

Pragmatic R for Biologists

10/22/10



R

- An environment for statistical computing
 - Statistics
 - Visualization

Strengths and Weaknesses

- Great for
 - Statistics
 - Graphics
 - Tabular data
 - Reproducible research
- Not great for
 - Parsing, string manipulation, web
- Also...
 - Functional programming is weird
 - Monolithic memory
 - Confusing gotchas

Online Resources

- <http://stackoverflow.com/questions/tagged/r>
- <http://stackoverflow.com/questions/tagged/ggplot2>
- <http://stats.stackexchange.com/>
- <http://onertipaday.blogspot.com/>

Getting R

- <http://www.r-project.org/>
- Essential cheat sheet
 - <http://cran.r-project.org/doc/contrib/Short-refcard.pdf>

Getting help with R

- `?melt`
- `ls("package:plyr")`
- `vignette()`

Using R Effectively

- **Interactive conversation**

```
savehistory(file = "myWork.Rhistory")
```

- `q()` to quit

R Basic Operations

```
> 1+2+3
[1] 6
> 1+2*3
[1] 7
> (1+2)*3
[1] 9
> c(0,1,1,2,3,5,8)
[1] 0 1 1 2 3 5 8
> str(c(0,1,1,2,3,5,8))
num [1:7] 0 1 1 2 3 5 8
> 1:10
[1] 1 2 3 4 5 6 7 8 9 10
> str(1:10)
int [1:10] 1 2 3 4 5 6 7 8 9 10
> c(1,2,3,4)+1
[1] 2 3 4 5
```

```
> c(1,2,3,4)*2
[1] 2 4 6 8
> theCount<-c("one","two","three")
> str(theCount)
chr [1:3] "one" "two" "three"
> 3==4
[1] FALSE
> b<-(1:12)
> b
[1] 1 2 3 4 5 6 7 8 9 10 11 12
> b[7]
[1] 7
> b[-1]
[1] 2 3 4 5 6 7 8 9 10 11 12
> b[-2]
[1] 1 3 4 5 6 7 8 9 10 11 12
```

```
> b[-7]
[1] 1 2 3 4 5 6 8 9 10 11 12
> b[ b %% 2 == 0]
[1] 2 4 6 8 10 12
> b %% 2
[1] 1 0 1 0 1 0 1 0 1 0 1 0
> b %% 2 == 0
[1] FALSE TRUE FALSE TRUE FALSE
TRUE FALSE TRUE FALSE
TRUE FALSE TRUE
```


Getting stuff into R and out of R

`read.table()`

```
read.table(file, header = FALSE, sep = "", quote = "\"",  
          dec = ".", row.names, col.names,  
          as.is = !stringsAsFactors,  
          na.strings = "NA", colClasses = NA, nrows = -1,  
          skip = 0, check.names = TRUE, fill = !blank.lines.skip,  
          strip.white = FALSE, blank.lines.skip = TRUE,  
          comment.char = "#",  
          allowEscapes = FALSE, flush = FALSE,  
          stringsAsFactors = default.stringsAsFactors(),  
          fileEncoding = "", encoding = "unknown")
```

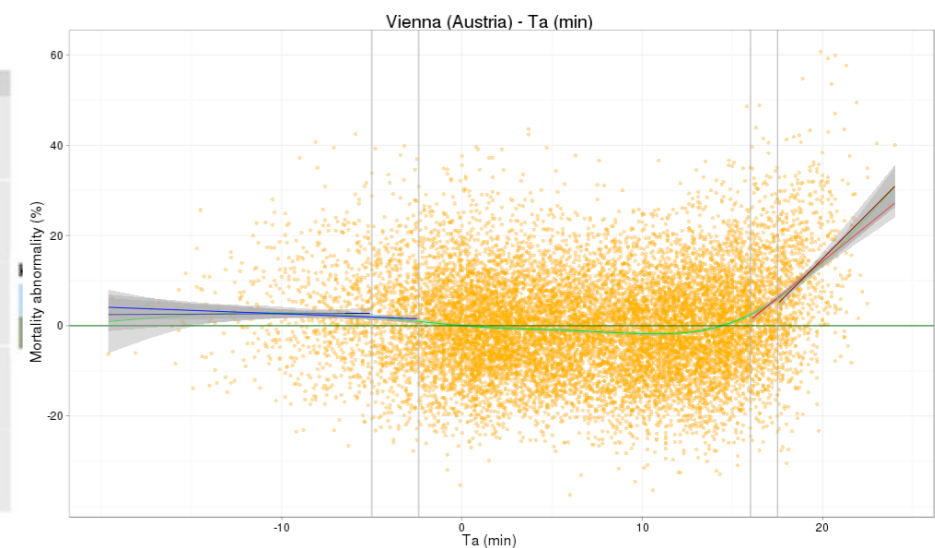
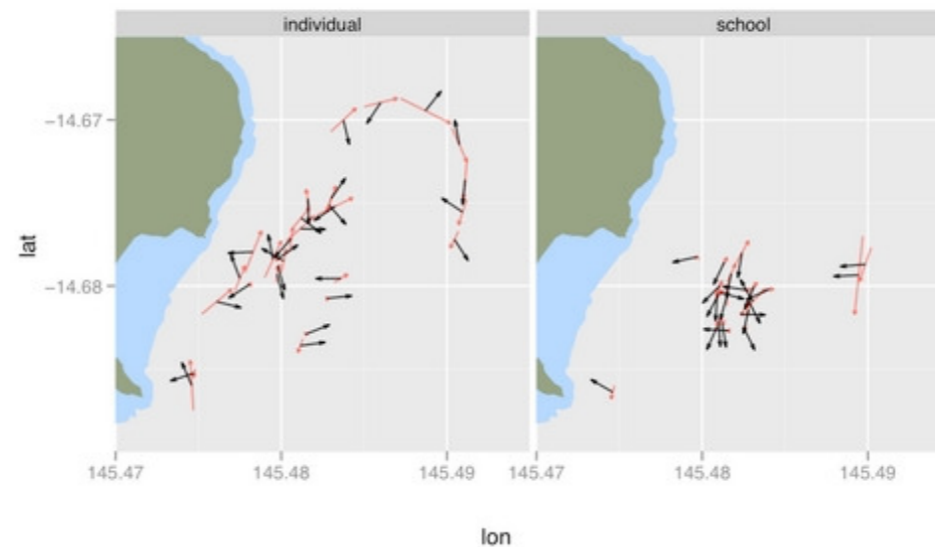
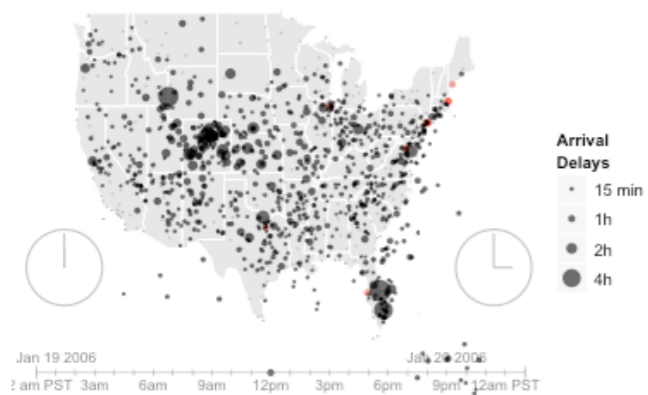
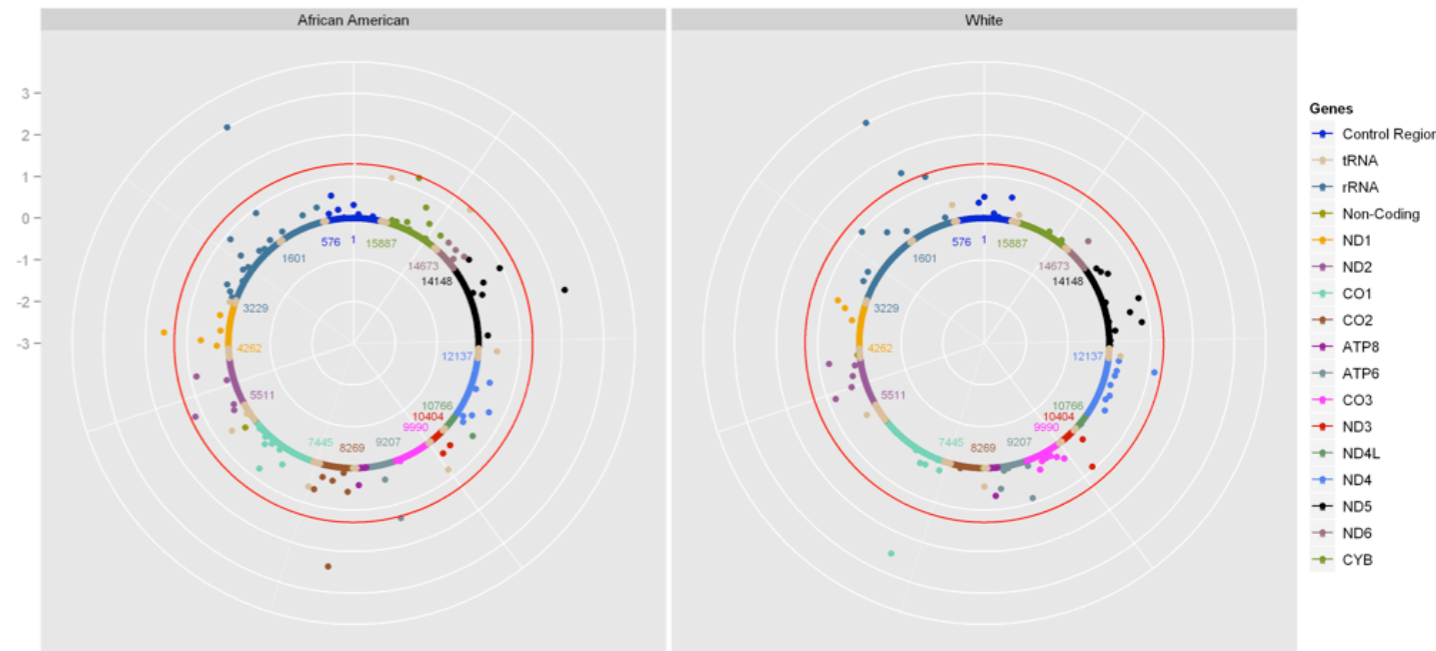
`load()`

`save`

```
save(..., list = character(0L),  
      file = stop("'file' must be specified"),  
      ascii = FALSE, version = NULL, envir = parent.frame(),  
      compress = !ascii, compression_level,  
      eval.promises = TRUE, precheck = TRUE)
```

ggplot2

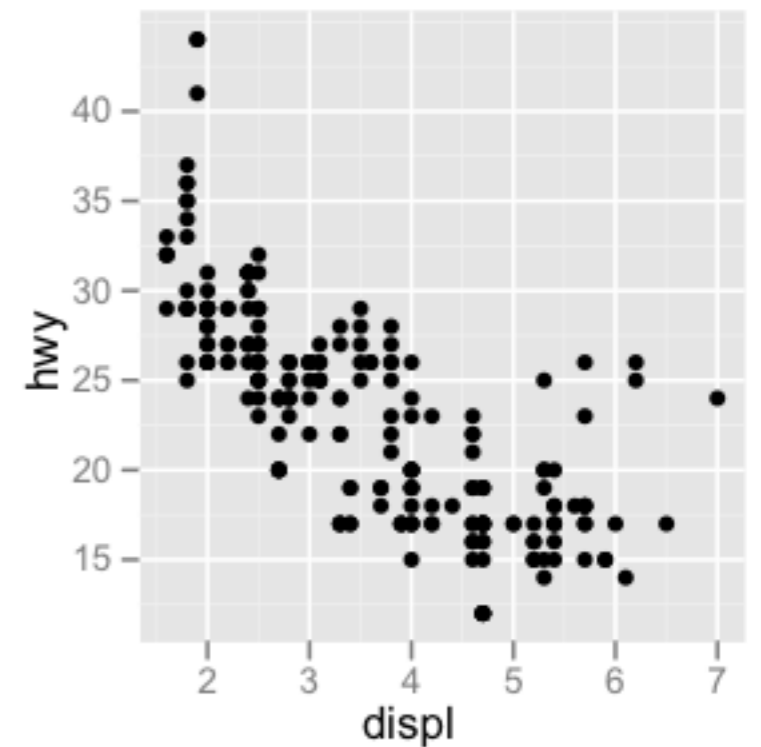
- Layered
- Sensible defaults



<http://github.com/hadley/ggplot2/wiki>

Getting ggplot2

- > `install.packages("ggplot2")`
- > `library("ggplot2")`
- > `data()`
- > `?mpg`
- > `head(mpg)`
- > `str(mpg)` *this is like Data Dumper*
- > `qplot(displ, hwy, data=mpg)`



Aesthetics and facetting

- `qplot(displ,hwy,data=mpg,color=class)`
- `qplot(displ,hwy,data=mpg)+facet_grid(. ~ cyl)`

Reorder

- `qplot(class,hwy,data=mpg)`
- `qplot(reorder(class,hwy),hwy,data=mpg)`
- `qplot(reorder(class,hwy),hwy,data=mpg,geom="boxplot")`

Fun with Diamonds

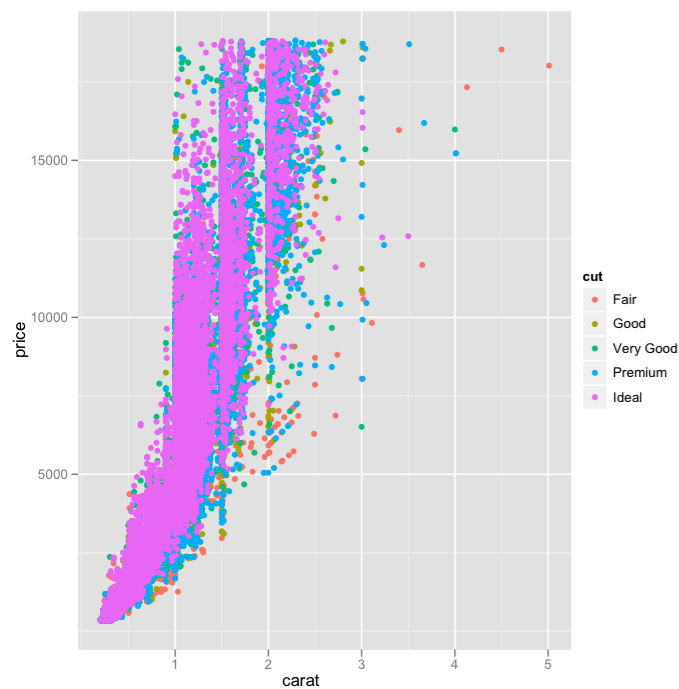
- `# With only one variable, qplot guesses that # you want a bar chart or histogram qplot(cut, data = diamonds)`
- `qplot(carat, data = diamonds)`
- `qplot(carat, data = diamonds, binwidth = 1)`
- `qplot(carat, data = diamonds, binwidth = 0.1)`
- `qplot(carat, data = diamonds, binwidth = 0.01)`
- `last_plot() + xlim(0, 3)`
- `qplot(depth, data=diamonds, binwidth=0.2) + xlim(55, 70) + facet_wrap(~ cut)`

ggplot2 grammar

```
qplot(carat, price, data = diamonds, color=cut)
```

is equivalent to

```
p<-ggplot(diamonds, aes(carat, price, color=cut)) + layer(geom="point")
```



Sweave

$$R + \text{LaTeX} = \text{.pdf}$$

- A pretentious mix of R and LaTeX designed to intimidate reviewers
- in R: `Sweave("myReport.Rnw",output="myReport.tex")`
- `pdflatex myReport.tex`

From shell:

```
echo "Sweave(\"myReport.Rnw\",output=\"myReport.tex\");" | R --no-save --quiet
```


2009 RNAseq Standard Report

```
\documentclass{article}
\usepackage{longtable}
\usepackage{rotating}
\begin{document}

\title{RNAseq Standard Report}
\author{Benjamin Blackman\\Aurelie Bonin\\Suzanne Joneson\\Eduardo Reis\\ \TA: Jeremy Leipzig}

\maketitle

\tableofcontents
\pagebreak
\section{Introduction}
The objective of this project was to align and analyze a short
read dataset obtained by Illumina cDNA sequencing. The study
species is Mimulus guttatus (monkey flower).

<<basicStuff,echo=FALSE>>=
library(xtable)
library(ggplot2)
@
```

RNAseq Standard Report

Benjamin Blackman
Aurelie Bonin
Suzanne Joneson
Eduardo Reis

TA: Jeremy Leipzig

October 27, 2009

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1 Introduction

The objective of this project was to align and analyze a short read dataset obtained by Illumina cDNA sequencing. The study species is *Mimulus guttatus* (monkey flower).

Report_1.txt

rdName	totRds	bpReads	refName	refBpLength	mappedRds	bpmappedRds	predName	numPredGenes	bpPredGenes	rdsMapPredGe	bpmmapPredGen	knownName	numKnownGen	bpKnownGenes	rdsMapKnownC	bpmmapKnownG
mimulus_scaff1	30246800	2268510000	scaffold_1	4921665	113123	8484225	scaffold_1.pred	330	453541	11390	854250	scaffold_1.annot	701	1133679	122646	9198450
mimulus_scaff1	30246800	2268510000	scaffold_1	4921665	79273	5945475	scaffold_1.pred	330	453541	8131	609825	scaffold_1.annot	701	1133679	86286	6471450

```
\section{Short read dataset and reference sequence - Basic
information}
Here is some basic information on the short read dataset and the
reference sequence used to perform the alignment:\\
```

```
\\
```

```
<<table1,echo=FALSE,fig=FALSE>>=
table1<-read.table("Report_1.txt")
totRds <- table1[2,2]
bpReads <-table1[2,3]
RefbpLength <-table1[2,5]
NumPredicted <-table1[2,9]
LengthPredicted <-table1[2,10]
NumKnown <-table1[2,14]
LengthKnown <-table1[2,15]
```

```
@
\textbf{Short read dataset}\\
Number of reads: \Sexpr{totRds}\\
Total length in bp: \Sexpr{bpReads}\\
\\
\textbf{Reference sequence}\\
Total length in bp: \Sexpr{RefbpLength}\\
Number of predicted genes: \Sexpr{NumPredicted}\\
Total length of predicted gene sequence in bp: \Sexpr
{LengthPredicted}\\
Number of annotated genes: \Sexpr{NumKnown}\\
Total length of annotated gene sequence in bp: \Sexpr
{LengthKnown}\\
```

```
\section{Comparison of alignments obtained with different
programs}
We tested two programs (Bowtie and BWA) to align the short reads
to the reference sequence. BWA allows for indels whereas Bowtie
doesn't. Bowtie was used for the rest of the analyses.\\
```

```
<<table1,echo=FALSE,fig=FALSE>>=
tableReport <- subset (table1, select = c(rdName, mappedRds,
bpmappedRds))
colnames (tableReport)<-c('Program','Number of aligned
reads','Total length in bp ')
tableReport [,1] <-c('BWA', 'Bowtie')
@
```

```
<<tableSetup2,results=tex,echo=FALSE>>=
myXtable<-xtable(tableReport,type=tex,caption="BWA and Bowtie -
Performance comparison")
print(myXtable,include.rownames=FALSE)
@
```

2 Short read dataset and reference sequence - Basic information

Here is some basic information on the short read dataset and the reference sequence used to perform the alignment:

Short read dataset

Number of reads: 30246800

Total length in bp: 2268510000

Reference sequence

Total length in bp: 4921665

Number of predicted genes: 330

Total length of predicted gene sequence in bp: 453541

Number of annotated genes: 701

Total length of annotated gene sequence in bp: 1133679

3 Comparison of alignments obtained with different programs

We tested two programs (Bowtie and BWA) to align the short reads to the reference sequence. BWA allows for indels whereas Bowtie doesn't. Bowtie was used for the rest of the analyses.

Program	Number of aligned reads	Total length in bp
BWA	113123	8484225
Bowtie	79273	5945475

Table 1: BWA and Bowtie - Performance comparison

```
\pagebreak
\section{Read coverage - Genes}
The following histogram presents the distribution of read coverages for genes,
as calculated by the percentage of a gene's coding sequences with at least one
read aligned.
```

```
<<genereads,echo=FALSE,fig=TRUE>>=
table3<-read.table("Jerm_table3.txt",header=TRUE)
p<-qplot(X.CDS_COVERAGE,data=table3,geom="histogram",xlab="Read coverage (%)
for genes", ylab= "Gene count")
print(p)
@
```

```
\pagebreak
\section{Read coverage - Intergenic regions}
The following histogram presents the distribution of read coverages for
intergenic regions, as calculated by the percentage of the region with at least
one read aligned.
```

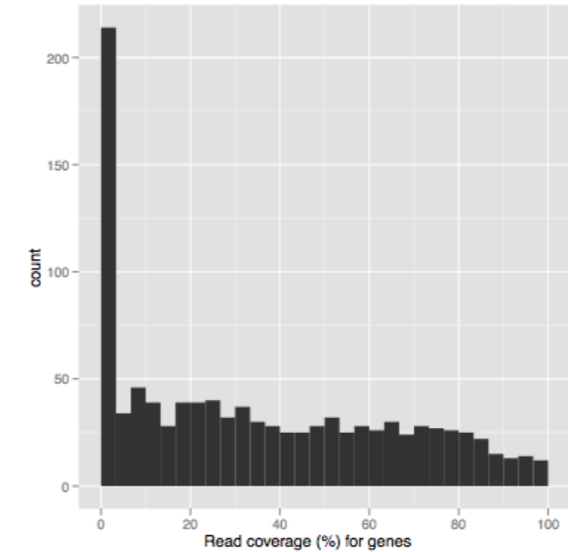
```
<<intergenicreads,echo=FALSE,fig=TRUE>>=
table4<-read.table("table4intergenicredo.txt",header=TRUE)
r<-qplot((INTERGENIC_COVERAGE)*100,data=table4,geom="histogram",xlab="Read
coverage (%) for intergenic regions", ylab= "Intergenic region count")
print(r)
@
```

```
\pagebreak
\section{Length of intergenic regions}
The following histogram presents the distribution of intergenic lengths.
```

```
<<intergenicregion,echo=FALSE,fig=TRUE>>=
table4<-read.table("table4intergenicredo.txt",header=TRUE)
r<-qplot(INTERGENIC_LENGTH,data=table4,geom="histogram",xlab="Intergenic length
in bp", ylab= "Intergenic region count")
print(r)
@
```

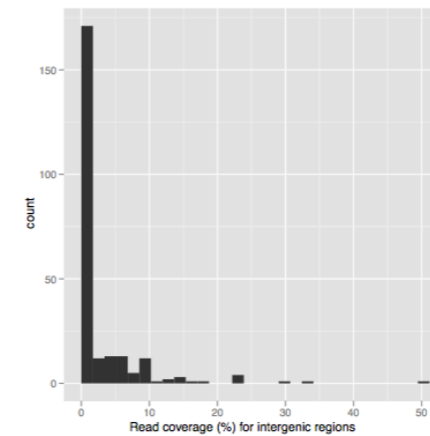
4 Read coverage - Genes

The following histogram presents the distribution of read coverages for genes, as calculated by the percentage of a gene's coding sequences with at least one read aligned.



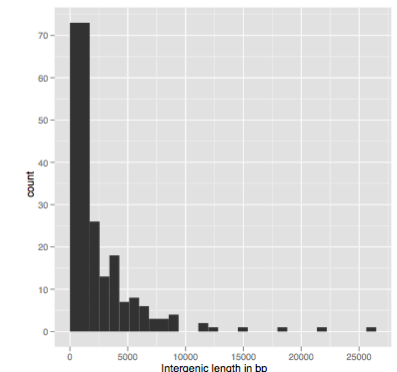
5 Read coverage - Intergenic regions

The following histogram presents the distribution of read coverages for intergenic regions, as calculated by the percentage of the region with at least one read aligned.



6 Length of intergenic regions

The following histogram presents the distribution of intergenic lengths.



READ_NAME	GENE_NAME	GENE_LENGTH	CDS_LENGTH	%CDS_COVER
Mimulus_reads	mgf021408m	607	576	36.805555556
Mimulus_reads	mgf024713m	623	265	0
Mimulus_reads	mgf003544m	244	243	67.489711934
Mimulus_reads	mg021480m	366	366	0
Mimulus_reads	mgf023543m	410	349	0
Mimulus_reads	mg012117m	298	297	0
Mimulus_reads	mgf019623m	440	409	0
Mimulus_reads	mgf022738m	393	393	0
Mimulus_reads	mgf019826m	504	473	0
Mimulus_reads	mgf022195m	604	321	43.613707165
Mimulus_reads	mgf012634m	744	742	94.339622642
Mimulus_reads	mg014291m	849	262	100
Mimulus_reads	mg015640m	677	677	11.225997046
Mimulus_reads	mgf001000m	557	557	0

Jerm_table3.txt

```
\pagebreak
\section{Number of genes per read count}
The following histogram presents the number of genes having a given read count.
```

```
<<genenumber,echo=FALSE,fig=TRUE>>=
table5<-read.table("Table5.txt", header=TRUE)
q<-qqplot(Read_count,log (Genes_number), data=table5,xlab="Read count", ylab="log
(Number of genes)")
print(q)
@
```

Read_set_name	Read_count	Genes_number
Mimulus_reads	0	202
Mimulus_reads	1	19
Mimulus_reads	2	46
Mimulus_reads	3	24
Mimulus_reads	4	30
Mimulus_reads	5	24
Mimulus_reads	6	17
Mimulus_reads	7	30
Mimulus_reads	8	19
Mimulus_reads	9	21
Mimulus_reads	10	21
Mimulus_reads	11	16
Mimulus_reads	12	14
Mimulus_reads	13	15
Mimulus_reads	14	11
Mimulus_reads	15	13
Mimulus_reads	16	10

```
\pagebreak
\section{Distribution of contig sizes - Genes}
The following histogram presents the distribution of contig sizes for genes.
```

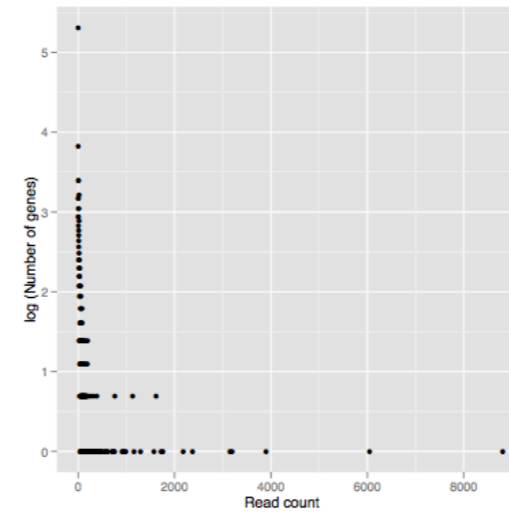
```
<<genecontigs,echo=FALSE,fig=TRUE>>=
ContigRNA<-read.table("table4contigs_size_output_bowtie_mRNAs.txt",header=TRUE)
s<-qqplot(Contig_Size,log (Number_Contigs),data=ContigRNA,xlab="Contig size for genes",
ylab= "Log (Number of contigs)")
print(s)
@
\pagebreak
```

```
\section{Distribution of contig sizes - Intergenic regions}
The following histogram presents the distribution of contig sizes for intergenic
regions.
```

```
<<intercontigs,echo=FALSE,fig=TRUE>>=
ContigInter<-read.table("table4contigs_size_output_bowtie_intergenic.txt",header=TRUE)
t<-qqplot(Contig_Size,log (Number_Contigs),data=ContigInter,xlab="Contig size for
intergenic regions", ylab= "Log (Number of contigs)")
print(t)
@
\pagebreak
\end{document}
```

7 Number of genes per read count

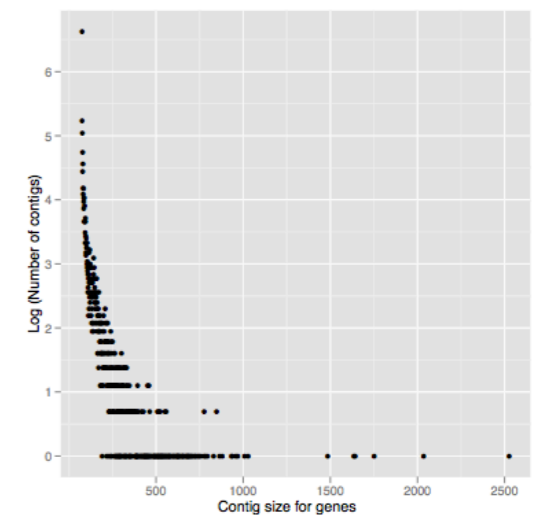
The following histogram presents the number of genes having a given read count.



Contig_Size	Number_Contig
76	755
77	188
78	155
79	115
80	85
81	96
82	60
83	66
84	65

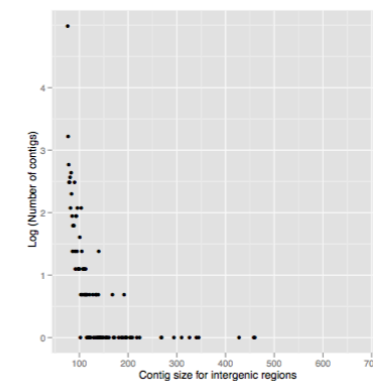
8 Distribution of contig sizes - Genes

The following histogram presents the distribution of contig sizes for genes.



9 Distribution of contig sizes - Intergenic regions

The following histogram presents the distribution of contig sizes for intergenic regions.



Contig_Size	Number_Contig
76	147
77	25
78	16
79	12
80	12
81	13
82	8
83	14
84	10
85	7

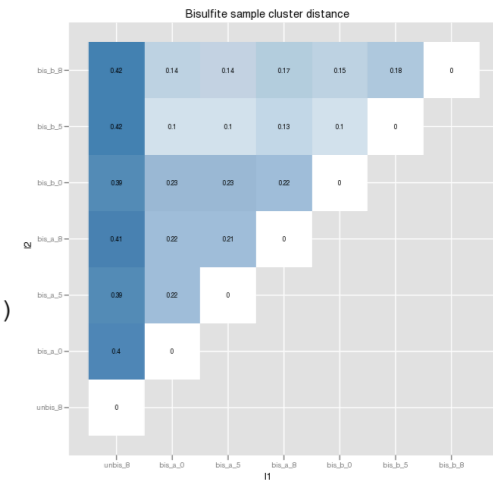
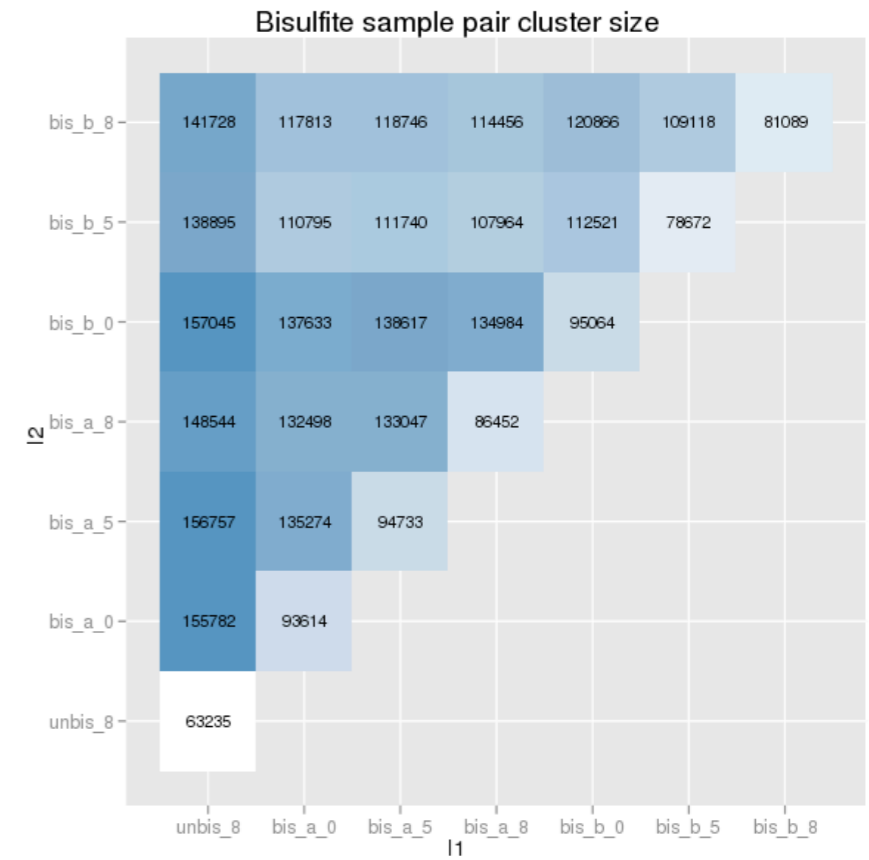
Bisulfite Analysis

lane	bis	phu
1	1	63235
1	2	155782
1	3	156757
1	5	148544
1	6	157045
1	7	138895
1	8	141728
2	2	93614
2	3	135274
2	5	132498
2	6	137633
2	7	110795
2	8	117813
3	3	94733

matrix.txt

lanes.txt

lane	bis	phu
1	unbis	8
2	bis_a	0
3	bis_a	5
5	bis_a	8
6	bis_b	0
7	bis_b	5
8	bis_b	8



```

library(ggplot2)
bisulfite<-read.table("matrix.txt",col.names=c("l1","l2","clusters"))
bisulfite$l1<-as.factor(bisulfite$l1)
bisulfite$l2<-as.factor(bisulfite$l2)

samples<-read.table("lanes.txt",col.names=c("lane","bis","phu"))
scale_labels<-paste(samples$bis,samples$phu,sep="_")

#you'll need this on mac
X11(type ="cairo")

#let's build this slowly
p<-ggplot(bisulfite,aes(l1,l2))+geom_tile(aes(fill=clusters))
p<-p+scale_fill_gradient(limits = c(min(bisulfite$clusters), max(bisulfite$clusters)), low="white",high="steelblue")
p<-p+scale_x_discrete(breaks=levels(bisulfite$l1),labels=scale_labels)
p<-p+scale_y_discrete(breaks=levels(bisulfite$l2),labels=scale_labels)
p<-p+geom_text(aes(size=3,label=clusters))+opts(title = "Bisulfite sample pair cluster size")+opts(legend.position = "none")

print(p)

savePlot(filename="plot.png",type="png")

selfLane<-function(x){bisulfite$clusters[bisulfite$l1 == x & bisulfite$l2 == x]}

bisulfite$allCor<-mapply(max,lapply(bisulfite$l1,selfLane),lapply(bisulfite$l2,selfLane))
bisulfite$noCor<-lapply(bisulfite$l1,selfLane)+lapply(bisulfite$l2,selfLane)
bisulfite$dist<-(bisulfite$clusters-bisulfite$allCor)/bisulfite$noCor

p<-ggplot(bisulfite,aes(l1,l2))+geom_tile(aes(fill=dist))
print(p+scale_fill_gradient(limits = c(min(bisulfite$dist), max(bisulfite$dist)), low="white",high="steelblue")+scale_x_discrete(breaks=levels(bisulfite
$l1),labels=scale_labels)+scale_y_discrete(breaks=levels(bisulfite$l2),labels=scale_labels)+geom_text(aes(size=3,label=round(dist,2)))+opts
(legend.position = "none")+opts(title = "Bisulfite sample cluster distance"))

```

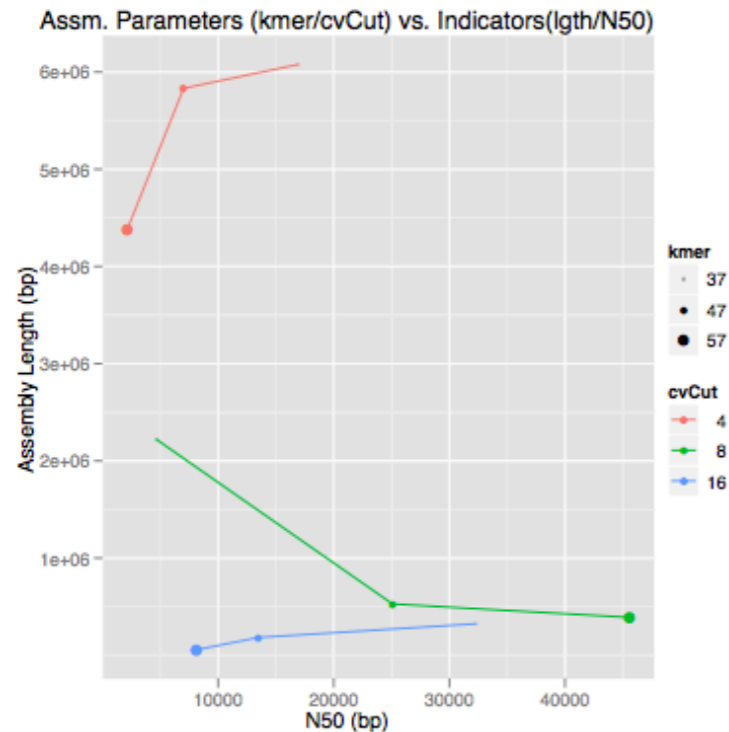
SVAR

3 Effect of kmer and cvCut on Assembly Profile

3.1 Assembly coherency

The following scatterplot illustrates the effect of these variables on N50 and assembly length. N50 refers to the length of the shortest contig in an assembly such that the sum of contigs of equal length or longer is at least 50% of the total length of all contigs.

We can plot this to show the effect of kmer value (as progressively larger points) and coverage cutoff (strata connected by lines) on N50 and assembly length:



```
> load("BD574-2.frame.RData")
> ls()
[1] "myDataFrame"
> myDataFrame
  totBP  reads  tiles goodContigs kmer cvCut expCov groupDir ctgs meanLgth medianLgth
1 323399 1263928 170121      57 37 16 32 out_BD574-2_37_16_32_dir 80 4057.925 143.5
2 6058426 1263928 1092568      998 37 4 8 out_BD574-2_37_4_8_dir 1390 4372.006 370.0
3 2214471 1263928 547583      1176 37 8 16 out_BD574-2_37_8_16_dir 1296 1713.209 606.0
4 182324 1263928 111783      33 47 16 32 out_BD574-2_47_16_32_dir 43 4256.372 596.0
5 5820563 1263928 985530      1584 47 4 8 out_BD574-2_47_4_8_dir 1772 3291.328 1773.5
6 525714 1263928 208144      212 47 8 16 out_BD574-2_47_8_16_dir 252 2097.060 325.5
7 56002 1263928 42878      19 57 16 32 out_BD574-2_57_16_32_dir 22 2556.727 613.0
8 4379805 1263928 725921      3020 57 4 8 out_BD574-2_57_4_8_dir 3094 1417.416 907.0
9 388091 1263928 161205      54 57 8 16 out_BD574-2_57_8_16_dir 76 5130.447 187.0
  totalCoverage maxLgth over1k N50
1 324634 61354 24 32297
2 6077088 75922 585 16918
3 2220319 101303 488 4677
4 183024 60374 19 13476
5 5832234 46530 1139 7018
6 528459 101732 51 25073
7 56248 13848 9 8116
8 4385486 63866 1438 2166
9 389914 101691 22 45544
```

```
<<plotCov, fig=TRUE, echo=FALSE>>=
kmerDotSizes<-seq(1:length(levels(as.factor(myDataFrame$kmer))))*1
p<-ggplot(myDataFrame[order(myDataFrame$cvCut),], aes(N50, totalCoverage))+
geom_point(aes(size=as.factor(kmer))) +
aes(colour=as.factor(cvCut)) +
scale_size_manual(name="kmer",value = kmerDotSizes) +
geom_line() +
scale_colour_discrete("cvCut") +
xlab("N50 (bp)") +
ylab("Assembly Length (bp)") +
opts(title="Assm. Parameters (kmer/cvCut) vs. Indicators(lgth/N50)")
print(p)
@
```

Five Prime Enrichment

leftReads

pco	pos	tLength	depth
pco500123	124	686	1
pco500123	125	686	1
pco500123	126	686	1
pco500123	127	686	1
pco500123	128	686	1
pco500123	129	686	1
pco500123	130	686	1
pco500123	131	686	1

```
library(xtable)
geneDefs<-read.table("geneDefs.txt",sep="\t",header=TRUE,quote='')
geneDefs$desc<-gsub("Top Blast","",geneDefs$desc)
top20left<-sort(xtabs(depth ~ pco,data=leftReads),decreasing=TRUE)[1:20]
top20leftpcos<-attr(top20left,"dimnames")
displayTableLeft<-merge(as.data.frame(top20leftpcos),geneDefs)
displayTableLeft$desc<-substr(displayTableLeft$desc,1,50)
```

geneDefs

```
pco desc
pco500123 top blast hypothetical protein
pco500134 zinc ion binding
pco644893 alcohol dehydrogenase
```

```
<<top20leftcounts,echo=FALSE,results=tex>>=
xtable(as.data.frame(top20left))
@
```

With gene definitions:

```
<<top20leftdisp,echo=FALSE,results=tex>>=
xtable(displayTableLeft)
@
```

2.1.1 Left reads

The counts here represent total base pairs aligned to that pco. To obtain the reads aligned, divide by average read length.

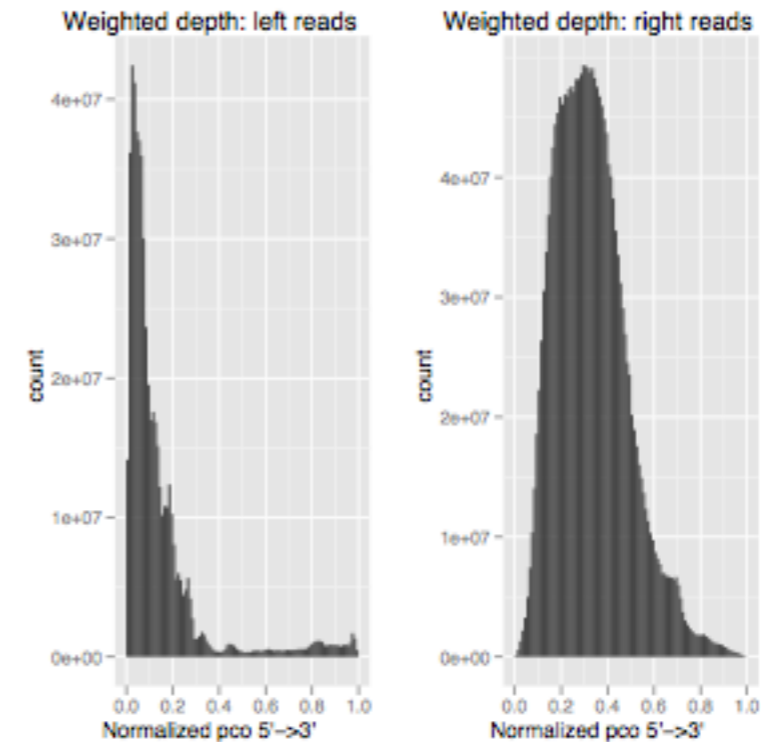
	top20left
pco598314	12610568.00
pco644849	10710320.00
pco640072	7495096.00
pco651827	7476770.00
pco647057	7234518.00
pco642907	6980828.00
pco664216	6651398.00
pco591868	5847596.00

With gene definitions:

	pco	desc
1	pco522245	Q8W1C9 Ribosomal protein L35A [Zea mays] E=3e-73
2	pco591868	P45633 60S ribosomal protein L10 [Zea mays] E=1e-
3	pco595782	Q6ZJC7 Early nodulin 75-like protein [Oryza sativ
4	pco598225	Q41852 40S ribosomal protein S21 [Zea mays] E=6e-

Five Prime Enrichment

```
<<prepHisto,echo=FALSE>>=  
library("ggplot2")  
vplot <- function(x, y){viewport(layout.pos.row = x, layout.pos.col = y)}  
  
w<-function(myReads,mySide){  
  qplot(nPos,data=myReads,binwidth=.01,weight=depth,xlim=c(0,1),xlab="Normalized pco 5'→3'",main=(paste("Weighted depth:",mySide,"reads")))}  
  
@  
  
<<sidesWeighted,fig=TRUE,echo=FALSE>>=  
grid.newpage()  
pushViewport(viewport(layout=grid.layout(1,2)))  
  
leftPlot<-w(leftReads,"left")  
print(leftPlot, vp=vplot(1,1))  
  
rightPlot<-w(rightReads,"right")  
print(rightPlot, vp=vplot(1,2))  
  
@
```



kmer analysis

	kmer	count	sample	conc
1	1	2	b73_mcrbc	200
2	2	10	b73_mcrbc	200
3	3	32	b73_mcrbc	200
4	4	136	b73_mcrbc	200
5	5	512	b73_mcrbc	200
6	6	2080	b73_mcrbc	200
7	7	8192	b73_mcrbc	200
8	8	32896	b73_mcrbc	200
9	9	131072	b73_mcrbc	200
10	10	524800	b73_mcrbc	200
11	11	2093412	b73_mcrbc	200
12	12	7639104	b73_mcrbc	200
13	13	18675851	b73_mcrbc	200
14	14	28596584	b73_mcrbc	200
15	15	33438709	b73_mcrbc	200
16	16	35081294	b73_mcrbc	200
17	17	35452389	b73_mcrbc	200
18	18	35377437	b73_mcrbc	200
19	19	35136629	b73_mcrbc	200

```
load("sampkmer.results.RData")
```

```
sampkmer$conc<-factor(sampkmer$conc, levels=kmerLevels)  
q<-qplot(kmer, count, color=sample, data=sampkmer, facets = conc ~ .)  
print(q)
```

