NGS, From data to table to figure

From downloading data to final publication ready figures.

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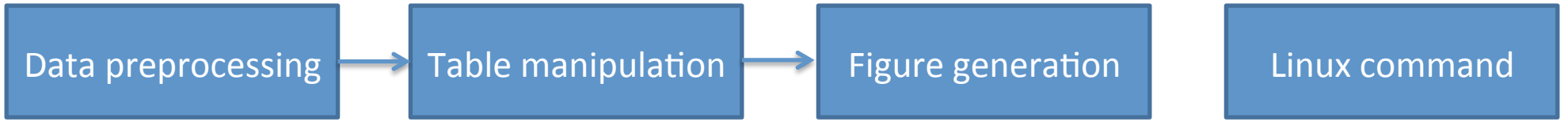
Object

1. I hope everyone can follow me, and I ensure you will meet these problems if you do NGS analysis or data analysis.
2. If you have any question, please don't hesitate to ask a question, actually some of the materials are very difficult to me.
3. Although it may take some time to learn, but it will save a lot of time in the future.
4. All the course martials can be download from https://github.com/limeng12/from_data_2_table_2_figure.

Workflow of the report

- 1. Data preprocessing (First we need to know where can we got the data and how to preprocess the data)
- 2. Table manipulation (We usually don't use the raw data directly, we need process them into a desired format for further investigation)
- 3. Figure generation (Human beings can't read thousands of rows in table simultaneously, we need figure to visualize them)
- 4. Linux (Besides R, we also need Linux to facilitate some work)

Let's learn together



The required knowledge include:

Biology (the most important one, all the analysis around this)

Programming skill (understand the code)

Statistic graphic (understand the figure)

The example problem

We will follow a problem during the course.

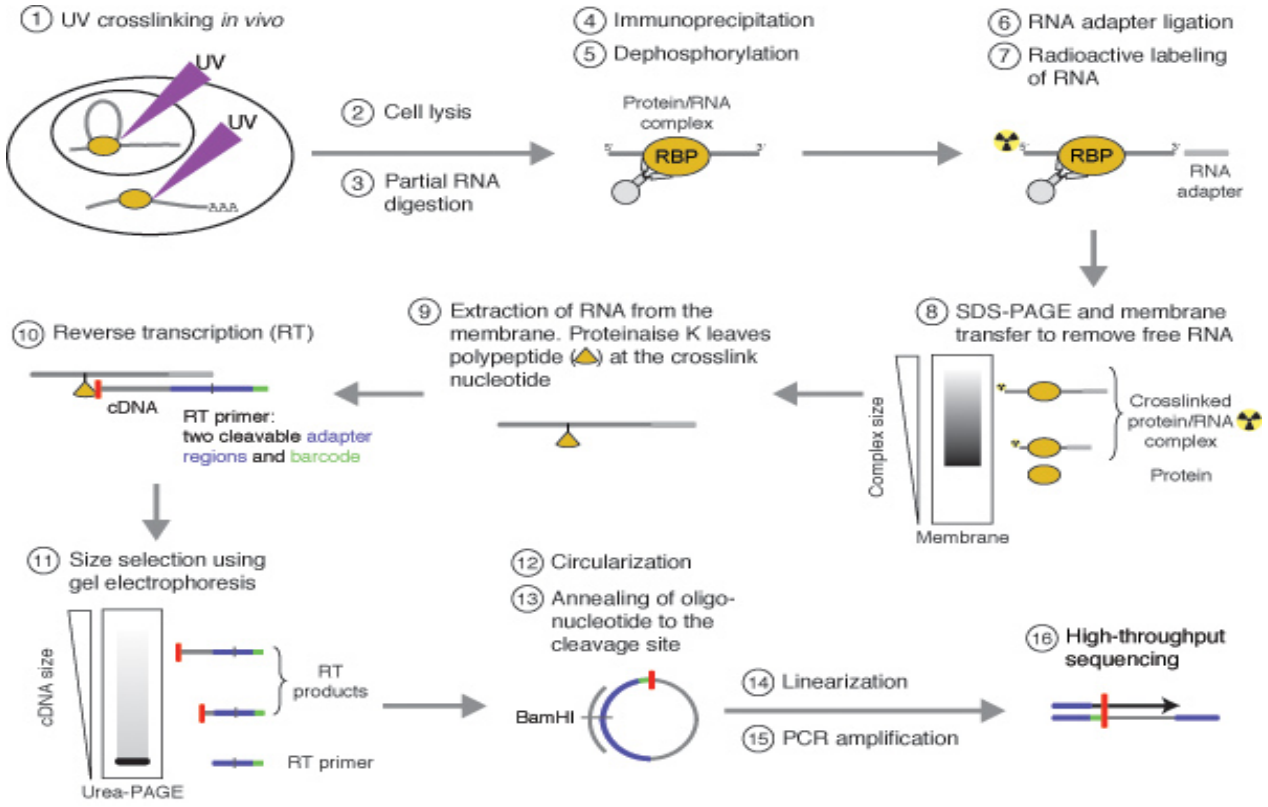
Problem to solve: RNA binding protein binding gene analysis. **We want to study the relationship between multi RBPs and alternative splicing event regulation. The object is to study which RBP enhance gene expression, which RBP repress gene expression.**

The CLIP-seq technique

- First we need to know where RBP bind?

The technique is called CLIP-seq, which locate the target of RBP binding site in the pre-mRNA.

CLIP-seq experiment



CLIP-seq workflow

It is a technique just like the CHIP-seq, whereas work on pre-mRNA.

The goal of the technique is extracting the sequence in the RBP binding sites.

We just need to know that the reads we will see later come from the binding sites.

I will show you the standard protocol later.

Getting data

- Data can be download from Database or come from sequencer directly.

- There are various kinds of database, eg: Encode, modEncode, GEO, SRA, EBI, GTEx, TCGA, 1000 Genome Project, ESP6500. (NCBI)

- Here we use data from Encode project.(SRSF1.web)

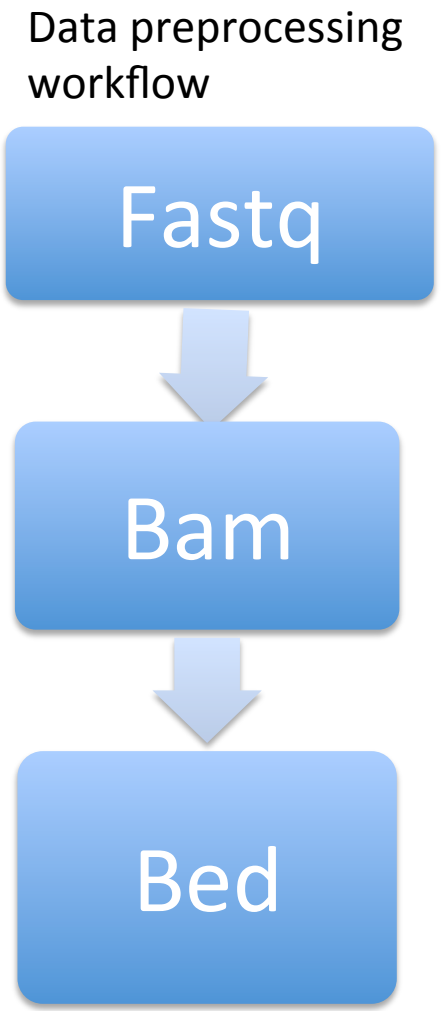
https://www.encodeproject.org/experiments/ENCSR432XUP/

Data format

The download data can be many different formats.

Including: fastq format (original reads),
bam format (alignment file),
bed format (peaks region file).

Here we assume the raw format is fastq format. (UCSC format help: http://genome.ucsc.edu/FAQ/FAQformat.html)



From fastq to bam

- The main command here is **read alignment**.

```
   e.g  bowtie2 [options]* -x <bowtie-index> {-1 read1 -2 read2 |
-U <r>} [-S <sam>]
```

- But there are possibly other command, eg:

1. Demultiplexing (sometimes, multi samples are sequenced once a time, multiplexing is a technique to index them)
2. Cutadaptor (remove the adapters from head or tail of the adaptor)
3. FastQC (quality control)
4. rmRep (remove repetitive elements)

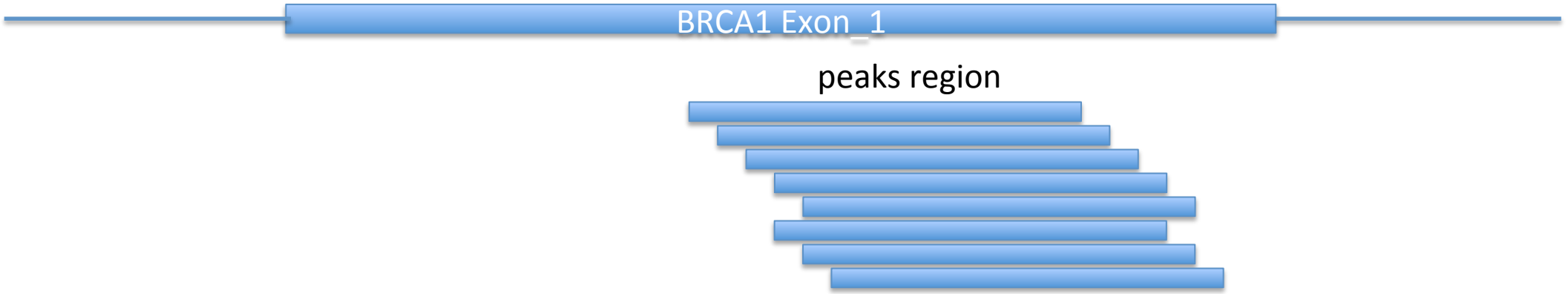
What we got

A bam file looks like below:

```
chr1    13232    33421    ATAAFCFAD   &%dfd2$@$ …
chr2    61323    62121    ATCTTGCGT   *&dfd2)a# …
…..

…
```

The bam file looks like this, it can be view through IGV viewer.



Quality control

- Usually, we need to check the quality of the result bam file.

- The criterion include:
- % of reads mapped to the genome.
- % of reads mapped to the exon region.
- % of reads mapped to the positive strand.
- % of reads mapped to the negative strand.

From bam to bed

- Next we need to call peaks in the bam file.
- The main command is the **Clipper**.

- It also include:
- Samtools sort (sort the bam file by postion)
- Samtools index (index the bam file to do fast retrieve)
- BigbedTobed (conver Bigbed to bed file)

The full pipeline here

- eCLIP_analysisSOP_v1.P.pdf (the computation pipeline)
- eCLIP_SOP_v1.P_110915.pdf. (the experiment pipeline)
- I will show you the standard analysis pipeline in the bam file.

The data we got (SRSF_rep1.bed)

chr1	15212	15250	ENSG00000227232.4_0_3	15	-	-1	-1	0.0292794233248	15230
chr1	16239	16287	ENSG00000227232.4_1_4	19	-	-1	-1	0.0104649442093	16263
chr1	16441	16485	ENSG00000227232.4_2_5	25	-	-1	-1	0.00256249972915	16462
chr1	17451	17517	ENSG00000227232.4_3_10	63	-	-1	-1	4.50322291742e-07	17481
chr1	90235	90280	ENSG00000239945.1_0_4	19	-	-1	-1	0.0105097488596	90256
chr1	90240	90275	ENSG00000238009.2_0_4	20	-	-1	-1	0.00879079737544	90256
chr1	109158	109192	ENSG00000238009.2_1_5	29	-	-1	-1	0.00115927397829	109175
chr1	113824	113881	ENSG00000238009.2_2_8	49	-	-1	-1	1.21214630854e-05	113848
chr1	115708	115784	ENSG00000238009.2_3_22	145	-	-1	-1	2.6562287759e-15	115737
chr1	116372	116428	ENSG00000238009.2_4_13	74	-	-1	-1	3.4175167505e-08	116397
chr1	135196	135226	ENSG00000237683.5_0_3	15	-	-1	-1	0.0270886550409	135213
chr1	135196	135226	ENSG00000268903.1_0_3	15	-	-1	-1	0.0274502908066	135213

Each row is a peak region.

Column 9 is the p-value.

Column 4 is the Ensembl gene id.

Basic quality control

- Sometimes, we need to check the file to ensure the file we got is right, this can be done by basic linux command.

- Some possible criterion is:
- The number of peaks in the files.
- How many chromosomes in the file.
- The number of peaks in the chromosome 1.
- The p-values of the peaks.

Most useful Linux command

```
#Count how many peaks in the file:
wc –l SRSF1_rep1.bed;

#Count how many peaks in chr1:
cut –f 1 SRSF1_rep1.bed | grep chr1 | wc –l;

#Get the p-value column:
cut –f 9 SRSF1_rep1.bed > SRSF1_rep1_pvalue.txt
```

Most useful Linux command

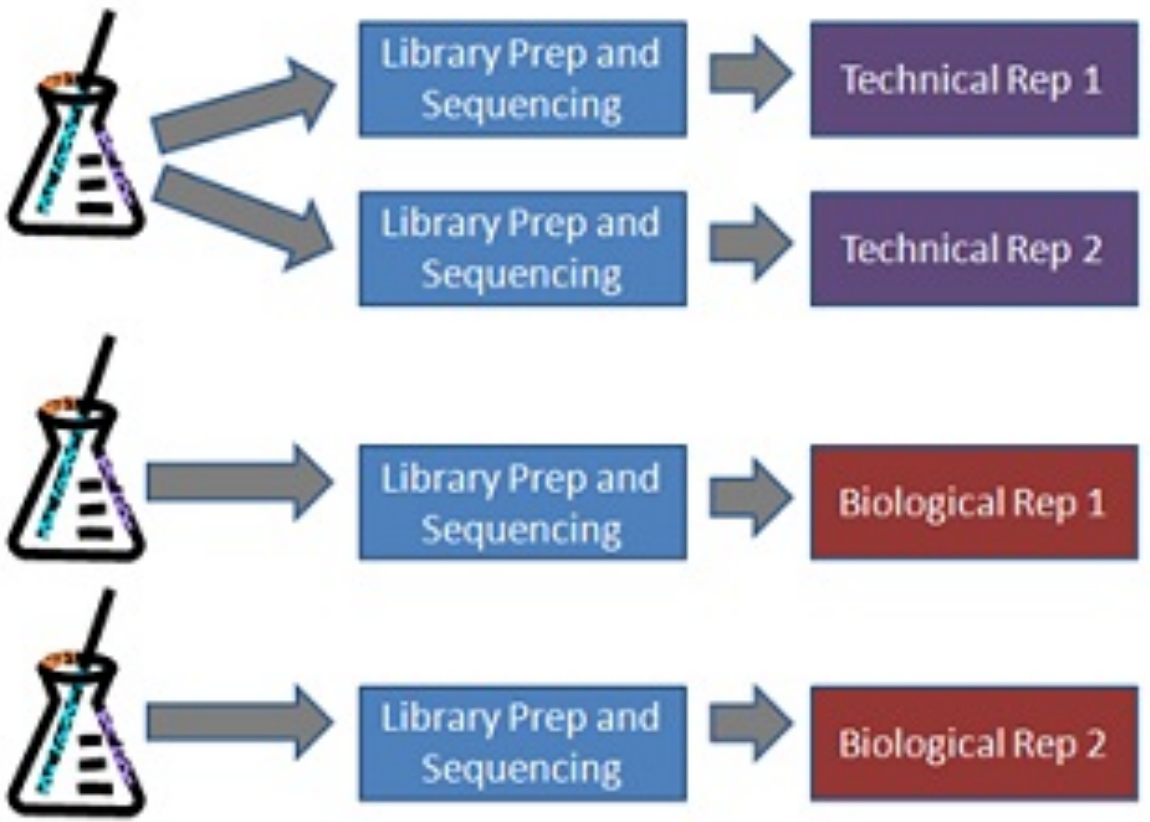
```
#how many chromosome in it
cut –f 1 SRSF1_rep1.bed | sort –u | wc –l

#remove the ‘chr’ in each line
cut –c 4- SRSF1_rep1.bed >SRSF1_rep1_noChr.bed

#count the peaks in each of the file and output to a new file
for i in `ls *.bed`
do
   `wc –l $i >> peaks_for_each_file.txt`;
done;
```

Biology replicate

- Usually we use at least one biological replicate to ensure the peaks we found are not due to variance.



Overlap of two biological replicates

- One possible solution is finding the overlap regions between the two biology replicates, and treat the overlapped region as peaks.

- But how to find the overlap region between two bed files.

The two bed files

Bed_rep_1

chr1	14924	14954	ENSG00000227232.4_0_3	15	-	-1	-1	0.0286089808565	14938
chr1	17454	17567	ENSG00000227232.4_1_15	95	-	-1	-1	2.52804288143e-10	17488
chr1	89861	89871	ENSG00000238009.2_0_3	16	-	-1	-1	0.020756022461	89862
chr1	89861	89871	ENSG00000239945.1_0_3	16	-	-1	-1	0.0211020769049	89862
chr1	89871	89904	ENSG00000238009.2_1_3	16	-	-1	-1	0.0246792521386	89885
chr1	89871	89904	ENSG00000239945.1_1_3	15	-	-1	-1	0.0258746724881	89885

Bed_rep_2

chr1	15212	15250	ENSG00000227232.4_0_3	15	-	-1	-1	0.0292794233248	15230
chr1	16239	16287	ENSG00000227232.4_1_4	19	-	-1	-1	0.0104649442093	16263
chr1	16441	16485	ENSG00000227232.4_2_5	25	-	-1	-1	0.00256249972915	16462
chr1	17451	17517	ENSG00000227232.4_3_10	63	-	-1	-1	4.50322291742e-07	17481
chr1	90235	90280	ENSG00000239945.1_0_4	19	-	-1	-1	0.0105097488596	90256
chr1	90240	90275	ENSG00000238009.2_0_4	20	-	-1	-1	0.00879079737544	90256

The general problem of overlapping

Here we need to find the overlap between the two bed files, actually sometimes we also need to overlap with different kinds of files.

The most common file formats we encountered are:

BAM, BED, GTF, VCF

They are all interval range formats, i.e. they all contain a chromosome and at least a start position, a **generic solution to this problem** is need.

Find overlap of biology replicates

- R package GenomicRanges:
- Bed file is table range format, also some other file formats like VCF, GFF, GTF. We need efficient methods to overlap between these kinds of files.
- 1. One Linux solution is using **Tabix**, while a java solution a **tribble**.
- 2. A more powerful solution is using R package **GenomicRanges**. It can efficient map between ranges format files. GenomicRangesIntroduction.pdf

VCF, BED, GTF file format

- I will show you the above VCF, BED, GTF file formats in UCSC, and show that they are all the general chromosome, coordinate format.

- https://genome.ucsc.edu/FAQ/FAQformat.html#format10.1

R package GenomicRanges

First converting Bed, VCF, GFF, GTF to **GenomicRange** objects. Reference:

Codes:

```
#First read bed file into table biology_rep_x_b
SRSF1_rep_1_range<-with(SRSF1_rep_1_bed,Granges(seqnames=chr,
+ranges=IRanges(start=start+1,end=end),strand=strand)


SRSF1_rep_2_range<-with(SRSF1_rep_2_bed,Granges(seqnames=chr,
+ranges=IRanges(start=start+1,end=end),strand=strand)
```

Bed format is 0-based and its left coordinate is inclusive, while the right coordinate is exclusive

R package GenomicRanges

Find the peaks which overlap between the two biology replicates.

```
Codes:
SRSF1_overlap_mch<-findoverlaps(SRSF1_rep_1_range, SRSF1_rep_2_range);

SRSF1_rep_1_overlap<-
SRSF1_rep_1_range[unique(queryHits(SRSF1_overlap_mch)),];

SRSF1_rep_2_overlap<-
SRSF1_rep_2_range[unique(subjectHits(SRSF1_overlap_mch)),]
```

what the overlap result looks like

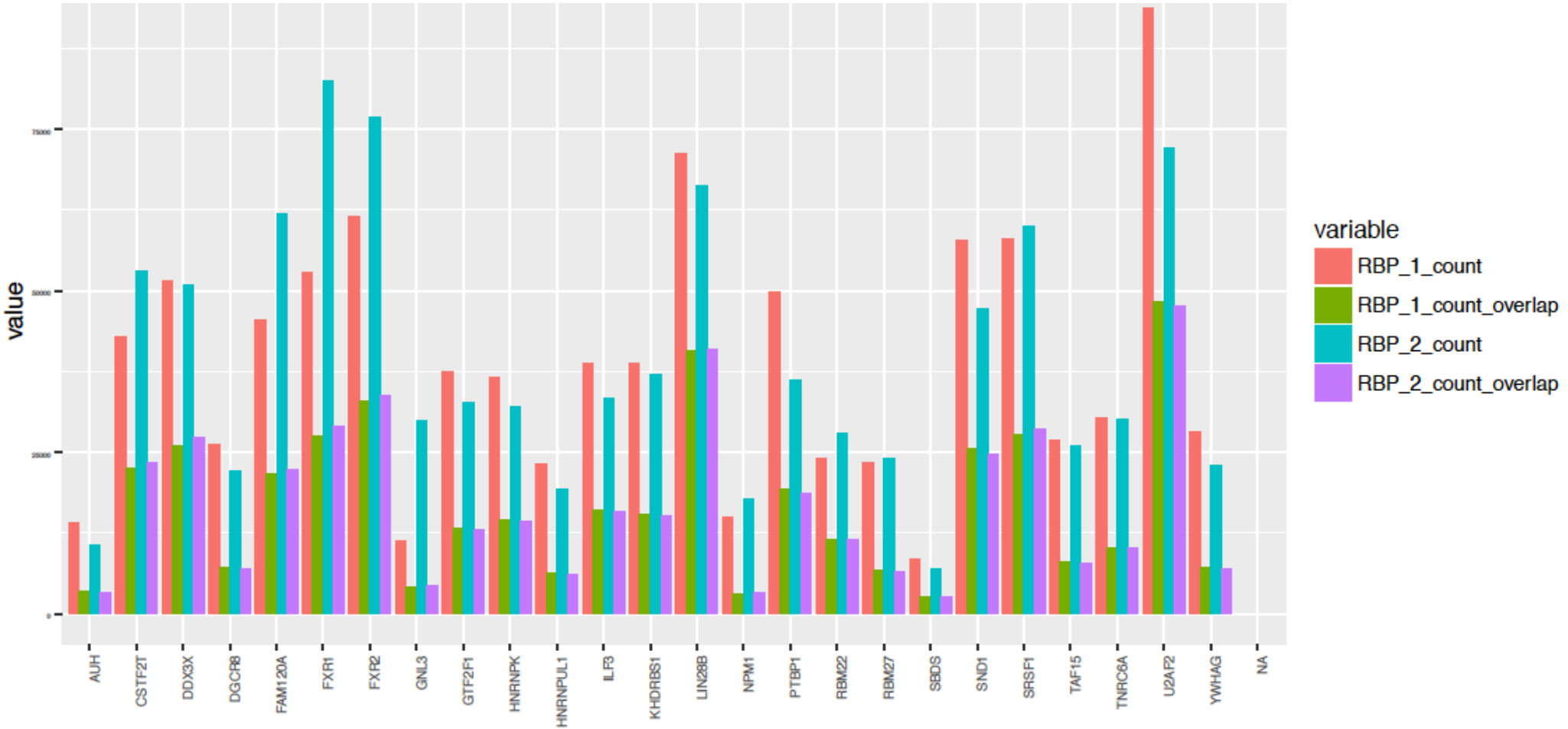
```
#RBP_overlap_mtch is revord the line number of each overlap.
>RBP_overlap_mtch
      queryHits      subjectHits
 [1,]         2                1
 [2,]         3                1
 [3,]         4                1
 [4,]         5                2
 [5,]         6                2
```

What we got currently

What we got is the overlapped peak region between the two biological replicates.

chr1	1212	1321
chr2	1312	1521
chr1	2212	3321
…		

How many peaks are overlapped between the two biological replicates



R package GenomicRanges

Besides overlap between two genome interval regions. GenomicRanges also include many additional functions. These include:

```
Set operations: ()
  union, setdiff, intersect;
Interval operation: (resize the interval, change the flank region)
  resize, flank, width,shift;
Overlp operations: (overlap with a region)
  countOverlaps,subsetByOverlaps,findOverlaps;
Split operations:split, c(); split a GenomicRanges into multi ones.
Basic operations:tail,head,rev; get subset of the genomicRanges
```

Multi RBPs CLIP-seq bed files

Sometimes, we need to analysis two or more RBPs to study their interaction. We need **GRangesList** structure. Here we take HNRNPK as example.

```
HNRNPK_rep_1_overlap<-
HNRNPK_rep_1_range[unique(queryHits(HNRNPK_overlap_mch)),];

RBPs_range_list<-GRangesList<-
C(SRSF1_rep_1_overlap, HNRNPK_rep_1_overlap);
```

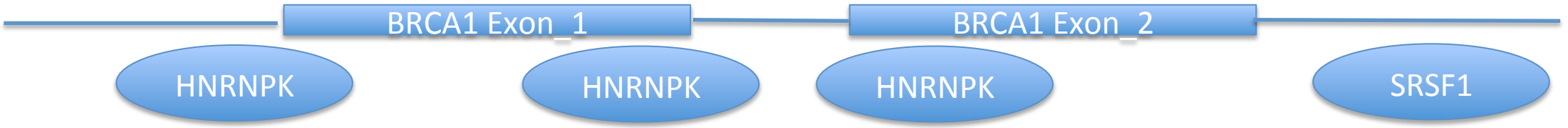
Count RBP binding sites in each gene

Currently we have two RBPs (SRSF1, HNRNPK) in a GRangeList object. Next, we want to count RBP binding sites in each transcript. We need human genome annotation, here we use hg19. GTF which can be download from UCSC table Browser. File: Table Browser

GTF is 1-based system and both the left and right coordinate are inclusive.

1	pseudogene	gene11869		14412	.	+	.	gene_id "ENSG00000223972";
2	processed_transcript	transcript11869		14409	.	+	.	gene_id
……								
……								

Count RBP binding sites in each gene



Example: BRCA1 contain 3 HNRNPK binding sites and 1 SRSF1 binding sites.

```
Codes: gRBP_frame<-read.table(“hg19_ens.gRBP”,header=F,as.is=T);

gRBP_range<-with(gRBP_frame,Granges(seqnames=chr,
+ranges=IRanges(start=start,end=end),strand=strand)
```

Overlap with RBPs binding sites and genome annotation

Next we overlap the **RBPs_range_list** with **gRBP_range** to get number of RBPs in each gene.

Codes:

```
GeneName_RBPName<-data.frame(gene_name,RBP_name)
for(i in 1:2){
RBP_overlap_mtch<-
findOverlaps(RBPs_range_list[[i]], gRBP_range);

RBP_gRBP_overlap<-
RBPs_range_list[[i]][queryHits(RBP_overlap_mtch),];

GRBP_RBP_overlap<-
gRBP_range[[1]][subjectHits(RBP_overlap_mtch),];
```

Overlap with RBPs binding sites and genome annotation

```
Code:
gene_names<-seqnames(GRBP_RBP_overlap);
RBP_names<-names(RBPs_range_list)[i];
GeneName_RBPName<-
cbind(GeneName_RBPName,c(gene_names, RBP_names) );
}

>GeneName_RBPName
#each line represent a overlap
    Gene_names  RBP_names
    TP53        SFRS1
    TP53        SFRS1
    TP53        HNRNPK
```

Bioconductor

- R package GenomicRanges is part of Bioconductor project, which contains many packages related to bioinformatics, including NGS analysis, microarray analysis and so on.
- Besides the **GenomicRanges**, another general use package I found very usefull is the **BSGenome**, which provide different annotation for many genomes.
- There are also other packages in it which target on different aspect, like Chip-seq, RNA-seq (EdgeR) and so on.
- I list two papers which describe the bioconductor packages in the supplement files.

R Package reshape2

Next we need to do transformation to count each gene's binding sites.

Here, we first introduce the **long table format** and **short table format**.

Long table and short table

Short Table format

Gene_name	RBP_name
TP53	HNRNPK
TP53	HNRNPK
BRCA1	SRSF1
TP53	HNRNPK
TP53	SRSF1

As you can see,
The data in the short table is redundant. I use more cell to store the same data.

Long Table format

Gene_name	HNRNPK	SRSF1
TP53	xxx	xxx
BRCA1	xxx	xxx

Column name become header

R package reshape2

R package reshape contain two fundamental function:

melt: Convert **long table to short table**.

cast: Convert **short table to long table**.

R package reshape2

```
>GeneName_RBPName
#each line represent a overlap
   Gene_names     RBP_names
   TP53           SFRS1
   TP53           SFRS1
   TP53           HNRNPK
```

Codes:

```
GeneName_RBPName_cast<-
Cast(GeneName_RBPName_melt,Gene_names~RBP_names,length)
#Gene_names~RBP_names is folumar, where the left side is #row id and
left side is column label
#Where length is the function to count.
```

R package reshape2

```
>GeneName_RBPName_cast

Gene_name      HNRNPK      SRSF1
TP53           3           1
BRCA1          1           3

#Now we have the table we need, we got each gene’s RBP #binding sites
for each RBP.
#
#
#
#
#
```

From table to figure

Humans can't read multi data at same time, we need visulization method to check the dataset.

We now have the table, we want to represent it using figures to do visualization. Maybe it not very useful for small table, but for large table, it means a lot.

We need R package ggplot2 to convert table to figure.

R package ggplot2

ggplot2 receive **short table format** data and convert it into **statistic figures**. Its main advantage is productive and high versatile. It make use of **graphic grammer.**

```
Codes:
#ggplot2 receive short table format, we need to melt the #table.

>GeneName_RBPName_cast

Gene_name     HNRNPK     SRSF1
TP53             3          1
BRCA1            1          3
```

melt table to short table format

```
Codes:
GeneName_RBPName_melt<-
melt(GeneName_RBPName_cast);

>GeneName_RBPName_melt

Gene_name       RBP_name        count
TP53            HNRNPK             3
TP53            SRSF1           1
BRCA1           HNRNPK             1
BRCA1           SRSF1           3
```

Give some ggplot figure example

Codes:

```
#count the number RBP binding in each gene’s exon region.

GeneName_RBPName_point2<-ggplot(se_target_data_melt)+
   geom_point( aes(x=gene_name,y=value,color=variable) )

GeneName_RBPName_bar<-ggplot(se_target_data_melt)+
geom_bar( aes(x=gene_name,y=value,fill=variable),stat="identity",posit
ion="dodge" )+

GeneName_RBPName_tile<-ggplot(se_target_data_melt)+
   geom_tile( aes(x=gene_name,y=variable, fill=value) )

print(GeneName_RBPName_p1);
```

Figure_1

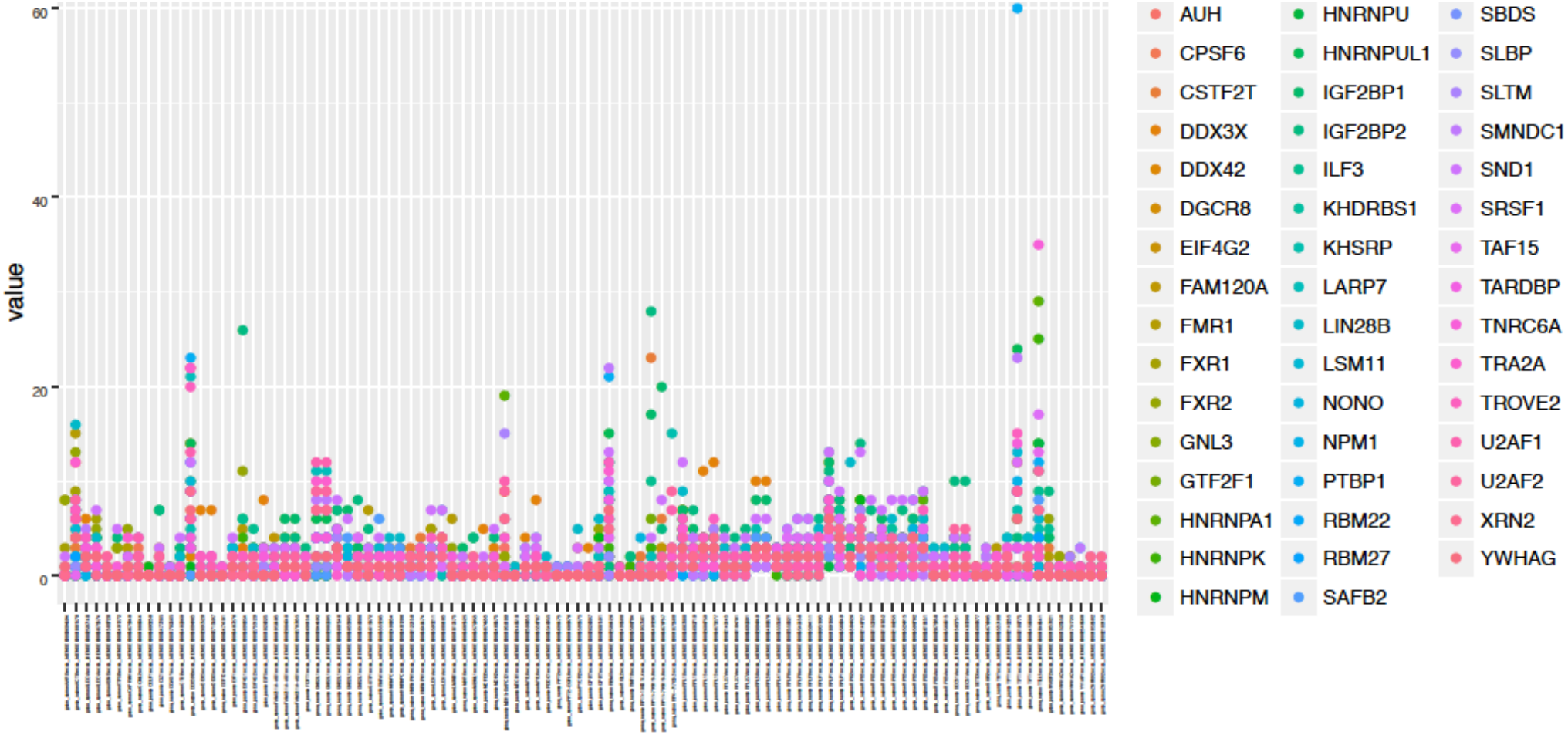

value
60
40
20
0
AUH
CPSF6
CSTF2T
DDX3X
DDX42
DGCR8
EIF4G2
FAM120A
FMR1
FXR1
FXR2
GNL3
GTF2F1
HNRNPA1
HNRNPK
HNRNPM
HNRNPU
HNRNPUL1
IGF2BP1
IGF2BP2
ILF3
KHDRBS1
KHSRP
LARP7
LIN28B
LSM11
NONO
NPM1
PTBP1
RBM22
RBM27
SAFB2
SBDS
SLBP
SLTM
SMNDC1
SND1
SRSF1
TAF15
TARDBP
TNRC6A
TRA2A
TROVE2
U2AF1
U2AF2
XRN2
YWHAG

Figure_2

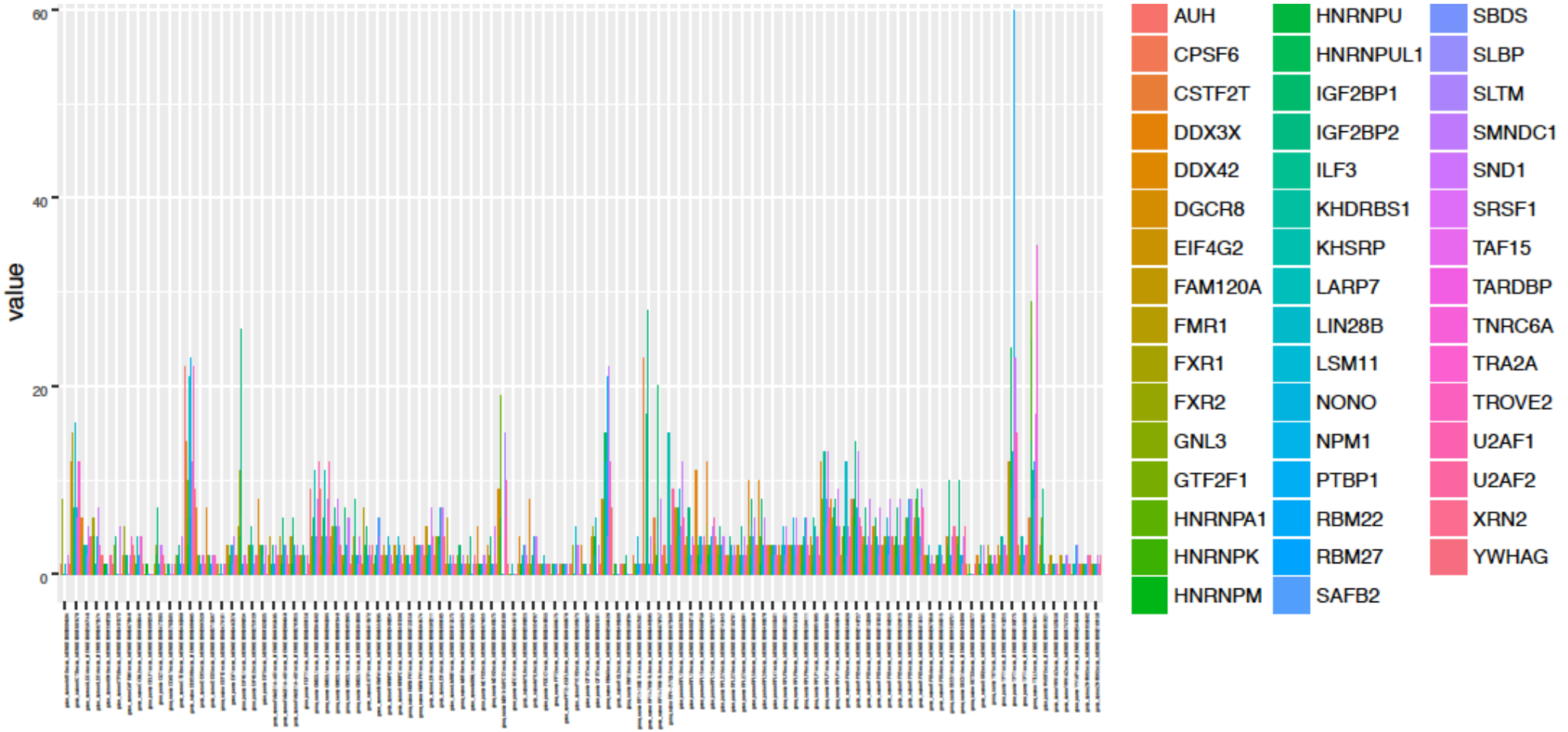
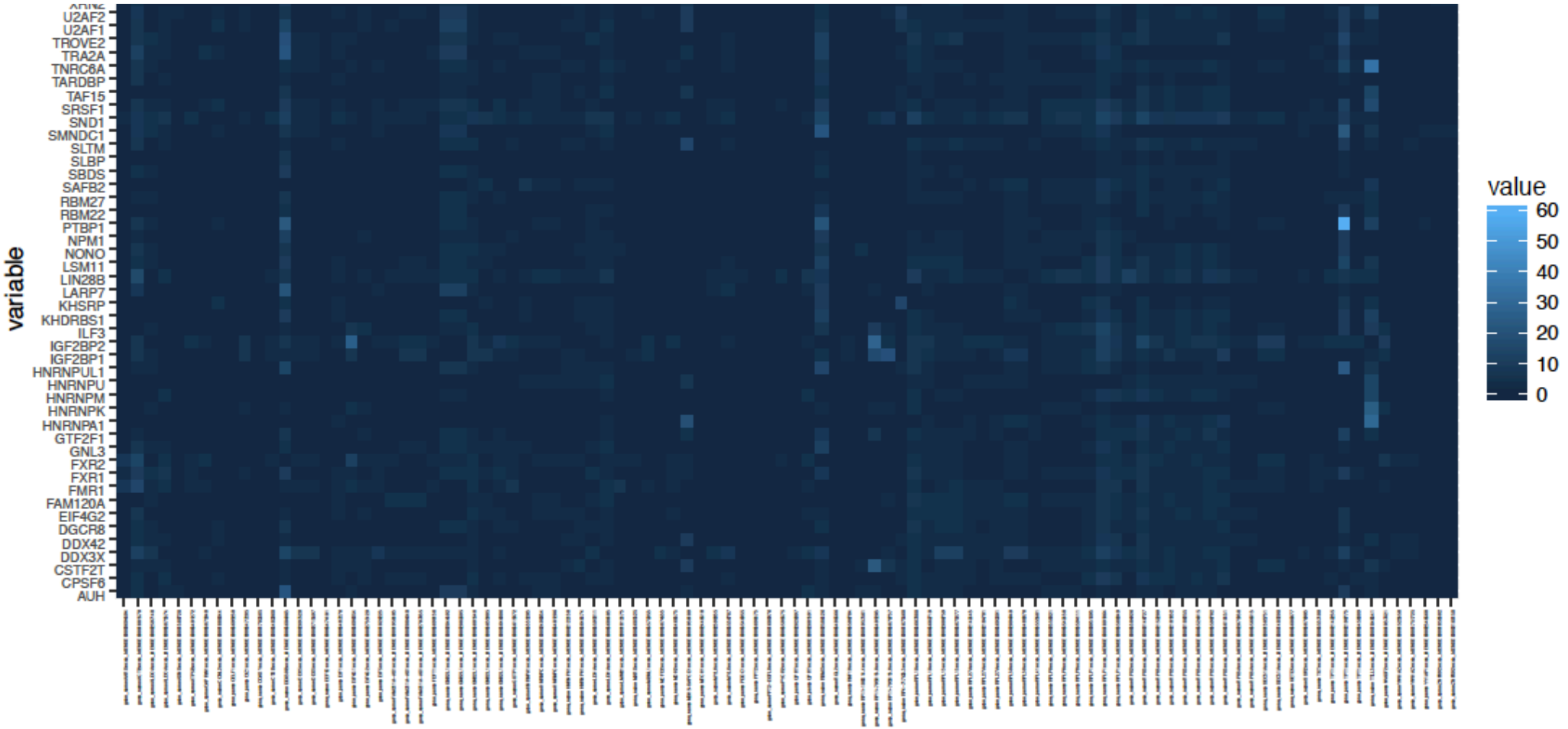

value
60
40
20
0
AUH
CPSF6
CSTF2T
DDX3X
DDX42
DGCR8
EIF4G2
FAM120A
FMR1
FXR1
FXR2
GNL3
GTF2F1
HNRNPA1
HNRNPK
HNRNPM
HNRNPU
HNRNPUL1
IGF2BP1
IGF2BP2
ILF3
KHDRBS1
KHSRP
LARP7
LIN28B
LSM11
NONO
NPM1
PTBP1
RBM22
RBM27
SAFB2
SBDS
SLBP
SLTM
SMNDC1
SND1
SRSF1
TAF15
TARDBP
TNRC6A
TRA2A
TROVE2
U2AF1
U2AF2
XRN2
YWHAG

Figure_3


variable
U2AF2
U2AF1
TROVE2
TRA2A
TNRC6A
TARDBP
TAF15
SRSF1
SND1
SMNDC1
SLTM
SLBP
SBDS
SAFB2
RBM27
RBM22
PTBP1
NPM1
NONO
LSM11
LIN28B
LARP7
KHSRP
KHDRBS1
ILF3
IGF2BP2
IGF2BP1
HNRNPUL1
HNRNPU
HNRNPM
HNRNPK
HNRNPA1
GTF2F1
GNL3
FXR2
FXR1
FMR1
FAM120A
EIF4G2
DGCR8
DDX42
DDX3X
CSTF2T
CPSF6
AUH
value
60
50
40
30
20
10
0

Which one is better?

- **I will show you the raw PDF here**, which is much clear than this one.
- Scatter plot is very easy to check which gene contain highest count, but if two RBP has same count in the gene, they will overlap. So scatter plot is wrong.
- Bar plot avoid the overlap problem by putting each point beside each other, but the 47 colors is very hard to distinguish.
- So I think the best one is the heatmap.

R package ggplot2

You can see the power of ggplot2. only change a few cods can do different kinds of plots, very efficient and productivity.

R package ggplot2

ggplot is multi layers figure, each '+' add a layer to the final figure.

aes: map variable to figure variable.

geom: The figure type, eg. Bar_plot, histogram, scatter plot.

stat: the statistic transformation, eg. bin, identity.

R package ggplot2

- I list all the figure types that support by ggplot2.

  Histogram, boxplot, barplot, contour, density, scatter, tileplot, hex plot, violin, pieplot, line and so on.

- If you want to combine two or ggplot2 object in one figure, you may need R function multiplot.r, which can be search and got from web.

R package ggplot2

- Besides productivity, ggplot is very versatile.
- These include:
- geom_xx (different kinds of figures)
- stat_xx (different kinds of statistics transformation)
- Scales (change the scales like color, legend, fill et.al.)
- Coordiante (cartesian, polar,flip,trans)
- Faceting (multi plots in one figure)
- Position adjustments (dodge, fill, identity, stack, jitter)
- Annotation (custom, logticks, map, raste)
- Fortify
- Themes (like font, size, label orientation et.al.)
- Aesthetics (map from variable to plot)
- Others ()

Calculate number of gene for each RBP

We want to summarize which RBP binds most genes.

```
>GeneName_RBPName
#each line represent a overlap
   Gene_names     RBP_names
   TP53           SRSF1
   TP53           SRSF1
   TP53           HNRNPK
```

Generalize the problem

The generalize of the problem is split-aggregate problem, what we want is split the data.frame into muliti small ones, and do some operation on each group.

There is a R package called ‘plyr’ which can be used to do these kinds of job.

The ‘for’ loop equivalent

R function :

sapply: apply a function to each element in list.

apply: apply a function to row or column.

lapply: apply a function to each element in list.

R package plyr

It includes many functions:

Where d means table, l means list, _ means nothing,a means vector, so:

d_ply means input is data.frame, output nothing.

ddply means input is data.frame, output data.frame.

dlply means input is data.frame, output list.

daply means input is data.frame, output vector.

R package plyr

R package plyr is a more powerful solution for this kind of analysis. **It group the table and apply a function.(also called split+modify+aggregate)**

For our problem, we need to treat each RBP a separate group and count the gene in each group.

R package plyr

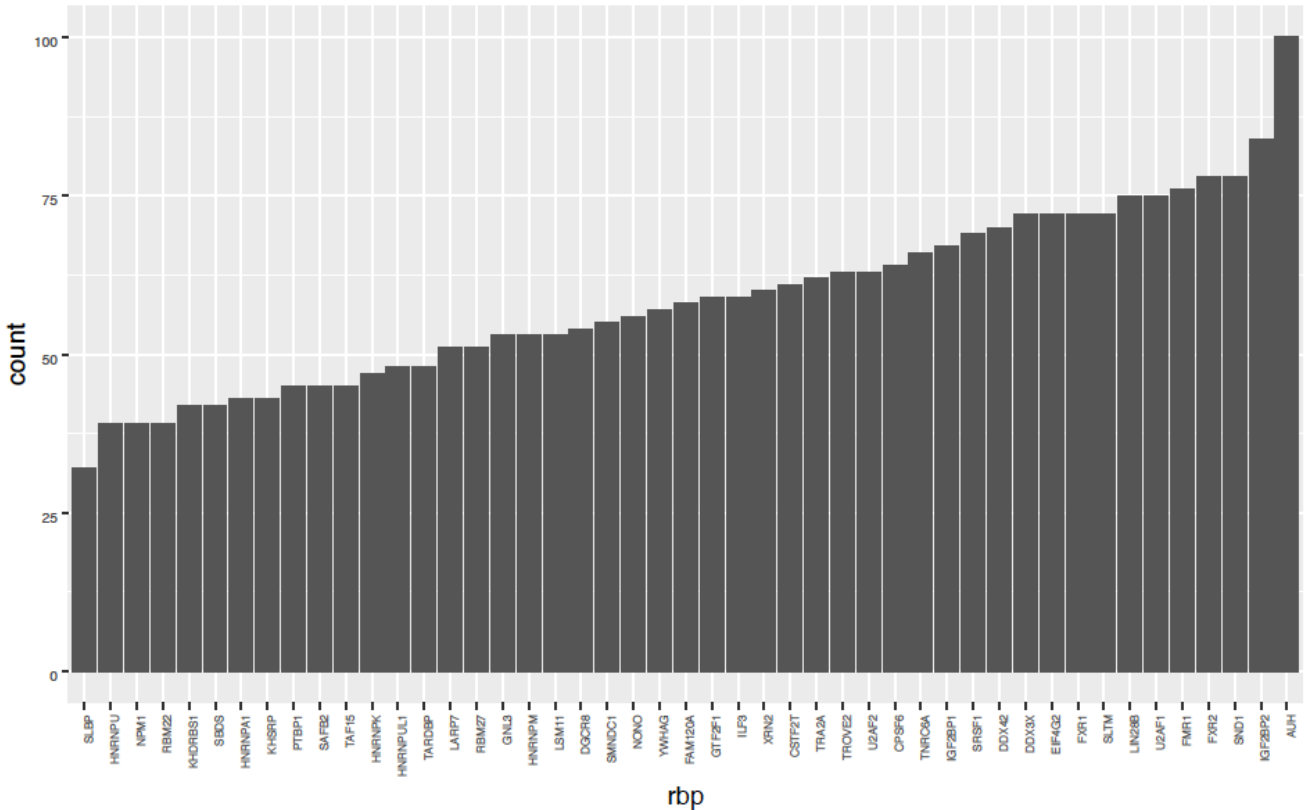
```
RBP_bind_fre<-ddply(GeneName_RBPName,.(RBP_names),nrow)
#each line represent a overlap
   Gene_names     RBP_names
   TP53           SRSF1
   TP53           SRSF1
   TP53           HNRNPK


>RBP_bind_fre
RBP_names         Var1
SRSF1          2
HNRNPK            2
```

Calculate number of gene for each RBP

With a little ggplot2, we can do the below figure.

```
rbp_gene_count_plot<-ggplot(rbp_gene_count)+
  geom_bar(aes(x=rbp,y=count),stat="identity")+
```



R package plyr

- Besides group analysis, R package plyr can also do .
- 1. data.frame operations, like rbind.fill, progress bar,
- 2.generalized for loop, executing a function multi times and return something (like data.frame).

Add gene expression

Next, we want to add gene expression information into our analysis. We want to the correlation between gene expression and RBP binding information

Here, we use **NGSUtils** to calculate each sample's FPKM, we have 115 samples and we need to write a small **pipeline** to do it.

How to write linux pipeline

A lot of language can be used to write pipeline, here, I use bash shell, I think write pipeline in **bash shell** is a nature way (bash.quickref.pdf).

How to write pipeline

```
for i in `ls –d dirContainBAMFiles`
do
  echo $i;
  sample=`basename $i`;
  my i_dir=${i/.bam};
  mkdir $i_dir;

  samtools sort $i $i.sort;
  samtools index $i.sort;

  script="bamutils count –gRBP Homo_sapiens.GRCh37.75_4.gRBP -rpkm -norm all $i.sort >
$sample.count";
  echo $script > $sample.count.job;

  `$script`
  #qsub -l nodes=1:ppn=1,walltime=4:00:00,mem=4gb -M li487@iupui.edu -d /N/dc2/scratch/liulab/
limeng/RBP_network/ -m ae -N $sample.count $sample.count.job
```

Process Gene expression

For each sample, we got a gene expression table.

#gene	geneid	isoid	chrom	strand	txstart	txend	length	count	count (CPM)	RPKM
WASH7P	ENSG00000227232	ENSG00000227232	chr1	-	14362	29806	2073	309	1.76326905472	0.850588063058
DDX11L1	ENSG00000223972	ENSG00000223972	chr1	+	11868	14412	1756	41	0.23396126616	0.133235345194
MIR1302-10	ENSG00000243485	ENSG00000243485	chr1	+	29553	31109	1021	65	0.370914202449	0.363285212977
FAM138A	ENSG00000237613	ENSG00000237613	chr1	-	34553	36081	1219	224	1.27822740536	1.04858687889
OR4G4P	ENSG00000268020	ENSG00000268020	chr1	+	52472	54936	947	10	0.0570637234537	0.0602573637315
OR4G11P	ENSG00000240361	ENSG00000240361	chr1	+	62947	63887	940	0	0.0	0.0
OR4F5	ENSG00000186092	ENSG00000186092	chr1	+	69090	70008	918	3	0.0171191170361	0.0186482756385
RP11-34P13.7	ENSG00000238009	ENSG00000238009	chr1	-	89294	133566	3569	2050	11.698063308	3.2776865531
RP11-34P13.8	ENSG00000239945	ENSG00000239945	chr1	-	89550	91105	1319	561	3.20127488575	2.42704691869

Process Gene expression

Next, we need to combine multi sample's gene expression into one table. This can be done in multi ways since each file is already ordered by the gene name, including using R read each file. Use linux command cut and paste.

X.gene	ENCFF000DV	ENCFF000DV	ENCFF000DV	ENCFF000DV	ENCFF000DY	ENCFF000DY
WASH7P	148.049887	167.275891	401.776058	428.998374	19.3728753	68.0487607
DDX11L1	2.33384883	2.42390186	0	0.61342398	1.11666424	0.25012929
MIR1302-10	0.90201027	1.44107576	0.36055293	2.11003431	3.88415134	2.11511547
FAM138A	0.83104818	1.16389709	0	1.59057468	0.2938757	0
OR4G4P	0.09724947	0	0	0	0.11945795	0.23190445
OR4G11P	0	0	0	0	0.00668597	0
OR4F5	0	0	0	0	0.02738482	0
RP11-34P13.	25.8042164	24.1905387	83.6506025	74.306493	2.14483193	4.22530905
RP11-34P13.	6.07451601	8.4458933	0	0	2.5301266	0.38850003
RP11-34P13.	59.1633562	73.6970671	155.569658	144.734635	11.2270541	13.258401
RP11-34P13.	30.8065091	33.6749976	81.0133226	75.8572195	15.2915755	4.76860331
RP11-34P13.	32.7517539	46.144623	30.7176767	29.3904024	14.7089653	1.35743453
CICP27	0.06039825	0.01378484	0.19313984	0	1.07329875	1.67072191
AL627309.1	16.6470554	22.1368215	17.3617548	16.4348757	8.526187	1.07289579

Gene expression quality control

Usually, before we do a experiment, we need to validate the result we got and the feasibility of next step. (quality control)

Here we use heatmap and boxplot to do quality control.

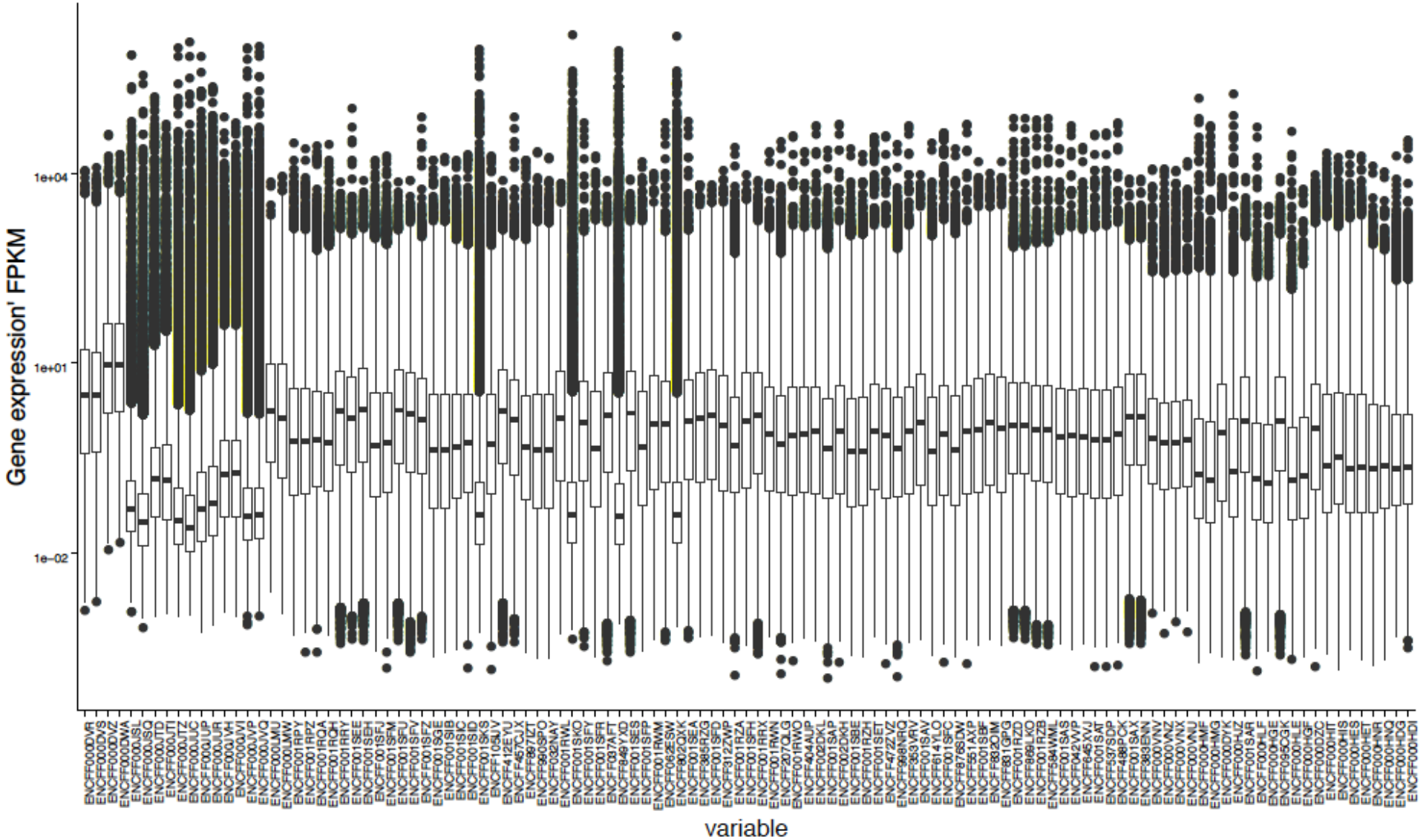
Box Plot code

```
gene_expression_tbl<-
read.table("gene.expression.tsv",sep="\t",as.is=TRUE);

#ggplot receive short format table
gene_expression_tbl_melt<-melt(gene_expression_tbl);

gene_exp_box_plot<-ggplot(gene_expression_tbl_melt)+
 #geom_boxplot are used to plot box plot.
  geom_boxplot(aes(x=variable, y=value  ) ) +
  theme_classic()+scale_y_log10();
```

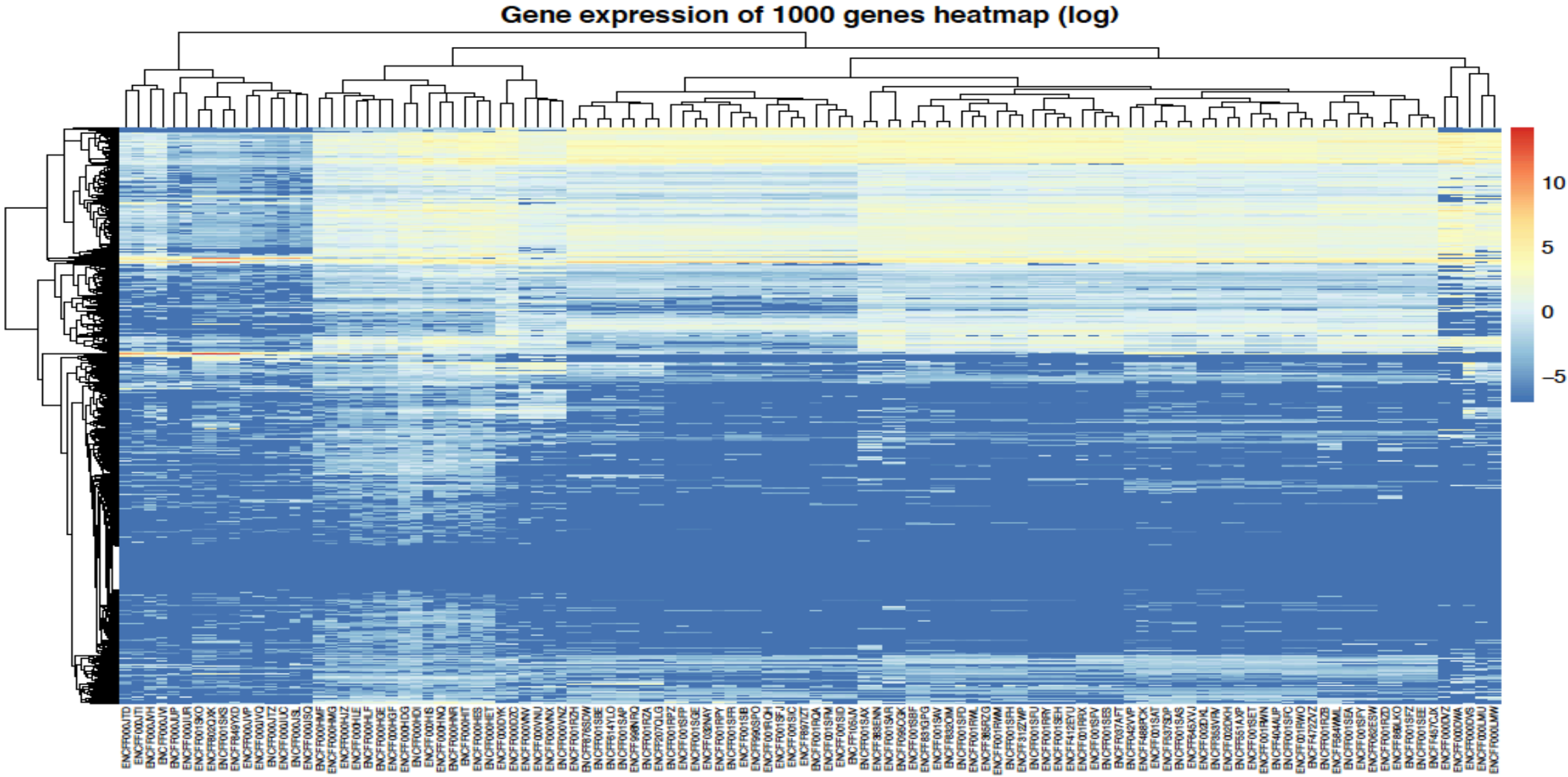
Figure



heatmap

A more generalize way to check gene expression is using heatmap, however, ggplot2 doesn’t provide this, here I use R package ‘pheatmap’.

Figure



What we can see from the figures?

- 1. Seems the there are two different groups of samples, although they come from only one cell line ‘K562’.

- 2.

Gene Expression

Now we take the median of gene expression among samples.

```
And the gene expression.
Gene_name   Sample1  Sample2  Sample3  …
TP53        113.0    313      112
BRCA1       313      113.1    132
…...
```

```
And the gene expression.
Gene_name   Median(FPKM)   …
TP53        213.4
BRCA1       313.5
…...
```

Combine the RBP and gene expression table

```
Now we have a table contain RBP binding information.
Gene_name   SRSF1    BX1
TP53        1        3
BRCA1       3        1
…...
```

```
And the gene expression.
Gene_name   Median(FPKM)   …
TP53        213.4
BRCA1       313.5
…...
```

Generalize of the problem

- Combine two tables is a daily task for SQL, and it include many kinds of operations, like left_join, inner_join, right_join and full_join.
- Left_join: keep all the data in left table in the result table.
- Right_join: keep all the data in the right table in the result table.
- Inner_join: keep the data in both the tables.
- Full_join:keep the data in either the table.

R package dplyr

Here we use R package dplyr. Table manupilation language. A very powerful tool to process tables in R. It implement the above join operations in R, and make multi table analysis more easily.

R package dplyr

Its function include:

filter – select a subset of the rows of a data frame.

arrange – works similarly to filter, except that instead of filtering or selecting rows, it reorders them.

select – select columns of a data frame.

mutate – add new columns to a data frame that are functions of existing columns.

summarize – summarize values.

group_by – describe how to break a data frame into groups of rows.

SQL JOIN – sql style joining multi tables together.

Join two tables

```
>geneName_RBPName_cast
Gene_name    SRSF1    BX1
TP53         1            3
BRCA1        3            1


>gene_expression_tbl_melt
Gene_name    Median(FPKM)…
TP53         213.4
BRCA1        313.5


Gene_RBP_exp_join<-
inner_join(geneName_RBPName_cast,
gene_expression_tbl_melt,by=c(“Gene_name”=“Gene_name”));
```

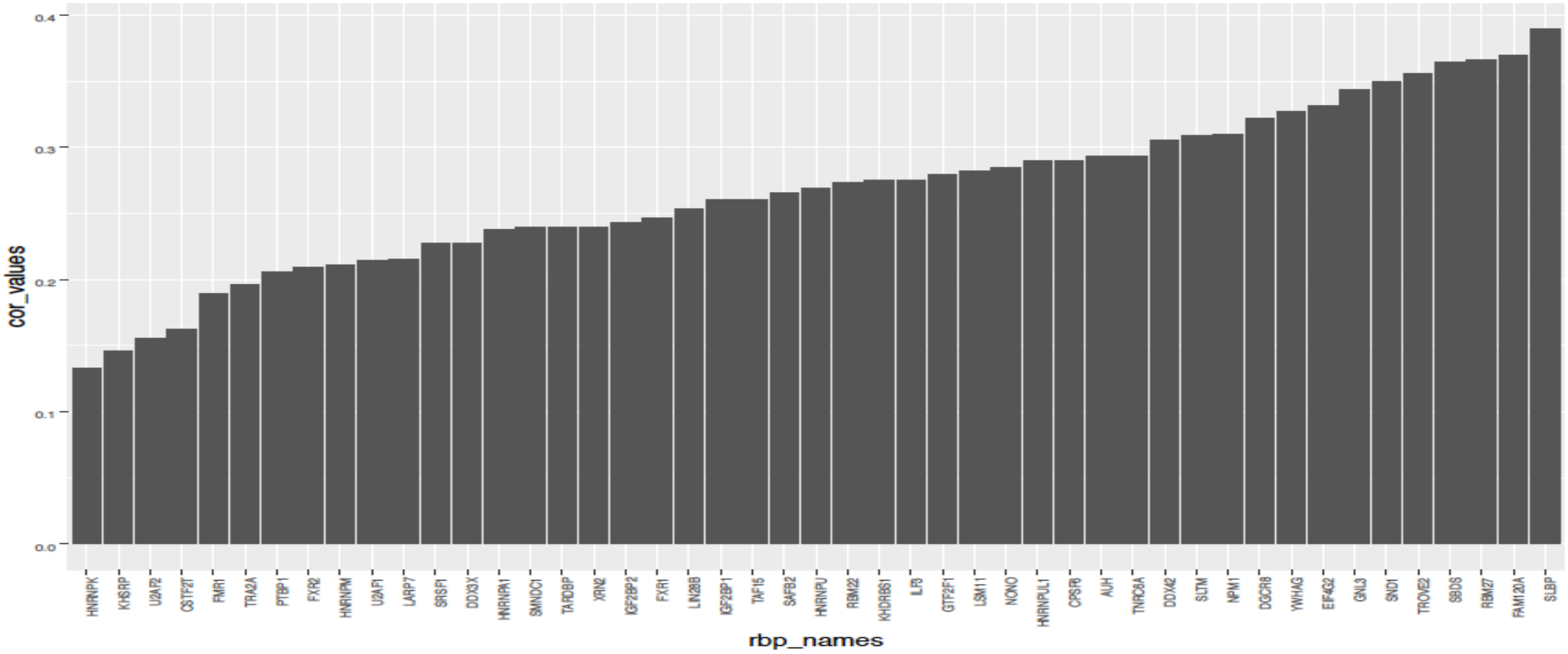
Join two tables

Gene_name	Median(FPKM)	SRSF1	BX1
TP53	213.4	1	3
BRCA1	313.5	3	1
…..			

Now we have all the data in one table.

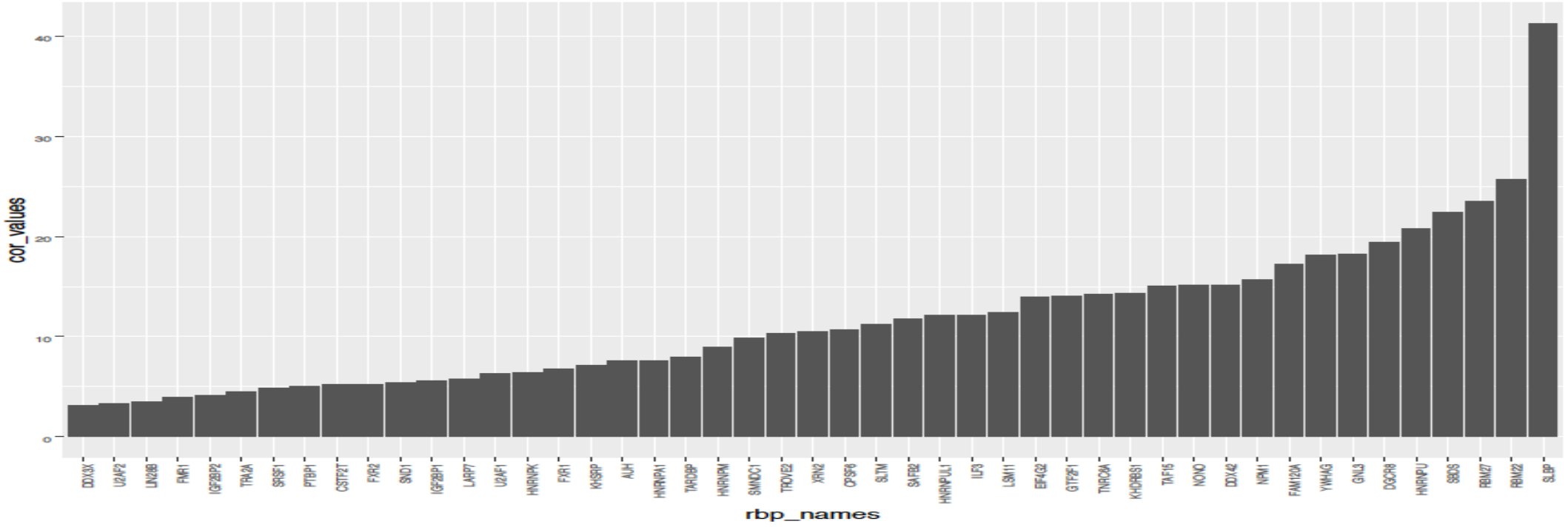
Correlation of RBP and Gene expression

```
With a little ggplot:
rbp_cor_values_data_plot<-ggplot(rbp_exp_data)
+geom_bar(aes(x=rbp_names,y=cor_values),stat="identity”)
```

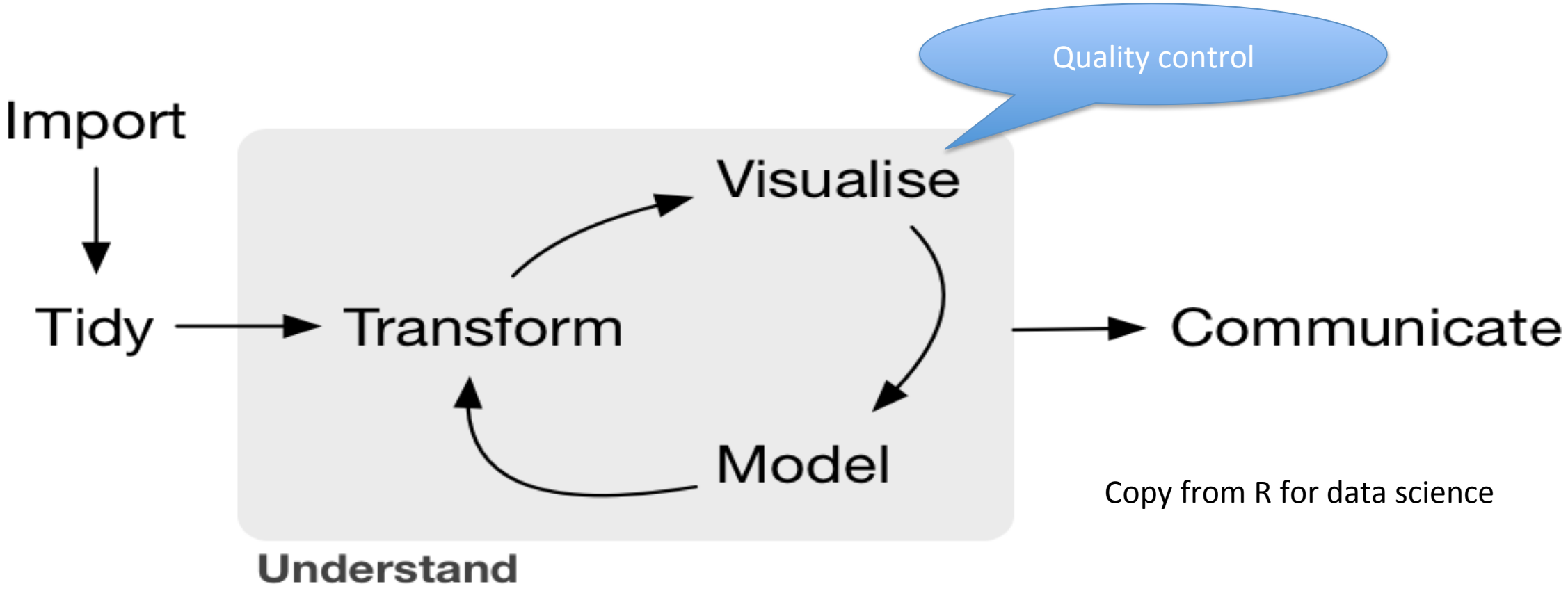


Linear regression of RBP and Gene expression

Another consideration is longer gene region has higher possibility to contain more RBPs, so we need to consider the gene's length.



Summary: a daily life for data scientist



Copy from R for data science

Further reading

http://hadley.nz

Advance R.

R for data science.

ggplot2: elegant graphics for data analysis.

ggplot2:http://docs.ggplot2.org/0.9.3.1/geom_bar.html

Bimedical data science: http://genomicsclass.github.io/book/

R packages.

Bioconductor tutorial:
https://www.bioconductor.org/help/course-materials/

致谢

冯伟兴教授