Raw Data

Report

April 2022

Humanizing Genomics
macrogen
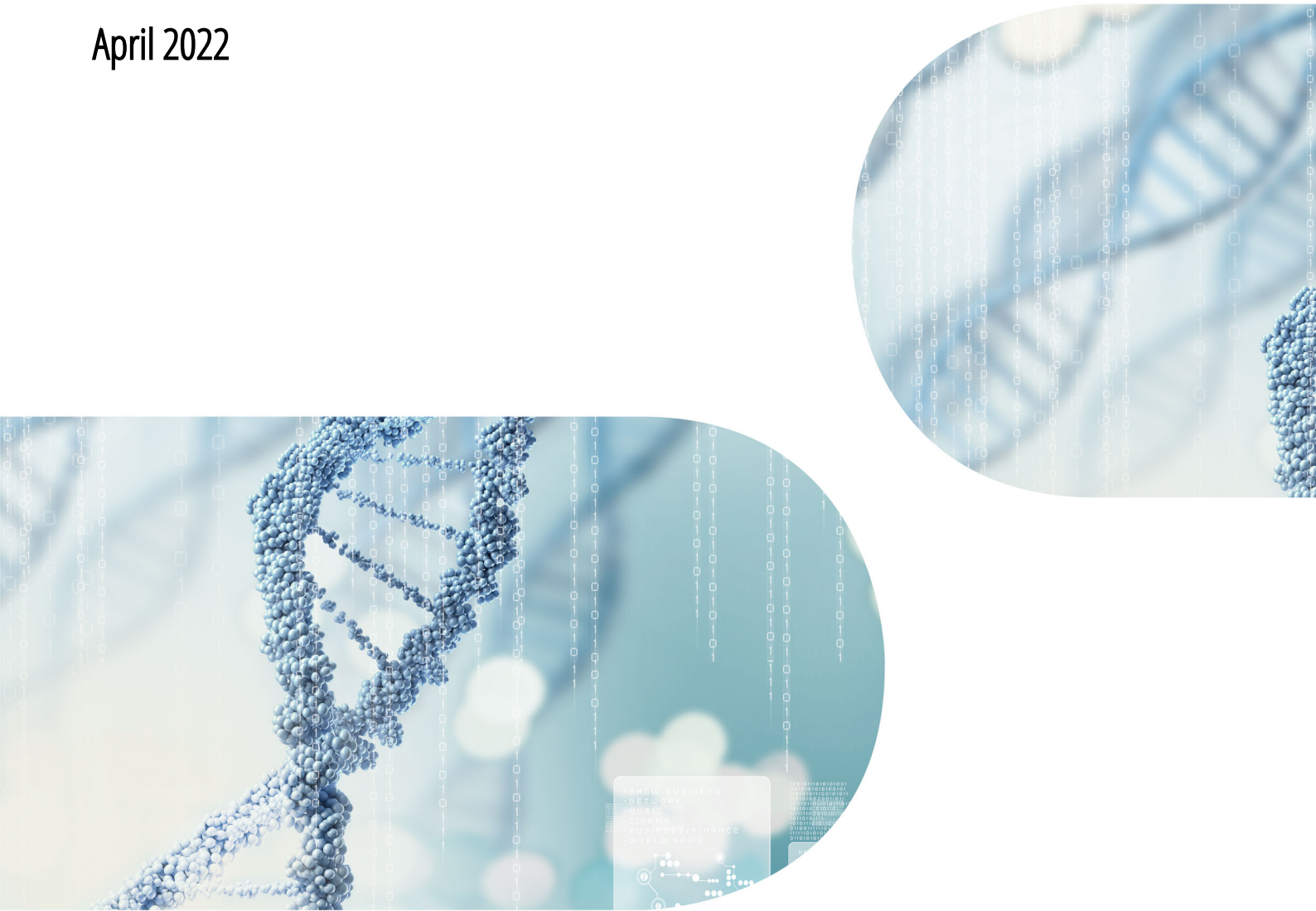
Project Information

Client Name	Ben Wainwright
Company / Institution	National University of Singapore
Order Number	2202KMI-0003
Type of Read	Paired-end
Read Length	301
Number of Samples	195
Library Kit	Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2
Library Protocol	16S Metagenomic Sequencing Library Preparation Part # 15044223 Rev. B
Type of Sequencer	Illumina platform

Table of Contents

1. Data Download Information

1. 1. Raw Data and Analysis Results

Download link	File size	md5sum
2202KMI-0003.zip	3.9G	4cd09f66be9df05c1105d7324e9498ed

- ⊙ *.zip : Read1/Read2 of All Samples (- One compressed file of all samples)

Download link	File size	md5sum
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Derawan_15_R2.fastq.gz	14.1M	7ff2b3679cc3b2bd4a083f784b374275
Derawan_16_R1.fastq.gz	8.1M	dfdbc9d739635f6f3bf59d9b53a4ab64
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Halmahera_02_R1.fastq.gz	9.5M	a4b320753a451f0304c42f97fca92e90
Halmahera_02_R2.fastq.gz	13.4M	8fac716902827e4dec560635a798deed
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Halmahera_06_R1.fastq.gz	9.6M	e6eaa57fa39c9de8395f9726aa1b8e90
Halmahera_06_R2.fastq.gz	13.5M	fc1acc5942e051b576c9bd2be9f1aed2
Halmahera_07_R1.fastq.gz	7.9M	9cbe15ac258cf96a90b56c18488ee99f
Halmahera_07_R2.fastq.gz	11.3M	6bbfecb629857b67410026508745a3a5
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Karimunjawa_12_R2.fastq.gz	11.8M	6caa511af0b7e95d198db6ba34e741ae
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Wakatobi_02_R1.fastq.gz	7.0M	2e8ac3ca359549c8c2ca26001e718c84
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Wakatobi_05_R2.fastq.gz	10.3M	7e03b35fdc7b6fbb363d01c78b7a1f5f
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Wakatobi_06_R2.fastq.gz	12.1M	685f02b6c9845ce851cb580fc09fa9b4
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Wakatobi_07_R2.fastq.gz	7.1M	be9dcde822c5b832a51db5205a4e3d99
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Wakatobi_14_R2.fastq.gz	13.8M	5ee779604e227b3ffcebd8962ba3be97
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Wakatobi_16_R2.fastq.gz	10.9M	bd6416f4435ae41a81b0ddce75762781

- fastq.gz : This is a zip file of raw data used in analysis.
- md5sum : In order to verify the integrity of files, md5sum is used. If the values of md5sum are the same, there is no forgery, modification or omission.

Your data will be retained in our server for 3 months. Should you wish to extend the retention period, please contact us.

2. Experimental Methods and Workflow
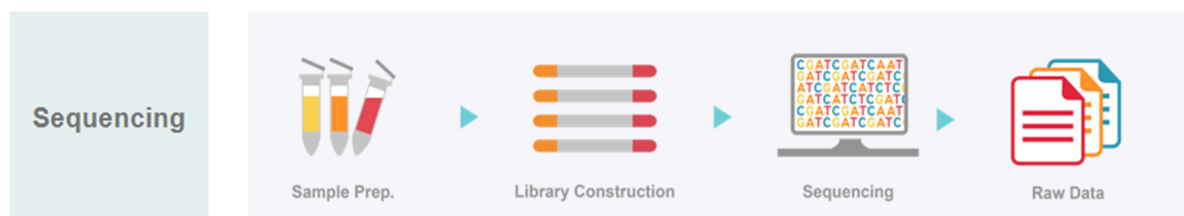
2. 1. Experiment Overview

Fig1. Experiment overview

The Illumina NGS workflow includes 4 basic steps :

1) Sample Preparation

     For library construction, DNA/RNA is extracted from a sample. After performing quality control
     (QC), qualified samples proceed to library construction.

2) Library Construction

     The sequencing library is prepared by random fragmentation of the DNA or cDNA sample, followed
     by 5' and 3' adapter ligation. Alternatively, "tagmentation" combines the fragmentation and ligation
     reactions into a single step that greatly increases the efficiency of the library preparation process.
     Adapter-ligated fragments are then PCR amplified and gel purified.

3) Sequencing

     For cluster generation, the library is loaded into a flow cell where fragments are captured on a lawn
     of surface-bound oligos complementary to the library adapters. Each fragment is then amplified
     into distinct, clonal clusters through bridge amplification. When cluster generation is complete, the
     templates are ready for sequencing.

     Illumina SBS technology utilizes a proprietary reversible terminator-based method that detects
     single bases as they are incorporated into DNA template strands. As all 4 reversible,
     terminator-bound dNTPs are persent during each sequencing cycle, natural competition minimizes
     incorporation bias and greatly reduces raw error rates compared to other technologies. The result
     is highly accurate base-by-base sequencing that virtually eliminates sequence-context-specific
     errors, even within repetitive sequence regions and homopolymers.

4) Raw data

     Sequencing data is converted into raw data for the analysis.

2. 2. Generation of Raw Data

The Illumina sequencer generates raw images utilizing sequencing control software for system control and base calling through an integrated primary analysis software called RTA (Real Time Analysis). The BCL (base calls) binary is converted into FASTQ utilizing illumina package bcl2fastq. Adapters are not trimmed away from the reads.

3. Summary of Produced Data

3. 1. Raw Data Statistics

The total number of bases, reads, GC (%), Q20 (%), and Q30 (%) are calculated for the 20 samples. For example, in Alor_01, 82,526 reads are produced, and total read bases are 24.8M bp. The GC content (%) is 53.38% and Q30 is 78.13%.

✔ **The following table only shows maximum of 20 samples. If your samples are more than 20, please refer to the attached excel file.** View full table : 2202KMI-0003_RawData_Stat.xlsx

Table 1. Raw data Stats (maximum 20 samples)

Sample ID	Total read bases (bp)	Total reads	GC(%)	AT(%)	Q20(%)	Q30(%)
Alor_01	24,840,326	82,526	53.38	46.62	88.68	78.13
Alor_02	53,455,192	177,592	53.76	46.24	89.25	78.99
Alor_03	27,779,290	92,290	51.83	48.17	90.28	80.51
Alor_04	31,227,546	103,746	53.66	46.34	89.18	78.9
Alor_05	36,997,716	122,916	53.57	46.43	88.64	78.04
Alor_06	31,410,554	104,354	51.83	48.17	88.44	77.73
Alor_07	31,641,722	105,122	51.99	48.01	89.52	79.36
Alor_08	48,163,612	160,012	54.71	45.29	89.22	79.01
Alor_09	23,990,904	79,704	52.82	47.18	89.45	79.34
Alor_10	27,841,898	92,498	54.18	45.82	88.84	78.46
Alor_11	28,682,892	95,292	53.63	46.37	88.05	77.36
Alor_12	30,159,598	100,198	50.26	49.74	89.62	79.44
Alor_13	27,825,644	92,444	53.37	46.63	89.83	80.01
Alor_14	34,797,406	115,606	53.87	46.13	89.54	79.39
Alor_15	25,855,298	85,898	49.82	50.18	90.6	81.03
Alor_16	51,295,818	170,418	49.34	50.66	89.5	79.31
Bali_01	26,566,862	88,262	54.07	45.93	88.68	78.19
Bali_02	40,356,274	134,074	54.33	45.67	89.33	79.21
Bali_03	25,475,436	84,636	53.36	46.64	89.56	79.45
Bali_04	28,524,566	94,766	53.5	46.5	89.84	79.88

- ◉ Sample ID : Sample name.
- ◉ Total read bases : Total number of bases sequenced.
- ◉ Total reads : Total number of reads. For Illumina paired-end sequencing, this value refers to the sum of read 1 and read 2.
- ◉ GC(%) : GC content.
- ◉ AT(%) : AT content.
- ◉ Q20(%) : Ratio of bases that have phred quality score of over 20.
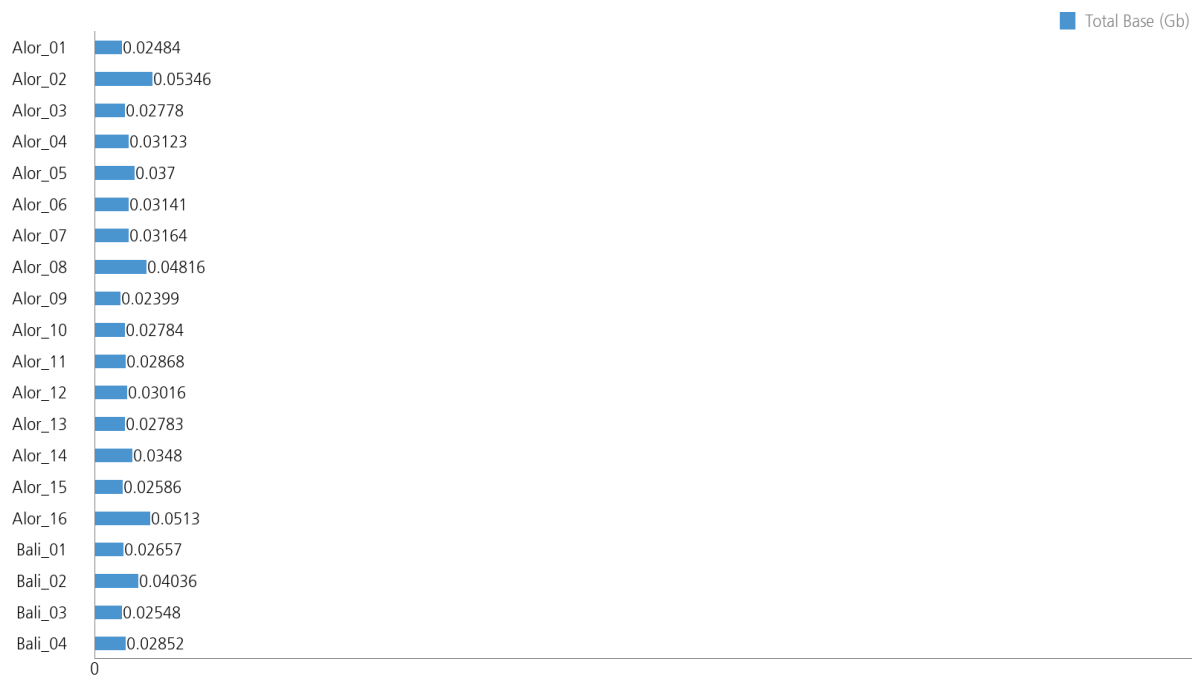- ◉ Q30(%) : Ratio of bases that have phred quality score of over 30.

3. 2. Total Read Bases
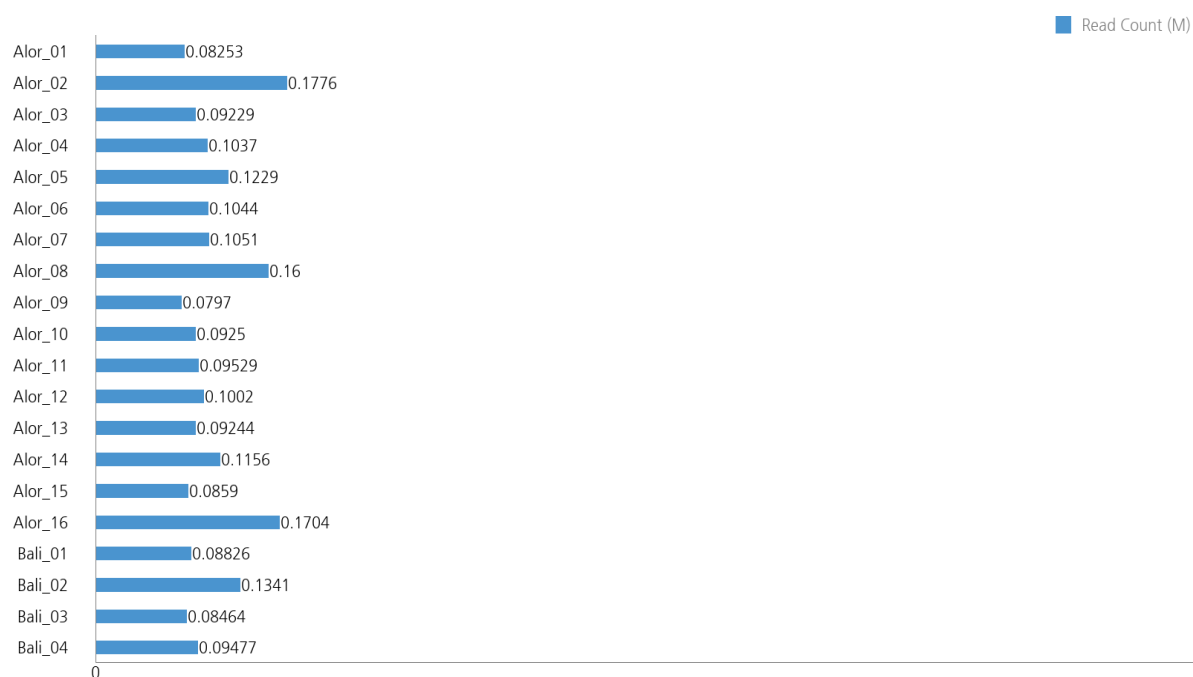

Figure 2.Throughput of Raw data

3. 3. Total Reads

Sample	Read Count (M)
Alor_01	0.08253
Alor_02	0.1776
Alor_03	0.09229
Alor_04	0.1037
Alor_05	0.1229
Alor_06	0.1044
Alor_07	0.1051
Alor_08	0.16
Alor_09	0.0797
Alor_10	0.0925
Alor_11	0.09529
Alor_12	0.1002
Alor_13	0.09244
Alor_14	0.1156
Alor_15	0.0859
Alor_16	0.1704
Bali_01	0.08826
Bali_02	0.1341
Bali_03	0.08464
Bali_04	0.09477

Figure 3. Total read count of Raw data
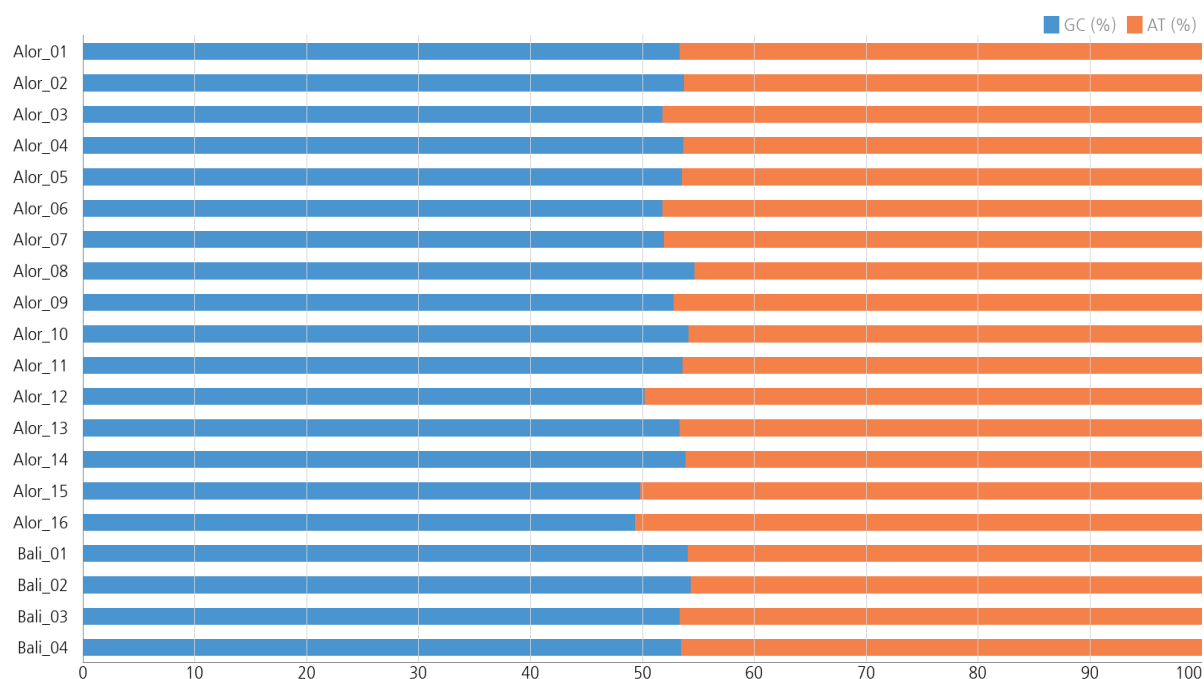
3. 4. GC/AT Content

Figure 4. GC/AT Content of Raw data
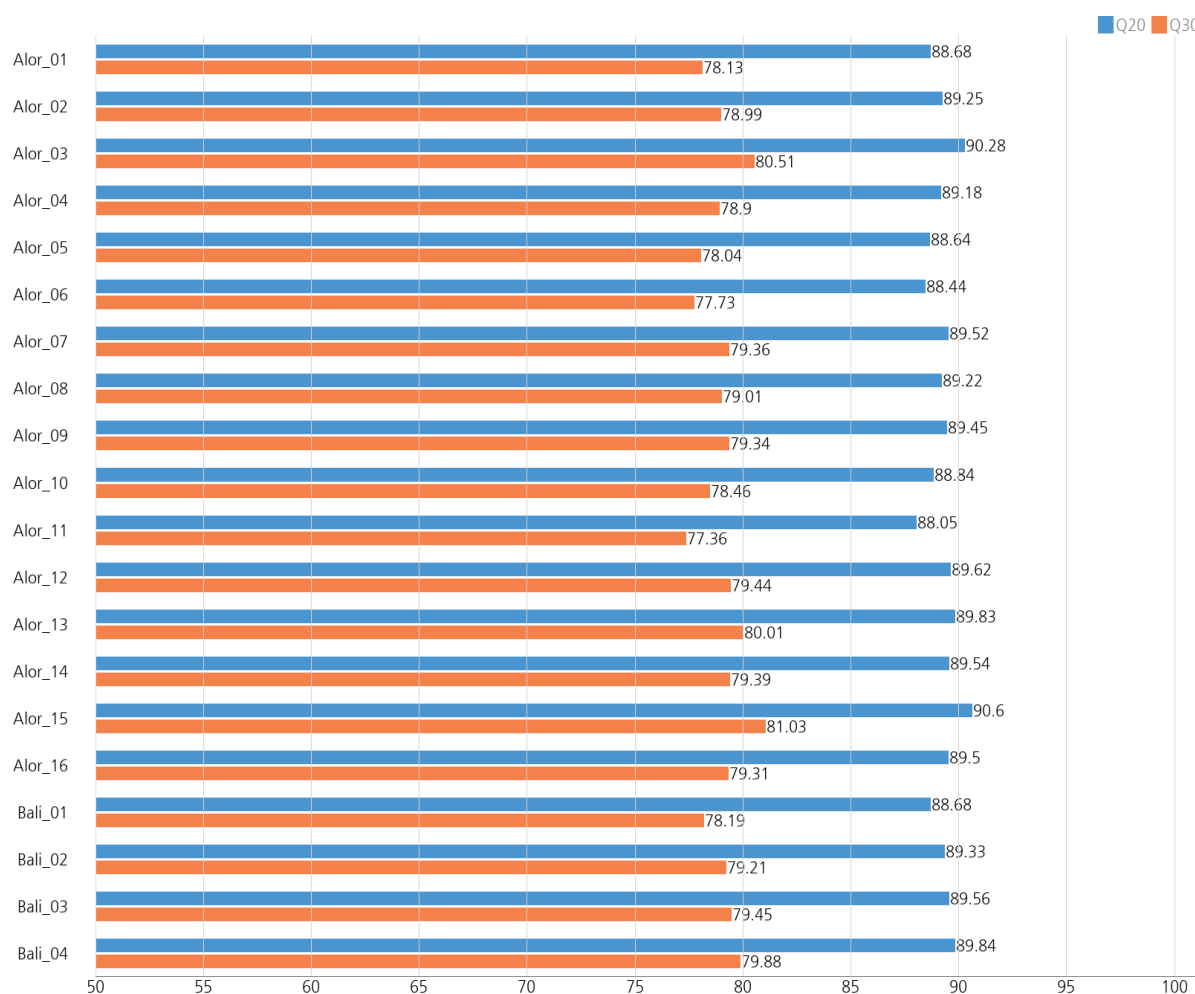
3. 5. Q20/Q30 (%)

Figure 5. Q20/Q30 scores of Raw data

4. Appendix

4. 1. FAQ

Q: I want to see the produced data. How can I open the files?

A: As the large size zip files provided by our company are hard to process in the Windows environment, we highly recommend using Linux environment for a smoother operation.

4. 2. FASTQ File

Example of FASTQ

```
@HISEQ-MFG:501:HB0TFADXX:1:1101:1247:2183 1:N:0:
CTCAGCTAAATACTTTGACACCNGTANNANNNNNNNNNNNTNNNNNNNNNNNNN
+
@@@BDDDDHHHHFHIIIIIIII#3AC########################
```

FASTQ file is composed of four lines.
Line 1 : ID line includes information such as flow cell lane information.
Line 2 : Sequences line.
Line 3 : Separator line (+ mark).
Line 4 : Quality values line about sequences.

4. 3. Phred Quality Score Chart

Phred quality score numerically expresses the accuracy of each nucleotide. Higher Q number signifies higher accuracy. For example, if Phred assigns a quality score of 30 to a base, the chances of having base call error are 1 in 1000.

Phred Quality Score Q is calculated with $-10\log_{10}P$, where P is probability of erroneous base call.

Quality of phred score	Probability of incorrect base call	Base call accuracy	Characters
10	1 in 10	90%	!"#$%&'()*+
20	1 in 100	99%	,-./012345
30	1 in 1000	99.9%	6789:;h=i?
40	1 in 10000	99.99%	@ABCDEFGHIJ

⊙ Encoding : Sanger Quality (ASCII Character Code=Phred Quality Value + 33)

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