

Supplementary Figure 4

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```
##load the library
library(motifStack)
colorSet <- c("Dm"="#00FC00", "b1h"="#00FC00", "sw"="#008080", "bml"="darkgreen",
            "Mm"="brown", "MmDREAM"="blue", "Ms"="#F69156",
            "Hs"="#D900D9")

## function to read example data
getMatAlignOut <- function(pcopath, outpath="output", groupDistance=2.5, trim=0.2){
  pcms <- readPCM(pcopath)
  pfms<-lapply(pcms,pcm2pfm)
  matalign_path <- "./app/matalign-v4a"
  neighbor_path <- "./app/neighbor.app/Contents/MacOS/neighbor"
  system(paste("perl MatAlign2tree.pl --in . --pcopath", pcopath, "--out", outpath,
              "--matalign", matalign_path, "--neighbor", neighbor_path, "--tree", "UPGMA"))
  newickstrUPGMA <- readLines(con=file.path(outpath, "NJ.matalign.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))
}

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

getMotIVOut <- function(pcopath, cc, align, groupDistance=.005, trim=0.2){
  pcms <- readPCM(pcopath)
  pfms<-lapply(pcms,pcm2pfm)
  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)
  phylog <- hclust2phylog(hc)
  pfms <- pfms[[hc$order]]
  aligned.pfms <- DNAmotifAlignment(pfms)
  leaveNames <-names(phylog$leaves)
  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))
}

```

Supplementary Figure 4A. alignment of *Drosophila* motifs by matAlign

```

gpDis <- 2
Fly_B1H <- getMatAlignOut("pcmsDatasetFly", groupDistance=gpDis)
attach(Fly_B1H)

leaveNames <- gsub("^Dm_ ", "", leaves)

motifPiles(phylog=phylog, motifs, sig,
           col.pfms=gpCol, col.pfms.width=.01,

```

```

col.pfms2=gpCol, col.pfms2.width=.01,
labels.leaves=leaveNames,
plotIndex=c(FALSE, TRUE), IndexCex=1,
groupDistance=gpDis, clabel.leaves=1)

detach(Fly_B1H)

```

Supplementary Figure 4B. alignment of *Drosophila* motifs by MotIV

```

gpDis <- 0.005
Fly_B1H.MotIV <- getMotIVOut("pcmsDatasetFly",
                               cc="ALLR", align="SWU",
                               groupDistance=gpDis)
attach(Fly_B1H.MotIV)

leaveNames <- gsub("^Dm_ ", "", leaves)
motifPiles(phylog=phylog, motifs, sig,
           col.pfms=gpCol, col.pfms.width=.01,
           col.pfms2=gpCol, col.pfms2.width=.01,
           labels.leaves=leaveNames,
           plotIndex=c(FALSE, TRUE), IndexCex=1,
           groupDistance=gpDis, clabel.leaves=1)

detach(Fly_B1H.MotIV)

```

Supplementary Figure 4C. fly vs mouse, by matAlign

```

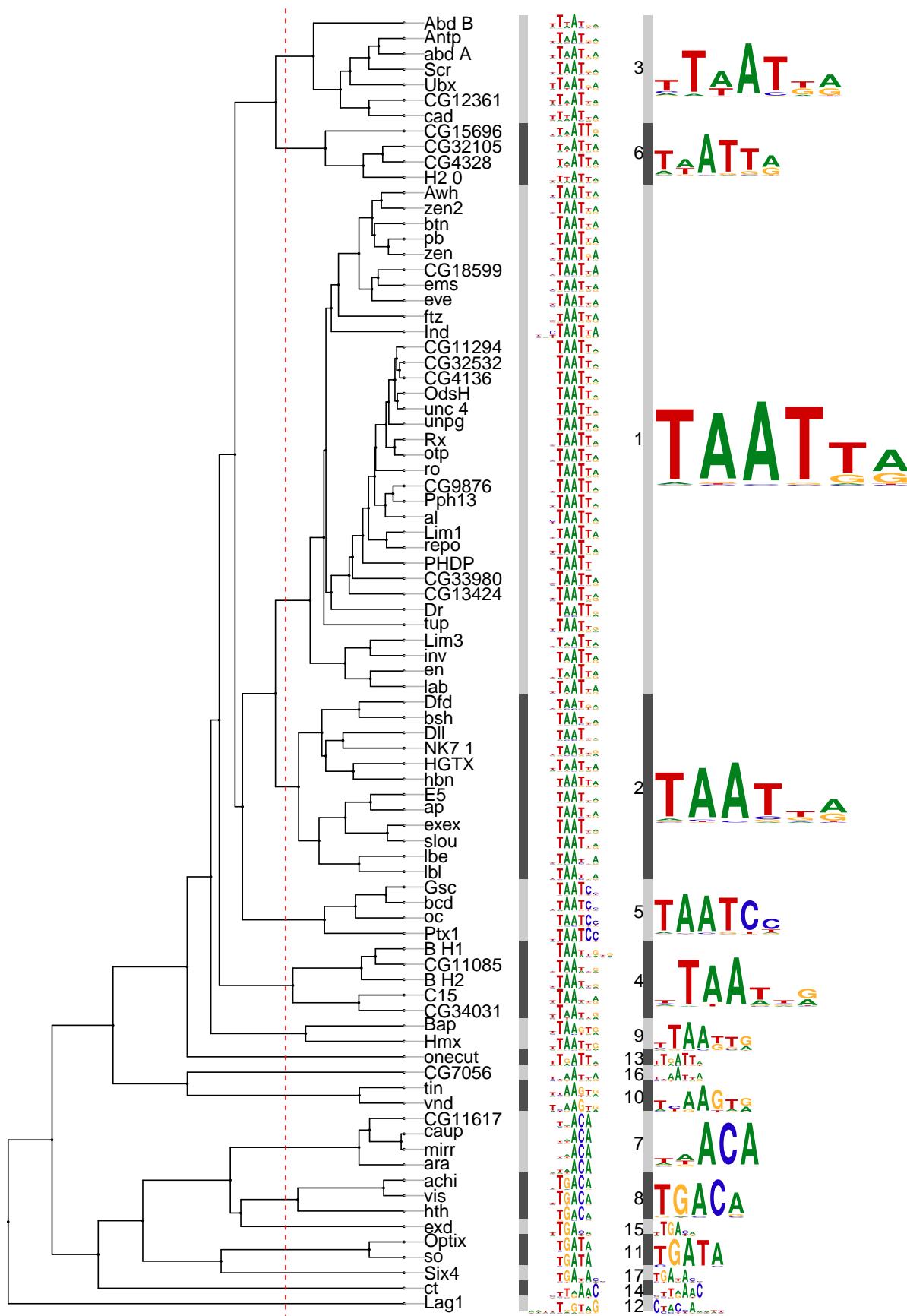
gpDis <- 2
FlyMousePBMSAW <- getMatAlignOut("pcmsDatasetFlyMousePBM.SAW",
                                    groupDistance=gpDis, trim=0.2)
attach(FlyMousePBMSAW)

##set methods color
species <- gsub("(Dm|Mm)_.*$", "\\\1", leaves)
species <- colorSet[species]##c("Dm/FFS/B1H", "Mm/Uniprobe/PBM/Seed-And-Wobble")

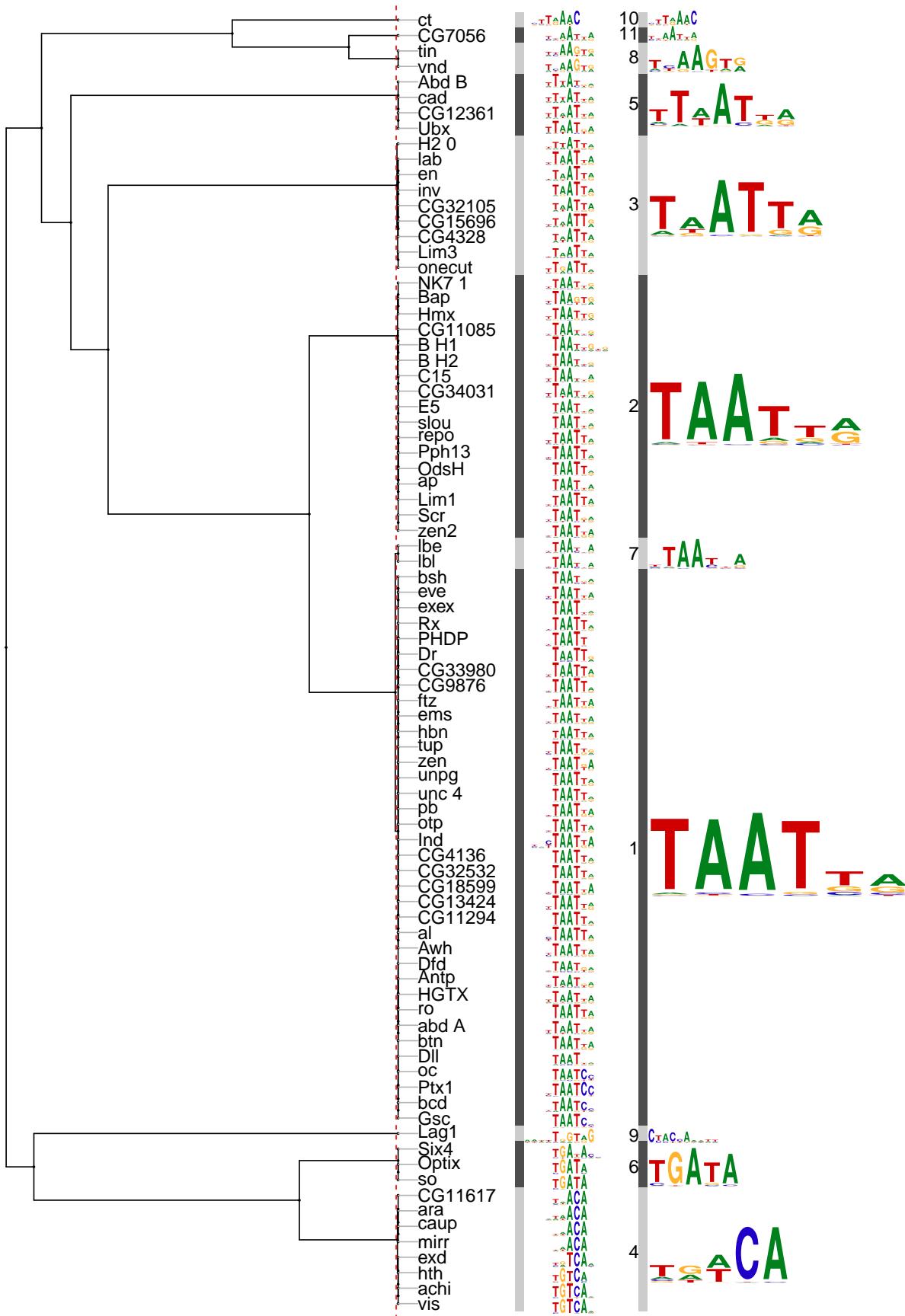
leaveNames <- gsub("(Dm|Mm)_", "", leaves)

plotMotifStackWithRadialPhylog(phylog=phylog, pfms=sig,
                               col.bg=species, col.bg.alpha=.3,
                               col.inner.label.circle=gpCol,
                               inner.label.circle.width=0.02,
                               labels.leaves=leaveNames,
                               cleaves=.1, circle=1.35, circle.motif=1.6,
                               clabel.leaves=.5, motifScale="logarithmic",
                               angle=358, plotIndex=TRUE, IndexCex=.6,
                               groupDistance=gpDis)

```



Supplementary Figure 4A: by matAlign

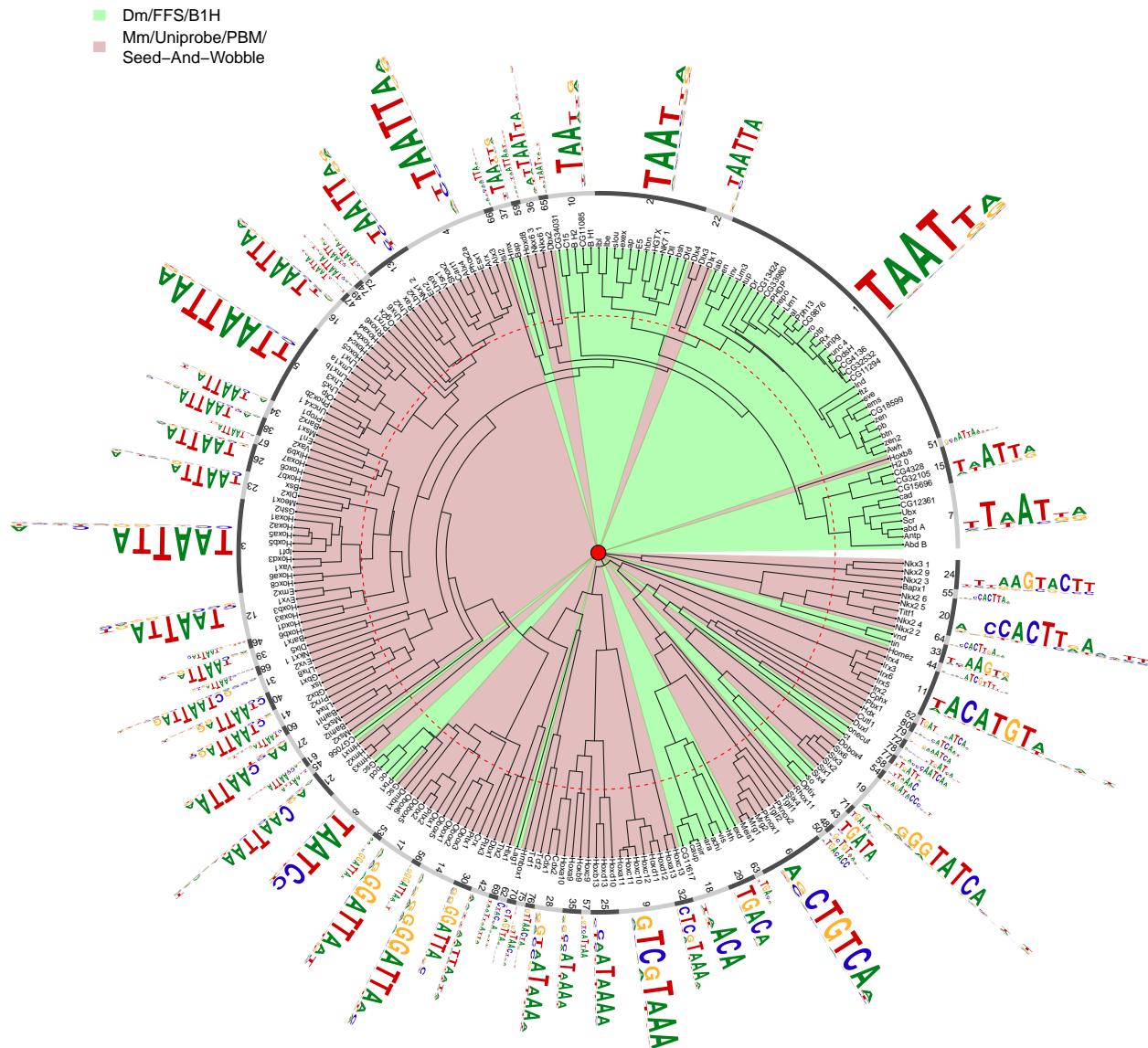


Supplementary Figure 4B: by MotIV

```

legend(-2.2, 2.4, legend=c("Dm/FFS/B1H", "Mm/Uniprobe/PBM/\nSeed-And-Wobble"),
      fill= highlightCol(colorSet[c("Dm", "Mm")], alpha=.3),
      border="white", lty=NULL, bty = "n")

```



Supplementary Figure 4C: Fly vs Mouse

```

detach(FlyMousePBMSAW)

```

Supplementary Figure 4D. fly vs human, by matAlign

```

gpDis <- 2
FlyHuman <- getMatAlignOut("pcmsDatasetFlyHuman", groupDistance=gpDis)
attach(FlyHuman)

```

```

##set methods color
species <- gsub("^(Dm|Hs)_.*$", "\\\1", leaves)
species <- colorSet[species] ##c("Dm/FFS/B1H", "Hs/CIS-BP/SELEX")

leaveNames <- gsub("^(Dm|Hs)_", "", leaves)

plotMotifStackWithRadialPhylog(phylog=phylog, pfms=sig, col.bg=species,
                               col.bg.alpha=.3, col.inner.label.circle=gpCol,
                               inner.label.circle.width=0.04,
                               labels.leaves=leaveNames,
                               cleaves=.1, circle=1.35, circle.motif=1.8,
                               clabel.leaves=.5, motifScale="logarithmic",
                               angle=358, plotIndex=TRUE, IndexCex=.6,
                               groupDistance=gpDis)

legend(-2, 2.2, legend=c("Dm/FFS/B1H", "Hs/CIS-BP/SELEX"),
       fill=highlightCol(colorSet[c("Dm", "Hs")], alpha=.3),
       border="white", lty=NULL, bty = "n")

detach(FlyHuman)

```

Supplementary Figure 4E. mouse selex vs mouse pbm/sw vs mouse pbm dream5

```

gpDis <- 2
MmPBM_SELEX <- getMatAlignOut("pcmsDatasetMmPBM_SELEX", groupDistance=gpDis)
attach(MmPBM_SELEX)

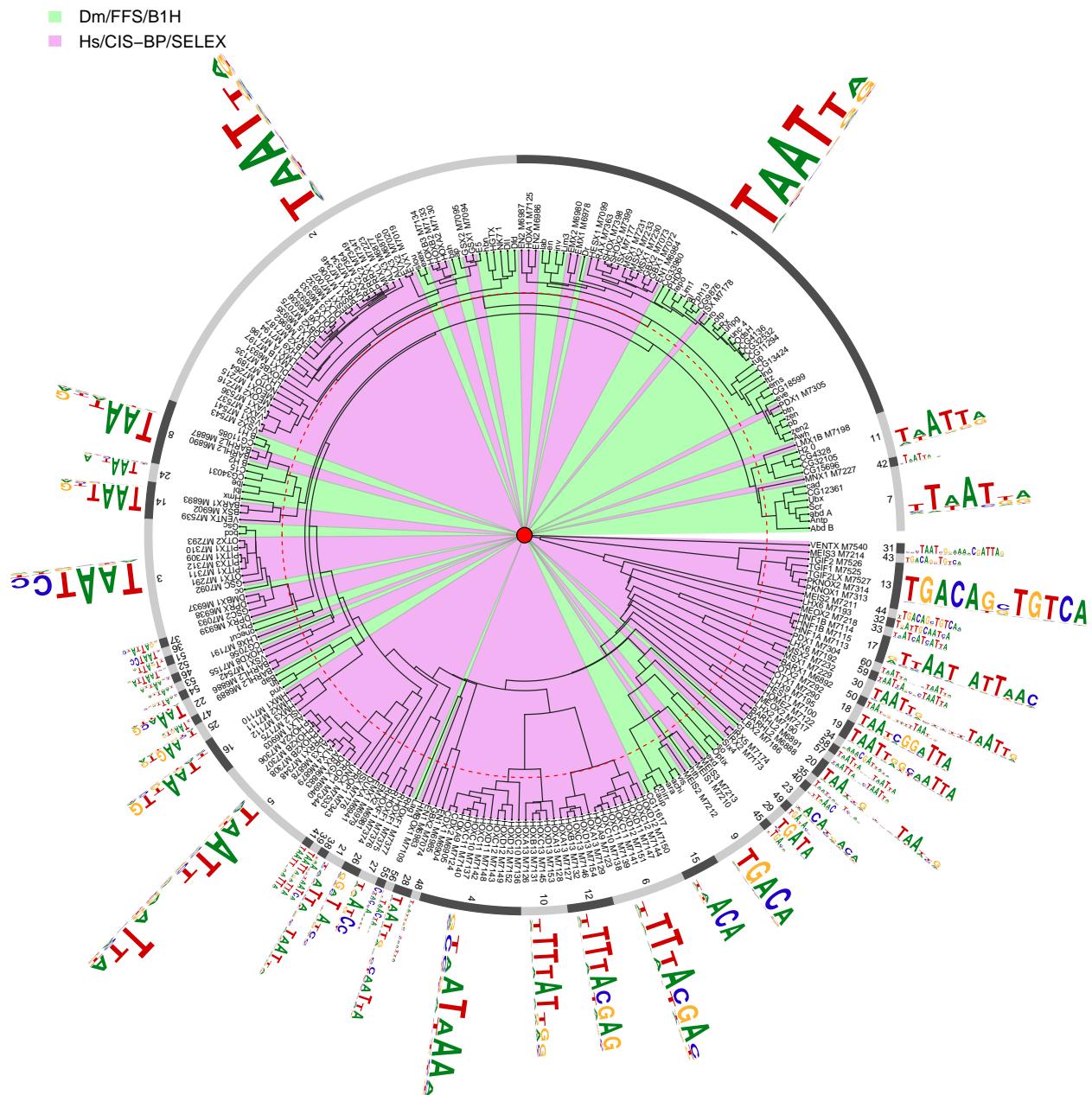
##set methods color
methods <- factor(grep("_SELEX", leaves) + grep("_M\d{4}", leaves))
levels(methods) <- c("Mm", "MmDREAM", "Ms")
##c("PBM/Uniprobe/Seed-And-Wobble", "PBM/CIS-BP/DREAM5", "SELEX/CIS-BP")
levels(methods) <- colorSet[levels(methods)]
methods <- as.character(methods)

leaveNames <- gsub("_SELEX|PBM", "", gsub("_Jolma|Berger", "", leaves))

## calculate average of top 8 position information content for each motif
icgp <- sapply(sapply(unaligned.pfms, getIC), function(.ele)
  mean(sort(.ele, decreasing=TRUE)[1:min(8, length(.ele))]))
icgp.ranges <- range(icgp)
icgp <- cut(icgp, 10, labels=colorRampPalette(c("green", "black", "red"))(10))
icgp.image <- as.raster(matrix(rev(levels(icgp)), ncol=1))
icgp <- as.character(icgp)

plotMotifStackWithRadialPhylog(phylog=phylog, pfms=sig,
                               col.bg=methods, col.bg.alpha=.3,
                               col.inner.label.circle=gpCol,
                               inner.label.circle.width=0.02,
                               labels.leaves=leaveNames, cleaves=.1,
                               circle=.95, circle.motif=1.8, clabel.leaves=.8,

```

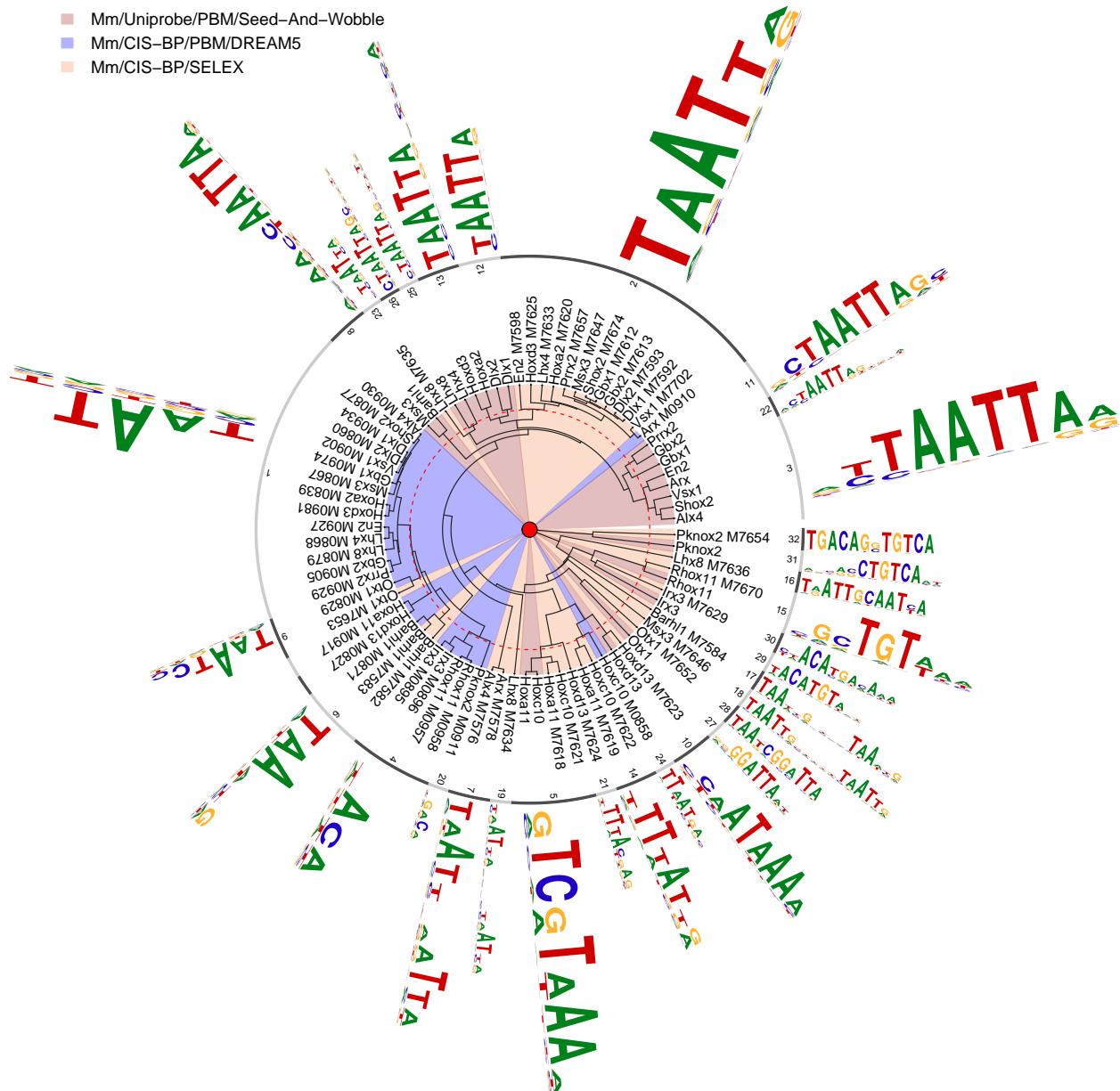


```

motifScale="logarithmic", angle=358,
plotIndex=TRUE, IndexCex=.6, groupDistance=gpDis)

legend(-2, 2.2, legend=c("Mm/Uniprobe/PBM/Seed-And-Wobble",
                        "Mm/CIS-BP/PBM/DREAM5", "Mm/CIS-BP/SELEX"),
       fill=highlightCol(colorSet[c("Mm", "MmDREAM", "Ms")], .3),
       border="white", lty=NULL, bty = "n")

```



Supplementary Figure 4E: Mouse data from different methods

```

motifCircos(phylog=phylog, pfms=motifs, col.tree.bg=methods,
            col.tree.bg.alpha=.3, r.rings=c(.1, .1),
            col.rings=list(icgp, rep("white", length(icgp))),
            col.inner.label.circle=gpCol, inner.label.circle.width=0.02,

```

```

    labels.leaves=leaveNames, cleaves=.1, r.tree=1, r.leaves=.5,
    clabel.leaves=.9, motifScale="logarithmic", angle=358,
    plotIndex=TRUE, IndexCex=.6, groupDistance=gpDis)

legend(-2.4, 2.4, legend=c("Mm/Uniprobe/PBM/Seed-And-Wobble",
                           "Mm/CIS-BP/PBM/DREAM5", "Mm/CIS-BP/SELEX"),
       fill=highlightCol(colorSet[c("Mm", "MmDREAM", "Ms")], .3),
       border="white", lty=NULL, bty = "n")

text(2.05, 2.3, labels="Information Content")
rasterImage(icgp.image, 2, 1.8, 2.04, 2.2, interpolate = FALSE)
text(c(2.05, 2.05), c(1.8, 2.2), labels=format(icgp.ranges, digits=3), adj=0)

detach(MmPBM_SELEX)

```

Supplementary Figure 4F. mouse selex vs mouse pbm/sw vs mouse pbm dream5, by motIV

```

gpDis <- 0.005
MmPBM_SELEX.MotIV <- getMotIVOut("pcmsDatasetMmPBM_SELEX",
                                      cc="ALLR", align="SWU",
                                      groupDistance=gpDis)
attach(MmPBM_SELEX.MotIV)

##set methods color
methods <- factor(grep("_SELEX", leaves) + grep("_M\\d{4}", leaves))
levels(methods) <- c("Mm", "MmDREAM", "Ms")
##c("PBM/Uniprobe/Seed-And-Wobble", "PBM/CIS-BP/DREAM5", "SELEX/CIS-BP")
levels(methods) <- colorSet[levels(methods)]
methods <- as.character(methods)

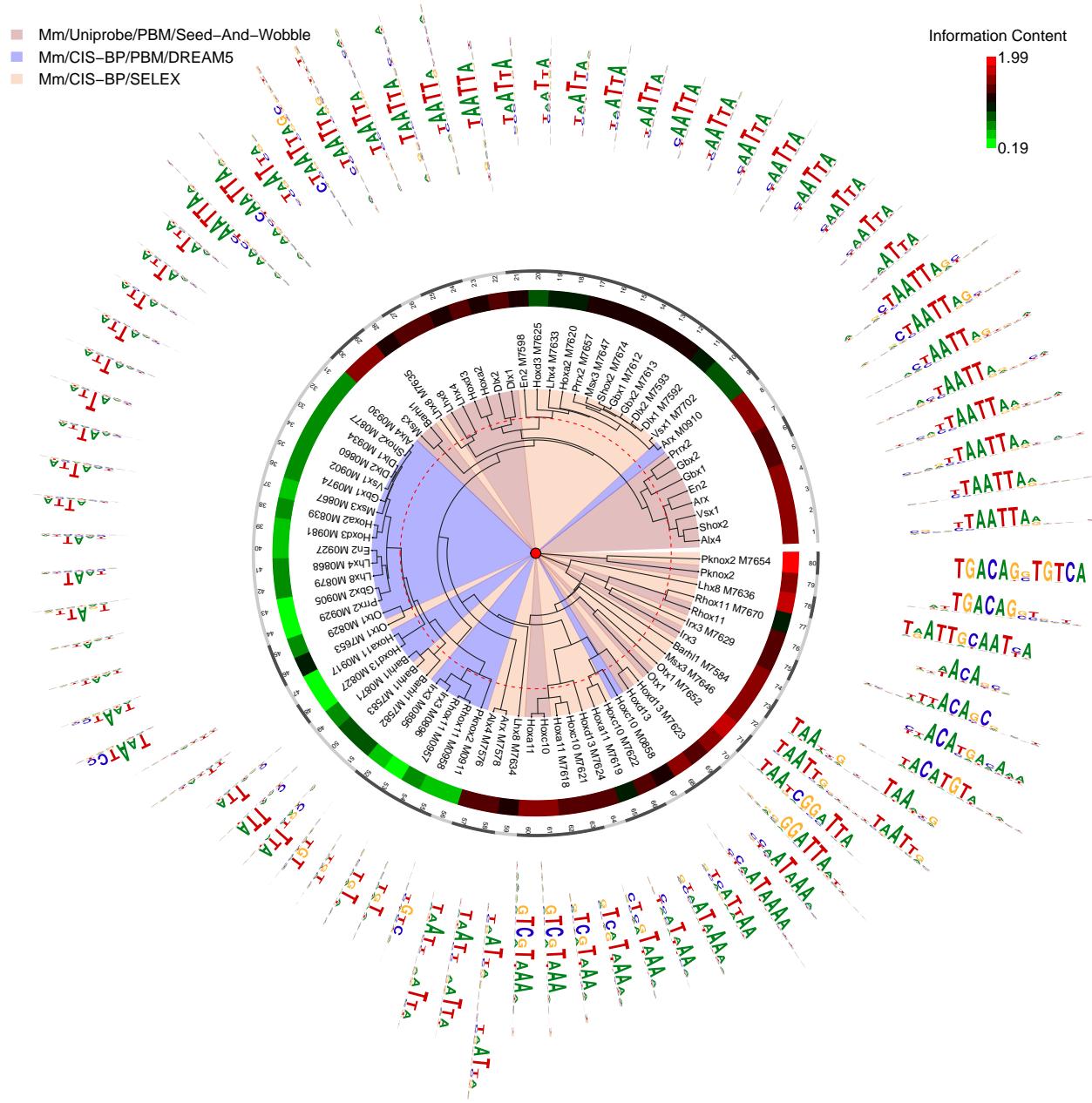
leaveNames <- gsub("_SELEX|PBM)", "", gsub("_Jolma|Berger)", "", leaves))

## calculate average of top 8 position information content for each motif
icgp <- sapply(sapply(unaligned.pfms, getIC), function(.ele)
               mean(sort(.ele, decreasing=TRUE)[1:min(8, length(.ele))]))
icgp.ranges <- range(icgp)
icgp <- cut(icgp, 10, labels=colorRampPalette(c("green", "black", "red"))(10))
icgp.image <- as.raster(matrix(rev(levels(icgp)), ncol=1))
icgp <- as.character(icgp)

plotMotifStackWithRadialPhylog(phylog=phylog, pfms=sig,
                               col.bg=methods, col.bg.alpha=.3,
                               col.inner.label.circle=gpCol,
                               inner.label.circle.width=0.02,
                               labels.leaves=leaveNames, cleaves=.1,
                               circle=.95, circle.motif=1.8, clabel.leaves=.8,
                               motifScale="logarithmic", angle=358,
                               plotIndex=TRUE, IndexCex=.6, groupDistance=gpDis)

legend(-2, 2.2, legend=c("Mm/Uniprobe/PBM/Seed-And-Wobble",

```



Supplementary Figure 4E: Mouse data from different methods

```

    "Mm/CIS-BP/PBM/DREAM5", "Mm/CIS-BP/SELEX"),
fill=highlightCol(colorSet[c("Mm", "MmDREAM", "Ms")], .3),
border="white", lty=NULL, bty = "n")

motifCircos(phylog=phylog, pfms=motifs, col.tree.bg=methods,
            col.tree.bg.alpha=.3, r.rings=c(.1, .1),
            col.rings=list(icgp, rep("white", length(icgp))),
            col.inner.label.circle=gpCol, inner.label.circle.width=0.02,
            labels.leaves=leaveNames, cleaves=.1, r.tree=1, r.leaves=.5,
            clabel.leaves=.9, motifScale="logarithmic", angle=358,
            plotIndex=TRUE, IndexCex=.6, groupDistance=gpDis)

legend(-2.4, 2.4, legend=c("Mm/Uniprobe/PBM/Seed-And-Wobble",
                           "Mm/CIS-BP/PBM/DREAM5", "Mm/CIS-BP/SELEX"),
       fill=highlightCol(colorSet[c("Mm", "MmDREAM", "Ms")], .3),
       border="white", lty=NULL, bty = "n")

text(2.05, 2.3, labels="Information Content")
rasterImage(icgp.image, 2, 1.8, 2.04, 2.2, interpolate = FALSE)
text(c(2.05, 2.05), c(1.8, 2.2), labels=format(icgp.ranges, digits=3), adj=0)

detach(MmPBM_SELEX.MotIV)

```

Supplementary Figure 4G. Engrailed orthologs, by matAlign

```

gpDis <- 2
EN <- getMatAlignOut("pcmsDatasetEn", groupDistance=gpDis)
attach(EN)

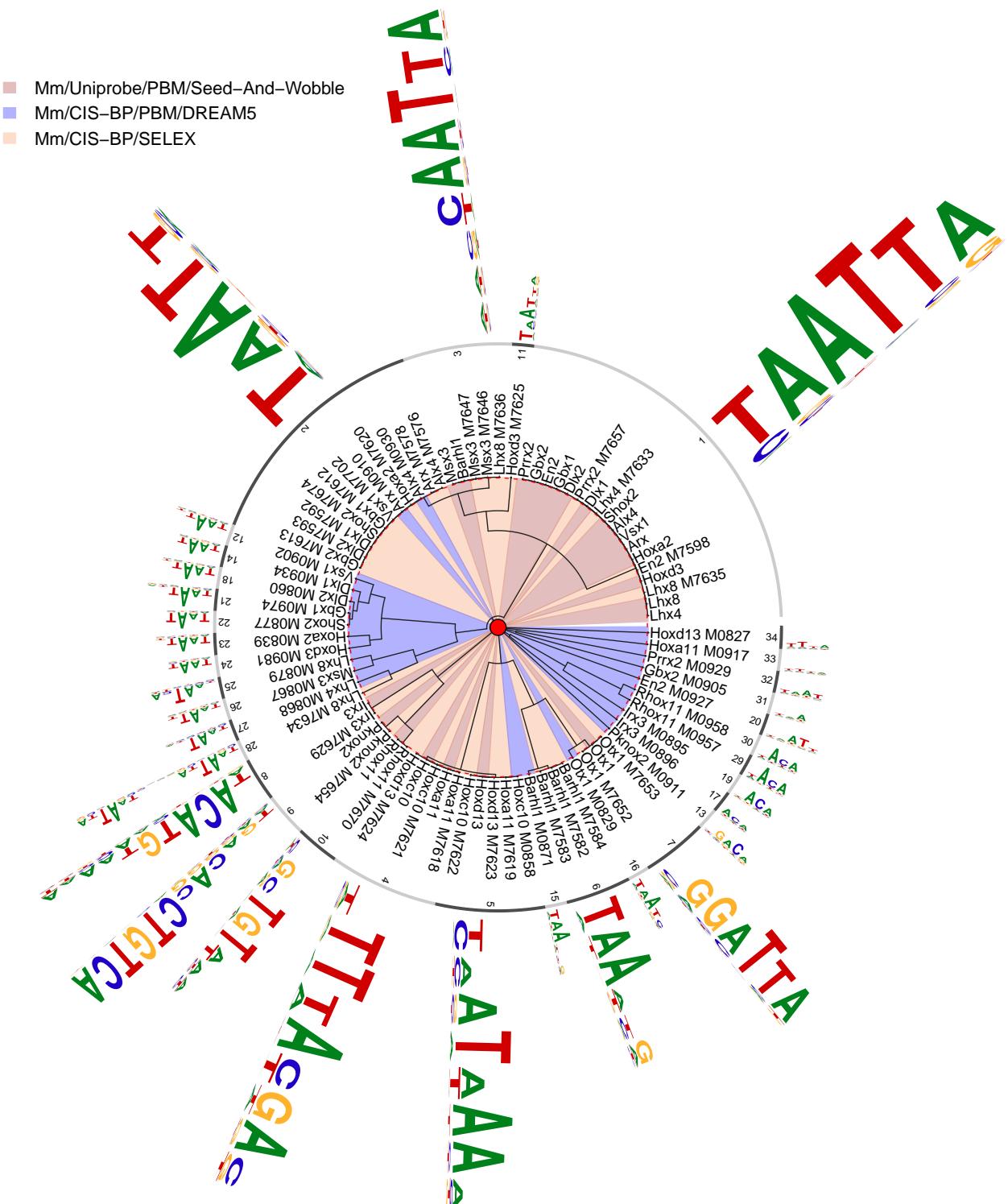
##set methods color
species <- gsub("(Dm|Hs|Mm).*?$", "\\\1", leaves)
species[grepl("_M\\d+", leaves) & species=="Mm"] <- "MmDREAM"
species <- colorSet[species] ##c("Dm/FFS/B1H", "Hs/CIS-BP/SELEX", "Mm/Uniprobe/PBM/\nSeed-And-Wobble")

leaveNames <- gsub("(Dm|Mm|Hs)_", "", leaves)

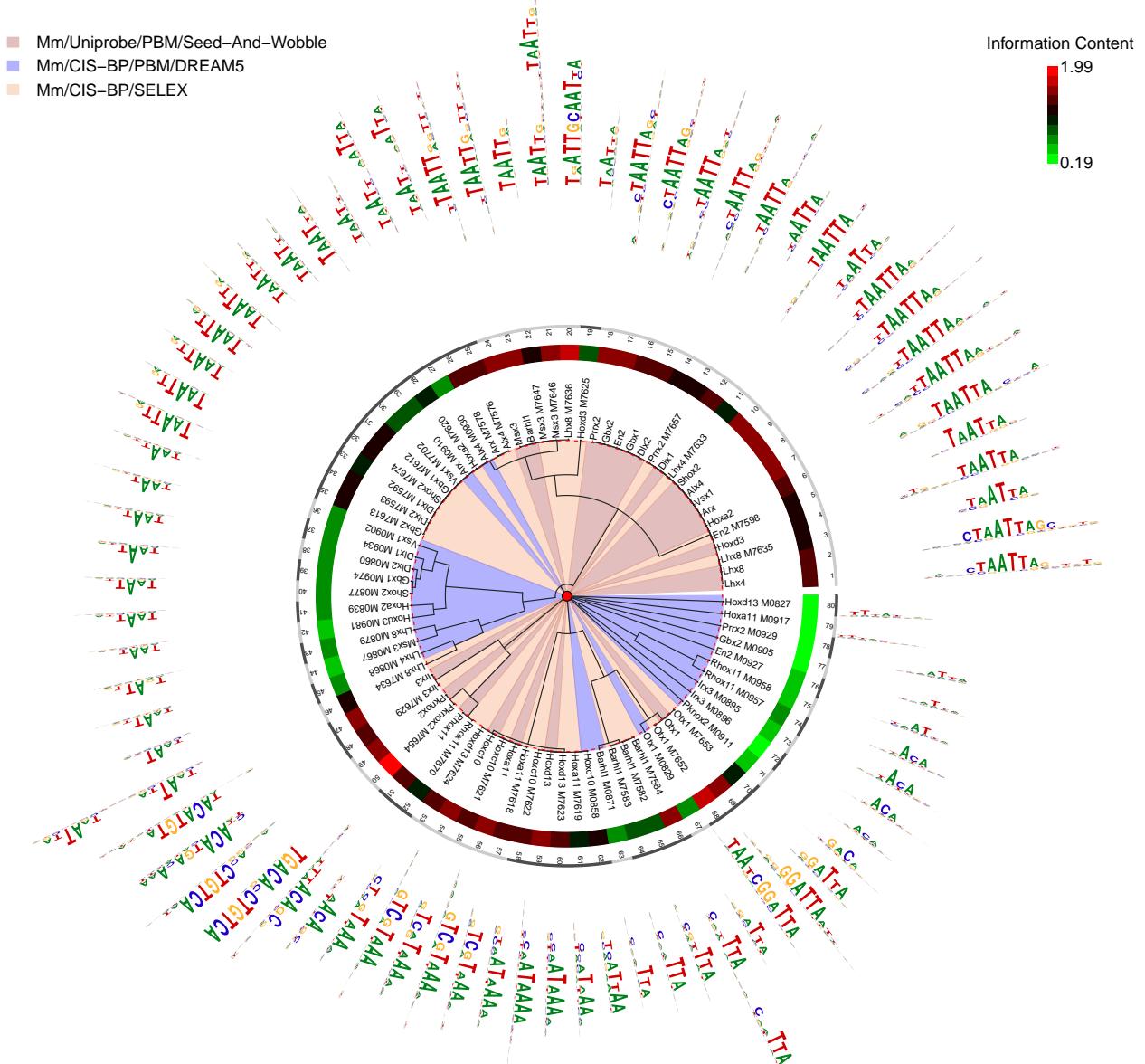
motifPiles(phylog=phylog, motifs, r.tree=.5, sig,
           col.tree=species, col.leaves=species,
           col.pfms2=gpCol, col.pfms2.width=.01,
           labels.leaves=leaveNames, plotIndex=TRUE,
           IndexCex=2, groupDistance=gpDis, clabel.leaves=2)
legend("topleft", legend=c("Dm/FFS/B1H", "Hs/CIS-BP/SELEX",
                           "Mm/Uniprobe/PBM/\nSeed-And-Wobble",
                           "Mm/CIS-BP/PBM/Dream5"),
       fill=colorSet[c("Dm", "Hs", "Mm", "MmDREAM")],
       border="white", lty=NULL, bty = "n", cex=.5)

detach(EN)

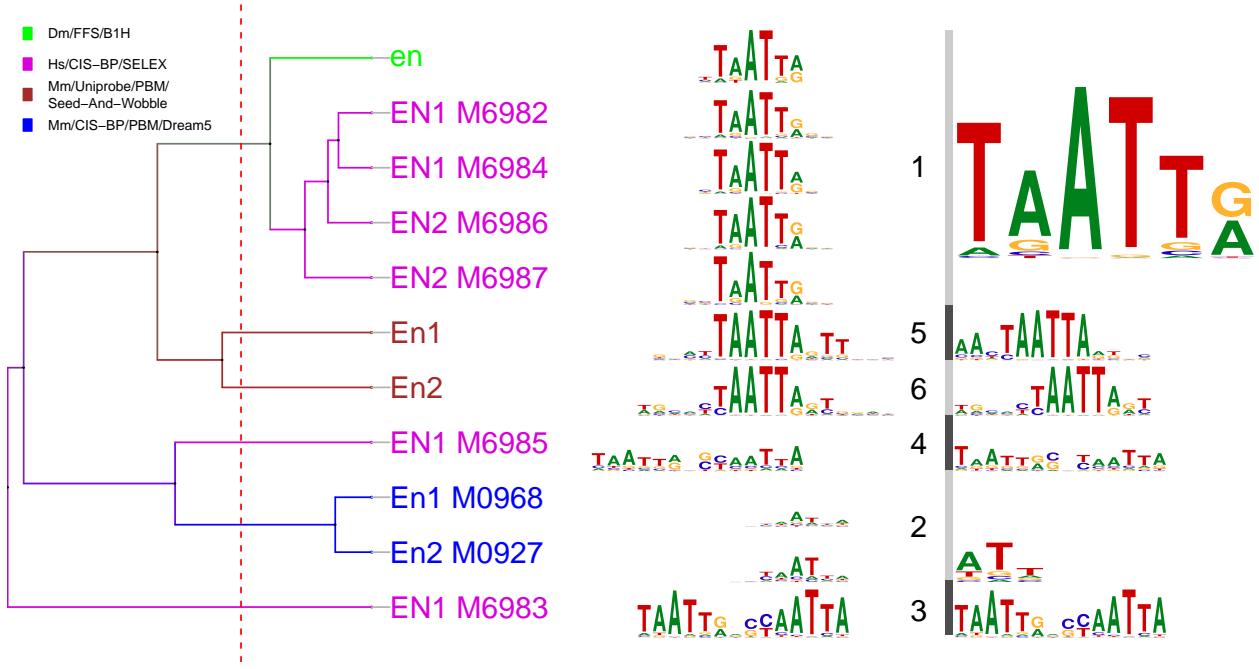
```



Supplementary Figure 4F: by MotIV



Supplementary Figure 4F: Mouse data from different methods



Supplementary Figure 4G: En

Supplementary Figure 4H. Engrailed orthologs, by motIV

```

gpDis <- 0.005
EN.MotIV <- getMotIVOut("pcmsDatasetEn",
                           cc="ALLR", align="SWU",
                           groupDistance=gpDis)
attach(EN.MotIV)

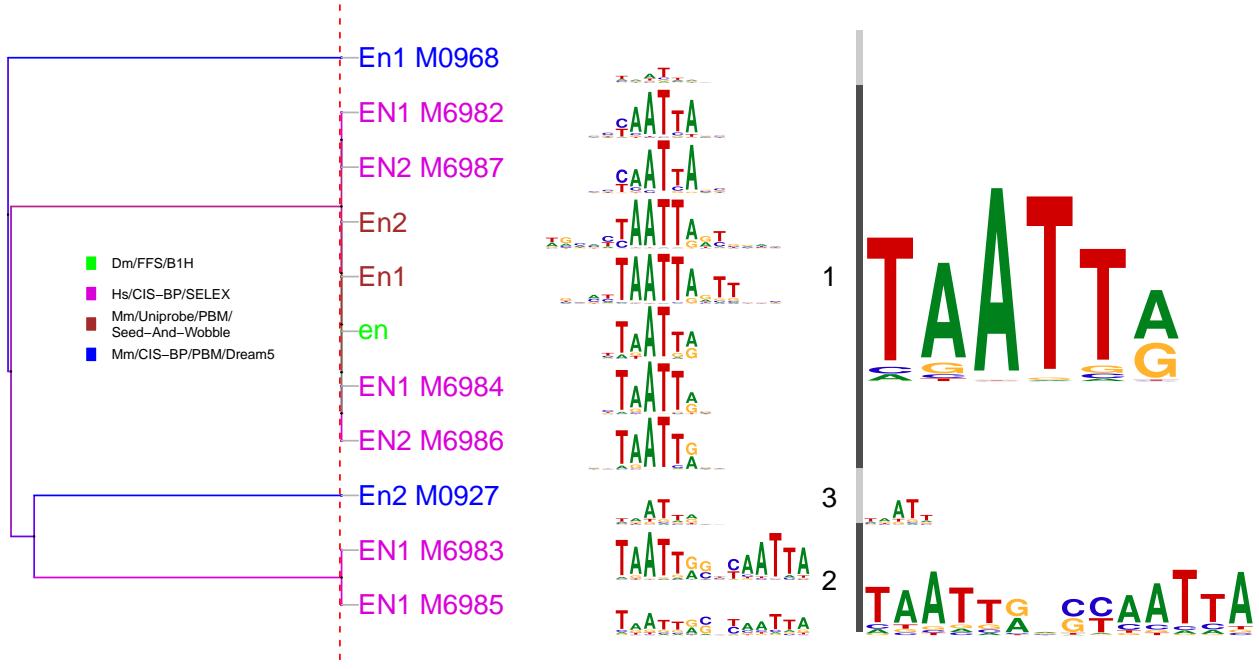
##set methods color
species <- gsub("(Dm|Hs|Mm).*?$", "\\\1", leaves)
species[grep("M\d+", leaves) & species=="Mm"] <- "MmDREAM"
##c("Dm/FFS/B1H", "Hs/CIS-BP/SELEX", "Mm/Uniprobe/PBM/\nSeed-And-Wobble")
species <- colorSet[species]

leaveNames <- gsub("(Dm|Mm|Hs)_", "", leaves)

motifPiles(phylog=phylog, motifs, r.tree=.5, sig,
           col.tree=species, col.leaves=species,
           col.pfms2=gpCol, col.pfms2.width=.01,
           labels.leaves=leaveNames, plotIndex=TRUE,
           IndexCex=2, groupDistance=gpDis, clabel.leaves=2)
legend(0.1, 0.65, legend=c("Dm/FFS/B1H", "Hs/CIS-BP/SELEX",
                           "Mm/Uniprobe/PBM/\nSeed-And-Wobble",
                           "Mm/CIS-BP/PBM/Dream5"),
      fill=colorSet[c("Dm", "Hs", "Mm", "MmDREAM")],
      border="white", lty=NULL, bty = "n", cex=.5)

detach(EN.MotIV)

```



Supplementary Figure 4H: En.MotIV

Supplementary Figure 4I. fly vs ms vs hum matAlign

```

gpDis <- 2.5
FlyMousePBMHuman <- getMatAlignOut("pcmsDatasetFlyMousePBMHuman",
                                      groupDistance=gpDis)
attach(FlyMousePBMHuman)

##set methods color
species <- factor(gsub("(Dm|Mm|Hs).*\\1", "", leaves))
##c("Dm/FFS/B1H", "Hs/CIS-BP/SELEX", "Mm/Uniprobe/PBM/Seed-And-Wobble")
levels(species) <- colorSet[c("Dm", "Hs", "Mm")]
species <- as.character(species)

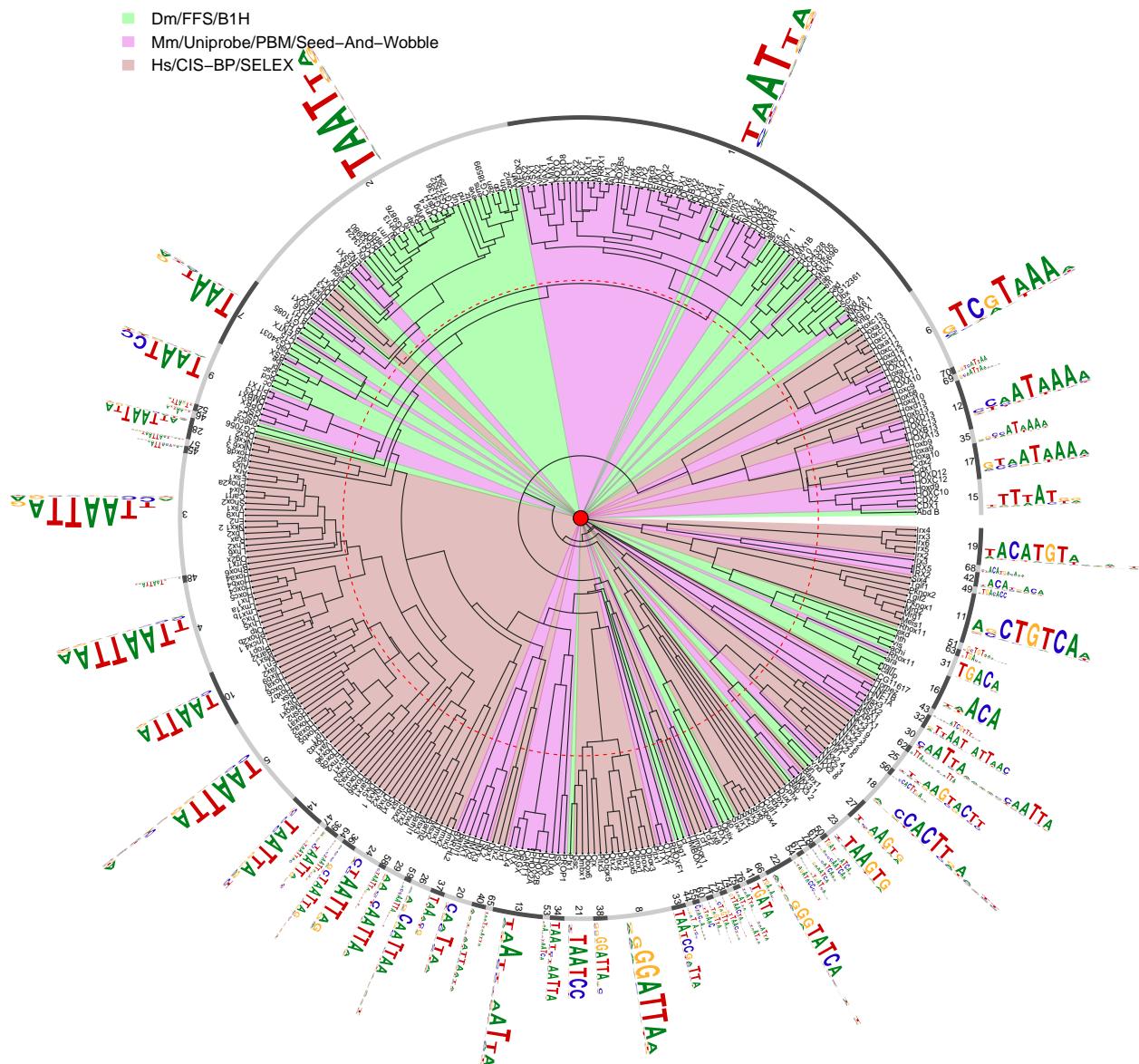
leaveNames <- gsub("(Dm|Mm|Hs)_", "", leaves)

plotMotifStackWithRadialPhylog(phylog=phylog, pfms=sig,
                                col.bg=species, col.bg.alpha=.3,
                                col.inner.label.circle=gpCol,
                                inner.label.circle.width=0.02,
                                labels.leaves=leaveNames,
                                cleaves=.1, circle=1.5, circle.motif=1.8,
                                clabel.leaves=.5, motifScale="logarithmic",
                                angle=358, plotIndex=TRUE, IndexCex=.6,
                                groupDistance=gpDis)

legend(-2, 2.2,
       legend=c("Dm/FFS/B1H",
               "Mm/Uniprobe/PBM/Seed-And-Wobble",
               "Hs/CIS-BP/SELEX"),

```

```
fill= highlightCol(colorSet[c("Dm", "Hs", "Mm")], alpha=.3),
border="white", lty=NULL, bty = "n")
```



Supplementary Figure 4I: Fly vs Mouse vs Human

```
detach(FlyMousePBMHuman)
```

Supplementary Figure 4J. fly vs ms vs hum motIV

```
gpDis <- 0.005
FlyMousePBMHuman.MotIV <- getMotIVOut("pcmsDatasetFlyMousePBMHuman",
                                         cc="ALLR", align="SWU",
                                         groupDistance=gpDis)
attach(FlyMousePBMHuman.MotIV)
```

```

##set methods color
species <- factor(gsub("(Dm|Mm|Hs).*$", "\\\1", leaves))
##c("Dm/FFS/B1H", "Hs/CIS-BP/SELEX", "Mm/Uniprobe/PBM/Seed-And-Wobble")
levels(species) <- colorSet[c("Dm", "Hs", "Mm")]
species <- as.character(species)

leaveNames <- gsub("(Dm|Mm|Hs)_", "", leaves)

plotMotifStackWithRadialPhylog(phylog=phylog, pfms=sig,
                                col.bg=species, col.bg.alpha=.3,
                                col.inner.label.circle=gpCol,
                                inner.label.circle.width=0.02,
                                labels.leaves=leaveNames,
                                cleaves=.1, circle=1.5, circle.motif=1.8,
                                clabel.leaves=.5, motifScale="logarithmic",
                                angle=358, plotIndex=TRUE, IndexCex=.6,
                                groupDistance=gpDis)

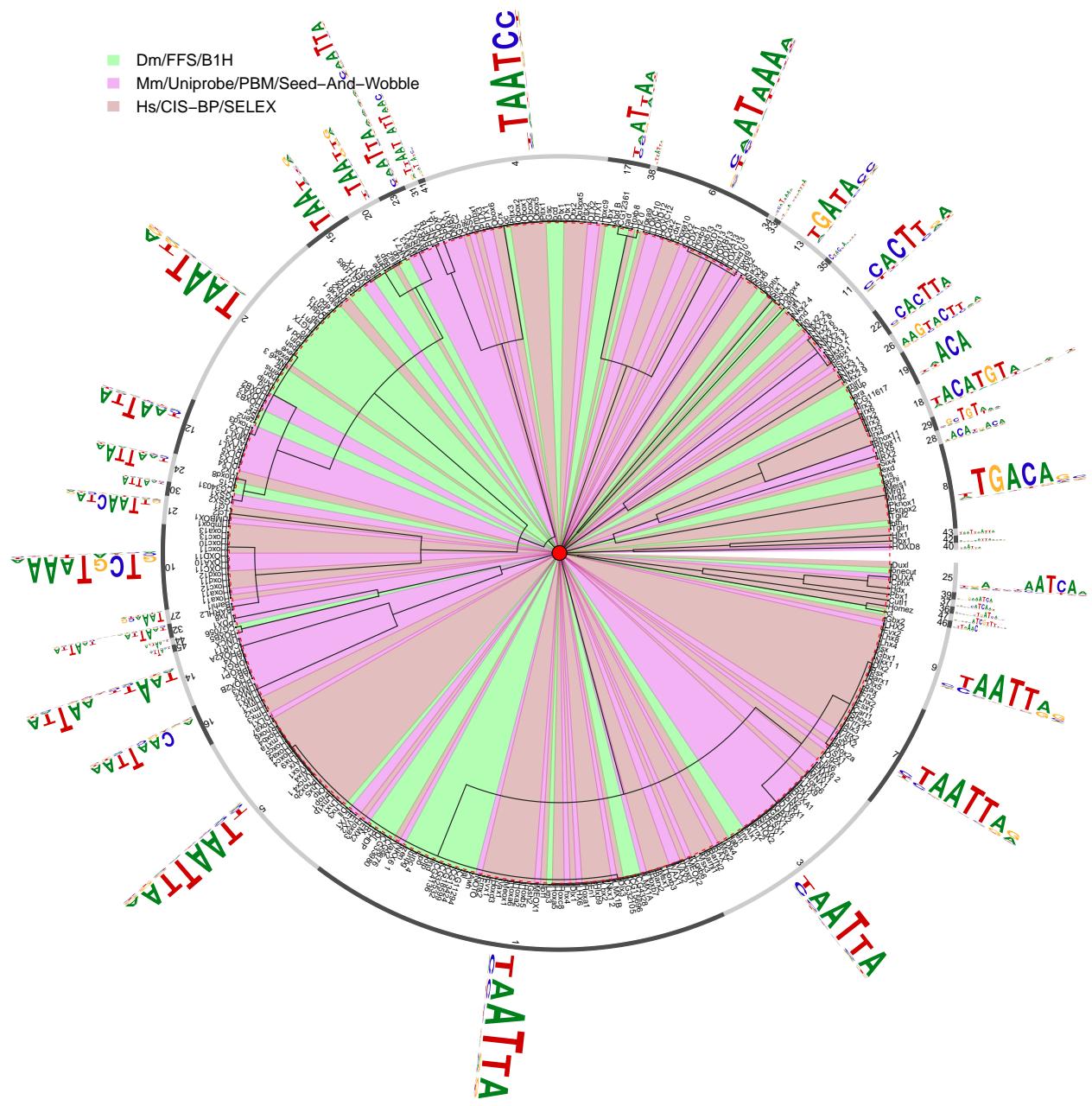
legend(-2, 2.2,
       legend=c("Dm/FFS/B1H",
               "Mm/Uniprobe/PBM/Seed-And-Wobble",
               "Hs/CIS-BP/SELEX"),
       fill= highlightCol(colorSet[c("Dm", "Hs", "Mm")], alpha=.3),
       border="white", lty=NULL, bty = "n")

detach(FlyMousePBMHuman.MotIV)

sessionInfo()

## R version 3.4.1 (2017-06-30)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: OS X El Capitan 10.11.6
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/3.4/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.4/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_1.20.1   Biostrings_2.44.2   XVector_0.16.0
## [4] IRanges_2.10.2     S4Vectors_0.14.3   ade4_1.7-6
## [7] MotIV_1.32.0      BiocGenerics_0.22.0 grImport_0.9-0
## [10] XML_3.98-1.9
##
## loaded via a namespace (and not attached):
## [1] Rcpp_0.12.12          highr_0.6
## [3] plyr_1.8.4            compiler_3.4.1
## [5] GenomeInfoDb_1.12.2   bitops_1.0-6

```



```
## [7] tools_3.4.1                  zlibbioc_1.22.0
## [9] digest_0.6.12                 evaluate_0.10.1
## [11] lattice_0.20-35              BSgenome_1.44.0
## [13] Matrix_1.2-10                DelayedArray_0.2.7
## [15] yaml_2.1.14                  seqLogo_1.42.0
## [17] GenomeInfoDbData_0.99.0     rtracklayer_1.36.4
## [19] stringr_1.2.0                knitr_1.16
## [21] htmlwidgets_0.9               rprojroot_1.2
## [23] Biobase_2.36.2              BiocParallel_1.10.1
## [25] rGADEM_2.24.0               rmarkdown_1.6
## [27] magrittr_1.5                 scales_0.4.1
## [29] backports_1.1.0              Rsamtools_1.28.0
## [31] htmltools_0.3.6              matrixStats_0.52.2
## [33] GenomicRanges_1.28.4         GenomicAlignments_1.12.1
## [35] SummarizedExperiment_1.6.3   colorspace_1.3-2
## [37] stringi_1.1.5               munsell_0.4.3
## [39] RCurl_1.95-4.8
```