NPD

Micro-synteny analysis using sub genomic regions (50 kbps)n from Fungi Ensembl database. The input files are results from the progressive alignment using the Mauve software. Install packages:

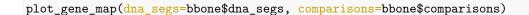
```
install.packages("ade4")
install.packages("grid")
install.packages("genoPlotR")
"'Call packages from library
library(ade4)
## Warning: package 'ade4' was built under R version 4.1.1
library(grid)
library(genoPlotR)
Call .backbone file from directory
bone_file <- "/Users/lavinialavin/Desktop/Ejemplos R/Genomica/npd_alin_7jun.backbone"
"'Determine the reference genome (longest genome is used as reference, in this case N. crassa genome)
bbone <- read_mauve_backbone(bone_file, ref=6, gene_type = "side_blocks", header = TRUE, filter_low = 0
Labels dna_segs objects using the species names.
names <- c("B_bas", "M_ory", "M_bru", "M_maj", "M_rob", "M_acr", "M_ril", "M_alb", "M_ani", "M_gui", "T
names(bbone$dna_segs)<-names
Attach objects to the bbone file.
dna_segs<-(bbone$dna_segs)</pre>
comparisons<-(bbone$comparisons)</pre>
Calculate the genome lengths.
for (i in 1:length(bbone$comparisons)){
  cmp <- bbone$comparisons[[i]]</pre>
```

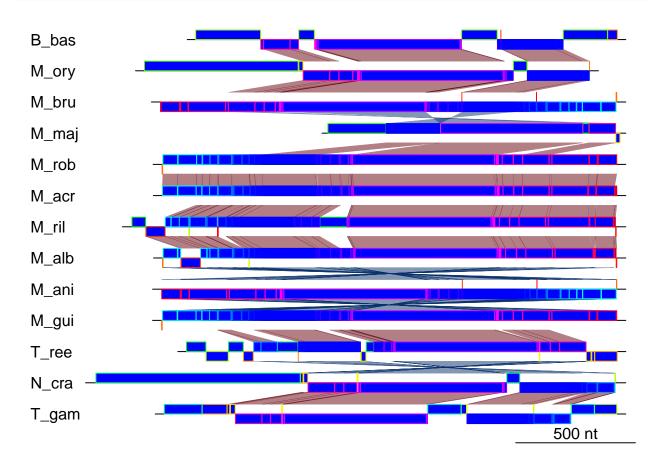
Initial plot, illustrates the comparison on the identified blocks.

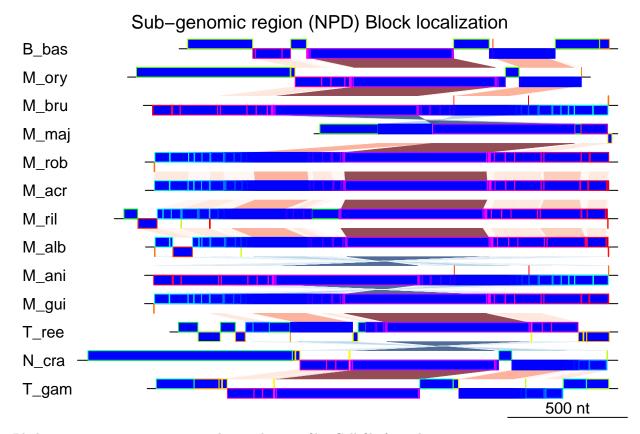
abs(cmp\$end1 - cmp\$start1) + abs(cmp\$end2 - cmp\$start2)

bbone\$comparisons[[i]]\$length <-

}







Phylogenetic reconstruction using the .guide_tree file. Call file from directory.

```
tree<- "/Users/lavinialavin/Desktop/Ejemplos R/Genomica/npd_alin_7jun.guidetree"
```

Using the calculated distances from each node, the tree is reconstructed; to do this you have to copy-paste the distances from the file .guide_tree and label each sequence with the corresponding name of each species.

```
tree_demo <- newick2phylog("(M_maj:0.200819,(M_ory:0.256736,(N_cra:0.294616,(B_bas:0.230773,((T_gam:0.1
names <- c("M_maj", "M_ory", "N_cra", "B_bas", "T_gam", "T_ree", "M_ril", "M_alb", "M_acr", "M_gui", "M_ril", "M_abb", "M_acr", "M_gui", "M_gui", "M_ril", "M_abb", "M_acr", "M_gui", "M_gui",
```

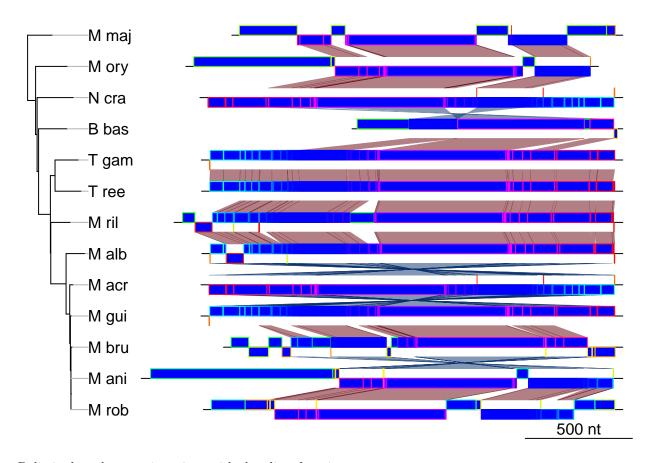
tree_str <- gsub(paste("seq", i, sep=""), names[i], tree_str) }</pre>

```
Plot
```

for (i in 1:length(names)){

tree <- newick2phylog(tree_demo)</pre>

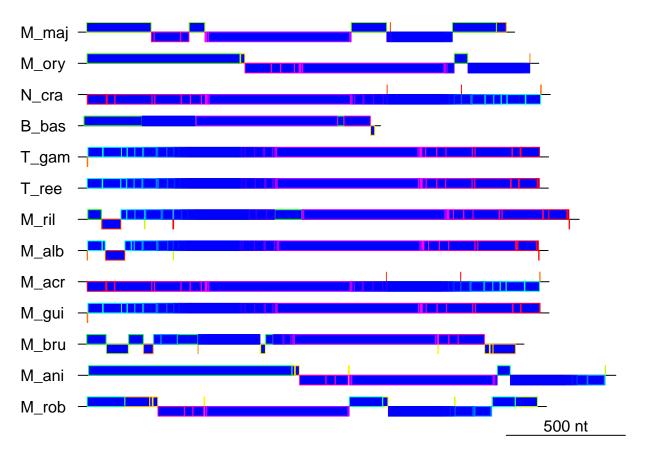
```
plot_gene_map(dna_segs=dna_segs, comparisons=bbone$comparisons, tree=tree_demo)
```



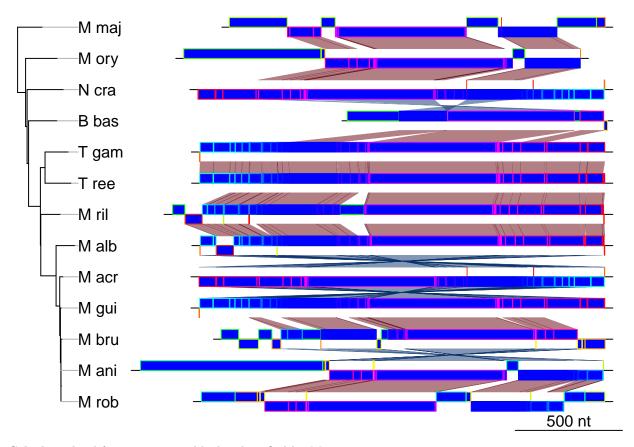
Delimit the sub-genomic regions with the xlims function.

Plot on the compared genes using the whole subgenomic region (xlims=null)

```
annotation_cex = 0.8,
seg_plots=NULL, # user-defined plots seg_plot_height=3, # height of plots (in lines) seg_
xlims = NULL,
offsets = NULL,
minimum_gap_size = 0.05,
fixed_gap_length = FALSE,
limit_to_longest_dna_seg = TRUE,
main = NULL,
main_pos = "centre",
dna_seg_labels = NULL,
dna_seg_label_cex=1,
dna_seg_label_col="black",
gene_type = NULL,
arrow_head_len = 200,
dna_seg_line = TRUE,
scale = TRUE,
n_scale_ticks=7,
scale_cex=0.6,
global_color_scheme = c("auto", "auto", "blue_red", 0.5), override_color_schemes = FALSE,
plot_new=TRUE,
debug = 0)
```



plot_gene_map(dna_segs=dna_segs, comparisons=comparisons, tree = tree_demo)



Calculate the diferences among blocks identified by Mauve.

```
df1 <- data.frame(name=c("feat1", "feat2", "feat3"), start=c(18000, 22000, 24000),
                 end=c(20000, 23000, 30000), strand=c(-1, 1, -1), col=c("red", "pink", "grey"))
dna_seg1 <- dna_seg(df1)</pre>
str(dna_seg1)
## Classes 'dna_seg' and 'data.frame': 3 obs. of 11 variables:
           : chr "feat1" "feat2" "feat3"
   $ start : num 18000 22000 24000
##
             : num 20000 23000 30000
  $ end
  $ strand : num -1 1 -1
##
##
   $ col
              : chr "red" "pink" "grey"
##
  $ fill
              : chr "blue" "blue" "blue"
## $ lty
              : num 1 1 1
              : num 1 1 1
## $ lwd
## $ pch
               : num 8888
## $ cex
               : num 1 1 1
   $ gene_type: chr "arrows" "arrows" "arrows"
df2 <- data.frame(name=c("feat1", "feat2", "feat3"),</pre>
                 start=c(15000, 22000, 24000), end=c(20000, 23000, 30000), strand=c(-1, 1, -1), col=c(
dna_seg2 <- dna_seg(df2)</pre>
str(dna_seg2)
```

```
## $ name : chr "feat1" "feat2" "feat3"
## $ start : num 15000 22000 24000
## $ end : num 20000 23000 30000
## $ strand : num -1 1 -1
## $ col
          : chr "blue" "grey" "grey"
## $ fill : chr "blue" "blue" "blue"
## $ lty
            : num 1 1 1
## $ lwd
             : num 1 1 1
         : num 888
: num 111
## $ pch
## $ cex
## $ gene_type: chr "arrows" "arrows"

df3 <- data.frame(name=c("feat1", "feat2", "feat3"),</pre>
                 start=c(18000, 22000, 24000), end=c(20000, 23000, 30000), strand=c(-1, -1, 1), col=re
dna_seg3 <- dna_seg(df3)</pre>
str(dna_seg3)
## Classes 'dna_seg' and 'data.frame': 3 obs. of 11 variables:
## $ name : chr "feat1" "feat2" "feat3"
## $ start : num 18000 22000 24000
## $ end : num 20000 23000 30000
## $ strand : num -1 -1 1
## $ col : chr "blue" "blue" "blue"
            : chr "blue" "blue" "blue"
## $ fill
## $ lty
            : num 1 1 1
## $ lwd
             : num 1 1 1
## $ pch
            : num 888
## $ cex : num 1 1 1
## $ gene_type: chr "arrows" "arrows" "arrows"
df4 <- data.frame(name=c("feat1", "feat2", "feat3"),</pre>
                start=c(18000, 22000, 24000), end=c(20000, 23000, 30000), strand=c(-1, -1, 1), col=re
dna_seg4 <- dna_seg(df4)</pre>
str(dna_seg4)
## Classes 'dna_seg' and 'data.frame': 3 obs. of 11 variables:
## $ name : chr "feat1" "feat2" "feat3"
## $ start : num 18000 22000 24000
           : num 20000 23000 30000
## $ end
## $ strand : num -1 -1 1
           : chr "green" "green" "green"
## $ col
## $ fill
            : chr "blue" "blue" "blue"
## $ lty
            : num 1 1 1
## $ lwd
             : num 1 1 1
## $ pch
            : num 888
           : num 1 1 1
## $ cex
## $ gene_type: chr "arrows" "arrows" "arrows"
df5 <- data.frame(name=c("feat1", "feat2", "feat3"),</pre>
                start=c(18000, 22000, 24000), end=c(20000, 23000, 30000), strand=c(-1, -1, 1), col=re
```

```
dna_seg5 <- dna_seg(df5)</pre>
str(dna_seg5)
## Classes 'dna_seg' and 'data.frame': 3 obs. of 11 variables:
## $ name : chr "feat1" "feat2" "feat3"
## $ start : num 18000 22000 24000
             : num 20000 23000 30000
## $ end
## $ strand : num -1 -1 1
## $ col : chr "yellow" "yellow" "yellow"
## $ fill : chr "blue" "blue" "blue"
## $ lty
             : num 1 1 1
## $ lwd
            : num 1 1 1
## $ pch
            : num 888
          : num 1 1 1
## $ cex
## $ gene_type: chr "arrows" "arrows" "arrows"
df6 <- data.frame(name=c("feat1", "feat2", "feat3"),</pre>
                start=c(5000, 10000, 24000), end=c(20000, 23000, 25000), strand=c(-1, -1, 1), col=rep
dna_seg6 <- dna_seg(df6)</pre>
str(dna_seg6)
## Classes 'dna_seg' and 'data.frame': 3 obs. of 11 variables:
## $ name : chr "feat1" "feat2" "feat3"
## $ start : num 5000 10000 24000
## $ end : num 20000 23000 25000
## $ strand : num -1 -1 1
## $ col
           : chr "cyan" "cyan" "cyan"
## $ fill
             : chr "blue" "blue" "blue"
## $ lty
            : num 1 1 1
## $ lwd
            : num 1 1 1
## $ pch
             : num 888
           : num 1 1 1
## $ cex
## $ gene_type: chr "arrows" "arrows" "arrows"
df7 <- data.frame(name=c("feat1", "feat2", "feat3"),</pre>
                start=c(20000, 22000, 30000), end=c(25000, 30000, 40000), strand=c(-1, -1, 1), col=re
dna_seg7 <- dna_seg(df7)</pre>
str(dna_seg7)
## Classes 'dna_seg' and 'data.frame': 3 obs. of 11 variables:
## $ name : chr "feat1" "feat2" "feat3"
## $ start
             : num 20000 22000 30000
            : num 25000 30000 40000
## $ end
## $ strand : num -1 -1 1
           : chr "pink" "pink" "pink"
## $ col
## $ fill
             : chr "blue" "blue" "blue"
## $ lty
            : num 1 1 1
## $ lwd
            : num 1 1 1
## $ pch
            : num 888
```

```
## $ cex : num 1 1 1
## $ gene_type: chr "arrows" "arrows" "arrows"
df8 <- data.frame(name=c("feat1", "feat2", "feat3"),</pre>
                 start=c(18000, 22000, 24000), end=c(20000, 23000, 30000), strand=c(-1, -1, 1), col=re
dna_seg8 <- dna_seg(df8)</pre>
str(dna_seg8)
## Classes 'dna_seg' and 'data.frame': 3 obs. of 11 variables:
## $ name : chr "feat1" "feat2" "feat3"
## $ start : num 18000 22000 24000
             : num 20000 23000 30000
## $ end
## $ strand : num -1 -1 1
## $ col
            : chr "tan" "tan" "tan"
## $ fill
             : chr "blue" "blue" "blue"
## $ lty
             : num 1 1 1
## $ lwd
              : num 1 1 1
          : num 888
: num 111
## $ pch
## $ cex
## $ gene_type: chr "arrows" "arrows"

df9 <- data.frame(name=c("feat1", "feat2", "feat3"),</pre>
                 start=c(18000, 22000, 24000), end=c(20000, 23000, 30000), strand=c(-1, -1, 1), col=re
dna_seg9 <- dna_seg(df9)</pre>
str(dna_seg9)
## Classes 'dna seg' and 'data.frame': 3 obs. of 11 variables:
## $ name : chr "feat1" "feat2" "feat3"
## $ start : num 18000 22000 24000
            : num 20000 23000 30000
## $ end
## $ strand : num -1 -1 1
           : chr "violet" "violet" "violet"
## $ col
## $ fill
             : chr "blue" "blue" "blue"
## $ lty : num 1 1 1
## $ lwd
             : num 1 1 1
## $ pch
             : num 888
             : num 1 1 1
## $ cex
## $ gene_type: chr "arrows" "arrows" "arrows"
df10 <- data.frame(name=c("feat1", "feat2", "feat3"),</pre>
                 start=c(18000, 22000, 24000), end=c(20000, 23000, 30000), strand=c(-1, -1, 1), col=re
dna_seg10 <- dna_seg(df10)</pre>
str(dna_seg10)
## Classes 'dna_seg' and 'data.frame': 3 obs. of 11 variables:
## $ name : chr "feat1" "feat2" "feat3"
## $ start : num 18000 22000 24000
            : num 20000 23000 30000
## $ end
## $ strand : num -1 -1 1
## $ col : chr "green4" "green4" "green4"
## $ fill : chr "blue"
## $ lty : num 1 1 1
             : chr "blue" "blue" "blue"
```

```
## $ lwd
             : num 1 1 1
## $ pch
             : num 888
## $ cex
            : num 1 1 1
## $ gene_type: chr "arrows" "arrows"

df11 <- data.frame(name=c("feat1", "feat2", "feat3"),</pre>
                 start=c(18000, 22000, 24000), end=c(20000, 23000, 30000), strand=c(-1, -1, 1), col=re
dna_seg11 <- dna_seg(df11)</pre>
str(dna_seg11)
## Classes 'dna_seg' and 'data.frame': 3 obs. of 11 variables:
## $ name : chr "feat1" "feat2" "feat3"
## $ start : num 18000 22000 24000
## $ end : num 20000 23000 30000
## $ strand : num -1 -1 1
           : chr "purple" "purple" "purple"
## $ col
             : chr "blue" "blue" "blue"
## $ fill
## $ lty
            : num 1 1 1
## $ lwd
             : num 1 1 1
## $ pch
             : num 888
## $ cex
             : num 1 1 1
## $ gene_type: chr "arrows" "arrows"

df12 <- data.frame(name=c("feat1", "feat2", "feat3"),</pre>
                start=c(18000, 22000, 24000), end=c(20000, 23000, 30000), strand=c(-1, -1, 1), col=re
dna_seg12 <- dna_seg(df12)</pre>
str(dna_seg12)
## Classes 'dna_seg' and 'data.frame': 3 obs. of 11 variables:
## $ name : chr "feat1" "feat2" "feat3"
## $ start : num 18000 22000 24000
## $ end : num 20000 23000 30000
## $ strand : num -1 -1 1
## $ col : chr "purple" "purple" "purple"
## $ fill
             : chr "blue" "blue" "blue"
             : num 1 1 1
## $ lty
             : num 1 1 1
## $ lwd
## $ pch
             : num 888
## $ cex
            : num 1 1 1
## $ gene_type: chr "arrows" "arrows" "arrows"
df13 <- data.frame(name=c("feat1", "feat2", "feat3"),</pre>
                start=c(18000, 22000, 24000), end=c(20000, 23000, 30000), strand=c(-1, -1, 1), col=re
dna_seg13 <- dna_seg(df13)</pre>
str(dna_seg13)
## Classes 'dna_seg' and 'data.frame': 3 obs. of 11 variables:
## $ name : chr "feat1" "feat2" "feat3"
## $ start
             : num 18000 22000 24000
## $ end : num 20000 23000 30000
## $ strand : num -1 -1 1
          : chr "purple" "purple" "purple"
## $ col
```

```
## $ fill : chr "blue" "blue" "blue"
## $ lty : num 1 1 1
## $ lwd : num 1 1 1
## $ pch : num 8 8 8
## $ cex : num 1 1 1
## $ gene_type: chr "arrows" "arrows" "arrows"
```

dna_segs <- list(dna_seg1, dna_seg2, dna_seg3, dna_seg4, dna_seg5, dna_seg6, dna_seg7, dna_seg8, dna_seg

Calculate comparisons among blocks, center the interest gene (25 kbp)

```
df4 <- data.frame(start1=dna_seg1$start, end1=dna_seg1$end,</pre>
                   start2=dna_seg2$start,
                   end2=dna_seg2$end)
comparison1 <- comparison(df4)</pre>
df5 <- data.frame(start1=c(20000, 38000), end1=c(35000, 41000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison2 <- comparison(df5)</pre>
comparisons <- list(comparison1, comparison2)</pre>
df5 \leftarrow data.frame(start1=c(20000, 38000), end1=c(35000, 41000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison2 <- comparison(df5)</pre>
df9 \leftarrow data.frame(start1=c(20000, 38000), end1=c(35000, 41000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison4 <- comparison(df9)</pre>
df3 \leftarrow data.frame(start1=c(10000, 20000), end1=c(15000, 30000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison5 <- comparison(df3)</pre>
df2 \leftarrow data.frame(start1=c(20000, 18000), end1=c(35000, 41000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison6 <- comparison(df2)</pre>
df2 \leftarrow data.frame(start1=c(20000, 18000), end1=c(35000, 41000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison6 <- comparison(df2)</pre>
```

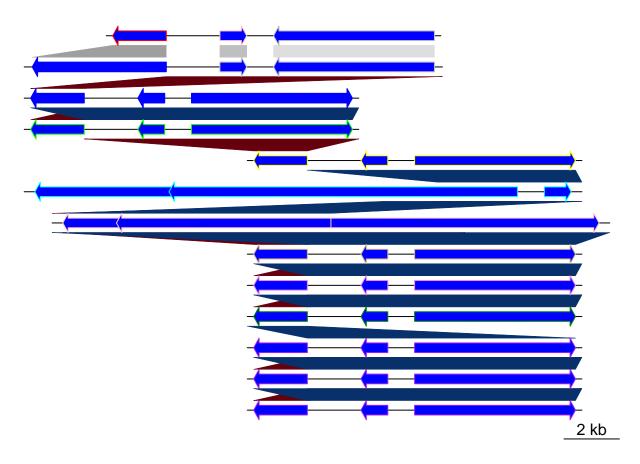
```
df7 <- data.frame(start1=c(30000, 34000), end1=c(35000, 41000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison3 <- comparison(df7)</pre>
df10 \leftarrow data.frame(start1=c(20000, 18000), end1=c(35000, 41000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison8 <- comparison(df10)</pre>
df11 <- data.frame(start1=c(20000, 18000), end1=c(35000, 41000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison9 <- comparison(df11)</pre>
df6 <- data.frame(start1=c(5000, 10000), end1=c(10000, 20000),
                    start2=c(18000, 20000),
                    end2=c(20000, 30000),
                    col=c("#67000D", "#08306B"))
comparison10 <- comparison(df6)</pre>
df1 \leftarrow data.frame(start1=c(20000, 18000), end1=c(35000, 41000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison7 <- comparison(df1)</pre>
df3 <- data.frame(start1=c(20000, 18000), end1=c(35000, 41000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison3 <- comparison(df3)</pre>
df12 <- data.frame(start1=c(20000, 18000), end1=c(35000, 41000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison12 <- comparison(df12)</pre>
df13 \leftarrow data.frame(start1=c(20000, 18000), end1=c(35000, 41000),
                   start2=c(18000, 20000),
                   end2=c(20000, 30000),
                   col=c("#67000D", "#08306B"))
comparison13 <- comparison(df13)</pre>
```

A new object is generated using the calculated comparisons.

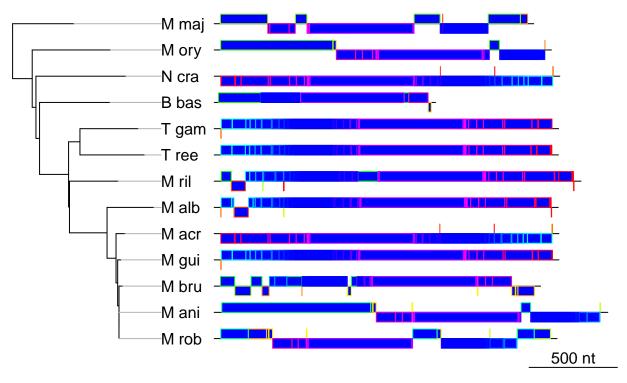
```
comparisons <- list(comparison1, comparison2, comparison3, comparison4, comparison5, comparison6, compa
comparisons[[1]]$col <- apply_color_scheme(c(0.6, 0.5, 0.4), "red")</pre>
```

Plot without names or block labels, arrows indicate the different LCBs.

```
plot_gene_map(dna_segs=dna_segs, comparisons=comparisons)
```



Comparison of Homologous Segments in 13 sub-genomic regions

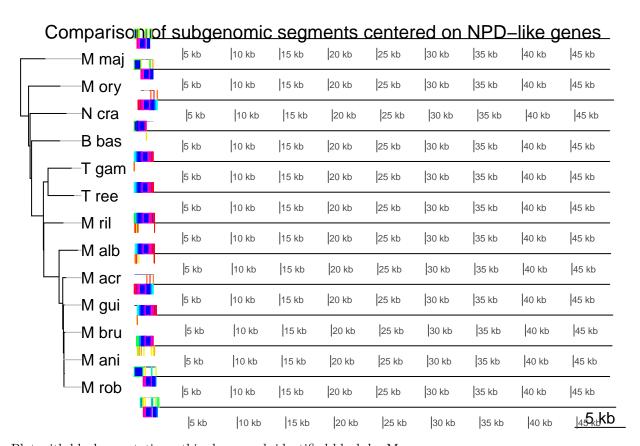


To delimit the sub genomic region of interest the xlims function is used. This xlims function contains the 50kbp sub genomic region of each species.

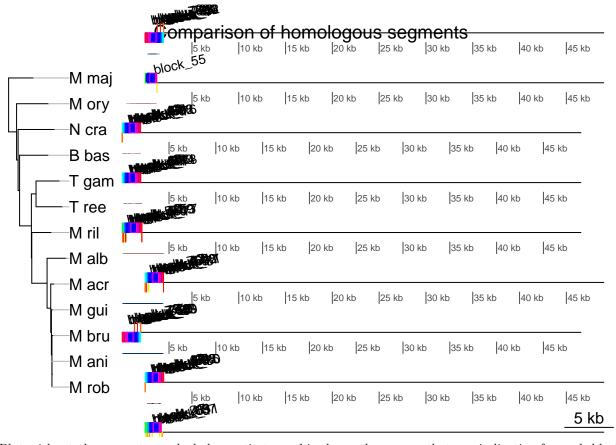
```
xlims <- list(c( 1, 49000),</pre>
               c(1, 49000),
               c(1,49000))
annots <- lapply(bbone$dna_segs, function(x){</pre>
  mid <- middle(x)
  annot <- annotation(x1=mid, text=x$name, rot=13)</pre>
  idx <- grep("^[^B]", annot$text, perl=TRUE)</pre>
  annot[idx[idx %% 4 == 0],])
```

Plot without annotations (block-annotations).

```
limit_to_longest_dna_seg=FALSE,
dna_seg_scale=TRUE, scale=TRUE,
main="Comparison of subgenomic segments centered on NPD-like genes")
```

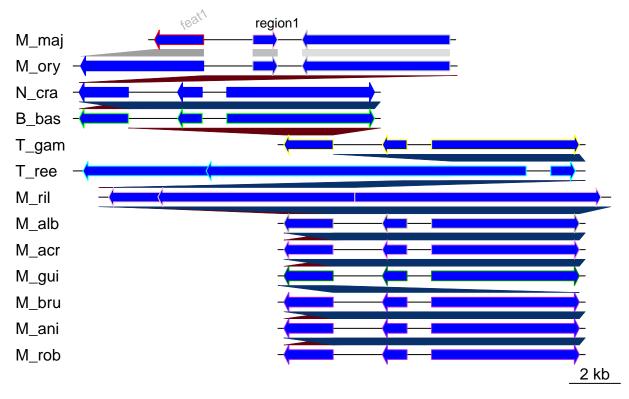


Plot with block annotations, this shows each identified block by Mauve.

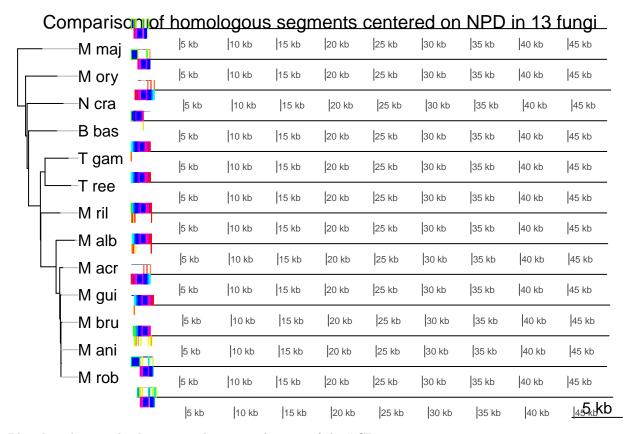


Plot without the reconstructed phylogenetic tree, this shows the arrows that are indicative for each block of interest.

Comparison of subgenomic regions centered on NPD



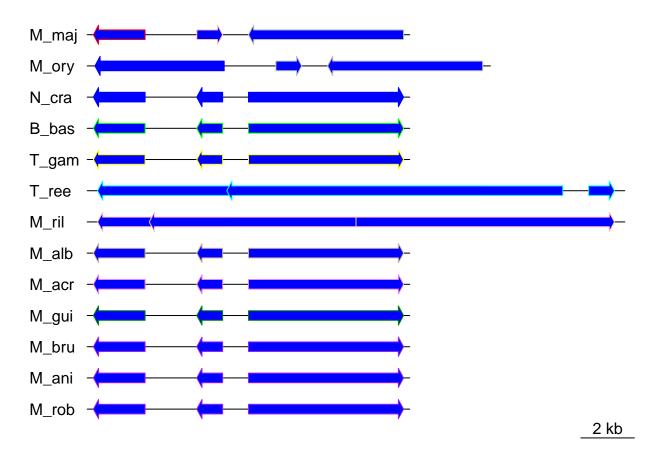
Plot that identifies the LCBs, the phylogenetic reconstruction and the comparisons between the 50kbp sub genomic regions.



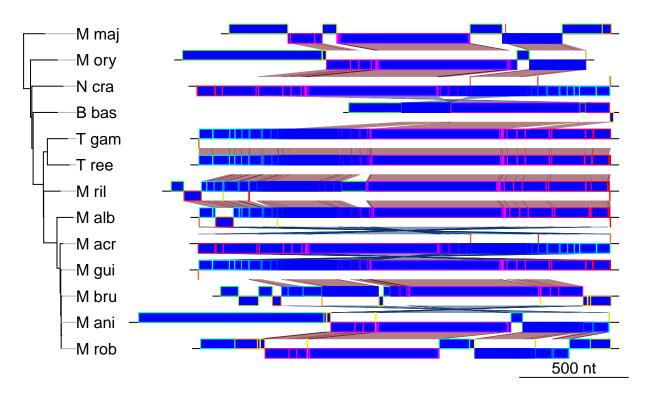
Plot that shows only the arrows that are indicative of the LCBs.

```
plot_gene_map(dna_segs,
              comparisons = NULL,
              tree = NULL,
              tree_width = NULL,
              tree_branch_labels_cex = NULL,
              tree scale = FALSE,
              legend = NULL,
              annotations = NULL,
              annotation_height = 1,
              annotation cex = 0.8,
              seg_plots=NULL, # user-defined plots
              seg_plot_height=3, # height of plots (in lines)
              seg_plot_height_unit="lines", # unit of preceding
              seg_plot_yaxis=3, # if non-null or non false, ticks
              seg_plot_yaxis_cex=scale_cex,
              xlims = NULL,
              offsets = NULL,
              minimum_gap_size = 0.05,
              fixed_gap_length = FALSE,
              limit_to_longest_dna_seg = TRUE,
              main = NULL,
              main_pos = "centre",
              dna_seg_labels = NULL,
              dna_seg_label_cex=1,
              dna seg label col="black",
```

```
gene_type = NULL,
arrow_head_len = 200,
dna_seg_line = TRUE,
scale = TRUE,
dna_seg_scale =FALSE,
n_scale_ticks=7,
scale_cex=0.6,
global_color_scheme = c("auto", "auto", "blue_red", 0.5),
override_color_schemes = FALSE,
plot_new=TRUE,
degub=0)
```



Comparison of homologous segments centered on NPD-like genes



To delimit the regions used to calculate the synteny, you can use the xlims function centering on the specific localization of the gene of interest to get a better view of the sub genomic context of each species.

```
xlims \leftarrow list(c(20000, 30000),
              c(20000, 30000),
              c(30000, 40000),
              c(20000, 30000),
              c(20000,
                        30000),
              c(20000,
                        30000),
              c(20000, 30000),
              c(20000, 30000),
              c(20000, 30000),
              c(20000, 30000),
              c( 20000, 30000),
              c(20000, 30000),
              c(20000, 30000))
plot_gene_map(bbone$dna_segs, bbone$comparisons, tree=tree_demo,
              xlims=xlims,
              limit_to_longest_dna_seg=FALSE,
              dna_seg_scale=TRUE, scale=TRUE,
              main="Comparison of homologous segments centered on NPD-like genes")
```

Comparison of homologous segments centered on NPD-like genes

