Working with ChIP-Seq Data in R/Bioconductor

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Contents

Disclaimer

This tutorial is a modified version of its original presented by **Tom Carroll** at the Bioconductor course, 2014 .

Reference: Carroll T. Assessing ChIP-seq sample quality with ChIPQC. BioC 2014, Boston. 2014. http://www.bioconductor.org/help/course-materials/2014/BioC2014/Bioc2014_ChIPQC_Practical.pdf

1 Introduction

This practical aims to introduce you to the analysis of ChIP-seq data in R. This will include loading aligned reads and peak call data into the R environment, performing various data analyses and visualisations and assessing ChIP-seq data quality using the ChIPQC package with real world datasets. R provides support for various sequencing data formats. Here we will work with aligned reads in indexed BAM files and BED files containing peaks called by MACS.

First we would need to load the libraries required:

```
library(GenomicAlignments)
library(ChIPQC)
library(GenomicRanges)
```

set your working directory to the Course_Materials folder:

```
setwd("/home/participant/Desktop/Course_Materials")
```

2 Working with aligned data

2.1 Reading in data

All files needed for this tutorial are contained within the directory 'Day4/data_for_practical/' including BAM files (.bam) and BAM index files (.bam.bai).

Firstly we create a *BamFileList* object for use within the R environment. Many packages assume the naming convention for BAM indicies to be '.bam.bai' although different names may be explicitly passed to these functions.

Use Case: Create a *BamFileList* object containing details of the BAM files we wish to analyse

```
dataDir <- "Day4/data_for_practical/"
bamFiles <- dir(file.path(getwd(), dataDir), pattern="*.bam$", full.name=T)
names(bamFiles) <- gsub(".bam","",basename(bamFiles))
bamFiles
bfList <- BamFileList(bamFiles)
bfList
```

Now that we have all the BAM files in a convenient list we can look at the information within the headers using `scanBamHeader()` function. This requires a path to the BAM file which we can get using the `path()` function.

Use Case: Extract the sam header for a single bam file and examine the structure of the list using the `str()` function

```
path(bfList)
samHeader <- scanBamHeader(path(bfList["TF_1"]))
str(samHeader,max.level=2)
```

We get a list containing two components: The first is 'targets' which contains a list of chromosomes used in the alignment. The second is 'text' which contains information including the species and alignment method used.

Use Case: Explore the information provided in the sam header. Find how the data has been sorted, the aligner used and what species the data has been aligned to.

```
samHeader[[1]]$targets
samHeader[[1]]$text
samHeader[[1]]$text["@HD"]
samHeader[[1]]$text["@PG"]
samHeader[[1]]$text["@CO"]
```

BAM files usually contain a lot of information and it is often more feasible to deal with a single chromosome at a time for processing and analysis.

Here we will set up parameters in order to select only reads aligning to Chromosome 1

Use Case: Extract information about chromosome 1 coordinates to set up filtering parameters using the `ScanBamParam()` function.

```
chr1dat <- samHeader[[1]]$targets["chr1"]
chr1dat
chr1range <- GRanges(seqnames=names(chr1dat),ranges=IRanges(1,chr1dat))
param <- ScanBamParam(which=chr1range)
param
```

Note that in this example, we use the default `ScanBamParam` 'flag' argument. However we could use this to build further filters (e.g. to remove duplicates or other potential artifacts) using the `scanBamFlag()` function. Look at the options for this function for more details using `?scanBamFlag`

Use Case: Having selected an area of interest, read in read data from a single BAM file using the parameters set using the `readGAlignments()` function. Convert this to a *GenomicRanges* object and inspect the data contained within. Calculate the average read length of the selected reads (in case some reads were trimmed).

```
alignDat <-  readGAlignments(path(bfList["TF_1"]),param=param)
alignGR <- granges(alignDat)
seqlevels(alignGR) <- "chr1"
median(width(alignGR))
```

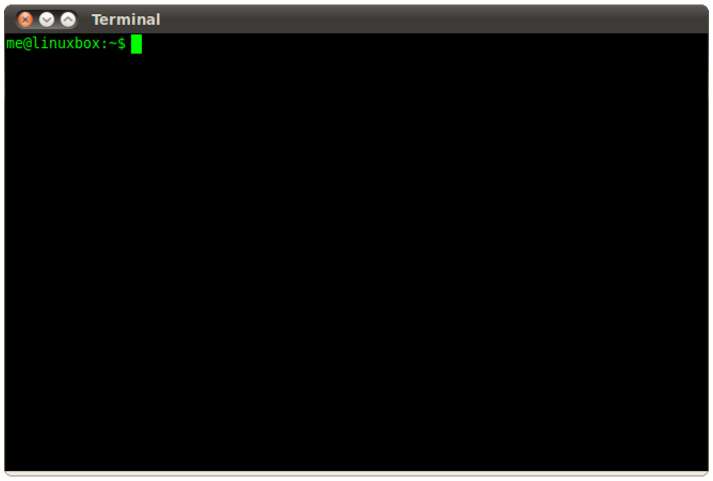
3 Calling peaks with MACS2

MACS takes mapped BAM files of ChIP-seq and control samples and calls peaks. To call peaks, we will use the main module in MACS2 called 'callpeak'. It can be invoked by 'macs2 callpeak' command. If you type this command without parameters, you will see a full description of command-line options. Here we only list commonly used ones, for more detail read MACS2 documentation https://github.com/taoliu/MACS/.

3.1 Calling peaks with MACS2 in the command line

Use Case: In the command-line (terminal), run MACS2 to call peaks. Use TF_1.bam and the Input.bam files located in the 'Day4/data_for_practical/' directory.

Use the terminal window



```
me@linuxbox:~$
```

```
cd /home/participant/Desktop/Course_Materials/Day4/data_for_practical
macs2 callpeak -t TF_1.bam -c Input.bam -n mypeaks
```

We used the following options:

```
-t: This is the only required parameter for MACS, refers to the name of the file with the ChIP-seq data
-c: The control or mock data file
-n: The name string of the experiment
```

MAC2 creates 4 files (mypeaks_peaks.narrowPeak, mypeaks_summits.bed, mypeaks_peaks.xls and mypeaks_model.r)

3.2 Loading peak call data into the R environment

Use Case: load the peaks file in R

The peak file contains the genomic intervals for each peaks and it is named mypeaks_peaks.narrowPeak (the prefix 'mypeaks' of the filename was given by the -n parameter). The NarrowPeak format is a extension of BED format. The USCS webpage contains information about what each column in the NarrowPeak format means (http://genome.ucsc.edu/FAQ/FAQformat.html#format12).

You can load theNarrowPeak file into R using the `read.delim` function. We specify header=FALSE to indicate that the file does not contain column names as its first line.

```
#Run this in R
setwd("/home/participant/Desktop/Course_Materials")
mypeaks <- read.delim("Day4/data_for_practical/mypeaks_peaks.narrowPeak", header=F)
colnames(mypeaks) <- c("chrom", "chromStart", "chromEnd", "name","score", "strand", "fold.enrichment","log
```

```
head(mypeaks)

##   chrom chromStart chromEnd           name score strand fold.enrichment
## 1  chr1     526349   526864 mypeaks_peak_1   239      .           14.31
## 2  chr1     750648   751354 mypeaks_peak_2   952      .           23.20
## 3  chr1    2342155  2342787 mypeaks_peak_3   572      .           23.11
## 4  chr1    2549147  2549618 mypeaks_peak_4   130      .            9.08
## 5  chr1    4657143  4657629 mypeaks_peak_5   336      .           17.16
## 6  chr1    4790217  4790582 mypeaks_peak_6    80      .            6.19
##   log10.pvalue log10.qvalue peak
## 1         27.3        23.95  247
## 2         99.5        95.29  423
## 3         61.1        57.27  328
## 4          16.2        13.09  216
## 5          37.1        33.61  228
## 6          11.0         8.01  143
```

Use Case: Create a genomic ranges object

Because the NarrowPeak format is a extension of BED format, you will need to convert the 0-base start coordinates encoded in the BED format to 1-base start coordinates (just sum +1 to every start coordinate). Remember, only the start coordinated have to be shifted, the end coordinates don't have to be shifted.

```
library(GenomicRanges)
GRanges(mypeaks$chrom, IRanges(mypeaks$chromStart+1, mypeaks$chromEnd))
```

```
## GRanges object with 1281 ranges and 0 metadata columns:
##          seqnames                 ranges strand
##             <Rle>              <IRanges>  <Rle>
##      [1]     chr1     [ 526350,  526864]      *
##      [2]     chr1     [ 750649,  751354]      *
##      [3]     chr1     [2342156, 2342787]      *
##      [4]     chr1     [2549148, 2549618]      *
##      [5]     chr1     [4657144, 4657629]      *
##      ...      ...                    ...    ...
##   [1277]     chr1 [246977471, 246977839]      *
##   [1278]     chr1 [247677836, 247678226]      *
##   [1279]     chr1 [247840780, 247841255]      *
##   [1280]     chr1 [248093673, 248094130]      *
##   [1281]     chr1 [249217644, 249217995]      *
##   -------
##   seqinfo: 1 sequence from an unspecified genome; no seqlengths
```

4 A simple example using ChIPQsample()

ChIPQC package allows for a quick assessment of the quality of your ChIP-seq data. It requires aligned data in the BAM format and a set of previously identified peak files in the BED format. The main function is `ChIPQCsample()`. This function can be run by simply indicating the location of a a BAM file.

First, set your working directory to the Course_Materials folder a load ChIPQC library:

```
setwd("/home/participant/Desktop/Course_Materials")
library(ChIPQC)
```

Use Case: Run `ChIPQCsample` on a single BAM file. As an example, we will use the TF_1.bam file located in the 'Day4/data_for_practical/' directory.

The chromosomes parameter specifies which chromosomes to examine when computing quality metrics. To increase the speed of computations and the reduce the size of the resulting ChIPCexperiment object we will limit the analysis in this example to chromosome 1.

```
bamFile <- file.path(getwd(), "Day4/data_for_practical/TF_1.bam")
exampleExp = ChIPQCsample(bamFile,peaks=NULL,annotation=NULL,chromosomes="chr1")
```

The function `ChIPQCsample` will return ChIPQC object

```
class(exampleExp)
```

```
## [1] "ChIPQCsample"
## attr(,"package")
## [1] "ChIPQC"
```

```
exampleExp
```

```
## ChIPQCsample
## Number of Mapped reads:  497994
## Number of Mapped reads passing MapQ filter:  420386
## Percentage Of Reads as Non-Duplicates (NRF): 96.33(0.04)
## Percentage Of Reads in Blacklisted Regions:  NA
## SSD: 1.59071199216826
## Fragment Length Cross-Coverage:  0.00533687290155682
## Relative Cross-Coverage:  2.80111111111111
## Percentage Of Reads in GenomicFeature:
```

```
##                          ProportionOfCounts
## 5utrs                                    NA
## 3UTRs                                    NA
## CountsinReads                            NA
## Introns                                  NA
## Transcripts                              NA
## Promoters500                             NA
## Promoters2000to500                       NA
## LongPromoter20000to2000                  NA
```

```
## Percentage Of Reads in Peaks:  NA
## Number of Peaks:  0
```

```
## GRanges object with 0 ranges and 0 metadata columns:
##    seqnames    ranges strand
##       <Rle> <IRanges>  <Rle>
##   -------
##   seqinfo: no sequences
```

Use Case: Use the function `QCmetrics` to show a summary of the main quality control metrics.

```
QCmetrics(exampleExp)
```

```
##    Reads     Map%    Filt%     Dup%    ReadL    FragL    RelCC      SSD
## 4.98e+05 1.00e+02 1.56e+01 4.35e+00 3.60e+01 1.55e+02 2.80e+00 1.59e+00
##     RiP%
##       NA
```

You can see there are 497,994 reads in this example BAM file. A large number of reads from a sufficiently complex library increases the chances of finding true binding sites [3]. However, the 'optimal' required number of reads for your experiment largely depends on enrichment, antibody quality, and the fraction of the genome containing the feature being measured. That is why it is so important to analyse the quality of your data using a variety of quality metrics.

Use Case: To take full advantage of ChIPQC features, run ChIPQCsample on a single BAM file including additional information on blacklisted regions, genome annotation and any peaks

```
peaksFile <- file.path(getwd(), "Day4/data_for_practical/TF_1_peaks.bed")
data(blacklist_hg19)
exampleExp = ChIPQCsample(bamFile,peaks=peaksFile,blacklist=blacklist.hg19,
                          annotation="hg19",chromosomes="chr1")
```

```
QCmetrics(exampleExp)
```

```
##    Reads     Map%    Filt%     Dup%    ReadL    FragL    RelCC      SSD 
## 4.98e+05 1.00e+02 1.56e+01 4.35e+00 3.60e+01 1.55e+02 2.80e+00 1.59e+00 
##     RiP%    RiBL% 
## 1.30e+01 2.92e+00
```

4.1 QCmetrics summary

Now the result of QCmetrics contains a number of quality metrics for your ChIP-seq BAM file. This is what they mean:

Reads total number of reads in the bam file.

Map% Percentage of total reads that were successfully mapped (aligned).

Filt% Percentage of mapped reads passing MapQ filter, in this case having a mapping quality score less than or equal to 15 (mapQCth=15 by default)

Dup% Percentage of mapped reads marked as duplicates.

ReadL Mean read length (as integer) derived from the data.

FragL Predicted fragment length by cross-coverage method. The fragment length is estimated by methods implemented in the `chipseq` package by systematically shifting the reads on each strand towards each other until the minimum genome coverage is achieved.

RelCC The relative cross-coverage score. The RelCC metric is calculated by comparing the maximum cross coverage peak (at the shift size corresponding to the fragment length) to the cross coverage at a shift size corresponding to the read length, with higher scores (generally 1 or greater) indicating good enrichment.

SSD Standardised standard deviation, or SSD score, as implemented in htSeqTools. This is another indication of evidence of enrichment. It is computed by looking at the standard deviation of signal pile-up along the genome normalised to the total number of reads. An enriched sample typically has regions of significant pile-up so a higher SSD is more indicative of better enrichment. SSD scores are dependent on the degree of total genome wide signal pile-up and so are sensitive to regions of high signal found with Blacklisted regions as well as genuine ChIP enrichment.

RiP% Reads mapped to peaks. This is another good indication of how "enriched" the sample is, and can be considered a "signal-to-noise" measure of what proportion of the library consists of fragments from binding sites vs. background reads. RiP% values for ChIPs around 5% or higher generally reflect successful enrichment.

RiBL% Reads mapped to blacklists. The signal from blacklisted has been shown to contribute to confound peak callers and fragment length estimation as well as contribute to the read length peak in cross coverage and cross coverage read length peak [1].

Now we have our full ChIPQCsample object we can start to review and visualise the metrics generated.

4.2 Cross-coverage and the FragmentLength/Relative cross-coverage scores (FragCC/RelCC)

For transcription factors and narrow epigenetic marks, an accumulation of Watson and Crick reads around the binding site/mark is often be seen. In ChIPQC we assess the reduction in total genome covered which occurs from shifting the Watson reads along the genome (from 5' to 3' of chromosome). This is performed by measuring measure total coverage after each every successive shift of 1bp. As the Watson reads overlap the Crick reads around peaks the total genome covered will be reduced. The total coverage after each successive shift is then converted to cross-coverage scores after each shift.

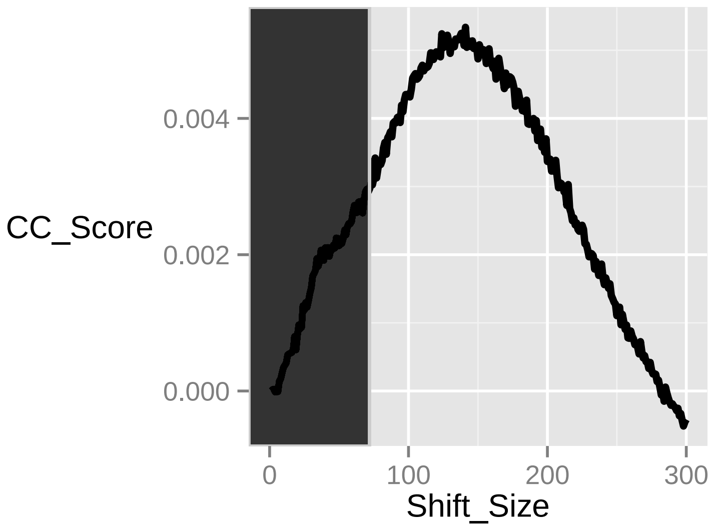
$CrossCoverageScoren = (TotalGenomeCoverage0 - TotalGenomeCoveragen)/TotalGenomeCoverage_0$

Where *n* is the bp shift of Watson reads and *0* is after no shift of Watson reads.

The cross-coverage scores after successive shifts can then be visualised and reviewed to identify the expected increase in cross-coverage scores around the fragment length as well as any evidence of artifacts by a peak in the cross-coverage score at the read length.

Use Case: Use the `plotCC` function to calculate cross-coverage scores and plot those after successive shifts.

```
plotCC(exampleExp)
```



The area shaded in gray (from 0 to 1.5*readlength) is excluded when identifying the fragment length peak. This is due to the presence of the artifact peak which, in less enriched samples, may have greater cross-coverage score than observed at the fragment length.

We can see that this ChIP has a peak in cross-coverage scores at 155bp, corresponding to the fragment size. This indicates that we have successfully enriched for signal around binding sites.

```
fragmentlength(exampleExp)

## [1] 155
```

Further to the visual inspection of the cross-coverage scores, we can extract RelCC and FragCC scores. These metrics can be considered to relate to efficiency of ChIP (FragCC) and efficiency of ChIP compared to artifact signal (RelCC).

$FragCC = CrossCoverageScore_{max}$

$RelCC = CrossCoverageScore_{max}/CrossCoverageScore_{readlength}$

Where *max* is shift with maximum cross-coverage score (excluding area 0 to 1.5*readlength) and *readlength* is the shift corresponding to the read length.

Use Case: Extract the RelCC score using the `RelativeCrossCoverage` function respectively.

```
FragmentLengthCrossCoverage(exampleExp) / ReadLengthCrossCoverage(exampleExp)

## [1] 2.8

RelativeCrossCoverage(exampleExp)

## [1] 2.8
```

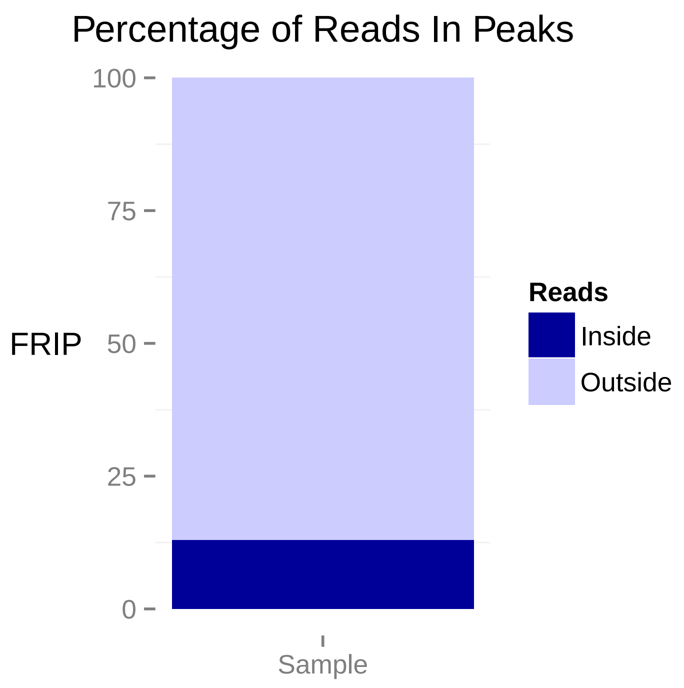
In this example we find, as expected from cross-coverage scores graph, that the the RelCC score is above 1 indicating a successful ChIP.

4.3 Distribution of signal within peaks

Another measurement of ChIP enrichment is the fraction of reads in peaks (FRiP or RiP%) metric. ChIPQC calculates the fraction of reads that fall in previously identified peak regions. Note that this metric is sensitive to the determination of enriched regions, comparison between samples is possible only when using the identical peak caller and parameters.

Use Case: To get the percentage of reads landing in peaks use the `frip` function as well as the `plotFrip` function for visualisation.

```
plotFrip(exampleExp)
```



```
frip(exampleExp)

## [1] 0.13
```

Larger FRiP values indicate higher signal to noise; 1 is the maximum possible value (all reads are signal) and 0 is the minimum possible value (all reads are noise). The FRiP metric shows that we have a percentage of signal in peaks of 13%; this is greater than 5% indicating a ChIP of acceptable quality.

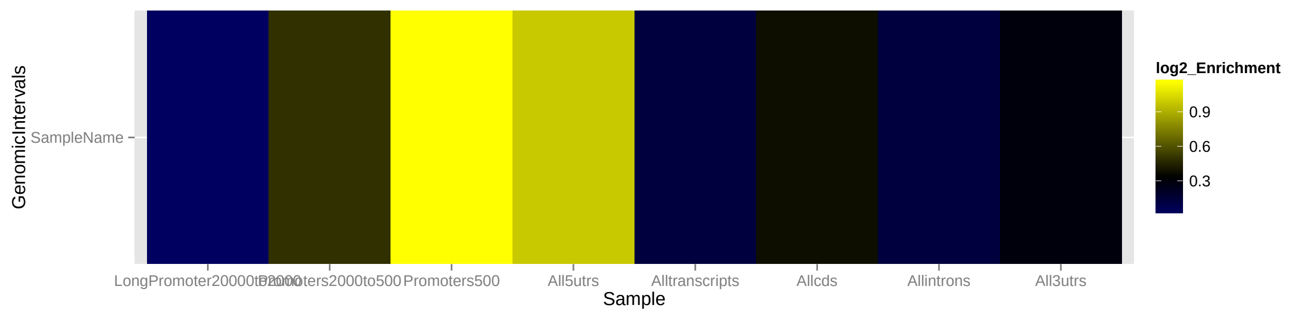
4.4 Distribution of signal in annotated regions

When provided with annotation in the form of genomic regions, ChIPQC can measure the enrichment of signal within them. The `regi` function provides a simple enrichment statistic which illustrates the distribution of signal within genomic interval annotation over that expected given their size.

$regi = ProportionOfReadsInInterval/ProportionOfGenomeInInterval$

Use Case: To review regi statistics, use the `regi` function or plot enrichment using `plotRegi`.

```
regi(exampleExp)

## LongPromoter20000to2000      Promoters2000to500           Promoters500
##                0.0274               0.4918               1.1518
##              All5utrs       Alltranscripts               Allcds
##                0.9837               0.1327               0.3780
##            Allintrons             All3utrs
##                0.1378               0.2939
plotRegi(exampleExp)
```



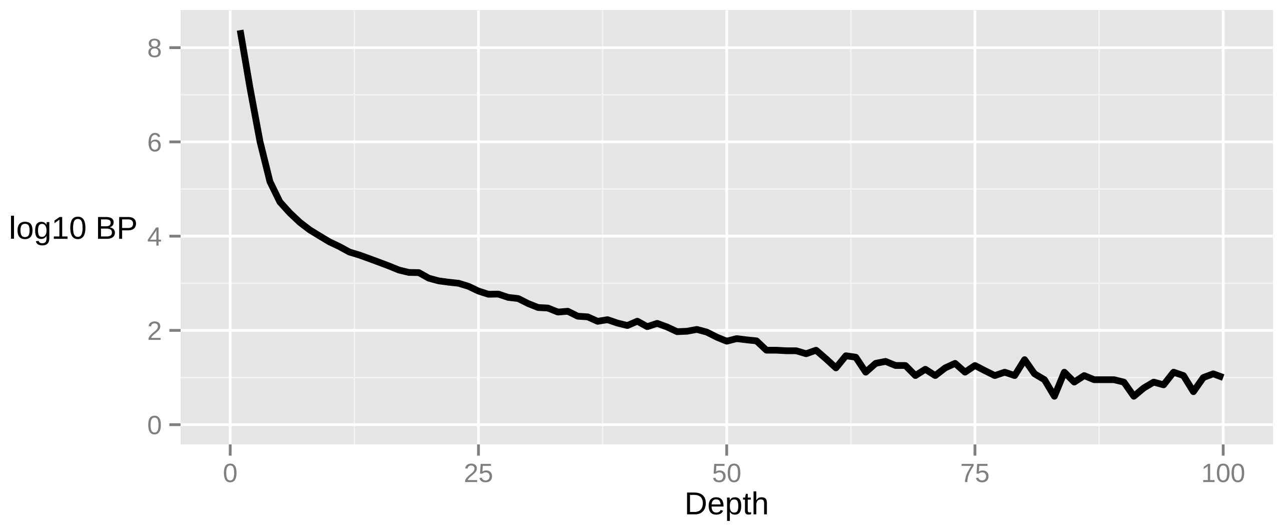
The regi scores and heatmap show an enrichment for regions around the TSS including 5'UTRs, and 500bp upstream regions. This suggests that the TF in question is a promoter associated transcription factor.

4.5 Dispersion of coverage across the genome

A hallmark of good ChIP data is the inequality of coverage that occurs when there is enrichment of reads to certain portions of the genome (e.g. transcription factor binding sites, peaks). We can evaluate the dispersion of coverage in two ways within ChIPQC, first by visualising the histogram of coverage depths and secondly by applying the SSD metric before and after removal of blacklisted regions.

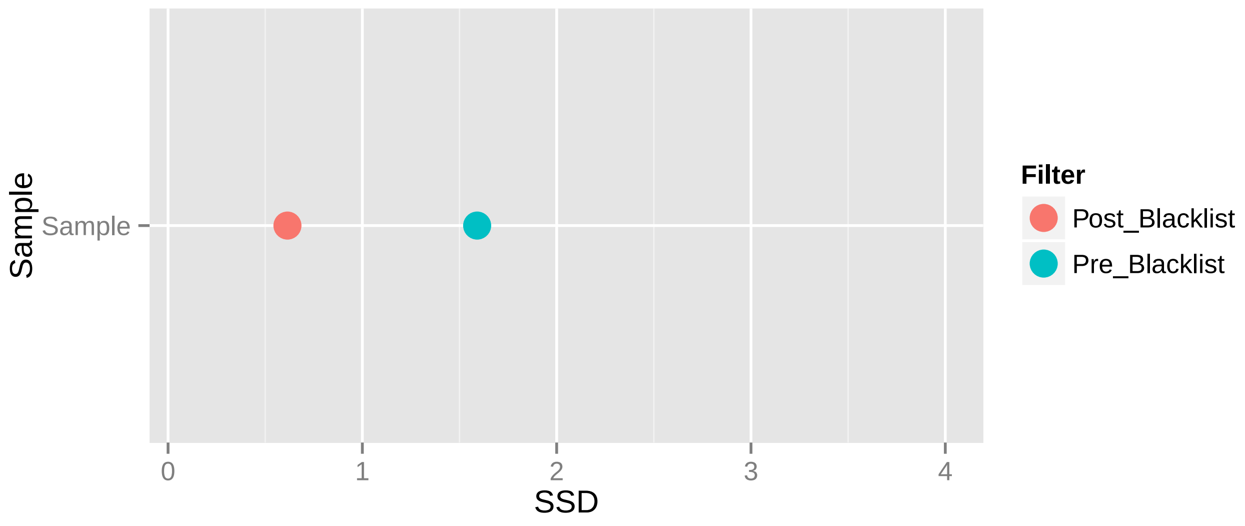
First we draw the coverage histogram using `plotCoverageHistogram`. Note the cut-off at 100bp for visualisation purposes. If you want to replot the whole histogram you can extract the data using coveragehistogram function or extend x-axis as shown in Advanced Topics section.

```
coveragehistogram(exampleExp)[1:10]

##       0       1       2       3       4       5       6       7
## 2.34e+08 1.36e+07 1.02e+06 1.44e+05 5.32e+04 3.11e+04 1.97e+04 1.36e+04
##       8       9
## 1.01e+04 7.55e+03

plotCoverageHist(exampleExp)
```



The coverage histogram shows that there is a significant stretch of high signal pile-up. This may indicate significant signal associated to binding events but could also be from signal seen within blacklisted regions. To assess the contribution of artifact signal to global distribution of signal pileup we measure SSD before and after exclusion of signal from known blacklisted regions.

```
plotSSD(exampleExp)
```



Here we find that the SSD signal is not greatly affected by blacklisted regions and so, taken together with the coverage histogram, indicates the TF ChIP has a clear ChIP-signal above background.

4.6 Conclusion

From the combination of metrics used here we can establish that this ChIP showed an enrichment for structured ChIP-signal, had a signal pile-up above that seen within artifact regions and that this ChIP is positively associated with TSS regions.

5 Assessing a ChIP-seq experiment using ChIPQC() - Example 1

The ChIPQC function wraps the functionality of ChIPQCsample to allow for the assessment of multiple datasets. We start by creating a samplesheet that contains the location for the necessary files. An example of the layout for a sample sheet can be seen in the "sample.csv" file that you can find under "Day4/data_for_practical".

5.1 Reading a sample sheet

Use Case: Load the samplesheet into R

```
samples = read.csv("Day4/data_for_practical/samples.csv")
```

```
samples

##       SampleID Tissue Factor Replicate                 bamReads
## 1 MCF7_GATA3_1   MCF7  GATA3         1 MCF7_GATA3_Myers_Rep1.bam
## 2 MCF7_GATA3_2   MCF7  GATA3         2 MCF7_GATA3_Myers_Rep2.bam
## 3  MCF7_CTCF_2   MCF7   CTCF         2  MCF7_CTCF_Myers_Rep2.bam
## 4  MCF7_CTCF_1   MCF7   CTCF         1  MCF7_CTCF_Myers_Rep1.bam
## 5 A549_GATA3_1   A549  GATA3         1 A549_GATA3_Myers_Rep1.bam
## 6 A549_GATA3_2   A549  GATA3         2 A549_GATA3_Myers_Rep2.bam
## 7  A549_CTCF_1   A549   CTCF         1  A549_CTCF_Myers_Rep1.bam
## 8  A549_CTCF_2   A549   CTCF         2  A549_CTCF_Myers_Rep2.bam
##                       Peaks
## 1 MCF7_GATA3_Myers_Rep1.bed
## 2 MCF7_GATA3_Myers_Rep2.bed
## 3  MCF7_CTCF_Myers_Rep2.bed
## 4  MCF7_CTCF_Myers_Rep1.bed
## 5 A549_GATA3_Myers_Rep1.bed
## 6 A549_GATA3_Myers_Rep2.bed
## 7  A549_CTCF_Myers_Rep1.bed
## 8  A549_CTCF_Myers_Rep2.bed
```

The sample sheet refers to data downloaded from the ENCODE portal [4]. The ChIP-seq data refers to GATA3 and CTCF ChIPs obtained in A549 and MCF7 cell lines. The BAM files were aligned the hg19 reference genome.

To reduce the size of the dataset being analysed in this practical, we have pre-computed the ChIPQC object:

```
#exampleExp2 = ChIPQC(samples,annotation="hg19")
#save(exampleExp2, file="ChIPQCexample1.RData")
```

You just need to load the resulting file using the following code:

```
Rdata <- file.path(getwd(), "Day4/data_for_practical/ChIPQCexample1.RData")
load(Rdata)
```

Now have a look at the resulting ChIPQC object:

```
exampleExp2

## Samples: 8 : MCF7_GATA3_1 MCF7_GATA3_2 ... A549_CTCF_1 A549_CTCF_2
##              Tissue Factor Replicate Peaks
## MCF7_GATA3_1   MCF7  GATA3         1 42381
## MCF7_GATA3_2   MCF7  GATA3         2 23604
## MCF7_CTCF_2    MCF7   CTCF         2 39765
## MCF7_CTCF_1    MCF7   CTCF         1 39464
## A549_GATA3_1   A549  GATA3         1  6075
## A549_GATA3_2   A549  GATA3         2 19702
## A549_CTCF_1    A549   CTCF         1 33001
## A549_CTCF_2    A549   CTCF         2 31725
##                Reads Map% Filt% Dup% ReadL FragL RelCC  SSD  RiP% RiBL%
## MCF7_GATA3_1 1878415  100     0    0    50   114 1.560 1.81 22.10 0.620
## MCF7_GATA3_2  826002  100     0    0    50   208 2.540 1.49 22.60 0.957
## MCF7_CTCF_2  1361078  100     0    0    50   110 1.840 2.30 24.50 0.770
## MCF7_CTCF_1  1974422  100     0    0    50   119 1.880 3.23 22.10 0.871
## A549_GATA3_1 1573136  100     0    0    50   104 0.983 1.38  2.78 0.972
## A549_GATA3_2 1896160  100     0    0    50   174 1.910 1.55 13.80 0.855
## A549_CTCF_1  2268054  100     0    0    36   151 3.080 3.31 15.20 4.060
## A549_CTCF_2  1439996  100     0    0    36   124 2.980 2.76 20.90 3.710
```

5.2 QCmetrics summary

The `QCmetrics` function now displays a table of metrics as seen before:

```
QCmetrics(exampleExp2)
```

```
##                Reads Map% Filt% Dup% ReadL FragL RelCC  SSD  RiP% RiBL%
## MCF7_GATA3_1 1878415  100     0    0    50   114 1.560 1.81 22.10 0.620
## MCF7_GATA3_2  826002  100     0    0    50   208 2.540 1.49 22.60 0.957
## MCF7_CTCF_2  1361078  100     0    0    50   110 1.840 2.30 24.50 0.770
## MCF7_CTCF_1  1974422  100     0    0    50   119 1.880 3.23 22.10 0.871
## A549_GATA3_1 1573136  100     0    0    50   104 0.983 1.38  2.78 0.972
## A549_GATA3_2 1896160  100     0    0    50   174 1.910 1.55 13.80 0.855
## A549_CTCF_1  2268054  100     0    0    36   151 3.080 3.31 15.20 4.060
## A549_CTCF_2  1439996  100     0    0    36   124 2.980 2.76 20.90 3.710
```

The table shows a summary of QC metrics, including the total number of peaks and reads in the correspondent files for each sample, the percentage of these that were successfully mapped (Map%), the percentage of filtered reads based on the mapping quality (15 by default) and the duplication rates (Dup%), the read length (ReadL) and the estimated fragment length (FragL).

Map%, Filt% and Dup%

Our downloaded BAM files contain only the good quality reads, so ChIPQC shows 100% alignment rate and 0% filtered or duplicated reads.

RelCC

The RelCC is a metric of ChIP-enrichment. For all samples, the RelCC score is greater than 1, indicating high quality, except for one of the GATA3 samples in A549 cells.

SSD

The SSD score is another metric of ChIP-enrichment. It is sensitive to regions of significant pile-up or reads, here the CTCF samples in both A549 and MCF7 show higher SSD scores $(2) than GATA3 samples and so greatest enrichment for depth of signal.$

RiP%

RiP reports the percentage of reads that overlap called peaks (%RiP, also known as FRiP). This is another good indication of how enriched the sample is. RiP% values for ChIPs around 5% or higher generally reflect successful enrichment (from ChIPQC vignette). Here, once again, one of the GATA3 samples in A549 cells jumps out with a low %RiP (2.78%) indicating that a big proportion of this library consists of background noise.

RiBL%

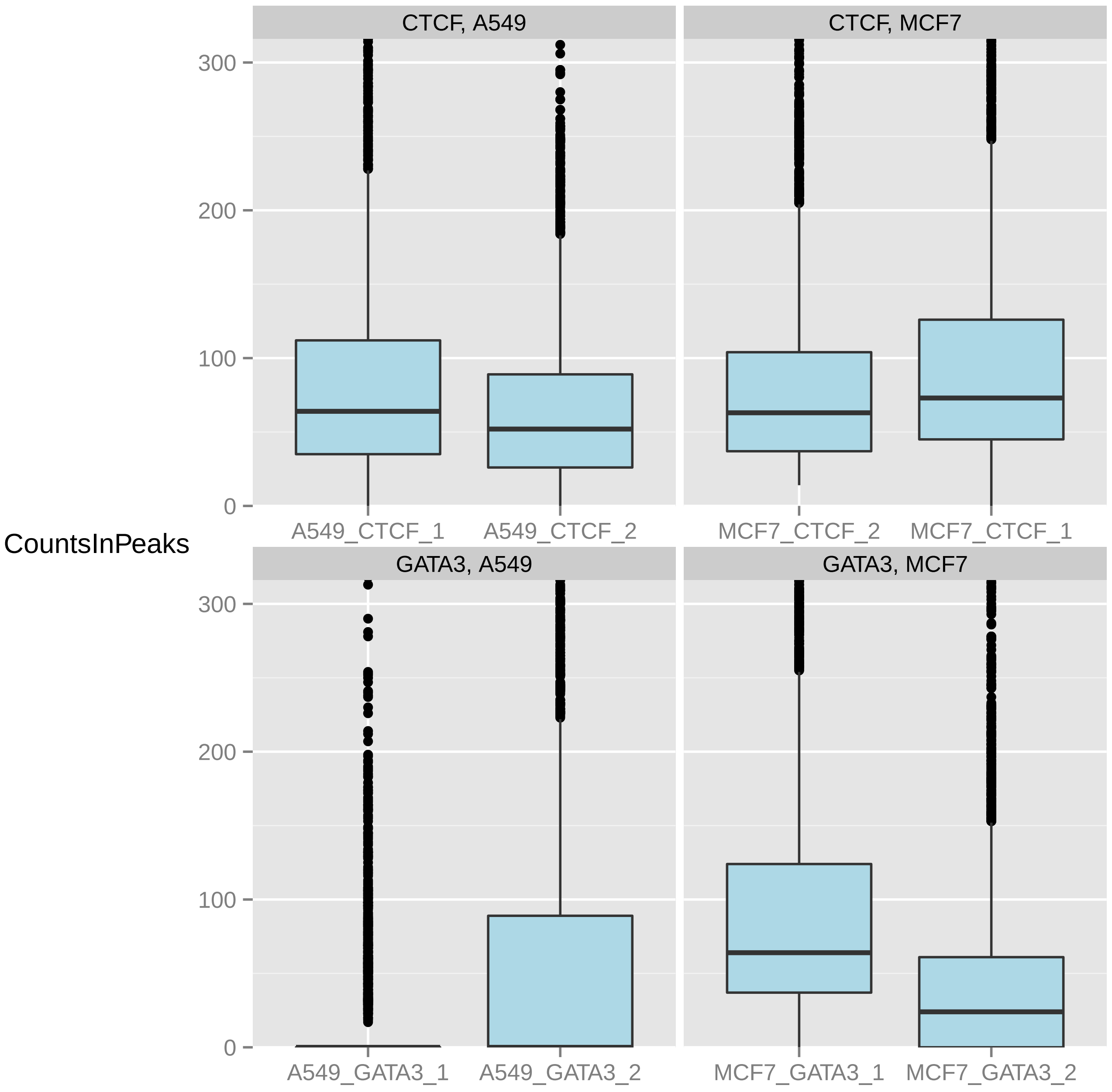
Finally, RiBL reports the percentage of reads that overlap blacklisted regions (%RiBL). The signal from blacklisted regions has been shown to influence the performance of peak callers [1].

5.3 Examining reads in peaks

Use Case: Visualise the number of reads in peaks A quick way to visualise the relative number of reads that overlap peaks vs. background reads is using the `plotRap` function.

Here its clearly visible the low %RiP for one of the GATA3 samples in A549 cells:

```
plotRap(exampleExp2,facetBy=c("Factor","Tissue"))
```



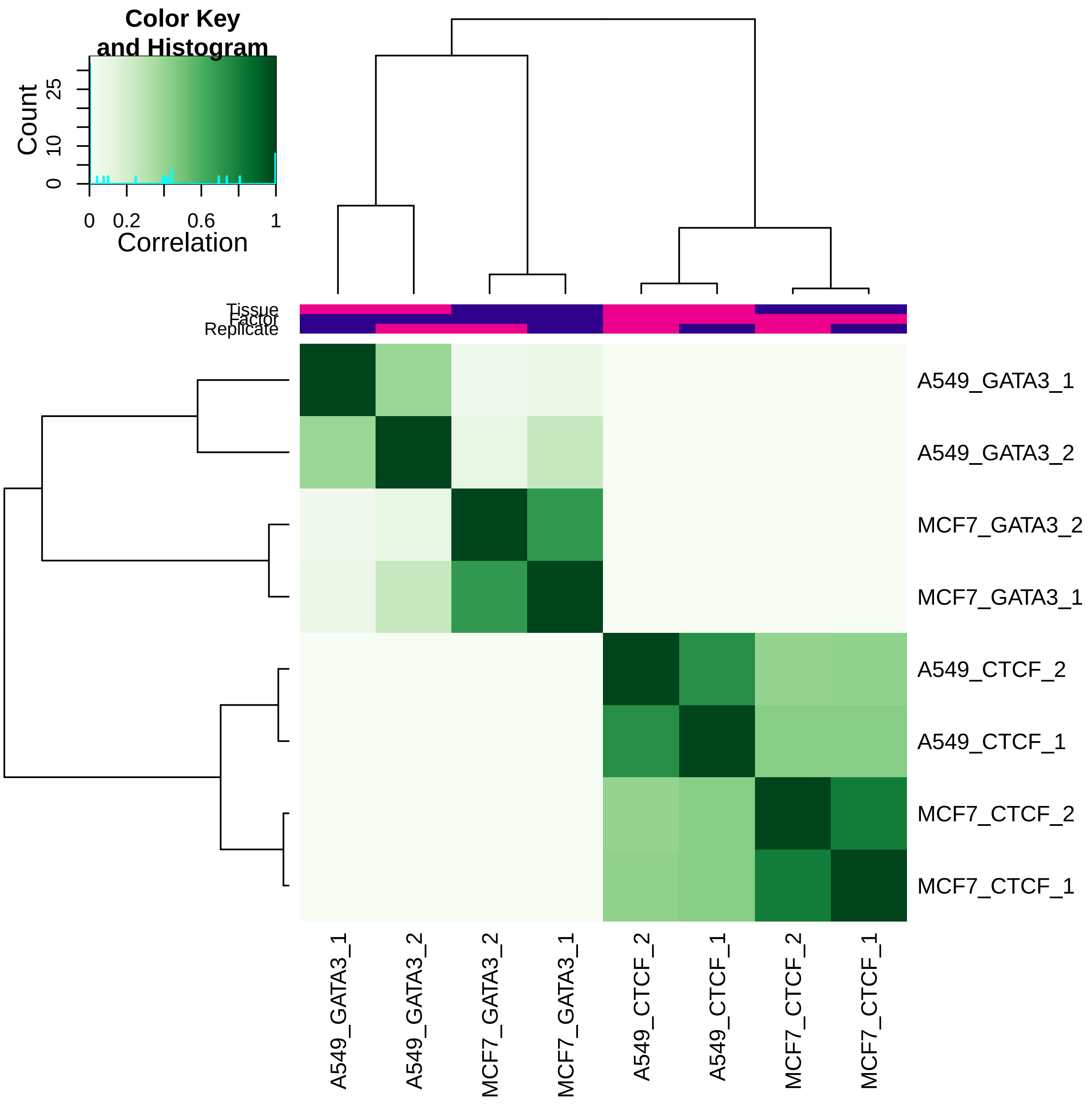
5.4 Assessing sample similarity with Diffbind

A final set of metrics useful for ChIP-quality relate to the correlation between binding events across samples within a ChIPQCexperiment. When analysing an experiment, ChIPQC will perform a sample clustering based on the co-occurrence of peaks using methods available within the `DiffBind` package.

Use Case: Examine clustering of samples based on the co-occurrence of peaks To produce the a sample heatmap, the `plotCorHeatmap` function can be used.

In this example (and has we would expect) the CTCF and GATA3 samples form two distinct clusters:

```
plotCorHeatmap(exampleExp2)
```



6 Assessing a ChIP-seq experiment using ChIPQC() - Example 2

For this section of the practical we have pre-computed the ChIPQC object. Load the ChIPQC object by typing:

```
#resExperiment = ChIPQC(SampleSheet,annotation="mm9")
Rdata <- file.path(getwd(), "Day4/data_for_practical/BCell_Examples.RData")
load(Rdata)
```

Now you can look at 'resExperiment', it contains a ChIPQCexperiment object:

```
resExperiment
```

```
## Samples: 19 : DNAse Ebf1 ... RNAPol2 RNAPol2ser2
##                    Tissue       Factor Replicate Peaks
## DNAse                Ch12        DNAse         1 72437
## Ebf1                 ProB         Ebf1         1  9841
## H3K4me3_IkNeg        ProB      H3K4me3         1 18462
## H3K4me3_IkPos        ProB      H3K4me3         2 20052
## H3K9ac_IkNeg         ProB       H3K9ac         1 27486
## H3K9ac_IkPos         ProB       H3K9ac         2 25902
## Ikaros_1_DPT          DPT       Ikaros         1 22253
## Ikaros_1_preproB preProB       Ikaros         1 16240
## Ikaros_1_proB        ProB       Ikaros         1 15377
## Ikaros_2_preproB preProB       Ikaros         2 16038
## Input_2_proB         ProB        Input         1     0
## Input_Ch12           Ch12        Input         1     0
## Irf                  ProB          Irf         1 39628
## Mi2b_IKneg           ProB         Mi2b         1     0
## Mi2b_IKpos           ProB         Mi2b         2     0
## Myc                  Ch12          Myc         1 44982
## Pu1                  ProB          Pu1         1 48472
## RNAPol2              Ch12      RNAPol2         1 59266
## RNAPol2ser2          Ch12 RNAPol2_Ser2         1 30787
##                     Reads Map% Filt%  Dup% ReadL FragL  RelCC  SSD  RiP% RiBL%
## DNAse            1.10e+08  100  15.5 27.60    36    73 0.9280 3.22 45.40  7.94
## Ebf1             2.91e+07  100  24.3  6.60    36   177 2.6400 2.67  3.07 11.00
## H3K4me3_IkNeg    7.61e+07  100  15.1 27.00    36   228 3.9300 2.98 45.90  7.94
## H3K4me3_IkPos    1.29e+08  100  14.0 50.00    36   243 3.8200 3.61 50.70  7.40
## H3K9ac_IkNeg     6.46e+07  100  14.8 13.00    36   215 3.9300 2.28 36.30  6.73
## H3K9ac_IkPos     6.91e+07  100  13.9 14.70    36   213 4.1700 2.01 37.90  5.84
## Ikaros_1_DPT     4.83e+07  100  23.0 15.30    36   173 1.8000 3.19  5.31 10.50
## Ikaros_1_preproB 3.59e+07  100  24.7 15.30    36   175 0.2780 3.43  3.50 12.50
## Ikaros_1_proB    2.48e+07  100  23.2 13.10    36   165 0.9210 2.28  3.49 10.50
## Ikaros_2_preproB 3.55e+07  100  24.7 15.20    36   181 0.3110 3.42  3.43 12.60
## Input_2_proB     2.35e+07  100  22.1  9.73    36   120 1.6500 1.93    NA  9.02
## Input_Ch12       1.91e+07  100  25.5 21.50    36   157 0.0556 2.06    NA  9.81
## Irf              2.79e+07  100  21.4 20.80    36   153 2.3200 2.24 10.50  9.91
## Mi2b_IKneg       3.85e+07  100  23.5 29.30    36   151 0.4990 3.09    NA 11.40
## Mi2b_IKpos       3.48e+07  100  23.8  6.08    36   152 0.6380 3.02    NA 11.60
## Myc              3.93e+07  100  19.4 11.60    36   150 1.5700 2.29 16.80  8.74
## Pu1              3.41e+07  100  20.8 20.30    36   204 3.3300 2.52 22.20  8.89
## RNAPol2          3.35e+07  100  12.3 17.90    36   152 1.9200 1.11 45.70  4.98
## RNAPol2ser2      4.82e+07  100  15.0  6.63    36   125 0.8050 1.61 30.30  5.86
```

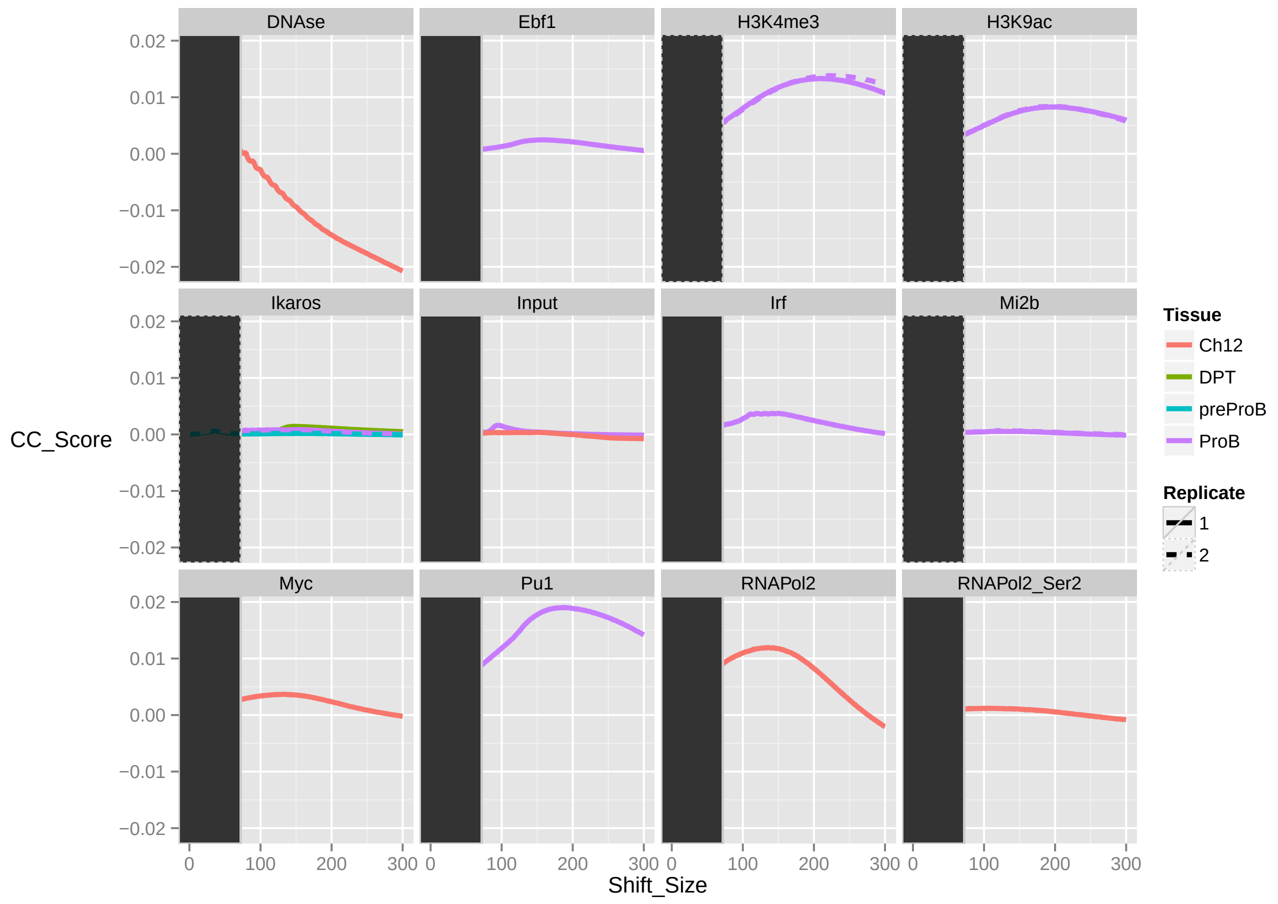
The `QCmetrics` function displays the following metrics table:

```
QCmetrics(resExperiment)

##                        Reads Map% Filt%  Dup% ReadL FragL  RelCC  SSD  RiP% RiBL%
## DNAse               1.10e+08  100  15.5 27.60    36    73 0.9280 3.22 45.40  7.94
## Ebf1                2.91e+07  100  24.3  6.60    36   177 2.6400 2.67  3.07 11.00
## H3K4me3_IkNeg       7.61e+07  100  15.1 27.00    36   228 3.9300 2.98 45.90  7.94
## H3K4me3_IkPos       1.29e+08  100  14.0 50.00    36   243 3.8200 3.61 50.70  7.40
## H3K9ac_IkNeg        6.46e+07  100  14.8 13.00    36   215 3.9300 2.28 36.30  6.73
## H3K9ac_IkPos        6.91e+07  100  13.9 14.70    36   213 4.1700 2.01 37.90  5.84
## Ikaros_1_DPT        4.83e+07  100  23.0 15.30    36   173 1.8000 3.19  5.31 10.50
## Ikaros_1_preproB 3.59e+07  100  24.7 15.30    36   175 0.2780 3.43  3.50 12.50
## Ikaros_1_proB       2.48e+07  100  23.2 13.10    36   165 0.9210 2.28  3.49 10.50
## Ikaros_2_preproB 3.55e+07  100  24.7 15.20    36   181 0.3110 3.42  3.43 12.60
## Input_2_proB        2.35e+07  100  22.1  9.73    36   120 1.6500 1.93    NA  9.02
## Input_Ch12          1.91e+07  100  25.5 21.50    36   157 0.0556 2.06    NA  9.81
## Irf                 2.79e+07  100  21.4 20.80    36   153 2.3200 2.24 10.50  9.91
## Mi2b_IKneg          3.85e+07  100  23.5 29.30    36   151 0.4990 3.09    NA 11.40
## Mi2b_IKpos          3.48e+07  100  23.8  6.08    36   152 0.6380 3.02    NA 11.60
## Myc                 3.93e+07  100  19.4 11.60    36   150 1.5700 2.29 16.80  8.74
## Pu1                 3.41e+07  100  20.8 20.30    36   204 3.3300 2.52 22.20  8.89
## RNAPol2             3.35e+07  100  12.3 17.90    36   152 1.9200 1.11 45.70  4.98
## RNAPol2ser2         4.82e+07  100  15.0  6.63    36   125 0.8050 1.61 30.30  5.86
```

6.1 Examining Cross-coverage and FragCC/RelCC scores across an experiment

As with the ChIPQCsample object, the cross coverage scores for a group of samples can be plotted using `plotCC()`. By default samples will be groups by their Tissue and Factor combinations and coloured by their Replicate number. Here we group by Factor, colour by Tissue and set the line type by the Replicate number.

```
FragmentLengthCrossCoverage(resExperiment)

##            DNAse             Ebf1    H3K4me3_IkNeg    H3K4me3_IkPos
##         0.000149         0.002461         0.013315         0.013781
##     H3K9ac_IkNeg     H3K9ac_IkPos     Ikaros_1_DPT Ikaros_1_preproB
##         0.008278         0.008374         0.001399         0.000167
##    Ikaros_1_proB Ikaros_2_preproB     Input_2_proB       Input_Ch12
##         0.000861         0.000191         0.001500         0.000331
##              Irf       Mi2b_IKneg       Mi2b_IKpos              Myc
##         0.003734         0.000533         0.000599         0.003622
##              Pu1          RNAPol2      RNAPol2ser2
##         0.018980         0.011852         0.001195

RelativeCrossCoverage(resExperiment)

##            DNAse             Ebf1    H3K4me3_IkNeg    H3K4me3_IkPos
##           0.9277           2.6369           3.9340           3.8202
##     H3K9ac_IkNeg     H3K9ac_IkPos     Ikaros_1_DPT Ikaros_1_preproB
##           3.9301           4.1730           1.7986           0.2776
##    Ikaros_1_proB Ikaros_2_preproB     Input_2_proB       Input_Ch12
##           0.9207           0.3106           1.6458           0.0556
##              Irf       Mi2b_IKneg       Mi2b_IKpos              Myc
##           2.3151           0.4991           0.6378           1.5726
##              Pu1          RNAPol2      RNAPol2ser2
##           3.3293           1.9199           0.8046

plotCC(resExperiment,colourBy="Tissue",facetBy="Factor",lineBy="Replicate")
```

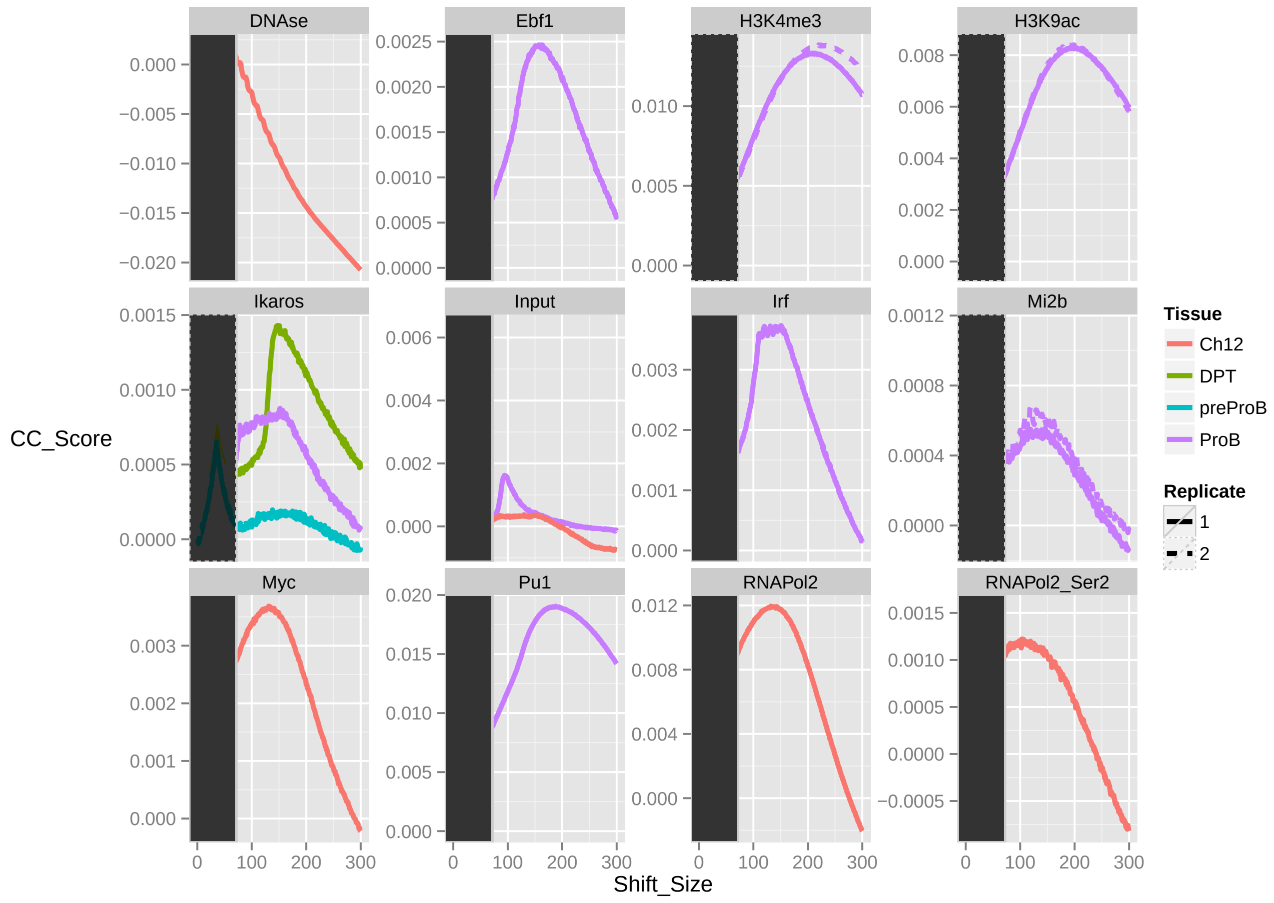


From this, it is immediately apparent that some samples not only have much higher scores, and hence efficiency, than others but that there fragment lengths appear to be very different from each other. The DNAse sample for example has a fragment length less than half of Pu1 sample.

Now we have established the difference in total efficiency, we can look at the overall shape of cross-coverage scores and the relationship between signal peaks and artifact peaks in the cross-coverage scores.

To help better visualise, we will apply a further facet wrap to the ggplot2 object returned by plotCC in order to compare within factors.

```
ccplot <- plotCC(resExperiment,colourBy="Tissue",facetBy="Factor",lineBy="Replicate")
ccplot +facet_wrap(~Factor,scales="free_y")
```



The free scaled cross-coverage score plots now reveal more about the distribution of signal within the samples. The Ebf1, Ikaros, Myc, Ifr and RNA Pol2 all show tight peaks within their cross-coverage score profiles illustrating their sharp binding profiles as expected for a transcription factor (and RNA Pol2 around TSS/Enhancers). The histone marks and Pu1 however show longer more diffused peaks reflecting the wider bredth of signal seen for these epigenetic marks.

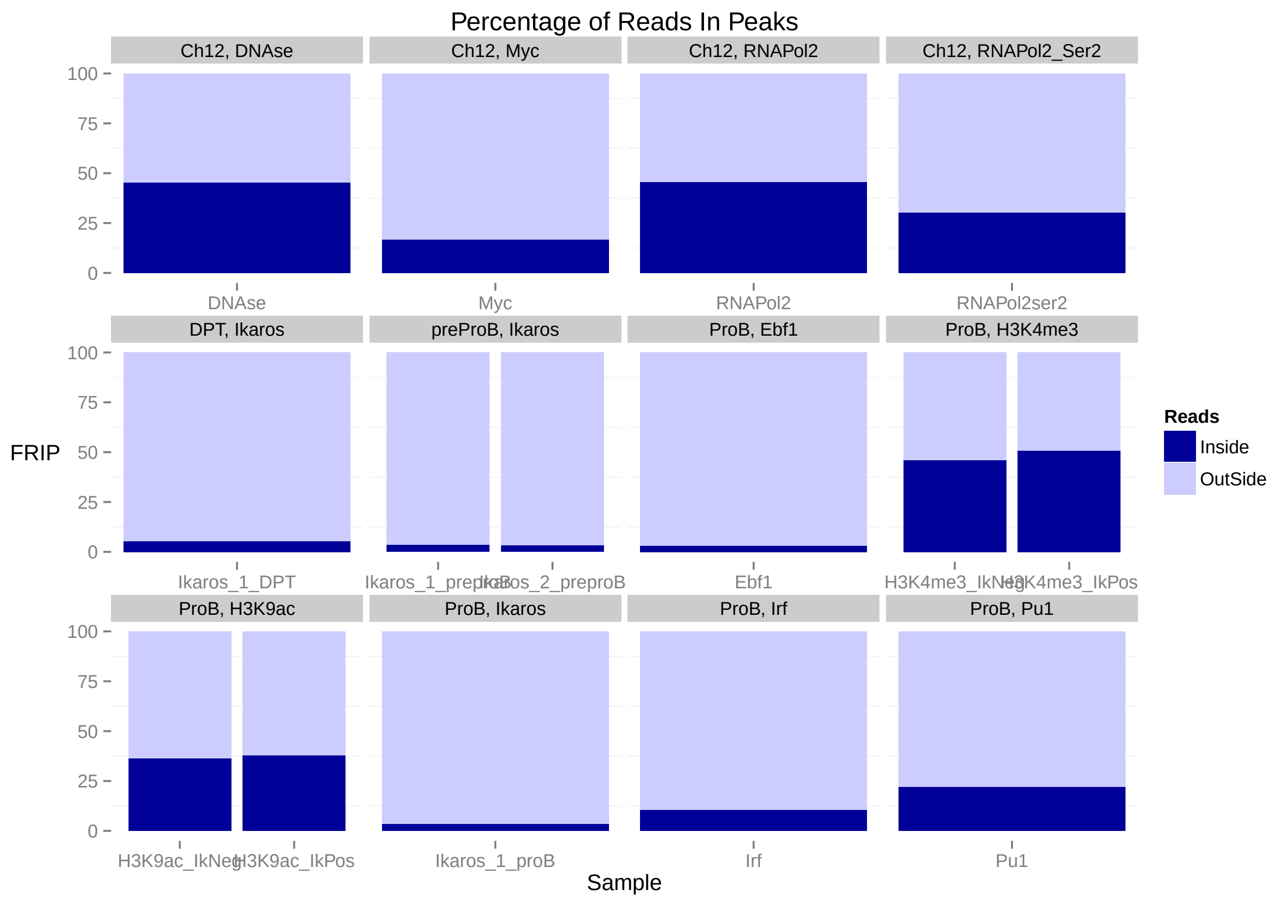
The signal of the Ikaros ChIP between cell lines can also be seen to be highly variable with DP thymocytes containing highest RelCC scores, ProB lower and preProB the lowest. This reflects the increased concentrations of Ikaros along haemopoetic differentiation with DP thymocytes having the highest Ikaros levels.

Finally, an enrichment for fragment length signal can be seen in the ProB input. The sharpness of this enrichment suggests a highly duplicated peak like signal within this track which would be cause for further investigation. This may be from the sono-seq effect commonly observed in input, where gel-selection of fragment lengths for input causes a small peak in cross coverage scores close to selected fragment length.

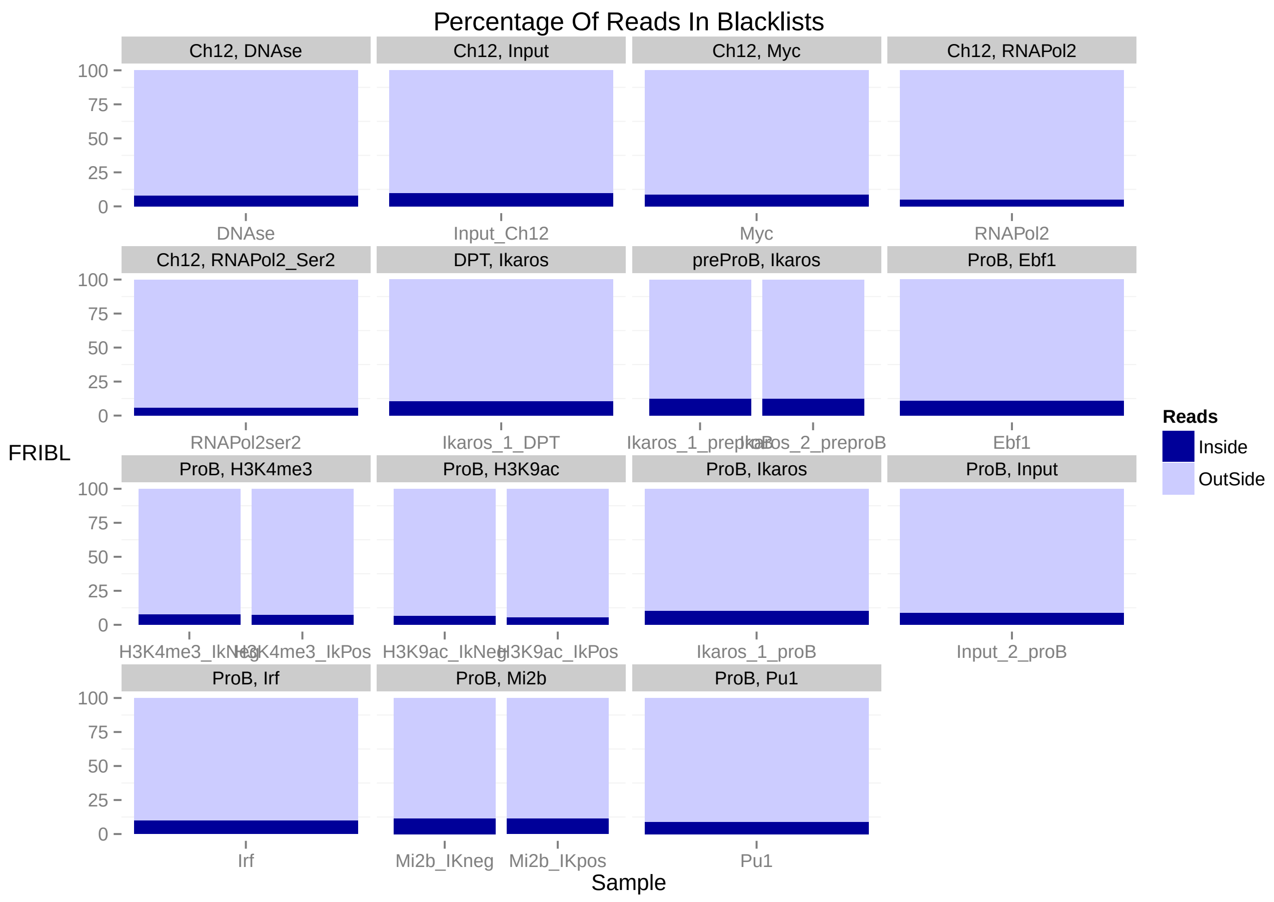
6.2 Distribution of signal within peaks and balcklists

As with the the ChIPQCsample object, the fraction of signal in peaks, blacklists and annotated genomic intervals can provide an understanding of the ChIPs' efficiency and pattern of enrichment.

```
plotFrip(resExperiment)

## Using Sample as id variables
```



```
plotFribl(resExperiment)

## Using Sample as id variables
```



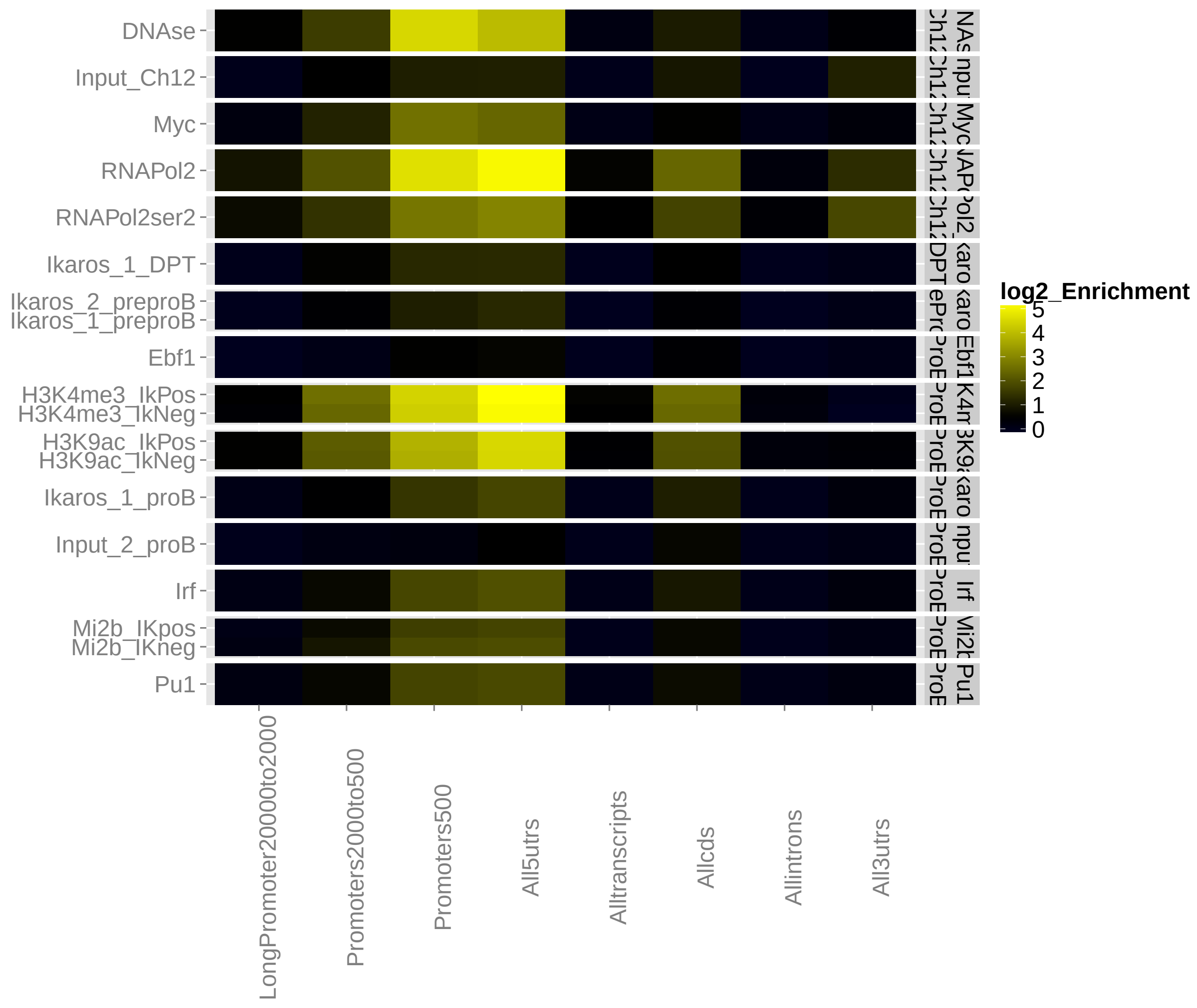
The output from plotFrip immediately identifies the Histone, RNA Pol2 and DNAse ChIP as having the highest enrichment (25% to 50%) for reads in peaks as expected for such epigenetic marks. Also apparent are the significant enrichment seen within Pu1, Myc and Irf whereas the Ikaros ChIPs all show considerably lower enrichment for signal within peaks.

The Fribl plot here shows that all samples have equivalent levels of signal so no outlier or need to investigate signal within known blacklisted regions.

6.3 Distribution of signal in annotated regions

Samples such as RNA Pol2, DNAse and Histone marks will have an expected enrichment for genomic locations. Here RNA Pol2 should be expected to have a stronger enrichment at the TSS than RNA Pol2ser2 where as RNA Pol2ser2 should show enrichment within the 3'UTRs regions.

```
plotRegi(resExperiment)
```



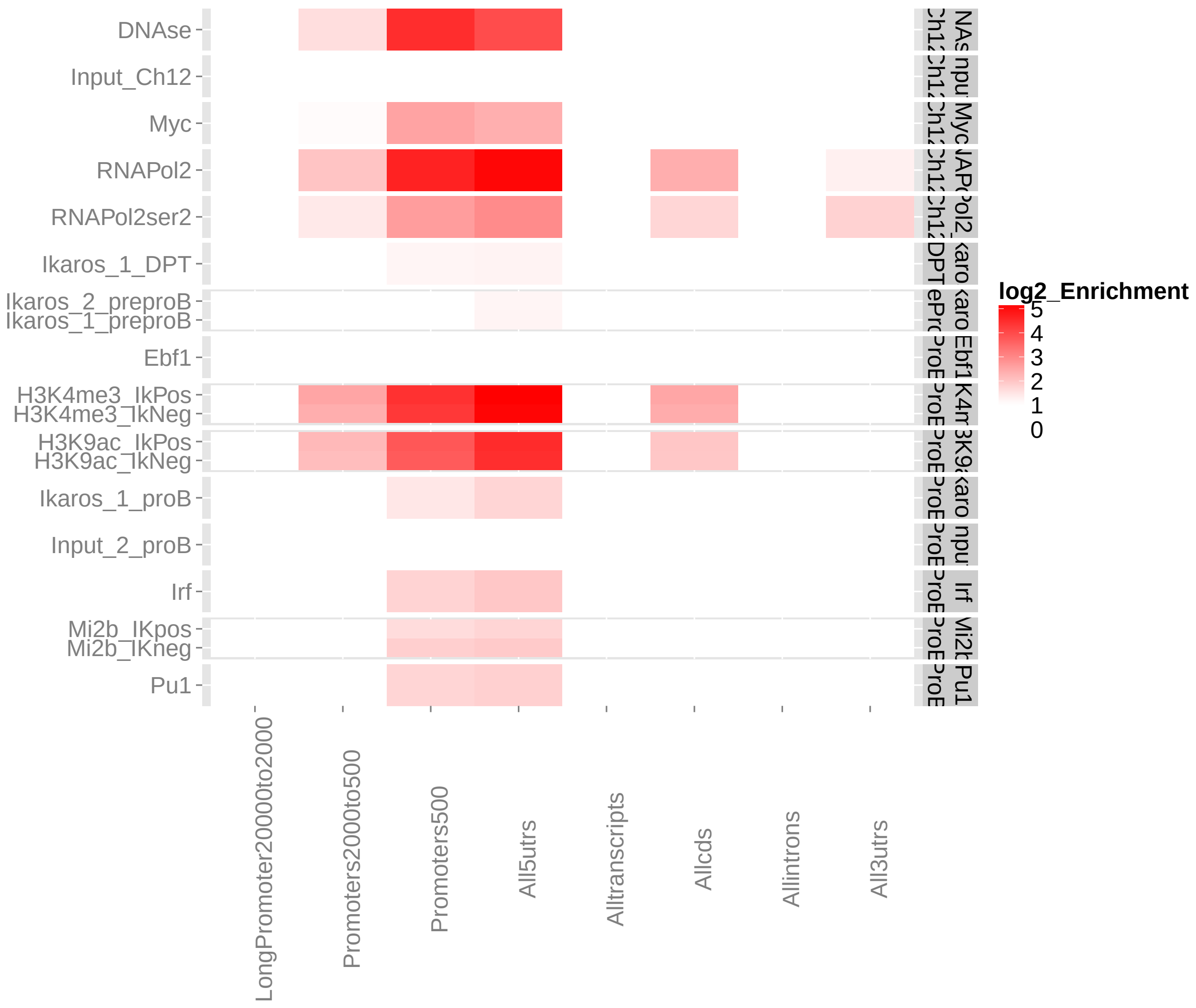
```
regi(resExperiment)["All3utrs",]
```

```
##          DNAse              Ebf1     H3K4me3_IkNeg     H3K4me3_IkPos
##         0.4215            0.0855           -0.0823            0.0188
##      H3K9ac_IkNeg      H3K9ac_IkPos      Ikaros_1_DPT Ikaros_1_preproB
##         0.3931            0.4044            0.1095            0.1080
##     Ikaros_1_proB Ikaros_2_preproB      Input_2_proB       Input_Ch12
##         0.2967            0.1083            0.1527            1.0896
##            Irf       Mi2b_IKneg        Mi2b_IKpos              Myc
##         0.2840            0.1622            0.1627            0.3285
##            Pu1           RNAPol2       RNAPol2ser2
##         0.2329            1.3167            1.8052
```

From the Regi plot it can seen that all histone marks, DNA and RNAPII show the expected enrichment across gene regions. Combined with the output from regi function, the RNA Pol2Ser2 has the greatest enrichment at 3'UTRs and so the expected pattern of enrichment.

To better visualise this enrichment we can adjust the scale to the enrichment seen in Ch12 input for 3'UTRs.

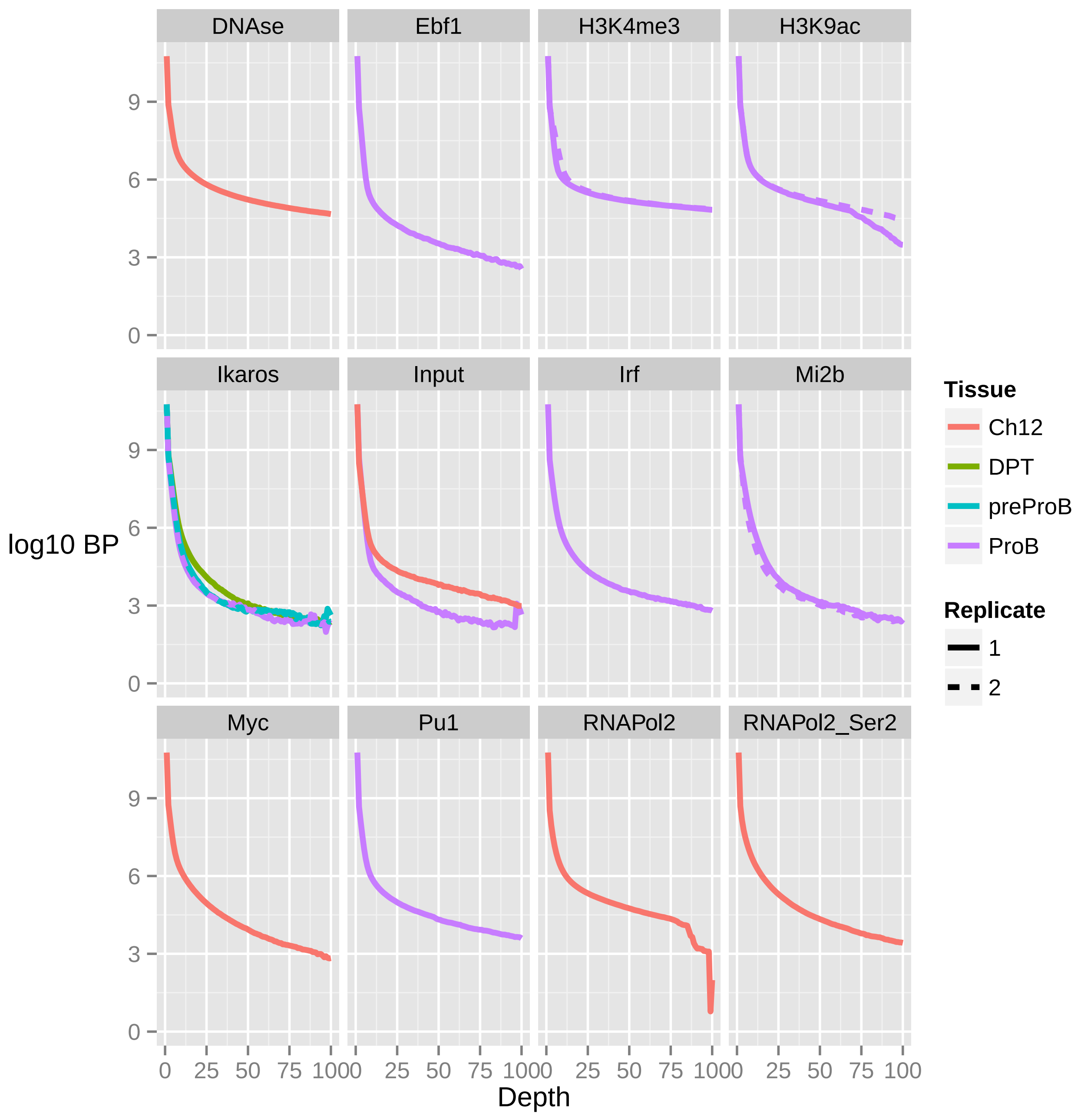
```
plotRegi(resExperiment)+scale_fill_gradient2(low="white",high="red",
    mid="white",midpoint=regi(resExperiment)["All3utrs","Input_Ch12"])

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



6.4 Dispersion of coverage across the genome

Both inputs showed a small but comparatively low enrichment for reads in genetic regions. Such enrichment around gene regions can typically be seen for input samples due to the increased accessibility of chromatin to fragmentation around TSSs. As with the ChIPQCsample object we can plot the coverage histogram and SSD before and after blacklisting by using the `plotCoverageHist` and `plotSSD` functions.

```
plotCoverageHist(resExperiment,facetBy="Factor",colourBy="Tissue",lineBy="Replicate")
```

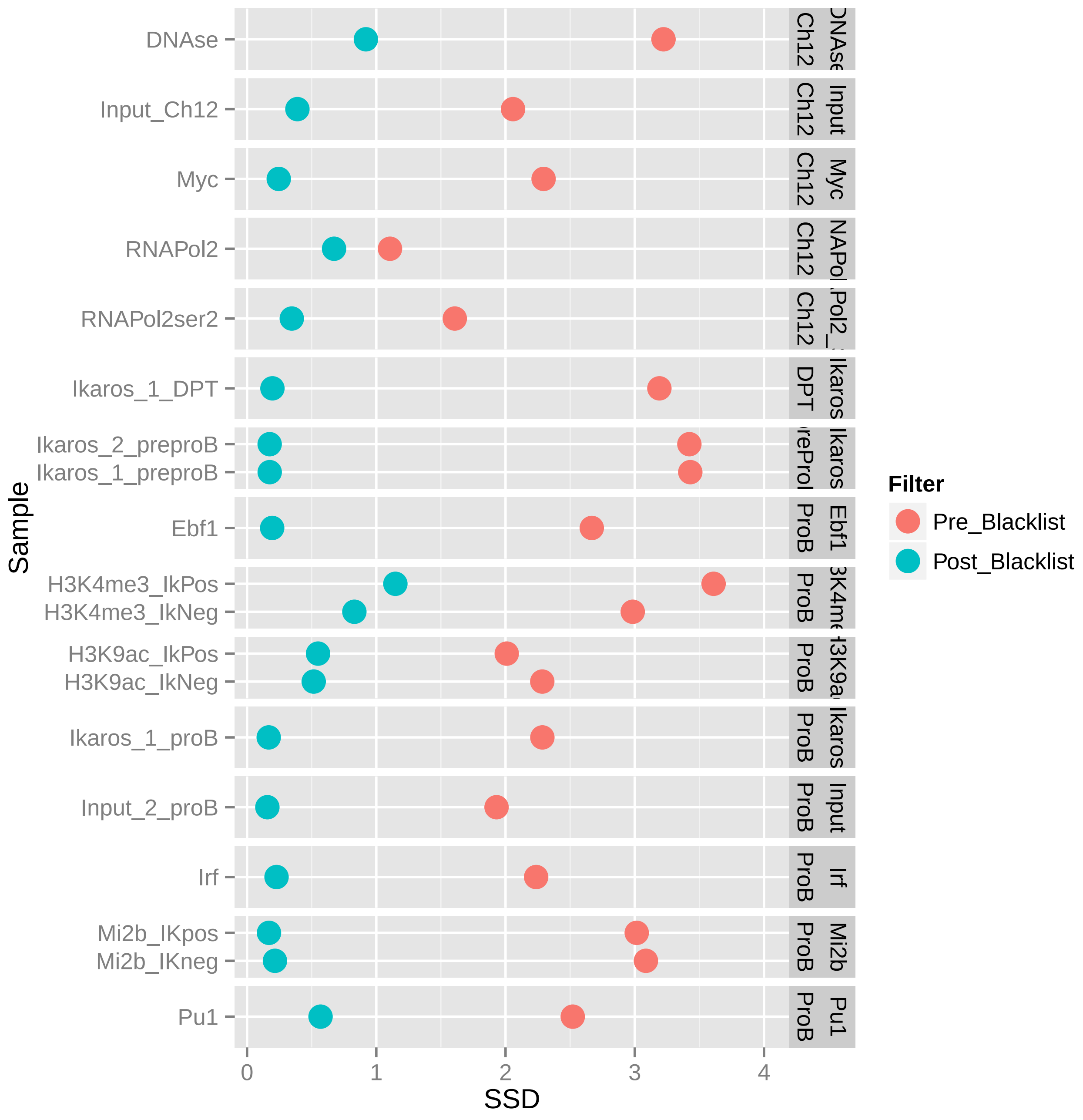


The coverage histogram shows the expected greater spans of high signal in Histone, RNA Pol2 and DNAse ChIPs as well as for transcription factors with both high Rip% and RelCC scores. Although most ProB transcription factors have greater spans of high depth than their input, enrichment for these transcription factors can be seen to be much smaller than observed for Histone,RNA Pol2 and DNAse ChIPs.

The Ikaros ChIPs universally had low enrichment and as seen with other metrics DP thymocytes had the greatest signal.

The two inputs samples show very different patterns of signal depth. The Ch12 input show considerable span of high signal where as the ProB shows a spike in signal at above 98 reads high, in keeping with observed high duplication rate for this sample and fragment length peak in cross-coverage scores.

```
plotSSD(resExperiment)

## Using Sample as id variables
```



The plot of SSD scores before and after blacklisting show that the effect of blacklisting on SSD scores is dramatic.

As with the coverage histogram plots, the histone, polymerase, DNAse marks as well as high scoring TFs have greatest SSDs after blacklisting.

The SSD for Ikaros after blacklisting can be seen to be just above background with again DP thymocytes having the greatest score.

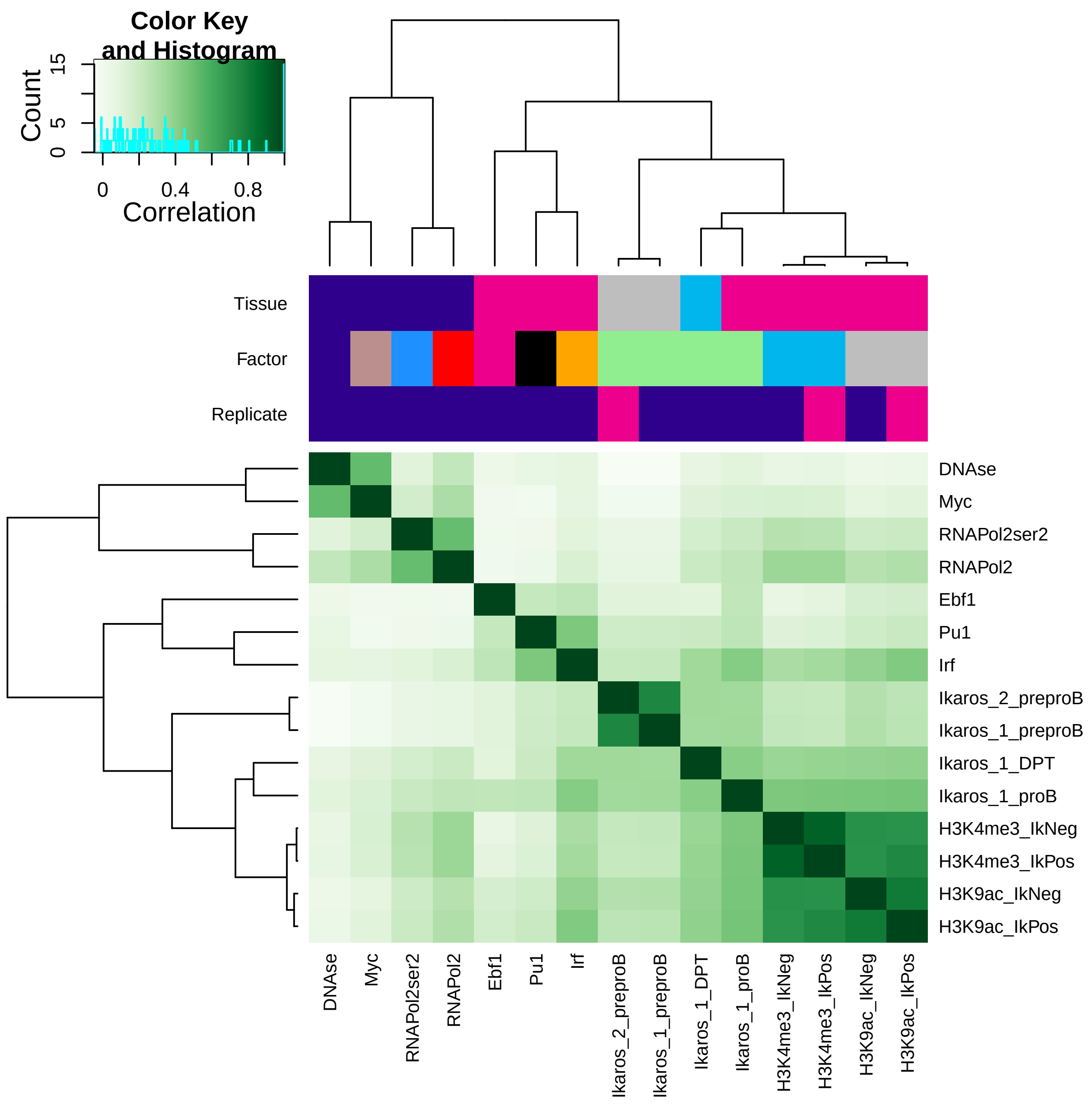
For the inputs, the ProB input can be seen to have its SSD score reduced to a background level of around 0.14 indicating the successful removal of artifact signal. The Ch12 input however can be seen to not drop to the background level after blacklisting suggesting remaining regions of artifact signal. The failure to reduce SSD after blacklisting suggests the persistent presence of artifacts within the Ch12 input and flags this sample for further blacklisting.

6.5 Assessing sample similarity with Diffbind

Use Case: Visualise the correlation between samples

The plotCorHeatmap shows the clustering in correlation heatmap based on correlation values for all the peak scores for each sample:

```
plotCorHeatmap(resExperiment))
```



The clustering and heatmaps generated from plotCorHeatmap allow us to identify which samples are most closely related and so both the reproducibilty of replicates and the similarity in binding profiles of different epigenetic marks. Here we see that all replicates cluster tightly together, illustrating the relative reproducibilty of our replicates. Further to this we see that the samples broadly group into their respective tissues with DNAse, RNA Pol2 and Myc forming the Ch12 cluster. The ProB transcription factor (Pu1, Irf and Ebf) are seen to group together as expected and are found to cluster away from ProB histone marks. This suggests that these transcription factors may be less associated to these histone marks than the Ikaros ChIPs.

6.6 Generating a summary HTML report

A summary HTML report can be generated for this example experiment by invoking ChIPQCreport

```
ChIPQCreport(resExperiment,colourBy="Tissue",facetBy="Factor",lineBy="Replicate")
```

ChIPQC will create a folder called 'ChIPQCreport' in your working directory that contains the html report. You can see the report in your browser by opening file '`ChIPQCreport/ChIPQC.html`'.

tip: to check your working directory type:

```
getwd()
```

6.7 Conclusion

The analysis of the quality of this experiment's ChIP quality using ChIPQC has identified several features of the data as well as highlighted variability in quality across ChIPs and cell-lines.

The Histone , RNA Pol2 and DNAse ChIPs all have high RIP% and SSD after blacklisting as expected from broader epigentic marks. Cross-coverage score profiles illustrate the broader regions of enrichment for the Histone marks, tighter profiles for the RNA Pol2 and RNA Pol2-ser2 marks, due to their narrow enrichment in TSSs and a sharp narrow profile for The DNAse ChIP. These epigenetic marks showed the characteristic enrichment within TSSs with RNApol2-ser2 most enriched for 3'UTRs.

The transcription factor ChIPs showed a much wider variability in RIP%, SSD and RelCC than seen for histone, pol2 and DNAse ChIPs. Pu1 and Irf were found to be highly efficient ChIPs and Pu1 was seen in its cross coverage scores to have a broader enrichment pattern than seen for for other TFs. The Ikaros ChIPs were found to have acceptable but low enrichment for signal by all metrics, and the enrichment for Ikaros ChIP signal was seen to fit known concentrations of Ikaros within these cell-lines. The ProB Pu1, Ebf and Irf transcription factors were found to cluster away from Ikaros ChIPs indicating a greater co-occurrence of binding among them than with Ikaros ChIPs.

The inputs used in this study showed different sources of artifact contamination. The Ch12 input showed a strong artifact, read length, peak in cross-coverage scores and significant pile-up of signal. Following blacklisting it's SSD score did not drop to that of a background level and so flagged this as a control for further blacklisting. The ProB input however showed peak like signal in its cross-coverage scores profile, a high level of duplication and a spike in its coverage histogram but removal of known blacklisted regions removed much of artifact signal as measured by SSD. Taken together, this suggests that the ProB input contains highly duplicated peak shaped spikes in signal which were contained within blacklisted regions.

7 Advanced Topics

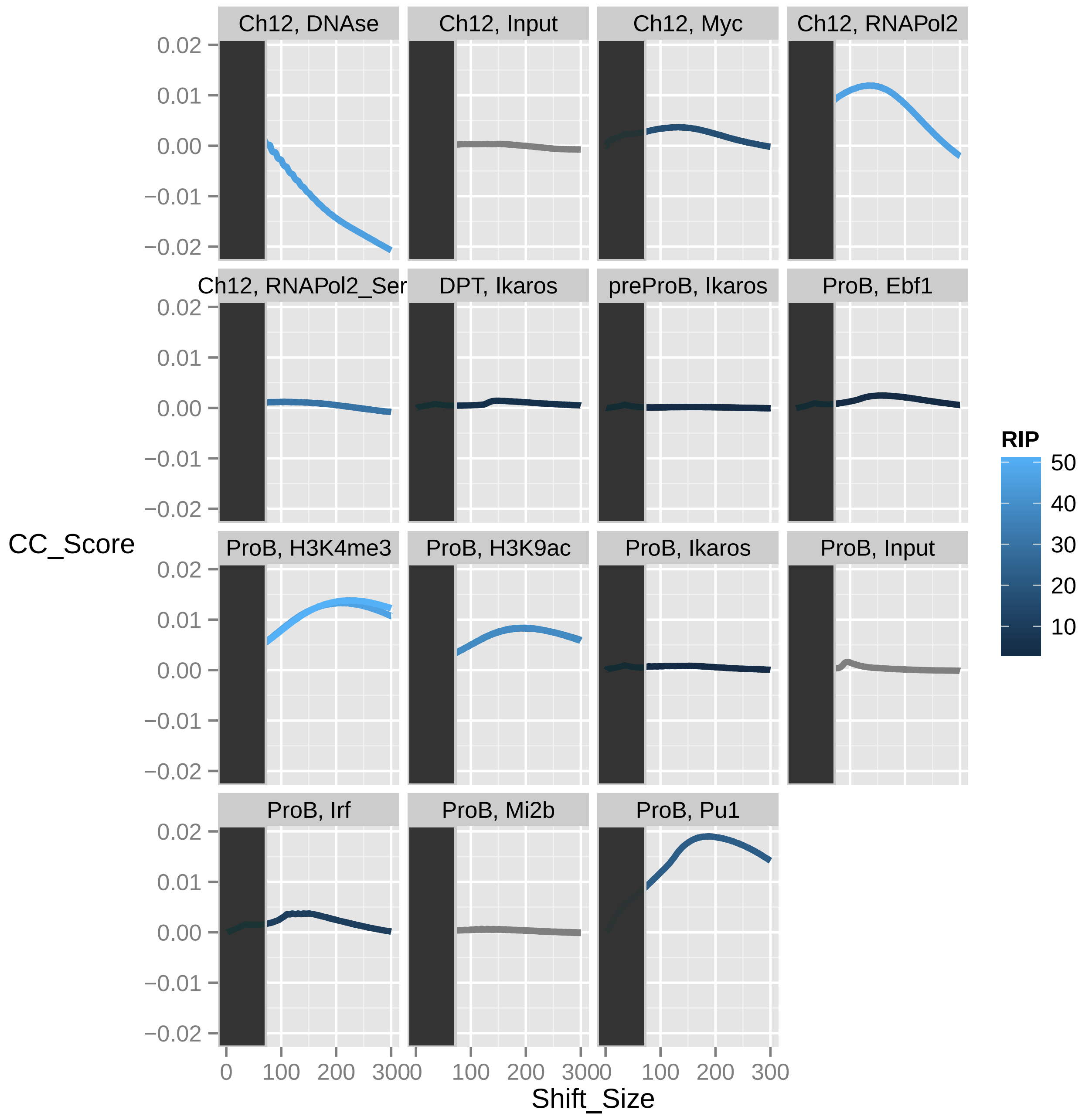
7.1 Providing additional data to ChIPQC plotting and reporting

The sample sheets for ChIPQC may contain the optional metadata columns Tissue,Factor,Condition and Treatment. In order to allow the user to specify custom metadata for their plotting and reporting, the additional addMetadata argument can be supplied with a data frame of sample IDs and associated additional metadata. The first column for addMetadata data frame must be SampleID and remaining columns may be categorical or discrete data.

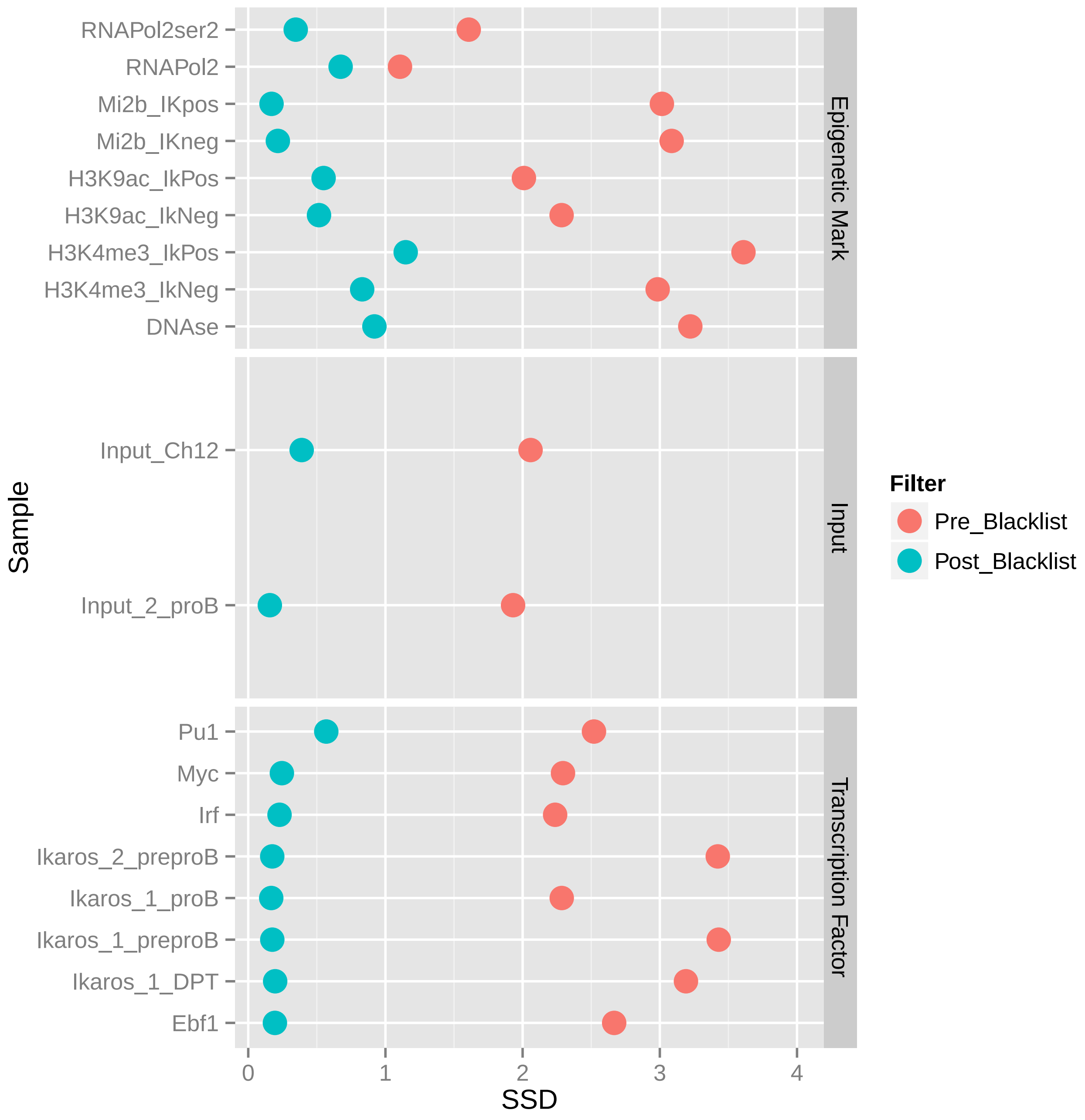
Here, first we illustrate the relationship between RIP and cross coverage scores by including RIP metrics as metadata and setting the column as colourBy argument.

Then we can use addMetadata argument to group SSD by their ChIP type to highlight higher SSD scores typically seen to epigenetic marks.

```
metrics <- QCmetrics(resExperiment)
metricsMetadata <- data.frame(SampleID=rownames(metrics),RIP =metrics[,"RiP%",drop=T])
plotCC(resExperiment,addMetaData = metricsMetadata,colourBy="RIP")
```



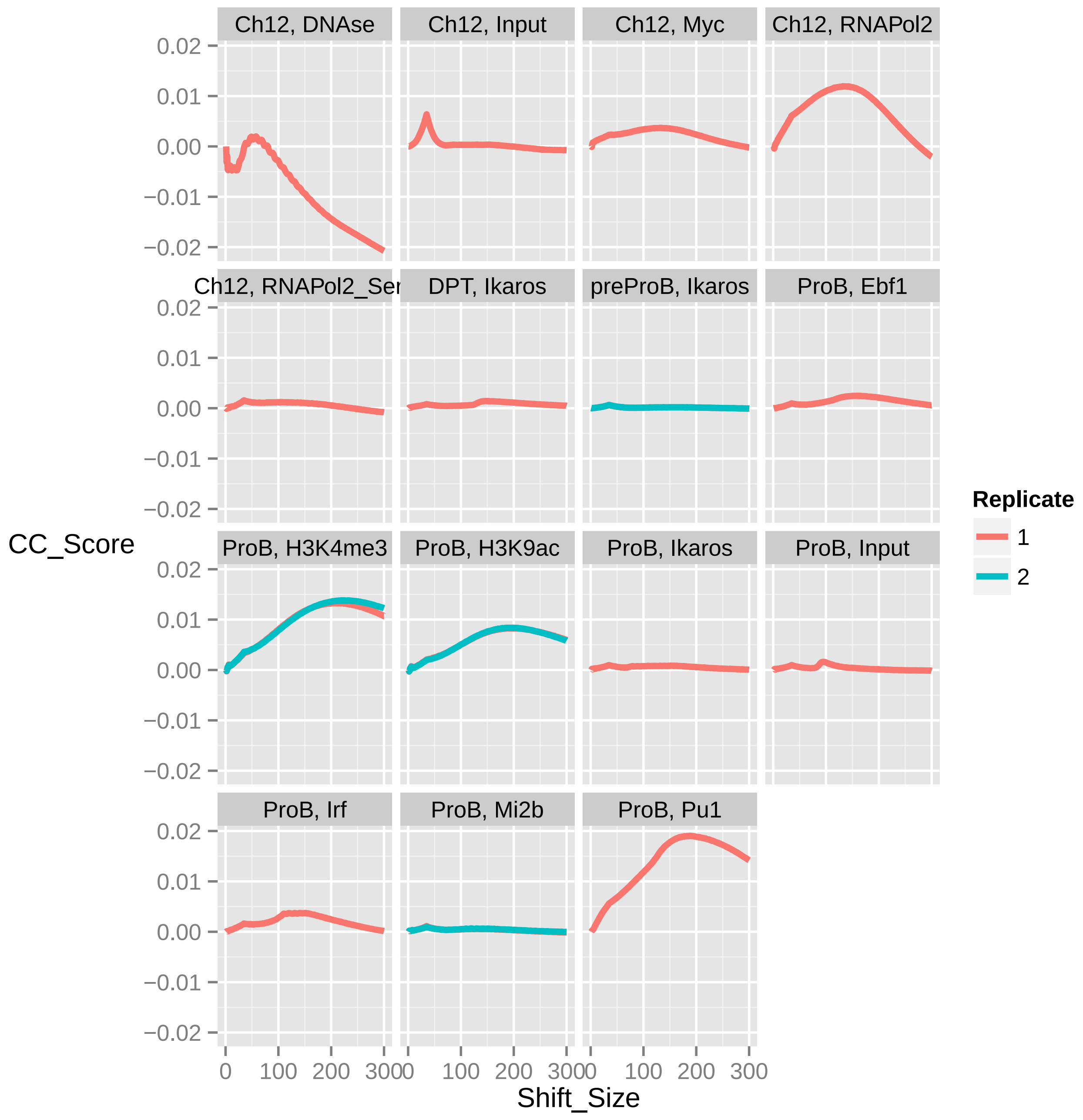
```
metricsMetadata <- data.frame(SampleID=rownames(metrics),ChIPType=
                    c(rep("Epigenetic Mark",1),
                    rep("Transcription Factor",1),
                    rep("Epigenetic Mark",4),
                    rep("Transcription Factor",4),
                    rep("Input",2),
                    rep("Transcription Factor",1),
                    rep("Epigenetic Mark",2),
                    rep("Transcription Factor",2),
                    rep("Epigenetic Mark",2)))
plotSSD(resExperiment,addMetaData = metricsMetadata,facetBy="ChIPType")

## Using Sample as id variables
```



7.2 Some plotting tips

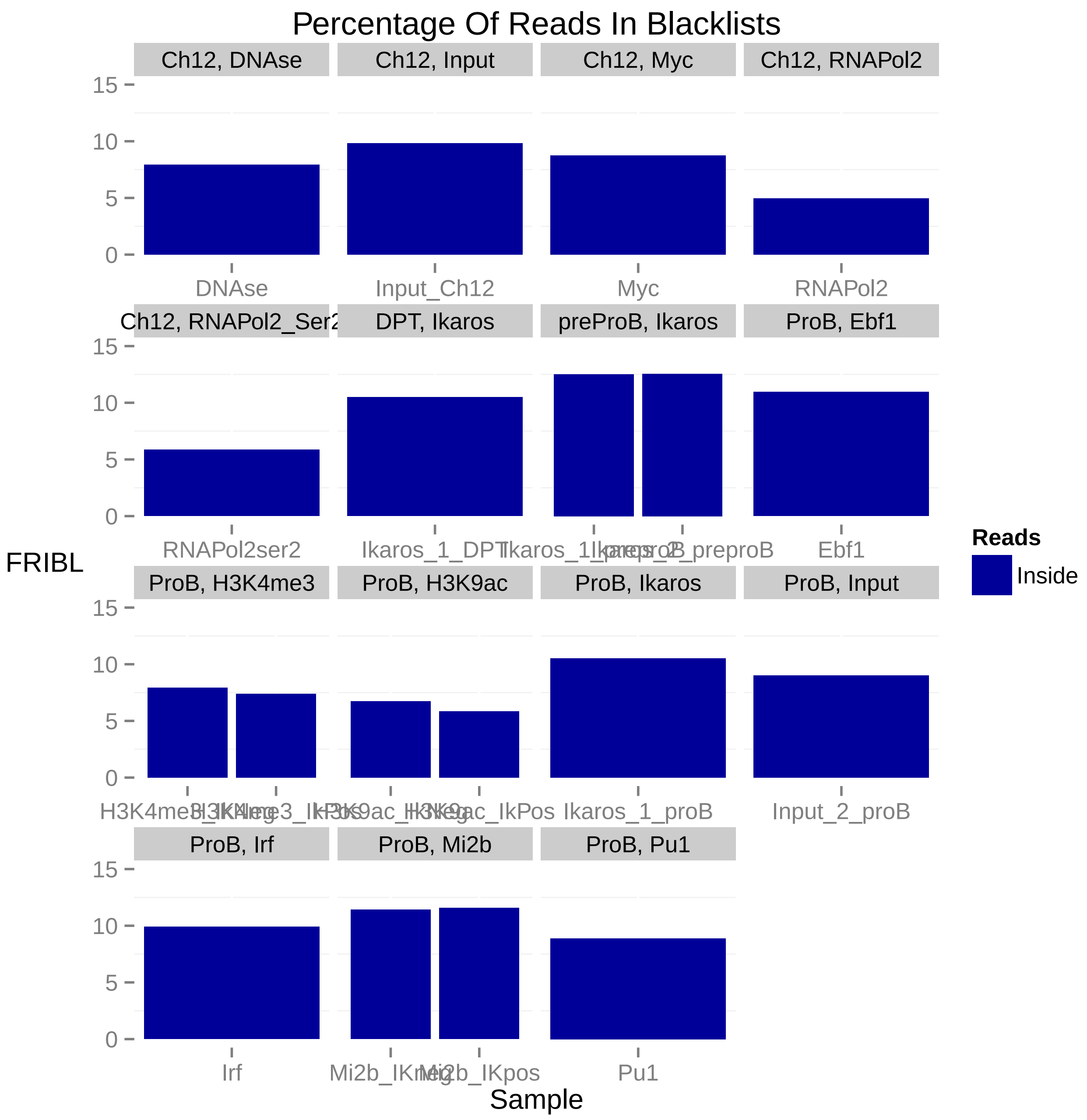
The plotCC function produces a line plot with the section excluded from identification of fragment length cross-coverage score shaded in grey. To remove this the plotCC ggplot object maybe altered and re-plotted.

```
ccplot <- plotCC(resExperiment)
ccplot$layers <- ccplot$layers[1]
ccplot
```

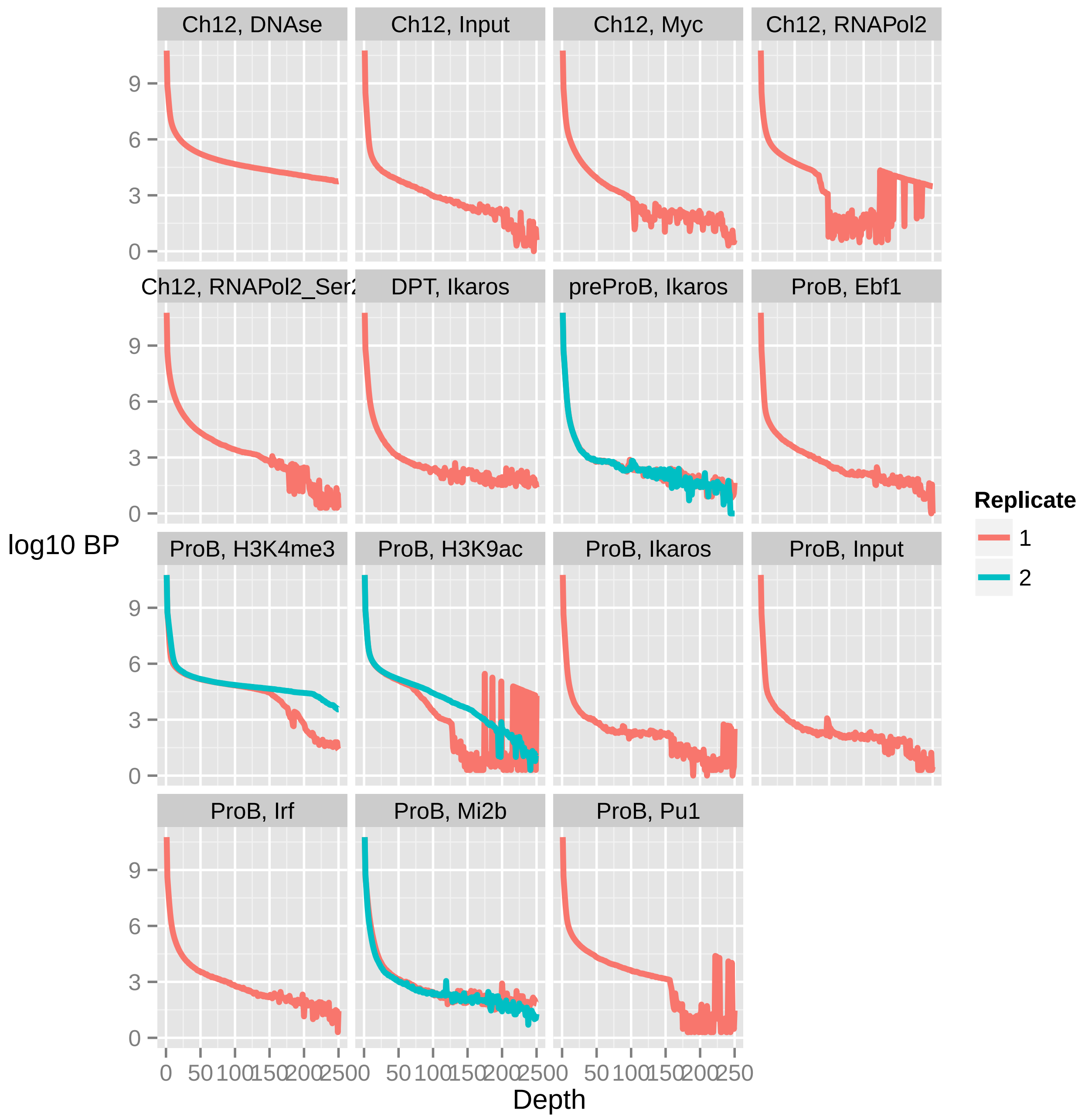


Another useful tip is expanding or narrowing the Y and X axis limits post plotting. This can be done by simply adding the xlim() and ylim() arguments

```
plotFribl(resExperiment) + ylim(0,15)

## Using Sample as id variables
```



```
plotCoverageHist(resExperiment) + xlim(0,250)

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



8 References

[1] Carroll T Liang Z , Salama R, Stark R and Santiago I Impact of artifact removal on ChIP quality metrics in ChIP-seq and ChIP-exo data, Frontiers in Genetics, April 2014.

[2] Carroll T. Assessing ChIP-seq sample quality with ChIPQC. BioC 2014, Boston. 2014 http://www.bioconductor.org/help/course-materials/2014/BioC2014/Bioc2014_ChIPQC_Practical.pdf

[3] Encode guidelines. http://genome.ucsc.edu/ENCODE/qualityMetrics.html

[4] ENCODE portal. https://genome.ucsc.edu/ENCODE/

9 Session Info

```
sessionInfo();
```

```
## R version 3.1.3 (2015-03-09)
## Platform: x86_64-apple-darwin10.8.0 (64-bit)
## Running under: OS X 10.7.5 (Lion)
##
## locale:
## [1] en_GB.UTF-8/en_GB.UTF-8/en_GB.UTF-8/C/en_GB.UTF-8/en_GB.UTF-8
##
## attached base packages:
## [1] stats4    parallel  stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] ChIPQC_1.2.2            DiffBind_1.12.3         limma_3.22.7
##  [4] ggplot2_1.0.1           GenomicAlignments_1.2.2 Rsamtools_1.18.3
##  [7] Biostrings_2.34.1       XVector_0.6.0           GenomicRanges_1.18.4
## [10] GenomeInfoDb_1.2.5      IRanges_2.0.1           S4Vectors_0.4.0
## [13] BiocGenerics_0.12.1     knitr_1.9
##
## loaded via a namespace (and not attached):
##  [1] amap_0.8-14         base64enc_0.1-2     BatchJobs_1.6
##  [4] BBmisc_1.9          Biobase_2.26.0      BiocParallel_1.0.3
##  [7] BiocStyle_1.4.1     bitops_1.0-6        brew_1.0-6
## [10] BSgenome_1.34.1     caTools_1.17.1      checkmate_1.5.2
## [13] chipseq_1.16.0      codetools_0.2-10    colorspace_1.2-6
## [16] DBI_0.3.1           digest_0.6.8        edgeR_3.8.6
## [19] evaluate_0.6        fail_1.2            foreach_1.4.2
## [22] formatR_1.1         gdata_2.13.3        gplots_2.16.0
## [25] grid_3.1.3          gtable_0.1.2        gtools_3.4.2
## [28] highr_0.4.1         hwriter_1.3.2       iterators_1.0.7
## [31] KernSmooth_2.23-14  labeling_0.3        lattice_0.20-30
## [34] latticeExtra_0.6-26 MASS_7.3-39         munsell_0.4.2
## [37] Nozzle.R1_1.1-1     plyr_1.8.2          proto_0.3-10
## [40] RColorBrewer_1.1-2  Rcpp_0.11.6         RCurl_1.95-4.6
## [43] reshape2_1.4.1      RSQLite_1.0.0       rtracklayer_1.26.3
## [46] scales_0.2.4        sendmailR_1.2-1     ShortRead_1.24.0
## [49] stringr_0.6.2       tools_3.1.3         XML_3.98-1.1
## [52] zlibbioc_1.12.0
```