Assignment 2

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Question 1. Coverage Analysis [20 pts]

Question 1a. How long is the reference genome?

In linux, use command

```
‘samtools faidx ref.fa’
‘cat ref.fa.fai’
```

```
jialinkang@DESKTOP-B9I1CPK: /mnt/d/Downloads/computational_genomics/comparative_genomics/assignment1/chrom_10
++> ls
README  dna-encode.pl  frag180.1.fq  frag180.2.fq  jump2k.1.fq  jump2k.2.fq  ref.fa  ref.fa.fai
++> samtools faidx ref.fa
++> cat ref.fa.fai
Halomonas       233806  11      70      71
++>
```

We can know the length of the reference genome is: 233806 bp

Question 1b. How many reads are provided and how long are they? Make sure to measure each file separately

In linux, use command

```
‘fastqc frag180.1.fq’
‘fastqc frag180.2.fq’
‘fastqc jump2k.1.fq’
‘fastqc jump2k.2.fq’
```

We can get a html file and zip file of each command.

Basic Statistics

Measure	Value
Filename	frag180.1.fq
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	35198
Sequences flagged as poor quality	0
Sequence length	100
%GC	54

Basic Statistics

Measure	Value
Filename	frag180.2.fq
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	35198
Sequences flagged as poor quality	0
Sequence length	100
%GC	54

Basic Statistics

Measure	Value
Filename	jump2k.1.fq
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	70396
Sequences flagged as poor quality	0
Sequence length	50
%GC	54

Basic Statistics

Measure	Value
Filename	jump2k.2.fq
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	70396
Sequences flagged as poor quality	0
Sequence length	50
%GC	54

From those result of basic statistics, we can get:

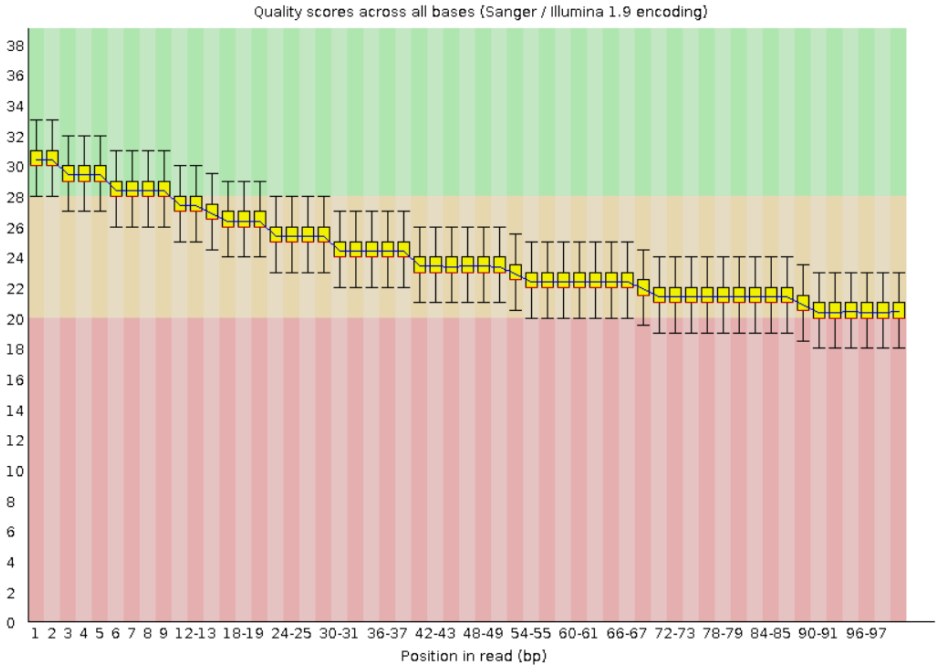
Name	Reads	Length	Coverage
frag180.1.fq	35198	100	15.05
frag180.2.fq	35198	100	15.05
Jump.2k.1.fq	70396	50	15.05
Jump.2k.2.fq	70396	50	15.04

Question 1c. How much coverage do you expect to have?

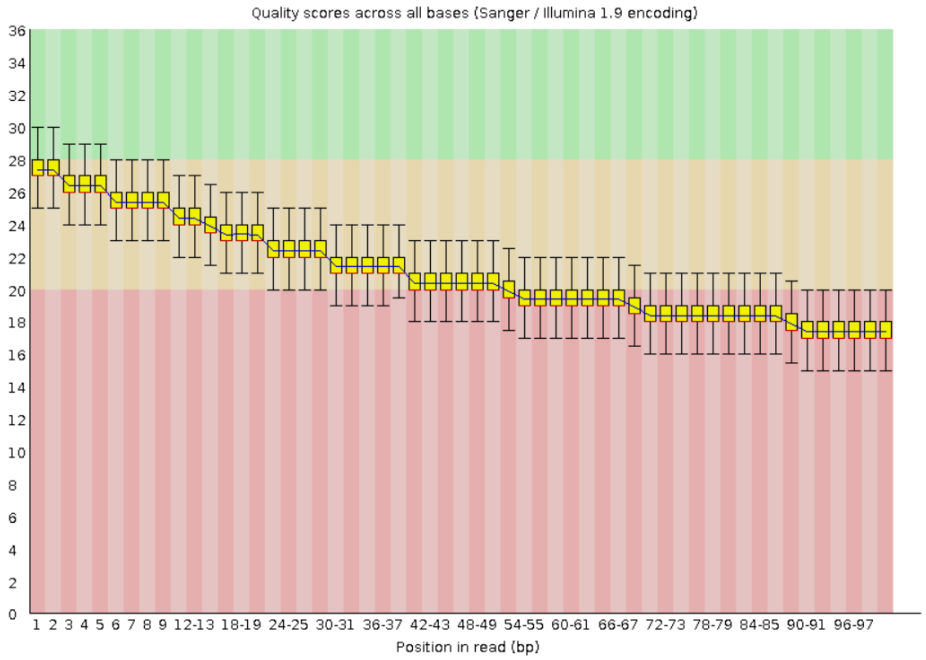
See the above chart. So the excepted coverage is 15.

Question 1d. Plot the average quality value across the length of the reads.

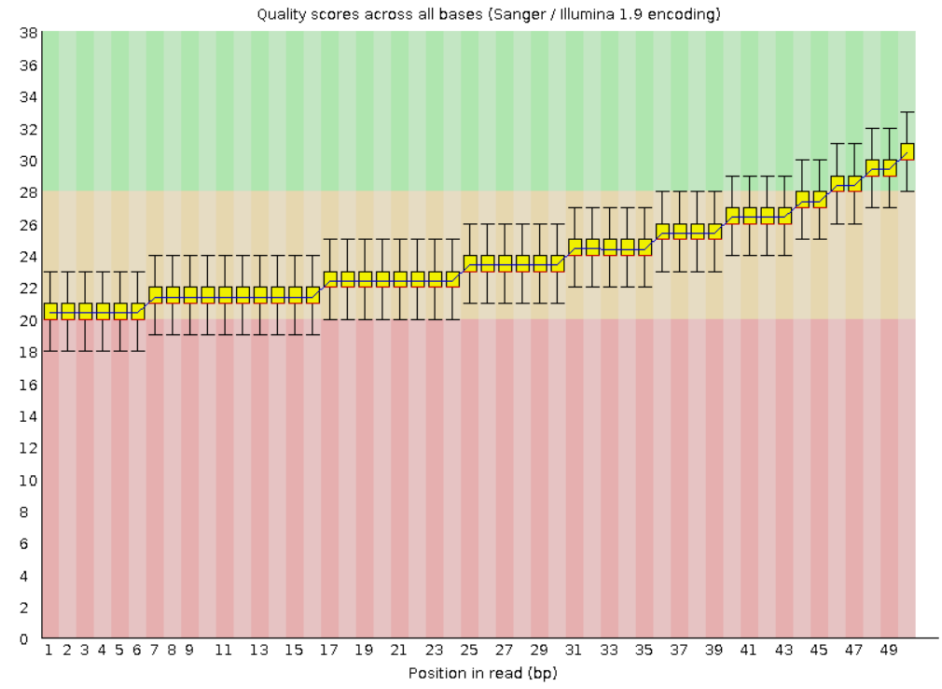
frag180.1.fq



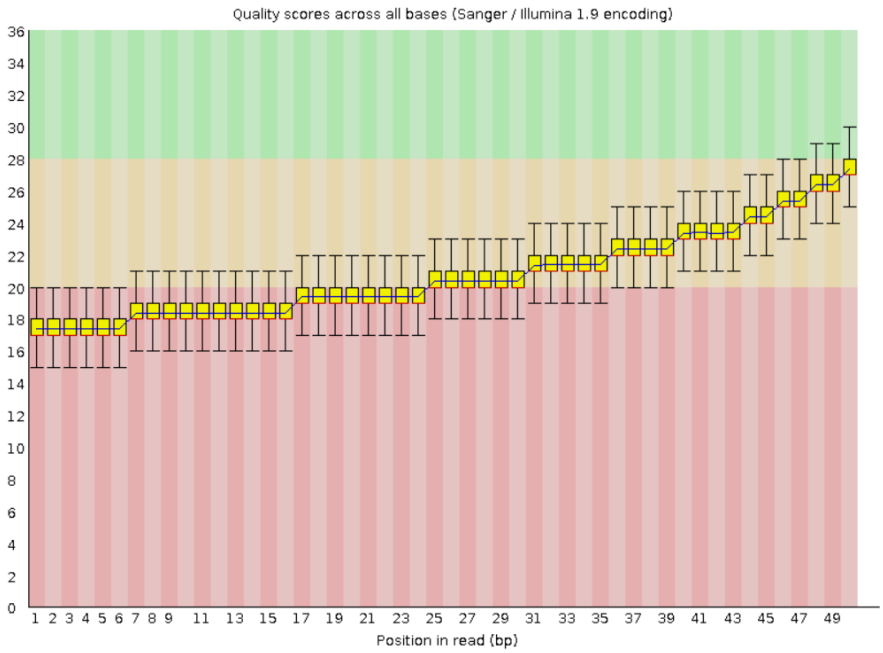
frag180.2.fq



Jump.2k.1.fq



Jump.2k.2.fq



Question 2. Kmer Analysis

Question 2a. How many kmers occur exactly 50 times?

Use the command:

```
jellyfish count -m 21 -s 100M -t 10 -C jump2k.1.fq jump2k.2.fq frag180.1.fq
frag180.2.fq
jellyfish histo mer_counts.jf
```

from the result we can know:

1062 kmers occur exactly 50 times

Question 2b. What are the top 10 most frequently occurring kmers?

Use command:

```
jellyfish dump -c -t mer_counts.jf > kmer_count.fasta
sort -rn -k2 kmer_count.fasta > sort.txt
head -n 10 sort.txt
```

CGCCCACTAATTAGTGGGCGC 94

CCCACTAATTAGTGGGCGCCG 94

GCAGGAATTGAACCTGCGACC 93

GCCCACTAATTAGTGGGCGCC 92

ACGGCGCCCACTAATTAGTGG 92

GGCAGGAATTGAACCTGCGAC 88

GCGCGCCCGGCAGGAATTGAA 87

CGCGCCCGGCAGGAATTGAAC 87

GCGCCCGGCAGGAATTGAACC 86

AGGTCGCAGGTTCAATTCCTG 86

Question 2c. What is the estimated genome size based on the kmer frequencies?

Use command:

```
jellyfish histo -t 10 mer_counts.jf > reads.histo
put the reads.histo file into genomescope website
```

the genomescope result link:
http://genomescope.org/analysis.php?code=YDrXUbdH3pNuOZz2nw4m

Results

```
GenomeScope version 1.0
k = 21

property                  min              max
Heterozygosity            -0.00321515%     0.0115018%
Genome Haploid Length     233,837 bp       234,211 bp
Genome Repeat Length      -72 bp           -72 bp
Genome Unique Length      233,909 bp       234,283 bp
Model Fit                 98.6444%         98.6455%
Read Error Rate           0.800445%        0.800445%
```

From the results table, we can know that the min Genome haploid length is: 233837 bp

the length of the reference genome is: 233806 bp and the estimation is : 233837~234283, the estimation is very near to the really result. It is a good estimation to the reference genome.

Question 3. De novo assembly

Question 3a. How many contigs were produced?

```
Sudo apt install spades
spades --pe1-1 frag180.1.fq --pe1-2 frag180.2.fq --mp1-1 jump2k.1.fq --mp1-2
jump2k.2.fq -o asm -t 4 -k 31
grep -c '>' contigs.fasta
```

we the number of contigs is 4

Question 3b. What is the total length of the contigs?

```
samtools faidx contigs.fasta
datamash sum 2 < contigs.fasta.fai
```

we can get the total length of contigs is 234596

Question 3c. What is the size of your large contig?

```
sort -rn -k2 contigs.fasta.fai
```

from the sort result we can know the large contig size is 105834

Question 3d. What is the contig N50 size?

```python
import sys

def fafile2dict():
    '''
    read a single FASTA file (SHH.fa) into a dictionary object
    and calculate the contig N50 size of this FASTA file
    run as :
    python3 N50.py < ./asm/contigs.fasta

    Rerurn
    --------------
    N50:int
    the N50 number of this FASTA file
    --------------
    '''
    # read the file context into a dict
    line = sys.stdin.readline().replace('\n','')
    seq = {}
    while line != '':
```

```
        if line[0] == '>':
            name = line.replace('>','')
            seq[name] = ''
        else:
            seq[name] += line.replace('\n','').strip()
        line = sys.stdin.readline()

    val_list = []
    for val in seq.values():
        val_list.append(len(val))
    val_list.sort(reverse=True)

    val_half = sum(val_list)/2
    for i in range(len(val_list)):
        if val_half > 0:
            val_half -= val_list[i]
        else:
            N_50 = val_list[i-1]
            break

    return N_50

if __name__ == "__main__":
    N_50 = fafile2dict()
    print('N50 is ', N_50)
```

the N50 of configs.fasts is: 47851

Question 4. Whole Genome Alignment

Question 4a. What is the average identify of your assembly compared to the reference?

Run the command:

```
dnadiff ./ref.fa ./asm/scaffolds.fasta
nucmer ./ref.fa ./asm/scaffolds.fasta
show-coords out.delta
```

the result is as followings:

```
jialinkang@DESKTOP-B9I1CPK:/mnt/d/Downloads/computational_genomics/comparative_genomics/assignment2/asm$ show-coords out.delta
/mnt/d/Downloads/computational_genomics/comparative_genomics/assignment2/asm/ref.fa /mnt/d/Downloads/computational_genomics/comparative_genomics/assignment2/asm/asm/scaffolds.fasta
NUCMER

    [S1]     [E1]  |     [S2]     [E2]  |  [LEN 1]  [LEN 2]  |  [% IDY]  | [TAGS]
================================================================================
       11    26789  |        1    26779  |    26779    26779  |   100.00  | Halomonas    NODE_1_length_234626_cov_20.511980
   26790   233794  |    27628   234626  |   207005   206999  |    99.98  | Halomonas    NODE_1_length_234626_cov_20.511980
```

And from the out.report file we can know:

the average identify is 99.98(1-to-1) and 99.98(M-to-M)

Question 4b. What is the length of the longest alignment

The longest alignment is 207005.

Question 4c. How many insertions and deletions are in the assembly?

There is 1 insertion in the assembly.

Insertions	2	1
InsertionSum	22	848
InsertionAvg	11.00	848.00

The insertion length is 848 bp.

No deletions.

Question 5. Decoding the insertion

Question 5a. What is the position of the insertion on the reference?

The position is scaffolds.fasta NODE_1_length_234626_cov_20.511980 26780-27627

Question 5b. How long is the novel insertion?

848bp

Question 5c. What is the DNA sequence of the encoded message?

The DNA sequence is:

>NODE_1_length_234626_cov_20.511980:26780-27627

CTAACATTCGTCGGTGATGCTTTCATTCCTTGCTGTCCTAAGTCCACTCTGTATCAATGG

CTAGCGTATGCAAGTACAATAGGTCGACCGGCGCAGCGTCGTGTAGGCTTGCCTGTCAGG

ACTAACACAGTTATCACTTATGGTAATCCACCAGGTCGAACGGCGCAACTTCAGCGACTC

CCCACTATCCGGATGGCAACATTTCCGACGGCTAATAGGCTGTAAGGCATTTAATCCCCC

AAGTCATAAAGTAAACCAGGACTCACTTCCCCACGCACAACTACTATCATCCGCCCAGAT

ATAGACGAACAACGCCACCGCGTTCAACCTGTACACCTTCTGAACGTAGCCGAGGCAGAT

ATGACTACCCGCAACACGACCGTTATTCCTAGCTTATGTAATGCTTGGCGGCTGAGCGGA

GCCGCGTCCATTCGTGCAGTAAGACCAACGGACAGGATTAGTTTATGTCGAGAGGGCCGC

CTTGAATGCGTCCGATCCTCGGTACCGCTTTCAATATTGCAAGAAACCAATCAAACCTAC

GCTGCGGCCGCGCGGAATATCTGGCCCCAATCCACCAGGCGTGGAGTCGTGAAAGAAACA

CTTATTAAATGCTTGGATGCGGGGAGGCTATTATGCTCAGATTATTTAAGGAAGTTCCGA

CAAAAGGACTTAAGTCTGGTATGTCCTACAGCCCAACGGCGGCAATTCTAATCTGTATCC

CTGGCTCACGTCTGACAGTAATCTAAGCCAAACTTCCTTTCTGGACGAATGAAGAGCGGC

ATAGCGTATTTCCATCACCCCGTCCCAGCGTATTAAAGTAGCATCGTAATCTAGGATTGC

ATGTAAGG

Question 5d. What is the secret message?

```
samtools faidx ./asm/scaffolds.fasta NODE_1_length_234626_cov_20.511980:26780-27627 > seq.fa
./dna-encode.pl -d seq.fafol
```

The secret message is:

Congratulations to the Spring 2020 JHU Applied Genomics course... Keep on looking for little green aliens