

motifStack guide

Jianhong Ou, Lihua Julie Zhu

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1 Introduction

A sequence logo, based on information theory, has been widely used as a graphical representation of sequence conservation (aka motif) in multiple amino acid or nucleic acid sequences. Sequence motif represents conserved characteristics such as DNA binding sites, where transcription factors bind, and catalytic sites in enzymes. Although many tools, such as seqlogo[1], have been developed to create sequence motif and to represent it as individual sequence logo, software tools for depicting the relationship among multiple sequence motifs are still lacking. We developed a flexible and powerful open-source R/Bioconductor package, motifStack, for visualization of the alignment of multiple sequence motifs.

2 Prepare environment

You will need ghostscript: the full path to the executable can be set by the environment variable `R_GSCMD`. If this is unset, a GhostScript executable will be searched by name on your path. For example, on a Unix, linux or Mac "gs" is used for searching, and on Windows the setting of the environment variable `GSC` is used, otherwise commands "gswi64c.exe" then "gswin32c.exe" are tried.

Example on Windows: assume that the `gswin32c.exe` is installed at `C:\Program Files\gs\gs9.06\bin`, then open R and try:

```
> Sys.setenv(R_GSCMD="\C:\Program Files\gs\gs9.06\bin\gswin32c.exe\")
```

3 Examples of using motifStack

3.1 plot a DNA sequence logo with different fonts and colors

Users can select different fonts and colors to draw the sequence logo (Figure 1).

```
> library(motifStack)
> pcm <- read.table(file.path(find.package("motifStack"),
+                             "extdata", "bin_SOLEXA.pcm"))
> pcm <- pcm[,3:ncol(pcm)]
> rownames(pcm) <- c("A", "C", "G", "T")
> motif <- new("pcm", mat=as.matrix(pcm), name="bin_SOLEXA")
> ##pfm object
> #motif <- pcm2pfm(pcm)
> #motif <- new("pfm", mat=motif, name="bin_SOLEXA")
> opar<-par(mfrow=c(4,1))
> plot(motif)
> #plot the logo with same height
> plot(motif, ic.scale=FALSE, ylab="probability")
> #try a different font
> plot(motif, font="mono,Courier")
> #try a different font and a different color group
> motif@color <- colorset(colorScheme='basepairing')
> plot(motif,font="Times")
> par(opar)
```

3.2 plot an amino acid sequence logo

Given that motifStack allows to use any letters as symbols, it can also be used to draw amino acid sequence logos (Figure 2).

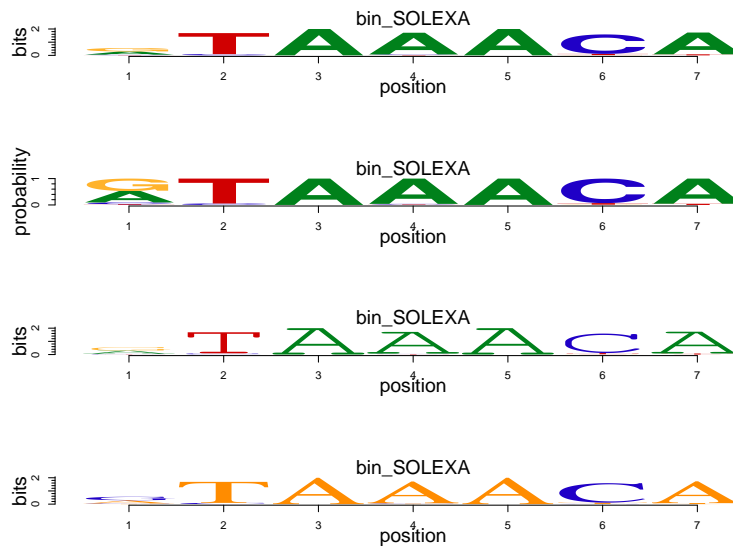


Figure 1: **DNA sequence logo**. Plot a DNA sequence logo with different fonts and colors.

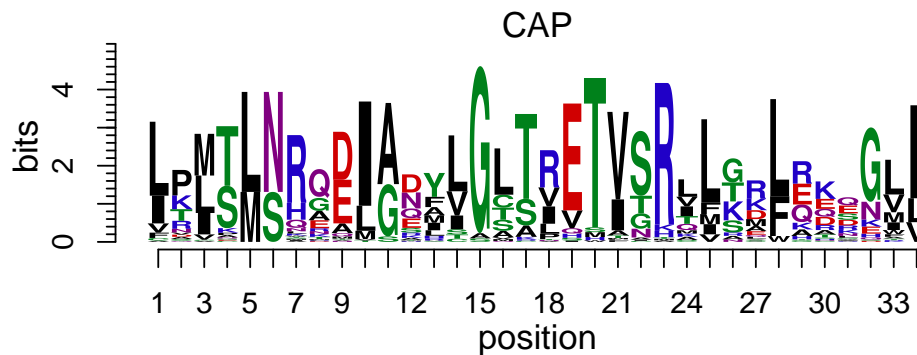


Figure 2: **Amino acid sequence logo**. Plot an amino acid sequence logo with any symbols as you want such as amino acid sequence logo

```
> library(motifStack)
> protein<-read.table(file.path(find.package("motifStack"),"extdata","cap.txt"))
> protein<-t(protein[,1:20])
> motif<-pcm2pfm(protein)
> motif<-new("pfm", mat=motif, name="CAP",
+           color=colorset(alphabet="AA",colorScheme="chemistry"))
> plot(motif)
```

3.3 plot sequence logo stack

motifStack is designed to show multiple motifs in same canvas. To show the sequence logo stack, the distance of motifs need to be calculated first for example by using `MotIV[2]::motifDistances`, which implemented STAMP[3]. After alignment, users can use `plotMotifLogoStack` function to draw sequence logos stack (Figure 3) or use `plotMotifLogoStackWithTree` function to show the distance tree with the sequence logos stack (Figure 4) or use `plotMotifStackWithRadialPhylog` function to plot sequence logo stack in radial style (Figure 5) in the same canvas. There is a shortcut function named as `motifStack`. Use `stack` layout to call `plotMotifLogoStack`, `treeview` layout to call `plotMotifLogoStackWithTree` and `radialPhylog` to call `plotMotifStackWithRadialPhylog`.

```
> library(motifStack)
> #####Input#####
> pcms<-readPCM(file.path(find.package("motifStack"), "extdata"),"pcm$")
> motifs<-lapply(pcms,pcm2pfm)
> ## plot stacks
> motifStack(motifs, layout="stack", ncex=1.0)

> ## plot stacks with hierarchical tree
> motifStack(motifs, layout="tree")

> ## When the number of motifs is too much to be shown in a vertical stack,
> ## motifStack can draw them in a radial style.
> ## random sample from MotifDb
> library("MotifDb")
> matrix.fly <- query(MotifDb, "Dmelanogaster")
> motifs2 <- as.list(matrix.fly)
> ## use data from FlyFactorSurvey
> motifs2 <- motifs2[grepl("Dmelanogaster\\-FlyFactorSurvey\\-",
+                          names(motifs2))]
> ## format the names
> names(motifs2) <- gsub("Dmelanogaster_FlyFactorSurvey_", "",
+                       gsub("_FBgn\\d+$", "",
+                             gsub("[^a-zA-Z0-9]", "_",
+                                   gsub("(\\d+)+$", "", names(motifs2))))))
> motifs2 <- motifs2[unique(names(motifs2))]
> pfms <- sample(motifs2, 50)
> ## creat a list of object of pfm
> motifs2 <- lapply(names(pfms),
+                  function(.ele, pfms){new("pfm",mat=pfms[[.ele]], name=.ele)}
+                  ,pfms)
> ## trim the motifs
> motifs2 <- lapply(motifs2, trimMotif, t=0.4)
> ## setting colors
> library(RColorBrewer)
> color <- brewer.pal(12, "Set3")
```

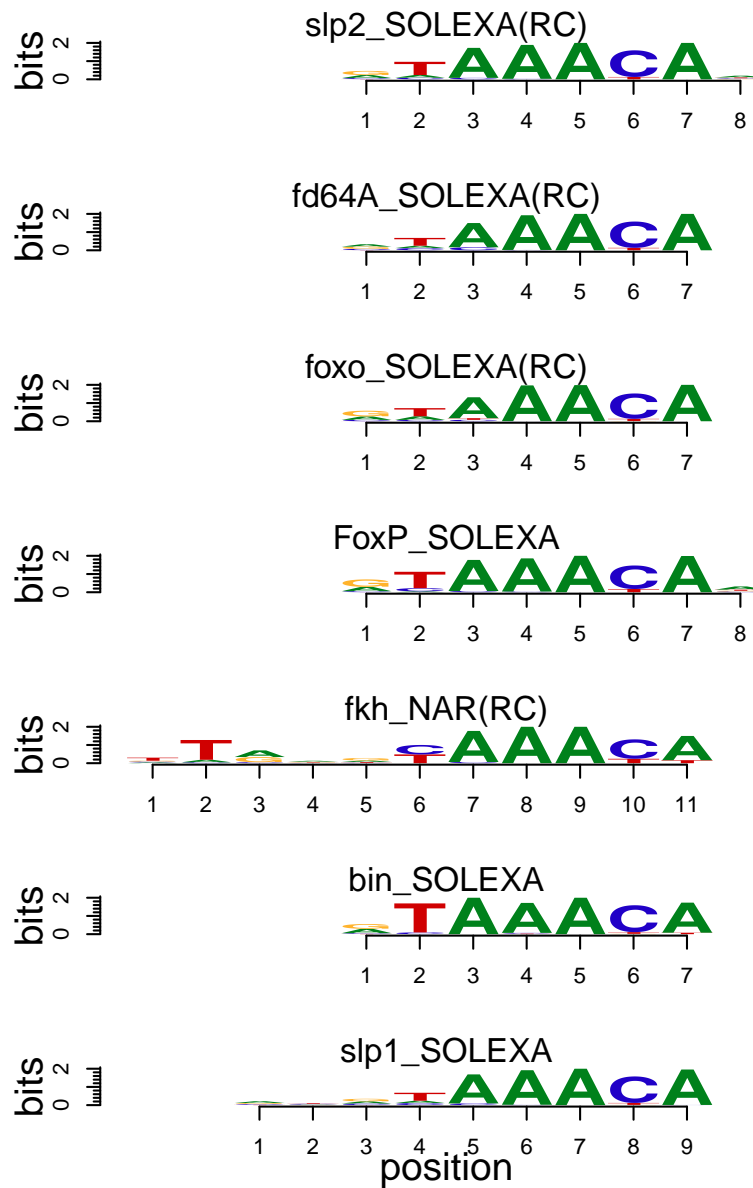


Figure 3: **Sequence logo stack.** Plot motifs with sequence logo stack style.

```
> ## plot logo stack with radial style
> motifStack(motifs2, layout="radialPhylog",
+           circle=0.3, cleaves = 0.2,
+           clabel.leaves = 0.5,
+           col.bg=rep(color, each=5), col.bg.alpha=0.3,
+           col.leaves=rep(color, each=5),
+           col.inner.label.circle=rep(color, each=5),
+           inner.label.circle.width=0.05,
+           col.outer.label.circle=rep(color, each=5),
+           outer.label.circle.width=0.02,
```

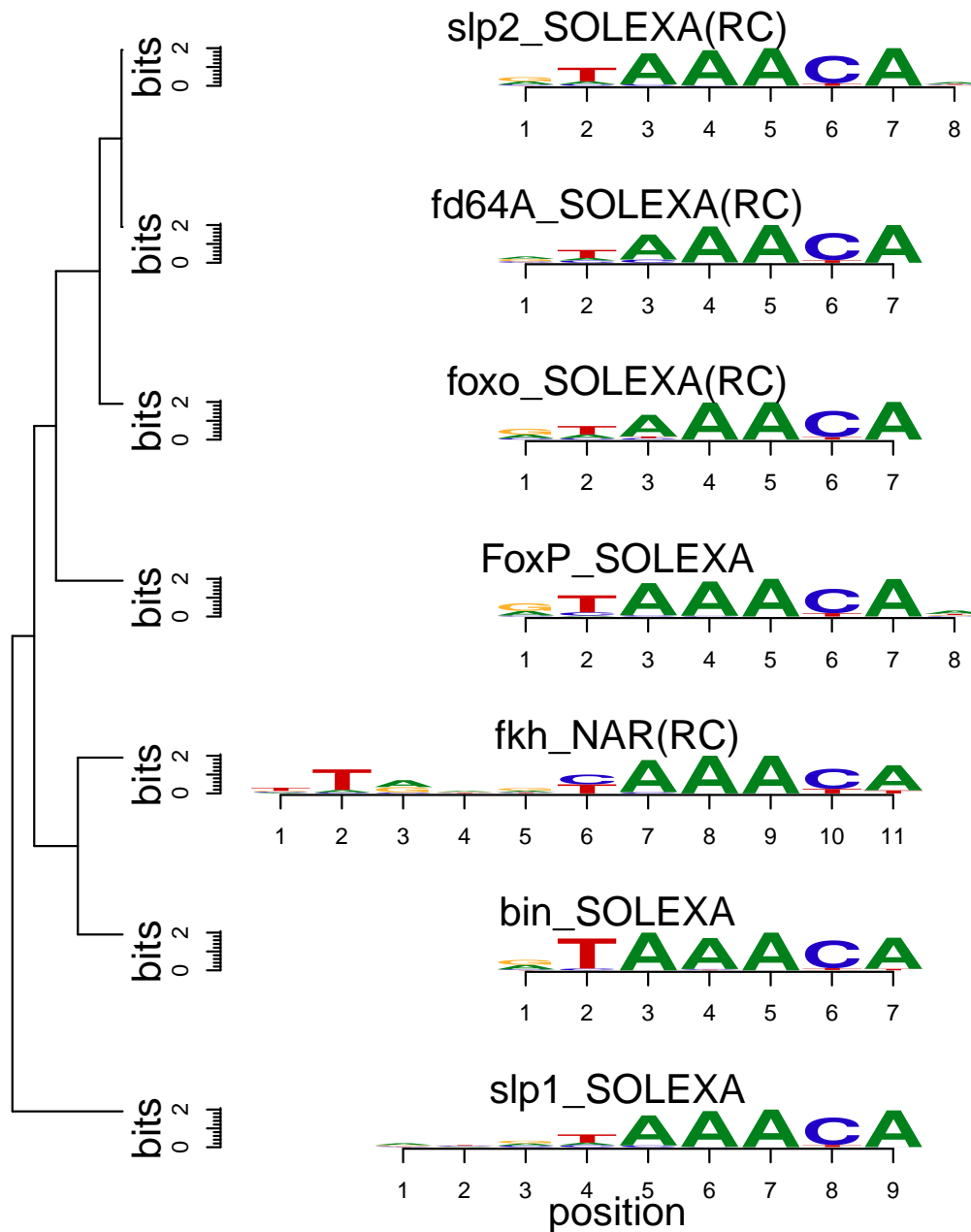


Figure 4: **Treeview layout logo stack.** Sequence logo stack with hierarchical cluster tree.

```
+      circle.motif=1.2,
+      angle=350)
```

3.4 plot a sequence logo cloud

We can also plot a sequence logo cloud for DNA sequence logo (Figure 6).

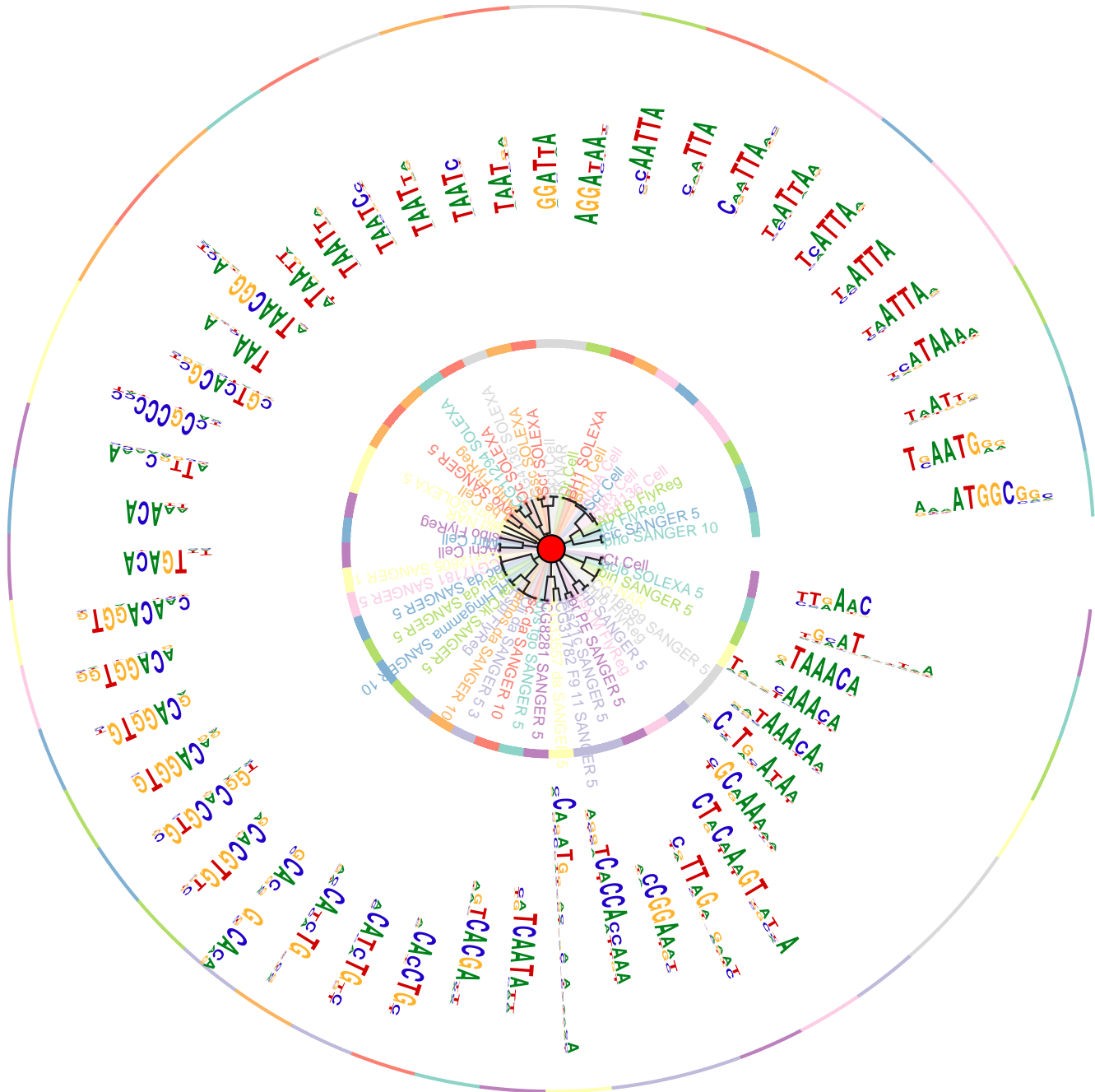


Figure 5: **Sequence logo stack in radial style** Plot motifs in a radial style when the number of motifs is too much to be shown in a vertical stack.

```

> ## assign groups for motifs
> groups <- rep(paste("group",1:5,sep=""), each=10)
> names(groups) <- names(pfms)
> ## assign group colors
> group.col <- brewer.pal(5, "Set3")
> names(group.col) <- paste("group",1:5,sep="")

```




Figure 6: **Sequence logo cloud with rectangle packing layout** Like tag-cloud, the sequence logo size is determined by the number of motifs of the signature. The group sources of the motifs for each signature are shown as a pie graph in topleft corner.

3.6 motifCircos

We can also plot it with circos style (Figure 8). In circos style, we can plot two group of motifs and with multiple color rings.

```
> ## plot the logo stack with radial style.
> motifCircos(phylog=phylog, pfms=pfms, pfms2=sig,
+             col.tree.bg=rep(color, each=5), col.tree.bg.alpha=0.3,
+             col.leaves=rep(rev(color), each=5),
+             col.inner.label.circle=gpCol,
+             inner.label.circle.width=0.03,
+             col.outer.label.circle=gpCol,
```

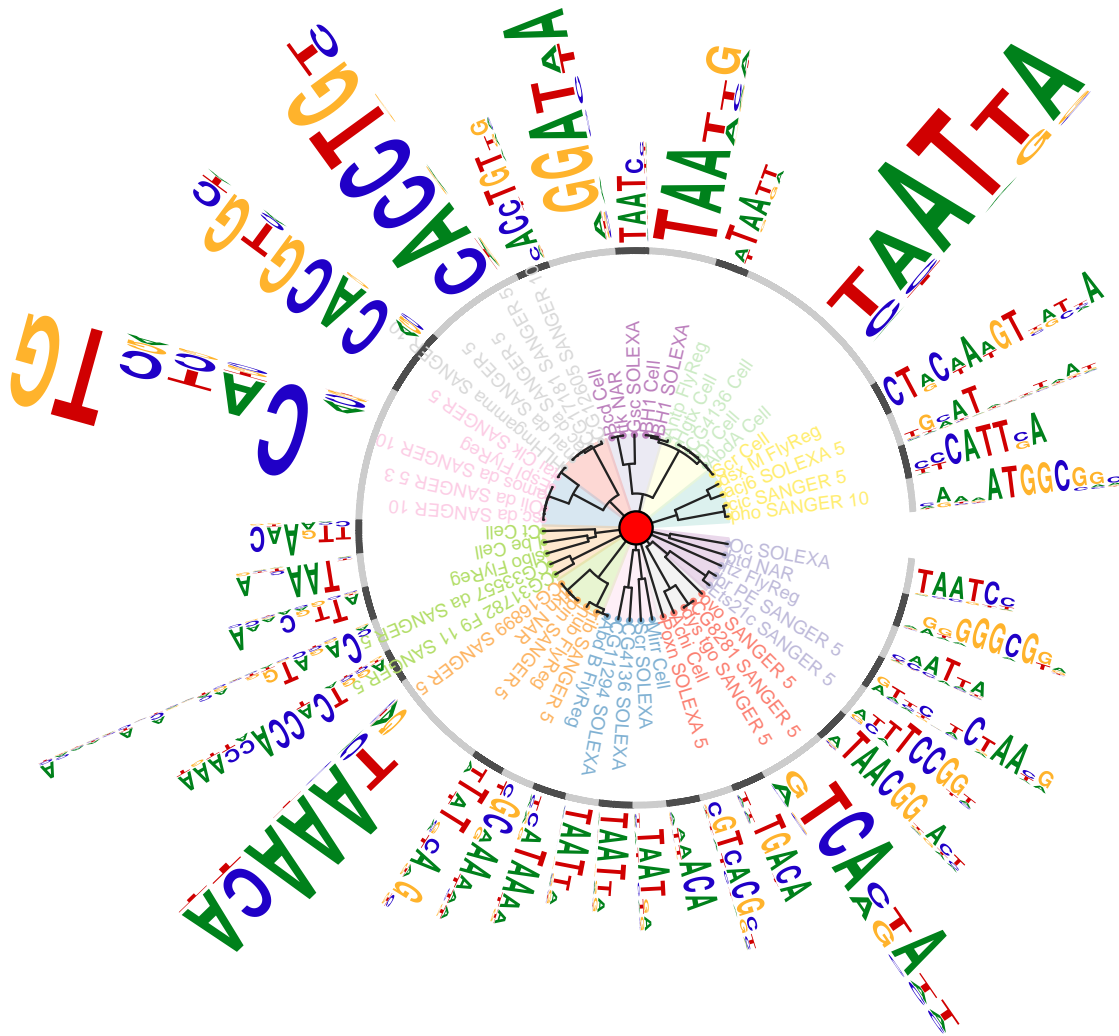


Figure 7: **Grouped sequence logo with radialPhylog style layout.** Like tag-cloud, the sequence logo size is determined by the number of motifs for the signature. The gray-black circle indicates the range of each signature.

```
+      outer.label.circle.width=0.03,
+      r.rings=c(0.02, 0.03, 0.04),
+      col.rings=list(sample(colors(), 50),
+                    sample(colors(), 50),
+                    sample(colors(), 50)),
```



Figure 8: **Grouped sequence logo with circos style layout.** more color sets with more motifs.

```
+      angle=350, motifScale="logarithmic")
```

3.7 motifPiles

We can also plot it with pile style (Figure 9). In pile style, we can plot two group of motifs and with multiple color annotations.

```

> ## plot the logo stack with radial style.
> motifPiles(phylog=phylog, pfms=pfms, pfms2=sig,
+           col.tree=rep(color, each=5),
+           col.leaves=rep(rev(color), each=5),
+           col.pfms2=gpCol,
+           r.anno=c(0.02, 0.03, 0.04),
+           col.anno=list(sample(colors(), 50),
+                         sample(colors(), 50),
+                         sample(colors(), 50)),
+           motifScale="logarithmic",
+           plotIndex=TRUE,
+           groupDistance=0.01)

```

4 References

References

- [1] seqLogo: Sequence logos for DNA sequence alignments. R package version 1.22.0.
- [2] MotIV: Motif Identification and Validation. Eloi Mercier and Raphael Gottardo (2010). R package version 1.10.0.
- [3] STAMP: a web tool for exploring DNA-binding motif similarities. Mahony S, Benos PV, Nucleic Acids Res. 2007, 35(Web Server issue): W253-W258.

5 Session Info

```

> toLatex(sessionInfo())

```

- R version 3.2.0 (2015-04-16), x86_64-unknown-linux-gnu
- Locale: LC_CTYPE=en_US.UTF-8, LC_NUMERIC=C, LC_TIME=en_US.UTF-8, LC_COLLATE=C, LC_MONETARY=en_US.UTF-8, LC_MESSAGES=en_US.UTF-8, LC_PAPER=en_US.UTF-8, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C, LC_MEASUREMENT=en_US.UTF-8, LC_IDENTIFICATION=C
- Base packages: base, datasets, grDevices, graphics, grid, methods, parallel, stats, stats4, utils
- Other packages: BiocGenerics 0.14.0, Biostrings 2.36.0, IRanges 2.2.0, MotIV 1.24.0, MotifDb 1.10.0, RColorBrewer 1.1-2, S4Vectors 0.6.0, XML 3.98-1.1, XVector 0.8.0, ade4 1.7-2, grImport 0.9-0, motifStack 1.12.0
- Loaded via a namespace (and not attached): BSgenome 1.36.0, BiocParallel 1.2.0, BiocStyle 1.6.0, GenomInfoDb 1.4.0, GenomicAlignments 1.4.0, GenomicRanges 1.20.0, RCurl 1.95-4.5, Rsamtools 1.20.0, bitops 1.0-6, futile.logger 1.4, futile.options 1.0.0,

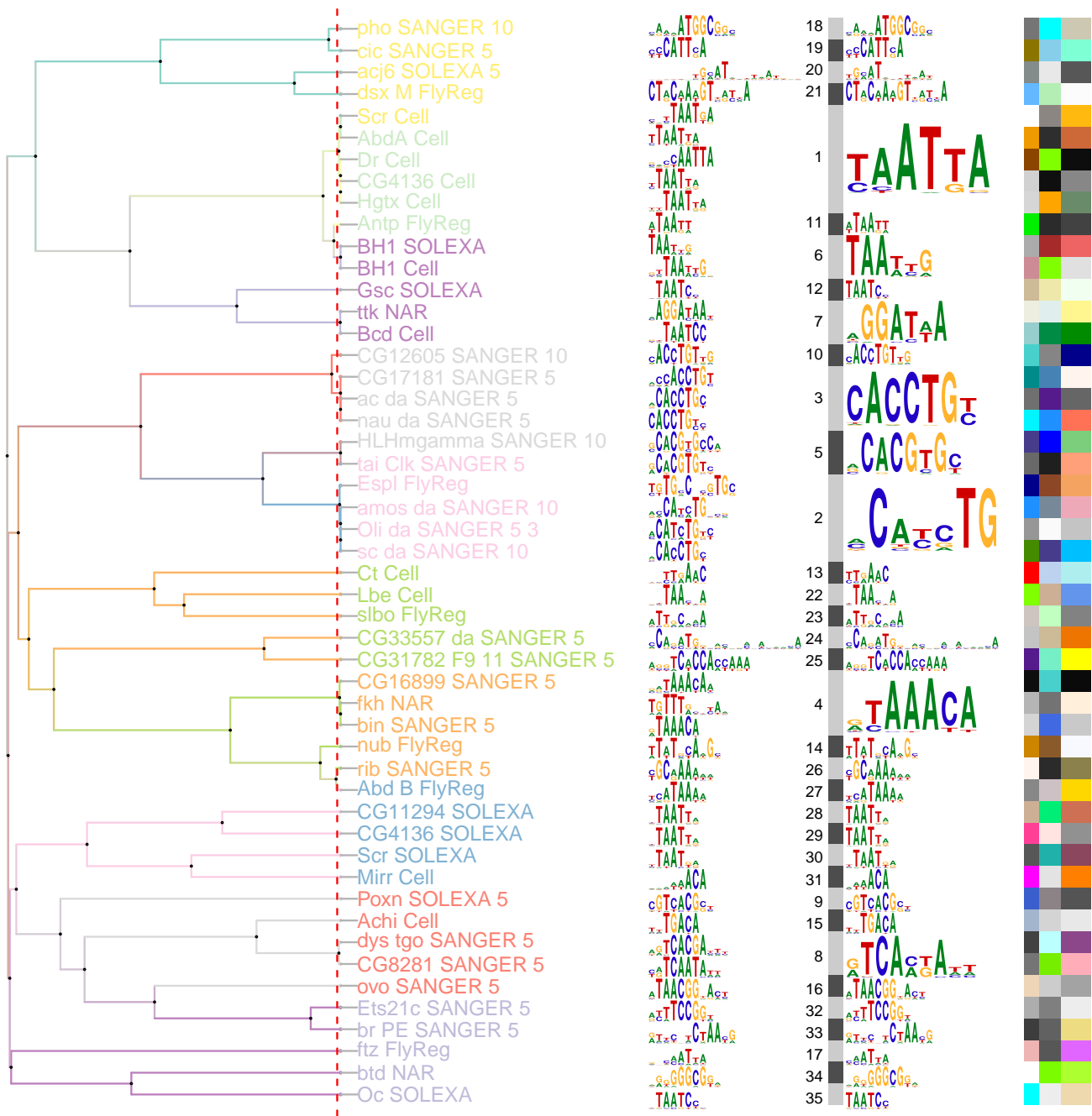


Figure 9: **Grouped sequence logo with piles style layout.** more color sets with more motifs.

lambda.r 1.1.7, lattice 0.20-31, rGADEM 2.16.0, rtracklayer 1.28.0, seqLogo 1.34.0, tools 3.2.0, zlibbioc 1.14.0