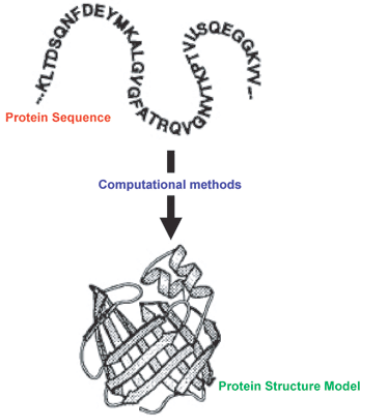
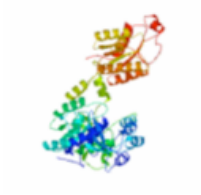
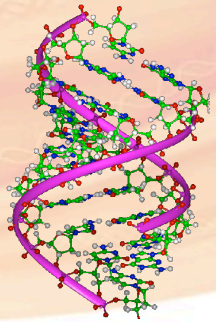




Bioinformatics Computational Methods 1 - BIOL 6308



October 29th 2013

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

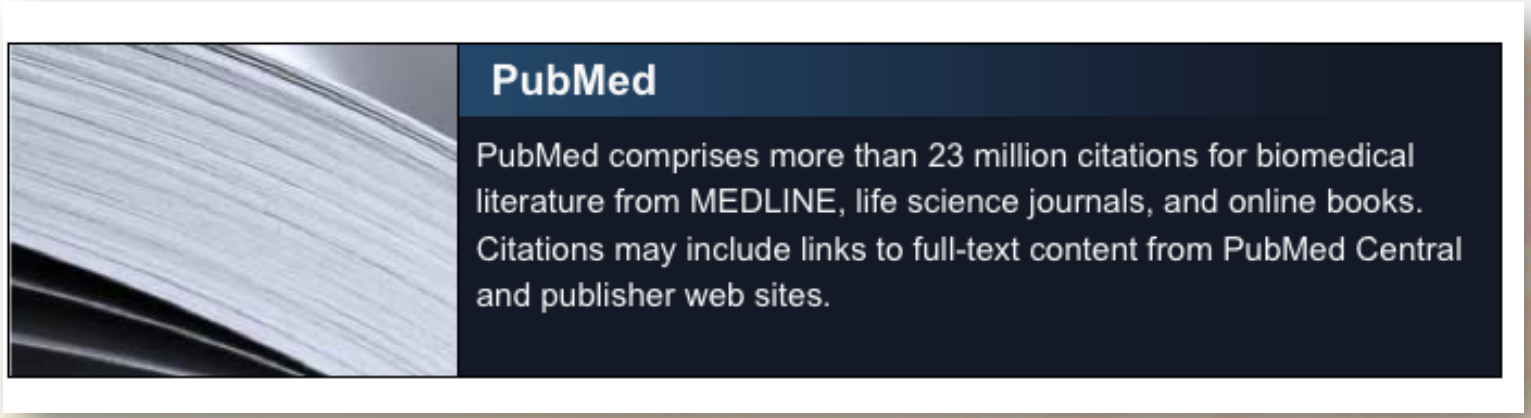
Last Time

- What is an Accession Number
- Examples of RefSeq Accession #'s
- Databases
  - Gene
  - GenPepts
  - Unigene
  - Structure
  - MMDB
  - Vast
  - CDD
- What are SNPs
  - Formal Definition
  - Linked and Causative SNPs
  - Functional Categories
  - Nomenclature
  - dbSNP
- Homework to "discover" the The Discovery Process

NCBI II
Access to Biomedical Literature

PubMed

- PubMed is the NCBI gateway to MEDLINE
- +23 million citations



- As a bioinformatics **scientist,** you will have to use PubMed
- Watch this video
  - https://webmeeting.nih.gov/p71971540/?launcher=false&fcsContent=true&pbMode=normal

Medical Subject Headings

- MeSH : "Medical Subject Headings"
  - National Library of Medicine's (NLM) controlled vocabulary thesaurus for indexing articles for PubMed
  - List of the vocabulary terms used for subject analysis of biomedical literature at NLM
  - Used for indexing journal articles for MEDLINE
  - Imposes uniformity and consistency to the indexing of biomedical literature

The MeSH Tree Structure
Organized by 19 main branches:

- Anatomy
- Organisms
- Diseases
- Chemical and Drugs Analytical, Diagnostic and Therapeutic Techniques and Equipment
- Psychiatry and Psychology
- Biological Sciences Natural Sciences
- Anthropology, Education, Sociology and Social Phenomena
- Technology, Industry, Agriculture
- Humanities
- Information Science
- Named Groups
- Health Care
- Publication Characteristics
- Geographic Location
- **And a few more**

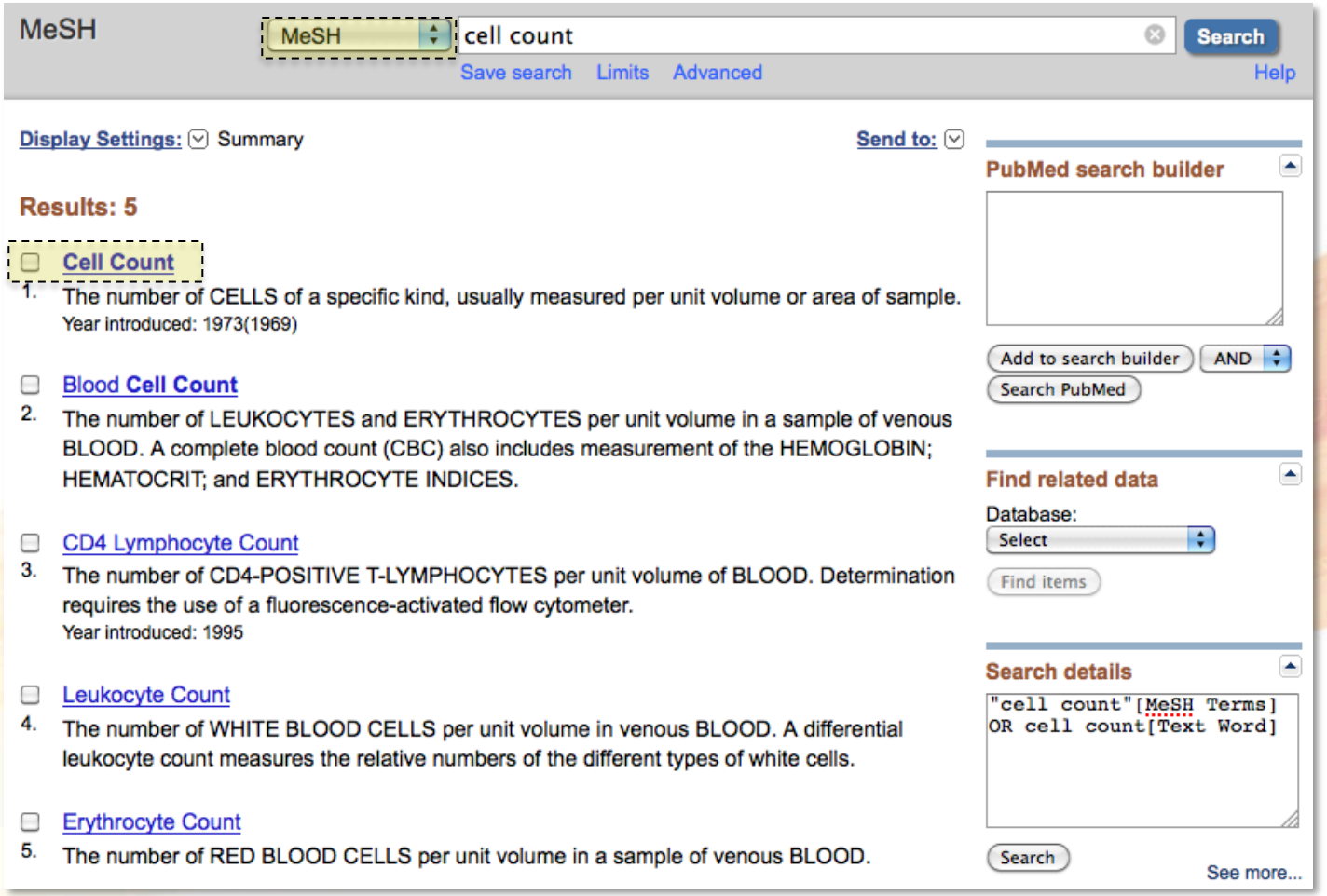
http://www.ncbi.nlm.nih.gov/mesh/1000048

You do not have to know these!

MeSH is an Entrez database

- http://www.ncbi.nlm.nih.gov/mesh
- MeSH is provided to assist PubMed users locate appropriate terms for MEDLINE searches
- This database provides information about MeSH terms including:
  - Definitions
  - Synonyms for the concept
  - Related terms
  - The position of the term in the MeSH hierarchy

MeSH Example



Cell Count - MeSH

Cell Count

The number of CELLS of a specific kind, usually measured per unit volume or area of sample.
Year introduced: 1973(1969)

MeSH term, definition, and year

PubMed search builder options

Subheadings:

1

- [ ] analysis
- [x] classification
- [ ] drug effects
- [ ] economics
- [ ] ethics
- [ ] history
- [ ] instrumentation
- [ ] methods
- [ ] organization and administration
- [ ] pharmacology
- [ ] physiology
- [ ] physiopathology
- [ ] radiation effects
- [ ] standards
- [ ] statistics and numerical data
- [ ] supply and distribution
- [ ] therapeutic use
- [ ] trends
- [ ] utilization
- [ ] veterinary

Select Subheadings

- [ ] Restrict to MeSH Major Topic.
- [ ] Do not include MeSH terms found below this term in the MeSH hierarchy.

Major MeSH terms & Explosion of MeSH terms

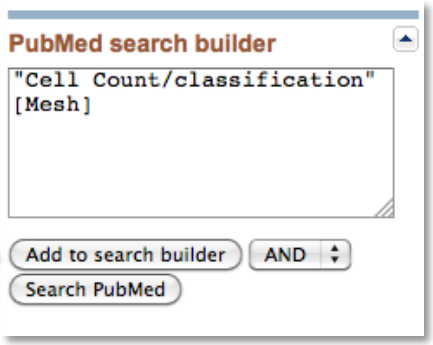
Tree Number(s): E01.370.225.500.195, E05.200.500.195, E05.242.195, G04.170

Entry Terms:

- Cell Counts
- Count, Cell
- Counts, Cell
- Cell Number
- Cell Numbers
- Number, Cell
- Numbers, Cell
- Cell Density
- Cell Densities
- Densities, Cell
- Density, Cell

"Synonyms" for this term.

2



Cell Count – MeSH - Subheadings

- Select specific *subheadings* to describe a particular aspect of a subject (diagnosis, prognosis, treatment etc.)
  - Select as many subheadings as you like to focus your search results
  - The more subheadings you select the broader your search results will be

Cell Count – MeSH - Major MeSH Terms

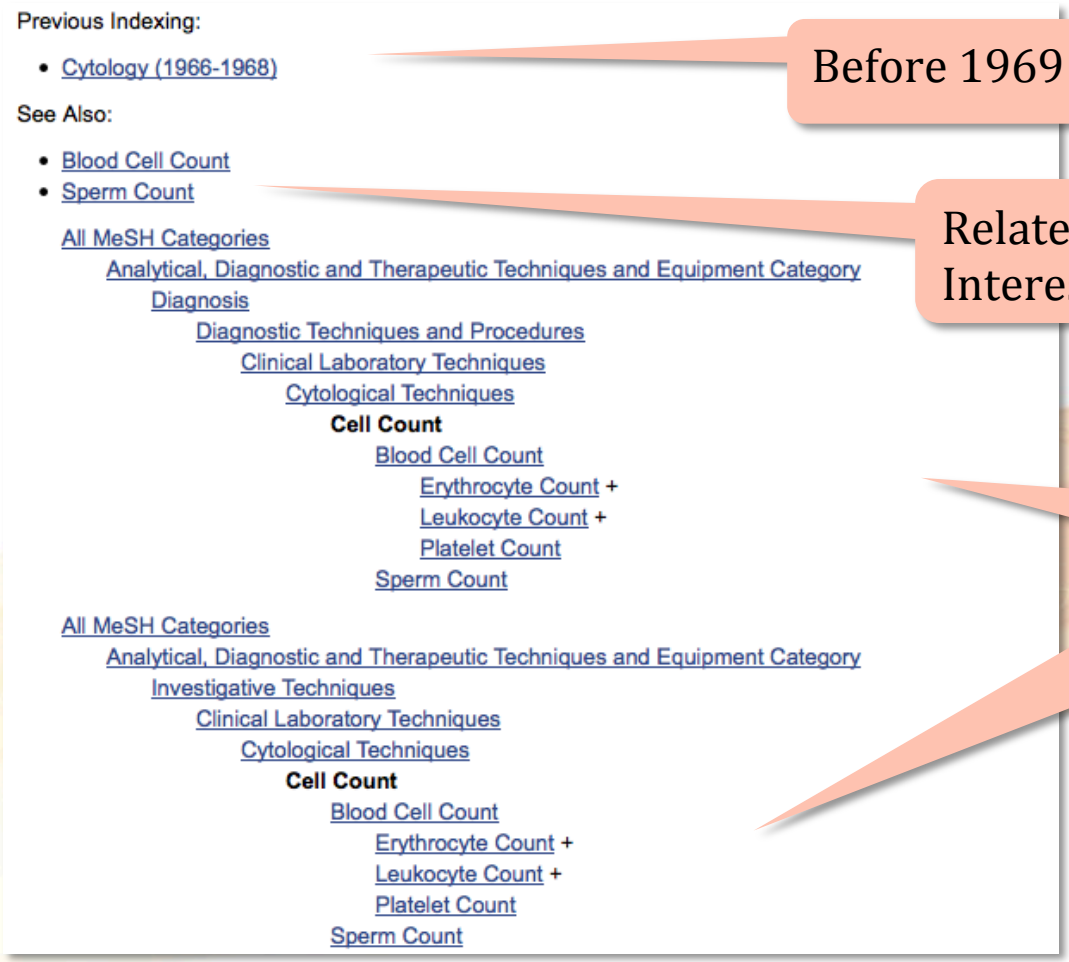
- Below the subheading list you will see a box where you can “Restrict Search To MeSH Major Topic”
- This will narrow your search results so that the MeSH term you search is the major focus of references retrieved

Darlene Chapman

Cell Count – MeSH - Explosion of MeSH Terms

- Also see a hierarchical arrangement of terms related to the MeSH term you selected
- Display shows how your term relates to other MeSH terms
- PubMed **automatically *explodes* MeSH terms** to include all narrower terms in the hierarchical list
- Check box beside "*Do Not Include MeSH Term…*"
  - Do not want to include the more specific subject headings indexed below your term in the list

Cell Count - MeSH



Previous Indexing:

- Cytology (1966-1968)

See Also:

- Blood Cell Count
- Sperm Count

All MeSH Categories
Analytical, Diagnostic and Therapeutic Techniques and Equipment Category
Diagnosis
Diagnostic Techniques and Procedures
Clinical Laboratory Techniques
Cytological Techniques
Cell Count
Blood Cell Count
Erythrocyte Count +
Leukocyte Count +
Platelet Count
Sperm Count

All MeSH Categories
Analytical, Diagnostic and Therapeutic Techniques and Equipment Category
Investigative Techniques
Clinical Laboratory Techniques
Cytological Techniques
Cell Count
Blood Cell Count
Erythrocyte Count +
Leukocyte Count +
Platelet Count
Sperm Count

Before 1969 …

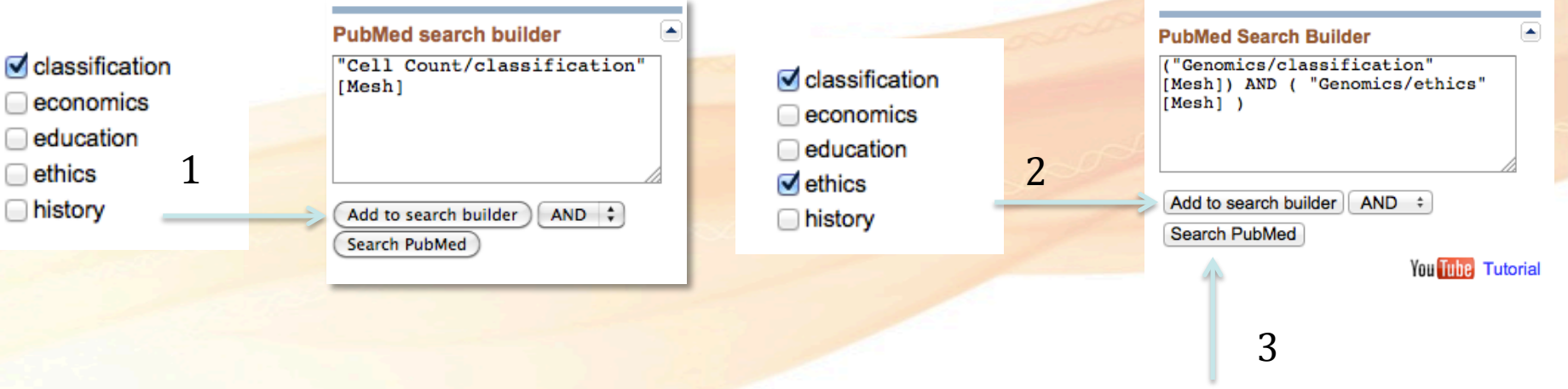
Related Terms of Possible Interest

Where its at in the tree (multiple branches)

You can choose other subject headings from this hierarchical list to broaden or narrow your search results

PubMed Search Builder

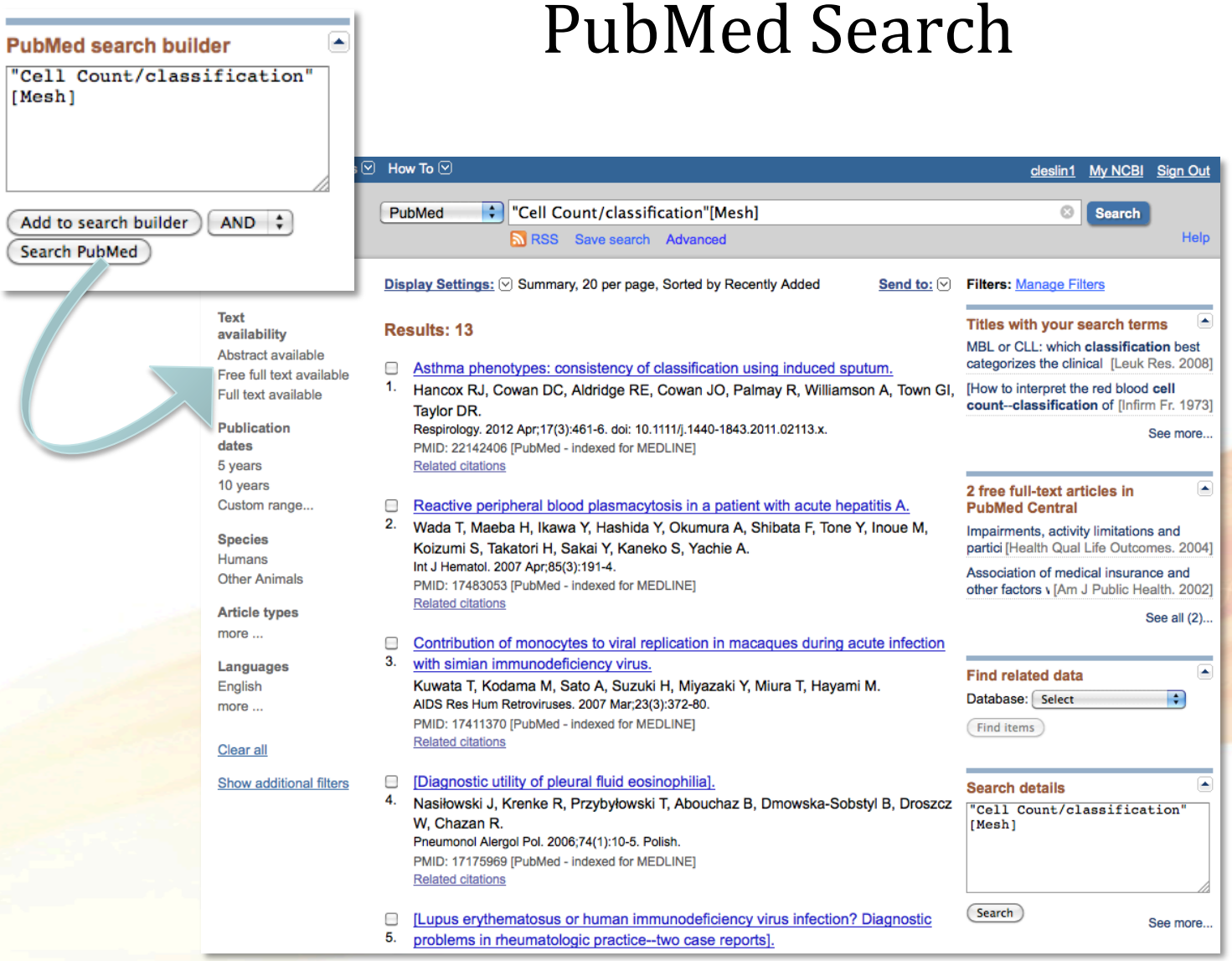
- After you've selected MeSH terms, decided if your term should be a Major MeSH term, and considered subheadings and explosions
- with basic searching, you can combine MeSH terms together with connector words "AND", "OR and "NOT"



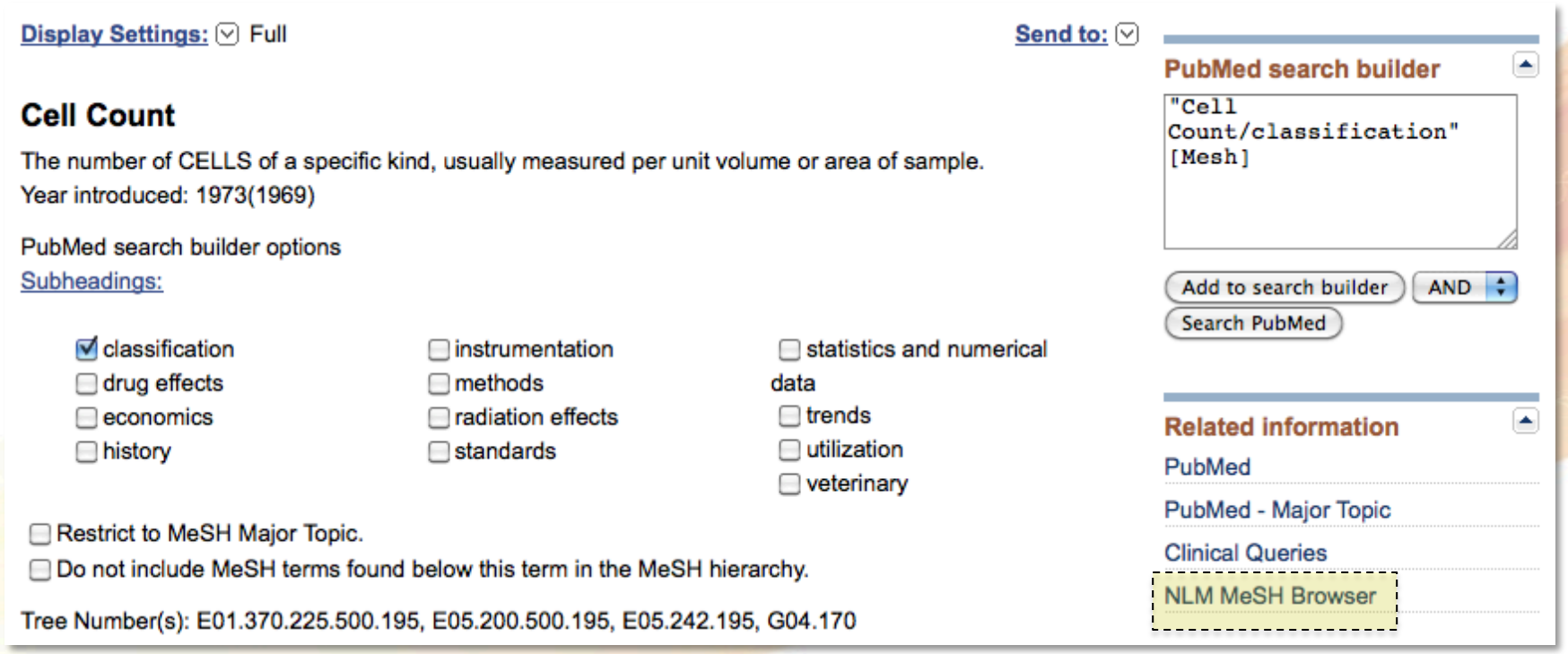
Check Our MeSH Terms For Bioinformatics

- http://www.ncbi.nlm.nih.gov/mesh/?term=bioinformatics

PubMed Search

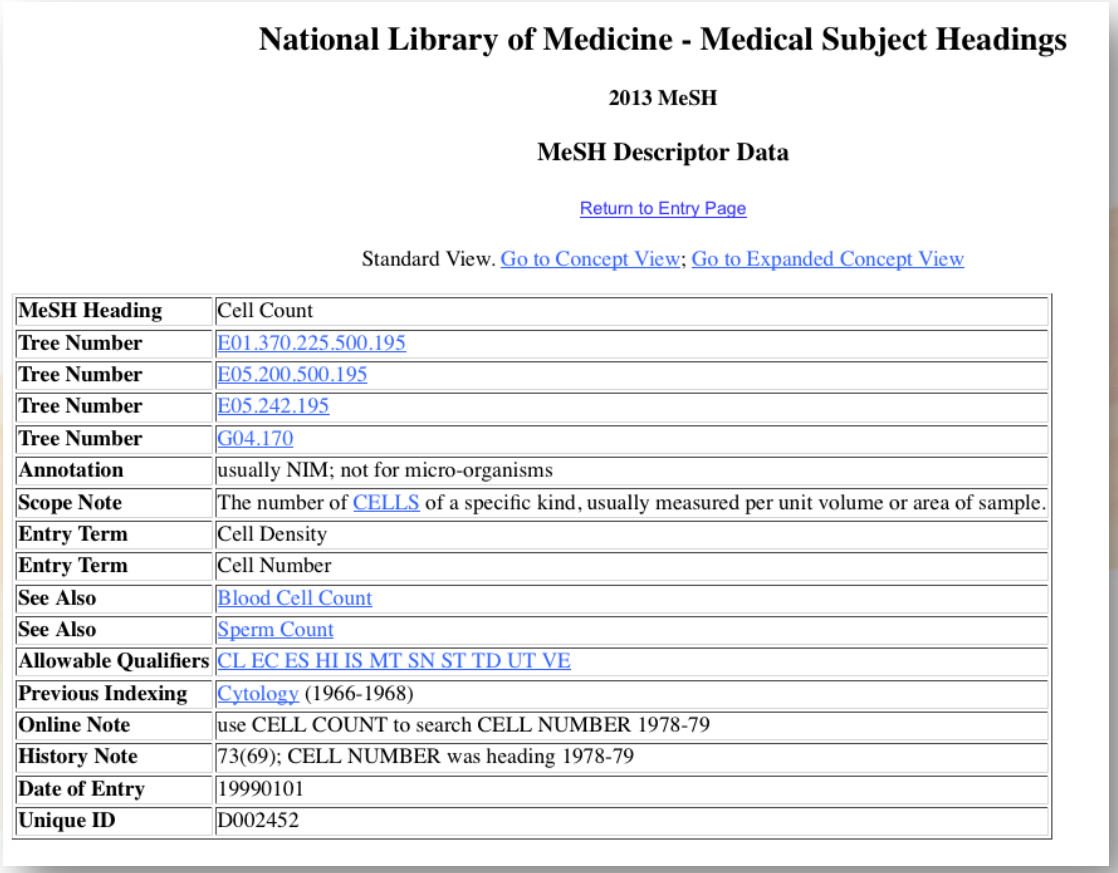


NLM MeSH Broswer



NLM MeSH Browser

- Is the tool used by MEDLINE indexers and catalogers
- Great to determine terms



National Library of Medicine - Medical Subject Headings

2013 MeSH

MeSH Descriptor Data

Return to Entry Page

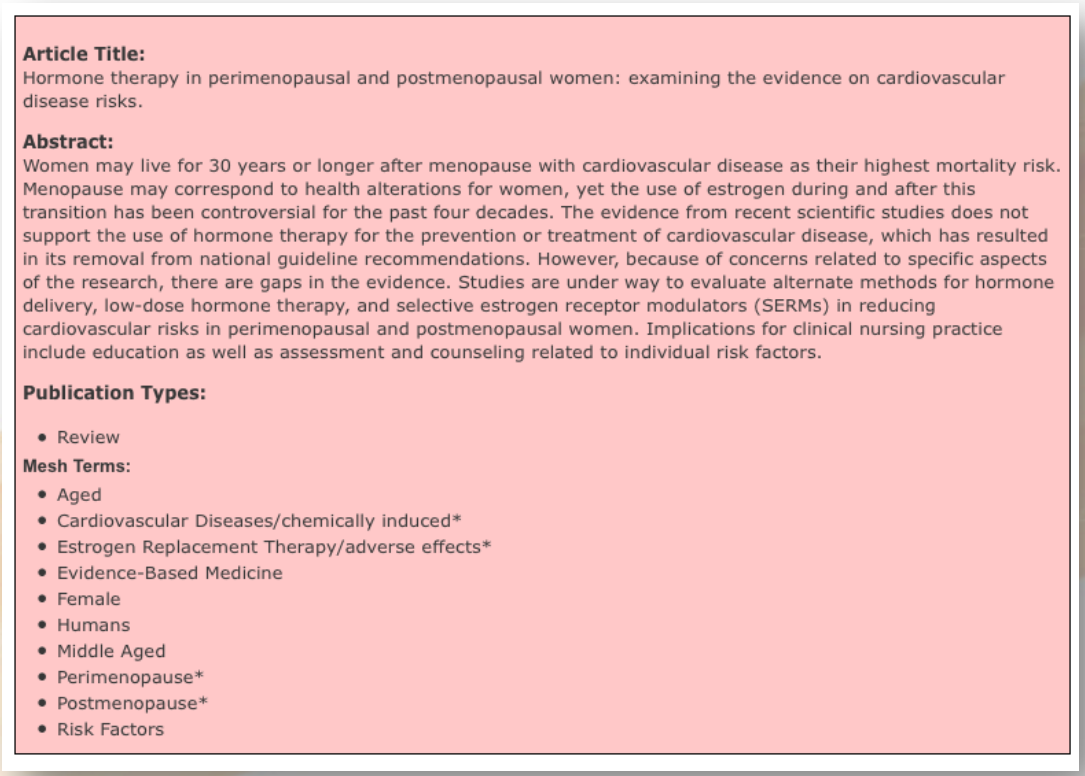
Standard View. Go to Concept View; Go to Expanded Concept View

MeSH Heading	Cell Count
Tree Number	E01.370.225.500.195
Tree Number	E05.200.500.195
Tree Number	E05.242.195
Tree Number	G04.170
Annotation	usually NIM; not for micro-organisms
Scope Note	The number of CELLS of a specific kind, usually measured per unit volume or area of sample.
Entry Term	Cell Density
Entry Term	Cell Number
See Also	Blood Cell Count
See Also	Sperm Count
Allowable Qualifiers	CL EC ES HI IS MT SN ST TD UT VE
Previous Indexing	Cytology (1966-1968)
Online Note	use CELL COUNT to search CELL NUMBER 1978-79
History Note	73(69); CELL NUMBER was heading 1978-79
Date of Entry	19990101
Unique ID	D002452

http://www.nlm.nih.gov/mesh/2013/mesh_browser/MBrowser.html

Indexing with MeSH Vocabulary

- Indexers examine articles
  - Assign the most specific MeSH heading(s) appropriate to describe the main concepts discussed
  - When there's no single specific MeSH heading for a concept
    - Indexer usess closest, more general MeSH heading available
  - Assign as many MeSH headings as appropriate to cover the topics of the article (generally 5 to 15)
- MeSH terms that reflect the major points of the article are marked with an asterisk (*) by indexers



Article Title:
Hormone therapy in perimenopausal and postmenopausal women: examining the evidence on cardiovascular disease risks.

Abstract:
Women may live for 30 years or longer after menopause with cardiovascular disease as their highest mortality risk. Menopause may correspond to health alterations for women, yet the use of estrogen during and after this transition has been controversial for the past four decades. The evidence from recent scientific studies does not support the use of hormone therapy for the prevention or treatment of cardiovascular disease, which has resulted in its removal from national guideline recommendations. However, because of concerns related to specific aspects of the research, there are gaps in the evidence. Studies are under way to evaluate alternate methods for hormone delivery, low-dose hormone therapy, and selective estrogen receptor modulators (SERMs) in reducing cardiovascular risks in perimenopausal and postmenopausal women. Implications for clinical nursing practice include education as well as assessment and counseling related to individual risk factors.

Publication Types:

- Review

Mesh Terms:

- Aged
- Cardiovascular Diseases/chemically induced*
- Estrogen Replacement Therapy/adverse effects*
- Evidence-Based Medicine
- Female
- Humans
- Middle Aged
- Perimenopause*
- Postmenopause*
- Risk Factors

Subheadings

- Subheadings further describe a particular aspect of a MeSH heading
- Examples: diagnosis, metabolism, adverse effects

Subheading Groupings

- Related subheadings have been grouped
- Not all subheadings have been placed in these groupings – some do not logically fit

Families of Subheading Explosions

etiology	physiology
chemically induced	genetics
complications	growth & development
secondary	immunology
congenital	metabolism
embryology	biosynthesis
genetics	blood
immunology	cerebrospinal fluid
microbiology	deficiency
virology	enzymology
parasitology	pharmacokinetics
transmission	urine
	physiopathology

Pharmacologic Action Terms

- Every drug and chemical MeSH heading has been assigned one or more headings that describe known pharmacological actions [PA]
- Beginning in 1996
  - Indexers add the appropriate pharmacological action MeSH heading
  - As well as the specific chemical MeSH heading to a citation when the action of the chemical is being discussed in the article

Example:
Pharmacologic Action Terms

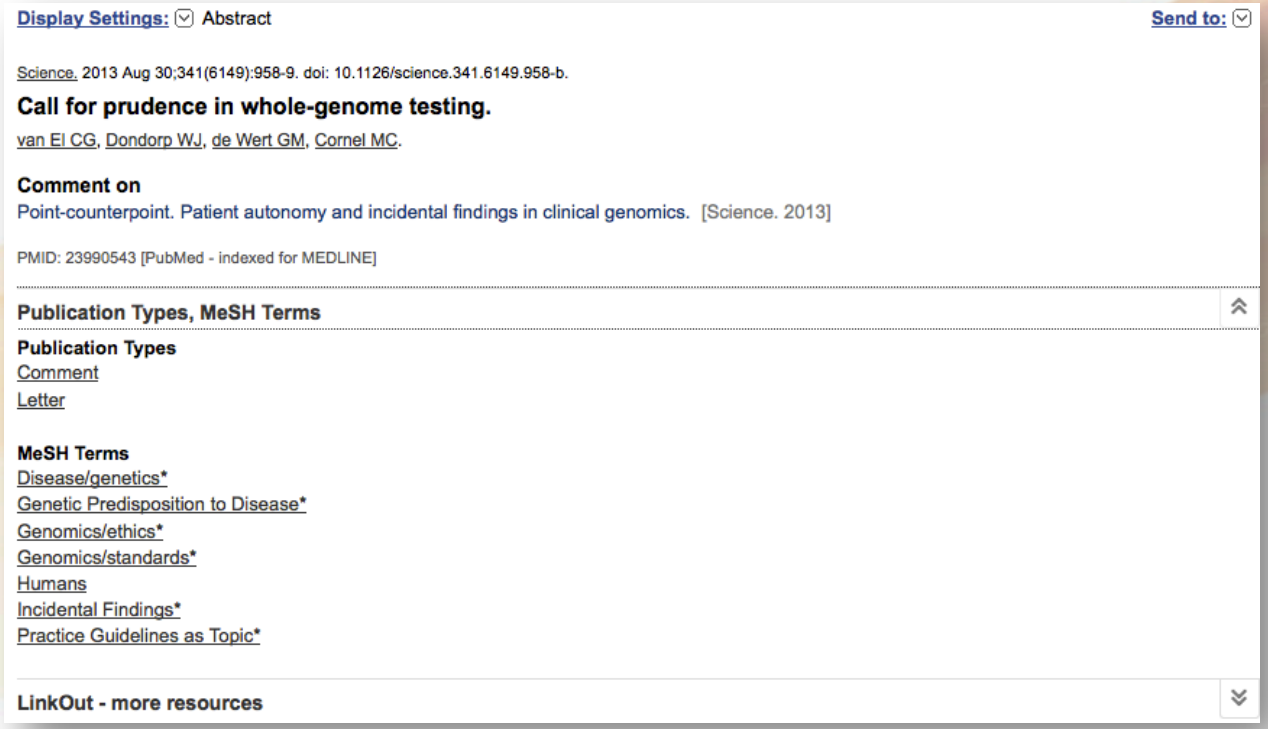
The pharmacological actions established for the MeSH Heading, ***Aspirin****:*

Pharmacological Action	Anti-Inflammatory Agents, Non-Steroidal
Pharmacological Action	Cyclooxygenase Inhibitors
Pharmacological Action	Fibrinolytic Agents
Pharmacological Action	Platelet Aggregation Inhibitors

- A citation to an article that discusses **aspirin used as an anti-inflammatory agent** will be assigned:
  - Aspirin
    Anti-Inflammatory Agents, Non-Steroidal
- A citation to an article that discusses **aspirin used to inhibit blood clotting** will be assigned:
  - Aspirin
    Platelet Aggregation Inhibitors

How Was It Indexed

- If you've found the perfect reference in PubMed
  - May want to take a look at how this paper was indexed with MeSH terms to revise your search strategy to find other, similar (and relevant) references



PubMed - Developing a Search Strategy

- Before you can search for any information, you should first develop a search strategy
- **What is a Search Strategy?**
  - A search strategy is a plan that helps you look for the information you need
- **Search Strategy Tips**
  - Identify the key concepts
  - Determine alternative terms for these concepts, if needed
    - Use the MeSH browser for this
    - Find those terms
  - Understand Boolean Logic (coming slides)
  - Refine your search to dates, study groups, etc., as appropriate
  - Practice helps
    - Strategies and styles will differ according to personal choice and professional discipline

What Happens When Conduct Search from PubMed

- PubMed uses an Automatic Term Mapping feature to search for **unqualified terms**
- When you click Search, PubMed will look for a match in several lists
- It looks first for a match for your phrase as a
  - **Subject** in the
    - MeSH Translation Table. If it doesn't find a match, it looks for your phrase as a
  - **Journal** in the
    - Journals Translation Table, then it searches for
  - **Author and Investigator** names in the
    - Full Author Translation Table in the
    - Author Index, in the
    - Full Investigator Translation Table, and in the
    - Investigator Index

Search from PubMed

- As soon as PubMed finds a match:
  - The mapping stops
  - If a term matches in the MeSH Translation Table
    - PubMed does not continue looking in the next table
- If no match is found
  - PubMed breaks apart the phrase and repeats the process until a match is found

FYI - Automatic Term Mapping

- The process used by PubMed to find a match to unqualified terms that are entered into the query box
- Untagged terms are matched (in this order) against subjects using the MeSH (Medical Subject Headings) translation table, journals using the Journals translation table, and authors and investigators, using the the Full Author translation table, Author index, Full Investigator translation table and Investigator index
- If a match is found in any translation table, the mapping stops
- When subject or journal matches are found, the query and individual terms are also searched in All Fields
- If no match is found in any tables, terms are searched in All Fields and AND'd together

FYI - Unqualified Terms

- Terms that are entered into the PubMed query box without a search field tag (e.g., [mh])

FYI - MeSH Translation Table

- The PubMed MeSH Translation Table is an alphabetical list of MeSH Terms, Subheadings, See-References for MeSH Terms, Unified Medical Language System mappings, and Names of Substances and synonyms to the Names of Substances

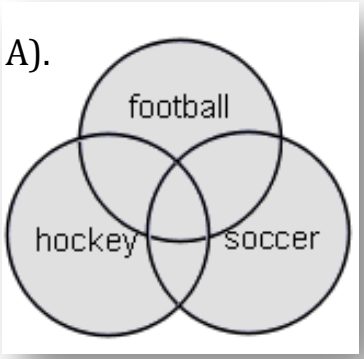
FYI - Journals Translation Table

- The PubMed Journals Translation Table is an alphabetical list of full journal titles, MEDLINE journal title abbreviations, and International Standard Serial Numbers (ISSNs)

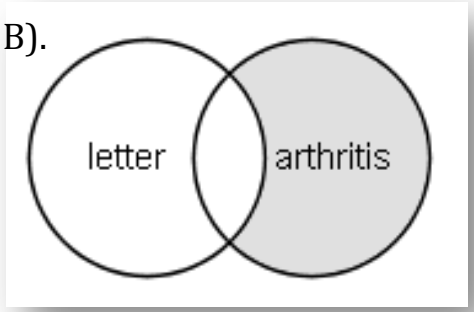
FYI - Additional

- **Full Author Translation Table** - A list of full author names, in direct and inverse order, that appear in PubMed citations
- **Author Index** - In PubMed there are several indexes including an All Fields Index as well as indexes for specific search fields including Author Name, Title Word, and Text Word indexes among others
- **Full Investigator Translation Table** - A list of the full names of investigators or collaborators, in direct and inverse order, that appear in PubMed citations
- **Investigator Index** - The list of all values that appear in the investigator name field in PubMed citations

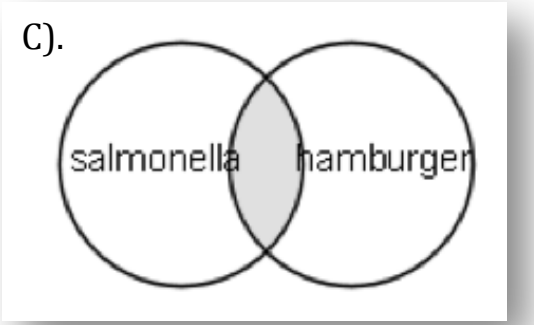
A).



B).



C).



Introduction to Boolean Logic

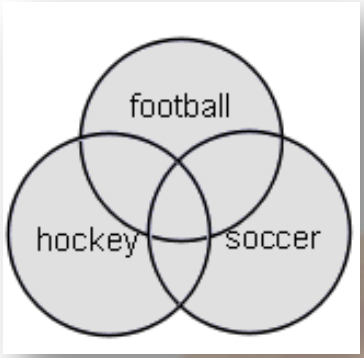
Introduction to Boolean Logic

- In the context of database searching:
  - Boolean logic refers to the logical relationships among search terms
  - Operators AND, OR, NOT can be used to combine search terms in PubMed
  - In PubMed, Boolean operators **must be entered in uppercase letters**

- **OR:**
  - Used to retrieve a set in which each citation contains *at least one* of the search terms
  - Use OR when you want to pull together articles on similar topics.

Example OR:

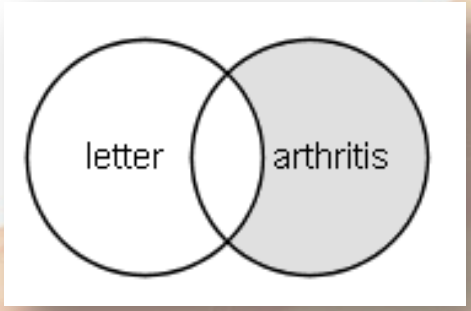
- Search "*football OR hockey OR soccer* "
- Each circle in the diagram to the right represents the retrieval for each ter.
- The grey areas represent the retrieval for this example – all records that include any one of these terms



Search terms	Results
football	**6325**
hockey	**2060**
soccer	**5498**
football OR hockey OR soccer	**12108**

Example NOT:

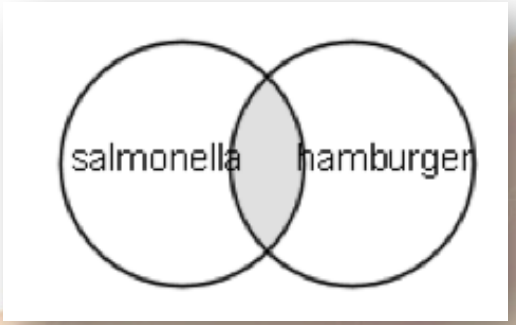
- **NOT:**
  - Retrieves a set from which citations to articles containing specified search terms following the NOT operator are eliminated
  - **Example**: *arthritis NOT letter*
  - the retrieval is a portion of the total retrieval for arthritis – that portion not including the term letter



Search terms	Results
arthritis	**243053**
letter	**859692**
arthritis NOT letter	**230685**

Example AND:

- **AND:**
  - Used to retrieve a set in which each citation contains *all* search terms
  - **Example**: *salmonella AND hamburger*
  - the retrieval is only the overlap of the results for each term – those records in which both terms appear



Search terms	Results
salmonella	**74677**
hamburger	**3142**
salmonella AND hamburger	**18**

Example Nesting:

- When using multiple Boolean operators in PubMed, they are processed left to right
  - **Example:** *salmonella AND hamburger OR eggs*
  - Retrieve records that include both terms *salmonella* AND *hamburger* as well as all records with the term *eggs*, whether or not they contain the other two terms
- Maybe what you wanted? If not…
  - Need Nesting

Example Nesting:

- To change the order in which terms are processed, enclose the terms(s) in parentheses
- The terms inside the set of parentheses will be processed as a unit and then incorporated into the overall strategy
- **This is called nesting**
- **Example:** *salmonella AND (hamburger OR eggs)*
- Retrieve records that contain the term *salmonella*, as well as one or both of the terms *hamburger* OR *eggs*

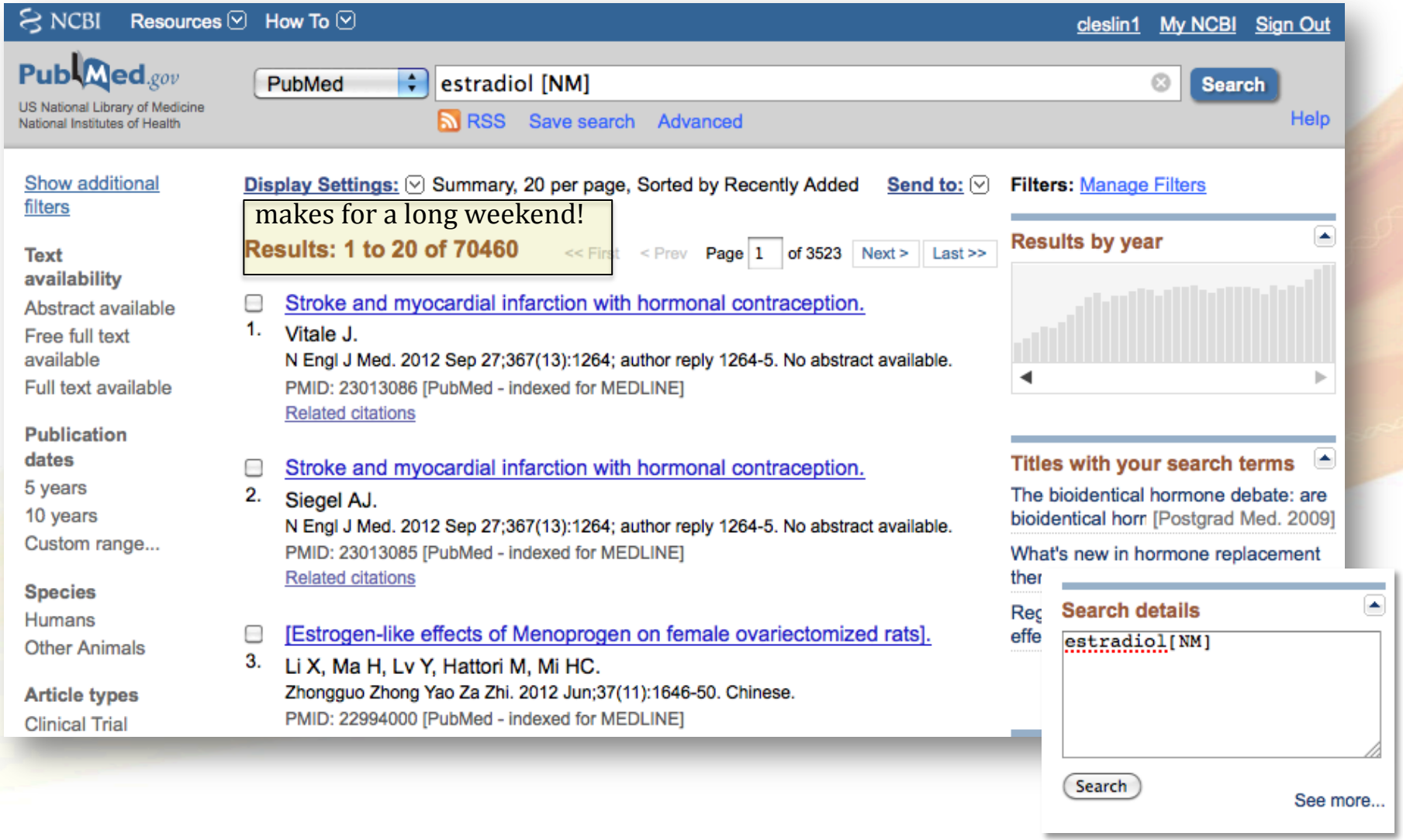
("Cell Count/classification"[Mesh]) AND ("Child, Preschool"[Mesh])

Boolean Rules and Syntax

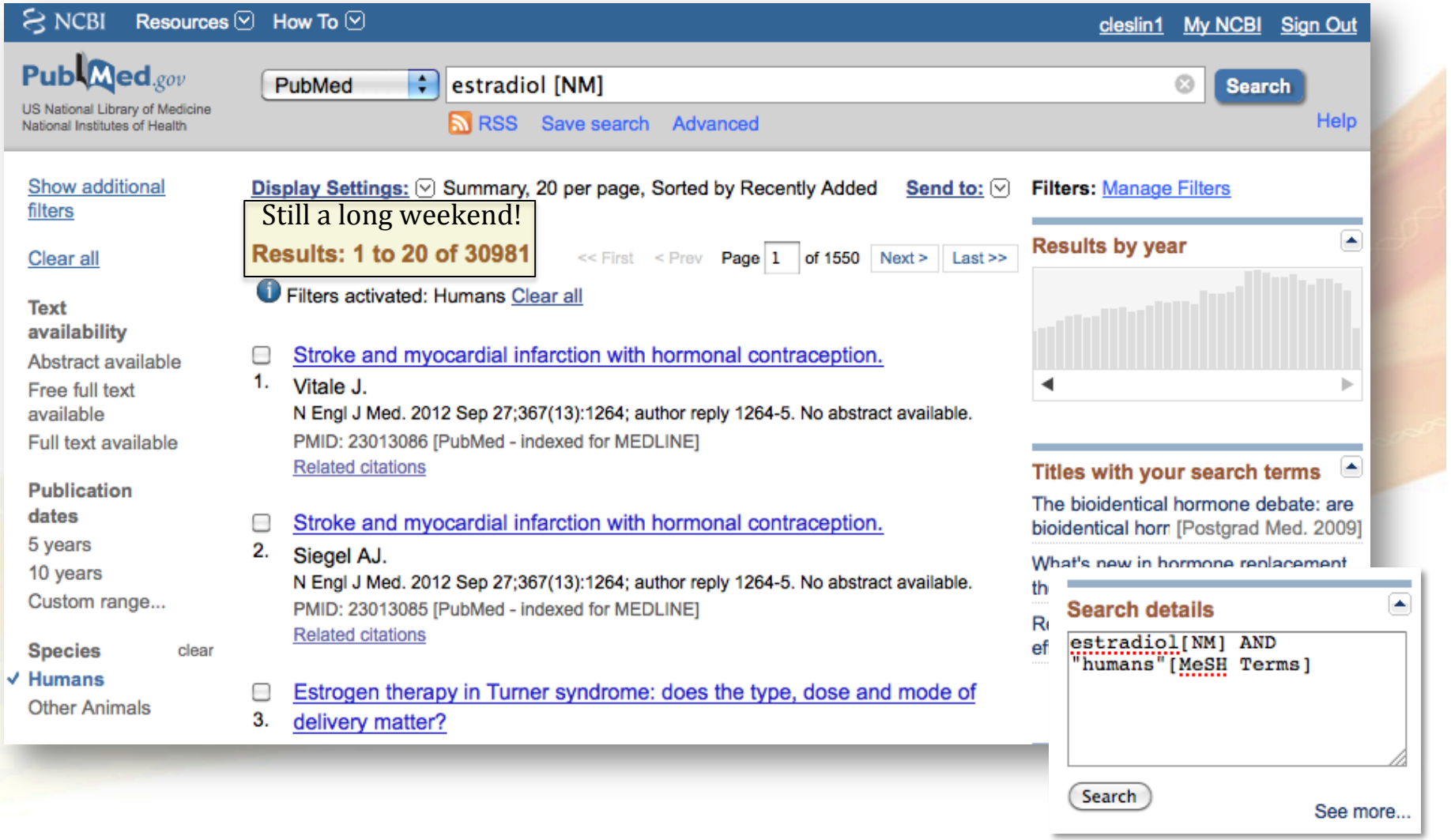
- Boolean operators: AND, OR, and NOT:
  - Must be entered in UPPERCASE
  - AND is the **default operator** used in
    - If you do not include Boolean operators in your search
    - PubMed will automatically use AND between terms

- PubMed processes Boolean connectors in a left-to-right sequence
- You can change order in which PubMed processes a search statement by nesting an **individual concept in parentheses**
  - Terms inside parentheses will be **processed as a unit** and **then incorporated** into the overall strategy

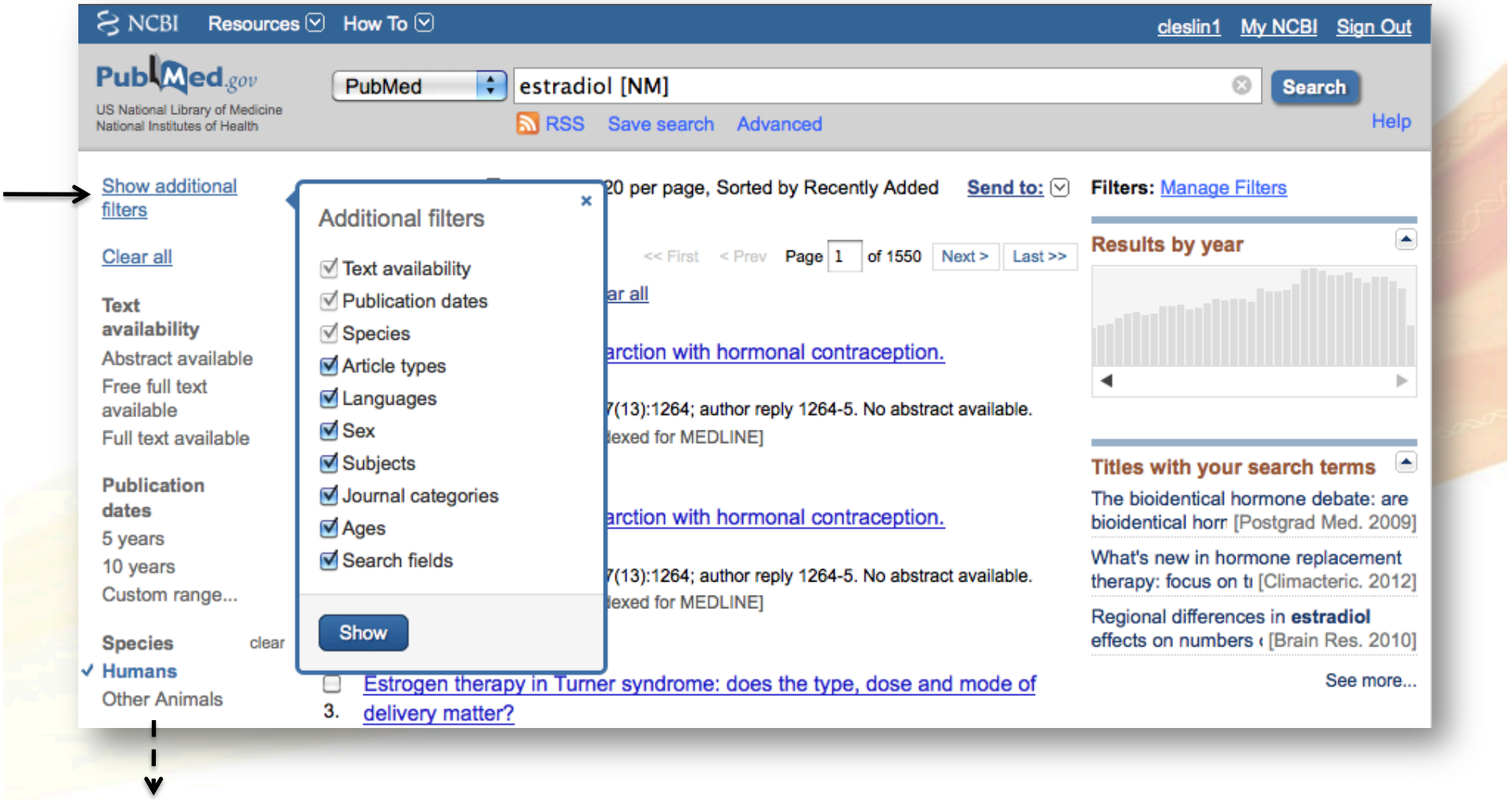
Searching PubMed



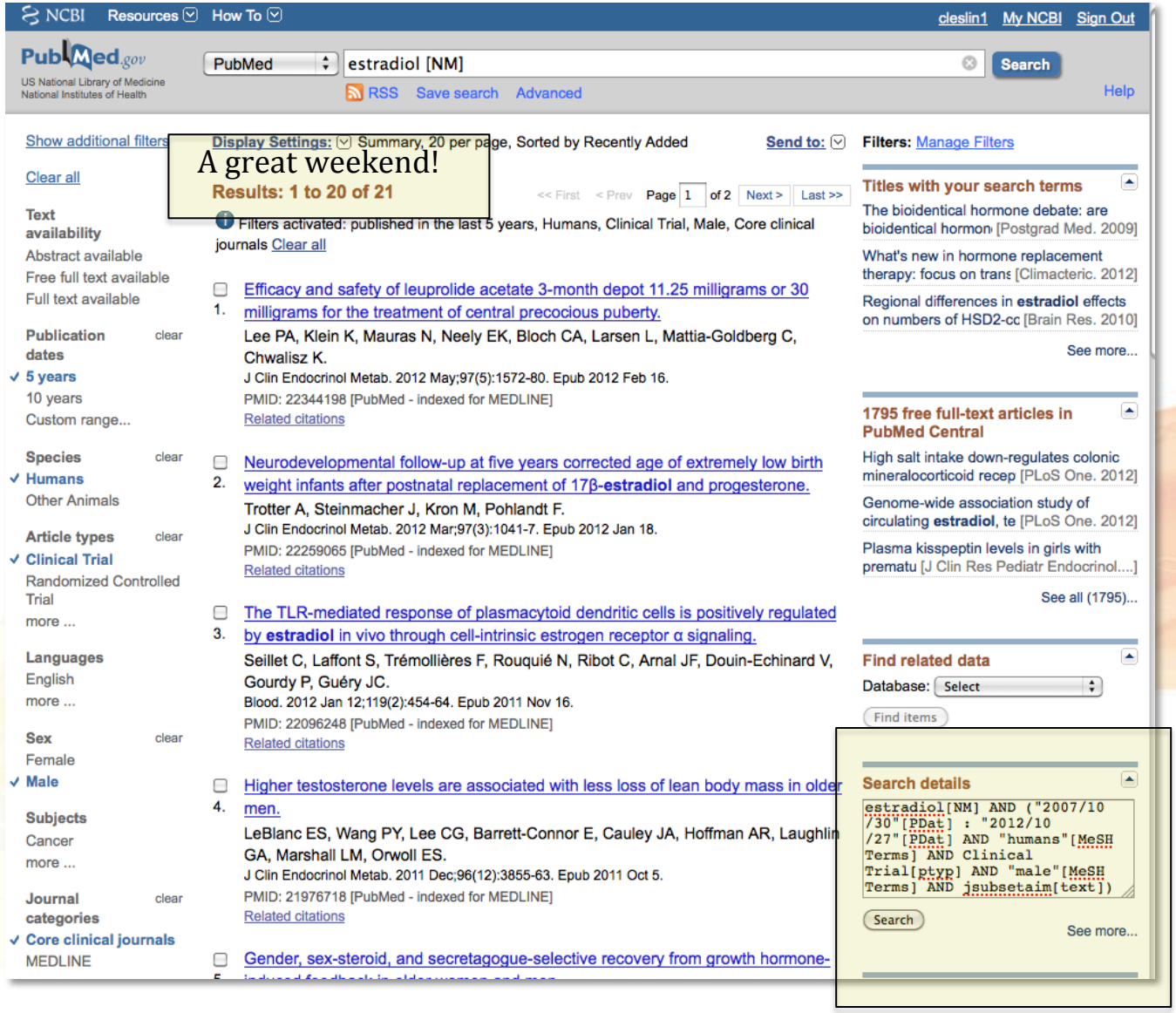
Searching PubMed



Searching PubMed



Searching PubMed



Now have query for an automated search!

Wait… How?

Search Field Descriptions and Tags

Search Field Descriptions and Tags
Affiliation [AD]
Article Identifier [AID]
All Fields [ALL]
Author [AU]
Author Identifier [AUID]
Book [book]
Comment Corrections
Corporate Author [CN]
Create Date [CRDT]
Completion Date [DCOM]
EC/RN Number [RN]
Editor [ED]
Entrez Date [EDAT]
Filter [FILTER]
First Author Name [1AU]
Full Author Name [FAU]
Full Investigator Name [FIR]
Grant Number [GR]
Investigator [IR]
ISBN [ISBN]
Issue [IP]
Journal [TA]
Language [LA]
Last Author [LASTAU]
Location ID [LID]
MeSH Date [MHDA]
MeSH Major Topic [MAJR]
MeSH Subheadings [SH]
MeSH Terms [MH]
Modification Date [LR]
NLM Unique ID [JID]
Other Term [OT]
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Pagination [PG]
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Place of Publication [PL]
PMID [PMID]
Publisher [PUBN]
Publication Date [DP]
Publication Type [PT]
Secondary Source ID [SI]
Subset [SB]
Supplementary Concept[NM]
Text Words [TW]
Title [TI]
Title/Abstract [TIAB]
Transliterated Title [TT]
UID [PMID]
Version
Volume [VI]

http://www.ncbi.nlm.nih.gov/books/NBK3827/

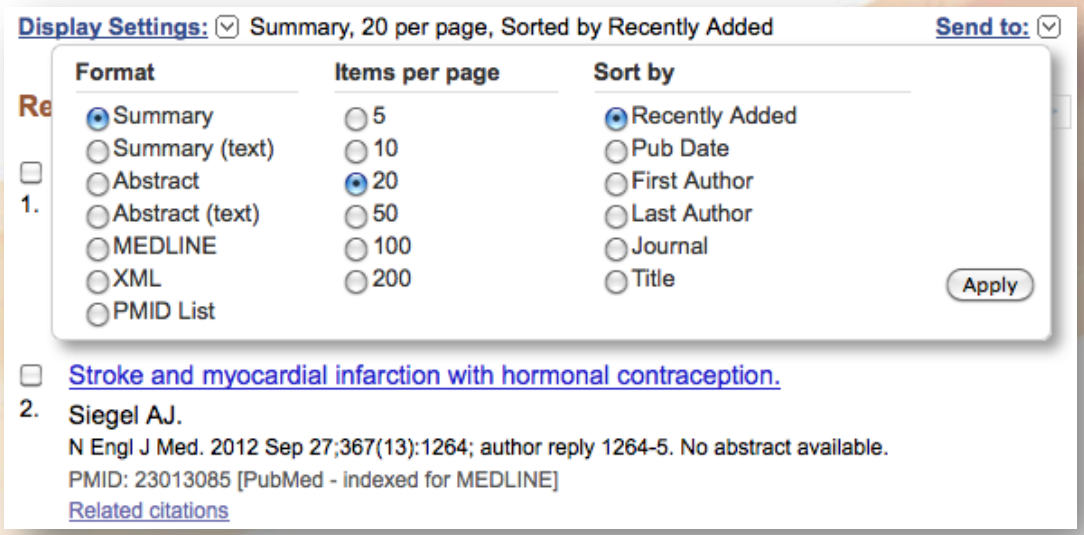
NCBI Entrez

- We can do a lot using a web-browser
  - Look up a specific record for PubMed
  - Search for matches to a gene or disease name
  - Find the search query needed for an automated process
- **Sometimes we need to automate the process**
  - Use Entrez:
    - to select and return the items of interest
    - rather than download, parse, and select

Automagically!

- http://www.ncbi.nlm.nih.gov/entrez/query/static/eutils_example.pl
- Download and Run from a terminal window:
- `perl eutils_example.pl` Accept the defaults and inspect the output
  - `Database [Pubmed]:`
  - `Query [zanzibar]:`
  - `Report [abstract]:`

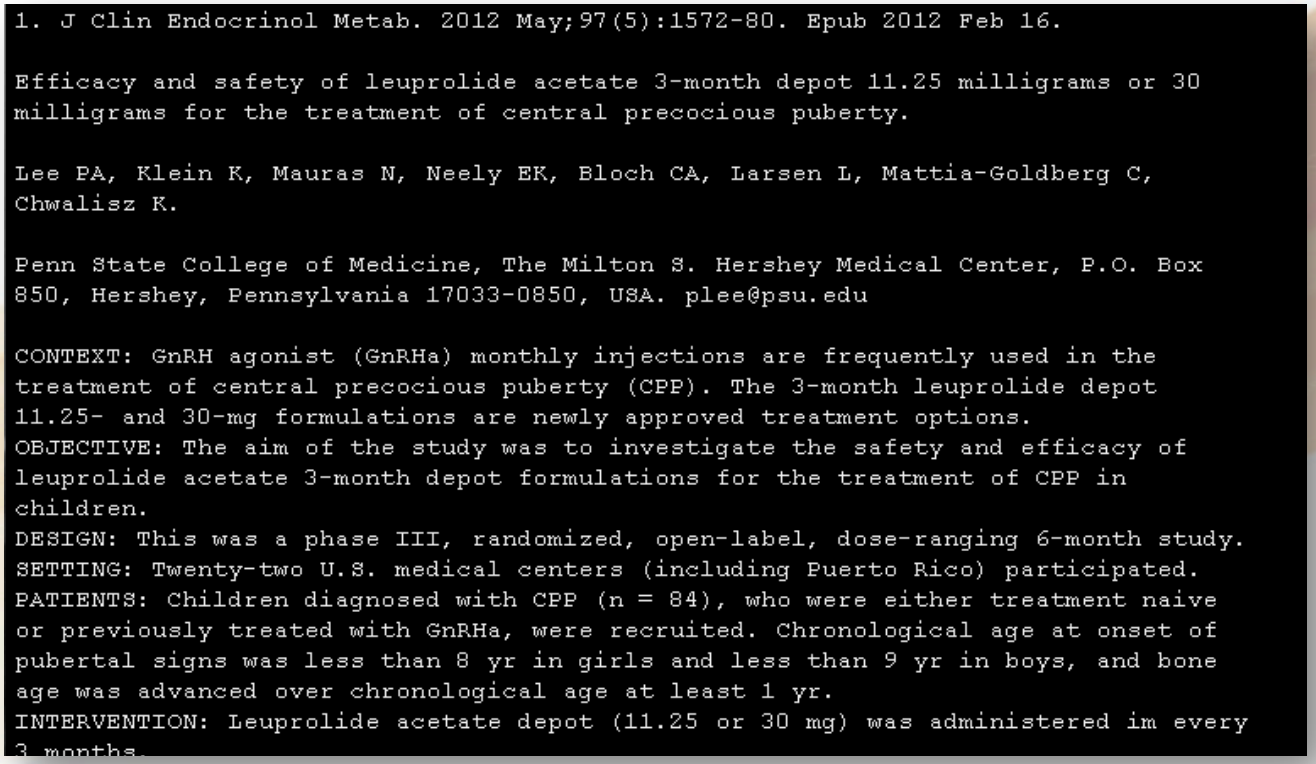
→



- **Different outputs!**
- **Try them with the Perl program**

Output

```
MacBook-Pro-SSD:~ cleslin$ perl eutils_example.pl
Database [Pubmed]:
Query [zanzibar]: estradiol[NM] AND ("2007/10/30"[PDat] : "2013/10/27"[PDat] AND
    "humans"[MeSH Terms] AND Clinical Trial[ptyp] AND "male"[MeSH Terms] AND
    jsubsetaim[text])
Report [abstract]:
```

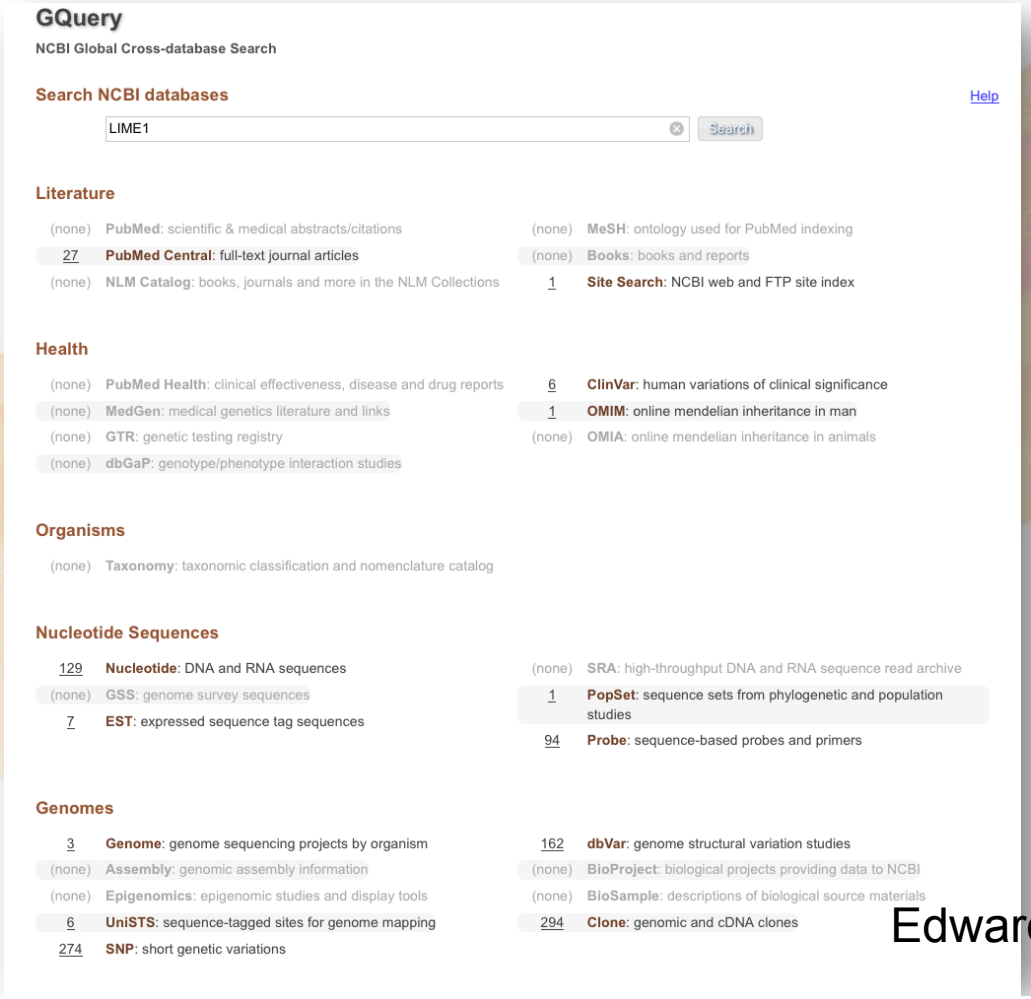


```
1. J Clin Endocrinol Metab. 2012 May;97(5):1572-80. Epub 2012 Feb 16.

Efficacy and safety of leuprolide acetate 3-month depot 11.25 milligrams or 30
milligrams for the treatment of central precocious puberty.

Lee PA, Klein K, Mauras N, Neely EK, Bloch CA, Larsen L, Mattia-Goldberg C,
Chwalisz K.

Penn State College of Medicine, The Milton S. Hershey Medical Center, P.O. Box
850, Hershey, Pennsylvania 17033-0850, USA. plee@psu.edu

CONTEXT: GnRH agonist (GnRHa) monthly injections are frequently used in the
treatment of central precocious puberty (CPP). The 3-month leuprolide depot
11.25- and 30-mg formulations are newly approved treatment options.
OBJECTIVE: The aim of the study was to investigate the safety and efficacy of
leuprolide acetate 3-month depot formulations for the treatment of CPP in
children.
DESIGN: This was a phase III, randomized, open-label, dose-ranging 6-month study.
SETTING: Twenty-two U.S. medical centers (including Puerto Rico) participated.
PATIENTS: Children diagnosed with CPP (n = 84), who were either treatment naive
or previously treated with GnRHa, were recruited. Chronological age at onset of
pubertal signs was less than 8 yr in girls and less than 9 yr in boys, and bone
age was advanced over chronological age at least 1 yr.
INTERVENTION: Leuprolide acetate depot (11.25 or 30 mg) was administered im every
3 months.
```

Entrez

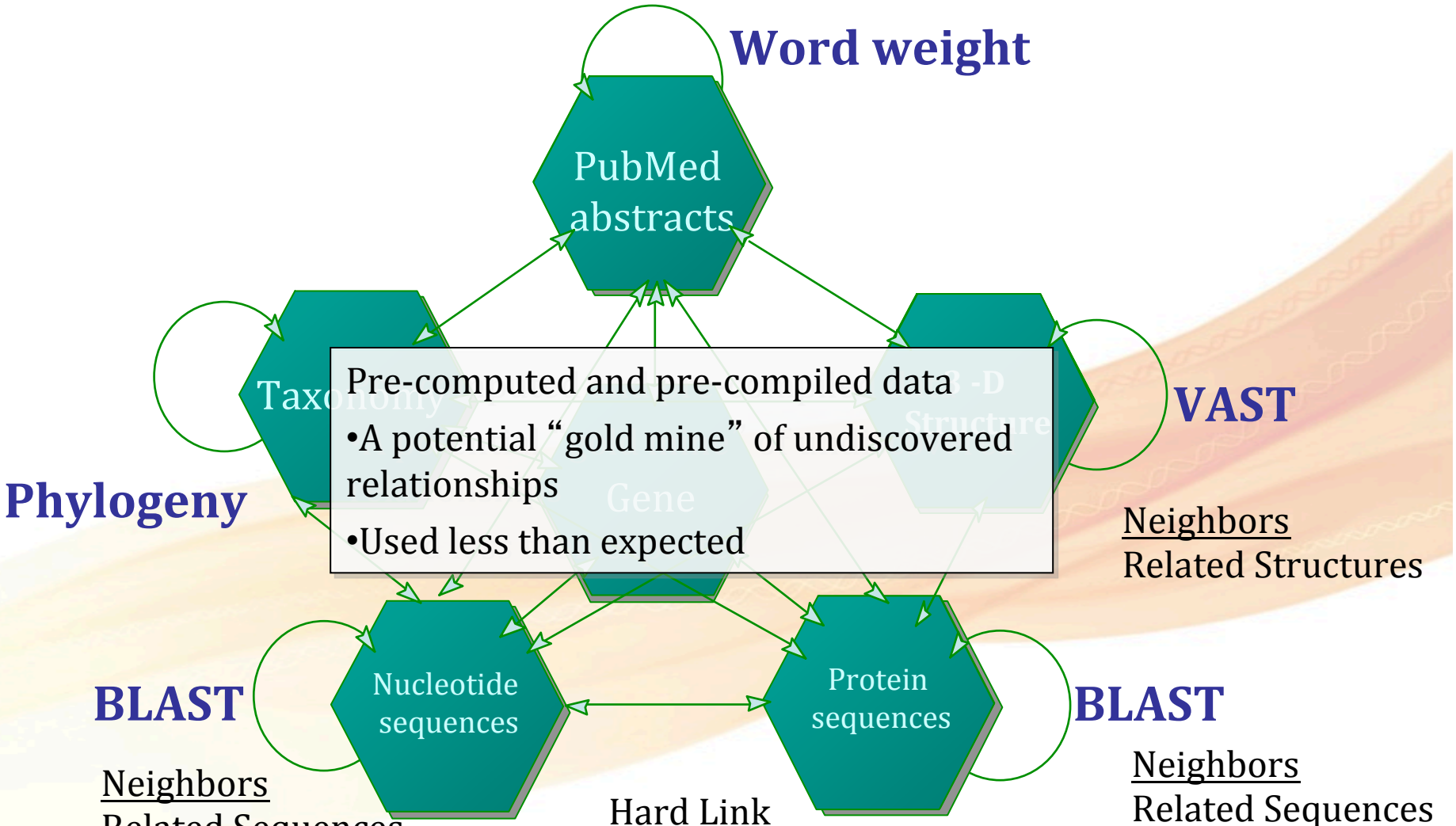
NCBI Entrez

- Powerful web-portal for NCBI's online databases
  - Nucleotide
  - Protein
  - PubMed
  - Gene
  - Structure
  - Taxonomy
  - OMIM
  - etc…



Edwards

Entrez: A Discovery System



The Discovery Initiative

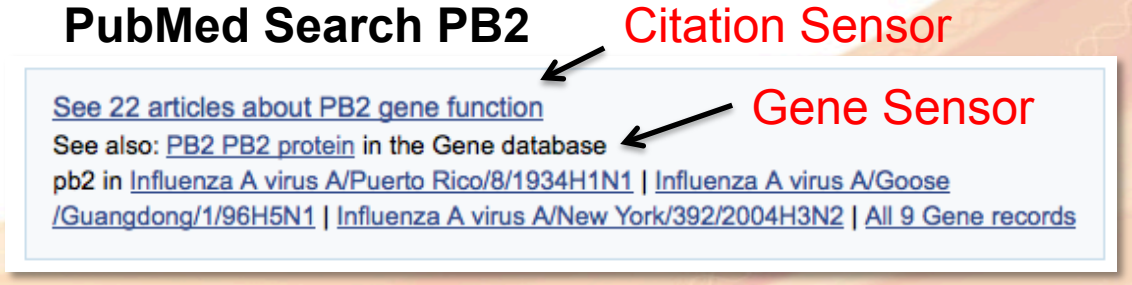
- Make the most relevant and interesting results more obvious and readily accessible
  - Through easier to use interfaces
  - Promote higher quality resources
    - Gene – A great place to start!
    - RefSeqs
- Expose the power of pre-computed similarities and pre-compiled links
- There are three main categories of discovery components :
  - Sensors
  - Database Ads
  - Analysis Tools

http://www.ncbi.nlm.nih.gov/books/NBK7039/

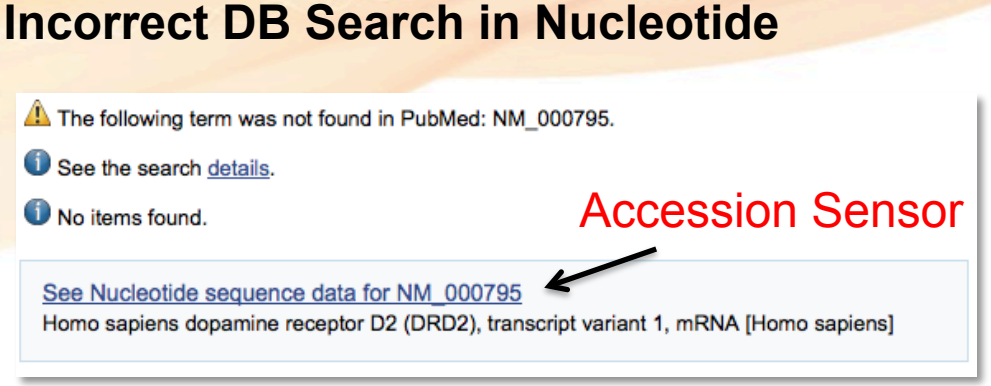
Sensors

- Detects certain types of search terms & provides access to **potentially** more relevant results

- For ex. PubMed:
  - Citation & Gene Sensor activated when someone searches with a literature citation



- For ex. Nucleotide Search
  - Accession Sensor that provides a direct link to the sequence databases when someone searches with an NCBI sequence identifier



Database Ads

- Promotes related information in other databases that may be more useful or may provide unexpected connections

Analysis Tools

- Provide on-the-fly analysis
- Important components of the discovery initiative
- Ex. Sequence records include a direct link:
  - BLAST search with the sequence as well as a link to run a conserved domain search for protein records

What is Entrez

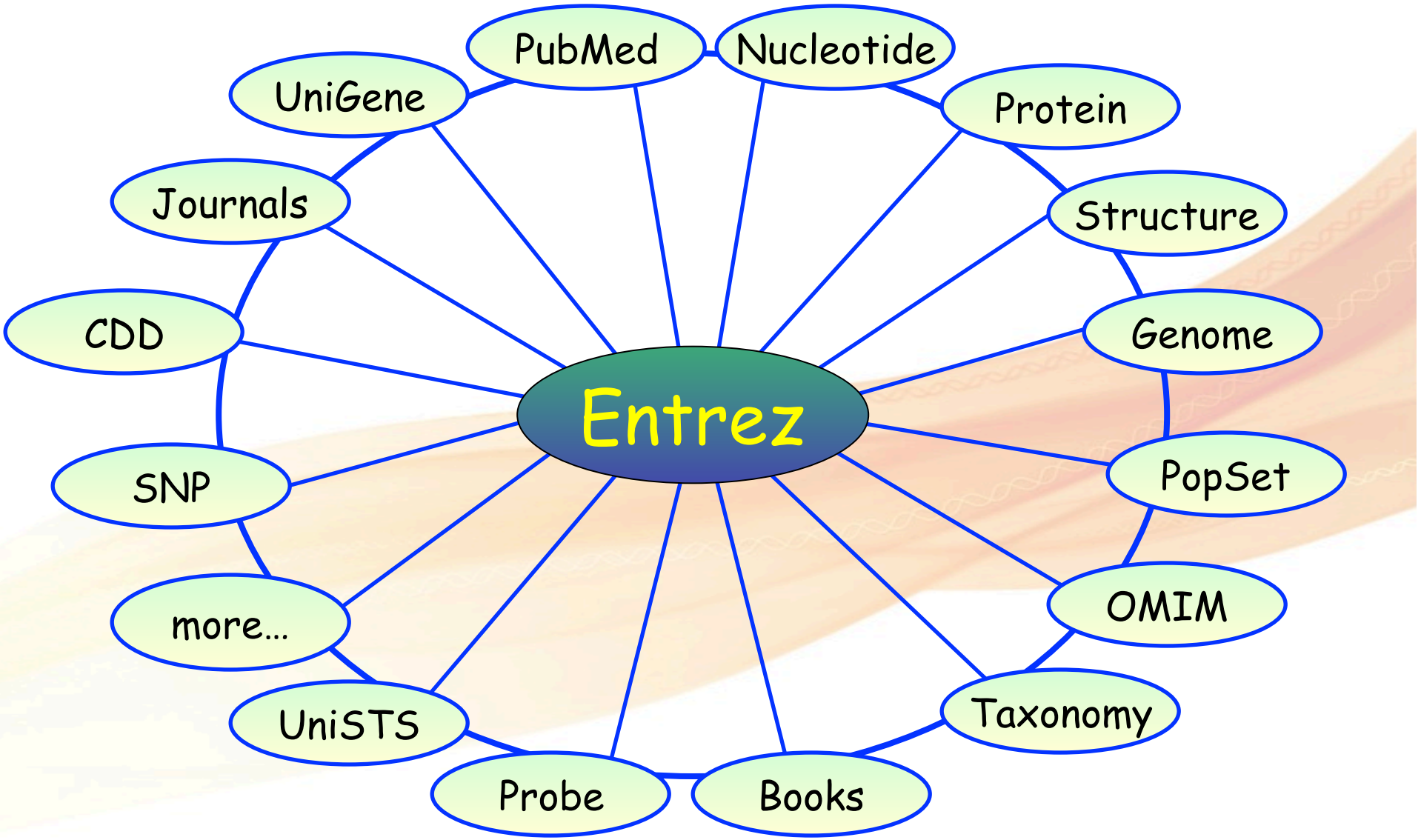
- "The life sciences search engine"
- Text-based search and retrieval system at NCBI
  - Single query string
  - Supports boolean operators
  - Search term tags to limit parts of the search statement of particular fields
- Covers all major databases at National Center for Biotechnology Information (NCBI)
  - PubMed; Nucleotide; Protein; Structure; Genome; Taxonomy
- More than one way to do things

- Go through the following:
  - http://www.ncbi.nlm.nih.gov/books/NBK3837/

Entrez Databases

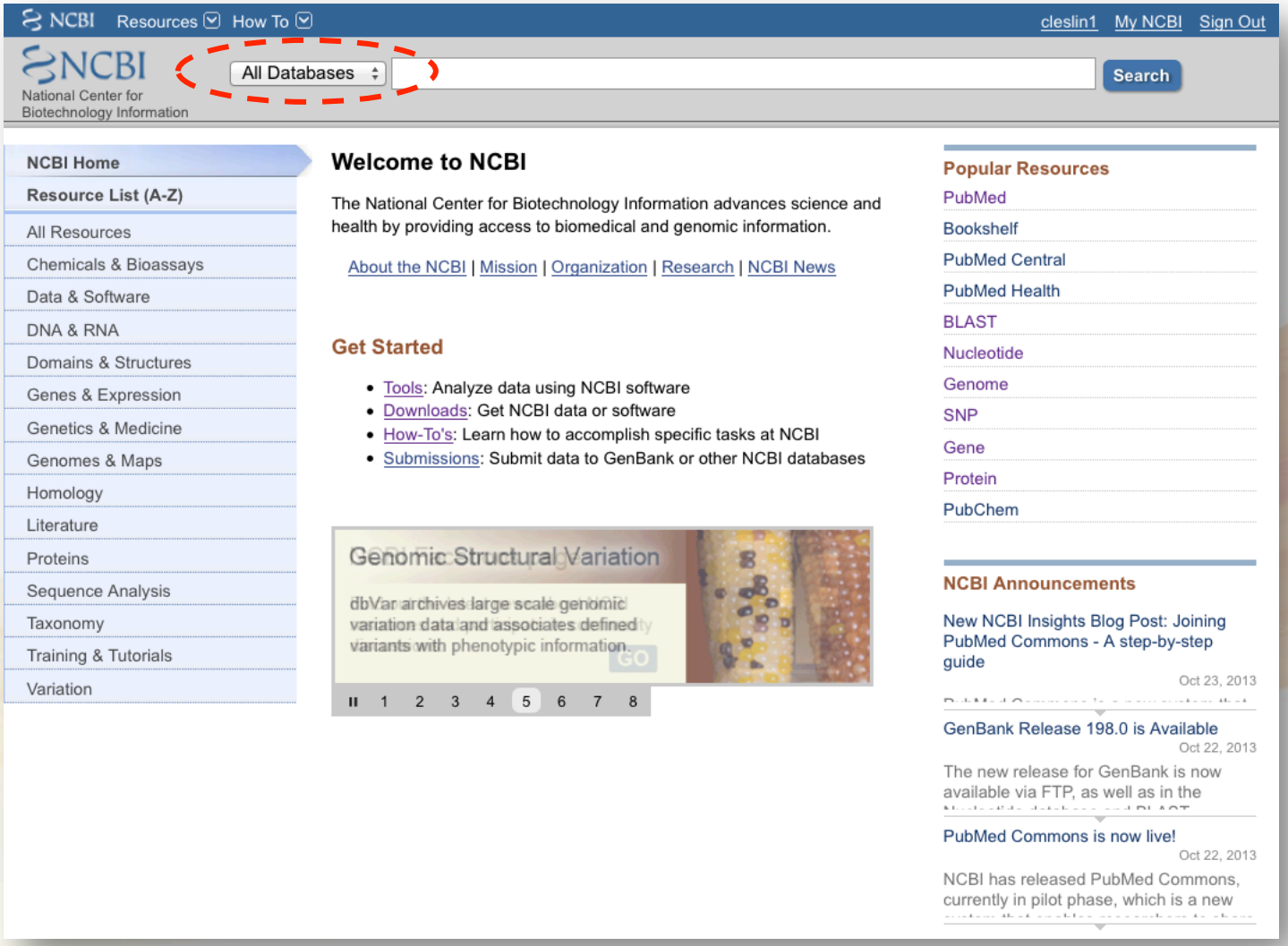
- All Molecular Database entries are organized by organism ***(Taxonomy Database)***
- Each record is assigned a UID
  - A "unique integer identifier" for internal tracking
- Each record is indexed by data fields
  - [author], [title], [organism], and many others
- Each record is given a Document Summary
  - A summary of the record's content (DocSum)
- Each record is manually or computationally assigned links to biologically related UIDs in and across databases

The (ever) Expanding Entrez System

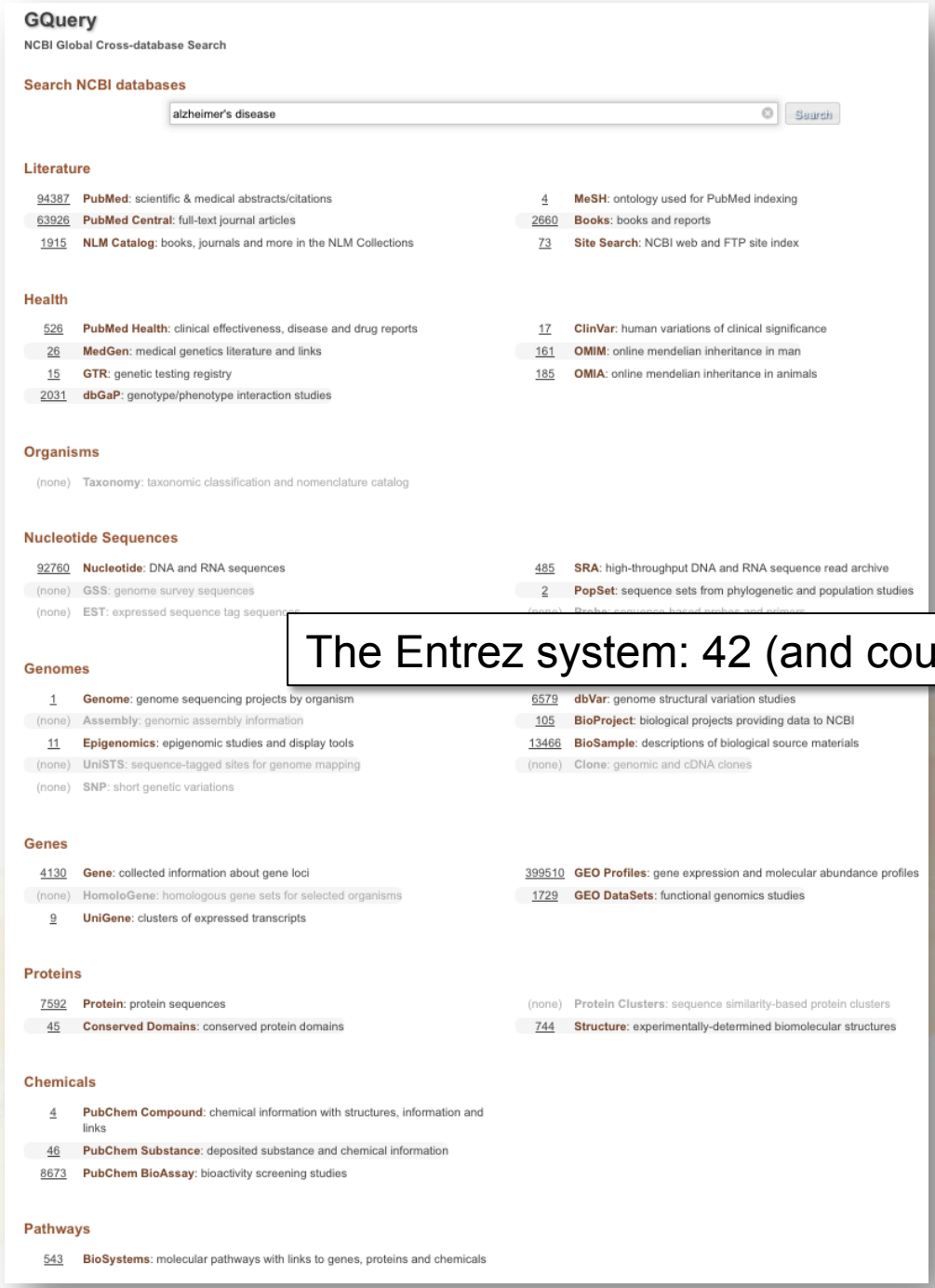


and more….

Accessing Entrez



Global Query: All NCBI Databases



GQuery

NCBI Global Cross-database Search

Search NCBI databases

alzheimer's disease

Search

Literature

94387 **PubMed**: scientific & medical abstracts/citations
63926 **PubMed Central**: full-text journal articles
1915 **NLM Catalog**: books, journals and more in the NLM Collections
4 **MeSH**: ontology used for PubMed indexing
2660 **Books**: books and reports
73 **Site Search**: NCBI web and FTP site index

Health

526 **PubMed Health**: clinical effectiveness, disease and drug reports
26 **MedGen**: medical genetics literature and links
15 **GTR**: genetic testing registry
2031 **dbGaP**: genotype/phenotype interaction studies
17 **ClinVar**: human variations of clinical significance
161 **OMIM**: online mendelian inheritance in man
185 **OMIA**: online mendelian inheritance in animals

Organisms

(none) **Taxonomy**: taxonomic classification and nomenclature catalog

Nucleotide Sequences

92760 **Nucleotide**: DNA and RNA sequences
(none) **GSS**: genome survey sequences
(none) **EST**: expressed sequence tag sequences
485 **SRA**: high-throughput DNA and RNA sequence read archive
2 **PopSet**: sequence sets from phylogenetic and population studies

Genomes

1 **Genome**: genome sequencing projects by organism
(none) **Assembly**: genomic assembly information
11 **Epigenomics**: epigenomic studies and display tools
(none) **UniSTS**: sequence-tagged sites for genome mapping
(none) **SNP**: short genetic variations
6579 **dbVar**: genome structural variation studies
105 **BioProject**: biological projects providing data to NCBI
13466 **BioSample**: descriptions of biological source materials
(none) **Clone**: genomic and cDNA clones

Genes

4130 **Gene**: collected information about gene loci
(none) **HomoloGene**: homologous gene sets for selected organisms
9 **UniGene**: clusters of expressed transcripts
399510 **GEO Profiles**: gene expression and molecular abundance profiles
1729 **GEO DataSets**: functional genomics studies

Proteins

7592 **Protein**: protein sequences
45 **Conserved Domains**: conserved protein domains
(none) **Protein Clusters**: sequence similarity-based protein clusters
744 **Structure**: experimentally-determined biomolecular structures

Chemicals

4 **PubChem Compound**: chemical information with structures, information and links
46 **PubChem Substance**: deposited substance and chemical information
8673 **PubChem BioAssay**: bioactivity screening studies

Pathways

543 **BioSystems**: molecular pathways with links to genes, proteins and chemicals

The Entrez system: 42 (and counting) integrated databases

Current Data in Entrez

2010 - Oct

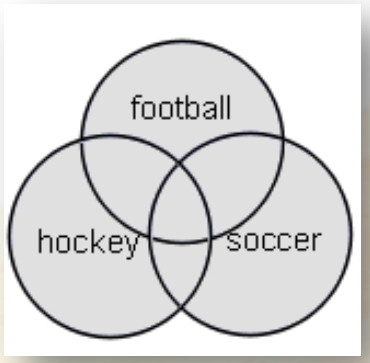
2013 - Oct

Searching NCBI Databases with Entrez

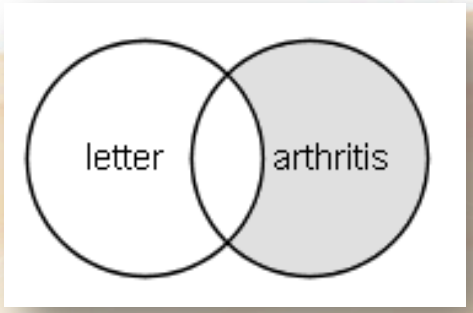
- Use words and phrases to construct precise searches of NCBI databases, either individually or searching across multiple databases
  - **Many features can be used in many or all databases**
  - **Others are specialized for specific databases**
- **Global Query**
  - Search across all Entrez databases by following the "All Databases" link from the NCBI homepage
  - Quick way to search NCBI content
    - But the search interface lacks many of the features we will discuss
- **Limits**
  - This tab is different for each database and provides an easy way to focus your search
  - Click the tab to see the options for the database you are using
    - e.g. limit to a particular species/taxonomy in Gene, limit to mRNA in Nucleotide, or limit to a particular chromosome in OMIM

Entrez - Boolean Operators

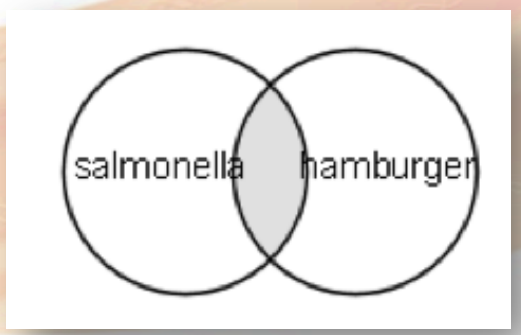
- As with PubMed we can use Booleans
- Use the upper case Boolean operators AND, OR and NOT in conjunction with parentheses to narrow or expand your search.
  - e.g. breast cancer AND (brca1 OR brca2)



OR



NOT



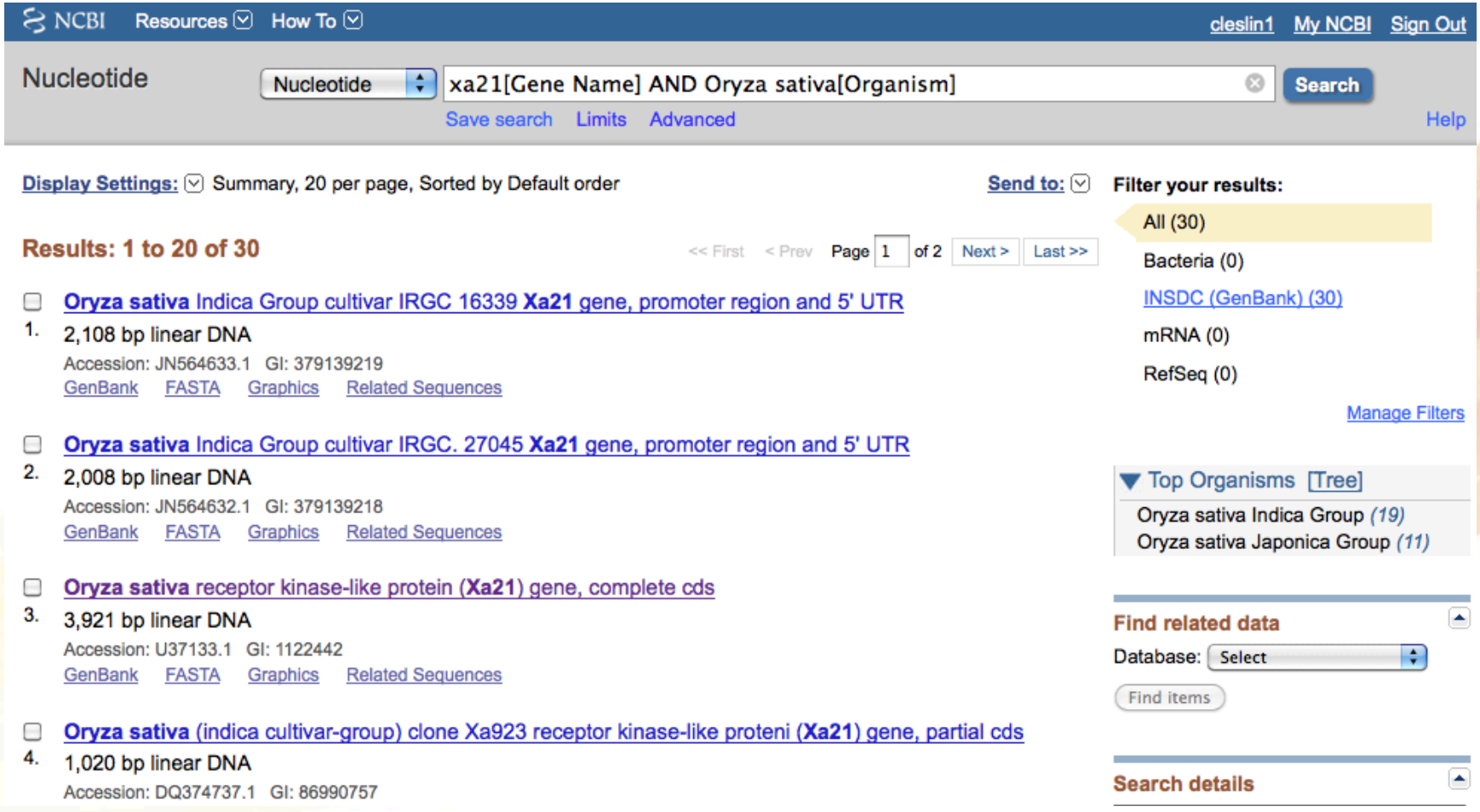
AND

Search Terms

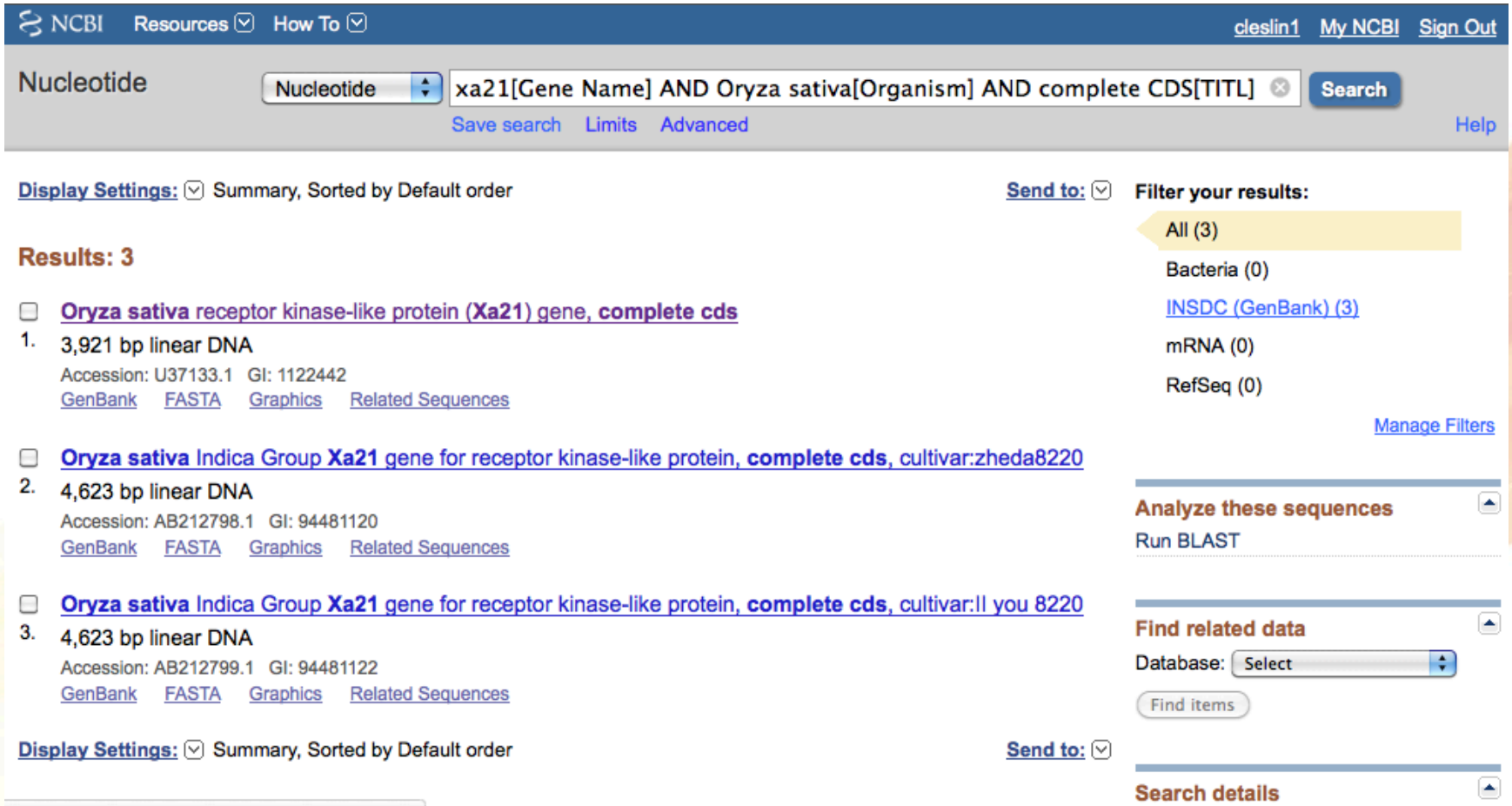
- Databases consist of “records” and “fields.”
- Records represent genes, proteins, journal articles or other objects depending on the database
- Each record is composed of fields that describe the object
- Fields can include Title, Accession Number, Organism, Author and more, depending on the record
- Using search “terms” to specify fields allows you to increase search precision, for example **mouse[ORGN] AND polya signal[FKEY]**
  - **Do nucleotide search for:**
    - **mouse AND polya signal**
    - **mouse[ORGN] AND polya signal[FKEY]**

http://dl.dropboxusercontent.com/u/1304874/entrez/index.html

Gene Search


NCBI Resources How To
cleslin1 My NCBI Sign Out
Nucleotide
Nucleotide
xa21[Gene Name] AND Oryza sativa[Organism]
Search
Save search Limits Advanced
Help
Display Settings: Summary, 20 per page, Sorted by Default order
Send to:
Results: 1 to 20 of 30
<< First < Prev Page 1 of 2 Next > Last >>
1. Oryza sativa Indica Group cultivar IRGC 16339 Xa21 gene, promoter region and 5' UTR
2,108 bp linear DNA
Accession: JN564633.1 GI: 379139219
GenBank FASTA Graphics Related Sequences
2. Oryza sativa Indica Group cultivar IRGC. 27045 Xa21 gene, promoter region and 5' UTR
2,008 bp linear DNA
Accession: JN564632.1 GI: 379139218
GenBank FASTA Graphics Related Sequences
3. Oryza sativa receptor kinase-like protein (Xa21) gene, complete cds
3,921 bp linear DNA
Accession: U37133.1 GI: 1122442
GenBank FASTA Graphics Related Sequences
4. Oryza sativa (indica cultivar-group) clone Xa923 receptor kinase-like proteni (Xa21) gene, partial cds
1,020 bp linear DNA
Accession: DQ374737.1 GI: 86990757
Filter your results:
All (30)
Bacteria (0)
INSDC (GenBank) (30)
mRNA (0)
RefSeq (0)
Manage Filters
Top Organisms [Tree]
Oryza sativa Indica Group (19)
Oryza sativa Japonica Group (11)
Find related data
Database: Select
Find items
Search details

A Better Gene Search



Writing Advanced Search Statements

FIELD		Entrez Search Tips
LOCUS	GenBank division	`gbdiv_est[PROP],` `gbdiv_pln[PROP], etc.`
	Molecule type	`biomol_genomic[PROP],` `biomol_mRNA[PROP], etc.`
DEFINITION		`complete CDS[TITL]`
ACCESSION		`AP001111 [ACCN]`
SOURCE	Organism	`Oryza sativa[ORGN]`
FEATURES		`TATA_signal [FKEY],` `CDS[FKEY], D-loop[FKEY], etc.`

Writing Advanced Search Statements

```
LOCUS       OSU37133                3921 bp    DNA     linear   PLN 14-DEC-1995
DEFINITION  Oryza sativa receptor kinase-like protein (Xa21) gene, complete
            cds.
ACCESSION   U37133
VERSION     U37133.1  GI:1122442
KEYWORDS    .
SOURCE      Oryza sativa Indica Group
  ORGANISM  Oryza sativa Indica Group
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; BEP
            clade; Ehrhartoideae; Oryzeae; Oryza.
REFERENCE   1  (bases 1 to 3921)
  AUTHORS   Song,W.-Y., Wang,G.-L., Chen,L.-L., Kim,H.-S., Pi,L.-Y.,
            Holsten,T., Wang,G.B., Zhai,W.-X., Zhu,L.-H., Fauquet,C. and
            Ronald,P.
  TITLE     A receptor kinase-like protein encoded by the rice disease
            resistance gene, Xa21
  JOURNAL   Science 270 (5243), 1804-1806 (1995)
   PUBMED   8525370
REFERENCE   2  (bases 1 to 3921)
  AUTHORS   Ronald,P., Song,W.-Y., Wang,G.-L., Kim,H.-S. and Pi,L.-Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-SEP-1995) Pamela Ronald, Plant Pathology, UC Davis,
            Hutchison Hall, Davis, CA 95616, USA
FEATURES             Location/Qualifiers
     source          1..3921
                     /organism="Oryza sativa Indica Group"
                     /mol_type="genomic DNA"
                     /strain="IRBB21"
                     /db_xref="taxon:39946"
                     /chromosome="11"
                     /map="11q, RG103"
     gene            1..3921
                     /gene="Xa21"
     CDS             join(1..2677,3521..3921)
                     /gene="Xa21"
                     /note="Xa21 disease resistance gene"
                     /codon_start=1
```

Some Common Search Fields

Some Common Search Fields

Field Name	Field Tag	Database Examples	Features and Examples
Accession number	[ACCN]	Gene, Nucleotide, Protein, Structure, SNP, Probe	Searches sequence accession numbers for nucleic acids and proteins. Use a colon to search a range of accession numbers.
Organism	[ORGN]	Gene, Nucleotide, Protein, Structure, EST, GSS, Probe	Use either common names or scientific names.
Creation Date	[CDAT]	All	Date the record was created. Use a colon to search for a range of dates, e.g. **2003/15/01:2004/15/01[CDAT]**. Month and day are optional.
Gene Name	[GENE NAME]	Gene, Nucleotide, Protein, OMIM, Probe, UniGene	Retrieves your search term as the exact gene name. Use a * to truncate your search; e.g. **lrh*[GENE NAME]** retrieves LRH1, LRHA, LRHR, etc.
Disease Name	[DIS]	Gene, OMIM, Probe, UniGene	Searches for the name of a disease or phenotype associated with genetic mutations.
Gene Ontology	[GO]	Gene, SNP, UniGene, Probe	Searches for the term in Gene Ontology annotations.
Molecular Weight	[MOLWT]	Protein	Use a colon to indicate a range of molecular weights; e.g. **1500:1550[MOLWT]**.
Sequence Length	[SLEN]	Gene, Nucleotide, Protein	Use a colon to indicate a range of sequence lengths; e.g. **900:1000[SLEN]**.

Entrez Search Terms

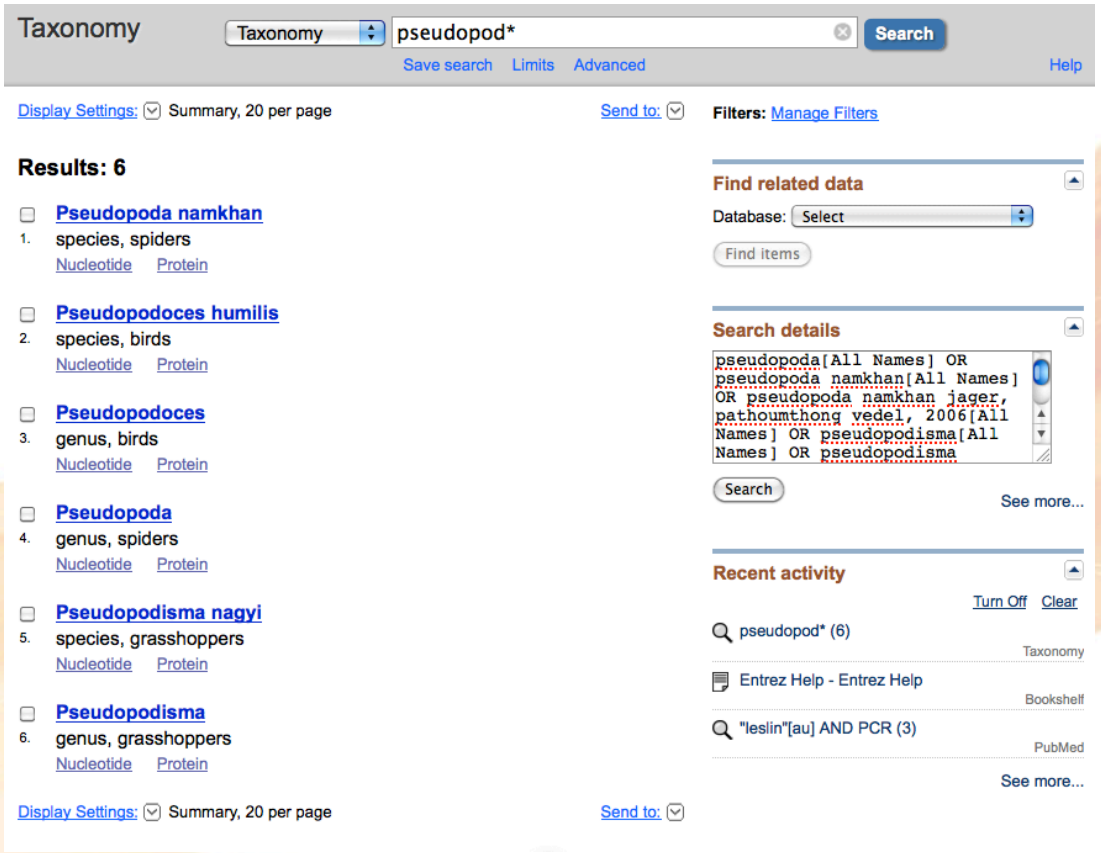
ALL, All Fields, All terms from all searchable fields
UID, UID, Unique number assigned to each sequence
FILT, Filter, Limits the records
WORD, Text Word, Free text associated with record
TITL, Title, Words in definition line
KYWD, Keyword, Nonstandardized terms provided by submitter
AUTH, Author, Author(s) of publication
JOUR, Journal, Journal abbreviation of publication
VOL, Volume, Volume number of publication
ISS, Issue, Issue number of publication
PAGE, Page Number, Page number(s) of publication
ORGN, Organism, Scientific and common names of organism, and all higher levels of taxonomy
ACCN, Accession, Accession number of sequence
PACC, Primary Accession, Does not include retired secondary accessions
GENE, Gene Name, Name of gene associated with sequence
PROT, Protein Name, Name of protein associated with sequence
ECNO, EC/RN Number, EC number for enzyme or CAS registry number
PDAT, Publication Date, Date sequence added to GenBank
MDAT, Modification Date, Date of last update
SUBS, Substance Name, CAS chemical name or MEDLINE Substance Name
PROP, Properties, Classification by source qualifiers and molecule type
SQID, SeqID String, String identifier for sequence
GPRJ, Genome Project, Genome Project
SLEN, Sequence Length, Length of sequence
FKEY, Feature key, Feature annotated on sequence
RTYP, Replicon type, Replicon type
RNAM, Replicon name, Replicon name
ORGL, Organelle, Organelle

http://www.ncbi.nlm.nih.gov/entrez/query/static/help/Summary_Matrices.html

http://155.33.203.128/teaching/BIOL6308-Fall2013/local/Literature/entrez_fields.pdf

Using Truncation to Expand Field Searches

- Incorporating Entrez's truncation symbol *
  - An asterisk
  - e.g. pseudopod* @ Taxonomy
- Will retrieve the following:
- Pseudopoda namkhan
- Pseudopodoces humilis
- Pseudopodoces
- Pseudopoda
- Pseudopodisma nagyi
- Pseudopodisma



Properties and Filters

- In addition to search fields like [ACCN] and [ORGN]
  - Entrez databases use properties and filters
  - These search features limit searches in many different ways depending on the database
- Some examples are searching in Gene to retrieve only records that have a link to Protein, or a searching in Nucleotide to retrieve only tRNA molecule records

Some Examples of Properties and Filters

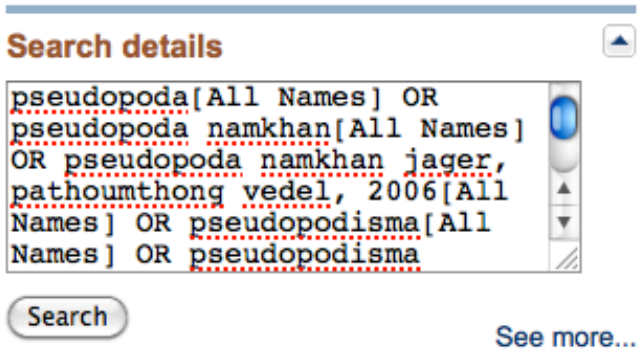
Search	Databases	Features and Examples
gene nucleotide[FILTER]	Gene	Gene records with links to Nucleotide records
genetype protein coding[PROPERTIES]	Gene	Gene records for protein coding genes
biomol trna[PROPERTIES]	Nucleotide	Nucleotide records for tRNA molecules
uncultured[FILTER]	Nucleotide	Nucleotide records for sequences from uncultured microorganisms
gene in plasmid[PROPERTIES]	Protein	Protein records in which the coding gene is on a plasmid
protein homologene[FILTER]	Protein	Protein records with links to the Homologene database

human[Organism]) AND RNR1[Gene Name]

human[Organism]) AND RNR1[Gene Name] AND gene nucleotide[FILTER]

Details & History

- Some databases use “Automatic Term Mapping” for your searches
  - "Search details" allows you to see how your term has been mapped and make any adjustments necessary
    - search for “human” in the Gene database
      - "Homo sapiens" [Organism] OR human[All Fields]



- Search History – Found in Advanced tab
  - Displays all of your recent searches in chronological order
  - Use the History tab to rerun, combine or modify previous searches
  - Searches are lost after 8 hours of inactivity on NCBI unless they are saved to a myNCBI account

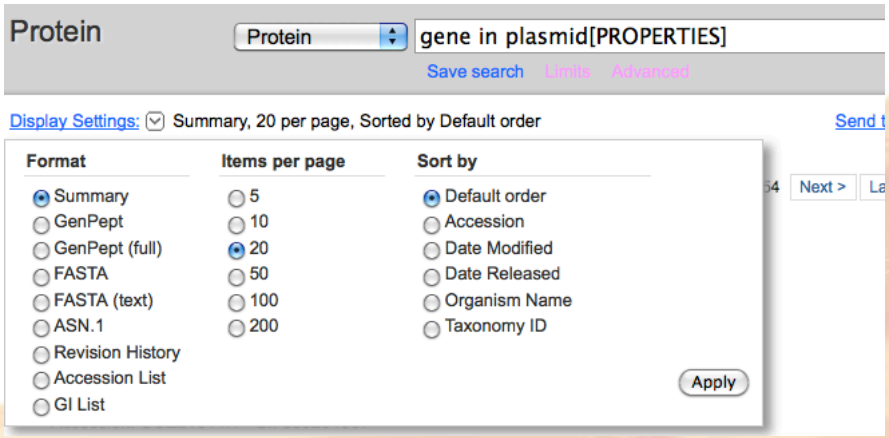
" – not needed

MyNCBI

- MyNCBI allows you to:
  - Save results and search strategies indefinitely
  - As well as set up email alerts and change displays to your preferences
- After registering:
  - You can access your results and preferences at any computer you use to access NCBI database

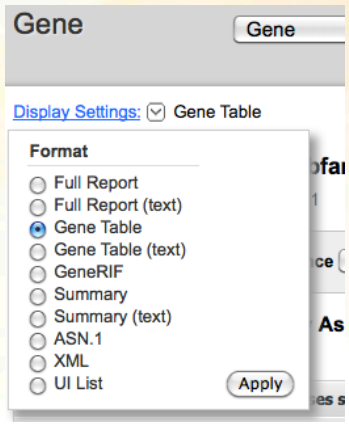
Display Setting

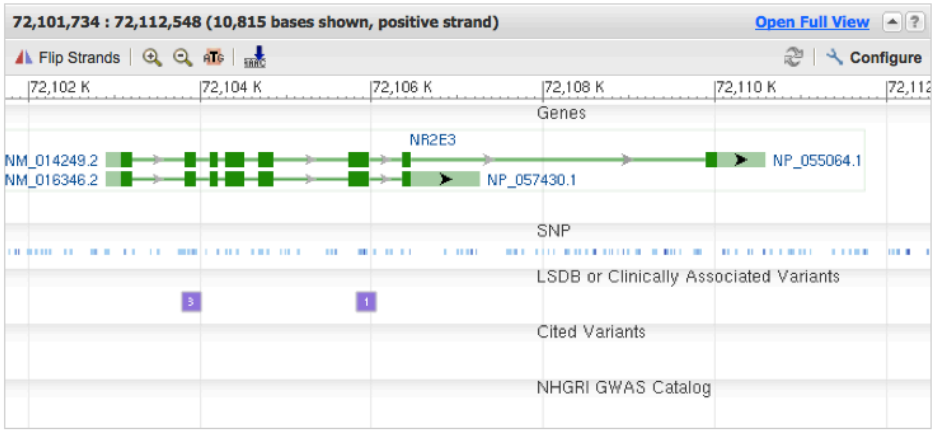
- The Display drop-down menu
  - View your search results in different formats depending on the database
  - Typically, displays are available that show:
    - Record summaries,
    - Links to other databases
    - Sequence data



Display Setting

- Some databases have specialized displays;
  - Such as the Gene Table display in Entrez Gene
    - Provides a graphical overview of the gene and includes intron and exon information
    - Ex: search for **NR2E3** in Gene





Top of page | Gene Table help

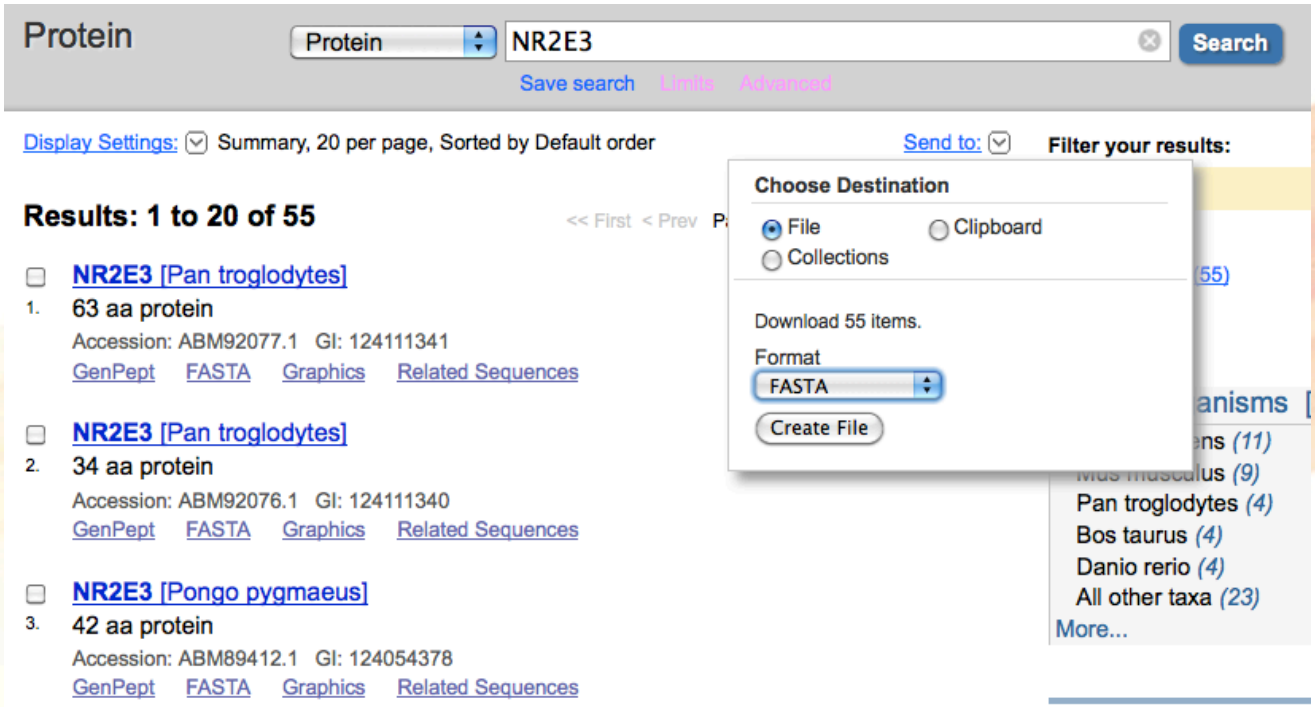
Reference transcript variant 2 mRNA NM_014249.2, 8 exons, total spliced exon length: 1999
Reference isoform b Protein NP_055064.1, 8 coding exons, AA length: 410

Exon table for mRNA NM_014249.2 and protein NP_055064.1

Interval (exons 5' to 3')		Length (bp)		
Exon	Coding	Exon	Coding	Intron
72102894-72103201	72103084-72103201	308	118	621
72103823-72103949	72103823-72103949	127	127	156
72104106-72104209	72104106-72104209	104	104	85
72104295-72104516	72104295-72104516	222	222	159
72104676-72104851	72104676-72104851	176	176	877
72105729-72105976	72105729-72105976	248	248	376
72106353-72106458	72106353-72106458	106	106	3434
72109893-72110600	72109893-72110025	708	133	

Send To

- The Send to drop-down menu allows you to send your results (in your chosen Display format) to a file, clipboard or collection



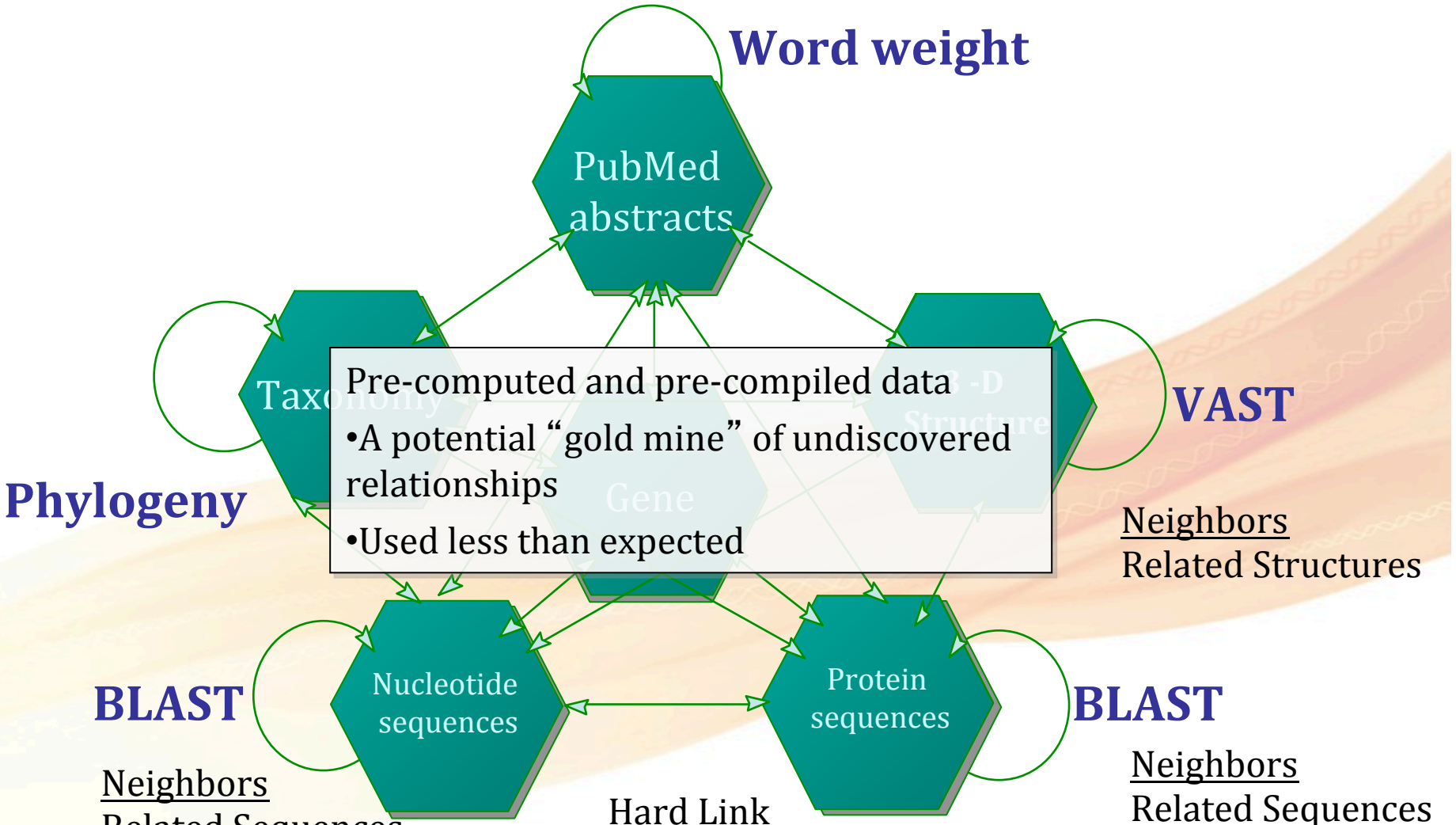
Output

```
MacBook-Pro-SSD:~ cleslin$ perl eutils_example.pl
Database [Pubmed]: protein
Query [zanzibar]: NR2E3
Report [abstract]: fasta
```

```
>gi|7706515|ref|NP_057430.1| photoreceptor-specific nuclear receptor isoform
 a [Homo sapiens]
METRPTALMSSTVAAAAPAAGAASRKESPGRWGLGEDPTGVSPSLQCRVCGDSSSGKHYGIYACNGCSGF
FKRSVRRRLIYRCQVGAGMCPVDKAHRNQCQACRLKKCLQAGMNQDAVQNERQPRSTAQVHLDSMESNTE
SRPESLVAPPAPAGRSPRGPTPMSAARALGHHFMASLITAETCAKLEPEDADENIDVTSNDPEFPSSPYS
SSSPCGLDSIHETSARLLFMAVKWAKNLPVFSSLPFRDQVILLEEAWSELFLLGAIQWSLPLDSCPLLAP
PEASAAGGAQGRLTLASMETRVLQETISRFRALAVDPTEFACMKALVLFKPETRGLKDPEHVEALQDQSQ
VMLSQHSKAHHPSQPVR

>gi|122132423|sp|Q08E02.1|NR1D1_BOVIN RecName: Full=Nuclear receptor subfami
ly 1 group D member 1; AltName: Full=Rev-erbA-alpha; AltName: Full=V-erbA-re
lated protein 1; Short=EAR-1
MTTLDSNNNTGGVITYIGSSGSSPNRTSPESLYSDSSNGSFQSLTQGCPTYFPPSPTGSLTQDPARSFGS
IPPSLGDDGSPSSSSSSSSSSSSSFYNGSPPGGLQVALEDSNRVSPSKSTSNITKLNGMVLLCKVCGDVA
SGFHYGVHACEGCKGFFRRSIQQNIQYKRCLKNENCSIVRINRNRCQQCRFKKCLSVGMSRDAVRFGRIP
KREKQRMLAEMQSAMNLANNQLSSQCPLETPPTQHPTPGPMGPSPPPAPAPSPLVGFSQFPQQLTPPRSP
SPEPTVEDVISQVARAHREIFTYAHDKLGTSPGNFNANHASGNRPATTPHRWESQGCPPANDNIMAAQRH
NEALNSLRQASSSYPPPWPPGAAHHSCHQPNSNGHRLCPTHVYPAPEGEAPVNSPRQGNSKNILLACPMN
MYPHGRSGRTVQEIWEDFSMSFTPAVREVVEFAKHIPGFRDLSQHDQVTLLKAGTFEVLMVRFASLFNVK
DQTVMFLSRTTYSLQELGAMGMGDLLNAMFDFSEKLNSLALTEEELGLFTAVVLVSADRSGMENSASVEQ
LQETLLRALRALVLKNRPSETSRFTKLLLKLPDLRTLNNMHSEKLLSFRVDAQ
```

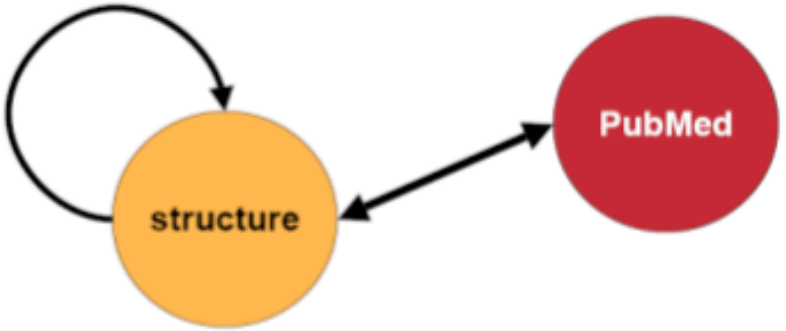
http://www.ncbi.nlm.nih.gov/entrez/query/static/eutils_example.pl

Entrez: A Discovery System



Following Links

Follow links to related data in the same database or in others!



"Hard" Links: ***Curated links based on biology***

for example:

nucleotide → Taxonomy (based on organism identifier)
protein → Domain relatives (based on domain assignment)
domains → PubMed (based on supporting literature)

"Soft" Links: ***Pre-computed analyses***

for example:

nucleotide → Related sequences (BLASTn neighbors)
protein → Conserved domains (RPS-BLAST search)
gene → Map viewer (map position of annotated gene)

Precomputed Services

Take a look at protein CAA18341

Some Entrez Links:

A List of Similar Sequences:	**Related Sequences**
Structures with Similar Sequences:	**Related Structures**
The Multifunctional Blast Link:	**Blink**
Gene Records	**Gene**
CDD *(Functional Domains)*	**Conserved Domains**
Taxonomic information	**Taxonomy**
Domain Relatives	**Domain Relatives**

What is BLink

- BLink stands for "BLAST Link"
- Foundation is protein sequence similarity information derived from protein BLAST search
- It is a link available for existing protein record
- It displays the proteins that most similar to that entry
- In addition, it also contains a collection of precomputed results/links to external data pertaining to that given protein sequence

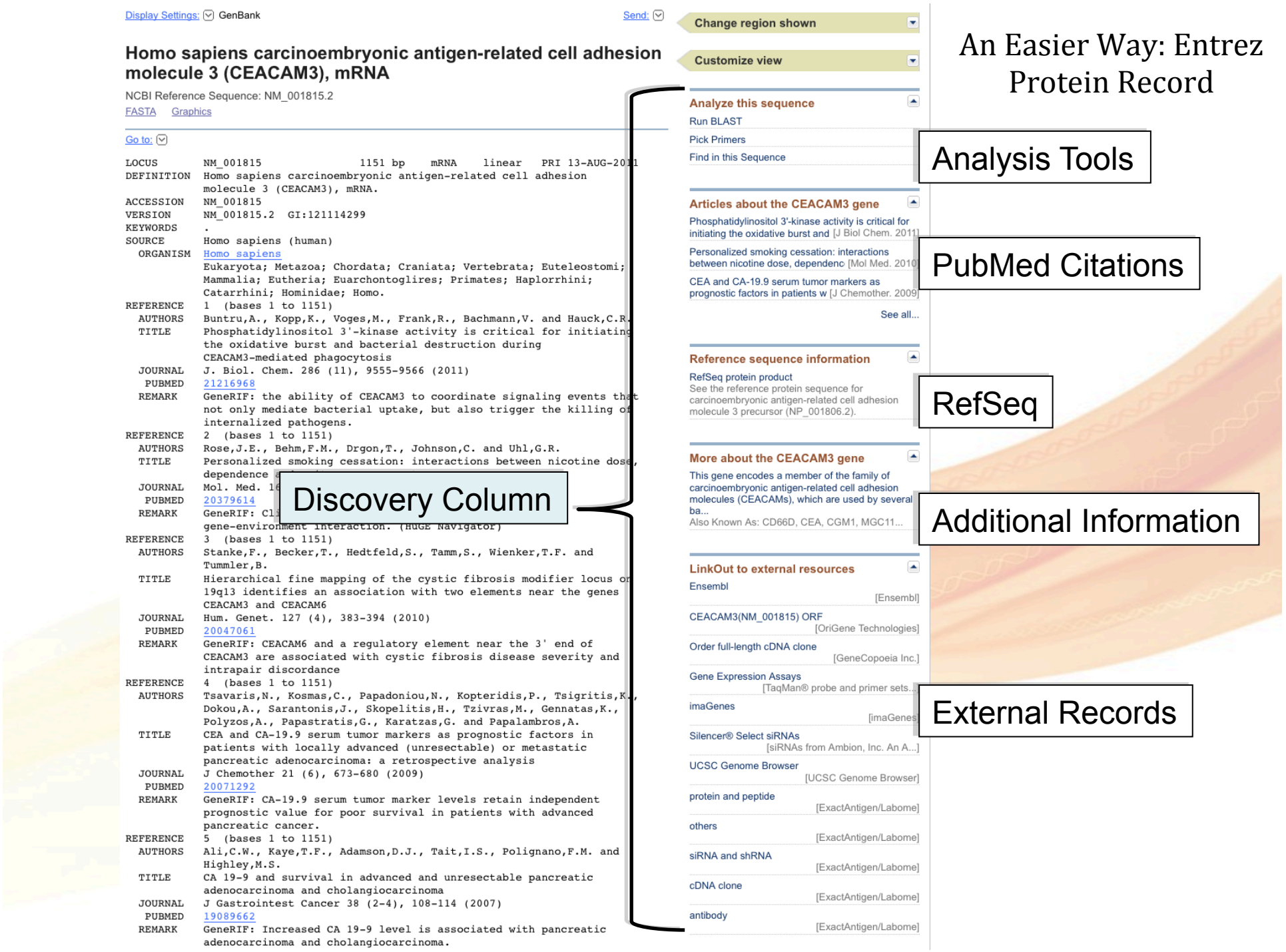
How Does NCBI Generate the BLink Data

- NCBI performs:
  - Scheduled BLAST searches against other existing proteins
    - for **every protein** record released into Entrez Protein database
  - Results are saved for "Related Sequences" Entrez links
- Additional information also saved to form the complete dataset for Blink
  - Taxonomic distribution
  - 3D structure matches
  - Conserved domain matches

Searching for Sequences: Hard and Soft Links

- Go to Entrez
  - Do a search for "U41100.1"
  - Click on nucleotide database
    - View the GenBank FlatFile
      - Investigate the **hard links** available from this nucleotide sequence record
        - » Full Text in PMC
        - » PubMed
        - » Protein
        - » Taxonomy
      - Investigate the **soft links**
        - » PubMed (Weighted) - identified using a word weight algorithm- similar words in their title, abstracts and Medical Subject Headings
        - » Related Sequences

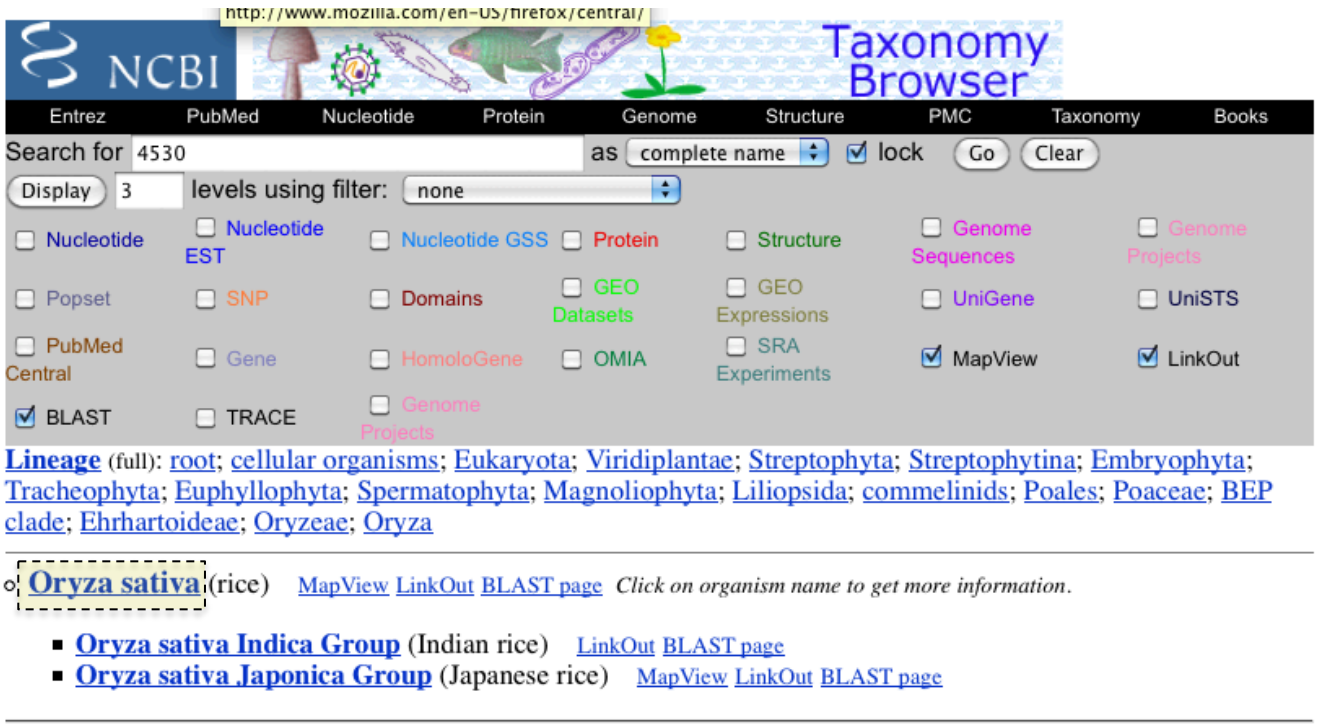
An Easier Way: Entrez Protein Record



Taxonomy

Taxonomy Database

- Browser for the major divisions of living organisms
  - archaea, bacteria, eukaryota, and viruses
- Taxonomy information such as genetic codes



Compiled Data at Taxonomy DB

- Subtree links
  - Entries linked to any taxa in this subtree of the taxonomy database
- Direct links
  - Entreis linked directly to this node in the taxonomy database

Oryza sativa

Taxonomy ID: 4530
Genbank common name: **rice**
Inherited blast name: **monocots**
Rank: species
Genetic code: Translation table 1 (Standard)
Mitochondrial genetic code: Translation table 1 (Standard)
Other names:
common name: **red rice**
authority: **Oryza sativa L.**

Lineage(full)
cellular organisms; Eukaryota; Viridiplantae; Streptophyta; Streptophytina; Embryophyta; Tracheophyta; Euphyllophyta; Spermatophyta; Magnoliophyta; Liliopsida; commelinids; Poales; Poaceae; BEP clade; Ehrhartoideae; Oryzeae; Oryza

Entrez records		
Database name	Subtree links	Direct links
Nucleotide	247,110	99,901
Nucleotide EST	1,253,557	62,231
Nucleotide GSS	500,493	3,769
Protein	281,241	17,717
Structure	108	34
Genome	1	1
Popset	854	754
SNP	11,023,172	11,023,172
Domains	19	11
GEO Datasets	7,300	4,928
UniGene	44,118	44,118
UniSTS	1,073	1,073
PubMed Central	9,517	9,517
Gene	84,179	1
HomoloGene	10,431	10,431
SRA Experiments	2,126	1,692
Probe	182,866	89,901
Assembly	6	-
Bio Project	452	298
Bio Sample	32,386	3,296
Bio Systems	1,130	661
GEO Profiles	670,939	670,939
PubChem BioAssay	106	70
Protein Clusters	12,990	-
Taxonomy	5	1

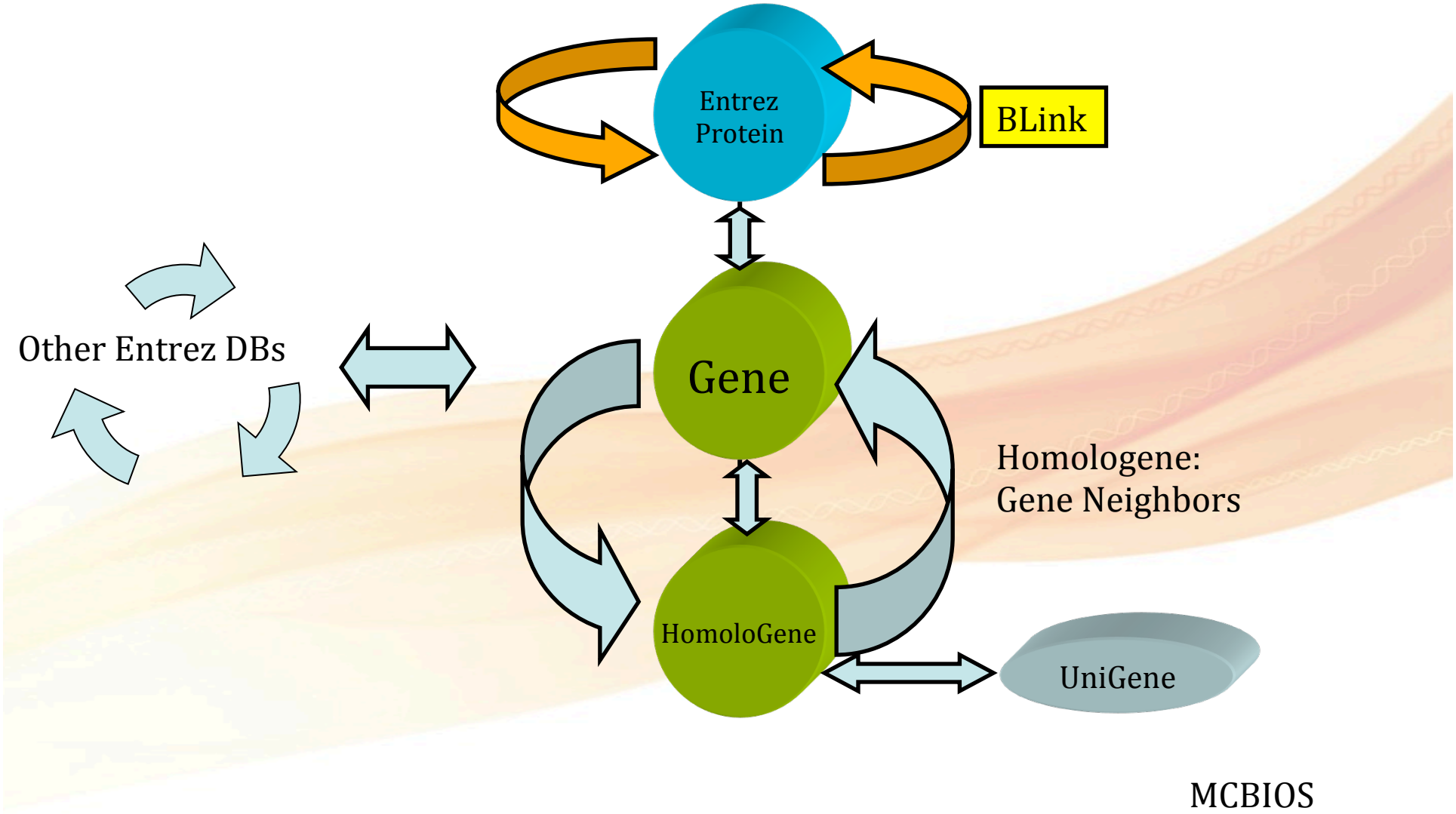
http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=4530&lvl=3&lin=f&keep=1&srchmode=1&unlock

LinkOut at Entrez

LinkOut

- LinkOut is a service of Entrez:
  - Link directly from PubMed and other Entrez databases to a wide range of information and services beyond the Entrez system
  - Aims to facilitate access to relevant online resources in order to extend, clarify, and supplement information found in the Entrez databases
- Examples of LinkOut Resources include:
  - Full-text publications
  - Biological databases
  - Consumer health information
  - Research tools
  - and more

FYI - Entrez Tip: Start Searches in Gene



MCBIOS

Where Else Can I get Help?

- Service Addresses
  - General Help info@ncbi.nlm.nih.gov
  - BLAST blast-help@ncbi.nlm.nih.gov
  - Telephone support: 301- 496- 2475

Bioinformatics Sequence Formats

Sequence Formats

- Microsoft WORD format is not a sequence format!!!
- Sequences can be read and written in a variety of formats
  - These can be very confusing for users
    - Ex. converting from one sequence editor to another
      - Now have your existing sequences in a format that is specific for that package
    - If you don't hold your sequence in a recognized standard format, you will not be able to analyze your sequence easily
- As a bioinformaticist you should be able to identify and use different formats

What a Sequence Format is NOT

- NOT any sort of program-specific format like a word processor format or text formatting language
  - Note: 'WORD', 'WORDPAD', 'PostScript', 'PDF', 'RTF', 'TeX', 'HTML'
- If you have somehow managed to type a sequence into a word-processor, you should:
  - # Save the sequence to a file as ASCII text (try selecting: File, Save As, Text)
  - # Stop using word-processors to write sequences
  - Investigate using simple text editor
    - like notepad Windows
    - Terminal or TextWrangler OSX

What a Sequence Format IS

- Sequence formats are ASCII TEXT
- They are the required arrangement of:
  - characters, symbols and keywords that specify:
    - What things such as the sequence, ID name, comments, etc. look like in the sequence entry
    - Where in the entry the program should look to find them
- There are generally no hidden, unprintable 'control' characters in any sequence format
- All standard sequence formats can be printed out or viewed simply by displaying their file (less or more)

Why So Many Formats?

- Dozens common sequence
- Some are much more common than others
- Formats were designed to be able to hold the sequence data and other information about the sequence
- Nearly every sequence analysis package has its own format
- Nearly every collection of sequences that dares call itself a database has stored its data in its own format

http://emboss.sourceforge.net/docs/themes/SequenceFormats.html

A Traditional GenBank Record

```
LOCUS       AF124527               2540 bp    mRNA    linear   PLN 29-JAN-2004
DEFINITION  Prunus persica ethylene receptor (ETR1) mRNA, complete cds.
ACCESSION   AF124527
VERSION     AF124527.1  GI:6841074
KEYWORDS    .
SOURCE      Prunus persica (peach)
  ORGANISM  Prunus persica
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core eudicotyledons;
            rosids; eurosids I; Rosales; Rosaceae; Amygdaloideae; Prunus.
REFERENCE   1  (bases 1 to 2540)
  AUTHORS   Bassett,C.L., Artlip,T.S. and Callahan,A.M.
  TITLE     Characterization of the peach homologue of the ethylene receptor,
            PpETR1, reveals some unusual features regarding transcript
            processing
  JOURNAL   Planta 215 (4), 679-688 (2002)
   PUBMED   12172852
REFERENCE   2  (bases 1 to 2540)
  AUTHORS   Bassett,C.B., Artlip,T.S. and Nickerson,M.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-JAN-1999) Appalachian Fruit Research Station,
            USDA-ARS, 45 Wiltshire Road, Kearneysville, WV 25430, USA
FEATURES             Location/Qualifiers
     source          1..2540
                     /organism="Prunus persica"
                     /mol_type="mRNA"
                     /cultivar="Loring"
                     /db_xref="taxon:3760"
                     /dev_stage="III B/C fruit"
     gene            1..2540
                     /gene="ETR1"
     CDS             269..2485
                     /gene="ETR1"
                     /codon_start=1
                     /product="ethylene receptor"
                     /protein_id="AAF28893.1"
                     /db_xref="GI:6841075"
                     /translation="MEACNCIEPQWPADELLMKYQYISDFFIALAYFSIPLELIYFVK
                     KSAVFPYRWVLVQFGAFIVLCGATHLINLWTFSMHSRTVAIVMTTAKVLTAVVSCATA
                     LMLVHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRH
                     TILKTTLVELGRTLALEECALWMPTRTGLELQLSYTLRQQNPVGYTVPIHLPVINQVF
                     SSNRALKISPNSPVARMRPLAGKHMPGEVVAVRVPLLHLSNFQINDWPELSTKRYALM
                     VLMLPSDSARQWHVHELELVEVVADQVAVALSHAAILEESMRARDLLMEQNIALDLAR
                     REAETAIRARNDFLAVMNHEMRTPMHAIIALSSLLQETELTPEQRLMVETILKSSHLL
                     ATLINDVLDLSRLEDGSLQLEIATFNLHSVFREVHNLIKPVASVKKLSVSLNLAADLP
                     VQAVGDEKRLMQIVLNVVGNAVKFSKEGSISITAFVAKSESLRDFRAPEFFPAQSDNH
                     FYLRVQVKDSGSGINPQDIPKLFTKFAQTQSLATRNSGGSGLGLAICKRFVNLMEGHI
                     WIESEGPGKGCTAIFIVKLGFAERSNESKLPFLTKVQANHVQTNFPGLKVLVMDDNGS
                     VTKGLLVHLGCDVTTVSSIDEFLHVISQEHKVVFMDVCMPGIDGYELAVRIHEKFTKR
                     HERPVLVALTGNIDKMTKENCMRVGMDGVILKPVSVDKMRSVLSELLEHRVLFEAM"
ORIGIN
        1 gcacgagggc tcaccgagcg agctagctct tcaggagtca aggcttctgg gtgaggggaa
       61 gaagaagaag cttctttgat gtgttggggt gccaatctaa agaggaagaa gaaggcctct
      121 aatgtattga ggtcggctgt ctgggctgcc gatctgtgtt gaatggatag tttggtagag
      181 atgcttcaac gacatagggt ggctgaaaag ggtttgaaga aagtgaagga ggaaaccaag
                                        ...
     2401 tatactgaaa cctgtctcag ttgataaaat gaggagtgtt ttatcagaac tgttggagca
     2461 tcgagtttta tttgaggcta tgtaagatat aggaaaattg ttctagtgaa ggaaagattt
     2521 aaatggaaaa aaaaaaaaaa
//
```

Header

The Flatfile Format

Feature Table

Sequence

FASTA format (a.k.a. Pearson Format)

```
>gi|5524211|gb|AAD44166.1| cytochrome b [Elephas maximus maximus]
LCLYTHIGRNIYYGSYLYSETWNTGIMLLLITMATAFMGYVLPWGQMSFWGATVITNLFSAIPYIGTNLV
EWIWGGFSVDKATLNRFFAFHFILPFTMVALAGVHLTFLHETGSNNPLGLTSDSDKIPFHPYYTIKDFLG
LLILILLLLLLALLSPDMLGDPDNHMPADPLNTPLHIKPEWYFLFAYAILRSVPNKLGGVLALFLSIVIL
GLMPFLHTSKHRSMMLRPLSQALFWTLTMDLLTLTWIGSQPVEYPYTIIGQMASILYFSIILAFLPIAGX
IENY
```

```
>sp_ac|P02769_WOSIG0 \ID=ALBU_BOVIN \DE="Serum albumin precursor
RGVFRRDTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQCPFDEHVKLVNELTEFAKTCV
ADESHAGCEKSLHTLFGDELCKVASLRETYGDMADCCEKQEPERNECFLSHKDDSPDLPK
LKPDPNTLCDEFKADEKKFWGKYLYEIARRHPYFYAPELLYYANKYNGVFQECCQAEDKG
ACLLPKIETMREKVLASSARQRLRCASIQKFGERALKAWSVARLSQKFPKAEFVEVTKLV
TDLTKVHKECCHGDLLECADDRADLAKYICDNQDTISSKLKECCDKPLLEKSHCIAEVEK
DAIPENLPPLTADFAEDKDVCKNYQEAKDAFLGSFLYEYSRRHPEYAVSVLLRLAKEYEA
TLEECCAKDDPHACYSTVFDKLKHLVDEPQNLIKQNCDQFEKLGEYGFQNALIVRYTRKV
PQVSTPTLVEVSRSLGKVGTRCCTKPESERMPCTEDYLSLILNRLCVLHEKTPVSEKVTK
CCTESLVNRRPCFSALTPDETYVPKAFDEKLFTFHADICTLPDTEKQIKKQTALVELLKH
KPKATEEQLKTVMENFVAFVDKCCAADDKEACFAVEGPKLVVSTQTALA
```

A sequence file in FASTA format can contain several sequences. With the **Header line,** beginning with '>', gives a name and/or unique identifier for the sequence, and other information as well

CLUSTAL W Format

```
CLUSTAL W (1.82) multiple sequence alignment


FOSB_MOUSE      MFQAFPGDYDSGSRCSSSPSAESQYLSSVDSFGSPPTAAASQECAGLGEMPGSFVPTVTA 60
FOSB_HUMAN      MFQAFPGDYDSGSRCSSSPSAESQYLSSVDSFGSPPTAAASQECAGLGEMPGSFVPTVTA 60
                ************************************************************

FOSB_MOUSE      ITTSQDLQWLVQPTLISSMAQSQGQPLASQPPAVDPYDMPGTSYSTPGLSAYSTGGASGS 120
FOSB_HUMAN      ITTSQDLQWLVQPTLISSMAQSQGQPLASQPPVVDPYDMPGTSYSTPGMSGYSSGGASGS 120
                ********************************.***************:*.**:****** 

FOSB_MOUSE      GGPSTSTTTSGPVSARPARARPRRPREETLTPEEEEKRRVRRERNKLAAAKCRNRRRELT 180
FOSB_HUMAN      GGPSTSGTTSGPGPARPARARPRRPREETLTPEEEEKRRVRRERNKLAAAKCRNRRRELT 180
                ****** ***** .********************************************** 

FOSB_MOUSE      DRLQAETDQLEEEKAELESEIAELQKEKERLEFVLVAHKPGCKIPYEEGPGPGPLAEVRD 240
FOSB_HUMAN      DRLQAETDQLEEEKAELESEIAELQKEKERLEFVLVAHKPGCKIPYEEGPGPGPLAEVRD 240
                ************************************************************

FOSB_MOUSE      LPGSTSAKEDGFGWLLPPPPPPPLPFQSSRDAPPNLTASLFTHSEVQVLGDPFPVVSPSY 300
FOSB_HUMAN      LPGSAPAKEDGFSWLLPPPPPPPLPFQTSQDAPPNLTASLFTHSEVQVLGDPFPVVNPSY 300
                ****:.******.**************:*:**************************.*** 

FOSB_MOUSE      TSSFVLTCPEVSAFAGAQRTSGSEQPSDPLNSPSLLAL 338
FOSB_HUMAN      TSSFVLTCPEVSAFAGAQRTSGSDQPSDPLNSPSLLAL 338
                ***********************:************** 
```

1. First line in the file must start with the words "CLUSTAL W". Other information in the first line is discarded
2. One or more empty lines
3. One or more blocks of sequence data. Each block consists of:
    * One line for each sequence in the alignment. Each line consists of:
        1. the sequence name
        2. white space
        3. up to 60 sequence symbols
        4. optional - white space followed by a cumulative count of residues for the sequences
    * A line showing the degree of conservation for the columns of the alignment in this block
    * One or more empty lines.

Phylip Format

```
 4 131
IXI_234    TSPASIRPPA GPSSRPAMVS SRRTRPSPPG PRRPTGRPCC SAAPRRPQAT
IXI_235    TSPASIRPPA GPSSR----- ----RPSPPG PRRPTGRPCC SAAPRRPQAT
IXI_236    TSPASIRPPA GPSSRPAMVS SR--RPSPPP PRRPPGRPCC SAAPPRPQAT
IXI_237    TSPASLRPPA GPSSRPAMVS SRR-RPSPPG PRRPT----C SAAPRRPQAT

           GGWKTCSGTC TTSTSTRHRG RSGWSARTTT AACLRASRKS MRAACSRSAG
           GGWKTCSGTC TTSTSTRHRG RSGW------ ----RASRKS MRAACSRSAG
           GGWKTCSGTC TTSTSTRHRG RSGWSARTTT AACLRASRKS MRAACSR--G
           GGYKTCSGTC TTSTSTRHRG RSGYSARTTT AACLRASRKS MRAACSR--G

           SRPNRFAPTL MSSCITSTTG PPAWAGDRSH E
           SRPNRFAPTL MSSCITSTTG PPAWAGDRSH E
           SRPPRFAPPL MSSCITSTTG PPPPAGDRSH E
           SRPNRFAPTL MSSCLTSTTG PPAYAGDRSH E
```

The PHYLIP format came from Joe Felsenstein's phylogeny inference package and is now used by several phylogenetics programs. PHYLIP file names often have have a .phy or .ph extension.

PIR Format

```
>P1; Cow
Cow
MAYPMQLGFQDATSPIMEELLHFHDHTLMIVFLISSLVLYIISLMLTTKLTHTSTMDAQEVETIWTILPAIILILI
ALPSLRILYMMDEINNPSLTVKTMGHQWYWSYEYTDYEDLSFDSYMIPTSELKPGELRLLEVDNRVVLPMEMTIRM
LVSSEDVLHSWAVPSLGLKTDAIPGRLNQTTLMSSRPGLYYGQCSEICGSNHSFMPIVLELVPLKYFEKWSASML-
------*

>P1; Carp
Carp
MAHPTQLGFKDAAMPVMEELLHFHDHALMIVLLISTLVLYIITAMVSTKLTNKYILDSQEIEIVWTILPAVILVLI
ALPSLRILYLMDEINDPHLTIKAMGHQWYWSYEYTDYENLGFDSYMVPTQDLAPGQFRLLETDHRMVVPMESPVRV
LVSAEDVLHSWAVPSLGVKMDAVPGRLNQAAFIASRPGVFYGQCSEICGANHSFMPIVVEAVPLEHFENWSSLMLE
DASLGS*

>P1; Chicken
Chicken
MANHSQLGFQDASSPIMEELVEFHDHALMVALAICSLVLYLLTLMLMEKLS-SNTVDAQEVELIWTILPAIVLVLL
ALPSLQILYMMDEIDEPDLTLKAIGHQWYWTYEYTDFKDLSFDSYMTPTTDLPLGHFRLLEVDHRIVIPMESPIRV
IITADDVLHSWAVPALGVKTDAIPGRLNQTSFITTRPGVFYGQCSEICGANHSYMPIVVESTPLKHFEAWSSL---
---LSS*
```

Protein Information Resource (PIR) is an annotated, non-redundant and cross-referenced database of protein sequences at the NBRF. PIR file names often have a .pir extension.

The header line of a nucleotide sequence file in pir-format begins with a greater than sign ">" followed by DL.
The header line of protein sequence file in pir-format begins with a greater than sign ">" followed by P1.

EMBL Format

```
ID   AB000263 standard; RNA; PRI; 368 BP.
XX
AC   AB000263;
XX
DE   Homo sapiens mRNA for prepro cortistatin like peptide, complete cds.
XX
SQ   Sequence 368 BP;
     acaagatgcc attgtccccc ggcctcctgc tgctgctgct ctccggggcc acggccaccg        60
     ctgccctgcc cctggagggt ggccccaccg gccgagacag cgagcatatg caggaagcgg       120
     caggaataag gaaaagcagc ctcctgactt tcctcgcttg gtggtttgag tggacctccc       180
     aggccagtgc cgggcccctc ataggagagg aagctcggga ggtggccagg cggcaggaag       240
     gcgcaccccc ccagcaatcc gcgcgccggg acagaatgcc ctgcaggaac ttcttctgga       300
     agaccttctc ctcctgcaaa taaaacctca cccatgaatg ctcacgcaag tttaattaca       360
     gacctgaa                                                               368
//
```

A sequence file in EMBL format can contain several sequences.
One sequence entry starts with an identifier line ("ID"), followed by further annotation lines. The start of the sequence is marked by a line starting with "SQ" and the end of the sequence is marked by two slashes ("//").

The EMBL flat format is a rich format for storing sequences and their associated meta-information, feature coordinates, and annotations. It shares details with the GenBank sequence format.

```
#NEXUS
[TITLE: NoName]

begin data;
dimensions ntax=3 nchar=384;
format interleave datatype=protein   gap=- symbols="FSTNKEYVQMCLAWPHDRIG";

matrix
CYS1_DICDI          -----MKVIL LFVLAVFTVF VSS------- --------RG IPPEEQ----
ALEU_HORVU          MAHARVLLLA LAVLATAAVA VASSSSFADS NPIRPVTDRA ASTLESAVLG
CATH_HUMAN          ------MWAT LPLLCAGAWL LGV------- -PVCGAAELS VNSLEK----


CYS1_DICDI          --------SQ FLEFQDKFNK KY-SHEEYLE RFEIFKSNLG KIEELNLIAI
ALEU_HORVU          ALGRTRHALR FARFAVRYGK SYESAAEVRR RFRIFSESLE EVRSTN----
CATH_HUMAN          --------FH FKSWMSKHRK TY-STEEYHH RLQTFASNWR KINAHN----


CYS1_DICDI          NHKADTKFGV NKFADLSSDE FKNYYLNNKE AIFTDDLPVA DYLDDEFINS
ALEU_HORVU          RKGLPYRLGI NRFSDMSWEE FQATRL-GAA QTCSATLAGN HLMRDA--AA
CATH_HUMAN          NGNHTFKMAL NQFSDMSFAE IKHKYLWSEP QNCSAT--KS NYLRGT--GP


CYS1_DICDI          IPTAFDWRTR G-AVTPVKNQ GQCGSCWSFS TTGNVEGQHF ISQNKLVSLS
ALEU_HORVU          LPETKDWRED G-IVSPVKNQ AHCGSCWTFS TTGALEAAYT QATGKNISLS
CATH_HUMAN          YPPSVDWRKK GNFVSPVKNQ GACGSCWTFS TTGALESAIA IATGKMLSLA


CYS1_DICDI          EQNLVDCDHE CMEYEGEEAC DEGCNGGLQP NAYNYIIKNG GIQTESSYPY
ALEU_HORVU          EQQLVDCAGG FNNF------ --GCNGGLPS QAFEYIKYNG GIDTEESYPY
CATH_HUMAN          EQQLVDCAQD FNNY------ --GCQGGLPS QAFEYILYNK GIMGEDTYPY


CYS1_DICDI          TAETGTQCNF NSANIGAKIS NFTMIP-KNE TVMAGYIVST GPLAIAADAV
ALEU_HORVU          KGVNGV-CHY KAENAAVQVL DSVNITLNAE DELKNAVGLV RPVSVAFQVI
CATH_HUMAN          QGKDGY-CKF QPGKAIGFVK DVANITIYDE EAMVEAVALY NPVSFAFEVT


CYS1_DICDI          E-WQFYIGGV F-DIPCN--P NSLDHGILIV GYSAKNTIFR KNMPYWIVKN
ALEU_HORVU          DGFRQYKSGV YTSDHCGTTP DDVNHAVLAV GYGVENGV-- ---PYWLIKN
CATH_HUMAN          QDFMMYRTGI YSSTSCHKTP DKVNHAVLAV GYGEKNGI-- ---PYWIVKN


CYS1_DICDI          SWGADWGEQG YIYLRRGKNT CGVSNFVSTS II--
ALEU_HORVU          SWGADWGDNG YFKMEMGKNM CAIATCASYP VVAA
CATH_HUMAN          SWGPQWGMNG YFLIERGKNM CGLAACASYP IPLV
```

Nexus Format

This is the file format used by many popular programs like GARLI, GDA, MacClade, Mesquite, ModelTest, MrBayes and PAUP*. Nexus file names often have a .nxs or .nex extension

GFF Format

- Latest version GFF3
- It's a tabular plain-text format for genome or sequence annotation
- Can contain also the sequences, alignments, and dependencies between features
- Is extensible
- One of the most recommended & "standard" format

```
##gff-version 3
ctg123 . operon          1300 15000  .  +  .  ID=operon001;Name=superOperon
ctg123 . mRNA            1300  9000  .  +  .  ID=mrna0001;Parent=operon001;Name=sonichedgehog
ctg123 . exon            1300  1500  .  +  .  Parent=mrna0001
ctg123 . exon            1050  1500  .  +  .  Parent=mrna0001
ctg123 . exon            3000  3902  .  +  .  Parent=mrna0001
ctg123 . exon            5000  5500  .  +  .  Parent=mrna0001
ctg123 . exon            7000  9000  .  +  .  Parent=mrna0001
ctg123 . mRNA           10000 15000  .  +  .  ID=mrna0002;Parent=operon001;Name=subsonicsquirrel
ctg123 . exon           10000 12000  .  +  .  Parent=mrna0002
ctg123 . exon           14000 15000  .  +  .  Parent=mrna0002
```

FASTQ

- FASTQ – FASTA "with an attitude" (embedded quality scores)
- Originally developed at the Sanger to couple (Phred) quality data with sequence
- Now common to specify raw read output data from NGS machines in this format
- Various flavors:
  - fastq-sanger
  - fastq-illumina
  - fastq-solexa

```
@NCYC361-11a03.q1k bases 1 to 1576
GCGTGCCCGAAAAAATGCTTTTGGAGCCGCGCGTGAAAT...
+NCYC361-11a03.q1k bases 1 to 1576
!)))))****(((***%%((((*(((+,**(((+**+,-...
```

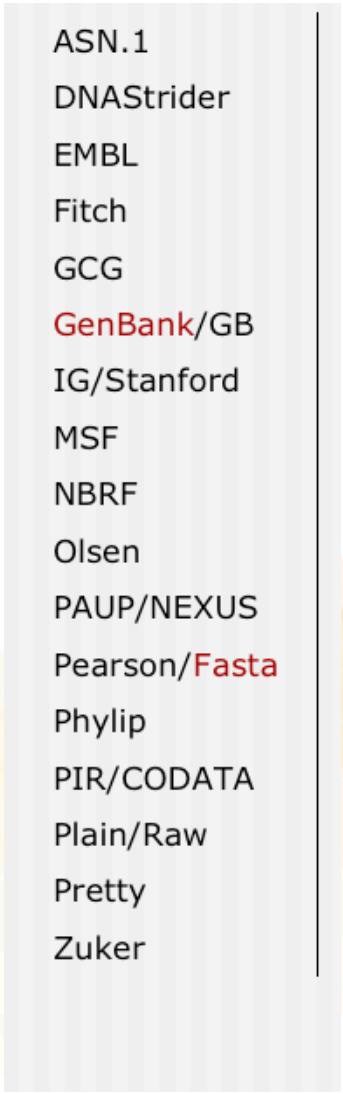
- "…the Sanger version of the FASTQ format has found the broadest acceptance, supported by many assembly and read mapping tools …Therefore, most users will do this conversion very early in their workflows…"

http://en.wikipedia.org/wiki/FASTQ_format
http://maq.sourceforge.net/fastq.shtml
http://nar.oxfordjournals.org/content/early/2009/12/16/nar.gkp1137.full

VCF (Variant Call Format) Files

```
##fileformat=VCFv4.0
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=1000GenomesPilot-NCBI36
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=.,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS     ID        REF ALT    QUAL FILTER INFO                              FORMAT      NA00001
20     14370   rs6054257 G   A       29   PASS   NS=3;DP=14;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1
20     17330   .         T   A       3    q10    NS=3;DP=11;AF=0.017               GT:GQ:DP:HQ 0|0:49:3
20     1110696 rs6040355 A   G,T     67   PASS   NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6
20     1230237 .         T   .       47   PASS   NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7
20     1234567 microsat1 GTCT G,GTACT 50   PASS   NS=3;DP=9;AA=G                    GT:GQ:DP    0/1:35:4
```

FYI - Sequence Formats



- Convert sequence formats
  - ReadSeq Web based
    - http://www.ebi.ac.uk/cgi-bin/readseq.cgi
  - SeqVerter (stand alone app Windows)
    - http://www.genestudio.com/seqverter.htm
- NOTE:
  - FASTA is a popular sequence format
  - It is also a sequence similarity tool used by EMBL-EBI and NCBI

Now we know where to get sequence data, and the formats, so what's next?

5 minutes

Introduction to Sequence Analysis and Alignment Methods

Pairwise Sequence Alignment Methods

- Primary methods of producing pairwise alignments are:
  - Manual & Dot plot
  - Automated Methods
    - Dynamic programming
    - Word Methods
      - FASTA
      - BLAST

Why Align Sequences?

- Many sequences:
  - Have no known ancestry, structure, or function
  - Some of them do
- If they align (with meaning), they are similar
  - If they are similar:
    - They might have the same:
      - ancestry
      - similar structure
      - function
    - **If one of them has known ancestry, structure, or function, then alignment to the others yields insight about them**

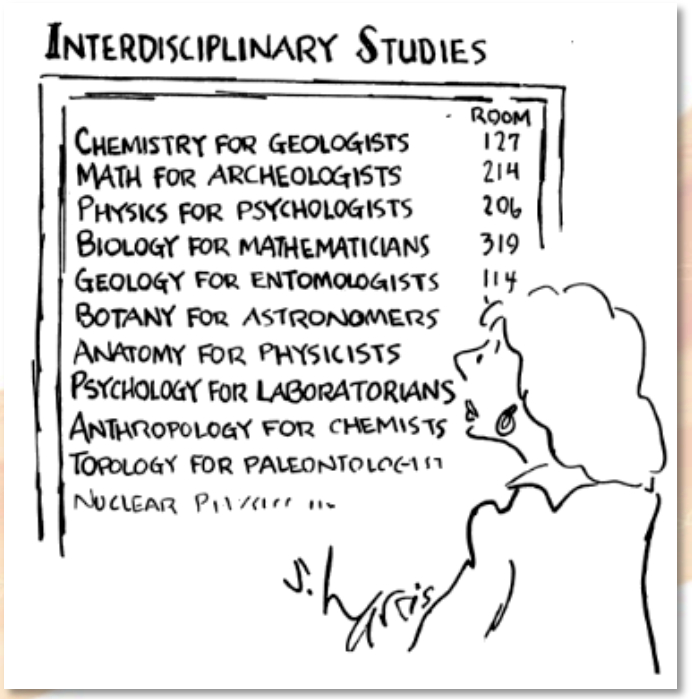
Sequence Alignment

- In bioinformatics
  - A Sequence alignment - way of arranging the sequences of DNA, RNA, or protein to identify regions of similarity
  - Similarities may be a consequence of:
    - functional
    - structural
    - or evolutionary relationships between sequence
      - molecular evolution

```
AAB24882        TYHMCQFHCRYVNNHSGEKLYECNERSKAFSCPSHLQCHKRRQIGEKTHEHNQCGKAFPT 60
AAB24881        --------------------YECNQCGKAFAQHSSLKCHYRTHIGEKPYECNQCGKAFSK 40
                                    ****: .***:  * *:** * :****.:* ********..

AAB24882        PSHLQYHERTHTGEKPYECHQCGQAFKKCSLLQRHKRTHTGEKPYE-CNQCGKAFAQ- 116
AAB24881        HSHLQCHKRTHTGEKPYECNQCGKAFSQHGLLQRHKRTHTGEKPYMNVINMVKPLHNS 98
                 **** *:***********:***:**.: .****************    :  *.: :
```

Molecular Evolution is an Interdisciplinary Endeavor

- **The discipline of Molecular Evolution deals only with events occurring after the emergence of biological systems that possess replicable genetic material**
- **Deals with two subjects**
  - The evolution of:
    - molecular entities, e.g., genes, genomes, proteins, introns, chromosomal arrangements
    - organisms and biological complexes as deduced from molecular data
- How is this done?
- Must perform Sequence Analysis



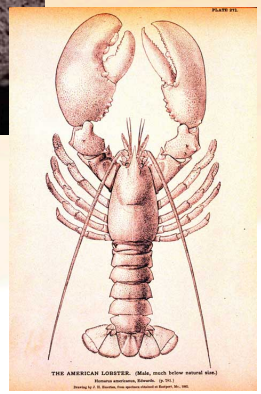
Adopted from Dan Graur, Ph.D.

Sequence Alignment

- One of the most common bioinformatics tasks
  - We will comprehensively learn about:
    - The different methods
    - Why we use BLAST and not dynamic programming for large datasets
    - How a simple dot plot provides information
    - Other things as well
- Remains **large area of research**
  - One of the major topics in bioinformatics
  - Present in almost every R&D activity:
    - Across the many industries in the area of life sciences
      - » Academia, biotech, services, software, pharma, and hospitals

End Results

- Analysis of **organismal** diversity/similarity




```
ARMADILLIDIUM   RIFDTSCKGFYDRGLFAQLDRVCEDCYNLYRKPH--
CARCINUS        QIYDTSCKGVYDRALF-DLEHVCDDCYNLYRTSYVA
HOMARUS         QVFDQACKGVYDRNLFKKLDRVCEDCYNLYRKPFVA
                ...* .*** *** **  *..**.******* .

ARMADILLIDIUM   AECRRDCYTTEVFESCLKDLMMHDFINEYKEMALMV
CARCINUS        SACRSNCYSNLVFRQCMDDLLMMDEFDQYARKVQMV
HOMARUS         TTCRENCYSNWVFRQCLDDLLL-NVIDEYVSNVQM-
                . ** .**.  **  *. **.. . ...*     *
```

images from Dan Graur, Ph.D.

Sequence Similarity Searching

- Availability of increasingly expanding databases poses a major challenge to bioinformatics experts:
  - Must develop effective programs/Web servers to extract maximum information from these databases
  - Possibly the fastest, cheapest, and most powerful experiment a biologist can conduct
- As databases become more *complete?*
  - Searches more likely to reveal database sequences with statistically significant similarity
  - Thus inferred **homology** to a query sequence

Homology: General Definition

- Homology designates a qualitative relationship of common descent between entities
- Two genes are either **homologous** or they are **not**!
  - it doesn't make sense to say:
    - "two genes are 43% homologous"
    - "Linda is 43% pregnant"

Homology Terms

- Two genes are **orthologs**
  - Originated from a single ancestral gene in the most recent common ancestor of their respective genomes
- Two genes are **paralogs**
  - Related by gene duplication
  - Two genes are **ohnologs** if they are related by gene duplication due to whole-genome duplication (WGD)
- **Xenologs**
  - Homologs resulting from horizontal gene transfer between two organisms are termed

- When comparing sequences, we are interested in positional homology,
  - We identify positional homology through?
    - sequence alignment

Interpretation

- If two sequences share a common ancestor:
  - Mismatches can be interpreted as point mutations
  - Gaps as indels
  - These have been introduced in one or both lineages since they diverged from one another
- In proteins:
  - The degree of similarity between a.a. occupying a particular position in the sequence can be interpreted as:
    - A rough measure of how conserved a particular region or sequence motif is among lineages
    - Absence of substitutions (or conserved substitutions) suggests this region has structural or functional importance

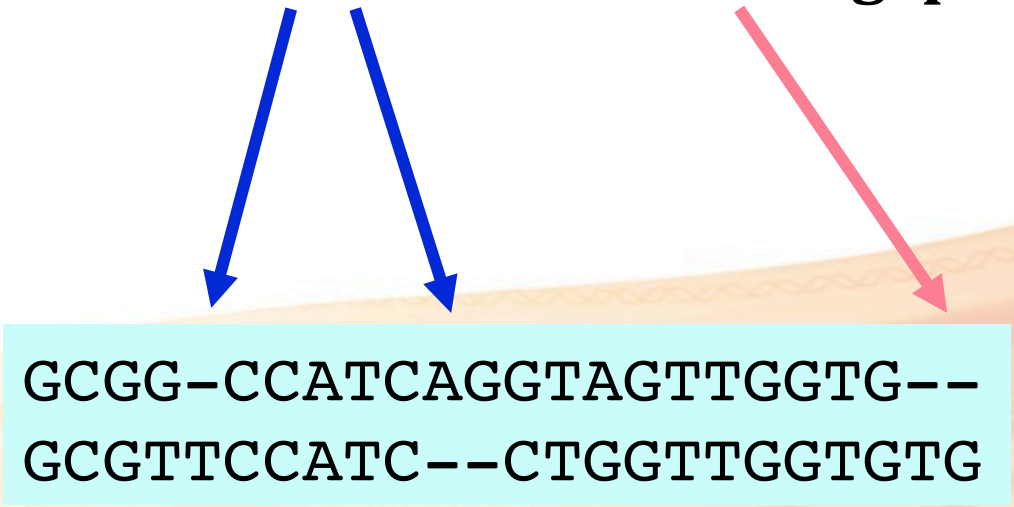
An Alignment Consists of a Series of Paired Bases, One Base from Each Sequence

- There are three types of pairs:

  (**1**) **matches** = the same nucleotide appears in both sequences
  (**2**) **mismatches** = different nucleotides are found in the two sequences.
  (**3**) **gaps** = a base in one sequence and a **null base** in the other

```
GCGGCCCATCAGGTAGTTGGTG-G
GCGTTCCATC--CTGGTTGGTGTG
***..*****   .*.******* *
```

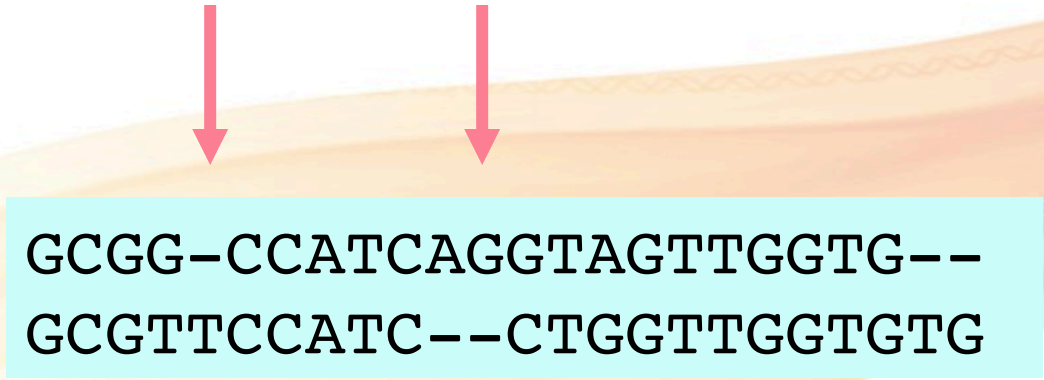
There are **internal** and **terminal** **gaps**



A terminal gap may indicate missing data

```
GCGG-CCATCAGGTAGTTGGTG--
GCGTTCCATC--CTGGTTGGTGTG
```

An internal gap indicates that a deletion or an insertion has occurred in one of the two lineages



A Word of Caution

- The alignment is the first step in many evolutionary and functional studies
- Errors in alignments tend to be amplified in later computational stages
  - Analyze the output from a method
    - Garbage in -> Garbage out
    - Does output seem correct?
    - Save time by doing this!

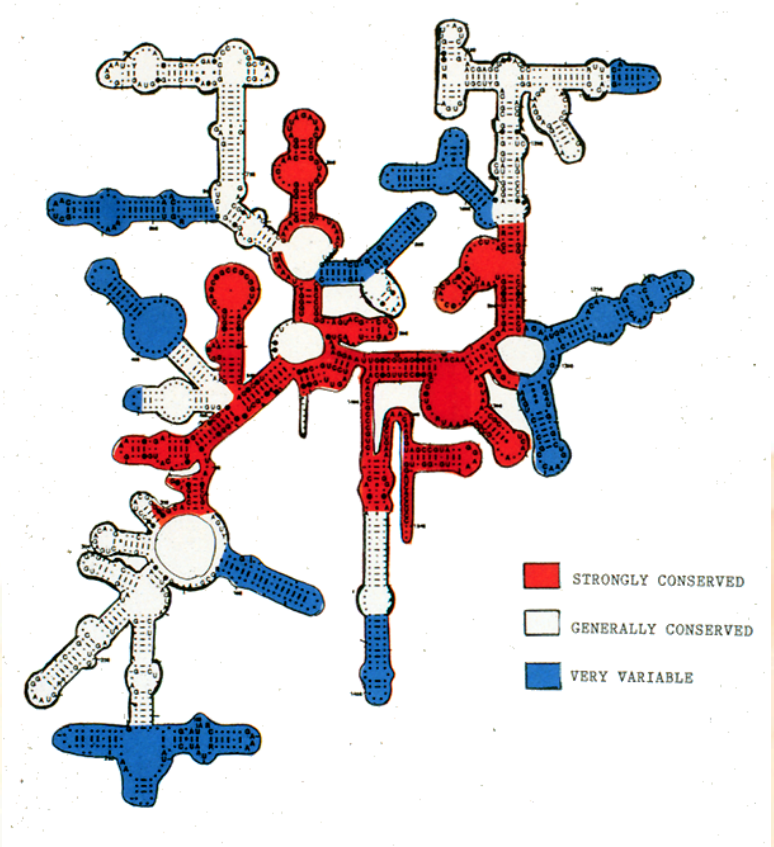
Manual Alignment

- When there are few gaps
- When two sequences are not too different from each other
- A reasonable alignment can be obtained by visual inspection

```
GCG-TCCATCAGGTAGTTGGTGTG
GCGTTCCATCAGGTGGTTGGTGTG
***  **********.*********
```

Advantages of Manual Alignments

- Using a powerful and trainable tool, your brain!
- Ability to integrate additional data:
  - **Domain structures**
  - **Biological function**
  - **Your knowledge**
- But…..
  - Too much to ask the brain to compare large, diverged sequences
  - The method is subjective and unscalable!



- Manually made ribosomal dna alignment – 2nd structure

- We can also visually inspect (manually) a Dot Plot

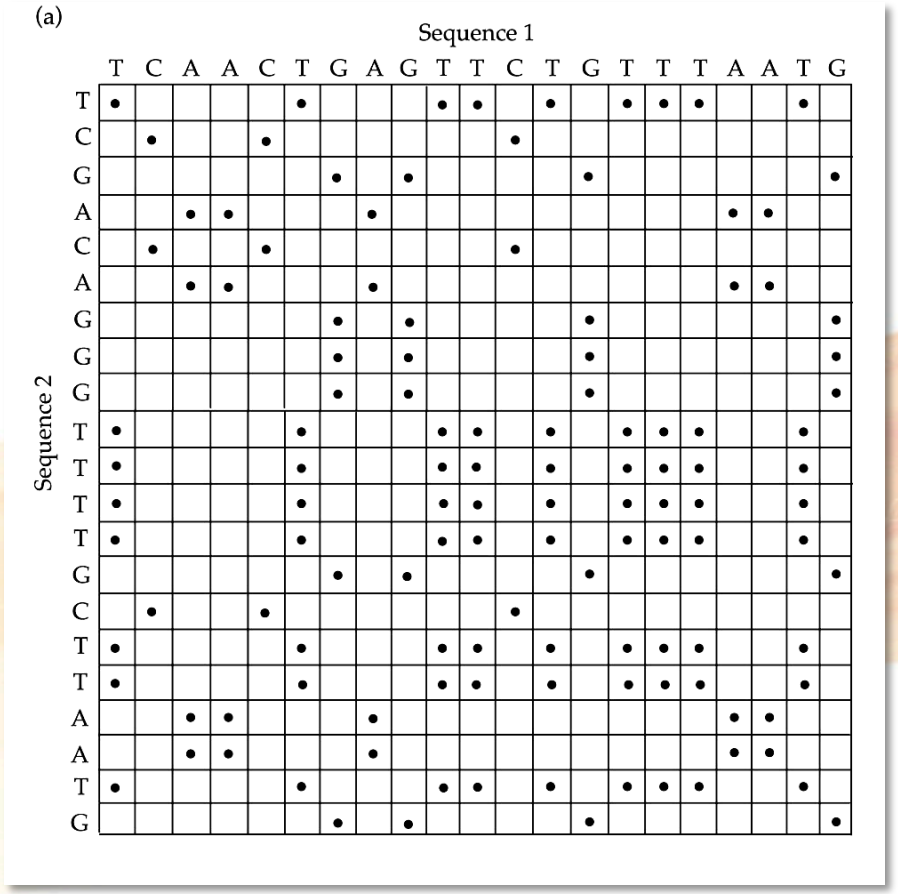
images from Dan Graur, Ph.D.

Dot Plots

Dot Plot

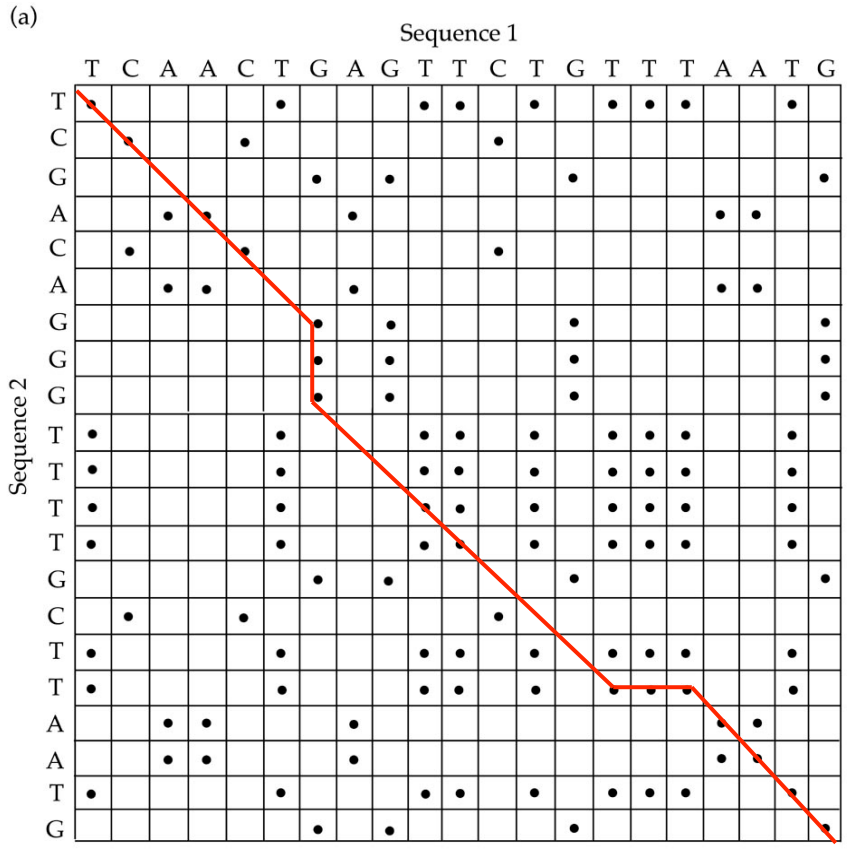
- Also known as a Dot Matrix
- Contact plot or Residue contact map
- Dot plots are a simple way of seeing alignments
  - We really like to see good visual demonstrations, not just tables of numbers
  - It's a grid: put one sequence along the top and the other down the side, and put a dot wherever they match
- Locates regions of similarity between two DNA or protein sequences
- Implicitly produces a family of alignments for individual regions
  - Its both qualitative and simple
  - Visualize recombinatorial events
  - But time consuming to analyze on a large scale

Dot Plot



Gibbs and McIntyre, 1970

Dot Plot

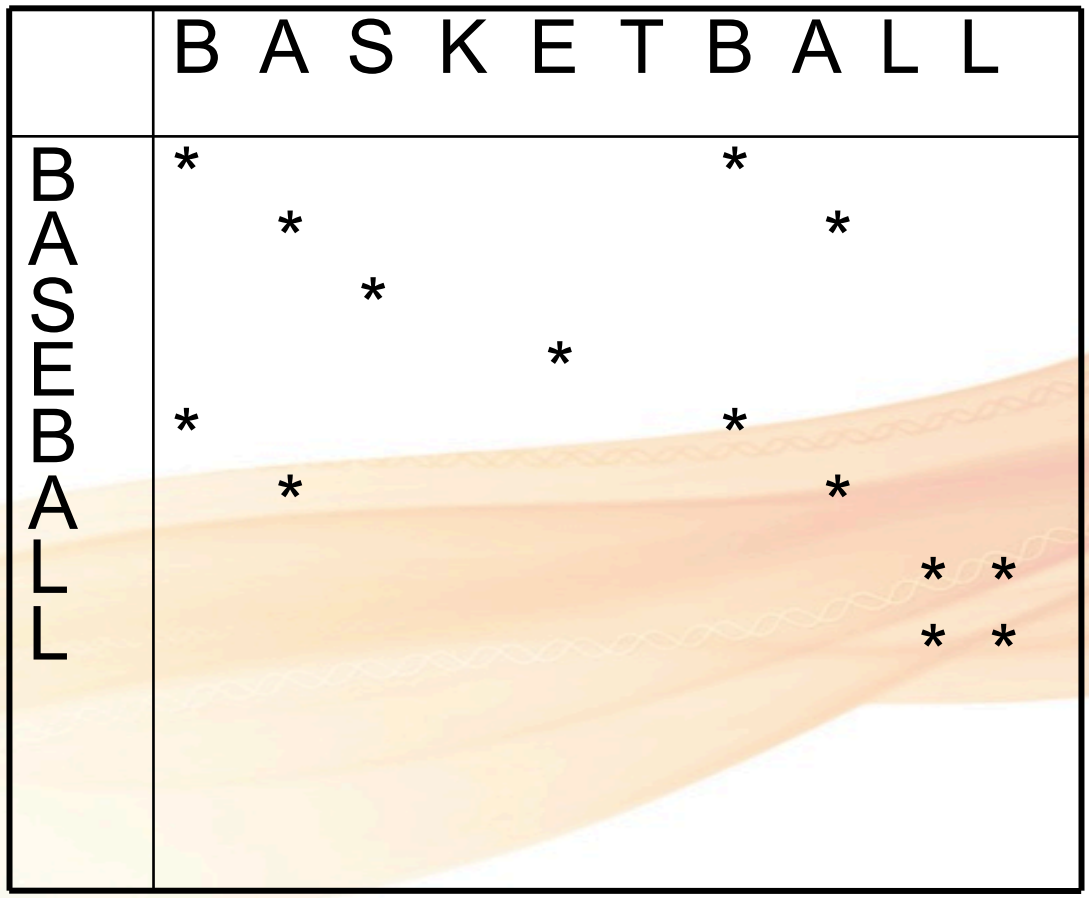


Gibbs and McIntyre, 1970

How to Generate a Dot Plot

- A Dot Plot is created by:
  - Designating one sequence to be the subject
    - Place it on the horizontal axis
  - Designate the second sequence to be the query
    - Place on the vertical axis of the matrix
- Each position within the matrix
  - A point is plotted if the horizontal and vertical elements are identical
  - Diagonal lines within the resulting matrix indicate regions of similarity

A Rather Simple Dot Plot



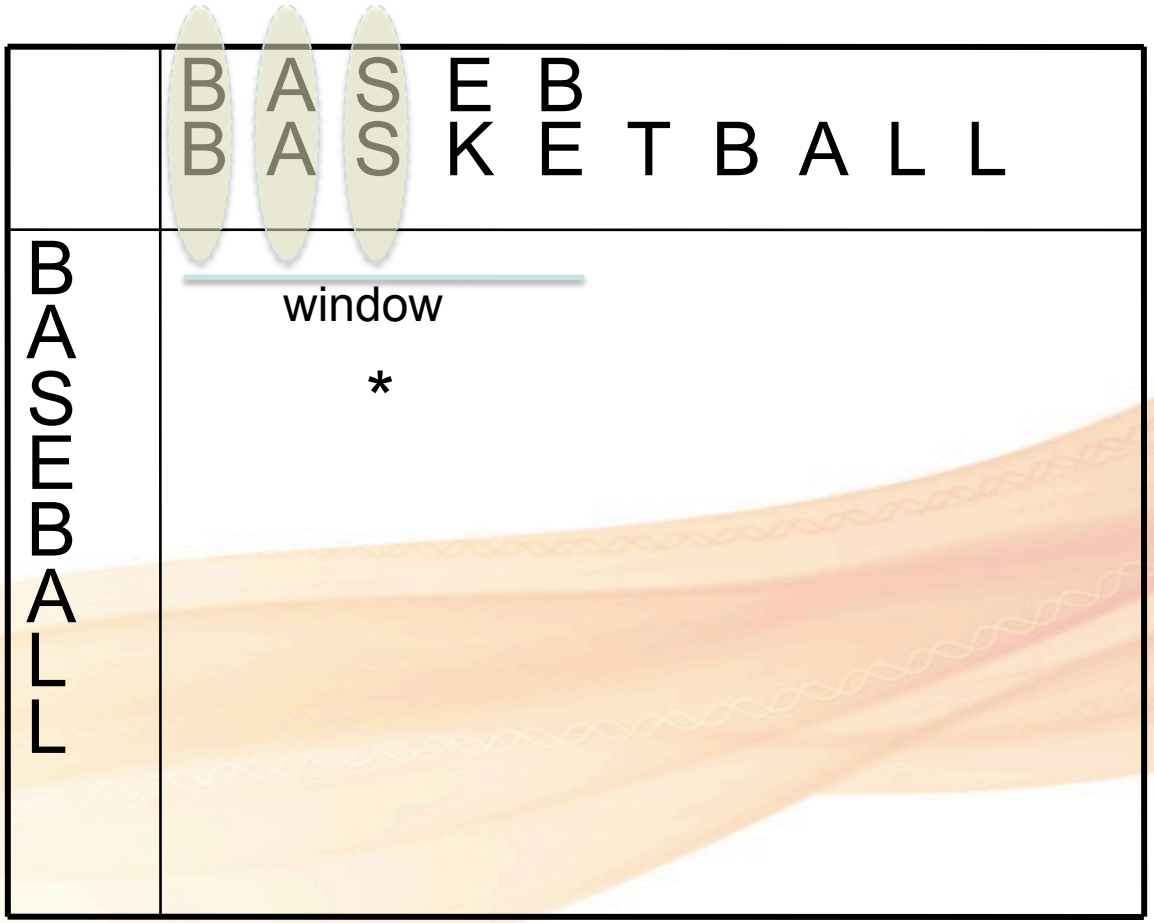
Dot Plot Visualization

- Using a Dot Plot, plot it's easy to visualize:
  - Insertions
  - Deletions
  - Repeats or Inverted Repeats
  - Dot plot of very closely related sequences will appear as a single line along the matrix's main diagonal
- Dot plots can also be used to assess repetitiveness in a single sequence?
  - Sequence plotted against itself
    - Regions sharing significant similarity will appears as lines off the main diagonal
    - Occur in proteins which consists of multiple similar structural domains

Dot Plot with Noise Reduction

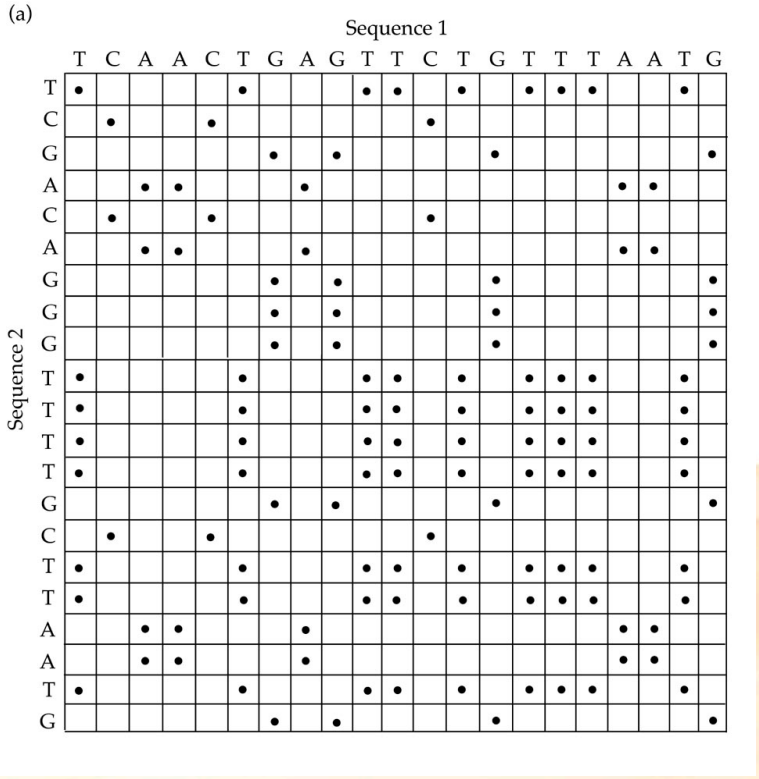
- A certain percentage of the matches between sequence elements can be expected to be random?
  - Random matches are considered “noise”
  - Need to be filtered out to enhance the diagonal lines “signal”
- How to reduce noise in a Dot Plot:
  - Center a substring of elements of the query sequence over each element in the subject sequence
  - Determining the number of corresponding elements within this “window”
    - If the number of corresponding elements >= a specified (“stringency”) threshold then a point is plotted for the center element

Dot Plot w/ Simple Example Filtering

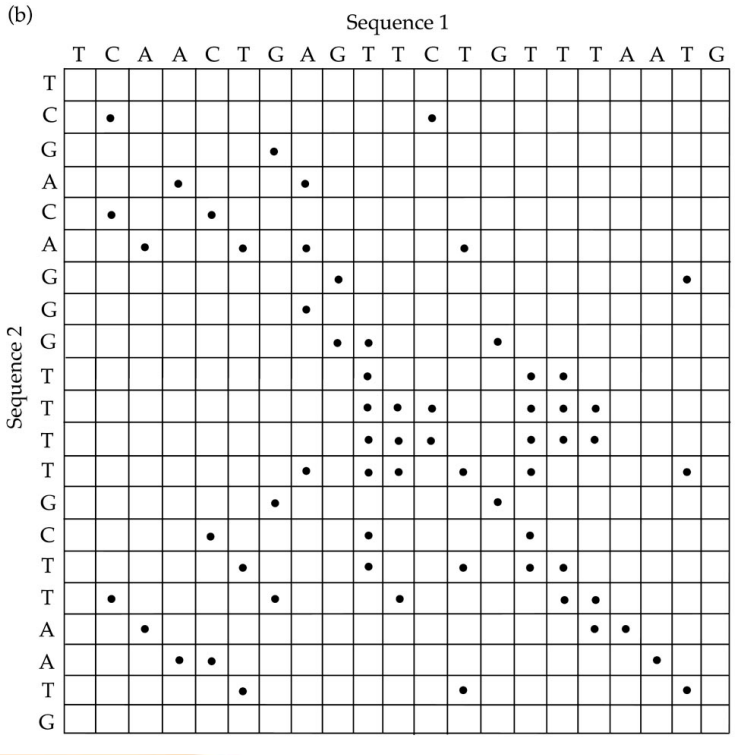


window size = 5
stringency = 3

Dot Plot w/ Actual Filtering



window size = 1
stringency = 1



window size = 3
stringency = 2

Dot Plot Advantages

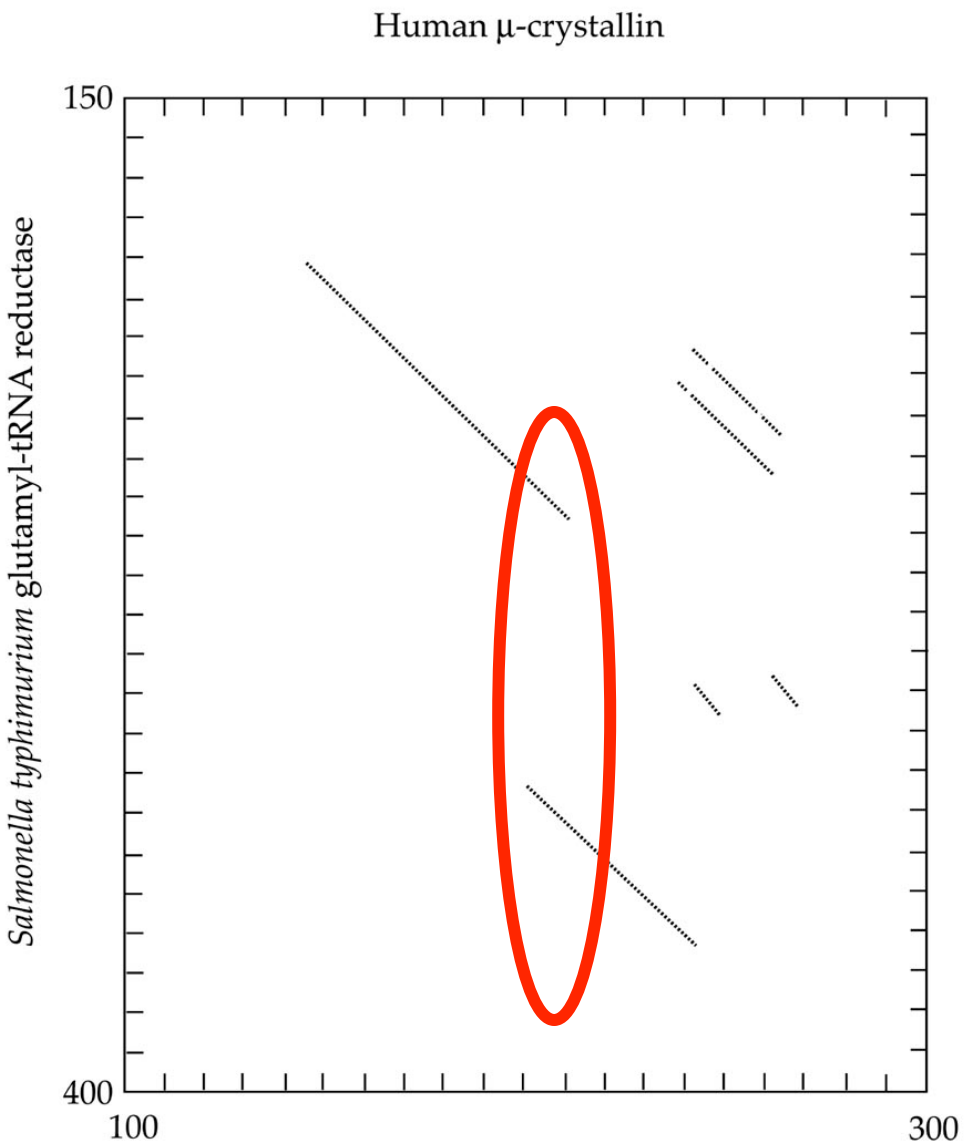
- Although simple to understand, and quite primitive in complexity
  - Dot Plot is extremely powerful
  - Visualization aspect may unravel information regarding the evolution of sequences
    - Highlighting this can be of great importance to a molecular biologist
    - Remember, the core of Bioinformatics is all about visualization!
  - Still used in current literature
- Useful for complete genome comparison b/t microbes
  - Since all possible matches of residues b/t two sequences are found
  - The investigator left with choice of identifying the most significant ones by examinations of long diagonals

- Logic led to FASTA algorithm (as we'll see)

Advantages: Highlighting Information

The vertical gap indicates that a coding region corresponding to ~75 amino acids has either been deleted from the human gene or inserted into the bacterial gene, which one is it?

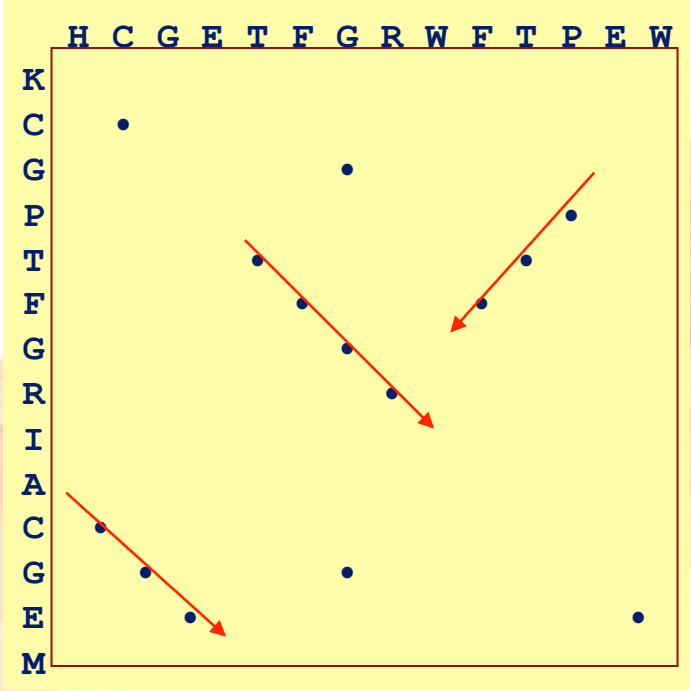
From a Dot Plot you really can't answer this question, ok why?



Window size = 60 amino acids; Stringency = 24 matches

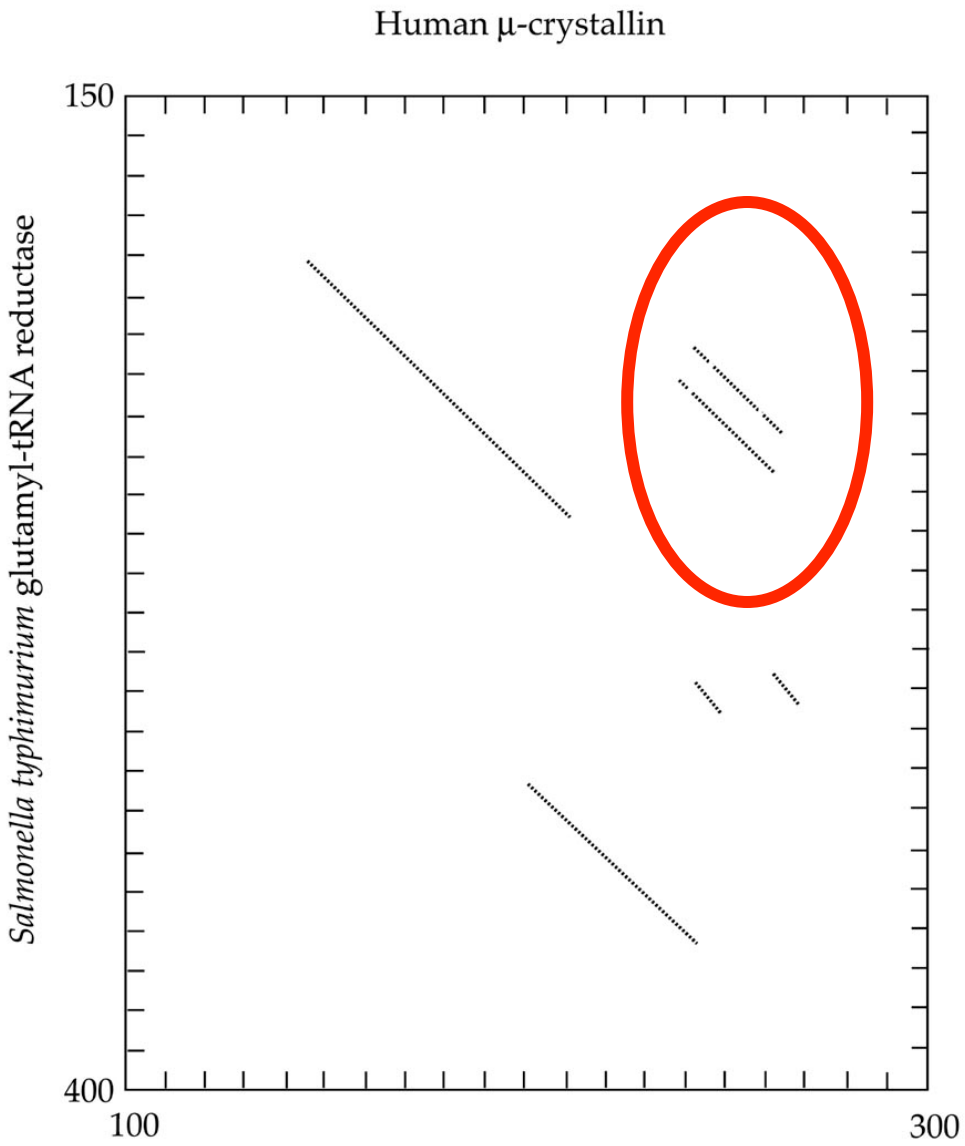
Dot Plot Display

- Diagonal rows (↘) of dots reveal sequence similarity or repeats
- Anti-diagonal rows (↙) of dots represent inverted repeats
- Isolated dots represent random similarity



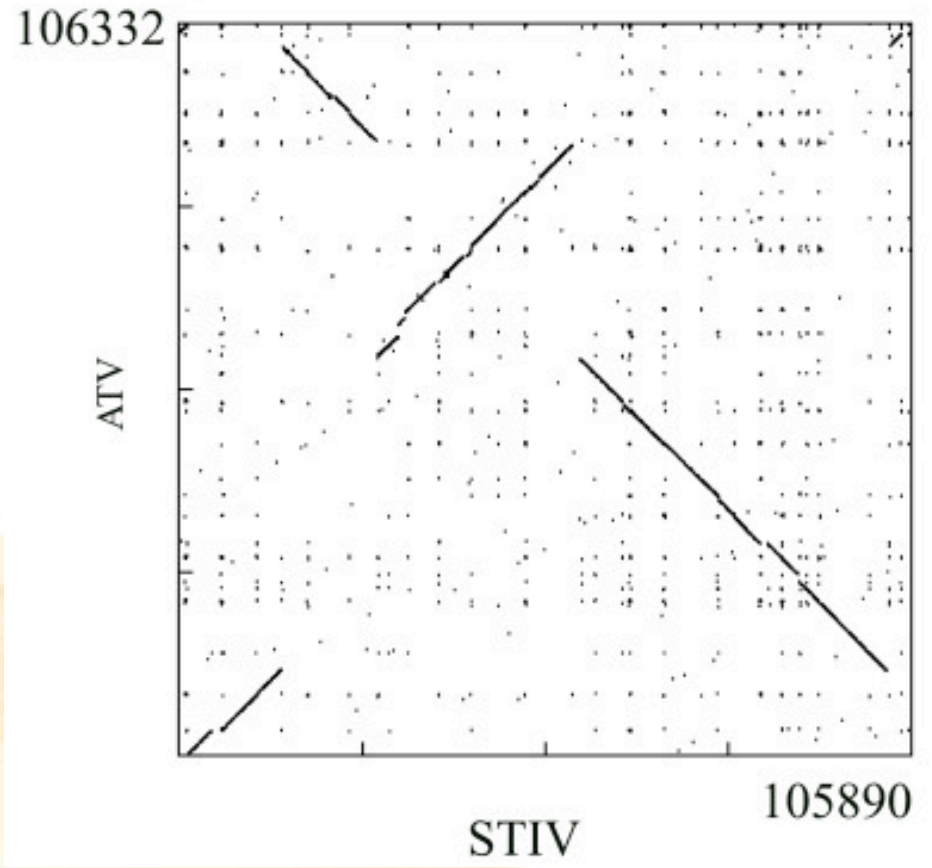
Advantages: Highlighting Information

The two diagonally oriented parallel lines most probably indicate that a small internal duplication has occurred in the bacterial gene



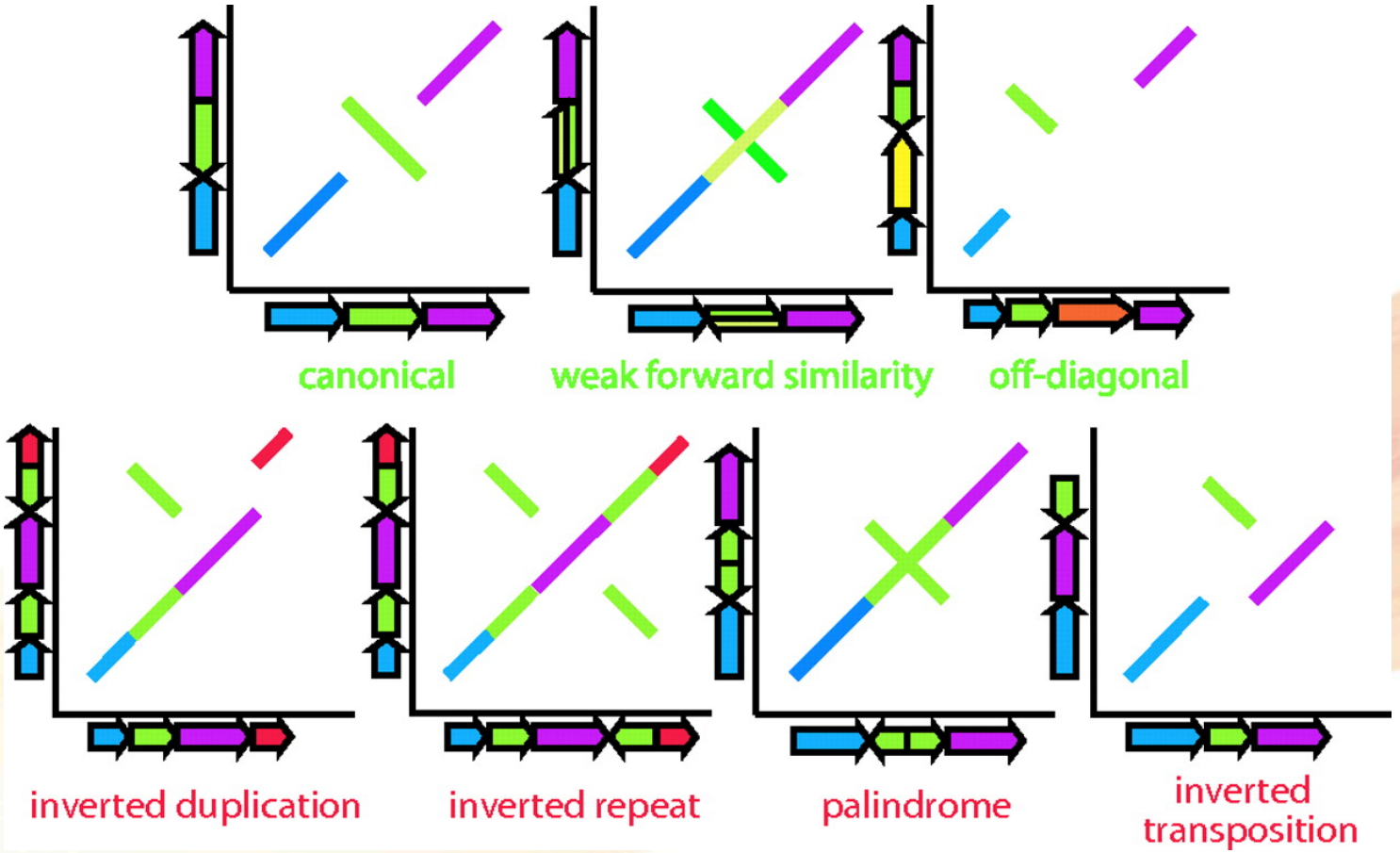
Window size = 60 amino acids; Stringency = 24 matches

Dot Plot with Inversions and Rearrangements



Huang et al. BMC Genomics 2009 10:224

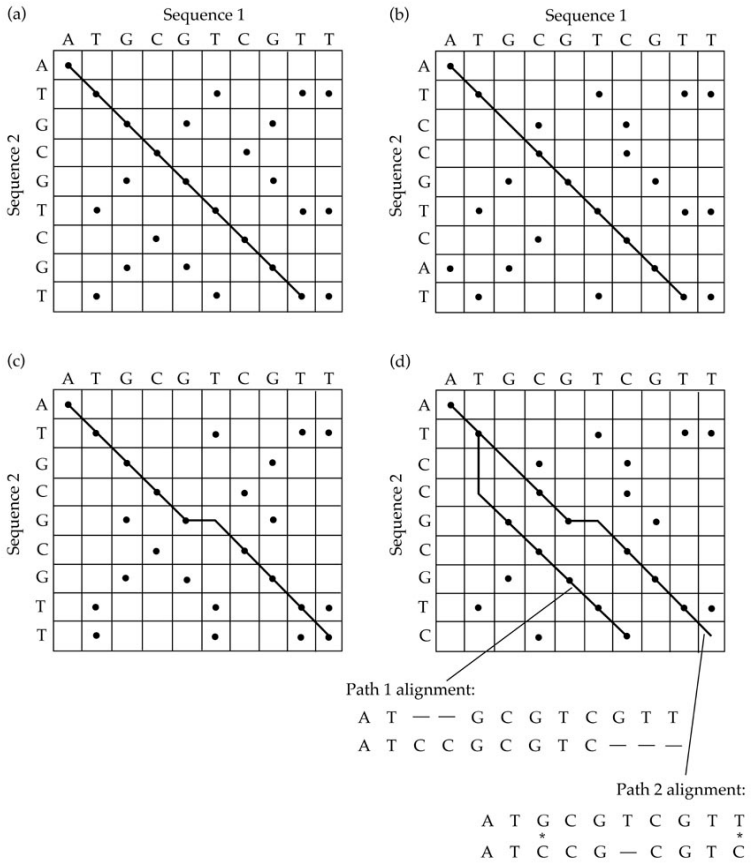
FYI - More Complex Situations in Dot Plot



Chaisson M J et al. PNAS 2006;103:19824-19829

Dot Plot Disadvantage:

- Relies on visual analysis
- Difficult to find optimal alignments
- Difficult to estimate significance of alignments
- Insensitive to conserved substitutions (e.g. L ↔ I or S ↔T) if no **substitution matrix** applied
- Compares only two sequences (vs. multiple alignment)
- Time consuming
  - 1,000 bp vs. 1,000 bp = 10^6 operations
  - 1,000,000 vs. 1,000,000 bp = 10^{12} operations



Advantages of Manual Alignments

- Using a powerful and trainable tool, your brain!
- Ability to integrate additional data:
  - **Domain structures**
  - **Biological function**
  - **Your knowledge**
- **But…..**
  - **Too much to ask the brain to compare large, diverged sequences**
  - **The method is subjective, irreproducible, and unscalable!**

Alignment Methods - Computational Complexity

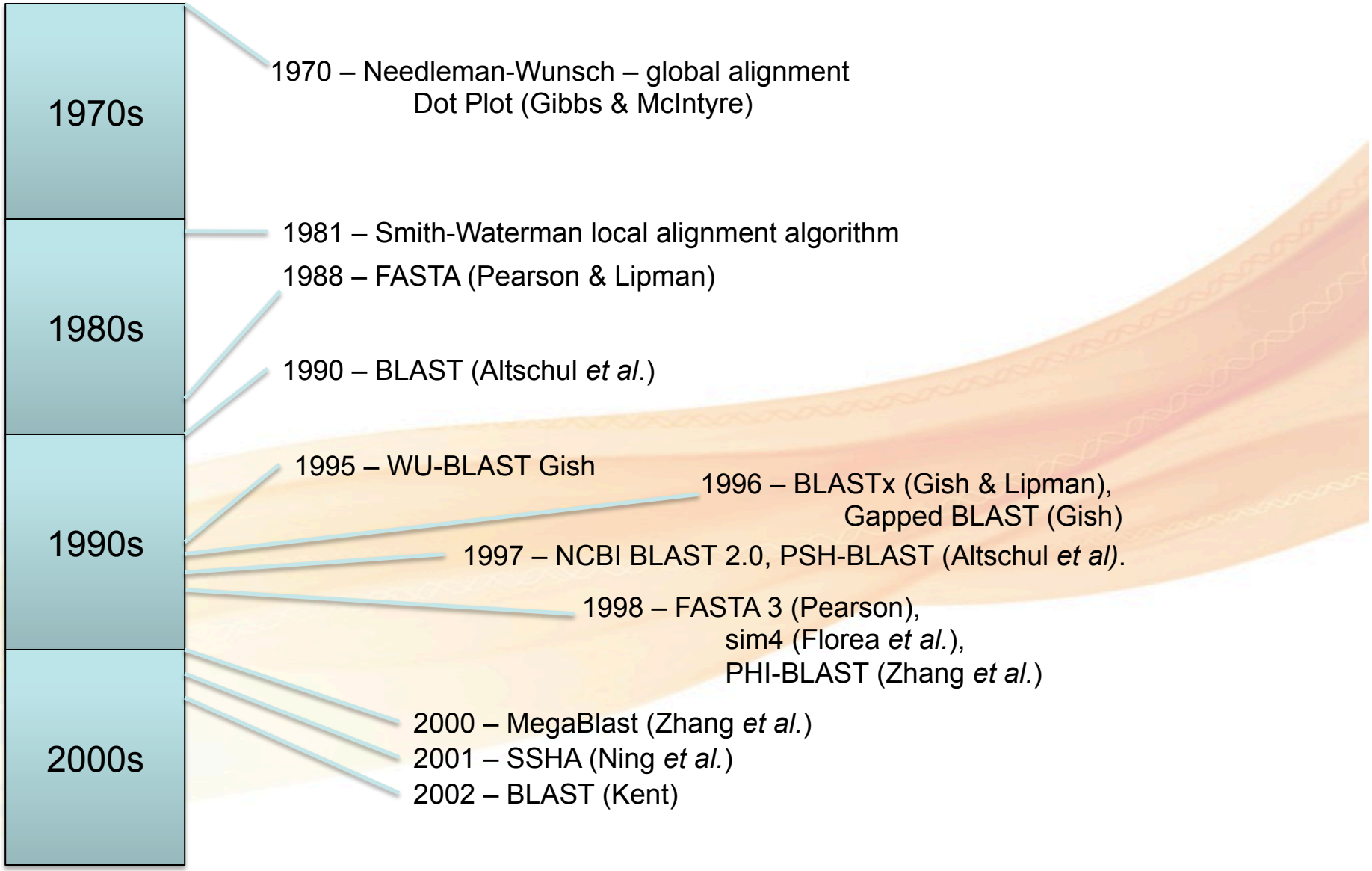
- Dynamic programming and Word Methods , as we'll see through coming lectures
- Can be divided into two categories:
  - For reasons of **computational complexity**
    - **Pairwise alignment** (i.e., the alignment of two sequences)
    - **Multiple-sequence alignment** (i.e., the alignment of three or more sequences)
- **Pairwise alignment** problems have **exact** solutions
- **Multiple-sequence alignment** problems only have approximate (**heuristic**) solutions
- We'll Discuss Pairwise first

Adopted from Dan Graur, Ph.D.

Why Do We Use Alignment Methods?

- Short sequences can be aligned by hand manually
  - Many sequences or long sequence require the use of computer algorithms
- Algorithmic details usually fall into two categories:
  - Global alignments
    - Form of global optimization that "forces" the alignment to span the entire length of all query sequences
    - Needleman-Wunsch algorithm
  - Local alignments
    - Identify regions of similarity with long sequences that are often widely divergent overall
    - Smith-Waterman algorithm

Sequence Alignment History



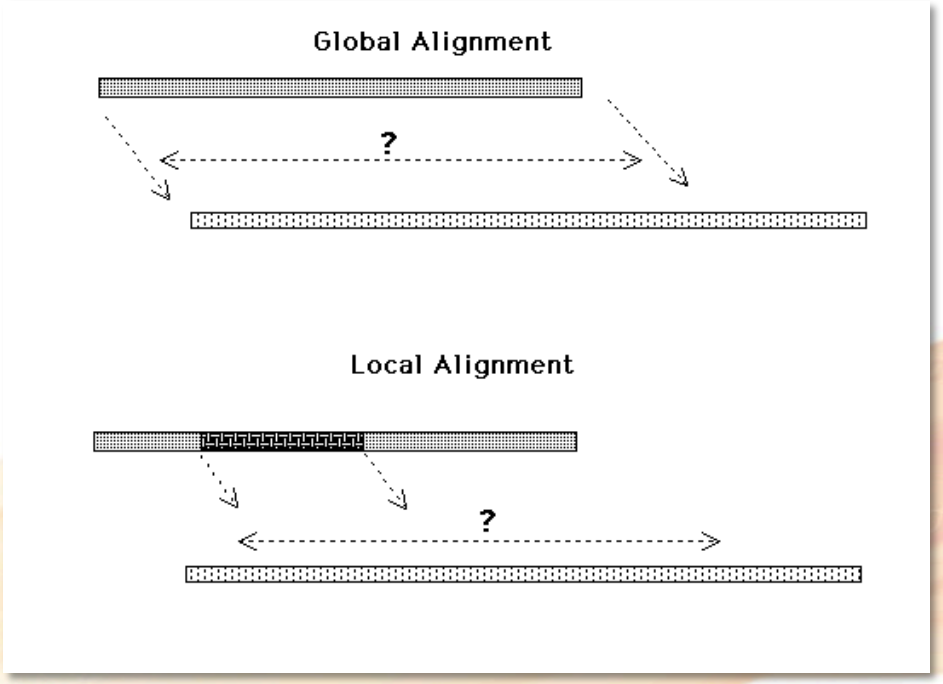
Global vs Local Similarity

- Global similarity uses completely aligned sequences - total % matches
  - Dot plots and Needleman & Wunch algorithm
- Local similarity looks for best internal matching region between 2 sequences
  - Dot plots
  - Smith-Waterman algorithm
  - BLAST and FASTA

Global vs. Local Similarity

- When should I use a certain method?
- Should result of alignment include all amino acids or just those that match?
  - If yes, a global alignment is desired
  - If no, a local alignment is desired

Global vs. Local Alignments



You Might Ask?

- Why do we need local alignments?
  - To compare a:
    - short sequence to a large one
    - single sequence to an entire database
    - partial sequence to the whole
  - Identify newly determined sequences
  - Compare new genes to known ones
  - Annotate functions for entire genomes full of ORFs of unknown function

For Thursday

- Go over tonight's lecture
- Understand code here:
  - http://www.ncbi.nlm.nih.gov/entrez/query/static/eutils_example.pl
  - Test it out!
- Get http://155.33.203.128/teaching/BIOL6308-Fall2013/local/Literature/entrez_fields.pdf

- Read
  - http://www.ncbi.nlm.nih.gov/books/NBK7039/