

# RFLPtools: Analysis of DNA fragment samples and standalone BLAST report files

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July 20, 2010

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## 1 Introduction

The package "RFLPtools" aims at

- the detection of similar band patterns based on DNA fingerprint fragment sizes (i.e. derived from RFLP-analysis)
- the analysis of standalone BLAST report files (i.e. DNA sequence analysis)

In this short vignette we describe and demonstrate the available functions.

> *library(RFLPtools)*

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## 2 RFLP data

We load example data and compute the Euclidean distance ...

```
> data(RFLPdata)
> res <- RFLPdist(RFLPdata)
> names(res) ## number of bands

[1] "3"  "4"  "5"  "6"  "7"  "8"  "9"  "10" "11" "12"

> str(res$"6")

Class 'dist'  atomic [1:210] 517.58 3.74 145.24 397.64 482.89 ...
.. attr(*, "Size")= int 21
.. attr(*, "Labels")= chr [1:21] "Ni_25_A3" "Ni_25_B1" "Ni_25_B3" "Ni_25_H5" ...
.. attr(*, "Diag")= logi FALSE
.. attr(*, "Upper")= logi FALSE
.. attr(*, "method")= chr "euclidean"
.. attr(*, "call")= language distfun(x = do.call("rbind", temp1))
```

Of course, we can also use other well-known distances ...

```
> res1 <- RFLPdist(RFLPdata, distfun = function(x) dist(x, method = "manhattan"))
> res2 <- RFLPdist(RFLPdata, distfun = function(x) dist(x, method = "maximum"))
> str(res[[1]])

Class 'dist'  atomic [1:28] 16.03 10.86 2.45 157.21 5.1 ...
.. attr(*, "Size")= int 8
.. attr(*, "Labels")= chr [1:8] "Ni_28_A6" "Ni_28_B2" "Ni_28_C2" "NI_28_D6" ...
.. attr(*, "Diag")= logi FALSE
.. attr(*, "Upper")= logi FALSE
.. attr(*, "method")= chr "euclidean"
.. attr(*, "call")= language distfun(x = do.call("rbind", temp1))

> str(res1[[1]])

Class 'dist'  atomic [1:28] 27 16 4 211 6 17 172 11 31 220 ...
.. attr(*, "Size")= int 8
.. attr(*, "Labels")= chr [1:8] "Ni_28_A6" "Ni_28_B2" "Ni_28_C2" "NI_28_D6" ...
.. attr(*, "Diag")= logi FALSE
.. attr(*, "Upper")= logi FALSE
.. attr(*, "method")= chr "manhattan"
.. attr(*, "call")= language dist(x = x, method = "manhattan")
```

```
> str(res2[[1]])

Class 'dist'  atomic [1:28] 12 9 2 147 5 7 103 6 13 154 ...
..- attr(*, "Size")= int 8
..- attr(*, "Labels")= chr [1:8] "Ni_28_A6" "Ni_28_B2" "Ni_28_C2" "Ni_28_D6" ...
..- attr(*, "Diag")= logi FALSE
..- attr(*, "Upper")= logi FALSE
..- attr(*, "method")= chr "maximum"
..- attr(*, "call")= language dist(x = x, method = "maximum")
```

Correlation distances

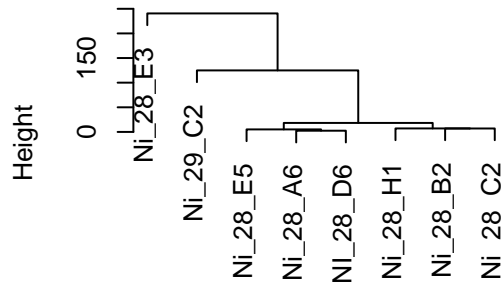
```
> library(MKmisc)
> res3 <- RFLPdists(RFLPdata, distfun = corDist)
> str(res3$"9")

Class 'dist'  atomic [1:21] 0.475 0.521 0.508 0.517 0.512 ...
..- attr(*, "Size")= int 7
..- attr(*, "Labels")= chr [1:7] "Ni_25_C4" "Ni_25_C5" "Ni_25_E4" "Ni_28_B9" ...
..- attr(*, "Diag")= logi FALSE
..- attr(*, "Upper")= logi FALSE
..- attr(*, "method")= chr "pearson"
..- attr(*, "call")= language distfun(x = do.call("rbind", temp1))
```

As we obtain a list of dist objects we can easily perform hierarchical clustering ...

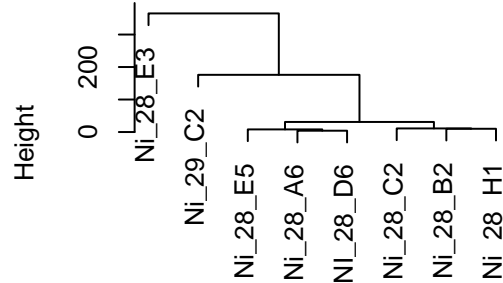
```
> par(mfrow = c(2,2))
> plot(hclust(res[[1]]), main = "Euclidean distance")
> plot(hclust(res1[[1]]), main = "Manhattan distance")
> plot(hclust(res2[[1]]), main = "Maximum distance")
> plot(hclust(res3[[1]]), main = "Pearson correlation distance")
```

**Euclidean distance**



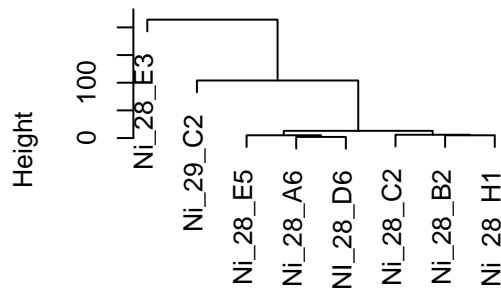
res[[1]]  
hclust (\*, "complete")

**Manhattan distance**



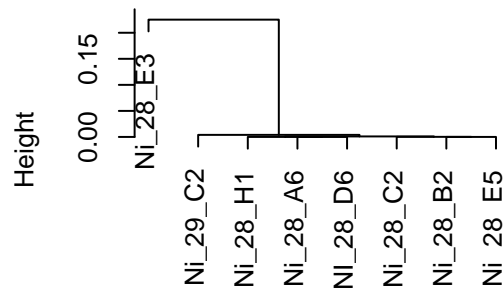
res1[[1]]  
hclust (\*, "complete")

**Maximum distance**



res2[[1]]  
hclust (\*, "complete")

**Pearson correlation distance**



res3[[1]]  
hclust (\*, "complete")

We easily can apply other functions ...

```
> clust4bd <- hclust(res[[2]])
> cgroups50 <- cutree(clust4bd, h=50)
> cgroups50
```

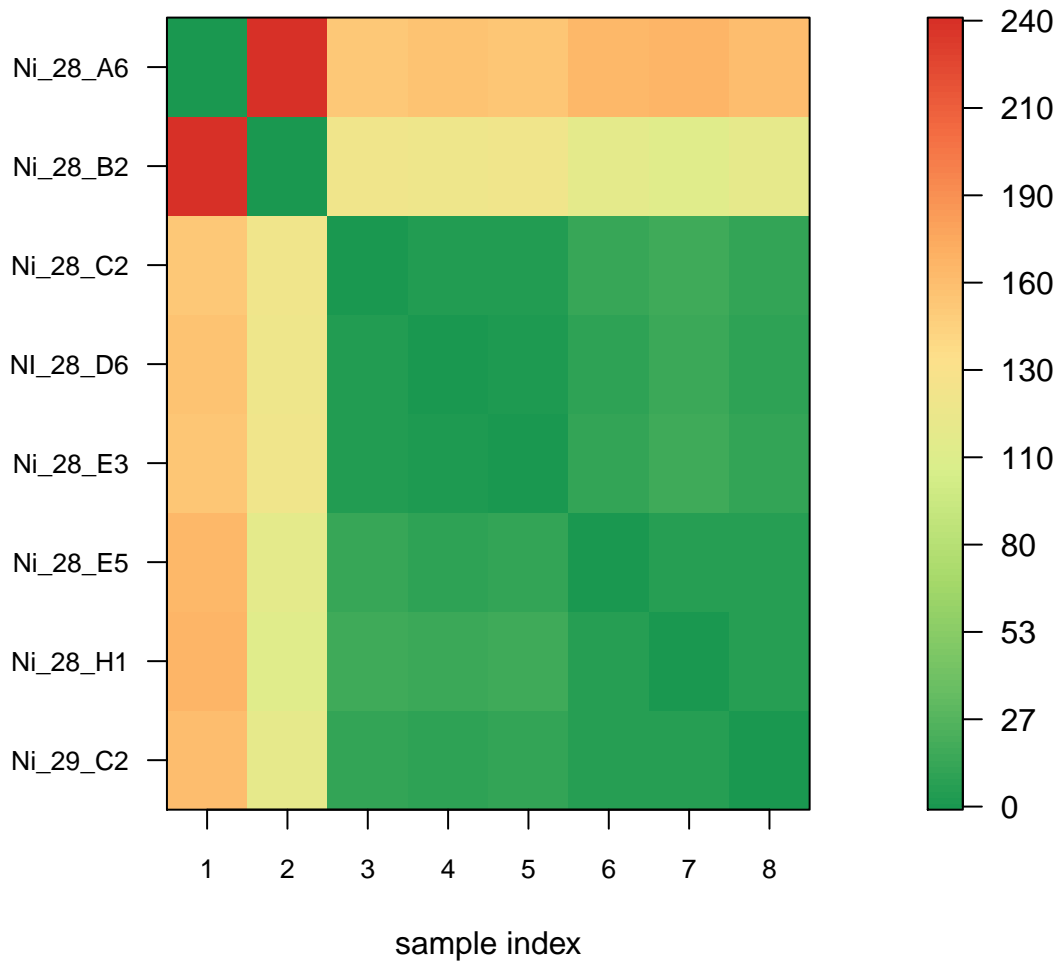
Ni_25_B2	Ni_25_B5	Ni_28_A2	Ni_28_A9	Ni_28_B4	Ni_28_B5	Ni_28_D4	Ni_28_E1
1	2	3	4	5	3	3	6
Ni_28_E4	Ni_28_F2	Ni_28_F4	Ni_28_G4	Ni_28_H4	Ni_29_A3	Ni_29_A4	Ni_29_A7

	3	3	3	3	3	7	8	8
Ni_29_B4	Ni_29_B5	Ni_29_C7	Ni_29_D1	Ni_29_D6	Ni_29_D7	Ni_29_E4	Ni_29_E5	
	9	10	11	12	13	14	8	9
Ni_29_F5	Ni_29_G1	Ni_29_G2	Ni_29_G4	Ni_29_H2	Ni_29_H4	Ni_29_H5		
	11	7	11	15	7	8	13	

Another possibility to display the similarity of the samples are so-called (dis-)similarity matrices ...

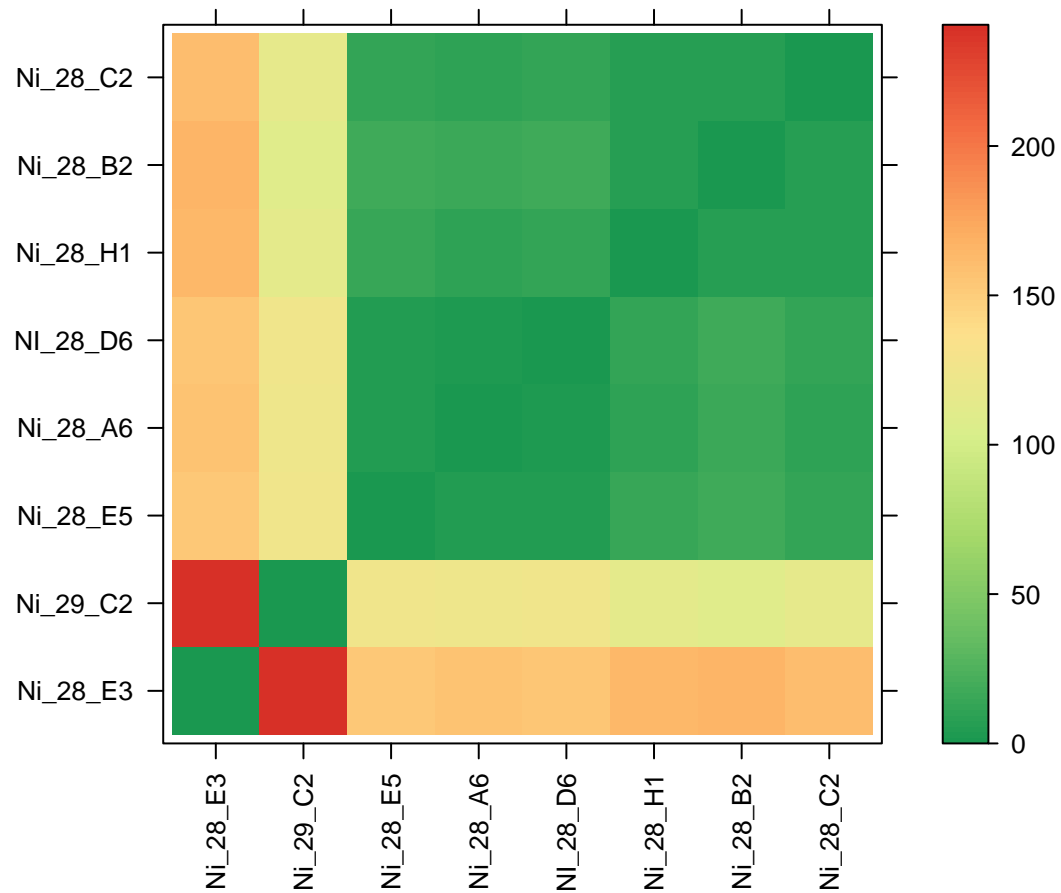
```
> library(RColorBrewer)
> library(MKmisc)
> myCol <- colorRampPalette(brewer.pal(8, "RdYlGn"))(128)
> ord <- order.dendrogram(as.dendrogram(hclust(res[[1]])))
> temp <- as.matrix(res[[1]])
> simPlot(temp[ord,ord], col = rev(myCol), minVal = 0,
+         labels = colnames(temp), title = "(Dis-)Similarity Plot")
```

### (Dis-)Similarity Plot



```
> library(lattice)
> print(levelplot(temp[ord,ord], col.regions = rev(myCol),
+               at = do.breaks(c(0, max(temp)), 128),
+               xlab = "", ylab = "",
+               ## Rotate labels of x-axis
+               scales = list(x = list(rot = 90)),
+               main = "(Dis-)Similarity Plot"))
```

## (Dis-)Similarity Plot



Some bands may be missing ...

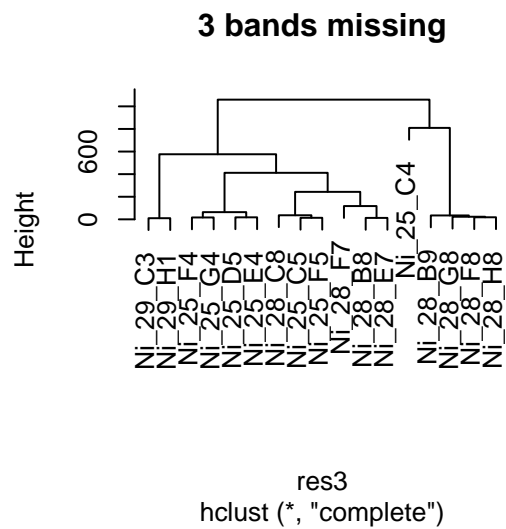
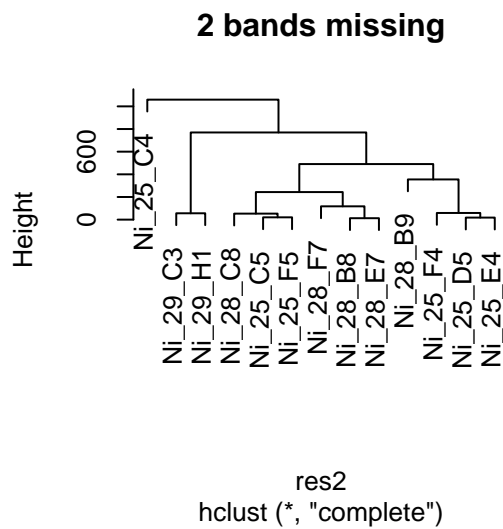
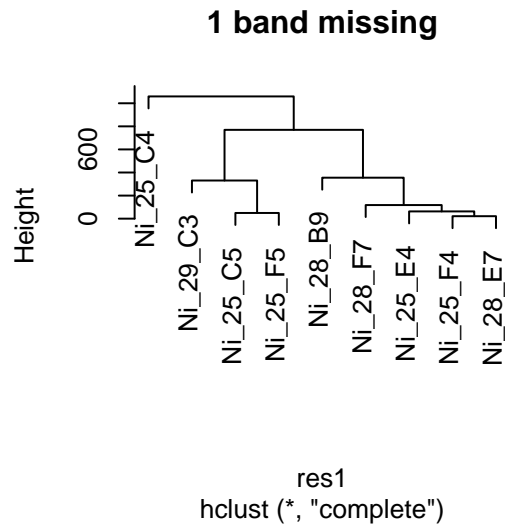
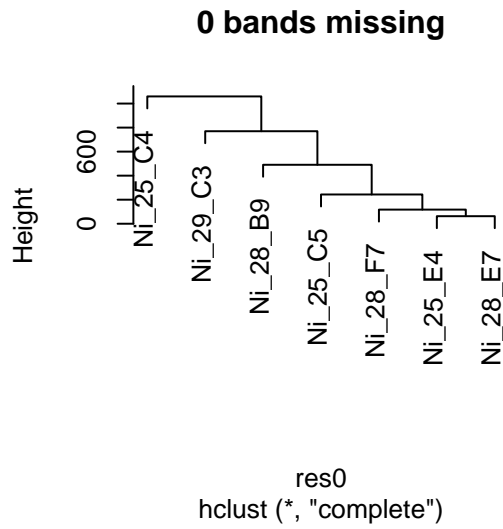
```
> ## Euclidean distance
> data(RFLPdata)
> data(RFLPref)
> nrBands(RFLPdata)
```

```
[1] 3 4 5 6 7 8 9 10 11 12
```

```
> res0 <- RFLPdist2(RFLPdata, nrBands = 9, nrMissing = 0)
> res1 <- RFLPdist2(RFLPdata, nrBands = 9, nrMissing = 1)
> res2 <- RFLPdist2(RFLPdata, nrBands = 9, nrMissing = 2)
> res3 <- RFLPdist2(RFLPdata, nrBands = 9, nrMissing = 3)

> ## hierarchical clustering
> par(mfrow = c(2,2))
> plot(hclust(res0), main = "0 bands missing")
> plot(hclust(res1), main = "1 band missing")
> plot(hclust(res2), main = "2 bands missing")
> plot(hclust(res3), main = "3 bands missing")
```

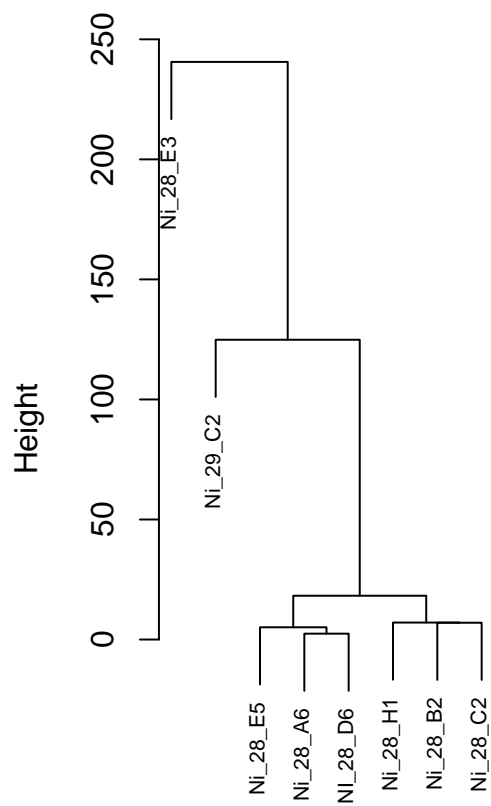




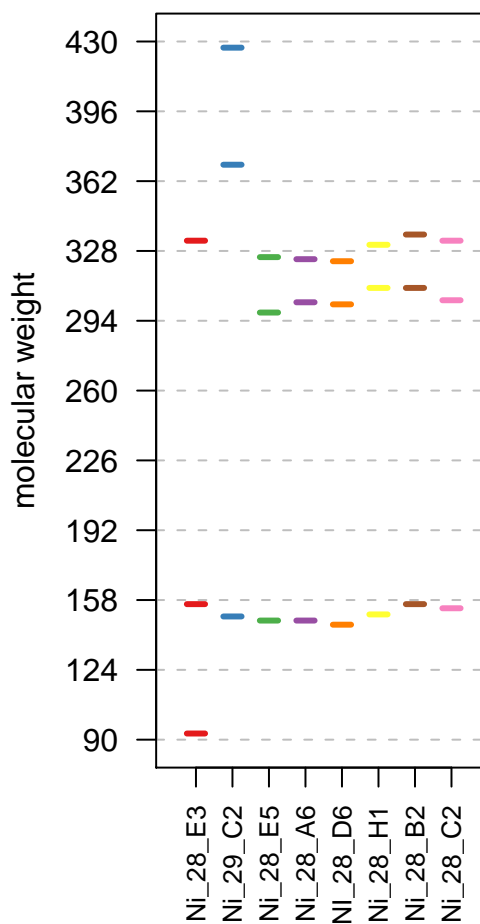
Another possible visualization ...

```
> par(mfrow = c(1,2))
> plot(hclust(RFLPdist(RFLPdata, nrBands = 3)), cex = 0.7)
> RFLPplot(RFLPdata, nrBands = 3, mar.bottom = 6, cex.axis = 0.8)
```

**Cluster Dendrogram**



**Samples with 3 bands**

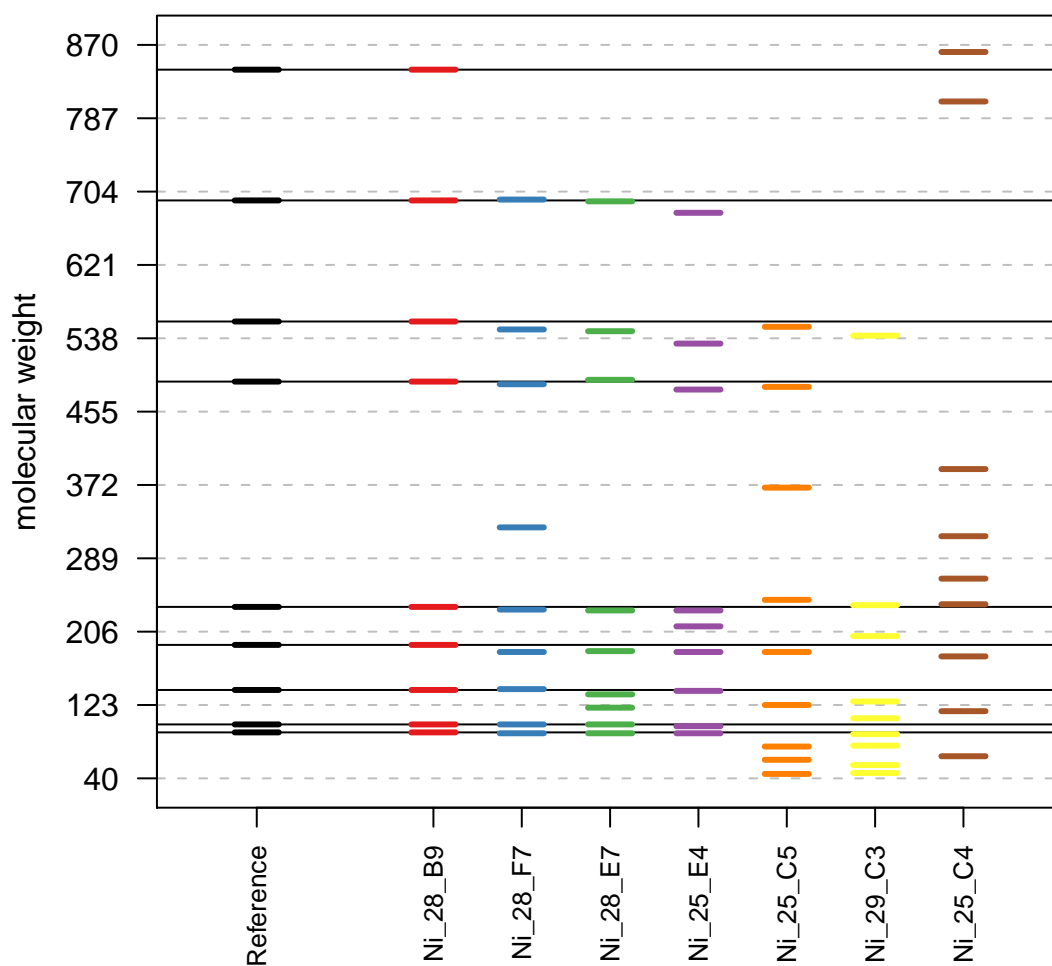


```
RFLPdlist(RFLPdata, nrBands = 3)
hclust (*, "complete")
```

Comparison to reference data ...

```
> RFLPrefplot(RFLPdata, RFLPref, nrBands = 9, cex.axis = 0.8)
```

## Reference sample: Zygomycota sp. (BS12346.1)



### 3 BLAST data

To analyze tabular report files generated with standalone BLAST from NCBI (cf. <ftp://ftp.ncbi.nlm.nih.gov/blast/executables/release>), a function for reading the BLAST report files is included (`read.blast`).

```
> Dir <- system.file("extdata", package = "RFLPtools") # input directory
> filename <- file.path(Dir, "BLASTexample.txt")
```

```

> BLAST1 <- read.blast(file = filename)
> str(BLAST1)

'data.frame':      4069 obs. of  12 variables:
 $ query.id       : chr  "agrFF002" "agrFF002" "agrFF002" "agrFF002" ...
 $ subject.id     : chr  "agrFF002" "agrFF148" "agrFF148" "agrFF176" ...
 $ identity       : num  100 93.4 100 91.4 100 ...
 $ alignment.length: int  544 243 11 255 11 255 11 256 11 256 ...
 $ mismatches     : int   0 14 0 20 0 20 0 18 0 18 ...
 $ gap.opens      : int   0 2 0 2 0 2 0 3 0 3 ...
 $ q.start        : int   1 199 462 187 462 187 462 187 462 187 ...
 $ q.end          : int  544 439 472 439 472 439 472 439 472 439 ...
 $ s.start        : int   1 671 785 123 250 121 248 121 248 126 ...
 $ s.end          : int  544 913 795 377 260 375 258 375 258 380 ...
 $ evalue         : num   0.0 6.0e-102 6.7 2.0e-100 6.7 ...
 $ bit.score      : num  944 360 21.1 354 21.1 354 21.1 352 21.1 352 ...

```

This example BLAST data is also available as loadable example data.

```
> data(BLASTdata)
```

The loaded `data.frame` can be used to compute similarities between the BLASTed sequences via function `simMatrix`. This function includes the following steps:

1. the length of each sequence (LS) comprised in the input data file is extracted.
2. if there is more than one comparison for one sequence including different parts of the respective sequence, that one with maximum base length is chosen.
3. the number of matching bases (mB) is calculated by multiplying two variables given in the BLAST output: the identity between sequences (%) and the number of nucleotides divided by 100.
4. the resulting value is rounded to the next integer.
5. the similarity is calculated by dividing mB by LS and saved in the corresponding similarity matrix.

If the similarity of a combination is not shown in the BLAST report file (because the similarity was lower than 70%), this comparison is included in the similarity matrix with the result zero.

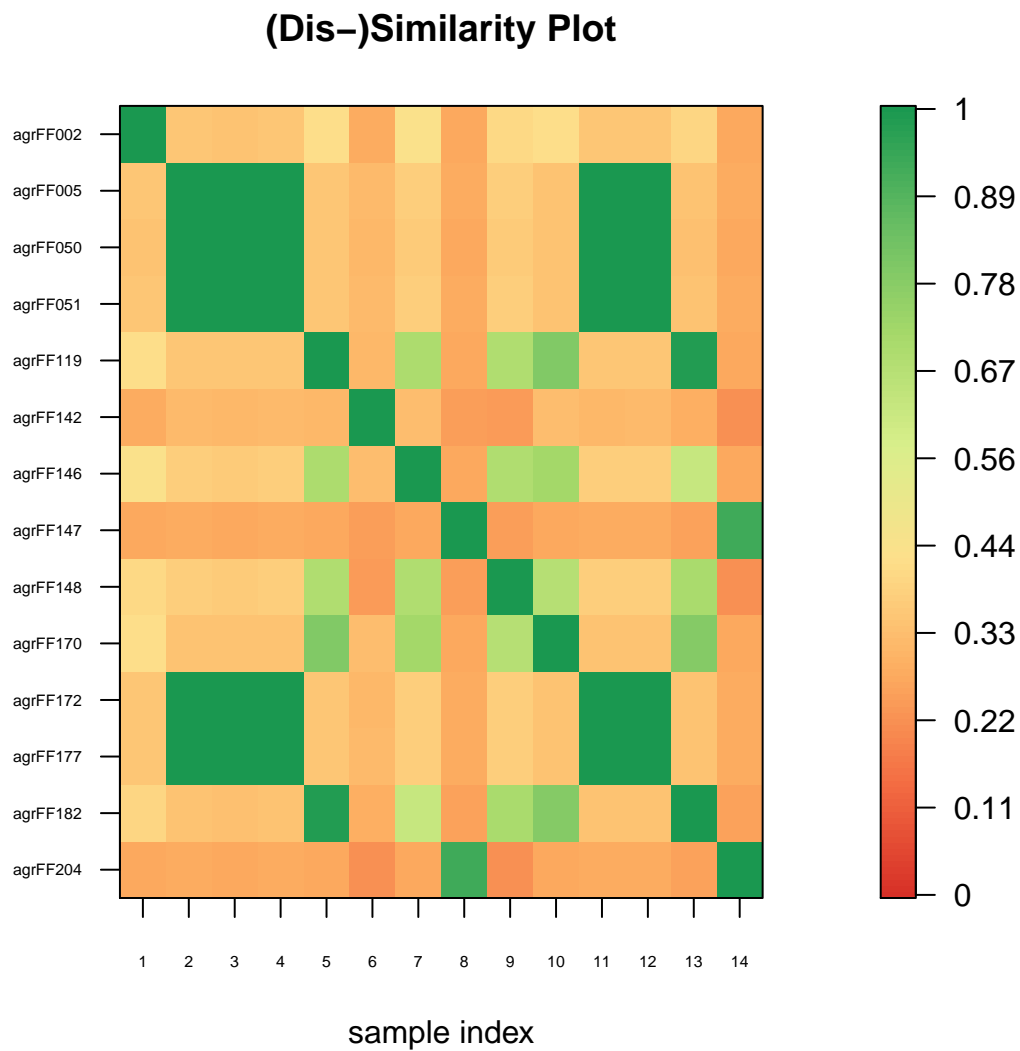
```
> res <- simMatrix(BLASTdata)
```

Optionally, the range of sequence length can be specified to exclude sequences which were too short or too long, respectively.

```
> res1 <- simMatrix(BLASTdata, sequence.range = TRUE, Min = 100, Max = 450)
> res2 <- simMatrix(BLASTdata, sequence.range = TRUE, Min = 500)
```

We visualize the similarity matrix ...

```
> library(MKmisc)
> simPlot(res2, col = myCol, minVal = 0, cex.axis = 0.5,
+         labels = colnames(res2), title = "(Dis-)Similarity Plot")
```



Alternatively, ...

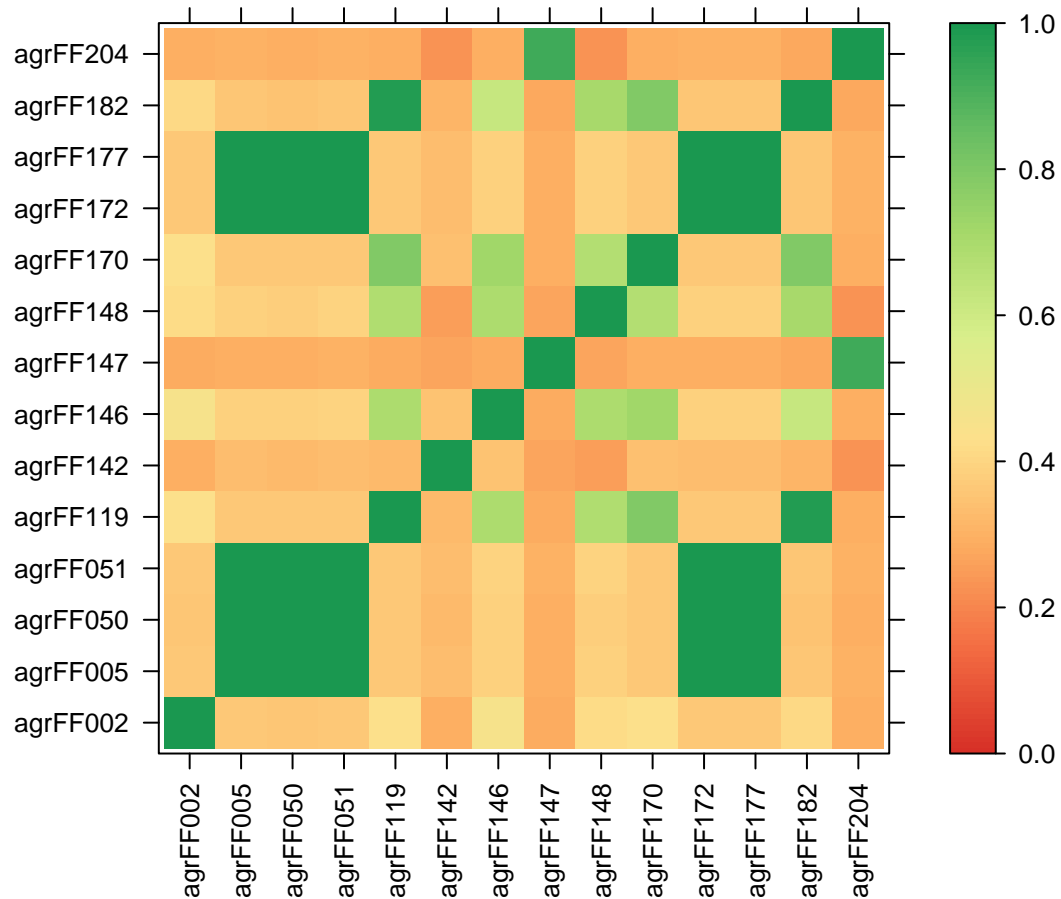
```

> library(lattice)
> txt <- trellis.par.get("add.text")
> txt$cex <- 0.5
> trellis.par.set("add.text" = txt)
> myCol <- colorRampPalette(brewer.pal(8, "RdYlGn"))(128)

> print(levelplot(res2, col.regions = myCol,
+               at = do.breaks(c(0, max(res2)), 128),
+               xlab = "", ylab = "",
+               ## Rotate labels of x axis
+               scales = list(x = list(rot = 90)),
+               main = "(Dis-)Similarity Plot"))

```

## (Dis-)Similarity Plot

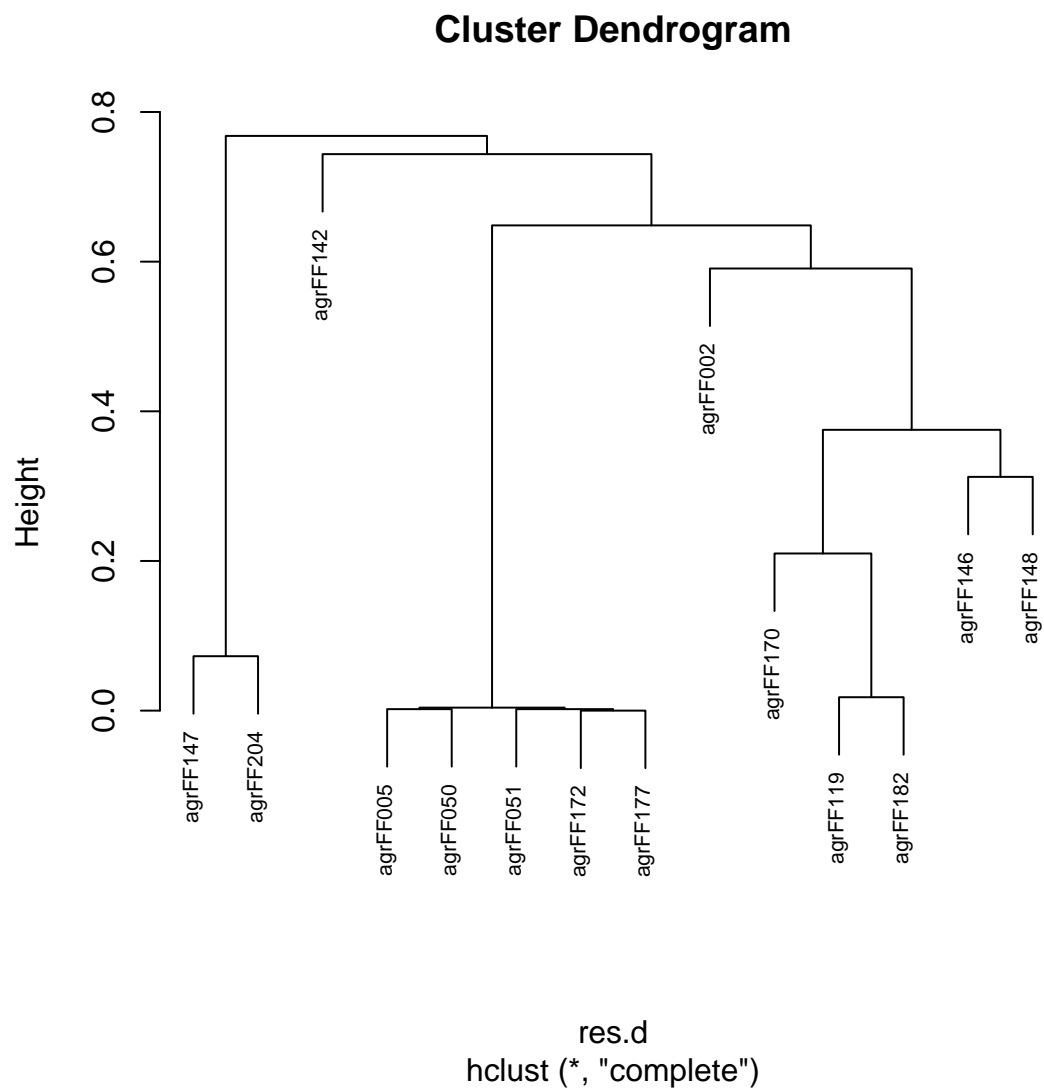


We can also convert the similarity matrix to an object of S3 class "dist".

```
> res.d <- sim2dist(res2)
```

After the conversion we can for instance perform hierarchical clustering ...

```
> ## hierarchical clustering
> plot(hclust(res.d), cex = 0.7)
```



## References

- [1] Poussier, Stephane; Trigalet-Demery, Danielle; Vandewalle, Peggy; Goffinet, Bruno; Luisetti, Jacques; Trigalet, Andre. Genetic diversity of *Ralstonia solanacearum* as assessed by PCR-RFLP of the *hrp* gene region, AFLP and 16S rRNA sequence analysis, and identification of an African subdivision. *Microbiology* 2000 146:1679-1692
- [2] Matsumoto, Masaru; Furuya, Naruto; Takanami, Yoichi; Matsuyama, Nobuaki. RFLP



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