Recruitment Data Challenge

The Bioinformatics & Biostatistics Group @ The Francis Crick Institute

Introduction

Here you will find the data from an RNA-Seq and ATAC-Seq experiment. Both experiments have the same design. There is a treatment and control group each containing three replicates making a total of six samples per experiment. The data files are defined as follows (all files are tab delimited text files):

RNA-Seq Data

- **rnaseq_design.txt**: Sample ids and corresponding condition labels.
- **rnaseq_gene_counts.txt**: Raw (not normalised) gene-level read counts for each sample.
- **rnaseq_annotation.txt**: Gene level annotation.

ATAC-Seq Data

- **atacseq_design.txt**: Sample ids and corresponding condition labels.
- **atacseq_peak_counts.txt**: Raw (not normalised) ATAC-Seq peak level counts for each sample.
- **atacseq_peaks.bed**: A bed file defining the peak loci

All sequence data were aligned to the human genome reference hg38.

The Challenge

The treatment here is thought to activate a transcriptional program via remodelling of the chromatin architecture. The aim here is to:
1. Identify genes that may be regulated in this fashion.
2. Identify the possible transcriptional programs involved.
3. Present candidate transcription factors that may be responsible for the underlying regulation.

Please produce a 20 minute presentation detailing your exploration of the data, your analysis approach and findings?

Analysis

Strategy

1. Identify genes with significant changes in expression.
2. Identify zones with significant changes in accessibility.
3. Detect hotspots in accessibility changes over gene regulatory areas of differentially expressed genes.
4. Detect enriched TF motifs in zones presenting accessibility changes.
5. Detect enriched TF motifs in hotspots.
6. Perform GO Analysis to put genes in context.

Differential Expression with DESeq2

Setup

[1]
```
library("DESeq2")
library("IHW")
```

```
Loading required package: S4Vectors

Loading required package: stats4

Loading required package: BiocGenerics

Loading required package: parallel


Attaching package: 'BiocGenerics'


The following objects are masked from 'package:parallel':

    clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
    clusterExport, clusterMap, parApply, parCapply, parLapply,
    parLapplyLB, parRapply, parSapply, parSapplyLB


The following objects are masked from 'package:stats':

    IQR, mad, sd, var, xtabs


The following objects are masked from 'package:base':

    anyDuplicated, append, as.data.frame, basename, cbind, colnames,
    dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
    grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
    order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
    rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
```

```
    union, unique, unsplit, which, which.max, which.min


Attaching package: 'S4Vectors'


The following object is masked from 'package:base':

    expand.grid


Loading required package: IRanges

Loading required package: GenomicRanges

Loading required package: GenomeInfoDb

Loading required package: SummarizedExperiment

Loading required package: Biobase

Welcome to Bioconductor

    Vignettes contain introductory material; view with
    'browseVignettes()'. To cite Bioconductor, see
    'citation("Biobase")', and for packages 'citation("pkgname")'.


Loading required package: DelayedArray

Loading required package: matrixStats


Attaching package: 'matrixStats'


The following objects are masked from 'package:Biobase':

    anyMissing, rowMedians


Loading required package: BiocParallel


Attaching package: 'DelayedArray'


The following objects are masked from 'package:matrixStats':

    colMaxs, colMins, colRanges, rowMaxs, rowMins, rowRanges
```

```
The following objects are masked from 'package:base':

    aperm, apply, rowsum
```

import data

[2]
```
#atac_cts <-
as.matrix(read.csv("data_challenge/atacseq_peak_counts.txt",sep="
\t",row.names="peakid"))
atac_fcts <-
as.matrix(read.csv("output/atacseq_fpeak_counts.txt",
                                  sep="\t",
                                  row.names="peakid"))
atac_col <- read.csv("data_challenge/atacseq_design.txt",
                     sep="\t",
                     row.names="sample.id")
dim(atac_fcts)
head(atac_fcts,2)
atac_col
```

```
173264   6
```

A matrix: 2 × 6 of type int

	s84	s85	s86	s93	s94	s95
1:10003-10507	278	195	292	255	287	284
1:20221-22634	66	56	90	67	66	120

A data.frame: 6 × 1

	condition
	<fct>
s84	control
s85	control
s86	control
s93	treated
s94	treated
s95	treated

[3]
```
rna_cts <-
as.matrix(read.csv("data_challenge/rnaseq_gene_counts.txt",
                                 sep="\t",
                                 row.names="featureid"))
rna_col <- read.csv("data_challenge/rnaseq_design.txt",
                    sep="\t",
                    row.names="sample.id")
dim(rna_cts)
head(rna_cts,2)
rna_col
```

58051 6

A matrix: 2 × 6 of type int

	s69	s70	s71	s75	s76	s77
ENSG00000000003	1	1	0	8	2	1
ENSG00000000005	0	0	0	0	0	0

A data.frame: 6 × 1

	condition
	<fct>
s69	control
s70	control
s71	control
s75	treated
s76	treated
s77	treated

Double check for entry into DESeq2

[4]
```
all(rownames(atac_col) == colnames(atac_fcts))
all(rownames(rna_col) == colnames(rna_cts))
```

TRUE
TRUE

[5]
```
atac_DDS <- DESeqDataSetFromMatrix(countData = atac_fcts,
```

```
                                        colData = atac_col,
                                        design = ~ condition)
atac_DDS
```

```
class: DESeqDataSet
dim: 173264 6
metadata(1): version
assays(1): counts
rownames(173264): 1:10003-10507 1:20221-22634 ... Y:56850334-56851158
  Y:56864434-56864700
rowData names(0):
colnames(6): s84 s85 ... s94 s95
colData names(1): condition
```

[6]
```
rna_DDS <- DESeqDataSetFromMatrix(countData = rna_cts,
                                  colData = rna_col,
                                  design = ~ condition)
rna_DDS
```

```
class: DESeqDataSet
dim: 58051 6
metadata(1): version
assays(1): counts
rownames(58051): ENSG00000000003 ENSG00000000005 ... ENSG00000283698
  ENSG00000283699
rowData names(0):
colnames(6): s69 s70 ... s76 s77
colData names(1): condition
```

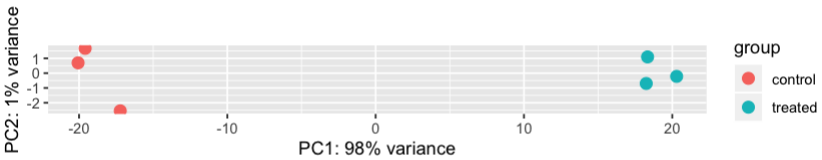
Set reference

[7]
```
atac_DDS$condition <- relevel(atac_DDS$condition,
                              ref = "control")
rna_DDS$condition <- relevel(rna_DDS$condition,
                             ref = "control")
```

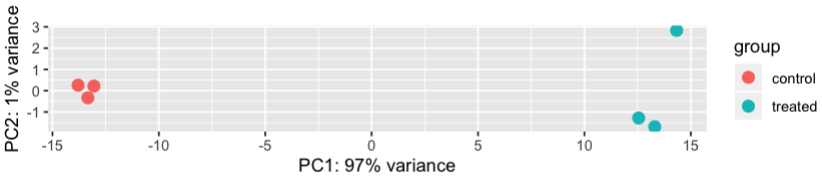
rLog normalisation and PCA

[8]
```
atac_DDS_Rlog <- rlog(atac_DDS)
rna_DDS_Rlog <- rlog(rna_DDS)
par(mfrow=c(1,2))
```

```
plotPCA(atac_DDS_Rlog, intgroup = "condition")
plotPCA(rna_DDS_Rlog, intgroup = "condition")
png("output/plot/atac_pca.png", width = 800, height = 800)
plotPCA(atac_DDS_Rlog, intgroup = "condition")
dev.off()
png("output/plot/rna_pca.png", width = 800, height = 800)
plotPCA(rna_DDS_Rlog, intgroup = "condition")
dev.off()
```



pdf: 2
pdf: 2



DESeq2 Analysis

[9]
```
atac_DDS_dea <- DESeq(atac_DDS)
rna_DDS_dea <- DESeq(rna_DDS)
```

```
estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing

estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship
```

```
final dispersion estimates

fitting model and testing
```

Quick look at results with Benjamini-Hochberg (BH) FDR < 0.05

[10]
```
atac_DDS_dea_res <- results(atac_DDS_dea)
summary(atac_DDS_dea_res)
sum(atac_DDS_dea_res$padj < 0.05, na.rm = TRUE)
```

```
out of 173264 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)       : 21543, 12%
LFC < 0 (down)     : 16786, 9.7%
outliers [1]       : 0, 0%
low counts [2]     : 0, 0%
(mean count < 3)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results

29556
```

[11]
```
rna_DDS_dea_res <- results(rna_DDS_dea)
summary(rna_DDS_dea_res)
sum(rna_DDS_dea_res$padj < 0.05, na.rm = TRUE)
```

```
out of 28704 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)       : 3587, 12%
LFC < 0 (down)     : 3327, 12%
outliers [1]       : 1, 0.0035%
low counts [2]     : 10531, 37%
(mean count < 3)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results

6073
```

Quick look at results with Independent Hypothesis Weighting (IHW) FDR < 0.05

[12]
```
atac_DDS_dea_resIHW <- results(atac_DDS_dea, filterFun=ihw)
```

```
summary(atac_DDS_dea_resIHW)
sum(atac_DDS_dea_resIHW$padj < 0.05, na.rm = TRUE)
```

```
out of 173264 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)       : 22782, 13%
LFC < 0 (down)     : 16128, 9.3%
outliers [1]       : 0, 0%
[1] see 'cooksCutoff' argument of ?results
see metadata(res)$ihwResult on hypothesis weighting
```

29636

[13]
```
rna_DDS_dea_resIHW <- results(rna_DDS_dea, filterFun=ihw)
summary(rna_DDS_dea_resIHW)
sum(rna_DDS_dea_resIHW$padj < 0.05, na.rm = TRUE)
```

```
out of 28704 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)       : 3586, 12%
LFC < 0 (down)     : 3388, 12%
outliers [1]       : 1, 0.0035%
[1] see 'cooksCutoff' argument of ?results
see metadata(res)$ihwResult on hypothesis weighting
```

6154

Plotting

Shrinkage

[14]
```
#check the coef term
resultsNames(atac_DDS_dea)
resultsNames(rna_DDS_dea)
```

'Intercept' 'condition_treated_vs_control'

'Intercept' 'condition_treated_vs_control'

[15]
```
atac_DDS_dea_resLFC <- lfcShrink(atac_DDS_dea, coef =
"condition_treated_vs_control", type = "apeglm")
```

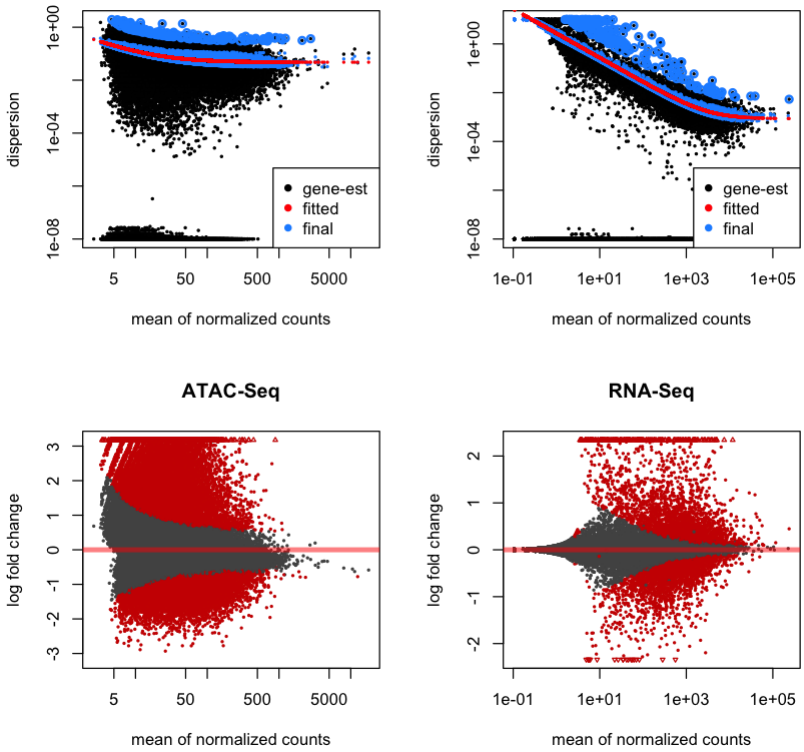
```
rna_DDS_dea_resLFC <- lfcShrink(rna_DDS_dea, coef =
"condition_treated_vs_control", type = "apeglm")
```

```
using 'apeglm' for LFC shrinkage. If used in published research, please
cite:
    Zhu, A., Ibrahim, J.G., Love, M.I. (2018) Heavy-tailed prior
distributions for
    sequence count data: removing the noise and preserving large
differences.
    Bioinformatics. https://doi.org/10.1093/bioinformatics/bty895

using 'apeglm' for LFC shrinkage. If used in published research, please
cite:
    Zhu, A., Ibrahim, J.G., Love, M.I. (2018) Heavy-tailed prior
distributions for
    sequence count data: removing the noise and preserving large
differences.
    Bioinformatics. https://doi.org/10.1093/bioinformatics/bty895
```

Scatter plots

[16]
```
par(mfrow=c(2,2))
plotDispEsts(atac_DDS_dea)
plotDispEsts(rna_DDS_dea)
plotMA(atac_DDS_dea_resLFC, main = "ATAC-Seq", alpha = 0.05)
plotMA(rna_DDS_dea_resLFC, main = "RNA-Seq", alpha = 0.05)
#export as png
png("output/plot/diffx.png", width = 1600, height = 1600)
par(mfrow=c(2,2))
plotDispEsts(atac_DDS_dea)
plotDispEsts(rna_DDS_dea)
plotMA(atac_DDS_dea_resLFC, main = "ATAC-Seq", alpha = 0.05)
plotMA(rna_DDS_dea_resLFC, main = "RNA-Seq", alpha = 0.05)
dev.off()
```

pdf: 2



Merge and export results

Intersect genes significantly differentially expressed in both tests (BH & IHW)

[17]

```
#atacseq
atac_DDF_BH <- as.data.frame(subset(atac_DDS_dea_res, padj <
0.05))
atac_DDF_IHW <- as.data.frame(subset(atac_DDS_dea_resIHW, padj <
0.05))
atac_DDF_FDR <- merge(atac_DDF_BH, atac_DDF_IHW, by=0)
#rnaseq
rna_DDF_BH <- as.data.frame(subset(rna_DDS_dea_res, padj < 0.05))
rna_DDF_IHW <- as.data.frame(subset(rna_DDS_dea_resIHW, padj <
0.05))
rna_DDF_FDR <- merge(rna_DDF_BH, rna_DDF_IHW, by=0)
head(atac_DDF_FDR,2)
dim(atac_DDF_FDR)
head(rna_DDF_FDR,2)
dim(rna_DDF_FDR)
```

Row.names	baseMean.x	log2FoldChange.x	lfcSE.x	stat.x	p
<l<chr>>	<dbl>	<dbl>	<dbl>	<dbl>	<

Row.names	baseMean.x	log2FoldChange.x	lfcSE.x	stat.x	p
<l<chr>>	<dbl>	<dbl>	<dbl>	<dbl>	<
1:10003-10507	272.09433	-0.842736	0.2584011	-3.261348	0
1:100046749-100047203	15.93489	1.460924	0.5002989	2.920102	0

28589 14

Row.names	baseMean.x	log2FoldChange.x	lfcSE.x	stat.x
<l<chr>>	<dbl>	<dbl>	<dbl>	<dbl>
ENSG00000000457	314.40428	0.4596662	0.1236178	3.718446
ENSG00000000938	32.65741	1.9510985	0.4074590	4.788454

5992 14

[18]
```
#removing redundant columns
atac_DDF_FDR <- atac_DDF_FDR[-c(4:5,8:12)]
rna_DDF_FDR <- rna_DDF_FDR[-c(4:5,8:12)]
head(atac_DDF_FDR,2)
head(rna_DDF_FDR,2)
```

A data.frame: 2 × 7

Row.names	baseMean.x	log2FoldChange.x	pvalue.x	padj.x
<l<chr>>	<dbl>	<dbl>	<dbl>	<dbl>
1:10003-10507	272.09433	-0.842736	0.001108837	0.01045958
1:100046749-100047203	15.93489	1.460924	0.003499171	0.02561063

A data.frame: 2 × 7

Row.names	baseMean.x	log2FoldChange.x	pvalue.x	padj.x
<l<chr>>	<dbl>	<dbl>	<dbl>	<dbl>
ENSG00000000457	314.40428	0.4596662	2.004525e-04	0.0009778

Row.names	baseMean.x	log2FoldChange.x	pvalue.x	padj.x
<I<chr>>	<dbl>	<dbl>	<dbl>	<dbl>
ENSG00000000938	32.65741	1.9510985	1.680711e-06	0.000011

Merging RNA DiffX Results

[19]
```
#merge with coordinates keeping only significant diffx
rna_bed <- read.csv("output/rnaseq_genes.bed6",
                    sep="\t",
                    header = FALSE,
                    col.names =
c("chr","fstart","fend","name","score","strand"))
rna_diffx <- merge(rna_bed,
                   rna_DDF_FDR,
                   by.x = "name",
                   by.y = "Row.names",
                   all = FALSE)
rna_diffx <- rna_diffx[c(2:4,1,8,6)] #back to bed6 format
head(rna_bed,2)
head(rna_diffx,2)
write.table(rna_diffx, file="output/rna_diffx.bed",
            sep = "\t",
            col.names = FALSE,
            row.names = FALSE,
            quote = FALSE)
```

A data.frame: 2 × 6

chr	fstart	fend	name	score	strand
<fct>	<int>	<int>	<fct>	<int>	<fct>
X	100628669	100636806	ENSG00000000003	8137	-
X	100584801	100599885	ENSG00000000005	15084	+

A data.frame: 2 × 6

chr	fstart	fend	name	log2FoldChange.x	s
<fct>	<int>	<int>	<fct>	<dbl>	<
1	169853073	169888888	ENSG00000000457	0.4596662	-
1	27612668	27626569	ENSG00000000938	1.9510985	-

Merging ATAC DiffA Results

[20]
```
#merge with coordinates keeping only significant diffx
atac_bed <- read.csv("output/atacseq_fpeaks.bed6",sep="\t",header
= FALSE, col.names =
c("chr","fstart","fend","name","score","strand"))
atac_diffx <- merge(atac_bed,
                    atac_DDF_FDR,
                    by.x = "name",
                    by.y = "Row.names",
                    all.x = FALSE)
atac_diffx <- atac_diffx[c(2:4,1,8,6)] #back to bed6 format
dim(atac_bed)
head(atac_bed,2)
dim(atac_diffx)
head(atac_diffx,2)
write.table(atac_diffx, file="output/atac_diffx.bed",
            sep = "\t",
            col.names = FALSE,
            row.names = FALSE,
            quote = FALSE)
```

173264 6

A data.frame: 2 × 6

chr	fstart	fend	name	score	strand
<fct>	<int>	<int>	<fct>	<int>	<fct>
1	10002	10507	1:10003-10507	505	.
1	20220	22634	1:20221-22634	2414	.

28589 6

A data.frame: 2 × 6

chr	fstart	fend	name	log2FoldChange.x	stranc
<fct>	<int>	<int>	<fct>	<dbl>	<fct>
1	10002	10507	1:10003-10507	-0.842736	.
1	100046748	100047203	1:100046749-100047203	1.460924	.

[21]
```
#split and export
```

```
atac_hi <- atac_diffx[atac_diffx$log2FoldChange.x > 0,]
write.table(atac_hi,
            file="output/atac_hi.bed",
            sep = "\t",
            col.names = FALSE,
            row.names = FALSE,
            quote = FALSE)
atac_lo <- atac_diffx[atac_diffx$log2FoldChange.x < 0,]
write.table(atac_lo,
            file="output/atac_lo.bed",
            sep = "\t",
            col.names = FALSE,
            row.names = FALSE,
            quote = FALSE)
rna_up <- rna_diffx[rna_diffx$log2FoldChange.x > 0,]
write.table(rna_up,
            file="output/rna_up.bed",
            sep = "\t",
            col.names = FALSE,
            row.names = FALSE,
            quote = FALSE)
rna_dw <- rna_diffx[rna_diffx$log2FoldChange.x < 0,]
write.table(rna_dw,
            file="output/rna_dw.bed",
            sep = "\t",
            col.names = FALSE,
            row.names = FALSE,
            quote = FALSE)
```

Cleaning Up

[22]
```
#don't need these anymore
detach("package:IHW", unload=TRUE)
detach("package:DESeq2", unload=TRUE)
```

[23]
```
sort(sapply(ls(),function(x){object.size(get(x))}))
```

atac_col	1656
rna_col	1656
rna_DDF_FDR	720776
rna_DDF_BH	730096
rna_DDF_IHW	789184
atac_DDF_FDR	3887888
atac_DDF_BH	4018872
atac_DDF_IHW	4267072
rna_dw	4276608
rna_up	4286144
rna_diffx	4353264

rna_DDS 4438032
rna_cts 5573768
rna_bed 5578712
rna_DDS_dea_re 6979000
s **rna_DDS_Rlog**
7458064 **rna_DDS_dea_re**
sIHW
10231120 **rna_DDS_dea**
13973632 **rna_DDS_dea_re**
sLFC
14876368 **atac_lo**
15615984 **atac_hi**
15787304 **atac_DDS**
15952552 **atac_diffx**
16044728 **atac_fcts**
19392552 **atac_bed**
19402528 **atac_DDS_dea_r**
es
23562880 **atac_DDS_Rlog**
24963656 **atac_DDS_dea_r**
esIHW
33269152 **atac_DDS_dea_r**
esLFC
52645888 **atac_DDS_dea**
58923520

ChIPseeker Analysis / Hotspot Identification

[24]
```
#load libraries
library(biomaRt)
library(GenomicFeatures)
library(ChIPseeker)
library(ggplot2)
```

```
Loading required package: AnnotationDbi
```

```
Registered S3 method overwritten by 'enrichplot':
  method               from
  fortify.enrichResult DOSE
```

```
ChIPseeker v1.20.0  For help: https://guangchuangyu.github.io/ChIPseeker

If you use ChIPseeker in published research, please cite:
Guangchuang Yu, Li-Gen Wang, Qing-Yu He. ChIPseeker: an R/Bioconductor
package for ChIP peak annotation, comparison and visualization.
```

Bioinformatics 2015, 31(14):2382-2383

Grabbing transcript level info via Biomart

[25]
```
listMarts(host="www.ensembl.org")
```

A data.frame: 4 × 2

biomart	version
<chr>	<chr>
ENSEMBL_MART_ENSEMBL	Ensembl Genes 98
ENSEMBL_MART_MOUSE	Mouse strains 98
ENSEMBL_MART_SNP	Ensembl Variation 98
ENSEMBL_MART_FUNCGEN	Ensembl Regulation 98

[26]
```
ensembl <- useMart("ENSEMBL_MART_ENSEMBL")
searchDatasets(mart = ensembl, pattern = "GRCh38")
```

A data.frame: 1 × 3

	dataset	description	version
	<I<chr>>	<I<chr>>	<I<chr>>
85	hsapiens_gene_ensembl	Human genes (GRCh38.p13)	GRCh38.p13

[27]
```
ensemblHs38 = useEnsembl(biomart = "ensembl", dataset =
"hsapiens_gene_ensembl")
gene2transcript <- getBM(mart = ensemblHs38, attributes =
c("ensembl_gene_id","ensembl_transcript_id"))
head(gene2transcript)
dim(gene2transcript)
```

A data.frame: 6 × 2

ensembl_gene_id	ensembl_transcript_id
<chr>	<chr>
ENSG00000210049	ENST00000387314
ENSG00000211459	ENST00000389680

ensembl_gene_id	ensembl_transcript_id
<chr>	<chr>
ENSG00000210077	ENST00000387342
ENSG00000210082	ENST00000387347
ENSG00000209082	ENST00000386347
ENSG00000198888	ENST00000361390

249158 2

We can generate custom TxDb objects for up/down regulated genes

[28]
```
rna_up_tids <- merge(rna_up, gene2transcript, by.x = "name", by.y
= "ensembl_gene_id", all.x = TRUE)
rna_up_tids <- na.omit(rna_up_tids[,"ensembl_transcript_id"])
head(rna_up_tids)
```

'ENST00000367771' 'ENST00000423670' 'ENST00000367772'
'ENST00000367770' 'ENST00000470238' 'ENST00000457296'

[29]
```
rna_dw_tids <- merge(rna_dw, gene2transcript, by.x = "name", by.y
= "ensembl_gene_id", all.x = TRUE)
rna_dw_tids <- na.omit(rna_dw_tids[,"ensembl_transcript_id"])
head(rna_dw_tids)
```

'ENST00000483528' 'ENST00000374409' 'ENST00000483261'
'ENST00000003583' 'ENST00000475760' 'ENST00000492753'

[30]
```
#this may take a while...
txdb_up <- makeTxDbFromBiomart(biomart = "ensembl",
dataset="hsapiens_gene_ensembl", transcript_ids=rna_up_tids)
txdb_dw <- makeTxDbFromBiomart(biomart = "ensembl",
dataset="hsapiens_gene_ensembl", transcript_ids=rna_dw_tids)
```

```
Download and preprocess the 'transcripts' data frame ...
OK

Download and preprocess the 'chrominfo' data frame ...
```

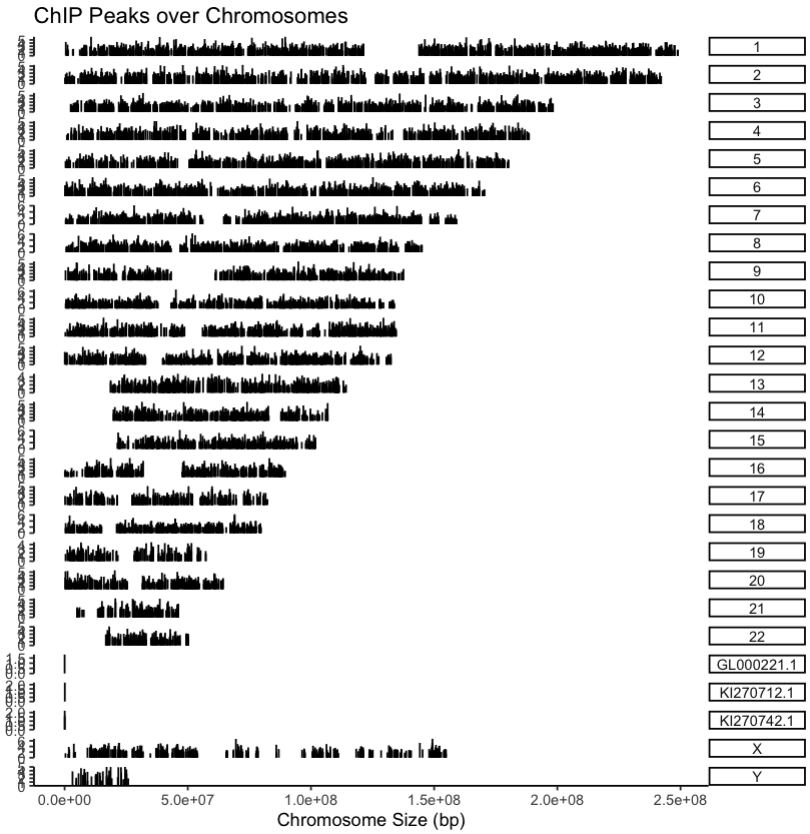
```
OK

Download and preprocess the 'splicings' data frame ...
OK

Download and preprocess the 'genes' data frame ...
OK

Prepare the 'metadata' data frame ...
OK

Make the TxDb object ...
OK

Download and preprocess the 'transcripts' data frame ...
OK

Download and preprocess the 'chrominfo' data frame ...
OK

Download and preprocess the 'splicings' data frame ...
OK

Download and preprocess the 'genes' data frame ...
OK

Prepare the 'metadata' data frame ...
OK

Make the TxDb object ...
OK
```

Import the data

[31]
```
atac_hi_GR <- readPeakFile("output/atac_hi.bed", as = "GRanges")
atac_lo_GR <- readPeakFile("output/atac_lo.bed", as = "GRanges")
```

[32]
```
covplot(atac_hi_GR, weightCol = "V5") #nice but not so
informative
```

```
GL000216.2 dosen't contain signal higher than 1

KI270711.1 dosen't contain signal higher than 1
```



Annotating peaks with information regarding their distance/overlaps with up or down regulated gene features

[33]
```
atac_hi_upreg <- annotatePeak(atac_hi_GR,
                              tssRegion = c(-10000,1000),
                              TxDb = txdb_up,
                              annoDb = "org.Hs.eg.db", #adds
extra info like ENTREZ ID and symbol
                              genomicAnnotationPriority =
c("Promoter",

 "5UTR",

 "3UTR",

 "Exon",

 "Intron",

 "Downstream"))
```

```
>> preparing features information...               2019-10-20 07:21:02
>> identifying nearest features...                 2019-10-20 07:21:02
>> calculating distance from peak to TSS...        2019-10-20 07:21:02
>> assigning genomic annotation...                 2019-10-20 07:21:02
>> adding gene annotation...                       2019-10-20 07:21:06
>> assigning chromosome lengths                    2019-10-20 07:21:06
```

```
>> done...                                          2019-10-20 07:21:06
Warning message in .Seqinfo.mergexy(x, y):
"Each of the 2 combined objects has sequence levels not in the other:
  - in 'x': GL000216.2, GL000221.1, KI270711.1, KI270712.1, KI270742.1
  - in 'y': MT
  Make sure to always combine/compare objects based on the same
reference
  genome (use suppressWarnings() to suppress this warning)."
Warning message in .Seqinfo.mergexy(x, y):
"Each of the 2 combined objects has sequence levels not in the other:
  - in 'x': GL000216.2, GL000221.1, KI270711.1, KI270712.1, KI270742.1
  - in 'y': MT
  Make sure to always combine/compare objects based on the same
reference
  genome (use suppressWarnings() to suppress this warning)."
Warning message in .Seqinfo.mergexy(x, y):
"Each of the 2 combined objects has sequence levels not in the other:
  - in 'x': GL000216.2, GL000221.1, KI270711.1, KI270712.1, KI270742.1
  - in 'y': MT
  Make sure to always combine/compare objects based on the same
reference
  genome (use suppressWarnings() to suppress this warning)."
Warning message in .Seqinfo.mergexy(x, y):
"Each of the 2 combined objects has sequence levels not in the other:
  - in 'x': GL000216.2, GL000221.1, KI270711.1, KI270712.1, KI270742.1
  - in 'y': MT
  Make sure to always combine/compare objects based on the same
reference
  genome (use suppressWarnings() to suppress this warning)."
Warning message in .Seqinfo.mergexy(x, y):
"Each of the 2 combined objects has sequence levels not in the other:
  - in 'x': GL000216.2, GL000221.1, KI270711.1, KI270712.1, KI270742.1
  - in 'y': MT
  Make sure to always combine/compare objects based on the same
reference
  genome (use suppressWarnings() to suppress this warning)."
Loading required package: org.Hs.eg.db
```

```
'select()' returned 1:many mapping between keys and columns
```

[34]

```
atac_hi_dwreg <- annotatePeak(atac_hi_GR,
                              tssRegion = c(-10000,1000),
                              TxDb = txdb_dw,
                              annoDb = "org.Hs.eg.db", #adds
extra info like ENTREZ ID and symbol
                              genomicAnnotationPriority =
c("Promoter",
```

```
 "5UTR",

 "3UTR",

 "Exon",

 "Intron",

 "Downstream"))
```

```
>> preparing features information...               2019-10-20 07:21:06
>> identifying nearest features...                 2019-10-20 07:21:07
>> calculating distance from peak to TSS...        2019-10-20 07:21:07
>> assigning genomic annotation...                 2019-10-20 07:21:07
>> adding gene annotation...                       2019-10-20 07:21:11
>> assigning chromosome lengths                    2019-10-20 07:21:11
>> done...                                         2019-10-20 07:21:11
'select()' returned 1:many mapping between keys and columns
```

[35]
```
atac_lo_upreg <- annotatePeak(atac_lo_GR,
                              tssRegion = c(-10000,1000),
                              TxDb = txdb_up,
                              annoDb = "org.Hs.eg.db", #adds
extra info like ENTREZ ID and symbol
                              genomicAnnotationPriority =
c("Promoter",

 "5UTR",

 "3UTR",

 "Exon",

 "Intron",

 "Downstream"))
```

```
>> preparing features information...               2019-10-20 07:21:11
>> identifying nearest features...                 2019-10-20 07:21:12
>> calculating distance from peak to TSS...        2019-10-20 07:21:12
>> assigning genomic annotation...                 2019-10-20 07:21:12
>> adding gene annotation...                       2019-10-20 07:21:15
>> assigning chromosome lengths                    2019-10-20 07:21:16
>> done...                                         2019-10-20 07:21:16
Warning message in .Seqinfo.mergexy(x, y):
"Each of the 2 combined objects has sequence levels not in the other:
  - in 'x': GL000205.2, GL000219.1, KI270435.1, KI270442.1, KI270519.1,
KI270581.1, KI270711.1, KI270713.1, KI270726.1, KI270727.1, KI270731.1,
KI270744.1, KI270751.1
```

```
  - in 'y': MT
  Make sure to always combine/compare objects based on the same
reference
  genome (use suppressWarnings() to suppress this warning)."
Warning message in .Seqinfo.mergexy(x, y):
"Each of the 2 combined objects has sequence levels not in the other:
  - in 'x': GL000205.2, GL000219.1, KI270435.1, KI270442.1, KI270519.1,
KI270581.1, KI270711.1, KI270713.1, KI270726.1, KI270727.1, KI270731.1,
KI270744.1, KI270751.1
  - in 'y': MT
  Make sure to always combine/compare objects based on the same
reference
  genome (use suppressWarnings() to suppress this warning)."
Warning message in .Seqinfo.mergexy(x, y):
"Each of the 2 combined objects has sequence levels not in the other:
  - in 'x': GL000205.2, GL000219.1, KI270435.1, KI270442.1, KI270519.1,
KI270581.1, KI270711.1, KI270713.1, KI270726.1, KI270727.1, KI270731.1,
KI270744.1, KI270751.1
  - in 'y': MT
  Make sure to always combine/compare objects based on the same
reference
  genome (use suppressWarnings() to suppress this warning)."
Warning message in .Seqinfo.mergexy(x, y):
"Each of the 2 combined objects has sequence levels not in the other:
  - in 'x': GL000205.2, GL000219.1, KI270435.1, KI270442.1, KI270519.1,
KI270581.1, KI270711.1, KI270713.1, KI270726.1, KI270727.1, KI270731.1,
KI270744.1, KI270751.1
  - in 'y': MT
  Make sure to always combine/compare objects based on the same
reference
  genome (use suppressWarnings() to suppress this warning)."
Warning message in .Seqinfo.mergexy(x, y):
"Each of the 2 combined objects has sequence levels not in the other:
  - in 'x': GL000205.2, GL000219.1, KI270435.1, KI270442.1, KI270519.1,
KI270581.1, KI270711.1, KI270713.1, KI270726.1, KI270727.1, KI270731.1,
KI270744.1, KI270751.1
  - in 'y': MT
  Make sure to always combine/compare objects based on the same
reference
  genome (use suppressWarnings() to suppress this warning)."
'select()' returned 1:many mapping between keys and columns
```

[36]

```
atac_lo_dwreg <- annotatePeak(atac_lo_GR,
                              tssRegion = c(-10000,1000),
                              TxDb = txdb_dw,
                              annoDb = "org.Hs.eg.db", #adds
extra info like ENTREZ ID and symbol
                              genomicAnnotationPriority =
c("Promoter",
```

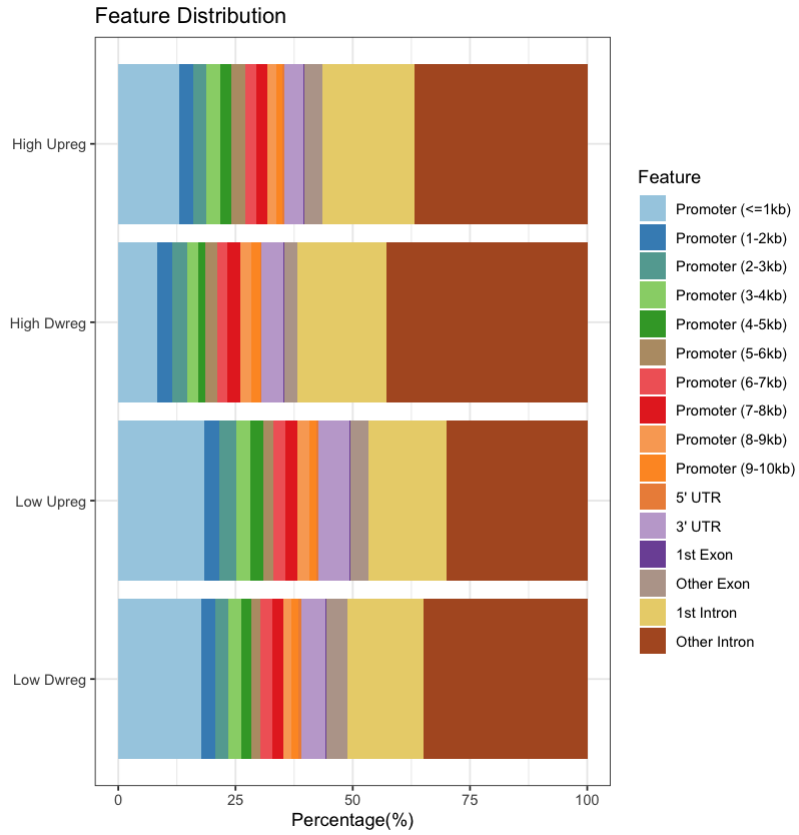
```
        "5UTR",

        "3UTR",

        "Exon",

        "Intron",

        "Downstream"))
```

```
>> preparing features information...               2019-10-20 07:21:16
>> identifying nearest features...                 2019-10-20 07:21:16
>> calculating distance from peak to TSS...        2019-10-20 07:21:16
>> assigning genomic annotation...                 2019-10-20 07:21:16
>> adding gene annotation...                       2019-10-20 07:21:20
>> assigning chromosome lengths                    2019-10-20 07:21:20
>> done...                                         2019-10-20 07:21:20
'select()' returned 1:many mapping between keys and columns
```

We can plot an overview of where the accessibility peaks land (distal/intergenic peaks have been purposefully left out but these simply appear as NAs)

[37]
```
PeakAnnoList <- list("High Upreg"=atac_hi_upreg,
                     "High Dwreg"=atac_hi_dwreg,
                     "Low Upreg"=atac_lo_upreg,
                     "Low Dwreg"=atac_lo_dwreg)
plotAnnoBar(PeakAnnoList)
#export as png
png("output/plot/atac_prox_bar.png", width = 1600, height = 1600)
plotAnnoBar(PeakAnnoList)
dev.off()
```

pdf: 2



These steps allow us to build the coverage profiles in the "promoter" region which we initially defined as -10000 to +1000 from TSS/GeneStart

[38]
```
upreg_prom_locs <- getPromoters(TxDb = txdb_up,
                                upstream = 10000,
                                downstream = 1000)
dwreg_prom_locs <- getPromoters(TxDb = txdb_dw,
                                upstream = 10000,
                                downstream = 1000)
atac_hi_upreg_prom <- getTagMatrix(atac_hi_GR,
                                   windows = upreg_prom_locs)
atac_hi_dwreg_prom <- getTagMatrix(atac_hi_GR,
                                   windows = dwreg_prom_locs)
atac_lo_upreg_prom <- getTagMatrix(atac_lo_GR,
                                   windows = upreg_prom_locs)
atac_lo_dwreg_prom <- getTagMatrix(atac_lo_GR,
                                   windows = dwreg_prom_locs)
PromTagMList <- list("High Upreg"=atac_hi_upreg_prom,
                     "High Dwreg"=atac_hi_dwreg_prom,
                     "Low Upreg"=atac_lo_upreg_prom,
                     "Low Dwreg"=atac_lo_dwreg_prom)
```

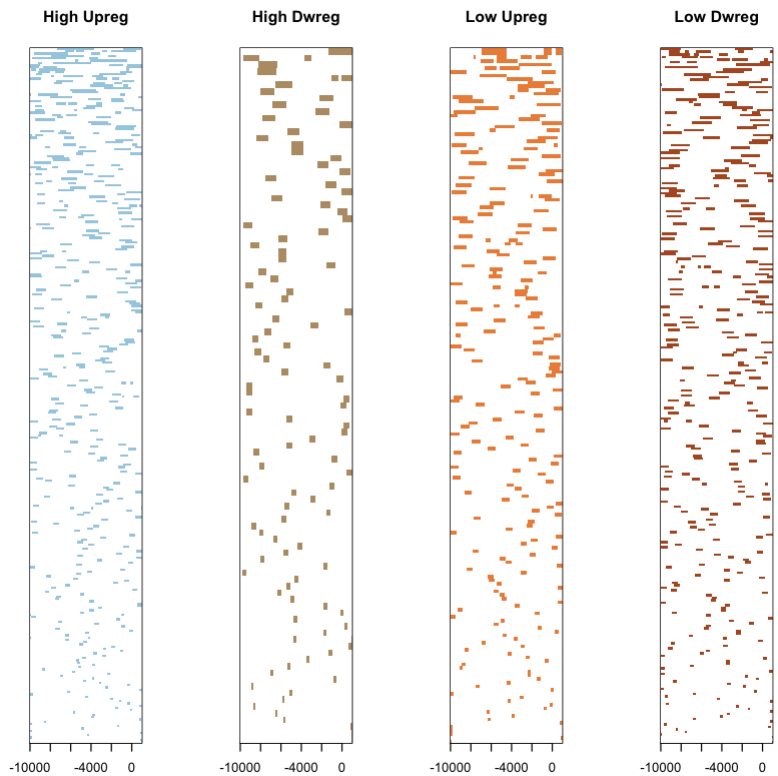
```
Warning message in .Seqinfo.mergexy(x, y):
"Each of the 2 combined objects has sequence levels not in the other:
  - in 'x': MT
  - in 'y': GL000216.2, GL000221.1, KI270711.1, KI270712.1, KI270742.1
  Make sure to always combine/compare objects based on the same
```

```
reference
  genome (use suppressWarnings() to suppress this warning)."
Warning message in .Seqinfo.mergexy(x, y):
"Each of the 2 combined objects has sequence levels not in the other:
  - in 'x': MT
  - in 'y': GL000205.2, GL000219.1, KI270435.1, KI270442.1, KI270519.1,
KI270581.1, KI270711.1, KI270713.1, KI270726.1, KI270727.1, KI270731.1,
KI270744.1, KI270751.1
  Make sure to always combine/compare objects based on the same
reference
  genome (use suppressWarnings() to suppress this warning)."
```

Heatmap views

[39]
```
tagHeatmap(PromTagMList,
           xlim=c(-10000, 1000),
           color=NULL)
#export as png
png("output/plot/atac_prom_heatmap.png", width = 1600, height =
1600)
tagHeatmap(PromTagMList, xlim=c(-10000, 1000), color=NULL)
dev.off()
```

pdf: 2

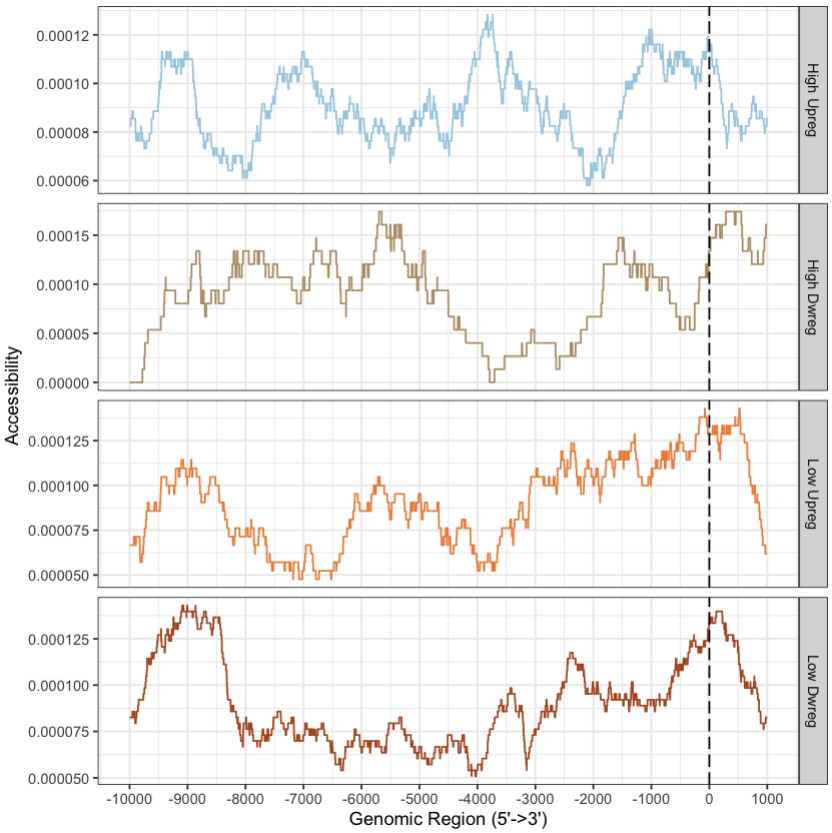


Next is the coverage plot, to identify hotspots visually

[40]
```
plotAvgProf(PromTagMList,
            xlim = c(-10000,1000),
            facet="row",
            ylab = "Accessibility") + scale_x_continuous(breaks =
round(seq(-10000, 1000, by = 1000),1))
#export as png
png("output/plot/atac_prom_graph.png", width = 1600, height =
1600)
plotAvgProf(PromTagMList, xlim = c(-10000,1000), facet="row",
ylab = "Accessibility") + scale_x_continuous(breaks =
round(seq(-10000, 1000, by = 1000),1))
dev.off()
```

```
Scale for 'x' is already present. Adding another scale for 'x', which
will
replace the existing scale.

Scale for 'x' is already present. Adding another scale for 'x', which
will
replace the existing scale.
```

pdf: 2



based on this output we can select Regions of Interest:

High Access / Upregulated

- Window 0: -2000 to 0
- Window 1: -4250 to -3250

- Window 2: -8000 to -4500
- Window 3: -9750 to -8500

High Access / Downregulated

- Window 0: -2000 to 0
- Window 1: -9500 to -4500

Low Access / Upregulated

- Window 0: -3000 to 0
- Window 1: -6000 to -4500
- Window 2: -9750 to -8500

Low Access / Downregulated

- Window 0: -3000 to 0
- Window 1: -9750 to -8250

[41]
```
atac_hi_upregDF <- as.data.frame(atac_hi_upreg)
atac_hi_dwregDF <- as.data.frame(atac_hi_dwreg)
atac_lo_upregDF <- as.data.frame(atac_lo_upreg)
atac_lo_dwregDF <- as.data.frame(atac_lo_dwreg)
write.table(atac_hi_upregDF, file="output/atac_hi_upreg.tsv", sep
= "\t", col.names = TRUE, row.names = FALSE, quote = FALSE)
write.table(atac_hi_dwregDF, file="output/atac_hi_dwreg.tsv", sep
= "\t", col.names = TRUE, row.names = FALSE, quote = FALSE)
write.table(atac_lo_upregDF, file="output/atac_lo_upreg.tsv", sep
= "\t", col.names = TRUE, row.names = FALSE, quote = FALSE)
write.table(atac_lo_dwregDF, file="output/atac_lo_dwreg.tsv", sep
= "\t", col.names = TRUE, row.names = FALSE, quote = FALSE)
```

Extracting the gene and coordinates that had hotspots in the "promoter" region

[42]
```
atac_hi_upreg_promDF <- atac_hi_upregDF[grep("Promoter",
atac_hi_upregDF$annotation),]
atac_hi_dwreg_promDF <- atac_hi_dwregDF[grep("Promoter",
atac_hi_dwregDF$annotation),]
atac_lo_upreg_promDF <- atac_lo_upregDF[grep("Promoter",
atac_lo_upregDF$annotation),]
atac_lo_dwreg_promDF <- atac_lo_dwregDF[grep("Promoter",
atac_lo_dwregDF$annotation),]
upregby_hiprom <- merge(rna_diffx, atac_hi_upreg_promDF, by.x =
"name", by.y = "geneId", all = FALSE)
dwregby_hiprom <- merge(rna_diffx, atac_hi_dwreg_promDF, by.x =
"name", by.y = "geneId", all = FALSE)
upregby_loprom <- merge(rna_diffx, atac_lo_upreg_promDF, by.x =
"name", by.y = "geneId", all = FALSE)
```

```
dwregby_loprom <- merge(rna_diffx, atac_lo_dwreg_promDF, by.x =
"name", by.y = "geneId", all = FALSE)
upregby_hiprom <-
upregby_hiprom[!duplicated(upregby_hiprom$name),]
dwregby_hiprom <-
dwregby_hiprom[!duplicated(dwregby_hiprom$name),]
upregby_loprom <-
upregby_loprom[!duplicated(upregby_loprom$name),]
dwregby_loprom <-
dwregby_loprom[!duplicated(dwregby_loprom$name),]
#back to bed6 format
upregby_hiprom <- upregby_hiprom[c(2:4,1,5,6)]
dwregby_hiprom <- dwregby_hiprom[c(2:4,1,5,6)]
upregby_loprom <- upregby_loprom[c(2:4,1,5,6)]
dwregby_loprom <- dwregby_loprom[c(2:4,1,5,6)]
dim(upregby_hiprom)
dim(dwregby_hiprom)
dim(upregby_loprom)
dim(dwregby_loprom)
#head(atac_hi_upreg_promDF,2)
#head(upregby_hiprom,2)
write.table(upregby_hiprom, file="output/upregby_hiprom.bed", sep
= "\t", col.names = FALSE, row.names = FALSE, quote = FALSE)
write.table(dwregby_hiprom, file="output/dwregby_hiprom.bed", sep
= "\t", col.names = FALSE, row.names = FALSE, quote = FALSE)
write.table(upregby_loprom, file="output/upregby_loprom.bed", sep
= "\t", col.names = FALSE, row.names = FALSE, quote = FALSE)
write.table(dwregby_loprom, file="output/dwregby_loprom.bed", sep
= "\t", col.names = FALSE, row.names = FALSE, quote = FALSE)
```

```
497   6
```

```
274   6
```

```
356   6
```

```
520   6
```