BANCseq KdApp Determination

Hannah Neikes

2022-11-29

This script is an example on how to determine absolute apparent binding affinities (K_d^{Apps}) in native chromatin by sequencing (BANC-seq).

```
library(foreach, quietly = T)
library(doParallel, quietly = T)
library(ComplexHeatmap, quietly = T)
library(circlize, quietly = T)
library(RColorBrewer, quietly = T)
```

1. Load the data

Load a count table of raw counts for each sample at each peak location, as well as information on spike-in reads in each sample.

```
mm10 <- read.csv("mm10_FLAG_YY1_1000nM_peaks.counts",
    stringsAsFactors = F, header = T, sep = "\t")
colnames(mm10)[7:ncol(mm10)] <- gsub("X([0-9]{4}_nM_YY1)",
    "\\1", colnames(mm10)[7:ncol(mm10)])
# make sure the column names in this df match
# with samples names in the spikeIn txt
spikeIn <- read.csv("readCounts_mm10_Yeast_FLAG_YY1.txt",
    stringsAsFactors = F, header = T, sep = "\t")
spikeIn$spikeIn_yeast <- 1e+06/spikeIn$yeast
print(paste("Number of sites before KdApp determination:",
    nrow(mm10)))
```

```
## [1] "Number of sites before KdApp determination: 22470"
```

```
print(paste("Number of tested concentrations:", nrow(spikeIn)))
```

```
## [1] "Number of tested concentrations: 9"
```

```
head(mm10)
```

```
##   Geneid  Chr   Start     End Strand Length 1000_nM_YY1 0564_nM_YY1 0500_nM_YY1
## 1      1 chr1 3670975 3671508      +    534          64          76          55
```

```
## 2      2 chr1 3671277 3671810      +    534          77          61          54
## 3      3 chr1 3671727 3672260      +    534          62          47          51
## 4      4 chr1 3672072 3672605      +    534          58          40          46
## 5      5 chr1 3681429 3681962      +    534          29          15          14
## 6      6 chr1 3852231 3852764      +    534          18          15          17
##   0250_nM_YY1 0125_nM_YY1 0050_nM_YY1 0010_nM_YY1 0001_nM_YY1 0000_nM_YY1
## 1          51          21          18          14          10          13
## 2          43          26          23          14          11          11
## 3          29          17          19           9           8          14
## 4          31          13          19           5           8          11
## 5          12           5          15           6          11           6
## 6          15          12          17           1           3           3
```

2. Normalisation

The following code normalizes reads in each sample to the spike-in DNA, and subsequently calculates the relative binding per tested concentration and site.

```
mm10_kd <- mm10
for (s in spikeIn$sample) {
    mm10_kd[, s] <- mm10[, s] * spikeIn$spikeIn_yeast[spikeIn$sample ==
        s]
}
# fold change of highest concentration over 0 nM
# sample
mm10_kd$fcOverControl <- log2((mm10_kd$`1000_nM_YY1` +
    2 * sd(mm10_kd$`0000_nM_YY1`))/(mm10_kd$`0000_nM_YY1` +
    2 * sd(mm10_kd$`0000_nM_YY1`)))
mm10_kd[, grep("nM", colnames(mm10_kd))] <- (mm10_kd[,
    grep("nM", colnames(mm10_kd))] + 1)/(mm10_kd$`0000_nM_YY1` +
    1)
# relative signal at each binding site for each
# concentration
mm10_kd[, grep("nM", colnames(mm10_kd))] <- mm10_kd[,
    grep("nM", colnames(mm10_kd))]/apply(mm10_kd[,
    grep("nM", colnames(mm10_kd))], 1, max)
head(mm10_kd)
```

```
##   Geneid  Chr   Start     End Strand Length 1000_nM_YY1 0564_nM_YY1 0500_nM_YY1
## 1      1 chr1 3670975 3671508      +    534    0.966694   1.0000000   0.6042279
## 2      2 chr1 3671277 3671810      +    534    1.000000   0.6902142   0.5101113
## 3      3 chr1 3671727 3672260      +    534    1.000000   0.6605065   0.5983055
## 4      4 chr1 3672072 3672605      +    534    1.000000   0.6009474   0.5768908
## 5      5 chr1 3681429 3681962      +    534    1.000000   0.4510472   0.3515890
## 6      6 chr1 3852231 3852764      +    534    1.000000   0.7263133   0.6871863
##   0250_nM_YY1 0125_nM_YY1 0050_nM_YY1 0010_nM_YY1 0001_nM_YY1 0000_nM_YY1
## 1   0.6375952   0.2731234   0.2525032  0.30983136  0.08608027  0.13522254
## 2   0.4622941   0.2906887   0.2773400  0.26641188  0.08138702  0.09843332
## 3   0.3873089   0.2361677   0.2845827  0.21282426  0.07361272  0.15546870
## 4   0.4425319   0.1931478   0.3042008  0.12657373  0.07868732  0.13066514
## 5   0.3429773   0.1490310   0.4802922  0.30348052  0.21598310  0.14286665
## 6   0.6900237   0.5740578   0.8763458  0.08257862  0.09583937  0.11570606
```

```
##   fcOverControl
## 1     0.4009686
## 2     0.4929090
## 3     0.3824430
## 4     0.3738932
## 5     0.2002631
## 6     0.1330309
```

3. K_d^{App} Determination

This is the actual K_d^{App} determination, based on (log10-transformed) concentrations (xValues) and relative binding per peak (yValues).

```
mm10_kd$kd <- NA
mm10_kd$p <- NA
mm10_kd$r <- NA
mm10_kd$n <- NA
xValues <- c(log10(as.numeric(gsub("([0-9]{4})_nM_YY1",
    "\\1", colnames(mm10_kd[, grep("*nM", colnames(mm10_kd))])))))
xValues <- xValues[xValues >= 0]

cl <- makeCluster(10)
registerDoParallel(cl)
ptm <- proc.time()
mm10_kd <- foreach(i = 1:nrow(mm10_kd), .combine = rbind,
    .errorhandling = "remove") %dopar% {
    library(minpack.lm)
    mm10_kdTemp <- mm10_kd[i, ]
    yValues <- c(as.numeric(mm10_kd[i, grep("*nM",
        colnames(mm10_kd[, colnames(mm10_kd) != "0000_nM_YY1"]))]))  # Don't use the 0nM value!
    myModel <- nlsLM(yValues ~ 1/(((kd/xValues)^n) +
        1), start = list(kd = 1, n = 1), control = nls.lm.control(maxiter = 50))
    myCoefs <- coef(myModel)
    myCor <- cor.test(yValues, predict(myModel))
    mm10_kdTemp$kd <- 10^(myCoefs[[1]])
    mm10_kdTemp$p <- myCor$p.value
    mm10_kdTemp$r <- myCor$estimate[[1]]
    mm10_kdTemp$n <- myCoefs[[2]]
    mm10_kdTemp
}
stopCluster(cl)
head(mm10_kd)
```

```
##   Geneid  Chr   Start     End Strand Length 1000_nM_YY1 0564_nM_YY1 0500_nM_YY1
## 1      1 chr1 3670975 3671508      +    534    0.966694   1.0000000   0.6042279
## 2      2 chr1 3671277 3671810      +    534    1.000000   0.6902142   0.5101113
## 3      3 chr1 3671727 3672260      +    534    1.000000   0.6605065   0.5983055
## 4      4 chr1 3672072 3672605      +    534    1.000000   0.6009474   0.5768908
## 5      5 chr1 3681429 3681962      +    534    1.000000   0.4510472   0.3515890
## 6      6 chr1 3852231 3852764      +    534    1.000000   0.7263133   0.6871863
##   0250_nM_YY1 0125_nM_YY1 0050_nM_YY1 0010_nM_YY1 0001_nM_YY1 0000_nM_YY1
## 1   0.6375952   0.2731234   0.2525032  0.30983136  0.08608027  0.13522254
```

```
## 2   0.4622941   0.2906887   0.2773400  0.26641188  0.08138702  0.09843332
## 3   0.3873089   0.2361677   0.2845827  0.21282426  0.07361272  0.15546870
## 4   0.4425319   0.1931478   0.3042008  0.12657373  0.07868732  0.13066514
## 5   0.3429773   0.1490310   0.4802922  0.30348052  0.21598310  0.14286665
## 6   0.6900237   0.5740578   0.8763458  0.08257862  0.09583937  0.11570606
##   fcOverControl        kd           p         r        n
## 1     0.4009686 174.36680 0.001891538 0.9066907 7.075834
## 2     0.4929090 225.38452 0.003841540 0.8810458 4.727067
## 3     0.3824430 272.16071 0.001310139 0.9176756 6.499369
## 4     0.3738932 279.05862 0.001364914 0.9165191 6.194282
## 5     0.2002631 383.61441 0.207707046 0.4993598 1.537376
## 6     0.1330309  36.16809 0.005221303 0.8677758 2.670069
```

4. Select high confidence sites

Select sites with high confidence K_d^{App} fit (based on r- and p-value), and remove outliers. For downstream analysis, we also safe the results.

```
mm10_kd$highConf <- F
mm10_kd$highConf[mm10_kd$p < 0.01 & mm10_kd$r > 0.9] <- T
mm10_kd$outlierMeanSd_n <- F
mm10_kd$outlierMeanSd_kd <- F
my_mean_n <- mean(mm10_kd$n[mm10_kd$highConf])
my_sd_n <- sd(mm10_kd$n[mm10_kd$highConf])
my_up_n <- my_mean_n + (2 * my_sd_n)  # Upper Range
my_low_n <- my_mean_n - (2 * my_sd_n)  # Lower Range
mm10_kd$outlierMeanSd_n[(mm10_kd$n > my_up_n | mm10_kd$n <
    my_low_n)] <- T
my_mean_kd <- mean(mm10_kd$kd[mm10_kd$highConf])
my_sd_kd <- sd(mm10_kd$kd[mm10_kd$highConf])
my_up_kd <- my_mean_kd + (2 * my_sd_kd)  # Upper Range
my_low_kd <- my_mean_kd - (2 * my_sd_kd)  # Lower Range
mm10_kd$outlierMeanSd_kd[(mm10_kd$kd > my_up_kd | mm10_kd$kd <
    my_low_kd)] <- T
mm10_kd$outlierMeanSd <- F
mm10_kd$outlierMeanSd[mm10_kd$outlierMeanSd_n == T &
    mm10_kd$outlierMeanSd_kd != T] <- "n"
mm10_kd$outlierMeanSd[mm10_kd$outlierMeanSd_n != T &
    mm10_kd$outlierMeanSd_kd == T] <- "kd"
mm10_kd$outlierMeanSd[mm10_kd$outlierMeanSd_n == T &
    mm10_kd$outlierMeanSd_kd == T] <- "n_kd"
rm(my_low_kd, my_up_kd, my_sd_kd, my_mean_kd, my_mean_n,
    my_low_n, my_up_n, my_sd_n)

mm10_kd_highConf <- mm10_kd[mm10_kd$highConf == T &
    mm10_kd$outlierMeanSd != "n_kd", ]
write.table(mm10_kd_highConf, file = "highConfPeaks_noOutliers.txt",
    quote = F, col.names = T, row.names = F, sep = "\t")
print(paste("Number of high confidence sites:", nrow(mm10_kd_highConf)))
```

```
## [1] "Number of high confidence sites: 10205"
```

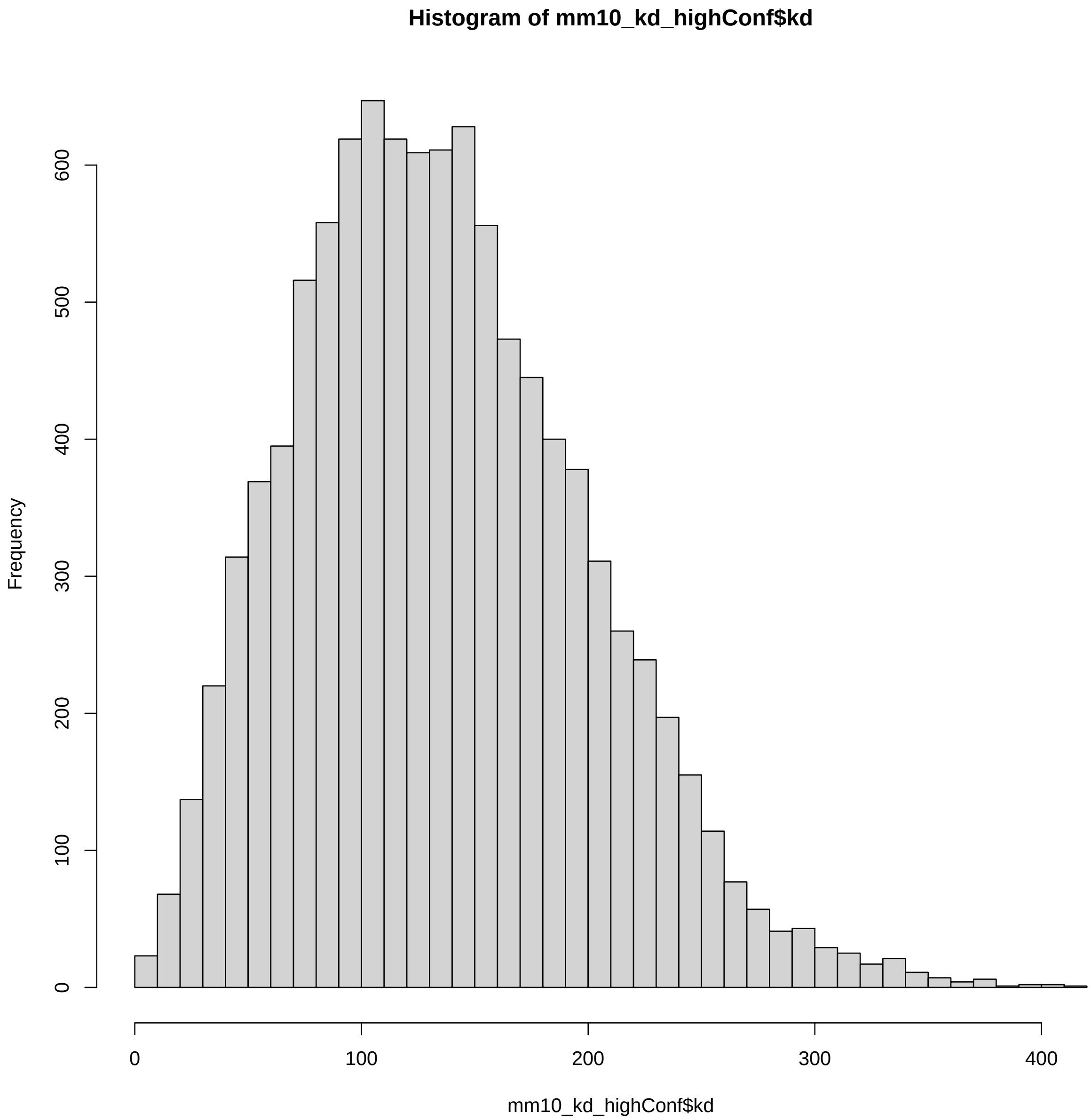
```r
head(mm10_kd_highConf)
```

```
##    Geneid  Chr   Start     End Strand Length 1000_nM_YY1 0564_nM_YY1
## 1       1 chr1 3670975 3671508      +    534   0.9666940   1.0000000
## 3       3 chr1 3671727 3672260      +    534   1.0000000   0.6605065
## 4       4 chr1 3672072 3672605      +    534   1.0000000   0.6009474
## 8       8 chr1 4492613 4493146      +    534   0.9316848   1.0000000
## 9       9 chr1 4493265 4493798      +    534   1.0000000   0.7658913
## 13     13 chr1 4807752 4808285      +    534   1.0000000   0.9170143
##    0500_nM_YY1 0250_nM_YY1 0125_nM_YY1 0050_nM_YY1 0010_nM_YY1 0001_nM_YY1
## 1    0.6042279   0.6375952   0.2731234   0.2525032  0.30983136  0.08608027
## 3    0.5983055   0.3873089   0.2361677   0.2845827  0.21282426  0.07361272
## 4    0.5768908   0.4425319   0.1931478   0.3042008  0.12657373  0.07868732
## 8    0.7501499   0.6472058   0.2435986   0.3089649  0.31690474  0.13257066
## 9    0.7511983   0.7366573   0.2837811   0.3467953  0.06460228  0.10628787
## 13   0.7145244   0.4938274   0.4529814   0.2932666  0.07744753  0.14975085
##    0000_nM_YY1 fcOverControl       kd            p         r        n highConf
## 1    0.1352225     0.4009686 174.3668 0.0018915380 0.9066907 7.075834     TRUE
## 3    0.1554687     0.3824430 272.1607 0.0013101391 0.9176756 6.499369     TRUE
## 4    0.1306651     0.3738932 279.0586 0.0013649143 0.9165191 6.194282     TRUE
## 8    0.1489352     0.3491947 163.4329 0.0012136108 0.9197929 7.383073     TRUE
## 9    0.0832938     0.5747209 138.7901 0.0002744890 0.9515267 5.564925     TRUE
## 13   0.2293404     0.3215770 140.8082 0.0002497142 0.9530494 5.537996     TRUE
##    outlierMeanSd_n outlierMeanSd_kd outlierMeanSd
## 1            FALSE            FALSE         FALSE
## 3            FALSE             TRUE            kd
## 4            FALSE             TRUE            kd
## 8            FALSE            FALSE         FALSE
## 9            FALSE            FALSE         FALSE
## 13           FALSE            FALSE         FALSE
```

5. Plot the data

To have a quick glance at the $K_d{}^{Apps}$ and relative enrichment, we can plot this histogram of $K_d{}^{Apps}$, as well as a Heatmap of the relative enrichment for each site and concentration, alongside the $K_d{}^{App}$. The data frame consists of the peak information, relative signal at each site and concentration, as well as $K_d{}^{Apps}$ and p- and r-values for the Hill curve fit.

```r
hist(mm10_kd_highConf$kd, breaks = 50)
```




```
mm10_kd_highConf <- mm10_kd_highConf[order(-mm10_kd_highConf$kd),
    ]  # sort the sites by KdApp for visualisation
Heatmap(mm10_kd_highConf[, 15:7], cluster_rows = F,
    cluster_columns = F, name = "Relative signal\nat peak",
    width = unit(5, "cm"), top_annotation = HeatmapAnnotation(`Flag-YY1 (nM)` = anno_barplot(as.numeric
        "\\1", colnames(mm10_kd_highConf[, 15:7]))),
        bar_width = 0.75, border = F, axis_param = list(side = "left",
            at = c(0, 500, 1000), labels = c(0, 500,
                1000))), height = unit(1.5, "cm")),
    col = colorRamp2(breaks = c(0, 0.25, 0.5, 0.75,
        1), colors = brewer.pal(5, "YlGnBu"))) + Heatmap(mm10_kd_highConf[,
    "kd"], name = "KdApp", width = unit(0.5, "cm"),
    col = colorRamp2(breaks = c(seq(min(mm10_kd_highConf$kd),
        max(mm10_kd_highConf$kd), length.out = 5)),
```

```
    brewer.pal(5, "Spectral")))
```