Heatmaps

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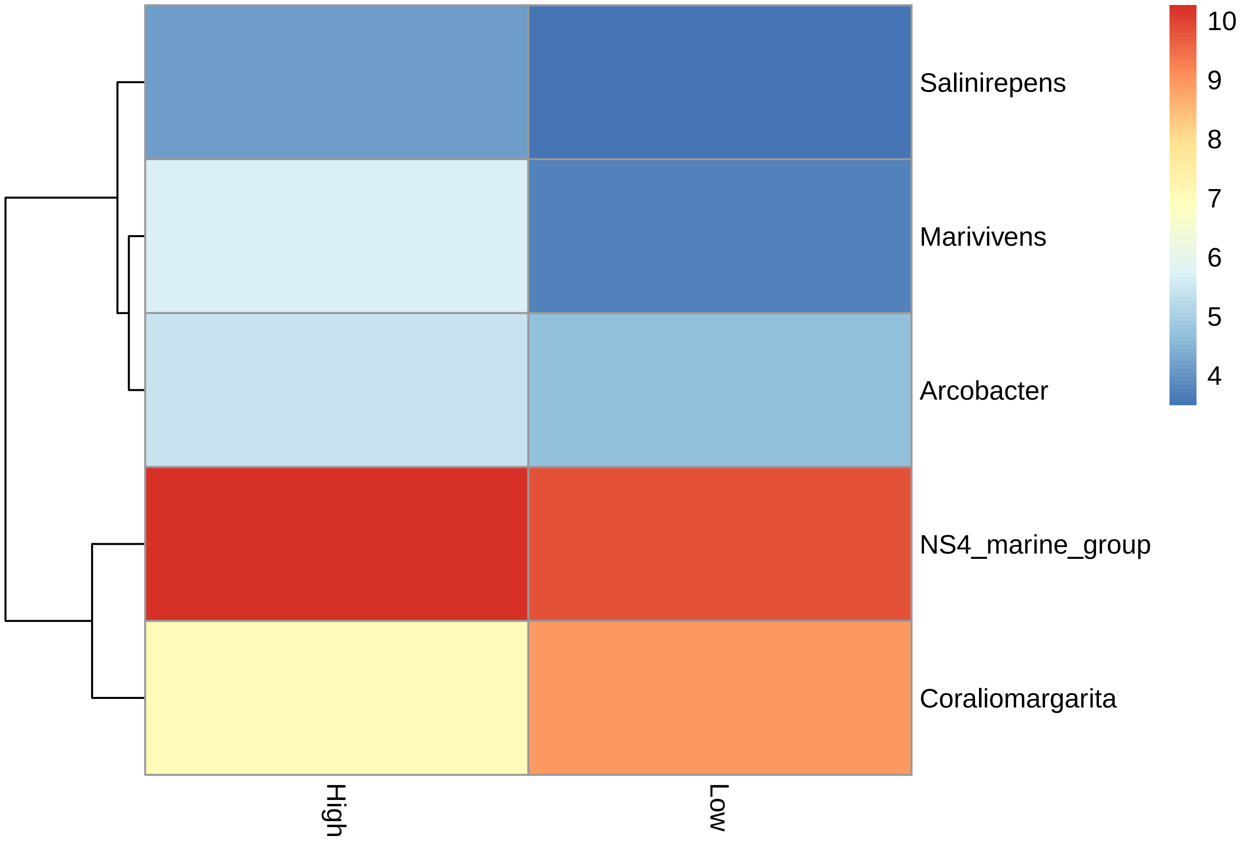
Load packages.

```
sapply(c("tidyverse", "ggplot2", "MuMIn", "gridExtra",
         "pheatmap", "ggpubr", "DESeq2", "phyloseq"),
         require, character.only = TRUE)
```

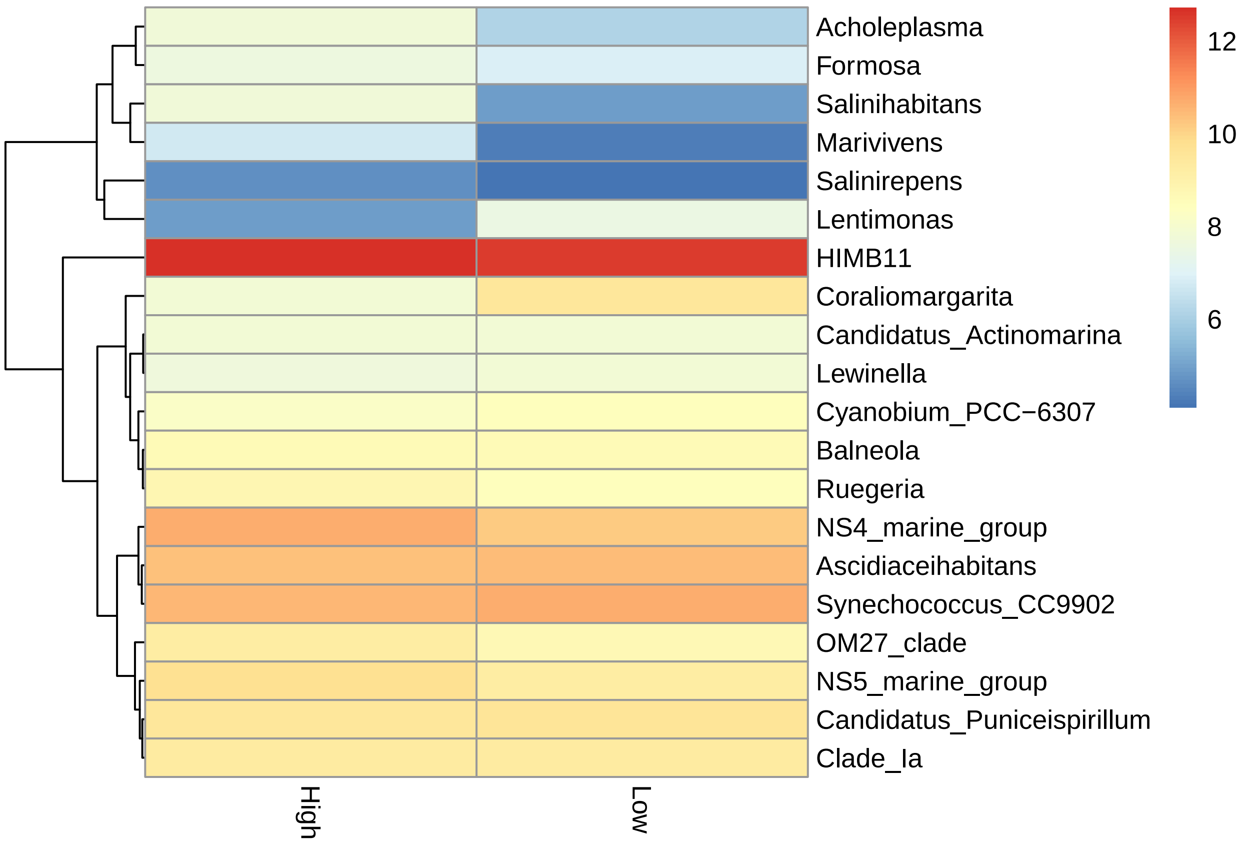
Heatmap of parasite load (binary) and significant Genera using DESeq2 normalised data.

```
sig_tax <- phyloseq_to_deseq2(ps3.Microbiome, ~ parasite_burden) %>%
    calc_geo_means() %>%
    deseq_filter() %>%
    DESeq(fitType = "local", test = "LRT", reduced = ~ 1) %>%
    get_deseq_res_lrt() %>%
    remove_rownames()

sig_tax <- ps3.Microbiome %>%
  tax_table() %>%
  unclass() %>%
  as.data.frame() %>%
  filter(Genus %in% sig_tax$Genus) %>%
  rownames_to_column()

phyloseq_to_deseq2(ps3.Microbiome, ~ parasite_burden) %>%
    calc_geo_means() %>%
    deseq_filter() %>%
    DESeq(fitType = "local", test = "LRT", reduced = ~ 1) %>%
    varianceStabilizingTransformation() %>%
    assay() %>%
    as.data.frame() %>% # counts of significant taxa
    rownames_to_column() %>%
    filter(rowname %in% sig_tax$rowname) %>%
    column_to_rownames("rowname") %>%
    t() %>%
    as.data.frame() %>%
    rownames_to_column("ID") %>%
    left_join(
      (sample_data(ps3.Microbiome) %>%
         unclass() %>%
         as.data.frame() %>%
         select(ID, parasite_burden) %>%
         mutate(ID = paste0("AM", ID))), by = "ID") %>%
    column_to_rownames("ID") %>%
    group_by(parasite_burden) %>%
    summarise_all(mean) %>%
    column_to_rownames("parasite_burden") %>%
    t() %>%
    as.data.frame() %>%
    rownames_to_column() %>%
    add_column( ID = c("NS4_marine_group", "Salinirepens", "Marivivens", "Coraliomargarita", "Arcobacte
    column_to_rownames("ID") %>%
    select(-rowname) %>%
    pheatmap(show_rownames = T, show_colnames = T, cluster_cols = F)# cluster by rows
```



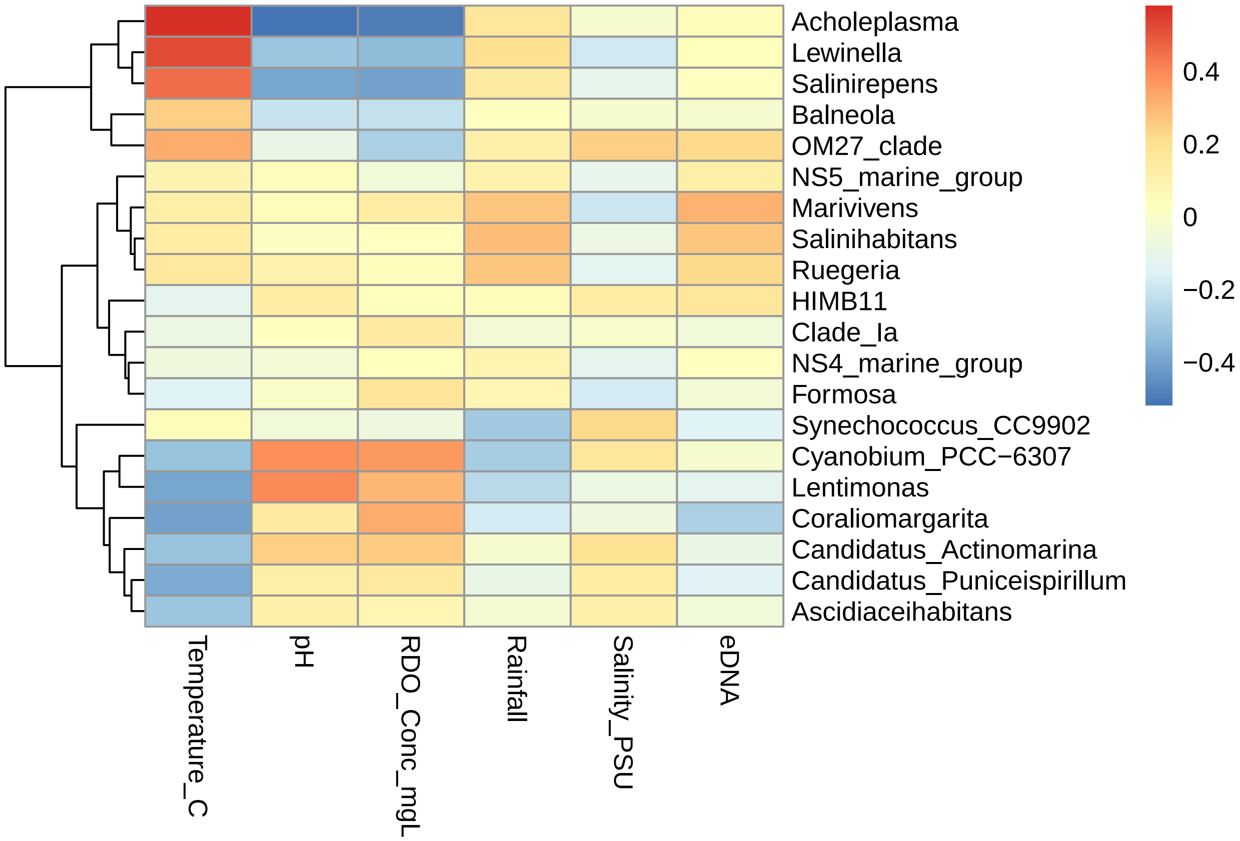
Heatmap of top20 Genera and parasite load (binary).

```
top20 <- names(sort(taxa_sums(ps3.Microbiome), decreasing=TRUE))[1:20]
ps.top20 <- prune_taxa(top20, ps3.Microbiome)

top20 <- phyloseq_to_deseq2(ps.top20, ~ parasite_burden) %>%
    calc_geo_means() %>%
    deseq_filter() %>%
    DESeq(fitType = "local", test = "LRT", reduced = ~ 1) %>%
    varianceStabilizingTransformation() %>%
    assay() %>%
    as.data.frame() %>%
    rownames_to_column()

top20 %>%
  left_join(
    ps3.Microbiome %>%
  tax_table() %>% # get the taxonomy table
  unclass() %>%
  as.data.frame() %>%
  rownames_to_column() %>%
  filter(rowname %in% top20$rowname) %>% # filter the tax table by ASV's in top20 taxa
  select(rowname, Genus)) %>%
  column_to_rownames("Genus") %>% # assign the genus at the rownames instead of the ASV
  select(-rowname) %>%
  t() %>%
  as.data.frame() %>%
  rownames_to_column("ID") %>%
  left_join(
    sample_data(ps.top20) %>% # join it with the sample data
      unclass() %>%
      as.data.frame() %>%
      mutate(ID = paste0("AM", ID)) %>%
      select(parasite_burden, ID), by = "ID") %>%
  group_by(parasite_burden) %>%
  summarise_all(mean) %>% # get means for high/low
  column_to_rownames("parasite_burden") %>%
  select(-ID) %>%
  t() %>%
  pheatmap(show_rownames = T, show_colnames = T, cluster_cols = F)
```



Heatmap of top20 Genera, eDNA and all environmental variables.

```
top20 %>%
  left_join(
    ps3.Microbiome %>%
  tax_table() %>% # get the taxonomy table
  unclass() %>%
  as.data.frame() %>%
  rownames_to_column() %>%
  filter(rowname %in% top20$rowname) %>% # filter tax table by ASV's that top20 taxa
  select(rowname, Genus)) %>%
  column_to_rownames("Genus") %>% # assign the genus as rownames instead of  ASVs
  select(-rowname) %>%
  t() %>%
  as.data.frame() %>%
  rownames_to_column("ID") %>%
  left_join(
    sample_data(ps.top20) %>% # join it with the sample data
      unclass() %>%
      as.data.frame() %>%
      mutate(ID = paste0("AM", ID)), by = "ID") %>%
  mutate(eDNA = log(ddPCR)) %>%
  select(-c(1, 22:26, 29, 31, 33, 35:36)) %>%
  cor(method = "kendall") %>%
  round(2) %>%
  as.data.frame() %>%
  select(21:26) %>%
  dplyr::slice(1:20) %>%
  pheatmap(show_rownames = T, show_colnames = T, cluster_cols = F)
```


Acholeplasma
Lewinella
Salinirepens
Balneola
OM27_clade
NS5_marine_group
Marivivens
Salinihabitans
Ruegeria
HIMB11
Clade_Ia
NS4_marine_group
Formosa
Synechococcus_CC9902
Cyanobium_PCC−6307
Lentimonas
Coraliomargarita
Candidatus_Actinomarina
Candidatus_Puniceispirillum
Ascidiaceihabitans
Temperature_C
pH
RDO_Conc_mgL
Rainfall
Salinity_PSU
eDNA