BIOS 7659 Homework 4

Tim Vigers

20 October 2020

1. RNA-seq Data and QC

a) Read information

The first entry in the .fastq file is:

`@SRR390924.1.1 COLUMBO:1:1:1:1926 length=36

AAAAAAAANAAAAAAAAAAAAAAAAAAAAAAAAAAA

+SRR390924.1.1 COLUMBO:1:1:1:1926 length=36

####################################`

The first line contains the sequence ID (after the @ symbol) and an optional description. Line 2 contains the read sequence and line 3 has the same sequence ID (this time after the + symbol) followed by another optional description. The final line encodes a quality score for each base pair (BP) in the sequence using the hexadecimal format. In this dataset, reads are 36 BP long.

For more recent Illumina systems, you can find the q-score for each BP by subtracting 33 from the ASCII code. From the q-score you can then calculate the probability that the BP call was incorrect using the formula $P = 10^{\frac{-Q}{10}}$. For for the first entry in this file, $q = 35 - 33 = 2$, so $P =$ 0.631. This is not a high quality read.

According to the SRA entry there are 3,614,610 reads in this file.

b) Summary statistics

"FASTQ Summary Statistics" returns a table where each row represents a BP position within a read (in this case 1-36). For each BP position, the table includes quality summary statistics such as minimum, maximum, and mean q-score. In general the summary statistics are fairly standard and self-explanatory, but the column descriptions from the usegalaxy.org documentation are as follows:

column = column number (1 to 36 for a 36-cycles read Solexa file)

count = number of bases found in this column.

min = Lowest quality score value found in this column.

max = Highest quality score value found in this column.

sum = Sum of quality score values for this column.

mean = Mean quality score value for this column.

Q1 = 1st quartile quality score.

med = Median quality score.

Q3 = 3rd quartile quality score.

IQR = Inter-Quartile range (Q3-Q1).

lW = ‘Left-Whisker’ value (for boxplotting).

rW = ‘Right-Whisker’ value (for boxplotting).

outliers = Scores falling beyond the left and right whiskers (comma separated list).

A_Count = Count of ‘A’ nucleotides found in this column.

C_Count = Count of ‘C’ nucleotides found in this column.

G_Count = Count of ‘G’ nucleotides found in this column.

T_Count = Count of ‘T’ nucleotides found in this column.

N_Count = Count of ‘N’ nucleotides found in this column.

Other_Nucs = Comma separated list of other nucleotides found in this column.

Other_Count = Comma separated count of other nucleotides found in this column.

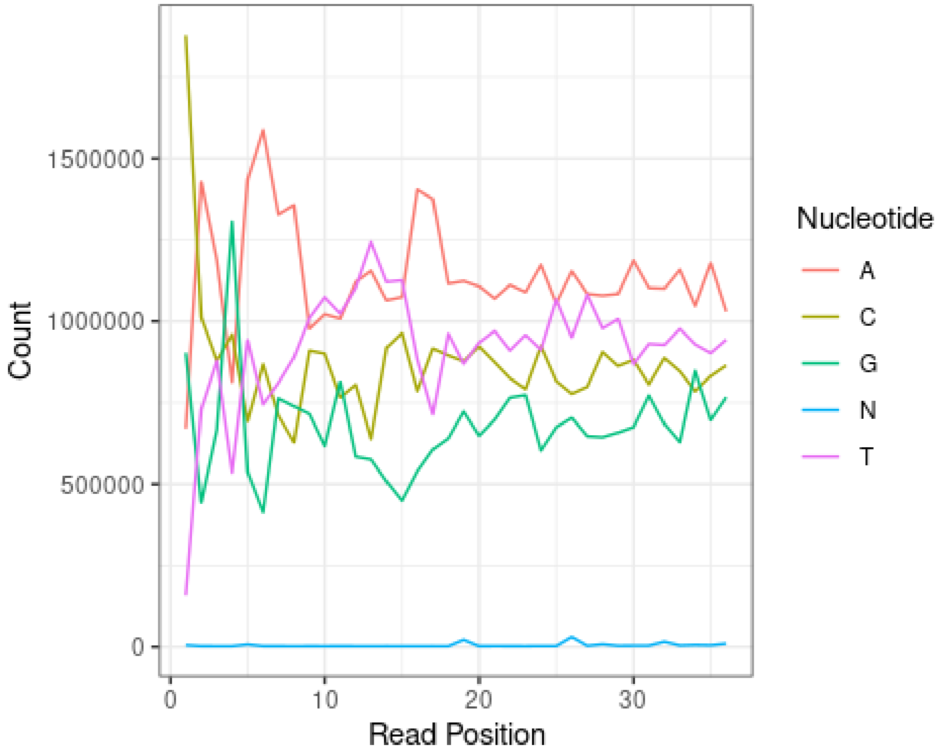
```r
sum_stat <- read.delim("./FASTQ_Summary_Statistics.tabular")
colnames(sum_stat)[1] <- "position"
kable(head(sum_stat,5))
```

position	count	min	max	sum	mean	Q1	med	Q3	IQR	lW	rW	outliers	A_Count	C_Count	G_Count	T_Count	N_Count	other_bases	other_base_count
1	3614610	2	33	113732847	31.46476	33	33	33	0	33	33	2,4,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32	670260	1877729	903145	158171	5305	NA	NA

2	3614610	2	34	114293473	31.61986	33	33	34	1	32	34	2,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31	1426604	1010947	444249	730545	2265	NA	NA
3	3614610	2	34	113169385	31.30888	33	33	33	0	33	33	2,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,34	1188225	877986	667041	879162	2196	NA	NA
4	3614610	2	34	113623731	31.43458	33	33	33	0	33	33	2,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,34	8130655	959180	1305248	534975	2142	NA	NA
5	3614610	2	34	111459496	30.83583	32	33	33	1	31	34	2,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30	1434968	694033	536934	941327	7348	NA	NA

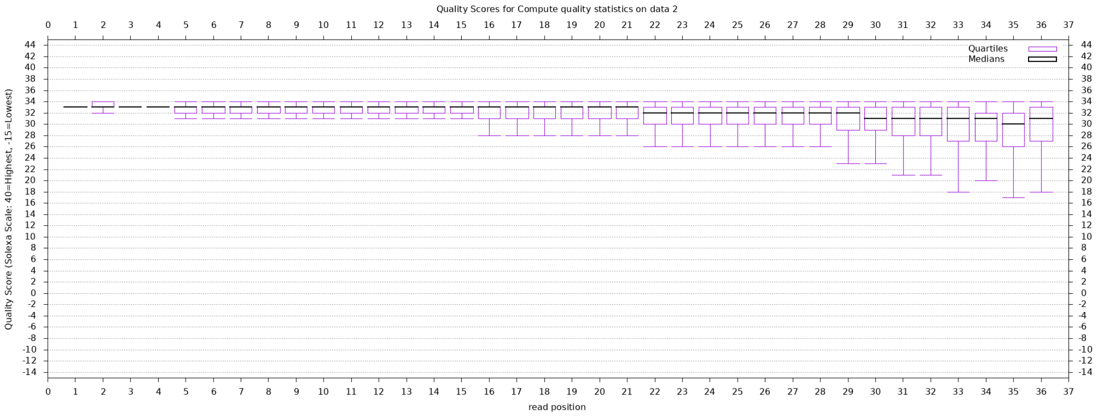
Nucleotide content by position

```
plot_sum_stat <- sum_stat %>%
  pivot_longer(cols = A_Count:N_Count)
ggplot(plot_sum_stat,aes(x=position,y=value,color=name)) +
  geom_line() +
  xlab("Read Position") + ylab("Count") +
  scale_color_discrete(name = "Nucleotide",
                       labels=c("A","C","G","N","T")) +
  theme_bw()
```



Early on in the reads there appear to be a large number of cytosines and not many thymines. However, as the read position increases the proportions seem to stabilize and there are generally more adenosines and thymines, as one might expect. Also, there appears to be more variability early on in the reads, and it decreases as the length of the read increases.

Quality score boxplot



As the read length increases, the quality tends to deteriorate. Based on visual inspection it seems that the drop in overall quality occurs around position 29. The variability in quality also increases as the read length increases.

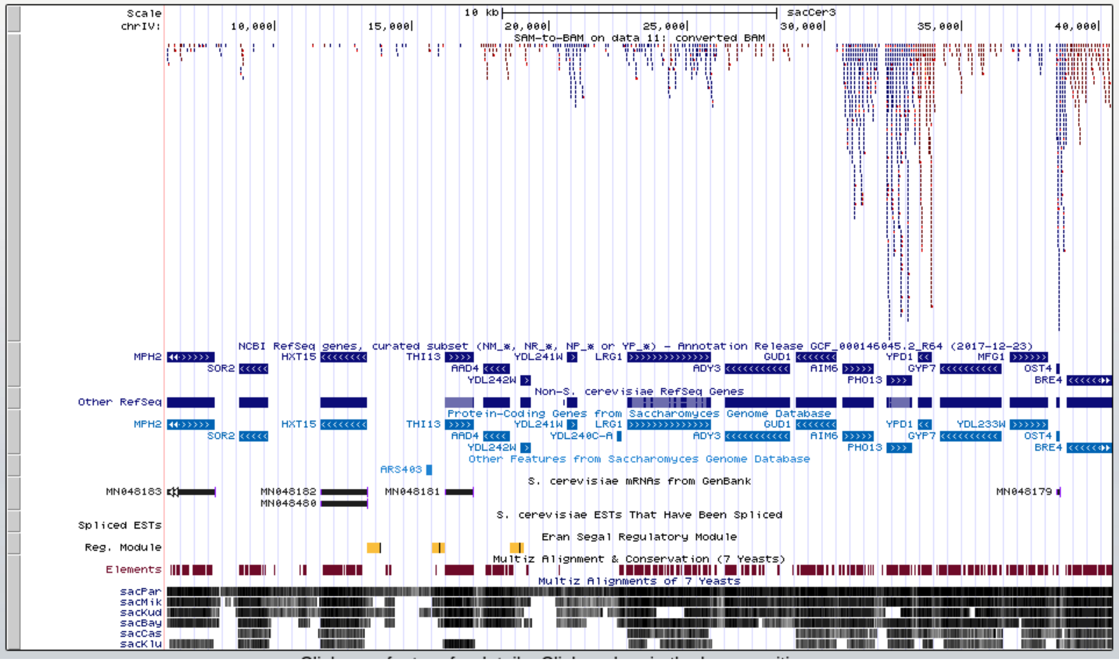
2. RNA-seq Mapping using Bowtie2

a) Bowtie2 Mapping

The first two reads have the flag 16, which corresponds to “SEQ being reverse complemented” (i.e. mapped to the reverse strand). However, the third read has flag 4 which corresponds to “segment unmapped.” Filtering out the unmapped reads and reads which failed quality control checks results in ~2,600,000 reads (which is about 72% of the original number of reads).

b) Visualization of Mapping

The UCSC squish view is showing chromosome IV. Based on the RefSeq track (the one directly below our data track), there appear to be 16 yeast genes in this section of the chromosome. The genes are MPH2 through BRE4, and it appears that the genes toward the right of the visualization have the highest coverage, particularly AIM6, PHO13, and YPD1. OST4 and BRE4 also appear to have good coverage, although not quite as much as the genes between 30kbp and 35kbp.


Scale
chrIV:
10 kb
sacCer3
10,000|
15,000|
20,000|
25,000|
30,000|
35,000|
40,000|
SAM-to-BAM on data 11: converted BAM
NCBI RefSeq genes, curated subset (NM_*, NR_*, NP_* or YP_*) - Annotation Release GCF_000146045.2_R64 (2017-12-23)
MPH2
SOR2
HXT15
THI13
AAD4
YDL242W
YDL241W
LRG1
ADY3
GUD1
AIM6
PHO13
YPD1
GYP7
MFG1
OST4
BRE4
Non-S. cerevisiae RefSeq Genes
Other RefSeq
Protein-Coding Genes from Saccharomyces Genome Database
MPH2
SOR2
HXT15
THI13
AAD4
YDL242W
YDL241W
YDL240C-A
LRG1
ADY3
GUD1
AIM6
PHO13
YPD1
GYP7
YDL233W
OST4
BRE4
Other Features from Saccharomyces Genome Database
ARS403
S. cerevisiae mRNAs from GenBank
MN048183
MN048182
MN048480
MN048181
MN048179
S. cerevisiae ESTs That Have Been Spliced
Spliced ESTs
Eran Segal Regulatory Module
Reg. Module
Multiz Alignment & Conservation (7 Yeasts)
Elements
Multiz Alignments of 7 Yeasts
sacPar
sacMik
sacKud
sacBay
sacCas
sacKlu

Screenshot of UCSC Genome Browser on S. cerevisiae Apr. 2011

c) Quantitation

The htseq function returns two tables, one with a summary of the number of aligned or ambiguous reads and another with read counts for specific features (in this case gene IDs).

```
# Import no features
no_feat <- read.delim("./htseq-count_no_feature.tabular",header = F)
```

```
kable(no_feat,col.names = c("Category","SAM-to-BAM on data 11: converted BAM"),
    caption = "htseq Summary Table")
```

htseq Summary Table

Category	SAM-to-BAM on data 11: converted BAM
__no_feature	197763
__ambiguous	7719
__too_low_aQual	582447
__not_aligned	0
__alignment_not_unique	0

```
# Counts per features
feat <- read.delim("./htseq-count.tabular",header = F)
kable(head(feat[order(feat$V2,decreasing = T),],10),
    caption = "htseq Top 10 Features by Read Count",
    col.names = c("Geneid","SAM-to-BAM on data 11: converted BAM"))
```

htseq Top 10 Features by Read Count

	Geneid	SAM-to-BAM on data 11: converted BAM
3013	YHR174W	39192
2640	YGR192C	37184
3794	YKL060C	34937
107	YAL038W	28748
4423	YLR249W	25601
4195	YLR044C	22660
729	YCR012W	20836
5710	YOL086C	19958
2166	YGL008C	19017
3894	YKL152C	16013