

Here we demonstrate the typical workflows for the analysis and visualization of TF DNA binding site motifs using *motifStack*. We provide examples of the specific commands to plot aligned motifs as a stack, a linear/radial tree, or a word cloud of sequence logos with various coloringoptions. The colors used here are recommended to improve accessibility for colorblind readers.

First, load the library, then read pcms (position count matrix), which is converted to pfm (position frequency matrices) subsequently.

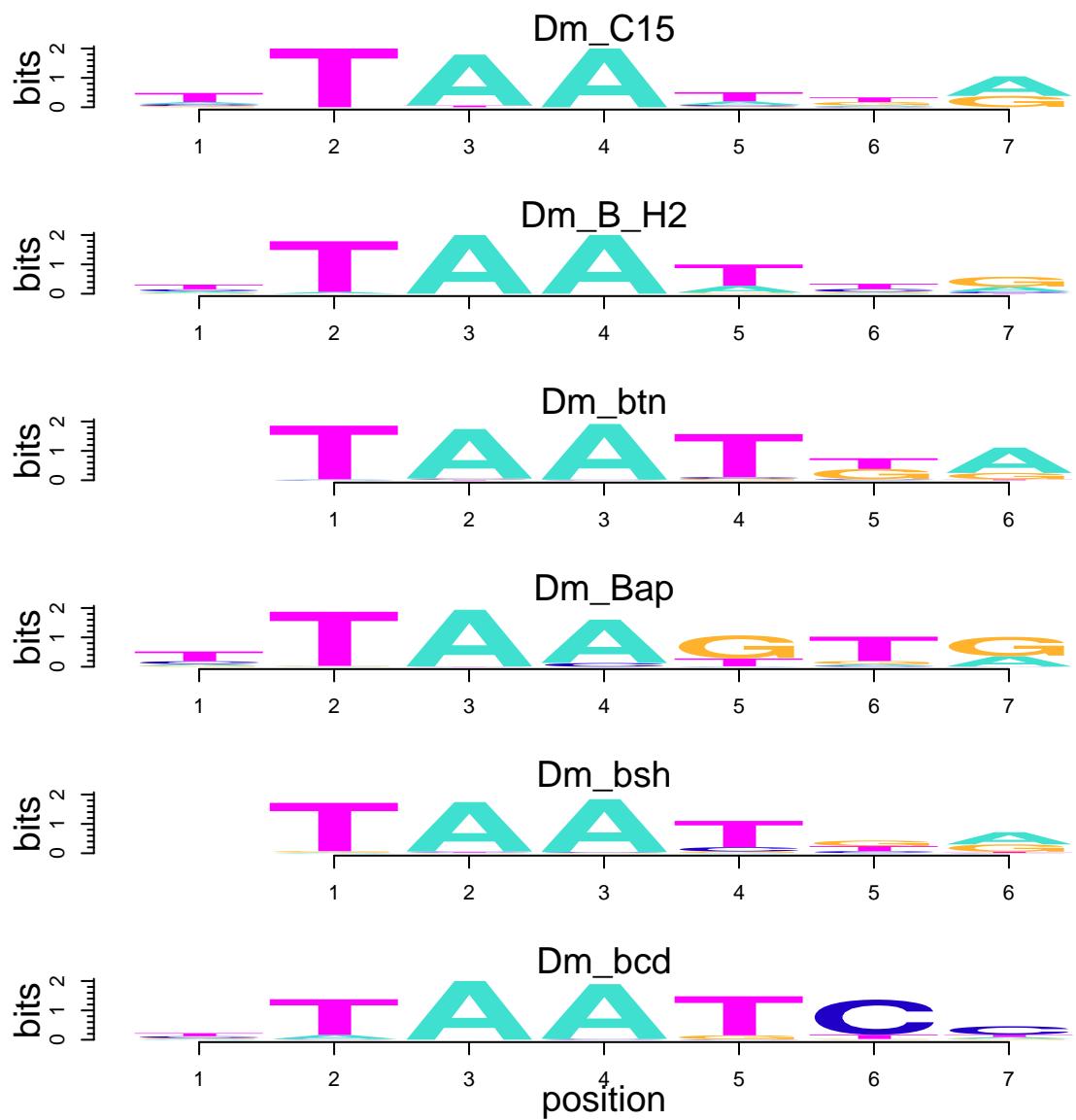
```
##load the library
library(motifStack)

##read pcms (position count matrices)
pcmpath <- "pcmsDatasetDM"
pcms <- readPCM(pcmpath)
## color blindness
source("safeColor.R")
pcms <- safeColor(pcms)
##convert to pfms (position frequency matrices)
pfms<-lapply(pcms,pcm2pfm)
```

## Examples of linear and radial dendograms and code to generate them.

Plot motifs as a stack with nucleotides drawn proportional to their information content (IC).

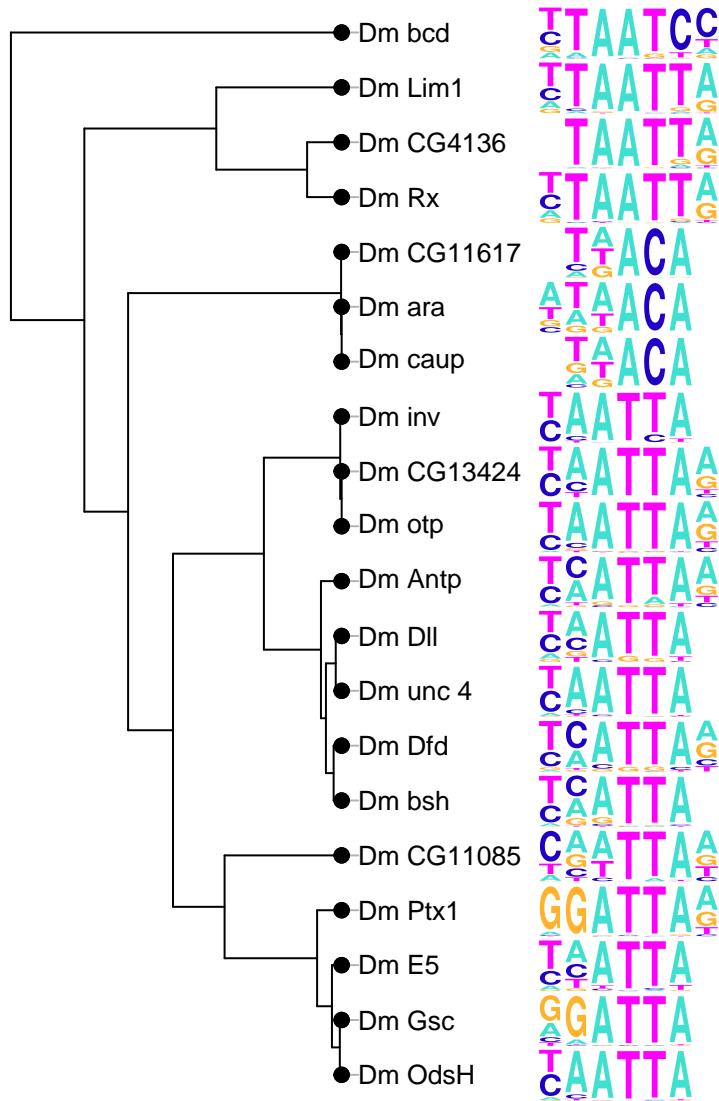
```
subset_pfms <- pfms[10:15]
motifStack(subset_pfms, layout="stack")
```



**Supplementary Figure 2A.** Plotted as a stack of motifs with nucleotides drawn proportional to their information content (IC)

Plot motifs as a linear tree with nucleotides drawn proportional to their frequency.

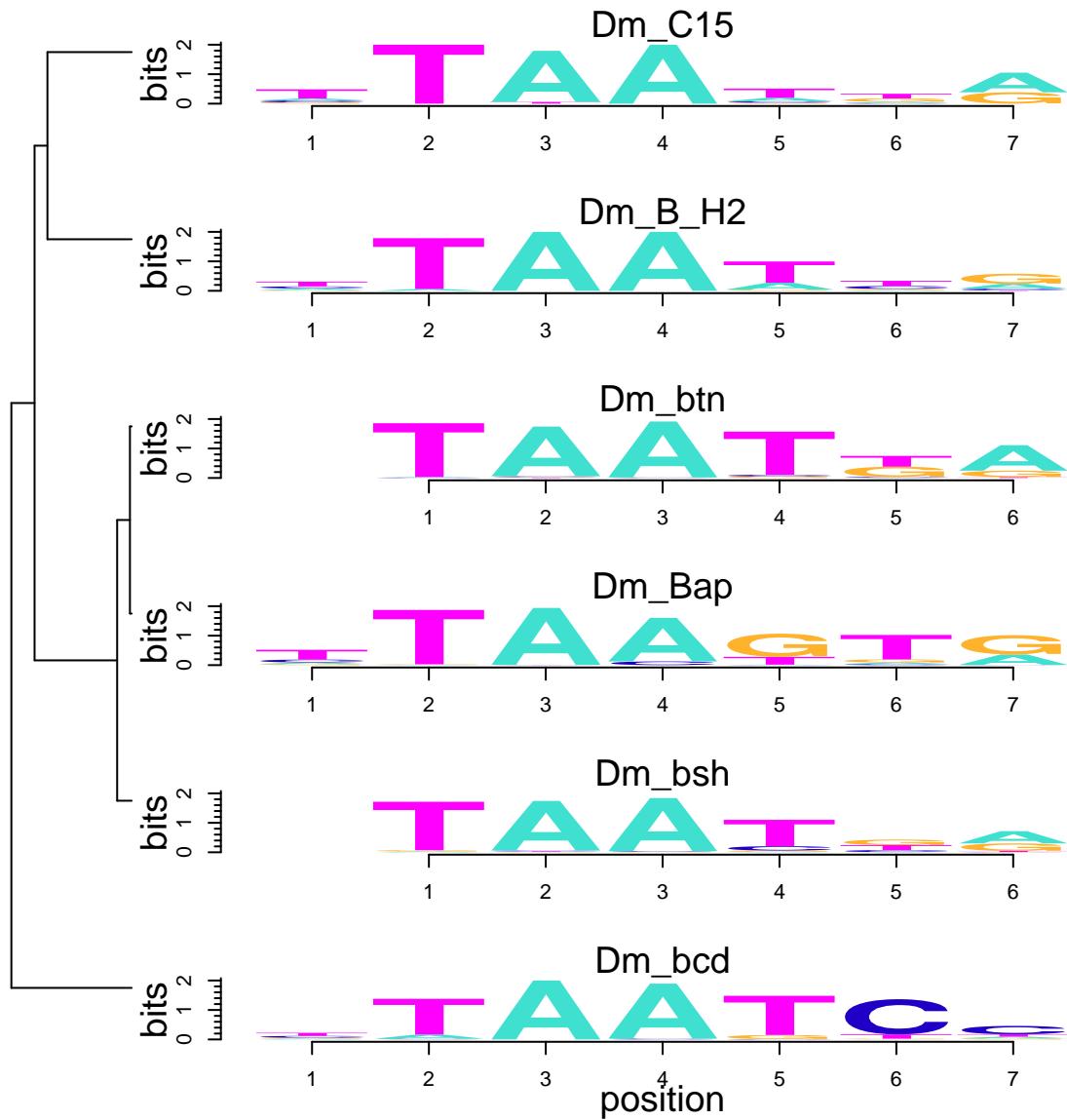
```
##try different style of sequence logo
motifStack(pfms[sample(1:length(pfms), 20)], layout="phylog", clabel.leaves=.8,
           f.logo=0.5, ic.scale=FALSE)
```



**Supplementary Figure 2B.** Plotted as a linear tree with nucleotides drawn proportional to their frequency

Plot motifs as a linear tree with nucleotides drawn proportional to their IC.

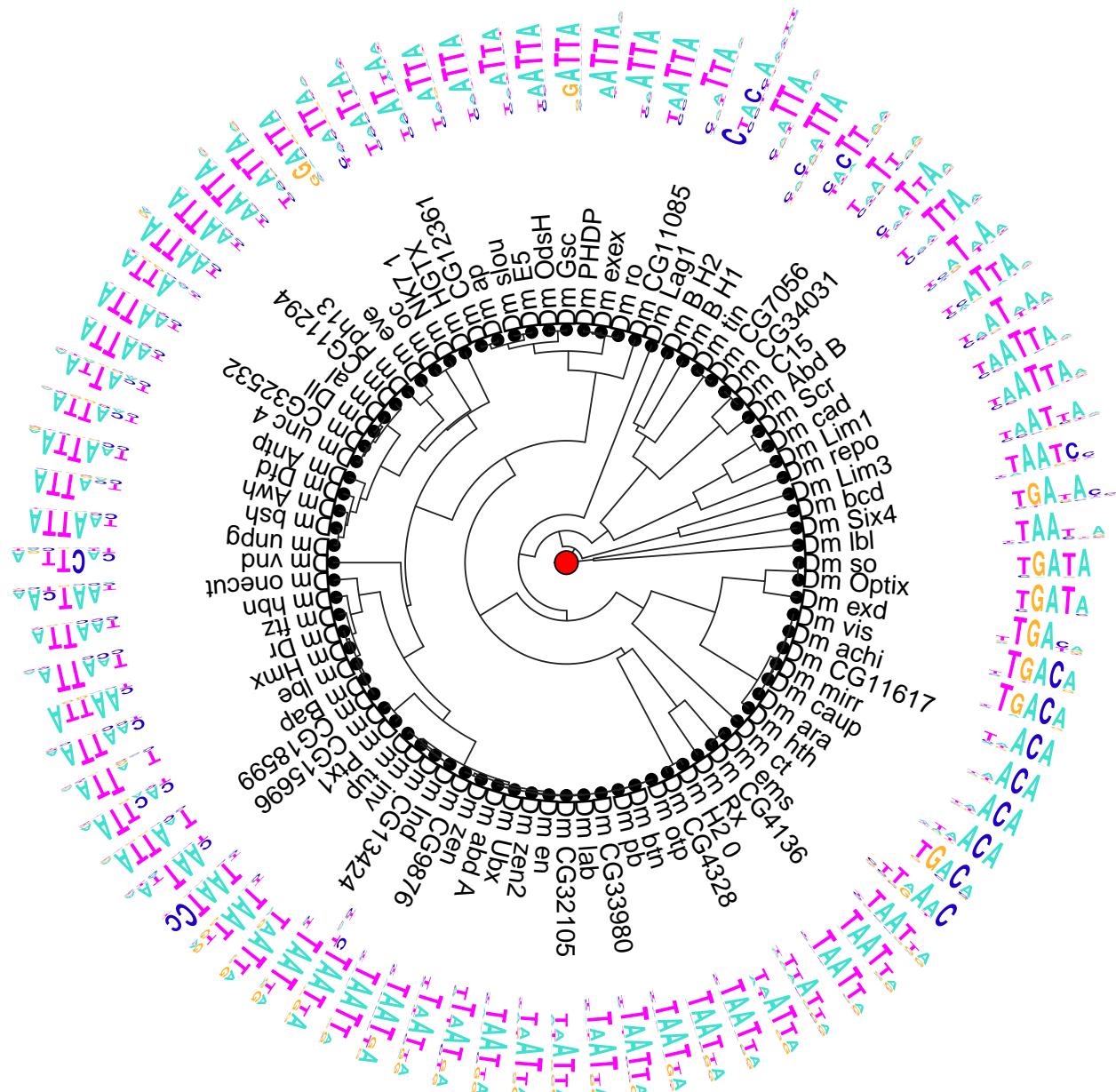
```
##By default, MotIV is used for clustering
motifStack(subset_pfms, layout="tree", trueDist=TRUE)
```



Supplementary Figure 2C. Plotted as a linear tree with nucleotides drawn proportional to their IC

Plot motifs as a radial tree.

```
motifStack(pfms, layout="radialPhylog")
```



Supplementary Figure 2D. Plotted as a radial tree

## Merge motifs with different distance cutoffs and display these motif signatures in various layouts.

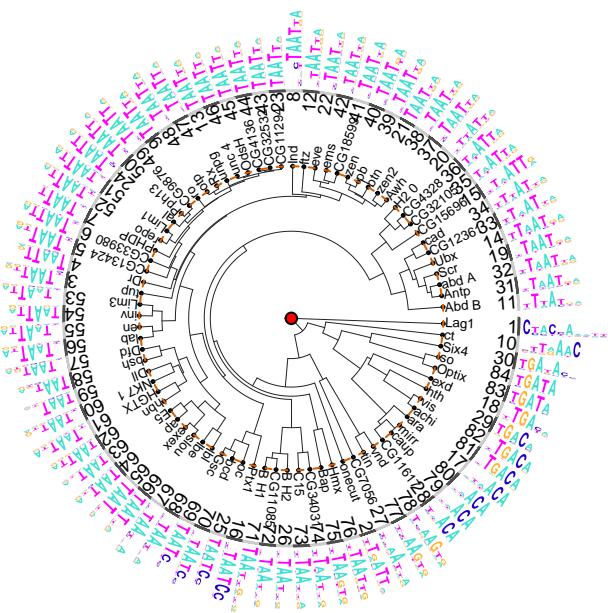
Before motifs are merged, the distances of the motifs are calculated using STAMP, MovIV or MatAlign. Here we show different ways to display the merged motifs and how different distance cutoffs affect the resulting motif signatures. The magenta dotted line indicates the distance cutoff used.

Merge motifs using different distance cutoffs.

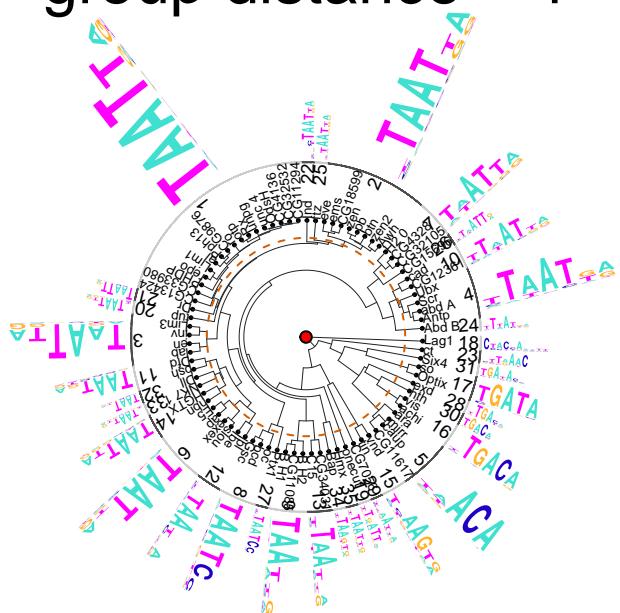
```
outpath <- "output"
matalign_path <- "./app/matalign-v4a"
neighbor_path <- "./app/neighbor.app/Contents/MacOS/neighbor"
MatAlign2tree_path <- "./MatAlign2tree.pl"
pcmpath <- "pcmsDatasetDM"
pcms <- readPCM(pcmpath)
pcms <- safeColor(pcms)
pfms<-lapply(pcms,pcm2pfm)
system(paste("perl", MatAlign2tree_path, "--in . --pcmpath", pcmpath,
            "--out", outpath,
            "--matalign", matalign_path,
            "--neighbor", neighbor_path,
            "--tree","UPGMA"))
newickstrUPGMA <- readLines(con=file.path(outpath, "NJ.matalign.distMX.nwk"))
phylogUPGMAmatAlign <- newick2phylog(newickstrUPGMA, FALSE)
##get the leaves of tree for ordering the pfms
matAlignLeaveNames <- names(phylogUPGMAmatAlign$leaves)
this_motifs <- pfms[matAlignLeaveNames]
matAlignLeaveNames <- gsub("^Dm_","", matAlignLeaveNames)

for(groupDistance in c(0, 1, 2, 3)){
  motifSig <- motifSignature(this_motifs,
                               phylogUPGMAmatAlign,
                               groupDistance=groupDistance,
                               min.freq=1)
  this_sig <- signatures(motifSig)
  ## get color set for the signature groups
  this_gpCol <- sigColor(motifSig)
  plotMotifStackWithRadialPhylog(phylog=phylogUPGMAmatAlign,
                                 pfms=this_sig,
                                 col.inner.label.circle=this_gpCol,
                                 inner.label.circle.width=0.02,
                                 labels.leaves=matAlignLeaveNames,
                                 cleaves=.2, circle=1, circle.motif=1.5,
                                 clabel.leaves=.4, motifScale="logarithmic",
                                 angle=358, plotIndex=TRUE, IndexCex=.6,
                                 groupDistance=groupDistance,
                                 groupDistanceLineCol="#D5E00")
  text(0, 2.3, label=paste("group distance =", groupDistance), cex=2)
}
```

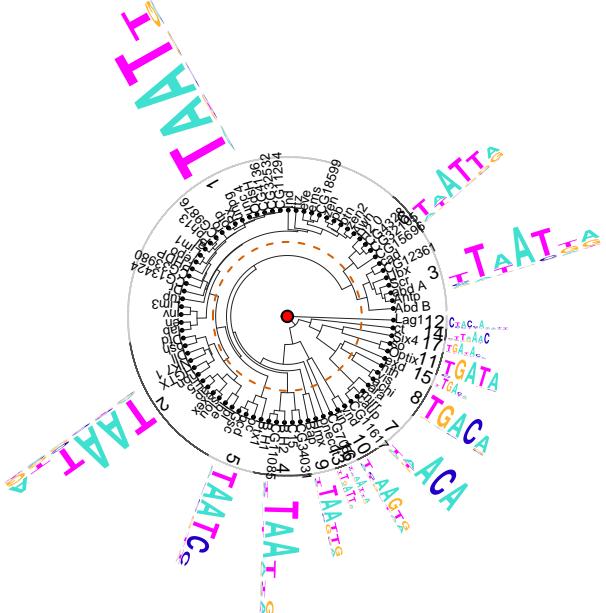
group distance = 0



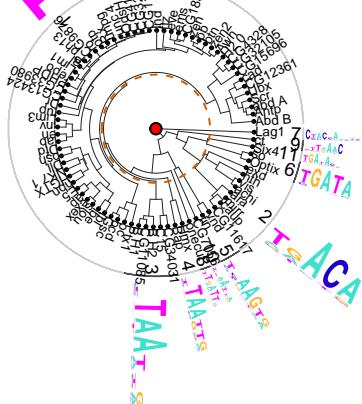
group distance = 1



group distance = 2



group distance = 3



Supplementary Figure 2E. Merged motif signatures plotted as radial trees with different distance cutoffs

Plot motif signatures as a circular word cloud.

```
## get signature
gpDist <- c(.5, 1)
motifSig <- lapply(gpDist, motifSignature,
                    pfms=this_motifs,
                    phylog=phylogUPGMAmatAlign,
                    min.freq=1)
## motif cloud, cloud style
motifCloud(motifSig[[1]], layout="cloud", scale=c(9, .75))
```



**Supplementary Figure 2F.** Motif signatures plotted as a circular word cloud with motif size representing the number of motifs that contributed to the signature. The larger the motif size, the larger the number of motifs within that motif signature

Plot motif signatures as rectangular word clouds.

```
## motif cloud, rectangle style
for(i in 1:2){
  motifCloud(motifSig[[i]], layout="rectangles", ic.scale=FALSE)
  op <- par(mar = c(0, 0, 0, 0))
  text(.5, .985, label= paste("group distance =", gpDist[i]))
  par(op)
}
```



**Supplementary Figure 2G.** Motif signature plotted as rectangular word clouds using different distance cutoffs

## Use various color options to highlight different motif features.

motifStack offers multiple options to color a radial tree: the background of the inner circle, the text of motif names, the background of the motif names, inner label rings and outer label rings. Here are a few examples using these options to show the motif's data source, the computational algorithm that generated the motifs, the motif's information content (IC) and the TF name.

```
getPairColor <- function(n=10L){
  if(n %% 2 != 0) n <- n+1
  n <- n/2
  n <- rainbow(n)#as.character(t(matrix(rainbow(n=n), ncol=2, byrow=FALSE)))
  n2 <- highlightCol(n, .5)
  as.character(t(cbind(n, n2)))
}

pairColor <- getPairColor(22)

pfmList2matrixList <- function(pfms){
  m <- lapply(pfms, function(.ele) as(.ele, "matrix"))
  names(m) <- unlist(lapply(pfms, function(.ele) .ele@name))
  m
}

getMotIVOut <- function(pfms, cc, align){
  jaspar.scores <-
    MotIV::readDBScores(
      file.path(".", "app", "scores",
      paste("JaspRand_", cc, "_", align, ".scores", sep="")))
  d <- MotIV::motifDistances(pfmList2matrixList(pfms), cc=cc, align=align)
  hc <- MotIV::motifHclust(d, method="average")
  phylog <- hclust2phylog(hc)
  pfms <- pfms[hc$order]
  aligned.pfms <- DNAmotifAlignment(pfms)
  leaveNames <- names(phylog$leaves)
  ## data source
  dataSource <- factor(grep("_M", leaveNames))
  levels(dataSource) <- c("#F0E442", "#56B4E9") ##c("Uniprobe", "CIS-BP")
  dataSource <- as.character(dataSource)
  ## algorithm
  algorithm <-
    factor(!grep("_M", leaveNames) + grep("_bml", leaveNames))
  levels(algorithm) <-
    c("#0072B2", "#CC79A7", "#E69F00") ##("DREAM5", "Seed-And-Wobble", "BEEML")
  algorithm <- as.character(algorithm)
  ## motifs from same PBM data
  motifGroup <- factor(gsub("(.*?)_.*$", "\\\1", leaveNames))
  levels.motifGroup <- levels(motifGroup)
  levels(motifGroup) <- pairColor[1:length(levels(motifGroup))]
  colors.motifGroup <- levels(motifGroup)
  motifGroup <- as.character(motifGroup)
  return(list(aligned.pfms=aligned.pfms, unaligned.pfms=pfms, phylog=phylog,
    leaveNames=leaveNames,
    dataSource=dataSource,
    algorithm=algorithm,
    motifGroup=motifGroup,
```

```

        levels.motifGroup=levels.motifGroup,
        colors.motifGroup=colors.motifGroup))
}

##read pcms
pcmpath <- "pcmsDatasetAlgorithm"
pcms <- readPCM(pcmpath)
pcms <- safeColor(pcms)
##convert to pfms
pfms<-lapply(pcms,pcm2pfm)

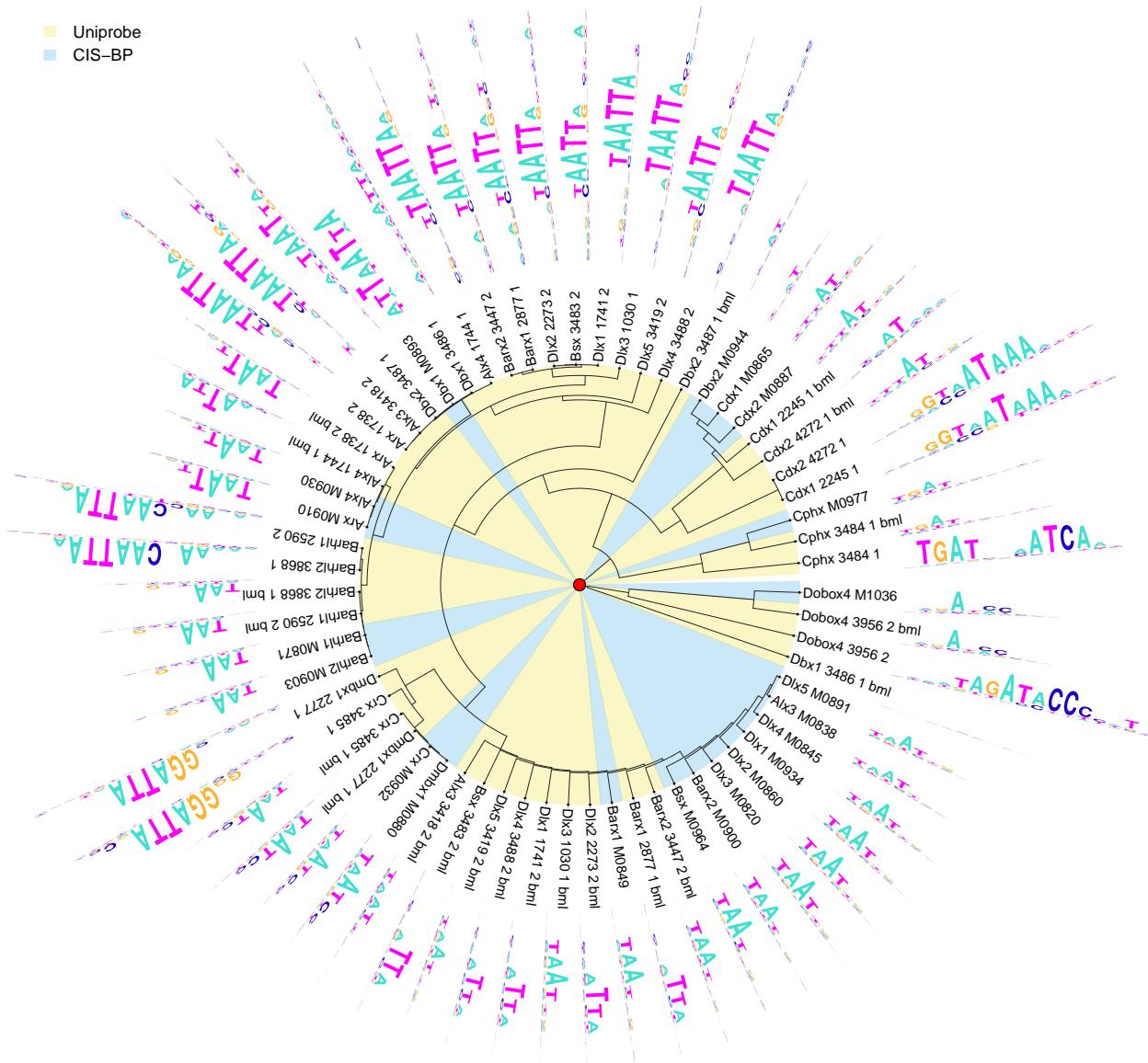
```

Use the background color of the inner circle to distinguish different data sources with the parameter col.bg.

```

motIVout <- getMotIVOut(pfms, "PCC", "SWU")
attach(motIVout)
plotMotifStackWithRadialPhylog(phylog=phylog, pfms=unaligned.pfms,
                                labels.leaves=leaveNames,
                                col.bg=dataSource, col.bg.alpha=.3,
                                cleaves=.2, circle=1.1, circle.motif=1.6,
                                clabel.leaves=.8, angle=358)
legend(-2.3, 2.4, legend=c("Uniprobe", "CIS-BP"),
       fill= highlightCol(c("#F0E442", "#56B4E9"), alpha=.3),
       border="white", lty=NULL, bty = "n", cex=1)

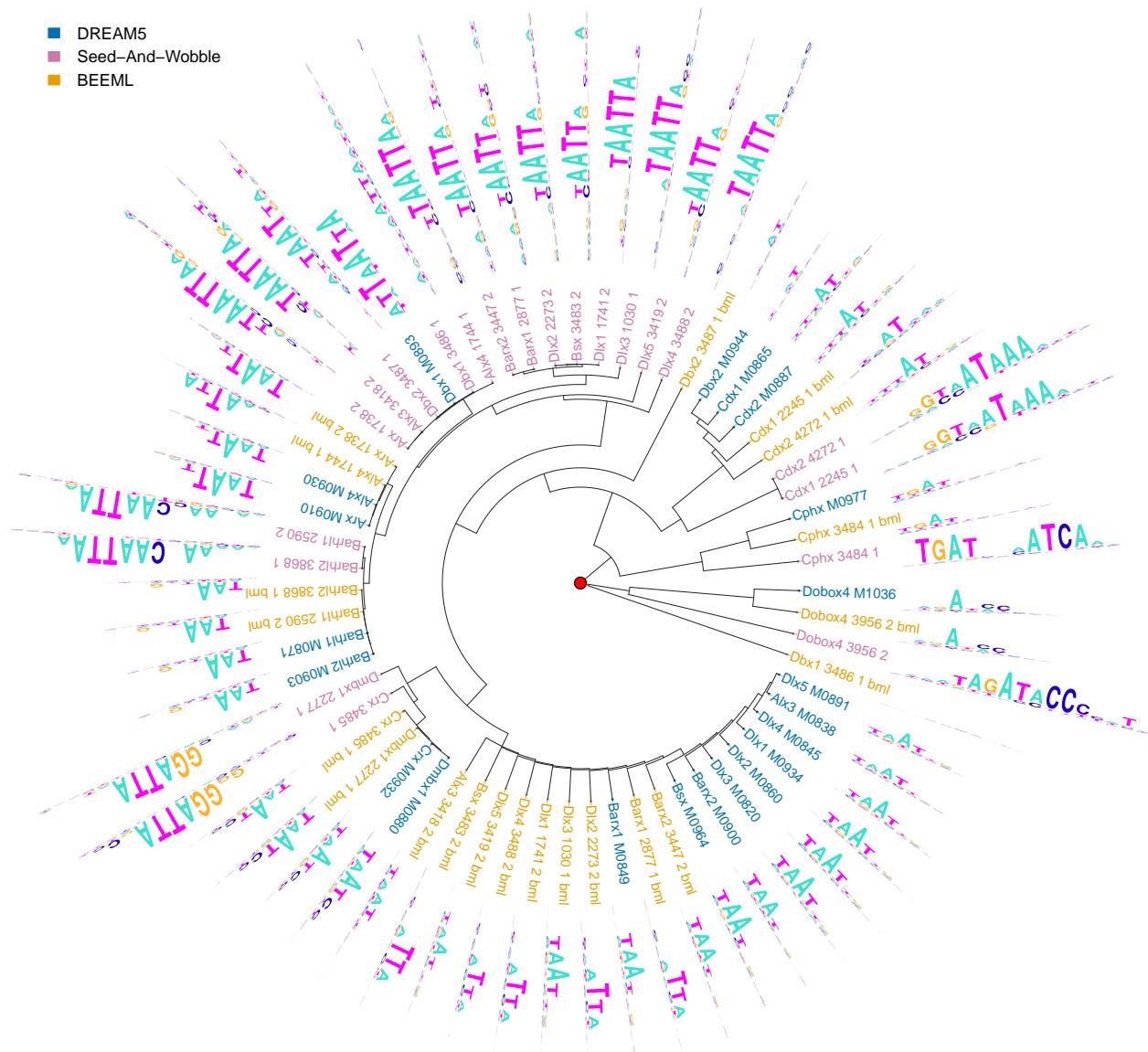
```



**Supplementary Figure 2H.** Use the background color of the inner circle to distinguish different data sources with the parameter col.bg

Use the text color of motif names to distinguish the computational algorithm with the parameter col.leaves.

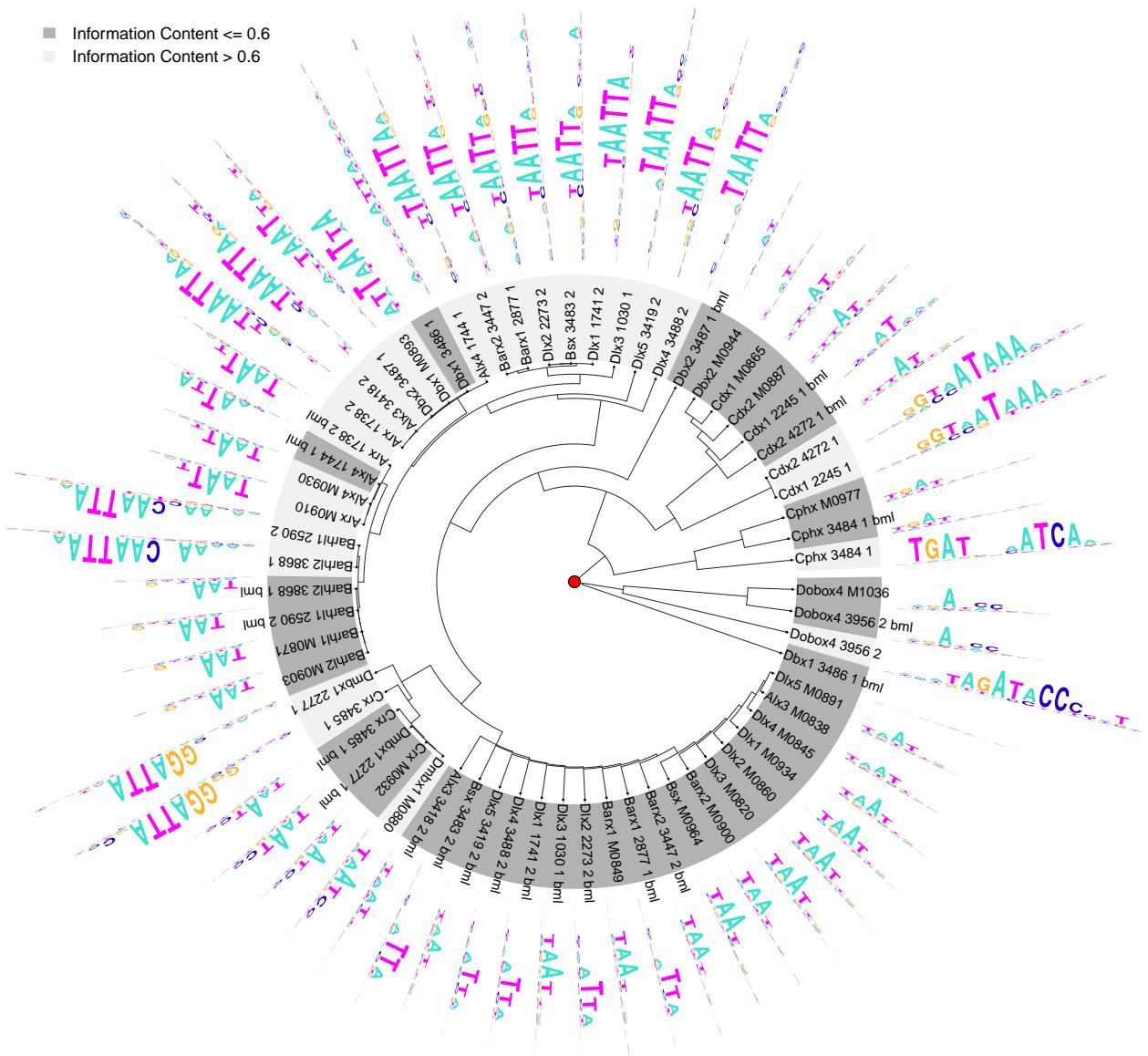
```
plotMotifStackWithRadialPhylog(phylog=phylog, pfms=unaligned.pfms,
                                labels.leaves=leaveNames,
                                col.leaves=algorithm,
                                cleaves=.2, circle=1.1, circle.motif=1.6,
                                clabel.leaves=.8, angle=358)
legend(-2.3, 2.4, legend=c("DREAM5", "Seed-And-Wobble", "BEEML"),
       fill= c("#0072B2", "#CC79A7", "#E69F00"),
       border="white", lty=NULL, bty = "n", cex=1)
```



**Supplementary Figure 2I.** Use the text color of motif names to distinguish the computational algorithms used with the parameter col.leaves

Use the background color of the motif names to distinguish motifs with high and low IC with the parameter `col.leaves.bg`.

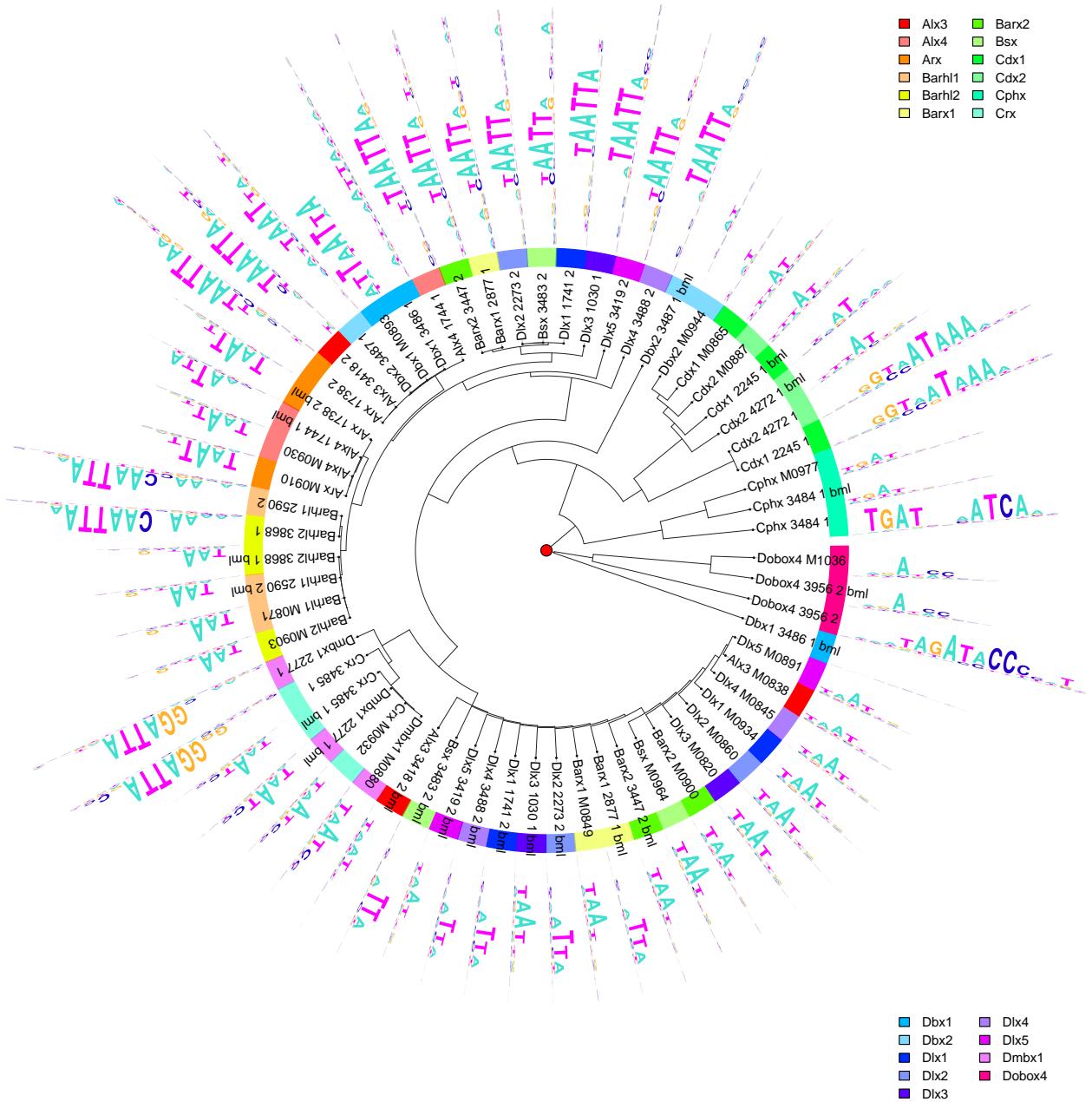
```
icgp <- ifelse(sapply(sapply(unaligned.pfms, getIC), mean) > 0.6,
                "lightgray", "black")
plotMotifStackWithRadialPhylog(phylog=phylog, pfms=unaligned.pfms,
                                labels.leaves=leaveNames,
                                col.leaves.bg=icgp,
                                col.leaves.bg.alpha=.3,
                                cleaves=.2, circle=1.1, circle.motif=1.6,
                                clabel.leaves=.8, angle=358)
legend(-2.3, 2.4,
       legend=c("Information Content <= 0.6", "Information Content > 0.6"),
       fill= highlightCol(c("black", "lightgray"), alpha=.3),
       border="white", lty=NULL, bty = "n", cex=1)
```



**Supplementary Figure 2J.** Use the background color of the motif names to distinguish motifs with high and low IC with the parameter col.leaves.bg

Use the inner ring color to distinguish the TF name with the parameter col.inner.label.circle.

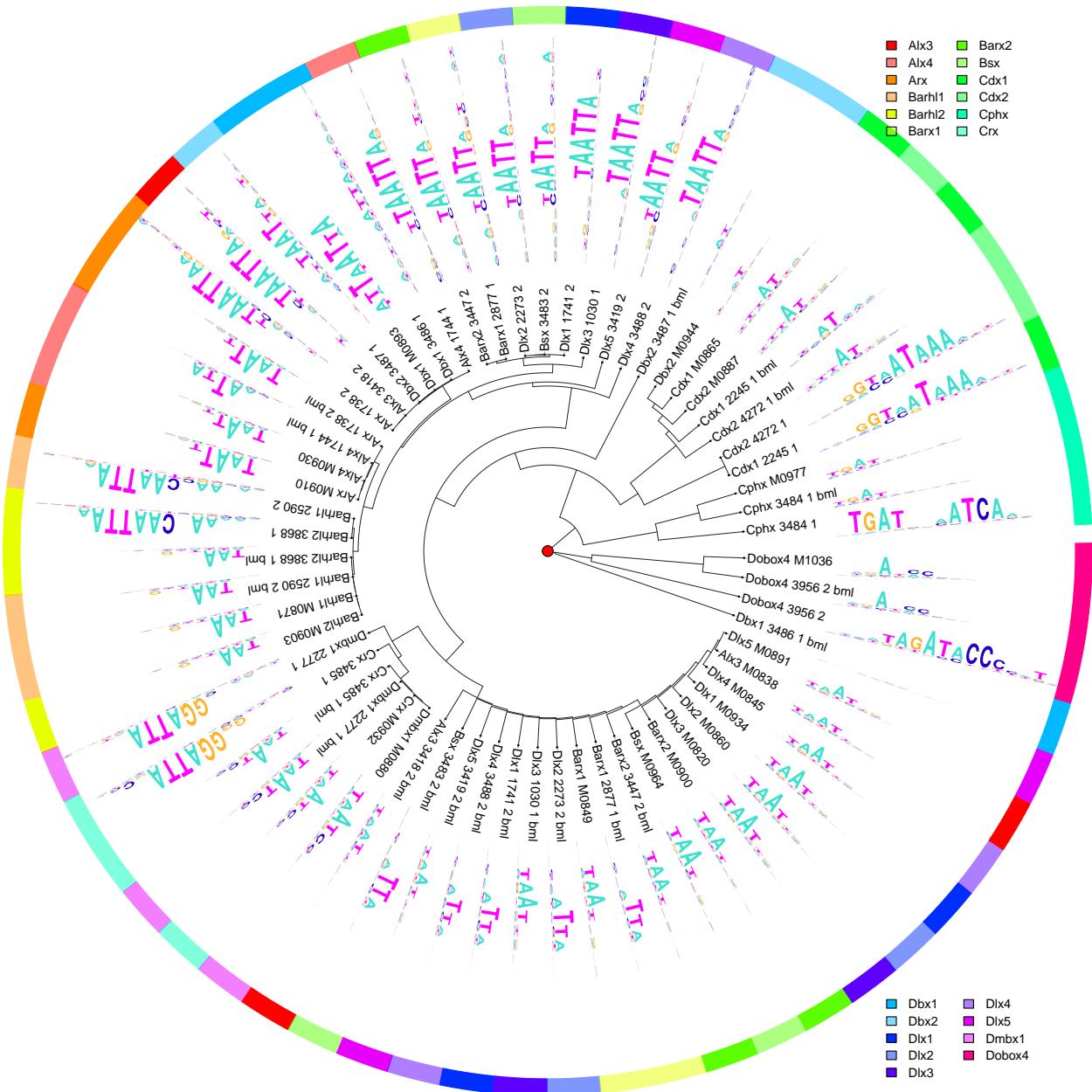
```
plotMotifStackWithRadialPhylog(phylog=phylog, pfms=unaligned.pfms,
                                labels.leaves=leaveNames,
                                col.inner.label.circle=motifGroup,
                                inner.label.circle.width=0.1,
                                cleaves=.2, circle=1.1, circle.motif=1.6,
                                clabel.leaves=.8, angle=358)
legend(1.5, 2.4, legend=levels.motifGroup[1:12],
       fill= colors.motifGroup[1:12],
       border="black", lty=NULL, bty = "n", ncol=2, cex=.8)
legend(1.5, -2, legend=levels.motifGroup[13:21],
       fill= colors.motifGroup[13:21],
       border="black", lty=NULL, bty = "n", ncol=2, cex=.8)
```



**Supplementary Figure 2K.** Use the inner ring color to distinguish the TFs with the parameter col.inner.label.circle

Use the outer ring color to distinguish the TF names with the parameter col.outer.label.circle.

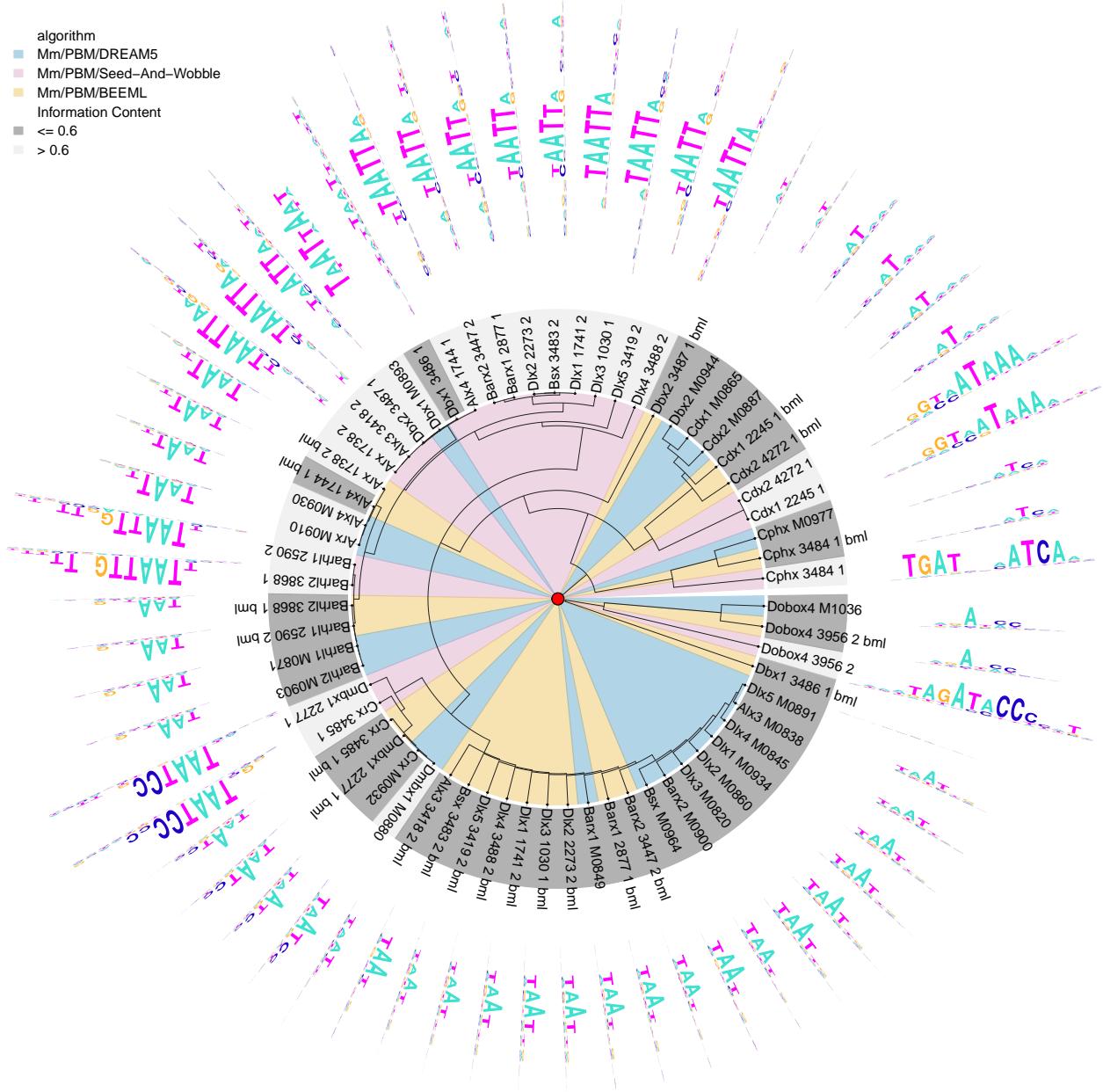
```
plotMotifStackWithRadialPhylog(phylog=phylog, pfms=unaligned.pfms,
                                labels.leaves=leaveNames,
                                col.outer.label.circle=motifGroup,
                                outer.label.circle.width=0.1,
                                cleaves=.2, circle=1.1, circle.motif=1.6,
                                clabel.leaves=.8, angle=358)
legend(1.5, 2.4, legend=levels.motifGroup[1:12],
       fill= colors.motifGroup[1:12],
       border="black", lty=NULL, bty = "n", ncol=2, cex=.8)
legend(1.5, -2, legend=levels.motifGroup[13:21],
       fill= colors.motifGroup[13:21],
       border="black", lty=NULL, bty = "n", ncol=2, cex=.8)
```



Use a combination of coloring options to visualize multiple motif features.

```
plotMotifStackWithRadialPhylog(phylog=phylog, pfms=aligned.pfms,
                                labels.leaves=leaveNames,
                                col.bg=algorithm, col.bg.alpha=.3,
                                col.leaves.bg=icgp,
                                col.leaves.bg.alpha=.3,
                                cleaves=.2, circle=1.1, circle.motif=1.6,
                                clabel.leaves=.8, angle=358)
legend(-2.3, 2.4,
       legend=c("algorithm", "Mm/PBM/DREAM5", "Mm/PBM/Seed-And-Wobble",
               "Mm/PBM/BEEML", "Information Content", "<= 0.6", "> 0.6"),
       fill= c("white",
              highlightCol(c("#0072B2", "#CC79A7", "#E69F00"), alpha=.3),
              "white", highlightCol(c("black", "lightgray"), alpha=.3)),
       border="white", lty=NULL, bty = "n", cex=.8)

detach(motIVout)
```



**Supplementary Figure 2M.** Use a combination of coloring options to visualize multiple motif features

## Compare different Column Comparison Metrics (CCM) and alignment methods.

Here we used a subset of mouse HD TFs with PBM data. For each TF, we generated different motif alignments using different computational methods. We compared how well different CCM and alignment methods could group together the motifs from the same TFs. This example illustrates how to select the CCM and the alignment method (MotIV or MatAlign) and how different methods can affect the resulting alignment.

When clustering using MotIV, Pearson Correlation Coefficient (PCC) or Average Log Likelihood Ratio (ALLR) was used as the CCM, and Smith-Waterman Ungapped (SWU) or Needleman-Wunsch (NW) was used as the alignment method. When clustering the motifs using MatAlign, ALLR and SWU were used as CCM and alignment respectively.

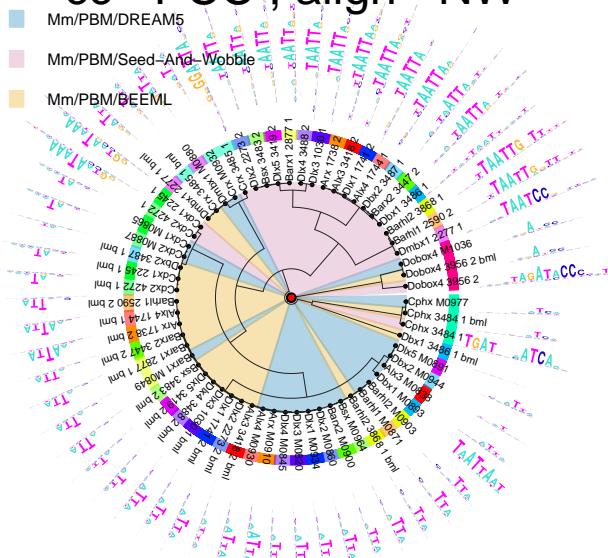
To run the MatAlign examples, phylib (<http://evolution.genetics.washington.edu/phylib/progs.data.dist.html>) and MatAlign (<http://stormo.wustl.edu/MatAlign/>) need to be installed first. Alternatively, the Docker container described in the Supplementary Notes contains all needed dependencies.

### Visualize motif alignments generated by MotIV with different CCM and alignment methods.

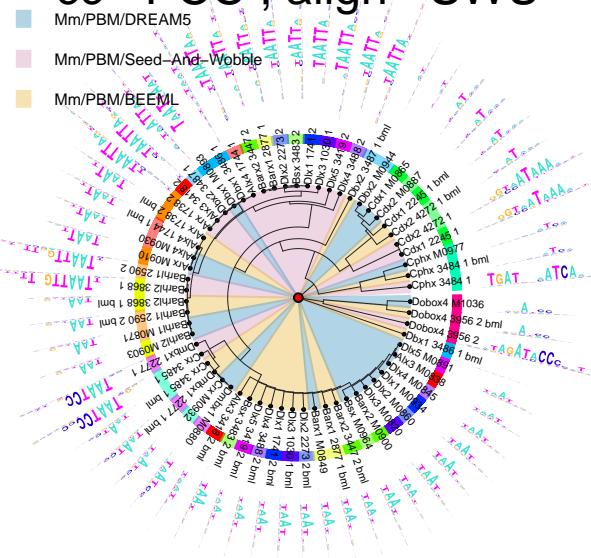
```
sta <- mapply(function(cc, align){
  motIVout <- getMotIVOut(pfms, cc, align)
  attach(motIVout)
  plotMotifStackWithRadialPhylog(phylog, pfms=aligned.pfms,
    labels.leaves=leaveNames,
    col.bg=algorithm, col.bg.alpha=.3,
    col.inner.label.circle=motifGroup,
    inner.label.circle.width=0.1,
    cleaves=.2, circle=1.1, circle.motif=1.6,
    clabel.leaves=.3, angle=358)

  legend(-2.3, 2.4,
    legend=c("Mm/PBM/DREAM5", "Mm/PBM/Seed-And-Wobble",
            "Mm/PBM/BEEML"),
    fill= highlightCol(c("#0072B2", "#CC79A7", "#E69F00"), alpha=.3),
    border="white", lty=NULL, bty = "n", cex=.5)
  text(0, 2.3, label=paste("cc=", cc, "; align=", align), cex=1.5)
  cnt <- rle(motifGroup)
  cnt <- split(algorithm, rep(1:length(cnt$lengths), cnt$lengths))
  cnt <- table(sapply(cnt, function(.ele) length(unique(.ele))))
  cnt.1 <- vector("integer", 3)
  names(cnt.1) <- 1:3
  cnt.1[names(cnt)] <- cnt
  cnt.1["1"] <- (cnt.1["1"]+cnt.1["2"]*2+cnt.1["3"]*3)/3 - cnt.1["2"] - cnt.1["3"]
  text(0, -2.3,
    label=paste(names(cnt.1), cnt.1, sep=":", collapse="; "), cex=1.5)
  detach(motIVout)
}, c("PCC", "PCC", "ALLR", "ALLR"), c("NW", "SWU", "NW", "SWU"))
```

**cc= PCC ; align= NW**

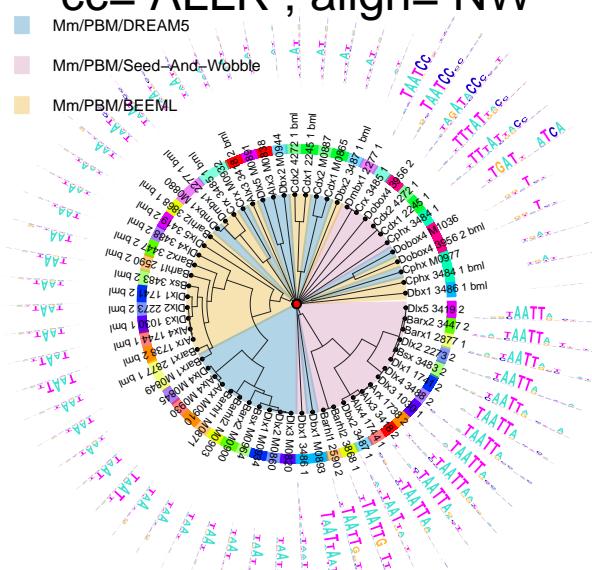


**cc= PCC ; align= SWU**



**1:17; 2:2; 3:2**

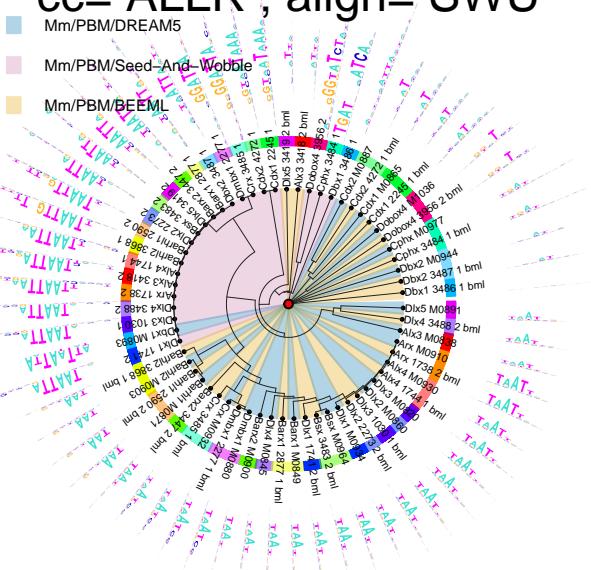
**cc= ALLR ; align= NW**



**1:15; 2.6; 3:0**

**1:10; 2:9; 3:2**

**cc= ALLR ; align= SWU**



**1:8; 2:13; 3:0**

**Supplementary Figure 2N.** Alignment of mouse PBM data by MotIV with different CCM and alignment methods

The number (1:X; 2:Y; 3:Z) on the bottom of the Supplementary Figures 2O and 2P represents the number of motifs from the same TF grouped by different algorithms. 3:Z means that Z number of TFs with 3 of 3 motifs clustered together; 2:Y means that Y number of TFs with 2 of 3 motifs clustered together; 1:X means that X number of TFs failed to cluster any of the 3 motifs together. Thus, the lower the X and the higher the Y and Z, the better the method performed. In this example, MatAlign performed better than MotIV even using the same CCM (ALLR) and alignment method (SWU).

Visualize motif alignments generated by MatAlign with ALLR as the MCC and SWU as the alignment method.

```

##read newick tree. Alignment is done by MatAlign
##The newick tree is generated by Neighbor, which is a part of phylip
outpath <- "output"
matalign_path <- "./app/matalign-v4a"
neighbor_path <- "./app/neighbor.app/Contents/MacOS/neighbor"
MatAlign2tree_path <- "./MatAlign2tree.pl"
system(paste("perl", MatAlign2tree_path, "--in . --pcmpath", pcmpath,
            "--out", outpath,
            "--matalign", matalign_path,
            "--neighbor", neighbor_path,
            "--tree", "UPGMA"))
newickstrUPGMA <- readLines(con=file.path(outpath, "NJ.matalign.distMX.nwk"))
##convert it to phylog object
phylogUPGMAmatAlign <- newick2phylog(newickstrUPGMA, FALSE)

##get the leaves of phylog for reordering the pfms
leaveNames <- names(phylogUPGMAmatAlign$leaves)
this_motifs <- pfms[leaveNames]

## data source
dataSource <- factor(grep("_M", leaveNames))
levels(dataSource) <- c("#F0E442", "#56B4E9") ##c("Uniprobe", "CIS-BP")
dataSource <- as.character(dataSource)
## algorithm
algorithm <- factor(!grep("_M", leaveNames) + grep("_bml", leaveNames))
levels(algorithm) <- c("#0072B2", "#CC79A7", "#E69F00") ##("DREAM5", "Seed-And-Wobble", "BEEML")
algorithm <- as.character(algorithm)
## motifs from the same PBM data source
motifGroup <- factor(gsub("(.*?)_.*$", "\\\1", leaveNames))
levels.motifGroup <- levels(motifGroup)
levels(motifGroup) <- pairColor[1:length(levels(motifGroup))]
colors.motifGroup <- levels(motifGroup)
motifGroup <- as.character(motifGroup)

## draw the motifs
plotMotifStackWithRadialPhylog(phylog=phylogUPGMAmatAlign,
                                pfms=DNAmotifAlignment(this_motifs),
                                labels.leaves=leaveNames,
                                col.bg=algorithm,
                                col.bg.alpha=.3,
                                col.inner.label.circle=motifGroup,
                                inner.label.circle.width=.1,
                                cleaves=.2, circle=1.1,
                                )

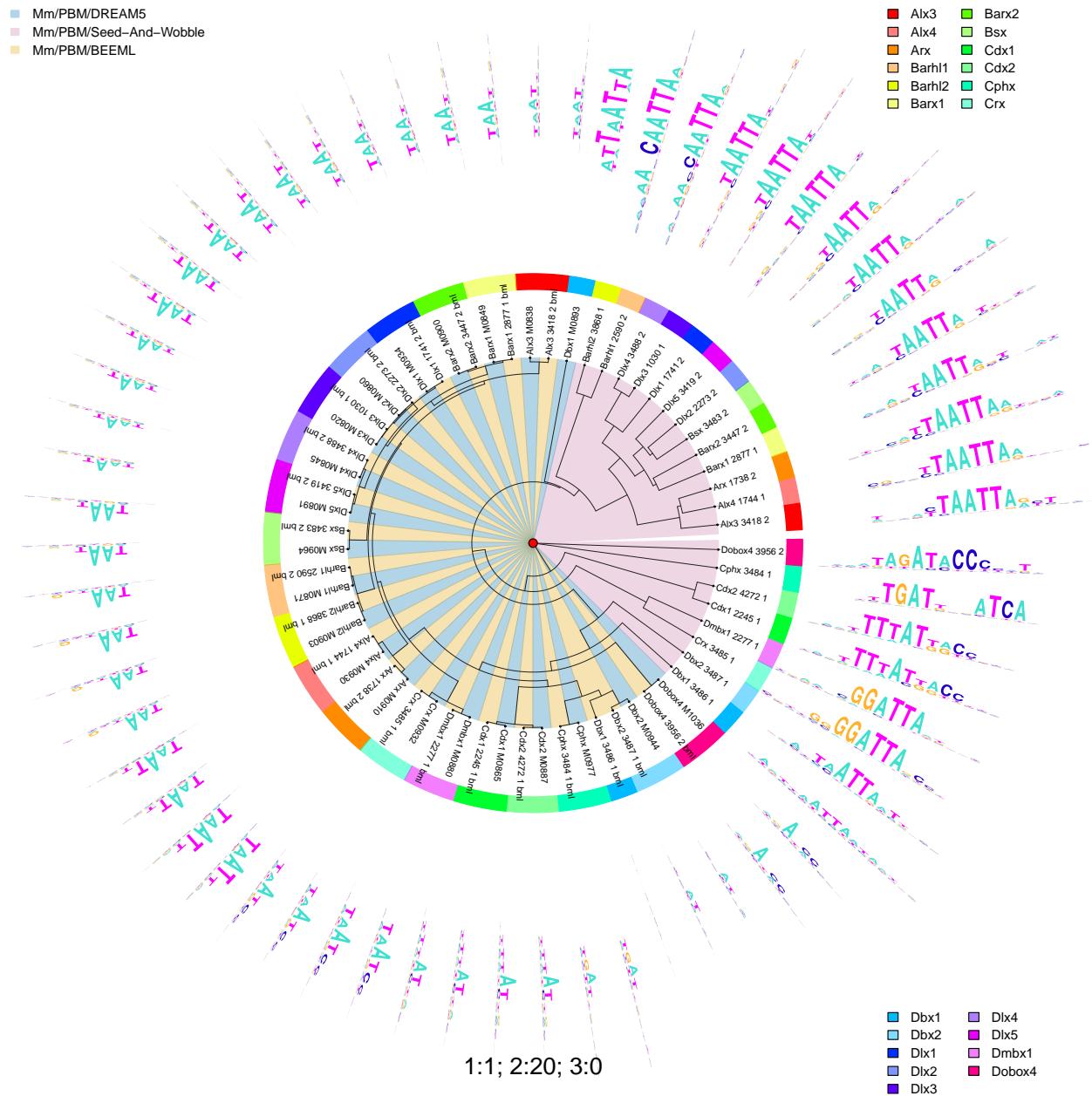
```

```

    circle.motif=1.6,
    clabel.leaves=.6, angle=358)

legend(1.5, 2.4, legend=levels.motifGroup[1:12],
       fill= colors.motifGroup[1:12],
       border="black", lty=NULL, bty = "n", ncol=2, cex=.8)
legend(1.5, -2, legend=levels.motifGroup[13:21],
       fill= colors.motifGroup[13:21],
       border="black", lty=NULL, bty = "n", ncol=2, cex=.8)
legend(-2.35, 2.4,
       legend=c("Mm/PBM/DREAM5", "Mm/PBM/Seed-And-Wobble", "Mm/PBM/BEEML"),
       fill= highlightCol(c("#0072B2", "#CC79A7", "#E69F00"), alpha=.3),
       border="white", lty=NULL, bty = "n", cex=.8)
cnt <- rle(motifGroup)
cnt <- split(algorithm, rep(1:length(cnt$lengths), cnt$lengths))
cnt <- table(sapply(cnt, function(.ele) length(unique(.ele))))
cnt.1 <- vector("integer", 3)
names(cnt.1) <- 1:3
cnt.1[names(cnt)] <- cnt
cnt.1["1"] <- (cnt.1["1"]+cnt.1["2"]*2+cnt.1["3"]*3)/3 - cnt.1["2"] - cnt.1["3"]
text(0, -2.3, label=paste(names(cnt.1), cnt.1, sep=":", collapse="; "), cex=1.5)

```

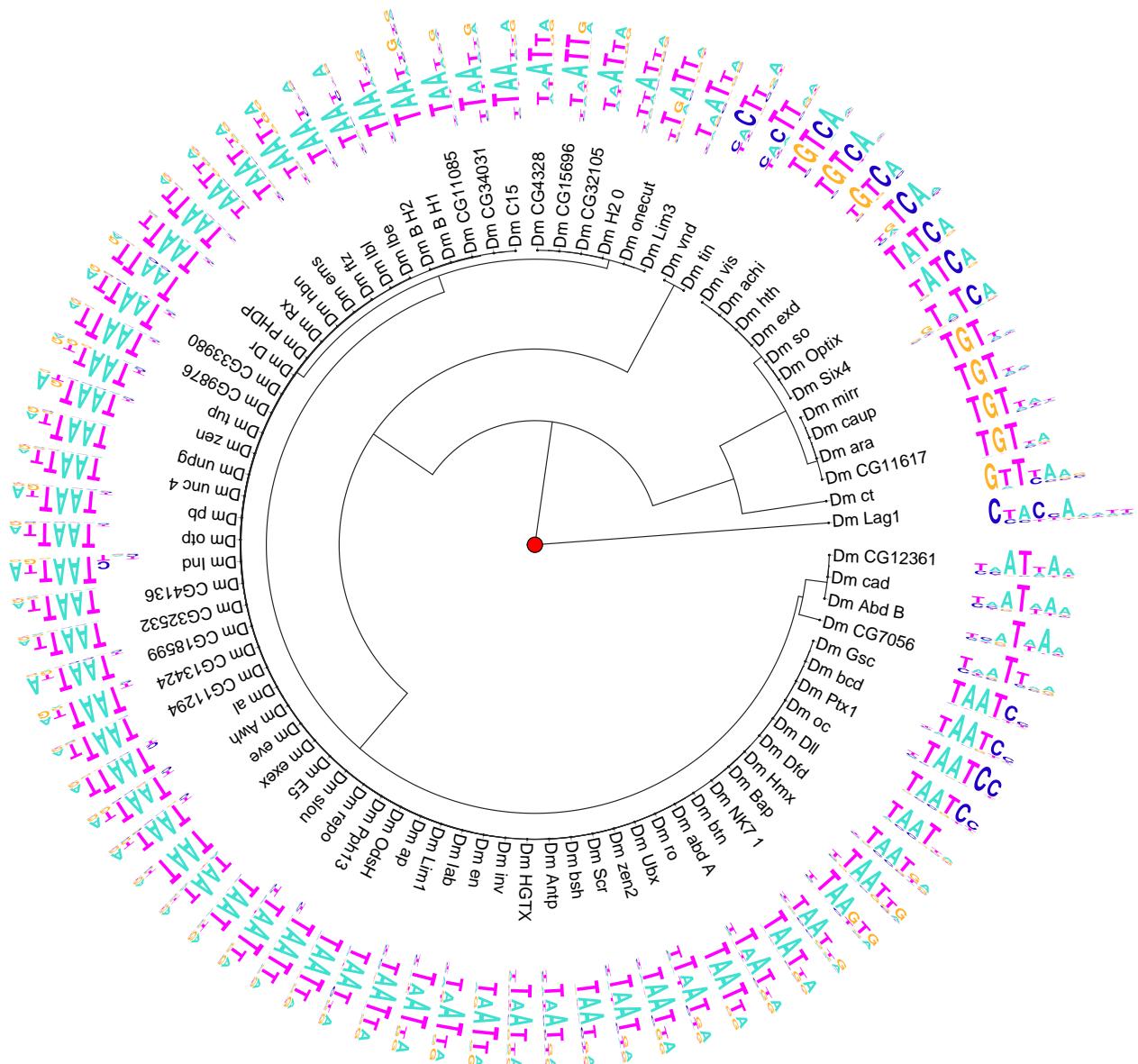


**Supplementary Figure 2O.** Alignment of mouse PBM data by MatAlign

Improved alignment of fly HD family motifs using MatAlign compared to MotIV. The MatAlign alignment method is superior for discriminating between the closely related motifs in the fly HD family.

```
pcmpath <- "pcmsDatasetDM"
pcms <- readPCM(pcmpath)
pcms <- safeColor(pcms)
pfms<-lapply(pcms,pcm2pfm)
motIVout <- getMotIVOut(pfms, "ALLR", "SWU")
plotMotifStackWithRadialPhylog(phylog=motIVout$phylog,
                                pfms=motIVout$aligned.pfms,
                                labels.leaves=motIVout$leaveNames,
                                cleaves=.2, circle=1.2, circle.motif=1.6,
                                clabel.leaves=1,
                                motifScale="logarithmic",
                                angle=358,
                                plotIndex=FALSE)
text(0, 2.4, label="motIV: cc=ALLR; align=SWU", cex=1.5)
```

motIV: cc=ALLR; align=SWU



**Supplementary Figure 2P.a.** MotIV(ALLR and SWU) as the alignment method

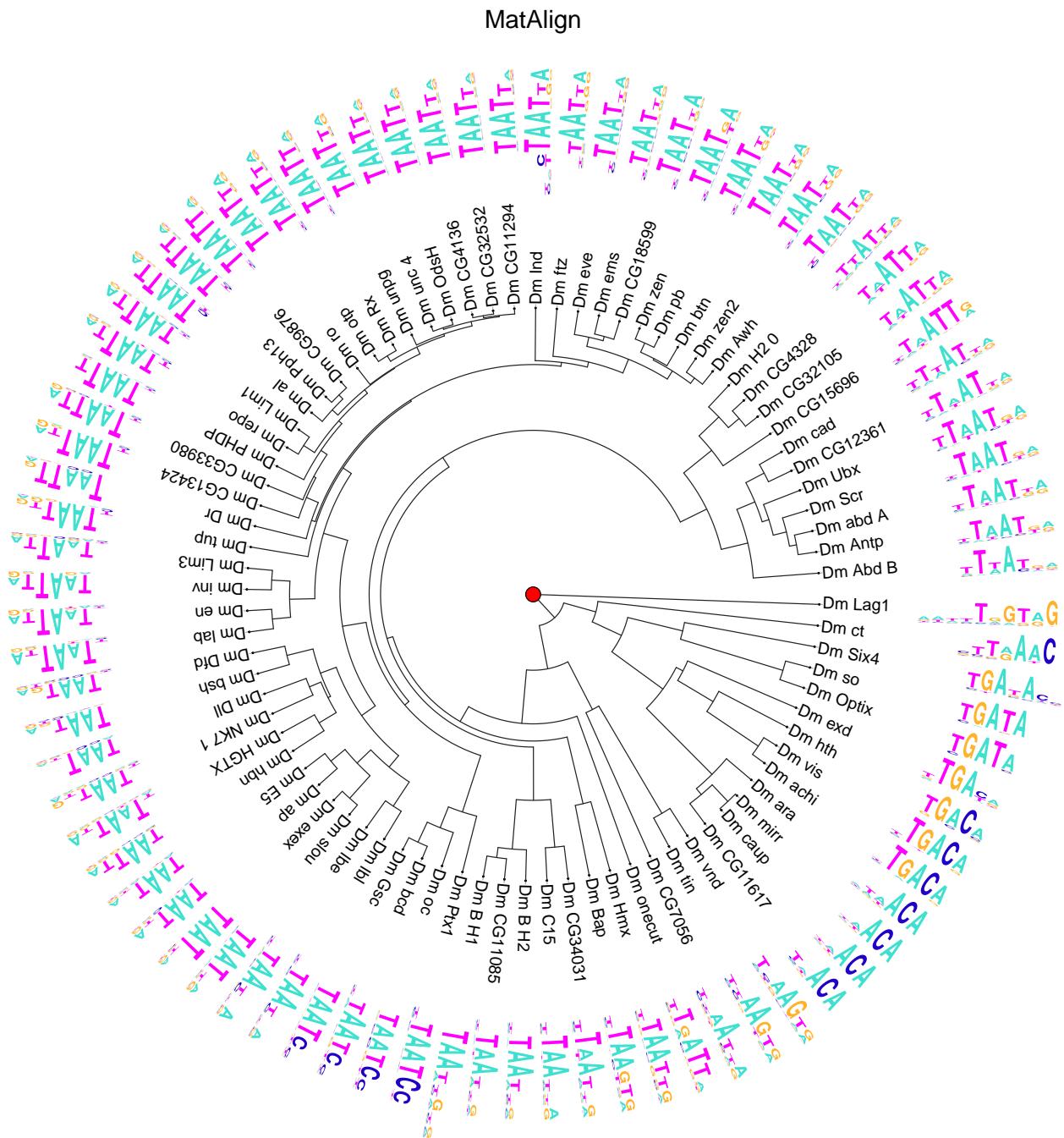
```

## function to read example data
getMatAlignOut <- function(pcopath, groupDistance=NA, trim=0.2){
  pcms <- readPCM(pcopath)
  pcms <- safeColor(pcms)
  pfms<-lapply(pcms,pcm2pfm)
  source("zzz.R")
  system(paste("perl", MatAlign2tree_path, "--in . --pcopath", pcopath,
              "--out", outpath,
              "--malign", malign_path,
              "--neighbor", neighbor_path,
              "--tree","UPGMA"))
  newickstrUPGMA <-
    readLines(con=file.path(outpath, "NJ.malign.distMX.nwk"))
  phylog <- newick2phylog(newickstrUPGMA, FALSE)
  leaves <- names(phylog$leaves)
  motifs <- pfms[leaves]
  if(!is.na(groupDistance)){
    motifSig <-
      motifSignature(motifs, phylog,
                     groupDistance=groupDistance,
                     min.freq=1, trim=trim)
    sig <- signatures(motifSig)
    gpCol <- sigColor(motifSig)
  }else{
    motifSig <- NA
    sig <- NA
    gpCol <- NA
  }

  return(list(phylog=phylog, sig=sig, gpCol=gpCol,
             motifs=DNAmotifAlignment(motifs),
             leaves=leaves,
             unaligned.pfms=motifs))
}

matAlignOut <- getMatAlignOut(pcopath)
plotMotifStackWithRadialPhylog(phylog=matAlignOut$phylog,
                               pfms=matAlignOut$motifs,
                               labels.leaves=matAlignOut$leaves,
                               cleaves=.2, circle=1.2, circle.motif=1.6,
                               xlabel.leaves=1, motifScale="logarithmic",
                               angle=358, plotIndex=FALSE)
text(0, 2.4, label="MatAlign", cex=1.5)

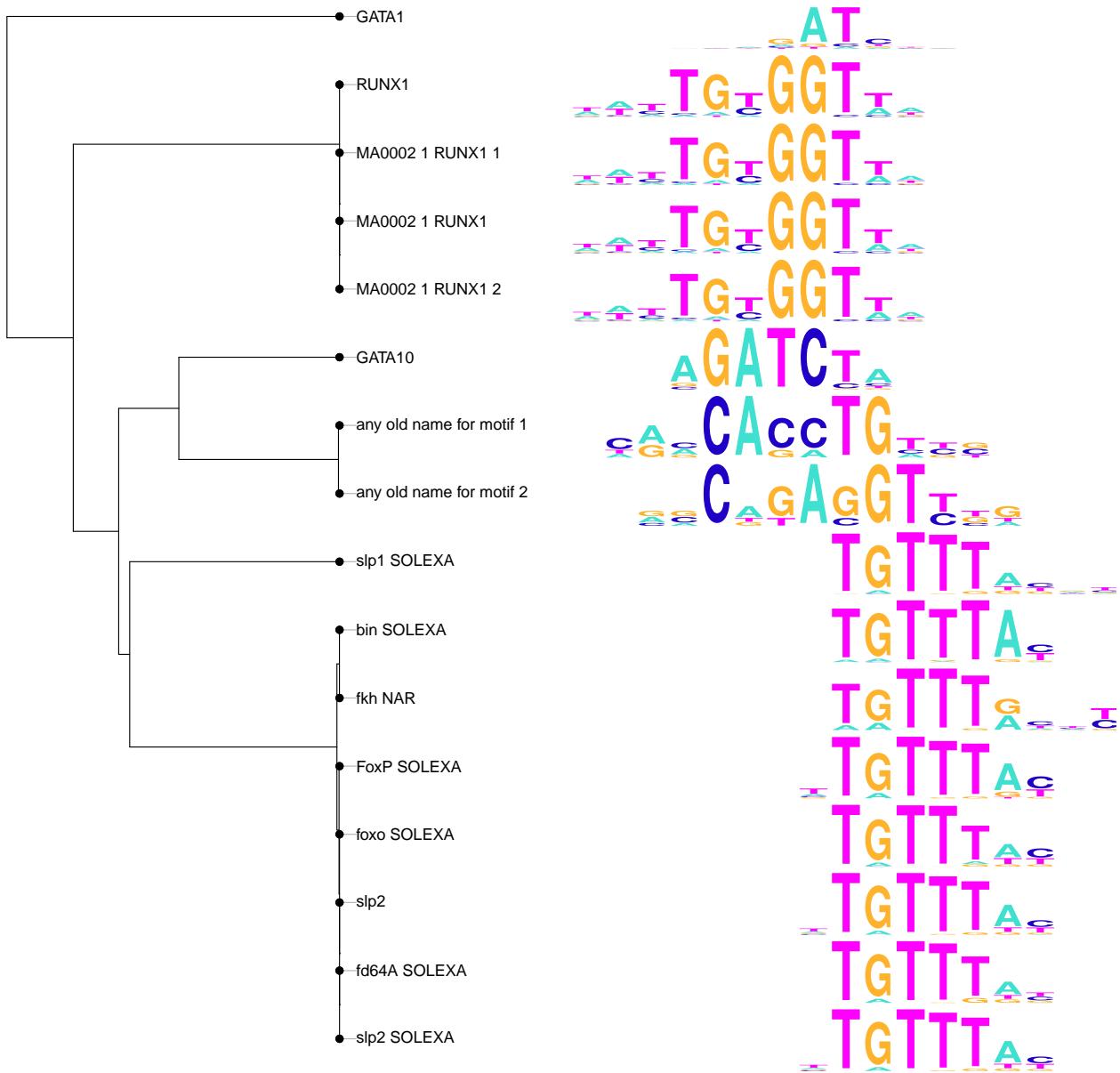
```



**Supplementary Figure 2P.b.** MatAlign (ALLR and SWU) as the alignment method

Import PFMs/PCMs files in Transfac, CisBP, or JASPAR format in batch mode.

```
path <- system.file("extdata", package = "motifStack", mustWork = TRUE)
pcms <- importMatrix(dir(path, "*.pcm", full.names = TRUE), format = "pcm", to = "pcm")
JASPAR <- importMatrix(dir(path, "*.jaspar", full.names = TRUE))
pfms <- importMatrix(dir(path, "*.pfm", full.names = TRUE))
transfac <- importMatrix(file.path(path, c("transfac.like.test.transfac", "RUNX1.transfac")))
cisbp <- importMatrix(file.path(path, "PWM.cisbp"))
motifs <- unlist(c(pcms, JASPAR, pfms, transfac, cisbp))
motifs[sapply(motifs, class)=="pcm"] <-
  lapply(motifs[sapply(motifs, class)=="pcm"], pcm2pfm)
motifs <- safeColor(motifs)
motifStack(motifs, layout = "phylog")
```



**Supplementary Figure 2Q.** Aligned motifs imported from PFM/PCMs files in Transfac, CisBP, or JASPAR format in batch mode.

Plot an affinity logo from a position specific affinity matrix (PSAM) as described by Foat et al. (Barrett C. Foat, Alexandre V. Morozov, Harmen J. Bussemaker; Statistical mechanical modeling of genome-wide transcription factor occupancy data by MatrixREDUCE, Bioinformatics, Volume 22, Issue 14, 15 July 2006, Pages e141-e149, <https://doi.org/10.1093/bioinformatics/btl223>).

```
psam <- importMatrix(file.path(path, "PSAM.mxr"), format = "psam")[[1]]
psam <- safeColor(psam)
motifStack(psam)
```



Supplementary Figure 2R. PSAM plotted as an affinity logo.

Session information including the version of R , motifStack and other packages.

```
sessionInfo()

## R Under development (unstable) (2017-11-29 r73795)
## Platform: x86_64-apple-darwin17.2.0 (64-bit)
## Running under: macOS High Sierra 10.13.1
##
## Matrix products: default
## BLAS: /System/Library/Frameworks/Accelerate.framework/Versions/A/Frameworks/vecLib.framework/Versions
## LAPACK: /Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats4     parallel   grid       stats      graphics   grDevices utils
## [8] datasets   methods    base
##
## other attached packages:
## [1] motifStack_1.23.5   Biostrings_2.47.0   XVector_0.19.1
## [4] IRanges_2.13.4      S4Vectors_0.17.12  ade4_1.7-8
## [7] MotIV_1.35.0       BiocGenerics_0.25.0 grImport_0.9-0
## [10] XML_3.98-1.9      knitr_1.17      rmarkdown_1.8
##
## loaded via a namespace (and not attached):
## [1] Rcpp_0.12.14          highr_0.6
## [3] plyr_1.8.4            compiler_3.5.0
## [5] GenomeInfoDb_1.15.1   bitops_1.0-6
## [7] tools_3.5.0           zlibbioc_1.25.0
## [9] digest_0.6.12         evaluate_0.10.1
## [11] lattice_0.20-35      BSgenome_1.47.0
## [13] Matrix_1.2-12        DelayedArray_0.5.6
## [15] yaml_2.1.15           seqLogo_1.45.0
## [17] GenomeInfoDbData_0.99.2 rtracklayer_1.39.2
## [19] stringr_1.2.0         htmlwidgets_0.9
## [21] rprojroot_1.2         Biobase_2.39.0
## [23] BiocParallel_1.13.0   rGADEM_2.27.0
## [25] magrittr_1.5           scales_0.5.0
## [27] Rsamtools_1.31.0      backports_1.1.1
## [29] matrixStats_0.52.2    htmltools_0.3.6
## [31] GenomicAlignments_1.15.2 GenomicRanges_1.31.1
## [33] SummarizedExperiment_1.9.2 colorspace_1.3-2
## [35] stringi_1.1.6          munsell_0.4.3
## [37] RCurl_1.95-4.8
```