Bioinformatics and Functional Genomics

Course Overview, Introduction of Bioinformatics, Biology Background

Biol4230 Thurs, Jan 17, 2017

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Goals of today's lecture:

- Overview of the course
- Introduction to Bioinformatics – questions, algorithms, resources, data types
- Introduction to Genome Biology – DNA, RNA, and protein (molecule types, sizes, and abundance), gene structure, protein structure
- Preparation for tomorrow's Unix Lecture/Lab

What should you do to reinforce the lecture material?

- Luscombe et al. (2001) "What is Bioinformatics? An introduction and overview" Methods Inf Med. 40:346-58. PMID: 11552348
- Pevsner, Ch. 1, 2
- Recombinant DNA, Ch. 1,2
- Basic Biology:
  - what is the DNA alphabet? the protein alphabet?
  - what is an "exon"? an "intron"? which sequences make mRNA?
  - what is an initiation codon (how many are there)? a termination codon (how many)?
- Visit the NCBI website (www.ncbi.nlm.nih.gov), and look up the plant protein alpha amylase in rice. (`alpha amylase AND rice[organism]`)
  - How many proteins are there? How many in RefSeq? What is the longest? The shortest? How many genes?
  - Pick a single rice alpha amylase (one longer than 400 aa) at the NCBI and check its domains (how many?), and gene structure (how many exons?, how many code for protein?).
- Look for rice alpha-amylase proteins at Uniprot (www.uniprot.org).
  - How many alpha-amylases are in SwissProt? In Trembl?
  - Can you find a long (>400 aa) rice alpha amylase in RefSeq that is not found in SwissProt? Can you find it in Trembl?
  - What information is available at the NCBI that is not available at Uniprot?
  - At Uniprot but not NCBI?

Bioinformatics and Functional Genomics – Overview

- Homology, Similarity searching, evolutionary tree reconstruction
  - BLAST and FASTA, scoring matrices, tree-building methods
- Unix at the command line, Python scripting
  - unix commands, directories and files, using an editor
  - writing/debugging Python scripts
- Gene expression analysis (RNAseq)
  - "NextGen" sequence analysis (cleaning, alignment, mapping)
  - 'R' and 'BioConductor'
- Identifying regulatory motifs

Why study/teach bioinformatics?

- The human genome project: 1991 – 2001 knowledge/assumptions before 2001
  - human genome size known (3 billion bp, haploid, 23 chromosomes)
  - E coli (4 million bp, had about 4,000 genes)
  - human gene estimates from 30,000 – 300,000 genes, with most estimates > 100,000
  - ~ 50% of genome was "single copy", 5 – 10% transcribed in most tissues (greater in brain)
- human genome, post 2001
  - correct genome size
  - 15,000 – 20,000 genes (smaller than plants)
  - <2% of genome transcribed into proteins
  - most individuals have 100 – 500 non-functional (truncated) protein coding genes
- Bioinformatics illustrates the shortcomings of "big data" approaches. The enormous increase in data "volume" seems to raise more questions than provide answers.

How to determine what's "true"?

Bioinformatics and Functional Genomics – What will you learn?

- Similarity searching, from the command line, and using scripts
- Multiple sequence alignment and phyogeny reconstruction
- Large-scale sequence mapping, and genome sequence manipulation
- (Regulatory) Motif finding
- Biological Pathway analysis

What are the algorithmic and biological reasons for errors and inconsistencies?

What can we trust?

What is Bioinformatics?

- Data organization
  - sequence/structure/expression/variation databases (resources)
  - Nucleic Acid Res. database, web server, issue
- Development of algorithms/statistics/tools
  - FASTA, BLAST, CLUSTAL, MUSCLE, PHYLIP, BALIPHY, MAC, TOPHAT, CUFFLINKS, BIOCONDUCTOR, DAVID,
- Application and evaluation of analysis methods to understand biological processes
  - what does an unknown protein do (activity)?
  - what genes are up/down-regulated in cancer?
  - what mutations increase/reduce heart disease?

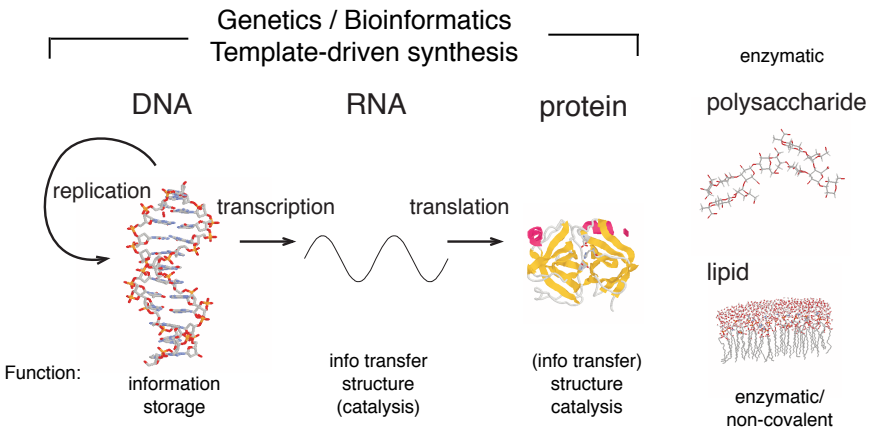
Luscombe et al. (2001) PMID: 11552348

What is Bioinformatics?

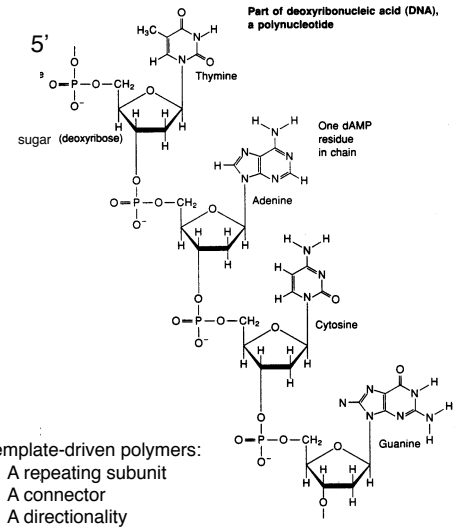
Bioinformatics explores differences (changes) in DNA, RNA, and protein sequence and abundance.

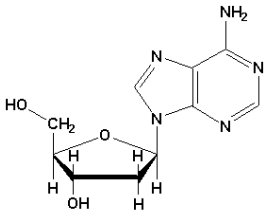
- genetic information – molecules made from a genetic template
- changes in DNA: variation
  - mutation (single site) or copy number variation (gene or multigene regions)
  - no changes in abundance, all cells have (almost) the same DNA content
- changes in RNA: structure and abundance
  - different cells express (make RNA for) different genes
  - different RNAs can be made from the same gene
- changes in protein:
  - abundance (dependent on RNA abundance, but other factors) – partially genetic
  - post-translational modification (non-template changes)
  - interactions and binding partners

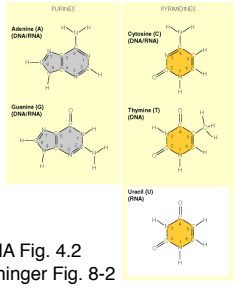
The Central Dogma of Molecular Biology
Molecules for Information transfer, storage, and function



Polymers and Monomers - DNA



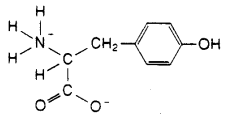




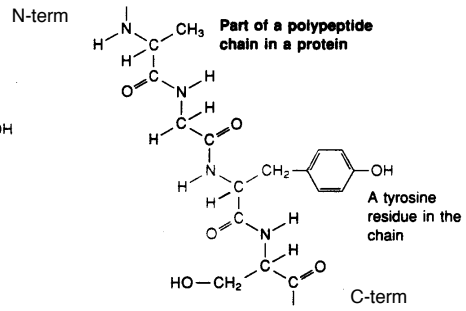
Template-driven polymers:
1. A repeating subunit
2. A connector
3. A directionality

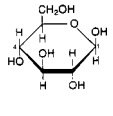
MvHA Fig. 4.2
Lehninger Fig. 8-2

Monomers and Polymers - proteins



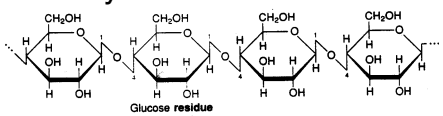
tyrosine monomer





β-D-glucose

carbohydrates



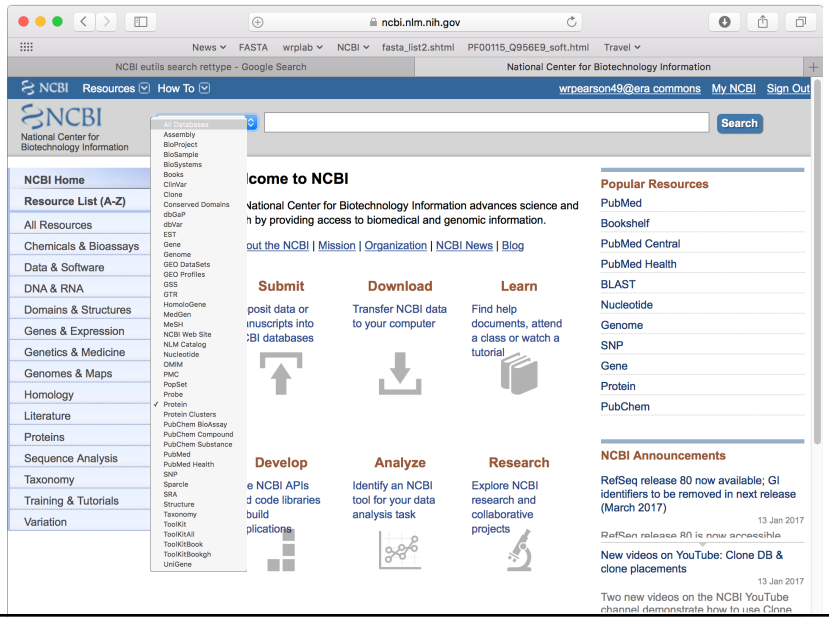
Basic biology you should know

- Central dogma DNA transcribed into RNA translated into protein
- Prokaryotes – bacteria, archaea
  - no nuclei
  - small genomes (1,000 – 5,000 genes), >90% of genome is protein coding
  - RNA transcript = mRNA (unspliced)
- Eukaryotes – higher organisms (yeast, plants, people)
  - nuclei, mitochondria, chloroplasts (plants)
  - small (yeast) to large (plants, metazoa) genomes
  - large genomes have similar numbers of genes (10,000 – 20,000), but < 5% of genome codes for protein
  - RNA transcripts can be spliced into mRNA
- proteins – (20 amino acids)
  - average size ~400 amino acids, range from 10 – 40,000 amino acids
  - are directional (start at N-terminus, initiation codon, AUG, end at C-terminus, stop codon, UAA, UAG, UGA)
  - fold into distinct 3-D structures, characterized by alpha-helices, beta-sheets
- mRNA – (4 nucleotides, 61 codons for amino acids + 3 termination)
  - average size ~2000 nucleotides (1200 nt code for protein, remainder short 5'-untranslated, long 3-untranslated), end with poly-A (added after transcription)
  - in prokaryotes, same as transcript
  - in eukaryotes, built from exons (separated by introns) from a much longer transcript
  - RNAs differ in abundance (>1000-fold) in different tissues

Protein Sequence and Structure Databases

1. NCBI/Entrez – Most comprehensive, linked to PubMed.
   - Best known: GenBank / GenPept , but probably least useful.
   - Most annotated: RefSeq
   - Best links to human disease: Entrez/Gene and OMIM.
2. Uniprot – Most information about proteins
   - Functional information (functional sites)
   - Links to other databases (InterPro for domains)
3. 1,500+ Biological/disease/genetic/variation databases
   - Nucleic Acids Research database issue nar.oxfordjournals.org/content/45/D1/D1.abstract

www.ncbi.nlm.nih.gov



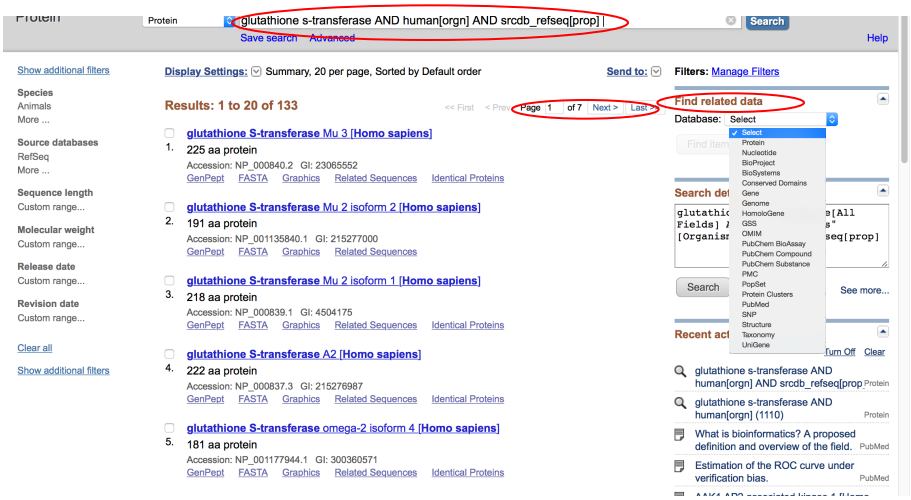
NCBI Databases and Services

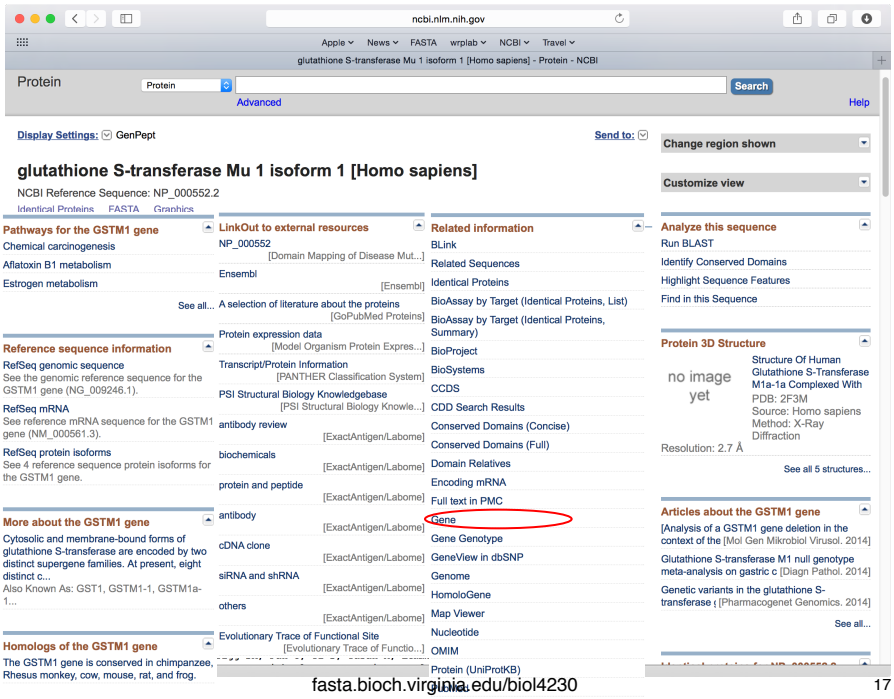
- GenBank primary sequence database
- Free public access to biomedical literature
  - PubMed free Medline
  - PubMed Central full text online access
- Entrez integrated molecular and literature databases
- BLAST highest volume sequence search service
- VAST structure similarity searches
- Software and databases for download

Types of Databases

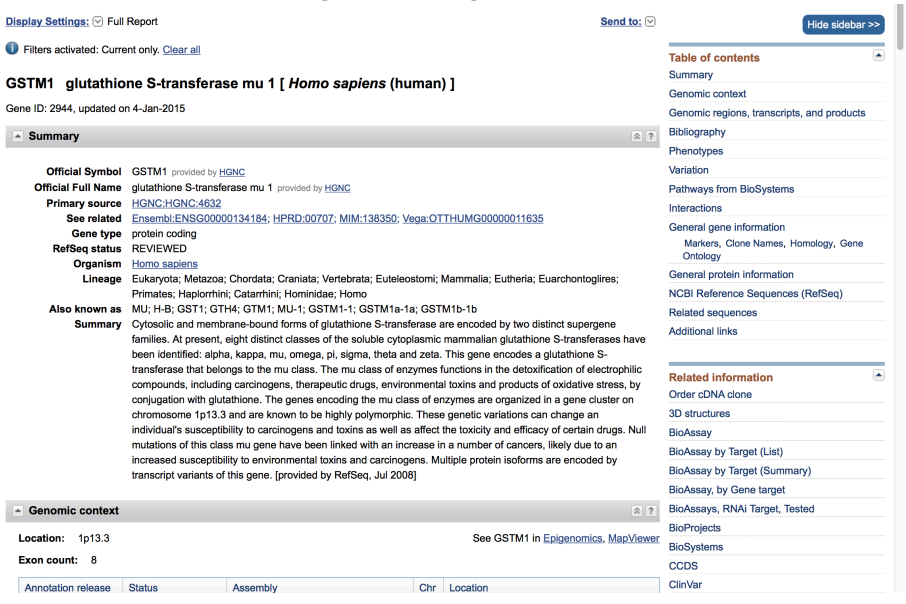
- Primary Databases (avoid)
  - Original submissions by experimentalists
  - Content controlled by the submitter
    - Examples: GenBank, SNP, GEO
- Derivative Databases (use)
  - Built from primary data
  - Content controlled by third party (NCBI)
    - Examples: NCBI Protein, Refseq, TPA, RefSNP, GEO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

Finding protein sequences with Entrez/Proteins

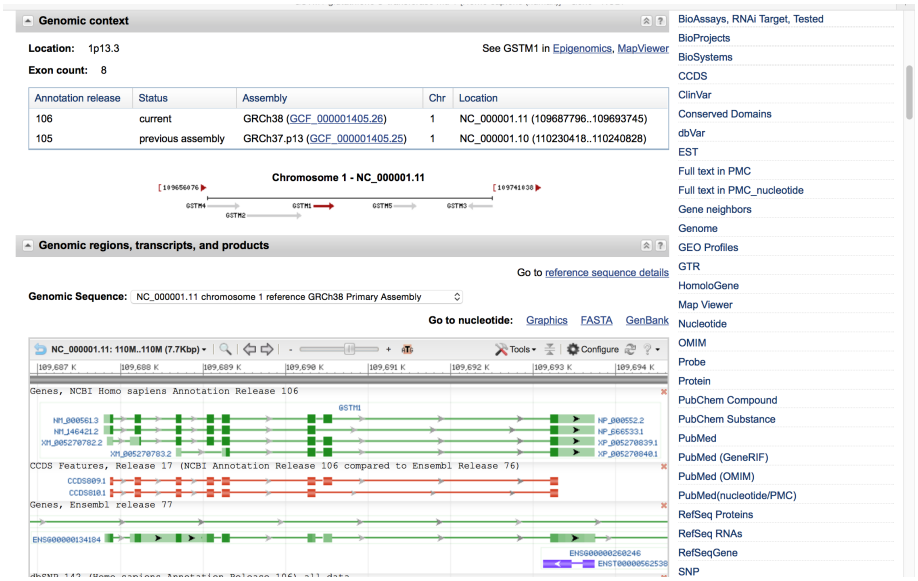




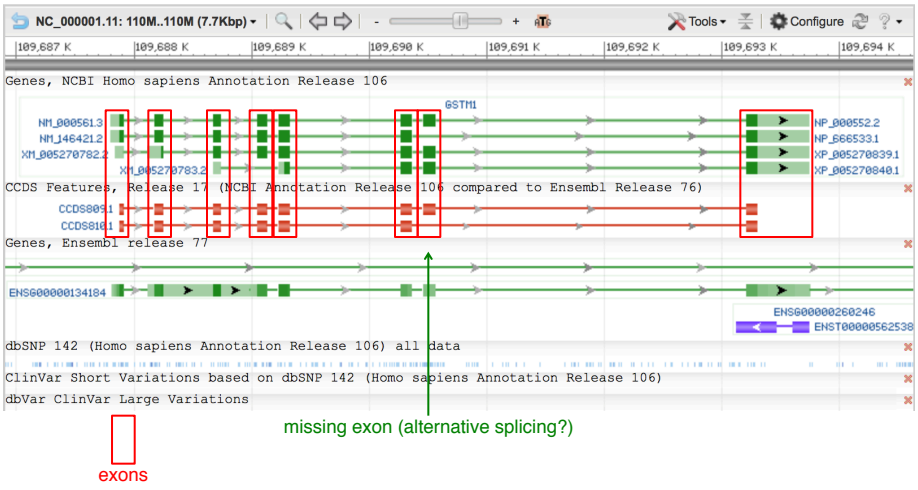
Entrez Gene: genetic/genomic information



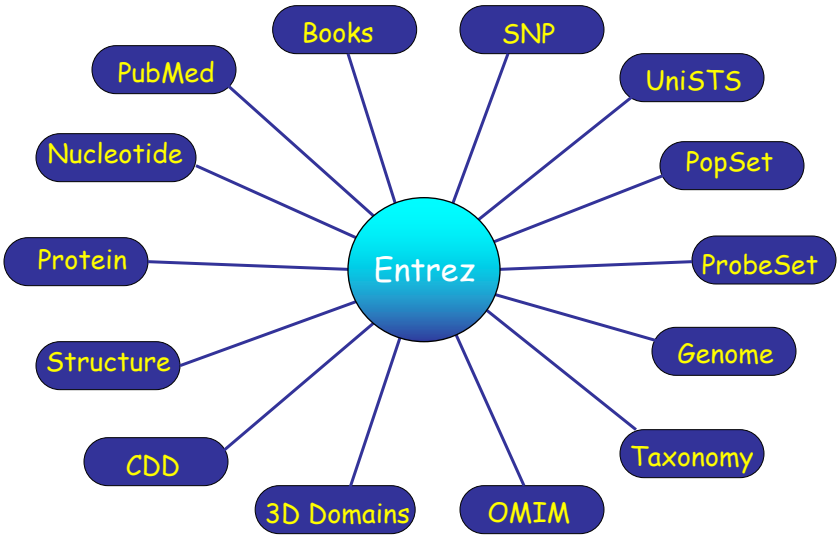
Entrez Gene: genetic/genomic information



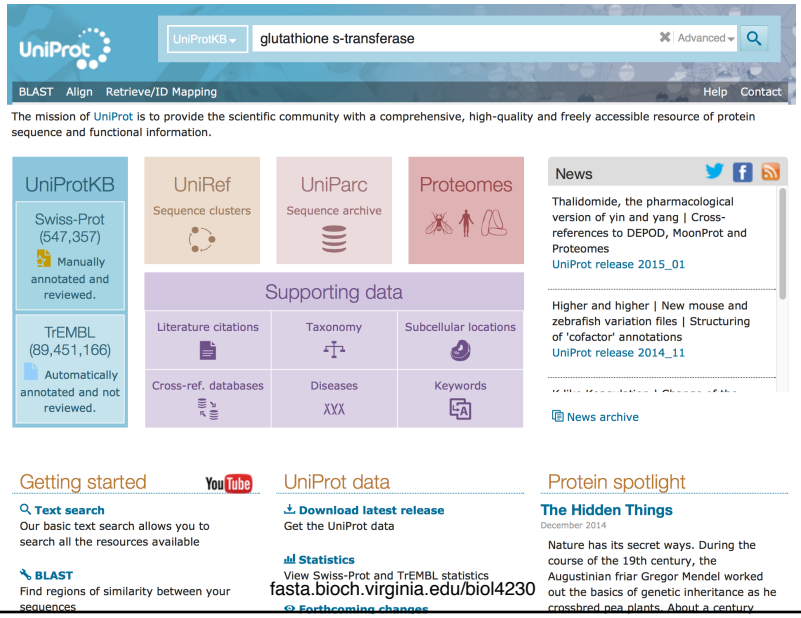
Entrez Gene: Genomic/transcript structure

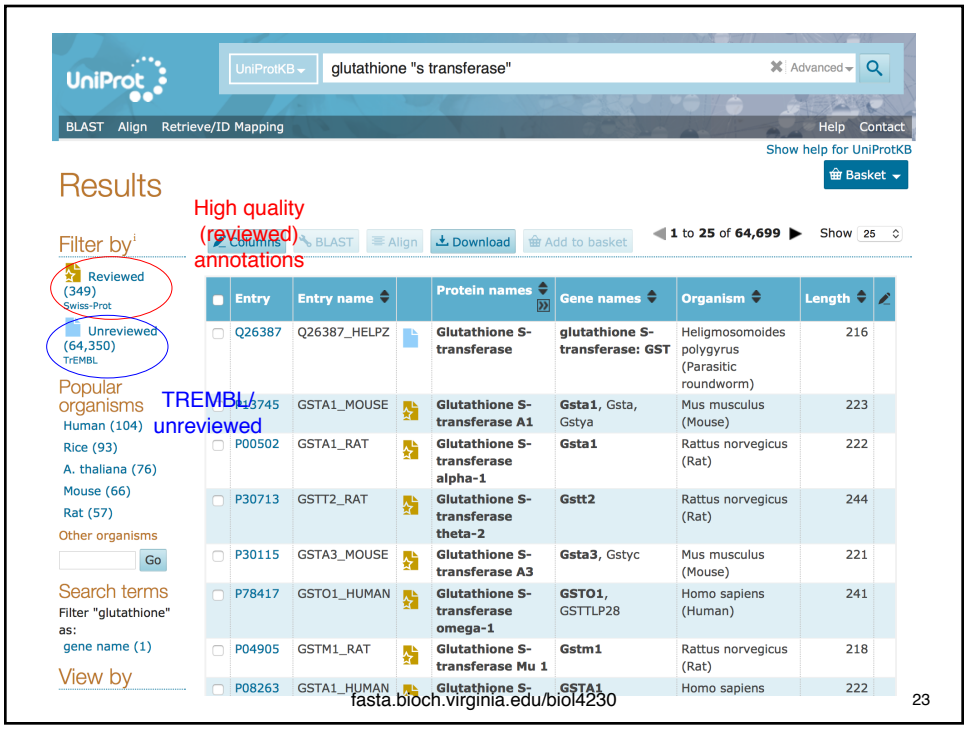


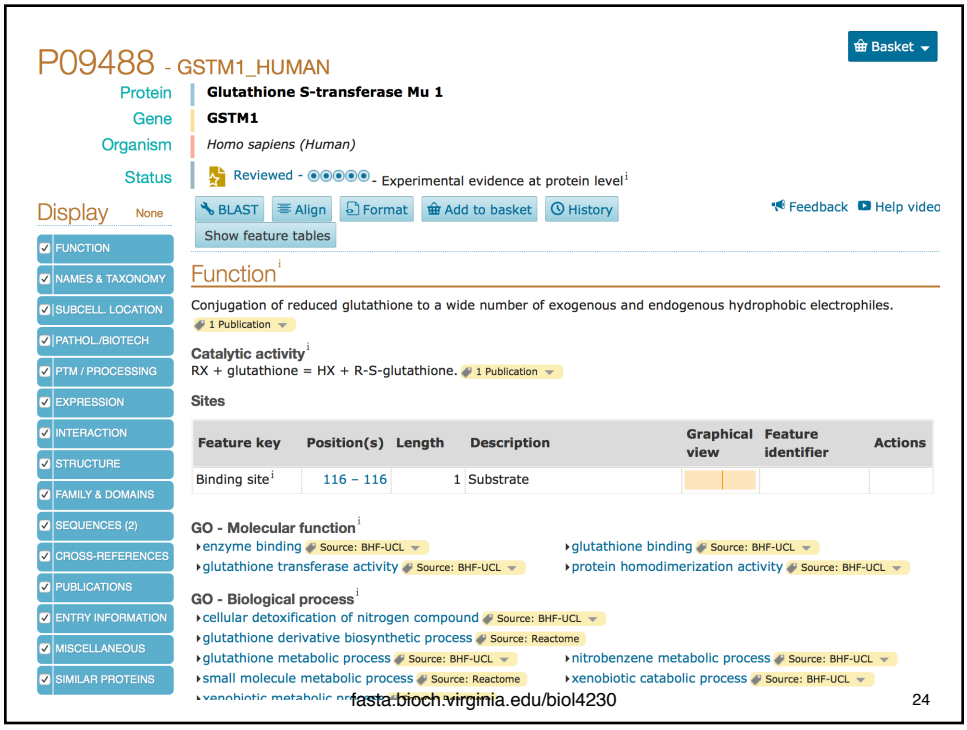
The (ever) Expanding Entrez System

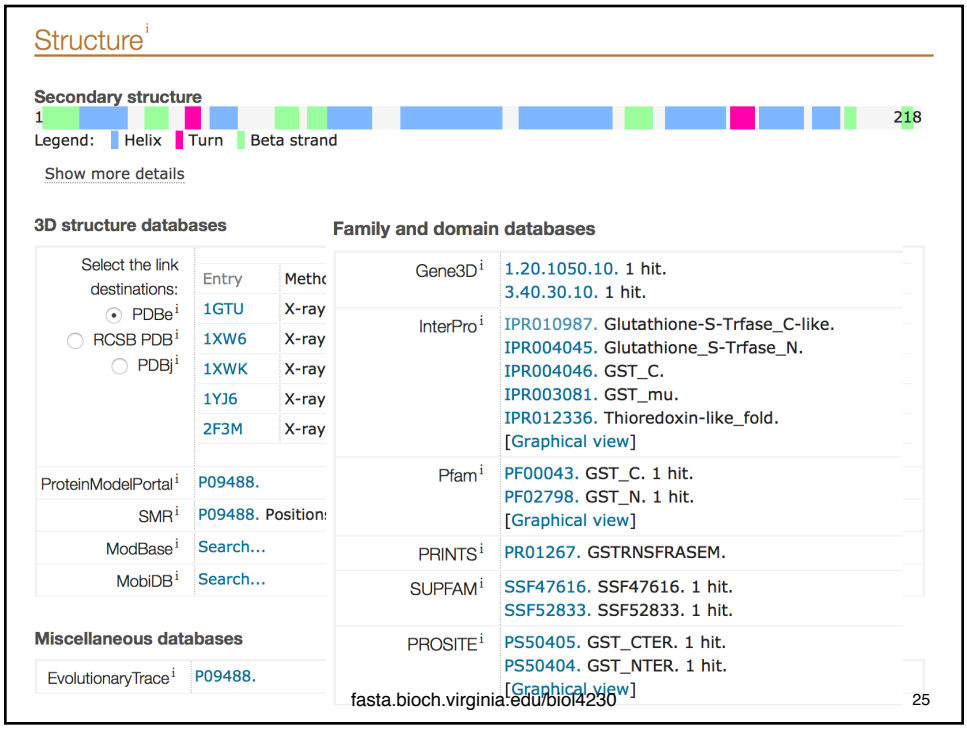


Uniprot/SwissProt (uniprot.org) Comprehensive (inclusive) Database links




UniProt
UniProtKB
glutathione "s transferase"
Advanced
BLAST Align Retrieve/ID Mapping
Help Contact
Show help for UniProtKB
Basket
Results
High quality (reviewed) annotations
Filter by
Reviewed (349) Swiss-Prot
Unreviewed (64,350) TrEMBL
TREMBL/ unreviewed
Popular organisms
Human (104)
Rice (93)
A. thaliana (76)
Mouse (66)
Rat (57)
Other organisms
Go
Search terms
Filter "glutathione" as:
gene name (1)
View by
Columns BLAST Align Download Add to basket
1 to 25 of 64,699
Show 25
Entry | Entry name | Protein names | Gene names | Organism | Length
Q26387 | Q26387_HELPZ | Glutathione S-transferase | glutathione S-transferase: GST | Heligmosomoides polygyrus (Parasitic roundworm) | 216
P13745 | GSTA1_MOUSE | Glutathione S-transferase A1 | Gsta1, Gsta, Gstya | Mus musculus (Mouse) | 223
P00502 | GSTA1_RAT | Glutathione S-transferase alpha-1 | Gsta1 | Rattus norvegicus (Rat) | 222
P30713 | GSTT2_RAT | Glutathione S-transferase theta-2 | Gstt2 | Rattus norvegicus (Rat) | 244
P30115 | GSTA3_MOUSE | Glutathione S-transferase A3 | Gsta3, Gstyc | Mus musculus (Mouse) | 221
P78417 | GSTO1_HUMAN | Glutathione S-transferase omega-1 | GSTO1, GSTTLP28 | Homo sapiens (Human) | 241
P04905 | GSTM1_RAT | Glutathione S-transferase Mu 1 | Gstm1 | Rattus norvegicus (Rat) | 218
P08263 | GSTA1_HUMAN | Glutathione S- | GSTA1 | Homo sapiens | 222


P09488 - GSTM1_HUMAN
Basket
Protein Glutathione S-transferase Mu 1
Gene GSTM1
Organism Homo sapiens (Human)
Status Reviewed - ●●●●● - Experimental evidence at protein level
BLAST Align Format Add to basket History
Feedback Help video
Show feature tables
Display None
FUNCTION
NAMES & TAXONOMY
SUBCELL. LOCATION
PATHOL./BIOTECH
PTM / PROCESSING
EXPRESSION
INTERACTION
STRUCTURE
FAMILY & DOMAINS
SEQUENCES (2)
CROSS-REFERENCES
PUBLICATIONS
ENTRY INFORMATION
MISCELLANEOUS
SIMILAR PROTEINS
Function
Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles.
1 Publication
Catalytic activity
RX + glutathione = HX + R-S-glutathione. 1 Publication
Sites
Feature key | Position(s) | Length | Description | Graphical view | Feature identifier | Actions
Binding site | 116 – 116 | 1 | Substrate
GO - Molecular function
enzyme binding Source: BHF-UCL
glutathione transferase activity Source: BHF-UCL
glutathione binding Source: BHF-UCL
protein homodimerization activity Source: BHF-UCL
GO - Biological process
cellular detoxification of nitrogen compound Source: BHF-UCL
glutathione derivative biosynthetic process Source: Reactome
glutathione metabolic process Source: BHF-UCL
small molecule metabolic process Source: Reactome
nitrobenzene metabolic process Source: BHF-UCL
xenobiotic catabolic process Source: BHF-UCL



Structure

Secondary structure

1 218

Legend: Helix Turn Beta strand

Show more details

3D structure databases

Select the link destinations:
PDBe
RCSB PDB
PDBj

Entry	Metho
1GTU	X-ray
1XW6	X-ray
1XWK	X-ray
1YJ6	X-ray
2F3M	X-ray

ProteinModelPortal	P09488.
SMR	P09488. Position
ModBase	Search...
MobiDB	Search...

Miscellaneous databases

EvolutionaryTrace	P09488.

Family and domain databases

Gene3D	1.20.1050.10. 1 hit. 3.40.30.10. 1 hit.
InterPro	IPR010987. Glutathione-S-Trfase_C-like. IPR004045. Glutathione_S-Trfase_N. IPR004046. GST_C. IPR003081. GST_mu. IPR012336. Thioredoxin-like_fold. [Graphical view]
Pfam	PF00043. GST_C. 1 hit. PF02798. GST_N. 1 hit. [Graphical view]
PRINTS	PR01267. GSTRNSFRASEM.
SUPFAM	SSF47616. SSF47616. 1 hit. SSF52833. SSF52833. 1 hit.
PROSITE	PS50405. GST_CTER. 1 hit. PS50404. GST_NTER. 1 hit. [Graphical view]

Glutathione S-transferase GSTM1

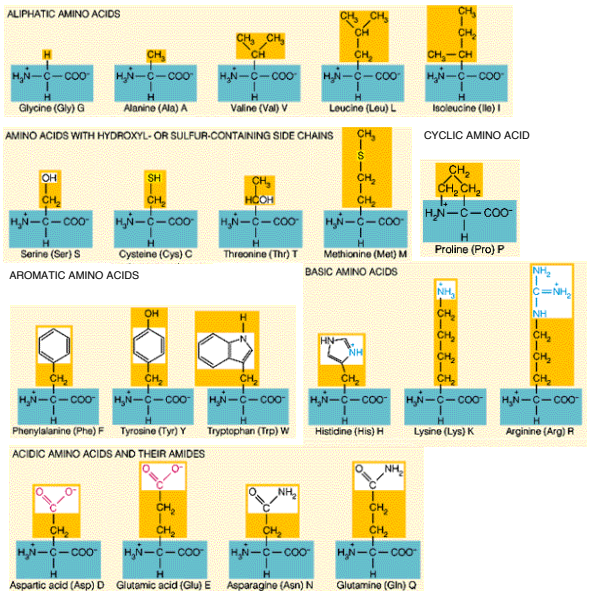
```
>sp|P09488|GSTM1_HUMAN Glutathione S-transferase Mu 1 GN=GSTM1
MPMILGYWDIRGLAHAIRLLLEYTDSSYEEKKYTMGDAPDYDRSQWLNEKFKLGLDFPNL
PYLIDGAHKITQSNAILCYIARKHNLCGETEEEKIRVDILENQTMDNHMQLGMICYNPEF
EKLKPKYLEELPEKLKLYSEFLGKRPWFAGNKITFVDFLVYDVLDLHRIFEPKCLDAFPN
LKDFISRFEGLEKISAYMKSSRFLPRPVFSKMAVWGNK
```

Sequence in "FASTA" format

Structure and properties of Amino-acids

Alanine	Ala	A	Leucine	Leu	L
Arginine	Arg	R	Lysine	Lys	K
Asparagine	Asn	N	Methionine	Met	M
Aspartic acid	Asp	D	Phenylalanine	Phe	F
Cysteine	Cys	C	Proline	Pro	P
Glutamine	Gln	Q	Serine	Ser	S
Glutamic acid	Glu	E	Threonine	Thr	T
Glycine	Gly	G	Tryptophan	Trp	W
Histidine	His	H	Tyrosine	Tyr	Y
Isoleucine	Ile	I	Valine	Val	V
Asp/Asn	Asx	B	Glu/Gln	Glx	Z

Figure 5.3: The amino acids found in proteins.



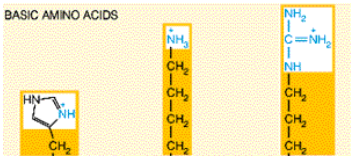
Some amino acids are more common than others:

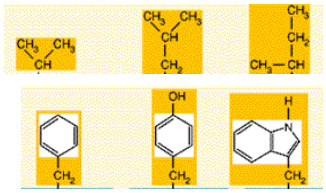
+	Ala	A	0.0780
	Arg	R	0.0512
	Asn	N	0.0448
	Asp	D	0.0536
-	Cys	C	0.0192
	Gln	Q	0.0426
+	Glu	E	0.0629
+	Gly	G	0.0737
-	His	H	0.0219
	Ile	I	0.0514
+	**Leu**	**L**	**0.0901**
	Lys	K	0.0574
-	Met	M	0.0224
-	Phe	F	0.0385
	Pro	P	0.0520
+	Ser	S	0.0711
	Thr	T	0.0584
-	***Trp***	***W***	***0.0132***
-	Tyr	Y	0.0321
+	Val	V	0.0644

Robinson and Robinson,
PNAS (1991) 88:8880

Amino acid Hydropathicity/Hydrophobicity

Hopp T.P., Woods K.R. (1981) PNAS. 78:3824-3828.
Kyte J., Doolittle R.F. (1982). J. Mol. Biol. 157:105-132
D. M. Engelman, T. A. Steitz, A. Goldman, (1986) Annu. Rev. Biophys. Biophys. Chem. 15, 321





Hopp/ Woods		Kyte/ Doolittle		GES	
Arg:	3.0	Arg:	-4.5	Arg:	12.3
Lys:	3.0	Lys:	-3.9	Asp:	9.2
Asp:	3.0	Asp:	-3.5	Lys:	8.8
Glu:	3.0	Glu:	-3.5	Glu:	8.2
Ser:	0.3	Gln:	-3.5	Asn:	4.8
Gln:	0.2	Asn:	-3.5	Gln:	4.1
Asn:	0.2	His:	-3.2	His:	3.0
Pro:	0.0	Pro:	-1.6	Tyr:	0.7
Gly:	0.0	Tyr:	-1.3	Pro:	0.2
Thr:	-0.4	Trp:	-0.9	Ser:	-0.6
His:	-0.5	Ser:	-0.8	Gly:	-1.0
Ala:	-0.5	Thr:	-0.7	Thr:	-1.2
Cys:	-1.0	Gly:	-0.4	Ala:	-1.6
Met:	-1.3	Ala:	1.8	Trp:	-1.9
Val:	-1.5	Met:	1.9	Cys:	-2.0
Leu:	-1.8	Cys:	2.5	Val:	-2.6
Ile:	-1.8	Phe:	2.8	Leu:	-2.8
Tyr:	-2.3	Leu:	3.8	Ile:	-3.1
Phe:	-2.5	Val:	4.2	Met:	-3.4
Trp:	-3.4	Ile:	4.5	Phe:	-3.7

Amino-acid classes from evolution/mutation

Given a set of (closely) related protein sequences...

```
GSTM1_HUMAN     MPMILGYWDIRGLAHAIRLLLEYTDSSYEEKKYTMGDAPDYDRSQWLNEKFKLGLD
GSTM2_HUMAN     MPMTLGYWNIRGLAHSIRLLLEYTDSSYEEKKYTMGDAPDYDRSQWLNEKFKLGLD
GSTM4_HUMAN     MPMILGYWDIRGLAHAIRLLLEYTDSSYEEKKYTMGGAPDYDRSQWLNEKFKLGLD
GSTM5_HUMAN     MPMTLGYWDIRGLAHAIRLLLEYTDSSYVEKKYTMGDAPDYDRSQWLNEKFKLGLD
GSTM1_MOUSE     MPMILGYWNVRGLTHPIRMLLEYTDSSYDEKRYTMGDAPDFDRSQWLNEKFKLGLD
GSTM2_MOUSE     MPMTLGYWDIRGLAHAIRLLLEYTDTSYEDKKYTMGDAPDYDRSQWLSEKFKLGLD
GSTM3_MOUSE     MPMTLGYWNTRGLTHSIRLLLEYTDSSYEEKRYVMGDAPNFDRSQWLSEKFNLGLD
GSTM4_MOUSE     MSMVLGYWDIRGLAHAIRMLLEFTDTSYEEKRYICGEAPDYDRSQWLDVKFKLDLD
GSTM3_RABIT     MPMTLGYWDVRGLALPIRMLLEYTDTSYEEKKYTMGDAPNYDQSKWLSEKFTLGLD
```

… how often is one amino-acid replaced by another?

REVIEW

Central dogma, databases, and amino-acids

- DNA, RNA, and proteins are template driven bio-polymers (what is the template for each?)
- Today, secondary, curated databases provide much more biological information than primary databases
- The 20 amino acids can be divided into different functional/chemical classes (they are not equally frequent)

Basic biology you should know

- Central dogma DNA transcribed into RNA translated into protein
- Prokaryotes – bacteria, archaea
  - no nuclei
  - small genomes (1,000 – 5,000 genes), >90% of genome is protein coding
  - RNA transcript = mRNA (unspliced)
- Eukaryotes – higher organisms (yeast, plants, people)
  - nuclei, mitochondria, chloroplasts (plants)
  - small (yeast) to large (plants, metazoa) genomes
  - large genomes similar numbers of genes (10,000 – 20,000), but < 5% of genome codes for protein
  - RNA transcripts can be spliced into mRNA
- proteins – (20 amino acids)
  - average size ~400 amino acids, range from 10 – 40,000 amino acids
  - are directional (start at N-terminus, initiation codon, AUG, end at C-terminus, stop codon, UAA, UAG, UGA)
  - fold into distinct 3-D structures, characterized by alpha-helices, beta-sheets
- mRNA – (4 nucleotides, 61 codons for amino acids + 3 termination)
  - average size ~2000 nucleotides (1200 nt code for protein, remainder short 5'-untranslated, long 3-untranslated), end with poly-A (added after transcription)
  - in prokaryotes, same as transcript
  - in eukaryotes, built from exons (separated by introns) from a much longer transcript
  - RNAs differ in abundance (>1000-fold) in different tissues

What should you do to reinforce the lecture material?

- Luscombe et al. (2001) "What is Bioinformatics? An introduction and overview" Methods Inf Med. 40:346-58. PMID: 11552348
- Pevsner, Ch. 1, 2
- Recombinant DNA, Ch. 1,2
- Basic Biology:
  - what is the DNA alphabet? the protein alphabet?
  - what is an "exon"? an "intron"? which sequences make mRNA?
  - what is an initiation codon (how many are there)? a termination codon (how many)?
- Visit the NCBI website (www.ncbi.nlm.nih.gov), and look up the plant protein alpha amylase in rice. (`alpha amylase AND rice[organism]`)
  - How many proteins are there? How many in RefSeq? What is the longest? The shortest? How many genes?
  - Pick a single rice alpha amylase (one longer than 400 aa) at the NCBI and check its domains (how many?), and gene structure (how many exons?, how many code for protein?).
- Look for rice alpha-amylase proteins at Uniprot (www.uniprot.org).
  - How many alpha-amylases are in SwissProt? In Trembl?
  - Can you find a long (>400 aa) rice alpha amylase in RefSeq that is not found in SwissProt? Can you find it in Trembl?
  - What information is available at the NCBI that is not available at Uniprot?
  - At Uniprot but not NCBI?

Before Unix Lab (tomorrow, Friday)

1. Make certain your laptop can use the "Cavalier" wireless
2. Windows: download and install SecureCRT
3. Know/reset your "`its`" eservices password
   `its.virginia.edu/accounts/createacct.html`
4. (For work outside UVA) Install UVA Anywhere VPN
5. Try to connect (ssh) to `interactive.hpc.virginia.edu`