R in NGS 实验 3

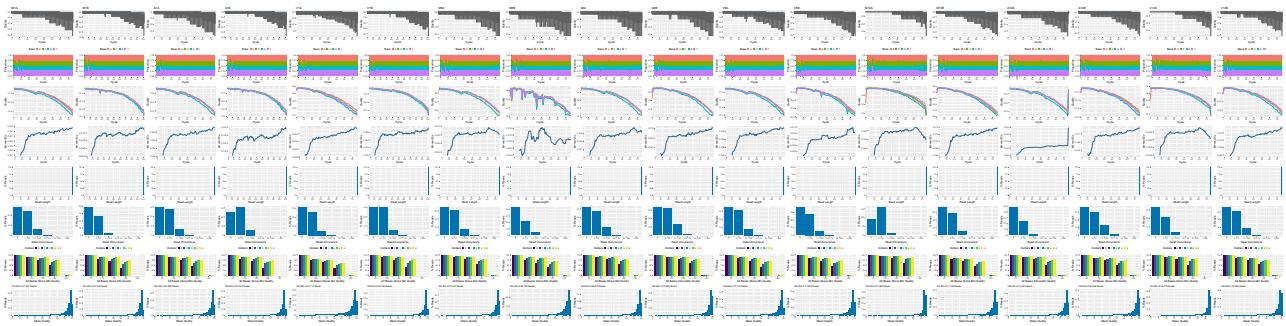
生信 2001 张子栋 2020317210101

1 实验步骤及结果

```
> library(systemPipeR)
> library(systemPipeRdata)
> setwd(choose.dir())
> genWorkenvir(workflow = "chipseq")
> targetsPath <- system.file("extdata", "targets_chip.txt", package =
"systemPipeR")
> targets <- read.delim(targetsPath, comment.char = "#")
> targets[1:4, -c(5, 6)]
                    FileName SampleName Factor SampleLong SampleReference
1 ./data/SRR446027_1.fastq.gz        M1A     M1  Mock.1h.A
2 ./data/SRR446028_1.fastq.gz        M1B     M1  Mock.1h.B
3 ./data/SRR446029_1.fastq.gz        A1A     A1   Avr.1h.A             M1A
4 ./data/SRR446030_1.fastq.gz        A1B     A1   Avr.1h.B             M1B
> dir_path <- system.file("extdata/cwl/preprocessReads/trim-se", package =
"systemPipeR")
> trim <- loadWF(targets = targetsPath, wf_file = "trim-se.cwl", input_file =
"trim-se.yml", dir_path = dir_path)
> trim <- renderWF(trim, inputvars = c(FileName = "_FASTQ_PATH1_", SampleName =
"_SampleName_"))
> output(trim)[1:2]
$M1A
$M1A$`trim-se`
[1] "./results/M1A.fastq_trim.gz"


$M1B
$M1B$`trim-se`
[1] "./results/M1B.fastq_trim.gz"
> filterFct <- function(fq, cutoff = 20, Nexceptions = 0) {
+     qcount <- rowSums(as(quality(fq), "matrix") <= cutoff, na.rm = TRUE)
+     fq[qcount <= Nexceptions]
+     # Retains reads where Phred scores are >= cutoff with N
+     # exceptions
+ }
> preprocessReads(args = trim, Fct = "filterFct(fq, cutoff=20, Nexceptions=0)",
+                 batchsize = 1e+05)
44022 processed reads written to file: ./results/M1A.fastq_trim.gz
44927 processed reads written to file: ./results/M1B.fastq_trim.gz
47793 processed reads written to file: ./results/A1A.fastq_trim.gz
41201 processed reads written to file: ./results/A1B.fastq_trim.gz
38549 processed reads written to file: ./results/V1A.fastq_trim.gz
```

```
49362 processed reads written to file: ./results/V1B.fastq_trim.gz
58018 processed reads written to file: ./results/M6A.fastq_trim.gz
41708 processed reads written to file: ./results/M6B.fastq_trim.gz
54241 processed reads written to file: ./results/A6A.fastq_trim.gz
62722 processed reads written to file: ./results/A6B.fastq_trim.gz
52165 processed reads written to file: ./results/V6A.fastq_trim.gz
50684 processed reads written to file: ./results/V6B.fastq_trim.gz
41583 processed reads written to file: ./results/M12A.fastq_trim.gz
49316 processed reads written to file: ./results/M12B.fastq_trim.gz
56692 processed reads written to file: ./results/A12A.fastq_trim.gz
58547 processed reads written to file: ./results/A12B.fastq_trim.gz
42475 processed reads written to file: ./results/V12A.fastq_trim.gz
56732 processed reads written to file: ./results/V12B.fastq_trim.gz
> writeTargetsout(x = trim, file = "targets_chip_trim.txt", step = 1,
+                 new_col = "FileName", new_col_output_index = 1, overwrite =
TRUE)
     Written content of 'targetsout(x)' to file: targets_chip_trim.txt
> library(BiocParallel)
> library(batchtools)
> f <- function(x) {
+     targets <- system.file("extdata", "targets_chip.txt", package =
"systemPipeR")
+     dir_path <- system.file("extdata/cwl/preprocessReads/trim-se",
+                             package = "systemPipeR")
+     trim <- loadWorkflow(targets = targets, wf_file = "trim-se.cwl",
+                          input_file = "trim-se.yml", dir_path = dir_path)
+     trim <- renderWF(trim, inputvars = c(FileName = "_FASTQ_PATH1_",
+                                          SampleName = "_SampleName_"))
+     seeFastq(fastq = infile1(trim)[x], batchsize = 1e+05, klength = 8)
+ }
> resources <- list(walltime = 120, ntasks = 1, ncpus = 4, memory = 1024)
> fqlist <- lapply(seq(along = trim), f)
> pdf("./results/fastqReport.pdf", height = 18, width = 4 * length(fqlist))
```

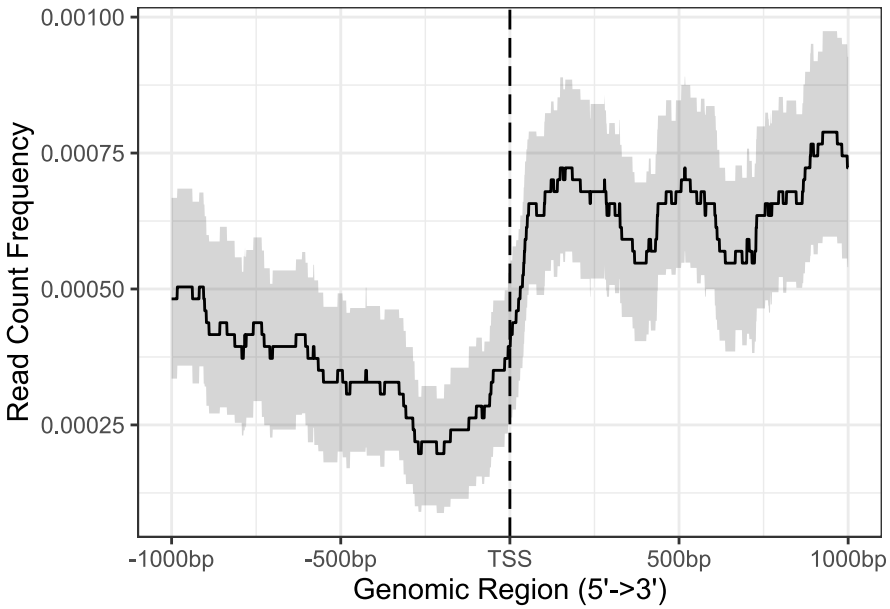


矢量图，可放大。

```
> seeFastqPlot(unlist(fqlist, recursive = FALSE))
> dev.off()
pdf
```

```
  2
> library(ChIPpeakAnno)
> library(GenomicFeatures)
> dir_path <- system.file("extdata/cwl/annotate_peaks", package = "systemPipeR")
> dir_path <- system.file("extdata/cwl/annotate_peaks", package = "systemPipeR")
> args <- loadWF(targets = "targets_macs.txt", wf_file = "annotate_peaks.cwl",
+                input_file = "annotate_peaks.yml", dir_path = dir_path)
> args <- renderWF(args, inputvars = c(FileName = "_FASTQ_PATH1_",
+                                      SampleName = "_SampleName_"))
>
> txdb <- makeTxDbFromGFF(file = "data/tair10.gff", format = "gff",
+                         dataSource = "TAIR", organism = "Arabidopsis thaliana")
> for (i in seq(along = args)) {
+     peaksGR <- as(read.delim(infile1(args)[i], comment = "#"),
+                   "GRanges")
+     annotatedPeak <- annotatePeakInBatch(peaksGR, AnnotationData = genes(txdb))
+     df <- data.frame(as.data.frame(annotatedPeak),
as.data.frame(values(ge[values(annotatedPeak)$feature,
+     ])))
+     df$tx_type <- unlist(df$tx_type)
+     tx_name <- c()
+     for (j in df$tx_name){
+         tx_name <- rbind(tx_name, j[1])
+     }
+     df$tx_name <- tx_name
+     outpaths <- subsetWF(args, slot = "output", subset = 1, index = 1)
+     write.table(df, outpaths[i], quote = FALSE, row.names = FALSE,
+                 sep = "\t")
+ }
> writeTargetsout(x = args, file = "targets_peakanno.txt", step = 1,
+                 new_col = "FileName", new_col_output_index = 1, overwrite =
TRUE)
> library(ChIPseeker)
> for (i in seq(along = args)) {
+     peakAnno <- annotatePeak(infile1(args)[i], TxDb = txdb, verbose = FALSE)
+     df <- as.data.frame(peakAnno)
+     outpaths <- subsetWF(args, slot = "output", subset = 1, index = 1)
+     write.table(df, outpaths[i], quote = FALSE, row.names = FALSE,
+                 sep = "\t")
+ }
> writeTargetsout(x = args, file = "targets_peakanno.txt", step = 1,
+                 new_col = "FileName", new_col_output_index = 1, overwrite =
TRUE)
> library(ChIPseeker)
> for (i in seq(along = args)) {
+     peakAnno <- annotatePeak(infile1(args)[i], TxDb = txdb, verbose = FALSE)
+     df <- as.data.frame(peakAnno)
```

```
+     outpaths <- subsetWF(args, slot = "output", subset = 1, index = 1)
+     write.table(df, outpaths[i], quote = FALSE, row.names = FALSE,
+                 sep = "\t")
+ }
> writeTargetsout(x = args, file = "targets_peakanno.txt", step = 1,
+                 new_col = "FileName", new_col_output_index = 1, overwrite =
TRUE)
     Written content of 'targetsout(x)' to file: targets_peakanno.txt
> peak <- readPeakFile(infile1(args)[1])
> covplot(peak, weightCol = "X.log10.pvalue.")
> outpaths <- subsetWF(args, slot = "output", subset = 1, index = 1)
> peakHeatmap(outpaths[1], TxDb = txdb, upstream = 1000, downstream = 1000,
+             palette = 'Reds')
> plotAvgProf2(outpaths[1], TxDb = txdb, upstream = 1000, downstream = 1000,
conf=0.05,
+              xlab = "Genomic Region (5'->3')", ylab = "Read Count Frequency")
```




```
> library(GenomicRanges)
> dir_path <- system.file("extdata/cwl/count_rangesets", package = "systemPipeR")
> args <- loadWF(targets = "targets_macs.txt", wf_file = "count_rangesets.cwl",
+                input_file = "count_rangesets.yml", dir_path = dir_path)
> args <- renderWF(args, inputvars = c(FileName = "_FASTQ_PATH1_",
+                                      SampleName = "_SampleName_"))
> targets <- system.file("extdata", "targets_chip.txt", package = "systemPipeR")
> dir_path <- system.file("extdata/cwl/bowtie2", package = "systemPipeR")
> args_bam <- loadWF(targets = targets, wf_file = "bowtie2-mapping-se.cwl",
+                    input_file = "bowtie2-mapping-se.yml", dir_path = dir_path)
> args_bam <- renderWF(args_bam, inputvars = c(FileName = "_FASTQ_PATH1_",
+                                              SampleName = "_SampleName_"))
> args_bam <- output_update(args_bam, dir = FALSE, replace = TRUE,
+                           extension = c(".sam", ".bam"))
```

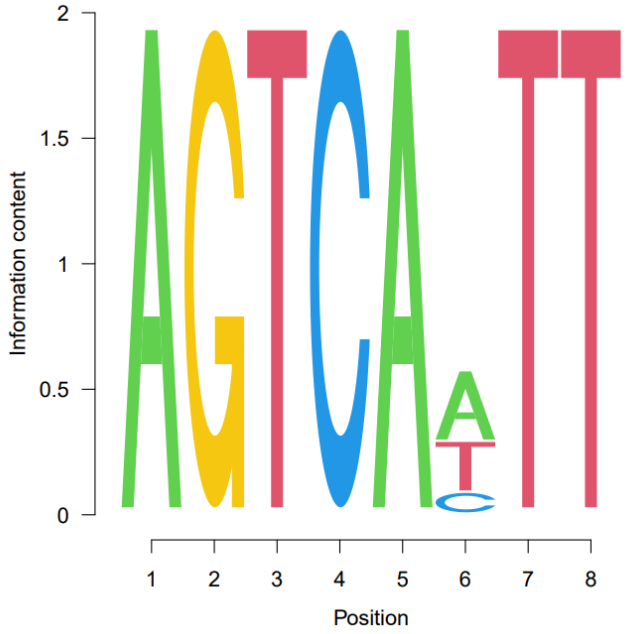
```
> outpaths <- subsetWF(args_bam, slot = "output", subset = 1, index = 1)
> bfl <- BamFileList(outpaths, yieldSize = 50000, index = character())
> # countDFnames <- countRangeset(bfl, args, mode = "Union", ignore.strand = TRUE) # skipped
> # writeTargetsout(x = args, file = "targets_countDF.txt", step = 1, new_col = "FileName", new_col_output_index = 1, overwrite = TRUE) # skipped
> library(GenomicRanges)
> dir_path <- system.file("extdata/cwl/count_rangesets", package = "systemPipeR")
> args <- loadWF(targets = "targets_macs.txt", wf_file = "count_rangesets.cwl",
+                input_file = "count_rangesets.yml", dir_path = dir_path)
> args <- renderWF(args, inputvars = c(FileName = "_FASTQ_PATH1_",
+                                      SampleName = "_SampleName_"))
>
> ## Bam Files
> targets <- system.file("extdata", "targets_chip.txt", package = "systemPipeR")
> dir_path <- system.file("extdata/cwl/bowtie2", package = "systemPipeR")
> args_bam <- loadWF(targets = targets, wf_file = "bowtie2-mapping-se.cwl",
+                    input_file = "bowtie2-mapping-se.yml", dir_path = dir_path)
> args_bam <- renderWF(args_bam, inputvars = c(FileName = "_FASTQ_PATH1_",
+                                              SampleName = "_SampleName_"))
> args_bam <- output_update(args_bam, dir = FALSE, replace = TRUE,
+                           extension = c(".sam", ".bam"))
> outpaths <- subsetWF(args_bam, slot = "output", subset = 1, index = 1)
> bfl <- BamFileList(outpaths, yieldSize = 50000, index = character())
> countDFnames <- countRangeset(bfl, args, mode = "Union", ignore.strand = TRUE)
> dir_path <- system.file("extdata/cwl/rundiff", package = "systemPipeR")
> args_diff <- loadWF(targets = "targets_countDF.txt", wf_file = "rundiff.cwl",
+                     input_file = "rundiff.yml", dir_path = dir_path)
> args_diff <- renderWF(args_diff, inputvars = c(FileName = "_FASTQ_PATH1_",
+                                                SampleName = "_SampleName_"))
>
> cmp <- readComp(file = args_bam, format = "matrix")
> dbrlist <- runDiff(args = args_diff, diffFct = run_edgeR, targets = targets.as.df(targets(args_bam)),
+                    cmp = cmp[[1]], independent = TRUE, dbrfilter = c(Fold = 2,
+                                                                      FDR = 1))
Disp = 0.25073 , BCV = 0.5007
Disp = 0.15223 , BCV = 0.3902
Disp = 0.19792 , BCV = 0.4449
Disp = 0.11692 , BCV = 0.3419
Disp = 0.09286 , BCV = 0.3047
Disp = 0.14312 , BCV = 0.3783
Disp = 0.17049 , BCV = 0.4129
Disp = 0.09671 , BCV = 0.311
Disp = 0.14805 , BCV = 0.3848
Wrote count result 1 to M1A_peaks.edgeR.xls
Saved plot 1 to M1A_peaks.edgeR.xls.pdf
```

```
Disp = 0.23843 , BCV = 0.4883
Disp = 0.11547 , BCV = 0.3398
Disp = 0.21655 , BCV = 0.4653
Disp = 0.04269 , BCV = 0.2066
Disp = 0.15216 , BCV = 0.3901
Disp = 0.12235 , BCV = 0.3498
Disp = 0.20886 , BCV = 0.457
Disp = 0.12322 , BCV = 0.351
Disp = 0.20853 , BCV = 0.4567
Wrote count result 2 to A1A_peaks.edgeR.xls
Saved plot 2 to A1A_peaks.edgeR.xls.pdf
Disp = 0.10458 , BCV = 0.3234
Disp = 0.13358 , BCV = 0.3655
Disp = 0.09085 , BCV = 0.3014
Disp = 0.06552 , BCV = 0.256
Disp = 0.13023 , BCV = 0.3609
Disp = 0.1174 , BCV = 0.3426
Disp = 0.09355 , BCV = 0.3059
Disp = 0.08648 , BCV = 0.2941
Disp = 0.0827 , BCV = 0.2876
Wrote count result 3 to V1A_peaks.edgeR.xls
Saved plot 3 to V1A_peaks.edgeR.xls.pdf
Disp = 0.19174 , BCV = 0.4379
Disp = 0.12306 , BCV = 0.3508
Disp = 0.15138 , BCV = 0.3891
Disp = 0.13047 , BCV = 0.3612
Disp = 0.09703 , BCV = 0.3115
Disp = 0.16206 , BCV = 0.4026
Disp = 0.17529 , BCV = 0.4187
Disp = 0.1089 , BCV = 0.33
Disp = 0.15879 , BCV = 0.3985
Wrote count result 4 to M6A_peaks.edgeR.xls
Saved plot 4 to M6A_peaks.edgeR.xls.pdf
Disp = 0.10874 , BCV = 0.3298
Disp = 0.17742 , BCV = 0.4212
Disp = 0.12824 , BCV = 0.3581
Disp = 0.24666 , BCV = 0.4967
Disp = 0.10608 , BCV = 0.3257
Disp = 0.20981 , BCV = 0.4581
Disp = 0.23416 , BCV = 0.4839
Disp = 0.10848 , BCV = 0.3294
Disp = 0.21298 , BCV = 0.4615
Wrote count result 5 to A6A_peaks.edgeR.xls
Saved plot 5 to A6A_peaks.edgeR.xls.pdf
Disp = 0.12458 , BCV = 0.353
Disp = 0.12455 , BCV = 0.3529
Disp = 0.1462 , BCV = 0.3824
```

```
Disp = 0.0498 , BCV = 0.2232
Disp = 0.05905 , BCV = 0.243
Disp = 0.06298 , BCV = 0.251
Disp = 0.10963 , BCV = 0.3311
Disp = 0.09929 , BCV = 0.3151
Disp = 0.10645 , BCV = 0.3263
Wrote count result 6 to V6A_peaks.edgeR.xls
Saved plot 6 to V6A_peaks.edgeR.xls.pdf
Disp = 0.19246 , BCV = 0.4387
Disp = 0.10992 , BCV = 0.3315
Disp = 0.15488 , BCV = 0.3935
Disp = 0.12168 , BCV = 0.3488
Disp = 0.09003 , BCV = 0.3
Disp = 0.14578 , BCV = 0.3818
Disp = 0.16995 , BCV = 0.4122
Disp = 0.09506 , BCV = 0.3083
Disp = 0.15563 , BCV = 0.3945
Wrote count result 7 to M12A_peaks.edgeR.xls
Saved plot 7 to M12A_peaks.edgeR.xls.pdf
Disp = 0.29779 , BCV = 0.5457
Disp = 0.16126 , BCV = 0.4016
Disp = 0.26423 , BCV = 0.514
Disp = 0.28419 , BCV = 0.5331
Disp = 0.11531 , BCV = 0.3396
Disp = 0.28049 , BCV = 0.5296
Disp = 0.27675 , BCV = 0.5261
Disp = 0.13993 , BCV = 0.3741
Disp = 0.25 , BCV = 0.5
Wrote count result 8 to A12A_peaks.edgeR.xls
Saved plot 8 to A12A_peaks.edgeR.xls.pdf
Disp = 0.22277 , BCV = 0.472
Disp = 0.19447 , BCV = 0.441
Disp = 0.17158 , BCV = 0.4142
Disp = 0.23746 , BCV = 0.4873
Disp = 0.14669 , BCV = 0.383
Disp = 0.24076 , BCV = 0.4907
Disp = 0.20954 , BCV = 0.4578
Disp = 0.11004 , BCV = 0.3317
Disp = 0.21206 , BCV = 0.4605
Wrote count result 9 to V12A_peaks.edgeR.xls
Saved plot 9 to V12A_peaks.edgeR.xls.pdf
> writeTargetsout(x = args_diff, file = "targets_rundiff.txt",
+                 step = 1, new_col = "FileName", new_col_output_index = 1,
+                 overwrite = TRUE)
     Written content of 'targetsout(x)' to file: targets_rundiff.txt
> writeTargetsout(x = args_diff, file = "targets_rundiff.txt",
+                 step = 1, new_col = "FileName", new_col_output_index = 1,
```

```
+                 overwrite = TRUE)
     Written content of 'targetsout(x)' to file: targets_rundiff.txt
> dir_path <- system.file("extdata/cwl/annotate_peaks", package = "systemPipeR")
> args <- loadWF(targets = "targets_bam_ref.txt", wf_file = "annotate_peaks.cwl",
+                input_file = "annotate_peaks.yml", dir_path = dir_path)
> args <- renderWF(args, inputvars = c(FileName1 = "_FASTQ_PATH1_",
+                                      SampleName = "_SampleName_"))
>
> args_anno <- loadWF(targets = "targets_macs.txt", wf_file =
"annotate_peaks.cwl",
+                     input_file = "annotate_peaks.yml", dir_path = dir_path)
> args_anno <- renderWF(args_anno, inputvars = c(FileName = "_FASTQ_PATH1_",
+                                                SampleName = "_SampleName_"))
> annofiles <- subsetWF(args_anno, slot = "output", subset = 1,
+                       index = 1)
> gene_ids <- sapply(names(annofiles), function(x)
unique(as.character(read.delim(annofiles[x])[,
+
              "geneId"])), simplify = FALSE)
> load("data/GO/catdb.RData")
> BatchResult <- GOCluster_Report(catdb = catdb, setlist = gene_ids,
+                                 method = "all", id_type = "gene", CLSZ = 2,
cutoff = 0.9,
+                                 gocats = c("MF", "BP", "CC"), recordSpecGO =
NULL)
> library(Biostrings)
> library(seqLogo)
> library(BCRANK)
> dir_path <- system.file("extdata/cwl/annotate_peaks", package = "systemPipeR")
> args <- loadWF(targets = "targets_macs.txt", wf_file = "annotate_peaks.cwl",
+                input_file = "annotate_peaks.yml", dir_path = dir_path)
> args <- renderWF(args, inputvars = c(FileName = "_FASTQ_PATH1_",
+                                      SampleName = "_SampleName_"))
>
> rangefiles <- infile1(args)
> for (i in seq(along = rangefiles)) {
+     df <- read.delim(rangefiles[i], comment = "#")
+     peaks <- as(df, "GRanges")
+     names(peaks) <- paste0(as.character(seqnames(peaks)), "_",
+                            start(peaks), "-", end(peaks))
+     peaks <- peaks[order(values(peaks)$X.log10.pvalue., decreasing = TRUE)]
+     pseq <- getSeq(FaFile("./data/tair10.fasta"), peaks)
+     names(pseq) <- names(peaks)
+     writeXStringSet(pseq, paste0(rangefiles[i], ".fasta"))
+ }
> set.seed(0)
> BCRANKout <- bcrank(paste0(rangefiles[1], ".fasta"), restarts = 25,
```

```
+                     use.P1 = TRUE, use.P2 = TRUE)
> toptable(BCRANKout)
     Consensus    Score
1     AGTCAHTT 87.08268
2     ATGTNAGA 78.86206
3   CACAHDBAAM 78.43056
4      MGGTATC 77.63990
5    AMRCABAAR 69.68058
6    AWAARBCAA 69.00625
7   DCCDDGAAAS 62.46803
8   RYNTGNCTCT 61.84035
9   DHGABWGGAA 61.19199
10  TGNSHTTCHT 60.77090
11  GHTGABNTTM 60.46803
12  GCHGHTVTVT 58.78979
13  CABKBTGBHA 58.76985
14  AAHTBHCTVC 57.98167
15  DDBCGBCCDT 57.18527
16 TTVGMNAGWTC 56.98050
17 GATTKGBNGAA 55.79763
18 DDHCNGHCTTG 52.71424
19  AKAGAHAAGC 52.56690
20  TBRTAKCTNC 52.18433
21  ACGAWNTBRK 51.60559
22 VWCGTVNDVTT 50.63590
23 CVDGDTCAVVC 50.49847
24  GAHNAMASAC 49.08524
25  CCBASGYNDG 46.22657
> topMotif <- toptable(BCRANKout, 1)
> weightMatrix <- pwm(topMotif, normalize = FALSE)
> weightMatrixNormalized <- pwm(topMotif, normalize = TRUE)
> pdf("results/seqlogo.pdf")
> seqLogo(weightMatrixNormalized)
> dev.off()
RStudioGD
        2
```


Information content
2
1.5
1
0.5
0
1
2
3
4
5
6
7
8
Position