output_PUL_fasta

-Identifies genomic islands for 178 HGGs that are convergently gained by captive ape-associated strains

-Output fastas for each island plus 100 genes upstream and downstream

Identify genomic islands

```r
source('scripts/pangenome_analyses/gene_gain_loss_functions.R')
#calls get_genomic_island function

Bxydir = 'results/pangenome/Bacteroides_xylanisolvens'
metadata_file = file.path(Bxydir,'metadata.txt')
Bxy_metadata = read_tsv(metadata_file,col_types = cols())
window_size = 5

#define outdir for fastas
dir.create(file.path(Bxydir,"output_PUL_fasta/gff_window5"),recursive = T)

#identify genomic islands for 178 HGGs across all captive ape isolates
get_captive_convergent_genes = function(isolate){
    #clusters genes within an isolate into genomic islands based on a given window size
    isolate_old = Bxy_metadata$isolate_old[Bxy_metadata$isolate==isolate]
    gff_file = paste0('results/pangenome/prokka/',isolate_old,'/',isolate_old,'.gff')
    genomic_islands_outfile =   file.path(
      Bxydir,'output_PUL_fasta', paste0('gff_window',window_size), paste0( #outfile dir
        isolate,'_window',window_size,'_island_gff.txt')) #outfile name
    gff_islands = get_genomic_island(pres_abs=pres_abs,
                               list_of_genes=captive_convergent_genes,
                               isolate=isolate,
                               gff_file=gff_file,
                               window_size = window_size,
                               outfile=genomic_islands_outfile)
}


pres_abs =  read_csv(file= file.path(Bxydir,'roary_nosplitparalogs/gene_presence_absence.csv'),
                      col_types = cols())
captive_convergent_genes = readRDS(file=file.path(Bxydir,'output_PUL_fasta/gene_list.RDS'))
print(length(captive_convergent_genes))
```

```
## [1] 178
```

```r
get_captive_convergent_genes('P17.C2')
```

```
## [1] "Found  178 of 178 genes families provided. 182 gene copies belonging to this families found on
```

```r
captive_isolates = Bxy_metadata$isolate[Bxy_metadata$human_ape=='ape']
#lapply(captive_isolates,get_captive_convergent_genes) #uncomment to run on all captive ape strains
```

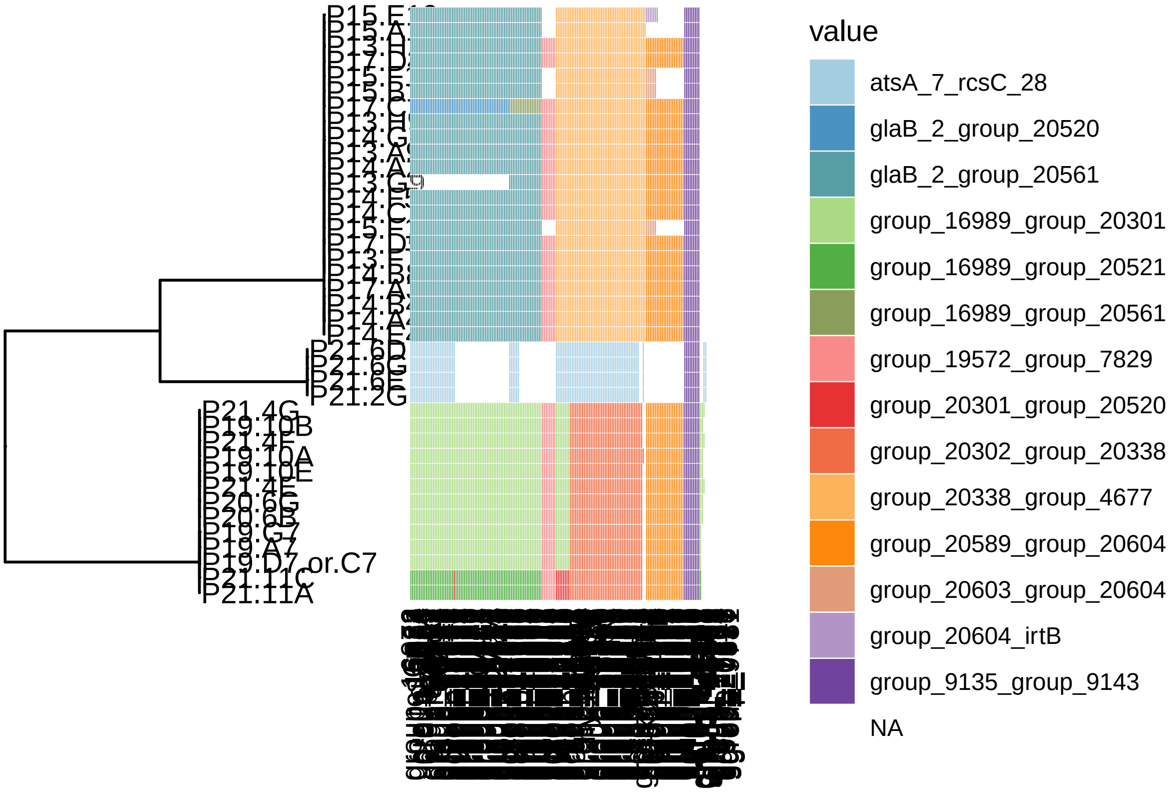
Visualize genomic islands

```r
#generate heatmap only with clusters longer than 5 genes
captive_convergent_table = data.frame(Gene=captive_convergent_genes)
for  (isolate in captive_isolates) {
  #read in output
  genomic_islands_outfile = file.path(
    Bxydir,'output_PUL_fasta',paste0('gff_window',window_size), #outfile dir
     paste0(isolate,'_window',window_size,'_island_gff.txt')) #outfile name
  iso = read_tsv(file=genomic_islands_outfile)
  iso = iso %>% filter(size>5) %>%
    select(Gene,gene_span) %>%
    mutate(Gene=make.names(Gene, unique=TRUE))
  colnames(iso) = c('Gene',isolate)
  captive_convergent_table = captive_convergent_table %>% full_join(iso,by='Gene')}

#heatmap matrix and tree
captive_convergent_tableM = captive_convergent_table %>% #format for heatmap
  column_to_rownames(var='Gene') %>%
  arrange_all() %>%
  t()

Bxy_tree = read.tree(file = file.path(Bxydir,'Bacteroides_xylanisolvens.tre'))
not_captive = Bxy_tree$tip.label[!Bxy_tree$tip.label %in% captive_isolates]
Bxy_tree_captive = drop.tip(Bxy_tree,not_captive) #remove not captive ape strains
write.tree(Bxy_tree_captive,file.path(Bxydir,'output_PUL_fasta/Bxy_tree_captive.tre'))

(captive_convergent_heatmap <- gheatmap(
                              ggtree(Bxy_tree_captive) + geom_tiplab() + ylim(-10,NA),
                              captive_convergent_tableM,
                              colnames_angle=90,
                              hjust=1,
                              offset = .01) +
    scale_fill_manual(values=colorRampPalette(brewer.pal(17, "Paired"))(17)))
```



subset to PUL region

```
table(captive_convergent_table$P21.4G) #appears the majority on genes are on two islands
```

```
##
## group_16989_group_20301  group_19572_group_7829 group_20302_group_20338
##                      87                       8                      42
## group_20589_group_20604   group_9135_group_9143
##                      22                       9
```

```
captive_PUL_table = captive_convergent_table %>% #select genes located on these 2 islands
  filter(P21.4G == 'group_16989_group_20301' | P21.4G == 'group_20302_group_20338') %>%
  separate(col = 'Gene',into=c('Gene'),extra='drop',sep='.1')
#get names of islands in other strains
(PUL_span = unique(unlist(select(captive_PUL_table,-Gene))))
```

```
##  [1] "group_16989_group_20561" "glaB_2_group_20520"
##  [3] "group_20338_group_4677"  NA
##  [5] "glaB_2_group_20561"      "group_16989_group_20301"
##  [7] "group_20302_group_20338" "atsA_7_rcsC_28"
##  [9] "group_16989_group_20521" "group_20301_group_20520"
```

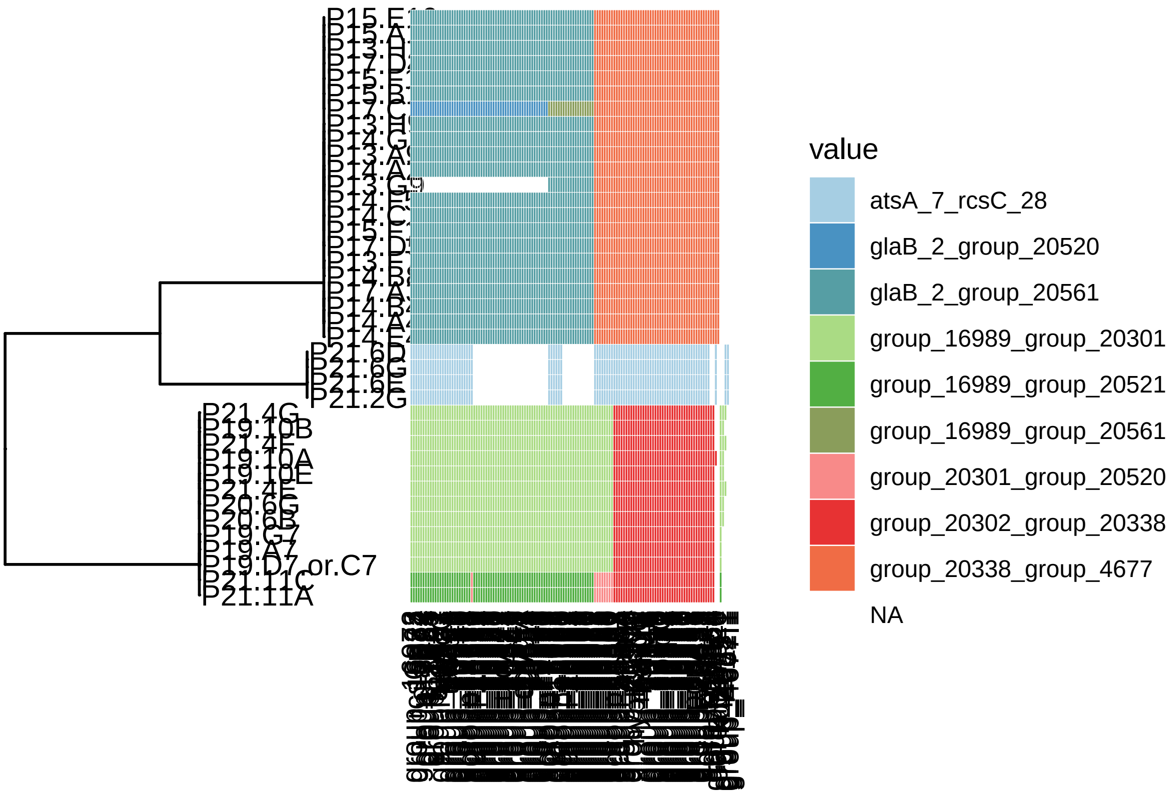
```
#reselect any island from any strain in that list
captive_PUL_table = captive_convergent_table %>%
  filter_at(vars(-Gene), all_vars(. %in% PUL_span))
#get rid of colnames that are all na
```

```
captive_PUL_table = captive_PUL_table %>%
  filter(rowSums(is.na(captive_PUL_table)) != ncol(captive_PUL_table)-1)
#number of HGGs in PUL
captive_PUL_table %>%
  separate(col = 'Gene',into=c('Gene'),extra='drop',sep='.1') %>%
  pull(Gene) %>% unique() %>% length()
```

```
## [1] 112
```

```
#plot heatmap of PUL subset
captive_PUL_tableM = captive_PUL_table %>%
  as.data.frame() %>%
  column_to_rownames(var='Gene') %>%
  arrange_all() %>%
  t()


(captive_convergent_heatmap <- gheatmap(
                              ggtree(Bxy_tree_captive) + geom_tiplab() + ylim(-10,NA),
                              captive_PUL_tableM,
                              colnames_angle=90,
                              hjust=1,
                              offset = .01) +
    scale_fill_manual(values=colorRampPalette(brewer.pal(17, "Paired"))(17)))
```



```
#examine gff file
isolate = 'P17.C2'
```

```r
P17.C2_gff_file = genomic_islands_outfile =
      file.path(Bxydir,'output_PUL_fasta',paste0('gff_window',window_size),
                    paste0(isolate,'_window',window_size,'_island_gff.txt'))
P17.C2_gff = read_tsv(P17.C2_gff_file)
P17.C2_gff = P17.C2_gff %>% filter(gene_span %in% PUL_span)
P17.C2_gff %>% group_by(contig,gene_span) %>%
  summarise(min(start),max(end))
```

```
## # A tibble: 3 x 4
## # Groups:   contig [2]
##   contig                gene_span                `min(start)` `max(end)`
##   <chr>                 <chr>                           <dbl>      <dbl>
## 1 scaffold11_size214356 glaB_2_group_20520             112010     214151
## 2 scaffold11_size214356 group_16989_group_20561         90457     107721
## 3 scaffold31_size67155  group_20338_group_4677           1775      67117
```

```r
#merge glaB_2_group_20520 and group_16989_group_20561 bc upstream and downstream
#regions will overlap
P17.C2_gff = P17.C2_gff %>% mutate(gene_span = recode(gene_span,
                                      'glaB_2_group_20520' = 'glaB_2_group_20561',
                                      'group_16989_group_20561' = 'glaB_2_group_20561'))
write_tsv(P17.C2_gff, file = P17.C2_gff_file)


#merge islands and report lengths
PUL_span = c(PUL_span,'atsA_7_rcsC_28','glaB_2_group_20561')

get_length_genomic_islands <- function(isolate) {
  gff_file = genomic_islands_outfile =
      file.path(Bxydir,'output_PUL_fasta',paste0('gff_window',window_size),
                    paste0(isolate,'_window',window_size,'_island_gff.txt'))
  df = read_tsv(gff_file)
  len = df %>% filter(gene_span %in% PUL_span) %>%
    group_by(contig,gene_span) %>%
    summarise(length=max(end)-min(start))
  len$isolate = isolate
  len = len %>% select(isolate,everything())
  return(len)
}

PUL_contig_summary = lapply(captive_isolates,get_length_genomic_islands) %>% bind_rows()
total_length = PUL_contig_summary %>%
  group_by(isolate) %>%
  summarise(total_length = sum(length))
#total length of the PUL
table(total_length$total_length)
```

```
##
##  82606 145992 145993 187316 187317 187588 187589 187590 189036
##      1      3      1      1      1      3      5      3     21
```

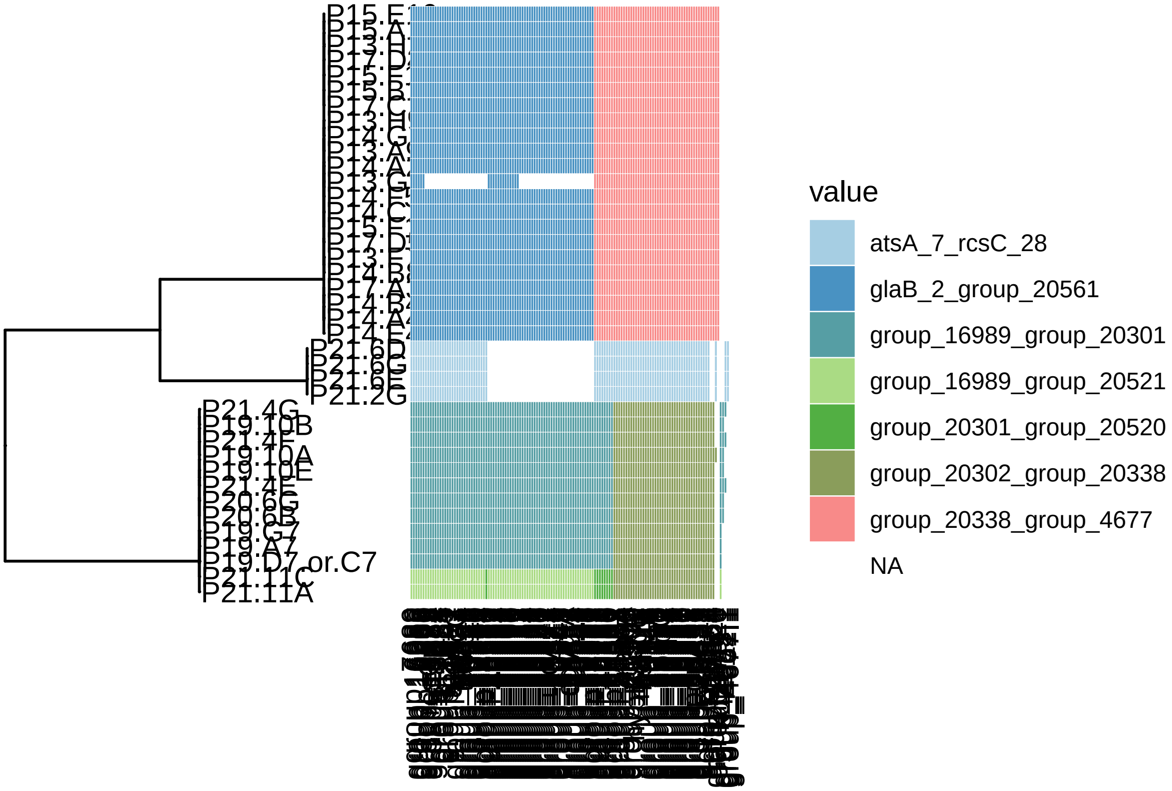
```r
write_tsv(PUL_contig_summary,
          file = file.path(Bxydir,'output_PUL_fasta/PUL_contig_summary.txt'))

##recode islands to make new heatmap
```

```
captive_PUL_table= captive_PUL_table %>%
  as.data.frame() %>%
  mutate_all(funs(recode(.,
                         'glaB_2_group_20520' = 'glaB_2_group_20561',
                         'group_16989_group_20561' = 'glaB_2_group_20561'
                         )))

captive_PUL_tableM = captive_PUL_table %>%
  column_to_rownames(var='Gene') %>%
  arrange_all() %>%
  t()
write.table(captive_PUL_tableM,
          file = file.path(Bxydir,'output_PUL_fasta/captive_PUL_tableM.txt'),
                    quote=F,sep='\t')

(captive_convergent_heatmap <- gheatmap(
                                ggtree(Bxy_tree_captive) + geom_tiplab() + ylim(-10,NA),
                                captive_PUL_tableM ,
                                colnames_angle=90,
                                hjust=1,
                                offset = .01) +
    scale_fill_manual(values=colorRampPalette(brewer.pal(17, "Paired"))(17)))
```



Output fasta

```
get_island_fna = function(isolate,gene_span,updown_size){
    #given isolate and name of genomic island outputs to fna plus X genes upstream and downstream
    isolate_old = Bxy_metadata$isolate_old[Bxy_metadata$isolate==isolate]
    gff_file = paste0('results/pangenome/prokka/',isolate_old,'/',isolate_old,'.gff')
    fna_file = paste0('results/pangenome/prokka/',isolate_old,'/',isolate_old,'.fna')
    genomic_islands_outfile =
      file.path(Bxydir,'output_PUL_fasta',paste0('gff_window',window_size),
                    paste0(isolate,'_window',window_size,'_island_gff.txt'))
    island_fasta_outfile =
      file.path(Bxydir,'output_PUL_fasta',
              paste0('fna_window',window_size,'updown',updown_size),
                 gene_span,paste0(isolate,'_',gene_span,'_updown_',updown_size,'.fna'))
    print(island_fasta_outfile)
    output_island_fasta(
          isolate=isolate,
          gene_span = gene_span,
          genomic_islands_outfile = genomic_islands_outfile,
          island_fasta_outfile  = island_fasta_outfile,
          updown_size = updown_size ,
          fna_file = fna_file,
          gff_file = gff_file
          )
}

#output fnas for test isolate
get_island_fna(isolate='P17.C2',gene_span='glaB_2_group_20561',updown=0)
```

```
## [1] "results/pangenome/Bacteroides_xylanisolvens/output_PUL_fasta/fna_window5updown0/glaB_2_group_205
## [1] "P17.C2"             "glaB_2_group_20561"
```

```
## [1] "output genomic island glaB_2_group_20561 containing 76 genes plus 0 genes on either side, total
```

```
#output fnas for all isolates
#mcmapply(get_island_fna,
#  PUL_contig_summary$isolate,PUL_contig_summary$gene_span,rep(100,nrow(PUL_contig_summary)) ,
#  mc.cores=8)
#mcmapply(get_island_fna,
#  PUL_contig_summary$isolate,PUL_contig_summary$gene_span,rep(0,nrow(PUL_contig_summary)) ,
#  mc.cores=8)
```