

An Introduction to the CSAR Package

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1 Introduction

We present here a R package for the statistical detection of protein-bound genomic regions, where, considering the average DNA fragment size submitted to sequencing, single-nucleotide enrichment values are obtained. After normalization, sample and control are compared using a test based on the Poisson distribution. Test statistic thresholds to control false discovery rate are obtained through random permutation. The computational efficiency is achieved implanting the most time-consuming functions in C++ language, and integrating them in the R package. Standard outputs of the package are tables of genomic coordinates of significantly enriched region locations, level of enrichment per nucleotide position and the distance of enriched regions to annotated genomic features. The algorithm is described in detail in [1], [2], [3].

2 Methods

Due to PCR artifacts a high number of reads can represent the same sequence. The elimination of these duplicated reads usually leads to a 15%-25% data reduction in a standard plant ChIP-seq experiment. This artifact is strand dependent, therefore CSAR requests that the number of extended reads that overlap a given nucleotide position should be supported by both strands independently. This is achieved by virtually extending the mapped reads to a length of 300 bp (the average DNA fragment length submitted to the sequence process) for each strand independently, and after taken the minimum value for both strand at each nucleotide position.

Before test enrichment between sample and control, the number of overlapped reads distribution of the sample is normalize to have the same mean and

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variance that the control.. Subsequently, a score enrichment is calculated based on the Poisson test or in the ratio.

Permutation is applied to calculate the FDR thresholds. The mapped reads are randomly permuted between the control and sample group, and new scores are calculated for this new permuted dataset. The procedure is repeated until have a enough number of permuted scores. This scores are used to calculate the FDR thresholds.

3 Example

We will use a dataset included in the CSAR package for this demonstration. The data represent a small subset of a SEP3 ChIP-seq experiment in *Arabidopsis* [1].

First, we load the CSAR package and the data *CSAR-dataset*. We use the *mappedReads2Nhits* function to calculate the number of hits (number of extended reads that overlap a particular position) per nucleotide position for the control and sample dataset. The results for each chromosome are saved in a file with the name of the chromosome and the tag used in the parameter *file*. See function *mappedReads2Nhits* for more information about the parameter values.

```
R> library(CSAR)
R> data("CSAR-dataset");
R> head(sampleSEP3_test)
```

	Nhits	lengthRead	strand	chr	pos
1	1	34	-	CHR1v01212004	20729
2	1	34	-	CHR1v01212004	62752
3	1	34	+	CHR1v01212004	8248
4	1	34	+	CHR1v01212004	48961
5	1	34	+	CHR1v01212004	8248
6	1	34	-	CHR1v01212004	55621

```
R> head(controlSEP3_test)
```

	Nhits	lengthRead	strand	chr	pos
1	1	35	-	CHR1v01212004	5714
2	1	35	+	CHR1v01212004	39819
3	1	35	-	CHR1v01212004	34149
4	1	35	+	CHR1v01212004	22988
5	1	35	-	CHR1v01212004	83202
6	1	35	-	CHR1v01212004	56583

```
R> nhitsS<-mappedReads2Nhits(sampleSEP3_test,file="sampleSEP3_test",chr=c("CHR1v01212004"),
R> nhitsC<-mappedReads2Nhits(controlSEP3_test,file="controlSEP3_test",chr=c("CHR1v01212004"),
R> nhitsC$filenames
```

```
[1] "CHR1v01212004_controlSEP3_test.CSARNhits"
```

```
R> nhitsS$filenames
```

```
[1] "CHR1v01212004_sampleSEP3_test.CSARNhits"
```

The variable `nhitsC` and `nhitsS` will have the needed information to use with the function `ChIPseqScore` in order to calculate the read-enrichment score of the sample compared to the control for each nucleotide position. The results are saved in one file per each chromosome. `sigWin` will generate candidate read-enriched regions, and `score2wig` will generate a wig file that can be read by standard genome browsers. `distance2Genes` function will report the relative position of candidate read-enriched regions regarding the start and end position of the annotated genes. `genesWithPeaks` function will report genes with a candidate enriched region located near them.

```
R> test<-ChIPseqScore(control=nhitsC,sample=nhitsS,file="test",times=10000)
```

```
R> test$filenames
```

```
[1] "CHR1v01212004_test.CSARScore"
```

```
R> win<-sigWin(test)
```

```
R> head(win)
```

GRanges object with 6 ranges and 2 metadata columns:

	seqnames	ranges	strand	posPeak	score
	<Rle>	<IRanges>	<Rle>	<numeric>	<numeric>
[1]	CHR1v01212004	1-31	*	1	180343
[2]	CHR1v01212004	332-516	*	332	536943
[3]	CHR1v01212004	974-1259	*	974	180343
[4]	CHR1v01212004	1368-1453	*	1368	180343
[5]	CHR1v01212004	1747-4525	*	2831	1071844
[6]	CHR1v01212004	4842-5004	*	4842	180343

seqinfo: 1 sequence from an unspecified genome

```
R> score2wig(test,file="test.wig",times=10000)
```

```
R> d<-distance2Genes(win=win,gff=TAIR8_genes_test)
```

```
R> d
```

	peakName	p1	p2	score	gene	le
1	CHR1v01212004_974	-2657	-4925	180342.6	AT1G01010	2268
2	CHR1v01212004_1368	-2263	-4531	180342.6	AT1G01010	2268
3	CHR1v01212004_2831	-800	-3068	1071844.4	AT1G01010	2268
4	CHR1v01212004_4842	1211	-1057	180342.6	AT1G01010	2268
5	CHR1v01212004_5108	1477	-791	180342.6	AT1G01010	2268
6	CHR1v01212004_6408	2777	509	180342.6	AT1G01010	2268
7	CHR1v01212004_6408	2329	382	180342.6	AT1G01020	1947
8	CHR1v01212004_6838	3207	939	358643.0	AT1G01010	2268

```

9 CHR1v01212004_6838 1899 -48 358643.0 AT1G01020 1947
10 CHR1v01212004_7422 1315 -632 180342.6 AT1G01020 1947
11 CHR1v01212004_8547 190 -1757 4281250.8 AT1G01020 1947
12 CHR1v01212004_8972 -235 -2182 180342.6 AT1G01020 1947

```

```

R> genes<-genesWithPeaks(d)
R> head(genes)

```

```

      name max3kb1kb      u3000 u2000      u1000      d0
1 AT1G01010  1071844 180342.6      0 1071844.4 180342.6
2 AT1G01020  4281251      0.0      0 180342.6 4281250.8
      d1000
1 358643.0
2 180342.6

```

With each run of the function *permutatedWinScores* one set of permuted scores is generated. Later, we can get the distribution of score values with the function *getPermutatedWinScores*. From this distribution, several cut-off values can be calculated to control the error of our test using functions implemented in R. In this package, it is implemented a control of the error based on FDR using the function *getThreshold*.

```

R> permutatedWinScores(nn=1,sample=sampleSEP3_test,control=controlSEP3_test,fileOutput="test1.txt")
R> permutatedWinScores(nn=2,sample=sampleSEP3_test,control=controlSEP3_test,fileOutput="test2.txt")
R> nulldist<-getPermutatedWinScores(file="test",nn=1:2)
R> getThreshold(winscores=values(win)$score,permutatedScores=nulldist,FDR=.05)

```

```

      threshold Error_type_I      FDR
25 83689.72 0.02755906 0.02755906

```

This is a very simple function to obtain the threshold value of our test statistic controlling FDR at a desired level. Basically, for each possible threshold value, the proportion of error type I is calculated assuming that the permuted score distribution is a optimal estimation of the score distribution under the null hypothesis, and FDR is obtained as the ratio of the proportion of error type I by the proportion of significant tests. Other functions implemented in R (eg: *multtest*) could be less conservative.

References

- [1] Kerstin Kaufmann, Jose M Muino, Ruy Jauregui, Chiara A Airoidi, Cezary Smaczniak, Pawel Krajewski, and Gerco C Angenent. Target genes of the mads transcription factor sepallata3: Integration of developmental and hormonal pathways in the *arabidopsis* flower. *PLoS Biol*, 7(4):e1000090, 04 2009.

- [2] Kerstin Kaufmann, Jose M Muino, Magne Osteras, Laurent Farinelli, Pawel Krajewski, and Gerco C Angenent. Chromatin immunoprecipitation (chip) of plant transcription factors followed by sequencing (chip-seq) or hybridization to whole genome arrays (chip-chip). *Nature Protocols*, (2010).
- [3] Jose M Muino, Kerstin Kaufmann, Roeland C van Ham, Gerco C Angenent, and P Krajewski. Plant chip-seq analyzer: An r package for the statistical detection of protein-bound genomic regions. (2011).

4 Details

This document was written using:

```
R> sessionInfo()
```

```
R version 4.5.0 (2025-04-11 ucrt)
Platform: x86_64-w64-mingw32/x64
Running under: Windows Server 2022 x64 (build 20348)
```

```
Matrix products: default
LAPACK version 3.12.1
```

```
locale:
```

```
[1] LC_COLLATE=C
[2] LC_CTYPE=English_United States.utf8
[3] LC_MONETARY=English_United States.utf8
[4] LC_NUMERIC=C
[5] LC_TIME=English_United States.utf8
```

```
time zone: America/New_York
tzcode source: internal
```

```
attached base packages:
```

```
[1] stats4      stats      graphics  grDevices  utils
[6] datasets    methods    base
```

```
other attached packages:
```

```
[1] CSAR_1.61.0      GenomicRanges_1.61.0
[3] GenomeInfoDb_1.45.4 IRanges_2.43.0
[5] S4Vectors_0.47.0 BiocGenerics_0.55.0
[7] generics_0.1.4
```

```
loaded via a namespace (and not attached):
```

```
[1] httr_1.4.7      compiler_4.5.0   R6_2.6.1
[4] XVector_0.49.0  tools_4.5.0      UCSC.utils_1.5.0
[7] jsonlite_2.0.0
```