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SGCP's
Guru Nanak Khalsa College
of Arts, Science & Commerce (Autonomous)

DEPARTMENT OF BIOINFORMATICS

CERTIFICATE

This is to certify that Ms. Prarthi Hrishit Kothari (Roll No: 115) of M. Sc. Bioinformatics (Part I) has satisfactorily completed the practical for Elective Paper: Genomics & Proteomics in Bioinformatics, NGS (GNKPSBI3P502) for Semester II course prescribed by the University of Mumbai during the academic year 2023-2024.

Teacher-in-Charge	Head of Department	External Examiner
(Mrs. Aparna Patil Kose)	(Dr. Gursimran Kaur Uppal)	

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DATE: 06/03/2024

WEBLEM 1

Introduction to Softberry Server

(URL: http://www.softberry.com/)

The Softberry server is a collection of software tools for genomic research that focuses on computational methods for high-throughput biomedical data analysis. Softberry provides services such as genome and transcript assembling, reads mapping, alternative transcripts, SNP discovery, bacterial gene identification, metagenomics, microbiome sequence analysis, gene identification using HMM FGENESH gene finder, genome annotation pipeline, RNA folding, miRNA identification, and more. The company works closely with clients to meet their computational genomics needs and offers custom genome annotation services, data set customization for gene prediction programs, expression data analysis tools, software customization, and more. Softberry is a leading developer of software tools for genomic research with a focus on computational methods for high-throughput biomedical data analysis. Their software supports next-generation sequencing technologies, transcriptome analysis, SNP detection, and disease-specific SNP subsets selection. The company's programs have been widely used in numerous research projects and publications.

Hundreds software applications developed in Softberry can be executed on-line at www.softberry.com or downloaded for free academic usage. Softberry started its commercial activities on May 1997 and is headquartered in Mount Kisco, NY, USA, with a satellite office in Novosibirsk, Russia. It participated in a number of academic collaborations on various research projects around the world.

Softberry's bioinformatics tools cover a wide range of functionalities beyond promoter prediction, including genome and transcript assembly, reads mapping, alternative transcript analysis, SNP discovery, metagenomics, microbiome sequence analysis, gene identification, RNA folding, miRNA target prediction, protein structure analysis, molecular docking, and much more. These tools are essential for comprehensive genomic analysis and functional annotation across various biological contexts. Softberry Programs available to academic users at no charge for occasional use in research projects. Below are some programs offered by Softberry.

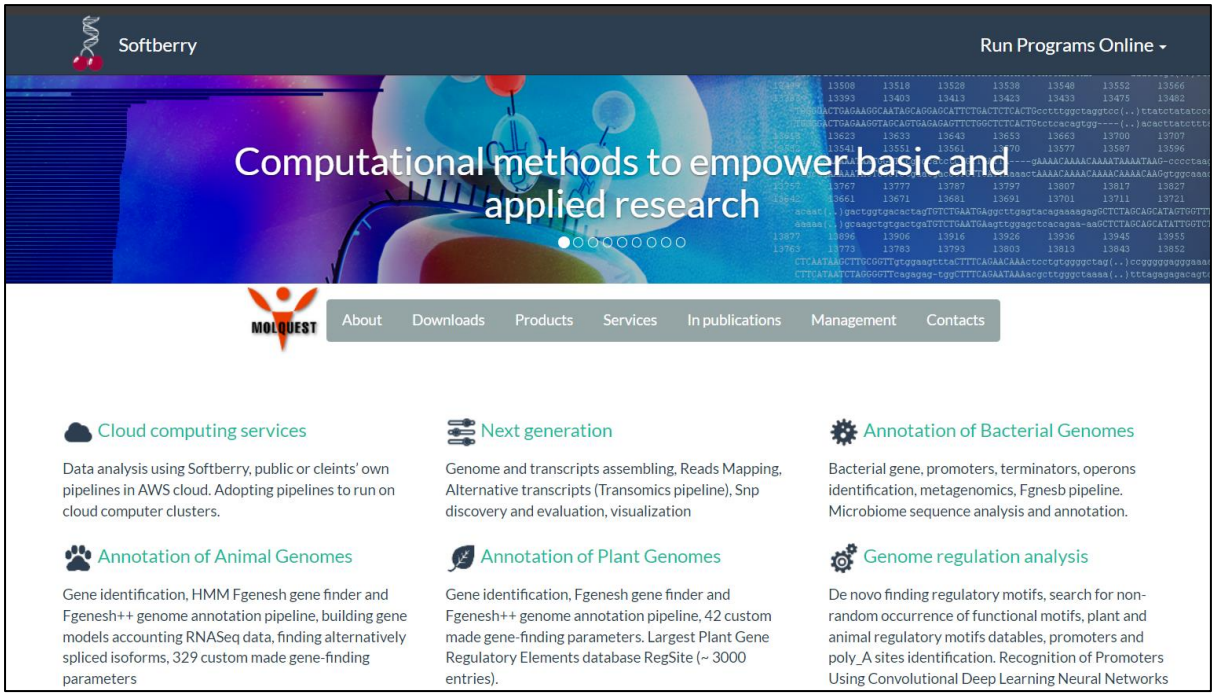

Fig 1: Homepage of Softberry server

Program 1: Gene Finding in Eukaryota

FGENESH - HMM-based gene structure prediction (multiple genes, both chains)

FGENESH is a highly accurate and fast gene prediction tool for eukaryotic genomes, outperforming other popular programs like GenScan and HMMGene in terms of sensitivity and specificity. Its efficacy has been notably demonstrated in rice genome sequencing projects, where it contributed significantly to the identification of high-confidence predicted genes. The comprehensive FGENESH++ package incorporates FGENESH and FGENESH+ programs, leveraging hidden Markov models (HMMs) and protein similarity to automate gene prediction in diverse eukaryotic genomes. This multifaceted tool enables users to predict genes through homology to known proteins, map existing mRNAs onto genomic sequences, and conduct ab-initio gene prediction in regions devoid of mapped mRNAs or homologous proteins.

In addition to its core functionalities, FGENESH offers a pseudogene annotation program (PSF) capable of identifying potential pseudogenes by analyzing exon-intron structures of annotated genes or known proteins. This feature enhances the comprehensiveness of gene discovery processes in eukaryotic genomes. Designed for versatility, FGENESH supports various organisms including humans, mice, Drosophila, nematodes, dicot plants, monocot plants, yeast (*S. pombe*), and Neurospora. Its adaptability across different species provides researchers with a robust platform for gene prediction and analysis.

The superiority of FGENESH is underscored by its performance in comparative evaluations. For instance, in a study assessing gene prediction accuracy in maize, FGENESH emerged as the top performer, outperforming other ab initio programs by a significant margin. Notably, it identified 11% more correct gene models compared to its closest competitor, GeneMark, on a set of 1353 test genes. FGENESH stands as a premier gene prediction tool renowned for its precision, speed, and adaptability across a wide range of eukaryotic genomes. Its advanced

features, including pseudogene annotation capabilities, make it an indispensable asset for researchers in the field of genomics and molecular biology.

Program 2: Operon and Gene Finding in Bacteria

A. FGENESB - Pattern/Markov chain-based bacterial operon and gene prediction:

The FGENESB program by Softberry is a suite of bacterial operon and gene finding programs known for its accuracy in prokaryotic gene prediction. It offers features such as automatic annotation of predicted genes by homology with COG and NR databases, prediction of promoters and terminators, and operon prediction based on distances between ORFs and frequencies of different genes neighboring each other. The FGENESB package includes options to work with a set of sequences like scaffolds of bacterial genomes or short sequencing data, making it a valuable tool for community sequence annotation.

The program follows a series of steps for gene prediction, including finding potential ribosomal RNA genes, predicting tRNA genes, and making initial predictions of long ORFs used as a starting point for calculating parameters for gene prediction. FGENESB utilizes highly accurate Markov chains-based gene prediction algorithms, based on coding regions and translation and termination sites, ensuring precise gene predictions in bacterial genomes. FGENESB automatically trains gene finding parameters for new bacterial genomes using genomic DNA as input, with the option to use pre-learned parameters from related organisms. FGENESB includes operon prediction based on distances between Open Reading Frames (ORFs) and frequencies of different genes neighboring each other, enhancing the understanding of gene organization in bacterial genomes.

FGENESB has been used in various projects and studies, including the first-ever published bacterial community annotation project by Tyson et al. in Nature 2004, showcasing its effectiveness in genome annotation and gene prediction tasks. The program's accuracy in gene prediction has been compared with other popular gene prediction programs like Glimmer and GeneMarkS, demonstrating high levels of accuracy in prokaryotic gene prediction. The final annotation can be presented in GeneBank format to be readable by visualization software such as Artemis or Softberry Bacterial Genome Explorer.

FGENESB from Softberry is a powerful tool for automatic annotation of bacterial genomes, offering accurate gene predictions, operon predictions, and various features that contribute to comprehensive genome annotation and analysis.

B. BPROM - Prediction of bacterial promoters

The BPROM program by Softberry is a bacterial promoter prediction tool that predicts potential transcription start positions of bacterial genes regulated by sigma70 promoters, a major E. coli promoter class. BPROM is bacterial sigma70 promoter recognition program with about 80% accuracy and specificity. It is best used in regions immediately upstream from ORF start for improved gene and operon prediction in bacteria. It uses a linear discriminant function (LDF) to combine characteristics describing functional motifs and oligonucleotide composition of these sites, achieving an accuracy of about 80% in E. coli promoter recognition. It is recommended to run BPROM on a region between two neighboring ORFs located on the same

strand or on a sequence upstream from an ORF, as most promoters are located within 150 bp from the protein coding sequence.

BPROM is part of the bacterial genome analysis suite of programs and complements operon and gene prediction by the FGENESB program. FGENESB is an automatic annotation tool for bacterial genomes that includes features like gene prediction, mapping of tRNA and rRNA genes, prediction of promoters and terminators, and operon prediction based on distances between predicted genes. The FGENESB package is known for its high accuracy in prokaryotic gene prediction and operon refinement using predicted promoters and terminators as additional evidence. BPROM is a specialized tool for predicting bacterial promoters, while FGENESB offers a comprehensive solution for gene prediction, operon identification, and annotation in bacterial genomes.

Program 3: Search for promoters/functional motifs

A. FPROM - Human promoter prediction

The FPROM program by Softberry is a bioinformatics tool specifically designed for human promoter prediction and the search for functional motifs within the human genome. This program is part of Softberry's suite of bioinformatics tools that aim to identify genes, functional signals, decipher gene expression data, and select disease-specific genes and drug target candidates. FPROM plays a crucial role in understanding gene regulation, transcriptional control, and gene expression patterns in humans.

FPROM focuses on predicting human promoters and functional motifs, providing valuable insights into the regulatory elements that govern gene expression in the human genome. It complements other tools within the Softberry suite, such as FGENESH for gene structure prediction and FGENESB for operon and gene finding in bacteria. By utilizing FPROM, researchers can gain a deeper understanding of the mechanisms underlying gene regulation in humans. FPROM is a specialized program developed by Softberry for human promoter prediction and the search for functional motifs within the human genome. It forms part of a comprehensive suite of bioinformatics tools that facilitate advanced genomic analysis and interpretation in the context of gene regulation and expression in humans.

B. TSSP - Prediction of PLANT Promoters

The TSSP program by Softberry is a tool designed for predicting potential transcription start positions in DNA sequences. It utilizes a linear discriminant function to combine characteristics and predict these positions. TSSP uses a file with selected factor binding sites from the RegSite DB (Plants) developed by Softberry Inc.

The TSSP program by Softberry has shown a high level of accuracy in predicting promoter regions and functional motifs. The TSSG program, which is closely related to TSSP, has been reported to achieve approximately 50-55% accuracy in recognizing true promoter regions with one false positive prediction for about 5000 bp. Additionally, TSSG is highlighted as the most accurate mammalian promoter prediction program, with significantly fewer false positive predictions compared to other programs like PROSCAN1.7 and NNPP2.0.

While specific accuracy metrics for TSSP are not explicitly mentioned in the search results provided, the overall performance of Softberry's promoter prediction programs, including

TSSP, is notable. These tools are designed to predict transcription start positions and functional motifs effectively, making them valuable resources for bioinformatics analysis, particularly in plant gene research.

The algorithm predicts transcription start positions and functional motifs, providing information on the first nucleotide of the transcript, TATA box positions, and functional motifs for each predicted region. TSSP is specifically used for searching for plant RNA Polymerase II TATA and TATA-less promoters (transcription start sites, TSSs). It takes single or multiple sequences in FASTA format as input, with an allowed sequence length of 251 - 100,000 bp. The output file is in classic Text format and can run on Linux or Unix systems. The program requires setting up an environmental variable and offers various options for customization such as selecting search criteria, neural network thresholds for promoters, and more. TSSP program is a valuable tool for bioinformatics analysis, particularly in predicting transcription start positions and functional motifs in DNA sequences, with a specific focus on plant RNA Polymerase II promoters.

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DATE: 06/03/2024

WEBLEM 1(A)

Gene Prediction in Eukaryotes: FGENESH

(URL:http://www.softberry.com/berry.phtml?topic=index&group=programs&subgroup=gfind)

AIM:

To predict the gene structure for query 'CDK1' (GenBank ID: NM_001320918.1) using HMM-based algorithm via FGENESH tool.

INTRODUCTION:

The FGENESH program by Softberry is a gene prediction tool used to predict multiple genes in genomic DNA sequences. It is known for its speed and accuracy, being 50-100 times faster than GenScan and highly precise in gene prediction. FGENESH has been recognized for its accuracy in various studies, outperforming other gene prediction programs like GenScan and HMMGene. It is widely used in different genomes including human, mouse, Drosophila, nematode, dicot plants, monocot plants, yeast, and Neurospora. The program offers features like predicting potential genes in genomic DNA, transcription start sites, coding sequences, and more. FGENESH++ is an advanced version of the FGENESH program that includes ab initio gene prediction using the FGENESH algorithm and additional steps for gene prediction. It is a pipeline for automatic gene prediction in eukaryotic genomes without human intervention. The FGENESH-C program is designed for predicting multiple genes in genomic DNA sequences using HMM gene models and similarity with known mRNA/EST sequences. It emphasizes the importance of using homologous mRNA information to improve the accuracy of gene finding.

FGENESH is the HMM-based gene-finding program with the algorithm similar to Genie (Kulp et al. 1996) and GENSCAN (Burge and Karlin 1997). The difference between FGENESH and analogous programs is that in the model of gene structure a signal term (such as splice site or start site score) has some advantage over a content term (such as coding potentials), reflecting the biological significance of the signals. Parameters of the program were trained on 1600 *D. melanogaster* entries from GenBank. The run time of FGENESH is approximately linear.

CDK1:

CDK1, a cyclin-dependent kinase, holds pivotal significance in cell cycle regulation, particularly governing the G2/M phase transition. Its heightened expression in various tumors signifies its involvement in tumorigenesis, implicating CDK1 as a potential target for cancer therapy. Pan-cancer analyses have illuminated CDK1's association with oncogenic signatures, immune cell infiltration, and survival rates across multiple cancers, suggesting its promise as a target for cancer immunotherapy. Additionally, CDK1's role extends beyond cell cycle regulation, encompassing the modulation of RNA polymerase activity, which may influence protein synthesis and cell cycle entry. In colorectal cancer, CDK1 emerges as a critical factor in oxaliplatin resistance, presenting a potential therapeutic target to overcome drug resistance

and inhibit tumor progression. Moreover, CDK1's predictive value in immune checkpoint inhibitor therapy response and its diverse impact on tumor immunity underscore its multifaceted role in tumor progression and treatment response across various cancer types.

METHODOLOGY:

1. Visit the Softberry Server, homepage. URL: http://www.softberry.com/
2. Click on "Run Programs Online" and select "Gene Finding in Eukaryota" from the available programs.
3. Choose "FGENESH - HMM-based gene structure prediction (multiple genes, both chains)" as the program.
4. Paste the sequence to the window for the query 'CDK1' gene (GenBank ID: NM_001320918.1) provide the nucleotide sequence in FASTA format, obtained from GenBank.
5. If the organism's name is given in your study, select it from the options provided.
6. Click on "Search" to initiate the analysis.
7. The tool will display the prediction of potential genes in the genomic DNA.

OBSERVATIONS:

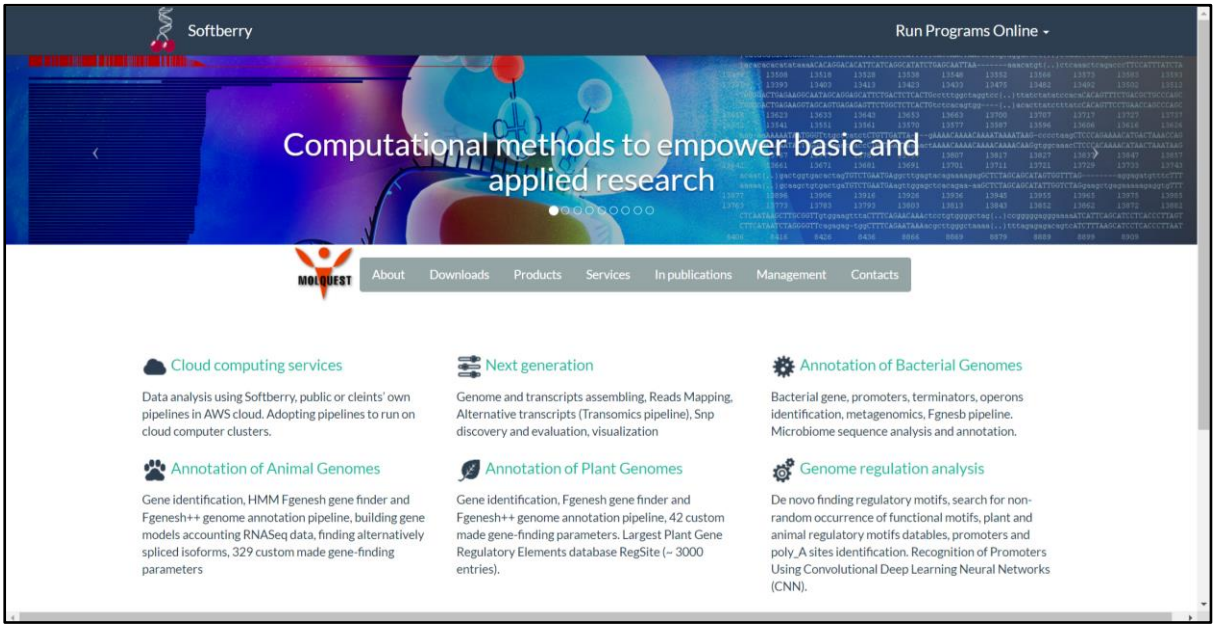

Fig 1: Homepage of Softberry server

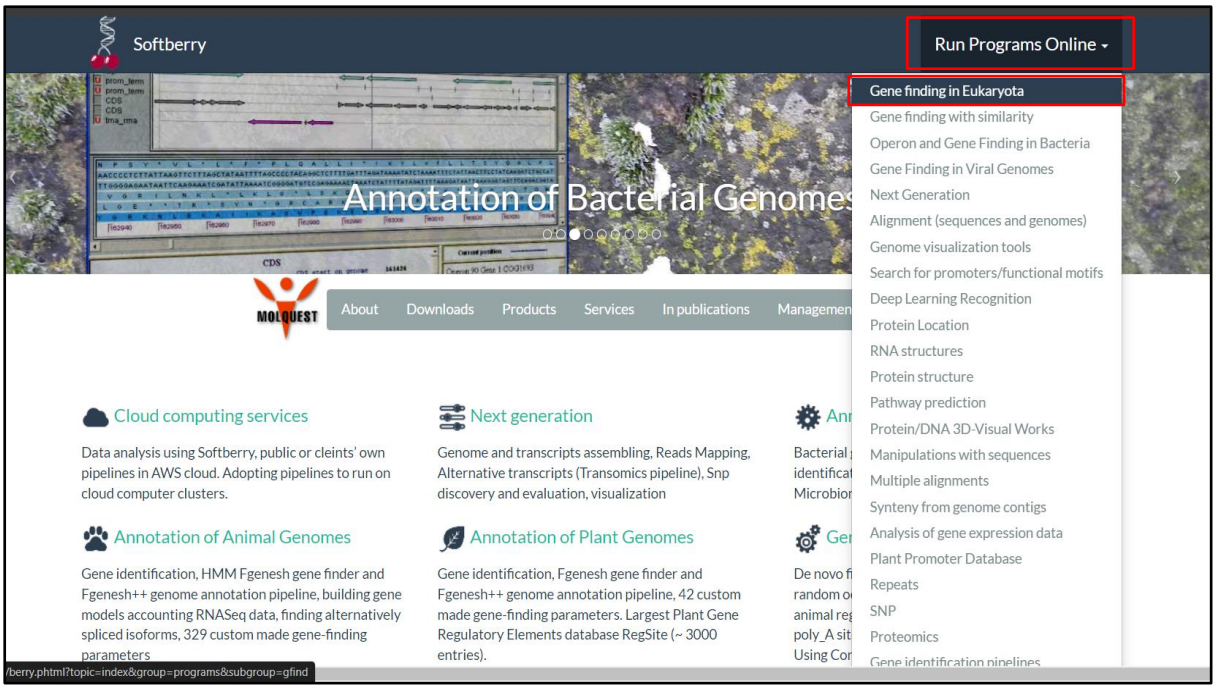

Fig 2: Select the program 'Gene Finding in Eukaryota'

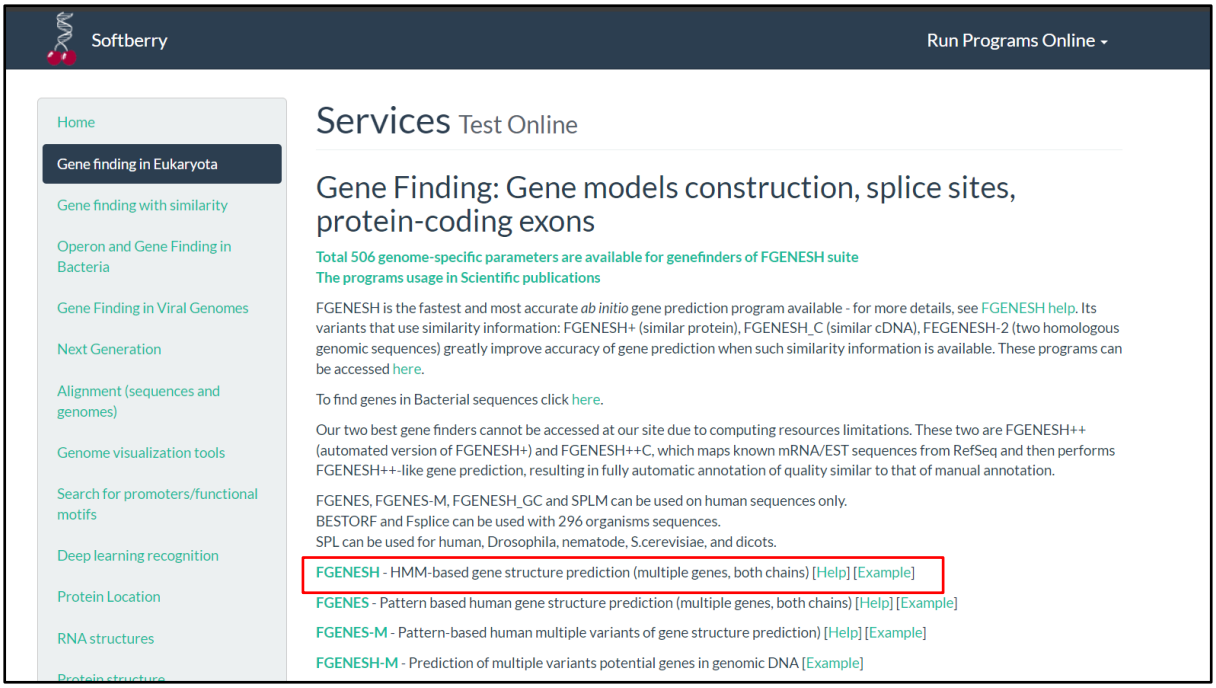

Fig 3: Selecting 'FGENESH - HMM-based gene structure prediction (multiple genes, both chains)'

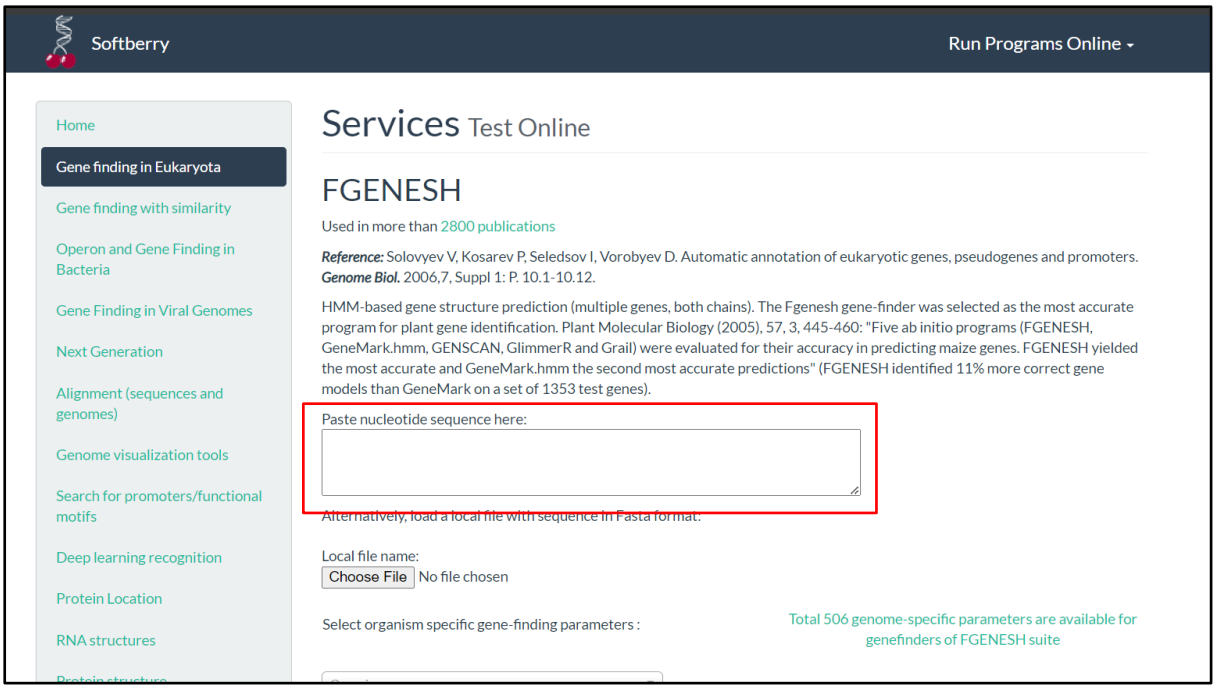

Fig 4: FGENESH Homepage where the query is to be pasted

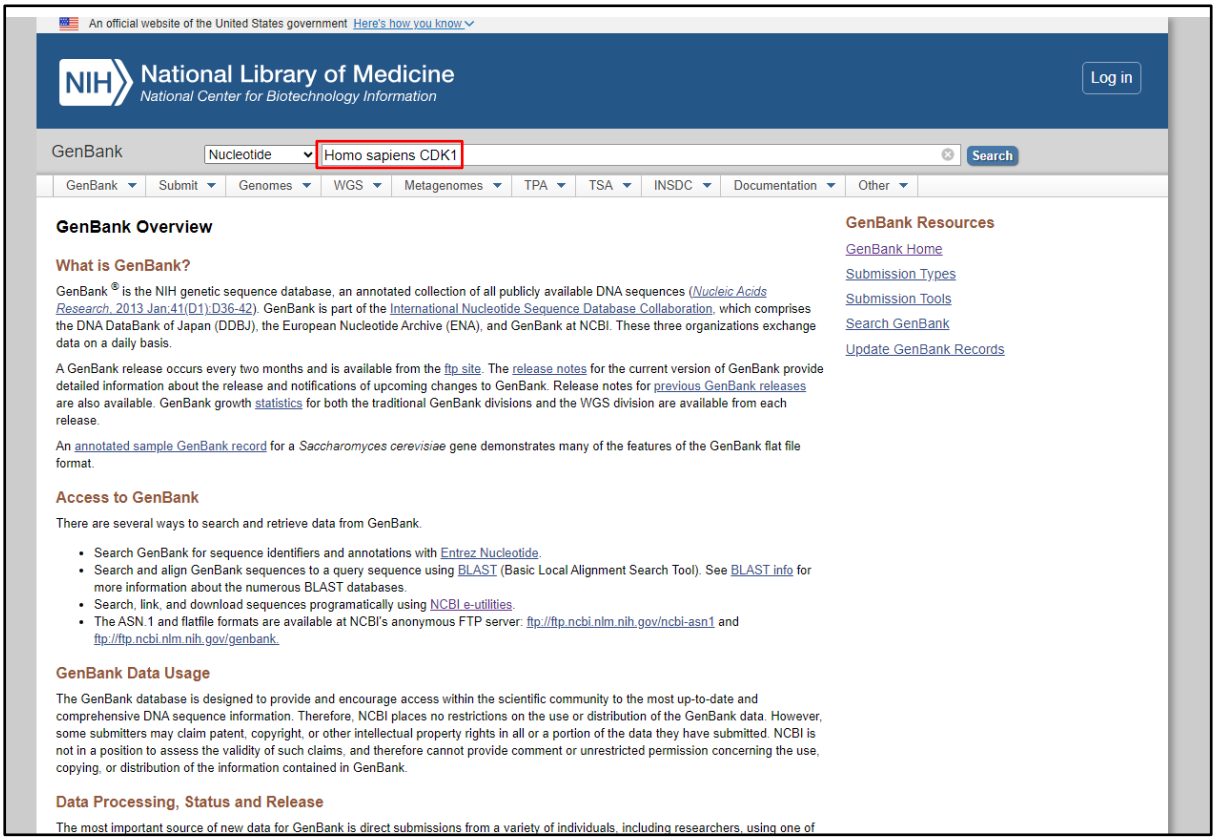

Fig 5: Homepage of GenBank database
Query searched for '*Homo sapiens* CDK1' Gene

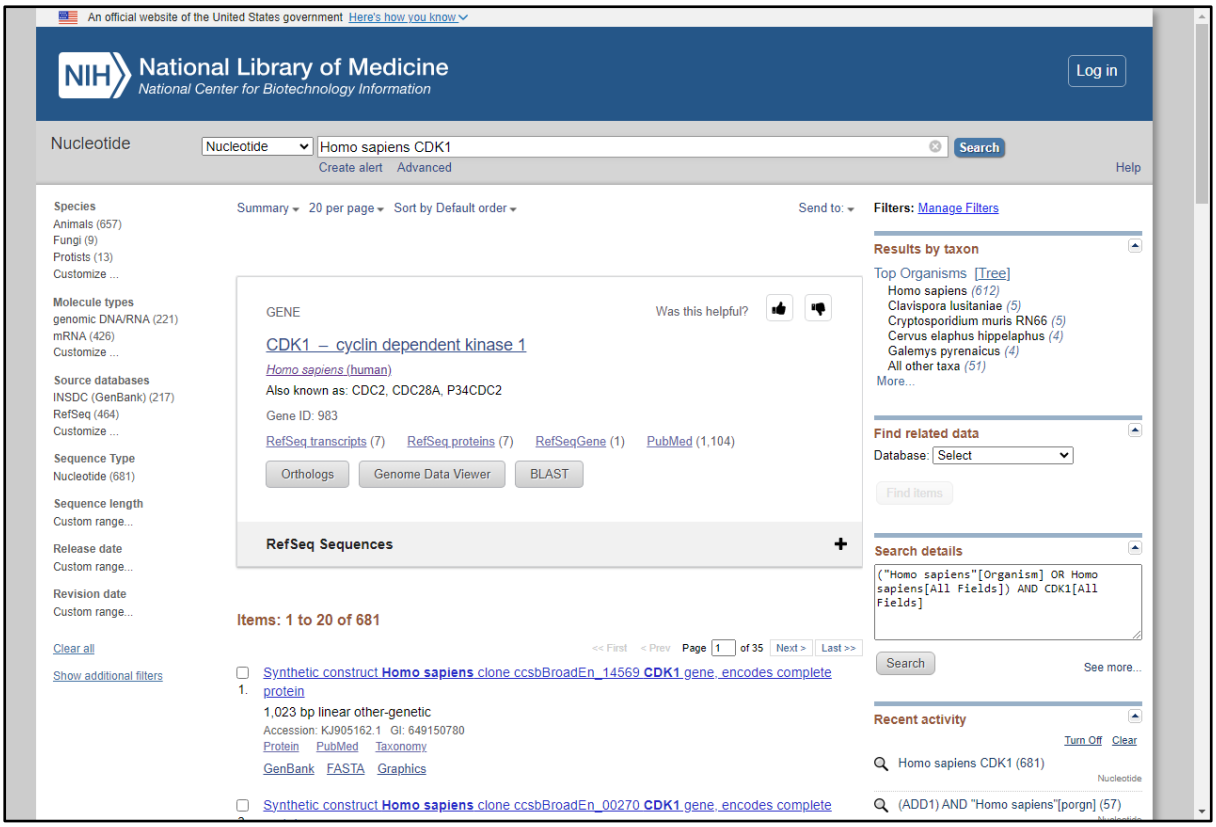

Fig 6: Result page

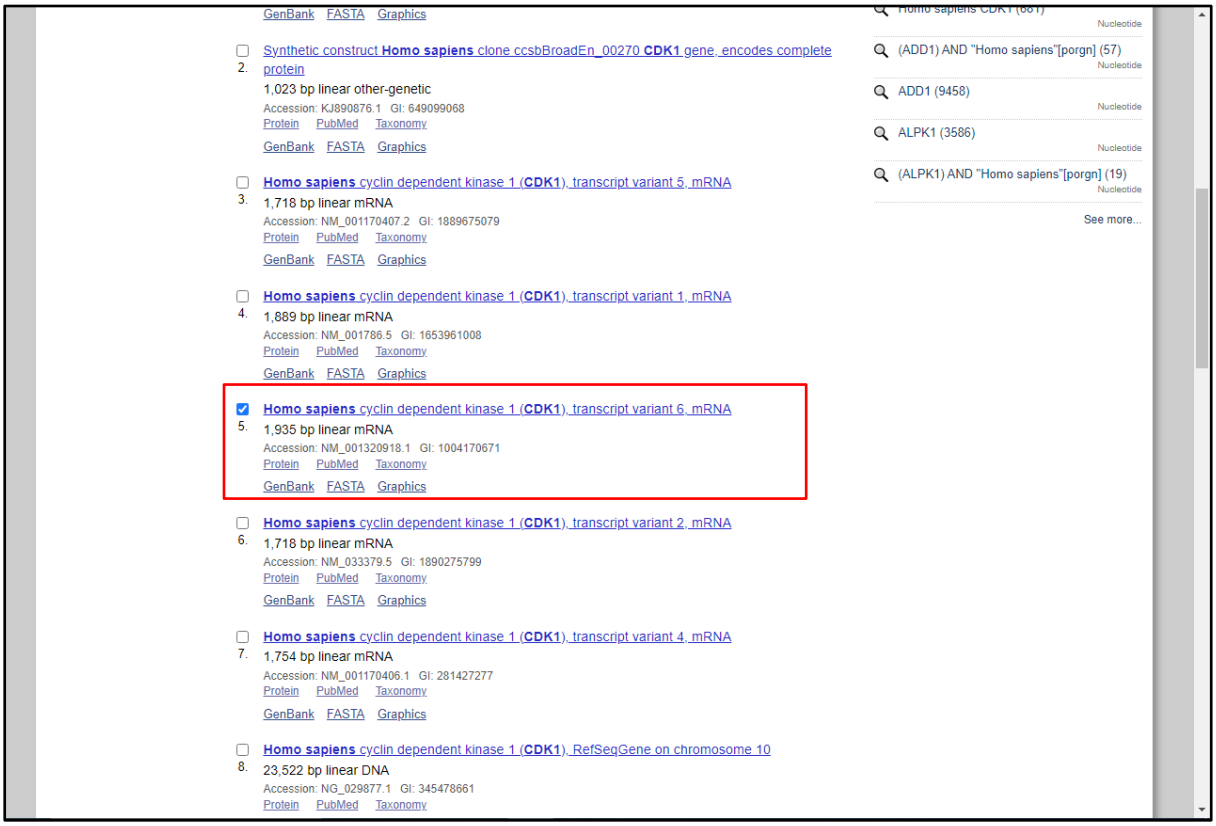

Fig 6.a: Query selected

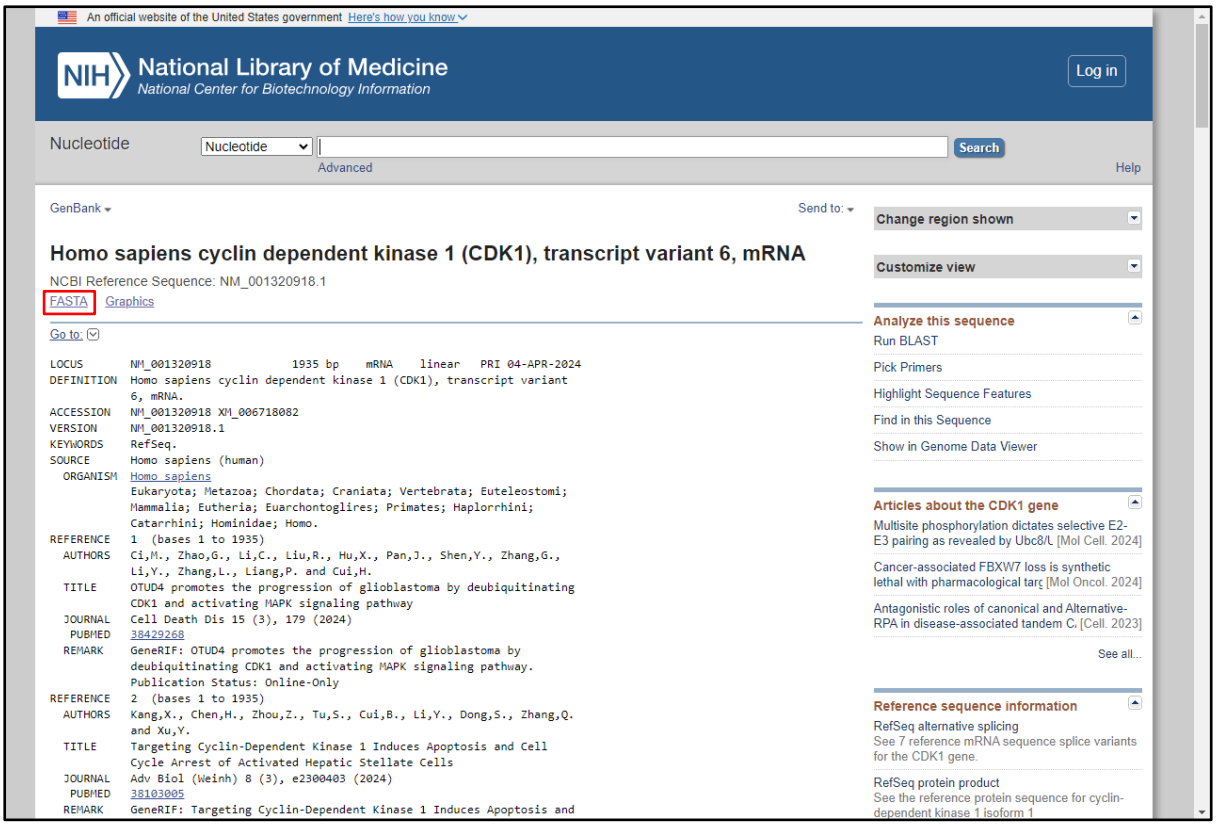

Fig 7: Entry Selected for the Query '*Homo sapiens* cyclin dependent kinase 1 (CDK1), transcript variant 6, mRNA'

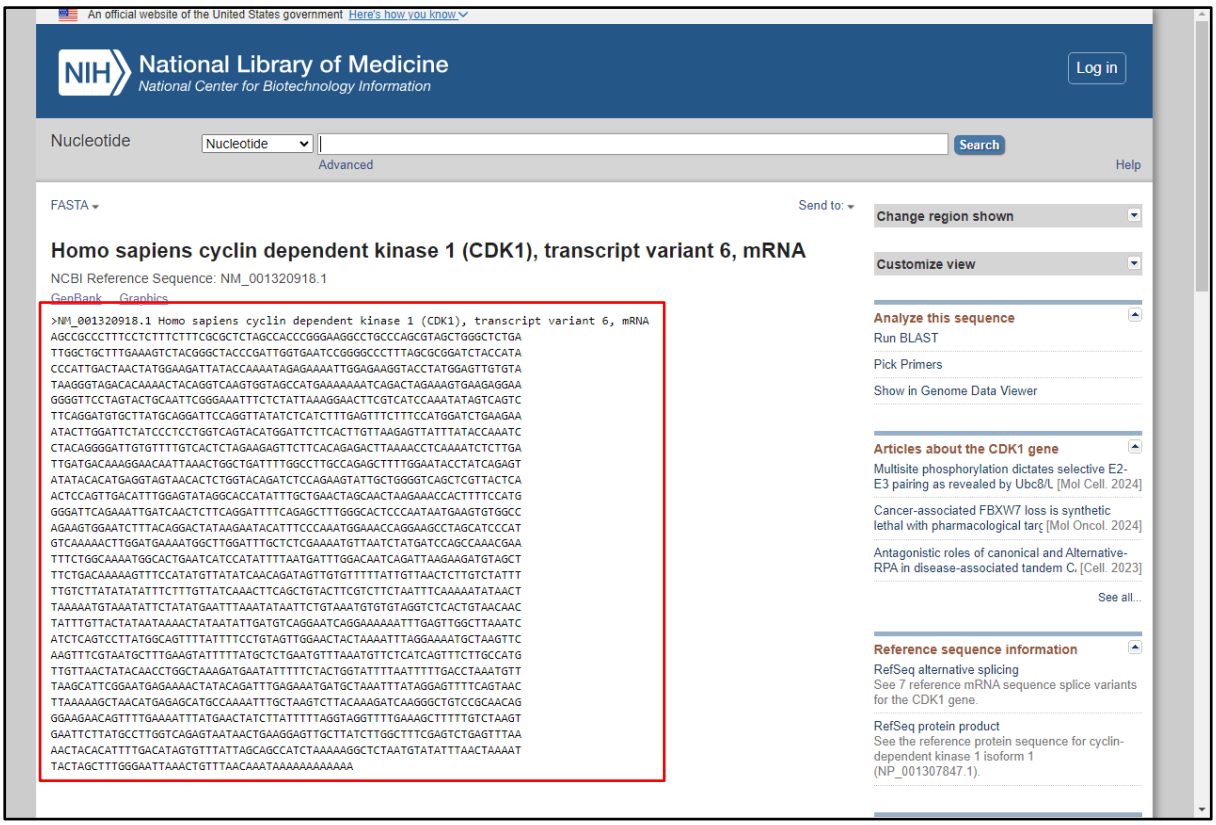

Fig 8: FASTA Format of the entry selected with header files

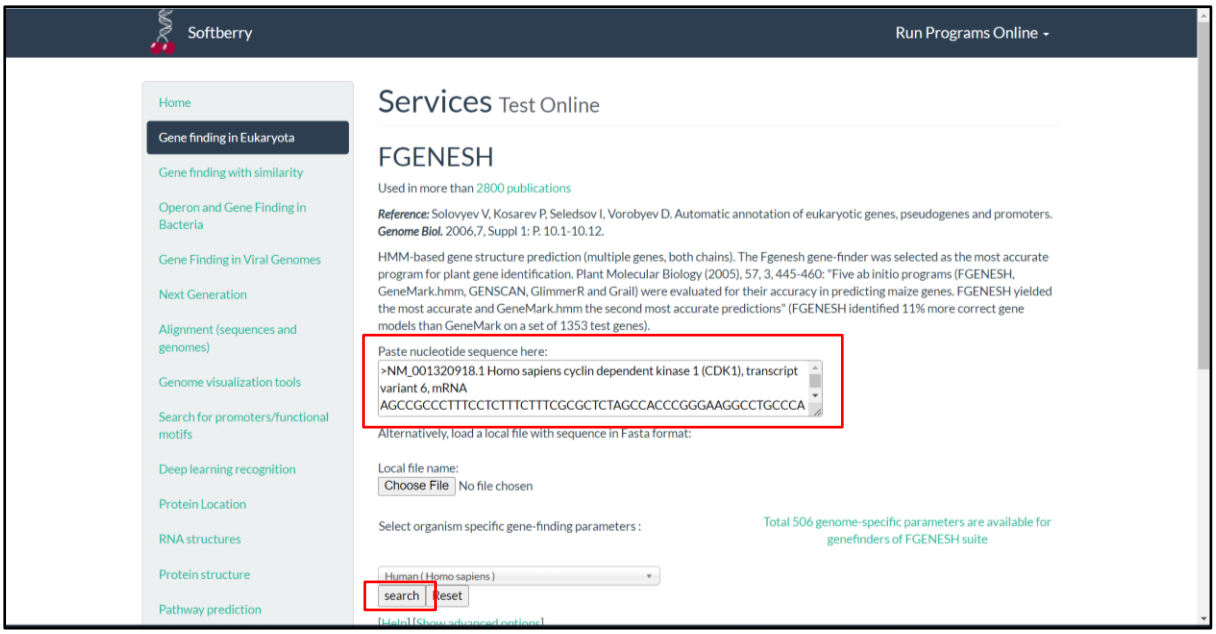

Fig 9: Paste the FASTA sequence in the Query box and click on search

Fig 10: FGENESH Output for the query '*Homo sapiens* CDK1' (GenBank ID: NM_001320918.1) gene

RESULTS:

The FGENESH program predicted one gene within the genomic DNA sequence of the CDK1 (GenBank ID: NM_001320918.1) gene. This gene is composed of one exon contributing to the protein sequence. The predicted protein sequence consists of 297 amino acids, translated from the exons, and exhibits characteristic features of the DDIT3 transcripts. The prediction is supported by high scores assigned to the exon, indicating a high degree of confidence in the gene prediction.

CONCLUSION:

FGENESH program is the fastest and most accurate, ab initio - HMM-based eukaryotic gene prediction program. The *Homo sapiens* CDK1 (GenBank ID: NM_001320918.1) likely encodes for a protein consisting of 297 amino acids. The predicted protein exhibits feature consistent with its role in cell cycle regulation and modulation of RNA polymerase activity, suggesting its potential involvement in processes such as protein synthesis and cell cycle entry. Further experiments are necessary for verification.

Abbreviation:

- G - predicted gene number, starting from start of sequence;
- Str - DNA strand (+ for direct or - for complementary);
- Feature - type of coding sequence:
- CDSf - First (Starting with Start codon);
- CDSi - internal (internal exon);
- CDSl - last coding segment, ending with stop codon);
- TSS - Position of transcription start (TATA-box position and score);
- Start and End - Position of the Feature;
- Weight - Log likelihood*10 score for the feature;
- ORF - start/end positions where the first complete codon starts and the last codon ends.

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DATE: 06/03/2024

WEBLEM 1(B)

FGENESB: Bacterial Operon and Gene Prediction

(URL:http://www.softberry.com/berry.phtml?topic=fgenesb&group=programs&subgroup=gfindb)

AIM:

To predict bacterial operon and gene for query *'Sulfobacillus thermosulfidooxidans'* (GenBank ID: NZ_MDZD01000033.1) using FGENESB tool.

INTRODUCTION:

FGENESB proposes an assortment of algorithms which can assign operon as well as to determine promoters, terminators and protein coding gene. Besides, the program can be used to determine both tRNA and rRNA genes. FGENESB pipeline that provides completely automatic and comprehensive annotation of microbial genomic sequence. The pipeline identifies protein, tRNA and rRNA genes, finds potential promoters, terminators and operons. Finally potential functions are assigned to predicted proteins using comparison with a set of databases such as COG, KEGG and NR. The package provides options to work with a set of sequences such as scaffolds of bacterial genomes or short reads of DNA extracted from an environmental sample of entire bacterial community. The gene prediction algorithm is based on Markov chain models of coding regions, start of translation and termination sites. Operon models are derived using distances between ORFs, frequencies of neighboring genes in known bacterial genomes and positions of predicted promoters and terminators. The parameters of gene prediction are automatically learned on initial steps of sequence analysis, so the only input necessary for annotation of a new genome is its sequence. The pipeline is actively used in many new genome-sequencing projects and provides a superior accuracy of gene finding (comparing with the other popular bacterial gene finding software) in bacterial contigs and especially in short sequences analyzed in metagenomic projects.

Sulfobacillus thermosulfidooxidans:

Sulfobacillus thermosulfidooxidans stands out as a thermophilic, acidophilic bacterium renowned for its capability to thrive in harsh environments such as acid hot springs, demonstrating a remarkable ability to biosorb heavy metal ions. Its potential in biotechnological recycling, particularly in the context of hazardous waste PCBs, has garnered attention, with ongoing efforts focused on optimizing processes and studying kinetics to enhance efficiency.

Furthermore, investigations into its interactions with other microorganisms, such as *Leptospirillum ferriphilum*, shed light on its biofilm formation and leaching performance dynamics. Coaggregation phenomena during dual-species biofilm formation contribute to its bioleaching efficacy, although outcomes vary depending on *L. ferriphilum's* preculture state. Additionally, studies exploring its tolerance mechanisms to nickel ions and the impact of iron

and media composition on arsenopyrite bioleaching highlight its versatility and potential for diverse industrial and environmental applications.

In essence, *Sulfobacillus thermosulfidooxidans* emerges as a promising candidate for biotechnological endeavors, offering insights into heavy metal biosorption, bioleaching, and microbial interactions, with implications spanning from waste management to resource recovery.

METHODOLOGY:

1. Visit the website http://www.softberry.com/
2. Go to the homepage, click on the 'Run Programs online' section and choose the 'Operon and Gene finding in bacteria' in it.
3. Within the "Services Test Online" section, select " FGENESB."
4. Proceed to the GenBank website and enter the query like '*Sulfobacillus thermosulfidooxidans*' and click on search.
5. Identify a relevant entry, extract its FASTA sequence.
6. Paste the acquired sequence into the nucleotide sequence box on the FGENESB page.
7. Pick a specific organism from the 'Choose closest organism' section and proceed by clicking on the process button.

OBSERVATIONS:

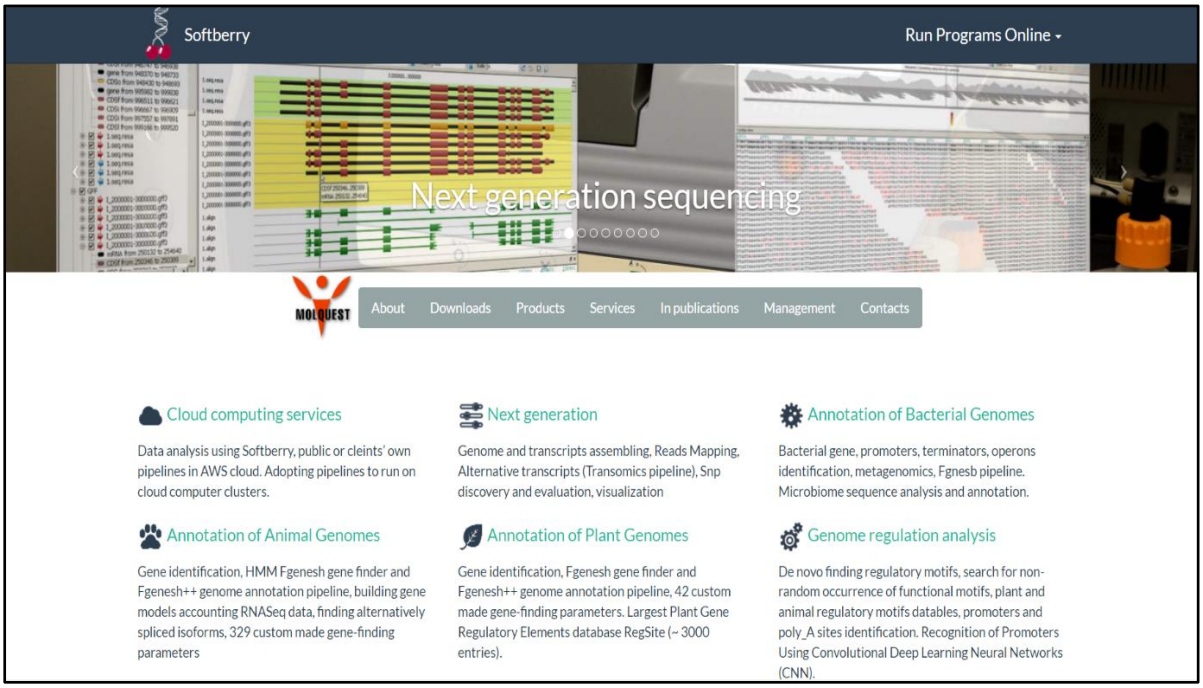


Fig 1: Homepage of Softberry server

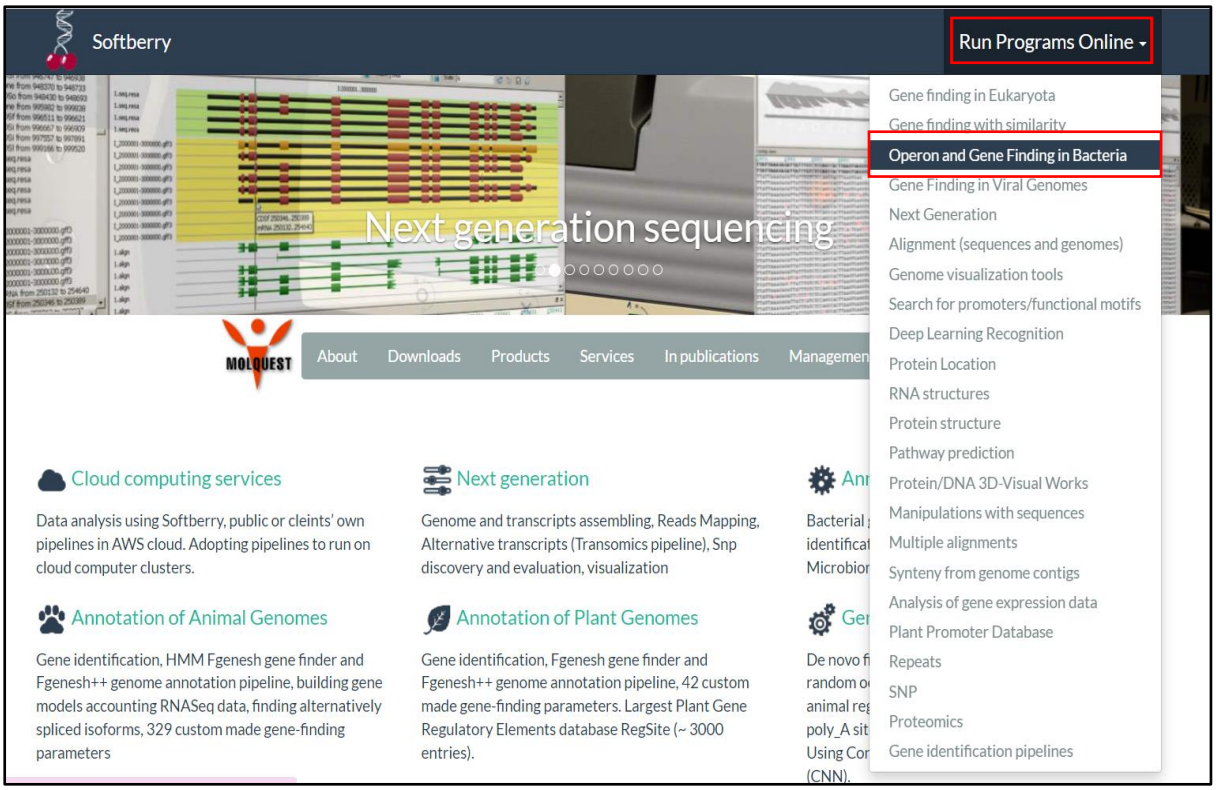


Fig 2: Select the option 'Operon and Gene Finding in Bacteria'

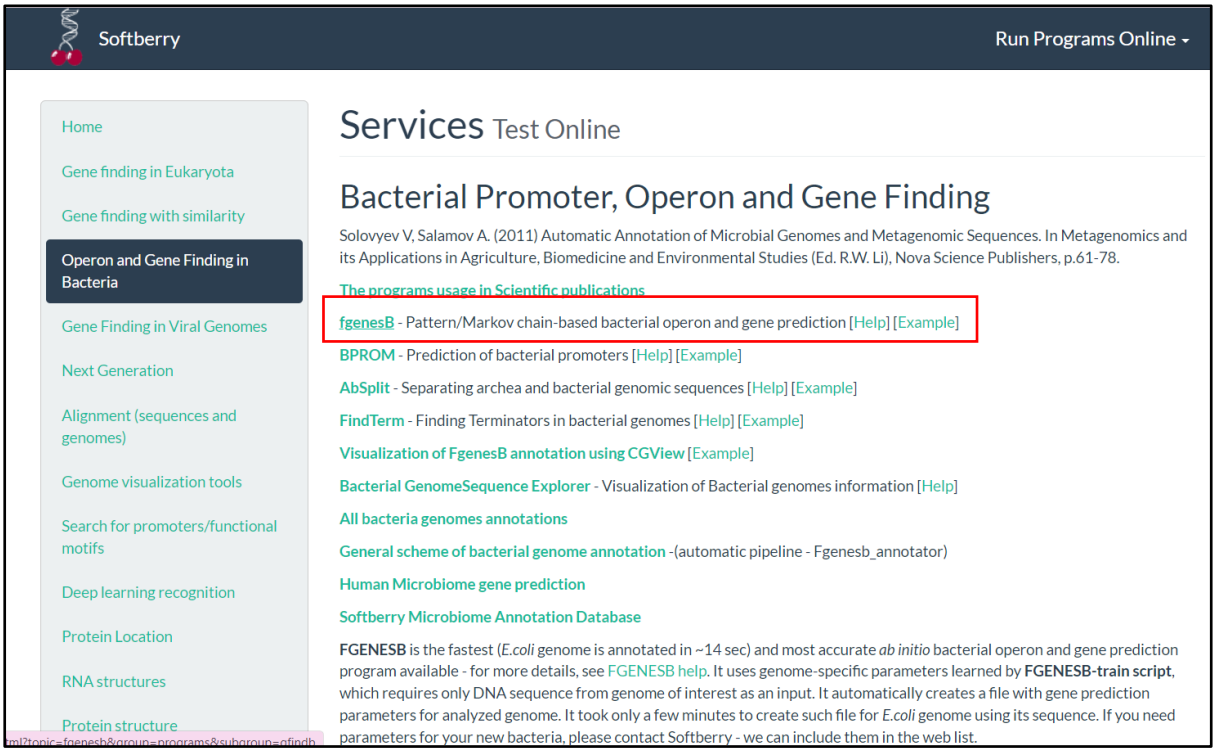


Fig 3: Select the 'FGENESB' option from the list of software

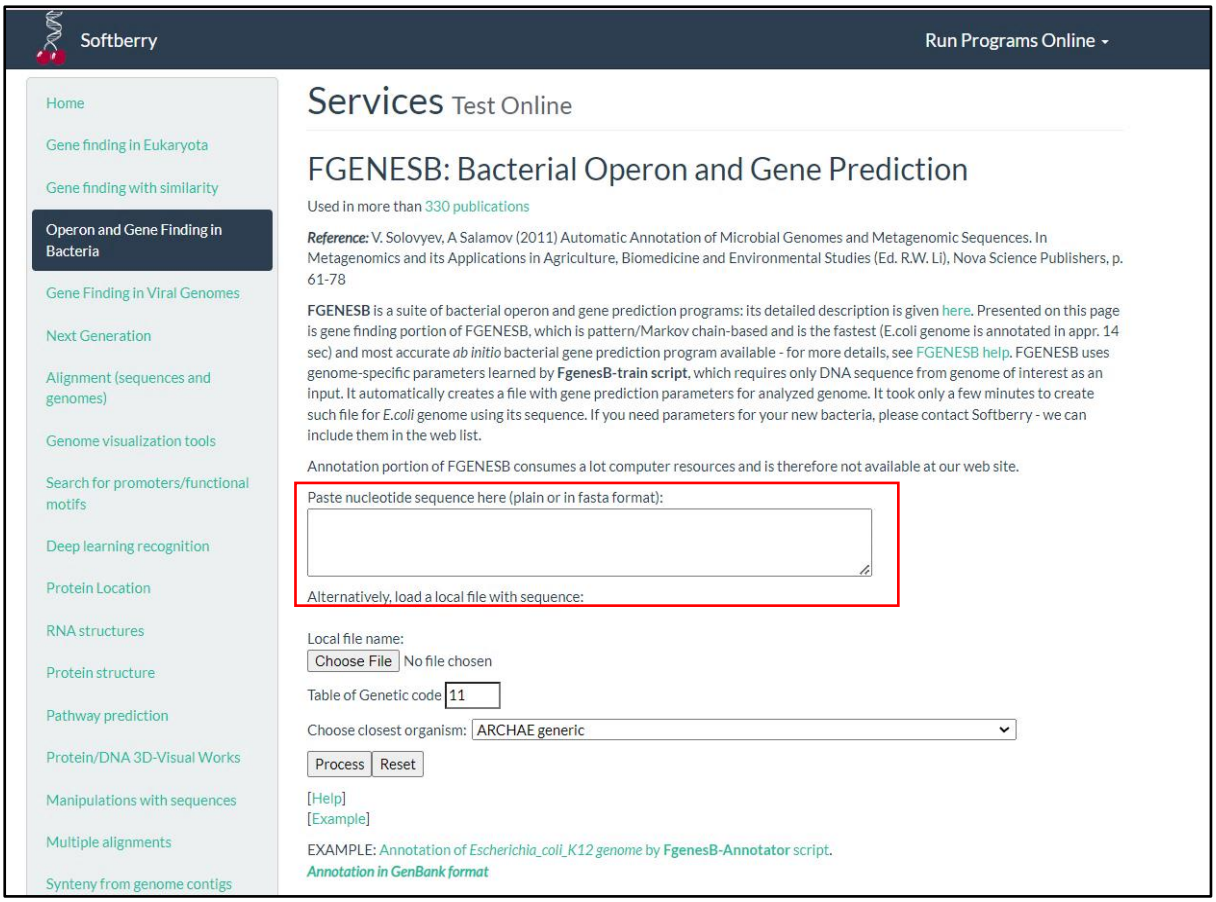


Fig 4: FGENESB software main page where the sequence is to be pasted and processed

Fig 5: Homepage of GenBank Database (with the query '*Sulfobacillus thermosulfidooxidans*')

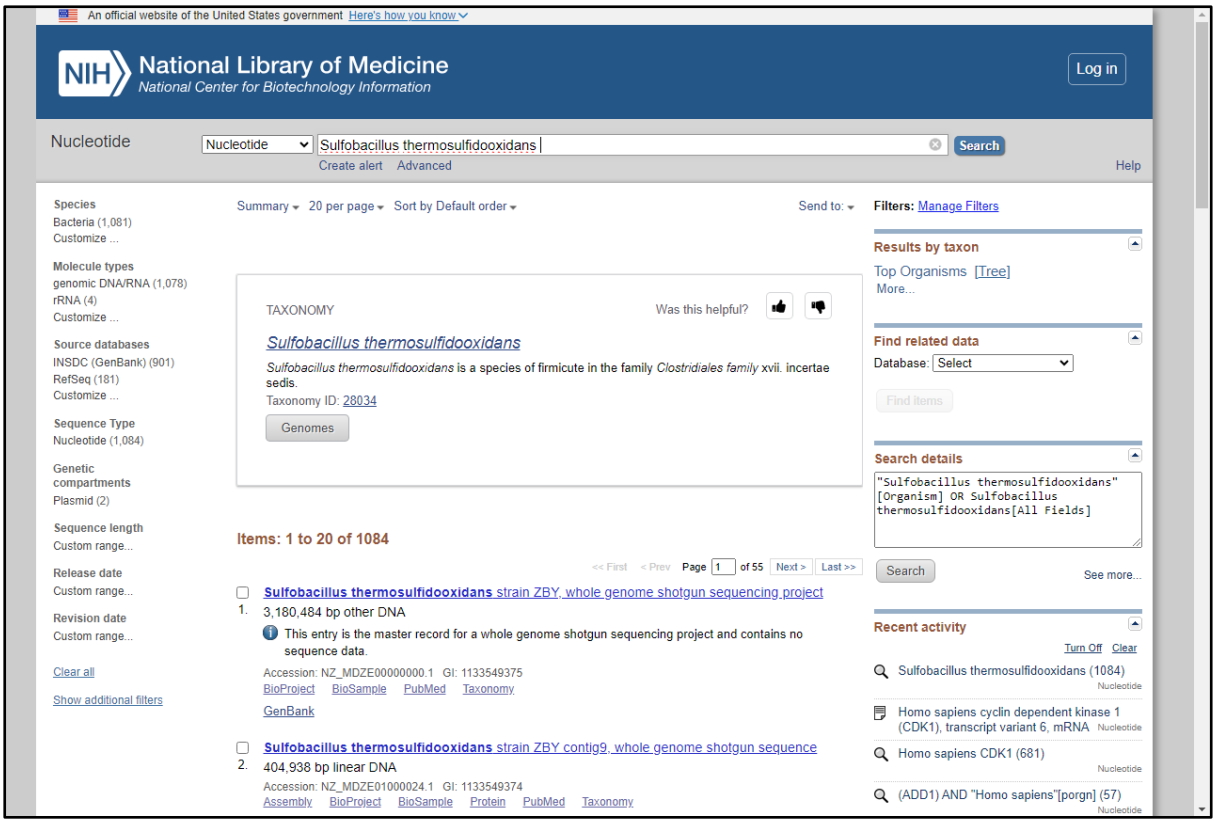


Fig 6: Result page obtained

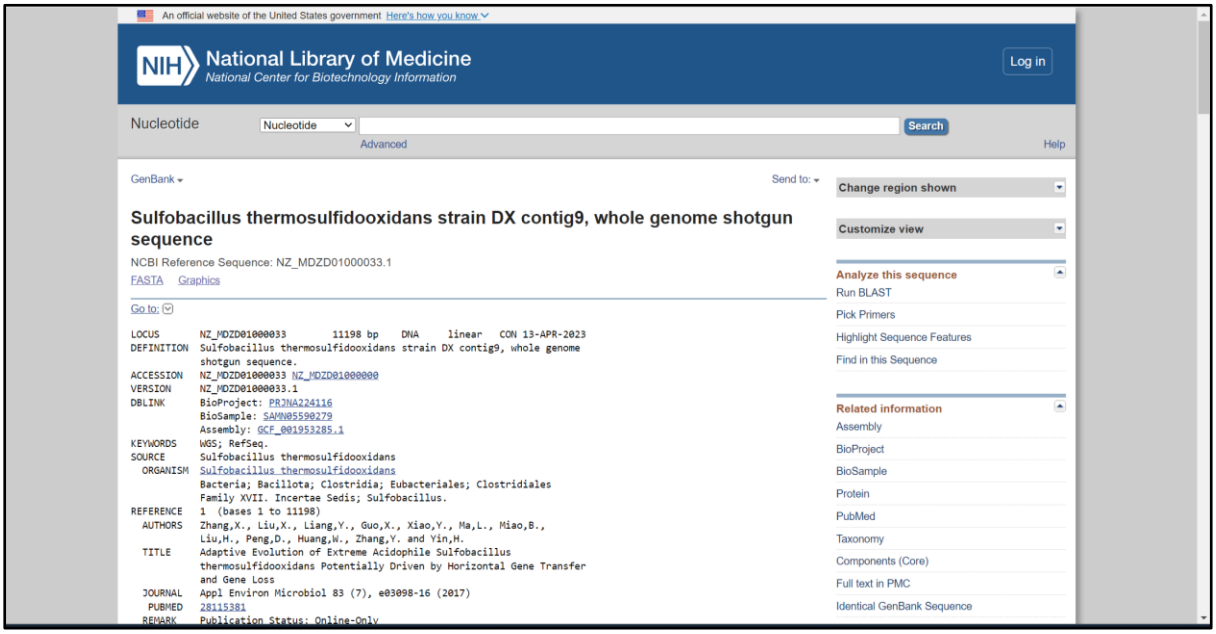


Fig 7: Query selected for study

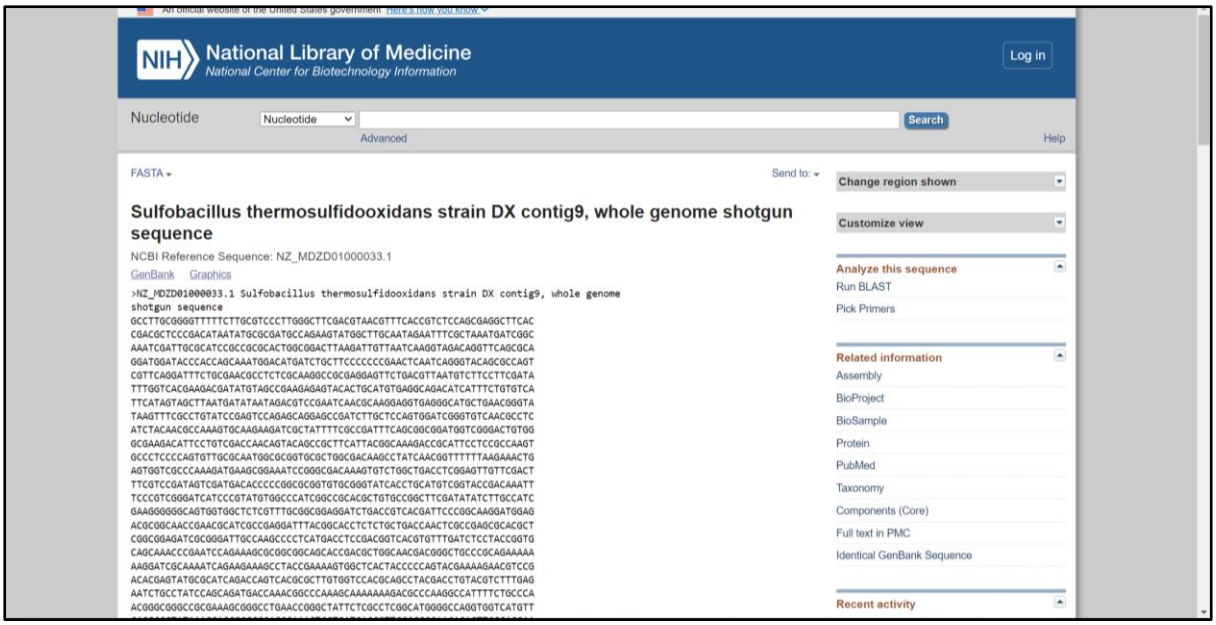


Fig 8: FASTA Sequence of the selected query

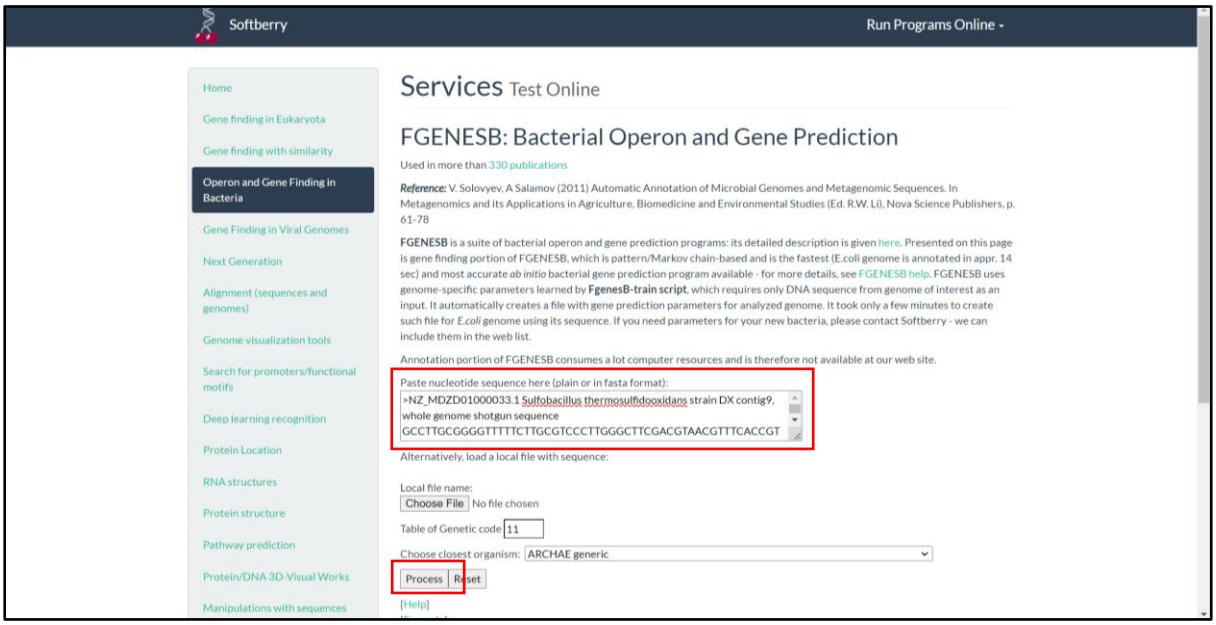
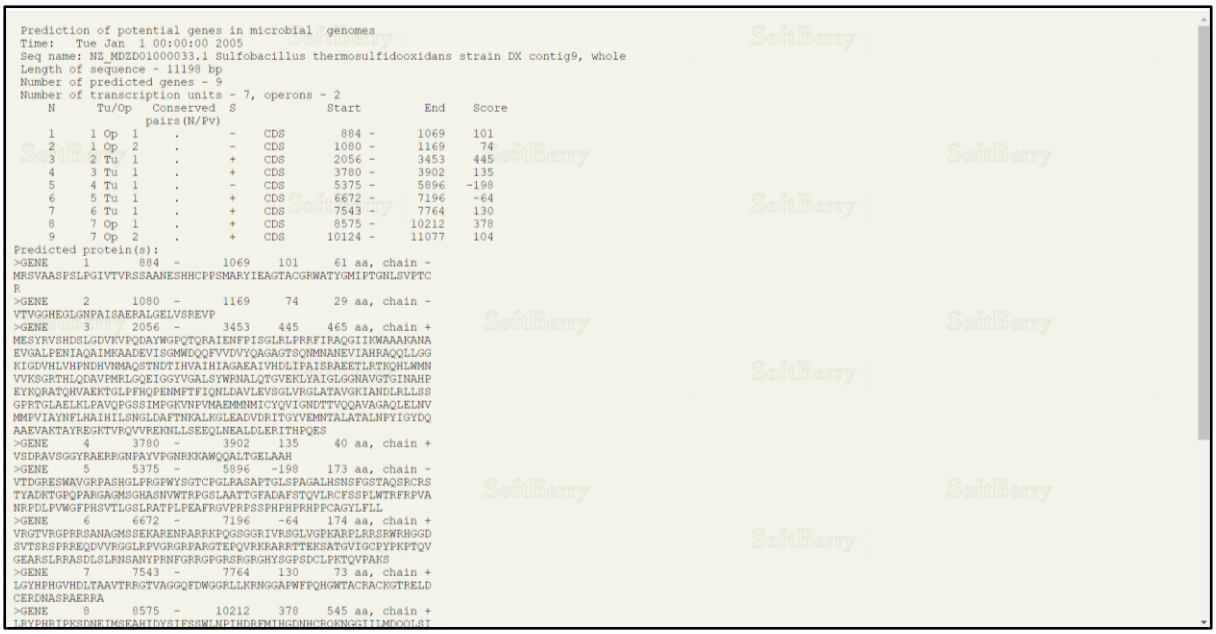


Fig 9: Paste sequence in the nucleotide sequence section, choose a closest organism and click on the process button



```
Prediction of potential genes in microbial genomes
Time:   Tue Jan  1 00:00:00 2005
Seq name: NZ_MDZD01000033.1 Sulfobacillus thermosulfidooxidans strain DX contig9, whole
Length of sequence - 11198 bp
Number of predicted genes - 9
Number of transcription units - 7, operons - 2
    N      Tu/Op   Conserved  S             Start         End    Score
                   pairs(N/Pv)
    1     1 Op  1     .       -    CDS        884 -      1069    101
    2     1 Op  2     .       -    CDS       1080 -      1169     74
    3     2 Tu  1     .       +    CDS       2056 -      3453    445
    4     3 Tu  1     .       +    CDS       3780 -      3902    135
    5     4 Tu  1     .       -    CDS       5375 -      5896   -198
    6     5 Tu  1     .       +    CDS       6672 -      7196    -64
    7     6 Tu  1     .       +    CDS       7543 -      7764    130
    8     7 Op  1     .       +    CDS       8575 -     10212    378
    9     7 Op  2     .       +    CDS      10124 -     11077    104
Predicted protein(s):
>GENE      1       884  -      1069     101      61 aa, chain -
MRSVAASPSLPGIVTVRSSAANESHHCPPSMARYIEAGTACGRWATYGMIPTGNLSVPTC
R
>GENE      2      1080  -      1169      74      29 aa, chain -
VTVGGHEGLGNPAISAERALGELVSREVP
>GENE      3      2056  -      3453     445     465 aa, chain +
MESYRVSHDSLGDVKVPQDAYWGPQTQRAIENFPISGLRLPRFFIRAQGIIKWAAAKANA
EVGALPENIAQAIMKAADEVISGMWDQQFVVDVYQAGAGTSQNMNANEVIAHRAQQLLGG
KIGDVHLVHPNDHVNMAQSTNDTIHVAIHIAGAEAIVHDLIPAISRAEETLRTKQHLWMN
VVKSGRTHLQDAVPMRLGQEIGGYVGALSYWRNALQTGVEKLYAIGLGGNAVGTGINAHP
EYKQRATQHVAEKTGLPFHQPENMFTFIQNLDAVLEVSGLVRGLATAVGKIANDLRLLSS
GPRTGLAELKLPAVQPGSSIMPGKVNPVMAEMMNMICYQVIGNDTTVQQAVAGAQLELNV
MMPVIAYNFLHAIHILSNGLDAFTNKALKGLEADVDRITGVVEMNTALATALNPYIGYDQ
AAEVAKTAYREGKTVRQVVREKNLLSEEQLNEALDLERITHPQES
>GENE      4      3780  -      3902     135      40 aa, chain +
VSDRAVSGGYRAERRGNPAYVPGNRKKAWQQALTGELAAH
>GENE      5      5375  -      5896    -198     173 aa, chain -
VTDGRESWAVGRPASHGLPRGPWYSGTCPGLRASAPTGLSPAGALHSNSFGSTAQSRCRS
TYADKTGPQPARGAGMSGHASNVWTRPGSLAATTGFADAFSTQVLRCFSSPLWTRFRPVA
NRPDLPVWGFPHSVTLGSLRATPLPEAFRGVPRPSSPHPHFRHPFCAGYLFLL
>GENE      6      6672  -      7196     -64     174 aa, chain +
VRGTVRGPRRSANAGMSSEKARENRARRKPQSGGGRIVRSGLVGPKARPLRRSRWRHGGD
SVTSRSPRREQDVVRGGLRPVGRGRPARGTEPQVRKRARRTTEKSATGVIGCPYPKPTQV
GEARSLRRASDLSLRNSANYPRNFGRRGPGRSRGRGHYSGPSDCLPKTQVPAKS
>GENE      7      7543  -      7764     130      73 aa, chain +
LGYHPHGVHDLTAAVTRRGTVAGGQFDWGGRLLKRNGGAPWFPQHGWTACRACKGTRELD
CERDNASRAERRA
>GENE      8      8575  -     10212     378     545 aa, chain +
LRYPHRIPKSDNEIMSEAHIDYSIFSSWLNPIHDRFMIHGDNHCPQKNGGIILMDQQLSI
TESGWVFGPAVKGIRMDSRLVQPGDVFIAVSGSLLDGHEFIQQAIDKGAVAIVGEKPMGT
LPVPYIQVQSSREAAADIATSFYRHPSKDLVTVAVTGTNGKTSVVYWLSAILNAANIKTG
LVSSVVNDIVCRKQPASLTTPESPDLQAYLDQIRNCGGKAAVVEVSSHGIVQQRVRGTIF
QLAILTNITREHLDFHGTMERYVDAKATLFKMLPPNSLGAVLNADDQHFETVAKASPAPV
TSYGIKAGDIRGQVLQETAWSTDIRVSGLNLDFITPVHHPGRYNVYIHVLAVTAAAIRLGV
TQEVLQREIPNLPSVPGRMQITGGQRGPMAVVDYAHTPDGLYQSLLTVRKPTEGKVWLIF
GARGGRDRGKRPEMGRIAAQYADYVIVTADSPNFEDPQKIADALIEGIRQVDDTKLKLVE
LNRALAIHYAVRHAEKNDVILITGRGPETIQHFGNEDVRLVDAEVVEEALQERWRQEGSH
VPGIS
>GENE      9     10124  -     11077     104     317 aa, chain +
MYDLSTPKWLKRPSKNAGVPREAMSPELVSVVIPAYNAESTIVEALESVYQQSHKNLEVI
VVDDGSTDLTRQIIERQFPQVILRANHNGGPSQARNTGIDMAHGEWIAFLDADDVWHPEK
IAQQLDVAHTDPEIGLVASDWIRDAHFLDVPRALPVSEVRYHDLLILNRFQTSTVLVKRS
IVQSLQGFDRRVDGAEDWDLWLRVAQVARVVKIDWPLVMYRDVPTGYSKDVWRVYITMLP
MLDKHREEVQVSHHEFRVIEAWHHLRFFVAFILMKQYDKAFRAVKPAFSLRLLSGLFPAL
YRYLFPFLWTRIKKKSA
```


Fig 10: FGENESB Output for the query *'Sulfobacillus thermosulfidooxidans'*
Key: 'Tu' denotes Transcription units
'Op' denotes Operons

Fig 10.1: FGENESB Output for the query '*Sulfobacillus thermosulfidooxidans*'

RESULTS:

Using FGENESB, the *Sulfobacillus thermosulfidooxidans* strain (GenBank ID: NZ_MDZD01000033.1) was analyzed for potential genes, revealing 9 predicted genes within a sequence length of 11198 bp. Among these, 7 transcription units and 2 operons were present. The 'tu' in the results denotes the Transcription units and 'op' in the results denotes the Operon. The analysis provided details on the start and end points of the genes, their scores, chain affiliations, and gene sequences.

CONCLUSION:

FGENESB gene prediction algorithm is based on Markov chain models and program uses genome-specific parameters learned by FGENESB-train script, which requires only DNA sequence from genome of interest as an input to give its prediction of termination units and operon for the given sequence. The required predictive result for the query *Sulfobacillus thermosulfidooxidans* strain (GenBank ID: NZ_MDZD01000033.1) was obtained.

REFERENCES:

1. FGENESB description. (n.d.). https://www.molquest.com/help/2.3/programs/FGENESB/description.html
2. Huang, Y., Li, M., Yang, Y., Zeng, Q., Loganathan, P., Hu, L., Zhong, H., & He, Z. (2020, February 12). Sulfobacillus thermosulfidooxidans: an acidophile isolated from acid hot spring for the biosorption of heavy metal ions. *International Journal of Environmental Science and Technology*, *17*(5), 2655–2666. https://doi.org/10.1007/s13762-020-02669-1
3. Li, Q., Zhu, J., Li, S., Zhang, R., Xiao, T., & Sand, W. (2020, January 29). Interactions Between Cells of *Sulfobacillus thermosulfidooxidans* and *Leptospirillum ferriphilum* During Pyrite Bioleaching. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.00044

Date: 06/03/2024

WEBLEM 1(C)

BPROM: Operon and Gene finding Bacteria

(URL:http://www.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb)

AIM:

To predict bacterial promoter for query '*Sulfobacillus thermosulfidooxidans*' (GenBank ID: NZ_MDZD01000033.1) using BPROM tool.

INTRODUCTION:

BPROM is a bacterial promoter prediction program developed by Softberry to identify potential transcription start positions of bacterial genes regulated by sigma70 promoters, particularly in the major E. coli promoter class. The algorithm utilizes a linear discriminant function (LDF) that combines characteristics describing functional motifs and oligonucleotide composition of these sites. BPROM demonstrates an accuracy of about 80% in recognizing E. coli promoters, with a similar specificity when tested on sets containing equal numbers of promoter and non-promoter sequences. To enhance specificity, it is recommended to run BPROM on a region between two neighboring ORFs located on the same strand or on a sequence upstream from an ORF, considering that most promoters are situated within a 150 bp region from the protein coding sequence. The output of BPROM includes the name of the sequence, LDF threshold, length of the presented sequence, number of predicted promoters, positions of predicted promoters, their scores, and the weights of two conserved promoter boxes. Promoter positions are assigned to the first nucleotide of the transcript (Transcription Start Site position). Additionally, for each predicted promoter, elements of Transcriptional factor binding sites are presented if found. This tool serves as a valuable resource for researchers involved in bacterial genome analysis and gene prediction, contributing to the understanding of gene regulation mechanisms in bacteria.

***Sulfobacillus thermosulfidooxidans*:**

Sulfobacillus thermosulfidooxidans stands out as a thermophilic, acidophilic bacterium renowned for its capability to thrive in harsh environments such as acid hot springs, demonstrating a remarkable ability to biosorb heavy metal ions. Its potential in biotechnological recycling, particularly in the context of hazardous waste PCBs, has garnered attention, with ongoing efforts focused on optimizing processes and studying kinetics to enhance efficiency.

Furthermore, investigations into its interactions with other microorganisms, such as *Leptospirillum ferriphilum*, shed light on its biofilm formation and leaching performance dynamics. Coaggregation phenomena during dual-species biofilm formation contribute to its bioleaching efficacy, although outcomes vary depending on *L. ferriphilum's* preculture state. Additionally, studies exploring its tolerance mechanisms to nickel ions and the impact of iron

and media composition on arsenopyrite bioleaching highlight its versatility and potential for diverse industrial and environmental applications.

In essence, *Sulfobacillus thermosulfidooxidans* emerges as a promising candidate for biotechnological endeavors, offering insights into heavy metal biosorption, bioleaching, and microbial interactions, with implications spanning from waste management to resource recovery.

METHODOLOGY:

1. Visit the website http://www.softberry.com/ .
2. Go to the Homepage, click on the 'Run Program Online' and choose the 'Operon and Gene finding Bacteria' in it.
3. Choose BPROM within the 'services test online'.
4. Obtain the FASTA sequence for query '*Sulfobacillus thermosulfidooxidans'* from GenBank and paste it into the nucleotide sequence box on the BPROM page.
5. Proceed by clicking on the process button.

OBSERVATIONS:

Fig 1: Homepage of Softberry server

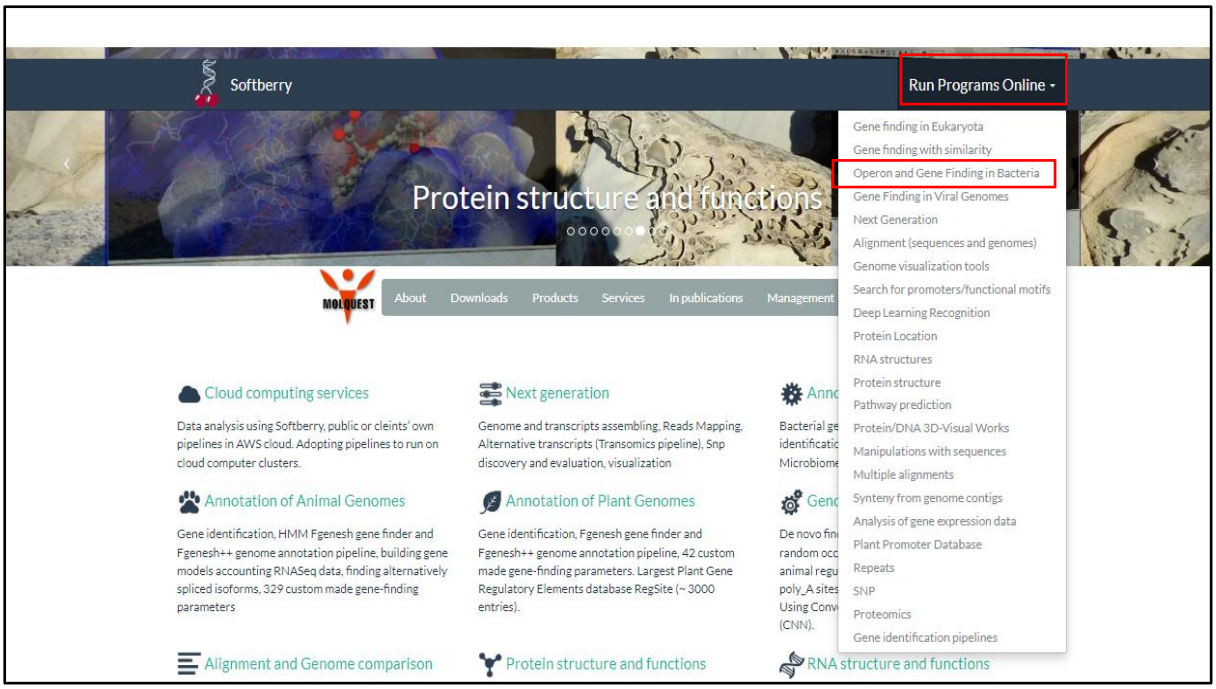


Fig 2: Select the option 'Operons and Gene finding in Bacteria'

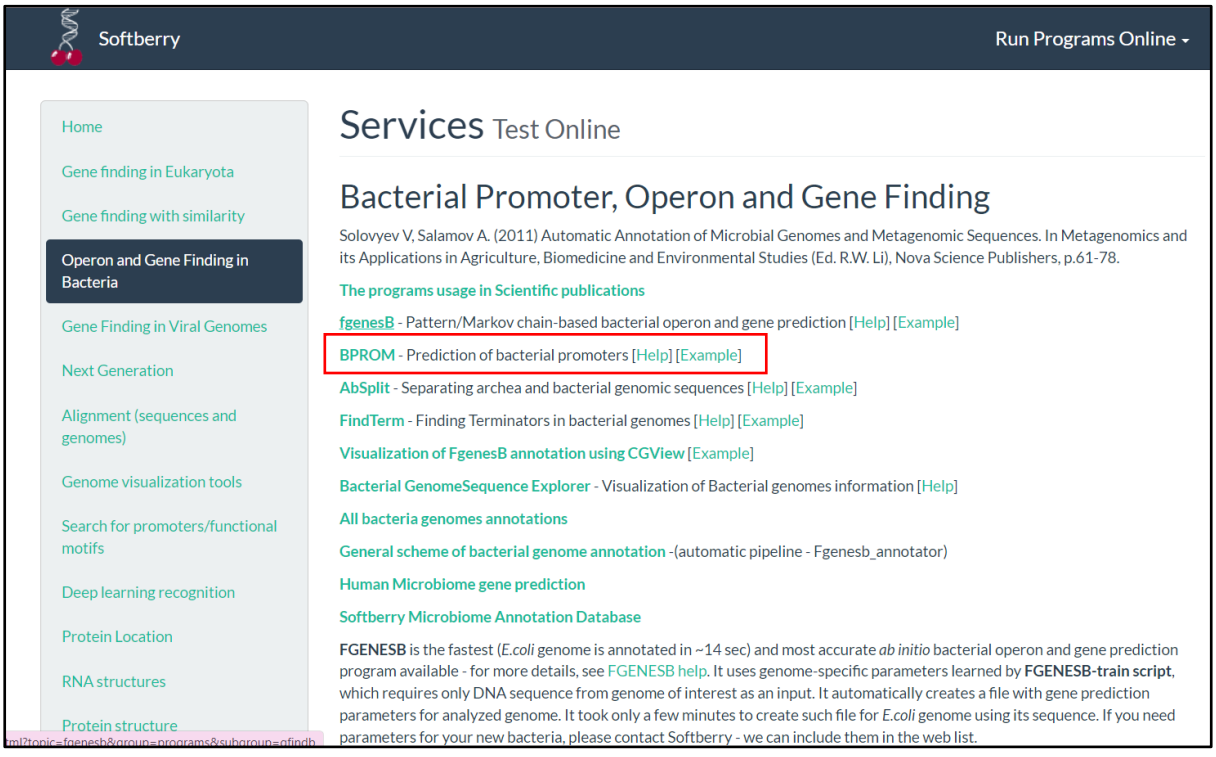


Fig 3: Select the BPROM option from the list of programs

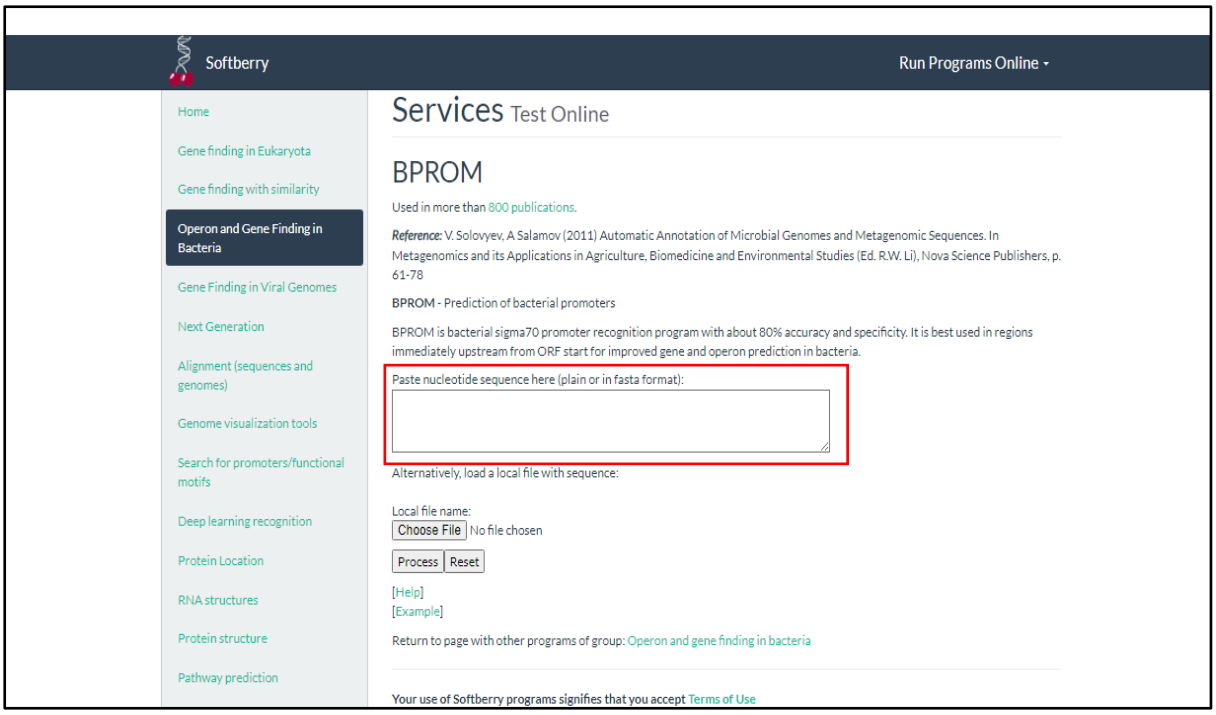


Fig 4: BPROM program main page where the sequence is to be pasted

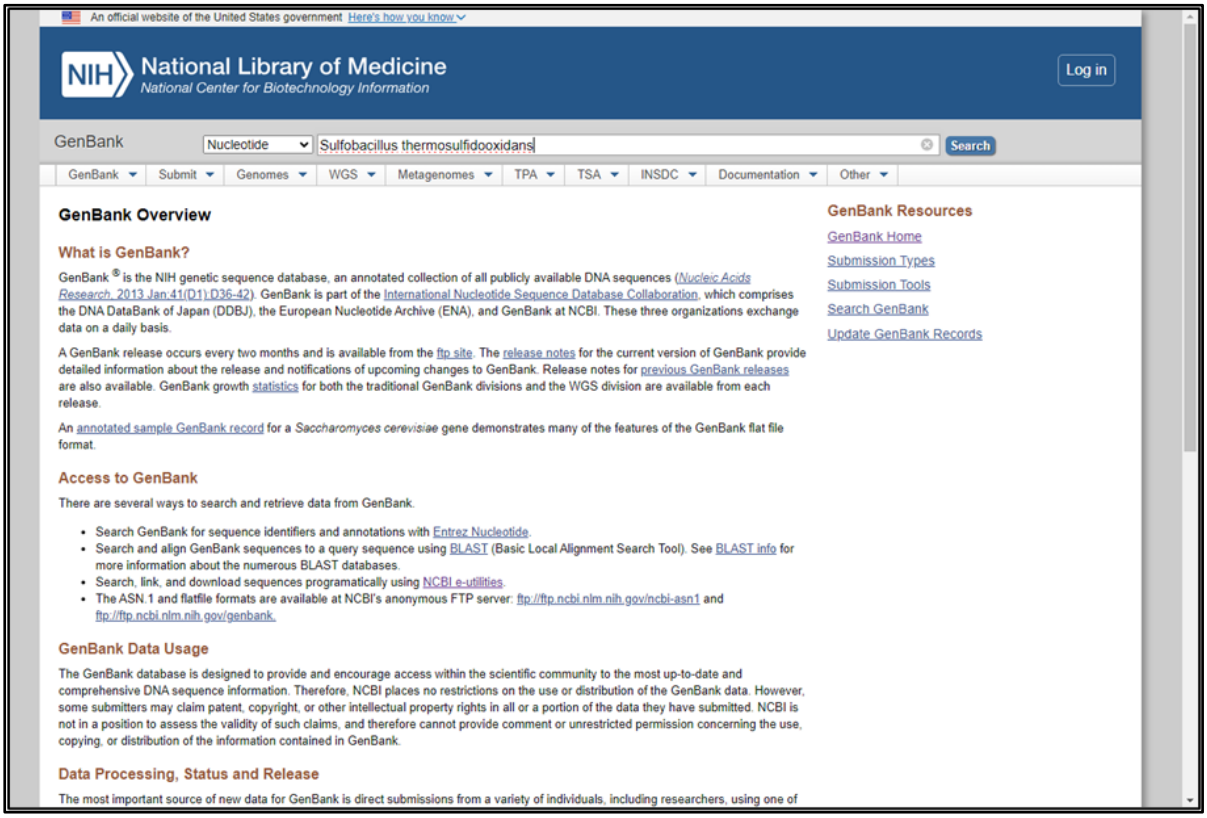


Fig 5: Homepage of GenBank Database (with the query '*Sulfobacillus thermosulfidooxidans*')

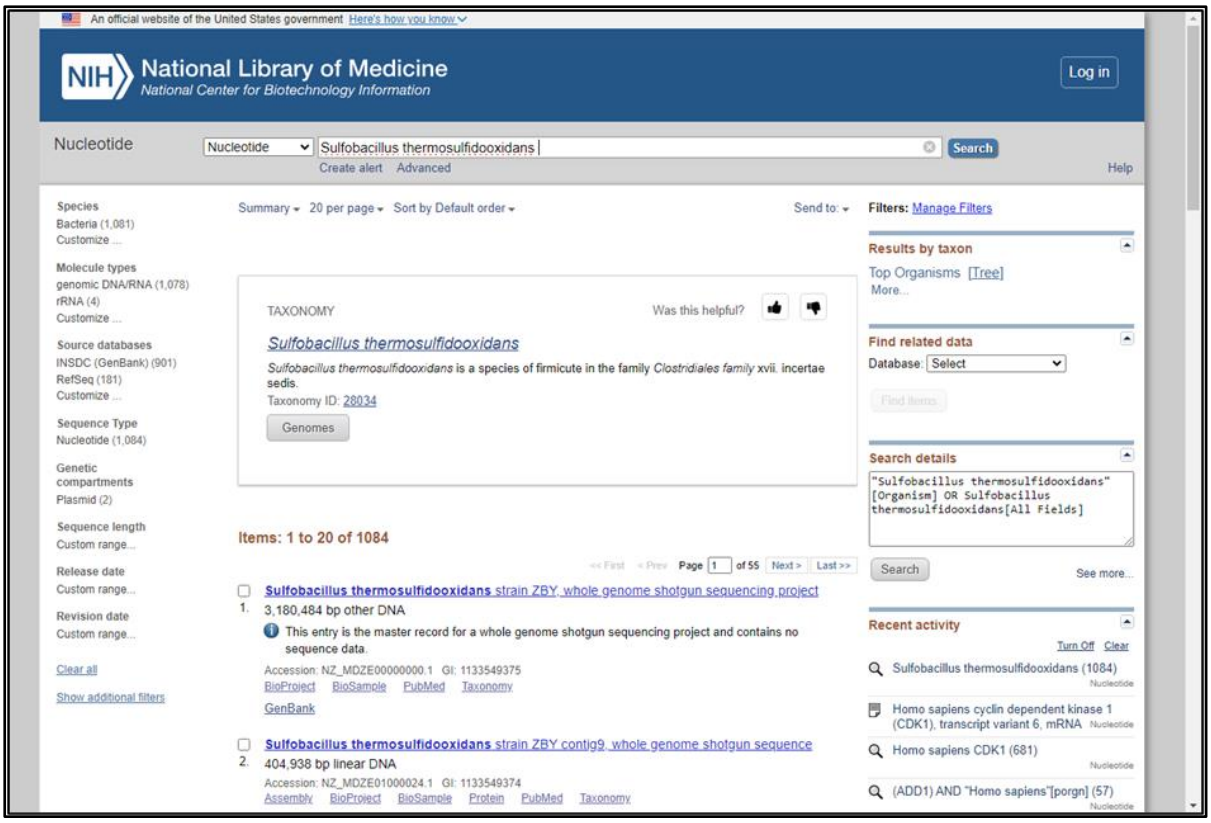


Fig 6: Result page obtained

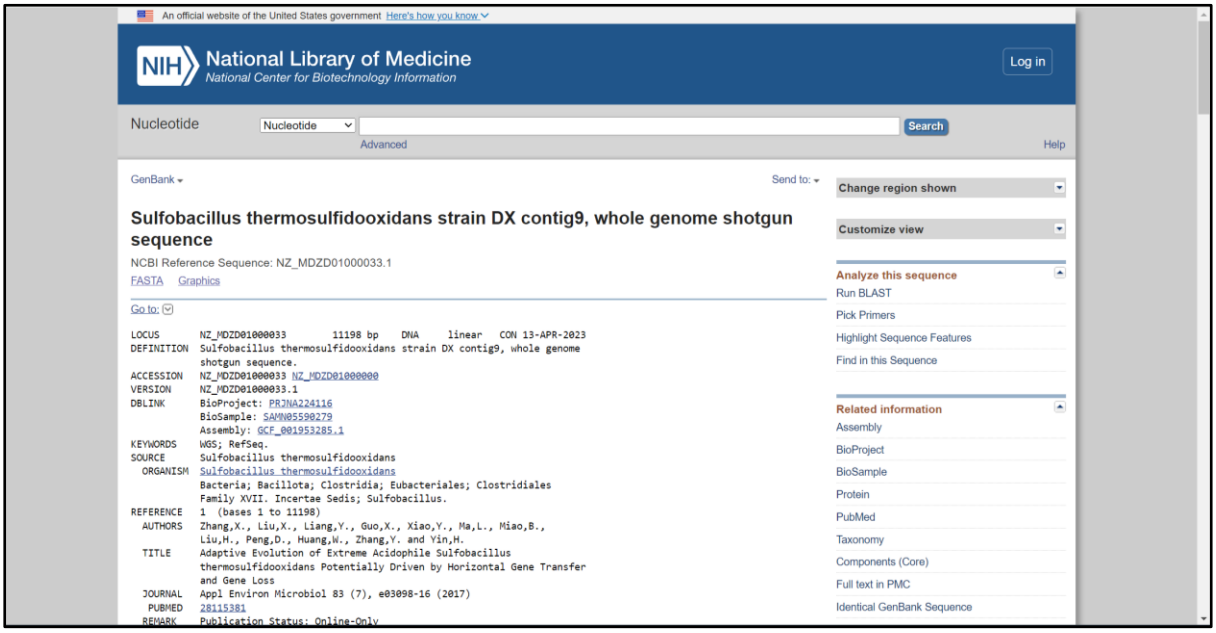


Fig 7: Query selected for study

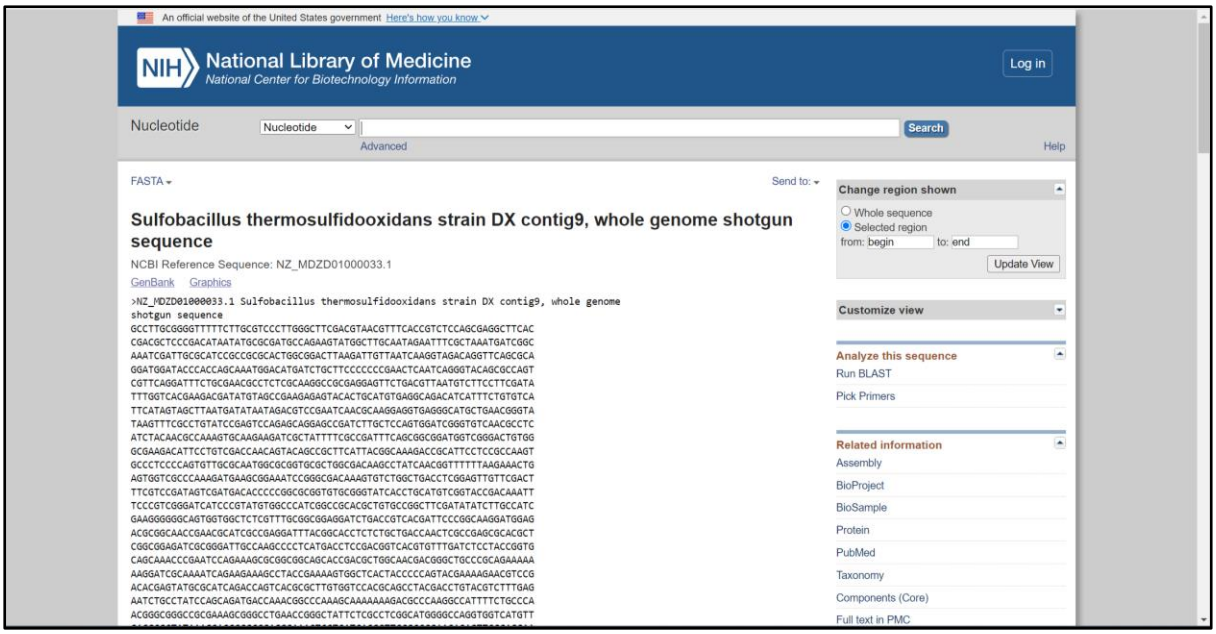


Fig 8: FASTA Sequence of the selected query

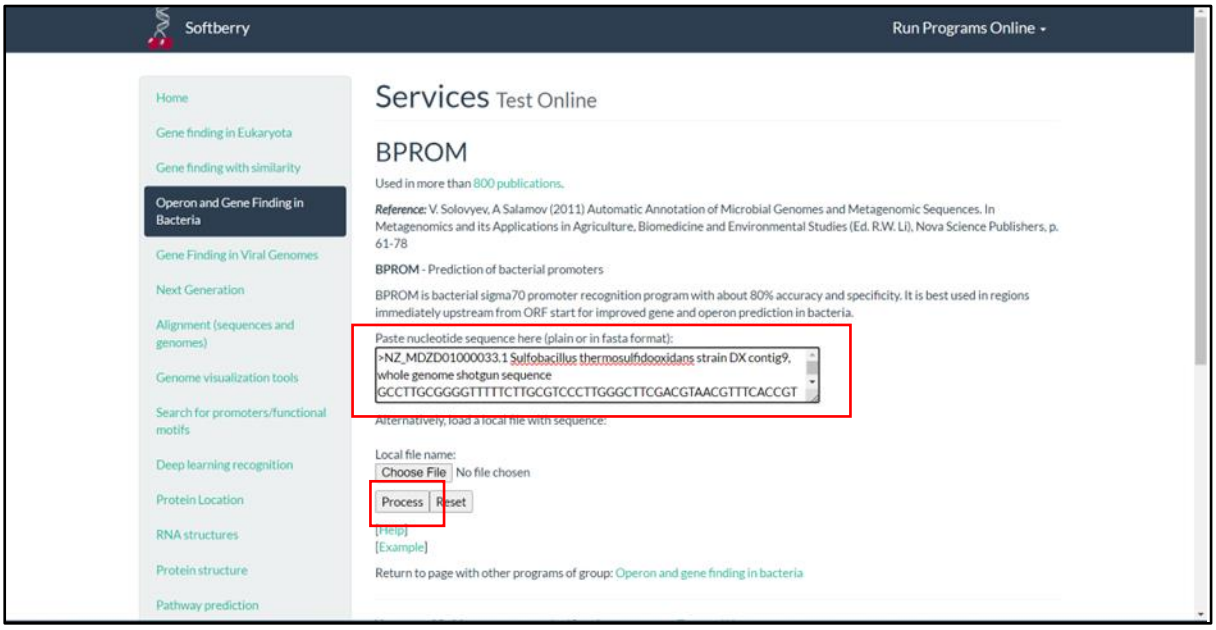
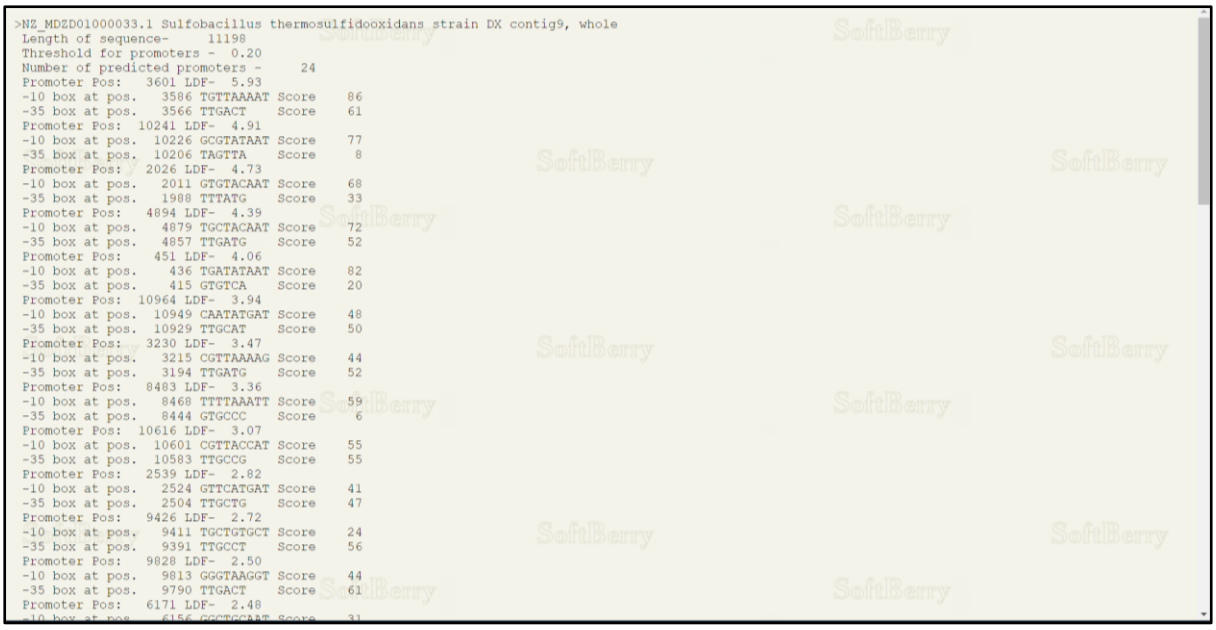
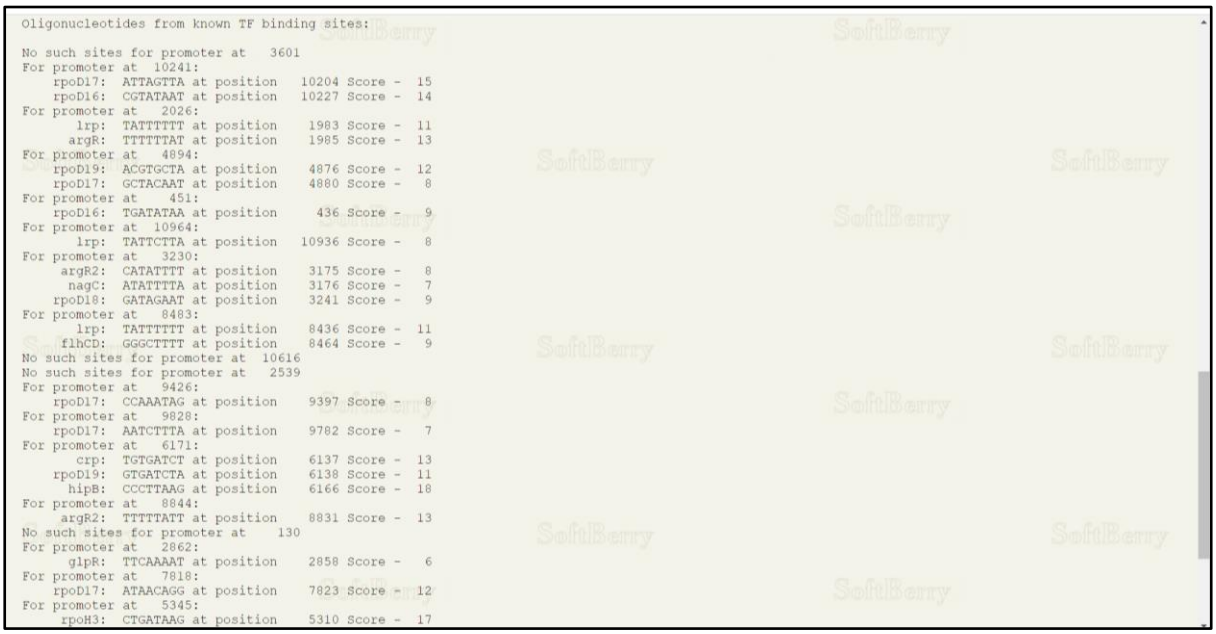


Fig 9: Paste the sequence in the nucleotide sequence section and click on process



```
>NZ_MDZD01000033.1 Sulfobacillus thermosulfidooxidans strain DX contig9, whole
 Length of sequence-     11198
 Threshold for promoters -  0.20
 Number of predicted promoters -     24
 Promoter Pos:   3601 LDF-  5.93
 -10 box at pos.   3586 TGTTAAAAT Score    86
 -35 box at pos.   3566 TTGACT    Score    61
 Promoter Pos:  10241 LDF-  4.91
 -10 box at pos.  10226 GCGTATAAT Score    77
 -35 box at pos.  10206 TAGTTA    Score     8
 Promoter Pos:   2026 LDF-  4.73
 -10 box at pos.   2011 GTGTACAAT Score    68
 -35 box at pos.   1988 TTTATG    Score    33
 Promoter Pos:   4894 LDF-  4.39
 -10 box at pos.   4879 TGCTACAAT Score    72
 -35 box at pos.   4857 TTGATG    Score    52
 Promoter Pos:    451 LDF-  4.06
 -10 box at pos.    436 TGATATAAT Score    82
 -35 box at pos.    415 GTGTCA    Score    20
 Promoter Pos:  10964 LDF-  3.94
 -10 box at pos.  10949 CAATATGAT Score    48
 -35 box at pos.  10929 TTGCAT    Score    50
 Promoter Pos:   3230 LDF-  3.47
 -10 box at pos.   3215 CGTTAAAAG Score    44
 -35 box at pos.   3194 TTGATG    Score    52
 Promoter Pos:   8483 LDF-  3.36
 -10 box at pos.   8468 TTTTAAATT Score    59
 -35 box at pos.   8444 GTGCCC    Score     6
 Promoter Pos:  10616 LDF-  3.07
 -10 box at pos.  10601 CGTTACCAT Score    55
 -35 box at pos.  10583 TTGCCG    Score    55
 Promoter Pos:   2539 LDF-  2.82
 -10 box at pos.   2524 GTTCATGAT Score    41
 -35 box at pos.   2504 TTGCTG    Score    47
 Promoter Pos:   9426 LDF-  2.72
 -10 box at pos.   9411 TGCTGTGCT Score    24
 -35 box at pos.   9391 TTGCCT    Score    56
 Promoter Pos:   9828 LDF-  2.50
 -10 box at pos.   9813 GGGTAAGGT Score    44
 -35 box at pos.   9790 TTGACT    Score    61
 Promoter Pos:   6171 LDF-  2.48
 -10 box at pos.   6156 GGCTGCAAT Score    31
```

Fig 10: BPROM Output for the query '*Sulfobacillus thermosulfidooxidans*'



```
 Oligonucleotides from known TF binding sites:

 No such sites for promoter at   3601
 For promoter at  10241:
     rpoD17:  ATTAGTTA at position   10204 Score -  15
     rpoD16:  CGTATAAT at position   10227 Score -  14
 For promoter at   2026:
        lrp:  TATTTTTT at position    1983 Score -  11
       argR:  TTTTTTAT at position    1985 Score -  13
 For promoter at   4894:
     rpoD19:  ACGTGCTA at position    4876 Score -  12
     rpoD17:  GCTACAAT at position    4880 Score -   8
 For promoter at    451:
     rpoD16:  TGATATAA at position     436 Score -   9
 For promoter at  10964:
        lrp:  TATTCTTA at position   10936 Score -   8
 For promoter at   3230:
      argR2:  CATATTTT at position    3175 Score -   8
       nagC:  ATATTTTA at position    3176 Score -   7
     rpoD18:  GATAGAAT at position    3241 Score -   9
 For promoter at   8483:
        lrp:  TATTTTTT at position    8436 Score -  11
      flhCD:  GGGCTTTT at position    8464 Score -   9
 No such sites for promoter at  10616
 No such sites for promoter at   2539
 For promoter at   9426:
     rpoD17:  CCAAATAG at position    9397 Score -   8
 For promoter at   9828:
     rpoD17:  AATCTTTA at position    9782 Score -   7
 For promoter at   6171:
        crp:  TGTGATCT at position    6137 Score -  13
     rpoD19:  GTGATCTA at position    6138 Score -  11
       hipB:  CCCTTAAG at position    6166 Score -  18
 For promoter at   8844:
      argR2:  TTTTTATT at position    8831 Score -  13
 No such sites for promoter at    130
 For promoter at   2862:
       glpR:  TTCAAAAT at position    2858 Score -   6
 For promoter at   7818:
     rpoD17:  ATAACAGG at position    7823 Score -  12
 For promoter at   5345:
      rpoH3:  CTGATAAG at position    5310 Score -  17
```

Fig 10.1: BPROM Output for the query '*Sulfobacillus thermosulfidooxidans*'

RESULTS:

The query *Sulfobacillus thermosulfidooxidans* (GenBank ID: NZ_MDZD01000033.1) with the sequence length 11198 base pairs was provided for predicting the promoter regions using the BPROM tool. A total of 24 promoters were predicted, with subsequent lines detailing the positions of these promoters and their scores, including the weights assigned to two conserved promoter boxes. The promoter positions were assigned to the first nucleotide of the transcription start site position.

CONCLUSION:

The result of gene *Sulfobacillus thermosulfidooxidans* strain (GenBank ID: NZ_MDZD01000033.1) highlights the predictive capabilities of BPROM in identifying potential promoter regions within a sequence based on specific criteria and scoring mechanisms. The program's ability to pinpoint transcription start sites and evaluate promoter strength is essential for understanding gene expression regulation and functional genomic.

REFERENCES:

1. BPROM - Prediction of bacterial promoters. (n.d.). http://www.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb
2. Huang, Y., Li, M., Yang, Y., Zeng, Q., Loganathan, P., Hu, L., Zhong, H., & He, Z. (2020, February 12). *Sulfobacillus thermosulfidooxidans:* an acidophile isolated from acid hot spring for the biosorption of heavy metal ions. *International Journal of Environmental Science and Technology*, *17*(5), 2655–2666. https://doi.org/10.1007/s13762-020-02669-1
3. Li, Q., Zhu, J., Li, S., Zhang, R., Xiao, T., & Sand, W. (2020, January 29). Interactions Between Cells of *Sulfobacillus thermosulfidooxidans* and *Leptospirillum ferriphilum* During Pyrite Bioleaching. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.00044

DATE: 06/03/2024

WEBLEM 1(D)

FPROM: Search for promoters/functional motifs

(URL:http://www.softberry.com/berry.phtml?topic=fprom&group=programs&subgroup=promoter)

AIM:

To predict human promoter for query 'JAK2' (GenBank ID: NM_001322195) using FPROM tool.

INTRODUCTION:

FPROM by Softberry is a program that predicts potential transcription start positions by combining characteristics describing functional motifs and oligonucleotide composition of these sites. It uses a linear discriminant function to achieve this. For approximately 50-55% true promoter region recognition, FPROM gives one false positive prediction for about 4000 bp. The program's sensitivity and specificity vary based on the threshold used, with higher thresholds leading to increased specificity but decreased sensitivity. FPROM is part of the FGENESB annotator pipeline, which provides automatic annotation of bacterial sequences, identifying protein and RNA genes, potential promoters, terminators, and operon units. The gene prediction algorithm in FPROM is based on Markov chain models of coding regions and translation sites. Operon models consider distances between ORFs, gene frequencies in known genomes, and information from predicted promoters and terminators. The program is highly accurate in predicting bacterial genes and operons.

JAK2:

JAK2, a tyrosine kinase, holds pivotal roles in cellular processes like proliferation, differentiation, and apoptosis, chiefly as part of the JAK2/STAT3 signaling pathway. This pathway transduces signals from extracellular cytokines and growth factors to the nucleus, impacting critical functions such as embryonic development, hemopoiesis, and immune system regulation. However, dysregulation of JAK2/STAT3 signaling, frequently observed in various tumors, contributes to oncogenesis, angiogenesis, and metastasis, often rendering cancers refractory to standard chemotherapy. Consequently, targeting the JAK2/STAT3 pathway emerges as a promising strategy in solid malignancy treatment.

Moreover, acquired mutations in JAK2 underlie several myeloproliferative disorders, including polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Unique clonal mutations, such as those causing constitutive signaling, are implicated in polycythemia vera, while gain-of-function mutations drive clonal expansion of hematopoietic cells in these disorders. Notably, the V617F mutation in JAK2, a somatic mutation found in hematopoietic cells, is strongly associated with myeloproliferative disorders. These insights into JAK2 mutations shed light on their significance in disease pathogenesis and underscore their potential as diagnostic and therapeutic targets.

METHODOLOGY:

1. Visit the website http://www.ssoftberry.com/.
2. Go to the Homepage, click on the 'Run Program Online' and choose the 'search for promoters/functional motifs' in it.
3. Choose FPROM within the service test online.
4. Obtain the FASTA sequence for query 'JAK2' from GenBank and paste it into the nucleotide sequence box on the FPROM page.
5. Set the threshold at 0.80 for TATA-box less promoters and proceed by clicking on the process button.

OBSERVATIONS:

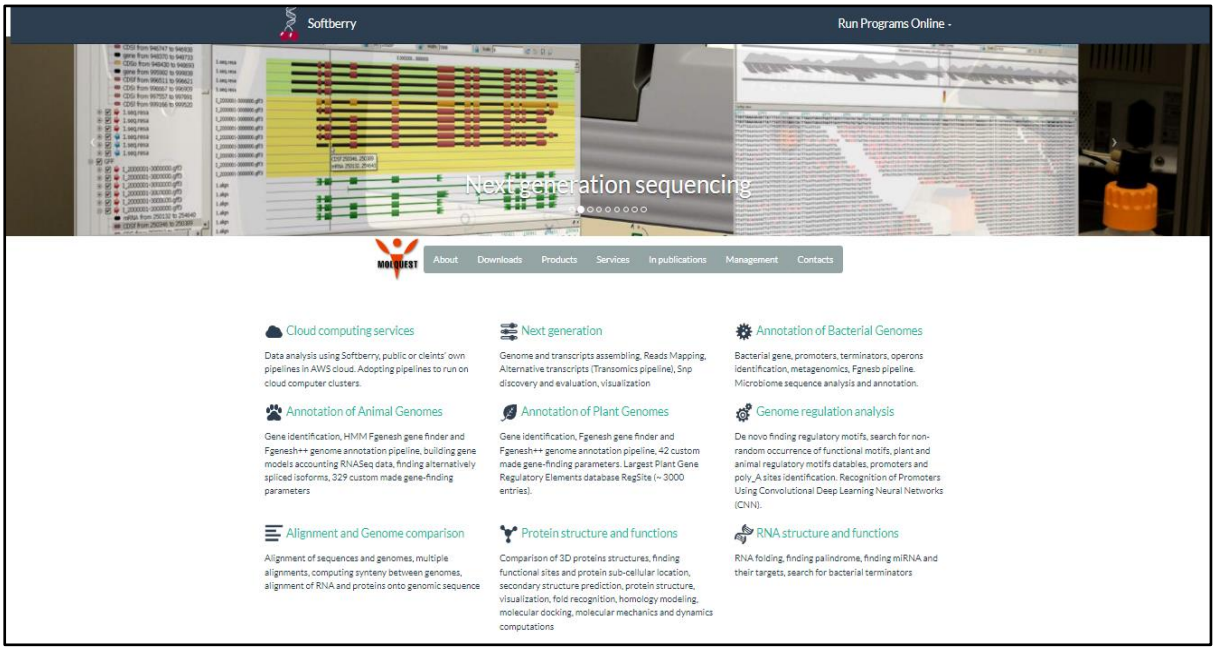


Fig 1: Homepage of Softberry server

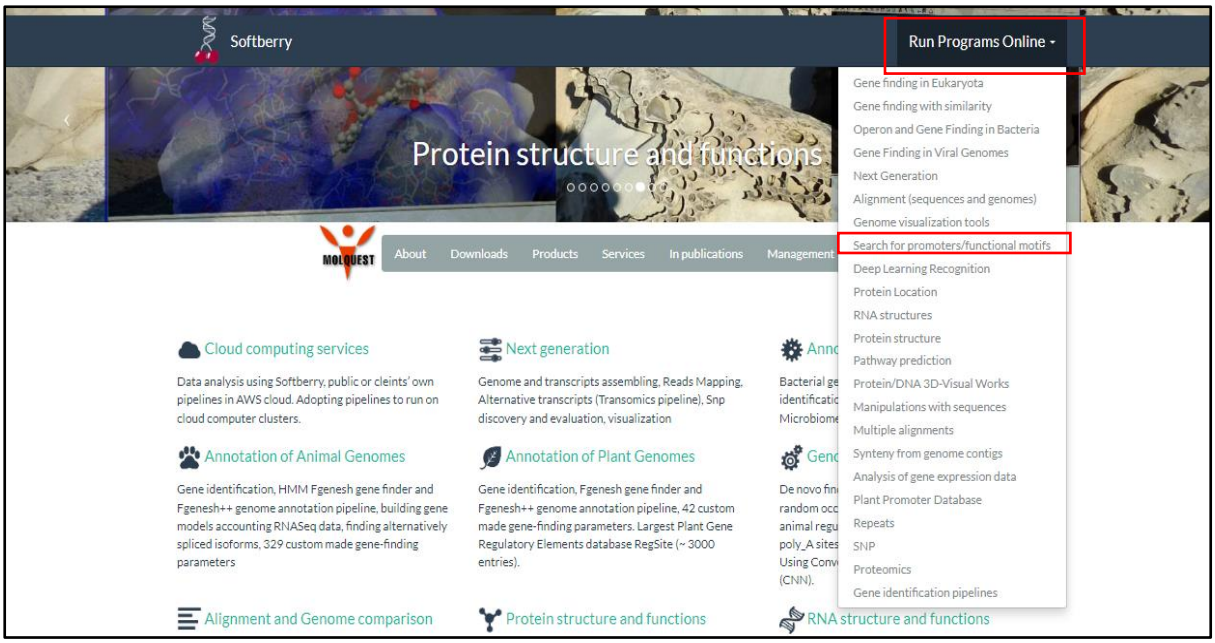


Fig 2: Select the option 'Search for promoters/functional motifs'

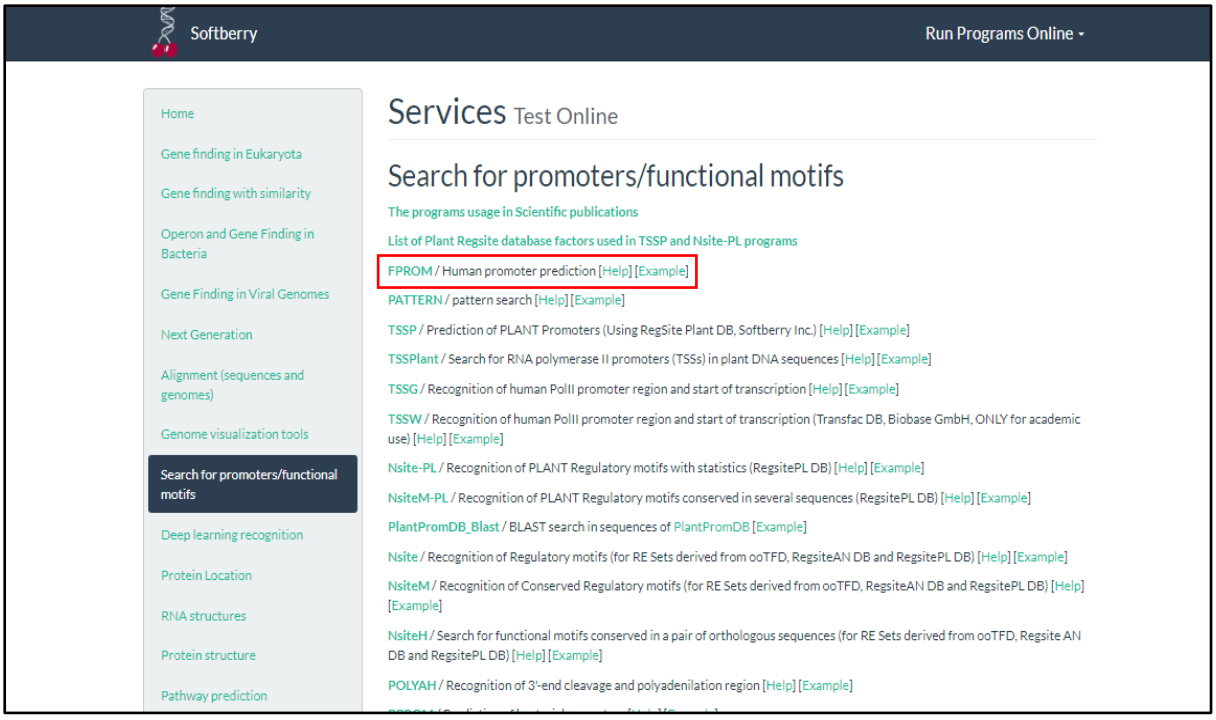


Fig 3: Select the FPROM option from the list of software

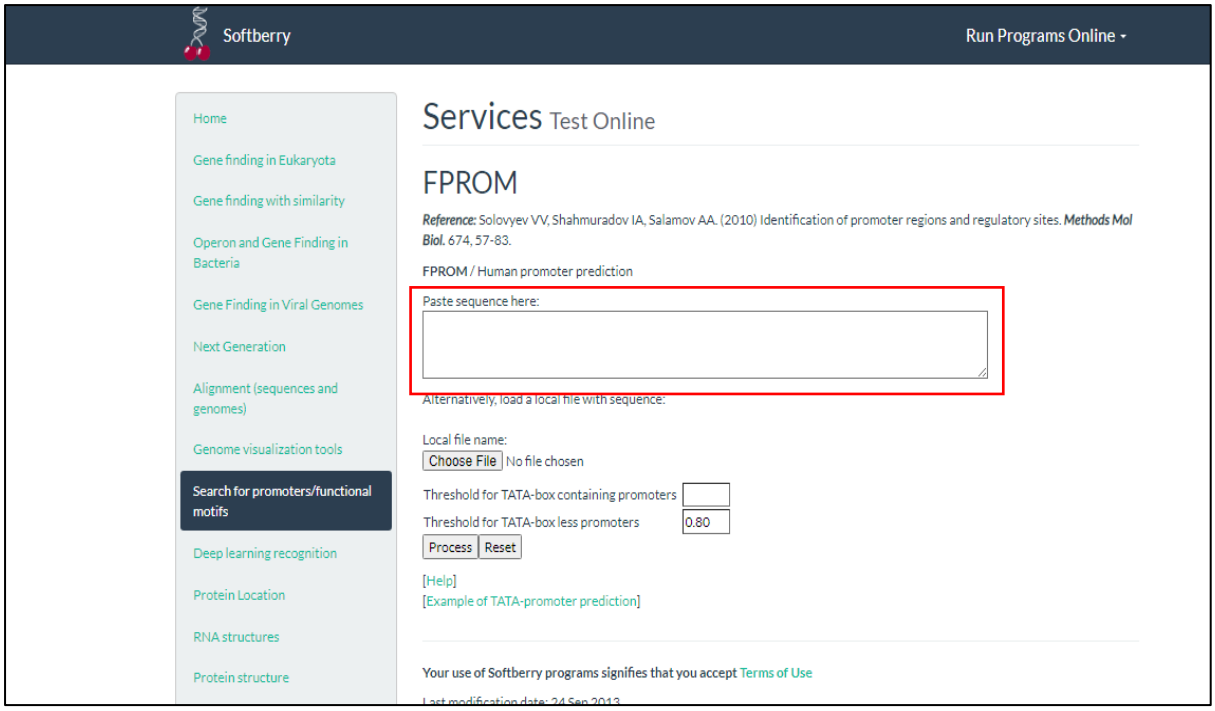


Fig 4: FPROM page where the sequence is to be pasted

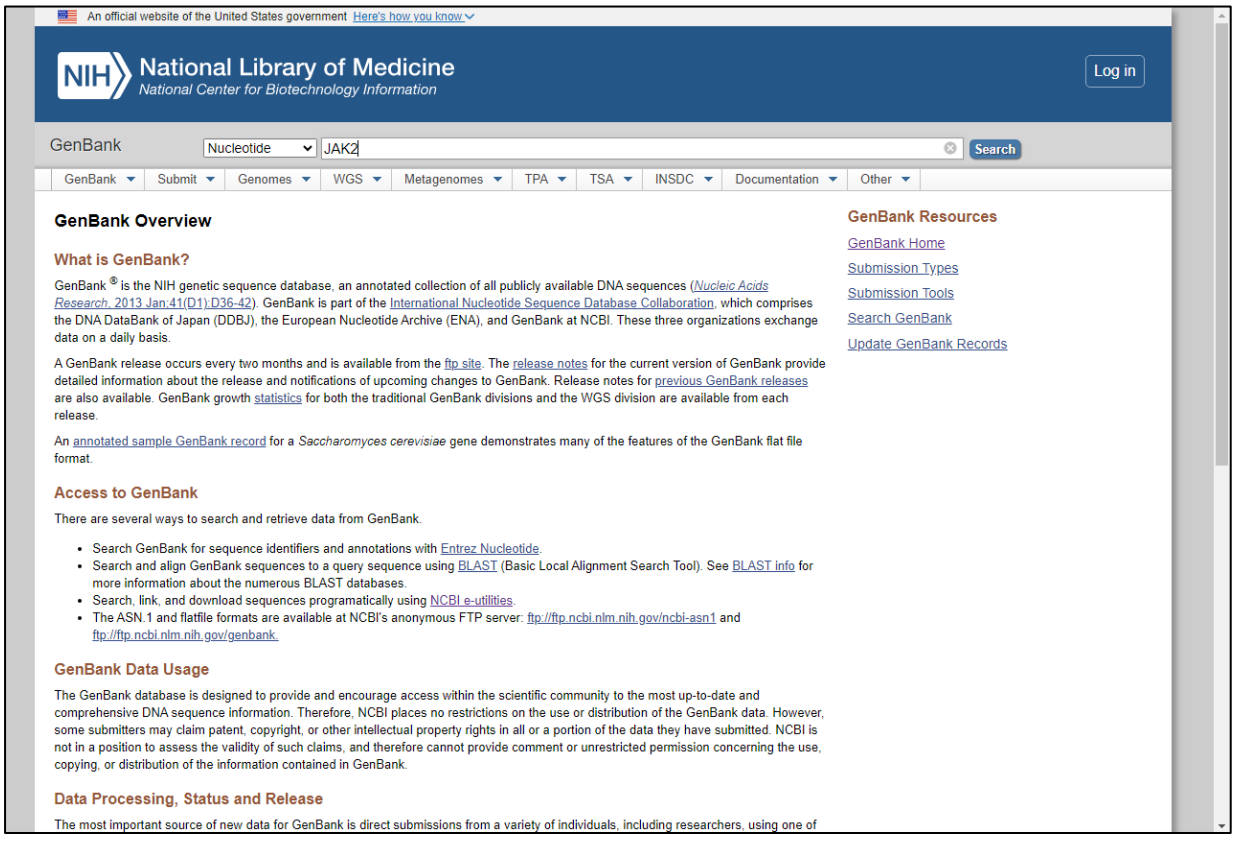


Fig 5: Homepage of GenBank database with the query 'JAK2'

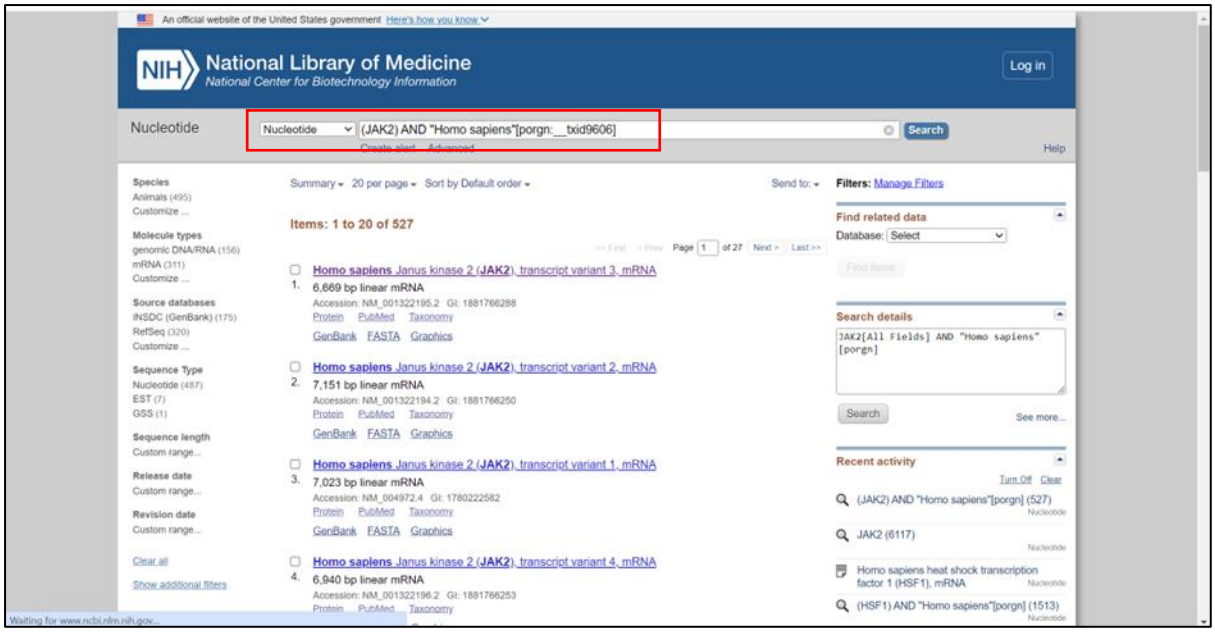


Fig 6: Results obtained

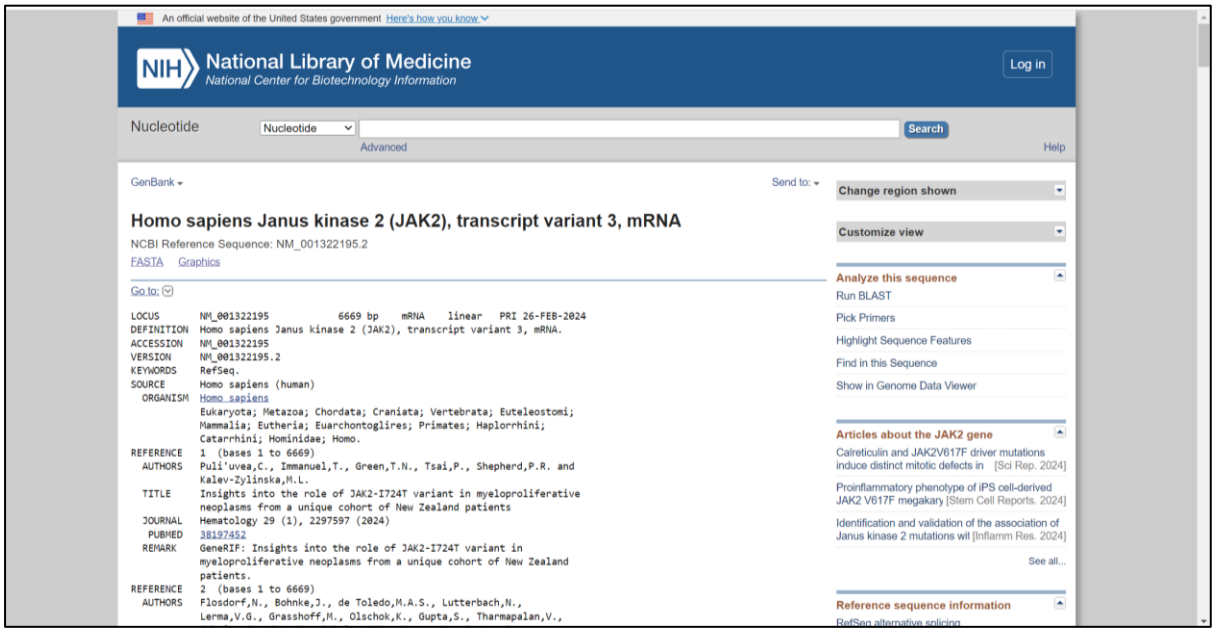


Fig 7: Entry selected for further study

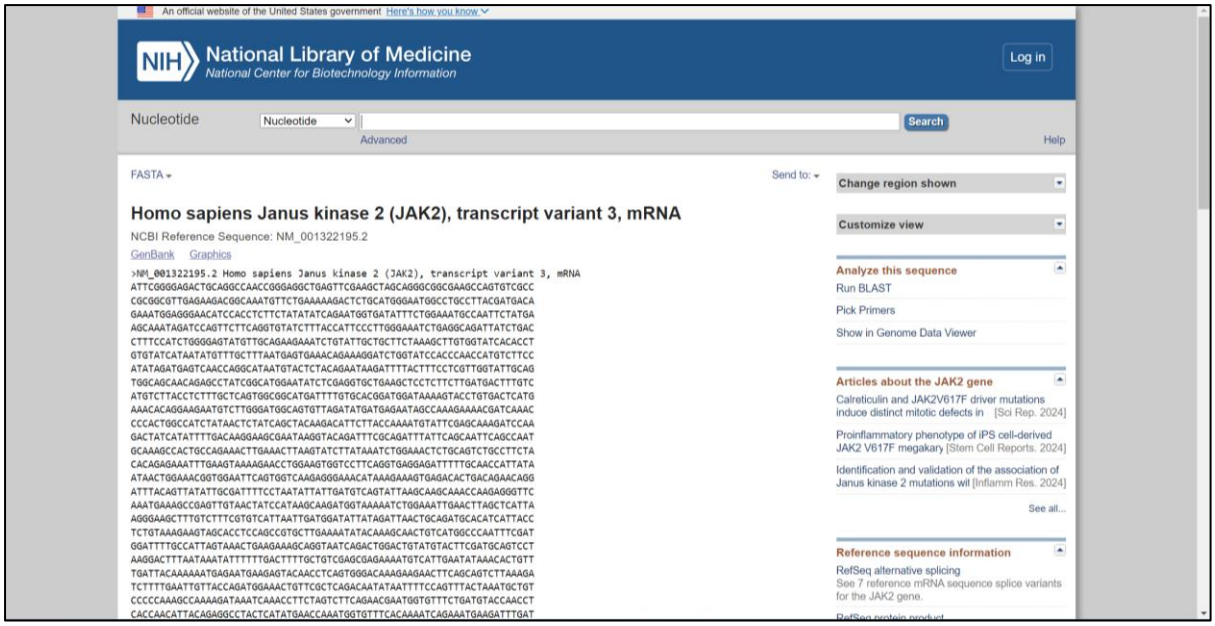


Fig 8: Extract the FASTA sequence

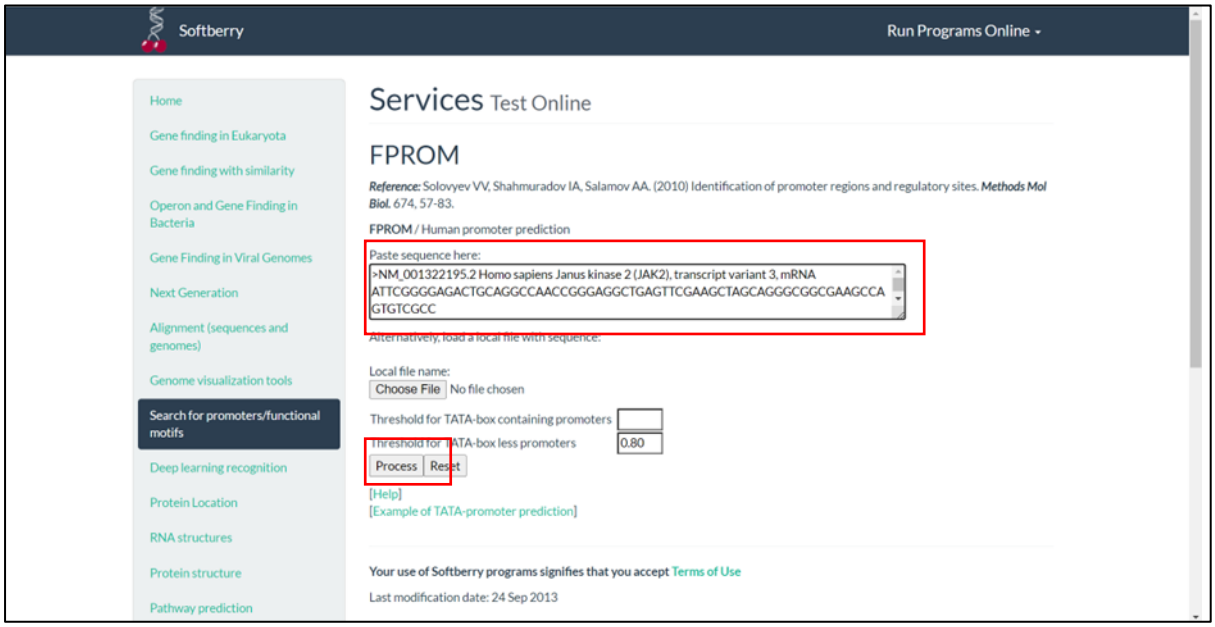


Fig 9: Paste the FASTA sequence into the nucleotide sequence section and click on process

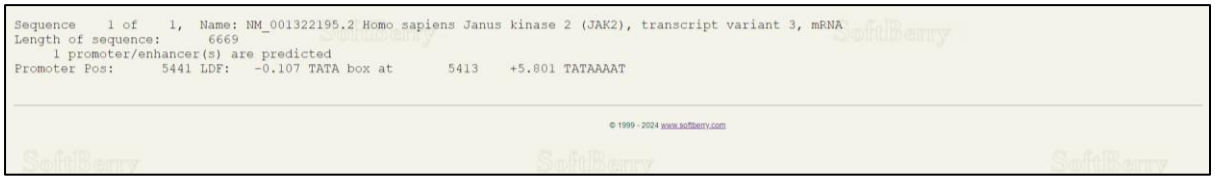


Fig 10: Result obtained for query 'JAK2'

RESULTS:

The results indicate the sequence name and its length of 6669 base pairs for the query 'JAK2' (GenBank ID: NM_001322195). Three promoters were predicted, with the TATA Box identified at positions 5413 within the promoter regions of 5441. This information highlights the presence of specific promoter elements within the sequence, aiding in the understanding of transcriptional regulation mechanisms in bacterial genomes.

CONCLUSION:

FPROM which is a robust tool used for query 'JAK2' (GenBank ID: NM_001322195) for predicting potential transcription start positions in bacterial genomes by utilizing a linear discriminant function that combines functional motifs and oligonucleotide composition.

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3. Levine, R. L., Wadleigh, M., Cools, J., Ebert, B. L., Wernig, G., Huntly, B. J., Boggon, T. J., Wlodarska, I., Clark, J. J., Moore, S., Adelsperger, J., Koo, S., Lee, J. C., Gabriel, S., Mercher, T., D'Andrea, A., Fröhling, S., Döhner, K., Marynen, P., . . . Gilliland, D. G. (2005, April). Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*, *7*(4), 387–397. https://doi.org/10.1016/j.ccr.2005.03.023

DATE: 06/03/2024

WEBLEM 1(E)

TSSP: Prediction of PLANT Promoters

(URL:http://www.softberry.com/berry.phtml?topic=tssp&group=programs&subgroup=promoter)

AIM:

To predict plant promoters for query 'Glutenin' (GenBank ID: X03346.1) using TSSP tool.

INTRODUCTION:

The TSSP software by Softberry is a comprehensive tool for various genomic analyses and data processing. It is used for Recognition of Pol II promoter region, enhancers and start of transcription. The algorithm used in the TSSP predicts potential transcription start positions by linear discriminant function combining characteristics describing functional motifs and oligonucleotide composition of these sites. TSSP uses file with selected factor binding sites from RegSite DB (Plants) developed by Softberry Inc.

Glutenin:

Glutenin, a key component of gluten found in wheat endosperms, contributes significantly to the elastic properties crucial for the texture and structure of wheat-based products. Comprising high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS), glutenin forms an elastic network within dough, providing strength and stability. Allelic variations at glutenin loci have been extensively studied, revealing associations between specific haplotypes and gluten strength, with observed changes in allelic frequencies over time coinciding with alterations in gluten strength. Additionally, the thermal stability and structural changes of glutenin and gliadin proteins in response to wheat bran dietary fiber (WBDF) incorporation have been investigated, highlighting modifications in protein characteristics and aggregation behavior induced by WBDF.

Moreover, nitrogen application during the wheat booting stage has been shown to enhance wheat gluten properties, including wet gluten content, sedimentation values, and dough strength, attributed to increased protein composition and modifications in glutenin polymerization and microstructure. Furthermore, the development of 3D porous scaffolds from wheat glutenin for cultured meat applications underscores the versatility of glutenin in novel food technologies. The gliadin/glutenin ratio has also been found to influence the pasting, thermal, and structural properties of wheat starch, offering insights into optimizing wheat-based product formulations. These studies collectively advance our understanding of glutenin's role in wheat quality and its potential applications in various food and biotechnological contexts.

METHODOLOGY:

1. Visit the website http://www.softberry.com/
2. Go to the homepage, click on the ‘Run Programs online’ section and choose the ‘Search for promoters/functional motifs’ in it.
3. Within the "Services Test Online" section, select "TSSP".
4. Proceed to the GenBank website and enter the query like ‘Glutenin’ and click on search.
5. Identify a relevant entry, extract its FASTA sequence.
6. Paste the acquired sequence into the nucleotide sequence box on the TSSP page.
7. Proceed by clicking on the process button.

OBSERVATIONS:

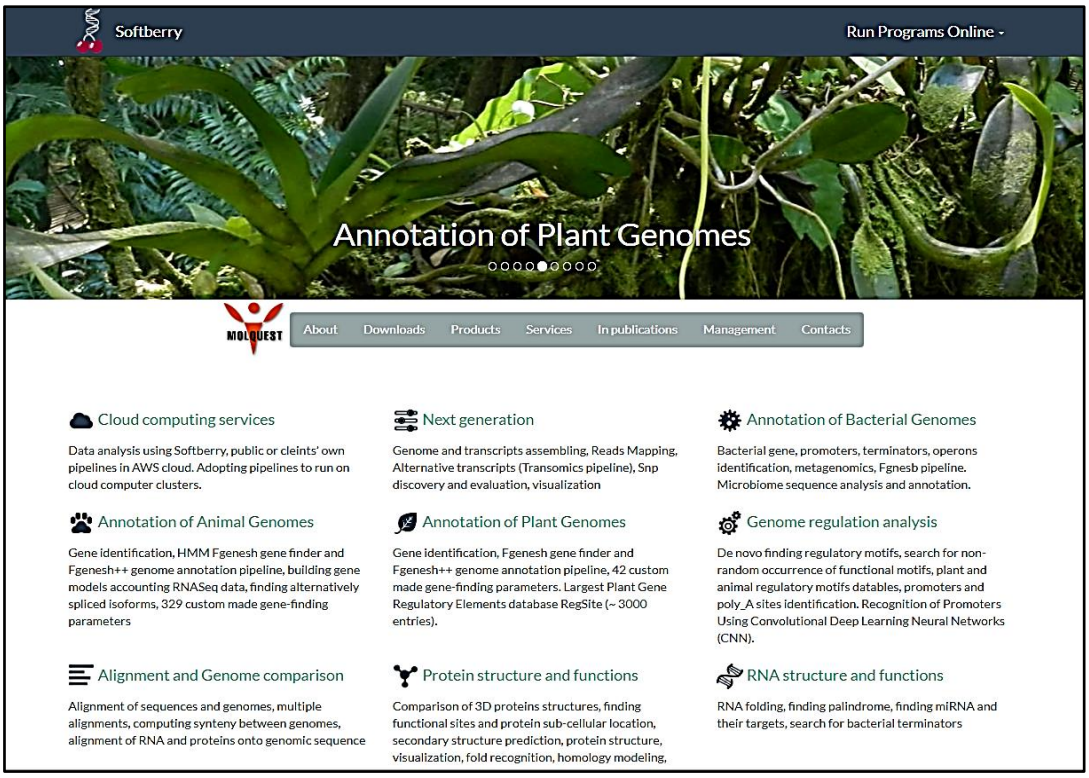

Fig 1: Homepage of Softberry Database

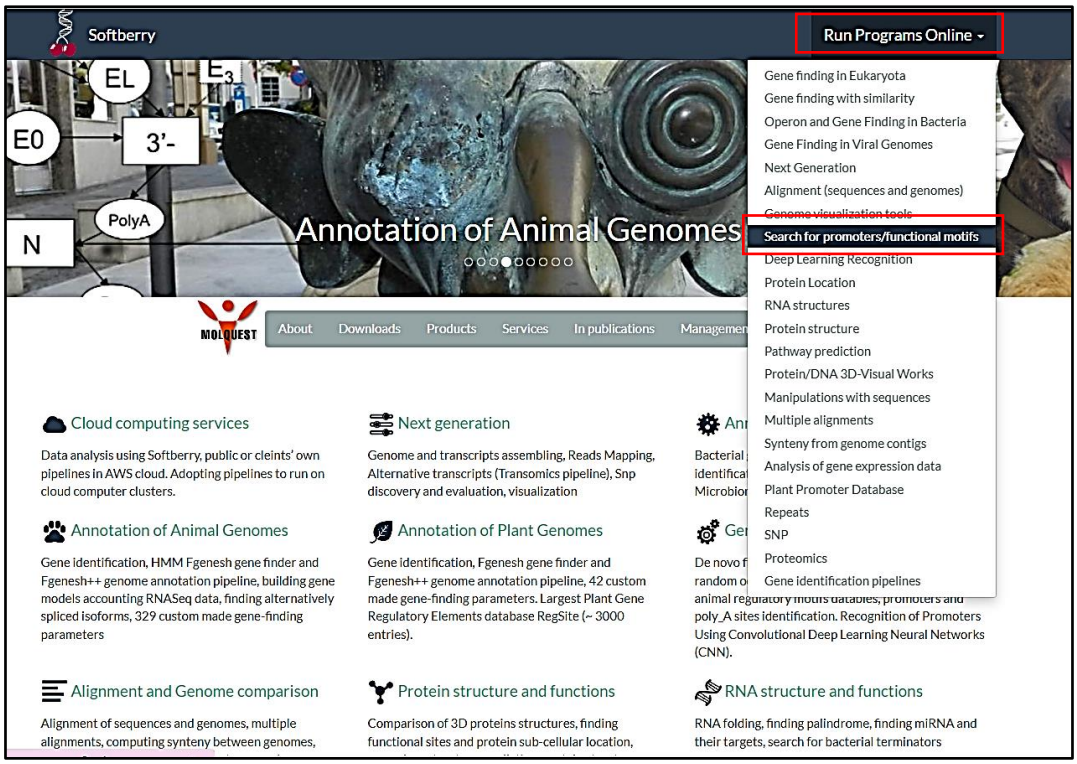

Fig 2: Select the option 'Search for promoters/functional motifs'

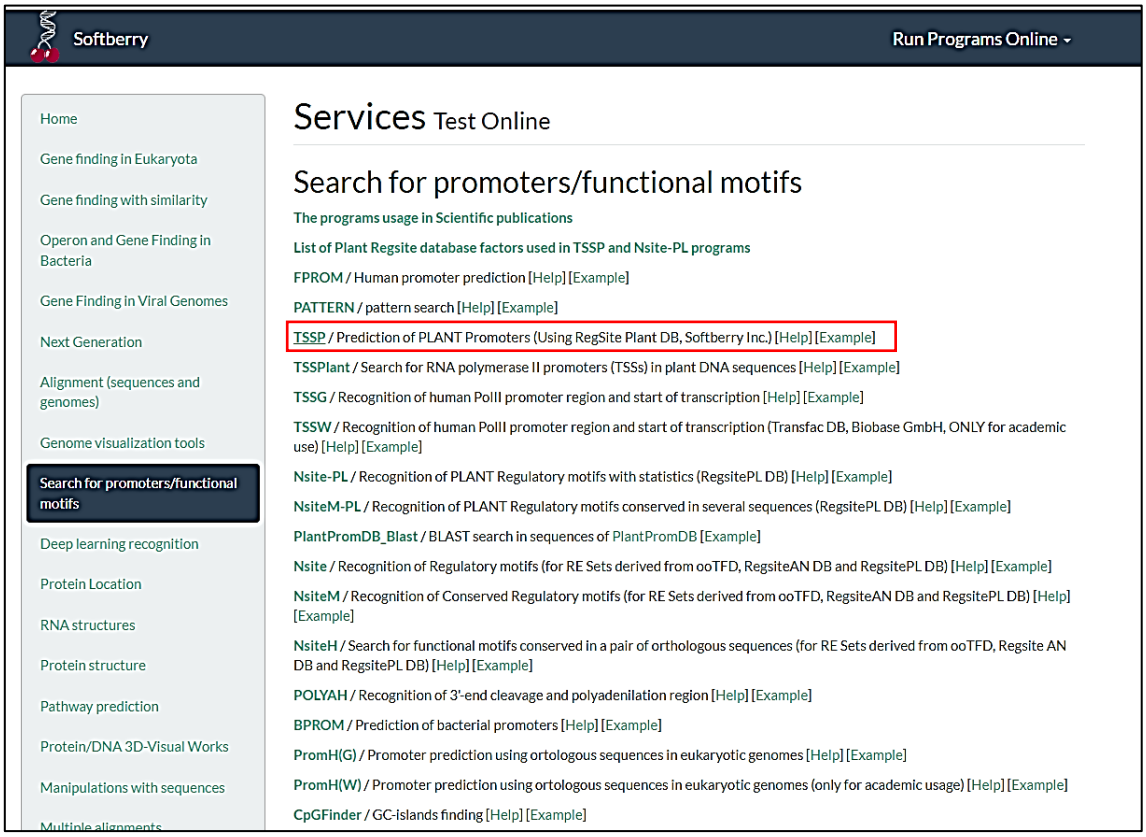

Fig 3: Select the 'TSSP' option from the list of software

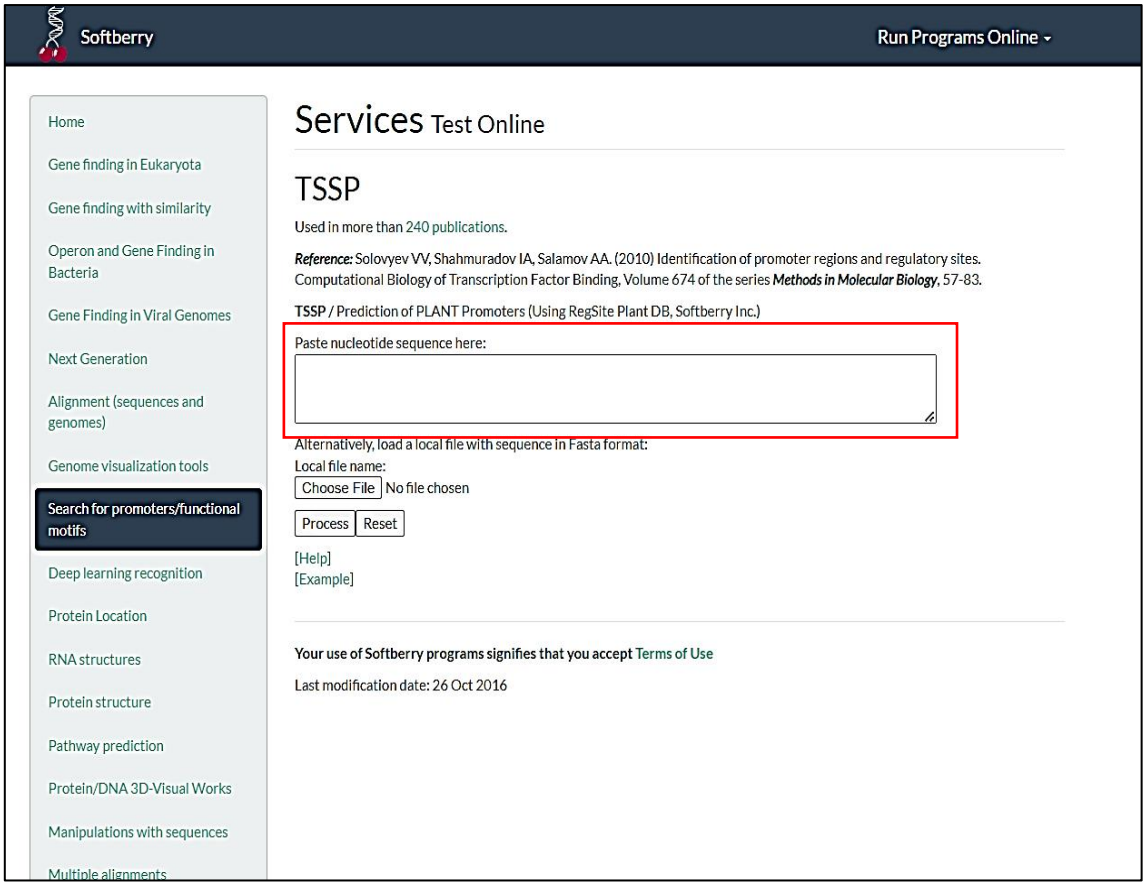

Fig 4: TSSP software main page where the sequence is to be pasted and processed

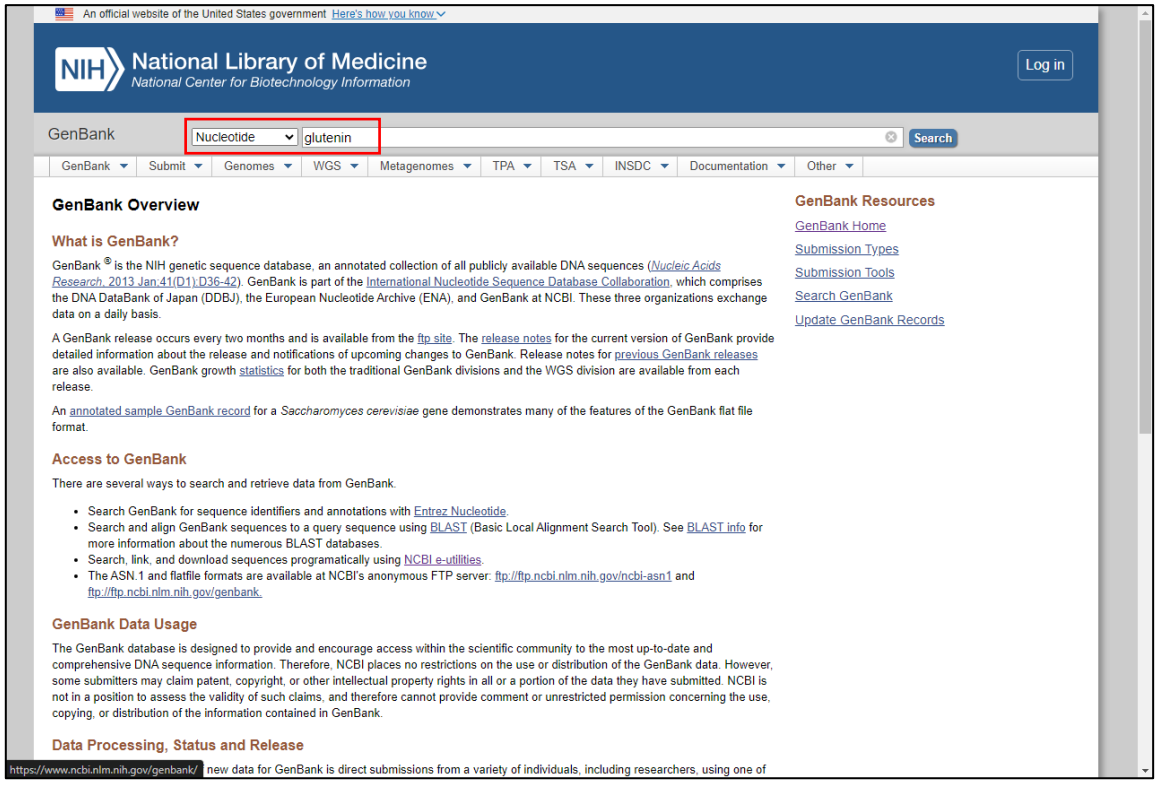

Fig 5: Homepage of GenBank Database (with the query 'glutenin')

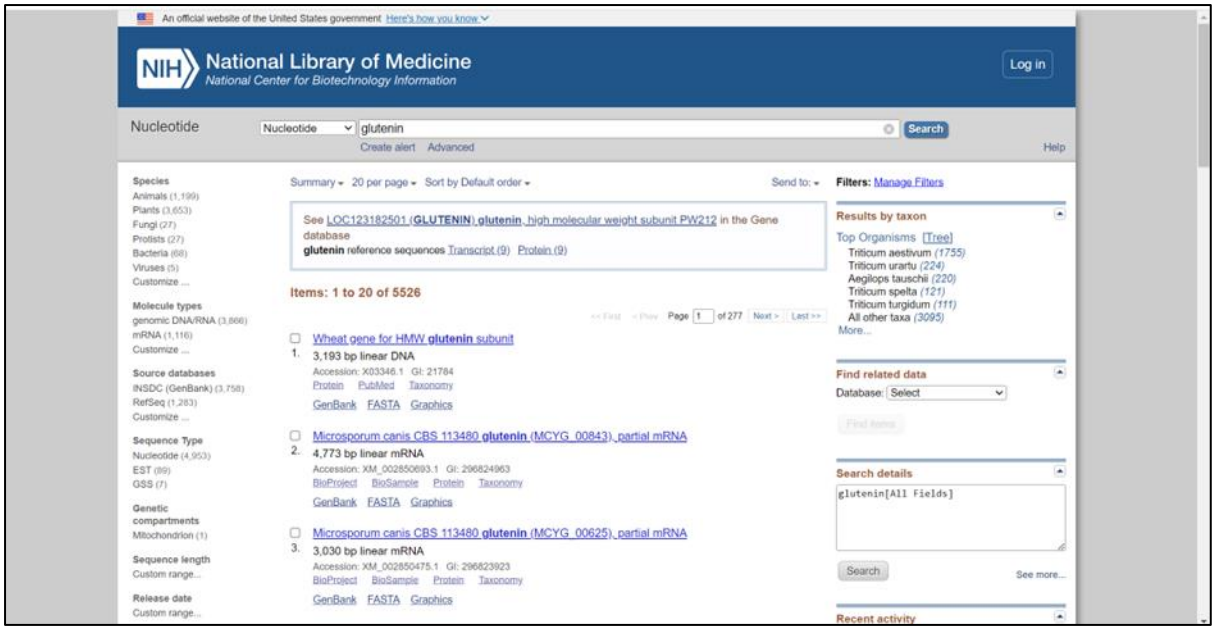

Fig 6: Results obtained for the query 'glutenin'

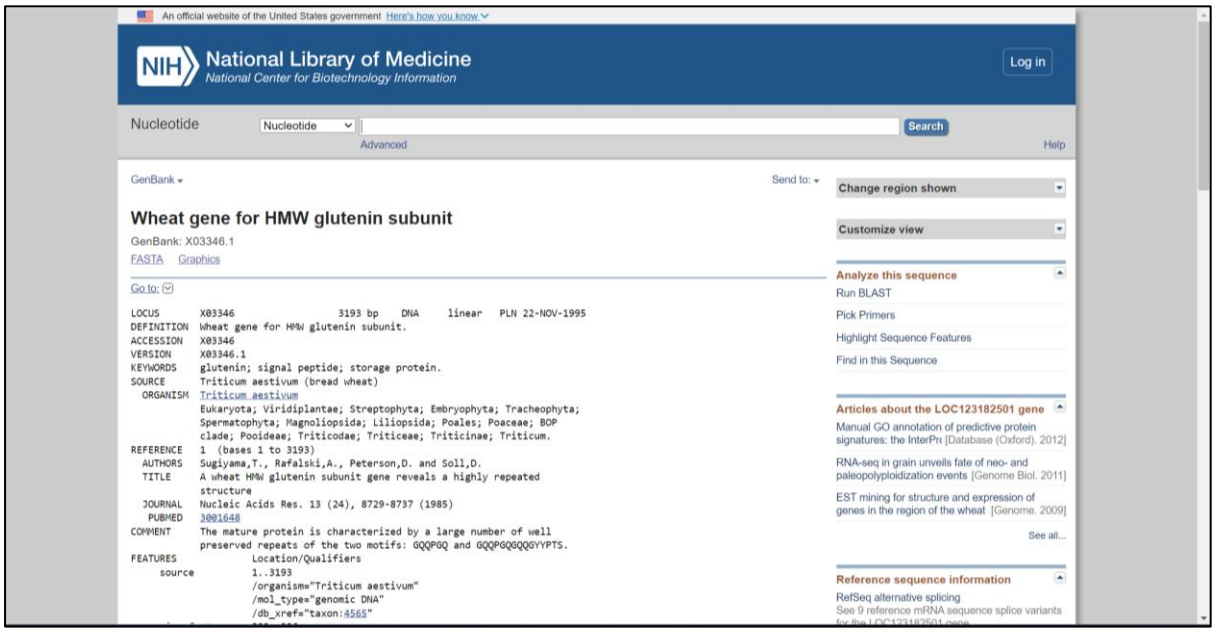

Fig 7: Entry selected for further study

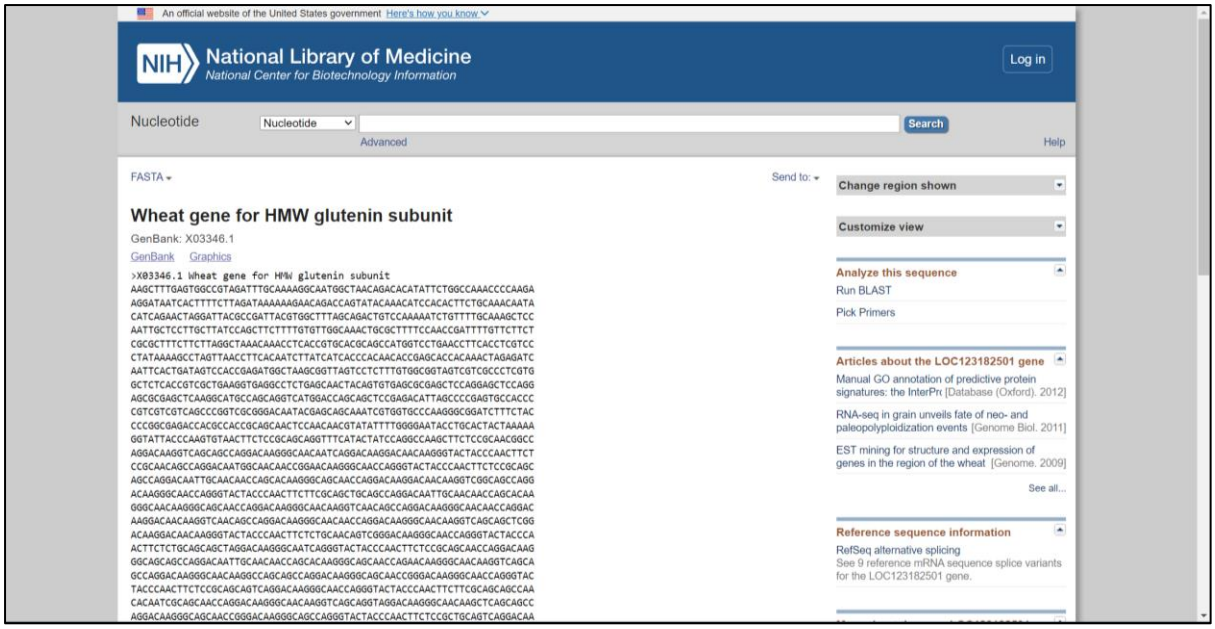

Fig 8: FASTA sequence of the selected entry

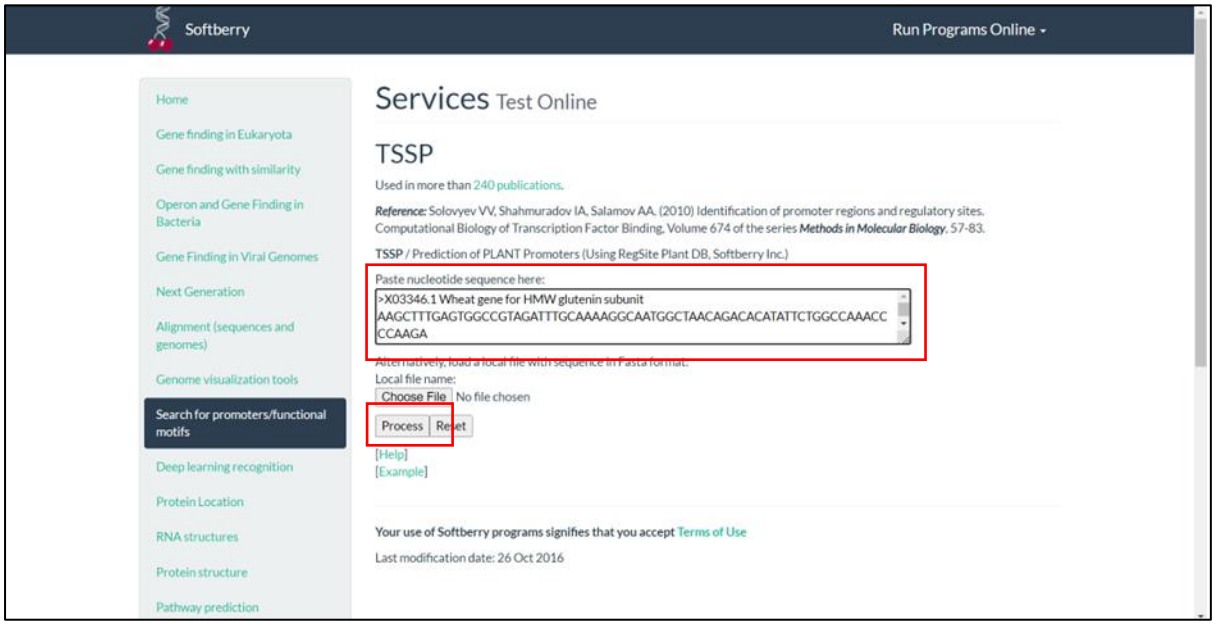

Fig 9: Paste sequence in the nucleotide sequence section and click on the process button

Fig 10: TSSP Output for the query 'Glutenin' (GenBank ID: X03346.1)
Key: Sequences in capitalized letters denote conserved domains
Sequences in small letters denote unconserved domains

RESULTS:

The TSSP was utilized to anticipate potential transcription start sites or promoters within the sequence of the 'glutenin' (GenBank ID: X03346.1) gene, which spans 3193 base pairs. The thresholds set for TATA+ promoters and TATA-enhancers were 0.02 and 0.04, respectively. The analysis identified a promoter at position 381 with a TATA box located at position 348. In the specific positions of the promoter, lowercase letters represent non-conserved sequences, while uppercase letters denote conserved sequences. The symbols + and – indicate the strands in the results.

CONCLUSION:

TSSP software by is used for Recognition of Pol II promoter region, enhancers and start of transcription. The predictions for the 'glutenin' (GenBank ID: X03346.1) gene were generated through the utilization of the TSSP software, yielding the intended outcomes.

REFERENCES:

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DATE: 07/03/2024

WEBLEM 2
INTRODUCTION TO GENE EXPRESSION OMNIBUS (GEO) DATABASE
(URL: https://www.ncbi.nlm.nih.gov/geo/)

Gene Expression Omnibus (GEO) is a database supported by the National Center for Biotechnology Information (NCBI) at the National Library of Medicine (NLM) that accepts raw and processed data with written descriptions of experimental design, sample attributes, and methodology for studies of high-throughput gene expression and genomics. The introduction of DNA microarrays and the Serial Analysis of Gene Expression (SAGE) protocol as methods of simultaneously assaying gene expression of multiple genes in 1995 enabled scientists to study gene expression of hundreds to thousands of genes, thereby vastly increasing the experimental scale and providing a far more complete understanding of biological processes compared to earlier single-gene studies. Microarray technology quickly dominated the field of high-throughput gene expression studies and with the genome sequencing of humans and many model organisms, genome-wide gene expression and other functional genomic studies became commonplace by the early 2000s. The accelerating pace of genomic-level data production and the bulky raw and processed data files they generated created a challenge for individual labs or journals to make the data available to the research community.

In 2000, NCBI launched the GEO database as a repository for high-throughput gene expression data. In 2002, major journals started to require deposit of microarray data into public repositories, and consequently, the content of GEO grew quickly. Furthermore, the nature of high-throughput genomic experiments expanded rapidly since the first microarrays used to analyze gene expression, and thus the GEO database similarly evolved to keep pace with the changing technologies and applications. Today, GEO accepts data from a wide variety of technologies, including DNA microarrays, protein or tissue arrays, high-throughput nucleic acid sequencing, SAGE, and RT-PCR. And while the majority, approximately 90%, of the data in GEO are indeed gene expression data, the applications have also expanded to include studies on genome methylation, genome binding/occupancy, protein profiling, chromosome conformation studies, and genome variation/copy number.

There are three types of GEO submitter records:

1. A **Platform** record describes an array or sequencer and, for array-based platforms, a data table defining the array template. Sample records are linked from Platform records.
2. A **Sample** record describes the sample source, the protocols used in its analysis, and the expression data derived from it. Samples can only reference a single Platform
3. A **Series** record links together a group of related Samples and describes a whole study.

These three types of records are organized into two higher-level categories for querying and analysis:

1. A **Dataset** represents a curated collection of biologically and statistically comparable GEO Samples. All Samples in a Dataset reference the same Platform. Datasets can be searched using the GEO Datasets database.
2. A **Profile** consists of the expression measurements for an individual gene across all Samples in a Dataset. Profiles can be searched using the GEO Profiles database.

The word "geo" is a prefix meaning "earth" because not only does GEO primarily host global gene expression data, GEO itself is indeed a global resource; at the time of this writing GEO contains submissions from 72 nations. There are no fees to submit data to GEO, download data, or use GEO tools. Scientists submit to GEO in order to share their data with the research community and/or as a requirement of publication or grant directives. GEO supports the Minimum Information About a Microarray Experiment (MIAME) and Minimum Information about a high-throughput SEQuencing Experiment (MINSEQE) guidelines set forth by the Functional Genomics Data Society for standardization of information about microarray and sequencing experiments that enable the data to be interpreted and replicated by the research community. The GEO database handles the majority of direct submissions from the research community and at the time of this writing holds 54,640 public studies, comprising over 1.3 million samples, derived from 2889 different organisms.

While the chief role of GEO is to serve as a public data archive, the database is not simply an online warehouse of data. GEO strives to make the data it contains accessible to the research community. Due to the complex nature of the data generated by genomic experiments most studies are analyzed by bioinformaticians and statisticians, or researchers with specialized analysis software. Researchers who lack these skills or software face a substantial challenge if they wish to analyze genomics experiments themselves. In order to make such data analysis accessible to all researchers, GEO has developed several tools for data query, visualization, and analysis that can be performed directly on the GEO website and do not require the download or manipulation of the data files.

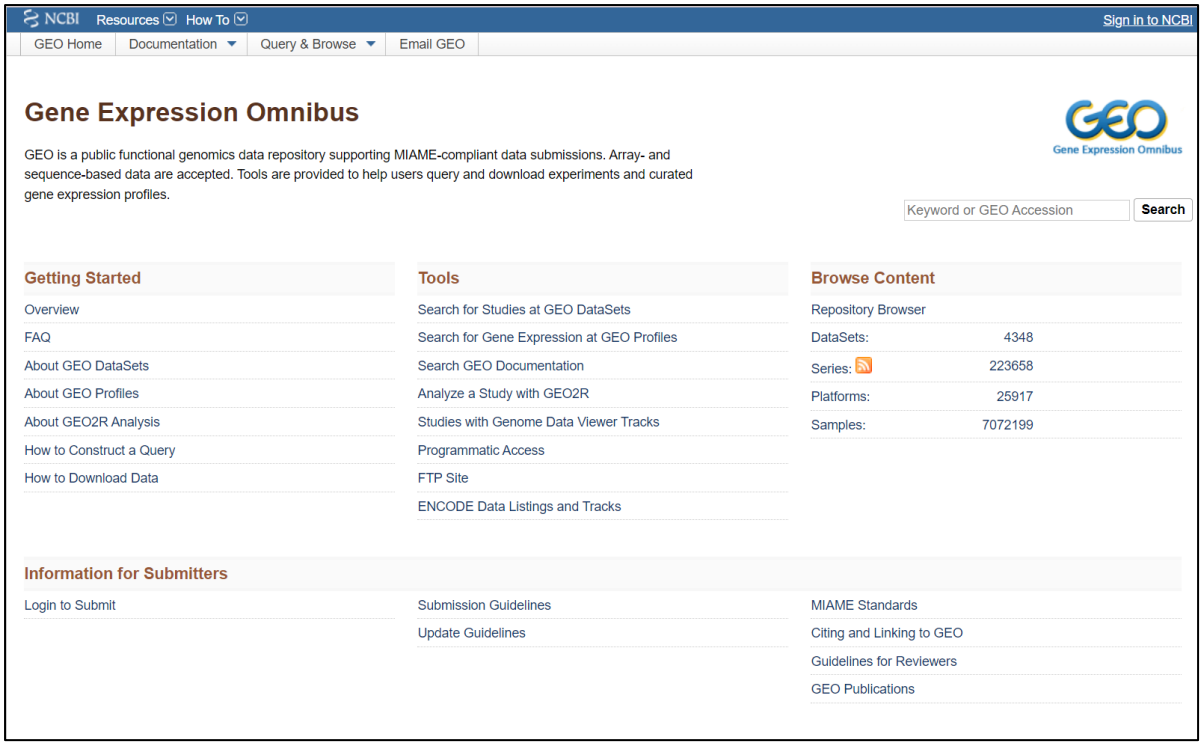

Fig 1: Homepage of Gene Expression Omnibus (GEO) Database

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1. Clough, E., & Barrett, T. (2016). The Gene Expression Omnibus Database. In Methods in molecular biology (pp. 93–110). https://doi.org/10.1007/978-1-4939-3578-9_5
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DATE: 07/03/2024

WEBLEM 2(A)
INTRODUCTION TO GENE EXPRESSION OMNIBUS (GEO) DATABASE
(URL: https://www.ncbi.nlm.nih.gov/geo/)

AIM:

To analyze gene expression data from the Gene Expression Omnibus (GEO) database to identify differentially expressed genes associated with 'Bladder tumor'.

INTRODUCTION:

The Gene Expression Omnibus (GEO) is a publicly available, comprehensive database hosted by the National Centre for Biotechnology Information (NCBI). It serves as a repository for high-throughput gene expression and functional genomics data submitted by researchers worldwide. The GEO database facilitates the dissemination and sharing of these valuable datasets, enabling researchers to explore and analyze gene expression patterns across a wide range of experimental conditions, biological processes, and organisms. Established in 2000, GEO has become an indispensable resource for the scientific community, promoting data sharing and collaboration in the field of genomics. It adheres to the Minimum Information About a Microarray Experiment (MIAME) standard, ensuring that submitted data is consistently annotated and described, enabling reproducibility and facilitating meta-analyses.

There are three types of GEO submitter records:

1. A **Platform** record describes an array or sequencer and, for array-based platforms, a data table defining the array template. Sample records are linked from Platform records.
2. A **Sample** record describes the sample source, the protocols used in its analysis, and the expression data derived from it. Samples can only reference a single Platform
3. A **Series** record links together a group of related Samples and describes a whole study.

These three types of records are organized into two higher-level categories for querying and analysis:

1. A **Dataset** represents a curated collection of biologically and statistically comparable GEO Samples. All Samples in a Dataset reference the same Platform. Datasets can be searched using the GEO Datasets database.
2. A **Profile** consists of the expression measurements for an individual gene across all Samples in a Dataset. Profiles can be searched using the GEO Profiles database.

GEO DataSets contain raw and processed data from individual studies, including sample characteristics, experimental design, and raw data files. GEO Profiles, on the other hand, provide a curated and compact representation of the gene expression data, allowing users to quickly identify interesting genes or compare expression patterns across different experimental conditions or sample types.

The Gene Expression Omnibus is a valuable resource for researchers in the field of genomics, providing a centralized repository for gene expression and functional genomics data. Its user-friendly interface, adherence to data standards, and powerful data analysis tools make it an essential tool for exploring gene expression patterns, identifying biomarkers, and advancing our understanding of biological processes and disease mechanisms.

Bladder cancer:

Bladder cancer develops in the lining of the bladder, a storage organ for urine. The most prevalent type, urothelial carcinoma (previously called transitional cell carcinoma), originates in cells lining the bladder and also affects similar structures like the renal pelvis, ureters, and urethra. Less common types include squamous cell carcinoma, adenocarcinoma, and sarcoma. Risk factors include smoking and exposure to certain chemicals. The disease is more frequent in individuals over 55, with men being four times more likely to develop it than women. Blood in the urine is a common symptom, but other conditions can cause it as well. Urinalysis, cytology, cystoscopy, and imaging studies are used for diagnosis.

The stage of the cancer, determined by whether it has invaded the bladder muscle wall, is crucial for treatment decisions. Non-muscle-invasive cancer, the most common type, is typically treated before it spreads. Muscle-invasive cancer has penetrated the lining and requires different treatment approaches. Surgery, radiation therapy, chemotherapy, and immunotherapy are all options, with the specific choice depending on the cancer's stage and type.

METHODOLOGY:

1. Access the GEO database through the National Centre for Biotechnology Information (NCBI) website: (ncbi.nlm.nih.gov/geo).
2. On the GEO homepage, use the search bar to look for specific keywords or GEO accession numbers related to your research topic or study of interest.
3. To find a specific dataset, search by keyword or GEO accession number in the search bar. For example, here searching for "bladder tumor" will display relevant datasets and also profiles. Further, click on ‘DataSets’.
4. Once found a dataset of interest, click on it to access its details. The dataset page will provide information such as the title, summary, organism, platform, citation, and sample count along with other information.
5. Under the "Data Analysis Tools" section, select the appropriate test (e.g. Two-tailed t-test) and set the desired significance level (e.g., 0.100) for comparing the sample groups.
6. Identify the sample groups you want to compare.
7. Assign the samples to their respective groups (Group A and Group B) based on the provided information. In this case,
   Group A: GSM2519, GSM2524, GSM2525
   Group B: GSM2544, GSM2506, GSM2509
8. Perform the selected statistical test (e.g., Two-tailed t-test) to compare the expression profiles between the two groups.

9. Analyze the results and visualize the data using various tools provided in the GEO Dataset Browser, such as hierarchical clustering, heatmaps, or correlation analysis.
10. Interpret the findings and draw conclusions based on the statistical analysis and visual representations.

OBSERVATIONS:

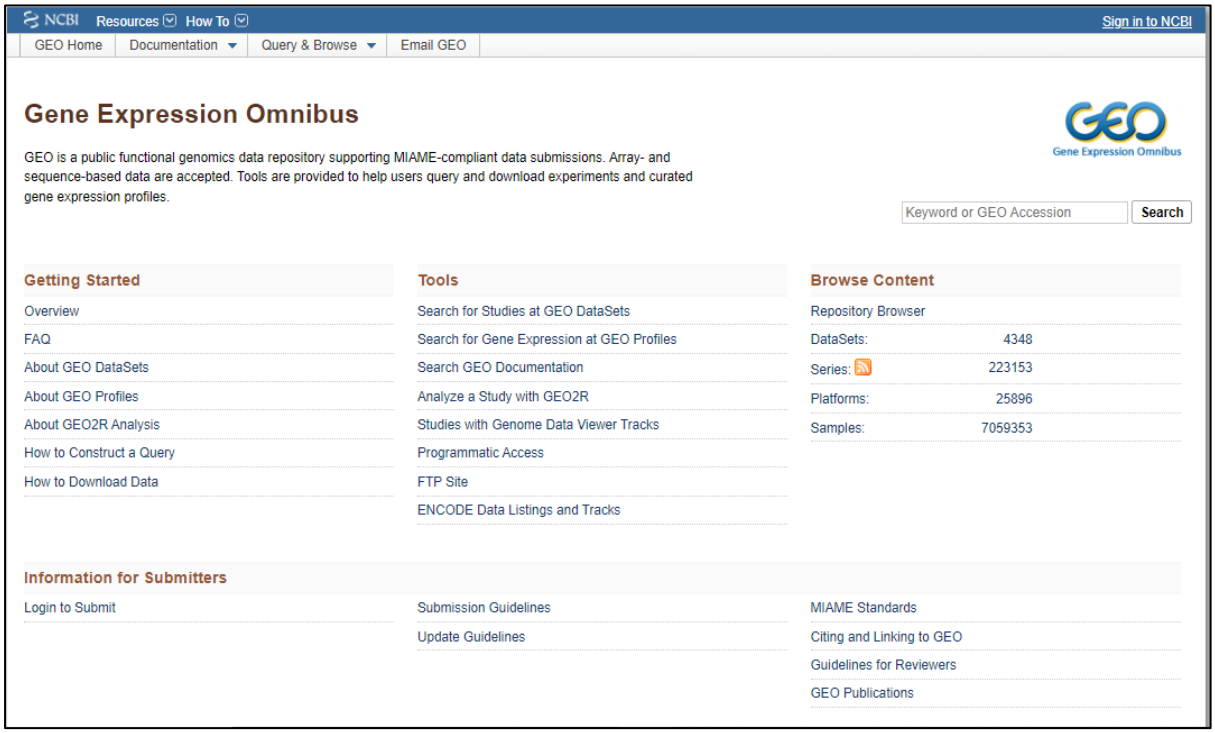

Fig 1: Homepage of Gene Expression Omnibus (GEO) Database

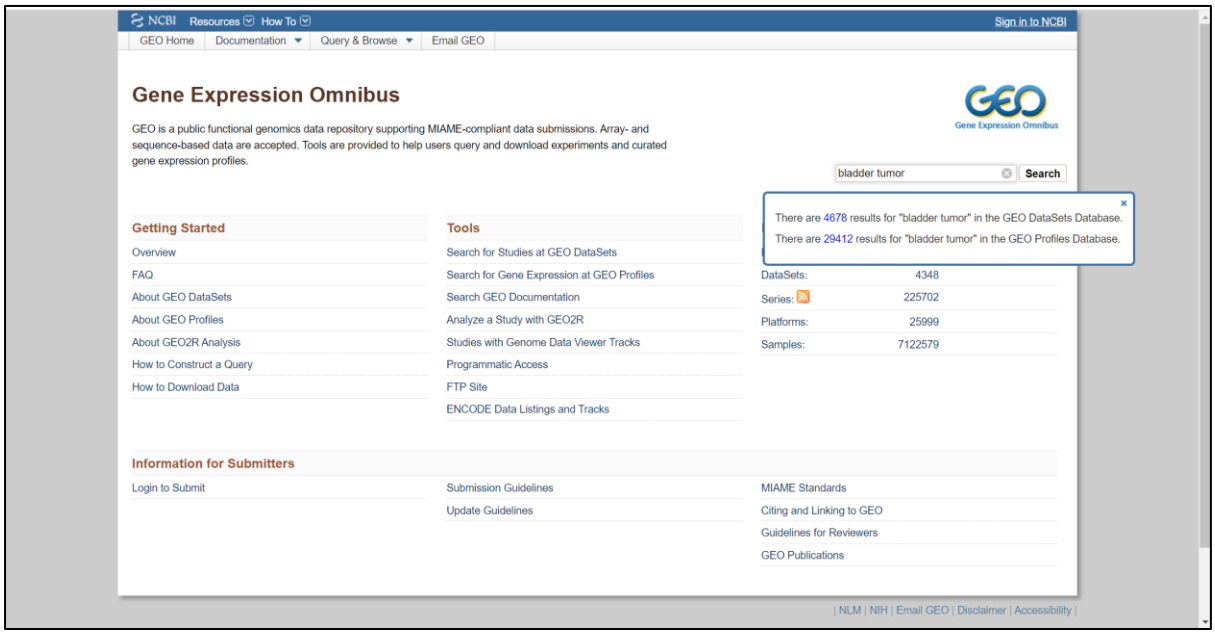

Fig 2: GEO Database search for the query 'Bladder tumor'

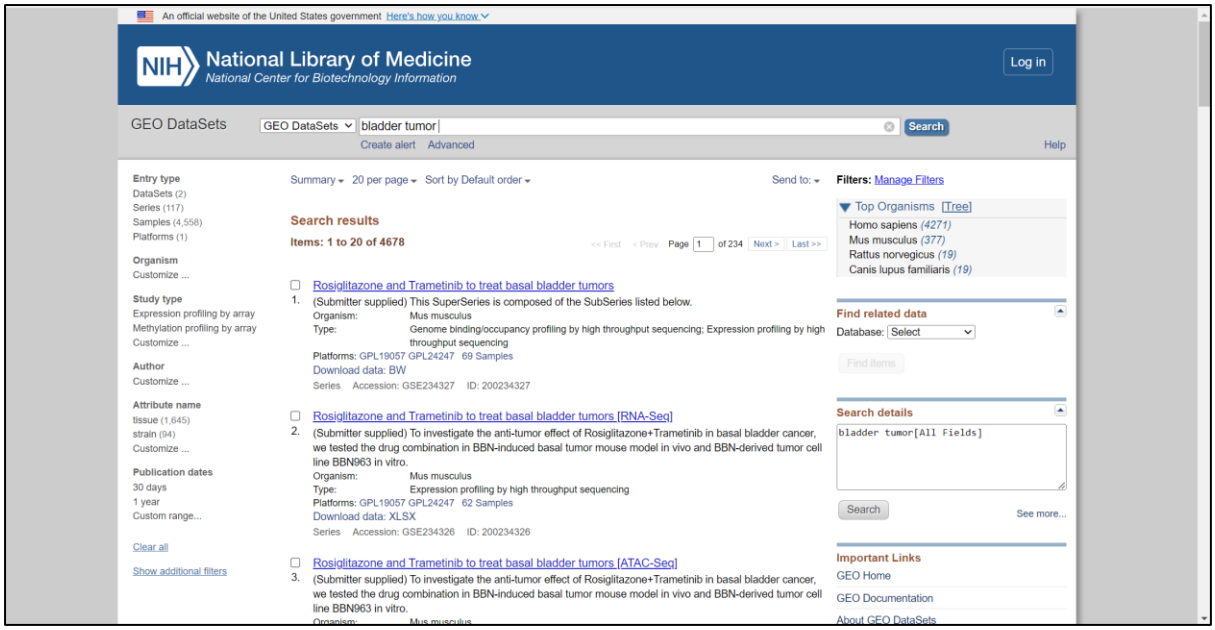

Fig 3: GEO DataSets obtained for Query 'Bladder tumor'

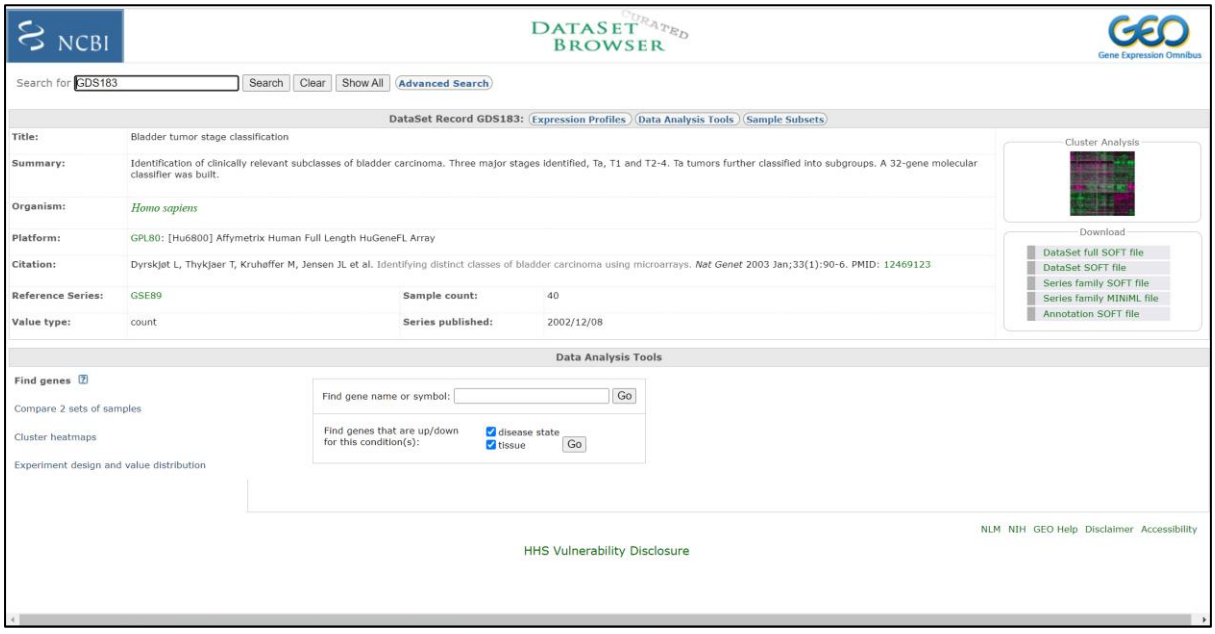

Fig 4: Results obtained for Dataset: 'GDS183'

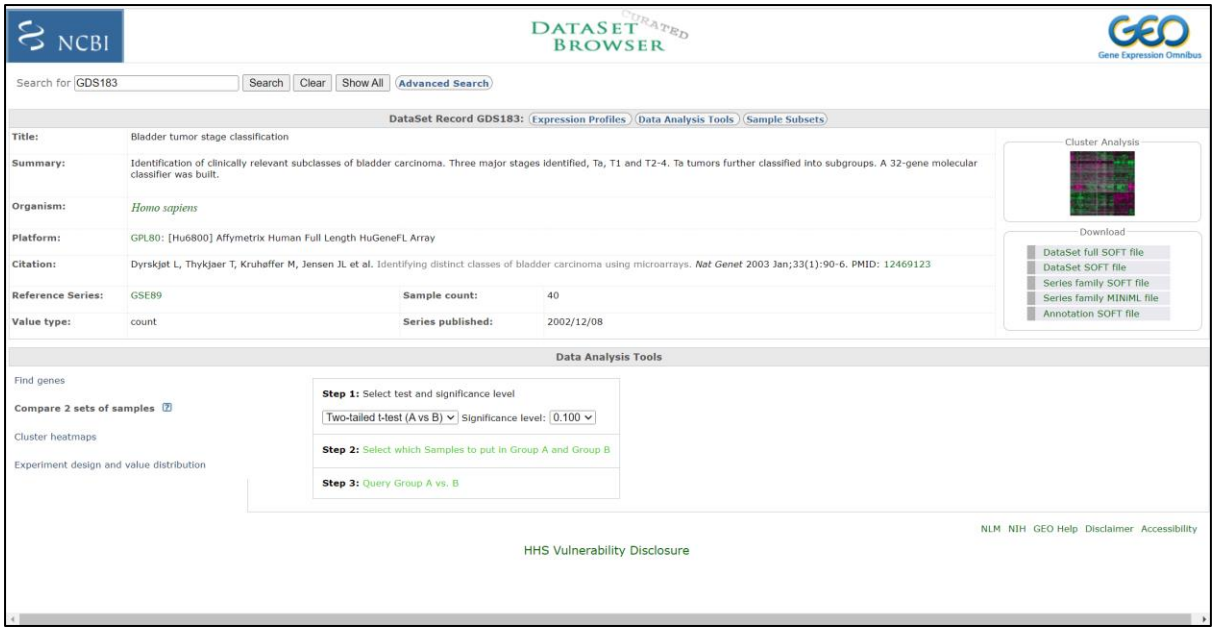

Fig 5: Data Analysis tool

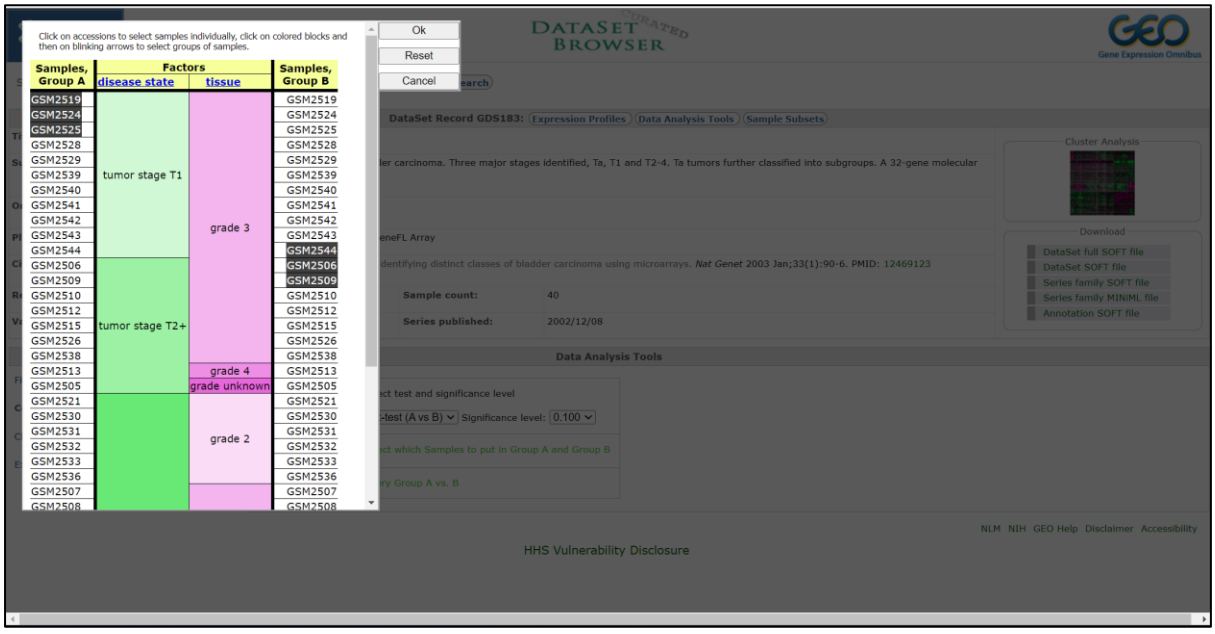

Fig 6: Selection of samples in Group A and Group B

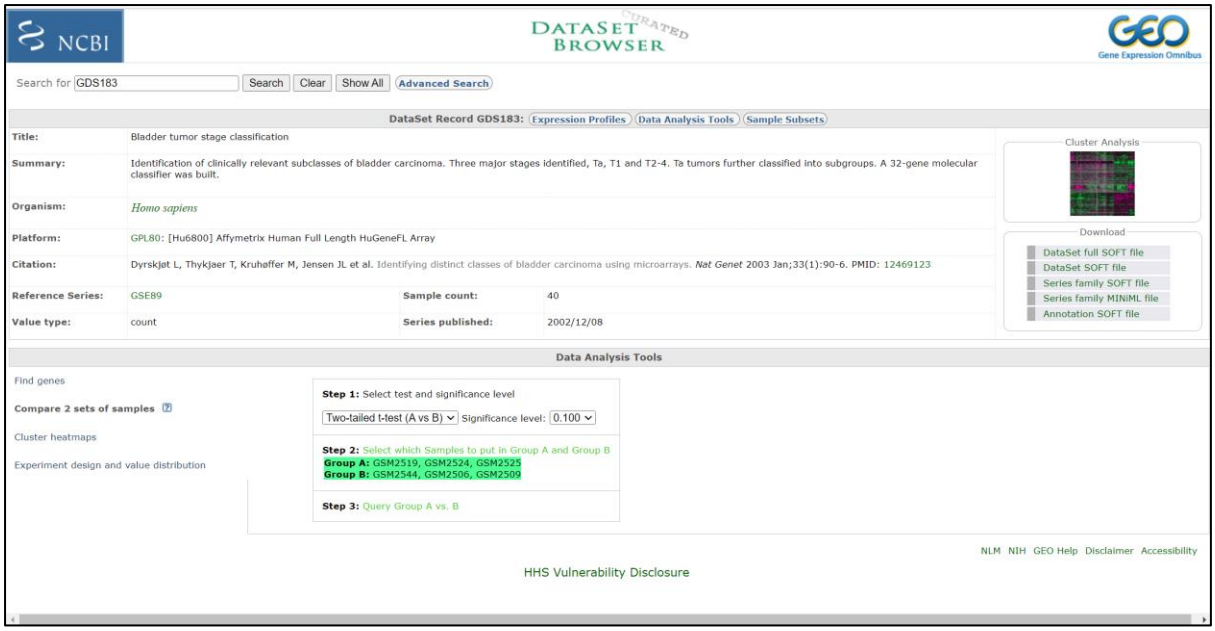

Fig 7: Data Analysis tool after selection of samples for comparison

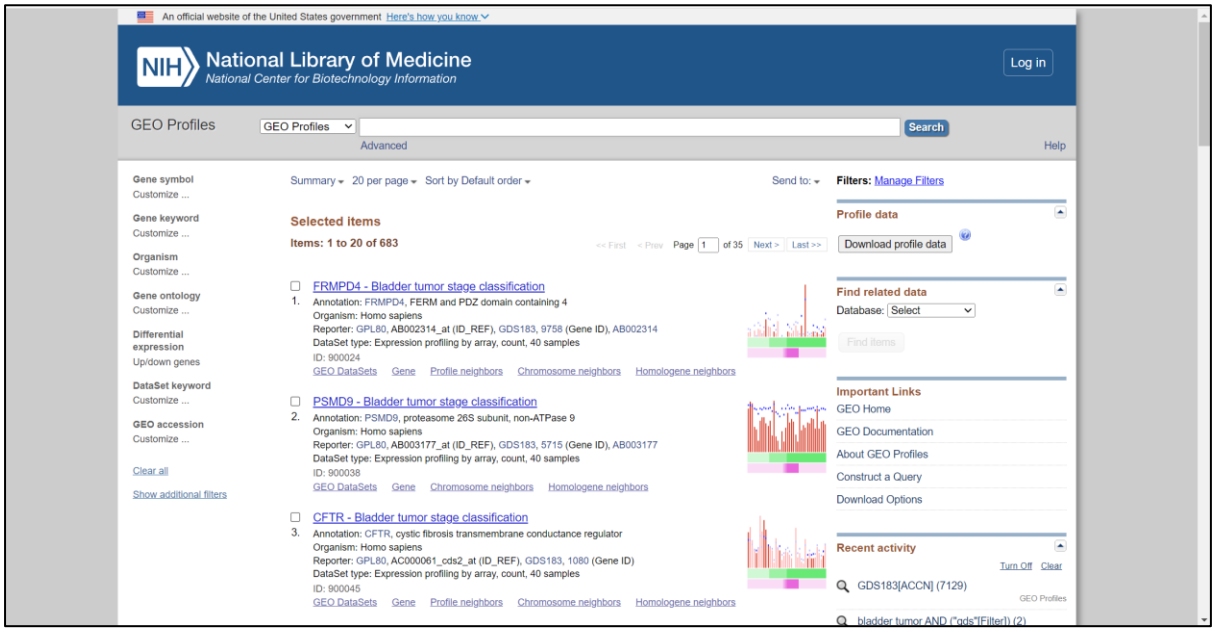

Fig 8: GEO Profiles page for selected samples

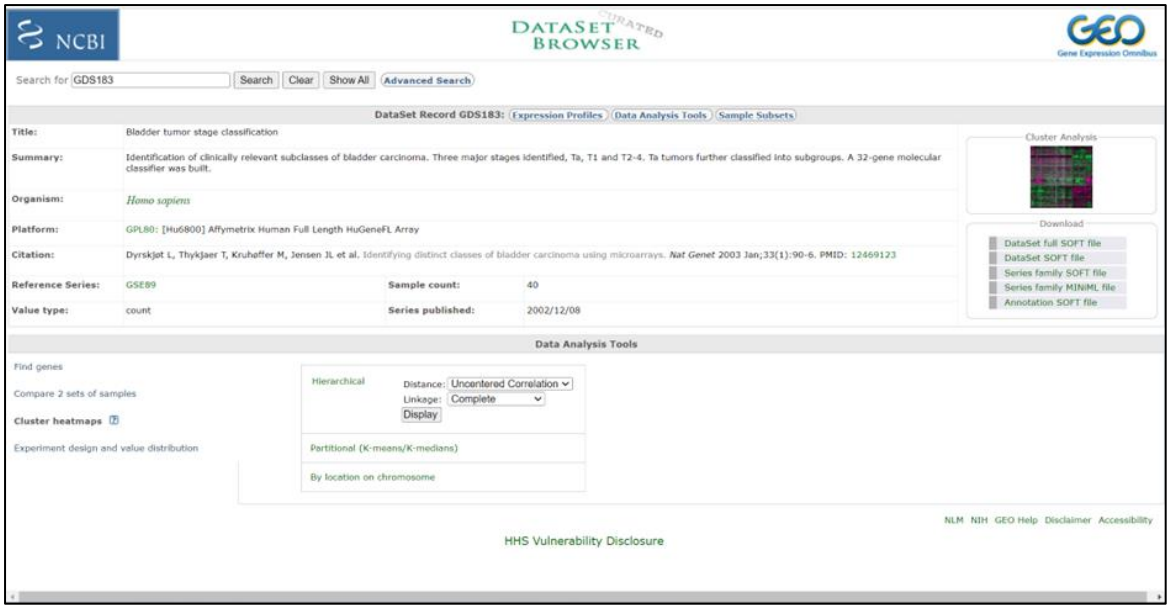

Fig 9: Option to analyze Query GDS183 using cluster maps

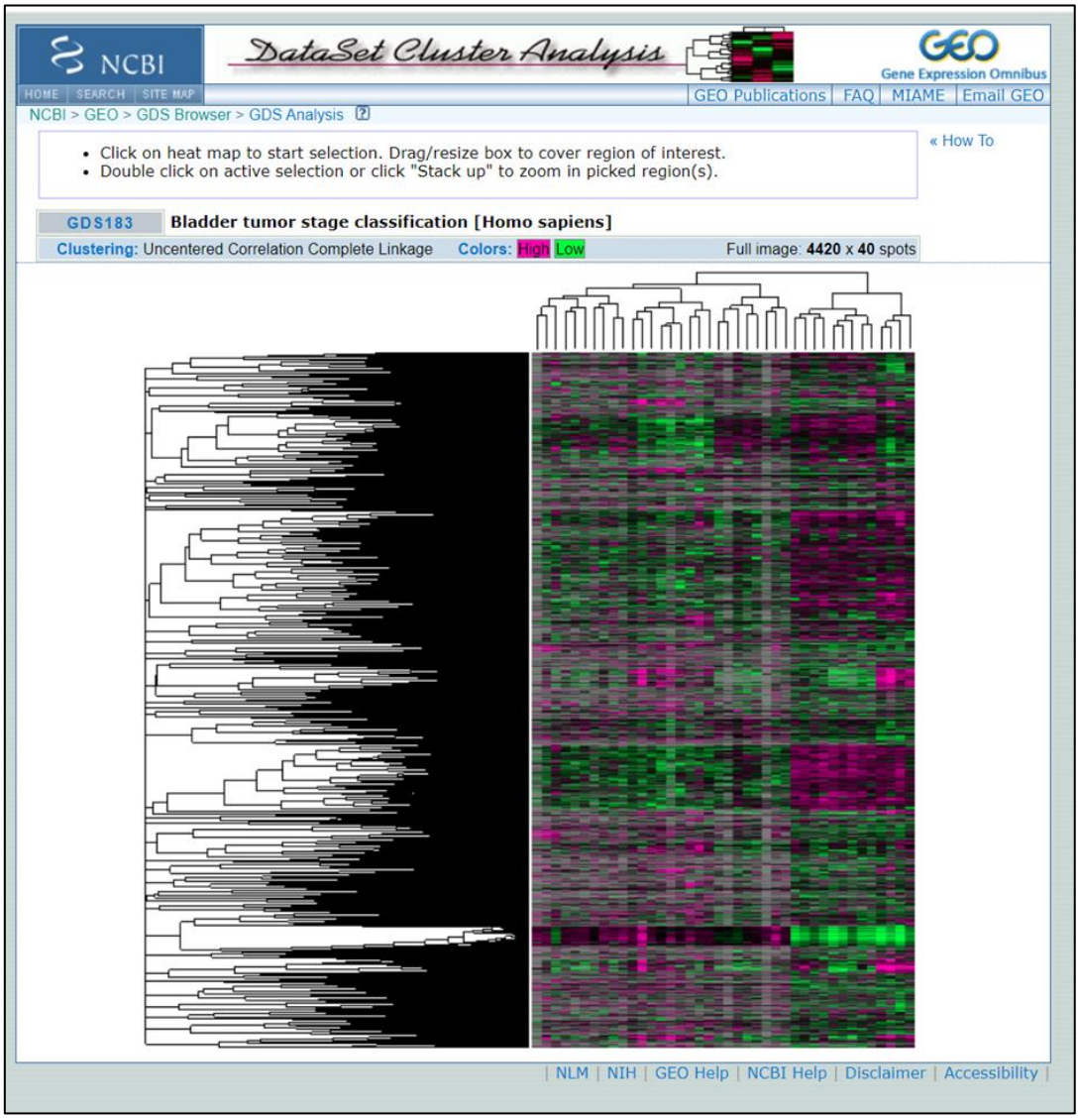

Fig 10: Cluster Heat maps for Query GDS183

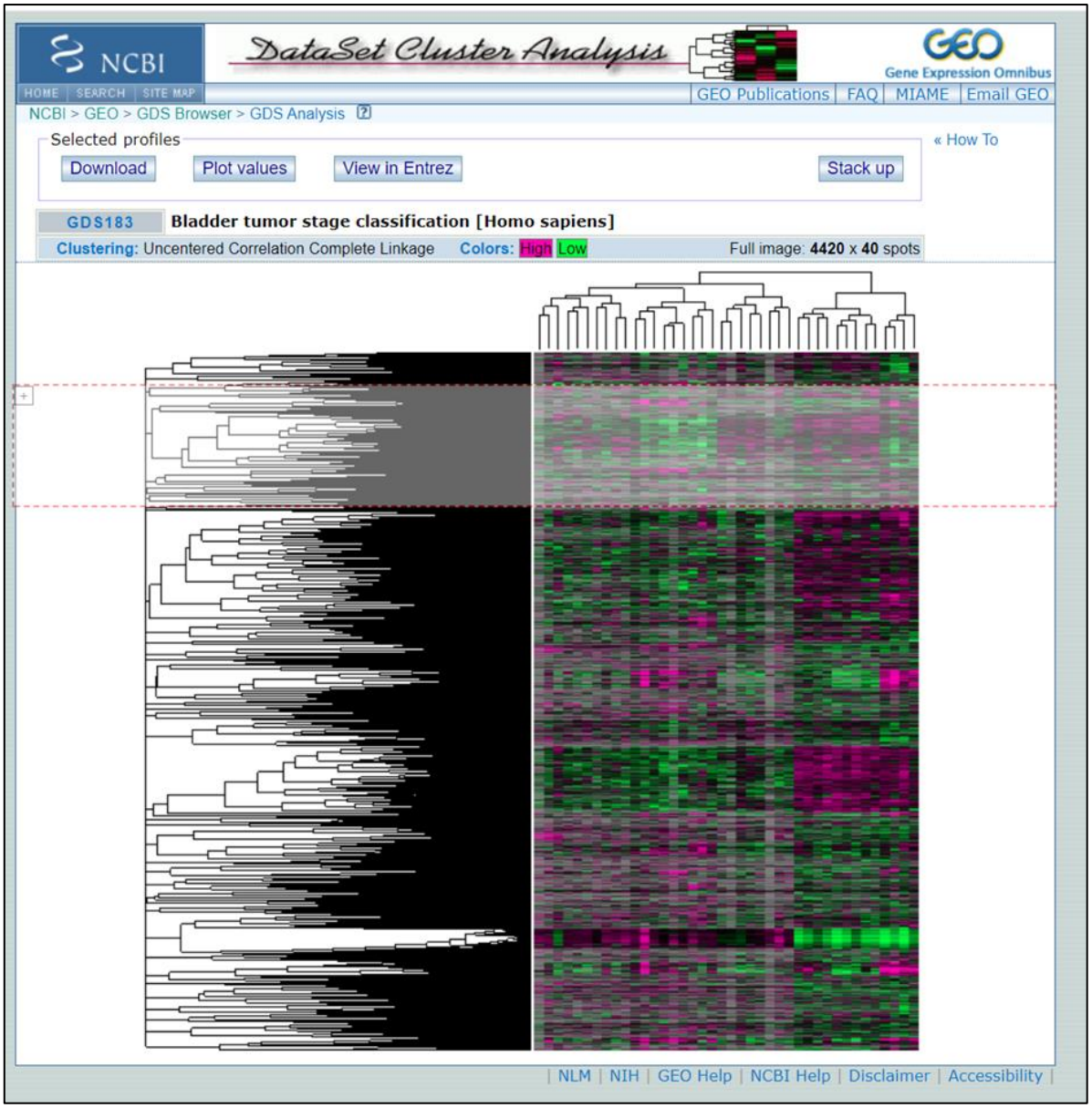

Fig 11: Selecting Region of Interest

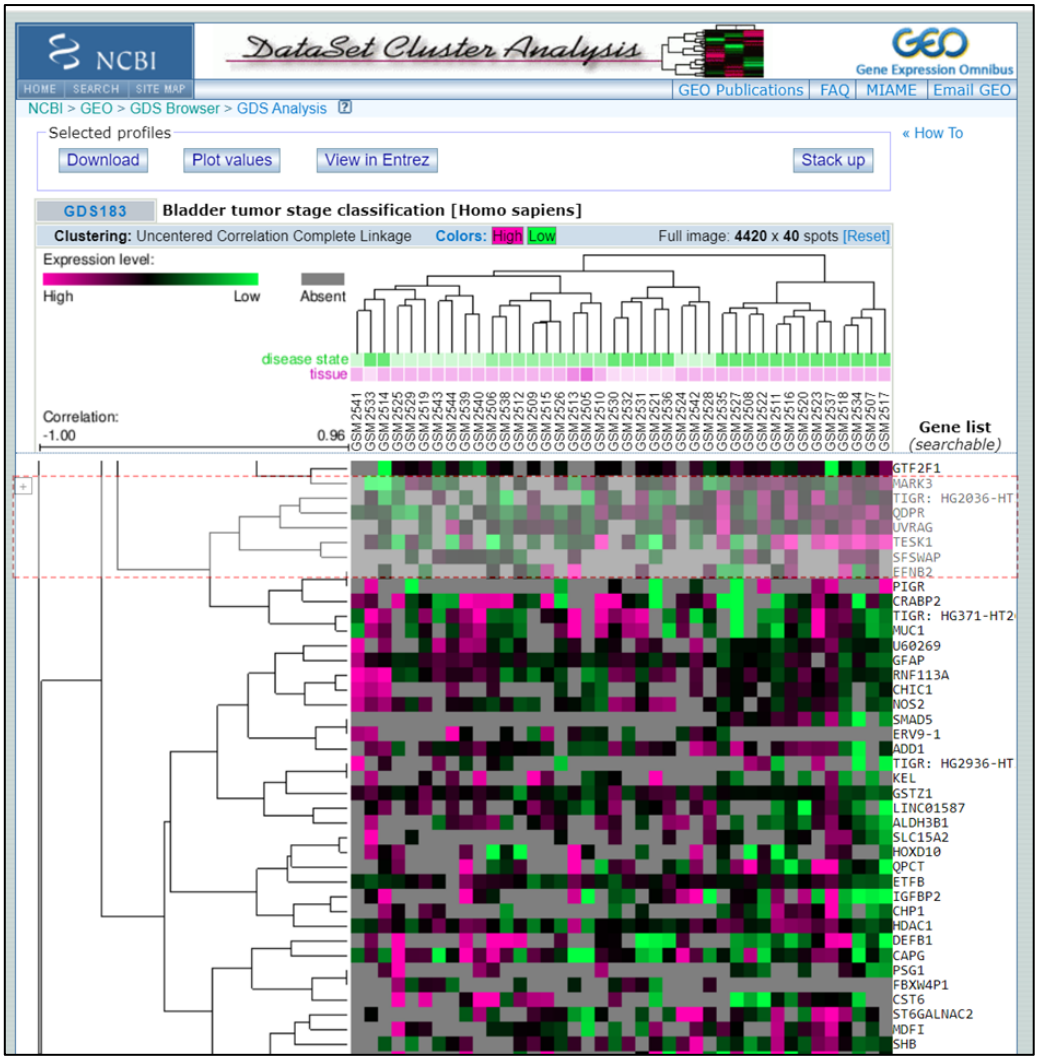

Fig 12: Cluster Heatmaps for selected region

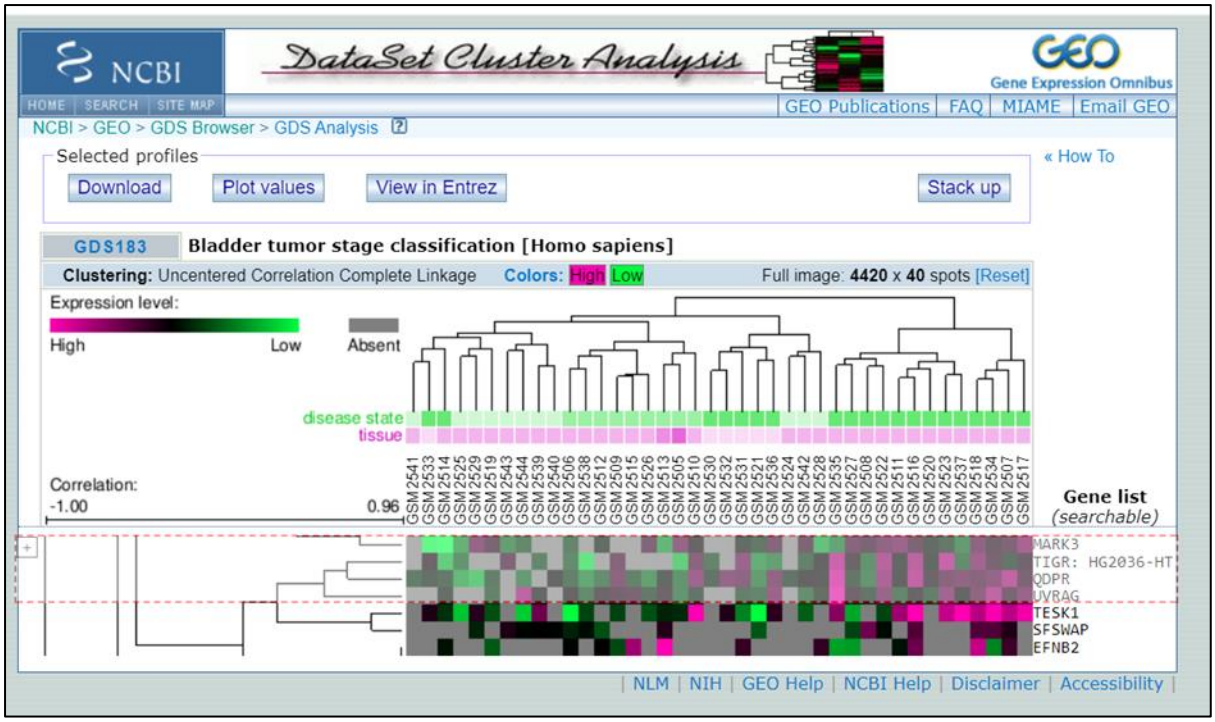

Fig 13: Cluster Heatmaps for Genes selected from Gene Lists in Fig 12

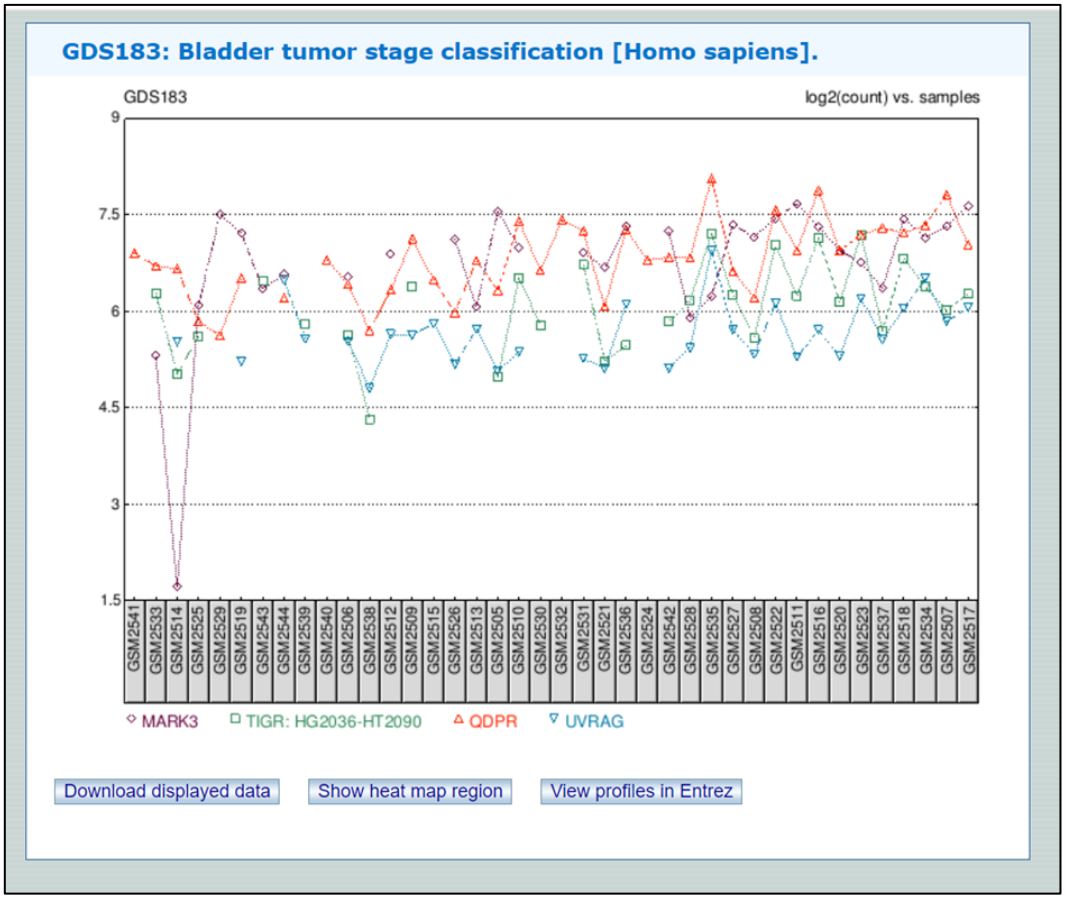

Fig 14: Plot Values of Genes selected in Fig 13

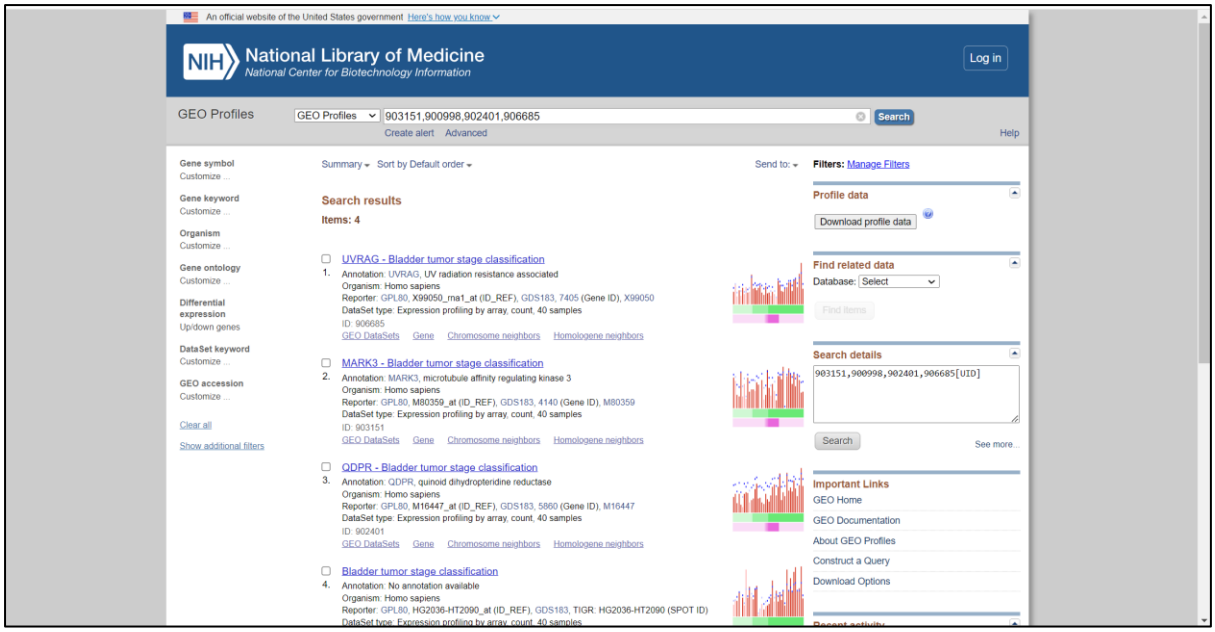

Fig 15: GEO Profiles in Entrez for the Selected Genes

RESULTS:

GEO Database showed differentially expressed genes for the query 'Bladder tumor'. Structured data files containing gene expression measurements and associated metadata were retrieved for the query. It also provided visualization tools and links to external analysis tools to analyze the data. Results were obtained into two categories which were 4678 in GEO DataSets Database and 29412 in GEO Profiles Database.

CONCLUSION:

GEO Database was explored in order to understand differential gene expression for the query 'Bladder tumor'.

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5. *What Is Bladder Cancer?* (2023, February 16). National Cancer Institute. https://www.cancer.gov/types/bladder

DATE: 17/03/2024

WEBLEM 3
GPCR Database
(URL: https://gpcrdb.org/)

The GPCR database, GPCRdb was started in 1993 by Gert Vriend, Ad IJzerman, Bob Bywater and Friedrich Rippmann. Over two decades, GPCRdb evolved to be a comprehensive information system storing and analyzing data. In 2013, the stewardship of GPCRdb was transferred to the David Gloriam group at the University of Copenhagen, backed up by an international team of contributors and developers from the EU COST Action 'GLISTEN'.

GPCRdb offers reference data and easy-to-use web tools and diagrams for a multidisciplinary audience investigating GPCR function, drug design or evolution. It stores a manual annotation of all GPCR crystal structures, the largest collections of receptor mutants and reference sequence alignments. The tools run directly in the web browser allowing for swift analysis of structures, sequence similarities, receptor relationships, and ligand target profiles. Diagrams illustrate receptor sequences (snake-plot and helix box diagrams) and relationships (phylogenetic trees). A visual overview can be seen in the GPCRdb poster.

The GPCRdb contains data, diagrams, and web tools for G protein-coupled receptors (GPCRs). Users can browse all GPCR crystal structures and the largest collection of receptor mutants. Diagrams can be produced and downloaded to illustrate receptor residues (snake-plot and helix box diagrams) and relationships (phylogenetic trees). Reference (crystal) structure-based sequence alignments taken into account helix bulges and constrictions, display statistics of amino acid conservation and have been assigned generic residue numbering for equivalent residues in different receptors.

GPCRs or G protein-coupled receptors are proteins located on the cell surface that recognize extracellular substances and transmit signals across the cell membrane. GPCRs do this by activating guanine nucleotide-binding proteins (G protein) that are responsible for signal transduction inside the cell. The signal delivery is very important in various cellular responses including cell growth, gene transcription, post-translational changes, and communication with other cells. This brings on adjustments in the body to adapt to the environmental changes such as increased heart rate when feeling threatened or change in vision in response to dim light. The human genome alone consists of at least 1,000 different GPCRs that detect hormones, lipids, amines, neurotransmitters, and light, among other things. A GPCR consists of three regions. The extracellular part detects and binds the ligand. Then, the seven-transmembrane region undergoes a conformational change.

OBJECTIVES COVERED UNDER (GPCR):

1. Sequence Alignment
2. To Retrieve Structures
3. Endogenous Ligand
4. Generating models
5. Receptor search
6. Mutation studies

1) Sequence Alignment:

1. **Structure-based alignments**: Users select receptors and sequence segments, then view an alignment with sequence and generic numbers. A consensus sequence and conservation statistics are displayed.
2. **Phylogenetic trees**: Users select receptors and sequence segments, then configure settings such as bootstrapping replicas and tree type. Trees are generated using PHYLIP and jsPhyloSVG, with an option to view the alignment.
3. **Similarity search -** GPCRdb: Users select a reference receptor, sequence segments, and a comparison set. The tool computes similarities, displaying an alignment ranked by similarity with computed properties.
4. **Similarity search -** BLAST: Users submit sequences for a standard BLAST search against a custom database containing GPCRdb sequences. results show the best BLAST hits.
5. **Similarity matrix:** Users select receptors and sequence segments, then view a table of identities and similarities, color-coded for easy interpretation. Each tool warns about potential performance issues with large datasets. These tools collectively offer a comprehensive toolkit for GPCR sequence analysis, covering alignment, phylogenetics, similarity search, and similarity visualization.

2) To Retrieve Structures:

The GPCRdb database provides comprehensive resources for G protein-coupled receptor (GPCR) structures, including a structure browser, refined structures, statistics, structure models, validation, and descriptors. The structure browser allows sorting and filtering of annotated GPCR structures, while refined structures incorporate missing segments and revert mutations. Structure statistics offer insights into available structures by year, resolution, and phylogenetic trees. Structure models include homology models for human GPCRs in various activation states. Model validation employs root-mean-square deviation (RMSD) calculations. Activation state definitions rely on Cα atom distances, while TM6 tilt measures assess receptor conformation. These resources aid in understanding GPCR structure-function relationships and are invaluable for research, with automated updates ensuring data currency and reliability.

3) Endogenous Ligand:

The Ligand Coverage section of GPCRdb provides an extensive overview of ligands associated with G protein-coupled receptors (GPCRs). A table displays ligand counts per receptor class, average ligands per GPCR, and the number of GPCRs with ligands. Phylogenetic trees visualize ligand data, with shaded inner circles indicating ligand counts per receptor. Users can explore ligands through receptor-based or ligand-based queries. Receptor-based queries allow selection of receptors and exploration of ligand bioactivities. Ligand-based queries enable searching by name, ID, or structure, leading to detailed ligand info pages. Additionally, an endogenous ligand browser offers data on endogenous ligands for human and rodent GPCRs. GPCRdb's Ligand Coverage section offers a user-friendly interface for comprehensive ligand exploration, serving both research and clinical needs.

4) Generating Models:

1. Structural data, such as crystallography or cryo-electron microscopy. These models serve as valuable tools for understanding the structural and functional properties of GPCRs, facilitating drug discovery and therapeutic development.
2. The generation of models typically involves several steps, including sequence alignment, homology modelling, and molecular dynamics simulations. Sequence alignment compares the amino acid sequences of the target receptor with known structures of related receptors, identifying conserved regions and structurally significant residues. Homology modelling then utilizes this alignment to predict the 3D structure of the target receptor based on the known structures of homologous proteins.
3. Molecular dynamics simulations refine the homology models by simulating the movement and interactions of atoms within the receptor structure, providing insights into its dynamic behavior and ligand binding properties. These computational models can be further validated and refined using experimental data, such as biochemical assays or mutagenesis studies.

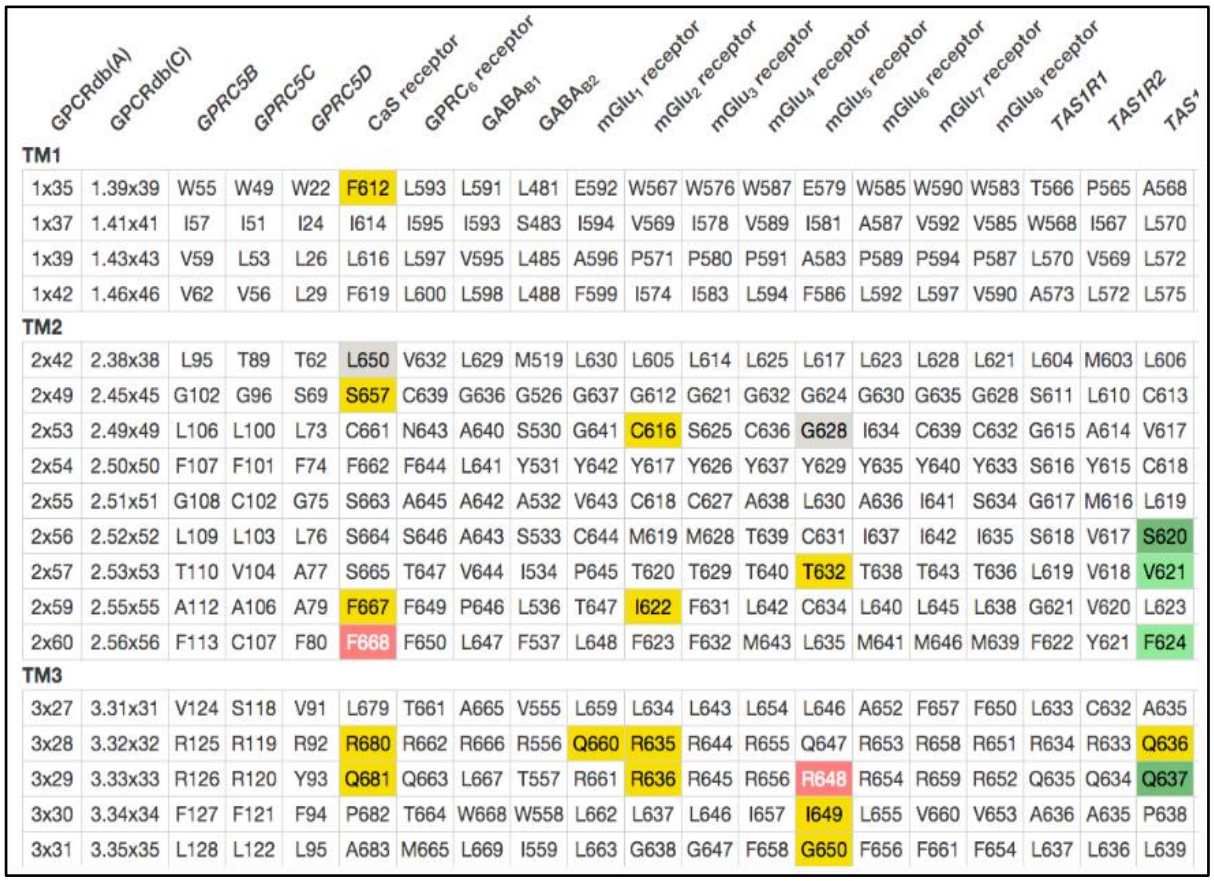
5) Receptor Search:

1. In the GPCR database, users can perform receptor searches to access detailed information on G protein-coupled receptors (GPCRs). By navigating to the receptor search functionality, users can input specific receptor names, aliases, or identifiers to retrieve relevant data. The search results typically include comprehensive information about the selected receptors, such as their names, classifications, structures, functions, and associated ligands.
2. Furthermore, users may encounter additional features within the receptor search interface, allowing for advanced filtering options or customization of search parameters. These features enable researchers and clinicians to tailor their searches to specific criteria, such as receptor families, species, or functional properties.
3. The receptor search functionality in the GPCR database serves as a valuable tool for accessing detailed information on GPCRs, facilitating research, drug discovery, and therapeutic development in the field of pharmacology and molecular biology. It provides a user-friendly interface for efficiently retrieving and exploring data related to GPCR receptors, contributing to advancements in understanding their roles in various physiological processes and diseases.

6) Mutational Studies:

The GPCRdb features a Mutation Browser enabling users to explore mutant data for specific receptors or receptor families. Users first select receptors and sequence segments, then view mutants in a table that can be sorted and filtered. Helix box and snake plots below highlight mutated residues on consensus sequences. Additionally, a table displays all residues with mutations highlighted. The database continuously welcomes contributions of mutational data, encouraging researchers to submit their findings, including data on pharmacological effects. A standardized Excel spreadsheet facilitates data submission, focusing on effects like ligand binding, function, surface expression, basal activity, and Emax. Future includes

expanding data collection to mutations affecting thermo-stabilization, biased signaling, G-protein binding, and dimerization. The Mutation Browser allows users to search mutations by receptor, sub-family, or generic numbering position, offering visualizations like snake and helix box diagrams. These tools aid in understanding mutational effects on ligand binding and function, with future to integrate mutational data into 3D structures.



	GPCRdb(A)	GPCRdb(C)	GPRC5B	GPRC5C	GPRC5D	CaS receptor	$GPRC_6$ receptor	$GABA_{B1}$	$GABA_{B2}$	$mGlu_1$ receptor	$mGlu_2$ receptor	$mGlu_3$ receptor	$mGlu_4$ receptor	$mGlu_5$ receptor	$mGlu_6$ receptor	$mGlu_7$ receptor	$mGlu_8$ receptor	TAS1R1	TAS1R2	TAS1
TM1																				
	1x35	1.39x39	W55	W49	W22	F612	L593	L591	L481	E592	W567	W576	W587	E579	W585	W590	W583	T566	P565	A568
	1x37	1.41x41	I57	I51	I24	I614	I595	I593	S483	I594	V569	I578	V589	I581	A587	V592	V585	W568	I567	L570
	1x39	1.43x43	V59	L53	L26	L616	L597	V595	L485	A596	P571	P580	P591	A583	P589	P594	P587	L570	V569	L572
	1x42	1.46x46	V62	V56	L29	F619	L600	L598	L488	F599	I574	I583	L594	F586	L592	L597	V590	A573	L572	L575
TM2																				
	2x42	2.38x38	L95	T89	T62	L650	V632	L629	M519	L630	L605	L614	L625	L617	L623	L628	L621	L604	M603	L606
	2x49	2.45x45	G102	G96	S69	S657	C639	G636	G526	G637	G612	G621	G632	G624	G630	G635	G628	S611	L610	C613
	2x53	2.49x49	L106	L100	L73	C661	N643	A640	S530	G641	C616	S625	C636	G628	I634	C639	C632	G615	A614	V617
	2x54	2.50x50	F107	F101	F74	F662	F644	L641	Y531	Y642	Y617	Y626	Y637	Y629	Y635	Y640	Y633	S616	Y615	C618
	2x55	2.51x51	G108	C102	G75	S663	A645	A642	A532	V643	C618	C627	A638	L630	A636	I641	S634	G617	M616	L619
	2x56	2.52x52	L109	L103	L76	S664	S646	A643	S533	C644	M619	M628	T639	C631	I637	I642	I635	S618	V617	S620
	2x57	2.53x53	T110	V104	A77	S665	T647	V644	I534	P645	T620	T629	T640	T632	T638	T643	T636	L619	V618	V621
	2x59	2.55x55	A112	A106	A79	F667	F649	P646	L536	T647	I622	F631	L642	C634	L640	L645	L638	G621	V620	L623
	2x60	2.56x56	F113	C107	F80	F668	F650	L647	F537	L648	F623	F632	M643	L635	M641	M646	M639	F622	Y621	F624
TM3																				
	3x27	3.31x31	V124	S118	V91	L679	T661	A665	V555	L659	L634	L643	L654	L646	A652	F657	F650	L633	C632	A635
	3x28	3.32x32	R125	R119	R92	R680	R662	R666	R556	Q660	R635	R644	R655	Q647	R653	R658	R651	R634	R633	Q636
	3x29	3.33x33	R126	R120	Y93	Q681	Q663	L667	T557	R661	R636	R645	R656	R648	R654	R659	R652	Q635	Q634	Q637
	3x30	3.34x34	F127	F121	F94	P682	T664	W668	W558	L662	L637	L646	I657	I649	L655	V660	V653	A636	A635	P638
	3x31	3.35x35	L128	L122	L95	A683	M665	L669	I559	L663	G638	G647	F658	G650	F656	F661	F654	L637	L636	L639

Fig 1: Sequence comparison of the 7TM domain binding pocket in the eight mGlu receptor subtypes with all residues that have been mutated

Color-coding: Green indicates increased binding/potency of >5-fold (light green) or >10-fold (dark green), red indicates reduced binding/potency of >5-fold (pink) or >10-fold(red), yellow indicates No/low effect (<5-fold), and grey indicates that no effect is annotated. The first two columns show generic GPCRdb residue numbers for each row of residues

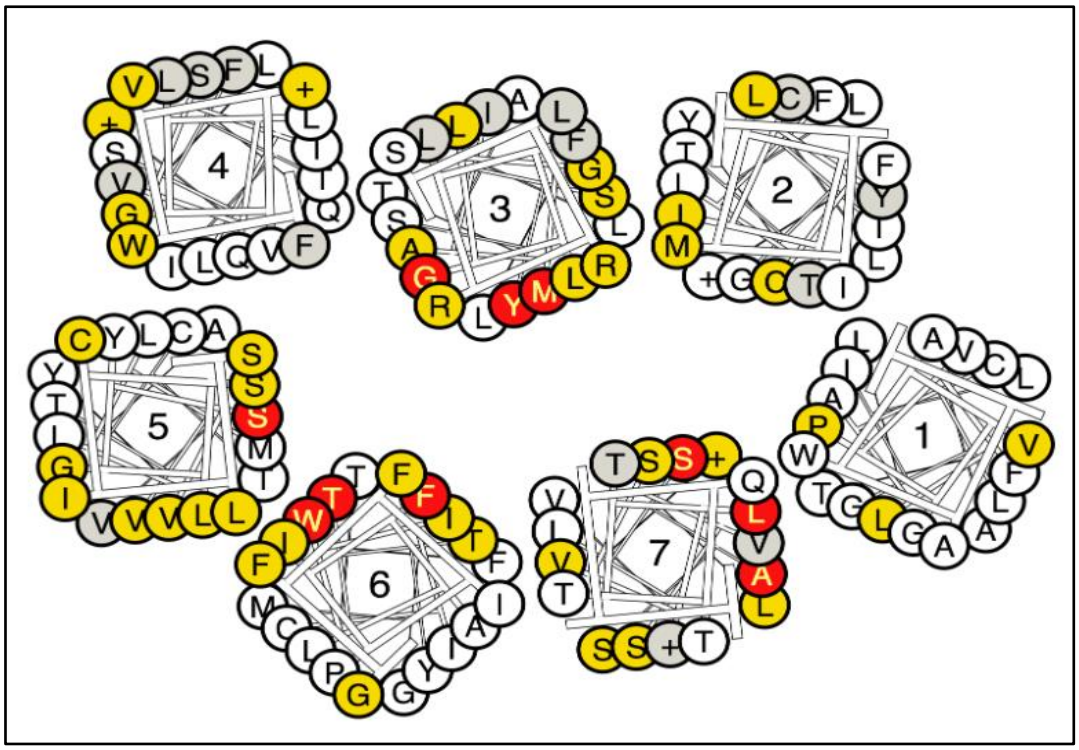


Fig 2: A helix box diagram of the metabotropic glutamate receptors displaying mutated residue positions from the extracellular side with all residues that have been mutated

Color-coding: Green indicates increased binding/potency of >5-fold (light green) or >10-fold (dark green), red indicates reduced binding/potency of >5-fold (pink) or >10-fold(red), yellow indicates No/low effect (<5-fold), and grey indicates that no effect is annotated

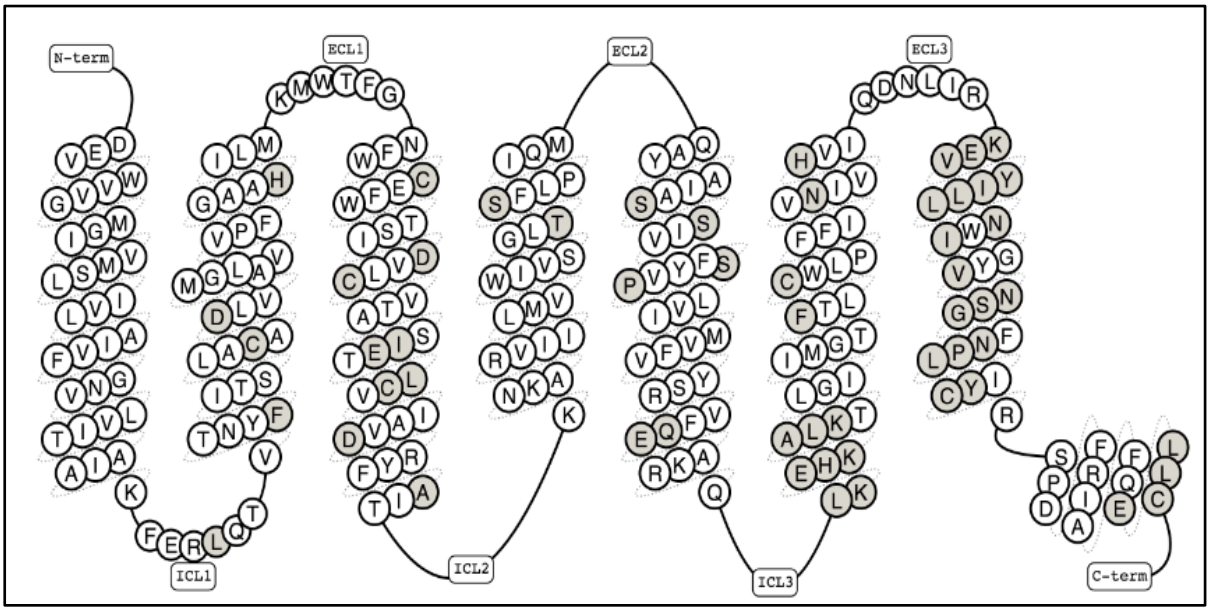


Fig 3: Snake diagram of the human β2 -adrenoceptor showing all residues (grey) for which mutational experiments have been deposited in the GPCRdb

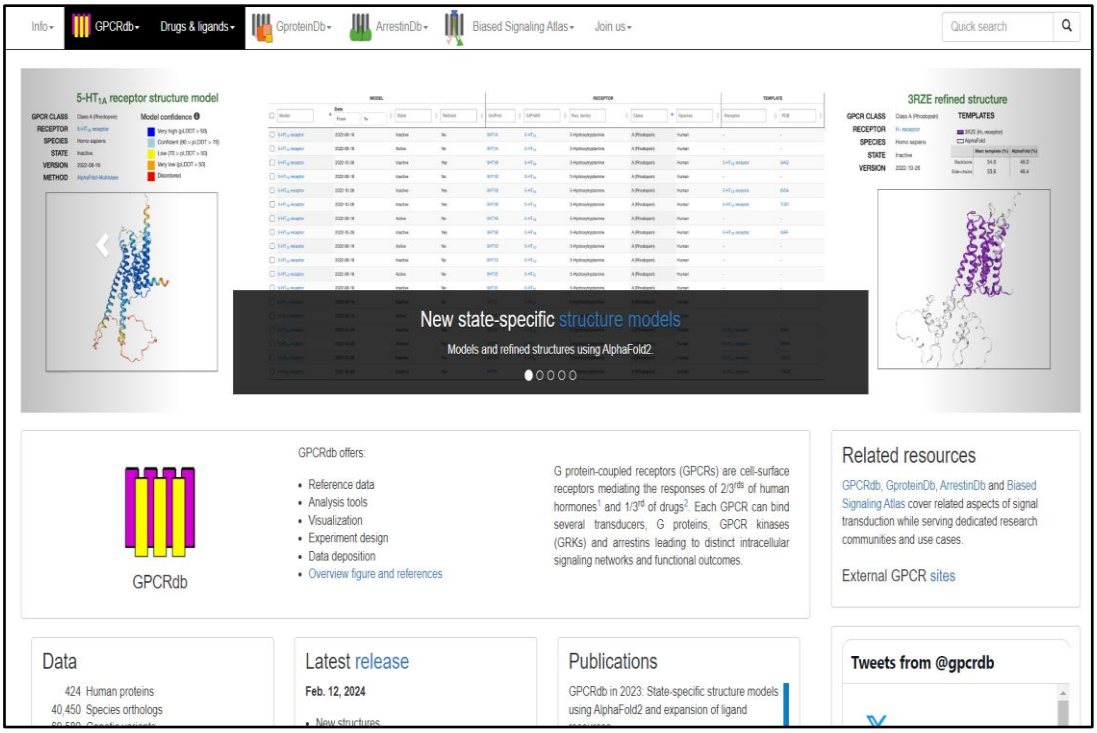


Fig 4: Homepage of GPCR Database

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DATE: 17/03/2024

WEBLEM 3(A)
GPCR DATABASE
(URL: https://gpcrdb.org/)

AIM:

To explore structure and functional characteristics of query 'PTH1 receptor' by using GPCR database.

INTRODUCTION:

The GPCR database, GPCRdb was started in 1993 by Gert Vriend, Ad IJzerman, Bob Bywater and Friedrich Rippmann. Over two decades, GPCRdb evolved to be a comprehensive information system storing and analyzing data. In 2013, the stewardship of GPCRdb was transferred to the David Gloriam group at the University of Copenhagen, backed up by an international team of contributors and developers from the EU COST Action 'GLISTEN'.

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PTH1:

PTHR1, or parathyroid hormone receptor-1, encodes a G protein-coupled receptor pivotal in bone biology, mediating the actions of parathyroid hormone (PTH) and PTH-related protein (PTHrP). Its roles in regulating bone formation and resorption make it a promising therapeutic target for osteoporosis treatment. PTHR1 exhibits distinct high-affinity conformations, R(0) and RG, with ligand analogs capable of selectively binding to these conformations, influencing signaling responses and calcemic effects. Abaloparatide, a synthetic PTHrP analog, demonstrates potential as an osteoporosis therapy by stimulating bone formation with reduced bone resorption and hypercalcemic effects compared to PTH (1-34), attributed to its selective binding to the RG conformation of PTHR1.

Beyond bone biology, PTHR1 involvement extends to cartilage differentiation regulation. Indian hedgehog (Ihh) regulates hypertrophic differentiation rate in cartilage elements by modulating PTHrP expression in the perichondrium, thereby blocking hypertrophic

differentiation in prehypertrophic chondrocytes. This mechanism forms a negative feedback loop that regulates chondrocyte differentiation rate, highlighting the intricate interplay between PTHR1, Ihh, and PTHrP in skeletal development and homeostasis. These insights deepen our understanding of PTHR1's multifaceted roles in bone and cartilage biology, offering potential avenues for therapeutic interventions in skeletal disorders.

METHODOLOGY:

1. Open homepage of GPCR database.
2. Search for query ‘PTH1’.
3. Under GPCRdb option select sequence alignment, structure models and interpret the results.
4. Under Drugs and ligand option select endogenous ligand (GtP), Mutations and interpret the results.

OBSERVATIONS:

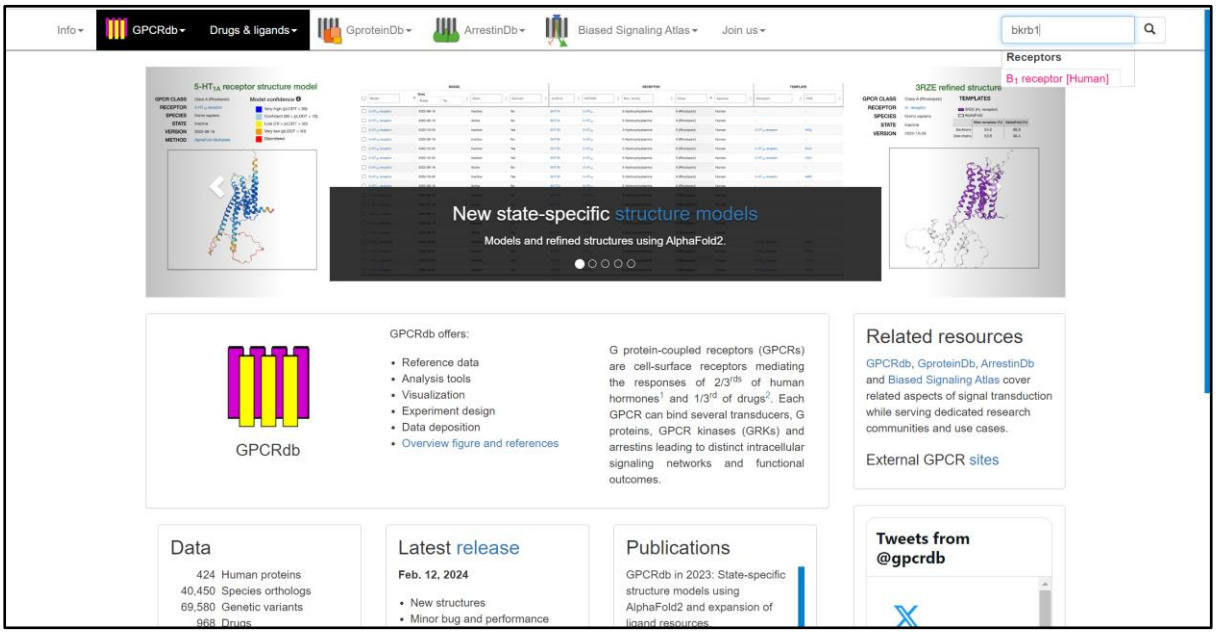


Fig 1: Homepage of GPCR database

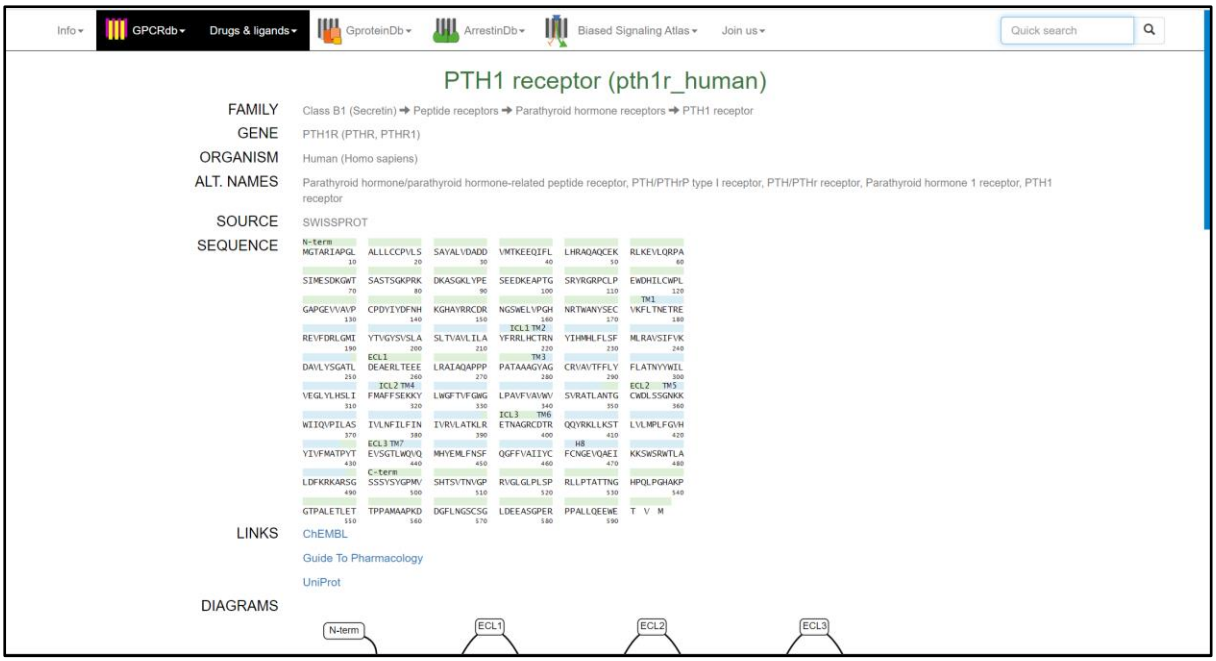


Fig 2: Result page for query 'PTH1 receptor'

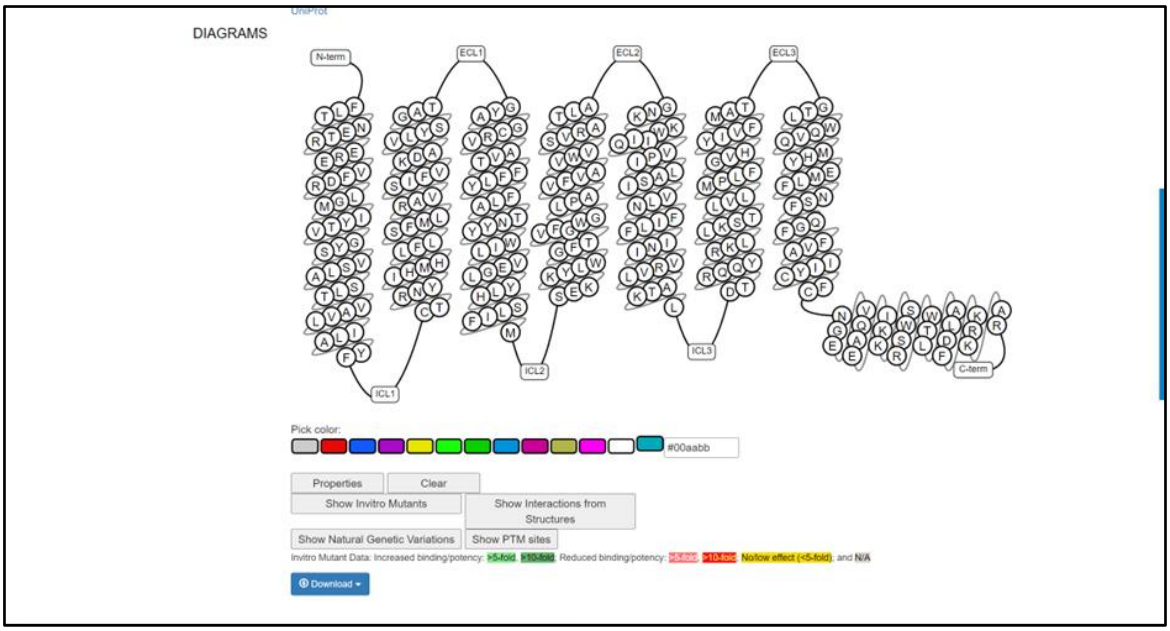


Fig 3: Snake diagram

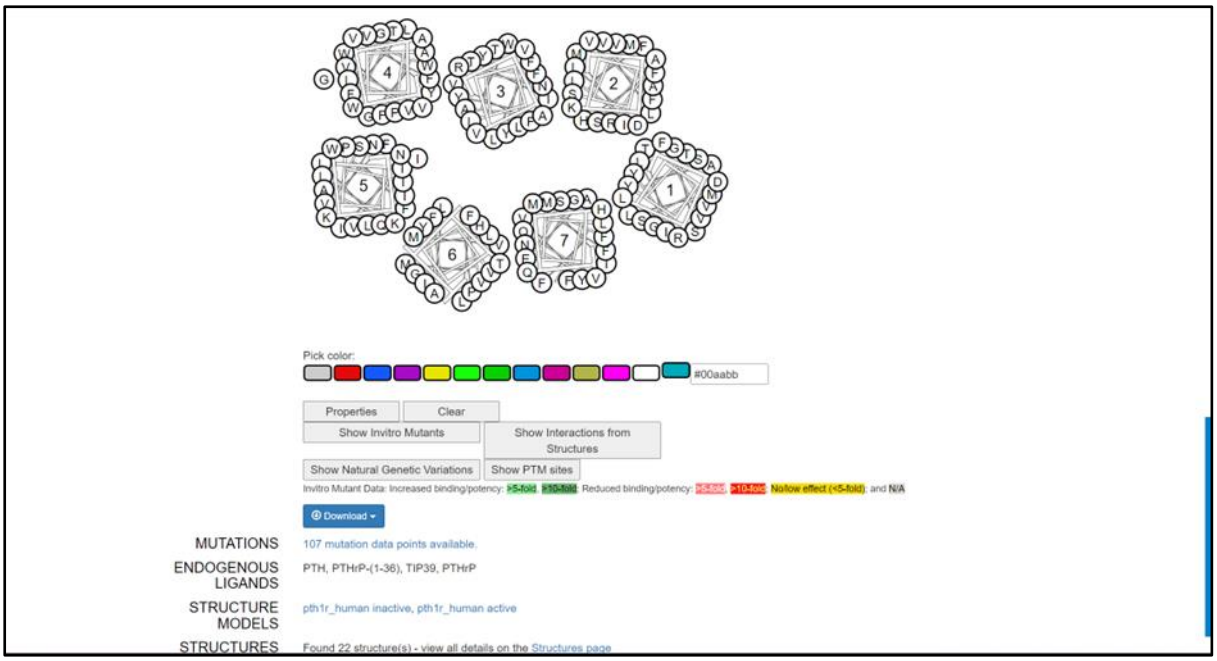


Fig 4: Necklace diagram

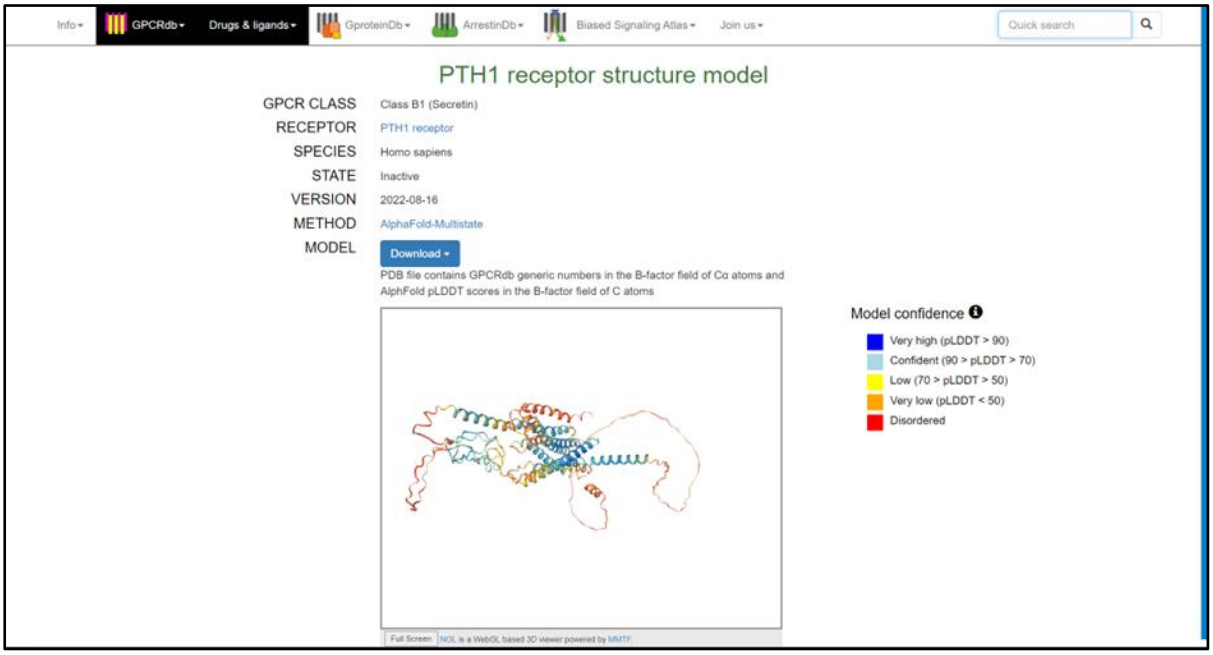


Fig 5: 'PTH1 receptor' structure model

Fig 6: Result for sequence alignment

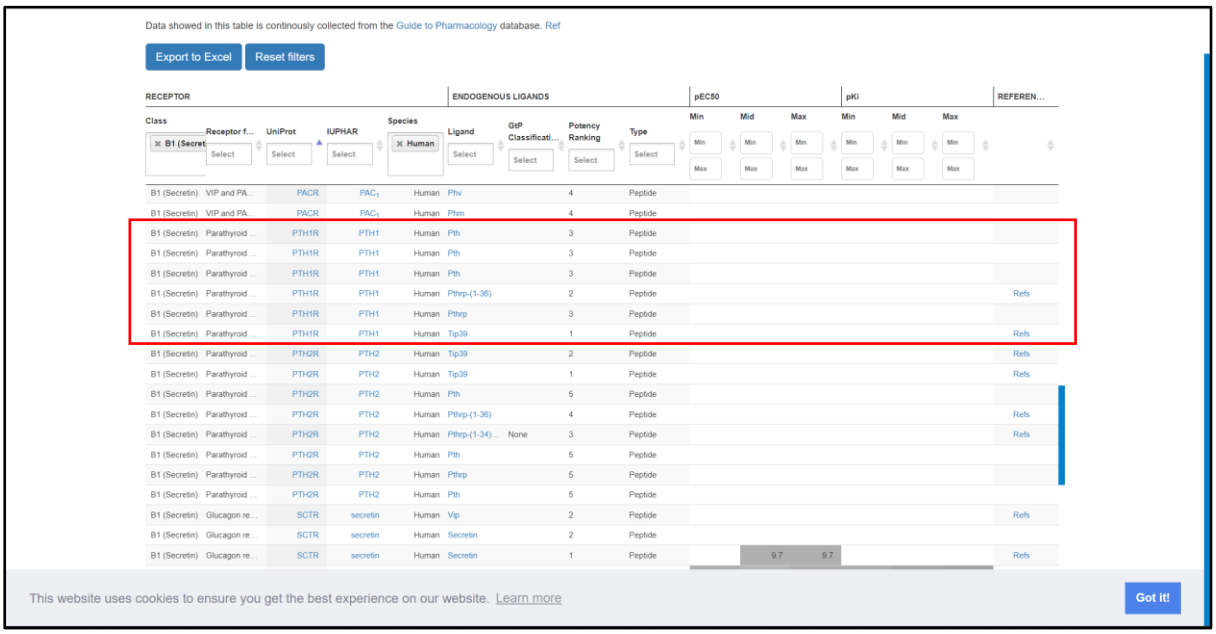
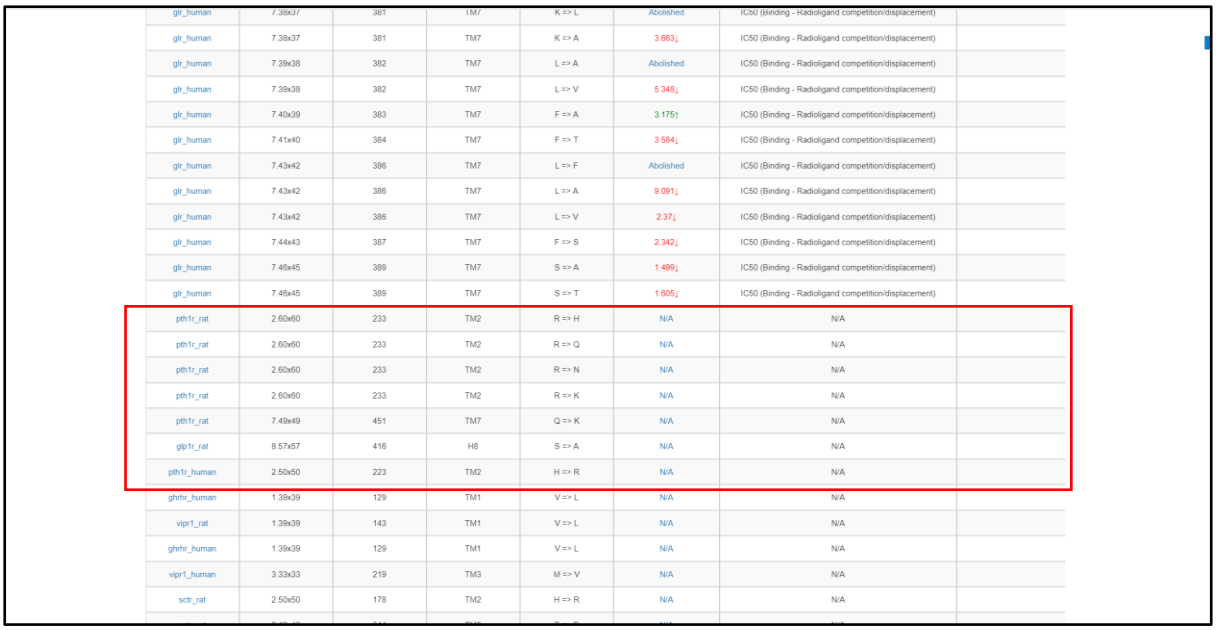


Fig 7: Result for Endogenous ligand



glr_human	7.38x37	381	TM7	K => L	Abolished	IC50 (Binding - Radioligand competition/displacement)
glr_human	7.38x37	381	TM7	K => A	3.663↓	IC50 (Binding - Radioligand competition/displacement)
glr_human	7.39x38	382	TM7	L => A	Abolished	IC50 (Binding - Radioligand competition/displacement)
glr_human	7.39x38	382	TM7	L => V	5.348↓	IC50 (Binding - Radioligand competition/displacement)
glr_human	7.40x39	383	TM7	F => A	3.175↑	IC50 (Binding - Radioligand competition/displacement)
glr_human	7.41x40	384	TM7	F => T	3.584↓	IC50 (Binding - Radioligand competition/displacement)
glr_human	7.43x42	386	TM7	L => F	Abolished	IC50 (Binding - Radioligand competition/displacement)
glr_human	7.43x42	386	TM7	L => A	9.091↓	IC50 (Binding - Radioligand competition/displacement)
glr_human	7.43x42	386	TM7	L => V	2.37↓	IC50 (Binding - Radioligand competition/displacement)
glr_human	7.44x43	387	TM7	F => S	2.342↓	IC50 (Binding - Radioligand competition/displacement)
glr_human	7.46x45	389	TM7	S => A	1.499↓	IC50 (Binding - Radioligand competition/displacement)
glr_human	7.46x45	389	TM7	S => T	1.605↓	IC50 (Binding - Radioligand competition/displacement)
pth1r_rat	2.60x60	233	TM2	R => H	N/A	N/A
pth1r_rat	2.60x60	233	TM2	R => Q	N/A	N/A
pth1r_rat	2.60x60	233	TM2	R => N	N/A	N/A
pth1r_rat	2.60x60	233	TM2	R => K	N/A	N/A
pth1r_rat	7.49x49	451	TM7	Q => K	N/A	N/A
glp1r_rat	8.57x57	416	H8	S => A	N/A	N/A
pth1r_human	2.50x50	223	TM2	H => R	N/A	N/A
ghrhr_human	1.39x39	129	TM1	V => L	N/A	N/A
vipr1_rat	1.39x39	143	TM1	V => L	N/A	N/A
ghrhr_human	1.39x39	129	TM1	V => L	N/A	N/A
vipr1_human	3.33x33	219	TM3	M => V	N/A	N/A
sctr_rat	2.50x50	178	TM2	H => R	N/A	N/A

Fig 8: Result for Mutations

RESULTS:

GPCR database were explored. Structural and functional characteristics of query 'PTH1 receptor' were observed. Sequence alignments, endogenous ligands, snake diagram, necklace diagram, structure models and mutations for query calcitonin were observed and studied.

CONCLUSION:

Structural and functional characteristics of query 'PTH1 receptor' was studied by exploring GPCR database.

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DATE: 16/03/2024

WEBLEM 4
INTRODUCTION TO EXPRESSED SEQUENCE TAGS DATABASE

Expressed Sequence Tags are small pieces of DNA sequence (usually 200 to 500 nucleotides long) that are generated by sequencing either one or both ends of an expressed gene. The idea is to sequence bits of DNA that represent genes expressed in certain cells, tissues, or organs from different organisms and use these "tags" to fish a gene out of a portion of chromosomal DNA by matching base pairs. The challenge associated with identifying genes from genomic sequences varies among organisms and is dependent upon genome size as well as the presence or absence of introns--the intervening DNA sequences interrupting the protein coding sequence of a gene.

ESTs and NCBI:

Expressed Sequence Tags, or ESTs, changed genomics because they were quick, cheap, and efficient. Scientists have produced hundreds of thousands of ESTs for public usage very quickly, including small-scale genome sequencing institutes and individual researchers. Nevertheless, the enormous number of ESTs being produced made it difficult to efficiently organize and retrieve this important data.

The National Institutes of Health (NIH) division, the National Center for Biotechnology Information (NCBI), realized the necessity of an EST-specific database in order to address this issue. In 1992, researchers at the NCBI created dbEST in response. This database was created especially to act as an EST central repository, making it simple for researchers to access and make use of this abundance of genetic data.

Before being deposited into dbEST, ESTs were subjected to screening and annotation procedures following submission to GenBank, the NIH's main sequence database. As a result, the database was guaranteed to have excellent, fully annotated EST sequences that were prepared for investigation and examination. The NCBI gave researchers an effective tool for genomic and gene discovery by centralizing EST data in dbEST.

Moreover, dbEST was more than just an EST repository; it was included into the larger ecosystem of genome data that the NCBI maintained. This integration made it easier to conduct thorough searches across a variety of genomic information types, which improved researchers' capacity to examine and interpret genetic data from a variety of perspectives.

dbEST: a descriptive catalog of ESTs

To arrange, preserve, and make accessible the vast amount of publicly available EST data that has previously been collected and is still growing every day, scientists at NCBI developed dbEST. A scientist can obtain information on ESTs from more than 300 different organisms, in addition to human ESTs, by using dbEST. Scientists at NCBI annotate the EST record with any information that they know, wherever possible. For instance, the name and function of a known gene are recorded on the EST record if an EST matches a DNA sequence that codes for that gene. Public scientists can utilize dbEST as a gene discovery tool by annotating EST

records. Using a database search engine like NCBI's BLAST, anybody with an interest can do sequence similarity searches against dbEST.

UniGene: an indistinguishable collection of gene-focused clusters

ESTs that are ultimately produced from mRNA may be redundant since a gene can express itself as mRNA several times. That is to say, multiple copies of the same EST that are identical or comparable may exist. Because of this redundancy and overlap, a user searching dbEST for a specific EST may get a lengthy list of tags, many of which could be for the same gene. Going through each of these similar ESTs can take a lot of time. UniGene was created by NCBI researchers as a solution to the redundancy and overlap issue.

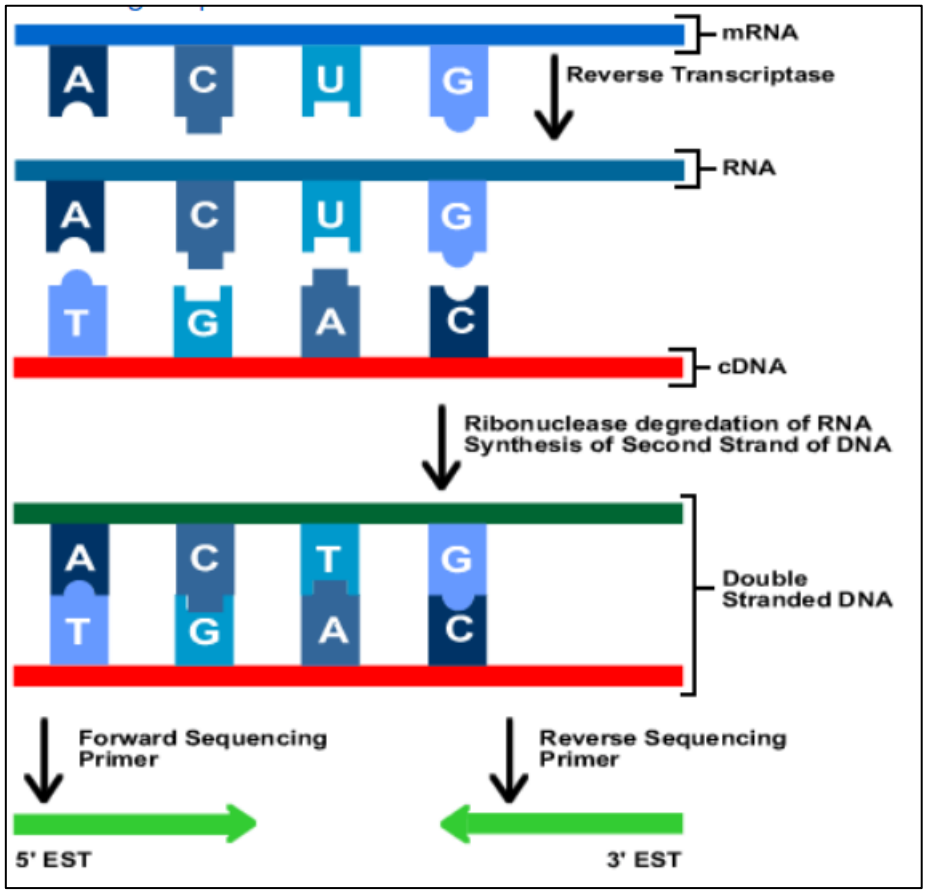

Fig 1: An overview of how Expressed Sequence Tags is generated

GenBank's "Expressed Sequence Tags" (or "single-pass" cDNA sequences) from various organisms are the subject of sequence data and other material in the EST Database (Nature Genetics 4:332-3;1993) subdivision. A synopsis of the development of human ESTs in GenBank can be found in Trends Biochemistry. Expressed Sequence Tags (ESTs) are single-pass sequence readings from mRNA (cDNA) that are brief (often less than 1000 bp). They are usually made in big quantities. They provide an image of the genes expressed in a particular tissue and/or at a particular stage of development. These are expression tags for the relevant cDNA library, some of which are coding and some of which are not.

The majority of EST projects produce a lot of sequences. These are typically uploaded in batches of dozens to thousands of entries, with shared submitter, source, and citation data, to GenBank and dbEST.

1. **Gene Discovery:** EST databases have proven invaluable in the search for new genes, particularly in organisms for which a complete genome sequence may not yet be accessible or adequately annotated. Researchers are able to discover new genes, examine their structures, and deduce their activities by sequencing segments of expressed genes.
2. **Gene Expression Analysis:** When examining patterns of gene expression in various tissues, developmental stages, or experimental settings, EST databases are invaluable resources. Through comparing the quantity of ESTs obtained from various libraries, scientists can learn which genes are expressed in particular biological circumstances.
3. **Comparative Genomics:** By offering sequences from several species, EST databases can also help in comparative genomics research. It is possible to determine conserved genes and regulatory elements as well as comprehend evolutionary changes in gene expression patterns by comparing ESTs from related animals.
4. **Functional Genomics:** By offering sequence data for relevant genes, EST databases can support functional genomics research. Utilizing ESTs, scientists can validate gene predictions from genome sequencing projects, create probes for gene expression microarrays, and investigate how genetic diversity affects gene expression.
5. **Database Resources:** There are numerous EST databases that are kept up to date by different organizations and academic institutions. ESTScan, TIGR Gene Indices, and dbEST (a component of the NCBI's Entrez system) are a few examples. Researchers from anywhere around the world can access EST data through these databases, which usually offer tools for browsing, searching, and analysis.

REFERENCES:

1. ESTs. (n.d.). http://www.cyto.purdue.edu/cdroms/cyto6/content/primer/est.htm
2. What is dbEST? (n.d.). https://www.ncbi.nlm.nih.gov/genbank/dbest/

DATE: 16-03-2024

WEBLEM 4(A)
EST DATABASE
(URL: https://www.ncbi.nlm.nih.gov/genbank/dbest/)

AIM:

To identify and characterize gene expression patterns for query 'DDIT3' (Accession ID: DN990078.1) using an EST database.

INTRODUCTION:

GenBank's "Expressed Sequence Tags" (or "single-pass" cDNA sequences) from various organisms are the subject of sequence data and other material in the EST Database (Nature Genetics 4:332-3;1993) subdivision. A synopsis of the development of human ESTs in GenBank can be found in Trends Biochemistry. Expressed Sequence Tags (ESTs) are single-pass sequence readings from mRNA (cDNA) that are brief (often less than 1000 bp). They are usually made in big quantities. They provide an image of the genes expressed in a particular tissue and/or at a particular stage of development. These are expression tags for the relevant cDNA library, some of which are coding and some of which are not.

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4. Functional Genomics: By offering sequence data for relevant genes, EST databases can support functional genomics research. Utilizing ESTs, scientists can validate gene predictions from genome sequencing projects, create probes for gene expression microarrays, and investigate how genetic diversity affects gene expression.
5. Database Resources: There are numerous EST databases that are kept up to date by different organizations and academic institutions. ESTScan, TIGR Gene Indices, and dbEST (a component of the NCBI's Entrez system) are a few examples. Researchers from anywhere around the world can access EST data through these databases, which usually offer tools for browsing, searching, and analysis.

DDIT3 gene:

The DDIT3 gene, also known as CHOP or GADD153, encodes a member of the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors. Located on chromosome 12, it regulates diverse biological processes such as transcriptional regulation, blood vessel maturation, and cellular responses to various stressors like DNA damage and endoplasmic reticulum stress. Its involvement extends to cell cycle regulation, Wnt signaling, and modulation of interleukin-8 production, among others.

Functionally, DDIT3 participates in transcriptional regulation by binding to specific DNA sequences near RNA polymerase II promoters and modulating gene expression. It acts as a transcriptional activator or repressor depending on the context, exerting its effects through protein-protein interactions and DNA binding. Moreover, DDIT3 plays roles in cellular processes such as protein binding, homodimerization, and interaction with other transcription factors like cAMP response element-binding protein (CREB). Its leucine zipper domain facilitates dimerization, essential for its transcriptional activity. Overall, DDIT3's multifaceted functions highlight its significance in cellular homeostasis and stress response pathways.

METHODOLOGY:

1. Launch a browser and go to the NCBI website.
2. By selecting the "All Databases" dropdown menu at the top of the NCBI webpage, you can gain access to the EST database. To search nucleotide sequences, including ESTs, choose "Nucleotide" from the dropdown menu.
3. Search query 'DDIT3'.
4. On the left-hand side of the screen, use filter EST to screen for ESTs for your query.
5. Interpret the results displayed for query 'DDIT3'.

OBSERVATIONS:

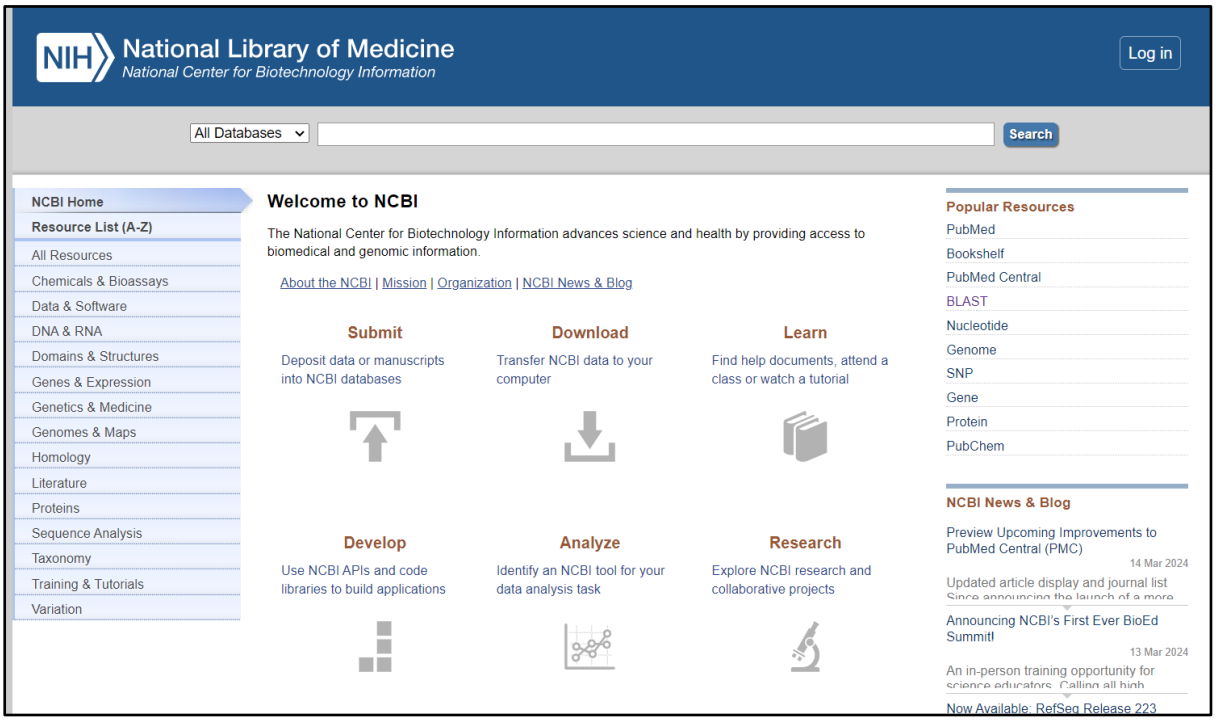

Fig 1: NCBI Homepage

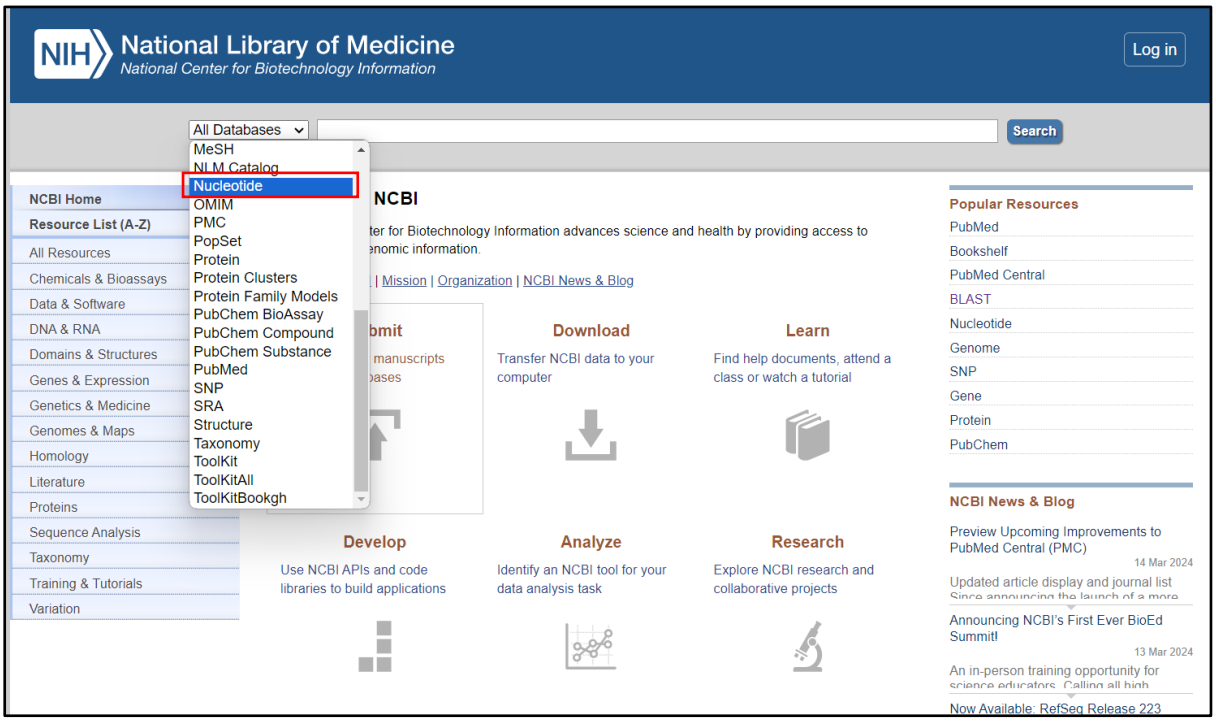

Fig 2: Select 'Nucleotide' option from all Databases

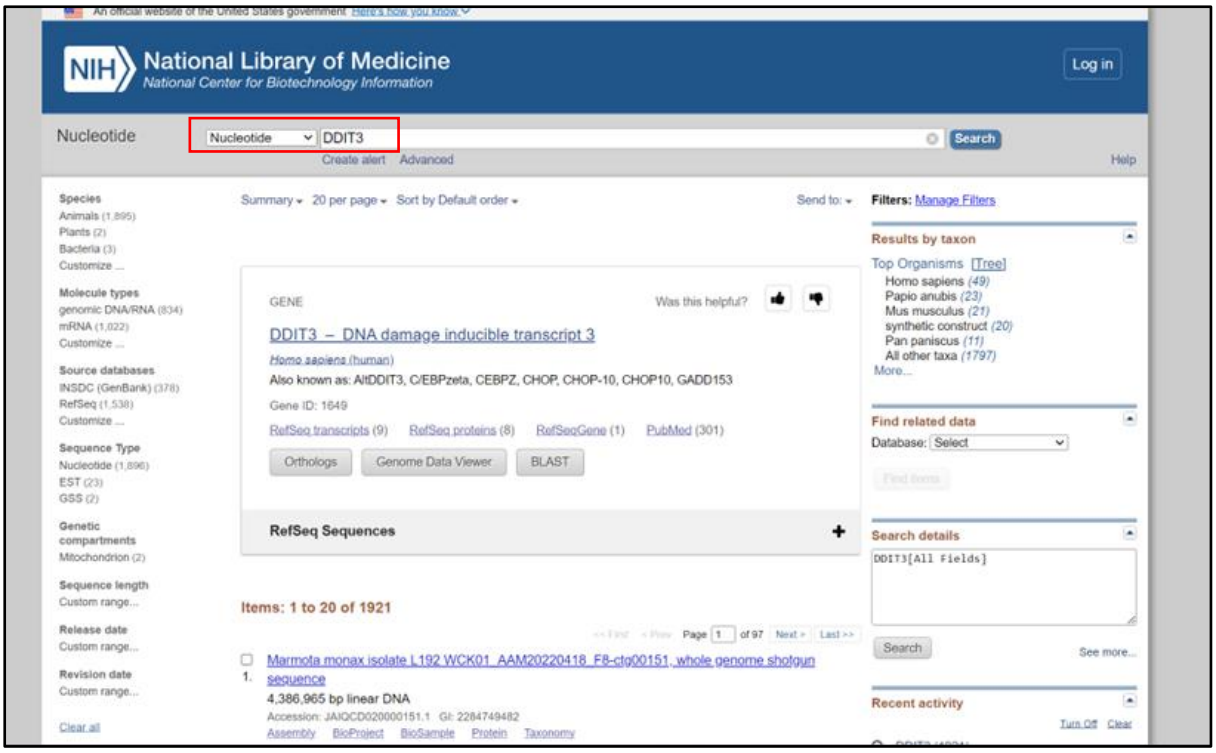

Fig 3: Results obtained after searching for the query 'DDIT3'

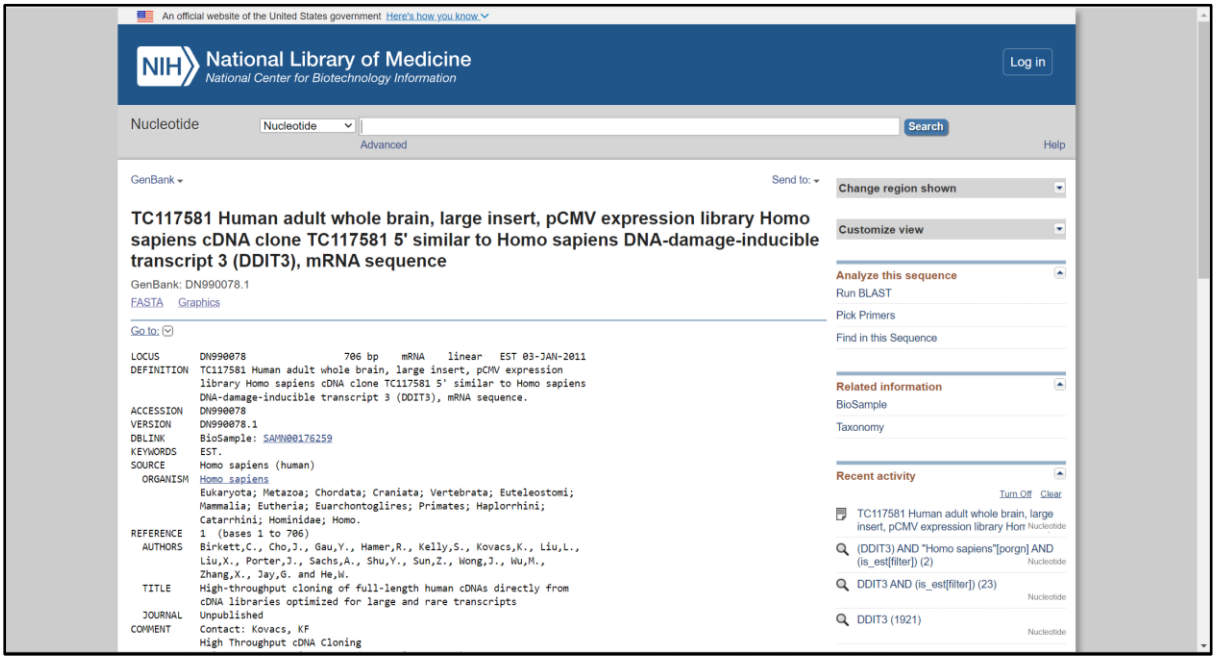

Fig 4: EST entry selected for further study: 'DDIT3' (Accession ID: DN990078.1)

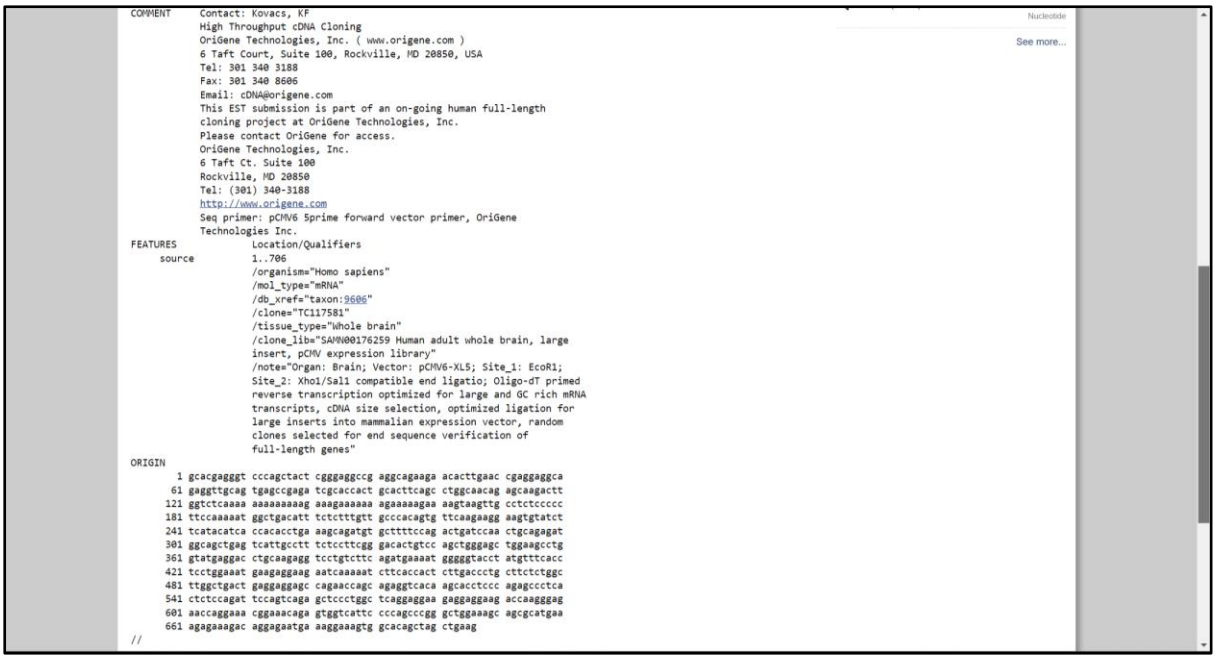

Fig 4.1: Comment and Features for 'DDIT3' (Accession ID: DN990078.1)

RESULTS:

Following results were obtained for the query 'DDIT3' (Accession ID: DN990078.1) gene which is a 706bp mRNA linear sequence having only 1 EST tag.

CONCLUSION:

The EST (Expressed Sequence Tag) database makes it easier to identify and describe the genes that are expressed in a variety of organisms. EST databases provide information on gene expression patterns, alternative splicing variations, and tissue-specific expression profiles by supplying brief sequences obtained from mRNA transcripts.

REFRENCES:

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DATE:15/03/2024

WEBLEM 5
INTRODUCTION TO BLAST2GO Tool
(URL: https://www.blast2go.com/)

BioBam's offerings lies BLAST2GO, a comprehensive bioinformatics platform tailored for functional annotation of genomic data. Leveraging cutting-edge algorithms and user-friendly interfaces, BLAST2GO enables researchers to unravel the functional significance of their sequences with ease. From sequence similarity searches using BLAST to mapping gene ontology terms and conducting annotation enrichment analysis, BLAST2GO offers a holistic approach to functional annotation, empowering researchers to glean insights into the biological roles of their sequences.

BLAST2GO tool research has revolutionized our understanding of biological systems, providing vast amounts of raw sequence data waiting to be deciphered. However, extracting meaningful biological insights from these sequences requires sophisticated tools for functional annotation. Enter BLAST2GO, a comprehensive bioinformatics solution designed to streamline the process of functional annotation for genomics data. BLAST2GO combines powerful algorithms with user-friendly interfaces, making it accessible to both seasoned bioinformaticians and bench scientists. At its core lies the integration of three essential steps: sequence similarity search (using BLAST), mapping of gene ontology (GO) terms, and annotation enrichment analysis. The first step involves comparing input sequences against vast databases of known sequences using the widely adopted BLAST algorithm. This process identifies homologous sequences, providing crucial insights into the functional conservation and evolutionary relationships of the query sequences. Once homologous sequences are identified, BLAST2GO maps them to Gene Ontology terms, a structured vocabulary that describes gene products in terms of their associated biological processes, molecular functions, and cellular components. This step assigns functional annotations to the query sequences based on the annotations of their homologs, greatly enhancing our understanding of their potential roles in biological systems. Finally, BLAST2GO offers annotation enrichment analysis, allowing researchers to identify overrepresented GO terms within their dataset compared to a reference set. This analysis unveils biological processes, molecular functions, and cellular components that are significantly enriched within the input data, shedding light on the underlying biological mechanisms at play. Beyond these fundamental features, BLAST2GO boasts a range of additional functionalities, including sequence filtering, statistical analysis tools, and customizable workflows, empowering users to tailor the annotation process to their specific research needs.

Blast2GO is a bioinformatics platform for high-quality functional annotation and analysis of genomic datasets. Main Application Features are:

1. **Easy start-up and low maintenance: Simply download the BLAST2GO from the portal and install the application and the updates are automatics.**
2. **User-friendly:** Blast2GO is designed for experimentalists. An intuitive interface, the many graphical parameters and the detailed user's manual makes the use of the tool possible from the first try.
3. **High-throughput and interactive:** Blast2GO can annotate thousands of sequences, in multiple projects. Users can follow and modify the annotation process at any stage.
4. **Highly configurable:** Blast2GO is a functional annotation workstation. You can design your custom annotation style through the many configurable parameters. Statistical charts are available to guide users in the annotation process.
5. **Datamining:** Blast2GO does not only generate functional annotations. You can interrogate the biological meaning of your data with different graphical and statistical functions.
6. **Vocabularies:** Gene Ontology Terms, InterPro Domains, RFAM IDs and Enzyme Codes are supported by Blast2GO.

INSTALLATION OF THE TOOL:

Installing BLAST2GO involves several steps to ensure proper setup and functionality. Here's a detailed guide on how to install the BLAST2GO application:

Step 1: Obtain the BLAST2GO Software: Visit the official BioBam website or the BLAST2GO website to obtain the software. BLAST2GO is available for download on Windows, macOS, and Linux platforms.

Step 2: Download the Installation Package: Once on the website, navigate to the download section and select the appropriate version of BLAST2GO for your operating system (Windows, macOS, or Linux). Ensure that you download the latest stable version of the software.

Step 3: Check System Requirements: Before proceeding with the installation, ensure that your system meets the minimum requirements specified by BLAST2GO. This includes requirements for operating system version, processor, RAM, and disk space.

Step 4: Installation Process: Locate the downloaded installation package (usually a .exe file) and double-click on it to initiate the installation process. Follow the on-screen instructions provided by the installation wizard. You may be prompted to specify the installation directory and agree to the terms of the license agreement. Once the installation is complete, BLAST2GO should be accessible from the Start menu or desktop shortcut.

Fig 1: Homepage of BIOBAM

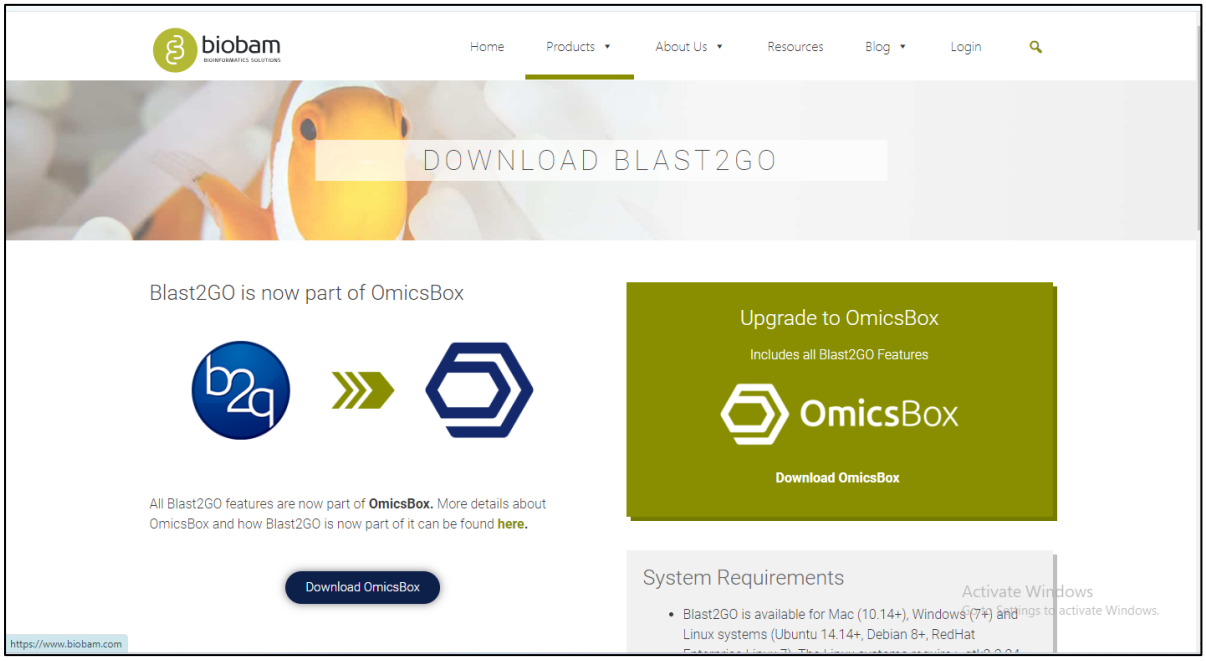

Fig 2: Download the latest version BLAST2GO tool for windows

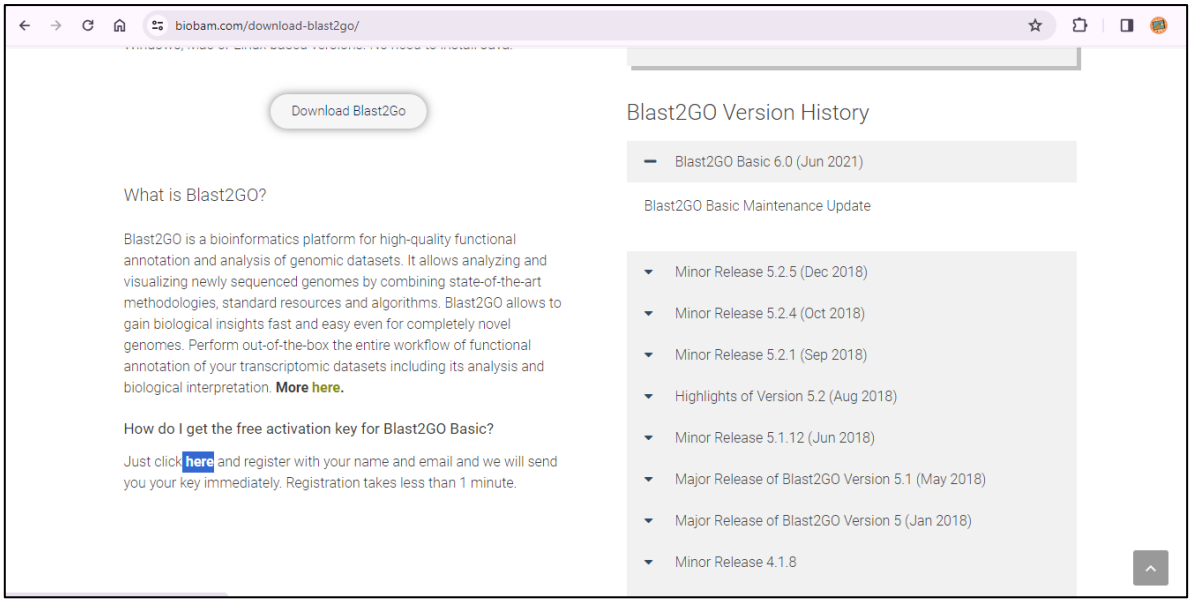

Fig 3: Click on the highlighted part for subscription key

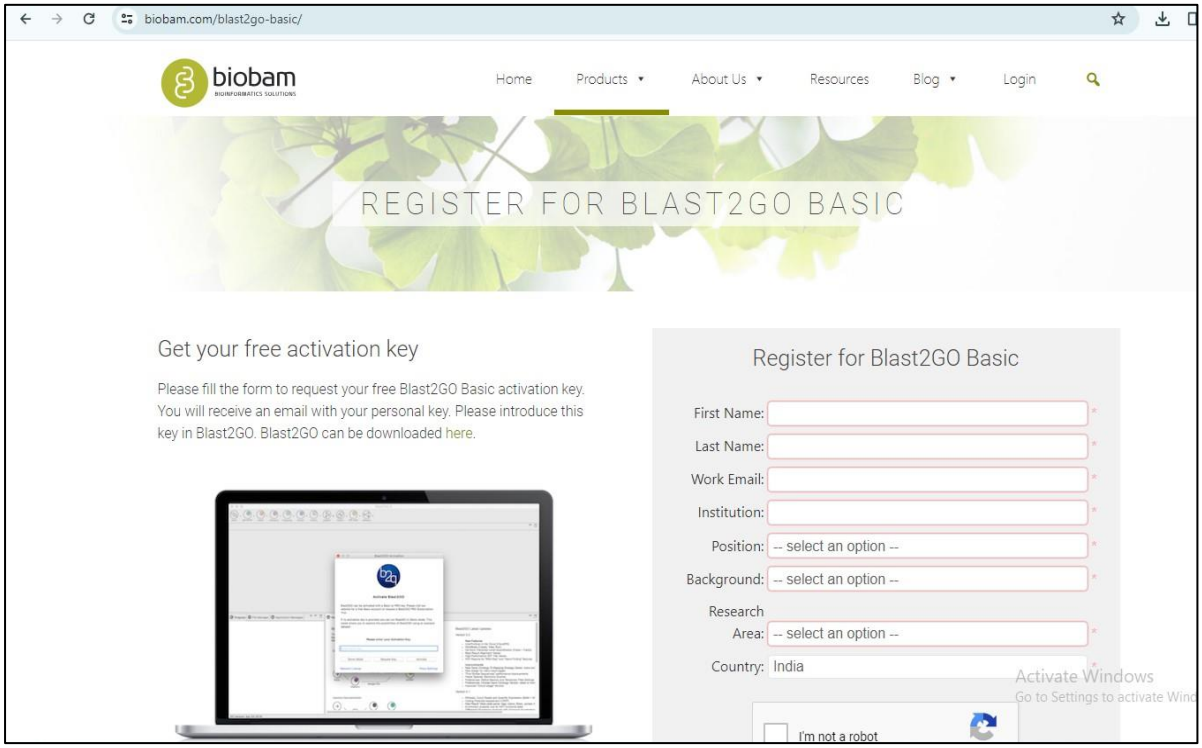

Fig 4: Registration page for subscription key

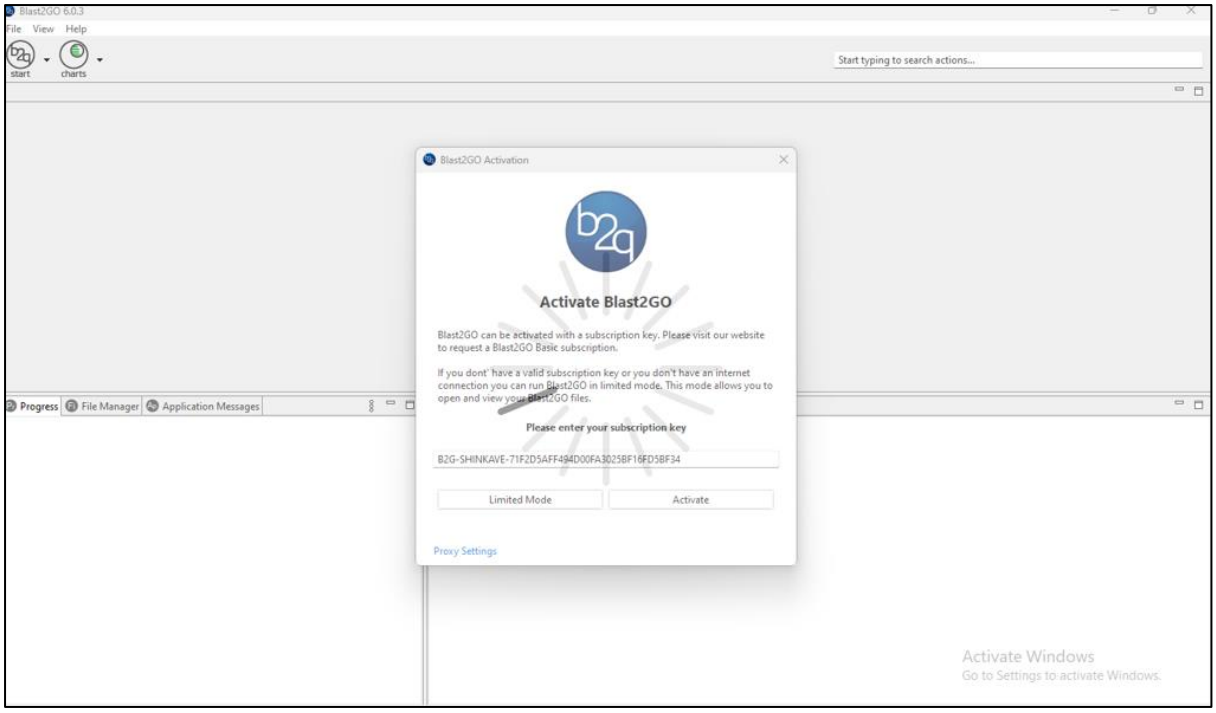

Fig 5: Enter the subscription received through the mail-ID

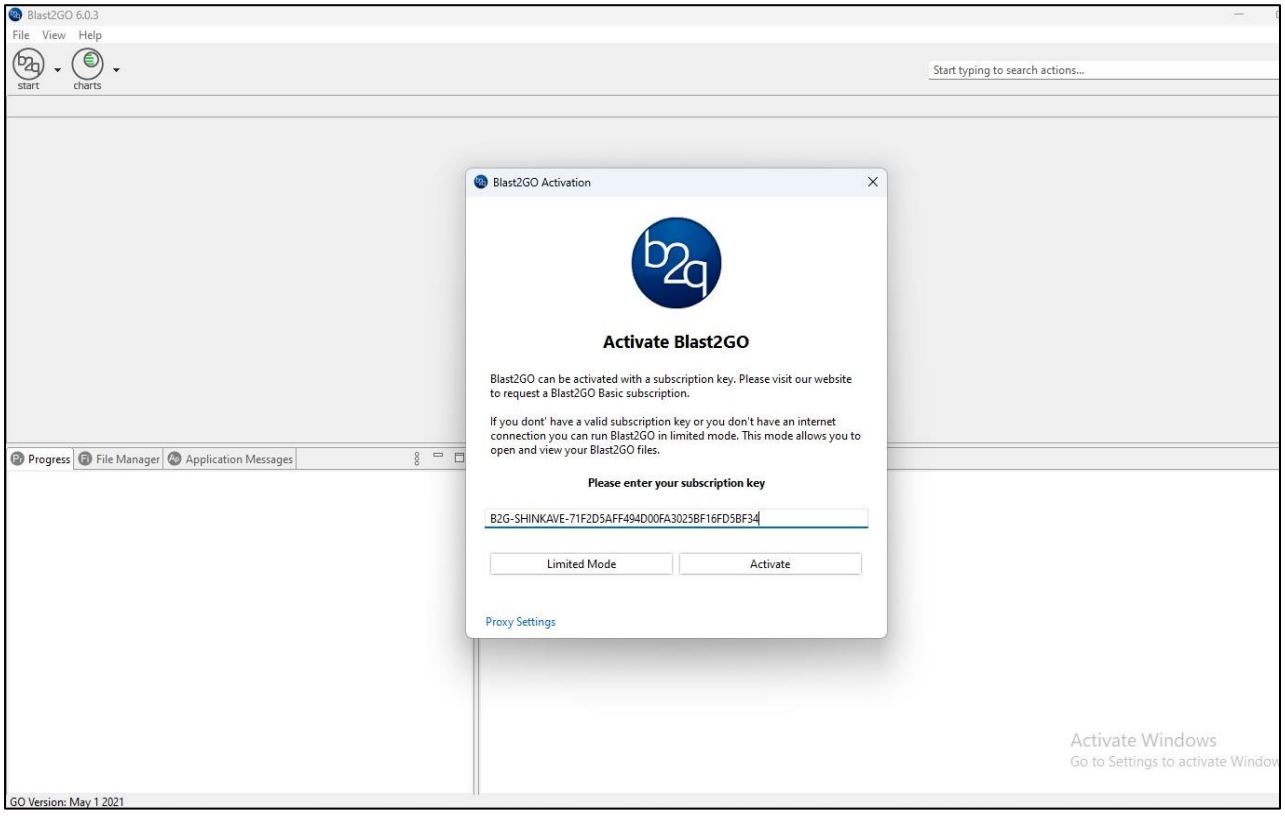

Fig 6: Activation after entering the subscription key

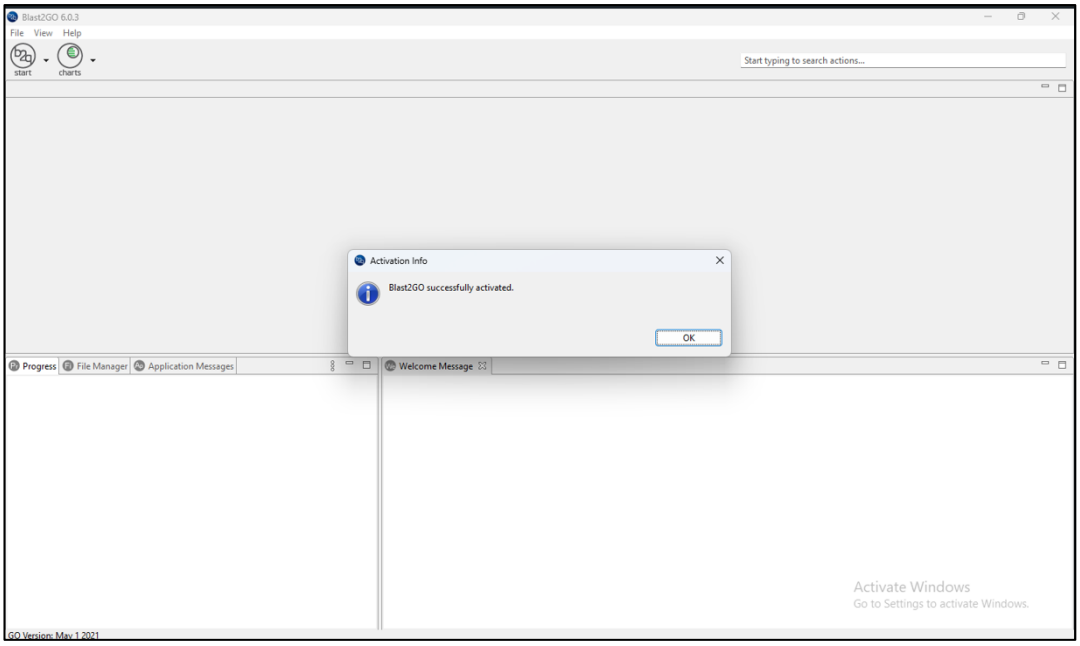

Fig 7: Your BLAST2GO is successfully activated

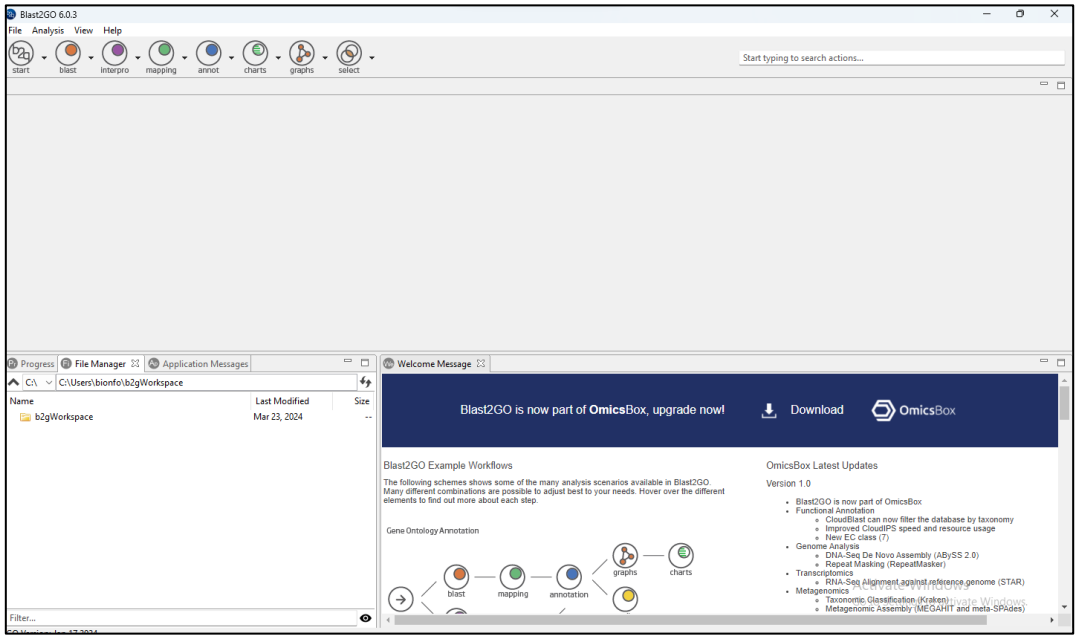

Fig 8: BLAST2GO

REFERENCES:

1. BioBam. (2024, February 28). BioBam - Bioinformatics Made Easy - Bioinformatics Made Easy. https://www.biobam.com.
2. Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M., & Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics, 21(18), 3674–3676. https://doi.org/10.1093/bioinformatics/bti610

DATE: 15/03/2024

WEBLEM 5(A)
Blast2GO
(URL: https://www.blast2go.com/)

AIM:

To annotate and analyze genomic or transcriptomic sequences for query 'LEPR' to understand their functional significance by using Blast2GO tool.

INTRODUCTION:

Blast2GO is a comprehensive bioinformatics tool for the functional annotation and analysis of genome-scale sequence datasets. The software was originally developed to provide a user-friendly interface for Gene Ontology annotation. Over the last years, many improvements have considerably increased the functionality of Blast2GO and many different functional genomics tools are now available. Additionally, the application offers a wide array of graphical and analytical tools for data manipulation and mining. The main concept behind the developments is the easiness for biological researchers: minimal set up requirements, automatic updates, simplicity in the usage and visual-oriented information display. Advanced functionality is instantly available requiring a minimal computational background. Basically, Blast2GO uses local or remote BLAST searches to find similar sequences to one or several input sequences. The program extracts the GO terms associated to each of the obtained hits and returns an evaluated GO annotation for the query sequence(s). Enzyme codes are obtained by mapping from equivalent GOs while InterPro motifs are directly queried at the InterProScan web service. GO annotation can be visualized reconstructing the structure of the Gene Ontology relationships.

A typical basic use case of Blast2GO consists of 5 steps: BLASTing, mapping, annotation, statistical analysis and visualization. These steps will be described in this document including installation instructions, further explanations and information on additional functions.

The program extracts GO terms to each obtained hit by mapping to existent annotation associations. An annotation rule finally assigns GO terms to the query sequence. Annotation and functional analysis can be visualized in a graph form reconstructing the GO relationships and color-highlighting the most relevant areas. B2G was conceived to be an attractive tool for research environments where genetic and/or computational resources are limited and where much work is still done in an explorative fashion. B2G is a user-friendly, easy to distribute and low maintenance tool. It allows monitoring and interaction at different steps of the analysis, and emphasizes visualization as an important component of knowledge acquisition. B2G is a Java application made available by Java Web Start. It is platform independent and has no further requirements than an Internet connection.

LEPR:

The LEPR gene, also known as the leptin receptor gene, is located on chromosome 1 and provides instructions for making a protein called the leptin receptor. This protein is found on the surface of cells in many organs and tissues of the body, including the hypothalamus, a part of the brain that controls hunger and energy balance. The leptin receptor plays a crucial role in the regulation of body weight by binding to the hormone leptin, which is produced by adipose tissue and signals available energy reserves to the brain.

In mammals, six isoforms of the leptin receptor (LepRa-f) have been identified, all of which have a common extracellular ligand binding domain. The long form of the leptin receptor (LepRb) has an approximately 300 amino acid cytoplasmic tail that mediates intracellular signaling, while the LepRa, -c, -d, and -f have short (~30-40) amino acid cytoplasmic extensions. The LepRe lacks transmembrane and cytoplasmic domains and may function as a secreted leptin binding protein. The LepRb is the only isoform that contains intracellular tyrosine residues necessary for signaling; the physiological functions, if any, for other LepR isoforms are unknown.

METHODOLOGY:

1. Open homepage of UNIPROT.
2. Extract 5 FASTA sequences of the same protein.
3. Save it in a notepad.
4. Open Blast2Go and load the file in sequences in FASTA file format.
5. After loading click on Blast and use NCBI Blast.
6. Set Blast program as BlastP and Blast DB as Swissprot.
7. Let the advanced configurations be as it is.
8. Run the file and save the results in XML file.
9. For interpro, let the configurations for the first page be default.
10. Only select Superfamily in other sequence features and let the others remain unchecked.
11. Run the file and save the results in XML file format.
12. After results are obtained and loaded onto the homepage, run the Blast2go statistics.
13. Select all four statistic options and hit run.\
14. For getting InterPro results, select InterProScan results and hit run.
15. Obtain results in graph formats.

OBSERVATIONS:

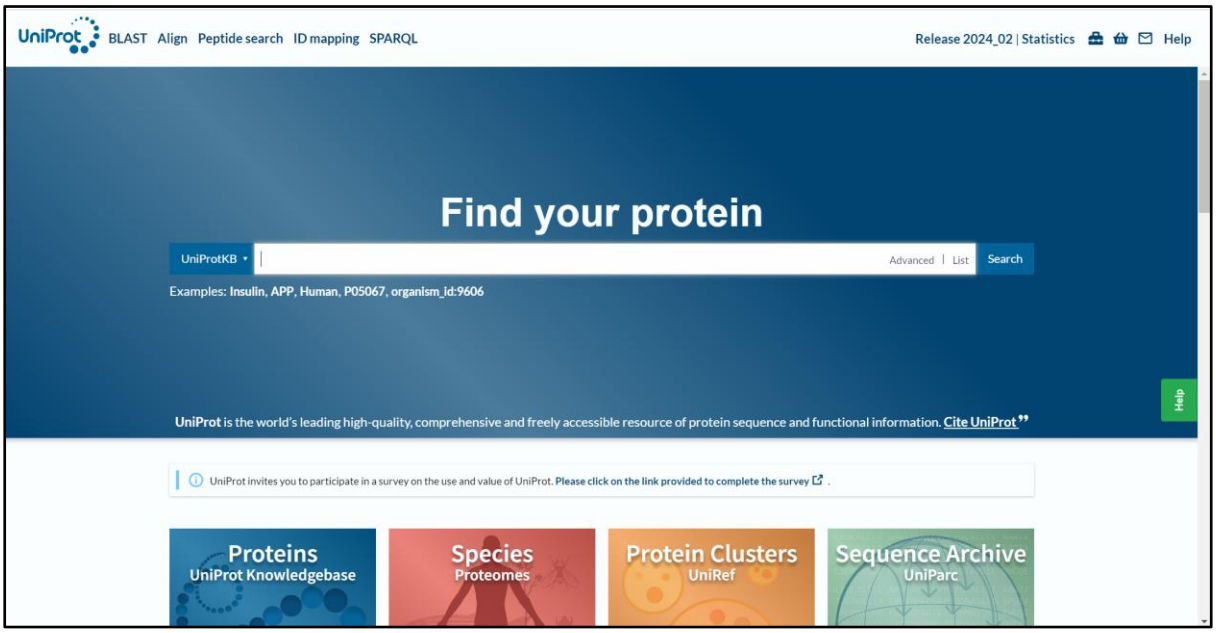

Fig 1: Homepage of UniProt database

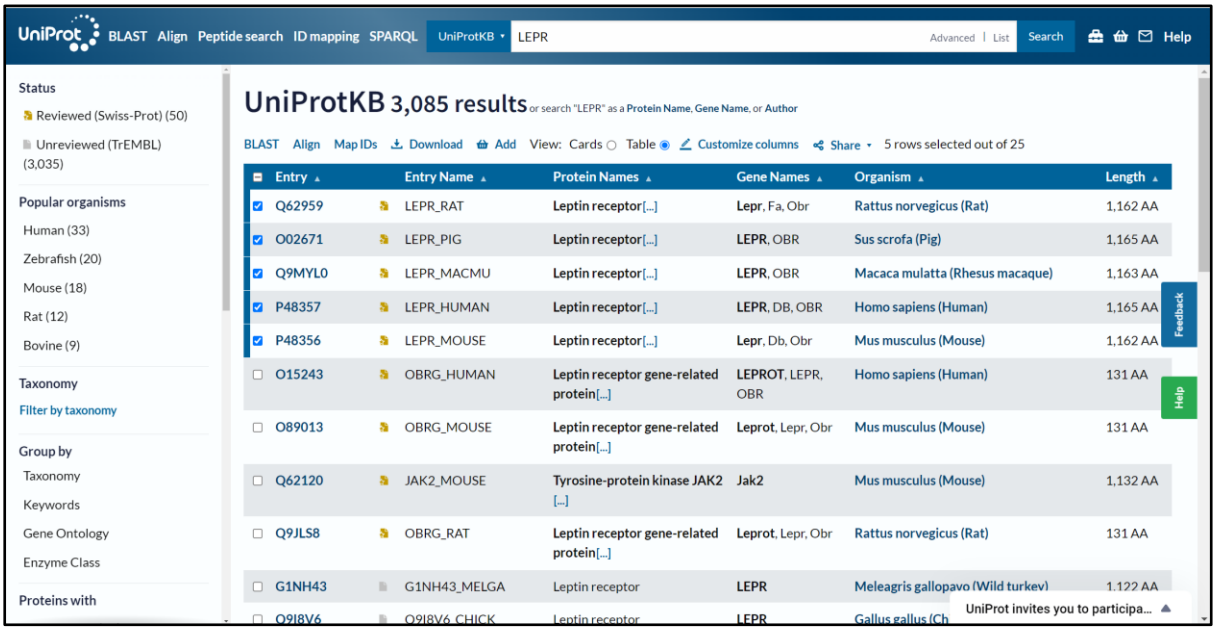

Fig 2: 5 selected sequences of query 'LEPR'

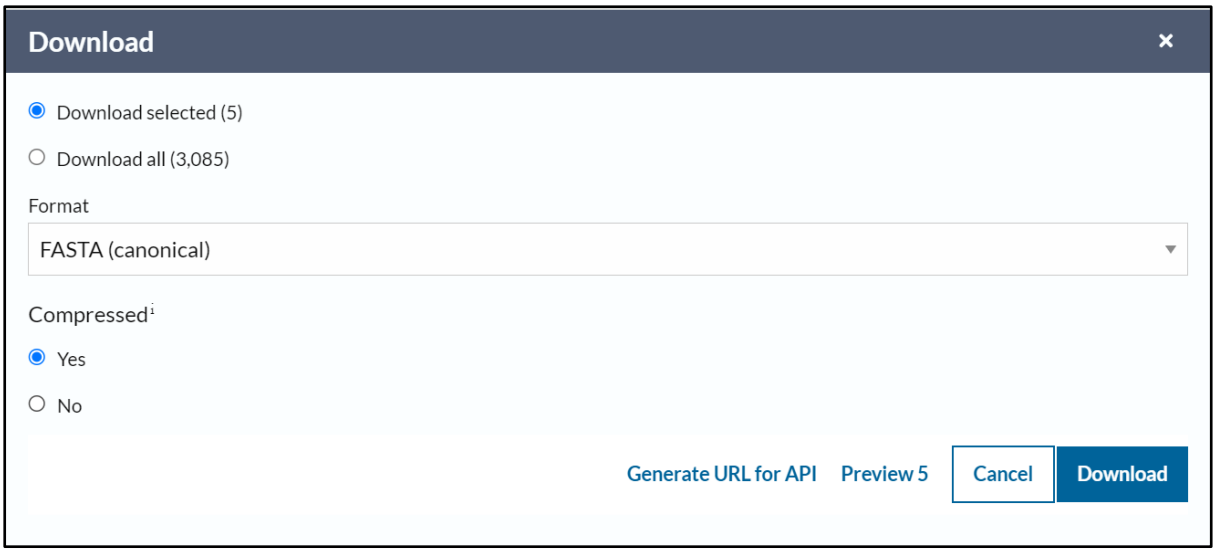

Fig 3: Downloading FASTA sequences of query 'LEPR'

```
>sp|P48357|LEPR_HUMAN Leptin receptor OS=Homo sapiens OX=9606 GN=LEPR PE=1 SV=2
MICQKFCVVLLHWEFIYVITAFNLSYPITPWRFKLSCMPPNSTYDYFLLPAGLSKNTSNS
NGHYETAVEPKFNSSGTHFSNLSKTTFHCCFRSEQDRNCSLCADNIEGKTFVSTVNSLVF
QQIDANWNIQCWLKGDLKLFICYVESLFKNLFRNYNYKVHLLYVLPEVLEDSPLVPQKGS
FQMVHCNCSVHECCECLVPVPTAKLNDTLLMCLKITSGGVIFQSPLMSVQPINMVKPDPP
LGLHMEITDDGNLKISWSSPPLVPFPLQYQVKYSENSTTVIREADKIVSATSLLVDSILP
GSSYEVQVRGKRLDGPGIWSDWSTPRVFTTQDVIYFPPKILTSVGSNVSFHCIYKKENKI
VPSKEIVWWMNLAEKIPQSQYDVVSDHVSKVTFFNLNETKPRGKFTYDAVYCCNEHECHH
RYAELYVIDVNINISCETDGYLTKMTCRWSTSTIQSLAESTLQLRYHRSSLYCSDIPSIH
PISEPKDCYLQSDGFYECIFQPIFLLSGYTMWIRINHSLGSLDSPPTCVLPDSVVKPLPP
SSVKAEITINIGLLKISWEKPVFPENNLQFQIRYGLSGKEVQWKMYEVYDAKSKSVSLPV
PDLCAVYAVQVRCKRLDGLGYWSNWSNPAYTVVMDIKVPMRGPEFWRIINGDTMKKEKNV
TLLWKPLMKNDSLCSVQRYVINHHTSCNGTWSEDVGNHTKFTFLWTEQAHTVTVLAINSI
GASVANFNLTFSWPMSKVNIVQSLSAYPLNSSCVIVSWILSPSDYKLMYFIIEWKNLNED
GEIKWLRISSSVKKYYIHDHFIPIEKYQFSLYPIFMEGVGKPKIINSFTQDDIEKHQSDA
GLYVIVPVIISSSILLLGTLLISHQRMKKLFWEDVPNPKNCSWAQGLNFQKPETFEHLFI
KHTASVTCGPLLLEPETISEDISVDTSWKNKDEMMPTTVVSLLSTTDLEKGSVCISDQFN
SVNFSEAEGTEVTYEDESQRQPFVKYATLISNSKPSETGEEQGLINSSVTKCFSSKNSPL
KDSFSNSSWEIEAQAFFILSDQHPNIISPHLTFSEGLDELLKLEGNFPEENNDKKSIYYL
GVTSIKKRESGVLLTDKSRVSCPFPAPCLFTDIRVLQDSCSHFVENNINLGTSSKKTFAS
YMPQFQTCSTQTHKIMENKMCDLTV
>sp|Q62959|LEPR_RAT Leptin receptor OS=Rattus norvegicus OX=10116 GN=Lepr PE=1 SV=1
MTCQKFYVVLLHWEFLYVITALNLAYPTSPWRFKLFCAPPSTTDDSFLSPAGVPNNTSSL
KGASEALVEAKFNSTGIYVSELSKTIFHCCFGNEQGQNCSALTGNTEGKTLASVVKPLVF
RQLGVNWDIECWMKGDLTLFICHMEPLLKNPFKNYDSKVHLLYDLPEVIDDLPLPPLKDS
FQTVQCNCSVRECECHVPVPRAKVNYALLMYLEITSAGVSFQSPLMSLQPMLVVKPDPPL
GLRMEVTDDGNLKISWDSQTKAPFPLQYQVKYLENSTIVREAAEIVSDTSLLVDSVLPGS
SYEVQVRSKRLDGSGVWSDWSLPQLFTTQDVMYFPPKILTSVGSNASFCCIYKNENQTIS
SKQIVWWMNLAEKIPETQYNTVSDHISKVTFSNLKATRPRGKFTYDAVYCCNEQACHHRY
AELYVIDVNINISCETDGYLTKMTCRWSPSTIQSLVGSTVQLRYHRRSLYCPDNPSIRPT
SELKNCVLQTDGFYECVFQPIFLLSGYTMWIRINHSLGSLDSPPTCVLPDSVVKPLPPSN
VKAEITINTGLLKVSWEKPVFPENNLQFQIRYGLNGKEIQWKTHEVFDAKSKSASLPVSD
LCAVYVVQVRCRRLDGLGYWSNWSSPAYTLVMDVKVPMRGPEFWRIMDGDITKKERNVTL
LWKPLMKNDSLCSVRRYVVKHRTAHNGTWSQDVGNQTNLTFLWAESAHTVTVLAINSIGA
SLVNFNLTFSWPMSKVNAVQSLSAYPLSSSCVILSWTLSPNDYSLLYLVIEWKNLNDDDG
MKWLRIPSNVNKYYIHDNFIPIEKYQFSLYPVFMEGVGKPKIINGFTKDDIAKQQNDAGL
YVIVPIIISSCVLLLGTLLISHQRMKKLFWDDVPNPKNCSWAQGLNFQKPETFEHLFTKH
AESVIFGPLLLEPEPVSEEISVDTAWKNKDEMVPAAMVSLLLTTPDSTRGSICISDQCNS
ANFSGAQSTQGTCEDECQSQPSVKYATLVSNVKTVETDEEQGAIHSSVSQCIARKHSPLR
QSFSSNSWEIEAQAFFLLSDHPPNVISPQLSFSGLDELLELEGNFPEENHGEKSVYYLGV
SSGNKRENDMLLTDEAGVLCPFPAHCLFSDIRILQESCSHFVENNLNLGTSGKNFVPYMP
QFQSCSTHSHKIIENKMCDLTV
>sp|P48356|LEPR_MOUSE Leptin receptor OS=Mus musculus OX=10090 GN=Lepr PE=1 SV=1
MMCQKFYVVLLHWEFLYVIAALNLAYPISPWKFKLFCGPPNTTDDSFLSPAGAPNNASAL
KGASEAIVEAKFNSSGIYVPELSKTVFHCCFGNEQGQNCSALTDNTEGKTLASVVKASVF
RQLGVNWDIECWMKGDLTLFICHMEPLPKNPFKNYDSKVHLLYDLPEVIDDSPLPPLKDS
```

Fig 4: Opening it in Notepad

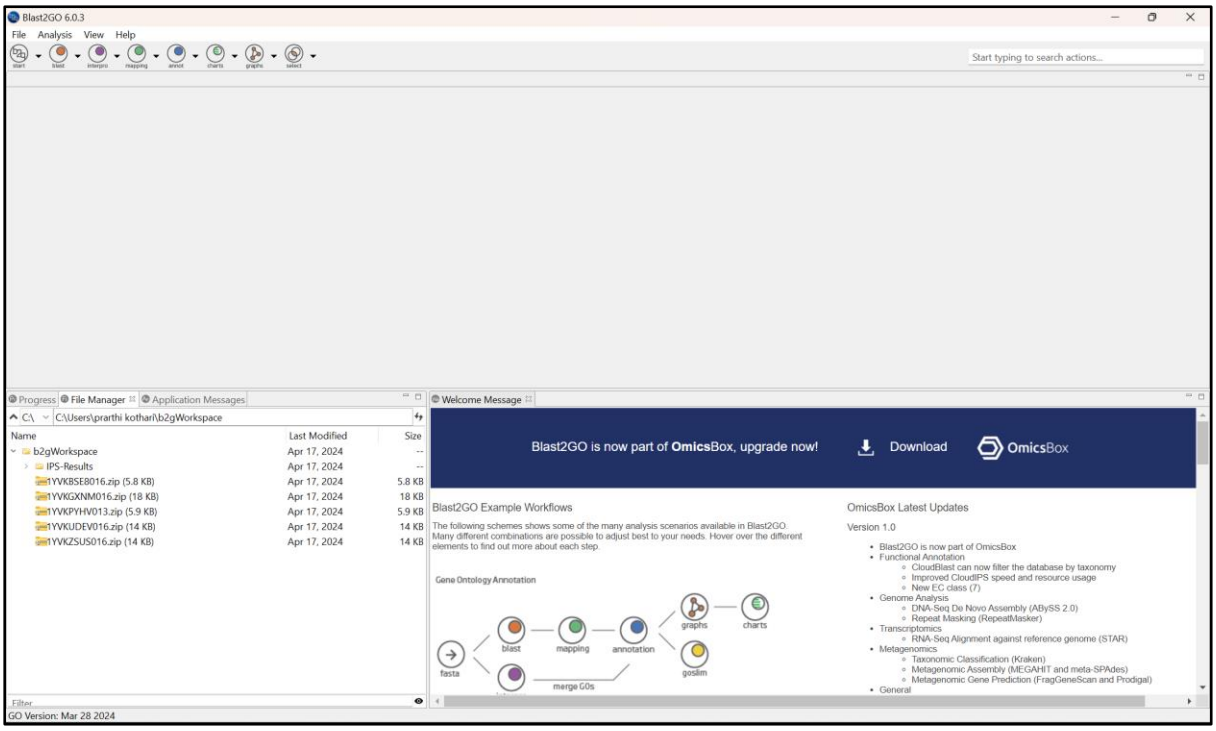

Fig 5: Opening page of Blast2GO

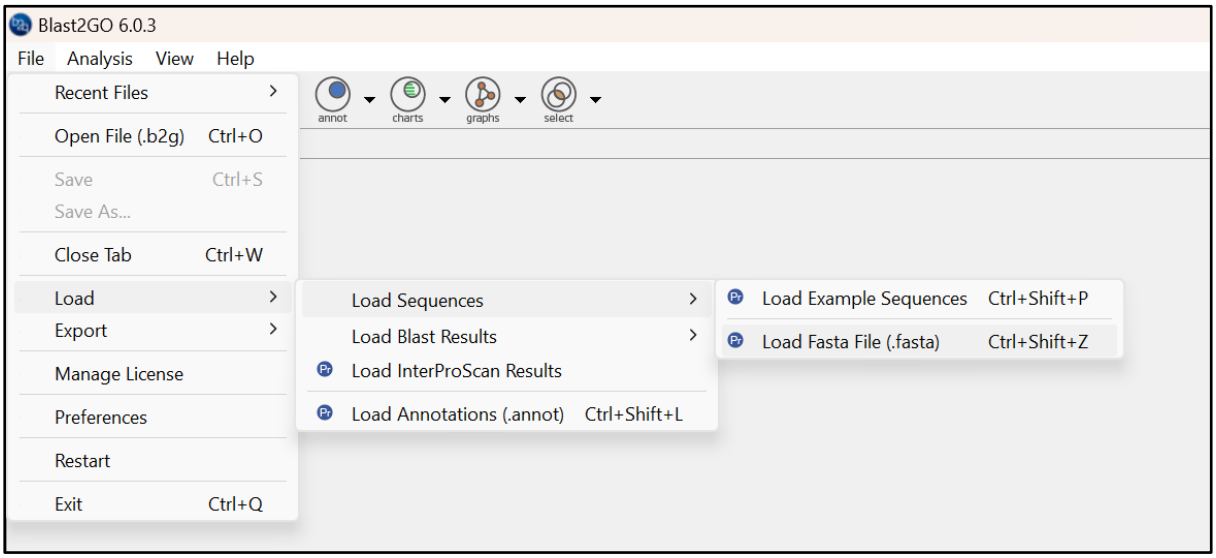

Fig 6: Pathway to load file

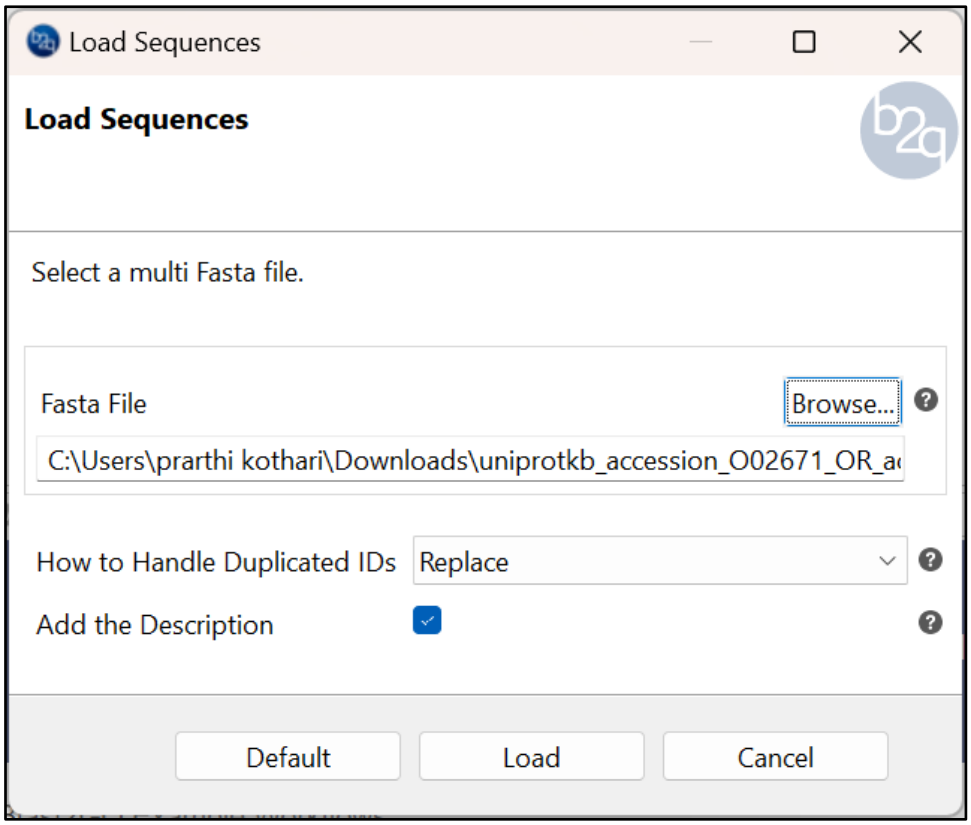

Fig 7: Loaded file

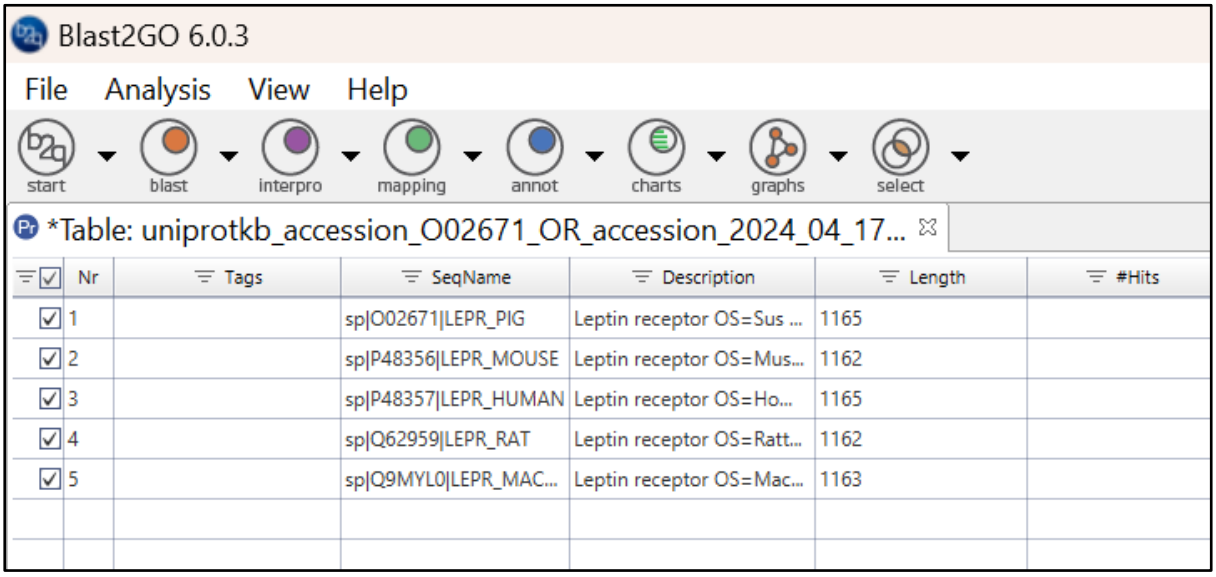

Fig 8: FASTA files displayed in Blast2GO

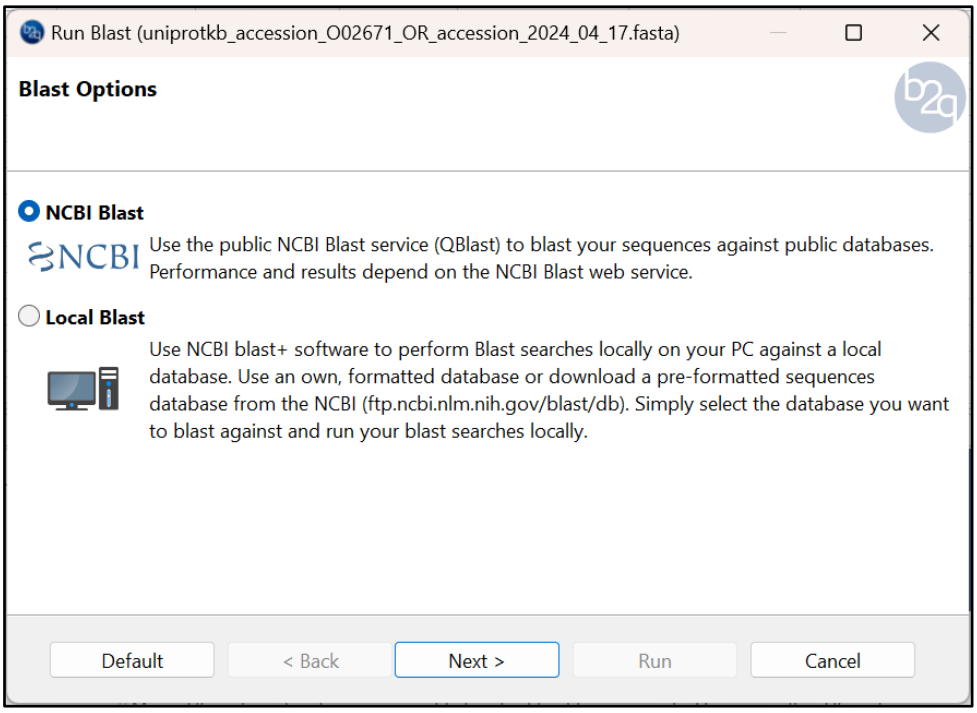

Fig 9: Running Blast

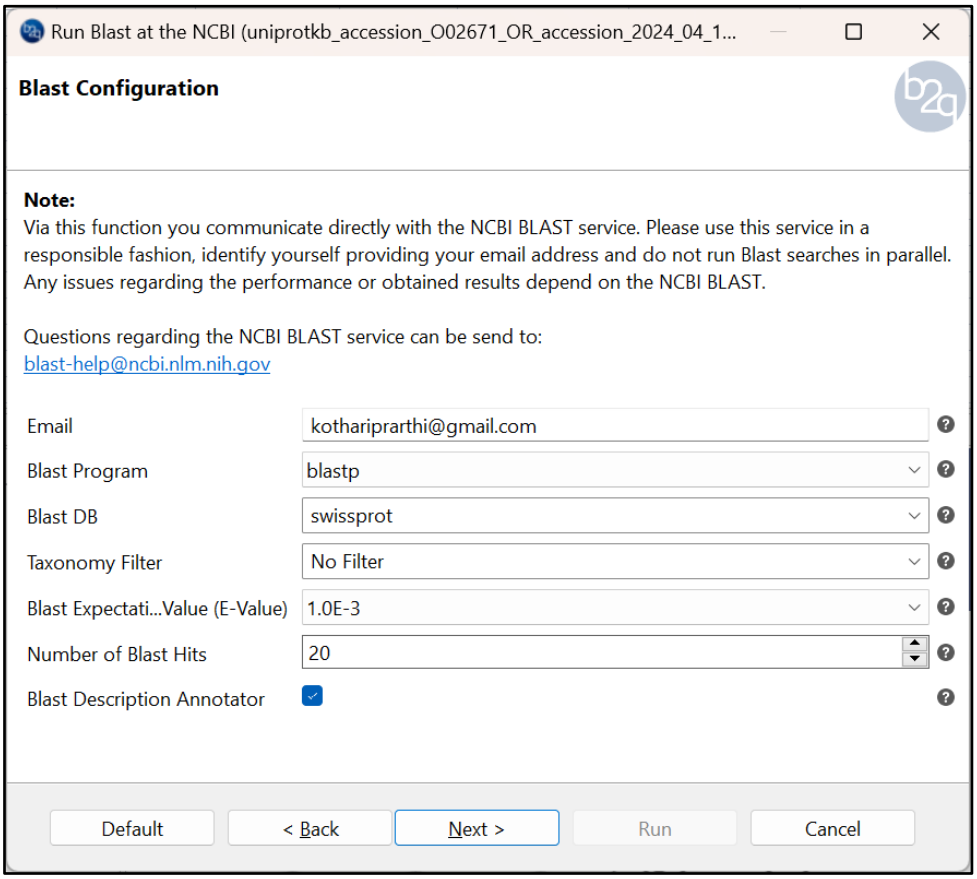

Fig 10: Blast input parameters

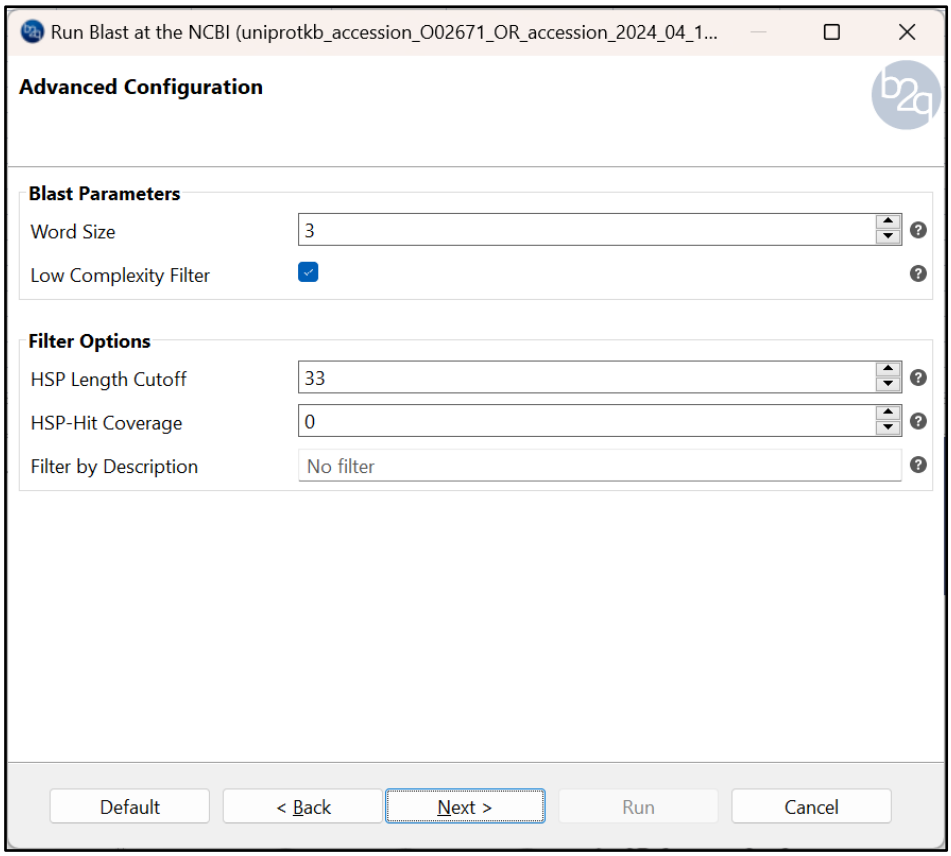

Fig 11: Advanced configurations for the tool

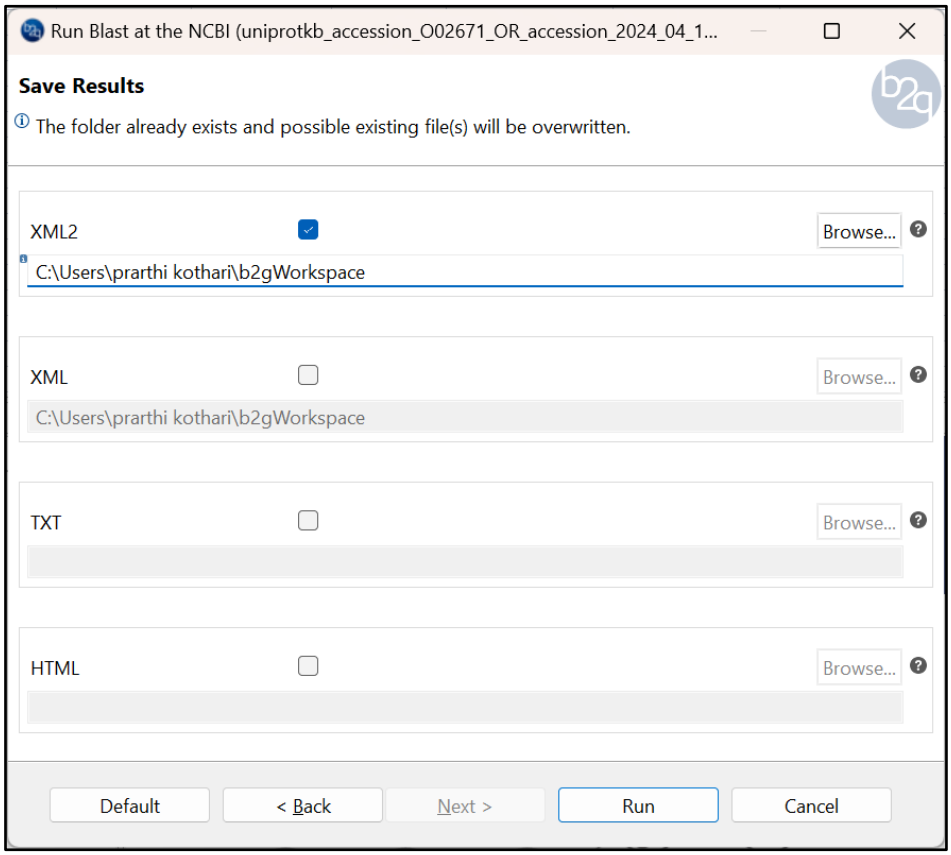

Fig 12: Running the file

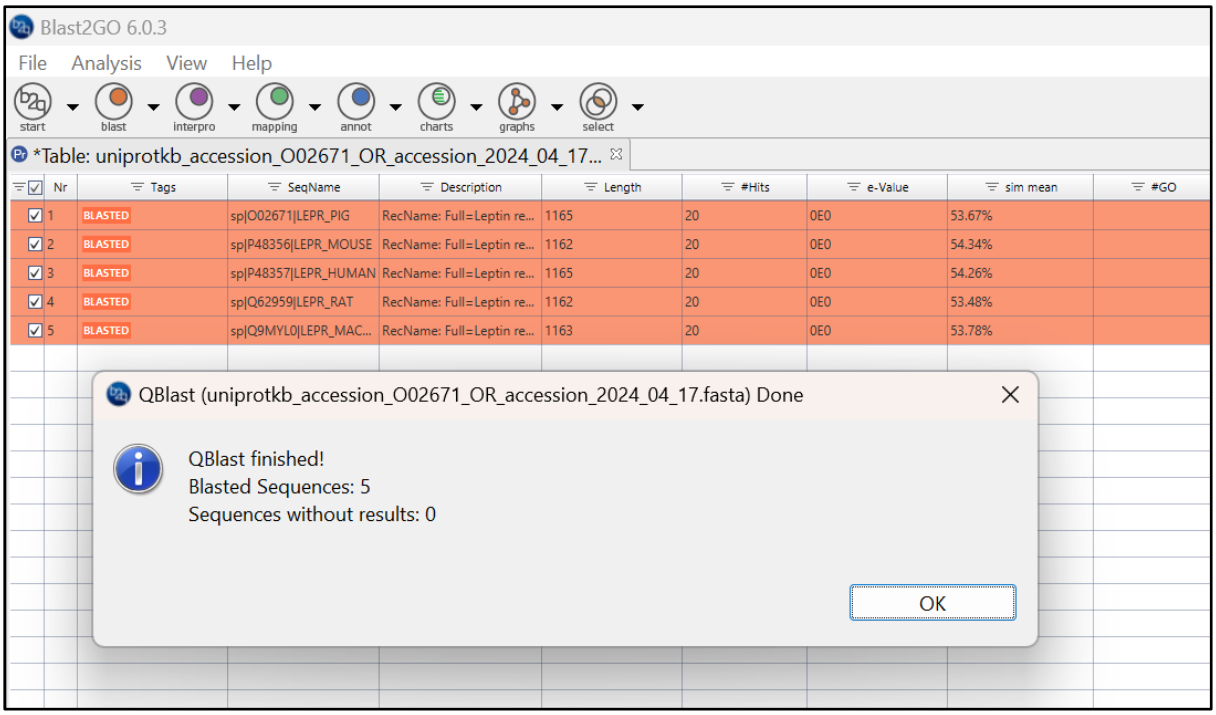

Fig 13: Results obtained for Blast

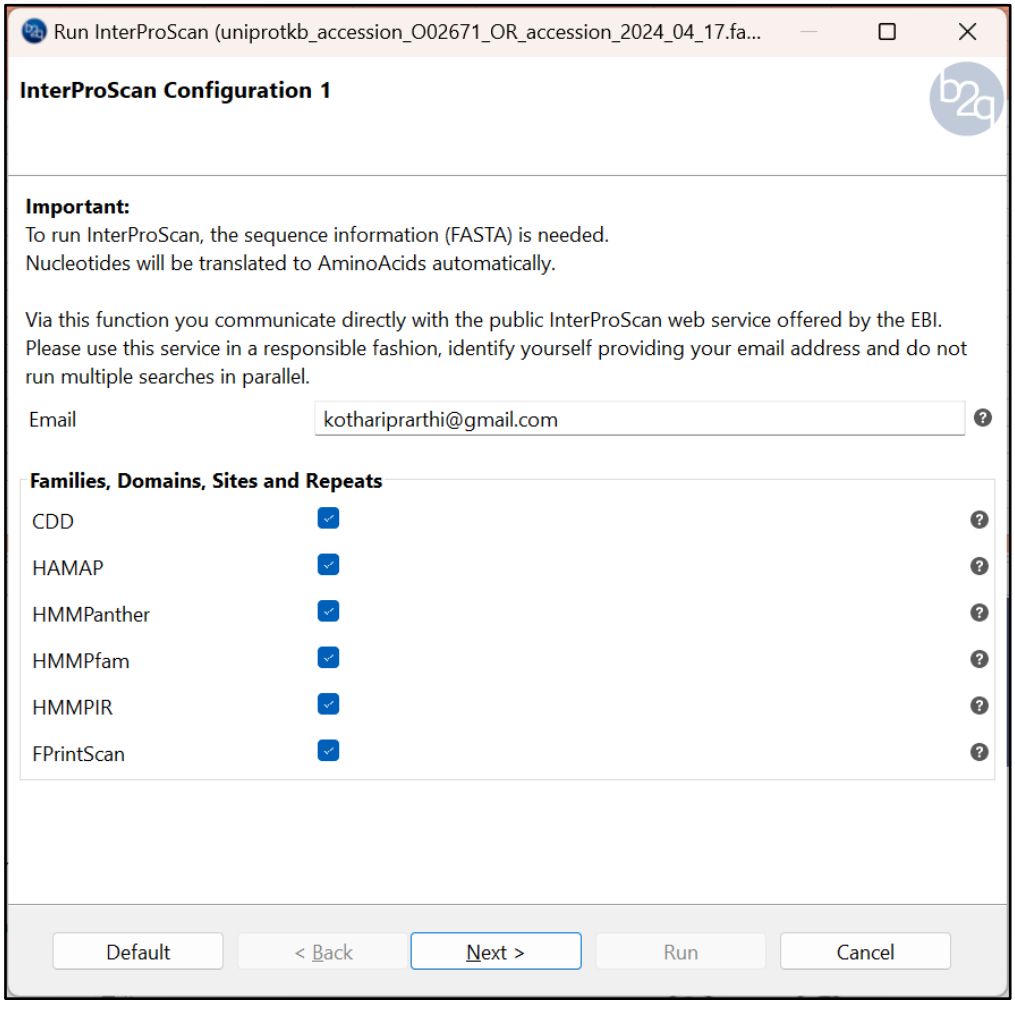

Fig 14: Input Parameters for Interpro

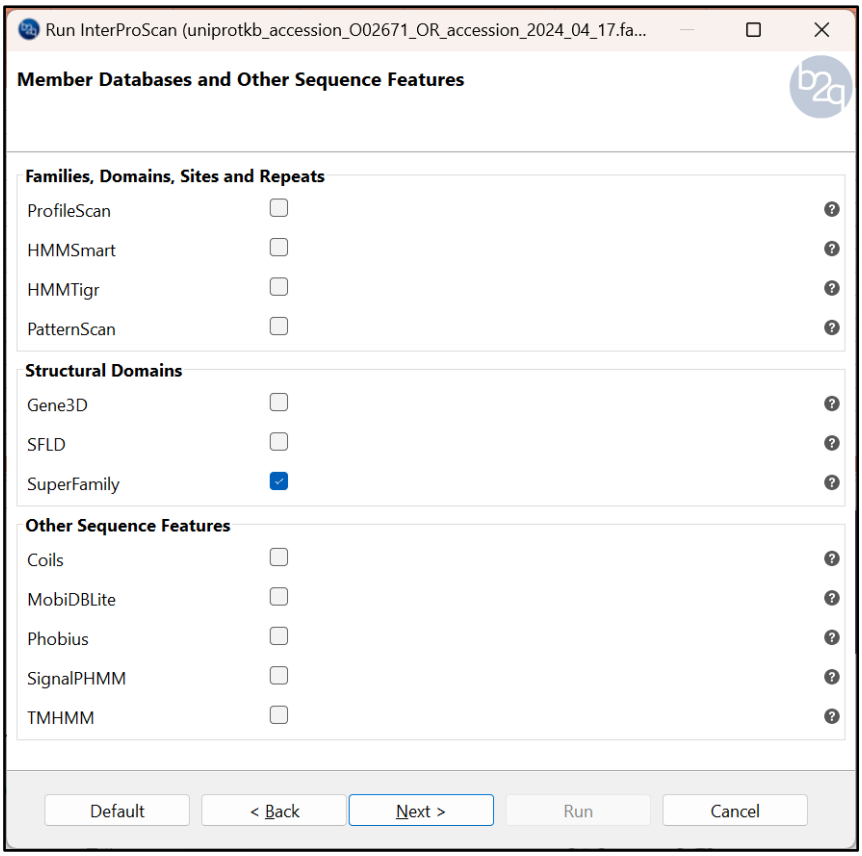

Fig 15: Ticking on Superfamily under structural domains

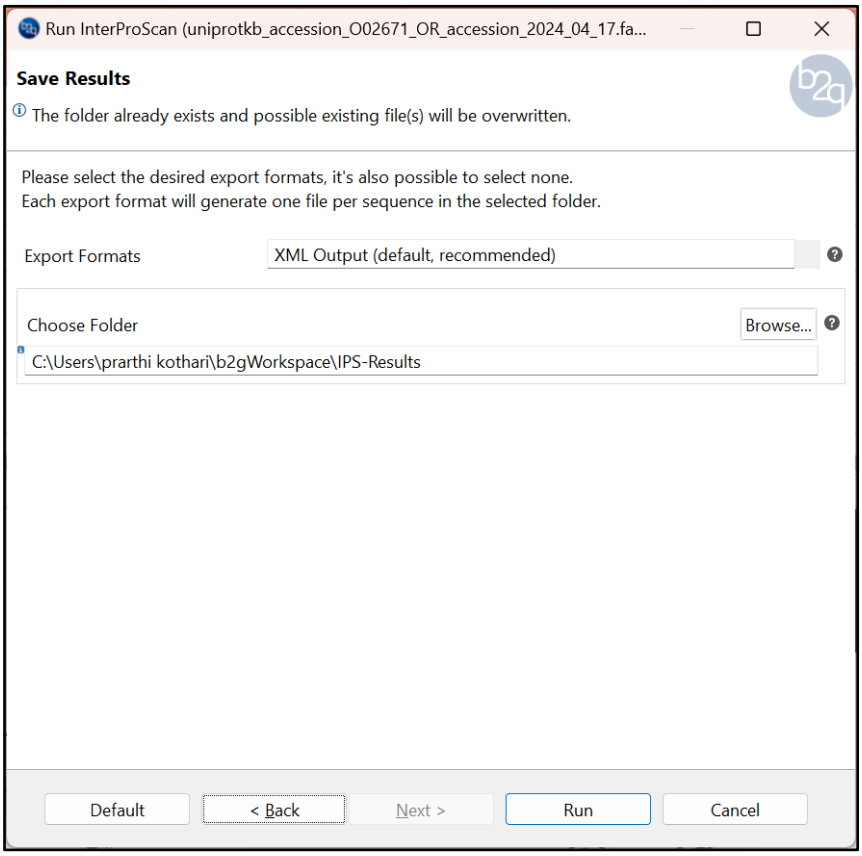

Fig 16: Running Interpro scan

InterPro IDs	InterPro GO IDs	InterPro GO Names
IPR041182 (PFAM); IPR010457 (PFAM); IPR050379 (PANTHER); IPR003961 (CDD); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY)	P:GO:0019221; F:GO:0004896; F:GO:0005515; F:GO:0019955; C:GO:0009897; C:GO:0043235	P:cytokine-mediated signaling pathway; F:cytokine receptor activity; F:protein binding; F:cytokine binding; C:external side of plasma membrane; C:receptor complex
IPR041182 (PFAM); IPR010457 (PFAM); IPR050379 (PANTHER); IPR003961 (CDD); IPR003961 (CDD); IPR003961 (CDD); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY)	P:GO:0019221; F:GO:0004896; F:GO:0005515; F:GO:0019955; C:GO:0009897; C:GO:0043235	P:cytokine-mediated signaling pathway; F:cytokine receptor activity; F:protein binding; F:cytokine binding; C:external side of plasma membrane; C:receptor complex
IPR010457 (PFAM); IPR041182 (PFAM); IPR050379 (PANTHER); IPR003961 (CDD); IPR003961 (CDD); IPR003961 (CDD); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY)	P:GO:0019221; F:GO:0004896; F:GO:0005515; F:GO:0019955; C:GO:0009897; C:GO:0043235	P:cytokine-mediated signaling pathway; F:cytokine receptor activity; F:protein binding; F:cytokine binding; C:external side of plasma membrane; C:receptor complex
IPR041182 (PFAM); IPR010457 (PFAM); IPR050379 (PANTHER); IPR003961 (CDD); IPR003961 (CDD); IPR003961 (CDD); IPR003961 (CDD); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY)	P:GO:0019221; F:GO:0004896; F:GO:0005515; F:GO:0019955; C:GO:0009897; C:GO:0043235	P:cytokine-mediated signaling pathway; F:cytokine receptor activity; F:protein binding; F:cytokine binding; C:external side of plasma membrane; C:receptor complex
IPR041182 (PFAM); IPR010457 (PFAM); IPR050379 (PANTHER); IPR003961 (CDD); IPR003961 (CDD); IPR003961 (CDD); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY); IPR036116 (SUPERFAMILY)	P:GO:0019221; F:GO:0004896; F:GO:0005515; F:GO:0019955; C:GO:0009897; C:GO:0043235	P:cytokine-mediated signaling pathway; F:cytokine receptor activity; F:protein binding; F:cytokine binding; C:external side of plasma membrane; C:receptor complex

Fig 17: Results obtained after InterPro Scan

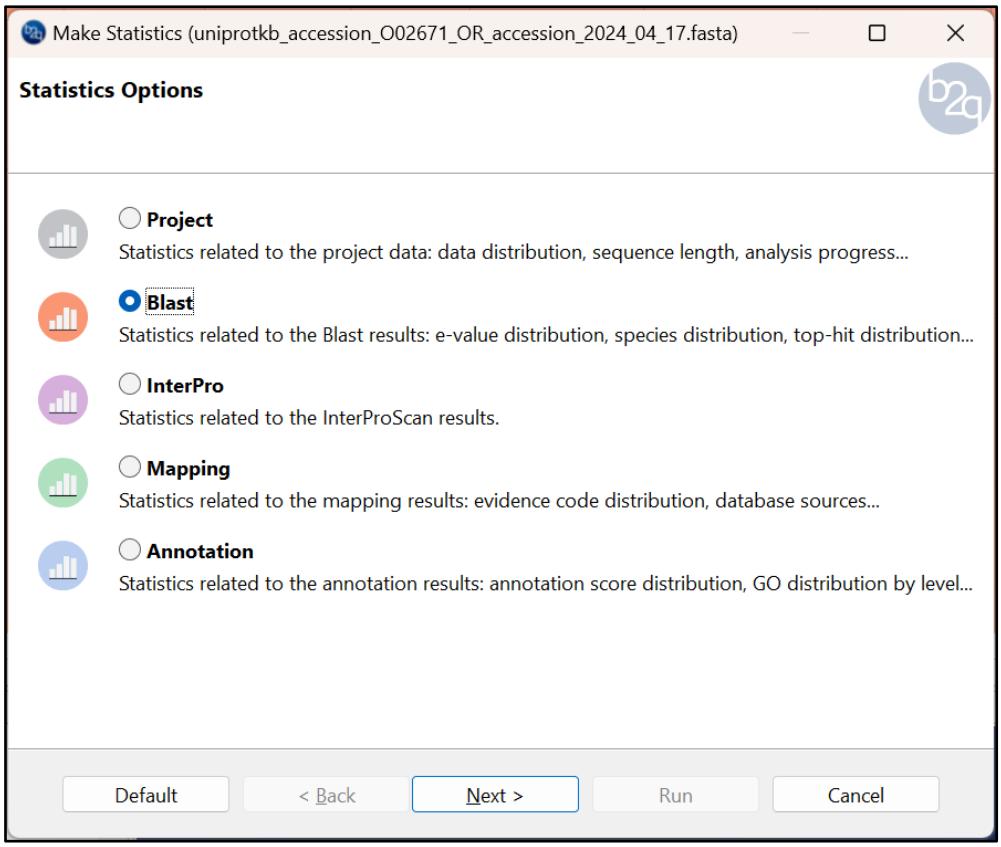

Fig 18: Statistics option after running Blast

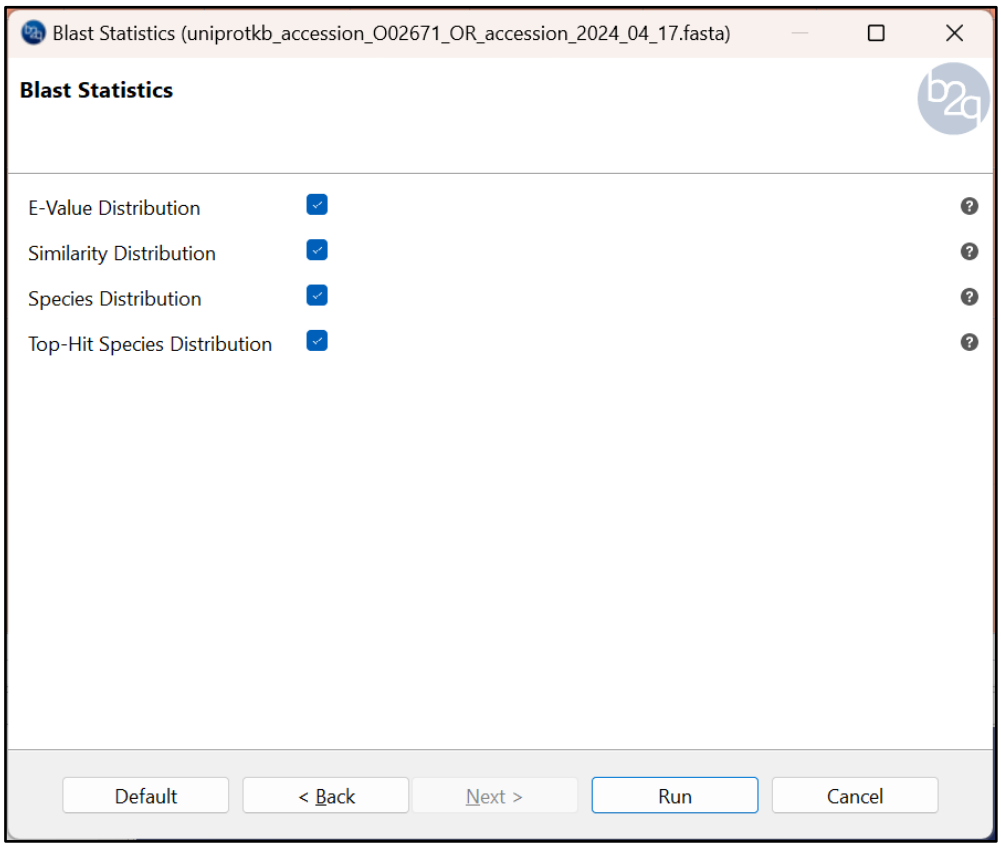

Fig 19: Adding input parameters and hitting run

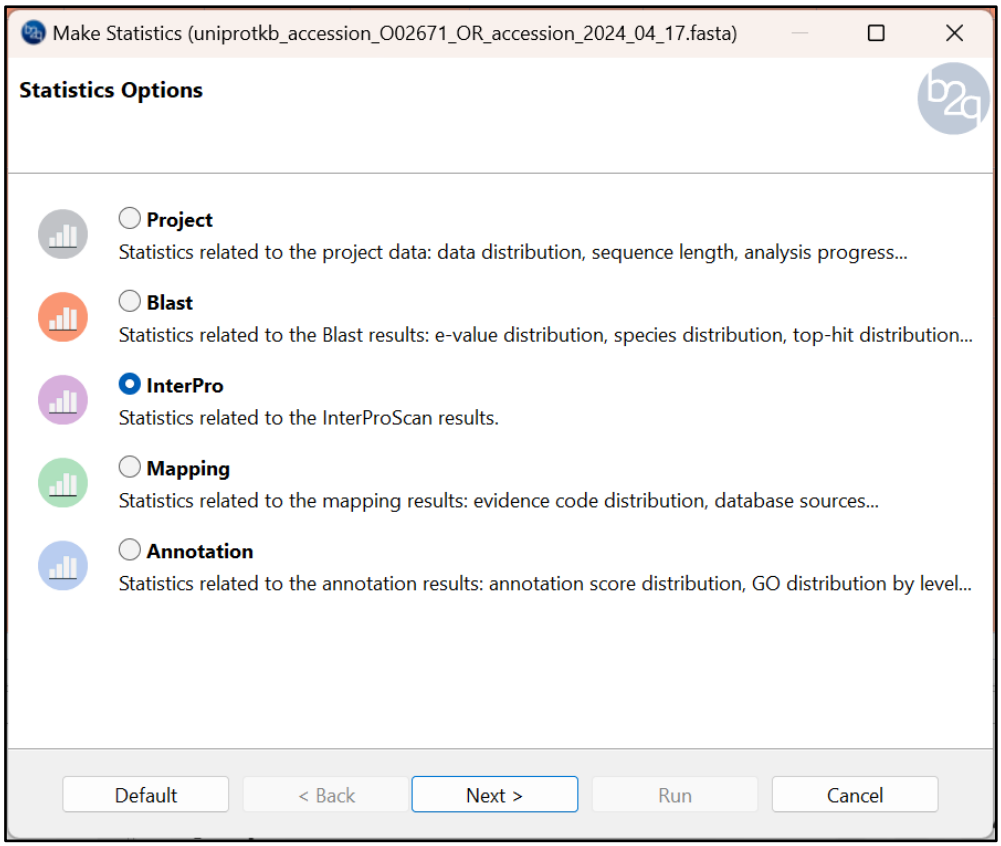

Fig 20: InterPro statistics option

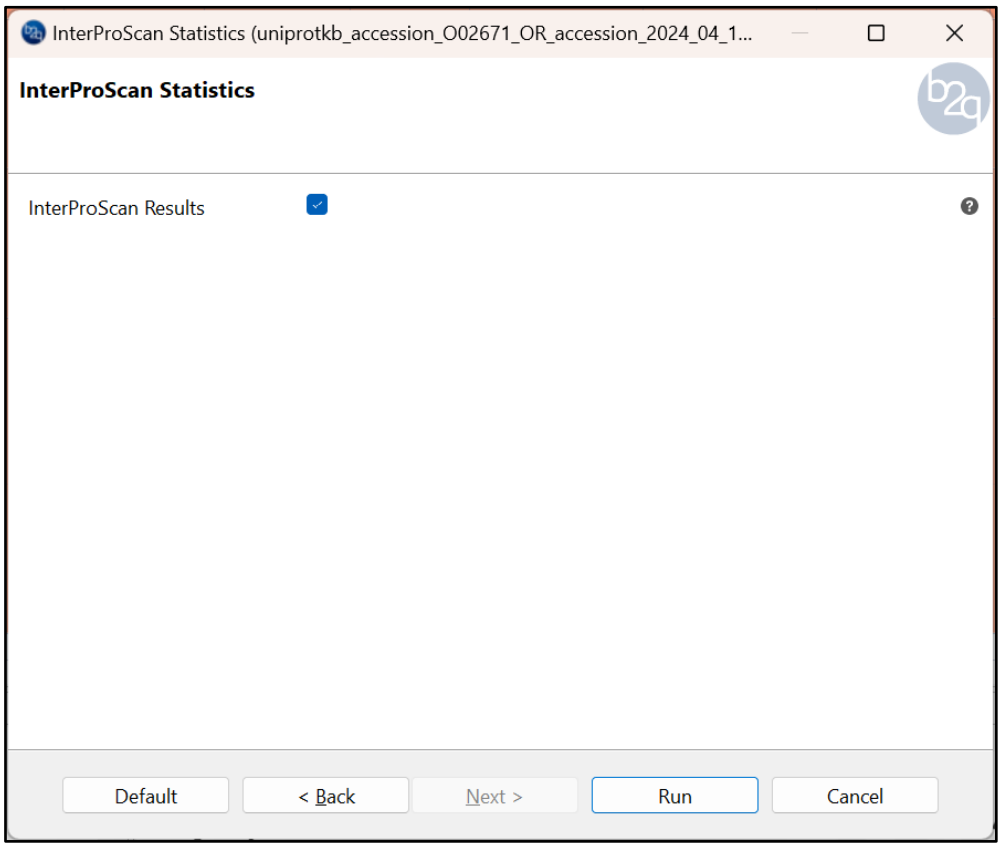

Fig 21: InterProScan Statistics and hit run

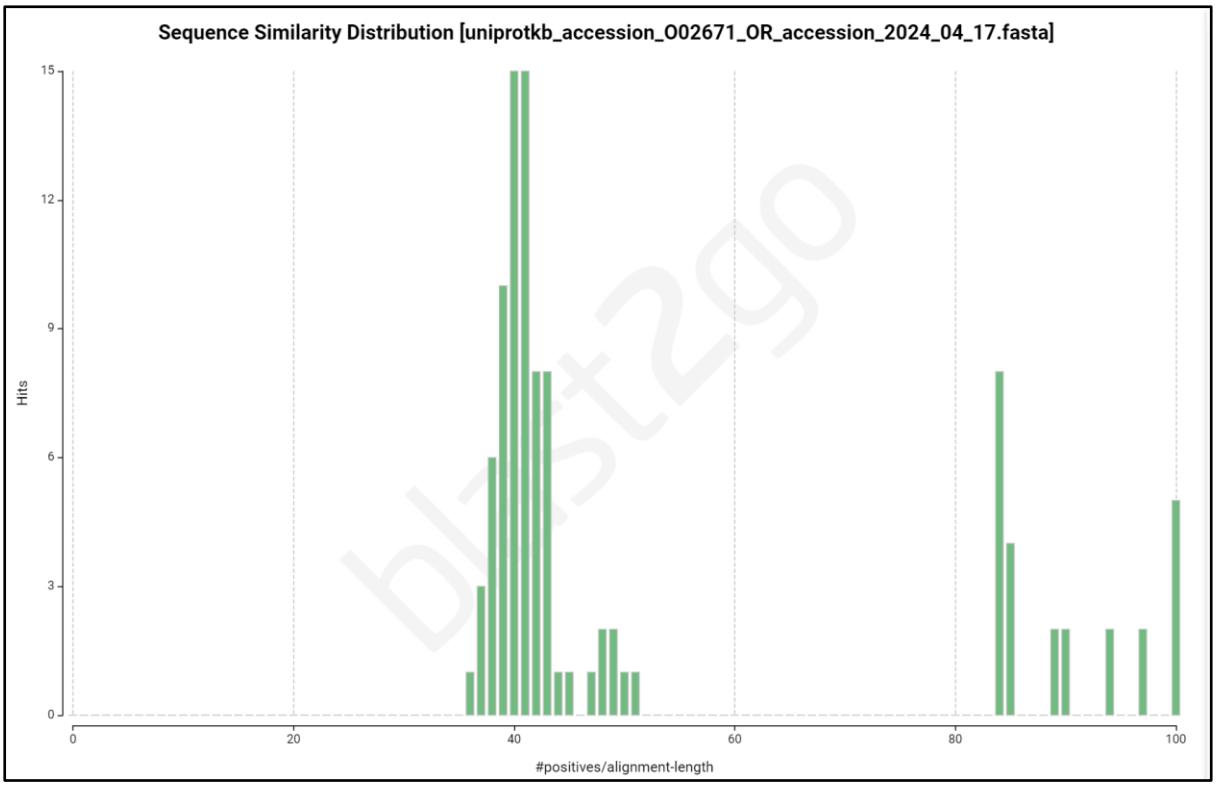

Fig 22: Sequence similarity distribution chart

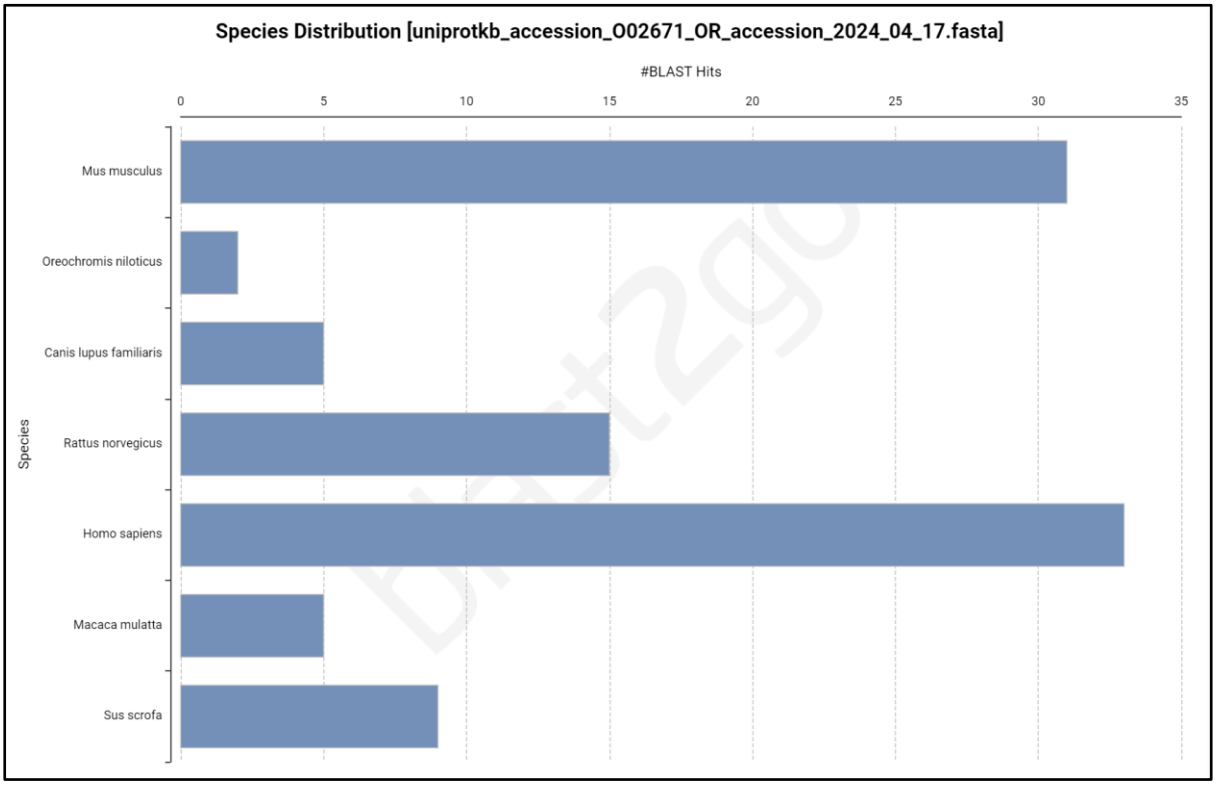

Fig 23: Species distribution chart

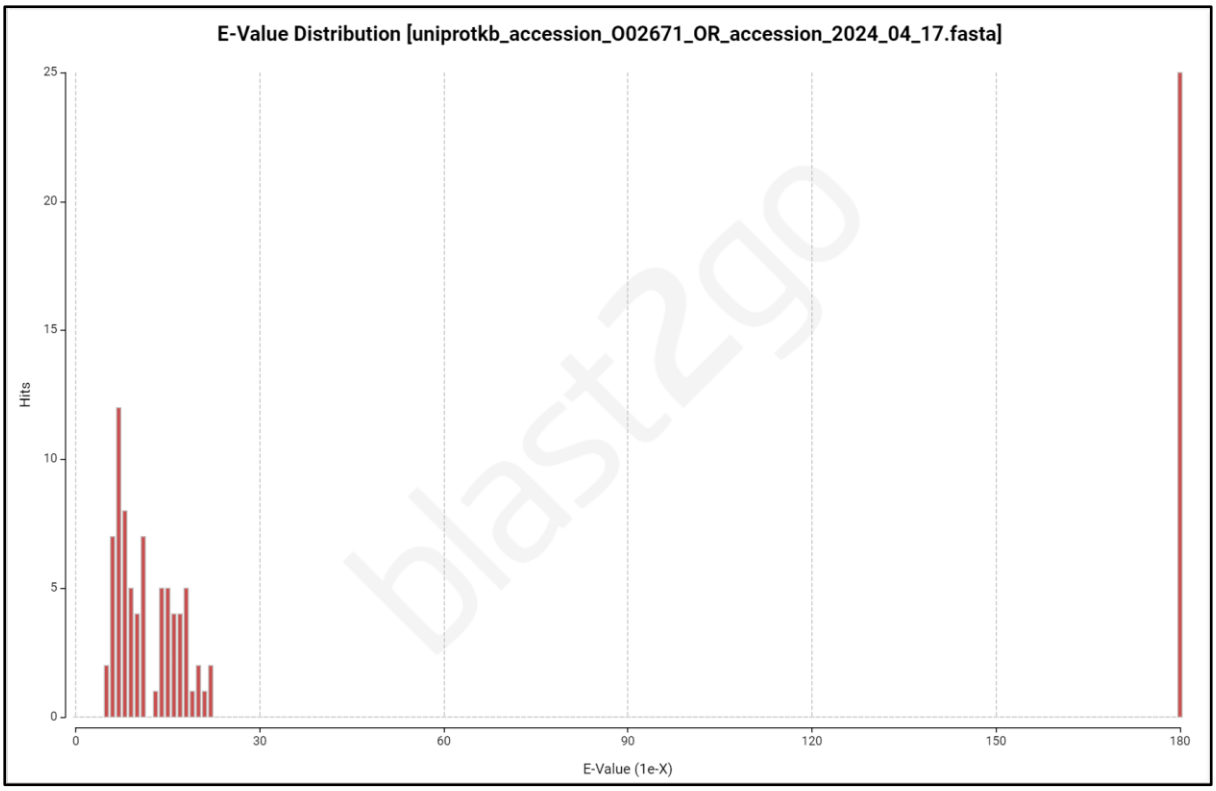

Fig 24: E-value distribution

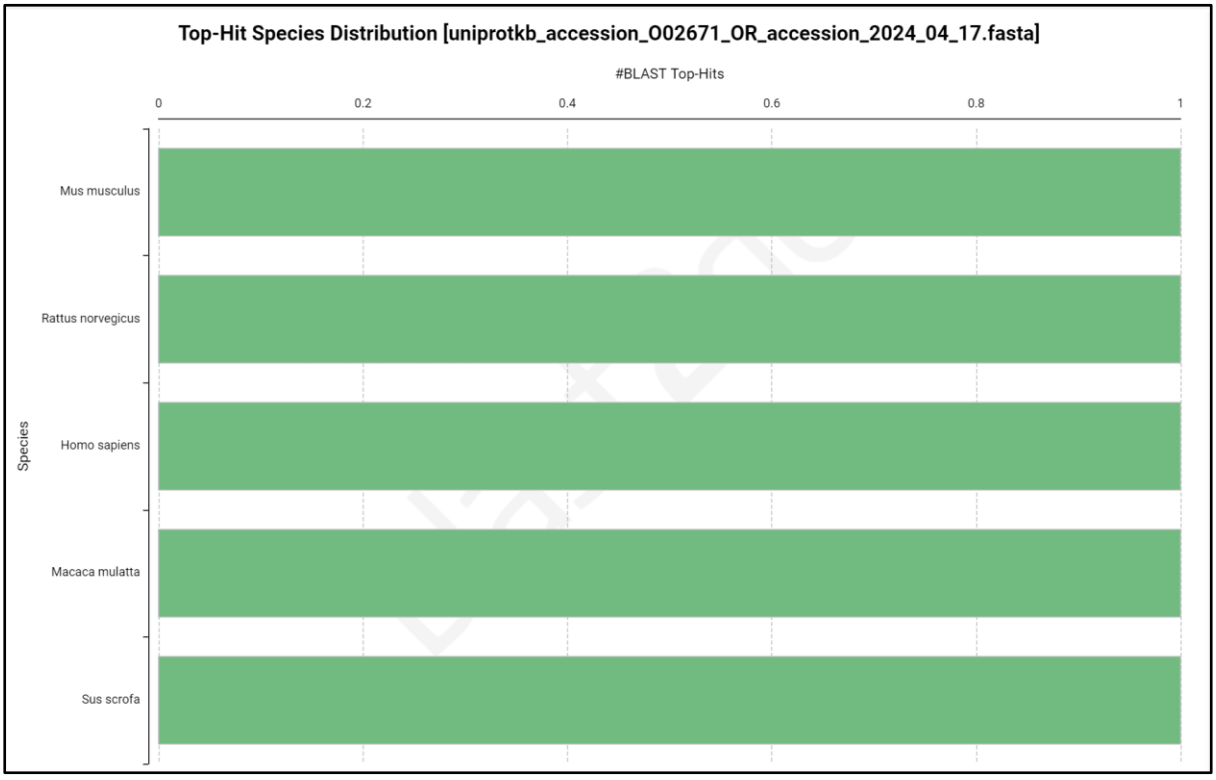

Fig 25: Top-Hit Species distribution

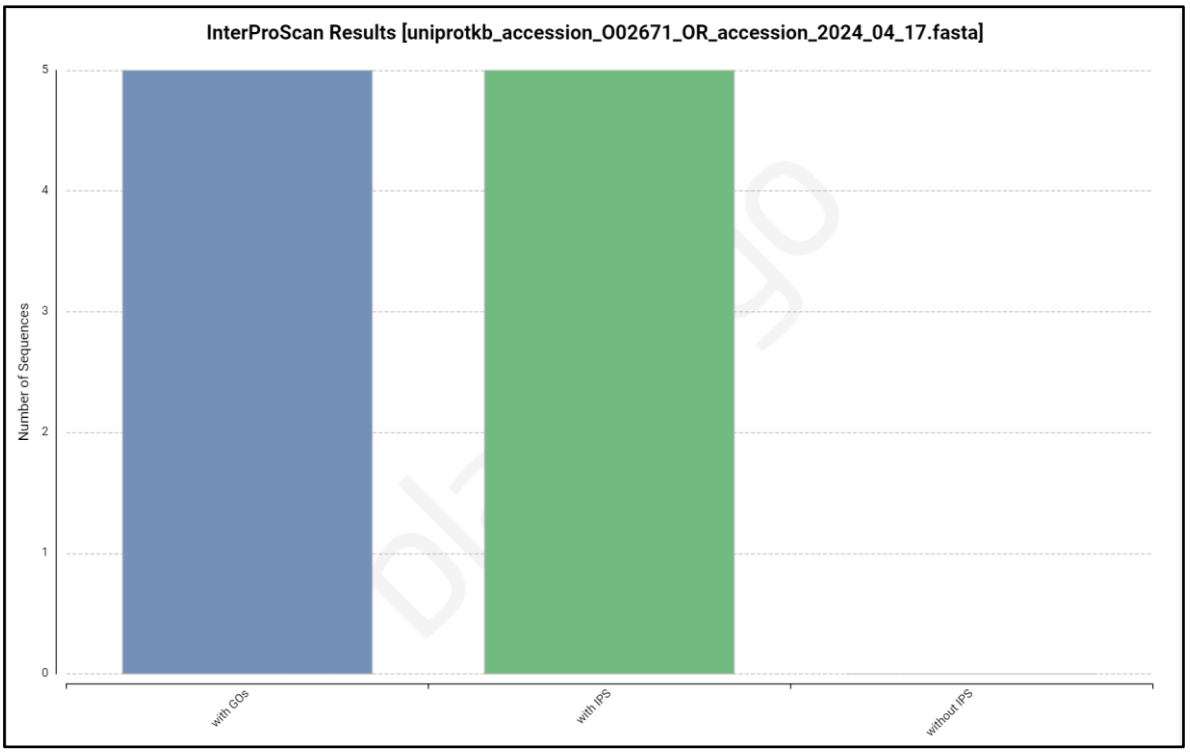

Fig 26: InterProScan results

RESULTS:

In the Blast2GO results that we have gotten via statistics, a sequence similarity chart was obtained and for which alignment length for 40 received the maximum amount of hits which was 15. In species distribution chart, 7 organisms were matched and *Homo sapiens* received 33 hits. A E-value distribution chart was also obtained and a top species was obtained with it. For InterPro, 5 sequences were obtained for both with GOs and with IPS.

CONCLUSION:

Blast2GO database was explored for annotating and analysing our query 'LEPR'.

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DATE: 16/03/2024

WEBLEM 6
INTRODUCTION OF SINGLE NUCLEOTIDE POLYMORPHISM DATABASE (dbSNP)

SNP database is indeed a crucial resource in genetics and genomics, serving as a repository for various genetic variations such as single nucleotide polymorphisms (SNPs), insertions, deletions, and structural variations. This database plays a significant role in understanding genetic diversity within populations, identifying disease associations, and studying variations in genes that can influence health and disease outcomes.

The dbSNP database includes a broad collection of simple genetic polymorphisms, encompassing single-base nucleotide substitutions (SNPs), small-scale multi-base deletions or insertions (DIPs), retroposable element insertions, and microsatellite repeat variations (STRs). Each entry in dbSNP provides essential information such as the sequence context of the polymorphism, the frequency of occurrence (by population or individual), and details about the experimental methods used to assay the variation.

Researchers utilize dbSNP for gene mapping, defining population structure, conducting functional studies, and exploring the genetic basis of complex disorders like heart disease and cancer. By leveraging the data in dbSNP, scientists can gain insights into the genetic underpinnings of diseases, population genetics, and the relationship between genetic variations and phenotypic characteristics.

Based on the provided sources, entries in dbSNP contain detailed information on nucleotide variations, flanking sequences, genotypes, frequencies, and more. Each submission in dbSNP is uniquely identified by an ID starting with "ss" (submitted SNP). The database offers effective search methods through Entrez, including text queries, a Limits page for refined searches, and a Preview/Index page. Additionally, dbSNP integrates submitted and computed data, requiring a minimum sequence length of 100 nucleotides to ensure accurate mapping on the reference genome. Computed information is obtained through a process called the "build," enhancing the database's comprehensiveness and usefulness in genetic research.

Originally intended to support large-scale polymorphism discovery projects like the HapMap project, dbSNP has expanded into a comprehensive repository for various genetic variations. Despite its name, dbSNP houses both common and rare variations, including clinically significant human variations and benign polymorphisms. The database not only stores genetic variant information but also provides data on population-specific allele frequencies, individual genotypes, validation status, and significant genome-wide association results. Furthermore, dbSNP collaborates with locus-specific databases and clinical diagnostic laboratories to integrate genetic variant data with clinical relevance. For those interested in contributing to or accessing information from dbSNP, an automated web submission portal is available along with user support services for inquiries.

dbSNP Reference SNP (rs or RefSNP) number is a locus accession for a variant type assigned by dbSNP. The RefSNP catalog is a non-redundant collection of submitted variants which were clustered, integrated and annotated. RefSNP number is the stable accession regardless of the differences in genomic assemblies. RefSNP numbers facilitate large-scale studies in

association genetics, medical genetics, functional and pharmaco-genomics, population genetics and evolutionary biology, personal genomics, and precision medicine. They provide a stable variant notation for mutation and polymorphism analysis, annotation, reporting, data mining, and data integration.

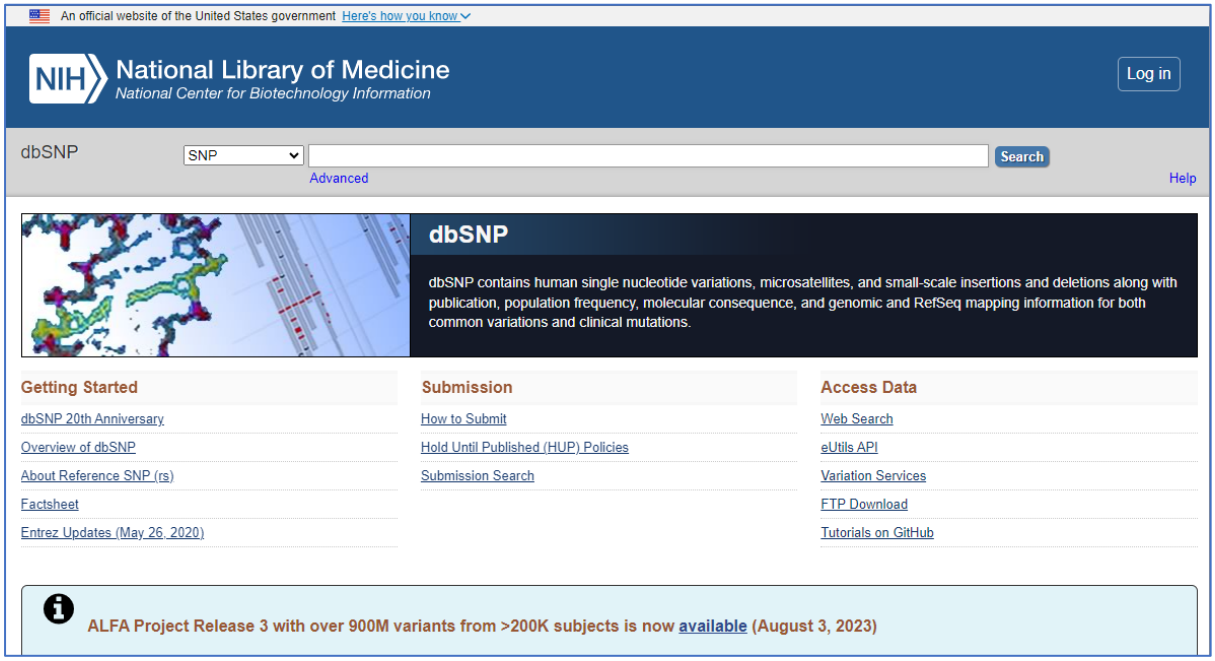

Fig 1: Homepage of dpSNP

Distinguishing RefSNP Features:

1. Non-redundancy and globally unique accession series.
2. Composed from over 2 billion Submitted SNP (ss) from thousands of submitters.
3. More than 20 years of tracking histories for all assigned, merged, and deleted RefSNP.
4. Annotated and linked to the latest human assembly and RefSNP nucleotide and protein sequences.
5. Updates to reflect current knowledge of sequence data and biology data validation.
6. Ongoing curation and annotation by NCBI staff and collaborators.
7. Searchable across variation and genomic databases
8. Supported and reported in open-source and commercial software and tools. Over 400K RefSNP are in CLIN Var
9. Cited in over 51K publications with biological, functional, disease, and clinical information for variants across the genomes.
10. Linked to many NCBI internal and external resources such as CLIN Var, PubMed, PubMed Central, RefSeq, UCSC, EBI, Top Med, and GnomAD.

Supports consistent reporting and non-redundant variation annotations across related sequences including alternate haplotypes, GRC patches, and future graph genomes if the alignment or sequence relationship is known.

dbSNP having two Accession Types:

1. **Submitted SNP (ss):** Submitted variant based on asserted location or flanking sequences.
2. **Reference SNP (rs):** Non-redundant set of variations based on clustering of SS'es of same variant type and sequence position.

SNP database provides various search methods, including text-based and sequence-based searches, enabling researchers to effectively navigate and retrieve genetic information. Detailed data on nucleotide variations, flanking sequences, genotypes, and frequencies are available for in-depth genetic analysis.

This database integrates submissions with other NCBI resources such as GenBank, PubMed, Locus Link, and the Human Genome Project data. This integration enhances the depth and utility of genetic data available for research purposes.

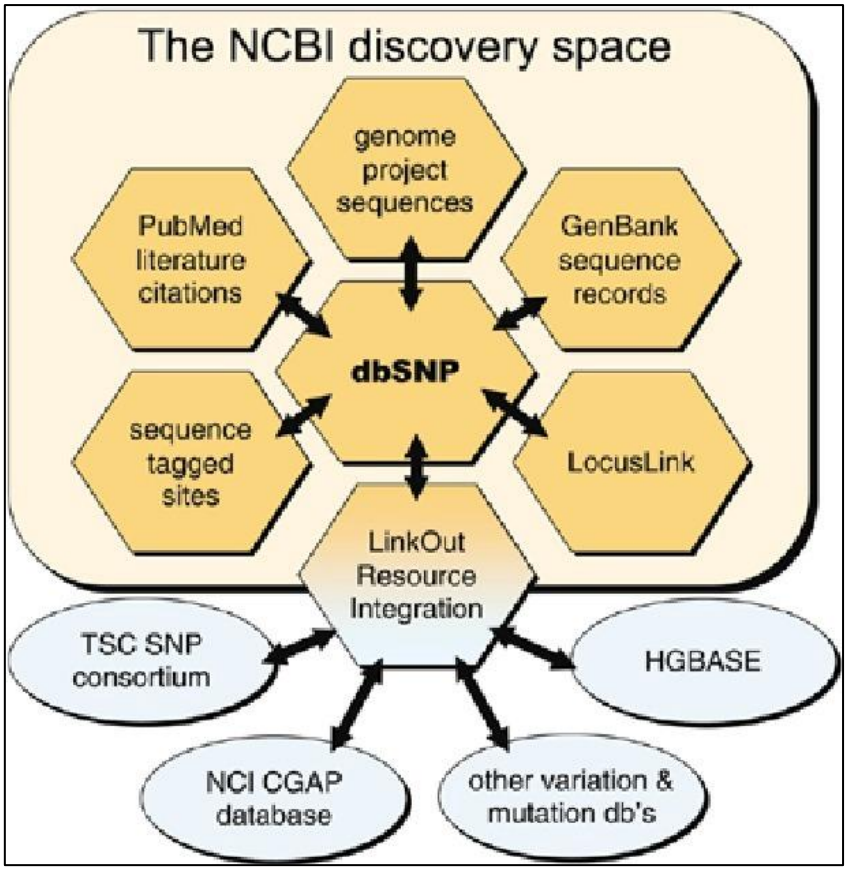

Fig 2: Other NCBI resources and out link resources for dbSNP integration

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2. BMC Bioinformatics. (2024, March 21). BioMed Central. https://bmcbioinformatics.biomedcentral.com/

DATE: 16/03/2024

WEBLEM 6(A)

dbSNP (Single Nucleotide Polymorphism database)

(URL:https://www.ncbi.nlm.nih.gov/snp/)

AIM:

To identify and analyze genetic variations (mutational gene) in the query 'DDIT3' (Reference SNP Report: rs28382352) using dbSNP (SNP database).

INTRODUCTION:

The dbSNP (Single Nucleotide Polymorphism Database) curated by the National Center for Biotechnology Information (NCBI) is indeed a pivotal resource in genetic research. It serves as a comprehensive repository of genetic variations, including single nucleotide polymorphisms (SNPs), insertions, deletions, and structural variations, offering researchers a rich dataset for genetic analysis. DbSNP plays a crucial role in supporting large-scale polymorphism discovery and provides detailed information on population-specific allele frequencies, individual genotypes, validation status, and significant genome-wide association results. This database integrates with other NCBI resources like GenBank, PubMed, LocusLink, and the Human Genome Project data, enhancing the utility and depth of genetic data available for research purpose. Researchers and students exploring genetic diversity, disease association studies, and population genetics benefit significantly from dbSNP's wealth of information. It offers valuable insights into the genetic landscape of populations and the underlying genetic basis of traits and diseases, making it an indispensable tool in the field of genetic research.

DDIT3:

The DDIT3 gene, also known as CHOP or GADD153, encodes a member of the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors. Located on chromosome 12, it regulates diverse biological processes such as transcriptional regulation, blood vessel maturation, and cellular responses to various stressors like DNA damage and endoplasmic reticulum stress. Its involvement extends to cell cycle regulation, Wnt signaling, and modulation of interleukin-8 production, among others.

Functionally, DDIT3 participates in transcriptional regulation by binding to specific DNA sequences near RNA polymerase II promoters and modulating gene expression. It acts as a transcriptional activator or repressor depending on the context, exerting its effects through protein-protein interactions and DNA binding. Moreover, DDIT3 plays roles in cellular processes such as protein binding, homodimerization, and interaction with other transcription factors like cAMP response element-binding protein (CREB). Its leucine zipper domain facilitates dimerization, essential for its transcriptional activity. Overall, DDIT3's multifaceted functions highlight its significance in cellular homeostasis and stress response pathways.

METHODOLOGY:

1. Go to NCBI dbSNP web page.
2. Use the search box in dbSNP to input ‘DDIT3’ query. You can search using text words or phrases related to genetic variations, SNPs, or specific genes.
3. Click on a specific SNP ID or genetic variation from the search results to open an entry.
4. Now interpret the results to understand how the genetic variant found in dbSNP could be linked to biological functions, diseases, or other important factors related to the query.

OBSERVATIONS:

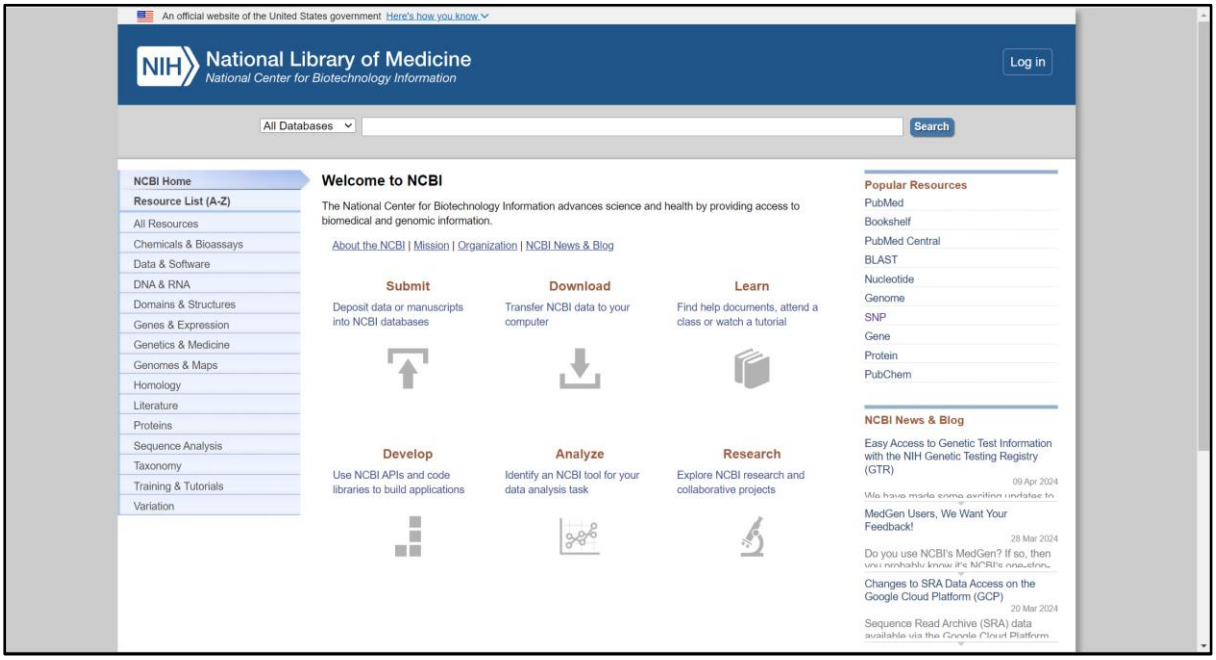

Fig 1: Homepage of NCBI database

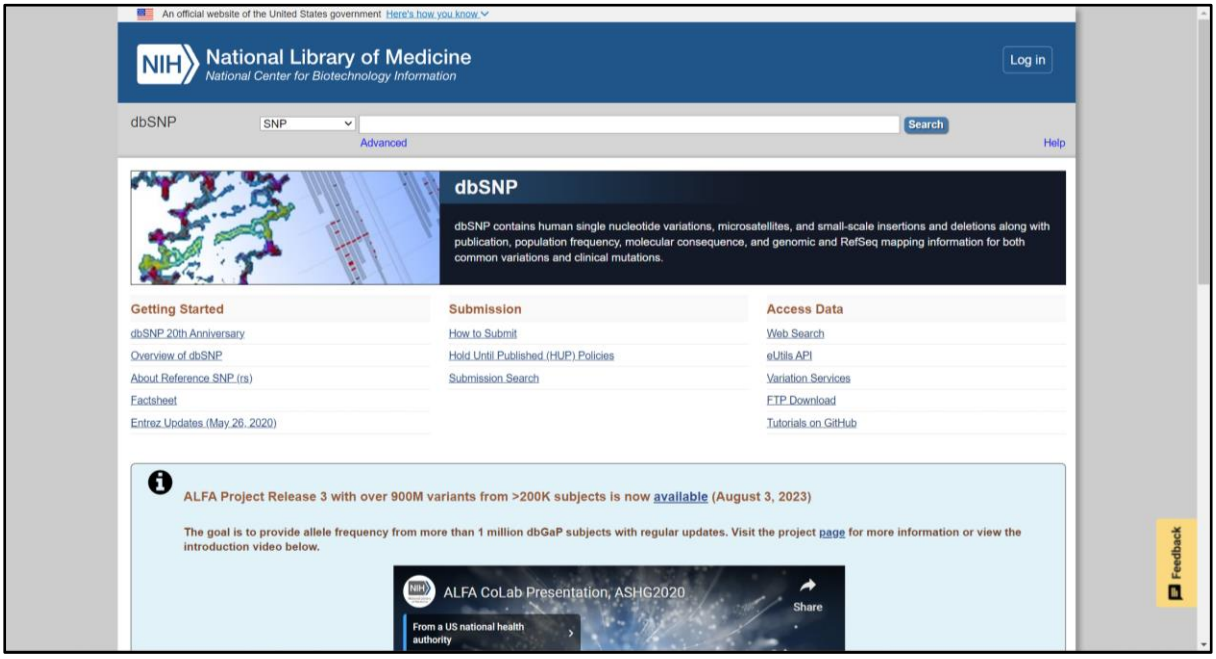

Fig 2: Homepage of dbSNP database

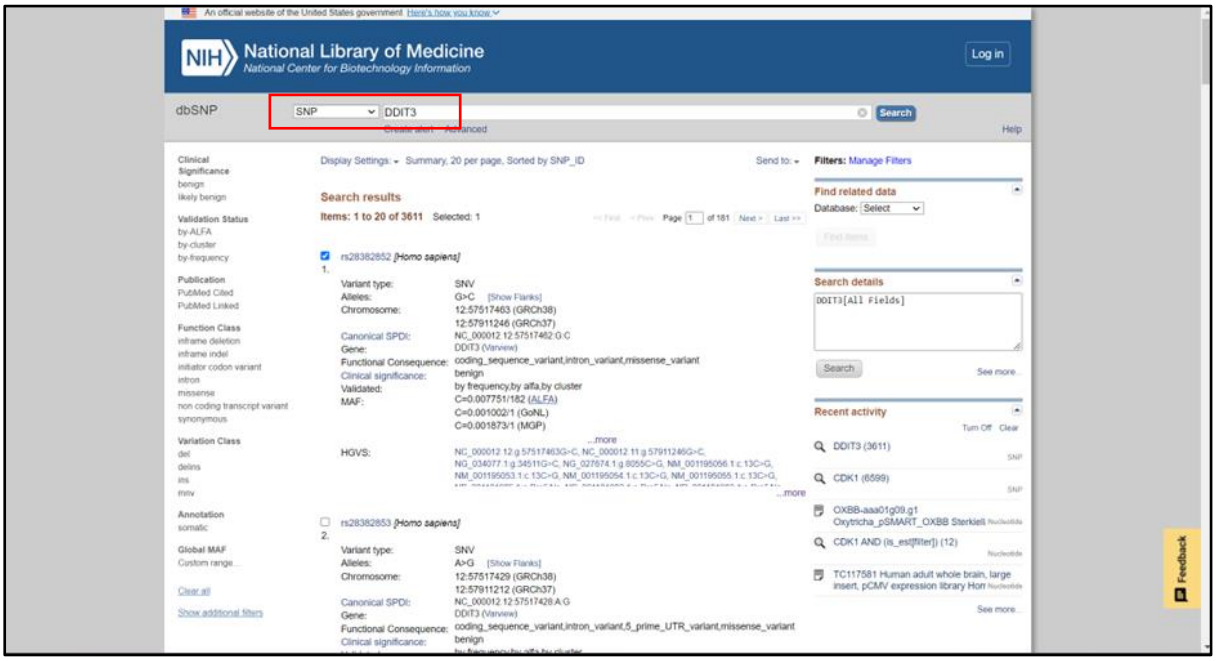

Fig 3: Results obtained after searching for the query 'DDIT3'

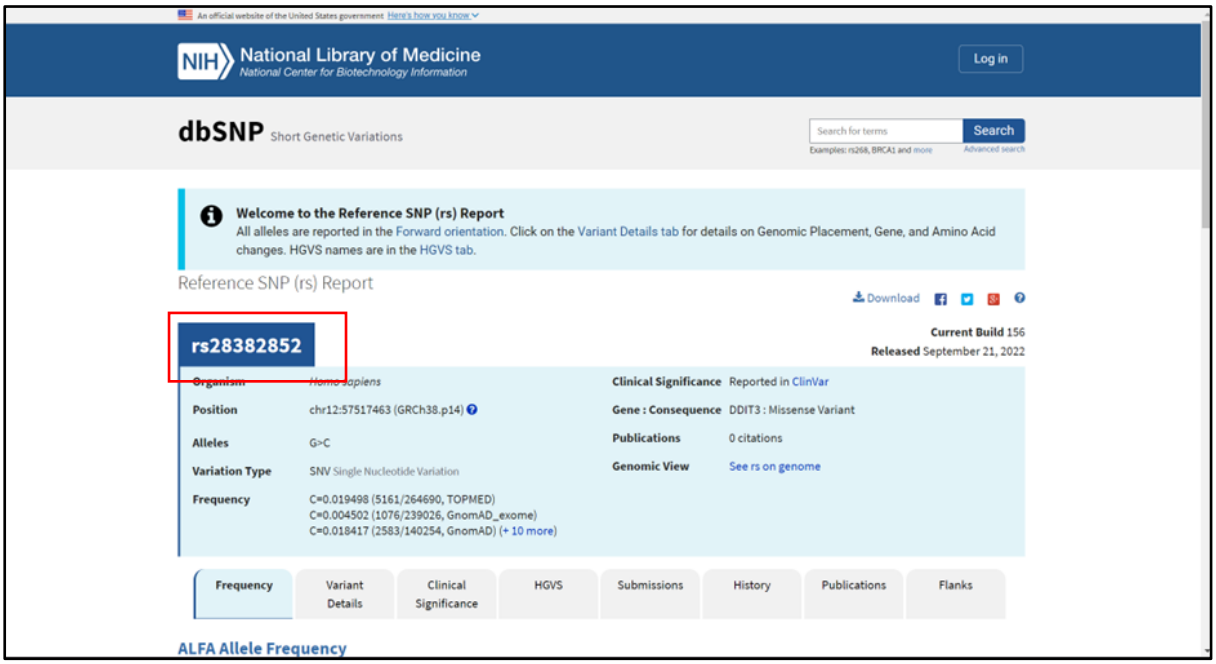

Fig 4: Studying the entry: rs28382352

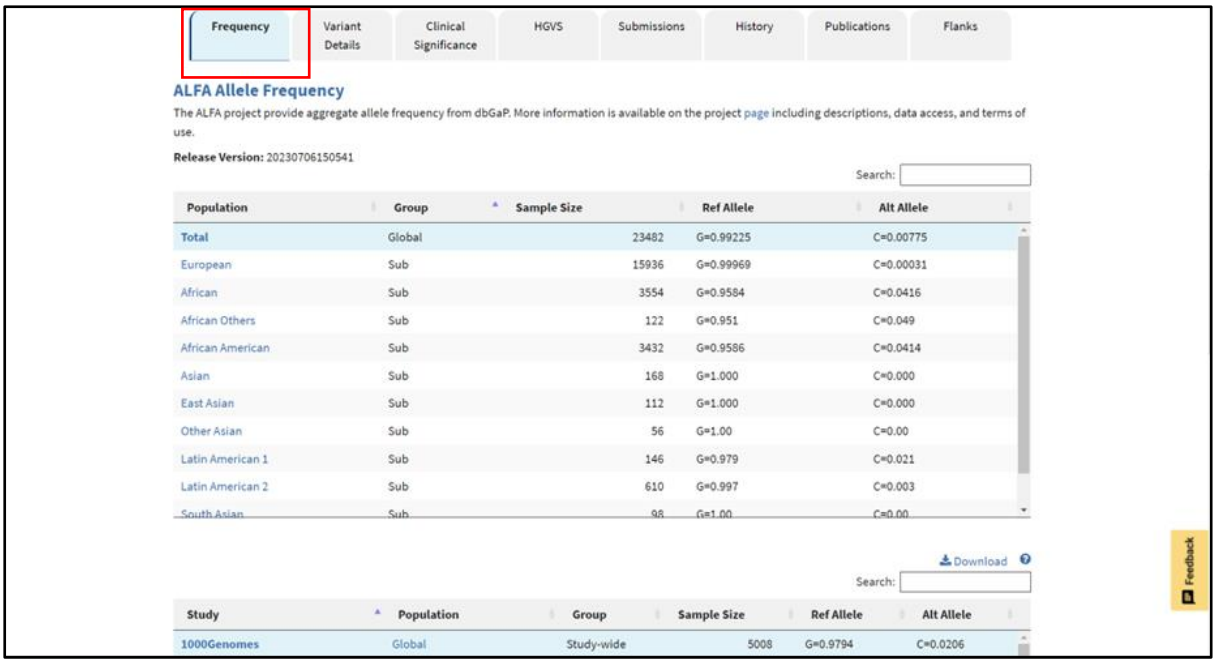

Fig 5: View of the 'Frequency' section

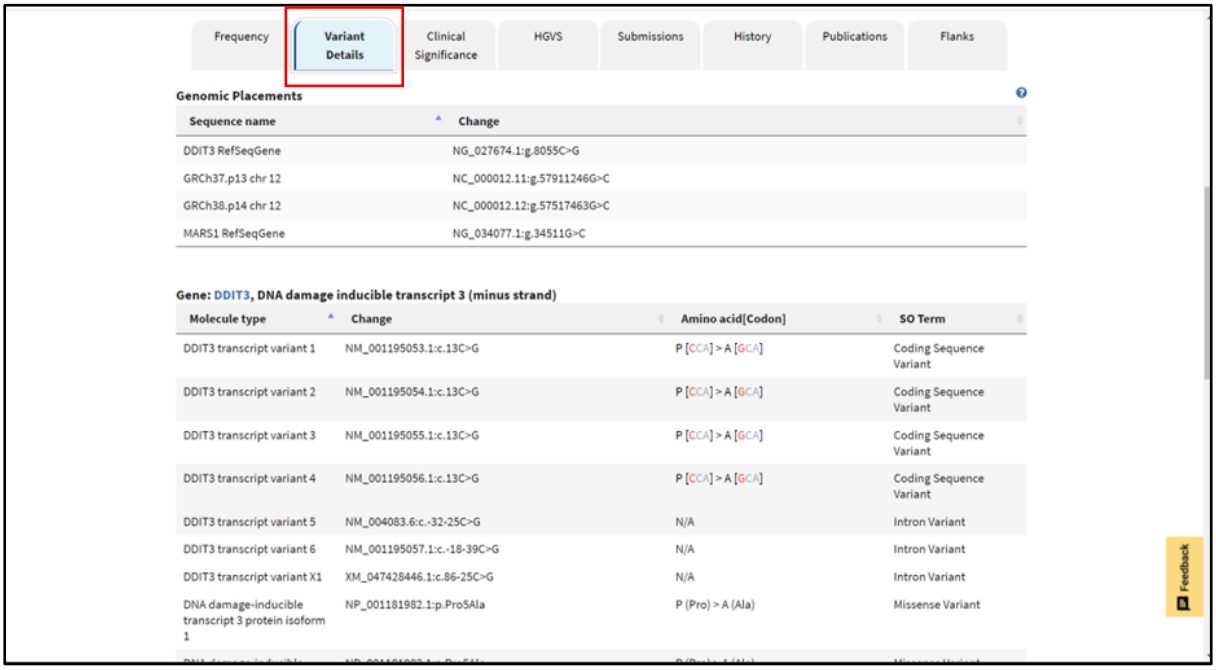

Fig 6: View of the 'Variant details' section

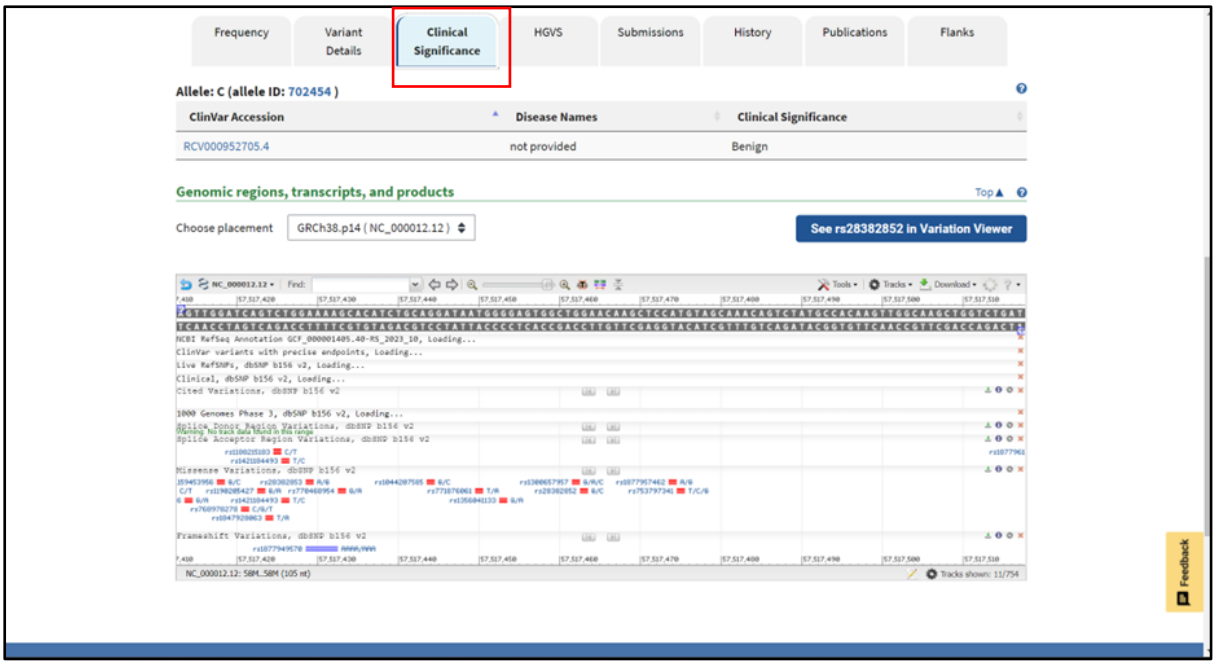

Fig 7: View of the 'Clinical Significance' section

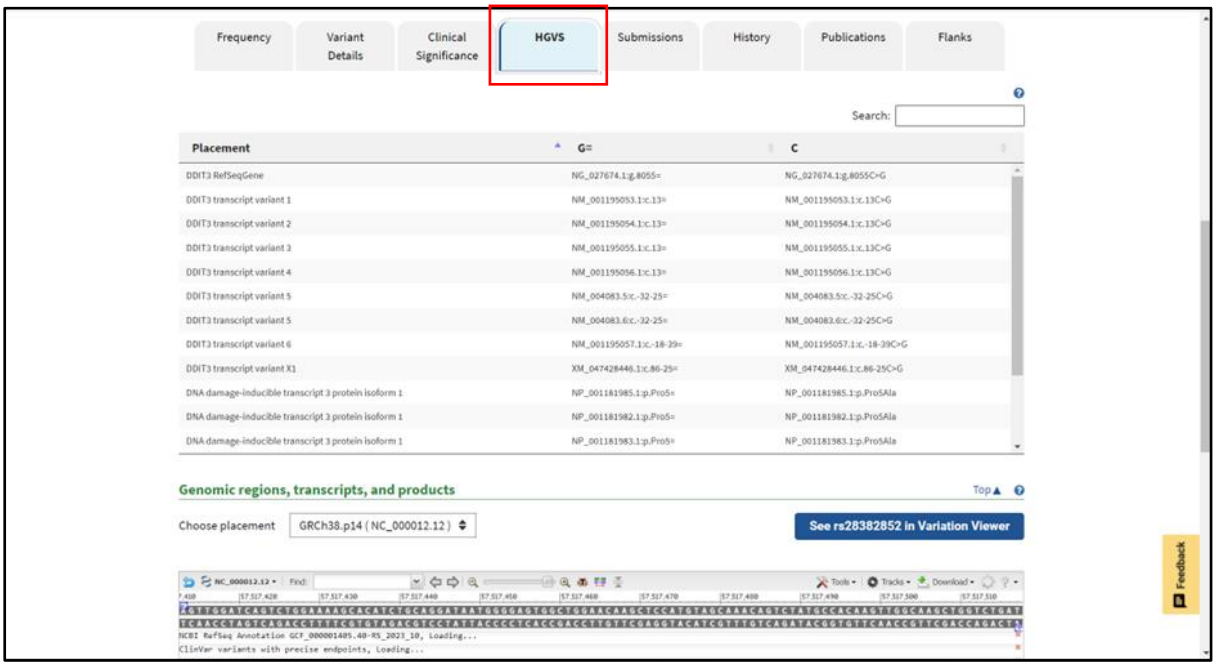

Fig 8: View of the 'HGVS' section

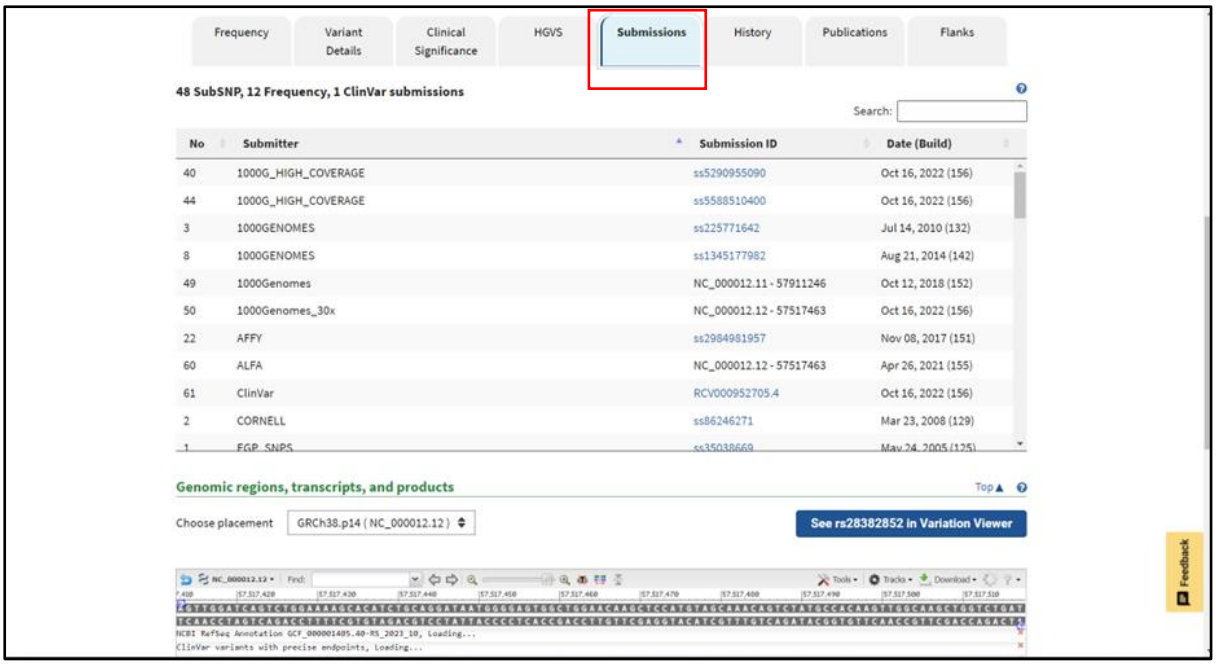

Fig 9: View of the 'Submission' section

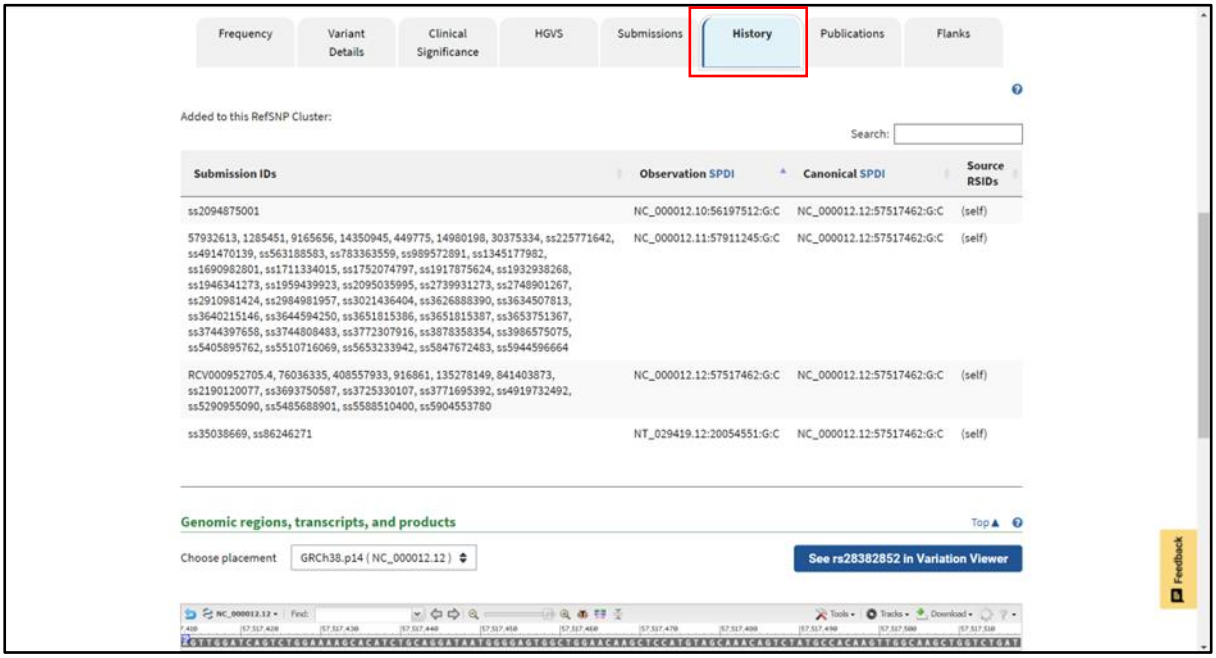

Fig 10: View of the 'History' section

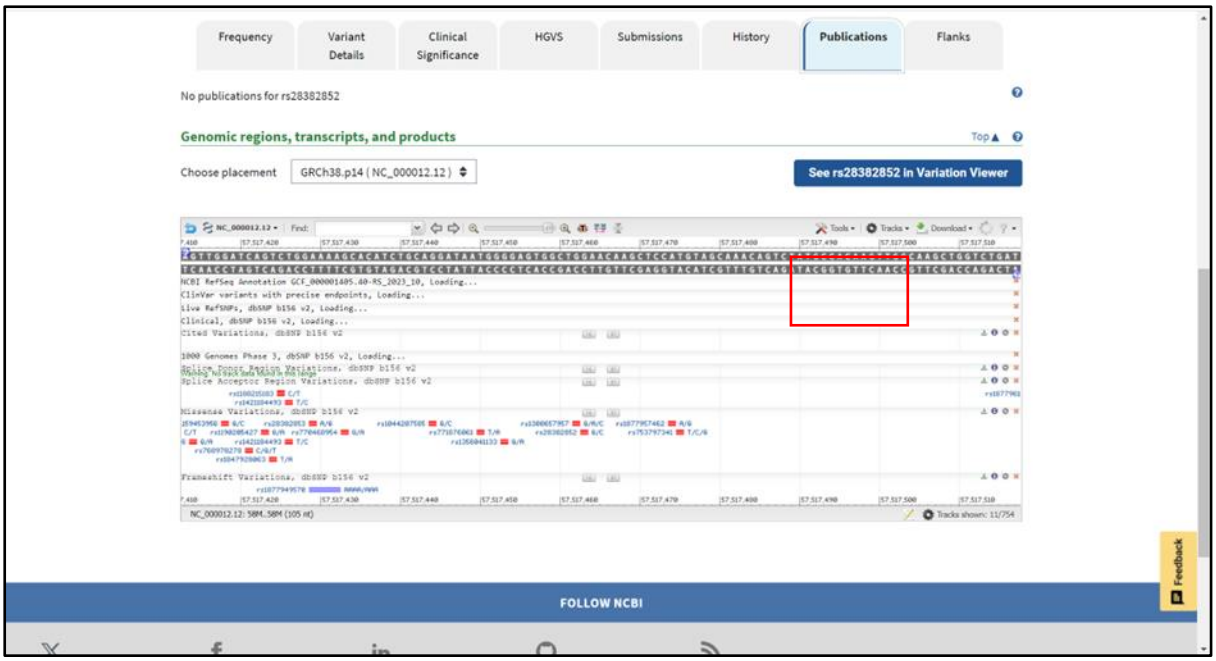

Fig 11: View of the 'Publication' section

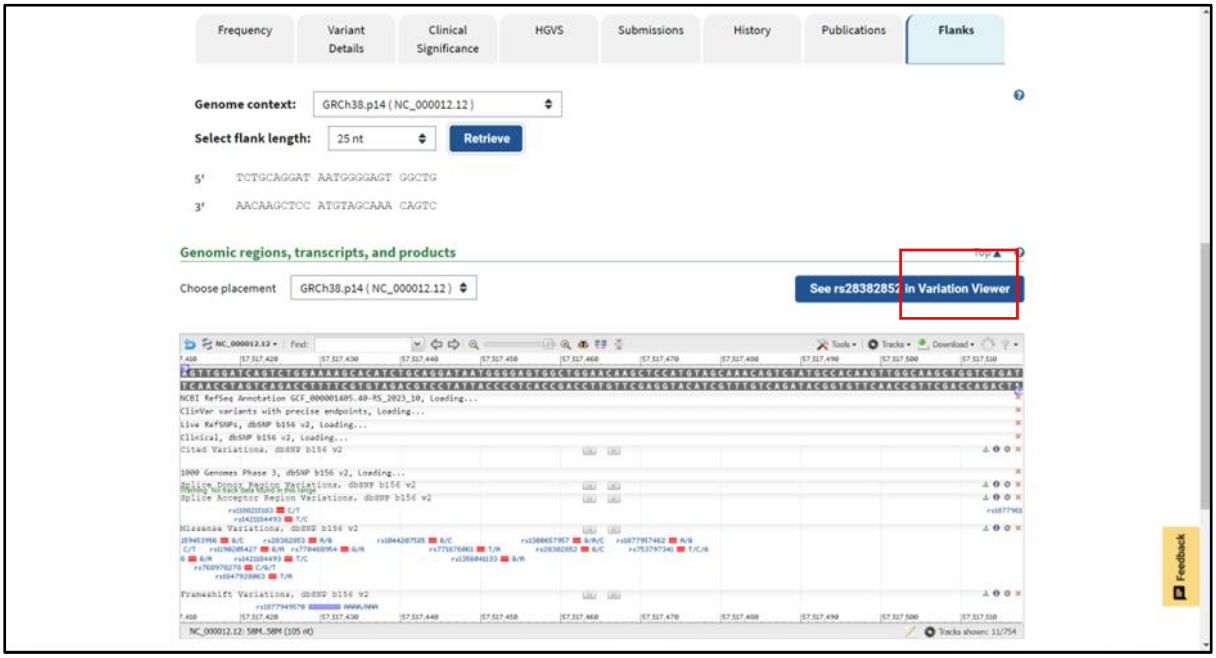

Fig 13: View of the 'Flanks' section

RESULTS:

We gained valuable insights into the genetic variations of interest for the query 'DDIT3' (Reference SNP Report: rs28382352)'. We examined the allele frequencies in various populations to understand the variant's prevalence across ethnicities. Pinpointing the exact genetic change was achieved by identifying the specific location and nucleotide change within the SNP(s) on the chromosome. Additionally, dbSNP annotations and external sources were used to assess the potential impact of the SNP(s) on gene function and human health, providing clues about disease or trait associations. For variants with potential clinical relevance, we

investigated their presence in the Human Disease Variant Submission (HDVS) database. Furthermore, the history section offered a timeline of the SNP(s) within dbSNP, including submission dates, updates, and annotation changes. To delve deeper into the functional significance, we identified relevant scientific publications associated with the SNP(s). Finally, analyzing the flanking DNA sequences surrounding the SNP(s) provided insights into potential regulatory elements or functional motifs affected by the variant.

CONCLUSION:

This comprehensive analysis of the Frequency section, Variant details, Clinical Significance, HDVS, History and Flanks section within dbSNP allowed for a thorough understanding of the SNP(s) for the query 'DDIT3' (Reference SNP Report: rs28382352)'. This knowledge can be a springboard for further research endeavours in disease association studies, personalized medicine, and functional genomics. However, it's important to acknowledge that dbSNP annotations may have limitations, and further exploration using external resources is recommended for a complete picture. Depending on the specific research question, future directions could involve functional assays or computational tools to analyze the SNP(s) in greater detail.

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DATE: 18/03/2024

WEBLEM 7
INTRODUCTION TO WHOLE GENOME SEQUENCING

Whole genome sequencing (WGS) is a comprehensive method that involves analyzing entire genomes, providing detailed genetic information crucial for identifying inherited disorders, characterizing cancer mutations, and tracking disease outbreaks. This technique is not limited to human genomes but can be applied to various species, including livestock, plants, and microbes. Unlike targeted approaches, WGS offers a comprehensive view of the entire genome, making it ideal for various applications such as identifying causative variants and assembling novel genomes. WGS can detect different types of genetic variations like single nucleotide variants, insertions/deletions, copy number changes, and large structural variants. Recent technological advancements have made whole-genome sequencing more efficient than ever before, enabling detailed insights into genetic codes.

WGS has significantly improved disease detection and outbreak investigations by providing precise data for identifying outbreaks early and characterizing bacteria to enhance surveillance efforts. It has become a standard method for detecting foodborne outbreaks associated with various bacteria like Campylobacter, E. coli, Salmonella, Vibrio, and Listeria since 2019. By comparing sequences from multiple bacteria and identifying differences, scientists can determine the relatedness of bacteria and detect outbreaks more effectively. The implementation of WGS in public health laboratories has enhanced surveillance for foodborne diseases and antimicrobial resistance, leading to quicker diagnoses and improved outbreak prevention.

Types of tools used for Whole Genome Sequencing:

A. MUMmer:

The MUMmer tool is a versatile software package designed for the rapid alignment of large DNA and amino acid sequences, offering a range of functionalities for genome analysis. It consists of various components that work together to produce desired outputs, with the main program being 'mummer,' which can quickly find exact matches of a specified length between sequences. Additionally, MUMmer includes scripts like 'run-mummer1,' 'run-mummer3,' 'nucmer,' and 'promer' that cluster matches and align non-exact regions using a modified Smith-Waterman algorithm, enhancing the analysis of genetic data. The tool is open-source, allowing free access to its source code for both non-profit and for-profit users, making it a valuable resource for genetic analysis and research.

MUMmer is particularly useful for aligning entire genomes, whether complete or in draft form, and can efficiently handle large datasets, making it suitable for various applications such as identifying genetic variations, comparing genomes, and detecting similarities between sequences. The tool's ability to align incomplete genomes, handle numerous contigs from sequencing projects, and generate alignments based on translations of input sequences makes it a powerful resource for genetic analysis and comparison. Overall, MUMmer is a valuable

tool in genomics, offering rapid and accurate alignment capabilities for a wide range of genetic analyses.

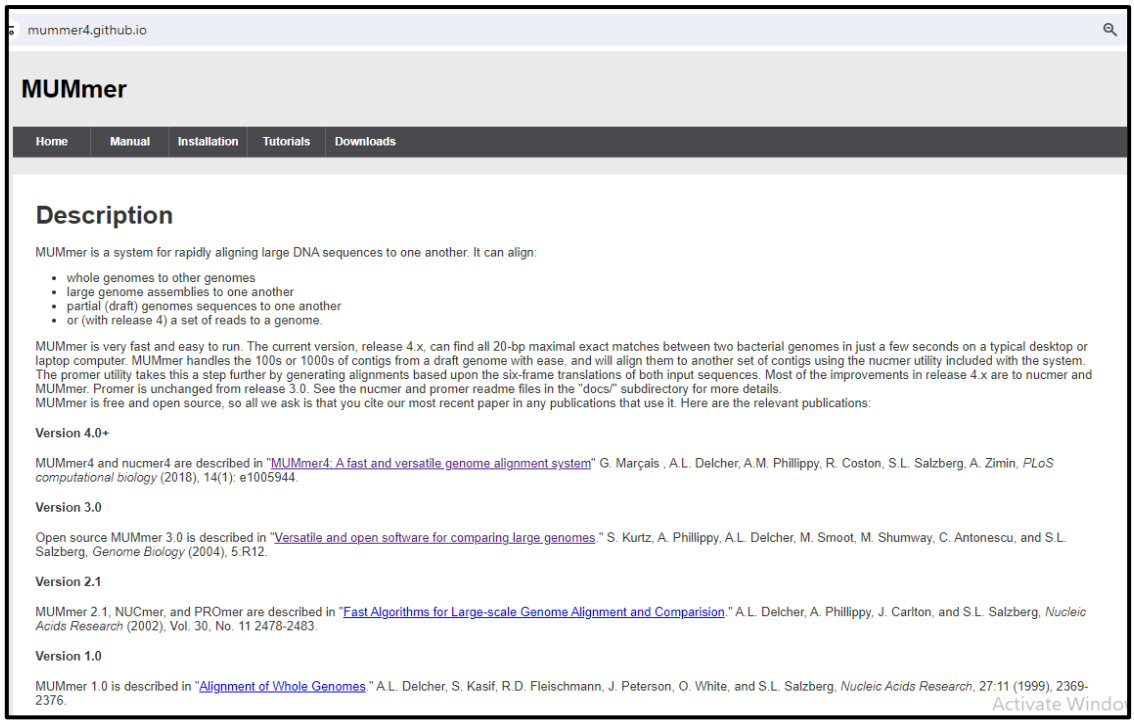

Fig 1: Homepage of MUMmer tool (Command Line tool, download from here)

B. PipMaker:

PipMaker is a web-based tool designed for aligning and comparing two long genomic DNA sequences to identify conserved segments between them. This tool, available at the World-Wide Web site bio.cse.psu.edu, offers an efficient method for aligning genomic sequences and produces informative output in the form of percent identity plots (pip). The percent identity plot generated by PipMaker displays aligning segments between two sequences, showing both the position relative to the first sequence and the degree of similarity, aiding in the interpretation of alignments. Optional annotations on the plot provide additional information to enhance alignment interpretation, making PipMaker a valuable resource for researchers analyzing genomic sequences. PipMaker utilizes default parameters from the blastz alignment program, which are optimized for human-mouse alignments, providing users with a reliable and accurate method for comparing large genomic sequences. This tool is particularly useful for identifying conserved regions between genomes, facilitating comparative genomics studies and aiding in the understanding of genetic similarities and differences across species. Overall, PipMaker serves as a user-friendly platform for researchers to perform detailed genomic sequence alignments efficiently and interpret the results effectively.

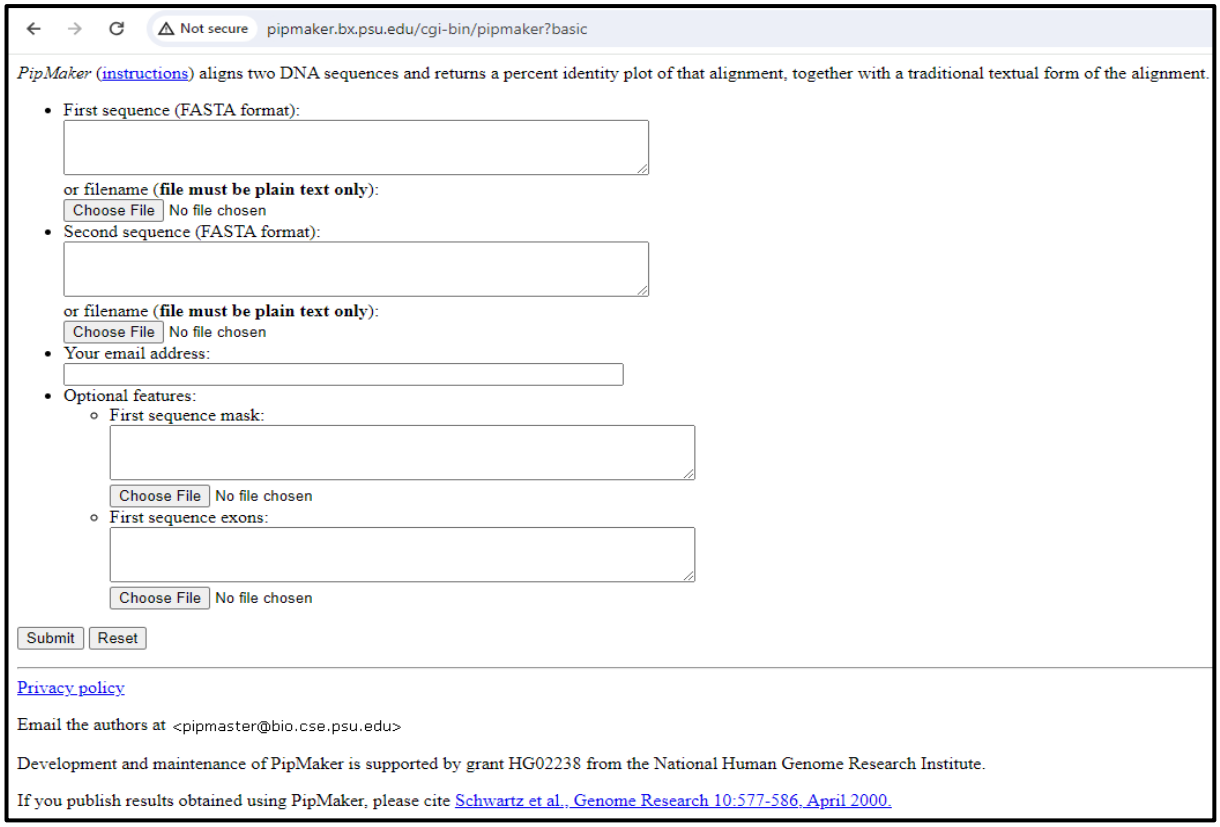

Fig 2: Homepage of Pipmaker tool

C. VISTA:

The VISTA tool is a comprehensive suite of programs and databases designed for comparative analysis of genomic sequences, offering rich capabilities for researchers in the field of genomics. It provides users with the ability to browse pre-computed whole-genome alignments of large vertebrate genomes and other organisms, submit their sequences for various types of comparative analysis, and obtain detailed comparative analysis results for specific genes of interest. VISTA offers a range of servers like mVISTA, rVISTA, GenomeVISTA, and VISTA Browser, each serving different purposes such as aligning sequences, identifying regulatory transcription factor binding sites, comparing whole genome assemblies, and examining pre-computed alignments among various species. The tool allows researchers to analyze multiple DNA sequence alignments from different species while considering their phylogenetic relationships, align sequences up to 10Mb long, and compare sequences with whole genome assemblies, making it a versatile resource for genetic analysis and comparison. With more than 28 searchable genomes, including vertebrates, non-vertebrates, plants, fungi, bacteria, and others, VISTA continues to expand its database to provide a comprehensive platform for genomic research and analysis. Developed and hosted at the Genomics Division of Lawrence Berkeley National Laboratory, VISTA is supported by various grants and collaborations, making it a valuable tool for researchers in the genomics field.

Fig 3: Homepage of VISTA tool

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DATE:18/03/2024

WEBLEM 7(A)
PipMaker Tool
(URL: http://pipmaker.bx.psu.edu/cgi-bin/pipmaker?basic)

AIM:

To compare genomic sequences, identify conserved regions, and visualize sequence alignments for query 'ADD1' (Accession ID: NM_001354759.2 and NM_001354756.2) using PipMaker Tool.

INTRODUCTION:

PipMaker is a web server used for aligning two genomic DNA sequences, allowing for the comparison of DNA sequences and identification of conserved segments between them. It provides functionalities such as generating alignments and percent identity plots (pips) to visualize similarities between sequences.

PipMaker is a World-Wide Web site for comparing two long DNA sequences to identify conserved segments and for producing informative, high-resolution displays of the resulting alignments. One display is a percent identity plot (pip), which shows both the position in one sequence and the degree of similarity for each aligning segment between the two sequences in a compact and easily understandable form. Positions along the horizontal axis can be labeled with features such as exons of genes and repetitive elements, and colors can be used to clarify and enhance the display. The web site also provides a plot of the locations of those segments in both species (similar to a dot plot). PipMaker is appropriate for comparing genomic sequences from any two related species, although the types of information that can be inferred (e.g., protein-coding regions and cis-regulatory elements) depend on the level of conservation and the time and divergence rate since the separation of the species. Gene regulatory elements are often detectable as similar, noncoding sequences in species that diverged as much as 100–300 million years ago, PipMaker supports analysis of unfinished or "working draft" sequences by permitting one of the two sequences to be in unoriented and unordered contigs.

ADD1 gene:

The ADD1 gene, encoding the α-adducin protein, plays significant roles in both animal and human physiology. In mice, it influences oocyte chromosome meiosis, indicating its involvement in embryonic development. In cattle, polymorphisms within the gene have been linked to growth traits, suggesting its potential as a marker for selective breeding in beef cattle populations.

In humans, genetic variations in ADD1 have been associated with hypertension and economic traits. Studies in North Indian populations and cattle-derived populations in Jeju-do revealed correlations between ADD1 polymorphisms and hypertension, along with economic traits such as meat color and carcass weight. Additionally, in essential hypertension, the G460T polymorphism in the ADD1 gene has been implicated in the new onset of diabetes, especially under the influence of diuretic and other antihypertensive therapies. A meta-analysis further

confirmed the association of the T allele in the ADD1 gene with essential hypertension susceptibility in Asian populations.

These findings underscore the diverse roles of the ADD1 gene in regulating physiological processes across species and its potential implications for health and breeding programs in both animals and humans.

METHODOLOGY:

1. Open Homepage of PipMaker Tool.
2. Take 2 FASTA Sequence from NCBI.
3. Paste the sequence in PipMaker Tool.

OBSERVATIONS:

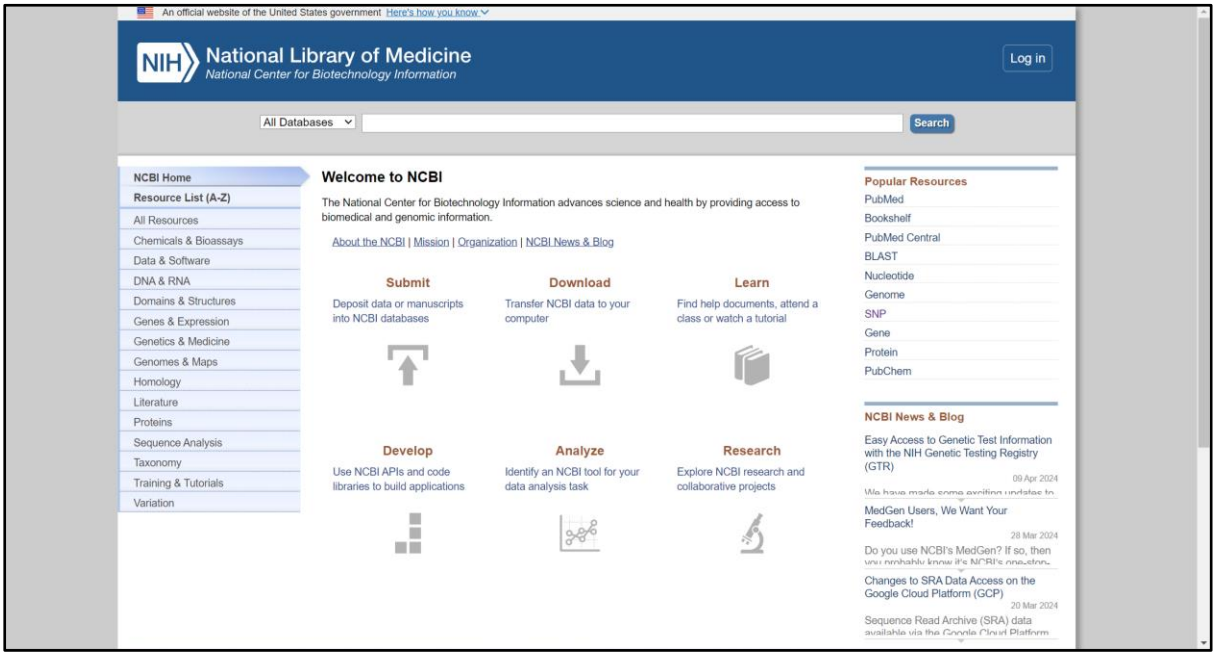

Fig 1: Homepage of NCBI database

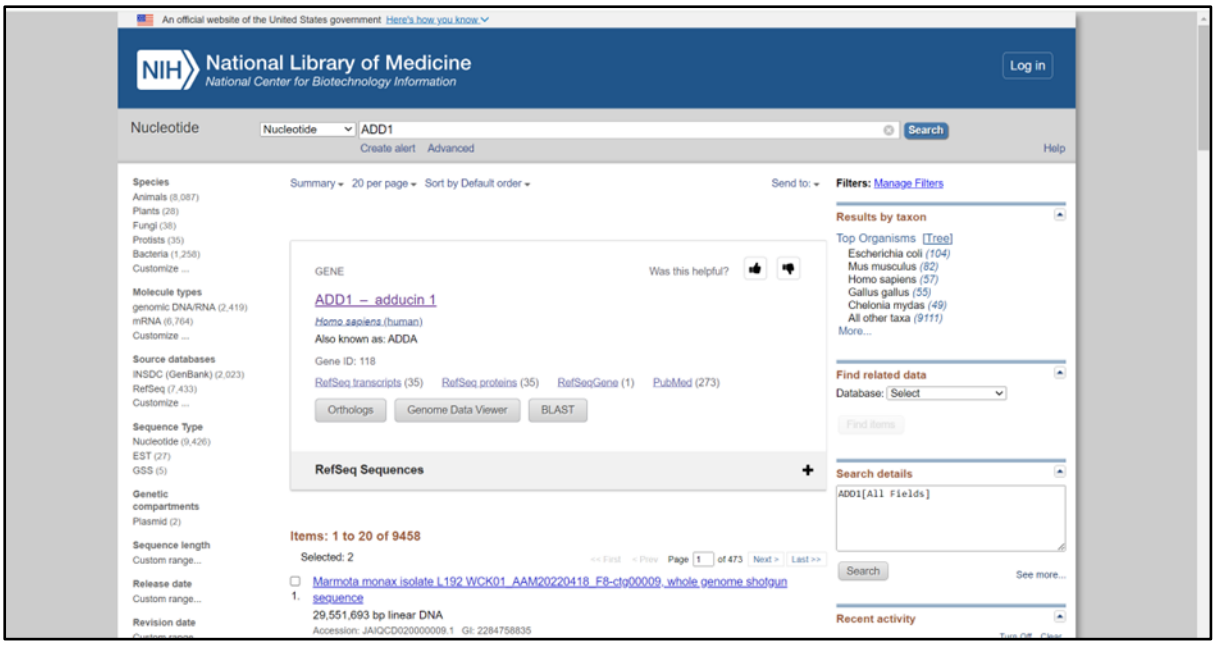

Fig 2: Results obtained for the query 'ADD1'

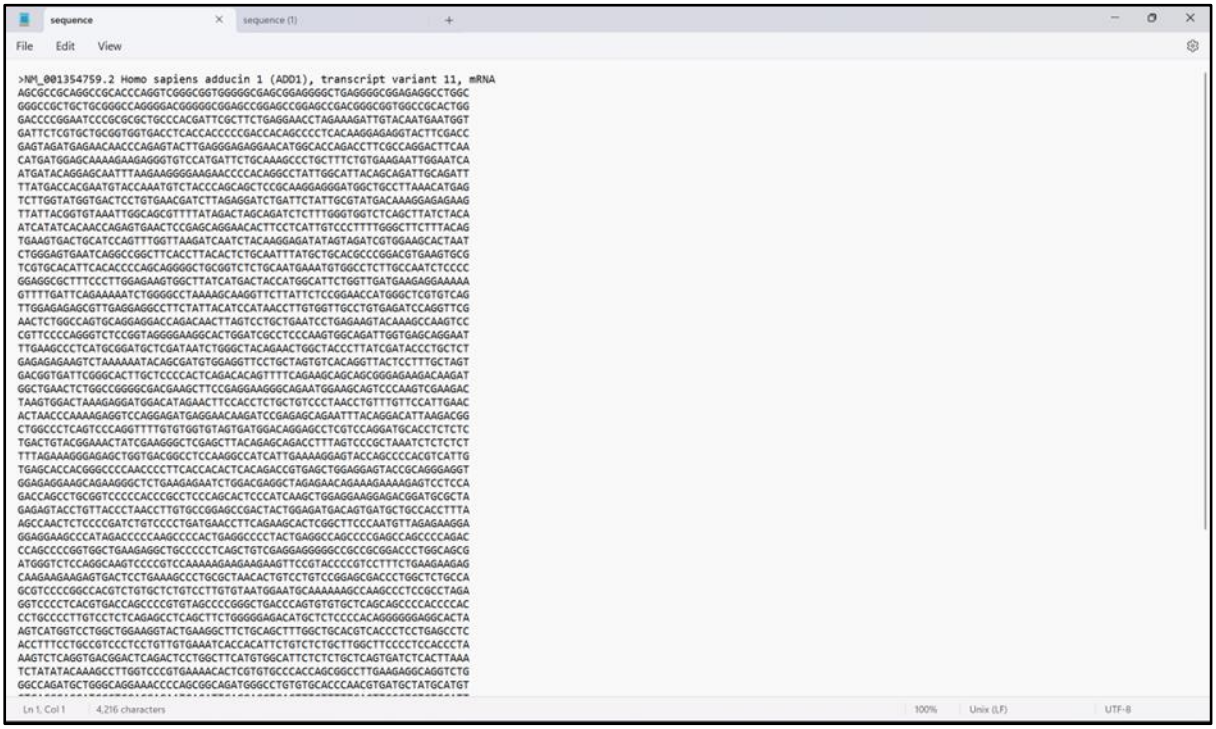

Fig 3: FASTA Sequence for the query 'ADD1' with Accession ID: NM_001354759.2

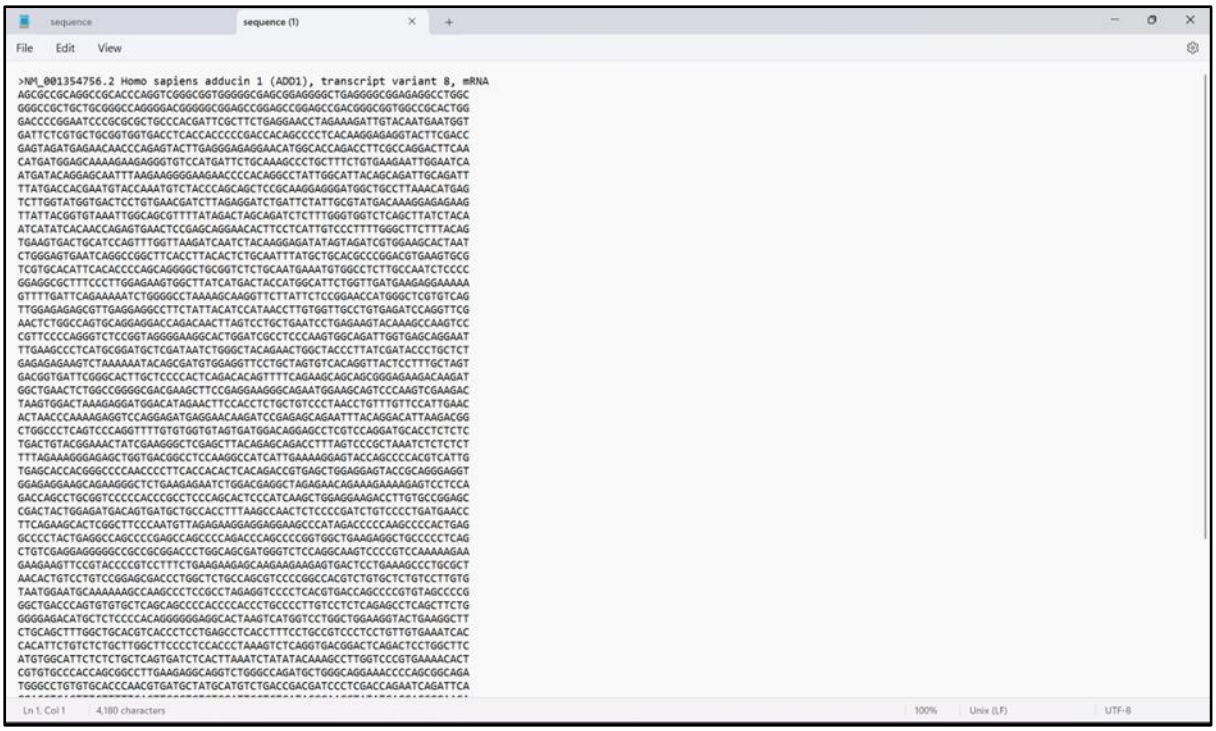

Fig 4: FASTA Sequence for the query 'ADD1' with Accession ID: NM_001354756.2

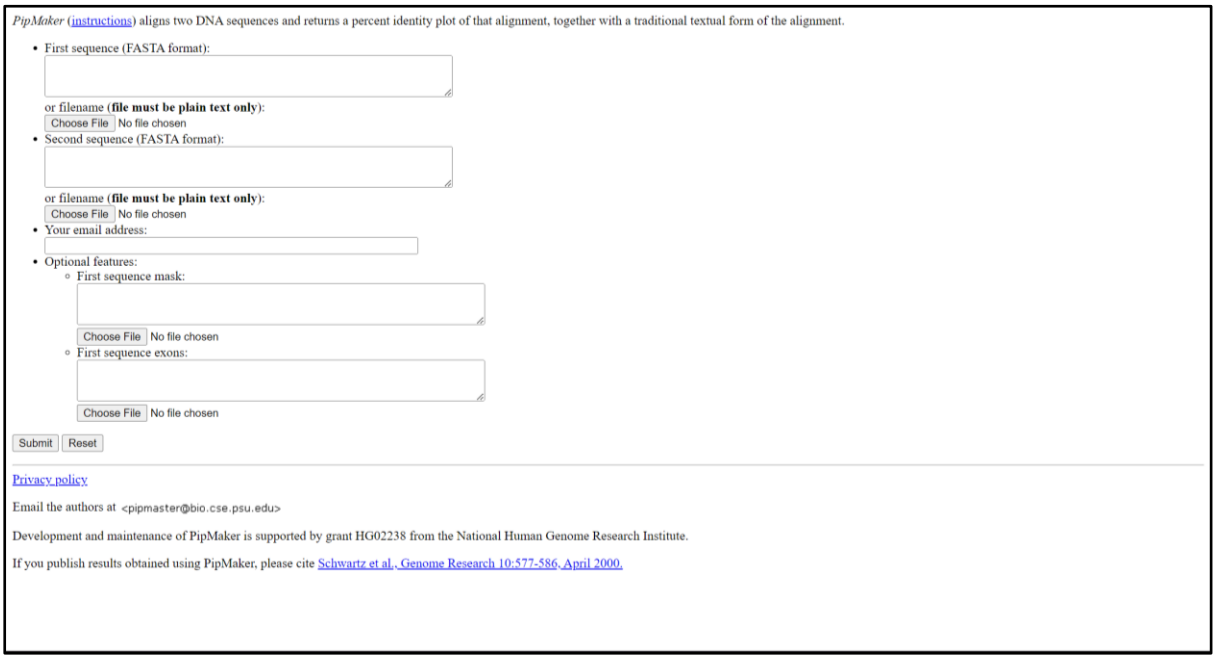

Fig 5: Homepage of Pipmaker tool

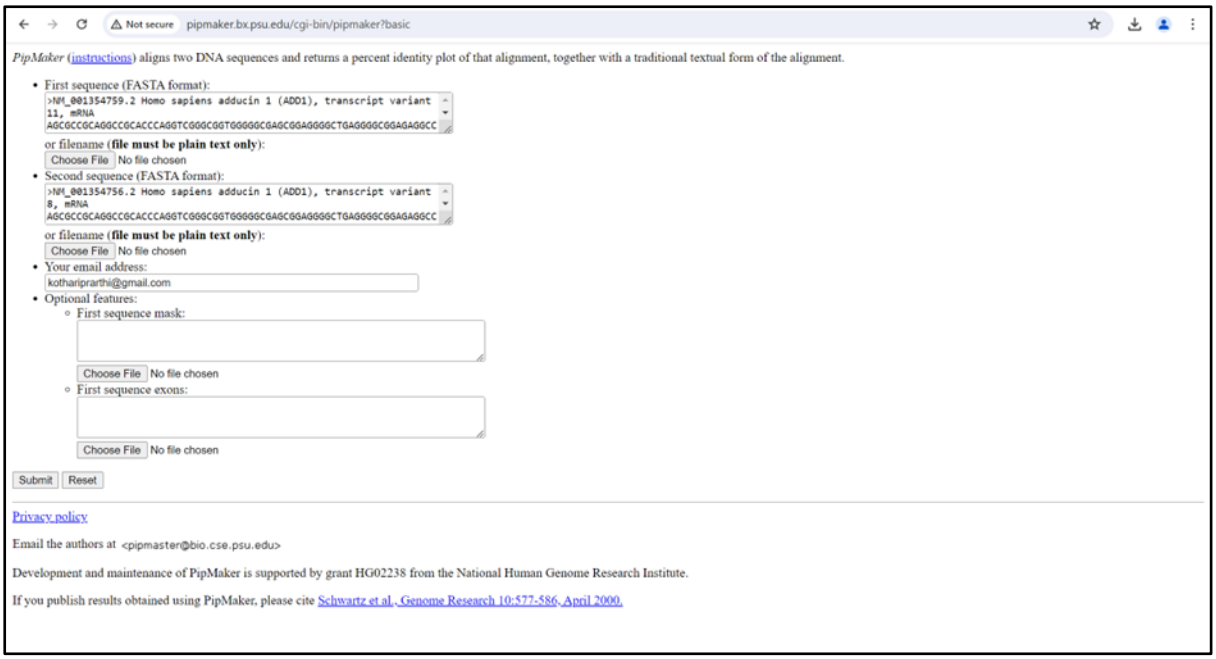

Fig 6: Sequences pasted in PipMaker tool

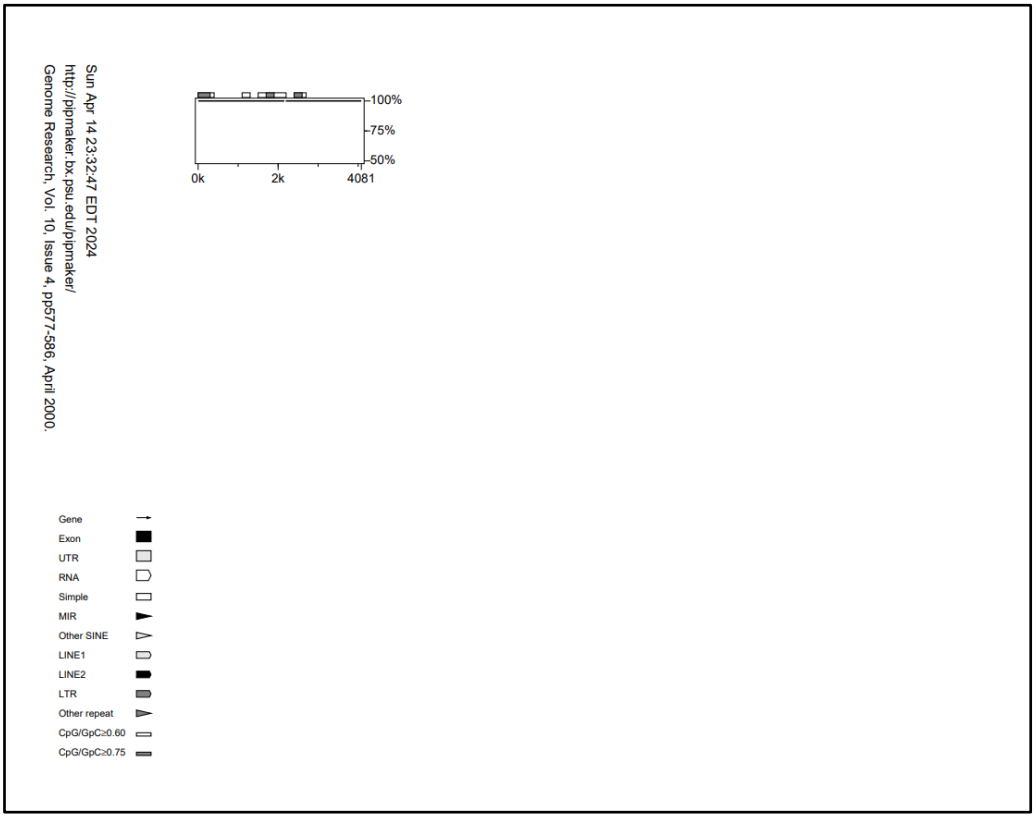

Fig 7: Results obtained for 'Sequence alignment'

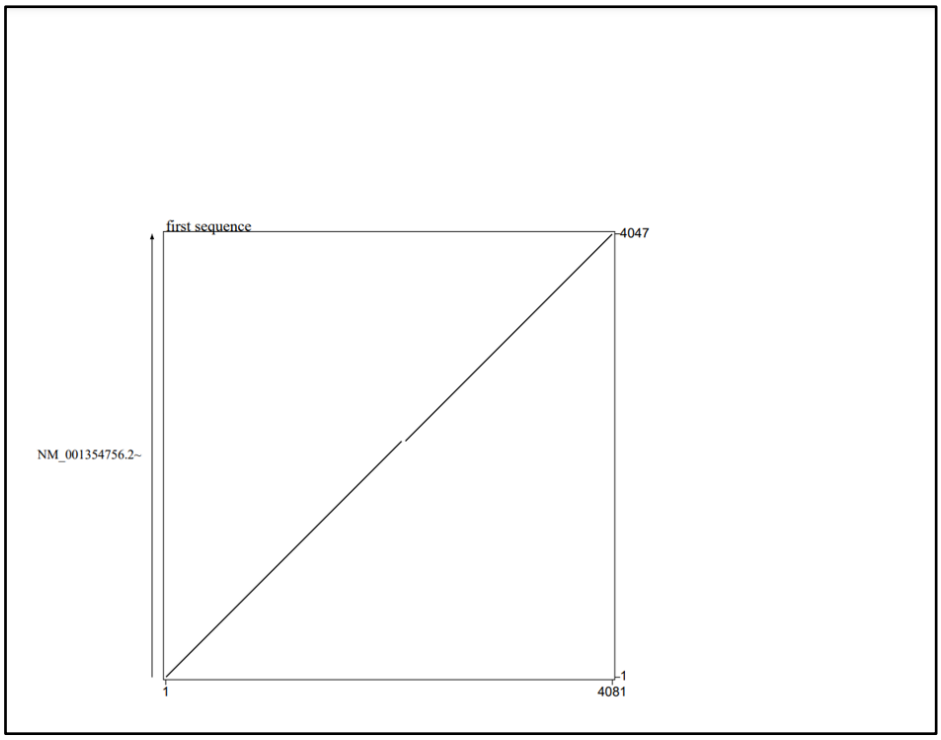

Fig 8: Results obtained for 'Dot plot'

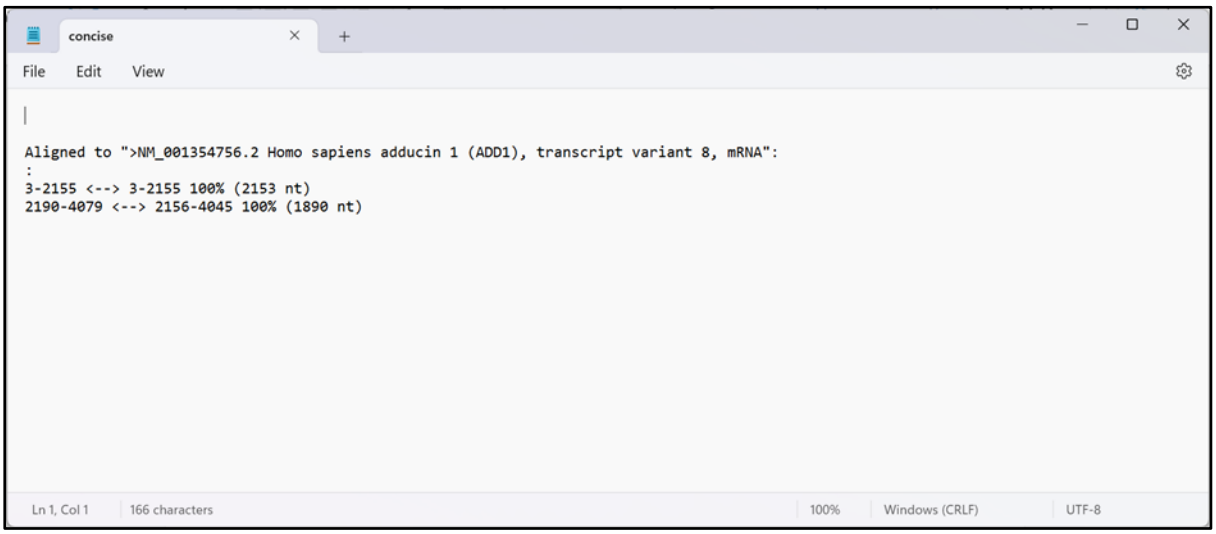

Fig 9: Results obtained for 'concise'

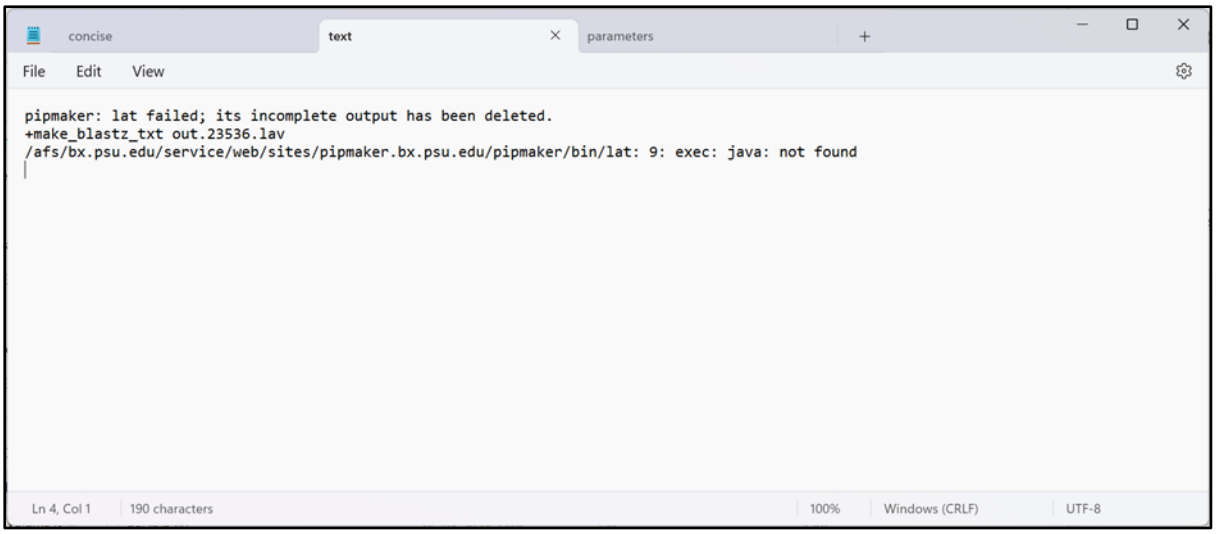

Fig 10: Results obtained for 'text'

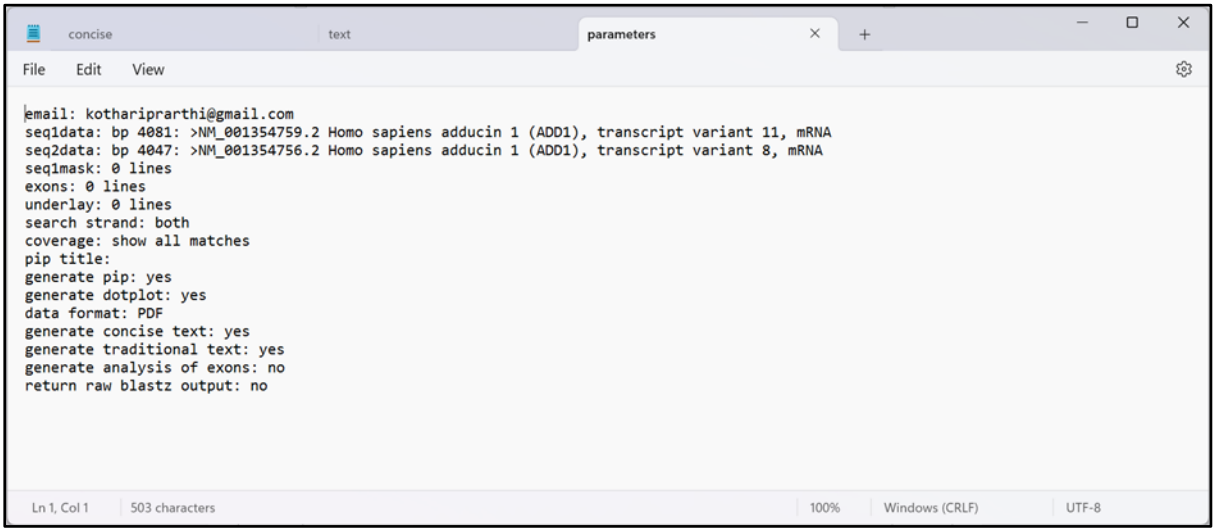

Fig 11: Results obtained for 'parameters'

RESULTS:

By exploring PipMaker tool for the query 'ADD1' (Accession ID: NM_001354759.2 and NM_001354756.2), files were generated as result of sequence of two entries for the query 'ADD1'. 100% ,75% 50% sequence alignment were observed. The dot plot showed a long diagonal line indicating similarity with a gap in between. The parameter file showed the information about the program. The query 'ADD1' gene sequence showed similarity and number of nucleotides aligned highest percent similarity was found to be 100% with nucleotides aligned.

CONCLUSION:

Genome sequence was compared and conserved regions were identified and sequence alignments were visualized for the query 'ADD1' (Accession ID: NM_001354759.2 and NM_001354756.2) by exploring PipMaker tool.

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1. PipMaker-A Web Server for Aligning Two Genomic DNA Sequences. (n.d.). PMC. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC310868/
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3. PipMaker and MultiPipMaker (n.d.). http://pipmaker.bx.psu.edu/pipmaker/
4. Han, S. H., Oh, H. S., Lee, J. B., Jwa, E. S., Kang, Y. J., Kim, S. G., Yang, S. N., Kim, Y. K., Cho, I. C., Cho, W. M., Ko, M. S., & Baek, K. S. (2015, January 30). Effects of Genetic Polymorphisms of ADD1 Gene on Economic Traits in Hanwoo and Jeju Black Cattle-derived Commercial Populations in Jeju-do. *Journal of Life Science*, *25*(1), 21–28. https://doi.org/10.5352/jls.2015.25.1.21
5. Huang, Y. Z., Qian, L. N., Wang, J., Zhang, C. L., Fang, X. T., Lei, C. Z., Lan, X. Y., Ma, Y., Bai, Y. Y., Lin, F. P., & Chen, H. (2018, March 12). Genetic Variants inADD1Gene and their Associations with Growth Traits in Cattle. *Animal Biotechnology*, *30*(1), 7–12. https://doi.org/10.1080/10495398.2017.1398754
6. Gupta, S., Jhawat, V., Agarwal, B. K., Roy, P., & Saini, V. (2019, May 29). Alpha Adducin (ADD1) Gene Polymorphism and New Onset of Diabetes Under the Influence of Selective Antihypertensive Therapy in Essential Hypertension. *Current Hypertension Reviews*, *15*(2), 123–134. https://doi.org/10.2174/1573402114666180731111453

DATE:18/03/2024

WEBLEM 7(B)
VISTA Tool
(URL: https://genome.lbl.gov/vista/index.shtml)

AIM:

To perform comparative genomics analysis to identify conserved regions and its functional elements for query 'ADD1' (Accession ID: NM_001354759.2) using VISTA tool.

INTRODUCTION:

The VISTA tool in bioinformatics is a powerful set of computational tools designed to facilitate comparative genomics studies. Developed to assist biologists in analyzing DNA sequences from different species, VISTA offers a range of tools for aligning sequences, visualizing conservation patterns, and identifying functional elements in genomes. The VISTA family of tools includes various servers like mVISTA, rVISTA, GenomeVISTA, and VISTA Browser, each serving different purposes such as aligning sequences, discovering regulatory transcription factor binding sites, comparing sequences with whole genome assemblies, and analyzing multiple DNA sequence alignments while considering phylogenic relationships. VISTA has been widely utilized by the biological community for tasks like comparing gene families, identifying non-coding functional elements, and studying conservation patterns on a whole-genome scale. This tool integrates global alignment strategies and curve-based visualization techniques, making it a valuable resource for researchers in the field of bioinformatics.

There are multiple VISTA servers, each allowing different types of searches.

1. mVISTA can be used to align and compare your sequences to those of multiple other species
2. rVISTA (regulatory VISTA) combines transcription factor binding sites database search with a comparative sequence analysis, the discovery of possible regulatory transcription factor binding sites in regions of their genes of interest. It can be used directly or through mVISTA, Genome VISTA, or VISTA Browser. A database of tissue-specific human enhancers is available through VISTA Enhancer Browser.
3. GenomeVISTA allows the comparison of sequences with whole genome assemblies. It will automatically find the ortholog, obtain the alignment and VISTA plot. It allows the viewing of an alignment together with pre-computed alignments of other species in the same interval.
4. Phylo-VISTA allows the analysis of multiple DNA sequence alignments of sequences from different species while considering their phylogenic relationships.
5. wgVISTA allows the alignment of sequences up to 10Mb long (finished or draft) including microbial whole-genome assemblies.

Researchers can use the VISTA Browser:

1. To examine pre-computed alignments among a variety of species
2. To submit sequences of their own (not limited by the species collection already in the database)

ADD1 gene:

The ADD1 gene, encoding the α-adducin protein, plays significant roles in both animal and human physiology. In mice, it influences oocyte chromosome meiosis, indicating its involvement in embryonic development. In cattle, polymorphisms within the gene have been linked to growth traits, suggesting its potential as a marker for selective breeding in beef cattle populations.

In humans, genetic variations in ADD1 have been associated with hypertension and economic traits. Studies in North Indian populations and cattle-derived populations in Jeju-do revealed correlations between ADD1 polymorphisms and hypertension, along with economic traits such as meat color and carcass weight. Additionally, in essential hypertension, the G460T polymorphism in the ADD1 gene has been implicated in the new onset of diabetes, especially under the influence of diuretic and other antihypertensive therapies. A meta-analysis further confirmed the association of the T allele in the ADD1 gene with essential hypertension susceptibility in Asian populations.

These findings underscore the diverse roles of the ADD1 gene in regulating physiological processes across species and its potential implications for health and breeding programs in both animals and humans.

METHODOLOGY:

1. Go to GenBank database and search for the query ‘ADD1’ gene and copy the fast sequence.
2. Open Homepage of Vista tool.
3. Paste the FASTA sequence of query ‘ADD1’ on gVISTA tool in the search bar.
4. Click on submit and wait for the results.
5. Interpret the results.

OBSERVATIONS:

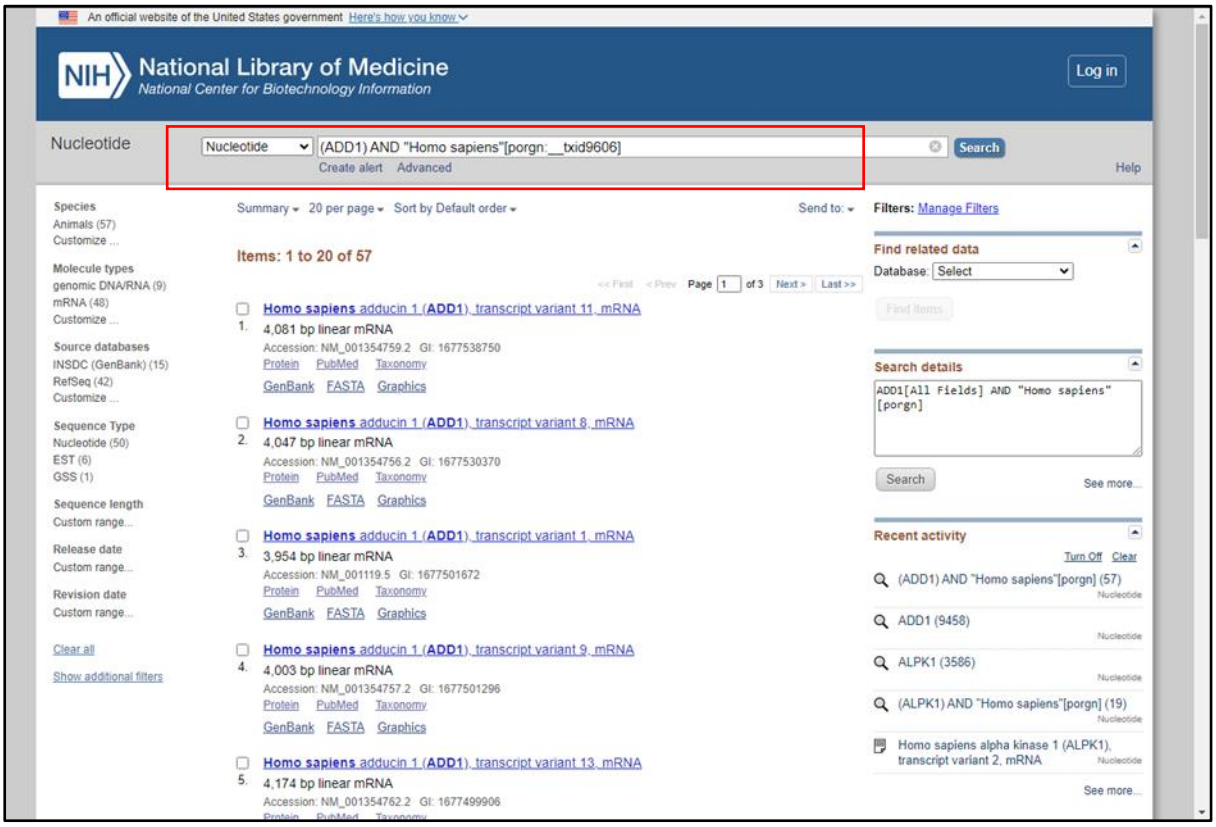

Fig 1: Query searched for the ADD1 gene in the GenBank database

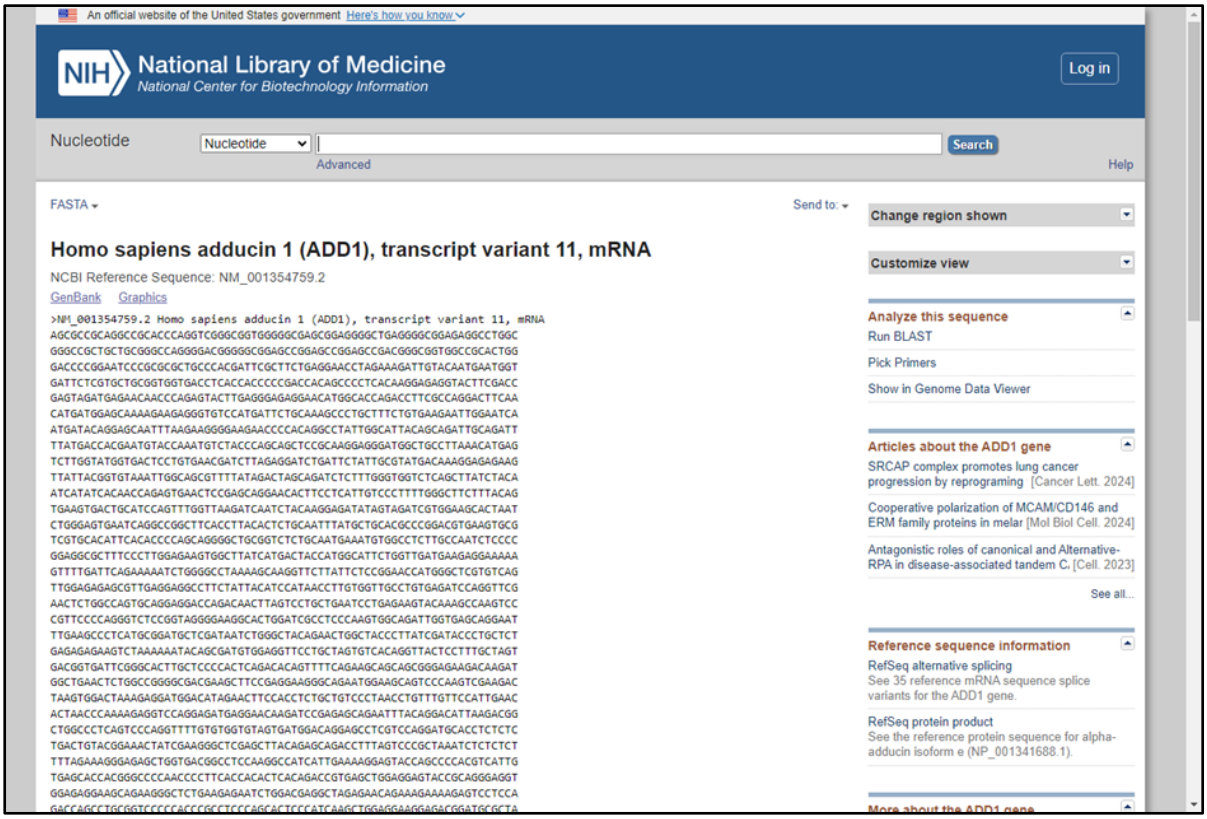

Fig 2: Copy the FASTA sequence of selected entry with Accession ID: NM_001354759.2

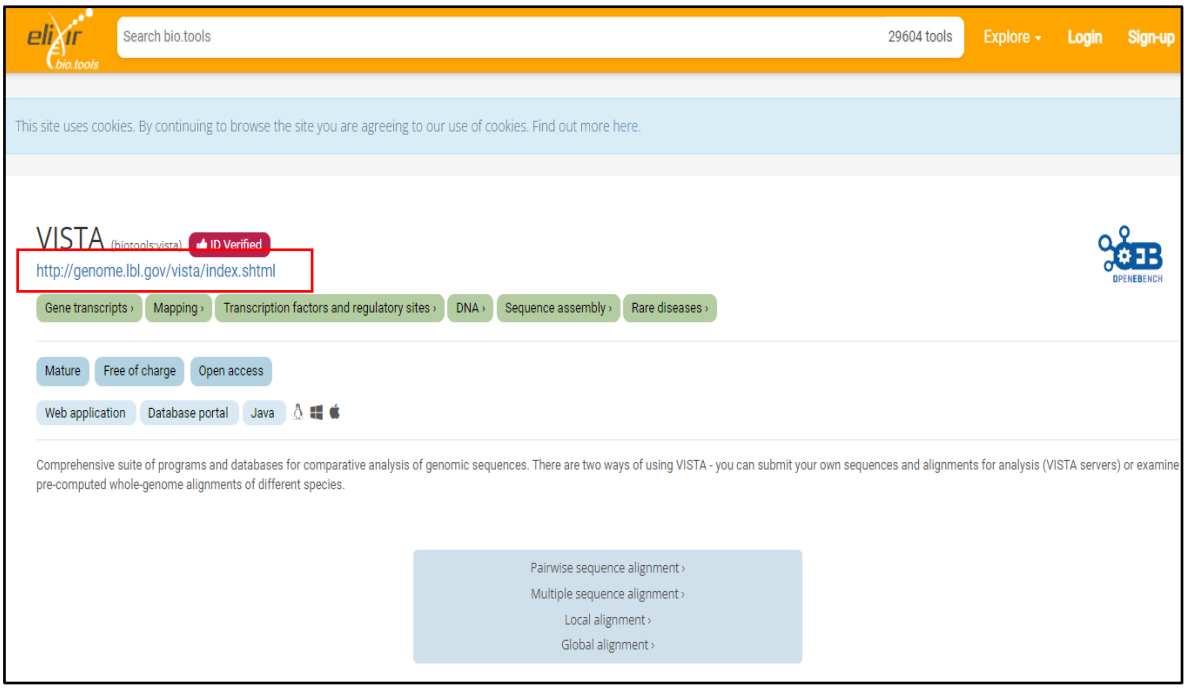

Fig 3: Link for VISTA tool

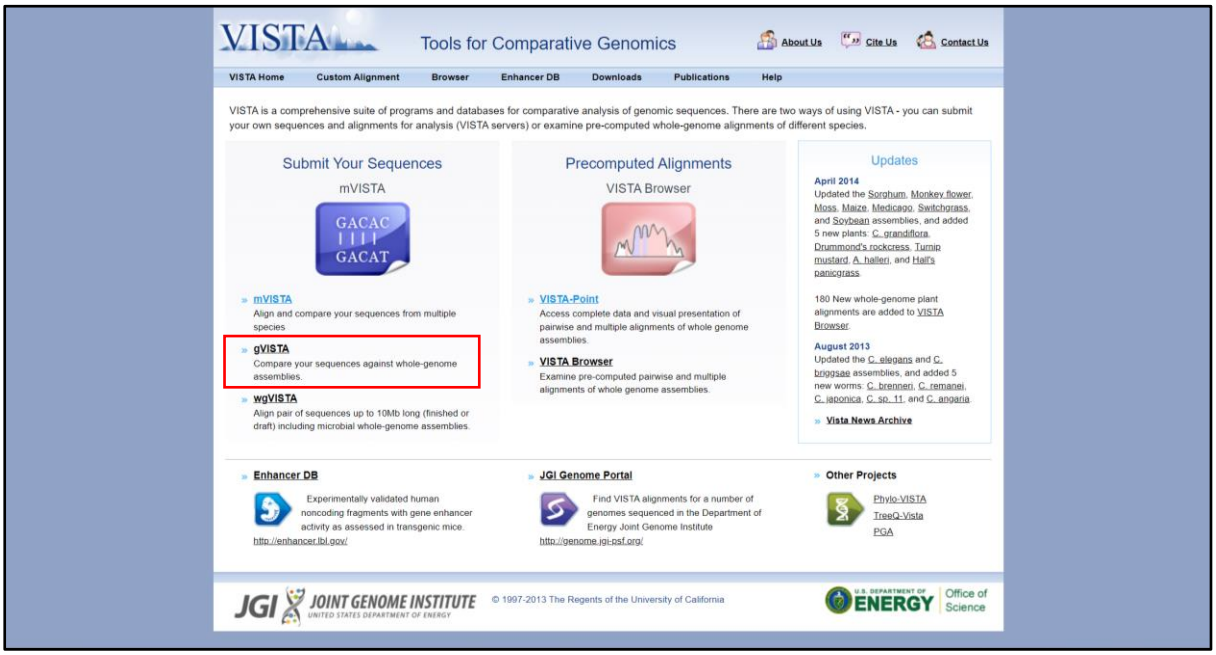

Fig 4: Homepage of VISTA tool

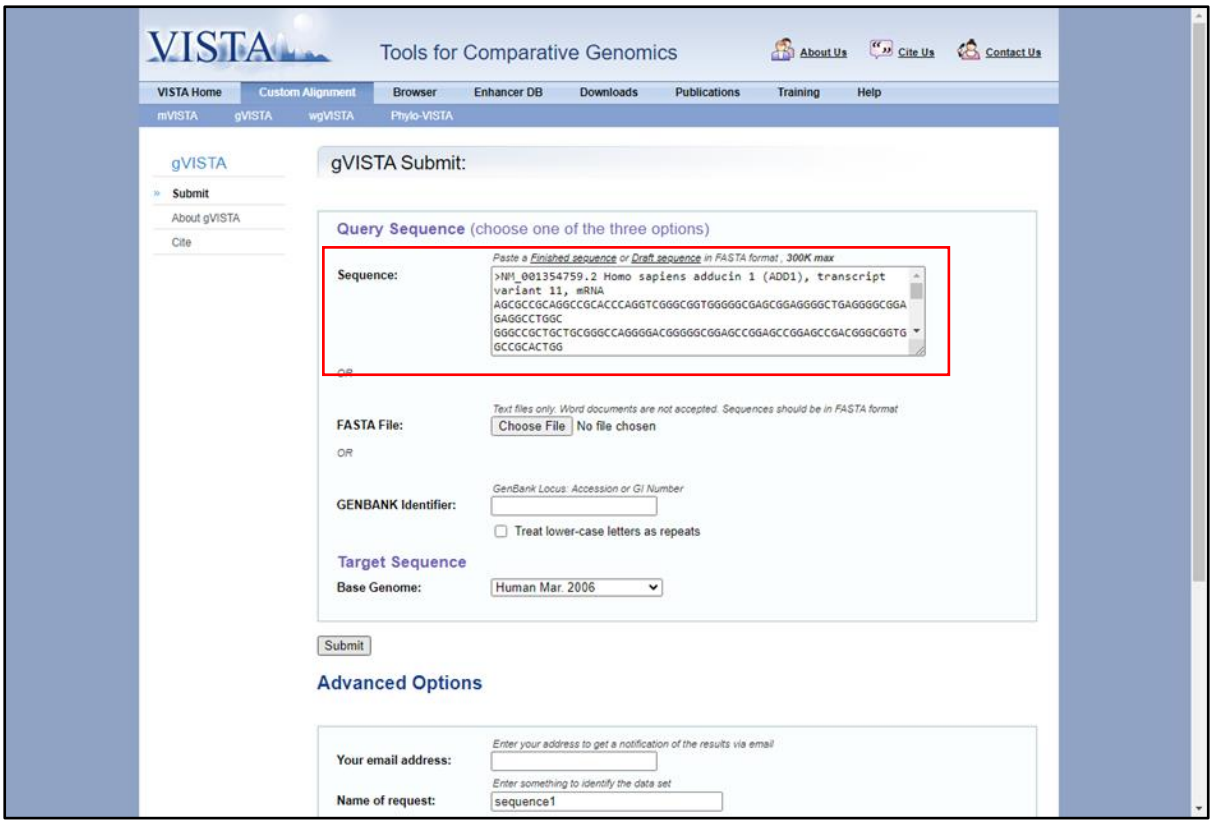

Fig 5: Paste the FASTA sequence on gVISTA tool

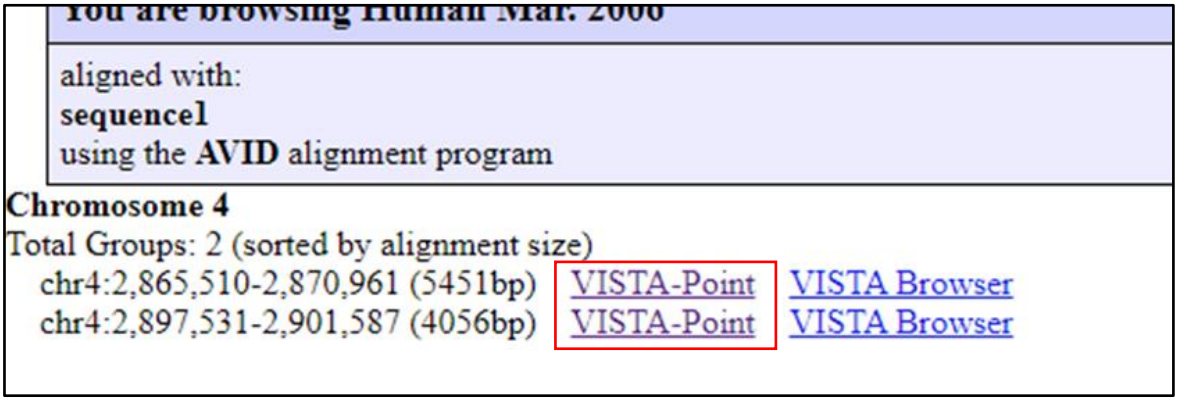

Fig 6: Result page for query ALPK1

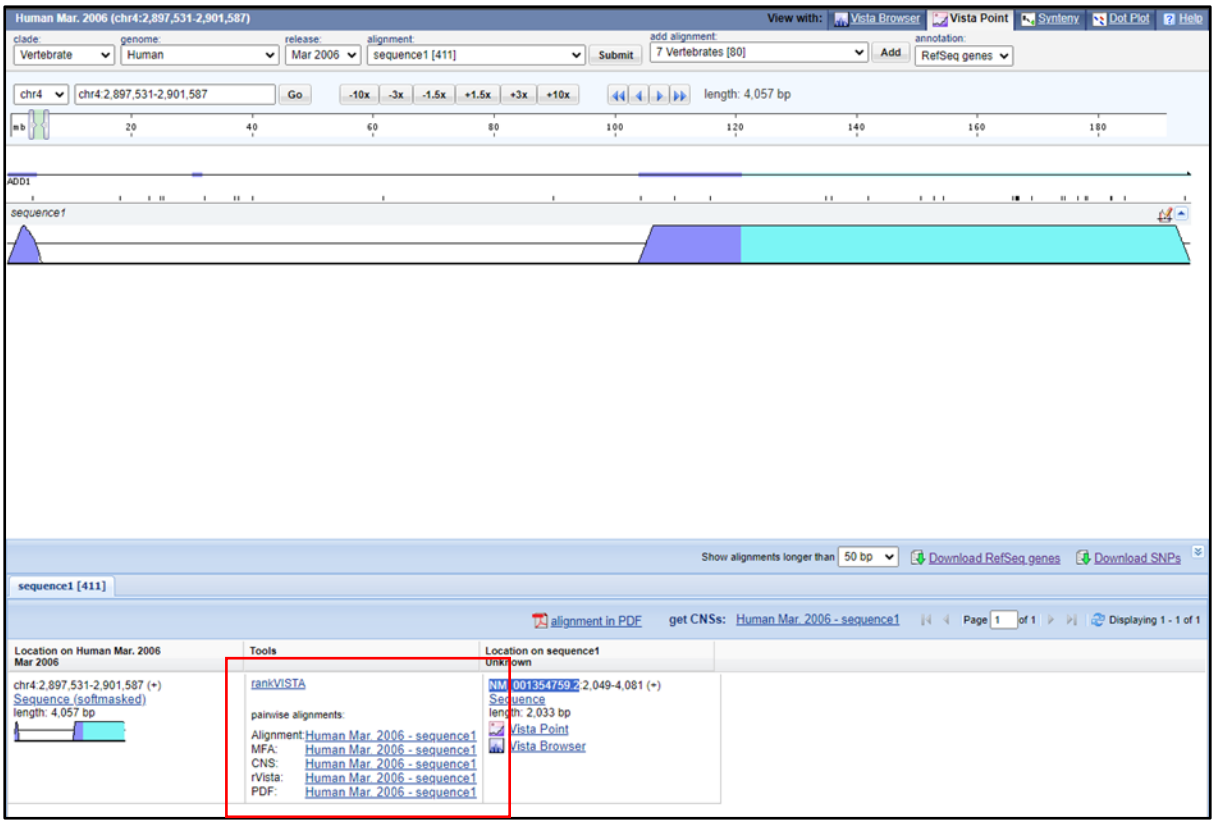

Fig 7: VISTA Point

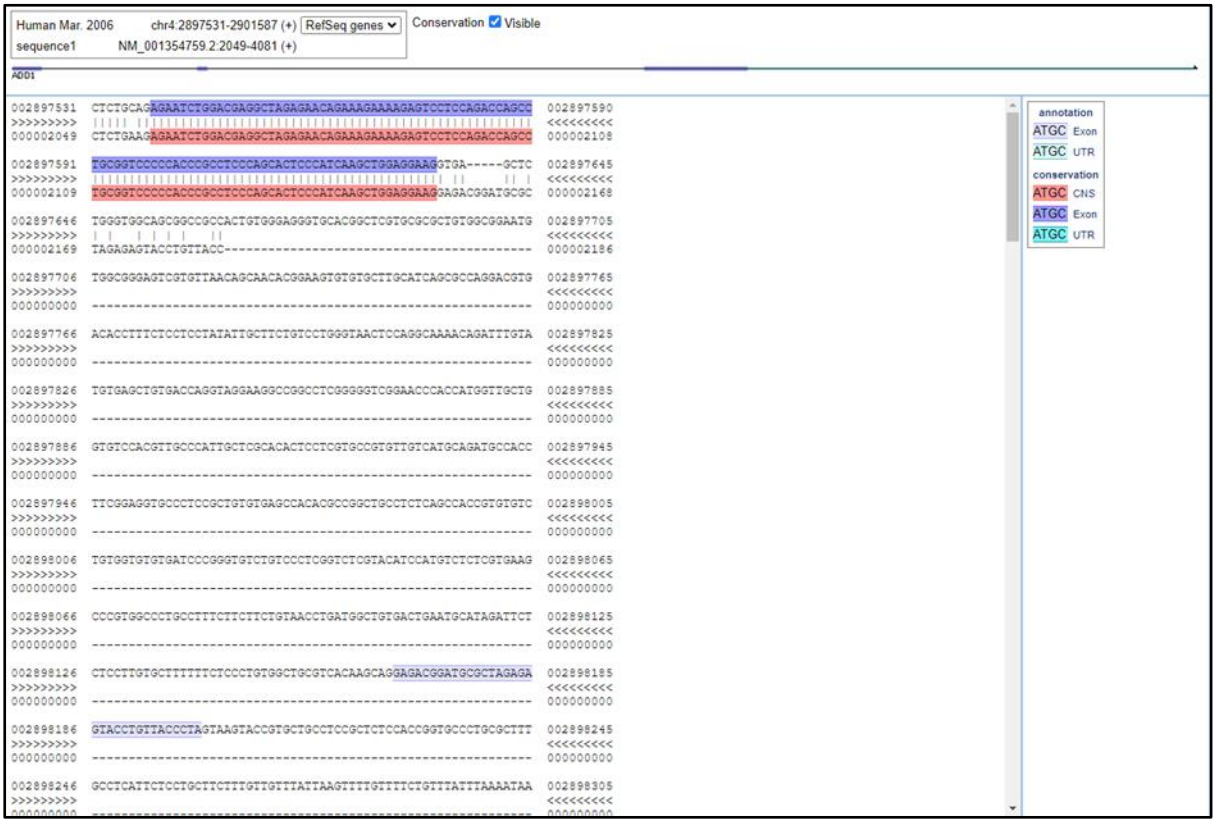

Fig 8: Sequence Alignment for query ADD1

Fig 9: MFA (Multiple Feature Alignment)

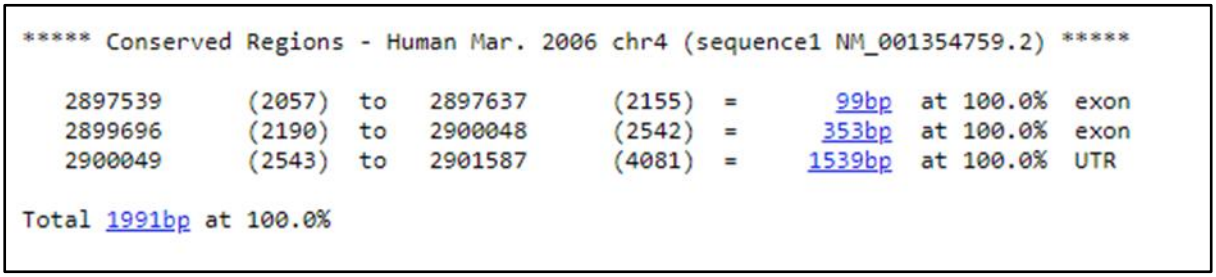

Fig 10: CNS (Conserved Regions of Sequence)

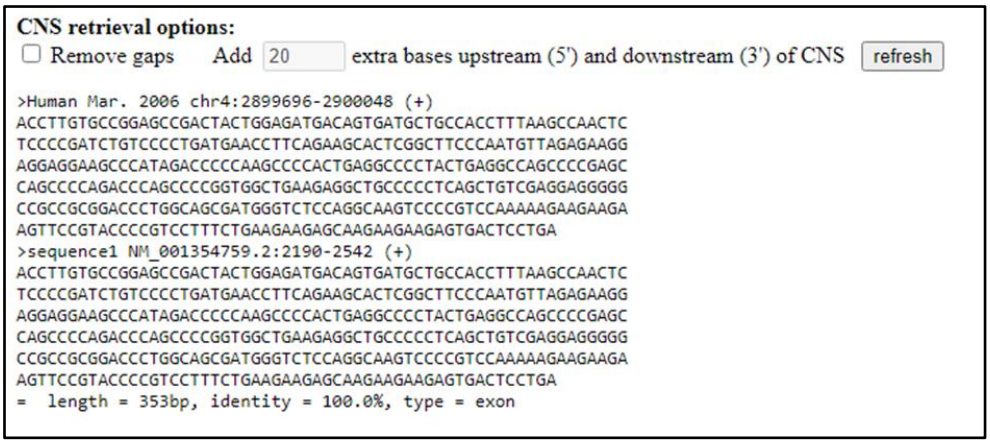

Fig 11: An entry of length 353bp with 100% identity of CNS

Fig 12: PDF format of result page

RESULTS:

The VISTA tool was used for the query 'ADD1' (Accession ID: NM_001354759.2) to interpret results by analyzing alignment plots, which highlighted regions of conservation or divergence among sequences. Areas of high similarity were identified by long stretches of aligned sequences or conserved regions, while areas of divergence indicated potential functional differences or evolutionary changes. Metrics or scores provided quantified the similarity or conservation levels between sequences, aiding in understanding evolutionary relationships, identifying functional elements within genomes, or studying genetic variation within populations.

CONCLUSION:

VISTA tool was used for the query 'ADD1' (Accession ID: NM_001354759.2) for the comparison of sequences against whole-genome assemblies. This analysis helps in understanding the similarities and differences between the sequences and provides valuable insights into their functional significance.

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DATE: 20/03/2024

WEBLEM 8
INTRODUCTION TO IDENTIFICATION OF REPETATIVE ELEMENTS: REPEATMASKER TOOL
(URL: http://galaxy.org/)

INTRODUCTION:

"Repeats" represent a mosaic of nucleotide patterns that have intrigued biologists for decades. Often seemingly mundane in their nature, "Repeats" also known as "Repetitive elements" hold profound significance in shaping and offering insights into genetic architecture, genomic stability, and evolutionary relationships across all forms of life.

The analysis of genetic diversity and relatedness within and between the different species and populations has been a major theme of research for many biologists. With the availability of whole-genome sequencing for an increasing number of species, focus has been shifted to the development of molecular markers based on DNA or protein polymorphism. DNA sequences originate and undergo evolutionary metamorphoses' and thus may be used as powerful genetic markers to characterize genomes of wide range of species.

The mammals have approximately 3 billion base pairs per haploid genome harbouring about 20,000-25000 genes. A minor part of the genome (5-10%) is coding sequences and the remaining part is non-coding (Heterochromatin) representing repetitive DNA. Comparison of the genome size of different eukaryotes shows that the amount of non-coding DNA is highly variable and constitutes 30% to about 99% of the total genome.

Repetitive sequences are dynamic elements exhibiting a high degree of polymorphism due to variation in the number of their repeat units caused by mutations involving several mechanisms. They reshape their host's genome by generating rearrangements, shuffling of genes and modulating pattern of expression. This dynamism of repeats leads to evolutionary divergence that can be used in species identification, phylogenetic inference and for studying process of sporadic mutations and natural selection. These repetitive sequences are mainly composed of "Interspersed" and "Tandem Repeats". However, the majority of repetitive DNA sequences have not been sequenced and/or are not identifiable by currently applied methods.

TYPES OF REPETITIVE ELEMENTS:

Tandem Repeats are small sequences (<60 base pairs) arranged one after the other in the genome in a head-to-tail organization. Tandemly repeated DNA is common in eukaryotic genomes, in some cases in short sequences 1–10 bp long and in other cases associated with genes and in much longer sequences. Tandem repeats may be further classified according to the length and copy number of the basic repeat units as well as its genomic localization. The greatest amount of tandemly repeated DNA is associated with centromeres and telomeres.

Interspersed Repeats or Dispersed Repeats consist of families of repeated sequences interspersed through the genome with unique-sequence DNA. Each family consists of a set of related sequences characteristic of the family. Often, small numbers of families have very high copy numbers and make up most of the dispersed repeated sequences in the genome. By the mechanism of their transposition, interspersed repeats are classified into two classes:

1. **RNA transposons:** RNA transposons also known as retroelements found in eukaryotic genome require reverse transcription for their activity. Based on their structural relationship, RNA transposons are divided into two general categories: _LTR elements and non-LTR elements. The non-LTR elements consist of Long Interspersed Elements (LINEs), in which the sequences in the families are about 1,000–7,000 bp long and Short Interspersed Elements (SINEs), in which the sequences in the families are 100–400 bp long.
2. **DNA Transposons:** DNA transposons do not require RNA intermediate and transpose in a direct DNA-to-DNA manner.

All eukaryotic organisms have LINEs and SINEs, with a wide variation in their relative proportions.

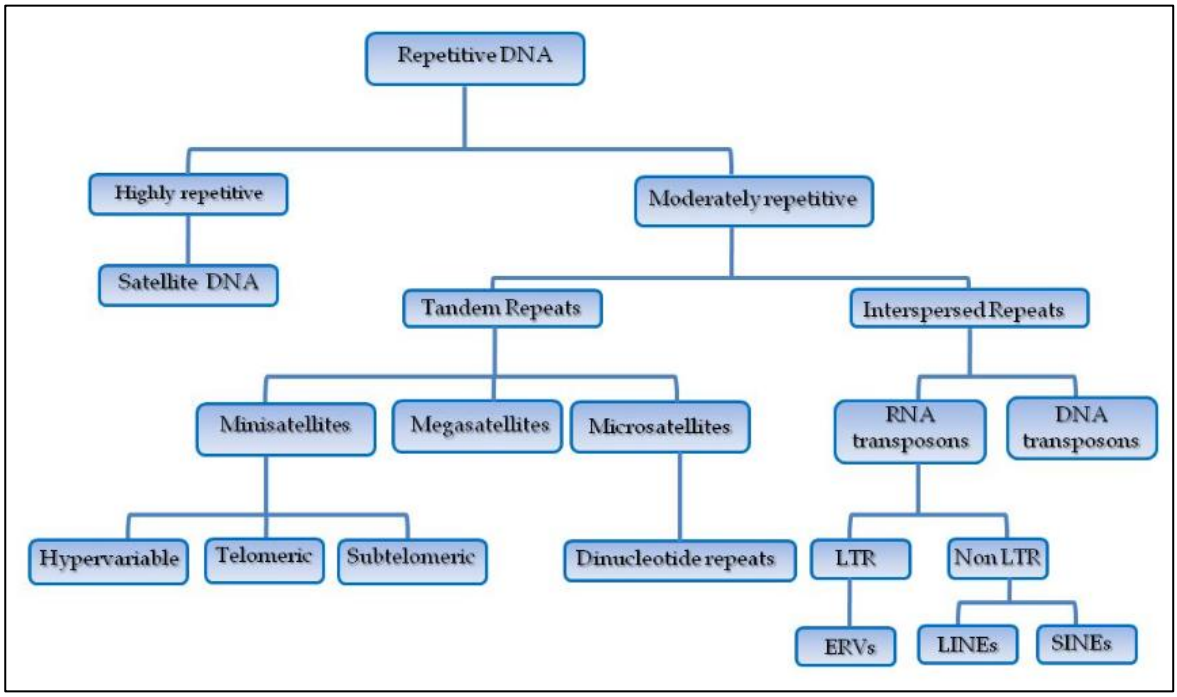

Fig 1: Schematic diagram showing biological categories of the different repetitive sequences

Repeats, however important, the source of a lot of trouble when you work on genomics data. The widespread occurrence of repetitive stretches of DNA in genomes of imposes fundamental challenges for sequencing, genome assembly, and automated annotation of genes and proteins. This multi-level problem can lead to errors in genome and protein databases that are often not recognized or acknowledged. Repetitive elements complicate the accurate determination of gene boundaries, regulatory regions, and structural variations. In sequencing efforts, repetitive sequences can confound read alignment algorithms, leading to misassembly or fragmentation of genomic contigs. This, in turn, undermines the reliability of downstream genomic analyses and annotations. To address these challenges, tools like RepeatMasker are employed.

REPEATMASKER TOOL:

RepeatMasker is a program that screens DNA sequences and detects transposable elements, satellites, and low-complexity DNA sequences. The output of the program is a detailed annotation of the repeats that are present in the query sequence as well as a modified version

of the query sequence in which all the annotated repeats have been masked (default: replaced by Ns). Currently over 56% of human genomic sequence is identified and masked by the program.

There are two types of masking, producing slightly different FASTA output:

1. **Soft-masking**: Repeat elements are written in lower case, e.g. ATATA = atata.
2. **Hard-masking**: Repeat elements are replaced by stretches of the letter N

The non-repeated sequences are always in uppercase. Hard-masking is destructive as large parts of the sequence which are replaced by stretches of N, therefore, soft masking is preferred as it allows to perform annotation.

The tool can be accessed from Galaxy Server's tool repository among the available options. Galaxy is an open source, web-based platform for data intensive biomedical research. It designed to facilitate accessible, reproducible, and collaborative computational analysis in the field of bioinformatics. It provides a user-friendly interface for researchers, enabling them to perform a wide range of genomic analyses without requiring advanced computational expertise or software installation. The development and maintenance of the site are supported by NIH NHGRI award U24 HG006620.

Repeat identification and masking is usually a previous step to the gene prediction and annotation phase. The masking step signals to downstream sequence alignment and gene prediction tools that these regions are repeats. Identifying repeats is complicated by the fact that repeats are often poorly conserved; thus, accurate repeat detection usually requires a repeat library for the species of interest. RepeatMasker relies on existing databases of repeated elements signatures like Dfam and RepBase.

The Dfam database is an open collection of DNA Transposable Element sequence alignments, Hidden Markov Models (HMMs), consensus sequences, and genome annotations. It represents a collection of multiple sequence alignments, each containing a set of representative members of a specific transposable element family. These alignments (seed alignments) are used to generate HMMs and consensus sequences for each family. While RepBase is a database of representative repetitive sequences from eukaryotic species.

Sequences can be pasted in or uploaded as files, both in FASTA format. The program returns four or five output files for each query:

1. Masked Sequence: This is the FASTA file that is used for future analysis. When displayed it is observed that some portions of the sequence are in lowercase or series of Ns.
2. Repeat Statistics: This one contains some statistics on the number of repeats found in each category, and the total number of base pairs masked.
3. Output Log: This is a tabular file listing all repeats.
4. Repeat Catalogue: This one contains the list of all repeat sequences that were identified, with their position, and their similarity with known repeats from the Dfam database.
5. Repeat Annotation: This one contains the coordinate of each repeat element in GFF2 format.

REFERENCES:

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DATE: 20/08/2024

WEBLEM 8(A)
REPEATMASKER TOOL
(URL: http://galaxy.org/)

AIM:

To mask repeats using RepeatMasker tool on dataset imported from Zenodo.

INTRODUCTION:

Repetitive sequences, refer to segments of DNA that occur multiple times within a genome. These sequences can range in length from a few base pairs to thousands of base pairs and can be categorized into different types based on their structure, function, and evolutionary origin. Repeats are ubiquitous across all domains of life and play diverse roles in genome organization, stability, and evolution.

They can contribute to genome plasticity and adaptation by promoting genetic diversity through recombination, mutation, and gene duplication. However, repeats can also pose challenges for genome assembly, annotation, and stability, as they can lead to errors in sequencing and alignment and can contribute to genomic instability and disease. Therefore, tools like RepeatMasker are used before downstream analysis of sequences and gene prediction.

RepeatMasker is a popular software tool widely used in computational genomics to identify, classify, and mask repetitive elements, including low-complexity sequences and interspersed repeats. The tool utilizes the Dfam database as a reference library for identifying known repetitive element families, improving the accuracy of repeat annotation in genomic analyses. RepeatMasker can be accessed from the toolkit of Galaxy Server.

Dfam is a comprehensive database of repetitive DNA elements, specifically focusing on transposable elements (TEs) and other repetitive sequences that proliferate within genomes. Created and maintained by the Gardner Lab at the University of California, Santa Cruz, Dfam serves as a valuable resource for researchers studying the structure, function, and evolution of repetitive elements in genomes.

The following weblem is based on a tutorial given by Trainings in Galaxy Project. The data is imported from Zenodo (link: https://zenodo.org/record/7085837/files/genome_raw.fasta).

Zenodo is an open-access digital repository and platform designed to facilitate the sharing, preservation, and citation of research outputs across all fields of science. Developed and maintained by CERN (the European Organization for Nuclear Research), Zenodo serves as a centralized repository where researchers, scientists, and scholars can deposit and archive a wide range of scholarly outputs, including datasets, software, papers, posters, presentations, and other research artifacts.

METHODOLOGY:

1. Open Galaxy Server Webpage (http://galaxy.org/) and log into your account.
2. Search for RepeatMasker Tool in the Galaxy Toolkit
3. Select “masked sequence on data 1”.

4. Go to Galaxy Trainings and click on Genome annotation, under that click on Repeat Masking with RepeatMasker.
5. Copy the Zenodo link for the dataset and import the files on RepeatMasker.
6. Using the genome sequence run the tool.
7. Six files are generated, interpret the results.

OBSERVATIONS:

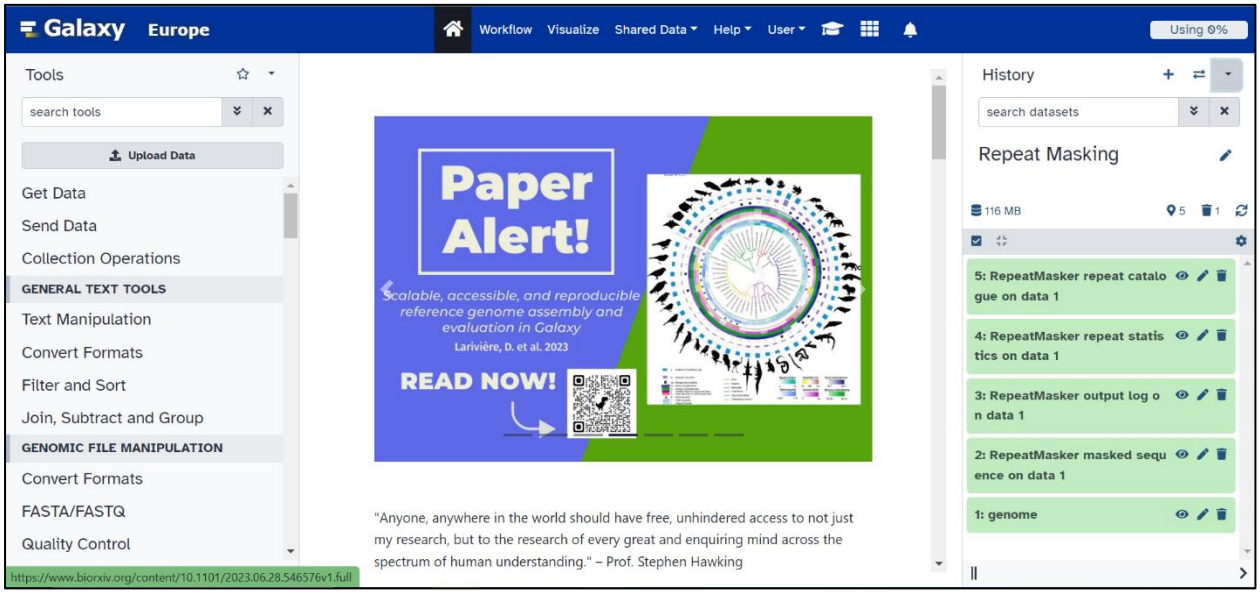

Fig 1: Homepage of Galaxy Server

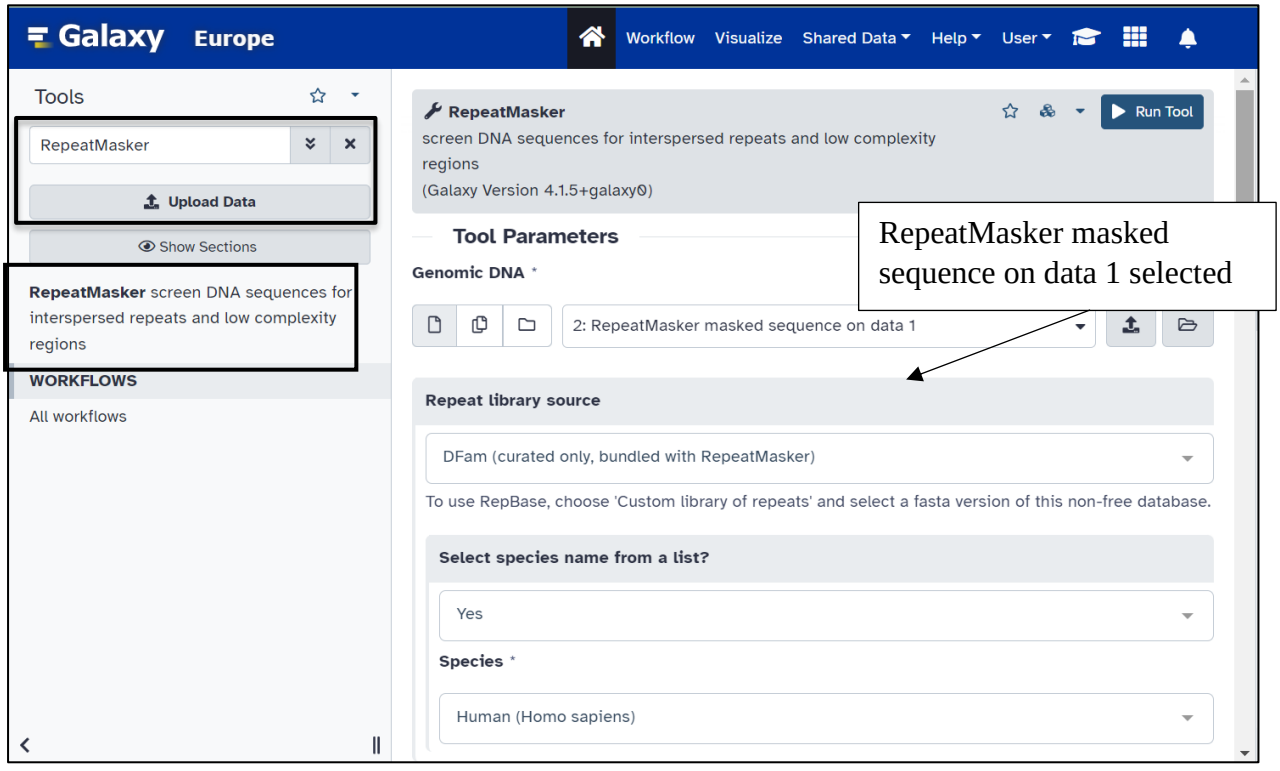

Fig 2: RepeatMasker Searched in Galaxy Toolkit

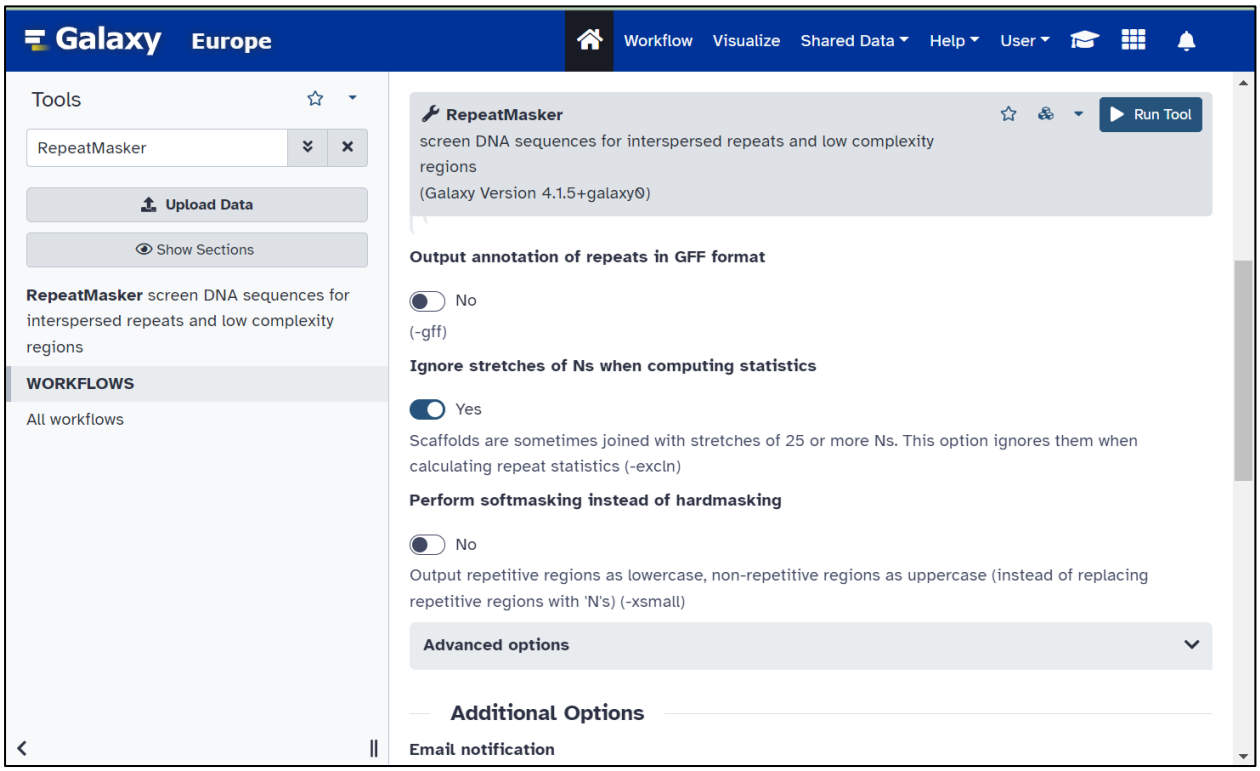

Fig 3: Default Criteria before Running tool

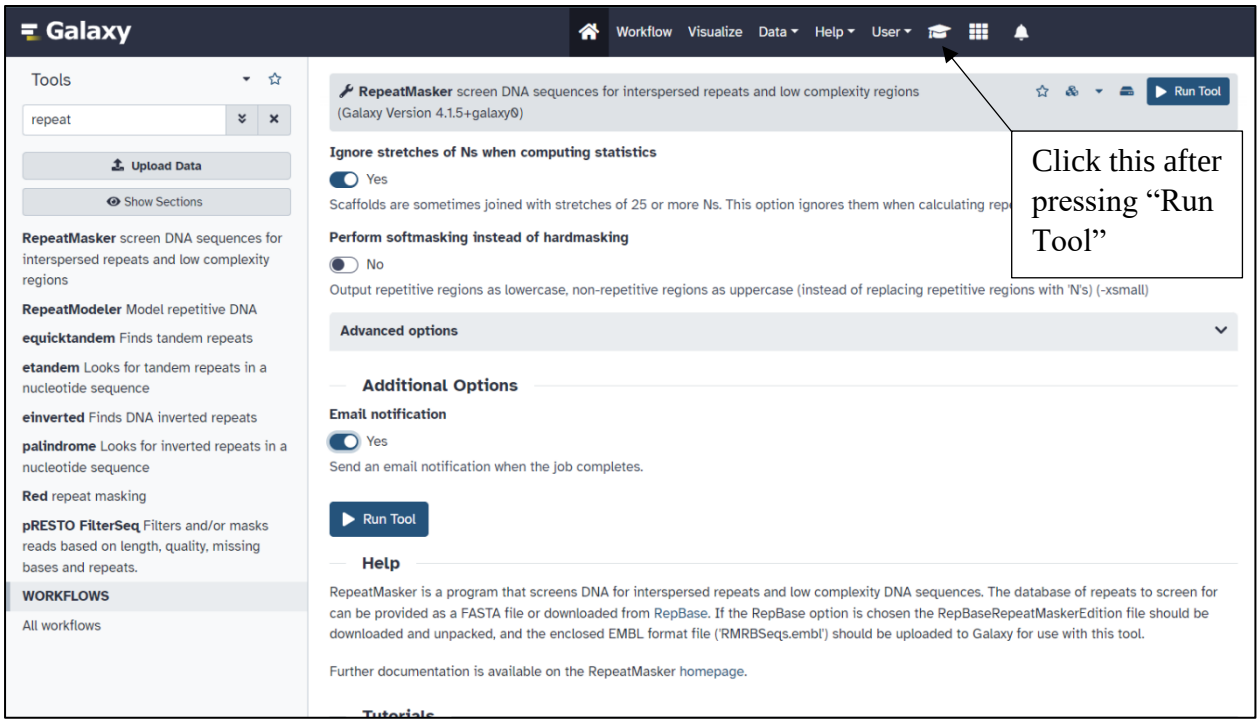

Fig 4: E-mail Notification Option Turned On

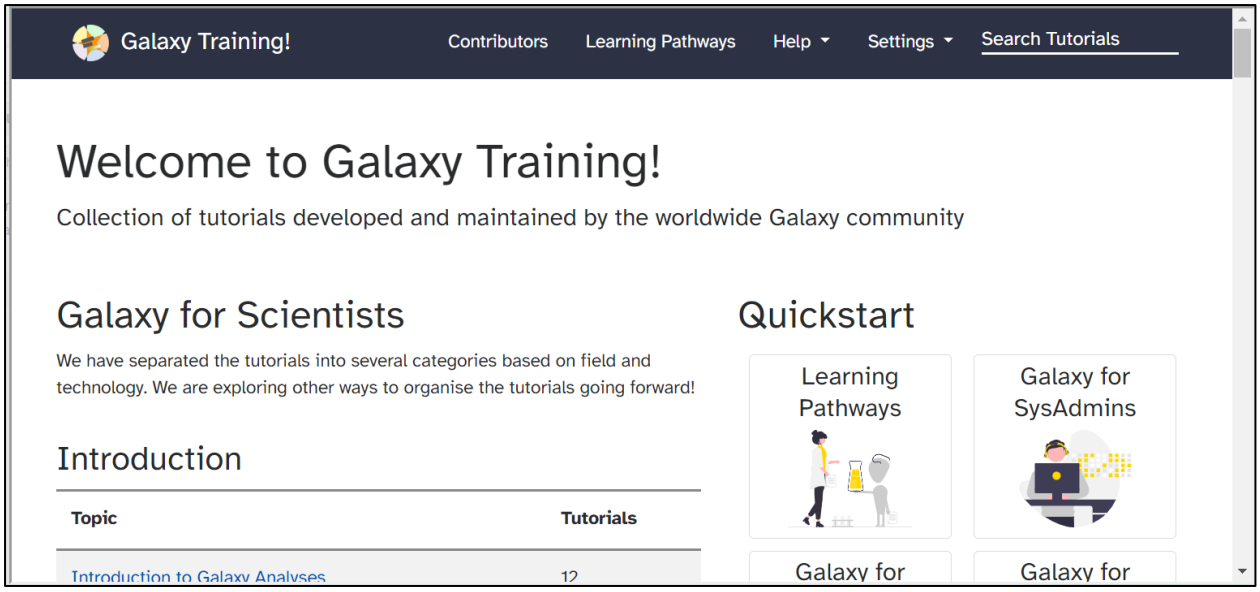

Fig 5: Galaxy Training Page Opened

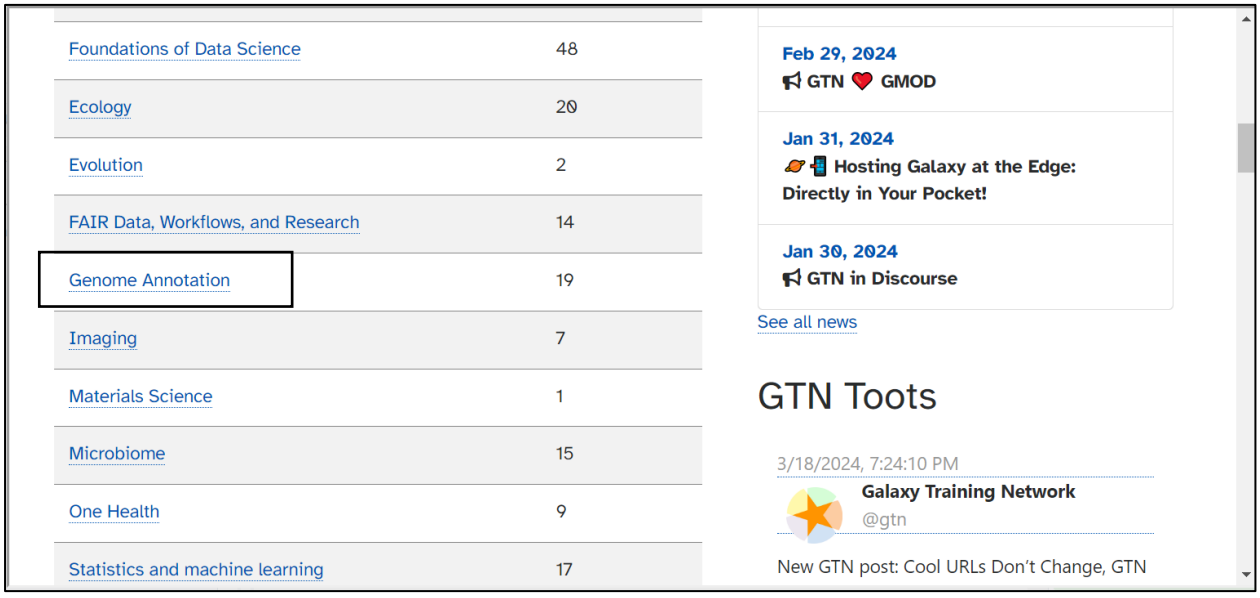

Fig 6: Click on Genome Annotation

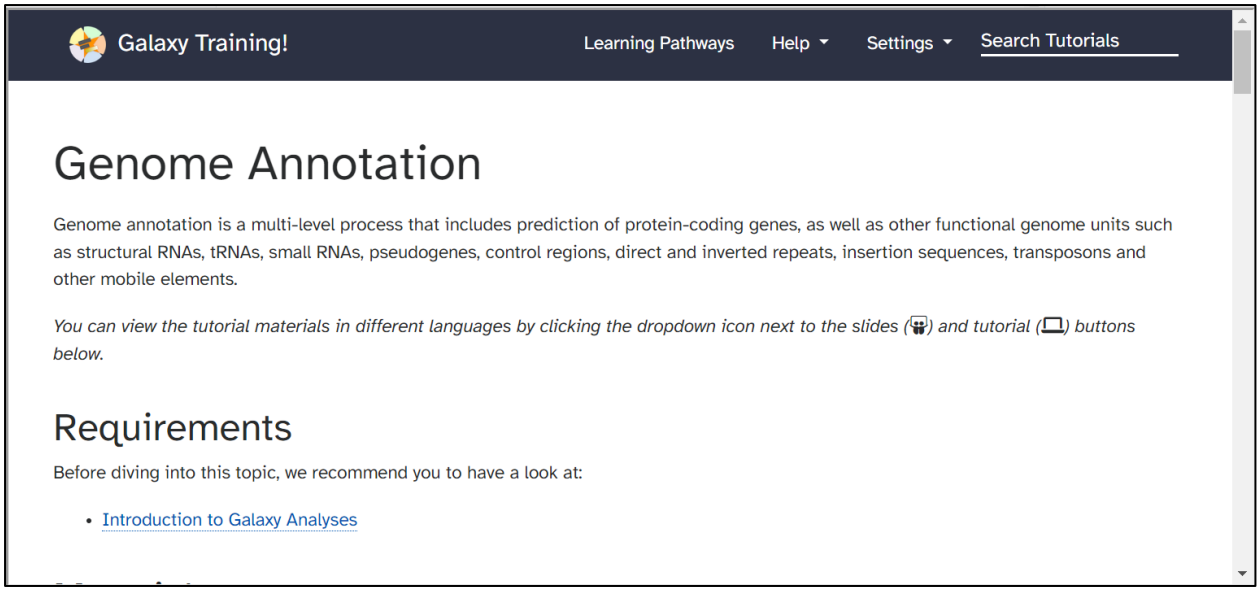

Fig 7: Genome Annotation Page Opened

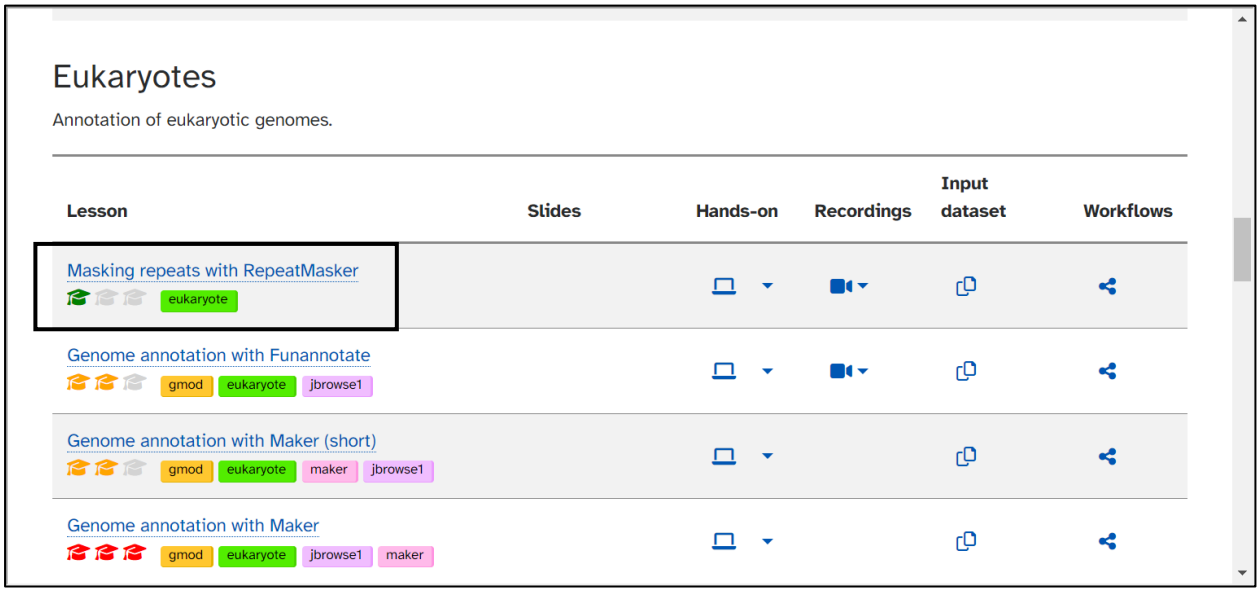

Fig 8: Click on "Masking Repeats with RepeatMasker" tutorial

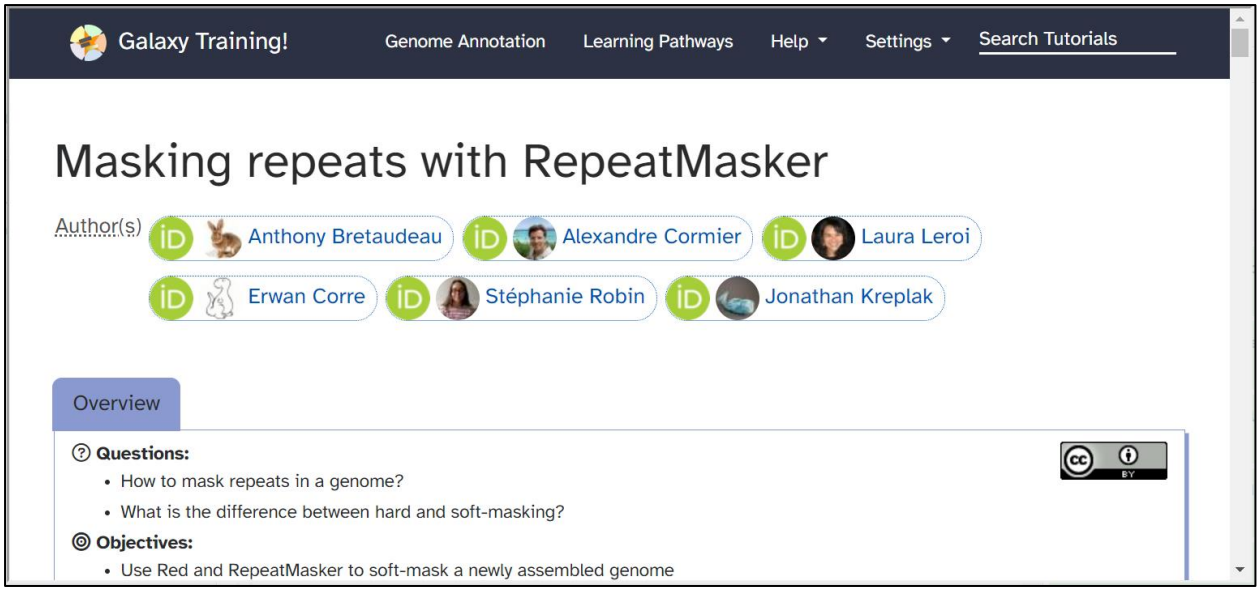

Fig 9: "Masking Repeats with RepeatMasker" tutorial opened

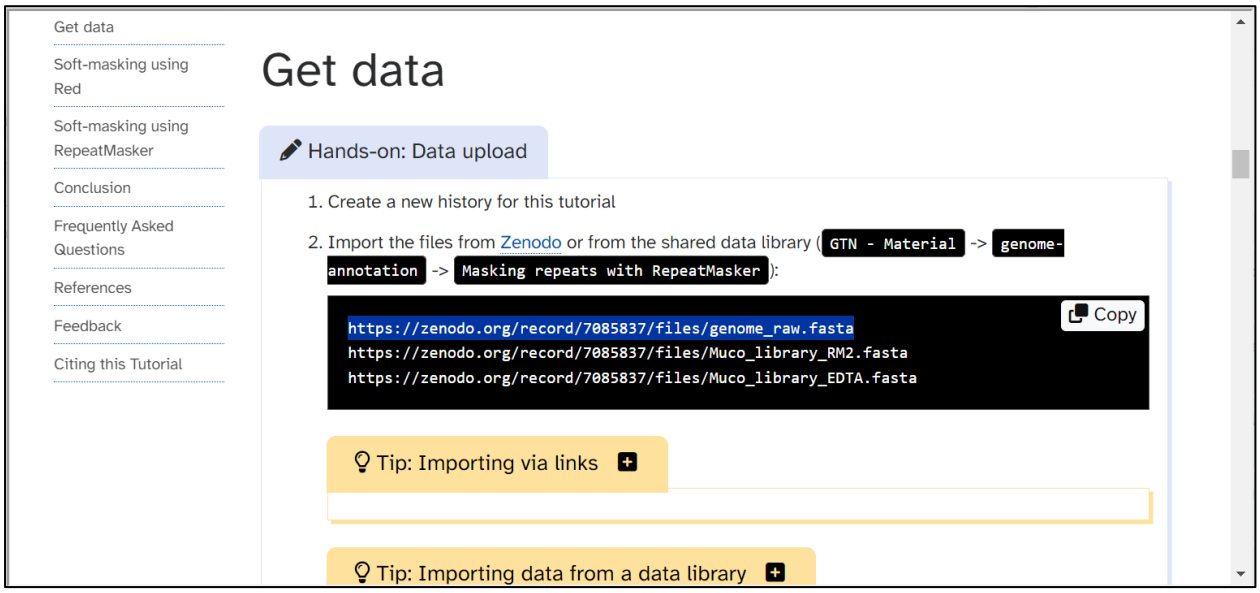

Fig 10: Copy the first link to import dataset

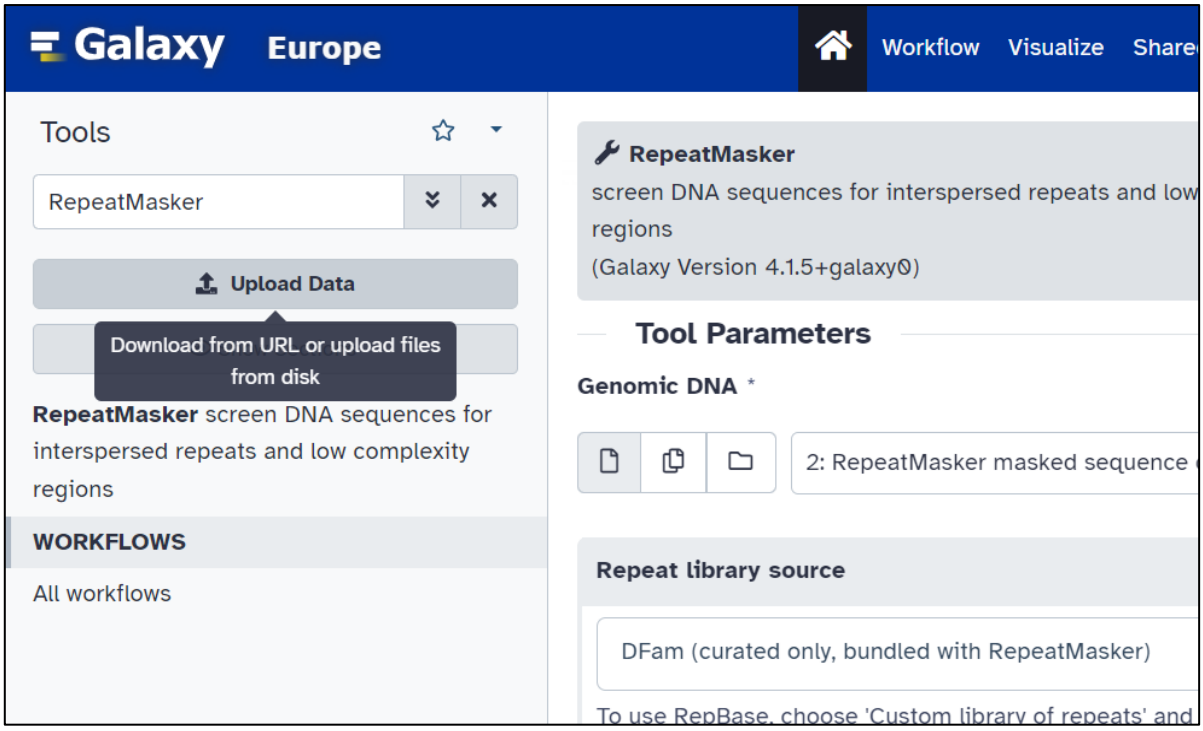

Fig 11: Click on Upload Data

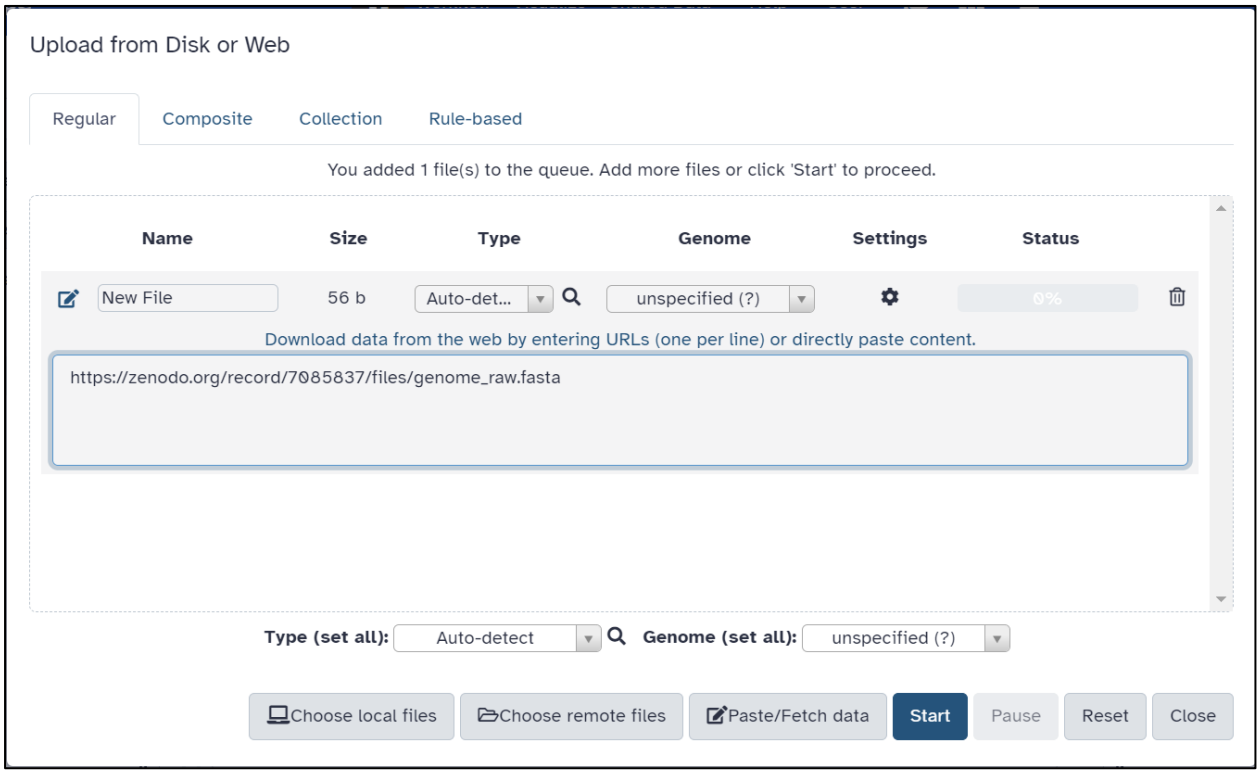

Fig 12: Paste the link and click “Start”

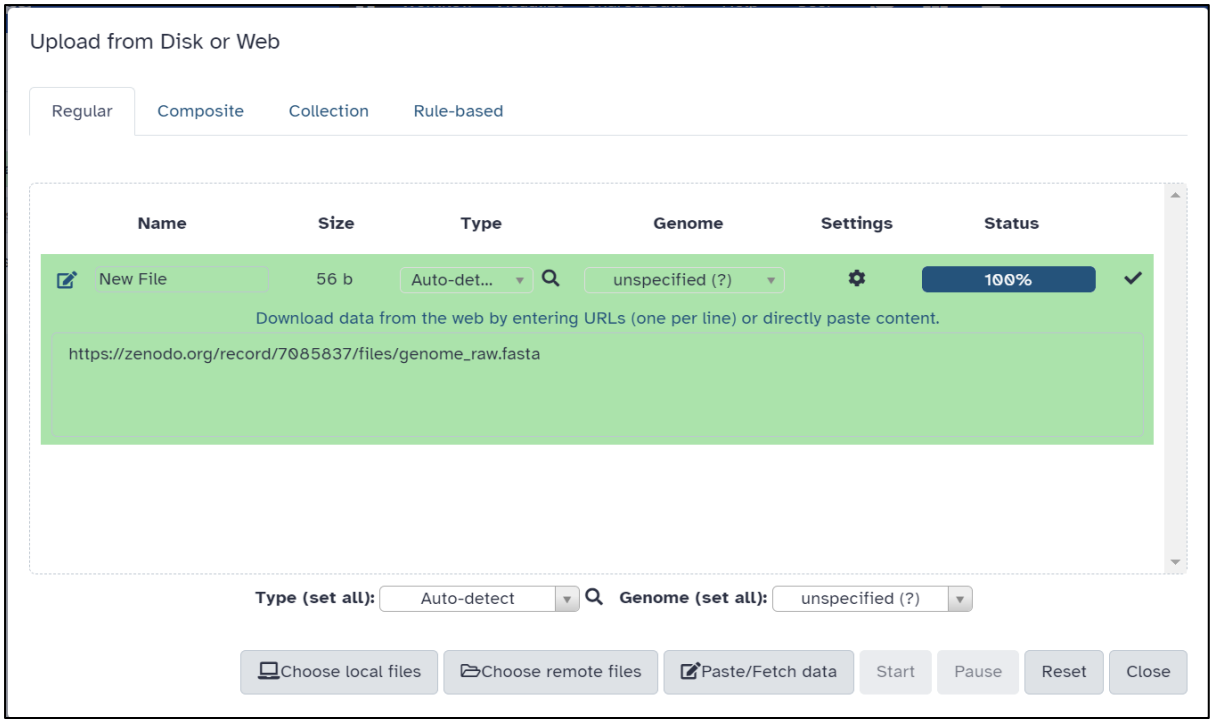

Fig 13: New File successfully loaded

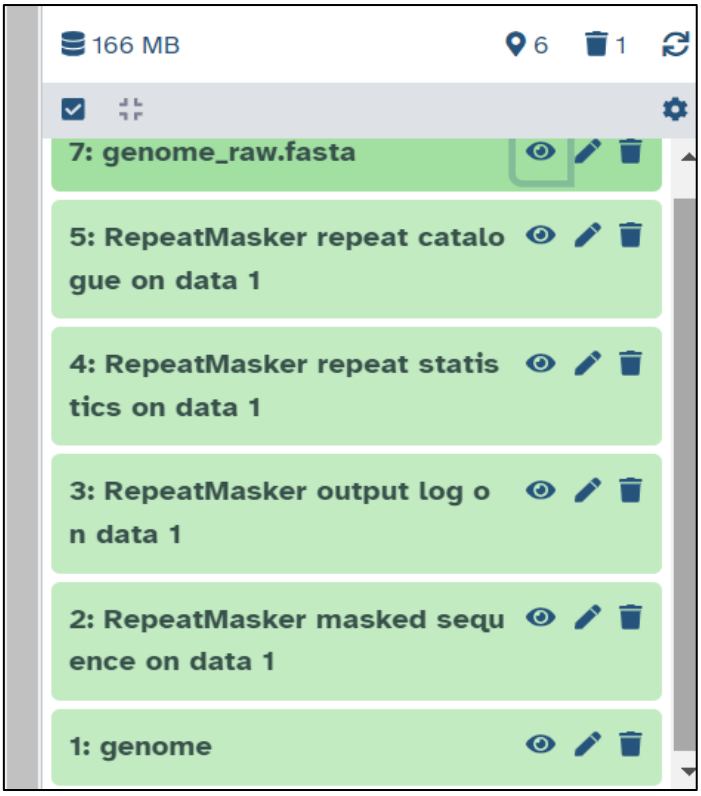

Fig 14: Six Files are generated

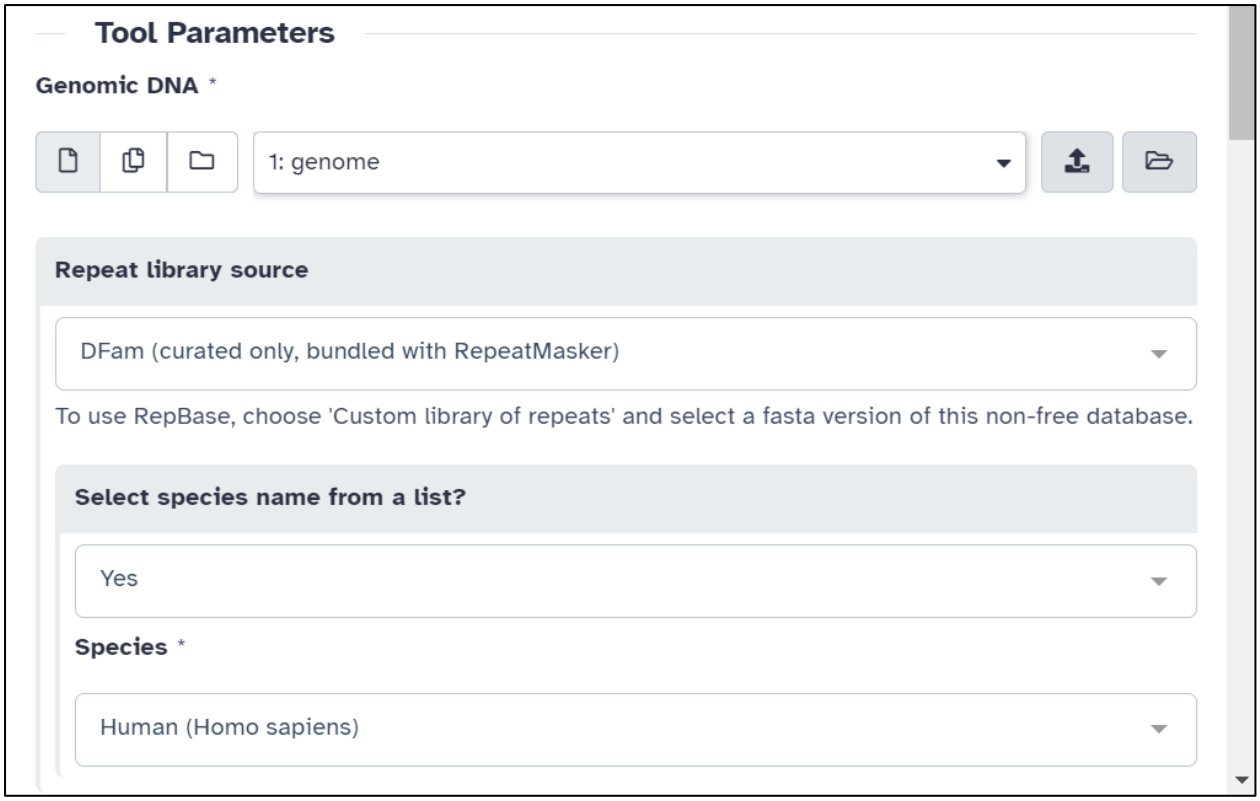

Fig 15: Import the Genome file

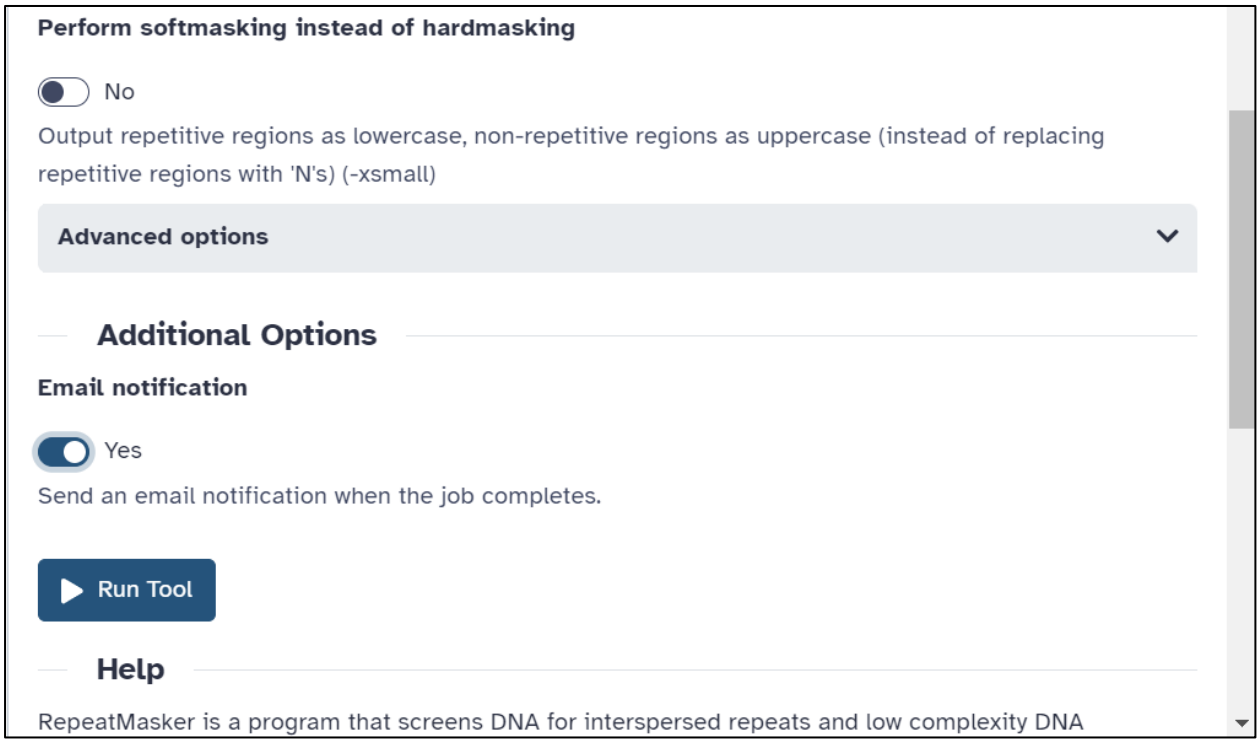

Fig 16: Turn On e-mail notification and Run Tool

```
>contig_1000
GTCTTGTACAGTCCCCTCCAAGATAAAAGTATCATTTTACAACTCCGCGATACATCACAG
ATGTATGATTAATATACTTTGAAGGATATACTCGACATTCAAATATTTAAAATTAGTGAG
ATGTATATGGAATCCATAAGGAACCTTTACTAAATGTGTTCATACAATTTTTTACAAAAA
AAAATAAAAAATCCATTTTTTTCATTTACTCAATAATCGATGGGGTATCCTTTACTTCGG
ATCACATCCTTCATTCGTTTTGGCATTGAATCGATTAACTTATTAATCTTTTCAACGCCC
ATTAAGTTCCATTCAGATTCTACGAGAATCCTCAATGATTCTATGTTTCGAACATACTCA
AAACGTCTTTCTTTTGCTCGTTTCAAGATCATCCAAAGATTCTCGATTGGGTTCAAATCT
GGTGATTGAGGTGGCCAATTCAATCGAAGAATTTGGTGATTGTCTTTCCATTCACGAGCA
ACTTTTGCAGTATGGATTGGAGCATTATCCTCCATCAATACTGGTGTGCTGCAGCTGTTG
TAAAAATCCAACAACACTGGTTCATAAACCTGCTTTATGAAGTCGTTGCTAGATCGCTGG
CCTTTGTTGAAAAAAACCAAACTGGTTCGACGATTATAGGTTATTCCTCCCCAAATAGAC
AACGACGACCTTCCACTCTTAAATGTTGGTGCTAAACAACTAGGAAGCATTTGTTCCTTT
GGGCCTCGTCGAATTCTAATAGGACAAGAAGAACTGCAAGTTTGGAAGCTGCATTCATCT
GTCCAGATTACTCGACGCCAGTCTTCCAAAGTCCAACTTTCGTGGTCCCTGGCAAACTGA
AGACGTCTTTTCCTCTGTTCCTCGTTCACAAAAGGCTTTTTCTTCGCGATACGGCTTCTC
AAGTTGAGGCGGTGAAGCTCTTTTCTAAAGGTGTTCTTTGTAGCATTAACCGCCACATTA
GCCATGATCTCGGCAAGAGTACTTCGTCTATGAGTAATCACATAGTATCGTAAGGCTCGT
CTGTCTCTTTCAGTAAAGATCGTACGTCTACCAGTGCCTTTTTTTCTCTCAAATGAACCT
GATTTCTCACAACGTTCAATCGCACGCTTAATTGCAGCATGGGTAAACTTCAGTTCGCGA
GCAATTTGGGCAATAGTTACGCCTTCATTCTTTTTCGCGACTGCATAGGCTTTTTGAGTG
GGACTTAATTGAGGCATTGTAAAGATCTTTATTTTTAAAAATGAGAAACTTAACGAATAA
AAGCTTTCTTATTTCGGGGGGTCCTTTGAAGAAAATGACGCGGAGTTGCAAAATGATACT
TTTATCTTGGAGGGGACTGTACATCCTCTTGCTAATATCGCCCCTGATCCGGACCCTGGT
```

Fig 17: Genome File

```
GGC
>contig_1001
AGGTTGTTCTCAAGCAAAAAAAATCTTCAGATCTGATCTGATCTGAAGTG
CGGAGTTACCTTCTATGGGATTTTGTGTAATAAAAACTTCAGTAAACTTC
AGATGAAAAAAATCCATCTGAAGTCTACCGAAGTGATATAAAAGGAAATT
TCCCATGATTTCTAATTTCACGAATATTATCCAATTTTTTTAAAAAATCC
AAATTTTCTTTTCTTTCTTTTTCATAAATACATTACCATGGAGCAACAAA
ACATATTCGAACAAAAGGAAAAGATTATTACTAAGAAACAGTATACCAAA
GATGATATGTACCATATTGCAGAATTATATGGACGCTACTATTTGGCTAT
CAATACTCACAACAATATTTTGTCACCAAACAGAGTTCAAATCAACAATC
AGCATTGGAAAGCGTTGACGTTGGAATACAACGAGCGCCAAGCCCTCCTT
CCCAACAATGAAGGTTGTTTCAGGTCCGAAAAAGCTCTTCTCGAGAAATA
TGGTGCTATGAAAACAAAGTATCAGAACAAAAAGGAGACCATTAAGTCGA
CCGGTTCGGGAGGTGGCCCACTTCTTGGGTACCTTCAAAAAATGCATGAT
TATTTGCATAAGGACCCCCAGCTTCACCCAAAGGCCACCTATAGCTCCGT
CAAAAGAGCTTATAGTGTTCGAGATGACGAGGAGAACAGTGATGATGTTG
TCGTCTCCGAAGATTCTTGNNNNNNNNNNNNNNNNNNNNNNNNNNGCCTCC
ATAGGCGAACAAAAAAAGAAGAGGAAATCTGTCAAGGAGGAAAAGGAGCT
AGCTCGAGTGAAGTCTAGAGATGAAGCTTTGGAACAGATGAAGCAAATCA
CGGAAGGCATGACAAGCAAGCTCGAGGAGATTATGAGCAAGCCTCTTGTT
GTTCAACACGTTGAATCTGAAGAGAAGAAGGAGTTTTACAAGAAGTTTCT
TGAATTGATGGAAAATGGTTTAAAATAAATTTCATGTTAAATATTTTTTA
TTGAAACTAGTTCAAGAACGTGTTAACATACACGTTTAAAAGATCTCGTC
GTTGTCCTCCAGCAGTTCTTTCCAAAGGAGTGTTTGTTTCAAATGTTGCT
GGACGATCACTTCCTGAAGCAAGTGACAACTCGACTAATTCTTCATCAGT
TATACCAAAAGTATCATTAGCATTGATGCAAAAGTTATGCAAAATACAGC
TGGTATTAACGATTTTGACCAAACGTAATTGGTCTTGGATGTACACATAC
TTCCAAAGGAATCTCCATCGCTTCACAAGGATACCAAATGCTCTTTCAAC
AACCTGGCGCATTGCACTAAACTTGGCGTTGAATCTTGCTTTGTTGGGAT
```

Hard-masking

Fig 18: Masked Sequence File

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9
SW score	% div.	% del.	% ins.	query sequence	pos in query: begin	end	(left)	repeat
13	13.7	0.0	0.0	contig_1001	720	744	(2078)	(CCTC)n
11	7.3	10.3	0.0	contig_1008	2423	2451	(564)	(GATGAC)n
12	7.5	6.7	3.2	contig_101	1447	1476	(126812)	(TACTTT)n
15	10.0	0.0	8.6	contig_101	13270	13307	(114981)	A-rich
37	0.0	0.0	0.0	contig_101	15274	15307	(112981)	(CAT)n
18	14.9	0.0	0.0	contig_101	18515	18544	(109744)	(T)n
18	33.6	0.0	0.0	contig_101	18894	18960	(109328)	(CTG)n
15	24.0	0.0	0.0	contig_101	19841	19879	(108409)	(ACA)n
37	0.0	0.0	0.0	contig_101	22257	22290	(105998)	(T)n
29	23.6	2.1	2.1	contig_101	23907	23999	(104289)	(AGAAGT)n
26	0.0	0.0	0.0	contig_101	24455	24479	(103809)	(CATCCT)n
25	0.0	0.0	0.0	contig_101	25516	25542	(102746)	(A)n

Fig 19: Sequence Output Log File

```
==================================================
file name: rm_input.fasta
sequences:           1461
total length:   48645285 bp  (48645285 bp excl N/X-runs)
GC level:         36.60 %
bases masked:    1173324 bp ( 2.41 %)
==================================================
               number of      length   percentage
               elements*    occupied  of sequence
--------------------------------------------------
SINEs:               26         1259 bp    0.00 %
      ALUs            0            0 bp    0.00 %
      MIRs            5          265 bp    0.00 %

LINEs:             160        10580 bp    0.02 %
      LINE1           6          321 bp    0.00 %
      LINE2          39         2382 bp    0.00 %
      L3/CR1         59         4081 bp    0.01 %

LTR elements:        15         2216 bp    0.00 %
      ERVL            2          106 bp    0.00 %
      ERVL-MaLRs      0            0 bp    0.00 %
      ERV_classI      0            0 bp    0.00 %
      ERV_classII     0            0 bp    0.00 %

DNA elements:        34         2431 bp    0.00 %
     hAT-Charlie      3          149 bp    0.00 %
     TcMar-Tigger     4          227 bp    0.00 %

Unclassified:         6          395 bp    0.00 %

Total interspersed repeats:    16881 bp    0.03 %
```

Fig 20: Sequence Statistics File

```
13 13.72 0.00 0.00 contig_1001 720 744 (2078) (CCTC)n#Simple_repeat 1 25 (0) m_b1s252i1

  contig_1001          720 CCCCCCTCCCTCTTTCCCTCCCTCC 744
                             i         ii
  (CCTC)n#Simpl          1 CCTCCCTCCCTCCCTCCCTCCCTCC 25

Matrix = Unknown
Transitions / transversions = 1.00 (3/0)
Gap_init rate = 0.00 (0 / 24), avg. gap size = 0.0 (0 / 0)

11 7.28 10.34 0.00 contig_1008 2423 2451 (564) (GATGAC)n#Simple_repeat 1 32 (0) m_b1s252i0

  contig_1008         2423 GATGACGATGAGGATGTC-ATGA-G-TGACGA 2451
                                          v    v -    - -
  (GATGAC)n#Sim          1 GATGACGATGACGATGACGATGACGATGACGA 32

Matrix = Unknown
Transitions / transversions = 0.00 (0/2)
Gap_init rate = 0.11 (3 / 28), avg. gap size = 1.00 (3 / 3)

12 7.52 6.67 3.23 contig_101 1447 1476 (126812) (TACTTT)n#Simple_repeat 1 31 (0) m_b2s252i0

  contig_101          1447 TACTTTT-CTTTTA-GTTTAGTTTTAACTTTT 1476
                                 -      -v    v    -
  (TACTTT)n#Sim          1 TACTTTTACTTTTACTTTTACTTTT-ACTTTT 31

Matrix = Unknown
Transitions / transversions = 0.00 (0/2)
```

Fig 21: Sequence Catalog File

RESULTS:

RepeatMasker tool was used (via Galaxy Server toolkit) to mask repeats in DNA sequences of dataset imported from Zenodo. The tool generated an output of 4 files namely: Masked Sequence file, Output Log File, Statistics file and Catalog file. The masked sequence file contains the input sequence with the identified repetitive elements masked, replacing them with Ns for nucleotides (hard-masking was carried out). The masked sequences are crucial for downstream analyses where repetitive elements might interfere, such as gene prediction or genome assembly. The output log file records the procedural events that occurred during the execution of RepeatMasker. This includes the list of repeats in the contigs, and their positions along with the SW score (Smith-Waterman score, represents a measure of similarity between the sequence being analyzed and the repetitive elements identified in the Dfam database). The statistics file summarizes the process of masking repeats by stating the number of sequences (1461), total number of base pairs (48645285), length occupied by LINEs, SINEs, LTR elements, DNA elements and other unclassified repeats, all giving a total interspersed repeat of 16881bp. The catalog file provides a detailed annotation of the repeats identified in each sequence, including the type of repeat, its location, and its score. The catalog file effectively maps all identified repeats within the sequences.

CONCLUSION:

The RepeatMasker tool is widely used in genomics for identifying and masking repetitive elements in DNA sequences. Repeat masking is followed through before proceeding with any downstream analysis of sequences like gene prediction and genome annotation as a thumb rule; because repetitive sequences can complicate sequence alignments and gene identification.

RepeatMasker tool in the Galaxy server, typically generates four output files, each providing different insights into the analyzed sequences.

The analysis concludes with repeats in the contig sequences masked, a list of repeats observed, and statistics on the repeats, useful for both specific studies and general genomic characterization. The diversity of repetitive elements found and mapped in the catalog file within the sequences can reveal predominant repeat families, potential regions of interest due to high repeat content, and evolutionary insights into the dataset used.

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DATE: 21/03/2024

WEBLEM 9
INTRODUCTION TO SEQUENCING METHODS

Sequencing is the process of determining the precise order of nucleotides in a DNA or RNA molecule. It is a basic molecular biology approach that aids in the understanding of genetic information at the molecular level. Researchers can investigate microbial diversity, examine gene expression patterns, and learn more about the genetic makeup of organisms through sequencing. Wide-ranging uses of sequencing can be found in domains including as biotechnology, evolutionary biology, medicine, and agriculture. Sequencing fosters innovation and changes our perception of the complexity of life.

Applications of Sequencing:

Sequencing, a pivotal technique in molecular biology, unveils the genetic makeup of organisms, providing insights into genetic variations and molecular processes. Its applications span diverse fields, driving advancements in personalized medicine, agriculture, evolutionary biology, and biotechnology, which have been explained below:

1. **Medical Research:** Sequencing aids in personalized medicine, disease diagnosis, and drug development by identifying genetic variations.
2. **Agricultural Research:** Enhances crop improvement through genomics-assisted breeding, optimizing plant traits for better yields and resilience.
3. **Evolutionary Biology:** Unravels evolutionary histories and genetic adaptations across species, shedding light on biodiversity and speciation processes.
4. **Biotechnology:** Enables genetic engineering, synthetic biology applications, and bioprospecting for novel bioactive compounds and enzymes.

Significance of Sequencing:

Sequencing holds immense significance in various fields due to its pivotal role in unraveling genetic information, understanding diseases, and advancing scientific knowledge. Studying sequencing is crucial as it enables:

1. **Precision Medicine:** By identifying genetic variations through sequencing, tailored treatments can be developed for individuals, enhancing therapeutic outcomes and minimizing adverse effects
2. **Disease Diagnosis:** Sequencing aids in diagnosing genetic disorders, predicting disease risks, and understanding the molecular basis of illnesses, leading to early interventions and personalized healthcare
3. **Biological Research:** Sequencing contributes to exploring evolutionary relationships, studying gene expression patterns, and uncovering novel biological mechanisms, driving advancements in diverse scientific disciplines
4. **Therapeutic Development:** Understanding genetic sequences through sequencing facilitates the development of targeted therapies, precision drugs, and gene editing techniques, revolutionizing medical treatments and interventions

In essence, the study of sequencing is essential for unlocking the mysteries of genetics, advancing medical treatments, and driving scientific discoveries that shape our understanding of life at a molecular level.

Types of Sequencing Methods:

Sequencing methodologies encompass various techniques for determining the order of nucleotides in DNA or RNA molecules. Three main methodologies include:

1. **First-Generation Sequencing (Sanger's Sequencing):** Utilizes chain-termination methods to determine DNA sequences, known for accuracy but limited throughput and high cost.
2. **Next-Generation Sequencing (NGS):** Employs parallel sequencing of DNA fragments, offering high throughput and cost-effectiveness, despite limitations like short reads.
3. **Third-Generation Sequencing:** Features long-read capabilities, enhancing analysis of complex genomic regions and improving genome assembly, demonstrating clinical application value.

First-Generation Sequencing:

First-generation sequencing, exemplified by Sanger's sequencing, involves chain-termination methods to determine DNA sequences. This method, known for its accuracy, was pivotal in completing the "working draft" of the human genome. Despite its precision, first-generation sequencing had limitations such as low throughput and high costs, which restricted its widespread application. Overall, first-generation sequencing laid the foundation for subsequent advancements in sequencing technologies, setting the stage for more efficient and cost-effective methodologies in genetic research.

Methods of First-Generation Sequencing:

1. **Sanger's Sequencing:**

   Sanger's Sequencing, also known as the first-generation sequencing technique, is a method that determines DNA sequences by incorporating dideoxynucleotides during DNA replication, by following sequencing by chain-termination principle.

   The Sanger's sequencing involves selectively incorporating chain-terminating dideoxynucleotides (ddNTPs) during DNA replication. This method allows for the generation of a series of DNA fragments with different lengths, which are then separated by gel electrophoresis to determine the sequence. By using these chain-terminating nucleotides, Sanger's sequencing enables the identification of the precise order of nucleotides in a DNA molecule, making it a foundational technique in molecular biology and genetic research.

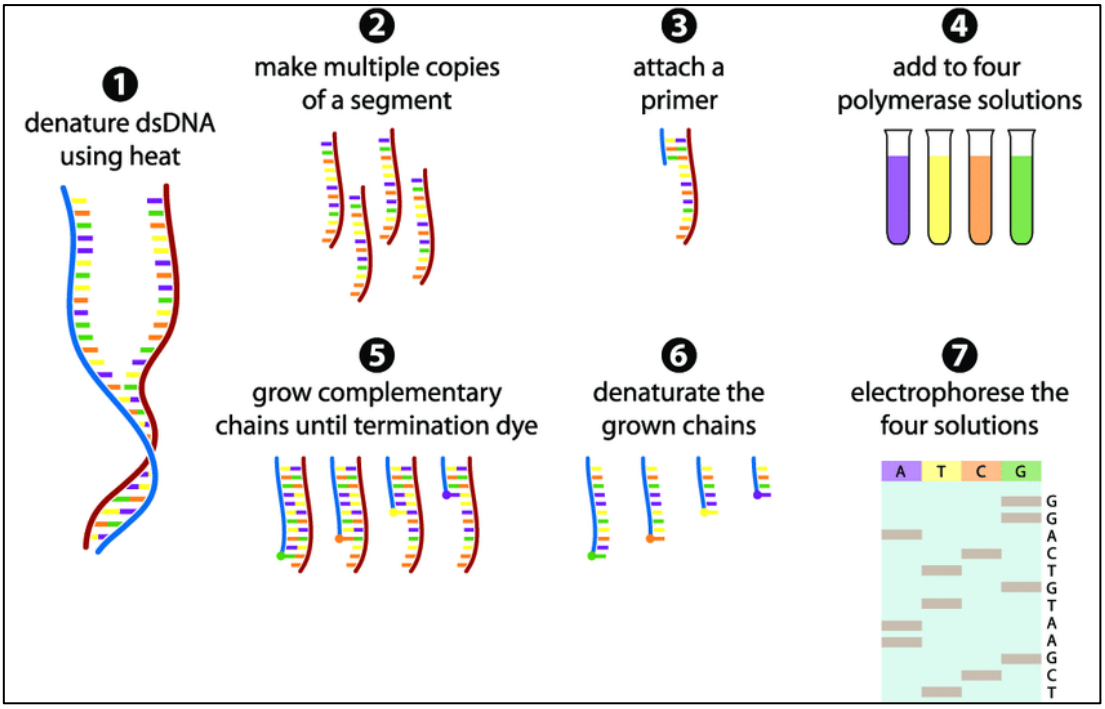

Fig 1: Sanger's Sequencing Method Workflow

2. **Maxam-Gilbert's Sequencing:**

Maxam-Gilbert sequencing is a method developed in the 1970s for DNA sequencing. It involves chemical cleavage of DNA at specific bases, followed by gel electrophoresis to determine the sequence. This technique, along with Sanger's sequencing, was instrumental in early genetic research and contributed to the completion of the human genome project.

The principle behind Maxam-Gilbert sequencing involves chemical cleavage of DNA at specific bases using chemicals like dimethyl sulfate, hydrazine, and piperidine. This method allows for the identification of nucleotide sequences by creating breaks at specific positions in the DNA molecule. By analyzing the fragments produced after cleavage through gel electrophoresis, the sequence of the DNA can be determined.

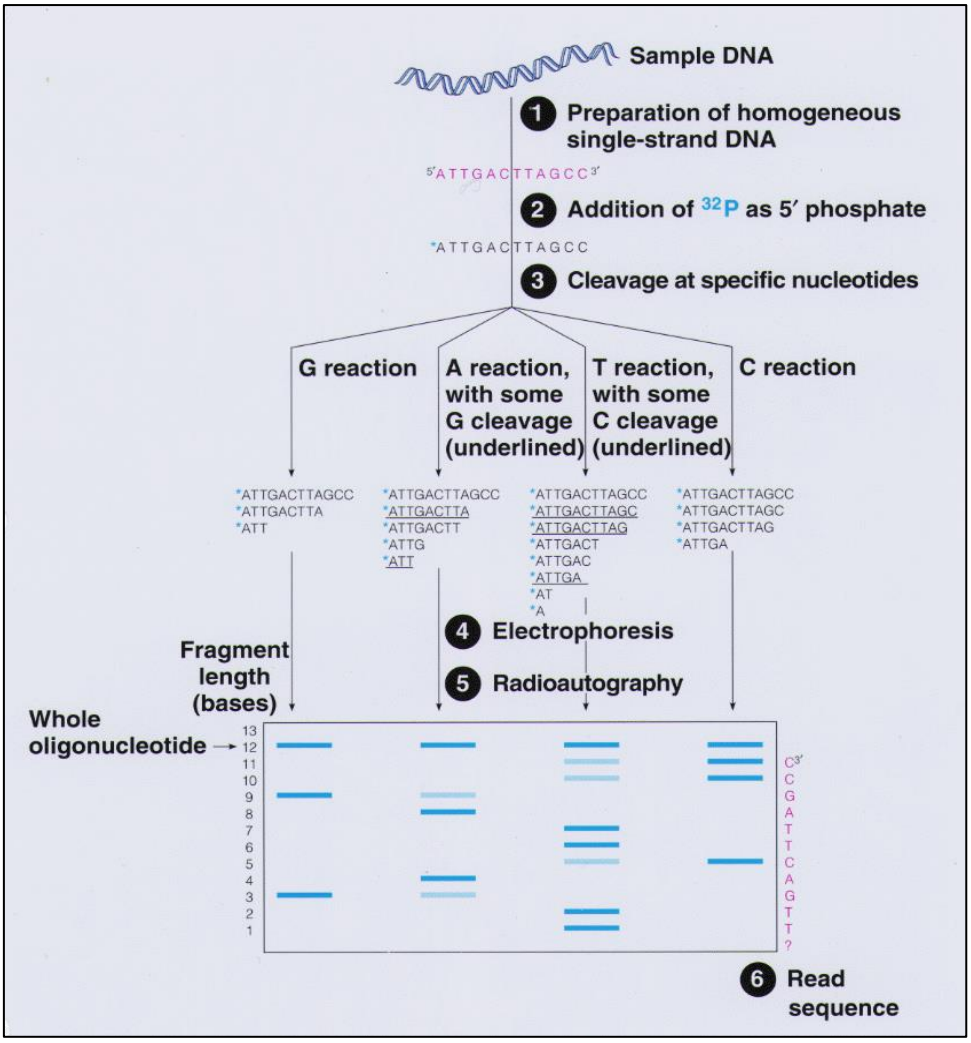

Fig 2: Maxam-Gilbert's Sequencing Method Workflow

Second-Generation Sequencing:

Second-generation sequencing, also known as next-generation sequencing (NGS), revolutionized genomics by enabling high-throughput sequencing of DNA and RNA. This technology, characterized by parallel sequencing of millions of DNA fragments, offers increased speed and cost-effectiveness compared to first-generation Sanger's sequencing. Despite its advantages in high throughput, second-generation sequencing is limited by short read lengths, which can pose challenges in analyzing complex genomic regions and repetitive sequences.

Methods of Second-Generation Sequencing:

1. **Roche 454:**

   The Roche 454 sequencing platform is a technology used for DNA sequencing that was compared with Illumina sequencing technologies in metagenomic studies. Despite differences in read length and sequencing protocols, Roche 454 provided a comparable view of microbial communities sampled. Studies have shown that the Roche 454 platform offers valuable insights into complex microbial communities and genetic diversity, contributing to advancements in genomic research.

The principle behind Roche 454 sequencing involves emulsion PCR and pyrosequencing. In this method, DNA fragments are amplified in water-in-oil emulsions to generate DNA beads, which are then sequenced using a pyrosequencing approach. During pyrosequencing, nucleotides are added one at a time, and the release of pyrophosphate generates a light signal that is detected and used to determine the DNA sequence. This process allows for the generation of longer reads compared to other sequencing technologies, making Roche 454 sequencing valuable for applications requiring detailed genetic analysis and longer sequences.

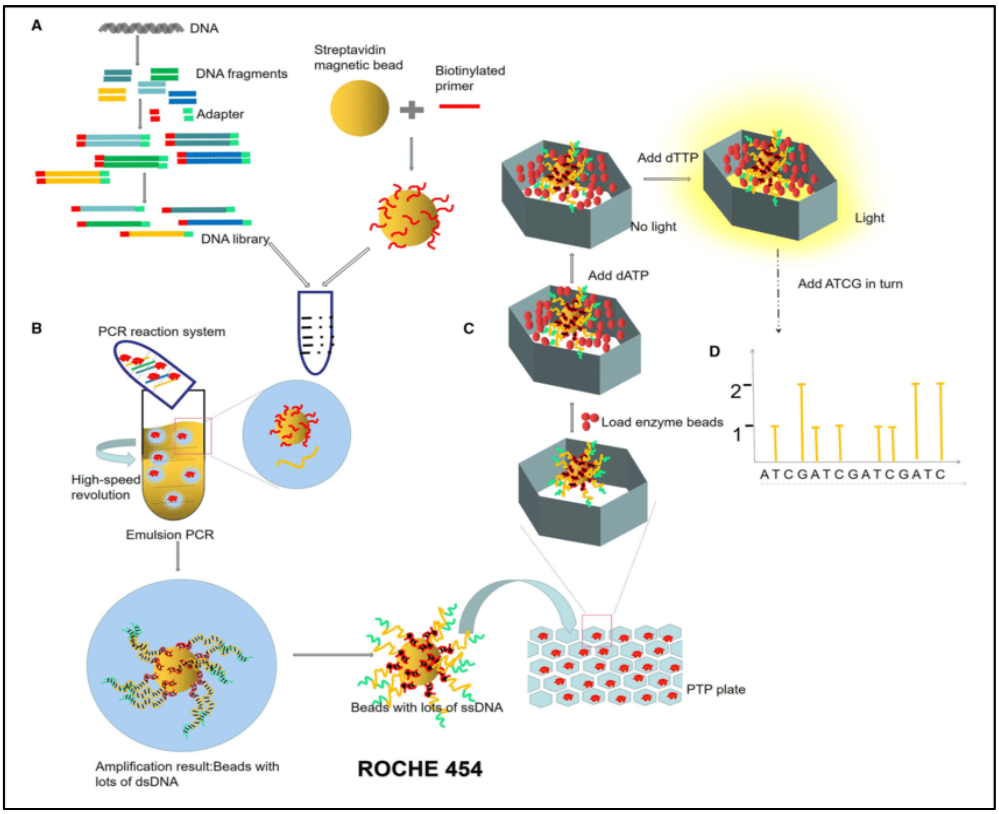

Fig 3 : Roche 454 Sequencing Workflow

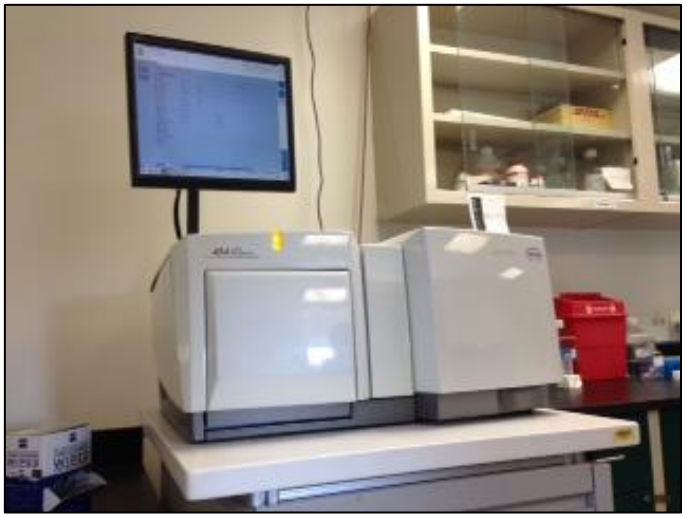

Fig 4: Roche 454 Instrument

2. ABI SOLiD Sequencing:

ABI SOLiD (Sequencing by Oligonucleotide Ligation and Detection) sequencing was a technology developed by Applied Biosystems for determining the sequence of DNA.

The ABI SOLiD sequencing method is based on a unique principle involving ligation of fluorescently labeled oligonucleotides to the DNA template. This approach allows for the determination of the DNA sequence by detecting the fluorescent signals emitted during the ligation process. The technology behind ABI SOLiD sequencing enables accurate and high-throughput sequencing, making it a valuable tool in genomic research and analysis.

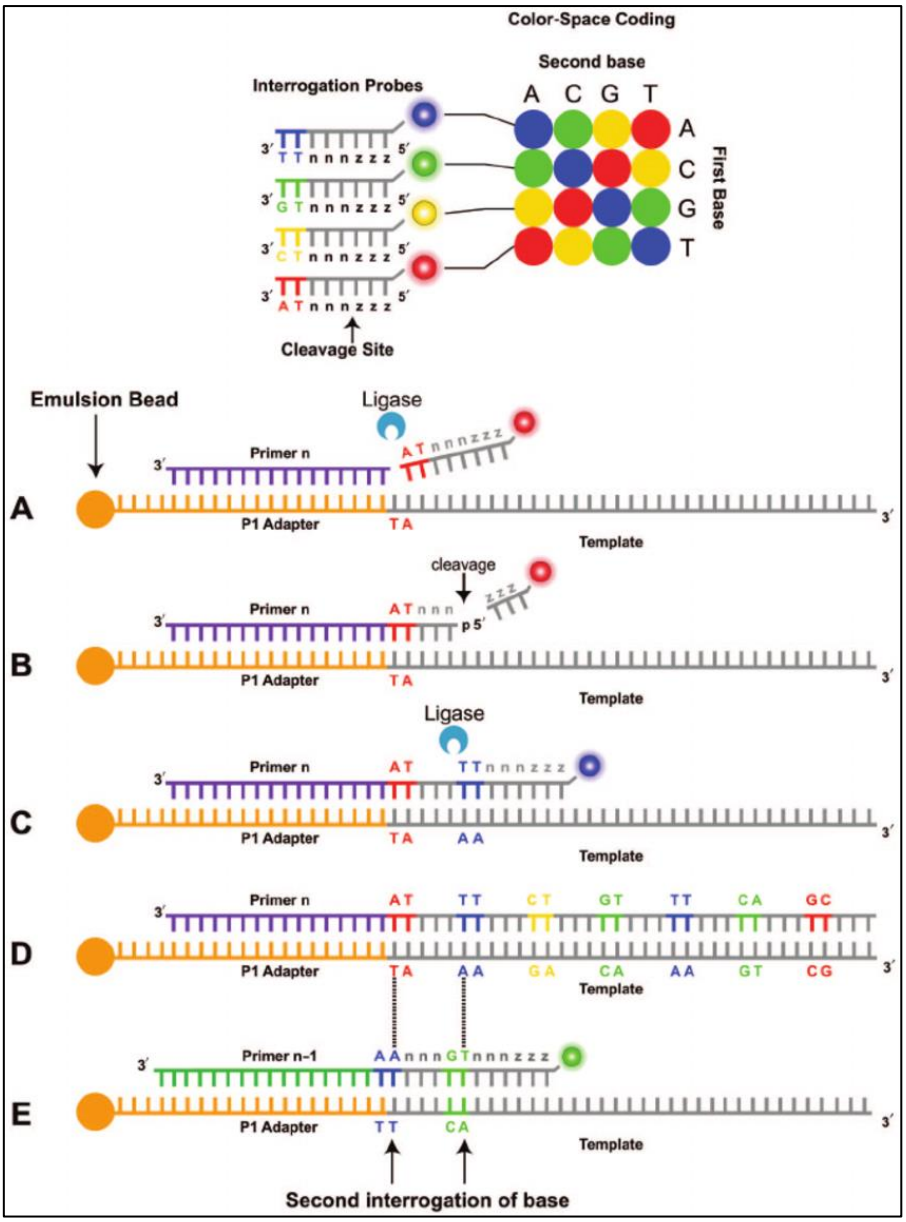

Fig 5: ABI SOLiD Sequencing Workflow

Fig 6: ABI SOLiD Instrument

3. **Ion Torrent:**

The Ion Torrent sequencing platform, specifically the Ion Torrent Personal Genome Machine (PGM) system, has been validated for detecting gene mutations in biopsy specimens from patients with non-small-cell lung cancer.

The principle behind the Ion Torrent sequencing platform involves detecting changes in pH that occur when nucleotides are incorporated into a DNA strand during sequencing. This technology is based on the release of hydrogen ions as nucleotides are added to the growing DNA strand, leading to a change in pH that is detected by a sensor. By measuring these pH changes, the Ion Torrent platform can determine the DNA sequence, offering a rapid and cost-effective method for DNA sequencing compared to traditional methods like Sanger's sequencing.

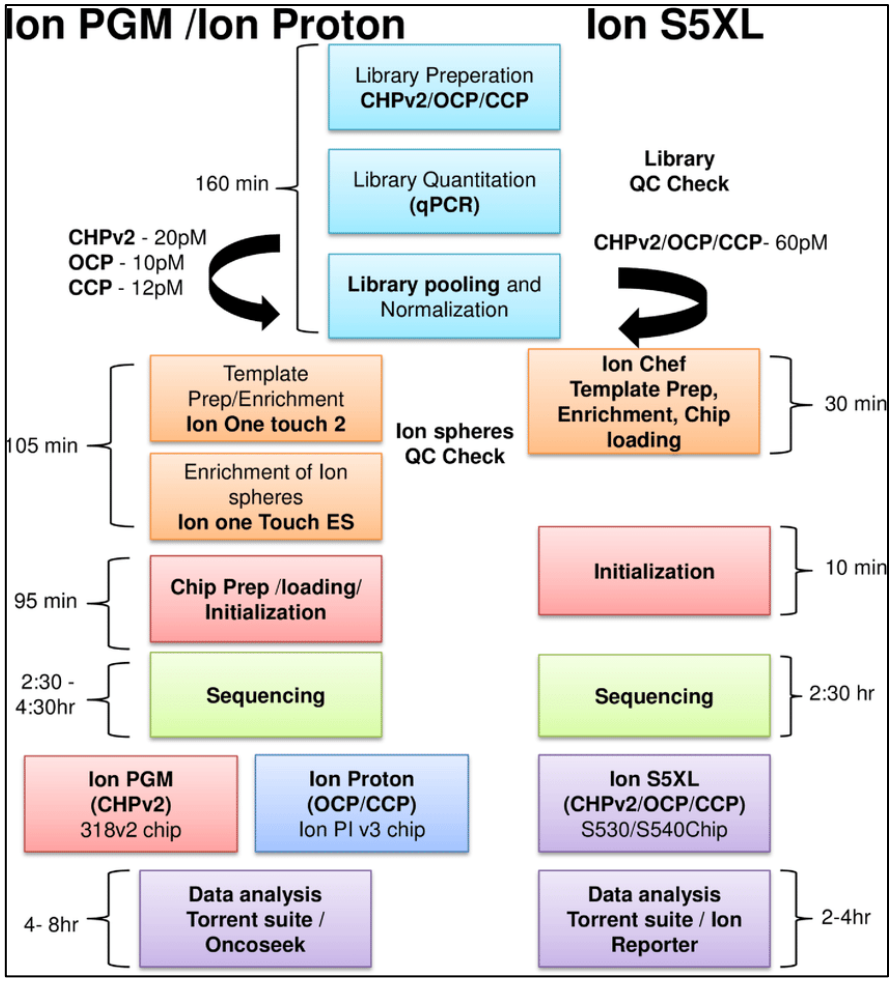

Fig 7: Ion Torrent Sequencing Method Workflow

Fig 8: Ion Torrent Instrument

4. Illumina:

Illumina sequencing is a widely used high-throughput sequencing technology that has revolutionized the field of genomics. It is based on the principle of sequencing-by-synthesis, where a DNA molecule is sequentially read by adding fluorescently labeled nucleotides to the growing DNA strand. The emitted light signals are then captured and used to determine the DNA sequence. Illumina sequencing offers several advantages, such as high-throughput capabilities, cost-effectiveness, and the ability to generate short reads with high accuracy. It is widely used in various applications, including whole-genome sequencing, transcriptome analysis, and metagenomics.

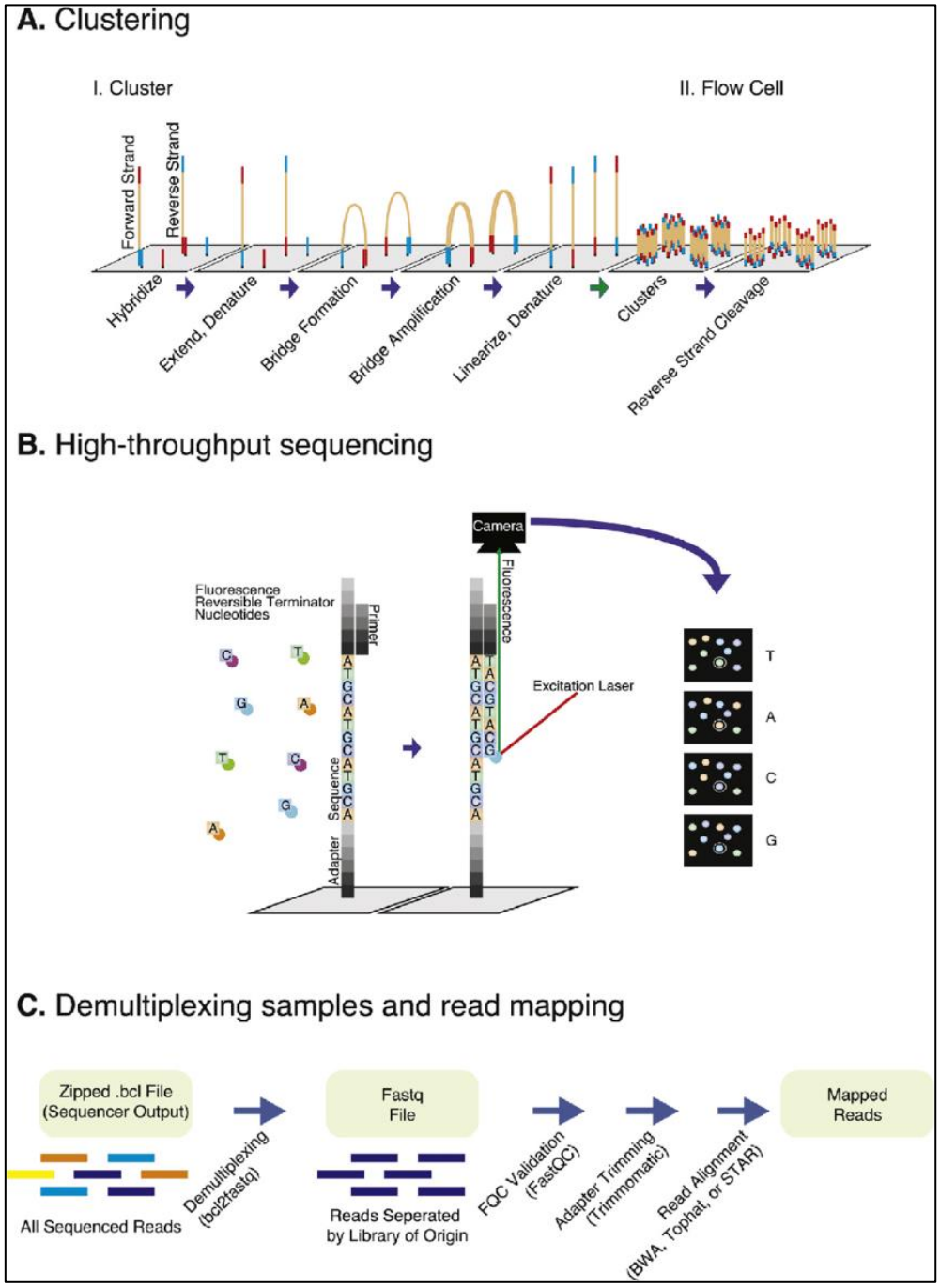

Fig 9: Illumina Sequencing Method Workflow

Fig10: Illumina Sequencing Instrument

Third-Generation Sequencing:

Third-generation sequencing refers to a newer generation of sequencing technologies that offer advantages over previous generations, such as longer read lengths, reduced amplification bias, and the ability to directly sequence native DNA molecules. These technologies, including platforms like PacBio and Oxford Nanopore, have revolutionized genetic research by enabling the sequencing of long DNA fragments without the need for fragmentation or amplification.

Methods of Third-Generation Sequencing:

1. **PacBio's Single-Molecule Real-Time (SMRT) Sequencing:**

   Single-molecule real-time (SMRT) sequencing is a third-generation sequencing technology developed by Pacific Biosciences (PacBio).

   The principle behind Single-Molecule Real-Time (SMRT) sequencing, involves the real-time detection of individual nucleotides as they are incorporated into a growing DNA strand. This technology utilizes zero-mode waveguides (ZMWs) to trap and sequence single DNA molecules, enabling long reads and the detection of complex genomic features like repetitive sequences and structural variants. SMRT sequencing offers advantages such as high accuracy, long read lengths, and the ability to directly sequence native DNA molecules without the need for fragmentation or amplification. This innovative approach has significantly advanced genetic research by providing high-throughput and high-sensitivity capabilities that were previously challenging with older sequencing technologies.

This technology has been instrumental in various applications, including genome assembly, transcriptome analysis, and CRISPR-Cas9 gene editing, offering high-throughput and high-sensitivity capabilities that are advantageous over previous sequencing technologies.

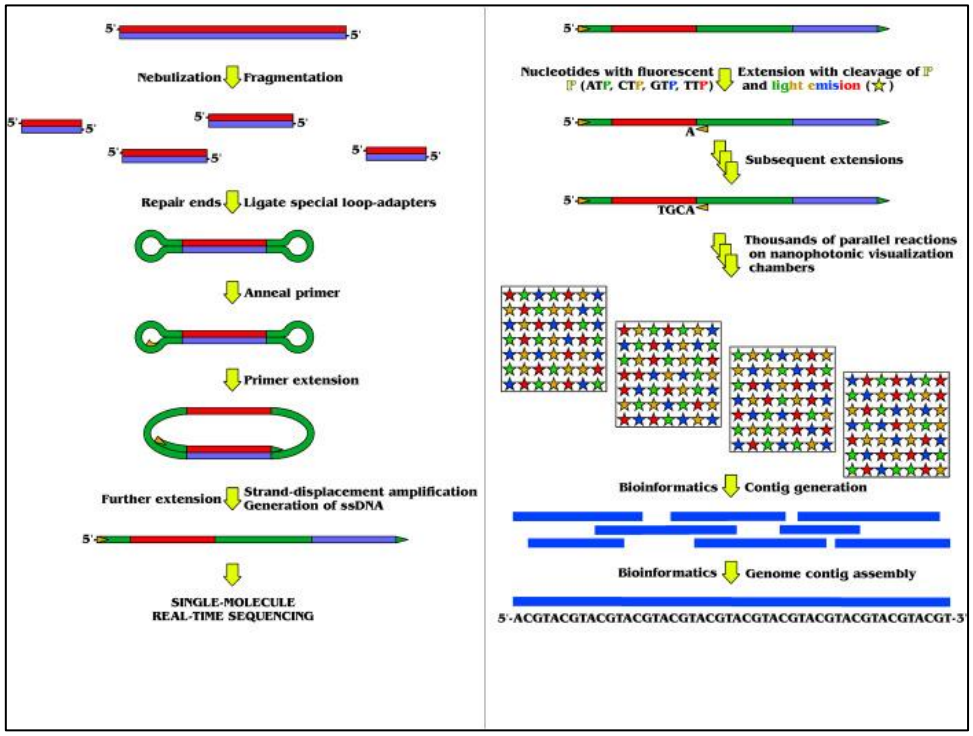

Fig 11: Single-Molecule Real-Time (SMRT) Sequencing Workflow

2. Oxford Nanopore Sequencing:

Oxford Nanopore sequencing is a cutting-edge technology that involves monitoring the progress of DNA molecules through a membrane pore for sequencing. This method utilizes nanopore-based sequencing instruments like the Oxford Nanopore MinION, which can sequence very long DNA molecules in real-time. Despite an accuracy of around 90%, Oxford Nanopore sequencing offers the advantage of generating long reads, sequencing RNA directly, and detecting modified bases.

The principle behind Oxford Nanopore sequencing involves passing DNA molecules through a nanopore embedded in a membrane and measuring changes in electrical current as individual nucleotides pass through the pore. This technology allows for the real-time sequencing of DNA or RNA molecules by detecting the unique electrical signals produced by different nucleotides. Detection of modified bases, makes it a valuable tool for various genomic applications due to its ability to provide real-time data and sequence long molecules.

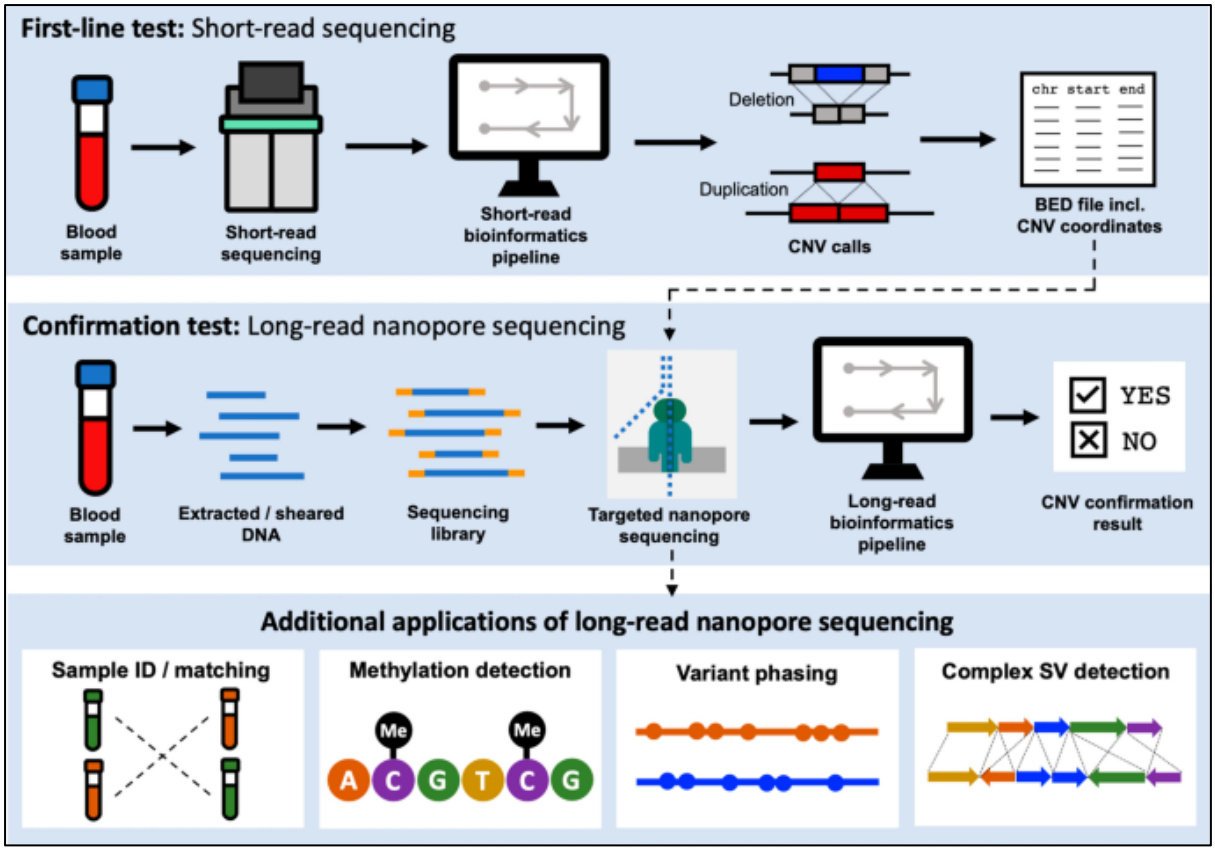

Fig 12: Oxford Nanopore Sequencing Workflow

Fig 13: Oxford Nanopore Instrument

Galaxy Europe Server:

The Galaxy Server, accessible at https://usegalaxy.eu and maintained by the Freiburg Galaxy Team as well as a global community of researchers, developers, and infrastructure providers as, is a part of the larger Galaxy Project, which is a robust platform for bioinformatics analysis. It offers a user-friendly web interface, enabling researchers to upload, analyze, and visualize

data without extensive programming skills. Collaboration is encouraged through the sharing of workflows, tools, and datasets. The platform promotes scalability and reproducibility, allowing users to create customized workflows and integrate diverse bioinformatics tools and databases for a wide range of analyses.

The Galaxy Server provides access to:

1. A huge compute and storage resource without any charge
2. More than 2500 different, well-documented and constantly maintained scientific tools
3. 250 GB per user (500 GB for ELIXIR members)
4. Free registration

Data Analysis and Visualization

Galaxy, accessible via web browser, offers powerful data analysis tools without requiring programming knowledge. It automates computation on a cluster and cloud, providing ample storage (250 GB per user) and API access for advanced users. It supports publication-ready visualizations and interactive tools like Trackster and Phinch.

Reproducibility and Transparency

Galaxy ensures reproducibility through its history feature, capturing inputs, parameters, and tool versions, shareable even outside Galaxy. It facilitates workflow creation from histories or scratch, downloadable and shareable, promoting transparency and avoiding vendor lock-in. It hosts thousands of tools with fixed versions managed by Bioconda and BioContainers, with 4 TB of reference data available**.**

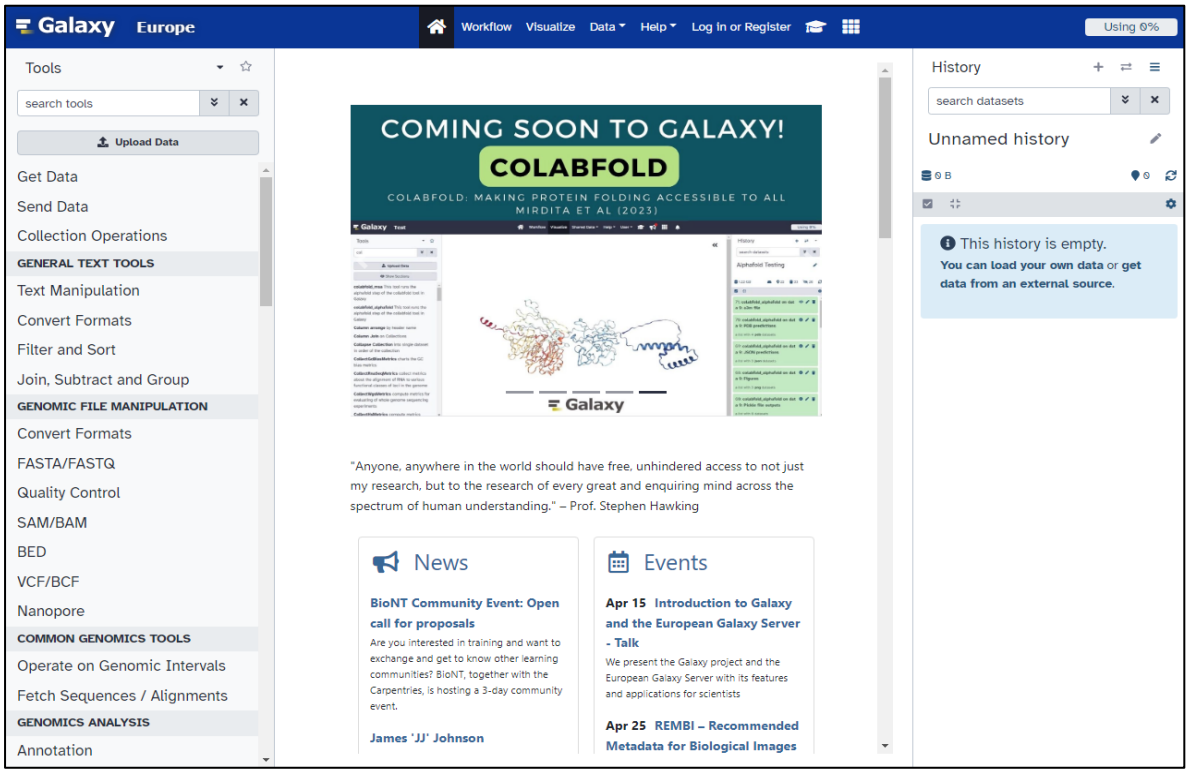

Fig 14: Homepage of Galaxy Server (Europe)

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DATE: 23/03/2024

WEBLEM 9(A)
NGS FILE FORMATS

1. **NCBI SRA DATABASE (URL: https://www.ncbi.nlm.nih.gov/sra)**
2. **GALAXY EUROPE TOOL (URL: https://usegalaxy.org/)**

AIM:

To study the NGS file format for the sequence read with the Accession ID: 'SRR26208365' for the query '*Klebsiella pneumoniae*' using the NCBI Sequence Read Archive (SRA) database and Galaxy Europe platform.

INTRODUCTION:

Sequence Read Archive (SRA) Database

The SRA is NIH's archive of high-throughput sequencing data and is part of the International Nucleotide Sequence Database Collaboration (INSDC) that includes the NCBI Sequence Read Archive (SRA), the European Bioinformatics Institute (EBI), and the DNA Database of Japan (DDBJ). Data submitted to any of the three organizations are shared among them. The SRA is a publicly available repository of high throughput sequencing data. The archive accepts data from all branches of life as well as metagenomic and environmental surveys. SRA stores raw sequencing data and alignment information to enhance reproducibility and facilitate new discoveries through data analysis.

SRA accepts data from all kinds of sequencing projects including clinically important studies that involve human subjects or their metagenomes, which may contain human sequences. These data often utilize NIH controlled access via dbGaP (the database of Genotypes and Phenotypes). Data submitters need to determine if their data is suitable for public distribution or if it needs controlled access. It is the responsibility of submitting parties to ensure that they have appropriate consent for human sequence data to be distributed publicly without access controls. Following submission, data are subject to automated and manual processing to ensure data integrity and quality and are subsequently made available to the public.

Galaxy Europe Platform

The Galaxy Europe platform is an open, web-based tool designed for accessible, reproducible, and transparent computational biological research. It offers users the ability to run over 2500 scientific tools without the need for coding or command-line interfaces, all through a user-friendly web interface. This open-source platform facilitates the exploration of extensive biological datasets through a comprehensive suite of bioinformatics tools, accessible via a drag-and-drop interface. This platform emphasizes reproducibility by capturing all metadata from analyses, making them completely reproducible. Users can easily share and publish analyses through interactive pages, enhancing them with annotations. Galaxy Europe is scalable, capable of running on various systems from laptops to large clusters or the cloud. It provides free registration, 250 GB per user (500 GB for ELIXIR members), and on-demand training capacity, making it a valuable resource for researchers in the field of bioinformatics.

Klebsiella pneumoniae

Klebsiella pneumoniae is a type of bacteria commonly found in the intestines, typically harmless in healthy individuals but can pose serious risks if it enters other parts of the body, especially in individuals with certain health conditions. This bacterium can lead to severe infections like pneumonia, urinary tract infections, bloodstream infections, and more, particularly affecting individuals in healthcare settings or with compromised immune systems. *Klebsiella pneumoniae* infections are often acquired in hospitals and can be challenging to treat due to increasing antibiotic resistance, with some strains being resistant to common antibiotics. Preventing the spread of *Klebsiella* infections involves strict infection control measures, including hand hygiene and proper cleaning procedures in healthcare facilities. Early diagnosis and appropriate antibiotic treatment are crucial for managing *Klebsiella pneumoniae* infections effectively.

METHODOLOGY:

1. Open the NCBI SRA Homepage (https://www.ncbi.nlm.nih.gov/sra).
2. Search for the query '*Klebsiella pneumoniae*' and apply the filter – Source: DNA.
3. Select a particular sequence read from the list of hits obtained and note its Accession ID ('SRR26208365').
4. Open the Galaxy Europe Platform (https://usegalaxy.org/).
5. Search for the 'fasterq' tool in the Search dialog box.
6. After selecting the first tool '**Faster Download and Extract Reads in FASTQ format from NCBI SRA',** enter the Accession ID of the selected sequence read. ('SRR26208365').
7. Click on 'Run Tool'.
8. Note the results obtained in the form of fasterq-dump files and a log file.

OBSERVATIONS:

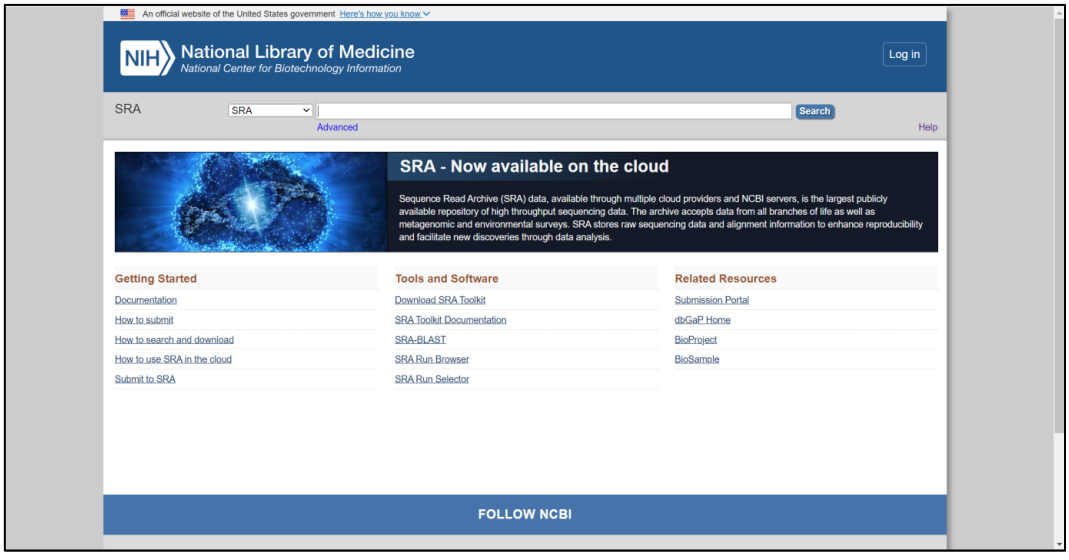

Fig 1: Homepage of NCBI SRA Database

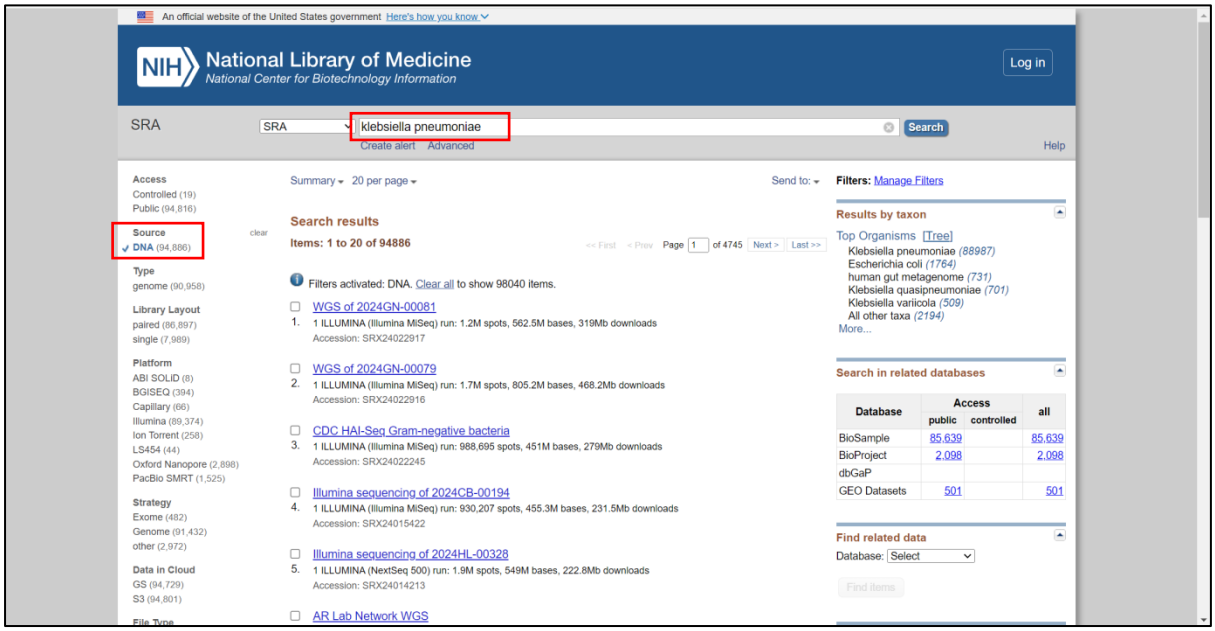

Fig 2: Query – '*Klebsiella pneumoniae*' searched and filter applied (Source: DNA) and 94886 hits were obtained

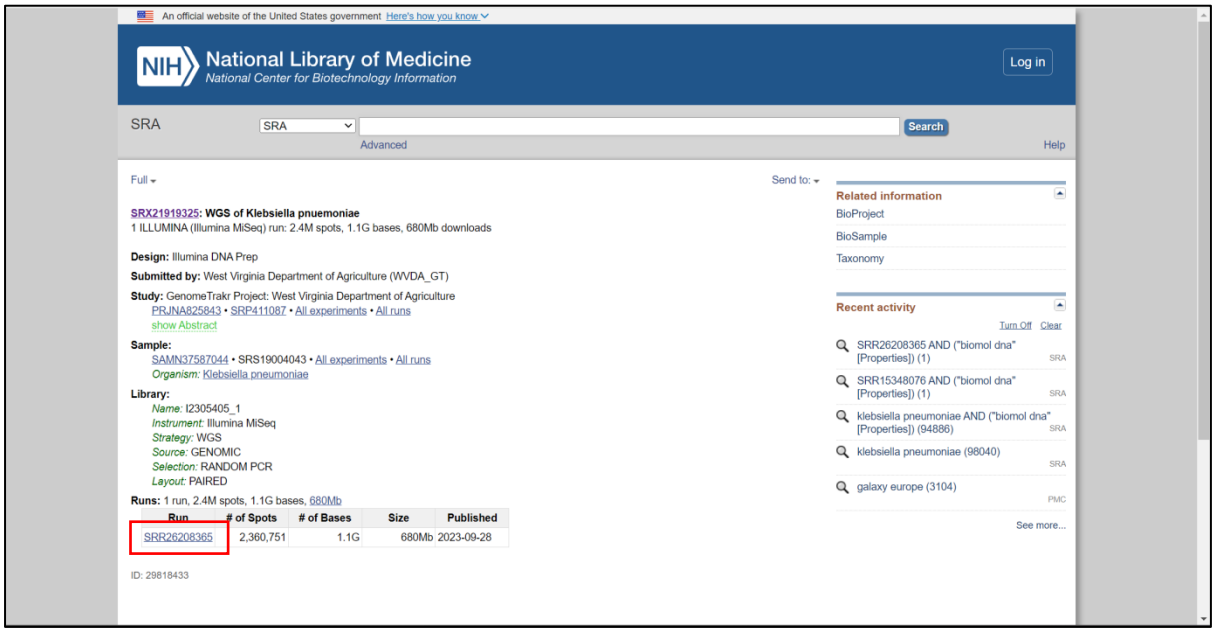

Fig 3: Information displayed for Run 'SRR26208365' to be used for further analysis

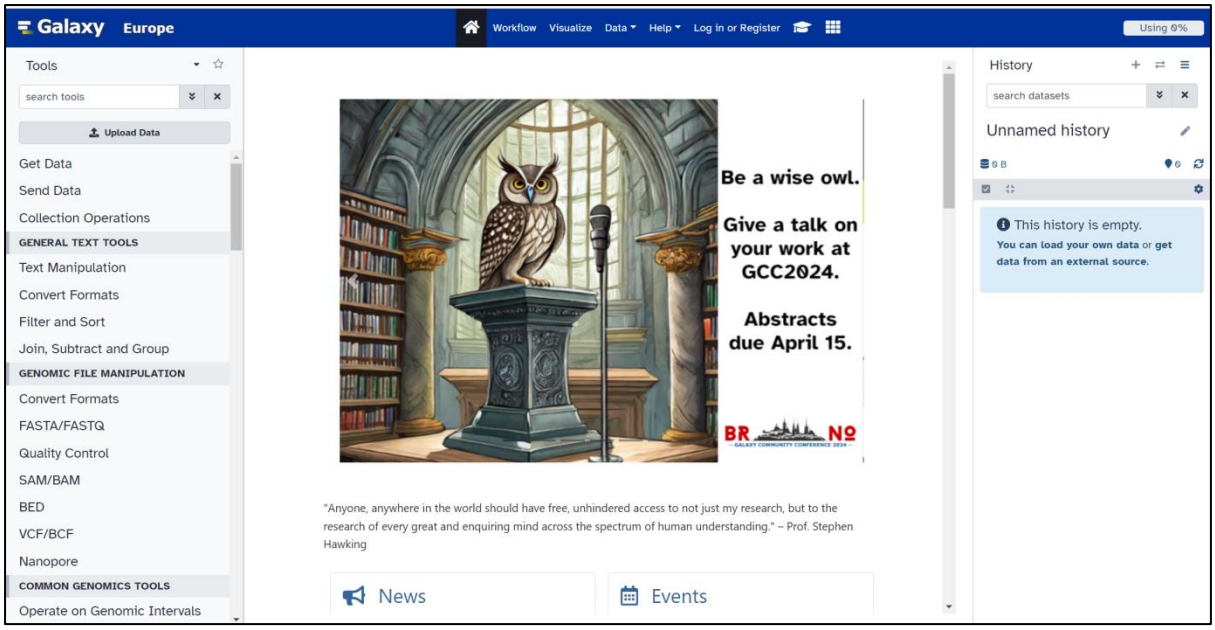

Fig 4: Homepage of Galaxy Europe tool

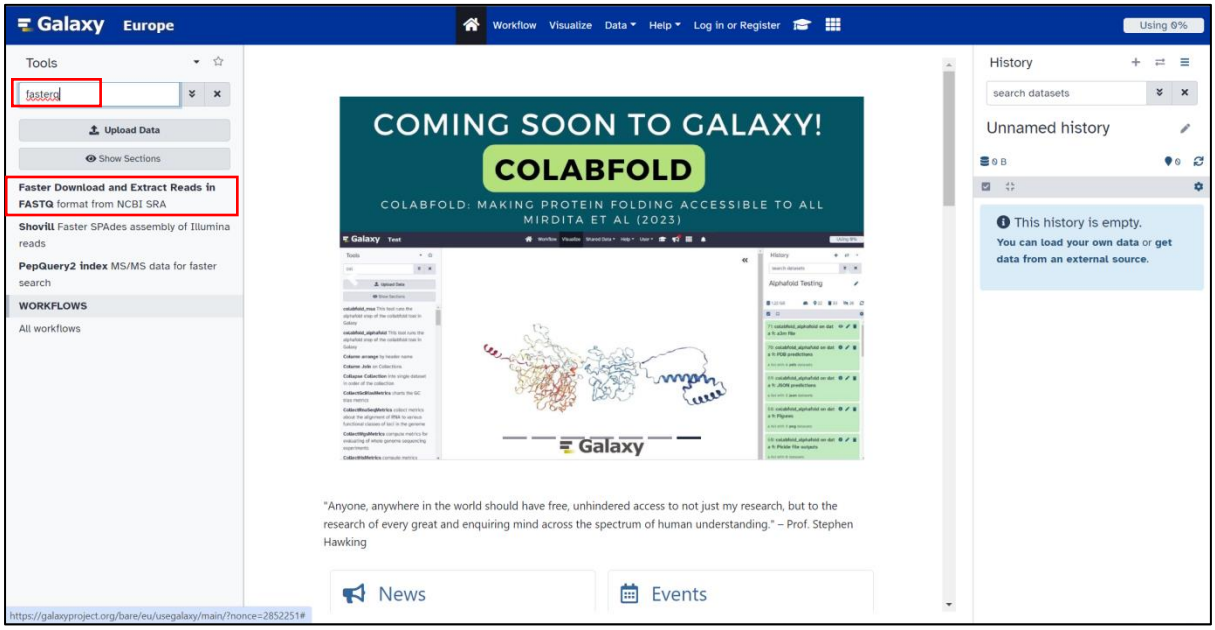

Fig 5: Searching for the 'fasterq' tool

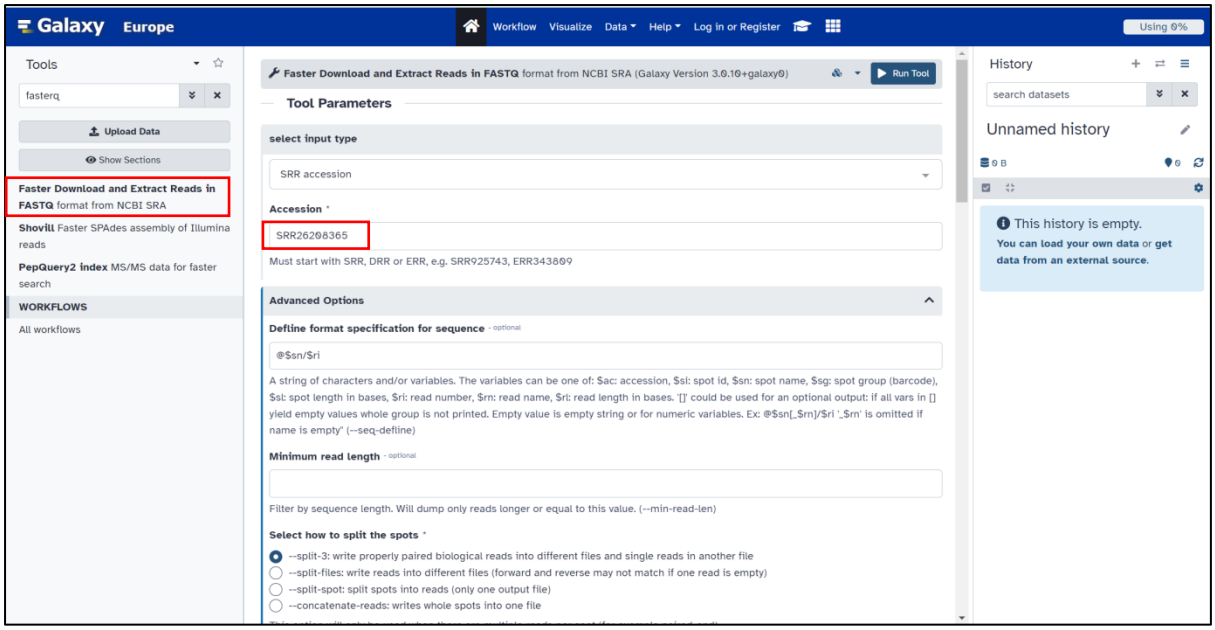

Fig 6: Entering the Accession ID of the selected run 'SRR26208365'

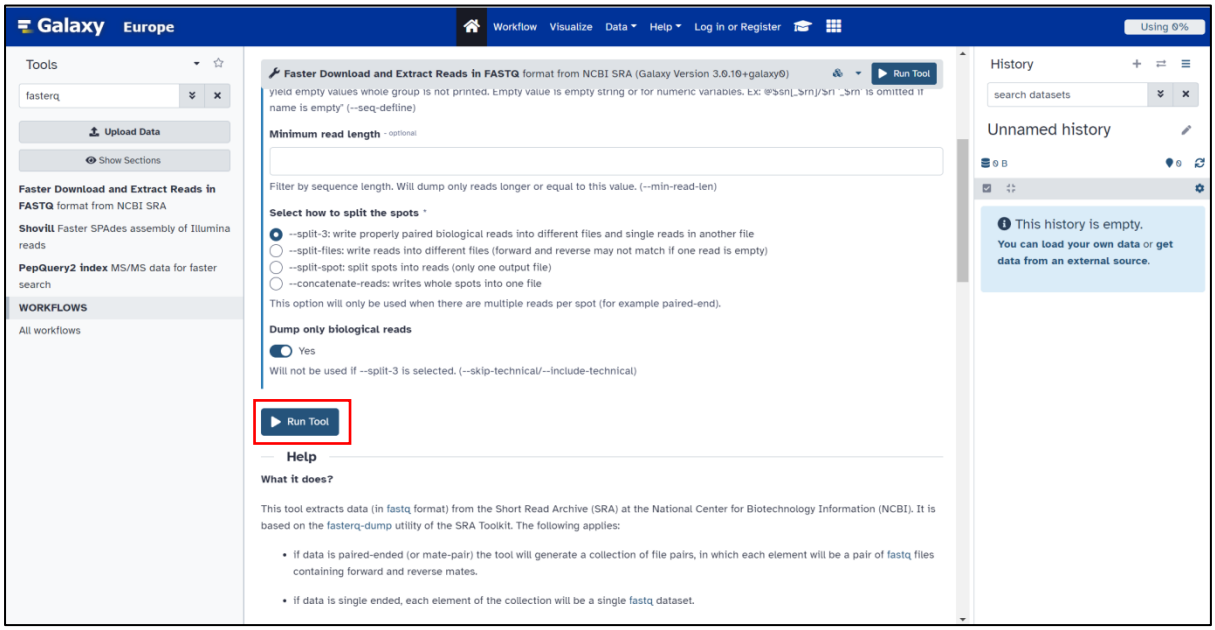

Fig 6.1: Display of the Advanced Options

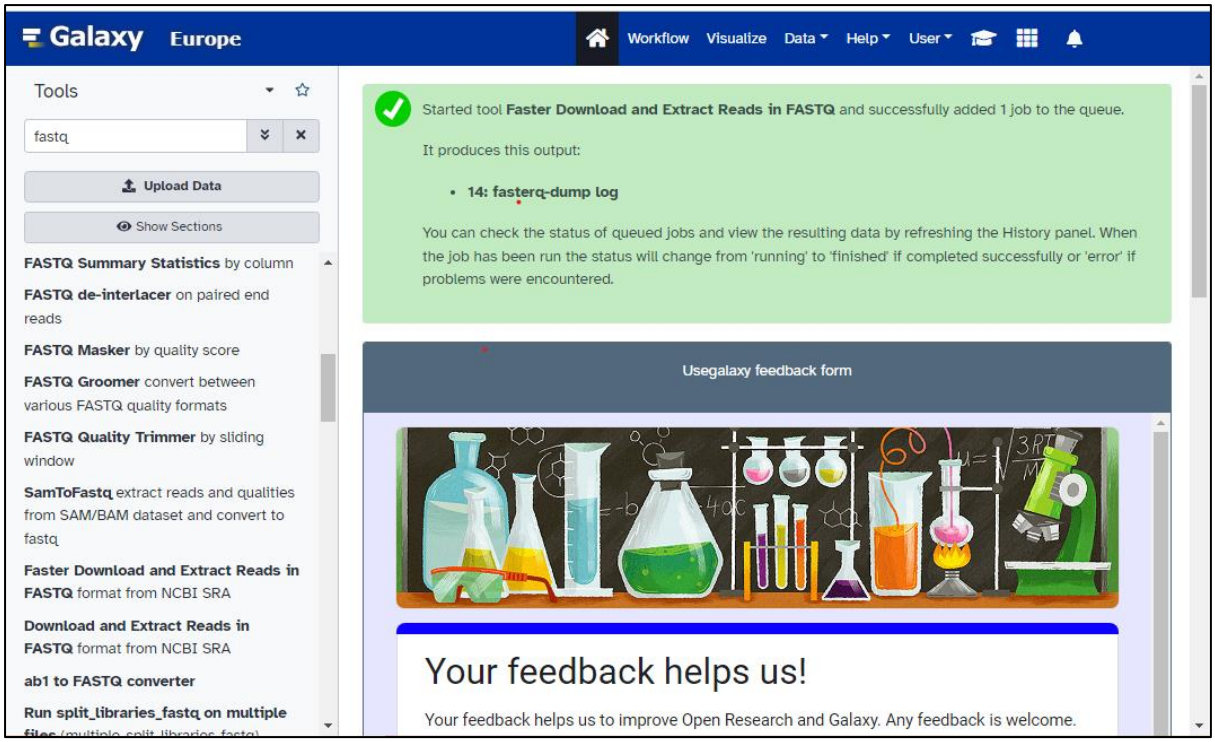

Fig 7: Job Running Image for Fastq

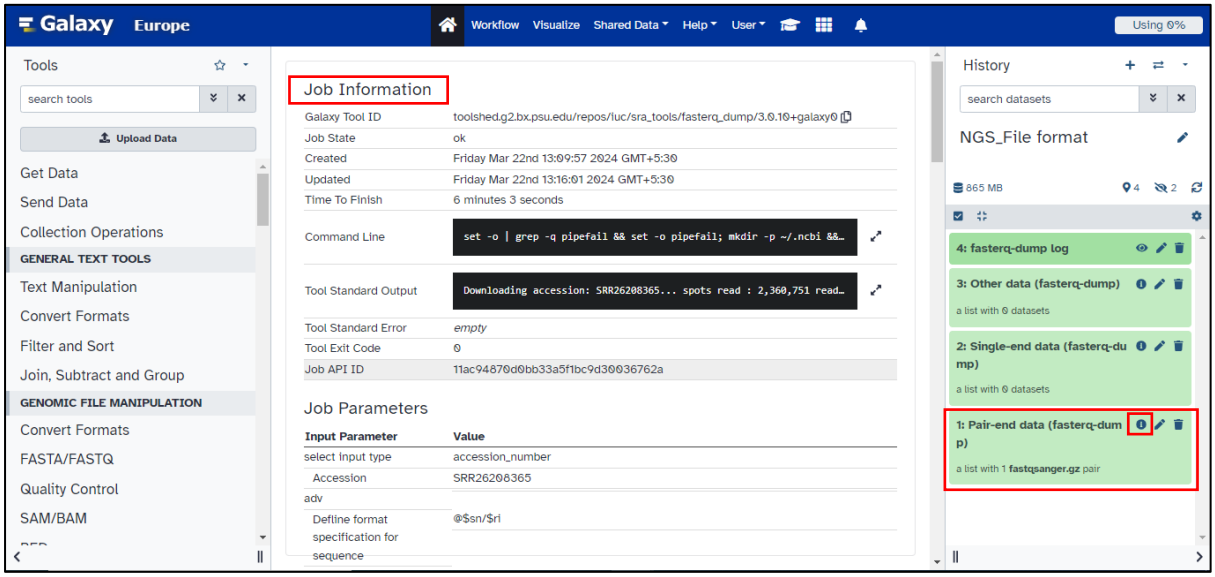

Fig 8: Viewing the results obtained in the 'Pair-end data (fasterq-dump)' file: Job Information

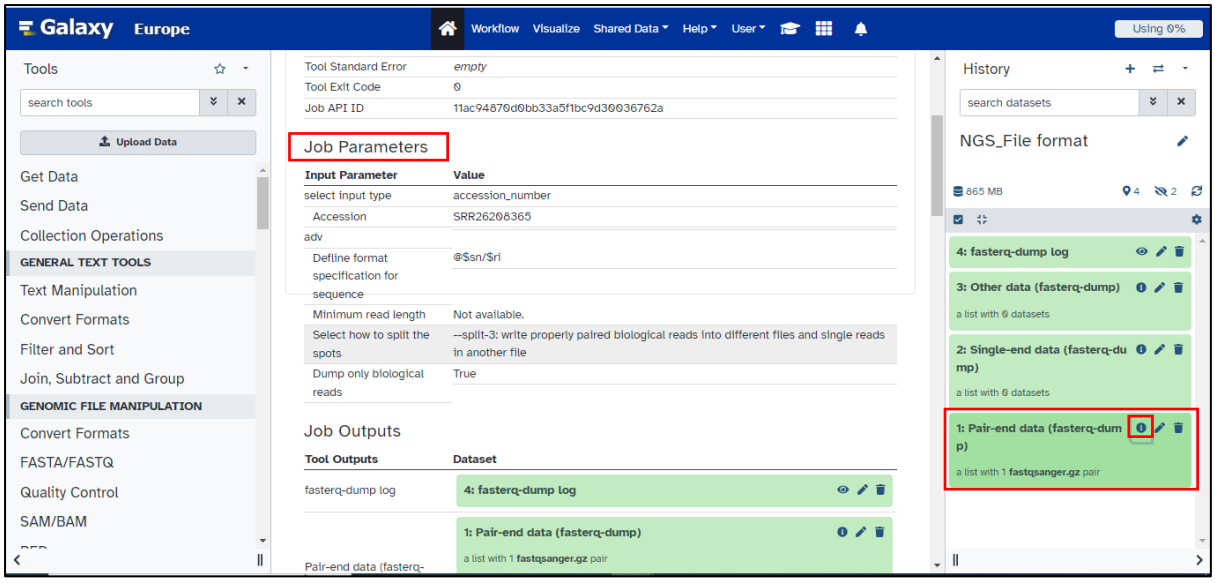

Fig 8.1: Viewing the results obtained in the 'Pair-end data (fasterq-dump)' file: Job Parameters

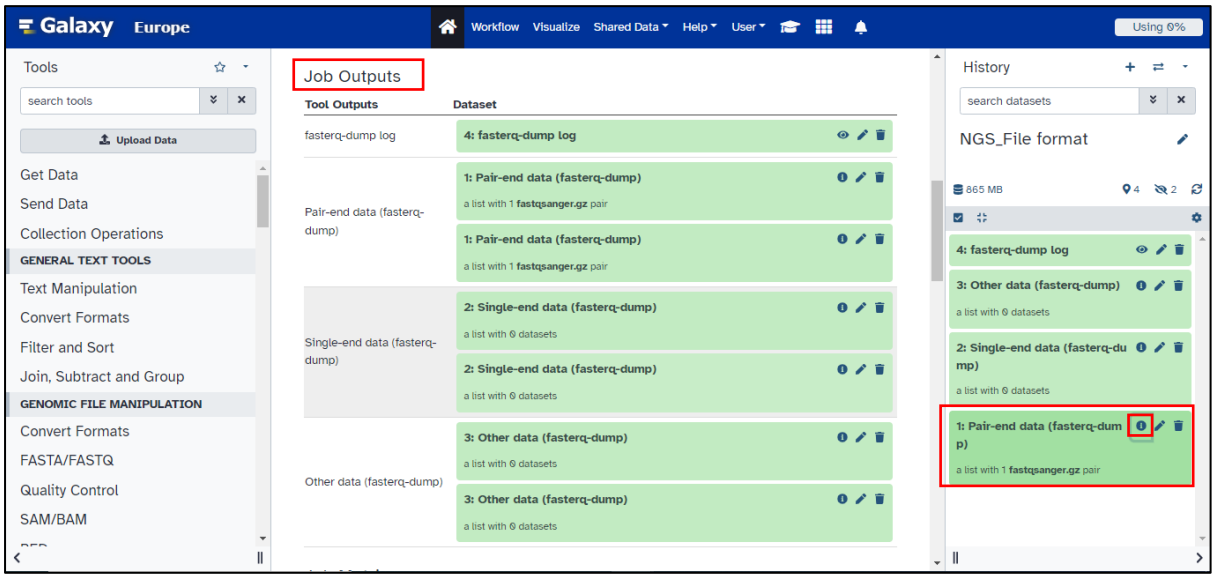

Fig 8.2: Viewing the results obtained in the 'Pair-end data (fasterq-dump)' file: Job Outputs

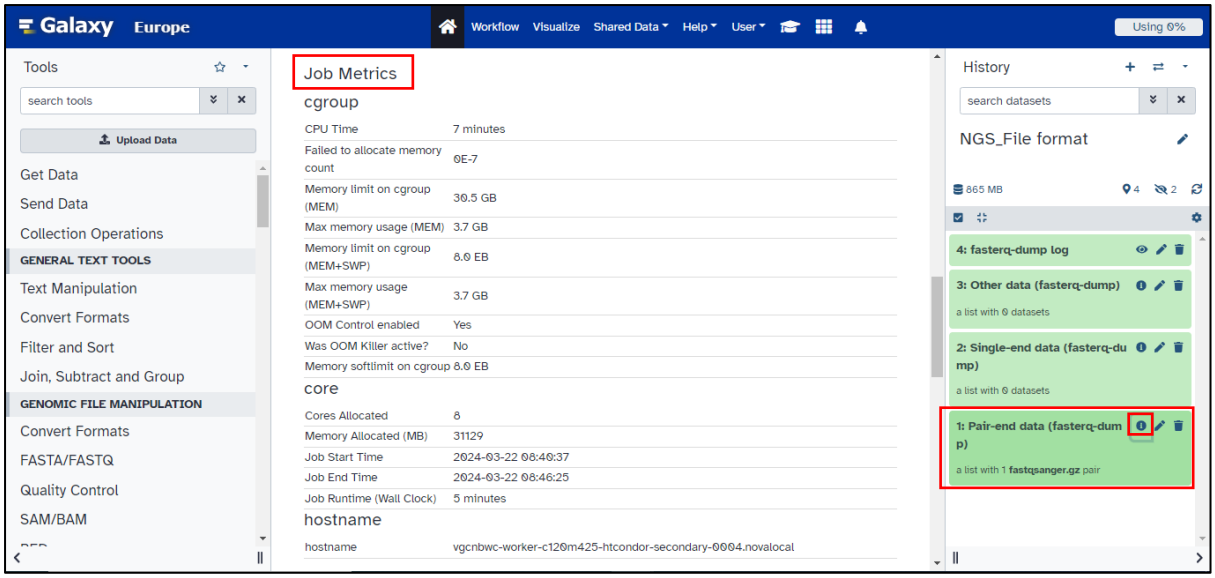

Fig 8.3: Viewing the results obtained in the 'Pair-end data (fasterq-dump)' file:Job Metrics

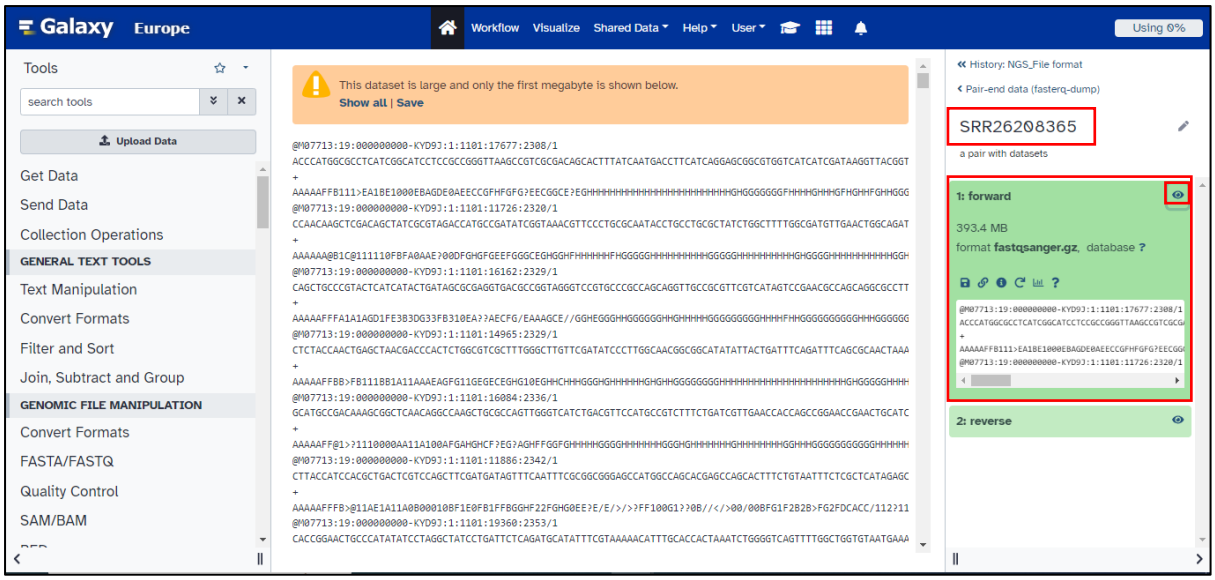

Fig 8.4: Viewing the forward read sequence after clicking on the 'Pair-end data (fasterq-dump)' tab

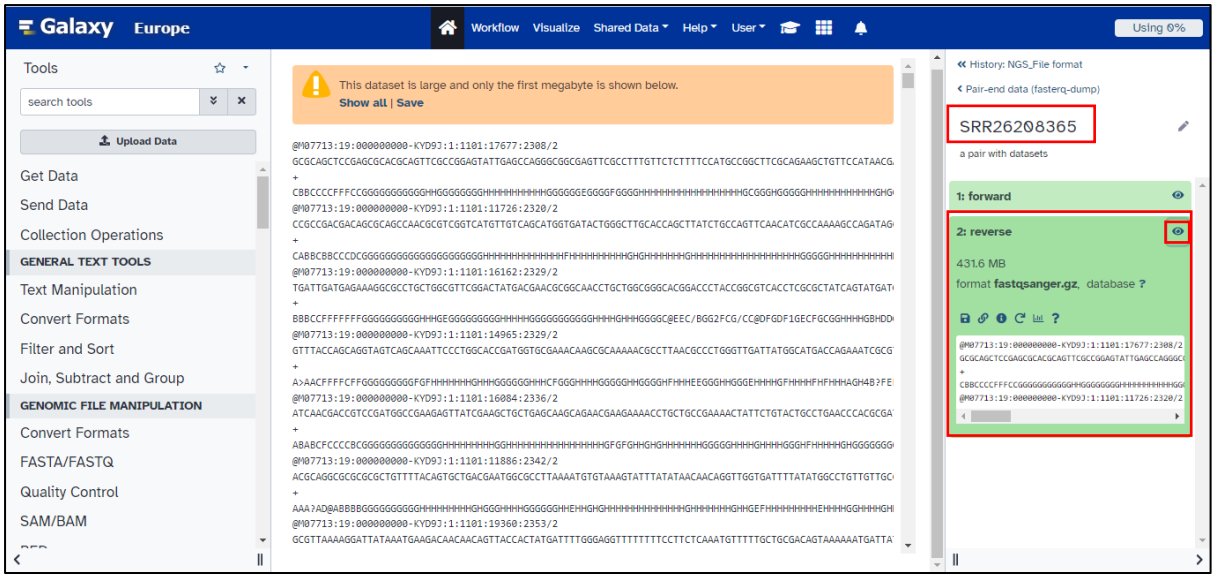

Fig 8.5: Viewing the reverse read sequence after clicking on the 'Pair-end data (fasterq-dump)' tab

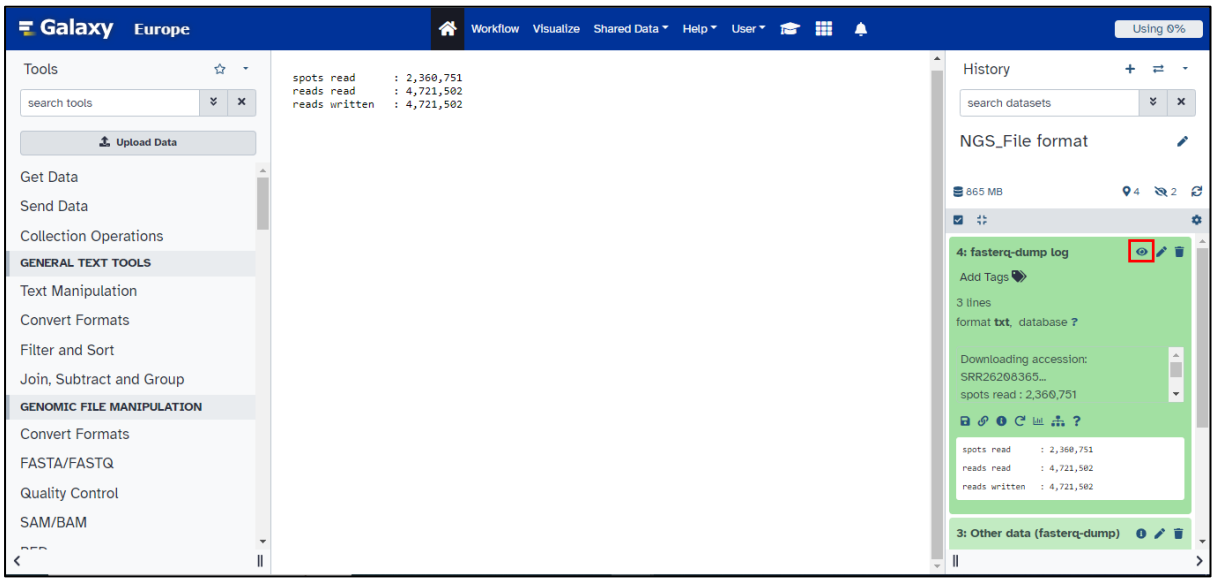

Fig 9: Viewing the log file of the complete run

RESULTS:

Using the NCBI Sequence Read Archive (SRA) database and Galaxy Europe platform, the fasterq-dump NGS file format was obtained and further studied for the sequence read with the Accession ID: 'SRR26208365' for the query '*Klebsiella pneumoniae*'. Job 'information, Job Parameters, Job Outputs and Job Metrics were observed when viewing the 'Pair-end data (fasterq-dump' file, along with viewing the sequences for the forward read and the reverse read. When viewing the log file of the run, it was found that 2,360,751 spots were read, 4,721,502 reads were read and 4,721,502 reads were written.

CONCLUSION:

The NGS file format was obtained and further studied for the sequence read with the Accession ID: 'SRR26208365' for the query '*Klebsiella pneumoniae*' using the NCBI Sequence Read Archive (SRA) database and Galaxy Europe platform.

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2. Afgan, E., Baker, D., Batut, B., van den Beek, M., Bouvier, D., Cech, M., Chilton, J., Clements, D., Coraor, N., Grüning, B. A., Guerler, A., Hillman-Jackson, J., Hiltemann, S., Jalili, V., Rasche, H., Soranzo, N., Goecks, J., Taylor, J., Nekrutenko, A., & Blankenberg, D. (2018). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic acids research, 46(W1), W537–W544. https://doi.org/10.1093/nar/gky379
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5. Klebsiella pneumoniae in Healthcare Settings | HAI | CDC. (n.d.). https://www.cdc.gov/hai/organisms/klebsiella/klebsiella.html

DATE: 10/04/2024

WEBLEM 9(B)
TRIMMOMATIC
(URL: https://usegalaxy.org/)

AIM:

To improve overall quality of NGS raw data by using trimmomatic tool for the given query.

INTRODUCTION:

Trimming refers to removing unwanted or low-quality sequences from high-throughput sequencing data. Trimming helps to remove the regions of low confidence, sequencing artifacts, adapter sequences, and low-quality bases. This means that these artifacts and errors have to be removed, and this process of removal is known as trimming. By performing trimming data, bioinformaticians can obtain cleaner, more accurate, and more reliable sequencing data. This is further important in obtaining high-quality results in various downstream bioinformatics applications, such as genome assembly, variant calling, gene expression analysis, and other biological investigations.

Trimmomatic is one of the most popular bioinformatics tools for quality control (QC) and next-generation sequencing (NGS) data preprocessing. It is widely used due to its efficiency, flexibility, and ability to work with various sequencing data formats. Trimmomatic's main functionality is to remove low-quality regions and sequencing artifacts from raw NGS reads, ensuring that only high-quality, reliable data is used for the downstream analysis. Trimmomatic is a widely-used tool for this purpose, as it can handle both single-end and paired-end data and supports various types of adapter sequences.

Before trimming data, you may observe sequences with varying lengths, potentially containing adapter sequences, low-quality bases, and regions with poor sequencing quality. The data may also contain artifacts introduced during the sequencing process, such as sequencing errors or biases. After trimming the data, you would typically see cleaner and more uniform sequencing reads. Adapter sequences would have been removed from the reads, ensuring they do not interfere with downstream analysis. Low-quality bases at the ends of reads would have been trimmed, improving the overall quality of the data. Regions of poor sequencing quality would have been identified and trimmed using Trimmomatic's sliding window approach, resulting in more reliable sequencing reads for subsequent analysis.

In Trimmomatic, various parameters can be set to customize the trimming process for NGS data. Some of the key parameters include:

1. **ILLUMINACLIP:** Cut adapter and other illumina-specific sequences from the read.
2. **SLIDINGWINDOW:** Perform a sliding window trimming, cutting once the average quality within the window falls below a threshold.
3. **MINLEN:** Drop the read if it is below a specified length.
4. **LEADING:** Cut bases off the start of a read, if below a threshold quality.
5. **TRAILING:** Cut bases off the end of a read, if below a threshold quality.
6. **CROP:** Cut the read to a specified length.

7. **HEADCROP:** Cut the specified number of bases from the start of the read.
8. **AVGQUAL:** Drop the read if the average quality is below a specified value.
9. **MAXINFO:** Trim reads adaptively, balancing read length and error rate to maximise the value of each read.

METHODOLOGY:

FastQC was performed but the read quality was not so good, thus this tool is used to improve the quality of the reads.

1. Open homepage of Galaxy Tool.
2. Search Trimomatic in search tool box.
3. Set different parameters of the tool.
4. Then set the different operations.
5. Run the tool and interpret the result which are come in four differnent sections.

OBSERVATIONS:

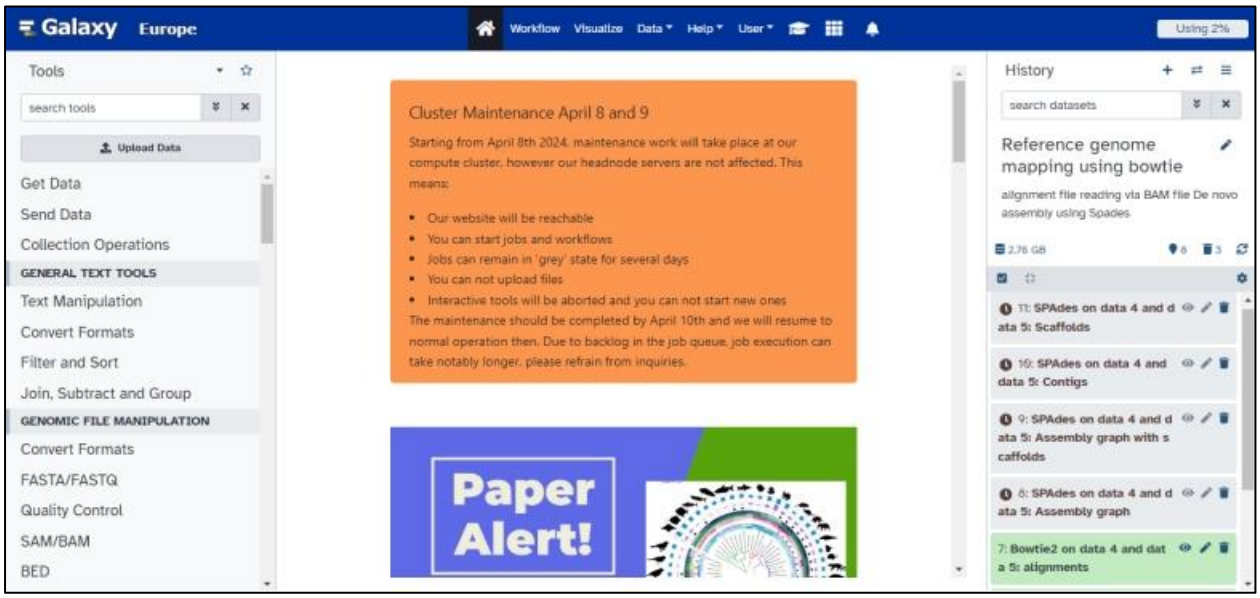

Fig 1: Homepage of Galaxy Europe Tool

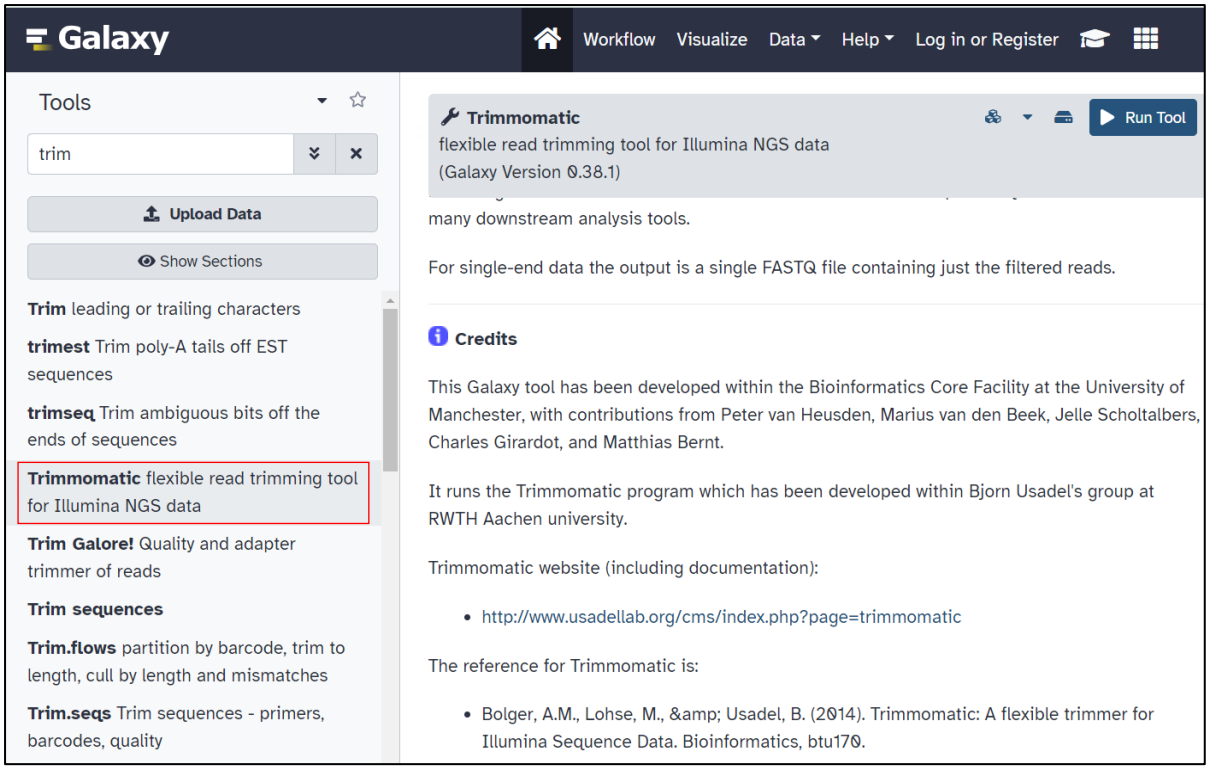

Fig 2: Select the Trimmomatic option in search tool box

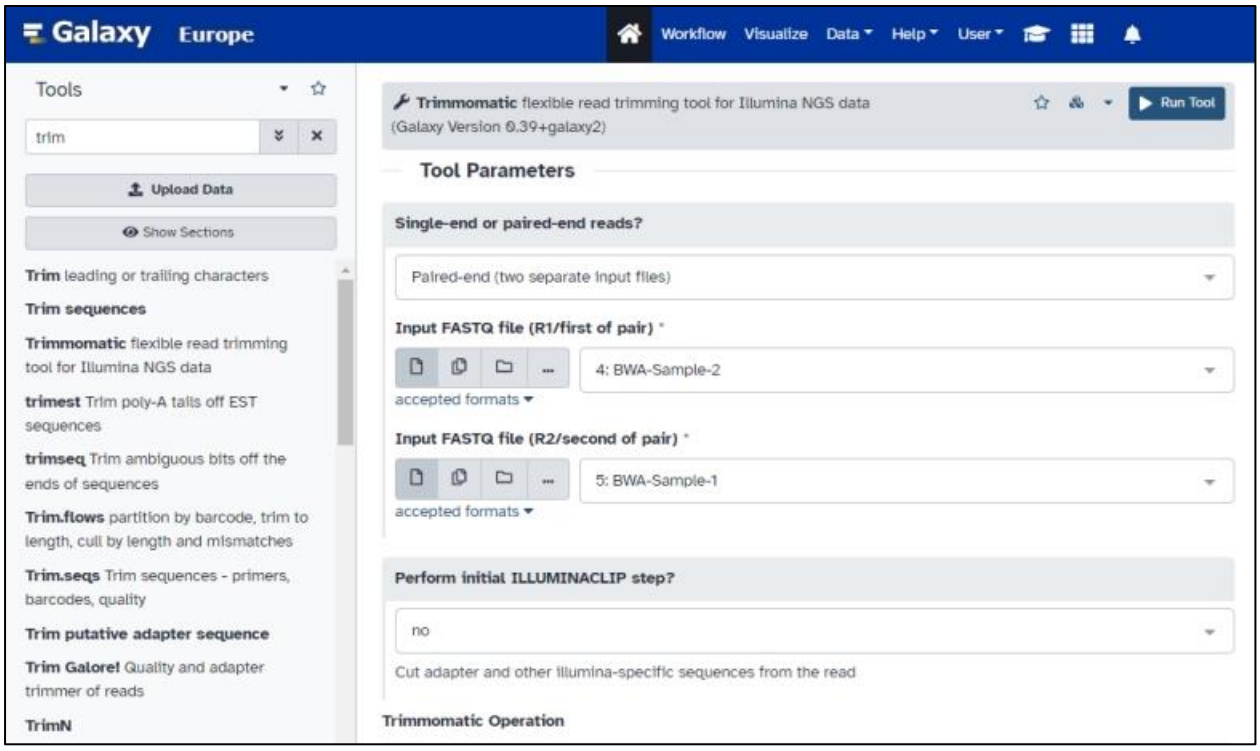

Fig 3: Set the tool parameters

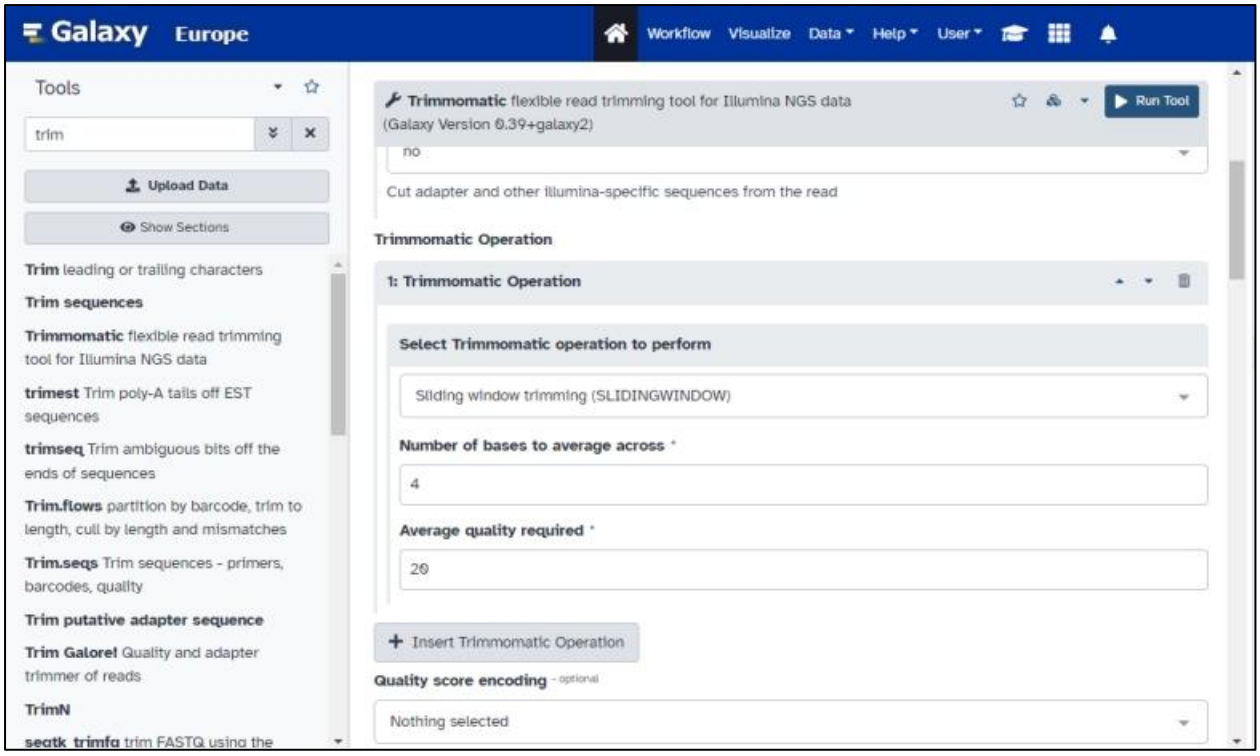

Fig 4: Set the trimmomatic operations

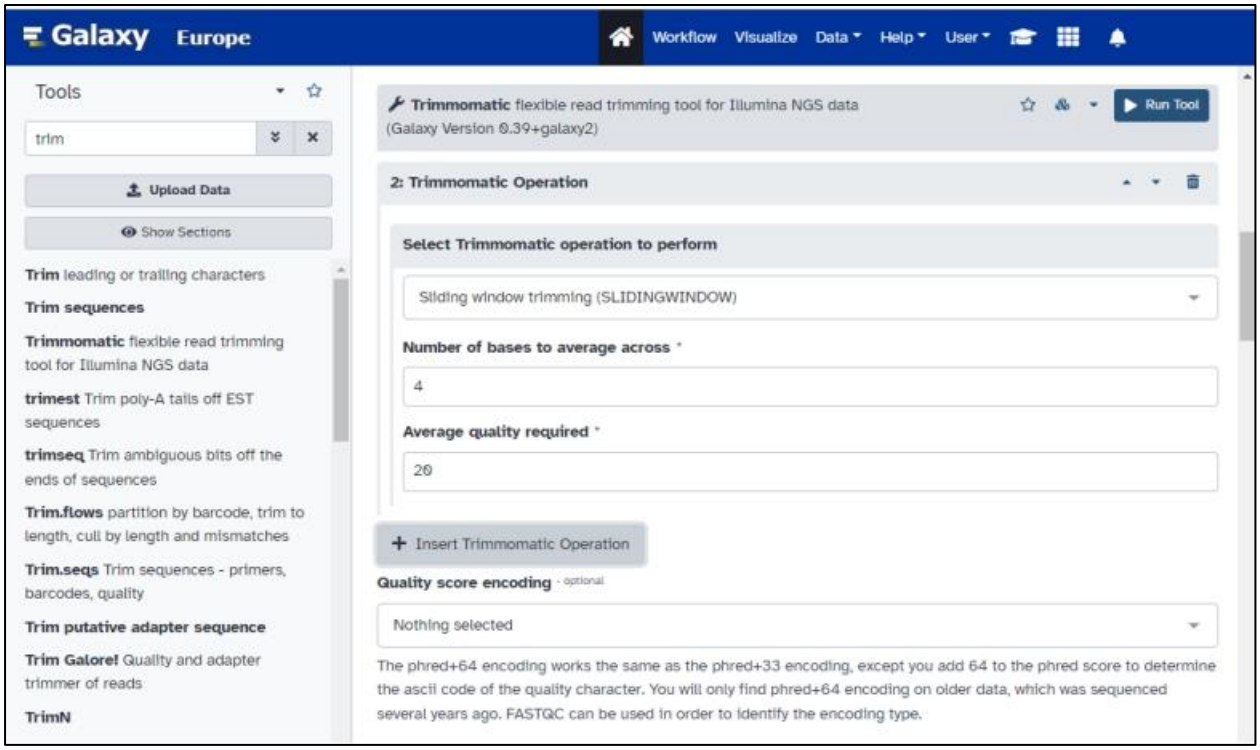

Fig 4.1: Set the trimmomatic operations

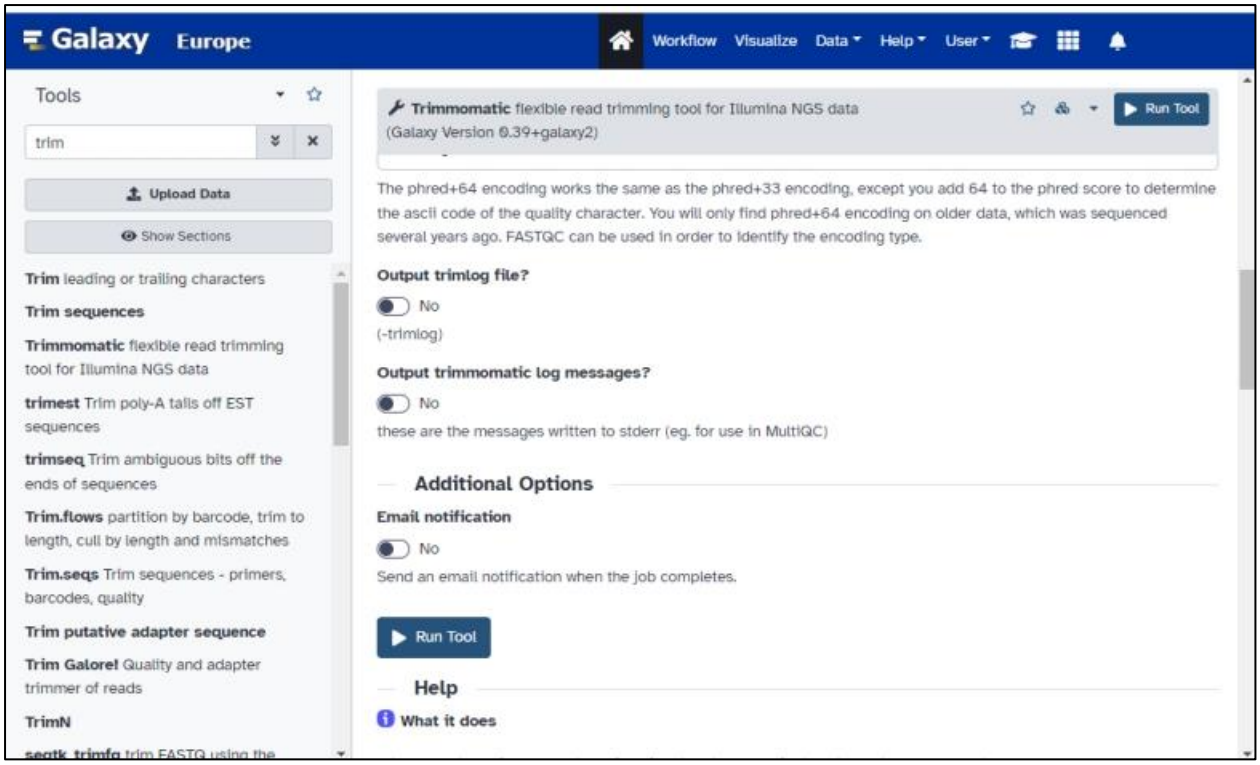

Fig 5: Run the tool

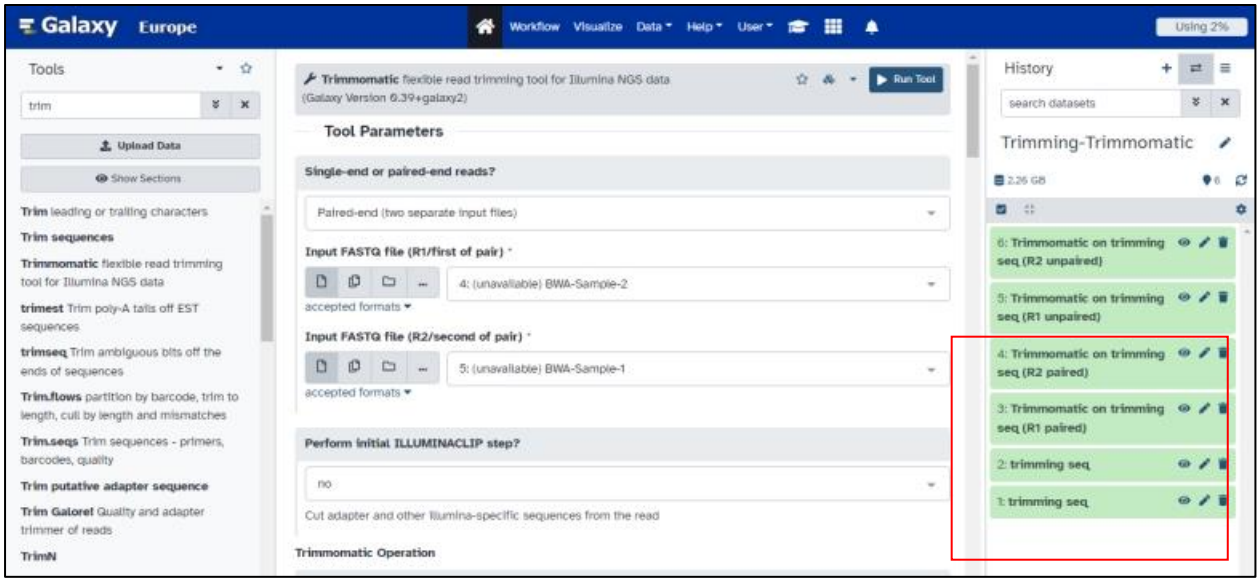

Fig 6: Results appears in 4 different sections

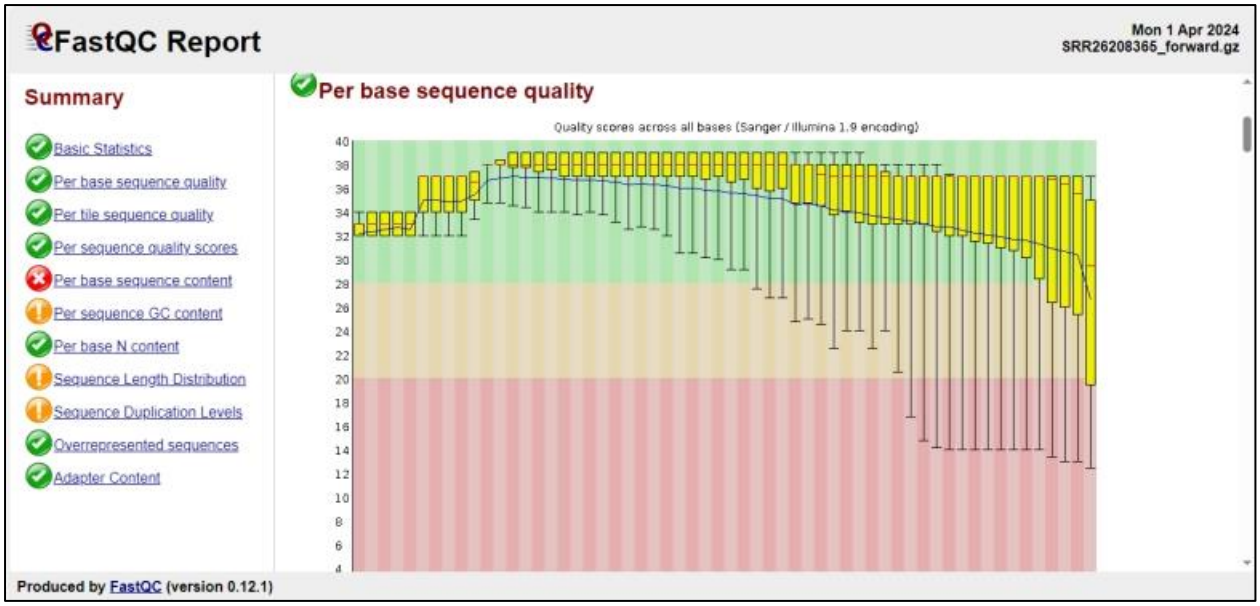

Fig 7: Pre-Trimming data

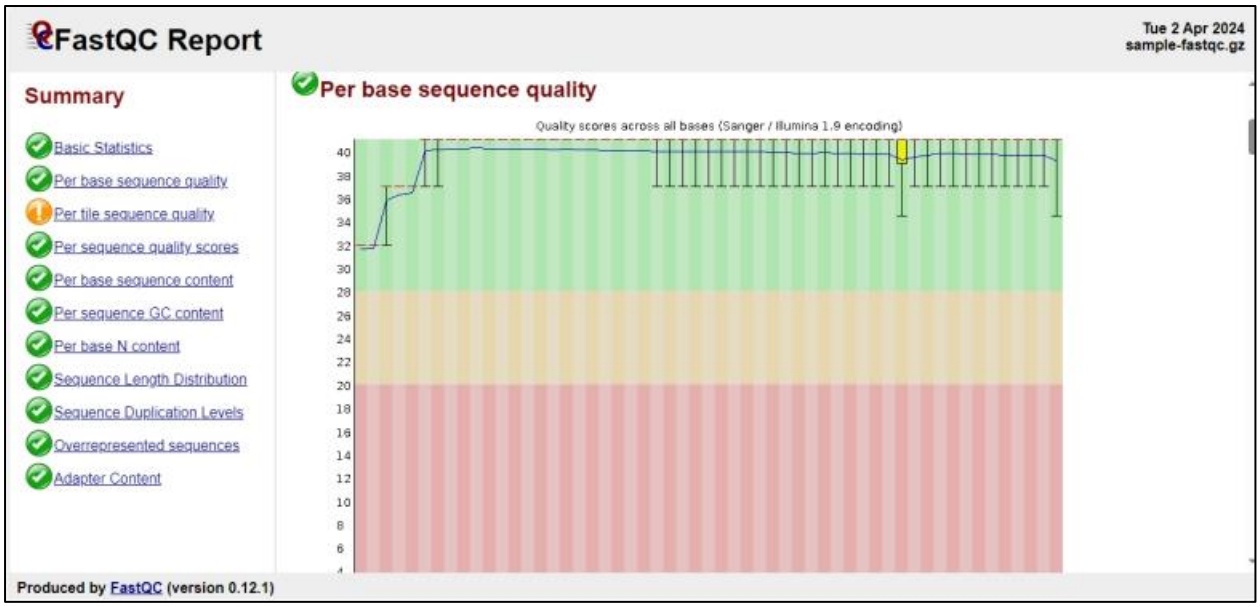

Fig 7.1: Post-Trimming data

RESULTS:

The trimmomatic produces trimmed FASTQ files containing sequences that pass the specified quality thresholds by generates quality control metrics such as the number of reads processed, the number of reads kept after trimming, the average quality scores before and after trimming, and the percentage of reads trimmed. Thus it provide insights into the quality of the sequencing data before and after trimming. Higher retention of reads and improvement in quality scores indicate effective trimming. The generated the in form of .bam will be further used for assembly studies.

CONCLUSION:

Trimmomatic tool has effectively removed low-quality regions and adapters for the query sequence.

REFERENCES:

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2. https://www.researchgate.net/publication/261327470_Trimmomatic_A_Flexible_Trimmer_for_Illumina_Sequence_Data
3. Hamzić, E. (2024, January 3). All You Need To Know About Trimmomatic & NGS Data Trimming. BioComputiX. https://www.biocomputix.com/post/trimming-ngs-data-trimmomatic

DATE: 04/04/2024

WEBLEM 9(C)
NGS- FASTQC
(URL: https://usegalaxy.eu/)

AIM:

To assess the quality of raw sequencing data and identify potential issues or biases using FASTQC for quality control.

INTRODUCTION:

Quality control (QC) is critical in next-generation sequencing (NGS) to ensure the reliability and integrity of the vast amounts of sequencing data generated. QC measures are essential to identify and address any anomalies, errors, and biases that may arise during library preparation, sequencing, and data analysis. Not only do QC measures enhance the accuracy of biological interpretations, but they also contribute to the cost-effectiveness, reproducibility, and comparability of results. Furthermore, QC enables the detection and quantification of biases and artifacts, optimization of experimental parameters, and ultimately the generation of high-quality data essential for advancing biological research, clinical diagnostics, and various applications of NGS technology.

FastQC is a highly versatile quality control tool designed specifically for analyzing sequencing data, particularly FastQ files, generated by modern high-throughput sequencers. Its primary function is to identify potential issues or biases in the raw data before downstream analysis. Unlike many sequencer-generated QC reports that focus solely on issues originating from the sequencer itself, FastQC aims to detect problems originating from both the sequencer and the initial library material. It offers two operational modes: an interactive standalone application for analyzing small numbers of files and a non-interactive mode suitable for processing large numbers of files within larger analysis pipelines.

One of FastQC's fundamental operations is opening and analyzing sequence files. Users can open files interactively, and newly opened files appear as tabs, allowing for easy navigation and analysis. FastQC supports various file formats, including FastQ, Casava FastQ, Colorspace FastQ, SAM, BAM, and GZip compressed FastQ. It also provides options for users to manually specify the file format if needed. Upon analysis, FastQC generates a summary report indicating whether the results are normal, slightly abnormal, or very unusual, enabling users to quickly identify potential issues in their data.

METHODOLOGY:

1. Open the Galaxy Server homepage and search for FastQC tool.
2. Use the result file of trimmomatic as the input for FastQC analysis.
3. Set the tool parameters and submit the file.
4. 4 result files are generated (for paired end sequence) out of which 2 are of raw data and 2 of webpage.

5. Open the webpage in which FastQC report is generated.
6. Interpret the results accordingly.

OBSERVATIONS:

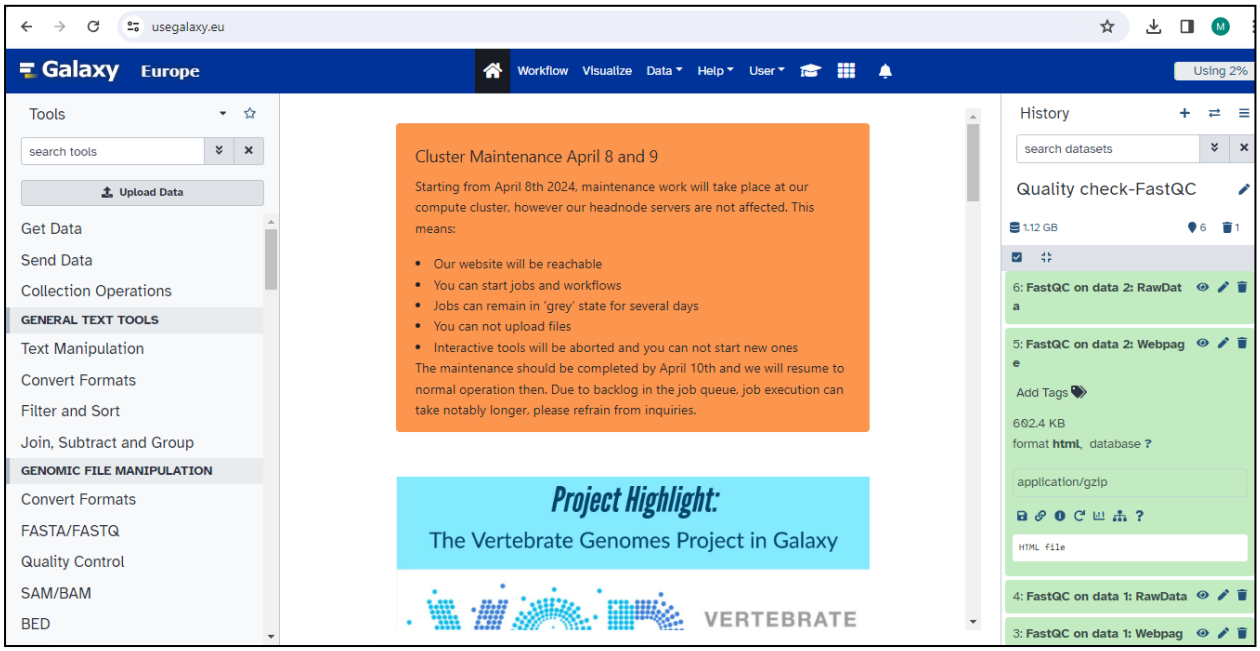


Fig 1: Galaxy Server Homepage

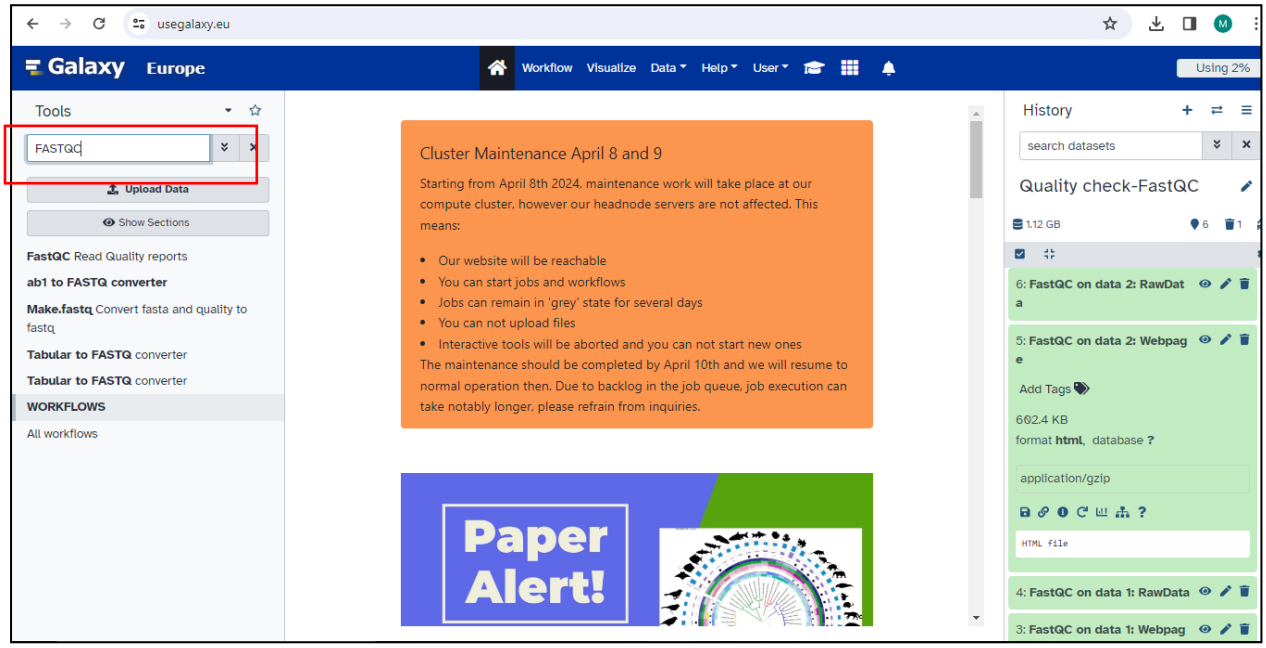
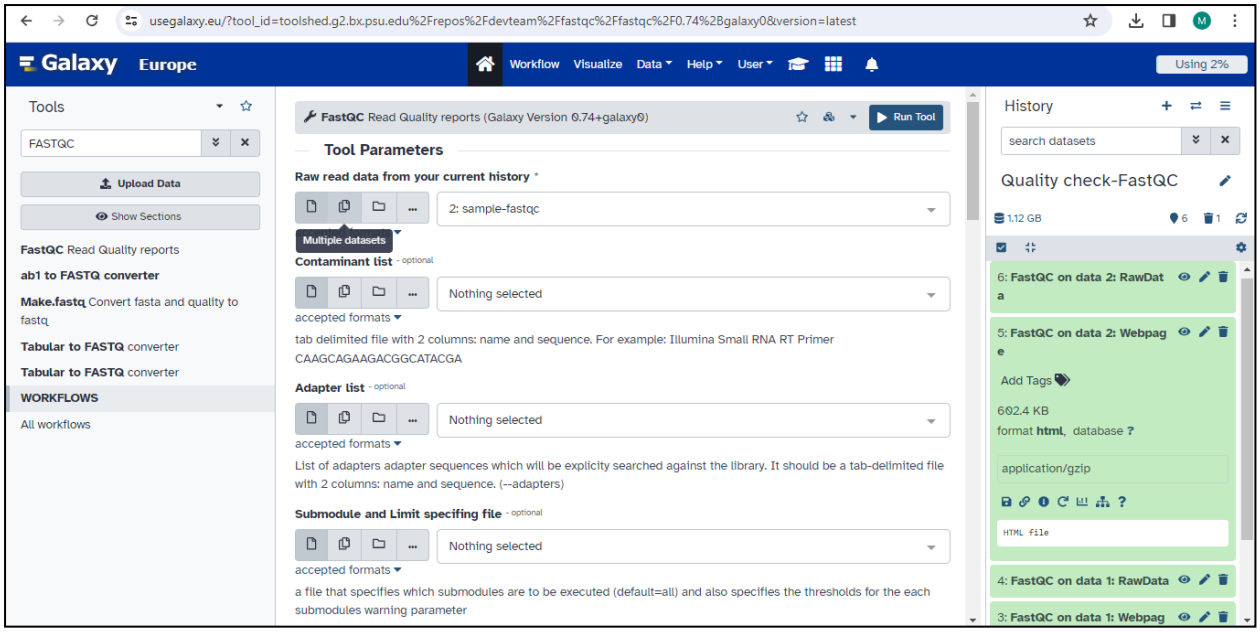
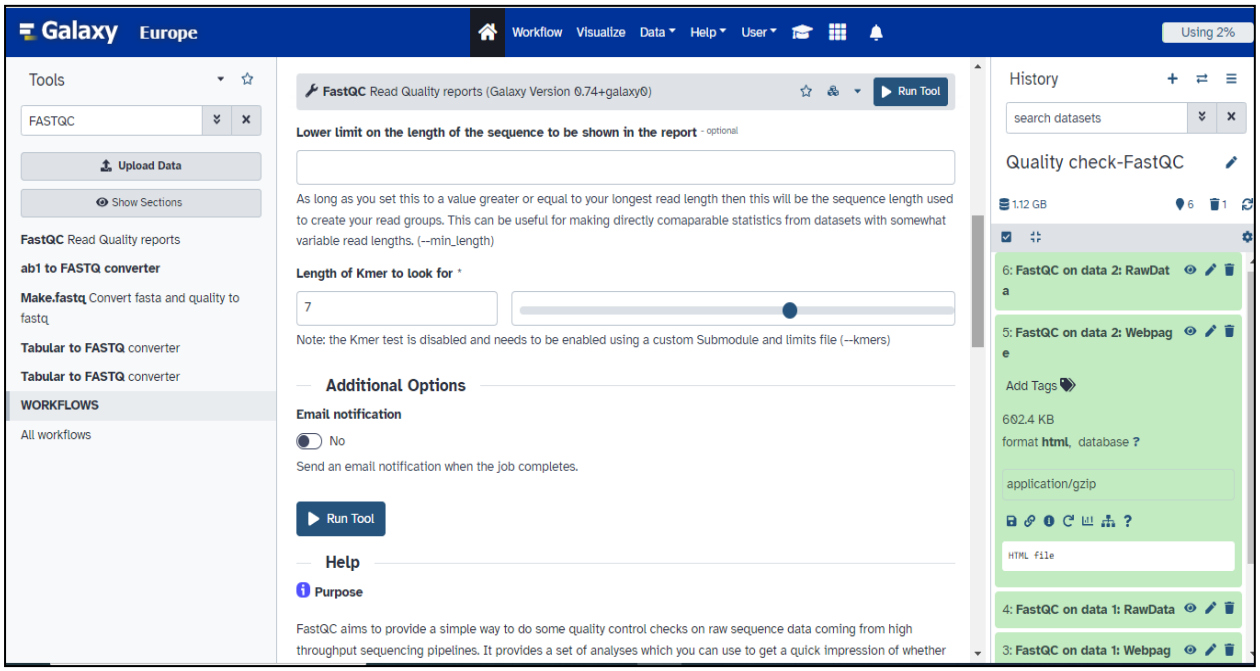


Fig 2: Searching for FastQC tool on Galaxy server



3a



3b

Fig 3a & 3b: Select Tool Parameters in the FastQC and submit

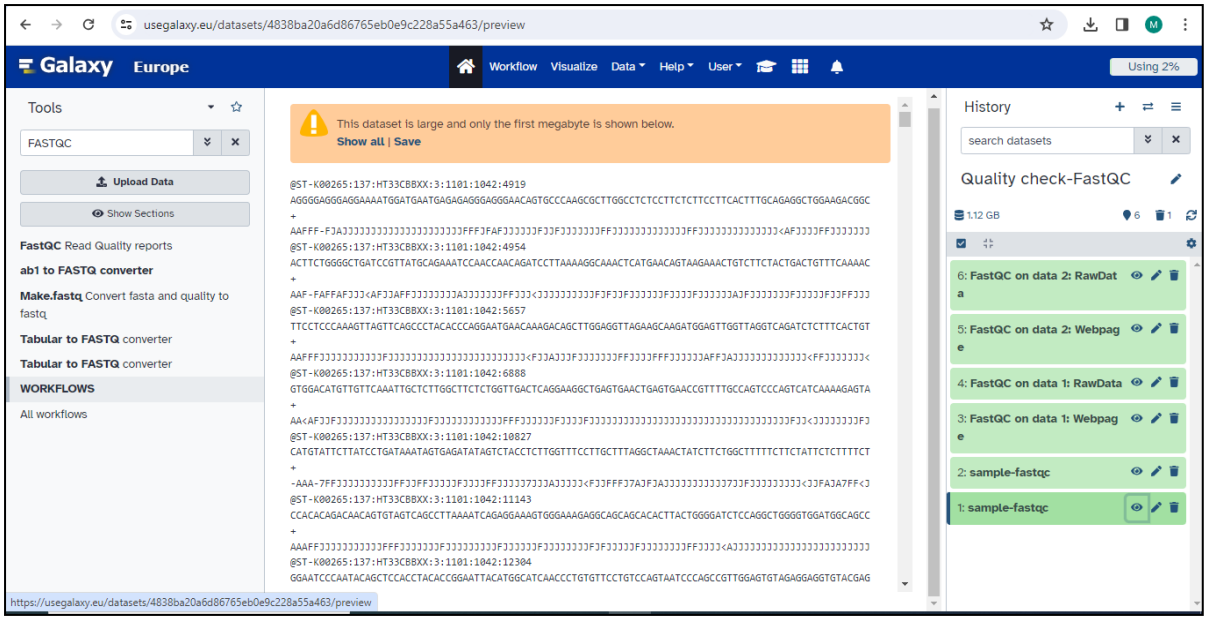


Fig 4: Sequence result for the forward strand

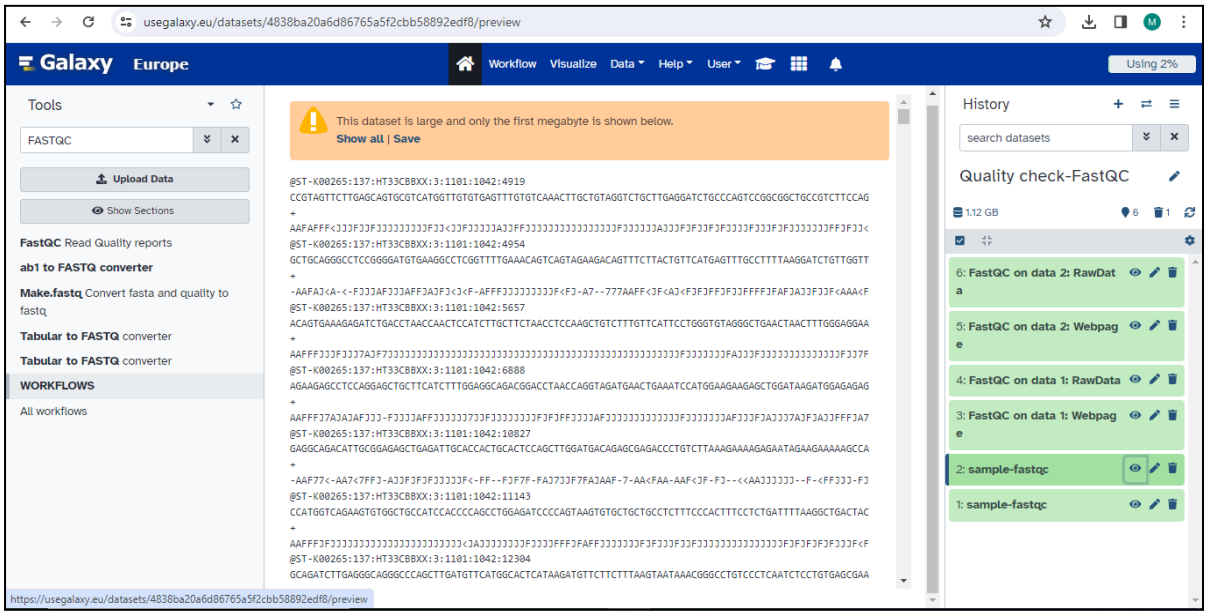


Fig 5: Sequence result of reverse strand

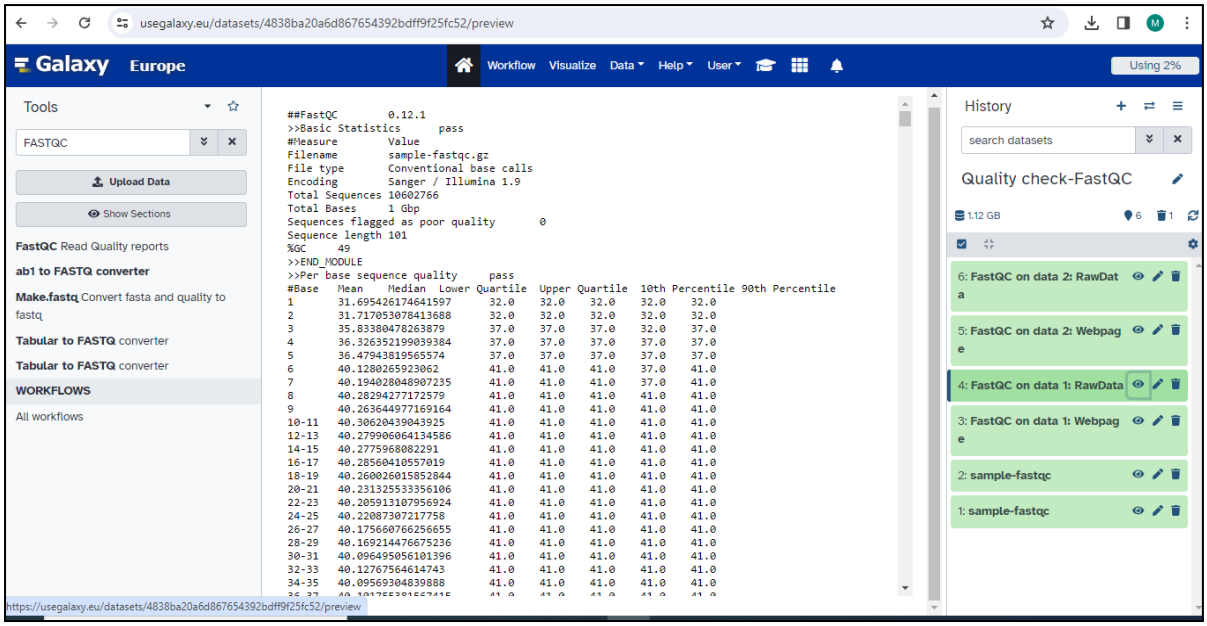


Fig 6: Raw data file of forward strand

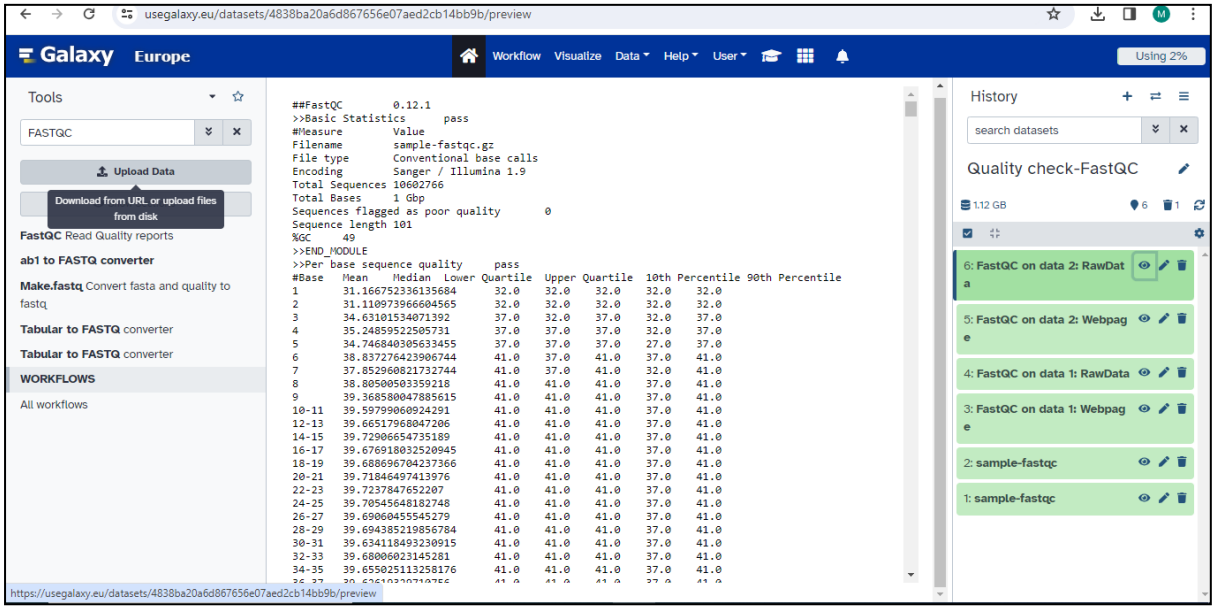


Fig 7: Raw data file of reverse strand

The results analysis of FastQC report (forward and reverse strand) is based on 11 analysis modules :

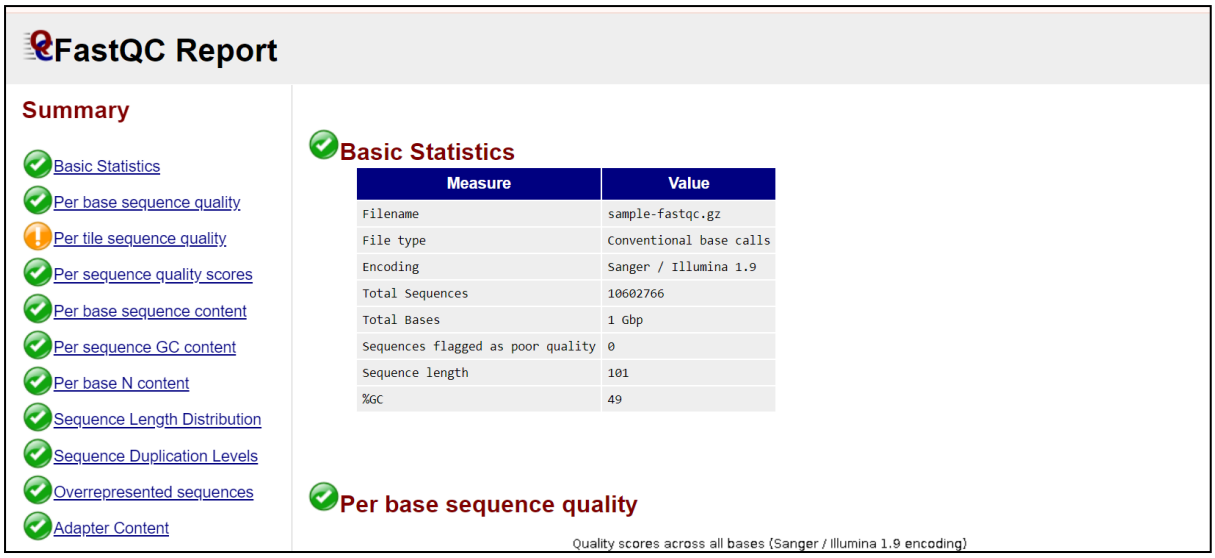


Fig 8: Basic statistics results

i) Basic Statistics: Provides composition statistics for the analyzed file, including the filename, file type, encoding, total sequences, filtered sequences, sequence length, and % GC. No warnings or failures are raised in this module.

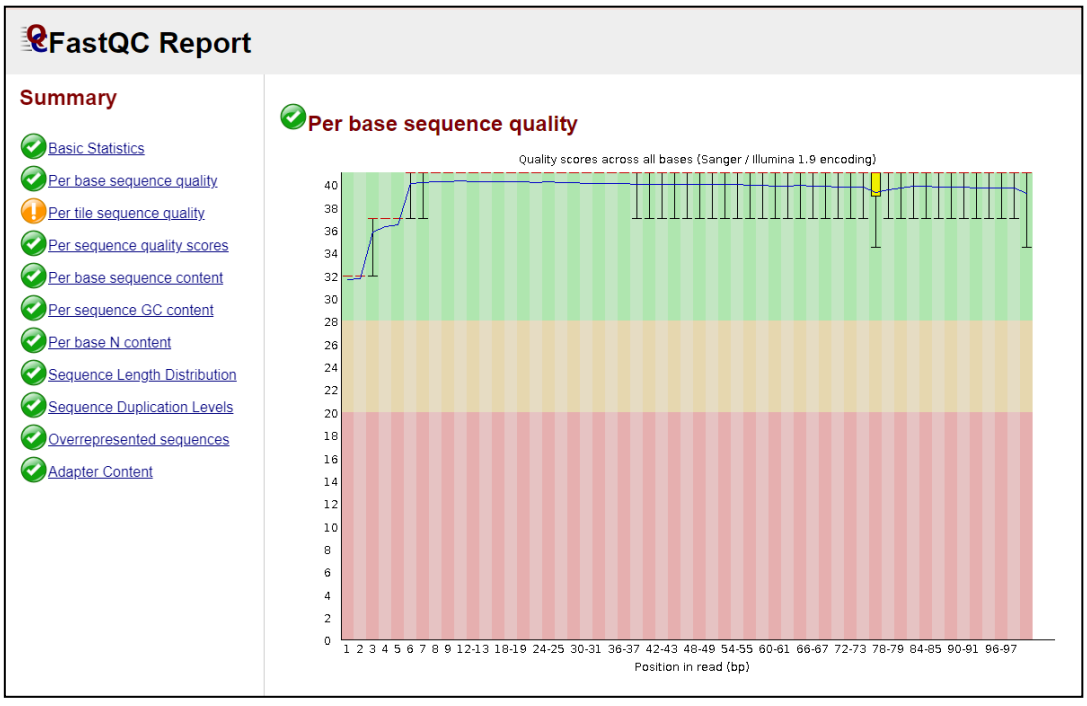


Fig 9: Per base sequence quality

ii) Per Base Sequence Quality: Presents a Box Whisker plot for the range of quality values across all bases at each position in the FastQ file. The initial position are of better quality which seems to be decreasing at the end of the read but still lies in the green zone.

Warning: Lower quartile - less than 10 or median for any base is less than 25.
Failure: Lower quartile - less than 5 or median for any base is less than 20.

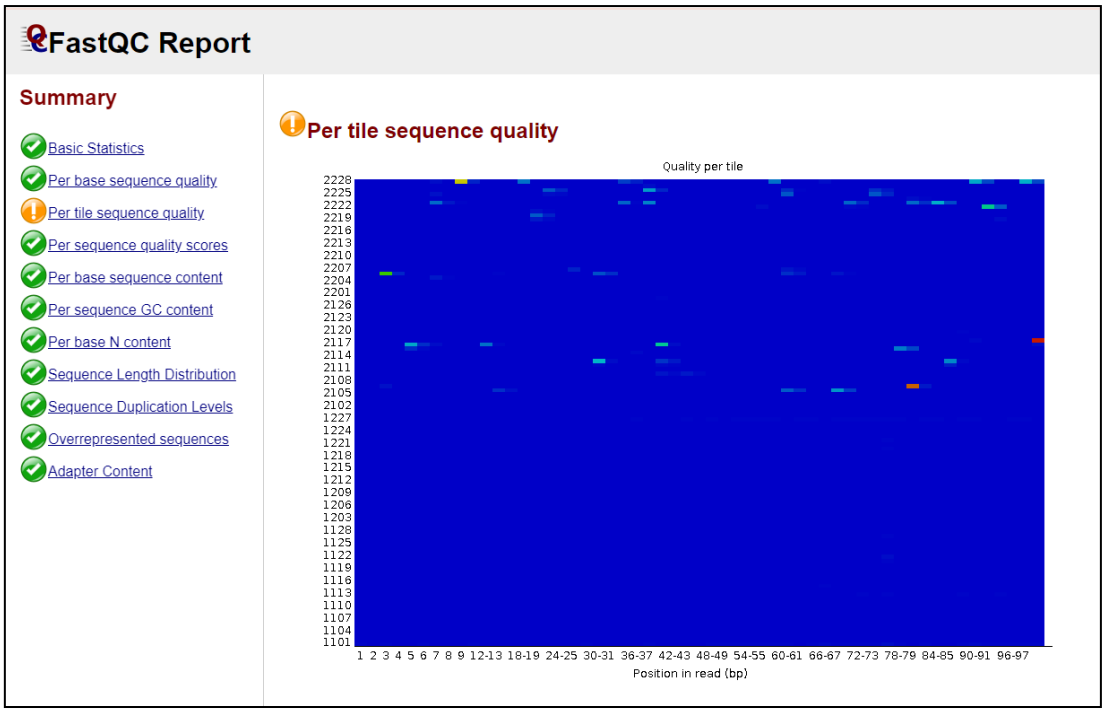


Fig 10: Per tile sequence quality

iii) Per Tile Sequence Quality: Evaluates sequencing quality across individual tiles, issuing a warning for excessive variability and passes if quality remains consistent, without specific failure criteria. Here marked tiles shows poor quality of reads or deviation from average quality of the reads.
Warning: Mean Phred score > 2 and less than the mean (base across all tiles).
Failure: Mean Phred score > 5 and less than the mean (base across all tiles).

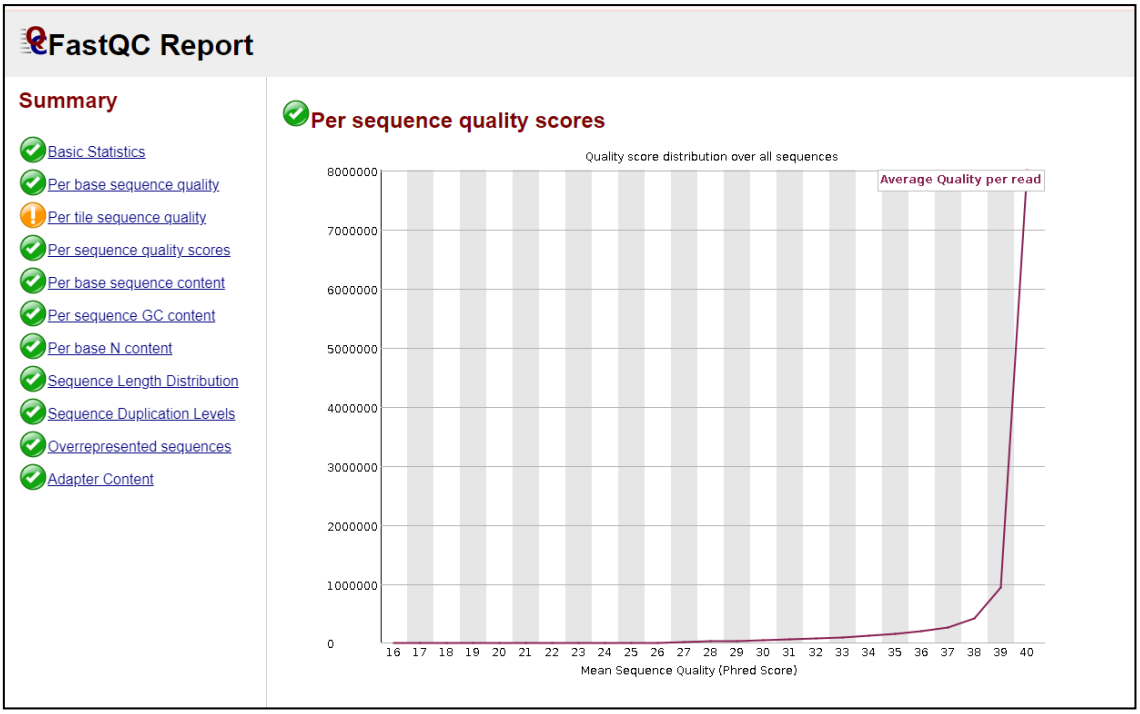


Fig 11: Per sequence quality score

iv) Per Sequence Quality Scores: Allows the assessment of universally low-quality values across a subset of sequences. Here the graph shows stable quality score distribution for overall sequences.

Warning: Mean quality < 27 - this equates to a 0.2% error rate.

Failure: Mean quality < 20 - this equates to a 1% error rate.

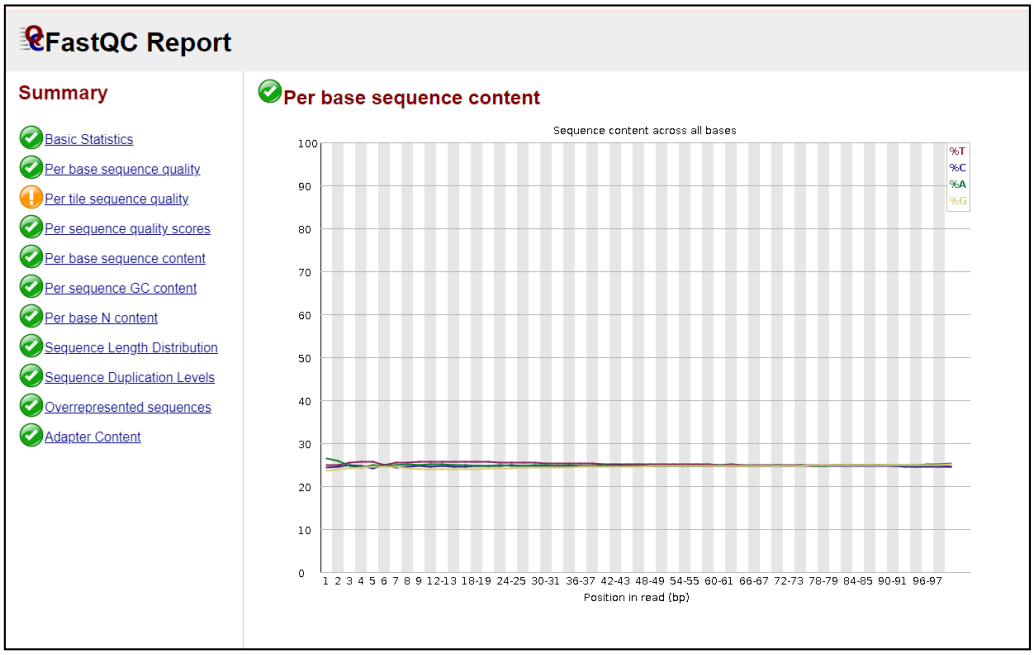


Fig 12: Per base sequence content

v) Per Base Sequence Content: Plots the proportion of each base position in a file for which each of the four normal DNA bases has been called. Here in the start (1-12) some unparallel lines are seen indicating difference in the proportion. Further the proportion of each base position is stable and parallel lines are seen indicating no difference in the base proportion.
Warning: Difference between A and T, or G and C is greater than 10% in any position.
Failure: Difference between A and T, or G and C is greater than 20% in any position.

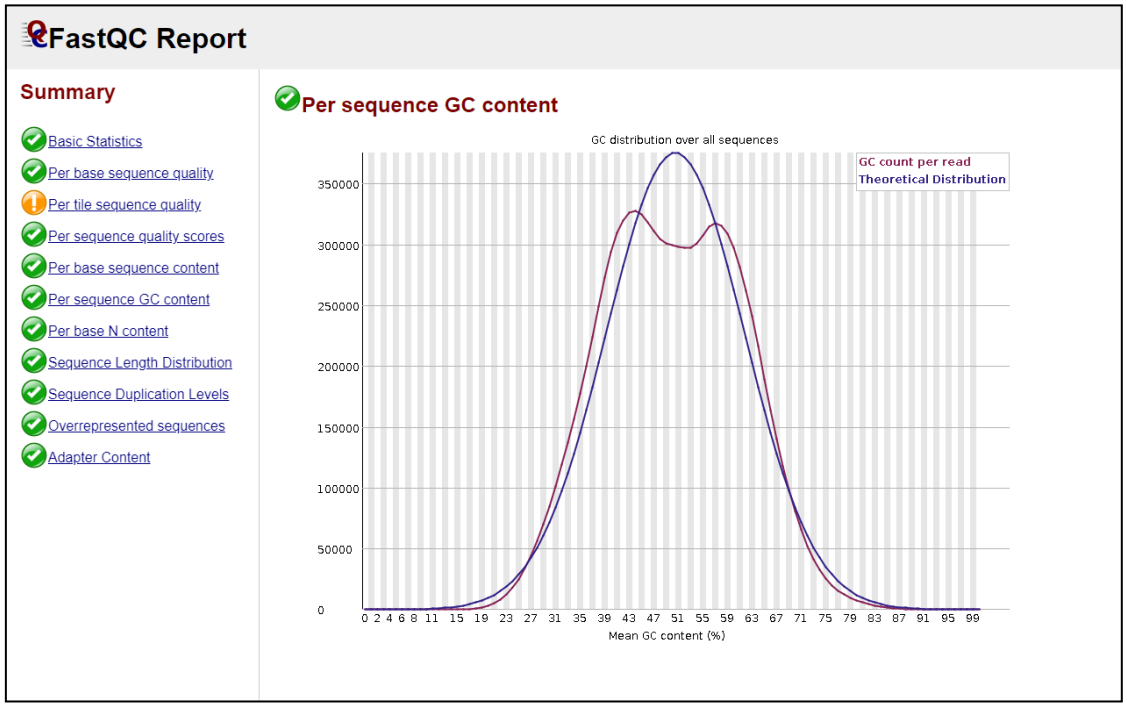


Fig 13: Per sequence GC content

vi) Per Sequence GC Content: Measures the GC content across the whole length of each sequence and compares it to a modeled normal distribution. Here the GC content across the whole length of sequence is upto the set theoretical value with little difference at the peak.
Warning: Sum of the deviations from the normal distribution > 15% of the reads.
Failure: Sum of the deviations from the normal distribution > 30% of the reads.

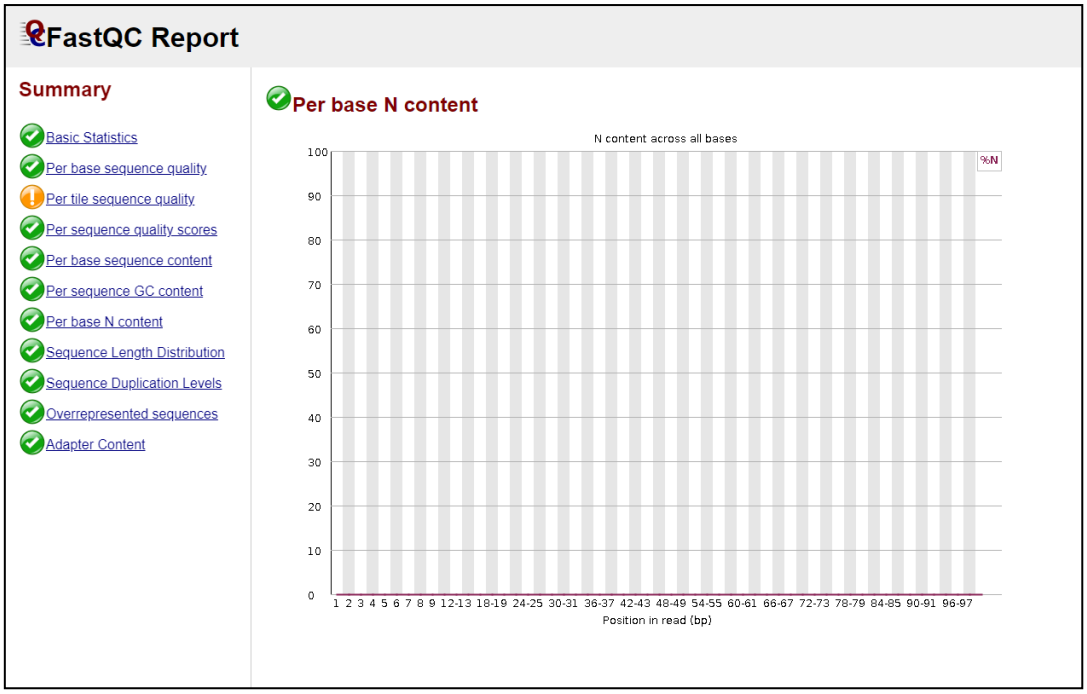


Fig 14: Per base N content

vii) Per Base N Content: Plots the percentage of base calls at each position for which an N was called. Here no presence of N content anywhere in the sequence indicating the base were properly called with confidence during sequencing

Warning: Position shows an N content of >5%.

Failure: Position shows an N content of >20%.

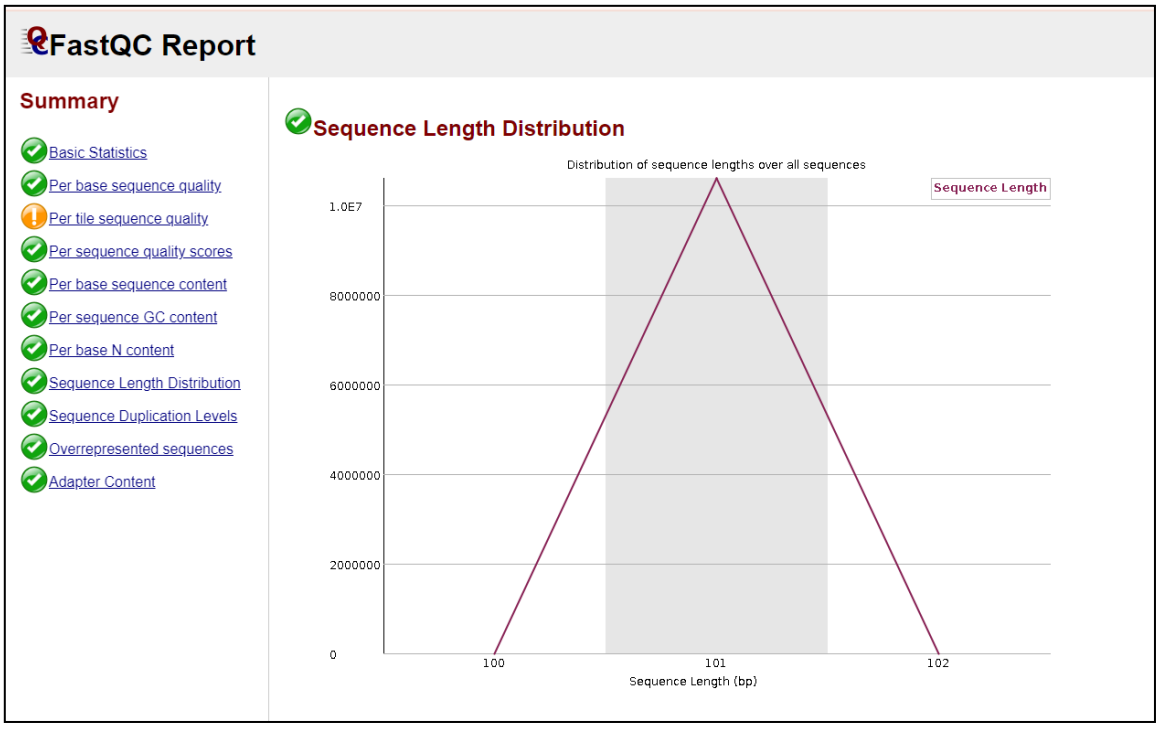


Fig 15: Sequence length distribution

viii) Sequence Length Distribution: Generates a graph showing the distribution of fragment sizes in the analyzed file. Graph shows constant sequence length (100bp-102bp) for overall sequences. Indicating proper fragmentation of the sequence.
Warning: if all sequences are not the same length.
Failure: if any of the sequences have zero length.

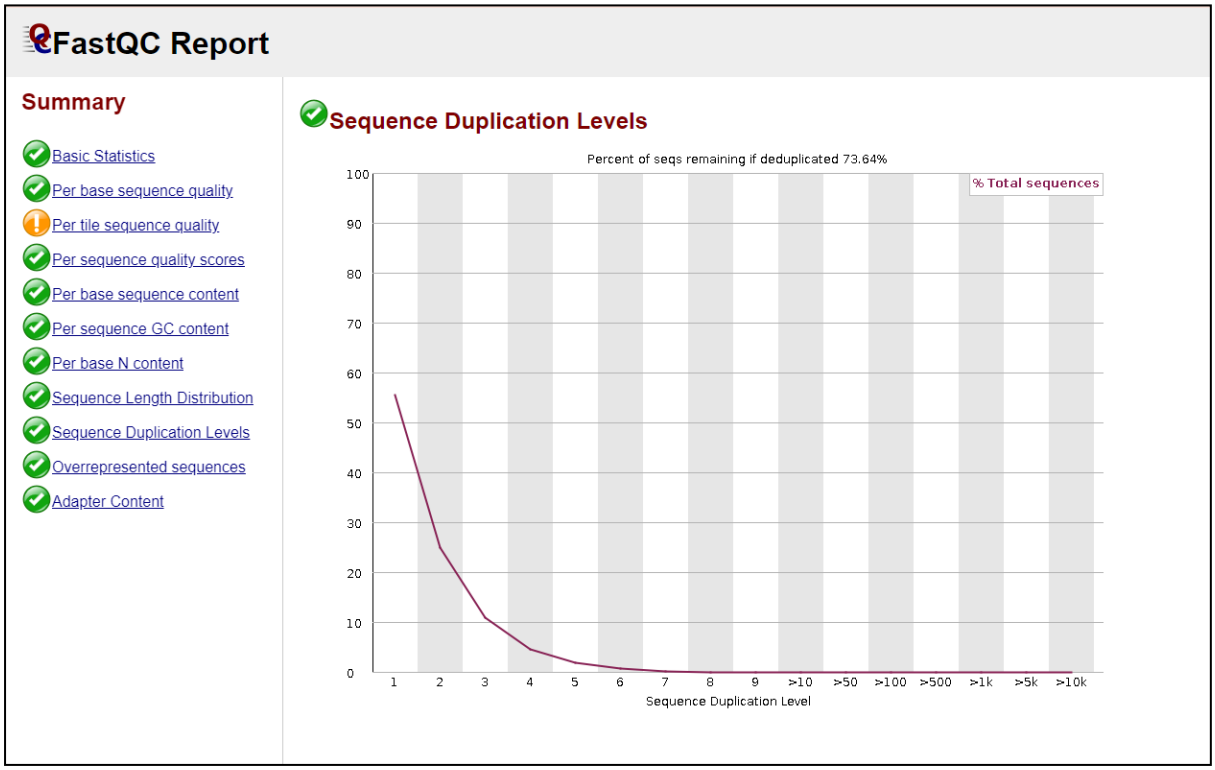


Fig 16: Sequence duplication levels

ix) Duplicate Sequences: Counts the degree of duplication for every sequence in the set. Shows some duplicate sequences but the duplication level is less than 10 which is acceptable.
Warning: if non-unique sequences make up more than 20% of the total.
Failure: if non-unique sequences make up more than 50% of the total.

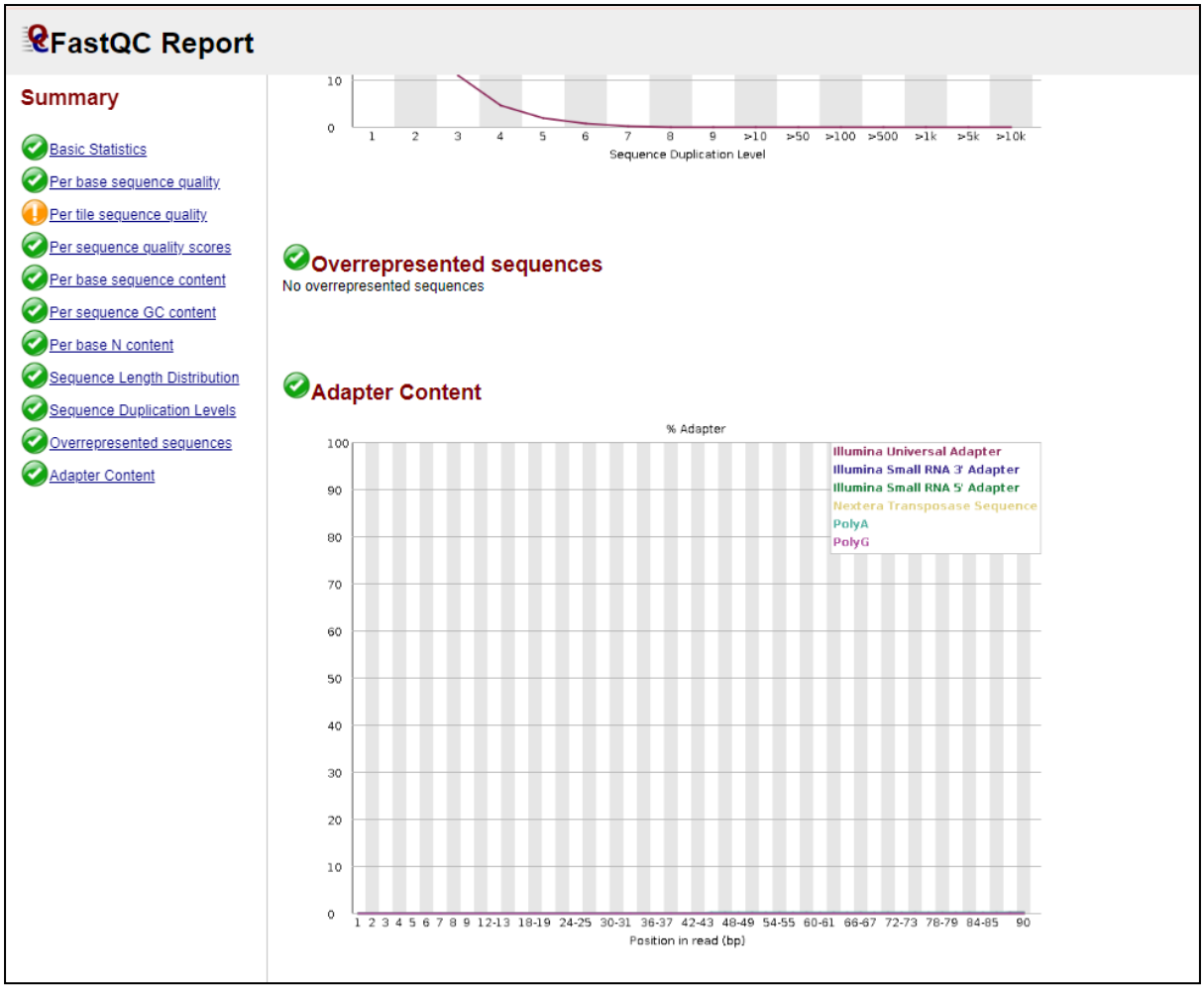


Fig 17: Overrepresented sequences and adapter content

x) Overrepresented Sequences: Lists all sequences representing more than 0.1% of the total. No overrepresented sequences were present.
Warning: sequence is found to represent more than 0.1% of the total.
Failure: sequence is found to represent more than 1% of the total.

xi) Adapter Content: A cumulative plot that shows the fraction of reads that contain a sequence library adapter at a given base position. Here there is no presence of adapter content in the sequence.
Warning: Any sequence is present in more than 5% of all reads.
Failure: Any sequence is present in more than 10% of all reads.

RESULTS:

The analysis in FastQC is performed by a series of analysis modules. The left hand side of the main interactive display or the top of the HTML report show a summary of the modules which were run, and a quick evaluation of whether the results of the module seem entirely normal (green tick), slightly abnormal (orange triangle) or very unusual (red cross). The specific module details like the graph, warning and failures have been discussed above in detail.

CONCLUSION:

The quality of raw sequencing data was checked using FastQC tool. All the potential issues were looked into. FastQC has been done to get accurate biological interpretations and check whether our sequence is of good quality or not to do further process.

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3. Index of /projects/fastqc/Help. (n.d.). https://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/
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DATE: 10/04/2024

WEBLEM 9(D)
Bowtie Tool
(URL: https://usegalaxy.org/)

AIM:

To map reads against reference genome using bowtie 2 tool for the given query.

INTRODUCTION:

Bowtie package is used to align short sequencing reads, such as those output by second-generation sequencing instruments. It also includes protocols for building a genome index and calling consensus sequences from Bowtie alignments using SAMtools. The Bowtie package enables ultrafast and memory-efficient alignment of large sets of sequencing reads to a reference sequence, such as the human genome. The package contains tools for building indexes of reference genomes and for aligning short reads using the index as a guide. This is the first step of many comparative genomics workflows, including variant detection and digital gene expression. In what follows, the term read refers to a short DNA sequence, typically as output by a sequencing instrument. A read may be accompanied by a corresponding string of quality values, where each value estimates the probability that the corresponding base was miscalled by the instrument software. The term subject sequence refers to the true sequence of the sample(s) from which the reads were drawn. The term reference sequence or reference genome refers to the sequence to which the subject is to be compared.

Bowtie 2 is an ultrafast and memory-efficient tool for aligning sequencing reads to long reference sequences. It is particularly good at aligning reads of about 50 up to 100s of characters to relatively long (e.g. mammalian) genomes. Bowtie 2 indexes the genome with an FM Index (based on the Burrows-Wheeler Transform or BWT) to keep its memory footprint small: for the human genome, its memory footprint is typically around 3.2 gigabytes of RAM. Bowtie 2 supports gapped, local, and paired-end alignment modes. Multiple processors can be used simultaneously to achieve greater alignment speed. Bowtie 2 outputs alignments in SAM format, enabling interoperation with a large number of other tools (e.g. SAMtools, GATK) that use SAM. Bowtie 2 is distributed under the GPLv3 license, and it runs on the command line under Windows, Mac OS X and Linux and BSD. Bowtie 2 is often the first step in pipelines for comparative genomics, including for variation calling, ChIP-seq, RNA-seq, BS-seq. Bowtie 2 and Bowtie (also called "Bowtie 1" here) are also tightly integrated into many other tools.

METHODOLOGY:

1. Open homepage of Galaxy Tool.
2. Search Bowtie in search tool box.
3. Set different parameters of the tool.
4. Set the different operations.
5. Run the tool and interpret the result which are come in four different sections.

OBSERVATIONS:

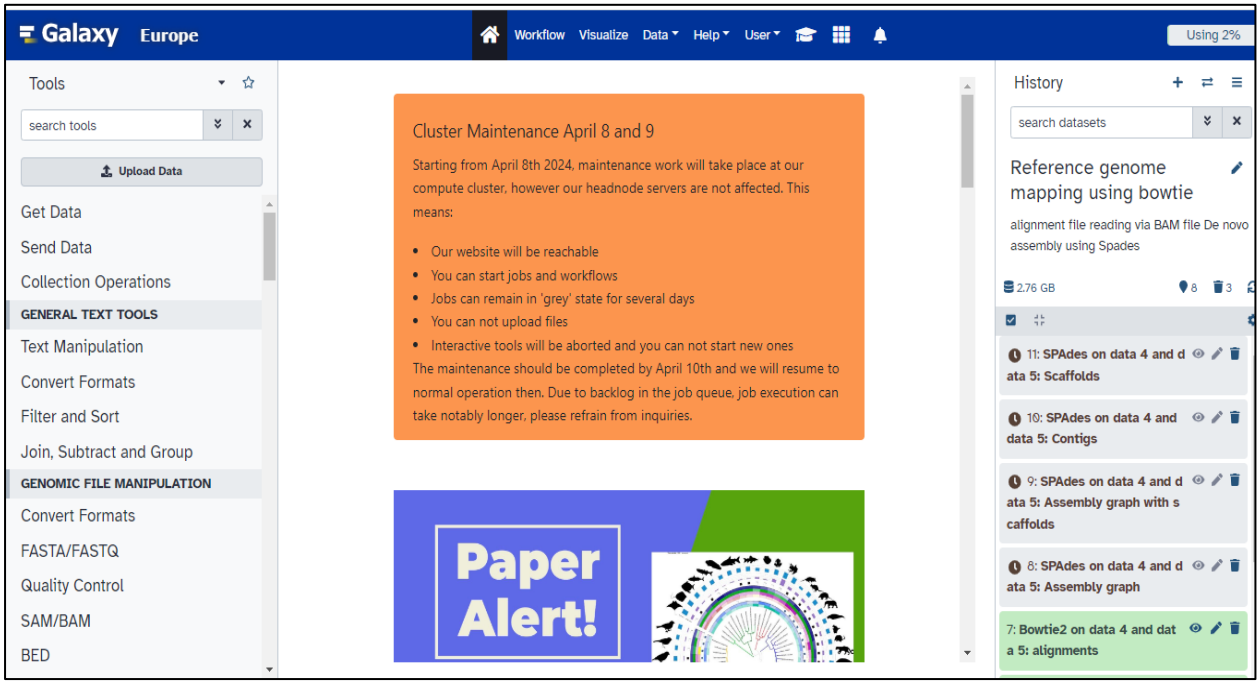


Fig 1: Homepage of Galaxy Tool

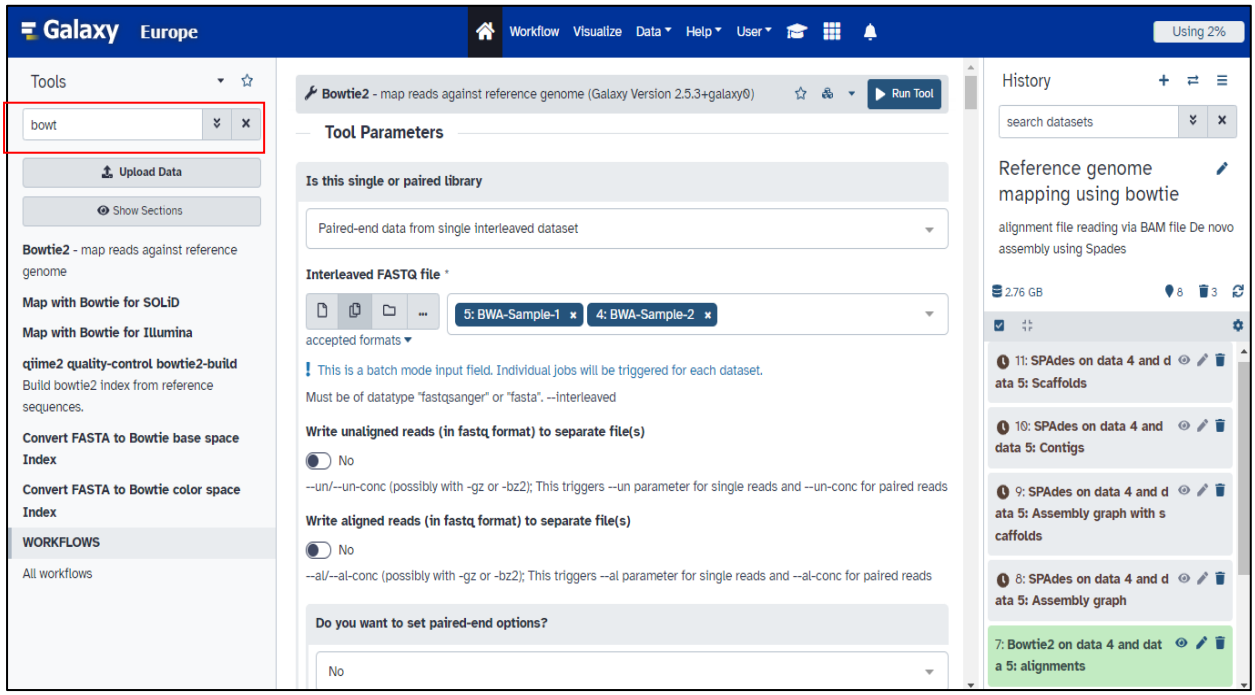


Fig 2: Select the Bowtie option in search tool box

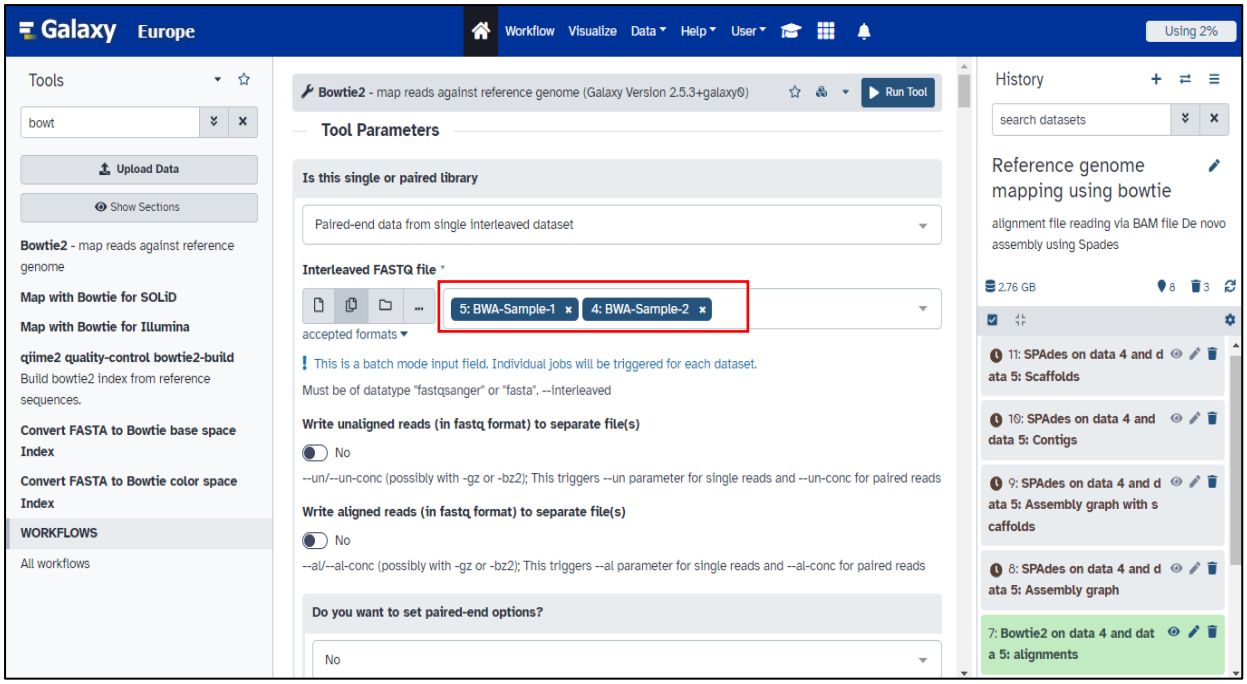


Fig 3: Set the tool parameters

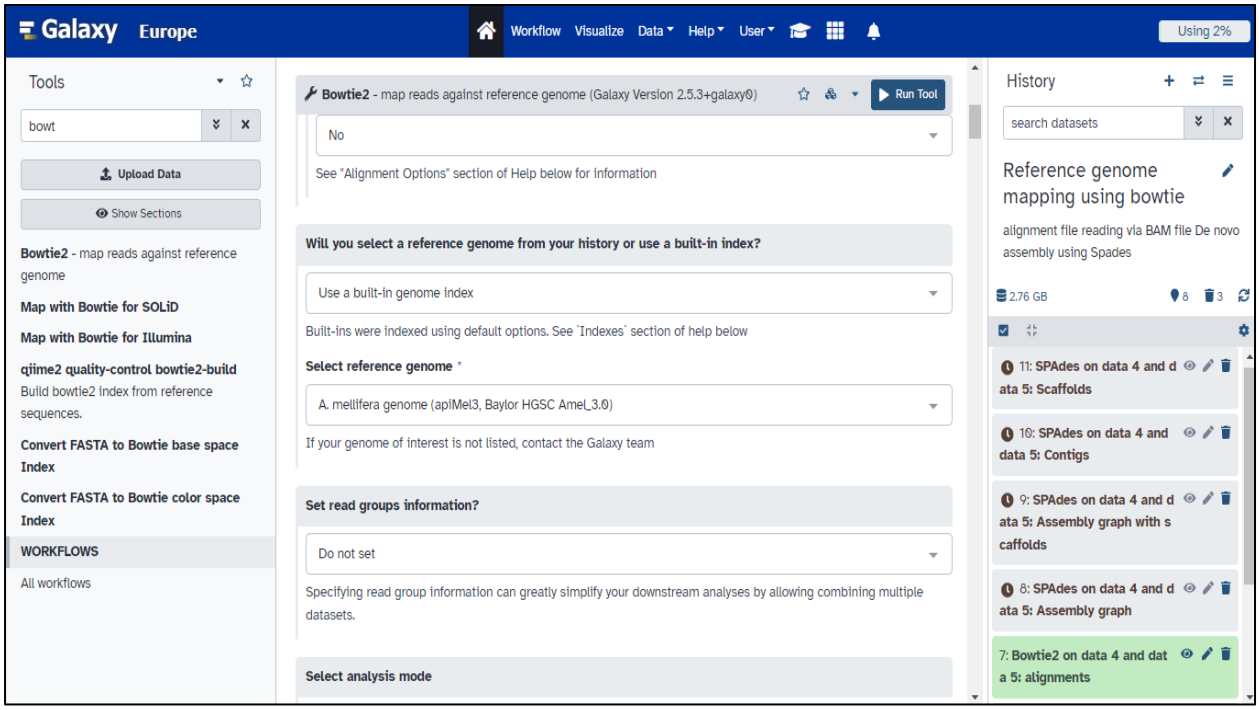


Fig 3.1: Set the tool parameters

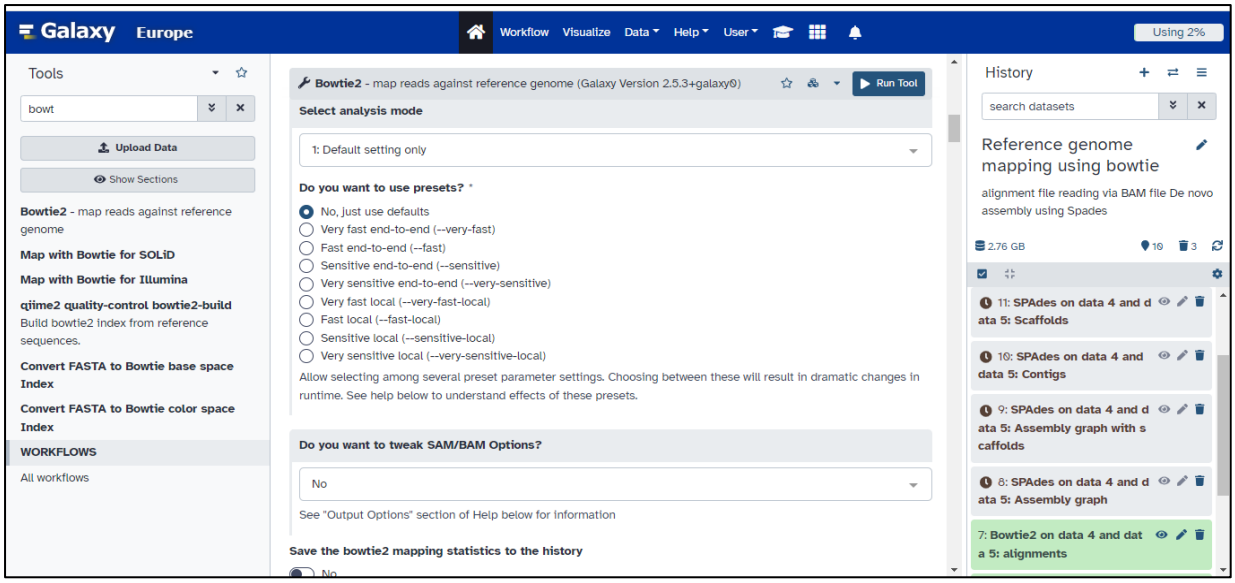


Fig 4: Set the analysis mode

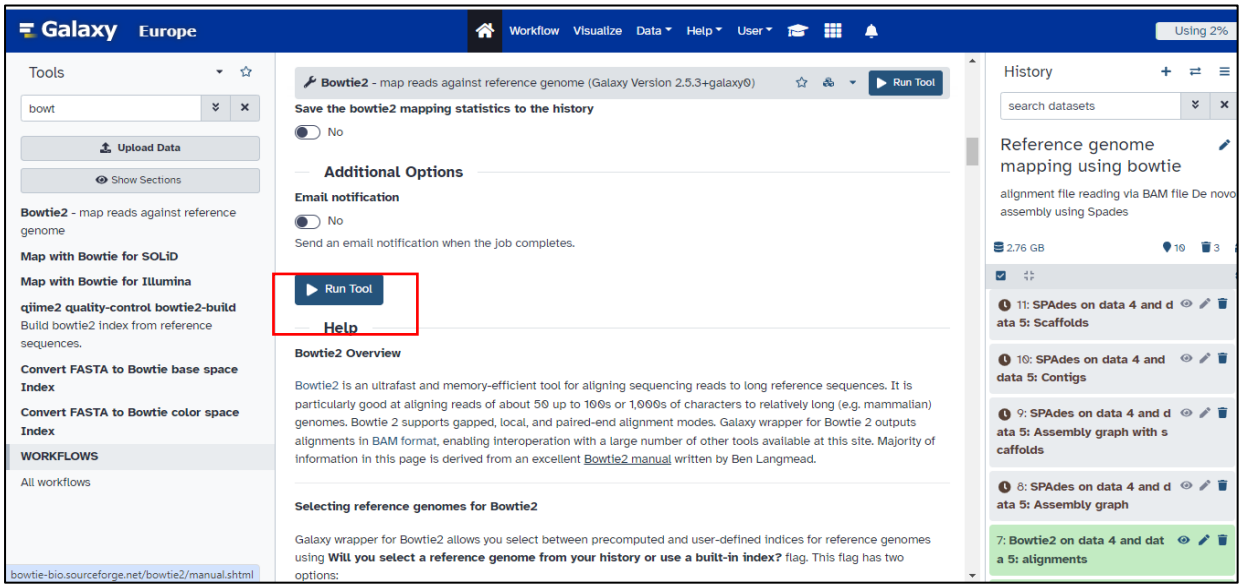


Fig 5: Run the Tool

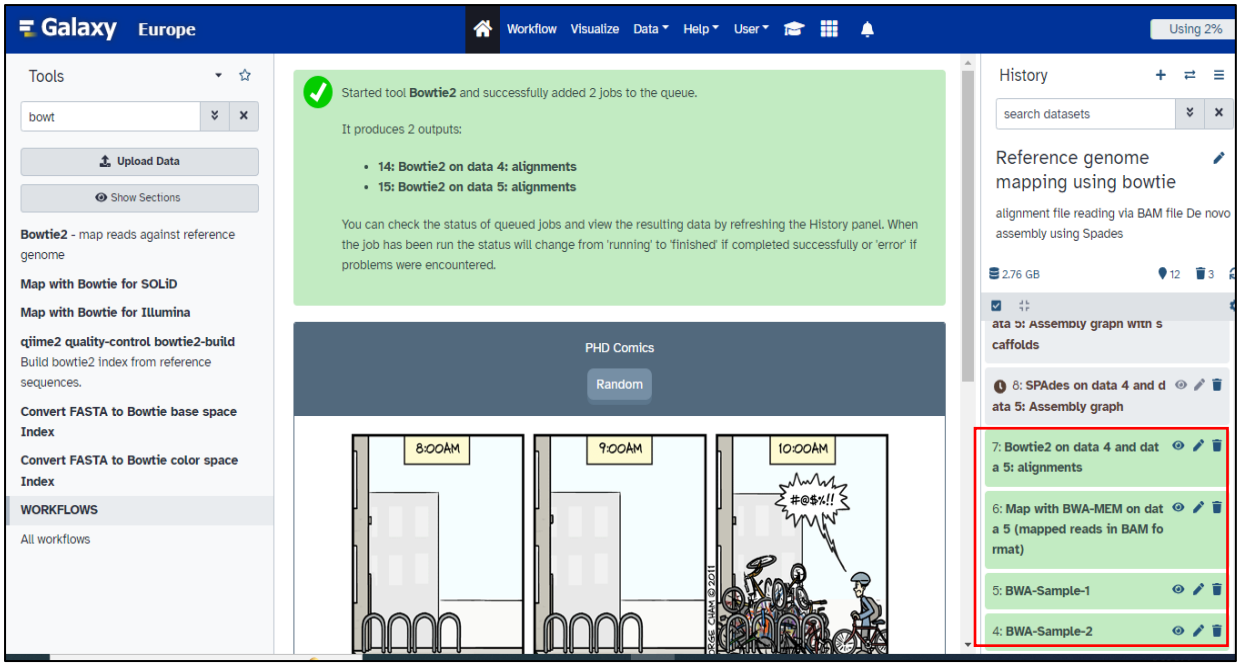


Fig 6: Results shown in four different options

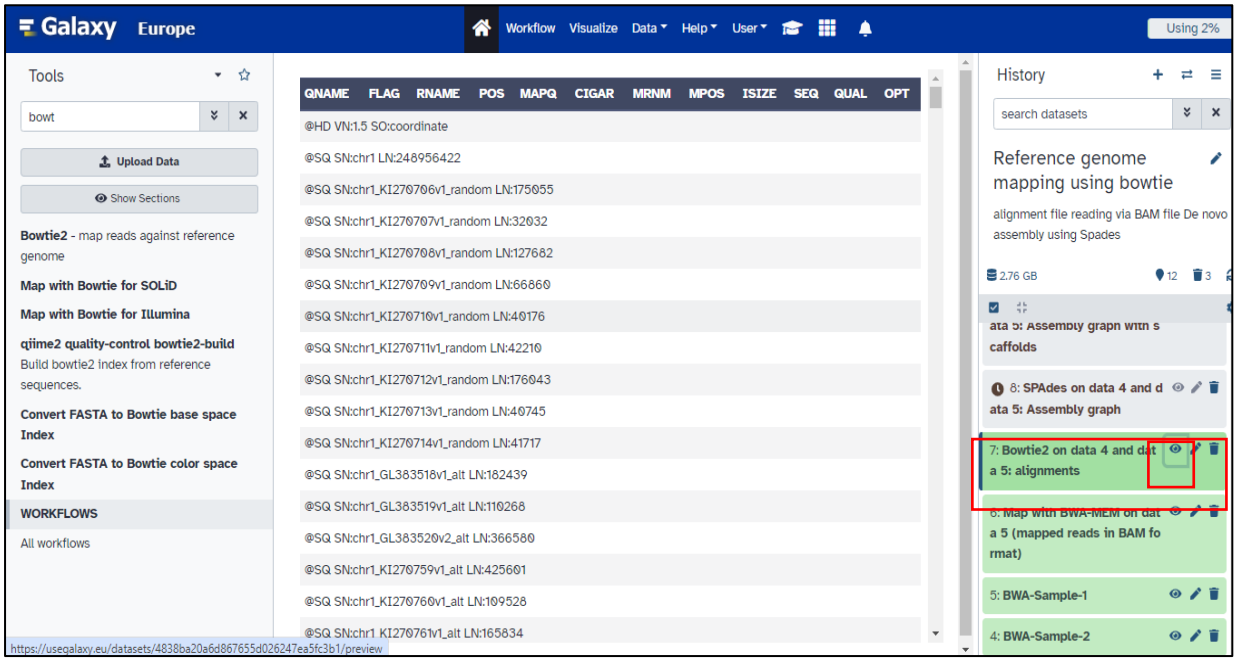


Fig 7: Result Page

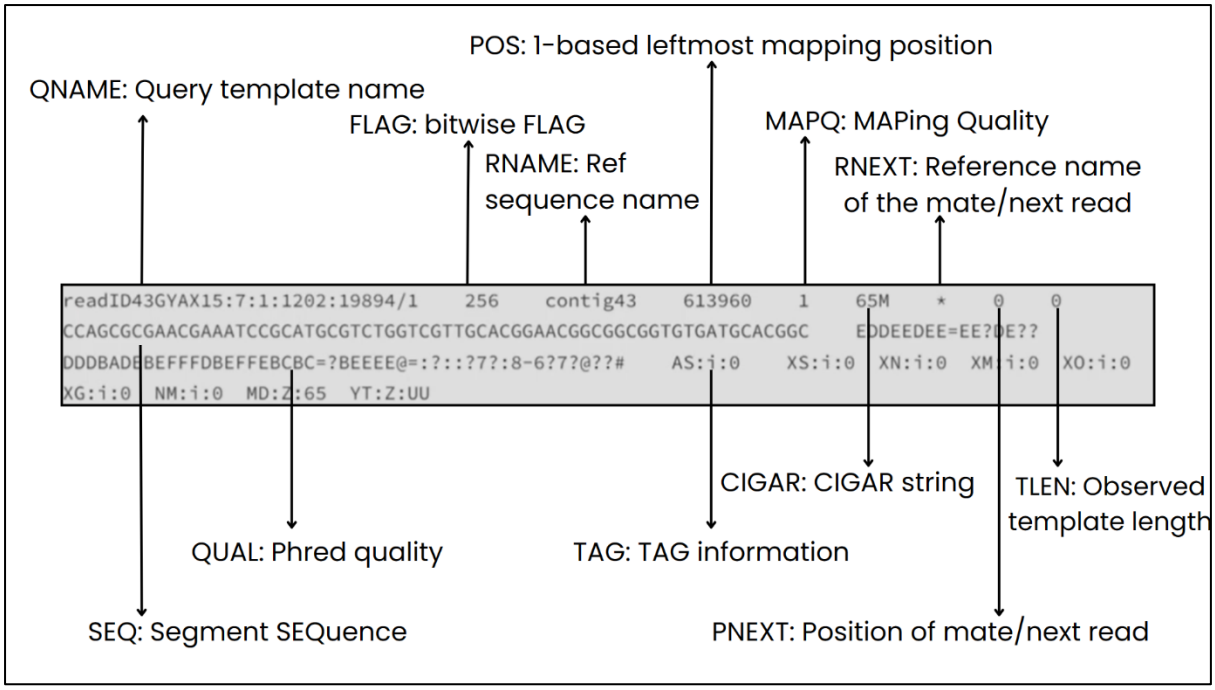


Fig 7.1 Detail result of the alignment

RESULTS:

Bowtie generates an alignment report summarizing the results of the alignment process. This report includes information such as the number of reads processed, the number of reads aligned to the reference genome, the alignment rate (percentage of reads aligned), and any potential issues encountered during the alignment process. In addition to the basic alignment report, Bowtie can generate more detailed statistics about the alignment process, such as the distribution of alignment quality scores, the distribution of alignment positions across the reference genome, and the frequency of mismatches or indels between the reads and the reference genome.

CONCLUSION:

For the given sequence, mapping was done with reference using Bowtie.

REFERENCES:

1. Trapnell C, et al. Nat. Biotechnol. 2010;28:511–515. [PMC free article] [PubMed] [Google Scholar]
2. Langmead, B., Salzberg, S. Fast gapped-read alignment with Bowtie 2. Nat Methods 9, 357–359 (2012). https://doi.org/10.1038/nmeth.1923
3. Bowtie 2: Manual. (n.d.). https://bowtie-bio.sourceforge.net/bowtie2/manual.shtml

DATE: 13/04/2024

WEBLEM 9(E)
VELVETH
(URL:https://usegalaxy.org/)

AIM:

To perform de novo assembly of sequencing reads into contigs using Velveth for genome assembly.

INTRODUCTION:

Velvet is software to perform DNA assembly from short reads by manipulating de Bruijn graphs. It is capable of forming long contigs (n50 of in excess of 150kb) from paired end short reads. It has several input parameters for controlling the structure of the de Bruijn graph and these must be set optimally to get the best assembly possible. Velvet can read Fasta, FastQ, sam or bam files. However, it ignores any quality scores and simply relies on sequencing depth to resolve errors. The Velvet Optimiser software performs many Velvet assemblies with various parameter sets and searches for the optimal assembly automatically. It is designed to handle short read data with high coverage and is suitable for assembling genomes of organisms that do not have a reference genome available. The algorithm implemented in Velvet is capable of manipulating de Bruijn graphs to remove sequencing errors and resolve repeats, allowing for the reconstruction of the original genome sequence.

In the context of the Galaxy platform, Velvet is available as a tool in the Galaxy platform, where it can be used as part of a larger bioinformatics workflow. The tool is particularly useful for assembling short DNA sequences and can be used to resolve repeats with the addition of long reads.

Galaxy is an open-source web-based platform for data analysis that provides a user-friendly interface for running a wide range of bioinformatics tools, including Velveth. By using Velveth in Galaxy, researchers can easily perform de novo genome assembly and integrate it with other analysis steps in their workflow.

To use Velveth in Galaxy, users can navigate to the tool's page in the Galaxy interface and provide the necessary input parameters, such as the location of the input reads and the desired k-mer size. Once the tool is executed, the resulting assembly can be further analyzed using other tools available in Galaxy.

Functions:

1. Velveth breaks down each read into k-mers of a specified length, where a k-mer is a subsequence of the read.
2. These k-mers and their reverse complements are then added to a hash table for categorization, with each k-mer stored once along with the number of times it appears.
3. This hashing process is crucial for organizing the reads and preparing them for the construction of the de Bruijn graph, which is essential for the subsequent steps in the assembly process.

Input formats:

1. Velveth can handle sequence files in formats such as fasta, fastq, raw, sam, and bam.
2. For paired-end reads, the assumption is that each read is paired with its mate read, facilitating the assembly process.

Usage in velveth assembly:

1. Velveth plays a fundamental role in the Velvet assembly process by preparing the input reads for further analysis and assembly.
2. It is a critical step in the de novo assembly of short read sequences, enabling the creation of high-quality unique contigs that form the basis of the reconstructed genome sequence.

METHODOLOGY:

1. Open homepage of Galaxy server.
2. In the search panel search VELVETH tool.
3. Select the required parameters and upload the dataset
   a. "Hash Length": 21
   b. "Input Files"
      i. Click on param-repeat "Input Files"
      ii. "Choose the input type": interleaved paired end
      iii. "read type": shortPaired reads
      iv. param-files "Dataset": Upload sample 'Velveth sample'
4. The tool takes our reads and break them into k-mers.
5. Click on 'Run Tool'.
6. Three output files will be generated.

OBSERVATIONS:

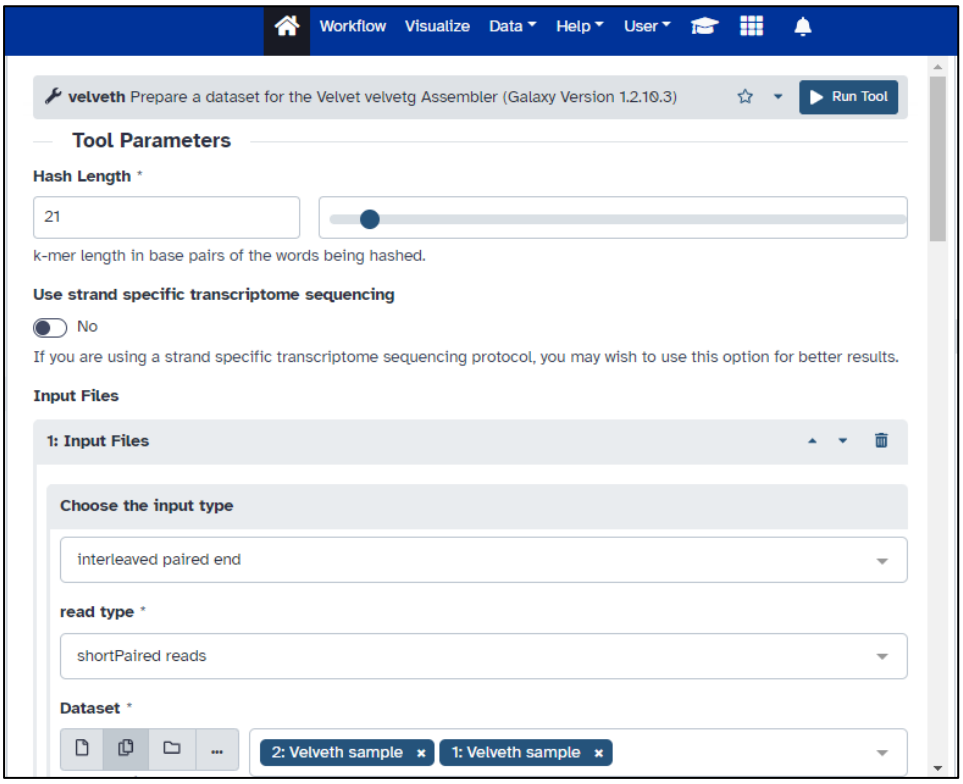


Fig 1.1: Select the parameters and upload the Dataset

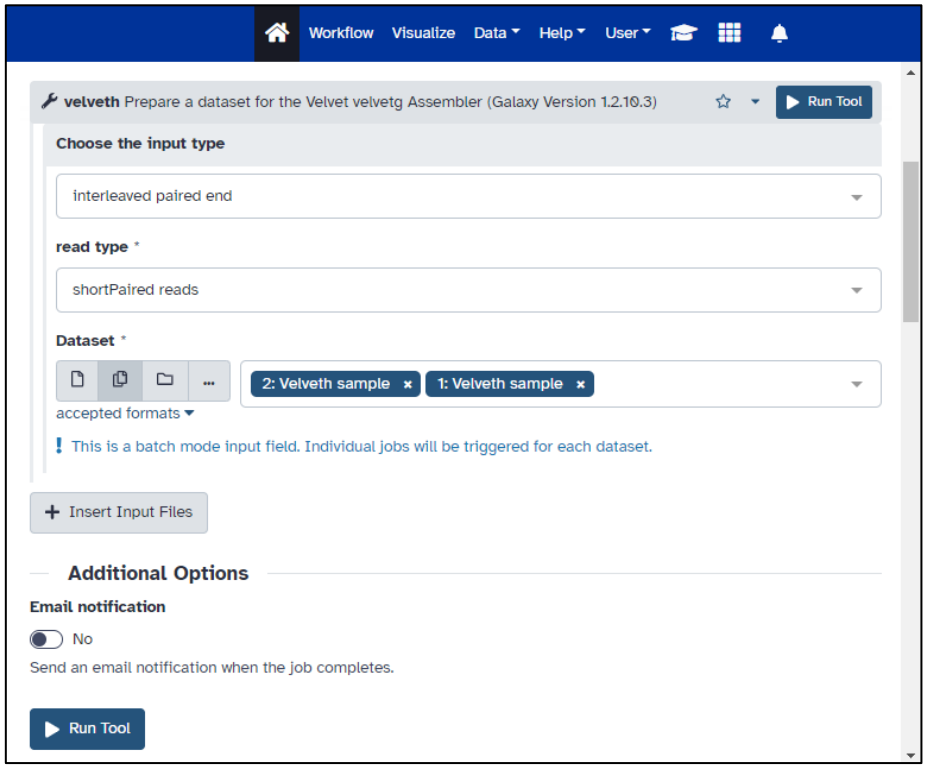


Fig 1.2: Click on 'Run Tool'

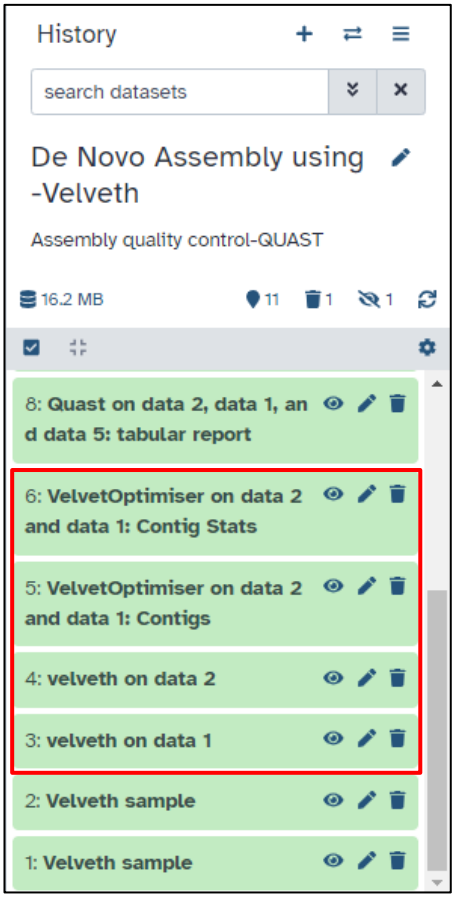
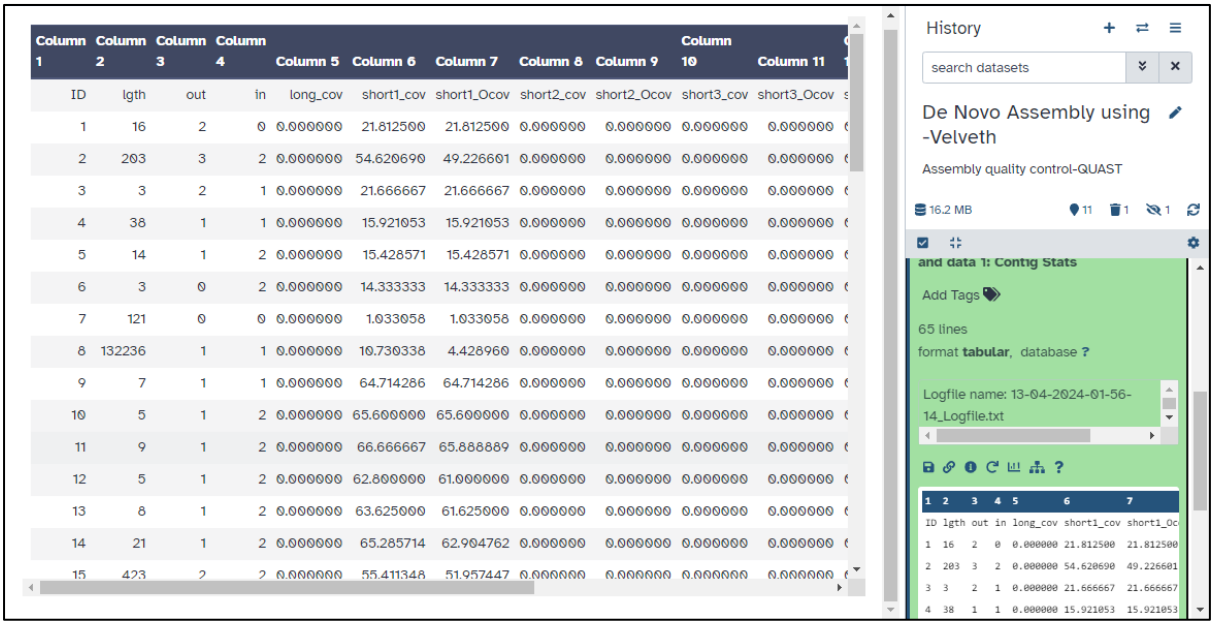


Fig 2: Output files (Sequence File, Contigs File, Contig Statistics File)



Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11
ID	lgth	out	in	long_cov	short1_cov	short1_Ocov	short2_cov	short2_Ocov	short3_cov	short3_Ocov
1	16	2	0	0.000000	21.812500	21.812500	0.000000	0.000000	0.000000	0.000000
2	203	3	2	0.000000	54.620690	49.226601	0.000000	0.000000	0.000000	0.000000
3	3	2	1	0.000000	21.666667	21.666667	0.000000	0.000000	0.000000	0.000000
4	38	1	1	0.000000	15.921053	15.921053	0.000000	0.000000	0.000000	0.000000
5	14	1	2	0.000000	15.428571	15.428571	0.000000	0.000000	0.000000	0.000000
6	3	0	2	0.000000	14.333333	14.333333	0.000000	0.000000	0.000000	0.000000
7	121	0	0	0.000000	1.033058	1.033058	0.000000	0.000000	0.000000	0.000000
8	132236	1	1	0.000000	10.730338	4.428960	0.000000	0.000000	0.000000	0.000000
9	7	1	1	0.000000	64.714286	64.714286	0.000000	0.000000	0.000000	0.000000
10	5	1	2	0.000000	65.600000	65.600000	0.000000	0.000000	0.000000	0.000000
11	9	1	2	0.000000	66.666667	65.888889	0.000000	0.000000	0.000000	0.000000
12	5	1	2	0.000000	62.800000	61.000000	0.000000	0.000000	0.000000	0.000000
13	8	1	2	0.000000	63.625000	61.625000	0.000000	0.000000	0.000000	0.000000
14	21	1	2	0.000000	65.285714	62.904762	0.000000	0.000000	0.000000	0.000000
15	423	2	2	0.000000	55.411348	51.957447	0.000000	0.000000	0.000000	0.000000

Fig 3: Tabular file giving for each contig the k-mer lengths, k-mer coverages and other measures

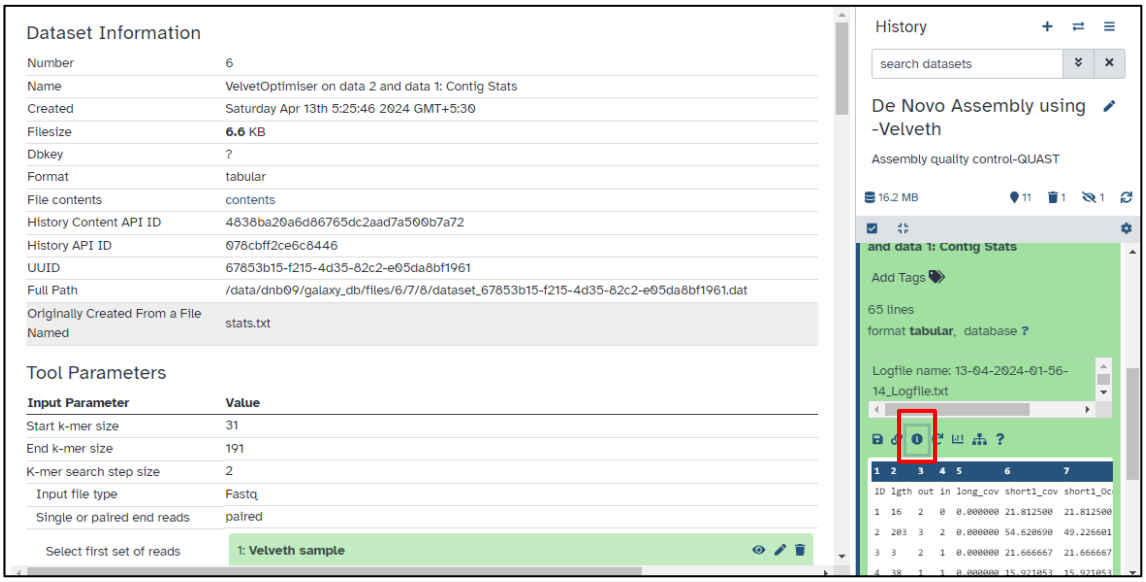


Fig 4: Dataset Information of the Contig Statistic File

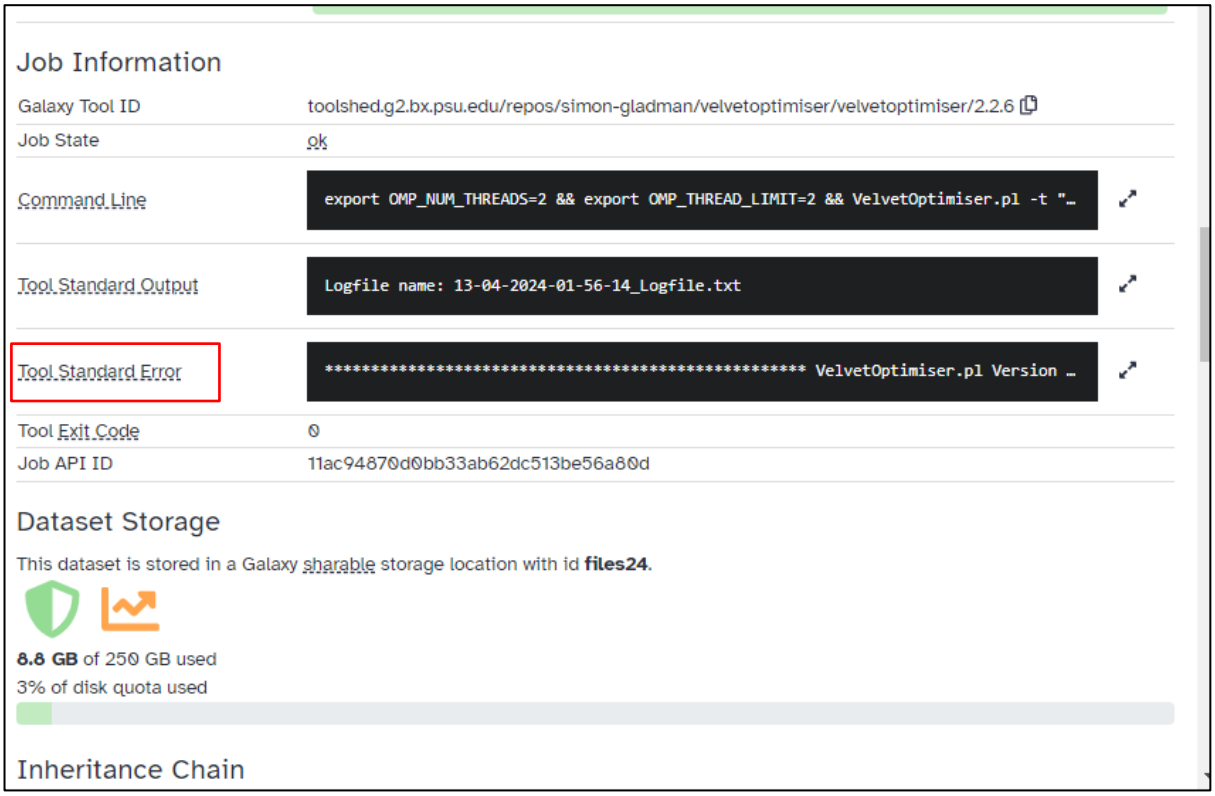


Fig 5: Click on 'Total Standard Error'

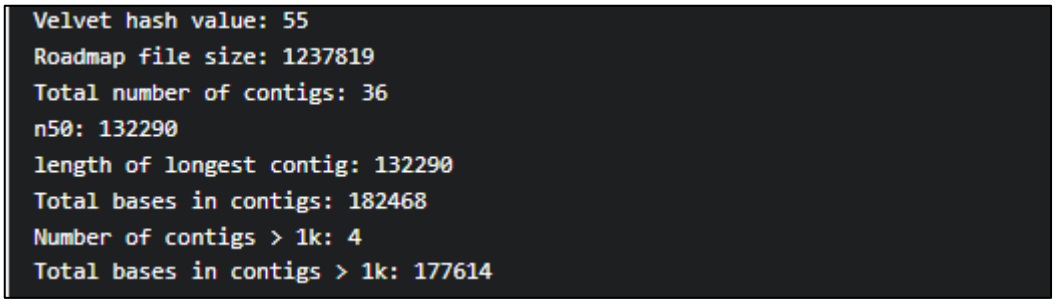


Fig 6: Size, Length and Number of Contigs built from the Sample

RESULTS:

There is total 36 contigs built. The length of the longest contig is 132290. Total bases in contigs are 182468.

CONCLUSION:

De Novo Assembly was performed on query using Velveth Tool.

REFERENCES:

1. Introduction to de novo assembly with Velvet - Bioinformatics Documentation. (n.d.). https://www.melbournebioinformatics.org.au/tutorials/tutorials/assembly/assembly-background/
2. Introduction to de novo genome assembly for Illumina reads - Bioinformatics Documentation. (n.d.). https://www.melbournebioinformatics.org.au/tutorials/tutorials/assembly/assembly-protocol/

DATE:13/04/2024

WEBLEM 9(F)
Quality Assessment Tool-QUAST
(URL: https://usegalaxy.eu/)

AIM:

To assess the quality of the sequence assemblies using QUAST tool

INTRODUCTION:

Modern DNA sequencing technologies cannot produce the complete sequence of a chromosome. Instead, they generate large numbers of reads, ranging from dozens to thousands of consecutive bases, sampled from different parts of the genome. Genome assembly software combines the reads into larger regions called contigs. However, current sequencing technologies and software face many complications that impede reconstruction of full chromosomes, including errors in reads and large repeats in the genome.

Different assembly programs use different heuristic approaches to tackle these challenges, resulting in many differences in the contigs they output. This leads to the questions of how to assess the quality of an assembly and how to compare different assemblies.

QUAST—a quality assessment tool for evaluating and comparing genome assemblies. This tool improves on leading assembly comparison software with new ideas and quality metrics. QUAST can evaluate assemblies both with a reference genome, as well as without a reference. QUAST produces many reports, summary tables and plots to help scientists in their research and in their publications. It is rather fast, and its most time-consuming steps are parallelized; therefore, it can be effectively run on multi-core processors

QUAST aggregates methods and quality metrics from existing software, such as Plantagora, GAGE, GeneMark.hmm and GlimmerHMM, and it extends these with new metrics. For example, the well-known N50 statistic can be artificially increased by concatenating contigs, at the expense of increasing the number of misassemblies. QUAST also computes metrics that are useful for assessing assemblies of previously unsequenced species, whereas most other assembly assessment software require a reference genome.

Many assembly algorithms have been developed for the challenging problem of genome assembly from short reads. QUAST will help scientists to assess different assembly software to choose the best pipeline for their research, and it will help developers of genome assemblers to improve their software and algorithms.

METHODOLOGY:

1. Open home page of Galaxy server.
2. In the search panel search QUAST tool
3. Upload the files of BAM and SAM data (Alignment mapping step) in Assembly mode
4. Select Co-assembly from the two given options
5. Set the parameters accordingly
6. Click on “RUN TOOL”.

OBSERVATIONS:

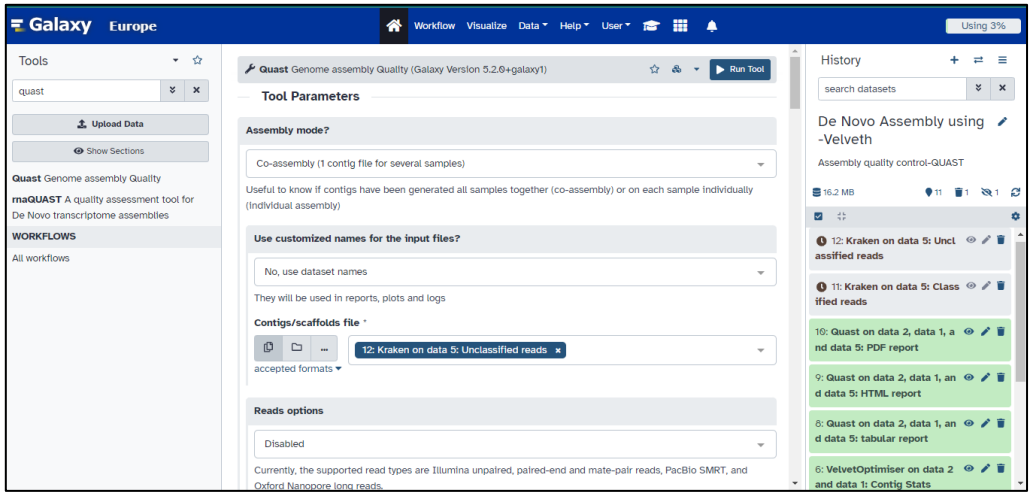


Fig 1: Home page of QUAST Tool

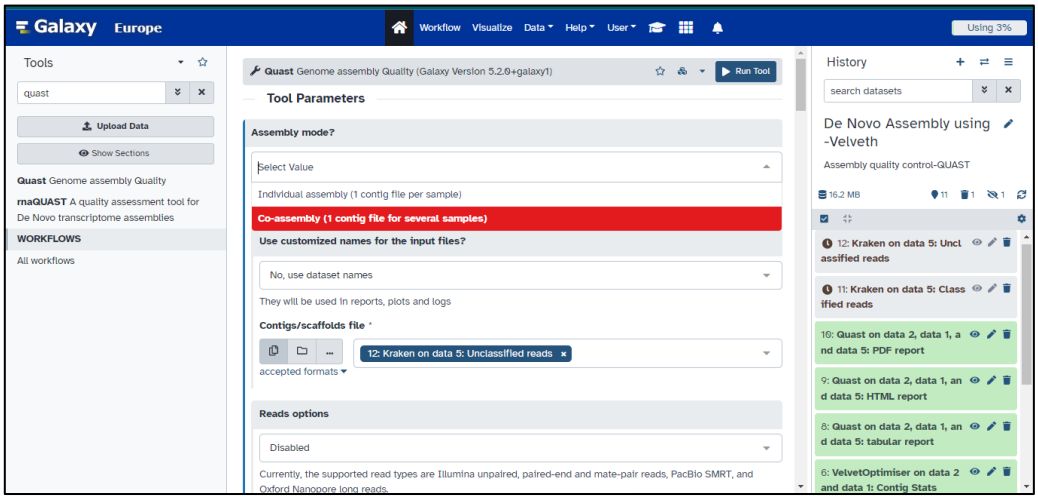


Fig 2.1: Upload the BAM and SAM files obtained from Alignment mapping

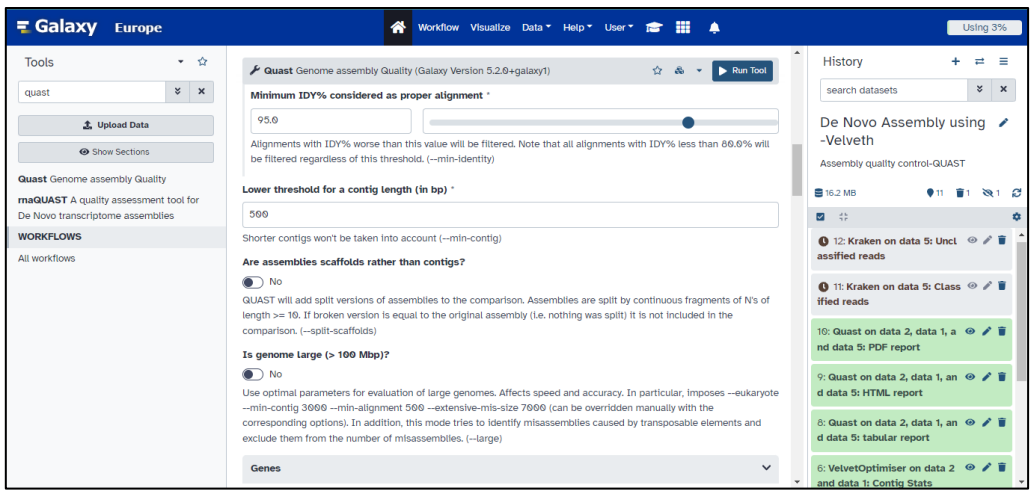


Fig 2.2: Set the parameters accordingly

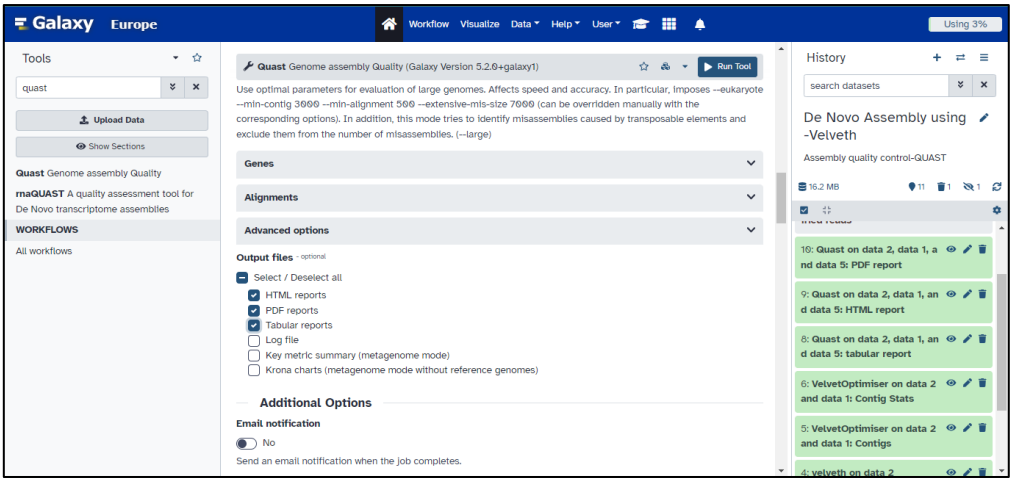


Fig 2.3: Select the output files i.e. HTML Reports, PDF Reports, Tabular Reports

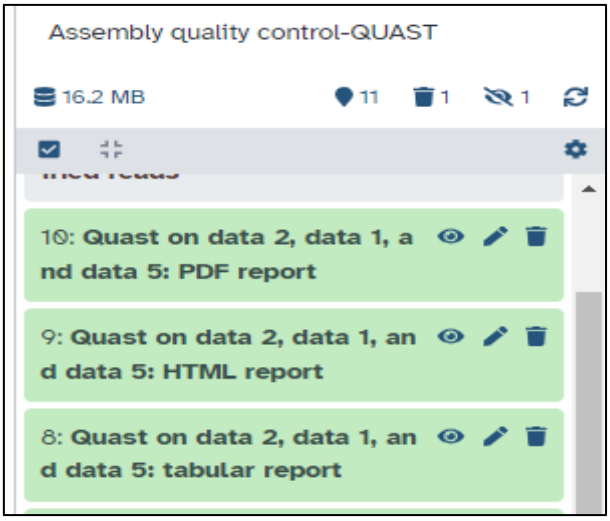


Fig 3: The PDF report, HTML Report and Tabular Report can be visualized

	VelvetOptimiser_on_data_2_and_data_1__Contigs
# contigs (>= 0 bp)	36
# contigs (>= 1000 bp)	4
Total length (>= 0 bp)	182468
Total length (>= 1000 bp)	177614
# contigs	5
Largest contig	132290
Total length	178145
GC (%)	33.60
N50	132290
N90	34980
auN	105411.6
L50	1
L90	2
# N's per 100 kbp	67.36

Fig 4: Tabular data showing sequence information

1. Contigs: is the total number of contigs in the assembly.
2. Total length: is the total number of bases in the assembly.
3. GC (%): is the total number of G and C nucleotides in the assembly, divided by the total length of the assembly.
4. N50 is the length for which the collection of all contigs of that length or longer covers at least half an assembly.
5. L50 (Lx, LG50, LGx) is the number of contigs equal to or longer than N50 (Nx, NG50, NGx)
6. In other words, L50, for example, is the minimal number of contigs that cover half the assembly.

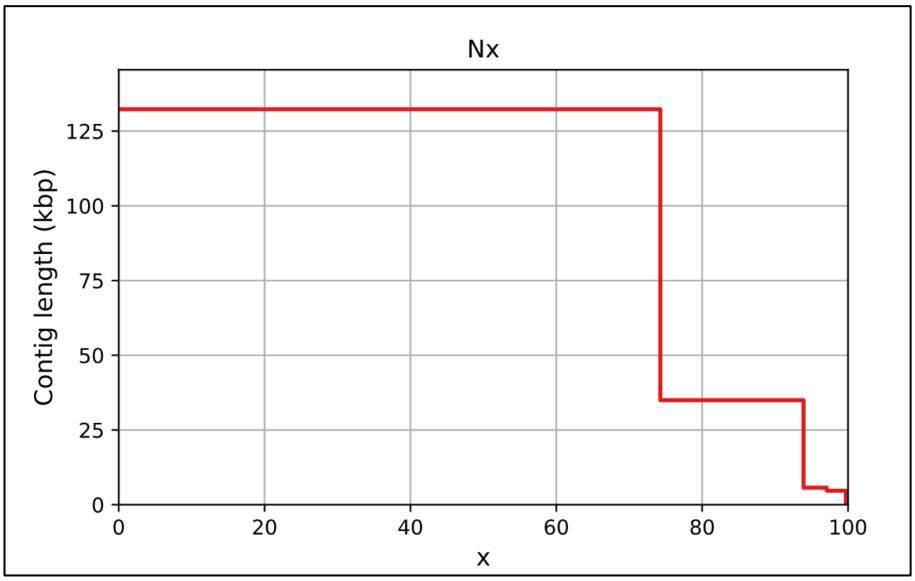


Fig 5: Nx-like plot

1. Nx plot shows Nx values as x varies from 0 to 100 %.
2. Plot of contig number vs. contig length.
3. NGx plot shows NGx values as x varies from 0 to 100 %.

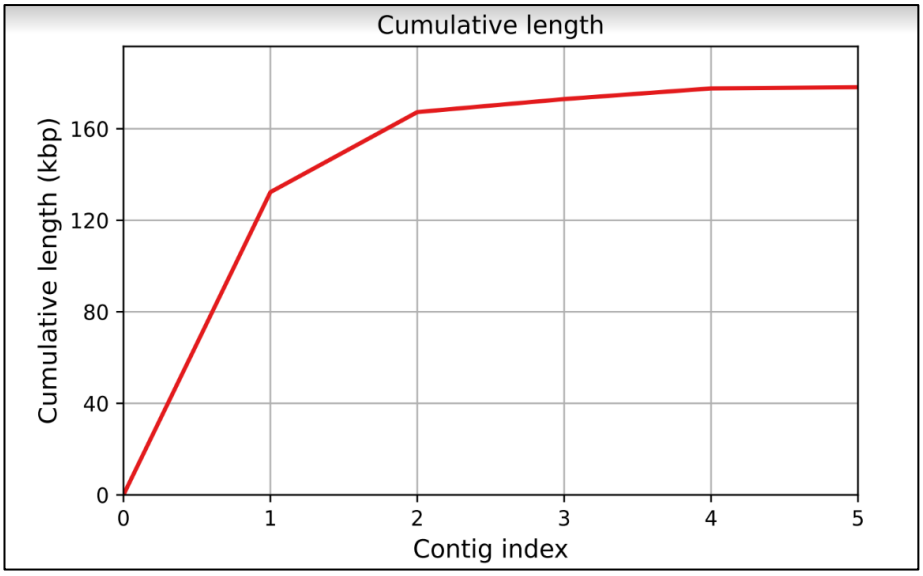


Fig 6: Graph of cumulative length

1. Cumulative length plot shows the growth of contig lengths. On the x-axis, contigs are ordered from the largest to smallest.
2. Graph of contig index vs cumulative length.
3. The y-axis gives the size of the x largest contigs in the assembly.

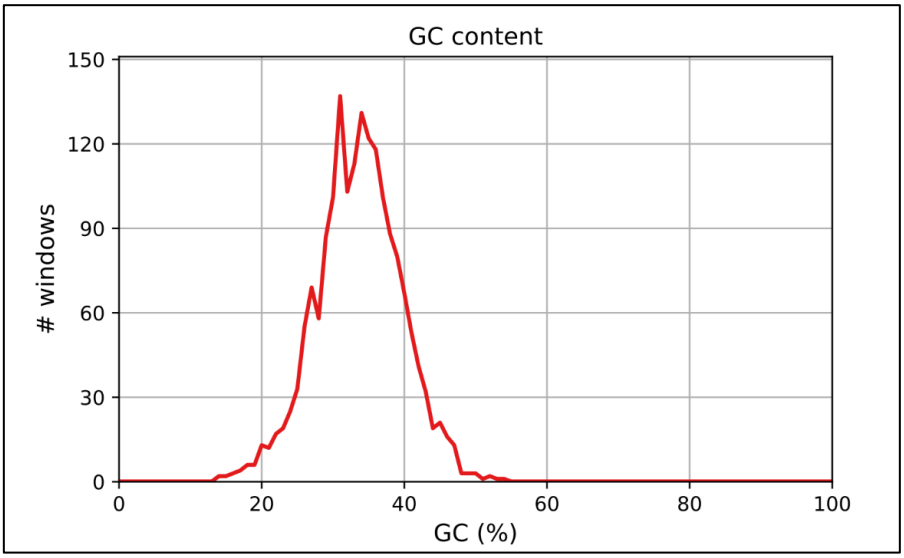


Fig 7: Graph of GC Content

1. GC content plot shows the distribution of GC content in the contigs.
2. Graph of GC% vs #hash windows
3. The x value is the GC percentage (0 to 100 %).
4. The y value is the number of non-overlapping 100 bp windows which GC content equals x %.

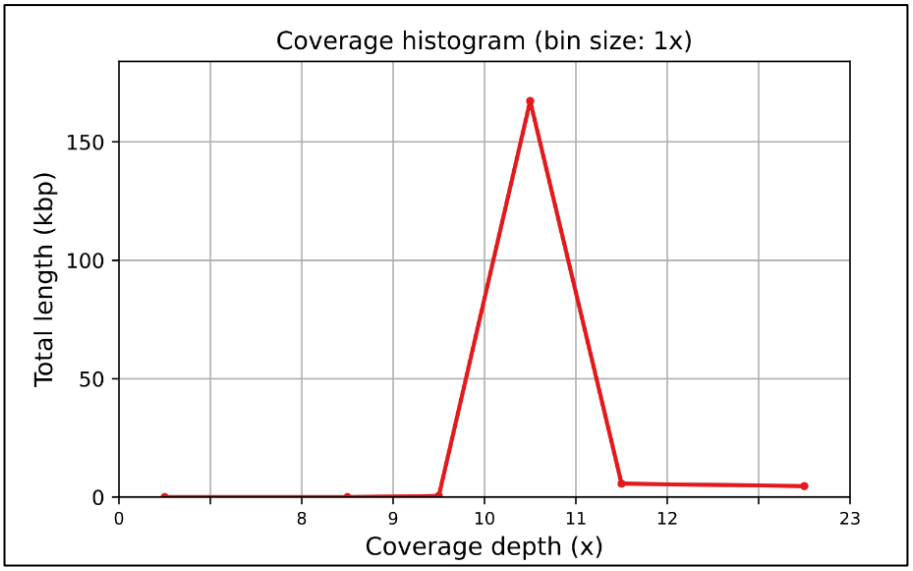


Fig 8: Graph of Coverage histogram

1. Coverage histogram shows distribution of total contig lengths (y-axis) at different read coverage depths (x-axis, grouped in bins).
2. Coverage bin size is automatically selected based on the number of contigs and coverage deviation.

Note: These histograms are only available for assemblies with SPAdes/ Velvet-like contig naming style (...length_X_cov_Y...).

RESULTS:

The QUAST tool was explored and studied to evaluate the quality of genome assemblies with the help of metrics like N50, L50, and GC content was studied to assess the completeness and accuracy of the assemblies.

CONCLUSION:

The completeness and accuracy of the assemblies were assessed by exploring and studying the QUAST tool.

REFERENCES:

1. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. Bioinformatics. 2013 Apr 15;29(8):1072-5. doi: 10.1093/bioinformatics/btt086. Epub 2013 Feb 19. PMID: 23422339; PMCID: PMC3624806.
2. Alexey Gurevich, Vladislav Saveliev, Nikolay Vyahhi, Glenn Tesler Author NotesBioinformatics, Volume 29, Issue 8, April 2013, Pages 1072–1075, https://doi.org/10.1093/bioinformatics/btt086
3. QUAST 5.2.0 manual. (n.d.). https://quast.sourceforge.net/docs/manual.html