Package ‘DegNorm’

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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.

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Depends R (>= 4.0.0), methods

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VignetteBuilder knitr

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DegNorm-package

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

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**

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`: data.frame 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 calculated 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.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 list of the envelop curves for all genes.

Examples

```r
# coverage_res_chr21 is a 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.
**plot_coverage**

Usage

```r
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

```r
## 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

```r
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`.  

```r
# res_DegNorm_chr21 is degnorm output stored in sysdata.Rda
data(res_DegNorm_chr21)
plot_coverage(res_DegNorm_chr21$gene_name, res_DegNorm_chr21$coverage_output, res_DegNorm_chr21$degnorm_output)
```
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_coverage outputs the coverage curves before- and after-degradation normalization.

Value

The coverage curve before and after degradation normalization.

Examples

```r
## 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

```r
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

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 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**

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