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

```
> 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')
> fnames <- dir(datdir)
> example.agff <- read.resultsGFF(fnames,path=datdir)
```

```
[1] "Reading /tmp/RtmpDitvOc/Rinst3a0b44d21cb8/ACME/extdata/testsamp1.gff"
[1] "Reading /tmp/RtmpDitvOc/Rinst3a0b44d21cb8/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.

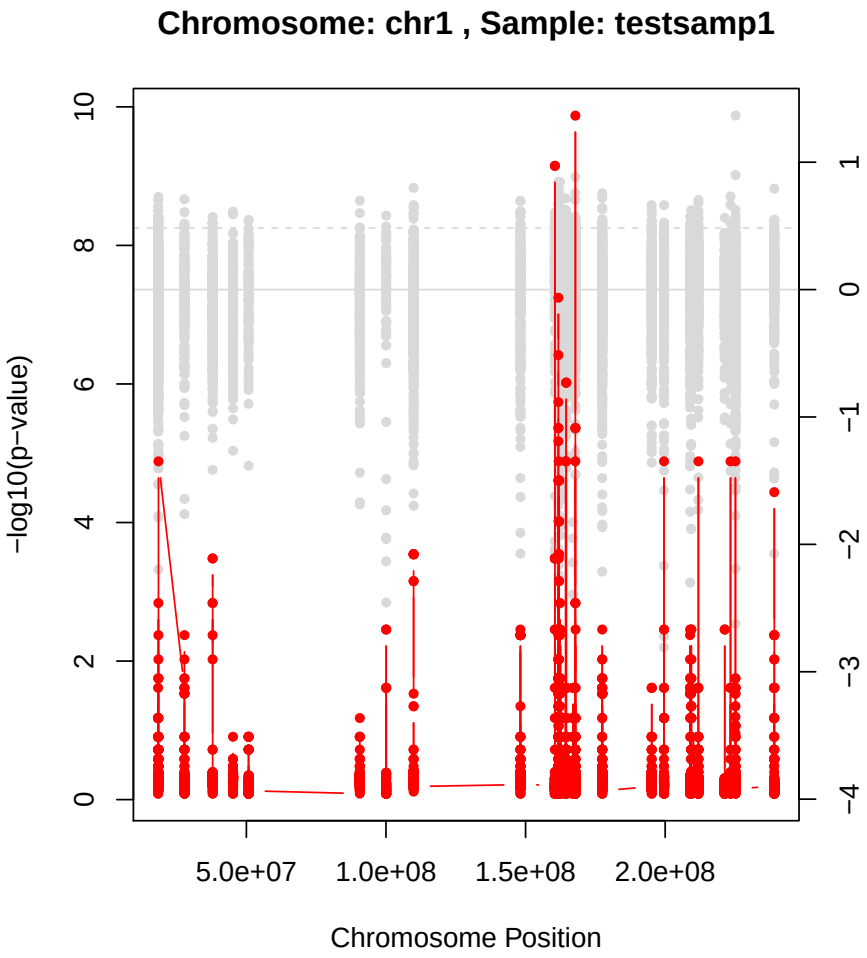
```
> calc <- do.aGFF.calc(example.agff,window=1000,thresh=0.95)
```

```
Working on sample 1
Working on chromosome:
chr1  chr10  chr11  chr12  chr13  chr14  chr15  chr16  chr17  chr18  chr19  chr2  chr20
Working on chromosome:
chr1  chr10  chr11  chr12  chr13  chr14  chr15  chr16  chr17  chr18  chr19  chr2  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)
```



And one can find significant regions of interest using:

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

```
                 Length    TF StartInd EndInd    Sample Chromosome     Start
testsamp1.chr1.1    918 FALSE        1    918 testsamp1       chr1  18370933
testsamp1.chr1.2      1  TRUE      919    919 testsamp1       chr1  18515429
testsamp1.chr1.3   1806 FALSE      920   2725 testsamp1       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
testsamp1.chr1.1  18514188 5.164139e-01 5.003686e-01
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
```