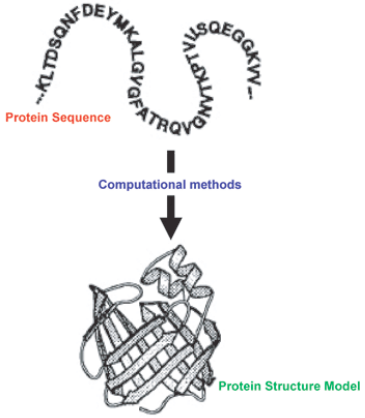
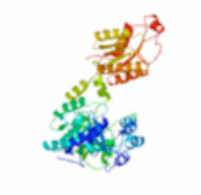
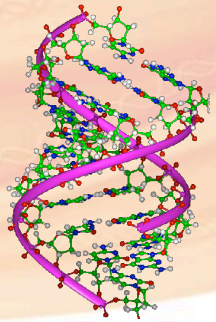




Bioinformatics Computational Methods 1 - BIOL 6308



October 22nd 2013

http://155.33.203.128/cleslin/home/teaching6308F2013.php

Last Time

- Why Study Genomes
  - **What About Other Genomic Features**
  - **And now the Genomes…**
- Other Genomic Features
  - Regulatory proteins
    - TF
  - Regulatory regions
    - TFBS
- Consensus
- Genomes
- Gene Ontology
  - Structure of GO
  - How to use GO

NCBI

The National Institutes of Health

National Center for Biotechnology Information NCBI

- Created by Public Law 100-607 in 1988 as part of National Library of Medicine at NIH to:
  - Create automated systems for knowledge about molecular biology, biochemistry, and genetics
  - Perform research into advanced methods of analyzing and interpreting molecular biology data
  - Enable biotechnology researchers and medical care personnel to use the systems and methods developed
- **NCBI is an important scientific portal**

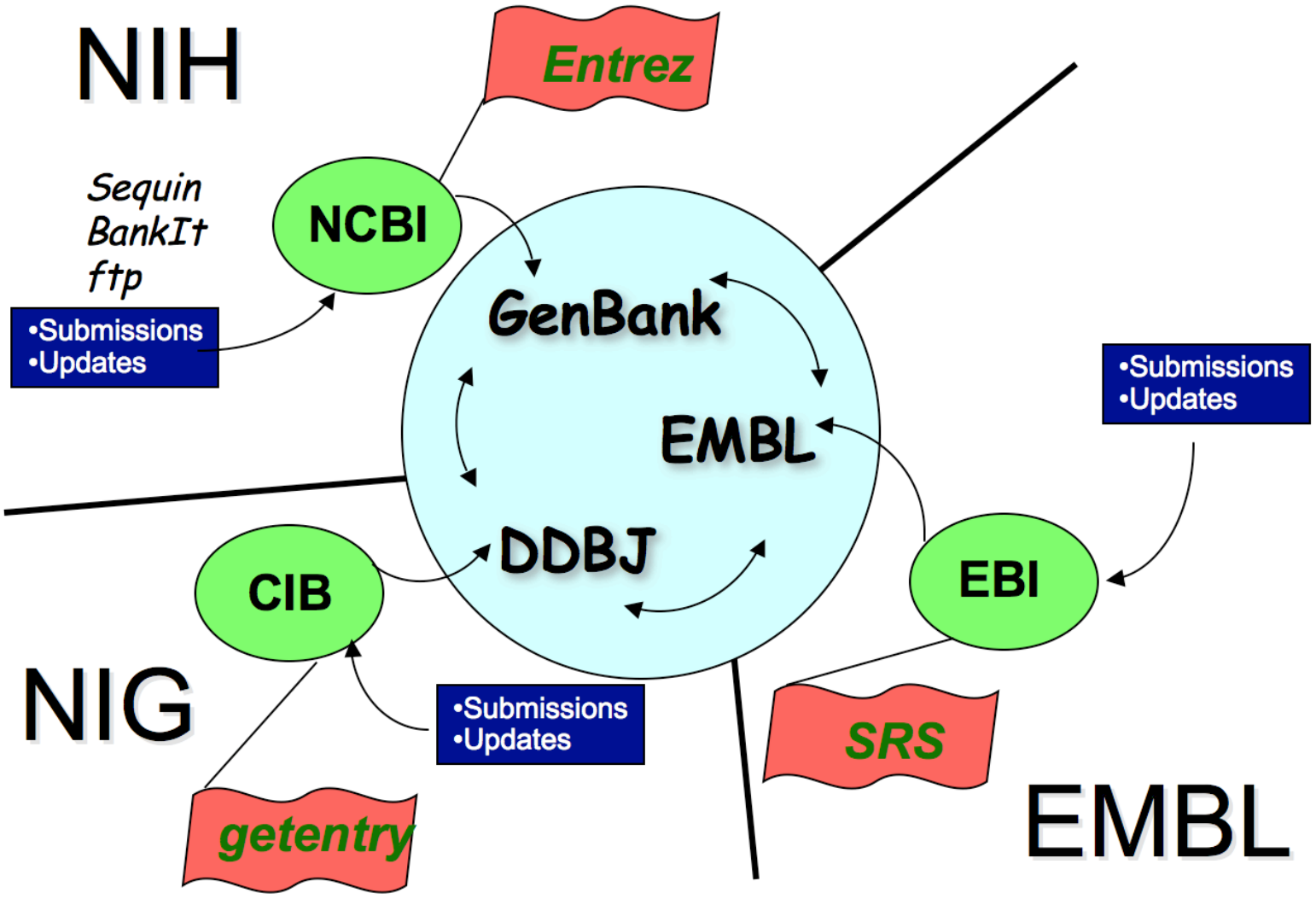
NCBI has a Facebook Page!!!

http://www.facebook.com/ncbi.nlm

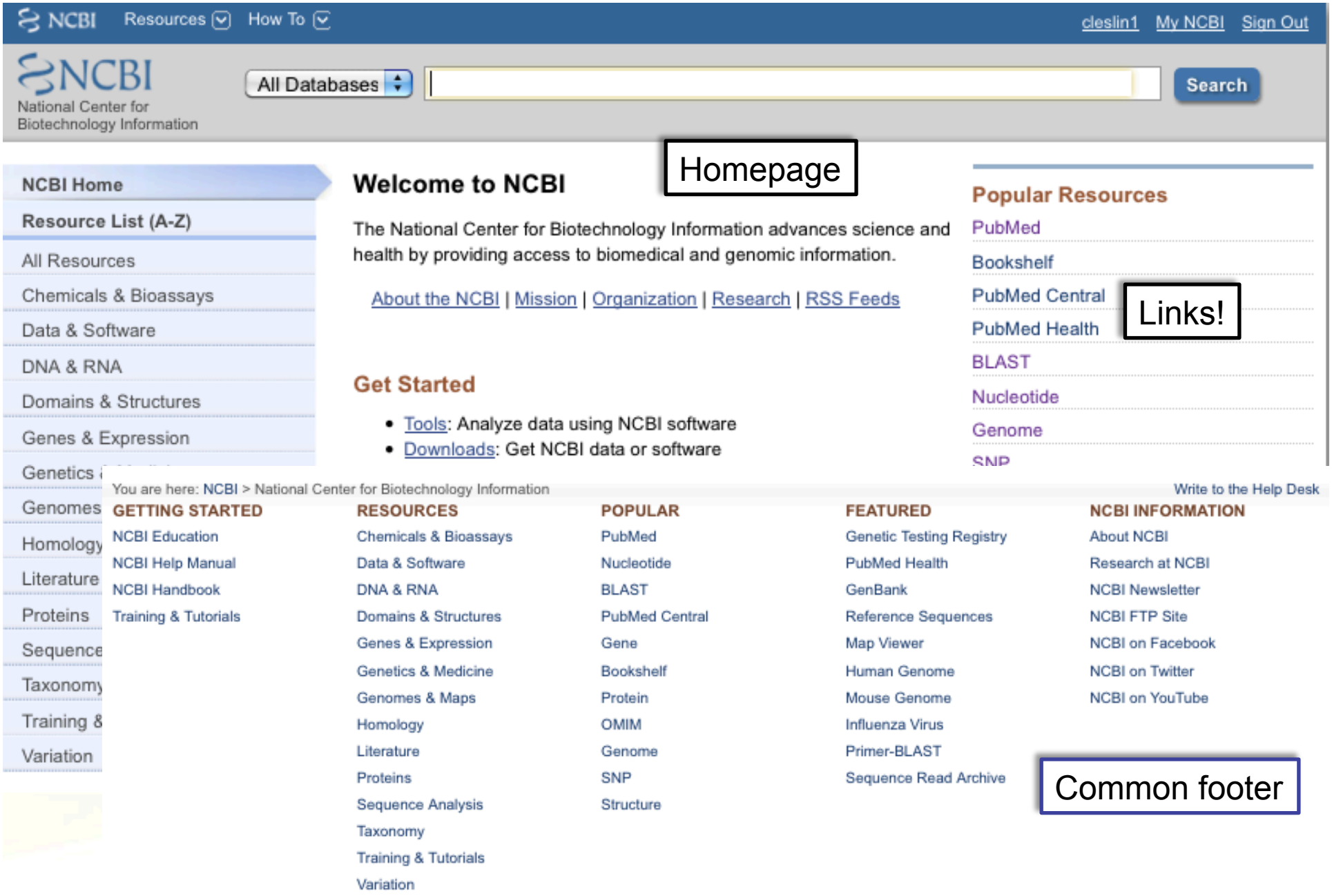
The International Nucleotide Sequence Database Collaboration

- Sequence data shared by INSD
- INSD developed and maintained collaboratively between DDBJ, EMBL, and GenBank for over 18 years
- International Advisory Committee
  - The INSDC advisory board
  - Made up of members of each of the databases advisory bodies
  - Members of committee unanimously endorsed the existing data-sharing policy of the three databases that make up the INSDC
  - http://www.insdc.org/

The International Nucleotide Sequence Database Collaboration



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

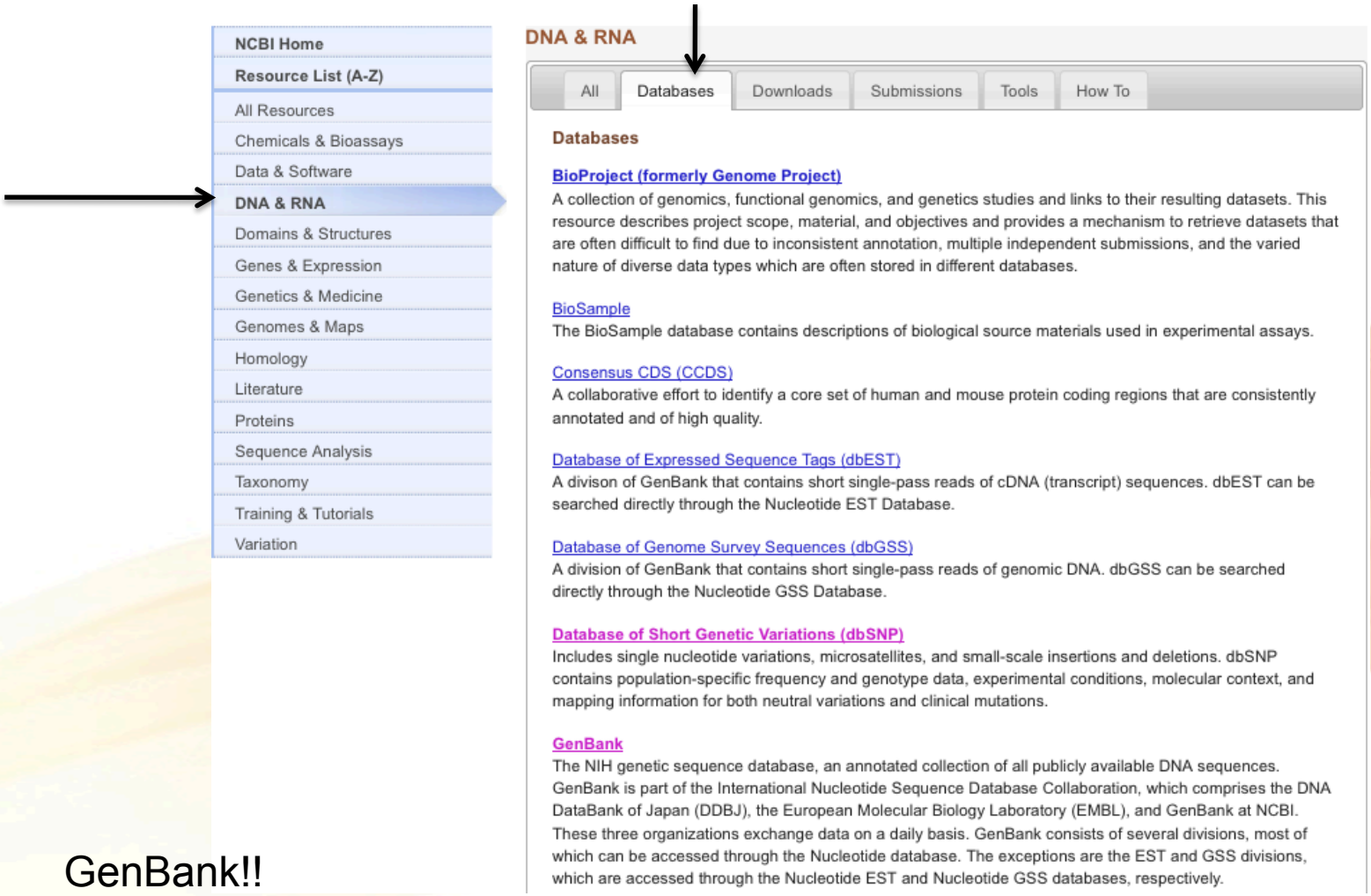


NCBI Databases and Services

- GenBank primary sequence database
- Free public access to biomedical literature
  - PubMed free Medline (3.5 million searches per day)
  - PubMed Central full text online access
- Entrez integrated molecular and literature databases
- BLAST highest volume sequence search service
  - 1997 – 38,000 searches per day
  - Now ~ 200 K searches per day
- Software and databases for download

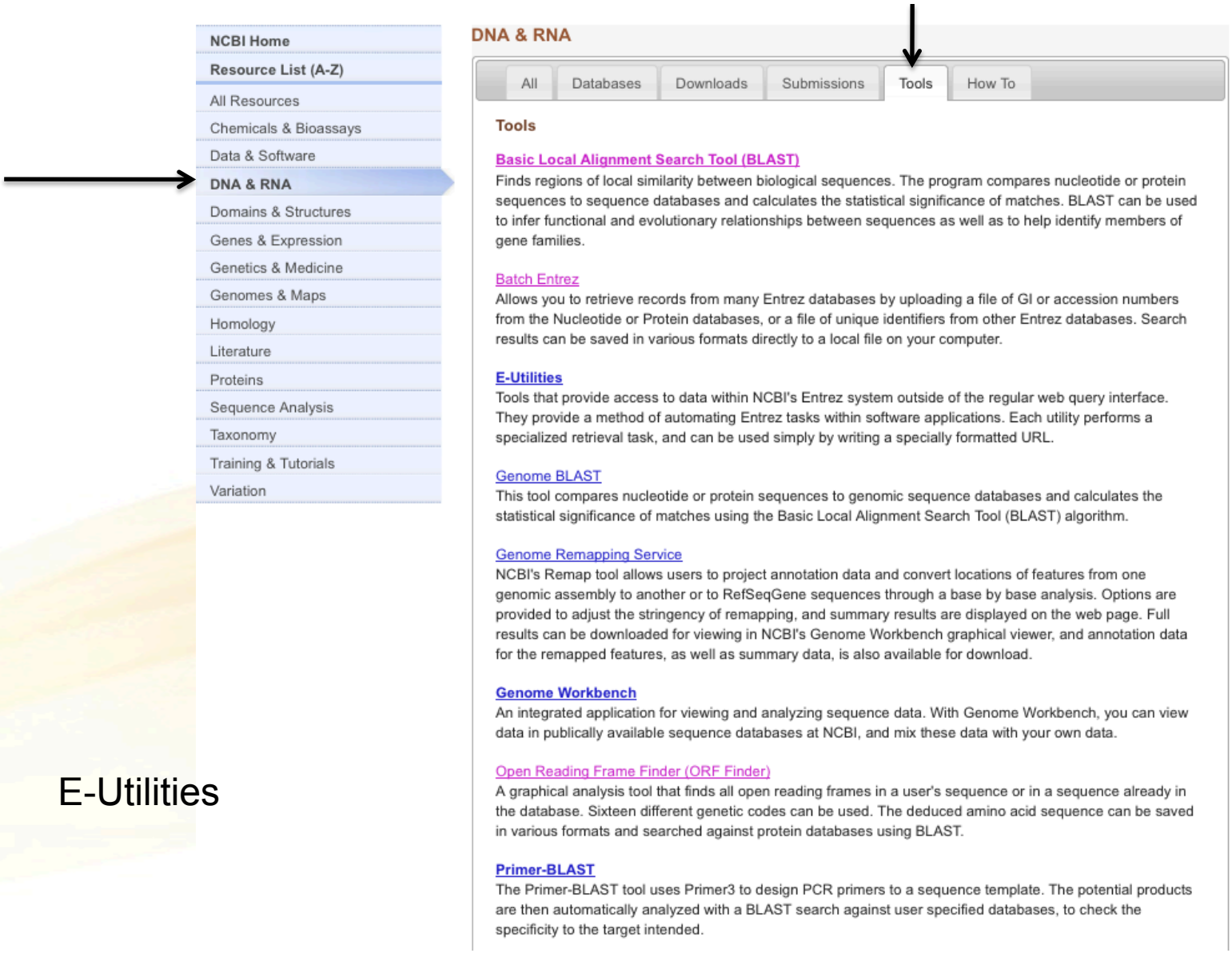
http://duncan.hull.name/2010/07/27/pubmed-20-million/

http://www.ncbi.nlm.nih.gov/guide/all/



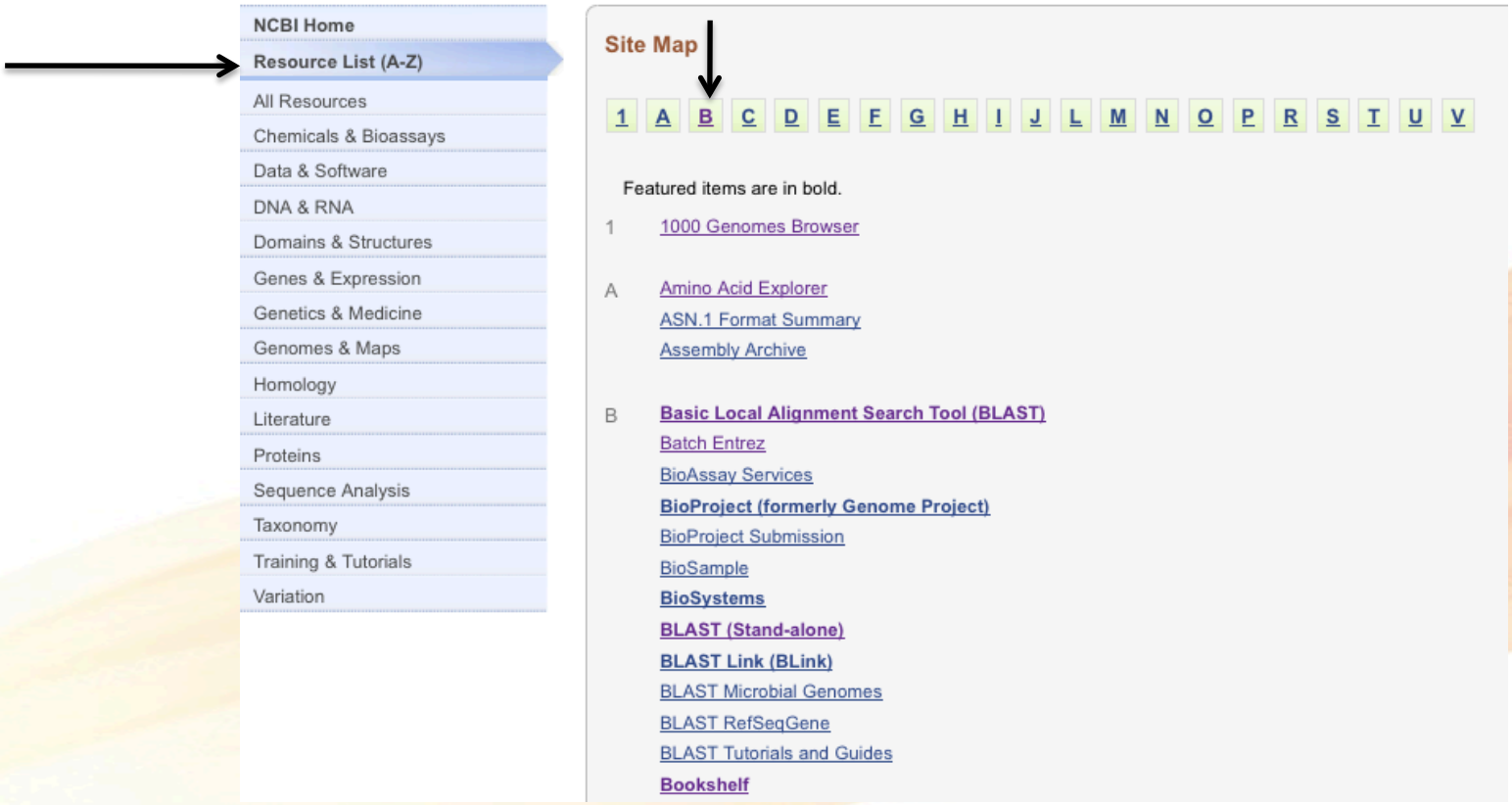
GenBank!!

http://www.ncbi.nlm.nih.gov/guide/all/



E-Utilities

http://www.ncbi.nlm.nih.gov/guide/all/

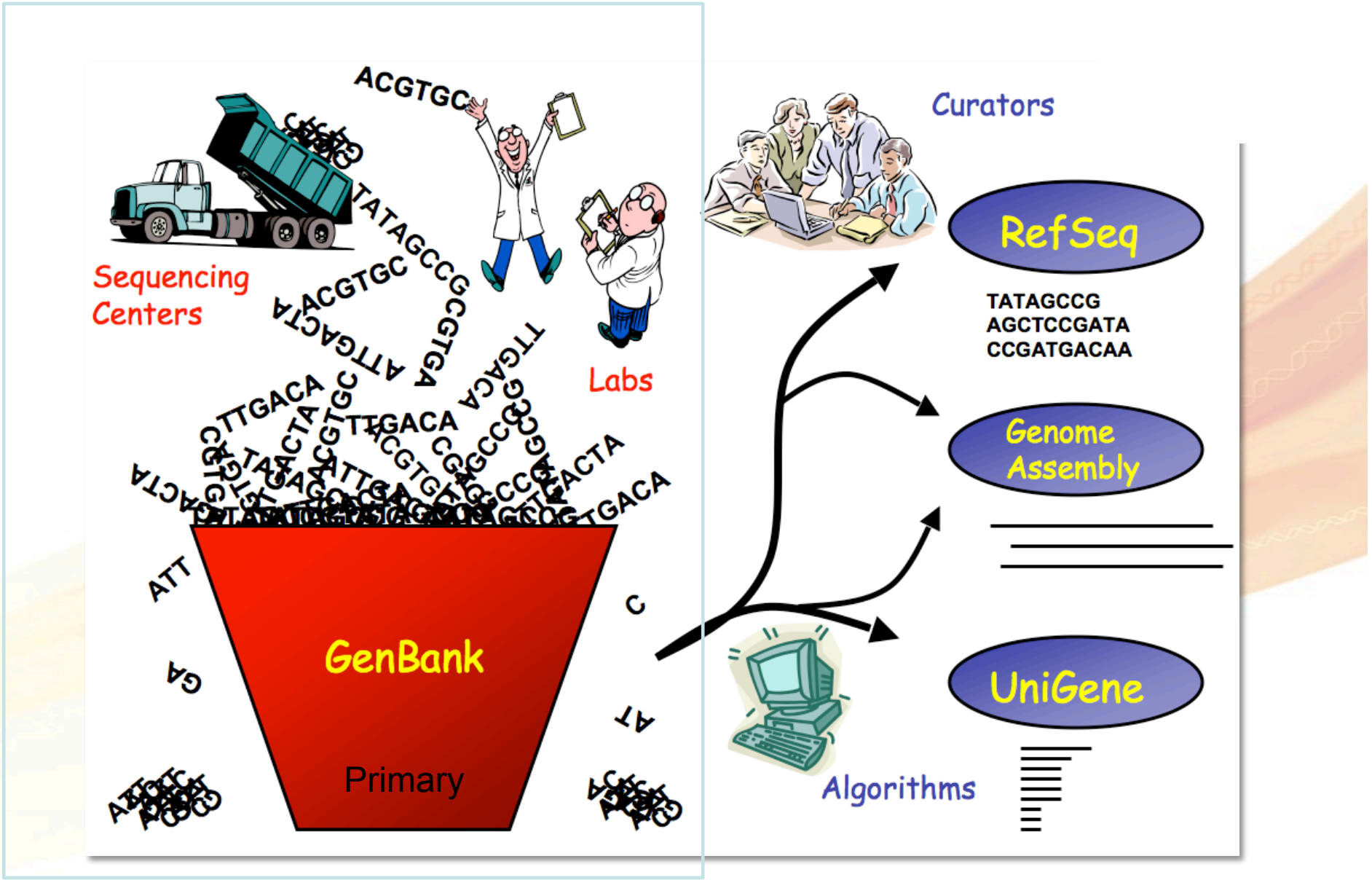


Batch Entrez

Types of Databases at NCBI

- **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
    - RefSNP
    - UniGene
    - NCBI protein, structure and Conserved Domain

Primary Vs Derivative Databases

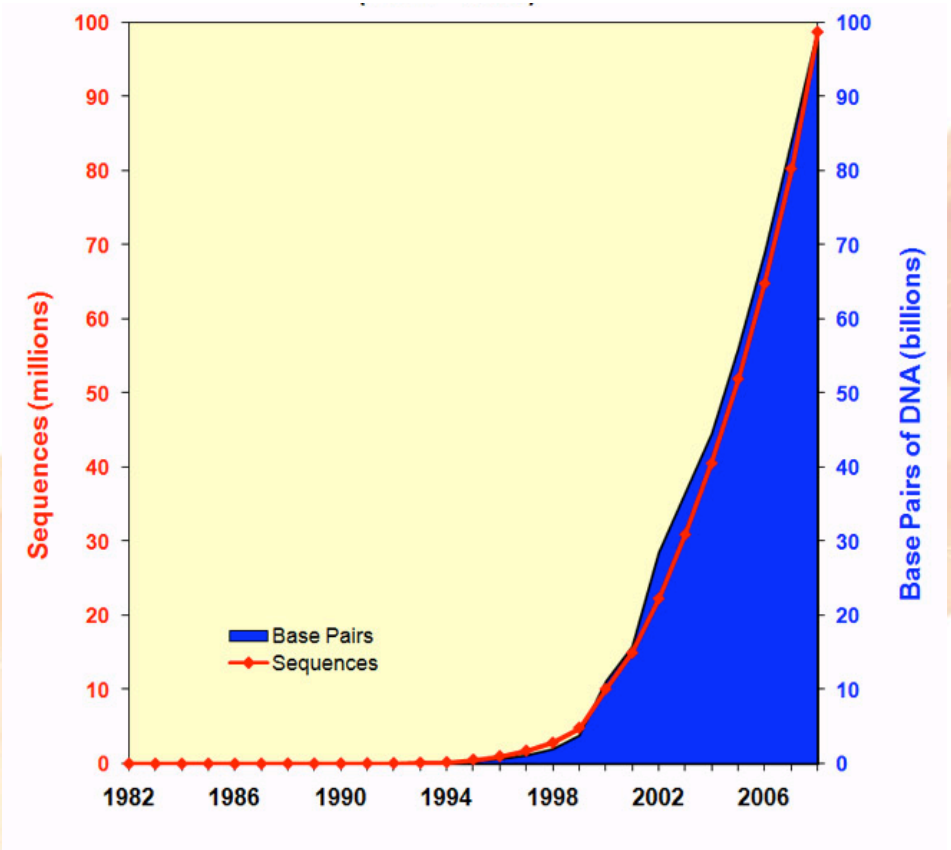


What is GenBank?

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

Last Year 2012 - Growth of GenBank

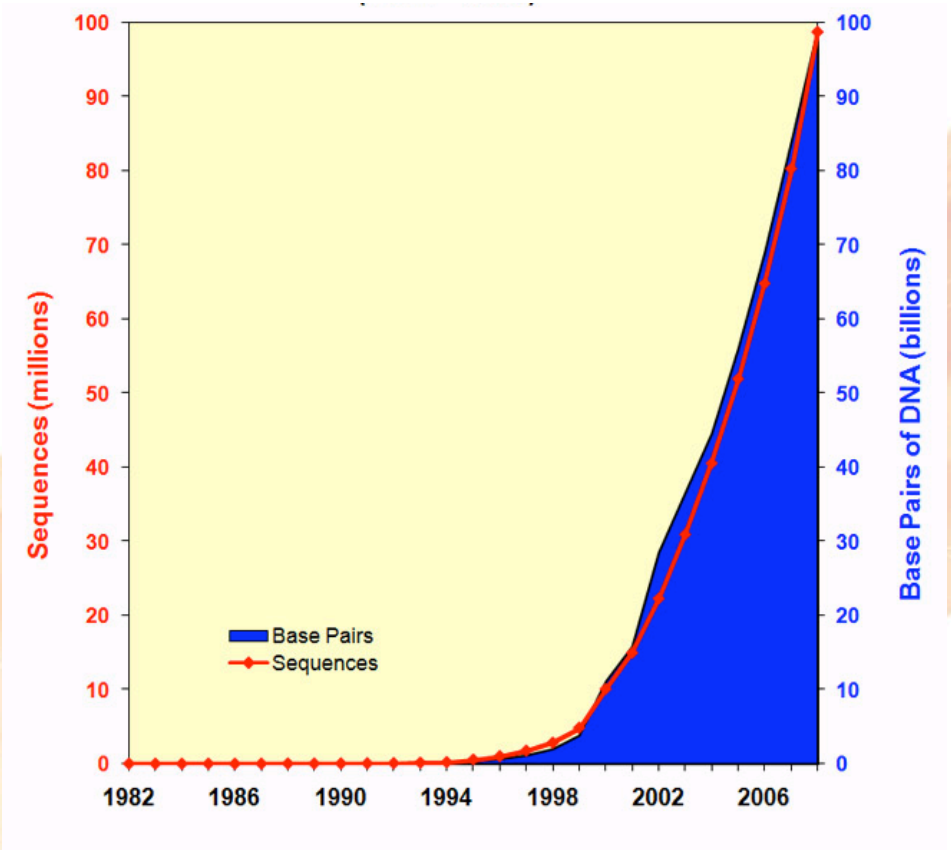
- Get the GBREL.TXT
- Genetic Sequence Data Bank **October 15 2012** NCBI GenBank Flat File Release **192.0** Distribution Release Notes
- 157,889,737 loci
- **145,430,961,262 bases**
- **From 157,889,737** reported sequences



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

2013 - Growth of GenBank

- Get the GBREL.TXT
- Genetic Sequence Data Bank **October 19 2013** NCBI GenBank Flat File Release **197.0** Distribution Release Notes
- 167,295,840 loci
- **154,192,921,011 bases**
- **From 167,295,840 reported sequences**



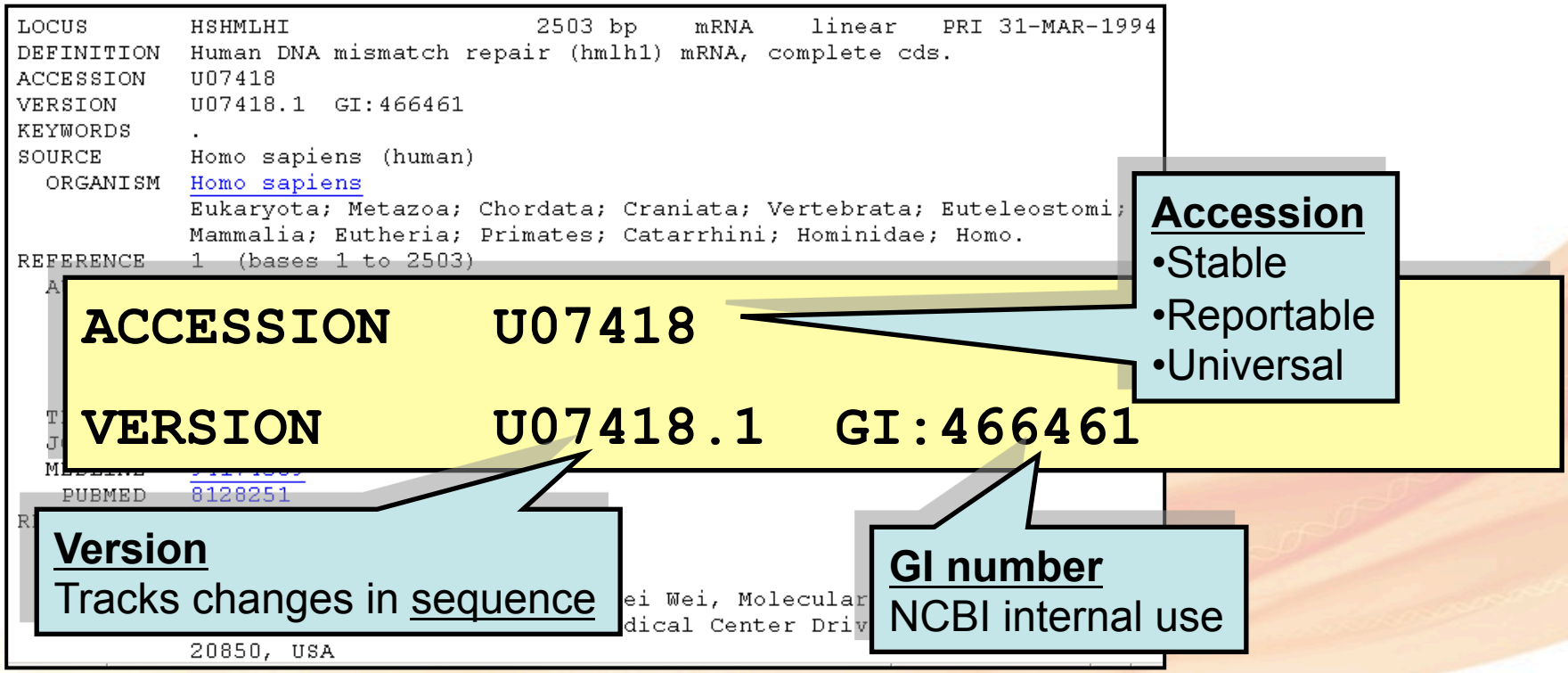
ftp://ftp.ncbi.nih.gov/genbank/gbrel.txt

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

Traditional GenBank Record

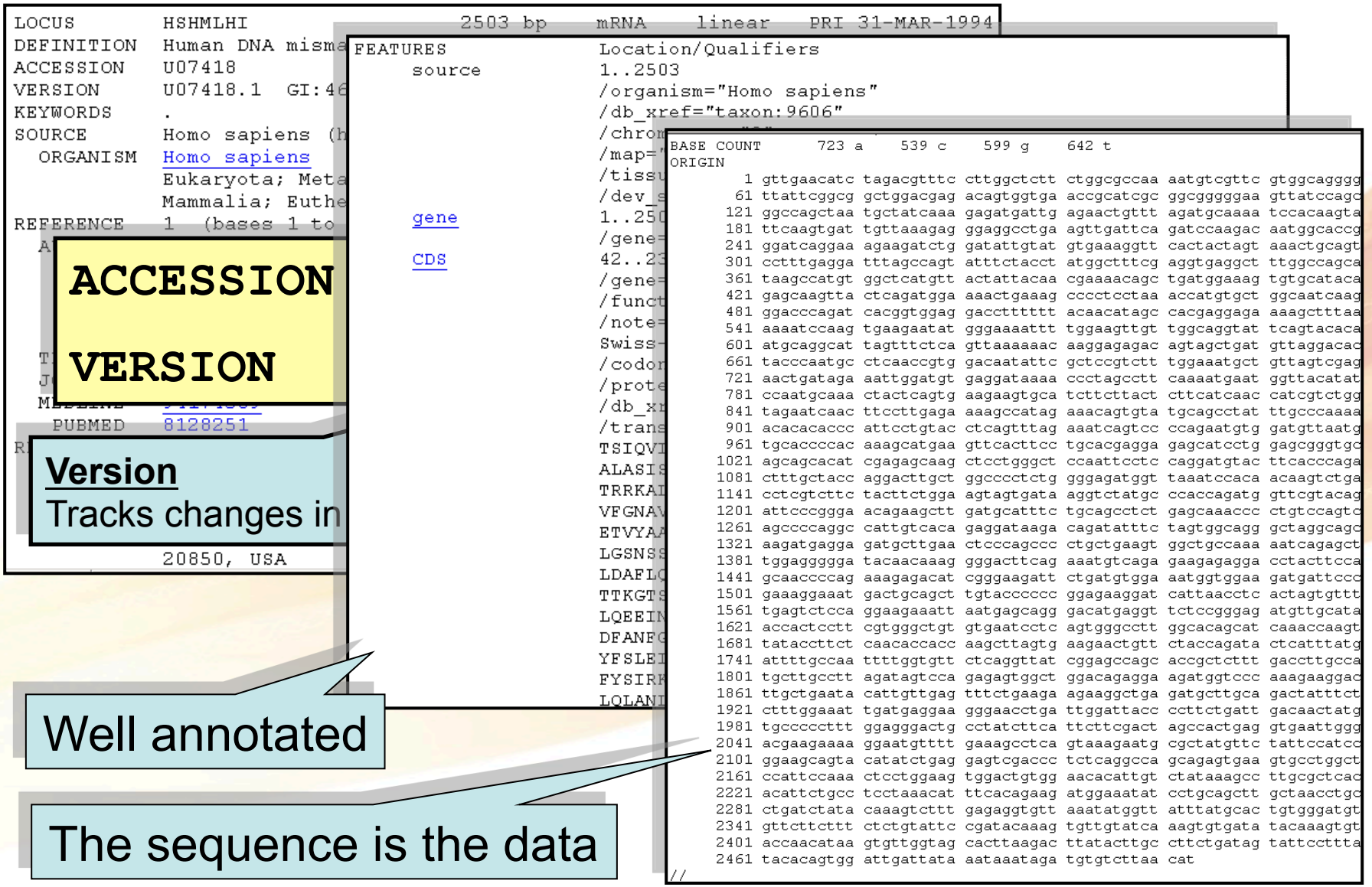
```
LOCUS       HSHMLHI                 2503 bp    mRNA    linear   PRI 31-MAR-1994
DEFINITION  Human DNA mismatch repair (hmlh1) mRNA, complete cds.
ACCESSION   U07418
VERSION     U07418.1  GI:466461
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 2503)
  AUTHORS   Papadopoulos,N., Nicolaides,N.C., Wei,Y.F., Ruben,S.M.,
            Carter,K.C., Rosen,C.A., Haseltine,W.H., Fleischmann,R.D.,
            Fraser,C.M., Adams,M.D., Venter,J.C., Hamilton,S.R., Petersen,G.M.,
            Watson,P., Lynch,H.T., Peltomaki,P., Mecklin,J.P., Chapelle,A.D.,
            Kinzler,K.W. and Vogelstein,B.
  TITLE     Mutation of a mutL homolog in hereditary colon cancer
  JOURNAL   Science 263 (5153), 1625-1629 (1994)
  MEDLINE   94174309
   PUBMED   8128251
REFERENCE   2  (bases 1 to 2503)
  AUTHORS   Wei,Y.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAR-1994) Ying-Fei Wei, Molecular Biology, Human
            Genome Sciences, Inc., 9620 Medical Center Drive, Rockville, MD
            20850, USA
```

Traditional GenBank Record



Traditional GenBank Record

Traditional GenBank Record



What is an Accession Number?

- Label used to identify a sequence
- String of letters and/or numbers that corresponds to a molecular sequence
- Examples (all for retinol-binding protein, RBP4):

X02775	GenBank genomic DNA sequence	**DNA**
NT_030059	Genomic contig	
Rs7079946	dbSNP (single nucleotide polymorphism)	
N91759.1	An expressed sequence tag (1 of 170)	**RNA**
NM_006744	RefSeq DNA sequence (from a transcript)	
NP_007635	RefSeq protein	**Protein**
AAC02945	GenBank protein	
Q28369	SwissProt protein	
1KT7	Protein Data Bank structure record	

- READ: http://www.ncbi.nlm.nih.gov/Sitemap/sequenceIDs.html

Organization of GenBank: Traditional (Taxonomy) Divisions

- Records are divided into 20 Divisions
  - 12 Traditional
  - 8 Bulk

- Traditional Divisions:
  - Direct Submissions
    - (Sequin and BankIt)
  - Accurate
  - Well characterized

1. PRI - primate sequences
2. ROD - rodent sequences
3. MAM - other mammalian sequences
4. VRT - other vertebrate sequences
5. INV - invertebrate sequences
6. PLN - plant, fungal, and algal sequences
7. BCT - bacterial sequences
8. VRL - viral sequences
9. PHG - bacteriophage sequences
10. SYN - synthetic sequences
11. UNA - unannotated sequences
12. ENV Environmental Samples

Entrez query: gbdiv_xxx[Properties]

Organization of GenBank: Bulk (Functional) Divisions

- Records are divided into 20 Divisions
  - 12 Traditional
  - 8 Bulk

1. EST - EST sequences (expressed sequence tags)
2. PAT - patent sequences
3. STS - STS sequences (sequence tagged sites)
4. GSS - GSS sequences (genome survey sequences)
5. HTG - HTG sequences (high throughput genomic sequences)
6. HTC - HTC sequences (high throughput cDNA sequences)
7. WGS - Whole Genome Shotgun (WGS) sequencing projects
8. TSA - Transcriptome Shotgun Assembly sequences

- Bulk Divisions:
  - collect sequences generated by a particular method
  - Batch Submissions
    - (Email and FTP)
  - Can be Inaccurate
  - Sometimes poorly characterized

Entrez query: gbdiv_xxx[Properties] e.g. gbdiv_**est**[Properties]

Growth of GenBank Divisions

Division	Description	Release 191 (8/2012)	Annual increase (%)[a]
Taxnomic divisons			
SYN	Synthetic	928 200 038	494.2%
PHG	Phages	84 079 451	34.4%
ENV	Environmental samples	3 374 433 548	32.1%
VRL	Viruses	1 429 464 786	21.1%
BCT	Bacteria	8 439 854 434	21.0%
PLN	Plants	5 481 470 133	15.6%
MAM	Other mammals	863 036 872	6.9%
VRT	Other vertebrates	2 886 594 595	6.7%
PRI	Primates	6 317 656 773	3.3%
UNA	Unannotated	127 803	1.5%
ROD	Rodents	4 435 106 948	0.9%
INV	Invertebrates	2 493 058 927	−1.7%
Functional divisions			
TSA	Transcriptome shotgun data	5 759 588 580	207.3%
WGS	Whole-genome shotgun data	308 196 411 905	47.9%
PAT	Patented sequences	12 118 622 726	8.6%
GSS	Genome survey sequences	21 947 780 105	5.7%
EST	Expressed sequence tags	40 888 051 100	4.8%
HTG	High-throughput genomic	24 359 210 558	0.1%
STS	Sequence tagged sites	636 262 446	0.1%
HTC	High-throughput cDNA	639 165 410	−3.5%
TOTAL	All GenBank sequences	451 278 177 138	33.1%

[a]Measured relative to Release 185 (8/2011).

Current - GenBank Divisions (**release 197**)

PRI	**(46)**	Primate
ROD	**(31)**	Rodent
PLN	**(63)**	Plant and Fungal
BCT	**(106)**	Bacteria/Archaea
INV	**(35)**	Invertebrate
VRT	**(31)**	Other Vertebrate
VRL	**(26)**	Viral
MAM	**(8)**	Mammalian
PHG	**(2)**	Phage
SYN	**(7)**	Synthetic
ENV	**(62)**	Envir. samples
UNA	**(1)**	Unannotated

EST	**(474)**	Expressed Sequence Tag
GSS	**(278)**	Genome Survey Sequence
HTG	**(141)**	High Throughput Genomic
PAT	**(195)**	Patent
STS	**(20)**	Sequence Tagged Site
HTC	**(141)**	HTC sequences (high throughput cDNA sequences)
TSA	**(145)**	Transcriptome Shotgun Assembly sequences

"Traditional"

(Taxonomy)

- Organized by taxonomy (sort of)
- Direct submissions (Sequin/Bankit)
- Accurate (~1 error per 10,000 bp)
- Well characterized

"Bulk"

(Functional)

- Organized by sequence type
- Batch submissions (ftp/email)
- Less accurate
- Poorly characterized

CON* (215) Contigs, virtual

ENV Division

- Environmental sample sequences
- The ENV division of GenBank accommodates sequences obtained via environmental sampling methods in which the source organism is unknown
- Many ENV sequences arise from metagenome samples derived from microbiota in various animal tissues, such as within the gut or skin, or from particular environments, such as freshwater sediment, hot springs or areas of mine drainage
- Environmental sample sequences are generally submitted for whole metagenomic shotgun sequencing experiments or surveys of sequences from targeted genes, like 16S rRNA

GenBank (Functional) Divisions

- **Expressed Sequence Tag**
  - 1st (single) pass single read cDNA
- **Genome Survey Sequence**
  - 1st (single) pass single read gDNA
- **High Throughput Genomic**
  - Incomplete sequences of genomic clones
- **High-Throughput cDNA/mRNA**
  - Similar to EST's but often contain more information
- **Sequence Tagged Site**
  - PCR-based mapping reagents
- **Patent**
  - Sequence which have corresponding patents
- **Transcriptome Shotgun Assembly Sequence Database**
  - **Archive of computationally assembled sequences from primary data such as ESTs, traces and Next Generation Sequencing Technologies**

*CON

CON - Contigs

- Do not contain any sequence data
- Instead, they utilize a CONTIG line type with a join() statement which describes how component sequences can be assembled to form the larger constructed sequence
- CON records are used to represent very long sequences, such as a eukaryotic chromosome, where the sequence is not complete but consists of several contig records with uncharacterized gaps between them
- Rather than listing the sequence itself, CON records contain assembly instructions involving the several component se- quences
- An example of such a CON record is CM000663 for human chromosome 1

STS Division

- Is a relatively short, easily PCR-amplified sequence (200 to 500 bp) which can be specifically amplified by PCR and detected in the presence of all other genomic sequences and whose location in the genome is mapped
- http://www.ncbi.nlm.nih.gov/genome/probe/doc/TechSTS.shtml

GenBank WGS Projects (The Other Functional Division)

WGS

Sequences from WGS sequencing projects are not represented in GenBank release, b/c all WGS project data are made available on a per-project basis

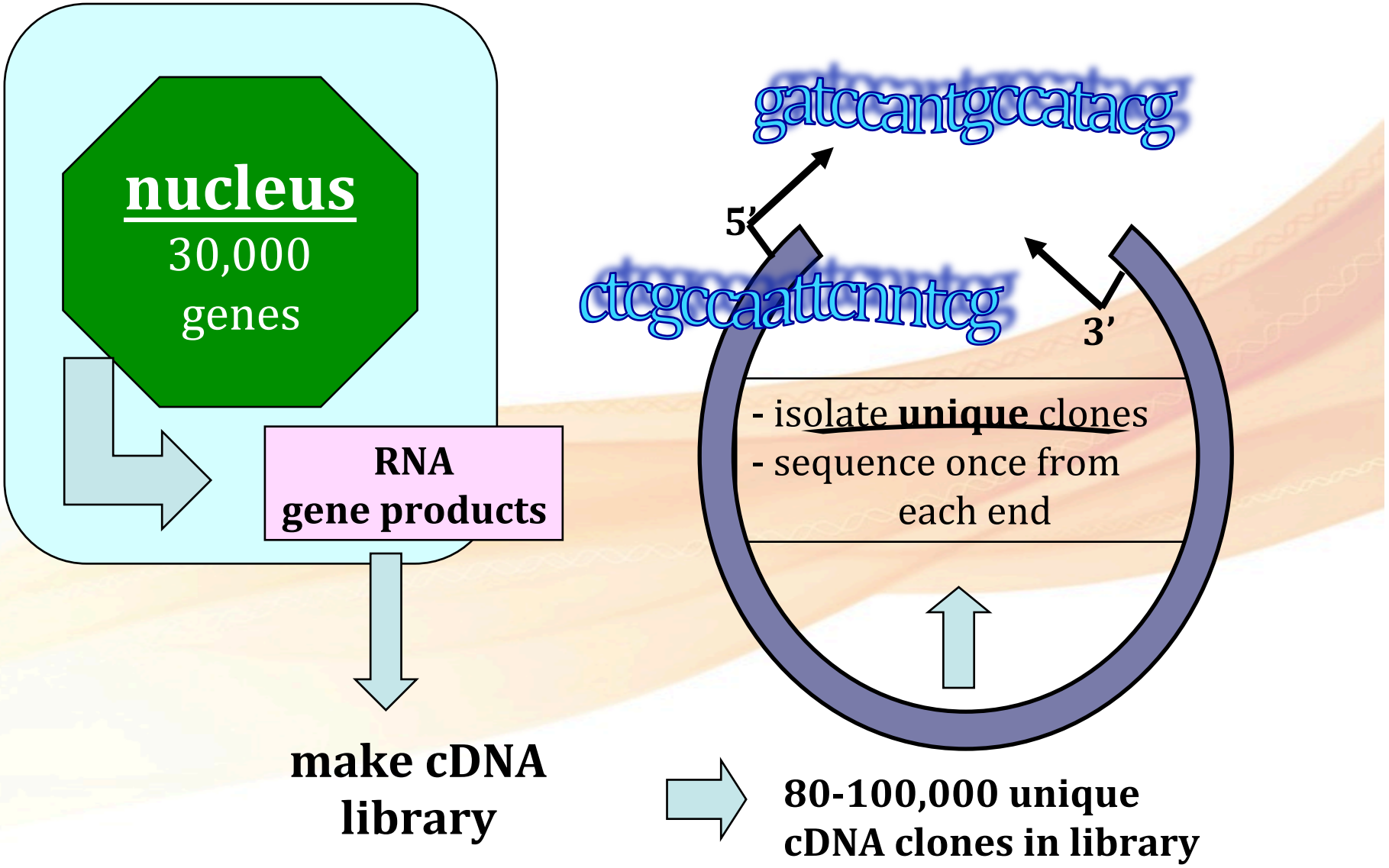
- DDBJ/EMBL/GenBank accepts both complete and incomplete genomes. Whole Genome Shotgun (WGS) sequencing projects are incomplete genomes or incomplete chromosomes that are being sequenced by a whole genome shotgun strategy
- WGS projects may be annotated, but annotation is not required
- The nucleotide data from all WGS projects go into the BLAST wgs database since the fall of 2011
- Proteins from: most WGS projects go into the BLAST nr database
- Proteins from environmental projects are present in either the BLAST nr or env_nr database, depending upon whether that sequence has been identified as a particular organism (nr), or if the organism is not yet known (env_nr)

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

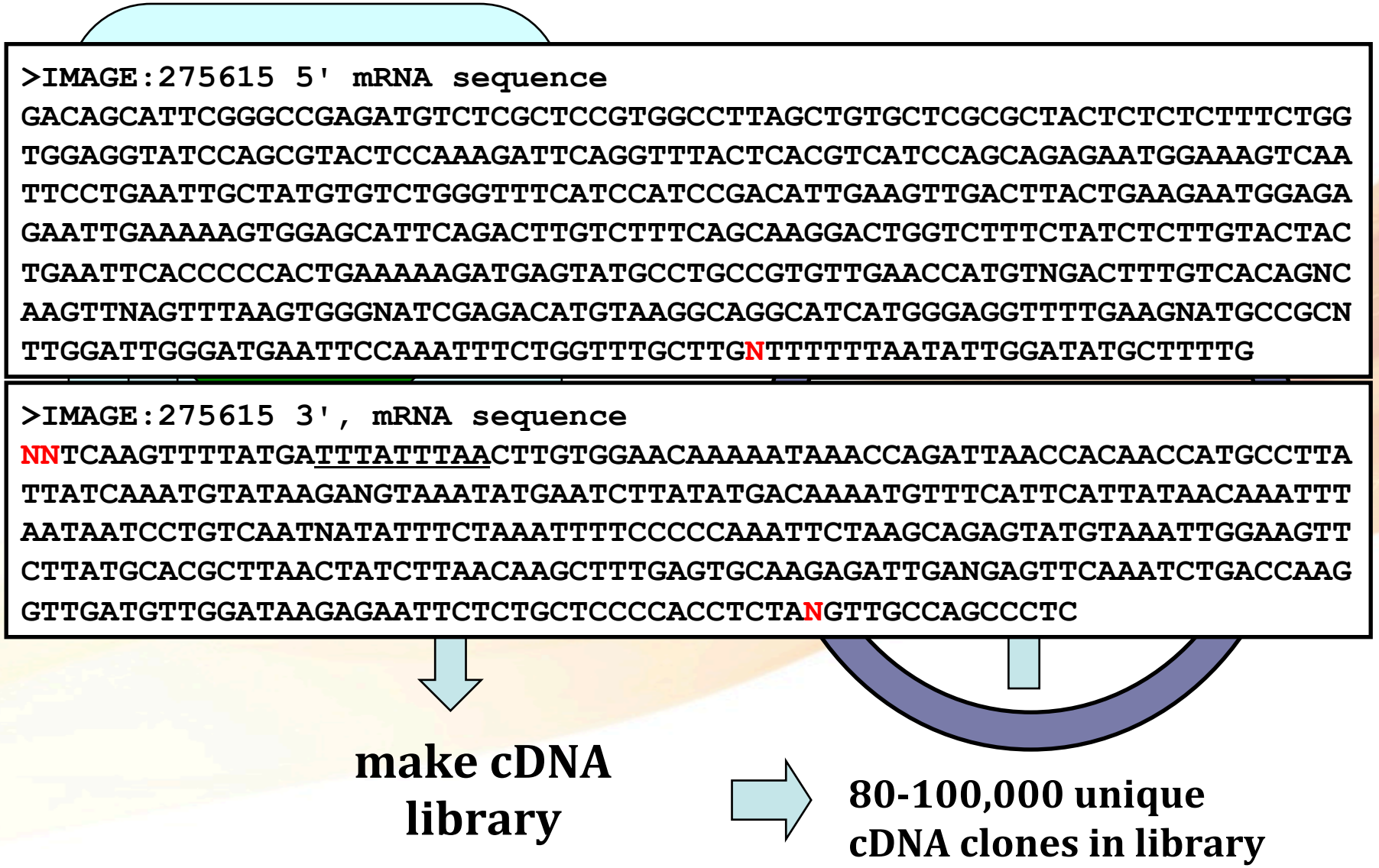
http://www.ncbi.nlm.nih.gov/projects/WGS/WGSprojectlist.cgi

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

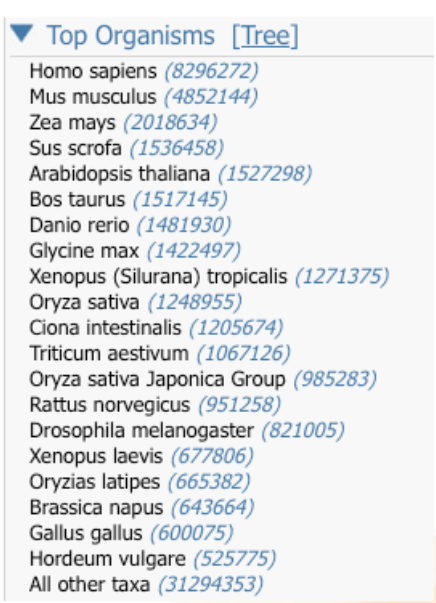
EST Division: Expressed Sequence Tags



EST Division: Expressed Sequence Tags



ESTs in Entrez (query gbdiv_est)



2009 - Oct

2010 - Oct

2011 - Oct

2012 - Oct

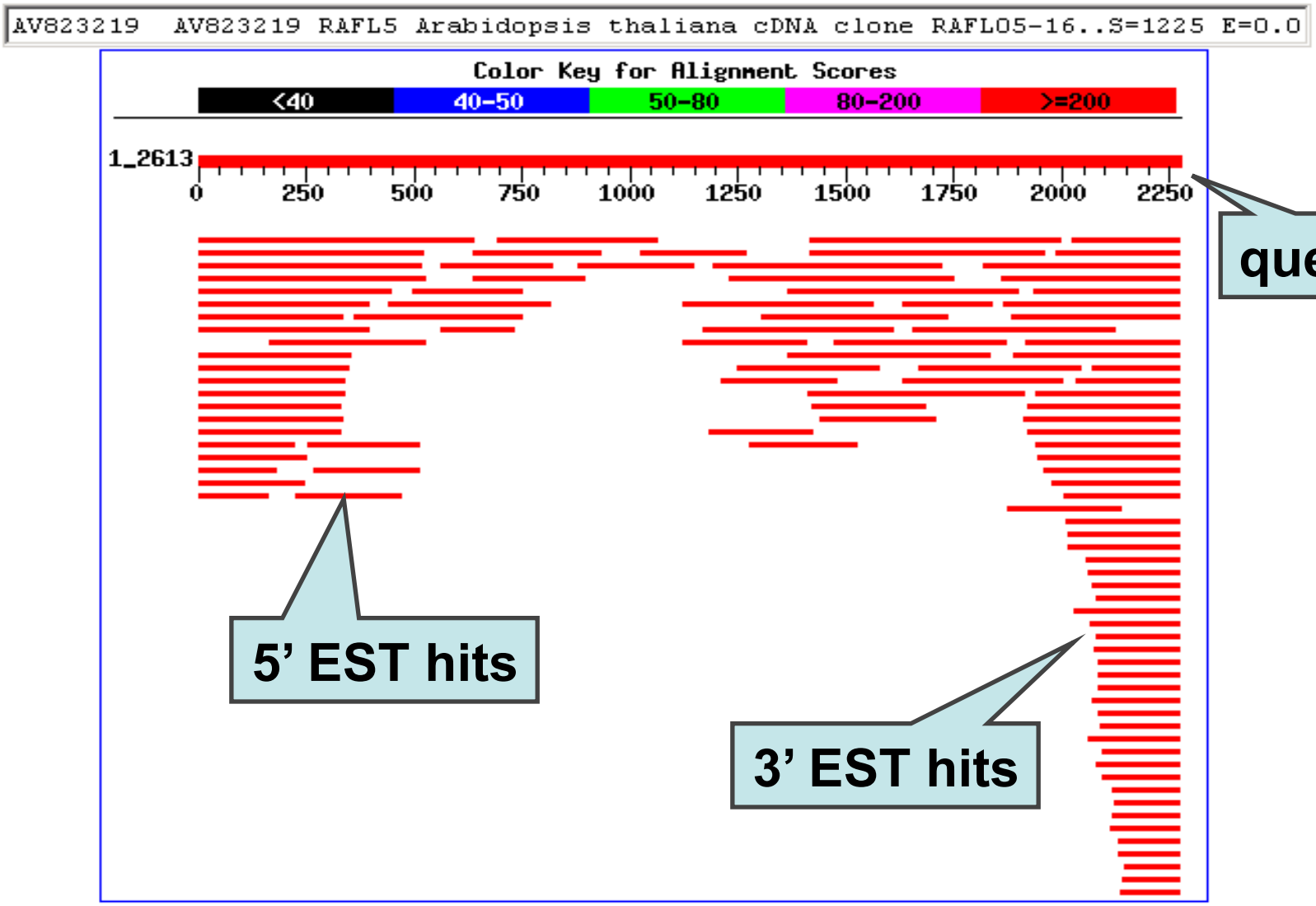
EST Division

- ESTs continue to be a major source of data for gene expression and annotation studies
- At almost 41 billion base pairs, it remains the largest non-WGS division in GenBank
- EST data are available for download from
- ftp.ncbi.nlm.nih.gov/repository/dbEST/
- The data in dbEST are clustered using the BLAST programs to produce the UniGene database (www.ncbi.nlm.nih.gov/unigene)
  - >5.8 million gene-oriented sequence clusters
  - representing 142 organisms

GSS Division

- Similar to the EST division – exception, most of the sequences are genomic in origin, rather than cDNA (mRNA)
- It should be noted that two classes (exon trapped products and gene trapped products) may be derived via a cDNA intermediate
  - Care should be taken when analyzing sequences from either of these classes, as a splicing event could have occurred and the sequence represented in the record may be interrupted when compared to genomic sequence
- The GSS division contains (but is not limited to) the following types of data:
  - random "single pass read" genome survey sequences.
  - cosmid/BAC/YAC end sequences
  - exon trapped genomic sequences
  - Alu PCR sequences
  - transposon-tagged sequences

A Cluster of ESTs



GenBank Bulk Sequence: EST

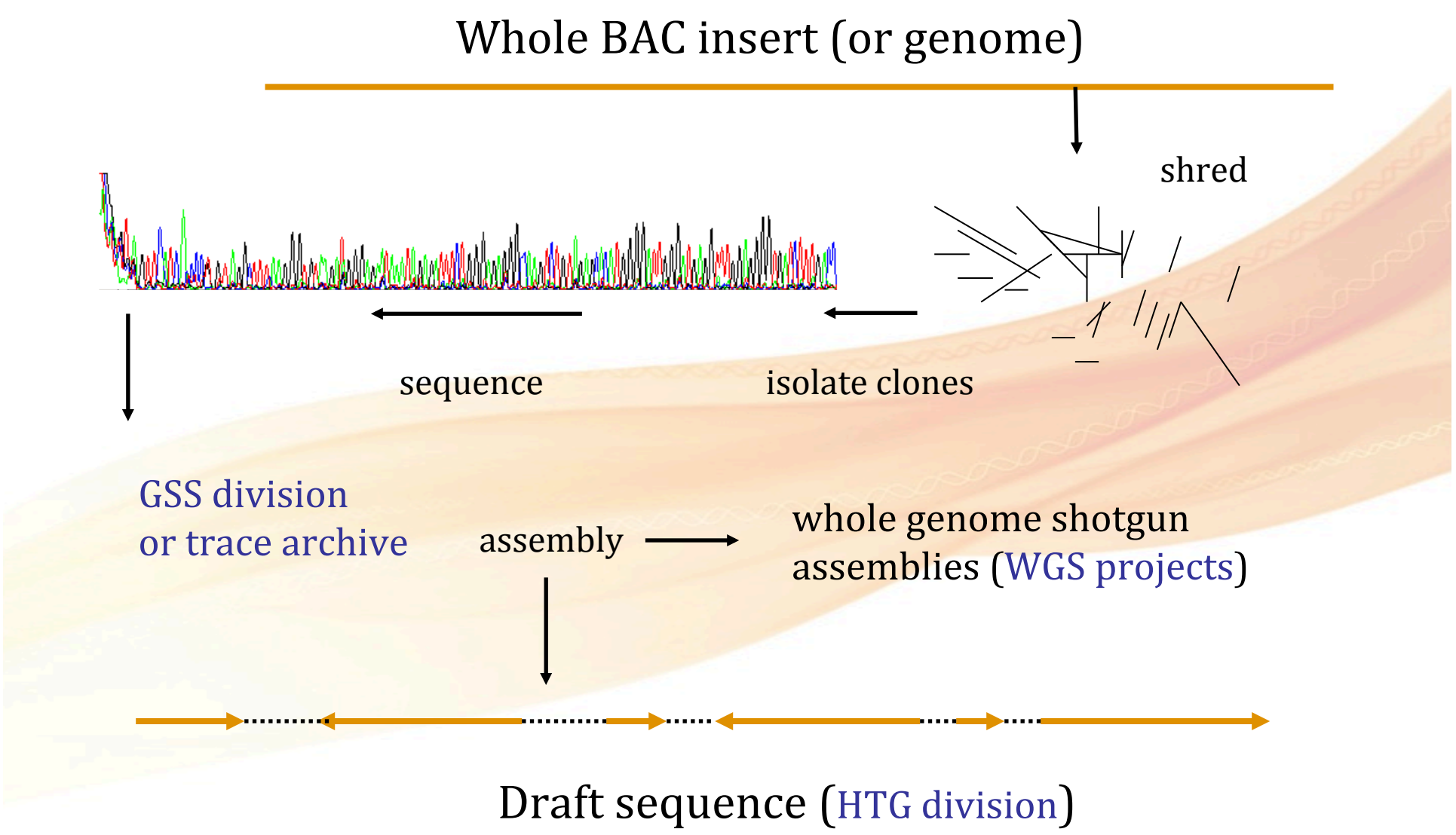
1: C75338. Reports C75338 Human panc...[gi:2366400] Links

```
LOCUS       C75338                  449 bp    mRNA    linear   EST 31-AUG-2006
DEFINITION  C75338 Human pancreatic islet Homo sapiens cDNA clone hbc7582
            similar to X-linked phosphoglycerated kinase, mRNA sequence.
ACCESSION   C75338
VERSION     C75338.1  GI:2366400
KEYWORDS    EST.
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 449)
  AUTHORS   Takeda,J.
  TITLE     Large scale collection of expressed sequence tags (ESTs) from human
            pancreatic islet cDNA library
  JOURNAL   Unpublished (1997)
COMMENT     Contact: Jun Takeda
            Institute for Molecular and Cellular Regulation, Gunma University
            Showa-machi, Maebashi, Gunma 371, Japan
            TEL: 027-220-8830
            FAX: 027-220-8889
            akeda@news.sb.gunma-u.ac.jp
            Human pancreatic islet cDNA'
            vector=Lambda ZAP II
            primer=T7 primer
            Rsite1=EcoR I
            Rsite2=Xho I
            mRNA was prepared from normal adult human islets. cDNA was
            directionally synthesized from the Xho I in the vector to the EcoRI
            site. cDNA was size fractionated to remove sequences <1000 bp in
            size.
FEATURES             Location/Qualifiers
     source          1..449
                     /organism="Homo sapiens"
                     /mol_type="mRNA"
                     /db_xref="taxon:9606"
                     /clone="hbc7582"
                     /clone_lib="Human pancreatic islet"
                     /note="Vector: Lambda ZAP II; Site_1: EcoRI; Site_2: Xho
                     I; mRNA was prepared from normal adult human islets. cDNA
                     was directionally synthesized from the Xho I in the vector
                     to the EcoRI site. cDNA was size fractionated to remove
                     sequences <1000 bp in size."
BASE COUNT  121 a  121 c  141 g  176 t
ORIGIN
        1 tttttttttt tttttttttc acggtttcaa tggacacttt tattgtttac ttaatggatc atcaattttg
       61 ttcctcactacc tacaaatgga atttcatctt gtttccatgc tgagtagtga aacagtgaca
      121 aagctaatca taataaccta catcaaaaga gaactaagct aacactgctc actttctttt
      181 taacaggcaa aatataaata tatgcactct agaatgcaca atggtttagt cactaaaaaa
      241 ttcaaatggg atcttgaaga tgtatgcaaa atccagggtg cagtgaagat gagctgagat
      301 gctgtgaacc tgttaaggg ttcctggcac tgcatcttt ggcactagc tgaatcttga
      361 catgaaagct ttagctaat gccaagtga gatgcaaaa atggcaagtt gacttagggg
      421 ctgtgcacag gaactaaaag caggaaagt
//
```

Gene?

poorly characterized

GSS, HTG, WGS


Whole BAC insert (or genome)
shred
sequence
isolate clones
GSS division
or trace archive
assembly
whole genome shotgun
assemblies (WGS projects)
Draft sequence (HTG division)

HTG Division

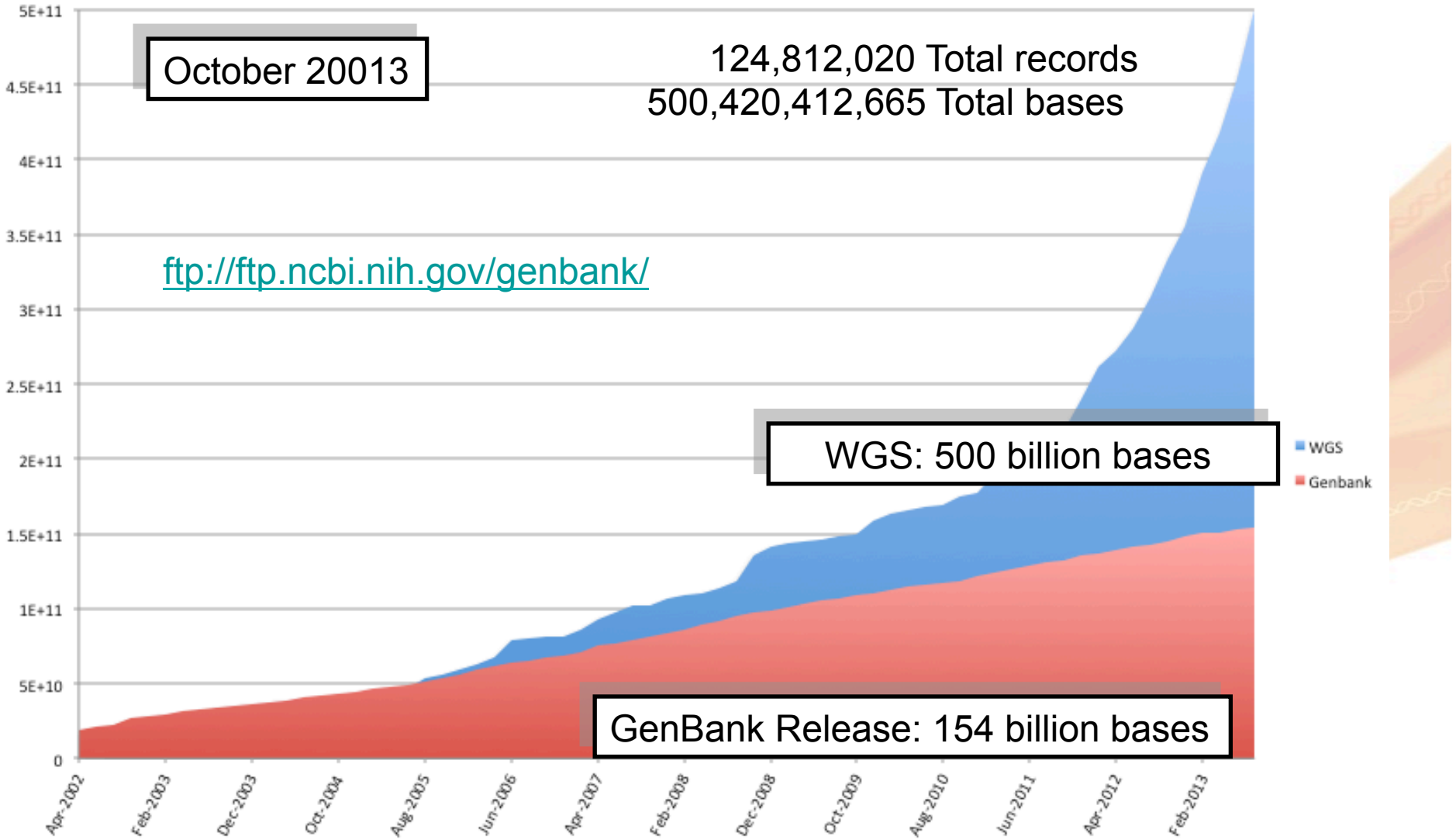
- The HTG division of GenBank (www.ncbi.nlm.nih.gov/genbank/htgs/)
- Contains unfinished large-scale genomic records, which are in transition to a finished state
- These records are designated as belonging to Phases 0 to 3 depending on the quality of the data, with Phase 3 being the finished state
- On reaching Phase 3, HTG records are moved into the appropriate organism division of GenBank

HTC Division

- The HTC division of GenBank contains high- throughput cDNA sequences that are of draft quality but may contain:
  - 5' untranslated regions (UTRs)
  - 3' UTRs, partial coding regions and introns
- HTC sequences that are finished and of high quality are moved to the appropriate organism division of GenBank
- A project generating HTC data is described in (9)*

Kawai,J., Shinagawa,A., Shibata,K., Yoshino,M., Itoh,M.,
Ishii,Y., Arakawa,T., Hara,A., Fukunishi,Y., Konno,H. et al. (2001) Functional annotation of a full-length mouse cDNA collection. Nature, 409, 685–690.

The Growth of WGS



Shotgun Assembly Sequences: Genome (WGS)

#	Prefix	Div	BioProject	BioSample	Organism	Contigs			Scaffolds			# of Proteins	Complete	Update Date	Create Date
						#	Mbases	Annot	#	Mbases	Annot				
1	AAAA02	PLN	PRJNA361		Oryza sativa Indica Group	50,231	410.7		3,095	769.7	Y	37,358		2011-02-25	2004-10-21
2	AAAB01	INV	PRJNA1438		Anopheles gambiae str. PEST	69,724	328.9	Y	5	230.5		14,089		2011-05-19	2002-03-22
3	AAAC01	BCT	PRJNA299		Bacillus anthracis str. A2012	1	5.1							2007-01-17	2002-05-09
4	AAAK03	BCT	PRJNA71		Enterococcus faecium DO	163	2.8	Y				2,721		2007-01-17	2004-06-07

- Each WGS project assigned a stable 4-letter WGS accession prefix, which does not change as the project is updated
- Ex. WGS accession number is **XXXX**00000000, project's first assembly version would be XXXX**01**000000, and first contig of that version: XXXX01000000**1**
- When there is more sequencing and the genome is reassembled
  - Contigs are submitted as the 02 version of the WGS project
  - No linkage or relationship is expected between the old and new contigs, and the new contigs are given new accession numbers beginning with XXXX02000001
  - The 01 contigs are suppressed when the 02 contigs are released

http://www.ncbi.nlm.nih.gov/Traces/wgs/?

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

TSA Division

- The HTC division of GenBank contains high- throughput cDNA sequences that are of draft quality but may contain:
  - 5' untranslated regions (UTRs)
  - 3' UTRs, partial coding regions and introns
- HTC sequences that are finished and of high quality are moved to the appropriate organism division of GenBank
- A project generating HTC data is described in (9)*

Kawai,J., Shinagawa,A., Shibata,K., Yoshino,M., Itoh,M.,
Ishii,Y., Arakawa,T., Hara,A., Fukunishi,Y., Konno,H. et al. (2001) Functional annotation of a full-length mouse cDNA collection. Nature, 409, 685–690.

Shotgun Assembly Sequences: Transcriptome (TSA)

#	Prefix	Div	BioProject	BioSample	Organism	Contigs			Scaffolds			# of Proteins	Complete	Update Date	Create Date
						#	Mbases	Annot	#	Mbases	Annot				
1	GAAA01	VRT	PRJNA77699	SRS283232	Latimeria chalumnae	93,507	72.6							2012-09-27	2012-05-15
2	GAAB01	INV	PRJNA173782		Agrilus planipennis	11,957	6.1							2012-12-10	2012-12-10
3	GAAC01	PLN	PRJNA167330		Camellia sinensis var. sinensis	52,919	23.9							2012-09-25	2012-09-25
4	GAAD01	VRT	PRJNA173219	SRS358712	Misgurnus anguillicaudatus	11,327	9.9							2013-03-11	2013-03-11
5	GAAF01	INV	PRJNA173897		Macrosiphum euphorbiae	550	.7							2012-09-26	2012-09-26

- Each TSA project is assigned a stable 4-letter TSA accession prefix, which does not change as the project is updated
- E.g. TSA accession number is XXXX00000000, then that project's first transcript version would be XXXX**01**000000, and the first assembly of that version would be XXXX01000000**1**
- When a project is reassembled
  - New assemblies are submitted as the 02 version of the TSA project
  - No linkage or relationship is expected between the old and new assemblies, and the new assemblies are given new accession numbers beginning with XXXX02000001
  - The 01 transcripts are suppressed when the 02 transcripts are released.# contigs in each project is listed

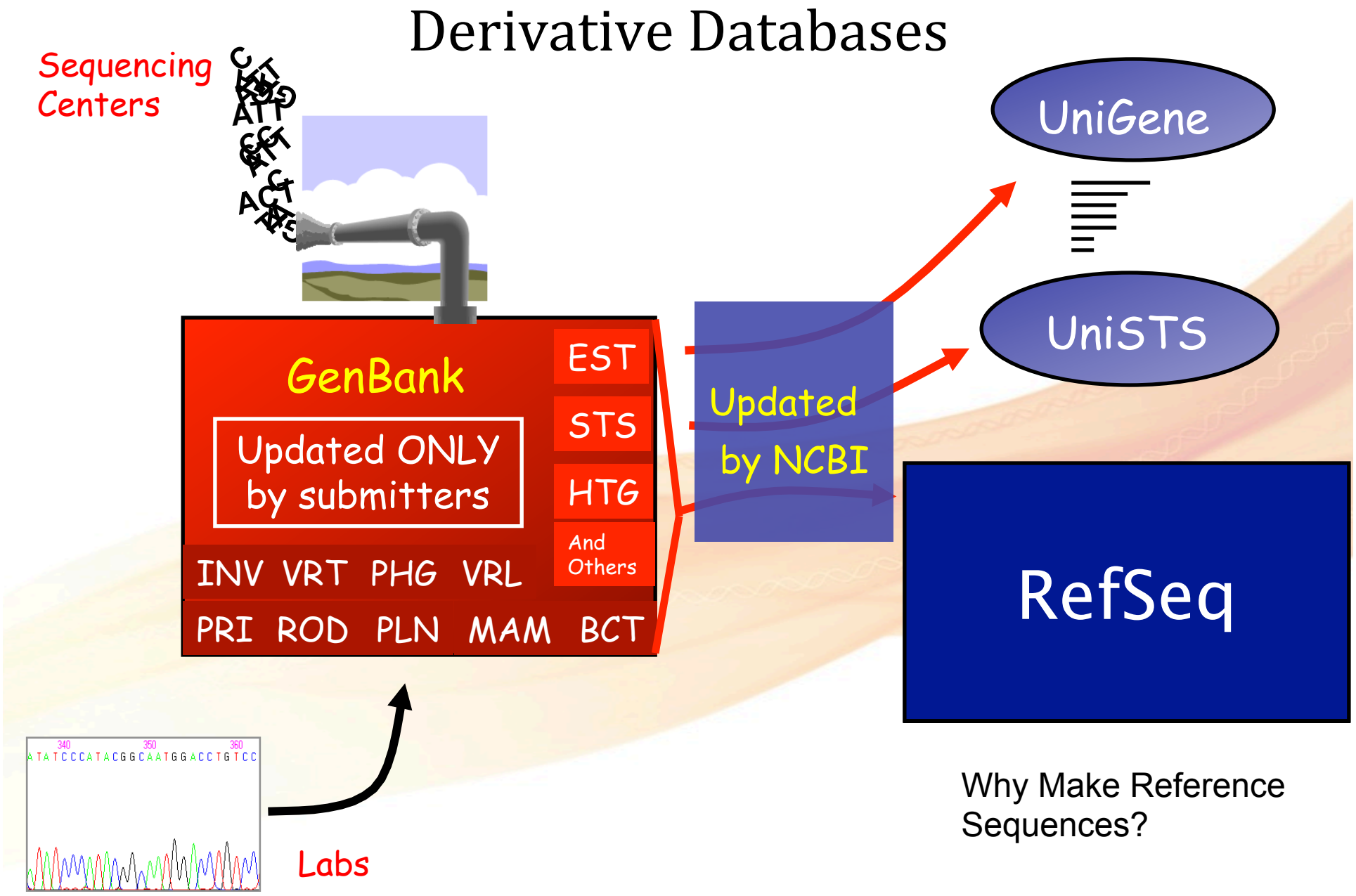
http://www.ncbi.nlm.nih.gov/Traces/wgs/?

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

FYI - Obtaining GenBank by FTP

- NCBI distributes GenBank releases in the traditional flat file format as well as in the ASN.1 format used for internal maintenance
- The full bimonthly GenBank release along with the daily updates
  - Which incorporate sequence data from EMBL-Bank and DDBJ
  - Available by anonymous FTP from NCBI at ftp.ncbi.nlm.nih.gov/genbank
- GenBank is also available for high-speed download using an Aspera client:
  - www.ncbi.nlm.nih.gov/public/
- Data are partitioned into multiple files; for **release 191**, there are **1852** files requiring **604GB** of uncompressed disk storage
- A script is provided in ftp.ncbi.nlm.nih.gov/genbank/tools/ convert a set of daily updates into a cumulative update

Derivative Databases



Why Make Reference Sequences?

RefSeq: NCBI Reference Sequence Database

A comprehensive, integrated, non-redundant, well-annotated set of reference sequences including genomic, transcript, and protein.

Why Make Reference Sequences?

Entrez Nucleotide query:
human[organism] AND lipase[title]

Results: 1 to 20 of 294

<< First < Prev Page 1 Next > Last >>

1. lipoprotein lipase LPL {promoter} [human, peripheral blood leukocytes, familial combined hyperlipidemia FCHL, mRNA Partial Mutant, 123 nt]
   123 bp linear mRNA
   S78266.1 GI:999330
   GenBank FASTA Graphics Related Sequences

2. LPL (Arita)=lipoprotein lipase [human, Genomic Mutant, 18 nt]
   18 bp linear DNA
   S81338.1 GI:245362
   GenBank FASTA Graphics

3. lipoprotein lipase {exon 9} [human, Genomic Mutant, 18 nt]
   18 bp linear DNA
   S75964.1 GI:242490
   GenBank FASTA Graphics

....

325 records as of Oct 2013

Entrez Nucleotide query:
human[organism] AND lipase[title]

Results: 8

1. Homo sapiens lipase, endothelial (LIPG) gene, complete cds — 34815 bp
   34,815 bp linear DNA
   EU332856.1 GI:166706794
   GenBank FASTA Graphics Related Sequences

2. Homo sapiens lipase, endothelial (LIPG), mRNA — 4143 bp
   4,143 bp linear mRNA
   NM_006033.2 GI:62422575
   GenBank FASTA Graphics Related Sequences

3. Homo sapiens lipase, endothelial, mRNA (cDNA clone MGC:71687 IMAGE:30338950), complete cds — 4150 bp
   4,150 bp linear mRNA
   BC060825.1 GI:38174525
   GenBank FASTA Graphics Related Sequences

4. Homo sapiens endothelial lipase mRNA, complete cds — 3927 bp
   3,927 bp linear mRNA
   AF118767.1 GI:4836418
   GenBank FASTA Graphics Related Sequences

5. Homo sapiens cDNA FLJ57818 complete cds, highly similar to Endothelial lipase precursor (EC 3.1.1.3) — 1760 bp
   1,760 bp linear mRNA
   AK300333.1 GI:194390641
   GenBank FASTA Graphics Related Sequences

6. Homo sapiens cDNA, FLJ96256, highly similar to Homo sapiens lipase, endothelial (LIPG), mRNA — 1783 bp
   1,783 bp linear mRNA
   AK315252.1 GI:164694848
   GenBank FASTA Graphics Related Sequences

7. Homo sapiens cDNA FLJ77993 complete cds, highly similar to Homo sapiens endothelial lipase mRNA — 1975 bp
   1,975 bp linear mRNA
   AK291799.1 GI:158257029
   GenBank FASTA Graphics Related Sequences

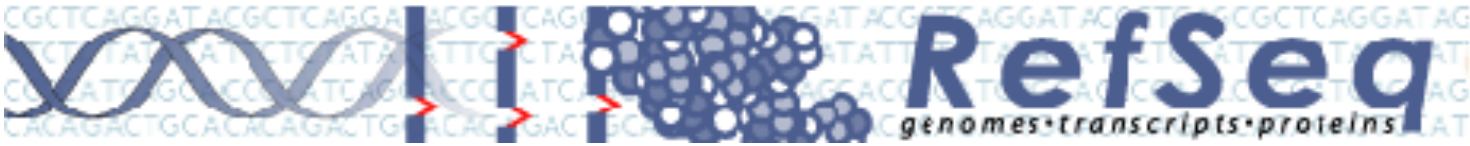
8. ANTISENSE MODULATION OF ENDOTHELIAL LIPASE EXPRESSION — 3927 bp
   3,927 bp linear DNA
   DD265273.1 GI:109245105
   GenBank FASTA Graphics

....

human[organism] AND lipase[title] AND endothelial[title]

Look at all these different sizes? Which one do we use?

11 records as of Oct 2013



genomes → transcripts → proteins

September 11, 2009: RefSeq Release 37 available for FTP

This release includes:

Proteins: 8,835,796
Organisms: 9,005
Available at: ftp://ftp.ncbi.nih.gov/refseq/release/

September 16, 2010: RefSeq Release 43 available for FTP

This release includes:

Proteins: 11,223,078
Organisms: 10,854
Available at: ftp://ftp.ncbi.nih.gov/refseq/release/

September 13, 2011: RefSeq Release 49 available for FTP

This release includes:

Proteins: 13,137,813
Organisms: 16,248

September 21, 2012: RefSeq Release 55 available for FTP

This release includes:

Proteins: 17,368,769
Organisms: 17,994
Available at: ftp://ftp.ncbi.nlm.nih.gov/refseq/release/

To receive announcements of future RefSeq releases and incremental large updates please subscribe to NCBI's refseq-announce mail list: refseq-announce

Announcements

September 17, 2013
RefSeq release 61 available for FTP

This release includes:

Proteins 33,139,114
Organisms 29,414
Available at ftp://ftp.ncbi.nlm.nih.gov/refseq/release/
Documentation Release Notes

Additional information is available in previous announcements, follow NCBI on Twitter, or subscribe to NCBI's refseq-announce mail list.

Growth!!!

ftp://ftp.ncbi.nih.gov/refseq/release/release-notes/RefSeq-release61.txt

RefSeq

A subdirectory exists for each sub-section as follows:

Release 55

complete	17994
fungi	332
invertebrate	934
microbial	11368
mitochondrion	3071
plant	285
plasmid	1258
plastid	287
protozoa	157
vertebrate_mammalian	395
vertebrate_other	1474
viral	3011

Release 61

complete	29414
fungi	812
invertebrate	1130
microbial	20859
mitochondrion	3896
plant	363
plasmid	1562
plastid	372
protozoa	182
vertebrate_mammalian	608
vertebrate_other	1830
viral	3629

ftp://ftp.ncbi.nih.gov/refseq/release/release-notes/RefSeq-release61.txt

The Reference Sequence – RefSeq

- Aims provide a:
  - Comprehensive
  - Integrated
  - Non-redundant
  - Well-annotated set of sequences,
  - Including genomic DNA, transcripts, and proteins
- Foundation for medical, functional, and diversity studies
- NCBI provides RefSeqs for taxonomically diverse organisms including **eukaryotes, bacteria, and viruses**
- Records are added to the collection as data become publicly available

RefSeq Scope and Access

- Scope
  - NCBI provides RefSeqs for taxonomically diverse organisms including eukaryotes, bacteria, and viruses
  - Additional records are added to the collection as data become publicly available
- **Data Access and Availability**
  - RefSeq is accessible via BLAST, Entrez, and the NCBI FTP site
  - Information is also available in Entrez Genomes and Entrez Gene, and for some genomes additional information is available in the Map Viewer
  - Special properties have been defined to facilitate Entrez-based retrieval
    - Next Slide

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

ftp://ftp.ncbi.nlm.nih.gov

Entrez Queries to Retrieve Sets of RefSeq Records

Query	Accession prefix	RefSeq status retrieved
srcdb_refseq[prop]	All RefSeq accessions	All
srcdb_refseq_known[prop]	NC_, AC_, NG_, NM_, NR_, NP_, AP_	REVIEWED, PROVISIONAL, PREDICTED, INFERRED, and VALIDATED
srcdb_refseq_reviewed[prop]	NC_, AC_,NG_, NM_, NR_, NP_, AP_	REVIEWED
srcdb_refseq_validated[prop]	NC_, NM_,NR_,NP_	VALIDATED
srcdb_refseq_provisional[prop]	NC_, AC_, NG_, NM_, NR_, NP_, AP_	PROVISIONAL
srcdb_refseq_predicted[prop]	NM_, NR_, NP_	PREDICTED
srcdb_refseq_inferred[prop]	AC_, AP_, NM_,NR_,NP_	INFERRED
srcdb_refseq_model[prop]	NT_, NW_, XM_, XR_, XP_, ZP_	Genome annotation models

human[organism] AND srcdb_refseq[prop]
human[organism] AND srcdb_refseq_known[prop]

http://www.ncbi.nlm.nih.gov/books/NBK21091/table/ch18.T.entrez_queries_to_retrieve_sets_o/?report=objectonly

Reference Sequence: RefSeq

- Every sequence is assigned:
  - A stable accession
  - Version
  - And GI
  - All older versions remain available over time
- RefSeq accessions have a distinct format
  - **Underscore** ("_") is the primary distinguishing feature of a RefSeq accession

Accession	Method	Sequence Type
NM_123456	**Mixed**	**mRNA**
NM_123456789		
NP_123456789	Mixed	protein, from NM_
NR_123456	Mixed	non-coding RNA
XM_123456	**Automated**	**predicted mRNA**
XM_123456789		
XP_123456	**Automated**	**predicted protein**
XP_123456789		
XR_123456	Automated	predicted non-coding RNA
ZP_12345678	Automated	predicted protein from NZ_
AP_123456	Mixed	Protein products; alternate protein record
YP_123456	Mixed	Protein products; no corresponding
YP_123456789	transcript record provided	
NC_123456	**Mixed**	**genomic, *e.g.*, chromosomes**
NG_123455	**Mixed**	**genomic, incomplete region**
NT_123456	**Automated**	**genomic, BAC assembly**
NW_123456	**Automated**	**genomic, WGS assembly**
NW_123456789		
NZ_ABCD12345678	Automated	genomic, WGS collection
NS_123456	Automated	genomic, assembly which does not reflect the structure of a real biological molecule
AC_123456	Mixed	genomic, Alternate complete genomic molecule

http://www.ncbi.nlm.nih.gov/RefSeq/key.html#accession

Method

- *Mixed*:
  - Indicates the process flow includes both automated processing and expert review for some of the records
  - Curation analysis may be provided either by NCBI staff or collaborators.

- *Automated*:
  - Indicates records that are not individually reviewed
  - Updates are released in bulk for a genome

COMMENT = Status

- Indicates Status of the record and the GenBank sequence data that was used to provide the record
- May identify a collaboration that supplied the defining sequence information for the genome, gene, or protein
- Level of curation may differ b/t different collaborating groups

DNA topoisomerase III [Pseudoalteromonas flavipulchra JG1]

NCBI Reference Sequence: ZP_11232112.1

FASTA Graphics

Go to:

```
LOCUS       ZP_11232112              640 aa            linear   BCT 19-OCT-2012
DEFINITION  DNA topoisomerase III [Pseudoalteromonas flavipulchra JG1].
ACCESSION   ZP_11232112
VERSION     ZP_11232112.1  GI:409203909
DBSOURCE    REFSEQ: accession NZ_AJMP01000069.1
KEYWORDS    .
SOURCE      Pseudoalteromonas flavipulchra JG1
  ORGANISM  Pseudoalteromonas flavipulchra JG1
            Bacteria; Proteobacteria; Gammaproteobacteria; Alteromonadales;
            Pseudoalteromonadaceae; Pseudoalteromonas.
COMMENT     WGS REFSEQ: This record is provided to represent a collection of
            whole genome shotgun sequences. The reference sequence was derived
            from AJMP01000069.
            Method: conceptual translation.
```

Status Keys

Code	Description
MODEL	The RefSeq record is provided by the NCBI Genome Annotation pipeline and is not subject to individual review or revision between annotation runs.
INFERRED	The RefSeq record has been predicted by genome sequence analysis, but it is not yet supported by experimental evidence. The record may be partially supported by homology data.
PREDICTED	The RefSeq record has not yet been subject to individual review, and some aspect of the RefSeq record is predicted.
PROVISIONAL	The RefSeq record has not yet been subject to individual review. The initial sequence-to-gene association has been established by outside collaborators or NCBI staff.
REVIEWED	The RefSeq record has been reviewed by NCBI staff or by a collaborator. The NCBI review process includes assessing available sequence data and the literature. Some RefSeq records may incorporate expanded sequence and annotation information.
VALIDATED	The RefSeq record has undergone an initial review to provide the preferred sequence standard. The record has not yet been subject to final review at which time additional functional information may be provided.
WGS	The RefSeq record is provided to represent a collection of whole genome shotgun sequences. These records are not subject to individual review or revisions between genome updates.

http://www.ncbi.nlm.nih.gov/books/NBK21091/table/ch18.T.refseq_status_codes/?report=objectonly

Why is RefSeq So Useful

- RefSeq provides a biologically **non-redundant set of sequences** for database searching and gene characterization
- Provides a valuable sequence resource for:
  - Similarity searching
  - Gene identification
  - Protein classification
  - Comparative genomics
  - Selection of probes for gene expression
- Acts as molecular "white pages"
  - Provides:
    - a **single**, uniform point of access for searching at the sequence level
    - by **connecting** the results with a diversity of organism-specific databases

Non-redundant Set of Sequences

- Provides an objective and experimentally verifiable definition of "non-redundant" in supplying one example of each natural biomolecule per organism
- Small amount of sequence redundancy introduced from:
  - Close paralogs
  - Alternate splicing products
  - Genome assembly intermediates

Additional RefSeq Benefits

- Updates to reflect current sequence data and biology
- Data validation (next slide)
- Format consistency (next slide)
- Distinct accession series
- Stewardship by NCBI staff and collaborators

Data Validation (1)

- Sequences are validated in several ways
- To confirm that genomic sequence from the region of the mRNA feature really does match the mRNA sequence itself
- That the annotated coding region features really can be translated into the protein sequences they refer to
- Checks for valid ASN.1 format
- Ensures that consistency is maintained in descriptive information (symbols, gene and protein names) between RefSeq and Gene records

Data Validation (2)

- Each molecule is annotated as accurately as possible with:
  - Correct organism name
  - Correct gene symbol for that organism
  - Reasonable names for proteins where possible
- When available, nomenclature provided by official nomenclature groups is used
- **Note that gene symbols are not required or expected to be unique either across species or within a species**

RefSeq: NCBI's Derivative Sequence Datatabase

- Curated transcripts and proteins
  - Reviewed
  - Human, mouse, rat, fruit fly, zebrafish, arabidopsis, microbial genomes, and more
- Model transcripts and proteins
- Assembled Genomes Regions (contigs)
- Chromosome records
  - Human genome
  - microbial
  - organelle
- ftp://ftp.ncbi.nih.gov/refseq/release

srcdb_refseq[Properties] = 5,846,009 nt, 17,698,431 protein, 10,965,669 gene (Oct 2010)

Reference Sequence: RefSeq

Accession	Method	Sequence Type
NM_123456	**Mixed**	**mRNA**
NM_123456789		
NP_123456789	Mixed	protein, from NM_
NR_123456	Mixed	non-coding RNA
XM_123456	**Automated**	**predicted mRNA**
XM_123456789		
XP_123456	**Automated**	**predicted protein**
XP_123456789		
XR_123456	Automated	predicted non-coding RNA
ZP_12345678	Automated	predicted protein from NZ_
AP_123456	Mixed	Protein products; alternate protein record
YP_123456	Mixed	Protein products; no corresponding
YP_123456789	transcript record provided	
NC_123456	**Mixed**	**genomic, *e.g.*, chromosomes**
NG_123455	**Mixed**	**genomic, incomplete region**
NT_123456	**Automated**	**genomic, BAC assembly**
NW_123456	**Automated**	**genomic, WGS assembly**
NW_123456789		
NZ_ABCD12345678	Automated	genomic, WGS collection
NS_123456	Automated	genomic, assembly which does not reflect the structure of a real biological molecule
AC_123456	Mixed	genomic, Alternate complete genomic molecule

Bold are explained next slides

RefSeq Chromosomes: NC_ , NG_
genomic: Chromosomes & , Incomplete region

Escherichia coli O157:H7 str. Sakai, complete genome

NCBI Reference Sequence: NC_002695.1

FASTA Graphics

Go to:

```
LOCUS       NC_002695             5498450 bp    DNA     circular BCT 24-MAY-2011
DEFINITION  Escherichia coli O157:H7 str. Sakai, complete genome.
ACCESSION   NC_002695
VERSION     NC_002695.1  GI:15829254
DBLINK      Project: 57781
KEYWORDS    .
SOURCE      Escherichia coli O157:H7 str. Sakai
  ORGANISM  Escherichia coli O157:H7 str. Sakai
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
            Enterobacteriaceae; Escherichia.
REFERENCE   1  (bases 1 to 5498450)
  AUTHORS   Bergholz,T.M., Wick,L.M., Qi,W., Riordan,J.T., Ouellette,L.M. and
            Whittam,T.S.
  TITLE     Global transcriptional response of Escherichia coli O157:H7 to
            growth transitions in glucose minimal medium
  JOURNAL   BMC Microbiol. 7, 97 (2007)
   PUBMED   17967175
  REMARK    Publication Status: Online-Only
REFERENCE   2  (sites)
  AUTHORS   Hayashi,T., Makino,K., Ohnishi,M., Kurokawa,K., Ishii,K.,
            Yokoyama,K., Han,C.G., Ohtsubo,E., Nakayama,K., Murata,T.,
            Tanaka,M., Tobe,T., Iida,T., Takami,H., Honda,T., Sasakawa,C.,
            Ogasawara,N., Yasunaga,T., Kuhara,S., Shiba,T., Hattori,M. and
            Shinagawa,H.
```

RefSeq Chromosomes: NT_ , **NW_**
genomic: BAC assembly & **WGS assembly**

Mus musculus chromosome 6 genomic contig, alternate assembly (based on MGSCv3)

NCBI Reference Sequence: NW_000272.1

⚠ **Record removed.** This record was removed as a result of standard genome annotation processing. See the genome build documentation at http://www.ncbi.nlm.nih.gov/genome/guide/build.html for further information, or contact info@ncbi.nlm.nih.gov.

FASTA Graphics

Go to:

```
LOCUS       NW_000272           27755676 bp    DNA     linear   CON 27-APR-2006
DEFINITION  Mus musculus chromosome 6 genomic contig, alternate assembly (based
            on MGSCv3).
ACCESSION   NW_000272
VERSION     NW_000272.1  GI:20915425
KEYWORDS    .
SOURCE      Mus musculus (house mouse)
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
            Sciurognathi; Muroidea; Muridae; Murinae; Mus.
  TITLE     Initial sequencing and comparative analysis of the mouse genome
  JOURNAL   Nature 420 (6915), 520-562 (2002)
   PUBMED   12466850
REFERENCE   2  (sites)
  AUTHORS   Lowe,T.M. and Eddy,S.R.
  TITLE     tRNAscan-SE: a program for improved detection of transfer RNA genes
            in genomic sequence
  JOURNAL   Nucleic Acids Res. 25 (5), 955-964 (1997)
   PUBMED   9023104
  REMARK    This is the methods paper for tRNAscan-SE.
COMMENT     GENOME ANNOTATION REFSEQ: Features on this sequence have been
            produced for build 36 version 1 of the NCBI's genome annotation
            [see documentation].
            This DNA sequence represents the MGSCv3, the publicly available
            Whole Genome Assembly of the mouse genome (C57BL/6J) that was made
            available in November of 2002.
```

Alignment Generated Transcripts: XM_ , XP_
Predicted mRNA & Predicted protein

PREDICTED: Mus musculus lipoprotein lipase, transcript variant 3 (Lpl), mRNA

NCBI Reference Sequence: XM_921447.1

⚠ **This sequence has been replaced by NM_008509.**

FASTA Graphics

Go to:

```
LOCUS       XM_921447               1923 bp    mRNA    linear   ROD 01-DEC-2005
DEFINITION  PREDICTED: Mus musculus lipoprotein lipase, transcript variant 3
            (Lpl), mRNA.
ACCESSION   XM_921447
VERSION     XM_921447.1  GI:82917585
KEYWORDS    .
SOURCE      Mus musculus (house mouse)
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
            Sciurognathi; Muroidea; Muridae; Murinae; Mus.
COMMENT     MODEL REFSEQ:  This record is predicted by automated computational
            analysis. This record is derived from a genomic sequence
            (NW_001030897) annotated using gene prediction method: GNOMON,
            supported by mRNA and EST evidence.
            Also see:
                Documentation of NCBI's Annotation Process

            [WARNING] On Dec 7, 2005 this sequence was replaced by gi:6678709.
FEATURES             Location/Qualifiers
     source          1..1923
                     /organism="Mus musculus"
                     /mol_type="mRNA"
                     /strain="mixed"
                     /db_xref="taxon:10090"
                     /chromosome="8"
     gene            1..1923
                     /gene="Lpl"
                     /note="Derived by automated computational analysis using
                     gene prediction method: GNOMON. Supporting evidence
                     includes similarity to: 74 mRNAs, 785 ESTs, 22 Proteins"
                     /db_xref="GeneID:16956"
                     /db_xref="MGI:96820"
     CDS             234..1658
                     /gene="Lpl"
                     /codon_start=1
                     /product="similar to Lipoprotein lipase precursor (LPL)
                     isoform 3"
                     /protein_id="XP_926540.1"
                     /db_xref="GI:82917586"
                     /db_xref="GeneID:16956"
                     /db_xref="MGI:96820"
```

Curated RefSeq Records: NM_ , NP_
mRNA & Protein from NM_

Mus musculus lipoprotein lipase (Lpl), mRNA

NCBI Reference Sequence: NM_008509.2

FASTA Graphics

Go to:

```
LOCUS       NM_008509               4049 bp    mRNA    linear   ROD 17-SEP-2011
DEFINITION  Mus musculus lipoprotein lipase (Lpl), mRNA.
ACCESSION   NM_008509 XM_909793 XM_921447
VERSION     NM_008509.2  GI:126723005
KEYWORDS    .
SOURCE      Mus musculus (house mouse)
  ORGANISM  Mus musculus
COMMENT     VALIDATED REFSEQ: This record has undergone validation or
            preliminary review. The reference sequence was derived from
            AK152014.1, AK150375.1 and AC158236.2.
            On Mar 8, 2007 this sequence version replaced gi:6678709.

            Publication Note:  This RefSeq record includes a subset of the
            publications that are available for this gene. Please see the Gene
            record to access additional publications.
            COMPLETENESS: complete on the 3' end.
```

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CDS             199..1623
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                /EC_number="3.1.1.34"
                /note="O 1-4-5"
                /codon_start=1
                /product="lipoprotein lipase precursor"
                /protein_id="NP_032535.2"
                /db_xref="GI:126723006"
                /db_xref="CCDS:CCDS40357.1"
                /db_xref="GeneID:16956"
                /db_xref="MGI:96820"
                /translation="MESKALLLVVLGVWLQSLTAFRGGVAAADAGRDFSDIESKFALR
                TPEDTAEDTCHLIPGLADSVSNCHFNHSSKTFVVIHGWTVTGMYESWVPKLVAALYKR
                EPDSNVIVVDWLYRAQQHYPVSAGYTKLVGNDVARFINWMEEEFNYPLDNVHLLGYSL
                GAHAAGVAGSLTNKKVNRITGLDPAGPNFEYAEAPSRLSPDDADFVDVLHTFTRGSPG
                RSIGIQKPVGHVDIYPNGGTFQPGCNIGEAIRVIAERGLGDVDQLVKCSHERSIHLFI
                DSLLNEENPSKAYRCNSKEAFEKGLCLSCRKNRCNNLGYEINKVRAKRSSKMYLKTRS
                QMPYKVFHYQVKIHFSGTEDGKQHNQAFEISLYGTVAESENIPFTLPEVSTNKTYSFL
                IYTEVDIGELLMMKLKWISDSYFSWPDWWSSPSFVIERIRVKAGETQKKVIFCAREKV
                SHLQKGKDSAVFVKCHDKSLKKSG"
```

Things to Read

- ftp://ftp.ncbi.nih.gov/refseq/release/release-notes/RefSeq-release61.txt
  - 4.1.3 NUCLEOTIDE FEATURE ANNOTATION
  - 4.1.4 PROTEIN FEATURE ANNOTATION
  - 4.2 Tracking Identifiers
  - 4.2.1 GeneID
  - 4.2.2 Transcript ID
  - 4.2.3 Protein ID
  - 4.2.4 Conserved Domain Database (CDD) ID
  - 5.2 RefSeq Distribution Formats
- RefSeq FAQ
  - http://www.ncbi.nlm.nih.gov/books/NBK50679/

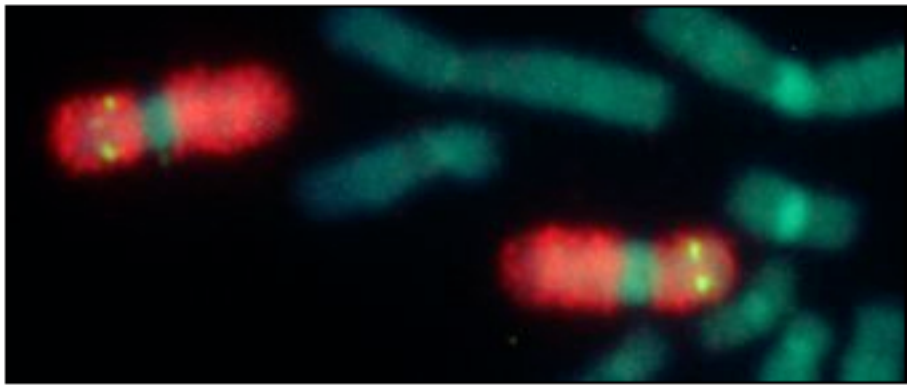
- Now for the Gene Database

NCBI Gene

Gene integrates information from a wide range of species

A record may include nomenclature, Reference Sequences (RefSeqs), maps, pathways, variations, phenotypes, and links to genome-, phenotype-, and locus-specific resources worldwide

2010 = The Gene Database

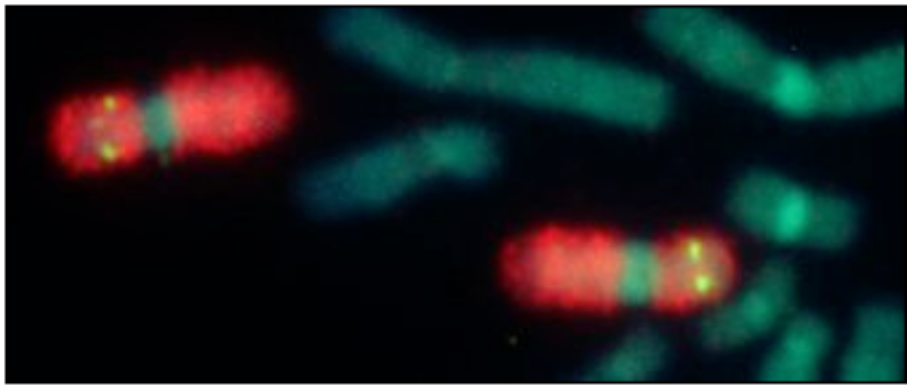


- Gene Centered Information
- Unifies NCBI-annotated and Submitted Genomes
- 8.19 million records for 7806 taxa (Oct 2010)
- Great place to start your searches
  - You'll see with the take home part of this lecture

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

http://www.ncbi.nlm.nih.gov/projects/Gene/gentrez_stats.cgi

2013 = The Gene Database

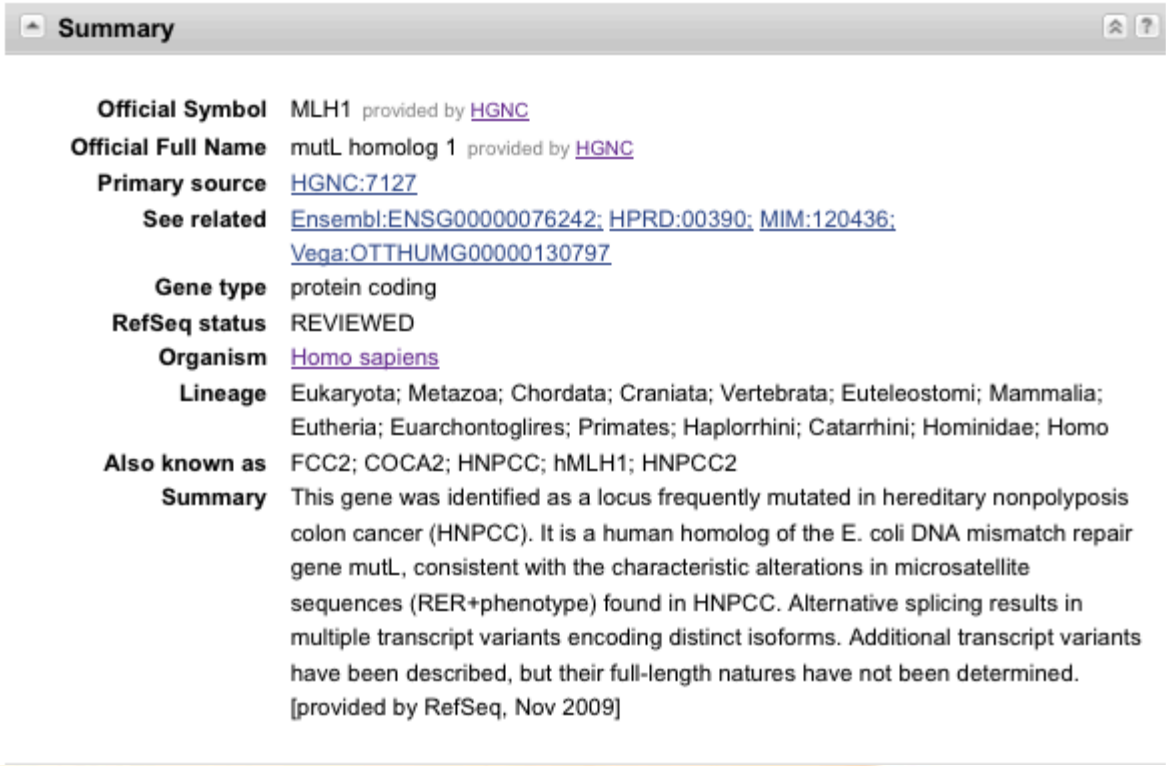


- Gene Centered Information
- Unifies NCBI-annotated and Submitted Genomes
- 13.366 million records for 11306 taxa (Oct 2013)
- Great place to start your searches
  - You'll see with the take home part of this lecture

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

http://www.ncbi.nlm.nih.gov/projects/Gene/gentrez_stats.cgi

Genes MLH1: One Stop Shopping

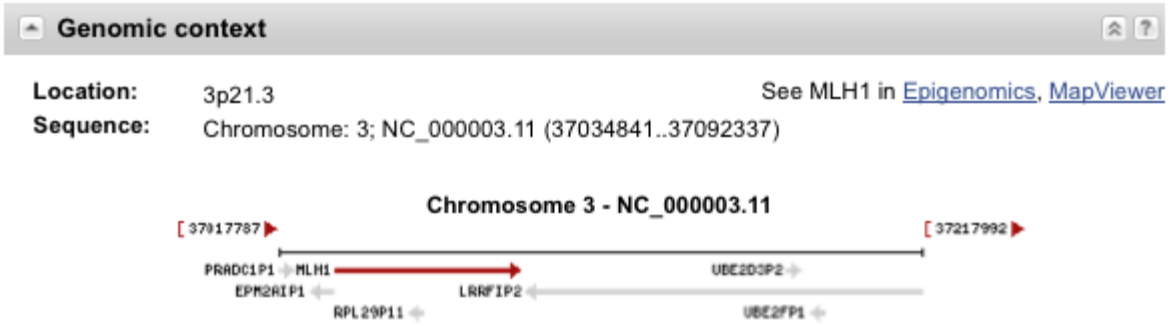


Summary

Official Symbol	MLH1 provided by HGNC
Official Full Name	mutL homolog 1 provided by HGNC
Primary source	HGNC:7127
See related	Ensembl:ENSG00000076242; HPRD:00390; MIM:120436; Vega:OTTHUMG00000130797
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Homo sapiens
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as	FCC2; COCA2; HNPCC; hMLH1; HNPCC2
Summary	This gene was identified as a locus frequently mutated in hereditary nonpolyposis colon cancer (HNPCC). It is a human homolog of the E. coli DNA mismatch repair gene mutL, consistent with the characteristic alterations in microsatellite sequences (RER+phenotype) found in HNPCC. Alternative splicing results in multiple transcript variants encoding distinct isoforms. Additional transcript variants have been described, but their full-length natures have not been determined. [provided by RefSeq, Nov 2009]

Gene Search: MLH1[All Fields] AND Homo Sapiens[Organism]
Great Place to Start a Search - Additional data also available

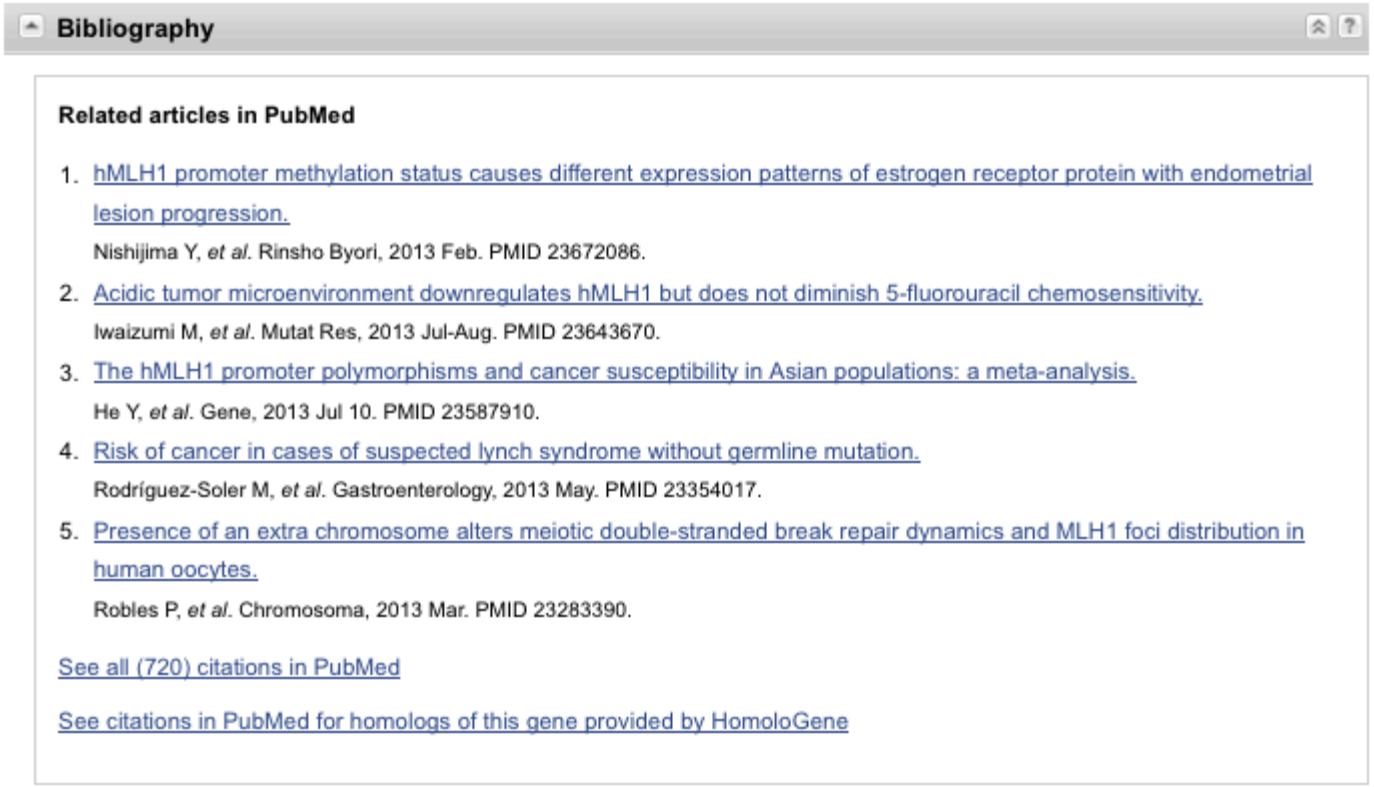
Genes MLH1: One Stop Shopping



Gene Search: MLH1[All Fields] AND Homo Sapiens[Organism]
Great Place to Start a Search - Additional data also available

Genes MLH1: One Stop Shopping

Genes MLH1: One Stop Shopping



Bibliography

Related articles in PubMed

1. hMLH1 promoter methylation status causes different expression patterns of estrogen receptor protein with endometrial lesion progression.
   Nishijima Y, *et al*. Rinsho Byori, 2013 Feb. PMID 23672086.
2. Acidic tumor microenvironment downregulates hMLH1 but does not diminish 5-fluorouracil chemosensitivity.
   Iwaizumi M, *et al*. Mutat Res, 2013 Jul-Aug. PMID 23643670.
3. The hMLH1 promoter polymorphisms and cancer susceptibility in Asian populations: a meta-analysis.
   He Y, *et al*. Gene, 2013 Jul 10. PMID 23587910.
4. Risk of cancer in cases of suspected lynch syndrome without germline mutation.
   Rodríguez-Soler M, *et al*. Gastroenterology, 2013 May. PMID 23354017.
5. Presence of an extra chromosome alters meiotic double-stranded break repair dynamics and MLH1 foci distribution in human oocytes.
   Robles P, *et al*. Chromosoma, 2013 Mar. PMID 23283390.

See all (720) citations in PubMed

See citations in PubMed for homologs of this gene provided by HomoloGene

Gene Search: MLH1[All Fields] AND Homo Sapiens[Organism]
Great Place to Start a Search - Additional data also available

NCBI Reference Sequences (RefSeq)

RefSeqs maintained independently of Annotated Genomes

These reference sequences exist independently of genome builds. Explain

Genomic

NG_007109.2 RefSeqGene

Range	5001..62497
Download	GenBank, FASTA, Sequence Viewer (Graphics), LRG_216

mRNA and Protein(s)

NM_000249.3 → NP_000240.1 DNA mismatch repair protein Mlh1 isoform 1

See proteins identical to NP_000240.1

Status: REVIEWED

Description	Transcript Variant: This variant (1) represents the longest transcript and encodes the longest isoform (1).
Source sequence(s)	BC006850, CN414955
Consensus CDS	CCDS2663.1
UniProtKB/Swiss-Prot	P40692
Related	ENSP00000231790, OTTHUMP00000161361, ENST00000231790, OTTHUMT00000253337

Conserved Domains (3) summary

cd00075 Location:31 – 122 Blast Score: 107	HATPase_c; Histidine kinase-like ATPases; This family includes several ATP-binding proteins for example: histidine kinase, DNA gyrase B, topoisomerases, heat shock protein HSP90, phytochrome-like ATPases and DNA mismatch repair proteins
cd03483 Location:211 – 335 Blast Score: 542	MutL_Trans_MLH1; MutL_Trans_MLH1: transducer domain, having a ribosomal S5 domain 2-like fold, found in proteins similar to yeast and human MLH1 (MutL homologue 1). This transducer domain is homologous to the second domain of the DNA gyrase B subunit, which is known to ...
TIGR00585 Location:6 – 315 Blast Score: 1082	mutl; DNA mismatch repair protein MutL

Gene Ontology Provided by GOA

Function	Evidence Code	Pubs
ATP binding	IEA	
ATPase activity	IBA	
contributes_to MutSalpha complex binding	IDA	PubMed
guanine/thymine mispair binding	IEA	
protein binding	IPI	PubMed
contributes_to protein binding	IPI	PubMed
contributes_to single-stranded DNA binding	IDA	PubMed

Process	Evidence Code	Pubs
ATP catabolic process	IBA	
double-strand break repair via nonhomologous end joining	IEA	
intrinsic apoptotic signaling pathway in response to DNA damage	IEA	
isotype switching	IEA	
male meiosis chromosome segregation	IEA	
meiotic metaphase I plate congression	IEA	
mismatch repair	IBA	
negative regulation of mitotic recombination	IEA	
nuclear-transcribed mRNA poly(A) tail shortening	IEA	
oogenesis	IEA	
reciprocal meiotic recombination	IBA	
resolution of meiotic recombination intermediates	IEA	
somatic hypermutation of immunoglobulin genes	IBA	
spermatogenesis	IEA	
spindle midzone assembly involved in meiosis	IEA	
synapsis	IEA	

Component	Evidence Code	Pubs
MutLalpha complex	IBA	
MutLbeta complex	IBA	
chiasma	IBA	
male germ cell nucleus	IEA	
mismatch repair complex	IBA	
nucleus	IC	PubMed
synaptonemal complex	IBA	

Other Data Sources include:
Phenotypes
Variation
Interactions
Pathways
General Protein Information
Related Sequences
Additional Links

NCBI Protein

The Protein database is a collection of sequences from several sources, including translations from annotated coding regions in GenBank, RefSeq and TPA, as well as records from SwissProt, PIR, PRF, and PDB. Protein sequences are the fundamental determinants of biological structure and function.

GenPept Database

- Database of GenBank gene products
  - Part of NCBI Protein Database
  - Contains:
    - a.a. translations from GenBank/EMBL/DDBJ records
      - that have a **coding region feature** annotated on them
- Any nucleotide sequence:
  - With **CDS region** is submitted to the GenBank database
  - Automatically translated into a.a sequence
- Genpept record is created based on the information included in **source record**

GenPept: GenBank CDS translations

```
FEATURES             Location/Qualifiers
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                     /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
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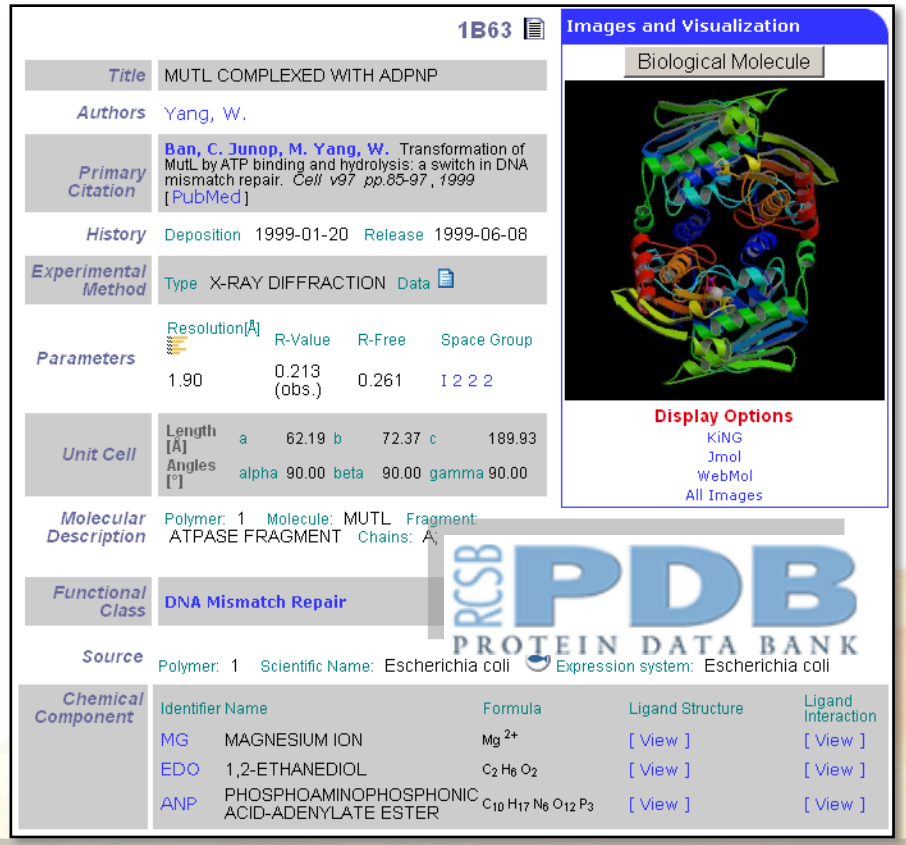
                     /db_xref="GI:463989"
                     /translation="MSFVAGVIRRLDETVVNRIAAGEVIQRPANAIKEMIENCLDAKS
                     TSIQVIVKEGGLKLIQIQDNGTGIRKEDLDIVCERFTTSKLQSFEDLASISTYGFRGE
                     ALASISHVAHVTITTKTADGKCAYRASYSDGKLKAPPKPCAGNQGTQITVEDLFYNIA
                     TRRKALKNPSEEYGKILEVVGRYSVHNAGISFSVKKQGETVADVRTLPNASTVDNIRS
```

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

At Home

- Check out the following
  - http://www.ncbi.nlm.nih.gov/protein/NP_004922
- Note is link to a RefSeq
- Look Over the Features

Protein Sequences from Structures



1B63

Title	MUTL COMPLEXED WITH ADPNP
Authors	Yang, W.
Primary Citation	Ban, C. Junop, M. Yang, W. Transformation of MutL by ATP binding and hydrolysis: a switch in DNA mismatch repair. *Cell v97 pp.85-97 , 1999* [PubMed]
History	Deposition 1999-01-20 Release 1999-06-08
Experimental Method	Type X-RAY DIFFRACTION Data

Parameters	Resolution[Å]	R-Value	R-Free	Space Group
	1.90	0.213 (obs.)	0.261	I 2 2 2

Unit Cell						
Length [Å]	a	62.19	b	72.37	c	189.93
Angles [°]	alpha	90.00	beta	90.00	gamma	90.00

Molecular Description: Polymer: 1 Molecule: MUTL Fragment: ATPASE FRAGMENT Chains: A

Functional Class: DNA Mismatch Repair

Source: Polymer: 1 Scientific Name: Escherichia coli Expression system: Escherichia coli

Chemical Component

Identifier	Name	Formula	Ligand Structure	Ligand Interaction
MG	MAGNESIUM ION	Mg^{2+}	[View]	[View]
EDO	1,2-ETHANEDIOL	$C_2 H_6 O_2$	[View]	[View]
ANP	PHOSPHOAMINOPHOSPHONIC ACID-ADENYLATE ESTER	$C_{10} H_{17} N_6 O_{12} P_3$	[View]	[View]

Images and Visualization

Biological Molecule

Display Options

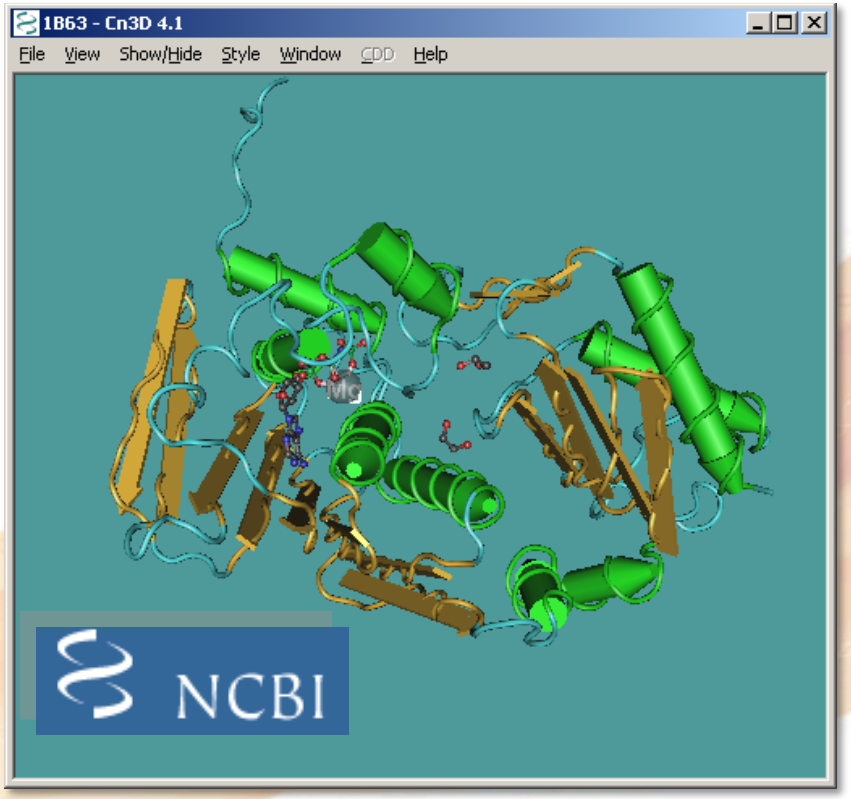
KiNG

Jmol

WebMol

All Images

RCSB PDB PROTEIN DATA BANK




```
>gi|5542073|pdb|1B63|A Chain A, Mutl Complexed With Adpnp
SHMPIQVLPPQLANQIAAGEVVERPASVVKELVENSLDAGATRIDIDIERGGAKLIRIRDNGCGIKKDEL
ALALARHATSKIASLDDLEAIISLGFRGEALASISSVSRLTLTSRTAEQQEAWQAYAEGRDMNVTVKPAA
HPVGTTLEVLDLFYNTPARRKFLRTEKTEFNHIDEIIRRIALARFDVTINLSHNGKIVRQYRAVPEGGQK
ERRLGAICGTAFLEQALAIEWQHGDLTLRGWVADPNHTTPALAEIQYCYVNGRMMRDRLINHAIRQACED
KLGADQQPAFVLYLEIDPHQVDVNVHPAKHEVRFHQSRLVHDFIYQGVLSVLQ
```

The Problems with Genbank and Genpept

- Lot of redundancy
- Same gene could be deposited into the database many times with different name
- **Different version** of the **same gene** could be **submitted many times** with different accession number
- The features of GenBank record could be chaotic

NCBI UniGene

UniGene computationally identifies transcripts from the same locus; analyzes expression by tissue, age, and health status; and reports related proteins (protEST) and clone resources.

Gene Expression is Regulated in Several Basic Ways

- By region (e.g. brain versus kidney)
- In development (e.g. fetal versus adult tissue)
- In dynamic response to environmental signals
  - e.g. immediate-early response genes)
- In disease states
- By gene activity

- UniGene data come from many cDNA libraries
  - Thus, when you look up a gene in UniGene you get information on its abundance and its regional distribution

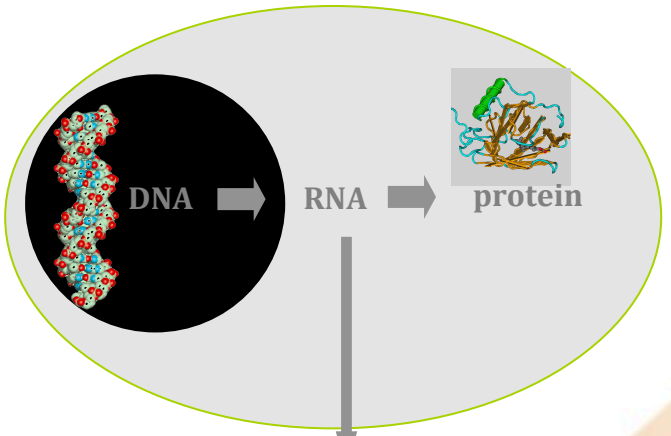
What is UniGene?

- Ongoing effort at NCBI to cluster EST sequences with traditional gene sequences
- A gene-oriented view of sequence entries
  - Organized view of the **Transcriptome**
- Blast based automated sequence clustering
  - Nonredundant set of gene oriented clusters
  - Each cluster a unique gene
- For each cluster, there is a lot of additional information included
  - Information on tissue types and map locations
  - Includes known genes and uncharacterized ESTs
  - Useful for gene discovery and selection of mapping reagents

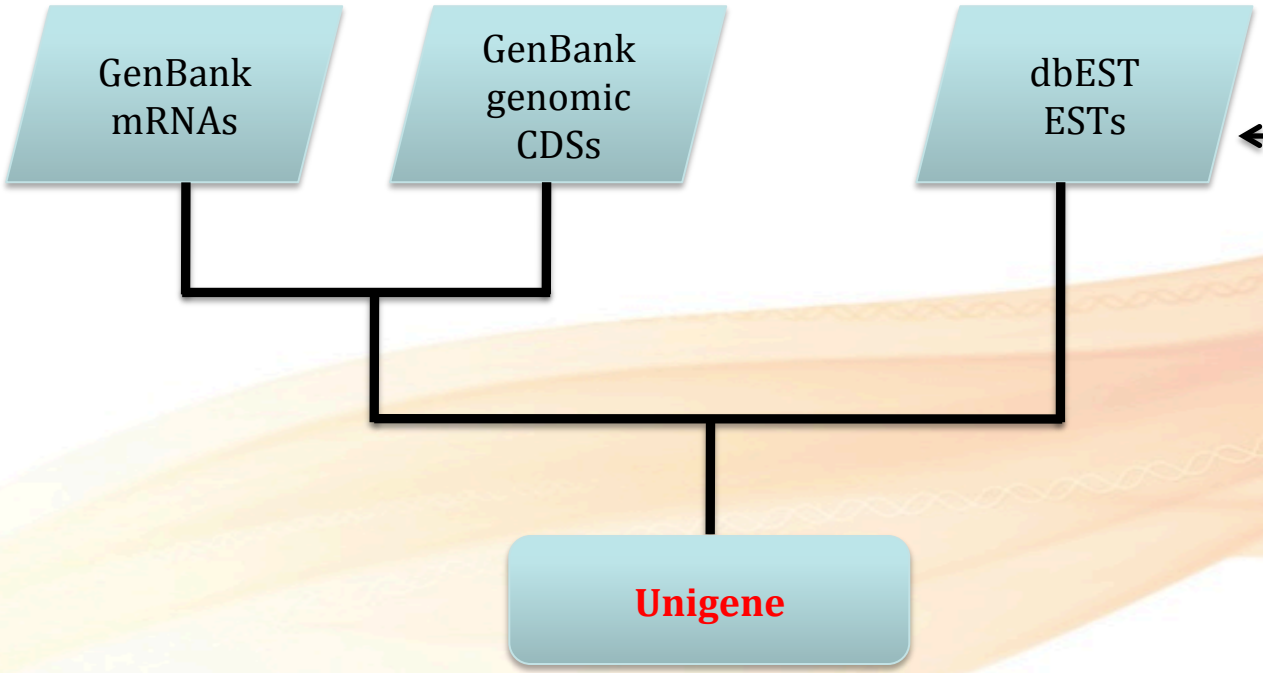
http://www.ncbi.nlm.nih.gov/unigene/

http://www.ncbi.nlm.nih.gov/UniGene/help.cgi?item=FAQ

UniGene

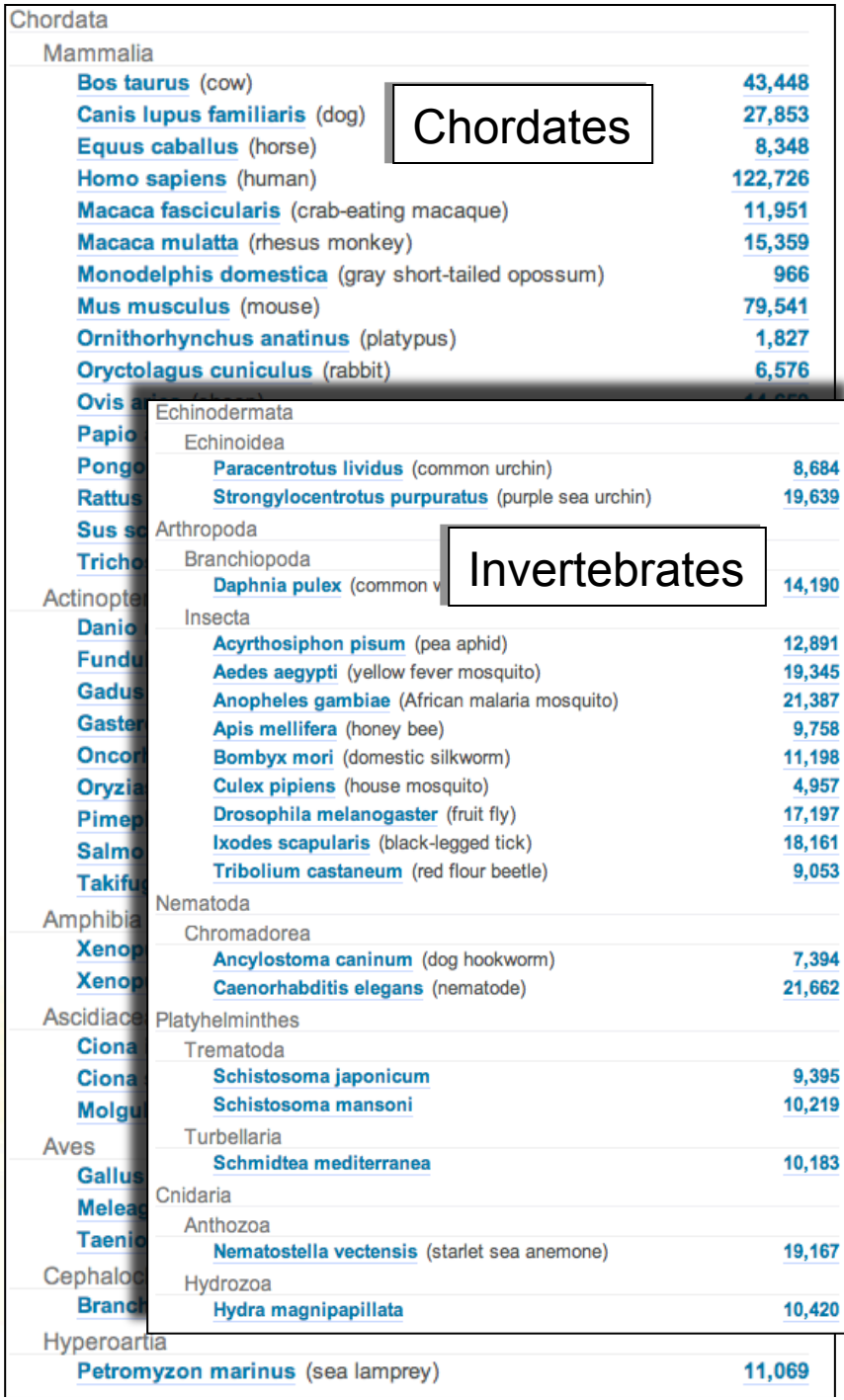


- A non-redundant set of gene-oriented clusters



- Organized view of the Transcriptome

UniGene


Chordata
Mammalia
Bos taurus (cow) 43,448
Canis lupus familiaris (dog) 27,853
Equus caballus (horse) 8,348
Homo sapiens (human) 122,726
Macaca fascicularis (crab-eating macaque) 11,951
Macaca mulatta (rhesus monkey) 15,359
Monodelphis domestica (gray short-tailed opossum) 966
Mus musculus (mouse) 79,541
Ornithorhynchus anatinus (platypus) 1,827
Oryctolagus cuniculus (rabbit) 6,576
Chordates
Echinodermata
Echinoidea
Paracentrotus lividus (common urchin) 8,684
Strongylocentrotus purpuratus (purple sea urchin) 19,639
Arthropoda
Branchiopoda
Daphnia pulex 14,190
Insecta
Acyrthosiphon pisum (pea aphid) 12,891
Aedes aegypti (yellow fever mosquito) 19,345
Anopheles gambiae (African malaria mosquito) 21,387
Apis mellifera (honey bee) 9,758
Bombyx mori (domestic silkworm) 11,198
Culex pipiens (house mosquito) 4,957
Drosophila melanogaster (fruit fly) 17,197
Ixodes scapularis (black-legged tick) 18,161
Tribolium castaneum (red flour beetle) 9,053
Invertebrates
Nematoda
Chromadorea
Ancylostoma caninum (dog hookworm) 7,394
Caenorhabditis elegans (nematode) 21,662
Platyhelminthes
Trematoda
Schistosoma japonicum 9,395
Schistosoma mansoni 10,219
Turbellaria
Schmidtea mediterranea 10,183
Cnidaria
Anthozoa
Nematostella vectensis (starlet sea anemone) 19,167
Hydrozoa
Hydra magnipapillata 10,420
Hyperoartia
Petromyzon marinus (sea lamprey) 11,069


Streptophyta
Bryopsida
Physcomitrella patens 20,137
Coniferopsida
Picea glauca (white spruce) 17,809
Picea sitchensis (Sitka spruce) 16,755
Pinus taeda (loblolly pine) 18,921
Plants
Eudicotyledons
Aquilegia formosa x Aquilegia pubescens 8,063
Arabidopsis thaliana (thale cress) 30,383
Artemisia annua (sweet wormwood) 9,462
Brassica napus (rape) 26,733
Brassica oleracea 5,617
Brassica rapa (field mustard) 14,497
Citrus clementina 6,107
Citrus sinensis (Valencia orange) 15,815
Glycine max (soybean) 30,248
Gossypium hirsutum (upland cotton) 21,743
Gossypium raimondii 3,297
Helianthus annuus (sunflower) 7,846
Lactuca sativa (garden lettuce) 7,940
Lotus japonicus 14,493
Malus x domestica (apple) 16,932
Medicago truncatula
Nicotiana tabacum
Populus tremula x Populu
Populus trichocarpa
Prunus persica (peach)
Raphanus raphanistrum
Raphanus sativus (radish)
Solanum lycopersicum
Solanum tuberosum
Vigna unguiculata
Vitis vinifera (wine grape)
Liliopsida
Hordeum vulgare (barley)
Oryza sativa (rice)
Saccharum officinarum
Sorghum bicolor
Triticum aestivum (Wheat)
Zea mays (maize)
Chlorophyta
Chlorophyceae
Chlamydomonas reinhardt
Volvox carteri
Dictyosteliida
Dictyostelium
Dictyostelium discoideum (slime mold) 5,957
Apicomplexa
Coccidia
Toxoplasma gondii 6,623
Fungi et al.
Ascomycota
Eurotiomycetes
Coccidioides posadasii 3,994
Sordariomycetes
Gibberella moniliformis 5,259
Magnaporthe grisea 5,380
Neurospora crassa 2,216
Basidiomycota
Heterobasidiomycetes
Filobasidiella neoformans 4,838
Oomycetes
Peronosporales
Phytophthora infestans (potato late blight agent) 7,257
Bacillariophyta
Bacillariophyceae
Phaeodactylum tricornutum 6,778

Unigene Identfier

- **Hs** for human
- **Mm** for mouse
- **Rn** for rat
- **Bt f**or cow
- **Dr** for zebrafish
- **Dm** for fruitfly
- **Aga** for malaria mosquito
- **Xl** for African clawed frog (Xenopus laevis)
- **At** for thale cress (*Arabidopsis thaliana)*
- **Os** for rice
- **Ta** for wheats
- **Zm** for maize

Examples:
Mm.213407
Hs.214142
At.138
Ppr.17865

UniGene build RSS

Should be noted that the procedures for automated sequence clustering are still under development and the results may change from time to time as improvements are made

Unigene is regularly rebuilt. Therefore, cluster identifiers are not stable gene indices

ftp://ftp.ncbi.nlm.nih.gov/repository/UniGene/

Homo sapiens: UniGene Build #236

Sequences Included in UniGene

Known genes are from GenBank 09 Mar 2013
ESTs are from dbEST through 09 Mar 2013

221,081	mRNAs
96	Models
20,073	HTC
1,695,050	EST, 3'reads
4,029,704	EST, 5'reads
1,034,785	EST, other/unknown
7,000,789	total sequences in clusters

Build Method: Genome Based

Alignments between transcript sequences and genomic sequences are used to generate clusters of sequences originating from the same gene. More...

Final Number of Clusters (sets)

130,056	sets total
34,637	sets contain at least one mRNA
11,925	sets contain at least one HTC sequence
124,724	sets contain at least one EST
29,805	sets contain both mRNAs and ESTs

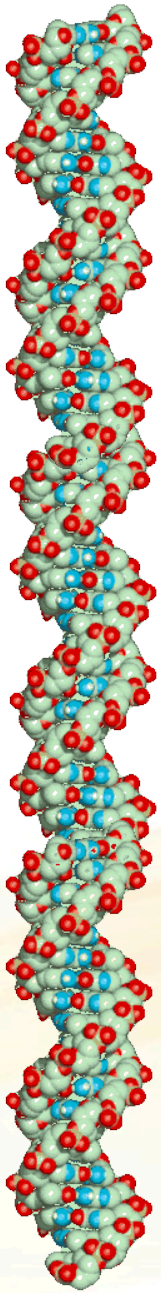
Hs for human

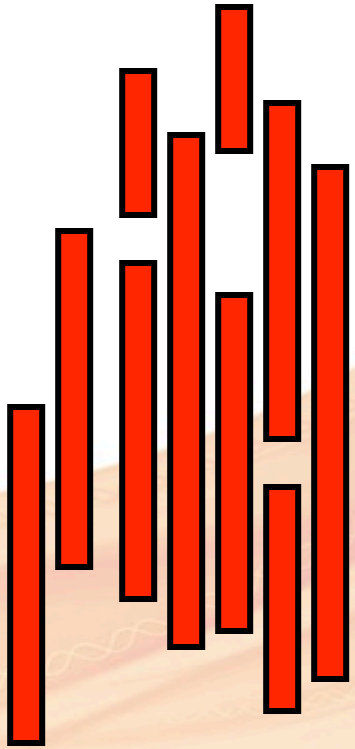
Histogram of cluster sizes for UniGene Hs build 236

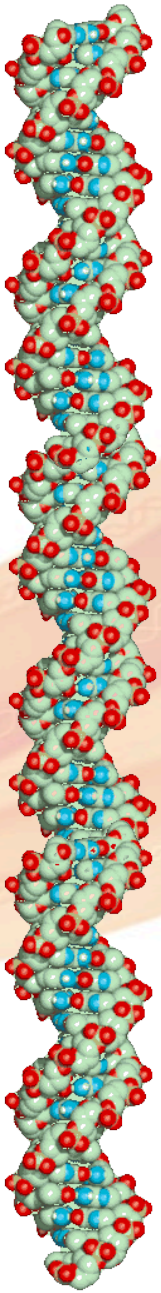
Cluster size	Number of clusters
32769-65536	1
16385-32768	4
8193-16384	20
4097-8192	56
2049-4096	224
1025-2048	729
513-1024	1982
257-512	4117
129-256	4557
65-128	3889
33-64	3858
17-32	4815
9-16	7177
5-8	10637
3-4	12760
2	10859
1	64371

sequences

Cluster Sizes in UniGene







This is a gene with 10 ESTs associated; the cluster size is 10

Tom Peavy

Gene Catalog: Fathead Minnow MLH1Cluster

UGID:2056516 UniGene Ppr.17865 *Pimephales promelas* (fathead minnow) Links

Transcribed locus, strongly similar to NP_956953.1 DNA mismatch repair protein Mlh1 [Danio rerio]

Ppr.17865

SELECTED PROTEIN SIMILARITIES

Comparison of cluster transcripts with RefSeq proteins. The alignments can suggest function of the cluster.

Best Hits and Hits from model organisms		Species	Id(%)	Len(aa)
NP_956953.1	DNA mismatch repair protein Mlh1	*D. rerio*	94.4	723
NP_001090545.1	mutL homolog 1,			
NP_000240.1	DNA mismatch re			
NP_081086.2	DNA mismatch re			
XP_001690102.1	mismatch repair p			
NP_477022.1	Mlh1			
XP_962522.1	hypothetical prote			
NP_013890.1	Mlh1p			
NP_567345.2	DNA mismatch re			
	Othe			
XP_002933455.1	PREDICTED: LO			
XP_001170433.1	PREDICTED: DN			

SEQUENCES

Sequences representing this gene; mRNAs, ESTs, and gene predictions supported by transcribed sequences.

ESTs

EST sequences (10 of 22) [Show all sequences]

DT170650.1	Clone ANNO42204	whole body	3' read **P**
DT170651.1	Clone ANNO42204	whole body	5' read **P**
DT182780.1	Clone ANNO49223	whole body	3' read **P**
DT182781.1	Clone ANNO49223	whole body	5' read **P**
DT191378.1	Clone ANNO54379	whole body	5' read **P**
DT206388.1	Clone CAAS13911	brain	3' read **PA**
DT206389.1	Clone CAAS13911	brain	5' read **P**
DT225851.1	Clone CAAT2335	brain	3' read **PA**
DT225852.1	Clone CAAT2335	brain	5' read **P**
DT237339.1	Clone CAAT9738	brain	3' read **P**

Download Sequences

Key to Symbols

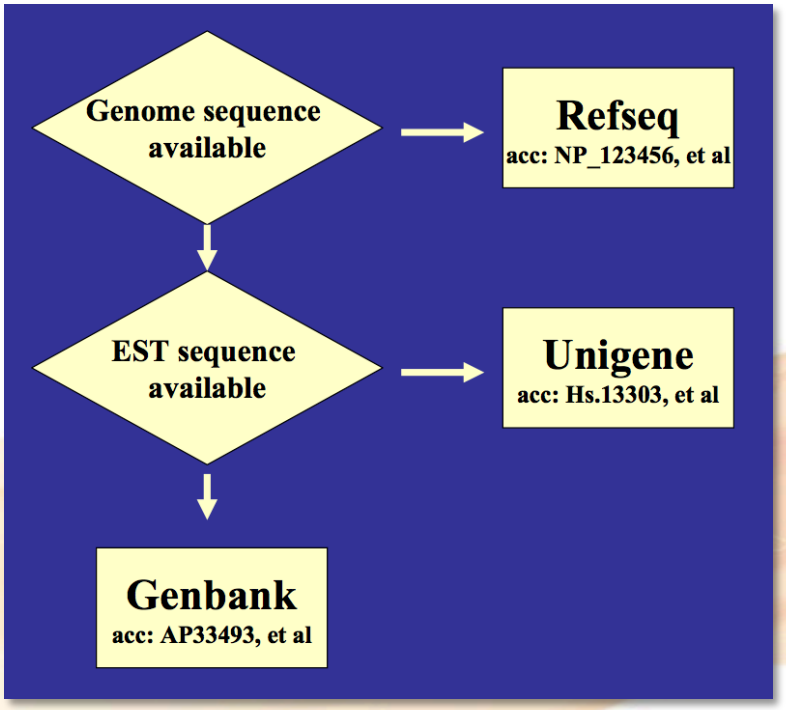
P Has similarity to known **P**roteins (after translation)
A Contains a poly-**A**denylation signal
S Sequence is a **S**uboptimal member of this cluster
M Clone is putatively CDS-complete by **M**GC criteria

http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?UGID=2056516&TAXID=90988&SEARCH=Ppr.17865

FYI – How is Unigene Clustered

- Transcript-Based Build Procedure
  - http://www.ncbi.nlm.nih.gov/UniGene/help.cgi?item=build1
- Genome-Based UniGene Build Procedure
  - http://www.ncbi.nlm.nih.gov/UniGene/help.cgi?item=build2
- Pontius JU, Wagner L, Schuler GD. UniGene: a unified view of the transcriptome. In: The NCBI Handbook. Bethesda (MD): National Center for Biotechnology Information; 2003.

Refseq? Unigene? Genbank?



Conclusion: UniGene

- UniGene is a useful tool to look up information about expressed genes
- UniGene displays information about:
  - The abundance of a transcript (expressed gene)
  - As well as its regional distribution of expression (e.g. brain vs. liver)

FYI - NCBI - MMDB

MMDB: Molecular Modeling DataBase

- Derived from experimentally determined PDB records
- Value added to PDB records including:
  - Explicit chemical graphs
  - Computationally identified similar 3D structure
  - Links:
    - literature
    - Similar sequences
    - information about chemicals bound to the structure
  - Validation (secondary structure elements)
  - Inclusion of Taxonomy, Citation
  - Conversion to ASN.1 data description language
- Structure neighbors determined by
- Vector Alignment Search Tool (VAST)

http://www.ncbi.nlm.nih.gov/Structure/MMDB/mmdb.shtml

Connections in MMDB

- Make it possible to find 3D structures for homologs of a protein sequence of interest then interactively view:
  - Sequence-structure relationships
  - Active sites
  - Bound chemicals
  - Journal articles
- How do you search?
  - If you don't know a PDB accession ID
    - Search MMDB using the gene and or protein name (e.g. COMT)
  - If you know the PDB
    - Search MMDB 1VID for example

http://www.ncbi.nlm.nih.gov/structure/?term=COMT&SITE=NcbiHome&submit.x=0&submit.y=0

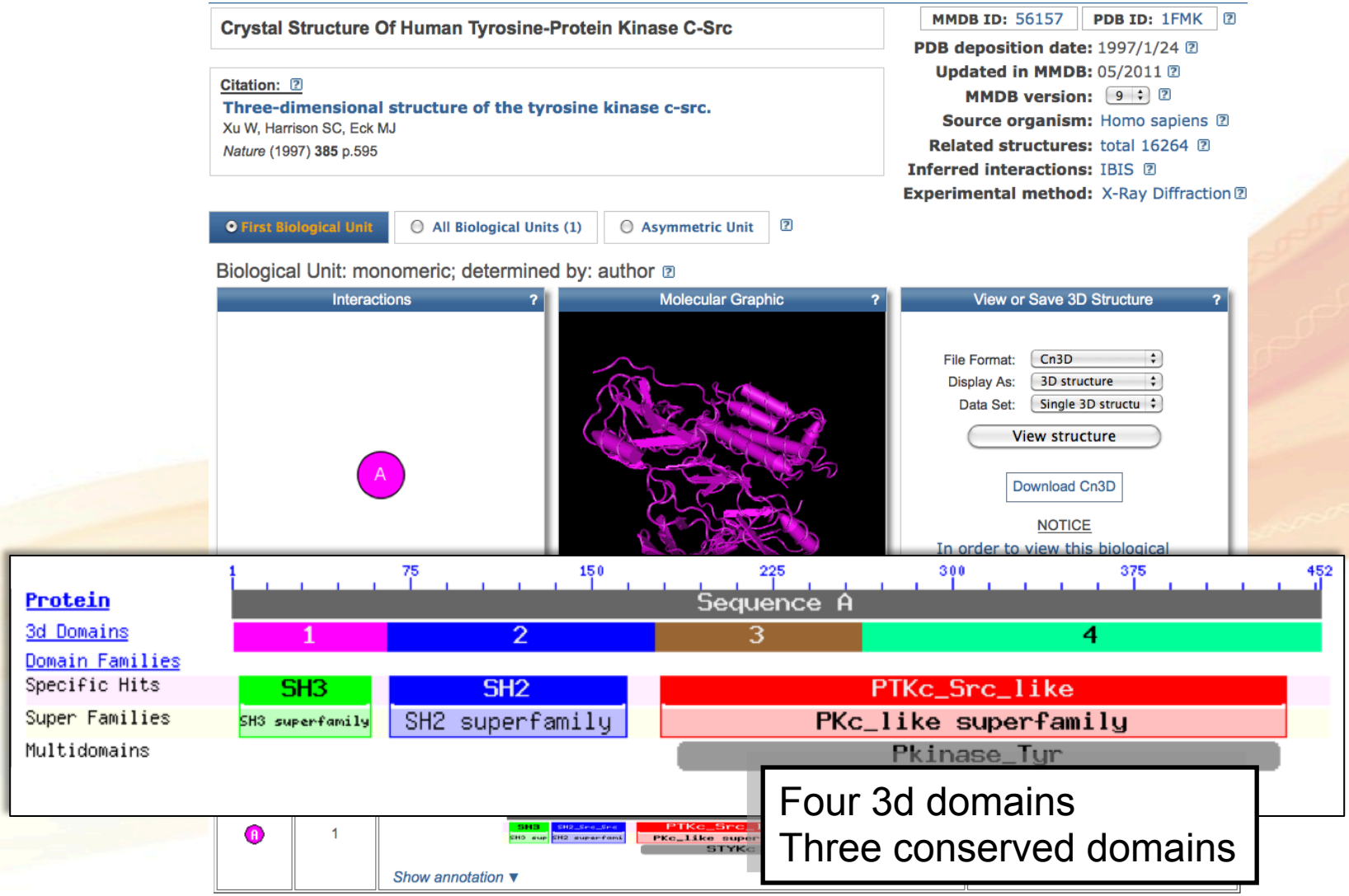
http://www.ncbi.nlm.nih.gov/structure/?term=1VID&SITE=NcbiHome&submit.x=0&submit.y=0

Exercise - Links to Explore

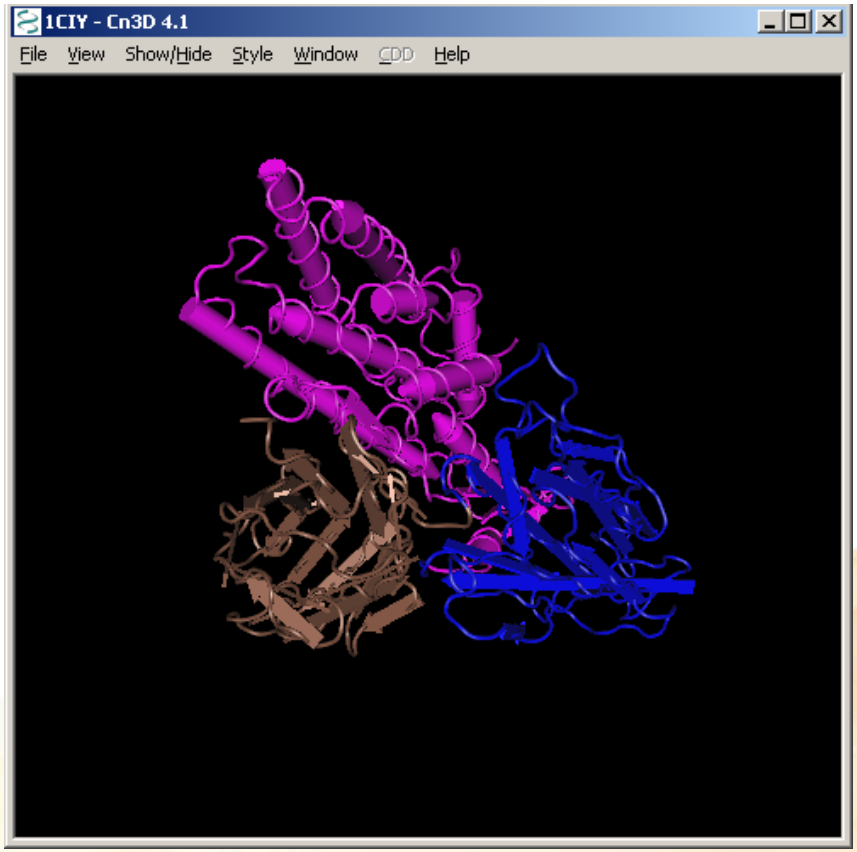
- Search MMDB 1FMK for example
  - Click PDB ID link: 1FMK at the top
  - Click the View in Cn3D, to view the structure on your computer
    - Install Cn3D first
  - Click the Similar Structures: How many related structures to Domain 1
  - Click the domain image to view the CDD neighbors (click Show annotation first)
    - Click the Protein to see the PDB entry in GenPept
      - Scan the file contents
    - Click the Conserved Domains "Domain Families" link to see entry in the Conserved Domain Database

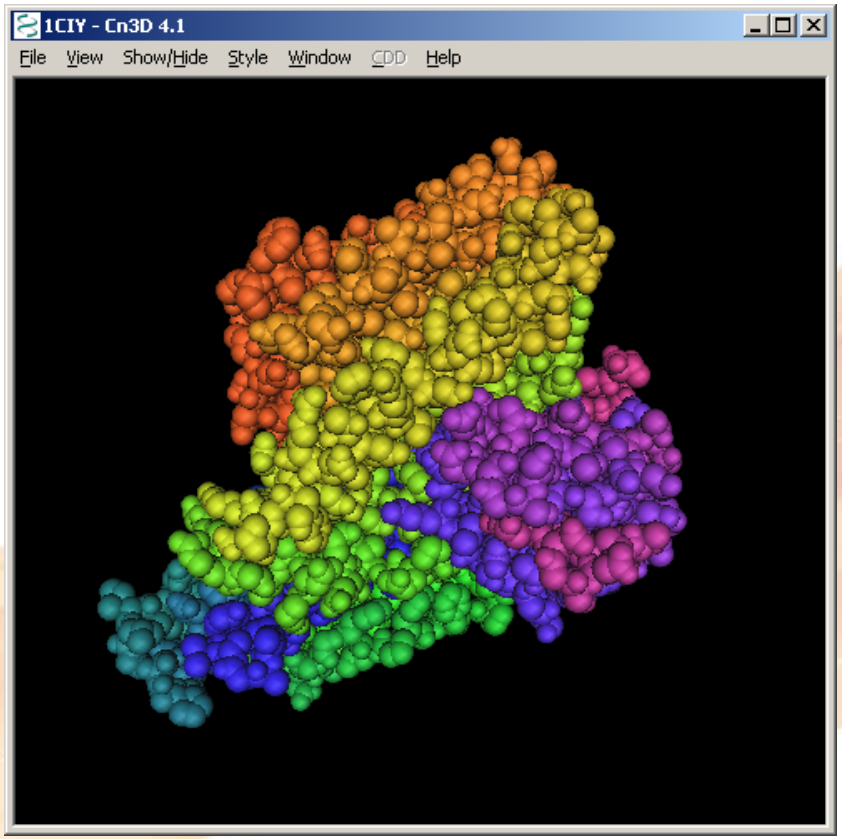
http://www.ncbi.nlm.nih.gov/Structure/MMDB/mmdb.shtml

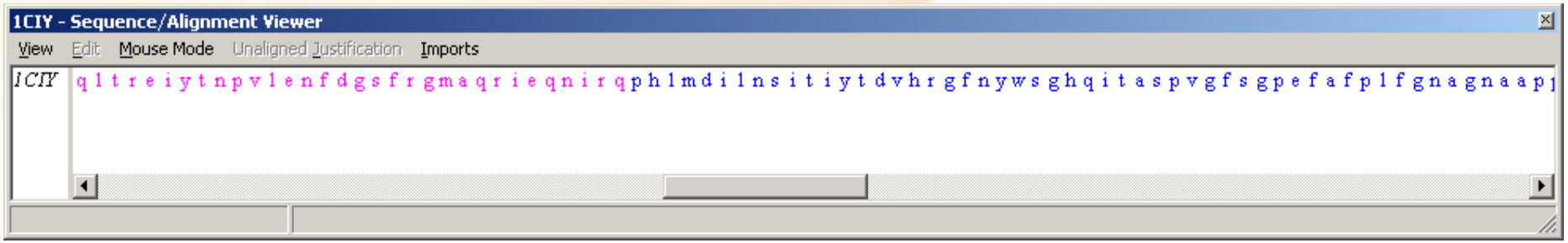
Human Tyrosine-Protein Kinase C-Src Domains



Cn3D 4.1: *Bacillus thuringiensis* Toxin





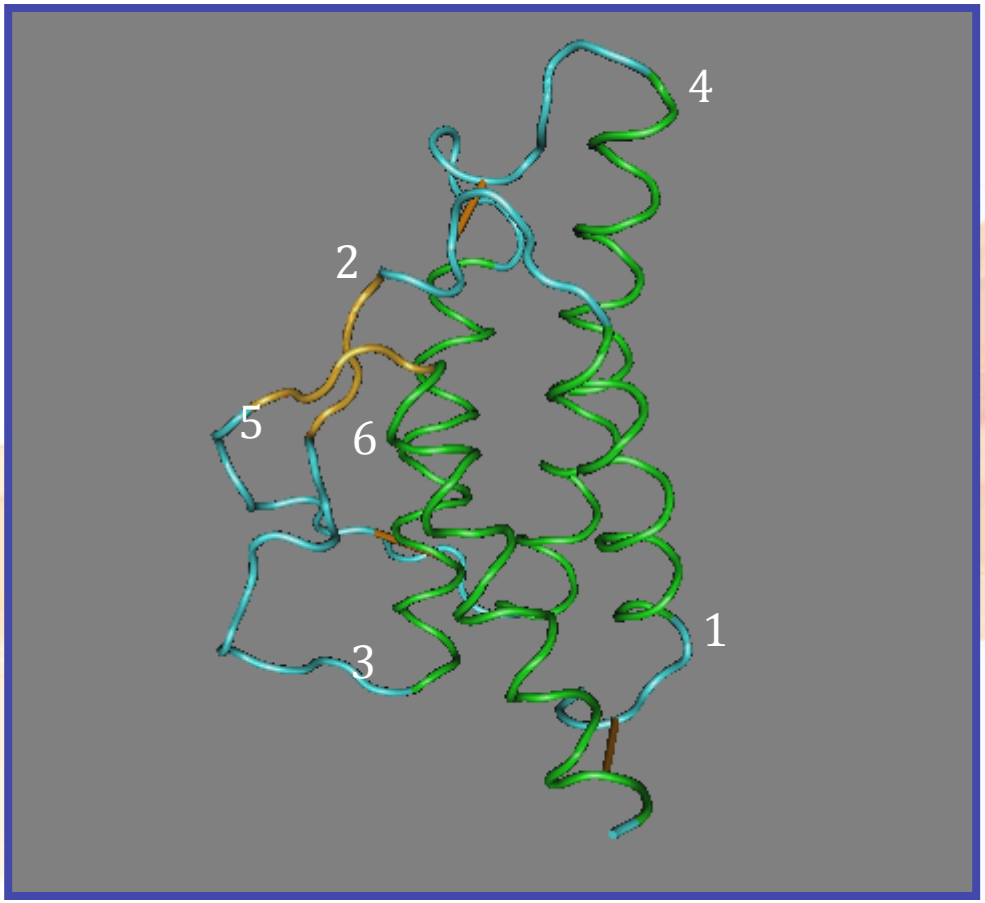


FYI - Structure - VAST

VAST: Related Structures

Vector Alignment Search Tool

For each protein chain



Human IL-4

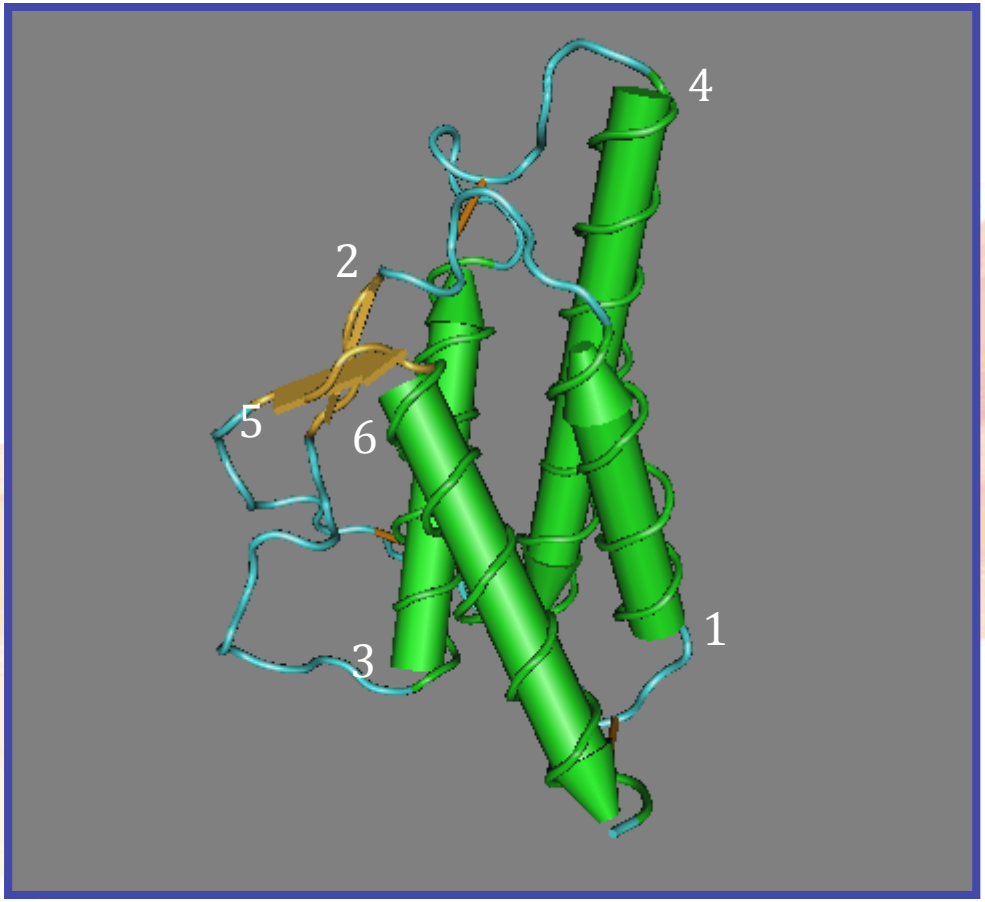
http://www.ncbi.nlm.nih.gov/Structure/VAST/vast.shtml

VAST: Related Structures

Vector Alignment Search Tool

For each protein chain

locate SSEs (secondary structure elements)



Human IL-4

http://www.ncbi.nlm.nih.gov/Structure/VAST/vast.shtml

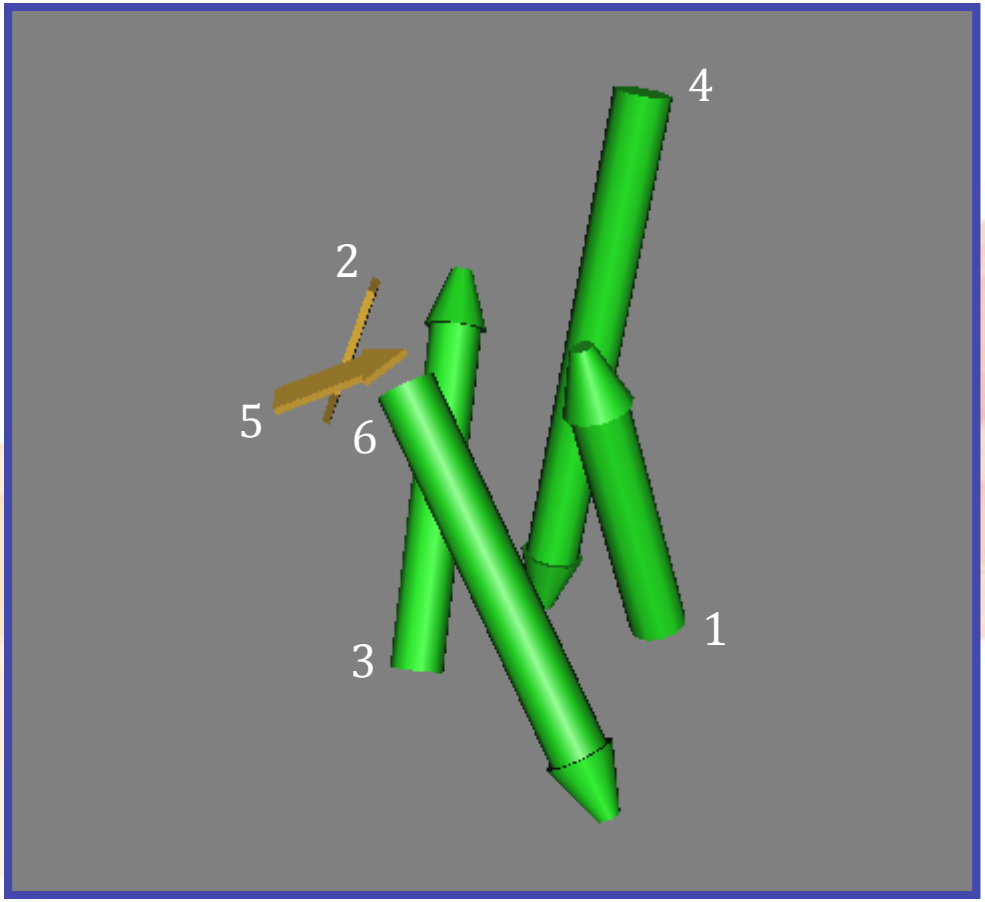
VAST: Related Structures

Vector Alignment Search Tool

For each protein chain

locate SSEs (secondary structure elements)

and represent them as individual vectors



Human IL-4

http://www.ncbi.nlm.nih.gov/Structure/VAST/vast.shtml

VAST: Related Structures

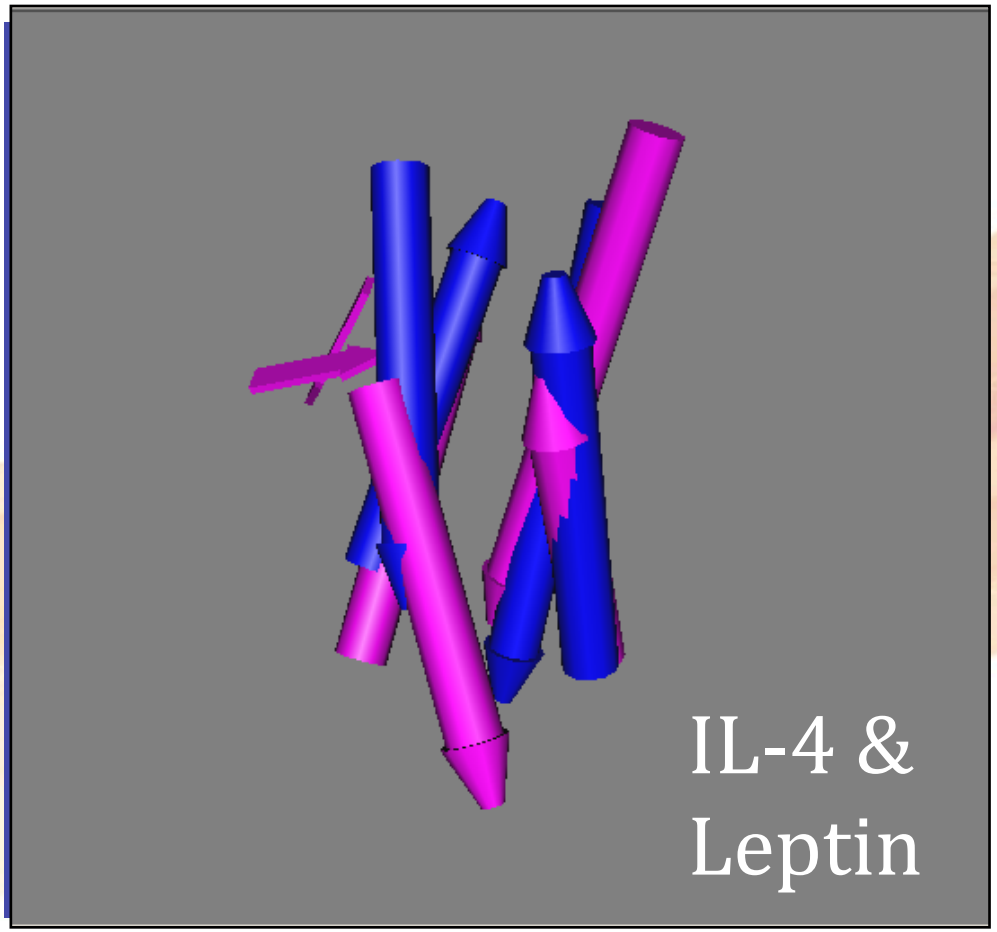
Vector Alignment Search Tool

For each protein chain

locate SSEs (secondary structure elements)

and represent them as individual vectors

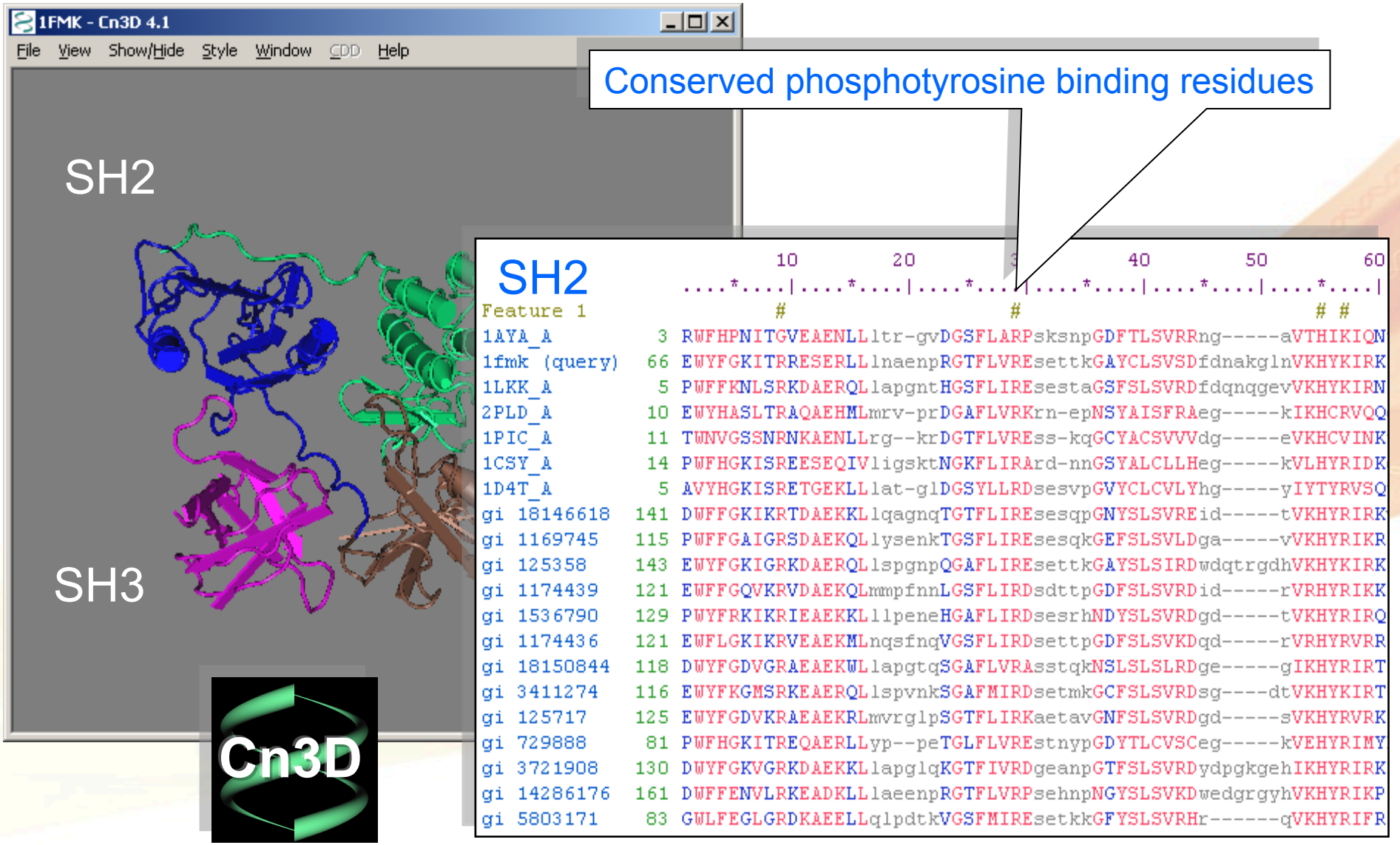
align the vectors



Human IL-4

http://www.ncbi.nlm.nih.gov/Structure/VAST/vast.shtml

Structure vs Conserved Domain



Protein Domains

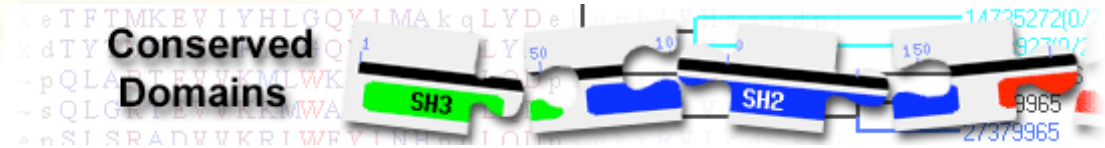
- What are Protein Domains?
  - Structural Domain
    - Discrete independently folding unit of a protein
  - Conserved Domain (sequence-based)
    - Protein region with recognizable position-specific pattern of sequence conservation
- Sequence-based domains often roughly correspond to structural domains
- Domains often have distinct, identifiable functions

NCBI Conserved Domain Database (CDD)

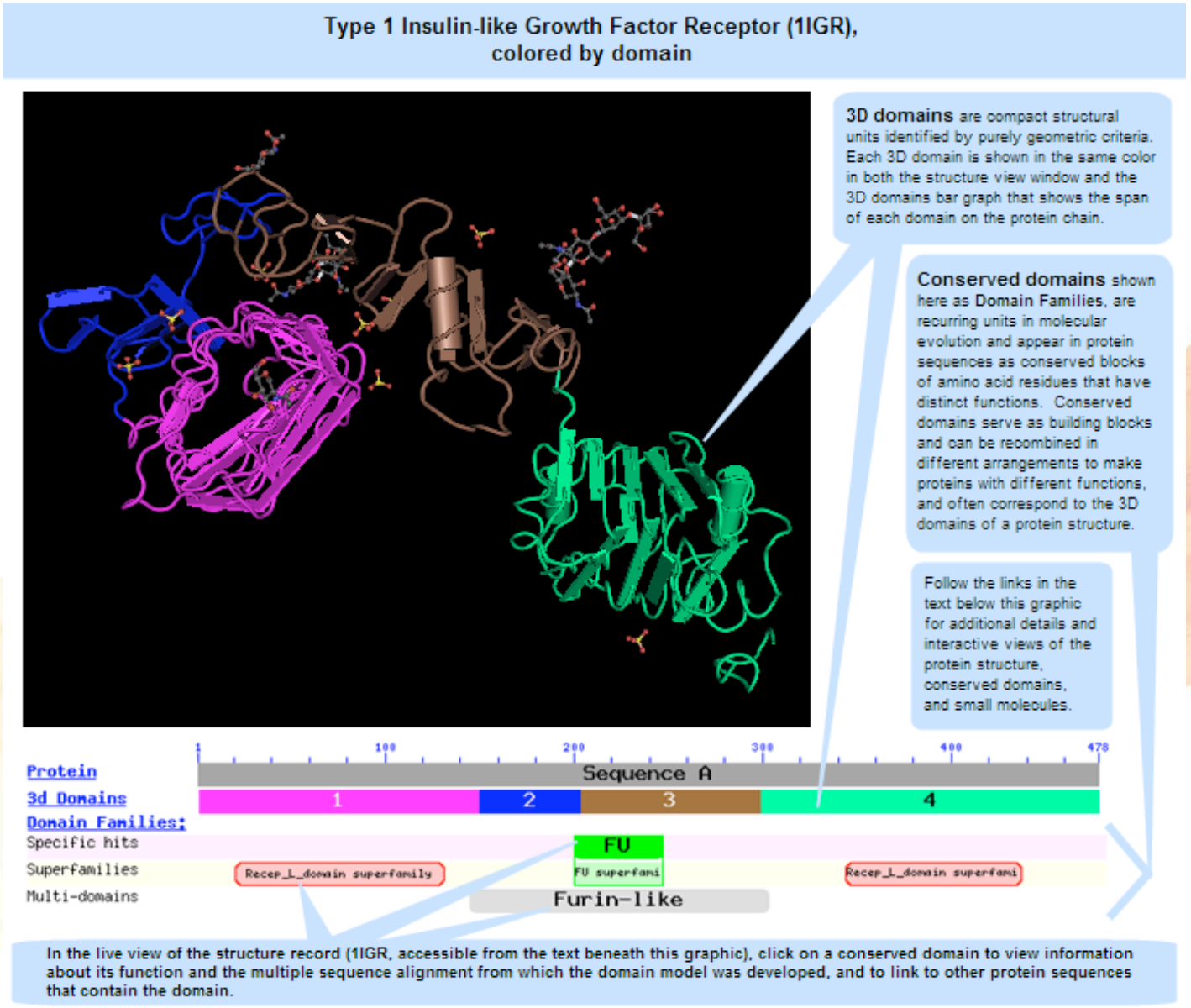
NCBI's Conserved Domain Database

- Protein annotation resource that consists of a collection of well-annotated multiple sequence alignment models for ancient domains and full-length proteins
- Available as Position-specific scoring matrices (PSSM)
  - For Fast identification of conserved domains in protein sequences via RPS-BLAST
- Sources
  - SMART
  - Pfam
  - COGs
  - NCBI curated domains
    - Structure-informed alignments

http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml



What is a Conserved Domain

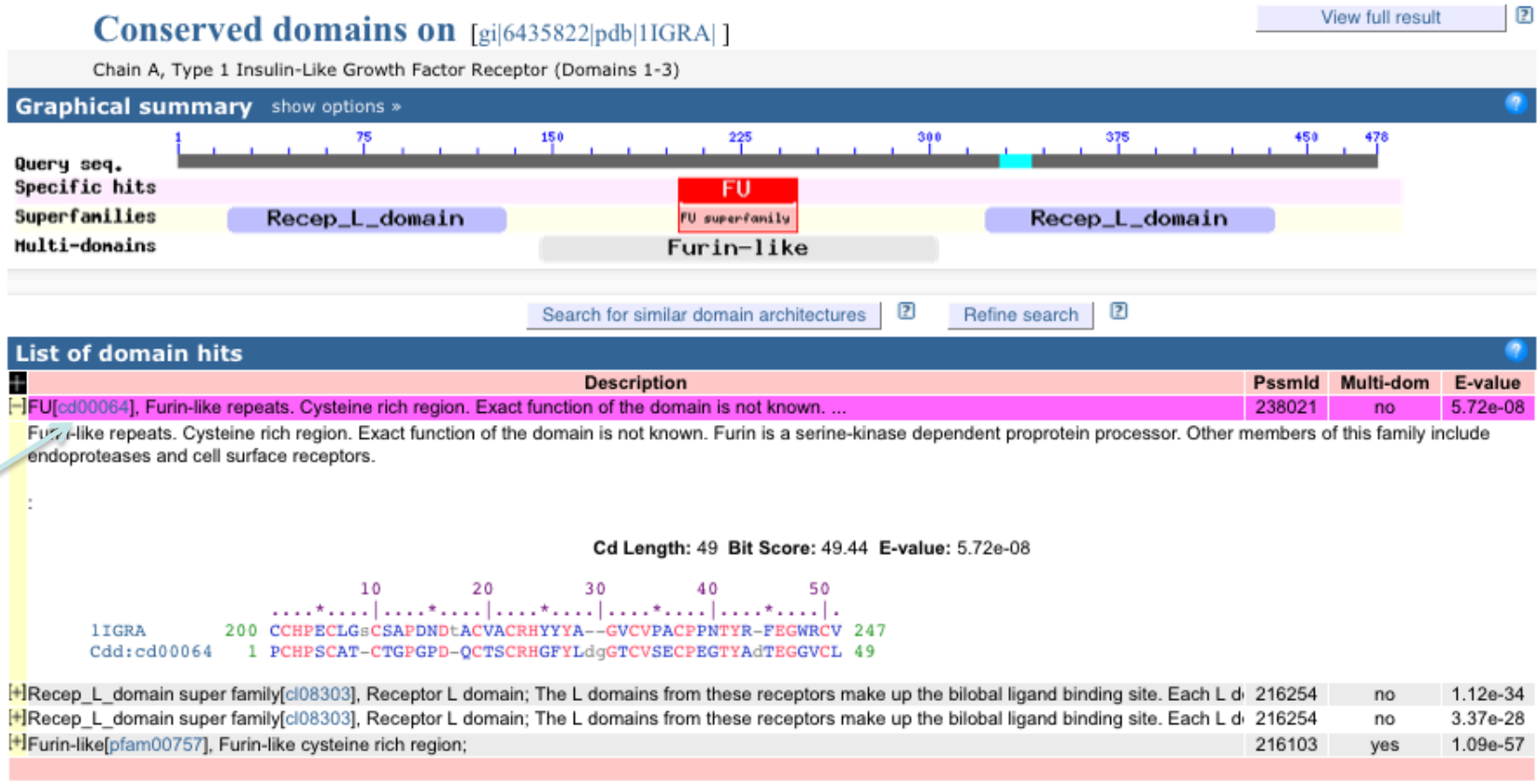


What is a Conserved Domain @ NCBI

- Distinct functional and/or structural units of a protein
- These two classifications coincide often
- Domains are often identified as recurring (sequence or structure) units
  - May exist in various contexts
  - Previous slide, four "domains" colored in magenta, blue, brown, and green
- In molecular evolution such domains may have been:
  - **Utilized as building blocks**
  - **Recombined in different arrangements** to modulate protein function

- NCBI defines conserved domains - recurring units in molecular evolution, the extents of which can be determined by sequence and structure analysis

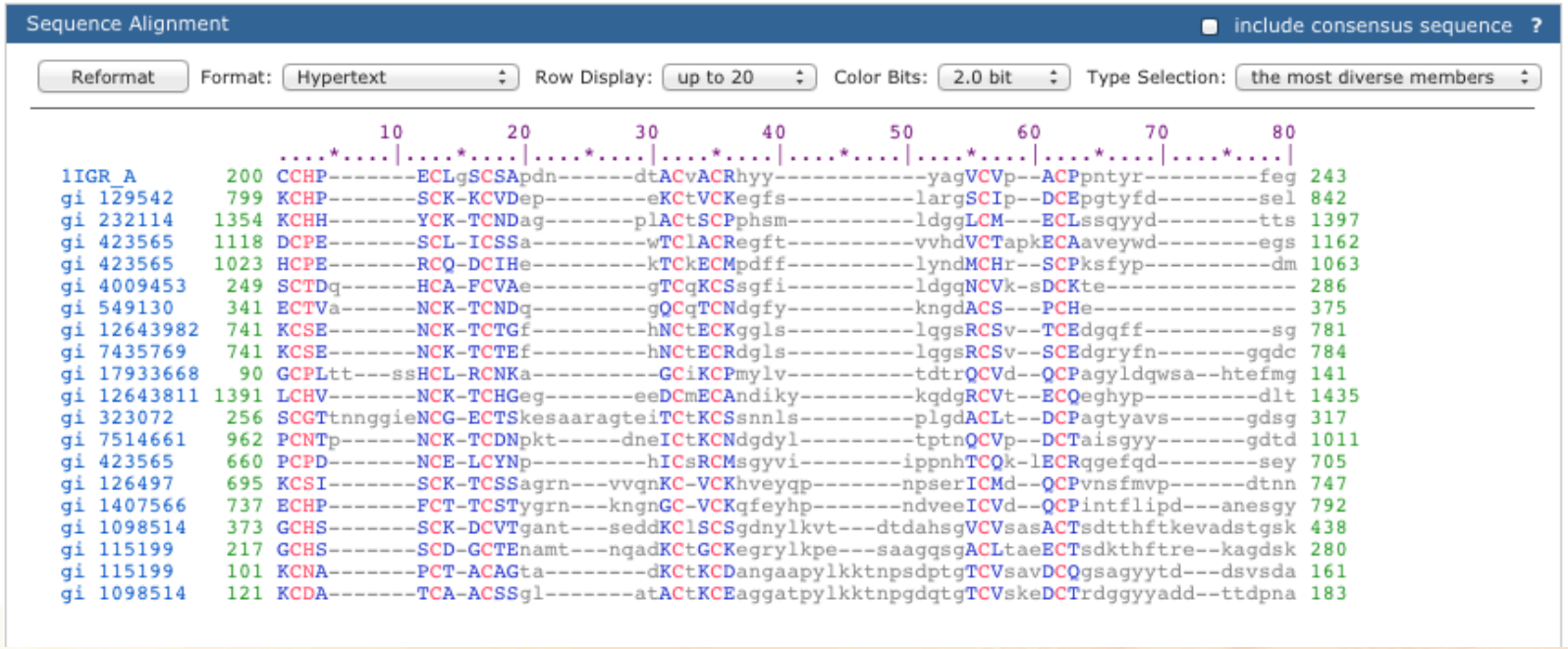
Conserved Domain Model



Click here

http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?INPUT_TYPE=precalc&SEQUENCE=6435822

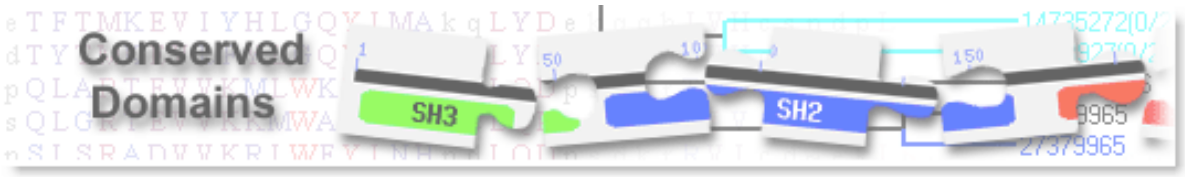
Conserved Domain Model



Based on MSA of related protein spanning a variety of organisms to reveal sequence regions that contain the same, or similar patterns of amino acids

http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?ascbin=8&maxaln=10&seltype=2&uid=238021

Search with Protein Query



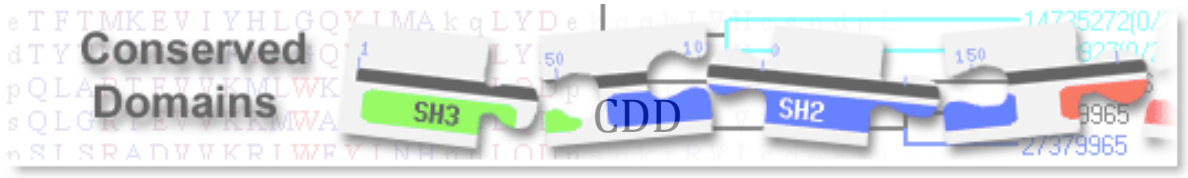
Protein or Nucleotide Query Sequence

Enter a protein or nucleotide query as an accession or GI number (e.g., AAC50285 or 463989), or as a sequence in FASTA format, to identify the protein's conserved domains and therefore its putative function:

Submit Query Search Database CDD v3.10 – 44354 PSSMs

>gi|45549418|gb|AAS67634.1| ATP7A [Solenodon paradoxus]
IVYQPHLITVEEIKKQIKAVGFPAFIKKQPKYLKLGAIDIERLKNIPVKSSEGSQQMS
PSSTNDSKVTLTIDGMHCNSCVSNIESALSTLHYVSSIVVSLQNKSAIIKYNANSVT
PEILKKAIEAISPGQYRVSITSEVESTSNSPSSSSQKAPLNVVSQPLTQVTVINING
MTCNSCVQSIEGVMSKKAGVKSIQVSLANRNGTVEYDPLLTSPEILRE

http://www.ncbi.nlm.nih.gov/Structure/cdd/docs/cdd_search.html



Click here 1st

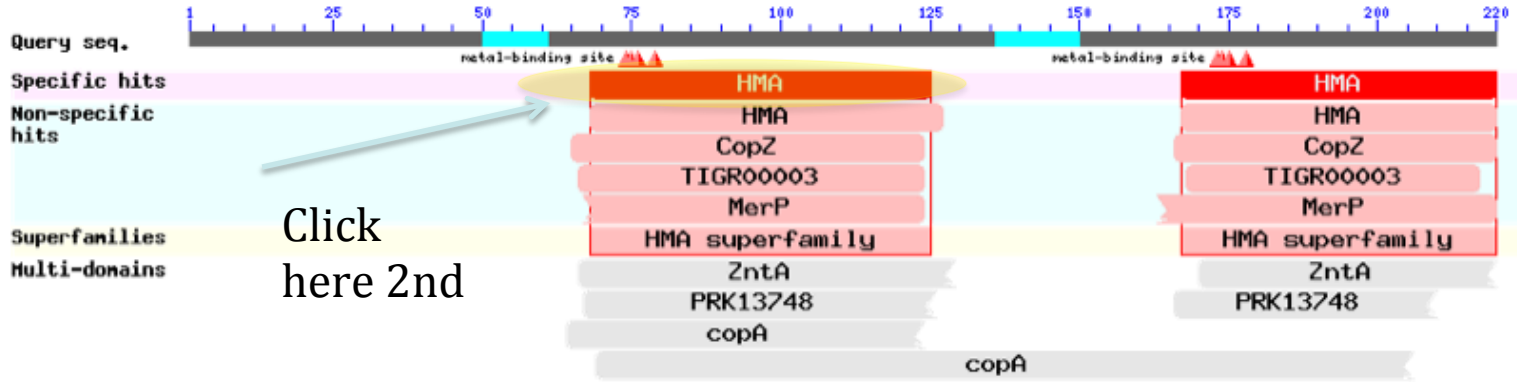
Previous protein query – Found conserved domains

Conserved domains on [gi|45549418|gb|AAS67634|]

View concise result

ATP7A, partial [Solenodon paradoxus]

Graphical summary show options »

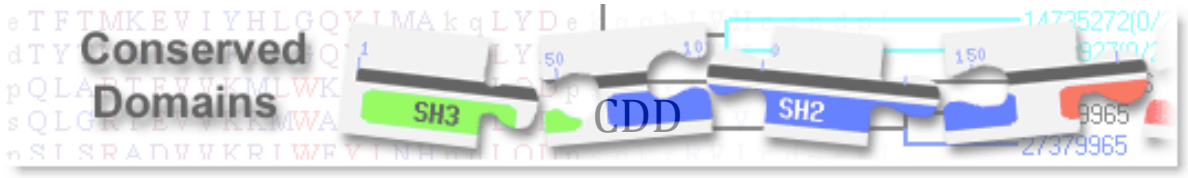


Click here 2nd

Search for similar domain architectures | Refine search

List of domain hits

Description	Pssmid	Multi-dom	E-value
[+]HMA[cd00371], Heavy-metal-associated domain (HMA) is a conserved domain of approximately 30 amino acid ...	238219	no	9.81e-16
[+]HMA[cd00371], Heavy-metal-associated domain (HMA) is a conserved domain of approximately 30 amino acid ...	238219	no	1.69e-13
[+]HMA[pfam00403], Heavy-metal-associated domain;	215902	no	2.10e-13
[+]CopZ[COG2608], Copper chaperone [Inorganic ion transport and metabolism]	225328	no	2.43e-10
[+]HMA[pfam00403], Heavy-metal-associated domain;	215902	no	7.75e-10
[+]TIGR00003[TIGR00003], copper ion binding protein; This model describes an apparently copper-specific subfamily of the metal-binding domain ...	188014	no	1.96e-08
[+]CopZ[COG2608], Copper chaperone [Inorganic ion transport and metabolism]	225328	no	2.41e-07
[+]MerP[TIGR02052], mercuric transport protein periplasmic component; This model represents the periplasmic mercury (II) binding protein of the bac	131107	no	3.92e-04
[+]TIGR00003[TIGR00003], copper ion binding protein; This model describes an apparently copper-specific subfamily of the metal-binding domain ...	188014	no	4.03e-04
[+]MerP[TIGR02052], mercuric transport protein periplasmic component; This model represents the periplasmic mercury (II) binding protein of the bac	131107	no	7.44e-04
[+]ZntA[COG2217], Cation transport ATPase [Inorganic ion transport and metabolism]	225127	yes	1.18e-08
[+]ZntA[COG2217], Cation transport ATPase [Inorganic ion transport and metabolism]	225127	yes	9.62e-07
[+]PRK13748[PRK13748], putative mercuric reductase; Provisional	184298	yes	1.96e-06
[+]PRK13748[PRK13748], putative mercuric reductase; Provisional	184298	yes	4.54e-06
[+]copA[PRK10671], copper exporting ATPase; Provisional	182635	yes	1.54e-05
[+]copA[PRK10671], copper exporting ATPase; Provisional	182635	yes	1.19e-04



Previous protein query – Found conserved domains

Sequence Alignment

include consensus sequence ?

Reformat | Format: Hypertext | Row Display: up to 20 | Color Bits: 2.0 bit | Type Selection: the most similar members

```
                      10        20        30        40        50        60
                ....*....|....*....|....*....|....*....|....*....|....*....|....*...
Feature 1             ###  #
1CPZ_A          3 EFSVKgMSCNHCVARIEEAVGRIsGVkKVKVQLkkeKAVVKFDea----nvQATEICQAINELGYQAE 66
query          68 TLTIDgMHCNSCVSNIESALSTLhYVsSIVVSLqnkSAIIKYNan----svTPEILKKAIEAispgqy 131
1AFJ            6 TLAVPgMTCAACPITVKKALSKVeGVsKVDVGFekrEAVVTFDdt----kaSVQKLTKATADAGYPSS 69
1K0V_A          5 TLQVEgMSCQHCVKAVETSVGELdGVsAVHVNLeagKVDVSFDad----kvSVKDIADAIEDQGYDVA 68
gi 2635862      7 EYVLDgLDCSNCARKIENGVKGIkGInGCAVNFaasTLTVSADgke--eqwVTNKVEKKVKSIDPHVT 72
gi 120199      40 ELSVPnAYCGTCIATIEGALRAKpEVeRARVNLssrRVSIVWKeevggrrtNPCDFLHAIAERGYQTH 107
gi 10764865    17 AVYKVhLHCRKCACDIKKPLLRFqGVqNVDFDLeknEIKVKGKi------eVVKIHKQIEKWSKKKVE 78
gi 3108347     24 EFMVD-MTCEGCVNAVKNKLETIeGIeKVEVDLsnqVVRILGSs-------PVKAMTQALEQTGRKAR 83
gi 2159998      8 GLSVAgMTCPSCTRHVEDALLAVpGVtRAAVDYpsnKAQVTGNr------lDVSALVAAVGALGYGAT 69
gi 3929319     12 EFMVD-MTCEGCVSAVKNSMLKLdGVsGVDVDLsnqLVRVIGSv-------PVKTMLKALEQTGRNAR 71
gi 3122077     39 DLSVSdVHCGGCISTIERALLTLpFVkTARVNLtarRVTCVYQeeiearatDPSKILGEINSAGYRAH 106
gi 2493001      6 NLQLEgMRCAACASSIERAIAKVpGVqSCQVNFaleQAVVSYHge-----tTPQILTDAVERAGYHAR 68
gi 2493016    102 QLLLSgMSCASCVTRVQNALQSVpGVtQARVNLaerTALVMGSa-------SPQDLVQAVEKAGYGAE 162
gi 1703455    260 QLRIDgMHCKSCVLNIEENIGQLlGVqSIQVSLenkTAQVKYDps----ctSPVALQRAIEALPPGNF 323
gi 5759320     95 EFMVD-MSCQGCVSAVKSKLQTVeGVkNVDVDLdnqVVRILGSs-------PVKTMTEALEQTGRKAR 154
gi 231677      15 AYRVQgFTCANCAGKFEKNVKQLsGVeDAKVNFgasKIAVYGNa-------TIEELEKAGAFENLKVT 75
gi 3080439     35 ELKVR-MDCDGCVLKIKNSLSSLkGVkTVEINKkqqKVTVSGYa-------DASKVLKKAKATGKKAE 94
gi 728935      83 LLSVQgMTCGSCVSTVTKQVEGIeGVeSVVVSLvteECHVIYEps----ktTLETAREMIEDCGFDSN 146
gi 12643938   403 MLAIAgMTCKSCVQSIEGLISQRvGVhQISVFLaegTAVVLYDps----rtHPEELRAAVEDMGFEAS 466
gi 1703455     61 TVRILgMTCQSCVKSIEDRISNLkGIiSMKVSLeqdSATVKYVps----vvCLQQVCHQIGDMGFEAS 124
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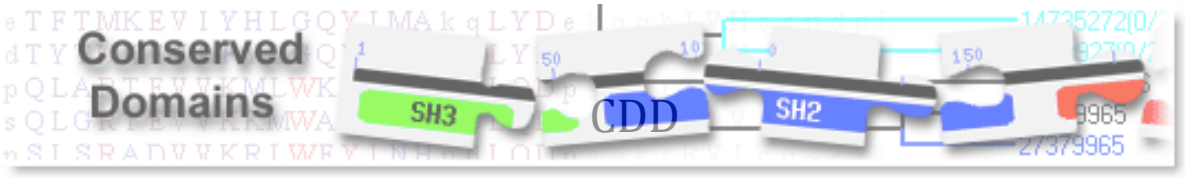
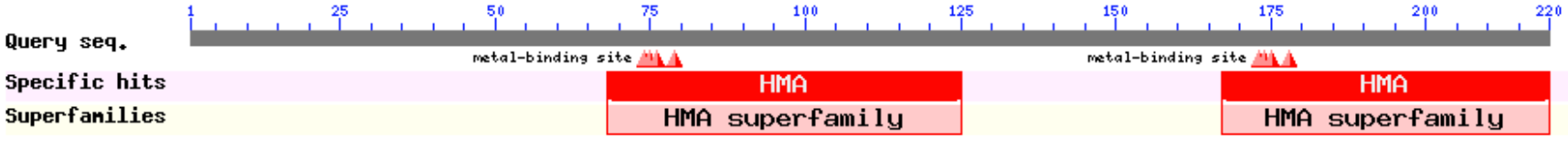


Previous protein query – Found conserved domains

Graphical summary show options »



Search for similar domain architectures | Refine search

List of domain hits

	Description	Pssmid	Multi-dom	E-value
[–]	HMA[cd00371], Heavy-metal-associated domain (HMA) is a conserved domain of approximately 30 amino acid ...	29471	no	1.11e-10

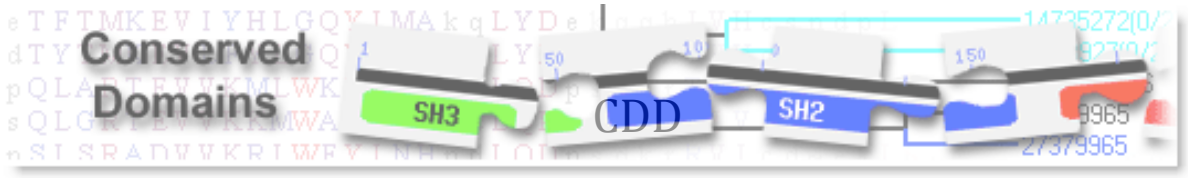
Heavy-metal-associated domain (HMA) is a conserved domain of approximately 30 amino acid residues found in a number of proteins that transport or detoxify heavy metals, for example, the CPx-type heavy metal ATPases and copper chaperones. HMA domain contains two cysteine residues that are important in binding and transfer of metal ions, such as copper, cadmium, cobalt and zinc. In the case of copper, stoichiometry of binding is one Cu+ ion per binding domain. Repeats of the HMA domain in copper chaperone has been associated with Menkes/Wilson disease due to binding of multiple copper ions.

:

Cd Length: 63 **Bit Score:** 61.78 **E-value:** 1.11e-10

```
                  10        20        30        40        50
                ....*....|....*....|....*....|....*....|....*....|....*...
gi 45549418   68 TLTIDGMHCNSCVSNIESALSTLHYVSSIVVSLQNKSAIIKYNAnSVTPEILKKAIEA 125
Cdd:cd00371    1 ELSVEGMTCAGCVSKIEKALEKLPGVESVEVDLETGKATVEYDP-EVSPEELLEAIED 57
```

[+]	HMA[cd00371], Heavy-metal-associated domain (HMA) is a conserved domain of approximately 30 amino acid ...	29471	no	2.86e-09



Go Back To Search

Previous protein query – Found conserved domains

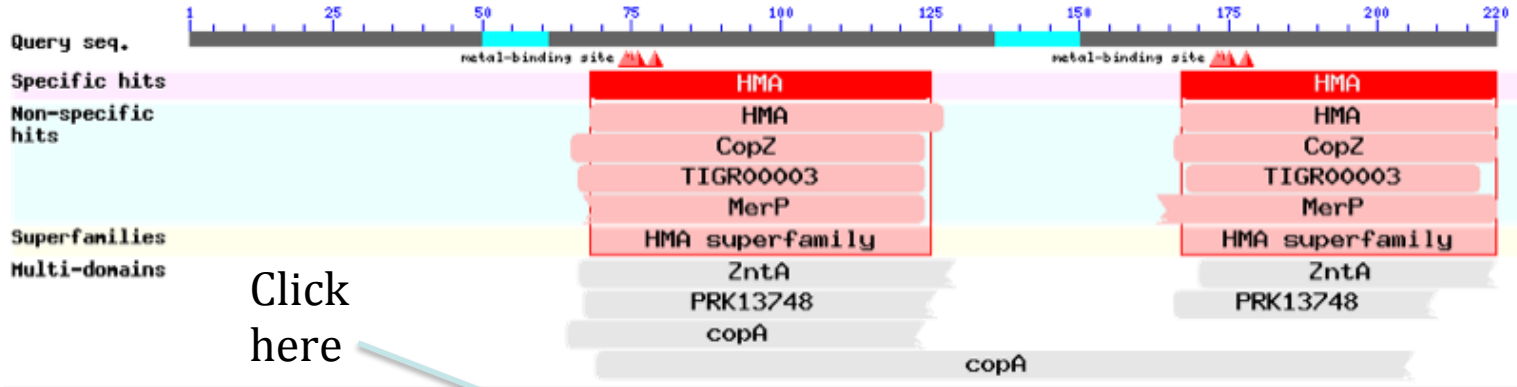
Conserved domains on [gi|45549418|gb|AAS67634|]

View concise result

ATP7A, partial [Solenodon paradoxus]

Graphical summary

show options »



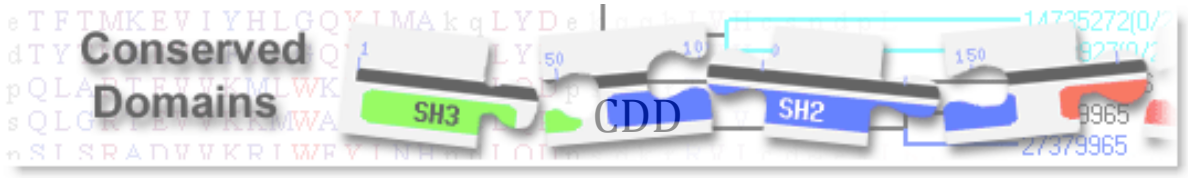
Click here

Search for similar domain architectures

Refine search

List of domain hits

Description	Pssmid	Multi-dom	E-value
[+]HMA[cd00371], Heavy-metal-associated domain (HMA) is a conserved domain of approximately 30 amino acid ...	238219	no	9.81e-16
[+]HMA[cd00371], Heavy-metal-associated domain (HMA) is a conserved domain of approximately 30 amino acid ...	238219	no	1.69e-13
[+]HMA[pfam00403], Heavy-metal-associated domain;	215902	no	2.10e-13
[+]CopZ[COG2608], Copper chaperone [Inorganic ion transport and metabolism]	225328	no	2.43e-10
[+]HMA[pfam00403], Heavy-metal-associated domain;	215902	no	7.75e-10
[+]TIGR00003[TIGR00003], copper ion binding protein; This model describes an apparently copper-specific subfamily of the metal-binding domain ...	188014	no	1.96e-08
[+]CopZ[COG2608], Copper chaperone [Inorganic ion transport and metabolism]	225328	no	2.41e-07
[+]MerP[TIGR02052], mercuric transport protein periplasmic component; This model represents the periplasmic mercury (II) binding protein of the bac	131107	no	3.92e-04
[+]TIGR00003[TIGR00003], copper ion binding protein; This model describes an apparently copper-specific subfamily of the metal-binding domain ...	188014	no	4.03e-04
[+]MerP[TIGR02052], mercuric transport protein periplasmic component; This model represents the periplasmic mercury (II) binding protein of the bac	131107	no	7.44e-04
[+]ZntA[COG2217], Cation transport ATPase [Inorganic ion transport and metabolism]	225127	yes	1.18e-08
[+]ZntA[COG2217], Cation transport ATPase [Inorganic ion transport and metabolism]	225127	yes	9.62e-07
[+]PRK13748[PRK13748], putative mercuric reductase; Provisional	184298	yes	1.96e-06
[+]PRK13748[PRK13748], putative mercuric reductase; Provisional	184298	yes	4.54e-06
[+]copA[PRK10671], copper exporting ATPase; Provisional	182635	yes	1.54e-05
[+]copA[PRK10671], copper exporting ATPase; Provisional	182635	yes	1.19e-04



CDART – Conserved Domain Architecture Retrieval Tool

[Query] >gi|45549418|gb|AAS67634.1| ATP7A [Solenodon paradoxus] IVYQPHLITVEEIKKQIKAVGFPAFIKKQPKYLI
Total architectures: 44

[+] **ATP7A**
taxonomy span: cellular organisms
Similarity score: 1
Total nr sequences: 4118

[+] **heavy metal-transporting ATPase**
taxonomy span: cellular organisms
Similarity score: 1
Total nr sequences: 3885

[+] **zinc/cadmium/mercury/lead-transporting**
taxonomy span: cellular organisms
Similarity score: 1
Total nr sequences: 486

[+] **heavy metal translocating P-type ATPase**
taxonomy span: cellular organisms
Similarity score: 1
Total nr sequences: 457

[+] **mercuric ion reductase**
taxonomy span: Bacteria
Similarity score: 1
Total nr sequences: 189

[+] **copper chaperone for superoxide**
taxonomy span: Eukaryota
Similarity score: 1
Total nr sequences: 183

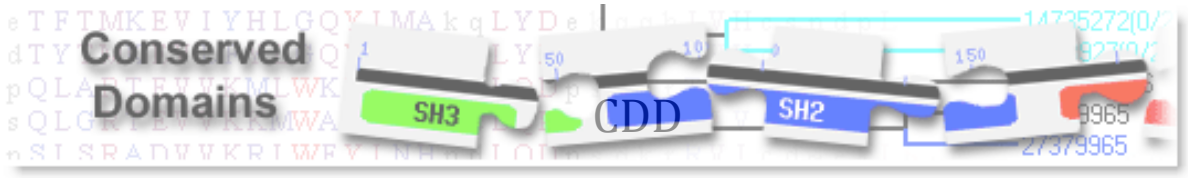
[+] **cation-transporting ATPase, P-type**
taxonomy span: cellular organisms
Similarity score: 1
Total nr sequences: 158

[+] **pit1**
taxonomy span: Bacteria
Similarity score: 1
Total nr sequences: 79

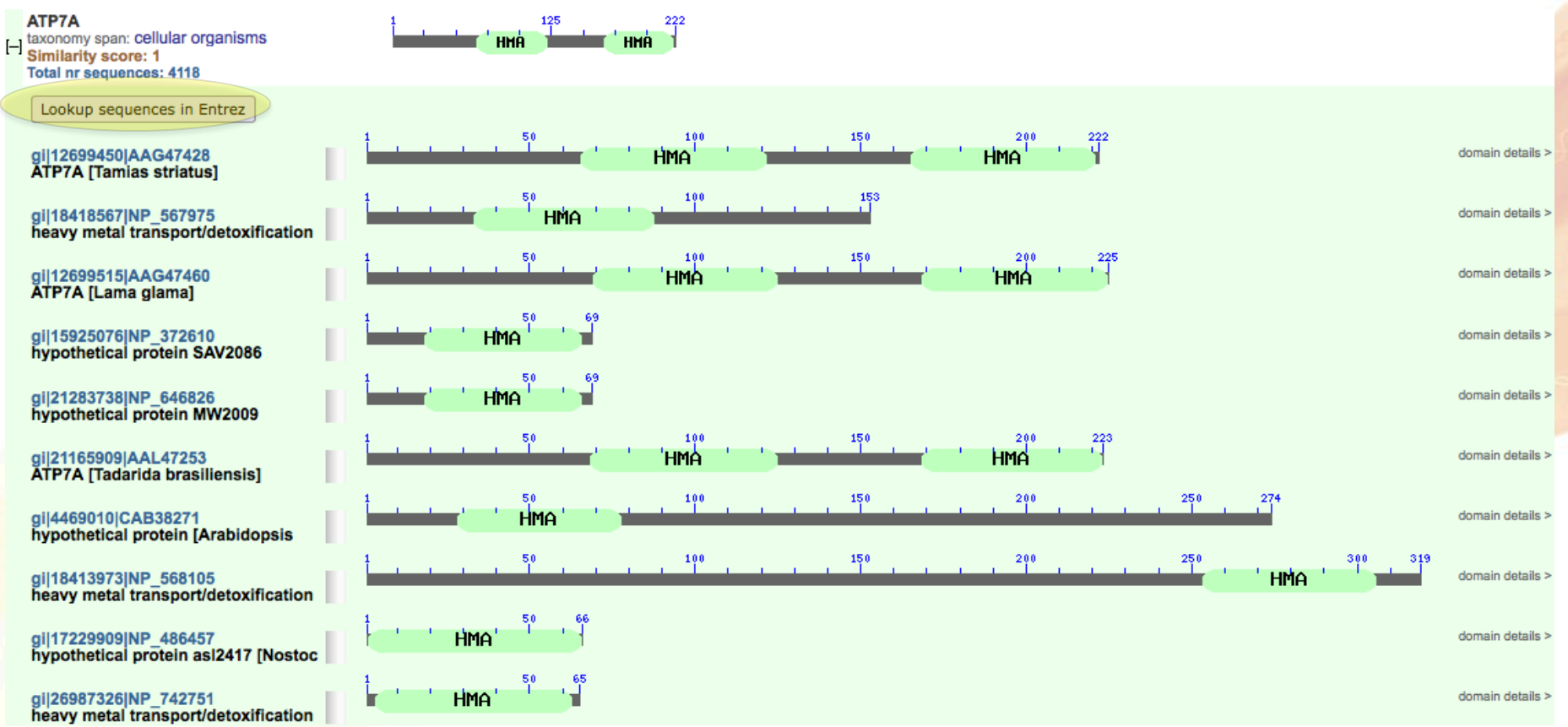
[+] **ATPase P**
taxonomy span: cellular organisms
Similarity score: 1
Total nr sequences: 36

[+] **probable copper-transporting ATPase**
taxonomy span: cellular organisms
Similarity score: 1
Total nr sequences: 33

Page 1



CDART – Conserved Domain Architecture Retrieval Tool



5 Minutes..

NCBI – dbSNP Short Genetic Variations

Finding SNPs in the Human Genome

- Challenge
  - Identify SNPs that correlate with a particular effect in patients
  - Reliable SNPs could serve as **predictive markers**
    - **Inform our decisions** about numerous aspects of medical care, including:
      - specific diseases
      - effectiveness of various drugs
      - adverse reactions to specific drug
- Scientists **approach** the problem of **identifying**, **cataloging** and **characterizing** SNPs in **two** main ways:
  - Genomic approaches
  - Functional approaches

http://learn.genetics.utah.edu/content/health/pharma/snips/

Genomic Approaches
Finding SNPs in the Human Genome

- Used by scientists who want to see **the big picture**
- Several large-scale projects - combined the efforts of many institutions:
  - To identify and catalog all of the SNPs in the 3-billion-base pair human genome
  - Each project involves **hundreds** of scientists
    - **Compare genomes** of numerous individuals to identify the differences
    - Comparisons require great deal of computer-powered data analysis
- Scientists sort and catalog results in databases available to anyone over the internet
  - **dbSNP at NCBI**

http://learn.genetics.utah.edu/content/health/pharma/snips/

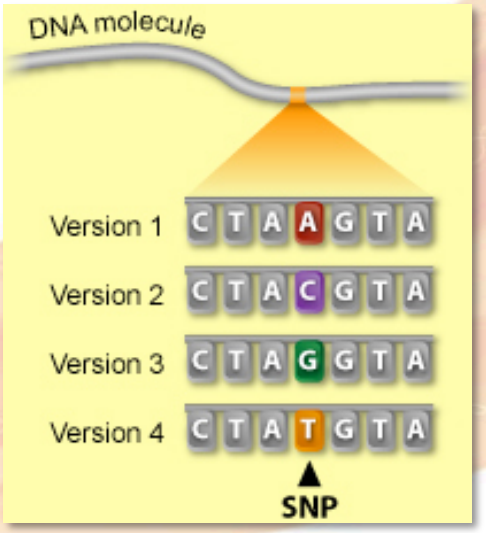
Functional Approaches
Finding SNPs in the Human Genome

- Used by scientists who are interested in a **particular disease** or **drug response**
  - The biological processes involved in diseases and drug responses are controlled by the activities of many genes
  - **Scientists interested** in **a particular process** select **genes known** to be **involved** in the **process** and **examine** them in people who:
    - Have a **response** or **disease**
    - As well as **those** who do **not**
- By comparing people's DNA sequences
  - Scientists can identify SNPs that correspond with a particular **function** or response
  - **This is where we will make major changes in the way we live**
    - **Many biotech in the area are going this!**

http://learn.genetics.utah.edu/content/health/pharma/snips/

SNP Quick Reference

- SNPs are single-nucleotide substitutions of one base for another
- Each SNP location in the genome can have up to four versions:
  - A, C, G and T
  - A SNP and its distribution in a population might look like the image right
- Not all single-nucleotide changes are SNPs
  - **To be classified as a SNP:**
    - **Two** or **more** versions of a **sequence** must each be **present**
    - **>= one percent of the general population**
- SNPs occur throughout the human genome
  - **One** in every **300** nucleotide base pairs
  - ~**10 million SNPs** within the **3-billion-nucleotide** human genome
  - So much work to do!



http://learn.genetics.utah.edu/content/health/pharma/snips/

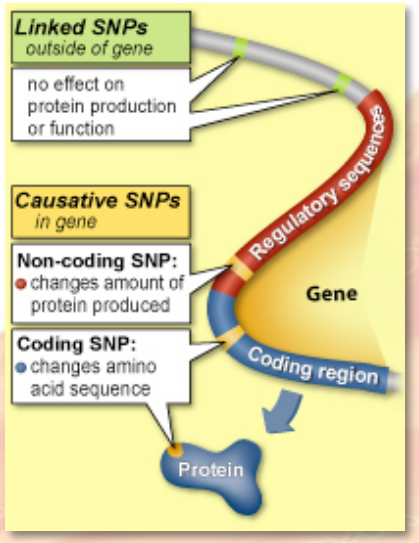
SNPs and Disease-Causing Mutations: Not the Same!

- If you know what a point mutation is, then description of a SNP might sound similar
- Both are single-nucleotide differences in a DNA sequence
- But SNPs should not be confused with disease-causing mutations
  - **First**:
    - SNP - one percent of the general population
    - **No known disease-causing mutation is this common**
  - **Second**:
    - **Most disease-causing mutations** occur within a gene's **coding** or **regulatory regions**
    - **Affect** the **function** of the protein encoded by the gene
- Unlike mutations:
  - **SNPs are not necessarily located within genes**
  - Do not always affect the way a protein functions

http://learn.genetics.utah.edu/content/health/pharma/snips/

Linked and Causative SNPs

- **Linked SNPs**
  - **Do not reside** within genes and **do not affect** protein function
  - **Correspond to a particular drug response** or to the risk for getting a certain disease
- **Causative SNPs**
  - **Affect** the way **a protein functions**:
    - Correlating with a disease or influencing a person's response to medication
  - Causative SNPs come in two forms:
    - **Coding SNPs**
      - Located within coding region of a gene
      - Change amino acid sequence of gene's protein product
    - **Non-coding SNPs**
      - Located within gene's regulatory sequences
      - Change level of gene expression and, therefore, how much RNA and protein is produced



http://learn.genetics.utah.edu/content/health/pharma/snips/

SNPs – Intragenic and Extragenic

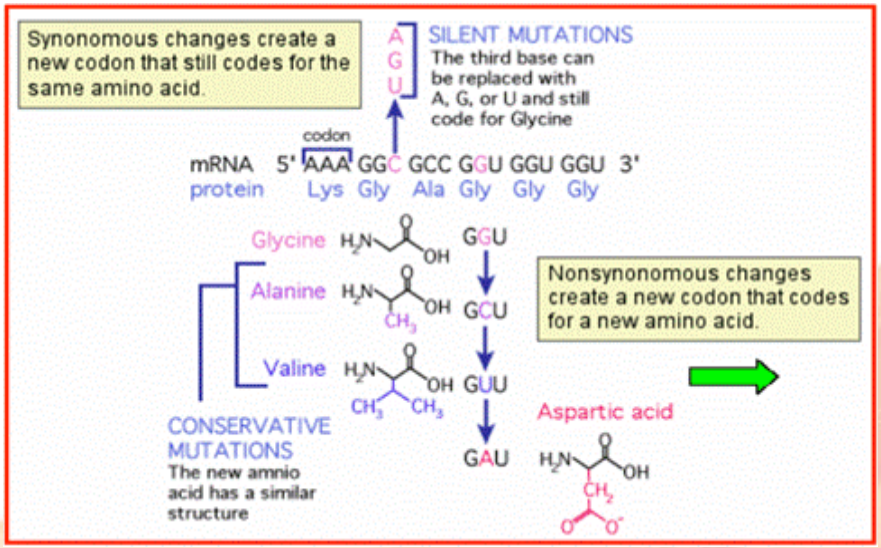
- Intragenic SNPs are often categorized by function
  - Are they in a coding region, an intron, part of the mRNA, outside the mRNA but still in the gene locus (i.e., in the promoter)
- Extragenic SNPs
  - Simply considered 'genomic' or might be labeled relative to the nearest gene
    - ie. 5' or 3' to a gene
  - An 'extragenic' SNP may affect regulatory regions important in gene expression or other DNA functions such as DNA replication

SNP Functional Categories

- Coding nonsynonymous
  - Missense, nonsense, frame shift
- Coding synonymous
- Intronic
  - splice site
- mRNA utr
  - 5' utr or 3' utr
- (gene) locus region (5’ or 3’ to the gene)
  - ‘near gene’ usually means within ~2000bp of gene
- genomic/extragenic (distant from any gene)

Coding Nonsynonymous SNPs

- Missense – change an aa
- Nonsense
  - Change an aa to a stop codon
  - Results in a shortened protein
- Frame Shift
  - Are really single-base indels
  - Drop or add one base and the triplet reading frame is thrown out of shift, altering all downstream aa's and usually resulting in an earlier stop codon



Jennifer Lyon

SNP Nomenclature

- The Human Genome Variation Society
  - http://www.hgvs.org/mutnomen/recs.html
  - Proposed some guidelines for SNP nomenclature, but at the moment, there is minimal consistency
- Different sources will refer to the same SNP in different ways
- While dbSNP identifiers (rs#12345678) are becoming common, they are not required of publishing authors and not used in all cases

SNPs at Base-Pair Level

- The base-pair change is given in various forms:
  - A/C
  - T→G
  - C>T
  - 432G>C
  - T73C
- The HGVS nomenclature recommendations:
  - "c." for a coding DNA sequence (like c.76A>T)
  - "g." for a genomic sequence (like g.476A>T)
  - "m." for a mitochondrial sequence (like m.8993T>C)
  - "r." for an RNA sequence (like r.76a>u)

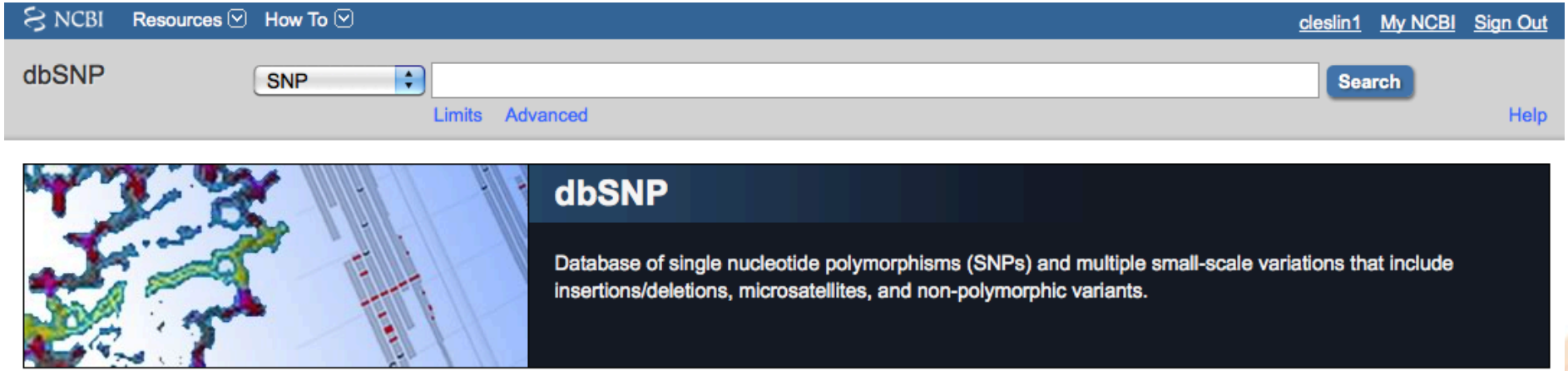
Position, position, position!

- The big issue with SNPs is identifying their location (**numerically**)
- Position can be specified:
  - Number location within a specific sequence
  - Relative to another genetic landmark
    - Start site for a coding region of a gene
    - Start or end of an exon or intron
    - Relative to a marker
    - Position in structure
- Published articles are not always clear on this!!!
- Different resources may use different landmarks/numbering
- Numbering is always relative to the chosen sequence/structure

Coding SNPs

- These are easier because they can be identified by the amino acid position rather than the base-pair position
- Most common nomenclature uses either 3-letter or single amino acid codes:
  - **Asn332Asp** OR **A95V**
- The HGVS recommendation is similar:
  - "p." for a protein sequence (like p.Lys76Asn)
- Amino Acid (protein) coding sequence positions becoming more consistent, but are not always consistent

dbSNP



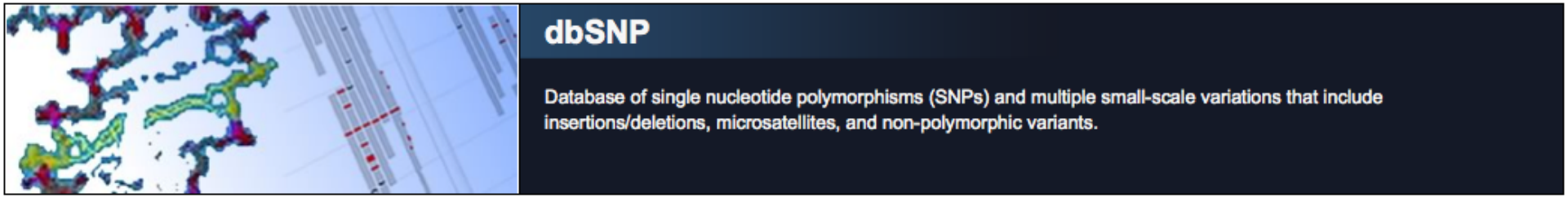
Short Genetic Variations
Database of ~~Single Nucleotide Polymorphisms~~

- Variation
  - Reflects the wide range of dbSNP's variation content
  - Hopefully prevents further misunderstandings about scope of the resource
- dbSNP also stores common and rare variations along with their genotypes and allele frequencies.
- Includes clinically significant variations

Stephen Sherry, PhD

NCBI's dbSNP Database

- International central repository for both **single base nucleotide substitutions** and **short deletion** and **insertion polymorphisms**
- Accepts data submissions from scientists
- Integrated with the NCBI's Entrez system
- Primary Database and Derivative (RefSNP)
- **Single Nucleotide Polymorphisms**
- **Repeat polymorphisms**
- **Insertion-Deletion Polymorphisms**
- **dbSNP build 131 (Mar 25, 2010)**
- **15 Organisms**
- **180,113,082 submissions (submitted SNPs)**
- **validated 24,735,618 reference SNPs**



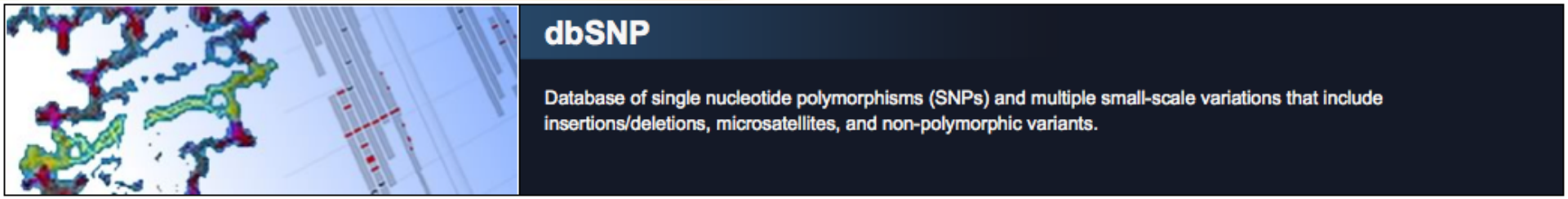
NCBI's dbSNP Database

http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp

http://www.ncbi.nlm.nih.gov/SNP/snp_summary.cgi

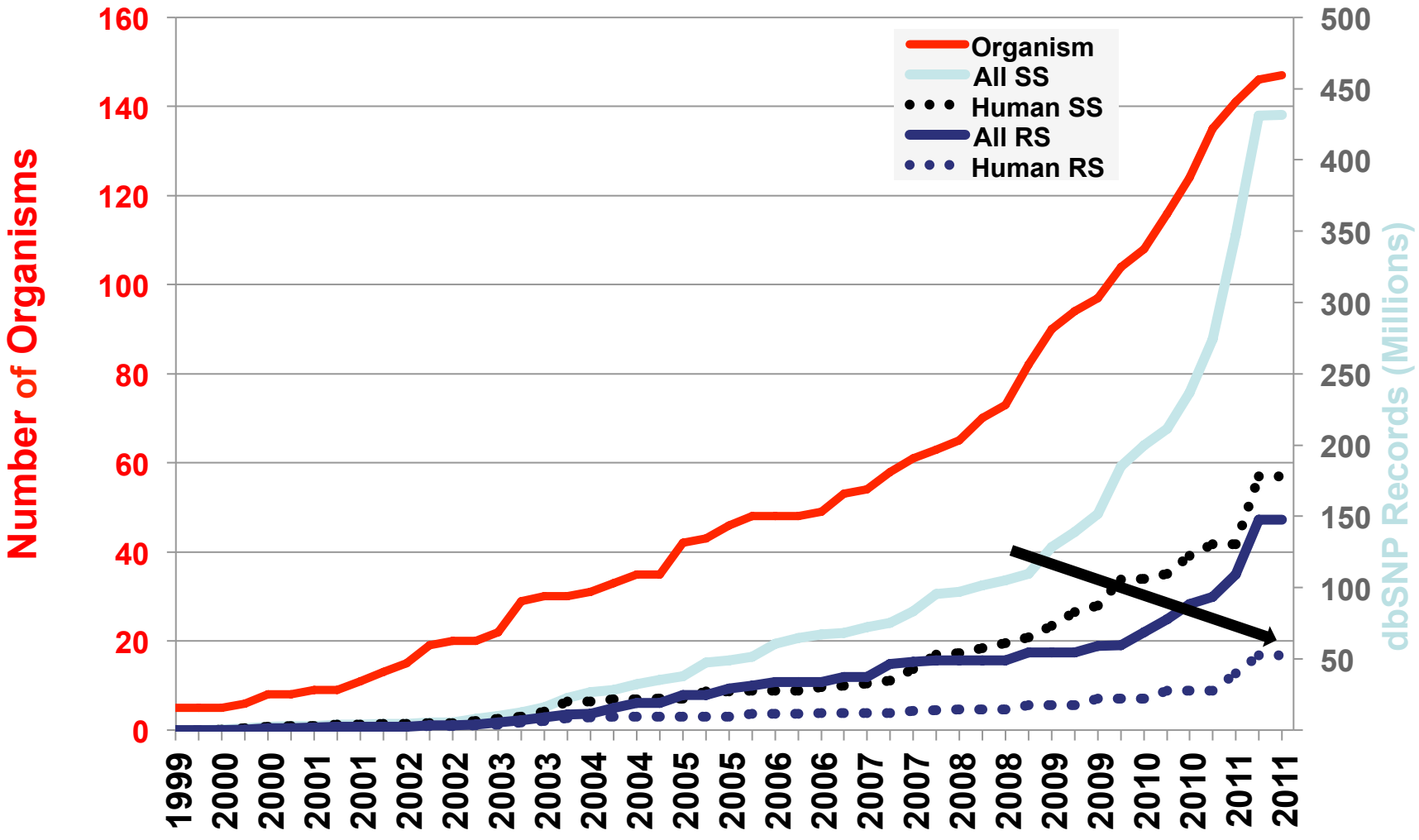
- **dbSNP build 138 (Apr 35, 2013)**
- **131 Organisms**
- **505,719,558 submissions (submitted SNPs)**
- **validated 70,316,939 reference SNPs**

Tremendous Growth in 3 years



dbSNP Content

- Two major classes of content:
- Submitted data, i.e., original observations of sequence variation:
  - Submitted SNPs (SS) with ss# (ss 5586300)
- Computed/curated data:
  - Reference SNP Clusters (Ref SNP) with rs# (rs 4986582)
- Ref SNP clusters are computer-generated and **curated by NCBI staff**
  - Ref SNP Clusters define a non-redundant set of SNPs
  - All individual SNPs:
    - Submitted by a researcher are given a submitter SNP number (ss#)
    - Then redundant (repetitive) submitter SNPs are combined into a RefSNP cluster record
      - With a unique rs#
      - Ref SNP clusters may contain multiple submitted SNPs



505,719,558 submissions (submitted SNPs) - **232,952,851 - Human**
validated 70,316,939 reference SNPs - **44,278,189 - Human**

Adopted Stephen Sherry, PhD

Types of Genetic Variations

- **Single Nucleotide Polymorphisms** (SNP)
  - Single base pair changes

  GTCA**T**TCGATT

  GTCA**G**TCGATT
- **Indels**
  - Small insertion/deletions

  CTT---GATC

  CTT**ACG**GATC
- **Small variable repeats – microsatellites**

  ACGACGACGACGACGACG (6 copies)

  ACGACGACGACGACGACGACG (7 copies)
- **Variable Long tandem repeats (can be dozens to hundreds to thousands)**
- **Chromosomal Aberrations: Translocations, Inversions, etc.**

Variation Classes

Abbreviation	Description	Example
SNP	Single Nucleotide Variation	A/G
MNP	Multiple Nucleotide Variation, where all alleles are same length, and length > 1	AT/GA
IN DEL	Deletion/Insertion Variation	-/T
MIXED	Variation has unknown sequence composition but is observed to be heterozygous	HETEROZYGOUS
MICROSATELLITE	Microsatellite/simple sequence repeat	(GACA)3/4/5
NAMED	Allele sequences defined by name tag instead of raw sequence	(Alu)/-
NO VARIATION	Submission reports invariant region in surveyed sequence	NOVARIATION
MIXED	Mixture of two or more of the above classes	A/G/-

New SNP Attributes (March 15, 2011)

New attributes have been added to dbSNP to allow searching and filtering of human variation by the following characteristics:

Allele Origin: The rs report summarizes the reported origin(s) of the variant allele asserted by each submitter for the submitted SNP (ss) . Current values are germline, somatic, and unknown. Additional attributes will be added in the future release to include:

- not-tested
- tested-inconclusive
- other

Clinical significance: The significance supplied by the submitter for the submitted SNP (ss).

The supported values are:

- unknown
- untested
- non-pathogenic
- probable-non-pathogenic
- probable-pathogenic
- pathogenic
- other

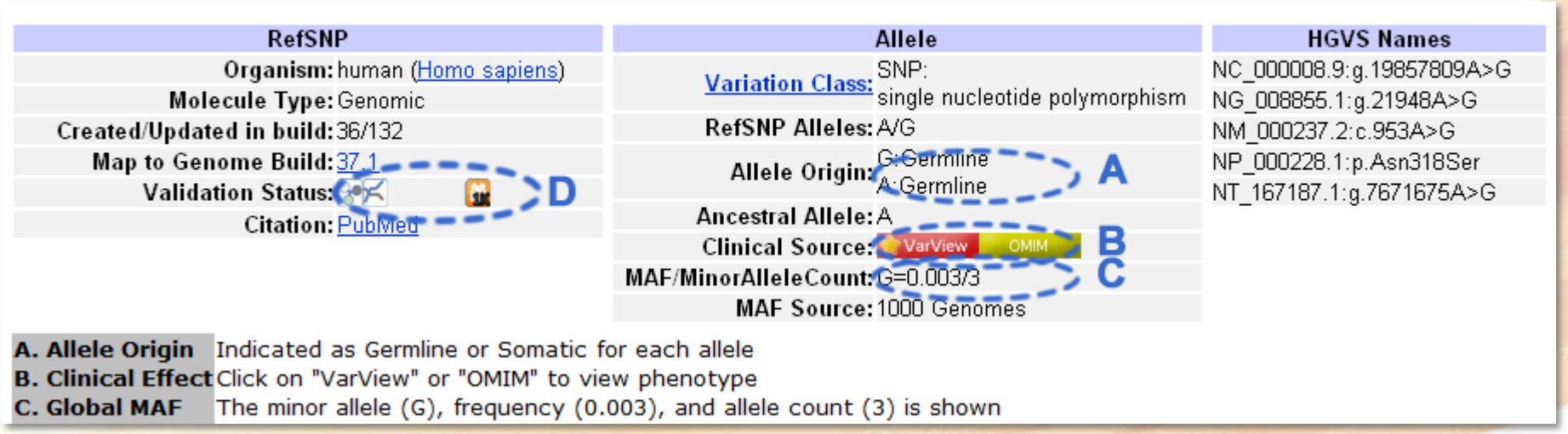
FYI

Global minor allele frequency (MAF): dbSNP is reporting the minor allele frequency for each rs included in a default global population. Since this is being provided to distinguish common polymorphism from rare variants, the MAF is actually the second most frequent allele value. In other words, if there are 3 alleles, with frequencies of 0.50, 0.49, and 0.01, the MAF will be reported as 0.49. The current default global population is 1000Genome phase 1 genotype data from 629 worldwide individuals.

Suspect: Variation suspected to be false positive due to artifacts of the presence of a paralogous sequence in the genome (Musumeci et al. 2010) (Sudmant et al. 2010) or evidence suggested sequencing error or computation artifacts.

Stephen Sherry, PhD

RefSNP Summary (Example)



SNP Gene View (Example)

Region	Chr. position	mRNA pos	dbSNP rs# cluster id	Hetero-zygosity	Validation	MAF	Allele origin	3D	Clinically Associated	Function	dbSNP allele	Protein residue	Codon pos	Amino acid pos	PubMed
	19805708	476	rs1801177	0.025		0.0175	A:Germline	Yes		missense	A	Asn [N]	1	36	
							G:Germline			contig reference	G	Asp [D]	1	36	
	19805815	583	rs11542065	0.024		0.0172		Yes		missense	G	Gln [Q]	3	71	
										contig reference	C	His [H]	3	71	
	19809435	775	rs1121923	0.121		0.0863		Yes		synonymous	A	Val [V]	3	135	
										contig reference	G	Val [V]	3	135	

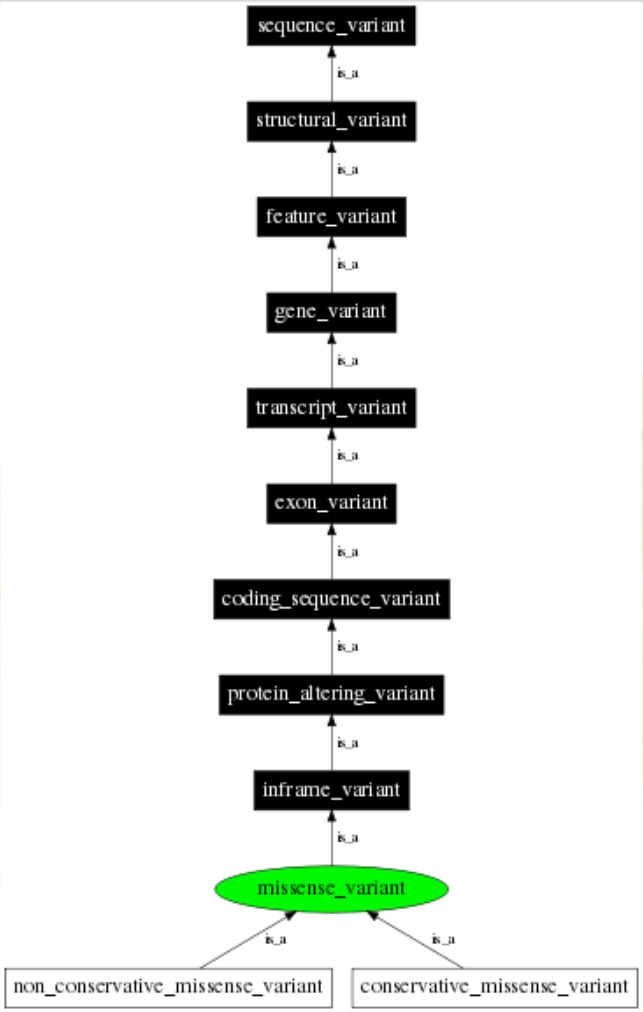
A
B
C
D

A. Allele Origin	Indicated as Germline or Somatic for each allele
B. Clinical Effect	Click on icon under "Clinical Source" to view effect in Variation Viewer
C. Global MAF	MAF is shown for the corresponding allele
D. Suspected	A red "?" icon is shown for suspected SNP under the "Validation" column

Stephen Sherry, PhD

Functional Terms = Sequence Ontology Standard

- **Function**: dbSNP's predicted functional effect of variant on RefSeq transcripts
- **Coding - Non-Synonymous** - change in peptide for allele with respect to the reference assembly (**dbSNP terms: nonsense, missense, stop-loss, frameshift, cds-indel;** Sequence Ontology terms: stop_gained, **missense_variant**, stop_lost, frameshift_variant, inframe_indel)
  - http://sequenceontology.org/browser/current_release/term/SO:0001587
  - **http://sequenceontology.org/browser/current_release/term/SO:0001583**
  - http://sequenceontology.org/browser/current_release/term/SO:0001578
  - http://sequenceontology.org/browser/current_release/term/SO:0001589
  - http://sequenceontology.org/browser/current_release/term/SO:0001820



missense_variant (CURRENT_RELEASE)	
SO Accession:	SO:0001583 (SOWiki)
Definition:	A sequence variant, where the change may be longer than 3 bases, and at least one base of a codon is changed resulting in a codon that encodes for a different amino acid.
Synonyms:	missense, missense codon, missense_variant, non synonymous codon, non synonymous variant, non_synonymous_coding, SO:0001584, SO:0001783
DB Xrefs:	EBI: gr
	SO: ke
Parent:	inframe_variant (SO:0001650)
Children:	non_conservative_missense_variant (SO:0001586)
	conservative_missense_variant (SO:0001585)

http://sequenceontology.org/browser/current_release/term/SO:0001583

Functional Terms = Sequence Ontology Standard

- **Function**: dbSNP's predicted functional effect of variant on RefSeq transcripts
- **Locus Region** - variation is 3' to and within 500 bases of a transcript, or is 5' to and within 2000 bases of a transcript (**dbSNP terms: near-gene-3, near-gene-5;** Sequence Ontology terms: downstream_gene_variant, upstream_gene_variant)
    - http://sequenceontology.org/browser/current_release/term/SO:0001632
    - http://sequenceontology.org/browser/current_release/term/SO:0001631

Functional Terms = Sequence Ontology Standard

- **Function**: dbSNP's predicted functional effect of variant on RefSeq transcripts
- **Coding - Synonymous** - no change in peptide for allele with respect to the reference assembly (**dbSNP term: coding-synon;** Sequence Ontology term: synonymous_variant)
  - http://sequenceontology.org/browser/current_release/term/SO:0001819

Functional Terms = Sequence Ontology Standard

- **Function**: dbSNP's predicted functional effect of variant on RefSeq transcripts
- **Untranslated** - variation is in a transcript, but not in a coding region interval (**dbSNP terms: untranslated-3, untranslated-5;** Sequence Ontology terms: 3_prime_UTR_variant, 5_prime_UTR_variant)
  - http://sequenceontology.org/browser/current_release/term/SO:0001624
  - http://sequenceontology.org/browser/current_release/term/SO:0001623

Functional Terms = Sequence Ontology Standard

- **Function**: dbSNP's predicted functional effect of variant on RefSeq transcripts
- **Intron** - variation is in an intron, but not in the first two or last two bases of the intron (**dbSNP term: intron;** Sequence Ontology term: intron_variant)
  - http://sequenceontology.org/browser/current_release/term/SO:0001627

Functional Terms = Sequence Ontology Standard

- **Function**: dbSNP's predicted functional effect of variant on RefSeq transcripts
- **Splice Site** - variation is in the first two or last two bases of an intron (**dbSNP terms: splice-3, splice-5;** Sequence Ontology terms: splice_acceptor_variant, splice_donor_variant)
  - http://sequenceontology.org/browser/current_release/term/SO:0001574
  - http://sequenceontology.org/browser/current_release/term/SO:0001575

Functional Terms = Sequence Ontology Standard

- **Function**: dbSNP's predicted functional effect of variant on RefSeq transcripts
- **Unknown** - no known functional classification

Now Let's Look at a Gene Search

Nitric Oxide Synthase 2

Display Settings: Summary, 20 per page, Sorted by Relevance Send to:

Results: 1 to 20 of 26 << First < Prev Page 1 of 2 Next > Last >>

1. nos2a – nitric oxide synthase 2a, inducible [Danio rerio (zebrafish)]
   nitric oxide synthase 2a, inducible
   Official Symbol: **nos2a**
   Other Aliases: inosa, nos2, si:dkey-57m14.4
   Other Designations: inducible nitric oxide synthase a; nitric oxide synthase 2, inducible, macrophage
   Annotation: Chromosome 5, NC_007116.5 (45400854..45415665)
   ID: 404036

2. **Nos2a*** – nitric oxide synthase 2, inducible, macrophage [Rattus norvegicus (Norway rat)]
   nitric oxide synthase 2, inducible, macrophage
   Other Aliases: **Nos2a**
   Chromosome: 10
   ⚠ **This record was replaced with GeneID: 24599**
   ID: 117133

3. **Nos2a** – nitric oxide synthase 2, inducible, macrophage [Rattus norvegicus (Norway rat)]
   nitric oxide synthase 2, inducible, macrophage
   Location: 10
   ⚠ **This record was replaced with GeneID: 24599**
   ID: 116798

4. NOS2 – nitric oxide synthase 2, inducible [Homo sapiens (human)]
   nitric oxide synthase 2, inducible
   Official Symbol: NOS2
   Other Aliases: HEP-NOS, INOS, NOS, **NOS2A**
   Other Designations: NOS type II; NOS, type II; hepatocyte NOS; inducible NO synthase; inducible NOS; nitric oxide synthase 2A (inducible, hepatocytes); nitric oxide synthase, inducible; nitric oxide synthase, macrophage; peptidyl-cysteine S-nitrosylase NOS2
   Location: 17q11.2-q12
   Annotation: Chromosome 17, NC_000017.10 (26083792..26127555, complement)
   MIM: 163730
   ID: 4843
   Order cDNA clone

Nitric Oxide Synthase 2

Display Settings: Full Report Send to:

NOS2 nitric oxide synthase 2, inducible [*Homo sapiens*]

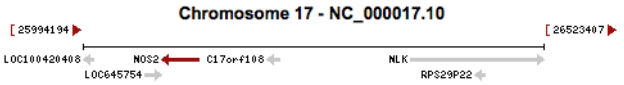
Gene ID: 4843, updated on 7-Oct-2012

Summary

Official Symbol NOS2 provided by HGNC
Official Full Name nitric oxide synthase 2, inducible provided by HGNC
Primary source HGNC:7873
See related Ensembl:ENSG00000007171; HPRD:01225; MIM:163730; Vega:OTTHUMG00000132445
Gene type protein coding
RefSeq status REVIEWED
Organism Homo sapiens
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as NOS; INOS; NOS2A; HEP-NOS
Summary Nitric oxide is a reactive free radical which acts as a biologic mediator in several processes, including neurotransmission and antimicrobial and antitumoral activities. This gene encodes a nitric oxide synthase which is expressed in liver and is inducible by a combination of lipopolysaccharide and certain cytokines. Three related pseudogenes are located within the Smith-Magenis syndrome region on chromosome 17. [provided by RefSeq, Jul 2008]

Genomic context

Location: 17q11.2-q12 See NOS2 in Epigenomics, MapViewer
Sequence: Chromosome: 17; NC_000017.10 (26083792..26127555, complement)



Genomic regions, transcripts, and products

Genomic Sequence NC_000017 chromosome 17 reference GRCh37.p9 Primary Assembl Go to reference sequence details

Go to nucleotide Graphics FASTA GenBank

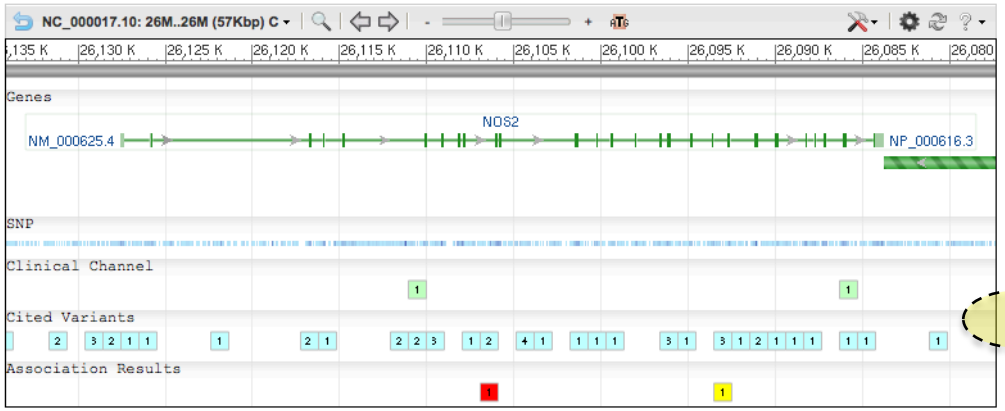
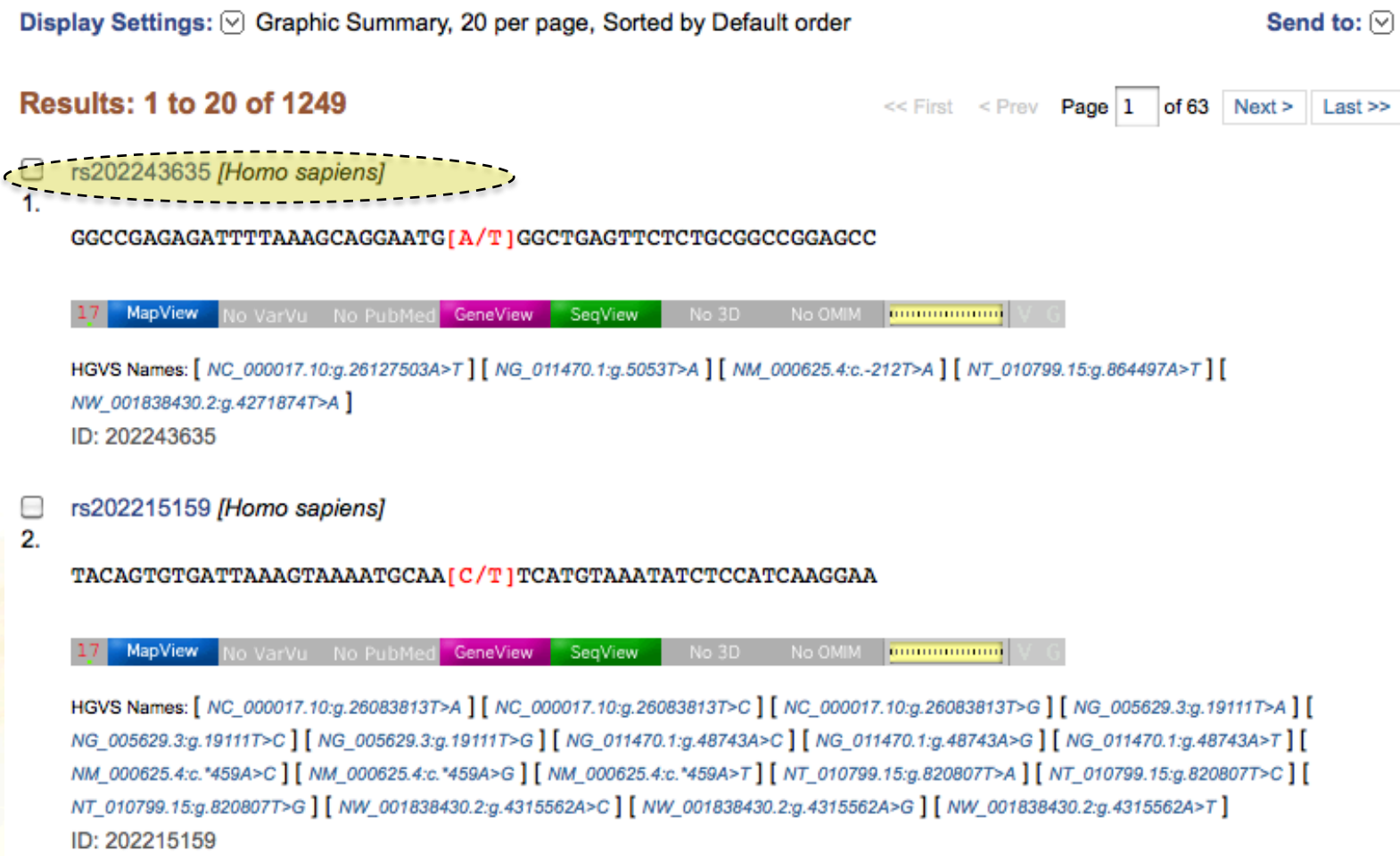


Table of contents

Related information

Nitric Oxide Synthase 2**



**Results are now in different order

Nitric Oxide Synthase 2

Reference SNP(refSNP) Cluster Report: rs202243635

RefSNP		Allele		HGVS Names	Links
Organism:	human (Homo sapiens)	**Variation Class:**	SNV: single nucleotide variation	NC_000017.10:g.26127503A>T	
Molecule Type:	Genomic	**RefSNP Alleles:**	A/T	NG_011470.1:g.5053T>A	
Created/Updated in build:	137/137	**Allele Origin:**		NM_000625.4:c.-212T>A	
Map to Genome Build:	37.3	**Ancestral Allele:**	Not available	NT_010799.15:g.864497A>T	
Validation Status:		**Clinical Channel:**	unknown	NW_001838430.2:g.4271874T>A	
		Clinical Significance:	NA		
		MAF/MinorAlleleCount:	NA		
		MAF Source:			

SNP Details are organized in the following sections:

GeneView | Map | Submission | Fasta | Resource | Diversity | Validation

Integrated Maps (Hint: click on 'Chr Pos' or 'Contig Pos' column value to see variation in NCBI sequence viewer)

Assembly	Genome Build	Chr	Chr Pos	Contig	Contig Pos	SNP to Chr	Contig allele	Contig to Chr	Neighbor SNP	Map Method
GRCh37.p5	37.3	17	26127503	NT_010799.15	864497	Fwd	A	Fwd	view	blast
HuRef	37.3	17	22334447	NW_001838430.2	4271874	Fwd	T	Rev	view	blast

GeneView

GeneView via analysis of contig annotation: NOS2 *nitric oxide synthase 2, inducible*

View more variation on this gene (click to hide).

Clinical Source: in gene region cSNP has frequency double hit Go

Primary Assembly Mapping

Assembly	SNP to Chr	Chr	Chr position	Contig	Contig position	Allele
GRCh37.p5	Fwd	17	26127503	NT_010799.15	864497	A

RefSeqGene Mapping

RefSeqGene	Gene (ID)	SNP to RefSeqGene	Position	Allele
NG_011470.1	NOS2 (4843)	Rev	5053	T

http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=202243635

Nitric Oxide Synthase 2

Gene Model (mRNA alignment) information from genome sequence

Total gene model (contig mRNA transcript):				6		
mrna	transcript	protein	mrna orientation	Contig	Contig Label	List SNP
NM_000625.4	plus strand	NP_000616.3	forward	NT_010799.15	GRCh37.p10	<- currently shown
NM_000625.4	minus strand	NP_000616.3	reverse	NT_010799.15	GRCh37.p10	<- currently shown
NM_000625.4	plus strand	NP_000616.3	forward	NW_001838430.2	HuRef	View snp on GeneModel
NM_000625.4	minus strand	NP_000616.3	reverse	NW_001838430.2	HuRef	View snp on GeneModel
NM_000625.4	plus strand	NP_000616.3	forward	NW_004078092.1	CHM1_1.0	View snp on GeneModel
NM_000625.4	minus strand	NP_000616.3	reverse	NW_004078092.1	CHM1_1.0	View snp on GeneModel

☑ Clinical Source ○ in gene region ◉ cSNP ○ has frequency ○ double hit [refresh]

gene model (contig mRNA transcript):	Contig Label	Contig	mrna	protein	mrna orientation	transcript	snp count
	GRCh37.p10	NT_010799.15	NM_000625.4	NP_000616.3	forward	plus strand	329, coding

Region	Chr. position	mRNA pos	dbSNP rs# cluster id	Hetero-zygosity	Validation	MAF	Allele origin	3D	Clinically Associated	Clinical Significance	Function	dbSNP allele	Protein residue	Codon pos	Amino acid pos	PubMed
	26084276	3722	rs201526410	N.D.							missense	C	Pro [P]	2	1153	
											contig reference	T	Leu [L]	2	1153	
	26084279	3719	rs199955383	N.D.							missense	T	Val [V]	2	1152	
											contig reference	C	Ala [A]	2	1152	
	26084286	3712	rs199748579	0.001		0.0005					missense	G	Val [V]	1	1150	
											contig reference	A	Met [M]	1	1150	
	26084307	3691	rs149327839	0.000							missense	A	Met [M]	1	1143	
											contig reference	G	Val [V]	1	1143	

Validation status description

- Validated by multiple, independent submissions to the refSNP cluster
- Validated by frequency or genotype data: minor alleles observed in at least two chromosomes.
- Validated by submitter confirmation
- All alleles have been observed in at least two chromosomes apiece
- H — Genotyped by HapMap project
- SNP has been sequenced in 1000Genome project.
- Suspect SNPs: snp suspected from paralogous region (PMID: 21030649). Added to dbSNP on 01/21/2011.

Finding SNPs - Entrez SNP Summary

- dbSNP is useful for investigating detailed information on:
  - Small number SNPs
  - And it's good for a picture of the gene
- Entrez SNP is a direct, fast database for querying SNP data
  - Data can be:
    - retrieved in batches for many SNPs
    - "limited" to specific subsets of SNPs
      - And formatted in plain text for easy parsing and manipulation
- More detailed queries can be formed using specific "field tags"
  - For retrieving SNP data

Searching dbSNP

- dbSNP is searched like any other Entrez db
- Specialized fields include:

Field	Tag	Notes
Allele	[Allele]	Uses IUPAC codes for bases
Chromosomal Location	[chrpos]	Uses chromosomal base-pair locations
Contig Position	[ctpos]	Uses contig base-pair locations
Function Class	[Func]	Includes coding synonymous, missense, nonsense, intron, utr, etc.
SNP Class	[SNP_Class]	Includes snp, indel, mixed

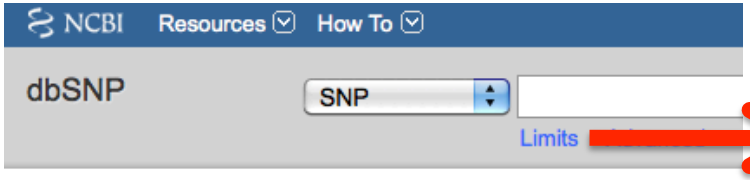
More Complex dbSNP Search

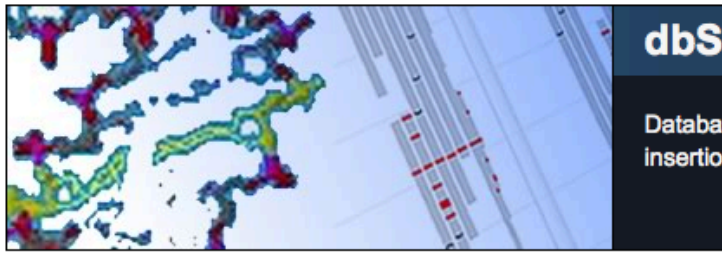
- Retrieve all:
  - synonymous coding reference SNPs
  - for the human norepinephrine transporter gene (Slc6a2) from dbSNP
- Utlize the **limits** like we did for lab with the Gene Database
- Search Strategy:

"Homo sapiens"[Organism] AND Slc6a2[gene] AND "synonymous codon"[Function_Class]

- Note: To use the (gene name) [gene] field, it is necessary to have the official gene name or gene symbol as per the Human Gene Nomenclature Committee
  - Entrez Gene can be used to find these
- More on search fileds can be found here:
  - http://www.ncbi.nlm.nih.gov/snp

More Specific Approach





Organism
- [x] Homo sapiens
- [ ] Agelaius phoeniceus
- [ ] Alectoris
- [ ] Alectoris chukar
- [ ] Alectoris rufa
- [ ] Allium cepa
- [ ] Amaranthus caudatus
- [ ] Anopheles funestus
- [ ] Anopheles gambiae
- [ ] Apis mellifera
- [ ] Artemisia tridentata

Chromosomes
- [x] 1
- [ ] 2
- [ ] 2a
- [ ] 2b
- [ ] 3
- [ ] 4
- [ ] 5
- [ ] 6
- [ ] 7
- [ ] 8
- [ ] 9

Chromosome Range
From
To

Map Weight
- [ ] 1
- [ ] 2
- [ ] 3-10
- [ ] 10+

Function Class
- [x] coding nonsynonymous
  - [x] nonsense
  - [x] missense
  - [x] frame shift
- [ ] stop gained
- [ ] intron
- [ ] coding synonymous
- [ ] locus region
- [ ] mrna utr
  - [ ] 5' utr
  - [ ] 3' utr

SNP Class
- [ ] het
- [x] in del
- [ ] microsatellite
- [ ] mixed
- [ ] mnp
- [ ] named
- [ ] no variation
- [x] snp

Method Class
- [ ] computed
- [ ] dhplc
- [ ] hybridize
- [ ] other
- [ ] rflp
- [ ] sequence
- [ ] sscp
- [ ] unknown

Validation Status
- [ ] by-HapMap
- [x] by-1000 Genomes
- [ ] by-cluster
- [ ] by-frequency
- [ ] by-submitter
- [ ] by-2hit-2allele
- [ ] no-info
- [ ] Suspected
  - [ ] Paralogous or SND
  - [ ] other

Variation Allele

Allele Origin
- [ ] Germline
- [ ] Somatic

IUPAC Variation Allele
- [ ] A
- [ ] C
- [ ] G
- [ ] T

Annotation
- [ ] Clinical/LSDB Submissions
- [ ] Clinical significance
  - [ ] pathogenic
  - [ ] probable pathogenic
  - [ ] non pathogenic
  - [ ] probable non pathogenic
  - [ ] unknown
  - [ ] other
- [ ] nucleotide
- [ ] OMIM
- [ ] protein

Heterozygosity
- [ ] 0-10
- [ ] 40-50
- [ ] 10-20
- [ ] 20-30
- [ ] 30-40

Success Rate
- [ ] 80-85
- [ ] 85-90
- [ ] 90-95
- [ ] 95+

Created Build ID
- [ ] Current Build ID(137)
- [ ] Last Build ID(136)

Updated Build ID
- [ ] Current Build ID(137)
- [ ] Last Build ID(136)

Individual SNP
- [ ] Venter
- [ ] Watson
- [ ] Chinese_YH1

Minor Allele Freq

Minor Allele Frequency:

>= <=

HapMap Populations
- [ ] ASW
- [ ] CEU
- [ ] CHB
- [ ] CHD
- [ ] GIH

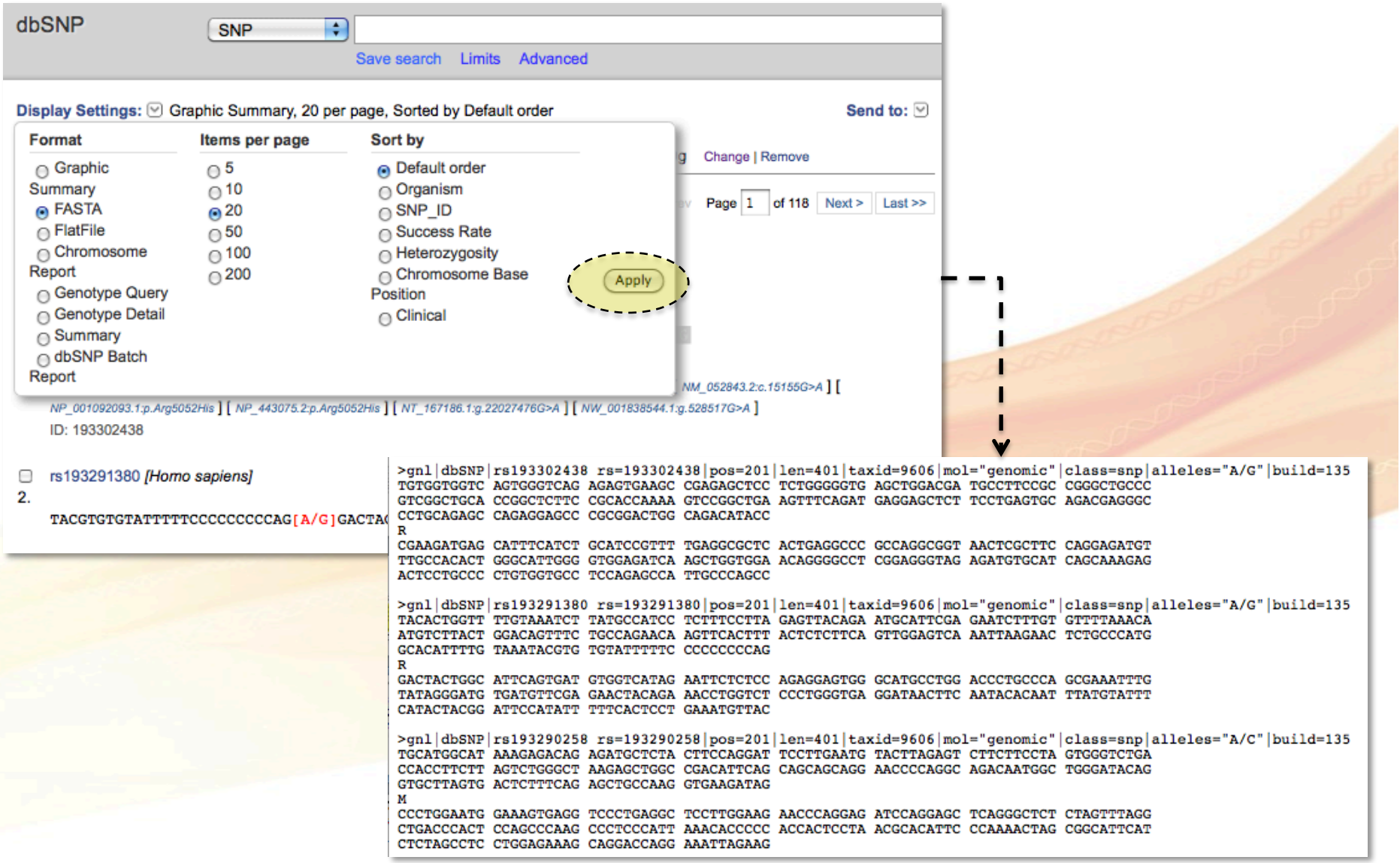
Global MAF

Global Minor Allele Frequency:

Min Max

Genome Project
- [ ] 1000 Genomes
  - [ ] Pilot 1
  - [ ] Pilot 2
  - [ ] Pilot 3
  - [ ] Has Frequency

More Specific Approach



FASTA Format and Data Structure for a Submitted SNP (ss) Record

```
            defline for FASTA records start with ">"
            | object-type=general
            | |                             total length
            | |   database name             of sequence                                                  list of
            | |    |                 offset of SNP |    Submitter                      organism    molecule   class of   alleles
            | |    |unique id  ss# in sequence  |         |         SubmitterSNPID         |         type     variation       |
            | |    |    |        |        |     |         |              |                 |           |           |         |
    defline: >gnl|dbSNP|ss271 ss=271|pos=51|len=101|handle="DEBNICK"|subid="lp03022"|taxid=9606|mol="Genomic"|class=1|alleles="G/A"
5' sequence: CTGCATCACATGTACTGATTCTGTCCATTGGAACAGAGATGATGACTGGT
  variation: R
3' sequence: TTACTAAACCCTGAGCCCTGGTGTTTCTGTTGATAGGGGGTTGCATTGAT
```

- Notes for ss FASTA records:
  - If variation is a SNP then **appropriate IUPAC nucleotide ambiguity** letter is selected to **represent the reported possible allele states**
  - microsatellite, insertion/deletion:
    - variation is represented as a single "N" on the variation line of the FASTA report
  - If the string of alleles is more than 30 characters, the list of alleles is replaced by the tag "lengthTooLong"

FASTA Format and Data Structure for a Reference SNP (rs) Record

```
            defline for FASTA records start with ">"
            | object-type=general
            | |                          total length
            | |   database name             of sequence                                  list of
            | |     |                 offset of SNP|    organism    molecule   class of   alleles     source of
            | |     |unique id  rs#  in sequence |         |         type     variation      |          sequence
            | |     |    |       |       |       |         |          |          |          |           |
    defline: >gnl|dbSNP|rs271 rs=271|pos=51|len=101|taxid=9606|mol="Genomic"|class=1|alleles="G/A"|source="dbSNP"
5' sequence: CTGCATCACATGTACTGATTCTGTCCATTGGAACAGAGATGATGACTGGT
  variation: R
3' sequence: TTACTAAACCCTGAGCCCTGGTGTTTCTGTTGATAGGGGGTTGCATTGAT
```

- rs FASTA records do not have the submitter/local SNP ID on the definition line (defline) - since they are clustered data objects constructed from one or more ss records
- SNP classes defined in: ftp://ftp.ncbi.nih.gov/snp/specs/docsum_3.0.asn
  - Remember the classes?

ftp://ftp.ncbi.nih.gov/snp/00readme.txt
ftp://ftp.ncbi.nih.gov/snp/database/README.create_local_dbSNP.txt
http://www.ncbi.nlm.nih.gov/books/NBK44378/

NCBI Supports the Public Distribution of dbSNP

- Provide zip-compressed data dumps in four different data formats
- Given this current dynamic environment
  - Periodically release builds and build updates to various organisms
  - Announcement of the build release or update on the dbSNP home site
- Access to the NCBI FTP site is available via the web or anonymous FTP
- The URL/host addresses are:
- World Wide Web: ftp://ftp.ncbi.nih.gov/snp/
- Anonymous FTP:
  - `ftp ftp.ncbi.nih.gov`

FTP

```
MacBook-Pro-SSD:~ cleslin$ ftp ftp.ncbi.nih.gov
Connected to ftp.wip.ncbi.nlm.nih.gov.
220-
 Warning Notice!

 You are accessing a U.S. Government information system which includes this
 computer, network, and all attached devices. This system is for
 Government-authorized use only. Unauthorized use of this system may result in
 disciplinary action and civil and criminal penalties. System users have no
 expectation of privacy regarding any communications or data processed by this
 system. At any time, the government may monitor, record, or seize any
 communication or data transiting or stored on this information system.
 ---
 Welcome to the NCBI ftp server! The anonymous access URL is ftp://ftp.ncbi.nlm.nih.gov/

 Public data may be downloaded by logging in as "anonymous" using your E-mail address as a password.

 Please see ftp://ftp.ncbi.nlm.nih.gov/README.ftp for hints on large file transfers
220 FTP Server ready.
Name (ftp.ncbi.nih.gov:cleslin): anonymous
331 Anonymous login ok, send your complete email address as your password
Password:
230 Anonymous access granted, restrictions apply
Remote system type is UNIX.
Using binary mode to transfer files.
ftp> cd snp
250 CWD command successful
ftp>
```

FTP

```
ftp> dir
229 Entering Extended Passive Mode (|||50308|)
150 Opening ASCII mode data connection for file list
-r--r--r--   1 ftp      anonymous    57152 Mar 23  2012 00readme.txt
dr-xr-xr-x   3 ftp      anonymous     4096 Mar 10  2010 Entrez
-r--r--r--   1 ftp      anonymous      157 Feb  7  2012 FTP_Getting_Started.txt
dr-xr-xr-x   2 ftp      anonymous     4096 Apr 21  2005 HapExampleDir
dr-xr-xr-x   5 ftp      anonymous    53248 Oct 20 16:34 batch
dr-xr-xr-x   9 ftp      anonymous     4096 Mar  5  2010 database
dr-xr-xr-x   2 ftp      anonymous     4096 Aug 29  2003 hs-data-freeze-index
dr-xr-xr-x  11 ftp      anonymous     4096 Feb  8  2012 last_minute_correction
dr-xr-xr-x 113 ftp      anonymous     4096 Feb  8  2012 organisms
dr-xr-xr-x   2 ftp      anonymous     4096 May 10  2010 osiris
dr-xr-xr-x   5 ftp      anonymous     4096 Nov  8  2010 release-notes
dr-xr-xr-x   3 ftp      anonymous     4096 Oct  1 19:33 specs
dr-xr-xr-x   2 ftp      anonymous     4096 Apr 28  2008 status
dr-xr-xr-x  22 ftp      anonymous     4096 Oct 15 16:35 temp
-r--r--r--   1 ftp      anonymous  3170070 May 29 16:14 temp.fromClinVar.30051
dr-xr-xr-x   3 ftp      anonymous     4096 May  1 19:04 test
dr-xr-xr-x   3 ftp      anonymous     4096 Apr 20  2005 webdata
226 Transfer complete
ftp> cd organisms/human_9606/rs_fasta
```

FTP

```
ftp> dir
229 Entering Extended Passive Mode (|||50295|)
150 Opening ASCII mode data connection for file list
-r--r--r--   1 ftp      anonymous 850305870 Jun  8 22:40 rs_ch1.fas.gz
-r--r--r--   1 ftp      anonymous 507372005 Jun  8 22:58 rs_ch10.fas.gz
-r--r--r--   1 ftp      anonymous 515659936 Jun  8 23:11 rs_ch11.fas.gz
-r--r--r--   1 ftp      anonymous 485748640 Jun  8 23:24 rs_ch12.fas.gz
-r--r--r--   1 ftp      anonymous 357265542 Jun  8 23:36 rs_ch13.fas.gz
-r--r--r--   1 ftp      anonymous 322122121 Jun  8 23:46 rs_ch14.fas.gz
-r--r--r--   1 ftp      anonymous 301860380 Jun  8 23:54 rs_ch15.fas.gz
-r--r--r--   1 ftp      anonymous 331279473 Jun  9 00:03 rs_ch16.fas.gz
-r--r--r--   1 ftp      anonymous 289118287 Jun  9 00:11 rs_ch17.fas.gz
-r--r--r--   1 ftp      anonymous 279678636 Jun  9 00:20 rs_ch18.fas.gz
..
..
-r--r--r--   1 ftp      anonymous 556362264 Jun  9 03:39 rs_ch8.fas.gz
-r--r--r--   1 ftp      anonymous 462457170 Jun  9 04:04 rs_ch9.fas.gz
-r--r--r--   1 ftp      anonymous       72 Jun  9 04:10 rs_chAltOnly.fas.gz
-r--r--r--   1 ftp      anonymous   161121 Jun  9 04:10 rs_chMT.fas.gz
-r--r--r--   1 ftp      anonymous 144427703 Jun  9 04:13 rs_chMulti.fas.gz
-r--r--r--   1 ftp      anonymous 43975102 Jun  9 04:15 rs_chNotOn.fas.gz
-r--r--r--   1 ftp      anonymous 16415699 Jun  9 04:16 rs_chPAR.fas.gz
-r--r--r--   1 ftp      anonymous 53189087 Jun  9 04:17 rs_chUn.fas.gz
-r--r--r--   1 ftp      anonymous 413074086 Jun  9 04:23 rs_chX.fas.gz
-r--r--r--   1 ftp      anonymous 22522600 Jun  9 04:29 rs_chY.fas.gz
226 Transfer complete
ftp> prompt
Interactive mode off.
ftp> mget rs_ch*
```

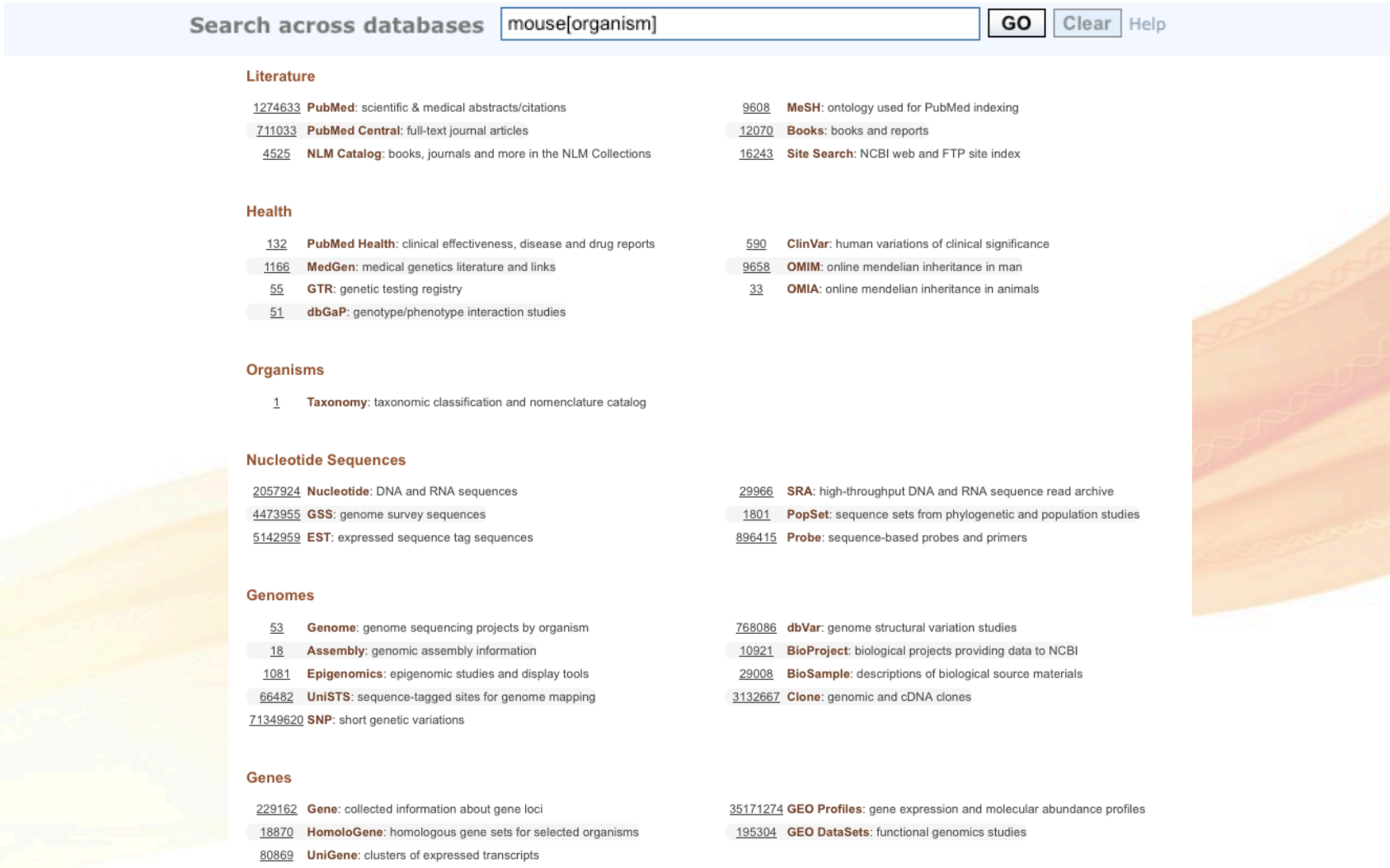
Turn off the prompting

Things to Do for Thursday

- Read
  - Featured Resource: An Expanded Set of Discovery Components in the Entrez System
    - http://www.ncbi.nlm.nih.gov/books/NBK7039/
  - Sequence Identifiers: A Historical Note
    - http://www.ncbi.nlm.nih.gov/Sitemap/sequenceIDs.html
  - Slide 55 "Things to Read"
- Try FTP
- Do the "Go Through These Slides After Lecture or At Home" – next slide
- Get started on the lab - posted later tonight

Go Through These Slides After Lecture or At Home

Perform an Organism Search – Across Databases



2013 - Oct

Apolipoprotein E (APOE)

- Important serum lipid transport protein
- Defects implicated in cardiovascular disease and late-onset Alzheimer disease (LOAD).
- Three common isoforms (alleles)

Isoform	**Position 112 (130)**	**Position 158 (176)**
e3	Cysteine (C)	Arginine (R)
e4	Arginine (R)	Arginine (R)
e2	Cysteine (C)	Cysteine (C)

The e4 isoform (allele) is associated with increased risk of LOAD

Human APOE Data Goals

- Reference Sequences
  - transcript (mRNA)
  - protein
  - gene (gDNA)
- Sites of Expression
  - UniGene Expression profile
  - GEO profiles
- View genomic assemblies and Maps
  - Comparative Maps
- Disease gene polymorphisms
- Genotypes
  - Reference Genome
  - HuRef (JC Venter genome)
- Homologs in other species
  - HomoloGene
  - BLAST to find Panda homologs

Human APOE Gene and RefSeqs

Gene Database

1. Search All Databases with APOE from NCBI Homepage
2. Retrieve PubMed results
3. Follow Gene Ad to Gene ID 348
4. Follow link to "reference sequence details"

Gene Database

1. Look for the Transcript and protein
    1. NM_000041.2
    2. NP_000032.1
2. Look at the Genomic
    1. RefSeq Gene NG_007084.2
3. Look for the Genome Builds
    1. Reference NC_000019.9
    2. HuRef

Keep this Gene page open for the rest of the searching, right click and "Open Link In New Window" for the rest of the slides

Genome Maps

APO Gene Cluster

1. Gene "Links" menu (on the right) to Map Viewer
2. Maps and Options
    1. Remove all but Gene
3. Zoom out 10X using graphic on right
4. Zoom in 2X by clicking on map
5. What other genes are part of the APO gene cluster?

Comparative Maps

1. Map Viewer "Maps and Options"
    1. Add Chimp and Mouse Gene Maps

Allelic Variants and Disease

OMIM

1. Gene links menu to OMIM
2. Retrieve APOLIPOPROTEIN E; APOE
3. Click allelic variants
4. Jump to .0016 ALZHEIMER DISEASE 2 [APOE, CYS112ARG]

dbSNP

1. Gene links menu to SNP
2. Click the "Gene View" on once of the graphic overives
3. Check “Clinical Source” and click “Refresh”
4. Look for position 130 Cys->Arg
5. Click on rs429358 in table
6. Note HuRef has C allele

Expression Information

UniGene

1. Gene links menu to UniGene
2. Look at the EST profile
3. What is the expression pattern, widespread or confined to a certain organ?

GEO Profiles

1. Gene links menu to GEO profiles
2. Use History to combine link from Gene AND GDS596
3. Predominate expression in liver, some in brain

Homlogous Genes / Proteins

HomoloGene

1. Gene links menu to HomoloGene
2. Use "pairwise alignments" device to compare Human and Chimp proteins at position 130
3. Use UniGene portion to find rabbit (*Oryctolagus cuniculus*) homolog
4. What's the Identity?

Using BLAST

1. Gene link to RefSeq protein NP_000032
2. "Run BLAST" from Discovery Column
3. Choose Reference Proteins database
4. Organism limit Giant Panda
5. BLAST button
6. Any homologs found in Panda?

Finding a Structure

Related Structures

1. Gene link to NP_000032
2. Links menu to Related Structures (Summary)
3. Change to "All similar MMDB" and click "Refresh Display"
4. Link to sequence alignment from 1B68_A
5. Do you see the polymorphism at position 112? Is so what is it?

Structure and Cn3D

1. Click the 1B68 from the "Related Structures" page you are at
2. Click the MMDB ID: 16994
3. Related structures link to structure for 1B68
4. Click Structure View in Cn3D (Cn3D must be installed)
5. Manipulate structure
6. Add side chains using Style -> Edit Global Style
7. Highlight Arg 112