

Gastric Cancer Microbiome

```
library(tidyverse)
library(reshape2)
library(knitr)
library(kableExtra)
library(venn)
library(ggsignif)
library(dunn.test)
```

score calculation

```
calc_score = function (tab){
  tab[tab == 0] = NA
  pos = sapply(tab, FUN = as.numeric)
  for (i in 1:ncol(tab)){
    o = order(tab[,i], decreasing = T, na.last = NA)
    names(o) = row.names(tab)[o]
    pos[o,i] = 1:length(o)
  }
  k = !is.na(pos)
  k = rowSums(k)
  freq = k/ncol(tab)
  pos[is.na(pos)] = nrow(pos)
  medianas = apply(pos, 1, FUN = median, na.rm = T)
  media = rowMeans(pos, na.rm = T)
  score = media * (1.1 - freq)
  sc = data.frame(score=score, freq=freq, row.names = row.names(tab), stringsAsFactors = F)
  return(sc)
}
```

Reading microbiome data, treating table, calculating score

```
tabela <- read.delim ("genusclosed.txt", sep = '\t', header = T, stringsAsFactors=F, row.names = 1)

samples = data.frame(sample = colnames(tabela), condition = unlist(tabela[1,]), stringsAsFactors = F)
samples$condition[samples$condition == "peri"] = "ADJ"

tabela = tabela[2:nrow(tabela),]
m = sapply(tabela, as.numeric)
m[m < 20] = 0

tabela[,] = as.data.frame(m[,])

tabela.m = data.frame(genus = character(), score = numeric(), freq = numeric(), sample = character(), counts = numeric(), condition = character(), relative = NULL, stringsAsFactors = F)
for (i in unique(samples$condition)){
  a = select(as.data.frame(tabela), samples$sample[samples$condition == i])
  t = as.data.frame(prop.table(sapply(a, as.numeric)), margin = 2)
  b = t
  b.m = melt(b)
  sc = calc_score(a)
  a$score = sc$score
  a$freq = sc$freq
  a$genus <- row.names(a)
  a.m <- melt(a, id.vars = c("genus", "score", "freq"))
  a.m$condition <- i
  colnames(a.m) <- c("genus", "score", "freq", "sample", "counts", "condition")
  a.m$relative = b.m$value
  tabela.m = rbind(tabela.m, a.m)
}
```

Creating top15 bacteria table by score

```

top15 <- filter(tabela.m) %>%
  group_by(condition, genus, score, freq) %>%
  summarize(abundance = sum(relative, na.rm = T)) %>%
  arrange(condition, score) %>%
  group_by(condition) %>%
  slice_min(n = 15, order_by = score, with_ties = F)

df = data.frame(position = 1:15, HC = NA, FD = NA, CG = NA, IM= NA, IN= NA, GC= NA, ADJ= NA )
df.allvalues = df

for (i in unique(top15$condition)){
  a = filter(top15, condition == i)
  a[,3:5] = format(as.data.frame(a[,3:5]), digits = 2, nsmall=2)
  a$valor = ""
  for (j in 1:nrow(a)){
    a$valor[j] = paste(c(a$genus[j], a$score[j], a$freq[j], a$abundance[j]), collapse = "|")
  }
  df[,i] = a$genus
  df.allvalues[,i] = a$valor
}

kable(df, caption = "Top 15 bacterias by Score") %>% kable_styling()

```

Top 15 bacterias by Score

position	HC	FD	CG	IM	IN	GC	ADJ
1	Prevotella	Methylobacterium	Helicobacter	Lactobacillus	Halomonas	Helicobacter	Helicobacter
2	Agrobacterium	Sphingomonas	Prevotella	Sphingomonas	Shewanella	Prevotella	Prevotella
3	Sphingobium	Streptococcus	Lactobacillus	Halomonas	Prevotella	Streptococcus	Sphingomonas
4	Helicobacter	Helicobacter	Streptococcus	Helicobacter	Neisseria	Halomonas	Halomonas
5	Porphyromonas	Prevotella	Pseudomonas	Prevotella	Lactobacillus	Pseudomonas	Pseudomonas
6	Pseudomonas	Acinetobacter	Sphingomonas	Bradyrhizobium	Helicobacter	Sphingomonas	Bacteroides
7	Halomonas	Enhydrobacter	Halomonas	Neisseria	Rothia	Lactobacillus	Streptococcus
8	Neisseria	Sediminibacterium	Neisseria	Vibrio	Haemophilus	Shewanella	Acinetobacter
9	Zoogloea	Fusobacterium	Porphyromonas	Pseudomonas	Bacillus	Acinetobacter	Shewanella
10	Shewanella	Corynebacterium	Bacillus	Porphyromonas	Actinobacillus	Corynebacterium	Faecalibacterium
11	Rhodobacter	Bacteroides	Acinetobacter	Shewanella	Actinomyces	Bacillus	Bifidobacterium
12	Vogesella	Lactobacillus	Haemophilus	Haemophilus	Sphingomonas	Neisseria	Chryseobacterium
13	Sphingomonas	Veillonella	Bradyrhizobium	Actinobacillus	Aggregatibacter	Leptotrichia	Bacillus
14	Erythromicrobium	Brevibacterium	[Prevotella]	Aggregatibacter	Granulicatella	Veillonella	Oscillospira
15	HB2-32-21	Chryseobacterium	Corynebacterium	Rothia	Porphyromonas	Bacteroides	Veillonella

```

kable(df.allvalues[,1:4], caption = "Top 15 bacterias by Score: genus|score|frequency|abundance")

```

Top 15 bacterias by Score: genus|score|frequency|abundance

position	HC	FD	CG
1	Prevotella 61.27 0.78 0.0487	Methylobacterium 6.76 0.95 0.0930	Helicobacter 150.73 0.63 0.2782
2	Agrobacterium 127.73 0.66 0.0180	Sphingomonas 32.89 0.85 0.0497	Prevotella 321.65 0.44 0.0148
3	Sphingobium 150.07 0.63 0.0402	Streptococcus 136.60 0.65 0.0202	Lactobacillus 394.16 0.37 0.0147
4	Helicobacter 174.94 0.60 0.4234	Helicobacter 172.38 0.60 0.6064	Streptococcus 397.98 0.37 0.0115
5	Porphyromonas 251.85 0.51 0.0060	Prevotella 213.51 0.55 0.0245	Pseudomonas 442.53 0.33 0.0426
6	Pseudomonas 286.51 0.47 0.0525	Acinetobacter 308.65 0.45 0.0071	Sphingomonas 485.19 0.30 0.0153
7	Halomonas 286.57 0.47 0.0322	Enhydrobacter 419.14 0.35 0.0068	Halomonas 518.98 0.27 0.0361

```

8Neisseria|287.08|0.47|0.0389 Sediminibacterium|419.33|0.35|0.0167Neisseria|570.01|0.23|0.0121
9Zoogloea|287.72|0.47|0.0223 Fusobacterium|481.56|0.30|0.0052 Porphyromonas|570.10|0.23|0.0026
10Shewanella|287.90|0.47|0.0145 Corynebacterium|481.68|0.30|0.0013 Bacillus|582.41|0.23|0.0052
11Rhodobacter|288.30|0.47|0.0151 Bacteroides|548.04|0.25|0.0028 Acinetobacter|592.90|0.22|0.0185
12Vogesella|314.91|0.45|0.0122 Lactobacillus|617.22|0.20|0.0095 Haemophilus|623.49|0.20|0.0032
13Sphingomonas|332.77|0.43|0.0077 Veillonella|617.22|0.20|0.0131 Bradyrhizobium|631.90|0.19|0.0032
14Erythromicrobium|334.13|0.43|0.0058Brevibacterium|617.58|0.20|0.0033 [Prevotella]|656.78|0.17|0.0026
15HB2-32-21|352.17|0.41|0.0057 Chryseobacterium|617.58|0.20|0.0033 Corynebacterium|661.35|0.17|0.0067

```

```
kable(df.allvalues[,5:8])
```

IM	IN	GC	ADJ
Lactobacillus 0.93 1.00 0.0123	Halomonas 0.20 1.00 0.2059	Helicobacter 132.21 0.66 0.2880	Helicobacter 150.86 0.63 0.5477
Sphingomonas 1.87 0.99 0.0293	Shewanella 0.41 1.00 0.0669	Prevotella 183.60 0.59 0.0422	Prevotella 196.28 0.57 0.0379
Halomonas 2.20 0.99 0.0727	Prevotella 1.82 0.99 0.0835	Streptococcus 203.19 0.56 0.0231	Sphingomonas 314.69 0.44 0.0218
Helicobacter 23.13 0.88 0.2660	Neisseria 3.43 0.97 0.1010	Halomonas 238.81 0.52 0.0324	Halomonas 343.32 0.42 0.0309
Prevotella 28.42 0.87 0.0131	Lactobacillus 3.99 0.97 0.0247	Pseudomonas 298.57 0.46 0.0351	Pseudomonas 360.35 0.40 0.0170
Bradyrhizobium 29.08 0.87 0.0052	Helicobacter 8.04 0.95 0.2812	Sphingomonas 343.12 0.42 0.0122	Bacteroides 367.92 0.39 0.0306
Neisseria 49.61 0.81 0.0143	Rothia 8.25 0.95 0.0243	Lactobacillus 345.89 0.41 0.0428	Streptococcus 438.32 0.33 0.0166
Vibrio 79.67 0.75 0.0027	Haemophilus 8.70 0.95 0.0189	Shewanella 366.99 0.40 0.0100	Acinetobacter 452.72 0.32 0.0093
Pseudomonas 91.29 0.73 0.0073	Bacillus 12.74 0.93 0.0048	Acinetobacter 372.16 0.39 0.0212	Shewanella 456.55 0.32 0.0129
Porphyromonas 92.95 0.73 0.0024	Actinobacillus 14.06 0.92 0.0300	Corynebacterium 447.03 0.33 0.0073	Faecalibacterium 461.04 0.32 0.0165
Shewanella 125.36 0.67 0.0217	Actinomyces 18.52 0.91 0.0090	Bacillus 461.49 0.32 0.0254	Bifidobacterium 583.03 0.22 0.0063
Haemophilus 137.15 0.65 0.0026	Sphingomonas 26.12 0.88 0.0074	Neisseria 479.80 0.30 0.0111	Chryseobacterium 593.67 0.22 0.0048
Actinobacillus 143.02 0.64 0.0069	Aggregatibacter 30.54 0.87 0.0057	Leptotrichia 527.23 0.27 0.0071	Bacillus 598.07 0.21 0.0086
Aggregatibacter 189.23 0.58 0.0020	Granulicatella 30.82 0.87 0.0052	Veillonella 539.87 0.26 0.0069	Oscillospira 619.87 0.20 0.0081
Rothia 200.49 0.57 0.0012	Porphyromonas 38.12 0.84 0.0117	Bacteroides 552.23 0.25 0.0059	Veillonella 650.39 0.18 0.0045

```
write.table(x = df, file = "top15.bac.score.txt", quote = F, sep = "\t", row.names = T, col.names = T)
```

```
write.table(x = df.allvalues, file = "top15.bac.score.freq.abund.txt", quote = F, sep = "\t", row.names = T, col.names = T)
```

top 15 frequency and score - long table

```

tabela.m %>%
  group_by(condition, genus, score, freq) %>%
  summarize(abundance = sum(relative, na.rm = T)) %>%
  arrange(condition, score) %>%
  group_by(condition) %>%
  slice_min(n = 15, order_by = score, with_ties = F) %>%
  kable(digits = 2)

```

```
## `summarise()` regrouping output by 'condition', 'genus', 'score' (override with `.groups` argument)
```

condition	genus	score	freq	abundance
ADJ	Helicobacter	150.86	0.63	0.55
ADJ	Prevotella	196.28	0.57	0.04
ADJ	Sphingomonas	314.69	0.44	0.02
ADJ	Halomonas	343.32	0.42	0.03
ADJ	Pseudomonas	360.35	0.40	0.02
ADJ	Bacteroides	367.92	0.39	0.03
ADJ	Streptococcus	438.32	0.33	0.02
ADJ	Acinetobacter	452.72	0.32	0.01
ADJ	Shewanella	456.55	0.32	0.01
ADJ	Faecalibacterium	461.04	0.32	0.02
ADJ	Bifidobacterium	583.03	0.22	0.01
ADJ	Chryseobacterium	593.67	0.22	0.00
ADJ	Bacillus	598.07	0.21	0.01
ADJ	Oscillospira	619.87	0.20	0.01
ADJ	Veillonella	650.39	0.18	0.00
CG	Helicobacter	150.73	0.63	0.28
CG	Prevotella	321.65	0.44	0.01
CG	Lactobacillus	394.16	0.37	0.01
CG	Streptococcus	397.98	0.37	0.01
CG	Pseudomonas	442.53	0.33	0.04
CG	Sphingomonas	485.19	0.30	0.02

CG	Halomonas	518.980.27	0.04
CG	Neisseria	570.010.23	0.01
CG	Porphyromonas	570.100.23	0.00
CG	Bacillus	582.410.23	0.01
CG	Acinetobacter	592.900.22	0.02
CG	Haemophilus	623.490.20	0.00
CG	Bradyrhizobium	631.900.19	0.00
CG	[Prevotella]	656.780.17	0.00
CG	Corynebacterium	661.350.17	0.01
FD	Methylobacterium	6.760.95	0.09
FD	Sphingomonas	32.890.85	0.05
FD	Streptococcus	136.600.65	0.02
FD	Helicobacter	172.380.60	0.61
FD	Prevotella	213.510.55	0.02
FD	Acinetobacter	308.650.45	0.01
FD	Enhydrobacter	419.140.35	0.01
FD	Sediminibacterium	419.330.35	0.02
FD	Fusobacterium	481.560.30	0.01
FD	Corynebacterium	481.680.30	0.00
FD	Bacteroides	548.040.25	0.00
FD	Lactobacillus	617.220.20	0.01
FD	Veillonella	617.220.20	0.01
FD	Brevibacterium	617.580.20	0.00
FD	Chryseobacterium	617.580.20	0.00
GC	Helicobacter	132.210.66	0.29
GC	Prevotella	183.600.59	0.04
GC	Streptococcus	203.190.56	0.02
GC	Halomonas	238.810.52	0.03
GC	Pseudomonas	298.570.46	0.04
GC	Sphingomonas	343.120.42	0.01
GC	Lactobacillus	345.890.41	0.04
GC	Shewanella	366.990.40	0.01
GC	Acinetobacter	372.160.39	0.02
GC	Corynebacterium	447.030.33	0.01
GC	Bacillus	461.490.32	0.03
GC	Neisseria	479.800.30	0.01
GC	Leptotrichia	527.230.27	0.01
GC	Veillonella	539.870.26	0.01
GC	Bacteroides	552.230.25	0.01
HC	Prevotella	61.270.78	0.05
HC	Agrobacterium	127.730.66	0.02
HC	Sphingobium	150.070.63	0.04
HC	Helicobacter	174.940.60	0.42
HC	Porphyromonas	251.850.51	0.01
HC	Pseudomonas	286.510.47	0.05
HC	Halomonas	286.570.47	0.03
HC	Neisseria	287.080.47	0.04
HC	Zoogloea	287.720.47	0.02
HC	Shewanella	287.900.47	0.01
HC	Rhodobacter	288.300.47	0.02
HC	Vogesella	314.910.45	0.01
HC	Sphingomonas	332.770.43	0.01
HC	Erythromicrobium	334.130.43	0.01
HC	HB2-32-21	352.170.41	0.01
IM	Lactobacillus	0.931.00	0.01
IM	Sphingomonas	1.870.99	0.03
IM	Halomonas	2.200.99	0.07
IM	Helicobacter	23.130.88	0.27
IM	Prevotella	28.420.87	0.01
IM	Bradyrhizobium	29.080.87	0.01
IM	Neisseria	49.610.81	0.01
IM	Vibrio	79.670.75	0.00
IM	Pseudomonas	91.290.73	0.01
IM	Porphyromonas	92.950.73	0.00
IM	Shewanella	125.360.67	0.02
IM	Haemophilus	137.150.65	0.00

IM	Actinobacillus	143.020.64	0.01
IM	Aggregatibacter	189.230.58	0.00
IM	Rothia	200.490.57	0.00
IN	Halomonas	0.201.00	0.21
IN	Shewanella	0.411.00	0.07
IN	Prevotella	1.820.99	0.08
IN	Neisseria	3.430.97	0.10
IN	Lactobacillus	3.990.97	0.02
IN	Helicobacter	8.040.95	0.28
IN	Rothia	8.250.95	0.02
IN	Haemophilus	8.700.95	0.02
IN	Bacillus	12.740.93	0.00
IN	Actinobacillus	14.060.92	0.03
IN	Actinomyces	18.520.91	0.01
IN	Sphingomonas	26.120.88	0.01
IN	Aggregatibacter	30.540.87	0.01
IN	Granulicatella	30.820.87	0.01
IN	Porphyromonas	38.120.84	0.01

Creating top15 bacteria table by abundance

```

top15.rel <- filter(tabela.m) %>%
  group_by(condition, genus, score, freq) %>%
  summarize(abundance = sum(relative, na.rm = T)) %>%
  arrange(condition, score) %>%
  group_by(condition) %>%
  slice_max(n = 15, order_by = abundance, with_ties = F)

df.rel = data.frame(position = 1:15, HC = NA, FD = NA, CG = NA, IM= NA, IN= NA, GC= NA, ADJ= NA )
df.allvalues = df.rel

for (i in unique(top15.rel$condition)){
  a = filter(top15.rel, condition == i)
  df.rel[,i] = a$genus
}

kable(df.rel, caption = "Top 15 bacterias por Abundancia") %>% kable_styling()

```

Top 15 bacterias por Abundancia

position	HC	FD	CG	IM	IN	GC	ADJ
1	Helicobacter	Helicobacter	Ochrobactrum	Ochrobactrum	Helicobacter	Helicobacter	Helicobacter
2	Pseudomonas	Methylobacterium	Helicobacter	Helicobacter	Halomonas	Ochrobactrum	Prevotella
3	Prevotella	Virgibacillus	Pseudomonas	Halomonas	Neisseria	Lactobacillus	Halomonas
4	Sphingobium	Sphingomonas	Halomonas	Sphingomonas	Prevotella	Prevotella	Bacteroides
5	Neisseria	Prevotella	Arthrobacter	Arthrobacter	Shewanella	Pseudomonas	Sphingomonas
6	Halomonas	Streptococcus	Acinetobacter	Shewanella	Actinobacillus	Halomonas	Pseudomonas
7	Zoogloea	Sediminibacterium	Sphingomonas	Neisseria	Lactobacillus	Bacillus	Streptococcus
8	Psychrobacter	Halomonas	Prevotella	Acinetobacter	Rothia	Streptococcus	Faecalibacterium
9	Macroccoccus	Veillonella	Lactobacillus	Prevotella	Haemophilus	Acinetobacter	Cupriavidus
10	Agrobacterium	Lactobacillus	Neisseria	Lactobacillus	Porphyromonas	Enhydrobacter	Shewanella
11	Rhodobacter	Acinetobacter	Polymorphum	Polymorphum	Actinomyces	Sphingomonas	Lactobacillus
12	Shewanella	Enhydrobacter	Streptococcus	Tsukamurella	Sphingomonas	Serratia	Acinetobacter
13	Vogesella	Fusobacterium	Shewanella	Salinispora	Staphylococcus	Arthrobacter	Bacillus
14	Dechloromonas	Bradyrhizobium	Tsukamurella	Gordonia	Aggregatibacter	Neisseria	Oscillospira

```
write.table(x = df.rel, file = "top15.bac.abund.txt", quote = F, sep = "\t", row.names = T, col.names = T)
```

Comparing score vs. relative abundance

```
par(mfrow = c(2,4))
for (i in 2:ncol(df)){
  venn(list(score = df[,i], abundance = df.rel[,i]), zcolor = "style", ilcs = 1.3, sncs = 1)
  text(-20, 60, colnames(df)[i], pos = 4, cex = 1.3)
  print(colnames(df)[i])
  print(intersect(df[,i],df.rel[,i]))
}
```

```
## [1] "HC"
## [1] "Prevotella" "Agrobacterium" "Sphingobium" "Helicobacter"
## [5] "Pseudomonas" "Halomonas" "Neisseria" "Zoogloea"
## [9] "Shewanella" "Rhodobacter" "Vogesella"
```

```
## [1] "FD"
## [1] "Methylobacterium" "Sphingomonas" "Streptococcus"
## [4] "Helicobacter" "Prevotella" "Acinetobacter"
## [7] "Enhydrobacter" "Sediminibacterium" "Fusobacterium"
## [10] "Lactobacillus" "Veillonella" "Chryseobacterium"
```

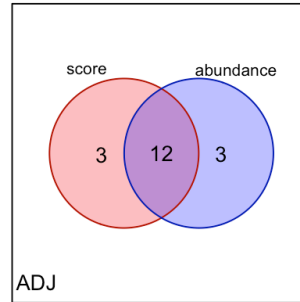
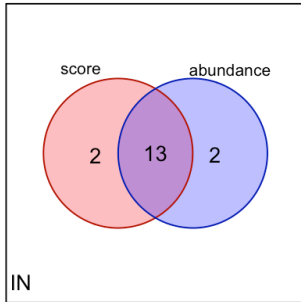
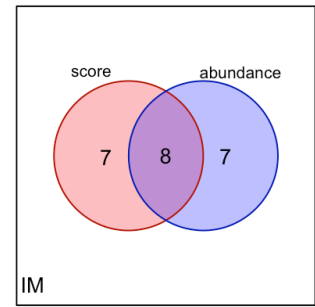
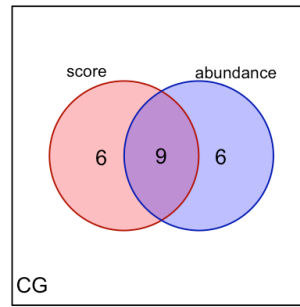
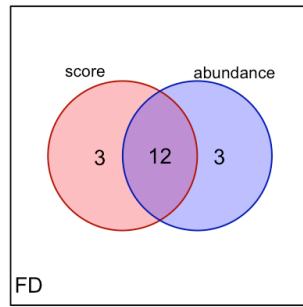
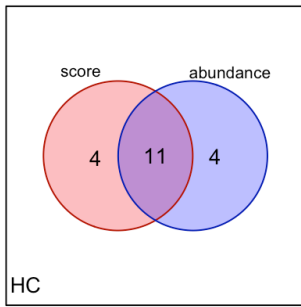
```
## [1] "CG"
## [1] "Helicobacter" "Prevotella" "Lactobacillus" "Streptococcus"
## [5] "Pseudomonas" "Sphingomonas" "Halomonas" "Neisseria"
## [9] "Acinetobacter"
```

```
## [1] "IM"
## [1] "Lactobacillus" "Sphingomonas" "Halomonas" "Helicobacter"
## [5] "Prevotella" "Neisseria" "Pseudomonas" "Shewanella"
```

```
## [1] "IN"
## [1] "Halomonas" "Shewanella" "Prevotella" "Neisseria"
## [5] "Lactobacillus" "Helicobacter" "Rothia" "Haemophilus"
## [9] "Actinobacillus" "Actinomyces" "Sphingomonas" "Aggregatibacter"
## [13] "Porphyromonas"
```

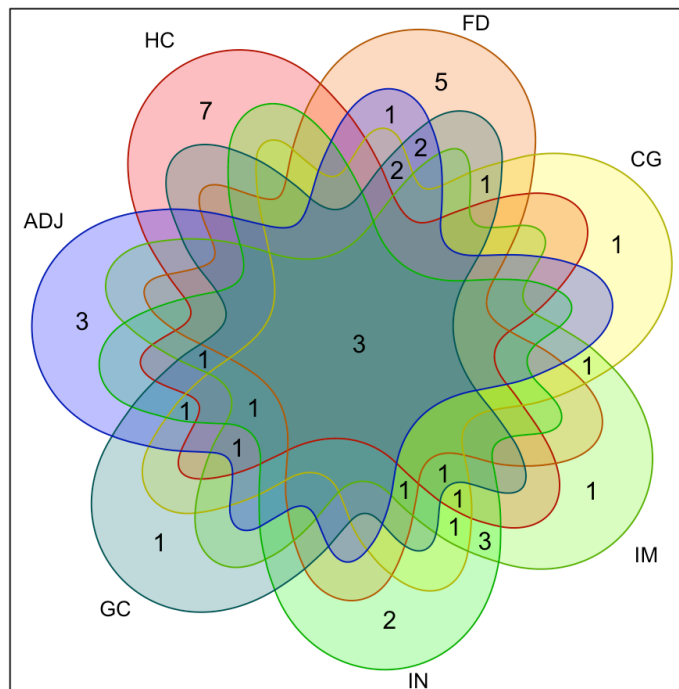
```
## [1] "GC"
## [1] "Helicobacter" "Prevotella" "Streptococcus" "Halomonas"
## [5] "Pseudomonas" "Sphingomonas" "Lactobacillus" "Shewanella"
## [9] "Acinetobacter" "Bacillus" "Neisseria"
```

```
## [1] "ADJ"
## [1] "Helicobacter" "Prevotella" "Sphingomonas" "Halomonas"
## [5] "Pseudomonas" "Bacteroides" "Streptococcus" "Acinetobacter"
## [9] "Shewanella" "Faecalibacterium" "Bacillus" "Oscillospira"
```



top 15 venn diagram - all conditions

```
v = list(HC = df$HC, FD = df$FD, CG = df$CG, IM = df$IM, IN = df$IN, GC = df$GC, ADJ = df$ADJ)
venn(v, zcolor = "style", ilcs = 1)
```



```
tiff(filename = "venn.75.tiff", compression = "lzw", units = "in", res = 300, width = 6.5, height = 4, type = "cairo")
par(mfrow=c(1,2))
venn(v, zcolor = "style", ilcs = 1)
```

```
Reduce(intersect, v)
```

```
## [1] "Prevotella" "Helicobacter" "Sphingomonas"
```

kruskal-wallis - alpha diversity - test and plot

```

alfa = read.delim ("tabelaalfa.txt", sep = '\t', header = T, stringsAsFactors=F, row.names = 1)
alfa$condition = samples$condition
alfa = filter(alfa, Shannon != 0)

```

```

dunn.test(alfa$Shannon, alfa$condition, kw=T, method = "hs")

```

```

##   Kruskal-Wallis rank sum test
##
## data: x and group
## Kruskal-Wallis chi-squared = 165.7184, df = 6, p-value = 0
##
##
##           Comparison of x by group
##           (Holm-Šidák)
## Col Mean-|
## Row Mean |          ADJ          CG          FD          GC          HC          IM
## -----+-----
##   CG |      7.834741
##       |      0.0000*
##
##   FD |      1.973800  -0.539631
##       |      0.0934      0.6492
##
##   GC |     -0.160446  -8.375789  -2.027052
##       |      0.4363      0.0000*      0.1022
##
##   HC |     -3.086344  -8.271732  -3.177515  -3.065098
##       |      0.0111*      0.0000*      0.0089*      0.0097*
##
##   IM |     -2.105638  -6.704259  -2.815206  -2.059964   0.473391
##       |      0.1170      0.0000*      0.0193*      0.1125      0.5348
##
##   IN |     -6.195299  -10.45889  -4.854898  -6.219164  -3.078576  -3.281700
##       |      0.0000*      0.0000*      0.0000*      0.0000*      0.0104*      0.0067*
##
## alpha = 0.05
## Reject Ho if p <= alpha/2

```

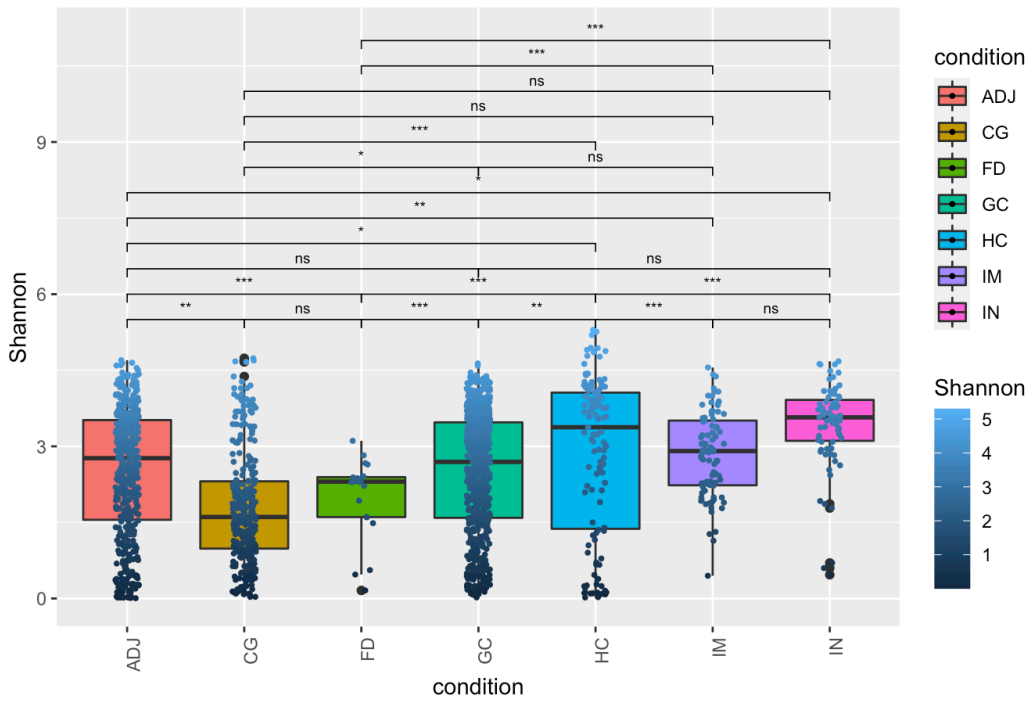
```

g = ggplot(alfa, mapping = aes(x = condition, y = Shannon, fill=condition))+
  geom_boxplot() +
  geom_jitter(aes(color=Shannon), width = 0.1, size=0.7) +
  ggtitle("Alpha Diversity")+ ylim(0,11.1)+
  theme(axis.text.x = element_text(angle = 90, hjust = 1))+
  geom_signif(annotations = "***",y_position=5.5, xmin=1, xmax=2,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "ns",y_position=5.5, xmin=2, xmax=3,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "****",y_position=5.5, xmin=3, xmax=4,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "***",y_position=5.5, xmin=4, xmax=5,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "****",y_position=5.5, xmin=5, xmax=6,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "ns",y_position=5.5, xmin=6, xmax=7,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "****",y_position=6, xmin=1, xmax=3,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "****",y_position=6, xmin=3, xmax=5,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "****",y_position=6, xmin=5, xmax=7,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "ns",y_position=6.5, xmin=1, xmax=4,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "ns",y_position=6.5, xmin=4, xmax=7,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "*",y_position=7, xmin=1, xmax=5,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "***",y_position=7.5, xmin=1, xmax=6,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "*",y_position=8, xmin=1, xmax=7,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "*",y_position=8.5, xmin=2, xmax=4,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "ns",y_position=8.5, xmin=4, xmax=6,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "****",y_position=9, xmin=2, xmax=5,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "ns",y_position=9.5, xmin=2, xmax=6,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "ns",y_position=10, xmin=2, xmax=7,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "****",y_position=10.5, xmin=3, xmax=6,size = 0.3, textsize = 2.5)+
  geom_signif(annotations = "****",y_position=11, xmin=3, xmax=7,size = 0.3, textsize = 2.5)

```

g

Alpha Diversity



```
ggsave(g, filename = "alfa.diversity.kkw.tiff", width = 6, height = 6, units = "in", dpi = 300, compression = "lz  
w", type="cairo")
```