of degnorm output (DegNormlass)
data(res_DegNorm_chr21)
summary_DegNormClass(res_DegNorm_chr21)
```

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