Certain manually curated clusters

Looking more closely to clusters to see for patterns

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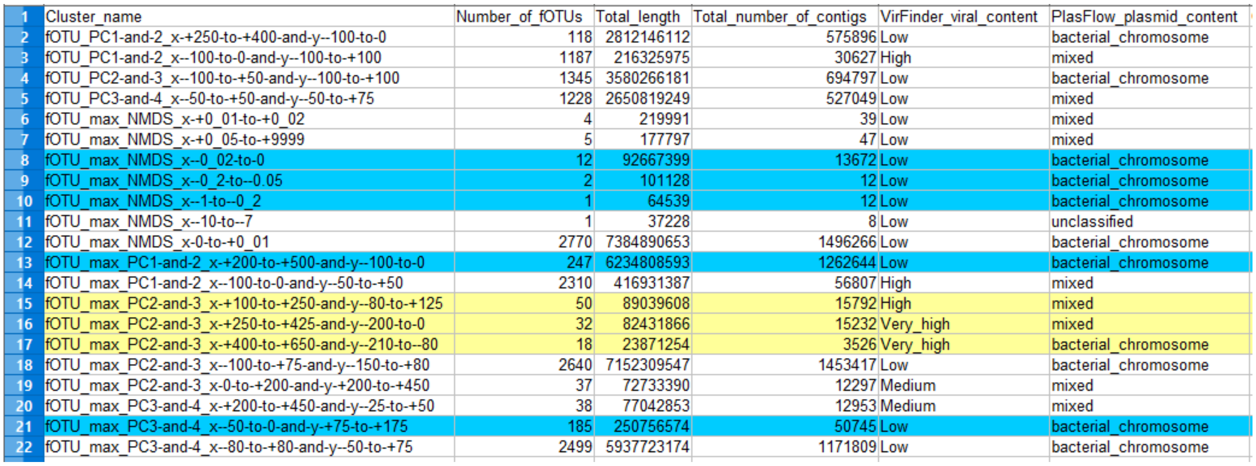
2020-01-06

Contents



1	Cluster_name	Number_of_fOTUs	Total_length	Total_number_of_contigs	VirFinder_viral_content	PlasFlow_plasmid_content
2	fOTU_PC1-and-2_x-+250-to-+400-and-y--100-to-0	118	2812146112	575896	Low	bacterial_chromosome
3	fOTU_PC1-and-2_x--100-to-0-and-y--100-to-+100	1187	216325975	30627	High	mixed
4	fOTU_PC2-and-3_x--100-to-+50-and-y--100-to-+100	1345	3580266181	694797	Low	bacterial_chromosome
5	fOTU_PC3-and-4_x--50-to-+50-and-y--50-to-+75	1228	2650819249	527049	Low	mixed
6	fOTU_max_NMDS_x-+0_01-to-+0_02	4	219991	39	Low	mixed
7	fOTU_max_NMDS_x-+0_05-to-+9999	5	177797	47	Low	mixed
8	fOTU_max_NMDS_x--0_02-to-0	12	92667399	13672	Low	bacterial_chromosome
9	fOTU_max_NMDS_x--0_2-to--0.05	2	101128	12	Low	bacterial_chromosome
10	fOTU_max_NMDS_x--1-to--0_2	1	64539	12	Low	bacterial_chromosome
11	fOTU_max_NMDS_x--10-to--7	1	37228	8	Low	unclassified
12	fOTU_max_NMDS_x-0-to-+0_01	2770	7384890653	1496266	Low	bacterial_chromosome
13	fOTU_max_PC1-and-2_x-+200-to-+500-and-y--100-to-0	247	6234808593	1262644	Low	bacterial_chromosome
14	fOTU_max_PC1-and-2_x--100-to-0-and-y--50-to-+50	2310	416931387	56807	High	mixed
15	fOTU_max_PC2-and-3_x-+100-to-+250-and-y--80-to-+125	50	89039608	15792	High	mixed
16	fOTU_max_PC2-and-3_x-+250-to-+425-and-y--200-to-0	32	82431866	15232	Very_high	mixed
17	fOTU_max_PC2-and-3_x-+400-to-+650-and-y--210-to--80	18	23871254	3526	Very_high	bacterial_chromosome
18	fOTU_max_PC2-and-3_x--100-to-+75-and-y--150-to-+80	2640	7152309547	1453417	Low	bacterial_chromosome
19	fOTU_max_PC2-and-3_x-0-to-+200-and-y-+200-to-+450	37	72733390	12297	Medium	mixed
20	fOTU_max_PC3-and-4_x-+200-to-+450-and-y--25-to-+50	38	77042853	12953	Medium	mixed
21	fOTU_max_PC3-and-4_x--50-to-0-and-y-+75-to-+175	185	250756574	50745	Low	bacterial_chromosome
22	fOTU_max_PC3-and-4_x--80-to-+80-and-y--50-to-+75	2499	5937723174	1171809	Low	bacterial_chromosome

Figure 1: Two by rough appreciation curated clusters. Blue with putatively more bacterial gene content and yellow with putatively more viral gene content.

1 Introduction

In this document will be studied further what features are present in mainly two groups:

1. VirFinder viral content is low and PlasFlow result suggests bacterial chromosomal material (putatively predominantly bacterial chromosomal content) (blue rows in Figure 1)
2. VirFinder viral content is high or very high and PlasFlow result suggests bacterial chromosomal material or mixed (putatively predominantly viral content) (yellow rows in Figure 1)

These estimation as based on previewing `summary_statistics.csv` file.

2 Gathering non-dublicate lists

First task is to gather lists of fOTUs of both of the above mentioned groups.

```
LIST_DIR="../analyses/HMMsearch/lists_of_clusters/"
COMM="../../../scripts/find_common.pl"
UNIQ="../../../scripts/uniquefy.pl"

# Array for holding predominantly chrom bacterial clusters (putative)
declare -a BACT
BACT[0]="fOTU_max_NMDS_x--0_02-to-0.txt"
BACT[1]="fOTU_max_NMDS_x--0_2-to--0_05.txt"
BACT[2]="fOTU_max_NMDS_x--1-to--0_2.txt"
BACT[3]="fOTU_max_PC1-and-2_x-+200-to-+500-and-y--100-to-0.txt"
BACT[4]="fOTU_max_PC3-and-4_x--50-to-0-and-y-+75-to-+175.txt"
# Array for holding predominantly viral clusters (putative)
declare -a VIR
VIR[0]="fOTU_max_PC2-and-3_x-+100-to-+250-and-y--80-to-+125.txt"
VIR[1]="fOTU_max_PC2-and-3_x-+250-to-+425-and-y--200-to-0.txt"
VIR[2]="fOTU_max_PC2-and-3_x-+400-to-+650-and-y--210-to--80.txt"

cd "$LIST_DIR"
# Let's now find a common non-dublicated list for both the viral and bacterial fOTUs
perl "$UNIQ" ${BACT[0]} ${BACT[1]} ${BACT[2]} ${BACT[3]} ${BACT[4]} > "b_fOTUs.txt"
perl "$UNIQ" ${VIR[0]} ${VIR[1]} ${VIR[2]} > "v_fOTUs.txt"

echo "The fOTUs in common between the two identified clusters:"

perl "$COMM" "b_fOTUs.txt" "v_fOTUs.txt" "bact_fOTUs.txt" "vir_fOTUs.txt"

BACT_NUM=$(echo $(wc -l "bact_fOTUs.txt")| awk '{print $1}')
VIR_NUM=$(echo $(wc -l "vir_fOTUs.txt") | awk '{print $1}')

echo -e "\nThere are $BACT_NUM fOTUs in the putatively bacterial cluster."
echo "There are $VIR_NUM fOTUs in the putatively viral cluster."

# Remove temporary files
rm "b_fOTUs.txt" "v_fOTUs.txt"
```

3 Gather grouped text files

From the fOTU wise data files it might be interesting to see if there are any patterns in these two groups (putatively more viral or bacterial). These pieces of information are now gathered into files one for each group:

- COGs functional category
- Description of the COG (in eggNOG database)
- Taxonomic order
- Taxonomic family
- Taxonomic species
- Scientific name

3.1 Create directories with soft links of the two groups

It will be maybe somewhat more easier to handle all the fOTUs of same type when they are in own directories (as symbolic links). Then one could just use globbing to access all the fOTUs with one expression. Let's use AWK to create the directories with relevant links inside them.

```
BACT_LIST="../../lists_of_clusters/bact_fOTUs.txt"
VIR_LIST="../../lists_of_clusters/vir_fOTUs.txt"
OUT_DIR_BACT="../analyses/HMMsearch/validation_and_further_analyses/bacts_lns/"
OUT_DIR_VIR="../virs_lns/"
SCRIPT="../../../../scripts/groupSymLinkify.awk"
cd $OUT_DIR_BACT
awk -f "$SCRIPT" "$BACT_LIST"
cd "$OUT_DIR_VIR"
awk -f "$SCRIPT" "$VIR_LIST"
```

In the AWK scripts above the current working directories are the same as the shell that started the script.

3.2 Gather grouped data files

```
OUT_DIR_BACT="../analyses/HMMsearch/validation_and_further_analyses/bacts_lns/"
OUT_DIR_VIR="../analyses/HMMsearch/validation_and_further_analyses/virs_lns/"
OUT="../analyses/HMMsearch/validation_and_further_analyses/cluster_data/"
SCRIPT_COGcat="../scripts/groupCOGcat.awk"
SCRIPT_Names="../scripts/groupNames.awk"
SCRIPT_Descs="../scripts/groupDescs.awk"
SCRIPT_Taxons="../scripts/groupTaxons.awk"

awk -F"\t" -f "$SCRIPT_COGcat" "$OUT_DIR_BACT"*".tsv" >> "$OUT""bact_COGcats.txt"

awk -F"\t" -v header="sci_names" -v field="8" \
-f "$SCRIPT_Names" "$OUT_DIR_BACT"*".tsv" >> "$OUT""bact_Sci_Names.txt"
awk -F"\t" -v header="species" -v field="14" \
-f "$SCRIPT_Names" "$OUT_DIR_BACT"*".tsv" >> "$OUT""bact_Spec_Names.txt"

awk -F"\t" -f "$SCRIPT_Descs" "$OUT_DIR_BACT"*".tsv" >> "$OUT""bact_Descs.txt"

awk -F"\t" -v header="orders" -v field="10" \
-f "$SCRIPT_Taxons" "$OUT_DIR_BACT"*".tsv" >> "$OUT""bact_Orders.txt"
awk -F"\t" -v header="families" -v field="11" \
-f "$SCRIPT_Taxons" "$OUT_DIR_BACT"*".tsv" >> "$OUT""bact_Families.txt"




awk -F"\t" -f "$SCRIPT_COGcat" "$OUT_DIR_VIR"*".tsv" >> "$OUT""vir_COGcats.txt"

awk -F"\t" -v header="sci_names" -v field="8" \
-f "$SCRIPT_Names" "$OUT_DIR_VIR"*".tsv" >> "$OUT""vir_Sci_Names.txt"
awk -F"\t" -v header="species" -v field="14" \
-f "$SCRIPT_Names" "$OUT_DIR_VIR"*".tsv" >> "$OUT""vir_Spec_Names.txt"

awk -F"\t" -f "$SCRIPT_Descs" "$OUT_DIR_VIR"*".tsv" >> "$OUT""vir_Descs.txt"

awk -F"\t" -v header="orders" -v field="10" \
-f "$SCRIPT_Taxons" "$OUT_DIR_VIR"*".tsv" >> "$OUT""vir_Orders.txt"
```

```
awk -F"\t" -v header="families" -v field="11" \
-f "$SCRIPT_Taxons" "$OUT_DIR_VIR"*".tsv" >> "$OUT""vir_Families.txt"
```

4 Check what is in the clusters

Now that we have these twelve text files. Let's read them in a form wordclouds from them.

```
#install.packages("wordcloud")
#install.packages("RColorBrewer")
#install.packages("tm")
library(wordcloud)
library(RColorBrewer)
library(tm)
library(tidyverse)
```

Let's define some functions. These functions will do the hard work of producing the visualisations.

```
de_underscore <- function(underscored) {
  de_underscored <- gsub("_",
                         " ",
                         underscored)
  de_underscored
  }

wordCloudify <- function(input_filename, output_filename){
  raw_text <- readLines(input_filename,
                        warn = F)
  docs <- Corpus(VectorSource(raw_text))
  # build a term-document matrix:
  tdm <- TermDocumentMatrix(docs)
  mat <- as.matrix(tdm)
  named_num <- sort(rowSums(mat),decreasing=TRUE)
  freq_data <- tibble(entity = names(named_num),
                      freq=named_num) %>%
    mutate_at(vars(entity),
              funs(de_underscore))

  #head(d, 10)

  # generate the wordcloud:
  png(filename = output_filename)
  wordcloud(words = freq_data$entity,
            freq = freq_data$freq,
            min.freq = 1,
            max.words=200,
            random.order=FALSE,
            rot.per=0.35,
            colors=brewer.pal(8, "Dark2"))
  dev.off()
}
```

The input files are stored next.

```
dir_path <- "../analyses/HMMsearch/validation_and_further_analyses/cluster_data/"
files <- list.files(path = dir_path,
                    pattern = ".+.txt")
```

Lastly, let's create word clouds to visualise which entities are most common with respect to the aforementioned aspects.

```
output_dir <- "../visualisations/HMMsearch/wordClouds/"
for (text_file in files) {
  in_file_name <- paste(dir_path,
                        text_file,
                        sep = "")
  extensionless <- gsub(".txt","",text_file)
  out_file_name <- paste(output_dir,
                         extensionless,
                         ".png",
                         sep = "")
  wordCloudify(in_file_name, out_file_name)
}
```