

Package ‘systemPipeR’

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Type Package

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Description R package for building and running automated end-to-end analysis workflows for a wide range of next generation sequence (NGS) applications such as RNA-Seq, ChIP-Seq, VAR-Seq and Ribo-Seq. Important features include a uniform workflow interface across different NGS applications, automated report generation, and support for running both R and command-line software, such as NGS aligners or peak/variant callers, on local computers or compute clusters. Efficient handling of complex sample sets and experimental designs is facilitated by a consistently implemented sample annotation infrastructure. Instructions for using systemPipeR are given in the Overview Vignette (HTML). The remaining Vignettes, linked below, are workflow templates for common NGS use cases.

Depends Rsamtools (>= 1.31.2), Biostrings, ShortRead (>= 1.37.1), methods

Imports GenomicRanges, GenomicFeatures (>= 1.31.3), SummarizedExperiment, VariantAnnotation (>= 1.25.11), rjson, ggplot2, limma, edgeR, DESeq2, GOstats, GO.db, annotate, pheatmap, batchtools, yaml, stringr, assertthat, magrittr, DOT, rsvg, IRanges, testthat, S4Vectors, crayon

Suggests BiocGenerics, ape, BiocStyle, knitr, rmarkdown, biomaRt, BiocParallel, BiocManager, systemPipeRdata, GenomicAlignments, grid, DT, dplyr, kableExtra

VignetteBuilder knitr

SystemRequirements systemPipeR can be used to run external command-line software (e.g. short read aligners), but the corresponding tool needs to be installed on a system.

License Artistic-2.0

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systemPipeR-package *systemPipeR package for Workflow Environment*

Description

The systemPipeR package provides a suite of R/Bioconductor for designing, building and running end-to-end analysis workflows on local machines, HPC clusters and cloud systems, while generating at the same time publication quality analysis reports.

For detailed information on usage, see the package vignette, by typing `vignette("systemPipeR")`, or more information on the project here: <https://systempipe.org/spr>

All software-related questions should be posted to the Bioconductor Support Site: <https://support.bioconductor.org>

The code can be viewed at the GitHub repository: <https://github.com/tgirke/systemPipeR>

Author(s)

Daniela Cassol, Tyler Backman, Thomas Girke

References

Backman TWH, Girke T (2016) systemPipeR: NGS workflow and report generation environment. BMC Bioinformatics 17 (1). <https://doi.org/10.1186/s12859-016-1241-0>

addAssay-methods

Extension accessor methods for SummarizedExperiment object

Description

Accessors for adding new data to the 'assay' and 'metadata' slot of a SummarizedExperiment object

Usage

```
addAssay(x, ...)  
addMetadata(x, ...)
```

Arguments

x Object of class SummarizedExperiment.
... dots, name of the object.

Methods

addAssay signature(x = "SummarizedExperiment"): add new dataset to assays slot

addMetadata signature(x = "SummarizedExperiment"): add new dataset to metadata slot

Author(s)

Daniela Cassol

alignStats	<i>Alignment statistics</i>
------------	-----------------------------

Description

Generate data frame containing important read alignment statistics such as the total number of reads in the FASTQ files, the number of total alignments, as well as the number of primary alignments in the corresponding BAM files.

Usage

```
alignStats(args, output_index = 1, subset="FileName1")
```

Arguments

args	Object of class SYSargs or SYSargs2.
output_index	A numeric index positions of the file in SYSargs2 object, slot output. Default is output_index=1.
subset	subset are the variables defined in the param.yml file, for example "FileName1".

Value

data.frame with alignment statistics.

Author(s)

Thomas Girke

See Also

clusterRun and runCommandline and output_update

Examples

```
#####
## Examples with \code{SYSargs} object ##
#####
## Construct SYSargs object from param and targets files
param <- system.file("extdata", "tophat.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
args
names(args); modules(args); cores(args); outpaths(args); sysargs(args)

## Not run:
## Execute SYSargs on single machine
runCommandline(args=args)
```

```

## Alignment stats
read_statsDF <- alignStats(args)
read_statsDF <- cbind(read_statsDF[targets$FileName,], targets)
write.table(read_statsDF, "results/alignStats.xls", row.names=FALSE, quote=FALSE, sep="\t")

## End(Not run)

#####
## Examples with \code{SYSargs2} object ##
#####
## Construct SYSargs2 object from CWL input, and targets files
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl",
                  input_file="hisat2-mapping-se.yml", dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
WF
targets <- system.file("extdata", "targets.txt", package="systemPipeR")

names(WF); modules(WF); targets(WF)[1]; cmdlist(WF)[1:2]; output(WF)

## Not run:
## Execute SYSargs2 on single machine
WF <- runCommandline(args=WF)

## Alignment stats
read_statsDF <- alignStats(WF)
read_statsDF <- cbind(read_statsDF[targets$FileName,], targets)
write.table(read_statsDF, "results/alignStats.xls", row.names=FALSE, quote=FALSE, sep="\t")

## End(Not run)

```

catDB-class

Class "catDB"

Description

Container for storing mappings of genes to annotation categories such as gene ontologies (GO), pathways or conserved sequence domains. The `catmap` slot stores a list of data frames providing the direct assignments of genes to annotation categories (e.g. gene-to-GO mappings); `catlist` is a list of lists of all direct and indirect associations to the annotation categories (e.g. genes mapped to a pathway); and `idconv` allows to store a lookup-table for converting identifiers (e.g. array feature ids to gene ids).

Objects from the Class

Objects can be created by calls of the form `new("catDB", ...)`.

Slots

catmap: Object of class "list" list of data.frames

catlist: Object of class "list" list of lists

idconv: Object of class "ANY" list of data.frames

Methods

catlist signature(x = "catDB"): extracts data from catlist slot

catmap signature(x = "catDB"): extracts data from catmap slot

coerce signature(from = "list", to = "catDB"): as(list, "catDB")

idconv signature(x = "catDB"): extracts data from idconv slot

names signature(x = "catDB"): extracts slot names

show signature(object = "catDB"): summary view of catDB objects

Author(s)

Thomas Girke

See Also

makeCATdb, GOHyperGAll, GOHyperGAll_Subset, GOHyperGAll_Simplify, GOcluster_Report, goBarplot

Examples

```
showClass("catDB")
## Not run:
## Obtain annotations from BioMart
listMarts() # To choose BioMart database
m <- useMart("ENSEMBL_MART_PLANT"); listDatasets(m)
m <- useMart("ENSEMBL_MART_PLANT", dataset="athaliana_eg_gene")
listAttributes(m) # Choose data types you want to download
go <- getBM(attributes=c("go_accession", "tair_locus", "go_namespace_1003"), mart=m)
go <- go[go[,3]!="",]; go[,3] <- as.character(go[,3])
write.table(go, "GOannotationsBiomart_mod.txt", quote=FALSE, row.names=FALSE, col.names=FALSE, sep="\t")

## Create catDB instance (takes a while but needs to be done only once)
catdb <- makeCATdb(myfile="GOannotationsBiomart_mod.txt", lib=NULL, org="", colno=c(1,2,3), idconv=NULL)
catdb

## End(Not run)
```

catmap

catDB accessor methods

Description

Methods to access information from catDB object.

Usage

```
catmap(x)
```

Arguments

x object of class catDB

Value

various outputs

Author(s)

Thomas Girke

Examples

```
## Not run:
## Obtain annotations from BioMart
m <- useMart("ENSEMBL_MART_PLANT"); listDatasets(m)
m <- useMart("ENSEMBL_MART_PLANT", dataset="athaliana_eg_gene")
listAttributes(m) # Choose data types you want to download
go <- getBM(attributes=c("go_accession", "tair_locus", "go_namespace_1003"), mart=m)
go <- go[go[,3]!="",,]; go[,3] <- as.character(go[,3])
write.table(go, "GOannotationsBiomart_mod.txt", quote=FALSE, row.names=FALSE, col.names=FALSE, sep="\t")

## Create catDB instance (takes a while but needs to be done only once)
catdb <- makeCATdb(myfile="GOannotationsBiomart_mod.txt", lib=NULL, org="", colno=c(1,2,3), idconv=NULL)
catdb

## Access methods for catDB
catmap(catdb)$D_MF[1:4,]
catlist(catdb)$L_MF[1:4]
idconv(catdb)

## End(Not run)
```

check.output	<i>Checking if the output files exist</i>
--------------	---

Description

This function returns a logical vector indicating whether the expected output files for each workflow step exist.

Usage

```
check.output(args, subset = 1, index = 1)
```

Arguments

args	object of class SYSargs2.
subset	name or numeric position of the values to be subsetting in the slot. The subset is the path for the expected output files for all the steps in the workflow. Default is subset=1
index	A numeric index positions of the file in SYSargs2 object, slot output. It requires a subset to be defined. Default is index=1.

Author(s)

Daniela Cassol and Thomas Girke

See Also

subsetWF

Examples

```
## Construct SYSargs2 object
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl",
                  input_file="hisat2-mapping-se.yml", dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
WF

check.output(WF)
```

 clusterRun

Submit command-line tools to cluster

Description

Submits non-R command-line software to queueing/scheduling systems of compute clusters using run specifications defined by functions similar to `runCommandline`. `clusterRun` can be used with most queueing systems since it is based on utilities from the `batchtools` package which supports the use of template files (`*.tmpl`) for defining the run parameters of the different schedulers. The path to the `*.tmpl` file needs to be specified in a conf file provided under the `conffile` argument.

Usage

```
clusterRun(args, FUN = runCommandline, more.args = list(args = args, make_bam = TRUE), conffile = ".batchtools.conf.R")
```

Arguments

<code>args</code>	Object of class <code>SYSargs</code> or <code>SYSargs2</code> .
<code>FUN</code>	Accepts functions such as <code>runCommandline(args, ...)</code> where the <code>args</code> argument is mandatory and needs to be of class <code>SYSargs</code> or <code>SYSargs2</code> .
<code>more.args</code>	Object of class <code>list</code> , which provides the arguments that control the <code>FUN</code> function.
<code>conffile</code>	Path to conf file (default location <code>./batchtools.conf.R</code>). This file contains in its simplest form just one command, such as this line for the Slurm scheduler: <code>cluster.functions <-makeClusterFunctionsSlurm(template="batchtools.slurm.tmpl")</code> . For more detailed information visit this page: https://mllg.github.io/batchtools/index.html
<code>template</code>	The template files for a specific queueing/scheduling systems can be downloaded from here: https://github.com/mllg/batchtools/tree/master/inst/templates . Slurm, PBS/Torque, and Sun Grid Engine (SGE) templates are provided.
<code>Njobs</code>	Integer defining the number of cluster jobs. For instance, if <code>args</code> contains 18 command-line jobs and <code>Njobs=9</code> , then the function will distribute them across 9 cluster jobs each running 2 command-line jobs. To increase the number of CPU cores used by each process, one can do this under the corresponding argument of the command-line tool, e.g. <code>-p</code> argument for Tophat.
<code>runid</code>	Run identifier used for log file to track system call commands. Default is <code>"01"</code> .
<code>resourceList</code>	List for reserving for each cluster job sufficient computing resources including memory (Megabyte), number of nodes, CPU cores, walltime (minutes), etc. For more details, one can consult the template file for each queueing/scheduling system.

Value

Object of class `Registry`, as well as files and directories created by the executed command-line tools.

Author(s)

Daniela Cassol and Thomas Girke

References

For more details on batchtools, please consult the following page: <https://github.com/mlg/batchtools/>

See Also

clusterRun replaces the older functions getQsubargs and qsubRun.

Examples

```
#####
## Examples with \code{SYSargs} object ##
#####
## Construct SYSargs object from param and targets files
param <- system.file("extdata", "hisat2.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
args
names(args); modules(args); cores(args); outpaths(args); sysargs(args)

## Not run:
## Execute SYSargs on multiple machines of a compute cluster. The following
## example uses the conf and template files for the Slurm scheduler. Please
## read the instructions on how to obtain the corresponding files for other schedulers.
file.copy(system.file("extdata", ".batchtools.conf.R", package="systemPipeR"), ".")
file.copy(system.file("extdata", "batchtools.slurm.tpl", package="systemPipeR"), ".")
resources <- list(walltime=120, ntasks=1, ncpus=cores(args), memory=1024)
reg <- clusterRun(args, FUN = runCommandline, more.args = list(args = args, make_bam = TRUE), conffile=".batchtools

## Monitor progress of submitted jobs
getStatus(reg=reg)
file.exists(outpaths(args))

## End(Not run)

#####
## Examples with \code{SYSargs2} object ##
#####
## Construct SYSargs2 object from CWL param, CWL input, and targets files
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl",
  input_file="hisat2-mapping-se.yml", dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
WF
names(WF); modules(WF); targets(WF)[1]; cmdlist(WF)[1:2]; output(WF)

## Not run:
## Execute SYSargs2 on multiple machines of a compute cluster. The following
```

```

## example uses the conf and template files for the Slurm scheduler. Please
## read the instructions on how to obtain the corresponding files for other schedulers.
file.copy(system.file("extdata", ".batchtools.conf.R", package="systemPipeR"), ".")
file.copy(system.file("extdata", "batchtools.slurm.tmpl", package="systemPipeR"), ".")
resources <- list(walltime=120, ntasks=1, ncpus=4, memory=1024)
reg <- clusterRun(WF, FUN = runCommandline, more.args = list(args = WF, make_bam = TRUE), conffile=".batchtools.conf.R")

## Monitor progress of submitted jobs
getStatus(reg=reg)

## Updates the path in the object \code{output(WF)}
WF <- output_update(WF, dir=FALSE, replace=TRUE, extension=c(".sam", ".bam"))

## Alignment stats
read_statsDF <- alignStats(WF)
read_statsDF <- cbind(read_statsDF[targets$FileName,], targets)
write.table(read_statsDF, "results/alignStats.xls", row.names=FALSE, quote=FALSE, sep="\t")

## End(Not run)

```

config.param

Adding param file

Description

Replace or adding a input configuration setting at "YML" param file

Usage

```
config.param(input_file = NULL, param, file = "default", silent = FALSE)
```

Arguments

input_file	a list of parameters, param file path, or SYSargs2 object.
param	object of class list, expressing the values and names to be added or replace at the command-line definition or the configuration files.
file	name and path of the new file. If set to default, the name of the write file will have the pattern: <Date-Time>_<OriginalName>.yaml. If set to append, the param information it will be append on the same file.
silent	if set to TRUE, all messages returned by the function will be suppressed.

Author(s)

Daniela Cassol

Examples

```
## Not run:
input_file <- system.file("extdata", "cwl/hisat2/hisat2-se/hisat2-mapping-se.yml", package="systemPipeR")
param <- list(thread=10, fq=list(class="File", path="./results2"))
input <- config.param(input_file=input_file, param, file="default")

## End(Not run)
```

 configWF

Workflow Steps Selection

Description

This function allows the user to control of which step of the workflow will be run and the generation of the new RMarkdown.

Usage

```
configWF(x, input_steps = "ALL", exclude_steps = NULL, silent = FALSE, ...)
```

Arguments

x	object of class SYSargsList.
input_steps	a character vector of all steps desires to preserve on the output file. Default is ALL. It can be used the symbol ":" to select many steps in sequence, for example, input_steps=1:5.2, from step 1 to step 5.2. The symbol "." represents the substeps and symbol "," is used to separate selections. Jump from a major step to sub-step is supported, but if a major step is selected/excluded, all sub-steps of this major step will be selected/excluded. Repeatedly selected steps will only result in a unique step. It is recommended to put major steps in input_steps, like 1:4, 6:8, 10; and unwanted sub-steps in exclude_step, like 1.1, 3.1.1-3.1.3, 6.5. Reverse selecting is supported e.g. 10:1.
exclude_steps	a character vector of all steps desires to remove on the output file.
silent	if set to TRUE, all messages returned by the function will be suppressed.
...	Additional arguments to pass on to configWF().

Author(s)

Daniela Cassol

Examples

```
## Not run:
library(systemPipeRdata)
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
script <- system.file("extdata/workflows/rnaseq", "systemPipeRNaseq.Rmd", package="systemPipeRdata")
SYSconfig <- initProject(projPath=".", targets=targets, script=script, overwrite=TRUE, silent=TRUE)

sysargslist <- configWF(x=sysargslist)

## End(Not run)
```

countRangeset

Read counting for several range sets

Description

Convenience function to perform read counting iteratively for several range sets, e.g. peak range sets or feature types. Internally, the read counting is performed with the `summarizeOverlaps` function from the `GenomicAlignments` package. The resulting count tables are directly saved to files.

Usage

```
countRangeset(bf1, args, format="tabular", ...)
```

Arguments

<code>bf1</code>	BamFileList object containing paths to one or more BAM files.
<code>args</code>	Object of class <code>SYSargs</code> or <code>SYSargs2</code> where <code>infile1(args)</code> specifies the paths to the tabular range data files (e.g. peak ranges) used for counting.
<code>format</code>	Format of input range files. Currently, supported are <code>tabular</code> or <code>bed</code> . If <code>tabular</code> is selected then the input range files need to contain the proper column titles to coerce with <code>as(..., "GRanges")</code> to <code>GRanges</code> objects after importing them with <code>read.delim</code> . The latter is the case for the peak files (<code>*peaks.xls</code>) generated by the MACS2 software.
<code>...</code>	Arguments to be passed on to internally used <code>summarizeOverlaps</code> function.

Value

Named character vector containing the paths from `outpaths(args)` to the resulting count table files.

Author(s)

Thomas Girke

See Also

summarizeOverlaps

Examples

```
## Paths to BAM files
param <- system.file("extdata", "bowtieSE.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args_bam <- systemArgs(sysma=param, mytargets=targets)
bfl <- BamFileList(outpaths(args_bam), yieldSize=50000, index=character())

## Not run:
#####
## Examples with \code{SYSargs} object ##
#####
## Construct SYSargs object
## SYSargs with paths to range data and count files
args <- systemArgs(sysma="param/count_rangesets.param", mytargets="targets_mac3.txt")

## Iterative read counting
countDFnames <- countRangeset(bfl, args, mode="Union", ignore.strand=TRUE)
writeTargetsout(x=args, file="targets_countDF.txt", overwrite=TRUE)

#####
## Examples with \code{SYSargs2} object ##
#####
## Construct SYSargs2 object
## SYSargs2 with paths to range data and count files
dir_path <- system.file("extdata/cwl/count_rangesets", package="systemPipeR")
args <- loadWF(targets = "targets_mac3.txt", wf_file = "count_rangesets.cwl",
  input_file = "count_rangesets.yml", dir_path = dir_path)
args <- renderWF(args, inputvars = c(FileName = "_FASTQ_PATH1_", SampleName = "_SampleName_"))

## Iterative read counting
countDFnames <- countRangeset(bfl, args, mode="Union", ignore.strand=TRUE)

## End(Not run)
```

createParamFiles

createParamFiles

Description

The constructor function creates an `SYSargs2` S4 class object from command-line string. Also, the function creates and saves the CWL param files. The latest storages all the parameters required for running command-line software, following the standard and specification defined on Common Workflow Language (CWL).

Usage

```
createParamFiles(commandline, cwlVersion = "v1.1", class = "CommandLineTool",
  results_path = "./results", module_load = "baseCommand", file = "default",
  writeParamFiles = FALSE, overwrite = FALSE, silent = FALSE)
writeParamFiles(sysargs, file = "default", overwrite = TRUE, silent = FALSE)
```

Arguments

commandline	string. Original command-line to create the CWL files from. Please see Details for more information.
cwlVersion	string. The version of the Common Workflow Language. More information here: https://www.commonwl.org/ .
class	character. Name of Common Workflow Language Description class. The following is accepted: CommandLineTool.
results_path	Path to the results folder. Default is results.
module_load	string, Name of software to load by Environment Modules system. Default is "baseCommand", which creates a subfolder and two files: *.cwl and *.yml at ./param/cwl/.
file	character. Name and path of output files. If set to "default" then the name of the output files will have the pattern <software>.cwl and <software>.yml, where <software> will be what baseCommand(x) returns, when x is an object of class SYSargs2. Also, it creates a subfolder at ./param/cwl/ with name <software>.
writeParamFiles	logical. If set to TRUE, it will write to file the content of the CWL files:*.cwl and *.yml. Default is FALSE.
overwrite	logical. If set to TRUE, existing files of the same name will be overwritten. Default is FALSE.
silent	logical. If set to TRUE, all messages returned by the function will be suppressed. Default is FALSE.
sysargs	Object of class SYSargs2. Output from the createParamFiles function.

Details

- First line of the command-line object will be treated as the baseCommand;
- For argument lines (starting from the second line), any word before the first space with leading '-' or '--' in each will be treated as a prefix, like -S or --min. Any line without this first word will be treated as no prefix;
- All defaults are placed inside <...>;
- First argument is the input argument type. F for "File", int for integer, string for string;
- Optional: use the keyword out followed the type with a , comma separation to indicate if this argument is also a CWL output;
- Then, use : to separate keywords and default values, any non-space value after the : will be treated as the default value;
- If any argument has no default value, just a flag, like --verbose, there no need to add any <...>.
- The \ is not required, however for consistency it is recommended to add.

Value

SYSargs2 object

Author(s)

Le Zhang and Daniela Cassol

References

For more details on CWL, please consult the following page: <https://www.commonwl.org/>

See Also

writeParamFiles printParam subsetParam replaceParam renameParam appendParam loadWorkflow
renderWF showClass("SYSargs2")

Examples

```
command <- "
hisat2 \
  -S <F, out: ./results/M1A.sam> \
  -x <F: ./data/tair10.fasta> \
  -k <int: 1> \
  -min-intronlen <int: 30> \
  -max-intronlen <int: 3000> \
  -threads <int: 4> \
  -U <F: ./data/SRR446027_1.fastq.gz> \
  --verbose
"
cmd <- createParamFiles(command)
cmdlist(cmd)
```

EnvModules-class

Class "EnvModules"

Description

The function module enables use of the Environment Modules system (<http://modules.sourceforge.net/>) from within the R environment. By default the user's login shell environment (ie. `bash -l`) will be used to initialize the current session. The module function can also; load or unload specific software, list all the loaded software within the current session, and list all the applications available for loading from the module system. Lastly, the module function can remove all loaded software from the current session.

Objects from the Class

Objects can be created by calls of the form `new("EnvModules", ...)`.

Slots

available_modules: Object of class "list" ~~
 loaded_modules: Object of class "list" ~~
 default_modules: Object of class "list" ~~
 modulecmd: Object of class "character" ~~

Methods

[signature(x = "EnvModules"): ...
 [[signature(x = "EnvModules", i = "ANY", j = "missing"): ...
 [[<- signature(x = "EnvModules"): ...
 \$ signature(x = "EnvModules"): ...
available_modules signature(x = "EnvModules"): ...
coerce signature(from = "EnvModules", to = "list"): ...
coerce signature(from = "list", to = "EnvModules"): ...
default_modules signature(x = "EnvModules"): ...
EnvModules signature(x = "EnvModules"): ...
loaded_modules signature(x = "EnvModules"): ...
modulecmd signature(x = "EnvModules"): ...
names signature(x = "EnvModules"): ...
show signature(object = "EnvModules"): ...

Author(s)

Jordan Hayes and Daniela Cassol

Examples

```
showClass("EnvModules")
```

 evalCode

Toggles option eval on the RMarkdown files

Description

Function to evaluate (eval=TRUE) or not evaluate (eval=FALSE) R chunk codes in the Rmarkdown file. This function does not run the code, just toggles between TRUE or FALSE the option eval and writes out a new file with the chosen option.

Usage

```
evalCode(infile, eval = TRUE, output)
```

Arguments

`infile` name and path of the infile file, format Rmd.
`eval` whether to evaluate the code and include its results. The default is TRUE.
`output` name and path of the output file. File format Rmd.

Value

Writes Rmarkdown file containing the exact copy of the `infile` file with the option choose on the `eval` argument. It will be easy to toggle between run all the R chunk codes or not.

Author(s)

Daniela Cassol

Examples

```
## Not run:  
library(systemPipeRdata)  
file <- system.file("extdata/workflows/rnaseq", "systemPipeRNaseq.Rmd", package="systemPipeRdata")  
evalCode(infile=file, eval=FALSE, output="test.Rmd")  
  
## End(Not run)
```

featureCoverage *Genome read coverage by transcript models*

Description

Computes read coverage along single and multi component features based on genomic alignments. The coverage segments of component features are spliced to continuous ranges, such as exons to transcripts or CDSs to ORFs. The results can be obtained with single nucleotide resolution (e.g. around start and stop codons) or as mean coverage of relative bin sizes, such as 100 bins for each feature. The latter allows comparisons of coverage trends among transcripts of variable length. The results can be obtained for single or many features (e.g. any number of transcripts) at once. Visualization of the coverage results is facilitated by a downstream `plotfeatureCoverage` function.

Usage

```
featureCoverage(bfl, grl, resizereads = NULL, readlengthrange = NULL, Nbins = 20,  
               method = mean, fixedmatrix, resizefeatures, upstream, downstream,  
               outfile, overwrite = FALSE)
```

Arguments

<code>bf1</code>	Paths to BAM files provided as <code>BamFileList</code> object. The name slot of the BAM files will be used for naming samples in the results.
<code>gr1</code>	Genomic ranges provided as <code>GRangesList</code> typically generated from <code>txdb</code> instances with operations like: <code>cdsBy(txdb, "tx")</code> or <code>exonsBy(txdb, "tx")</code> . Single component features will be processed the same way as multi component features.
<code>resizereads</code>	Positive integer defining the length alignments should be resized to prior to the coverage calculation. NULL will omit the resizing step.
<code>readlengthrange</code>	Positive integer of length 2 determining the read length range to use for the coverage calculation. Reads falling outside of the specified length range will be excluded from the coverage calculation. For instance, <code>readlengthrange=c(30:40)</code> will base the coverage calculation on reads between 30 to 40 bps. Assigning NULL will skip this filtering step.
<code>Nbins</code>	Single positive integer defining the number of segments the coverage of each feature should be binned into in order to obtain coverage summaries of constant length, e.g. for plotting purposes.
<code>method</code>	Defines the summary statistics to use for binning. The default is <code>method=mean</code> .
<code>fixedmatrix</code>	If set to TRUE, a coverage matrix with single nucleotide resolution will be returned for any number of transcripts centered around precise anchor points in a genome annotation, such a stop/start codons or transcription start sites. For instance, a matrix with coverage information 20bps upstream and downstream of the stop/start codons can be obtained with <code>fixedmatrix=TRUE, upstream=20, downstream=20</code> along with a <code>gr1</code> instance containing the CDS exon ranges required for this operation, e.g. generated with <code>cdsBy(txdb, "tx")</code> .
<code>resizefeatures</code>	Needs to be set to TRUE when <code>fixedmatrix=TRUE</code> . Internally, this will use the <code>systemPipeR::resizeFeature</code> function to extend single and multi component features at their most left and most right end coordinates. The corresponding extension values are specified under the <code>upstream</code> and <code>downstream</code> arguments.
<code>upstream</code>	Single positive integer specifying the upstream extension length relative to the orientation of each feature in the genome. More details are given above.
<code>downstream</code>	Single positive integer specifying the downstream extension length relative to the orientation of each feature in the genome. More details are given above.
<code>outfile</code>	Default NULL omits writing of the results to a file. If a file name is specified then the results are written to a tabular file. If <code>bf1</code> contains the paths to several BAM files then the results will be appended to the same file where the first column specifies the sample labels. Redirecting the results to file is particularly useful when processing large files of many sample where computation times can be significant.
<code>overwrite</code>	If set to TRUE any existing file assigned to <code>outfile</code> will be overwritten.

Value

The function allows to return the following four distinct outputs. The settings to return these instances are illustrated below in the example section.

- (A) data.frame containing binned coverage where rows are features and columns coverage bins. The first four columns contain (i) the sample names, (ii) the number of total aligned reads in the corresponding BAM files (useful for normalization), (iii) the feature IDs, (iv) strand of the coverage. All following columns are numeric and contain the actual coverage data for the sense and antisense strand of each feature.
- (B) data.frame containing coverage with single nucleotide resolution around anchor points such as start and stop codons. The two matrix components are appended column-wise. To clearly distinguish the two data components, they are separated by a specialty column containing pipe characters. The first four columns are the same as described under (A). The column title for the anchor point is 0. For instance, if the features are CDSs then the first 0 corresponds to the first nucleotide of the start codon and the second 0 to the last nucleotide of the stop codon. Upstream and downstream positions are indicated by negative and positive column numbers, respectively.
- (C) data.frame containing combined results of (A) and (B) where the first set of columns contains to the coverage around the start codons, the second one the binned coverage of the CDSs and the third one the coverage around the stop codons separated by the same pipe columns mentioned under (B).
- (D) Rle list containing the nucleotide level coverage of each feature

Author(s)

Thomas Girke

See Also

plotfeatureCoverage

Examples

```
## Construct SYSargs object from param and targets files
param <- system.file("extdata", "tophat.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)

## Not run:
## Features from sample data of systemPipeRdata package
library(GenomicFeatures)
file <- system.file("extdata/annotation", "tair10.gff", package="systemPipeRdata")
txdb <- makeTxDbFromGFF(file=file, format="gff3", organism="Arabidopsis")

## (A) Generate binned coverage for two BAM files and 4 transcripts
gr1 <- cdsBy(txdb, "tx", use.names=TRUE)
fcov <- featureCoverage(bf1=BamFileList(outputpaths(args)[1:2]), gr1=gr1[1:4], resizereads=NULL,
```

```

        readlengthrange=NULL, Nbins=20, method=mean, fixedmatrix=FALSE,
        resizefeatures=TRUE, upstream=20, downstream=20)
plotfeatureCoverage(covMA=fcov, method=mean, scales="fixed", scale_count_val=10^6)

## (B) Coverage matrix upstream and downstream of start/stop codons
fcov <- featureCoverage(bfl=BamFileList(outpaths(args)[1:2]), grl=grl[1:4], resizereads=NULL,
        readlengthrange=NULL, Nbins=NULL, method=mean, fixedmatrix=TRUE,
        resizefeatures=TRUE, upstream=20, downstream=20)
plotfeatureCoverage(covMA=fcov, method=mean, scales="fixed", scale_count_val=10^6)

## (C) Combined matrix for both binned and start/stop codon
fcov <- featureCoverage(bfl=BamFileList(outpaths(args)[1:2]), grl=grl[1:4], resizereads=NULL,
        readlengthrange=NULL, Nbins=20, method=mean, fixedmatrix=TRUE,
        resizefeatures=TRUE, upstream=20, downstream=20)
plotfeatureCoverage(covMA=fcov, method=mean, scales="fixed", scale_count_val=10^6)

## (D) Rle coverage objects one for each query feature
fcov <- featureCoverage(bfl=BamFileList(outpaths(args)[1:2]), grl=grl[1:4], resizereads=NULL,
        readlengthrange=NULL, Nbins=NULL, method=mean, fixedmatrix=FALSE,
        resizefeatures=TRUE, upstream=20, downstream=20)

## End(Not run)

```

featuretypeCounts

Plot read distribution across genomic features

Description

Counts how many reads in short read alignment files (BAM format) overlap with entire annotation categories. This utility is useful for analyzing the distribution of the read mappings across feature types, e.g. coding versus non-coding genes. By default the read counts are reported for the sense and antisense strand of each feature type separately. To minimize memory consumption, the BAM files are processed in a stream using utilities from the `Rsamtools` and `GenomicAlignment` packages. The counts can be reported for each read length separately or as a single value for reads of any length. Subsequently, the counting results can be plotted with the associated `plotfeaturetypeCounts` function.

Usage

```
featuretypeCounts(bfl, grl, singleEnd = TRUE, readlength = NULL, type = "data.frame")
```

Arguments

bfl BamFileList object containing the paths to the target BAM files stored on disk. Note, memory-efficient processing is achieved by streaming through BAM files rather than reading entire files into memory at once. The maximum number of alignments to process in each iteration is determined by the `yieldSize` value passed on to the `BamFileList` function. For details see `?BamFileList`.

grl	GRangesList object containing in each list component the range set of a feature type. Typically, this object is generated with the genFeatures function. For details see the example section below and ?genFeatures.
singleEnd	Specifies whether the targets BAM files contain alignments for single-end (SE) or paired-end read data. TRUE is for SE and FALSE for PE data.
readlength	Integer vector specifying the read length values for which to report counts separately. If readlength=NULL the length of the reads will be ignored resulting in a single value for each feature type and strand. Note, for PE data the two reads in a pair may differ in length. In those cases the length of the two reads is averaged and then assigned to the corresponding length category after rounding the mean length to the closest integer. This is not an ideal solution but a reasonable compromise for the purpose of the summary statistics generated by featuretypeCounts.
type	Determines whether the results are returned as data.frame (type="data.frame") or as list (type="list"). Each list component contains the counting results for one BAM file and is named after the corresponding sample. The data.frame result contains this sample assignment information in a separate column.

Value

The results are returned as data.frame or list of data.frames. For details see above under types argument. The result data.frames contain the following columns in the given order:

SampleName	Sample names obtained from BamFileList object.
Strand	Sense or antisense strand of read mappings.
Featuretype	Name of feature type provided by GRangesList object. Note, the total number of aligned reads is reported under the special feature type 'N_total_aligned'. This value is useful for scaling/normalization purposes in plots, e.g. counts per million reads.
Featuretypelength	Total genomic length of each reduced feature type in bases. This value is useful to normalize the read counts by genomic length units, e.g. in plots.
Subsequent columns	Counts for reads of any length or for individual read lengths.

Author(s)

Thomas Girke

See Also

plotfeaturetypeCounts, genFeatures

Examples

```
## Construct SYSargs object from param and targets files
param <- system.file("extdata", "tophat.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
```

```

args <- systemArgs(sysma=param, mytargets=targets)

## Not run:
## Features from sample data of systemPipeRdata package
library(GenomicFeatures)
file <- system.file("extdata/annotation", "tair10.gff", package="systemPipeRdata")
txdb <- makeTxDbFromGFF(file=file, format="gff3", organism="Arabidopsis")
feat <- genFeatures(txdb, featuretype="all", reduce_ranges=TRUE, upstream=1000, downstream=0, verbose=TRUE)

## Generate and plot feature counts for specific read lengths
fc <- featuretypeCounts(bfl=BamFileList(outpaths(args), yieldSize=50000), grl=feat, singleEnd=TRUE, readlength=c(
p <- plotfeaturetypeCounts(x=fc, graphicsfile="featureCounts.pdf", graphicsformat="pdf", scales="fixed", anyread

## Generate and plot feature counts for any read length
fc2 <- featuretypeCounts(bfl=BamFileList(outpaths(args), yieldSize=50000), grl=feat, singleEnd=TRUE, readlength=c(
p2 <- plotfeaturetypeCounts(x=featureCounts2, graphicsfile="featureCounts2.pdf", graphicsformat="pdf", scales="f

## End(Not run)

```

filterDEGs

Filter and plot DEG results

Description

Filters and plots DEG results for a given set of sample comparisons. The gene identifiers of all (i) Up_or_Down, (ii) Up and (iii) Down regulated genes are stored as separate list components, while the corresponding summary statistics, stored in a fourth list component, is plotted in form of a stacked bar plot.

Usage

```
filterDEGs(degDF, filter, plot = TRUE)
```

Arguments

degDF	data.frame generated by run_edgeR
filter	Named vector with filter cutoffs of format $c(\text{Fold}=2, \text{FDR}=1)$ where Fold refers to the fold change cutoff (unlogged) and FDR to the p-value cutoff.
plot	Allows to turn plotting behavior on and off with default set to TRUE.

Details

Currently, there is no community standard available how to calculate fold changes (here logFC) of genomic ranges, such as gene or feature ranges, to unambiguously refer to them as features with increased or decreased read abundance; or in case of gene expression experiments to up or down regulated genes, respectively. To be consistent within systemPipeR, the corresponding functions, such as filterDEGs, use here the following definition. Genomic ranges with positive logFC values are classified as up and those with negative logFC values as down. This means if a comparison

among two samples a and b is specified in the corresponding targets file as a-b then the feature with a positive logFC has a higher `_normalized_` read count value in sample a than in sample b, and vice versa. To inverse this assignment, users want to change the specification of their chosen sample comparison(s) in the targets file accordingly, e.g. change a-b to b-a. Alternatively, one can swap the column order of the matrix assigned to the `cmp` argument of the `run_edgeR` or `run_DESeq2` functions. Users should also be aware that for logFC values close to zero (noise range), the direction of the fold change (sign of logFC) can be very sensitive to minor differences in the normalization method, while this assignment is much more robust for more pronounced changes or higher absolute logFC values.

Value

Returns list with four components

UporDown	List of up or down regulated gene/transcript identifiers meeting the chosen filter settings for all comparisons defined in data frames <code>pval</code> and <code>log2FC</code> .
Up	Same as above but only for up regulated genes/transcript.
Down	Same as above but only for down regulated genes/transcript.

Author(s)

Thomas Girke

See Also

`run_edgeR`

Examples

```
targetspath <- system.file("extdata", "targets.txt", package="systemPipeR")
targets <- read.delim(targetspath, comment.char = "#")
cmp <- readComp(file=targetspath, format="matrix", delim="-")
countfile <- system.file("extdata", "countDFeByg.xls", package="systemPipeR")
countDF <- read.delim(countfile, row.names=1)
edgeDF <- run_edgeR(countDF=countDF, targets=targets, cmp=cmp[[1]], independent=FALSE, mdsplot="")
pval <- edgeDF[, grep("_FDR$", colnames(edgeDF)), drop=FALSE]
fold <- edgeDF[, grep("_logFC$", colnames(edgeDF)), drop=FALSE]
DEG_list <- filterDEGs(degDF=edgeDF, filter=c(Fold=2, FDR=10))
names(DEG_list)
DEG_list$Summary
```

filterVars

Filter VCF files

Description

Convenience function for filtering VCF files based on user definable quality parameters. The function imports each VCF file into R, applies the filtering on an internally generated `VRanges` object and then writes the results to a new VCF file.

Usage

```
filterVars(args, filter, varcaller, organism)
```

Arguments

args	Object of class SYSargs or SYSargs2. The paths of the input VCF files are specified under <code>infile1(args)</code> and the paths of the output files under <code>outfile1(args)</code> or <code>output(args)</code> .
filter	Character vector of length one specifying the filter syntax that will be applied to the internally created VRanges object.
varcaller	Character vector of length one specifying the variant caller used for generating the input VCFs. Currently, this argument can be assigned 'gatk', 'bcftools' or 'vartools'.
organism	Character vector specifying the organism name of the reference genome.

Value

Output files in VCF format. Their paths can be obtained with `outpaths(args)` or `output(args)`.

Author(s)

Thomas Girke

See Also

`variantReport` `combineVarReports`, `varSummar`

Examples

```
## Alignment with BWA (sequentially on single machine)
param <- system.file("extdata", "bwa.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
sysargs(args)[1]

## Not run:
system("bwa index -a bwtsv ./data/tair10.fasta")
bampaths <- runCommandline(args=args)

## Alignment with BWA (parallelized on compute cluster)
resources <- list(walltime="20:00:00", nodes=paste0("1:ppn=", cores(args)), memory="10gb")
reg <- clusterRun(args, conffile=".BatchJobs.R", template="torque.tpl", Njobs=18, runid="01",
  resourceList=resources)

## Variant calling with GATK
## The following creates in the initial step a new targets file
## (targets_bam.txt). The first column of this file gives the paths to
## the BAM files created in the alignment step. The new targets file and the
## parameter file gatk.param are used to create a new SYSargs
## instance for running GATK. Since GATK involves many processing steps, it is
```

```

## executed by a bash script gatk_run.sh where the user can specify the
## detailed run parameters. All three files are expected to be located in the
## current working directory. Samples files for gatk.param and
## gatk_run.sh are available in the subdirectory ./inst/extdata/ of the
## source file of the systemPipeR package.
writeTargetsout(x=args, file="targets_bam.txt")
system("java -jar CreateSequenceDictionary.jar R=./data/tair10.fasta O=./data/tair10.dict")
# system("java -jar /opt/picard/1.81/CreateSequenceDictionary.jar R=./data/tair10.fasta O=./data/tair10.dict")
args <- systemArgs(sysma="gatk.param", mytargets="targets_bam.txt")
resources <- list(walltime="20:00:00", nodes=paste0("1:ppn=", 1), memory="10gb")
reg <- clusterRun(args, conffile=".BatchJobs.R", template="torque.tmpl", Njobs=18, runid="01",
  resourceList=resources)
writeTargetsout(x=args, file="targets_gatk.txt")

## Variant calling with BCFtools
## The following runs the variant calling with BCFtools. This step requires in
## the current working directory the parameter file sambcf.param and the
## bash script sambcf_run.sh.
args <- systemArgs(sysma="sambcf.param", mytargets="targets_bam.txt")
resources <- list(walltime="20:00:00", nodes=paste0("1:ppn=", 1), memory="10gb")
reg <- clusterRun(args, conffile=".BatchJobs.R", template="torque.tmpl", Njobs=18, runid="01",
  resourceList=resources)
writeTargetsout(x=args, file="targets_sambcf.txt")

## Filtering of VCF files generated by GATK
args <- systemArgs(sysma="filter_gatk.param", mytargets="targets_gatk.txt")
filter <- "totalDepth(vr) >= 2 & (altDepth(vr) / totalDepth(vr) >= 0.8) & rowSums(softFilterMatrix(vr))==4"
# filter <- "totalDepth(vr) >= 20 & (altDepth(vr) / totalDepth(vr) >= 0.8) & rowSums(softFilterMatrix(vr))==6"
filterVars(args, filter, varcaller="gatk", organism="A. thaliana")
writeTargetsout(x=args, file="targets_gatk_filtered.txt")

## Filtering of VCF files generated by BCFtools
args <- systemArgs(sysma="filter_sambcf.param", mytargets="targets_sambcf.txt")
filter <- "rowSums(vr) >= 2 & (rowSums(vr[,3:4])/rowSums(vr[,1:4]) >= 0.8)"
# filter <- "rowSums(vr) >= 20 & (rowSums(vr[,3:4])/rowSums(vr[,1:4]) >= 0.8)"
filterVars(args, filter, varcaller="bcftools", organism="A. thaliana")
writeTargetsout(x=args, file="targets_sambcf_filtered.txt")

## Annotate filtered variants from GATK
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_gatk_filtered.txt")
txdb <- loadDb("./data/tair10.sqlite")
fa <- FaFile(systemPipeR::reference(args))
variantReport(args=args, txdb=txdb, fa=fa, organism="A. thaliana")

## Annotate filtered variants from BCFtools
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_sambcf_filtered.txt")
txdb <- loadDb("./data/tair10.sqlite")
fa <- FaFile(systemPipeR::reference(args))
variantReport(args=args, txdb=txdb, fa=fa, organism="A. thaliana")

## Combine results from GATK
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_gatk_filtered.txt")
combinedDF <- combineVarReports(args, filtercol=c(Consequence="nonsynonymous"))

```

```

write.table(combinedDF, "./results/combinedDF_nonsyn_gatk.xls", quote=FALSE, row.names=FALSE, sep="\t")

## Combine results from BCFtools
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_sambcf_filtered.txt")
combinedDF <- combineVarReports(args, filtercol=c(Consequence="nonsynonymous"))
write.table(combinedDF, "./results/combinedDF_nonsyn_sambcf.xls", quote=FALSE, row.names=FALSE, sep="\t")

## Summary for GATK
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_gatk_filtered.txt")
write.table(varSummary(args), "./results/variantStats_gatk.xls", quote=FALSE, col.names = NA, sep="\t")

## Summary for BCFtools
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_sambcf_filtered.txt")
write.table(varSummary(args), "./results/variantStats_sambcf.xls", quote=FALSE, col.names = NA, sep="\t")

## Venn diagram of variants
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_gatk_filtered.txt")
varlist <- sapply(names(outpaths(args))[1:4], function(x) as.character(read.delim(outpaths(args)[x])$VARID))
vennset_gatk <- overLapper(varlist, type="vennsets")
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_sambcf_filtered.txt")
varlist <- sapply(names(outpaths(args))[1:4], function(x) as.character(read.delim(outpaths(args)[x])$VARID))
vennset_bcf <- overLapper(varlist, type="vennsets")
vennPlot(list(vennset_gatk, vennset_bcf), mymain="", mysub="GATK: red; BCFtools: blue", colmode=2, ccol=c("blue",

## End(Not run)

```

genFeatures

Generate feature ranges from TxDb

Description

Function to generate a variety of feature types from TxDb objects using utilities provided by the GenomicFeatures package. The feature types are organized per gene and can be returned on that level in their non-reduced or reduced form.

Currently, supported features include intergenic, promoter, intron, exon, cds, 5'/3'UTR and different transcript types. The latter contains as many transcript types as available in the tx_type column when extracting transcripts from TxDb objects as follows: transcripts(txdb, c("tx_name", "gene_id", "tx_type"

Usage

```
genFeatures(txdb, featuretype = "all", reduce_ranges, upstream = 1000, downstream = 0, verbose = TRUE)
```

Arguments

txdb	TxDb object
featuretype	Feature types can be specified by assigning a character vector containing any of the following: c("tx_type", "promoter", "intron", "exon", "cds", "fiveUTR", "threeUTR", "intergenic"). The default all is a shorthand to select all supported features.

reduce_ranges	If set to TRUE the feature ranges will be reduced on the gene level. As a result overlapping feature components of the same type and from the same gene will be merged to a single range, e.g. two overlapping exons from the same gene are merged to one. Intergenic ranges are not affected by this setting. Note, all reduced feature types are labeled with the suffix '_red'.
upstream	Defines for promoter features the number of bases upstream from the transcription start site.
downstream	Defines for promoter features the number of bases downstream from the transcription start site.
verbose	verbose=FALSE turns off all print messages.

Value

The results are returned as a GRangesList where each component is a GRanges object containing the range set of each feature type. Intergenic ranges are assigned unique identifiers and recorded in the featuretype_id column of the metadata block. For this the ids of their adjacent genes are concatenated with two underscores as separator. If the adjacent genes overlap with other genes then their identifiers are included in the id string as well and separated by a single underscore.

Author(s)

Thomas Girke

See Also

transcripts and associated TxDb accessor functions from the GenomicFeatures package.

Examples

```
## Sample from GenomicFeatures package
library(GenomicFeatures)
gffFile <- system.file("extdata", "GFF3_files", "a.gff3", package="GenomicFeatures")
txdb <- makeTxDbFromGFF(file=gffFile, format="gff3", organism="Solanum lycopersicum")
feat <- genFeatures(txdb, featuretype="all", reduce_ranges=FALSE, upstream=1000, downstream=0)

## List extracted feature types
names(feat)

## Obtain feature lists by genes, here for promoter
split(feat$promoter, unlist(mcols(feat$promoter)$feature_by))

## Return all features in single GRanges object
unlist(feat)

## Not run:
## Sample from systemPipeRdata package
file <- system.file("extdata/annotation", "tair10.gff", package="systemPipeRdata")
txdb <- makeTxDbFromGFF(file=file, format="gff3", organism="Arabidopsis")
feat <- genFeatures(txdb, featuretype="all", reduce_ranges=TRUE, upstream=1000, downstream=0)

## End(Not run)
```

 getQsubargs

Arguments for qsub

Description

Note: This function has been deprecated. Please use `clusterRun` instead. `getQsubargs` defines arguments to submit runX job(s) to queuing system (e.g. Torque) via `qsub`.

Usage

```
getQsubargs(software = "qsub", queue = "batch", Nnodes = "nodes=1", cores = as.numeric(gsub("^.* ", "",
```

Arguments

<code>software</code>	Software to use for submission to queuing system. Default is <code>qsub</code> .
<code>queue</code>	Name of queue to use. Default is <code>batch</code> .
<code>Nnodes</code>	Number of compute nodes to use for processing. Default is <code>nodes=1</code> .
<code>cores</code>	Number of CPU cores to use per compute node. Default will use what is provided by <code>under -p</code> in <code>myargs</code> of <code>systemArgs()</code> output.
<code>memory</code>	Amount of RAM to reserve per node.
<code>time</code>	Walltime limit each job is allowed to run per node.

Value

list

Author(s)

Thomas Girke

Examples

```
## Construct SYSargs object from param and targets files
param <- system.file("extdata", "tophat.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
args
names(args); modules(args); cores(args); outpaths(args); sysargs(args)
```

```
## Not run:
## Execute SYSargs on single machine
runCommandline(args=args)
```

```
## Execute SYSargs on multiple machines
qsubargs <- getQsubargs(queue="batch", Nnodes="nodes=1", cores=cores(tophat), memory="mem=10gb", time="walltime="
qsubRun(args=args, qsubargs=qsubargs, Nqsubs=1, package="systemPipeR")
## Alignment stats
```

```

read_statsDF <- alignStats(fqpaths=tophatargs$infile1, bampaths=bampaths, fqgz=TRUE)
read_statsDF <- cbind(read_statsDF[targets$FileName,], targets)
write.table(read_statsDF, "results/alignStats.xls", row.names=FALSE, quote=FALSE, sep="\t")

## End(Not run)

```

GOHyperGAll

GO term enrichment analysis for large numbers of gene sets

Description

To test a sample population of genes for over-representation of GO terms, the core function GOHyperGAll computes for all nodes in the three GO networks (BP, CC and MF) an enrichment test based on the hypergeometric distribution and returns the corresponding raw and Bonferroni corrected p-values. Subsequently, a filter function supports GO Slim analyses using default or custom GO Slim categories. Several convenience functions are provided to process large numbers of gene sets (e.g. clusters from partitioning results) and to visualize the results.

Note: GOHyperGAll provides similar utilities as the GOHyperG function in the GOstats package. The main difference is that GOHyperGAll simplifies processing of large numbers of gene sets, as well as the usage of custom array-to-gene and gene-to-GO mappings.

Usage

```

## Generate gene-to-GO mappings and store as catDB object
makeCATdb(myfile, lib = NULL, org = "", colno = c(1, 2, 3), idconv = NULL, rootUK=FALSE)

## Enrichment function
GOHyperGAll(catdb, gocat = "MF", sample, Nannot = 2)

## GO slim analysis
GOHyperGAll_Subset(catdb, GOHyperGAll_result, sample = test_sample, type = "goSlim", myslimv)

## Reduce GO term redundancy
GOHyperGAll_Simplify(GOHyperGAll_result, gocat = "MF", cutoff = 0.001, correct = TRUE)

## Batch analysis of many gene sets
GOCluster_Report(catdb, setlist, id_type = "affy", method = "all", CLSZ = 10, cutoff = 0.001, gocats = c

## Bar plot of GOCluster_Report results
goBarplot(GOBatchResult, gocat)

```

Arguments

myfile File with gene-to-GO mappings. Sample files can be downloaded from geneontology.org (<http://geneontology.org/GO.downloads.annotations.shtml>) or from BioMart as shown in example below.

<code>colno</code>	Column numbers referencing in <code>myfile</code> the three target columns containing GOID, GeneID and GOCAT, in that order.
<code>org</code>	Optional argument. Currently, the only valid option is <code>org="Arabidopsis"</code> to get rid of transcript duplications in this particular annotation.
<code>lib</code>	If the gene-to-GO mappings are obtained from a <code>*.db</code> package from Bioconductor then the package name can be specified under the <code>lib</code> argument of the <code>sampleDFgene2GO</code> function.
<code>idconv</code>	Optional id conversion data <code>.frame</code>
<code>catdb</code>	<code>catdb</code> object storing mappings of genes to annotation categories. For details, see <code>?SYSargs-class</code> .
<code>rootUK</code>	If the argument <code>rootUK</code> is set to <code>TRUE</code> then the root nodes are treated as terminal nodes to account for the new unknown terms.
<code>sample</code>	character vector containing the test set of gene identifiers
<code>Nannot</code>	Defines the minimum number of direct annotations per GO node from the sample set to determine the number of tested hypotheses for the p-value adjustment.
<code>gocat</code>	Specifies the GO type, can be assigned one of the following character values: "MF", "BP" and "CC".
<code>GOHyperGAll_result</code>	<code>data.frame</code> generated by <code>GOHyperGAll</code>
<code>type</code>	The function <code>GOHyperGAll_Subset</code> subsets the <code>GOHyperGAll</code> results by directly assigned GO nodes or custom <code>goSlim</code> categories. The argument <code>type</code> can be assigned the values <code>goSlim</code> or <code>assigned</code> .
<code>myslimv</code>	optional argument to provide custom <code>goSlim</code> vector
<code>cutoff</code>	p-value cutoff for GO terms to show in result <code>data.frame</code>
<code>correct</code>	If <code>TRUE</code> the function will favor the selection of terminal (informationich) GO terms that have at the same time a large number of sample matches.
<code>setlist</code>	list of character vectors containing gene IDs (or array feature IDs). The names of the <code>list</code> components correspond to the set labels, e.g. DEG comparisons or cluster IDs.
<code>id_type</code>	specifies type of IDs in input, can be assigned <code>gene</code> or <code>affy</code>
<code>method</code>	Specifies analysis type. Current options are <code>all</code> for <code>GOHyperGAll</code> , <code>slim</code> for <code>GOHyperGAll_Subset</code> or <code>simplify</code> for <code>GOHyperGAll_Simplify</code> .
<code>CLSZ</code>	minimum gene set (cluster) size to consider. Gene sets below this cutoff will be ignored.
<code>gocats</code>	Specifies GO type, can be assigned the values "MF", "BP" and "CC".
<code>recordSpecGO</code>	argument to report in the result <code>data.frame</code> specific GO IDs for any of the 3 ontologies disregarding whether they meet the specified p-value cutoff, e.g: <code>recordSpecGO=c("GO:0003674", "GO:0008150", "GO:0005575")</code>
<code>GOBatchResult</code>	<code>data.frame</code> generated by <code>GOCluster_Report</code>
<code>...</code>	additional arguments to pass on

Details

GOHyperGAll_Simplify: The result data frame from GOHyperGAll will often contain several connected GO terms with significant scores which can complicate the interpretation of large sample sets. To reduce this redundancy, the function GOHyperGAll_Simplify subsets the data frame by a user specified p-value cutoff and removes from it all GO nodes with overlapping children sets (OFFSPRING), while the best scoring nodes are retained in the result data.frame.

GOCluster_Report: performs the three types of GO term enrichment analyses in batch mode: GOHyperGAll, GOHyperGAll_Subset or GOHyperGAll_Simplify. It processes many gene sets (e.g. gene expression clusters) and returns the results conveniently organized in a single result data frame.

Value

makeCATdb generates catDB object from file.

Author(s)

Thomas Girke

References

This workflow has been published in Plant Physiol (2008) 147, 41-57.

See Also

GOHyperGAll_Subset, GOHyperGAll_Simplify, GOCluster_Report, goBarplot

Examples

```
## Not run:

## Obtain annotations from BioMart
listMarts() # To choose BioMart database
m <- useMart("ENSEMBL_MART_PLANT"); listDatasets(m)
m <- useMart("ENSEMBL_MART_PLANT", dataset="athaliana_eg_gene")
listAttributes(m) # Choose data types you want to download
go <- getBM(attributes=c("go_accession", "tair_locus", "go_namespace_1003"), mart=m)
go <- go[go[,3]!="",]; go[,3] <- as.character(go[,3])
write.table(go, "GOannotationsBiomart_mod.txt", quote=FALSE, row.names=FALSE, col.names=FALSE, sep="\t")

## Create catDB instance (takes a while but needs to be done only once)
catdb <- makeCATdb(myfile="GOannotationsBiomart_mod.txt", lib=NULL, org="", colno=c(1,2,3), idconv=NULL)
catdb

## Create catDB from Bioconductor annotation package
# catdb <- makeCATdb(myfile=NULL, lib="ath1121501.db", org="", colno=c(1,2,3), idconv=NULL)

## AffyID-to-GeneID mappings when working with AffyIDs
# affy2locusDF <- systemPipeR::.AffyID2GeneID(map = "ftp://ftp.arabidopsis.org/home/tair/Microarrays/Affymetrix")
# catdb_conv <- makeCATdb(myfile="GOannotationsBiomart_mod.txt", lib=NULL, org="", colno=c(1,2,3), idconv=list(affy2locusDF))
# systemPipeR::.AffyID2GeneID(catdb=catdb_conv, affyIDs=c("244901_at", "244902_at"))
```

```

## Next time catDB can be loaded from file
save(catdb, file="catdb.RData")
load("catdb.RData")

## Perform enrichment test on single gene set
test_sample <- unique(as.character(catmap(catdb)$D_MF[1:100,"GeneID"]))
GOHyperGAll(catdb=catdb, gocat="MF", sample=test_sample, Nannot=2)[1:20,]

## GO Slim analysis by subsetting results accordingly
GOHyperGAll_result <- GOHyperGAll(catdb=catdb, gocat="MF", sample=test_sample, Nannot=2)
GOHyperGAll_Subset(catdb, GOHyperGAll_result, sample=test_sample, type="goSlim")

## Reduce GO term redundancy in 'GOHyperGAll_results'
simplifyDF <- GOHyperGAll_Simplify(GOHyperGAll_result, gocat="MF", cutoff=0.001, correct=T)
# Returns the redundancy reduced data set.
data.frame(GOHyperGAll_result[GOHyperGAll_result[,1]

## Batch Analysis of Gene Clusters
testlist <- list(Set1=test_sample)
GOBatchResult <- GOCluster_Report(catdb=catdb, setlist=testlist, method="all", id_type="gene", CLSZ=10, cutoff=0.

## Plot 'GOBatchResult' as bar plot
goBarplot(GOBatchResult, gocat="MF")

## End(Not run)

```

initWF

Workflow Project Initiation

Description

Collection of functions to construct `SYSargsList` workflow control environment (S4 object). `initWF` function constructs `SYSargsList` S4 class objects from a script file. The latter is an RMarkdown file containing all the workflow steps. In the future, it will be accepting other formats, as `Rscript` and `bash`. This file is defined either by the `sysconfig` file or `script` argument. Internally, `initWF` function calls `initProject` function, which creates a storage folder for all the logs, and the output to `stdout` or `stderr`. This function also creates the `SYSconfig.yml` on the fly to register all the parameters of the workflow. This function checks the project structure, which by default is:

- `SPRproject/`
 - `data/`
 - `param/`
 - `results/`

The default structure of the project directories can be modified here.

Usage

```
initWF(sprconfig = NULL, script = NULL, targets = NULL, silent = FALSE, overwrite = FALSE)
```

```
initProject(projPath = "./", data = "data", param = "param", results = "results",
script = NULL, targets = NULL, filename = "SPRconfig.yml",
overwrite = FALSE, silent = FALSE, tempdir = FALSE)
```

```
SPRproject(projPath = "./", overwrite = FALSE, silent = FALSE, tempdir = FALSE)
```

Arguments

sprconfig	a character vector of a full sprconfig file path name. Default is NULL, which the function understand that the Rmd will be provide.
script	a character vector of a full script file path name. This file containing all the information to run all the steps on the workflow analysis. The current format accepted is Rmd.
targets	a character vector of a full sample annotation file path name. Default is NULL. The targets file can be either a simple tabular or yml file. Also, it is possible to assign NULL to run the pipeline without the 'targets' file. This can be useful for running specific workflows that do not require input files.
silent	if set to TRUE, all messages returned by the function will be suppressed.
overwrite	if set to TRUE, existing files or directories of same name will be overwritten.
projPath	a character vector of a full project path name. Default is the current path.
data	a character vector of a data directory name. Default is data. This subdirectory in the project stores all the raw data, reference, and annotation files.
param	a character vector of a param directory name. Default is param. This subdirectory in the project stores all the parameter and configuration files.
results	a character vector of a results directory name. Default is results. This subdirectory in the project stores all the analysis results, including but not limited: alignment, variant, and peak files (BAM, VCF, BED); tabular result files; and image/plot files.
filename	name and path of the output file which will store all the project configuration information. Default is SYSconfig.yml.
tempdir	if set to TRUE, the temporary directory for the workflow management files will be created.

Details

SPRproject function detects and creates the project directory to store all the flat files from the workflow management. If `overwrite` is TRUE, the previous folder will be deleted and replace for a new one. Also, the user can choose to store all this information on a temporary folder, setting `tempdir = TRUE`.

initProject function detects an existing project or creates a project structure on the path provide.

Value

SPRproject will return a character vector containing the path name. initProject will return a list containing all the information in the sysconfig file. initWF will return a SYSargsList object.

Author(s)

Daniela Cassol and Thomas Girke

See Also

See also as SYSargsList-class.

Examples

```
## Not run:
## Construct SYSargsList object from Rmd file
library(systemPipeRdata)
script <- system.file("extdata/workflows/rnaseq", "systemPipeRNAseq.Rmd", package="systemPipeRdata")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- tempdir()
SPRconfig <- initProject(projPath=dir_path, targets=targets, script=script, overwrite = TRUE)
sysargslist <- initWF(sprconfig =file.path(dir_path, "SPRconfig.yml"))

## End(Not run)
```

INTERSECTset-class	<i>Class "INTERSECTset"</i>
--------------------	-----------------------------

Description

Container for storing standard intersect results created by the overLapper function. The setlist slot stores the original label sets as vectors in a list; intersectmatrix organizes the label sets in a present-absent matrix; complexitylevels represents the number of comparisons considered for each comparison set as vector of integers; and intersectlist contains the standard intersect vectors.

Objects from the Class

Objects can be created by calls of the form `new("INTERSECTset", ...)`.

Slots

setlist: Object of class "list": list of vectors
 intersectmatrix: Object of class "matrix": binary matrix
 complexitylevels: Object of class "integer": vector of integers
 intersectlist: Object of class "list": list of vectors

Methods

as.list signature(x = "INTERSECTset"): coerces INTERSECTset to list

coerce signature(from = "list", to = "INTERSECTset"): as(list, "INTERSECTset")

complexitylevels signature(x = "INTERSECTset"): extracts data from complexitylevels slot

intersectlist signature(x = "INTERSECTset"): extracts data from intersectlist slot

intersectmatrix signature(x = "INTERSECTset"): extracts data from intersectmatrix slot

length signature(x = "INTERSECTset"): returns number of original label sets

names signature(x = "INTERSECTset"): extracts slot names

setlist signature(x = "INTERSECTset"): extracts data from setlist slot

show signature(object = "INTERSECTset"): summary view of INTERSECTset objects

Author(s)

Thomas Girke

See Also

overLapper, vennPlot, olBarplot, VENNset-class

Examples

```
showClass("INTERSECTset")

## Sample data
setlist <- list(A=sample(letters, 18), B=sample(letters, 16),
               C=sample(letters, 20), D=sample(letters, 22),
               E=sample(letters, 18), F=sample(letters, 22))

## Create VENNset
interset <- overLapper(setlist[1:5], type="intersects")
class(interset)

## Accessor methods for VENNset/INTERSECTset objects
names(interset)
setlist(interset)
intersectmatrix(interset)
complexitylevels(interset)
intersectlist(interset)

## Coerce VENNset/INTERSECTset object to list
as.list(interset)
```

`loadWorkflow`*Constructs SYSargs2 object from CWL param and targets files*

Description

The constructor functions create an SYSargs2 S4 class object from three input files: a CWL param and input files, and one simple tabular or yml file, a targets file. The latter is optional for workflow steps lacking input files. The CWL param provides all the parameters required for running command-line software, following the standard and specification defined on [Common Workflow Language \(CWL\)](#). The input file provides additional information for the command-line, allowing each sample level input/outfile operation uses its own SYSargs2 instance. In the targets file users could provide the paths to the initial sample input files (e.g. FASTQ) along with sample labels, and if appropriate biological replicate and contrast information for controlling differential abundance analyses.

Usage

```
loadWorkflow(targets = NULL, wf_file, input_file, dir_path = ".")
```

Arguments

<code>targets</code>	either the path to targets file or an object of SYSargs2 class. The targets file can be either a simple tabular or yml file. Also, it is possible to assign NULL to run the pipeline without the 'targets' file. This can be useful for running specific workflows that do not require input files.
<code>wf_file</code>	name and path to CWL param file.
<code>input_file</code>	name and path to input file.
<code>dir_path</code>	full path to the directory with the CWL param and input files.

Value

SYSargs2 object

Author(s)

Daniela Cassol and Thomas Girke

See Also

```
renderWF showClass("SYSargs2")
```

Examples

```
## Construct SYSargs2 object from CWL param, CWL input, and targets files
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl",
                  input_file="hisat2-mapping-se.yml", dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
WF
```

mergeBamByFactor	<i>Merge BAM files based on factor</i>
------------------	--

Description

Merges BAM files based on sample groupings provided by a factor using internally the mergeBam function from the Rsamtools package. The function also returns an updated SYSargs or SYSargs2 object containing the paths to the merged BAM files as well as to the unmerged BAM files if there are any. All rows of merged parent samples are removed.

The functionality provided by mergeBamByFactor is useful for experiments where pooling of replicates is advantageous to maximize the depth of read coverage, such as prior to peak calling in ChIP-Seq or miRNA gene prediction experiments.

Usage

```
mergeBamByFactor(args, mergefactor = "Factor", overwrite = FALSE, silent = FALSE, ...)
```

Arguments

args	An instance of SYSargs or SYSargs2 constructed from a targets file where the first column (targetsin(args) or targets.as.df(targets(args))) contains the paths to the BAM files along with the column title FileName.
mergefactor	factor containing the grouping information required for merging the BAM files referenced in the first column of targetsin(args) or targets.as.df(targets(args)). The default uses Factor column from the targets files as factor. The latter merges BAM files for which replicates are specified in the Factor column.
overwrite	If overwrite=FALSE existing BAM files of the same name will not be overwritten.
silent	If silent=TRUE print statements will be suppressed.
...	To pass on additional arguments to the internally used mergeBam function from Rsamtools.

Value

The merged BAM files will be written to output files with the following naming convention: <first_BAM_file_name>_<group>. In addition, the function returns an updated SYSargs or SYSargs2 object where all output file paths contain the paths to the merged BAM files. The rows of the merged parent samples are removed and the rows of the unmerged samples remain unchanged.

Author(s)

Thomas Girke

See Also

writeTargetsout, writeTargetsRef

Examples

```
## Construct initial SYSargs object
targetspath <- system.file("extdata", "targets_chip.txt", package="systemPipeR")
parampath <- system.file("extdata", "bowtieSE.param", package="systemPipeR")
args <- systemArgs(sysma=parampath, mytargets=targetspath)

## Not run:
## After running alignmets (e.g. with Bowtie2) generate targets file
## for the corresponding BAM files. The alignment step is skipped here.
writeTargetsout(x=args, file="targets_bam.txt", overwrite=TRUE)
args <- systemArgs(sysma=NULL, mytargets="targets_bam.txt")

## Merge BAM files and return updated SYSargs object
args_merge <- mergeBamByFactor(args, overwrite=TRUE, silent=FALSE)

## Export modified targets file
writeTargetsout(x=args_merge, file="targets_mergeBamByFactor.txt", overwrite=TRUE)

## End(Not run)
```

moduleload

Interface to allow full use of the Environment Modules system for Unix

Description

The function `module` enables use of the Environment Modules system (<http://modules.sourceforge.net/>) from within the R environment. By default the user's login shell environment (ie. `bash -l`) will be used to initialize the current session. The `module` function can also; load or unload specific software, list all the loaded software within the current session, and list all the applications available for loading from the module system. Lastly, the `module` function can remove all loaded software from the current session.

Usage

```
module(action_type, module_name="")
moduleload(module_name)
moduleUnload(module_name)
modulelist()
moduleAvail()
moduleClear()
moduleInit()
```


Arguments

`action_type` Name of the action to be executed as character vector. The following switches are accepted: `avail`, `list`, `init`, `load`, `unload`, and `clear`.

`module_name` Name of software to load as character vector.

Author(s)

Tyler Backman, Jordan Hayes and Thomas Girke

Examples

```
## Not run:
## List all available software from the module system
avail <- moduleAvail()

## List loaded software in the current session
modulelist()

## Example for loading a software into the shell environment
moduleload("hisat2")
moduleload(modules(args))

## Example for removing software from the shell environment
moduleUnload("hisat2")

## Clear all of the software from the shell's initialization files
moduleClear()

## List and load all the software loaded in users default login shell into the current session (default)
moduleInit()

## End(Not run)
```

olBarplot

Bar plot for intersect sets

Description

Generates bar plots of the intersect counts of `VENNset` and `INTERSECTset` objects generated by the `overLapper` function. It is an alternative to Venn diagrams (e.g. `vennPlot`) that scales to larger numbers of label sets. By default the bars in the plot are colored and grouped by complexity levels of the intersect sets.

Usage

```
olBarplot(x, mincount = 0, complexity="default", myxlabel = "default", myylabel="Counts", mytitle = "de
```

Arguments

<code>x</code>	Object of class <code>VENNset</code> or <code>INTERSECTset</code> .
<code>mincount</code>	Sets minimum number of counts to consider in the bar plot. Default <code>mincount=0</code> considers all counts.
<code>complexity</code>	Allows user to limit the bar plot to specific complexity levels of intersects by specifying the chosen ones with an integer vector. Default <code>complexity="default"</code> considers all complexity levels.
<code>myxlabel</code>	Defines label of x-axis.
<code>myylabel</code>	Defines label of y-axis.
<code>mytitle</code>	Defines main title of plot.
<code>...</code>	Allows to pass on additional arguments to <code>geom_bar</code> from <code>ggplot2</code> . For instance, <code>fill=seq(along=vennlist(x))</code> or <code>fill=seq(along=intersectlist(x))</code> will assign a different color to each bar, or <code>fill="blue"</code> will color all of them blue. The default bar coloring is by complexity levels of the intersect sets.

Value

Bar plot.

Note

The functions provided here are an extension of the Venn diagram resources on this site: <http://manuals.bioinformatics.ucr.edu/Venn-Diagrams>

Author(s)

Thomas Girke

See Also

`overLapper`, `vennPlot`

Examples

```
## Sample data: list of vectors with object labels
setlist <- list(A=sample(letters, 18), B=sample(letters, 16),
               C=sample(letters, 20), D=sample(letters, 22),
               E=sample(letters, 18), F=sample(letters, 22))

## 2-way Venn diagram
vennset <- overLapper(setlist[1:2], type="vennsets")
vennPlot(vennset)

## 3-way Venn diagram
vennset <- overLapper(setlist[1:3], type="vennsets")
vennPlot(vennset)

## 4-way Venn diagram
```

```

vennset <- overLapper(setlist[1:4], type="vennsets")
vennPlot(list(vennset, vennset))

## Pseudo 4-way Venn diagram with circles
vennPlot(vennset, type="circle")

## 5-way Venn diagram
vennset <- overLapper(setlist[1:5], type="vennsets")
vennPlot(vennset)

## Alternative Venn count input to vennPlot (not recommended!)
counts <- sapply(vennlist(vennset), length)
vennPlot(counts)

## 6-way Venn comparison as bar plot
vennset <- overLapper(setlist[1:6], type="vennsets")
olBarplot(vennset, mincount=1)

## Bar plot of standard intersect counts
interaset <- overLapper(setlist, type="intersects")
olBarplot(interaset, mincount=1)

## Accessor methods for VENNset/INTERSECTset objects
names(vennset)
names(interaset)
setlist(vennset)
intersectmatrix(vennset)
complexitylevels(vennset)
vennlist(vennset)
intersectlist(interaset)

## Coerce VENNset/INTERSECTset object to list
as.list(vennset)
as.list(interaset)

## Pairwise intersect matrix and heatmap
olMA <- sapply(names(setlist),
function(x) sapply(names(setlist),
function(y) sum(setlist[[x]] %in% setlist[[y]])))
olMA
heatmap(olMA, Rowv=NA, Colv=NA)

## Presence-absence matrices for large numbers of sample sets
interaset <- overLapper(setlist=setlist, type="intersects", complexity=2)
(paMA <- intersectmatrix(interaset))
heatmap(paMA, Rowv=NA, Colv=NA, col=c("white", "gray"))

```

Description

Function for identifying consensus peak among two peaks sets sharing a minimum relative overlap.

Usage

```
olRanges(query, subject, output = "gr")
```

Arguments

query	Object of class GRanges, which is a vector of genomic locations and associated annotations.
subject	Object of class GRanges.
output	By default "gr" returns any overlap with OL length information in an object of class GRanges. Also, can returns an object of class data.frame with "df".

Author(s)

Thomas Girke

Examples

```
## Sample Data Sets
grq <- GRanges(seqnames = Rle(c("chr1", "chr2", "chr1", "chr3"), c(1, 3, 2, 4)), ranges = IRanges(seq(1, 100, by=10)
grs <- shift(grq[c(2,5,6)], 5)
## Run olRanges function
olRanges(query=grq, subject=grs, output="df")
olRanges(query=grq, subject=grs, output="gr")
```

output_update

Updates the output files paths in the SYSargs2 object

Description

After executing all the command-lines by the runCommandline function, the output files can be created in specific directories rather than results in a particular directory. Also, the runCommandline function allows converting the SAM file outputs to sorted and indexed BAM files. Thus, the output_update function allows updating the location of these files in the output of the SYSargs2 object.

Usage

```
output_update(args, dir = FALSE, dir.name = NULL, replace = FALSE, extension = NULL, make_bam=FALSE)
```

Arguments

args	object of class SYSargs2.
dir	assign TRUE to update the location of the output files in the args object accordingly with the workflow name directory. Default is dir=FALSE.
dir.name	if the results directory name is not specified in the input file, it is possible to specify here the name. This argument is required if the path name return NULL from the input file. Default is dir.name=NULL.
replace	replace the extension for selected output files in the args object. Default is replace=FALSE.
extension	object of class "character" storing the current extension of the files and the respective replacement. For example, runCommandLine function by default autodetects SAM file outputs in the args object and create the BAM files. In order to update the output of args object, the extension argument should be set: extension = c(".sam", ".bam").
make_bam	Auto detects SAM file outputs and update them on the SYSargs2 object for sorted and indexed BAM files. Default is make_bam=FALSE. This argument should be used in integration with runCommandLine function.

Value

SYSargs2 object with output location files updated.

Author(s)

Daniela Cassol and Thomas Girke

See Also

To check directory name in the input file: `yamlinput(WF)$results_path$path`.

Examples

```
## Construct SYSargs2 object from CWL param, CWL input, and targets files
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl",
                  input_file="hisat2-mapping-se.yml", dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
WF
output(WF)

## Not run:
runCommandLine(args=WF, make_bam=TRUE)
## Output paths update
WF <- output_update(WF, dir=FALSE, replace=TRUE, extension=c(".sam", ".bam"))

runCommandLine(args=WF, make_bam=TRUE, dir=TRUE)
## Output paths update
WF <- output_update(WF, dir=TRUE, replace=TRUE, extension=c(".sam", ".bam"))
```

```
## End(Not run)
```

```
overLapper
```

```
Set Intersect and Venn Diagram Functions
```

Description

Function for computing Venn intersects or standard intersects among large numbers of label sets provided as list of vectors. The resulting intersect objects can be used for plotting 2-5 way Venn diagrams or intersect bar plots using the functions `vennPlot` or `olBarplot`, respectively. The `overLapper` function scales to 2-20 or more label vectors for Venn intersect calculations and to much larger sample numbers for standard intersects. The different intersect types are explained below under the definition of the `type` argument. The upper Venn limit around 20 label sets is unavoidable because the complexity of Venn intersects increases exponentially with the label set number n according to this relationship: $2^n - 1$. The current implementation of the plotting function `vennPlot` supports Venn diagrams for 2-5 label sets. To visually analyze larger numbers of label sets, a variety of intersect methods are introduced in the `olBarplot` help file. These methods are much more scalable than Venn diagrams, but lack their restrictive intersect logic.

Usage

```
overLapper(setlist, complexity = "default", sep = "_", cleanup = FALSE, keepdups = FALSE, type)
```

Arguments

<code>setlist</code>	Object of class <code>list</code> where each list component stores a label set as vector and the name of each label set is stored in the name slot of each list component. The names are used for naming the label sets in all downstream analysis steps and plots.
<code>complexity</code>	Complexity level of intersects specified as integer vector. For Venn intersects it needs to be assigned <code>1:length(setlist)</code> (default). If <code>complexity=2</code> the function returns all pairwise intersects.
<code>sep</code>	Character used to separate set labels.
<code>cleanup</code>	If set to <code>TRUE</code> then all characters of the label sets are set to upper case, and leading and trailing spaces are removed. The default <code>cleanup=FALSE</code> omits this step.
<code>keepdups</code>	By default all duplicates are removed from the label sets. The setting <code>keepdups=TRUE</code> will retain duplicates by appending a counter to each entry.
<code>type</code>	With the default setting <code>type="vennsets"</code> the <code>overLapper</code> function computes the typical Venn intersects for the label sets provided under <code>setlist</code> . With the setting <code>type="intersects"</code> the function will compute pairwise intersects (not compatible with Venn diagrams). Venn intersects follow the typical 'only in' intersect logic of Venn comparisons, such as: labels present only in set A, labels present only in the intersect of A & B, etc. Due to this restrictive intersect

logic, the combined Venn sets contain no duplicates. In contrast to this, regular intersects follow this logic: labels present in the intersect of A & B, labels present in the intersect of A & B & C, etc. This approach results usually in many duplications of labels among the intersect sets.

Details

Additional Venn diagram resources are provided by the packages `limma`, `gplots`, `venerable`, `eVenn` and `VennDiagram`, or online resources such as `shapes`, `Venn Diagram Generator` and `Venny`.

Value

`overLapper` returns standard intersect and Venn intersect results as `INTERSECTset` or `VENNset` objects, respectively. These S4 objects contain the following components:

<code>setlist</code>	Original label sets accessible with <code>setlist()</code> .
<code>intersectmatrix</code>	Present-absent matrix accessible with <code>intersectmatrix()</code> , where each overlap set in the <code>vennlist</code> data component is labeled according to the label names provided under <code>setlist</code> . For instance, the composite name 'ABC' indicates that the entries are restricted to A, B and C. The separator used for naming the intersect sets can be specified under the <code>sep</code> argument.
<code>complexitylevels</code>	Complexity levels accessible with <code>complexitylevels()</code> .
<code>vennlist</code>	Venn intersects for <code>VENNset</code> objects accessible with <code>vennlist()</code> .
<code>intersectlist</code>	Standard intersects for <code>INTERSECTset</code> objects accessible with <code>intersectlist()</code> .

Note

The functions provided here are an extension of the Venn diagram resources on this site: <http://manuals.bioinformatics.ucr.edu/Venn-Diagrams>

Author(s)

Thomas Girke

References

See examples in 'The Electronic Journal of Combinatorics': <http://www.combinatorics.org/files/Surveys/ds5/VennSymmExam>

See Also

`vennPlot`, `olBarplot`

Examples

```

## Sample data
setlist <- list(A=sample(letters, 18), B=sample(letters, 16),
               C=sample(letters, 20), D=sample(letters, 22),
               E=sample(letters, 18), F=sample(letters, 22))

## 2-way Venn diagram
vennset <- overLapper(setlist[1:2], type="vennsets")
vennPlot(vennset)

## 3-way Venn diagram
vennset <- overLapper(setlist[1:3], type="vennsets")
vennPlot(vennset)

## 4-way Venn diagram
vennset <- overLapper(setlist[1:4], type="vennsets")
vennPlot(list(vennset, vennset))

## Pseudo 4-way Venn diagram with circles
vennPlot(vennset, type="circle")

## 5-way Venn diagram
vennset <- overLapper(setlist[1:5], type="vennsets")
vennPlot(vennset)

## Alternative Venn count input to vennPlot (not recommended!)
counts <- sapply(vennlist(vennset), length)
vennPlot(counts)

## 6-way Venn comparison as bar plot
vennset <- overLapper(setlist[1:6], type="vennsets")
olBarplot(vennset, mincount=1)

## Bar plot of standard intersect counts
intersect <- overLapper(setlist, type="intersects")
olBarplot(intersect, mincount=1)

## Accessor methods for VENNset/INTERSECTset objects
names(vennset)
names(intersect)
setlist(vennset)
intersectmatrix(vennset)
complexitylevels(vennset)
vennlist(vennset)
intersectlist(intersect)

## Coerce VENNset/INTERSECTset object to list
as.list(vennset)
as.list(intersect)

## Pairwise intersect matrix and heatmap
olMA <- sapply(names(setlist),

```



```

function(x) sapply(names(setlist),
function(y) sum(setlist[[x]] %in% setlist[[y]])))
olMA
heatmap(olMA, Rowv=NA, Colv=NA)

## Presence-absence matrices for large numbers of sample sets
intersect <- overLapper(setlist=setlist, type="intersects", complexity=2)
(paMA <- intersectmatrix(intersect))
heatmap(paMA, Rowv=NA, Colv=NA, col=c("white", "gray"))

```

plotfeatureCoverage *Plot feature coverage results*

Description

Plots the 3 tabular data types (A-C) generated by the featureCoverage function. It accepts data from single or many features (e.g. CDSs) and samples (BAM files). The coverage from multiple features will be summarized using methods such as mean, while the data from multiple samples will be plotted in separate panels.

Usage

```
plotfeatureCoverage(covMA, method = mean, scales = "fixed", extendylim=2, scale_count_val = 10^6)
```

Arguments

covMA	Object of class <code>data.frame</code> generated by featureCoverage function.
method	Defines the summary statistics to use when covMA contains coverage data from multiple features (e.g. transcripts). The default calculates the mean coverage for each position and/or bin of the corresponding coverage vectors.
scales	Scales setting passed on to the <code>facet_wrap</code> function of <code>ggplot2</code> . For details see <code>ggplot2::facet_wrap</code> . The default <code>fixed</code> assures a constant scale across all bar plot panels, while <code>free</code> uses the optimum scale within each bar plot panel. To evaluate plots in all their details, it may be necessary to generate two graphics files one for each scaling option.
extendylim	Allows to extend the upper limit of the y axis when <code>scales=fixed</code> . Internally, the function identifies the maximum value in the data and then multiplies this maximum value by the value provided under <code>extendylim</code> . The default is set to <code>extendylim=2</code> .
scale_count_val	Scales (normalizes) the read counts to a fixed value of aligned reads in each sample such as counts per million aligned reads (default is 10^6). For this calculation the <code>N_total_aligned</code> values are used that are reported in the input <code>data.frame</code> generated by the upstream featureCoverage function. Assign <code>NULL</code> to turn off scaling.

Value

Currently, the function returns ggplot2 bar plot graphics.

Author(s)

Thomas Girke

See Also

featureCoverage

Examples

```
## Construct SYSargs object from param and targets files
param <- system.file("extdata", "tophat.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)

## Not run:
## Features from sample data of systemPipeRdata package
library(GenomicFeatures)
file <- system.file("extdata/annotation", "tair10.gff", package="systemPipeRdata")
txdb <- makeTxDbFromGFF(file=file, format="gff3", organism="Arabidopsis")

## (A) Generate binned coverage for two BAM files and 4 transcripts
grl <- cdsBy(txdb, "tx", use.names=TRUE)
fcov <- featureCoverage(bfl=BamFileList(outpaths(args)[1:2]), grl=grl[1:4], resizereads=NULL,
  readlengthrange=NULL, Nbins=20, method=mean, fixedmatrix=FALSE,
  resizefeatures=TRUE, upstream=20, downstream=20)
fcov <- featureCoverage(bfl=BamFileList(outpaths(args)[1:2]), grl=grl[1:4], resizereads=NULL,
  readlengthrange=NULL, Nbins=20, method=mean, fixedmatrix=TRUE,
  resizefeatures=TRUE, upstream=20, downstream=20)
plotfeatureCoverage(covMA=fcov, method=mean, scales="fixed", scale_count_val=10^6)

## (B) Coverage matrix upstream and downstream of start/stop codons
fcov <- featureCoverage(bfl=BamFileList(outpaths(args)[1:2]), grl=grl[1:4], resizereads=NULL,
  readlengthrange=NULL, Nbins=NULL, method=mean, fixedmatrix=TRUE,
  resizefeatures=TRUE, upstream=20, downstream=20)
plotfeatureCoverage(covMA=fcov, method=mean, scales="fixed", scale_count_val=10^6)

## (C) Combined matrix for both binned and start/stop codon
fcov <- featureCoverage(bfl=BamFileList(outpaths(args)[1:2]), grl=grl[1:4], resizereads=NULL,
  readlengthrange=NULL, Nbins=20, method=mean, fixedmatrix=TRUE,
  resizefeatures=TRUE, upstream=20, downstream=20, outfile="results/test.xls")
plotfeatureCoverage(covMA=fcov, method=mean, scales="fixed", scale_count_val=10^6)

## (D) Rle coverage objects one for each query feature
fcov <- featureCoverage(bfl=BamFileList(outpaths(args)[1:2]), grl=grl[1:4], resizereads=NULL,
  readlengthrange=NULL, Nbins=NULL, method=mean, fixedmatrix=FALSE,
  resizefeatures=TRUE, upstream=20, downstream=20)
```

```
## End(Not run)
```

plotfeaturetypeCounts *Plot read distribution across genomic features*

Description

Function to visualize the distribution of reads across different feature types for many alignment files in parallel. The plots are stacked bar plots representing the raw or normalized read counts for the sense and antisense strand of each feature. The graphics results are generated with ggplot2. Typically, the expected input is generated with the affiliated featuretypeCounts function.

Usage

```
plotfeaturetypeCounts(x, graphicsfile, graphicsformat = "pdf", scales = "fixed", anyreadlength = FALSE,  
                      drop_N_total_aligned = TRUE, scale_count_val = 10^6, scale_length_val = NULL)
```

Arguments

- | | |
|----------------------|---|
| x | data.frame with feature counts generated by the featuretypeCounts function. |
| graphicsfile | Path to file where to write the output graphics. Note, the function returns the graphics instructions from ggplot2 for interactive plotting in R. However, due to the complexity of the graphics generated here, the finished results are written to a file directly. |
| graphicsformat | Graphics file format. Currently, supported formats are: pdf, png or jpeg. Argument accepts one of them as character string. |
| scales | Scales setting passed on to the facet_wrap function of ggplot2. For details see ggplot2::facet_wrap. The default fixed assures a constant scale across all bar plot panels, while free uses the optimum scale within each bar plot panel. To evaluate plots in all their details, it may be necessary to generate two graphics files one for each scaling option. |
| anyreadlength | If set to TRUE read length specific read counts will be summed up to a single count value to plot read counts for any read length. Otherwise the bar plots will show the counts for each read length value. |
| drop_N_total_aligned | If set to TRUE the special feature count N_total_aligned will not be included as a separate feature in the plots. However, the information will still be used internally for scaling the read counts to a fixed value if this option is requested under the scale_count_val argument. |
| scale_count_val | Scales (normalizes) the read counts to a fixed value of aligned reads in each sample such as counts per million aligned reads (default is 10^6). For this calculation the N_total_aligned values are used that are reported in the input data.frame generated by the upstream featuretypeCounts function. Assign NULL to turn off scaling by aligned reads. |

scale_length_val

Allows to adjust the raw or scaled read counts to a constant length interval (e.g. `scale_length_val=10^3` in bps) considering the total genomic length of the corresponding feature type. The required genomic length information for each feature type is obtained from the `Featuretypelength` column of the input `data.frame` generated by the `featuretypeCount` function. To turn off feature length adjustment, assign `NULL` (default).

Value

The function returns bar plot graphics for aligned read counts with read length resolution if the input contains this information and argument `anyreadlength` is set to `FALSE`. If the input contains counts for any read length and/or `anyreadlength=TRUE` then there will be only one bar per feature and sample. Due to the complexity of the plots, the results are directly written to file in the chosen graphics format. However, the function also returns the plotting instructions returned by `ggplot2` to display the result components using R's plotting device.

Author(s)

Thomas Girke

See Also

`featuretypeCounts`, `genFeatures`

Examples

```
## Construct SYSargs object from param and targets files
param <- system.file("extdata", "tophat.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)

## Not run:
## Features from sample data of systemPipeRdata package
library(GenomicFeatures)
file <- system.file("extdata/annotation", "tair10.gff", package="systemPipeRdata")
txdb <- makeTxDbFromGFF(file=file, format="gff3", organism="Arabidopsis")
feat <- genFeatures(txdb, featuretype="all", reduce_ranges=TRUE, upstream=1000, downstream=0, verbose=TRUE)

## Generate and plot feature counts for specific read lengths
fc <- featuretypeCounts(bfl=BamFileList(outpaths(args), yieldSize=50000), grl=feat, singleEnd=TRUE, readlength=c(
p <- plotfeaturetypeCounts(x=fc, graphicsfile="featureCounts.pdf", graphicsformat="pdf", scales="fixed", anyread

## Generate and plot feature counts for any read length
fc2 <- featuretypeCounts(bfl=BamFileList(outpaths(args), yieldSize=50000), grl=feat, singleEnd=TRUE, readlength=
p2 <- plotfeaturetypeCounts(x=featureCounts2, graphicsfile="featureCounts2.pdf", graphicsformat="pdf", scales="f

## End(Not run)
```

plotWF	<i>Graphviz Plot Workflow</i>
--------	-------------------------------

Description

Graphviz Plot Workflow

Usage

```
plotWF(sysargslist, plot_style = "detect", out_type = "html", out_path = "default", height = NULL, width = NULL)
```

Arguments

sysargslist	data frame, the standard df generated from subsetRmd function
plot_style	one of 'detect', 'none', 'linear'
out_type	choose from 'html', 'svg', 'png'
out_path	string, path of output plot, only apply from svg or png
height	int, height of svg or png in pixels, default NULL, automatic
width	int, width of svg or png in pixels, default NULL, automatic

Author(s)

Le Zhang and Daniela Cassol

Examples

```
## Not run:  
## Construct SYSargsList object from Rmd file  
library(systemPipeRdata)  
script <- system.file("extdata/workflows/rnaseq", "systemPipeRNAseq.Rmd", package="systemPipeRdata")  
targets <- system.file("extdata", "targets.txt", package="systemPipeR")  
dir_path <- tempdir()  
SYSconfig <- initProject(projPath=dir_path, targets=targets, script=script, overwrite = TRUE)  
sysargslist <- initWF(sysconfig = "SYSconfig.yml")  
plotWF(sysargslist, plot_style = "none", out_type = "png")  
  
## End(Not run)
```

predORF *Predict ORFs*

Description

Predicts open reading frames (ORFs) and coding sequences (CDSs) in DNA sequences provided as DNASTring or DNASTringSet objects.

Usage

```
predORF(x, n = 1, type = "gr1", mode = "orf", strand = "sense", longest_disjoint=FALSE, startcodon = "ATG")
```

Arguments

x	DNA query sequence(s) provided as DNASTring or DNASTringSet object.
n	Defines the maximum number of ORFs to return for each input sequence. The ORFs identified are sorted decreasingly by their length. For instance, n=1 (default) returns the longest ORF, n=2 the two longest ones, and so on.
type	One of three options provided as character values: 'df' returns results as data.frame, while 'gr' and 'gr1' (default) return them as GRanges or GRangesList objects, respectively.
mode	The setting mode='ORF' returns a continuous reading frame that begins with a start codon and ends with a stop codon. The setting mode='CDS' return continuous reading frames that do not need to begin or end with start or stop codons, respectively.
strand	One of three options passed on as character vector of length one: 'sense' performs the predictions only for the sense strand of the query sequence(s), 'antisense' does it only for the antisense strand and 'both' does it for both strands.
longest_disjoint	If set to TRUE and n='all', the results will be subsetted to non-overlapping ORF set containing longest ORF.
startcodon	Defines the start codon(s) for ORF predictions. The default is set to the standard start codon 'ATG'. Any custom set of triplet DNA sequences can be assigned here.
stopcodon	Defines the stop codon(s) for ORF predictions. The default is set to the three standard stop codons 'TAA', 'TAG' and 'TGA'. Any custom set of triplet DNA sequences can be assigned here.

Value

Returns ORF/CDS ranges identified in query sequences as GRanges or data.frame object. The type argument defines which one of them will be returned. The objects contain the following columns:

- seqnames: names of query sequences

- `subject_id`: identified ORF/CDS ranges numbered by query
- `start/end`: start and end positions of ORF/CDS ranges
- `strand`: strand of query sequence used for prediction
- `width`: length of subject range in bases
- `inframe2end`: frame of identified ORF/CDS relative to 3' end of query sequence. This can be important if the query sequence was extracted directly upstream of an ORF (e.g. 5' UTR upstream of main ORF). The value 1 stands for in-frame with downstream ORF, while 2 or 3 indicates a shift of one or two bases, respectively.

Author(s)

Thomas Girke

See Also

`scaleRanges`

Examples

```
## Load DNA sample data set from Biostrings package
file <- system.file("extdata", "someORF.fa", package="Biostrings")
dna <- readDNAStringSet(file)

## Predict longest ORF for sense strand in each query sequence
(orf <- predORF(dna[1:4], n=1, type="gr", mode="orf", strand="sense"))

## Not run:
## Usage for more complex example
library(GenomicFeatures); library(systemPipeRdata)
gff <- system.file("extdata/annotation", "tair10.gff", package="systemPipeRdata")
txdb <- makeTxDbFromGFF(file=gff, format="gff3", organism="Arabidopsis")
futr <- fiveUTRsByTranscript(txdb, use.names=TRUE)
genome <- system.file("extdata/annotation", "tair10.fasta", package="systemPipeRdata")
dna <- extractTranscriptSeqs(FaFile(genome), futr)
uorf <- predORF(dna, n="all", mode="orf", longest_disjoint=TRUE, strand="sense")
grl_scaled <- scaleRanges(subject=futr, query=uorf, type="uORF", verbose=TRUE)
export.gff3(unlist(grl_scaled), "uorf.gff")

## End(Not run)
```

```
preprocessReads
```

```
Run custom read preprocessing functions
```

Description

Applies custom read preprocessing functions to single-end or paired-end FASTQ files. The function uses the `FastqStreamer` function from the `ShortRead` package to stream through large files in a memory-efficient manner.

Usage

```
preprocessReads(args, Fct, batchsize = 1e+05, overwrite = TRUE, ...)
```

Arguments

args	Object of class SYSargs or SYSargs2.
Fct	character string of custom read preprocessing function call where both the input and output needs to be an object of class ShortReadQ. The name of the input ShortReadQ object needs to be fq.
batchsize	Number of reads to process in each iteration by the internally used FastqStreamer function.
overwrite	If TRUE existing file will be overwritten.
...	To pass on additional arguments to the internally used writeFastq function.

Value

Writes to files in FASTQ format. Their names are specified by `outpaths(args)`.

Author(s)

Thomas Girke

See Also

FastqStreamer

Examples

```
## Preprocessing of single-end reads
param <- system.file("extdata", "trim.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
## Not run:
preprocessReads(args=args, Fct="trimLRPatterns(Rpattern='GCCCGGTAA', subject=fq)", batchsize=100000, overwrite=TRUE)
## End(Not run)

## Preprocessing of paired-end reads
param <- system.file("extdata", "trimPE.param", package="systemPipeR")
targets <- system.file("extdata", "targetsPE.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
## Not run:
preprocessReads(args=args, Fct="trimLRPatterns(Rpattern='GCCCGGTAA', subject=fq)", batchsize=100000, overwrite=TRUE)
## End(Not run)
```

printParam *Accessories function to modify the Command-line*

Description

Accessories function to modify the Command-line

Usage

```
printParam(sysargs, position, index = NULL)
subsetParam(sysargs, position, index = NULL, trim = TRUE, mute = FALSE)
replaceParam(sysargs, position, index = NULL, replace, mute = FALSE)
renameParam(sysargs, position, index = FALSE, rename, mute = FALSE)
appendParam(sysargs, position, index = NULL, append, after, mute = FALSE)
```

Arguments

sysargs	Object of class SYSargs2. Output from the createParamFiles function.
position	string, one of baseCommand, inputs, outputs to view or apply a modification.
index	numeric or character vector, index to view or change a single item in baseCommand, inputs, outputs.
trim	logical, only keep arguments specified by index. Default is "TRUE".
replace	named list, replace arguments in different positions. Replace list length must be the same as index. Different positions will have different requirements.
rename	character vector, rename items in different positions. rename vector length must be the same as index.
append	named list, same requirements as replace, however it cannot append baseCommand.
after	a subscript, after which the values are to be appended. If NULL will be after the last argument or specify a numeric integer.
mute	logical, print the raw command-line string and output after replacing or rename.

Details

- printParam: prints its arguments defined by position and index.
- subsetParam: returns subsets of command-line, keeping the arguments defined by position and index.
- replaceParam: replaces the values in command-line with indices given in list by those given in values
- renameParam: rename the names of the arguments.
- appendParam: Add arguments to the original command line.

Value

SYSargs2 object

Author(s)

Le Zhang and Daniela Cassol

References

For more details on CWL, please consult the following page: <https://www.commonwl.org/>

See Also

`writeParamFiles createParamFiles loadWorkflow renderWF showClass("SYSargs2")`

Examples

```
command <- "
hisat2 \
  -S <F, out: ./results/M1A.sam> \
  -x <F: ./data/tair10.fasta> \
  -k <int: 1> \
  -min-intronlen <int: 30> \
  -max-intronlen <int: 3000> \
  -threads <int: 4> \
  -U <F: ./data/SRR446027_1.fastq.gz> \
  --verbose
"
cmd <- createParamFiles(command)
cmdlist(cmd)
```

qsubRun

Submit command-line tools to cluster

Description

Note: This function has been deprecated. Please use `clusterRun` instead. `qsubRun` submits command-line tools to queue (e.g. Torque) of compute cluster using run specifications defined by `runX` and `getQsubargs` functions.

Usage

```
qsubRun(appfct="runCommandline(args=args, runid='01')", args, qsubargs, Nqsubs = 1, package = "systemP
```

Arguments

<code>appfct</code>	Accepts <code>runX</code> functions, such as <code>appfct="runCommandline(args,runid)"</code>
<code>args</code>	Argument list returned by <code>systemArgs()</code> .
<code>qsubargs</code>	Argument list returned by <code>getQsubargs()</code> .

Nqsubs	Integer defining the number of qsub processes. Note: the function will not assign more qsub processes than there are FASTQ files. E.g. if there are 10 FASTQ files and Nqsubs=20 then the function will generate only 10 qsub processes. To increase the number of CPU cores used by each process, one can increase the p value under systemArgs().
package	Package to load. Name provided as character vector of length one. Default is sytemPipeR.
shebang	defines shebang (fist line) used in submission shell script; default is set to #!/bin/bash.

Value

Returns list where list components contain FASTQ file names and their names are the qsub process IDs assigned by the queuing system. In addition, three files will be generated for each qsub submission process: submitargs0X (R object containing appargs), submitargs0X.R (R script using appargs) and submitargs0X.sh (shell submission script). In addition, the chosen runX function will output a submitargs0X_log file for each qsub process containing the executable commands processed by each qsub instance.

Author(s)

Thomas Girke

Examples

```
## Construct SYSargs object from param and targets files
param <- system.file("extdata", "tophat.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
args
names(args); modules(args); cores(args); outpaths(args); sysargs(args)

## Not run:
## Execute SYSargs on single machine
runCommandline(args=args)

## Execute SYSargs on multiple machines
qsubargs <- getQsubargs(queue="batch", Nnodes="nodes=1", cores=cores(tophat), memory="mem=10gb", time="walltime=")
qsubRun(args=args, qsubargs=qsubargs, Nqsubs=1, package="systemPipeR")
## Alignment stats
read_statsDF <- alignStats(fqpaths=tophatargs$infile1, bampaths=bampaths, fqgz=TRUE)
read_statsDF <- cbind(read_statsDF[targets$FileName,], targets)
write.table(read_statsDF, "results/alignStats.xls", row.names=FALSE, quote=FALSE, sep="\t")

## End(Not run)
```

`readComp`*Import sample comparisons from targets file*

Description

Parses sample comparisons specified in <CMP> line(s) of targets file or in targetsheader slot of SYSargs object. All possible comparisons can be specified with 'CMPset: ALL'.

Usage

```
readComp(file, format = "vector", delim = "-")
```

Arguments

file	Path to targets file. Alternatively, a SYSargs or SYSargs2 object can be assigned.
format	Object type to return: vector or matrix.
delim	Delimiter to use when sample comparisons are returned as vector.

Value

list where each component is named according to the name(s) used in the <CMP> line(s) of the targets file. The list will contain as many sample comparisons sets (list components) as there are sample comparisons lines in the corresponding targets file.

Author(s)

Thomas Girke

Examples

```
## Return comparisons from targets file
targetspath <- system.file("extdata", "targets.txt", package="systemPipeR")
read.delim(targetspath, comment.char = "#")
readComp(file=targetspath, format="vector", delim="-")

## Return comparisons from SYSargs object
param <- system.file("extdata", "tophat.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
readComp(args, format = "vector", delim = "-")
```

renderReport	<i>Render RMarkdown Report</i>
--------------	--------------------------------

Description

Render the input file to the specified output format using pandoc.

Usage

```
renderReport(sysargslist, type = c("html_document"), silent = FALSE)
```

Arguments

sysargslist	object of class SYSargsList.
type	The R Markdown output format to convert to. Default is "ALL".The option can be the name of a format (e.g. "pdf_document" or "html_document").
silent	If set to TRUE, all messages returned by the function will be suppressed.

Value

It will return an SYSargsList updated.

Author(s)

Daniela Cassol and Thomas Girke

See Also

See also as SYSargsList-class.

Examples

```
## Not run:
## Construct SYSargsList object from Rmd file
library(systemPipeRdata)
script <- system.file("extdata/workflows/rnaseq", "systemPipeRNAseq.Rmd", package="systemPipeRdata")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- tempdir()
SYSconfig <- initProject(projPath=dir_path, targets=targets, script=script, overwrite = TRUE)
sysargslist <- initWF(sysconfig ="SYSconfig.yml")
sysargslist <- configWF(x=sysargslist, input_steps = "1:3")
sysargslist <- runWF(sysargslist = sysargslist, steps = "ALL")
renderReport(x=sysargslist)

## End(Not run)
```

renderWF	<i>Populate all the command-line in an SYSargs2 object</i>
----------	--

Description

The SYSargs2 S4 class object is constructed from the loadWorkflow, which stores all the information and instructions needed for processing a set of input files with a specific command-line or a series of command-line within a workflow. The renderWF function populates all the command-line for each sample in each step of the particular workflow. Each sample level input/outfile operation uses its own SYSargs2 instance. The output of SYSargs2 define all the expected output files for each step in the workflow, which usually it is the sample input for the next step in an SYSargs2 instance. Between different instances, this connectivity is established by writing the subsetting output with the writeTargetsout function to a new targets file that serves as input to the next loadWorkflow and renderWF call. By chaining several SYSargs2 steps together one can construct complex workflows involving many sample-level input/output file operations with any combination of command-line or R-based software.

Usage

```
renderWF(WF, inputvars = NULL)
```

Arguments

WF	Object of class SYSargs2.
inputvars	variables list defined in the input file that matches the column names defined in the targets file.

Value

SYSargs2 object

Author(s)

Daniela Cassol and Thomas Girke

See Also

showClass("SYSargs2") loadWorkflow writeTargetsout

Examples

```
## Construct SYSargs2 object from CWL param, CWL input, and targets files
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl",
                  input_file="hisat2-mapping-se.yml", dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
WF
```

returnRPKM	<i>RPKM Normalization</i>
------------	---------------------------

Description

Converts read counts to RPKM normalized values.

Usage

```
returnRPKM(counts, ranges)
```

Arguments

counts	Count data frame, e.g. from an RNA-Seq experiment.
ranges	GRangesList object, e.g. generated by exonsBy(txdb, by="gene").

Value

data.frame

Author(s)

Thomas Girke

Examples

```
## Not run:  
countDFrpkm <- apply(countDF, 2, function(x) returnRPKM(counts=x, gffsub=eByg))  
  
## End(Not run)
```

runCommandline	<i>Execute SYSargs and SYSargs2</i>
----------------	-------------------------------------

Description

Function to execute system parameters specified in SYSargs and SYSargs2 object.

Usage

```
runCommandline(args, runid = "01", make_bam = TRUE, del_sam=TRUE, dir = FALSE, dir.name = NULL, force=FA
```

Arguments

args	object of class SYSargs or SYSargs2.
runid	Run identifier used for log file to track system call commands. Default is "01".
make_bam	Auto detects SAM file outputs and converts them to sorted and indexed BAM files. Default is make_bam=TRUE.
del_sam	This option allows deleting the SAM files created when the make_BAM converts the SAM files to sorted and indexed BAM files. Default is del_sam=TRUE.
dir	This option allows creating an exclusive results folder for each step in the workflow and a sub-folder for each sample defined in the targets file. All the outputs and log files for the particular step will be created in the respective folders. Default is dir=FALSE. Option available only for an object of class SYSargs2.
dir.name	Name of the workflow directory. Default is dir.name=FALSE. Note: This argument is required when the dir=TRUE.
force	Internally, the function checks if the expected output files exist, and it skips the command lines when the respective files exist. If the argument force is set to TRUE, the command line will be executed and the files overwrite. Default is force=FALSE.
...	Additional arguments to pass on to runCommandline().

Value

Output files, their paths can be obtained with `outpaths()` from `SYSargs` container or `output()` from `SYSargs2`. In addition, a character vector is returned containing the same paths.

Author(s)

Daniela Cassol and Thomas Girke

Examples

```
#####
## Examples with \code{SYSargs} object ##
#####
## Construct SYSargs object from param and targets files
param <- system.file("extdata", "hisat2.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
args
names(args); modules(args); cores(args); outpaths(args); sysargs(args)

## Not run:
## Execute SYSargs on single machine
runCommandline(args=args)

## Execute SYSargs on multiple machines of a compute cluster.
file.copy(system.file("extdata", ".batchtools.conf.R", package="systemPipeR"), ".")
file.copy(system.file("extdata", "batchtools.slurm.tmpl", package="systemPipeR"), ".")
resources <- list(walltime=120, ntasks=1, ncpus=cores(args), memory=1024)
```



```

reg <- clusterRun(args, FUN = runCommandline, conffile=".batchtools.conf.R", template="batchtools.slurm.tpl", Nj

## Monitor progress of submitted jobs
getStatus(reg=reg)
file.exists(outpaths(args))

## Alignment stats
read_statsDF <- alignStats(args)
read_statsDF <- cbind(read_statsDF[target$FileName,], targets)
write.table(read_statsDF, "results/alignStats.xls", row.names=FALSE, quote=FALSE, sep="\t")

## End(Not run)

#####
## Examples with \code{SYSargs2} object ##
#####
## Construct SYSargs2 object from CWL param, CWL input, and targets files
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl",
  input_file="hisat2-mapping-se.yml", dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
WF
names(WF); modules(WF); targets(WF)[1]; cmdlist(WF)[1:2]; output(WF)

## Not run:
## Execute SYSargs2 on single machine
WF <- runCommandline(args=WF)

## Execute SYSargs on multiple machines of a compute cluster.
file.copy(system.file("extdata", ".batchtools.conf.R", package="systemPipeR"), ".")
file.copy(system.file("extdata", "batchtools.slurm.tpl", package="systemPipeR"), ".")
resources <- list(walltime=120, ntasks=1, ncpus=4, memory=1024)
reg <- clusterRun(WF, FUN = runCommandline, more.args = list(args = WF, make_bam = TRUE), conffile=".batchtools.conf.R", Nj

## Monitor progress of submitted jobs
getStatus(reg=reg)

## Updates the path in the object \code{output(WF)}
WF <- output_update(WF, dir=FALSE, replace=TRUE, extension=c(".sam", ".bam"))

## Alignment stats
read_statsDF <- alignStats(WF)
read_statsDF <- cbind(read_statsDF[target$FileName,], targets)
write.table(read_statsDF, "results/alignStats.xls", row.names=FALSE, quote=FALSE, sep="\t")

## End(Not run)

```

Description

Convenience wrapper function for `run_edgeR` and `run_DESeq2` to perform differential expression or abundance analysis iteratively for several count tables. The latter can be peak calling results for several samples or counts generated for different genomic feature types. The function also returns the filtering results and plots from `filterDEGs`.

Usage

```
runDiff(args, diffFct, targets, cmp, dbrfilter, ...)
```

Arguments

<code>args</code>	Object of class <code>SYSargs</code> or <code>SYSargs2</code> where <code>infile1(args)</code> specifies the paths to the tabular read count data files and outputs files to the result files.
<code>diffFct</code>	Defines which function should be used for the differential abundance analysis. Can be <code>diffFct=run_edgeR</code> or <code>diffFct=run_DESeq2</code> .
<code>targets</code>	<code>targets data.frame</code>
<code>cmp</code>	character matrix where comparisons are defined in two columns. This matrix should be generated with <code>readComp()</code> from the targets file. Values used for comparisons need to match those in the <code>Factor</code> column of the targets file.
<code>dbrfilter</code>	Named vector with filter cutoffs of format <code>c(Fold=2, FDR=1)</code> where <code>Fold</code> refers to the fold change cutoff (unlogged) and <code>FDR</code> to the p-value cutoff. Those values are passed on to the <code>filterDEGs</code> function.
<code>...</code>	Arguments to be passed on to the internally used <code>run_edgeR</code> or <code>run_DESeq2</code> function.

Value

Returns list containing the `filterDEGs` results for each count table. Each result set is a list with four components which are described under `?filterDEGs`. The result files contain the `edgeR` or `DESeq2` results from the comparisons specified under `cmp`. The base names of the result files are the same as the corresponding input files specified under `countfiles` and the value of extension appended.

Author(s)

Thomas Girke

See Also

`run_edgeR`, `run_DESeq2`, `filterDEGs`

Examples

```
## Paths to BAM files
param <- system.file("extdata", "bowtieSE.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args_bam <- systemArgs(sysma=param, mytargets=targets)
```

```
bfl <- BamFileList(outpaths(args_bam), yieldSize=50000, index=character())

## Not run:
## SYSargs with paths to range data and count files
args <- systemArgs(sysma="param/count_rangesets.param", mytargets="targets_mac3.txt")

## Iterative read counting
countDFnames <- countRangeset(bfl, args, mode="Union", ignore.strand=TRUE)
writeTargetsout(x=args, file="targets_countDF.txt", overwrite=TRUE)

## Run differential abundance analysis
cmp <- readComp(file=args_bam, format="matrix")
args_diff <- systemArgs(sysma="param/rundiff.param", mytargets="targets_countDF.txt")
dbrlist <- runDiff(args, diffFct=run_edgeR, targets=targetsin(args_bam), cmp=cmp[[1]], independent=TRUE, dbrfilt
writeTargetsout(x=args_diff, file="targets_rundiff.txt", overwrite=TRUE)

## End(Not run)
```

runWF

Execute SYSargsList

Description

Function to execute all the code list specified in SYSargsList object.

Usage

```
runWF(args, warning.stop=FALSE, error.stop=TRUE, silent=FALSE)
```

Arguments

args	object of class SYSargsList.
warning.stop	If set to TRUE, the process will be interrupted when a warning is detected.
error.stop	If set to TRUE, the process will be interrupted when a error is detected.
silent	If set to TRUE, all messages returned by the function will be suppressed.

Value

It will return an SYSargsList updated.

Author(s)

Daniela Cassol and Thomas Girke

See Also

See also as SYSargsList-class.

Examples

```
## Not run:
## Construct SYSargsList object from Rmd file
library(systemPipeRdata)
script <- system.file("extdata/workflows/rnaseq", "systemPipeRNAseq.Rmd", package="systemPipeRdata")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- tempdir()
SYSconfig <- initProject(projPath=dir_path, targets=targets, script=script, overwrite = TRUE)
sysargslist <- initWF(sprconfig ="SPRconfig.yml")
sysargslist <- configWF(x=sysargslist, input_steps = "1:3")
sal <- runWF(args = sysargslist)

## End(Not run)
```

run_DESeq2

Runs DESeq2

Description

Convenience wrapper function to identify differentially expressed genes (DEGs) in batch mode with DESeq2 for any number of pairwise sample comparisons specified under the `cmp` argument. Users are strongly encouraged to consult the DESeq2 vignette for more detailed information on this topic and how to properly run DESeq2 on data sets with more complex experimental designs.

Usage

```
run_DESeq2(countDF, targets, cmp, independent = FALSE, lfcShrink=FALSE, type="normal")
```

Arguments

<code>countDF</code>	date.frame containing raw read counts
<code>targets</code>	targets data.frame
<code>cmp</code>	character matrix where comparisons are defined in two columns. This matrix should be generated with the <code>readComp()</code> function from the targets file. Values used for comparisons need to match those in the Factor column of the targets file.
<code>independent</code>	If <code>independent=TRUE</code> then the <code>countDF</code> will be subsetting for each comparison. This behavior can be useful when working with samples from unrelated studies. For samples from the same or comparable studies, the setting <code>independent=FALSE</code> is usually preferred.
<code>lfcShrink</code>	logical. If <code>TRUE</code> adds shrunken log2 fold changes (LFC) to the object.
<code>type</code>	please check <code>DESeq2:lfcShrink()</code> documentation. Available character alternatives: "apeglm"; "ashr"; "normal".

Value

data.frame containing DESeq2 results from all comparisons. Comparison labels are appended to column titles for tracking.

Author(s)

Thomas Girke

References

Please properly cite the DESeq2 papers when using this function: <http://www.bioconductor.org/packages/devel/bioc/html/DESeq2>

See Also

run_edgeR, readComp and DESeq2 vignette

Examples

```
targetspath <- system.file("extdata", "targets.txt", package="systemPipeR")
targets <- read.delim(targetspath, comment.char = "#")
cmp <- readComp(file=targetspath, format="matrix", delim="-")
countfile <- system.file("extdata", "countDFeByg.xls", package="systemPipeR")
countDF <- read.delim(countfile, row.names=1)
degseqDF <- run_DESeq2(countDF=countDF, targets=targets, cmp=cmp[[1]], independent=FALSE)
pval <- degseqDF[, grep("_FDR$", colnames(degseqDF)), drop=FALSE]
fold <- degseqDF[, grep("_logFC$", colnames(degseqDF)), drop=FALSE]
DEG_list <- filterDEGs(degDF=degseqDF, filter=c(Fold=2, FDR=10))
names(DEG_list)
DEG_list$Summary
```

run_edgeR

*Runs edgeR***Description**

Convenience wrapper function to identify differentially expressed genes (DEGs) in batch mode with the edgeR GML method for any number of pairwise sample comparisons specified under the `cmp` argument. Users are strongly encouraged to consult the edgeR vignette for more detailed information on this topic and how to properly run edgeR on data sets with more complex experimental designs.

Usage

```
run_edgeR(countDF, targets, cmp, independent = TRUE, paired = NULL, mdsplot = "")
```

Arguments

countDF	data.frame containing raw read counts
targets	targets data.frame
cmp	character matrix where comparisons are defined in two columns. This matrix should be generated with readComp() from the targets file. Values used for comparisons need to match those in the Factor column of the targets file.

independent	If independent=TRUE then the countDF will be subsetted for each comparison. This behavior can be useful when working with samples from unrelated studies. For samples from the same or comparable studies, the setting independent=FALSE is usually preferred.
paired	Defines pairs (character vector) for paired analysis. Default is unpaired (paired=NULL).
mdsplot	Directory where plotMDS should be written to. Default setting mdsplot="" will omit the plotting step.

Value

data.frame containing edgeR results from all comparisons. Comparison labels are appended to column titles for tracking.

Author(s)

Thomas Girke

References

Please properly cite the edgeR papers when using this function: <http://www.bioconductor.org/packages/devel/bioc/html/edgeR/>

See Also

run_DESeq2, readComp and edgeR vignette

Examples

```
targetspath <- system.file("extdata", "targets.txt", package="systemPipeR")
targets <- read.delim(targetspath, comment.char = "#")
cmp <- readComp(file=targetspath, format="matrix", delim="-")
countfile <- system.file("extdata", "countDFeByg.xls", package="systemPipeR")
countDF <- read.delim(countfile, row.names=1)
edgeDF <- run_edgeR(countDF=countDF, targets=targets, cmp=cmp[[1]], independent=FALSE, mdsplot="")
pval <- edgeDF[, grep("_FDR$", colnames(edgeDF)), drop=FALSE]
fold <- edgeDF[, grep("_logFC$", colnames(edgeDF)), drop=FALSE]
DEG_list <- filterDEGs(degDF=edgeDF, filter=c(Fold=2, FDR=10))
names(DEG_list)
DEG_list$Summary
```

scaleRanges

Scale spliced ranges to genome coordinates

Description

Function to scale mappings of spliced features (query ranges) to their corresponding genome coordinates (subject ranges). The method accounts for introns in the subject ranges that are absent in the query ranges. A use case example are uORFs predicted in the 5' UTRs sequences using predORF. These query ranges are given relative to the 5' UTR sequence. The scaleRanges function will scale them to the corresponding genome coordinates. This way they can be used in RNA-Seq expression experiments like other gene ranges.

Usage

```
scaleRanges(subject, query, type = "custom", verbose = TRUE)
```

Arguments

subject	Genomic ranges provided as GRangesList object. Their name and length requirements are described under query.
query	Feature level ranges provided as GRangesList object. The names of the query ranges need to match the names of the GRangesList object assigned to the subject argument. In addition, the length of each query range cannot exceed the total length of the corresponding subject range set.
type	Feature name to use in type column of GRangesList result.
verbose	The setting verbose=FALSE suppresses all print messages.

Value

Object of class GRangesList

Author(s)

Thomas Girke

See Also

predORF

Examples

```
## Usage for simple example
subject <- GRanges(seqnames="Chr1", IRanges(c(5,15,30),c(10,25,40)), strand="+")
query <- GRanges(seqnames="myseq", IRanges(1, 9), strand="+")
scaleRanges(GRangesList(myid1=subject), GRangesList(myid1=query), type="test")

## Not run:
## Usage for more complex example
library(GenomicFeatures); library(systemPipeRdata)
gff <- system.file("extdata/annotation", "tair10.gff", package="systemPipeRdata")
txdb <- makeTxDbFromGFF(file=gff, format="gff3", organism="Arabidopsis")
futr <- fiveUTRsByTranscript(txdb, use.names=TRUE)
genome <- system.file("extdata/annotation", "tair10.fasta", package="systemPipeRdata")
dna <- extractTranscriptSeqs(FaFile(genome), futr)
uorf <- predORF(dna, n="all", mode="orf", longest_disjoint=TRUE, strand="sense")
grl_scaled <- scaleRanges(subject=futr, query=uorf, type="uORF", verbose=TRUE)
export.gff3(unlist(grl_scaled), "uorf.gff")

## End(Not run)
```

`seeFastq`*Quality reports for FASTQ files*

Description

The following `seeFastq` and `seeFastqPlot` functions generate and plot a series of useful quality statistics for a set of FASTQ files including per cycle quality box plots, base proportions, base-level quality trends, relative k-mer diversity, length and occurrence distribution of reads, number of reads above quality cutoffs and mean quality distribution. The functions allow processing of reads with variable length, but most plots are only meaningful if the read positions in the FASTQ file are aligned with the sequencing cycles. For instance, constant length clipping of the reads on either end or variable length clipping on the 3' end maintains this relationship, while variable length clipping on the 5' end without reversing the reads erases it.

The function `seeFastq` computes the summary stats and stores them in a relatively small list object that can be saved to disk with `save()` and reloaded with `load()` for later plotting. The argument 'klength' specifies the k-mer length and 'batchsize' the number of reads to random sample from each fastq file.

Usage

```
seeFastq(fastq, batchsize, klength = 8)
```

```
seeFastqPlot(fqlist, arrange = c(1, 2, 3, 4, 5, 8, 6, 7), ...)
```

Arguments

<code>fastq</code>	Named character vector containing paths to FASTQ file in the data fields and sample labels in the name slots.
<code>batchsize</code>	Number of reads to random sample from each FASTQ file that will be considered in the QC analysis. Smaller numbers reduce the memory footprint and compute time.
<code>klength</code>	Specifies the k-mer length in the plot for the relative k-mer diversity.
<code>fqlist</code>	list object returned by <code>seeFastq()</code> .
<code>arrange</code>	Integer vector from 1 to 7 specifying the row order of the QC plot. Dropping numbers eliminates the corresponding plots.
<code>...</code>	Additional plotting arguments to pass on to <code>seeFastqPlot()</code> .

Value

The function `seeFastq` returns the summary stats in a list containing all information required for the quality plots. The function `seeFastqPlot` plots the information generated by `seeFastq` using `ggplot2`.

Author(s)

Thomas Girke

Examples

```
## Not run:
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
args <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl",
                    input_file="hisat2-mapping-se.yml", dir_path=dir_path)
args <- renderWF(args, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
fqlist <- seeFastq(fastq=infile1(args), batchsize=10000, klength=8)
pdf("fastqReport.pdf", height=18, width=4*length(fastq))
seeFastqPlot(fqlist)
dev.off()

## End(Not run)
```

 SPRdata

SPRdata

Description

Integrates targets files and count table from **systemPipeR** to a SummarizedExperiment object

Usage

```
SPRdata(counts = SimpleList(), rowData = NULL, rowRanges = GRangesList(),
        cmp = FALSE, targetspath = NULL, SEobj = NULL, SEobjName = "default")
```

Arguments

counts	counts A list or S4Vector::SimpleList() of matrix-like elements, or a matrix-like object (e.g. an ordinary matrix, a data frame, a DataFrame object from the S4Vectors package, etc...). All elements of the list must have the same dimensions, and dimension names (if present) must be consistent across elements and with the row names of rowRanges and colData.
rowData	A DataFrame object describing the rows. Row names, if present become the row names of the SummarizedExperiment object. The number of rows of the DataFrame must equal the number of rows of the matrices in assays.
rowRanges	A GRanges or GRangesList object describing the ranges of interest. Names, if present, become the row names of the SummarizedExperiment object. The length of the GRanges or GRangesList must equal the number of rows of the matrices in assays. If rowRanges is missing, a SummarizedExperiment instance is returned.
cmp	character matrix where comparisons are defined in two columns. This matrix should be generated with the readComp() function from the targets file. Values used for comparisons need to match those in the Factor column of the targets file.
targetspath	targets data.frame

SEobj A SummarizedExperiment object.
SEobjName character vector name for the added assays

Value

A SummarizedExperiment object.

Author(s)

Daniela Cassol

Examples

```
## Not run:
library(systemPipeR)
# Targets file
targetspath <- system.file("extdata", "targets.txt", package="systemPipeR")
targets <- read.delim(targetspath, comment.char="#")
cmp <- readComp(file=targetspath, format="matrix", delim="-")

## Count table file
countMatrixPath <- system.file("extdata", "countDFeByg.xls", package="systemPipeR")
countMatrix <- read.delim(countMatrixPath, row.names=1)

## Create empty SummarizedExperiment
sprSE <- SPRdata()
class(sprSE)
sprSE
metadata(sprSE)

## Create an object with targets file and comparison
sprSE <- SPRdata(cmp=TRUE, targetspath = targetspath)
sprSE
metadata(sprSE)
colData(sprSE)
assays(sprSE)

## Create an object with targets file and comparison and count table
sprSE <- SPRdata(counts = countMatrix, cmp=TRUE, targetspath = targetspath)
sprSE
metadata(sprSE)
colData(sprSE)
assays(sprSE)

## End(Not run)
```

subsetWF	<i>Subsetting SYSargs2 class slots</i>
----------	--

Description

Return subsets of character for the input, output or the list of command-line for each workflow step.

Usage

```
subsetWF(args, slot, subset=NULL, index=NULL, delete=FALSE)
```

Arguments

args	object of class SYSargs2.
slot	three options available: type="input" returns input slot from SYSargs2 object; type="output" returns output slot from SYSargs2 object; and type="step" returns all the command-line for each workflow step from SYSargs2 object.
subset	name or numeric position of the values to be subsetting in the slot. If slot="input", the subset are the variables defined in the param.yml file. If slot="step", the subset is the command line defined on the SYSargs2 object for all the steps of the workflow. If slot="output", the subset is the path for the expected output files for all the steps in the workflow. Default is subset=NULL
index	A numeric index positions of the file in SYSargs2 object, slot output. It requires a subset to be defined. Default is index=NULL.
delete	allows to delete a subset of files in the case of slot="output". Default is delete=NULL.

Author(s)

Daniela Cassol and Thomas Girke

See Also

loadWorkflow renderWF

Examples

```
## Construct SYSargs2 object
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl",
                  input_file="hisat2-mapping-se.yml", dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
WF

## Testing subset_wf function
```

```
input <- subsetWF(WF, slot="input", subset='FileName')
output <- subsetWF(WF, slot="output", subset=1, index=1)
step.cmd <- subsetWF(WF, slot="step", subset=1) ## subset all the HISAT2 commandline
# subsetWF(WF, slot="output", subset=1, index=1, delete=TRUE) ## in order to delete the subset files list
```

symLink2bam

Symbolic links for IGV

Description

Function for creating symbolic links to view BAM files in a genome browser such as IGV.

Usage

```
symLink2bam(sysargs, command="ln -s", htmlDir, ext = c(".bam", ".bai"), urlbase, urlfile)
```

Arguments

sysargs	Object of class SYSargs or SYSargs2
command	Shell command, defaults to "ln -s"
htmlDir	Path to HTML directory with http access.
ext	File name extensions to use for BAM and index files.
urlbase	The base URL structure to use in URL file.
urlfile	Name and path of URL file.

Value

symbolic links and url file

Author(s)

Thomas Girke

Examples

```
## Construct SYSargs object from param and targets files
param <- system.file("extdata", "tophat.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
```

Not run:

```
## Construct SYSargs2 object from cwl and targets files
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
args <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl", input_file="hisat2-mapping-se.yml", dir_p
args <- renderWF(args, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
```

```
## Create sym links and URL file for IGV
```

```
symLink2bam(sysargs=args, command="ln -s", htmlDir=c("~/html/", "somedir/"), ext=c(".bam", ".bai"), urlbase="http://")  
## End(Not run)
```

sysargs

SYSargs accessor methods

Description

Methods to access information from SYSargs object.

Usage

```
sysargs(x)
```

Arguments

x object of class SYSargs

Value

various outputs

Author(s)

Thomas Girke

Examples

```
## Construct SYSargs object from param and targets files  
param <- system.file("extdata", "hisat2.param", package="systemPipeR")  
targets <- system.file("extdata", "targets.txt", package="systemPipeR")  
args <- systemArgs(sysma=param, mytargets=targets)  
args  
names(args); modules(args); cores(args); outpaths(args); sysargs(args)
```

 SYSargs-class

 Class "SYSargs"

Description

S4 class container for storing parameters of command-line- or R-based software. *SYSargs* instances are constructed by the `systemArgs` function from two simple tabular files: a `targets` file and a `param` file. The latter is optional for workflow steps lacking command-line software. Typically, a *SYSargs* instance stores all sample-level inputs as well as the paths to the corresponding outputs generated by command-line- or R-based software generating sample-level output files. Each sample level input/outfile operation uses its own *SYSargs* instance. The outpaths of *SYSargs* usually define the sample inputs for the next *SYSargs* instance. This connectivity is achieved by writing the outpaths with the `writeTargetsout` function to a new `targets` file that serves as input to the next `systemArgs` call. By chaining several *SYSargs* steps together one can construct complex workflows involving many sample-level input/output file operations with any combination of command-line or R-based software.

Objects from the Class

Objects can be created by calls of the form `new("SYSargs", ...)`.

Slots

`targetsin`: Object of class "data.frame" storing tabular data from targets input file
`targetsout`: Object of class "data.frame" storing tabular data from targets output file
`targetsheader`: Object of class "character" storing header/comment lines of targets file
`modules`: Object of class "character" storing software versions from module system
`software`: Object of class "character" name of executable of command-line software
`cores`: Object of class "numeric" number of CPU cores to use
`other`: Object of class "character" additional arguments
`reference`: Object of class "character" path to reference genome file
`results`: Object of class "character" path to results directory
`infile1`: Object of class "character" paths to first FASTQ file
`infile2`: Object of class "character" paths to second FASTQ file if data is PE
`outfile1`: Object of class "character" paths to output files generated by command-line software
`sysargs`: Object of class "character" full commands used to execute external software
`outpaths`: Object of class "character" paths to final outputs including postprocessing by `Rsamtools`

Methods

SampleName signature(x = "SYSargs"): extracts sample names
 [signature(x = "SYSargs"): subsetting of class with bracket operator
coerce signature(from = "list", to = "SYSargs"): as(list, "SYSargs")
cores signature(x = "SYSargs"): extracts data from cores slot
infile1 signature(x = "SYSargs"): extracts data from infile1 slot
infile2 signature(x = "SYSargs"): extracts data from infile2 slot
modules signature(x = "SYSargs"): extracts data from modules slot
names signature(x = "SYSargs"): extracts slot names
length signature(x = "SYSargs"): extracts number of samples
other signature(x = "SYSargs"): extracts data from other slot
outfile1 signature(x = "SYSargs"): extracts data from outfile1 slot
outpaths signature(x = "SYSargs"): extracts data from outpath slot
reference signature(x = "SYSargs"): extracts data from reference slot
results signature(x = "SYSargs"): extracts data from results slot
show signature(object = "SYSargs"): summary view of SYSargs objects
software signature(x = "SYSargs"): extracts data from software slot
targetsheader signature(x = "SYSargs"): extracts data from targetsheader slot
targetsin signature(x = "SYSargs"): extracts data from targetsin slot
targetsout signature(x = "SYSargs"): extracts data from targetsout slot

Author(s)

Thomas Girke

See Also

systemArgs and runCommandline

Examples

```
showClass("SYSargs")
## Construct SYSargs object from param and targets files
param <- system.file("extdata", "tophat.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
args
names(args); targetsin(args); targetsout(args); targetsheader(args);
software(args); modules(args); cores(args); outpaths(args)
sysargs(args); other(args); reference(args); results(args); infile1(args)
infile2(args); outfile1(args); SampleName(args)

## Return sample comparisons
readComp(args, format = "vector", delim = "-")
```

```

## The subsetting operator '[' allows to select specific samples
args[1:4]

## Not run:
## Execute SYSargs on single machine
runCommandline(args=args)

## Execute SYSargs on multiple machines
qsubargs <- getQsubargs(queue="batch", Nnodes="nodes=1", cores=cores(args), memory="mem=10gb", time="walltime=20")
qsubRun(appfct="runCommandline(args=args)", appargs=args, qsubargs=qsubargs, Nqsubs=1, submitdir="results", pack

## Write outpaths to new targets file for next SYSargs step
writeTargetsout(x=args, file="default")

## End(Not run)

```

SYSargs2-class	<i>Class "SYSargs2"</i>
----------------	-------------------------

Description

SYSargs2 class stores all the information and instructions needed for processing a set of input files with a specific command-line or a series of command-line within a workflow. The SYSargs2 S4 class object is created from the loadWorkflow and renderWF function, which populates all the command-line for each sample in each step of the particular workflow. Each sample level input/outfile operation uses its own SYSargs2 instance. The output of SYSargs2 define all the expected output files for each step in the workflow, which usually it is the sample input for the next step in an SYSargs2 instance. Between different instances, this connectivity is established by writing the subsetting output with the writeTargetsout function to a new targets file that serves as input to the next loadWorkflow and renderWF call. By chaining several SYSargs2 steps together one can construct complex workflows involving many sample-level input/output file operations with any combination of command-line or R-based software.

Objects from the Class

Objects can be created by calls of the form `new("SYSargs2", ...)`.

Slots

targets: Object of class "list" storing data from each sample from targets file
targetsheader: Object of class "list" storing header/comment lines of targets file
modules: Object of class "list" storing software versions from module system
wf: Object of class "list" storing data from Workflow CWL param file
clt: Object of class "list" storing data from each CommandLineTool step in the Workflow or the single CommandLineTool CWL param file
yamlinput: Object of class "list" storing data from input file

cmdlist: Object of class "list" storing all command-line used to execute external software
input: Object of class "list" storing data from each target defined in inputvars
output: Object of class "list" paths to final outputs files
cwlfiles: Object of class "list" paths to input and CWL param files
inputvars: Object of class "list" storing data from each inputvars
cmdToCwl: Object of class "list" storing data from each cmdToCwl

Methods

[signature(x = "SYSargs2"): subsetting of class with bracket operator
[[signature(x = "SYSargs2", i = "ANY", j = "missing"): subsetting of class with bracket operator
[[<- signature(x = "SYSargs2"): replacement method for SYSargs2 class
\$ signature(x = "SYSargs2"): extracting slots elements by name
clt signature(x = "SYSargs2"): extracts data from clt slot
cmdlist signature(x = "SYSargs2"): extracts data from cmdlist slot
coerce signature(from = "list", to = "SYSargs2"): as(list, "SYSargs2")
cwlfiles signature(x = "SYSargs2"): extracts data from cwlfiles slot
infile1 signature(x = "SYSargs2"): extracting paths to first FASTQ file
infile2 signature(x = "SYSargs2"): extracting paths to second FASTQ file if data is PE
input signature(x = "SYSargs2"): extracts data from input slot
inputvars signature(x = "SYSargs2"): extracts data from inputvars slot
length signature(x = "SYSargs2"): extracts number of samples
modules signature(x = "SYSargs2"): extracts data from modules slot
names signature(x = "SYSargs2"): extracts slot names
output signature(x = "SYSargs2"): extracts data from cmdlist slot
show signature(object = "SYSargs2"): summary view of SYSargs2 objects
sysargs2 signature(x = "SYSargs2"): Coerce back to list as(SYSargs2, "list")
targets signature(x = "SYSargs2"): extract data from targets slot
targetsheader signature(x = "SYSargs2"): extracts data from targetsheader slot
wf signature(x = "SYSargs2"): extracts data from wf slot
yamlinput signature(x = "SYSargs2"): extracts data from yamlinput slot
cmdToCwl signature(x = "SYSargs2"): extracts data from cmdToCwl slot
yamlinput<- signature(x = "SYSargs2"): replacement method for yamlinput slot

Author(s)

Daniela Cassol and Thomas Girke

See Also

loadWorkflow and renderWF and runCommandline and clusterRun

Examples

```
showClass("SYSargs2")

## Construct SYSargs2 object from CWL param, CWL input, and targets files
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl",
                  input_file="hisat2-mapping-se.yml", dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
WF
names(WF); modules(WF); targets(WF)[1]; cmdlist(WF)[1:2]; output(WF)

## The subsetting operator '[' allows to select specific command-line
cmdlist(WF)[1:2]

## Not run:
## Execute SYSargs2 on single machine
WF <- runCommandline(args=WF)

## Execute SYSargs2 on multiple machines of a compute cluster. The following
## example uses the conf and template files for the Slurm scheduler. Please
## read the instructions on how to obtain the corresponding files for other schedulers.
file.copy(system.file("extdata", ".batchtools.conf.R", package="systemPipeR"), ".")
file.copy(system.file("extdata", "batchtools.slurm.tmpl", package="systemPipeR"), ".")
resources <- list(walltime=120, ntasks=1, ncpus=4, memory=1024)
reg <- clusterRun(args, FUN = runCommandline, conffile=".batchtools.conf.R", template="batchtools.slurm.tmpl", Nj

## Monitor progress of submitted jobs
getStatus(reg=reg)

## Updates the path in the object \code{output(WF)}
WF <- output_update(WF, dir=FALSE, replace=TRUE, extension=c(".sam", ".bam"))

## Alignment stats
read_statsDF <- alignStats(WF)
read_statsDF <- cbind(read_statsDF[targets$FileName,], targets)
write.table(read_statsDF, "results/alignStats.xls", row.names=FALSE, quote=FALSE, sep="\t")

## End(Not run)
```

SYSargsList

SYSargsList

Description

S4 class container for storing parameters of command-line- or R-based software. SYSargsList instances are constructed by the SYSargsList function.

Usage

```
SYSargsList(args = NULL, targets = NULL, wf_file = NULL, input_file = NULL, dir_path = ".", inputvars = N
```

Arguments

args	SYSargs2 object
targets	the path to targets file. The targets file can be either a simple tabular or yml file. Also, it is possible to assign NULL to run the pipeline without the 'targets' file. This can be useful for running specific workflows that do not require input files.
wf_file	name and path to CWL param file.
input_file	name and path to input file.
dir_path	full path to the directory with the CWL param and input files.
inputvars	variables list defined in the input file that matches the column names defined in the targets file.
step_index	character with the step index name.
silent	If set to TRUE, all messages returned by the function will be suppressed.
restartProject	restart previous workflow run.
rm_targets_col	targets file columns to be removed.
dependency	step previous dependency.

Value

SYSargsList object

Author(s)

Daniela Cassol

See Also

```
showClass("SYSargsList")
```

Examples

```
dir_path <- system.file("extdata/cwl/example/", package="systemPipeR")
sal <- SYSargsList(wf_file="example.cwl", input_file="example_single.yml", dir_path = dir_path)
sal
cmdlist(sal)
```

SYSargsList-class *Class "SYSargsList"*

Description

S4 class container for storing parameters and information of command-line- or R-based workflow.

Objects from the Class

Objects can be created by calls of the form `new("SYSargsList", ...)`.

Slots

sprconfig: Object of class "list" storing all the config information for the project
stepsWF: Object of class "list" storing all the SYSargs2 objects of the workflow
statusWF: Object of class "list" storing all the success and failure of each step in the workflow
dependency: Object of class "list" storing all the dependency steps in the workflow
projectWF: Object of class "list" storing all the projectWF information of the workflow
targetsWF: Object of class "list" storing all the targets files for each step in the workflow
SEobj: Object of class "list" storing all the SummarizedExperiment objects in the workflow
outfiles: Object of class "list" storing all the output files in the workflow
targets_connection: Object of class "list" storing all targets files connection in the workflow

Methods

[signature(x = "SYSargsList", i = "ANY", j = "ANY", drop = "ANY"): subsetting of class with bracket operator
[[signature(x = "SYSargsList", i = "ANY", j = "ANY"): subsetting of class with bracket operator
[<- signature(x = "SYSargsList", i = "ANY", j = "ANY", value = "ANY"): replacement method for SYSargsList class
\$ signature(x = "SYSargsList"): extracting slots elements by name
coerce signature(from = "list", to = "SYSargsList"): as(list, "SYSargsList")
coerce signature(from = "SYSargsList", to = "list"): as(SYSargsList, "list")
length signature(x = "SYSargsList"): extracts number of SYSargs2 objects
names signature(x = "SYSargsList"): extracts slot names
show signature(object = "SYSargsList"): summary view of SYSargsList steps
sprconfig signature(x = "SYSargsList"): extract data from sprconfig slot
stepsWF signature(x = "SYSargsList"): extract data from stepsWF slot
statusWF signature(x = "SYSargsList"): extract data from statusWF slot
dependency signature(x = "SYSargsList"): extract data from dependency slot

projectWF signature(x = "SYSargsList"): extract data from projectWF slot
targetsWF signature(x = "SYSargsList"): extract data from targetsWF slot
sysargslist signature(x = "SYSargsList"): Coerce back to list as(SYSargsList, "list")
SEobj signature(x = "SYSargsList"): extract data from SEobj slot
cmdlist signature(x = "SYSargsList"): extracts data from cmdlist slot for each step
appendStep<- signature(x = "SYSargsList"): replacement method for append a step
appendStep<- signature(x = "SYSargsList"): replacement method for append a step
replaceStep<- signature(x = "SYSargsList"): replacement method for replace a step
renameStep<- signature(x = "SYSargsList"): replacement method for rename a step
yamlinput<- signature(x = "SYSargs2"): replacement method for yamlinput slot for each step

Author(s)

Daniela Cassol and Thomas Girke

See Also

SYSargs2 and initWF

Examples

```
showClass("SYSargsList")
## Not run:
## Construct SYSargsList object from Rmd file
library(systemPipeRdata)
script <- system.file("extdata/workflows/rnaseq", "systemPipeRNAseq.Rmd", package="systemPipeRdata")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- tempdir()
SYSconfig <- initProject(projPath=dir_path, targets=targets, script=script, overwrite = TRUE)
sysargslist <- initWF(sysconfig = "SYSconfig.yml")

## End(Not run)
```

systemArgs

Constructs SYSargs object from param and targets files

Description

Constructs SYSargs S4 class objects from two simple tabular files: a targets file and a param file. The latter is optional for workflow steps lacking command-line software. Typically, a SYSargs instance stores all sample-level inputs as well as the paths to the corresponding outputs generated by command-line- or R-based software generating sample-level output files. Each sample level input/outfile operation uses its own SYSargs instance. The outpaths of SYSargs usually define the sample inputs for the next SYSargs instance. This connectivity is established by writing the outpaths with the writeTargetsout function to a new targets file that serves as input to the next systemArgs call. By chaining several SYSargs steps together one can construct complex workflows involving many sample-level input/output file operations with any combination of command-line or R-based software.

Usage

```
systemArgs(sysma, mytargets, type = "SYSargs")
```

Arguments

sysma	path to 'param' file; file structure follows a simple name/value syntax that converted into JSON format; for details about the file structure see sample files provided by package. Assign NULL to run the pipeline without 'param' file. This can be useful for running partial workflows, e.g. with pregenerated BAM files.
mytargets	path to targets file
type	type="SYSargs" returns SYSargs, type="json" returns param file content in JSON format (requires rjson library)

Value

SYSargs object or character string in JSON format

Author(s)

Thomas Girke

See Also

```
showClass("SYSargs")
```

Examples

```
## Construct SYSargs object from param and targets files
param <- system.file("extdata", "tophat.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
args
names(args); modules(args); cores(args); outpaths(args); sysargs(args)

## Not run:
## Execute SYSargs on single machine
runCommandline(args=args)

## Execute SYSargs on multiple machines of a compute cluster
resources <- list(walltime=120, ntasks=1, ncpus=cores(args), memory=1024)
reg <- clusterRun(args, conffile=".batchtools.conf.R", template="batchtools.slurm.tpl", Njobs=18, runid="01", re

## Monitor progress of submitted jobs
getStatus(reg=reg)
file.exists(outpaths(args))
sapply(1:length(args), function(x) loadResult(reg, x)) # Works once all jobs have completed successfully.

## Alignment stats
read_statsDF <- alignStats(args)
write.table(read_statsDF, "results/alignStats.xls", row.names=FALSE, quote=FALSE, sep="\t")
```

```
## Write outpaths to new targets file for next SYSargs step
writeTargetsout(x=args, file="default")

## End(Not run)
```

targets.as.df	<i>Convert targets list to data.frame</i>
---------------	---

Description

Convert list, which stores data from each target input file to data.frame object.

Usage

```
targets.as.df(x)
```

Arguments

x An object of the class "list" that stores data from each target input file, as targets(WF).

Value

data.frame containing all the input file information.

Author(s)

Daniela Cassol and Thomas Girke

Examples

```
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl",
                  input_file="hisat2-mapping-se.yml", dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
WF
targets.as.df(targets(WF))
```

`tryCL`*Collect information about the third-party software*

Description

Function to check if third-party software or utility is installed and set in the PATH.

Usage

```
tryCL(command)
```

Arguments

`command` a character vector containing the command line name to be tested.

Value

It will return a positive message if the software is set on the PATH or an error message if the software is not set it.

Note

Please note that not necessary the software is not installed if the message indicates an error, but it has not been exported on the current PATH.

Author(s)

Danela Cassol

Examples

```
## Not run:  
tryCL(command="R")  
tryCL(command="blastp")  
tryCL(command="hisat2")  
  
## End(Not run)
```

tryPath	<i>Validation of the files or directories</i>
---------	---

Description

Function to check if the full path (file or directory) exists.

Usage

```
tryPath(path)
```

Arguments

path a character vector of full path name.

Details

This function produces a character vector of the file or directory name defined on the path argument.

Value

A character vector containing the name of the file or directory. If the path does not exist, it will return an error message.

Author(s)

Daniela Cassol

Examples

```
## Not run:  
library(systemPipeRdata)  
file <- system.file("extdata/workflows/rnaseq", "systemPipeRNAseq.Rmd", package="systemPipeRdata")  
tryPath(path=file)  
  
## End(Not run)
```

variantReport	<i>Generate Variant Report</i>
---------------	--------------------------------

Description

Functions for generating tabular variant reports including genomic context annotations and confidence statistics of variants. The annotations are obtained with utilities provided by the `VariantAnnotation` package and the variant statistics are retrieved from the input VCF files.

Usage

```
## Variant report
variantReport(args, txdb, fa, organism)

## Combine variant reports
combineVarReports(args, filtercol, ncol = 15)

## Create summary statistics of variants
varSummary(args)
```

Arguments

args	Object of class <code>SYSargs</code> or <code>SYSargs2</code> . The paths of the input VCF files are specified under <code>infile1(args)</code> and the paths of the output files under <code>outfile1(args)</code> or <code>output(args)</code> .
txdb	Annotation data stored as <code>TranscriptDb</code> object, which can be obtained from GFF/GTF files, BioMart, Bioc Annotation packages, UCSC, etc. For details see the vignette of the <code>GenomicFeatures</code> package. It is important to use here matching versions of the <code>txdb</code> and <code>fa</code> objects. The latter is the genome sequence used for read mapping and variant calling.
fa	<code>FaFile</code> object pointing to the sequence file of the corresponding reference genome stored in FASTA format or a <code>BSgenome</code> instance.
organism	Character vector specifying the organism name of the reference genome.
filtercol	Named character vector containing in the <code>name</code> field the column titles to filter on, and in the <code>data</code> field the corresponding values to include in the report. For instance, the setting <code>filtercol=c(Consequence="nonsynonymous")</code> will include only nonsynonymous variances listed in the <code>Consequence</code> column. To omit the filtering step, one can use the setting <code>filtercol="All"</code> .
ncol	Integer specifying the number of columns in the tabular input files. Default is set to 15.

Value

Tabular output files.

Author(s)

Thomas Girke

See Also

filterVars

Examples

```
## Alignment with BWA (sequentially on single machine)
param <- system.file("extdata", "bwa.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)
sysargs(args)[1]

## Not run:
system("bwa index -a bwtsv ./data/tair10.fasta")
bampaths <- runCommandline(args=args)

## Alignment with BWA (parallelized on compute cluster)
resources <- list(walltime=120, ntasks=1, ncpus=cores(args), memory=1024)
reg <- clusterRun(args, conffile=".batchtools.conf.R", template="batchtools.slurm.tmpl", Njobs=18, runid="01", re

## Variant calling with GATK
## The following creates in the initial step a new targets file
## (targets_bam.txt). The first column of this file gives the paths to
## the BAM files created in the alignment step. The new targets file and the
## parameter file gatk.param are used to create a new SYSargs
## instance for running GATK. Since GATK involves many processing steps, it is
## executed by a bash script gatk_run.sh where the user can specify the
## detailed run parameters. All three files are expected to be located in the
## current working directory. Samples files for gatk.param and
## gatk_run.sh are available in the subdirectory ./inst/extdata/ of the
## source file of the systemPipeR package.
writeTargetsout(x=args, file="targets_bam.txt")
system("java -jar CreateSequenceDictionary.jar R=./data/tair10.fasta O=./data/tair10.dict")
# system("java -jar /opt/picard/1.81/CreateSequenceDictionary.jar R=./data/tair10.fasta O=./data/tair10.dict")
args <- systemArgs(sysma="gatk.param", mytargets="targets_bam.txt")
resources <- list(walltime=120, ntasks=1, ncpus=cores(args), memory=1024)
reg <- clusterRun(args, conffile=".batchtools.conf.R", template="batchtools.slurm.tmpl", Njobs=18, runid="01", re
writeTargetsout(x=args, file="targets_gatk.txt")

## Variant calling with BCFtools
## The following runs the variant calling with BCFtools. This step requires in
## the current working directory the parameter file sambcf.param and the
## bash script sambcf_run.sh.
args <- systemArgs(sysma="sambcf.param", mytargets="targets_bam.txt")
resources <- list(walltime=120, ntasks=1, ncpus=cores(args), memory=1024)
reg <- clusterRun(args, conffile=".batchtools.conf.R", template="batchtools.slurm.tmpl", Njobs=18, runid="01", re
writeTargetsout(x=args, file="targets_sambcf.txt")

## Filtering of VCF files generated by GATK
```

```

args <- systemArgs(sysma="filter_gatk.param", mytargets="targets_gatk.txt")
filter <- "totalDepth(vr) >= 2 & (altDepth(vr) / totalDepth(vr) >= 0.8) & rowSums(softFilterMatrix(vr))==4"
# filter <- "totalDepth(vr) >= 20 & (altDepth(vr) / totalDepth(vr) >= 0.8) & rowSums(softFilterMatrix(vr))==6"
filterVars(args, filter, varcaller="gatk", organism="A. thaliana")
writeTargetsout(x=args, file="targets_gatk_filtered.txt")

## Filtering of VCF files generated by BCFtools
args <- systemArgs(sysma="filter_sambcf.param", mytargets="targets_sambcf.txt")
filter <- "rowSums(vr) >= 2 & (rowSums(vr[,3:4])/rowSums(vr[,1:4]) >= 0.8)"
# filter <- "rowSums(vr) >= 20 & (rowSums(vr[,3:4])/rowSums(vr[,1:4]) >= 0.8)"
filterVars(args, filter, varcaller="bcftools", organism="A. thaliana")
writeTargetsout(x=args, file="targets_sambcf_filtered.txt")

## Annotate filtered variants from GATK
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_gatk_filtered.txt")
txdb <- loadDb("./data/tair10.sqlite")
fa <- FaFile(systemPipeR::reference(args))
variantReport(args=args, txdb=txdb, fa=fa, organism="A. thaliana")

## Annotate filtered variants from BCFtools
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_sambcf_filtered.txt")
txdb <- loadDb("./data/tair10.sqlite")
fa <- FaFile(systemPipeR::reference(args))
variantReport(args=args, txdb=txdb, fa=fa, organism="A. thaliana")

## Combine results from GATK
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_gatk_filtered.txt")
combinedDF <- combineVarReports(args, filtercol=c(Consequence="nonsynonymous"))
write.table(combinedDF, "./results/combinedDF_nonsyn_gatk.xls", quote=FALSE, row.names=FALSE, sep="\t")

## Combine results from BCFtools
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_sambcf_filtered.txt")
combinedDF <- combineVarReports(args, filtercol=c(Consequence="nonsynonymous"))
write.table(combinedDF, "./results/combinedDF_nonsyn_sambcf.xls", quote=FALSE, row.names=FALSE, sep="\t")

## Summary for GATK
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_gatk_filtered.txt")
write.table(varSummary(args), "./results/variantStats_gatk.xls", quote=FALSE, col.names = NA, sep="\t")

## Summary for BCFtools
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_sambcf_filtered.txt")
write.table(varSummary(args), "./results/variantStats_sambcf.xls", quote=FALSE, col.names = NA, sep="\t")

## Venn diagram of variants
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_gatk_filtered.txt")
varlist <- sapply(names(outpaths(args))[1:4], function(x) as.character(read.delim(outpaths(args)[x])$VARID))
vennset_gatk <- overLapper(varlist, type="vennsets")
args <- systemArgs(sysma="annotate_vars.param", mytargets="targets_sambcf_filtered.txt")
varlist <- sapply(names(outpaths(args))[1:4], function(x) as.character(read.delim(outpaths(args)[x])$VARID))
vennset_bcf <- overLapper(varlist, type="vennsets")
vennPlot(list(vennset_gatk, vennset_bcf), mymain="", mysub="GATK: red; BCFtools: blue", colmode=2, ccol=c("blue",
## End(Not run)

```

vennPlot	<i>Plot 2-5 way Venn diagrams</i>
----------	-----------------------------------

Description

Plotting function of 2-5 way Venn diagrams from 'VENNset' objects or count set vectors. A useful feature is the possibility to combine the counts from several Venn comparisons with the same number of label sets in a single Venn diagram.

Usage

```
vennPlot(x, mymain = "Venn Diagram", mysub = "default", setlabels = "default", yoffset = seq(0, 10, by =
```

Arguments

x	VENNset or list of VENNset objects. Alternatively, a vector of Venn counts or a list of vectors of Venn counts can be provided as input. If several Venn comparisons are provided in a list then their results are combined in a single Venn diagram, where the count sets are organized above each other.
mymain	Main title of plot.
mysub	Subtitle of plot. Default mysub="default" reports the number of unique items in all sets, as well as the number of unique items in each individual set, respectively.
setlabels	The argument setlabels allows to provide a vector of custom sample labels. However, assigning the proper names in the name slots of the initial setlist is preferred for tracking purposes.
yoffset	The results from several Venn comparisons can be combined in a single Venn diagram by assigning to x a list with several VENNsets or count vectors. The positional offset of the count sets in the plot can be controlled with the yoffset argument. The argument setting colmode allows to assign different colors to each count set. For instance, with colmode=2 one can assign to ccol a color vector or a list, such as ccol=c("blue", "red") or ccol=list(1:8,8:1).
ccol	Character or numeric vector to define colors of count values, e.g. ccol=c("black", "black", "red").
colmode	See argument yoffset.
lcol	Character or numeric vector to define colors of set labels, e.g. lcol=c("red", "green")
lines	Character or numeric vector to define colors of lines in plot.
mylwd	Defines line width of shapes used in plot.
diacol	See argument type.
type	Defines shapes used to plot 4-way Venn diagram. Default type="ellipse" uses ellipses. The setting type="circle" returns an incomplete 4-way Venn diagram as circles. This representation misses two overlap sectors, but is sometimes easier to navigate than the default ellipse version. The missing Venn intersects are reported below the Venn diagram. Their font color can be controlled with the argument diacol.

ccex	Controls font size for count values.
lcex	Controls font size for set labels.
sepsplit	Character used to separate sample labels in Venn counts.
...	Additional arguments to pass on.

Value

Venn diagram plot.

Note

The functions provided here are an extension of the Venn diagram resources on this site: <http://manuals.bioinformatics.ucr.edu/Venn-Diagrams>

Author(s)

Thomas Girke

References

See examples in 'The Electronic Journal of Combinatorics': <http://www.combinatorics.org/files/Surveys/ds5/VennSymmExar>

See Also

overLapper, olBarplot

Examples

```
## Sample data
setlist <- list(A=sample(letters, 18), B=sample(letters, 16),
               C=sample(letters, 20), D=sample(letters, 22),
               E=sample(letters, 18), F=sample(letters, 22))

## 2-way Venn diagram
vennset <- overLapper(setlist[1:2], type="vennsets")
vennPlot(vennset)

## 3-way Venn diagram
vennset <- overLapper(setlist[1:3], type="vennsets")
vennPlot(vennset)

## 4-way Venn diagram
vennset <- overLapper(setlist[1:4], type="vennsets")
vennPlot(list(vennset, vennset))

## Pseudo 4-way Venn diagram with circles
vennPlot(vennset, type="circle")

## 5-way Venn diagram
vennset <- overLapper(setlist[1:5], type="vennsets")
vennPlot(vennset)
```

```

## Alternative Venn count input to vennPlot (not recommended!)
counts <- sapply(vennlist(vennset), length)
vennPlot(counts)

## 6-way Venn comparison as bar plot
vennset <- overLapper(setlist[1:6], type="vennsets")
olBarplot(vennset, mincount=1)

## Bar plot of standard intersect counts
intersect <- overLapper(setlist, type="intersects")
olBarplot(intersect, mincount=1)

## Accessor methods for VENNset/INTERSECTset objects
names(vennset)
names(intersect)
setlist(vennset)
intersectmatrix(vennset)
complexitylevels(vennset)
vennlist(vennset)
intersectlist(intersect)

## Coerce VENNset/INTERSECTset object to list
as.list(vennset)
as.list(intersect)

## Pairwise intersect matrix and heatmap
olMA <- sapply(names(setlist),
function(x) sapply(names(setlist),
function(y) sum(setlist[[x]] %in% setlist[[y]])))
olMA
heatmap(olMA, Rowv=NA, Colv=NA)

## Presence-absence matrices for large numbers of sample sets
intersect <- overLapper(setlist=setlist, type="intersects", complexity=2)
(paMA <- intersectmatrix(intersect))
heatmap(paMA, Rowv=NA, Colv=NA, col=c("white", "gray"))

```

VENNset-class

Class "VENNset"

Description

Container for storing Venn intersect results created by the `overLapper` function. The `setlist` slot stores the original label sets as vectors in a list; `intersectmatrix` organizes the label sets in a present-absent matrix; `complexitylevels` represents the number of comparisons considered for each comparison set as vector of integers; and `vennlist` contains the Venn intersect vectors.

Objects from the Class

Objects can be created by calls of the form `new("VENNset", ...)`.

Slots

setlist: Object of class "list": list of vectors
intersectmatrix: Object of class "matrix": binary matrix
complexitylevels: Object of class "integer": vector of integers
vennlist: Object of class "list": list of vectors

Methods

as.list signature(x = "VENNset"): coerces VENNset to list
coerce signature(from = "list", to = "VENNset"): as(list, "VENNset")
complexitylevels signature(x = "VENNset"): extracts data from complexitylevels slot
intersectmatrix signature(x = "VENNset"): extracts data from intersectmatrix slot
length signature(x = "VENNset"): returns number of original label sets
names signature(x = "VENNset"): extracts slot names
setlist signature(x = "VENNset"): extracts data from setlist slot
show signature(object = "VENNset"): summary view of VENNset objects
vennlist signature(x = "VENNset"): extracts data from vennset slot

Author(s)

Thomas Girke

See Also

overLapper, vennPlot, olBarplot, INTERSECTset-class

Examples

```

showClass("VENNset")

## Sample data
setlist <- list(A=sample(letters, 18), B=sample(letters, 16),
               C=sample(letters, 20), D=sample(letters, 22),
               E=sample(letters, 18), F=sample(letters, 22))

## Create VENNset
vennset <- overLapper(setlist[1:5], type="vennsets")
class(vennset)

## Accessor methods for VENNset/INTERSECTset objects
names(vennset)
setlist(vennset)
intersectmatrix(vennset)
complexitylevels(vennset)
vennlist(vennset)

## Coerce VENNset/INTERSECTset object to list
as.list(vennset)

```

writeTargetsout	<i>Write updated targets out to file</i>
-----------------	--

Description

Convenience write function for generating targets files with updated FileName columns containing the paths to files generated by input/output processes. These processes can be commandline- or R-based software. Typically, the paths to the inputs are stored in the targets infile (`targetsin(args)` for `SYSargs` objects or `targets.as.df(targets(WF))` for `SYSargs2` objects) and the outputs are stored in the targets outfile (`targetsout(args)` for `SYSargs` objects or `output(WF)` for `SYSargs2` objects). Note: by default the function cannot overwrite any existing files. If a file exists then the user has to explicitly remove it or set `overwrite=TRUE`.

Usage

```
writeTargetsout(x, file = "default", silent = FALSE, overwrite = FALSE, step = NULL, new_col=NULL, new_col_output_index=NULL)
```

Arguments

<code>x</code>	Object of class <code>SYSargs</code> or <code>SYSargs2</code> .
<code>file</code>	Name and path of the output file. If set to "default" then the name of the output file will have the pattern 'targets_<software>.txt', where <software> will be what <code>software(x)</code> returns, when <code>x</code> is an object of class <code>SYSargs</code> . For an object of class <code>SYSargs2</code> , the output file will have the pattern 'targets_<software>_<step>.txt', where <software> will be the workflow name (<code>cwlfiles(x)\$cwl</code>) and <step> will be the step chosen in the argument <code>step</code> .
<code>silent</code>	If set to <code>TRUE</code> , all messages returned by the function will be suppressed.
<code>overwrite</code>	If set to <code>TRUE</code> , existing files of same name will be overwritten.
<code>step</code>	Name or numeric position of the step in the workflow to write the output files. The names can be check running <code>names(x\$cwl)</code> .
<code>new_col</code>	A vector of character strings of the new column name to add to target file.
<code>new_col_output_index</code>	A vector of numeric index positions of the file in <code>SYSargs2</code> class output. It requires <code>new_col</code> definition.
<code>remove</code>	To remove the original columns. Default is <code>FALSE</code> .
<code>...</code>	To pass on additional arguments.

Value

Writes tabular targets files containing the header/comment lines from `targetsheader(x)` and the columns from `targetsout(x)`.

Author(s)

Daniela Cassol and Thomas Girke

See Also

writeTargetsRef

Examples

```
#####
## Examples with \code{SYSargs} object ##
#####
## Create SYSargs object
param <- system.file("extdata", "tophat.param", package="systemPipeR")
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
args <- systemArgs(sysma=param, mytargets=targets)

## Not run:
## Write targets out file
writeTargetsout(x=args, file="default")

## End(Not run)

#####
## Examples with \code{SYSargs2} object ##
#####
## Construct SYSargs2 object
targets <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl/hisat2/hisat2-se", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2-mapping-se.cwl",
                  input_file="hisat2-mapping-se.yml", dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
WF

## Not run:
## Write targets out file
names(WF$cלט)
writeTargetsout(x=WF, file="default", step=1, new_col=c("sam_file"), new_col_output_index=c(1))

## End(Not run)
```

writeTargetsRef

Generate targets file with reference

Description

Generates targets file with sample-wise reference as required for some NGS applications, such as ChIP-Seq containing input samples. The reference sample information needs to be provided in the input file in a column called SampleReference where the values reference the labels used in the SampleName column. Sample rows without reference assignments will be removed automatically.

Usage

```
writeTargetsRef(infile, outfile, silent = FALSE, overwrite = FALSE, ...)
```

Arguments

<code>infile</code>	Path to input targets file.
<code>outfile</code>	Path to output targets file.
<code>silent</code>	If set to TRUE, all messages returned by the function will be suppressed.
<code>overwrite</code>	If set to TRUE, existing files of same name will be overwritten.
<code>...</code>	To pass on additional arguments.

Value

Generates modified targets file with the paths to the reference samples in the second column named `FileName2`. Note, sample rows not assigned reference samples are removed automatically.

Author(s)

Thomas Girke

See Also

`writeTargetsout`, `mergeBamByFactor`

Examples

```
## Path to input targets file
targets <- system.file("extdata", "targets_chip.txt", package="systemPipeR")

## Not run:
## Write modified targets file with reference (e.g. input) sample
writeTargetsRef(infile=targets, outfile="~/targets_refsamle.txt", silent=FALSE, overwrite=FALSE)

## End(Not run)
```

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