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/Rinst2531774686/ACME/extdata/testsamp1.gff"
[1] "Reading /tmp/Rinst2531774686/ACME/extdata/testsamp2.gff"

> example.agff

ACMESet (storageMode: lockedEnvironment)
assayData: 190181 features, 2 samples
  element names: exprs
protocolData: none
phenoData
  sampleNames: testsamp1, testsamp2
  varLabels and varMetadata description:
    fullfnames: NA
featureData
  featureNames: 74065, 74066, ..., 103913  (190181 total)
  fvarLabels and fvarMetadata description:
    chromosome: NA
    source: NA
    ...: ...
    comment: NA
    (8 total)
experimentData: use 'experimentData(object)'
Annotation:
```

Now, a is an R data structure (of class *ACMESet*) that contains the data from two test GFF files.

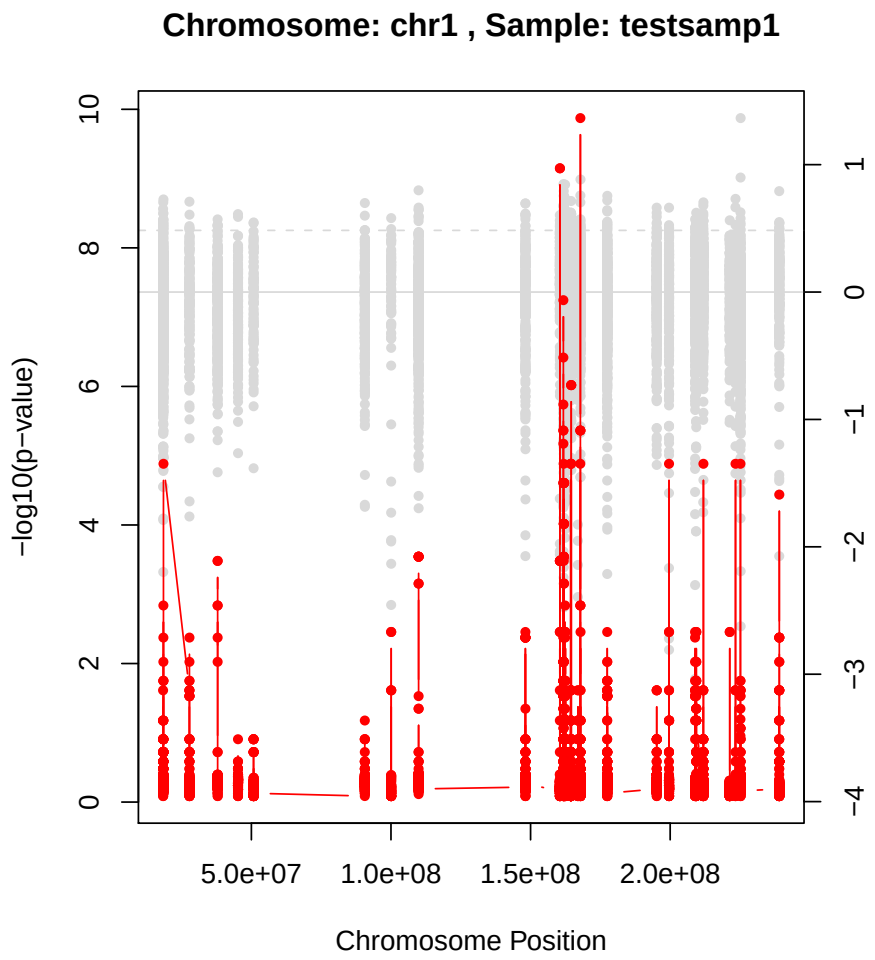
```
> calc <- do.aGFF.calc(example.agff, window = 1000, thresh = 0.95)
```

```
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 the Affymetrix Integrated Genome Browser

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 (such as those needed by the UCSC Genome Browser, another fantastic way to view results). With minor modifications, other formats can be generated.

```
> write.sgr(calc)
```

```
./testsamp1_thresh0.95.sgr
./testsamp1_raw.sgr
./testsamp2_thresh0.95.sgr
./testsamp2_raw.sgr
```

```
> write.sgr(calc, raw = FALSE)
```

```
./testsamp1_thresh0.95.sgr
./testsamp2_thresh0.95.sgr
```

The function also serves as a model for how to generate other file formats (such as those needed by the UCSC Genome Browser, another fantastic way to view results). With minor modifications, other formats can be generated.