Humanizing Genomics
macrogen

Metagenome Shotgun Sequencing

Report

2023.08

RAWDATA REPORT

Research use only

Table of Contents

Order Information

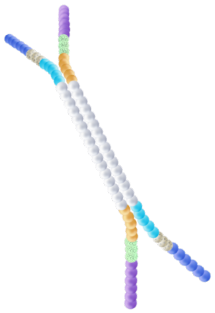
Client Name	Leonildo Torres
Client Organization	SUMINISTROS CLINICOS ISLA SAS
Order Number	HN00199640
Application	Metagenome Shotgun Sequencing
Type of Read	Paired-end
Read Length	151
Library Kit	TruSeq Nano DNA Kit
Library Protocol	TruSeq Nano DNA Sample Preparation Guide, Part # 15041110 Rev. D
Type of Sequencer	illumina system

Experimental Workflow

The samples are prepared according to NGS library preparation workflow, and sequenced using Illumina platform.
The workflow illustrated below shows the common ligation based method of library preparation. The process may differ based on the library preparation protocol followed.

Sample Preparation

DNA/RNA is first extracted from the sample, and samples which meet quality control standards proceed to library construction.



Ligate Adapters

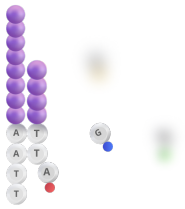
The sequencing library is prepared by random fragmentation of the DNA or cDNA sample, followed by 5' and 3' adapter ligation. Alternatively, "tagmentation" combines the fragmentation and ligation reactions into a single step which greatly increases the efficiency of the library preparation process.

Final library Construction

Adapter-ligated fragments are then PCR amplified with a PCR primer solution which anneals to the ends of each adapters.
The library templates undergo quality control and quantification process.

Cluster generation using bridge amplification

The library is loaded onto 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.
Once cluster generation is complete, the templates are ready for sequencing.



Sequencing by synthesis (SBS) technology

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 present 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.

Generation of Raw data

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

Raw Data Statistics

• The total number of bases, reads, GC (%), Q20 (%), and Q30 (%) are calculated for the 11 samples.
For example, in 4 sample, 82,346,786 reads are produced, and total read bases are 12.4 Gbp.
The GC content (%) is 57.2% and Q30 is 93.3%.

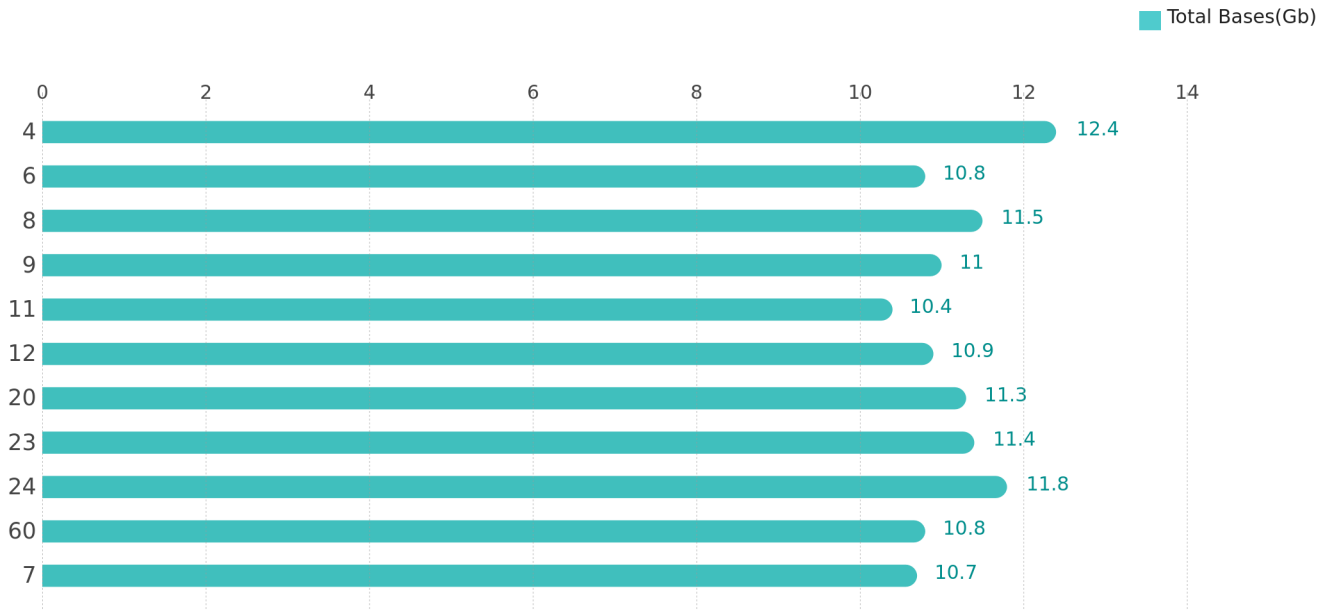
* Raw Data

Sample ID	Total bases(bp)	Total reads	GC(%)	AT(%)	Q20(%)	Q30(%)
4	12,434,364,686	82,346,786	57.2	42.8	97.5	93.3
6	10,847,768,124	71,839,524	52.8	47.2	97.4	93.1
8	11,510,200,896	76,226,496	59.0	41.0	97.6	93.4
9	11,014,234,148	72,941,948	59.1	40.9	97.7	93.8
11	10,368,798,842	68,667,542	46.2	53.8	97.2	92.5
12	10,890,153,522	72,120,222	45.0	55.0	97.2	92.5
20	11,286,049,550	74,742,050	60.5	39.5	97.7	93.8
23	11,361,986,544	75,244,944	57.2	42.8	97.6	93.4
24	11,819,963,202	78,277,902	56.0	44.0	97.9	93.9
60	10,845,632,682	71,825,382	44.6	55.4	97.1	92.3
7	10,672,027,378	70,675,678	50.5	49.5	97.5	92.9

- Sample ID : Sample name.
- Total bases(bp) : Total number of bases sequenced.
- Total reads : Total number of reads. For illumina paired-end sequencing, this value refers to the sum of read1 and read2.
- GC(%) : Ratio of GC content.
- AT(%) : Ratio of 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.

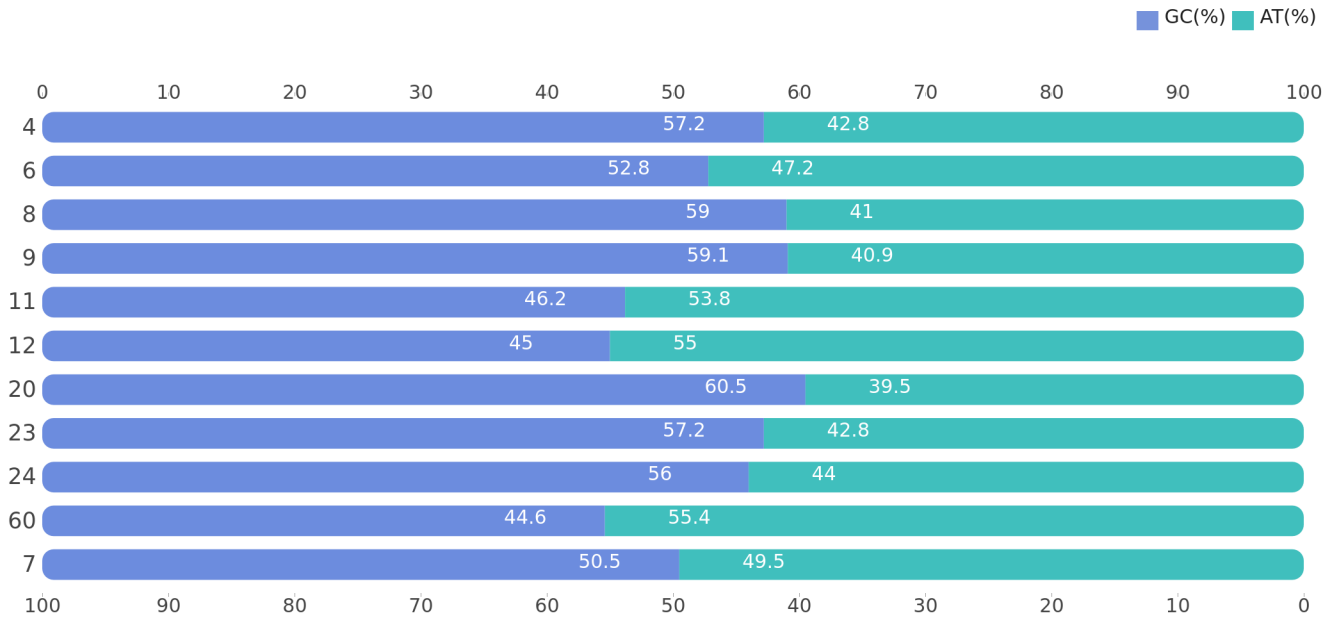
Total Bases Total number of samples : 11

* Raw Data



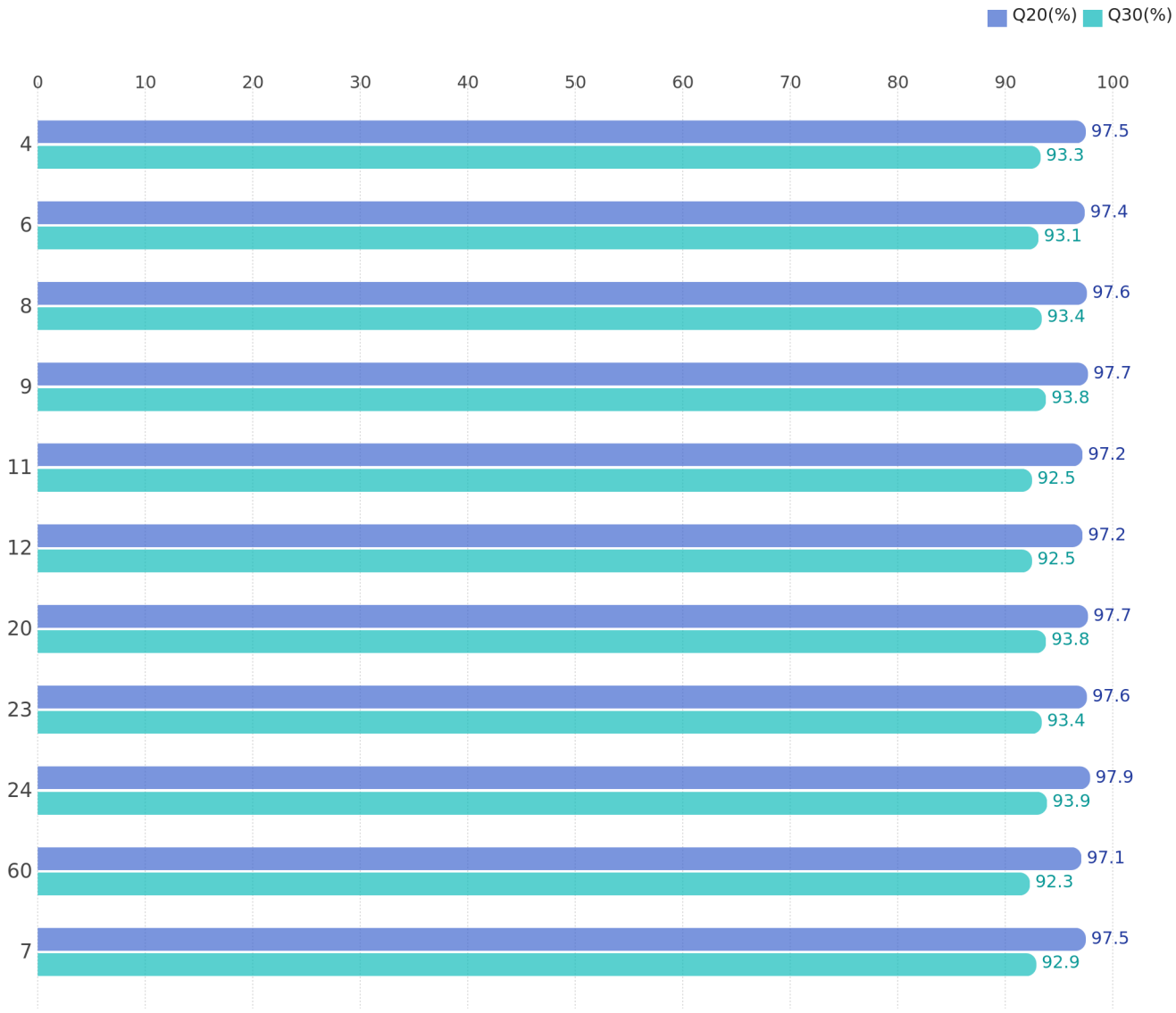
GC/AT Content Total number of samples : 11

* Raw Data



Q20/Q30 (%) Total number of samples : 11

* Raw Data



Download List

- The data can be downloaded from the links below. The download links are active for 2 weeks only, so please download your data within this period.
- Once you receive/download the data, please make sure to check the integrity of the files.
  Please note that the sequencing files will be deleted from our server 3 months after the analysis report is released;
  please contact us within 3 months if you encounter a problem with the data.

* Raw Data Download

File Name	File Size(byte)	md5sum
11_1.fastq.gz	2,673,737,799	69721d88917339e6fece657e70dbc129
11_2.fastq.gz	2,751,192,884	cfddadbaa91760ff3c264c04ea921e06
24_1.fastq.gz	3,017,311,362	0d39f4e71f950fa9c19e77f1d798d684
24_2.fastq.gz	3,079,927,871	6938c08c489fa64a18238b34e3761d67
12_1.fastq.gz	2,801,588,160	83081d5e43be1a3f40eb08a030016d28
12_2.fastq.gz	2,885,050,542	6102352519637bf82399062ac21ef620
20_1.fastq.gz	2,857,369,361	7ca6e5bc6ac3b18fde24a32cb877c01f
20_2.fastq.gz	2,901,380,018	95344a905918689f0a87adf0af1d1bae
23_1.fastq.gz	2,911,100,638	4bbed7e921fa8d1e1ffa7b380edf814f
23_2.fastq.gz	2,956,977,225	dd869f29e35208e3a9bcda3106b5ab25
60_1.fastq.gz	2,796,644,262	88448cb7911083cd76be3b8c5837df13
60_2.fastq.gz	2,875,781,204	89629189d11c4b638cf58138cf3b5163
4_1.fastq.gz	3,157,692,089	4195830b916ee47ec2bdf0f9384e7964
4_2.fastq.gz	3,242,313,845	fc1293336656bf07f59dfcdf70cd6c48
7_1.fastq.gz	2,759,842,492	75194a2f75c82f0f4cbd87283b043518
7_2.fastq.gz	2,863,153,886	2dddcb1b47802b46b67ba51981481cd8
6_1.fastq.gz	2,794,016,473	30efafbc9496e5b82b3fcf555742d17c
6_2.fastq.gz	2,863,132,600	c51b43feb8915be61c727bfd1d18f652
9_1.fastq.gz	2,788,290,450	296b1ee77d2268fa70cb3213600cadbc
9_2.fastq.gz	2,829,063,063	1793ba96fd0e97be202f5b31bf69408d
8_1.fastq.gz	2,954,572,391	36ba1c157302be85636c5197408fc0a6
8_2.fastq.gz	3,003,904,124	d329610d2d8d3c3c3af442481502d842

FAQ

Q **Why do I need to check the md5sum values, and how can I check it? (Windows system)**

A NGS data tend to have a large files size which makes them more likely to be corrupted during file transfer. So it's important that you check the md5sum of the files after receiving them to make sure what you received are what we gave.

Checking md5 hash in a Windows system

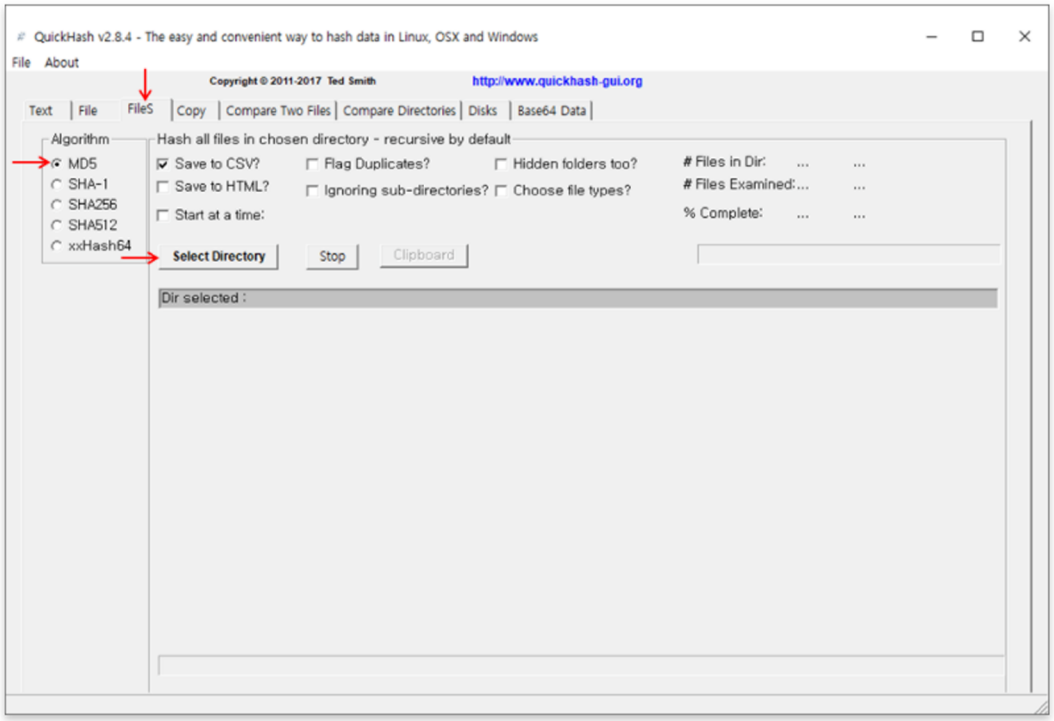
Windows does not provide a program for checking md5sum by default. An external program such as QuickHash-Windows can be used instead.

STEP 1 Download QuickHash-Windows from the website, and unzip the file.

STEP 2 Take a look at the UserManual.pdf file inside the zip file, and execute the .exe file.

Quickhash-GUI.exe	2,090,414	6,505,472
sqlite3-win32.dll	429,646	852,754
sqlite3-win64.dll	717,149	1,742,848
UserManual.pdf	512,697	576,987

STEP 3 Click on the "FileS" tab, and select [MD5] as the Algorithm.



STEP 4 Click "Select Directory" and choose the directory where the files to be checked are located in.
The output can be saved as a csv or txt file.
The process may take some time depending on the performance of the system being used.

STEP 5 Compare the newly calculated md5 value with the md5 value provided to you through the Analysis Report.

FAQ

Q **Why do I need to check the md5sum values, and how can I check it? (Linux system)**

A NGS data tend to have a large files size which makes them more likely to be corrupted during file transfer. So it's important that you check the md5sum of the files after receiving them to make sure what you received are what we gave.

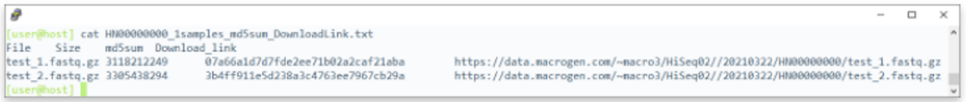
Checking md5 hash in a Linux system

Linux systems have an internal md5sum program under /user/bin/md5sum.
md5sum has a "-c" option, which reads the MD5 sums from the input file and checks them simultaneously.

Usage: $ md5sum -c [input file name]

STEP 1 Macrogen provides a text file containing the md5sum of deliverables you'll be receiving, which you can use to validate the integrity of the files. You can download this file by clicking on the "md5sum List" button in the "Download List" page. The text file will have the following name and format depending on how you're receiving your data:

- Via download link : **<OrderNumber>_#samples_md5sum_DownloadLink.txt**



- Via HDD : **<OrderNumber>_#samples_md5sum.txt**

- You can also find **"md5sum.txt"** located inside the HDD delivered to you.

STEP 2 Use "md5sum -c" to validate the integrity of the file you've received. The input file for md5sum -c has to be delimited by two spaces with the md5sum column appearing before the file name, just like the sample image of "md5sum.txt" file shown above. As you can see, the two other files above are not formatted this way and need to altered to be used as input for md5sum -c. You can manually exclude the header and cut out "File" and "md5sum" column from the files, or simply run the following command:

$ awk '{print $3 " " $1}' <md5sum_file> | grep -v File

STEP 3 **"md5sum -c"** reads the input containing the md5 value of a file, and checks whether the md5 value of that file matches what's written inside the input file. This action outputs **"OK"** if the md5 value matches, and **"FAILED"** if otherwise. Check if the command outputs **"OK"** for all the files. (Refer to image below)

FAQ

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

A NGS data tend to have large file sizes, and are not user-friendly to work with in a Windows environment.
We recommend that you use Linux system for smoother operation.

Q Where can I find information for Illumina adapter sequences?

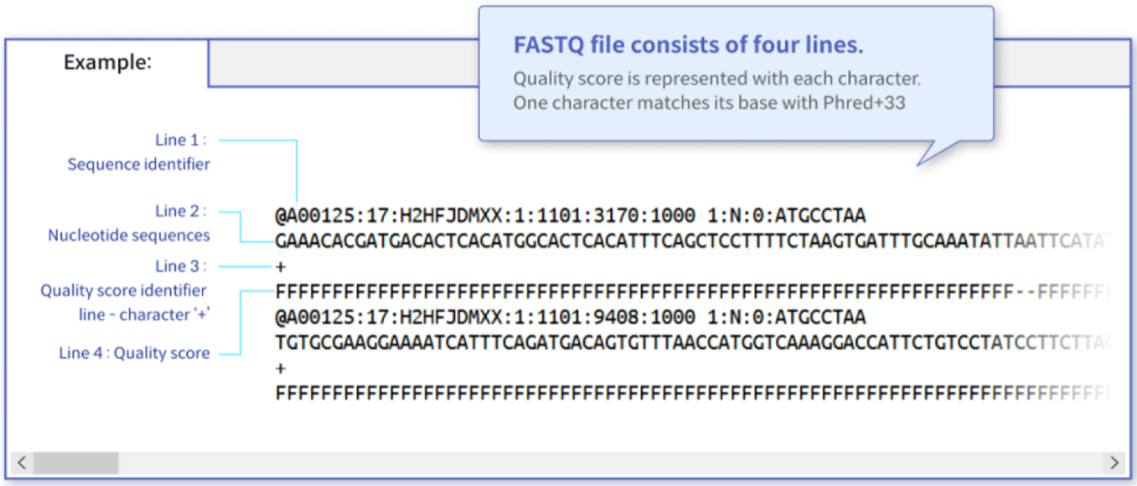
A Information on Illumina adapters can be found in this support document:
Adapter Sequences Intro

Result File Description

Deliverables List

File Type	File Name	Description
FASTQ	[Sample name]_[read1].fastq.gz	Raw read1 sequence data
	[Sample name]_[read2].fastq.gz	Raw read2 sequence data
md5sum	[Order#]_[#samples]_md5sum[_DownloadLink].txt	You can download this file by clicking on the "md5sum List" button found on the "Download List" page. The file is slightly different in terms content, depending on how you're receiving your data. If you're receiving via download link, the file contains the following information : File name, File size, md5sum, FTP link. Otherwise, if your receiving your data via HDD the file only contains : File name, File size, and md5sum. MD5 is a string of 32 hexadecimal values, which represents a 'fingerprint' of a file. By comparing the supplied MD5 value to the actual value computed by the MD5sums utility, you can make sure that the file that you downloaded off of the internet has not been tampered with or modified from the original file stored in our server.

FASTQ Format



Phred Quality Score

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
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10000	99.99%

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