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A Practical Guide to NCBI BLAST

NCBI Search Services and Tools

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- Entrez integrated literature and molecular databases
  - Viewers
    - BLink protein similarities
    - Graphical Sequence Viewer annotation viewer and analysis tool
- BLAST sequence similarity search service
- VAST structure similarity searches
- Tools, special services, standalone software
  - Entrez Utilities Entrez API <ncbi>/books/NBK25501/
  - Standalone BLAST BLAST programs + databases <ncbi>/books/NBK1762/
  - Cn3D 3D structure viewer <ncbi>/Structure/CN3D/cn3d.shtml
  - Genome Workbench sequence analysis / annotation platform <ncbi>/tools/gbench/
  - SRA Utilities <ncbi>/Traces/sra/
    - SRA Run Browser web access
    - SRA toolkit standalone SRA manipulator and client

Topics

- Basics of using NCBI BLAST
  - Motivation, Statistics, Scoring, Search Programs
- Using the Web Interface
- Other Web services
  - COBALT – protein multiple alignment
  - Primer BLAST
  - MOLE-BLAST
- Live Searches

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What is BLAST?

- Widely used sequence similarity search tool
- Finds high scoring local alignments between two sequences (protein or DNA)
- Includes a model of score distributions for random local alignments
- Provides statistical significance for alignments

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BLAST Fundamentals

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- BLAST tells you about non-chance similarities between biological sequences.
- If similarities are not due chance then they must be due to something else!
  - Homology
  - Simple identification
- All BLAST searches begin with a sequence
  - protein or nucleotide
  - experimentally determined or one from database

What BLAST tells you

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Here's my sequence.

1. What is it related to? (What does it do?)
   - Homology
   - Function
2. Is it already in the database? (Identification)
   - find the matching sequence in the database
   - organism of origin
3. Where is it located or how is it organized?
   - in a genome
   - other annotation problems
     - comparing sequences
     - looking for frame shifts

BLAST Statistics

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```
 Score = 18.5 bits (36),  Expect = 47992
 Identities = 5/5 (100%), Positives = 5/5 (100%), Gaps = 0/5 (0%)

Query  1   ELVIS  5
           ELVIS
Sbjct  8   ELVIS  12
```

- Number of chance alignments = 48 thousand!
- Indistinguishable from chance

The most important statistic: Expect value (e-value)
Expected number of random alignments with a particular score or better

```
 Score = 89.7 bits (204),  Expect = 7e-18
 Identities = 50/103 (49%), Positives = 54/103 (52%), Gaps = 18/103 (17%)

Query  1   MKLLAATVL---LLTICSLEGALVR
           MK L   VL   LL +CSLEGA V
Sbjct  1   MKVL---VLAMVLLCVCSLEGAVVM

Query  54  SPELQAEAKSYFEKSKEQLTPLIKKAGTELVNFLSYFVELGTQ  96
             E   +AK Y E   EQ  P  K   TE       F +L TQ
Sbjct  57  AEEIKTQAKAYLEQANEQFSPIAKRLHTE-------FMDLLTQ  92
```

- Number of chance alignments = 7 X 10^{-18}
- Not due to chance

- The e-value depends directly on the size of the search space (database)
- Search the smallest database likely to contain the sequence of interest

Scoring: Nucleotide

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Number of Chance Alignments = 2 X 10^{-73}

```
 Score =  288 bits (318),  Expect = 2e-73
 Identities = 262/325 (81%), Gaps = 8/325 (2%)
 Strand=Plus/Plus

Query  1923   TCAGCCTACCATGAGAATAAGAGAAAGA-AAATGAAGATCAAAAGCTTATTCATCTGTTT  1981
Sbjct  33774  TCAGACTACCCTGAGAATAAGAGAAAGAGAAATGAAGACCTAGA-CTTATCCATCTCTTT  33832
Query  1982   TTGTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCAT  2041
Sbjct  33833  TTGTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAAATAT  33892
Query  2042   TTTGCCTCTTTTCT                                GAATCTAATAGAGTGGT  2100
Sbjct  33893  TTTGCCTCTTTTCTCTGTGCTACAATTAATAAAAAAATGAAAAGAATCTAATTTAATTGT  33952
Query  2101   ACAGCACTGTTA-TT                                            TTCTGTGG  2159
Sbjct  33953  CTATGACTGTTATTT                                            TTCTATGA  34012
Query  2160   AAGTTCCAGTGTTCT                                            GTGGGCTA  2219
Sbjct  34013  AAATTCCACTATTCTCTCTTTCCCTATTTCAATGGAGGACTTCTAGTTCCTTCTGGATTA  34072
Query  2220   AT----TAAATAAATCATTAATACT  2240
Sbjct  34073  ATTGCATAAAAGAAACATTAATACT  34097
```

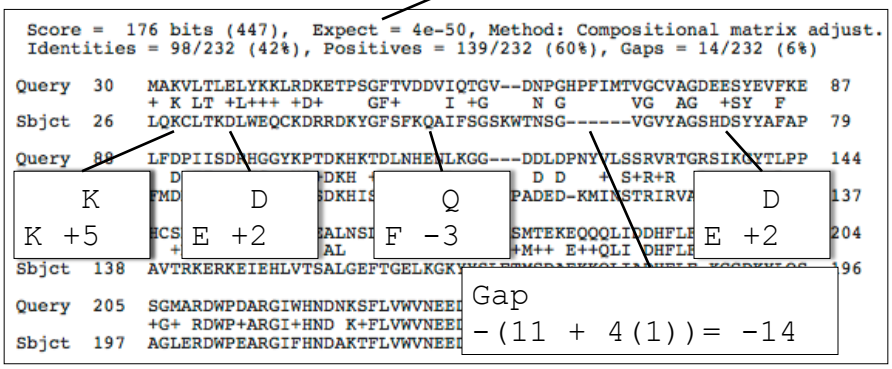
Match=+2

Mismatch=-3

Gap
-(5 + 4(2))= -13

Scoring: Protein

Number of Chance Alignments = 4 X 10^{-50}



Scores from BLOSUM62, a position independent matrix
- Same substitution gets the same score at all positions
- All positions equally likely to change

BLAST Search Programs

Nucleotide Search Programs

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- blastn
  - traditional BLAST algorithm
  - most sensitive nucleotide search
- megablast
  - larger word size than blastn
  - different gapping model
    - Contiguous megablast
      - Nearly identical sequences
    - Discontiguous megablast
      - Cross-species comparisons

- Default nucleotide search program
- Best for
  - Identification
  - Same-species annotation

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Protein Search Programs

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Position Independent scoring

- blastp
- translating searches
  - useful for unannotated protein coding regions
  - six frame translations of query, database or both
    - blastx – translated query
    - tblastn – translated database
    - tblastx – translated query and database

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Protein Domains and Position Specific Scoring

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Position-specific scoring model

- Multiple alignment based
- Substitution scores depend on the position in the protein.
- Some positions are more important (less likely to change)
- More accurate alignments
- More sensitive at identifying distant homologies
- Better at identifying structural / functional domain

Position-specific Programs
(protein only)

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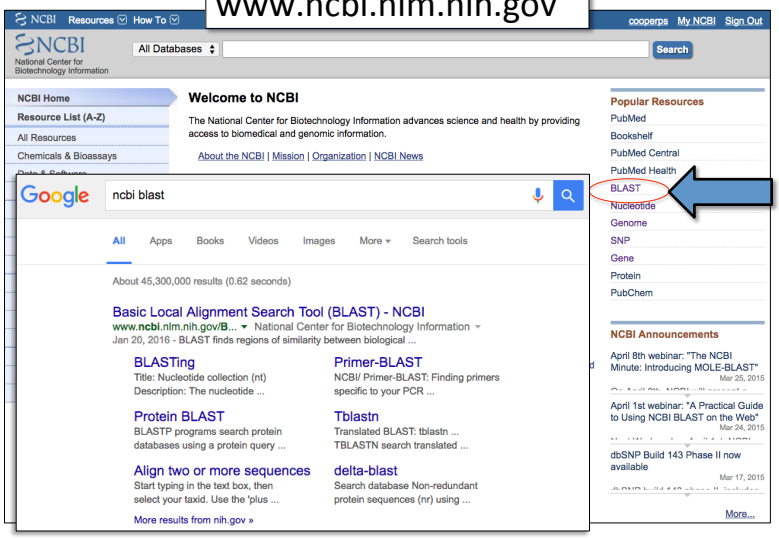
- **Position Specific Iterative BLAST (PSI-BLAST)**
  Automatically generates a position specific score matrix (PSSM) from initial set of BLAST alignments
- **Position-Hit Initiated BLAST (PHI-BLAST)**
  Focuses search around pattern (motif)
- **Domain Enhanced Lookup Time Accelerated (DELTA) BLAST**
  Uses conserved domain PSSM in first round of search
- **Reverse PSI-BLAST (RPS-BLAST)**
  Searches a database of PSI-BLAST PSSMs
  Conserved Domain Database Search
  Quickly identifies type of protein and potential function

  - Runs with all blastp searches at the NCBI
  - Identifies conserved domains in query

Web Access

www.ncbi.nlm.nih.gov



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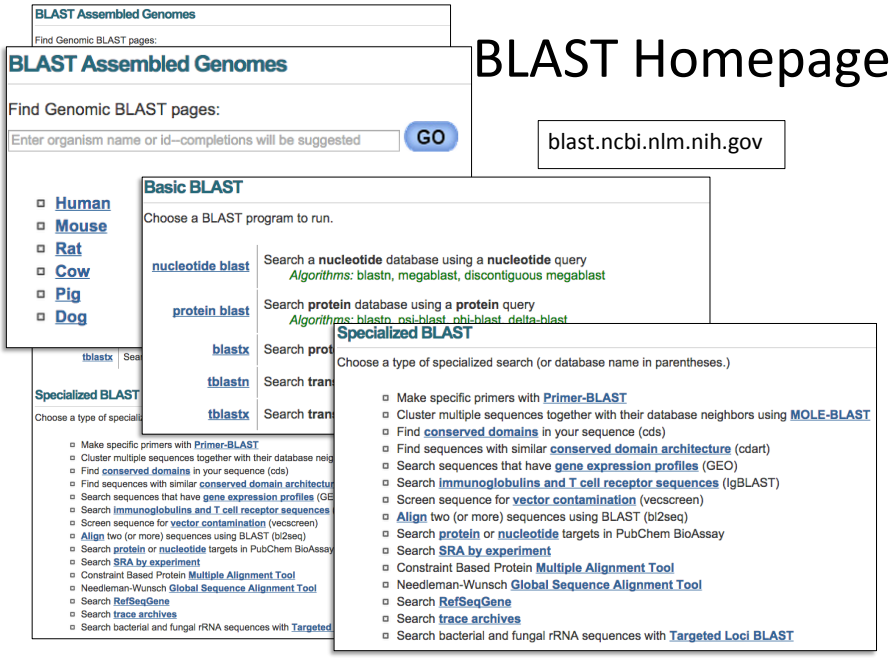
BLAST Homepage

blast.ncbi.nlm.nih.gov



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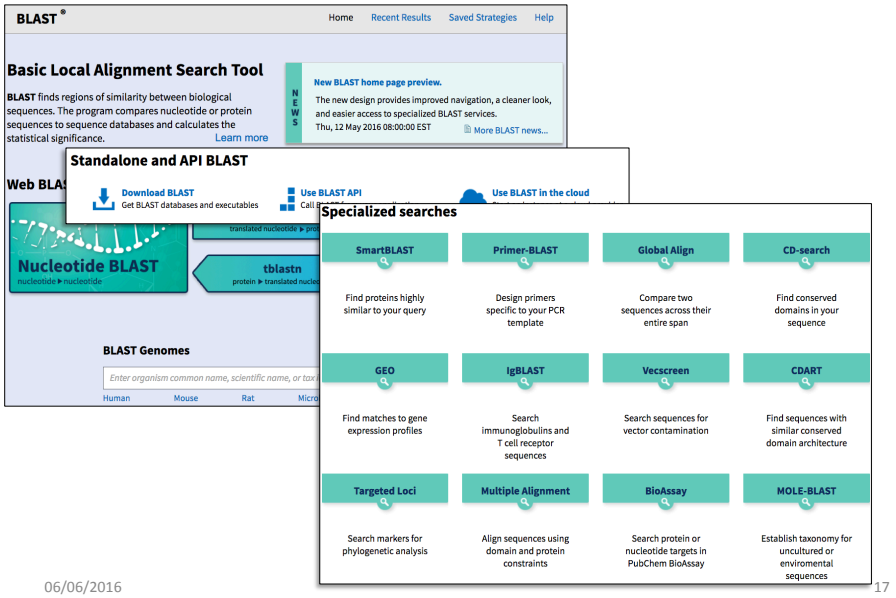
The New BLAST Homepage



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Query Sequences

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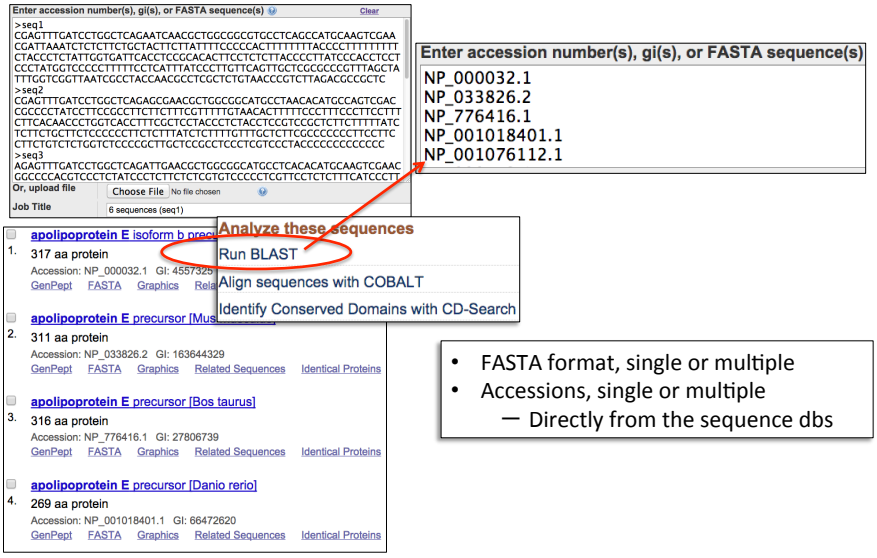
Queries



- FASTA format, single or multiple
- Accessions, single or multiple
  - Directly from the sequence dbs

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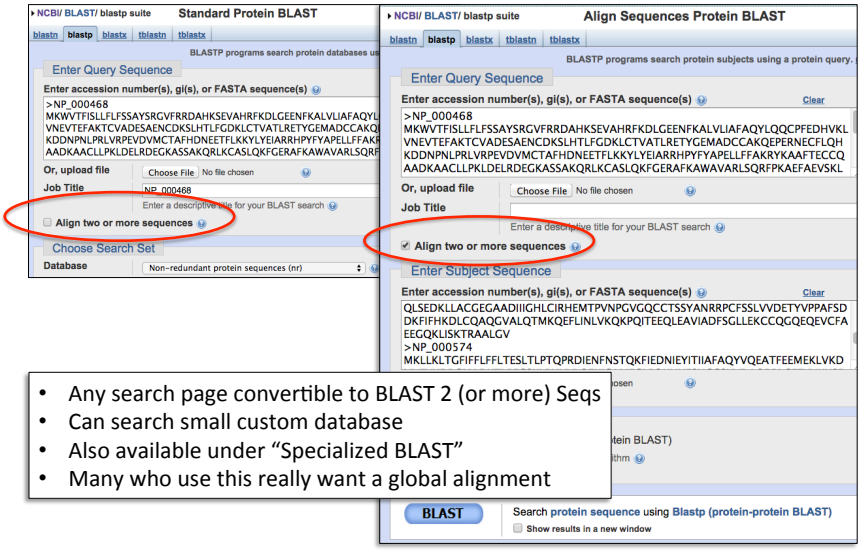
BLAST 2 (or more) Sequences



- Any search page convertible to BLAST 2 (or more) Seqs
- Can search small custom database
- Also available under “Specialized BLAST”
- Many who use this really want a global alignment

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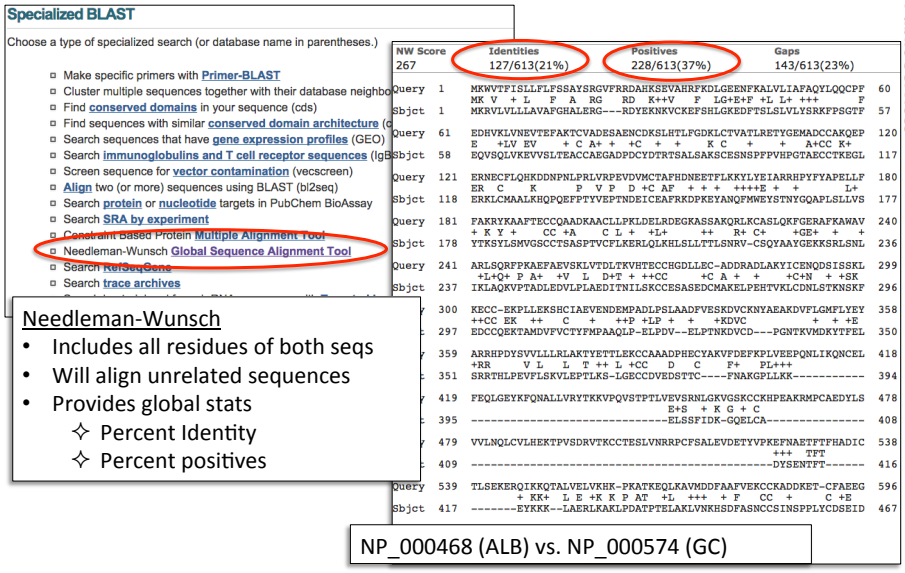
Global Alignment Tool



Needleman-Wunsch
- Includes all residues of both seqs
- Will align unrelated sequences
- Provides global stats
  - ✧ Percent Identity
  - ✧ Percent positives

NP_000468 (ALB) vs. NP_000574 (GC)

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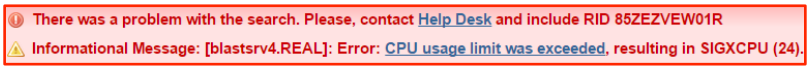
Restrictions on Web BLAST



- No fixed limit on size / number of queries
- Web BLAST limited by processing time (one hour) for single search
  - – Difficult to estimate processing time
  - – Factors
    - Size and number of queries
    - Size of database
    - Depends on size and complexity of results too

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BLAST Databases

Protein Databases

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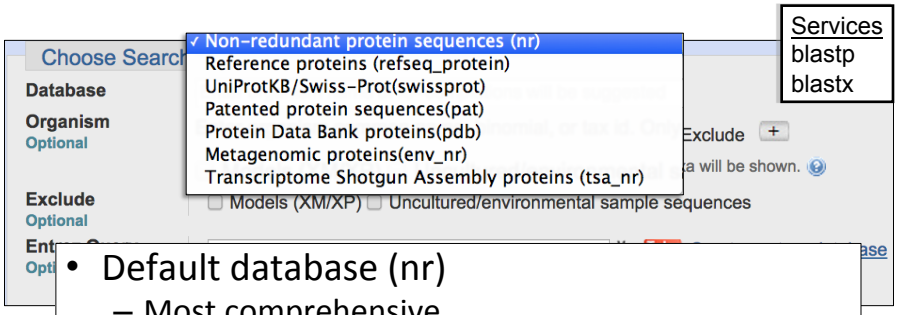
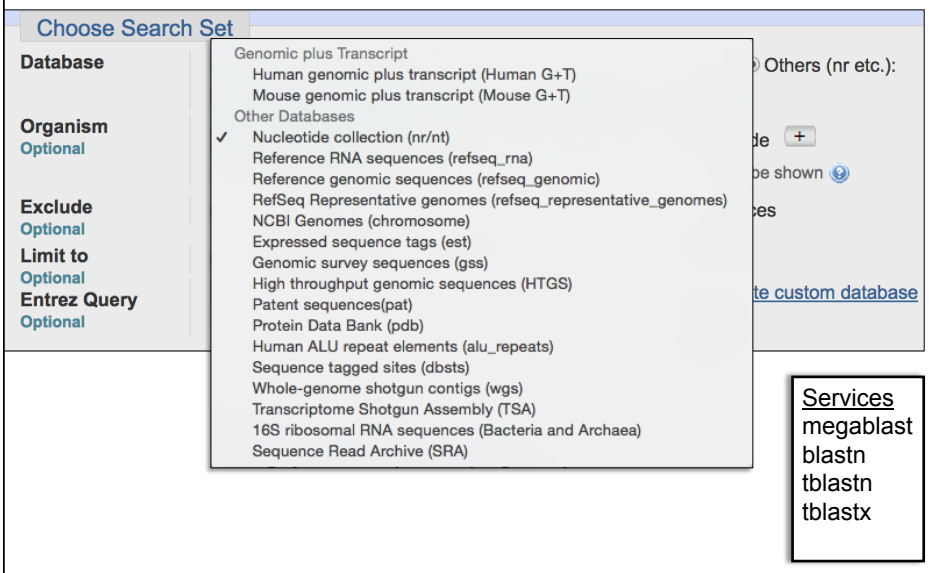


- Default database (nr)
  - Most comprehensive
  - Useful subsets: RefSeq, Swiss-Prot, PDB
- What's not in nr?
  - US , European and Asian Patents
  - Proteins from metagenomes
  - Proteins from Next-Gen assemblies

Nucleotide Databases



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Nucleotide Databases

- Default database (nr/nt) is not comprehensive
  - Contains traditional GenBank and RefSeq RNA
  - Useful subsets: RefSeq RNA, 16S rRNA reference sequences
- What is not in nr/nt? The majority of nucleotide data
  - Bulk sequences (EST, GSS, HTGS, STS)
  - RefSeq Genomic Sequences (Chromosome, RefSeq Genomic, RefSeq Representative Genomes)
  - US, European and Asian Patents (pat)
  - Whole Genome Shotgun Contigs (WGS) (Second Largest)
  - Transcriptome Shotgun Assemblies (TSA)
  - Next-Gen RNA-Seq, DNA-Seq Reads (SRA) (Largest set of data)

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Limiting Databases

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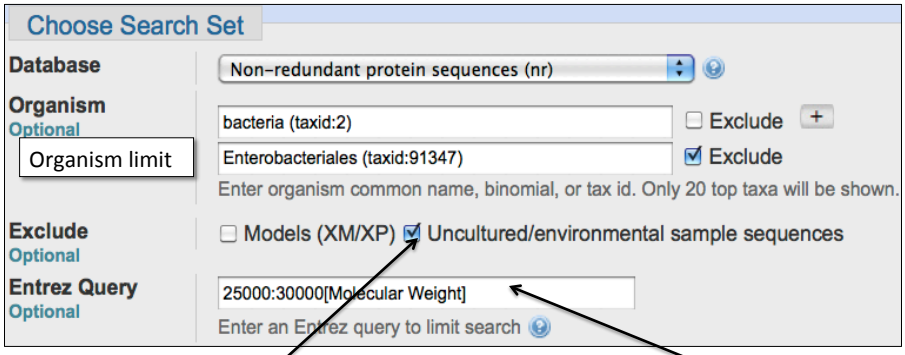
Search the smallest database likely to contain the sequence of interest.



Organism limit

Exclude predicted and uncultured

Limit with Entrez query

Genome Databases

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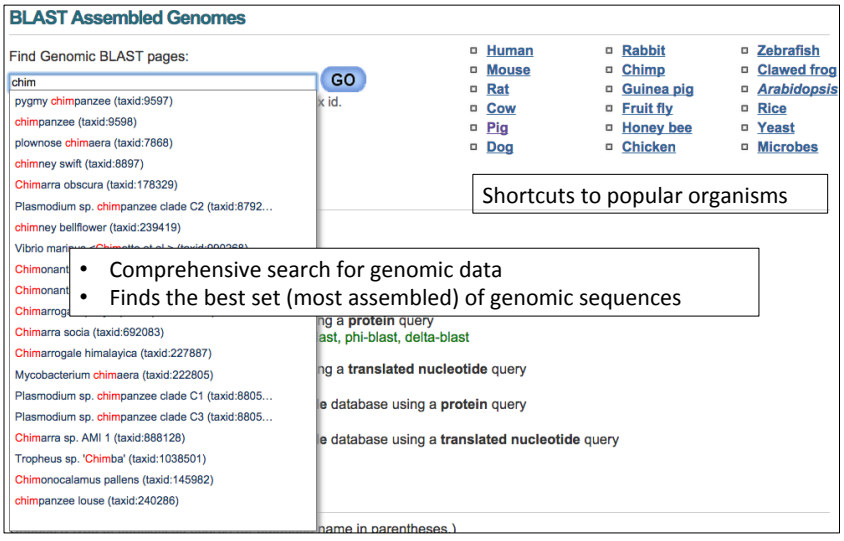

Shortcuts to popular organisms

- Comprehensive search for genomic data
- Finds the best set (most assembled) of genomic sequences

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Web Program Selection

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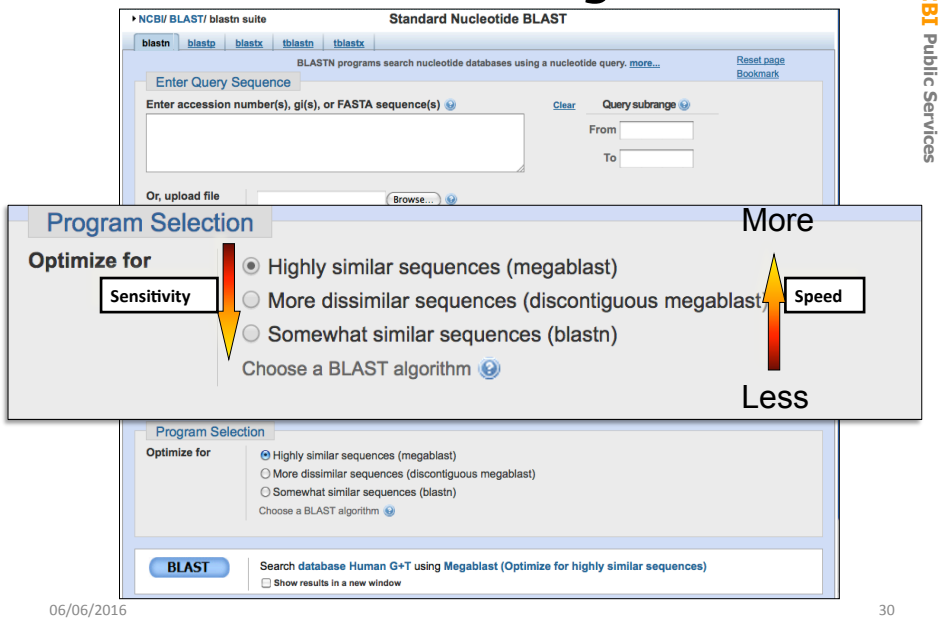
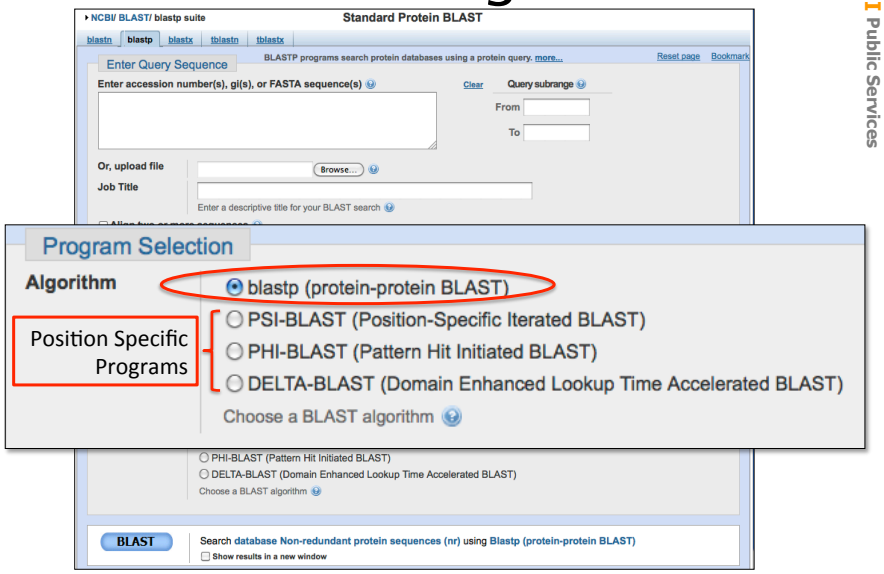
Nucleotide Programs



Protein Programs



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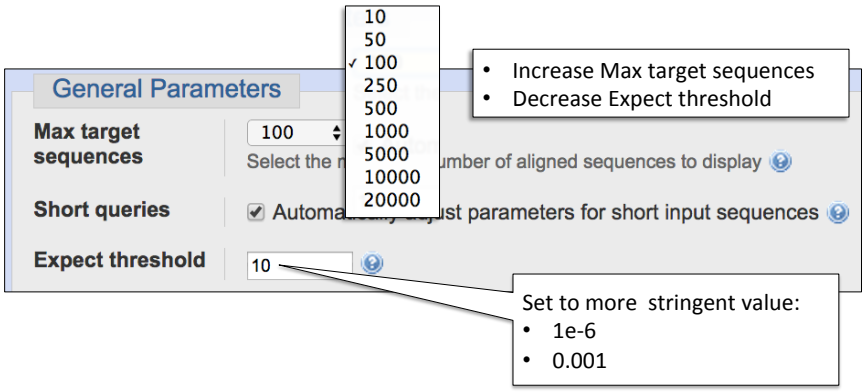
Algorithm Parameters: General



- Increase Max target sequences
- Decrease Expect threshold

Set to more stringent value:
- 1e-6
- 0.001

Let **Expect threshold** govern output not **Max target** sequences

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Nucleotide Repeat Filters

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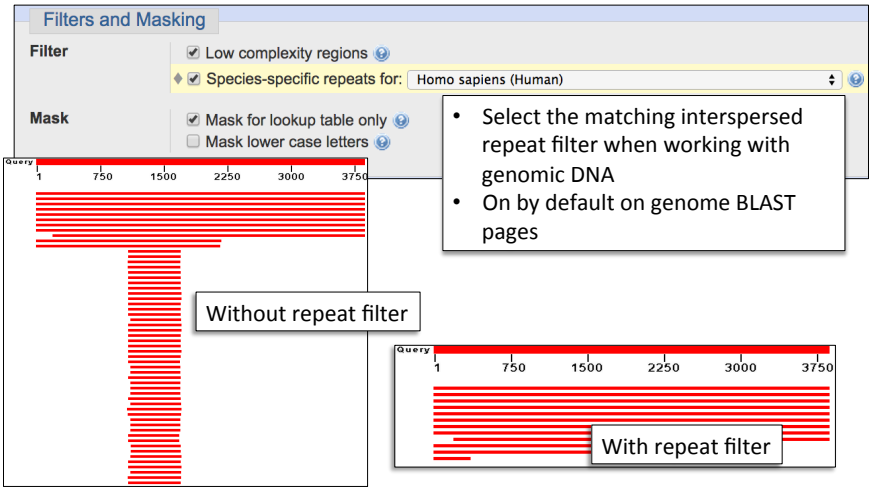

- Select the matching interspersed repeat filter when working with genomic DNA
- On by default on genome BLAST pages

Formatting options

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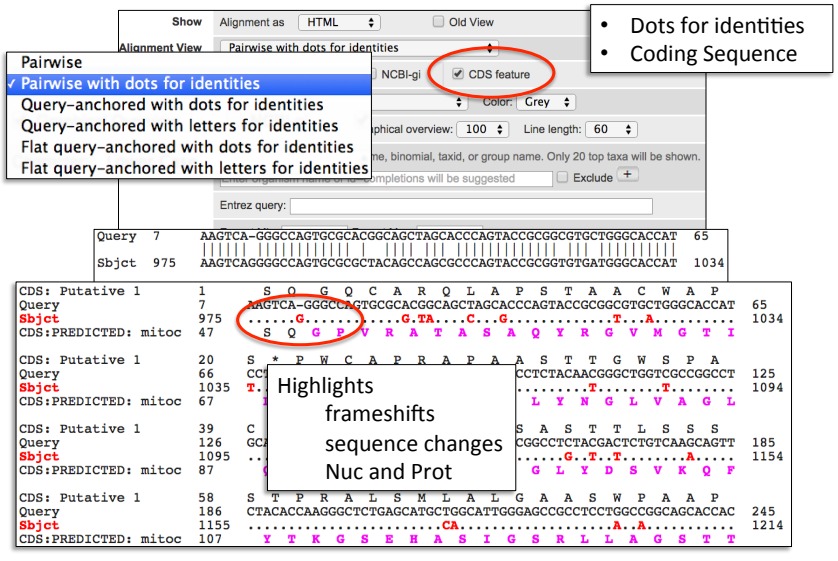

- Dots for identities
- Coding Sequence

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Managing Your Results

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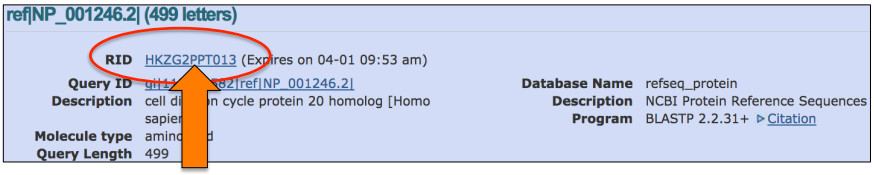
The Request ID (RID) is the key

The BLAST Request ID (RID) is the key



http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Get&RID=HKZG2PPT013

- Uniquely identifies search settings and results
- Persists at NCBI for 36 hours
- View through Recent Results, My NCBI
- Allows sharing results and reformatting
- Send the RID to blast-help@ncbi.nlm.nih.gov to ask about a search

Download Options

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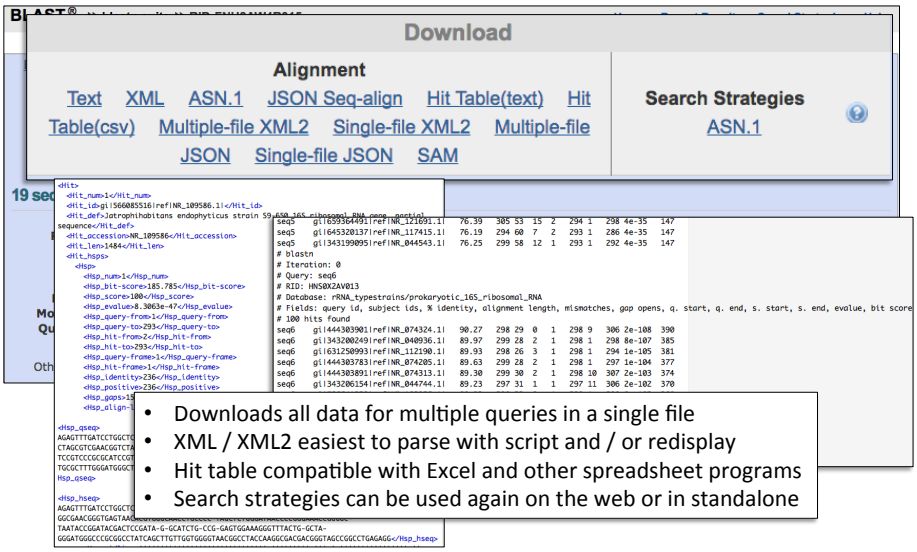

- Downloads all data for multiple queries in a single file
- XML / XML2 easiest to parse with script and / or redisplay
- Hit table compatible with Excel and other spreadsheet programs
- Search strategies can be used again on the web or in standalone

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Specialized BLAST Services

Nucleotide Services

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- PrimerBlast
  - primer designer / specificity checker
  - Primer3 primer design
  - Uses RefSeq annotation
    - exon boundaries
    - splice variants
    - SNPs
- MOLE-BLAST
  - Helps identify sources of 16S and other targeted sequences
  - BLAST followed by global multiple alignment
  - Clusters queries plus most similar database sequences
  - Identifies taxonomic units (neighbors)
  - Labels database sequences from type material for accurate ID

Protein Services

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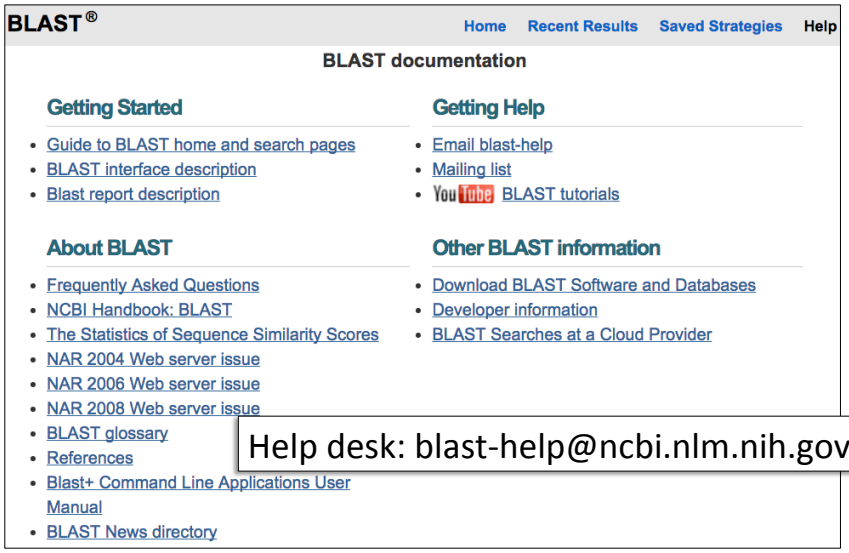
- COBALT – Constraint Based Alignment Tool
  - Protein global multiple alignment tool
  - Uses conserved domains to guide alignment
  - Extension to BLAST search
- SmartBLAST – Rapid protein identification tool
  - Uses fast k-mer search
  - Identifies closest match in reference organism database
  - Produces multiple alignment and protein tree
  - Prototype for on-the-fly protein similarity (BLink)

BLAST Help



Help desk: blast-help@ncbi.nlm.nih.gov

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More Help Links

- Help Manual: <ncbi>/books/NBK3831/
- Learn: <ncbi>/home/learn.shtml
- Factsheets: <ftp>/pub/factsheets/
- NCBI YouTube: <youtube>/ncbinlm
- NCBI Helpdesks
  - General: info@ncbi.nlm.nih.gov
  - BLAST: blast-help@ncbi.nlm.nih.gov

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Web Demonstrations

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- Basic BLAST
  - blastp, MLH1
    - COBALT extension
- Genome BLAST
  - blastn, macaque CDC20
    - Formatting options
    - Genome context
- SRA BLAST
  - Melanoma gene expression
- Primer BLAST
  - BRCA1 Exon Primers
- SmartBLAST
  - Finding yeast MLH1