systemPipeR: NGS workflow and report generation environment

Thomas Girke
Email contact: thomas.girke@ucr.edu

January 19, 2015

1 Introduction

systemPipeR provides utilities for building *end-to-end* analysis workflows with automated report generation for next generation sequence (NGS) applications such as RNA-Seq, ChIP-Seq, VAR-Seq and many others (Girke, 2014). 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. For instance, *systemPipeR* can be used with most command-line aligners such as `BWA` (Li, 2013; Li and Durbin, 2009), `TopHat 2` (Kim et al., 2013) and `Bowtie 2` (Langmead and Salzberg, 2012), as well as the R-based NGS aligner *Rsubread* (Liao et al., 2013). 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 (Lawrence et al., 2013).

Templates for setting up custom project reports are provided as `*.Rnw` files in the `vignettes` subdirectory of this package. The corresponding PDFs of these report templates are linked here: systemPipeRNAseq, systemPipeChIPseq and systemPipeVARseq.

Contents

2 Getting Started

2.1 Installation

The R software for running *systemPipeR* can be downloaded from CRAN (http://cran.at.r-project.org/). The *systemPipeR* package can be installed from R using the `biocLite` install command.

```
> source("http://bioconductor.org/biocLite.R") # Sources the biocLite.R installation script
> biocLite("systemPipeR") # 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

The mini sample FASTQ files used by this overview vignette as well as the associated workflow reporting vignettes can be downloaded from here. The chosen data set SRP010938 contains 18 paired-end (PE) read sets from *Arabidposis thaliana* (Howard et al., 2013). To minimize processing time during testing, each FASTQ file has been subsetted to 90,000-100,000 randomly sampled PE reads that map to the first 100,000 nucleotides of each chromosome of the *A. thalina* genome. The corresponding reference genome sequence (FASTA) and its GFF annotion 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

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. In target files for single end (SE) data sets, the first three columns are mandatory including their column names, while it is four mandatory columns for PE data. All subsequent columns are optional and any number of additional columns can be added as needed.

```
> library(systemPipeR)
> targetspath <- system.file("extdata", "targets.txt", package="systemPipeR")
> 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.

```
> targetspath <- system.file("extdata", "targetsPE.txt", package="systemPipeR")
> 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

Sample comparisons are defined in the header lines of the targets file starting with '# <CMP>'. The function readComp imports the comparison and stores them in a list. These lines are also optional. They are mainly useful for controlling comparative analysis according to certain biological expectations, such as simple pairwise comparisons in RNA-Seq experiments.

```
> 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 <- system.file("extdata", "tophat.param", package="systemPipeR")
> 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]
```

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

```
> 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()
```

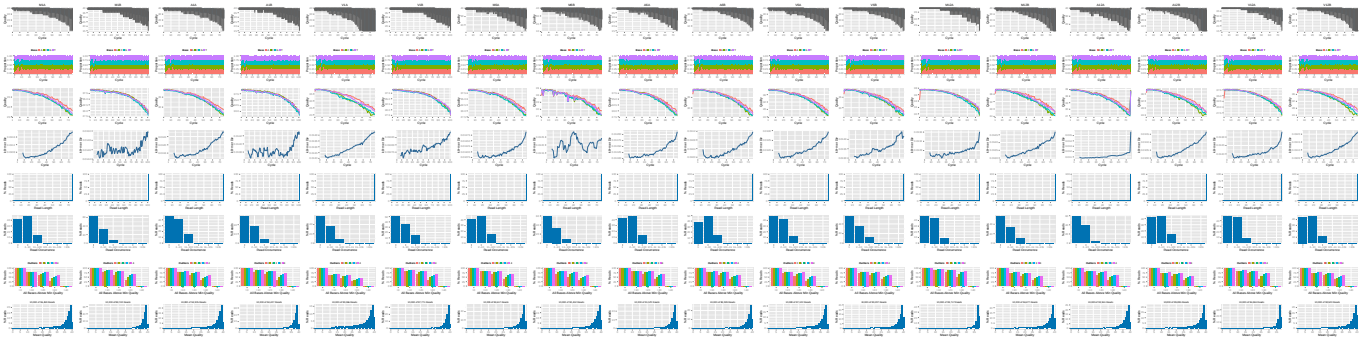

Figure 1: QC report for 18 FASTQ files.

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. This way the input FASTQ files will be processed sequentially. If available, multiple CPU cores can be used for processing each file. The number of CPU cores (here 4) to use for each process is defined in the *.param file. With cores(args) one can return this value from the SYSargs object. Note, if a module system is not installed or used, then the corresponding *.param file needs to be edited accordingly by either providing an empty field in the line(s) starting with module or by deleting these lines.

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

Alternatively, the computation can be greatly accelerated by processing many files in parallel using several compute nodes of a cluster, where a scheduling/queuing system is used for load balancing. To avoid over-subscription of CPU cores on the compute nodes, the value from cores(args) is passed on to the submission command, here nodes in the resources list object. The number of independent parallel cluster processes is defined under the Njobs argument. The following example will run 18 processes in parallel using for each 4 CPU cores. If the resources available on a cluster allow to run all 18 processes at the same time then the shown sample submission will utilize in total 72 CPU cores. Note, runCluster can be used with most queueing systems as it is based on utilities from the BatchJobs package which supports the use of template files (*.tmpl) for defining the run parameters of different schedulers. To run the following code, one needs

to have both a conf file (see .BatchJob samples here) and a template file (see *.tmpl samples here) for the queueing available on a system. The following example uses the sample conf and template files for the Torque scheduler provided by this package.

```
> file.copy(system.file("extdata", ".BatchJobs.R", package="systemPipeR"), ".")
> file.copy(system.file("extdata", "torque.tmpl", package="systemPipeR"), ".")
> 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)
```

Useful commands for monitoring progress of submitted jobs

```
> showStatus(reg)
> file.exists(outpaths(args))
> sapply(1:length(args), function(x) loadResult(reg, x)) # Works after job completion
```

5.4 Read and alignment count stats

Generate table of read and alignment counts for all samples.

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

The following shows the first four lines of the sample alignment stats file provided by the *systemPipeR* package. For simplicity the number of PE reads is multiplied here by 2 to approximate proper alignment frequencies where each read in a pair is counted.

```
> read.table(system.file("extdata", "alignStats.xls", package="systemPipeR"), header=TRUE)[1:4,]
```

	FileName	Nreads2x	Nalign	Perc_Aligned	Nalign_Primary	Perc_Aligned_Primary
1	M1A	192918	177961	92.24697	177961	92.24697
2	M1B	197484	159378	80.70426	159378	80.70426
3	A1A	189870	176055	92.72397	176055	92.72397
4	A1B	188854	147768	78.24457	147768	78.24457

5.5 Create symbolic links for viewing BAM files in IGV

The genome browser IGV supports reading of indexed/sorted BAM files via web URLs. This way it can be avoided to create unnecessary copies of these large files. To enable this approach, an HTML directory with http access needs to be available in the user account (*e.g.* ~/public_html) of a system. If this is not the case then the BAM files need to be moved or copied to the system where IGV runs. In the following, htmldir defines the path to the HTML directory with http access where the symbolic links to the BAM files will be stored. The corresponding URLs will be written to a text file specified under the urlfile argument.

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

5.6 Alternative NGS Aligners

5.6.1 Alignment with Bowtie 2 (e.g. for miRNA profiling)

The following example runs Bowtie 2 as a single process without submitting it to a cluster.

```
> args <- systemArgs(sysma="bowtieSE.param", mytargets="targets.txt")
> moduleload(modules(args)) # Skip if module system is not available
> bampaths <- runCommandline(args=args)
```

Alternatively, submit the job 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"))
```

5.6.2 Alignment with BWA-MEM (e.g. for VAR-Seq)

The following example runs BWA-MEM as a single process without submitting it to a cluster.

```
> args <- systemArgs(sysma="bwa.param", mytargets="targets.txt")
> moduleload(modules(args)) # Skip if module system is not available
> system("bwa index -a bwtsw ./data/tair10.fasta") # Indexes reference genome
> bampaths <- runCommandline(args=args)
```

5.6.3 Alignment with Rsubread (e.g. for RNA-Seq)

The following example shows how one can use within the *systemPipeR* environment the R-based aligner *Rsubread* or other R-based functions that read from input files and write to output files.

```
> library(Rsubread)
> args <- systemArgs(sysma="rsubread.param", mytargets="targets.txt")
> buildindex(basename=reference(args), reference=reference(args)) # Build indexed reference genome
> align(index=reference(args), readfile1=infile1(args), input_format="FASTQ",
+       output_file=outfile1(args), output_format="SAM", nthreads=8, indels=1, TH1=2)
> for(i in seq(along=outfile1(args))) asBam(file=outfile1(args)[i], destination=gsub(".sam", "", outfile1(a
```

5.7 Read counting for mRNA profiling experiments

Create `txdb` (needs to be done only once)

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

5.8 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")
```

5.9 Correlation analysis of samples

The following computes the sample-wise Spearman correlation coefficients from the RPKM normalized expression values. After transformation to a distance matrix, hierarchical clustering is performed with the `hclust` function and the result is plotted as a dendrogram (sample_tree.pdf).

```
> library(ape)
> rpkmDFeBygpath <- system.file("extdata", "rpkmDFeByg.xls", package="systemPipeR")
> rpkmDFeByg <- read.table(rpkmDFeBygpath, 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)
```

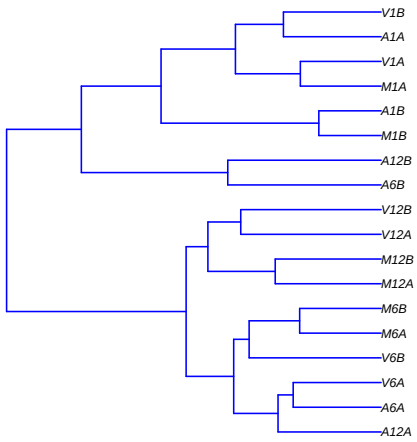

Figure 2: Correlation dendrogram of samples.

5.10 DEG analysis with 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"
```

Run *edgeR*

```
> countDFeBygpath <- system.file("extdata", "countDFeByg.xls", package="systemPipeR")
> countDFeByg <- read.delim(countDFeBygpath, row.names=1)
> edgeDF <- run_edgeR(countDF=countDFeByg, targets=targets, cmp=cmp[[1]], independent=FALSE, mdsplot="")
```

```
Disp = 0.20653 , BCV = 0.4545
```

Filter and plot DEG results for up and down regulated genes. Because of the small size of the toy data set used by this vignette, the FDR value has been set to a relatively high threshold (here 10%). More commonly used FDR cutoffs are 1% or 5%.

```
> DEG_list <- filterDEGs(degDF=edgeDF, filter=c(Fold=2, FDR=10))
```

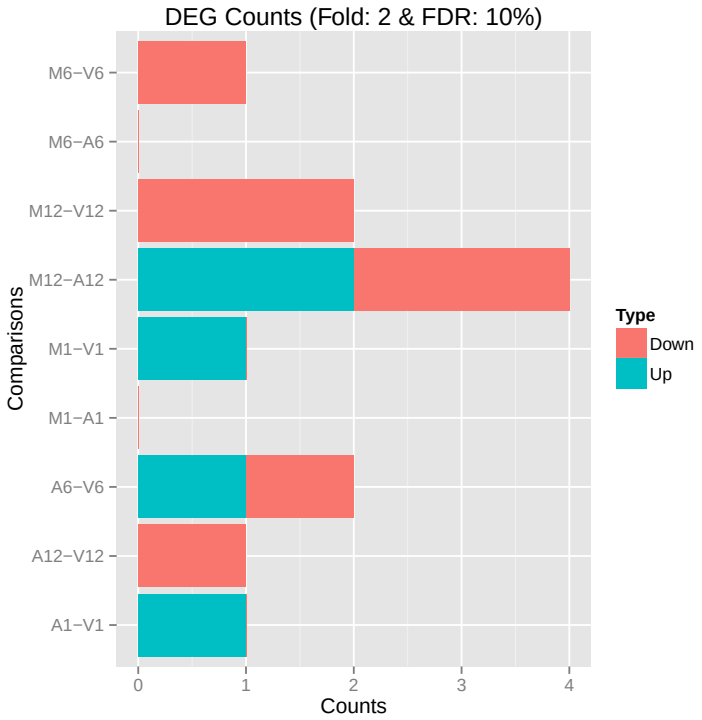

Figure 3: Up and down regulated DEGs.

```
> names(DEG_list)
```

```
[1] "UporDown" "Up"       "Down"     "Summary"
```

```
> DEG_list$Summary[1:4,]
```

```
      Comparisons Counts_Up_or_Down Counts_Up Counts_Down
M1-A1       M1-A1                 0         0           0
```

```
M1-V1       M1-V1              1         1         0
A1-V1       A1-V1              1         1         0
M6-A6       M6-A6              0         0         0
```

5.11 Venn Diagrams

The function `overLapper` can compute Venn intersects for large numbers of sample sets (up to 20 or more) and `vennPlot` can plot 2-5 way Venn diagrams. A useful feature is the possiblity to combine the counts from several Venn comparisons with the same number of sample sets in a single Venn diagram (here for 4 up and down DEG sets).

```
> vennsetup <- overLapper(DEG_list$Up[6:9], type="vennsets")
> vennsetdown <- overLapper(DEG_list$Down[6:9], type="vennsets")
> vennPlot(list(vennsetup, vennsetdown), mymain="", mysub="", colmode=2, ccol=c("blue", "red"))
```

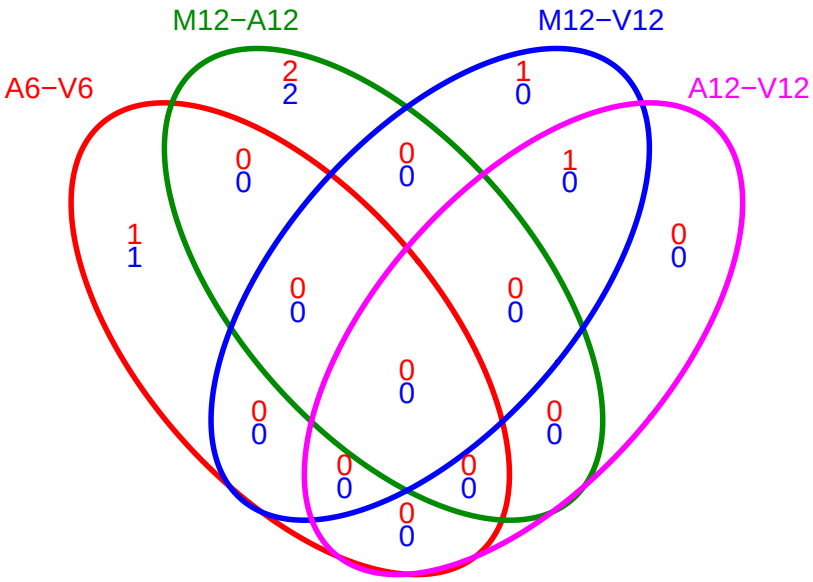

Figure 4: Venn Diagram for 4 Up and Down DEG Sets.

5.12 GO term enrichment analysis of DEGs

5.12.1 Obtain gene-to-GO mappings

The following shows how to obtain gene-to-GO mappings from *biomaRt* (here for *A. thaliana*) and how to organize them for the downstream GO term enrichment analysis. Alternatively, the gene-to-GO mappings can be obtained for many organisms from Bioconductor's `*.db` genome annotation packages or GO annotation files provided by various genome databases. For each annotation this relatively slow preprocessing step needs to be performed only once. Subsequently, the preprocessed data can be loaded with the `load` function as shown in the next subsection.

```
> library("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])
> dir.create("./data/GO")
> write.table(go, "data/GO/GOannotationsBiomart_mod.txt", quote=FALSE, row.names=FALSE, col.names=FALSE, se
> catdb <- makeCATdb(myfile="data/GO/GOannotationsBiomart_mod.txt", lib=NULL, org="", colno=c(1,2,3), idcol
> save(catdb, file="data/GO/catdb.RData")
```

5.12.2 Batch GO term enrichment analysis

Apply the enrichment analysis to the DEG sets obtained in the above differential expression analysis. Note, in the following example the `FDR` filter is set here to an unreasonably high value, simply because of the small size of the toy data set used in this vignette. Batch enrichment analysis of many gene sets is performed with the `GOCluster_Report` function. When `method="all"`, it returns all GO terms passing the p-value cutoff specified under the `cutoff` arguments. When `method="slim"`, it returns only the GO terms specified under the `myslimv` argument. The given example shows how one can obtain such a GO slim vector from BioMart for a specific organism.

```
> load("data/GO/catdb.RData")
> DEG_list <- filterDEGs(degDF=edgeDF, filter=c(Fold=2, FDR=50), plot=FALSE)
> up_down <- DEG_list$UporDown; names(up_down) <- paste(names(up_down), "_up_down", sep="")
> up <- DEG_list$Up; names(up) <- paste(names(up), "_up", sep="")
> down <- DEG_list$Down; names(down) <- paste(names(down), "_down", sep="")
> DEGlist <- c(up_down, up, down)
> DEGlist <- DEGlist[sapply(DEGlist, length) > 0]
> BatchResult <- GOCluster_Report(catdb=catdb, setlist=DEGlist, method="all", id_type="gene", CLSZ=2, cuto
> library("biomaRt"); m <- useMart("ENSEMBL_MART_PLANT", dataset="athaliana_eg_gene")
> goslimvec <- as.character(getBM(attributes=c("goslim_goa_accession"), mart=m)[,1])
> BatchResultslim <- GOCluster_Report(catdb=catdb, setlist=DEGlist, method="slim", id_type="gene", myslimv=
```

5.12.3 Plot batch GO term results

The `data.frame` generated by `GOCluster_Report` can be plotted with the `goBarplot` function. Because of the variable size of the sample sets, it may not always be desirable to show the results from different DEG sets in the same bar plot. Plotting single sample sets is achieved by subsetting the input data frame as shown in the first line of the following example.

```
> gos <- BatchResultslim[grep("M6-V6_up_down", BatchResultslim$CLID), ]
> gos <- BatchResultslim
> pdf("GOslimbarplotMF.pdf", height=8, width=10); goBarplot(gos, gocat="MF"); dev.off()
> goBarplot(gos, gocat="BP")
> goBarplot(gos, gocat="CC")
```

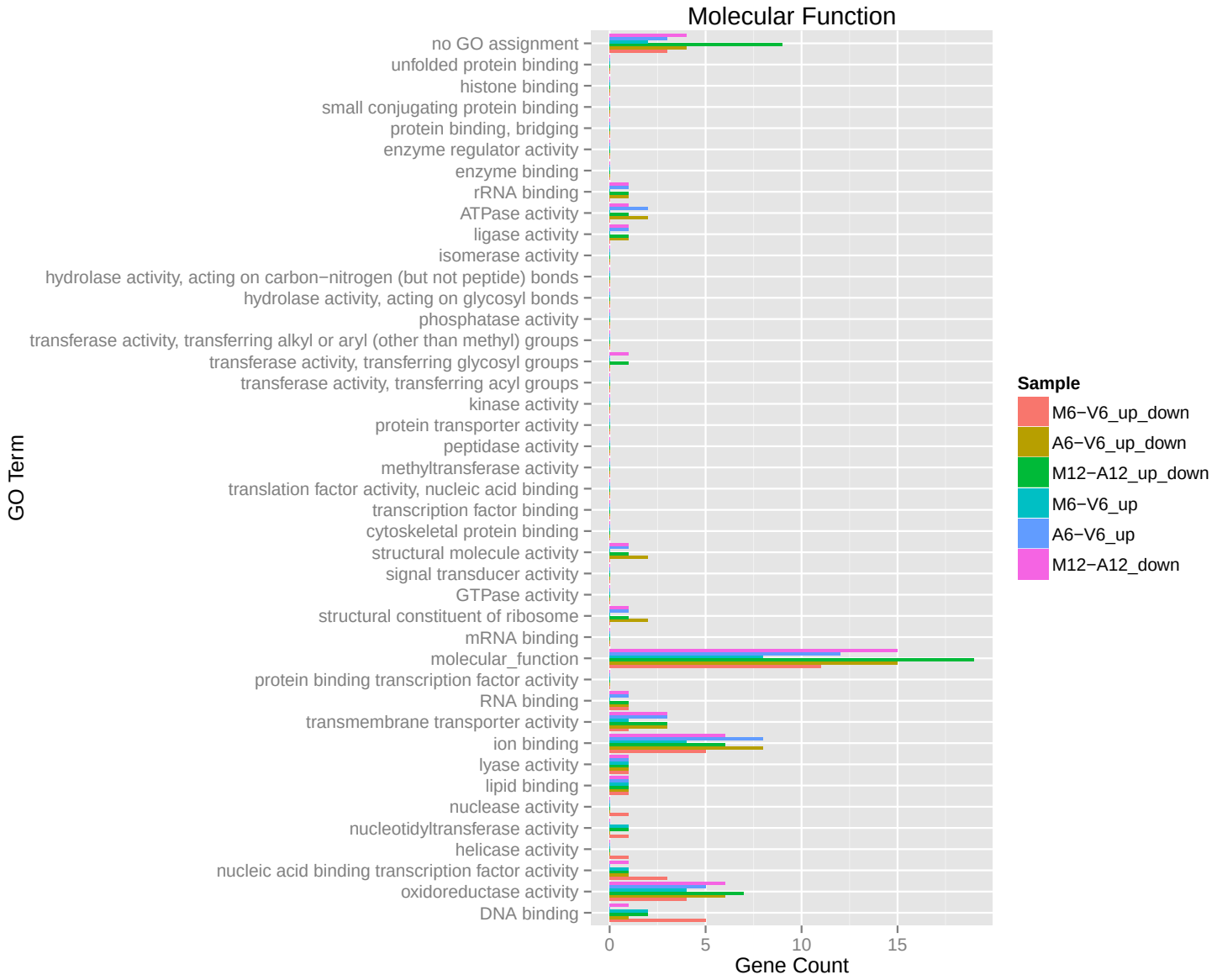

Figure 5: GO Slim Barplot for MF Ontology.

5.13 Clustering and heat maps

The following example performs hierarchical clustering on the RPKM normalized expression matrix subsetted by the DEGs identified in the above differential expression analysis. It uses a Pearson correlation-based distance measure and complete linkage for cluster joining.

```
> library(pheatmap)
> geneids <- unique(as.character(unlist(DEG_list[[1]])))
> y <- rpkmDFeByg[geneids, ]
> pdf("heatmap1.pdf")
> pheatmap(y, scale="row", clustering_distance_rows="correlation", clustering_distance_cols="correlation")
> dev.off()
```

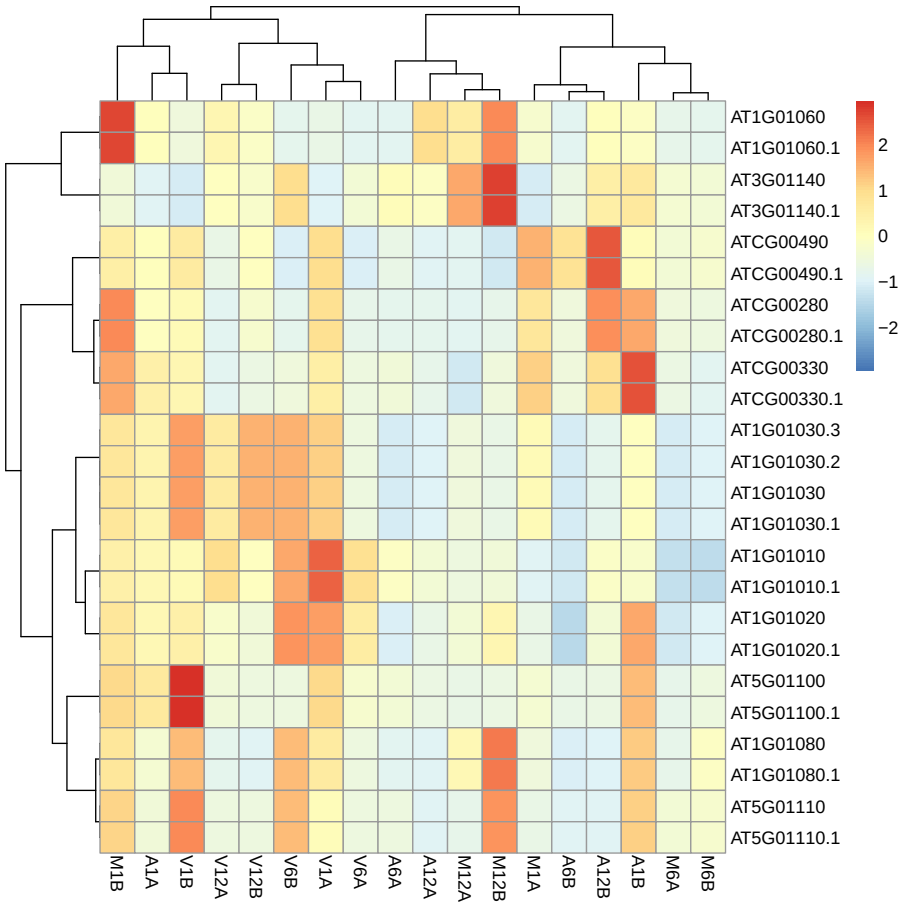

Figure 6: Heat map with hierarchical clustering dendrograms of DEGs.

6 Version Information

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

- R Under development (unstable) (2014-11-15 r66987), `x86_64-unknown-linux-gnu`
- Locale: `C`
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, stats4, utils
- Other packages: AnnotationDbi 1.29.1, Biobase 2.27.0, BiocGenerics 0.13.1, BiocParallel 1.1.5, Biostrings 2.35.4, DBI 0.3.1, GenomeInfoDb 1.3.7, GenomicAlignments 1.3.8, GenomicRanges 1.19.10, IRanges 2.1.13, RSQLite 1.0.0, Rsamtools 1.19.9, S4Vectors 0.5.6, ShortRead 1.25.5, XVector 0.7.2, ape 3.1-4, systemPipeR 1.1.6
- Loaded via a namespace (and not attached): AnnotationForge 1.9.2, BBmisc 1.8, BatchJobs 1.5, BiocStyle 1.5.3, Category 2.33.0, GO.db 3.0.0, GOstats 2.33.0, GSEABase 1.29.0, MASS 7.3-35, Matrix 1.1-4, RBGL 1.43.0, RColorBrewer 1.0-5, Rcpp 0.11.3, XML 3.98-1.1, annotate 1.45.0, base64enc 0.1-2, bitops 1.0-6, brew 1.0-6, checkmate 1.5.0, codetools 0.2-9, colorspace 1.2-4, digest 0.6.4, edgeR 3.9.6, fail 1.2, foreach 1.4.2, genefilter 1.49.2, ggplot2 1.0.0, graph 1.45.0, grid 3.2.0, gtable 0.1.2, hwriter 1.3.2, iterators 1.0.7, labeling 0.3, lattice 0.20-29, latticeExtra 0.6-26, limma 3.23.1, munsell 0.4.2, nlme 3.1-118, pheatmap 0.7.7, plyr 1.8.1, proto 0.3-10, reshape2 1.4, rjson 0.2.15, scales 0.2.4, sendmailR 1.2-1, splines 3.2.0, stringr 0.6.2, survival 2.37-7, tools 3.2.0, xtable 1.7-4, zlibbioc 1.13.0

7 Funding

This software was developed with funding from the Agriculture and Food Research Institute of the National Institute of Food and Agriculture of the USDA (2011-68004-30154), the National Science Foundation (MCB-1021969) and the National Institutes of Health/National Institute of Allergy and Infectious Diseases (5R01 AI036959).

8 References

Thomas Girke. systemPipeR: NGS workflow and report generation environment, 28 June 2014. URL https://github.com/tgirke/systemPipeR.

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. ISSN 1932-6203. doi: 10.1371/journal.pone.0074183. URL http://dx.doi.org/10.1371/journal.pone.0074183.

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. ISSN 1465-6906. doi: 10.1186/gb-2013-14-4-r36. URL http://dx.doi.org/10.1186/gb-2013-14-4-r36.

Ben Langmead and Steven L Salzberg. Fast gapped-read alignment with bowtie 2. *Nat. Methods*, 9(4):357–359, April 2012. ISSN 1548-7091. doi: 10.1038/nmeth.1923. URL http://dx.doi.org/10.1038/nmeth.1923.

Michael Lawrence, Wolfgang Huber, Hervé Pagès, Patrick Aboyoun, Marc Carlson, Robert Gentleman, Martin T Morgan, and Vincent J Carey. Software for computing and annotating genomic ranges. *PLoS Comput. Biol.*, 9(8):e1003118, 8 August 2013. ISSN 1553-734X. doi: 10.1371/journal.pcbi.1003118. URL http://dx.doi.org/10.1371/journal.pcbi.1003118.

H Li and R Durbin. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, 25(14): 1754–1760, July 2009. ISSN 1367-4803. doi: 10.1093/bioinformatics/btp324. URL http://dx.doi.org/10.1093/bioinformatics/btp324.

Heng Li. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. 03 2013. URL http://arxiv.org/abs/1303.3997.

Yang Liao, Gordon K Smyth, and Wei Shi. The subread aligner: fast, accurate and scalable read mapping by seed-and-vote. *Nucleic Acids Res.*, 41(10):e108, 4 April 2013. ISSN 0305-1048. doi: 10.1093/nar/gkt214. URL http://dx.doi.org/10.1093/nar/gkt214.