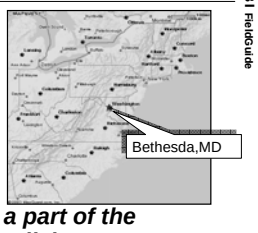
NCBI FieldGuide

NCBI Molecular Biology Resources

April 4, 2006

NCBI FieldGuide

The National Center for Biotechnology Information



Created in 1988 as a part of the National Library of Medicine at NIH

- Establish public databases
- Research in computational biology
- Develop software tools for sequence analysis
- Disseminate biomedical information

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Web Access: www.ncbi.nlm.nih.gov

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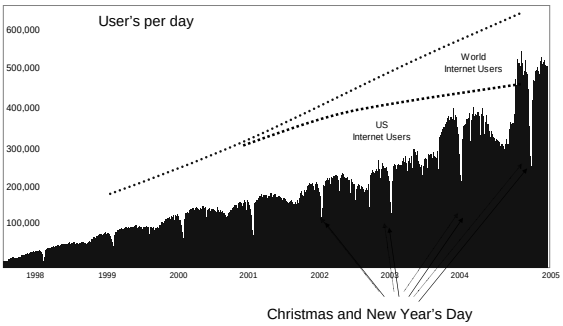
Website Traffic



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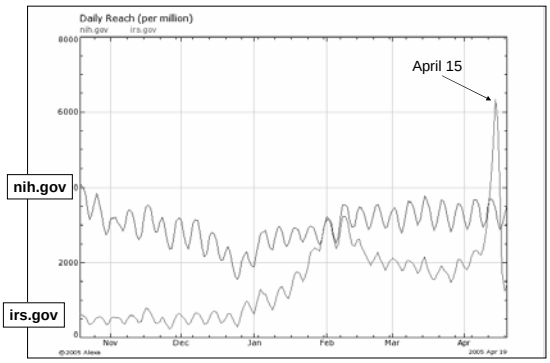
Government Web Traffic



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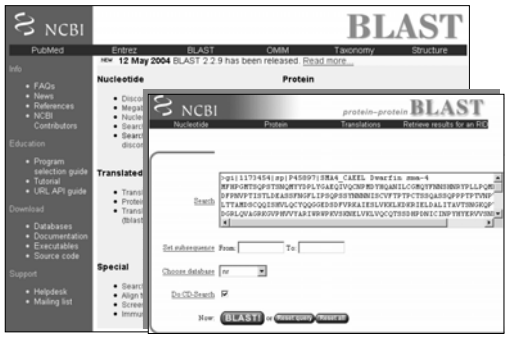
The Entrez System: Text Searches

BLAST: Sequence Similarity Searches



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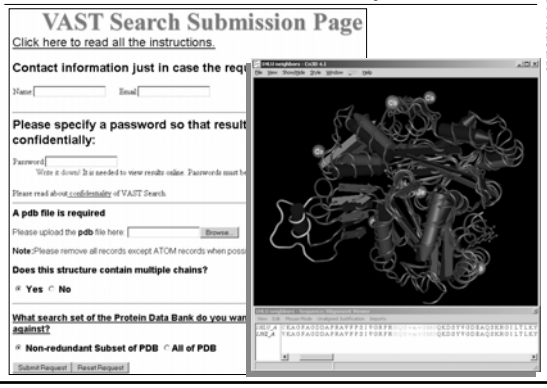
VAST: Structure Similarity Searches



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Types of Databases

- Primary Databases
  - Original submissions by experimentalists
  - Content controlled by the submitter
    - Examples: GenBank, SNP, GEO
- Derivative Databases
  - Built from primary data
  - Content controlled by third party (NCBI)
    - Examples: Refseq, TPA, RefSNP, UniGene, NCBI Protein, Structure, Conserved Domain

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What is GenBank?

NCBI's Primary Sequence Database

- Nucleotide only sequence database
- Archival in nature
  - Historical
  - Reflective of submitter point of view (subjective)
  - Redundant
- GenBank Data
  - Direct submissions (traditional records)
  - Batch submissions (EST, GSS, STS)
  - ftp accounts (genome data)
- Three collaborating databases
  - GenBank
  - DNA Database of Japan (DDBJ)
  - European Molecular Biology Laboratory (EMBL) Database

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GenBank: NCBI's Primary Sequence Database

Release 152	February 2006
54,584,635	Records (non-WGS)
59,750,386,305	Nucleotides (non-WGS)
63,183,065,091	Nucleotides (WGS)
122,933,451,396	Total

GenBank exceeds 100 GigaBases !

>140,000 Species

216 Gigabytes *908 files*

- full release every two months
- incremental and cumulative updates daily
- available only via ftp

ftp://ftp.ncbi.nih.gov/genbank/

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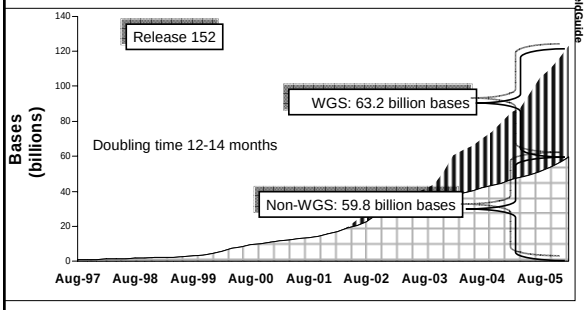
The Growth of GenBank



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Organization of GenBank: Traditional Divisions

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Records are divided into 17 Divisions.

- 12 Traditional
- 6 Bulk

Traditional Divisions:

- Direct Submissions (Sequin and BankIt)
- Accurate
- Well characterized

PRI Primate
PLN Plant and Fungal
BCT Bacterial and Archeal
INV Invertebrate
ROD Rodent
VRL Viral
VRT Other Vertebrate
MAM Mammalian
PHG Phage
SYN Synthetic (cloning vectors)
ENV Environmental Samples
UNA Unannotated

Entrez query: `gbdiv_xxx[Properties]`

Organization of GenBank: Bulk Divisions

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Records are divided into 17 Divisions.

- 11 Traditional
- 6 Bulk

BULK Divisions:

- Batch Submission (Email and FTP)
- Inaccurate
- Poorly characterized

EST Expressed Sequence Tag
GSS Genome Survey Sequence
HTG High Throughput Genomic
STS Sequence Tagged Site
HTC High Throughput cDNA
PAT Patent

Entrez query: `gbdiv_xxx[Properties]`

EST Division: Expressed Sequence Tags

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`gbdiv_est[Properties]`

```
>IMAGE:275615 5' mRNA sequence
GACAGCATTCGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGG
TGGAGGTATCCAGCGTACTCCAAAGATTCAGGTTTACTCACGTCATCCAGCAGAGAATGGAAAGTCAA
TTCCTGAATTGCTATGTGTCTGGGTTTCATCCATCCGACATTGAAGTTGACTTACTGAAGAATGGAGA
GAATTGAAAAAGTGGAGCATTCAGACTTGTCTTTCAGCAAGGACTGGTCTTTCTATCTCTTGTACTAC
TGAATTCACCCCCACTGAAAAAGATGAGTATGCCTGCCGTGTTGAACCATGTNGACTTTGTCACAGNC
AAGTTNAGTTTAAGTGGGNATCGAGACATGTAAGGCAGGCATCATGGGAGGTTTTGAAGNATGCCGCN
TTGGATTGGGATGAATTCCAAATTTCTGGTTTGCTTGNTTTTTTAATATTGGATATGCTTTTG
```

```
>IMAGE:275615 3', mRNA sequence
NNTCAAGTTTTATGATTTATTTAACTTGTGGAACAAAAATAAACCAGATTAACCACAACCATGCCTTA
TTATCAAATGTATAAGANGTAAATATGAATCTTATATGACAAAATGTTTCATTCATTATAACAAATTT
AATAATCCTGTCAATNATATTTCTAAATTTTCCCCCAAATTCTAAGCAGAGTATGTAAATTGGAAGTT
CTTATGCACGCTTAACTATCTTAACAAGCTTTGAGTGCAAGAGATTGANGAGTTCAAATCTGACCAAG
GTTGATGTTGGATAAGAGAATTCTCTGCTCCCCACCTCTANGTTGCCAGCCCTC
```

make cDNA library ⇨ **80-100,000 unique cDNA clones in library**

What is UniGene?

A gene-oriented view of sequence entries

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- MegaBlast based automated sequence clustering
- Now informed by genome hits *New!*
- Nonredundant set of gene oriented clusters
- Each cluster a unique gene
- Information on tissue types and map locations
- Includes well-characterized genes and novel ESTs
- Useful for gene discovery and selection of mapping reagents

EST hits: *Arabidopsis* serine protease mRNA

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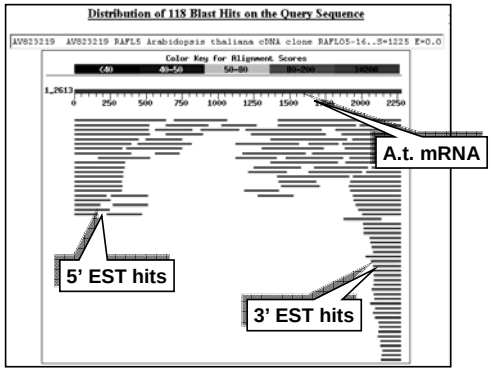

UniGene *March 2006*

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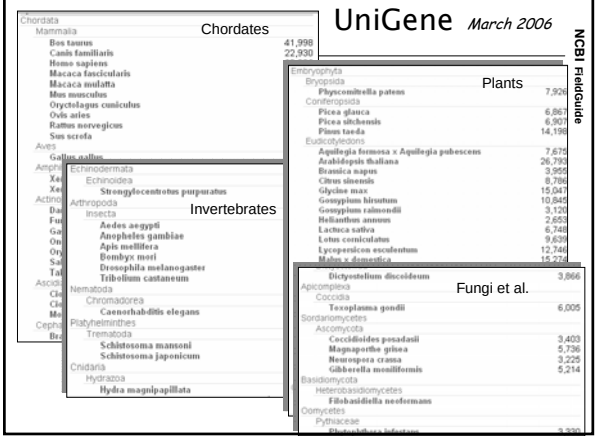

Genome Sequencing – HTG, GSS,(WGS)

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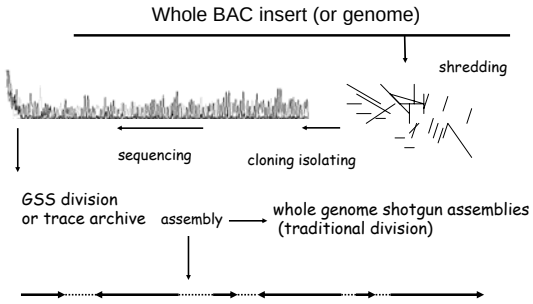

HTG Division: Rice Draft Sequences

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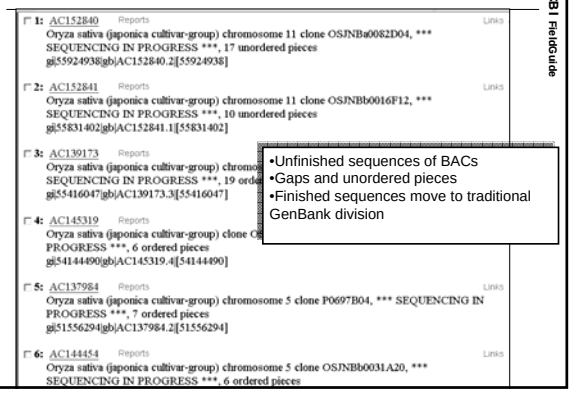

- Unfinished sequences of BACs
- Gaps and unordered pieces
- Finished sequences move to traditional GenBank division

Whole Genome Shotgun Projects

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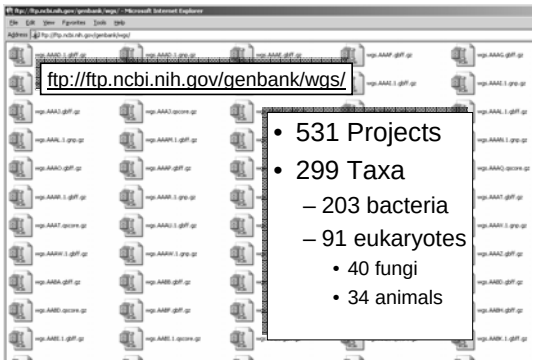

- 531 Projects
- 299 Taxa
  - 203 bacteria
  - 91 eukaryotes
    - 40 fungi
    - 34 animals

Mammalian WGS

NCBI FieldGuide

- Nine banded armadillo
- Hedgehog
- African elephant
- Dog
- Eurasian shrew
- Cow
- Domestic rabbit
- Guinea pig
- Mouse
- Rat
- Human
- Chimpanzee
- Gray short-tailed opossum

Accessing WGS Records: Entrez

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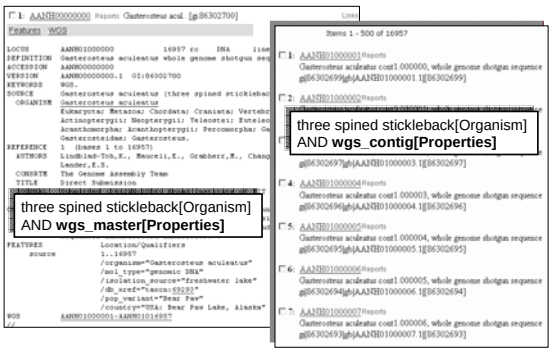

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Derivative Sequence Databases

GenPept
RefSeq

NCBI Protein: Derivative Database

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GenPept	**5,223,665**
RefSeq	**2,429,517**
Third Party Annotation	**4,495**
Swiss Prot	**207,038**
PIR	**39,615**
PRF	**12,079**
PDB	**76,628**
Total	**7,993,037**
BLAST nr total	**3,310,354**

GenPept: GenBank CDS translations

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```
FEATURES             Location/Qualifiers
     source          1..2484
                     /organism="Homo sapiens"
                     /mol_type="mRNA"
                     /db_xref="taxon:9606"
                     /chromosome="3"
                     /map="3p22-p23"
     gene            1..2484
                     /gene="M
     CDS             22..2292
                     /gene="M
                     /note="homolog of S. cerevisiae PMS1 (Swiss-Prot Accession
                     Number P14242), S. cerevisiae MLH1 (GenBank Accession
                     Number U07187), E. coli MUTL (Swiss-Prot Accession Number
                     P23367), Salmonella typhimurium MUTL (Swiss-Prot Accession
                     Number P14161) and Streptococcus pneumoniae (Swiss-Prot
                     Accession Number P14160)"
                     /codon_start=1
                     /product="DNA mismatch repair protein homolog"
                     /protein_id="AAC50285.1"
                     /db_xref="GI:463989"
                     /translation="MSFVAGVIRRLDETVVNRIAAGEVIQRPANAIKEMIENCLDAKS
                     TSIQVIVKEGGLKLIQIQDNGTGIRKEDLDIVCERFTTSKLQSFEDLASISTYGFRGE
                     ALASISHVAHVTITTKTADGKCAYRASYSDGKLKAPPKPCAGNQGTQITVEDLFYNIA
                     TRRKALKNPSEEYGKILEVVGRYSVHNAGISFSVKKQGETVADVRTLPNASTVDNIRS
```

```
>gi|463989|gb|AAC50285.1| DNA mismatch repair prote...
MSFVAGVIRRLDETVVNRIAAGEVIQRPANAIKEMIENCLDAKSTSIQVIV...
EDLDIVCERFTTSKLQSFEDLASISTYGFRGEALASISHVAHVTITTKTAD...
```

Redundant Proteins

NCBI FieldGuide

```
>gi|463989|gb|AAC50285.1| DNA mismatch repair prote...
MSFVAGVIRRLDETVVNRIAAGEVIQRPANAIKEMIENCLDAKSTSIQVIV...
EDLDIVCERFTTSKLQSFEDLASISTYGFRGEALASISHVAHVTITTKTAD...
```

```
>gi|13905126|gb|AAH06850.1| MutL protein homolog 1 ...
MSFVAGVIRRLDETVVNRIAAGEVIQRPANAIKEMIENCLDAKSTSIQVIV...
EDLDIVCERFTTSKLQSFEDLASISTYGFRGEALASISHVAHVTITTKTAD...
```

```
>gi|1079787|gb|AAA82079.1| DNA mismatch repair prot...
MSFVAGVIRRLDETVVNRIAAGEVIQRPANAIKEMIENCLDAKSTSIQVIV...
EDLDIVCERFTTSKLQSFEDLASISTYGFRGEALASISHVAHVTITTKTAD...
```

GenPept

```
>gi|4557757|ref|NP_000240.1| MutL protein homolog 1...
MSFVAGVIRRLDETVVNRIAAGEVIQRPANAIKEMIENCLDAKSTSIQVIV...
EDLDIVCERFTTSKLQSFEDLASISTYGFRGEALASISHVAHVTITTKTAD...
```

NCBI RefSeq

```
>gi|730028|sp|P40692|MLH1_HUMAN DNA mismatch repair...
MSFVAGVIRRLDETVVNRIAAGEVIQRPANAIKEMIENCLDAKSTSIQVIV...
EDLDIVCERFTTSKLQSFEDLASISTYGFRGEALASISHVAHVTITTKTAD...
```

Swiss-Prot

```
>gi|741682|prf||2007430A DNA mismatch repair protei...
MSFVAGVIRRLDETVVNRIAAGEVIQRPANAIKEMIENCLDAKSTSIQVIV...
EDLDIVCERFTTSKLQSFEDLASISTYGFRGEALASISHVAHVTITTKTAD...
```

PRF

Primary vs. Derivative Sequence Databases

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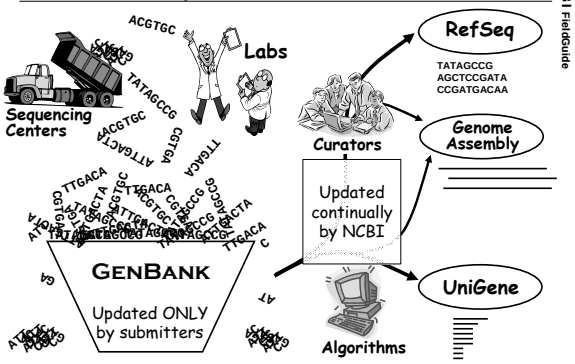

RefSeq: NCBI's Derivative Sequence Database

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- **Curated transcripts and proteins [NM_, NP_]**
  - reviewed
  - human, mouse, rat, cow, fruit fly, zebrafish, arabidopsis *C.elegans*
- **Model transcripts and proteins [XM_, XP_]**
- **Assembled Genomic Regions [NT_, NW_]**
  - draft human genome
  - mouse genome
- **Chromosome records [NC_]**
  - microbial
  - organelle

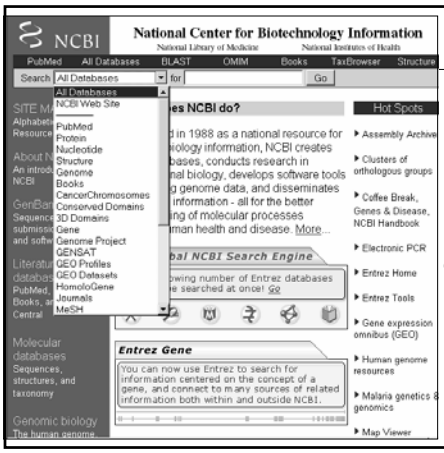
`srcdb_refseq[Properties]`

ftp://ftp.ncbi.nih.gov/refseq/release/

Other NCBI Databases

NCBI FieldGuide

•dbSNP:	nucleotide polymorphism
•Geo:	Gene Expression Omnibus microarray and other expression data
•Gene:	gene records Unifies LocusLink and Microbial Genomes
•Structure:	imported structures (PDB) Cn3D viewer, NCBI curation
•CDD:	conserved domain database Protein families (COGs and KOGs) Single domains (PFAM, SMART, CD)



WWW Access

Entrez & BLAST

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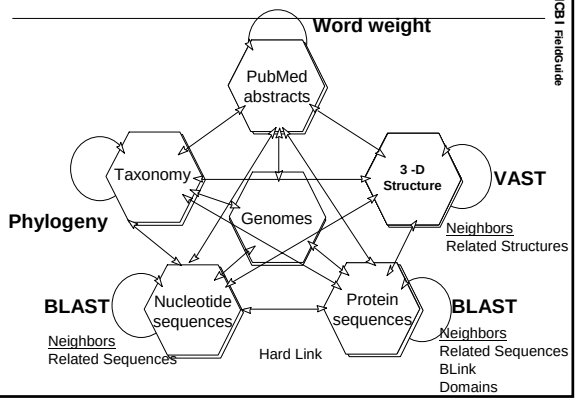
Entrez: Database Integration



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Database Searching with Entrez

- ◆Using limits and field restriction to find human MutL homolog
- ◆Linking and neighboring with MutL
- ◆Mapping SNPs onto structure and the genome

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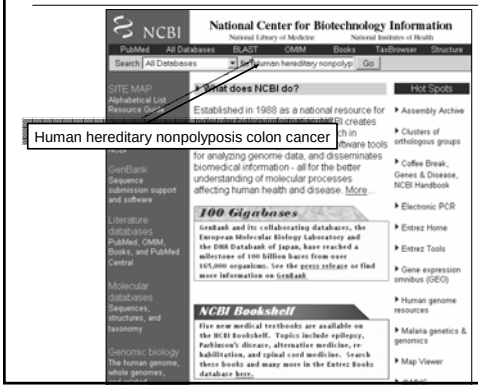
Global NCBI (Entrez) Search



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Global Entrez Search Results

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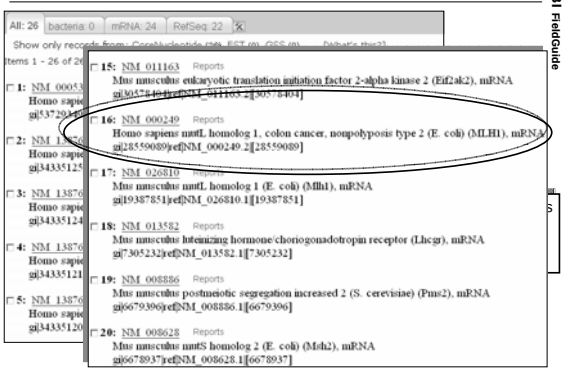
Nucleotide Sequences



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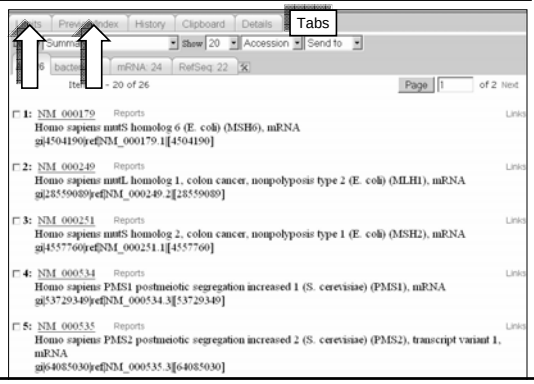
Advanced Search Options



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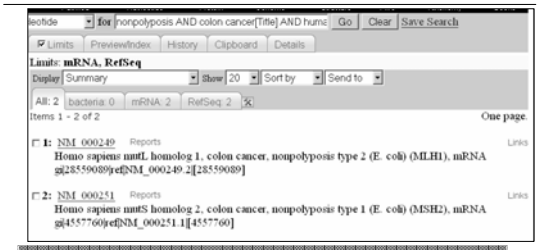
More Precise Nucleotides Search



nonpolyposis[All Fields] AND colon cancer[Title] AND human[Organism] AND biomol_mrna[Properties] AND srcdb_refseq[Properites]

NCBI FieldGuide

Useful Field Restrictions

[Title]: Definition line in GenBank / GenPept format shown in Summary format

glyceraldehyde 3 phosphate dehydrogenase[Title]

[Organism]: NCBI's taxonomy. Organizing system for molecular databases

mouse[organism]; green plants[organism]; Streptomyces coelicolor[organism]

[Properties]: molecule type, location, database source

biomol_mrna[properties]; biomol_genomic[properties]; gene_in_mitochondrion[properties]; srcdb pdb[properties]

[Filter]: subsets of data, Entrez links

all[filter]; nucleotide mapview[filter]; nucleotide omim[filter]

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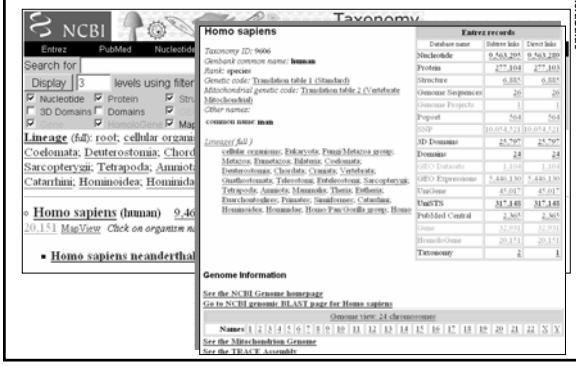
Organism Field: NCBI's Taxonomy



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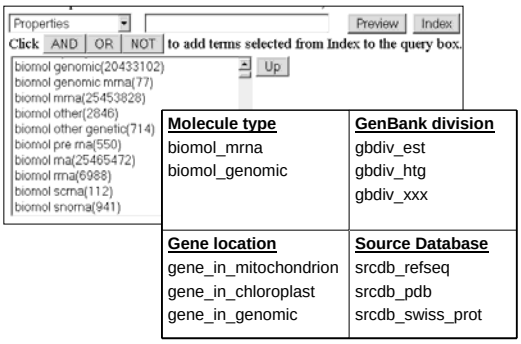
Useful Properties Field Terms



Molecule type	GenBank division
biomol_mrna	gbdiv_est
biomol_genomic	gbdiv_htg
	gbdiv_xxx
Gene location	**Source Database**
gene_in_mitochondrion	srcdb_refseq
gene_in_chloroplast	srcdb_pdb
gene_in_genomic	srcdb_swiss_prot

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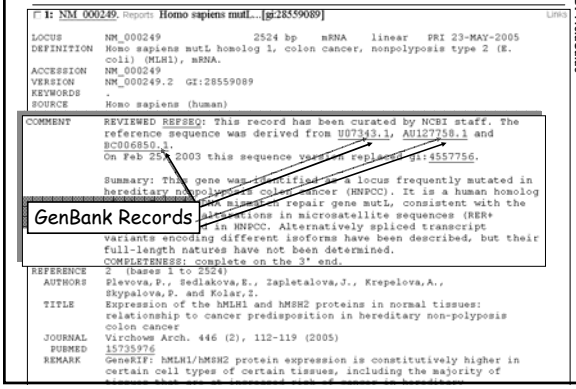
Human MutL RefSeq



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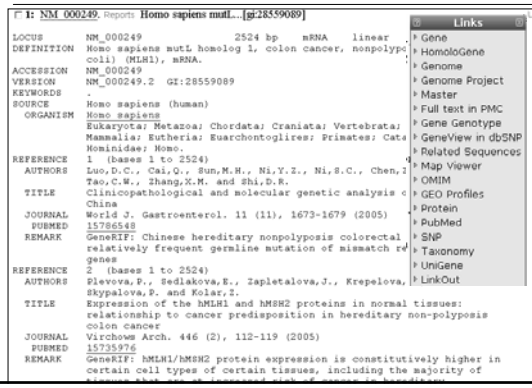
NM_000249: Links



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Literature Links

OMIM

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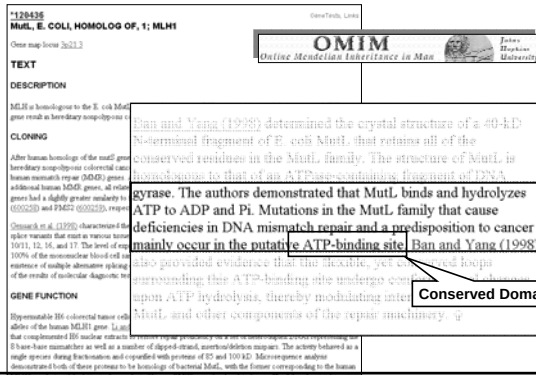
OMIM: Human Disease Genes



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Sequence Links

Finding Homologs and Structures

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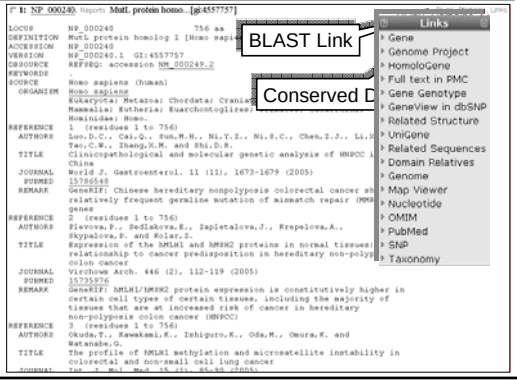
Protein Link



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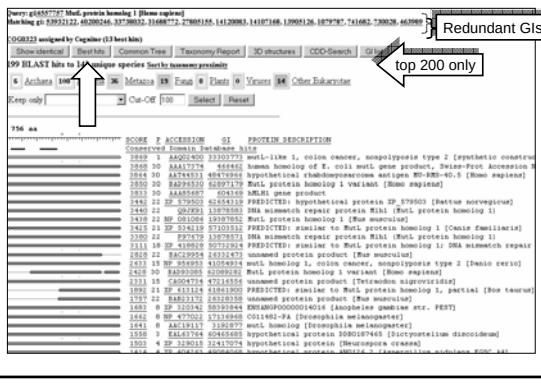
BLink: BLAST Link



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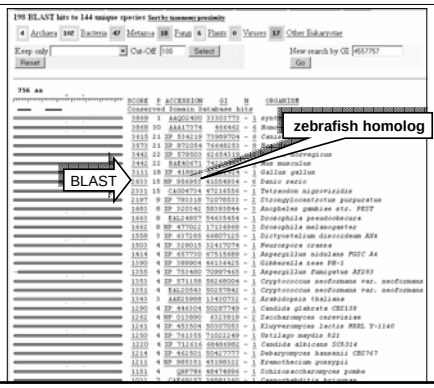
BLink: non-redundant relatives



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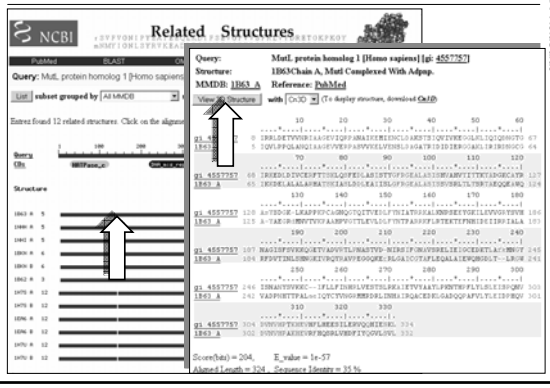
Related Structures



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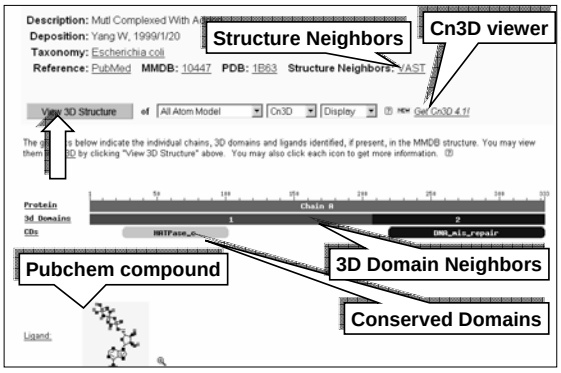
E. coli MutL Structure



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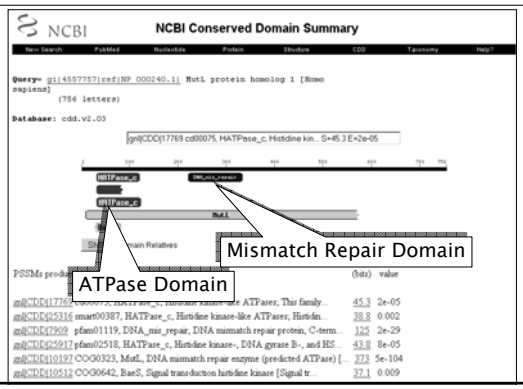
MLH1 Domain Structure: CDD



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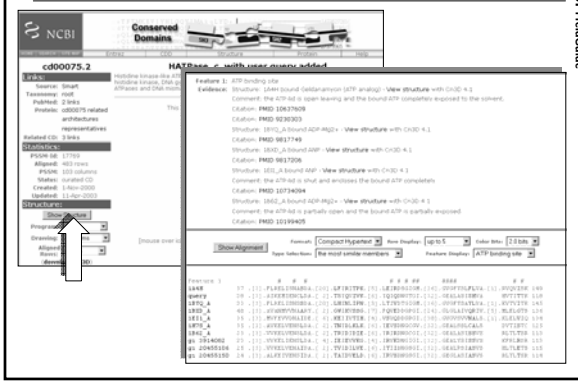
MLH1: ATPase Domain



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Mapping Polymorphisms onto Structure

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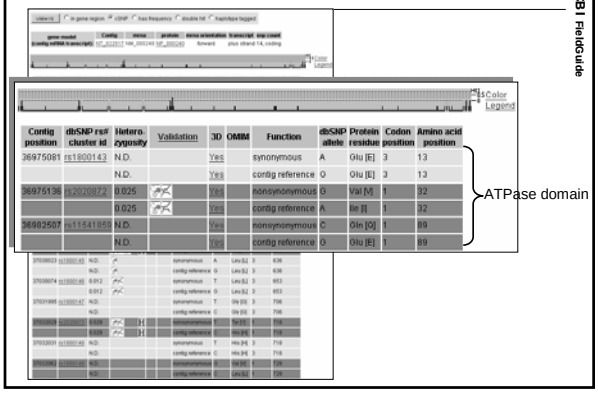
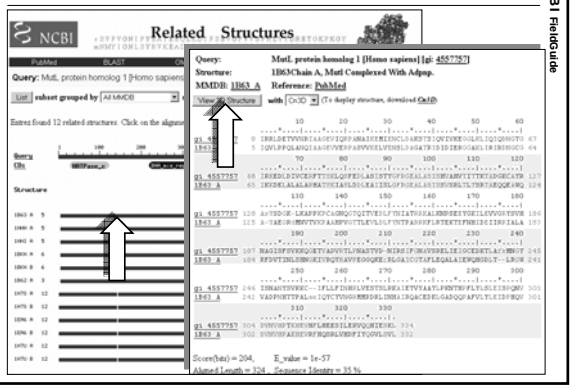
GeneView: Variations Human MLH1



NCBI FieldGuide

Related Structures



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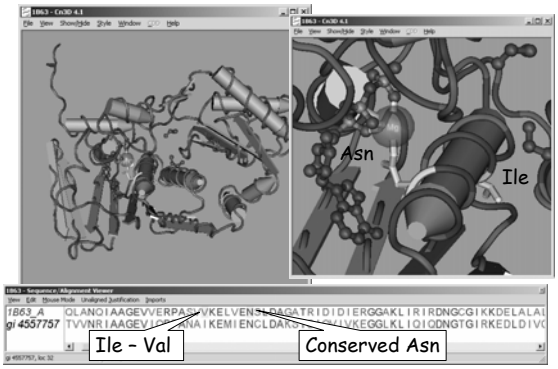
Mapping Variation Onto Structure

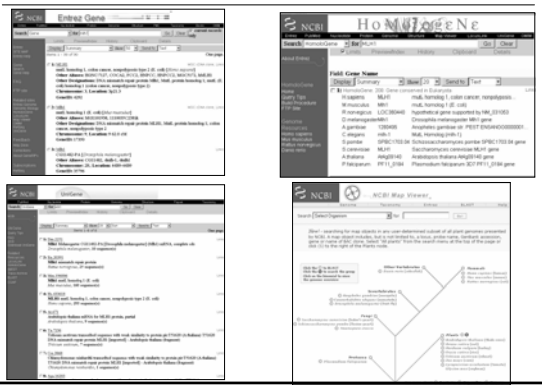


NCBI FieldGuide

Genome Resources

NCBI FieldGuide

Higher Genome Resources



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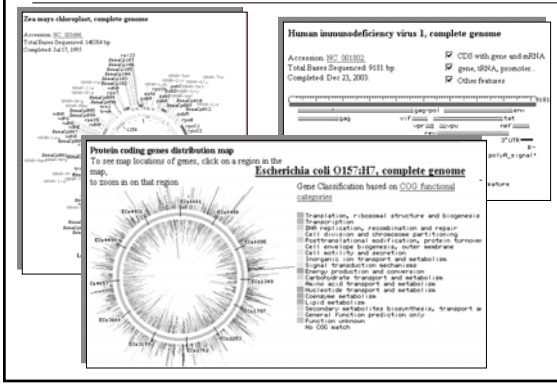
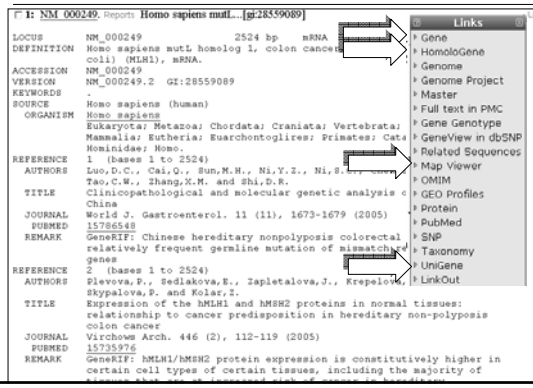
Microbial Genomes



NCBI FieldGuide

NM_000249: Genome Links



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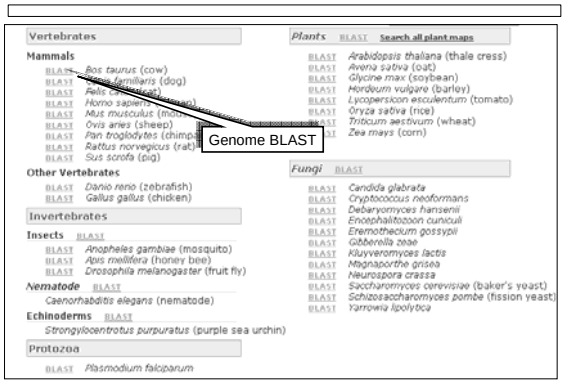
The Map Viewer



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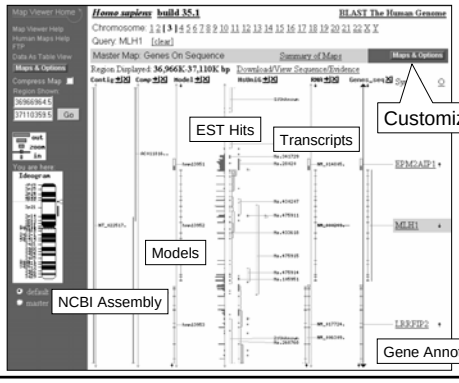
Map Viewer: Human MLH1



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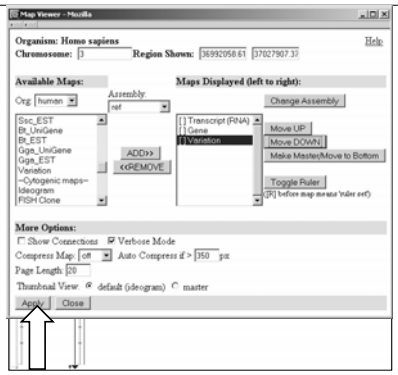
Maps and Options



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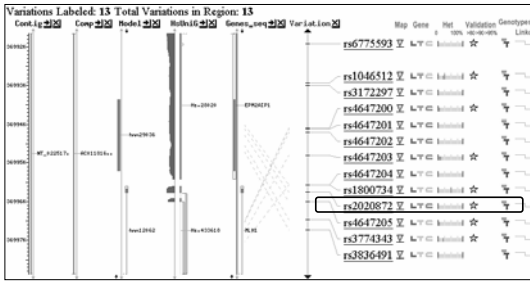
Mapped Variations



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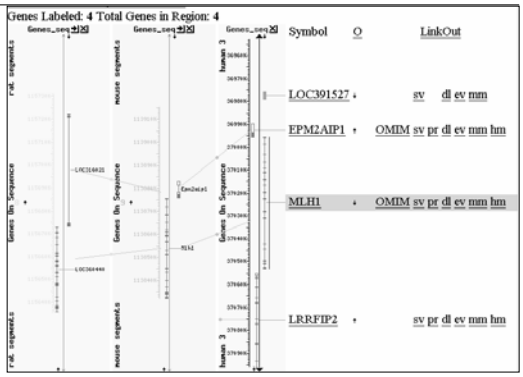
Synteny: Mammalian Genomes



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The New Homologene

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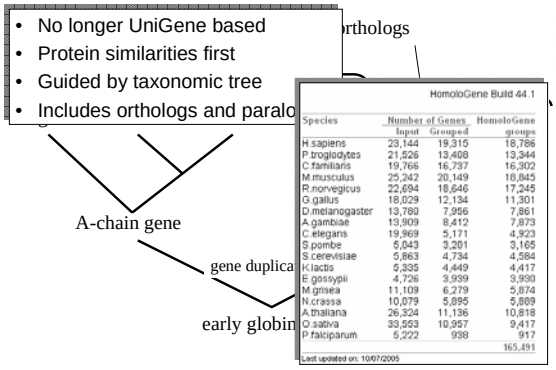
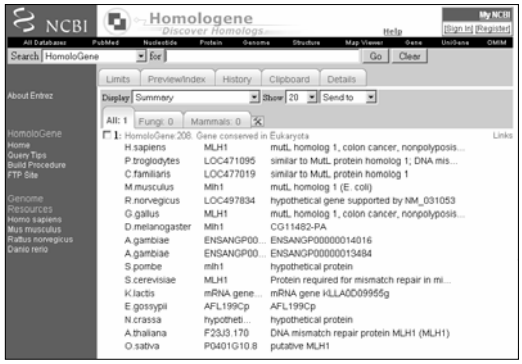
- No longer UniGene based
- Protein similarities first
- Guided by taxonomic tree
- Includes orthologs and paralo



The New Homologene

NCBI FieldGuide



Rice Homolog

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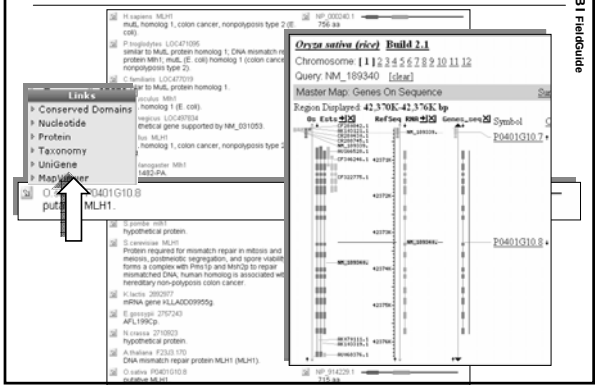

The Gene Database

NCBI FieldGuide

- Gene Centered Information
- Unifies LocusLink and microbial Genomes
- 1.5 million records for 3,038 taxa

Human	32,924	Chicken	18, 444
Chimpanzee	24,158	Zebrafish	37, 715
Mouse	47,988	Sea Urchin	20,636
Rat	28,584	Mosquito	13,897
Dog	20,153	Fruit Fly	20,763
Cow	23, 647	*C. elegans*	20,846

Genes MLH1: One Stop Shopping

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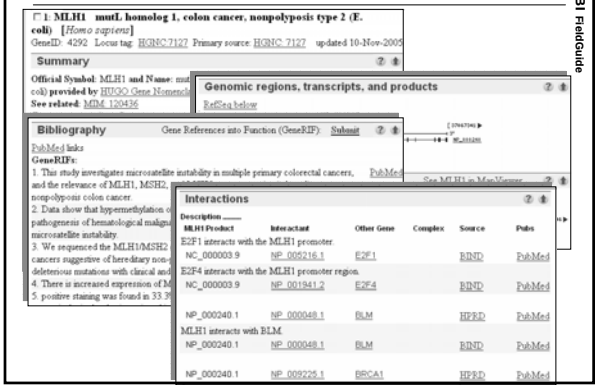

Genes MLH1: One Stop Shopping (cont.)

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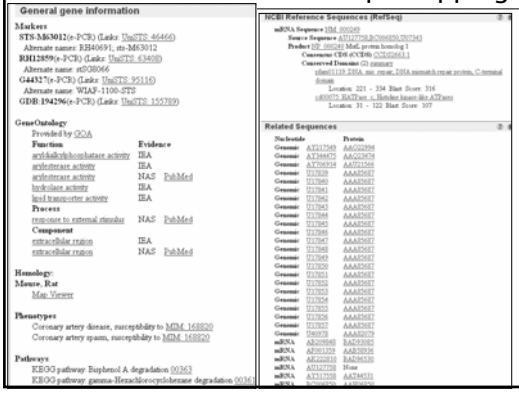
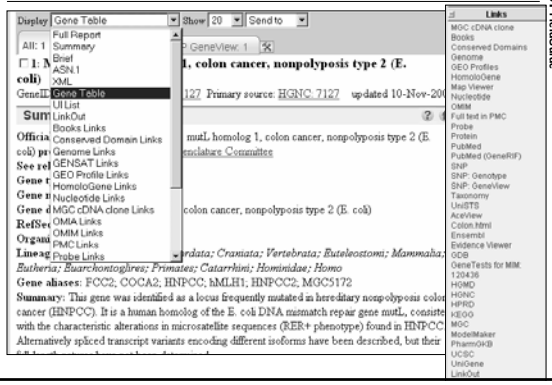


Genes: Display Options and Links



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Taxonomy Link

The Tax Browser

NCBI's Taxonomy

NCBI FieldGuide

Sequence Similarity Searching

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What BLAST tells you

- BLAST reports surprising alignments
  - Different than chance
- Assumptions
  - Random sequences
  - Constant composition
- Conclusions
  - Surprising similarities imply **evolutionary homology**

Evolutionary Homology: descent from a common ancestor
Does not always imply similar function

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Basic Local Alignment Search Tool

- Widely used similarity search tool
- Heuristic approach based on Smith Waterman algorithm
- Finds best **local** alignments
- Provides statistical **significance**
- All combinations (DNA/Protein) query and database.
  - DNA vs DNA
  - DNA translation vs Protein
  - Protein vs Protein
  - Protein vs DNA translation
  - DNA translation vs DNA translation
- www, standalone, and network clients

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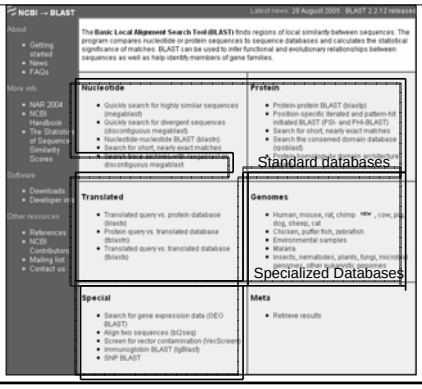
The BLAST homepage



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Finding Zebrafish mlh1

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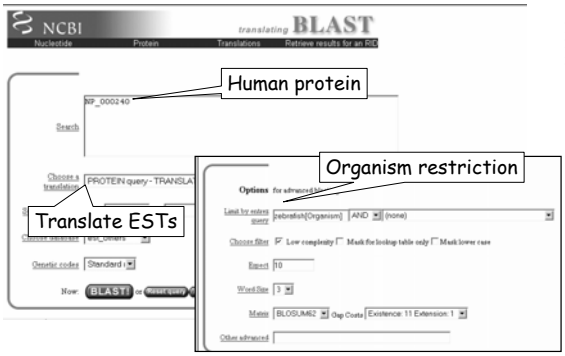

BLAST Results Zebrafish ESTs

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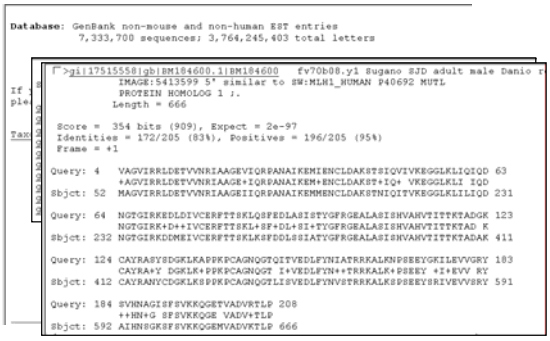

Service Addresses

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•***General Help***	`info@ncbi.nlm.nih.gov`
•***BLAST***	`blast-help@ncbi.nlm.nih.gov`