Supplementary Information:

Isolation of wheat bran-colonizing and metabolizing species from the human fecal microbiota

De Paepe et al., Submission to PeerJ

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Availability of raw data

This Supplementary Data file contains the R code to reproduce all analyses and plots presented in the manuscript entitled "Isolation of wheat bran-colonizing and metabolizing species from the human fecal microbiota". All the required datasets are uploaded as additional supplementary files (DataS3-S9).

The raw 16S rRNA next-generation amplicon sequencing data is, moreover, deposited in the NCBI sequence read archive (SRA) under the accession number SRP091975 https://trace.ncbi.nlm.nih.gov/Traces/study/?acc=SRP091975&go=go. Raw 16S rRNA gene Sanger sequences of the isolates are supplied as a compressed folder (Sanger_isolates.zip). This folder and the raw Data files should be present in the same working directory containing this .Rmd script.

Load the packages and datasets

The following packages are required to run the script:

```
# Formatting data
library(reshape2)
library(plyr)
library(dplyr)
library(tidyr)
library(stringi)
library(stringr)
library(splitstackshape)
library(tibble)
library(gtools)

# Plotting data
library(ggplot2)
library(gridExtra)
```

```
library(cowplot)
require(scales)
library(gplots)

# User defined functions (make sure that the R scripts below are present in t
he same working directory as this Rmarkdown script)
source('DataS11.R') # formatting of ggplot graphs
source('DataS12.R') # formatting mothur taxonomy file

# Processing of 16S rRNA gene Sanger sequencing data
library("ape")
library("phangorn", lib.loc="/usr/local/lib/R/site-library")
source("https://bioconductor.org/biocLite.R")
biocLite("DECIPHER")
biocLite("Biostrings")
biocLite("sangerseqR")
library(devtools)
install_github("Liliad/sangeranalyseR")
library(sangeranalyseR)

# Processing of next-generation 16S rRNA gene amplicon sequencing data
biocLite("phyloseq")
library("phyloseq")
library(vegan)

# Rmarkdown knitr
library(knitr)
```

Load the functional data (short chain fatty acid production in the enrichment series):

```
enrichment_SCFA <- read.csv("DataS3.csv")
```

Load the 16S rRNA gene amplicon sequencing data (read count table and taxonomy):

```
# READ COUNT DATA
shared3 <- read.csv("DataS4.csv",header=TRUE)
rownames(shared3) <- shared3[,1]
shared3 <- shared3[,-1]

# Transpose
shared3 <- as.data.frame(t(shared3))

# Apply filtering cut-offs from the waste not want not paper by McMurdie and
Holmes (2014)
shared3minsingleton <- shared3[rowSums(shared3)!=0,]
shared3minsingleton <- shared3minsingleton[rowSums(shared3minsingleton)!=1,]
minobs=1
prevalence <- apply(as.matrix(shared3minsingleton),1,function(x,minobs){sum(x
>=minobs)},minobs)/ncol(shared3minsingleton)
prevalencefilter <- prevalence>0.05
```

```r
shared3minsingletonwnwn <- shared3minsingleton[prevalencefilter,]
shared3minsingletonwnwn <- shared3minsingletonwnwn[rowSums(shared3minsingleto
nwnwn)>0.5*ncol(shared3minsingletonwnwn),]

# Transform to proportions
shared3minsingletonwnwnprop = sweep(shared3minsingletonwnwn,2,colSums(shared3
minsingletonwnwn),'/')

#TAXONOMY
RDPtax <- read.csv("DataS6.csv")
RDPtax <- splittax(RDPtax,"otu")
rownames(RDPtax) <- RDPtax$id
RDPtax <- RDPtax[,-1]
RDPtax_noprob <- dplyr::select(RDPtax,-contains("prob"))

# Formatting
RDPtax_noprob$Genus <- mapvalues(RDPtax_noprob$Genus,from=c("Enterobacteriace
ae_unclassified","Erysipelotrichaceae_unclassified","Lachnospiraceae_unclassi
fied","Lactobacillales_unclassified","Actinobacteria_unclassified","Burkholde
riales_unclassified","Clostridiales_unclassified","Ruminococcaceae_unclassifi
ed","Coriobacteriaceae_unclassified"),to=c("Enterobacteriaceae","Erysipelotri
chaceae","Lachnospiraceae","Lactobacillales","Actinobacteria","Burkholderiale
s","Clostridiales","Ruminococcaceae","Coriobacteriaceae"))

#COMBINE IN OTU TABLE
otutab3 <- dplyr::select(RDPtax_noprob,-Species)
otutab3 <- cbind(otutab3[rownames(shared3minsingletonwnwnprop),],shared3minsi
ngletonwnwnprop)

# Constructing Genus level otutable with RDP taxonomy
genusotutab3 <- aggregate(. ~ Genus, data=otutab3[,-c(1:5)], FUN=sum)
rownames(genusotutab3) <- genusotutab3[,1]
genusotutab3 <- genusotutab3[,-1]

# Constructing Family level otutable with with RDP taxonomy
familyotutab3 <- aggregate(. ~ Family, data=otutab3[,-c(1:4,6)], FUN=sum)
rownames(familyotutab3) <- familyotutab3[,1]
familyotutab3 <- familyotutab3[,-1]

# Constructing Order level otutable with with RDP taxonomy
orderotutab3 <- aggregate(. ~ Order, data=otutab3[,-c(1:3,5,6)], FUN=sum)
rownames(orderotutab3) <- orderotutab3[,1]
orderotutab3 <- orderotutab3[,-1]

# Constructing Class level otutable with with RDP taxonomy
classotutab3 <- aggregate(. ~ Class, data=otutab3[,-c(1:2,4:6)], FUN=sum)
rownames(classotutab3) <- classotutab3[,1]
classotutab3 <- classotutab3[,-1]
```

```
# Constructing Phylum level otutable with with RDP taxonomy
phylumotutab3 <- aggregate(. ~ Phylum, data=otutab3[,-c(1,3:6)], FUN=sum)
rownames(phylumotutab3) <- phylumotutab3[,1]
phylumotutab3 <- phylumotutab3[,-1]

# ABUNDANCE DATA
shared4 <- read.csv("DataS5.csv",header=TRUE)
rownames(shared4) <- shared4[,1]
shared4 <- shared4[,-1]

# Transpose
shared4 <- as.data.frame(t(shared4))

# Apply filtering cut-offs from the waste not want not paper by McMurdie and
Holmes (2014)
shared4minsingleton <- shared4[rowSums(shared4)!=0,]
shared4minsingleton <- shared4minsingleton[rowSums(shared4minsingleton)!=1,]
minobs=1
prevalence <- apply(as.matrix(shared4minsingleton),1,function(x,minobs){sum(x
>=minobs)},minobs)/ncol(shared4minsingleton)
prevalencefilter <- prevalence>0.05
shared4minsingletonwnwn <- shared4minsingleton[prevalencefilter,]
shared4minsingletonwnwn <- shared4minsingletonwnwn[rowSums(shared4minsingleto
nwnwn)>0.5*ncol(shared4minsingletonwnwn),]

# Transform to proportions
shared4minsingletonwnwnprop = sweep(shared4minsingletonwnwn,2,colSums(shared4
minsingletonwnwn),'/')

#TAXONOMY DATA
RDPtax4 <- read.csv("DataS7.csv")
RDPtax4 <- splittax(RDPtax4,"otu")
rownames(RDPtax4) <- RDPtax4$id
RDPtax4 <- RDPtax4[,-1]
RDPtax4_noprob <- dplyr::select(RDPtax4,-contains("prob"))

RDPtax4_noprob$Genus <- mapvalues(RDPtax4_noprob$Genus,from=c("Enterobacteria
ceae_unclassified","Erysipelotrichaceae_unclassified","Lachnospiraceae_unclas
sified","Lactobacillales_unclassified","Actinobacteria_unclassified","Burkhol
deriales_unclassified","Clostridiales_unclassified","Ruminococcaceae_unclassi
fied","Coriobacteriaceae_unclassified","Lactobacillaceae_unclassified","Micro
bacteriaceae_unclassified"),to=c("Enterobacteriaceae","Erysipelotrichaceae","
Lachnospiraceae","Lactobacillales","Actinobacteria","Burkholderiales","Clostr
idiales","Ruminococcaceae","Coriobacteriaceae","Lactobacillaceae","Microbacte
riaceae"))

#COMBINE IN OTU TABLE
otutab4 <- dplyr::select(RDPtax4_noprob,-Species)
otutab4 <- cbind(otutab4[rownames(shared4minsingletonwnwnprop),],shared4minsi
ngletonwnwnprop)
```

```
# Constructing Genus level otutable with RDP taxonomy
genusotutab4 <- aggregate(. ~ Genus, data=otutab4[,-c(1:5)], FUN=sum)
rownames(genusotutab4) <- genusotutab4[,1]
genusotutab4 <- genusotutab4[,-1]

# Constructing Family level otutable with with RDP taxonomy
familyotutab4 <- aggregate(. ~ Family, data=otutab4[,-c(1:4,6)], FUN=sum)
rownames(familyotutab4) <- familyotutab4[,1]
familyotutab4 <- familyotutab4[,-1]

# Constructing Order level otutable with with RDP taxonomy
orderotutab4 <- aggregate(. ~ Order, data=otutab4[,-c(1:3,5,6)], FUN=sum)
rownames(orderotutab4) <- orderotutab4[,1]
orderotutab4 <- orderotutab4[,-1]

# Constructing Class level otutable with with RDP taxonomy
classotutab4 <- aggregate(. ~ Class, data=otutab4[,-c(1:2,4:6)], FUN=sum)
rownames(classotutab4) <- classotutab4[,1]
classotutab4 <- classotutab4[,-1]

# Constructing Phylum level otutable with with RDP taxonomy
phylumotutab4 <- aggregate(. ~ Phylum, data=otutab4[,-c(1,3:6)], FUN=sum)
rownames(phylumotutab4) <- phylumotutab4[,1]
phylumotutab4 <- phylumotutab4[,-1]
```

Load the metadata:

```
sampleinfo3 <- read.csv('DataS8.csv',header=TRUE)
sampleinfo3$Lumen_bran <- factor(sampleinfo3$Lumen_bran,levels=c("FS","Lumen"
,"Bran"))
sampleinfo3$pH <-factor(sampleinfo3$pH,levels=c("5.8","6.8"))

sampleinfo4 <- read.csv('DataS9.csv',header=TRUE)
sampleinfo4$Lumen_bran <- factor(sampleinfo4$Lumen_bran,levels=c("FS","Lumen"
,"Bran"))
sampleinfo4$pH <-factor(sampleinfo4$pH,levels=c("5.8","6.8"))
```

Specify color vectors:

```
# Colors
fourcolors <- c("#DC143C","#8B5F65","#00008B","#EE9A00")
eightcolors=c("#7B4B71","#C5CD42","#C44F44","#91B7C0","#484934","#BA9C5F","#B
167C8","#69C467")

elevencolors = c("#000000","#CD6600","#36648B","#008B00","#7A378B","#838B83",
"#8B0000","#323C4D","#A03D44","#98B736","#C8ADA4")
fifteencolors <- c("#000000","#CD6600","#36648B","#008B00","#7A378B","#838B83
","#8B0000","#323C4D","#A03D44","#98B736","#C8ADA4","#BB5236","#7FCD50","#AB5
CC7","#C9A325")
```

```
thirtycolors=c("#323F24","#CB51D7","#72E245","#DE4F2D","#81DDC7","#6584C6","#
CFAE3C","#914261","#BFDC86","#CDC6BE","#D3469A","#3C315A","#607B30","#DD4469"
,"#5B9072","#CA9FC7","#7F592D","#5A656D","#D1867F","#4C2426","#79B6CE","#6E67
D1","#CBDC3F","#63D98B","#97362B","#CBB37D","#7E3F85","#CF8338","#5DA73A","#C
B80D0")

# Resolution of figures
dpi <- 300
```

Sanger sequences of the isolates are supplied as a compressed folder (Sanger_isolates.zip).

SCFA production during consecutive enrichment steps

The fecal sample (enrichment step 0) and the liquid broth in the enrichment Hungate tubes after each transfer (enrichment steps 1 to 4) were sampled for SCFA analysis.

```
SCFA <- melt(enrichment_SCFA,id.vars=c("Donor","pHstart","Enrichment","pHend"
))
SCFA1 <- subset(SCFA,Donor=="1")
SCFA2 <- subset(SCFA,Donor=="2")
SCFA3<- subset(SCFA,Donor=="3")
SCFA4<- subset(SCFA,Donor=="4")

p <- ggplot(data=SCFA1,aes(x=Enrichment,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pHstart~.,drop=TRUE,scale="free_x",space="free
_x") + scale_fill_manual(values=eightcolors,name=NULL)
p <- p+ ylab("Donor 1 SCFA (mM)") + xlab("Enrichment step") + theme(strip.tex
t.y = element_text(angle=0),legend.justification="center") +guides(fill=guide
_legend(nrow=2))
p <- p + geom_text(data=SCFA1,aes(x=Enrichment,y=100,label=pHend),size=6)
mylegendSCFA<-get_legend(p)
SCFAd1 <- p + theme(legend.position = "none",axis.title.x = element_text(colo
r="white"))

p <- ggplot(data=SCFA1,aes(x=Enrichment,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pHstart~.,drop=TRUE,scale="free_x",space="free
_x") + scale_fill_manual(values=eightcolors,name=NULL)
p <- p+ ylab("Donor 1 SCFA (mM)") + xlab("Enrichment step") + theme(strip.tex
t.y = element_text(angle=0),legend.justification="center") +guides(fill=guide
_legend(nrow=2))
p <- p + geom_text(data=SCFA1,aes(x=Enrichment,y=100,label=pHend),size=6)
mylegendSCFA<-get_legend(p)
SCFAd1 <- p + theme(legend.position = "none",axis.title.x = element_text(colo
r="white"))

p <- ggplot(data=SCFA2,aes(x=Enrichment,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
```

```
p <- papertheme(p) +facet_grid(pHstart~.,drop=TRUE,scale="free_x",space="free
_x") + scale_fill_manual(values=eightcolors)
p <- p+ ylab("Donor 2 SCFA (mM)") + xlab("Enrichment step") + theme(legend.te
xt=element_text(face='italic'),strip.text.y = element_text(angle=0)) +guides(
fill=guide_legend(nrow=4))
p <- p + geom_text(data=SCFA2,aes(x=Enrichment,y=85,label=pHend),size=6)
SCFAd2 <- p + theme(legend.position = "none",axis.title.x = element_text(colo
r="white"))

p <- ggplot(data=SCFA3,aes(x=Enrichment,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pHstart~.,drop=TRUE,scale="free_x",space="free
_x") + scale_fill_manual(values=eightcolors)
p <- p+ ylab("Donor 3 SCFA (mM)") + xlab("Enrichment step") + theme(legend.te
xt=element_text(face='italic'),strip.text.y = element_text(angle=0)) +guides(
fill=guide_legend(nrow=4))
p <- p + geom_text(data=SCFA3,aes(x=Enrichment,y=100,label=pHend),size=6)
SCFAd3 <- p + theme(legend.position = "none")

p <- ggplot(data=SCFA4,aes(x=Enrichment,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pHstart~.,drop=TRUE,scale="free_x",space="free
_x") + scale_fill_manual(values=eightcolors)
p <- p+ ylab("Donor 4 SCFA (mM)") + xlab("Enrichment step") + theme(legend.te
xt=element_text(face='italic'),strip.text.y = element_text(angle=0)) +guides(
fill=guide_legend(nrow=4))
p <- p + geom_text(data=SCFA4,aes(x=Enrichment,y=100,label=pHend),size=6)
SCFAd4 <- p + theme(legend.position = "none")

tiff("Figure2.tiff",width=10*dpi,height=16*dpi,res=dpi,compression="lzw")
cowplot::plot_grid(plot_grid(SCFAd1,SCFAd2,SCFAd3,SCFAd4,nrow=2,ncol=2,rel_he
ights =c(0.5,0.5,0.5,0.5),rel_widths=c(0.5,0.5,0.5,0.5),labels='AUTO',label_s
ize=20,label_fontfamily='sans'),mylegendSCFA,nrow=2,rel_heights=c(0.95,0.05))
dev.off()

## png
##   2

knitr::include_graphics("Figure2.tiff")
```

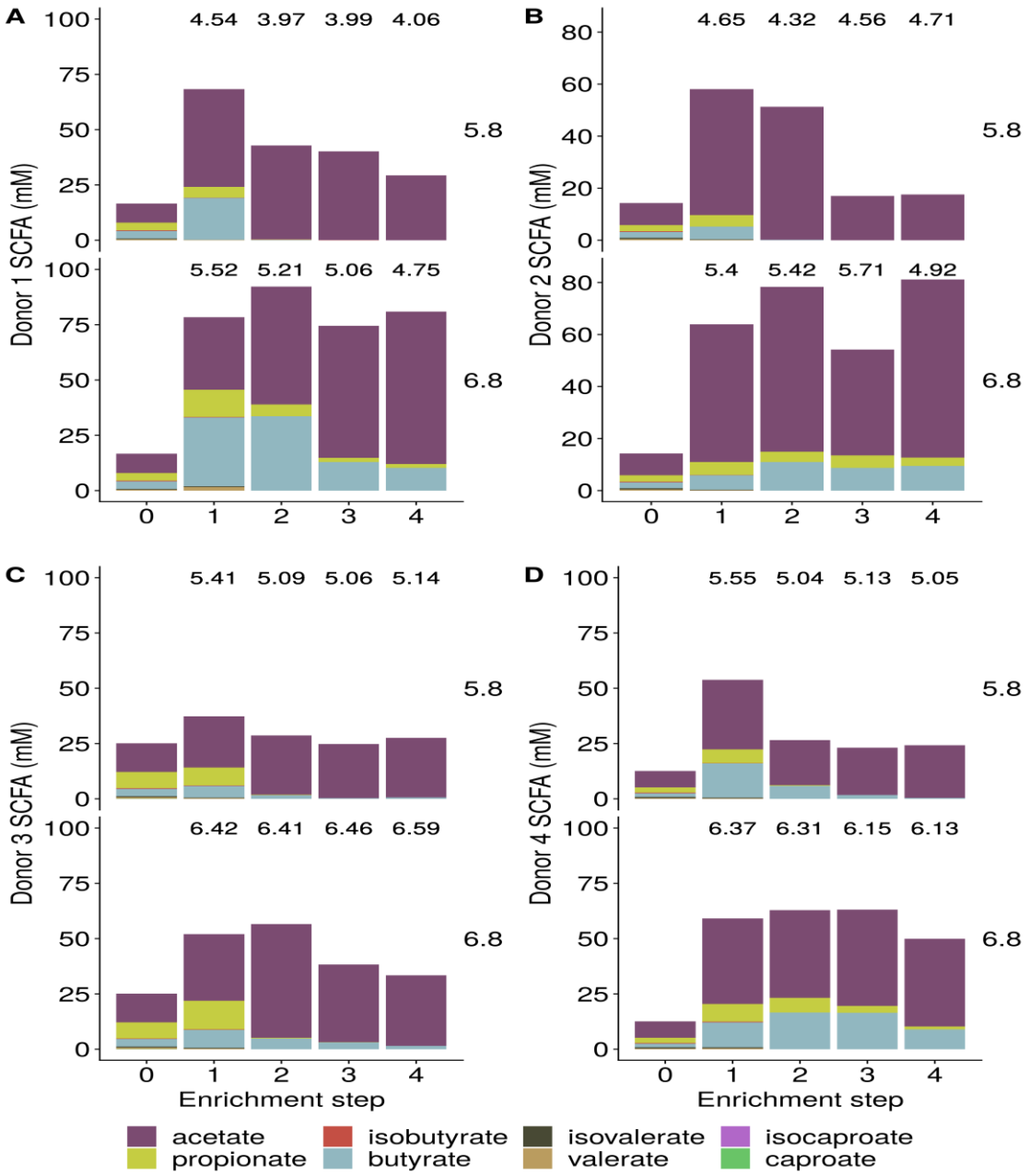

Figure 2: Short Chain Fatty Acid((SCFA) production by the fecal microbiota derived from four different donors during enrichment with wheat bran as the sole nutrient source. *The fecal sample (enrichment step 0) was incubated with wheat bran for 24 h (enrichment step 1), after which the wheat bran residue was washed to remove loosely attached bacteria and used to seed a new incubation (enrichment step 2). This procedure was repeated two more times (enrichment step 3 and 4). The pH is indicated on top of the stacked bars and decreased considerably comparing to the starting pH (5.8 and 6.8).*

Richness and Diversity indices calculated on the next-generation 16S rRNA gene amplicon sequencing confirm the 'enrichment'

The outcome of the enrichment procedure was assessed by computing richness (Chao1 Richness estimator) and diversity (Shannon, Simpson, inverse Simpson and Fisher alpha) estimators using vegan_2.4-4 (Oksanen et al. 2016).

```
dataunnormalised <- as.data.frame(t(shared3))
# Calculate the chao index
chaoest=apply(dataunnormalised,1,function(x) estimateR(x))[2,]
# Annotate the dataframe
richnessdf <- cbind(chaoest,sampleinfo3)
richnessdf$Enrichment <-as.numeric(as.character(richnessdf$Enrichment))
richness1 <- subset(richnessdf,Donor=="1"&Nameshared!="Donor2_low4_Brep.V4")
richness2 <- subset(richnessdf,Donor=="2")
richness3<- subset(richnessdf,Donor=="3")
# Repeat for donor 4
dataunnormalised <- as.data.frame(t(shared4))
chaoest=apply(dataunnormalised,1,function(x) estimateR(x))[2,]
richnessdf4 <- cbind(chaoest,sampleinfo4)
richnessdf4$Enrichment <- as.numeric(as.character(richnessdf4$Enrichment))
richness4 <- subset(richnessdf4,Donor=="4")

# Evolution of the chao richness during consecutive enrichment steps in donor
1 (Figure 3A in the manuscript)
p <- ggplot(data=richness1,aes(x=Enrichment,y=as.numeric(as.character(chaoest
))))
p <- p +geom_line(aes(group=Lumen_bran,linetype=Lumen_bran))+geom_point(aes(s
hape=Lumen_bran),size=5)
p <- papertheme(p) +facet_grid(pH~.,drop=TRUE,scale="free_x",space="free_x")
+ scale_fill_manual(values=eightcolors,name=NULL) + scale_shape_manual(name=N
ULL,values=c(15,16,17))+ scale_linetype_manual(name=NULL,values=c(0,1,2))
p <- p+ ylab("Chao1 richness estimator \n Donor1") + xlab("Enrichment step")
+ theme(strip.text.y = element_text(angle=0)) +guides(fill=guide_legend(nrow=
2))+ theme(legend.key.width = unit(2,"cm"))
richplot1 <- p + theme(legend.position = "none",axis.title.x = element_text(c
olor="white"))

# Evolution of the chao richness during consecutive enrichment steps in three
donors
richness <- rbind(richness1,richness2,richness3)
richness$Donor <- as.factor(richness$Donor)

p <- ggplot(data=richness,aes(x=Enrichment,y=as.numeric(as.character(chaoest)
)))
p <- p +geom_line(aes(group=interaction(Lumen_bran,Donor),color=Donor))+geom_
point(aes(shape=Lumen_bran,color=Donor),size=5)
p <- papertheme(p) +facet_grid(pH~.,drop=TRUE,scale="free_x",space="free_x")
+ scale_color_manual(values=fourcolors,name=NULL)
```

```r
p <- p+ ylab("Chao richness estimator") + xlab("Enrichment step") + theme(leg
end.text=element_text(face='italic'),strip.text.y = element_text(angle=0)) +g
uides(fill=guide_legend(nrow=2))
richplot <- p + theme(legend.position = "none",axis.title.x = element_text(co
lor="white"))

dataunnormalised <- as.data.frame(t(shared3))
# Calculate different diversity indices
divdf <- data.frame(shannon=apply(dataunnormalised,1,function(x) vegan::diver
sity(x)),simpson=apply(dataunnormalised,1,function(x) vegan::diversity(x,"sim
p")),invsimpson=apply(dataunnormalised,1,function(x) vegan::diversity(x,"inv"
)),fisher=apply(dataunnormalised,1,function(x) vegan::fisher.alpha(x)))
# Annotate the dataframe
divdf <- cbind(divdf,sampleinfo3)
divdf$Enrichment <- as.numeric(as.character(divdf$Enrichment))
divdf1 <- subset(divdf,Donor=="1"&Nameshared!="Donor2_low4_Brep.V4")
divdf1$Lumen_bran <- mapvalues(divdf1$Lumen_bran,from="FS",to="Fecal sample")

divdf2 <- subset(divdf,Donor=="2")
divdf3<- subset(divdf,Donor=="3")
# Repeat for donor 4
dataunnormalised <- as.data.frame(t(shared4))
divdf4 <- data.frame(shannon=apply(dataunnormalised,1,function(x) vegan::dive
rsity(x)),simpson=apply(dataunnormalised,1,function(x) vegan::diversity(x,"si
mp")),invsimpson=apply(dataunnormalised,1,function(x) vegan::diversity(x,"inv
")),fisher=apply(dataunnormalised,1,function(x) vegan::fisher.alpha(x)))
divdf4 <- cbind(divdf4,sampleinfo4)

# Evolution of the diversity indices during consecutive enrichment steps in d
onor 1 (Figure 3B in the manuscript)
p <- ggplot(data=divdf1,aes(x=Enrichment,y=as.numeric(as.character(shannon)))
)
p <- p +geom_line(aes(group=Lumen_bran,linetype=Lumen_bran))+geom_point(aes(s
hape=Lumen_bran),size=5)
p <- papertheme(p) +facet_grid(pH~.,drop=TRUE,scale="free_x",space="free_x")
+ scale_fill_manual(values=eightcolors,name=NULL) + scale_shape_manual(name=N
ULL,values=c(15,16,17)) + scale_linetype_manual(name=NULL,values=c(0,1,2))
p <- p+ ylab("Shannon diversity index \n Donor 1") + xlab("Enrichment step")
+ theme(strip.text.y = element_text(angle=0)) +guides(fill=guide_legend(nrow=
2)) + theme(legend.justification="center") + theme(legend.key.width = unit(2,
"cm"))
mylegendrichndiv <- get_legend(p)
divplot1 <- p + theme(legend.position = "none")
# Evolution of the diversity indices during consecutive enrichment steps thre
e donors
divdf <- rbind(divdf1,divdf2,divdf3)
divdf$Donor <- as.factor(divdf$Donor)

p <- ggplot(data=divdf,aes(x=Enrichment,y=as.numeric(as.character(shannon))))
p <- p +geom_line(aes(group=interaction(Lumen_bran,Donor),color=Donor))+geom_
```

```
point(aes(shape=Lumen_bran,color=Donor),size=5)
p <- papertheme(p) +facet_grid(pH~.,drop=TRUE,scale="free_x",space="free_x")
+ scale_color_manual(values=fourcolors,name=NULL)
p <- p+ ylab("Chao richness estimator") + xlab("Enrichment step") + theme(leg
end.text=element_text(face='italic'),strip.text.y = element_text(angle=0)) +g
uides(fill=guide_legend(nrow=2))
p <- p + theme(legend.position = "none",axis.title.x = element_text(color="wh
ite"))

tiff("Figure3.tiff",width=6*dpi,height=14*dpi,res=dpi,compression="lzw")
cowplot::plot_grid(plot_grid(richplot1,divplot1,ncol=1,rel_heights = c(0.5,0.
5),labels='AUTO',label_size=20,label_fontfamily='sans'),mylegendrichndiv,nrow
=2,rel_heights=c(0.95,0.05))
dev.off()

## png
##   2

include_graphics("Figure3.tiff")
```

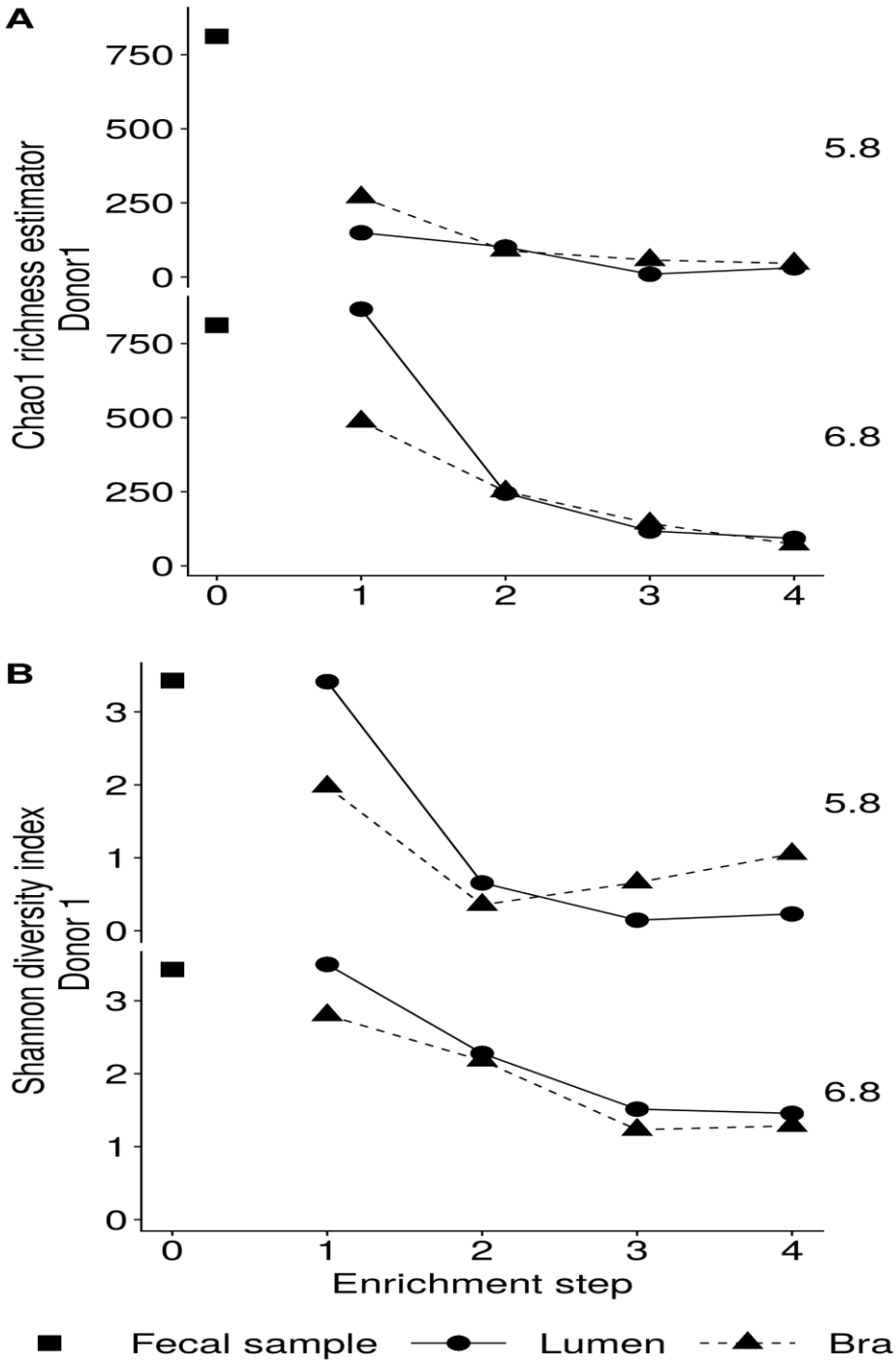

Figure 3: Reduction in the microbial community richness (Chao 1 richness estimator) and diversity (Shannon diversity index) during consecutive enrichment steps with wheat bran as the sole nutrient source, as shown for donor 1. *The fecal sample (enrichment step 0) was incubated with wheat bran for 24 h (enrichment step 1), after which the wheat bran residue was washed to remove loosely attached bacteria and used to seed a new incubation (enrichment step 2). This procedure was repeated two more times (enrichment step 3 and 4).*

Identification of the enriched species by next-generation 16S rRNA gene amplicon sequencing

Bar graphs of the proporional microbial community composition at phylum, genus, and species level were created to demonstrate community shifts during consecutive enrichment steps with wheat bran as the sole nutrient source.

```
otuspec <- phylumotutab3
otuspec <- 100*otuspec
otuspec <- rbind(names=colnames(otuspec),otuspec)
otuspec <- mutate(as.data.frame(t(otuspec)),donor=sampleinfo3$Donor,LBM=sampl
einfo3$Lumen_bran,enrichment=sampleinfo3$Enrichment,pH=sampleinfo3$pH)
otuspec <- melt(otuspec,id.vars=c('donor','LBM','enrichment','pH','names'))
otuspec1 <- subset(otuspec,donor=="1"&enrichment!="c"&names!="Donor2_low4_Bre
p.V4")
otuspec2 <- subset(otuspec,donor=="2"&enrichment!="c"&names!="Donor2_low4_Bre
p.V4")
otuspec3<- subset(otuspec,donor=="3"&enrichment!="c"&names!="Donor2_low4_Brep
.V4")

# Donor1
p <- ggplot(data=otuspec1,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~enrichment,drop=TRUE,scale="free_x",space="
free_x") + scale_fill_manual(values=elevencolors)
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),strip.text.y = element_text(angle=0)) +guides(fill=gui
de_legend(nrow=4))

# Donor2
p <- ggplot(data=otuspec2,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~enrichment,drop=TRUE,scale="free_x",space="
free_x") + scale_fill_manual(values=elevencolors)
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),strip.text.y = element_text(angle=0)) +guides(fill=gui
de_legend(nrow=4))

#Donor3
p <- ggplot(data=otuspec3,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~enrichment,drop=TRUE,scale="free_x",space="
free_x") + scale_fill_manual(values=elevencolors)
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),strip.text.y = element_text(angle=0)) +guides(fill=gui
de_legend(nrow=4))

otuspec<- genusotutab3
otuspec <- cbind(sampleinfo3,t(otuspec))
```

```
otuspec <- subset(otuspec,Enrichment!="c")
otuspec <- otuspec[,-c(1:length(colnames(sampleinfo3)))]
otuspec <- data.frame(t(otuspec))
selecttop20par <- rowSums(otuspec)
otuspec <- tibble::rownames_to_column(otuspec)
otuspec <- as.data.frame(otuspec  %>% top_n(13,selecttop20par))
other <- 1-colSums(otuspec[,-1])
rownames(otuspec) <- otuspec$rowname
otuspec <- otuspec[,-1]
otuspec <- rbind(otuspec,other)
rownames(otuspec)[nrow(otuspec)] <- "Other"
otuspec <- 100*otuspec

sampleinfosub3 <- subset(sampleinfo3,Enrichment!="c")
otuspec <- mutate(as.data.frame(t(otuspec)),donor=sampleinfosub3$Donor,LBM=sa
mpleinfosub3$Lumen_bran,enrichment=sampleinfosub3$Enrichment,pH=sampleinfosub
3$pH,names=colnames(otuspec))
otuspec <- melt(otuspec,id.vars=c('donor','LBM','enrichment','pH','names'))
otuspec1 <- subset(otuspec,donor=="1"&names!="Donor2_low4_Brep.V4")
otuspec2 <- subset(otuspec,donor=="2"&names!="Donor2_low4_Brep.V4")
otuspec3<- subset(otuspec,donor=="3"&names!="Donor2_low4_Brep.V4")

# Donor1
p <- ggplot(data=otuspec1,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~enrichment,drop=TRUE,scale="free_x",space="
free_x") + scale_fill_manual(values=fifteencolors,name="Genus")
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),strip.text.y = element_text(angle=0),legend.position="
right") +guides(fill=guide_legend(ncol=1))
bargraph1genus <- p

# Donor2
p <- ggplot(data=otuspec2,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~enrichment,drop=TRUE,scale="free_x",space="
free_x") + scale_fill_manual(values=fifteencolors,name="Genus")
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),strip.text.y = element_text(angle=0),legend.position="
right") +guides(fill=guide_legend(ncol=1))

# Donor3
p <- ggplot(data=otuspec3,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~enrichment,drop=TRUE,scale="free_x",space="
free_x") + scale_fill_manual(values=fifteencolors,name="Genus")
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),strip.text.y = element_text(angle=0),legend.position="
right") +guides(fill=guide_legend(ncol=1))
```

```r
# Donor4
otuspec<- genusotutab4
selecttop20par <- rowSums(otuspec)
otuspec <- tibble::rownames_to_column(otuspec)
otuspec <- as.data.frame(otuspec  %>% top_n(13,selecttop20par))
other <- 1-colSums(otuspec[,-1])
rownames(otuspec) <- otuspec$rowname
otuspec <- otuspec[,-1]
otuspec <- rbind(otuspec,other)
rownames(otuspec)[nrow(otuspec)] <- "Other"
otuspec <- otuspec*100

otuspec <- mutate(as.data.frame(t(otuspec)),donor=sampleinfo4$Donor,LBM=sampl
einfo4$Lumen_bran,enrichment=sampleinfo4$Enrichment,pH=sampleinfo4$pH,names=c
olnames(otuspec))
otuspec4 <- melt(otuspec,id.vars=c('donor','LBM','enrichment','pH','names'))

p <- ggplot(data=otuspec4,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~enrichment,drop=TRUE,scale="free_x",space="
free_x") + scale_fill_manual(values=fifteencolors,name="Genus")
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),strip.text.y = element_text(angle=0),legend.position="
right") +guides(fill=guide_legend(ncol=1))

# Species level annotation of OTUs (verified by BLAST and RDP)
label3donorsillu <- c("OTU1 Bifidobacterium sp.","OTU2 Enterobacteriaceae sp.
","OTU3 Escherichia/Shigella sp.","OTU4 Lactobacillus sp.","OTU5 Faecalibacte
rium prausnitzii","OTU6 Prevotella copri","OTU7 Faecalibacterium prausnitzii"
,"OTU8 Pediococcus sp.","OTU9 E. rectale/R. faecis","OTU10 Fusobacterium mort
iferum","OTU11 Bacteroides dorei","OTU12 Dialister sp.","OTU13 Sutterella wad
sworthensis","OTU14 Lactobacillus sp.","OTU15","OTU16 Bacteroides eggerthii",
"OTU17 Blautia wexlerae","OTU18 Lactobacillus fermentus","OTU19 Fusicatenibac
ter saccharivorans","OTU20 Roseburia faecis","Other")
otuspec<-otutab3[,-c(1:6)]
otuspec <- cbind(sampleinfo3,t(otuspec))
otuspec <- subset(otuspec,Enrichment!="c")
otuspec <- otuspec[,-c(1:length(colnames(sampleinfo3)))]
otuspec <- data.frame(t(otuspec))
selecttop20par <- rowSums(otuspec)
otuspec <- tibble::rownames_to_column(otuspec)
otuspec <- as.data.frame(otuspec  %>% top_n(20,selecttop20par))
other <- 1-colSums(otuspec[,-1])
rownames(otuspec) <- otuspec$rowname
otuspec <- otuspec[,-1]
otuspec <- rbind(otuspec,other)
rownames(otuspec)[nrow(otuspec)] <- "Other"
otuspec <- 100*otuspec

sampleinfosub3 <- subset(sampleinfo3,Enrichment!="c")
```

```
otuspec <- mutate(as.data.frame(t(otuspec)),donor=sampleinfosub3$Donor,LBM=sa
mpleinfosub3$Lumen_bran,enrichment=sampleinfosub3$Enrichment,pH=sampleinfosub
3$pH,names=colnames(otuspec))
otuspec <- melt(otuspec,id.vars=c('donor','LBM','enrichment','pH','names'))
otuspec1 <- subset(otuspec,donor=="1"&names!="Donor2_low4_Brep.V4")
otuspec2 <- subset(otuspec,donor=="2"&names!="Donor2_low4_Brep.V4")
otuspec3<- subset(otuspec,donor=="3"&names!="Donor2_low4_Brep.V4")

# Donor1
p <- ggplot(data=otuspec1,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~enrichment,drop=TRUE,scale="free_x",space="
free_x") + scale_fill_manual(values=thirtycolors,name="Species",labels=label3
donorsillu)
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),strip.text.y = element_text(angle=0),legend.position="
right") +guides(fill=guide_legend(ncol=1))
bargraph1species <- p

#Donor2
p <- ggplot(data=otuspec2,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~enrichment,drop=TRUE,scale="free_x",space="
free_x") + scale_fill_manual(values=thirtycolors,name="Species")
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),legend.position="right",strip.text.y = element_text(an
gle=0)) +guides(fill=guide_legend(ncol=1))

# Donor3
p <- ggplot(data=otuspec3,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~enrichment,drop=TRUE,scale="free_x",space="
free_x") + scale_fill_manual(values=thirtycolors,name="Species")
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),strip.text.y = element_text(angle=0),legend.position="
right") +guides(fill=guide_legend(ncol=1))

tiff("Figure4.tiff",width=16*dpi,height=14*dpi,res=dpi,compression="lzw")
cowplot::plot_grid(bargraph1genus,bargraph1species,nrow=2,rel_heights =c(0.5,
0.5),labels='AUTO',label_size=20,label_fontfamily='sans',align="v",axis="1")
dev.off()

## png
##   2

knitr::include_graphics("Figure4.tiff")
```

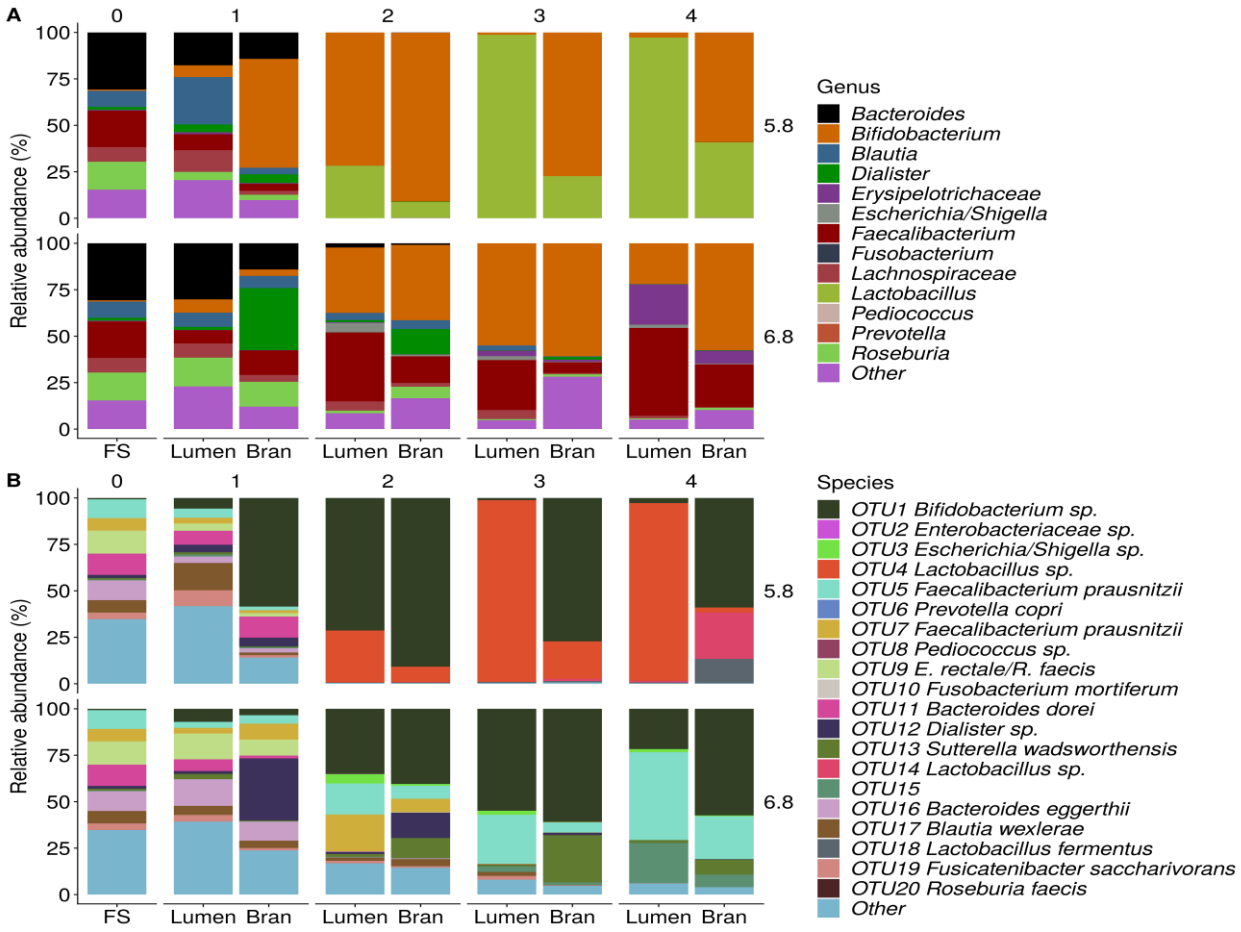

Figure 4: Shifts in genus (A) and species (B) level microbial community composition of donor 1 during consecutive enrichment steps with wheat bran as the sole nutrient source. *The fecal sample (FS; enrichment step 0) was incubated with wheat bran for 24 h (enrichment step 1), after which the wheat bran residue was washed to remove loosely attached bacteria and used to seed a new incubation (enrichment step 2). This procedure was repeated two more times (enrichment step 3 and 4). Family level taxa appearing in the genus level plots should be interpreted as unclassified genus belonging to the respective family.*

```
# Genus level
otuspec<- genusotutab3
otuspec <- cbind(sampleinfo3,t(otuspec))
otuspec <- subset(otuspec,Enrichment=="4"|Enrichment=="0")
otuspec <- otuspec[,-c(1:length(colnames(sampleinfo3)))]
otuspec <- data.frame(t(otuspec))
selecttop20par <- rowSums(otuspec)
otuspec <- tibble::rownames_to_column(otuspec)
otuspec <- as.data.frame(otuspec  %>% top_n(13,selecttop20par))
other <- 1-colSums(otuspec[,-1])
rownames(otuspec) <- otuspec$rowname
otuspec <- otuspec[,-1]
```

```
otuspec <- rbind(otuspec,other)
rownames(otuspec)[nrow(otuspec)] <- "Other"
otuspec <- 100*otuspec

sampleinfosub3 <- subset(sampleinfo3,Enrichment=="4"|Enrichment=="0")
otuspec <- mutate(as.data.frame(t(otuspec)),donor=sampleinfosub3$Donor,LBM=sa
mpleinfosub3$Lumen_bran,enrichment=sampleinfosub3$Enrichment,pH=sampleinfosub
3$pH,names=colnames(otuspec))
otuspec <- melt(otuspec,id.vars=c('donor','LBM','enrichment','pH','names'))
otuspec23 <- subset(otuspec,donor=="2"|donor=="3")
otuspec23$donor <- mapvalues(otuspec23$donor,from=c("2","3"),to=c("Donor2","D
onor3"))

p <- ggplot(data=otuspec23,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~donor,drop=TRUE,scale="free_x",space="free_
x") + scale_fill_manual(values=fifteencolors,name="Genus")
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),strip.text.y = element_text(angle=0),legend.position="
right") +guides(fill=guide_legend(ncol=1))
bargraph23genus <- p

# Species level
otuspec<- otutab3[,-c(1:6)]
otuspec <- cbind(sampleinfo3,t(otuspec))
otuspec <- subset(otuspec,Enrichment=="4"|Enrichment=="0")
otuspec <- otuspec[,-c(1:length(colnames(sampleinfo3)))]
otuspec <- data.frame(t(otuspec))
selecttop20par <- rowSums(otuspec)
otuspec <- tibble::rownames_to_column(otuspec)
otuspec <- as.data.frame(otuspec  %>% top_n(20,selecttop20par))
other <- 1-colSums(otuspec[,-1])
rownames(otuspec) <- otuspec$rowname
otuspec <- otuspec[,-1]
otuspec <- rbind(otuspec,other)
rownames(otuspec)[nrow(otuspec)] <- "Other"
otuspec <- 100*otuspec

sampleinfosub3 <- subset(sampleinfo3,Enrichment=="4"|Enrichment=="0")
otuspec <- mutate(as.data.frame(t(otuspec)),donor=sampleinfosub3$Donor,LBM=sa
mpleinfosub3$Lumen_bran,enrichment=sampleinfosub3$Enrichment,pH=sampleinfosub
3$pH,names=colnames(otuspec))
otuspec <- melt(otuspec,id.vars=c('donor','LBM','enrichment','pH','names'))
otuspec23 <- subset(otuspec,donor=="2"|donor=="3")
otuspec23$donor <- mapvalues(otuspec23$donor,from=c("2","3"),to=c("Donor2","D
onor3"))

p <- ggplot(data=otuspec23,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~donor,drop=TRUE,scale="free_x",space="free_
```

```
x") + scale_fill_manual(values=thirtycolors,name="Species",labels=label3donor
sillu)
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),strip.text.y = element_text(angle=0),legend.position="
right") +guides(fill=guide_legend(ncol=1))
bargraph23species <- p

tiff("Figure5.tiff",width=12*dpi,height=16*dpi,res=dpi,compression="lzw")
cowplot::plot_grid(bargraph23genus,bargraph23species,nrow=2,rel_heights =c(0.
5,0.5),labels='AUTO',label_size=20,label_fontfamily='sans',align="v",axis="1"
)
dev.off()

## png
##   2

include_graphics("Figure5.tiff")
```

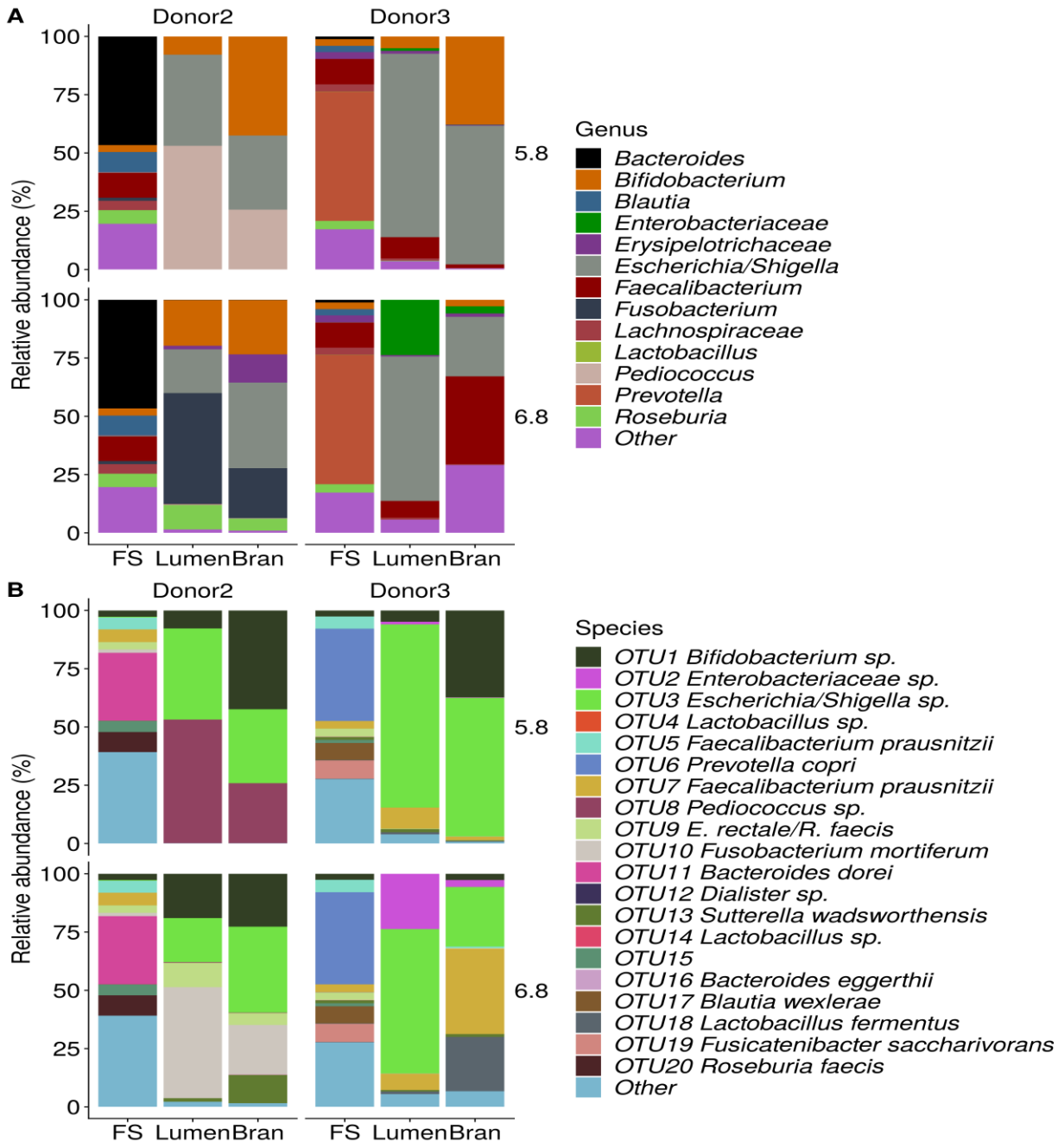

Figure 5: Shifts in genus (A) and species (B) level microbial community composition of donor 2 and 3 after four enrichment steps with wheat bran as the sole nutrient source. *The fecal sample (FS; enrichment step 0) was incubated with wheat bran for 24 h (enrichment step 1), after which the wheat bran residue was washed to remove loosely attached bacteria and used to seed a new incubation (enrichment step 2). This procedure was repeated two more times (enrichment step 3 and 4). Only the final enrichment step and FS are shown in this plot. Family level taxa appearing in the genus level plots should be interpreted as unclassified genus belonging to the respective family.*

```r
# Define additional colors
fifteencolorsbis <- c("#C9A325","#000000","#CD6600","#36648B","#008B00","#838B83","#8B0000","#323C4D","#A03D44","#98B736","#7FCD50","#BB5236","#C8ADA4","#AB5CC7","#7A378B")

# Species level annotation of OTUs (verified by BLAST and RDP)
label4donorsillu <- c("OTU1 Escherichia/Shigella sp.","OTU2 Bifidobacterium sp.","OTU3 Faecalibacterium prausnitzii","OTU4 Lactobacillus gasseri","OTU5 Clostridium XVII","OTU6 Lachnospiraceae sp.","OTU7 Fusicatenibacter saccharivorans","OTU8 Clostridium XIVa sp.","OTU9 Collinsella aerofaciens","OTU10 Eubacterium rectale","OTU11 Bacteroides vulgatus","OTU12 Bifidobacterium longum","OTU13 Gemmiger formicilis","OTU14 Dialister invisus","Other")

otuspec<- genusotutab4
selecttop20par <- rowSums(otuspec)
otuspec <- tibble::rownames_to_column(otuspec)
otuspec <- as.data.frame(otuspec  %>% top_n(13,selecttop20par))
other <- 1-colSums(otuspec[,-1])
rownames(otuspec) <- otuspec$rowname
otuspec <- otuspec[,-1]
otuspec <- rbind(otuspec,other)
rownames(otuspec)[nrow(otuspec)] <- "Other"
otuspec <- otuspec*100

otuspec <- mutate(as.data.frame(t(otuspec)),donor=sampleinfo4$Donor,LBM=sampleinfo4$Lumen_bran,enrichment=sampleinfo4$Enrichment,pH=sampleinfo4$pH,names=colnames(otuspec))
otuspec <- otuspec[c(5,6,9,10),]
otuspec4 <- melt(otuspec,id.vars=c('donor','LBM','enrichment','pH','names'))

p <- ggplot(data=otuspec4,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~.,drop=TRUE,scale="free_x",space="free_x")
+ scale_fill_manual(values=fifteencolorsbis,name="Genus")
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=element_text(face='italic'),legend.position="right",strip.text.y = element_text(angle=0)) +guides(fill=guide_legend(ncol=1))
bargraph4genus <-p

# Species
otuspec<- otutab4[,-c(1:6)]
selecttop20par <- rowSums(otuspec)
otuspec <- tibble::rownames_to_column(otuspec)
otuspec <- as.data.frame(otuspec  %>% top_n(14,selecttop20par))
other <- 1-colSums(otuspec[,-1])
rownames(otuspec) <- otuspec$rowname
otuspec <- otuspec[,-1]
otuspec <- rbind(otuspec,other)
rownames(otuspec)[nrow(otuspec)] <- "Other"
otuspec <- otuspec*100
```

```
otuspec <- mutate(as.data.frame(t(otuspec)),donor=sampleinfo4$Donor,LBM=sampl
einfo4$Lumen_bran,enrichment=sampleinfo4$Enrichment,pH=sampleinfo4$pH,names=c
olnames(otuspec))
otuspec <- otuspec[c(5,6,9,10),]
otuspec4 <- melt(otuspec,id.vars=c('donor','LBM','enrichment','pH','names'))

p <- ggplot(data=otuspec4,aes(x=LBM,y=as.numeric(as.character(value))))
p <- p +geom_bar(stat="identity",aes(fill=variable))
p <- papertheme(p) +facet_grid(pH~.,drop=TRUE,scale="free_x",space="free_x")
+ scale_fill_manual(values=thirtycolors,name="Species",label=label4donorsillu
)
p <- p+ ylab("Relative abundance (%)") + xlab(NULL) + theme(legend.text=eleme
nt_text(face='italic'),legend.position="right",strip.text.y = element_text(an
gle=0)) +guides(fill=guide_legend(ncol=1))
bargraph4species <- p

tiff("Figure6.tiff",width=12*dpi,height=10*dpi,res=dpi,compression="lzw")
cowplot::plot_grid(bargraph4genus,bargraph4species,nrow=2,rel_heights =c(0.5,
0.5),labels='AUTO',label_size=20,label_fontfamily='sans',align="v",axis="1")
dev.off()

## png
##   2

include_graphics("Figure6.tiff")
```

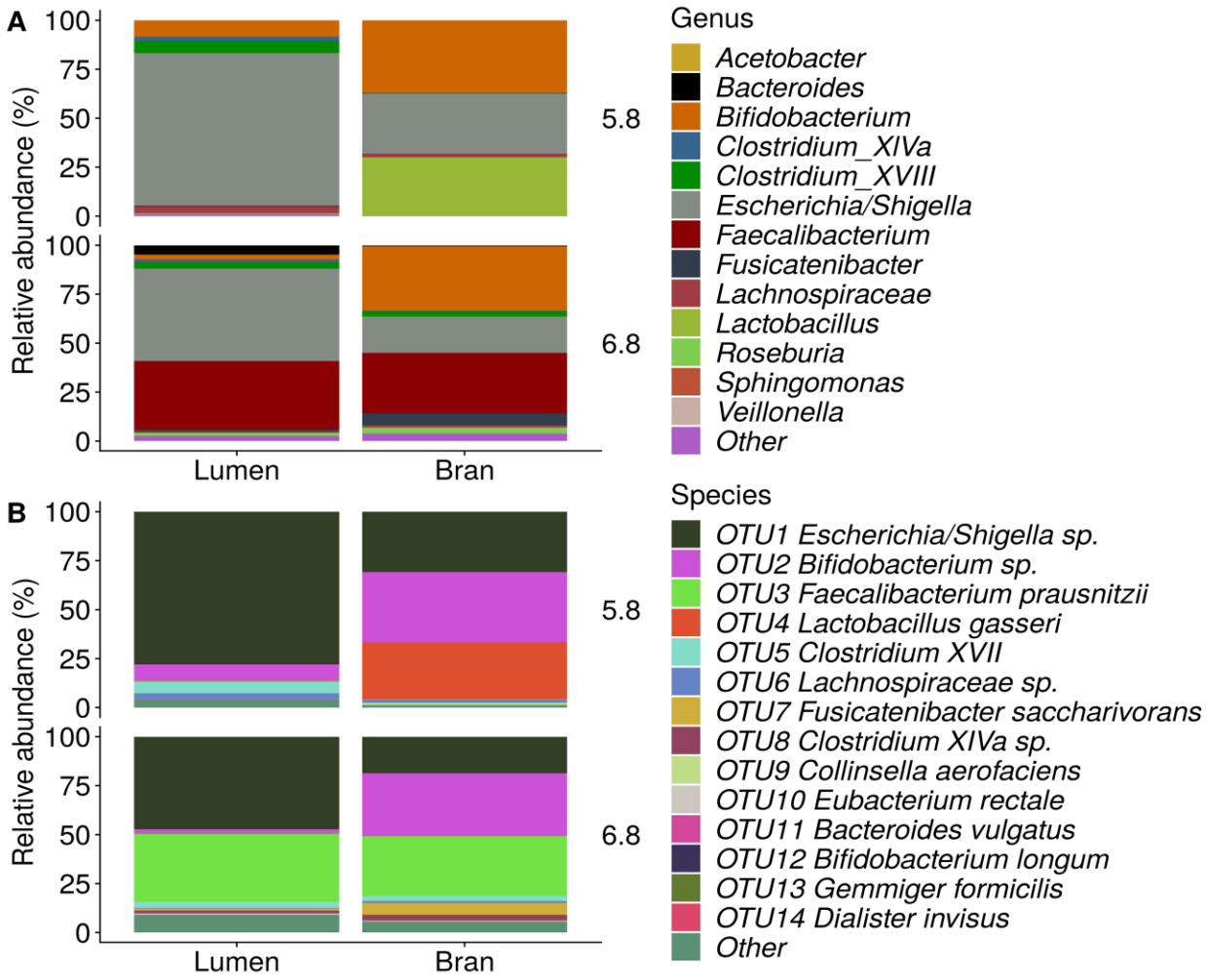

Figure 6: Genus (A) and species (B) level microbial community composition of donor 4 after the final enrichment step with wheat bran as the sole nutrient source. *The fecal sample (FS; enrichment step 0) was incubated with wheat bran for 24 h (enrichment step 1), after which the wheat bran residue was washed to remove loosely attached bacteria and used to seed a new incubation (enrichment step 2). This procedure was repeated two more times (enrichment step 3 and 4). Only the final enrichment step was shown in this plot, due to a low read number (<100 reads) in the fecal sample. Family level taxa appearing in the genus level plots should be interpreted as unclassified genus belonging to the respective family.*

Build consensus sanger sequences

The OTUs, resulting from 16S rRNA gene amplicon sequencing of the consecutive enrichments were compared to the 16S rRNA gene Sanger reads of the obtained isolates by means of a phylogenetic placement analysis. Forward and reverse Sanger sequences for each donor were grouped into separate files and reverse complements and summary statistics were obtained using the mothur software package (v.1.39.5) (Schloss et al. 2009). The 515F-806R primer pair, used for Illumina MiSeq 16S rRNA gene amplicon sequencing was located

in the Sanger reads (forward and reverse compliment). In case both primers were not present on one and the same read (either forward, or reverse), consensus sequences (contigs) were generated using the sangeranalyseR package (version 0.1.0) (Lanfear 2015). Contigs with more than 100 degenerated positions, indicative of a poor quality alignment, were omitted.

```
# Summarize data
sf = summarise.abi.folder("Sanger_isolates")

## [1] "Looking for .ab1 files..."
## [1] "Found 256 .ab1 files..."
## [1] "Loading reads..."
## [1] "Calculating read summaries..."
## [1] "Cleaning up"

sf$summaries

##                       file.path     folder.name  file.name raw.length
## 1   Sanger_isolates/100F.F.ab1 Sanger_isolates 100F.F.ab1        891
## 2   Sanger_isolates/100R.R.ab1 Sanger_isolates 100R.R.ab1        485
## 3   Sanger_isolates/101F.F.ab1 Sanger_isolates 101F.F.ab1        793
## 4   Sanger_isolates/101R.R.ab1 Sanger_isolates 101R.R.ab1       1128
## 5   Sanger_isolates/102F.F.ab1 Sanger_isolates 102F.F.ab1       1164
## 6   Sanger_isolates/102R.R.ab1 Sanger_isolates 102R.R.ab1       1100
## 7   Sanger_isolates/103F.F.ab1 Sanger_isolates 103F.F.ab1       1117
## 8   Sanger_isolates/103R.R.ab1 Sanger_isolates 103R.R.ab1       1115
## 9   Sanger_isolates/104F.F.ab1 Sanger_isolates 104F.F.ab1       1165
## 10  Sanger_isolates/104R.R.ab1 Sanger_isolates 104R.R.ab1       1166
## 11  Sanger_isolates/105F.F.ab1 Sanger_isolates 105F.F.ab1       1074
## 12  Sanger_isolates/105R.R.ab1 Sanger_isolates 105R.R.ab1        805
## 13  Sanger_isolates/106F.F.ab1 Sanger_isolates 106F.F.ab1        894
## 14  Sanger_isolates/106R.R.ab1 Sanger_isolates 106R.R.ab1       1116
## 15  Sanger_isolates/107F.F.ab1 Sanger_isolates 107F.F.ab1       1021
## 16  Sanger_isolates/107R.R.ab1 Sanger_isolates 107R.R.ab1       1133
## 17  Sanger_isolates/108F.F.ab1 Sanger_isolates 108F.F.ab1        962
## 18  Sanger_isolates/108R.R.ab1 Sanger_isolates 108R.R.ab1       1088
## 19  Sanger_isolates/109F.F.ab1 Sanger_isolates 109F.F.ab1       1126
## 20  Sanger_isolates/109R.R.ab1 Sanger_isolates 109R.R.ab1       1148
## 21   Sanger_isolates/10F.F.ab1 Sanger_isolates  10F.F.ab1       1045
## 22   Sanger_isolates/10R.R.ab1 Sanger_isolates  10R.R.ab1       1104
## 23  Sanger_isolates/110F.F.ab1 Sanger_isolates 110F.F.ab1        876
## 24  Sanger_isolates/110R.R.ab1 Sanger_isolates 110R.R.ab1       1033
## 25   Sanger_isolates/11F.F.ab1 Sanger_isolates  11F.F.ab1        546
## 26   Sanger_isolates/11R.R.ab1 Sanger_isolates  11R.R.ab1        683
## 27  Sanger_isolates/128F.F.ab1 Sanger_isolates 128F.F.ab1       1017
## 28  Sanger_isolates/128R.R.ab1 Sanger_isolates 128R.R.ab1       1196
## 29  Sanger_isolates/129F.F.ab1 Sanger_isolates 129F.F.ab1        711
## 30  Sanger_isolates/129R.R.ab1 Sanger_isolates 129R.R.ab1       1073
## 31  Sanger_isolates/130F.F.ab1 Sanger_isolates 130F.F.ab1        546
## 32  Sanger_isolates/130R.R.ab1 Sanger_isolates 130R.R.ab1        489
## 33  Sanger_isolates/131F.F.ab1 Sanger_isolates 131F.F.ab1        977
```

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## 34  Sanger_isolates/131R.R.ab1 Sanger_isolates 131R.R.ab1       1105
## 35  Sanger_isolates/132F.F.ab1 Sanger_isolates 132F.F.ab1        200
## 36  Sanger_isolates/132R.R.ab1 Sanger_isolates 132R.R.ab1        199
## 37  Sanger_isolates/133F.F.ab1 Sanger_isolates 133F.F.ab1        199
## 38  Sanger_isolates/133R.R.ab1 Sanger_isolates 133R.R.ab1       1001
## 39  Sanger_isolates/134F.F.ab1 Sanger_isolates 134F.F.ab1        287
## 40  Sanger_isolates/134R.R.ab1 Sanger_isolates 134R.R.ab1        341
## 41  Sanger_isolates/135F.F.ab1 Sanger_isolates 135F.F.ab1        993
## 42  Sanger_isolates/135R.R.ab1 Sanger_isolates 135R.R.ab1        129
## 43  Sanger_isolates/136F.F.ab1 Sanger_isolates 136F.F.ab1        920
## 44  Sanger_isolates/136R.R.ab1 Sanger_isolates 136R.R.ab1       1109
## 45  Sanger_isolates/137F.F.ab1 Sanger_isolates 137F.F.ab1        735
## 46  Sanger_isolates/137R.R.ab1 Sanger_isolates 137R.R.ab1        691
## 47  Sanger_isolates/138F.F.ab1 Sanger_isolates 138F.F.ab1        714
## 48  Sanger_isolates/138R.R.ab1 Sanger_isolates 138R.R.ab1        872
## 49  Sanger_isolates/139F.F.ab1 Sanger_isolates 139F.F.ab1        610
## 50  Sanger_isolates/139R.R.ab1 Sanger_isolates 139R.R.ab1        382
## 51   Sanger_isolates/13F.F.ab1 Sanger_isolates  13F.F.ab1        604
## 52   Sanger_isolates/13R.R.ab1 Sanger_isolates  13R.R.ab1        957
## 53  Sanger_isolates/140F.F.ab1 Sanger_isolates 140F.F.ab1        542
## 54  Sanger_isolates/140R.R.ab1 Sanger_isolates 140R.R.ab1        943
## 55  Sanger_isolates/141F.F.ab1 Sanger_isolates 141F.F.ab1       1060
## 56  Sanger_isolates/141R.R.ab1 Sanger_isolates 141R.R.ab1        779
## 57  Sanger_isolates/142F.F.ab1 Sanger_isolates 142F.F.ab1        680
## 58  Sanger_isolates/142R.R.ab1 Sanger_isolates 142R.R.ab1        707
## 59  Sanger_isolates/143F.F.ab1 Sanger_isolates 143F.F.ab1       1067
## 60  Sanger_isolates/143R.R.ab1 Sanger_isolates 143R.R.ab1       1019
## 61  Sanger_isolates/144F.F.ab1 Sanger_isolates 144F.F.ab1        905
## 62  Sanger_isolates/144R.R.ab1 Sanger_isolates 144R.R.ab1       1059
## 63  Sanger_isolates/145F.F.ab1 Sanger_isolates 145F.F.ab1       1049
## 64  Sanger_isolates/145R.R.ab1 Sanger_isolates 145R.R.ab1        650
## 65  Sanger_isolates/146F.F.ab1 Sanger_isolates 146F.F.ab1       1121
## 66  Sanger_isolates/146R.R.ab1 Sanger_isolates 146R.R.ab1       1145
## 67  Sanger_isolates/147F.F.ab1 Sanger_isolates 147F.F.ab1       1002
## 68  Sanger_isolates/147R.R.ab1 Sanger_isolates 147R.R.ab1       1050
## 69  Sanger_isolates/148F.F.ab1 Sanger_isolates 148F.F.ab1        736
## 70  Sanger_isolates/148R.R.ab1 Sanger_isolates 148R.R.ab1        390
## 71  Sanger_isolates/149F.F.ab1 Sanger_isolates 149F.F.ab1        660
## 72  Sanger_isolates/149R.R.ab1 Sanger_isolates 149R.R.ab1       1139
## 73  Sanger_isolates/150F.F.ab1 Sanger_isolates 150F.F.ab1        505
## 74  Sanger_isolates/150R.R.ab1 Sanger_isolates 150R.R.ab1        937
## 75  Sanger_isolates/151F.F.ab1 Sanger_isolates 151F.F.ab1        706
## 76  Sanger_isolates/151R.R.ab1 Sanger_isolates 151R.R.ab1        999
## 77  Sanger_isolates/152F.F.ab1 Sanger_isolates 152F.F.ab1        614
## 78  Sanger_isolates/152R.R.ab1 Sanger_isolates 152R.R.ab1        843
## 79  Sanger_isolates/153F.F.ab1 Sanger_isolates 153F.F.ab1        703
## 80  Sanger_isolates/153R.R.ab1 Sanger_isolates 153R.R.ab1        933
## 81  Sanger_isolates/154F.F.ab1 Sanger_isolates 154F.F.ab1        943
## 82  Sanger_isolates/154R.R.ab1 Sanger_isolates 154R.R.ab1       1221
## 83  Sanger_isolates/155F.F.ab1 Sanger_isolates 155F.F.ab1        752
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## 84  Sanger_isolates/155R.R.ab1 Sanger_isolates 155R.R.ab1       1122
## 85  Sanger_isolates/156F.F.ab1 Sanger_isolates 156F.F.ab1        610
## 86  Sanger_isolates/156R.R.ab1 Sanger_isolates 156R.R.ab1       1034
## 87  Sanger_isolates/157F.F.ab1 Sanger_isolates 157F.F.ab1        712
## 88  Sanger_isolates/157R.R.ab1 Sanger_isolates 157R.R.ab1       1115
## 89  Sanger_isolates/158F.F.ab1 Sanger_isolates 158F.F.ab1        874
## 90  Sanger_isolates/158R.R.ab1 Sanger_isolates 158R.R.ab1        990
## 91  Sanger_isolates/159F.F.ab1 Sanger_isolates 159F.F.ab1        702
## 92  Sanger_isolates/159R.R.ab1 Sanger_isolates 159R.R.ab1       1103
## 93   Sanger_isolates/15F.F.ab1 Sanger_isolates  15F.F.ab1        829
## 94   Sanger_isolates/15R.R.ab1 Sanger_isolates  15R.R.ab1        923
## 95  Sanger_isolates/160F.F.ab1 Sanger_isolates 160F.F.ab1        778
## 96  Sanger_isolates/160R.R.ab1 Sanger_isolates 160R.R.ab1       1157
## 97  Sanger_isolates/161F.F.ab1 Sanger_isolates 161F.F.ab1        796
## 98  Sanger_isolates/161R.R.ab1 Sanger_isolates 161R.R.ab1       1015
## 99  Sanger_isolates/162F.F.ab1 Sanger_isolates 162F.F.ab1        665
## 100 Sanger_isolates/162R.R.ab1 Sanger_isolates 162R.R.ab1        581
## 101 Sanger_isolates/163F.F.ab1 Sanger_isolates 163F.F.ab1        806
## 102 Sanger_isolates/163R.R.ab1 Sanger_isolates 163R.R.ab1        837
## 103 Sanger_isolates/181F.F.ab1 Sanger_isolates 181F.F.ab1       1149
## 104 Sanger_isolates/181R.R.ab1 Sanger_isolates 181R.R.ab1       1188
## 105 Sanger_isolates/182F.F.ab1 Sanger_isolates 182F.F.ab1       1050
## 106 Sanger_isolates/182R.R.ab1 Sanger_isolates 182R.R.ab1        815
## 107 Sanger_isolates/183F.F.ab1 Sanger_isolates 183F.F.ab1       1044
## 108 Sanger_isolates/183R.R.ab1 Sanger_isolates 183R.R.ab1       1190
## 109 Sanger_isolates/184F.F.ab1 Sanger_isolates 184F.F.ab1        916
## 110 Sanger_isolates/184R.R.ab1 Sanger_isolates 184R.R.ab1       1151
## 111 Sanger_isolates/185F.F.ab1 Sanger_isolates 185F.F.ab1        757
## 112 Sanger_isolates/185R.R.ab1 Sanger_isolates 185R.R.ab1       1147
## 113 Sanger_isolates/186F.F.ab1 Sanger_isolates 186F.F.ab1        595
## 114 Sanger_isolates/186R.R.ab1 Sanger_isolates 186R.R.ab1       1023
## 115 Sanger_isolates/187F.F.ab1 Sanger_isolates 187F.F.ab1       1142
## 116 Sanger_isolates/187R.R.ab1 Sanger_isolates 187R.R.ab1       1131
## 117 Sanger_isolates/188F.F.ab1 Sanger_isolates 188F.F.ab1       1095
## 118 Sanger_isolates/188R.R.ab1 Sanger_isolates 188R.R.ab1       1023
## 119 Sanger_isolates/189F.F.ab1 Sanger_isolates 189F.F.ab1       1049
## 120 Sanger_isolates/189R.R.ab1 Sanger_isolates 189R.R.ab1       1029
## 121  Sanger_isolates/18F.F.ab1 Sanger_isolates  18F.F.ab1        655
## 122  Sanger_isolates/18R.R.ab1 Sanger_isolates  18R.R.ab1        999
## 123 Sanger_isolates/190F.F.ab1 Sanger_isolates 190F.F.ab1       1063
## 124 Sanger_isolates/190R.R.ab1 Sanger_isolates 190R.R.ab1        709
## 125 Sanger_isolates/191F.F.ab1 Sanger_isolates 191F.F.ab1       1021
## 126 Sanger_isolates/191R.R.ab1 Sanger_isolates 191R.R.ab1       1153
## 127 Sanger_isolates/192F.F.ab1 Sanger_isolates 192F.F.ab1       1208
## 128 Sanger_isolates/192R.R.ab1 Sanger_isolates 192R.R.ab1       1157
## 129 Sanger_isolates/193F.F.ab1 Sanger_isolates 193F.F.ab1       1009
## 130 Sanger_isolates/193R.R.ab1 Sanger_isolates 193R.R.ab1       1121
## 131 Sanger_isolates/194F.F.ab1 Sanger_isolates 194F.F.ab1       1020
## 132 Sanger_isolates/194R.R.ab1 Sanger_isolates 194R.R.ab1       1137
## 133 Sanger_isolates/195F.F.ab1 Sanger_isolates 195F.F.ab1       1091
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## 134 Sanger_isolates/195R.R.ab1 Sanger_isolates 195R.R.ab1       1107
## 135 Sanger_isolates/196F.F.ab1 Sanger_isolates 196F.F.ab1       1175
## 136 Sanger_isolates/196R.R.ab1 Sanger_isolates 196R.R.ab1       1095
## 137 Sanger_isolates/197F.F.ab1 Sanger_isolates 197F.F.ab1        676
## 138 Sanger_isolates/197R.R.ab1 Sanger_isolates 197R.R.ab1       1179
## 139 Sanger_isolates/198F.F.ab1 Sanger_isolates 198F.F.ab1       1100
## 140 Sanger_isolates/198R.R.ab1 Sanger_isolates 198R.R.ab1        928
## 141 Sanger_isolates/199F.F.ab1 Sanger_isolates 199F.F.ab1        609
## 142 Sanger_isolates/199R.R.ab1 Sanger_isolates 199R.R.ab1        906
## 143  Sanger_isolates/19F.F.ab1 Sanger_isolates  19F.F.ab1       1060
## 144  Sanger_isolates/19R.R.ab1 Sanger_isolates  19R.R.ab1       1160
## 145 Sanger_isolates/200F.F.ab1 Sanger_isolates 200F.F.ab1       1217
## 146 Sanger_isolates/200R.R.ab1 Sanger_isolates 200R.R.ab1        368
## 147 Sanger_isolates/201F.F.ab1 Sanger_isolates 201F.F.ab1       1183
## 148 Sanger_isolates/201R.R.ab1 Sanger_isolates 201R.R.ab1        965
## 149 Sanger_isolates/202F.F.ab1 Sanger_isolates 202F.F.ab1       1227
## 150 Sanger_isolates/202R.R.ab1 Sanger_isolates 202R.R.ab1       1117
## 151 Sanger_isolates/203F.F.ab1 Sanger_isolates 203F.F.ab1        482
## 152 Sanger_isolates/203R.R.ab1 Sanger_isolates 203R.R.ab1        987
## 153 Sanger_isolates/204F.F.ab1 Sanger_isolates 204F.F.ab1        603
## 154 Sanger_isolates/204R.R.ab1 Sanger_isolates 204R.R.ab1        584
## 155 Sanger_isolates/205F.F.ab1 Sanger_isolates 205F.F.ab1        487
## 156 Sanger_isolates/205R.R.ab1 Sanger_isolates 205R.R.ab1        566
## 157 Sanger_isolates/206F.F.ab1 Sanger_isolates 206F.F.ab1       1183
## 158 Sanger_isolates/206R.R.ab1 Sanger_isolates 206R.R.ab1       1190
## 159  Sanger_isolates/21F.F.ab1 Sanger_isolates  21F.F.ab1        903
## 160  Sanger_isolates/21R.R.ab1 Sanger_isolates  21R.R.ab1        918
## 161  Sanger_isolates/25F.F.ab1 Sanger_isolates  25F.F.ab1        904
## 162  Sanger_isolates/25R.R.ab1 Sanger_isolates  25R.R.ab1        931
## 163  Sanger_isolates/26F.F.ab1 Sanger_isolates  26F.F.ab1       1127
## 164  Sanger_isolates/26R.R.ab1 Sanger_isolates  26R.R.ab1        813
## 165  Sanger_isolates/27F.F.ab1 Sanger_isolates  27F.F.ab1        966
## 166  Sanger_isolates/27R.R.ab1 Sanger_isolates  27R.R.ab1        824
## 167  Sanger_isolates/28F.F.ab1 Sanger_isolates  28F.F.ab1        908
## 168  Sanger_isolates/28R.R.ab1 Sanger_isolates  28R.R.ab1       1017
## 169  Sanger_isolates/29F.F.ab1 Sanger_isolates  29F.F.ab1        966
## 170  Sanger_isolates/29R.R.ab1 Sanger_isolates  29R.R.ab1       1109
## 171  Sanger_isolates/30F.F.ab1 Sanger_isolates  30F.F.ab1        914
## 172  Sanger_isolates/30R.R.ab1 Sanger_isolates  30R.R.ab1        945
## 173  Sanger_isolates/31F.F.ab1 Sanger_isolates  31F.F.ab1        970
## 174  Sanger_isolates/31R.R.ab1 Sanger_isolates  31R.R.ab1        999
## 175  Sanger_isolates/32F.F.ab1 Sanger_isolates  32F.F.ab1       1045
## 176  Sanger_isolates/32R.R.ab1 Sanger_isolates  32R.R.ab1       1234
## 177  Sanger_isolates/33F.F.ab1 Sanger_isolates  33F.F.ab1        996
## 178  Sanger_isolates/33R.R.ab1 Sanger_isolates  33R.R.ab1        825
## 179  Sanger_isolates/34F.F.ab1 Sanger_isolates  34F.F.ab1        933
## 180  Sanger_isolates/34R.R.ab1 Sanger_isolates  34R.R.ab1       1143
## 181  Sanger_isolates/35F.F.ab1 Sanger_isolates  35F.F.ab1        920
## 182  Sanger_isolates/35R.R.ab1 Sanger_isolates  35R.R.ab1       1161
## 183  Sanger_isolates/36F.F.ab1 Sanger_isolates  36F.F.ab1        843
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## 184  Sanger_isolates/36R.R.ab1 Sanger_isolates  36R.R.ab1       1091
## 185  Sanger_isolates/37F.F.ab1 Sanger_isolates  37F.F.ab1        942
## 186  Sanger_isolates/37R.R.ab1 Sanger_isolates  37R.R.ab1       1018
## 187  Sanger_isolates/38F.F.ab1 Sanger_isolates  38F.F.ab1        771
## 188  Sanger_isolates/38R.R.ab1 Sanger_isolates  38R.R.ab1        610
## 189  Sanger_isolates/39F.F.ab1 Sanger_isolates  39F.F.ab1        481
## 190  Sanger_isolates/39R.R.ab1 Sanger_isolates  39R.R.ab1        460
## 191  Sanger_isolates/40F.F.ab1 Sanger_isolates  40F.F.ab1       1039
## 192  Sanger_isolates/40R.R.ab1 Sanger_isolates  40R.R.ab1       1046
## 193  Sanger_isolates/41F.F.ab1 Sanger_isolates  41F.F.ab1       1172
## 194  Sanger_isolates/41R.R.ab1 Sanger_isolates  41R.R.ab1       1114
## 195  Sanger_isolates/42F.F.ab1 Sanger_isolates  42F.F.ab1        988
## 196  Sanger_isolates/42R.R.ab1 Sanger_isolates  42R.R.ab1        980
## 197  Sanger_isolates/43F.F.ab1 Sanger_isolates  43F.F.ab1       1119
## 198  Sanger_isolates/43R.R.ab1 Sanger_isolates  43R.R.ab1       1164
## 199  Sanger_isolates/44F.F.ab1 Sanger_isolates  44F.F.ab1        956
## 200  Sanger_isolates/44R.R.ab1 Sanger_isolates  44R.R.ab1       1067
## 201  Sanger_isolates/45F.F.ab1 Sanger_isolates  45F.F.ab1       1088
## 202  Sanger_isolates/45R.R.ab1 Sanger_isolates  45R.R.ab1        878
## 203  Sanger_isolates/46F.F.ab1 Sanger_isolates  46F.F.ab1        990
## 204  Sanger_isolates/46R.R.ab1 Sanger_isolates  46R.R.ab1       1086
## 205  Sanger_isolates/47F.F.ab1 Sanger_isolates  47F.F.ab1        752
## 206  Sanger_isolates/47R.R.ab1 Sanger_isolates  47R.R.ab1        582
## 207  Sanger_isolates/48F.F.ab1 Sanger_isolates  48F.F.ab1       1157
## 208  Sanger_isolates/48R.R.ab1 Sanger_isolates  48R.R.ab1        200
## 209  Sanger_isolates/76F.F.ab1 Sanger_isolates  76F.F.ab1       1138
## 210  Sanger_isolates/76R.R.ab1 Sanger_isolates  76R.R.ab1       1168
## 211  Sanger_isolates/77F.F.ab1 Sanger_isolates  77F.F.ab1       1095
## 212  Sanger_isolates/77R.R.ab1 Sanger_isolates  77R.R.ab1       1111
## 213  Sanger_isolates/78F.F.ab1 Sanger_isolates  78F.F.ab1        944
## 214  Sanger_isolates/78R.R.ab1 Sanger_isolates  78R.R.ab1       1180
## 215  Sanger_isolates/79F.F.ab1 Sanger_isolates  79F.F.ab1        528
## 216  Sanger_isolates/79R.R.ab1 Sanger_isolates  79R.R.ab1        966
## 217  Sanger_isolates/80F.F.ab1 Sanger_isolates  80F.F.ab1        638
## 218  Sanger_isolates/80R.R.ab1 Sanger_isolates  80R.R.ab1        999
## 219  Sanger_isolates/81F.F.ab1 Sanger_isolates  81F.F.ab1        801
## 220  Sanger_isolates/81R.R.ab1 Sanger_isolates  81R.R.ab1       1031
## 221  Sanger_isolates/82F.F.ab1 Sanger_isolates  82F.F.ab1        891
## 222  Sanger_isolates/82R.R.ab1 Sanger_isolates  82R.R.ab1       1129
## 223  Sanger_isolates/83F.F.ab1 Sanger_isolates  83F.F.ab1        637
## 224  Sanger_isolates/83R.R.ab1 Sanger_isolates  83R.R.ab1        915
## 225  Sanger_isolates/84F.F.ab1 Sanger_isolates  84F.F.ab1        515
## 226  Sanger_isolates/84R.R.ab1 Sanger_isolates  84R.R.ab1       1088
## 227  Sanger_isolates/85F.F.ab1 Sanger_isolates  85F.F.ab1        525
## 228  Sanger_isolates/85R.R.ab1 Sanger_isolates  85R.R.ab1       1072
## 229  Sanger_isolates/86F.F.ab1 Sanger_isolates  86F.F.ab1        513
## 230  Sanger_isolates/86R.R.ab1 Sanger_isolates  86R.R.ab1       1104
## 231  Sanger_isolates/87F.F.ab1 Sanger_isolates  87F.F.ab1        699
## 232  Sanger_isolates/87R.R.ab1 Sanger_isolates  87R.R.ab1       1022
## 233  Sanger_isolates/88F.F.ab1 Sanger_isolates  88F.F.ab1        990
```

```
## 234  Sanger_isolates/88R.R.ab1 Sanger_isolates  88R.R.ab1        820
## 235  Sanger_isolates/89F.F.ab1 Sanger_isolates  89F.F.ab1        628
## 236  Sanger_isolates/89R.R.ab1 Sanger_isolates  89R.R.ab1        916
## 237  Sanger_isolates/90F.F.ab1 Sanger_isolates  90F.F.ab1        950
## 238  Sanger_isolates/90R.R.ab1 Sanger_isolates  90R.R.ab1       1182
## 239  Sanger_isolates/91F.F.ab1 Sanger_isolates  91F.F.ab1        973
## 240  Sanger_isolates/91R.R.ab1 Sanger_isolates  91R.R.ab1       1112
## 241  Sanger_isolates/92F.F.ab1 Sanger_isolates  92F.F.ab1        988
## 242  Sanger_isolates/92R.R.ab1 Sanger_isolates  92R.R.ab1       1127
## 243  Sanger_isolates/93F.F.ab1 Sanger_isolates  93F.F.ab1        933
## 244  Sanger_isolates/93R.R.ab1 Sanger_isolates  93R.R.ab1       1049
## 245  Sanger_isolates/94F.F.ab1 Sanger_isolates  94F.F.ab1        986
## 246  Sanger_isolates/94R.R.ab1 Sanger_isolates  94R.R.ab1        802
## 247  Sanger_isolates/95F.F.ab1 Sanger_isolates  95F.F.ab1       1050
## 248  Sanger_isolates/95R.R.ab1 Sanger_isolates  95R.R.ab1        911
## 249  Sanger_isolates/96F.F.ab1 Sanger_isolates  96F.F.ab1       1167
## 250  Sanger_isolates/96R.R.ab1 Sanger_isolates  96R.R.ab1       1125
## 251  Sanger_isolates/97F.F.ab1 Sanger_isolates  97F.F.ab1        915
## 252  Sanger_isolates/97R.R.ab1 Sanger_isolates  97R.R.ab1        993
## 253  Sanger_isolates/98F.F.ab1 Sanger_isolates  98F.F.ab1        713
## 254  Sanger_isolates/98R.R.ab1 Sanger_isolates  98R.R.ab1        716
## 255  Sanger_isolates/99F.F.ab1 Sanger_isolates  99F.F.ab1       1178
## 256  Sanger_isolates/99R.R.ab1 Sanger_isolates  99R.R.ab1       1116
##     trimmed.length trim.start trim.finish raw.secondary.peaks
## 1              314         24         337                 264
## 2                2        484         485                 237
## 3              610         20         629                  27
## 4              750         21         770                   8
## 5              573         20         592                  38
## 6              474         73         546                  20
## 7              625         14         638                  25
## 8              492         14         505                  12
## 9              557         17         573                  32
## 10             569         26         594                  29
## 11             624         14         637                  39
## 12             327          5         331                  74
## 13             589         14         602                  16
## 14             499          6         504                   7
## 15             624         11         634                  33
## 16             498          5         502                  32
## 17             609         12         620                  23
## 18             493         13         505                  26
## 19             604         10         613                  20
## 20             490         15         504                  42
## 21             610         11         620                  55
## 22             752         16         767                  24
## 23             621         11         631                   8
## 24             491         15         505                  19
## 25             319        143         461                 209
## 26             546         39         584                 238
```

```
## 27              363          11          373                58
## 28              730          17          746                38
## 29              356          17          372                55
## 30              730          19          748                29
## 31              344          23          366                26
## 32              310          14          323                16
## 33              408          85          492               265
## 34              825          19          843                23
## 35                0           0            0               145
## 36                0           0            0               136
## 37                0           0            0               147
## 38              754          16          769                25
## 39                0           0            0               223
## 40                1         341          341               243
## 41              168          22          189               177
## 42                0           0            0                99
## 43              214          16          229               201
## 44              832          21          852                14
## 45              566          17          582                19
## 46              594          15          608                24
## 47              237         192          428               204
## 48              463          18          480                35
## 49              231          34          264                56
## 50              122          17          138                23
## 51              355         120          474                81
## 52              462          40          501                86
## 53              343          37          379                31
## 54              457          54          510               150
## 55              365          11          375                55
## 56              204          17          220                38
## 57              498          25          522                40
## 58              307          14          320                42
## 59              560          20          579               116
## 60              829          18          846                39
## 61              594          23          616                47
## 62              729          19          747                28
## 63              354          22          375                57
## 64              204          18          221                43
## 65               72          83          154               174
## 66              797          19          815                16
## 67              353          22          374                54
## 68              730          15          744                22
## 69              225         277          501               264
## 70               38          10           47               232
## 71              559          23          581                17
## 72              707          18          724                26
## 73              423          17          439                 6
## 74              753          17          769                23
## 75              256          13          268               271
## 76              770          19          788                14
```

```
## 77              294          85          378             104
## 78              606          18          623              16
## 79              407          78          484             121
## 80              719          14          732              22
## 81              408          85          492             156
## 82              718          19          736              29
## 83              526          12          537              38
## 84              753          16          768              22
## 85              295          85          379              88
## 86              737          20          756               8
## 87              295          85          379             154
## 88              606          19          624               7
## 89               68          87          154             161
## 90              730          18          747              40
## 91              295          81          375             124
## 92              715          19          733              26
## 93              404          86          489             131
## 94              482          30          511              36
## 95              351          26          376             149
## 96              732          18          749              23
## 97               68          86          153             160
## 98              617          18          634               5
## 99              352          27          378             112
## 100             299          12          310             135
## 101             111          20          130             223
## 102             612          68          679              32
## 103             759           7          765              17
## 104             727          18          744              33
## 105             706          14          719              28
## 106             536          11          546              25
## 107             287         240          526             242
## 108             847          20          866             111
## 109             403          21          423             286
## 110             589          17          605              21
## 111             110          17          126             313
## 112             588          17          604              19
## 113             100          31          130             105
## 114             589          15          603              11
## 115             713          18          730              11
## 116             830          16          845              10
## 117             695          17          711              18
## 118             839          14          852               9
## 119             679          16          694              32
## 120             818          22          839              20
## 121              48          74          121             115
## 122             506           6          511              24
## 123             712          15          726              34
## 124             539          15          553              33
## 125             782          17          798              22
## 126             840          10          849              14
```

```
## 127  712  18  729  41
## 128  890  11  900  15
## 129  678  21  698  16
## 130  788   8  795  43
## 131  623  12  634  49
## 132  499   8  506   9
## 133  534  18  551  12
## 134  693  14  706  13
## 135  708  18  725  34
## 136  689  18  706   7
## 137  345 325  669 461
## 138  830  15  844  22
## 139  703   6  708  47
## 140  319  10  328 267
## 141    4 388  391 438
## 142  690  17  706  28
## 143  629  11  639  77
## 144  516  15  530  25
## 145  592  18  609  31
## 146    1 368  368 243
## 147  713   8  720   9
## 148  687  20  706  32
## 149  705  15  719  22
## 150  922  18  939  13
## 151    0   0    0 331
## 152  440  13  452 118
## 153    1 603  603 431
## 154   28  14   41 361
## 155    0   0    0 380
## 156    0   0    0 416
## 157  718  15  732  13
## 158  963  17  979  21
## 159   75  20   94 411
## 160  465  11  475  48
## 161  561  74  634 107
## 162  748  19  766  30
## 163  697  14  710  24
## 164  610  13  622   5
## 165  539  39  577  48
## 166  349  15  363  31
## 167  245  34  278 262
## 168  619  17  635  14
## 169  588   7  594  44
## 170  738   1  738  24
## 171  615  14  628  83
## 172  716  11  726  14
## 173  621  19  639  25
## 174  582  21  602  30
## 175  642  25  666  24
## 176  831   9  839  33
```

```
## 177  586  11  596  24
## 178  616   9  624   7
## 179  360  99  458 126
## 180  761  23  783  35
## 181  560  21  580  29
## 182  763  10  772  19
## 183  512  16  527  33
## 184  643  19  661  24
## 185  633  21  653  25
## 186  727  16  742   4
## 187  519  10  528  63
## 188  411  17  427  20
## 189  342  15  356  31
## 190   72  15   86 207
## 191  645  10  654  29
## 192  783  18  800  11
## 193  713  14  726  71
## 194  747  17  763  12
## 195  629  11  639  44
## 196  586  14  599  54
## 197  641  15  655  17
## 198  828  14  841  22
## 199  499  23  521  56
## 200  740  15  754  14
## 201  585  16  600  39
## 202  317  34  350 199
## 203  550  17  566  31
## 204  700  30  729  29
## 205  547  20  566  28
## 206  314  13  326  33
## 207  524  14  537  57
## 208    0   0    0 129
## 209  706   9  714  11
## 210  839  14  852  14
## 211  215  46  260 367
## 212  636  33  668 268
## 213  633  14  646  30
## 214  716  28  743  12
## 215  225  15  239 107
## 216  751  23  773  12
## 217  441  76  516 114
## 218  275  26  300  47
## 219  567   7  573  64
## 220  796  21  816  17
## 221  556  10  565  28
## 222  710  17  726  15
## 223  594  21  614  12
## 224  709  33  741  25
## 225  501  14  514   0
## 226  769  24  792  30
```

```
## 227             291          10          300                   27
## 228             729          17          745                   12
## 229             277          22          298                    4
## 230             819          21          839                   26
## 231             474          25          498                   41
## 232             713          17          729                    9
## 233             617           8          624                   67
## 234             655          19          673                   42
## 235              40         139          178                  220
## 236              30          30           59                  340
## 237             283          58          340                  105
## 238             901          19          919                   13
## 239             709           9          717                   24
## 240             654          18          671                   16
## 241             614          16          629                   13
## 242             911          10          920                   10
## 243             705          17          721                   12
## 244             706          15          720                   10
## 245             199          76          274                  280
## 246             581          23          603                   33
## 247              81          70          150                  205
## 248             198          29          226                   33
## 249             610          14          623                   46
## 250             723          20          742                   13
## 251             476         217          692                  126
## 252              85          55          139                  204
## 253             534          15          548                   38
## 254             595          19          613                    3
## 255             680          17          696                   28
## 256             909          17          925                   14
##     trimmed.secondary.peaks raw.mean.quality trimmed.mean.quality
## 1                        68         23.52626              28.95860
## 2                         1         17.29835              41.00000
## 3                         2         45.56604              51.27049
## 4                         0         47.80229              52.50400
## 5                         5         38.44748              45.30890
## 6                         1         41.82790              50.24684
## 7                         0         44.18926              52.12320
## 8                         1         46.71632              52.29472
## 9                         6         42.88615              50.83124
## 10                        1         42.21843              48.74868
## 11                        0         43.04356              51.55609
## 12                        8         44.62283              53.20489
## 13                        0         45.51059              51.64686
## 14                        3         46.27200              51.16836
## 15                        0         42.58244              51.13141
## 16                        4         43.72024              51.44309
## 17                        0         44.96687              51.40558
## 18                        1         45.29670              52.06288
## 19                        0         43.70712              51.82616
```

```
## 20                    3         44.36121              51.89796
## 21                    1         41.73789              51.63607
## 22                    0         46.10872              52.54122
## 23                    1         46.69818              51.25282
## 24                    4         44.35714              49.69654
## 25                  122         19.52190              20.86834
## 26                  202         21.88141              22.33516
## 27                    2         41.69990              50.81267
## 28                    4         45.30661              51.90548
## 29                    2         44.29032              50.97472
## 30                    5         44.81320              51.05342
## 31                    1         42.53370              48.79651
## 32                    0         47.19084              57.95161
## 33                   65         25.31256              33.45588
## 34                    0         46.69182              52.10182
## 35                    0         11.50000                    NA
## 36                    0          9.68500                    NA
## 37                    0          9.54000                    NA
## 38                    0         46.45817              51.85942
## 39                    0         13.90941                    NA
## 40                    0         16.65982              51.00000
## 41                    0         33.66131              48.80952
## 42                    0         13.94574                    NA
## 43                   29         26.37595              35.11682
## 44                    0         47.07706              52.07332
## 45                    1         50.60893              56.80212
## 46                    0         47.13833              50.88047
## 47                   41         26.01257              33.25738
## 48                   11         44.81257              49.79482
## 49                   20         40.38725              44.09957
## 50                    5         40.43455              39.86885
## 51                   21         36.43493              43.17465
## 52                   12         39.42961              47.54762
## 53                    6         39.91544              44.66181
## 54                   69         35.06421              39.51204
## 55                    0         40.40994              51.49041
## 56                    1         43.90269              50.26961
## 57                    2         42.61054              48.40161
## 58                    0         41.78200              50.64169
## 59                    0         37.81471              51.78393
## 60                    9         45.50294              50.45838
## 61                    1         40.44714              48.74242
## 62                    3         44.63526              51.66255
## 63                    1         42.36053              50.97740
## 64                    0         42.45929              50.63725
## 65                   11         28.17019              35.50000
## 66                    0         46.61232              51.95107
## 67                    3         42.39801              50.27479
## 68                    5         45.96314              51.26849
## 69                   63         19.39213              23.85778
```

```
## 70                9          14.86667          23.94737
## 71                1          45.83409          49.19857
## 72                3          43.60435          51.69731
## 73                1          45.42436          48.10875
## 74                1          47.12354          51.25365
## 75               84          26.38418          27.64062
## 76                1          47.26456          52.04545
## 77               47          30.14821          32.12585
## 78                0          47.72393          51.65842
## 79               64          30.43121          33.80344
## 80                0          46.96162          51.80807
## 81               66          29.17797          33.41176
## 82                0          44.49638          53.04735
## 83                1          43.10359          48.40684
## 84                3          44.52515          51.00398
## 85               41          31.71031          34.21695
## 86                0          47.69884          52.05020
## 87               49          29.23843          33.68136
## 88                0          46.78559          52.96205
## 89               11          29.88686          34.75000
## 90                0          45.77387          52.00548
## 91               44          30.51278          33.48136
## 92                0          45.26036          52.20839
## 93               10          39.76145          53.92079
## 94                7          45.59307          50.04357
## 95               61          30.09487          32.08547
## 96                0          44.83675          52.36885
## 97               14          29.22585          34.32353
## 98                0          47.66243          52.77958
## 99               54          31.28186          31.79830
## 100               3          38.86575          52.85284
## 101               3          26.53713          47.02703
## 102               9          43.79380          48.55229
## 103               0          45.57021          51.87352
## 104               1          45.90696          52.61073
## 105               2          46.25922          51.52691
## 106               2          49.07506          57.05784
## 107              64          26.86437          30.41812
## 108              22          39.32750          48.58796
## 109              93          23.98364          30.43921
## 110               1          46.42845          52.27844
## 111              31          26.95376          32.40909
## 112               1          45.30882          51.76531
## 113              19          31.10756          33.68000
## 114               3          48.83965          52.37012
## 115               0          46.89791          52.07293
## 116               2          46.71604          51.49157
## 117               7          45.79581          51.01007
## 118               5          49.07415          50.99046
## 119               4          44.54502          50.10457
```

```
## 120   2  46.13967  50.09169
## 121   3  37.86280  50.04167
## 122  12  44.37313  48.00988
## 123   3  44.11610  50.15730
## 124   3  49.96474  56.99629
## 125   3  47.09756  51.26854
## 126   1  46.81638  51.57976
## 127   0  44.17473  52.06882
## 128   2  46.29256  51.57191
## 129   0  45.55676  51.15782
## 130   1  43.72099  50.80584
## 131   0  42.37877  51.09952
## 132   0  45.78534  51.83768
## 133   0  45.72338  51.79213
## 134   0  47.28150  52.48052
## 135   0  44.07912  51.60311
## 136   0  48.79946  53.03048
## 137 243  13.87130  13.66087
## 138   1  45.91261  52.04699
## 139   0  43.39892  50.79232
## 140 100  27.14393  29.44201
## 141   4  13.86864  32.25000
## 142   1  46.50494  51.06957
## 143  53  34.43218  37.30366
## 144   4  42.84799  51.32558
## 145   2  44.51629  52.33277
## 146   1  14.18699  45.00000
## 147   2  46.45987  51.45302
## 148   3  46.46591  51.60408
## 149   1  45.02753  51.66099
## 150   3  47.18482  51.46095
## 151   0  13.25934        NA
## 152  23  38.89889  48.61364
## 153   0  14.32338  42.00000
## 154   0  15.65925  46.64286
## 155   0  12.86242        NA
## 156   0  12.37633        NA
## 157   1  45.42989  52.26741
## 158   2  46.64190  52.01454
## 159   2  17.59757  39.37333
## 160  20  43.77935  46.73763
## 161  28  33.30795  39.86631
## 162   6  45.12286  49.24465
## 163   0  43.64877  52.24821
## 164   0  51.26779  57.74098
## 165   5  41.28306  48.91651
## 166   5  47.21540  51.14900
## 167  77  26.96061  30.80408
## 168   3  45.81703  50.77544
## 169   0  42.57407  52.03741
```

```
## 170                    0         44.61325             52.06967
## 171                    3         40.12092             50.23902
## 172                    3         47.72076             51.59916
## 173                    1         44.13673             52.07729
## 174                    2         45.81655             51.86770
## 175                    1         45.08815             52.33645
## 176                    1         43.88818             52.10469
## 177                    0         44.15400             51.71843
## 178                    0         47.25962             50.52922
## 179                   51         32.44124             35.23889
## 180                    6         44.07605             50.28252
## 181                    1         45.26030             51.95179
## 182                    0         45.71769             52.24771
## 183                    0         41.94208             48.58203
## 184                    1         43.80748             49.44946
## 185                    0         45.64942             52.08057
## 186                    0         47.88824             52.20083
## 187                    3         45.16516             52.90366
## 188                    4         45.52700             50.73966
## 189                    0         42.61411             48.48538
## 190                    0         23.30952             45.31944
## 191                    0         46.01811             52.24031
## 192                    0         47.42912             52.64112
## 193                    1         40.15228             50.86816
## 194                    0         47.17663             52.30254
## 195                    2         42.96177             49.65978
## 196                    2         45.09729             55.10922
## 197                    1         45.53771             52.27769
## 198                    2         45.97950             52.40580
## 199                    2         43.78778             54.23246
## 200                    0         46.84308             51.78784
## 201                    0         43.03297             51.89744
## 202                   30         25.50342             29.71609
## 203                   15         41.75907             44.21818
## 204                    9         41.05775             46.70000
## 205                    1         43.88977             49.17733
## 206                    1         42.77949             51.51592
## 207                    0         39.53012             49.66985
## 208                    0          9.80500                   NA
## 209                    0         44.99567             52.06516
## 210                    2         43.39237             50.66031
## 211                   68         20.19143             23.00465
## 212                  195         25.31847             25.66195
## 213                    4         48.50936             55.63507
## 214                    1         46.05228             51.41480
## 215                    2         40.14205             47.48889
## 216                    2         48.06082             51.01731
## 217                   72         32.29844             35.15873
## 218                    4         41.25222             47.86909
## 219                    1         48.68897             58.84656
```

```
## 220                        2          48.70281              52.08794
## 221                        3          49.28101              57.92086
## 222                        0          48.26678              53.06479
## 223                        5          45.04702              46.95286
## 224                       11          45.16576              47.93794
## 225                        0          51.21470              52.21557
## 226                        4          44.86447              49.32900
## 227                        2          45.89184              51.19931
## 228                        2          48.61860              52.56927
## 229                        1          48.41942              51.59567
## 230                        1          47.16937              52.70574
## 231                        0          42.68714              51.18987
## 232                        0          47.34732              49.84853
## 233                       13          42.83585              50.72123
## 234                        0          45.38929              51.47939
## 235                        4          23.82194              38.97500
## 236                        0          20.64130              50.10000
## 237                       26          34.88655              41.56184
## 238                        1          46.73662              51.73030
## 239                        0          46.37755              51.01693
## 240                        0          47.77907              53.06269
## 241                        0          48.38153              52.37296
## 242                        0          48.85915              52.58836
## 243                        0          47.27079              50.78440
## 244                        3          48.34414              51.65864
## 245                       71          28.73834              34.01005
## 246                       13          42.83602              45.78657
## 247                        2          29.74430              45.71605
## 248                        3          41.85137              46.61111
## 249                        2          40.89957              49.95738
## 250                        4          46.03530              51.77593
## 251                       54          33.62568              37.63445
## 252                       14          28.39196              32.38824
## 253                        1          43.49930              51.18539
## 254                        0          50.22981              52.82689
## 255                        4          44.20185              51.02059
## 256                        2          46.10993              51.00880
##     raw.min.quality trimmed.min.quality
## 1                 2                   8
## 2                 2                  41
## 3                 2                  15
## 4                 2                  10
## 5                 2                   2
## 6                 2                  23
## 7                 2                   7
## 8                 2                   7
## 9                 2                   7
## 10                2                   2
## 11                2                   7
## 12                2                   7
```

```
## 13                2                 7
## 14                2                 9
## 15                2                 7
## 16                2                10
## 17                2                 7
## 18                2                15
## 19                2                 7
## 20                2                10
## 21                2                10
## 22                2                 2
## 23                7                 7
## 24                2                 6
## 25                2                 7
## 26                2                 2
## 27                4                12
## 28                3                 3
## 29                2                 9
## 30                4                 8
## 31                4                16
## 32                5                 9
## 33                2                10
## 34                3                 9
## 35                4                NA
## 36                3                NA
## 37                3                NA
## 38                5                14
## 39                5                NA
## 40                6                51
## 41                5                20
## 42                5                NA
## 43                2                 7
## 44                2                22
## 45                5                 5
## 46                7                22
## 47                2                 8
## 48                5                 7
## 49                5                 5
## 50                5                 8
## 51                2                 7
## 52                2                 6
## 53                4                12
## 54                4                 4
## 55                2                12
## 56                3                 6
## 57                5                15
## 58                5                 7
## 59                5                20
## 60                3                10
## 61                4                15
## 62                5                14
```

```
## 63                5                20
## 64                5                 6
## 65                2                12
## 66                5                 9
## 67                4                 9
## 68                5                 8
## 69                2                 5
## 70                5                 9
## 71                4                14
## 72                5                14
## 73                5                 8
## 74                5                14
## 75                4                 8
## 76                5                 9
## 77                2                 8
## 78                5                 9
## 79                5                10
## 80                3                 9
## 81                2                10
## 82                2                 9
## 83                3                20
## 84                2                14
## 85                2                10
## 86                4                 8
## 87                5                12
## 88                4                 8
## 89                6                12
## 90                4                 8
## 91                2                10
## 92                4                 7
## 93                2                31
## 94                2                 7
## 95                2                 7
## 96                2                 8
## 97                6                12
## 98                2                14
## 99                3                 7
## 100               5                 9
## 101               6                14
## 102               5                12
## 103               5                 8
## 104               5                12
## 105               5                 5
## 106               5                 5
## 107               2                 8
## 108               5                 8
## 109               2                 7
## 110               5                10
## 111               5                10
## 112               2                14
```

```
## 113                5                 10
## 114                5                  8
## 115                4                 11
## 116                2                  2
## 117                5                  5
## 118                4                  4
## 119                3                  5
## 120                5                  5
## 121                2                 24
## 122                2                  8
## 123                2                  4
## 124                5                  8
## 125                5                  6
## 126                5                  9
## 127                5                 20
## 128                2                 18
## 129                2                  2
## 130                5                  5
## 131                5                 20
## 132                2                  5
## 133                6                 11
## 134                4                  9
## 135                5                 14
## 136                5                  9
## 137                5                  5
## 138                3                 10
## 139                5                  5
## 140                4                 10
## 141                5                 19
## 142                2                 12
## 143                2                  7
## 144                2                  7
## 145                6                 20
## 146                5                 45
## 147                6                  8
## 148                5                  5
## 149                4                  9
## 150                4                  4
## 151                5                 NA
## 152                5                  8
## 153                5                 42
## 154                5                 15
## 155                5                 NA
## 156                5                 NA
## 157                5                  8
## 158                5                  8
## 159                2                  8
## 160                2                  6
## 161                2                 10
## 162                2                  2
```

```
## 163                2                  20
## 164                2                  12
## 165                2                   7
## 166                2                   2
## 167                2                   8
## 168                2                   6
## 169                2                   7
## 170                2                  10
## 171                2                  12
## 172                2                   8
## 173                2                  20
## 174                2                   8
## 175                2                  10
## 176                2                   2
## 177                2                  15
## 178                2                   2
## 179                2                  10
## 180                2                   7
## 181                2                  20
## 182                2                   2
## 183                2                  14
## 184                2                   6
## 185                2                  20
## 186                2                   2
## 187                2                   9
## 188                2                   2
## 189                2                   7
## 190                5                  10
## 191                2                   7
## 192                2                  14
## 193                2                  20
## 194                2                  20
## 195                2                   6
## 196                2                   2
## 197                2                  14
## 198                2                   8
## 199                2                   6
## 200                2                   6
## 201                2                  14
## 202                2                   2
## 203                2                   8
## 204                2                   9
## 205                6                  15
## 206                6                   6
## 207                2                  20
## 208                3                  NA
## 209                2                   9
## 210                2                   2
## 211                2                   7
## 212                2                   8
```

```
## 213                2                   2
## 214                2                   7
## 215                2                   2
## 216                2                   2
## 217                6                   8
## 218                2                   7
## 219                2                  13
## 220                2                  10
## 221                2                  14
## 222                2                  12
## 223                6                   7
## 224                2                  14
## 225                2                  15
## 226                2                   8
## 227                2                  14
## 228                2                  12
## 229                7                  12
## 230                2                   9
## 231                2                  20
## 232                2                  15
## 233                2                   7
## 234                2                  22
## 235                2                  14
## 236                2                  40
## 237                2                  10
## 238                2                   2
## 239                2                  14
## 240                2                   7
## 241                2                  15
## 242                2                  13
## 243                2                  14
## 244                2                   7
## 245                2                  13
## 246                6                  10
## 247                2                  21
## 248                2                   2
## 249                2                   2
## 250                2                   6
## 251                2                  10
## 252                2                   2
## 253                2                  15
## 254                2                   8
## 255                2                   7
## 256                2                   2
```

```
p<- ggplot(sf$summaries, aes(x = folder.name, y = raw.mean.quality)) + geom_b
oxplot()
p <- ggplot(sf$summaries, aes(x = folder.name, y = trimmed.mean.quality)) + g
eom_boxplot()
p <- ggplot(sf$summaries, aes(x = folder.name, y = trimmed.secondary.peaks))
```

```
+ geom_boxplot() + geom_hline(yintercept = 2, linetype = 3, colour = 'red')
p <- ggplot(sf$summaries, aes(x = trimmed.mean.quality, y = trimmed.secondary
.peaks)) + geom_point()

# Make consensus by merging reads
fwd = readsangerseq("Sanger_isolates/13F.F.ab1")
rev = readsangerseq("Sanger_isolates/13R.R.ab1")
fwd = primarySeq(fwd)
rev = primarySeq(rev)
rev = reverseComplement(rev)
reads = DNAStringSet(c(as.character(fwd), as.character(rev)))
names(reads) = c('fwd', 'rev')
merged.reads = merge.reads(reads)

## [1] "Aligning reads"
## [1] "Calling consensus sequence"
## [1] "Calculating differences between reads and consensus"

names(merged.reads)

## [1] "consensus"              "alignment"
## [3] "differences"            "distance.matrix"
## [5] "dendrogram"             "indels"
## [7] "stop.codons"            "secondary.peak.columns"

merged.reads

## $consensus
##   1283-letter "DNAString" instance
## seq: GATGCAATCTACAGATTCTGCATACAGGCTACTA...TCGCCTACATGAAGTNCGGAATCGCTAGTAAT
C
##
## $alignment
##   A DNAStringSet instance of length 3
##     width seq                                             names
## [1]  1283 GATGCAATCTACAGATTCTGC...-------------------- fwd
## [2]  1283 ----------------------...GTNCGGAATCGCTAGTAATC rev
## [3]  1283 GATGCAATCTACAGATTCTGC...GTNCGGAATCGCTAGTAATC consensus
##
## $differences
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                          60            0
## 2  rev                          67            0
##
## $distance.matrix
##           fwd       rev
## fwd 0.0000000 0.2491807
## rev 0.2491807 0.0000000
## attr(,"correction")
## [1] "Jukes-Cantor"
##
```

```
## $dendrogram
## 'dendrogram' with 2 branches and 2 members total, at height 0.1245903
##
## $indels
## NULL
##
## $stop.codons
## NULL
##
## $secondary.peak.columns
## NULL
##
## attr(,"class")
## [1] "merged.read"
```

```
merged.reads$consensus
```

```
##   1283-letter "DNAString" instance
## seq: GATGCAATCTACAGATTCTGCATACAGGCTACTA...TCGCCTACATGAAGTNCGGAATCGCTAGTAAT
C
```

```
BrowseSeqs(merged.reads$alignment)
merged.reads$secondary.peak.columns
```

```
## NULL
```

```
merged.reads$differences
```

```
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                         60            0
## 2  rev                         67            0
```

```
write.dna(merged.reads,file="Sanger_isolates/13.fasta",format='fasta',nbcol =
-1, colsep = "", colw = 10000000)
```

```
fwd = readsangerseq("Sanger_isolates/30F.F.ab1")
rev = readsangerseq("Sanger_isolates/30R.R.ab1")
fwd = primarySeq(fwd)
rev = primarySeq(rev)
rev = reverseComplement(rev)
reads = DNAStringSet(c(as.character(fwd), as.character(rev)))
names(reads) = c('fwd', 'rev')
merged.reads = merge.reads(reads)
```

```
## [1] "Aligning reads"
## [1] "Calling consensus sequence"
## [1] "Calculating differences between reads and consensus"
```

```
names(merged.reads)
```

```
## [1] "consensus"              "alignment"
## [3] "differences"            "distance.matrix"
```

```
## [5] "dendrogram"             "indels"
## [7] "stop.codons"            "secondary.peak.columns"

merged.reads

## $consensus
##   1285-letter "DNAString" instance
## seq: GGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACG...CGACTCCATGAAGTCGGAATCGCTGTAATCGT
N
##
## $alignment
##   A DNAStringSet instance of length 3
##     width seq                                             names
## [1]  1285 GGCAGCTTGCTGCTTTGCTGA...-------------------- fwd
## [2]  1285 ----------------------...TCGGAATCGCTGTAATCGTN rev
## [3]  1285 GGCAGCTTGCTGCTTTGCTGA...TCGGAATCGCTGTAATCGTN consensus
##
## $differences
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                          40            0
## 2  rev                          35            0
##
## $distance.matrix
##            fwd        rev
## fwd 0.00000000 0.06081996
## rev 0.06081996 0.00000000
## attr(,"correction")
## [1] "Jukes-Cantor"
##
## $dendrogram
## 'dendrogram' with 2 branches and 2 members total, at height 0.03040998
##
## $indels
## NULL
##
## $stop.codons
## NULL
##
## $secondary.peak.columns
## NULL
##
## attr(,"class")
## [1] "merged.read"

merged.reads$consensus

##   1285-letter "DNAString" instance
## seq: GGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACG...CGACTCCATGAAGTCGGAATCGCTGTAATCGT
N
```

```
BrowseSeqs(merged.reads$alignment)
merged.reads$secondary.peak.columns

## NULL

merged.reads$differences

##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                          40            0
## 2  rev                          35            0

write.dna(merged.reads,file="Sanger_isolates/30.fasta",format='fasta',nbcol =
-1, colsep = "", colw = 10000000)

fwd = readsangerseq("Sanger_isolates/95F.F.ab1")
rev = readsangerseq("Sanger_isolates/95R.R.ab1")
fwd = primarySeq(fwd)
rev = primarySeq(rev)
rev = reverseComplement(rev)
reads = DNAStringSet(c(as.character(fwd), as.character(rev)))
names(reads) = c('fwd', 'rev')
merged.reads = merge.reads(reads)

## [1] "Aligning reads"
## [1] "Calling consensus sequence"
## [1] "Calculating differences between reads and consensus"

names(merged.reads)

## [1] "consensus"              "alignment"
## [3] "differences"            "distance.matrix"
## [5] "dendrogram"             "indels"
## [7] "stop.codons"            "secondary.peak.columns"

merged.reads

## $consensus
##   1289-letter "DNAString" instance
## seq: NNGCTCTTATGAGGTTAGCGGCGGACGGGTGAGT...TACATGAAGCTGGAATCGCTAGTAATCGCGAT
C
##
## $alignment
##   A DNAStringSet instance of length 3
##     width seq                                               names
## [1]  1289 NNGCTCTTATGAGGTTAGCGG...-------------------- fwd
## [2]  1289 ----------------------...AATCGCTAGTAATCGCGATC rev
## [3]  1289 NNGCTCTTATGAGGTTAGCGG...AATCGCTAGTAATCGCGATC consensus
##
## $differences
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                          66            0
## 2  rev                          62            0
```

```
##
## $distance.matrix
##            fwd        rev
## fwd 0.00000000 0.09751936
## rev 0.09751936 0.00000000
## attr(,"correction")
## [1] "Jukes-Cantor"
##
## $dendrogram
## 'dendrogram' with 2 branches and 2 members total, at height 0.04875968
##
## $indels
## NULL
##
## $stop.codons
## NULL
##
## $secondary.peak.columns
## NULL
##
## attr(,"class")
## [1] "merged.read"
```

```
merged.reads$consensus
```

```
##   1289-letter "DNAString" instance
## seq: NNGCTCTTATGAGGTTAGCGGCGGACGGGTGAGT...TACATGAAGCTGGAATCGCTAGTAATCGCGAT
C
```

```
BrowseSeqs(merged.reads$alignment)
merged.reads$secondary.peak.columns
```

```
## NULL
```

```
merged.reads$differences
```

```
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                          66            0
## 2  rev                          62            0
```

```
write.dna(merged.reads,file="Sanger_isolates/95.fasta",format='fasta',nbcol =
-1, colsep = "", colw = 10000000)

fwd = readsangerseq("Sanger_isolates/98F.F.ab1")
rev = readsangerseq("Sanger_isolates/98R.R.ab1")
fwd = primarySeq(fwd)
rev = primarySeq(rev)
rev = reverseComplement(rev)
reads = DNAStringSet(c(as.character(fwd), as.character(rev)))
names(reads) = c('fwd', 'rev')
merged.reads = merge.reads(reads)
```

```
## [1] "Aligning reads"
## [1] "Calling consensus sequence"
## [1] "Calculating differences between reads and consensus"
```

```
names(merged.reads)
```

```
## [1] "consensus"              "alignment"
## [3] "differences"            "distance.matrix"
## [5] "dendrogram"             "indels"
## [7] "stop.codons"            "secondary.peak.columns"
```

```
merged.reads
```

```
## $consensus
##   737-letter "DNAString" instance
## seq: NNAGCGAGKRRCSWCSGCKGMKTRRSWCSKGKAG...CWYSMMKWCSGARTYSCWTGYAMKCKMGRTYA
G
##
## $alignment
##   A DNAStringSet instance of length 3
##     width seq                                              names
## [1]   737 NNAGCGAGTGGCGACGGCTGA...NCCCATGCACGCTAGGTT-- fwd
## [2]   737 -------GGAACCTCCGCGGC...TTGCTNGTAATCGCGATCAG rev
## [3]   737 NNAGCGAGKRRCSWCSGCKGM...TYSCWTGYAMKCKMGRTYAG consensus
##
## $differences
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                          399            0
## 2  rev                          394            0
##
## $distance.matrix
##          fwd      rev
## fwd 0.000000 1.029787
## rev 1.029787 0.000000
## attr(,"correction")
## [1] "Jukes-Cantor"
##
## $dendrogram
## 'dendrogram' with 2 branches and 2 members total, at height 0.5148934
##
## $indels
## NULL
##
## $stop.codons
## NULL
##
## $secondary.peak.columns
## NULL
##
## attr(,"class")
## [1] "merged.read"
```

```
merged.reads$consensus
```

```
##   737-letter "DNAString" instance
## seq: NNAGCGAGKRRCSWCSGCKGMKTRRSWCSKGKAG...CWYSMMKWCSGARTYSCWTGYAMKCKMGRTYA
G
```

```
BrowseSeqs(merged.reads$alignment)
merged.reads$secondary.peak.columns
```

```
## NULL
```

```
merged.reads$differences
```

```
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                         399            0
## 2  rev                         394            0
```

```
write.dna(merged.reads,file="Sanger_isolates/98.fasta",format='fasta',nbcol =
-1, colsep = "", colw = 10000000)
```

```
fwd = readsangerseq("Sanger_isolates/137F.F.ab1")
rev = readsangerseq("Sanger_isolates/137R.R.ab1")
fwd = primarySeq(fwd)
rev = primarySeq(rev)
rev = reverseComplement(rev)
reads = DNAStringSet(c(as.character(fwd), as.character(rev)))
names(reads) = c('fwd', 'rev')
merged.reads = merge.reads(reads)
```

```
## [1] "Aligning reads"
## [1] "Calling consensus sequence"
## [1] "Calculating differences between reads and consensus"
```

```
names(merged.reads)
```

```
## [1] "consensus"              "alignment"
## [3] "differences"            "distance.matrix"
## [5] "dendrogram"             "indels"
## [7] "stop.codons"            "secondary.peak.columns"
```

```
merged.reads
```

```
## $consensus
##   1276-letter "DNAString" instance
## seq: GTTGGAGAGTTGCGAACGGGTGAGTAACGCGTAG...ATGAAGTCGGAATCGCTAGTAATCGCGGATCA
G
##
## $alignment
##   A DNAStringSet instance of length 3
##     width seq                                               names
## [1]  1276 GTTGGAGAGTTGCGAACGGGT...-------------------- fwd
## [2]  1276 ----------------------...CGCTAGTAATCGCGGATCAG rev
```

```
## [3]  1276 GTTGGAGAGTTGCGAACGGGT...CGCTAGTAATCGCGGATCAG consensus
##
## $differences
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                           6            0
## 2  rev                           6            0
##
## $distance.matrix
##            fwd        rev
## fwd 0.00000000 0.03922462
## rev 0.03922462 0.00000000
## attr(,"correction")
## [1] "Jukes-Cantor"
##
## $dendrogram
## 'dendrogram' with 2 branches and 2 members total, at height 0.01961231
##
## $indels
## NULL
##
## $stop.codons
## NULL
##
## $secondary.peak.columns
## NULL
##
## attr(,"class")
## [1] "merged.read"

merged.reads$consensus

##   1276-letter "DNAString" instance
## seq: GTTGGAGAGTTGCGAACGGGTGAGTAACGCGTAG...ATGAAGTCGGAATCGCTAGTAATCGCGGATCA
G

BrowseSeqs(merged.reads$alignment)
merged.reads$secondary.peak.columns

## NULL

merged.reads$differences

##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                           6            0
## 2  rev                           6            0

write.dna(merged.reads,file="Sanger_isolates/137.fasta",format='fasta',nbcol
= -1, colsep = "", colw = 10000000)

fwd = readsangerseq("Sanger_isolates/138F.F.ab1")
rev = readsangerseq("Sanger_isolates/138R.R.ab1")
```

```
fwd = primarySeq(fwd)
rev = primarySeq(rev)
rev = reverseComplement(rev)
reads = DNAStringSet(c(as.character(fwd), as.character(rev)))
names(reads) = c('fwd', 'rev')
merged.reads = merge.reads(reads)

## [1] "Aligning reads"
## [1] "Calling consensus sequence"
## [1] "Calculating differences between reads and consensus"

names(merged.reads)

## [1] "consensus"              "alignment"
## [3] "differences"            "distance.matrix"
## [5] "dendrogram"             "indels"
## [7] "stop.codons"            "secondary.peak.columns"

merged.reads

## $consensus
##   1297-letter "DNAString" instance
## seq: TGACTGCTCTGCCTCCTGATTGAATAATCGTTTG...TGAAGCCGGAATCGCTAGTAATCGCGGATCAG
C
##
## $alignment
##   A DNAStringSet instance of length 3
##     width seq                                              names
## [1]  1297 TGACTGCTCTGCCTCCTGATT...-------------------- fwd
## [2]  1297 ----------------------...GCTAGTAATCGCGGATCAGC rev
## [3]  1297 TGACTGCTCTGCCTCCTGATT...GCTAGTAATCGCGGATCAGC consensus
##
## $differences
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                          59            0
## 2  rev                          59            0
##
## $distance.matrix
##           fwd       rev
## fwd 0.0000000 0.2335444
## rev 0.2335444 0.0000000
## attr(,"correction")
## [1] "Jukes-Cantor"
##
## $dendrogram
## 'dendrogram' with 2 branches and 2 members total, at height 0.1167722
##
## $indels
## NULL
##
## $stop.codons
```

```
## NULL
##
## $secondary.peak.columns
## NULL
##
## attr(,"class")
## [1] "merged.read"
```

```
merged.reads$consensus
```

```
##   1297-letter "DNAString" instance
## seq: TGACTGCTCTGCCTCCTGATTGAATAATCGTTTG...TGAAGCCGGAATCGCTAGTAATCGCGGATCAG
C
```

```
BrowseSeqs(merged.reads$alignment)
merged.reads$secondary.peak.columns
```

```
## NULL
```

```
merged.reads$differences
```

```
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                          59            0
## 2  rev                          59            0
```

```
write.dna(merged.reads,file="Sanger_isolates/138.fasta",format='fasta',nbcol
= -1, colsep = "", colw = 10000000)
```

```
fwd = readsangerseq("Sanger_isolates/152F.F.ab1")
rev = readsangerseq("Sanger_isolates/152R.R.ab1")
fwd = primarySeq(fwd)
rev = primarySeq(rev)
rev = reverseComplement(rev)
reads = DNAStringSet(c(as.character(fwd), as.character(rev)))
names(reads) = c('fwd', 'rev')
merged.reads = merge.reads(reads)
```

```
## [1] "Aligning reads"
## [1] "Calling consensus sequence"
## [1] "Calculating differences between reads and consensus"
```

```
names(merged.reads)
```

```
## [1] "consensus"              "alignment"
## [3] "differences"            "distance.matrix"
## [5] "dendrogram"             "indels"
## [7] "stop.codons"            "secondary.peak.columns"
```

```
merged.reads
```

```
## $consensus
##   1288-letter "DNAString" instance
## seq: GCTCACCGGAAAGAGGAGTGGCGAACGGGTGAGT...TGAAGCCGGAATCGCTAGTAATCGCGGATCAG
```

```
C
##
## $alignment
##   A DNAStringSet instance of length 3
##     width seq                                               names
## [1]  1288 GCTCACCGGAAAGAGGAGTGG...-------------------- fwd
## [2]  1288 ----------------------...GCTAGTAATCGCGGATCAGC rev
## [3]  1288 GCTCACCGGAAAGAGGAGTGG...GCTAGTAATCGCGGATCAGC consensus
##
## $differences
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                           8            0
## 2  rev                           8            0
##
## $distance.matrix
##            fwd        rev
## fwd 0.00000000 0.04859999
## rev 0.04859999 0.00000000
## attr(,"correction")
## [1] "Jukes-Cantor"
##
## $dendrogram
## 'dendrogram' with 2 branches and 2 members total, at height 0.0243
##
## $indels
## NULL
##
## $stop.codons
## NULL
##
## $secondary.peak.columns
## NULL
##
## attr(,"class")
## [1] "merged.read"

merged.reads$consensus

##   1288-letter "DNAString" instance
## seq: GCTCACCGGAAAGAGGAGTGGCGAACGGGTGAGT...TGAAGCCGGAATCGCTAGTAATCGCGGATCAG
C

BrowseSeqs(merged.reads$alignment)
merged.reads$secondary.peak.columns

## NULL

merged.reads$differences
```

```
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                           8            0
## 2  rev                           8            0
```

```
write.dna(merged.reads,file="Sanger_isolates/152.fasta",format='fasta',nbcol
= -1, colsep = "", colw = 10000000)
```

```
fwd = readsangerseq("Sanger_isolates/142F.F.ab1")
rev = readsangerseq("Sanger_isolates/142R.R.ab1")
fwd = primarySeq(fwd)
rev = primarySeq(rev)
rev = reverseComplement(rev)
reads = DNAStringSet(c(as.character(fwd), as.character(rev)))
names(reads) = c('fwd', 'rev')
merged.reads = merge.reads(reads)
```

```
## [1] "Aligning reads"
## [1] "Calling consensus sequence"
## [1] "Calculating differences between reads and consensus"
```

```
names(merged.reads)
```

```
## [1] "consensus"              "alignment"
## [3] "differences"            "distance.matrix"
## [5] "dendrogram"             "indels"
## [7] "stop.codons"            "secondary.peak.columns"
```

```
merged.reads
```

```
## $consensus
##   733-letter "DNAString" instance
## seq: AGTCTTCTAGTAGMRGSKTRSTRSWTCGCYSASG...RAWTCGSYRWAATSGTRWWMAGATTGAACGCT
C
##
## $alignment
##   A DNAStringSet instance of length 3
##     width seq                                           names
## [1]   733 -----------AGCAGCTTGC...GGTATAAAGATTGAACGCTC fwd
## [2]   733 AGTCTTCTAGTAGAGGGGTAG...CGTGATCAG----------- rev
## [3]   733 AGTCTTCTAGTAGMRGSKTRS...SGTRWWMAGATTGAACGCTC consensus
##
## $differences
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                         367            0
## 2  rev                         367            0
##
## $distance.matrix
##          fwd      rev
## fwd 0.000000 1.011149
## rev 1.011149 0.000000
## attr(,"correction")
```

```
## [1] "Jukes-Cantor"
##
## $dendrogram
## 'dendrogram' with 2 branches and 2 members total, at height 0.5055747
##
## $indels
## NULL
##
## $stop.codons
## NULL
##
## $secondary.peak.columns
## NULL
##
## attr(,"class")
## [1] "merged.read"
```

```
merged.reads$consensus
```

```
##   733-letter "DNAString" instance
## seq: AGTCTTCTAGTAGMRGSKTRSTRSWTCGCYSASG...RAWTCGSYRWAATSGTRWWMAGATTGAACGCT
C
```

```
BrowseSeqs(merged.reads$alignment)
merged.reads$secondary.peak.columns
```

```
## NULL
```

```
merged.reads$differences
```

```
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                         367            0
## 2  rev                         367            0
```

```
write.dna(merged.reads,file="Sanger_isolates/142.fasta",format='fasta',nbcol
= -1, colsep = "", colw = 10000000)
```

```
fwd = readsangerseq("Sanger_isolates/184F.F.ab1")
rev = readsangerseq("Sanger_isolates/184R.R.ab1")
fwd = primarySeq(fwd)
rev = primarySeq(rev)
rev = reverseComplement(rev)
reads = DNAStringSet(c(as.character(fwd), as.character(rev)))
names(reads) = c('fwd', 'rev')
merged.reads = merge.reads(reads)
```

```
## [1] "Aligning reads"
## [1] "Calling consensus sequence"
## [1] "Calculating differences between reads and consensus"
```

```
names(merged.reads)
```

```
## [1] "consensus"              "alignment"
## [3] "differences"            "distance.matrix"
## [5] "dendrogram"             "indels"
## [7] "stop.codons"            "secondary.peak.columns"
```

```
merged.reads
```

```
## $consensus
##   1281-letter "DNAString" instance
## seq: GCTTGCACTGAGATGGCGACCGGCGCACGGGTGA...GTGAAGCTGGATTCGCTAGTAATCGCGCATCA
G
##
## $alignment
##   A DNAStringSet instance of length 3
##     width seq                                          names
## [1]  1281 GCTTGCACTGAGATGGCGACC...-------------------- fwd
## [2]  1281 --------------------...CGCTAGTAATCGCGCATCAG rev
## [3]  1281 GCTTGCACTGAGATGGCGACC...CGCTAGTAATCGCGCATCAG consensus
##
## $differences
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                         114            0
## 2  rev                         114            0
##
## $distance.matrix
##           fwd       rev
## fwd 0.0000000 0.1589253
## rev 0.1589253 0.0000000
## attr(,"correction")
## [1] "Jukes-Cantor"
##
## $dendrogram
## 'dendrogram' with 2 branches and 2 members total, at height 0.07946267
##
## $indels
## NULL
##
## $stop.codons
## NULL
##
## $secondary.peak.columns
## NULL
##
## attr(,"class")
## [1] "merged.read"
```

```
merged.reads$consensus
```

```
##   1281-letter "DNAString" instance
## seq: GCTTGCACTGAGATGGCGACCGGCGCACGGGTGA...GTGAAGCTGGATTCGCTAGTAATCGCGCATCA
G
```

```
BrowseSeqs(merged.reads$alignment)
merged.reads$secondary.peak.columns

## NULL

merged.reads$differences

##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                         114            0
## 2  rev                         114            0

write.dna(merged.reads,file="Sanger_isolates/184.fasta",format='fasta',nbcol
= -1, colsep = "", colw = 10000000)

fwd = readsangerseq("Sanger_isolates/185F.F.ab1")
rev = readsangerseq("Sanger_isolates/185R.R.ab1")
fwd = primarySeq(fwd)
rev = primarySeq(rev)
rev = reverseComplement(rev)
reads = DNAStringSet(c(as.character(fwd), as.character(rev)))
names(reads) = c('fwd', 'rev')
merged.reads = merge.reads(reads)

## [1] "Aligning reads"
## [1] "Calling consensus sequence"
## [1] "Calculating differences between reads and consensus"

names(merged.reads)

## [1] "consensus"              "alignment"
## [3] "differences"            "distance.matrix"
## [5] "dendrogram"             "indels"
## [7] "stop.codons"            "secondary.peak.columns"

merged.reads

## $consensus
##   1286-letter "DNAString" instance
## seq: GCACTGAGATGGCGACCGGCGCACGGGTGAGTAA...CGTGAAGCTGGATTCGCTAGTAATCGCGCATC
A
##
## $alignment
##   A DNAStringSet instance of length 3
##     width seq                                               names
## [1]  1286 GCACTGAGATGGCGACCGGCG...-------------------- fwd
## [2]  1286 ----------------------...TCGCTAGTAATCGCGCATCA rev
## [3]  1286 GCACTGAGATGGCGACCGGCG...TCGCTAGTAATCGCGCATCA consensus
##
## $differences
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                          45            0
## 2  rev                          45            0
```

```
##
## $distance.matrix
##            fwd        rev
## fwd 0.00000000 0.07544043
## rev 0.07544043 0.00000000
## attr(,"correction")
## [1] "Jukes-Cantor"
##
## $dendrogram
## 'dendrogram' with 2 branches and 2 members total, at height 0.03772021
##
## $indels
## NULL
##
## $stop.codons
## NULL
##
## $secondary.peak.columns
## NULL
##
## attr(,"class")
## [1] "merged.read"
```

```
merged.reads$consensus
```

```
##   1286-letter "DNAString" instance
## seq: GCACTGAGATGGCGACCGGCGCACGGGTGAGTAA...CGTGAAGCTGGATTCGCTAGTAATCGCGCATC
A
```

```
BrowseSeqs(merged.reads$alignment)
merged.reads$secondary.peak.columns
```

```
## NULL
```

```
merged.reads$differences
```

```
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                          45            0
## 2  rev                          45            0
```

```
write.dna(merged.reads,file="Sanger_isolates/185.fasta",format='fasta',nbcol
= -1, colsep = "", colw = 10000000)
```

```
fwd = readsangerseq("Sanger_isolates/186F.F.ab1")
rev = readsangerseq("Sanger_isolates/186R.R.ab1")
fwd = primarySeq(fwd)
rev = primarySeq(rev)
rev = reverseComplement(rev)
reads = DNAStringSet(c(as.character(fwd), as.character(rev)))
names(reads) = c('fwd', 'rev')
merged.reads = merge.reads(reads)
```

```
## [1] "Aligning reads"
## [1] "Calling consensus sequence"
## [1] "Calculating differences between reads and consensus"
```

```
names(merged.reads)
```

```
## [1] "consensus"              "alignment"
## [3] "differences"            "distance.matrix"
## [5] "dendrogram"             "indels"
## [7] "stop.codons"            "secondary.peak.columns"
```

```
merged.reads
```

```
## $consensus
##   1270-letter "DNAString" instance
## seq: GCAACTGAGATGGCGACCGGCGCACGGGTGAGTA...CGTGAAGCTGGATTCGCTAGTAATCGCGCATC
A
##
## $alignment
##   A DNAStringSet instance of length 3
##     width seq                                             names
## [1]  1270 GCAACTGAGATGGCGACCGGC...-------------------- fwd
## [2]  1270 ----------------------...TCGCTAGTAATCGCGCATCA rev
## [3]  1270 GCAACTGAGATGGCGACCGGC...TCGCTAGTAATCGCGCATCA consensus
##
## $differences
##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                          26            0
## 2  rev                          26            0
##
## $distance.matrix
##            fwd        rev
## fwd 0.00000000 0.07729607
## rev 0.07729607 0.00000000
## attr(,"correction")
## [1] "Jukes-Cantor"
##
## $dendrogram
## 'dendrogram' with 2 branches and 2 members total, at height 0.03864803
##
## $indels
## NULL
##
## $stop.codons
## NULL
##
## $secondary.peak.columns
## NULL
##
## attr(,"class")
## [1] "merged.read"
```

```
merged.reads$consensus

##   1270-letter "DNAString" instance
## seq: GCAACTGAGATGGCGACCGGCGCACGGGTGAGTA...CGTGAAGCTGGATTCGCTAGTAATCGCGCATC
A

BrowseSeqs(merged.reads$alignment)
merged.reads$secondary.peak.columns

## NULL

merged.reads$differences

##   name pairwise.diffs.to.consensus unused.chars
## 1  fwd                          26            0
## 2  rev                          26            0

write.dna(merged.reads,file="Sanger_isolates/186.fasta",format='fasta',nbcol
= -1, colsep = "", colw = 10000000)
```

Most similar OTUs for each Sanger read

A reference alignment was built from the Sanger reads (for each donor separately) applying the sina alinger (Pruesse et al. 2012). OTUs were aligned to this reference Sanger alignment in mothur (align.seqs), yielding a report with the closest Sanger reference for each OTU based on kmer searching (Schloss et al. 2009). The report for each donor was loaded into R, version 3.4.2 (2017-09-28) (R Core Team 2016). For each isolate in the report, the top two OTUs with the highest SearchScores were selected. A fasta file was constructed containing these OTUs.

```
# Donor1
donor1topotusanger <- read.table('DataS13.report',header=TRUE)
donor1topotusanger <- donor1topotusanger[order(donor1topotusanger$TemplateNam
e,-donor1topotusanger$SearchScore),]
donor1topotusanger <-donor1topotusanger%>%group_by(TemplateName) %>%dplyr::sl
ice(1:2)
# find these otu's in the fasta
donor1fastaotusforfylogeny <- read.table(DataS17.fasta')
donor1fastaotusforfylogeny <- donor1fastaotusforfylogeny[donor1fastaotusforfy
logeny$V1%in%donor1topotusanger$QueryName,]
wantedforfylogeny1 <-paste(RDPtax_noprob[donor1fastaotusforfylogeny$V1,]$Genu
s,donor1fastaotusforfylogeny$V1,sep=" ")
donor1fastaotusforfylogeny$V1 <- paste(RDPtax_noprob[donor1fastaotusforfyloge
ny$V1,]$Genus,donor1fastaotusforfylogeny$V1,sep="_")
write.csv2(donor1fastaotusforfylogeny,"donor1fastaotusforfylogeny.csv",row.na
mes=FALSE,col.names=FALSE)

# Donor2
donor2topotusanger <- read.table('DataS14.report',header=TRUE)
donor2topotusanger <- donor2topotusanger[order(donor2topotusanger$TemplateNam
e,-donor2topotusanger$SearchScore),]
```

```
donor2topotusanger <-donor2topotusanger%>%group_by(TemplateName) %>%dplyr::sl
ice(1:2)
donor2fastaotusforfylogeny <- read.table('DataS17.fasta')
donor2fastaotusforfylogeny <- donor2fastaotusforfylogeny[donor2fastaotusforfy
logeny$V1%in%donor2topotusanger$QueryName,]
wantedforfylogeny2 <-paste(RDPtax_noprob[donor2fastaotusforfylogeny$V1,]$Genu
s,donor2fastaotusforfylogeny$V1,sep=" ")
donor2fastaotusforfylogeny$V1 <- paste(RDPtax_noprob[donor2fastaotusforfyloge
ny$V1,]$Genus,donor2fastaotusforfylogeny$V1,sep="_")
write.csv2(donor2fastaotusforfylogeny,"donor2fastaotusforfylogeny.csv",row.na
mes=FALSE,col.names=FALSE)

# Donor3
donor3topotusanger <- read.table('DataS15.report',header=TRUE)
donor3topotusanger <- donor3topotusanger[order(donor3topotusanger$TemplateNam
e,-donor3topotusanger$SearchScore),]
donor3topotusanger <-donor3topotusanger%>%group_by(TemplateName) %>%dplyr::sl
ice(1:2)
donor3fastaotusforfylogeny <- read.table('DataS17.fasta')
donor3fastaotusforfylogeny <- donor3fastaotusforfylogeny[donor3fastaotusforfy
logeny$V1%in%donor3topotusanger$QueryName,]
wantedforfylogeny3 <-paste(RDPtax_noprob[donor3fastaotusforfylogeny$V1,]$Genu
s,donor3fastaotusforfylogeny$V1,sep=" ")
donor3fastaotusforfylogeny$V1 <- paste(RDPtax_noprob[donor3fastaotusforfyloge
ny$V1,]$Genus,donor3fastaotusforfylogeny$V1,sep="_")
write.csv2(donor3fastaotusforfylogeny,"donor3fastaotusforfylogeny.csv",row.na
mes=FALSE,col.names=FALSE)


# Donor4
donor4topotusanger <- read.table('DataS16.report',header=TRUE)
donor4topotusanger <- donor4topotusanger[order(donor4topotusanger$TemplateNam
e,-donor4topotusanger$SearchScore),]
donor4topotusanger <-donor4topotusanger%>%group_by(TemplateName) %>%dplyr::sl
ice(1:2)
donor4fastaotusforfylogeny <- read.table('DataS18.fasta')
donor4fastaotusforfylogeny <- donor4fastaotusforfylogeny[donor4fastaotusforfy
logeny$V1%in%donor4topotusanger$QueryName,]
wantedforfylogeny4 <-paste(RDPtax4_noprob[donor4fastaotusforfylogeny$V1,]$Gen
us,donor4fastaotusforfylogeny$V1,sep=" ")
donor4fastaotusforfylogeny$V1 <- paste(RDPtax4_noprob[donor4fastaotusforfylog
eny$V1,]$Genus,donor4fastaotusforfylogeny$V1,sep="_")
write.csv2(donor4fastaotusforfylogeny,"donor4fastaotusforfylogeny.csv",row.na
mes=FALSE,col.names=FALSE)
```

Create metadata for the annotation of ITOL phylogenetic trees

In order to compare the OTUs spanning the V4 region of the 16S rRNA gene with the near full-length Sanger reads, the RAxML implementation of the evolutionary placement algorithm of short reads, as introduced by Berger et al. (2011), was used (Stamatakis 2014). The bootstrap supported maximum likelihood (ML) phylogenetic reference tree was also constructed using RAxML, selecting the General Time Reversible model of nucleotide substitution under the Gamma model of rate heterogeneity (GTRGAMMA) with the parsimony random seed set to 12345. The rapid bootstrap analysis was conducted starting from N=1000 distinct randomized maximum parsimony trees and was followed by a search for the best-scoring ML tree with rapid bootstrap random number seed 123 (Stamatakis 2014). The best scoring ML tree with the OTU short read insertions was visualized in iTOL (Letunic & Bork 2016). Metadata for the annotation of the iTOL trees is created in the chuncks below.

```
currentotuname1 <- donor1fastaotusforfylogeny$V1
RAXMLotuname1 <- paste("QUERY___",currentotuname1,sep="")
otuname1df <- cbind(currentname1=RAXMLotuname1,wantedname1=wantedforfylogeny1
)

wantedsangername1 <- splittax(read.table('DataS19.taxonomy'),"seqs")
wantedsangername1$Genus <- mapvalues(wantedsangername1$Genus,from=c("Enteroba
cteriaceae_unclassified","Erysipelotrichaceae_unclassified","Lachnospiraceae_
unclassified","Lactobacillales_unclassified","Actinobacteria_unclassified","B
urkholderiales_unclassified","Clostridiales_unclassified"),to=c("Enterobacter
iaceae","Erysipelotrichaceae","Lachnospiraceae","Lactobacillales","Actinobact
eria","Burkholderiales","Clostridiales"))
currentsangername1 <-as.character(wantedsangername1$id)
wantedsangername1 <- as.character(paste(wantedsangername1$id,wantedsangername
1$Genus))
sangername1df <- cbind(currentname1=currentsangername1,wantedname1=wantedsang
ername1)

itolname1 <- data.frame(rbind(otuname1df,sangername1df))
itolname1$itolcode <- paste(itolname1$currentname1,",",itolname1$wantedname1,
sep="")
itollabel1<- c("LABELS","SEPARATOR COMMA","DATA",itolname1$itolcode)
write.table(itollabel1,"itollabel1.txt",row.names=FALSE,col.names=FALSE,quote
=FALSE)

#style labels
itollabelstyle1 <- paste(currentsangername1,",label",",#000000",",bold",",1",
sep="")
itollabelstyle1<- c("TREE_COLORS","SEPARATOR COMMA","DATA",itollabelstyle1)
write.table(itollabelstyle1,"itollabelstyle1.txt",row.names=FALSE,col.names=F
ALSE,quote=FALSE)

# Stacked bar charts of the relative abundance
propbar1 <- subset(shared3minsingletonwnwnprop*100,select=c(Donor1_high4_L.V4
```

```
,Donor1_low4_L.V4,FS_1_isolationhigh.V4))
propbar1 <- propbar1[rownames(propbar1)%in%donor1topotusanger$QueryName,]
propbar1 <- round(propbar1,2)
propbar1 <- rownames_to_column(propbar1)
propbar1$rowname <-paste(RDPtax_noprob[propbar1$rowname,]$Genus,propbar1$rown
ame,sep="_")
propbar1$rowname<- paste("QUERY___",propbar1$rowname,sep="")
propbar1$high <- paste(",",propbar1$Donor1_high4_L.V4,sep="")
propbar1$low<- paste(",",propbar1$Donor1_low4_L.V4,sep="")
propbar1$FS <- paste(",",propbar1$FS_1_isolationhigh.V4,sep="")
propbar1 <- propbar1[,-c(2:4)]
propbar1 <- rbind(c("DATA","","",""),propbar1)
propbar1 <- rbind(c("DATASET_SCALE",",10",",25",",50"),propbar1)
propbar1 <- rbind(c("ALIGN_FIELDS",",0","",""),propbar1)
propbar1 <- rbind(c("LEGEND_TITLE",",Relative abundance (%)","",""),propbar1)
propbar1 <- rbind(c("LEGEND_SHAPES",",1",",1",",1"),propbar1)
propbar1 <- rbind(c("LEGEND_COLORS",",#323C4D",",#A03D44",",#98B736"),propbar
1)
propbar1 <- rbind(c("LEGEND_LABELS",",pH6.8",",pH5.8",",Fecal slurry"),propba
r1)
propbar1 <- rbind(c("WIDTH",",350","",""),propbar1)
propbar1 <- rbind(c("FIELD_COLORS",",#323C4D",",#A03D44",",#98B736"),propbar1
)
propbar1 <- rbind(c("FIELD_LABELS",",f1",",f2",",f3"),propbar1)
propbar1 <- rbind(c("COLOR",",#ff0000","",""),propbar1)
propbar1 <- rbind(c("DATASET_LABEL",",barchart","",""),propbar1)
propbar1 <- rbind(c("SEPARATOR"," COMMA","",""),propbar1)
propbar1 <- rbind(c("DATASET_MULTIBAR","","",""),propbar1)
write.table(propbar1,"itolpropbar1.txt",col.names=FALSE,row.names=FALSE,sep="
",quote=FALSE)

currentotuname2 <- donor2fastaotusforfylogeny$V1
RAXMLotuname2 <- paste("QUERY___",currentotuname2,sep="")
otuname2df <- cbind(currentname2=RAXMLotuname2,wantedname2=wantedforfylogeny2
)

wantedsangername2 <- splittax(read.table('DataS20.taxonomy'),"seqs")
wantedsangername2$Genus <- mapvalues(wantedsangername2$Genus,from=c("Enteroba
cteriaceae_unclassified","Erysipelotrichaceae_unclassified","Lachnospiraceae_
unclassified","Lactobacillales_unclassified","Actinobacteria_unclassified","B
urkholderiales_unclassified","Clostridiales_unclassified"),to=c("Enterobacter
iaceae","Erysipelotrichaceae","Lachnospiraceae","Lactobacillales","Actinobact
eria","Burkholderiales","Clostridiales"))
currentsangername2 <-as.character(wantedsangername2$id)
wantedsangername2 <- as.character(paste(wantedsangername2$id,wantedsangername
2$Genus))
sangername2df <- cbind(currentname2=currentsangername2,wantedname2=wantedsang
ername2)

itolname2 <- data.frame(rbind(otuname2df,sangername2df))
```

```
itolname2$itolcode <- paste(itolname2$currentname2,",",itolname2$wantedname2,
sep="")
itollabel2<- c("LABELS","SEPARATOR COMMA","DATA",itolname2$itolcode)
write.table(itollabel2,"itollabel2.txt",row.names=FALSE,col.names=FALSE,quote
=FALSE)

#style labels
itollabelstyle2 <- paste(currentsangername2,",label",",#000000",",bold",",1",
sep="")
itollabelstyle2<- c("TREE_COLORS","SEPARATOR COMMA","DATA",itollabelstyle2)
write.table(itollabelstyle2,"itollabelstyle2.txt",row.names=FALSE,col.names=F
ALSE,quote=FALSE)

# Stacked bar charts of the relative abundance
propbar2 <- subset(shared3minsingletonwnwnprop*100,select=c(Donor2_high4_L.V4
,Donor2_low4_L.V4,FS_2_isolationhigh.V4))
propbar2 <- propbar2[rownames(propbar2)%in%donor2topotusanger$QueryName,]
propbar2 <- round(propbar2,2)
propbar2 <- rownames_to_column(propbar2)
propbar2$rowname <-paste(RDPtax_noprob[propbar2$rowname,]$Genus,propbar2$rown
ame,sep="_")
propbar2$rowname<- paste("QUERY___",propbar2$rowname,sep="")
propbar2$high <- paste(",",propbar2$Donor2_high4_L.V4,sep="")
propbar2$low<- paste(",",propbar2$Donor2_low4_L.V4,sep="")
propbar2$FS <- paste(",",propbar2$FS_2_isolationhigh.V4,sep="")
propbar2 <- propbar2[,-c(2:4)]
propbar2 <- rbind(c("DATA","","",""),propbar2)
propbar2 <- rbind(c("DATASET_SCALE",",5",",10",",25"),propbar2)
propbar2 <- rbind(c("ALIGN_FIELDS",",0","",""),propbar2)
propbar2 <- rbind(c("LEGEND_TITLE",",Relative abundance (%)","",""),propbar2)
propbar2 <- rbind(c("LEGEND_SHAPES",",1",",1",",1"),propbar2)
propbar2 <- rbind(c("LEGEND_COLORS",",#323C4D",",#A03D44",",#98B736"),propbar
2)
propbar2 <- rbind(c("LEGEND_LABELS",",pH6.8",",pH5.8",",Fecal slurry"),propba
r2)
propbar2 <- rbind(c("WIDTH",",350","",""),propbar2)
propbar2 <- rbind(c("FIELD_COLORS",",#323C4D",",#A03D44",",#98B736"),propbar2
)
propbar2 <- rbind(c("FIELD_LABELS",",f1",",f2",",f3"),propbar2)
propbar2 <- rbind(c("COLOR",",#ff0000","",""),propbar2)
propbar2 <- rbind(c("DATASET_LABEL",",barchart","",""),propbar2)
propbar2 <- rbind(c("SEPARATOR"," COMMA","",""),propbar2)
propbar2 <- rbind(c("DATASET_MULTIBAR","","",""),propbar2)
write.table(propbar2,"itolpropbar2.txt",col.names=FALSE,row.names=FALSE,sep="
",quote=FALSE)

currentotuname3 <- donor3fastaotusforfylogeny$V1
RAXMLotuname3 <- paste("QUERY___",currentotuname3,sep="")
otuname3df <- cbind(currentname3=RAXMLotuname3,wantedname3=wantedforfylogeny3
)
```

```
wantedsangername3 <- splittax(read.table('DataS21.taxonomy'),"seqs")
wantedsangername3$Genus <- mapvalues(wantedsangername3$Genus,from=c("Enteroba
cteriaceae_unclassified","Erysipelotrichaceae_unclassified","Lachnospiraceae_
unclassified","Lactobacillales_unclassified","Actinobacteria_unclassified","B
urkholderiales_unclassified","Clostridiales_unclassified"),to=c("Enterobacter
iaceae","Erysipelotrichaceae","Lachnospiraceae","Lactobacillales","Actinobact
eria","Burkholderiales","Clostridiales"))
currentsangername3 <-as.character(wantedsangername3$id)
wantedsangername3 <- as.character(paste(wantedsangername3$id,wantedsangername
3$Genus))
sangername3df <- cbind(currentname3=currentsangername3,wantedname3=wantedsang
ername3)

itolname3 <- data.frame(rbind(otuname3df,sangername3df))
itolname3$itolcode <- paste(itolname3$currentname3,",",itolname3$wantedname3,
sep="")
itollabel3<- c("LABELS","SEPARATOR COMMA","DATA",itolname3$itolcode)
write.table(itollabel3,"itollabel3.txt",row.names=FALSE,col.names=FALSE,quote
=FALSE)

#style labels
itollabelstyle3 <- paste(currentsangername3,",label",",#000000",",bold",",1",
sep="")
itollabelstyle3<- c("TREE_COLORS","SEPARATOR COMMA","DATA",itollabelstyle3)
write.table(itollabelstyle3,"itollabelstyle3.txt",row.names=FALSE,col.names=F
ALSE,quote=FALSE)

# Stacked bar charts of the relative abundance
propbar3 <- subset(shared3minsingletonwnwnprop*100,select=c(Donor3_high4_L.V4
,Donor3_low4_L.V4,FS_3_isolationhigh.V4))
propbar3 <- propbar3[rownames(propbar3)%in%donor3topotusanger$QueryName,]
propbar3 <- round(propbar3,2)
propbar3 <- rownames_to_column(propbar3)
propbar3$rowname <-paste(RDPtax_noprob[propbar3$rowname,]$Genus,propbar3$rown
ame,sep="_")
propbar3$rowname<- paste("QUERY___",propbar3$rowname,sep="")
propbar3$high <- paste(",",propbar3$Donor3_high4_L.V4,sep="")
propbar3$low<- paste(",",propbar3$Donor3_low4_L.V4,sep="")
propbar3$FS <- paste(",",propbar3$FS_3_isolationhigh.V4,sep="")
propbar3 <- propbar3[,-c(2:4)]
propbar3 <- rbind(c("DATA","","",""),propbar3)
propbar3 <- rbind(c("DATASET_SCALE",",25",",50",",100"),propbar3)
propbar3 <- rbind(c("ALIGN_FIELDS",",0","",""),propbar3)
propbar3 <- rbind(c("LEGEND_TITLE",",Relative abundance (%)","",""),propbar3)
propbar3 <- rbind(c("LEGEND_SHAPES",",1",",1",",1"),propbar3)
propbar3 <- rbind(c("LEGEND_COLORS",",#323C4D",",#A03D44",",#98B736"),propbar
3)
propbar3 <- rbind(c("LEGEND_LABELS",",pH6.8",",pH5.8",",Fecal slurry"),propba
r3)
```

```
propbar3 <- rbind(c("WIDTH",",350","",""),propbar3)
propbar3 <- rbind(c("FIELD_COLORS",",#323C4D",",#A03D44",",#98B736"),propbar3
)
propbar3 <- rbind(c("FIELD_LABELS",",f1",",f2",",f3"),propbar3)
propbar3 <- rbind(c("COLOR",",#ff0000","",""),propbar3)
propbar3 <- rbind(c("DATASET_LABEL",",barchart","",""),propbar3)
propbar3 <- rbind(c("SEPARATOR"," COMMA","",""),propbar3)
propbar3 <- rbind(c("DATASET_MULTIBAR","","",""),propbar3)
write.table(propbar3,"itolpropbar3.txt",col.names=FALSE,row.names=FALSE,sep="
",quote=FALSE)

currentotuname4 <- donor4fastaotusforfylogeny$V1
RAXMLotuname4 <- paste("QUERY___",currentotuname4,sep="")
otuname4df <- cbind(currentname4=RAXMLotuname4,wantedname4=wantedforfylogeny4
)

wantedsangername4 <- splittax(read.table('DataS22.taxonomy'),"seqs")
wantedsangername4$Genus <- mapvalues(wantedsangername4$Genus,from=c("Enteroba
cteriaceae_unclassified","Erysipelotrichaceae_unclassified","Lachnospiraceae_
unclassified","Lactobacillales_unclassified","Actinobacteria_unclassified","B
urkholderiales_unclassified","Clostridiales_unclassified","Ruminococcaceae_un
classified","Coriobacteriaceae_unclassified","Lactobacillaceae_unclassified",
"Microbacteriaceae_unclassified"),to=c("Enterobacteriaceae","Erysipelotrichac
eae","Lachnospiraceae","Lactobacillales","Actinobacteria","Burkholderiales","
Clostridiales","Ruminococcaceae","Coriobacteriaceae","Lactobacillaceae","Micr
obacteriaceae"))
currentsangername4 <-as.character(wantedsangername4$id)
wantedsangername4 <- as.character(paste(wantedsangername4$id,wantedsangername
4$Genus))
sangername4df <- cbind(currentname4=currentsangername4,wantedname4=wantedsang
ername4)

itolname4 <- data.frame(rbind(otuname4df,sangername4df))
itolname4$itolcode <- paste(itolname4$currentname4,",",itolname4$wantedname4,
sep="")
itollabel4<- c("LABELS","SEPARATOR COMMA","DATA",itolname4$itolcode)
write.table(itollabel4,"itollabel4.txt",row.names=FALSE,col.names=FALSE,quote
=FALSE)

#style labels
itollabelstyle4 <- paste(currentsangername4,",label",",#000000",",bold",",1",
sep="")
itollabelstyle4<- c("TREE_COLORS","SEPARATOR COMMA","DATA",itollabelstyle4)
write.table(itollabelstyle4,"itollabelstyle4.txt",row.names=FALSE,col.names=F
ALSE,quote=FALSE)

# Stacked bar charts of the relative abundance
propbar4 <- subset(shared4minsingletonwnwnprop*100,select=c(Kim8_Donor4_high4
_L,Kim4_Donor4_low4_L,Kim1_Donor4_FS_high))
propbar4 <- propbar4[rownames(propbar4)%in%donor4topotusanger$QueryName,]
```

```
propbar4 <- round(propbar4,2)
propbar4 <- rownames_to_column(propbar4)
propbar4$rowname <-paste(RDPtax4_noprob[propbar4$rowname,]$Genus,propbar4$row
name,sep="_")
propbar4$rowname<- paste("QUERY___",propbar4$rowname,sep="")
propbar4$high <- paste(",",propbar4$Kim8_Donor4_high4_L,sep="")
propbar4$low<- paste(",",propbar4$Kim4_Donor4_low4_L,sep="")
propbar4$FS <- paste(",",propbar4$Kim1_Donor4_FS_high,sep="")
propbar4 <- propbar4[,-c(2:4)]
propbar4 <- rbind(c("DATA","","",""),propbar4)
propbar4 <- rbind(c("DATASET_SCALE",",25",",50",",100"),propbar4)
propbar4 <- rbind(c("ALIGN_FIELDS",",0","",""),propbar4)
propbar4 <- rbind(c("LEGEND_TITLE",",Relative abundance (%)","",""),propbar4)
propbar4 <- rbind(c("LEGEND_SHAPES",",1",",1",",1"),propbar4)
propbar4 <- rbind(c("LEGEND_COLORS",",#323C4D",",#A03D44",",#98B736"),propbar
4)
propbar4 <- rbind(c("LEGEND_LABELS",",pH6.8",",pH5.8",",Fecal slurry"),propba
r4)
propbar4 <- rbind(c("WIDTH",",350","",""),propbar4)
propbar4 <- rbind(c("FIELD_COLORS",",#323C4D",",#A03D44",",#98B736"),propbar4
)
propbar4 <- rbind(c("FIELD_LABELS",",f1",",f2",",f3"),propbar4)
propbar4 <- rbind(c("COLOR",",#ff0000","",""),propbar4)
propbar4 <- rbind(c("DATASET_LABEL",",barchart","",""),propbar4)
propbar4 <- rbind(c("SEPARATOR"," COMMA","",""),propbar4)
propbar4 <- rbind(c("DATASET_MULTIBAR","","",""),propbar4)
write.table(propbar4,"itolpropbar4.txt",col.names=FALSE,row.names=FALSE,sep="
",quote=FALSE)
```