

# Package ‘DegNorm’

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**Type** Package

**Title** DegNorm: degradation normalization for RNA-seq data

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ImmunoOncology, QualityControl, DataImport

**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, 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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**VignetteBuilder** knitr

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DegNorm-package	<i>DegNorm: degradation normalization for RNA-seq data</i>
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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)

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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

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coverage\_res\_chr21      *Example CoverageClass data*

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### 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 coverage 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 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	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 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 is 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**

```
##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)
```

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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 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 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)
```

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plot_coverage	<i>Coverage plot functions for DegNorm</i>
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**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\_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

<code>bam_file</code>	The name of the bam file.
<code>all_genes</code>	An GRangesList object. It's the parsed genes annotation file from GTF file.
<code>cores</code>	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](#)

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read_coverage_batch	<i>Compute the read coverage score and read counts for all genes in batch mode.</i>
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## 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

```
## 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 coverage.

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

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