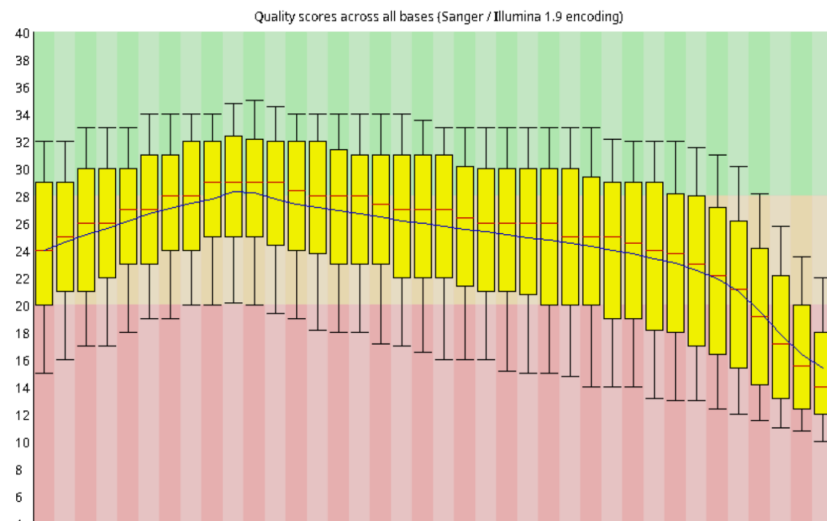


Результаты fastqc:

Summary

- ✓ Basic Statistics
- ✗ Per base sequence quality
- ! Per sequence quality scores
- ✓ Per base sequence content
- ✓ Per sequence GC content
- ✓ Per base N content
- ! Sequence Length Distribution
- ✓ Sequence Duplication Levels
- ✓ Overrepresented sequences
- ✓ Adapter Content

✗ Per base sequence quality



Команда для тримминга:

```
trimmomatic PE ILLUMINACLIP:./Sequencing_adaptors.fasta:2:30:10  
SLIDINGWINDOW:4:17 LEADING:20 TRAILING:20
```

```
1314447 reads; of these:  
  1314447 (100.00%) were paired; of these:  
    2560 (0.19%) aligned concordantly 0 times  
   1217284 (92.61%) aligned concordantly exactly 1 time  
    94603 (7.20%) aligned concordantly >1 times  
----  
  2560 pairs aligned concordantly 0 times; of these:  
    1055 (41.21%) aligned discordantly 1 time  
----  
  1505 pairs aligned 0 times concordantly or discordantly; of these:  
    3010 mates make up the pairs; of these:  
      1902 (63.19%) aligned 0 times  
       155 (5.15%) aligned exactly 1 time  
       953 (31.66%) aligned >1 times  
99.93% overall alignment rate
```

Общий скрипт:

```
#!/bin/bash
```

```
echo 'Write the path to bowtie2 index: $1'
read BOWTIE2_INDEX
echo "write the path to your fastq file 1"
read FASTQ_FILE_1
echo "write the path to your fastq file 2"
read FASTQ_FILE_2
echo "write number of threads for bowtie2"
read THREADS
echo "write path to output_dir "
read OUTPUT_DIR
echo "write the name for output SORTED BAM file (without .bam at the end, just name)"
read OUTPUT_FILE_NAME
```

```
echo "write the path to your ref sequence fasta"
read REFERENCE_FASTA
```

```
bowtie2 --threads $THREADS -x $BOWTIE2_INDEX --sensitive -1 $FASTQ_FILE_1 -2
$FASTQ_FILE_2 |
samtools view -@ $THREADS -S -b |
```

```
samtools sort -@ $THREADS > "${OUTPUT_DIR}/${OUTPUT_FILE_NAME}_sorted.bam"
```

```
samtools index -@ $THREADS "${OUTPUT_DIR}/${OUTPUT_FILE_NAME}_sorted.bam"
```

```
mkdir "${OUTPUT_DIR}/bam_file_stats"
```

```
samtools stats -@ $THREADS "${OUTPUT_DIR}/${OUTPUT_FILE_NAME}_sorted.bam" >
"${OUTPUT_DIR}/bam_file_stats/${OUTPUT_FILE_NAME}_stats_file.stats"
```

```
plot-bamstats -p
"${OUTPUT_DIR}/bam_file_stats/${OUTPUT_FILE_NAME}_stats_file_graph"
"${OUTPUT_DIR}/bam_file_stats/${OUTPUT_FILE_NAME}_stats_file"
```

```
samtools view -@ $THREADS -b -F 4
"${OUTPUT_DIR}/${OUTPUT_FILE_NAME}_sorted.bam" |
```

```
samtools sort -@ $THREADS >
"${OUTPUT_DIR}/${OUTPUT_FILE_NAME}_sorted_F4_flag.bam"
```

#надо установить gnuplot

```
samtools coverage "${OUTPUT_DIR}/${OUTPUT_FILE_NAME}_sorted.bam" >
"${OUTPUT_DIR}/bam_file_stats/${OUTPUT_FILE_NAME}_coverage_file.txt"
```

Samtools stats output:

```
SN    raw total sequences:  2626992      # excluding supplementary and secondary
reads
SN    filtered sequences:    0
SN    sequences:            2626992
SN    is sorted:            1
SN    1st fragments: 1313491
SN    last fragments: 1313501
SN    reads mapped: 2626992
SN    reads mapped and paired: 2626064    # paired-end technology bit set +
both mates mapped
SN    reads unmapped:        0
SN    reads properly paired: 2623774      # proper-pair bit set
SN    reads paired: 2626992      # paired-end technology bit set
SN    reads duplicated:      0          # PCR or optical duplicate bit set
SN    reads MQ0: 425          # mapped and MQ=0
```

$(\text{mapped\&paired-MQ0})/\text{total reads} = 0,99$

Coverage file:

#rname	startpos	endpos	numreads	covbases	coverage	meandepth	meanbaseq	meanmapq
NC_000964.3	1	4215606	2626992	4215600	99.9999	47.8217	27.7	40.3