Script R2: Whole Metagenome Contig and Sequencing Statistics

HANNIGAN GD, GRICE EA, ET AL.

Abstract

This protocol outlines the analysis used to plot contig coverage stats, as well as sequence count and length stats. We begin with visualizing contig length vs coverage. We then visualize the distributions of sequence counts per sample as a probability density plot (similar idea as a histogram), and then do the same for median sequence length. Based on the methods from the following publication:

Hannigan, Geoffrey D., et al. "The Human Skin Double-Stranded DNA Virome: Topographical and Temporal Diversity, Genetic Enrichment, and Dynamic Associations with the Host Microbiome." *mBio* 6.5 (2015): e01578-15.

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Guidelines

sessionInfo()

```
## R version 3.2.0 (2015-04-16)
## Platform: x86_64-apple-darwin13.4.0 (64-bit)
## Running under: OS X 10.10.4 (Yosemite)
## ## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats graphics grDevices utils datasets methods base
##
## loaded via a namespace (and not attached):
## [1] magrittr_1.5    formatR_1.2    tools_3.2.0    htmltools_0.2.6
## [5] yaml_2.1.13    stringi_0.4-1    rmarkdown_0.7    knitr_1.10.5
## [9] stringr_1.0.0    digest_0.6.8    evaluate_0.7
```

Before start

Supplemental informtion available at:

https://figshare.com/articles/The_Human_Skin_dsDNA_Virome_Topographical_and_Temporal_Diversity

_Genetic_Enrichment_and_Dynamic_Associations_with_the_Host_Microbiome/1281248

Protocol

Step 1.
Load the required R packages.

cmd COMMAND
```
library("plyr")
packageVersion("plyr")

library("ggplot2")
packageVersion("ggplot2")

library("hexbin")
packageVersion("hexbin")

library("reshape2")
packageVersion("reshape2")
```

EXPECTED RESULTS
```
## [1] '1.8.2'



## [1] '1.0.1'



## [1] '1.27.0'



## [1] '1.4.1'
```

Step 2.
Import the tab delimited file containing the virome contig statistics.

cmd COMMAND
```
contig_stats <-
 read.delim("../../IntermediateOutput/Whole_Microbiome_Sequence_Counts/contig_length_with_c
overage_for_graphing.tsv", header=TRUE, sep='\t')
head(contig_stats)
```

EXPECTED RESULTS

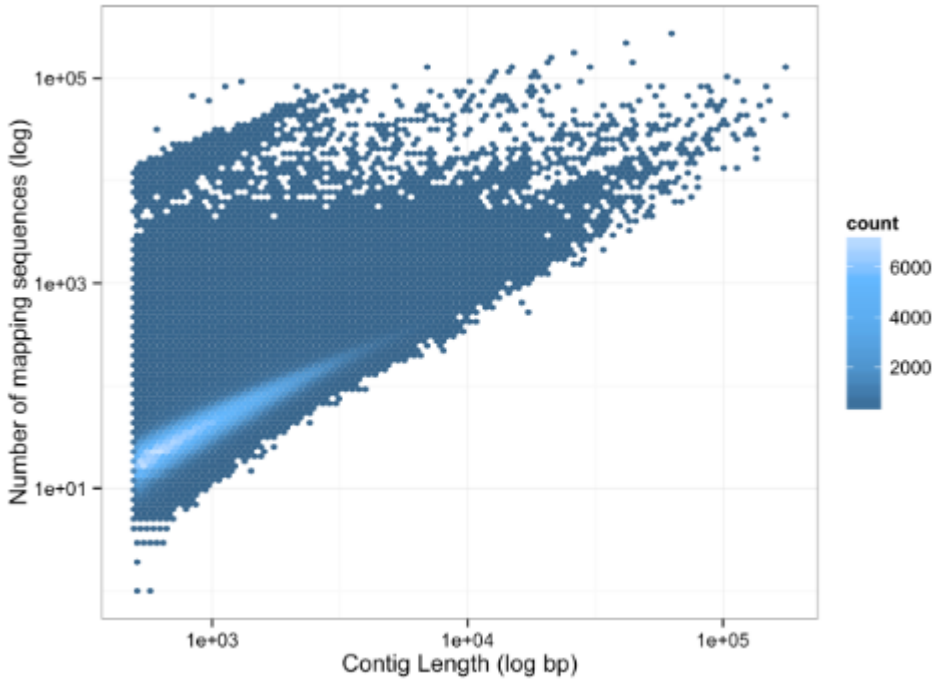
##	contig	count	contig_length
## 1	144983_	34	750
## 2	603659_	30	806
## 3	502067_	11	524
## 4	105059_	24	1091
## 5	454425_	53	1110
## 6	140762_	24	927

Step 3.
Generate a scatter plot comparing the number of mapped sequences to each contig, compared to the contig length.

cmd COMMAND

```
ggplot(contig_stats, aes(x=contig_length, y=count)) + theme_bw() + stat_binhex(bins=100)+ s
cale_fill_gradientn(colours=c("steelblue4", "steelblue3", "steelblue2","steelblue1","slateg
ray1")) + scale_y_log10() + scale_x_log10() + xlab("Contig Length (log bp)") + ylab("Number
 of mapping sequences (log)")
```

EXPECTED RESULTS



Step 4.
Next we will estimate contig coverage, using a sequence length value of 150 bp.

cmd COMMAND

```
contig_stats$coverage <- (contig_stats$count * 150) / contig_stats$contig_length
head(contig_stats)
```

EXPECTED RESULTS

##	contig	count	contig_length	coverage
## 1	144983_	34	750	6.800000
## 2	603659_	30	806	5.583127
## 3	502067_	11	524	3.148855
## 4	105059_	24	1091	3.299725
## 5	454425_	53	1110	7.162162
## 6	140762_	24	927	3.883495

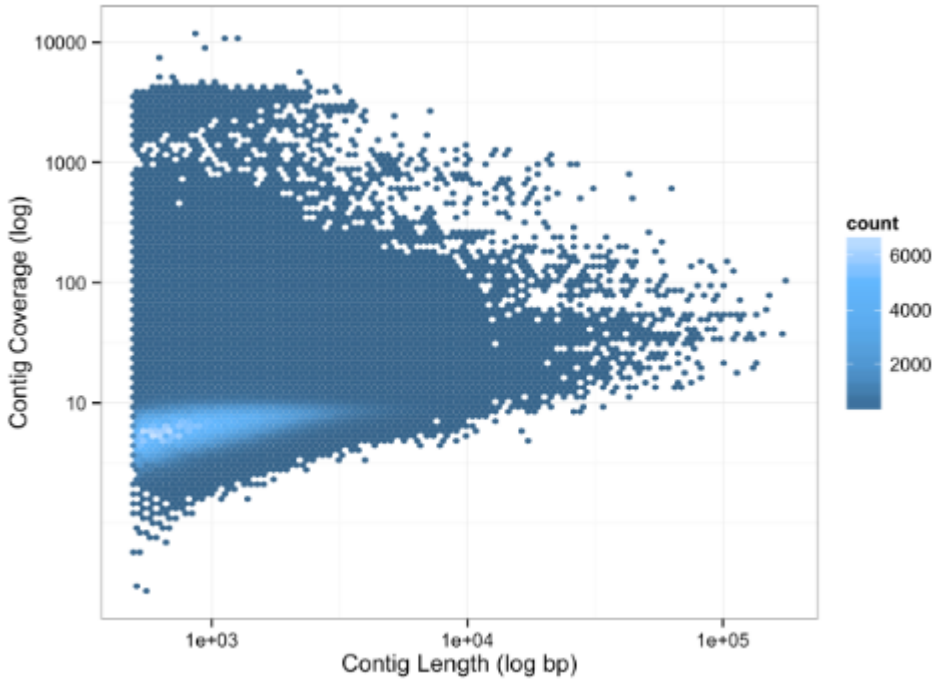
Step 5.

Like above, we will plot the contig coverage against the contig length.

cmd COMMAND

```
ggplot(contig_stats, aes(x=contig_length, y=coverage)) + theme_bw() + stat_binhex(bins=100)
+ scale_fill_gradientn(colours=c("steelblue4", "steelblue3", "steelblue2","steelblue1","sla
tegray1")) + scale_y_log10(breaks=c(1e+01, 1e+02, 1e+03, 1e+04, 1e+05)) + scale_x_log10() +
 xlab("Contig Length (log bp)") + ylab("Contig Coverage (log)")
```

EXPECTED RESULTS



Step 6.

Next we can calculate the sequence statistics. First import the sequence summary statistics table. Be sure to change the column names of the input data frames, and to merge the data frames together for downstream processing. Here we will also import and format the mapping file.

cmd COMMAND

```
INPUT_RAW <-
 read.delim("../../IntermediateOutput/Whole_Microbiome_Sequence_Counts/raw_sequence_counts.
tsv", header=FALSE, sep="\t")
colnames(INPUT_RAW) <- c("SampleID","Raw")
INPUT_TRIM <-
 read.delim("../../IntermediateOutput/Whole_Microbiome_Sequence_Counts/trimmed_sequence_cou
nts.tsv", header=FALSE, sep="\t")
colnames(INPUT_TRIM) <- c("SampleID","Trim")
INPUT_HUMAN <-
 read.delim("../../IntermediateOutput/Whole_Microbiome_Sequence_Counts/human_deconseq_seque
nce_counts.tsv", header=FALSE, sep="\t")
colnames(INPUT_HUMAN) <- c("SampleID","Human")
INPUT_NEG <-
 read.delim("../../IntermediateOutput/Whole_Microbiome_Sequence_Counts/phix_clean_sequence_
counts.tsv", header=FALSE, sep="\t")
colnames(INPUT_NEG) <- c("SampleID","Neg")

INPUT_MERGE <-
 merge(merge(merge(INPUT_RAW, INPUT_TRIM, by="SampleID"), INPUT_HUMAN, by="SampleID"), INPU
T_NEG, by="SampleID", all=TRUE)

INPUT_MAP <-
 read.delim("../../IntermediateOutput/Mapping_files/SkinMet_and_Virome_001_metadata.tsv", h
eader=TRUE)
```

Step 7.

Merge the mapping file and merged data frame.

cmd COMMAND

```
MERGE_MAP <- merge(INPUT_MERGE, INPUT_MAP, by.x="SampleID", by.y="NexteraXT_SampleID")
head(MERGE_MAP)[,c(1:5)]
```

EXPECTED RESULTS

##	SampleID	Raw	Trim	Human	Neg
## 1	MG100128	676336	674907	578071	578071
## 2	MG100129	675067	673546	392441	392441
## 3	MG100130	584346	582675	547615	547615
## 4	MG100131	585573	584404	550047	550047
## 5	MG100132	662992	662283	427419	427419
## 6	MG100133	730349	727636	655387	655387

Step 8.

We can now calculate the overall sequence statistics and plot the sequence count information after some more formatting and merging.

cmd COMMAND

```
MERGE_SUBSET <- MERGE_MAP[,c(2:5,1,10,11)]
MERGE_SUB_RAW <- MERGE_SUBSET[,c("Raw","SampleID","Site_Symbol")]
colnames(MERGE_SUB_RAW) <- c("Count","SampleID","Site_Symbol")
MERGE_SUB_RAW$GROUP <- "Raw"
MERGE_SUB_TRIM <- MERGE_SUBSET[,c("Trim","SampleID","Site_Symbol")]
colnames(MERGE_SUB_TRIM) <- c("Count","SampleID","Site_Symbol")
MERGE_SUB_TRIM$GROUP <- "Trim"
MERGE_SUB_HUMAN <- MERGE_SUBSET[,c("Human","SampleID","Site_Symbol")]
colnames(MERGE_SUB_HUMAN) <- c("Count","SampleID","Site_Symbol")
MERGE_SUB_HUMAN$GROUP <- "Human"
MERGE_SUB_NEG <- MERGE_SUBSET[,c("Neg","SampleID","Site_Symbol")]
colnames(MERGE_SUB_NEG) <- c("Count","SampleID","Site_Symbol")
MERGE_SUB_NEG$GROUP <- "Neg"
CAT_MERGE <- rbind(MERGE_SUB_RAW, MERGE_SUB_TRIM, MERGE_SUB_HUMAN, MERGE_SUB_NEG)
```

Step 9.

Remove the incomplete sites.

cmd COMMAND

```
CAT_MERGE_SUB <- CAT_MERGE[-which(CAT_MERGE$Site_Symbol %in% c("Ba","Ph","Vf","No")), ]
```

Step 10.

Check the head of this data frame.

cmd COMMAND

```
head(CAT_MERGE_SUB)
```

EXPECTED RESULTS

##	Count	SampleID	Site_Symbol	GROUP
## 1	676336	MG100128	Ra	Raw
## 2	675067	MG100129	Ra	Raw
## 3	584346	MG100130	Ra	Raw
## 4	585573	MG100131	Ra	Raw
## 5	662992	MG100132	Ra	Raw
## 6	730349	MG100133	Ra	Raw

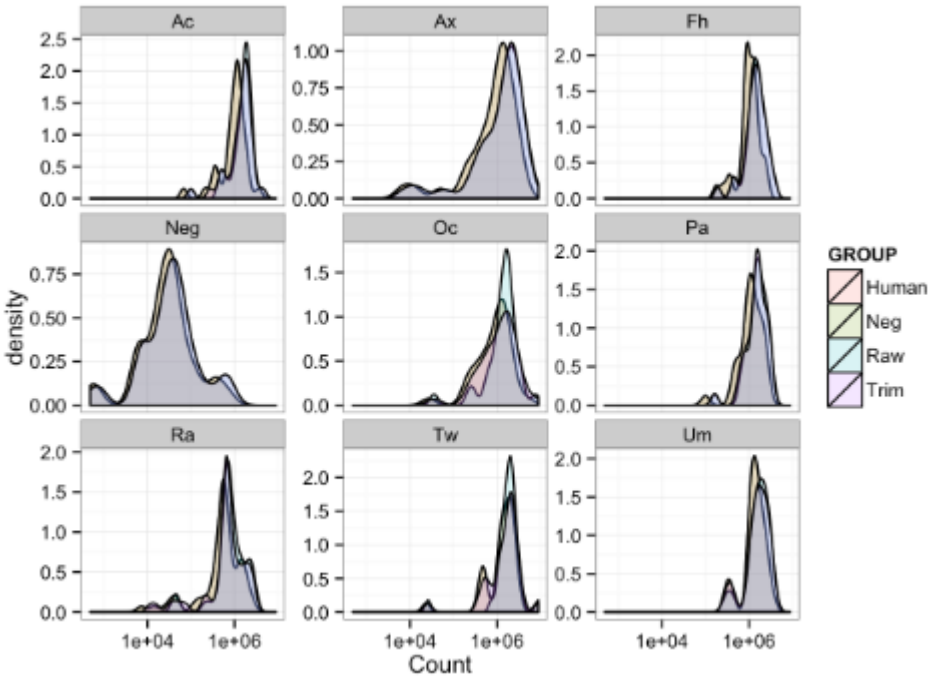
Step 11.

Now plot the sequence count information.

cmd COMMAND

```
ggplot(CAT_MERGE_SUB, aes(x=Count, fill=GROUP)) + theme_bw() + geom_density(alpha=0.2) + sc
ale_x_log10() + facet_wrap(~Site_Symbol, scale="free_y")
```

EXPECTED RESULTS



Step 12.

Now we are going to do the same thing as the above sequence count stats, except now we are using the sequence length stats.

cmd COMMAND

```
LENGTH_RAW <-
 read.delim("../../IntermediateOutput/Whole_Microbiome_Sequence_Counts/raw_median_seq_lengt
h_formatted.txt", header=FALSE, sep="\t")
colnames(LENGTH_RAW) <- c("SampleID","Raw")
LENGTH_TRIM <-
 read.delim("../../IntermediateOutput/Whole_Microbiome_Sequence_Counts/trimmed_median_seq_l
ength_formatted.txt", header=FALSE, sep="\t")
colnames(LENGTH_TRIM) <- c("SampleID","Trim")
LENGTH_HUMAN <-
 read.delim("../../IntermediateOutput/Whole_Microbiome_Sequence_Counts/human_cleaned_median
_seq_length_formatted.txt", header=FALSE, sep="\t")
colnames(LENGTH_HUMAN) <- c("SampleID","Human")
LENGTH_NEG <-
 read.delim("../../IntermediateOutput/Whole_Microbiome_Sequence_Counts/clean_phix_median_se
q_length_formatted.txt", header=FALSE, sep="\t")
colnames(LENGTH_NEG) <- c("SampleID","Neg")

LENGTH_MERGE <-
 merge(merge(merge(LENGTH_RAW, LENGTH_TRIM, by="SampleID"), LENGTH_HUMAN, by="SampleID"), L
ENGTH_NEG, by="SampleID", all=TRUE)

MERGE_MAP <- merge(LENGTH_MERGE, INPUT_MAP, by.x="SampleID", by.y="NexteraXT_SampleID")
```

Step 13.

Prepare the format to plot sequence length.

cmd COMMAND

```
MERGE_SUBSET <- MERGE_MAP[,c(2:5,1,10,11)]
```

```
MERGE_SUB_RAW <- MERGE_SUBSET[,c("Raw","SampleID","Site_Symbol")]
colnames(MERGE_SUB_RAW) <- c("Count","SampleID","Site_Symbol")
MERGE_SUB_RAW$GROUP <- "Raw"
MERGE_SUB_TRIM <- MERGE_SUBSET[,c("Trim","SampleID","Site_Symbol")]
colnames(MERGE_SUB_TRIM) <- c("Count","SampleID","Site_Symbol")
MERGE_SUB_TRIM$GROUP <- "Trim"
MERGE_SUB_HUMAN <- MERGE_SUBSET[,c("Human","SampleID","Site_Symbol")]
colnames(MERGE_SUB_HUMAN) <- c("Count","SampleID","Site_Symbol")
MERGE_SUB_HUMAN$GROUP <- "Human"
MERGE_SUB_NEG <- MERGE_SUBSET[,c("Neg","SampleID","Site_Symbol")]
colnames(MERGE_SUB_NEG) <- c("Count","SampleID","Site_Symbol")
MERGE_SUB_NEG$GROUP <- "Neg"
CAT_MERGE <- rbind(MERGE_SUB_RAW, MERGE_SUB_TRIM, MERGE_SUB_HUMAN, MERGE_SUB_NEG)
CAT_MERGE_SUB <- CAT_MERGE[-which(CAT_MERGE$Site_Symbol %in% c("Ba","Ph","Vf","No")), ]
```

Step 14.

Plot the sequence count information.

cmd COMMAND

```
ggplot(CAT_MERGE_SUB, aes(x=Count, fill=GROUP)) + theme_bw() + geom_density(alpha=0.2) + fa
cet_wrap(~Site_Symbol, scale="free_y")
```

EXPECTED RESULTS