

# Using the ACME package

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## Contents

<b>1</b>	<b>Overview of ACME</b>	<b>1</b>
<b>2</b>	<b>Getting Started using ACME</b>	<b>2</b>
2.1	Generating files for viewing in genome browsers . . . . .	4

## 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 determining 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/Rtmp8r9BXm/Rinst2af25958076/ACME/extdata/testsamp1.gff"
[1] "Reading /tmp/Rtmp8r9BXm/Rinst2af25958076/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
```