MinION Bioinformatic Analysis

Francesca Colavita Martina Rueca Emanuela Giombini

INMI «L. Spallanzani»





PRODUCTS SERVICES APPLICATIONS GET STARTED RESOURCES

Basecalling and primary data analysis with Guppy

Guppy is a data processing toolkit that contains the Oxford Nanopore Technologies' basecalling algorithms, and several bioinformatic post-processing features. It is provided as binaries to run on Windows, OS X and Linux platforms, as well as being integrated with MinKNOW, the Oxford Nanopore device control software.

Early downstream analysis components such as barcoding/demultiplexing, adapter trimming and alignment are contained within Guppy. Furthermore, Guppy now performs modified basecalling (5mC, 6mA and CpG) from the raw signal data, producing an additional FAST5 file of modified base probabilities.

Join the community >

Publications

A quality control tool for high throughput sequence data.

Program: FastQC

FastQC **aims** to provide a simple way to do some quality control checks on raw sequence data coming from high throughput sequencing pipelines.

Basic Statistics

Per Base Sequence Quality

Per Sequence Quality Scores

Per Base Sequence Content Summary

Per Sequence GC Content

Per Base N Content

Sequence Length Distribution

Duplicate Sequences

Overrepresented Sequences

Adapter Content

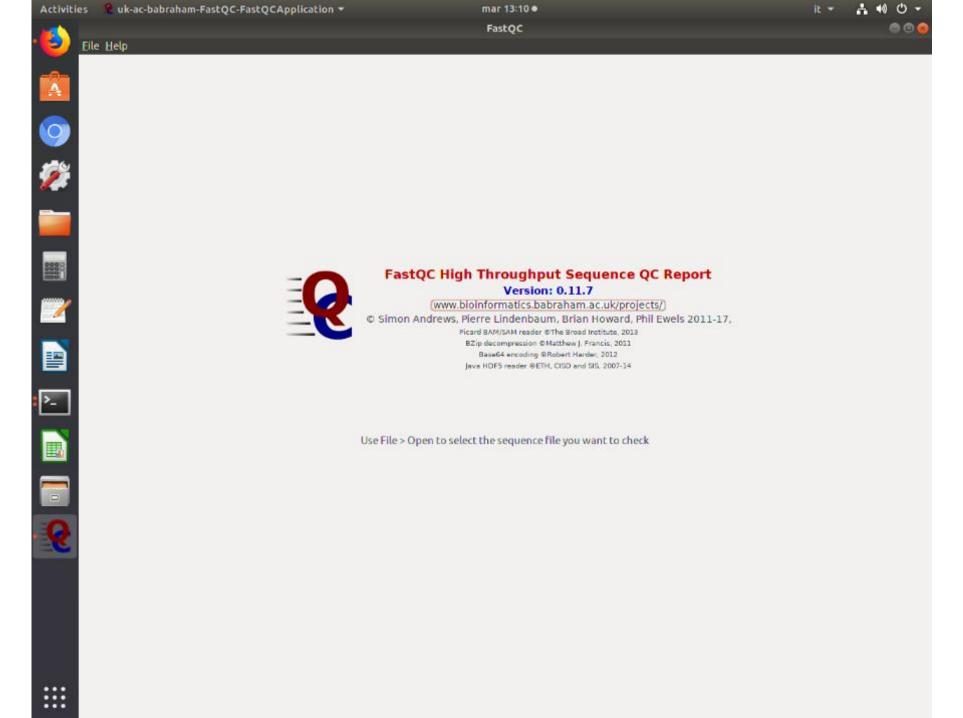
Kmer Content

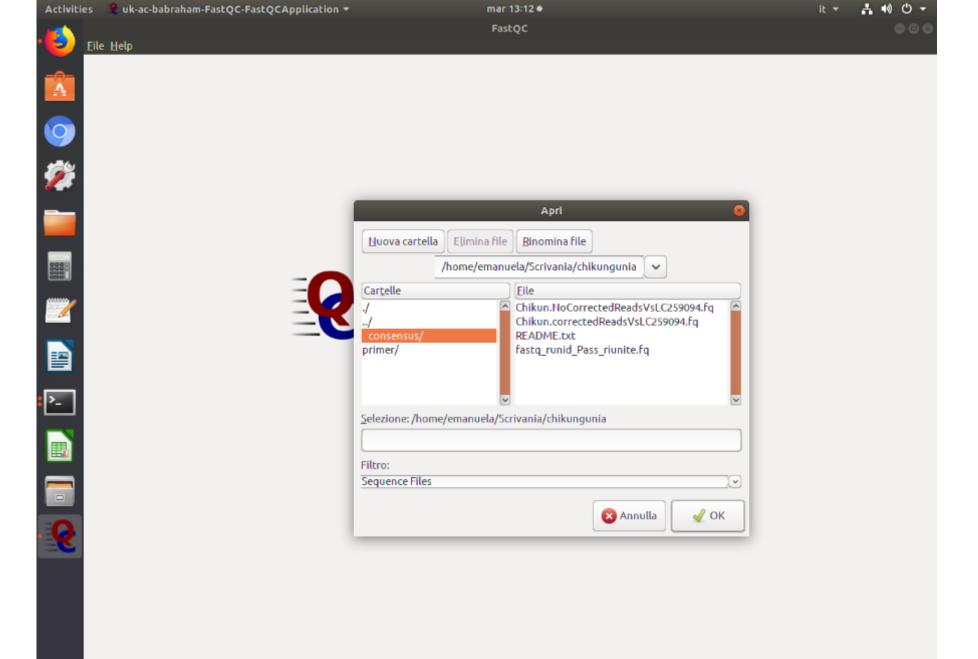
Per Tile Sequence Quality

Supported files:

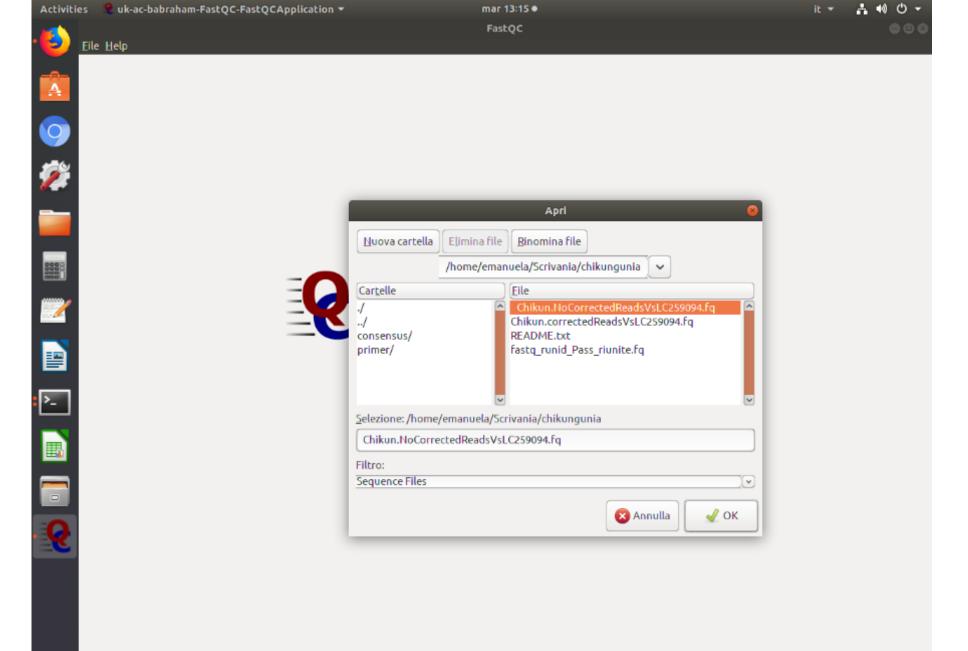
- FastQ (all quality encoding variants)
- Casava FastQ files*
- Colorspace FastQ
- GZip compressed FastQ
- •SAM
- •BAM
- •SAM/BAM Mapped only (normally used for colorspace data)

Documentation: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

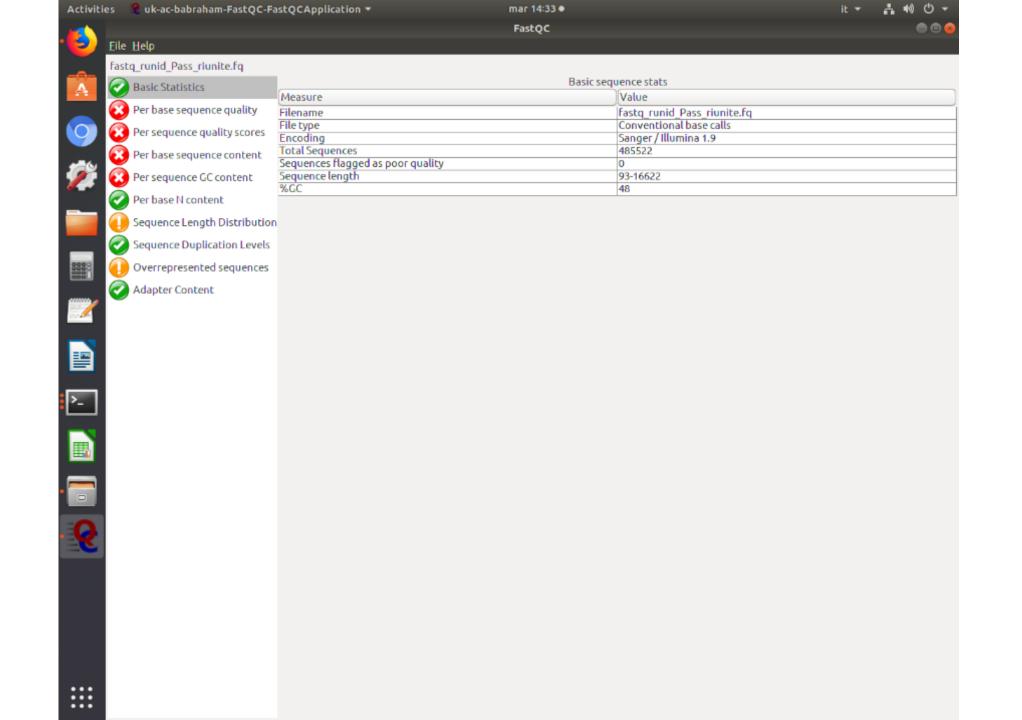


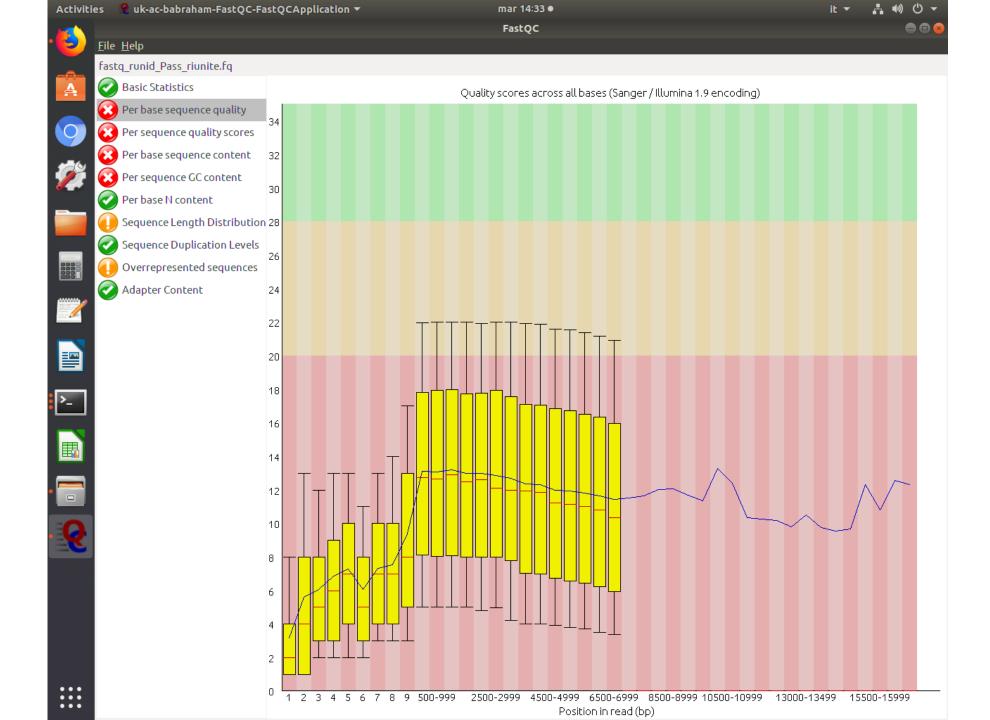




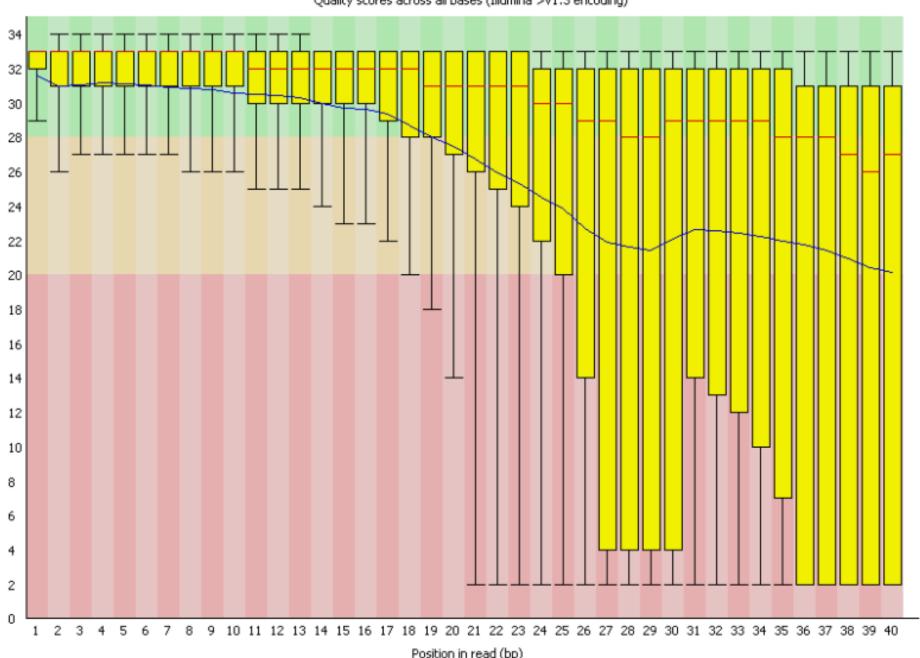


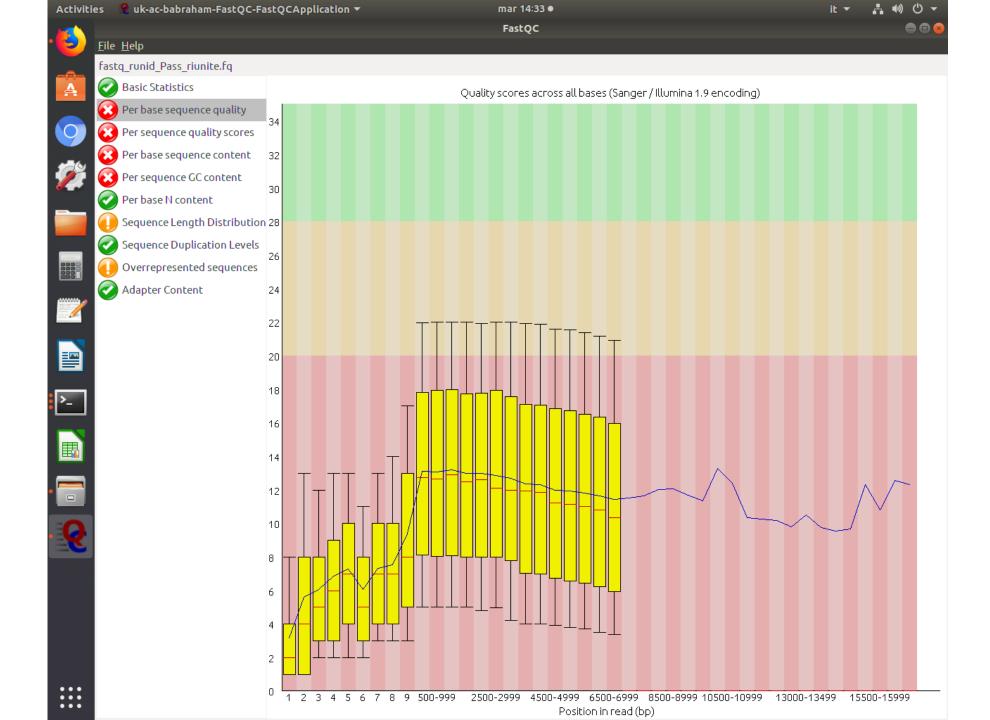


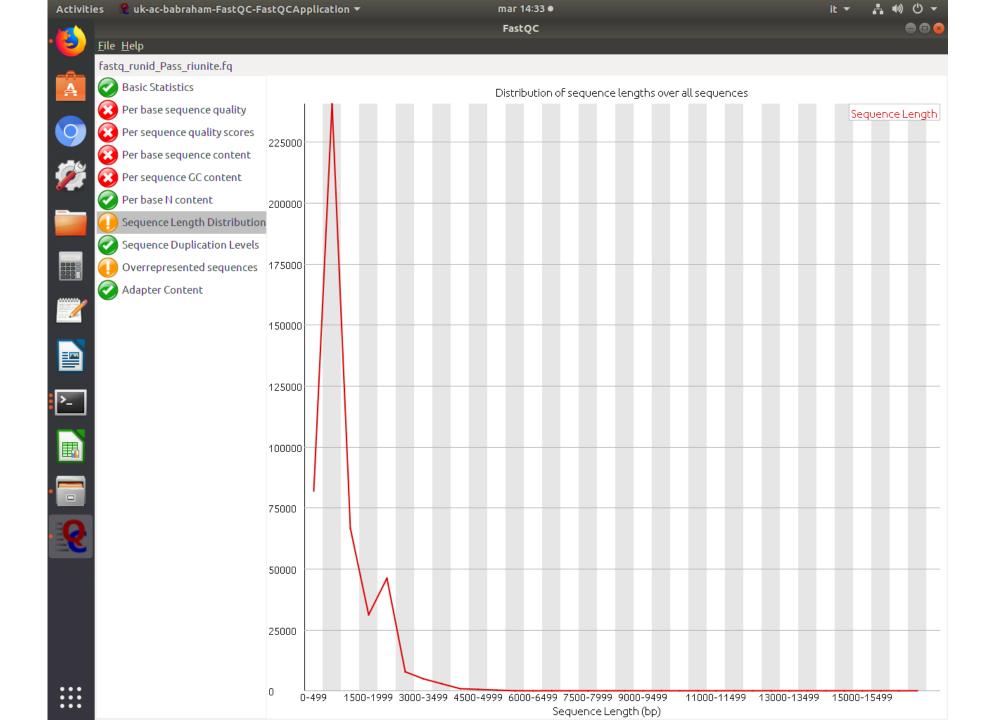


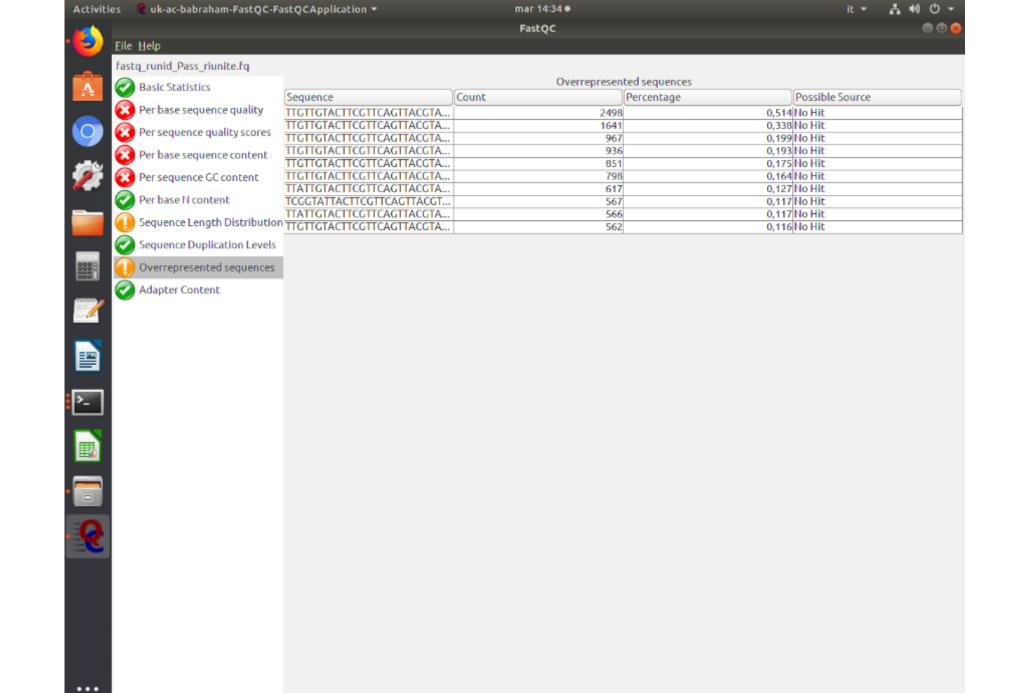


Quality scores across all bases (Illumina >v1.3 encoding)







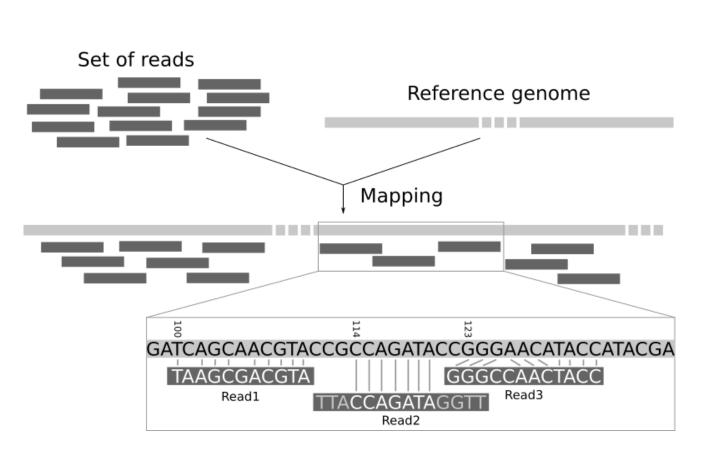


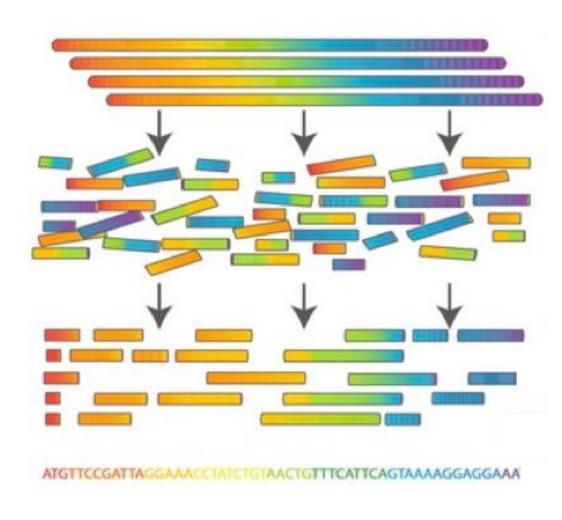
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Genome Recostruction

Mapping

De Novo Assemby



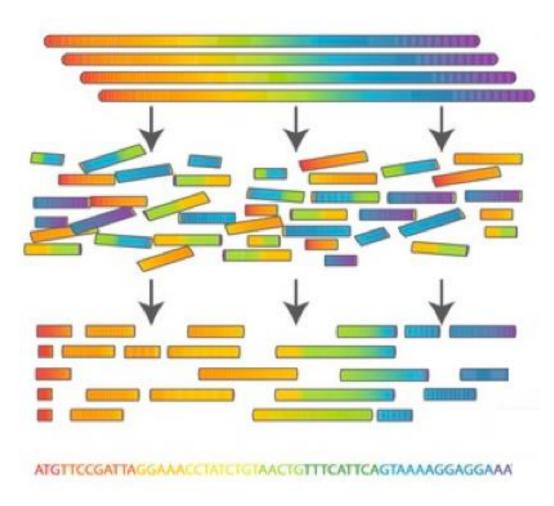


Mapping

Set of reads Reference genome Mapping Read3 Read1 Read2

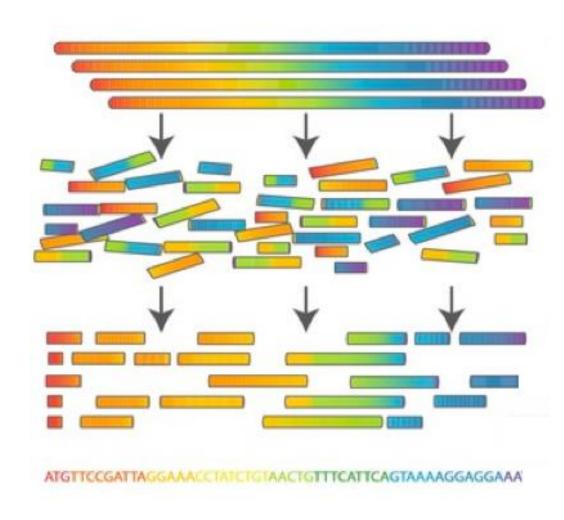
De Novo Assemby

Pre-correction (Canu)



Mapping Set of reads Reference genome Mapping TAAGCGACGTA Read3 Read1 Read2

De Novo Assemby

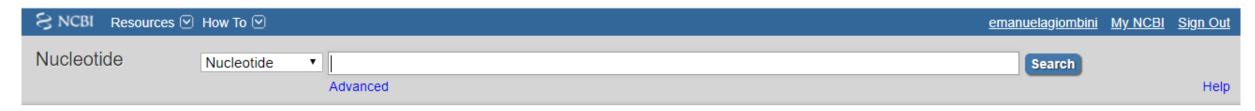


Identification of sequence reference

Literature
Standard reference
Bioinformatic analysis

Mapping Sequence nearest sequence our data

https://www.ncbi.nlm.nih.gov/nuccore





Nucleotide

The Nucleotide database is a collection of sequences from several sources, including GenBank, RefSeq, TPA and PDB. Genome, gene and transcript sequence data provide the foundation for biomedical research and discovery.

Using Nucleotide	Nucleotide Tools	Other Resources
Quick Start Guide	Submit to GenBank	GenBank Home
FAQ	<u>LinkOut</u>	RefSeq Home
<u>Help</u>	E-Utilities	Gene Home
GenBank FTP	BLAST	SRA Home
RefSeq FTP	Batch Entrez	INSDC

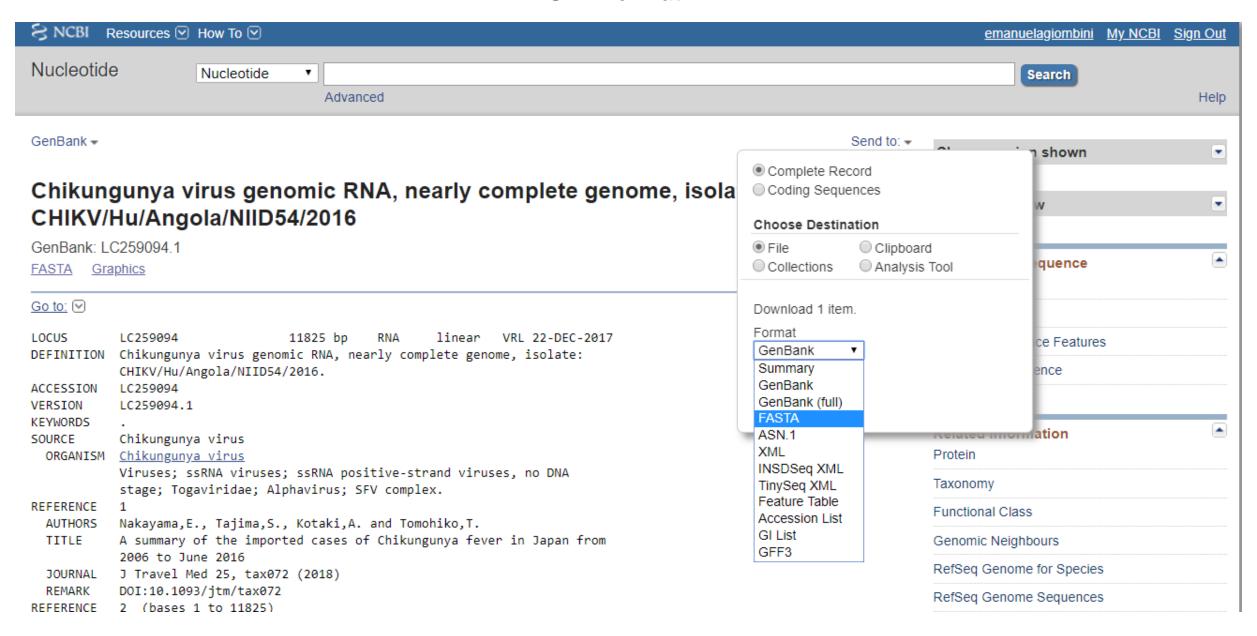


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FASTA Format

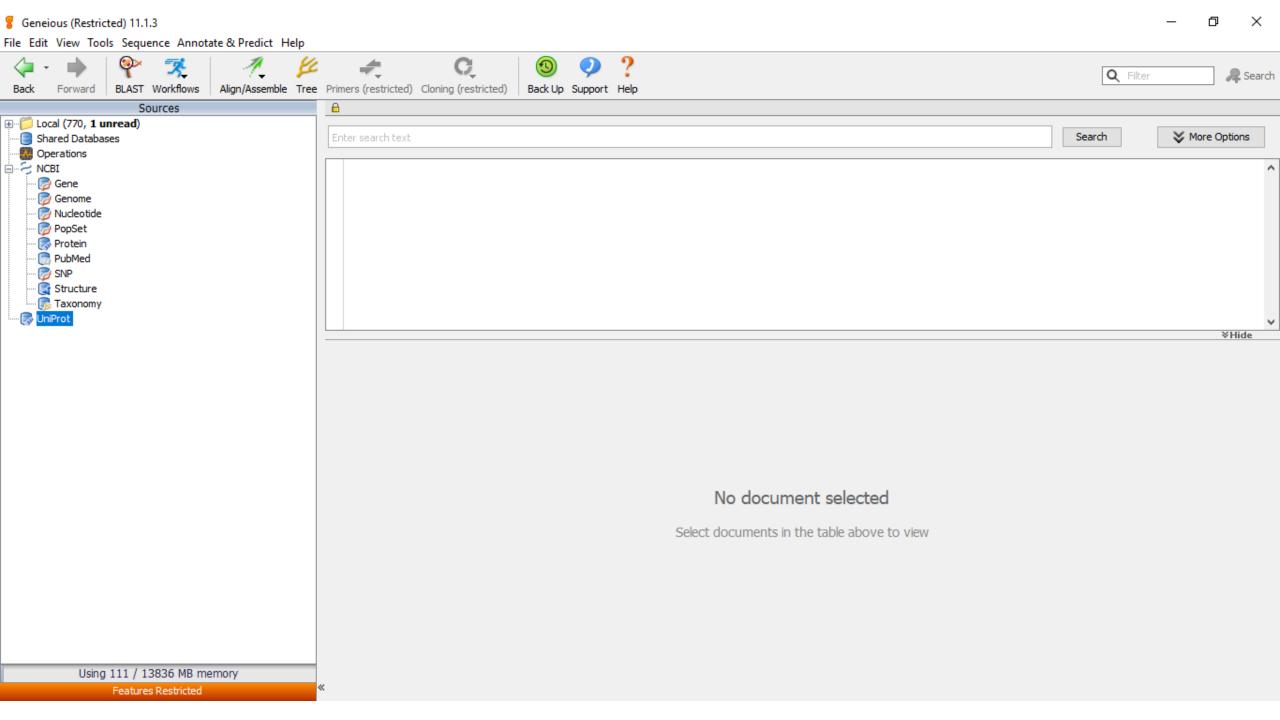


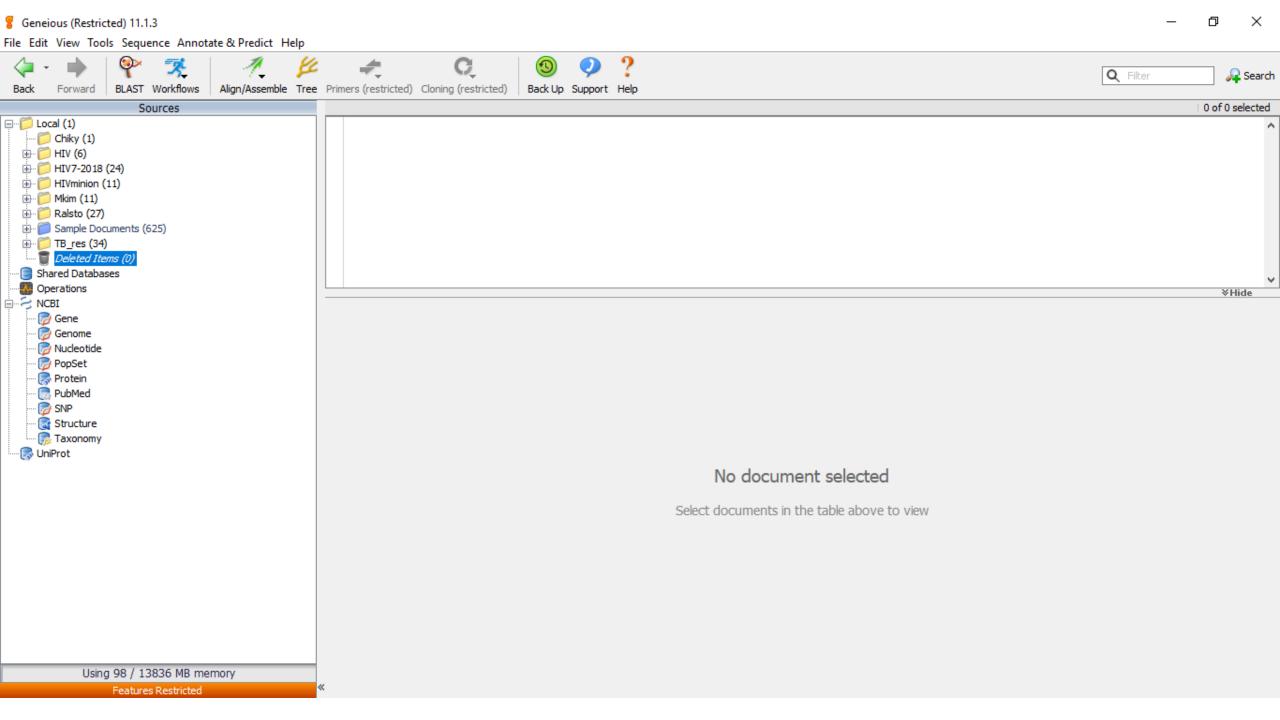
Alignment of reads on the reference

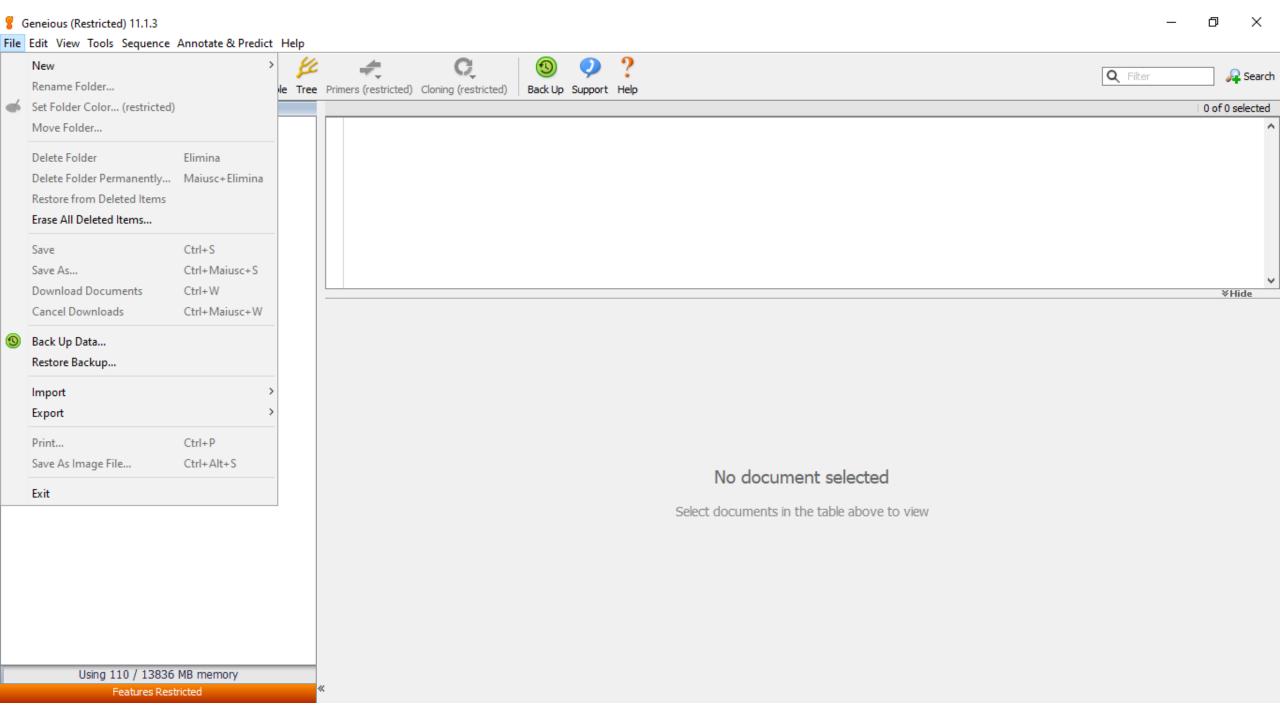


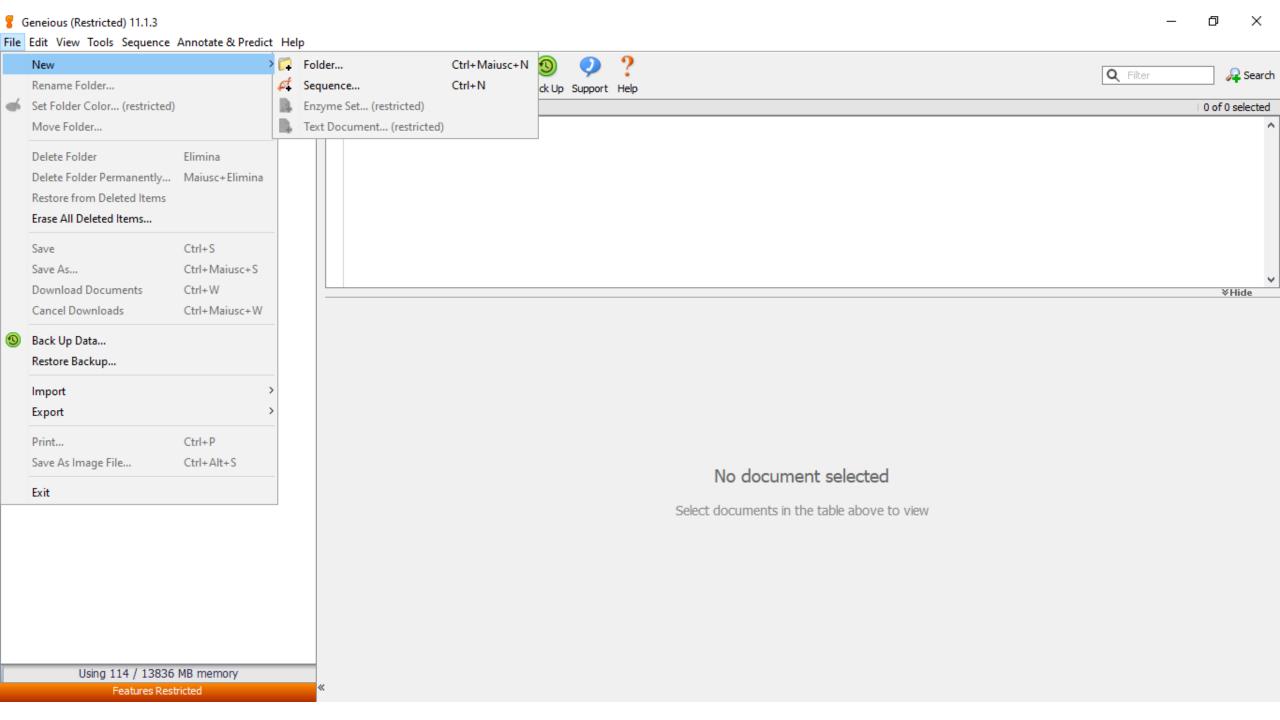
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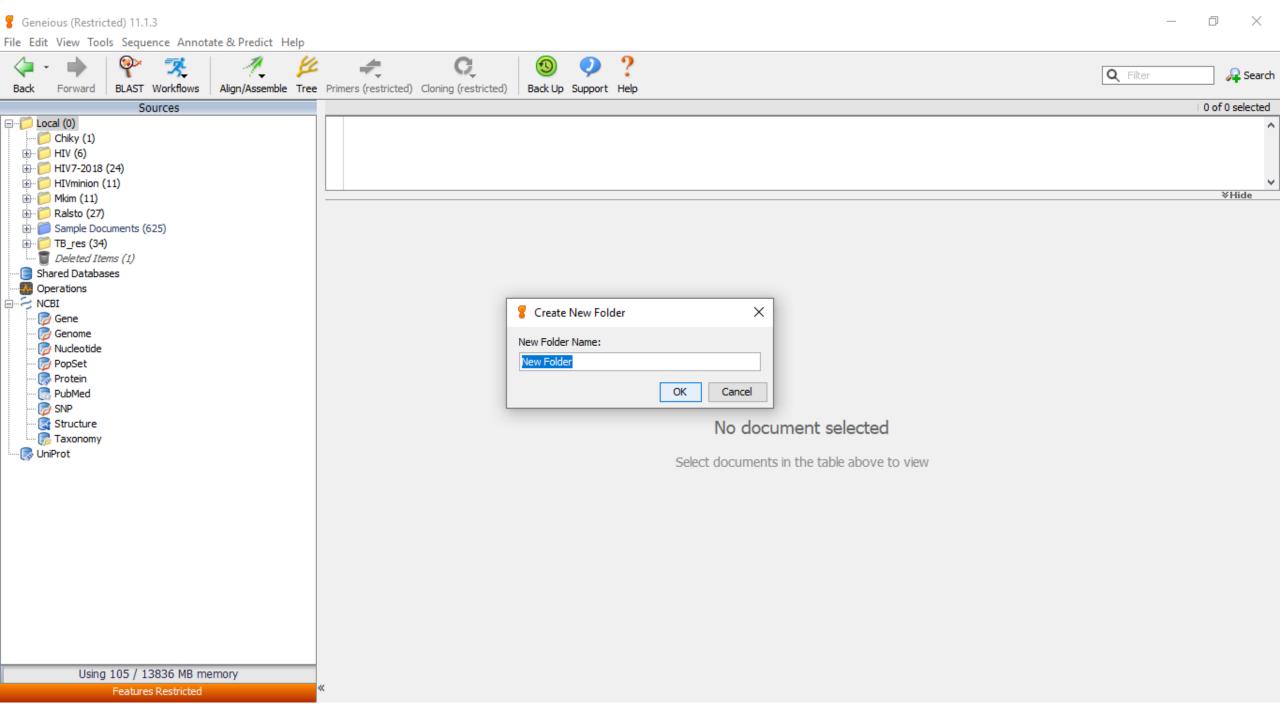
https://www.geneious.com/tutorials/

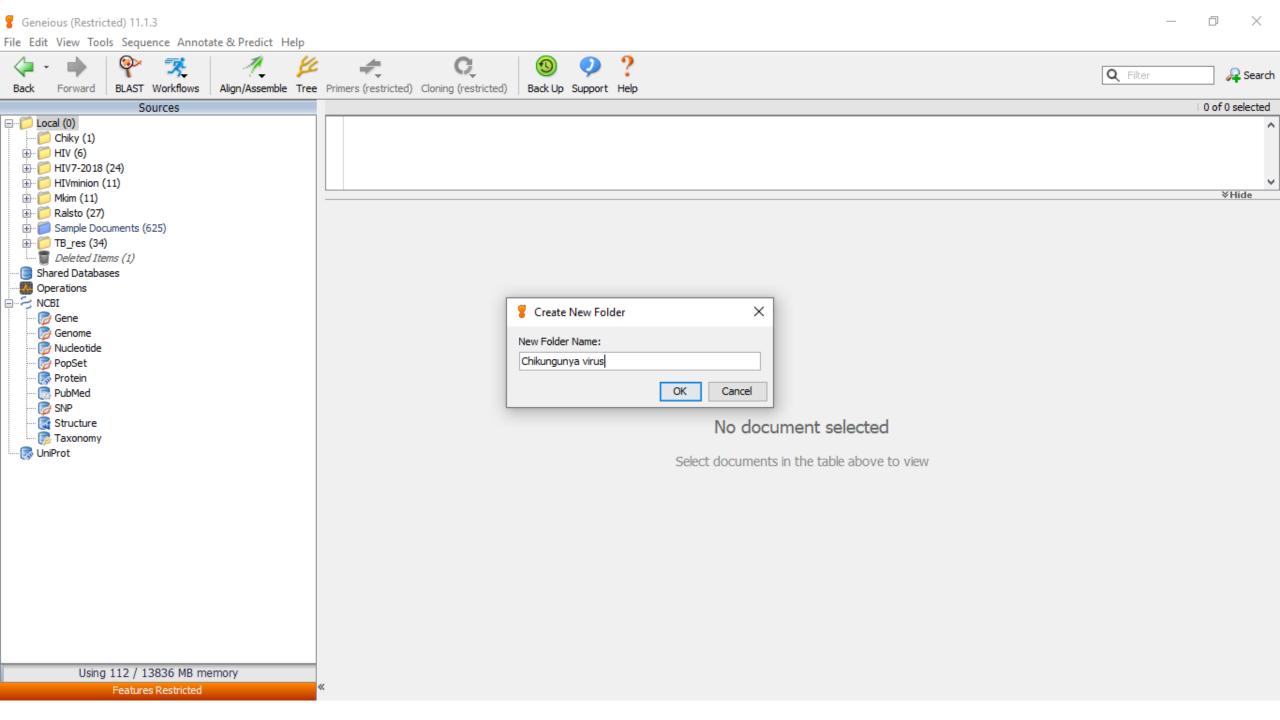


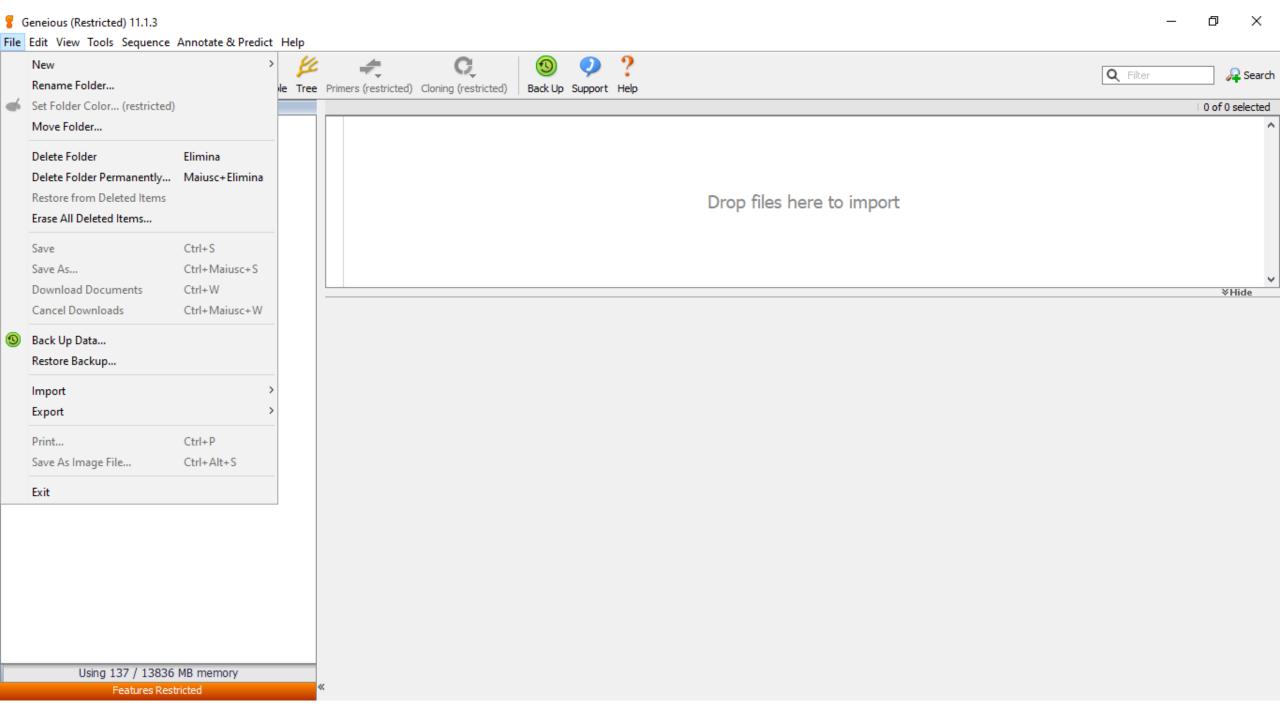


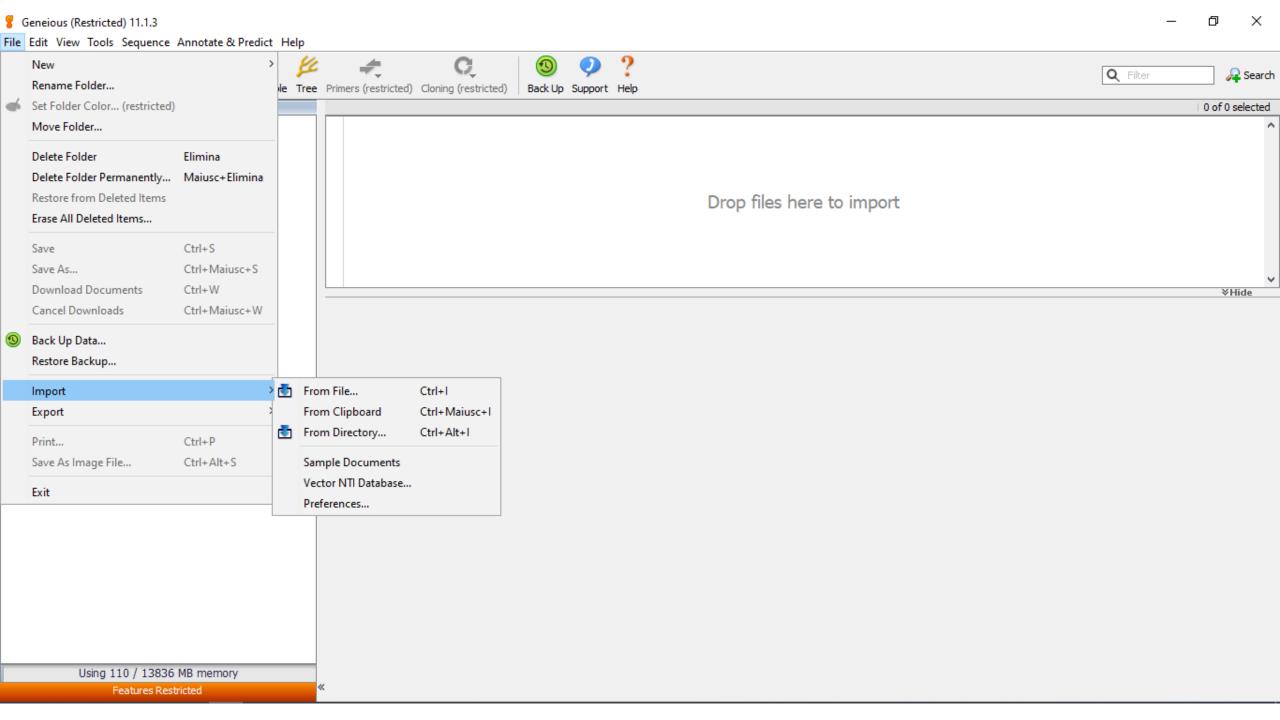


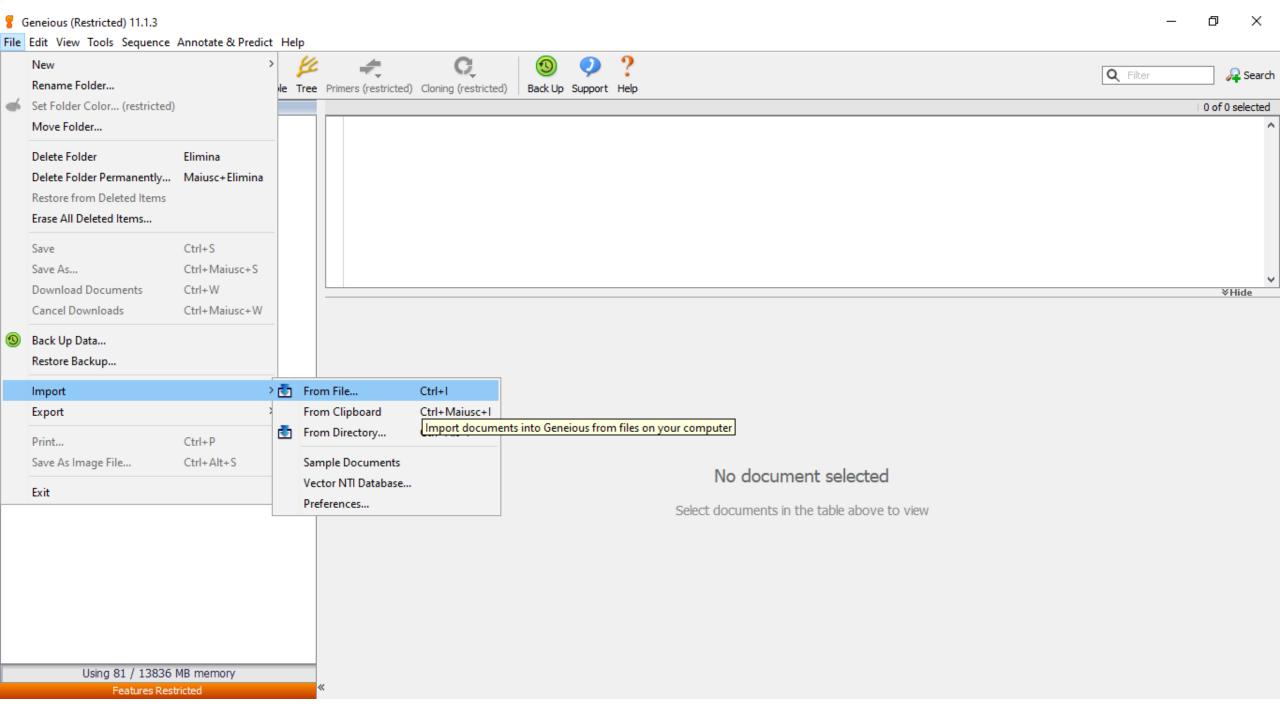


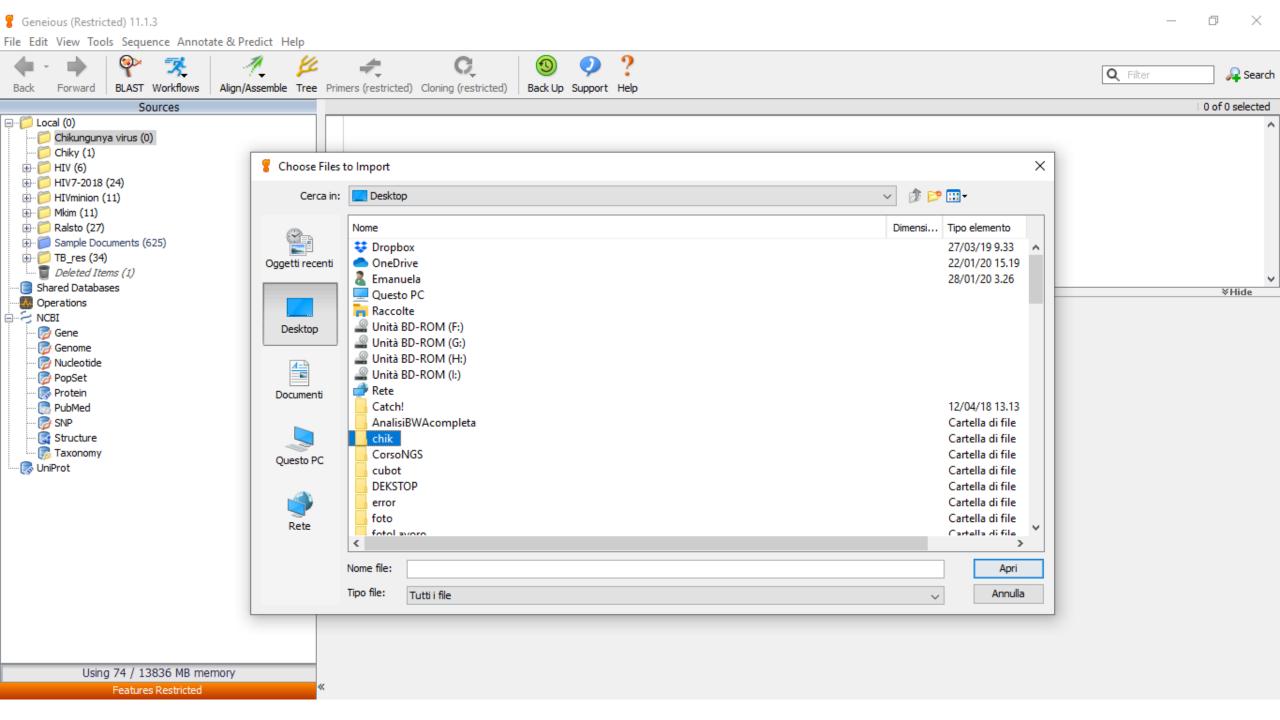


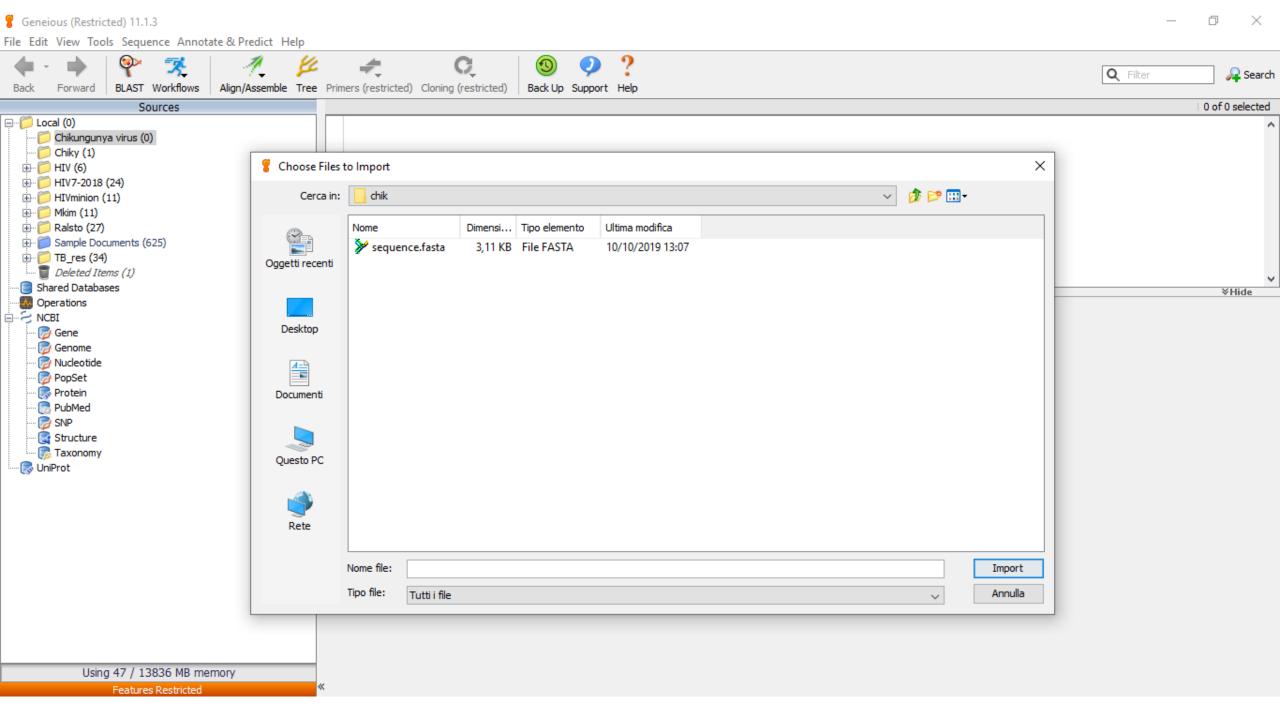










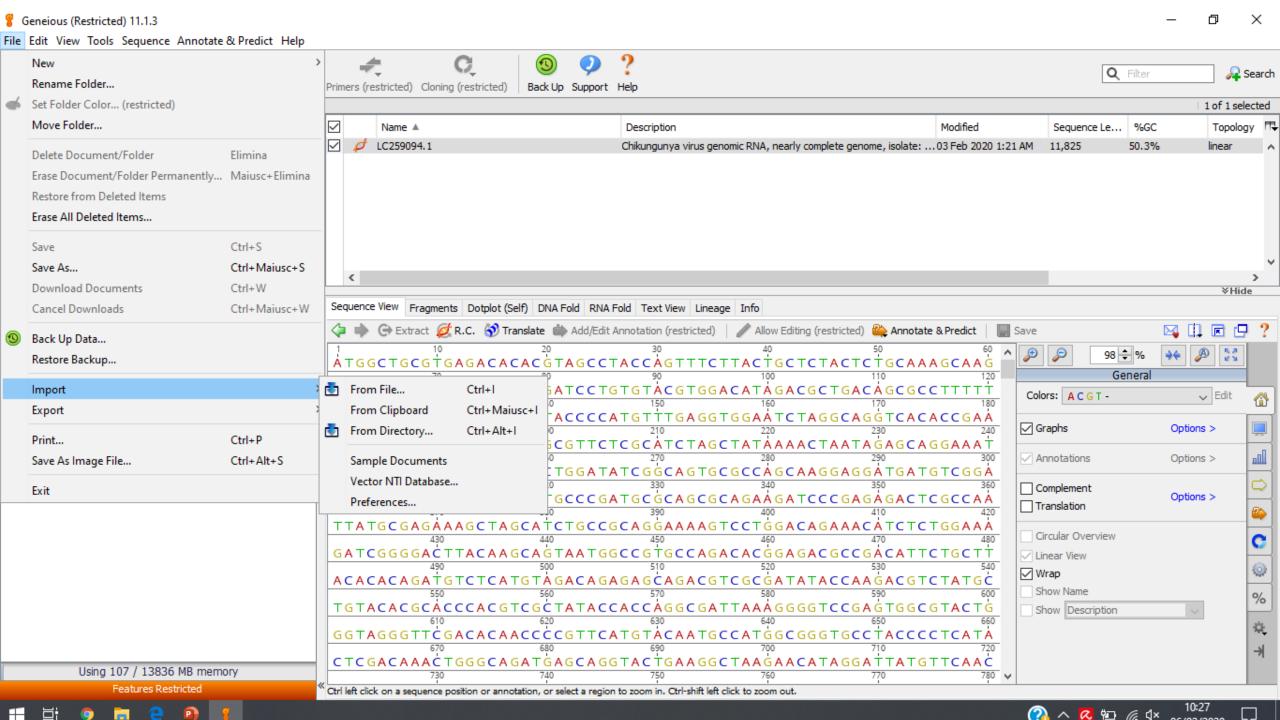


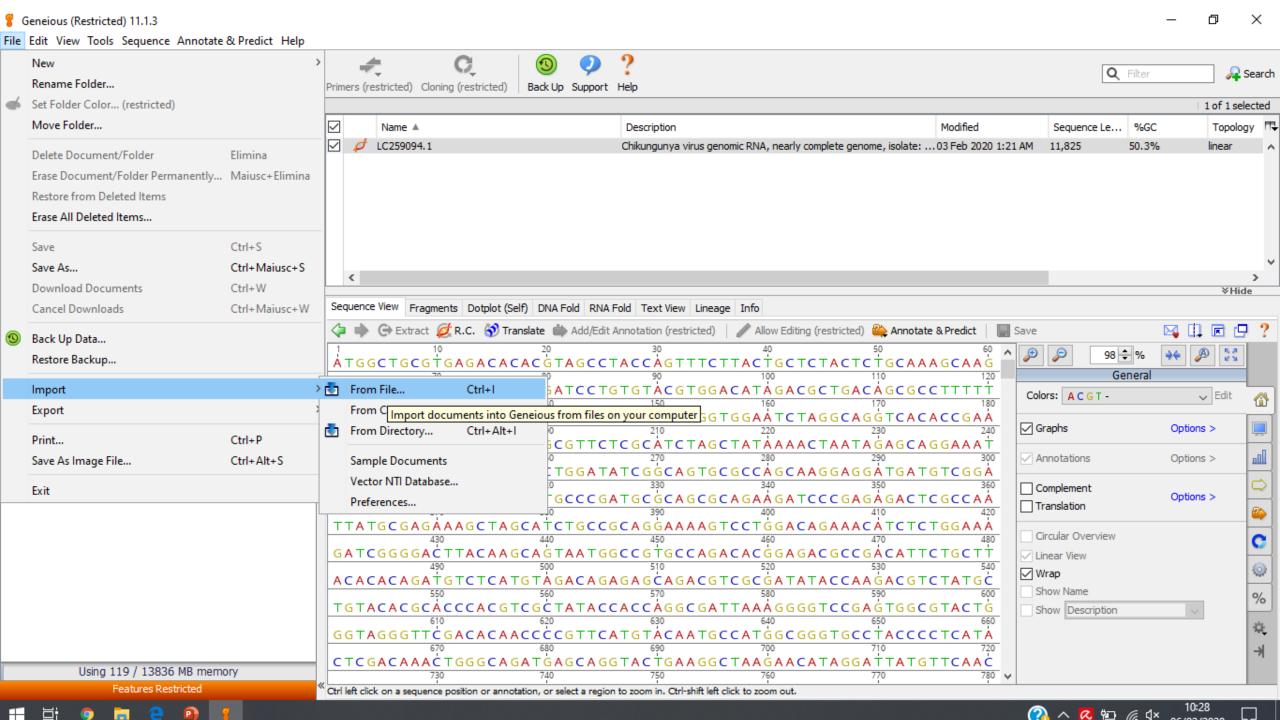
Geneious (Restricted) 11.1.3

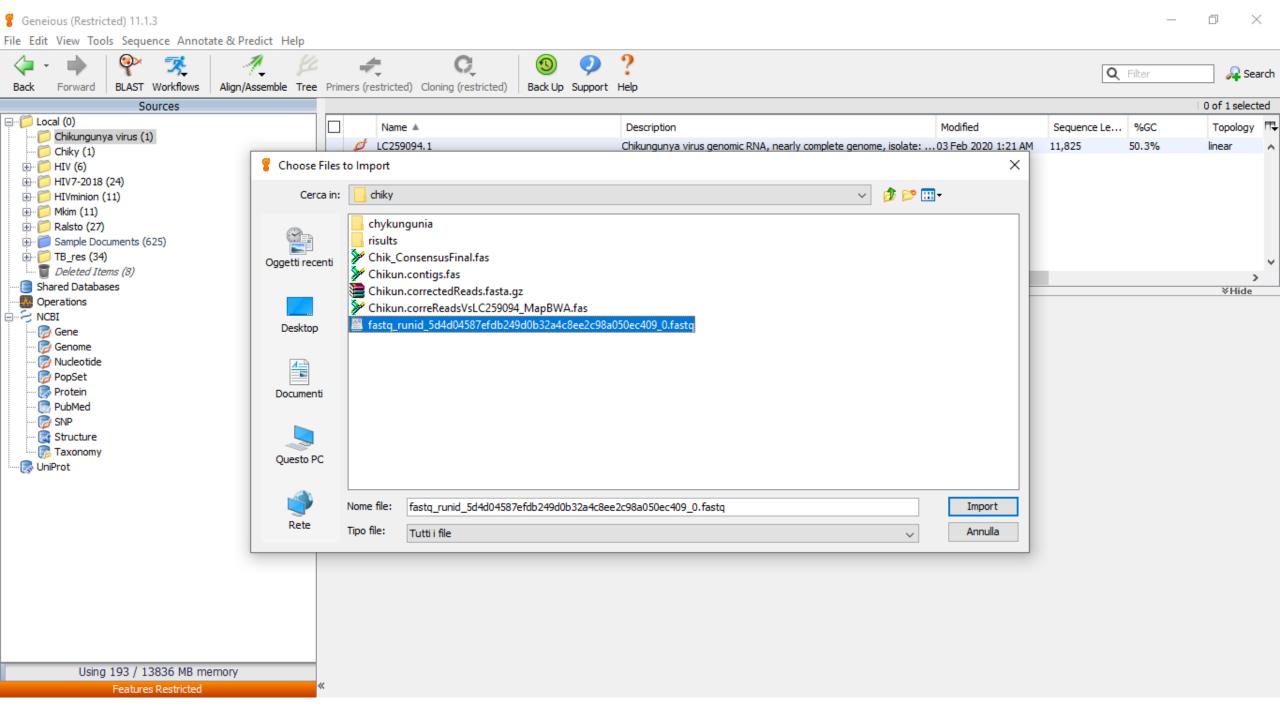
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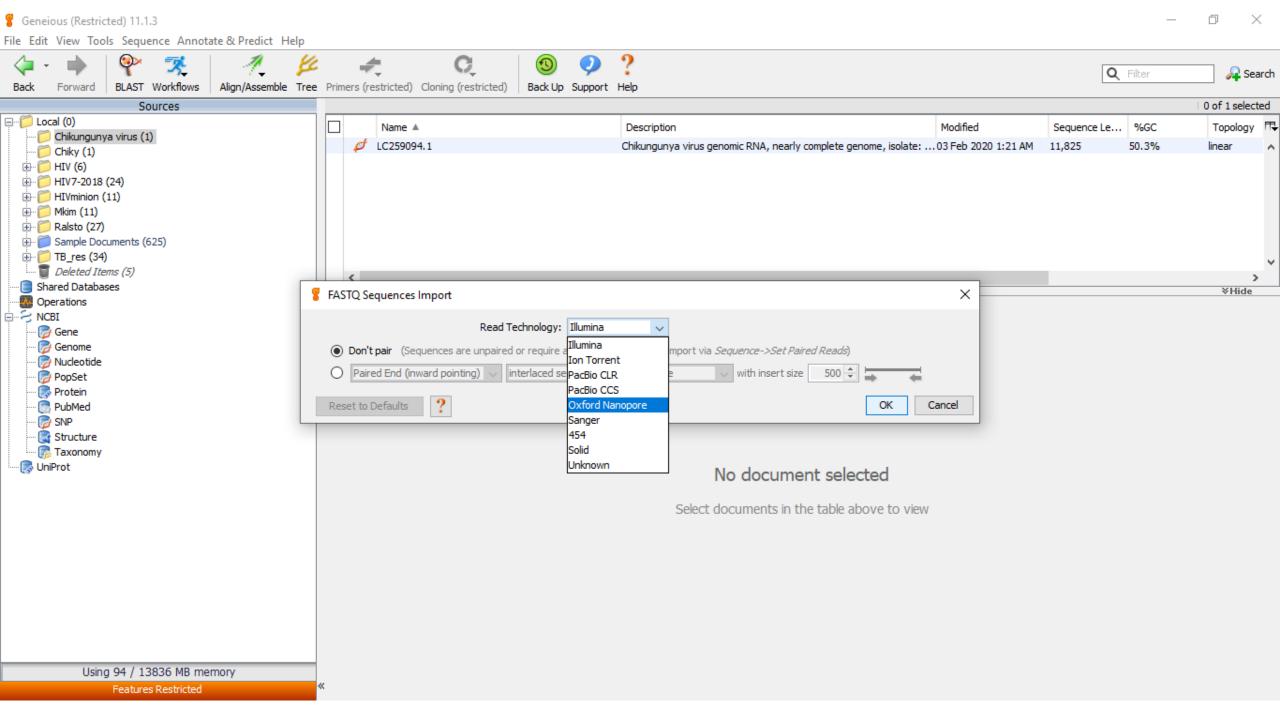
File Edit View Tools Sequence Annotate & Predict Help Q Filter Search BLAST Workflows Align/Assemble Tree Primers (restricted) Cloning (restricted) Back Up Support Help Sources 1 of 1 selected □··· Local (0) Modified Name A Description Seguence Le... %GC Topology Chikungunya virus (1) Chikungunya virus genomic RNA, nearly complete genome, isolate: ... 03 Feb 2020 1:21 AM 11,825 50.3% linear Chiky (1) HIVminion (11) Mkim (11) Ralsto (27) Deleted Items (5) < Shared Databases ¥Hide Operations Sequence View Fragments Dotplot (Self) DNA Fold RNA Fold Text View Lineage Info Extract R.C. Translate Add/Edit Annotation (restricted) · 🧺 Gene Allow Editing (restricted) Annotate & Predict Save Genome 98 🔷 % Nucleotide ATGGCTGCGTGAGACACACGTAGCCTACCAGTTTCTTACTGCTCTACTCTGCAAAGCAAG PopSet General Protein AGATTAAGAACCCATCATGGATCCTGTGTACGTGGACATAGACGCTGACAGCGCCTTTTT Colors: ACGT-√ Edit PubMed GAAGGCCCTGCAACGTGCGTACCCCATGTTTGAGGTGGAATCTAGGCAGGTCACACCGAA SNP ✓ Graphs Options > Structure · 🛜 Taxonomy Annotations Options > B UniProt TGATCCCGACTCAACCATCCTGGATATCGGCAGTGCGCCAGCAAGGAGGATGATGTCGGA \Leftrightarrow Complement CAGGAAGTACCACTGCGTCTGCCCGATGCGCAGCGCAGAAGATCCCGAGAGACTCGCCAA Options > Translation TTATGCGAGAAAGCTAGCATCTGCCGCAGGAAAAGTCCTGGACAGAAACATCTCTGGAAA Circular Overview GATCGGGGACTTACAAGCAGTAATGGCCGTGCCAGACACGGGAGACGCCGACATTCTGCTT ✓ Linear View ✓ Wrap ACACACAGATGTCTCATGTAGACAGAGAGCGTCGCGATATACCAAGACGTCTATGC Show Name % TGTACACGCACCCACGTCGCTATACCACCAGGCGATTAAAGGGGTCCGAGTGGCGTACTG Show Description GGTAGGGTTCGACACACCCCGTTCATGTACAATGCCATGGCGGGTGCCTACCCCTCATA CTCGACAAACTGGGCAGATGAGCAGGTACTGAAGGCTAAGAACATAGGATTATGTTCAAC Using 70 / 13836 MB memory Features Restricted Ctrl left click on a sequence position or annotation, or select a region to zoom in. Ctrl-shift left click to zoom out.

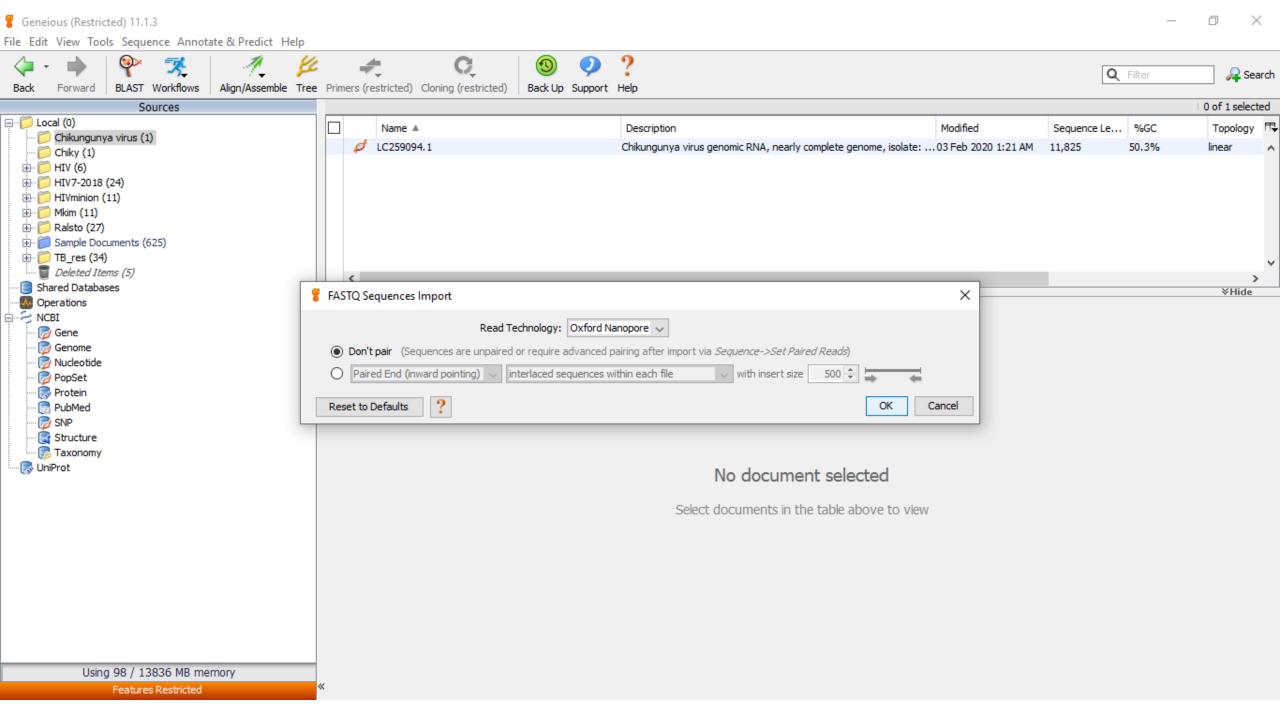
Geneious (Restricted) 11.1.3

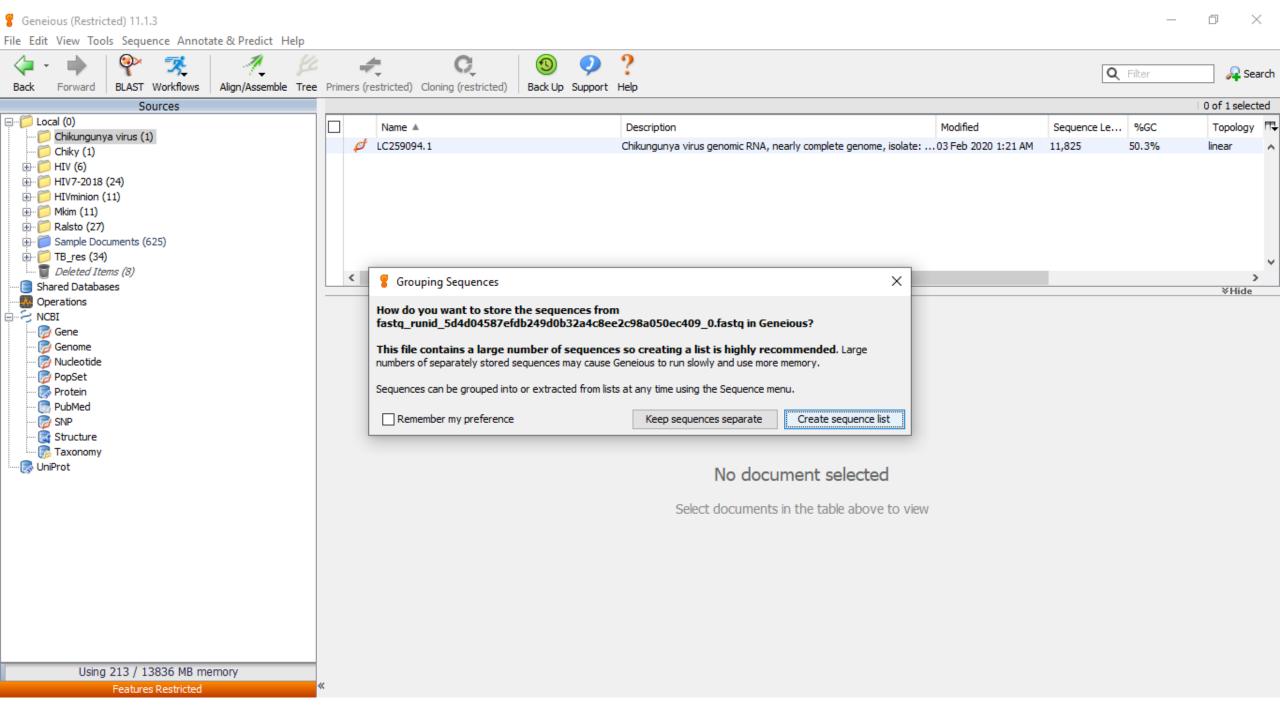


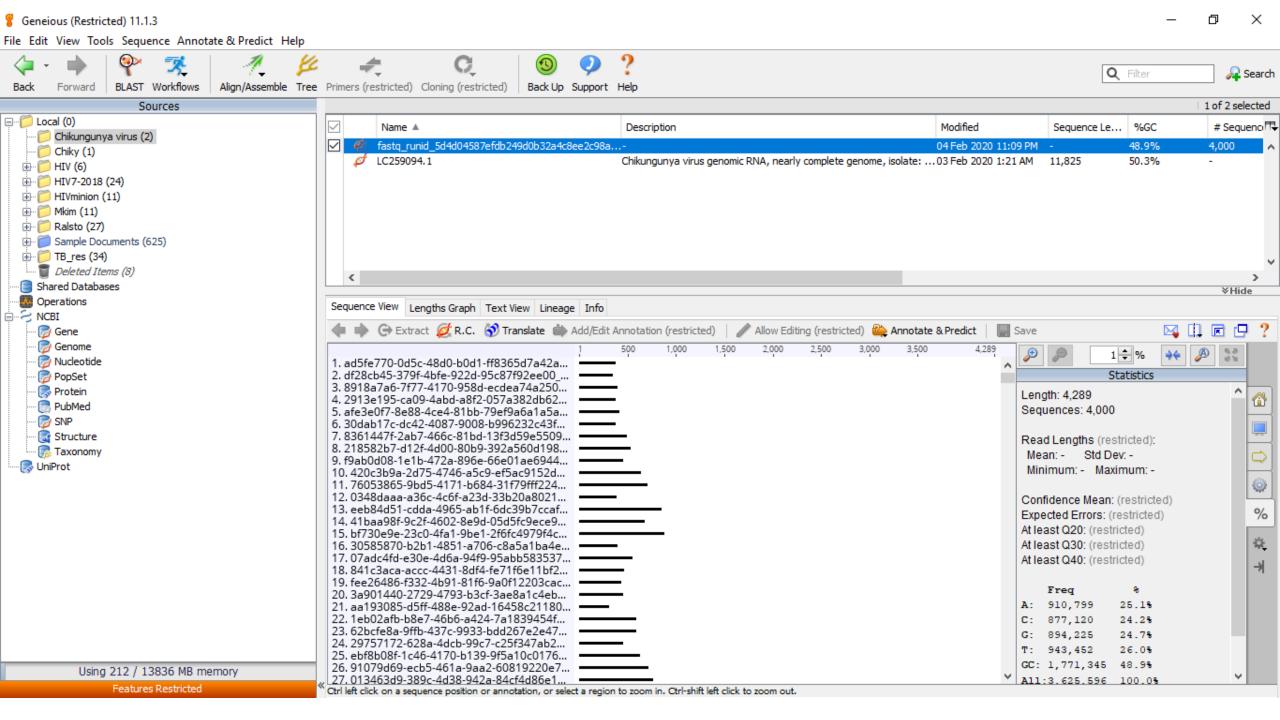


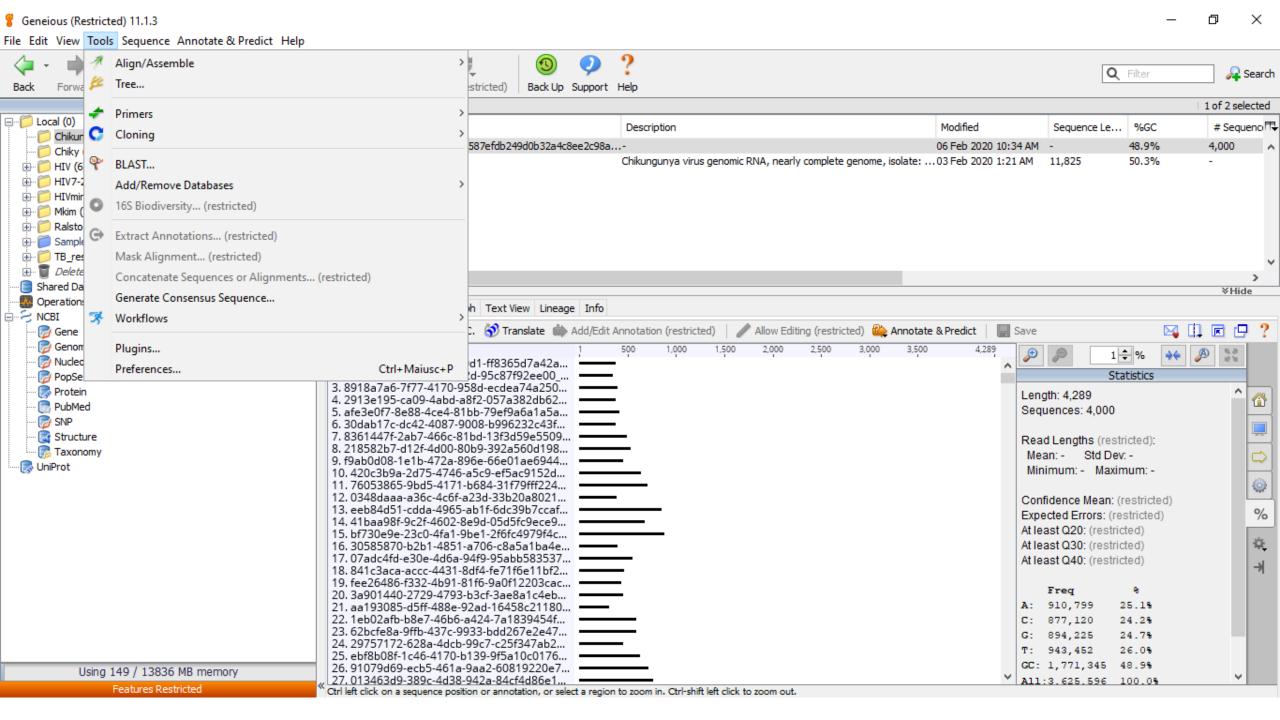


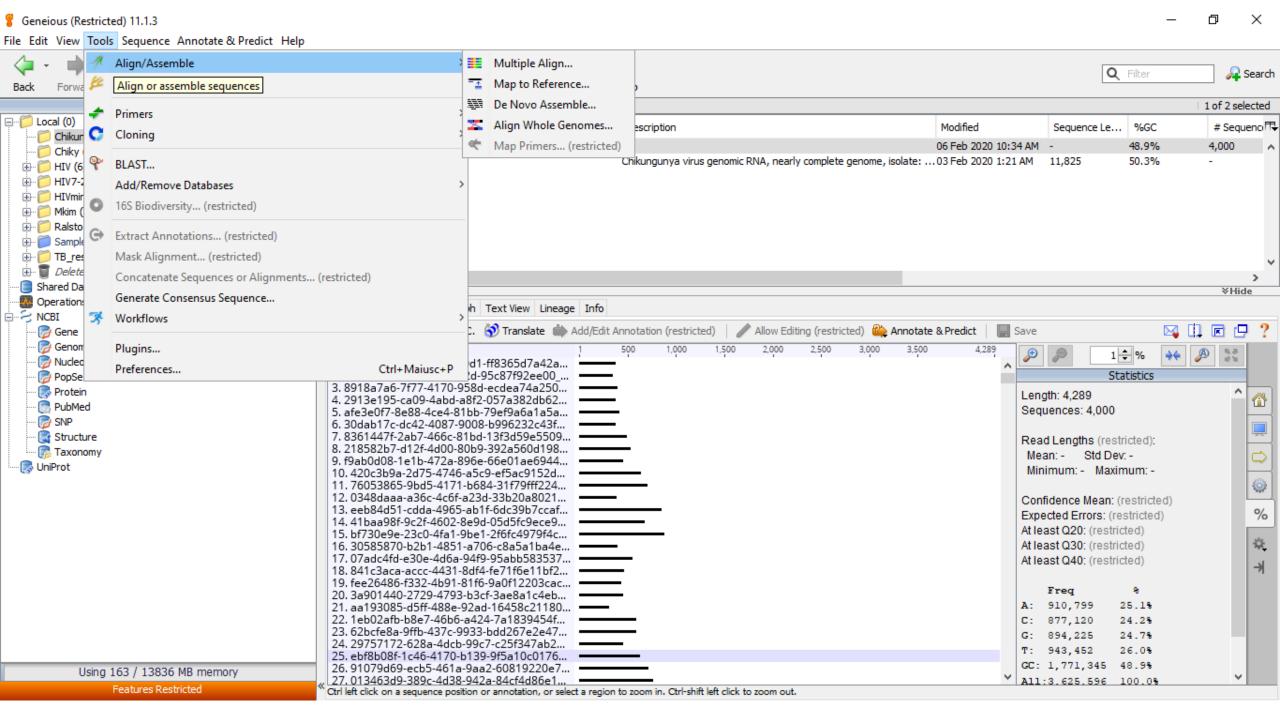


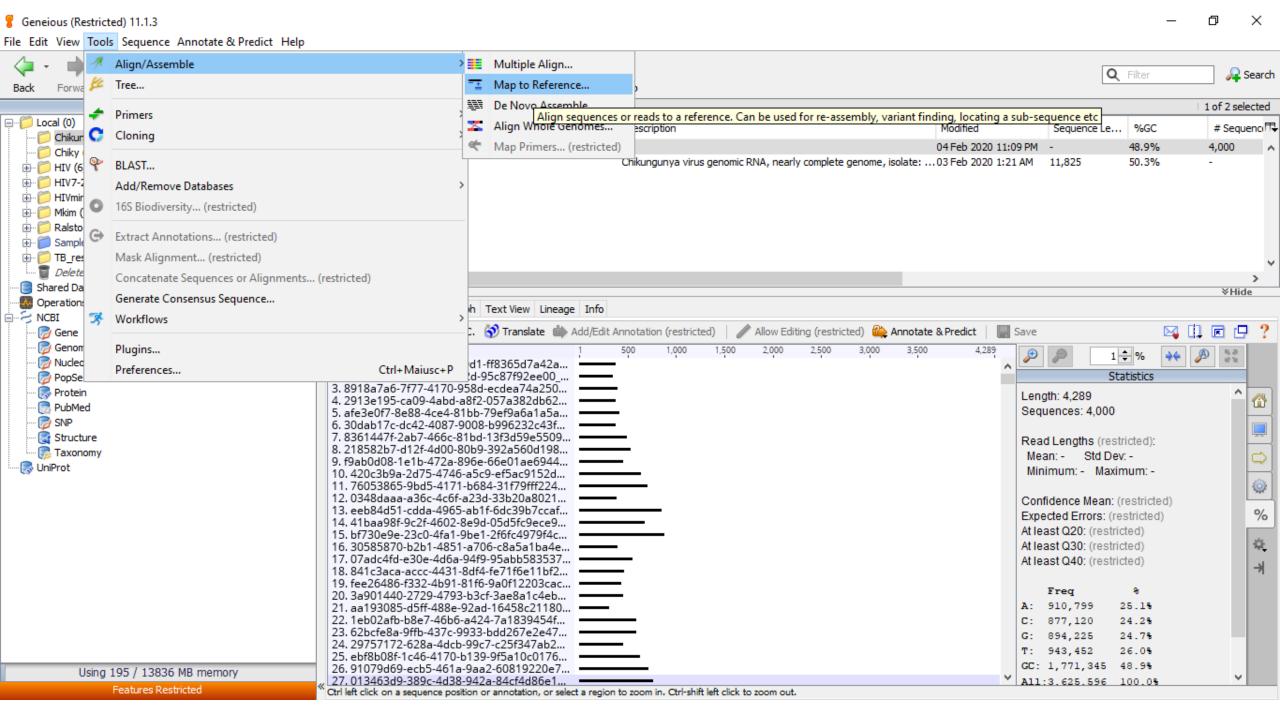












Geneious (Restricted) 11.1.3 File Edit View Tools Sequence Annotate & Predict Help Map to Reference 🛺 Search Data Align/Assemble BLAST Workflows Reference Sequence: None ▼ Choose... Sources 1 of 2 selected IsolatoTNscaffsConsensuCorr -consensus will be mapped to LC259094 □··· Local (0) # Seguenci 🖫 Chikungunya virus (2) Assemble by: 1st = part of name, separated by er). · Chiky (1) g Assemble each sequence list separately ⊞ (6) Method Mapper: Geneious ⊕ □ TB res (34) Deleted Items (5) Sensitivity: Highest Sensitivity / Slow Shared Databases ¥Hide Find structural variants, short insertions, and deletions of any size ? Operations Ė... € NCBI Find short insertions and large deletions up to 1,000 🖪 🗗 ? · 🧺 Gene Fine Tuning: None (fast / read mapping) Genome 16.20 Nucleotide Memory Required: 24 MB of 122 GB PopSet Note: Paired reads can be set up or changed using Sequence > Set Paired Reads Protein ∠ Edit PubMed · 🧺 SNP Structure Trim Before Mapping Results · 🔂 Taxonomy · 🔜 UniProt Assembly Name {Reads Name} assembled to {Reference Name} \Leftrightarrow Save assembly report Use existing trim regions 0 Save list of unused reads Remove existing trim regions from sequences Save list of used reads Include mates O Trim sequences Options Save in sub-folder Do not trim Save contigs Save consensus sequences Options Cancel ZJ. 1ZU47077*0434*43€0*DC00*D1Z0C300U7.. 26. e34d26bf-ed7c-49db-9092-ee049ab8fae... Using 131 / 13836 MB memory 27. 663ee3c1-ad7e-41f3-95fc-7bb8d074609... Features Restricted Otrl left click on a sequence position or annotation, or select a region to zoom in. Otrl-shift left click to zoom out.

Geneious (Restricted) 11.1.3 File Edit View Tools Sequence Annotate & Predict Help Map to Reference Q Filter 🛺 Search BLAST Workflows Select Nucleotide Sequences Forward Alig X 1 of 2 selected Sources Cocal Q Filter Subfolders not included. # Sequenci 🖫 %GC Seguence Le... Chikungunya virus Chikungunya virus (2) Name ▲ Sequence Length # Sequences Organism Description Chiky 49.7% 214 ... Chiky (1) Chikun . Reads 214 ı. i HIV 11,825 50.3% Ø LC259094.1 11825 Chikungunya viru... ⊕ ∏ HIVminion HIVminion (11) ⊞. Mkim ⊕ · i Ralsto ± Sample Documents (625) ⊞... 🎁 TB_res ⊞ TB_res (34) Deleted Items (5) Shared Databases ¥Hide · Operations ii... S NCBI 🖂 🗓 🖪 🗗 ? · 房 Gene · 房 Genome 0 💠 % Nucleotide PopSet General Rrotein ors: ACGT-↓ Edit PubMed · 🧺 SNP raphs Options > Structure · 🔂 Taxonomy nnotations Options > ... 🔜 UniProt \Leftrightarrow pmplement Options > anslation 0 rcular Overview % 贷 how Names now Description V how sequence numbers Select Use Folder Cancel More Options Cancel 25. 12d49699-8434-43e8-bc00-b120c306d9... 26. e34d26bf-ed7c-49db-9092-ee049ab8fae... Using 146 / 13836 MB memory 27. 663ee3c1-ad7e-41f3-95fc-7bb8d074609... -Features Restricted Ctrl left click on a sequence position or annotation, or select a region to zoom in. Ctrl-shift left click to zoom out.

Geneious (Restricted) 11.1.3 File Edit View Tools Sequence Annotate & Predict Help Map to Reference Search Data BLAST Workflows Align/Assemble Forward Reference Sequence: LC259094.1 - Chikungunya virus 💌 Choose... 📍 Sources 1 of 2 selected IsolatoTNscaffsConsensuCorr -consensus will be mapped to LC259094 # Seguenci 📆 Chikungunya virus (2) Assemble by: 1st - part of name, separated by 214 · 🦲 Chiky (1) Assemble each sequence list separately Method ⊞ ∏ HIVminion (11) Mapper: Geneious <u>+</u> TB_res (34) Deleted Items (5) Sensitivity: Highest Sensitivity / Slow Shared Databases ¥Hide Find structural variants, short insertions, and deletions of any size ? Operations Ė...≲ NCBI Find short insertions and large deletions up to 1,000 🗷 🗗 ? · 🕝 Gene Fine Tuning: None (fast / read mapping) Genome Mudeotide Memory Required: 24 MB of 122 GB PopSet Protein Note: Paired reads can be set up or changed using Sequence > Set Paired Reads Edit PubMed · 🛜 SNP · 🛜 Structure Trim Before Mapping Results-· 🛜 Taxonomy Assembly Name {Reads Name} assembled to {Reference Name} · 🔜 UniProt Save assembly report Use existing trim regions 0 Save list of unused reads Remove existing trim regions from sequences Save list of used reads Include mates O Trim sequences Options Save in sub-folder Do not trim Save contigs Save consensus sequences Options OK Cancel 26. e34d26bf-ed7c-49db-9092-ee049ab8fae... Using 141 / 13836 MB memory 27. 663ee3c1-ad7e-41f3-95fc-7bb8d074609... Features Restricted Otrl left click on a sequence position or annotation, or select a region to zoom in. Otrl-shift left click to zoom out.

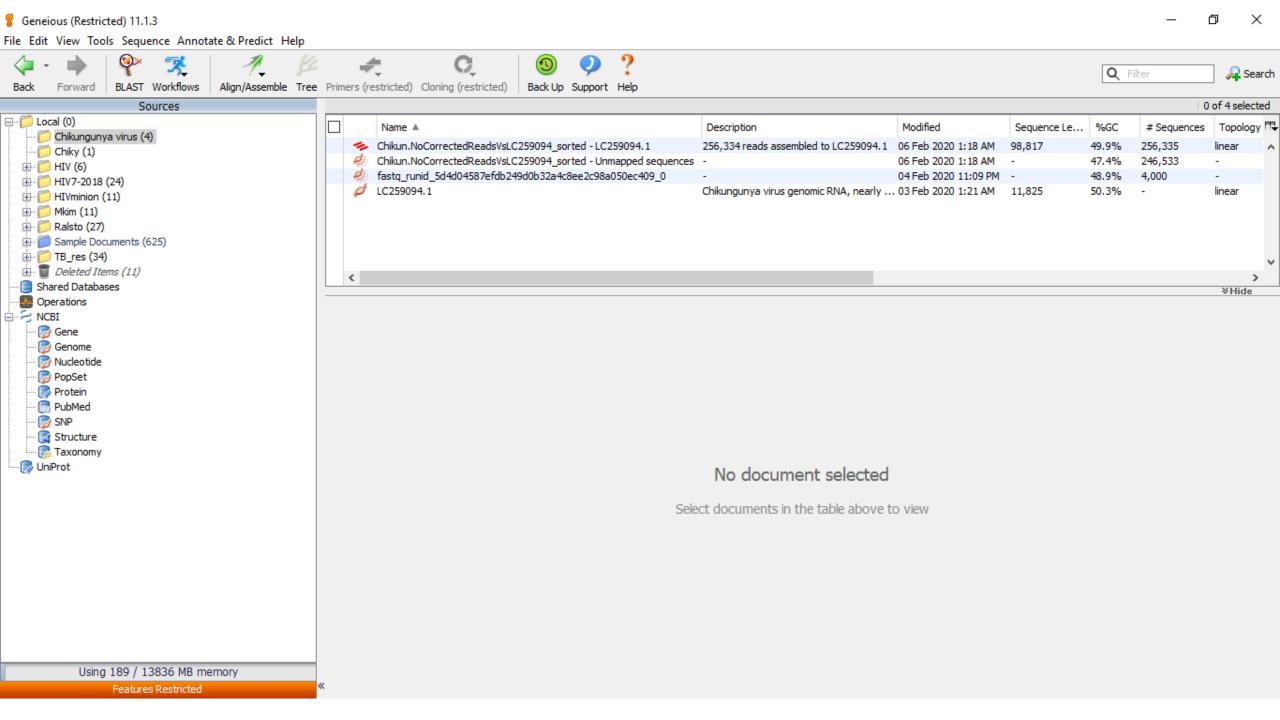
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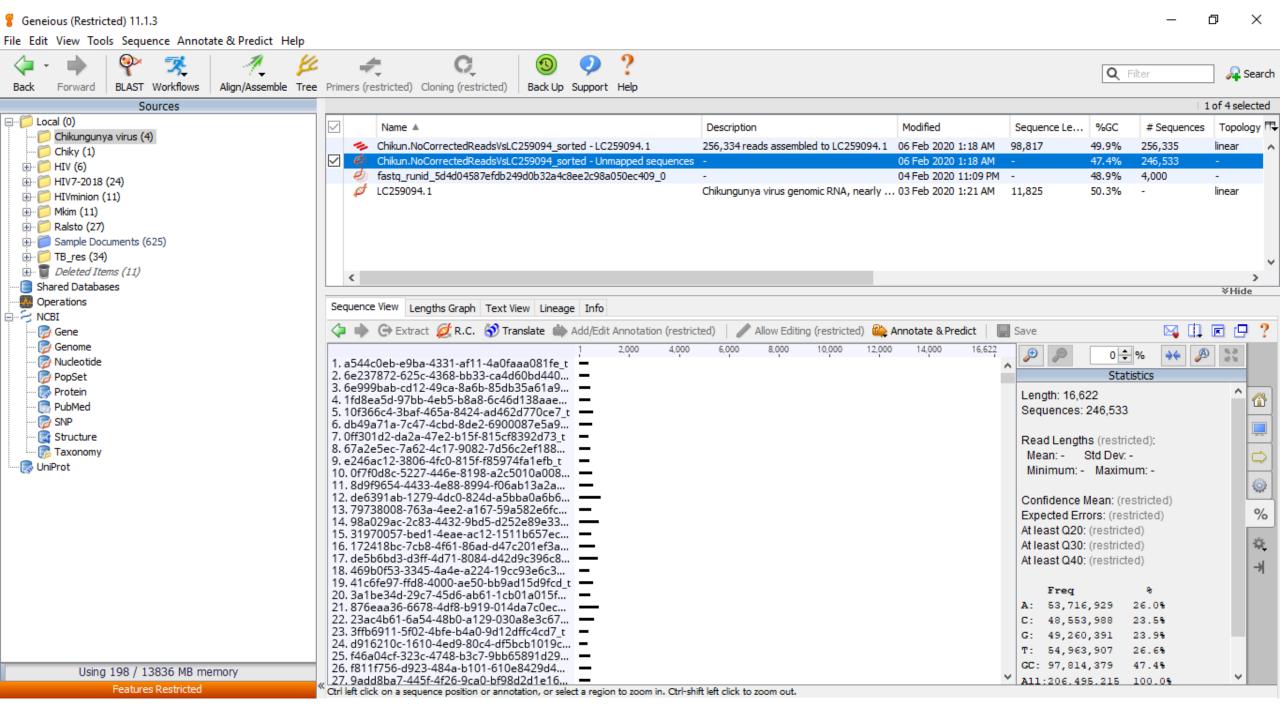
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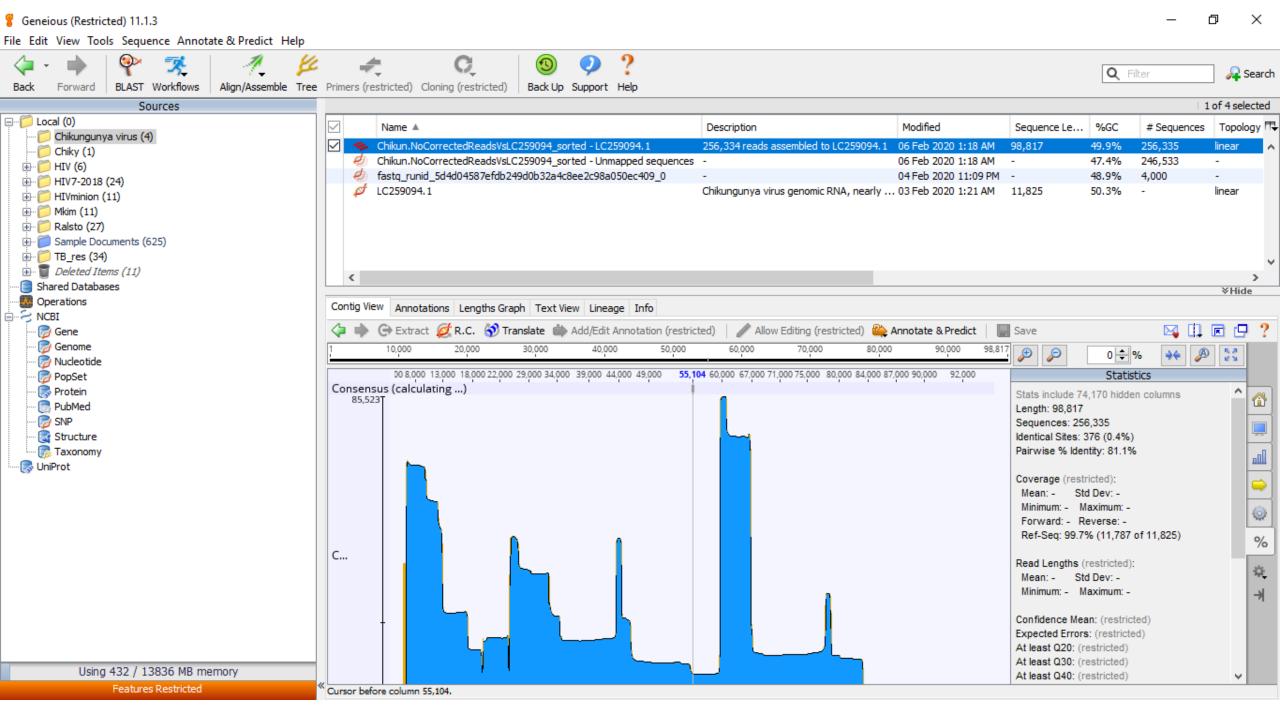


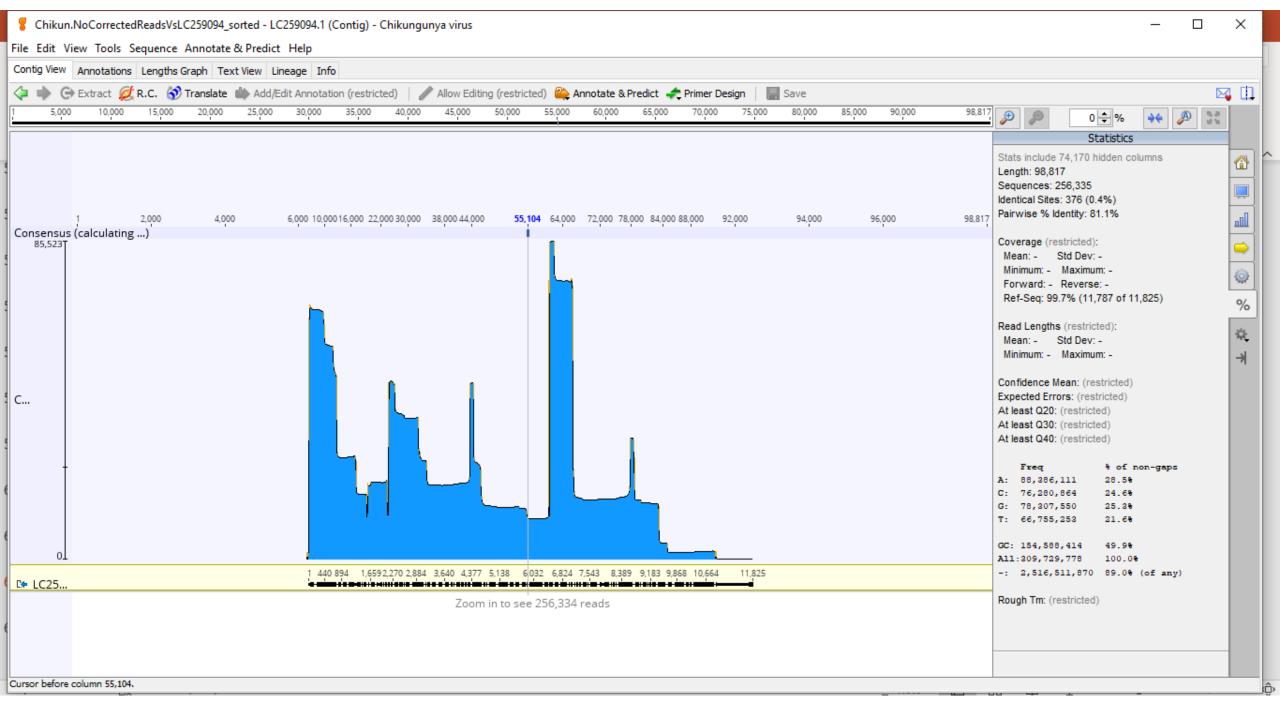
- Type of computer (laptop, workstation, server...)
- Number of the reads
- Length of the reads
- Number and type of the CPU
- Type of computer processor

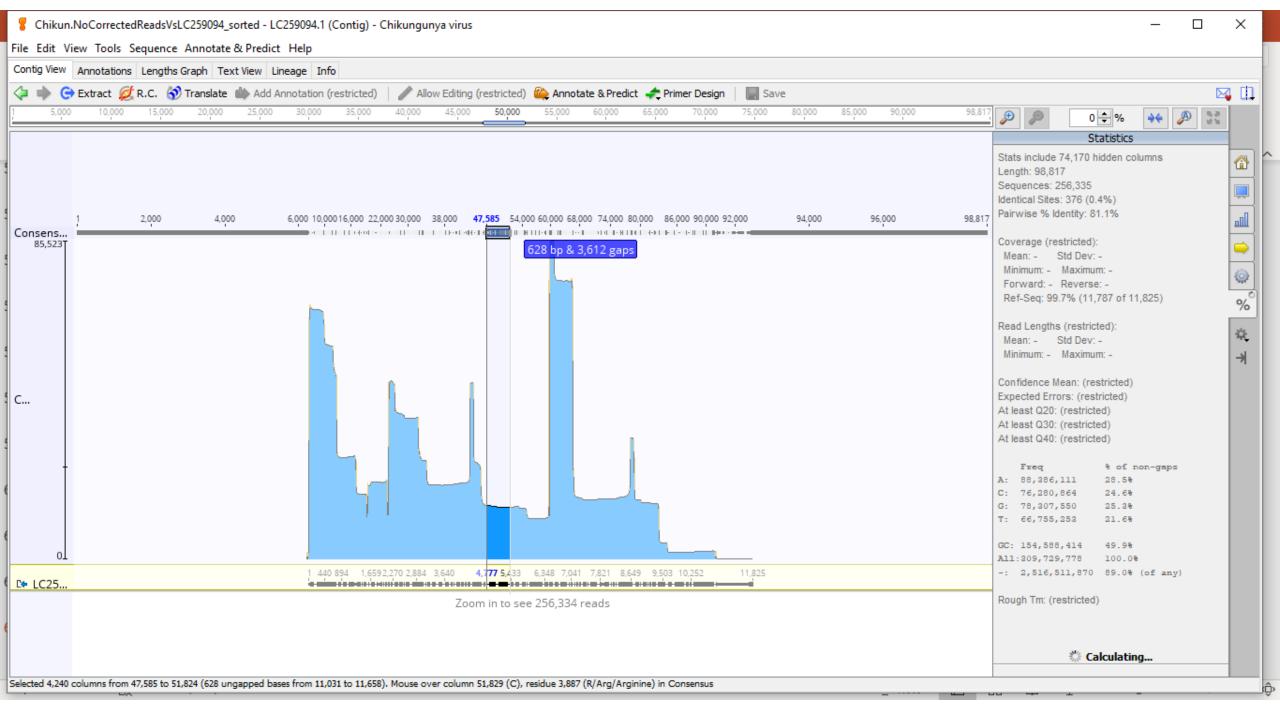
At the end....

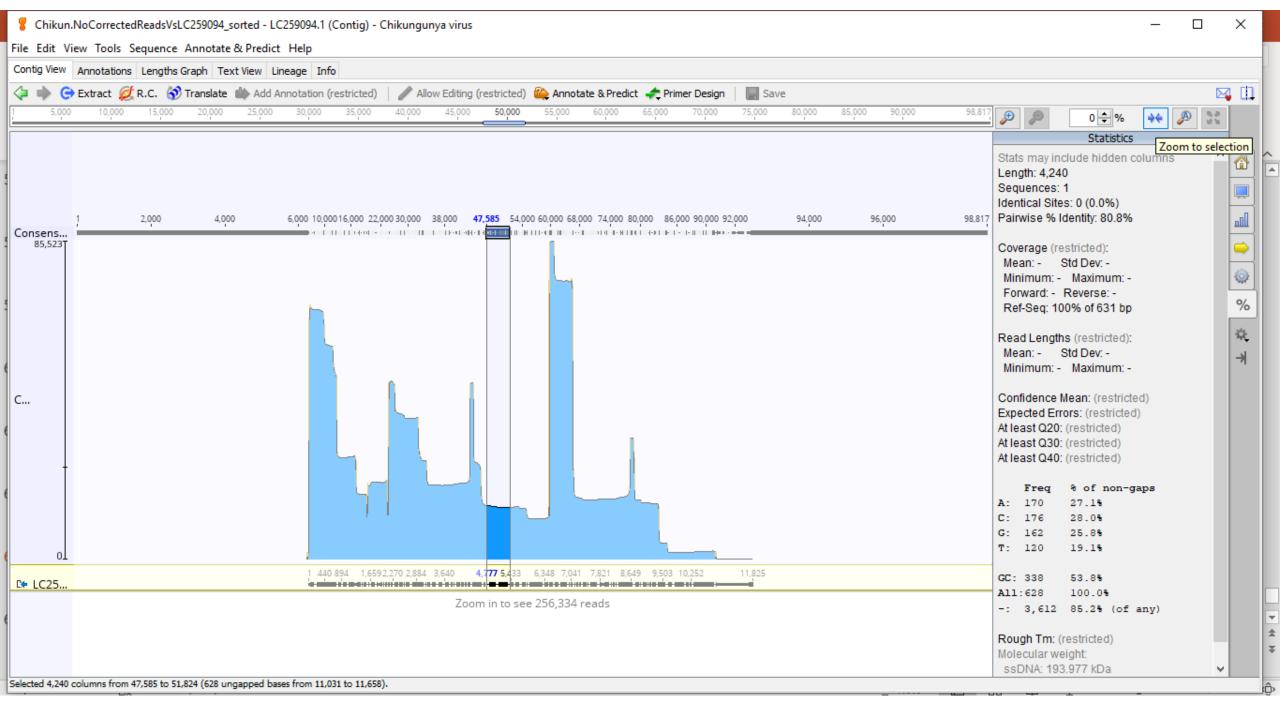


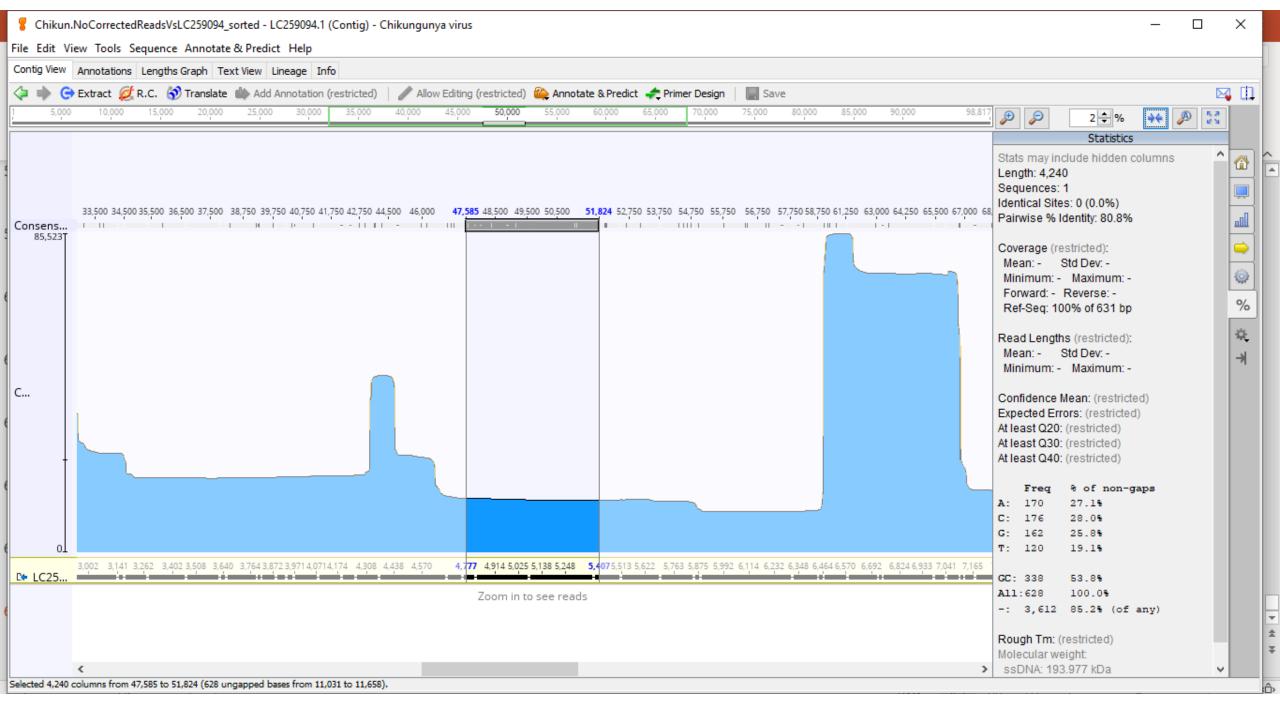


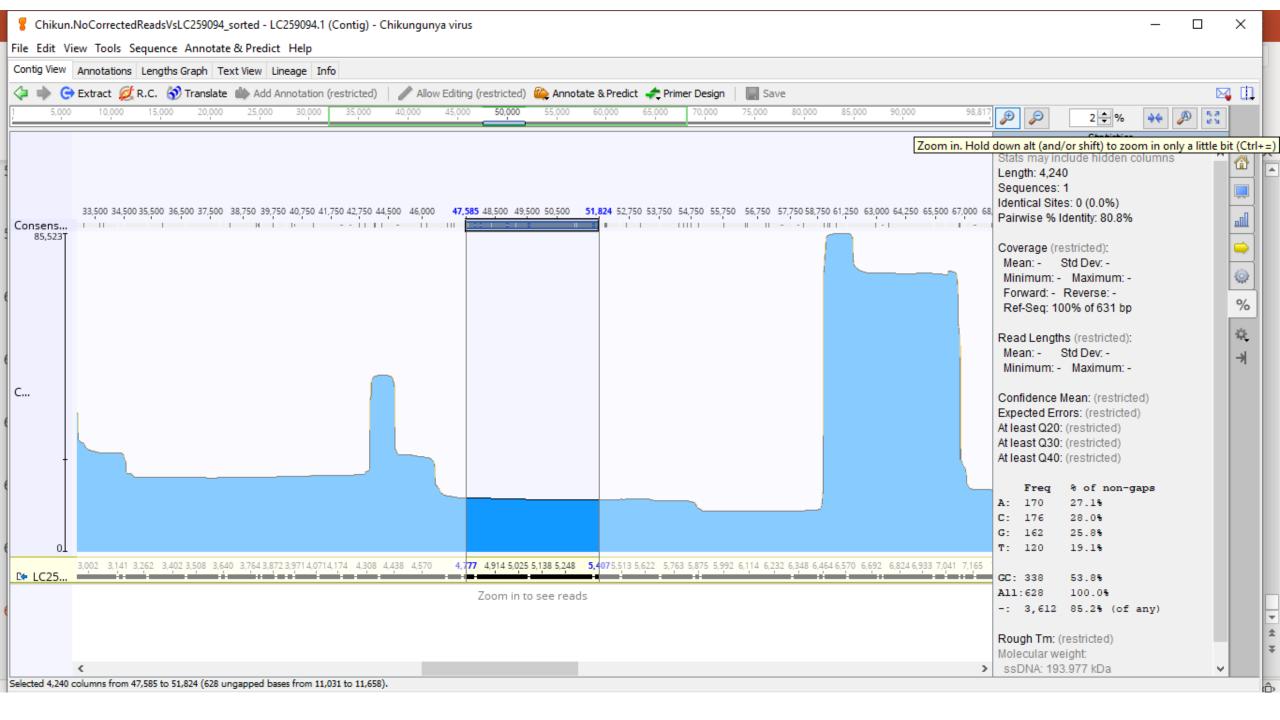


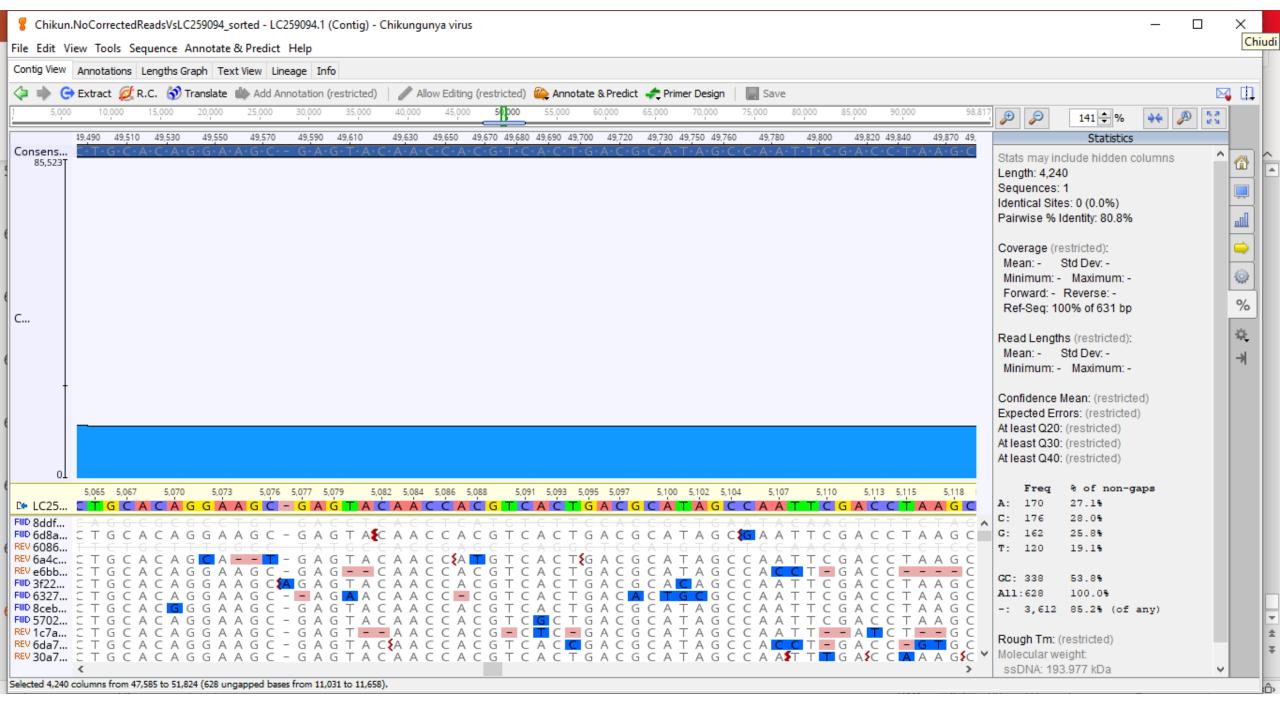












Thanks to

INMI Bioinformatic Group

Emanuela Giombini Francesco Messina Cesare Gruber



