

# Package ‘GRaNIE’

January 27, 2023

**Title** GRaNIE: Reconstruction cell type specific gene regulatory networks including enhancers using chromatin accessibility and RNA-seq data

**Version** 1.2.3

**Encoding** UTF-8

**Description** Genetic variants associated with diseases often affect non-coding regions, thus likely having a regulatory role. To understand the effects of genetic variants in these regulatory regions, identifying genes that are modulated by specific regulatory elements (REs) is crucial. The effect of gene regulatory elements, such as enhancers, is often cell-type specific, likely because the combinations of transcription factors (TFs) that are regulating a given enhancer have celltype specific activity. This TF activity can be quantified with existing tools such as diffTF and captures differences in binding of a TF in open chromatin regions. Collectively, this forms a gene regulatory network (GRN) with cell-type and data-specific TF-RE and RE-gene links. Here, we reconstruct such a GRN using bulk RNAseq and open chromatin (e.g., using ATACseq or ChIPseq for open chromatin marks) and optionally TF activity data. Our network contains different types of links, connecting TFs to regulatory elements, the latter of which is connected to genes in the vicinity or within the same chromatin domain (TAD). We use a statistical framework to assign empirical FDRs and weights to all links using a permutation-based approach.

**Imports** futile.logger, checkmate, patchwork, reshape2, data.table, matrixStats, Matrix, GenomicRanges, RColorBrewer, ComplexHeatmap, DESeq2, circlize, progress, utils, methods, stringr, scales, igraph, S4Vectors, ggplot2, rlang, Biostrings, GenomeInfoDb, SummarizedExperiment, forcats, gridExtra, limma, tidyselect, readr, grid, tidyr, dplyr, stats, grDevices, graphics, magrittr, tibble, viridis, colorspace, biomaRt, topGO

**Depends** R (>= 4.2.0)

**Suggests** knitr, BSgenome.Hsapiens.UCSC.hg19, BSgenome.Hsapiens.UCSC.hg38, BSgenome.Mmusculus.UCSC.mm10, BSgenome.Mmusculus.UCSC.mm9, TxDb.Hsapiens.UCSC.hg19.knownGene, TxDb.Hsapiens.UCSC.hg38.knownGene, TxDb.Mmusculus.UCSC.mm10.knownGene, TxDb.Mmusculus.UCSC.mm9.knownGene, org.Hs.eg.db, org.Mm.eg.db, IHW, clusterProfiler, ReactomePA, DOSE, BiocFileCache,

ChIPseeker, testthat (>= 3.0.0), BiocStyle, csaw, BiocParallel,  
robust, variancePartition, purrr, EDASeq

**VignetteBuilder** knitr

**biocViews** Software, GeneExpression, GeneRegulation, NetworkInference,  
GeneSetEnrichment, BiomedicalInformatics, Genetics,  
Transcriptomics, ATACSeq, RNASeq, GraphAndNetwork, Regression,  
Transcription, ChIPSeq

**License** Artistic-2.0

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**Author** Christian Arnold [cre, aut],  
Judith Zaugg [aut],  
Rim Moussa [aut],  
Armando Reyes-Palomares [ctb],  
Giovanni Palla [ctb],  
Maksim Kholmatov [ctb]

**Maintainer** Christian Arnold <chrarnold@web.de>

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---

addConnections\_peak\_gene

*Add peak-gene connections to a GRN object*

---

## Description

After the execution of this function, QC plots can be plotted with the function `plotDiagnosticPlots_peakGene` unless this has already been done by default due to `plotDiagnosticPlots = TRUE`

## Usage

```
addConnections_peak_gene(
  GRN,
  overlapTypeGene = "TSS",
  corMethod = "pearson",
  promoterRange = 250000,
  TADs = NULL,
```

```

TADs_mergeOverlapping = FALSE,
shuffleRNACounts = TRUE,
nCores = 4,
plotDiagnosticPlots = TRUE,
plotGeneTypes = list(c("all"), c("protein_coding")),
outputFolder = NULL,
addRobustRegression = FALSE,
forceRerun = FALSE
)

```

## Arguments

GRN	Object of class <a href="#">GRN</a>
overlapTypeGene	Character. "TSS" or "full". Default "TSS". If set to "TSS", only the TSS of the gene is used as reference for finding genes in the neighborhood of a peak. If set to "full", the whole annotated gene (including all exons and introns) is used instead.
corMethod	Character. pearson or spearman. Default pearson. Method for calculating the correlation coefficient. See <a href="#">cor</a> for details.
promoterRange	Integer $\geq 0$ . Default 250000. The size of the neighborhood in bp to correlate peaks and genes in vicinity. Only peak-gene pairs will be correlated if they are within the specified range. Increasing this value leads to higher running times and more peak-gene pairs to be associated, while decreasing results in the opposite.
TADs	Data frame with TAD domains. Default NULL. If provided, the neighborhood of a peak is defined by the TAD domain the peak is in rather than a fixed-sized neighborhood. The expected format is a BED-like data frame with at least 3 columns in this particular order: chromosome, start, end, the 4th column is optional and will be taken as ID column. All additional columns as well as column names are ignored. For the first 3 columns, the type is checked as part of a data integrity check.
TADs_mergeOverlapping	TRUE or FALSE. Default FALSE. Should overlapping TADs be merged? Only relevant if TADs are provided.
shuffleRNACounts	TRUE or FALSE. Default TRUE. Should the RNA sample labels be permuted in addition to testing random peak-gene pairs for the permuted background? When set to FALSE, only peak-gene pairs are shuffled, but for each pair, the counts from peak and RNA that are correlated are matched (i.e., sample 1 counts from peak data are compared to sample 1 counts from RNA). If set to TRUE, however, the RNA sample labels are in addition permuted so that sample 1 counts from peak data are compared to sample 4 data from RNA, for example. Permuting twice randomizes the resulting eGRN even more. Note that this parameter and its influence is still being investigated. Until version 1.0.7, this parameter (although not existent explicitly) was implicitly set to TRUE.

nCores	Integer >0. Default 1. Number of cores to use. A value >1 requires the BiocParallel package (as it is listed under Suggests, it may not be installed yet).
plotDiagnosticPlots	TRUE or FALSE. Default TRUE. Run and plot various diagnostic plots? If set to TRUE, PDF files will be produced and saved in the output directory (in a sub-folder called plots).
plotGeneTypes	List of character vectors. Default <code>list(c("all"), c("protein_coding"))</code> . Each list element may consist of one or multiple gene types that are plotted collectively in one PDF. The special keyword "all" denotes all gene types that are found (be aware: this typically contains 20+ gene types, see <a href="https://www.encodegenes.org/pages/biotypes.html">https://www.encodegenes.org/pages/biotypes.html</a> for details).
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
addRobustRegression	TRUE or FALSE. EXPERIMENTAL. Default FALSE. Use a robust regression in addition to a non-robust one? Significantly increases overall running time. If set to TRUE, the package robust is required (as it is listed under Suggests, it may not be installed).
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

**Value**

An updated [GRN](#) object, with additional information added from this function.

**See Also**

[plotDiagnosticPlots\\_peakGene](#)

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = addConnections_peak_gene(GRN, promoterRange=10000, plotDiagnosticPlots = FALSE)
```

---

addConnections\_TF\_peak

*Add TF-peak connections to a [GRN](#) object*

---

**Description**

After the execution of this function, QC plots can be plotted with the function [plotDiagnosticPlots\\_TFPeaks](#) unless this has already been done by default due to `plotDiagnosticPlots = TRUE`

**Usage**

```

addConnections_TF_peak(
  GRN,
  plotDiagnosticPlots = TRUE,
  plotDetails = FALSE,
  outputFolder = NULL,
  corMethod = "pearson",
  connectionTypes = c("expression"),
  removeNegativeCorrelation = c(FALSE),
  maxFDRToStore = 0.3,
  addForPermuted = TRUE,
  useGCCorrection = FALSE,
  percBackground_size = 75,
  percBackground_resample = TRUE,
  forceRerun = FALSE
)

```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
plotDiagnosticPlots	TRUE or FALSE. Default TRUE. Run and plot various diagnostic plots? If set to TRUE, PDF files will be produced and saved in the output directory (in a sub-folder called plots).
plotDetails	TRUE or FALSE. Default FALSE. Print additional plots that may help for debugging and QC purposes? Note that these plots are currently less documented or not at all.
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
corMethod	Character. <code>pearson</code> or <code>spearman</code> . Default <code>pearson</code> . Method for calculating the correlation coefficient. See <a href="#">cor</a> for details.
connectionTypes	Character vector. Default <code>expression</code> . Vector of connection types to include for the TF-peak connections. If an additional connection type is specified here, it has to be available already within the object (EXPERIMENTAL). See the function <a href="#">addData_TFActivity</a> for details.
removeNegativeCorrelation	Vector of TRUE or FALSE. Default FALSE. EXPERIMENTAL. Must be a logical vector of the same length as the parameter <code>connectionType</code> . Should negatively correlated TF-peak connections be removed for the specific connection type? For connection type <code>expression</code> , the default is FALSE, while for any TF Activity related connection type, we recommend setting this to TRUE.
maxFDRToStore	Numeric[0,1]. Default 0.3. Maximum TF-peak FDR value to permanently store a particular TF-peak connection in the object? This parameter has a large influ-

ence on the overall memory size of the object, and we recommend not storing connections with a high FDR due to their sheer number.

addForPermuted	TRUE or FALSE. Default FALSE. Add connections also for permuted data. Leave at TRUE unless you know what you are doing.
useGCCorrection	TRUE or FALSE. Default FALSE. EXPERIMENTAL. Should a GC-matched background be used when calculating FDRs?
percBackground_size	Numeric[0,100]. Default 75. EXPERIMENTAL. Description will follow. Only relevant if useGCCorrection is set to TRUE, ignored otherwise.
percBackground_resample	TRUE or FALSE. Default TRUE. EXPERIMENTAL. Should resampling be enabled for those GC bins for which not enough background peaks are available?. Only relevant if useGCCorrection is set to TRUE, ignored otherwise.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

**Value**

An updated [GRN](#) object, with additional information added from this function.

**See Also**

[plotDiagnosticPlots\\_TFPeaks](#)

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = addConnections_TF_peak(GRN, plotDiagnosticPlots = FALSE, forceRerun = FALSE)
```

---

addData                      *Add data to a [GRN](#) object.*

---

**Description**

This function adds both RNA and peak data to a [GRN](#) object, along with data normalization. In addition, and highly recommended, sample metadata can be optionally provided.

**Usage**

```
addData(
  GRN,
  counts_peaks,
  normalization_peaks = "DESeq2_sizeFactors",
  idColumn_peaks = "peakID",
  counts_rna,
```

```

normalization_rna = "limma_quantile",
idColumn_RNA = "ENSEMBL",
sampleMetadata = NULL,
additionalParams.l = list(),
allowOverlappingPeaks = FALSE,
keepOriginalReadCounts = FALSE,
forceRerun = FALSE
)

```

## Arguments

GRN	Object of class <a href="#">GRN</a>
counts_peaks	Data frame. No default. Counts for the peaks, with raw or normalized counts for each peak (rows) across all samples (columns). In addition to the count data, it must also contain one ID column with a particular format, see the argument <code>idColumn_peaks</code> below. Row names are ignored, column names must be set to the sample names and must match those from the RNA counts and the sample metadata table.
normalization_peaks	Character. Default <code>DESeq2_sizeFactors</code> . Normalization procedure for peak data. Must be one of <code>limma_cyclicloess</code> , <code>limma_quantile</code> , <code>limma_scale</code> , <code>csaw_cyclicLoess_orig</code> , <code>csaw_TMM</code> , <code>EDASeq_GC_peaks</code> , <code>gcqn_peaks</code> , <code>DESeq2_sizeFactors</code> , <code>none</code> .
idColumn_peaks	Character. Default <code>peakID</code> . Name of the column in the <code>counts_peaks</code> data frame that contains peak IDs. The required format must be <code>chr:start-end</code> , with <code>chr</code> denoting the abbreviated chromosome name, and <code>start</code> and <code>end</code> the begin and end of the peak coordinates, respectively. End must be bigger than start. Examples for valid peak IDs are <code>chr1:400-800</code> or <code>chrX:20-25</code> .
counts_rna	Data frame. No default. Counts for the RNA-seq data, with raw or normalized counts for each gene (rows) across all samples (columns). In addition to the count data, it must also contain one ID column with a particular format, see the argument <code>idColumn_rna</code> below. Row names are ignored, column names must be set to the sample names and must match those from the RNA counts and the sample metadata table.
normalization_rna	Character. Default <code>limma_quantile</code> . Normalization procedure for peak data. Must be one of <code>limma_cyclicloess</code> , <code>limma_quantile</code> , <code>limma_scale</code> , <code>csaw_cyclicLoess_orig</code> , <code>csaw_TMM</code> , <code>DESeq2_sizeFactors</code> , <code>none</code> .
idColumn_RNA	Character. Default <code>ENSEMBL</code> . Name of the column in the <code>counts_rna</code> data frame that contains Ensembl IDs.
sampleMetadata	Data frame. Default <code>NULL</code> . Optional, additional metadata for the samples, such as age, sex, gender etc. If provided, the <code>@seealso [plotPCA_all()]</code> function can then incorporate and plot it. Sample names must match with those from both peak and RNA-Seq data. The first column is expected to contain the sample IDs, the actual column name is irrelevant.
additionalParams.l	Named list. Default <code>list()</code> . Additional parameters for the chosen normalization method. Currently, only the GC-aware normalization methods <code>EDASeq_GC_peaks</code>



and `gcqn_peaks` are supported here. Both support the parameters `roundResults` (logical flag, TRUE or FALSE) and `nBins` (Integer > 0), and `EDASeq_GC_peaks` supports three additional parameters: `withinLane_method` (one of: "loess", "median", "upper", "full") and `betweenLane_method` (one of: "median", "upper", "full"). For more information, see the EDASeq vignette.

`allowOverlappingPeaks`

TRUE or FALSE. Default FALSE. Should overlapping peaks be allowed (then only a warning is issued when overlapping peaks are found) or (the default) should an error be raised?

`keepOriginalReadCounts`

TRUE or FALSE. Default FALSE. Should the original read counts as provided to the function be kept in addition to storing the read counts after a (if any) normalization? This increases the memory footprint of the object because 2 additional count matrices have to be stored.

`forceRerun`

TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

## Details

If the `ChIPseeker` package is installed, additional peak annotation is provided in the annotation slot and a peak annotation QC plot is produced as part of peak-gene QC. This is fully optional, however, and has no consequences for downstream functions. Normalizing the data sensibly is very important. When quantiles are chosen, `limma::normalizeQuantiles` is used, which in essence does the following: Each quantile of each column is set to the mean of that quantile across arrays. The intention is to make all the normalized columns have the same empirical distribution. This will be exactly true if there are no missing values and no ties within the columns: the normalized columns are then simply permutations of one another.

## Value

An updated `GRN` object, with added data from this function (e.g., slots `GRN@data$peaks` and `GRN@data$RNA`)

## See Also

[plotPCA\\_all](#)

## Examples

```
# See the Workflow vignette on the GRaNIE website for examples
# library(readr)
# rna.df = read_tsv("https://www.embl.de/download/zaugg/GRaNIE/rna.tsv.gz")
# peaks.df = read_tsv("https://www.embl.de/download/zaugg/GRaNIE/peaks.tsv.gz")
# meta.df = read_tsv("https://www.embl.de/download/zaugg/GRaNIE/sampleMetadata.tsv.gz")
# GRN = loadExampleObject()
# We omit sampleMetadata = meta.df in the following line, becomes too long otherwise
# GRN = addData(GRN, counts_peaks = peaks.df, counts_rna = rna.df, forceRerun = FALSE)
```

---

addData\_TFActivity      *Add TF activity data to GRN object using a simplified procedure for estimating it. EXPERIMENTAL.*

---

### Description

We do not yet provide full support for this function. It is currently being tested. Use at our own risk.

### Usage

```
addData_TFActivity(
  GRN,
  normalization = "cyclicLoess",
  name = "TF_activity",
  forceRerun = FALSE
)
```

### Arguments

GRN	Object of class <a href="#">GRN</a>
normalization	Character. Default cyclicLoess. One of cyclicLoess, sizeFactors, quantile, or none. Normalization procedure. When set to cyclicLoess, the csaw package is required (as it is listed under Suggests, it may not be installed).
name	Name in object under which it should be stored. This corresponds to the connectionType afterwards that some functions iterate over.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

### Value

An updated [GRN](#) object, with added data from this function (GRN@data\$TFs[[name]]) in particular, with name referring to the value of the name parameter)

---

addTFBS      *Add TFBS to a [GRN](#) object.*

---

### Description

For this, a folder that contains one TFBS file per TF in bed or bed.gz format must be given (see details). The folder must also contain a so-called translation table, see the argument translationTable for details. We provide example files for all supported genome assemblies (hg19, hg38 and mm10) that are fully compatible with GRaNIE as separate downloads. For more information, check <https://diffTF.readthedocs.io/en/latest/chapter2.html#dir-tfbs>.

**Usage**

```

addTFBS(
  GRN,
  motifFolder,
  TFs = "all",
  translationTable = "translationTable.csv",
  translationTable_sep = " ",
  nTFMax = NULL,
  filesTFBSPattern = "_TFBS",
  fileEnding = ".bed",
  forceRerun = FALSE
)

```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
motifFolder	Character. No default. Path to the folder that contains the TFBS predictions. The files must be in BED format, 6 columns, one file per TF. See the other parameters for more details. The folder must also contain a so-called translation table, see the argument <code>translationTable</code> for details.
TFs	Character vector. Default <code>all</code> . Vector of TF names to include. The special keyword <code>all</code> can be used to include all TF found in the folder as specified by <code>motifFolder</code> . If <code>all</code> is specified anywhere, all TFs will be included. TF names must otherwise match the file names that are found in the folder, without the file suffix.
translationTable	Character. Default <code>translationTable.csv</code> . Name of the translation table file that is also located in the folder along with the TFBS files. This file must have the following structure: at least 2 columns, called ENSEMBL and ID. ID denotes the ID for the TF that is used throughout the pipeline (e.g., AHR) and the prefix of how the corresponding file is called (e.g., AHR.0.B if the file for AHR is called AHR.0.B_TFBS.bed.gz), while ENSEMBL denotes the ENSEMBL ID (dot suffix; e.g., ENSG00000106546, are removed automatically if present).
translationTable_sep	Character. Default <code>" "</code> (whitespace character). The column separator for the <code>translationTable</code> file.
nTFMax	NULL or Integer[1,]. Default NULL. Maximal number of TFs to import. Can be used for testing purposes, e.g., setting to 5 only imports 5 TFs even though the whole <code>motifFolder</code> has many more TFs defined.
filesTFBSPattern	Character. Default <code>"_TFBS"</code> . Suffix for the file names in the TFBS folder that is not part of the TF name. Can be empty. For example, for the TF CTCF, if the file is called CTCF.all.TFBS.bed, set this parameter to <code>".all.TFBS"</code> .
fileEnding	Character. Default <code>".bed"</code> . File ending for the files from the motif folder.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

**Value**

An updated [GRN](#) object, with additional information added from this function (GRN@annotation\$TFs in particular)

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
```

---

```
add_featureVariation  Quantify and interpret multiple sources of biological and technical
                      variation for features (TFs, peaks, and genes) in a GRN object
```

---

**Description**

Runs the main function `fitExtractVarPartModel` of the package `variancePartition`: Fits a linear (mixed) model to estimate contribution of multiple sources of variation while simultaneously correcting for all other variables for the features in a GRN object (TFs, peaks, and genes) given particular metadata. The function reports the fraction of variance attributable to each metadata variable. **Note: The results are not added to** `GRN@connections$all.filtered`, **rerun the function** `getGRNConnections` **and set** `include_variancePartitionResults` **to TRUE to do so**. The results object is stored in `GRN@stats$variancePartition` and can be used for the various diagnostic and plotting functions from `variancePartition`.

**Usage**

```
add_featureVariation(
  GRN,
  formula = "auto",
  metadata = c("all"),
  features = "all_filtered",
  nCores = 1,
  forceRerun = FALSE,
  ...
)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
formula	Character(1). Either <code>auto</code> or a manually defined formula to be used for the model fitting. Default <code>auto</code> . Must include only terms that are part of the metadata as specified with the <code>metadata</code> parameter. If set to <code>auto</code> , the formula will be build automatically based on all metadata variables as specified with the <code>metadata</code> parameter. By default, numerical variables will be modeled as fixed effects, while variables that are defined as factors or can be converted to factors (characters and logical variables) are modeled as random effects as recommended by the <code>variancePartition</code> package.

metadata	Character vector. Default all. Vector of column names from the metadata data frame that was provided when using the function <code>addData</code> . Must either contain the special keyword <code>all</code> to denote that all (!) metadata columns from <code>GRN@data\$metadata</code> are taken or if not, a subset of the column names from <code>GRN@data\$metadatato</code> to include in the model fitting for <code>fitExtractVarPartModel</code> .
features	Character(1). Either <code>all_filtered</code> or <code>all</code> . Default <code>all_filtered</code> . Should <code>variancePartition</code> only be run for the features (TFs, peaks and genes) from the filtered set of connections (the result of <code>filterGRNAndConnectGenes</code> ) or for all genes that are defined in the object? If set to <code>all</code> , the running time is greatly increased.
nCores	Integer >0. Default 1. Number of cores to use. A value >1 requires the <code>BiocParallel</code> package (as it is listed under Suggests, it may not be installed yet).
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.
...	Additional parameters passed on to <code>variancePartition::fitExtractVarPartModel</code> beyond <code>exprObj</code> , <code>formula</code> and <code>data</code> . See the function help for more information

## Details

The normalized count matrices are used as input for `fitExtractVarPartModel`.

## Value

An updated `GRN` object, with additional information added from this function to `GRN@stats$variancePartition` as well as the elements `genes`, `consensusPeaks` and `TFs` within `GRN@annotation`. As noted above, the results are not added to `GRN@connections$all.filtered`; rerun the function `getGRNConnections` and set `include_variancePartitionResults` to `TRUE` to include the results in the eGRN output table.

## See Also

[addData](#)

[getGRNConnections](#)

## Examples

```
# See the Workflow vignette on the GRaNIE website for examples
# GRN = loadExampleObject()
# GRN = add_featureVariation(GRN, metadata = c("mt_frac"), forceRerun = TRUE)
```

---

`add_TF_gene_correlation`*Add TF-gene correlations to a [GRN](#) object.*

---

### Description

The information is currently stored in `GRN@connections$TF_genes.filtered`. Note that raw p-values are not adjusted.

### Usage

```
add_TF_gene_correlation(  
  GRN,  
  corMethod = "pearson",  
  addRobustRegression = FALSE,  
  nCores = 1,  
  forceRerun = FALSE  
)
```

### Arguments

<code>GRN</code>	Object of class <a href="#">GRN</a>
<code>corMethod</code>	Character. <code>pearson</code> or <code>spearman</code> . Default <code>pearson</code> . Method for calculating the correlation coefficient. See <a href="#">cor</a> for details.
<code>addRobustRegression</code>	TRUE or FALSE. EXPERIMENTAL. Default FALSE. Use a robust regression in addition to a non-robust one? Significantly increases overall running time. If set to TRUE, the package <code>robust</code> is required (as it is listed under <code>Suggests</code> , it may not be installed).
<code>nCores</code>	Integer >0. Default 1. Number of cores to use. A value >1 requires the <code>BiocParallel</code> package (as it is listed under <code>Suggests</code> , it may not be installed yet).
<code>forceRerun</code>	TRUE or FALSE. Default FALSE. Force execution, even if the <code>GRN</code> object already contains the result. Overwrites the old results.

### Value

An updated [GRN](#) object, with additional information added from this function.

### Examples

```
# See the Workflow vignette on the GRaNIE website for examples  
GRN = loadExampleObject()  
GRN = add_TF_gene_correlation(GRN, forceRerun = FALSE)
```

---

 AR\_classification\_wrapper

*Run the activator-repressor classification for the TFs for a [GRN](#) object*


---

## Description

Run the activator-repressor classification for the TFs for a [GRN](#) object

## Usage

```
AR_classification_wrapper(
  GRN,
  significanceThreshold_Wilcoxon = 0.05,
  plot_minNoTFBS_heatmap = 100,
  deleteIntermediateData = TRUE,
  plotDiagnosticPlots = TRUE,
  outputFolder = NULL,
  corMethod = "pearson",
  forceRerun = FALSE
)
```

## Arguments

GRN	Object of class <a href="#">GRN</a>
significanceThreshold_Wilcoxon	Numeric[0,1]. Default 0.05. Significance threshold for Wilcoxon test that is run in the end for the final classification. See the Vignette and <i>*diffTF*</i> paper for details.
plot_minNoTFBS_heatmap	Integer[1,]. Default 100. Minimum number of TFBS for a TF to be included in the heatmap that is part of the output of this function.
deleteIntermediateData	TRUE or FALSE. Default TRUE. Should intermediate data be deleted before returning the object after a successful run? Due to the size of the produced intermediate data, we recommend setting this to TRUE, but if memory or object size are not an issue, the information can also be kept.
plotDiagnosticPlots	TRUE or FALSE. Default TRUE. Run and plot various diagnostic plots? If set to TRUE, PDF files will be produced and saved in the output directory (in a subfolder called plots).
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
corMethod	Character. pearson or spearman. Default pearson. Method for calculating the correlation coefficient. See <a href="#">cor</a> for details.

forceRerun      TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

### Value

An updated [GRN](#) object, with additional information added from this function.

### Examples

```
# See the Workflow vignette on the GRaNIE website for examples
# GRN = loadExampleObject()
# GRN = AR_classification_wrapper(GRN, outputFolder = ".", forceRerun = FALSE)
```

---

build\_eGRN\_graph      *Builds a graph out of a set of connections*

---

### Description

This function requires a filtered set of connections in the [GRN](#) object as generated by [filterGRNAndConnectGenes](#)

### Usage

```
build_eGRN_graph(
  GRN,
  model_TF_gene_nodes_separately = FALSE,
  allowLoops = FALSE,
  removeMultiple = FALSE,
  directed = FALSE,
  forceRerun = FALSE
)
```

### Arguments

GRN	Object of class <a href="#">GRN</a>
model_TF_gene_nodes_separately	TRUE or FALSE. Default FALSE. Should TF and gene nodes be modeled separately? If set to TRUE, this may lead to unwanted effects in case of TF-TF connections (i.e., a TF regulating another TF)
allowLoops	TRUE or FALSE. Default FALSE. Allow loops in the network (i.e., a TF that regulates itself)
removeMultiple	TRUE or FALSE. Default FALSE. Remove loops with the same start and end point? This can happen if multiple TF originate from the same gene, for example.
directed	TRUE or FALSE. Default FALSE. Should the network be directed?
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.



**Value**

An updated [GRN](#) object, with the graph(s) being stored in the slot 'graph' (i.e., 'GRN@graph' for both TF-gene and TF-peak-gene graphs)

**See Also**

[filterGRNAndConnectGenes](#)

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = build_eGRN_graph(GRN, forceRerun = FALSE)
```

---

calculateCommunitiesEnrichment

*Run an enrichment analysis for the genes in each community in the filtered [GRN](#) object*

---

**Description**

The enrichment analysis is based on the subset of the network connected to a particular community as identified by [calculateCommunitiesStats](#), see [calculateTFEnrichment](#) and [calculateGeneralEnrichment](#) for TF-specific and general enrichment, respectively. This function requires the existence of the eGRN graph in the [GRN](#) object as produced by [build\\_eGRN\\_graph](#) as well as community information as calculated by [calculateCommunitiesStats](#). Results can subsequently be visualized with the function [plotCommunitiesEnrichment](#).

**Usage**

```
calculateCommunitiesEnrichment(
  GRN,
  ontology = c("GO_BP", "GO_MF"),
  algorithm = "weight01",
  statistic = "fisher",
  background = "neighborhood",
  background_geneTypes = "all",
  selection = "byRank",
  communities = seq_len(10),
  pAdjustMethod = "BH",
  forceRerun = FALSE
)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
ontology	Character vector of ontologies. Default <code>c("GO_BP", "GO_MF")</code> . Valid values are "GO_BP", "GO_MF", "GO_CC", "KEGG", "DO", and "Reactome", referring to <i>GO Biological Process</i> , <i>GO Molecular Function</i> , <i>GO Cellular Component</i> , <i>KEGG Disease Ontology</i> , and <i>Reactome Pathways</i> , respectively. GO ontologies require the topGO, "KEGG" the clusterProfiler, "DO" the DOSE, and "Reactome" the ReactomePA packages, respectively. As they are listed under Suggests, they may not yet be installed, and the function will throw an error if they are missing.
algorithm	Character. Default "weight01". One of: "classic", "elim", "weight", "weight01", "lea", "parentchild". Only relevant if ontology is GO related (GO_BP, GO_MF, GO_CC), ignored otherwise. Name of the algorithm that handles the GO graph structures. Valid inputs are those supported by the topGO library. For general information about the algorithms, see <a href="https://academic.oup.com/bioinformatics/article/22/13/1600/193669">https://academic.oup.com/bioinformatics/article/22/13/1600/193669</a> . weight01 is a mixture between the elim and the weight algorithms.
statistic	Character. Default "fisher". One of: "fisher", "ks", "t", "globaltest", "sum", "ks.ties". Statistical test to be used. Only relevant if ontology is GO related (GO_BP, GO_MF, GO_CC), and valid inputs are those supported by the topGO library, ignored otherwise. For the other ontologies the test statistic is always Fisher.
background	Character. Default "neighborhood". One of: "all_annotated", "all_RNA", "all_RNA_filtered", "neighborhood". Set of genes to be used to construct the background for the enrichment analysis. This can either be all annotated genes in the reference genome (all_annotated), all genes from the provided RNA data (all_RNA), all genes from the provided RNA data excluding those marked as filtered after executing filterData (all_RNA_filtered), or all the genes that are within the neighborhood of any peak (before applying any filters except for the user-defined promoterRange value in addConnections_peak_gene) (neighborhood).
background_geneTypes	Character vector of gene types that should be considered for the background. Default "all". Only gene types as defined in the <a href="#">GRN</a> object, slot <code>GRN@annotation\$genes\$gene.type</code> are allowed. The special keyword "all" means no filter on gene type.
selection	Character. Default "byRank". One of: "byRank", "byLabel". Specify whether the communities enrichment will be calculated based on their rank, where the largest community (with most vertices) would have a rank of 1, or by their label. Note that the label is independent of the rank.
communities	Numeric vector. Default <code>c(1:10)</code> . Depending on what was specified in the display parameter, this parameter would indicate either the rank or the label of the communities to be plotted. i.e. for <code>communities = c(1,4)</code> , if <code>display = "byRank"</code> the GO enrichment for the first and fourth largest communities will be calculated if <code>display = "byLabel"</code> , the results for the communities labeled "1", and "4" will be plotted.
pAdjustMethod	Character. Default "BH". One of: "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr". This parameter is only relevant for the following ontologies:

	KEGG, DO, Reactome. For the other ontologies, the algorithm serves as an adjustment.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

### Details

All enrichment functions use the TF-gene graph as defined in the ‘GRN’ object. See the ‘ontology’ argument for currently supported ontologies. Also note that some parameter combinations for ‘algorithm’ and ‘statistic’ are incompatible, an error message will be thrown in such a case.

### Value

An updated [GRN](#) object, with the enrichment results stored in the `stats$Enrichment$byCommunity` slot.

### See Also

[plotCommunitiesEnrichment](#)  
[plotGeneralEnrichment](#)  
[calculateGeneralEnrichment](#)  
[calculateCommunitiesStats](#)

### Examples

```
# See the Workflow vignette on the GRaNIe website for examples
GRN = loadExampleObject()
GRN = calculateCommunitiesEnrichment(GRN, ontology = c("GO_BP"), forceRerun = FALSE)
```

---

calculateCommunitiesStats

*Generate graph communities and their summarizing statistics*

---

### Description

The results can subsequently be visualized with the function [plotCommunitiesStats](#). This function requires a filtered set of connections in the [GRN](#) object as generated by [filterGRNAndConnectGenes](#). It then generates the TF-gene graph from the filtered connections, and clusters its vertices into communities using established community detection algorithms.

### Usage

```
calculateCommunitiesStats(GRN, clustering = "louvain", forceRerun = FALSE, ...)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
clustering	Character. Default <code>louvain</code> . One of: <code>louvain</code> , <code>leiden</code> , <code>leading_eigen</code> , <code>fast_greedy</code> , <code>optimal</code> , <code>walktrap</code> . The community detection algorithm to be used. Please bear in mind the robustness and time consumption of the algorithms when opting for an alternative to the default.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.
...	Additional parameters for the used clustering method, see the <code>igraph::cluster_*</code> methods for details on the specific parameters and what they do. For <code>leiden</code> clustering, for example, you may add a <code>resolution_parameter</code> to control the granularity of the community detection or <code>n_iterations</code> to modify the number of iterations.

**Value**

An updated [GRN](#) object, with a table that consists of the connections clustered into communities stored in the `GRN@graph$TF_gene$clusterGraph` slot as well as within the `igraph` object in `GRN@graph$TF_gene$graph` (retrievable via `igraph` using `igraph::vertex.attributes(GRN@graph$TF_gene$graph)$c` for example.)

**See Also**

[plotCommunitiesStats](#)  
[calculateCommunitiesEnrichment](#)

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = calculateCommunitiesStats(GRN, forceRerun = FALSE)
```

---

`calculateGeneralEnrichment`

*Run an enrichment analysis for the genes in the whole network in the filtered [GRN](#) object*

---

**Description**

The enrichment analysis is based on the whole network, see [calculateCommunitiesEnrichment](#) and [calculateTFEnrichment](#) for community- and TF-specific enrichment, respectively. This function requires the existence of the eGRN graph in the [GRN](#) object as produced by [build\\_eGRN\\_graph](#). Results can subsequently be visualized with the function [plotGeneralEnrichment](#).

**Usage**

```
calculateGeneralEnrichment(
  GRN,
  ontology = c("GO_BP", "GO_MF"),
  algorithm = "weight01",
  statistic = "fisher",
  background = "neighborhood",
  background_geneTypes = "all",
  pAdjustMethod = "BH",
  forceRerun = FALSE
)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
ontology	Character vector of ontologies. Default c("GO_BP", "GO_MF"). Valid values are "GO_BP", "GO_MF", "GO_CC", "KEGG", "DO", and "Reactome", referring to <i>GO Biological Process</i> , <i>GO Molecular Function</i> , <i>GO Cellular Component</i> , <i>KEGG Disease Ontology</i> , and <i>Reactome Pathways</i> , respectively. GO ontologies require the topGO, "KEGG" the clusterProfiler, "DO" the DOSE, and "Reactome" the ReactomePA packages, respectively. As they are listed under Suggests, they may not yet be installed, and the function will throw an error if they are missing.
algorithm	Character. Default "weight01". One of: "classic", "elim", "weight", "weight01", "lea", "parentchild". Only relevant if ontology is GO related (GO_BP, GO_MF, GO_CC), ignored otherwise. Name of the algorithm that handles the GO graph structures. Valid inputs are those supported by the topGO library. For general information about the algorithms, see <a href="https://academic.oup.com/bioinformatics/article/22/13/1600/193669">https://academic.oup.com/bioinformatics/article/22/13/1600/193669</a> . weight01 is a mixture between the elim and the weight algorithms.
statistic	Character. Default "fisher". One of: "fisher", "ks", "t", "globaltest", "sum", "ks.ties". Statistical test to be used. Only relevant if ontology is GO related (GO_BP, GO_MF, GO_CC), and valid inputs are those supported by the topGO library, ignored otherwise. For the other ontologies the test statistic is always Fisher.
background	Character. Default "neighborhood". One of: "all_annotated", "all_RNA", "all_RNA_filtered", "neighborhood". Set of genes to be used to construct the background for the enrichment analysis. This can either be all annotated genes in the reference genome (all_annotated), all genes from the provided RNA data (all_RNA), all genes from the provided RNA data excluding those marked as filtered after executing filterData (all_RNA_filtered), or all the genes that are within the neighborhood of any peak (before applying any filters except for the user-defined promoterRange value in addConnections_peak_gene) (neighborhood).
background_geneTypes	Character vector of gene types that should be considered for the background. Default "all". Only gene types as defined in the <a href="#">GRN</a> object, slot GRN@annotation\$genes\$gene.type are allowed. The special keyword "all" means no filter on gene type.

pAdjustMethod	Character. Default "BH". One of: "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr". This parameter is only relevant for the following ontologies: KEGG, DO, Reactome. For the other ontologies, the algorithm serves as an adjustment.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

### Details

All enrichment functions use the TF-gene graph as defined in the 'GRN' object. See the 'ontology' argument for currently supported ontologies. Also note that some parameter combinations for 'algorithm' and 'statistic' are incompatible, an error message will be thrown in such a case.

### Value

An updated [GRN](#) object, with the enrichment results stored in the stats\$Enrichment\$general slot.

### See Also

[plotGeneralEnrichment](#)  
[calculateCommunitiesEnrichment](#)  
[calculateTFEnrichment](#)  
[plotCommunitiesEnrichment](#)

### Examples

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = calculateGeneralEnrichment(GRN, ontology = "GO_BP", forceRerun = FALSE)
```

---

`calculateTFEnrichment` *Run an enrichment analysis for the set of genes connected to a particular TF or sets of TFs in the filtered GRN object*

---

### Description

The enrichment analysis is based on the subset of the network connected to particular TFs (TF regulons), see [calculateCommunitiesEnrichment](#) and [calculateGeneralEnrichment](#) for community- and general enrichment, respectively. This function requires the existence of the eGRN graph in the [GRN](#) object as produced by [build\\_eGRN\\_graph](#). Results can subsequently be visualized with the function [plotTFEnrichment](#).

**Usage**

```
calculateTFEnrichment(
  GRN,
  rankType = "degree",
  n = 3,
  TF.names = NULL,
  ontology = c("GO_BP", "GO_MF"),
  algorithm = "weight01",
  statistic = "fisher",
  background = "neighborhood",
  background_geneTypes = "all",
  pAdjustMethod = "BH",
  forceRerun = FALSE
)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
rankType	Character. Default "degree". One of: "degree", "EV", "custom". This parameter will determine the criterion to be used to identify the "top" TFs. If set to "degree", the function will select top TFs based on the number of connections to genes they have, i.e. based on their degree-centrality. If set to "EV" it will select the top TFs based on their eigenvector-centrality score in the network. If set to custom, a set of TF names will have to be passed to the "TF.names" parameter.
n	Numeric. Default 3. If this parameter is passed as a value between 0 and 1, it is treated as a percentage of top nodes. If the value is passed as an integer it will be treated as the number of top nodes. This parameter is not relevant if rankType = "custom".
TF.names	Character vector. Default NULL. If the rank type is set to "custom", a vector of TF names for which the GO enrichment should be calculated should be passed to this parameter.
ontology	Character vector of ontologies. Default c("GO_BP", "GO_MF"). Valid values are "GO_BP", "GO_MF", "GO_CC", "KEGG", "DO", and "Reactome", referring to <i>GO Biological Process</i> , <i>GO Molecular Function</i> , <i>GO Cellular Component</i> , <i>KEGG Disease Ontology</i> , and <i>Reactome Pathways</i> , respectively. GO ontologies require the topGO, "KEGG" the clusterProfiler, "DO" the DOSE, and "Reactome" the ReactomePA packages, respectively. As they are listed under Suggests, they may not yet be installed, and the function will throw an error if they are missing.
algorithm	Character. Default "weight01". One of: "classic", "elim", "weight", "weight01", "lea", "parentchild". Only relevant if ontology is GO related (GO_BP, GO_MF, GO_CC), ignored otherwise. Name of the algorithm that handles the GO graph structures. Valid inputs are those supported by the topGO library. For general information about the algorithms, see <a href="https://academic.oup.com/bioinformatics/article/22/13/1600/193669">https://academic.oup.com/bioinformatics/article/22/13/1600/193669</a> . weight01 is a mixture between the elim and the weight algorithms.
statistic	Character. Default "fisher". One of: "fisher", "ks", "t", "globaltest", "sum", "ks.ties". Statistical test to be used. Only relevant if ontology is GO

	related (GO_BP, GO_MF, GO_CC), and valid inputs are those supported by the topGO library, ignored otherwise. For the other ontologies the test statistic is always Fisher.
background	Character. Default "neighborhood". One of: "all_annotated", "all_RNA", "all_RNA_filtered", "neighborhood". Set of genes to be used to construct the background for the enrichment analysis. This can either be all annotated genes in the reference genome (all_annotated), all genes from the provided RNA data (all_RNA), all genes from the provided RNA data excluding those marked as filtered after executing filterData (all_RNA_filtered), or all the genes that are within the neighborhood of any peak (before applying any filters except for the user-defined promoterRange value in addConnections_peak_gene) (neighborhood).
background_geneTypes	Character vector of gene types that should be considered for the background. Default "all". Only gene types as defined in the GRN object, slot GRN@annotation\$genes\$gene.type are allowed. The special keyword "all" means no filter on gene type.
pAdjustMethod	Character. Default "BH". One of: "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr". This parameter is only relevant for the following ontologies: KEGG, DO, Reactome. For the other ontologies, the algorithm serves as an adjustment.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

## Details

All enrichment functions use the TF-gene graph as defined in the 'GRN' object. See the 'ontology' argument for currently supported ontologies. Also note that some parameter combinations for 'algorithm' and 'statistic' are incompatible, an error message will be thrown in such a case.

## Value

An updated GRN object, with the enrichment results stored in the stats\$Enrichment\$byTF slot.

## See Also

[plotTFEnrichment](#)

## Examples

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = calculateTFEnrichment(GRN, n = 5, ontology = "GO_BP", forceRerun = FALSE)
```



---

changeOutputDirectory *Change the output directory of a GRN object*

---

**Description**

Change the output directory of a GRN object

**Usage**

```
changeOutputDirectory(GRN, outputDirectory = ".")
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
outputDirectory	Character. Default <code>..</code> . New output directory for all output files unless overwritten via the parameter <code>outputFolder</code> .

**Value**

An updated [GRN](#) object, with the output directory being adjusted accordingly

**Examples**

```
GRN = loadExampleObject()
GRN = changeOutputDirectory(GRN, outputDirectory = ".")
```

---

deleteIntermediateData

*Optional convenience function to delete intermediate data from the function [AR\\_classification\\_wrapper](#) and summary statistics that may occupy a lot of space*

---

**Description**

Optional convenience function to delete intermediate data from the function [AR\\_classification\\_wrapper](#) and summary statistics that may occupy a lot of space

**Usage**

```
deleteIntermediateData(GRN)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
-----	-------------------------------------

**Value**

An updated [GRN](#) object, with some slots being deleted (GRN@data\$TFs\$classification as well as GRN@stats\$connectionDetails.l)

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = deleteIntermediateData(GRN)
```

---

 filterData

---

*Filter RNA-seq and/or peak data from a [GRN](#) object*


---

**Description**

This function marks genes and/or peaks as filtered depending on the chosen filtering criteria and is based on the count data AFTER potential normalization as chosen when using the [addData](#) function. Most of the filters may not be meaningful and useful anymore to apply after using particular normalization schemes that can give rise to, for example, negative values such as cyclic loess normalization. If normalized counts do not represent counts anymore but rather a deviation from a mean or something a like, the filtering criteria usually do not make sense anymore. Filtered genes / peaks will then be disregarded when adding connections in subsequent steps via [addConnections\\_TF\\_peak](#) and [addConnections\\_peak\\_gene](#). **This function does NOT (re)filter existing connections when the GRN object already contains connections. Thus, upon re-execution of this function with different filtering criteria, all downstream steps have to be re-run.**

**Usage**

```
filterData(
  GRN,
  minNormalizedMean_peaks = NULL,
  maxNormalizedMean_peaks = NULL,
  minNormalizedMeanRNA = NULL,
  maxNormalizedMeanRNA = NULL,
  chrToKeep_peaks = NULL,
  minSize_peaks = 20,
  maxSize_peaks = 10000,
  minCV_peaks = NULL,
  maxCV_peaks = NULL,
  minCV_genes = NULL,
  maxCV_genes = NULL,
  forceRerun = FALSE
)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
minNormalizedMean_peaks	Numeric[0,] or NULL. Default 5. Minimum mean across all samples for a peak to be retained for the normalized counts table. Set to NULL for not applying the filter. Be aware that depending on the chosen normalization, this filter may not make sense and should NOT be applied. See the notes for this function.
maxNormalizedMean_peaks	Numeric[0,] or NULL. Default NULL. Maximum mean across all samples for a peak to be retained for the normalized counts table. Set to NULL for not applying the filter. Be aware that depending on the chosen normalization, this filter may not make sense and should NOT be applied. See the notes for this function.
minNormalizedMeanRNA	Numeric[0,] or NULL. Default 5. Minimum mean across all samples for a gene to be retained for the normalized counts table. Set to NULL for not applying the filter. Be aware that depending on the chosen normalization, this filter may not make sense and should NOT be applied. See the notes for this function.
maxNormalizedMeanRNA	Numeric[0,] or NULL. Default NULL. Maximum mean across all samples for a gene to be retained for the normalized counts table. Set to NULL for not applying the filter. Be aware that depending on the chosen normalization, this filter may not make sense and should NOT be applied. See the notes for this function.
chrToKeep_peaks	Character vector or NULL. Default NULL. Vector of chromosomes that peaks are allowed to come from. This filter can be used to filter sex chromosomes from the peaks, for example (e.g, c(paste0("chr", 1:22), "chrX", "chrY"))
minSize_peaks	Integer[1,] or NULL. Default 20. Minimum peak size (width, end - start) for a peak to be retained. Set to NULL for not applying the filter.
maxSize_peaks	Integer[1,] or NULL. Default 10000. Maximum peak size (width, end - start) for a peak to be retained. Set to NULL for not applying the filter.
minCV_peaks	Numeric[0,] or NULL. Default NULL. Minimum CV (coefficient of variation, a unitless measure of variation) for a peak to be retained. Set to NULL for not applying the filter. Be aware that depending on the chosen normalization, this filter may not make sense and should NOT be applied. See the notes for this function.
maxCV_peaks	Numeric[0,] or NULL. Default NULL. Maximum CV (coefficient of variation, a unitless measure of variation) for a peak to be retained. Set to NULL for not applying the filter. Be aware that depending on the chosen normalization, this filter may not make sense and should NOT be applied. See the notes for this function.
minCV_genes	Numeric[0,] or NULL. Default NULL. Minimum CV (coefficient of variation, a unitless measure of variation) for a gene to be retained. Set to NULL for not applying the filter. Be aware that depending on the chosen normalization, this filter may not make sense and should NOT be applied. See the notes for this function.

maxCV_genes	Numeric[0,] or NULL. Default NULL. Maximum CV (coefficient of variation, a unitless measure of variation) for a gene to be retained. Set to NULL for not applying the filter. Be aware that depending on the chosen normalization, this filter may not make sense and should NOT be applied. See the notes for this function.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

### Details

All this function does is setting (or modifying) the filtering flag in `GRN@data$peaks$counts_metadata` and `GRN@data$RNA$counts_metadata`, respectively.

### Value

An updated [GRN](#) object, with added data from this function.

### Examples

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = filterData(GRN, forceRerun = FALSE)
```

---

filterGRNAndConnectGenes

*Filter TF-peaks and peak-gene connections and combine them to TF-peak-gene connections to construct an eGRN.*

---

### Description

This is one of the main integrative functions of the GRaNIE package. It has two main functions: First, filtering both TF-peak and peak-gene connections according to different criteria such as FDR and other properties. Second, joining the three major elements that an eGRN consist of (TFs, peaks, genes) into one data frame, with one row per unique TF-peak-gene connection. **After successful execution, the connections (along with additional feature metadata) can be retrieved with the function `getGRNConnections`. Note that a previously stored eGRN graph is reset upon successful execution of this function along with printing a descriptive warning, and re-running the function `build_eGRN_graph` is necessary when any of the network functions of the package shall be executed. If the filtered connections changed, all network related enrichment functions also have to be rerun.** Internally, before joining them, both TF-peak links and peak-gene connections are filtered separately for reasons of memory and computational efficacy: First filtering out unwanted links dramatically reduces the memory needed for the full eGRN. Peak-gene p-value adjustment is only done after all filtering steps on the remaining set of connections to lower the statistical burden of multiple-testing adjustment; therefore, this may lead to initially counter-intuitive effects such as a particular connections not being included anymore as compared to a filtering based on different thresholds, or the FDR being different for the same reason.

**Usage**

```

filterGRNAndConnectGenes(
  GRN,
  TF_peak.fdr.threshold = 0.2,
  TF_peak.connectionTypes = "all",
  peak_gene.p_raw.threshold = NULL,
  peak_gene.fdr.threshold = 0.2,
  peak_gene.fdr.method = "BH",
  peak_gene.IHW.covariate = NULL,
  peak_gene.IHW.nbins = "auto",
  gene.types = c("protein_coding"),
  allowMissingTFs = FALSE,
  allowMissingGenes = TRUE,
  peak_gene.r_range = c(0, 1),
  peak_gene.selection = "all",
  peak_gene.maxDistance = NULL,
  filterTFs = NULL,
  filterGenes = NULL,
  filterPeaks = NULL,
  TF_peak_FDR_selectViaCorBins = FALSE,
  filterLoops = TRUE,
  outputFolder = NULL,
  resetGraphAndStoreInternally = TRUE,
  silent = FALSE
)

```

**Arguments**

**GRN** Object of class [GRN](#)

**TF\_peak.fdr.threshold** Numeric[0,1]. Default 0.2. Maximum FDR for the TF-peak links. Set to 1 or NULL to disable this filter.

**TF\_peak.connectionTypes** Character vector. Default all. TF-peak connection types to consider. The special keyword all denotes all connection types (e.g., expression and TFActivity) that are found in the [GRN](#) object. By default, only expression is present in the object, so all and expression are usually equivalent unless calculation of TF-peak links based on TF activity has also been enabled.

**peak\_gene.p\_raw.threshold** Numeric[0,1]. Default NULL. Threshold for the peak-gene connections, based on the raw p-value. All peak-gene connections with a larger raw p-value will be filtered out.

**peak\_gene.fdr.threshold** Numeric[0,1]. Default 0.2. Threshold for the peak-gene connections, based on the FDR. All peak-gene connections with a larger FDR will be filtered out.

**peak\_gene.fdr.method** Character. Default "BH". One of: "holm", "hochberg", "hommel", "bonferoni", "BH", "BY", "fdr", "none", "IHW". Method for adjusting p-values for

multiple testing. If set to "IHW", the package IHW is required (as it is listed under Suggests, it may not be installed), and independent hypothesis weighting will be performed, and a suitable covariate has to be specified for the parameter `peak_gene.IHW.covariate`.

<code>peak_gene.IHW.covariate</code>	Character. Default NULL. Name of the covariate to use for IHW (column name from the table that is returned with the function <code>getGRNConnections</code> ). Only relevant if <code>peak_gene.fdr.method</code> is set to "IHW". You have to make sure the specified covariate is suitable or IHW, see the diagnostic plots that are generated in this function for this. For many datasets, the peak-gene distance (called <code>peak_gene.distance</code> in the object) seems suitable.
<code>peak_gene.IHW.nbins</code>	Integer or "auto". Default "auto". Number of bins for IHW. Only relevant if <code>peak_gene.fdr.method</code> is set to "IHW".
<code>gene.types</code>	Character vector of supported gene types. Default <code>c("protein_coding", "lincRNA")</code> . Filter for gene types to retain, genes with gene types not listed here are filtered. The special keyword "all" indicates no filter and retains all gene types. The specified names must match the names as stored in the GRN object (see <code>GRN@annotation\$genes\$gene.type</code> ) and correspond 1:1 to the gene type names as provided by biomaRt, with the exception of lincRNAs, which is internally renamed to lincRNAs when first fetching all gene types. This is done due to a recent change in biomaRt and aims at keeping backwards compatibility with GRN objects.
<code>allowMissingTFs</code>	TRUE or FALSE. Default FALSE. Should connections be returned for which the TF is NA (i.e., connections consisting only of peak-gene links?). If set to TRUE, this generally greatly increases the number of connections but it may not be what you aim for.
<code>allowMissingGenes</code>	TRUE or FALSE. Default TRUE. Should connections be returned for which the gene is NA (i.e., connections consisting only of TF-peak links?). If set to TRUE, this generally increases the number of connections.
<code>peak_gene.r_range</code>	Numeric(2). Default <code>c(0,1)</code> . Filter for lower and upper limit for the peak-gene links. Only links will be retained if the correlation coefficient is within the specified interval. This filter is usually used to filter out negatively correlated peak-gene links.
<code>peak_gene.selection</code>	"all" or "closest". Default "all". Filter for the selection of genes for each peak. If set to "all", all previously identified peak-gene are used, while "closest" only retains the closest gene for each peak that is retained until the point the filter is applied.
<code>peak_gene.maxDistance</code>	Integer >0. Default NULL. Maximum peak-gene distance to retain a peak-gene connection.
<code>filterTFs</code>	Character vector. Default NULL. Vector of TFs (as named in the GRN object) to retain. All TFs not listed will be filtered out.

filterGenes	Character vector. Default NULL. Vector of gene IDs (as named in the GRN object) to retain. All genes not listed will be filtered out.
filterPeaks	Character vector. Default NULL. Vector of peak IDs (as named in the GRN object) to retain. All peaks not listed will be filtered out.
TF_peak_FDR_selectViaCorBins	TRUE or FALSE. Default FALSE. Use a modified procedure for selecting TF-peak links that is based on the user-specified FDR but that retains also links that may have a higher FDR but a more extreme correlation.
filterLoops	TRUE or FALSE. Default TRUE. If a TF regulates itself (i.e., the TF and the gene are the same entity), should such loops be filtered from the GRN?
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
resetGraphAndStoreInternally	TRUE or FALSE. Default TRUE. If set to TRUE, the stored eGRN graph (slot graph) is reset due to the potentially changed connections that would otherwise cause conflicts in the information stored in the object. Also, a GRN object is returned. If set to FALSE, only the new filtered connections are returned and the object is not altered.
silent	TRUE or FALSE. Default FALSE. Print progress messages and filter statistics.

### Value

An updated [GRN](#) object, with additional information added from this function. The filtered and merged TF-peak and peak-gene connections in the slot `GRN@connections$all.filtered` and can be retrieved (along with other feature metadata) using the function [getGRNConnections](#).

### See Also

[visualizeGRN](#)  
[addConnections\\_TF\\_peak](#)  
[addConnections\\_peak\\_gene](#)  
[build\\_eGRN\\_graph](#)  
[getGRNConnections](#)

### Examples

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = filterGRNAndConnectGenes(GRN)
```

---

`generateStatsSummary` *Generate a summary for the number of connections for different filtering criteria for a [GRN](#) object.*

---

### Description

This functions calls [filterGRNAndConnectGenes](#) repeatedly and stores the total number of connections and other statistics each time to summarize them afterwards. All arguments are identical to the ones in [filterGRNAndConnectGenes](#), see the help for this function for details. The function [plot\\_stats\\_connectionSummary](#) can be used afterwards for plotting.

### Usage

```
generateStatsSummary(
  GRN,
  TF_peak.fdr = c(0.001, 0.01, 0.05, 0.1, 0.2),
  TF_peak.connectionTypes = "all",
  peak_gene.fdr = c(0.001, 0.01, 0.05, 0.1, 0.2),
  peak_gene.r_range = c(0, 1),
  gene.types = c("protein_coding"),
  allowMissingGenes = c(FALSE, TRUE),
  allowMissingTFs = c(FALSE),
  forceRerun = FALSE
)
```

### Arguments

<code>GRN</code>	Object of class <a href="#">GRN</a>
<code>TF_peak.fdr</code>	Numeric vector[0,1]. Default <code>c(0.001, 0.01, 0.05, 0.1, 0.2)</code> . TF-peak FDR values to iterate over.
<code>TF_peak.connectionTypes</code>	Character vector. Default <code>all</code> . TF-peak connection types to consider. The special keyword <code>all</code> denotes all connection types (e.g., <code>expression</code> and <code>TFActivity</code> ) that are found in the <a href="#">GRN</a> object. By default, only <code>expression</code> is present in the object, so <code>all</code> and <code>expression</code> are usually equivalent unless calculation of TF-peak links based on TF activity has also been enabled.
<code>peak_gene.fdr</code>	Numeric vector[0,1]. Default <code>c(0.001, 0.01, 0.05, 0.1, 0.2)</code> . Peak-gene FDR values to iterate over.
<code>peak_gene.r_range</code>	Numeric vector of length 2[-1,1]. Default <code>c(0,1)</code> . The correlation range of peak-gene connections to keep.
<code>gene.types</code>	Character vector of supported gene types. Default <code>c("protein_coding", "lincRNA")</code> . Filter for gene types to retain, genes with gene types not listed here are filtered. The special keyword <code>"all"</code> indicates no filter and retains all gene types. The specified names must match the names as stored in the <a href="#">GRN</a> object (see <code>GRN@annotation\$genes\$gene.type</code> ) and correspond 1:1 to the gene type names



as provided by biomaRt, with the exception of lincRNAs, which is internally re-named to lincRNAs when first fetching all gene types. This is done due to a recent change in biomaRt and aims at keeping backwards compatibility with [GRN](#) objects.

allowMissingGenes

Logical vector of length 1 or 2. Default `c(FALSE, TRUE)`. Allow genes to be missing for peak-gene connections? If both FALSE and TRUE are given, the code loops over both

allowMissingTFs

Logical vector of length 1 or 2. Default `c(FALSE)`. Allow TFs to be missing for TF-peak connections? If both FALSE and TRUE are given, the code loops over both

forceRerun

TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

### Value

An updated [GRN](#) object, with additional information added from this function.

### See Also

[plot\\_stats\\_connectionSummary](#)

[filterGRNAndConnectGenes](#)

### Examples

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = generateStatsSummary(GRN, TF_peak.fdr = c(0.01, 0.1), peak_gene.fdr = c(0.01, 0.1))
```

---

getCounts

*Get counts for the various data defined in a [GRN](#) object*

---

### Description

Get counts for the various data defined in a [GRN](#) object. **Note: This function, as all get functions from this package, does NOT return a [GRN](#) object.**

Get counts for the various data defined in a [GRN](#) object. **Note: This function, as all get functions from this package, does NOT return a [GRN](#) object.**

**Usage**

```
getCounts(
  GRN,
  type,
  permuted = FALSE,
  asMatrix = FALSE,
  includeIDColumn = TRUE,
  includeFiltered = FALSE
)
```

```
getCounts(
  GRN,
  type,
  permuted = FALSE,
  asMatrix = FALSE,
  includeIDColumn = TRUE,
  includeFiltered = FALSE
)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
type	Character. Either peaks or rna. peaks corresponds to the counts for the open chromatin data, while rna refers to the RNA-seq counts. If set to rna, both permuted and non-permuted data can be retrieved, while for peaks, only the non-permuted one (i.e., 0) can be retrieved.
permuted	TRUE or FALSE. Default FALSE. Should the permuted data be taken (TRUE) or the non-permuted, original one (FALSE)?
asMatrix	Logical. TRUE or FALSE. Default FALSE. If set to FALSE, counts are returned as a data frame with or without an ID column (see includeIDColumn). If set to TRUE, counts are returned as a matrix with the ID column as row names.
includeIDColumn	Logical. TRUE or FALSE. Default TRUE. Only relevant if asMatrix = FALSE. If set to TRUE, an explicit ID column is returned (no row names). If set to FALSE, the IDs are in the row names instead.
includeFiltered	Logical. TRUE or FALSE. Default FALSE. If set to FALSE, genes or peaks marked as filtered (after running the function filterData) will not be returned. If set to TRUE, all elements are returned regardless of the currently active filter status.

**Value**

Data frame of counts, with the type as indicated by the function parameters. This function does **NOT** return a [GRN](#) object.

Data frame of counts, with the type as indicated by the function parameters. This function does **NOT** return a [GRN](#) object.

## Examples

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
counts.df = getCounts(GRN, type = "peaks", permuted = FALSE)
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
counts.df = getCounts(GRN, type = "peaks", permuted = FALSE)
```

---

getGRNConnections	<i>Extract connections or links from a GRN object as da data frame.</i>
-------------------	---

---

## Description

Returns stored connections/links (either TF-peak, peak-genes or the filtered set of connections as produced by [filterGRNAndConnectGenes](#)). **Note: This function, as all get functions from this package, does NOT return a GRN object.**

Returns stored connections/links (either TF-peak, peak-genes or the filtered set of connections as produced by [filterGRNAndConnectGenes](#)). **Note: This function, as all get functions from this package, does NOT return a GRN object.**

## Usage

```
getGRNConnections(  
  GRN,  
  type = "all.filtered",  
  permuted = FALSE,  
  include_TF_gene_correlations = FALSE,  
  include_TFMetadata = FALSE,  
  include_peakMetadata = FALSE,  
  include_geneMetadata = FALSE,  
  include_variancePartitionResults = FALSE  
)
```

```
getGRNConnections(  
  GRN,  
  type = "all.filtered",  
  permuted = FALSE,  
  include_TF_gene_correlations = FALSE,  
  include_TFMetadata = FALSE,  
  include_peakMetadata = FALSE,  
  include_geneMetadata = FALSE,  
  include_variancePartitionResults = FALSE  
)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
type	Character. One of TF_peaks, peak_genes, TF_genes or all.filtered. Default all.filtered. The type of connections to retrieve.
permuted	TRUE or FALSE. Default FALSE. Should the permuted data be taken (TRUE) or the non-permuted, original one (FALSE)?
include_TF_gene_correlations	Logical. TRUE or FALSE. Default FALSE. Should TFs and gene correlations be returned as well? If set to TRUE, they must have been computed beforehand with <a href="#">add_TF_gene_correlation</a> . Only relevant for type = "all.filtered"
include_TFMetadata	Logical. TRUE or FALSE. Default FALSE. Should TF metadata be returned as well? Only relevant for type = "all.filtered"
include_peakMetadata	Logical. TRUE or FALSE. Default FALSE. Should peak metadata be returned as well? Only relevant for type = "all.filtered"
include_geneMetadata	Logical. TRUE or FALSE. Default FALSE. Should gene metadata be returned as well? Only relevant for type = "all.filtered"
include_variancePartitionResults	Logical. TRUE or FALSE. Default FALSE. Should the results from the function <a href="#">add_featureVariation</a> be included? If set to TRUE, they must have been computed beforehand with <a href="#">add_featureVariation</a> ; otherwise, an error is thrown. Only relevant for type = "all.filtered"

**Value**

A data frame with the requested connections. This function does **\*\*NOT\*\*** return a [GRN](#) object.

A data frame with the requested connections. This function does **\*\*NOT\*\*** return a [GRN](#) object.

**See Also**

[filterGRNAndConnectGenes](#)  
[add\\_featureVariation](#)  
[add\\_TF\\_gene\\_correlation](#)  
[filterGRNAndConnectGenes](#)  
[add\\_featureVariation](#)  
[add\\_TF\\_gene\\_correlation](#)

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN_con.all.df = getGRNConnections(GRN)
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN_con.all.df = getGRNConnections(GRN)
```

---

getParameters	<i>Retrieve parameters for previously used function calls and general parameters for a <a href="#">GRN</a> object.</i>
---------------	--

---

### Description

**Note: This function, as all get functions from this package, does NOT return a [GRN](#) object.**

### Usage

```
getParameters(GRN, type = "parameter", name = "all")
```

### Arguments

GRN	Object of class <a href="#">GRN</a>
type	Character. Either function or parameter. Default parameter. When set to function, a valid GRaNIE function name must be given that has been run before. When set to parameter, in combination with name, returns a specific parameter (as specified in GRN@config).
name	Character. Default all. Name of parameter or function name to retrieve. Set to the special keyword all to retrieve all parameters.

### Value

The requested parameters. This function does **\*\*NOT\*\*** return a [GRN](#) object.

### Examples

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
params.l = getParameters(GRN, type = "parameter", name = "all")
```

---

getTopNodes	<i>Retrieve the top nodes (TFs or genes) with respect to either degree or Eigenvector centrality in the filtered <a href="#">GRN</a> object.</i>
-------------	--

---

### Description

This function requires a filtered set of connections in the [GRN](#) object as generated by [filterGRNAndConnectGenes](#).

**Note: This function, as all get functions from this package, does NOT return a [GRN](#) object.**

### Usage

```
getTopNodes(GRN, nodeType, rankType, n = 0.1, use_TF_gene_network = TRUE)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
nodeType	Character. One of: "gene" or "TF". Node type.
rankType	Character. One of: "degree", "EV". This parameter will determine the criterion to be used to identify the "top" nodes. If set to "degree", the function will select top nodes based on the number of connections they have, i.e. based on their degree-centrality. If set to "EV" it will select the top nodes based on their eigenvector-centrality score in the network.
n	Numeric. Default 0.1. If this parameter is passed as a value between [0,1], it is treated as a percentage of top nodes. If the value is passed as an integer $\geq 1$ it will be treated as the number of top nodes.
use_TF_gene_network	TRUE or FALSE. Default TRUE. Should the TF-gene network be used (TRUE) or the TF-peak-gene network (FALSE)?

**Value**

A data frame with the node names and the corresponding scores used to rank them

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
topGenes = getTopNodes(GRN, nodeType = "gene", rankType = "degree", n = 3)
topTFs = getTopNodes(GRN, nodeType = "TF", rankType = "EV", n = 5)
```

---

GRaNIE	<b>GRaNIE</b> ( <i>Gene Regulatory Network Inference including Enhancers</i> ): Reconstruction and evaluation of data-driven, cell type specific gene regulatory networks including enhancers using chromatin accessibility and RNAseq data (general package information)
--------	---

---

**Description**

Genetic variants associated with diseases often affect non-coding regions, thus likely having a regulatory role. To understand the effects of genetic variants in these regulatory regions, identifying genes that are modulated by specific regulatory elements (REs) is crucial. The effect of gene regulatory elements, such as enhancers, is often cell-type specific, likely because the combinations of transcription factors (TFs) that are regulating a given enhancer have celltype specific activity. This TF activity can be quantified with existing tools such as `diffTF` and captures differences in binding of a TF in open chromatin regions. Collectively, this forms a gene regulatory network (eGRN) with cell-type and data-specific TF-RE and RE-gene links. Here, we reconstruct such a eGRN using bulk RNAseq and open chromatin (e.g., using ATACseq or ChIPseq for open chromatin marks) and optionally TF activity data. Our network contains different types of links, connecting TFs to regulatory elements, the latter of which is connected to genes in the vicinity or within the same chromatin domain (TAD). We use a statistical framework to assign empirical FDRs and weights to all links using a permutation-based approach.

## Package functions

See the Vignettes for a workflow example and more generally <https://grp-zaugg.embl-community.io/GRaNIE> for all project-related information.

## GRN object

The GRaNIE package works with GRN objects. See [GRN](#) for details.

## Contact Information

Please check out <https://grp-zaugg.embl-community.io/GRaNIE> for how to get in contact with us.

---

GRN-class	<i>Create, represent, investigate, quantify and visualize enhancer-mediated gene regulatory networks (eGRNs)</i>
-----------	--

---

## Description

The class [GRN](#) stores data and information related to our eGRN approach to construct enhancer-mediated gene regulatory networks out of open chromatin and RNA-Seq data. See the description below for more details, and **visit our project website at <https://grp-zaugg.embl-community.io/GRaNIE> and have a look at the various Vignettes.**

## Slots

data Currently stores 4 different types of data:

- peaks:
  - counts:
  - counts\_metadata:
- RNA:
  - counts:
  - counts\_metadata:
  - counts\_permuted\_index:
- TFs:
  - TF\_activity:
  - TF\_peak\_overlap:
  - classification:

config Contains general configuration data and parameters such as parameters, files, directories, flags, and recorded function parameters.

connections Stores various types of connections

annotation Stores annotation data for peaks and genes

stats Stores statistical and summary information for a GRN network. Currently, connection details are stored here.

graph Stores the eGRN graph related information and data structures

## Constructors

Currently, a [GRN](#) object is created by executing the function `initializeGRN`.

## Accessors

In the following code snippets, GRN is a [GRN](#) object.

```
# Get general annotation of a GRN object from the GRaNIE package
nPeaks(GRN), nTFs(GRN) and nGenes(GRN): Retrieve the number of peaks, TFs and genes,
respectively, that have been added to the object (both before and after filtering)
```

---

```
importTFData          Import externally derived TF Activity data. EXPERIMENTAL.
```

---

## Description

We do not yet provide full support for this function. It is currently being tested. Use at our own risk.

## Usage

```
importTFData(
  GRN,
  data,
  name,
  idColumn = "ENSEMBL",
  nameColumn = "TF.name",
  normalization = "none",
  forceRerun = FALSE
)
```

## Arguments

GRN	Object of class <a href="#">GRN</a>
data	Data frame. No default. Data with TF data.
name	Name in object under which it should be stored. This corresponds to the <code>connectionType</code> afterwards that some functions iterate over.
idColumn	Character. Default ENSEMBL. Name of the ID column. Must not be unique as some TFs may correspond to the same ID.
nameColumn	Character. Default TF.name. Must be unique for each TF / row.
normalization	Character. Default cyclicLoess. One of cyclicLoess, sizeFactors, quantile, or none. Normalization procedure. When set to cyclicLoess, the csaw package is required (as it is listed under Suggests, it may not be installed).
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.



**Value**

An updated [GRN](#) object, with added data from this function.

---

initializeGRN	<i>Create and initialize a <a href="#">GRN</a> object.</i>
---------------	--

---

**Description**

Executing this function is the very first step in the \*GRaNIE\* workflow. After its execution, data can be added to the object. **Depending on the genome assembly version, additional genome annotation packages are required, as follows:** For hg19 and hg38, the packages `org.Hs.eg.db` as well as `BSgenome.Hsapiens.UCSC.hg19+TxDb.Hsapiens.UCSC.hg19.knownGene` or `BSgenome.Hsapiens.UCSC.hg38+TxDb.Hsapiens.UCSC.hg38.knownGene` are required, respectively. For mm9 and mm10, the packages `org.Mm.eg.db` as well as `BSgenome.Mmusculus.UCSC.mm9+TxDb.Mmusculus.UCSC.mm9.knownGene` or `BSgenome.Mmusculus.UCSC.mm10+TxDb.Mmusculus.UCSC.mm10.knownGene` are required, respectively. For more information, see the error message if any of these packages is missing or the [Package Details Vignette](#).

**Usage**

```
initializeGRN(objectMetadata = list(), outputFolder = ".", genomeAssembly)
```

**Arguments**

objectMetadata	List. Default <code>list()</code> . Optional (named) list with an arbitrary number of elements, all of which capture metadata for the object. <b>Only atomic data types are allowed for each list element (see <code>?is.atomic</code> for more help: logical, integer, numeric, complex, character, raw, and NULL), and this slot is not supposed to store real data.</b> This is mainly used to distinguish GRN objects from one another by storing object-specific metadata along with the data.
outputFolder	Output folder, either absolute or relative to the current working directory. Default <code>"."</code> . Default output folder where all pipeline output will be put unless specified otherwise. We recommend specifying an absolute path. Note that for Windows-based systems, the path must be correctly specified with <code>"\"</code> as path separator.
genomeAssembly	Character. No default. The genome assembly of all data that to be used within this object. Currently, supported genomes are: hg19, hg38, mm9 and mm10. See function description for further information and notes.

**Value**

Empty [GRN](#) object

**Examples**

```
meta.l = list(name = "exampleName", date = "01.03.22")
GRN = initializeGRN(objectMetadata = meta.l, outputFolder = "output", genomeAssembly = "hg38")
```

---

loadExampleObject	<i>Load example GRN dataset</i>
-------------------	---------------------------------

---

### Description

Loads an example GRN object with 6 TFs, ~61.000 peaks, ~19.000 genes, 259 filtered connections and pre-calculated enrichments. This function uses BiocFileCache if installed to cache the example object, which is considerably faster than re-downloading the file anew every time the function is executed. If not, the file is re-downloaded every time anew. Thus, to enable caching, you may install the package BiocFileCache.

### Usage

```
loadExampleObject(  
  forceDownload = FALSE,  
  fileURL = "https://git.embl.de/grp-zaugg/GRaNIE/-/raw/master/data/GRN.rds"  
)
```

### Arguments

**forceDownload** TRUE or FALSE. Default FALSE. Should the download be enforced even if the local cached file is already present?

**fileURL** Character. Default <https://git.embl.de/grp-zaugg/GRaNIE/-/raw/master/data/GRN.rds>. URL to the GRN example object in rds format.

### Value

An small example [GRN](#) object

### Examples

```
GRN = loadExampleObject()
```

---

nGenes	<i>Get the number of genes for a <a href="#">GRN</a> object.</i>
--------	--

---

### Description

Returns the number of genes (all or only non-filtered ones) from the provided RNA-seq data in the [GRN](#) object.

### Usage

```
nGenes(GRN, filter = TRUE)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
filter	TRUE or FALSE. Default TRUE. Should genes marked as filtered be included in the count?

**Value**

Integer. Number of genes that are defined in the [GRN](#) object, either by excluding (filter = TRUE) or including (filter = FALSE) genes that are currently marked as *filtered*.

**See Also**

[nTFs](#)  
[nPeaks](#)

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
nGenes(GRN, filter = TRUE)
nGenes(GRN, filter = FALSE)
```

---

nPeaks	<i>Get the number of peaks for a <a href="#">GRN</a> object.</i>
--------	--

---

**Description**

Returns the number of peaks (all or only non-filtered ones) from the provided peak data in the [GRN](#) object.

**Usage**

```
nPeaks(GRN, filter = TRUE)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
filter	TRUE or FALSE. Default TRUE. Should peaks marked as filtered be included in the count?

**Value**

Integer. Number of peaks that are defined in the [GRN](#) object, either by excluding (filter = TRUE) or including (filter = FALSE) peaks that are currently marked as *filtered*.

**See Also**[nTFs](#)[nGenes](#)**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
nPeaks(GRN, filter = TRUE)
nPeaks(GRN, filter = FALSE)
```

---

nTFs

*Get the number of TFs for a [GRN](#) object.*

---

**Description**

Returns the number of TFs from the provided TFBS data in the [GRN](#) object.

**Usage**

```
nTFs(GRN)
```

**Arguments**

GRN                      Object of class [GRN](#)

**Value**

Integer. Number of TFs that are defined in the [GRN](#) object.

**See Also**[nGenes](#)[nPeaks](#)**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
nTFs(GRN)
```

---

overlapPeaksAndTFBS     *Overlap peaks and TFBS for a GRN object*

---

### Description

Overlap peaks and TFBS for a [GRN](#) object

### Usage

```
overlapPeaksAndTFBS(GRN, nCores = 2, forceRerun = FALSE)
```

### Arguments

GRN	Object of class <a href="#">GRN</a>
nCores	Integer >0. Default 1. Number of cores to use. A value >1 requires the BiocParallel package (as it is listed under Suggests, it may not be installed yet).
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

### Value

An updated [GRN](#) object, with added data from this function (GRN@data\$TFs\$TF\_peak\_overlap in particular)

### Examples

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = overlapPeaksAndTFBS(GRN, nCores = 2, forceRerun = FALSE)
```

---

performAllNetworkAnalyses

*Perform all network-related statistical and descriptive analyses, including community and enrichment analyses. See the functions it executes in the @seealso section below.*

---

### Description

A convenience function that calls all network-related functions in one-go, using selected default parameters and a set of adjustable ones also. For full adjustment, run the individual functions separately. This function requires a filtered set of connections in the [GRN](#) object as generated by [filterGRNAndConnectGenes](#)

**Usage**

```
performAllNetworkAnalyses(
  GRN,
  ontology = c("GO_BP", "GO_MF"),
  algorithm = "weight01",
  statistic = "fisher",
  background = "neighborhood",
  clustering = "louvain",
  communities = seq_len(10),
  display = "byRank",
  topnGenes = 20,
  topnTFs = 20,
  maxWidth_nchar_plot = 50,
  display_pAdj = FALSE,
  outputFolder = NULL,
  forceRerun = FALSE
)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
ontology	Character vector of ontologies. Default c("GO_BP", "GO_MF"). Valid values are "GO_BP", "GO_MF", "GO_CC", "KEGG", "DO", and "Reactome", referring to <i>GO Biological Process</i> , <i>GO Molecular Function</i> , <i>GO Cellular Component</i> , <i>KEGG Disease Ontology</i> , and <i>Reactome Pathways</i> , respectively. GO ontologies require the topGO, "KEGG" the clusterProfiler, "DO" the DOSE, and "Reactome" the ReactomePA packages, respectively. As they are listed under Suggests, they may not yet be installed, and the function will throw an error if they are missing.
algorithm	Character. Default "weight01". One of: "classic", "elim", "weight", "weight01", "lea", "parentchild". Only relevant if ontology is GO related (GO_BP, GO_MF, GO_CC), ignored otherwise. Name of the algorithm that handles the GO graph structures. Valid inputs are those supported by the topGO library. For general information about the algorithms, see <a href="https://academic.oup.com/bioinformatics/article/22/13/1600/193669">https://academic.oup.com/bioinformatics/article/22/13/1600/193669</a> . weight01 is a mixture between the elim and the weight algorithms.
statistic	Character. Default "fisher". One of: "fisher", "ks", "t", "globaltest", "sum", "ks.ties". Statistical test to be used. Only relevant if ontology is GO related (GO_BP, GO_MF, GO_CC), and valid inputs are those supported by the topGO library, ignored otherwise. For the other ontologies the test statistic is always Fisher.
background	Character. Default "neighborhood". One of: "all_annotated", "all_RNA", "all_RNA_filtered", "neighborhood". Set of genes to be used to construct the background for the enrichment analysis. This can either be all annotated genes in the reference genome (all_annotated), all genes from the provided RNA data (all_RNA), all genes from the provided RNA data excluding those marked as filtered after executing filterData (all_RNA_filtered), or all the genes that are within the neighborhood of any peak (before applying any filters

	except for the user-defined promoterRange value in addConnections_peak_gene) (neighborhood).
clustering	Character. Default louvain. One of: louvain, leiden, leading_eigen, fast_greedy, optimal, walktrap. The community detection algorithm to be used. Please bear in mind the robustness and time consumption of the algorithms when opting for an alternative to the default.
communities	Numeric vector. Default seq_len(10). Depending on what was specified in the display parameter, this parameter would indicate either the rank or the label of the communities to be plotted. i.e. for communities = c(1,4), if display = "byRank" the results for the first and fourth largest communities will be plotted. if display = "byLabel", the results for the communities labeled "1", and "4" will be plotted. If set to NULL, all communities will be plotted
display	Character. Default "byRank". One of: "byRank", "byLabel". Specify whether the communities will be displayed based on their rank, where the largest community (with most vertices) would have a rank of 1, or by their label. Note that the label is independent of the rank.
topnGenes	Integer > 0. Default 20. Number of genes to plot, sorted by their rank or label.
topnTFs	Integer > 0. Default 20. Number of TFs to plot, sorted by their rank or label.
maxWidth_nchar_plot	Integer (>=10). Default 50. Maximum number of characters for a term before it is truncated.
display_pAdj	TRUE or FALSE. Default FALSE. Is the p-value being displayed in the plots the adjusted p-value? This parameter is relevant for KEGG, Disease Ontology, and Reactome enrichments, and does not affect GO enrichments.
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in link{initializeGRN} will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

**Value**

An updated [GRN](#) object, with added data from this function.

**See Also**

[build\\_eGRN\\_graph](#)  
[plotGeneralGraphStats](#)  
[calculateGeneralEnrichment](#)  
[plotGeneralEnrichment](#)  
[calculateCommunitiesStats](#)  
[plotCommunitiesStats](#)  
[calculateCommunitiesEnrichment](#)

```
plotCommunitiesEnrichment
calculateTFEnrichment
plotTFEnrichment
```

### Examples

```
# See the Workflow vignette on the GRaNIE website for examples
# GRN = loadExampleObject()
# GRN = performAllNetworkAnalyses(GRN, outputFolder = ".", forceRerun = FALSE)
```

---

```
plotCommunitiesEnrichment
```

*Plot community-based enrichment results for a filtered GRN object*

---

### Description

Similarly to [plotGeneralEnrichment](#) and [plotTFEnrichment](#), the results of the community-based enrichment analysis are plotted. This function produces multiple plots. First, one plot per community to summarize the community-specific enrichment. Second, a summary heatmap of all significantly enriched terms across all communities and for the whole eGRN. The latter allows to compare the results with the general network enrichment. Third, a subset of the aforementioned heatmap, showing only the top most significantly enriched terms per community and for the whole eGRN (as specified by nID) for improved visibility

### Usage

```
plotCommunitiesEnrichment(
  GRN,
  outputFolder = NULL,
  basenameOutput = NULL,
  display = "byRank",
  communities = NULL,
  topn_pvalue = 30,
  p = 0.05,
  nSignificant = 2,
  nID = 10,
  maxWidth_nchar_plot = 50,
  display_pAdj = FALSE,
  plotAsPDF = TRUE,
  pdf_width = 12,
  pdf_height = 12,
  pages = NULL,
  forceRerun = FALSE
)
```



**Arguments**

GRN	Object of class <a href="#">GRN</a>
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
basenameOutput	NULL or character. Default NULL. Basename of the output files that are produced. If set to NULL, a default basename is chosen. If a custom basename is specified, all output files will have the chosen basename as file prefix, be careful with not overwriting already existing files (if <code>forceRerun</code> is set to TRUE)
display	Character. Default "byRank". One of: "byRank", "byLabel". Specify whether the communities will be displayed based on their rank, where the largest community (with most vertices) would have a rank of 1, or by their label. Note that the label is independent of the rank.
communities	NULL or numeric vector. Default NULL. If set to NULL, the default, all communities enrichments that have been calculated before are plotted. If a numeric vector is specified: Depending on what was specified in the <code>display</code> parameter, this parameter indicates either the rank or the label of the communities to be plotted. i.e. for <code>communities = c(1, 4)</code> , if <code>display = "byRank"</code> the results for the first and fourth largest communities are plotted. if <code>display = "byLabel"</code> , the results for the communities labeled "1", and "4" are plotted.
topn_pvalue	Numeric. Default 30. Maximum number of ontology terms that meet the p-value significance threshold to display in the enrichment dot plot
p	Numeric. Default 0.05. p-value threshold to determine significance.
nSignificant	Numeric > 0. Default 3. Threshold to filter out an ontology term with less than nSignificant overlapping genes.
nID	Numeric > 0. Default 10. For the reduced summary heatmap, number of top terms to select per community / for the general enrichment.
maxWidth_nchar_plot	Integer (>=10). Default 50. Maximum number of characters for a term before it is truncated.
display_pAdj	TRUE or FALSE. Default FALSE. Is the p-value being displayed in the plots the adjusted p-value? This parameter is relevant for KEGG, Disease Ontology, and Reactome enrichments, and does not affect GO enrichments.
plotAsPDF	TRUE or FALSE. Default TRUE. Should the plots be printed to a PDF file? If set to TRUE, a PDF file is generated, the name of which depends on the value of <code>basenameOutput</code> . If set to FALSE, all plots are printed to the currently active device. Note that most functions print more than one plot, which means you may only see the last plot depending on your active graphics device.
pdf_width	Number>0. Default 12. Width of the PDF, in cm.
pdf_height	Number>0. Default 12. Height of the PDF, in cm.
pages	Integer vector or NULL. Default NULL. Page number(s) to plot. Can be used to plot only specific pages to a PDF or the currently active graphics device.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

**Value**

The same [GRN](#) object, without modifications.

**See Also**

[plotGeneralEnrichment](#)

[plotTFEnrichment](#)

[calculateCommunitiesEnrichment](#)

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = plotCommunitiesEnrichment(GRN, plotAsPDF = FALSE, pages = 1)
```

---

`plotCommunitiesStats` *Plot general structure & connectivity statistics for each community in a filtered [GRN](#)*

---

**Description**

Similarly to the statistics produced by [plotGeneralGraphStats](#), summaries regarding the vertex degrees and the most important vertices per community are generated. Note that the communities need to first be calculated using the [calculateCommunitiesStats](#) function

**Usage**

```
plotCommunitiesStats(  
  GRN,  
  outputFolder = NULL,  
  basenameOutput = NULL,  
  display = "byRank",  
  communities = seq_len(5),  
  topnGenes = 20,  
  topnTFs = 20,  
  plotAsPDF = TRUE,  
  pdf_width = 12,  
  pdf_height = 12,  
  pages = NULL,  
  forceRerun = FALSE  
)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
basenameOutput	NULL or character. Default NULL. Basename of the output files that are produced. If set to NULL, a default basename is chosen. If a custom basename is specified, all output files will have the chosen basename as file prefix, be careful with not overwriting already existing files (if <code>forceRerun</code> is set to TRUE)
display	Character. Default "byRank". One of: "byRank", "byLabel". Specify whether the communities will be displayed based on their rank, where the largest community (with most vertices) would have a rank of 1, or by their label. Note that the label is independent of the rank.
communities	Numeric vector. Default <code>seq_len(10)</code> . Depending on what was specified in the <code>display</code> parameter, this parameter would indicate either the rank or the label of the communities to be plotted. i.e. for <code>communities = c(1, 4)</code> , if <code>display = "byRank"</code> the results for the first and fourth largest communities will be plotted. if <code>display = "byLabel"</code> , the results for the communities labeled "1", and "4" will be plotted. If set to NULL, all communities will be plotted
topnGenes	Integer > 0. Default 20. Number of genes to plot, sorted by their rank or label.
topnTFs	Integer > 0. Default 20. Number of TFs to plot, sorted by their rank or label.
plotAsPDF	TRUE or FALSE. Default TRUE. Should the plots be printed to a PDF file? If set to TRUE, a PDF file is generated, the name of which depends on the value of <code>basenameOutput</code> . If set to FALSE, all plots are printed to the currently active device. Note that most functions print more than one plot, which means you may only see the last plot depending on your active graphics device.
pdf_width	Number > 0. Default 12. Width of the PDF, in cm.
pdf_height	Number > 0. Default 12. Height of the PDF, in cm.
pages	Integer vector or NULL. Default NULL. Page number(s) to plot. Can be used to plot only specific pages to a PDF or the currently active graphics device.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

**Value**

The same [GRN](#) object, without modifications.

**See Also**

[plotGeneralGraphStats](#)

[calculateCommunitiesStats](#)

[calculateCommunitiesEnrichment](#)

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = plotCommunitiesStats(GRN, plotAsPDF = FALSE, pages = 1)
```

---

```
plotDiagnosticPlots_peakGene
```

*Plot diagnostic plots for peak-gene connections for a [GRN object](#)*

---

**Description**

Plot diagnostic plots for peak-gene connections for a [GRN object](#)

**Usage**

```
plotDiagnosticPlots_peakGene(
  GRN,
  outputFolder = NULL,
  basenameOutput = NULL,
  gene.types = list(c("protein_coding", "lincRNA")),
  useFiltered = FALSE,
  plotDetails = FALSE,
  plotPerTF = FALSE,
  plotAsPDF = TRUE,
  pdf_width = 12,
  pdf_height = 12,
  pages = NULL,
  forceRerun = FALSE
)
```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
basenameOutput	NULL or character. Default NULL. Basename of the output files that are produced. If set to NULL, a default basename is chosen. If a custom basename is specified, all output files will have the chosen basename as file prefix, be careful with not overwriting already existing files (if <code>forceRerun</code> is set to TRUE)
gene.types	List of character vectors. Default <code>list(c("protein_coding", "lincRNA"))</code> . Vectors of gene types to consider for the diagnostic plots. Multiple distinct combinations of gene types can be specified. For example, if set to <code>list(c("protein_coding",</code>

"lincRNA"), c("protein\_coding"), c("all")), 3 distinct PDFs will be produced, one for each element of the list. The first file would only consider protein-coding and lincRNA genes, while the second plot only considers protein-coding ones. The special keyword "all" denotes all gene types found (usually, there are many gene types present, also more exotic and rare ones).

useFiltered	Logical. TRUE or FALSE. Default FALSE. If set to FALSE, the diagnostic plots will be produced based on all peak-gene connections. This is the default and will usually be best to judge whether the background behaves as expected. If set to TRUE, the diagnostic plots will be produced based on the filtered set of connections. For this, the function <code>link{filterGRNAndConnectGenes}</code> must have been run before.
plotDetails	TRUE or FALSE. Default FALSE. Print additional plots that may help for debugging and QC purposes? Note that these plots are currently less documented or not at all.
plotPerTF	Logical. TRUE or FALSE. Default FALSE. If set to FALSE, the diagnostic plots will be done across all TF (the default), while setting it to TRUE will generate the QC plots TF-specifically, including "all" TF, sorted by the number of connections.
plotAsPDF	TRUE or FALSE. Default TRUE. Should the plots be printed to a PDF file? If set to TRUE, a PDF file is generated, the name of which depends on the value of <code>basenameOutput</code> . If set to FALSE, all plots are printed to the currently active device. Note that most functions print more than one plot, which means you may only see the last plot depending on your active graphics device.
pdf_width	Number > 0. Default 12. Width of the PDF, in cm.
pdf_height	Number > 0. Default 12. Height of the PDF, in cm.
pages	Integer vector or NULL. Default NULL. Page number(s) to plot. Can be used to plot only specific pages to a PDF or the currently active graphics device.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

**Value**

An updated [GRN](#) object.

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
types = list(c("protein_coding"))
GRN = plotDiagnosticPlots_peakGene(GRN, gene.types=types, plotAsPDF = FALSE, pages = 1)
```

---

plotDiagnosticPlots\_TFPeaks

*Plot diagnostic plots for TF-peak connections for a GRN object*


---

## Description

Due to the number of plots that this functions produces, we currently provide only the option to plot as PDF. This may change in the future.

## Usage

```
plotDiagnosticPlots_TFPeaks(
  GRN,
  outputFolder = NULL,
  basenameOutput = NULL,
  plotDetails = FALSE,
  dataType = c("real", "permuted"),
  nTFMax = NULL,
  plotAsPDF = TRUE,
  pdf_width = 12,
  pdf_height_base = 8,
  pages = NULL,
  forceRerun = FALSE
)
```

## Arguments

GRN	Object of class <a href="#">GRN</a>
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
basenameOutput	NULL or character. Default NULL. Basename of the output files that are produced. If set to NULL, a default basename is chosen. If a custom basename is specified, all output files will have the chosen basename as file prefix, be careful with not overwriting already existing files (if <code>forceRerun</code> is set to TRUE)
plotDetails	TRUE or FALSE. Default FALSE. Print additional plots that may help for debugging and QC purposes? Note that these plots are currently less documented or not at all.
dataType	Character vector. One of, or both of, "real" or "permuted". For which data type, real or permuted data, to produce the diagnostic plots?
nTFMax	NULL or Integer > 0. Default NULL. Maximum number of TFs to process. Can be used for testing purposes by setting this to a small number i(e., 10)

plotAsPDF	TRUE or FALSE. Default TRUE. Should the plots be printed to a PDF file? If set to TRUE, a PDF file is generated, the name of which depends on the value of <code>basenameOutput</code> . If set to FALSE, all plots are printed to the currently active device. Note that most functions print more than one plot, which means you may only see the last plot depending on your active graphics device.
pdf_width	Number > 0. Default 12. Width of the PDF, in cm.
pdf_height_base	Number. Default 8. Base height of the PDF, in cm, per connection type. The total height is automatically determined based on the number of connection types that are found in the object (e.g., expression or TF activity). For example, when two connection types are found, the base height is multiplied by 2.
pages	Integer vector or NULL. Default NULL. Page number(s) to plot. Can be used to plot only specific pages to a PDF or the currently active graphics device.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

**Value**

An updated [GRN](#) object.

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = plotDiagnosticPlots_TFPeaks(GRN, outputFolder = ".", dataType = "real", nTFMax = 2, pages = 1)
```

---

plotGeneralEnrichment *Plot the general enrichment results*

---

**Description**

This function plots the results of the general enrichment analysis for every specified ontology.

**Usage**

```
plotGeneralEnrichment(
  GRN,
  outputFolder = NULL,
  basenameOutput = NULL,
  ontology = NULL,
  topn_pvalue = 30,
  p = 0.05,
  display_pAdj = FALSE,
  maxWidth_nchar_plot = 50,
  plotAsPDF = TRUE,
  pdf_width = 12,
```

```

pdf_height = 12,
pages = NULL,
forceRerun = FALSE
)

```

## Arguments

GRN	Object of class <a href="#">GRN</a>
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
basenameOutput	NULL or character. Default NULL. Basename of the output files that are produced. If set to NULL, a default basename is chosen. If a custom basename is specified, all output files will have the chosen basename as file prefix, be careful with not overwriting already existing files (if <code>forceRerun</code> is set to TRUE)
ontology	Character. NULL or vector of ontology names. Default NULL. Vector of ontologies to plot. The results must have been previously calculated otherwise an error is thrown.
topn_pvalue	Numeric. Default 30. Maximum number of ontology terms that meet the p-value significance threshold to display in the enrichment dot plot
p	Numeric. Default 0.05. p-value threshold to determine significance.
display_pAdj	TRUE or FALSE. Default FALSE. Is the p-value being displayed in the plots the adjusted p-value? This parameter is relevant for KEGG, Disease Ontology, and Reactome enrichments, and does not affect GO enrichments.
maxWidth_nchar_plot	Integer ( $\geq 10$ ). Default 50. Maximum number of characters for a term before it is truncated.
plotAsPDF	TRUE or FALSE. Default TRUE. Should the plots be printed to a PDF file? If set to TRUE, a PDF file is generated, the name of which depends on the value of <code>basenameOutput</code> . If set to FALSE, all plots are printed to the currently active device. Note that most functions print more than one plot, which means you may only see the last plot depending on your active graphics device.
pdf_width	Number $> 0$ . Default 12. Width of the PDF, in cm.
pdf_height	Number $> 0$ . Default 12. Height of the PDF, in cm.
pages	Integer vector or NULL. Default NULL. Page number(s) to plot. Can be used to plot only specific pages to a PDF or the currently active graphics device.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

## Value

The same [GRN](#) object, without modifications.



**See Also**

[plotCommunitiesEnrichment](#)  
[plotTFEnrichment](#)  
[calculateGeneralEnrichment](#)

**Examples**

```

# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = plotGeneralEnrichment(GRN, plotAsPDF = FALSE, pages = 1)

```

---

plotGeneralGraphStats *Plot general structure and connectivity statistics for a filtered [GRN](#) object*

---

**Description**

This function generates graphical summaries about the structure and connectivity of the TF-peak-gene and TF-gene graphs. These include, distribution of vertex types (TF, peak, gene) and edge types (tf-peak, peak-gene), the distribution of vertex degrees, and the most "important" vertices according to degree centrality and eigenvector centrality scores.

**Usage**

```

plotGeneralGraphStats(
  GRN,
  outputFolder = NULL,
  basenameOutput = NULL,
  plotAsPDF = TRUE,
  pdf_width = 12,
  pdf_height = 12,
  pages = NULL,
  forceRerun = FALSE
)

```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
basenameOutput	NULL or character. Default NULL. Basename of the output files that are produced. If set to NULL, a default basename is chosen. If a custom basename is specified, all output files will have the chosen basename as file prefix, be careful with not overwriting already existing files (if <code>forceRerun</code> is set to TRUE)

plotAsPDF	TRUE or FALSE. Default TRUE. Should the plots be printed to a PDF file? If set to TRUE, a PDF file is generated, the name of which depends on the value of <code>basenameOutput</code> . If set to FALSE, all plots are printed to the currently active device. Note that most functions print more than one plot, which means you may only see the last plot depending on your active graphics device.
pdf_width	Number > 0. Default 12. Width of the PDF, in cm.
pdf_height	Number > 0. Default 12. Height of the PDF, in cm.
pages	Integer vector or NULL. Default NULL. Page number(s) to plot. Can be used to plot only specific pages to a PDF or the currently active graphics device.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

**Value**

The same [GRN](#) object, without modifications.

**See Also**

[plotGeneralEnrichment](#)

[plotCommunitiesStats](#)

[plotCommunitiesEnrichment](#)

**Examples**

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = plotGeneralGraphStats(GRN, plotAsPDF = FALSE, pages = 1)
```

---

plotPCA\_all

*Produce a PCA plot of the data from a [GRN](#) object*

---

**Description**

Produce a PCA plot of the data from a [GRN](#) object

**Usage**

```
plotPCA_all(
  GRN,
  outputFolder = NULL,
  basenameOutput = NULL,
  data = c("rna", "peaks"),
  topn = c(500, 1000, 5000),
  type = "normalized",
  removeFiltered = TRUE,
  plotAsPDF = TRUE,
```

```

    pdf_width = 12,
    pdf_height = 12,
    pages = NULL,
    forceRerun = FALSE
)

```

## Arguments

GRN	Object of class <a href="#">GRN</a>
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
basenameOutput	NULL or character. Default NULL. Basename of the output files that are produced. If set to NULL, a default basename is chosen. If a custom basename is specified, all output files will have the chosen basename as file prefix, be careful with not overwriting already existing files (if <code>forceRerun</code> is set to TRUE)
data	Character. Either "peaks" or "rna" or "all". Default <code>c("rna", "peaks")</code> . Type of data to plot a PCA for. "peaks" corresponds to the the open chromatin data, while "rna" refers to the RNA-seq counts. If set to "all", PCA will be done for both data modalities. In any case, PCA will be based on the original provided data before any additional normalization has been run (i.e., usually the raw data).
topn	Integer vector. Default <code>c(500, 1000, 5000)</code> . Number of top variable features to do PCA for. Can be a vector of different numbers (see default).
type	Character. Must be "normalized". On which data type (raw or normalized) should the PCA plots be done? We removed support for raw and currently only support normalized.
removeFiltered	Logical. TRUE or FALSE. Default TRUE. Should features marked as filtered as determined by <code>filterData</code> be removed?
plotAsPDF	TRUE or FALSE. Default TRUE. Should the plots be printed to a PDF file? If set to TRUE, a PDF file is generated, the name of which depends on the value of <code>basenameOutput</code> . If set to FALSE, all plots are printed to the currently active device. Note that most functions print more than one plot, which means you may only see the last plot depending on your active graphics device.
pdf_width	Number >0. Default 12. Width of the PDF, in cm.
pdf_height	Number >0. Default 12. Height of the PDF, in cm.
pages	Integer vector or NULL. Default NULL. Page number(s) to plot. Can be used to plot only specific pages to a PDF or the currently active graphics device.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

## Value

An updated [GRN](#) object.

## Examples

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = plotPCA_all(GRN, topn = 500, data = "rna", type = "normalized", plotAsPDF = FALSE, pages = 1)
```

---

plotTFEnrichment      *Plot TF-based GO enrichment results*

---

## Description

Similarly to [plotGeneralEnrichment](#) and [plotCommunitiesEnrichment](#), the results of the TF-based enrichment analysis are plotted. This function produces multiple plots. First, one plot per community to summarize the TF-specific enrichment. Second, a summary heatmap of all significantly enriched terms across all TFs and for the whole eGRN. The latter allows to compare the results with the general network enrichment. Third, a subset of the aforementioned heatmap, showing only the top most significantly enriched terms per TF and for the whole eGRN (as specified by nID) for improved visibility .

## Usage

```
plotTFEnrichment(
  GRN,
  rankType = "degree",
  n = NULL,
  TF.names = NULL,
  topn_pvalue = 30,
  p = 0.05,
  nSignificant = 2,
  nID = 10,
  display_pAdj = FALSE,
  maxWidth_nchar_plot = 50,
  outputFolder = NULL,
  basenameOutput = NULL,
  plotAsPDF = TRUE,
  pdf_width = 12,
  pdf_height = 12,
  pages = NULL,
  forceRerun = FALSE
)
```

## Arguments

GRN	Object of class <a href="#">GRN</a>
rankType	Character. One of: "degree", "EV", "custom". This parameter will determine the criterion to be used to identify the "top" nodes. If set to "degree", the function will select top nodes based on the number of connections they have, i.e. based on their degree-centrality. If set to "EV" it will select the top nodes based on their eigenvector-centrality score in the network.

n	NULL or numeric. Default NULL. If set to NULL, all previously calculated TF enrichments will be plotted. If set to a value between (0,1), it is treated as a percentage of top nodes. If the value is passed as an integer it will be treated as the number of top nodes. This parameter is not relevant if rankType = "custom".
TF.names	NULL or character vector. Default NULL. For rankType="custom" the names of the TFs to plot. Ignored otherwise.
topn_pvalue	Numeric. Default 30. Maximum number of ontology terms that meet the p-value significance threshold to display in the enrichment dot plot
p	Numeric. Default 0.05. p-value threshold to determine significance.
nSignificant	Numeric > 0. Default 3. Threshold to filter out an ontology term with less than nSignificant overlapping genes.
nID	Numeric > 0. Default 10. For the reduced summary heatmap, number of top terms to select per community / for the general enrichment.
display_pAdj	TRUE or FALSE. Default FALSE. Is the p-value being displayed in the plots the adjusted p-value? This parameter is relevant for KEGG, Disease Ontology, and Reactome enrichments, and does not affect GO enrichments.
maxWidth_nchar_plot	Integer (>=10). Default 50. Maximum number of characters for a term before it is truncated.
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
basenameOutput	NULL or character. Default NULL. Basename of the output files that are produced. If set to NULL, a default basename is chosen. If a custom basename is specified, all output files will have the chosen basename as file prefix, be careful with not overwriting already existing files (if <code>forceRerun</code> is set to TRUE)
plotAsPDF	TRUE or FALSE. Default TRUE. Should the plots be printed to a PDF file? If set to TRUE, a PDF file is generated, the name of which depends on the value of <code>basenameOutput</code> . If set to FALSE, all plots are printed to the currently active device. Note that most functions print more than one plot, which means you may only see the last plot depending on your active graphics device.
pdf_width	Number > 0. Default 12. Width of the PDF, in cm.
pdf_height	Number > 0. Default 12. Height of the PDF, in cm.
pages	Integer vector or NULL. Default NULL. Page number(s) to plot. Can be used to plot only specific pages to a PDF or the currently active graphics device.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

### Value

The same [GRN](#) object, without modifications.

**See Also**

[plotGeneralEnrichment](#)  
[plotCommunitiesEnrichment](#)  
[calculateTFEnrichment](#)

**Examples**

```

# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = plotTFEnrichment(GRN, n = 5, plotAsPDF = FALSE, pages = 1)

```

---

plot\_stats\_connectionSummary

*Plot various network connectivity summaries for a [GRN](#) object*

---

**Description**

Plot various network connectivity summaries for a [GRN](#) object

**Usage**

```

plot_stats_connectionSummary(
  GRN,
  type = "heatmap",
  outputFolder = NULL,
  basenameOutput = NULL,
  plotAsPDF = TRUE,
  pdf_width = 12,
  pdf_height = 12,
  pages = NULL,
  forceRerun = FALSE
)

```

**Arguments**

GRN	Object of class <a href="#">GRN</a>
type	Character. Either "heatmap" or "boxplot". Default "heatmap". Which plot type to produce?
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
basenameOutput	NULL or character. Default NULL. Basename of the output files that are produced. If set to NULL, a default basename is chosen. If a custom basename is specified, all output files will have the chosen basename as file prefix, be careful with not overwriting already existing files (if <code>forceRerun</code> is set to TRUE)

plotAsPDF	TRUE or FALSE. Default TRUE. Should the plots be printed to a PDF file? If set to TRUE, a PDF file is generated, the name of which depends on the value of <code>basenameOutput</code> . If set to FALSE, all plots are printed to the currently active device. Note that most functions print more than one plot, which means you may only see the last plot depending on your active graphics device.
pdf_width	Number > 0. Default 12. Width of the PDF, in cm.
pdf_height	Number > 0. Default 12. Height of the PDF, in cm.
pages	Integer vector or NULL. Default NULL. Page number(s) to plot. Can be used to plot only specific pages to a PDF or the currently active graphics device.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

### Value

The same [GRN](#) object, without modifications.

### Examples

```
# See the Workflow vignette on the GRaNIE website for examples
GRN = loadExampleObject()
GRN = plot_stats_connectionSummary(GRN, forceRerun = FALSE, plotAsPDF = FALSE, pages = 1)
```

---

visualizeGRN	<i>Visualize a filtered eGRN in a flexible manner.</i>
--------------	--

---

### Description

This function can visualize a filtered eGRN in a very flexible manner and requires a [GRN](#) object as generated by [build\\_eGRN\\_graph](#).

### Usage

```
visualizeGRN(
  GRN,
  outputFolder = NULL,
  basenameOutput = NULL,
  plotAsPDF = TRUE,
  pdf_width = 12,
  pdf_height = 12,
  title = NULL,
  maxEdgesToPlot = 500,
  nCommunitiesMax = 8,
  graph = "TF-gene",
  colorby = "type",
  layout = "fr",
  vertice_color_TFs = list(h = 10, c = 85, l = c(25, 95)),
```

```

vertice_color_peaks = list(h = 135, c = 45, l = c(35, 95)),
vertice_color_genes = list(h = 260, c = 80, l = c(30, 90)),
vertexLabel_cex = 0.4,
vertexLabel_dist = 0,
forceRerun = FALSE
)

```

## Arguments

GRN	Object of class <a href="#">GRN</a>
outputFolder	Character or NULL. Default NULL. If set to NULL, the default output folder as specified when initiating the object in <code>link{initializeGRN}</code> will be used. Otherwise, all output from this function will be put into the specified folder. We recommend specifying an absolute path.
basenameOutput	NULL or character. Default NULL. Basename of the output files that are produced. If set to NULL, a default basename is chosen. If a custom basename is specified, all output files will have the chosen basename as file prefix, be careful with not overwriting already existing files (if <code>forceRerun</code> is set to TRUE)
plotAsPDF	TRUE or FALSE. Default TRUE. Should the plots be printed to a PDF file? If set to TRUE, a PDF file is generated, the name of which depends on the value of <code>basenameOutput</code> . If set to FALSE, all plots are printed to the currently active device. Note that most functions print more than one plot, which means you may only see the last plot depending on your active graphics device.
pdf_width	Number > 0. Default 12. Width of the PDF, in cm.
pdf_height	Number > 0. Default 12. Height of the PDF, in cm.
title	NULL or Character. Default NULL. Title to be assigned to the plot.
maxEdgesToPlot	Integer > 0. Default 500. Refers to the maximum number of connections to be plotted. If the network size is above this limit, nothing will be drawn. In such a case, it may help to either increase the value of this parameter or set the filtering criteria for the network to be more stringent, so that the network becomes smaller.
nCommunitiesMax	Integer > 0. Default 8. Maximum number of communities that get a distinct coloring. All additional communities will be colored with the same (gray) color.
graph	Character. Default TF-gene. One of: TF-gene, TF-peak-gene. Whether to plot a graph with links from TFs to peaks to gene, or the graph with the inferred TF to gene connections.
colorby	Character. Default type. Either type or community. Color the vertices by either type (TF/peak/gene) or community. See <a href="#">calculateCommunitiesStats</a>
layout	Character. Default fr. One of star, fr, sugiyama, kk, lgl, graphopt, mds, sphere
vertice_color_TFs	Named list. Default <code>list(h = 10, c = 85, l = c(25, 95))</code> . The list must specify the color in hcl format (hue, chroma, luminence). See the <code>colorspace</code> package for more details and examples



vertice_color_peaks	Named list. Default <code>list(h = 135, c = 45, l = c(35, 95))</code> .
vertice_color_genes	Named list. Default <code>list(h = 260, c = 80, l = c(30, 90))</code> .
vertexLabel_cex	Numeric. Default <code>0.4</code> . Font size (multiplication factor, device-dependent)
vertexLabel_dist	Numeric. Default <code>0</code> vertex. Distance between the label and the vertex.
forceRerun	TRUE or FALSE. Default FALSE. Force execution, even if the GRN object already contains the result. Overwrites the old results.

**Value**

The same [GRN](#) object, without modifications.

**See Also**

[build\\_eGRN\\_graph](#)

**Examples**

```
GRN = loadExampleObject()
GRN = visualizeGRN(GRN, maxEdgesToPlot = 700, graph = "TF-gene", colorby = "type")
```

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