Raw Data

Report

January 2022

Humanizing Genomics
macrogen
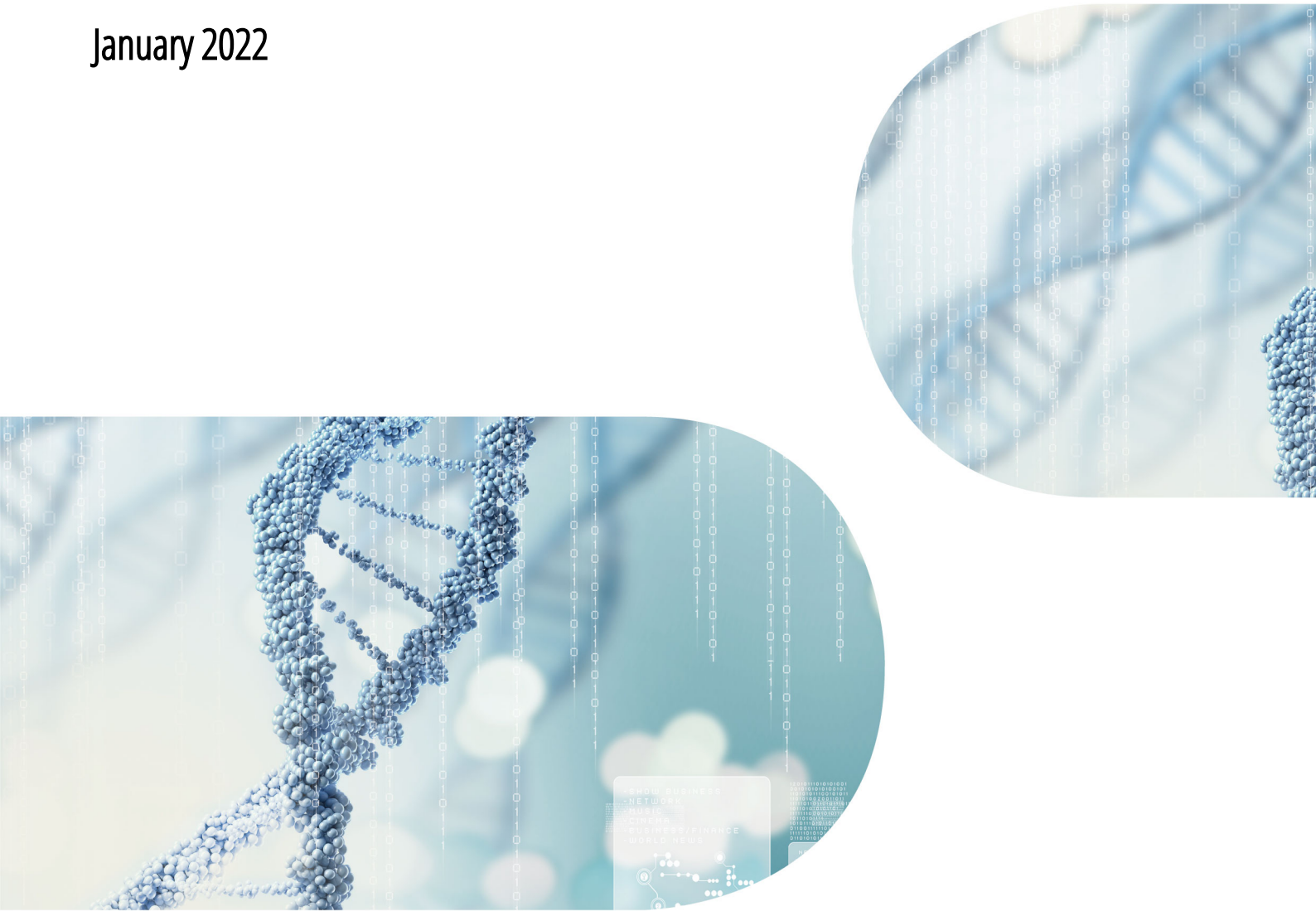
Project Information

Client Name	Macrogen Europe
Company / Institution	Macrogen Europe
Order Number	HN00163855
Type of Read	Paired-end
Read Length	151
Number of Samples	4
Type of Sequencer	Illumina platform

Table of Contents

1. Data Download Information

1. 1. Raw Data and Analysis Results

Download link	File size	md5sum
DN1_KO_Dec1_1.fastq.gz	994.2M	c3e04f75f561517638b7a80dd8635322
DN1_KO_Dec1_2.fastq.gz	937.4M	7c13a2dfdbb703e7c3aa269c1bfcd5c0
DN1_KO_Jul1_1.fastq.gz	1.0G	c0ae66f209be0f4cf374026ec51c99d7
DN1_KO_Jul1_2.fastq.gz	1.0G	6c5e04466d99d9255a7b80d5c62a8a24
DN1_KO_Jul2_1.fastq.gz	718.3M	2039a5b5ba37bba5cb9ebe9eecdc73bc
DN1_KO_Jul2_2.fastq.gz	710.5M	606175b18dcb8f2dc2c4fd55dc792a7c
DN1_WT_Dec1_1.fastq.gz	998.7M	39053aa0dac78196b0827969a20e1f7e
DN1_WT_Dec1_2.fastq.gz	905.2M	f1e692d66da4f116d70340e9fde100c5

- 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
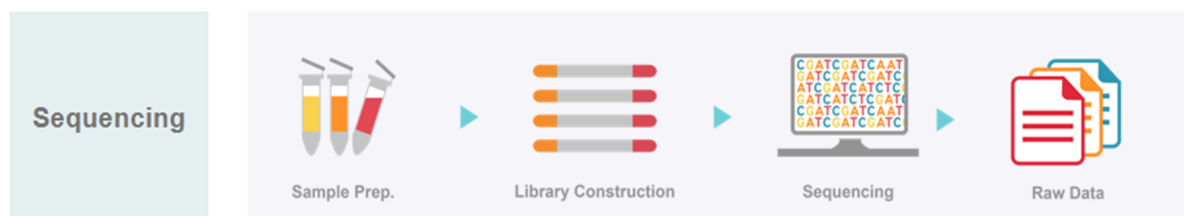
2. 1. Experiment Overview

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 4 samples. For example, in DN1_KO_Dec1, 33,240,456 reads are produced, and total read bases are 5.0G bp. The GC content (%) is 55.03% and Q30 is 87.84%.

Table 1. Raw data Stats (maximum 20 samples)

Sample ID	Total read bases (bp)	Total reads	GC(%)	AT(%)	Q20(%)	Q30(%)
DN1_KO_Dec1	5,019,308,856	33,240,456	55.03	44.97	93.25	87.84
DN1_KO_Jul1	5,001,671,452	33,123,652	48.62	51.38	94.78	88.81
DN1_KO_Jul2	3,143,836,308	20,820,108	49.76	50.24	95.01	89.16
DN1_WT_Dec1	5,007,650,146	33,163,246	53.29	46.71	92.15	86.49

- 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
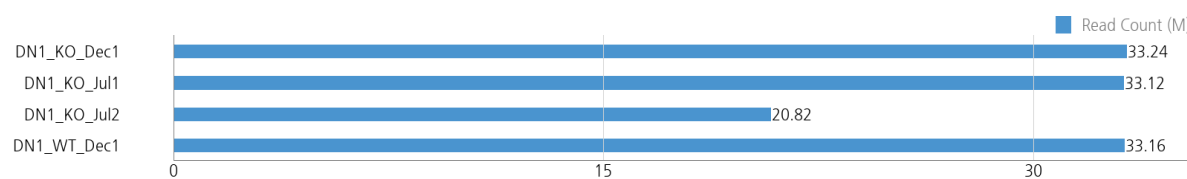

Figure 2.Throughput of Raw data

3. 3. Total Reads

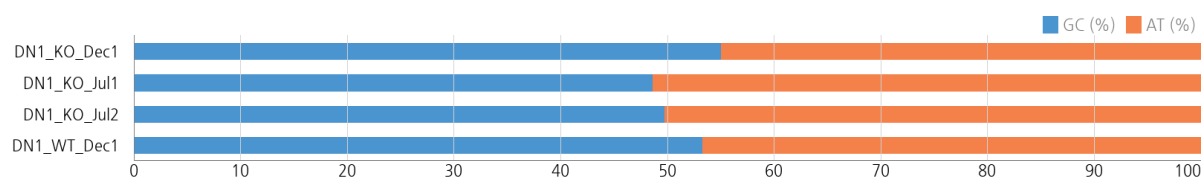Figure 3. Total read count of Raw data

3. 4. GC/AT Content

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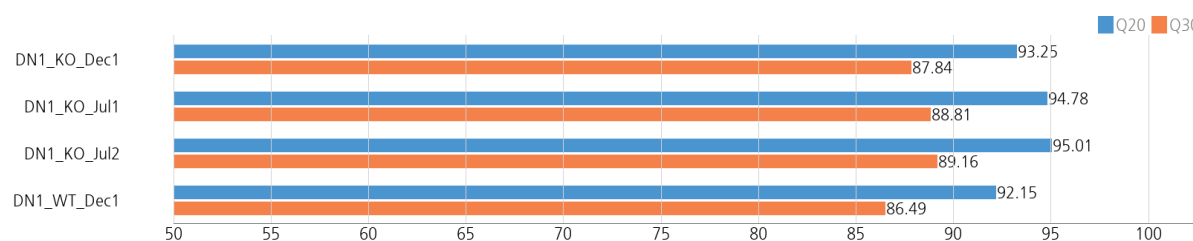
3. 5. Q20/Q30 (%)

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:
CTCAGCTAAATACTTTGACACCNGTANNANNNNNNNNNNTNNNNNNNNNNNN
+
@@@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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