Protein Clustering

Gabriel Ramirez-Vilchis & Santiago Orozco-Barrera

2025-03-07

Contents

Introduction

Protein clustering is a fundamental step in the analysis of protein sequences. It allows to group proteins with similar functions, structures, or evolutionary relationships. In this project, we will perform a protein clustering analysis using a dataset of protein sequences from the ABC transporter family.

The ABC transporter family is a group of proteins that are involved in the transport of various molecules across the cell membrane. These proteins are found in all living organisms and play a crucial role in many biological processes. The ABC transporter family is divided into several subfamilies, each with specific functions and characteristics. The main problematic of this project is to cluster the proteins of the ABC transporter family into groups based on their sequence similarity.

Main Part

BlastP Alignment

The first step in protein clustering analysis is to perform an all-against-all protein sequence alignment to obtain the bit score with BlastP. We did this using the local server *chaac* of the Bachelor of Genomic Sciences of the UNAM.

```
# Create BLAST database
makeblastdb -in ABC.faa -dbtype prot -out ABC_blastdb

# Execute BlastP
blastp -query ABC.faa -db ABC_blastdb -outfmt 7 -max_hsps 1 -use_sw_tback -out ABC.blastp
```

Data formatting

The output of the BlastP alignment was processed to obtain a matrix of dissimilarity with the bit scores gotten from BLAST. This matrix was used to perform a hierarchical clustering analysis of the proteins.

```r
# Import libraries
library(cluster)
suppressPackageStartupMessages(library(tidyverse))
suppressPackageStartupMessages(library(factoextra))
suppressPackageStartupMessages(library(dendextend))
suppressPackageStartupMessages(library(ape))
suppressPackageStartupMessages(library(corrplot))
```

```r
# Read the data
data <- read.table("data/ABC.blastp", sep = "\t", header = FALSE, comment.char = "#")

# Assign names to the columns
colnames(data) <- c("query",
                    "subject",
                    "identity",
                    "alignment_length",
                    "mismatches",
                    "gap_opens",
                    "q_start",
                    "q_end",
                    "s_start",
                    "s_end",
                    "evalue",
                    "bit_score")
```

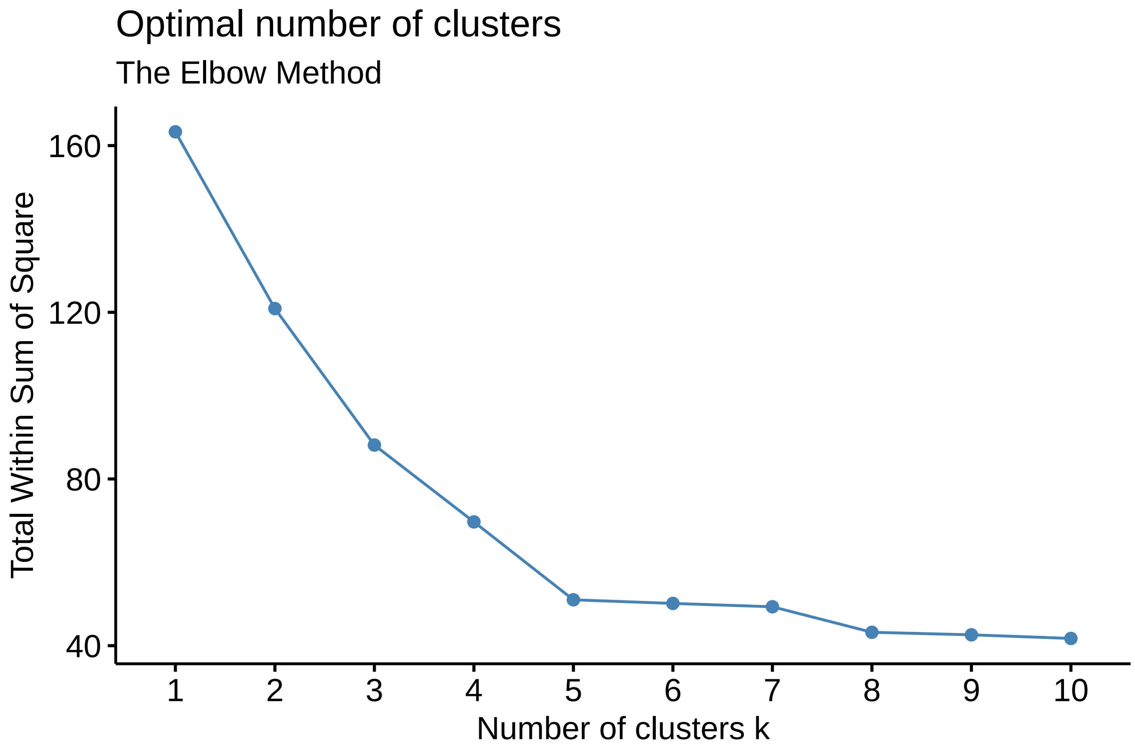
```r
# Calculate the normalized similarity
similarity <- select(data, query, subject, bit_score)
similarity <- mutate(similarity, normalized_bit_score = bit_score / max(data$bit_score))

# Regularize diagonal in matrix
for(row in 1:nrow(similarity)) {
  if(similarity[row, "query"] == similarity[row, "subject"]) {
    similarity[row, "normalized_bit_score"] <- 1
  }
}
```

```r
# Calculate dissimilarity
dissimilarity <- mutate(similarity, dissimilarity = 1 - normalized_bit_score)

# Create a dissimilarity matrix
dissimilarity_matrix <- dissimilarity %>%
  select(query, subject, dissimilarity) %>%
  spread(key = subject, value = dissimilarity) %>%
  column_to_rownames(var = "query")
```

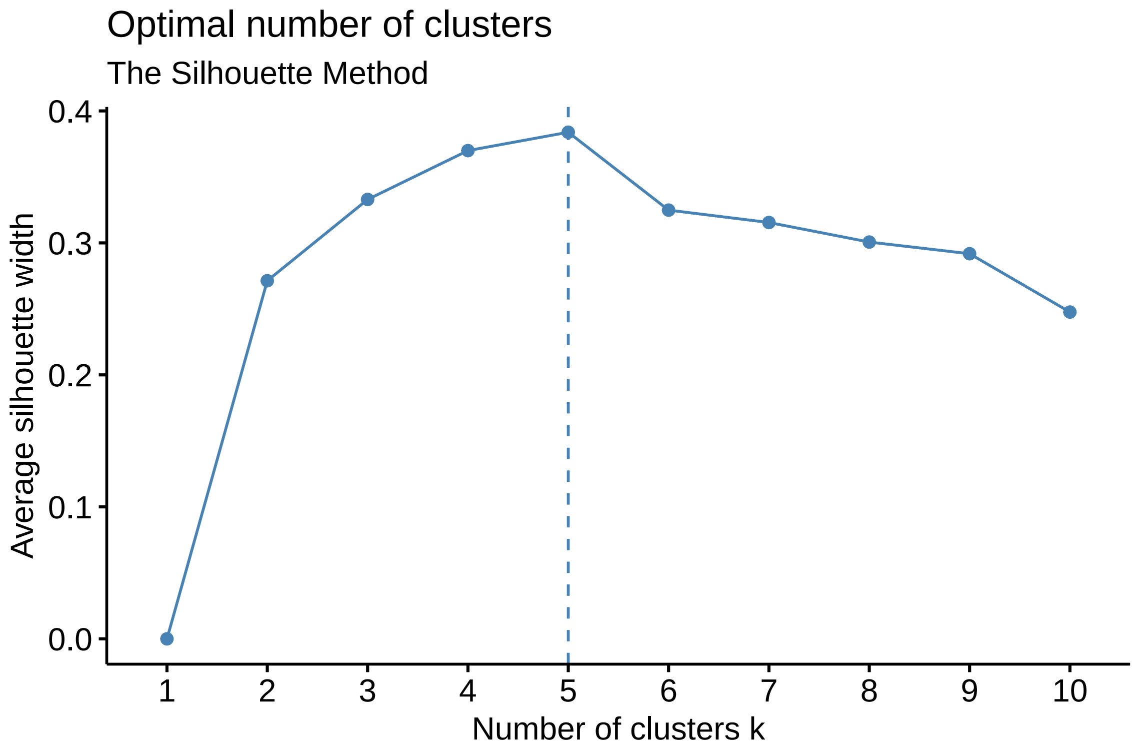
Best number of clusters

We have learned that the determining an optimal number of clusters isn't trivial, we use different methods and plots in order to deciding the best number of clusters fitting our data. We will use the Elbow Method and the Silhouette Method to determine the optimal number of clusters.

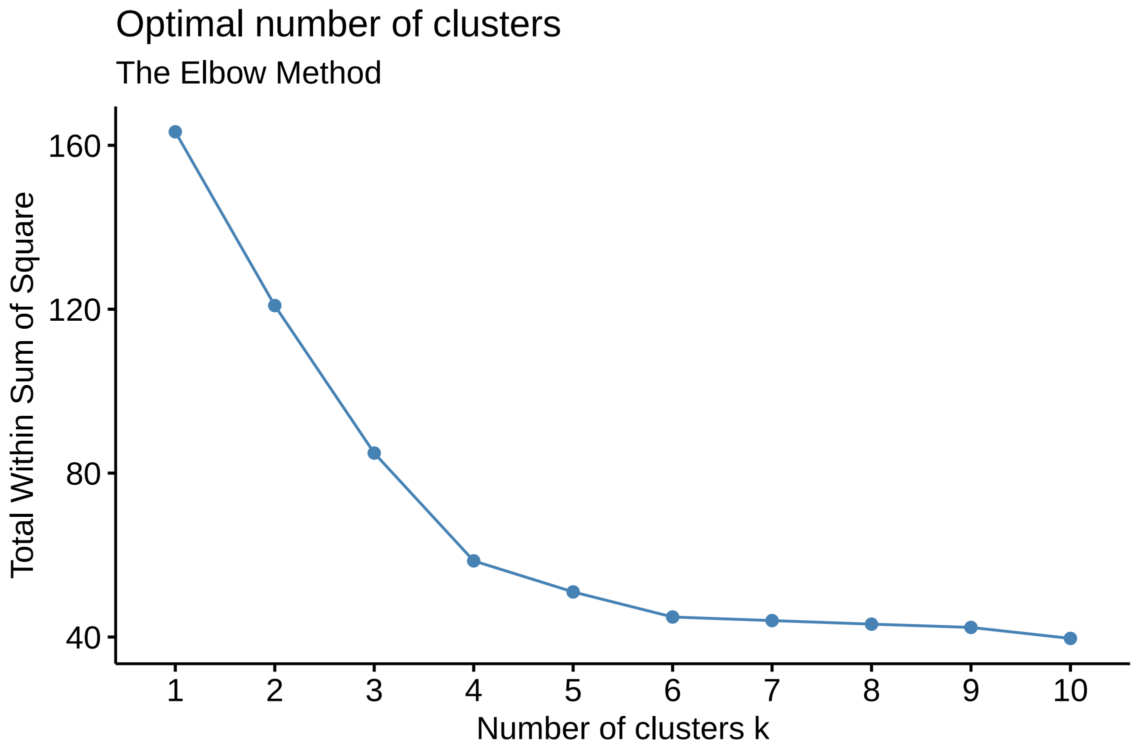
```
fviz_nbclust(dissimilarity_matrix, FUN = hcut, hc_method = "single", method = "wss", k.max = 10) +
  labs(subtitle = "The Elbow Method")
```



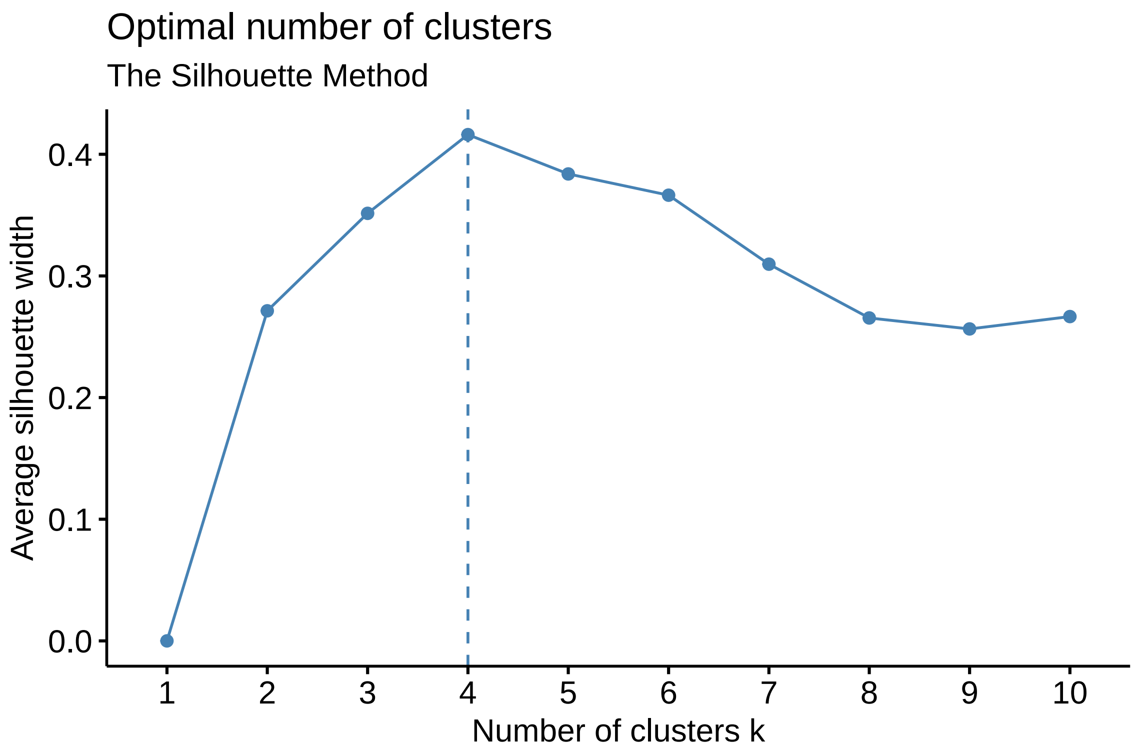
```
fviz_nbclust(dissimilarity_matrix, FUN = hcut, hc_method = "single", method = "silhouette", k.max = 10)
  labs(subtitle = "The Silhouette Method")
```



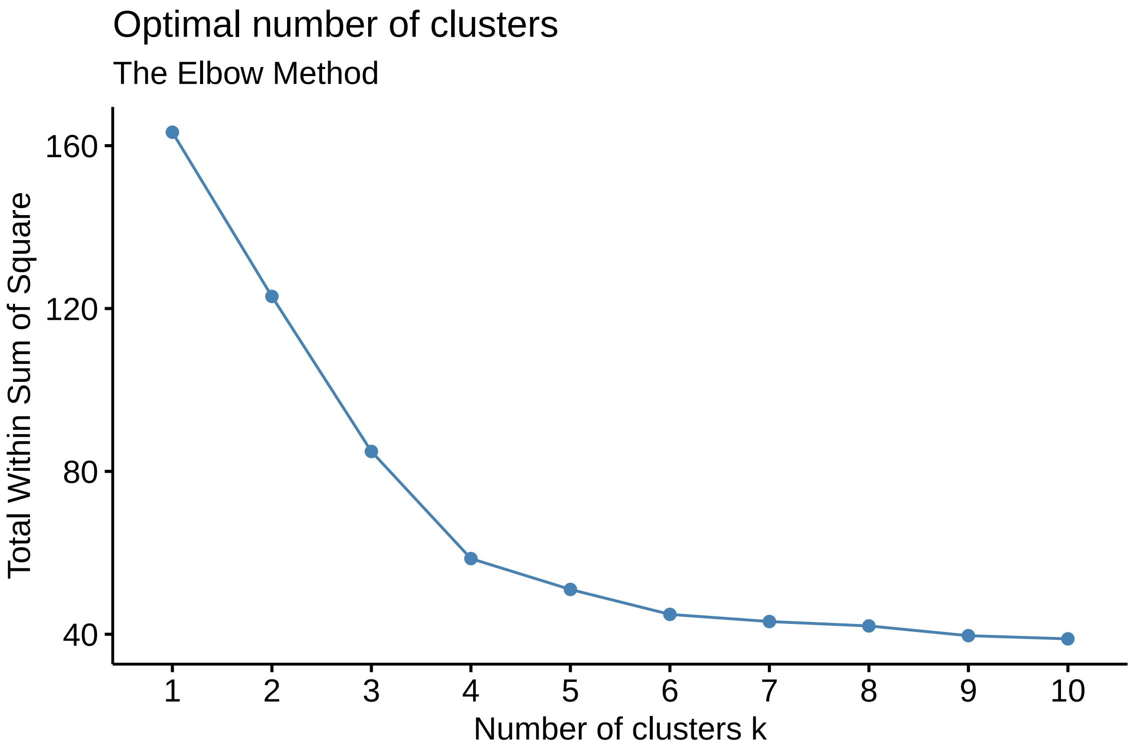
```r
fviz_nbclust(dissimilarity_matrix, FUN = hcut, hc_method = "average", method = "wss", k.max = 10) +
  labs(subtitle = "The Elbow Method")
```



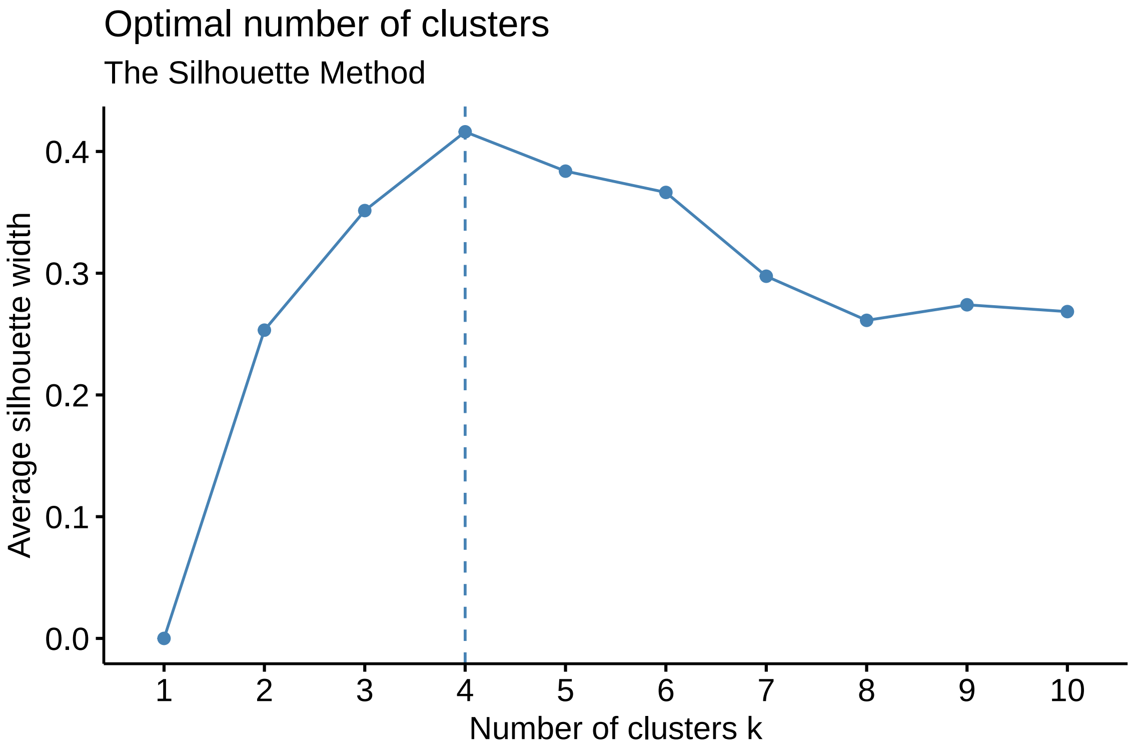
```
fviz_nbclust(dissimilarity_matrix, FUN = hcut, hc_method = "average", method = "silhouette", k.max = 10)
  labs(subtitle = "The Silhouette Method")
```



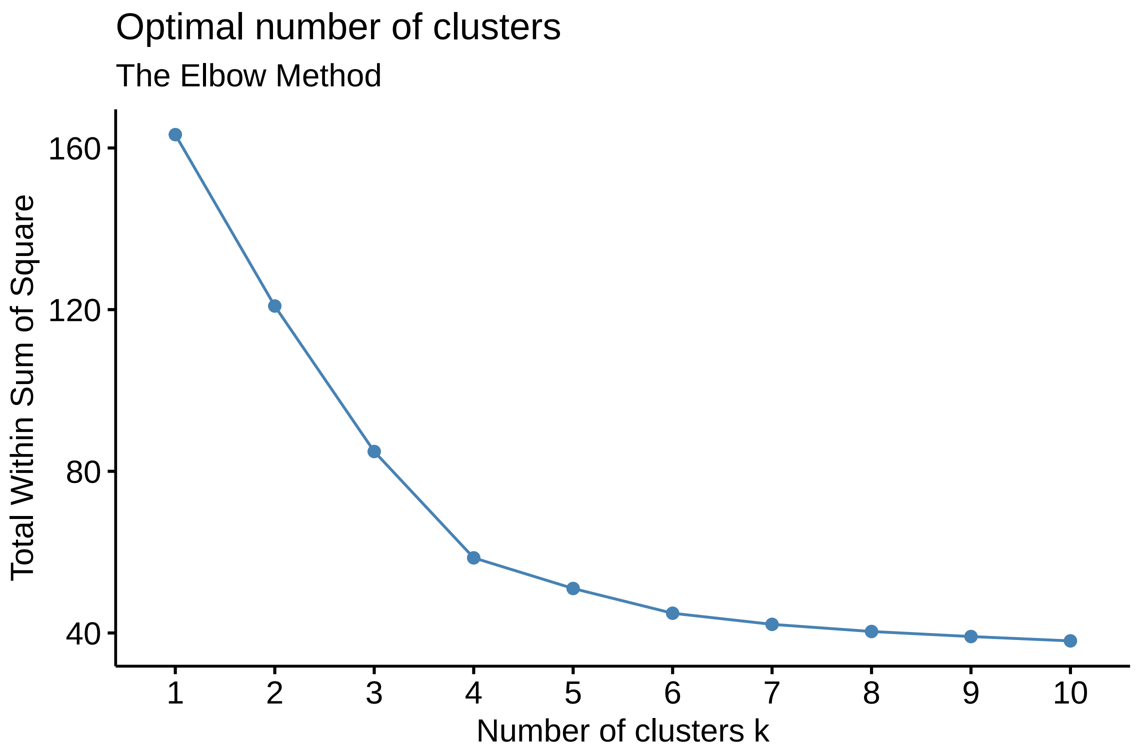
```
fviz_nbclust(dissimilarity_matrix, FUN = hcut, hc_method = "complete", method = "wss", k.max = 10) +
  labs(subtitle = "The Elbow Method")
```



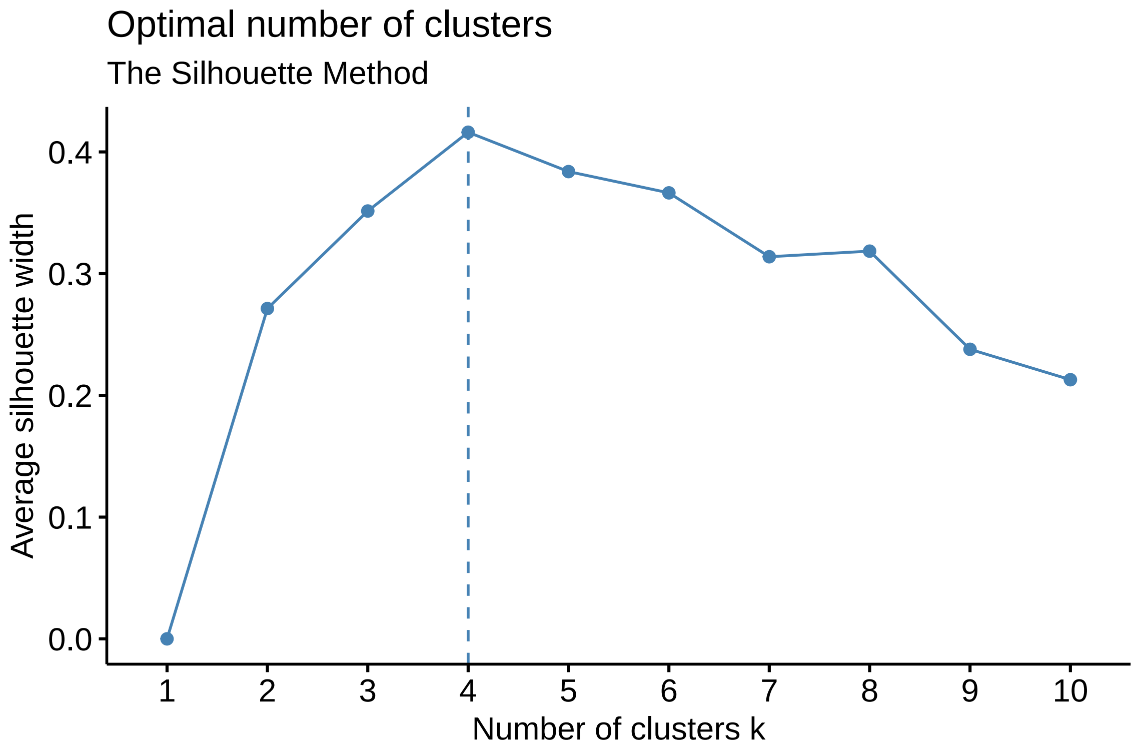
```
fviz_nbclust(dissimilarity_matrix, FUN = hcut, hc_method = "complete", method = "silhouette", k.max = 10
  labs(subtitle = "The Silhouette Method")
```



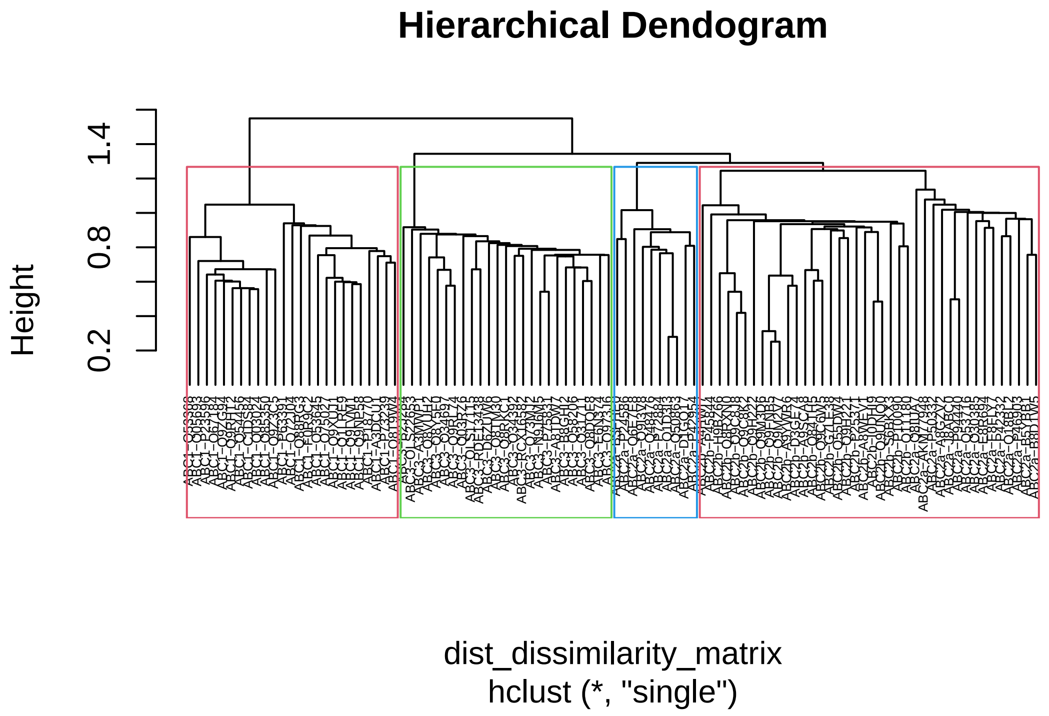
```r
fviz_nbclust(dissimilarity_matrix, FUN = hcut, hc_method = "ward.D", method = "wss", k.max = 10) +
  labs(subtitle = "The Elbow Method")
```



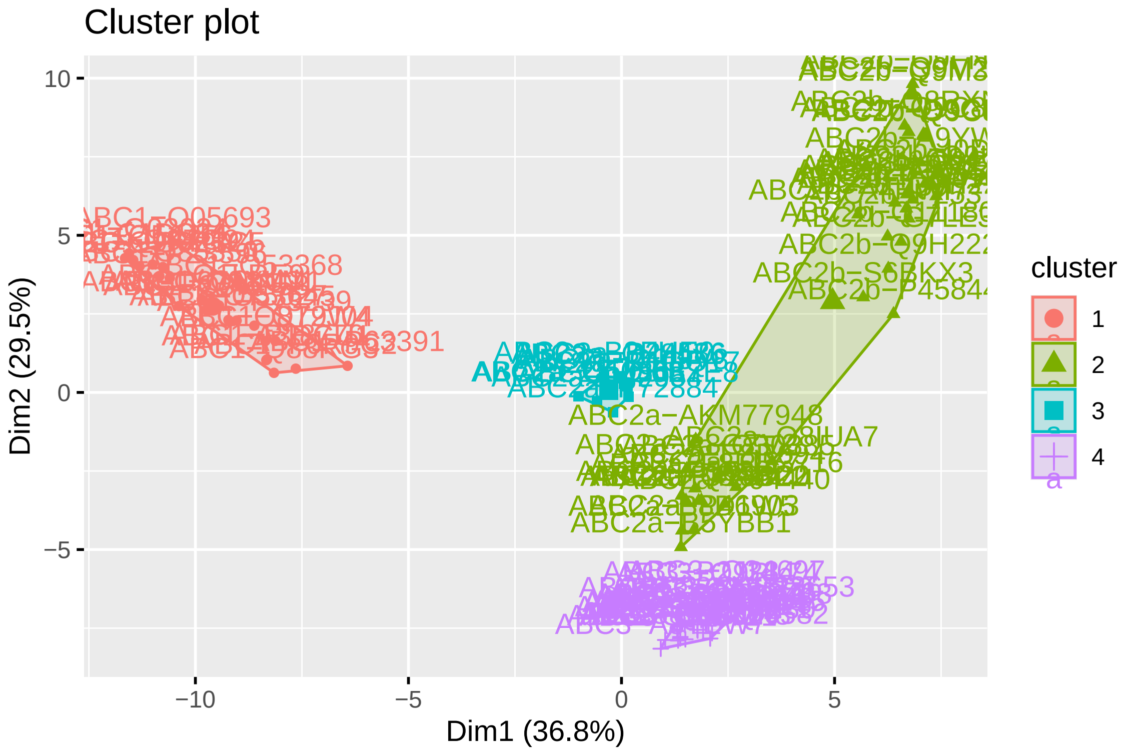
```r
fviz_nbclust(dissimilarity_matrix, FUN = hcut, hc_method = "ward.D", method = "silhouette", k.max = 10)
  labs(subtitle = "The Silhouette Method")
```



```r
dist_dissimilarity_matrix <- dist(dissimilarity_matrix)

# Cluster by hierarchical method
csin <- hclust(dist_dissimilarity_matrix, method = "single")

plot(csin, hang = -1, main = "Hierarchical Dendogram", cex=0.4)

#cut the dendogram such that 4 clusters are produced
rect.hclust(csin, k=4, border=2:4)
```



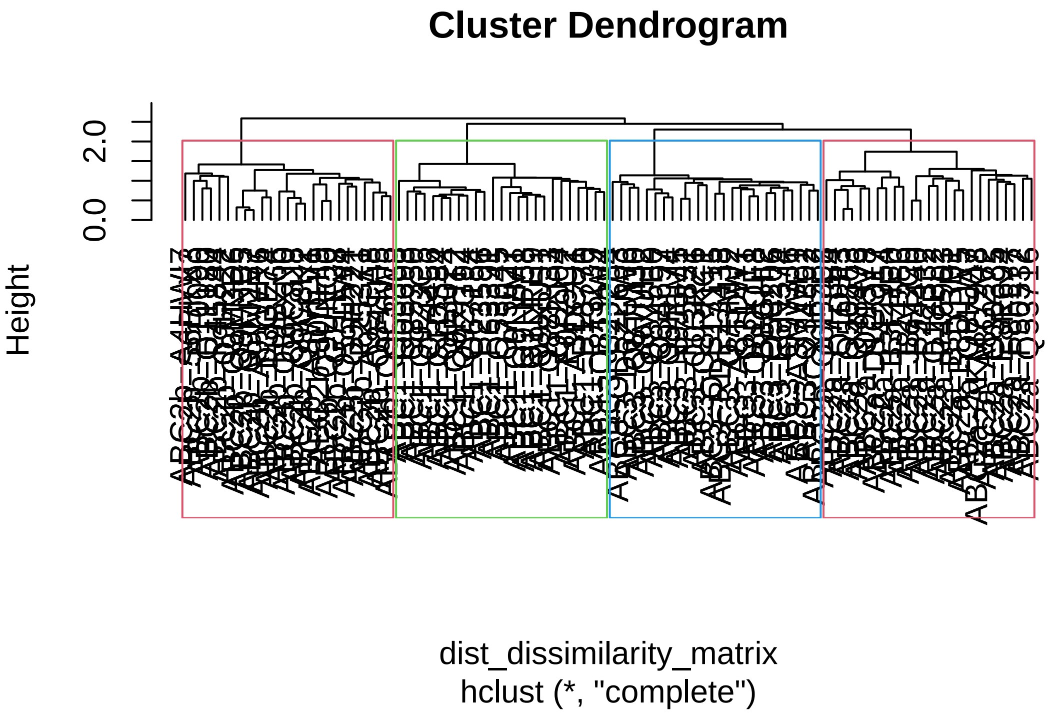
```
#get the data points within each cluster
cls3 <- cutree(csin, k=4)

#Scatter plot to visualize the data points in each cluster
#plot(dist_dissimilarity_matrix, xlim=c(0,8), ylim=c(0,8), col=cls3)
fviz_cluster(list(data = dist_dissimilarity_matrix, cluster = cls3))
```



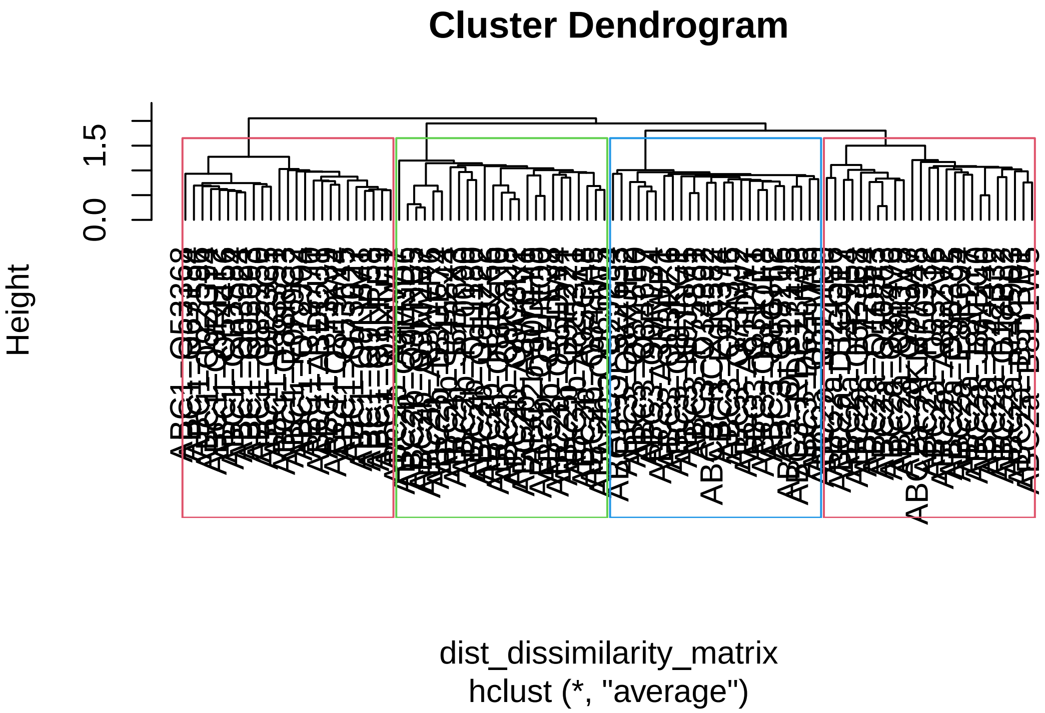
Using several methods, we can see that the optimal number of clusters is 4 more repeatedly. Both single method results supports 5 clusters, but the other 6 methods finds an optimum in 4 clusters. Nevertheless, in the cluster plot it looks more obvious that 5 clusters are the best option, same as the dendogram. Because we know somethings about the nature of the data, we will use 4 clusters for the next steps.

Comparing different hierarchical clustering methods

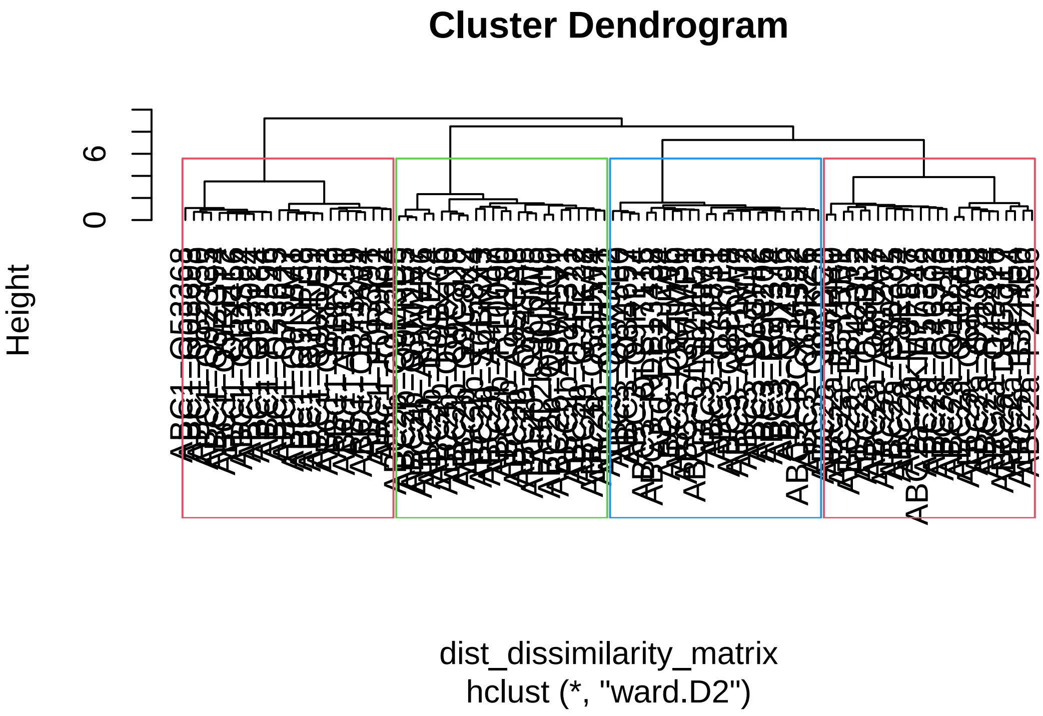
```
# compare with method complete linkage

ccom <- hclust(dist_dissimilarity_matrix, method = "complete")
plot (ccom, hang = -1)
rect.hclust(ccom, k=4, border=2:4)
```



```
dend1 <- as.dendrogram (csin)
dend2 <- as.dendrogram(ccom)


#Now compare with method average and Ward.

cave <- hclust(dist_dissimilarity_matrix, method = "average")
plot (cave, hang = -1)
rect.hclust(cave, k=4, border=2:4)
```



```
dend3 <- as.dendrogram(cave)


cward <- hclust(dist_dissimilarity_matrix, method = "ward.D2")
plot (cward, hang = -1)
rect.hclust(cward, k=4, border=2:4)
```



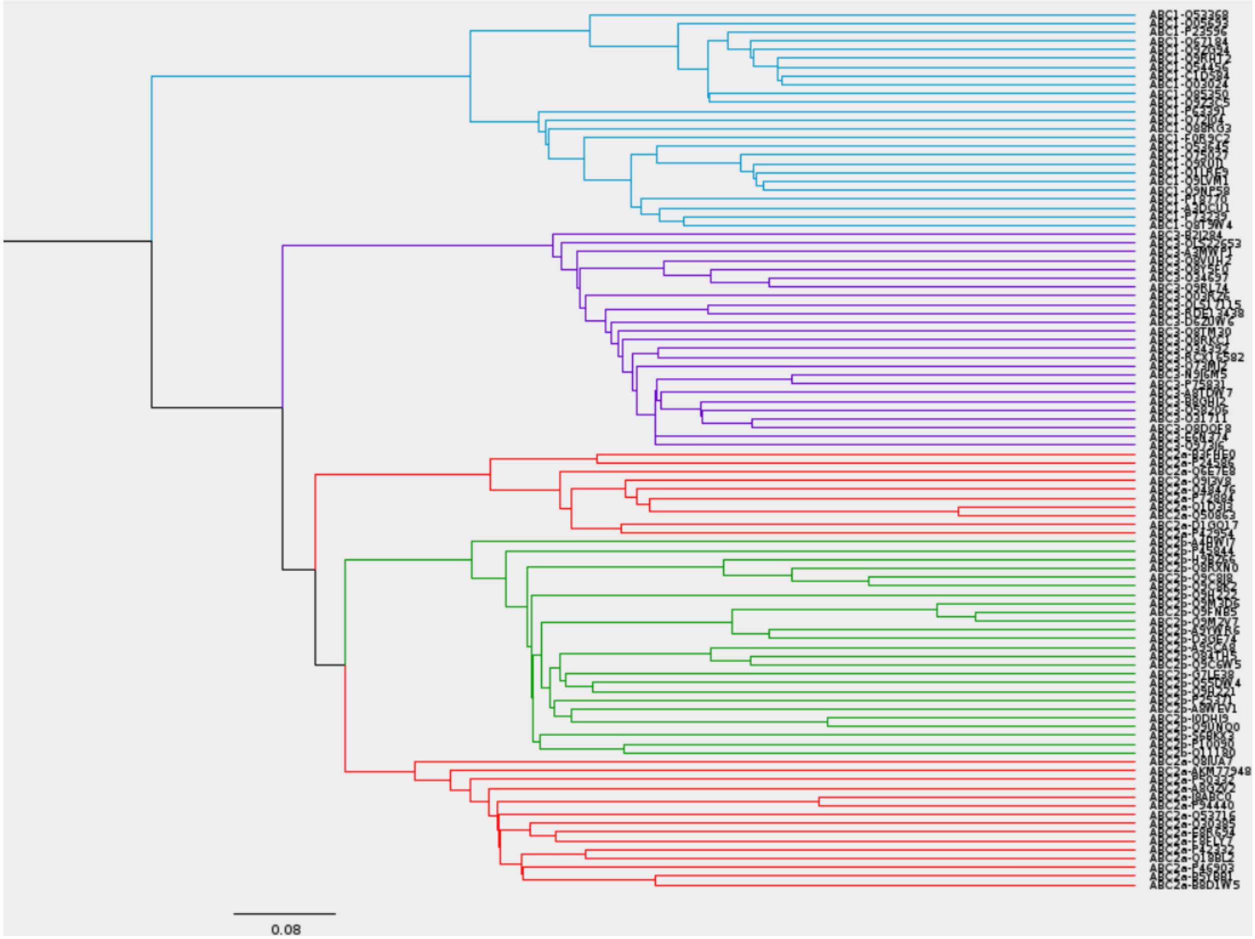
```
dend4 <- as.dendrogram(cward)
```

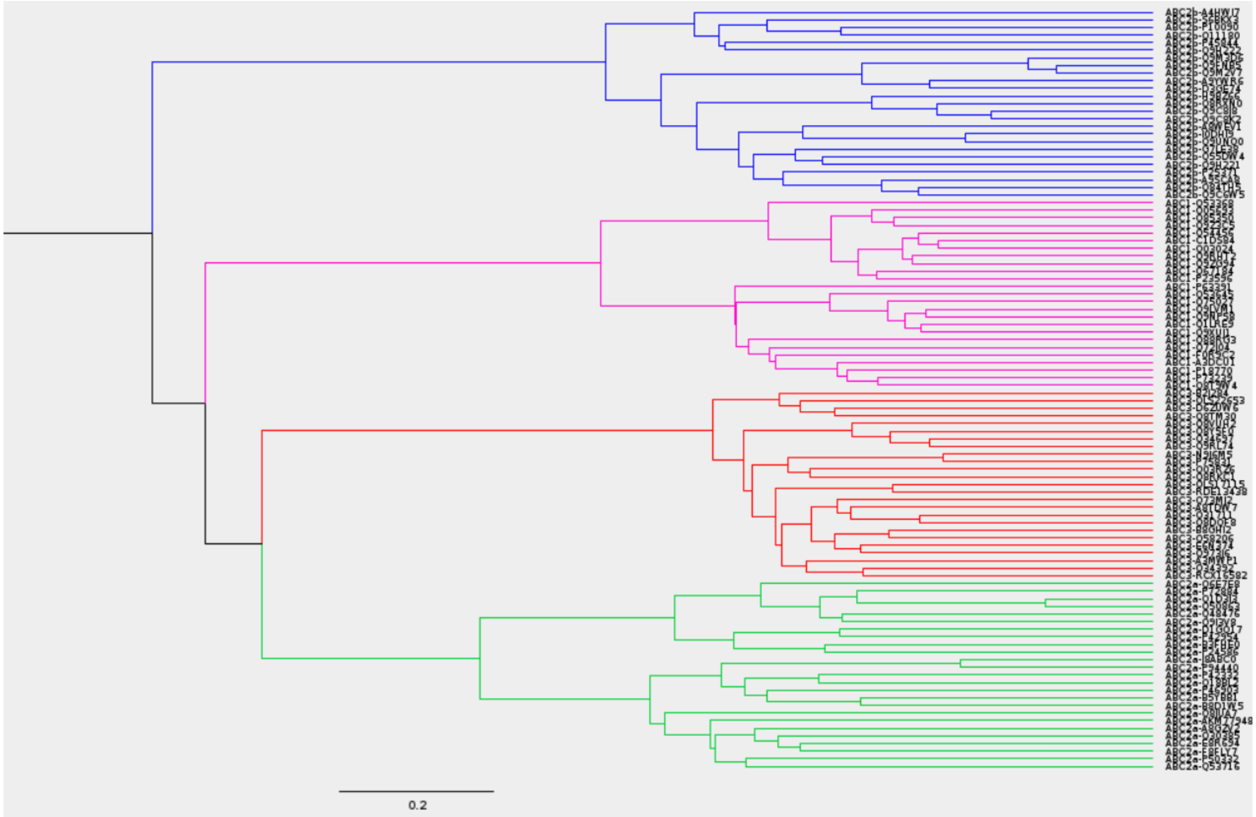
From a first sight, we can see that the ward method may be the best option, as it has the lower inner distances within each clusters, and the higher distances between clusters, representing a better separation of the clusters and a better classification.

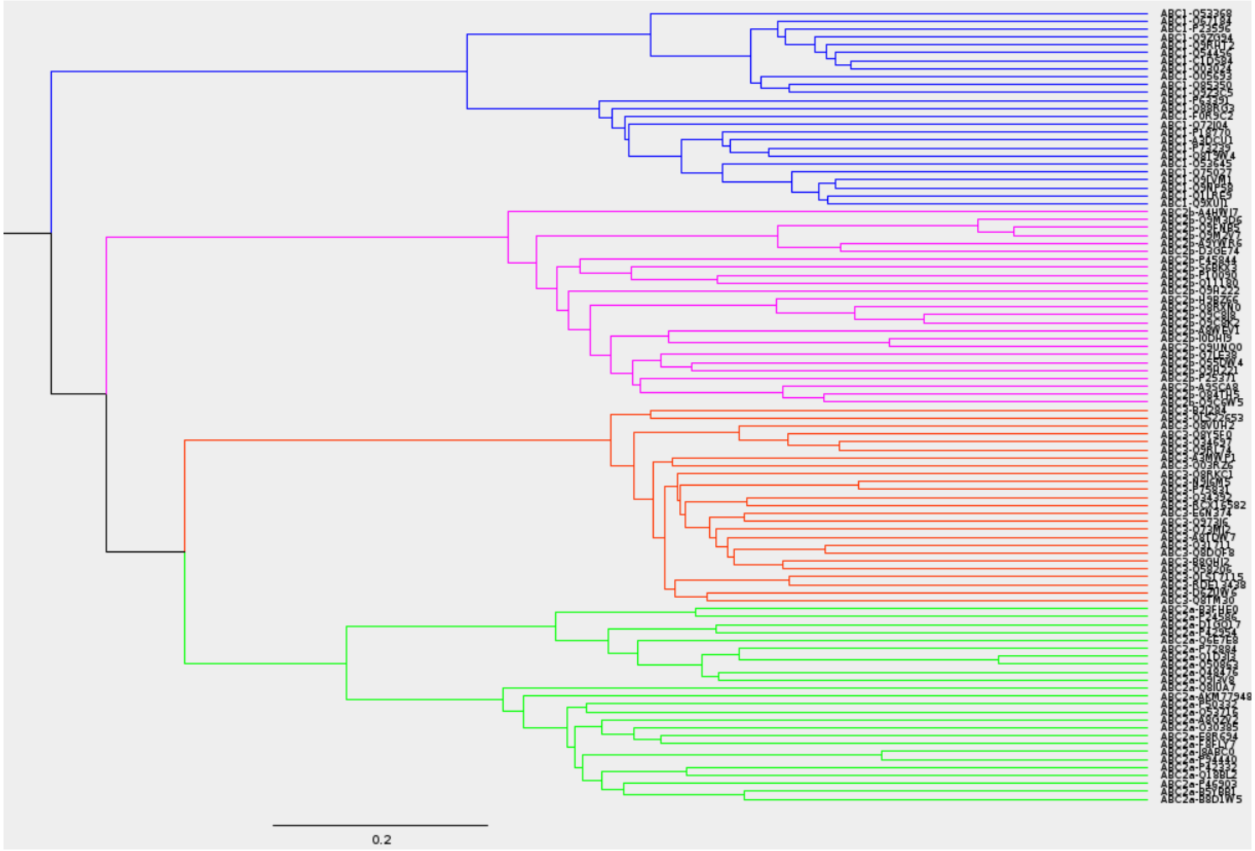
```
trees_names <- c("csin", "ccom", "cave", "cward")
trees <- list(csin, ccom, cave, cward)
for (i in seq_along(trees)) {
  my_tree <- as.phylo(trees[[i]])  # Convertir hclust a phylo
  write.tree(phy = my_tree, file = paste0(trees_names[i], ".tree"))  # Nombre correcto
             #Save single tree in Newick format
}
```

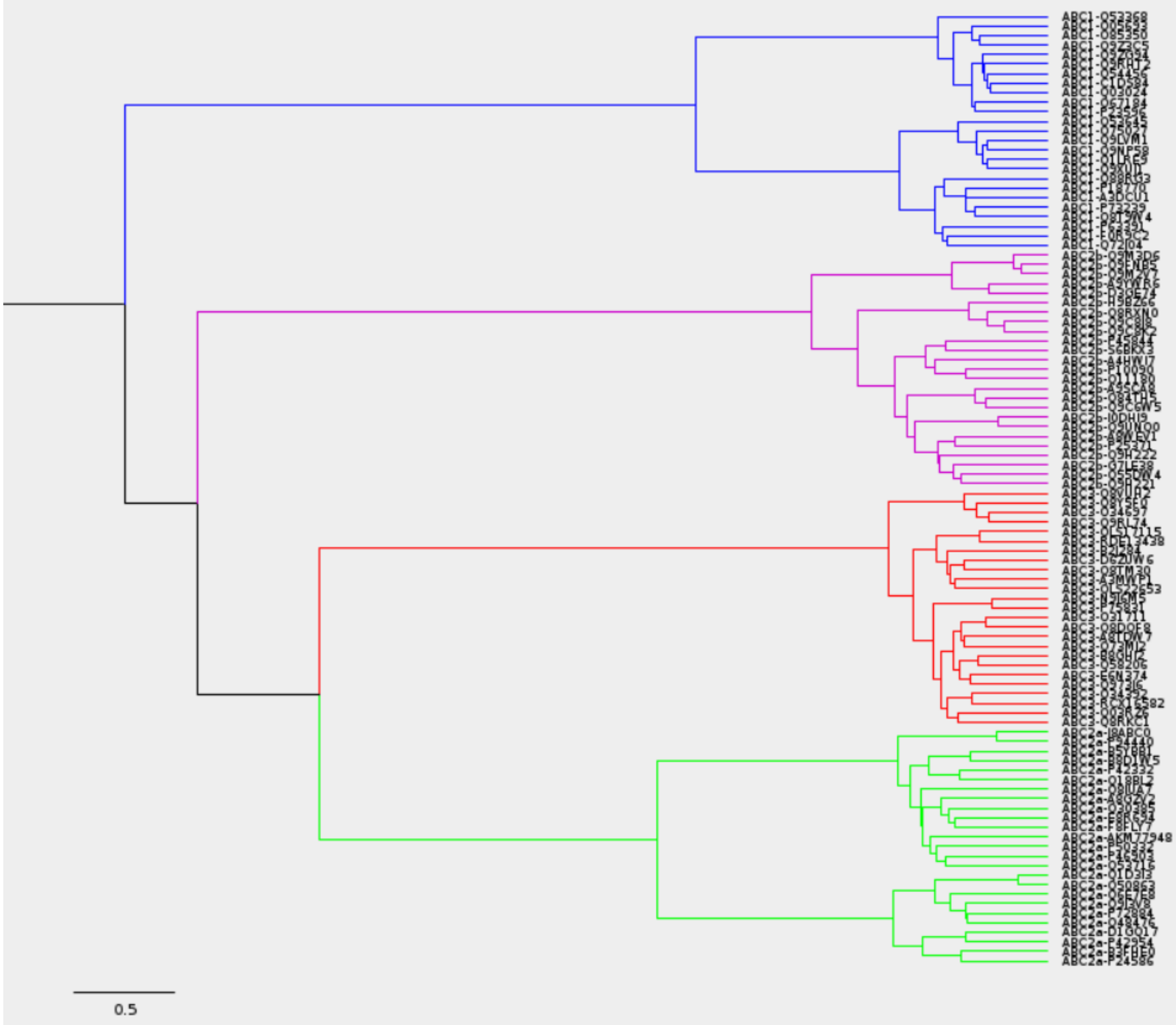
```
figtree csin.tree
figtree ccom.tree
figtree cave.tree
figtree cward.tree
```

Through the visualization of the trees with FigTree, we can see that in general all the methods cluster the data in a carrect way, knowing that each proteins ID has marker of its group. Only the single method has a cluster that is not well defined, it splited the ABC2a group in two, it didn't do the best clustering.


ABC1-Q52368
ABC1-Q05593
ABC1-P23596
ABC1-Q67184
ABC1-Q9ZG94
ABC1-Q9RHT2
ABC1-Q54456
ABC1-C1D584
ABC1-Q03024
ABC1-Q85350
ABC1-Q9Z3C5
ABC1-P63391
ABC1-Q72I04
ABC1-Q88RG3
ABC1-F0R9C2
ABC1-Q53645
ABC1-Q75027
ABC1-Q9XUI1
ABC1-Q1LRE9
ABC1-Q9LVM1
ABC1-Q9NP58
ABC1-P18770
ABC1-A3DCU1
ABC1-P73239
ABC1-Q8T9W4
ABC3-B2I284
ABC3-OLS22653
ABC3-A3MWP1
ABC3-Q8VUH2
ABC3-Q8Y5E0
ABC3-O34697
ABC3-Q9RL74
ABC3-Q03R76
ABC3-OLS17115
ABC3-RDE13438
ABC3-D6Z0W6
ABC3-Q8TM30
ABC3-Q8RKC1
ABC3-O34392
ABC3-RCX16582
ABC3-O73MJ2
ABC3-N9J6M5
ABC3-P75831
ABC3-A8TDW7
ABC3-B8GBI2
ABC3-O58206
ABC3-O31711
ABC3-Q8DQF8
ABC3-E6N374
ABC3-Q973I6
ABC2a-B3FHE0
ABC2a-P24586
ABC2a-Q6E7E8
ABC2a-Q9I3V8
ABC2a-Q48476
ABC2a-P72884
ABC2a-Q1D3I3
ABC2a-Q50863
ABC2a-D1GQ17
ABC2a-P42954
ABC2b-A4BWI7
ABC2b-P45844
ABC2b-H9BZ66
ABC2b-Q8RXN0
ABC2b-Q9C8I8
ABC2b-Q9C8K2
ABC2b-Q9H222
ABC2b-Q9M3D6
ABC2b-Q9FNB5
ABC2b-Q9MZV7
ABC2b-A5YWR6
ABC2b-D3GE74
ABC2b-A5SCA8
ABC2b-Q84TH5
ABC2b-Q9C6W5
ABC2b-G7LE38
ABC2b-Q55DW4
ABC2b-Q9H221
ABC2b-P25371
ABC2b-A8WEV1
ABC2b-I0DHI9
ABC2b-Q9UNQ0
ABC2b-S6BKX3
ABC2b-P10090
ABC2b-Q11180
ABC2a-Q8IUA7
ABC2a-AKM77948
ABC2a-P50332
ABC2a-A8GZV2
ABC2a-I8ABC0
ABC2a-P94440
ABC2a-O53716
ABC2a-Q30385
ABC2a-E8R694
ABC2a-E8ELY7
ABC2a-P42332
ABC2a-Q18BL2
ABC2a-P46903
ABC2a-B5YBB1
ABC2a-B8D1W5
0.08

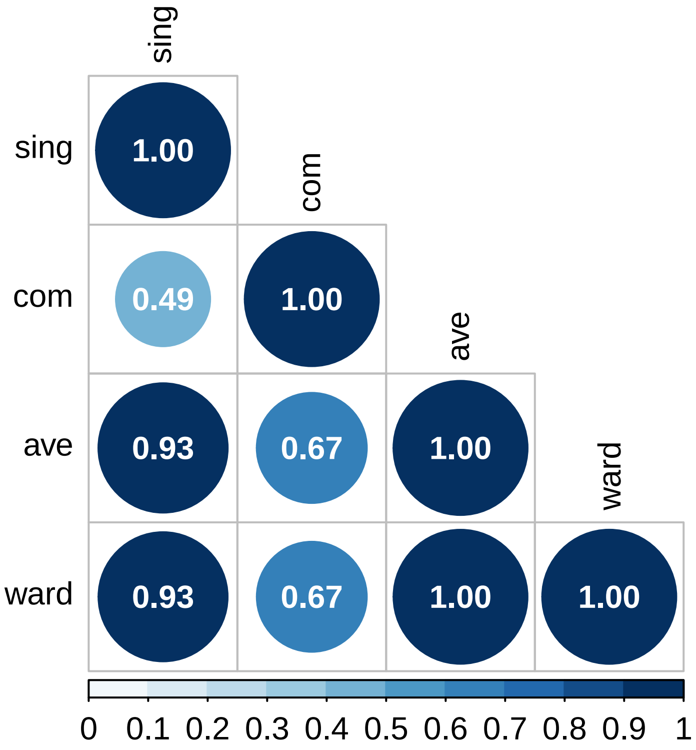

ABC2b-A4HWI7
ABC2b-S6BKX3
ABC2b-P10090
ABC2b-O11180
ABC2b-P45844
ABC2b-Q9H222
ABC2b-Q9M3D6
ABC2b-Q9FNB5
ABC2b-Q9M2V7
ABC2b-A9YWR6
ABC2b-D3GE74
ABC2b-H9BZ66
ABC2b-Q8RXN0
ABC2b-Q9C8I8
ABC2b-Q9C8K2
ABC2b-A8WEV1
ABC2b-I0DHI9
ABC2b-Q9UNQ0
ABC2b-Q7LE38
ABC2b-Q55DW4
ABC2b-Q9H221
ABC2b-P25371
ABC2b-A9SCA8
ABC2b-Q84TH5
ABC2b-Q9C6W5
ABC1-Q53368
ABC1-O05593
ABC1-Q85350
ABC1-Q9Z2C5
ABC1-O54456
ABC1-C1DS84
ABC1-O03024
ABC1-Q9RHT2
ABC1-Q9ZG94
ABC1-Q67184
ABC1-P23596
ABC1-P63391
ABC1-O53645
ABC1-O75027
ABC1-Q9LVM1
ABC1-Q9NP58
ABC1-Q1LRE9
ABC1-Q9XUI1
ABC1-Q88RG3
ABC1-Q72I04
ABC1-F0R9C2
ABC1-A3DCU1
ABC1-P18770
ABC1-P73239
ABC1-Q8T9W4
ABC3-B2I284
ABC3-OLS22653
ABC3-D6ZUW6
ABC3-Q8TM30
ABC3-Q8VUH2
ABC3-Q8Y5E0
ABC3-O34697
ABC3-Q9RL74
ABC3-N9I6M5
ABC3-P75831
ABC3-Q03R26
ABC3-Q8RKC1
ABC3-OLS17115
ABC3-RDE13438
ABC3-O73MI2
ABC3-A8TQW7
ABC3-O31711
ABC3-Q8DQF8
ABC3-B8GHI2
ABC3-O58206
ABC3-E6N374
ABC3-Q97JI6
ABC3-A3MWP1
ABC3-O34392
ABC3-RCX16582
ABC2a-Q6E7E8
ABC2a-P72884
ABC2a-Q1D3I3
ABC2a-Q50863
ABC2a-Q48476
ABC2a-Q9I3V8
ABC2a-D1GQI7
ABC2a-P42954
ABC2a-B3FHE0
ABC2a-P24586
ABC2a-I8ABC0
ABC2a-P94440
ABC2a-P42332
ABC2a-Q18BL2
ABC2a-P46903
ABC2a-B5YBB1
ABC2a-B8D1W5
ABC2a-Q8I0A7
ABC2a-AKM77948
ABC2a-A8GZV2
ABC2a-O30385
ABC2a-E8R694
ABC2a-F8FLY7
ABC2a-P50332
ABC2a-Q53716
0.2


ABC1-Q53368
ABC1-Q67184
ABC1-P23596
ABC1-Q9ZG94
ABC1-Q9RHT2
ABC1-O54456
ABC1-C1DS84
ABC1-O03024
ABC1-O05693
ABC1-Q85350
ABC1-Q9Z3C5
ABC1-P63391
ABC1-Q88RG3
ABC1-F0R9C2
ABC1-Q72I04
ABC1-P18770
ABC1-A3DCU1
ABC1-P73239
ABC1-Q8T9W4
ABC1-O53645
ABC1-O75027
ABC1-Q9LVM1
ABC1-Q9NP58
ABC1-Q1LRE9
ABC1-Q9XUI1
ABC2b-A4HWI7
ABC2b-Q9M3D6
ABC2b-Q9FNB5
ABC2b-Q9M2V7
ABC2b-A9YWR6
ABC2b-D3GE74
ABC2b-P45844
ABC2b-S6BKX3
ABC2b-P10090
ABC2b-O11180
ABC2b-Q9H222
ABC2b-H9BZ66
ABC2b-Q8RXN0
ABC2b-Q9C8I8
ABC2b-Q9C8K2
ABC2b-A8WEV1
ABC2b-I0DHI9
ABC2b-Q9UNQ0
ABC2b-Q7LE38
ABC2b-Q55DW4
ABC2b-Q9H221
ABC2b-P25371
ABC2b-A9SCA8
ABC2b-Q84TH5
ABC2b-Q9C6W5
ABC3-B2I284
ABC3-OLS22653
ABC3-Q8VUH2
ABC3-Q8Y5E0
ABC3-O34697
ABC3-Q9RL74
ABC3-A3MWP1
ABC3-Q03R26
ABC3-Q8RKC1
ABC3-N9I6M5
ABC3-P75831
ABC3-O34392
ABC3-RCX16582
ABC3-E6N374
ABC3-Q97JI6
ABC3-O73MI2
ABC3-A8TQW7
ABC3-O31711
ABC3-Q8DQF8
ABC3-B8GHI2
ABC3-O58206
ABC3-OLS17115
ABC3-RDE13438
ABC3-D6ZUW6
ABC3-Q8TM30
ABC2a-B3FHE0
ABC2a-P24586
ABC2a-D1GQI7
ABC2a-P42954
ABC2a-Q6E7E8
ABC2a-P72884
ABC2a-Q1D3I3
ABC2a-Q50863
ABC2a-Q48476
ABC2a-Q9I3V8
ABC2a-Q8I0A7
ABC2a-AKM77948
ABC2a-P50332
ABC2a-Q53716
ABC2a-A8GZV2
ABC2a-O30385
ABC2a-E8R694
ABC2a-F8FLY7
ABC2a-I8ABC0
ABC2a-P94440
ABC2a-P42332
ABC2a-Q18BL2
ABC2a-P46903
ABC2a-B5YBB1
ABC2a-B8D1W5
0.2



Comparing different hierarchical clustering methods

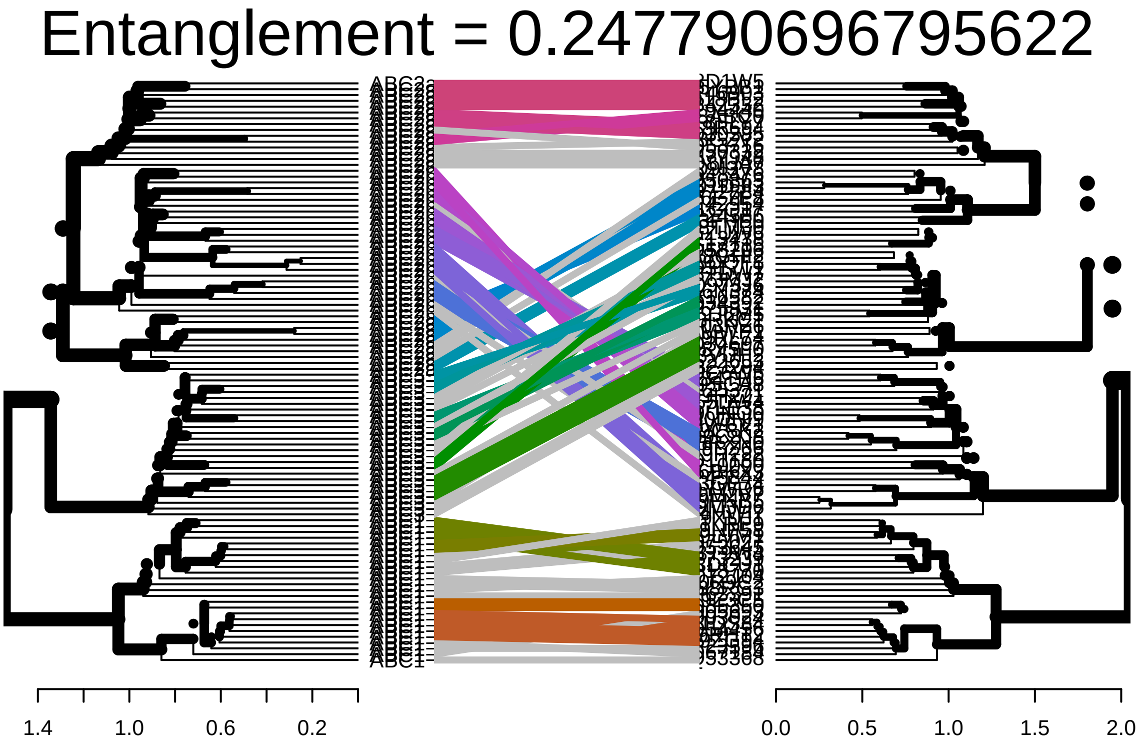
```
trees = dendlist("sing"=dend1, "com"=dend2, "ave"=dend3, "ward"=dend4)
baker = cor.dendlist(trees,method="baker")

corrplot(baker,
         method="circle",
         type="lower",
         tl.col = "black",
         tl.cex = 1.0,
         cl.cex = 1.0,
         addCoef.col = "white",
         number.cex = 1.0,
         col.lim=c(0.0,1),
         col=COL2("RdBu",n = 20)
)
```



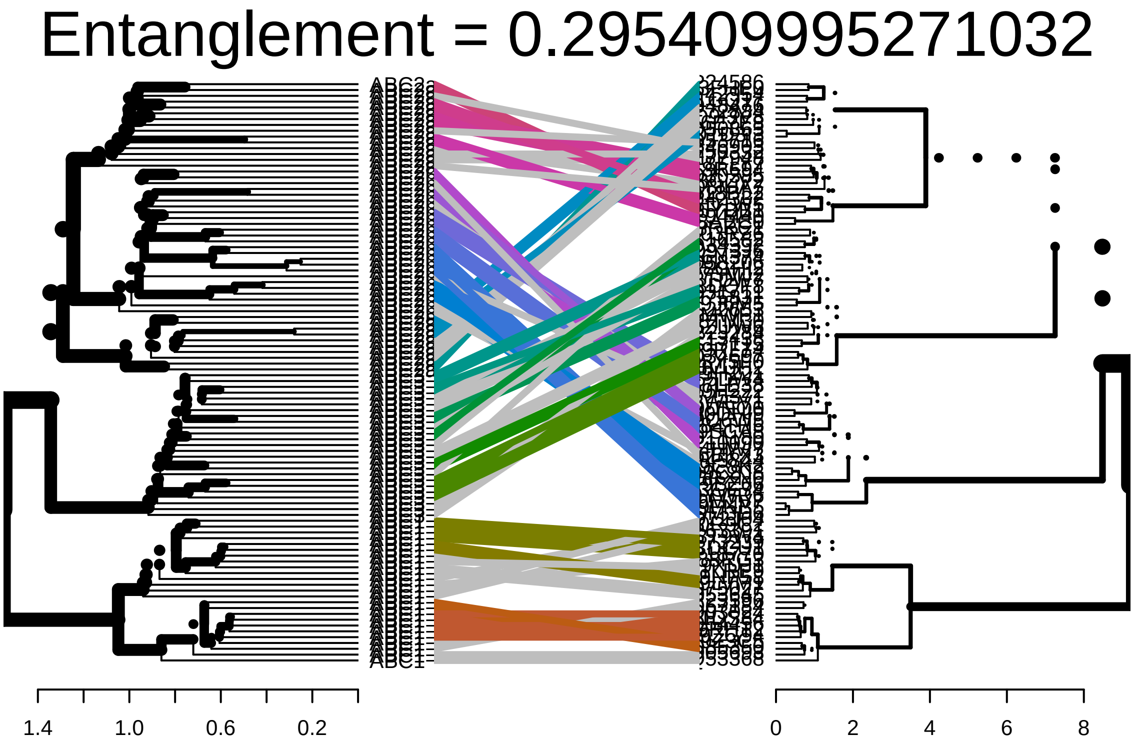
The correlation plot shows that the ward method shares a perfect correlation with average method, additionally, it has a great correlation with the single method. Intuitively, average and single methods has a great correlation too.

Because of this we'll explore the comparison of the dendograms of this 3 pairs of methods. It showed what expected, the entaglement value is congruent with the correlation plot, the ward and average methods are the most similar, and both, ward and average methods have a good correlation with the single method.

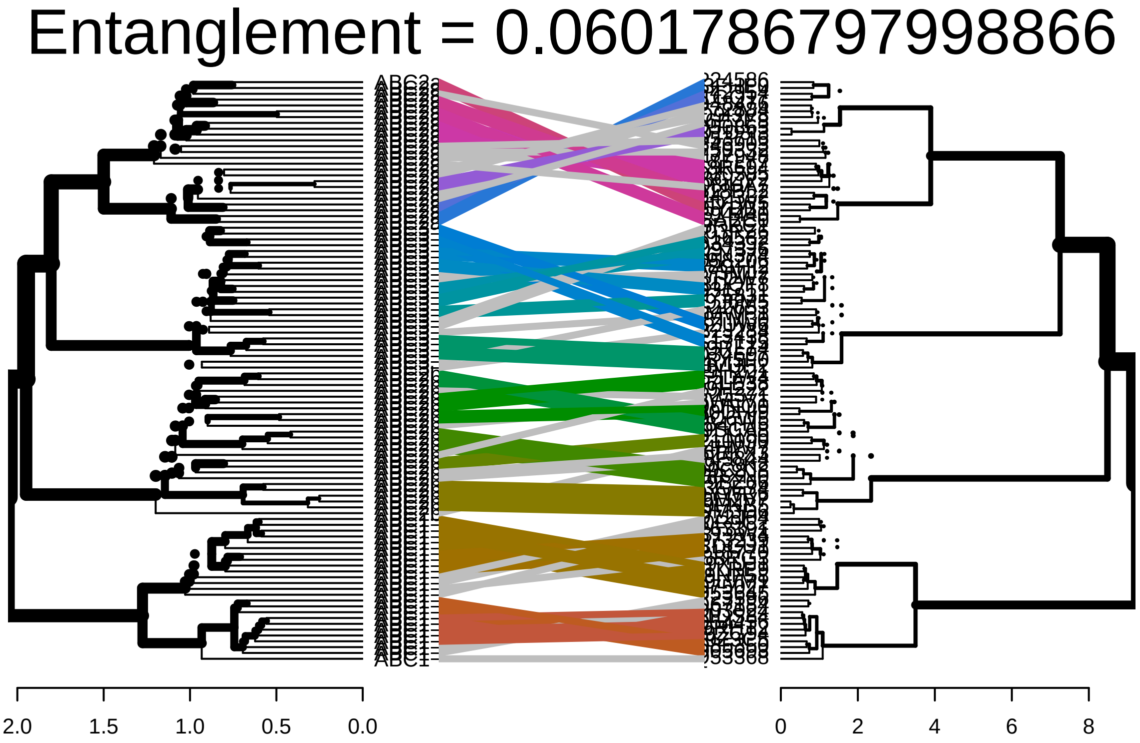
```
tanglegram(dend1, dend3, main = paste("Entanglement =", entanglement(dendlist(dend1, dend3))))
```



```
tanglegram(dend1, dend4, main = paste("Entanglement =", entanglement(dendlist(dend1, dend4))))
```



```
tanglegram(dend3, dend4, main = paste("Entanglement =", entanglement(dendlist(dend3, dend4))))
```



Tree Agglomerative Coefficient

At last, we will use the agnes function to perform the hierarchical clustering analysis using the different methods and compare the agglomerative coefficient of each method. We see that ward method is the best options for this data, as it has the highest agglomerative coefficient, of 0.9181161.

```
hc_sin <- agnes(dist_dissimilarity_matrix, method = "single")
hc_sin$ac
```

```
## [1] 0.531044
```

```
hc_com <- agnes(dist_dissimilarity_matrix, method = "complete")
hc_com$ac
```

```
## [1] 0.7093296
```

```
hc_ave <- agnes(dist_dissimilarity_matrix, method = "average")
hc_ave$ac
```

```
## [1] 0.6370328
```

```
hc_ward <- agnes(dist_dissimilarity_matrix, method = "ward")
hc_ward$ac
```

```
## [1] 0.9181161
```

Conclusion

In conclusion, we have performed a protein clustering analysis using a dataset of protein sequences from the ABC transporter family. We have obtained a dendogram with 4 clusters using the hierarchical clustering method. We have also compared different hierarchical clustering methods, and through several comparison values and agglomerative coefficient, we found that the ward method is the best option from 4 different clustering methods (single, complete, average and ward) for this data.

Additionally, through this project, we have learned the importance of clustering, due to it is a powerful strategy to solve a problem as ancient and human characteristic as the classification of things. Clustering propose a way to study study objects, that many time its classification nor its properties are clear, so common in biology problematic.

We learnt how to perform a protein clustering analysis using R and the different methods and plots that can be used to determine the optimal number of clusters and compare different hierarchical clustering methods.

For more details about the project, please refer to the GitHub repository.