

# Package ‘systemPipeR’

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**Type** Package

**Title** systemPipeR: NGS workflow and report generation environment

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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, SummarizedExperiment, ggplot2, yaml, stringr, magrittr, S4Vectors, crayon, BiocGenerics, htmlwidgets

**Suggests** BiocStyle, knitr, rmarkdown, systemPipeRdata, GenomicAlignments, grid, dplyr, testthat, rjson, annotate, AnnotationDbi, kableExtra, GO.db, GenomeInfoDb, DT, rtracklayer, limma, edgeR, DESeq2, IRanges, batchtools, GenomicFeatures (>= 1.31.3), VariantAnnotation (>= 1.25.11)

**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

**URL** <https://systempipe.org/>

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## R topics documented:

systemPipeR-package . . . . .	3
addAssay-methods . . . . .	4
alignStats . . . . .	5
catDB-class . . . . .	6
catmap . . . . .	8
check.output . . . . .	9
clusterRun . . . . .	10
config.param . . . . .	12
configWF . . . . .	13
countRangeset . . . . .	14
createParam . . . . .	16
cwlFilesUpdate . . . . .	18
EnvModules-class . . . . .	19
evalCode . . . . .	20
featureCoverage . . . . .	20
featuretypeCounts . . . . .	23
filterDEGs . . . . .	25
filterVars . . . . .	27
genFeatures . . . . .	30
GOHyperGAll . . . . .	31
importWF . . . . .	35
INTERSECTset-class . . . . .	36
LineWise-class . . . . .	37
loadWorkflow . . . . .	40
mergeBamByFactor . . . . .	42
moduleload . . . . .	43
olBarplot . . . . .	44
olRanges . . . . .	47
output_update . . . . .	48
overLapper . . . . .	49
plotfeatureCoverage . . . . .	52
plotfeaturetypeCounts . . . . .	54
plotWF . . . . .	56
predORF . . . . .	59
preprocessReads . . . . .	61

printParam . . . . .	62
readComp . . . . .	64
renderLogs . . . . .	65
renderReport . . . . .	66
returnRPKM . . . . .	67
runCommandline . . . . .	68
runDiff . . . . .	70
runWF . . . . .	72
run_DESeq2 . . . . .	73
run_edgeR . . . . .	74
sal2bash . . . . .	76
sal2rmd . . . . .	77
scaleRanges . . . . .	78
seeFastq . . . . .	79
showDF . . . . .	80
SPRproject . . . . .	81
subsetWF . . . . .	83
symLink2bam . . . . .	84
sysargs . . . . .	85
SYSargs-class . . . . .	86
SYSargs2-class . . . . .	88
SYSargsList . . . . .	91
systemArgs . . . . .	96
targets.as.df . . . . .	97
tryCMD . . . . .	98
tryPath . . . . .	99
variantReport . . . . .	100
vennPlot . . . . .	103
VENNset-class . . . . .	105
writeTargetsout . . . . .	107
writeTargetsRef . . . . .	109
write_SYSargsList . . . . .	110

**Index****111**


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systemPipeR-package    *systemPipeR package for Workflow Environment*

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**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

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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, fqpaths, pairEnd = TRUE, output_index = 1, subset="FileName1")
```

### Arguments

args	Object of class SYSargs or SYSargs2 or named character with BAM files PATH and the names should be the sampleID.
fqpaths	named vector with raw FASTQ files PATH and the names should be the sampleID. Required when class(args) is "character".
pairEnd	logical. For pair-end libraries, select TRUE.
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{SYSargs2} object ##
#####
## Construct SYSargs2 object from CWL input, and targets files
targetspath <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl", package="systemPipeR")
WF <- loadWorkflow(targets=targetspath, wf_file="hisat2/hisat2-mapping-se.cwl",
                  input_file="hisat2/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, make_bam=TRUE)

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

## End(Not run)

#####
## 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)

## Alignment stats
read_statsDF <- alignStats(args)
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
library("biomaRt")
listMarts() # To choose BioMart database
listMarts(host = "plants.ensembl.org")
m <- useMart("plants_mart", host = "plants.ensembl.org")
listDatasets(m)
m <- useMart("plants_mart", dataset = "athaliana_eg_gene", host = "plants.ensembl.org")
listAttributes(m) # Choose data types you want to download
go <- getBM(attributes = c("go_id", "tair_locus", "namespace_1003"), mart = m)
go <- go[go[, 3] != "", ]
go[, 3] <- as.character(go[, 3])
go[go[, 3] == "molecular_function", 3] <- "F"
go[go[, 3] == "biological_process", 3] <- "P"
go[go[, 3] == "cellular_component", 3] <- "C"
go[1:4, ]

dir.create("./data/GO", recursive = TRUE)
write.table(go, "data/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 = "data/GO/GOannotationsBiomart_mod.txt", lib = NULL, org = "",
```

```

                                colno = c(1, 2, 3), idconv = NULL)
catdb
save(catdb, file = "data/GO/catdb.RData")
load("data/GO/catdb.RData")

## 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

*Checking if the outfiles files exist*

---

## Description

This function returns a data.frame indicating the number of existing files and how many files are missing.

## Usage

```
check.output(sysargs, type="data.frame")
check.outfiles(sysargs, type="data.frame")
```

## Arguments

sysargs            object of class SYSargs2 or SYSargsList.  
type                return object option. It can be data.frame or list.

## Value

data.frame or list containing all the outfiles file information.

## Author(s)

Daniela Cassol and Thomas Girke

## See Also

- [SYSargs2-class](#)  
- [SYSargsList-class](#)

## Examples

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

```
## Construct SYSargsList object
sal <- SPRproject(overwrite=TRUE)
targetspath <- system.file("extdata/cwl/example/targets_example.txt", package="systemPipeR")
appendStep(sal) <- SYSargsList(step_name = "echo",
                              targets=targetspath, dir=TRUE,
                              wf_file="example/workflow_example.cwl", input_file="example/example.yml",
                              dir_path = system.file("extdata/cwl", package="systemPipeR"),
                              inputvars = c(Message = "_STRING_", SampleName = "_SAMPLE_"))
## Check outfiles
check.outfiles(sal)
```

---

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",
           template = "batchtools.slurm.tmpl",
           Njobs,
           runid = "01",
           resourceList)
```

## 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 &lt;- makeClusterFunctionsSlurm(template="batchtools.slurm.tmpl")</code> . For more detailed information visit this page: <a href="https://mllg.github.io/batchtools/index.html">https://mllg.github.io/batchtools/index.html</a>
<code>template</code>	The template files for a specific queueing/scheduling systems can be downloaded from here: <a href="https://github.com/mllg/batchtools/tree/master/inst/templates">https://github.com/mllg/batchtools/tree/master/inst/templates</a> . Slurm, PBS/Torque, and Sun Grid Engine (SGE) templates are provided.

Njobs	Integer defining the number of cluster jobs. For instance, if args contains 18 command-line jobs and Njobs=9, 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. -p argument for Tophat.
runid	Run identifier used for log file to track system call commands. Default is "01".
resourceList	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.conf.R",
  template="batchtools.slurm.tpl",
```

```

        Njobs=18, runid="01",
        resourceList=resources)

## 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", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2/hisat2-mapping-se.cwl",
                  input_file="hisat2/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",
                 template="batchtools.slurm.tmpl",
                 Njobs=18, runid="01", resourceList=resources)

## 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)

```

**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-mapping-se.yaml", 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(bfl, args, format="tabular", ...)
```

**Arguments**

bfl	BamFileList object containing paths to one or more BAM files.
args	Object of class SYSargs or SYSargs2 where infile1(args) specifies the paths to the tabular range data files (e.g. peak ranges) used for counting.
format	Format of input range files. Currently, supported are tabular or bed. If tabular is selected then the input range files need to contain the proper column titles to coerce with as(..., "GRanges") to GRanges objects after importing them with read.delim. The latter is the case for the peak files (*peaks.xls) generated by the MACS2 software.
...	Arguments to be passed on to internally used summarizeOverlaps 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{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_mac2.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)

#####
## 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_macs.txt")

## Iterative read counting
countDFnames <- countRangeset(bf1, args, mode="Union", ignore.strand=TRUE)
writeTargetsout(x=args, file="targets_countDF.txt", overwrite=TRUE)

## End(Not run)
```

---

createParam	<i>createParam</i>
-------------	--------------------

---

## 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 = TRUE, confirm = 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: <a href="https://www.commonwl.org/">https://www.commonwl.org/</a> .
class	character. Name of Common Workflow Language Description class. The following is accepted: <code>CommandLineTool</code> .
results_path	Path to the results folder. Default is <code>results</code> .
module_load	string, Name of software to load by Environment Modules system. Default is <code>"baseCommand"</code> , which creates a subfolder and two files: <code>*.cwl</code> and <code>*.yaml</code> at <code>./param/cwl/</code> .
file	character. Name and path of output files. If set to <code>"default"</code> then the name of the output files will have the pattern <code>&lt;software&gt;.cwl</code> and <code>&lt;software&gt;.yaml</code> , where <code>&lt;software&gt;</code> will be what <code>baseCommand(x)</code> returns, when <code>x</code> is an object of class <code>SYSargs2</code> . Also, it creates a subfolder at <code>./param/cwl/</code> with name <code>&lt;software&gt;</code> .
writeParamFiles	logical. If set to <code>TRUE</code> , it will write to file the content of the CWL files: <code>*.cwl</code> and <code>*.yaml</code> . Default is <code>TRUE</code> .



confirm	If set to FALSE and in an interactive section, it will prompt a question to proceed or not.
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 <- createParam(command, writeParamFiles=FALSE)
cmdlist(cmd)

```

---

cwlFilesUpdate

*Update CWL description files*


---

### Description

Function to sync and download the most updated CWL description files from the systemPipeR package.

### Usage

```
cwlFilesUpdate(destdir, force = FALSE, verbose = TRUE)
```

### Arguments

destdir	a character string with the directory path where the param are stored.
force	logical. Force the download of the CWL files.
verbose	logical. The setting FALSE suppresses all print messages.

### Author(s)

Daniela Cassol

### Examples

```

## Not run:
destdir <- "param/"
cwlFilesUpdate(destdir)

## End(Not run)

```

---

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	<i>Toggles option eval on the RMarkdown files</i>
----------	---

---

### 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

```
library(systemPipeRdata)
file <- system.file("extdata/workflows/rnaseq", "systemPipeRNaseq.Rmd", package="systemPipeRdata")
evalCode(infile=file, eval=FALSE, output=file.path(tempdir(), "test.Rmd"))
```

---

featureCoverage	<i>Genome read coverage by transcript models</i>
-----------------	--

---

### 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**

bfl	Paths to BAM files provided as BamFileList object. The name slot of the BAM files will be used for naming samples in the results.
grl	Genomic ranges provided as GRangesList typically generated from txdb instances with operations like: cdsBy(txdb, "tx") or exonsBy(txdb, "tx"). Single component features will be processed the same way as multi component features.
resizereads	Positive integer defining the length alignments should be resized to prior to the coverage calculation. NULL will omit the resizing step.
readlengthrange	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, readlengthrange=c(30:40) will base the coverage calculation on reads between 30 to 40 bps. Assigning NULL will skip this filtering step.
Nbins	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.
method	Defines the summary statistics to use for binning. The default is method=mean.
fixedmatrix	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 fixedmatrix=TRUE, upstream=20, downstream=20 along with a grl instance containing the CDS exon ranges required for this operation, e.g. generated with cdsBy(txdb, "tx").
resizefeatures	Needs to be set to TRUE when fixedmatrix=TRUE. Internally, this will use the systemPipeR::.resizeFeature 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 upstream and downstream arguments.
upstream	Single positive integer specifying the upstream extension length relative to the orientation of each feature in the genome. More details are given above.
downstream	Single positive integer specifying the downstream extension length relative to the orientation of each feature in the genome. More details are given above.
outfile	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 bfl 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.

`overwrite` If set to TRUE any existing file assigned to `outfile` 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) R1e list containing the nucleotide level coverage of each feature

### Author(s)

Thomas Girke

### See Also

`plotfeatureCoverage`

### Examples

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

```

## 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")

targetspath <- system.file("extdata", "targets.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl", package="systemPipeR")
args <- loadWorkflow(targets=targetspath, wf_file="hisat2/hisat2-mapping-se.cwl",
                    input_file="hisat2/hisat2-mapping-se.yml", dir_path=dir_path)
args <- renderWF(args, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
args <- runCommandline(args, make_bam = TRUE, dir = TRUE)
outpaths <- subsetWF(args , slot="output", subset=1, index=1)
file.exists(outpaths)

## (A) Generate binned coverage for two BAM files and 4 transcripts
grl <- cdsBy(txdb, "tx", use.names=TRUE)
fcov <- featureCoverage(bfl=BamFileList(outpaths[1:2]), grl=grl[1:4], resizereads=NULL,
                      readlengthrange=NULL, Nbins=20, method=mean, fixedmatrix=FALSE,
                      resizefeatures=TRUE, upstream=20, downstream=20,
                      outfile="results/featureCoverage.xls", overwrite=TRUE)
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[1:2]), grl=grl[1:4], resizereads=NULL,
                      readlengthrange=NULL, Nbins=NULL, method=mean, fixedmatrix=TRUE,
                      resizefeatures=TRUE, upstream=20, downstream=20,
                      outfile="results/featureCoverage_up_down.xls", overwrite=TRUE)
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[1:2]), grl=grl[1:4], resizereads=NULL,
                      readlengthrange=NULL, Nbins=20, method=mean, fixedmatrix=TRUE,
                      resizefeatures=TRUE, upstream=20, downstream=20,
                      outfile="results/featureCoverage_binned.xls", overwrite=TRUE)
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[1:2]), grl=grl[1:4], resizereads=NULL,
                      readlengthrange=NULL, Nbins=NULL, method=mean, fixedmatrix=FALSE,
                      resizefeatures=TRUE, upstream=20, downstream=20,
                      outfile="results/featureCoverage_query.xls", overwrite=TRUE)

## End(Not run)

```

**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**

<code>bfl</code>	<code>BamFileList</code> 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 <code>yieldSize</code> value passed on to the <code>BamFileList</code> function. For details see <code>?BamFileList</code> .
<code>grl</code>	<code>GRangesList</code> object containing in each list component the range set of a feature type. Typically, this object is generated with the <code>genFeatures</code> function. For details see the example section below and <code>?genFeatures</code> .
<code>singleEnd</code>	Specifies whether the targets BAM files contain alignments for single-end (SE) or paired-end read data. <code>TRUE</code> is for SE and <code>FALSE</code> for PE data.
<code>readlength</code>	Integer vector specifying the read length values for which to report counts separately. If <code>readlength=NULL</code> 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 <code>featuretypeCounts</code> .
<code>type</code>	Determines whether the results are returned as <code>data.frame</code> ( <code>type="data.frame"</code> ) or as <code>list</code> ( <code>type="list"</code> ). Each list component contains the counting results for one BAM file and is named after the corresponding sample. The <code>data.frame</code> result contains this sample assignment information in a separate column.

**Value**

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

<code>SampleName</code>	Sample names obtained from <code>BamFileList</code> object.
<code>Strand</code>	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 <code>c(Fold=2, FDR=1)</code> 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(files, args=files, filter, varcaller="gatk", organism, out_dir="results")
```

**Arguments**

files	Object of class SYSargs or SYSargs2. The paths of the input VCF files are specified under infile1(args) and the paths of the output files under outfile1(args) or output(args).
args	Deprecated. Object of class SYSargs or SYSargs2. The paths of the input VCF files are specified under infile1(args) and the paths of the output files under outfile1(args) or output(args).
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.
out_dir	Character vector of a results directory name. Default is results.

**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:
library(VariantAnnotation)
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.tmpl", 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)
```

---

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("MF", "BP", "CC"), myslimv = "default",
                 correct = TRUE, recordSpecGO = NULL, ...)

## 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 ( <a href="http://geneontology.org/GO.downloads.annotations.shtml">http://geneontology.org/GO.downloads.annotations.shtml</a> ) or from BioMart as shown in example below.
colno	Column numbers referencing in myfile the three target columns containing GOID, GeneID and GOCAT, in that order.
org	Optional argument. Currently, the only valid option is org="Arabidopsis" to get rid of transcript duplications in this particular annotation.
lib	If the gene-to-GO mappings are obtained from a *.db package from Bioconductor then the package name can be specified under the lib argument of the sampleDFgene2GO function.
idconv	Optional id conversion data.frame
catdb	catdb object storing mappings of genes to annotation categories. For details, see ?"SYSargs-class".
rootUK	If the argument rootUK is set to TRUE then the root nodes are treated as terminal nodes to account for the new unknown terms.
sample	character vector containing the test set of gene identifiers
Nannot	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.
gocat	Specifies the GO type, can be assigned one of the following character values: "MF", "BP" and "CC".



GOHyperGAll_result	data.frame generated by GOHyperGAll
type	The function GOHyperGAll_Subset subsets the GOHyperGAll results by directly assigned GO nodes or custom goSlim categories. The argument type can be assigned the values goSlim or assigned.
myslimv	optional argument to provide custom goSlim vector
cutoff	p-value cutoff for GO terms to show in result data.frame
correct	If TRUE the function will favor the selection of terminal (informationich) GO terms that have at the same time a large number of sample matches.
setlist	list of character vectors containing gene IDs (or array feature IDs). The names of the list components correspond to the set labels, e.g. DEG comparisons or cluster IDs.
id_type	specifies type of IDs in input, can be assigned gene or affy
method	Specifies analysis type. Current options are all for GOHyperGAll, slim for GOHyperGAll_Subset or simplify for GOHyperGAll_Simplify.
CLSZ	minimum gene set (cluster) size to consider. Gene sets below this cutoff will be ignored.
gocats	Specifies GO type, can be assigned the values "MF", "BP" and "CC".
recordSpecGO	argument to report in the result data.frame specific GO IDs for any of the 3 ontologies disregarding whether they meet the specified p-value cutoff, e.g: recordSpecGO=c("GO:0003674", "GO:0008150", "GO:0005575")
GOBatchResult	data.frame generated by GOCluster_Report
...	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/
                                     download=TRUE)
# catdb_conv <- makeCATdb(myfile="GOannotationsBiomart_mod.txt", lib=NULL, org="",
                         colno=c(1,2,3), idconv=list(affy=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=0.001,
                                gocats=c("MF", "BP", "CC"),
                                recordSpecGO=c("GO:0003674", "GO:0008150", "GO:0005575"))

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

## End(Not run)
```

---

importWF

---

*Import R Markdown file as workflow*


---

## Description

Import R Markdown file as workflow. Each R code chunk will be set as a step in the workflow. This operation requires a few extra settings on the R Markdown chunk options, to include a particular code chunk in the workflow analysis. Please check Details.

## Usage

```
importWF(sysargs, file_path, ignore_eval = TRUE, verbose = TRUE)
```

## Arguments

sysargs	<a href="#">SYSargsList</a> empty object. More information on Details.
file_path	string, file path of the workflow file.
ignore_eval	logical, treat all R chunks' eval option as TRUE in workflow Rmd file even if some chunks have eval=FALSE.
verbose	logical, print out verbose message while function running.

## Details

To include a particular code chunk from the R Markdown file in the workflow analysis, please use the following code chunk options:

- spr = 'r': for code chunks with R code lines; - spr = 'sysargs': for code chunks with an 'SYSargsList' object; - spr.dep = <StepName>: for specify the previous dependency. If this options is not found, it will automaticly add the previous step.

For spr = 'sysargs', the last object assigned needs to be the [SYSargsList](#). If the spr flag is not found, the R chunk will not be included in the workflow.

It is required to start a project using SPRproject() function, and use the object to populate the steps from R Markdown file.

**Value**

importWF will return an SYSargsList object.

**Author(s)**

Le Zhang and Daniela Cassol

**See Also**

[SYSargsList](#)

**Examples**

```
file_path <- system.file("extdata/spr_simple_lw.Rmd", package="systemPipeR")
sal <- SPRproject(overwrite = TRUE)
sal <- importWF(sal, file_path)
```

---

INTERSECTset-class      *Class "INTERSECTset"*

---

**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)
```

---

LineWise-class

*Class "LineWise"*

---

**Description**

S4 class container for storing R-based code for a workflow step. LineWise class instances are constructed by the LineWise function, based on the R-based code, step name, and dependency tree. When the container is built from the R Markdown, using `importWF` function, two other slots are populated: `codeChunkStart` and `rmdPath`. `codeChunkStart` will store the first line of each R chunk, and `rmdPath` will store the R Markdown file path.

**Usage**

```
## Constructor
```

```
LineWise(code, step_name = "default", codeChunkStart = integer(),
         rmdPath = character(), dependency="",
         run_step = "mandatory",
         run_session = "management")
```

**Arguments**

code	R code separated either by a semi-colon (\;), or by a newline, and enclosed by braces ({ }).
codeChunkStart	integer. R Markdown code chunk line start. This element will be populated when the object is built by <code>importWF</code> .
rmdPath	character. Path of R Markdown file used by <code>importWF</code> .
step_name	character. Step name needs to be unique and is required when appending this step to the workflow.
dependency	character. Dependency tree, required when appending this step to the workflow. Character name of a previous step in the workflow. Default is empty string "".
run_step	character. If the step has "mandatory" or "optional" flag for the execution.
run_session	character. If the step has "management" or "compute" flag for the execution.

**Objects from the Class**

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

**Slots**

**codeLine:** Object of class "expression" storing R-based code.

**codeChunkStart:** Object of class "integer" storing start line from the rmdPath file, when the "LineWise" is built from R Markdown.

**stepName:** Object of class "character" storing step name.

**dependency:** Object of class "list" storing dependency tree.

**status:** Object of class "list" storing status steps.

**files:** Object of class "list" storing file for R Markdown file and the file containing stdout and stderr after running the R-based code.

**runInfo:** Object of class "list" storing all the runInfo information of the workflow

**Functions and Methods**

See 'Usage' for details on invocation.

Constructor:

**LineWise:** Returns a LineWise object.

Accessors:

**codeLine** Printing method for the CodeLine slot.  
**codeChunkStart** Extract start line of the R Markdown R chunk.  
**rmdPath** Extract Rmarkdown file path.  
**stepName** Extract the step name.  
**dependency** Extract the dependency tree.  
**status** Extract status of the step.  
**files** Extract log file path storing stdout and stderr after running step.  
**appendCodeLine<-** Replacement method for append a R code line.  
**replaceCodeLine<-** Replacement method for replace a R code line.

Methods:

[ Return a new LineWise object made of the selected R code lines.  
[[ Extract the slot information from LineWise object.  
[[<- Replacement method for LineWise slots.  
\$ Extract slots elements by name.  
**length** Extract number of R-based code lines.  
**names** Extract slot names.  
**show** Summary view of LineWise elements.  
**coerce** signature(from = "LineWise", to = "list")as(LineWise, "list")  
**coerce** signature(from = "list", to = "LineWise")as(list, "LineWise")  
**linewise** Coerce back to list as(LineWise, "list")

### Author(s)

Daniela Cassol

### See Also

[SYSargsList](#)

### Examples

```
showClass("LineWise")
lw <- LineWise(code = {
  log_out <- log(10)
},
step_name = "R_log")

codeLine(lw)

## ImportWF option
file_path <- system.file("extdata/spr_simple_lw.Rmd", package="systemPipeR")
sal <- SPRproject(overwrite = TRUE)
```

```

# file_path <- "../inst/extdata/spr_simple_lw.Rmd"
sal <- importWF(sal, file_path)
sal <- runWF(sal)
lw2 <- sal$stepsWF[[2]]
lw2
names(lw2)
length(lw2)

## Accessors
codeLine(lw2)
codeChunkStart(lw2)
rmdPath(lw2)
stepName(lw2)
dependency(lw2)
status(lw2)
files(lw2)

## Replacement
appendCodeLine(lw2, after = 0) <- "log <- log(10)"
codeLine(lw2)
replaceCodeLine(lw2, 1) <- "plot(iris)"
codeLine(lw2)

## Coerce
lw2 <- linewise(lw2) ## OR lw2 <- as(lw2, "list")
lw2 <- as(lw2, "LineWise")

```

---

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.

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. 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. Between different instances, this connectivity is established by ``appendStep<-`` method. Please check more details from [SYSargsList-class](#) class.



**Usage**

```
loadWorkflow(targets = NULL, wf_file, input_file, dir_path = "param/cwl", id = "SampleName")

renderWF(WF, inputvars = NULL)

updateWF(WF, write.yaml=FALSE, name.yaml="default", new_targets=NULL,
         new_targetsheader=NULL, inputvars=NULL, silent=FALSE)
```

**Arguments**

targets	either the path to targets file or an object of SummarizedExperiment 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.
wf_file	name and path to CWL parameters file.
input_file	name and path to input parameters file.
dir_path	path to the parameters directory with the wf_file and input_file files. It is recommended to keep both files in the same directory.
id	A column from targets file, which will be used as an id for each one of the samples. It is required to be unique.
WF	Object of class SYSargs2, generated by loadWF .
inputvars	named character vector. Variables defined in the input file that matches the column names defined in the targets file.
write.yaml	logical. If set to TRUE, it will write to file the content of the CWL files: *.yaml. Default is FALSE.
name.yaml	name and path to input parameters file, if write.yaml is set to TRUE. Default value will write a file at the same directory of dir_path appending to the file name the current date.
new_targets	new targets files as list. 'targets' data.frame can be converted by targets.as.list function. Default is NULL, and it will maintain the original.
new_targetsheader	character. New header/comment lines of targets file. Default is NULL, and it will maintain the original.
silent	If set to TRUE, all messages returned by the function will be suppressed.

**Value**

SYSargs2 object.

**Author(s)**

Daniela Cassol and Thomas Girke

**See Also**

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

## If required to update the object
yamlinput(WF, "thread") <- 6L
WF <- updateWF(WF)
cmdlist(WF)[1]
yamlinput(WF)$thread
```

---

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 alignments (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)
```

**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
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
intersec <- overLapper(setlist=setlist, type="intersects", complexity=2)
(paMA <- intersectmatrix(intersec))
heatmap(paMA, Rowv=NA, Colv=NA, col=c("white", "gray"))

```

---

olRanges

*Identify Range Overlaps for IRanges and GRanges Object*


---

## 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::IRanges(seq(1, 10), 5))
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	<i>Updates the output files paths in the SYSargs2 object</i>
---------------	--

---

### 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, del
```

### 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.
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.

### 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", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2/hisat2-mapping-se.cwl",
                  input_file="hisat2/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**

setlist	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.
complexity	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.
sep	Character used to separate set labels.
cleanup	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.
keepdups	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.
type	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`, `vennerable`, `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:

setlist	Original label sets accessible with <code>setlist()</code> .
intersectmatrix	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 set 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.
complexitylevels	Complexity levels accessible with <code>complexitylevels()</code> .
vennlist	Venn intersects for <code>VENNset</code> objects accessible with <code>vennlist()</code> .
intersectlist	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/VennSymmExar>

**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(interset, mincount=1)

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

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

## 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
interset <- overLapper(setlist=setlist, type="intersects", complexity=2)
(paMA <- intersectmatrix(interset))
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 data.frame 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 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.
extendylim	Allows to extend the upper limit of the y axis when scales=fixed. Internally, the function identifies the maximum value in the data and then multiplies this maximum value by the value provided under extendylim. The default is set to extendylim=2.
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 <sup>6</sup> ). For this calculation the N_total_aligned values are used that are reported in the input data.frame generated by the upstream featureCoverage function. Assign NULL 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=c
p2 <- plotfeaturetypeCounts(x=featureCounts2, graphicsfile="featureCounts2.pdf", graphicsformat="pdf", scales="f

## End(Not run)

```

---

plotWF

*Visualize SPR workflow and status*


---

## Description

Visualize SPR workflow and status. plotWF is the general function that creates the plot. plotwfOutput and renderPlotwf are used in Shiny UI and server respectively, similar to plotOutput and renderPlot.

## Usage

```

plotWF(
  sysargs,
  width = NULL, height = NULL,
  elementId = NULL,
  responsive = TRUE,
  branch_method = "auto",
  branch_no = NULL,
  layout = "compact",
  no_plot = FALSE,
  plot_method = "svg",
  out_format = "plot",
  out_path = NULL,
  show_legend = TRUE,
  mark_main_branch = FALSE,
  rstudio = FALSE,
  in_log = FALSE,
  rmarkdown = "detect",
  verbose = FALSE,
  show_warns = FALSE,
  plot_ctr = TRUE,
  pan_zoom = FALSE,
  exit_point = 0

```



```

)

plotwfOutput(
  outputId,
  width = '100%',
  height = '400px'
)

renderPlotwf(
  expr,
  env = parent.frame(),
  quoted = FALSE
)

```

### Arguments

sysargs	object of class SYSargsList.
width	string, a valid CSS string for width, like "500px", "100%".
height	string, a valid CSS string for height, like "500px", "100%".
elementId	string, optional ID value for the plot.
responsive	bool, should the plot be responsive? useful in Rstudio built-in viewer, Rmarkdown, Shiny or embed it into other web pages.
branch_method	string, one of "auto", "choose". How to determine the main branch of the workflow. "auto" will be determined by internal algorithm: Branches connecting the first and last step and/or the longest will be favored. "choose" will list all possible branches and you can make a choice.
branch_no	numeric, only works if branch_method == "choose". Specify a branch number to be the main branch instead of choosing from the prompt. This option can be good if you are in a non-interactive mode, e.g. rendering Rmd.
layout	string, one of "compact", "vertical", "horizontal", "execution".
no_plot	bool, if you want to assign the plot to a variable and do not want to see it interactively, change this to FALSE.
plot_method	string, one of "svg", "png", how to make plot, use svg or png to embed the plot.
out_format	string, one of "plot", "html", "dot", "dot_print" <ul style="list-style-type: none"> <li>• plot: directly open your viewer or browser of the plot</li> <li>• html: save the plot to a html file</li> <li>• dot: save the plot in DOT language, need a dot engine to remake the plot</li> <li>• dot_print: directly cat the DOT code on console</li> </ul>
out_path	string, if the out_format is not "plot" or "dot_print", provide a path of where to save the plot.
show_legend	bool, show plot legend?
mark_main_branch	bool, color the main branch on the plot?

<code>rstudio</code>	bool, if you are using Rstudio, open the built-in viewer to see the plot? Default is no, open the browser tab to see it plot. The default viewer is too small to see the full plot clearly, so we recommend to use the browser tab. However, if you are using this plot in Shiny apps, always turn <code>rstudio = TRUE</code> .
<code>in_log</code>	bool, is this plot been made in a SPR log file? If TRUE will add links of steps to the corresponding log sections.
<code>rmarkdown</code>	are you rendering this plot in a Rmarkdown document? default value is "detect", this function will determine based on current R environment, or you can force it to be TRUE or FALSE.
<code>verbose</code>	bool, turn on verbose mode will give you more information.
<code>show_warns</code>	bool, print the warning messages on the plot?.
<code>plot_ctr</code>	bool, add the plot control panel to the plot? This requires you to have internet connection. It will download some additional javascript libraries, and allow you to save the plot as png, jpg, svg, pdf or graphviz directly from the browser.
<code>pan_zoom</code>	bool, allow panning and zooming of the plot? Use mouse wheel or touch pad to zoom in and out of the plot. You need to have internet connection, additional javascript libraries will be loaded automatically online. Cannot be used with <code>responsive = TRUE</code> together. If both TRUE, <code>responsive</code> will be automatically set to FALSE.
<code>exit_point</code>	numeric, for advanced debugging only, see details
<code>outputId</code>	string, shiny output ID
<code>expr</code>	An expression that generates a plotwf, like <code>plotWF(sal)</code>
<code>env</code>	The environment in which to evaluate <code>expr</code> .
<code>quoted</code>	Is <code>expr</code> a quoted expression (with <code>quote()</code> )? This is useful if you want to save an expression in a variable.

## Details

### layout:

- compact: try to plot steps as close as possible.
- vertical: main branch will be placed vertically and side branches will be placed on the same horizontal level and sub steps of side branches will be placed vertically.
- horizontal: main branch is placed horizontally and side branches and sub steps will be placed vertically.
- execution: a linear plot to show the workflow execution order of all steps.

### exit\_point:

return intermediate results at different points and exit the function

- 0: no early exit
- 1: after all branches are found, return tree
- 2: after the new tree has been built, return new nodes
- 3: after dot translation, return graph string

**Rmarkdown:**

Rmarkdown will change some of the format and cause conflicts. If the plot can be rendered outside Rmd but cannot within Rmd, try to turn this option on. Some additional javascript processing will be performed to avoid the conflict but may cause unknown issues.

**Shiny:**

When the plot is rendered in a Shiny app, the `rstudio` option must be turned on, `plotWF(sal, rstudio = TRUE, ...)`.

**Value**

see `out_format` and `exit_point`

---

predORF	<i>Predict ORFs</i>
---------	---------------------

---

**Description**

Predicts open reading frames (ORFs) and coding sequences (CDSs) in DNA sequences provided as `DNAStr` or `DNAStrSet` objects.

**Usage**

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

**Arguments**

<code>x</code>	DNA query sequence(s) provided as <code>DNAStr</code> or <code>DNAStrSet</code> object.
<code>n</code>	Defines the maximum number of ORFs to return for each input sequence. The ORFs identified are sorted decreasingly by their length. For instance, <code>n=1</code> (default) returns the longest ORF, <code>n=2</code> the two longest ones, and so on.
<code>type</code>	One of three options provided as character values: <code>'df'</code> returns results as <code>data.frame</code> , while <code>'gr'</code> and <code>'gr1'</code> (default) return them as <code>GRanges</code> or <code>GRangesList</code> objects, respectively.
<code>mode</code>	The setting <code>mode='ORF'</code> returns a continuous reading frame that begins with a start codon and ends with a stop codon. The setting <code>mode='CDS'</code> return continuous reading frames that do not need to begin or end with start or stop codons, respectively.
<code>strand</code>	One of three options passed on as character vector of length one: <code>'sense'</code> performs the predictions only for the sense strand of the query sequence(s), <code>'antisense'</code> does it only for the antisense strand and <code>'both'</code> does it for both strands.
<code>longest_disjoint</code>	If set to <code>TRUE</code> and <code>n='all'</code> , 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")
```

```
gr1_scaled <- scaleRanges(subject=futr, query=uorf, type="uORF", verbose=TRUE)
export.gff3(unlist(gr1_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 = NULL,
                FileName1 = NULL, FileName2 = NULL,
                outfile1 = NULL, outfile2 = NULL,
                Fct, batchsize = 100000, overwrite = TRUE, ...)
```

### Arguments

<code>args</code>	Object of class <code>SYSargs</code> or <code>SYSargs2</code> .
<code>FileName1</code>	Path to input forward fastq file. Default is <code>NULL</code> .
<code>FileName2</code>	Path to input reverse fastq file. Default is <code>NULL</code> .
<code>outfile1</code>	Path to output forward fastq file. Default is <code>NULL</code> .
<code>outfile2</code>	Path to output reverse fastq file. Default is <code>NULL</code> .
<code>Fct</code>	character string of custom read preprocessing function call where both the input and output needs to be an object of class <code>ShortReadQ</code> . The name of the input <code>ShortReadQ</code> object needs to be <code>fq</code> .
<code>batchsize</code>	Number of reads to process in each iteration by the internally used <code>FastqStreamer</code> function.
<code>overwrite</code>	If <code>TRUE</code> existing file will be overwritten.
<code>...</code>	To pass on additional arguments to the internally used <code>writeFastq</code> function.

### Value

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

### Author(s)

Thomas Girke

**See Also**

FastqStreamer

**Examples**

```
## Preprocessing of single-end reads
dir_path <- system.file("extdata/cwl/preprocessReads/trim-se", package="systemPipeR")
targetspath <- system.file("extdata", "targets.txt", package="systemPipeR")
trim <- loadWorkflow(targets=targetspath, wf_file="trim-se.cwl", input_file="trim-se.yml", dir_path=dir_path)
trim <- renderWF(trim, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
## Not run:
preprocessReads(args=trim[1], Fct="trimLRPatterns(Rpattern='GCCCGGTAA', subject=fq)", batchsize=100000, overwr

## End(Not run)

## Preprocessing of paired-end reads
dir_path <- system.file("extdata/cwl/preprocessReads/trim-pe", package="systemPipeR")
targetspath <- system.file("extdata", "targetsPE.txt", package="systemPipeR")
trim <- loadWorkflow(targets=targetspath, wf_file="trim-pe.cwl", input_file="trim-pe.yml", dir_path=dir_path)
trim <- renderWF(trim, inputvars=c(FileName1="_FASTQ_PATH1_", FileName2="_FASTQ_PATH2_", SampleName="_SampleName_"))
trim
## Not run:
preprocessReads(args=trim[1], Fct="trimLRPatterns(Rpattern='GCCCGGTAA', subject=fq)", batchsize=100000, overwr

## 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)
```

---

readComp	<i>Import sample comparisons from targets file</i>
----------	--

---

### 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 = "-")
```



---

renderLogs	<i>Render RMarkdown Logs Report</i>
------------	-------------------------------------

---

## Description

Render the logs report file to the specified output format using pandoc.

## Usage

```
renderLogs(sysargs, type = c("html_document", "pdf_document"),
           fileName = "default", quiet = FALSE,
           open_file = TRUE)
```

## Arguments

sysargs	object of class SYSargsList.
type	The R Markdown output format to convert to. The option can be the name of a format (e.g. "pdf_document" or "html_document").
fileName	character string naming a file output. Default is "logs_<date>.Rmd".
quiet	If set to TRUE, all messages returned by the function will be suppressed.
open_file	Default is TRUE.

## Value

It will return an SYSargsList updated.

## Author(s)

Daniela Cassol

## See Also

See also as SYSargsList-class.

## Examples

```
## Construct SYSargsList object from Rmd file
sal <- SPRproject(overwrite=TRUE)
targetspath <- system.file("extdata/cwl/example/targets_example.txt", package="systemPipeR")

## Constructor and `appendStep`-`
appendStep(sal) <- SYSargsList(step_name = "echo",
                              targets=targetspath, dir=TRUE,
                              wf_file="example/workflow_example.cwl", input_file="example/example.yml",
                              dir_path = system.file("extdata/cwl", package="systemPipeR"),
                              inputvars = c(Message = "_STRING_", SampleName = "_SAMPLE_"))
appendStep(sal) <- LineWise(code = {
```

```

      hello <- lapply(getColumn(sal, step=1, 'outfiles'), function(x) yaml::read_yaml(x))
      },
      step_name = "R_read",
      dependency = "echo")
sal <- runWF(sal)
sal <- renderLogs(sal, open_file = FALSE)

```

---

renderReport

*Render RMarkdown Report*


---

## Description

Render the technical report file to the specified output format using pandoc.

## Usage

```

renderReport(sysargs, fileName = "spr_report",
             rmd_title = "SPR workflow Template - Report",
             rmd_author = "Author",
             rmd_date= "Last update: `r format(Sys.time(), '%d %B, %Y')`",
             type = c("html_document"),
             desc = "This is a workflow template.",
             quiet = FALSE, open_file = TRUE)

```

## Arguments

sysargs	object of class SYSargsList.
fileName	character string naming a file output. Default is "spr_report.Rmd".
rmd_title	string, title of the Rmd.
rmd_author	string, author(s) of the Rmd, put all authors in a single character string.
rmd_date	string, date header of Rmd.
type	The R Markdown output format to convert to. The option can be the name of a format (e.g. "pdf_document" or "html_document").
desc	string, or character vector of strings, some description text in format Rmarkdown that will be added to the document before the workflow steps start. It can be a single line or multiple lines by providing a character vector, each item is one line.
quiet	If set to TRUE, all messages returned by the function will be suppressed.
open_file	Default is TRUE.

## Value

It will return an SYSargsList updated, with the file path location.

**Author(s)**

Daniela Cassol

**See Also**

See also as SYSargsList-class.

**Examples**

```
sal <- SPRproject(overwrite = TRUE)
file_path <- system.file("extdata", "spr_simple_wf.Rmd", package = "systemPipeR")
sal <- importWF(sal, file_path = file_path, verbose = FALSE)
targetspath <- system.file("extdata/cwl/example/targets_example.txt", package = "systemPipeR")
appendStep(sal) <- SYSargsList(step_name = "echo",
                             targets = targetspath, dir = TRUE,
                             wf_file = "example/workflow_example.cwl", input_file = "example/example.yml",
                             dir_path = system.file("extdata/cwl", package = "systemPipeR"),
                             inputvars = c(Message = "_STRING_", SampleName = "_SAMPLE_"))
sal <- renderReport(sal, open_file = FALSE)
```

returnRPKM

*RPKM Normalization***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      *Execute SYSargs and SYSargs2*

---

### Description

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

### Usage

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

### 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=FALSE.
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=TRUE. 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.
input_targets	This option allows selecting which targets file and, by consequence which command line will be executed. Default is NULL, in which all command lines will be executed.
...	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{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", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2/hisat2-mapping-se.cwl",
                  input_file="hisat2/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", template="batchtools.slurm.tpl",
                 Njobs=18, runid="01", resourceList=resources)

## 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)

#####
## 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.tpl", 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", Njobs=18,
                  runid="01", resourceList=resources)

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

```

---

runDiff

*Differential abundance analysis for many range sets*


---

## 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</code> data.frame
<code>cmp</code>	character matrix where comparisons are defined in two columns. This matrix should be generated with <code>readComp()</code> from the <code>targets</code> file. Values used for comparisons need to match those in the <code>Factor</code> column of the <code>targets</code> file.

dbrfilter      Named vector with filter cutoffs of format `c(Fold=2,FDR=1)` where `Fold` refers to the fold change cutoff (unlogged) and `FDR` to the p-value cutoff. Those values are passed on to the `filterDEGs` function.

...              Arguments to be passed on to the internally used `run_edgeR` or `run_DESeq2` 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_macs.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, dbrfilter=
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(sysargs, steps = NULL, targets = NULL,
      force = FALSE, saveEnv = TRUE,
      run_step = "ALL", ignore.dep = FALSE,
      warning.stop = FALSE, error.stop = TRUE, silent = FALSE, ...)
```

**Arguments**

sysargs	object of class SYSargsList.
steps	character or numeric. Step name or index. If NULL, all the steps will be executed.
targets	This option allows selecting which targets file and, by consequence which command line will be executed for each SYSargs2 class step. Default is NULL, in which all command lines will be executed.
force	Internally, the option 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.
saveEnv	If set to TRUE, the environment will be saved to an RDS file. To check the RDS file location, please use <code>projectInfo(sysargs)[["envir"]]</code> .
run_step	character. If the step has "mandatory" or "optional" flag for the execution. When ALL, all the steps will be executed.
ignore.dep	logical. This option allow to ignore the dependency tree, when TRUE.
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.
...	Additional arguments to pass on from <code>runCommandline()</code> .

**Value**

It will return an SYSargsList updated.

**Author(s)**

Daniela Cassol and Thomas Girke



**See Also**

See also as SYSargsList-class.

**Examples**

```
## Construct SYSargsList object from Rmd file
sal <- SPRproject(overwrite=TRUE)
targetspath <- system.file("extdata/cwl/example/targets_example.txt", package="systemPipeR")

## Constructor and `appendStep<-`
appendStep(sal) <- SYSargsList(step_name = "echo",
                              targets=targetspath, dir=TRUE,
                              wf_file="example/workflow_example.cwl", input_file="example/example.yml",
                              dir_path = system.file("extdata/cwl", package="systemPipeR"),
                              inputvars = c(Message = "_STRING_", SampleName = "_SAMPLE_"))
appendStep(sal) <- LineWise(code = {
  hello <- lapply(getColumn(sal, step=1, 'outfiles'), function(x) yaml::read_yaml(x))
},
  step_name = "R_read",
  dependency = "echo")

## Not run:
sal <- runWF(sal)

## 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>	data.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 <code>Factor</code> 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.
lfcShrink	logiactal. If TRUE adds shrunken log2 fold changes (LFC) to the object.
type	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, mdspplot = "")
```

**Arguments**

countDF	date.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).
mdspplot	Directory where plotMDS should be written to. Default setting mdspplot="" 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, mdspplot="")
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
```

---

sal2bash	<i>Translate SYSargsList back to a bash workflow</i>
----------	--

---

### Description

This function takes a `SYSargsList` object and translate it to an executable bash script, so one can run the workflow without loading SPR or using an R console.

### Usage

```
sal2bash(sal, out_dir = ".", bash_path = "/bin/bash", stop_on_error = TRUE)
```

### Arguments

<code>sal</code>	<code>SYSargsList</code> object.
<code>out_dir</code>	string, a relative or absolute path to a directory. If the directory does not exist, this function will first try to create it.
<code>bash_path</code>	string, the path to the bash executable program
<code>stop_on_error</code>	bool, should the bash script stop if any error happens in running

### Details

## out files

1. The main executable bash file will be created to the root of `'out_dir'`
2. All R steps will be stored as R scripts and along with other supporting files inside a folder called `'spr_bash'` under `'out_dir'`
3. Not all R steps will have an individual file. This function will "collapse" adjacent R steps into one file as much as possible. Namely, if there is no `sysArgs` steps in between, R steps will be merged into one file, otherwise they will be in different files.

## R steps

Similarly as running the workflow in R console, all R steps will share the same environment variables and loaded packages. This is done by loading and saving the R environment into a file `'spr_wf.RData'` before and after the R script execution. Therefore, it will be good to keep all R steps bundle together as much as possible to avoid the package and environment loading/saving overhead time.

Initially, this environment only contains the `SYSargsList` object that was used to create the bash script. Note: the `SYSargsList` object name will be the same as what you pass to `'sal2bash'`. If you have `'sal2bash(my_sal)'`, then in the `'spr_wf.RData'` there will be an object called `'my_sal'` saved there. It is important to keep using the same name for the `SYSargsList` object managing the workflow and in workflow running. For example, if you have an R step that requires to query a column from the outfiles of the SAL, `'getColumn(sal, "FileName1")'`, but you pass `'my_sal'` to `'sal2bash(my_sal)'`, this will cause this R step cannot find the SAL object when run the workflow from bash.

## Execution

This way of execution is not able to handle complex dependency graphs. The original step dependencies from SAL object will be ignored, so all steps will be executed in a linear manner. It is recommended to adjust the workflow order before using this function.

### Value

no return

### Author(s)

Le Zhang and Daniela Cassol

### Examples

```
file_path <- system.file("extdata/spr_simple_wf.Rmd", package="systemPipeR")
sal <- SPRproject(overwrite = TRUE)
sal <- importWF(sal, file_path)
sal2bash(sal)
```

---

sal2rmd	<i>Translate SYSargsList back to a workflow template Rmarkdown file</i>
---------	---

---

### Description

This function takes a SYSargsList object and translate it to SPR workflow template Rmarkdown format.

### Usage

```
sal2rmd(sal, out_path = "spr_template.Rmd", rmd_title = "SPR workflow template",
        rmd_author = "my name",
        rmd_date = "Last update: `r format(Sys.time(), '%d %B, %Y')`",
        rmd_output = "html_document",
        desc = "This is a workflow template.", verbose = TRUE)
```

### Arguments

sal	SYSargsList object.
out_path	string, output file name.
rmd_title	string, title of the Rmd.
rmd_author	string, author(s) of the Rmd, put all authors in a single character string.
rmd_date	string, date header of Rmd.
rmd_output	string, output format of Rmd, used in header.
desc	string, or character vector of strings, some description text in format Rmarkdown that will be added to the document before the workflow steps start. It can be a single line or multiple lines by providing a character vector, each item is one line.
verbose	logical. If TRUE will show you more information as the function runs.

**Value**

no return

**Author(s)**

Le Zhang and Daniela Cassol

**Examples**

```
file_path <- system.file("extdata/spr_simple_wf.Rmd", package="systemPipeR")
sal <- SPRproject(overwrite = TRUE)
sal <- importWF(sal, file_path)
sal2rmd(sal)
```

---

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**

```

library(IRanges)
## 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**

fastq	Named character vector containing paths to FASTQ file in the data fields and sample labels in the name slots.
batchsize	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.
klength	Specifies the k-mer length in the plot for the relative k-mer diversity.
fqlist	list object returned by seeFastq().
arrange	Integer vector from 1 to 7 specifying the row order of the QC plot. Dropping numbers eliminates the corresponding plots.
...	Additional plotting arguments to pass on to seeFastqPlot().

**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", package="systemPipeR")
args <- loadWorkflow(targets=targets, wf_file="hisat2/hisat2-mapping-se.cwl",
                    input_file="hisat2/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)
```

---

showDF

*Create an HTML table using DT package with fixed columns*

---

**Description**

Create an HTML table using DT package with fixed columns



**Usage**

```
showDF(data, ...)
```

**Arguments**

data            data object (either a matrix or a data frame).  
...            Additional arguments used by dDT::atatable() function.

**Value**

returns an object of datatables and htmlwidget.

**Examples**

```
showDF(iris)
```

---

SPRproject

*Workflow Project Initiation*

---

**Description**

Function to construct SYSargsList workflow control environment (S4 object). This function creates and checks the directory structure. If the expected directories are not available, it is possible to create those. The project directory structure expected is:

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

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

**Usage**

```
SPRproject(projPath = getwd(), data = "data", param = "param", results = "results",  
          logs.dir= ".SPRproject", sys.file="SYSargsList.yml",  
          envir = new.env(),  
          restart = FALSE, resume=FALSE,  
          load.envir = FALSE,  
          overwrite = FALSE, silent = FALSE)
```

**Arguments**

<code>projPath</code>	a character vector of a full project path name. Default is the current path.
<code>data</code>	a character vector of a data directory name. Default is <code>data</code> . This subdirectory in the project stores all the raw data, reference, and annotation files.
<code>param</code>	a character vector of a param directory name. Default is <code>param</code> . This subdirectory in the project stores all the parameter and configuration files.
<code>results</code>	a character vector of a results directory name. Default is <code>results</code> . 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.
<code>logs.dir</code>	a character vector of a logs directory name. Default is <code>.SPRproject</code> .
<code>sys.file</code>	name and path of the <code>SYSargsList</code> file which will store all the project configuration information. Default is <code>.SPRproject/SYSargsList.yml</code> .
<code>envir</code>	the environment in which <code>expr</code> is to be evaluated. Default will create a new <code>env()</code> . Another option is to use <code>globalenv()</code> .
<code>restart</code>	if set to <code>TRUE</code> , existing <code>SYSargsList</code> save in the directory will be used to restart the workflow. This option requires <code>overwrite=TRUE</code> . All the log will be deleted.
<code>resume</code>	if set to <code>TRUE</code> , existing <code>SYSargsList</code> save in the directory will be used to resume the workflow.
<code>load.envir</code>	after running the workflow, the environment can be saved on disk. This argument allows to load the environment and recover all the objects. This argument can be set as <code>TRUE</code> when <code>restart = TRUE</code> .
<code>overwrite</code>	if set to <code>TRUE</code> , existing logs files or directory of same name will be overwritten.
<code>silent</code>	if set to <code>TRUE</code> , all messages returned by the function will be suppressed.

**Details**

If an `SYSargsList` instance was created before or independent of the project initialization, it is possible to append this instance after the project is created. Please see `checkAppendStep<-` function.

**Value**

`SPRproject` will return a `SYSargsList` object.

**Author(s)**

Daniela Cassol

**See Also**

See also as `SYSargsList-class`.

**Examples**

```
sal <- SPRproject(projPath = tempdir())
sal
```

---

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", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2/hisat2-mapping-se.cwl",
                  input_file="hisat2/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", package="systemPipeR")
args <- loadWorkflow(targets=targets, wf_file="hisat2/hisat2-mapping-se.cwl", input_file="hisat2/hisat2-mapping-se.cwl")
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=20:00:00")
qsubRun(appfct="runCommandline(args=args)", appargs=args, qsubargs=qsubargs,
        Nqsubs=1, submitdir="results", package="systemPipeR")

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

## End(Not run)

```

---

SYSargs2-class	Class "SYSargs2"
----------------	------------------

---

## 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 loadWF 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. 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 parameters file  
**clt:** Object of class "list" storing data from each CommandLineTool substep in the Workflow or the single codeCommandLineTool CWL parameters file  
**yamlinput:** Object of class "list" storing data from input (\*.yaml) 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  
**files:** Object of class "list" paths to input and CWL parameters files  
**inputvars:** Object of class "list" storing data from each inputvars  
**cmdToCwl:** Object of class "list" storing data from each cmdToCwl  
**status:** Object of class "list" storing data from each status  
**internal\_outfiles:** Object of class "list" storing raw data from each output

## Methods

**[** Subsetting of class with bracket operator.  
**[[** Subsetting of class with bracket operator.  
**[[<-** Replacement method for "SYSargs2" class.  
**\$** Extracting slots elements by name.  
**length** Extracts number of samples.  
**names** Extracts slot names.  
**show** Summary view of SYSargs2 objects.  
**coerce** signature(from = "list", to = "SYSargs2"): as(list, "SYSargs2")  
**coerce** signature(from = "SYSargs2", to = "list")as(SYSargs2, "list")  
**coerce** signature(from = "SYSargs2", to = "DataFrame"): as(x, "DataFrame"); for targets slot.  
**sysargs2** Coerce back to list as(SYSargs2, "list")  
**targets** Extract data from targets slot.  
**targetsheader** Extracts data from targetsheader slot.  
**modules** Extracts data from modules slot.  
**wf** Extracts data from wf slot.  
**clt** Extracts data from clt slot.  
**yamlinput** Extracts data from yamlinput slot.  
**cmdlist** Extracts data from cmdlist slot.  
**input** Extracts data from input slot.  
**output** Extracts data from cmdlist slot.  
**files** Extracts data from files slot.  
**inputvars** Extracts data from inputvars slot.  
**cmdToCwl** Extracts data from cmdToCwl slot.  
**status** Extracts data from status slot.  
**infile1** extracting paths to first FASTQ file.  
**infile2** extracting paths to second FASTQ file if data is PE.  
**baseCommand** Extracts baseCommand from command-line used to execute external software.  
**SampleName** Extracts all samples names.  
**yamlinput<-** Replacement method for yamlinput slot input.

**Author(s)**

Daniela Cassol and Thomas Girke

**See Also**

loadWF and renderWF and runCommandline and clusterRun

**Examples**

```
showClass("SYSargs2")

## Construct SYSargs2 object from CWL param, CWL input, and targets files
targetspath <- system.file("extdata/cwl/example/targets_example.txt", package="systemPipeR")
dir_path <- system.file("extdata/cwl", package="systemPipeR")
WF <- loadWorkflow(targets=targetspath,
                  wf_file="example/workflow_example.cwl",
                  input_file="example/example.yml",
                  dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(Message = "_STRING_", SampleName = "_SAMPLE_"))
WF

## Methods
names(WF)
length(WF)
baseCommand(WF)
SampleName(WF)

## Accessors
targets(WF)
targetsheader(WF)
modules(WF)
yamlinput(WF)
cmdlist(WF)
input(WF)
output(WF)
files(WF)
inputvars(WF)
cmdToCwl(WF)
status(WF)

## The subsetting operator '[' allows to select specific command-line/sample
WF2 <- WF[1:2]

## Not run:
## Execute SYSargs2 on single machine
WF2 <- runCommandline(WF2)

## End(Not run)
## 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.tpl", package="systemPipeR"), ".")
resources <- list(walltime=120, ntasks=1, ncpus=4, memory=1024)
reg <- clusterRun(WF, FUN = runCommandline, conffile=".batchtools.conf.R",
                 template="batchtools.slurm.tpl", Njobs=2, runid="01", resourceList=resources)

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

## End(Not run)

```

---

SYSargsList	<i>Class "SYSargsList"</i>
-------------	----------------------------

---

### Description

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

### Usage

## Constructor

```

SYSargsList(sysargs = NULL, step_name = "default",
            targets = NULL, wf_file = NULL, input_file = NULL, dir_path = ".",
            id = "SampleName",
            inputvars = NULL, rm_targets_col = NULL,
            dir = TRUE, dependency = NA,
            run_step = "mandatory",
            run_session = "management",
            run_remote_resources = NULL,
            silent = FALSE,
            projPath = getOption("projPath", getwd()))

```

## Accessors

```

stepsWF(x)
statusWF(x)
targetsWF(x)
outfiles(x)
SE(x)
dependency(x)
projectInfo(x)
runInfo(x)

```

## Methods

```

baseCommand(x, ...)
SampleName(x, ...)
stepName(x)
targetsheader(x, ...)
yamlinput(x, ...)
cmdlist(x, ...)
codeLine(x, ...)
copyEnvir(x, list = character(), new.env = globalenv(), silent = FALSE)
viewEnvir(x, silent = FALSE)

## Subset Methods

subset(x, ...)
getColumn(x, step, position = c("outfiles", "targetsWF"), column = 1,
         names = SampleName(x, step))

## Replacement

dependency(x, step, ...) <- value
appendStep(x, after = length(x), ...) <- value
replaceStep(x, step, step_name = "default") <- value
renameStep(x, step, ...) <- value
yamlinput(x, paramName, ...) <- value
appendCodeLine(x, after = length(x), ...) <- value
replaceCodeLine(x, line, ...) <- value
updateColumn(x, step, position = c("outfiles", "targetsWF")) <- value

```

## Arguments

<code>sysargs</code>	SYSargs2 object.
<code>step_name</code>	character with the step index name.
<code>targets</code>	the path to targets file. The targets file can be either a simple tabular or yaml 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 parameters file.
<code>input_file</code>	name and path to input parameters file.
<code>dir_path</code>	full path to the directory with the CWL parameters and input files.
<code>id</code>	A column from targets file, which will be used as an id for each one of the samples. It is required to be unique.
<code>inputvars</code>	variables list defined in the input file that matches the column names defined in the targets file.
<code>rm_targets_col</code>	targets file columns to be removed.
<code>dir</code>	This option allows creating an exclusive results folder for each step in the workflow. All the outfiles and log files for the particular step will be created in the respective folders. Default is <code>dir=TRUE</code> .

dependency	character. Dependency tree, required when appending this step to the workflow. Character name of a previous step in the workflow. Default is NA.
run_step	character. If the step has "mandatory" or "optional" flag for the execution.
run_session	character. If the step has "management" or "compute" flag for the execution.
run_remote_resources	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 queuing/scheduling system.
silent	If set to TRUE, all messages returned by the function will be suppressed.
projPath	a character vector of a full project path name. Default is the current path.
x	An instance of class SYSargsList.
step	character or numeric. Step name or index.
list	a character vector naming objects to be copied from the environment.
new.env	An environment to copy to. Default is globalenv().
position	character. Options are "outfiles" or "targetsWF" slots.
column	character or numeric. Which column will be replaced from the position argument.
names	character vector. Names of the new column.
after	A subscript, after which the values are to be appended.
paramName	character. Input name from yamlinput(x), which value should be replaced.
line	An instance of class LineWise.
value	object containing the values to be replaced to x. Values may be of the same class as the original values. For updateColumn<-a dataframe that must have the same rows as the modifying dataframe. However, if there is no column in the original dataframe. This new df will replace the empty one. If there is a non-empty dataframe, any existing columns with the same name as this new df will be replaced. Any columns that do not exist in the original column but this new df will be added to the original data.frame.
...	object containing the values to be replaced to x. Values may be of the same class as the original values.

### Objects from the Class

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

### Slots

**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

**targetsWF:** Object of class "list" storing all the targets files for each step in the workflow

**outfiles:** Object of class "list" storing all the output files in the workflow

**SE:** Object of class "list" storing all the SummarizedExperiment objects in the workflow

**dependency:** Object of class "list" storing all the dependency steps in the workflow  
**targets\_connection:** Object of class "list" storing all targets files connection in the workflow  
**projectInfo:** Object of class "list" storing all the projectInfo information of the workflow  
**runInfo:** Object of class "list" storing all the runInfo information of 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  
**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  
**projectInfo** signature(x = "SYSargsList"): extract data from projectInfo slot  
**runInfo** signature(x = "SYSargsList"): extract data from runInfo slot  
**targetsWF** signature(x = "SYSargsList"): extract data from targetsWF slot  
**sysargslist** signature(x = "SYSargsList"): Coerce back to list as(SYSargsList, "list")  
**SE** signature(x = "SYSargsList"): extract data from SE slot  
**cmdlist** signature(x = "SYSargsList", sample=NULL): 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 = "SYSargsList"): replacement method for yamlinput slot for each step  
**dependency<-** signature(x = "SYSargsList"): replacement method for dependency slot for each step  
**updateStatus<-** signature(x = "SYSargsList"): updateStatus method for update a step status

## Author(s)

Daniela Cassol and Thomas Girke

**See Also**

SYSargs2, LineWise, and SPRproject

**Examples**

```
sal <- SPRproject(overwrite=TRUE)
targetspath <- system.file("extdata/cwl/example/targets_example.txt",
                           package="systemPipeR")

## Constructor and `appendStep<-`
appendStep(sal) <- SYSargsList(step_name = "echo",
                              targets=targetspath, dir=TRUE,
                              wf_file="example/workflow_example.cwl",
                              input_file="example/example.yml",
                              dir_path = system.file("extdata/cwl",
                                                      package="systemPipeR"),
                              inputvars = c(Message = "_STRING_",
                                             SampleName = "_SAMPLE_"))

appendStep(sal) <- LineWise(code = {
  hello <- lapply(getColumn(sal, step=1, 'outfiles'), function(x) yaml::read_yaml(x))
},
  step_name = "R_read",
  dependency = "echo")

## Accessors

stepsWF(sal)
statusWF(sal)
targetsWF(sal)
outfiles(sal)
SE(sal)
dependency(sal)
projectInfo(sal)
runInfo(sal)

## Methods

baseCommand(sal, 1) ## SYSargs2 step
SampleName(sal, step="echo")
stepName(sal)
targetsheader(sal, step=1) ## SYSargs2 step
yamlinput(sal, step=1) ## SYSargs2 step
cmdlist(sal, step=1, targets=1:2) ## SYSargs2 step
codeline(sal, step=2) ## SYSargs2 step
copyEnvir(sal, list = character(), new.env = globalenv())
viewEnvir(sal)

## Subset Methods

sal_sub <- subset(sal, subset_steps=1, input_targets=1:2, keep_steps = TRUE)
targetsIn <- getColumn(sal, step=1, position = c("outfiles"))
```

```

## Replacement
renameStep(sal, step=1) <- "new_echo"
dependency(sal, step=2) <- "new_echo"
updateColumn(sal, step=2, position = c("targetsWF")) <- data.frame(targetsIn)
targetsWF(sal)

replaceStep(sal, step=2) <- LineWise(code = {
  hello <- "Printing a new message"
},
  step_name = "R_hello",
  dependency = "new_echo")
#yamlinput(sal, step=1, paramName="results_path") <- list(results_path=list(
#
#               class="Directory", path="./data"))
appendCodeLine(sal, step=2, after = 0) <- "log <- log(10)"
codeLine(sal, 2)
replaceCodeLine(sal, step=2, line=1) <- LineWise(code = {
  log <- log(50)
})

codeLine(sal, 2)

```

---

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.tmpl", Njobs=18, runid="01", r

## 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

*Convert targets to list or data.frame*

---

**Description**

Convert targets files to list or data.frame object.

**Usage**

```
targets.as.df(x)
targets.as.list(x, id="SampleName")
```

**Arguments**

x	An object of the class "list" or "data.frame" that stores data from each target file, as targets(WF).
id	A column from targets file, which will be used as an id for each one of the samples. It is required to be unique.

**Value**

data.frame or list containing all the targets file information.

**Author(s)**

Daniela Cassol

**See Also**

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

**Examples**

```
targetspath <- system.file("extdata", "targets.txt", package="systemPipeR")
targets <- read.delim(targetspath, comment.char = "#")
targetslst <- targets.as.list(x=targets)
targets.as.df(x=targetslst)
```

---

tryCMD

*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**

```
tryCMD(command, silent = FALSE)
```

**Arguments**

command	a character vector containing the command line name to be tested.
silent	If set to TRUE, all messages returned by the function will be suppressed.

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

---

tryPath

*Validation of the files or directories*

---

**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**

```
file <- system.file("extdata/", "targets.txt", package="systemPipeR")
tryPath(path=file)
```

---

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(files, args=files, txdb, fa, organism, out_dir = "results")

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

## Create summary statistics of variants
varSummary(files, args=files)
```

**Arguments**

<code>files</code>	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> .
<code>args</code>	Deprecated. 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> .
<code>txdb</code>	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.
<code>fa</code>	<code>FaFile</code> object pointing to the sequence file of the corresponding reference genome stored in FASTA format or a <code>BSeqGenome</code> instance.
<code>organism</code>	Character vector specifying the organism name of the reference genome.
<code>filtercol</code>	Named character vector containing in the name field the column titles to filter on, and in the data 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 Consequence 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.
out_dir	Character vector of a results directory name. Default is results.

**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:
library(VariantAnnotation)
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

*Plot 2-5 way Venn diagrams***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/VennSymmExa>

**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"))

```

**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_c
```

## 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>files(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\$c1t)</code> .
<code>new_col</code>	A vector of character strings of the new column name to add to target file.

```

new_col_output_index
    A vector of numeric index positions of the file in SYSargs2 class output. It
    requires new_col definition.

remove
    To remove the original columns. Default is FALSE.

...
    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", package="systemPipeR")
WF <- loadWorkflow(targets=targets, wf_file="hisat2/hisat2-mapping-se.cwl",
                  input_file="hisat2/hisat2-mapping-se.yml", dir_path=dir_path)
WF <- renderWF(WF, inputvars=c(FileName="_FASTQ_PATH1_", SampleName="_SampleName_"))
WF
## 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))

```

---

writeTargetsRef	<i>Generate targets file with reference</i>
-----------------	---

---

### 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

infile	Path to input targets file.
outfile	Path to output targets file.
silent	If set to TRUE, all messages returned by the function will be suppressed.
overwrite	If set to TRUE, existing files of same name will be overwritten.
...	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_refsampl.txt", silent=FALSE, overwrite=FALSE)

## End(Not run)
```

---

write\_SYSargsList      *Writeout SYSargsList object*

---

### Description

Function to writeout SYSargsList workflow control environment (S4 object) object.

### Usage

```
write_SYSargsList(sysargs, sys.file=".SPRproject/SYSargsList.yml", silent=TRUE)
```

### Arguments

sysargs	object of class SYSargsList.
sys.file	name and path of the SYSargsList file which will store all the project configuration information. Default is .SPRproject/SYSargsList.yml.
silent	if set to TRUE, all messages returned by the function will be suppressed.

### Value

write\_SYSargsList will return a sys.file path.

### Author(s)

Daniela Cassol

### See Also

See also as SYSargsList-class.

### Examples

```
## Construct SYSargsList object from Rmd file
sal <- SPRproject(overwrite=TRUE)
targetspath <- system.file("extdata/cwl/example/targets_example.txt", package="systemPipeR")

## Constructor and `appendStep<-`
appendStep(sal) <- SYSargsList(step_name = "echo",
                               targets=targetspath, dir=TRUE,
                               wf_file="example/workflow_example.cwl", input_file="example/example.yml",
                               dir_path = system.file("extdata/cwl", package="systemPipeR"),
                               inputvars = c(Message = "_STRING_", SampleName = "_SAMPLE_"))
appendStep(sal) <- LineWise(code = {
  hello <- lapply(getColumn(sal, step=1, 'outfiles'), function(x) yaml::read_yaml(x))
},
  step_name = "R_read",
  dependency = "echo")

sal <- write_SYSargsList(sal)
sal
```

# Index

## \* classes

- catDB-class, 6
- EnvModules-class, 19
- INTERSECTset-class, 36
- LineWise-class, 37
- SYSargs-class, 86
- SYSargs2-class, 88
- SYSargsList, 91
- VENNset-class, 105

## \* methods

- addAssay-methods, 4

## \* package

- systemPipeR-package, 3

## \* utilities

- alignStats, 5
- catmap, 8
- check.output, 9
- clusterRun, 10
- config.param, 12
- configWF, 13
- countRangeset, 14
- createParam, 16
- cwlFilesUpdate, 18
- evalCode, 20
- featureCoverage, 20
- featuretypeCounts, 23
- filterDEGs, 25
- filterVars, 27
- genFeatures, 30
- GOHyperGAll, 31
- importWF, 35
- loadWorkflow, 40
- mergeBamByFactor, 42
- moduleload, 43
- olBarplot, 44
- olRanges, 47
- output\_update, 48
- overLapper, 49
- plotfeatureCoverage, 52

- plotfeaturetypeCounts, 54

- predORF, 59

- preprocessReads, 61

- printParam, 62

- readComp, 64

- renderLogs, 65

- renderReport, 66

- returnRPKM, 67

- run\_DESeq2, 73

- run\_edgeR, 74

- runCommandline, 68

- runDiff, 70

- runWF, 72

- sal2bash, 76

- sal2rmd, 77

- scaleRanges, 78

- seeFastq, 79

- SPRproject, 81

- subsetWF, 83

- symlink2bam, 84

- sysargs, 85

- systemArgs, 96

- targets.as.df, 97

- tryCMD, 98

- tryPath, 99

- variantReport, 100

- vennPlot, 103

- write\_SYSargsList, 110

- writeTargetsout, 107

- writeTargetsRef, 109

- [, EnvModules, ANY, ANY, ANY-method  
(EnvModules-class), 19

- [, LineWise, ANY, ANY, ANY-method  
(LineWise-class), 37

- [, SYSargs, ANY, ANY, ANY-method  
(SYSargs-class), 86

- [, SYSargs2, ANY, ANY, ANY-method  
(SYSargs2-class), 88

- [, SYSargsList, ANY, ANY, ANY-method

- (SYSargsList), 91
- [[, EnvModules, ANY, ANY-method (EnvModules-class), 19
- [[, EnvModules, ANY, missing-method (EnvModules-class), 19
- [[, LineWise, ANY, missing-method (LineWise-class), 37
- [[, SYSargs2, ANY, missing-method (SYSargs2-class), 88
- [[, SYSargsList, ANY, missing-method (SYSargsList), 91
- [[<- , EnvModules, ANY, ANY, ANY-method (EnvModules-class), 19
- [[<- , EnvModules, ANY, ANY-method (EnvModules-class), 19
- [[<- , EnvModules-method (EnvModules-class), 19
- [[<- , LineWise, ANY, ANY, ANY-method (LineWise-class), 37
- [[<- , LineWise, ANY, ANY-method (LineWise-class), 37
- [[<- , SYSargs2, ANY, ANY, ANY-method (SYSargs2-class), 88
- [[<- , SYSargs2, ANY, ANY-method (SYSargs2-class), 88
- [[<- , SYSargsList, ANY, ANY, ANY-method (SYSargsList), 91
- [[<- , SYSargsList, ANY, ANY-method (SYSargsList), 91
- \$, EnvModules-method (EnvModules-class), 19
- \$, LineWise-method (LineWise-class), 37
- \$, SYSargs2-method (SYSargs2-class), 88
- \$, SYSargsList-method (SYSargsList), 91
  
- addAssay (addAssay-methods), 4
- addAssay, SummarizedExperiment-method (addAssay-methods), 4
- addAssay-methods, 4
- addMetadata (addAssay-methods), 4
- addMetadata, SummarizedExperiment-method (addAssay-methods), 4
- addMetadata-methods (addAssay-methods), 4
- addResources (SYSargsList), 91
- addResources, SYSargsList-method (SYSargsList), 91
- alignStats, 5
  
- appendCodeLine, LineWise-method (LineWise-class), 37
- appendCodeLine<- (SYSargsList), 91
- appendCodeLine<- , LineWise-method (LineWise-class), 37
- appendCodeLine<- , SYSargsList-method (SYSargsList), 91
- appendParam (printParam), 62
- appendStep<- (SYSargsList), 91
- appendStep<- , SYSargsList-method (SYSargsList), 91
- as.list, INTERSECTset-method (INTERSECTset-class), 36
- as.list, VENNset-method (VENNset-class), 105
- available\_modules, EnvModules-method (EnvModules-class), 19
  
- baseCommand (SYSargsList), 91
- baseCommand, SYSargs2-method (SYSargs2-class), 88
- baseCommand, SYSargsList-method (SYSargsList), 91
  
- catDB-class, 6
- catlist (catmap), 8
- catlist, catDB-method (catDB-class), 6
- catlist-methods (catmap), 8
- catmap, 8
- catmap, catDB-method (catDB-class), 6
- catmap-methods (catmap), 8
- check.outfiles (check.output), 9
- check.output, 9
- clt (SYSargs2-class), 88
- clt, SYSargs2-method (SYSargs2-class), 88
- clusterRun, 10
- cmdlist (SYSargsList), 91
- cmdlist, SYSargs2-method (SYSargs2-class), 88
- cmdlist, SYSargsList-method (SYSargsList), 91
- cmdToCwl (SYSargs2-class), 88
- cmdToCwl, SYSargs2-method (SYSargs2-class), 88
- cmdToCwl<- (SYSargs2-class), 88
- cmdToCwl<- , SYSargs2-method (SYSargs2-class), 88
- codeChunkStart (LineWise-class), 37



- codeChunkStart, LineWise-method  
(LineWise-class), 37
- codeLine (SYSargsList), 91
- codeLine, LineWise-method  
(LineWise-class), 37
- codeLine, SYSargsList-method  
(SYSargsList), 91
- coerce, EnvModules, list-method  
(EnvModules-class), 19
- coerce, LineWise, list-method  
(LineWise-class), 37
- coerce, list, catDB-method (catDB-class),  
6
- coerce, list, EnvModules-method  
(EnvModules-class), 19
- coerce, list, INTERSECTset-method  
(INTERSECTset-class), 36
- coerce, list, LineWise-method  
(LineWise-class), 37
- coerce, list, SYSargs-method  
(SYSargs-class), 86
- coerce, list, SYSargs2-method  
(SYSargs2-class), 88
- coerce, list, SYSargsList-method  
(SYSargsList), 91
- coerce, list, VENNset-method  
(VENNset-class), 105
- coerce, SYSargs2, list-method  
(SYSargs2-class), 88
- coerce, SYSargsList, list-method  
(SYSargsList), 91
- combineVarReports (variantReport), 100
- complexitylevels (overLapper), 49
- complexitylevels, INTERSECTset-method  
(INTERSECTset-class), 36
- complexitylevels, VENNset-method  
(VENNset-class), 105
- complexitylevels-methods (overLapper),  
49
- config.param, 12
- configWF, 13
- copyEnvir (SYSargsList), 91
- copyEnvir, SYSargsList-method  
(SYSargsList), 91
- cores (sysargs), 85
- cores, SYSargs-method (SYSargs-class), 86
- cores-methods (sysargs), 85
- countRangeset, 14
- createParam, 16
- createParamFiles (createParam), 16
- cwlFilesUpdate, 18
- default\_modules, EnvModules-method  
(EnvModules-class), 19
- dependency (SYSargsList), 91
- dependency, LineWise-method  
(LineWise-class), 37
- dependency, SYSargsList-method  
(SYSargsList), 91
- dependency<- (SYSargsList), 91
- dependency<- , SYSargsList-method  
(SYSargsList), 91
- DESeq2::lfcShrink(), 74
- EnvModules, EnvModules-method  
(EnvModules-class), 19
- EnvModules-class, 19
- evalCode, 20
- featureCoverage, 20
- featuretypeCounts, 23
- files (SYSargs2-class), 88
- files, LineWise-method (LineWise-class),  
37
- files, SYSargs2-method (SYSargs2-class),  
88
- filterDEGs, 25
- filterVars, 27
- genFeatures, 30
- getColumn (SYSargsList), 91
- getColumn, SYSargsList-method  
(SYSargsList), 91
- goBarplot (GOHyperGAll), 31
- GOCluster\_Report (GOHyperGAll), 31
- GOHyperGAll, 31
- GOHyperGAll\_Simplify (GOHyperGAll), 31
- GOHyperGAll\_Subset (GOHyperGAll), 31
- idconv (catmap), 8
- idconv, catDB-method (catDB-class), 6
- idconv-methods (catmap), 8
- importWF, 35, 37, 38
- infile1 (sysargs), 85
- infile1, SYSargs-method (SYSargs-class),  
86
- infile1, SYSargs2-method  
(SYSargs2-class), 88

- infile1-methods (sysargs), 85
- infile2 (sysargs), 85
- infile2, SYSargs-method (SYSargs-class), 86
- infile2, SYSargs2-method (SYSargs2-class), 88
- infile2-methods (sysargs), 85
- input (SYSargs2-class), 88
- input, SYSargs2-method (SYSargs2-class), 88
- inputvars (SYSargs2-class), 88
- inputvars, SYSargs2-method (SYSargs2-class), 88
- internal\_outfiles (SYSargs2-class), 88
- internal\_outfiles, SYSargs2-method (SYSargs2-class), 88
- intersectlist (overLapper), 49
- intersectlist, INTERSECTset-method (INTERSECTset-class), 36
- intersectlist-methods (overLapper), 49
- intersectmatrix (overLapper), 49
- intersectmatrix, INTERSECTset-method (INTERSECTset-class), 36
- intersectmatrix, VENNset-method (VENNset-class), 105
- intersectmatrix-methods (overLapper), 49
- INTERSECTset-class, 36
- length, INTERSECTset-method (INTERSECTset-class), 36
- length, LineWise-method (LineWise-class), 37
- length, SYSargs-method (SYSargs-class), 86
- length, SYSargs2-method (SYSargs2-class), 88
- length, SYSargsList-method (SYSargsList), 91
- length, VENNset-method (VENNset-class), 105
- LineWise (LineWise-class), 37
- linewise (LineWise-class), 37
- linewise, LineWise-method (LineWise-class), 37
- LineWise-class, 37
- loaded\_modules, EnvModules-method (EnvModules-class), 19
- loadWF (loadWorkflow), 40
- loadWorkflow, 40
- makeCATdb (GOHyperGAll), 31
- mergeBamByFactor, 42
- module (moduleload), 43
- moduleAvail (moduleload), 43
- moduleClear (moduleload), 43
- modulecmd, EnvModules-method (EnvModules-class), 19
- moduleInit (moduleload), 43
- modulelist (moduleload), 43
- moduleload, 43
- modules (SYSargs2-class), 88
- modules, SYSargs-method (SYSargs-class), 86
- modules, SYSargs2-method (SYSargs2-class), 88
- modules-methods (sysargs), 85
- moduleUnload (moduleload), 43
- names, catDB-method (catDB-class), 6
- names, EnvModules-method (EnvModules-class), 19
- names, INTERSECTset-method (INTERSECTset-class), 36
- names, LineWise-method (LineWise-class), 37
- names, SYSargs-method (SYSargs-class), 86
- names, SYSargs2-method (SYSargs2-class), 88
- names, SYSargsList-method (SYSargsList), 91
- names, VENNset-method (VENNset-class), 105
- olBarplot, 44
- olRanges, 47
- other (sysargs), 85
- other, SYSargs-method (SYSargs-class), 86
- other-methods (sysargs), 85
- outfile1 (sysargs), 85
- outfile1, SYSargs-method (SYSargs-class), 86
- outfile1-methods (sysargs), 85
- outfiles (SYSargsList), 91
- outfiles, SYSargsList-method (SYSargsList), 91
- outpaths (sysargs), 85
- outpaths, SYSargs-method (SYSargs-class), 86
- outpaths-methods (sysargs), 85

- output (SYSargs2-class), 88
- output, SYSargs2-method
  - (SYSargs2-class), 88
- output\_update, 48
- overLapper, 49
  
- plotfeatureCoverage, 52
- plotfeaturetypeCounts, 54
- plotWF, 56
- plotwfOutput (plotWF), 56
- predORF, 59
- preprocessReads, 61
- printParam, 62
- projectInfo (SYSargsList), 91
- projectInfo, SYSargsList-method
  - (SYSargsList), 91
  
- readComp, 64
- reference (sysargs), 85
- reference, SYSargs-method
  - (SYSargs-class), 86
- reference-methods (sysargs), 85
- renameParam (printParam), 62
- renameStep<- (SYSargsList), 91
- renameStep<-, SYSargsList-method
  - (SYSargsList), 91
- renderLogs, 65
- renderPlotwf (plotWF), 56
- renderReport, 66
- renderWF (loadWorkflow), 40
- replaceCodeLine, LineWise-method
  - (LineWise-class), 37
- replaceCodeLine<- (SYSargsList), 91
- replaceCodeLine<-, LineWise-method
  - (LineWise-class), 37
- replaceCodeLine<-, SYSargsList-method
  - (SYSargsList), 91
- replaceParam (printParam), 62
- replaceStep<- (SYSargsList), 91
- replaceStep<-, SYSargsList-method
  - (SYSargsList), 91
- results (sysargs), 85
- results, SYSargs-method (SYSargs-class), 86
- results-methods (sysargs), 85
- returnRPKM, 67
- rmdPath (LineWise-class), 37
- rmdPath, LineWise-method
  - (LineWise-class), 37
  
- run\_DESeq2, 73
- run\_edgeR, 74
- runCommandline, 68
- runDiff, 70
- runInfo (SYSargsList), 91
- runInfo, LineWise-method
  - (LineWise-class), 37
- runInfo, SYSargsList-method
  - (SYSargsList), 91
- runInfo<- (SYSargsList), 91
- runInfo<-, SYSargsList-method
  - (SYSargsList), 91
- runWF, 72
  
- sal2bash, 76
- sal2rmd, 77
- SampleName (SYSargsList), 91
- SampleName, SYSargs-method
  - (SYSargs-class), 86
- SampleName, SYSargs2-method
  - (SYSargs2-class), 88
- SampleName, SYSargsList-method
  - (SYSargsList), 91
- SampleName-methods (sysargs), 85
- scaleRanges, 78
- SE (SYSargsList), 91
- SE, SYSargsList-method (SYSargsList), 91
- seeFastq, 79
- seeFastqPlot (seeFastq), 79
- setlist (overLapper), 49
- setlist, INTERSECTset-method
  - (INTERSECTset-class), 36
- setlist, VENNset-method (VENNset-class), 105
- setlist-methods (overLapper), 49
- show, catDB-method (catDB-class), 6
- show, EnvModules-method
  - (EnvModules-class), 19
- show, INTERSECTset-method
  - (INTERSECTset-class), 36
- show, LineWise-method (LineWise-class), 37
- show, SYSargs-method (SYSargs-class), 86
- show, SYSargs2-method (SYSargs2-class), 88
- show, SYSargsList-method (SYSargsList), 91
- show, VENNset-method (VENNset-class), 105
- showDF, 80

- software (sysargs), 85
- software, SYSargs-method (SYSargs-class), 86
- software-methods (sysargs), 85
- SPRproject, 81
- status (SYSargs2-class), 88
- status, LineWise-method (LineWise-class), 37
- status, SYSargs2-method (SYSargs2-class), 88
- statusWF (SYSargsList), 91
- statusWF, SYSargsList-method (SYSargsList), 91
- stepName (SYSargsList), 91
- stepName, LineWise-method (LineWise-class), 37
- stepName, SYSargsList-method (SYSargsList), 91
- stepsWF (SYSargsList), 91
- stepsWF, SYSargsList-method (SYSargsList), 91
- subset (SYSargsList), 91
- subset, SYSargsList-method (SYSargsList), 91
- subsetParam (printParam), 62
- subsetWF, 83
- symLink2bam, 84
- sysargs, 85
- sysargs, SYSargs-method (SYSargs-class), 86
- SYSargs-class, 86
- sysargs-methods (sysargs), 85
- sysargs2 (SYSargs2-class), 88
- sysargs2, SYSargs2-method (SYSargs2-class), 88
- SYSargs2-class, 88
- SYSargsList, 35, 36, 39, 91
- sysargslist (SYSargsList), 91
- sysargslist, SYSargsList-method (SYSargsList), 91
- SYSargsList-class (SYSargsList), 91
- systemArgs, 96
- systemPipeR-package, 3
- targets (SYSargs2-class), 88
- targets, SYSargs2-method (SYSargs2-class), 88
- targets.as.df, 97
- targets.as.list (targets.as.df), 97
- targets\_connection (SYSargsList), 91
- targets\_connection, SYSargsList-method (SYSargsList), 91
- targetsheader (SYSargsList), 91
- targetsheader, SYSargs-method (SYSargs-class), 86
- targetsheader, SYSargs2-method (SYSargs2-class), 88
- targetsheader, SYSargsList-method (SYSargsList), 91
- targetsheader-methods (sysargs), 85
- targetsin (sysargs), 85
- targetsin, SYSargs-method (SYSargs-class), 86
- targetsin-methods (sysargs), 85
- targetout (sysargs), 85
- targetout, SYSargs-method (SYSargs-class), 86
- targetout-methods (sysargs), 85
- targetWF (SYSargsList), 91
- targetWF, SYSargsList-method (SYSargsList), 91
- tryCL (tryCMD), 98
- tryCMD, 98
- tryPath, 99
- updateColumn<- (SYSargsList), 91
- updateColumn<-, SYSargsList-method (SYSargsList), 91
- updateStatus<- (SYSargsList), 91
- updateStatus<-, SYSargsList-method (SYSargsList), 91
- updateWF (loadWorkflow), 40
- variantReport, 100
- varSummary (variantReport), 100
- vennlist (overLapper), 49
- vennlist, VENNset-method (VENNset-class), 105
- vennlist-methods (overLapper), 49
- vennPlot, 103
- VENNset-class, 105
- viewEnvir (SYSargsList), 91
- viewEnvir, SYSargsList-method (SYSargsList), 91
- wf (SYSargs2-class), 88
- wf, SYSargs2-method (SYSargs2-class), 88
- write\_SYSargsList, 110

writeParamFiles (createParam), 16  
writeTargetsout, 107  
writeTargetsRef, 109

yamlinput (SYSargsList), 91  
yamlinput, SYSargs2-method  
    (SYSargs2-class), 88  
yamlinput, SYSargsList-method  
    (SYSargsList), 91  
yamlinput<- (SYSargsList), 91  
yamlinput<-, SYSargs2, ANY, ANY-method  
    (SYSargs2-class), 88  
yamlinput<-, SYSargs2-method  
    (SYSargs2-class), 88  
yamlinput<-, SYSargsList-method  
    (SYSargsList), 91