Consiglio Nazionale delle Ricerche

Laboratorio di bioinformatica

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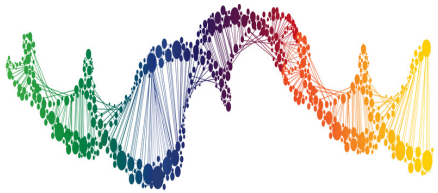
ISTITUTO
DI INFORMATICA
E TELEMATICA

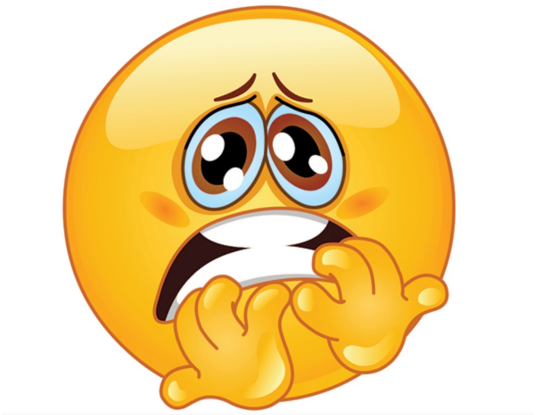
Perché la bioinformatica è difficile?



- Professionisti con competenze molto diverse
- Protocolli complessi anche per attività «semplici»
  - richiedono molte tecnologie contemporaneamente

Cosa facciamo oggi

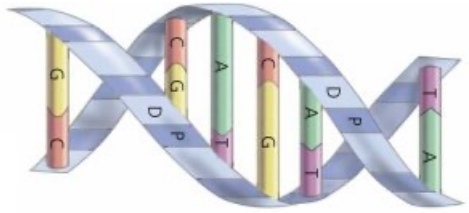
- Scarichiamo il genoma di riferimento del SARS-COV-2 e il sequenziamento di alcuni campioni, poi cerchiamo delle mutazioni



Cosa facciamo oggi

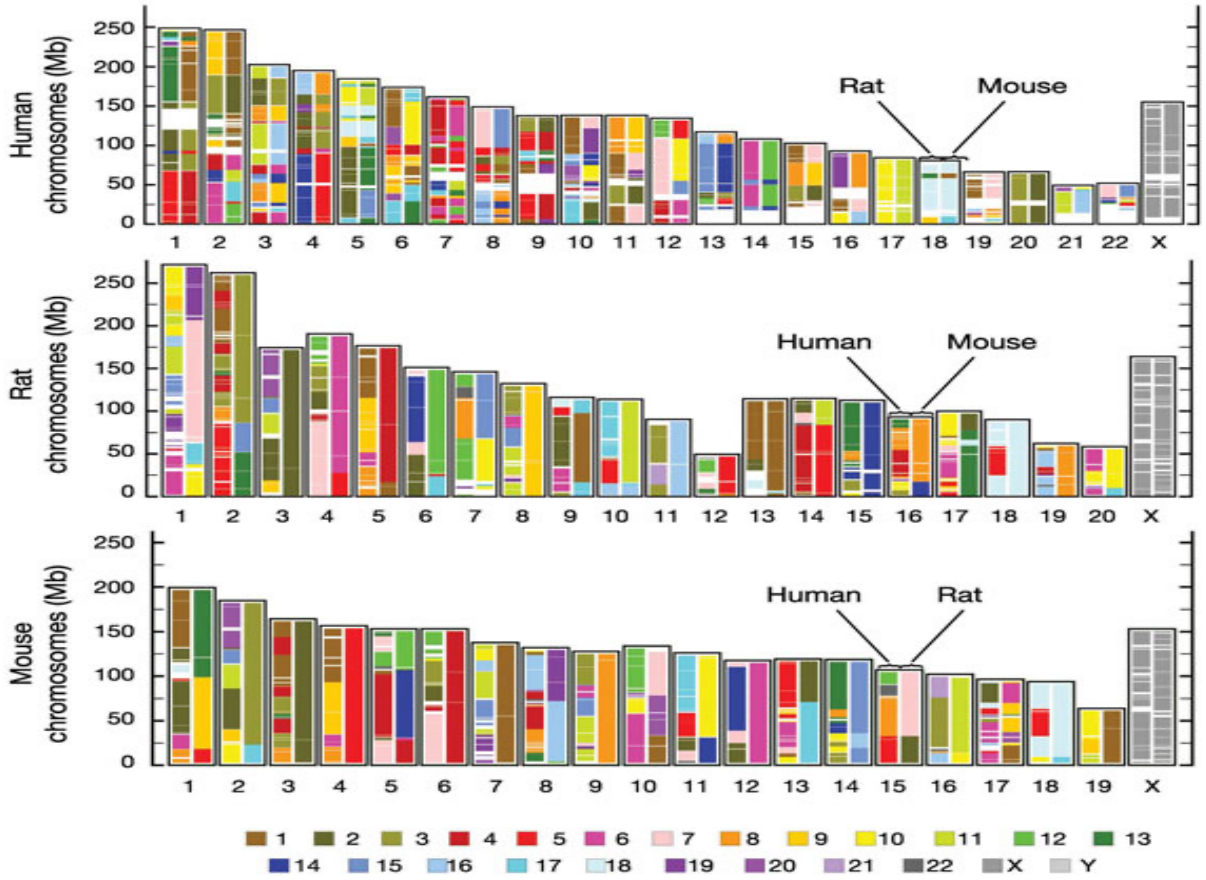
1. Comprendiamo come sono fatti i dati genomici e come vengono analizzati
2. Ci cimentiamo nello scaricare e visualizzare un genoma
3. Scarichiamo un sequenziamento e visualizziamo il suo allineamento

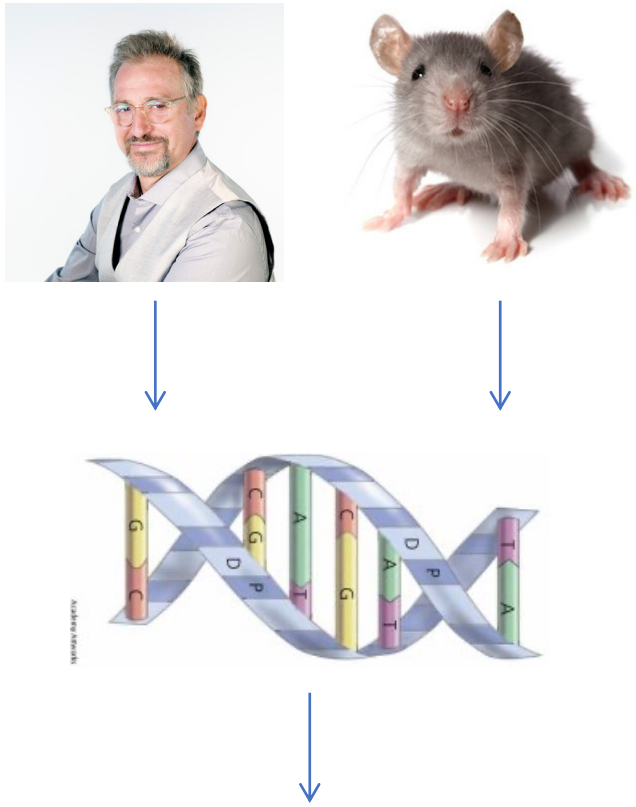
Le varianti genomiche (mutazioni): dal fenotipo al genotipo



Simili al 99%

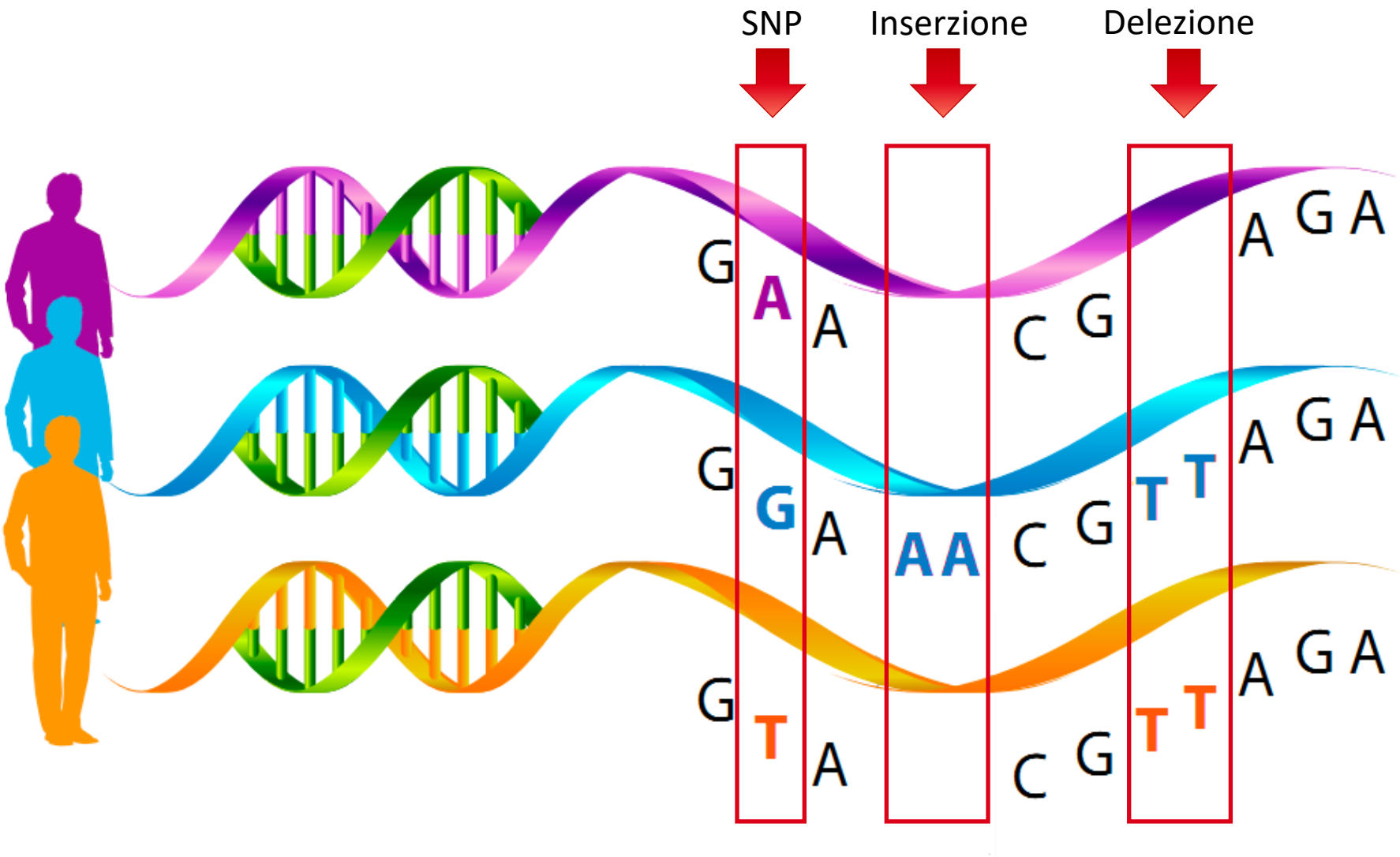
Le varianti genomiche: dal fenotipo al genotipo





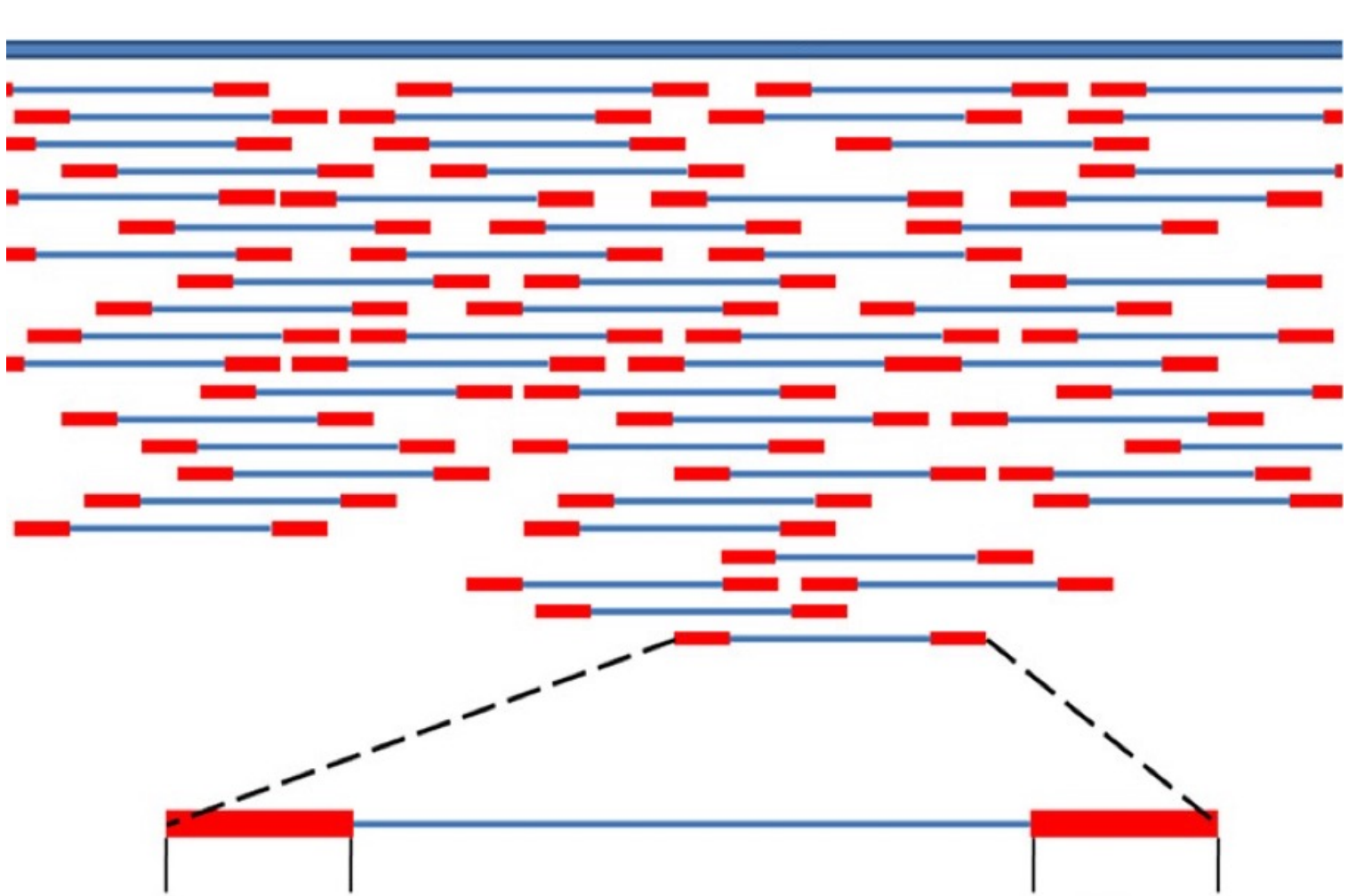
Simili al 98%

Le mutazioni

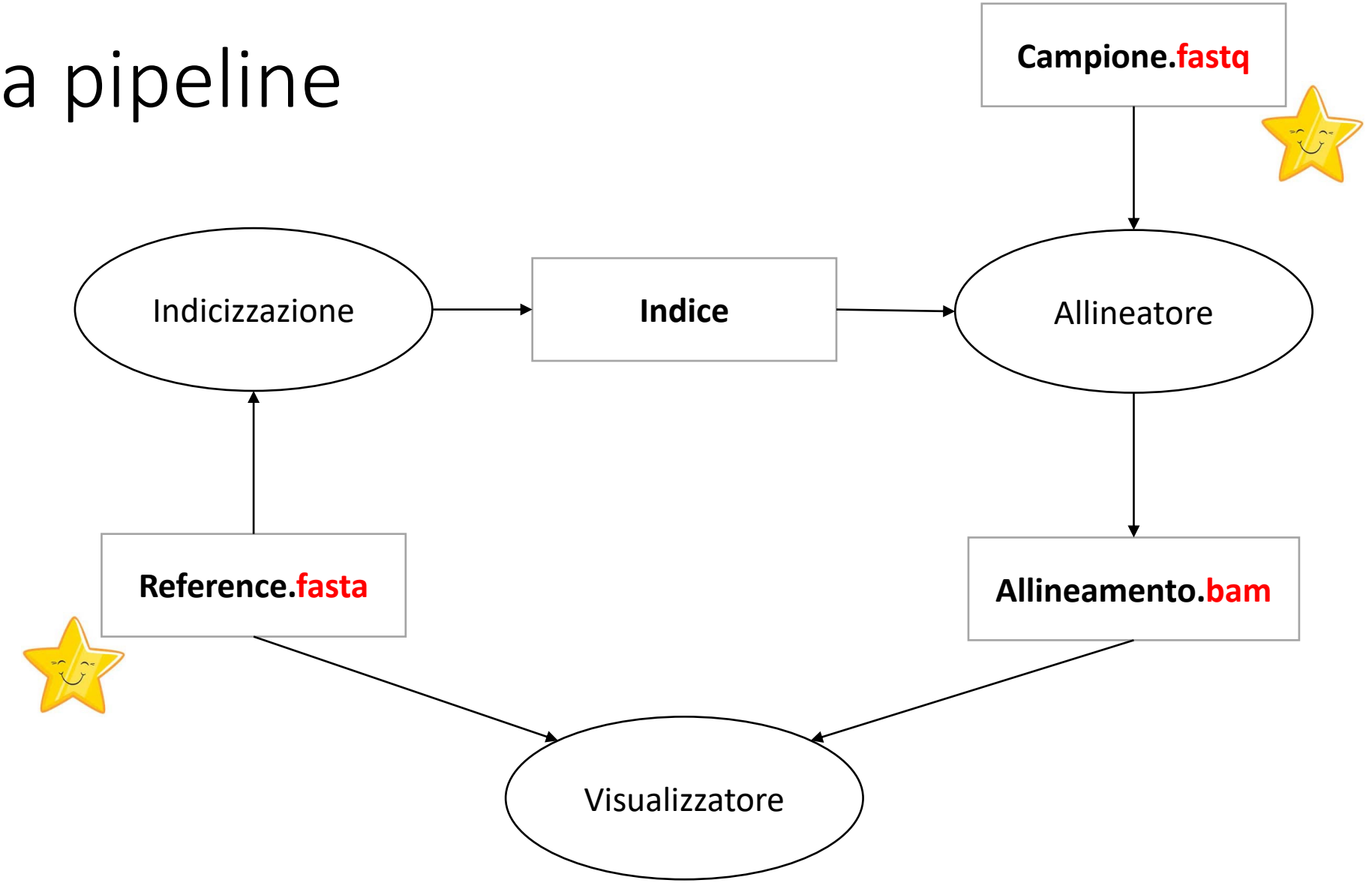


Genoma di riferimento e sequenziamento

Genoma di riferimento



La pipeline


Campione.fastq
Indicizzazione
Indice
Allineatore
Reference.fasta
Allineamento.bam
Visualizzatore

I formati dei file che ci servono

- Fasta: memorizzano una o più sequenze genomiche
- Fastq: memorizzano i frammenti provenienti dal sequenziamento
  - I sequenziatori assegnano un punteggio ad ogni nucleotide in base alla probabilità di errore nella lettura della sequenza
- SAM/BAM: memorizzano i frammenti provenienti dal sequenziamento oltre informazioni sull'allineamento
  - Coordinate genomiche, etc.

fasta

```
>gi|511344723|gb|KE141102.1| Homo sapiens CAGGAGAATCGCTTGAACCCAGGAGGTGGAGGTTGCGGTGA
GCCAAGATCACACCATTGCAGTCCAGCCTGAGCAACAGAGC
AAGACTCTCTCTCGAGACAATAAAAACACACAAAAAATTAA
CTCGCCATGATGGCACAGCCACGTCAGGCGGCACAGACTCC
GCTCCCCAAGCCTGGCTCCCTTAGAGTTTGGGCTCAAAGGA
TGTCG
>gi|511344722|gb|KE141103.1| Homo sapiens GGCTCACTGCATCATGCGCCTCCCTGGTTCAAGCAATTCTG
ATGCCTGAGCCTCCCAAGTAGGAGGATTACAGGTGCACGCC
ACCACACCCAGCTAATTCTTATATTTTCAGGAGAAATGGGG
TTTCACCATGTTGCCTACATGCTCTCATTTGAGAAGAGCAT
GGAGTTAAAGGTGAATGAAAATATTTGTCTCCCTCTCCCTC
TCCCTCTCCCTCTCCCTCTCCCTCTCCCCACGGTCTCCCTC
TCATGC
```

FastQ

```
@SRR21643281.1
GCCTCAACTTTGTCAAGCCGTGAAAGGATATCATTTAAAACGTTTTAAATGATATCCTTACACGTCTTGACAAAGTTGAGGC
+
AA6/AEAEAAAEEE6/E/EAEEE/AEE6A//AEEEEEEA/EAAAAA6EEAEAEEEEEAE/EEEAEEE//EE/AEE//E<A/E
@SRR21643281.2
CCAGCCCTTGAGACAACTACAGCAACTGGTNNNNNACCAGTTGCTGTAGTTGTCTCAAGGGCTGTNNNNN
+
/AAAAEEAAEAEEE/AAEEEEEEEEEEEEE#####AAAAAEEEEEEEAAEEEEEEEA/AEEEEEE#####
@SRR21643281.3
ACTGTTGTCCAGCATATCGTAAANNNNNNNNNNNNTTTACGATATGCTGGACAACAGTNNNNNNNNNNN
+
AAAAAEEEEEEEEEEEEEEEE###########AAAAAEEEEEEEEAAAEEEEE###########
@SRR21643281.4
GTAGGGCTGTTCAAGTTGAGGCAAAACGCCTTTTTCAACTTCTAC
+
/A6AAA/EEEEEEEEEEEEEEE//EEE/EEEEEEAAEEAAEEE
```

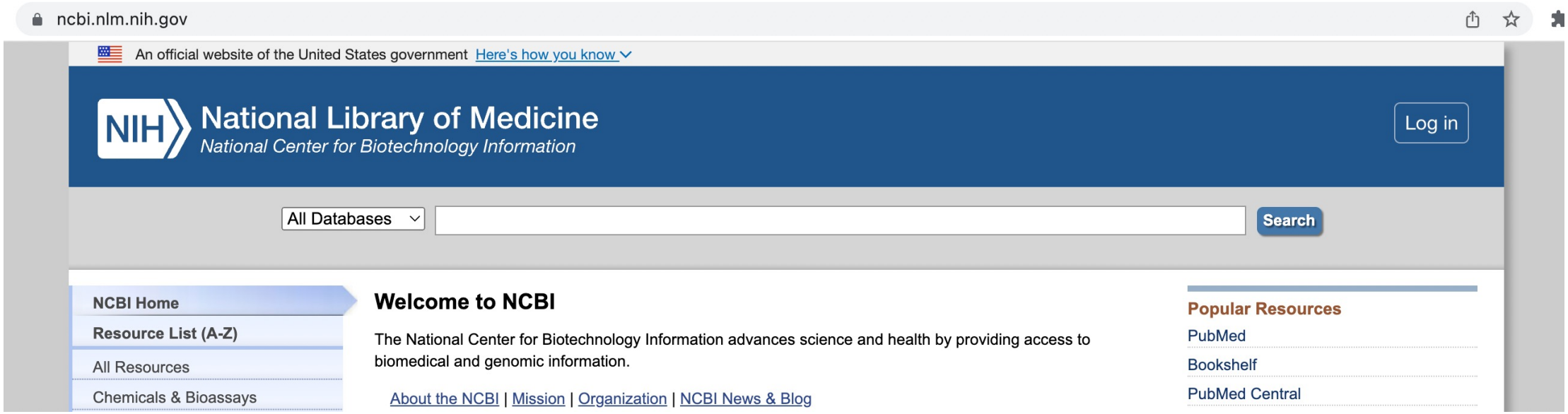
Qualità

- Phred score:

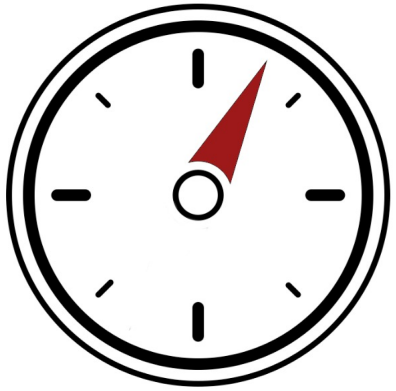
$$Q_{\mathbf{sanger}} = -10 \log_{10} p$$

- dove p è la probabilità che la chiamata sia corretta
- Il Phred score viene codificato in una scala da 0 a 93 usando I caratteri ASCII da 33 a 126
  - !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~

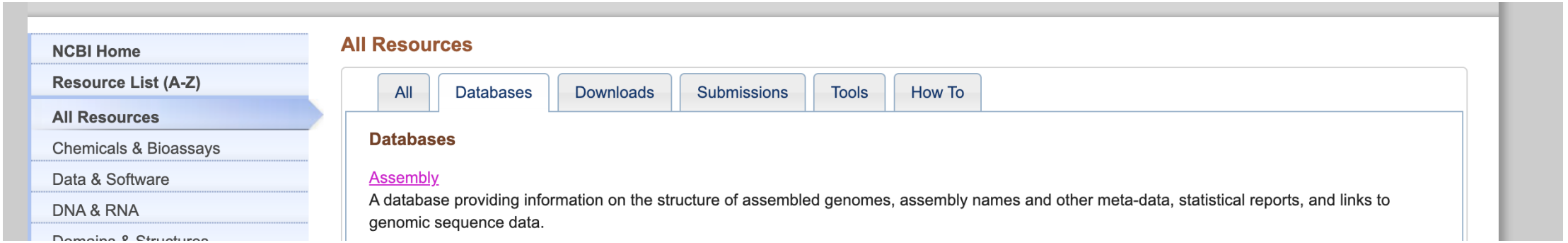
Il sito di NCBI… la sorgente di tutti i nostri dati



Il sito di NCBI… la sorgente di tutti i nostri dati



Le risorse sono suddivise in database tematici ed hanno un numero identificativo chiamato **Accession number**. La pagina «All Resources» contiene la descrizione di tutti i database.



I database di cui abbiamo bisogno

- Assembly A database providing information on the structure of assembled genomes, assembly names and other meta-data, statistical reports, and links to genomic sequence data.

- Sequence Read Archive (SRA) The Sequence Read Archive (SRA) stores sequencing data from the next generation of sequencing platforms including Roche 454 GS System®, Illumina Genome Analyzer®, Life Technologies AB SOLiD System®, Helicos Biosciences Heliscope®, Complete Genomics®, and Pacific Biosciences SMRT®.

I database di cui abbiamo bisogno

- Assembly A d… assembled geno… reports, and link…

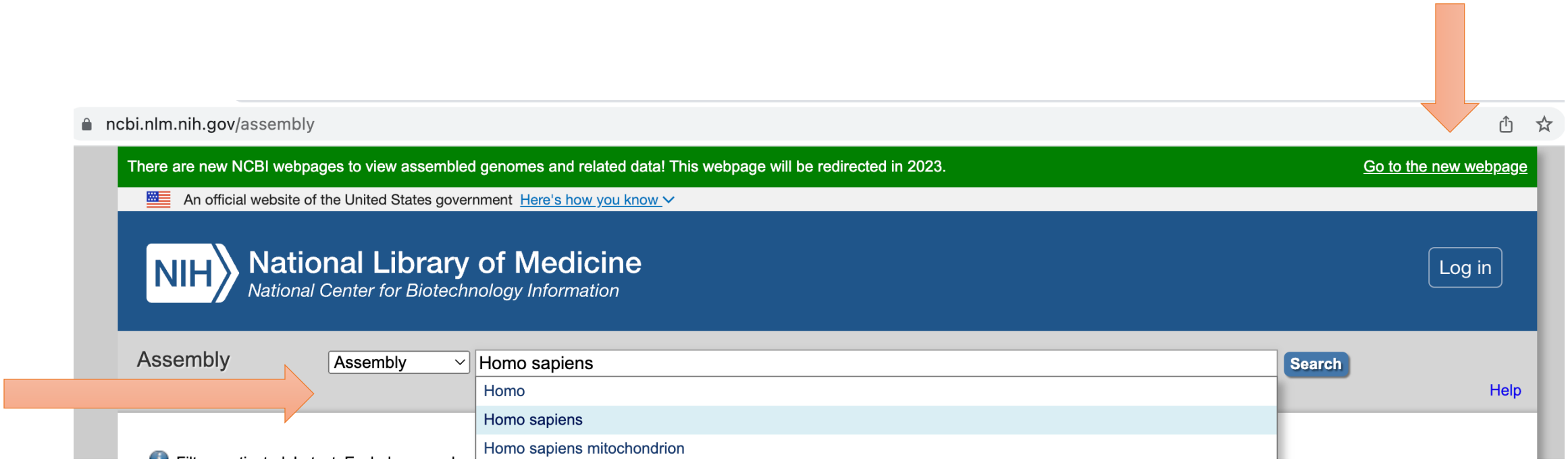
Il database da cui si scarica un genoma GENERICO ASSEMBLATO da usare come mappa per ricostruire il genoma dell'individuo che vogliamo studiare

- Sequence Read Archive (SRA) The Sequence Read Archive (SRA) stores sequencing dat… from the next generation of sequencing platforms including Roc… …S System®, Illumina Genome Analyzer®, Life … Heliscope®, Co…

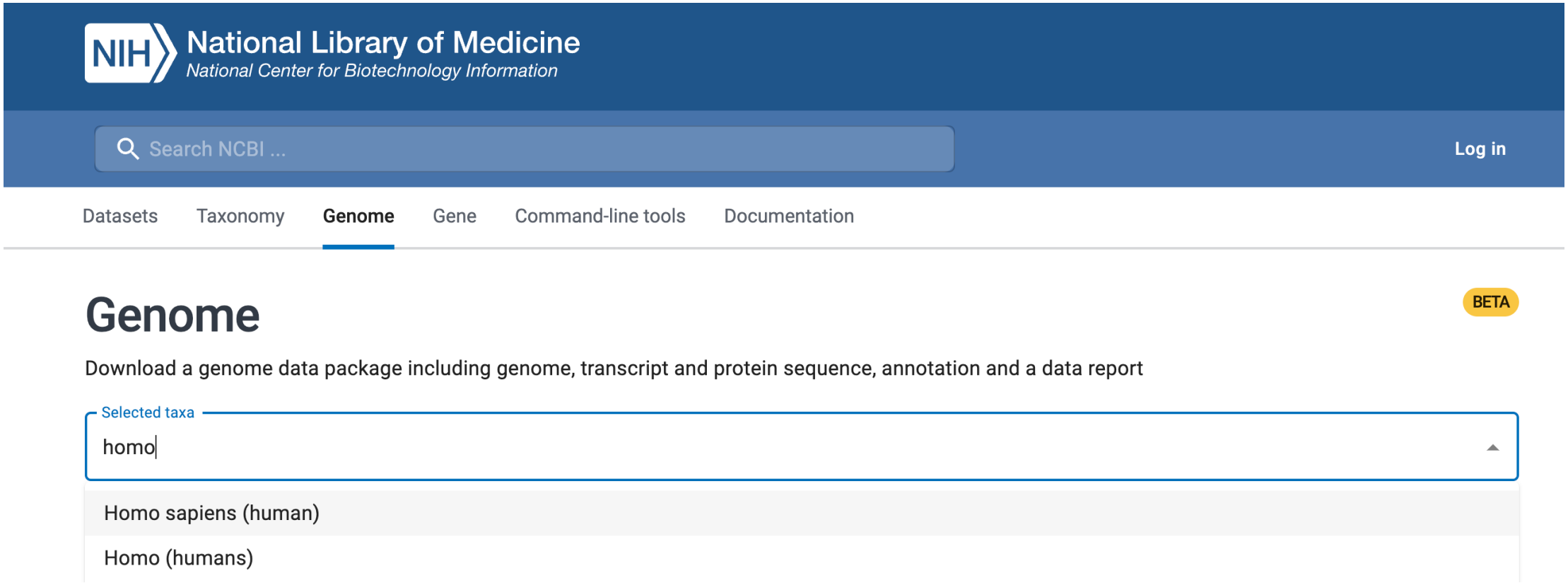
Il database da cui si scaricano i genomi degli individui che vogliamo studiare (In questo DB i dati provengono da sequenziamenti NGS quindi sono ~~frullati~~ frammentati)

Esempio: il reference del genoma umano

- Il sito di NCBI si sta rinnovando
  - Possiamo ancora fare la ricerca sul vecchio, ma conviene imparare direttamente ad usare il nuovo



Esempio: il reference del genoma umano



Esempio: il reference del genoma umano

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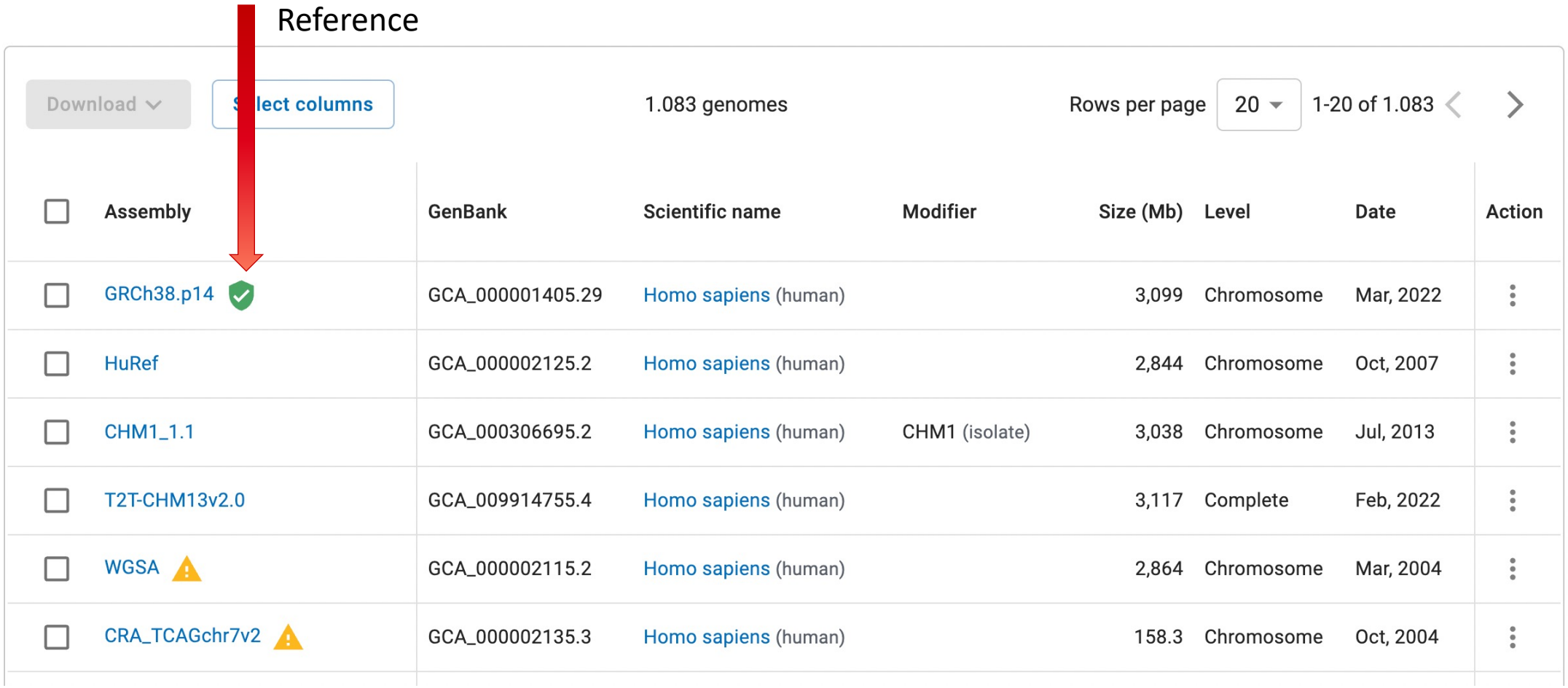
Assembly	GenBank	Scientific name	Modifier	Size (Mb)	Level	Date	Action
GRCh38.p14	GCA_000001405.29	Homo sapiens (human)		3,099	Chromosome	Mar, 2022	
HuRef	GCA_000002125.2	Homo sapiens (human)		2,844	Chromosome	Oct, 2007	
CHM1_1.1	GCA_000306695.2	Homo sapiens (human)	CHM1 (isolate)	3,038	Chromosome	Jul, 2013	
T2T-CHM13v2.0	GCA_009914755.4	Homo sapiens (human)		3,117	Complete	Feb, 2022	
WGSA	GCA_000002115.2	Homo sapiens (human)		2,864	Chromosome	Mar, 2004	
CRA_TCAGchr7v2	GCA_000002135.3	Homo sapiens (human)		158.3	Chromosome	Oct, 2004	

Esempio: il reference del genoma umano



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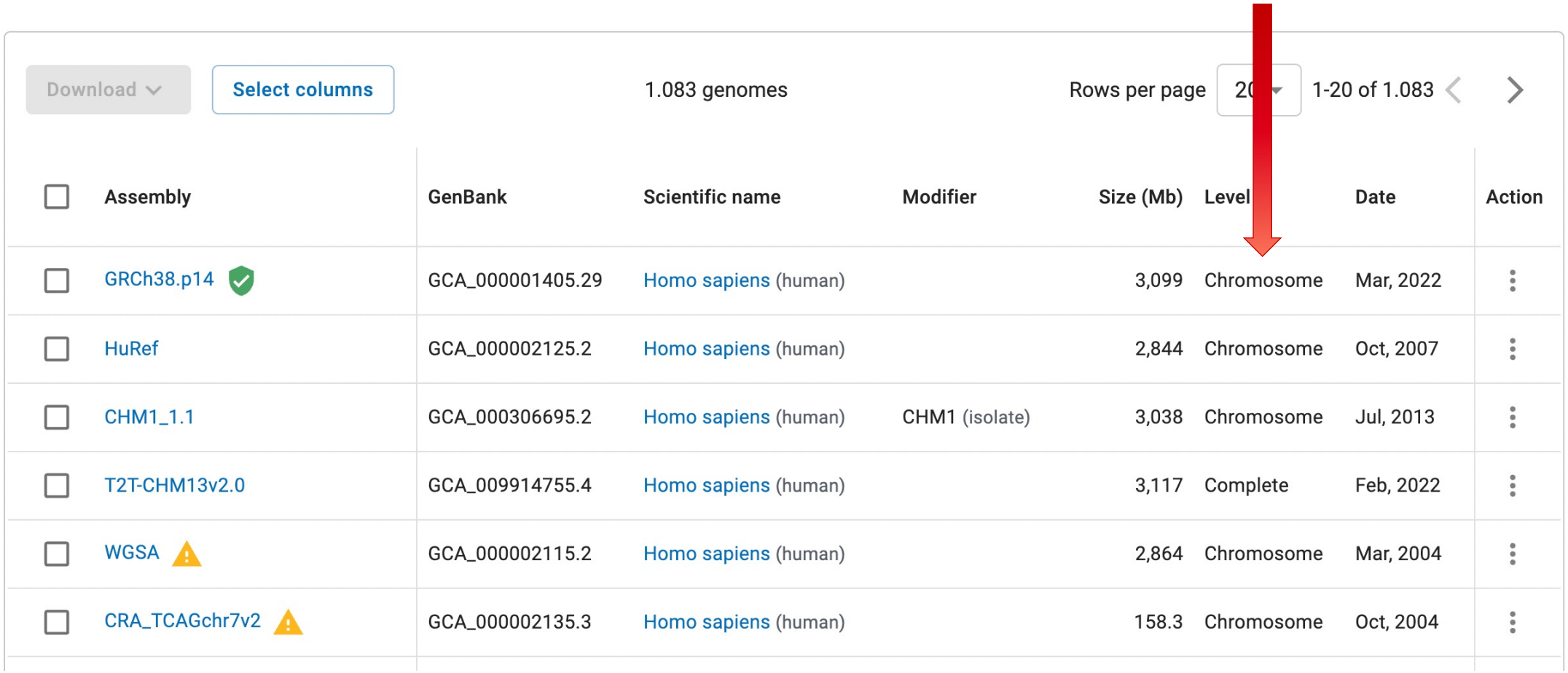
Altri genomi non reference

- La disponibilità di altri genomi assemblati consente di fare studi di popolazione o di usare come riferimento individui piu' simili a quello che voglio studiare (esempio della stessa etnia)
  - **HuRef** – J. Craig Venter Institute (Genoma di J. C. Venter)
  - **YH2** - Beijing Genomics Institute (Cinese)
  - **CHM1_1.1** - Washington University School of Medicine
  - **BGIAF** - Beijing Genomics Institute (Africano Yoruba)
  - **Watson-partial** - Baylor College of Medicine (Genoma di J. Watson)
  - **WGSA** - Celera Genomics
  - **KOREF 1.0** – Genome research foundation (Coreano)
  - **MSB_human_1b** - University of Luebeck (Egyptian)

Esempio: il reference del genoma umano

Livello di assemblaggio



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T2T-CHM13v2.0	GCA_009914755.4	Homo sapiens (human)		3,117	Complete	Feb, 2022	
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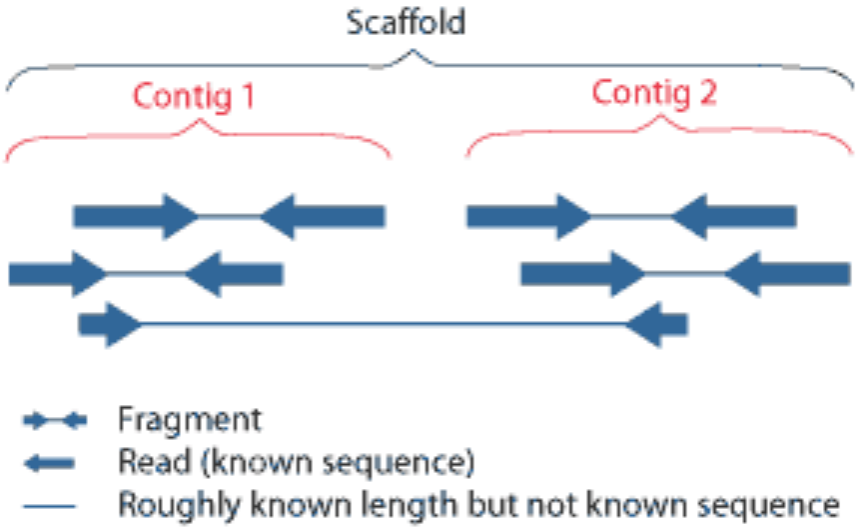
Assembly level

- **Complete genome** - tutti i cromosomi sono senza buchi e non hanno sequenze ambigue più lunghe di 10 nucleotidi.
- **Chromosome** - è presente la sequenza di uno o piú cromosomi, ma possono esserci dei buchi.
- **Scaffold** - l'assemblato contiene per lo piú frammenti di «grandi» dimensioni
- **Contig** - l'assemblato contiene solo frammenti di piccole dimensioni



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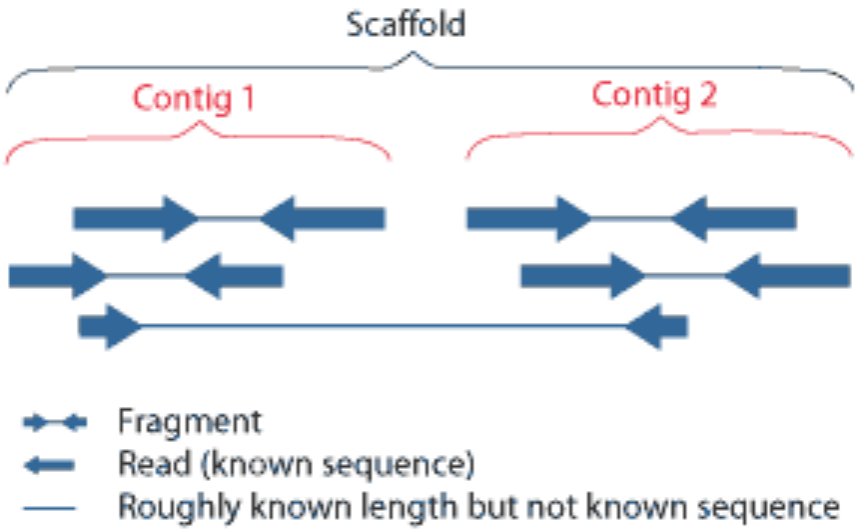

Esempio: il reference del genoma umano

Datasets / Genome / GRCh38.p14

Genome assembly GRCh38.p14

reference

Download | datasets | curl

Reference sequence	RefSeq GCF_000001405.40
Submitted sequence	GenBank GCA_000001405.29
Taxon	*Homo sapiens* (human)
Synonym	hg38
Assembly type	haploid-with-alt-loci
Submitter	Genome Reference Consortium
Date	Feb 3, 2022

View the legacy Assembly page

Esempio: il reference del genoma umano

Opzioni di download

Datasets / Genome / GRCh38.p14

Genome assembly GRCh38.p14

reference

Download | datasets | curl

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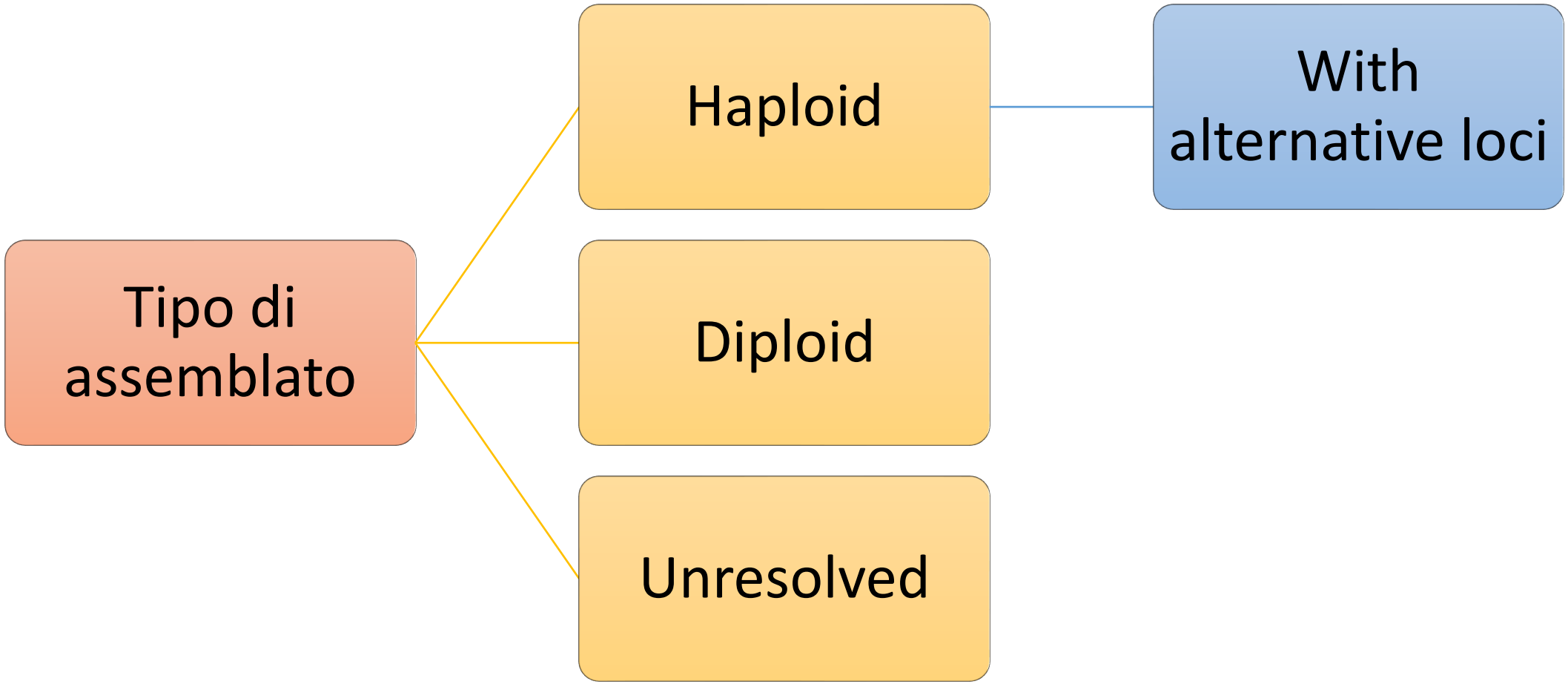
View the legacy Assembly page

Accession ID

Tipo di assemblato

Tipo di assemblato



Esempio: il reference del genoma umano

Assembly statistics

These statistics describe the RefSeq genome sequence GCF_000001405.40

Genome size	3.1 Gb
Number of chromosomes	24
Number of scaffolds	470
Scaffold N50	67.8 Mb
Scaffold L50	16
Number of contigs	996
Contig N50	57.9 Mb
Contig L50	18
GC percent	40.5
Assembly level	Chromosome

Esempio: il reference del genoma umano

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Esempio: il reference del genoma umano

Chromosomes

Chromosome	GenBank	RefSeq	Size (bp)	GC content (%)
1	CM000663.2	NC_000001.11	248.956.422	41,5
2	CM000664.2	NC_000002.12	242.193.529	40
3	CM000665.2	NC_000003.12	198.295.559	39,5
4	CM000666.2	NC_000004.12	190.214.555	38
5	CM000667.2	NC_000005.10	181.538.259	39
6	CM000668.2	NC_000006.12	170.805.979	39,5
7	CM000669.2	NC_000007.14	159.345.973	40,5

Esempio: il reference del genoma umano

Accession ID

Dimensione

Chromosomes

Chromosome	GenBank	RefSeq	Size (bp)	GC content (%)
1	CM000663.2	NC_000001.11	248.956.422	41,5
2	CM000664.2	NC_000002.12	242.193.529	40
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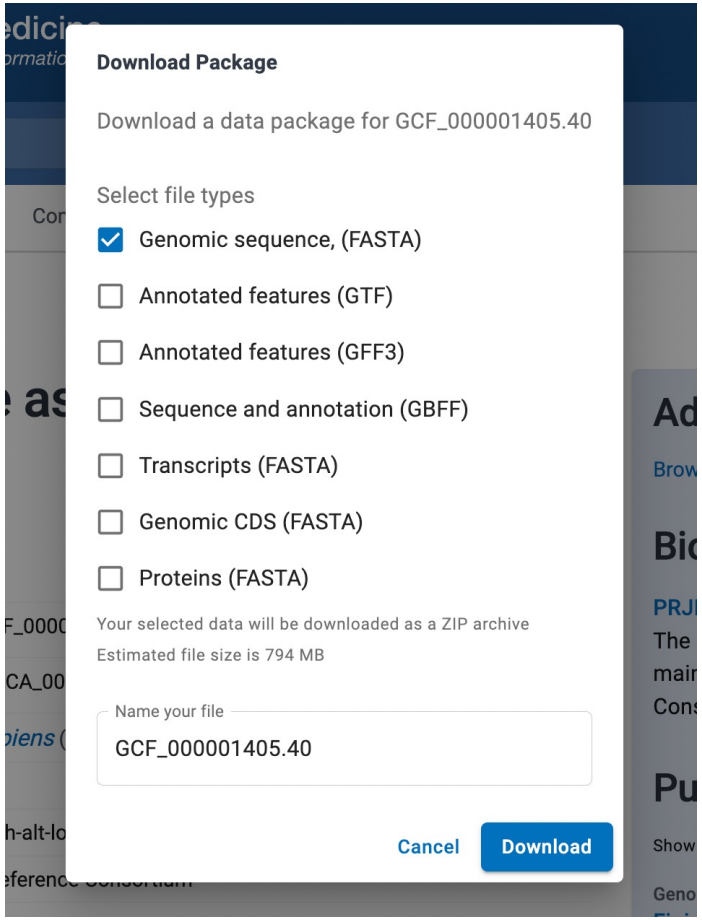
6	CM000668.2	NC_000006.12	170.805.979	39,5
7	CM000669.2	NC_000007.14	159.345.973	40,5

Esempio: il reference del genoma umano

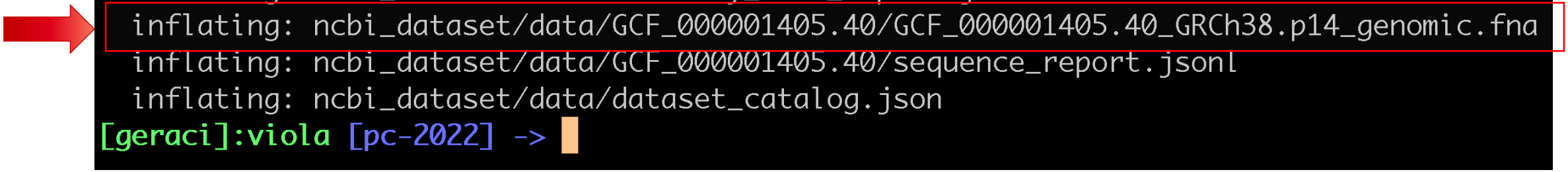
Revision History

GenBank	RefSeq	Name	Level	Date	Action
GCA_000001405.29	GCF_000001405.40	GRCh38.p14	Chromosome	2022-02-03	⋮
GCA_000001405.28	GCF_000001405.39	GRCh38.p13	Chromosome	2019-02-28	⋮
GCA_000001405.27	GCF_000001405.38	GRCh38.p12	Chromosome	2017-12-21	⋮
GCA_000001405.26	GCF_000001405.37	GRCh38.p11	Chromosome	2017-06-14	⋮

Esempio: il reference del genoma umano



Esempio: il reference del genoma umano



Domande

- In che formato sarà il file?

- Come lo apro?

Domande

- In che formato sarà il file?
  - Trattandosi di sequenze di DNA assemblate mi aspetto un file in formato (multi)fasta
- Come lo apro?
  - Mi serve un editor di testi, non posso usare un word processor

- Editor grafico adeguato
- Prompt dei comandi
  - Il comando «more» è disponibile sia su Windows che su MacOS che su Linux

Esempio: il reference del genoma umano

Accession ID

```
>NC_000001.11 Homo sapiens chromosome 1, GRCh38.p14 Primary Assembly
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
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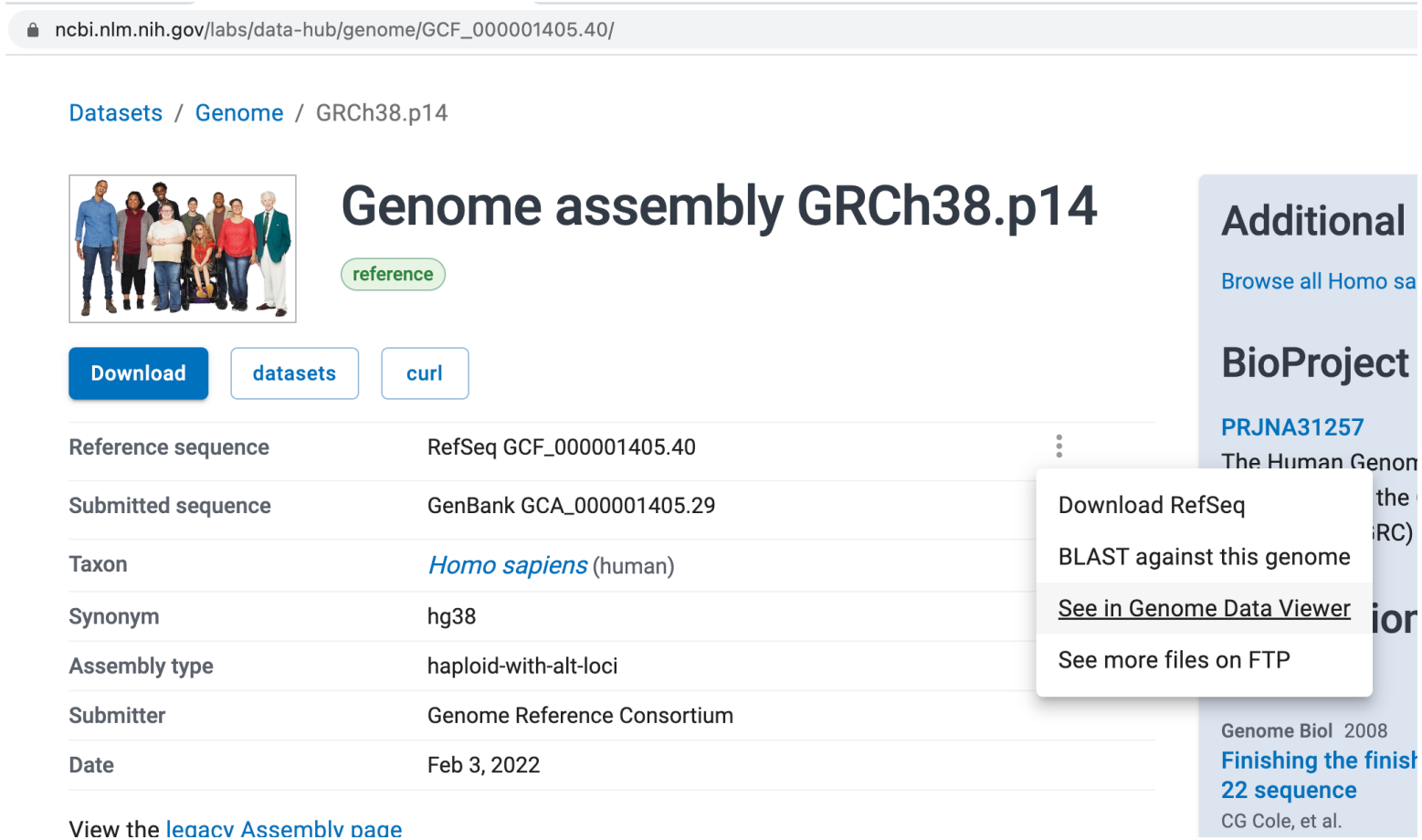
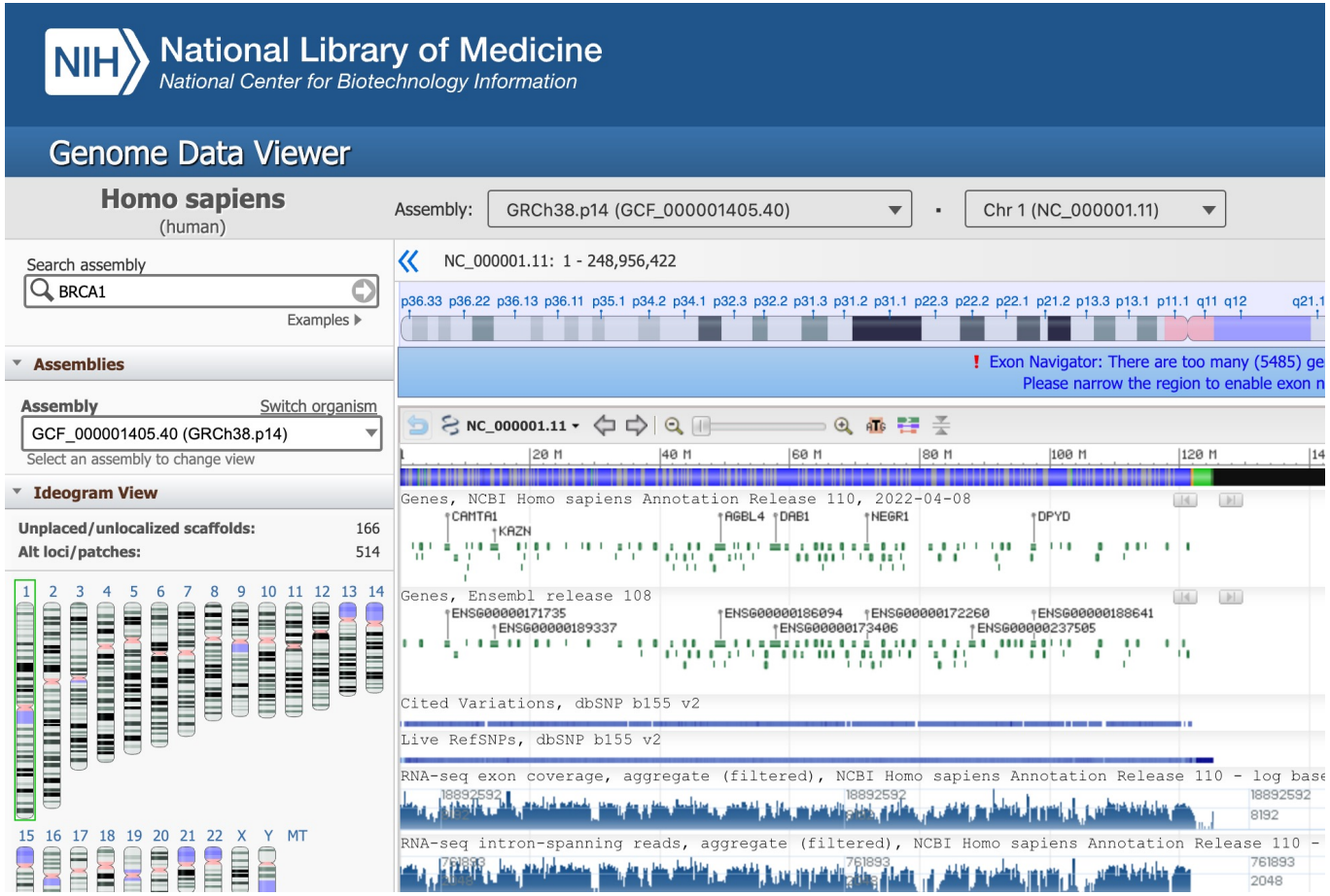
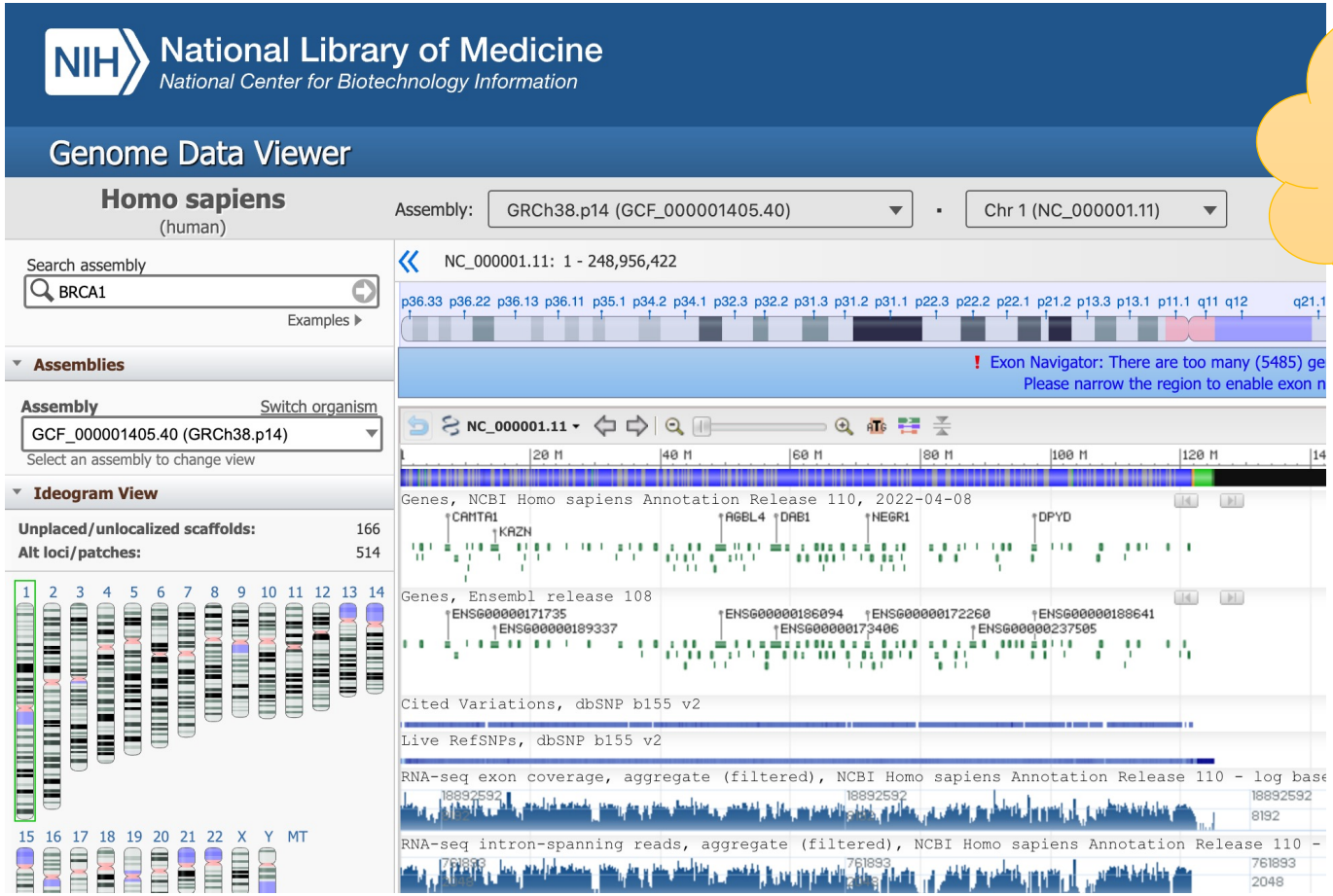
Visualizzare il reference

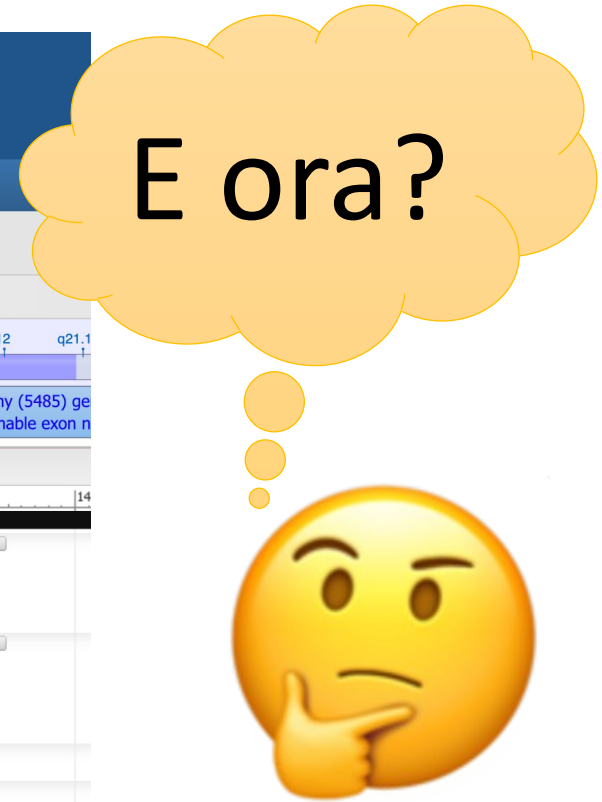


Visualizzare il reference



Visualizzare il reference



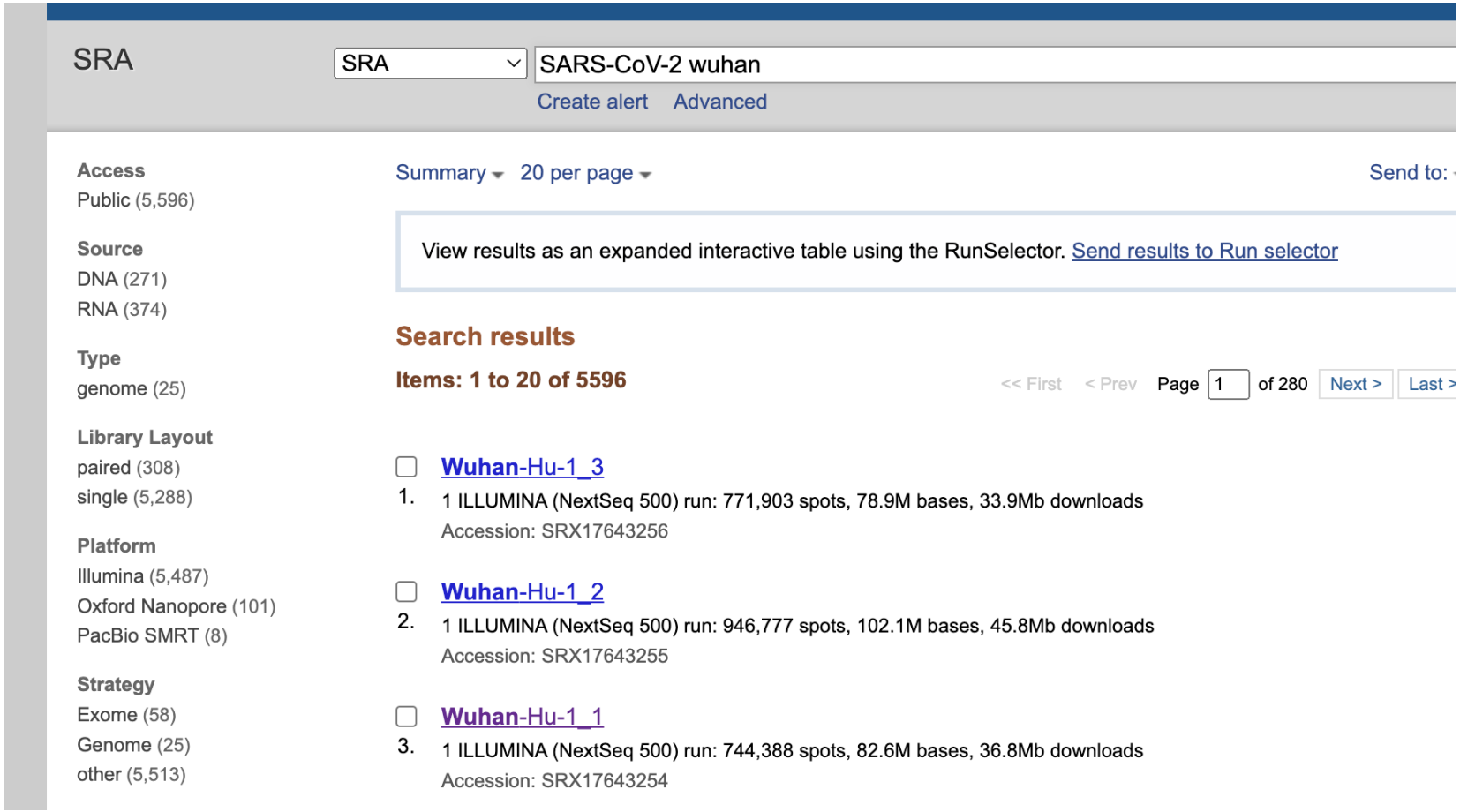


Esercizio

- Proviamo a scaricare il genoma del SARS-COV-2)severe acute respiratory syndrome coronavirus 2)

Suggerimento

Scarichiamo un campione da SRA



Scarichiamo un campione da SRA

Full ▾ Send to: ▾

SRX17643254: Wuhan-Hu-1_1
1 ILLUMINA (NextSeq 500) run: 744,388 spots, 82.6M bases, 36.8Mb downloads

Design: RNA was converted to cDNA using the NEBNextARTIC SARS-CoV-2 FSLibrary prep kit. The cDNA was amplified using the VarSkip short express protocol (NEB). The library was sequenced using the Illumina platform. Five hundred MB of data (2x 150 bp, paired ends) was produced per sample.

Submitted by: University of the Witwatersrand

Study: Whole genome sequences of SARS-CoV-2 variants
PRJNA882477 • SRP398285 • All experiments • All runs
show Abstract

Sample: Wuhan-Hu-1_1
SAMN30937604 • SRS15177384 • All experiments • All runs
Organism: Severe acute respiratory syndrome coronavirus 2

Library:
Name: CE-A1_S15
Instrument: NextSeq 500
Strategy: WGS
Source: VIRAL RNA
Selection: RT-PCR
Layout: PAIRED

Runs: 1 run, 744,388 spots, 82.6M bases, 36.8Mb

Run	# of Spots	# of Bases	Size	Published
SRR21643281	744,388	82.6M	36.8Mb	2022-09-20

Scarichiamo un campione da SRA

Wuhan-Hu-1_1 (SRR21643281)

Metadata | Analysis | Reads | Data access | FASTA/FASTQ download

Run

Run	Spots	Bases	Size	GC Content	Published	Access Type
SRR21643281	744.4k	82.6M	36.8M	38.5%	2022-09-20	public

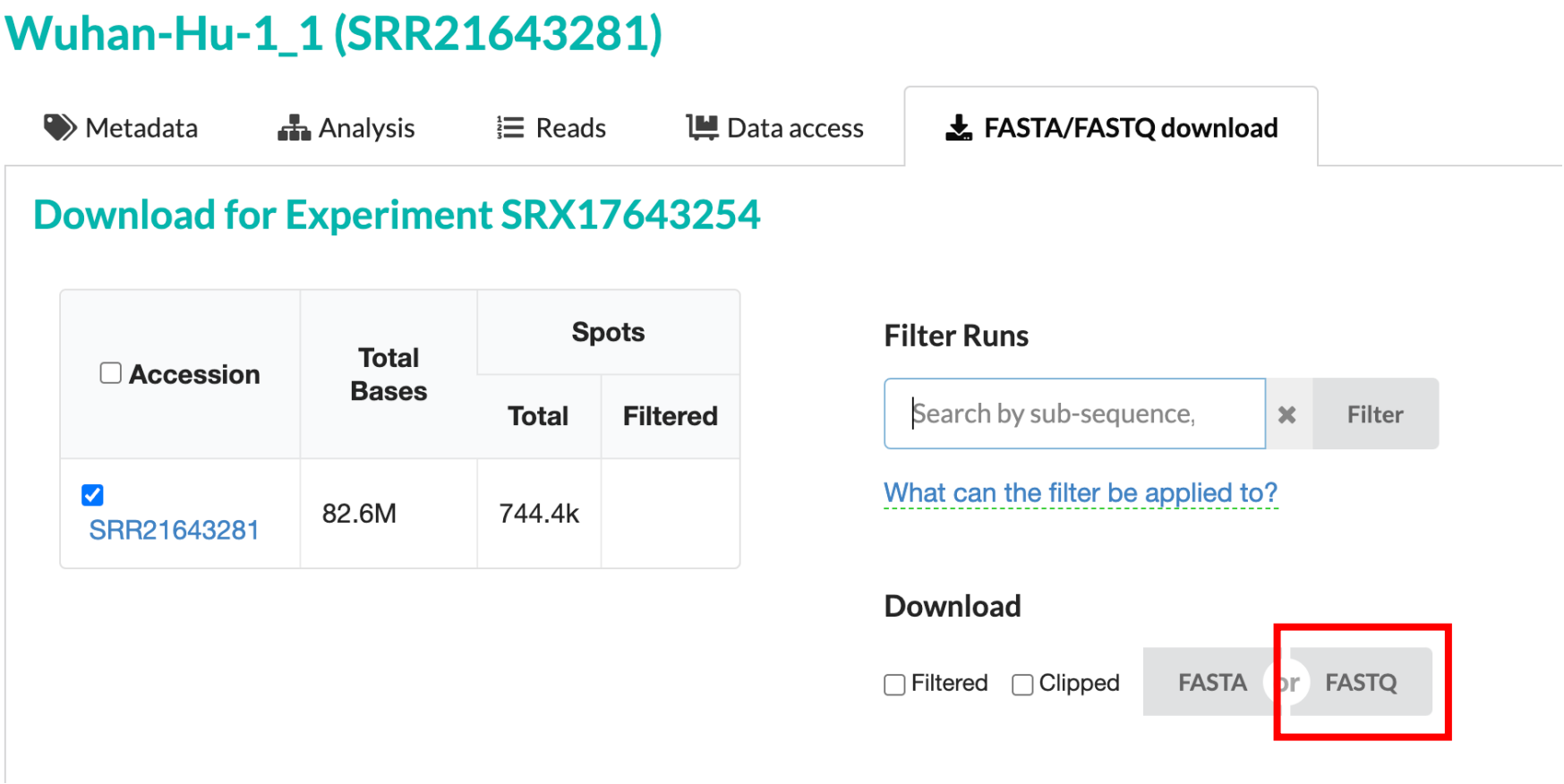
Quality graph (bigger)

This run has 2 reads per spot:

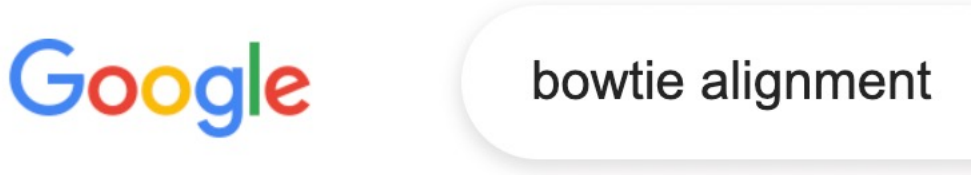
¯L=45, σ=22.8, 100%	¯L=66, σ=46.6, 100%

Legend

Scarichiamo un campione da SRA



Procuriamoci il materiale per l’allineamento



https://bowtie-bio.sourceforge.net · Traduci questa pagina

Bowtie: An ultrafast, memory-efficient short read aligner

Bowtie is an ultrafast, memory-efficient short read **aligner**. It aligns short DNA sequences (reads) to the human genome at a rate of over 25 million 35-bp reads ...

Manual · Tools that use Bowtie · Bowtie 1.3.1

Bowtie2

bowtie2-2.5.0-linux-aarch64.zip

bowtie2-2.5.0-source.zip

bowtie2-2.5.0-mingw-x86_64.zip

bowtie2-2.5.0-macos-arm64.zip

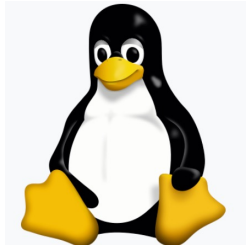
bowtie2-2.5.0-linux-x86_64.zip

bowtie2-2.5.0-macos-x86_64.zip

```
$ sudo apt-get install bowtie2
```

```
-> brew install bowtie2
```

Samtools



```
~$ sudo apt-get install samtools
```


```
[~] -> brew install samtools
```

- Scaricare da http://bioinformatics.iit.cnr.it/pc/samtools.zip
  - (Il link rimarrà attivo per qualche giorno)
- Scompattare il file zip
- samtools.exe va eseguito dalla PowerShell di windows

Creazione dell'indice

```
[lab] -> mkdir index
[lab] -> bowtie2-build GCF_009858895.2_ASM985889v3_genomic.fna index/GCF_009858895.2
```

```
[geraci]:viola [lab] -> ls index/
GCF_009858895.2.1.bt2     GCF_009858895.2.3.bt2     GCF_009858895.2.rev.1.bt2
GCF_009858895.2.2.bt2     GCF_009858895.2.4.bt2     GCF_009858895.2.rev.2.bt2
```

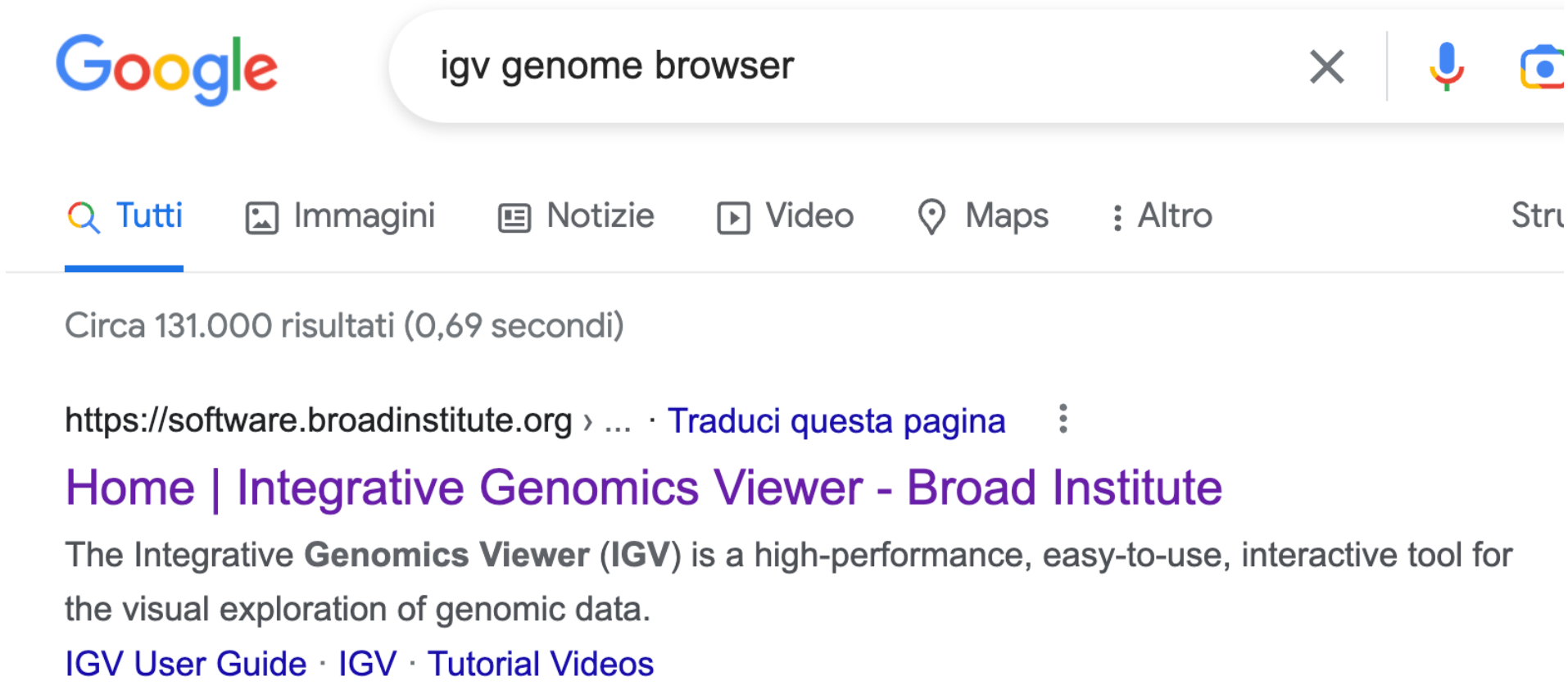
- Per poter allineare le sequenze di un campione dobbiamo creare un indice del genoma di riferimento.
- L'indice funziona come l'indice analitico di un libro: data una sequenza permette di trovare la sua posizione

Allineamento

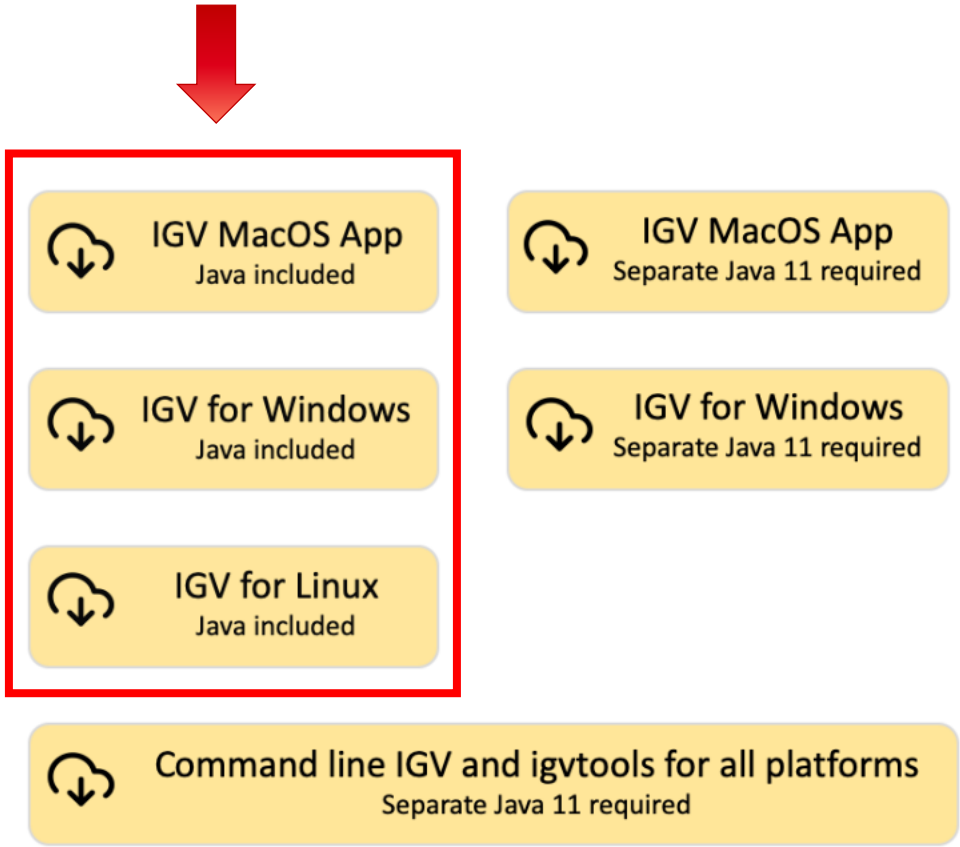
```
[geraci]:viola [lab] -> bowtie2 -x index/GCF_009858895.2 -U SRR21643281.fastq -S SRR21643281.sam
1488776 reads; of these:
  1488776 (100.00%) were unpaired; of these:
    986049 (66.23%) aligned 0 times
    502727 (33.77%) aligned exactly 1 time
    0 (0.00%) aligned >1 times
33.77% overall alignment rate
```

```
[geraci]:viola [lab] -> samtools sort SRR21643281.sam -o SRR21643281.sort.bam
[geraci]:viola [lab] -> samtools index SRR21643281.sort.bam
[geraci]:viola [lab] -> ls SRR21643281*
SRR21643281.fastq         SRR21643281.sort.bam
SRR21643281.sam           SRR21643281.sort.bam.bai
```

Visualizzare l’allineamento (IGV)

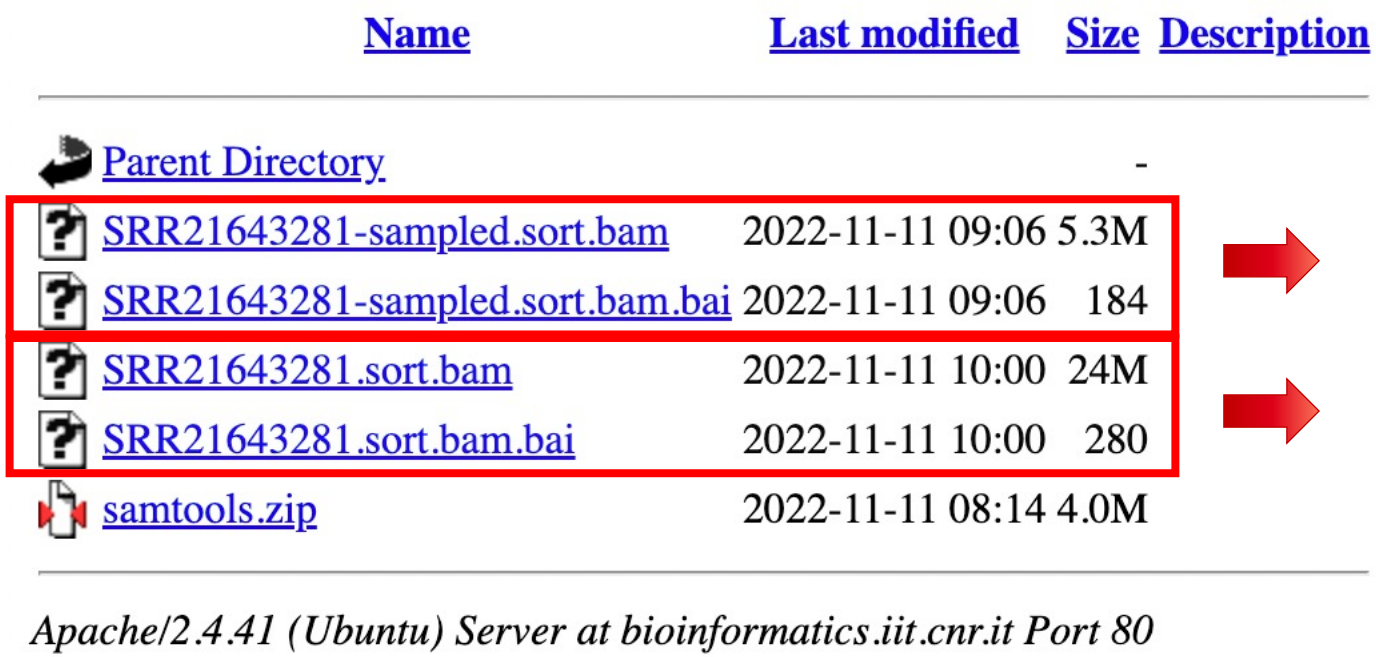


Visualizzare l’allineamento (IGV)



Materiale

- Potete scaricare i dati da questo link
  - http://bioinformatics.iit.cnr.it/pc/
- Il link rimane attio per qualche giorno



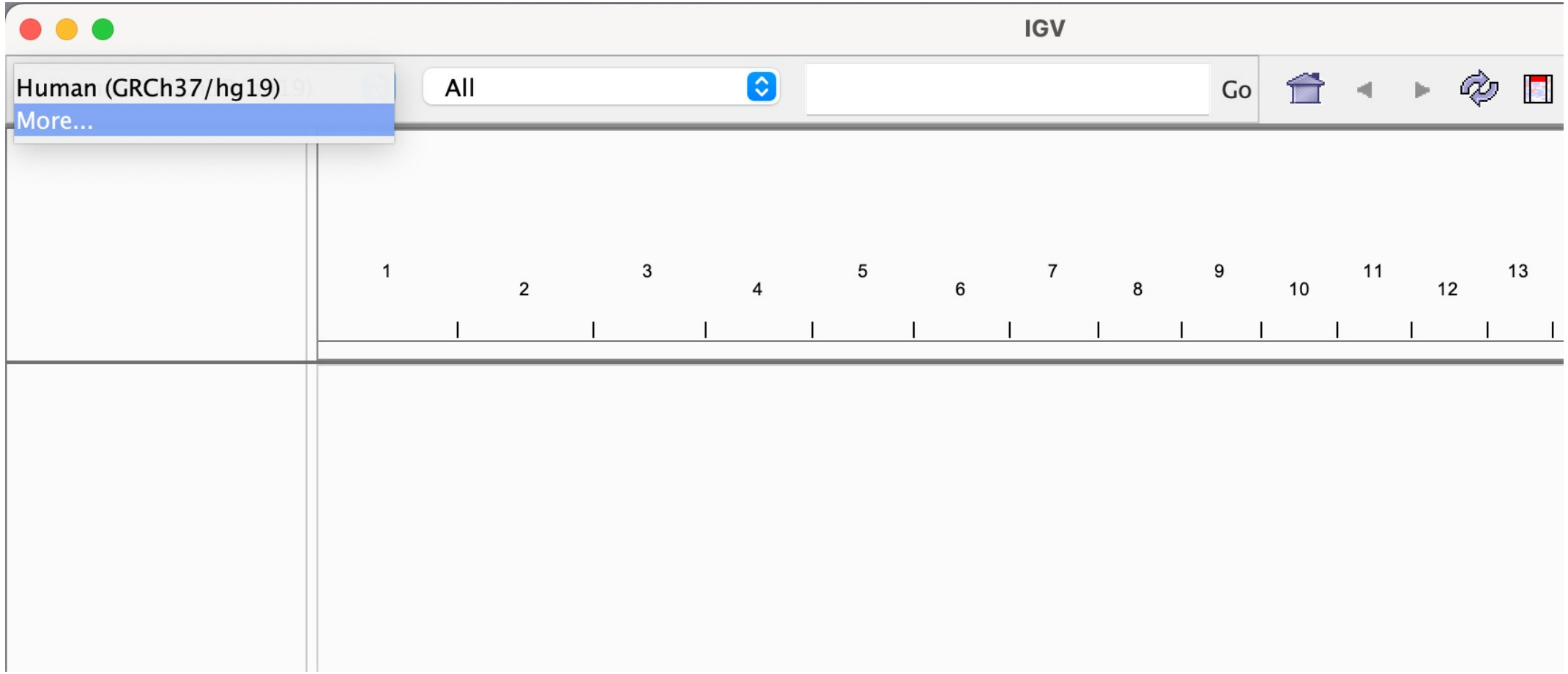
Name	Last modified	Size	Description
Parent Directory		-	
SRR21643281-sampled.sort.bam	2022-11-11 09:06	5.3M	
SRR21643281-sampled.sort.bam.bai	2022-11-11 09:06	184	
SRR21643281.sort.bam	2022-11-11 10:00	24M	
SRR21643281.sort.bam.bai	2022-11-11 10:00	280	
samtools.zip	2022-11-11 08:14	4.0M	

Apache/2.4.41 (Ubuntu) Server at bioinformatics.iit.cnr.it Port 80

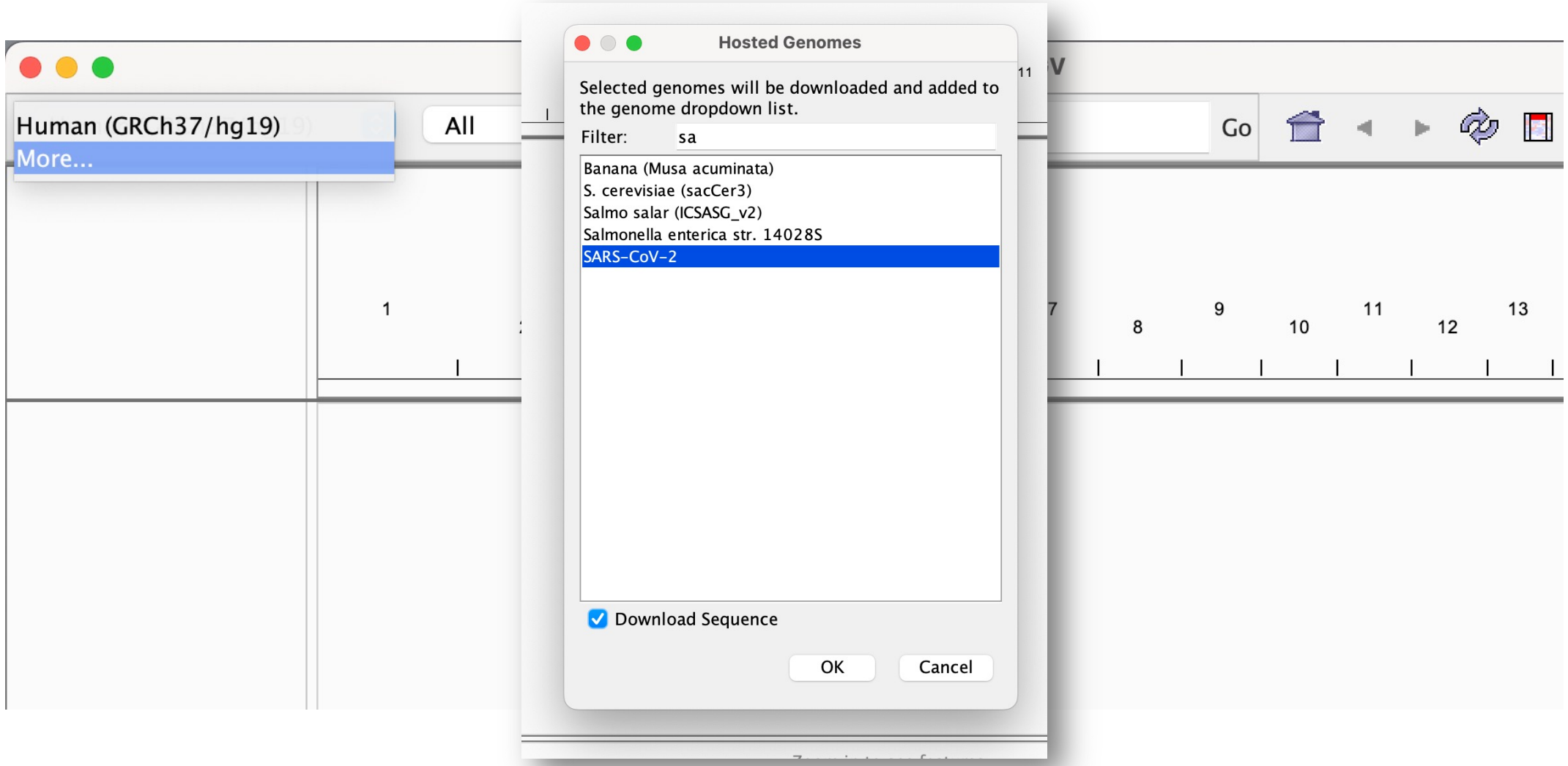
Computer lento

Computer veloce

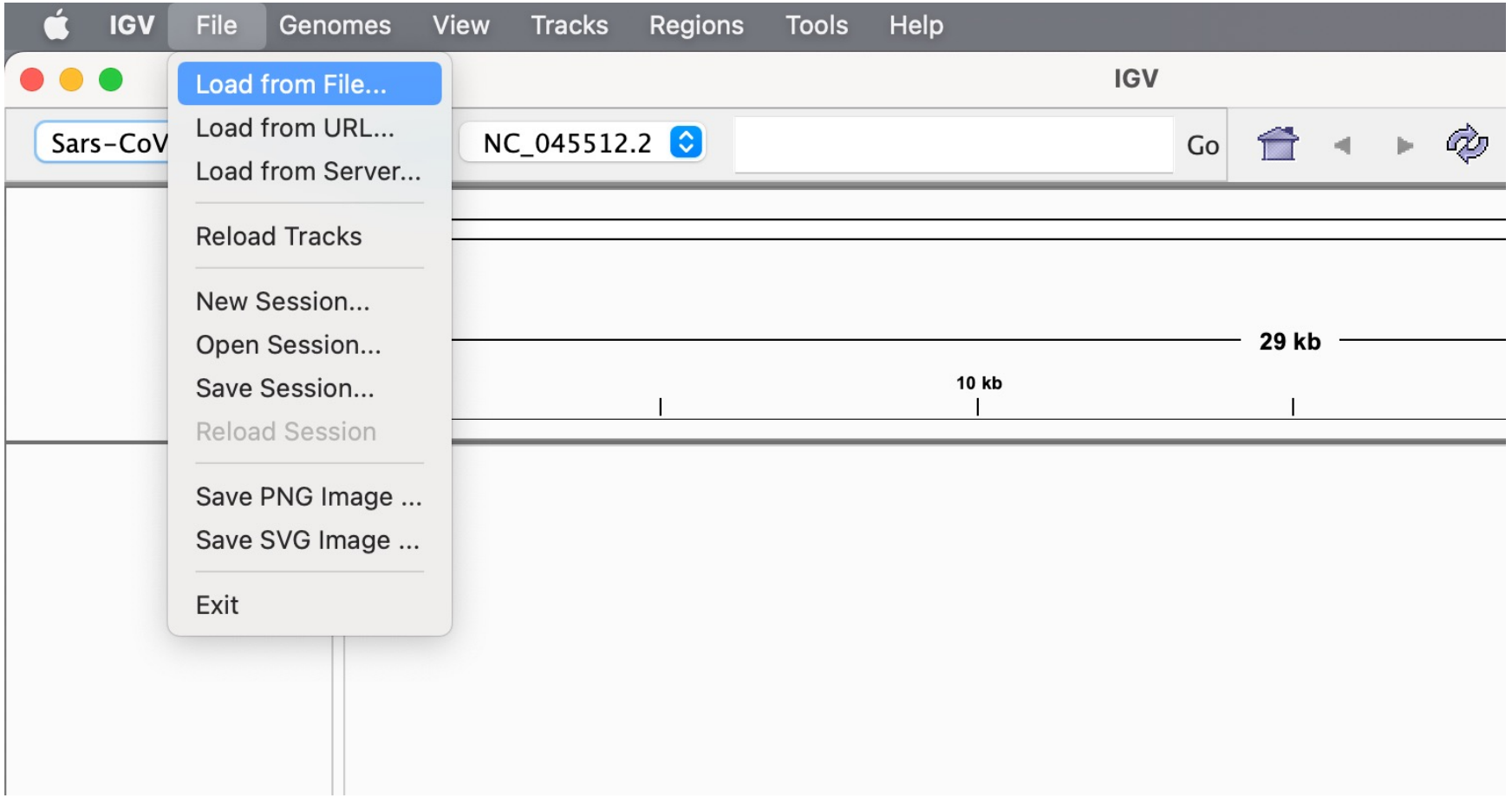
Caricare il genoma di riferimento in IGV



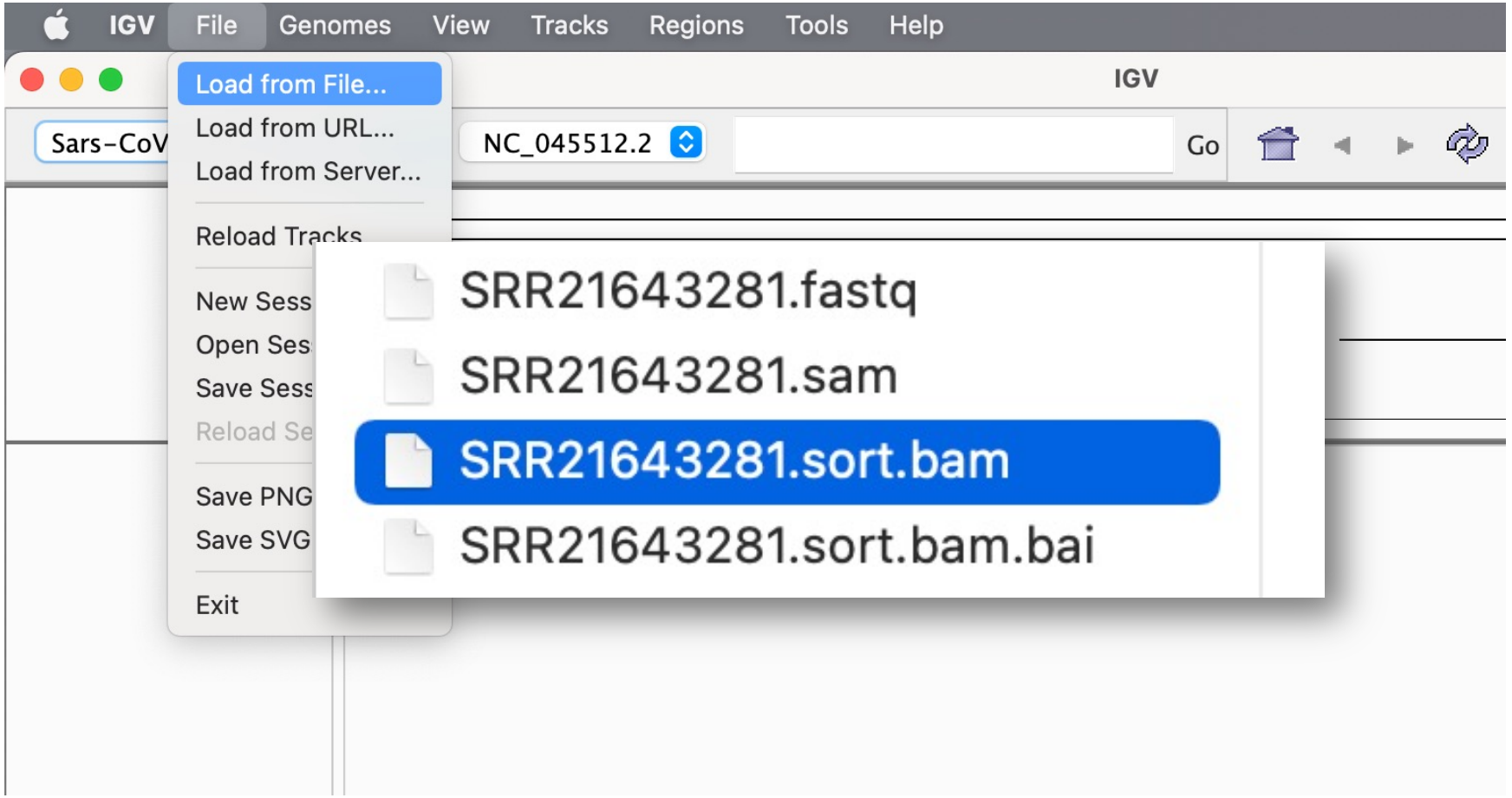
Caricare il genoma di riferimento in IGV



Carichiamo l’allineamento

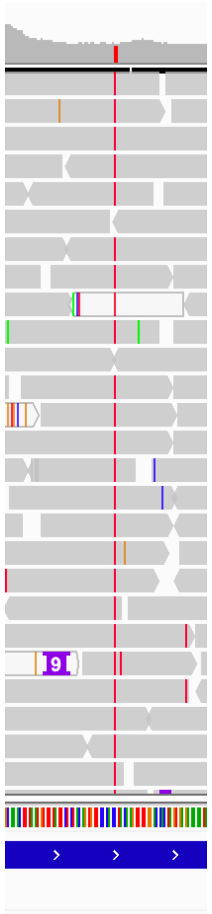


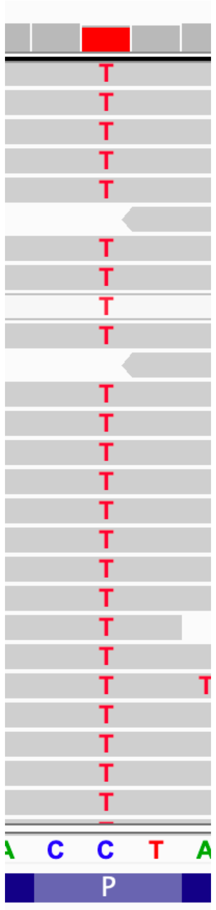
Carichiamo l’allineamento



Visualizziamo l’allineamento

Una variazione…finalmente





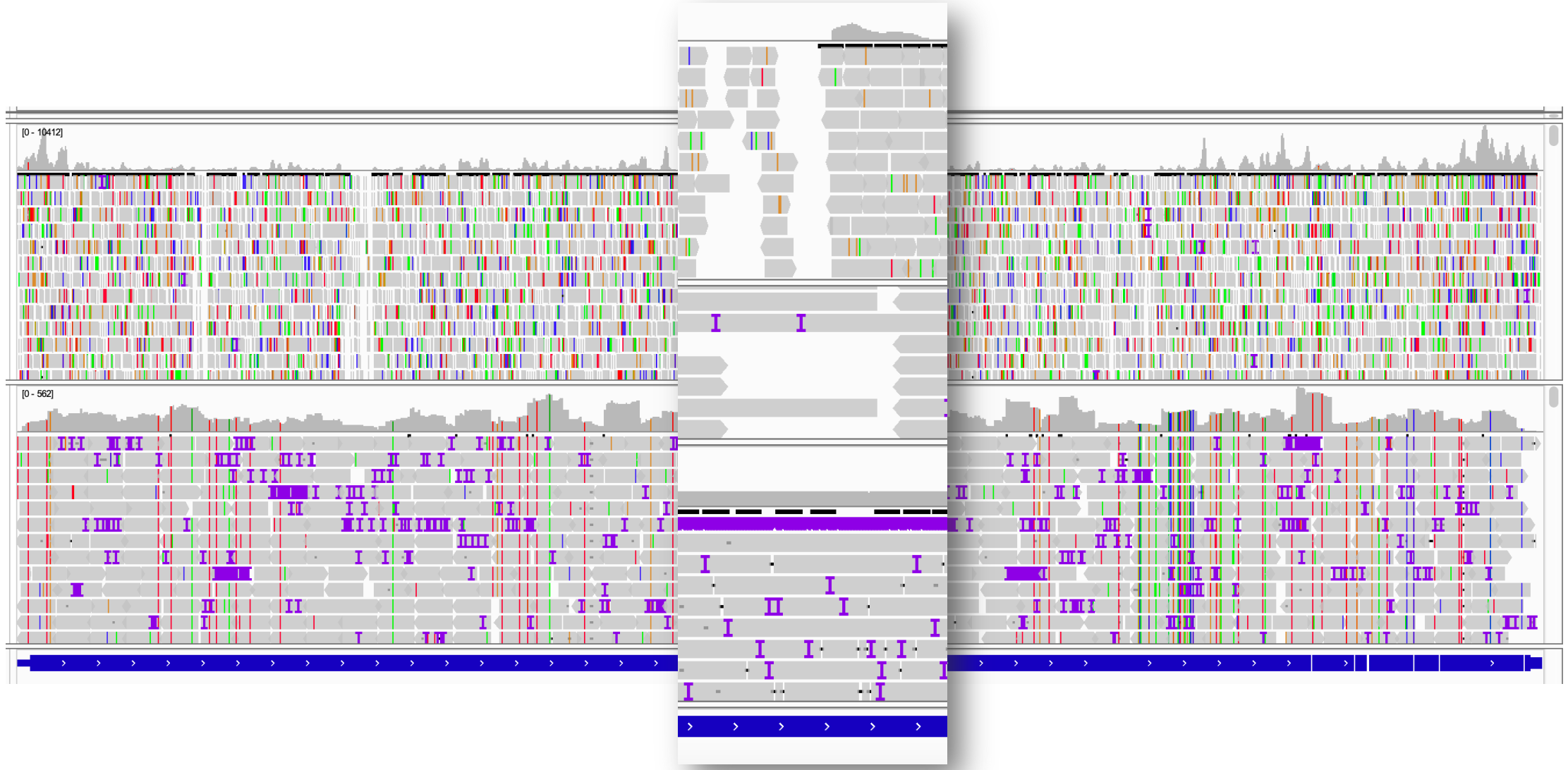
apolare	polare	basico	acido	codone di stop

Codice genetico standard

Prima base	Seconda base T		Seconda base C		Seconda base A		Seconda base G		Terza base
T	TTT	(Phe/F) Fenilalanina	TCT	(Ser/S) Serina	TAT	(Tyr/Y) Tirosina	TGT	(Cys/C) Cisteina	T
	TTC		TCC		TAC		TGC		C
	TTA	(Leu/L) Leucina	TCA		TAA	Stop (*Ocra*)	TGA	Stop (*Opale*)	A
	TTG		TCG		TAG	Stop (*Ambra*)	TGG	(Trp/W) Triptofano	G
C	CTT		CCT	(Pro/P) Prolina	CAT	(His/H) Istidina	CGT	(Arg/R) Arginina	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	(Gln/Q) Glutammina	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	(Ile/I) Isoleucina	ACT	(Thr/T) Treonina	AAT	(Asn/N) Asparagina	AGT	(Ser/S) Serina	T
	ATC		ACC		AAC		AGC		C
	ATA		ACA		AAA	(Lys/K) Lisina	AGA	(Arg/R) Arginina	A
	ATG	(Met/M) Metionina	ACG		AAG		AGG		G
G	GTT	(Val/V) Valina	GCT	(Ala/A) Alanina	GAT	(Asp/D) Acido aspartico	GGT	(Gly/G) Glicina	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	(Glu/E) Acido glutammico	GGA		A
	GTG		GCG		GAG		GGG		G

La ricerca scientifica al tempo della pandemia

La ricerca scientifica al tempo della pandemia



Grazie

Ogni diritto è l'effetto collaterale di un dovere

F. Geraci