

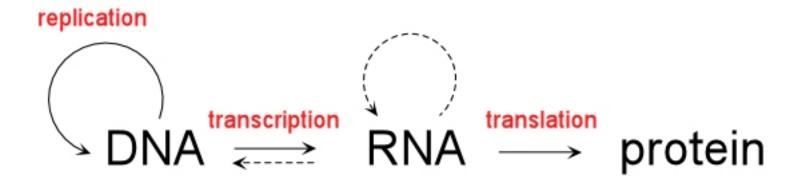
Outline

Nucleic acid sequence analysis

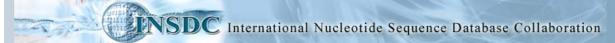
- Famous database of nucleic acid sequence
- > Analysis of nucleic acid sequence
- ➤ BLAST software
 - Basic concept of BLAST
 - BLAST use web manners
 - BLAST use local manners

PART I Famous database of nucleic acid sequence

- Nucleic acid sequence is important material of bioinformatics research.
- Nucleic acid sequences provide the fundamental starting point for describing and understanding the structure, function, and development of genetically diverse organisms.



http://www.insdc.org/



ABOUT INSDC

POLICY

ADVISORS

DOCUMENTS







International Nucleotide Sequence Database Collaboration

The International Nucleotide Sequence Database Collaboration (INSDC) is a long-standing foundational
initiative that operates between <u>DDBJ</u>, <u>EMBL-EBI</u> and <u>NCBI</u>. INSDC covers the spectrum of data raw reads,
though alignments and assemblies to functional annotation, enriched with contextual information relating to
samples and experimental configurations.

Data type	DDBJ	EMBL-EBI	NCBI
Next generation reads	Sequence Read Archive		Sequence Read Archive
Capillary reads	Trace Archive	European Nucleotide Archive (ENA)	Trace Archive
Annotated sequences	<u>DDBJ</u>		GenBank
Samples	BioSample		BioSample
Studies	BioProject	1	BioProject

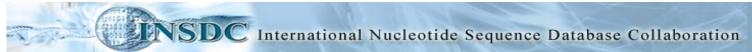
- The INSDC advisory board, the <u>International Advisory Committee</u>, is made up of members of each of the
 databases' advisory bodies. At their most recent meeting, members of this committee unanimously
 endorsed and reaffirmed the existing data-sharing policy of the three databases that make up the INSDC,
 which is stated below.
- . Individuals submitting data to the international sequence databases should be aware of INSDC policy.

How to submit data

- For full details of how to submit data to the databases, please select a collaborating partner.
- · DDBJ, ENA, GenBank
- . The INSDC Feature Table Definition Document is available here.

Feature table

http://www.insdc.org/documents/feature_table.html



ABOUT INSDC POLICY ADVISORS DOCUMENTS



The DDBJ/ENA/GenBank Feature Table Definition

The DDBJ/ENA/GenBank Feature Table Definition Feature Table:
Definition

Version 10.6 November 2016

DNA Data Bank of Japan, Mishima, Japan. EMBL-EBI, European Nucleotide Archive, Cambridge, UK. GenBank, NCBI, Bethesda, MD, USA.

2.1 Format Design

The format design is based on a tabular approach and consists of the following items:

Feature key - a single word or abbreviation indicating functional group Location - instructions for finding the feature Qualifiers - auxiliary information about a feature

http://www.ncbi.nlm.nih.gov/genbank/



GenBank Overview

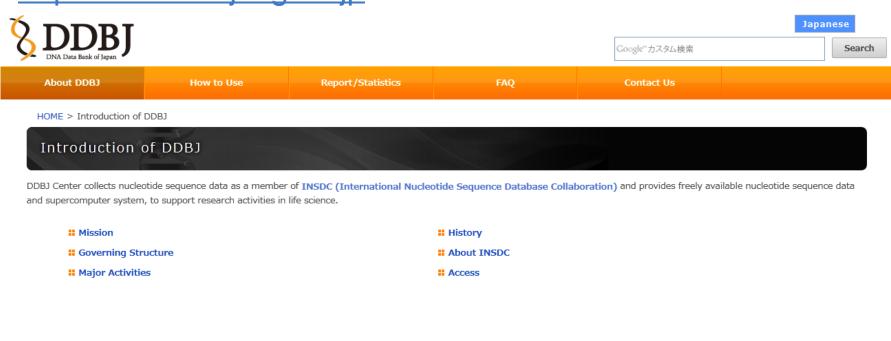
What is GenBank?

GenBank [®] is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (<u>Nucleic Acids Research</u>, 2013 Jan;41(D1):D36-42). GenBank is part of the <u>International Nucleotide Sequence Database Collaboration</u>, which comprises the DNA DataBank of Japan (DDBJ), the European Nucleotide Archive (ENA), and GenBank at NCBI. These three organizations exchange data on a daily basis.

A GenBank release occurs every two months and is available from the ftp site. The release notes for the current version of GenBank provide detailed information about the release and notifications of upcoming changes to GenBank. Release notes for previous GenBank releases are also available. GenBank growth statistics for both the traditional GenBank divisions and the WGS division are available from each release. GenBank growth statistics for both the traditional GenBank divisions and the WGS division are available from each release.

An <u>annotated sample GenBank record</u> for a *Saccharomyces cerevisiae* gene demonstrates many of the features of the GenBank flat file format.

http://www.ddbj.nig.ac.jp



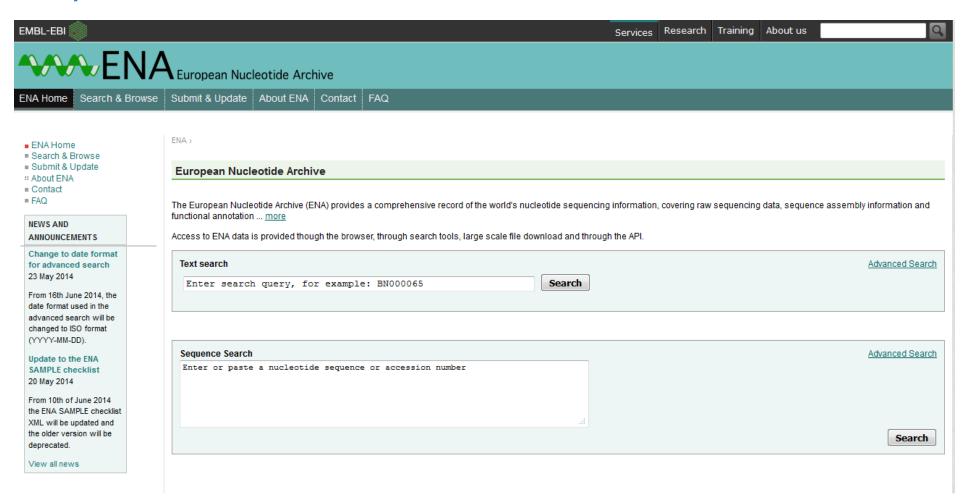
Mission

It is generally accepted that research in biology today requires both computer and experimental equipment equally well. Information achieved from enormous exhaustive data have greatly contributed to the paradigm shift in biology. Biology or life sciences are no longer restricted to wet-bench experiments. *In silico* and *in vitro / in vivo* analyses together will push back the frontiers of life sciences. In particular, researchers in life science must rely on computers to analyze nucleotide sequence data accumulating at a remarkably rapid rate. Actually, this triggered the birth and development of information biology. DDBJ Center is to play a major role in carrying out research in information biology and to run DDBJ operation in the world.

The principal purpose of DDBJ operations is to improve the quality of INSD, as public domains. When researchers make their data open to the public through INSD and commonly shared in world wide, we at DDBJ Center make efforts to describe information on the data as rich as possible, according to the unified rules of INSD, preferably without any stress by using DDBJ.

Nucleotide sequence records organismic evolution more directly than other biological materials and thus is invaluable not only for research in life sciences but also human welfare in general. The database is, so to speak, a common treasure of human beings. With this in mind, we make the database online accessible to anyone in the world.

http://www.ebi.ac.uk/ena/



Submit sequence (http://www.ncbi.nlm.nih.gov/genbank/submit_types)



GenBank Submission Types

Standard

GenBank accepts mRNA or genomic sequence data directly determined by the submitter. The submission must include information about the source organism and annotation provided by the submitter. More details about adding annotation and sample files can be found in the GenBank Submissions Handbook. If you have any questions about the best method for submitting your data, please contact our user services group at: info@ncbi.nlm.nih.gov.

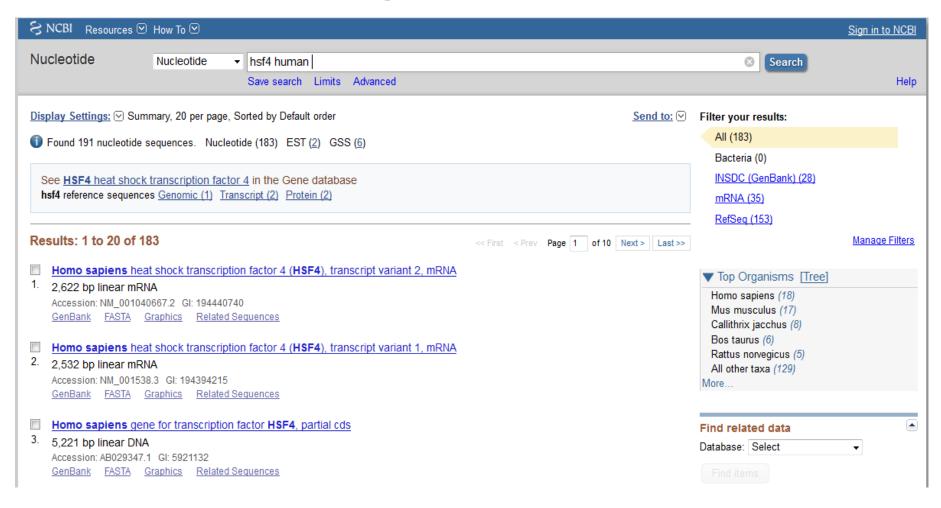
The following data is not accepted by GenBank:

- Noncontiguous sequences
- Primer sequences
- Protein sequences with no underlying nucleotide submission
- Sequence containing a mix of genomic and mRNA sequence
- · Sequences without a physical counterpart (consensus sequences)
- · Sequences with length less than 200 nucleotides

Raw sequence reads from next generation sequencing platforms should be submitted to the Sequence Read Archive (SRA).

Sequence data not directly obtained by the submitter may be acceptable for the Third Party Annotation database.

http://www.ncbi.nlm.nih.gov/nucleotide/



Data format of nucleic acid (I)

Homo sapiens heat shock transcription factor 4 (HSF4), transcript variant 2, mRNA

NCBI Reference Sequence: NM 001040667.2

FASTA Graphics

Go to: ♥

LOCUS NM 001040667 2622 bp mRNA linear PRI 03-MAY-2014 DEFINITION Homo sapiens heat shock transcription factor 4 (HSF4), transcript variant 2, mRNA. ACCESSION NM 001040667 XM 005255925 VERSION NM 001040667.2 GI:194440740 KEYWORDS RefSeq. SOURCE Homo sapiens (human) ORGANISM Homo sapiens Eukarvota: Metazoa: Chordata: Craniata: Vertebrata: Euteleostomi: Mammalia: Eutheria: Euarchontoglires: Primates: Haplorrhini: Catarrhini: Hominidae: Homo. REFERENCE 1 (bases 1 to 2622) AUTHORS Merath K, Ronchetti A and Sidjanin DJ. TITLE Functional analysis of HSF4 mutations found in patients with autosomal recessive congenital cataracts JOURNAL Invest. Ophthalmol. Vis. Sci. 54 (10), 6646-6654 (2013) PUBMED 24045990 REMARK GeneRIF: the transcriptional activation of HSF4 is mediated by interactions between activator and repressor domains within the C-terminal end. Publication Status: Online-Only

Data format of nucleic acid (II)

```
Location/Qualifiers
FEATURES
                      1..2622
     source
                     /organism="Homo sapiens"
                     /mol_type="mRNA"
                     /db_xref="taxon:9606"
                     /chromosome="16"
                     /map="16q21"
                     1..2622
     gene
                     /gene="HSF4"
                     /gene_synonym="CTM; CTRCT5"
                     /note="heat shock transcription factor 4"
                     /db_xref="GeneID:3299"
                     /db xref="HGNC:5227"
                     /db xref="MIM:602438"
                     1..468
     exon
                     /gene="HSF4"
                     /gene_synonym="CTM; CTRCT5"
                     /inference="alignment:Splign:1.39.8"
                     469..594
     exon
                     /gene="HSF4"
                     /gene synonym="CTM; CTRCT5"
                     /inference="alignment:Splign:1.39.8"
                      595..1088
     exon
                     /gene="HSF4"
                     /gene_synonym="CTM; CTRCT5"
                     /inference="alignment:Splign:1.39.8"
                     849..851
     misc_feature
                     /gene="HSF4"
                     /gene_synonym="CTM; CTRCT5"
                     /note="upstream in-frame stop codon"
                     898..2569
     STS
                     /gene="HSF4"
                     /gene synonym="CTM: CTRCT5"
                     /db xref="UniSTS:494796"
     CDS
                     966..2444
                     /gene="HSF4"
                     /gene_synonym="CTM; CTRCT5"
                     /note="isoform b is encoded by transcript variant 2; heat
                      shock factor protein 4: HSF 4: hHSF4: HSTF 4"
```

Data format of nucleic acid (III)

AGATGCACGCGCGCCCCCCCCCAACGAAGCCTGGGTCGCGTTGCGCCGCCGCCACCCTGGGCTGGCA

Homo sapiens heat shock transcription factor 4 (HSF4), transcript variant 2, mRNA

NCBI Reference Sequence: NM_001040667.2

GenBank Graphics

>gi|194440740|ref $|NM_001040667.2|$ Homo sapiens heat shock transcription factor 4 (HSF4), transcript variant 2, mRNA

TGGCTGCGCTCCACCTCTCAGGCGACACCGTAGGCCCAGTGCGCTTCGCAGCACACCACTACGC CGCAACCCTGTGCGCGCTCGAGGTGCGCGCAGCCGCTTCGGCCGAGCTGAACGCCGCGCTGGAGGAGCTG GCGGCGCGCGCGCGCGCGAGGTGCATTGTTTCTGCGTGGTGAGCCACTCGGTGCTGGACGCCT TCCGCGCGCACTGCCCGCGCCTGCGCACCTATACCCTCAAGCTCACGCGCGAGCCGCATCCCTGGAGGCC TACGCTCGTGGCGTGATTGGGCGACTTCTCTCCCCCGTCCCCGTGGACACGCCCCACCCGCTCGGTCCTG GACACACTGCCCCCTCTTTGCCTCCACCCCTCTGCGGACTCTGCAGCTCCGCGGCCCCGGCGCAGGGA GAGGGAGGCACGGCCGGGCCGGGCCTCAAGGGACTTGCCCAGCCCACACCAGGTCGCGCACCGGCGA TTTCTCCTGTAGAACAAAGAAGGAAATAGAGGGACCGAGAGGGGGTGGGACTCGAACCCAAGTCTCCCACT CATCTCACCCCACCCCACCCCACTCCACCCCACCCCTCCACTCCACTCCACTCCACACACACCAT CCCCGCCTGACCCGGCGCCCCGGGGCGGAGTAGGGCGGAGCGGGCAAACGCAGCACTTTCCGCGGC TTTGACGAGCCCGCAGCGGCCGGGCCCAGAGCCGCGGCCGAGACTGCACCATGCAGGAAGCGCCA GCTGCGCTGCCCACGGAGCCAGGCCCCAGCCCCGTGCCTTCCTCGGCAAGCTATGGGCGCTGGTGG GGGACCCAGGCACAGACCACCTGATCCGCTGGAGCCCGAGCGGGACCAGTTTCCTCGTAAGCGACCAGAG CCGTTTCGCCAAGGAAGTGCTGCCCCAGTATTTCAAGCATAGCAACATGGCGAGCTTCGTGCGCCAACTC AACATGTACGGTTTTCGGAAGGTGGTGAGCATCGAGCAGGGCGGCCTGCTTAGGCCGGAGCGCCACCA TCGAGTTCCAGCACCCGAGCTTCGTGCGCGGCCGCGAGCAGCTACTGGAGCGCGTGCGGCGCAAGGTGCC CGCGCTGCGCGGCGACGACGCCGCTGGCGCCCGGAGGACCTGGGTCGACTACTGGGCGAGGTGCAGGCT TTGCGGGGAGTGCAGGAGAGCACCGAGGCGCGGCTGCGGGAGCTCAGGCAGCAGAACGAGATCTTGTGGC GGGAGGTGGTGACACTTCGGCAGAGCCACGGTCAGCACCGGGTCATTGGCAAGCTGATCCAGTGTCT CTTTGGGCCACTTCAGGCGGGCCGAGCAATGCAGGAGGCAAGAGAAAGCTGTCCCTGATGCTGGATGAG GGGAGCTCATGCCCAACACCTGCCAAGTTCAACACCTGCCCTCTACCTGGTGCCCTTCTGCAGGACCCCT ACTTCATCCAGTCGCCTCTCCCAGAGACAAATTTGGGCCTTAGCCCTCACAGGGCCAGGGGCCCCATCAT GAGAAGGGCCTGGCACTGCTCAAAGAAGAGCCGGCCAGTCCAGGGGGGGATGGCCGGGCTGGCCC CCTGGAAGGGAAAGGGAGCTTCAGCCCCGAGGGGCCCAGGAATGCCCAACAGCCTGAACCAGGGGATCCC AGGGAGATACCTGACAGGGGGCCTCTGGGCCTGGAAAGCGGGGACAGGAGCCCAGAGAGTCTGCTGCCTC CGATGCTGCTTCAGCCCCCTCAAGAAAGTGTGGAACCTGCAGGGCCTCTAGATGTGCTGGGCCCCAGTCT CCAAGGGCGAGAATGGACCCTGATGGACTTGGACATGGAGCTGTCCTTGATGCAGCCCTTGGTTCCAGAG CGGGGTGAGCCTGAGCTGCGGTCAAGGGGTTAAATTCTCCAAGCCCAGGGAAGGACCCCACGCTCGGGG GCGCCTCTGAAGGGGCTTGGAACCAGTCCGCCGCTGCACATCCTTCTTGGCTTCCTGGCGCCCCCTATCG GGGGTGAGCGAAGCCCCCACTACTAAATGGCCTCTCTCCACTACCCCGACTATCCCTGCACATAAACTCC GTTTTTTTTTTCAAAAAAAAAAAAAAAAAAAAA

http://www.ncbi.nlm.nih.gov/books/NBK25501/



Introduction to the E-utilities

- . You Tube E-utilities Introduction
- Please see the Release Notes for details and changes.

The Entrez Programming Utilities (E-utilities) are a set of eight server-side programs that provide a stable interface into the Entrez query and database system at the National Center for Biotechnology Information (NCBI). The E-utilities use a fixed URL syntax that translates a standard set of input parameters into the values necessary for various NCBI software components to search for and retrieve the requested data. The E-utilities are therefore the structured interface to the Entrez system, which currently includes 38 databases covering a variety of biomedical data, including nucleotide and protein sequences, gene records, three-dimensional molecular structures, and the biomedical literature.

EFetch Go to: ♥

Base URL

http://eutils.ncbi.nlm.nih.gov/entrez/eutils/efetch.fcgi

Functions

- · Returns formatted data records for a list of input UIDs
- · Returns formatted data records for a set of UIDs stored on the Entrez History server

Required Parameters

db

Database from which to retrieve records. The value must be a valid <u>Entrez database name</u> (default = pubmed). Currently EFetch does not support all Entrez databases. Please see <u>Table 1</u> in Chapter 2 for a list of available databases

Required Parameter - Used only when input is from a UID list

id

UID list. Either a single UID or a comma-delimited list of UIDs may be provided. All of the UIDs must be from the database specified by **db**. There is no set maximum for the number of UIDs that can be passed to EFetch, but if more than about 200 UIDs are to be provided, the request should be made using the HTTP POST method.

efetch.fcgi?db=protein&id=15718680,157427902,119703751

Nucleotide/Nuccore

Fetch the first 100 bases of the plus strand of GI 21614549 in FASTA format:

http://eutils.ncbi.nlm.nih.gov/entrez/eutils/efetch.fcgi?db=nuccore&id=21614549&strand=1&seq_start=1&seq_stop=100&rettype=fasta&retmode=text

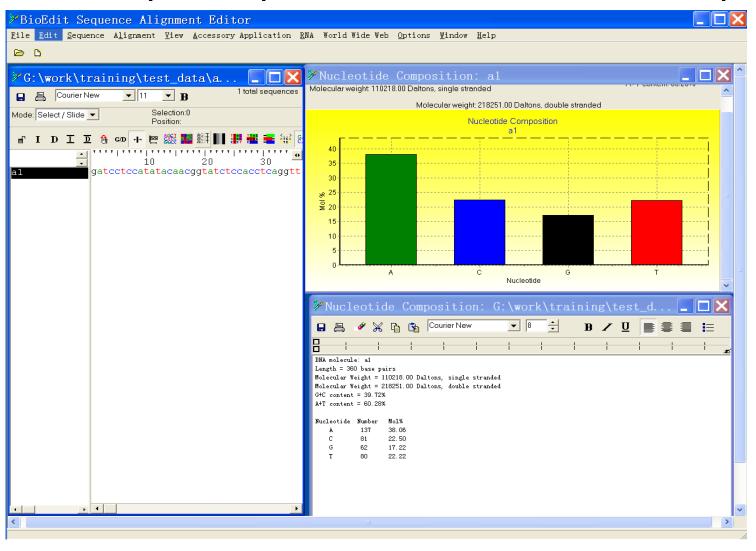
PART II Analysis of nucleic acid sequence

Analysis of nucleic acid sequence

- (1) Getting basic information of a nucleic acid sequence
- (2) Primer design
- (3) Tow sequences alignment
- (4) Multi sequences alignment
- (5) Finding open reading frame of a nucleic acid sequence
- (6) Gene prediction
- (7) Sequence localization in genome
- (8) Sequence assembly

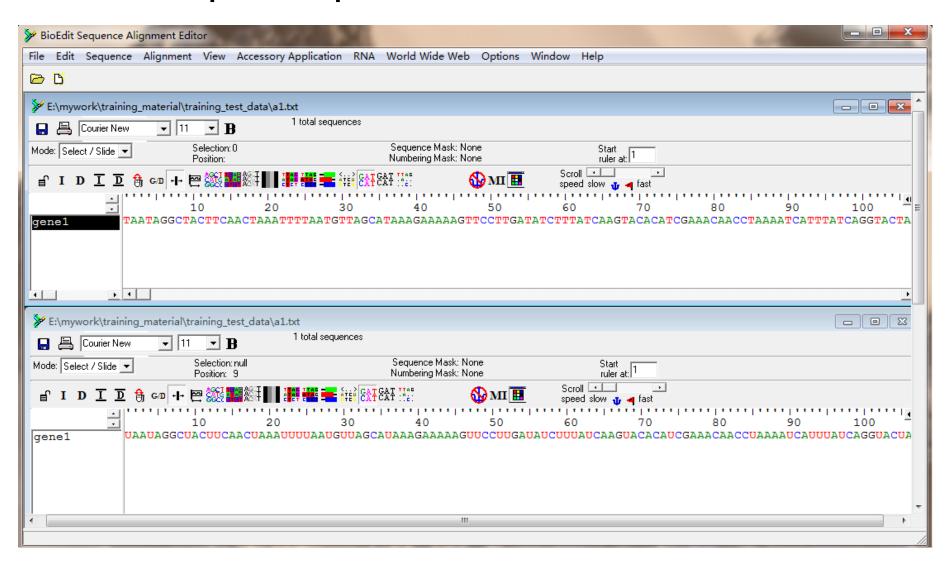
Obtain basic information of nucleic acid sequence

Software: BioEdit (http://www.mbio.ncsu.edu/bioedit/bioedit.html) menu: File -> open -> sequence -> nucleic acid -> nucleotide composition



- ➤ DNA -> RNA
- > Sequence -> Reverse complement
- ➤ DNA -> protein

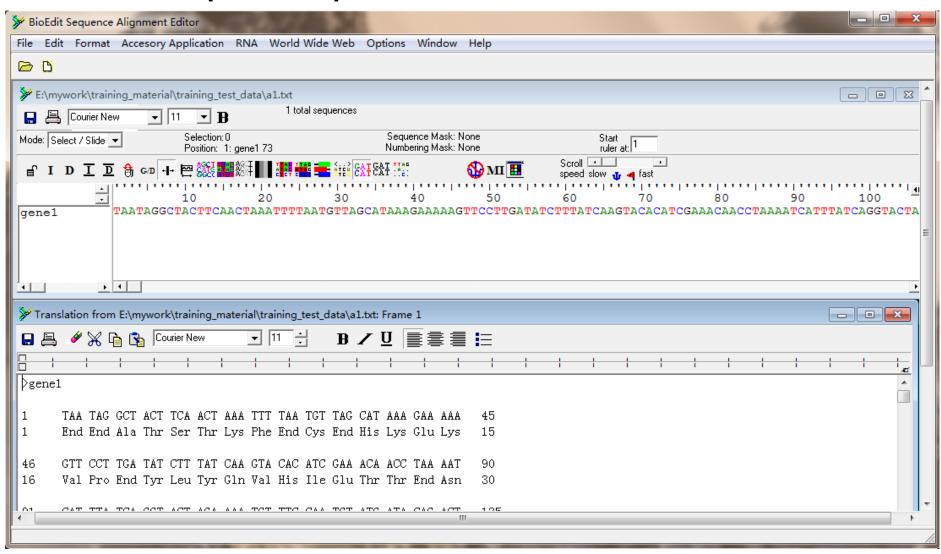
menu: File -> open -> sequence -> nucleic acid -> DNA-RNA



menu: File -> open -> sequence -> nucleic acid -> Reverse Complement

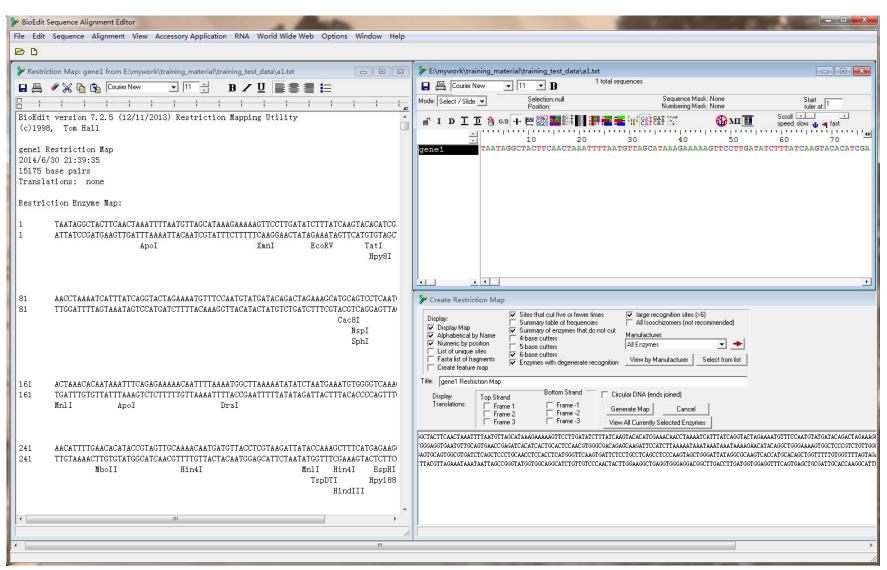


menu: File -> open -> sequence -> nucleic acid -> translate



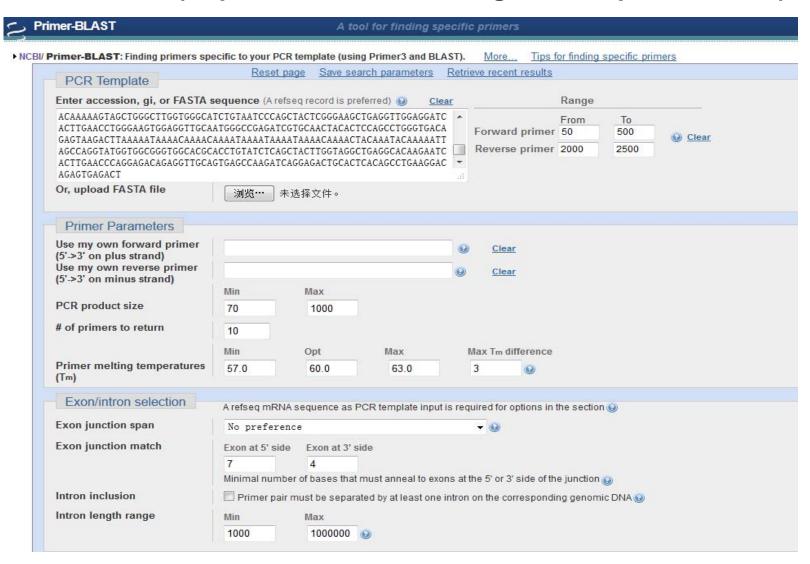
Analysis of enzyme mapping

menu: File -> open -> sequence -> nucleic acid -> Restriction Map

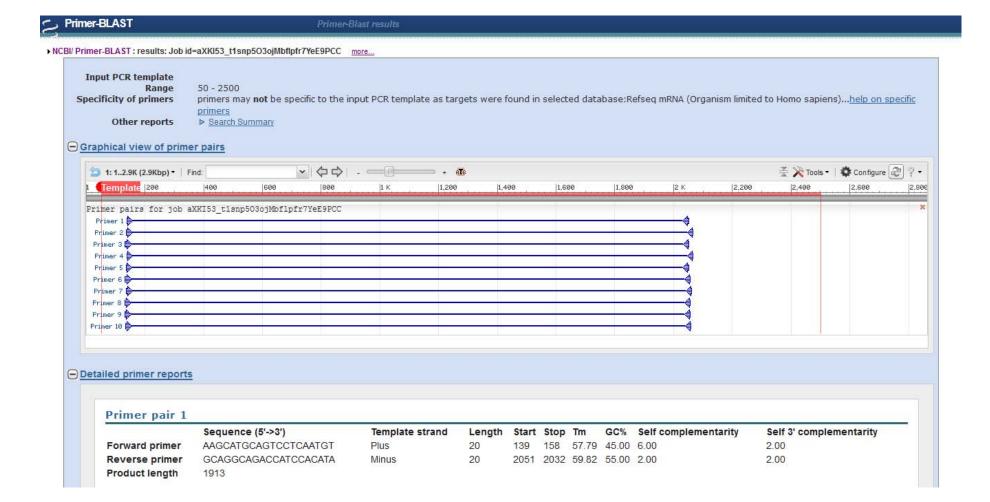


Primer design

NCBI website (http://www.ncbi.nlm.nih.gov/tools/primer-blast/)



Primer design



Primer design

Primer3 (http://primer3.wi.mit.edu/)

Primer3web version 4.0.0	O - Pick primers from a DNA sequence.		disclaimer cautions	code
Select the <u>Task</u> for primer selection ga	eneric •			
	ring of ACGTNacgtn other letters treated as N use a <u>Mispriming Library (repeat library)</u> NONE	numbers and blanks ignored). FASTA format ok.	Please N-out undesire	able
☑ Pick left primer, or use left primer below	Pick hybridization probe (internal oligo), or use oligo below	Pick right primer, or use right p (5' to 3' on opposite strand)	primer below	
Pick Primers Download Settings Reset	Form			

Primer3 codes (http://sourceforge.net/projects/primer3/)

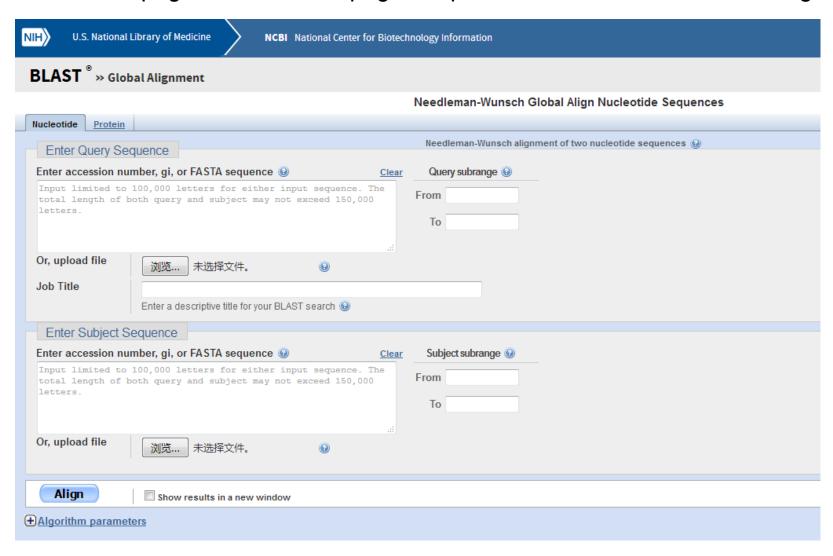
Primer3 - PCR primer design tool

Prought to you by: brantfaircloth, steverozen, untergasser

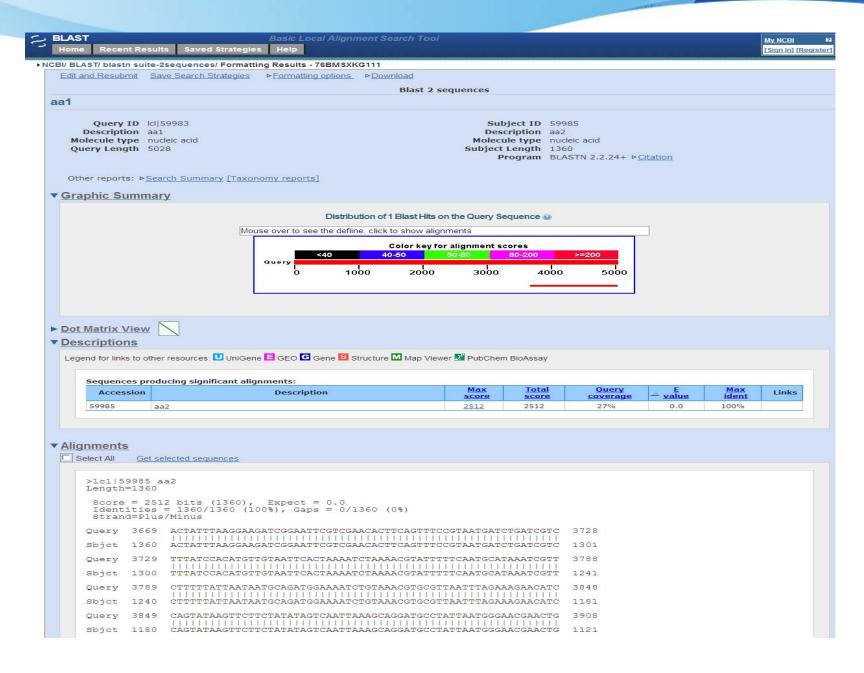


Sequences alignment (two sequence)

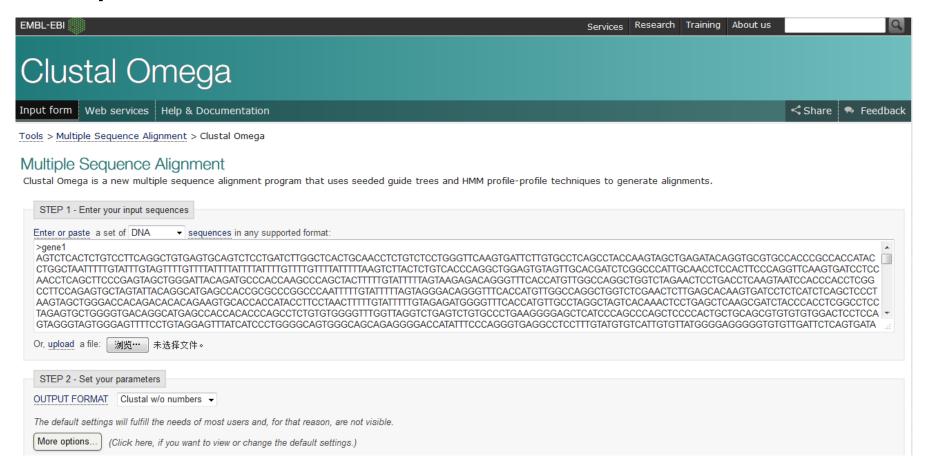
NCBI homepage -> Blast homepage -> specialized searches -> Global Align



Sequences alignment (two sequence)



http://www.ebi.ac.uk/Tools/msa/clustalo/





Software: BioEdit

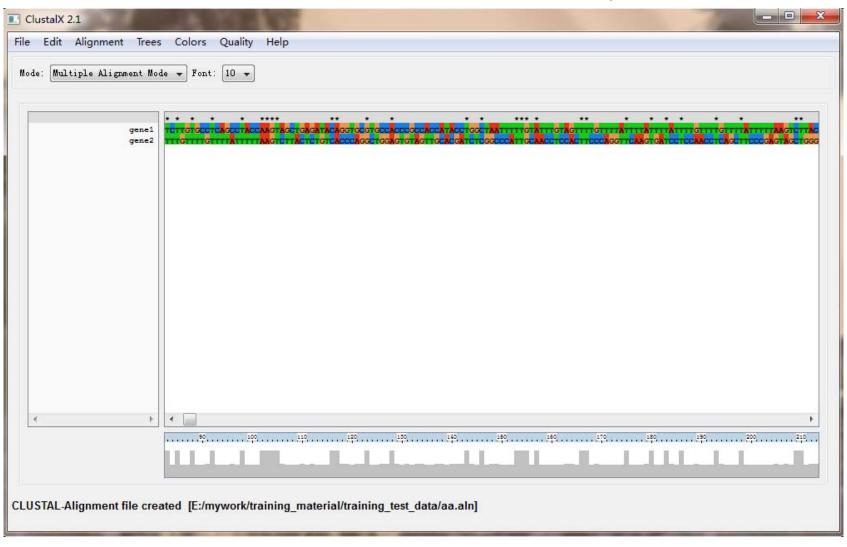
File -> open -> sequence -> Accessory Application -> clustalw



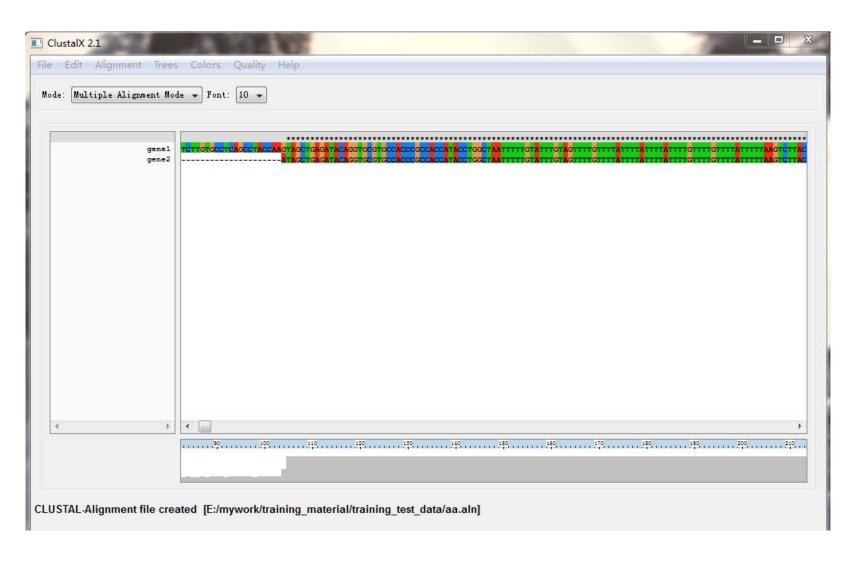
http://www.clustal.org/clustal2/



Software: clustalw / clustalX menu : load sequence

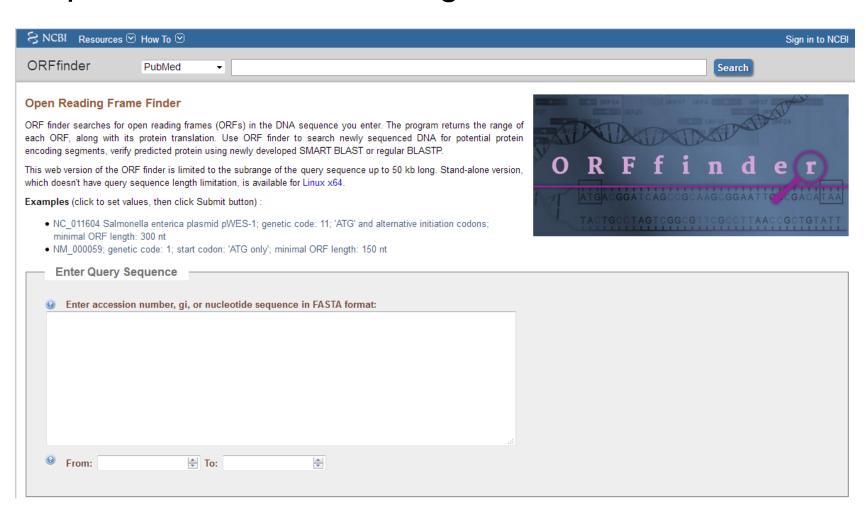


Software: clustalw / clustalX menu : Alignment -> do complete alignment

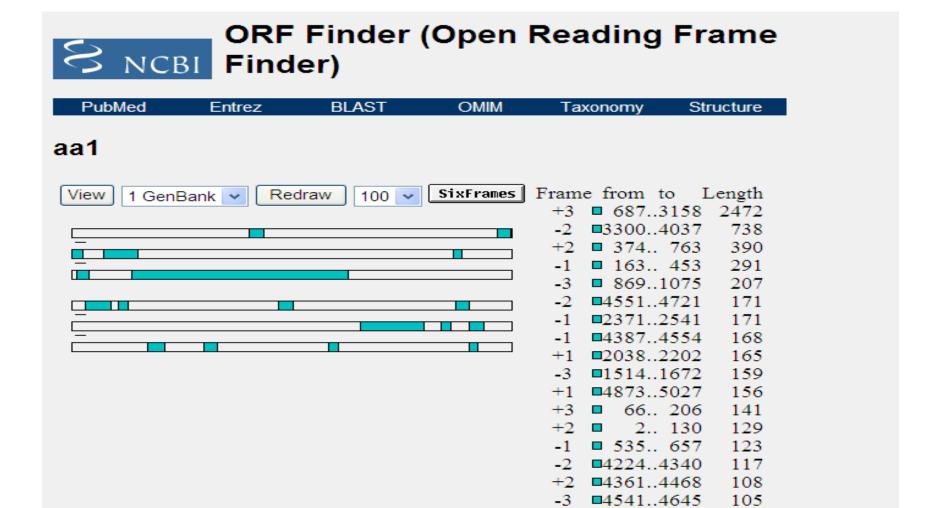


Analysis of open reading frame

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



Analysis of open reading frame

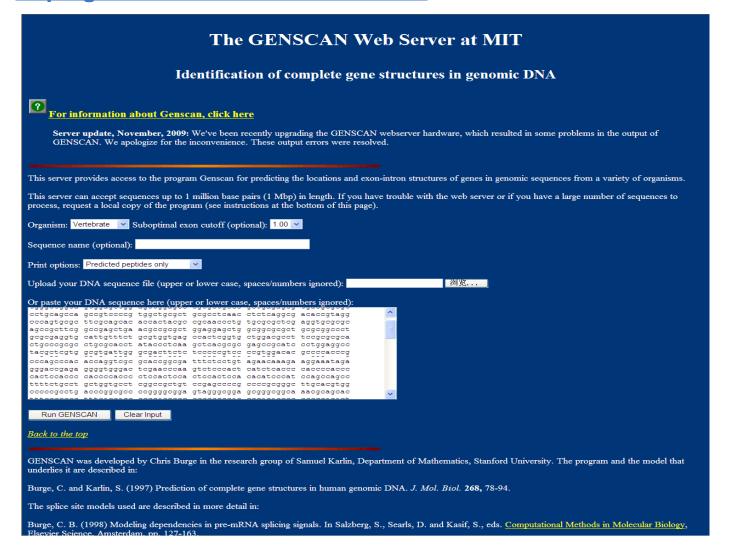


-3 ■2942..3046

105

Gene prediction

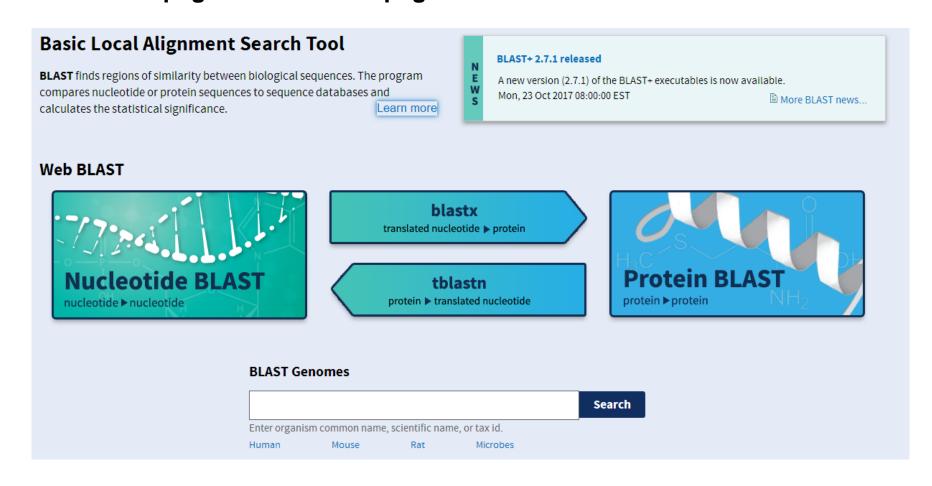
http://genes.mit.edu/GENSCAN.html

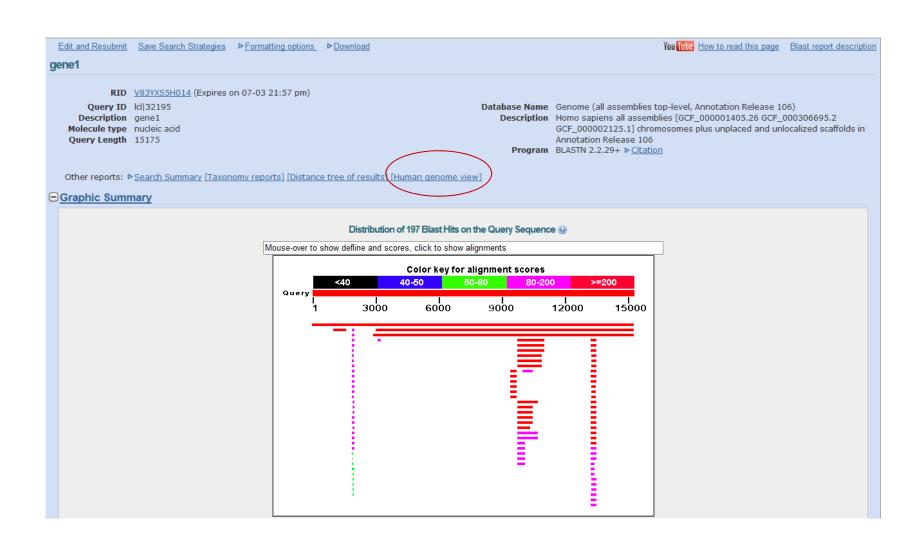


Gene prediction

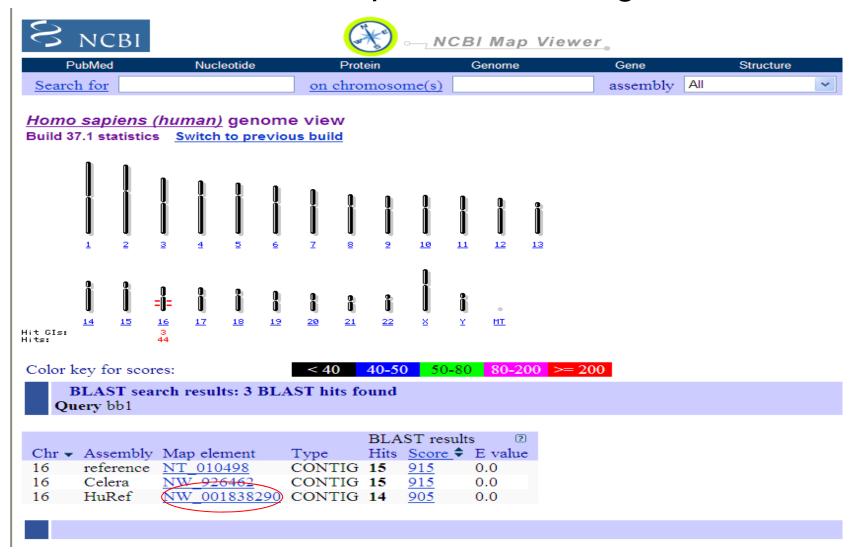
GENSCAN Output View gene model output: PS | PDF GENSCAN 1.0 Date run: 29-Aug-110 Time: 05:08:50 Predicted genes/exons: Gn.Ex Type S .Begin ... End .Len Fr Ph I/Ac Do/T CodRg P.... Tscr.. 1.01 Term + 72 436 365 1 2 15 38 583 0.999 41.79 1.02 PlyA + 643 648 6 Suboptimal exons with probability > 1.000 Exnum Type S .Begin ...End .Len Fr Ph B/Ac Do/T CodRg P.... Tscr.. NO EXONS FOUND AT GIVEN PROBABILITY CUTOFF XELELEPALPAESVTRVLQPAVPVAALRLNLSGDTVGPVRFAAHHYAATLCALEVRAAAS AELNAALEELAARCAALREVHCFCVVSHSVLDAFRAHCPRLRTYTLKLTREPHPWRPTLV QHRVIGKLIQCLFGPLQAGPSNAGGKRKLSLMLDEGSSCPTPAKFNTCPLPGALLQDPYF IOSPLPETNIGLSPHRARGPIISDIPEDSPSPEGTRLSPSSDGRREKGLALLKEEPASPG GDGEAGLALAPNECDFCVTAPPPLPVAVVQAILEGKGSFSPEGPRNAQQPEPGDPREIPD RGPLGLESGDRSPESLLPPMLLQPPQESVEPAGPLDVLGPSLQGREWTLMDLDMELSLMQ PLVPERGEPELAVKGLNSPSPGKDPTLGAPLLLDVOAALGGPALGLPGALTIYSTPESRT Back to GENSCAN

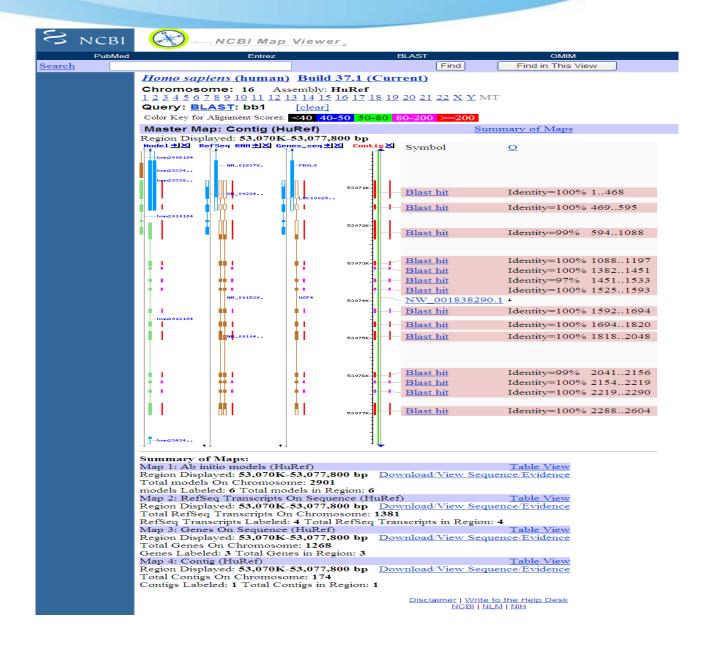
NCBI homepage -> Blast homepage -> BLAST Genomes -> Human





Blast result -> view report -> human genome view

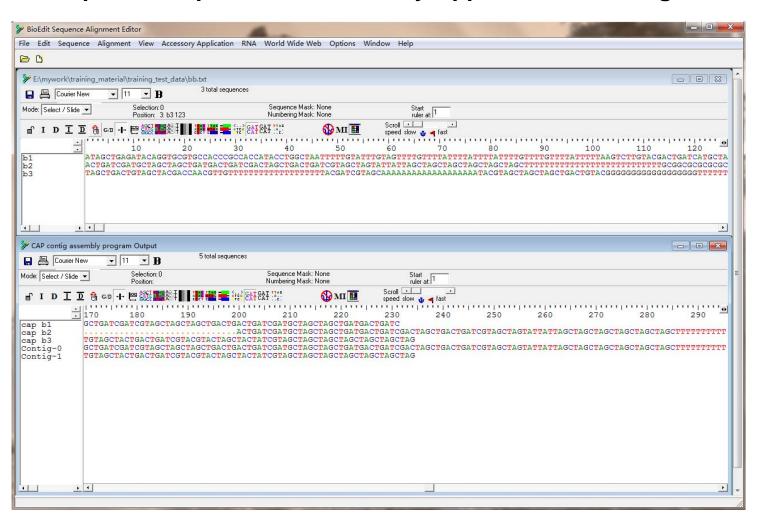




Sequence assembly

Software BioEdit

File -> open -> sequence -> Accessory Application -> contig assembly



Sequence assembly

CAP3 (contig assembly program) http://seq.cs.iastate.edu/cap3.html

CAP3 Assembly Program

- A version of CAP3 for a 32-bit Linux system with an Intel processor download tar file
- · A version of CAP3 for a 64-bit Linux system with an Opterron processor: download tar file
- · A version of CAP3 for a 64-bit Linux system with an Intel processor: download tar file
- · A version of CAP3 for an old version (2009) of 64-bit Linux system with an Intel processor: download tar file
- · A version of CAP3 for a 32-bit MacOSX system with an Intel processor: download tar file
- · A version of CAP3 for a 64-bit MacOSX system with an Intel processor: download tar file
- · A version of CAP3 for a 64-bit Solaris system with an Opterron processor: download tar file
- A version of CAP3 for a 32-bit Cygwin simulator on Windows: <u>download tar file</u> You need to download and install: Cygwin

PART III insight into BLAST

Concept of BLAST

Concept: The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

Purpose: predict function

infer evolutional tree

construct families

Concept of BLAST

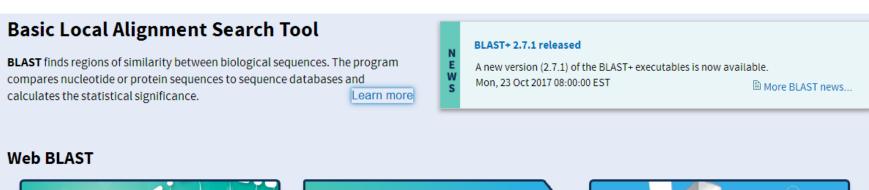
BLAST: word size -> high-scoring segment (HSP)

query sequence: (nucleotide/translated nucleotide, protein)

database: (nucleotide/translated nucleotide, protein)

BLAST HOME

http://blast.ncbi.nlm.nih.gov/Blast.cgi









BLAST Genomes

Search

Enter organism common name, scientific name, or tax id.

Human

Mouse

Rat

licrobes

Basic program of BLAST

nucleotide blast

Search a **nucleotide** database using a **nucleotide** query Algorithms: blastn, megablast, discontiguous megablast

protein blast

Search **protein** database using a **protein** query Algorithms: blastp, psi-blast, phi-blast

blastx

Search protein database using a translated nucleotide query

<u>tblastn</u>

Search translated nucleotide database using a protein query

tblastx

Search translated nucleotide database using a translated nucleotide query

PSI-BLAST

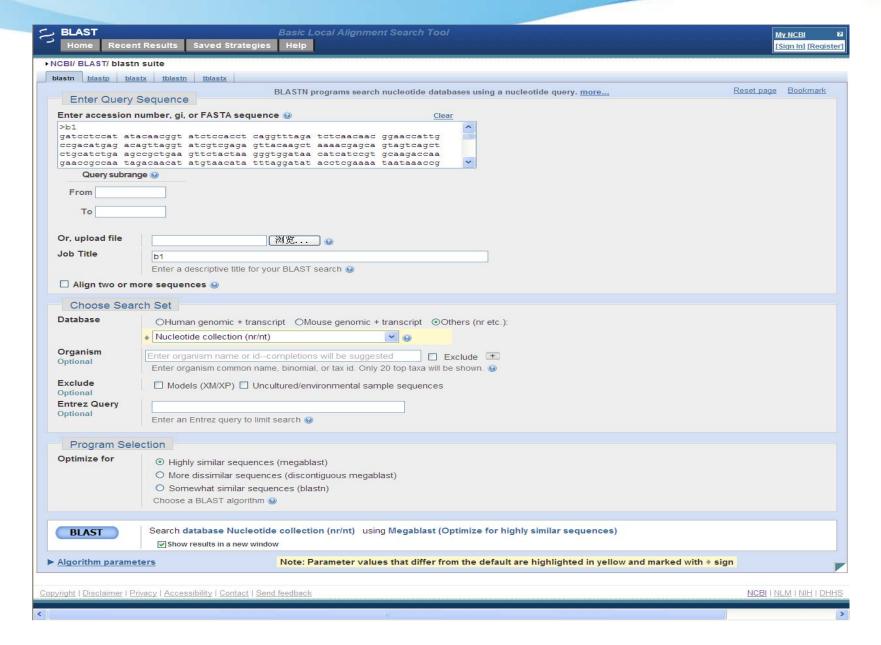
Position-Specific Iterated BLAST (PSI-BLAST, family protein)

- (1) PSI-BLAST takes as an input a single protein sequence and compares it to a protein database, using the gapped BLAST program
- (2) The program constructs a multiple alignment, and then a profile, from any significant local alignments found.
- (3) The profile is compared to the protein database, again seeking local alignments.
- (4) PSI-BLAST estimates the statistical significance of the local alignments found.
- (5) Finally, PSI-BLAST iterates, by returning to step (2), an arbitrary number of times or until convergence.

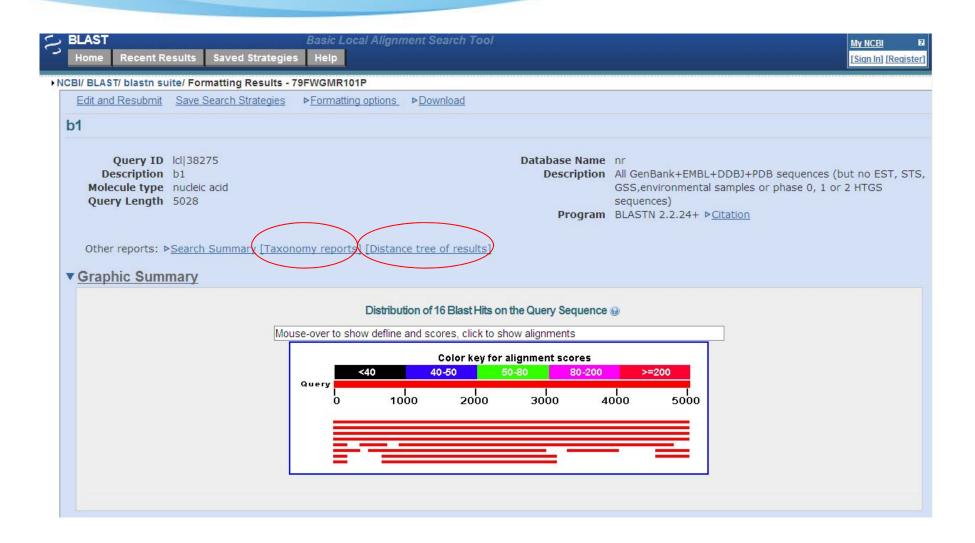
Summary of BLAST

- Traditional BLAST (formerly blastall) nucleotide, protein, translations
 - blastn nucleotide query vs. nucleotide database
 - blastp protein query vs. protein database
 - blastx nucleotide query vs. protein database
 - tblastn protein query vs. translated nucleotide database
 - tblastx translated nucleotide query vs. translated nucleotide database
- Position Specific BLAST Programs protein only
 - Position Specific Iterative BLAST (PSI-BLAST)
 Automatically generates a position specific score matrix (PSSM)

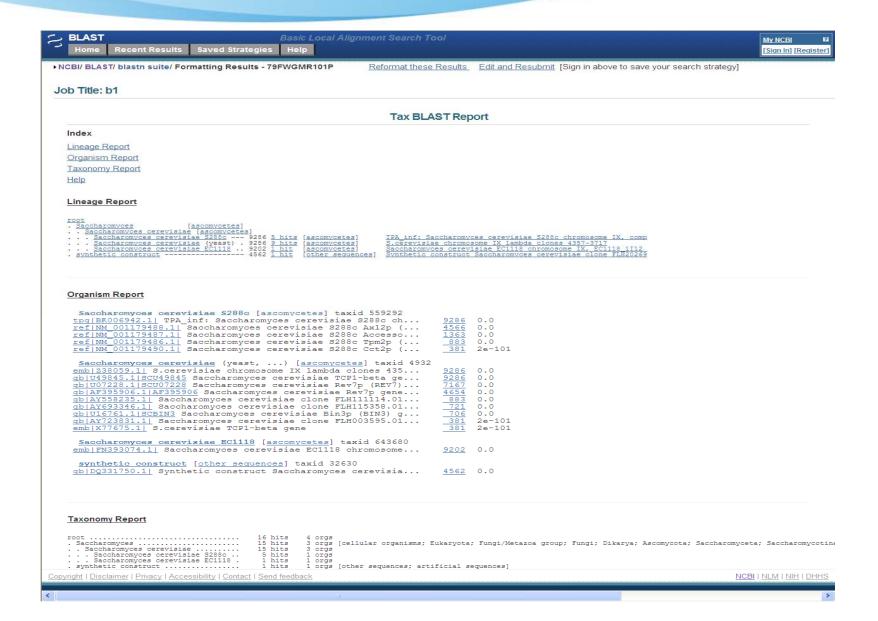
BLAST use - web means (nucleotide)



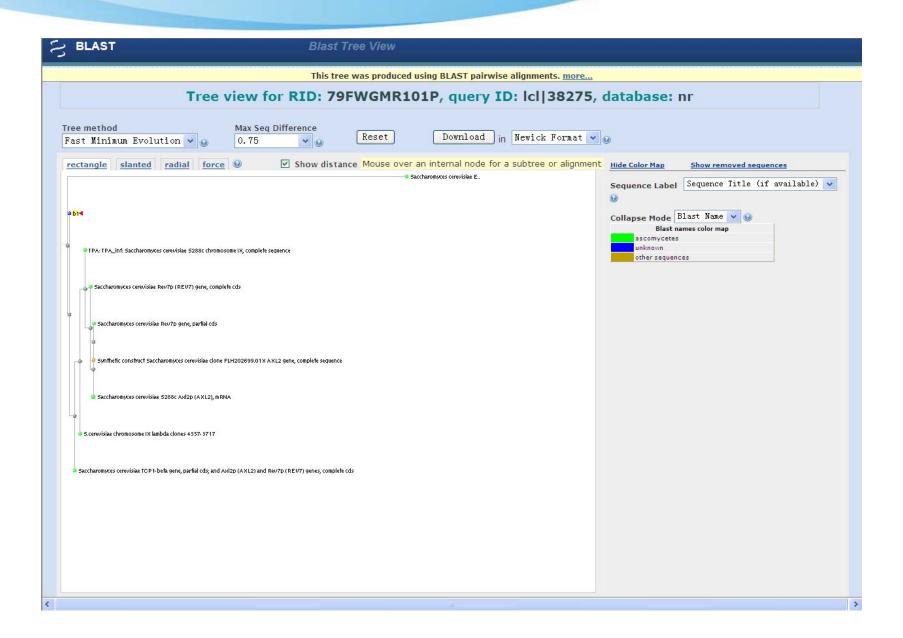
BLAST use - web means (nucleotide)



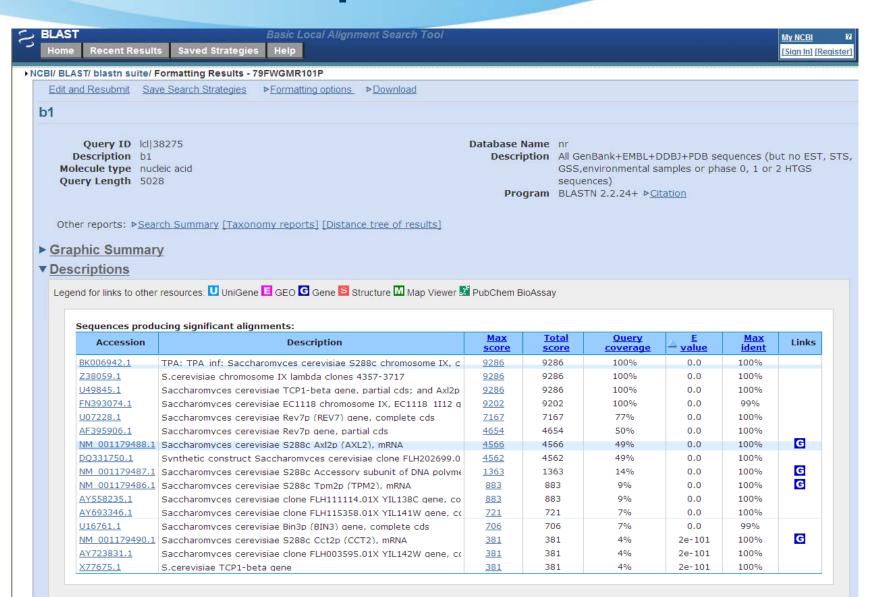
BLAST use – web means (taxonomy report)



BLAST use - infer evolution tree



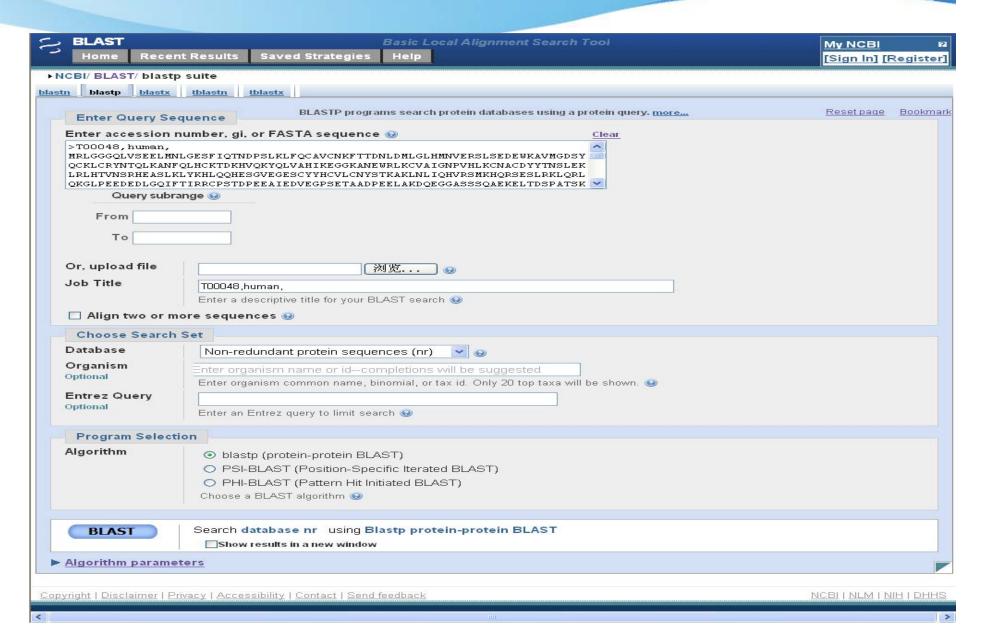
BLAST use - predict function-



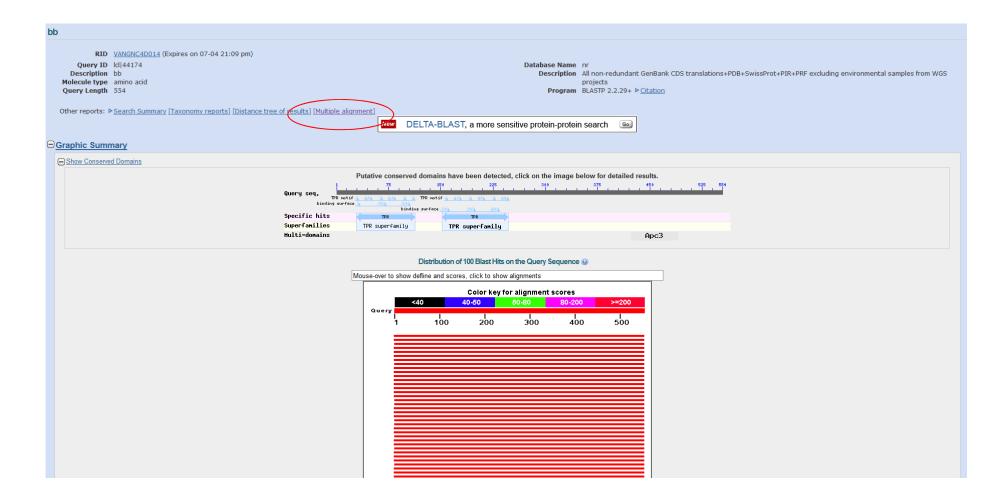
BLAST use – web manners (alignment report)

```
▼ Alignments
 Select All
         Get selected sequences Distance tree of results
    > tpg | BK006942.1 | TPA inf: Saccharomyces cerevisiae S288c chromosome IX, complete
    sequence
    Length=439885
    Features in this part of subject sequence:
      Cct2p
      Ax12p
    Score = 9286 \text{ bits } (5028), \text{ Expect} = 0.0
    Identities = 5028/5028 (100%), Gaps = 0/5028 (0%)
    Strand=Plus/Plus
              GATCCTCCATATACAACGGTATCTCCACCTCAGGTTTAGATCTCAACAACGGAACCATTG
    Query 1
               Sbjct 84680
              GATCCTCCATATACAACGGTATCTCCACCTCAGGTTTAGATCTCAACAACGGAACCATTG
    Query 61
              CCGACATGAGACAGTTAGGTATCGTCGAGAGTTACAAGCTAAAACGAGCAGTAGTCAGCT 120
              Sbjct 84740
              CCGACATGAGACAGTTAGGTATCGTCGAGAGTTACAAGCTAAAACGAGCAGTAGTCAGCT
              CTGCATCTGAAGCCGCTGAAGTTCTACTAAGGGTGGATAACATCATCCGTGCAAGACCAA
    Query 121
              Sbjct 84800
                                                             84859
    Query 181
              GAACCGCCAATAGACAACATATGTAACATATTTAGGATATACCTCGAAAATAATAAACCG
               Sbjct 84860
              GAACCGCCAATAGACAACATATGTAACATATTTAGGATATACCTCGAAAATAATAAACCG
              CCACACTGTCATTATTATAATTAGAAACAGAACGCAAAAATTATCCACTATATAATTCAA
    Query 241
               Sbjct 84920
              CCACACTGTCATTATTATAATTAGAAACAGAACGCAAAAATTATCCACTATATAATTCAA
    Query 301
              AGACGCGaaaaaaaaGAACAACGCGTCATAGAACTTTTGGCAATTCGCGTCACAAATAA
              Sbjct 84980
                                                            85039
              AGACGCGAAAAAAAAAAACAACGCGTCATAGAACTTTTGGCAATTCGCGTCACAAATAA
    Query 361
              ATTTTGGCAACTTATGTTTCCTCTTCGAGCAGTACTCGAGCCCTGTCTCAAGAATGTAAT
                                                             420
              ATTTTGGCAACTTATGTTTCCTCTTCGAGCAGTACTCGAGCCCTGTCTCAAGAATGTAAT
    Sbjct 85040
    Query 421
              AATACCCATCGTAGGTATGGTTAAAGATAGCATCTCCACAACCTCAAAGCTCCTTGCCGA
               Sbjct 85100
              AATACCCATCGTAGGTATGGTTAAAGATAGCATCTCCACAACCTCAAAGCTCCTTGCCGA
                                                             85159
    Query 481
              GAGTCGCCCTCCTTTGTCGAGTAATTTTCACTTTTCATATGAGAACTTATTTTCTTATTC
              Sbjct 85160
              GAGTCGCCCTCCTTTGTCGAGTAATTTTCACTTTTCATATGAGAACTTATTTTCTTATTC
    Query 541
              TTTACTCTCACATCCTGTAGTGATTGACACTGCAACAGCCACCATCACTAGAAGAACAGA
               Sbjct 85220
              TTTACTCTCACATCCTGTAGTGATTGACACTGCAACAGCCACCATCACTAGAAGAACAGA
    Query 601
              ACAATTACTTAATAGAAAAATTATATCTTCCTCGAAACGATTTCCTGCTTCCAACATCTA
               Sbjct 85280
              ACAATTACTTAATAGAAAAATTATATCTTCCTCGAAACGATTTCCTGCTTCCAACATCTA
                                                            85339
              CGTATATCAAGAAGCATTCACTTACCATGACACAGCTTCAGATTTCATTATTGCTGACAG
    Query 661
               Sbjct 85340
             CGTATATCAAGAAGCATTCACTTACCATGACACAGCTTCAGATTTCATTATTGCTGACAG
```

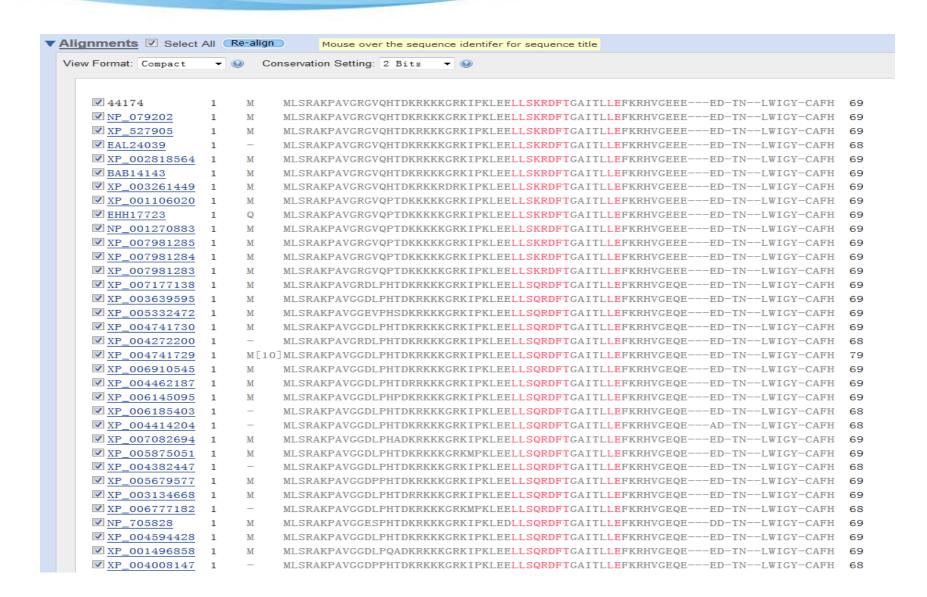
BLAST use – web manners (protein)



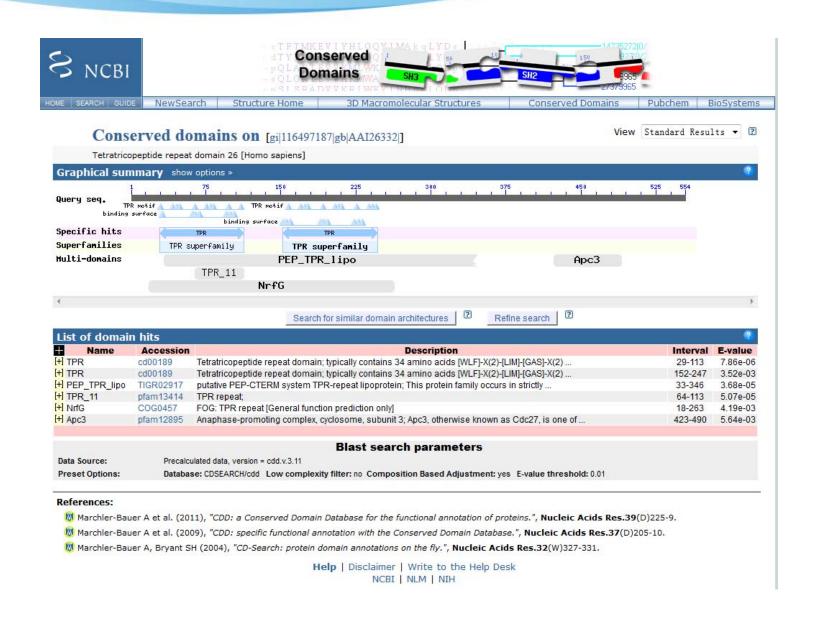
BLAST use - result summary -



BLAST use – multi alignment –



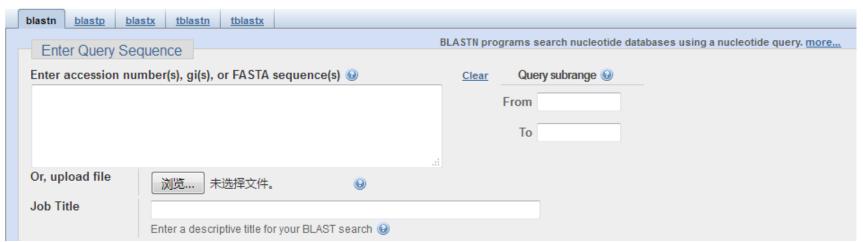
BLAST use – conserved domain



BLAST use

Batch BLAST jobs

(1) input "batches" of sequences into one form and retrieve the results



Standard Nucleotide BLAST

BLAST use

Batch BLAST jobs

(2) Utilize the standalone BLAST binaries.

You can retrieve BLAST execute files from NCBI ftp sites ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/

ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/

ftp://ftp.ncbi.nlm.nih.gov/blast/executables/	blast+/LATEST/ 的索引		
1 回到上一层文件夹			
名称	大小	修改时间	
A ChangeLog	1 KB	2017/10/19	3:07:00
ncbi-blast-2.7.1+-1.src.rpm	18531 KB	2017/10/19	3:05:00
ncbi-blast-2,7.1+-1.src.rpm.md5	1 KB	2017/10/19	3:08:00
ncbi-blast-2.7.1+-1.x86_64.rpm	185089 KB	2017/10/19	3:05:00
ncbi-blast-2.7.1+-1.x86_64.rpm.md5	1 KB	2017/10/19	3:08:00
ncbi-blast-2.7.1+-src.tar.gz	22710 KB	2017/10/19	3:08:00
ncbi-blast-2.7.1+-src.tar.gz.md5	1 KB	2017/10/19	3:08:00
ncbi-blast-2.7.1+-src.zip	26322 KB	2017/10/19	3:08:00
ncbi-blast-2.7.1+-src.zip.md5	1 KB	2017/10/19	3:08:00
ncbi-blast-2.7.1+-win64.exe	89536 KB	2017/10/19	3:07:00
ncbi-blast-2.7.1+-win64.exe.md5	1 KB	2017/10/19	3:08:00
ncbi-blast-2.7.1+-x64-linux.tar.gz	228821 KB	2017/10/19	3:07:00
ncbi-blast-2.7.1+-x64-linux.tar.gz.md5	1 KB	2017/10/19	3:08:00
ncbi-blast-2.7.1+-x64-macosx.tar.gz	129893 KB	2017/10/19	3:05:00
ncbi-blast-2.7.1+-x64-macosx.tar.gz.md5	1 KB	2017/10/19	3:08:00
ncbi-blast-2.7.1+-x64-win64.tar.gz	89217 KB	2017/10/19	3:08:00
ncbi-blast-2.7.1+-x64-win64.tar.gz.md5	1 KB	2017/10/19	3:08:00
ncbi-blast-2.7.1+.dmg	131554 KB	2017/10/19	3:04:00
ncbi-blast-2.7.1+.dmg.md5	1 KB	2017/10/19	3:09:00

BLAST use – command line means

List of the BLAST directory

- (1) bin binary files for BLAST execute
- (2) doc documents of BLAST
- (3) ncbi_package_info version information of BLAST

BLAST use

(1) Make a formatted database to use

execute command: makeblastdb

input: fasta format sequences (database sequences)

output: formatted database, used by BLAST program

Argument of makeblastdb

```
*** Input options
-in <File_In>
   Input file/database name
   Default = `-'
-input_type <String, `asnl_bin', `asnl_txt', `blastdb', `fasta'>
   Type of the data specified in input_file
   Default = `fasta'

*** Output options
-out <String>
   Name of BLAST database to be created
   Default = input file name provided to -in argumentRequired if multiple
   file(s)/database(s) are provided as input
-max_file_sz <String>
   Maximum file size for BLAST database files
   Default = `1GB'
```

BLAST use
(2) Carry out BLAST program

execute command: blastn, blastp

input: fasta sequences (query sequences), database

output : query result file

Usage of blastn

```
blastn [-h] [-help] [-import search strategy filename]
  [-export search strategy filename] [-task task name] [-db database name]
  [-dbsize num letters] [-gilist filename] [-seqidlist filename]
  [-negative gilist filename] [-entrez query entrez query]
  [-db soft mask filtering algorithm] [-db hard mask filtering algorithm]
  [-subject subject input file] [-subject loc range] [-query input file]
  [-out output file] [-evalue evalue] [-word size int value]
  [-gapopen open penalty] [-gapextend extend penalty]
  [-perc identity float value] [-xdrop ungap float value]
  [-xdrop gap float value] [-xdrop gap final float value]
  [-searchsp int value] [-max hsps int value] [-sum statistics]
  [-penalty penalty] [-reward reward] [-no greedy]
  [-min raw gapped score int value] [-template type type]
  [-template length int value] [-dust DUST options]
  [-filtering db filtering database]
  [-window masker taxid window masker taxid]
  [-window masker db window masker db] [-soft masking soft masking]
  [-ungapped] [-culling limit int value] [-best hit overhang float value]
  [-best hit score edge float value] [-window size int value]
  [-off diagonal range int value] [-use index boolean] [-index name string]
  [-lcase masking] [-query loc range] [-strand strand] [-parse deflines]
  [-outfmt format] [-show gis] [-num descriptions int value]
  [-num alignments int value] [-html] [-max target seqs num sequences]
  [-num threads int value] [-remote] [-version]
```

Usage of blastp

```
blastp [-h] [-help] [-import search strategy filename]
  [-export search strategy filename] [-task task name] [-db database name]
  [-dbsize num letters] [-gilist filename] [-seqidlist filename]
  [-negative gilist filename] [-entrez query entrez query]
  [-db soft mask filtering algorithm] [-db hard mask filtering algorithm]
  [-subject subject input file] [-subject loc range] [-query input file]
  [-out output file] [-evalue evalue] [-word size int value]
  [-gapopen open penalty] [-gapextend extend penalty]
  [-xdrop ungap float value] [-xdrop gap float value]
  [-xdrop gap final float value] [-searchsp int value] [-max hsps int value]
  [-sum statistics] [-seg SEG options] [-soft masking soft masking]
  [-matrix matrix name] [-threshold float value] [-culling limit int value]
  [-best hit overhang float value] [-best hit score edge float value]
  [-window size int value] [-lcase masking] [-query loc range]
  [-parse deflines] [-outfmt format] [-show gis]
  [-num descriptions int value] [-num alignments int value] [-html]
  [-max target seqs num sequences] [-num threads int value] [-ungapped]
  [-remote] [-comp based stats compo] [-use sw tback] [-version]
```

THANK YOU