# Using the ACME package

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## 1 Overview of ACME

Data obtained from high-density oligonucleotide tiling arrays present new computational challenges for users. ACME (Algorithm for Capturing Microarray Enrichment) is a method for determing genomic regions of enrichment in the context of tiling microarray experiments. ACME identifies signals or "peaks" in tiled array data using a user-defined sliding window of n-base-pairs and a threshold (again, user-defined) strategy to assign a probability value (p-value) of enrichment to each probe on the array. This approach has been applied successfully to at least two different genomic applications involving tiled arrays: ChIP-chip and DNase-chip. However, it can potentially be applied to tiling array data whenever regions of relative enrichment are expected.

The ACME algorithm is quite straightforward. Using a user-defined quantile of the data, called the threshold, any probes in the data that are above that threshold are considered positive probes. For example, if a user chooses a threshold of 0.95, then, of course, 5 percent of the total data are going to be positive probes. To look for enrichment, a sliding window of fix number of base pairs (the chosen window size) is examined centered on each probe. Enrichment is calculated using a chi-square of the number of expected positive probes in the window as compared to the expected number. A p-value is then assigned to each probe.

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Note that these p-values are not corrected for multiple comparisons and should be used as a guide to determining regions of interest rather than a strict statistical significance level.

# 2 Getting Started using ACME

chr1 chr10 chr11 chr12 chr13 chr14

Working on chromosome: chr1 chr10 chr11 ch

#### > library(ACME)

This loads the ACME library.

To illustrate the package, we begin by loading some example data from two nimblegen arrays. The arrays were custom-designed to assay HOX genes in a ChIP-chip experiment.

```
> datdir <- system.file('extdata',package='ACME')</pre>
> fnames <- dir(datdir)</pre>
> example.agff <- read.resultsGFF(fnames,path=datdir)</pre>
[1] "Reading /tmp/RtmpyGMafz/Rinst74d9174a89f/ACME/extdata/testsamp1.gff"
[1] "Reading /tmp/RtmpyGMafz/Rinst74d9174a89f/ACME/extdata/testsamp2.gff"
> example.agff
ACMESet (storageMode: lockedEnvironment)
assayData: 190181 features, 2 samples
  element names: exprs
protocolData: none
phenoData
  sampleNames: testsamp1 testsamp2
  varLabels: fullfnames
  varMetadata: labelDescription
featureData
  featureNames: 74065 74066 ... 103913 (190181 total)
  fvarLabels: chromosome source ... comment (8 total)
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
Annotation:
   Now, a is an R data structure (of class ACMESet) that contains the data from two test
GFF files.
> calc <- do.aGFF.calc(example.agff,window=1000,thresh=0.95)</pre>
Working on sample 1
Working on chromosome:
```

chr15 chr16

chr12 chr13 chr14 chr15 chr16 chr17 chr18

chr17

chr18

chr19

chr19

chr2

chr2

chr20

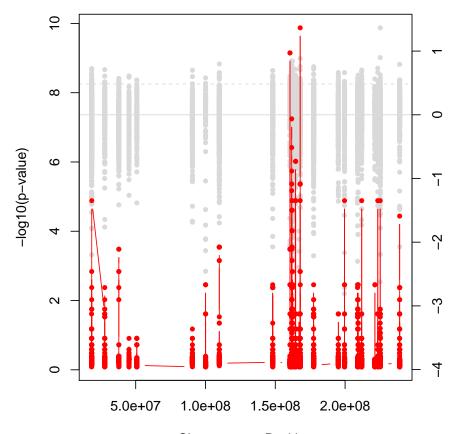
chr20

The function do.aGFF.calc takes as input an *ACMESet* object, a window size (usually 2-3 times the expected fragment size from the experiment and large enough to include about 10 probes, at least), and a threshold which will be used to determine which probes are counted as positive in the chi-square test.

If desired, the results can be plotted in an R graphics window. The raw signal intensities of each oligonucleotide (Chip/total genomic DNA) will be displayed as grey points; corresponding P values will be displayed in red. The dotted horizontal line represents the threshold as defined in the call to do.aGFF.calc. In the following example, R plots the results from an arbitrarily chosen region on chromosome 1, genome coordinates 10,000-50,000.

> plot(calc,chrom='chr1',sample=1)

#### Chromosome: chr1, Sample: testsamp1



Chromosome Position

And one can find significant regions of interest using:

- > regs <- findRegions(calc)</pre>
- > regs[1:5,]

```
Length
                            TF StartInd EndInd
                                                   Sample Chromosome
                                                                          Start
                    918 FALSE
                                       1
testsamp1.chr1.1
                                            918 testsamp1
                                                                 chr1
                                                                       18370933
                          TRUE
                                    919
                                            919 testsamp1
testsamp1.chr1.2
                       1
                                                                       18515429
                                                                 chr1
testsamp1.chr1.3
                    1806 FALSE
                                           2725 testsamp1
                                    920
                                                                 chr1
                                                                       27803112
testsamp1.chr1.4
                       2
                          TRUE
                                   2726
                                           2727 testsamp1
                                                                 chr1 160510960
testsamp1.chr1.5
                     183 FALSE
                                   2728
                                           2910 testsamp1
                                                                 chr1 160512520
                        End
                                  Median
                                                  Mean
                  18514188 5.164139e-01 5.003686e-01
testsamp1.chr1.1
testsamp1.chr1.2
                  18515429 1.308413e-05 1.308413e-05
testsamp1.chr1.3 160504694 4.912989e-01 5.041074e-01
testsamp1.chr1.4 160511031 7.101277e-10 7.101277e-10
testsamp1.chr1.5 161743150 6.079601e-01 5.724538e-01
```

### 2.1 Generating files for viewing in genome browsers

The Affymetrix Integrated Genome Browser (IGB) is a very fast, cross-platform (Java-based) genome browser that can display data in many formats. By generating so-called "sgr" files, one can view both the raw data and the calculated p-values in a fully interactive manner. A simple function, write.sgr, will generate such files that can then be loaded into that browser. The function also serves as a model for how to generate other file formats. With minor modifications, other formats can be generated.

```
> # write both calculated values and raw data
> write.sgr(calc)
./testsamp1_thresh0.95.sgr
./testsamp1_raw.sgr
./testsamp2_thresh0.95.sgr
./testsamp2_raw.sgr
> # OR write only calculated data
> write.sgr(calc,raw=FALSE)
./testsamp1_thresh0.95.sgr
./testsamp2_thresh0.95.sgr
   Export to the UCSC genome browser bedGraph format is also supported.
> # or for the UCSC genome browser
> write.bedGraph(calc)
./testsamp1_thresh0.95.bed
./testsamp1_raw.bed
./testsamp2_thresh0.95.bed
./testsamp2_raw.bed
```