Welcome to the Plant Breeding and Genomics Webinar Series

Today's Presenter: Dr. Candice Hansey

Presentation: http://www.extension.org/pages/60428

Host: Heather Merk

Technical Production: John McQueen

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http://www.extension.org/pages/60426

How to Align Sequences

Presenter: Candice Hansey

hansey @msu.edu

Michigan State University

Overview

- Navigating NCBI to obtain sequences
- Using BLAST for sequence alignment
- Using other programs for specialized sequence alignment
- Next generation sequence alignment programs

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- Navigating NCBI to obtain sequences
- Using BLAST for sequence alignment
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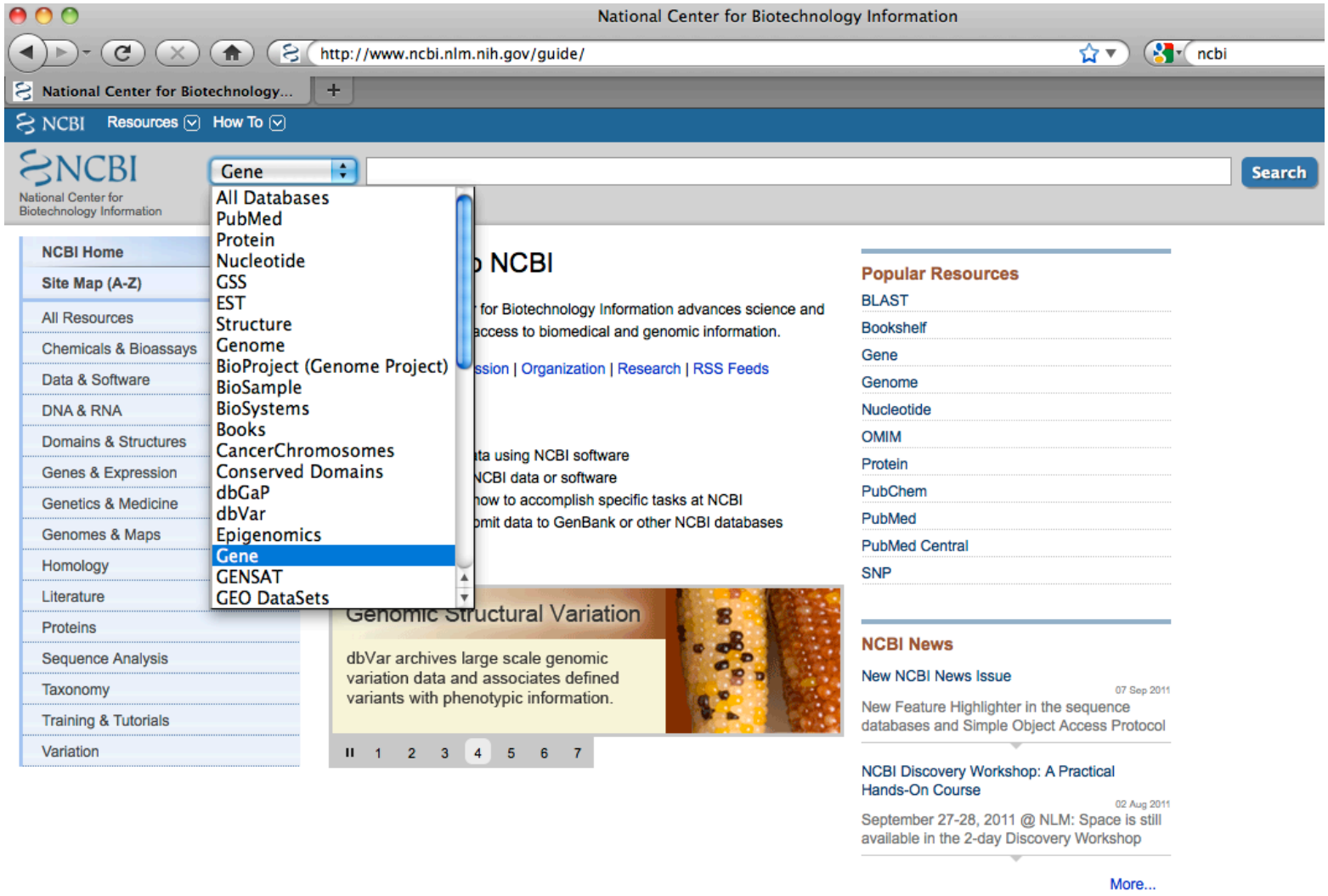
Goal

- Obtain sequence for the maize teosinte branched1 gene and then find organisms with orthologous genes

NCBI


National Center for Biotechnology Information
http://www.ncbi.nlm.nih.gov/guide/
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National Center for Biotechnology...
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Resources
How To
NCBI
National Center for Biotechnology Information
Gene
Search
All Databases
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Protein
Nucleotide
GSS
EST
Structure
Genome
BioProject (Genome Project)
BioSample
BioSystems
Books
CancerChromosomes
Conserved Domains
dbGaP
dbVar
Epigenomics
Gene
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DNA & RNA
Domains & Structures
Genes & Expression
Genetics & Medicine
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Literature
Proteins
Sequence Analysis
Taxonomy
Training & Tutorials
Variation
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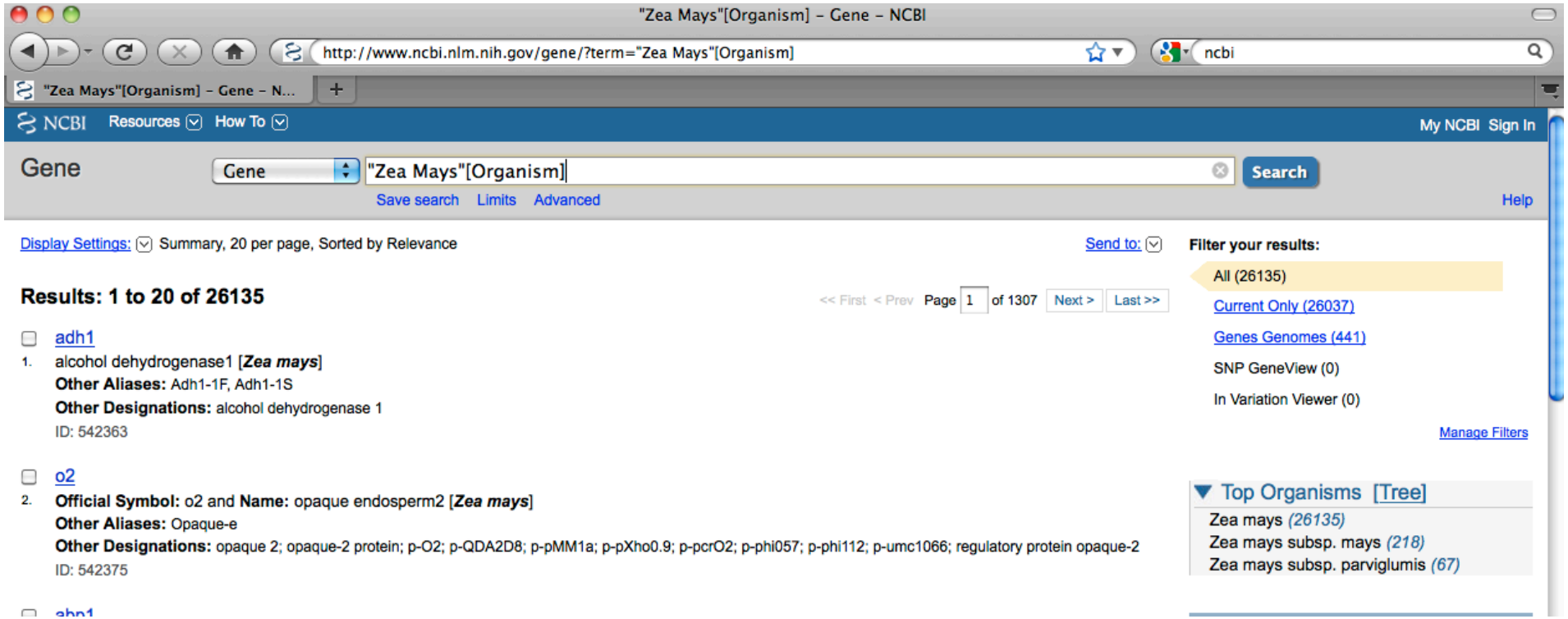
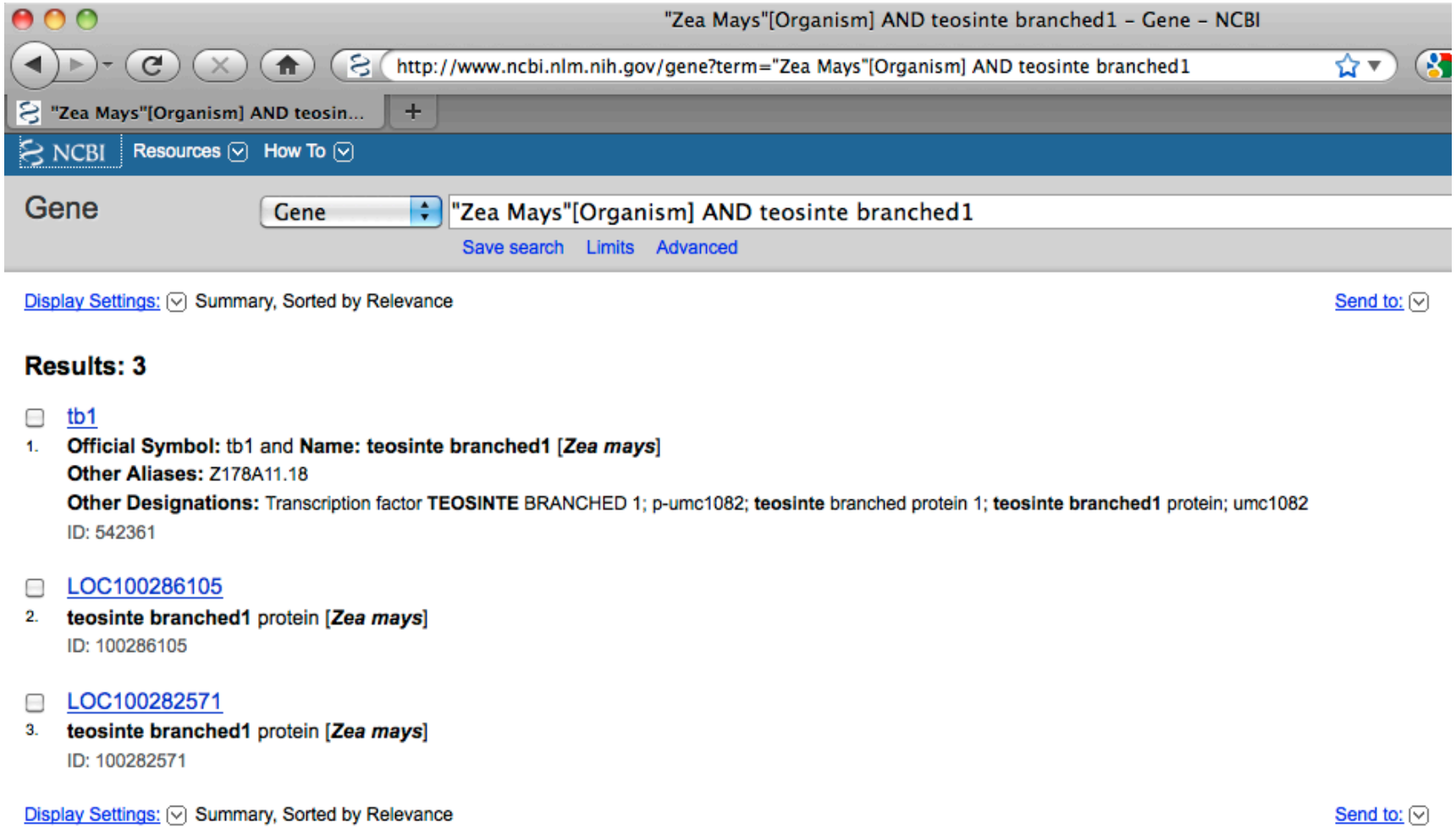
NCBI data or software
how to accomplish specific tasks at NCBI
omit data to GenBank or other NCBI databases
Genomic Structural Variation
dbVar archives large scale genomic variation data and associates defined variants with phenotypic information.
1 2 3 4 5 6 7
Popular Resources
BLAST
Bookshelf
Gene
Genome
Nucleotide
OMIM
Protein
PubChem
PubMed
PubMed Central
SNP
NCBI News
New NCBI News Issue
07 Sep 2011
New Feature Highlighter in the sequence databases and Simple Object Access Protocol
NCBI Discovery Workshop: A Practical Hands-On Course
02 Aug 2011
September 27-28, 2011 @ NLM: Space is still available in the 2-day Discovery Workshop
More...

NCBI



Search specifying Zea Mays as the organism resulted in thousands of sequences

NCBI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http://www.ncbi.nlm.nih.gov/gene?term="Zea Mays"[Organism] AND teosinte branched1
"Zea Mays"[Organism] AND teosin...
NCBI
Resources
How To
Gene
Gene
"Zea Mays"[Organism] AND teosinte branched1
Save search
Limits
Advanced
Display Settings: Summary, Sorted by Relevance
Send to:
Results: 3
tb1
1. Official Symbol: tb1 and Name: teosinte branched1 [Zea mays]
Other Aliases: Z178A11.18
Other Designations: Transcription factor TEOSINTE BRANCHED 1; p-umc1082; teosinte branched protein 1; teosinte branched1 protein; umc1082
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LOC100286105
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ID: 100286105
LOC100282571
3. teosinte branched1 protein [Zea mays]
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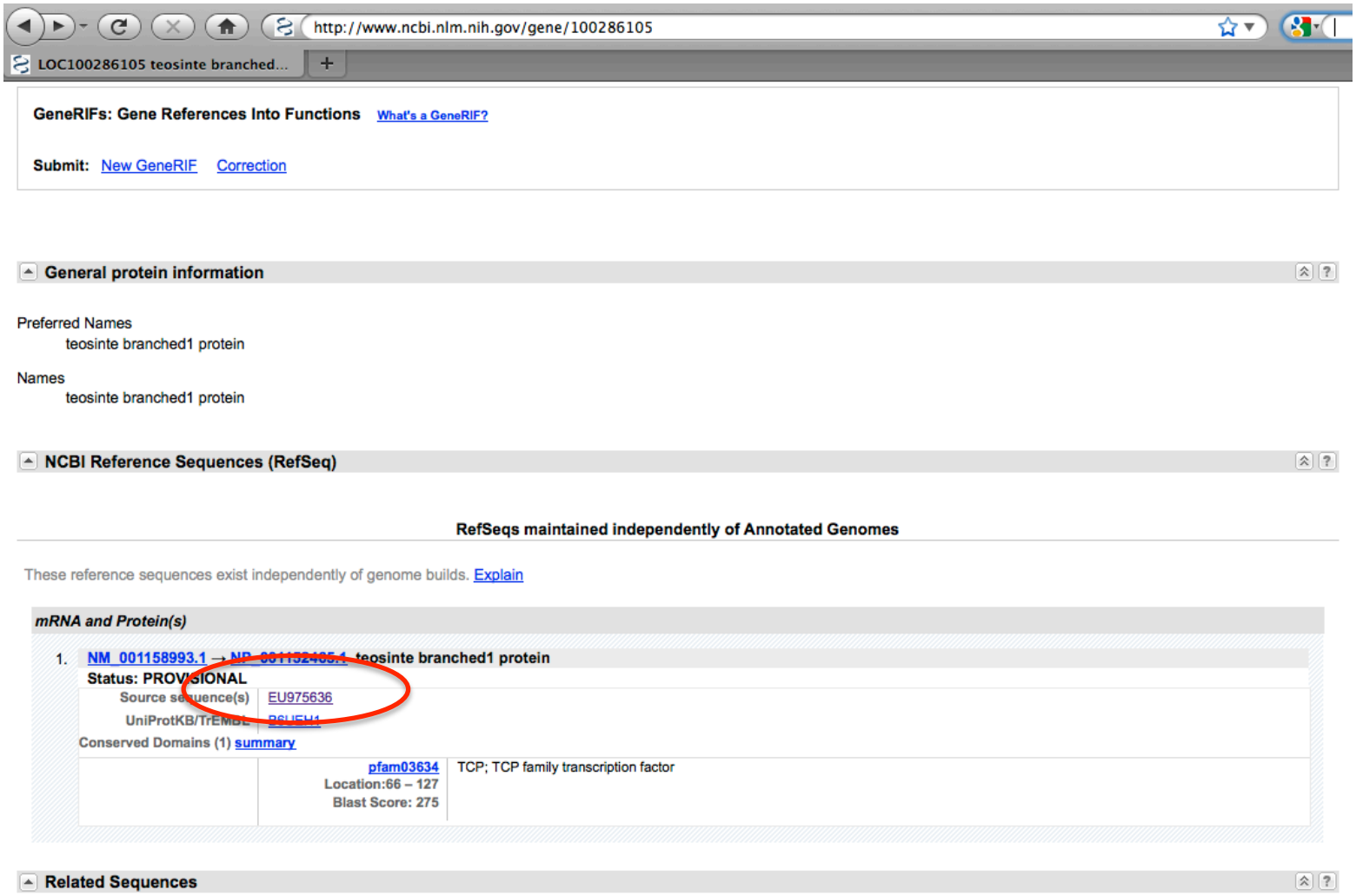
Send to:

NCBI


http://www.ncbi.nlm.nih.gov/gene/100286105
LOC100286105 teosinte branched...
GeneRIFs: Gene References Into Functions What's a GeneRIF?
Submit: New GeneRIF Correction
General protein information
Preferred Names
teosinte branched1 protein
Names
teosinte branched1 protein
NCBI Reference Sequences (RefSeq)
RefSeqs maintained independently of Annotated Genomes
These reference sequences exist independently of genome builds. Explain
mRNA and Protein(s)
1. NM_001158993.1 → NP_001152465.1 teosinte branched1 protein
Status: PROVISIONAL
Source sequence(s) EU975636
UniProtKB/TrEMBL B6UEH1
Conserved Domains (1) summary
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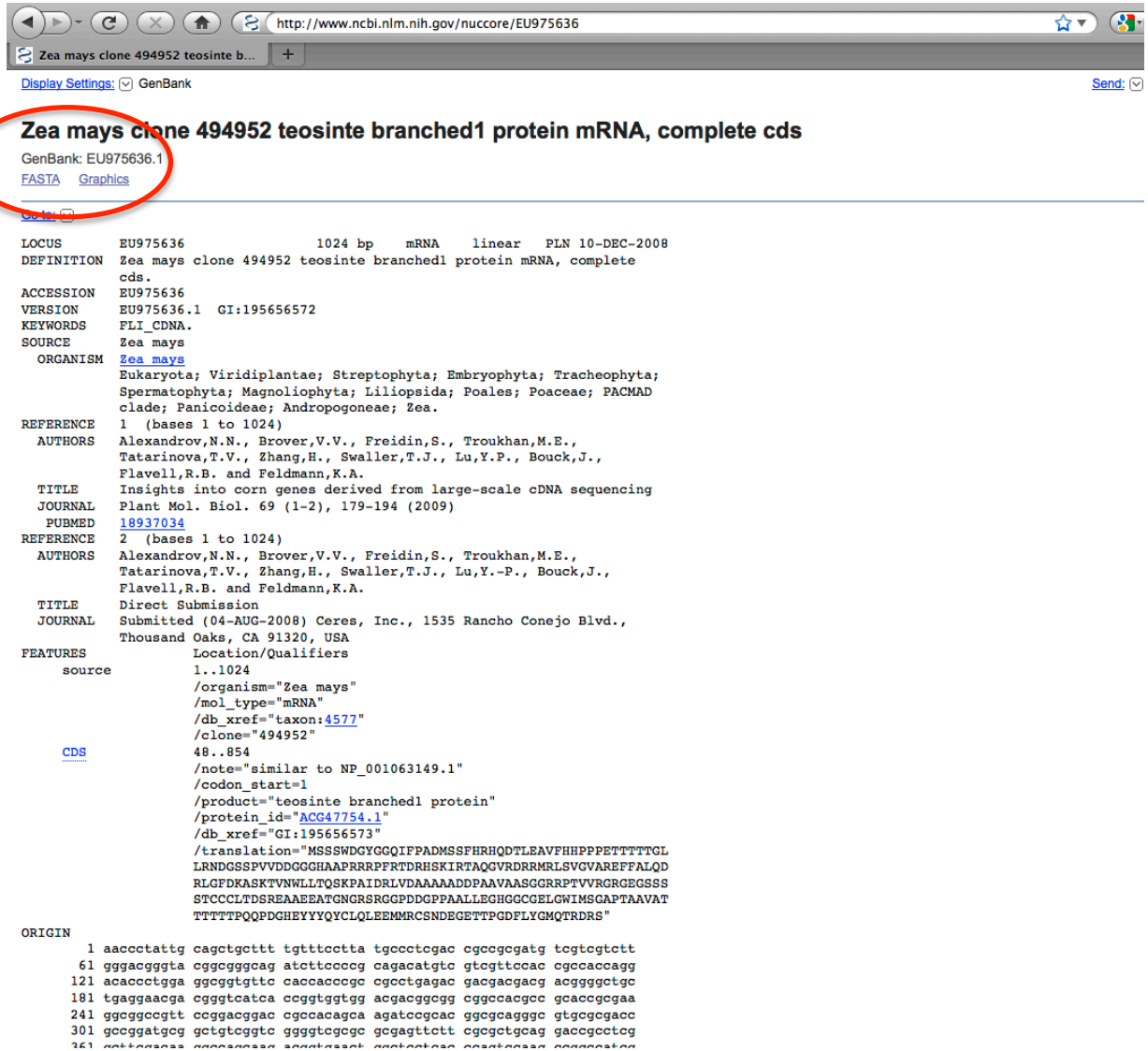
Location:66 – 127
Blast Score: 275
TCP; TCP family transcription factor
Related Sequences

NCBI


http://www.ncbi.nlm.nih.gov/nuccore/EU975636
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VERSION EU975636.1 GI:195656572
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SOURCE Zea mays
ORGANISM Zea mays
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REFERENCE 1 (bases 1 to 1024)
AUTHORS Alexandrov,N.N., Brover,V.V., Freidin,S., Troukhan,M.E., Tatarinova,T.V., Zhang,H., Swaller,T.J., Lu,Y.P., Bouck,J., Flavell,R.B. and Feldmann,K.A.
TITLE Insights into corn genes derived from large-scale cDNA sequencing
JOURNAL Plant Mol. Biol. 69 (1-2), 179-194 (2009)
PUBMED 18937034
REFERENCE 2 (bases 1 to 1024)
AUTHORS Alexandrov,N.N., Brover,V.V., Freidin,S., Troukhan,M.E., Tatarinova,T.V., Zhang,H., Swaller,T.J., Lu,Y.-P., Bouck,J., Flavell,R.B. and Feldmann,K.A.
TITLE Direct Submission
JOURNAL Submitted (04-AUG-2008) Ceres, Inc., 1535 Rancho Conejo Blvd., Thousand Oaks, CA 91320, USA
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/clone="494952"
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ORIGIN
1 aaccctattg cagctgcttt tgtttcctta tgccctcgac cgccgcgatg tcgtcgtctt
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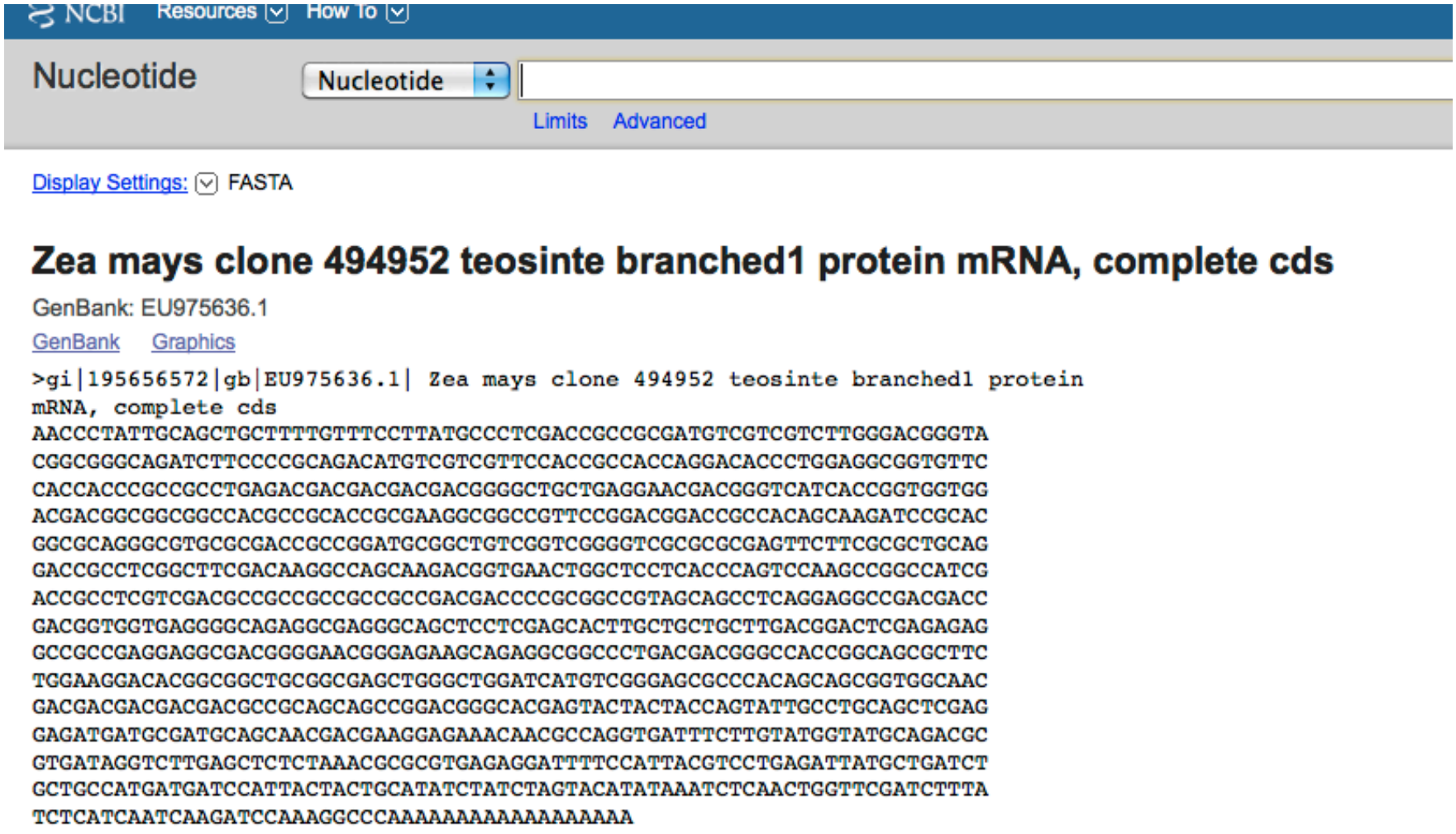
NCBI


NCBI
Resources
How To
Nucleotide
Nucleotide
Limits
Advanced
Display Settings:
FASTA
Zea mays clone 494952 teosinte branched1 protein mRNA, complete cds
GenBank: EU975636.1
GenBank
Graphics
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mRNA, complete cds
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CACCACCCGCCGCCTGAGACGACGACGACGACGGGGCTGCTGAGGAACGACGGGTCATCACCGGTGGTGG
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GCTGCCATGATGATCCATTACTACTGCATATCTATCTAGTACATATAAATCTCAACTGGTTCGATCTTTA
TCTCATCAATCAAGATCCAAAGGCCCAAAAAAAAAAAAAAAAAA

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- Two main versions: NCBI Blast and WU-BLAST

Local vs Global Alignments

```
Global  FTFTALILLAVAV
        F--TAL-LLA-AV

Local   FTFTALILL-AVAV
        --FTAL-LLAAV--
```

For sequences that are divergent, the optimal global alignment introduces gaps that can hide biologically relevant information such as motifs.

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- A gapped alignment is then performed using a modified Smith-Waterman algorithm – indels are added here.
- Only results scoring above a threshold (expect value or e value) are reported back to the user

BLAST Programs

- blastn - query DNA, subject DNA
- blastp- query Protein, subject Protein
- blastx - query DNA (6 frame translation) subject Protein
- tblastn - query protein - subject DNA (6 frame translation) - slow
- tblastx - query DNA (6 frame translation), subject DNA (6 frame translation) - very slow

Which Program Should You Use

What database do you have and how sensitive does your search has to be?

blastn, blastp – good for identifying sequences that are already in a database, finding local regions of similarity in closely related organisms.

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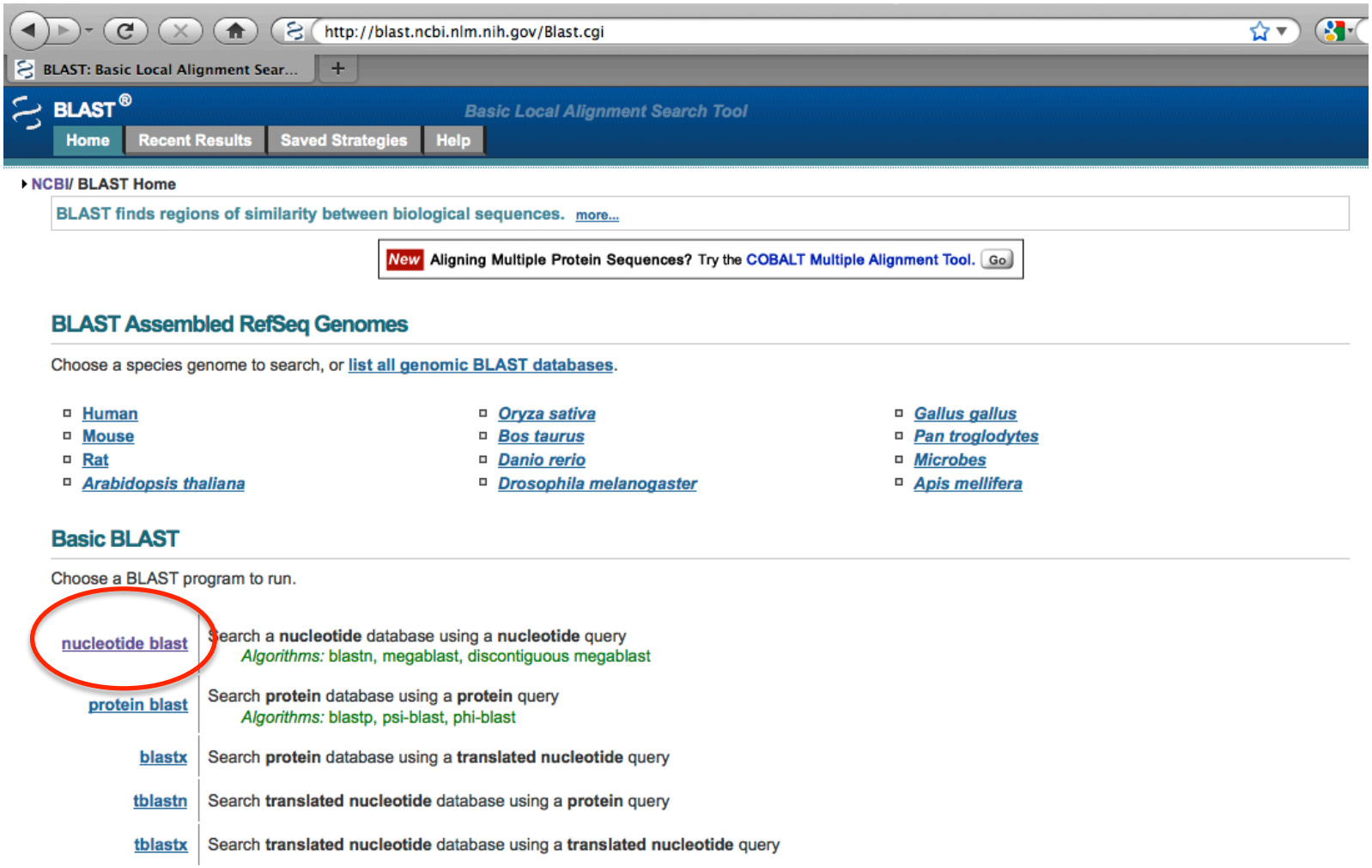
tblastn- useful for finding homologs in sequence where the frame is unknown or sequencing errors are likely to be present such as ESTs and draft sequence.

tblastx - used to detect novel ORFs/exons. Very Slow, use as the last resort.

Performing BLAST



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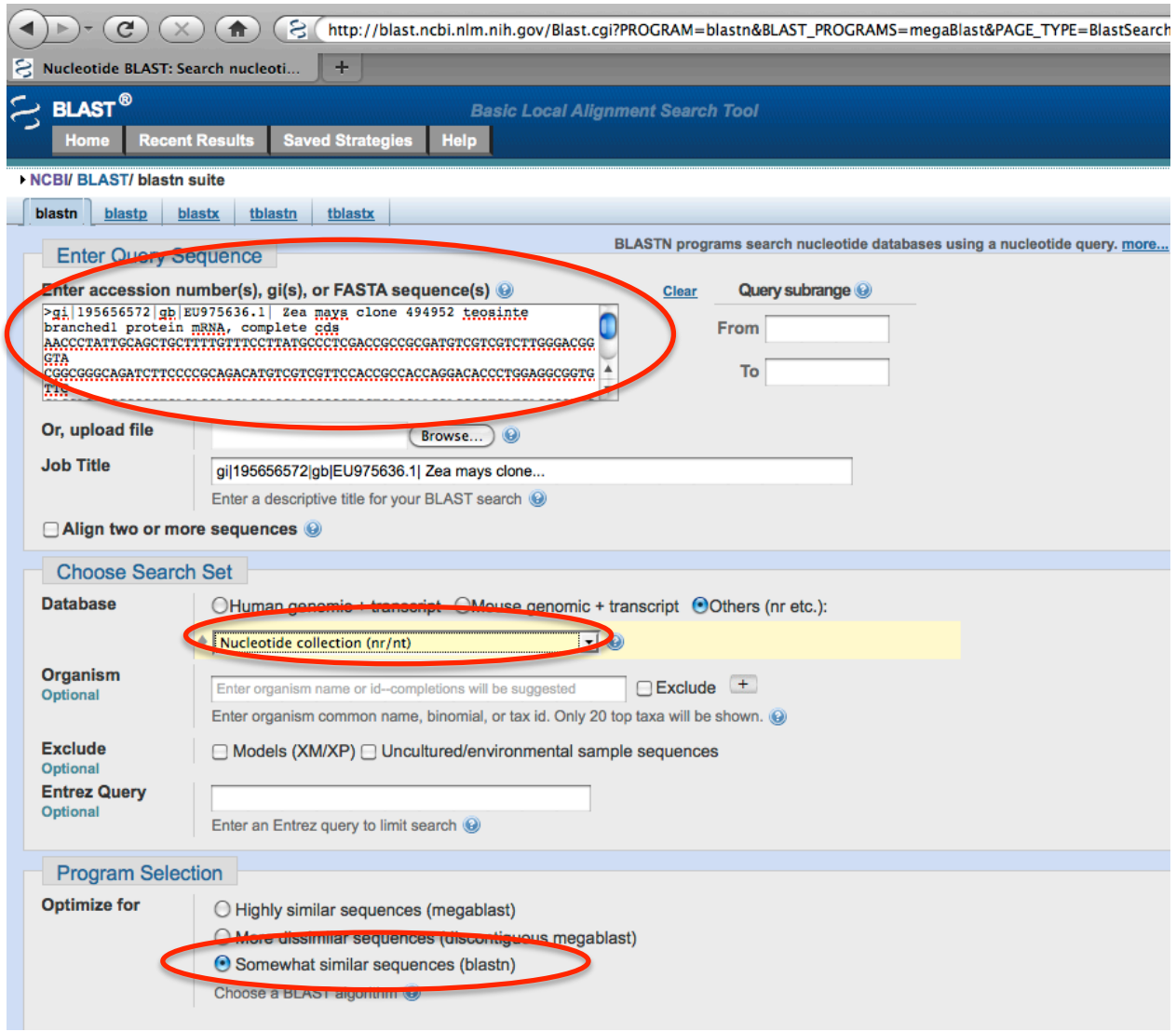

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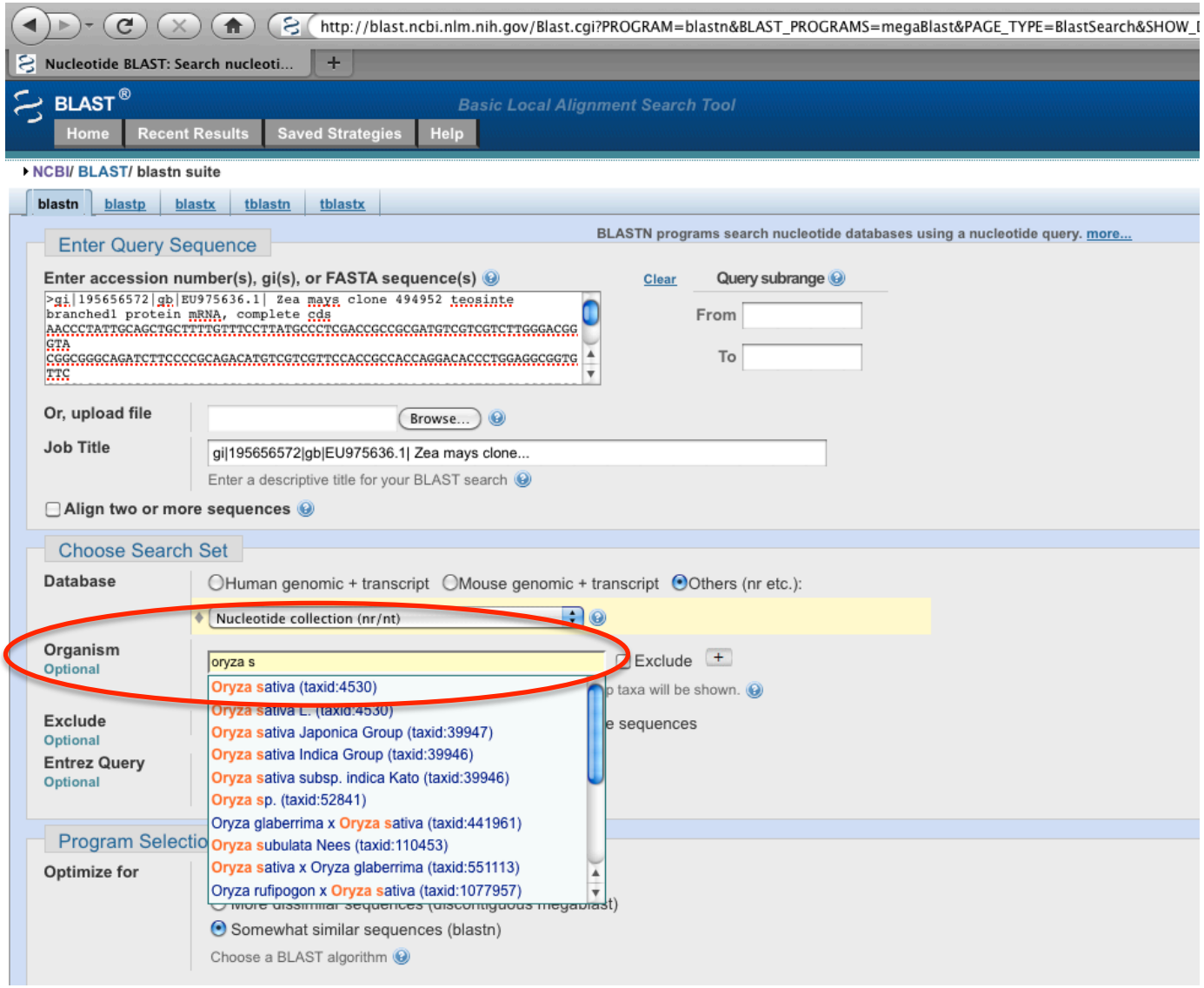
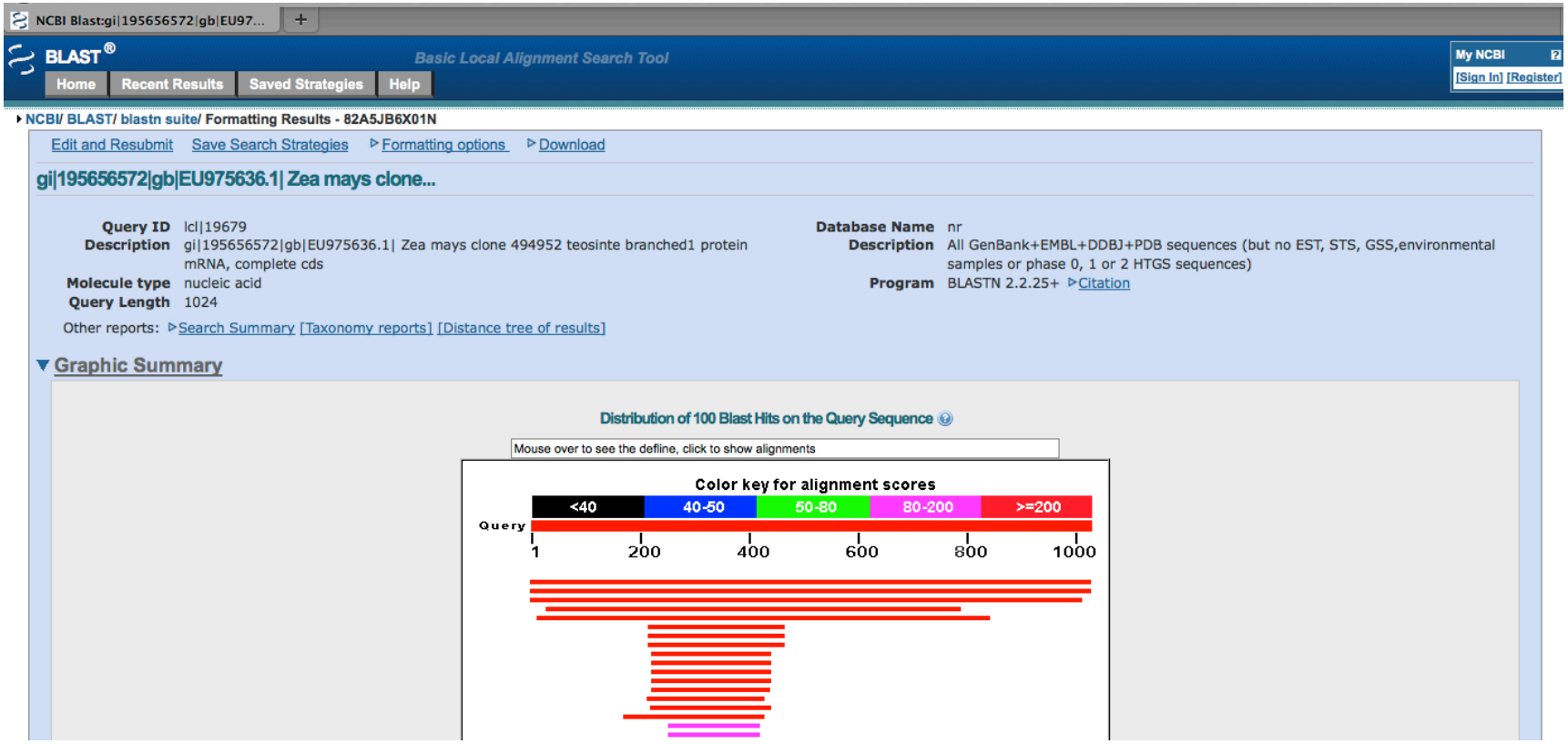


Output from BLAST



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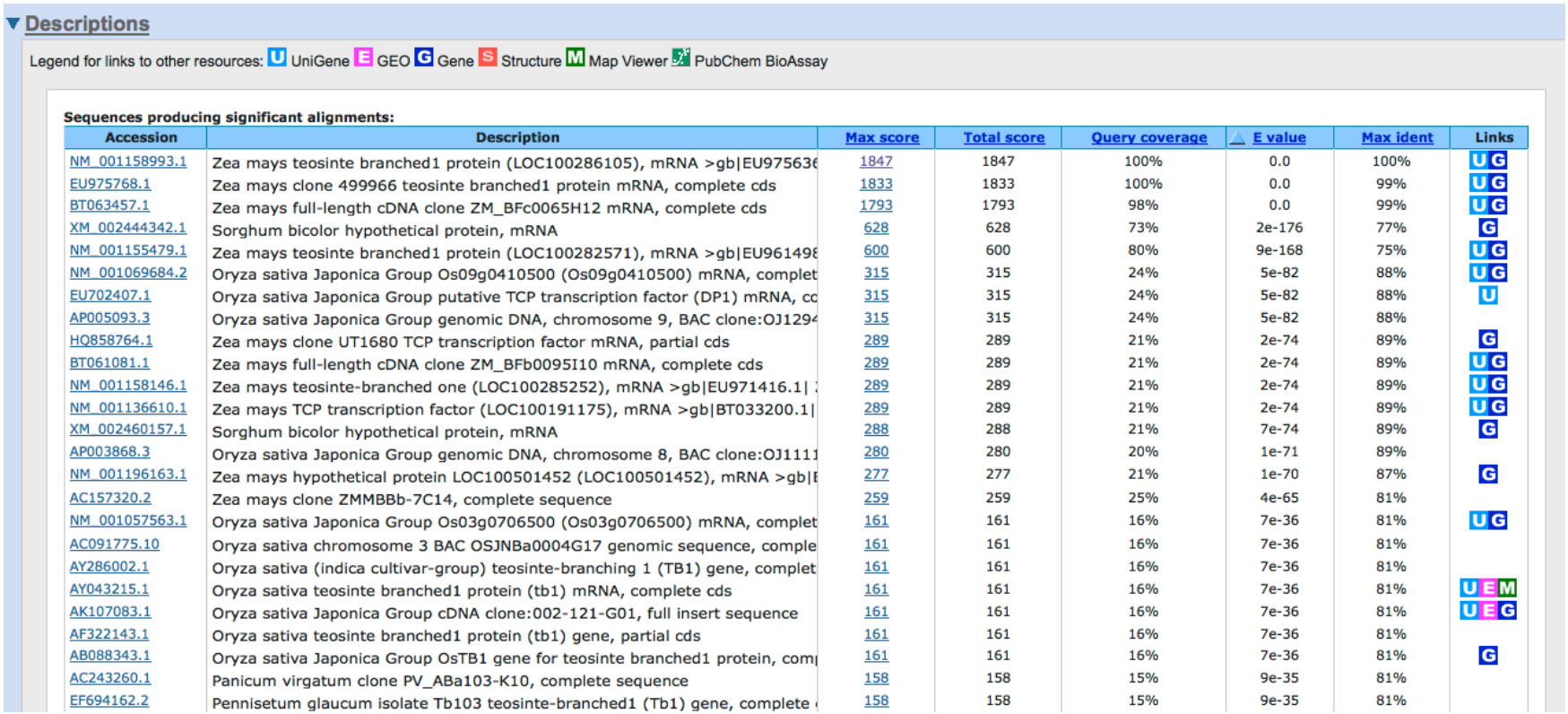


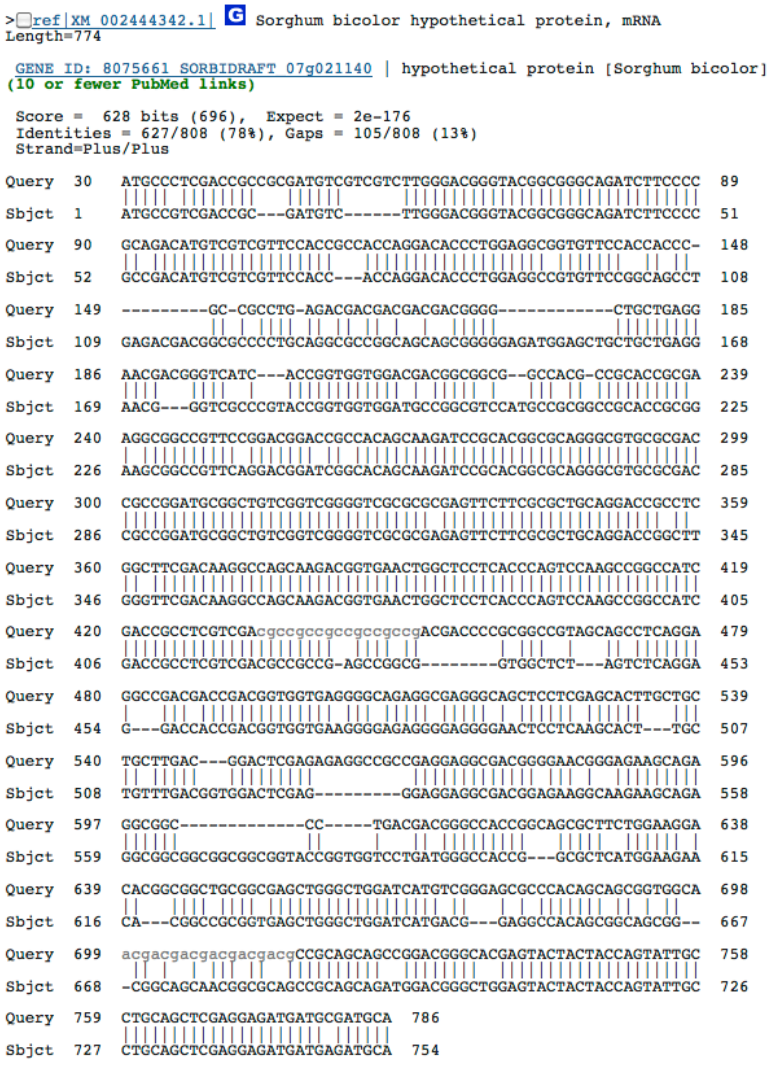
Descriptions

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer PubChem BioAssay

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
NM_001158993.1	Zea mays teosinte branched1 protein (LOC100286105), mRNA >gb|EU97563	1847	1847	100%	0.0	100%	U G
EU975768.1	Zea mays clone 499966 teosinte branched1 protein mRNA, complete cds	1833	1833	100%	0.0	99%	U G
BT063457.1	Zea mays full-length cDNA clone ZM_BFc0065H12 mRNA, complete cds	1793	1793	98%	0.0	99%	U G
XM_002444342.1	Sorghum bicolor hypothetical protein, mRNA	628	628	73%	2e-176	77%	G
NM_001155479.1	Zea mays teosinte branched1 protein (LOC100282571), mRNA >gb|EU96149	600	600	80%	9e-168	75%	U G
NM_001069684.2	Oryza sativa Japonica Group Os09g0410500 (Os09g0410500) mRNA, complet	315	315	24%	5e-82	88%	U G
EU702407.1	Oryza sativa Japonica Group putative TCP transcription factor (DP1) mRNA, co	315	315	24%	5e-82	88%	U
AP005093.3	Oryza sativa Japonica Group genomic DNA, chromosome 9, BAC clone:OJ129	315	315	24%	5e-82	88%	
HQ858764.1	Zea mays clone UT1680 TCP transcription factor mRNA, partial cds	289	289	21%	2e-74	89%	G
BT061081.1	Zea mays full-length cDNA clone ZM_BFb0095I10 mRNA, complete cds	289	289	21%	2e-74	89%	U G
NM_001158146.1	Zea mays teosinte-branched one (LOC100285252), mRNA >gb|EU971416.1|	289	289	21%	2e-74	89%	U G
NM_001136610.1	Zea mays TCP transcription factor (LOC100191175), mRNA >gb|BT033200.1|	289	289	21%	2e-74	89%	U G
XM_002460157.1	Sorghum bicolor hypothetical protein, mRNA	288	288	21%	7e-74	89%	G
AP003868.3	Oryza sativa Japonica Group genomic DNA, chromosome 8, BAC clone:OJ111	280	280	20%	1e-71	89%	
NM_001196163.1	Zea mays hypothetical protein LOC100501452 (LOC100501452), mRNA >gb|E	277	277	21%	1e-70	87%	G
AC157320.2	Zea mays clone ZMMBBb-7C14, complete sequence	259	259	25%	4e-65	81%	
NM_001057563.1	Oryza sativa Japonica Group Os03g0706500 (Os03g0706500) mRNA, complet	161	161	16%	7e-36	81%	U G
AC091775.10	Oryza sativa chromosome 3 BAC OSJNBa0004G17 genomic sequence, comple	161	161	16%	7e-36	81%	
AY286002.1	Oryza sativa (indica cultivar-group) teosinte-branching 1 (TB1) gene, complet	161	161	16%	7e-36	81%	
AY043215.1	Oryza sativa teosinte branched1 protein (tb1) mRNA, complete cds	161	161	16%	7e-36	81%	U E M
AK107083.1	Oryza sativa Japonica Group cDNA clone:002-121-G01, full insert sequence	161	161	16%	7e-36	81%	U E G
AF322143.1	Oryza sativa teosinte branched1 protein (tb1) gene, partial cds	161	161	16%	7e-36	81%	
AB088343.1	Oryza sativa Japonica Group OsTB1 gene for teosinte branched1 protein, com	161	161	16%	7e-36	81%	G
AC243260.1	Panicum virgatum clone PV_ABa103-K10, complete sequence	158	158	15%	9e-35	81%	
EF694162.2	Pennisetum glaucum isolate Tb103 teosinte-branched1 (Tb1) gene, complete	158	158	15%	9e-35	81%	

Output from BLAST



BLAST

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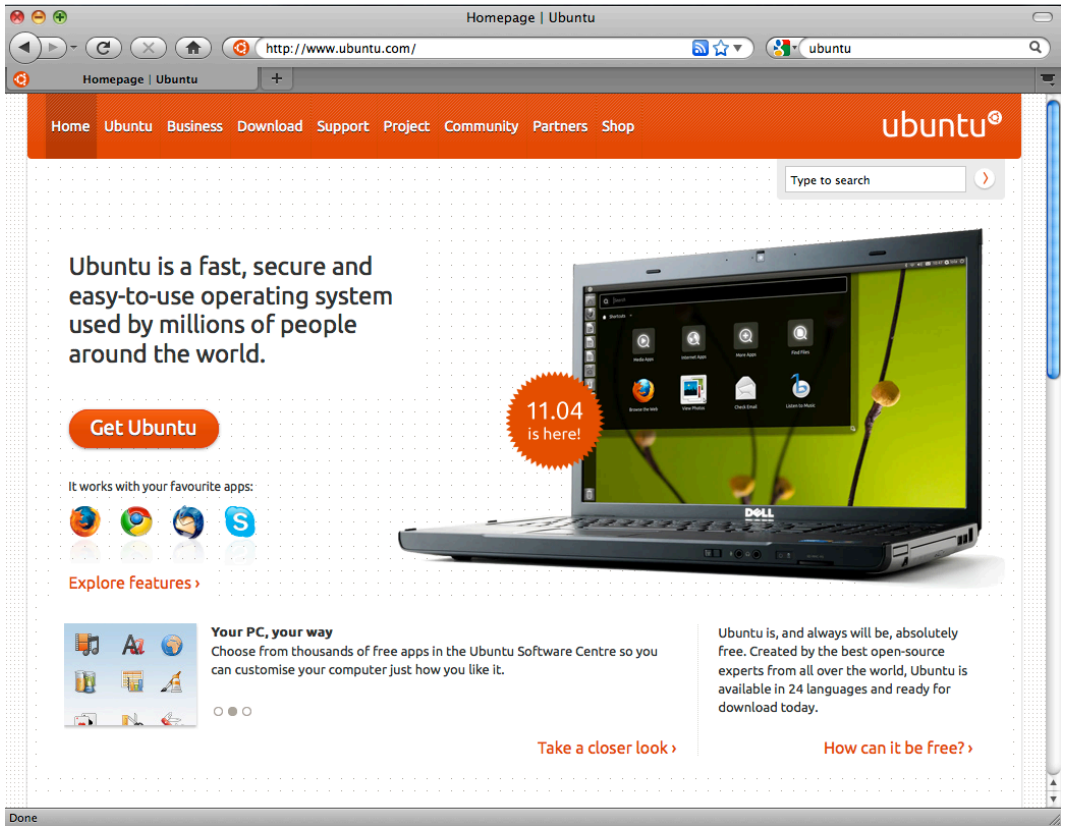
- Web based BLAST interfaces are designed for low throughput searching
- Personalized databases can be generated and subsequent BLASTs performed using the command line
- Using the command line BLAST, multiple sequences can be aligned simultaneously

Ubuntu



- For PC users I highly recommend getting Ubuntu
- This can be run as a virtual machine on your PC through programs such as VirtualBox https://www.virtualbox.org/

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EST and cDNA Alignment

EXONERATE

a generic tool for sequence alignment

- Exonerate is a generic tool for pairwise sequence comparison (http://www.ebi.ac.uk/~guy/exonerate/) and comes grid ready with the ability to chunk files directly through exonerate

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- The est2genom model can be used for alignment of EST sequences to genomic sequence and the cdna2genome model can be used to align full length cDNAs that can be flanked by UTRs

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- For additional models see the man page online at http://www.ebi.ac.uk/~guy/exonerate/exonerate.man.html or on the command line with the -h option
  - exonerate -h

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- Command line example
  - exonerate --query est_sequences.fasta --querytype DNA --target genome_sequence.fasta –targettype DNA --model est2genome –showalignment no --showvulgar no --showtargetgff no --ryo “%qi\t%ti\t%qab\t%qae\t%tab\t%tae \n” –minintron 10 --maxintron 1500 >exonerate_alignment.txt

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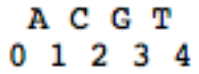
- Command line example
  - exonerate --query est_sequences.fasta --querytype DNA --target genome_sequence.fasta –targettype DNA --model est2genome –showalignment no --showvulgar no --showtargetgff no --ryo "%qi\t%ti\t%qab\t%qae\t%tab\t%tae \n" –minintron 10 --maxintron 1500 >exonerate_alignment.txt
  - The output from this command will be a tab delimited file with the following columns for each alignment: query_id, target_id, query_start, query_end, target_start, target_end
  - Note: using this output format the positions are in interbase coordinates



- For complete list of options see the man page online at http://www.ebi.ac.uk/~guy/exonerate/exonerate.man.html or on the command line with the -h option

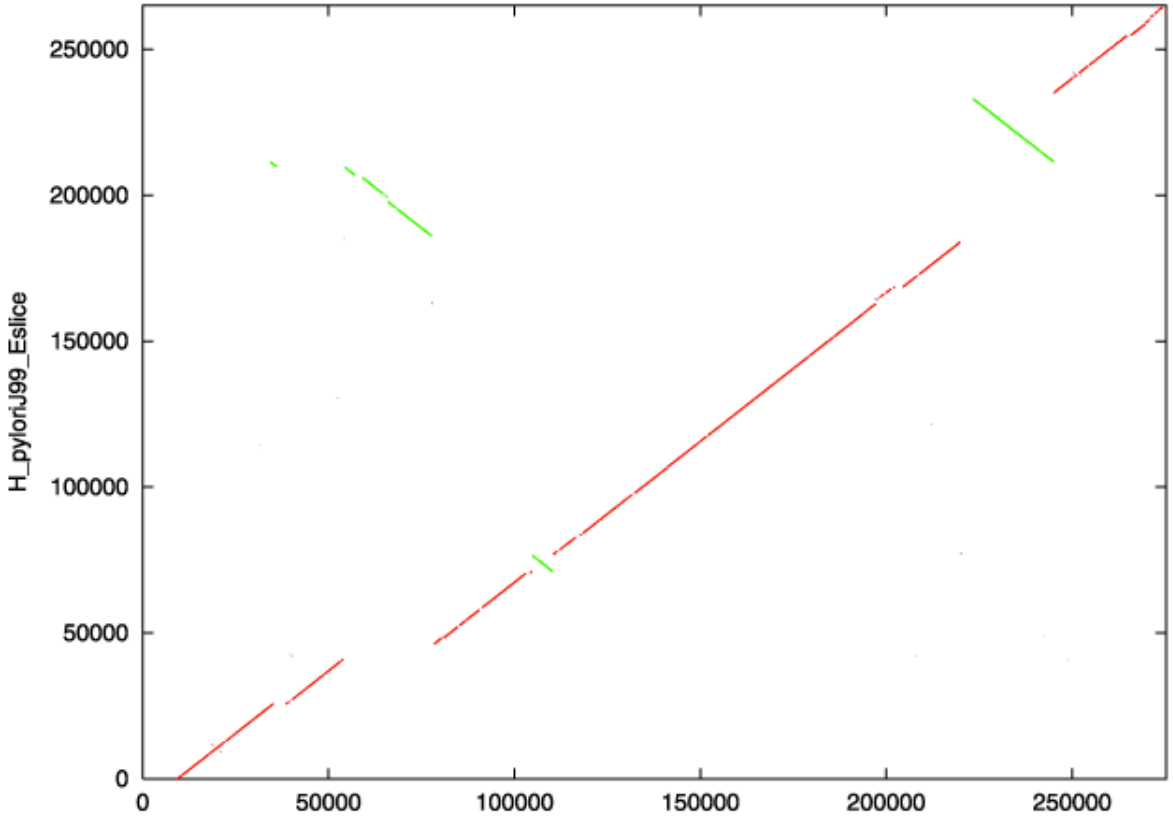
Whole Genome Alignment

- MUMmer – designed for rapid alignment of entire genomes
- NUCmer – designed for alignment of contigs to another set of contigs or a genome
- PROmer – designed for alignment of species too divergent for DNA alignment using six-frame translation of both input sequences
- http://mummer.sourceforge.net/

Whole Genome Alignment

- mummer can handle multiple reference and query sequences
- Command line usage
  - mummer [options] <reference-file> <query-files>
  - mummerplot [options] <match file>
- Example command line
  - mummer –mum –b –c genotype1.fasta genotype2.fasta > output.mums
    - -mum finds all maximal unique matches, -b will compute forward and reverse complement matches, -c reports all match positions relative to the forward starnd
  - mummerplot –x “[0,275287]” –y “[0,265111]” –png output.mums
    - -x sets the x-axis range in the plot, -y sets the y-axis range in the plot, --png outputs the plot in .png format
- Additional options are available in the manual or by using the -h option for both mummer and mummerplot
- Examples for NUCmer and PROmer are available at http://mummer.sourceforge.net/examples/

Whole Genome Alignment



Forward MUMs are red and reverse MUMs are green. Dots on a line with a slope = 1 are unchanged, and dots on a line with a slope = -1 are inverted.

http://mummer.sourceforge.net/examples/

Short Sequence Alignment

- Vmatch is a tool that is ideal for aligning short sequences such as probes, primers, and SNPs with short context sequence
- This program requires a license
- http://www.vmatch.de/

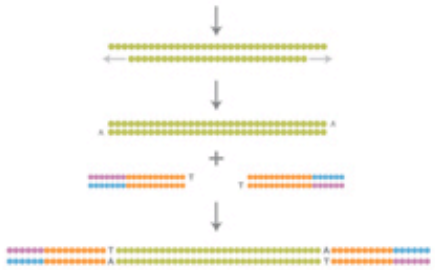
Overview

- Navigating NCBI to obtain sequences
- Using BLAST for sequence alignment
- Using other programs for specialized sequence alignment
- Next generation sequence alignment programs

High Throughput Sequencing Platforms

- Illumina HiSeq 1000 and HiSeq 2000
- Illumina Genome Analyzer IIx
- Life Sciences/Roche 454 pyrosequencing
- Pacific Biosciences
- Ion Torrent

High Throughput Sequencing



Library Preparation
~2 h [15 min hands-on (Nextera)]
< 6 h [< 3 h hands-on (TruSeq)]

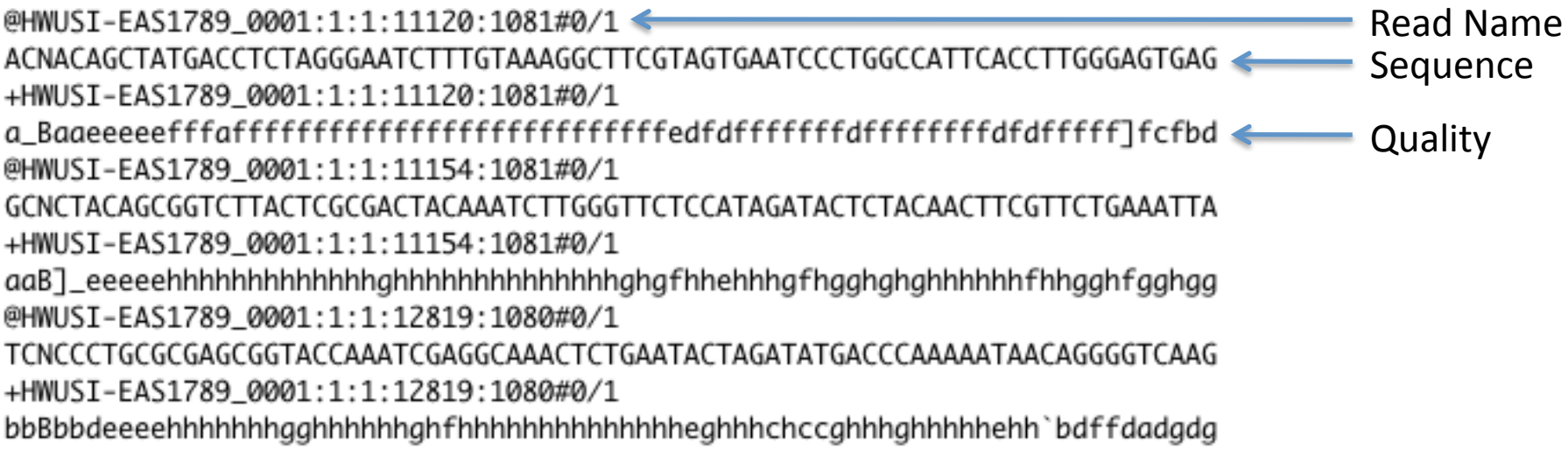
Cluster Generation
~5 h (<10 min hands-on)

Sequencing by Synthesis
~1.5 to 8.5 days

CASAVA
2 days (30 min hands-on)

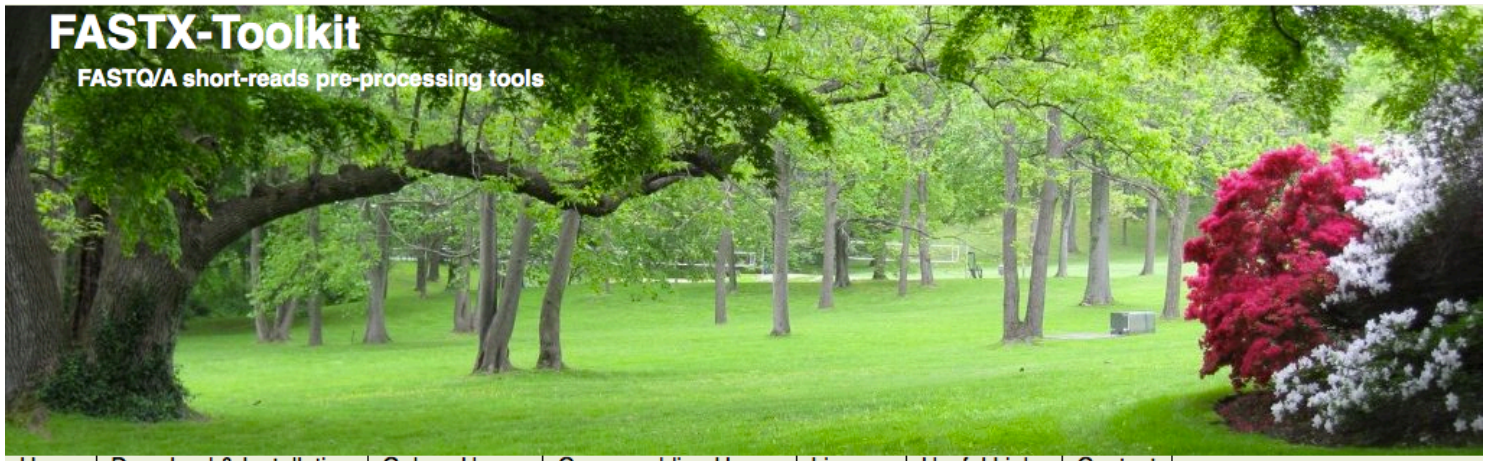
- HiSeq 2000
- Highly parallel sequencing by synthesis
- Single and paired-end reads between 50 bp and 100 bp
- 187 million single end or 374 million paired-end reads per lane
- High error rate in the 3’ end

Sequence File Format



Quality scores are in ASCII characters that are converted to Phred scores.
These scores provide a likelihood that the base was called incorrectly.
10 – 1 in 10 chance the base call is incorrect
20 – 1 in 100 chance the base call is incorrect
30 – 1 in 1000 chance the base call is incorrect
Suggest checking the quality of the reads prior to performing sequence alignments

Read Quality with the FASTX-Toolkit



Home | Download & Installation | Galaxy Usage | Command-line Usage | License | Useful Links | Contact |

Introduction

The FASTX-Toolkit is a collection of command line tools for Short-Reads FASTA/FASTQ files preprocessing.

Next-Generation sequencing machines usually produce FASTA or FASTQ files, containing multiple short-reads sequences (possibly with quality information).

The main processing of such FASTA/FASTQ files is mapping (aka aligning) the sequences to reference genomes or other databases using specialized programs. Example of such mapping programs are: Blat, SHRiMP, LastZ, MAQ and many many others.

However,
It is sometimes more productive to preprocess the FASTA/FASTQ files before mapping the sequences to the genome - manipulating the sequences to produce better mapping results.

The FASTX-Toolkit tools perform some of these preprocessing tasks.

http://hannonlab.cshl.edu/fastx_toolkit/

Read Quality with the FASTX-Toolkit

FASTX Statistics

>fastx_quality_stats –i sequence_file.fastq –o stats.txt

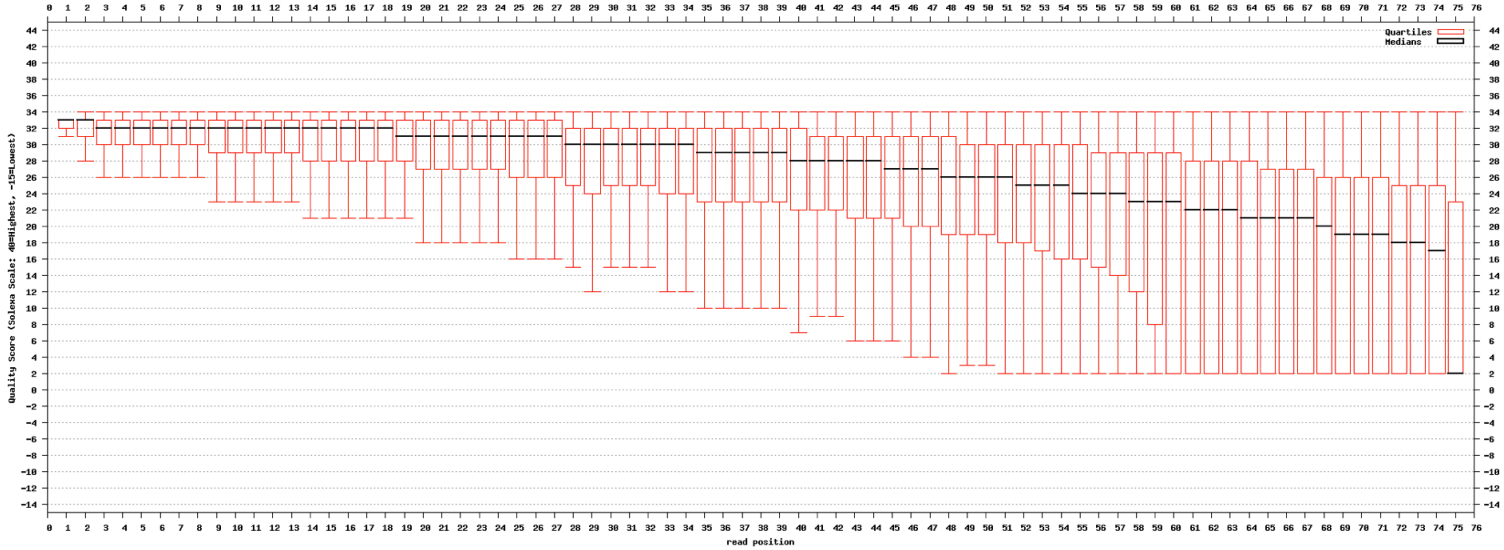
```
$ fastx_quality_stats -h
usage: fastx_quality_stats [-h] [-i INFILE] [-o OUTFILE]

version 0.0.6 (C) 2008 by Assaf Gordon (gordon@cshl.edu)
   [-h] = This helpful help screen.
   [-i INFILE]  = FASTA/Q input file. default is STDIN.
                  If FASTA file is given, only nucleotides
                  distribution is calculated (there's no quality info).
   [-o OUTFILE] = TEXT output file. default is STDOUT.

The output TEXT file will have the following fields (one row per column):
        column  = column number (1 to 36 for a 36-cycles read solexa file)
        count   = number of bases found in this column.
        min     = Lowest quality score value found in this column.
        max     = Highest quality score value found in this column.
        sum     = Sum of quality score values for this column.
        mean    = Mean quality score value for this column.
        Q1      = 1st quartile quality score.
        med     = Median quality score.
        Q3      = 3rd quartile quality score.
        IQR     = Inter-Quartile range (Q3-Q1).
        lW      = 'Left-Whisker' value (for boxplotting).
        rW      = 'Right-Whisker' value (for boxplotting).
        A_Count = Count of 'A' nucleotides found in this column.
        C_Count = Count of 'C' nucleotides found in this column.
        G_Count = Count of 'G' nucleotides found in this column.
        T_Count = Count of 'T' nucleotides found in this column.
        N_Count = Count of 'N' nucleotides found in this column.
        max-count = max. number of bases (in all cycles)
```

FASTQ Quality Chart

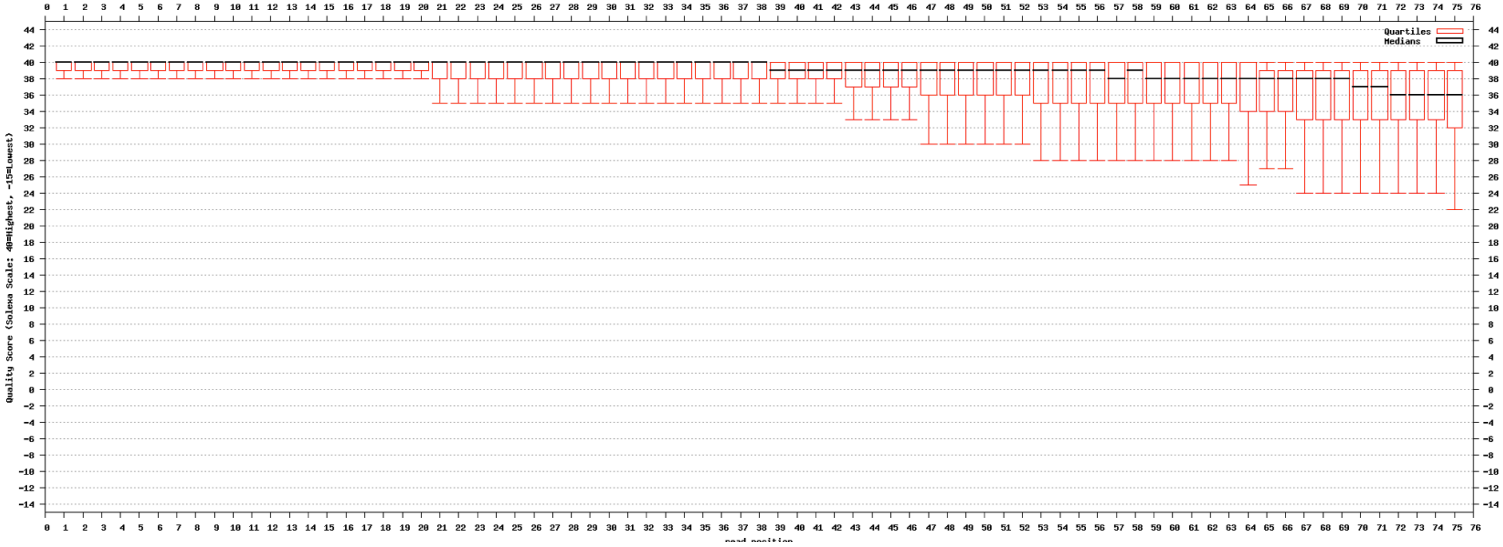
>fastx_quality_boxplot_graph.sh –i stats.txt –t sample1 –o quality.png

```
$ fastq_quality_boxplot_graph.sh -h
Solexa-Quality BoxPlot plotter
Generates a solexa quality score box-plot graph

Usage: /usr/local/bin/fastq_quality_boxplot_graph.sh [-i INPUT.TXT] [-t TITLE] [-p] [-o OUTPUT]

  [-p]           - Generate PostScript (.PS) file. Default is PNG image.
  [-i INPUT.TXT] - Input file. Should be the output of "solexa_quality_statistics" program.
  [-o OUTPUT]    - Output file name. default is STDOUT.
  [-t TITLE]     - Title (usually the solexa file name) - will be plotted on the graph.
```

Read Quality with the FASTX-Toolkit



Bad Sequence



Good Sequence

High Throughput Sequence Alignment

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- Utilize genome indexing such as Burrows-Wheeler for ultrafast and memory-efficient alignment programs

High Throughput Sequence Alignment

- Traditional sequence alignment algorithms cannot be scaled to align millions of reads
- Utilize genome indexing such as Burrows-Wheeler for ultrafast and memory-efficient alignment programs
- Next generation sequence alignment algorithms are rapidly evolving to accommodate the increasing sequence throughput

Tuxedo Suite

- Bowtie – fast and quality aware short read aligner for aligning DNA and RNA sequence reads (http://bowtie-bio.sourceforge.net/index.shtml)
- TopHat – fast, splice junction mapper for RNA-Seq reads built on the Bowtie aligner (http://tophat.cbcb.umd.edu/)
- Cufflinks – assembles transcripts, estimates their abundances, and test for differential expression and regulation using the alignments from Bowtie and TopHat

Bowtie

- Available for Windows, Mac OS X, Linux, and Solaris

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- Not a general-purpose alignment tool like MUMmer, BLAST, or Vmatch
- Ideal for aligning short reads to large genomes
- Forms the basis for TopHat, Cufflinks, Crossbow, and Myrna
- Online manual (http://bowtie-bio.sourceforge.net/manual.shtml) is very helpful to understand the options that are available

Bowtie

- Bowtie Index
  - To print a help screen with the optional parameters
    - bowtie-build –h
  - Command line usage
    - bowtie-build [options]* <reference_in> <ebwt_base>
  - Example command line
    - bowtie-build chr1.fa,chr2.fa,chr3.fa,chr4.fa test_index
    - Additional options can be used to improve performance

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    - Additional options can be used to improve performance
- Alignment with Bowtie
  - To print a help screen with the optional parameters
    - bowtie –h
  - Command line usage
    - bowtie [options]* <ebwt> {-1 <m1> -2 <m2> | --12 <r> | <s>} [<hit>]
  - Example command line for single end reads with our test_index
    - Bowtie test_index --solexa1.3-quals test_index sequence_file.fastq > output_file

Bowtie

- Additional Options:
- Quality aware MAQ-like mode is the default, Bowtie can also be run in a non-quality aware SOAP-like mode
  - Bowtie test_index --solexa1.3-quals –v 2 test_index sequence_file.fastq > output_file
  - -v 2 is specifying that there can only be two mismatches in an alignment
- -a report all valid alignments
- -k 2 report up to 2 valid alignments
- --best report the best alignment
- -m 2 do not report any alignments for reads with greater than 2 reportable alignments
- -S print alignments in SAM format which is compatible with SAMtools for subsequent variant calling and manipulation of the alignments
- Many other options available

TopHat

- Available for Linux and OS X

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- Built on Bowtie and uses the same genome index

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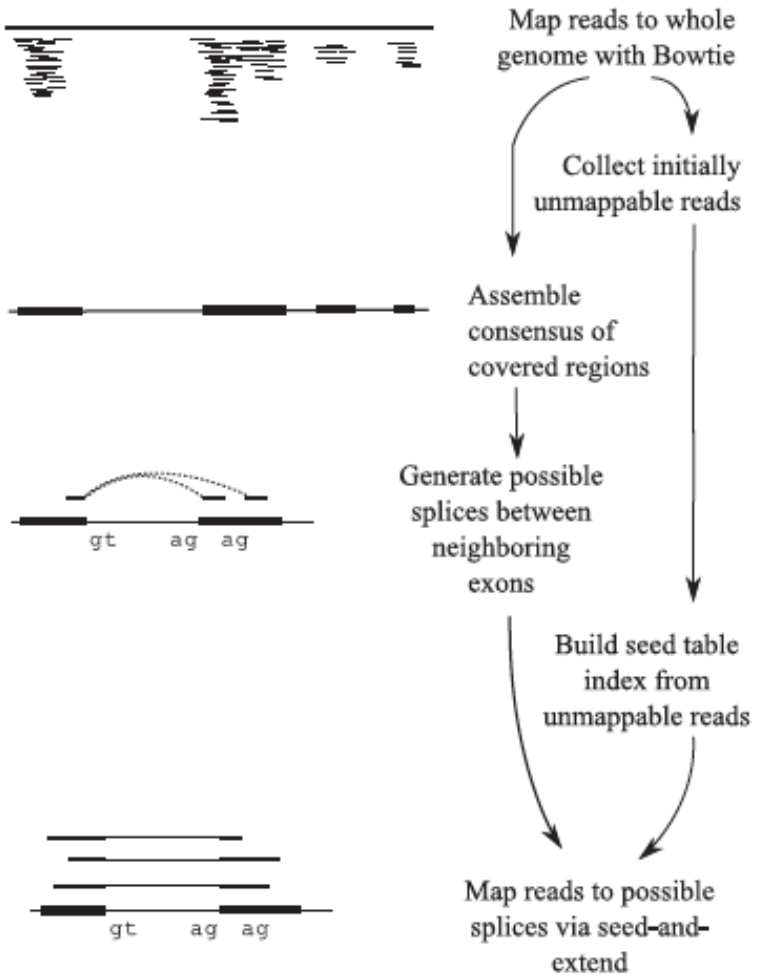
- Online manual (http://tophat.cbcb.umd.edu/manual.html) is very helpful to understand the options that are available

TopHat

TopHat
A spliced read mapper for RNA-Seq

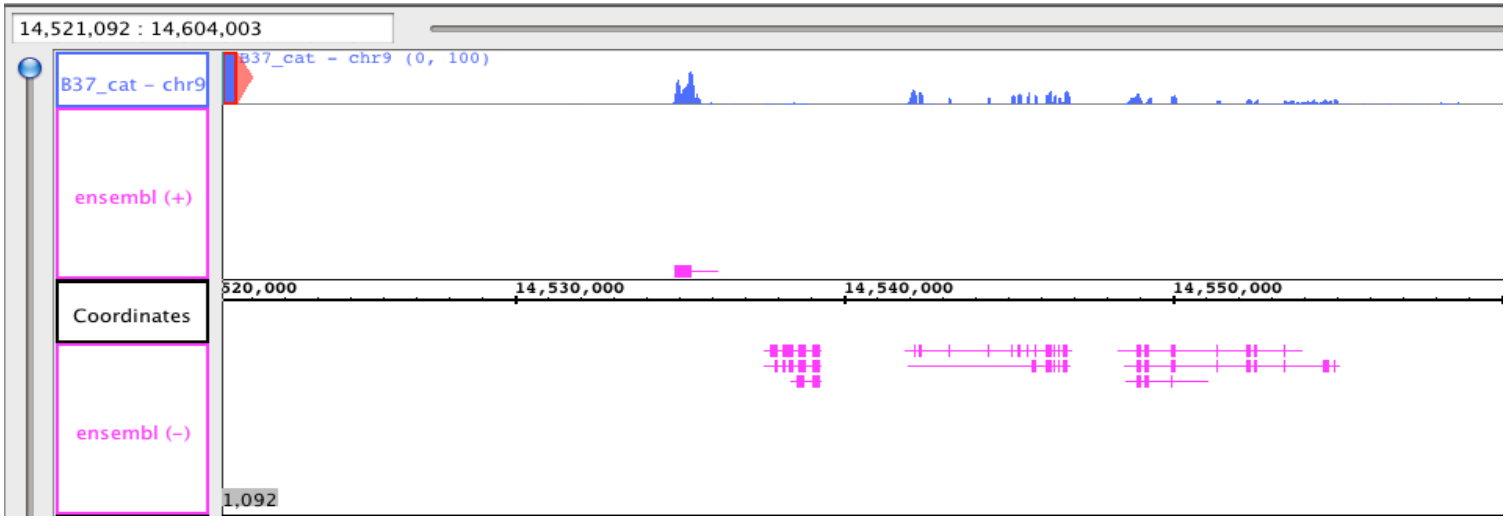


TopHat

- Alignment with TopHat
  - To print a help screen with the optional parameters
    - tophat –h
  - Command line usage
    - tophat [options]* <index_base> <reads1_1[,...,readsN_1]> [reads1_2,...readsN_2]
  - Example command line for single end reads with our test_index from before
    - Tophat –o output_directory --solexa1.3-quals test_index sequence_file.fastq
  - Additional options
    - --max-intron size (default is 500kb)
    - --min-intron size (default is 70bp)
    - --max-multihits (default is 40)
    - --mate-inner-dis (required for paired-end alignment mode)
    - --num_threads N (runs on N CPUs)

TopHat

- Wiggle tracks can be generated from the TopHat output file accepted_hits.bam
- Convert to SAM file
  - samtools view –h –o accepted_hits.sam accepted_hits.bam
- Generate wiggle file
  - wiggles accepted_hits.sam coverage.wig
- Wiggle tracks are viewable in programs such as Integrated Genome Browser (http://bioviz.org/igb/)



Additional Alignment Programs

How to map billions of short reads onto genomes

Cole Trapnell & Steven L Salzberg

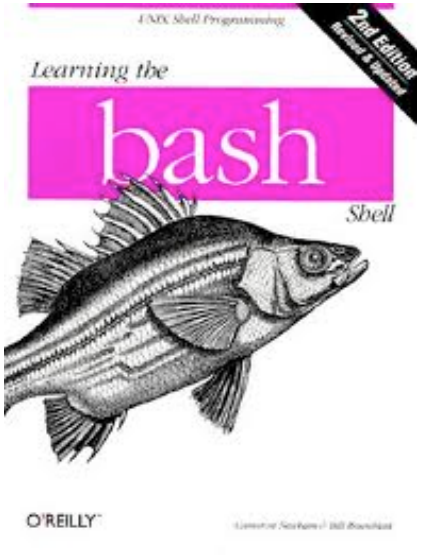
Mapping the vast quantities of short sequence fragments produced by next-generation sequencing platforms is a challenge. What programs are available and how do they work?

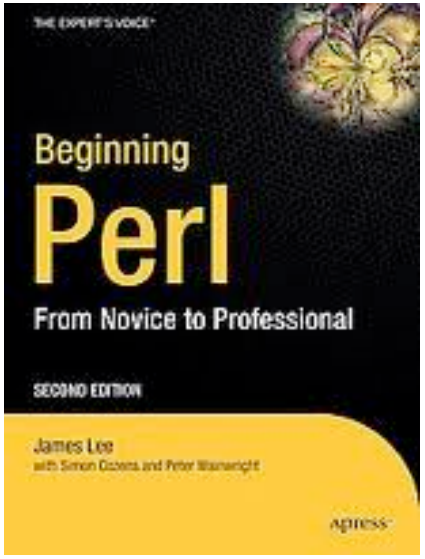
Nature Biotechnology **27**, 455-457 (2009)

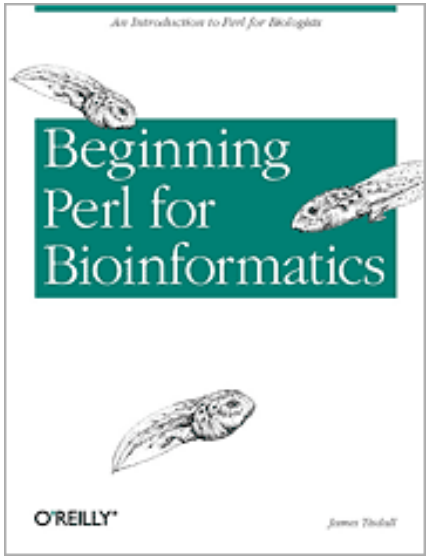
Table 1 A selection of short-read analysis software

Program	Website	Open source?	Handles ABI color space?	Maximum read length
Bowtie	http://bowtie.cbcb.umd.edu	Yes	No	None
BWA	http://maq.sourceforge.net/bwa-man.shtml	Yes	Yes	None
Maq	http://maq.sourceforge.net	Yes	Yes	127
Mosaik	http://bioinformatics.bc.edu/marthlab/Mosaik	No	Yes	None
Novoalign	http://www.novocraft.com	No	No	None
SOAP2	http://soap.genomics.org.cn	No	No	60
ZOOM	http://www.bioinfor.com	No	Yes	240

Valuable Resources







Questions