

12.PCA_Heatmap_field_lab_FACS

Fay

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Load libraries

```
library(tidyverse)
```

```
## Warning: package 'tidyverse' was built under R version 4.2.1
```

```
## -- Attaching packages ----- tidyverse 1.3.2 --
```

```
## v ggplot2 3.3.6      v purrr   0.3.5
## v tibble  3.1.8      v dplyr  1.0.10
## v tidyr   1.2.1      v stringr 1.4.1
## v readr   2.1.3      v forcats 0.5.2
```

```
## Warning: package 'tibble' was built under R version 4.2.1
```

```
## Warning: package 'tidyr' was built under R version 4.2.1
```

```
## Warning: package 'readr' was built under R version 4.2.1
```

```
## Warning: package 'purrr' was built under R version 4.2.1
```

```
## Warning: package 'dplyr' was built under R version 4.2.1
```

```
## Warning: package 'stringr' was built under R version 4.2.1
```

```
## Warning: package 'forcats' was built under R version 4.2.1
```

```
## -- Conflicts ----- tidyverse_conflicts() --
```

```
## x dplyr::filter() masks stats::filter()
```

```
## x dplyr::lag()     masks stats::lag()
```

```
library(dplyr)
library(stringr)
library(FactoMineR)
```

```
## Warning: package 'FactoMineR' was built under R version 4.2.1
```

```
library(reshape2)
```

```
##  
## Attaching package: 'reshape2'  
##  
## The following object is masked from 'package:tidyr':  
##  
## smiths
```

```
library(corrplot)
```

```
## corrplot 0.92 loaded
```

```
library(factoextra)
```

```
## Welcome! Want to learn more? See two factoextra-related books at https://goo.gl/ve3WBa
```

```
library(lmtest)
```

```
## Loading required package: zoo
```

```
## Warning: package 'zoo' was built under R version 4.2.1
```

```
##  
## Attaching package: 'zoo'  
##  
## The following objects are masked from 'package:base':  
##  
## as.Date, as.Date.numeric
```

```
library(ggpubr)  
library(janitor)
```

```
##  
## Attaching package: 'janitor'  
##  
## The following objects are masked from 'package:stats':  
##  
## chisq.test, fisher.test
```

```
library(pheatmap)  
library(visdat)
```

Load data

```
hm <- read.csv("output_data/imputed_mice.csv")
```

vectors for selecting

```
Facs_lab <- c("CD4", "Treg", "Div_Treg", "Treg17", "Th1",
             "Div_Th1", "Th17", "Div_Th17", "CD8", "Act_CD8",
             "Div_Act_CD8", "IFNy_CD4", "IFNy_CD8", "Treg_prop",
             "IL17A_CD4")

Facs_wild <- c("Treg", "CD4", "Treg17", "Th1", "Th17", "CD8",
             "Act_CD8", "IFNy_CD4", "IFNy_CD8") #"IL17A_CD4",
```

PCA on the facs *-imputed*

```
#####field
# somehow the field samples have the origin na,
# fix that
hm$origin[is.na(hm$origin)] <- "Field"

field <- hm %>%
  dplyr::filter(origin == "Field")

field <- unique(field)

#make a factor out of the melting curves (important for later visualization)
field <- field %>%
  dplyr::mutate(MC.Eimeria = as.factor(MC.Eimeria))

facs_mouse <- field %>%
  dplyr::select(c(Mouse_ID, all_of(Facs_wild)))

facs <- facs_mouse[,-1]

#remove rows with only nas
facs <- facs[,colSums(is.na(facs))<nrow(facs)]

#remove columns with only nas
facs <- facs[rowSums(is.na(facs)) != ncol(facs), ]

##select same rows in the first table
field <- field[row.names(facs), ]

#####lab
#select the facs and lab mice
```

```

lab <- hm %>%
  dplyr::filter(origin == "Lab", Position == "mLN") #selecting for mln to avoid
# duplicates

lab <- unique(lab)

facs_mouse <- lab %>%
  dplyr::select(c(Mouse_ID, all_of(Facs_wild))) #choosing the same with the wild

facs_mouse <- unique(facs_mouse)

facs_lab <- facs_mouse[, -1]

#remove rows with only nas
facs_lab <- facs_lab[, colSums(is.na(facs_lab)) < nrow(facs_lab)]

#remove columns with only nas
facs_lab <- facs_lab[rowSums(is.na(facs_lab)) != ncol(facs_lab), ]

#select same rows in the first table
facs_mouse <- facs_mouse[row.names(facs_lab), ]

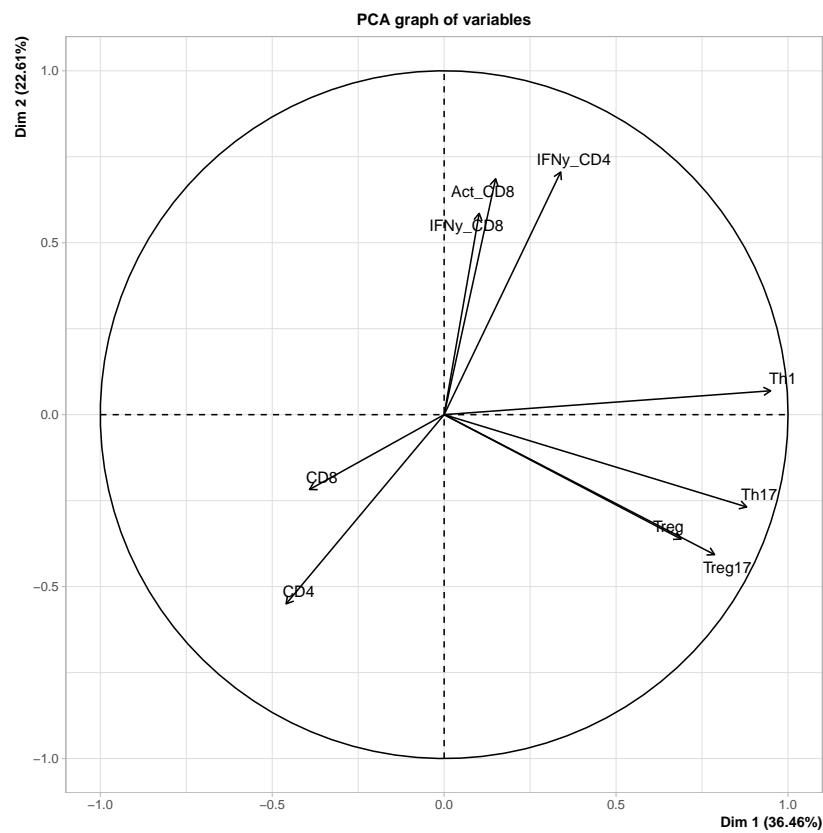
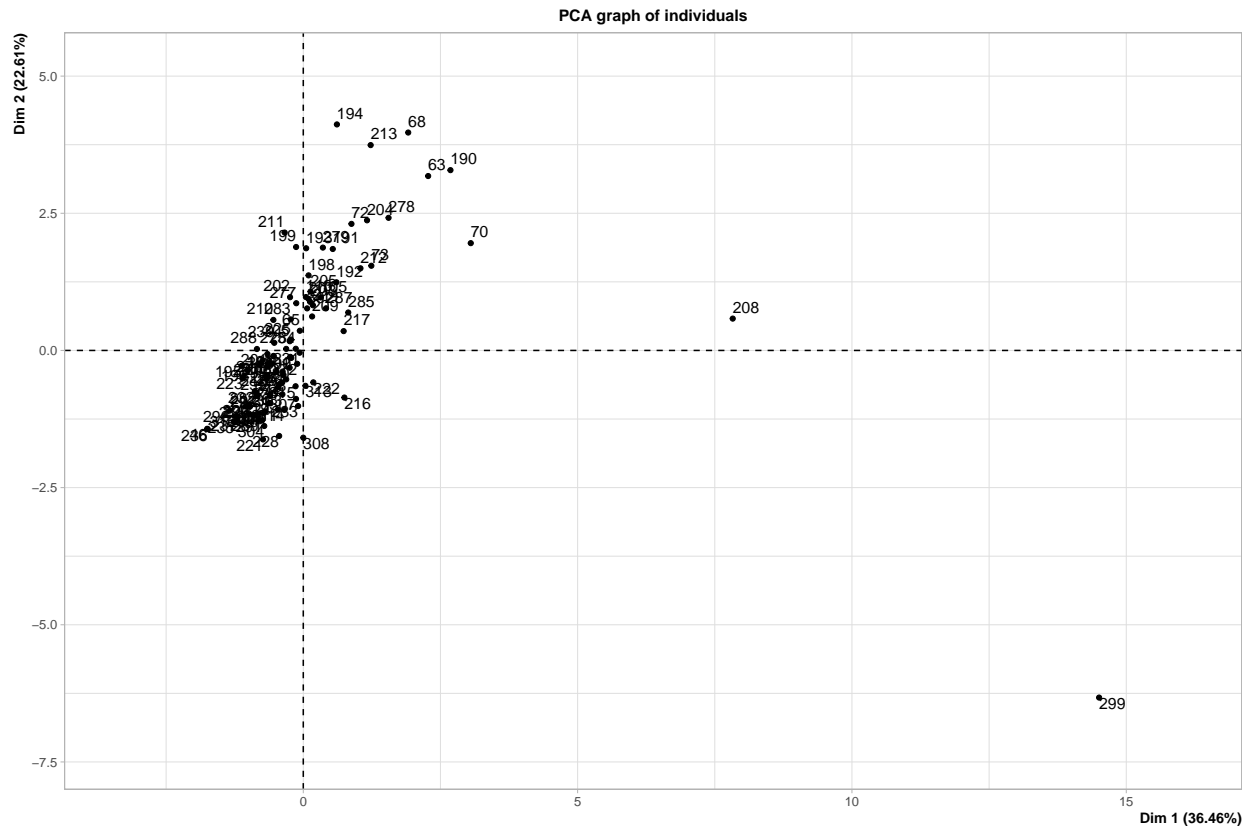
##select same rows in the first table
lab <- lab[row.names(facs_mouse), ]

hm_facs <- rbind(facs_lab, facs)

hm_selection_g <- rbind(lab, field)

# we can now run a normal pca on the complete data set
res.pca <- PCA(hm_facs)

```



Caution: When imputing data, the percentages of inertia associated with the first dimensions will be over-

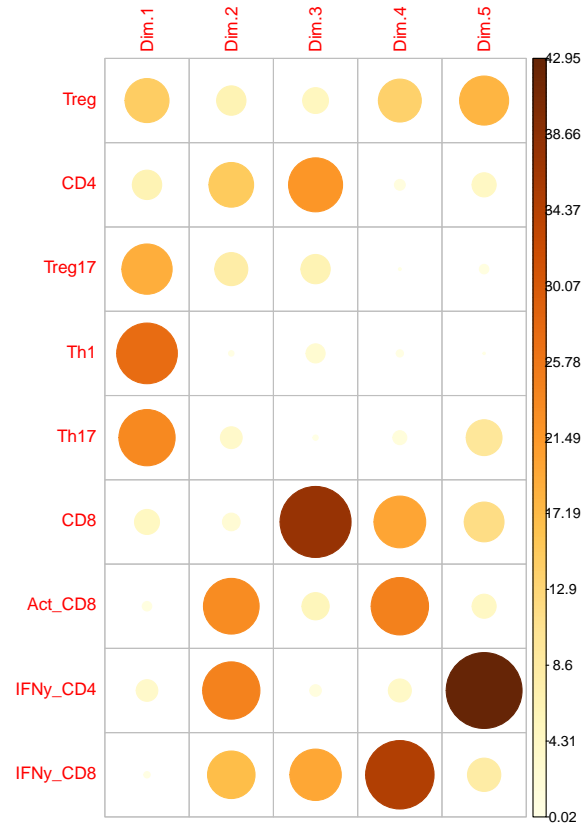
estimated.

Another problem: the imputed data are, when the pca is performed considered like real observations. But they are estimations!!

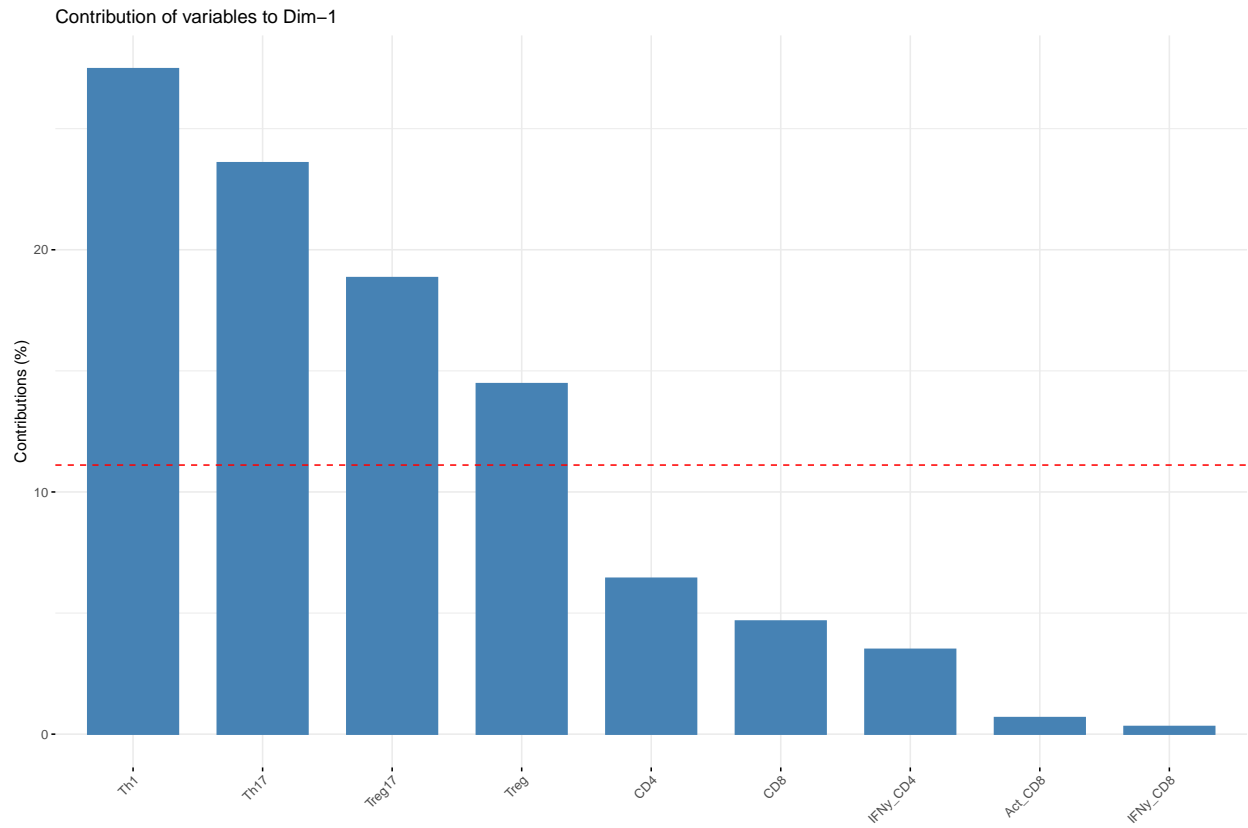
Visualizing uncertainty due to missing data:

→ multiple imputation: generate several plausible values for each missing data point

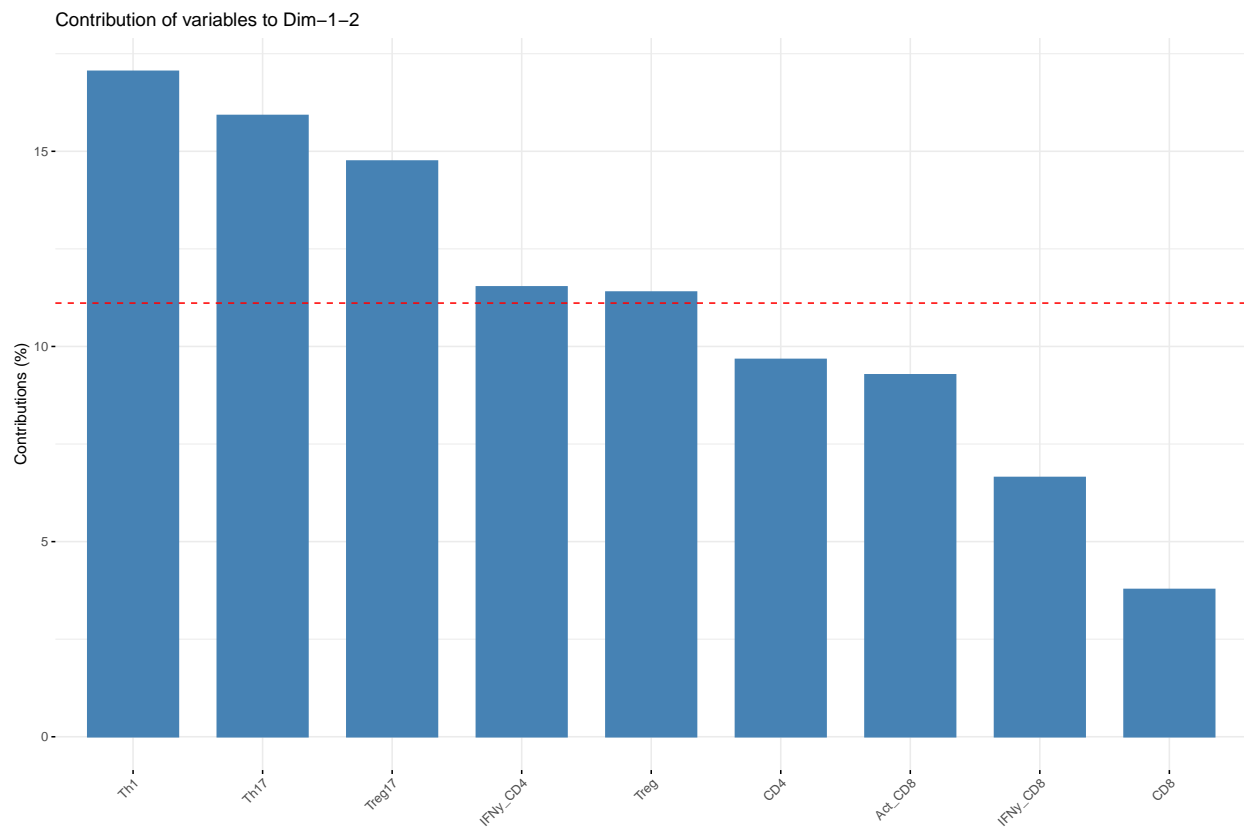
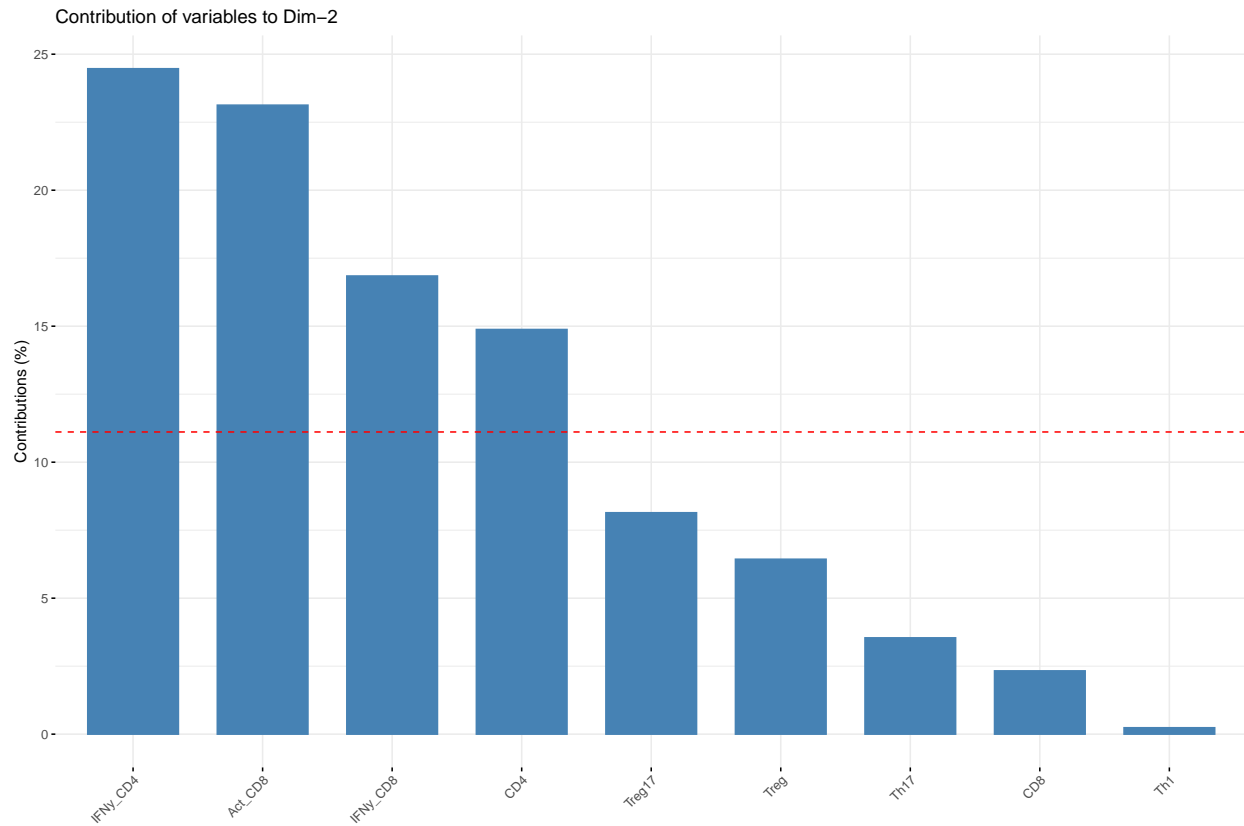
We here visualize the variability, that is uncertainty on the plane defined by two pca axes.



The function `fviz_contrib()` [factoextra package] can be used to draw a bar plot of variable contributions. If your data contains many variables, you can decide to show only the top contributing variables. The R code below shows the top 10 variables contributing to the principal components:



```
# Contributions of variables to PC2  
fviz_contrib(res.pca, choice = "var", axes = 2, top = 18)
```

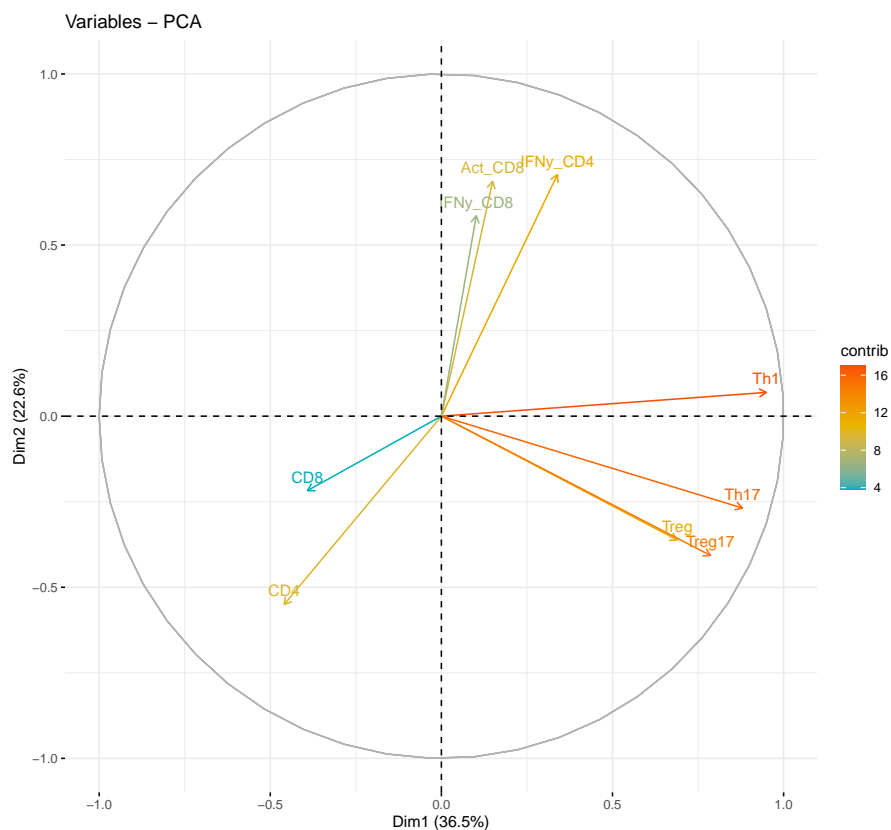


The red dashed line on the graph above indicates the expected average contribution. If the contribution of

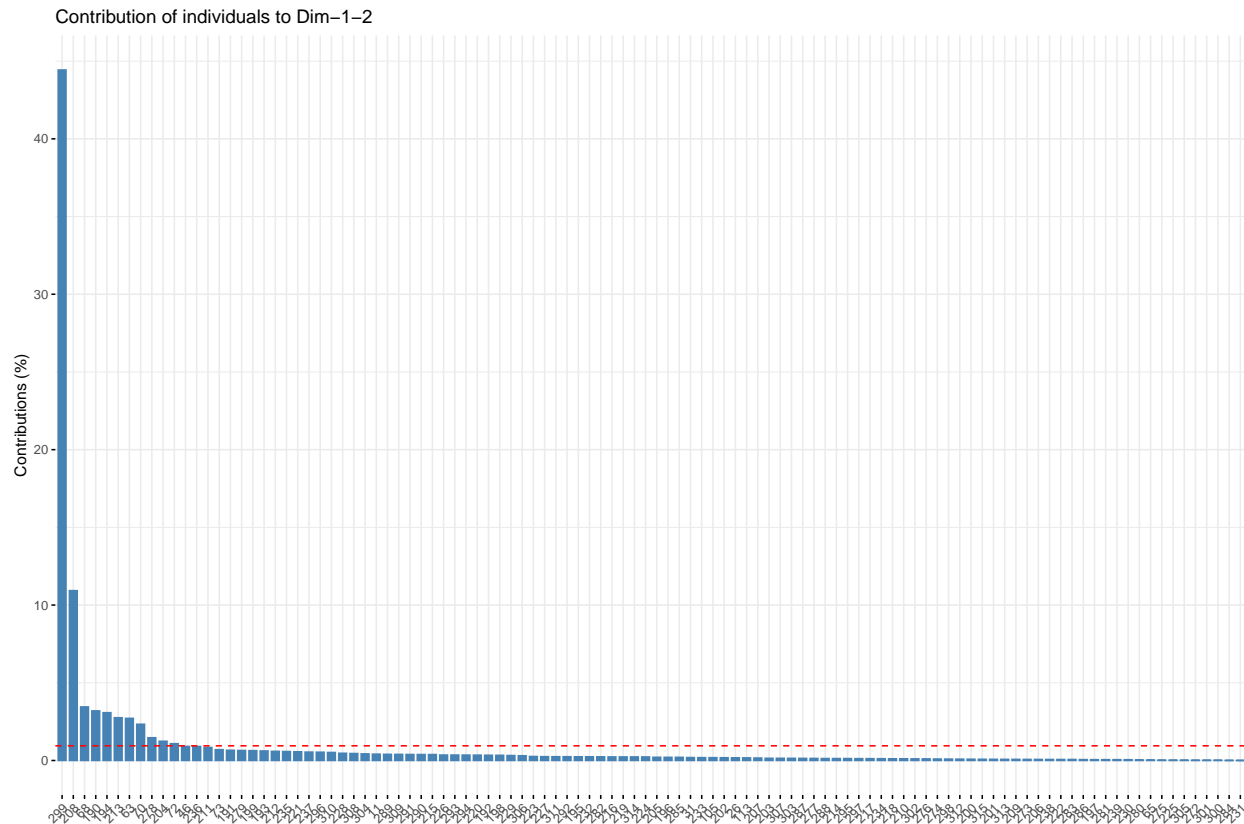
the variables were uniform, the expected value would be $1/\text{length}(\text{variables}) = 1/10 = 10\%$. For a given component, a variable with a contribution larger than this cutoff could be considered as important in contributing to the component.

Note that, the total contribution of a given variable, on explaining the variations retained by two principal components, say PC1 and PC2, is calculated as $\text{contrib} = [(C1 * \text{Eig1}) + (C2 * \text{Eig2})]/(\text{Eig1} + \text{Eig2})$, where

C1 and C2 are the contributions of the variable on PC1 and PC2, respectively Eig1 and Eig2 are the eigenvalues of PC1 and PC2, respectively. Recall that eigenvalues measure the amount of variation retained by each PC. In this case, the expected average contribution (cutoff) is calculated as follow: As mentioned above, if the contributions of the 10 variables were uniform, the expected average contribution on a given PC would be $1/10 = 10\%$. The expected average contribution of a variable for PC1 and PC2 is : $[(10 * \text{Eig1}) + (10 * \text{Eig2})]/(\text{Eig1} + \text{Eig2})$

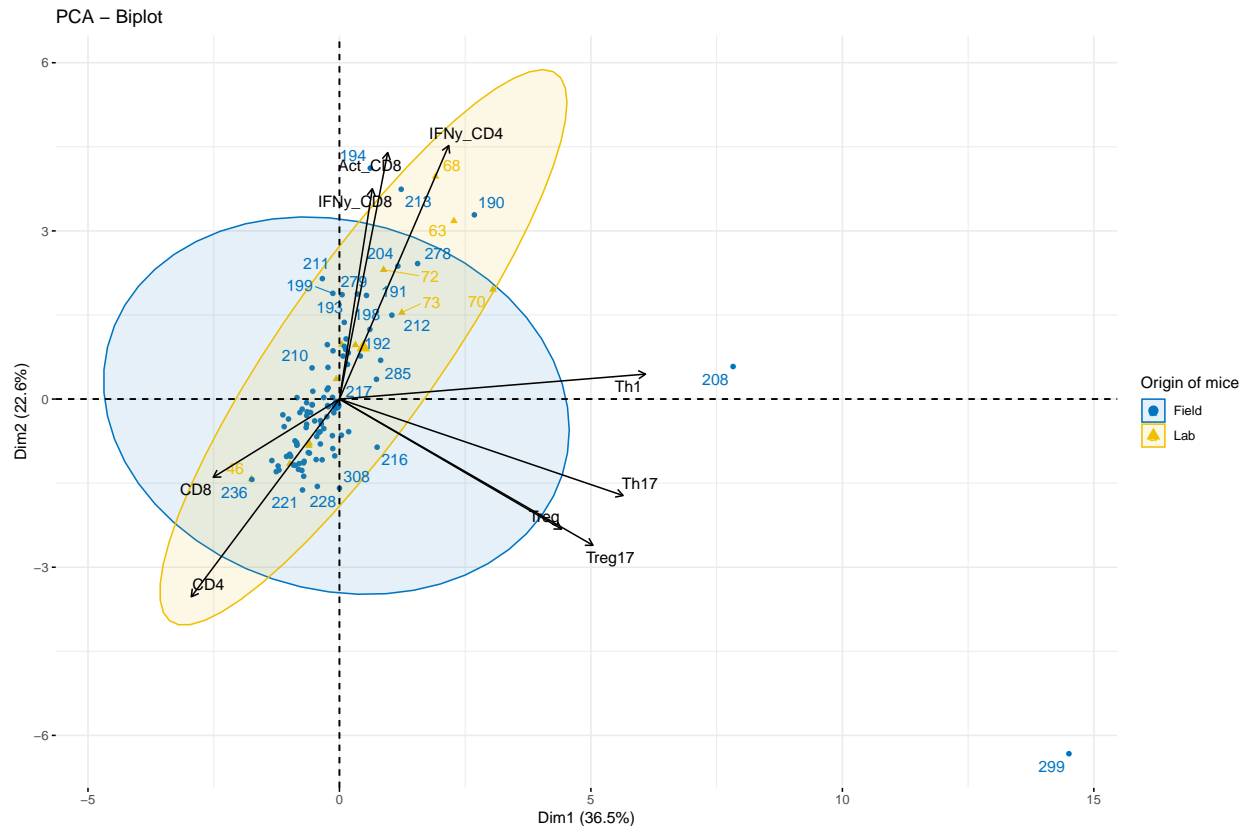


To visualize the contribution of individuals to the first two principal components:



PCA + Biplot combination

```
## Warning: ggrepel: 77 unlabeled data points (too many overlaps). Consider  
## increasing max.overlaps
```



In the following example, we want to color both individuals and variables by groups. The trick is to use `pointshape = 21` for individual points. This particular point shape can be filled by a color using the argument `fill.ind`. The border line color of individual points is set to “black” using `col.ind`. To color variable by groups, the argument `col.var` will be used.

repeating the heatmap on the now imputed data

```
mouse_id <- hm_selection_g %>%
  dplyr::select(Mouse_ID)

hm_facs <- cbind(mouse_id, hm_facs)

# turn the data frame into a matrix and transpose it. We want to have each cell
# type as a row name
hm_facs <- t(as.matrix(hm_facs))

# turn the first row into column names
hm_facs %>%
  row_to_names(row_number = 1) -> heatmap_data

heatmap_data <- as.data.frame(heatmap_data)

table(rowSums(is.na(heatmap_data)) == nrow(heatmap_data))
```

```
##
```

```
## FALSE
##      9

# turn the columns to numeric other wise the heatmap function will not work
heatmap_data[] <- lapply(heatmap_data, function(x) as.numeric(as.character(x)))

# remove columns with only NAs
heatmap_data <- Filter(function(x)!all(is.na(x)), heatmap_data)

#remove rows with only NAs
heatmap_data <- heatmap_data[, colSums(is.na(heatmap_data)) !=
                                nrow(heatmap_data)]

#Prepare the annotation data frame
annotation_df <- as_tibble(hm_selection_g) %>%
  dplyr::select(c("Mouse_ID", "origin"))

annotation_df <- unique(annotation_df)

annotation_df <- as.data.frame(annotation_df)

### Prepare the annotation columns for the heatmap

rownames(annotation_df) <- annotation_df$Mouse_ID

# Match the row names to the heatmap data frame
rownames(annotation_df) <- colnames(heatmap_data)

#remove the unnecessary column
annotation_df <- annotation_df %>% dplyr::select(-Mouse_ID, )
```

Heatmap on gene expression data:

