Package ‘DegNorm’

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Type Package

Title DegNorm: degradation normalization for RNA-seq data

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Author Bin Xiong and Ji-Ping Wang

Maintainer Ji-Ping Wang <jzwang@northwestern.edu>

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Description This package performs degradation normalization in bulk RNA-seq data to improve differential expression analysis accuracy.

License LGPL (>= 3)

Depends R (>= 4.0.0), methods

Imports Rcpp (>= 1.0.2), GenomicFeatures, 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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### Description
DegNorm is an R package for degradation normalization 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

### Author(s)
Bin Xiong, Ji-Ping Wang

Maintainer: Ji-Ping Wang <jzwang@northwestern.edu>

### 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**

```r
data(coverage_res_chr21)
```

**Format**

A CoverageClass list of the following

- `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.

**Examples**

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

---

**degnorm**  
*Main function to perform degradation normalization.*

---

**Description**

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

**Usage**

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

**Arguments**

- `read_coverage` a list of coverage 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.
degnorm outputs a list of following objects:

- **counts**: a data.frame of read counts for each gene within each sample.
- **counts_normed**: a data.frame 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

```r
##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]],
itration = 2,
down_sampling = 1,
grid_size=10,
loop = 20,
cores=2)
```

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

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 plotted 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_boxplot plots 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 output 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.
read_coverage

Details

plot_coverage outputs 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 socre 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 HTseq protocol for counting valid read or read pairs for each gene.
3. When reading alignment file, isSecondaryAlignment flag is set as FALSE to avoid possible redundant counting.
4. For paired-end data, isPaired is set as TRUE. We don’t recommend setting isProperPair as TRUE as some fragments length may exceed 200bp.
5. User can modify scanBamParam in the R codes below as needed.

Usage

read_coverage_batch(bam_file_list, gtf_file, cores=1)

Arguments

bam_file_list a character vector of bam file names.
gtf_file the gtf file that RNA-seq reads were aligned with reference to.
cores number of cores to be used. Default=1.

Value

A list of the following:

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

See Also

read_coverage
Examples

```r
# 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

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

### Format

A DegNormClass list of the following items:

- **counts**: a data.frame of read counts for each gene within each sample.
- **counts_normed**: a data.frame 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 converge.
- **envelop**: a list of the envelop curves for all genes.

### Examples

```r
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**
```r
summary_CoverageClass(object)
```

**Arguments**
- `object`  
  CoverageClass from `coderead_coverage_batch`.

**Value**
On-screen plot of summary of CoverageClass object.

**Examples**
```r
## 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**
```r
summary_DegNormClass(object)
```

**Arguments**
- `object`  
  DegNormClass from `degnorm` function.

**Value**
On-screen summary of DegNormClass object.

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