Introduction to Linux and BLAST

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UNIX

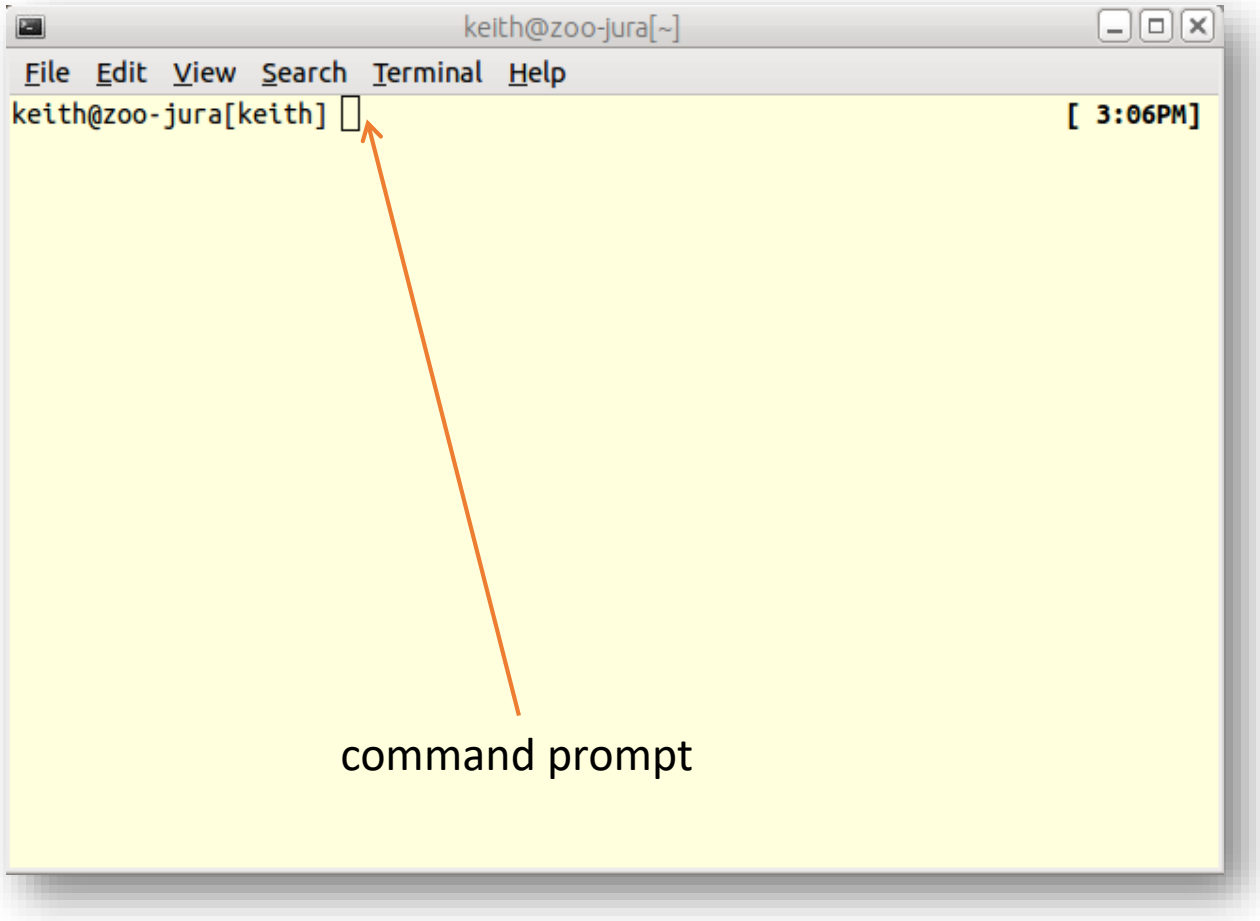
- Development dates back to 1960s.
- Philosophy is to use a large range of simple, dependable tools that each do one simple task.
- Combining tools facilitates complex analysis.
- Popular for high-performance computing.

Ken Thompson (sitting) and Dennis Ritchie
at PDP-11 (photo: Peter Hamer) circa 1970

UNIX/Linux

- UNIX is the operating system of choice for engineering and scientific computing.
- Linux is a free Unix clone.
- Linux runs on most hardware.
  - Embedded systems
  - Mobile phone
  - Desktop computers
  - Super-computing clusters

The command line

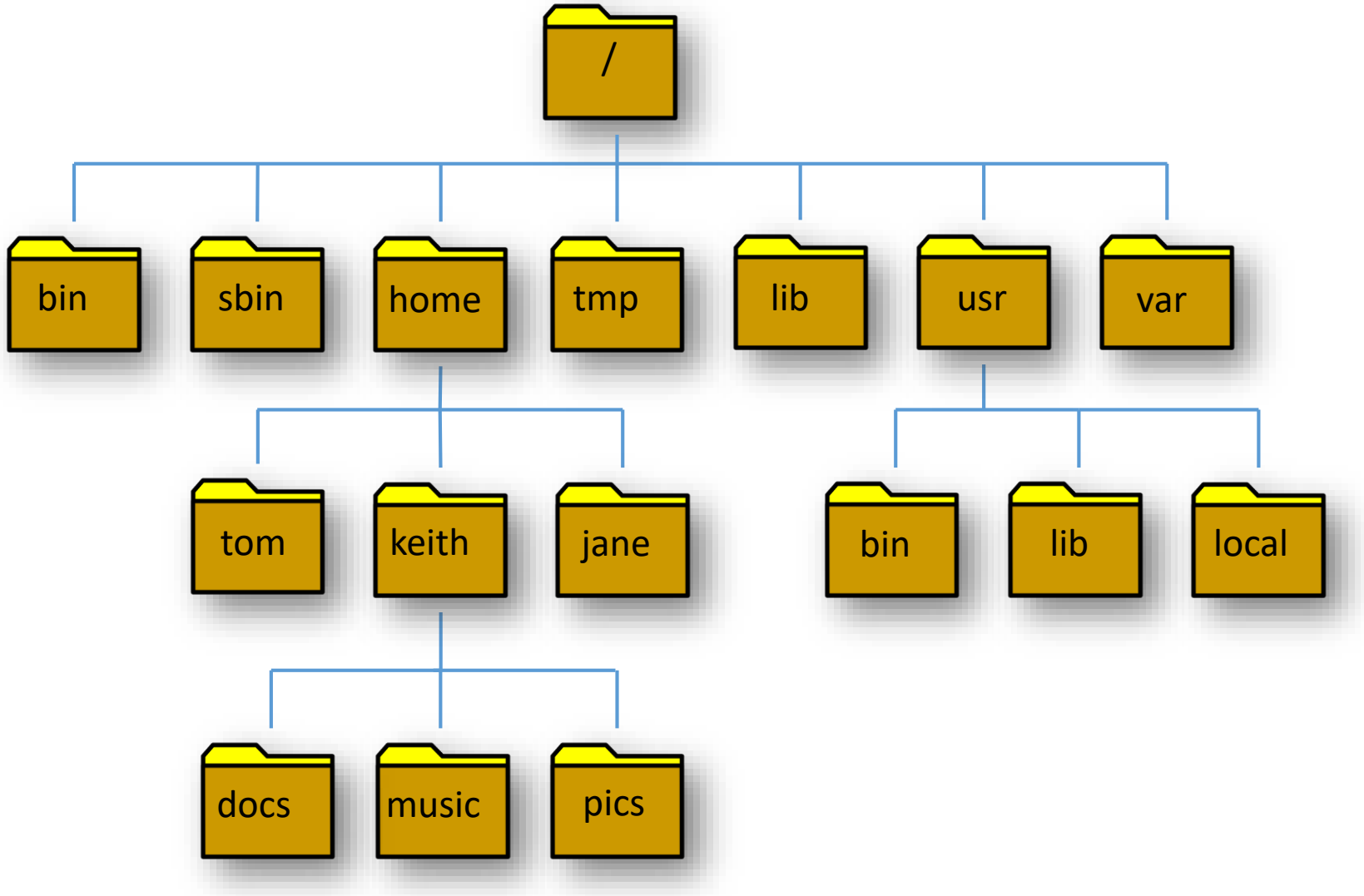


- A command line, or terminal, is a text-based interface to the system.
- You enter commands by typing them on the keyboard and feedback is given to you similarly as text.
- The command line usually presents you with a prompt.

The command line

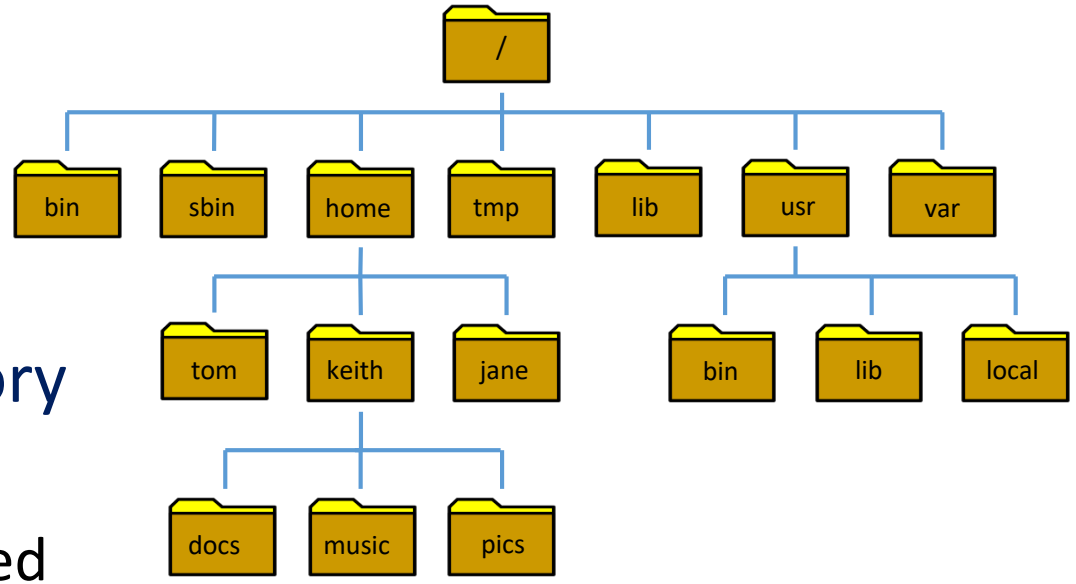
- Command syntax must be **exact!**
- Commands are **case-sensitive**.

The Linux file system is a hierarchy



Commands to navigate Linux filesystem

- ls (list)
  - Lists contents of current directory
- cd (change directory)
  - Change current focus to a new directory
  - Examples:
    - **`cd documents`** (enters directory called 'documents' in current directory)
    - **`cd ..`** (moves up a directory in the filesystem hierarchy)
    - **`cd`** (takes you to your home directory)
- pwd (print working directory)
  - Shows full path to current directory



Commands that operate of files

- mv (move file or directory)
  - Examples
    - **`mv file1 dir1`** (move ‘file1’ in to existing sub-directory ‘dir1’)
  - If destination does not exist, then file is renamed
    - **`mv file1 file2`** (renames ‘file1’ to ‘file2’)
- rm (remove file)
  - Examples
    - **`rm file1`** (remove ‘file1’)
    - **`rm -fr dir1`** (remove dir1 and all its contents)

BLAST

Basic Local Alignment Search Tool

What is BLAST?

- Basic BLAST search
  - What is BLAST?
  - Different BLAST programs
  - BLAST databases you can search
  - Where can I run BLAST on the web?
- The BLAST algorithm
  - Stand-alone program is used behind-the-scenes in most sequence comparison applications

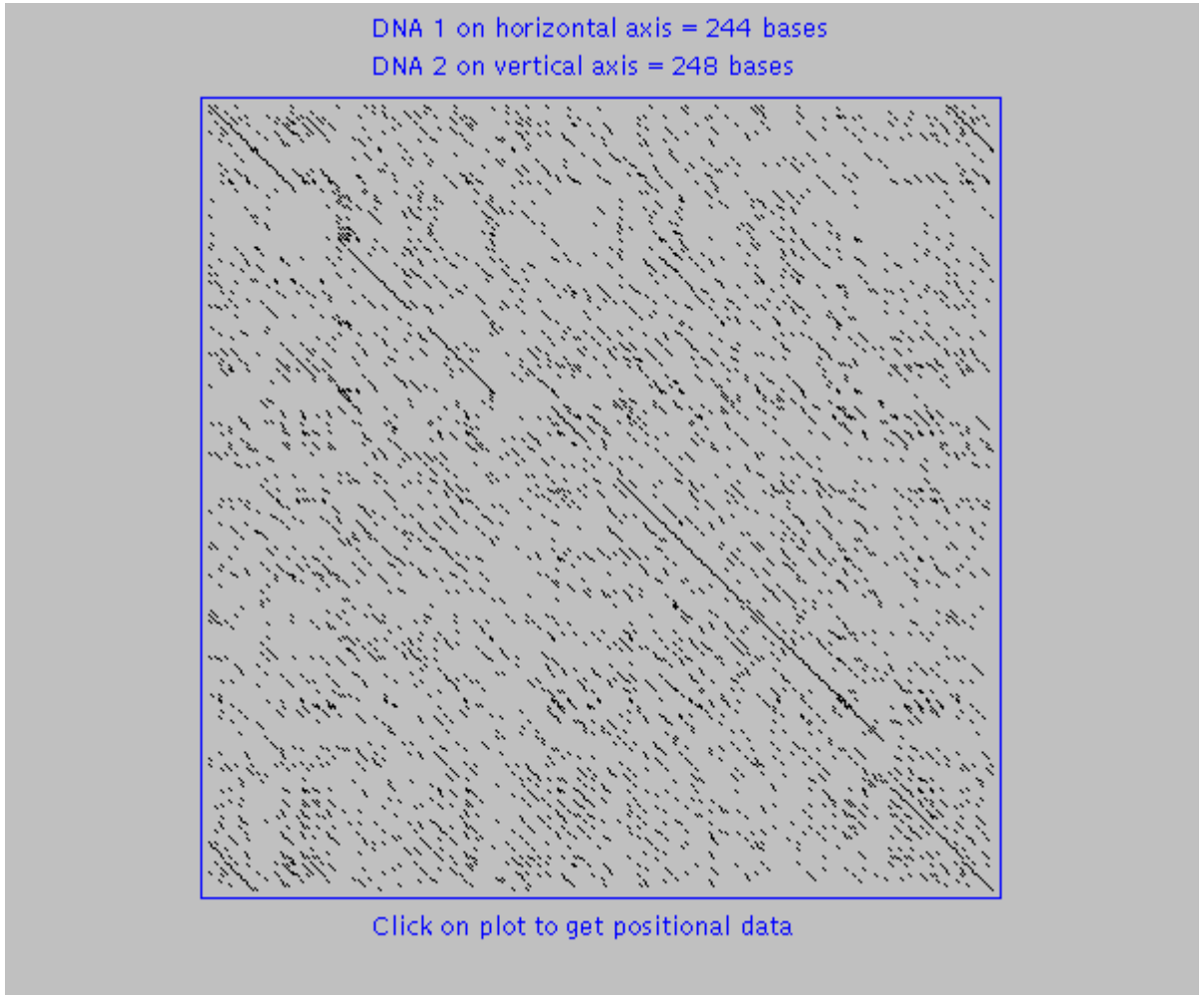
What is BLAST?

- BLAST stands for

  Basic Local Alignment Search Tool

- Why BLAST is popular?
  - Good balance of sensitivity and speed
  - Reliable
  - Flexible
- Local alignments; search query divided into short sequences ('words') and exact matches identified. Once found, these matches are extended

BLAST extends local alignments



- Many nucleotide matches purely by chance (1:4)
- BLAST only considers significant ‘seed’ matches (default word size: 11)
- Scoring system takes account of matches, mismatches, gap formation and gap extension

BLAST output

1. List of sequences with scores
   - Raw score
     - Higher is better
     - Depends on aligned length
   - Expect Value (E-value)
     - Smaller is better
     - Independent of length and database size
2. List of alignments

```
Score =  248 bits (129), Expect = 1e-63
Identities = 213/263 (80%), Gaps = 34/263 (12%)
Strand = Plus / Plus

Query: 161 atatcaccacgtcaaaggtgactccaactcca---ccactccattttgttcagataatgc 217
           ||||||||||||||||||||||||||||  |     | |   || ||||||||||||||
Sbjct: 481 atatcaccacgtcaaaggtgactccaact-tattgatagtgttttatgttcagataatgc 539

Query: 218 ccgatgatcatgtcatgcagctccaccgattgtgagaacgacagcgacttccgtcccagc 277
           |||||||   ||||||||||||||||||| || |            ||||||||||||
Sbjct: 540 ccgatgactttgtcatgcagctccaccgattttg-g-----------ttccgtcccagc 586

Query: 278 c-gtgcc--aggtgctgcctcagattcaggttatgccgctcaattcgctgcgtatatcgc 334
           |  ||  |  | ||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct: 587 caatgacgta-gtgctgcctcagattcaggttatgccgctcaattcgctgggtatatcgc 645

Query: 335 ttgctgattacgtgcagctttcccttcaggcggga-----------ccagccatccgtc 382
           ||||||||||||||||||||||||||||||||||           ||||||||||||
Sbjct: 646 ttgctgattacgtgcagctttcccttcaggcgggattcatacagcggccagccatccgtc 705

Query: 383 ctccatatc-accacgtcaaagg 404
            ||||||| ||||||||||||
Sbjct: 706 atccatatcaaccacgtcaaagg 728
```

BLAST Programs

Program	Database (Subject)	Query
BLASTN	Nucleotide	Nucleotide
BLASTP	Protein	Protein
BLASTX	Protein	Nt. → Protein
TBLASTN	Nt. → Protein	Protein
TBLASTX	Nt. → Protein	Nt. → Protein

The BLOSUM62 matrix is used by BLAST to align amino acids and determine if two non-identical amino acids are ‘positives’

```
#  Matrix made by matblas from blosum62.iij
#  * column uses minimum score
#  BLOSUM Clustered Scoring Matrix in 1/2 Bit Units
#  Blocks Database = /data/blocks_5.0/blocks.dat
#  Cluster Percentage: >= 62
#  Entropy =   0.6979, Expected =  -0.5209
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V  B  Z  X  *
A  4 -1 -2 -2  0 -1 -1  0 -2 -1 -1 -1 -1 -2 -1  1  0 -3 -2  0 -2 -1  0 -4
R -1  5  0 -2 -3  1  0 -2  0 -3 -2  2 -1 -3 -2 -1 -1 -3 -2 -3 -1  0 -1 -4
N -2  0  6  1 -3  0  0  0  1 -3 -3  0 -2 -3 -2  1  0 -4 -2 -3  3  0 -1 -4
D -2 -2  1  6 -3  0  2 -1 -1 -3 -4 -1 -3 -3 -1  0 -1 -4 -3 -3  4  1 -1 -4
C  0 -3 -3 -3  9 -3 -4 -3 -3 -1 -1 -3 -1 -2 -3 -1 -1 -2 -2 -1 -3 -3 -2 -4
Q -1  1  0  0 -3  5  2 -2  0 -3 -2  1  0 -3 -1  0 -1 -2 -1 -2  0  3 -1 -4
E -1  0  0  2 -4  2  5 -2  0 -3 -3  1 -2 -3 -1  0 -1 -3 -2 -2  1  4 -1 -4
G  0 -2  0 -1 -3 -2 -2  6 -2 -4 -4 -2 -3 -3 -2  0 -2 -2 -3 -3 -1 -2 -1 -4
H -2  0  1 -1 -3  0  0 -2  8 -3 -3 -1 -2 -1 -2 -1 -2 -2  2 -3  0  0 -1 -4
I -1 -3 -3 -3 -1 -3 -3 -4 -3  4  2 -3  1  0 -3 -2 -1 -3 -1  3 -3 -3 -1 -4
L -1 -2 -3 -4 -1 -2 -3 -4 -3  2  4 -2  2  0 -3 -2 -1 -2 -1  1 -4 -3 -1 -4
K -1  2  0 -1 -3  1  1 -2 -1 -3 -2  5 -1 -3 -1  0 -1 -3 -2 -2  0  1 -1 -4
M -1 -1 -2 -3 -1  0 -2 -3 -2  1  2 -1  5  0 -2 -1 -1 -1 -1  1 -3 -1 -1 -4
F -2 -3 -3 -3 -2 -3 -3 -3 -1  0  0 -3  0  6 -4 -2 -2  1  3 -1 -3 -3 -1 -4
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4  7 -1 -1 -4 -3 -2 -2 -1 -2 -4
S  1 -1  1  0 -1  0  0  0 -1 -2 -2  0 -1 -2 -1  4  1 -3 -2 -2  0  0  0 -4
T  0 -1  0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1  1  5 -2 -2  0 -1 -1  0 -4
W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1  1 -4 -3 -2 11  2 -3 -4 -3 -2 -4
Y -2 -2 -2 -3 -2 -1 -2 -3  2 -1 -1 -2 -1  3 -3 -2 -2  2  7 -1 -3 -2 -1 -4
V  0 -3 -3 -3 -1 -2 -2 -3 -3  3  1 -2  1 -1 -2 -2  0 -3 -1  4 -3 -2 -1 -4
B -2 -1  3  4 -3  0  1 -1  0 -3 -4  0 -3 -3 -2  0 -1 -4 -3 -3  4  1 -1 -4
Z -1  0  0  1 -3  3  4 -2  0 -3 -3  1 -1 -3 -1  0 -1 -3 -2 -2  1  4 -1 -4
X  0 -1 -1 -1 -2 -1 -1 -1 -1 -1 -1 -1 -1 -1 -2  0  0 -2 -1 -1 -1 -1 -1 -4
* -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4  1
```

Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance. Learn more

NEWS

BLAST+ 2.8.1 is released

New databases, better performance.
Wed, 19 Dec 2018 17:00:00 EST

More BLAST news...

Web BLAST

Nucleotide BLAST
nucleotide ▶ nucleotide

blastx
translated nucleotide ▶ protein

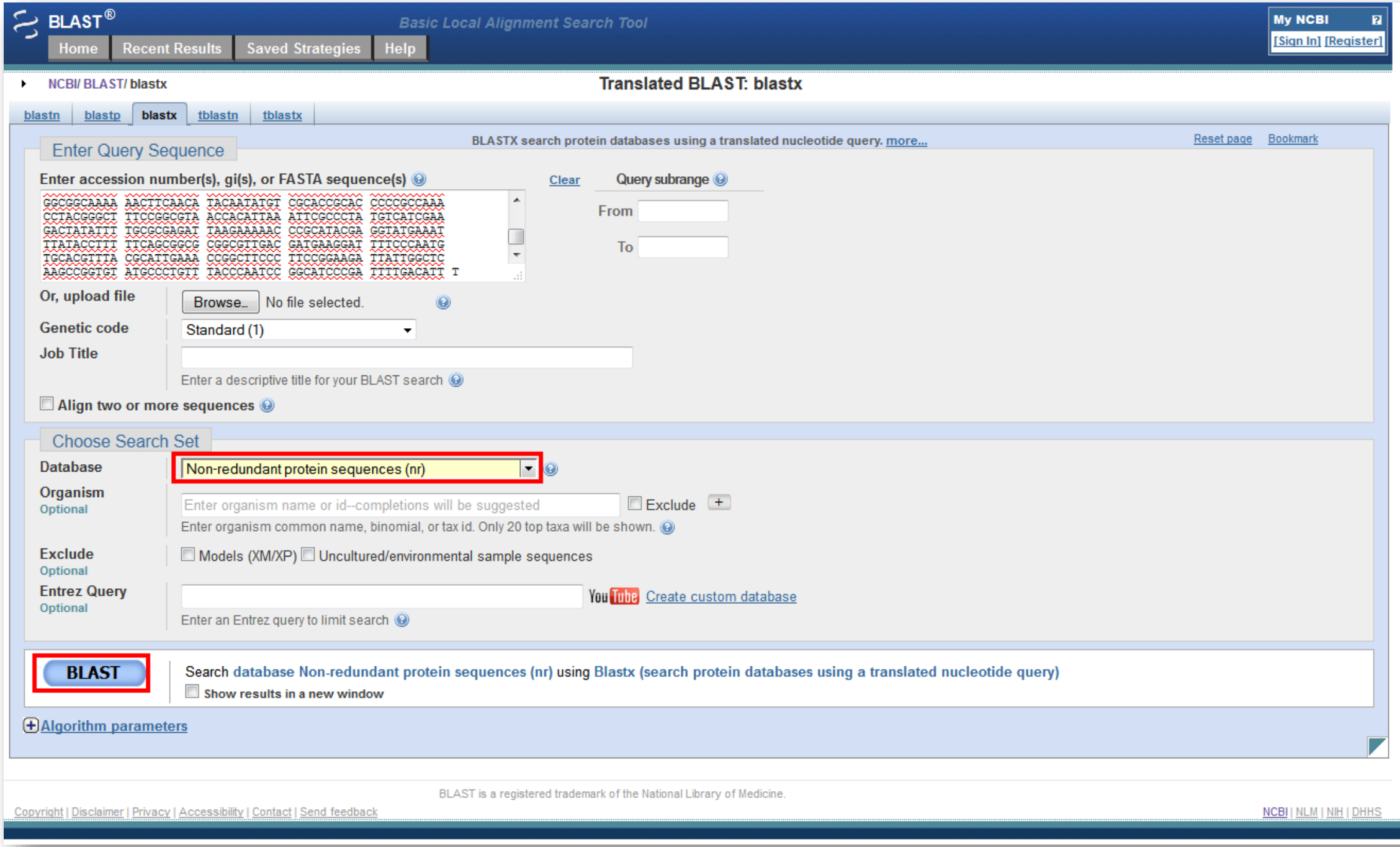
tblastn
protein ▶ translated nucleotide

Protein BLAST
protein ▶ protein

BLAST Genomes

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

Human Mouse Rat Microbes


BLAST®
Basic Local Alignment Search Tool
Home
Recent Results
Saved Strategies
Help
My NCBI
[Sign In] [Register]
NCBI/ BLAST/ blastx
Translated BLAST: blastx
blastn
blastp
blastx
tblastn
tblastx
BLASTX search protein databases using a translated nucleotide query. more...
Reset page
Bookmark
Enter Query Sequence
Enter accession number(s), gi(s), or FASTA sequence(s)
Clear
Query subrange
From
To
GGCGGCAAAA AACTTCAACA TACAATATGT CGCACCGCAC CCCCGCCAAA
CCTACGGGCT TTCCGGCGTA ACCACATTAA ATTCGCCCTA TGTCATCGAA
GACTATATTT TGCGCGAGAT TAAGAAAAAC CCGCATACGA GGTATGAAAT
TTATACCTTT TTCAGCGGCG CGGCGTTGAC GATGAAGGAT TTTCCCAATG
TGCACGTTTA CGCATTGAAA CCGGCTTCCC TTCCGGAAGA TTATTGGCTC
AAGCCGGTGT ATGCCCTGTT TACCCAATCC GGCATCCCGA TTTTGACATT T
Or, upload file
Browse...
No file selected.
Genetic code
Standard (1)
Job Title
Enter a descriptive title for your BLAST search
Align two or more sequences
Choose Search Set
Database
Non-redundant protein sequences (nr)
Organism
Optional
Enter organism name or id--completions will be suggested
Exclude
Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.
Exclude
Optional
Models (XM/XP)
Uncultured/environmental sample sequences
Entrez Query
Optional
YouTube
Create custom database
Enter an Entrez query to limit search
BLAST
Search database Non-redundant protein sequences (nr) using Blastx (search protein databases using a translated nucleotide query)
Show results in a new window
Algorithm parameters
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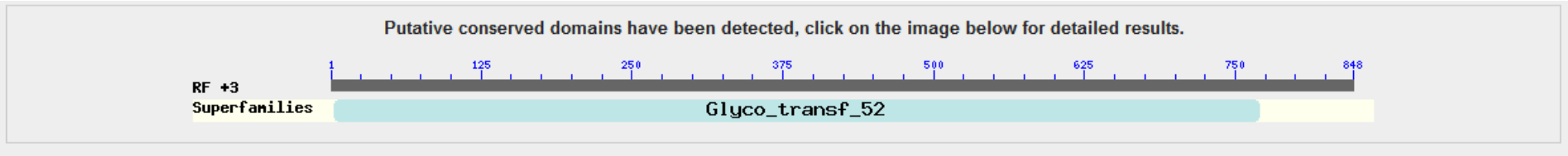
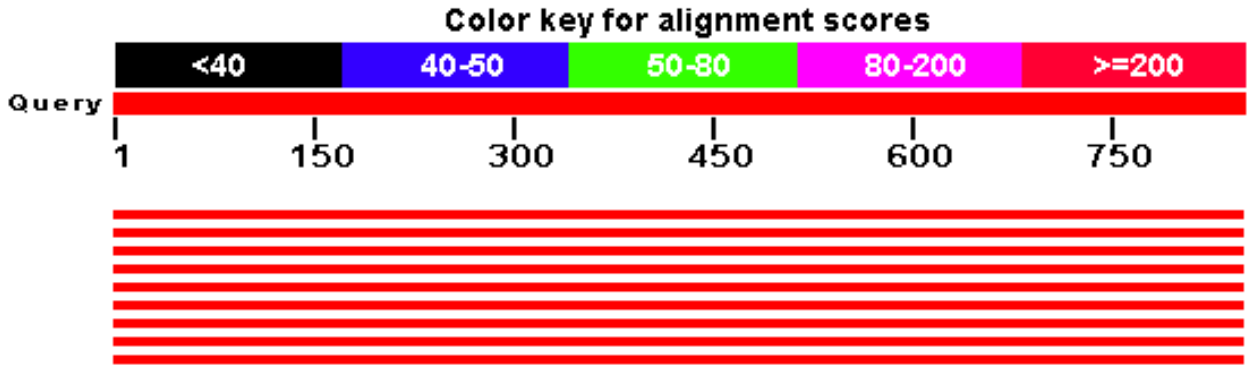
NCBI BLAST output





Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenPept Graphics

Description	Max score	Total score	Query cover	E value	Ident	Accession
alpha-2,3-sialyltransferase [Neisseria meningitidis]	576	576	99%	0.0	99%	AAC44543.1
CMP-N-acetylneuraminate-beta-galactosamide-alpha-2 3-sialyltransferase [Neisseria meningitidis]	576	576	99%	0.0	99%	WP_002234614.1
CMP-N-acetylneuraminate-beta-galactosamide-alpha-2 3-sialyltransferase [Neisseria meningitidis]	575	575	99%	0.0	99%	WP_014580539.1
CMP-N-acetylneuraminate-beta-galactosamide-alpha-2 3-sialyltransferase [Neisseria meningitidis]	574	574	99%	0.0	99%	WP_002236898.1
CMP-N-acetylneuraminate-beta-galactosamide-alpha-2 3-sialyltransferase [Neisseria meningitidis]	573	573	99%	0.0	98%	WP_002230437.1
CMP-N-acetylneuraminate-beta-galactosamide-alpha-2 3-sialyltransferase [Neisseria meningitidis]	573	573	99%	0.0	98%	WP_002239594.1

Sequence alignment output

CMP-N-acetylneuraminate-beta-galactosamide-alpha-2 3-sialyltransferase [Neisseria meningitidis]

Sequence ID: ref|WP_002234614.1| **Length:** 371 **Number of Matches:** 1

▶ See 29 more title(s)

Range 1: 86 to 367 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
576 bits(1485)	0.0	Compositional matrix adjust.	279/282(99%)	279/282(98%)	0/282(0%)	+3

```
Query  3    NEKYDYYFKQIKDKAERAYFFHLPYDLNKSFNVIPTMAELKVKSMLLPKVKRTYLASLEK  182
            NEKYDYYFKQIKDKAERAYFFHLPY LNKSFN IPTMAELKVKSMLLPKVKR YLASLEK
Sbjct  86   NEKYDYYFKQIKDKAERAYFFHLPYGLNKSFNFIPTMAELKVKSMLLPKVKRIYLASLEK  145

Query  183  VSIAAFLSTYPDAEIKTFDDGTGNLIQSSSYLGDEFSVNGTIKRNFARMMIGDWSIAKTR  362
            VSIAAFLSTYPDAEIKTFDDGTGNLIQSSSYLGDEFSVNGTIKRNFARMMIGDWSIAKTR
Sbjct  146  VSIAAFLSTYPDAEIKTFDDGTGNLIQSSSYLGDEFSVNGTIKRNFARMMIGDWSIAKTR  205

Query  363  NASDEHYTIFKGLKNIMDDGRRKMTYLPLFDASELKAGDETGGTVRILLGSPDKEMKEIS  542
            NASDEHYTIFKGLKNIMDDGRRKMTYLPLFDASELKAGDETGGTVRILLGSPDKEMKEIS
Sbjct  206  NASDEHYTIFKGLKNIMDDGRRKMTYLPLFDASELKAGDETGGTVRILLGSPDKEMKEIS  265

Query  543  EKAAKNFNIQYVAPHPRQTYGLSGVTTLNSPYVIEDYILREIKKNPHTRYEIYTFFSGAA  722
            EKAAKNFNIQYVAPHPRQTYGLSGVTTLNSPYVIEDYILREIKKNPHTRYEIYTFFSGAA
Sbjct  266  EKAAKNFNIQYVAPHPRQTYGLSGVTTLNSPYVIEDYILREIKKNPHTRYEIYTFFSGAA  325

Query  723  LTMKDFPNVHVYALKPASLPEDYWLKPVYALFTQSGIPILTF  848
            LTMKDFPNVHVYALKPASLPEDYWLKPVYALFTQSGIPILTF
Sbjct  326  LTMKDFPNVHVYALKPASLPEDYWLKPVYALFTQSGIPILTF  367
```

Gotchas

- BLAST will usually return a result!
  - The top match may not be significant
  - E-values up to 10 returned by default
- Domains are often shared by different proteins
  - Significant match to parts of the sequence, but not to others