

# systemPipeR: NGS analysis pipeline with automated report generation

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## 1 Introduction

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*systemPipeR* provides utilities for building *end-to-end* analysis pipelines with automated report generation for NGS applications such as RNA-Seq, ChIP-Seq, VAR-Seq and many others. An important feature is support for running command-line software, such as NGS aligners, on both single machines or compute clusters. This includes both interactive job submissions or batch submissions to queuing systems of clusters (tested only with Torque). For instance, *systemPipeR* can be used with most command-line aligners such as TopHat 2 [1] and Bowtie 2 [2]. Efficient handling of complex sample sets and experimental designs is facilitated by a well-defined sample annotation infrastructure which improves reproducibility and user-friendliness of many typical analysis workflows in the NGS area.

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## 2 Getting Started

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### 2.1 Installation

The R software can be downloaded from CRAN (<http://cran.at.r-project.org/>) and the *systemPipeR* package from GitHub (<https://github.com/tgirke/systemPipeR>). The *systemPipeR* package can be installed from R using the `install.packages` command after downloading and uncompressing the package directory.

```
> system("R CMD build systemPipeR") # Builds package
> install.packages("systemPipeR.X.X.X.tar.gz", repos=NULL, type="source") # Installs the package
```

### 2.2 Loading the Package and Documentation

```
> library("systemPipeR") # Loads the package
> library(help="systemPipeR") # Lists all functions and classes
> vignette("systemPipeR") # Opens this PDF manual from R
```

### 2.3 Sample FASTQ Files

Mini sample FASTQ files used by this overview vignette as well as the associated workflow reporting vignettes can be downloaded from [here](#). This data set (SRA: SRP010938) contains 18 paired-end (PE) read sets from *Arabidopsis thaliana* [3]. To minimize processing time during testing, each FASTQ file has been subsetting to 90,000-100,000 random sampled PE reads that map to the first 100,000 nucleotides of each chromosome of the *A. thaliana* genome. The corresponding reference genome sequence (FASTA) and its GFF annotation files (provided in the same download) have been truncated accordingly. This way the entire test sample data set is less than 200MB in storage space. A PE read set has been chosen for this test data set for flexibility, because it can be used for testing both types of analysis routines requiring either SE (single end) reads or PE reads.

## 3 Structure of targets file

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The targets file defines all FASTQ files and sample comparisons of an analysis workflow. The following shows the format of a sample targets file provided by this package.

```
> library(systemPipeR)
> targetspath <- paste0(system.file("extdata", package="systemPipeR"), "/targets.txt")
> read.delim(targetspath, comment.char = "#")
```

	FileName	SampleName	Factor	SampleLong	Experiment	Date
1	./data/SRR446027_1.fastq	M1A	M1	Mock.1h.A	1	23-Mar-2012
2	./data/SRR446028_1.fastq	M1B	M1	Mock.1h.B	1	23-Mar-2012
3	./data/SRR446029_1.fastq	A1A	A1	Avr.1h.A	1	23-Mar-2012
4	./data/SRR446030_1.fastq	A1B	A1	Avr.1h.B	1	23-Mar-2012
5	./data/SRR446031_1.fastq	V1A	V1	Vir.1h.A	1	23-Mar-2012
6	./data/SRR446032_1.fastq	V1B	V1	Vir.1h.B	1	23-Mar-2012
7	./data/SRR446033_1.fastq	M6A	M6	Mock.6h.A	1	23-Mar-2012
8	./data/SRR446034_1.fastq	M6B	M6	Mock.6h.B	1	23-Mar-2012
9	./data/SRR446035_1.fastq	A6A	A6	Avr.6h.A	1	23-Mar-2012
10	./data/SRR446036_1.fastq	A6B	A6	Avr.6h.B	1	23-Mar-2012
11	./data/SRR446037_1.fastq	V6A	V6	Vir.6h.A	1	23-Mar-2012
12	./data/SRR446038_1.fastq	V6B	V6	Vir.6h.B	1	23-Mar-2012
13	./data/SRR446039_1.fastq	M12A	M12	Mock.12h.A	1	23-Mar-2012

```

14 ./data/SRR446040_1.fastq      M12B    M12 Mock.12h.B      1 23-Mar-2012
15 ./data/SRR446041_1.fastq      A12A    A12  Avr.12h.A      1 23-Mar-2012
16 ./data/SRR446042_1.fastq      A12B    A12  Avr.12h.B      1 23-Mar-2012
17 ./data/SRR446043_1.fastq      V12A    V12  Vir.12h.A      1 23-Mar-2012
18 ./data/SRR446044_1.fastq      V12B    V12  Vir.12h.B      1 23-Mar-2012

```

Structure of targets file for paired end (PE) samples.

```

> library(systemPipeR)
> targetspath <- paste0(system.file("extdata", package="systemPipeR"), "/targetsPE.txt")
> read.delim(targetspath, comment.char = "#")[1:2,1:6]

```

	FileName1	FileName2	SampleName	Factor	SampleLong	Experiment
1	./data/SRR446027_1.fastq	./data/SRR446027_2.fastq	M1A	M1	Mock.1h.A	1
2	./data/SRR446028_1.fastq	./data/SRR446028_2.fastq	M1B	M1	Mock.1h.B	1

Comparisons are defined in the header lines of the targets starting with '# <CMP>'. The function readComp imports the comparison and stores them in a list.

```

> readComp(file=targetspath, format="vector", delim="-")
$CMPset1
[1] "M1-A1" "M1-V1" "A1-V1" "M6-A6" "M6-V6" "A6-V6" "M12-A12" "M12-V12" "A12-V12"

$CMPset2
[1] "M1-A1" "M1-V1" "M1-M6" "M1-A6" "M1-V6" "M1-M12" "M1-A12" "M1-V12" "A1-V1"
[10] "A1-M6" "A1-A6" "A1-V6" "A1-M12" "A1-A12" "A1-V12" "V1-M6" "V1-A6" "V1-V6"
[19] "V1-M12" "V1-A12" "V1-V12" "M6-A6" "M6-V6" "M6-M12" "M6-A12" "M6-V12" "A6-V6"
[28] "A6-M12" "A6-A12" "A6-V12" "V6-M12" "V6-A12" "V6-V12" "M12-A12" "M12-V12" "A12-V12"

```

## 4 Structure of param file and SYSargs container

The param file defines the parameters of the command-line software. The following shows the format of a sample param file provided by this package.

```

> parampath <- paste0(system.file("extdata", package="systemPipeR"), "/tophat.param")
> read.delim(parampath, comment.char = "#")

```

	PairSet	Name	Value
1	modules	<NA>	bowtie2/2.1.0
2	modules	<NA>	tophat/2.0.8b
3	software	<NA>	tophat
4	cores	-p	4
5	other	<NA>	-g 1 --segment-length 25 -i 30 -I 3000
6	outfile1	-o	<FileName1>
7	outfile1	path	./results/
8	outfile1	remove	<NA>
9	outfile1	append	.tophat
10	outfile1	outextension	.tophat/accepted_hits.bam
11	reference	<NA>	./data/tair10.fasta
12	infile1	<NA>	<FileName1>
13	infile1	path	<NA>
14	infile2	<NA>	<FileName2>
15	infile2	path	<NA>

The systemArgs function imports the definitions of both the param file and the targets file, and stores all relevant information as SYSargs object.

```
> args <- systemArgs(sysma=parampath, mytargets=targetspath)
> args
```

An instance of 'SYSargs' for running 'tophat' on 18 samples

Several accessor functions are available that are named after the slot names of the SYSargs object class.

```
> names(args)
[1] "modules"    "software"   "cores"      "other"      "reference"  "results"    "infile1"
[8] "infile2"    "outfile1"   "sysargs"    "outpaths"
```

```
> modules(args)
```

```
[1] "bowtie2/2.1.0" "tophat/2.0.8b"
```

```
> cores(args)
```

```
[1] 4
```

```
> outpaths(args)[1]
```

```
"/rhome/tgirke/Projects/github/systemPipeR/vignettes/results/SRR446027_1.fastq.tophat/accepted_hits.bam"
M1A
```

```
> sysargs(args)[1]
```

```
"tophat -p 4 -g 1 --segment-length 25 -i 30 -I 3000 -o /rhome/tgirke/Projects/github/systemPipeR/vignettes/
```

The content of the param file can be returned as JSON object as follows (requires *rjson* package).

```
> systemArgs(sysma=parampath, mytargets=targetspath, type="json")
```

```
[1] "{\"modules\":{\"n1\":\"\", \"v2\":\"bowtie2/2.1.0\", \"n1\":\"\", \"v2\":\"tophat/2.0.8b\"}, \"software\"
```

## 5 Workflow

---

### 5.1 Define environment settings and samples

Load package:

```
> library(systemPipeR)
```

Construct SYSargs object from param and targets files.

```
> args <- systemArgs(sysma="tophat.param", mytargets="targetsPE.txt")
```

### 5.2 FASTQ quality report

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.

```
> fqlist <- seeFastq(fastq=infile1(args), batchsize=10000, klength=8)
> pdf("./results/fastqReport.pdf", height=18, width=4*length(fqlist))
> seeFastqPlot(fqlist)
> dev.off()
```



Figure 1: QC report for 18 FASTQ files.

### 5.3 Alignment with Tophat 2

Build Bowtie 2 index.

```
> moduleload(modules(args)) # Skip if module system is not available
> system("bowtie2-build ./data/tair10.fasta ./data/tair10.fasta")
```

Execute SYSargs on a single machine without submitting to a queuing system of a compute cluster.

```
> bampaths <- runCommandline(args=args)
```

Submit to compute nodes.

```
> qsubargs <- getQsubargs(queue="batch", cores=cores(args), memory="mem=10gb", time="walltime=20:00:00")
> (joblist <- qsubRun(args=args, qsubargs=qsubargs, Nqsubs=18, package="systemPipeR"))
```

Alignment Stats

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

### 5.4 Create symbolic links for viewing BAM files in IGV

```
> symLink2bam(sysargs=args, htmldir=c("~/html/", "somedir/"),
+             urlbase="http://myserver.edu/~username/",
+             urlfile="IGVurl.txt")
```

### 5.5 Alignment with Bowtie 2 (here for miRNA profiling experiment)

Run as single process without submitting to cluster, e.g. via qsub -l.

```
> args <- systemArgs(sysma="bowtieSE.param", mytargets="targets.txt")
> bampaths <- runCommandline(args=args)
```

Submit to compute nodes

```
> qsubargs <- getQsubargs(queue="batch", cores=cores(args), memory="mem=10gb", time="walltime=20:00:00")
> (joblist <- qsubRun(args=args, qsubargs=qsubargs, Nqsubs=18, package="systemPipeR"))
```

### 5.6 Read counting for mRNA profiling experiments

Create txdb (do only once)

```
> library(GenomicFeatures)
> txdb <- makeTranscriptDbFromGFF(file="data/tair10.gff", format="gff", dataSource="TAIR", species="A. tha
> saveDb(txdb, file="./data/tair10.sqlite")
```

Read counting with summarizeOverlaps in parallel mode with multiple cores

```
> library(BiocParallel)
> txdb <- loadDb("./data/tair10.sqlite")
> eByg <- exonsBy(txdb, by="gene")
> bfl <- BamFileList(outpaths(args), yieldSize=50000, index=character())
> multicoreParam <- MulticoreParam(workers=4); register(multicoreParam); registered()
> counteByg <- bplapply(bfl, function(x) summarizeOverlaps(eByg, x, mode="Union", ignore.strand=TRUE, inter
> countDFeByg <- sapply(seq(along=counteByg), function(x) assays(counteByg[[x]])$counts)
> rownames(countDFeByg) <- names(rowData(counteByg[[1]])); colnames(countDFeByg) <- names(bfl)
> rpkmDFeByg <- apply(countDFeByg, 2, function(x) returnRPKM(counts=x, ranges=eByg))
> write.table(countDFeByg, "results/countDFeByg.xls", col.names=NA, quote=FALSE, sep="\t")
> write.table(rpkmDFeByg, "results/rpkmDFeByg.xls", col.names=NA, quote=FALSE, sep="\t")
```

## 5.7 Read counting for miRNA profiling experiments

Download miRNA genes from miRBase

```
> system("wget ftp://mirbase.org/pub/mirbase/19/genomes/My_species.gff3 -P ./data/")
> gff <- import.gff("./data/My_species.gff3", asRangedData=FALSE)
> gff <- split(gff, elementMetadata(gff)$ID)
> bams <- names(bampaths); names(bams) <- targets$SampleName
> bfl <- BamFileList(bams, yieldSize=50000, index=character())
> countDFmiR <- summarizeOverlaps(gff, bfl, mode="Union", ignore.strand=FALSE, inter.feature=FALSE) # Note
> rpkmDFmiR <- apply(countDFmiR, 2, function(x) returnRPKM(counts=x, gffsub=gff))
> write.table(assays(countDFmiR)$counts, "results/countDFmiR.xls", col.names=NA, quote=FALSE, sep="\t")
> write.table(rpkmDFmiR, "results/rpkmDFmiR.xls", col.names=NA, quote=FALSE, sep="\t")
```

## 5.8 Correlation analysis of samples

```
> library(ape)
> rpkmDFeByg <- read.table("./results/rpkmDFeByg.xls", check.names=FALSE)
> rpkmDFeByg <- rpkmDFeByg[rowMeans(rpkmDFeByg) > 50,]
> d <- cor(rpkmDFeByg, method="spearman")
> hc <- hclust(as.dist(1-d))
> plot.phylo(as.phylo(hc), type="p", edge.col="blue", edge.width=2, show.node.label=TRUE, no.margin=TRUE)
```

## 5.9 DEG analysis with edgeR

```
> library(edgeR)
> targets <- read.delim(targetspath, comment="#")
> cmp <- readComp(file=targetspath, format="matrix", delim="-")
> cmp[[1]]
```

```
      [,1] [,2]
[1,] "M1"  "A1"
[2,] "M1"  "V1"
[3,] "A1"  "V1"
[4,] "M6"  "A6"
```

```
[5,] "M6" "V6"
[6,] "A6" "V6"
[7,] "M12" "A12"
[8,] "M12" "V12"
[9,] "A12" "V12"
```

```
> edgeDF <- run_edgeR(countDF=countDFeByg, targets=targets, cmp=cmp[[1]], independent=TRUE, mdsplot="")
```

## 6 Version Information

---

```
> toLatex(sessionInfo())
```

- R version 3.1.0 (2014-04-10), x86\_64-unknown-linux-gnu
- Locale: C
- Base packages: base, datasets, grDevices, graphics, grid, methods, parallel, stats, utils
- Other packages: BSgenome 1.32.0, BiocGenerics 0.10.0, BiocParallel 0.6.1, Biostings 2.32.0, GenomInfoDb 1.0.2, GenomicAlignments 1.0.1, GenomicRanges 1.16.3, IRanges 1.22.8, Rsamtools 1.16.0, ShortRead 1.22.0, XVector 0.4.0, edgeR 3.6.2, ggplot2 1.0.0, limma 3.20.4, rjson 0.2.13, systemPipeR 1.0.9
- Loaded via a namespace (and not attached): BBmisc 1.6, BatchJobs 1.2, Biobase 2.24.0, BiocStyle 1.2.0, DBI 0.2-7, MASS 7.3-33, RColorBrewer 1.0-5, RSQLite 0.11.4, Rcpp 0.11.1, bitops 1.0-6, brew 1.0-6, codetools 0.2-8, colorspace 1.2-4, digest 0.6.4, fail 1.2, foreach 1.4.2, gtable 0.1.2, hwriter 1.3, iterators 1.0.7, lattice 0.20-29, latticeExtra 0.6-26, munsell 0.4.2, plyr 1.8.1, proto 0.3-10, reshape2 1.4, scales 0.2.4, sendmailR 1.1-2, stats4 3.1.0, stringr 0.6.2, tools 3.1.0, zlibbioc 1.10.0

## 7 Funding

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## References

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- [1] Daehwan Kim, Geo Pertea, Cole Trapnell, Harold Pimentel, Ryan Kelley, and Steven L Salzberg. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol.*, 14(4):R36, 25 April 2013. URL: <http://dx.doi.org/10.1186/gb-2013-14-4-r36>, doi:10.1186/gb-2013-14-4-r36.
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- [3] Brian E Howard, Qiwen Hu, Ahmet Can Babaoglu, Manan Chandra, Monica Borghi, Xiaoping Tan, Luyan He, Heike Winter-Sederoff, Walter Gassmann, Paola Veronese, and Steffen Heber. High-throughput RNA sequencing of pseudomonas-infected arabidopsis reveals hidden transcriptome complexity and novel splice variants. *PLoS One*, 8(10):e74183, 1 October 2013. URL: <http://dx.doi.org/10.1371/journal.pone.0074183>, doi:10.1371/journal.pone.0074183.