

Introduction to the plotAlongChrom function

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1 Introduction to the example data

The purpose of this vignette is to demonstrate some of the functionalities of the `plotAlongChrom` in the *tilingArray* package. We use a small subset data from an expression profiling paper [1]; The data only include the region from 35000bp to 50000bp in yeast chromosome one. Expression profiling is done in YPE and YPD conditions, 3 replicates each. Further information about the experimental design can be found at the paper website <http://steinmetzlab.embl.de/NFRsharing/>.

```
> library("grid")
> library("RColorBrewer")
> library("tilingArray")
```

```
> data("segnf")
> class(segnf)
```

```
[1] "environment"
```

```
> ls(segnf)
```

```
[1] "1.+" "1.-"
```

```
> segnf$"1.+"
```

```
Object of class 'segmentation':
```

```
Data matrix: 1775 x 6
```

```
Change point estimates for number of segments S = 1:17
```

```
Confidence intervals for 1 fits from S = 17 to 17
```

```
Selected S = 17
```

```
> head(segnf$"1.+"@y)
```

	YPE1	YPE2	YPE3	YPD1	YPD2	YPD3
[1,]	-1.55	-2.70	-2.52	-4.78	-6.66	-4.62
[2,]	-1.95	-4.20	-3.55	-3.93	-4.75	-4.74
[3,]	-2.24	-2.24	-1.86	-6.15	-4.74	-3.82
[4,]	-2.59	-2.52	-2.39	-4.31	-4.44	-4.40
[5,]	-2.62	-3.91	-4.41	-4.94	-5.19	-4.65
[6,]	-4.30	-4.82	-4.61	-5.62	-5.14	-5.60

```
> dim(segnf$"1.+"@y)
```

```
[1] 1775    6
```

```

> head(segnf$"1."+@"x")
[1] 35001 35009 35017 35025 35033 35041
> length(segnf$"1."+@"x")
[1] 1775
> segnf$"1."+@"logLik"
[1] -Inf -5136 -4772 -4627 -4562 -4498 -4428 -4372 -4314 -4280 -4235 -4198
[13] -4148 -4106 -4082 -4059 -4045
> segnf$"1."+@"nrSegments"
[1] 17
> head(segnf$"1."+@"breakpoints[[segnf$"1."+@"nrSegments]]")
  lower estimate upper
1    13         13   13
2   158        158  158
3   177        177  177
4   242        242  242
5   258        259  260
6   273        273  273

```

The **segnf** object is an environment which contains two objects of class *segmentation*. **segnf** is the output of the **segChrom** in the *tilingArray* package. The *segmentation* object in **segnf** stores the probe expression information in the slot **y**. As can be seen, it contains 1775 probes and 6 array hybes in two conditions. The genomic coordinates where the probes aligned to is stored in the slot **x**. The order of the slot **x** is the same as the probe row order in slot **y**. The segment boundary information is stored in the slot **breakpoints** which is a list that contains all the optimal placement of 1 segment to the designate number(here in this data set is 17) of segments for this data. A log likelihood score for each placement is stored in slot **logLik** from which the best one is choosen and stored in the slot **nrSegments**. Further information about how the segmentation algorithm works, please read the vignette segmentation demo.

```

> data(gffSub)
> head(gffSub)

```

	id	chr	start	end	strand	source	feature	Name	orf_classification
41	41	1	35156	36304	+	SGD	gene	YAL060W	Verified
42	42	1	35156	36304	+	SGD	CDS	YAL060W	Verified
43	43	1	36497	36919	-	SGD	gene	YAL059C-A	Dubious
44	44	1	36497	36919	-	SGD	CDS_dubious	YAL059C-A	Dubious
45	45	1	36510	37148	+	SGD	gene	YAL059W	Verified
46	46	1	36510	37148	+	SGD	CDS	YAL059W	Verified

```

  gene
41 BDH1
42 BDH1
43 <NA>
44 <NA>
45 ECM1
46 ECM1

```

The **gffSub** object is a data frame that contains the SGD annotated features of the region 35000bp-50000bp for yeast chromosome one.

2 Visualizing the expression profiling with the plotAlongChrom function

The function `plotAlongChrom` accepts an environment as its first argument, which is expected to contain objects of class *segmentation* with names given by `paste(chr, c("+", "-"), sep=".")`, where `chr` is the chromosome identifier.

The following code generates Figure 1, a dot plot that averaged across all hybes.

```
> grid.newpage()
> plotAlongChrom(segnf, chr=1, coord=c(35000, 50000), what="dots", gff=gffSub)
```

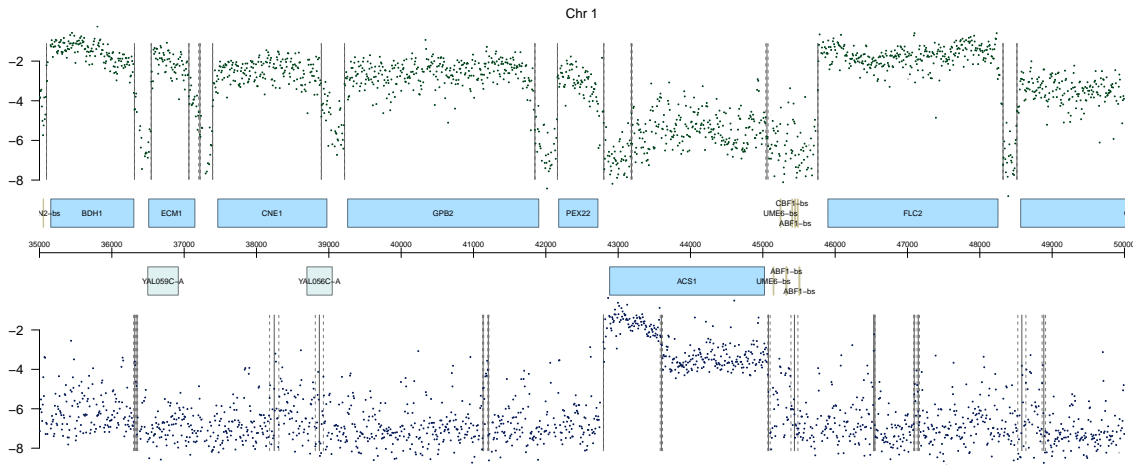


Figure 1: Along-chromosome dot plot of the averaged value across all hybes.

We could also make separate dot plot for different hybes by setting the parameter `sepPlot` as `TRUE`. The following code generates Figure 2 that plots the expression separately for the two conditions.

```
> segObj = new.env(parent = baseenv())
> nmLabel = colnames(segnf$"1."+@y)
> lab = gsub("\\d", "", nmLabel)
> for(nm in paste(1, c("+", "-"), sep=".")){
+   s = get(nm, env = segnf)
+   rpY = tapply(1:length(lab), lab, function(i) rowMeans(s@y[, i]))
+   s@y = do.call(cbind, rpY)
+   assign(nm, s, segObj)
+ }
> grid.newpage()
> plotAlongChrom(segObj, chr=1, coord=c(35000, 50000), what="dots", gff=gffSub, sepPlot = T)
```

However, with the number of hybes increases, it is very hard to see the difference in dot plots in a normal screen. Thus, if the number of hybes is more than 4, the function will force to take the average. A better alternative of displaying multiple hybes is to use the heatmap. The following code generates Figure 3 that makes the heatmap plot.

```
> grid.newpage()
> plotAlongChrom(segnf, chr=1, coord=c(35000, 50000), what="heatmap", gff=gffSub,
+   rowNamesHeatmap=nmLabel, makeRasterImage=FALSE)
```



Figure 2: Along-chromosome dot plot of the averaged value among different replicates for YPD and YPE condition.

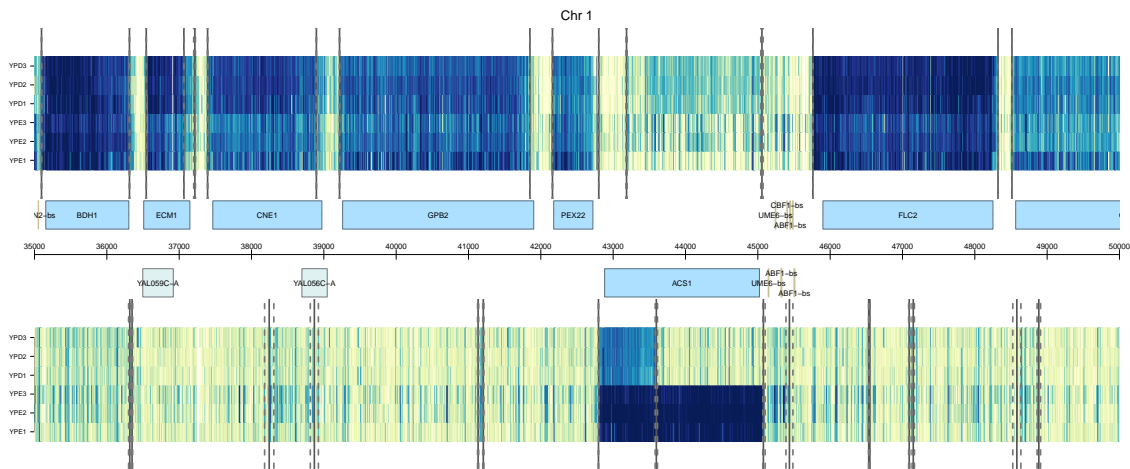


Figure 3: Along-chromosome heatmap plot of all the replicates in YPD and YPE condition.

Start with R 2.11.0, the *grid* package introduced the raster array image function `grid.raster` which is a faster and efficient way of generating heatmap images. From R 2.11.0, The *tilingArray* package will use the `grid.raster` function as default to make heatmap images replacing the previous `grid.rect` function. The choice between the two drawing functions can be changed by the parameter *makeRasterImage*. The following code generates Figure 4 that makes the raster heatmap plot.

```
> grid.newpage()
> plotAlongChrom(segnf,chr=1, coord=c(35000,50000),what="heatmap", gff=gffSub,
+               rowNamesHeatmap=nmLabel,makeRasterImage=TRUE)
```

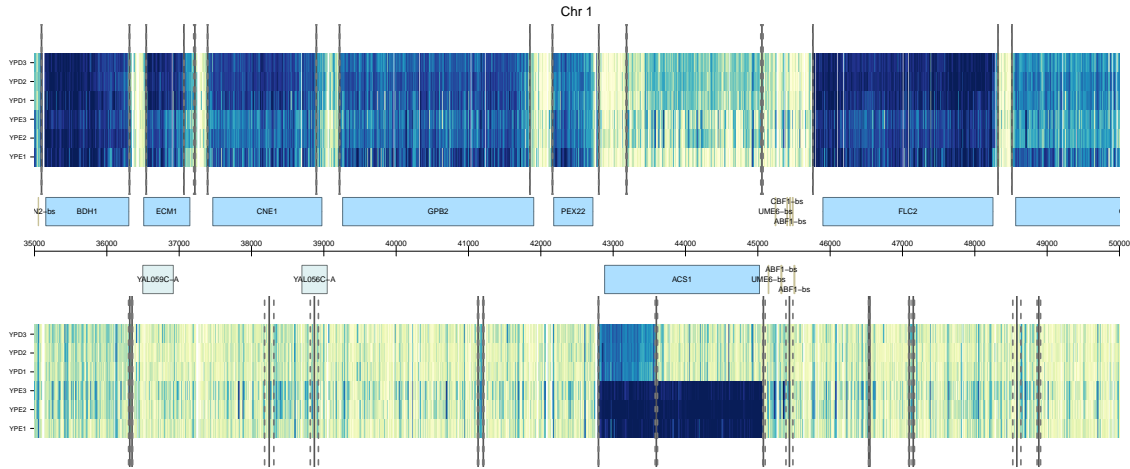


Figure 4: Along-chromosome raster heatmap plot of all the replicates in YPD and YPE condition.

The color gradient of the heatmap could be changed by the parameter *colHeatmap*. The following code generates Figure 5 that makes the raster heatmap plot using a blue color gradient.

```
> grid.newpage()
> plotAlongChrom(segnf,chr=1, coord=c(35000,50000),what="heatmap", gff=gffSub,
+               rowNamesHeatmap=nmLabel,makeRasterImage=TRUE,
+               colHeatmap = colorRamp(brewer.pal(9, "Blues")))
```

References

- [1] Zhenyu Xu, Wu Wei, Julien Gagneur, Fabiana Perocchi, Sandra Clauder-Munster, Jurgi Camblong, Elisa Guffanti, Françoise Stutz, Wolfgang Huber and Lars M. Steinmetz Bidirectional promoters generate pervasive transcription in yeast. *Nature*, 2009. [1](#)



Figure 5: Along-chromosome raster heatmap plot of all the replicates in YPD and YPE condition with a blue color gradient.