Introduction to sequence similarity searches and sequence alignment

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Overview of the presentation

PART 1

- An example showing how useful bioinformatics can be
- Searching sequence databases
- A walk-through of the BLAST search service

PART 2

- Alignments, sequence similarity and homology
- Significance of matches: What is a good match?
- How does BLAST work?

PART 3

Iterative searching with a family of proteins (PSI-BLAST)

PART 4:

Multiple sequence alignments

One example of how useful bioinformatics can be

- The protein AlkB was discovered in *E.coli* in 1984.
- It was known that it protected the bacterium when subjected to DNA-alkylation agents.
- No enzymatic activity was found.
- Perhaps some co-factors where missing?
- In 2001, a bioinformatics paper was published that shed light on the problem. Many similar sequences where found using advanced sequence similarity searches ...

http://genomebiology.com/2001/2/3/research/0007.1

Research

The DNA-repair protein AlkB, EGL-9, and leprecan define new families of 2-oxoglutarate- and iron-dependent dioxygenases L Aravind and Eugene V Koonin

Address: National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, USA.

Correspondence: L Aravind. E-mail: aravind@ncbi.nlm.nih.gov

Example...

Alignment showing conserved amino acids among many sequences

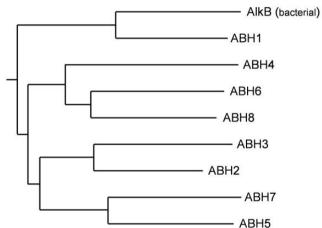
CAS Scla 322266	RSGTVYHDVYP-SPGAHHL-SSETSETLEFFERBMAYHRLOPNYYMLACSRADHERTANTLVASVRK70VTEAVYLEFG-DLLTVDNFRTTEARTPFSPRWDGKDRWLHRVYIRT 302\	
IPNS En 124825	TLASVVEIRYPYLDPYP3KTAADGTKLSPEWEBDVSABROSEPFFVNL 288	
FLAS Pet 421946	IVYLLKINYYP-PCPRPDLALGYVAHIDMS	
LDOX Pet 1730108	LLLOMKINYYPKCPQPELALGYEAHTDVSALTFILHNMYEGLOLFPEGOWVTAKCYPN-SIIMHIGDTIEILSNGKYKSILHRGVVNKEKVRFSWAIFCEP 311	
Srg At 479047	SVOSMRMNYYP-PCPOPDOVIGHTFESDSVGLTVLMOVNDVEGLOIKKDGKWVPVKPLPN-AFIVNIGDVLEIITNGTYRSIDFRGVVNSEKERLSIATFHNV 309	
EFE Le 398992	PNFGTKYSNYPPCPKPDLIKGLRAHTDAGGITLLFODDKVSGLCLLKDEQWIDVPPMRH-SIVVNLGDQLEVITNGKYKSVLHRYIAGTDGTRMSLASFYNP 253 Small	
Ga200x Sot 10800976	NESIMRLNYYPTCOKPDLALGTGFEODPTSLTILHODSVSGLOVFMDNOWRSISPNLS-AFVVNIGDTFMALSNGRYKSCLERAVVNNKTPRKSLAFFLCP 317 molecule	
PA0147 Pa 9945977	PVSVFRLIHTP-PASAROSADOPGAGAETDYGCVTLLYQDAAGGLCVONRQGEWIDAPPIDG-TFVVNIGDMMARWSNDRYRSTPERVISPRGVHRYSMPFFAEP 274 dioxygen	
PA4191 Pa 9950401	PLILFRUFNYPSOPVPEGLDVOMGYGBUTDYGLLTLLHODAIGGLOVRTPOGWLEAPPIPG-SFVCNLGDMLERMTGGLYRSTPUR VARNTSGRDRLSPPLFFDP 277	
ISP7_Sp_729862	PTTSIRLIRYPSSPNRLGYOBETDADALTIMSQDNVKGLEILDPVSNCFLSVSPAPG-ALIANLGDIMAILTNNRYKSSMERYCNNSGSDRYTIPFFLQG 353	
SPCC1494.01 Sc 7491815	BEDVLRLKYSI-PEGVERREDDEDAGAESDYGSITLLFQRDAAGLEIRPPNFVKDMDWLKVNVOPD-VVLVNIADMLQFWTSGKLRSTVERVRIDPGVKTROTIAYFVTP 267	
DAOCS_Ly1_769809	CDPVLRTRPDVPEDRCABCOPNRMAPHYDLSIVBLILOTPCPNGFVSLCVEDGRFVEVPPRPG-CVVVFCGSIAPLVSDGKIKAPCHRVVS-PGA4-GSNRTSSVLFLRP 268/	
RRPO SHVX 548840	TYNOCL V OKYE	
POL ASPV 487652	FYNOCL VOERS TOHGESMENDDESIVIDIN- HOVLTVNYS GDAIFCI ECIGSGF-EIL EGGO-MILMPFGFOXERHEIGIKSP SKGRISLTFRIJK 853	
POL BSV 409711	TYDOMLACRYGAGGKIGFBADNEEIFMRG	
RRPO PMV 139137	EFNOCITYOOFK	
POL GLV 1154656	YFNCVI BOKKD	
Pol GVA 1405615	SYDELLORYT	
RRPO ACLSV 1710717	NFNSALLOVYN	A
T13L16.2 At 2708738	VPDSCI VNI VD	
T19K4.220 At 3036813	IIKSCIWNIVE	
At2g48080 At 4249414	RPNGCVINFEDO P-FOKPPHIVD OPISTIVL SESTIWIFGHRLGVD NDGNFRGSL-TLFLKEGS-LLVWRGNSADMARHUWCPS PNKKVAITFFKLK 351	
AK000315.1 Hs 7020317	GFVNSAVINDYO	
CG17807 Dm 7291441	SPDOLTWINE POHG PPEND PHSAFL DPILELS OF SPUND FREGORD DDOV-OVELPRES - LLINSGEARYDM HGI PPHND1 3RGKRTS TFRELR 325 Bukaryot	ic
CG6144 Dm 7297712	NANHULWIEL PROGELPHIDGPLFH PIISTISTG AHTVLEFVKREDTTTETEAGDOTTREVLF-KLLLEPRS-LLILKDTLYTDYLHAISETSED24RSPRESLTIRNVP 213 Family o	
CG4036 Dm 7297561	OTIEOCSLEVEPSKGASIDPHVDDCWIWGERVVTVNCLGDSVBTLTPYEVOOSGKYNLDLVAS YEDELLAP-LLTDDDOLATFEGKVURIFMPNLS-LIVLYGPARYOFPHSVLREDVOBRAVCWAYREFT 278 ALKB	-
FLJ2001 Hs 38923019	RPVBOCNLDVCPERGSAIDPHUDDWLWGERLVSLNLLSTVLSMCREAPGSLLLCSAPSAAFEALVDSVIAPSRSVLCOEVEVAIPLPARS-LLVLTGAARHOWSHAIHRHIEARRVCVTFRELS 274 paralogs	ε
C14B1.10 Ce 6580210	RPDOVTANVESGHGIPSHYDTHSAFDDPIVSISLSDVVMEFKDGANSARIAPVLLKARS-LCLIOGESRYRWHGIVNRKYD10ROTRVSLTLRKIR 343	
SPAP8A3.02c Sp 7491301	DABAIIMOVYN	
L3377.4 Lm 9989036	WLNNOTANLYE	
MTCI237.14c Mtu 2052134	마는 마	
AlkB Cc 2055386	PPDSCLWNLWATGARMGLEODRDEADPRFPLLSISLGDTAVFRIGGVNRKDPTRSLRLASGDVCRLLGPARLAFIGWDRILPG6-GGGRINLTLRRAR 190	
ALKB Ec 113638	OPDACLINRYA	
AlkB Scoe 8894829	PYDIALINFYDADARMGMERDADERTDAPVVSLSLGDTCVFFGONPETTTRPYTDTELRSGD-LFVFGGPSRLAYEGVPRVHPG7-LRGRLNITLRVSG 215 AlkB	
AlkB At 4835778	RPEGALWNYPGIGDTLGGHLDDMEADWS	
AlkB Sp 3080529	KABAAIWNFYS	
AlkB Hs 2134723	RABAGIENYYRLDSTEGIEWDRSELDHSKPLLSFSFGOSAIFLEGGLORDEAPP-PMFMHSGD-IMIMSGFSRLLMEAYPRULPN39KTARVNMA ROVL 272/	
Consensus (85%):	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
	* *	

Example...

- By comparing E.coli AlkB to other sequences in the database it was found that AlkB had some features in common with more well-known enzymes
- Based on these similarities the following was suggested regarding AlkB:
 - That AlkB is a dioxygenase
 - That the enzyme is Iron(II) dependent
 - That the enzyme is 2-oxo-glutarate dependent
 - That AlkB repairs alkylated bases through a form of oxidation
 - That the enzyme could demethylate RNA as well (not just DNA)
 - That there were eukaryotic counterparts of the protein
- All of this was later verified in the lab and resulted in three publications in *Nature*.

Example...

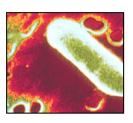
- By further sequence analysis 3 AlkB-like sequences were found in humans:
 - ALKBH1
 - ALKBH2
 - ALKBH3
- And by even more advanced analysis another 5 homologs were found in humans:
 - ALKBH4
 - ALKBH5
 - ALKBH6
 - ALKBH7
 - ALKBH8

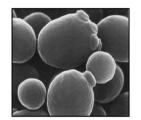


• The function of these 8 enzymes are now being studied in detail. Some of them may be related to human diseases.

Genomes are a huge source of information

- More than 7000 "completely" sequenced genomes available – an enormous source of information. Many thousands of other genomes in progress*
- More than 1 000 000 000 000 basepairs in GenBank (incl. WGS) (2015)*
- Database sizes are growing exponentially
 doubling in about 18 months since 1982
- Searching sequence databases for a similar sequence is fundamental in many types of analyses in bioinformatics
- Searching a sequence database with a new amino acid or nucleotide sequence allow us to find out more about:
 - Gene function
 - Conserved and probably important residues
 - 3D structure of a protein
 - Distribution of the gene among species
 - Gene structure
 - Chromosomal localisation
- Save time in the lab!
- Database searching is highly compute intensive and is probably the task consuming the largest amount of computing time within bioinformatics.

















^{*} Sources: genomesonline.org & NCBI (ftp://ftp.ncbi.nih.gov/genbank/gbrel.txt)

Searching sequence databases

- Goal: Identify which sequences in a database are significantly similar to a given DNA, RNA or protein sequence.
- How: The query sequence is compared (aligned) with each of the database sequences, and the amount of similarity is determined for each database sequence.

Example:

Query sequence: acgatcgattagcca

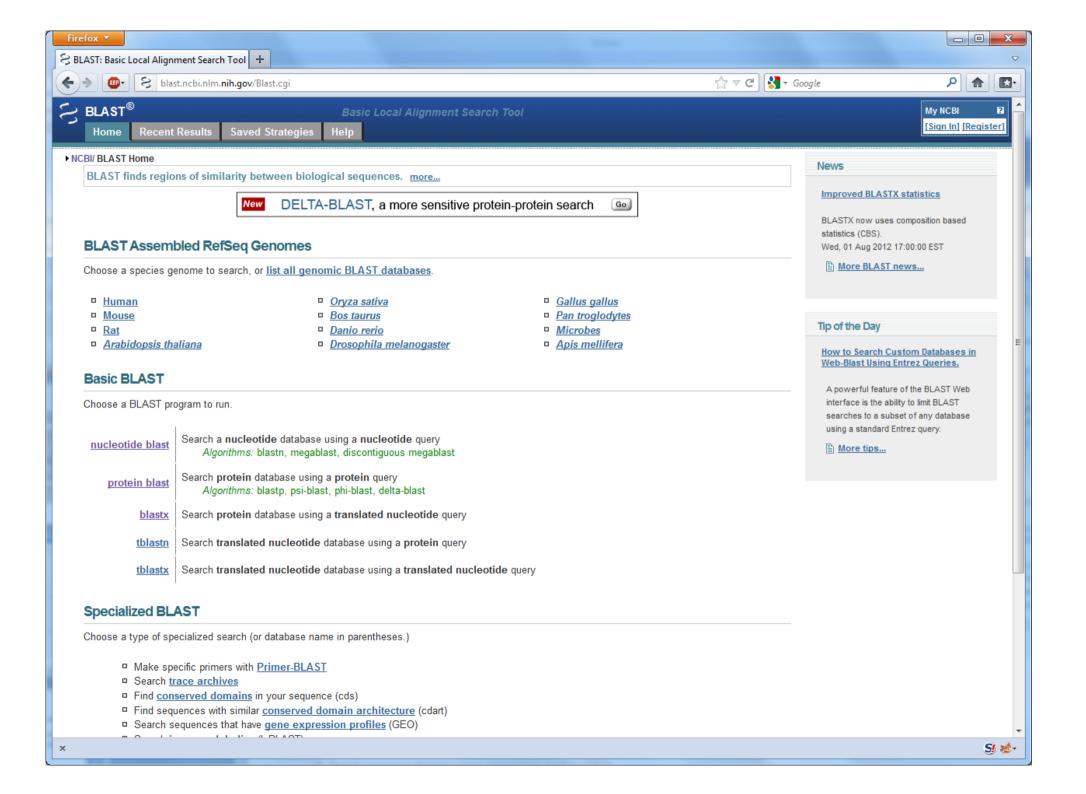
Database sequences:

Identical (trivial): acgatcgattagcca

Very similar (easy): acgaccgatgagcca

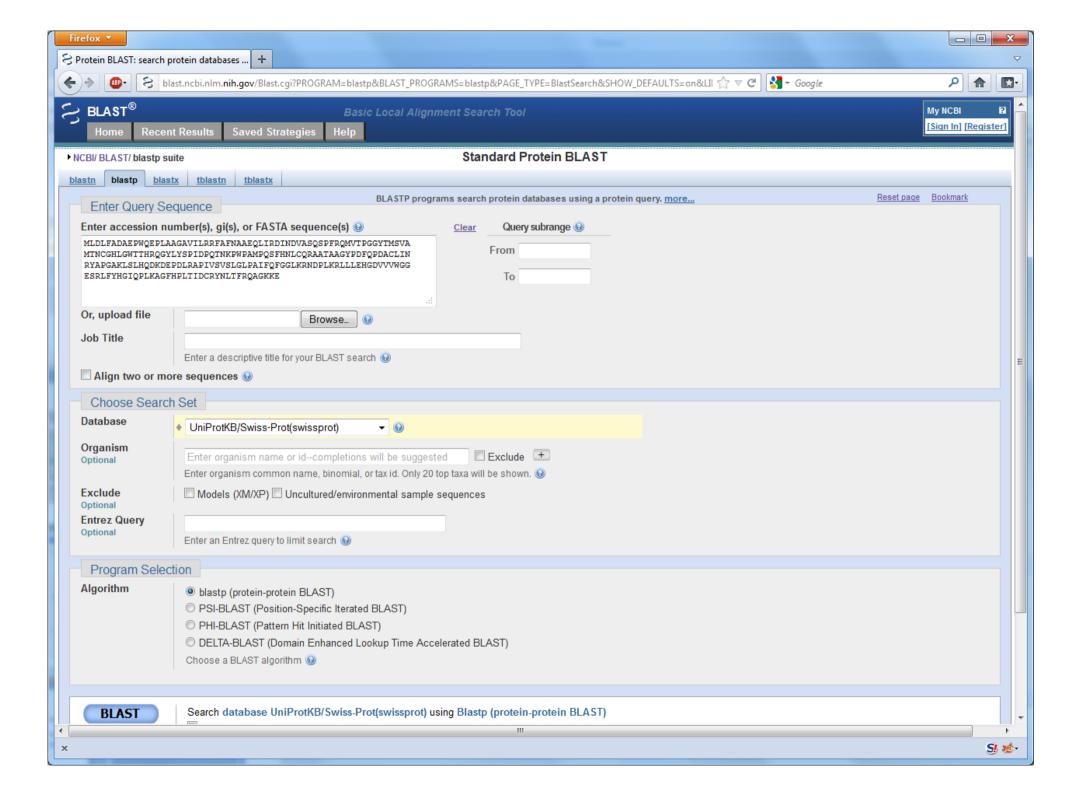
Similar (moderate): atgacggatgagcga

Very diverged (hard): atgacgggatgagcga



Search program variants

Query	Database	Comparisons	FASTA	BLAST	Description
Nucleotide	Nucleotide	Nucleotide (2)	fasta (fastn)	blastn	Compares directly both strands (forward and reverse complement) of the nucleotide query sequence to the nucleotide sequences in the database.
Amino acid	Amino acid	Amino acid (1)	fasta (fastp)	blastp	Compares the amino acid query sequence with the amino acid sequences in the database.
Amino acid	Nucleotide	Amino acid (6)	tfasta, tfastx, tfasty	tblastn	Translates the database nucleotide sequences into all six frames and compares the resulting amino acid sequences with the amino acid query sequences. tfasty allows intra-codon substitutions and frameshifts.
Nucleotide	Amino acid	Amino acid (6)	fastx, fasty	blastx	Translates the nucleotide query sequence into all six frames and compares the resulting amino acid sequences with the amino acid sequences in the database. fasty allows intra-codon substitutions and frameshifts.
Nucleotide	Nucleotide	Amino acid (36)	-	tblastx	Translates both the query nucleotide sequence and the database nucleotide sequences into all six frames and compares the resulting amino acid sequences with each other.



BLAST databases (protein)

nr: All non-redundant GenBank CDS translations + RefSeq Proteins +

PDB + UniProtKB/SwissProt + PIR + PRF

refseq: RefSeq protein sequences from NCBI's Reference Sequence Project.

swissprot: The SWISSPROT part of UniProt Knowledge Base (UniProtKB)

pat: Patented protein sequences

pdb: Sequences of proteins in the Protein Data Bank (PDB) containing the

3-dimensional structure of proteins

env_nr: Protein sequences from metagenomic projects and environmental

samples.

BLAST databases (nucleotides)

All GenBank + RefSeg Nucleotides + EMBL + DDBJ + PDB nr:

sequences (excluding HTGS0,1,2, EST, GSS, STS, PAT, WGS). No longer "non-redundant".

refseg rna: RNA entries from NCBI's Reference Sequence project

refsea genomic: Genomic entries from NCBI's Reference Sequence project

chromosome: A database with complete genomes and chromosomes from the

NCBI Reference Sequence project..

Database of GenBank + EMBL + DDBJ sequences from EST est:

Divisions

Genome Survey Sequence, includes single-pass genomic data, gss:

exon-trapped sequences, and Alu PCR sequences.

Unfinished High Throughput Genomic Sequences: phases 0, 1 htgs:

and 2 (finished, phase 3 HTG sequences are in nr)

Nucleotides from the Patent division of GenBank. pat:

pdb: Sequences derived from the 3-dimensional structure from

Protein Data Bank (PDB)

alu repeats: Human ALU repeat elements

dbsts: Database of GenBank+EMBL+DDBJ sequences from STS

Divisions.

A database for whole genome shotgun sequence entries wgs:

Transcriptome shotgun assembly tsa:

16S: 16S ribosomal RNA from Bacteria and Archaea

