Next Generation Sequence (NGS) Technologies

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Outlines

- Overview of Sequencing History
- Next Generation Sequencing (NGS) – Revolution in Genome Research
- Illumina leader of the field
- NCDC/Genome Center overview

Sequencing Past

Historical Timeline

1870 – DNA discuvery, Miescher

1940 – DNA was proposed as Genetic Material, Avery

1953 – Double helical structure, Watson and Crick

1977 – sequence by Chemical degradation, Gilbert

1977 – sequence by Dideoxy chain termination, Sanger

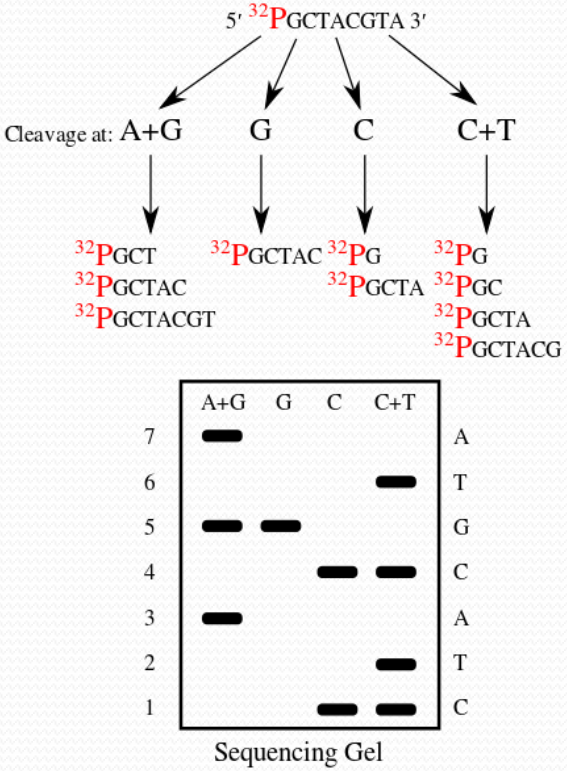
1986 – Partial Automation

1990 – Cycle Sequencing

2002 – NGS 454, pyrosequencing

Sequencing Past

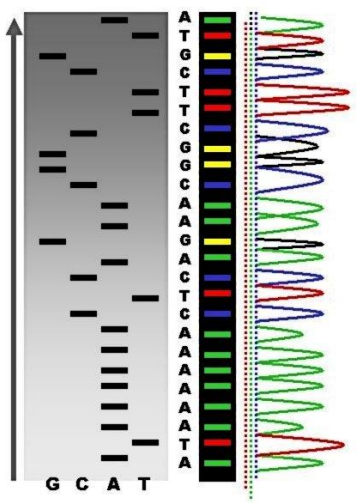
Maxam and Gilbert Sequencing technology



- Here is used purified DNA
- Chemical modification in the DNA
- The fragments are radioactively labeled at 5’ end
- Chemical treatment generates the nucleotide bases
- DNA is cleaved by hot piperidine at modified base
- Modifying chemicals is applied to the DNA
- Generates radiolabeled DNA fragments
- Electrophoresis and visualized in X-rays films

Sequencing Past

Sanger Chain Termination Sequencing technology



Frederick Sanger (1918 – 2013; age 95); Sanger sequencing is a method of DNA sequencing based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication; This machine, developed in 1987, uses the Sanger method for DNA sequencing for the first time.

Sequencing Past

Capillary Sequencing Instruments

ABI 3130-xl

ABI 3730-xl

ABI 3500-xl

Main Players on the Market

Companies

PACIFIC BIOSCIENCES

illumina

Roche 454 SEQUENCING

NASDAQ

Helicos Bioscience

HeliScope™

ThermoFisher SCIENTIFIC

NuGEN imagine more from less

华大基因 BGI

Oxford NANOPORE Technologies

ROCHE

Next Generation Instruments

454 pyrosequencing

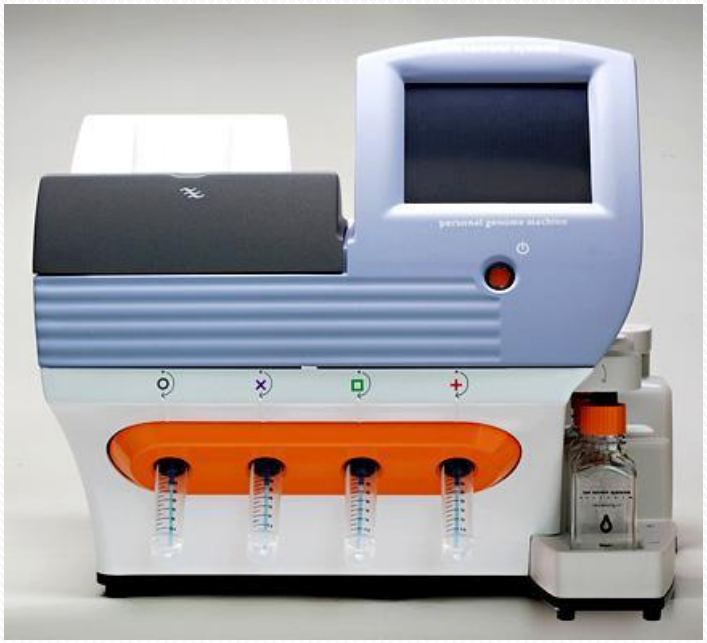
GS Junior

Roche and NuGEN Technologies

life Technologies

Next Generation Instruments

Ion Torrent PGM

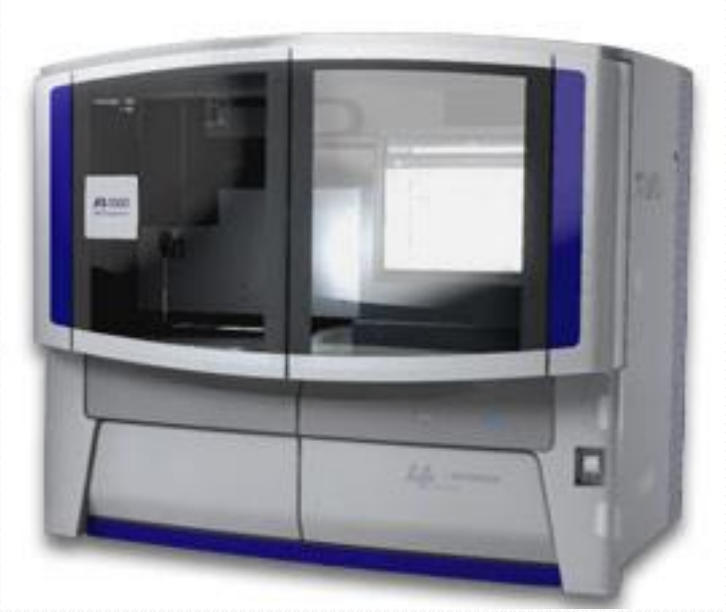


IonProton

life Technologies

Next Generation Instruments

SOLiD5500xl



SOLiD

PACBIO

Next Generation Instruments

BGI

Next Generation Instruments

BGISEQ 500

BGISEQ 50

BGI

Next Generation Instruments

Sequencing Present

Next Generation Sequencing – Illumina Platforms

GA (Solexa) sequencing

HiSeq 2000

HiSeq 3000

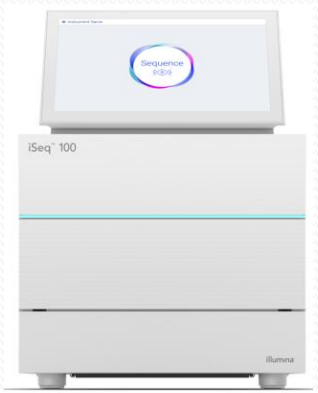
HiSeq X

NextSeq

NeoPrep

Sequencing Present

Next Generation Sequencing – Illumina Platform iSeq

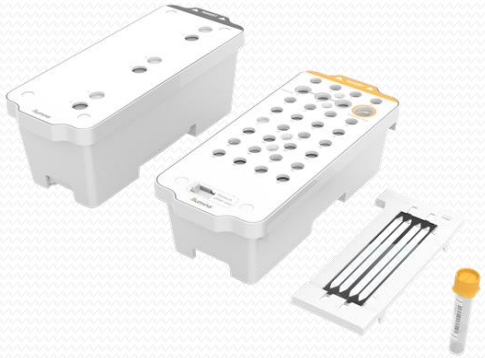


Run Configuration	Reads (Passing Filter)/Run	Output	Quality Scores[b]	Run Time[c]
1 x 36 bp	4 M	144 Mb	> 85%	9 hrs
1 x 50 bp	4 M	200 Mb	> 85%	9 hours
1 x 75 bp	4 M	300 Mb	> 80%	10 hours
2 x 75 bp	4 M	600 Mb	> 80%	13 hours
2 x 150 bp	4 M	1.2 Gb	> 80%	17.5 hours

a. Performance parameters may vary based on sample type, sample quality, and clusters passing filter.
b. The percentage of bases > Q30 is averaged over the entire run.
c. Times include cluster generation, sequencing, base calling, and quality scoring.

Sequencing Present

Next Generation Sequencing – Illumina Platform NovaSeq



Sequencing Present

Next Generation Sequencing – Illumina Platform MiSeq

MiSeq

MiSeqDx

MiSeqFGx

Sequencing Future

Oxford Nanopore Technologies

MinION

GridIONx5

SmidgION - coming soon

PromethION

Genome Center at National Center for Disease Control and Public Health

Genome Center Equipment

MiSeq Personal Sequencer

Applied Biosystems 3130xl Genetic Analyzer

M220™ Focused-ultrasonicator™
DNA Shearing for NGS

2100 Bioanalyzer

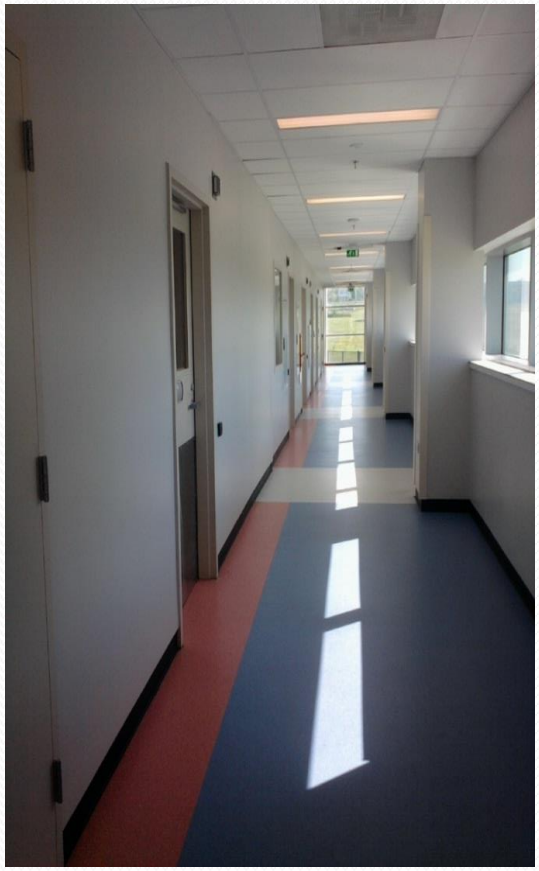
RT-PCR

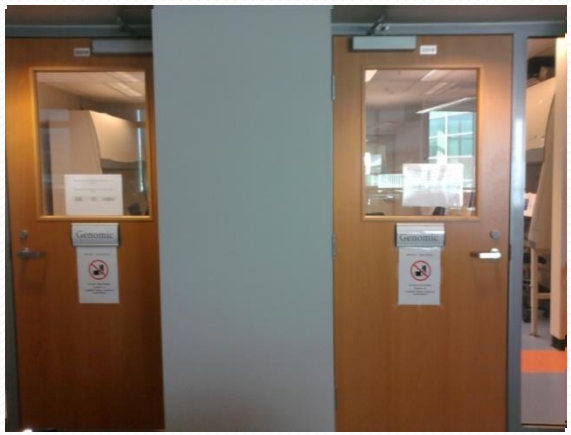
Qubit, Fluorometric Quantitation

Genome Center at National Center for Disease Control and Public Health

Lab Space







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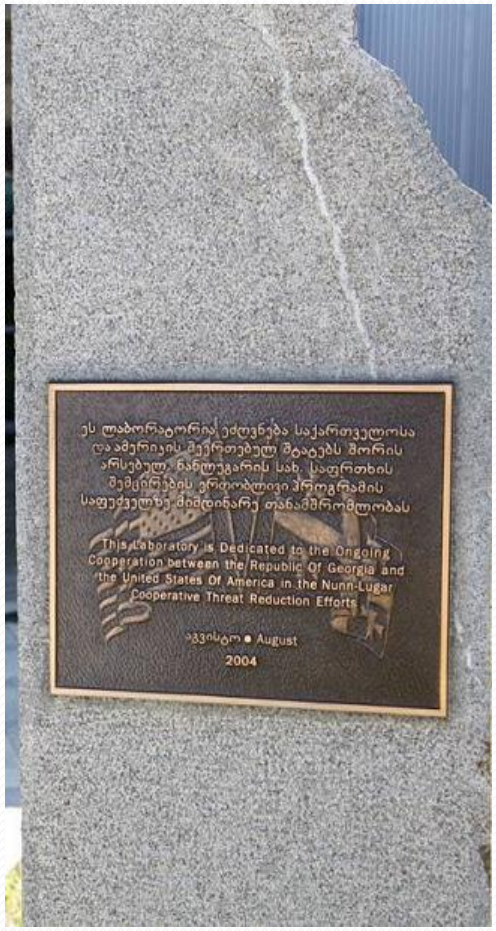
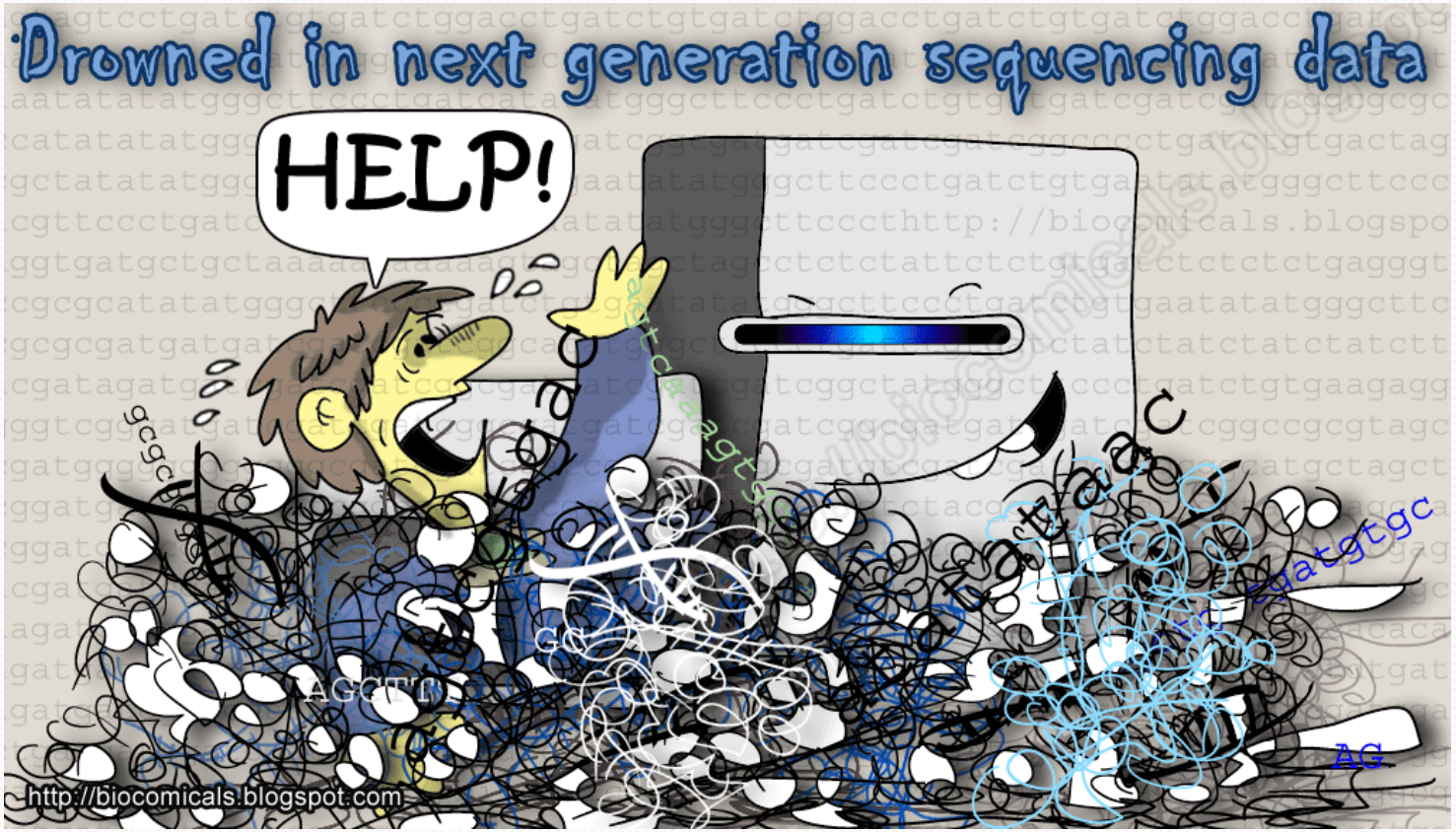
Lab Space



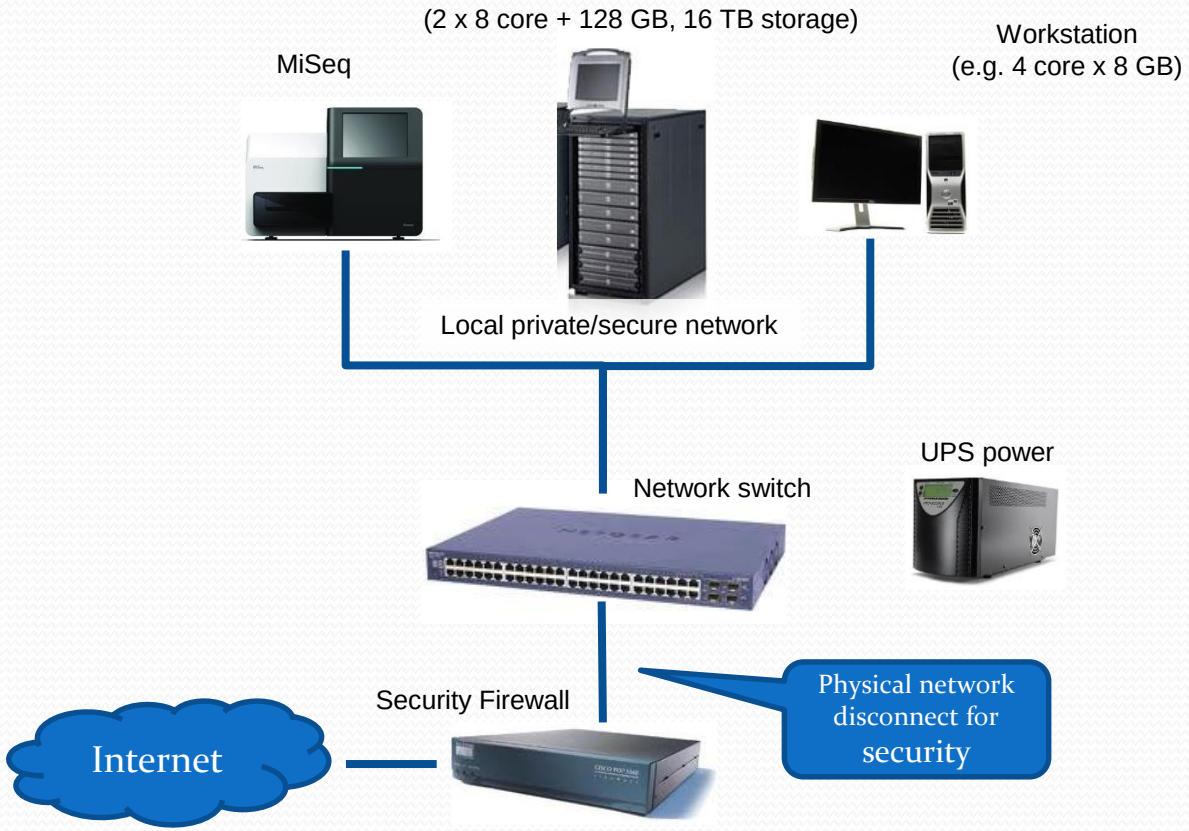
Genome Center at National Center for Disease Control and Public Health

Bioinformatics



Genome Center at National Center for Disease Control and Public Health

Bioinformatics



Genomics Machine Specs

-2U Rackmount w/ redundant power supply
-Dual Xeon with C602 chipset
-16 2.0Ghz E-5 series CPU cores
-128GB DDR3 1600Mhz ECC REG memory
-(8) 2TB Enterprise Hard Drive (2 x OS RAID 1, 6 x RAID 6 w/ 2 Hot Spare) roughly 3.6TB Accessible
-Slimline DVD +/- RW
-dual GigE NIC
-onboard basic graphics
-no sound
-CentOS 6.2 64-bit installed
-Storage

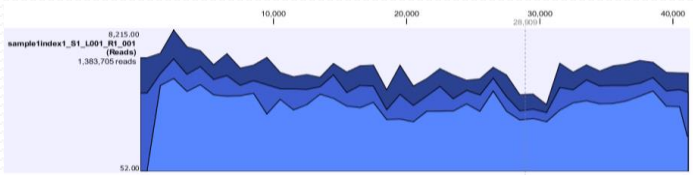
•Includes a 15U Rackmount Enclosure, pre-racked and cabled, shipping crate with ramp. Estimated value of $2,500.00.

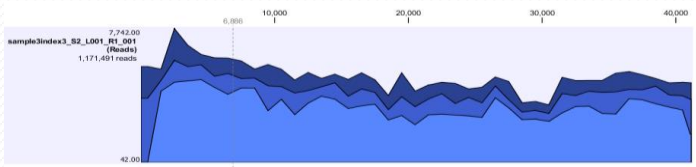
•Includes a 2200VA UPS with temperature monitoring card, racking and shipping. Estimated value of $2,300.00.

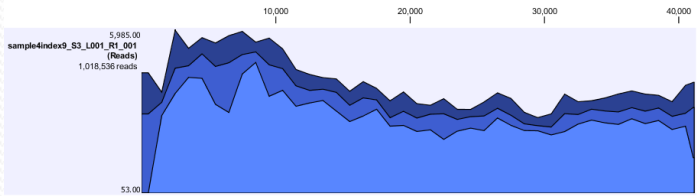
•Includes Hardware testing, installation of CLC Genomics Server, and shipping to end user. The server will be installed on the cabinet, you would just need to unpack the crate, plug in the UPS and then plug in the server.

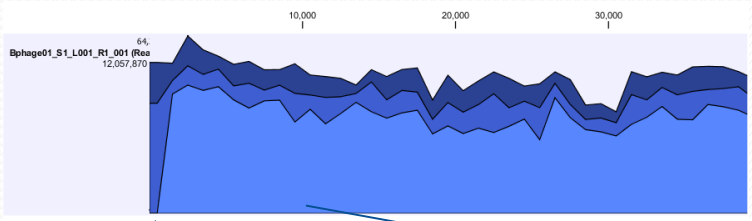
Genome Center at National Center for Disease Control and Public Health

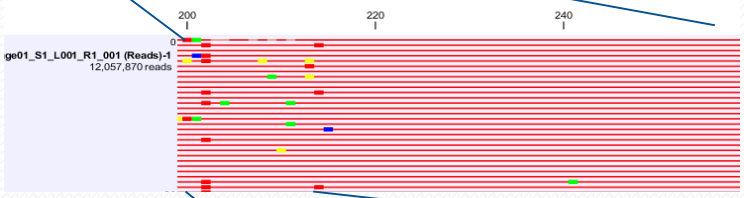
First run at Genome Center 23Jan2013

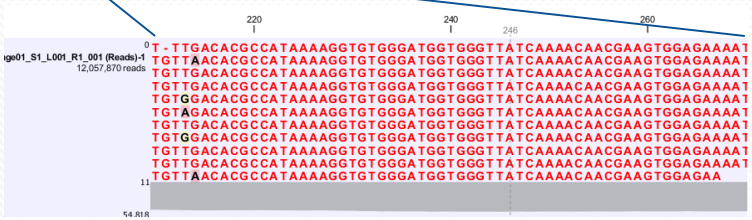












Thank you for your attention !