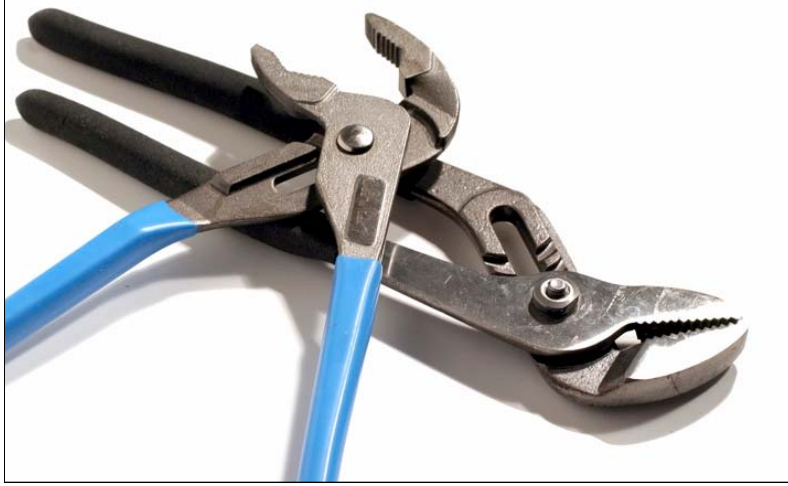
joanne@msl.ubc.ca

Bioinformatics

Common tools, useful databases, and tricks of the trade.



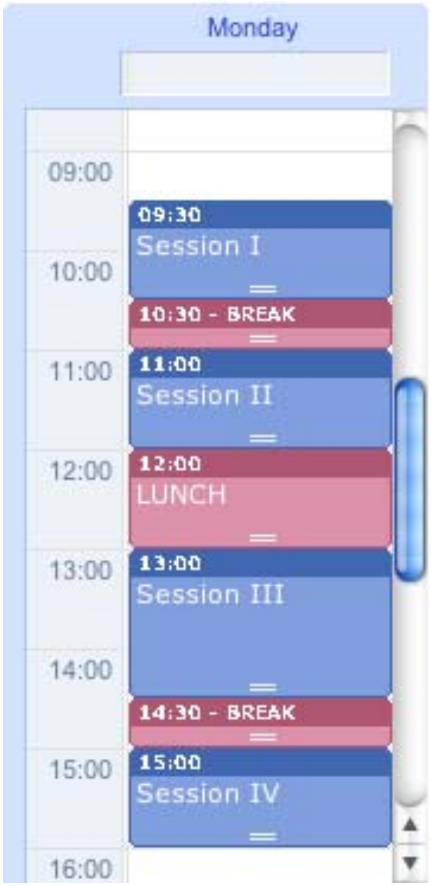
bioteach.ubc.ca/bioinfo2008

Workshop Schedule

- Laptops, available here for your use 9am - 4:30pm
- wireless login

  mslguest

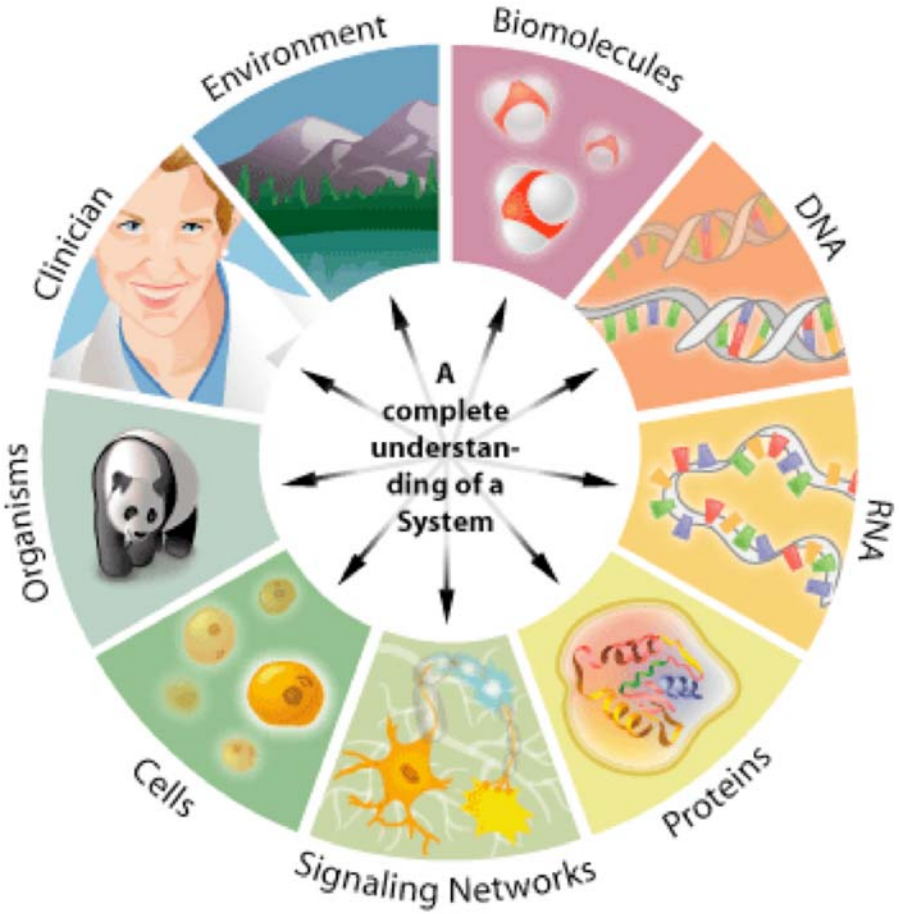
  4myguest
- Vancouver guide books available



Today's Topics

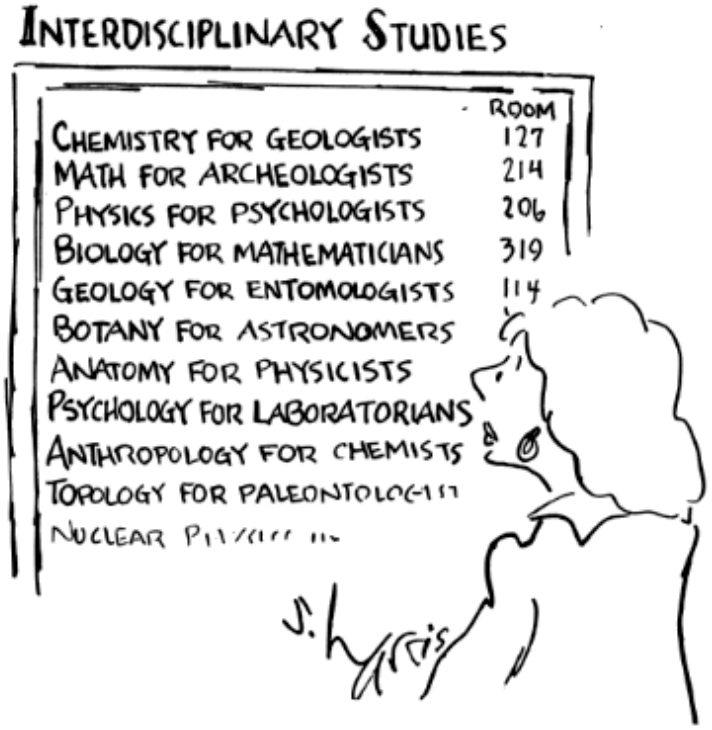
- **DNA Sequencing -** Generating Data & Emerging Technologies
- **Sequence Databases** - Public Resources at the NCBI
- **GUIDED TOUR** - Advanced Tips & Tricks for Searching Entrez
- **PRACTICAL EXERCISES** - Navigating Links, Retrieving Data with Entrez, and Searching PubMed

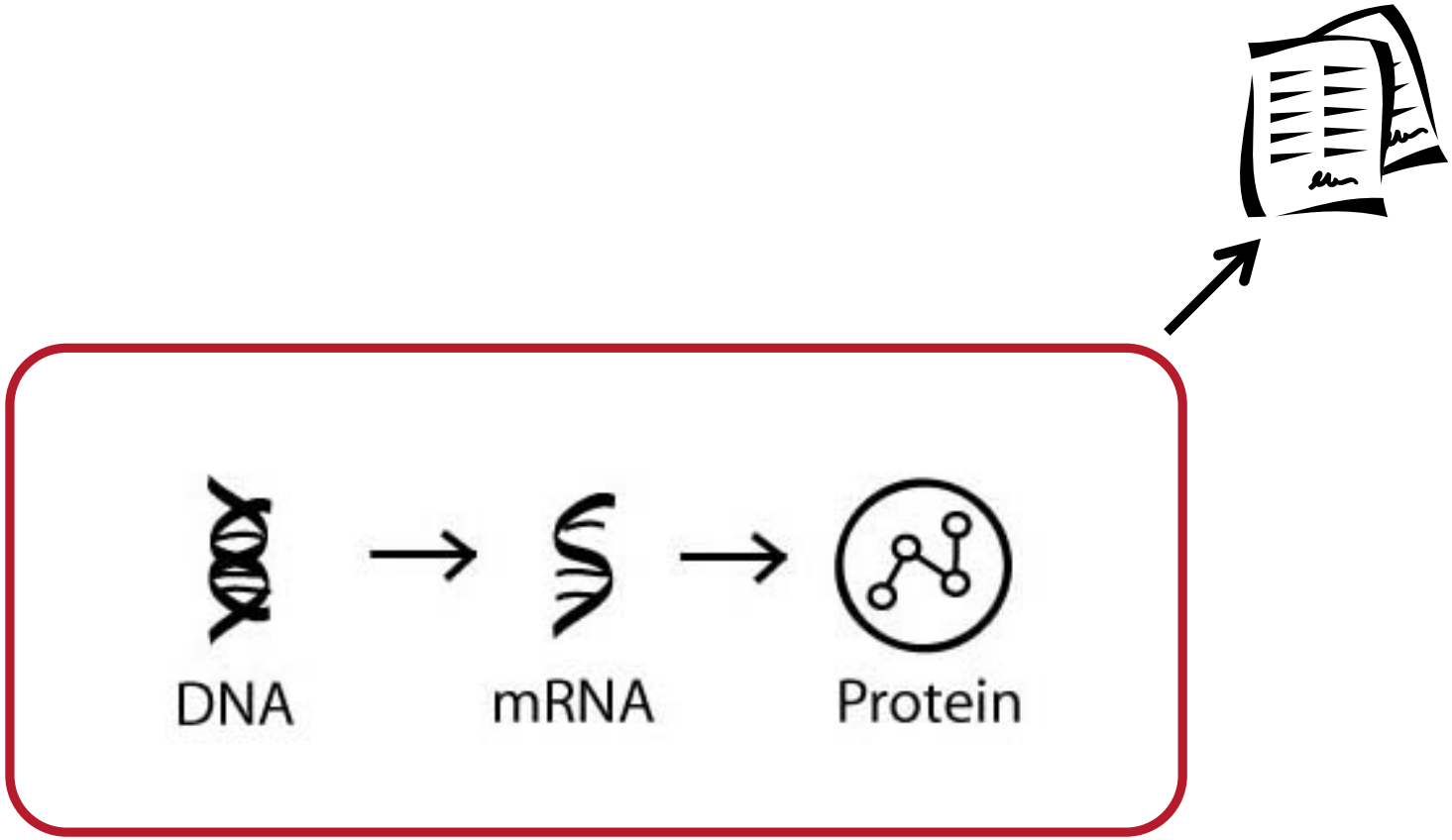
What is Bioinformatics?

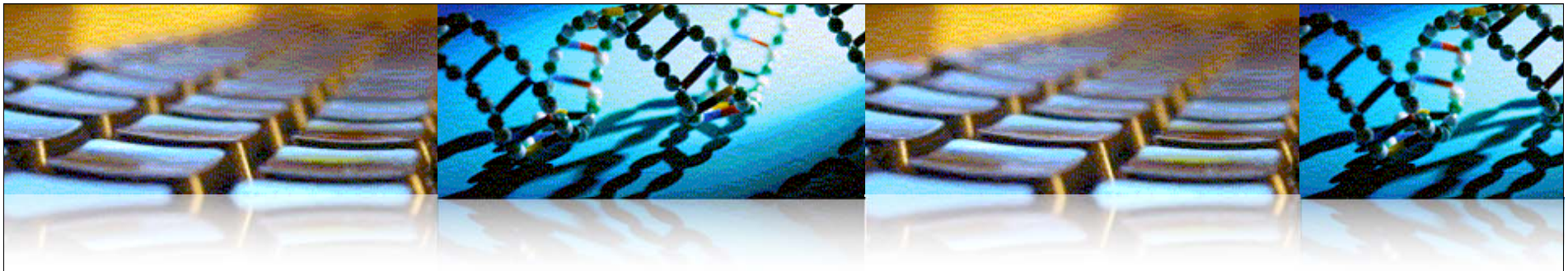


Bioinformatics for ~~Biologists~~

Government Employees

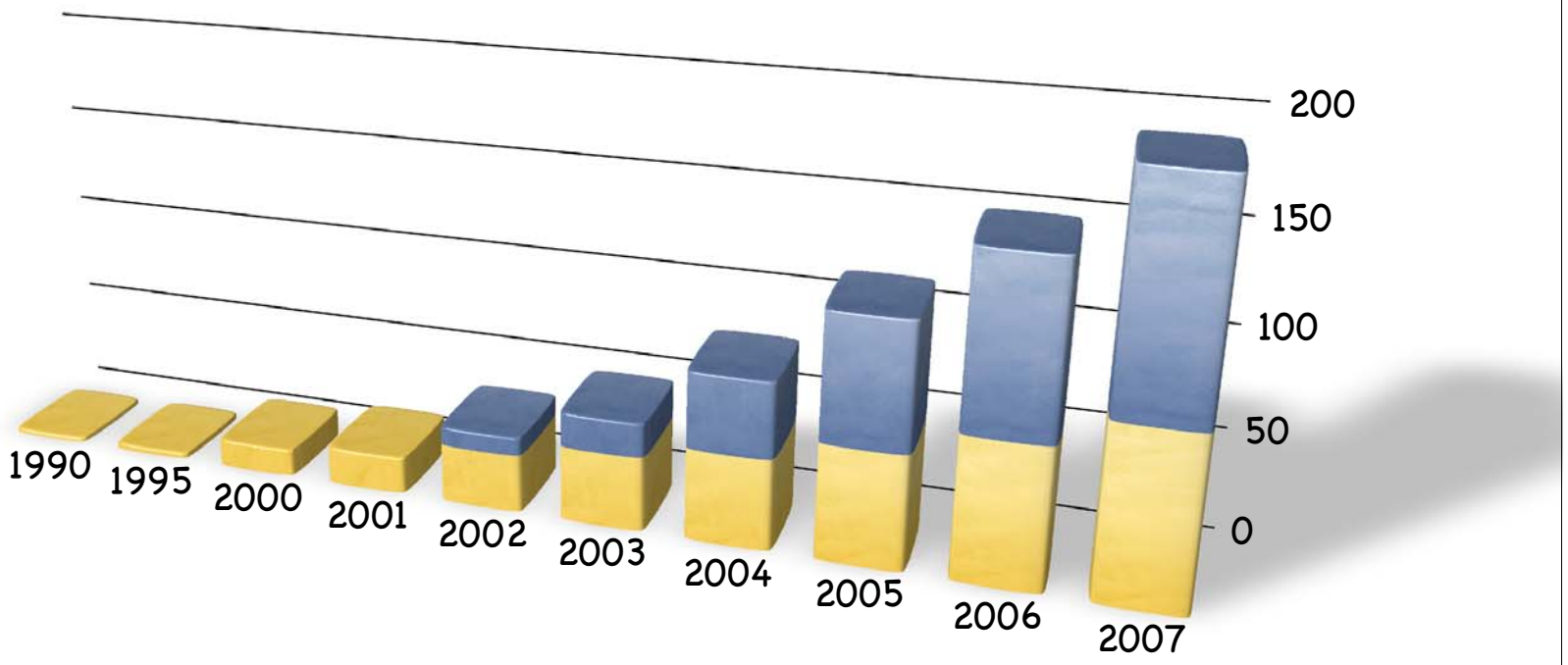



DNA
mRNA
Protein

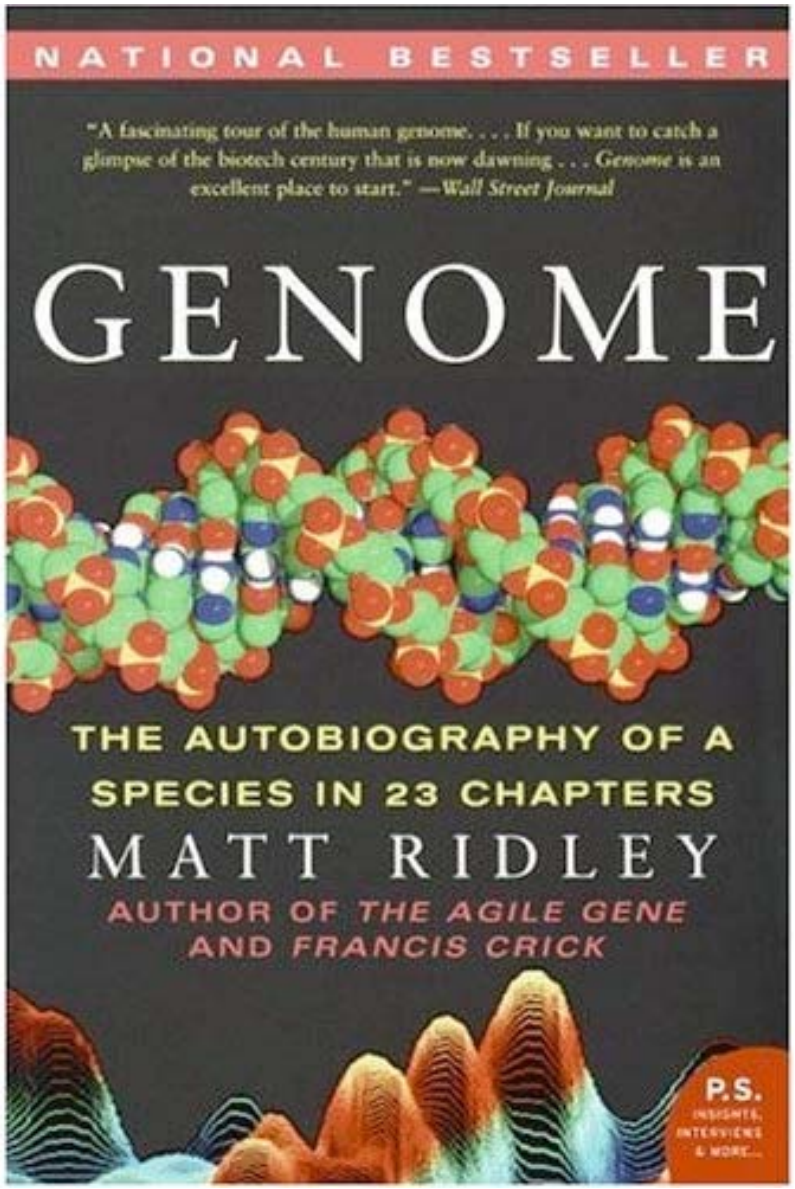


By the end of the morning session, you will define bioinformatics in your own words

Growth of GenBank

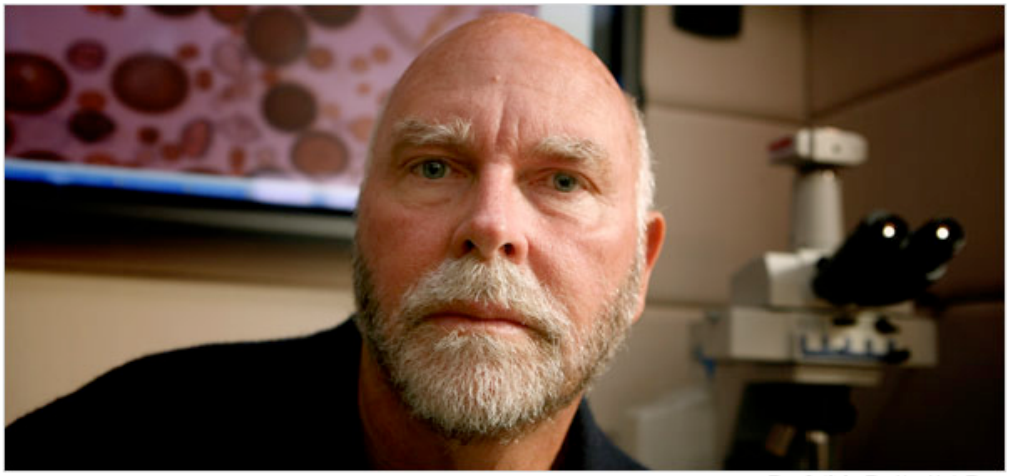


In 2005, International sequence databases exceed 100 gigabases


NATIONAL BESTSELLER
"A fascinating tour of the human genome. . . . If you want to catch a glimpse of the biotech century that is now dawning . . . Genome is an excellent place to start." —Wall Street Journal
GENOME
THE AUTOBIOGRAPHY OF A SPECIES IN 23 CHAPTERS
MATT RIDLEY
AUTHOR OF THE AGILE GENE AND FRANCIS CRICK
P.S.

Personalized Medicine?

In the Genome Race, the Sequel Is Personal

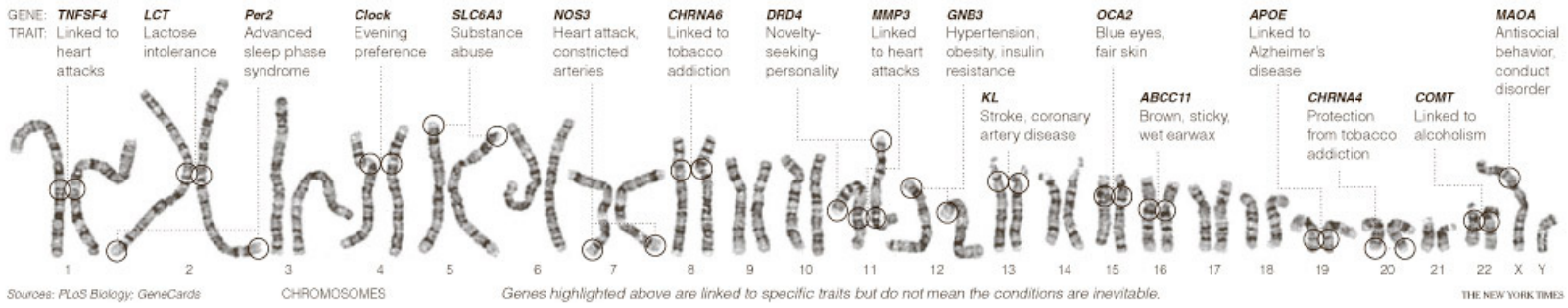


Thor Swift for The New York Times

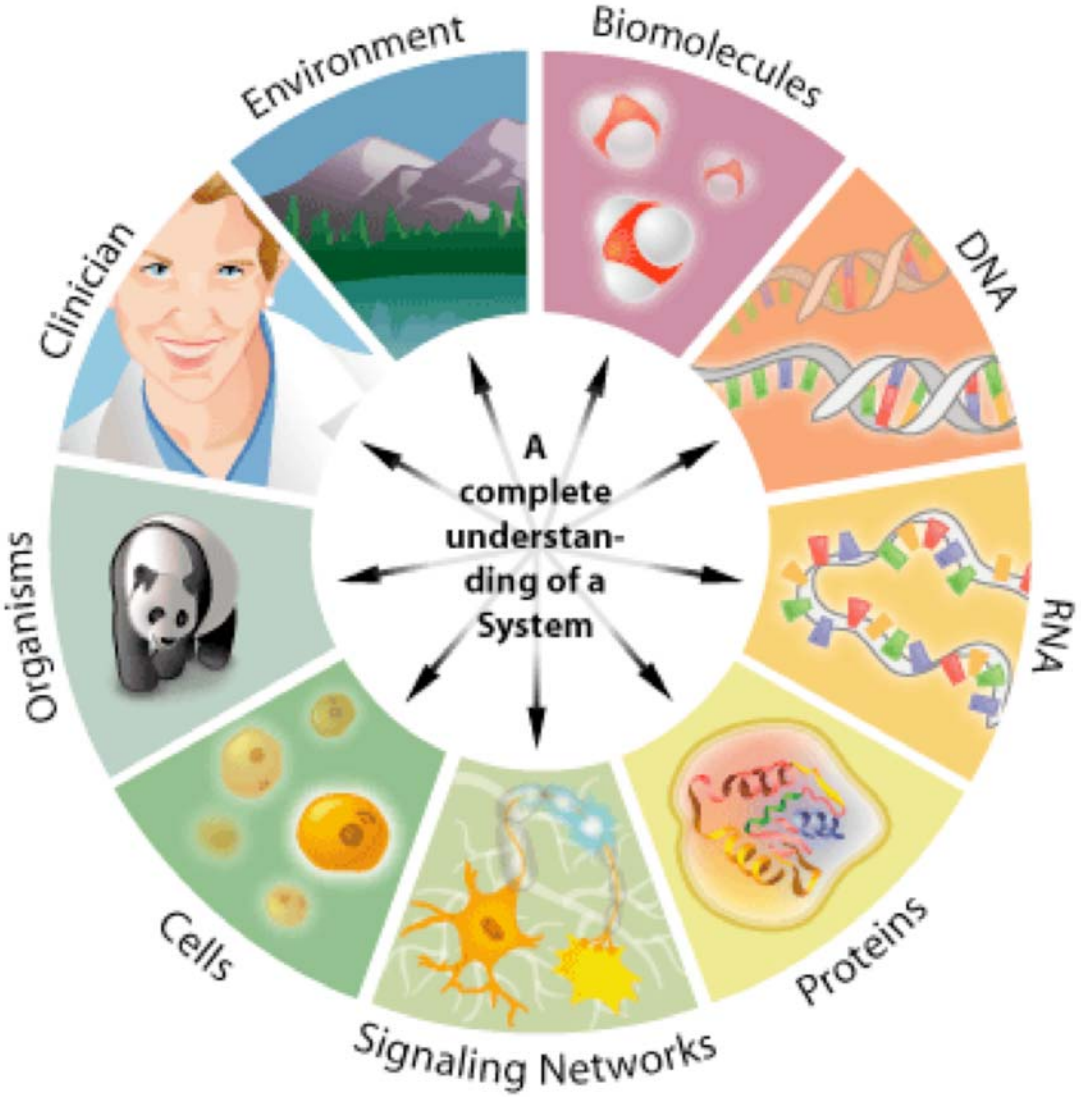
A team led by J. Craig Venter, above, has finished the first mapping of a full, or diploid, genome, made up of DNA inherited from both parents. The genome is Dr. Venter's own.

The New York Times — September 3, 2007

DECODING HIMSELF A team led by J. Craig Venter, above, has finished the first mapping of a full, or diploid, genome, made up of DNA inherited from both parents. The genome is Dr. Venter's own.

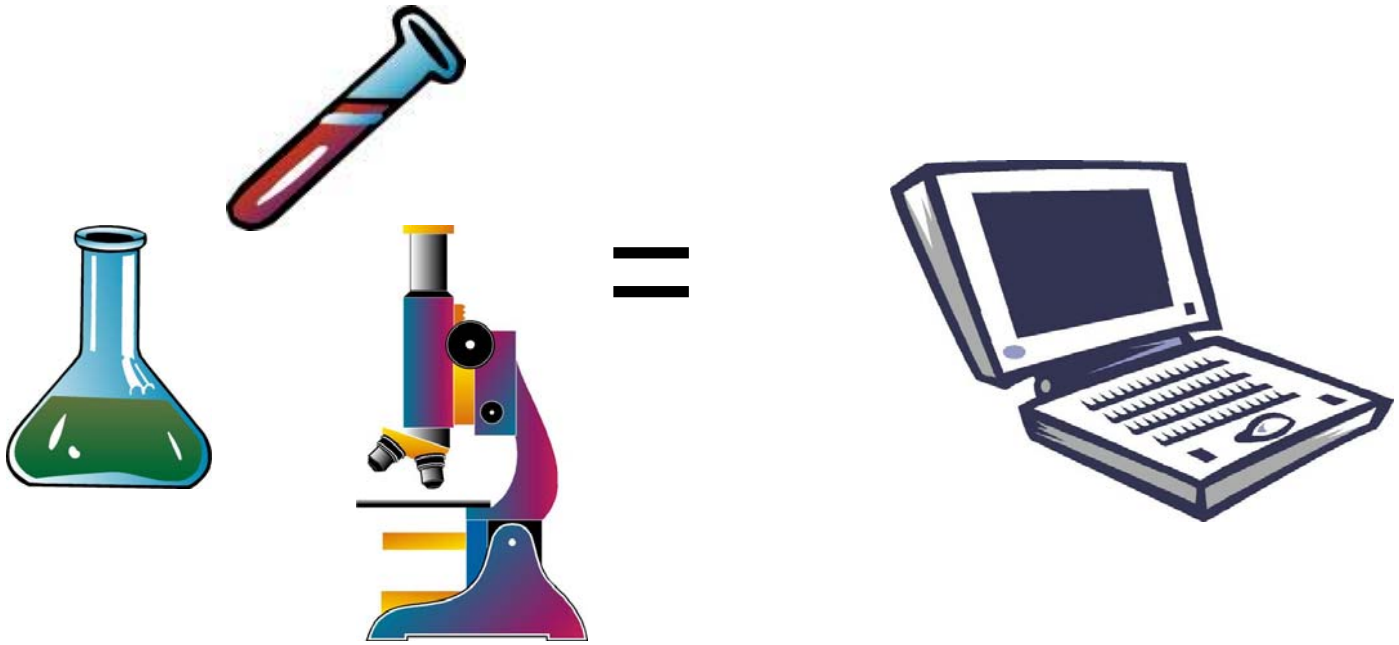


Sources: PLoS Biology; GeneCards — CHROMOSOMES — *Genes highlighted above are linked to specific traits but do not mean the conditions are inevitable.* — THE NEW YORK TIMES

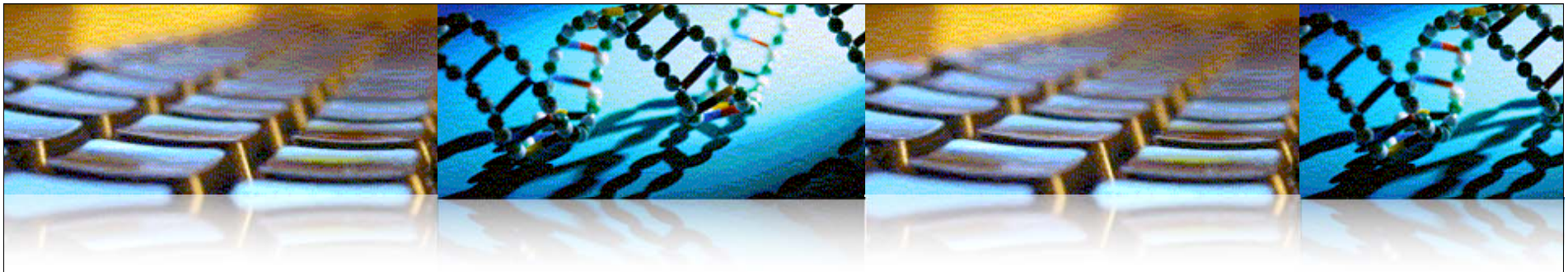

Environment
Biomolecules
Clinician
DNA
A complete understan-ding of a System
Organisms
RNA
Cells
Proteins
Signaling Networks

Linking Biological Information

- Nucleic acid & Protein Sequence data
- Sequence similarity implies homology or similar function
- Literature / Books
- Papers on related topics
- Macromolecular 3D Structure Data
- Similar protein fold implies homology
- Regulatory Pathways, Expression Data
- Two genes / enzymes in the same pathway
- Taxonomy
- Linkage of organisms by evolution



What is Bioinformatics?



Bioinformatics is an fast-paced interdisciplinary research field that involves the integration of computers, software tools, and databases in an effort to address biological questions.

Genomics refers to the analysis of all of the genes and transcripts included within the genome. Proteomics, on the other hand, refers to the analysis of the complete set of proteins or proteome.

Bioinformatics Questions

- What is encoded by the genome?
  - Genes, regulatory, and functional regions
- How is genome information expressed?
  - Function of genes and gene products (proteins)
  - Structure of proteins
- How can we interpret the information encoded in the genome?
  - Linking knowledge to the biological entities.
  - Systems biology approach
  - drugs, metabolites, ...
- How does the genome interact with its environment?

Summary

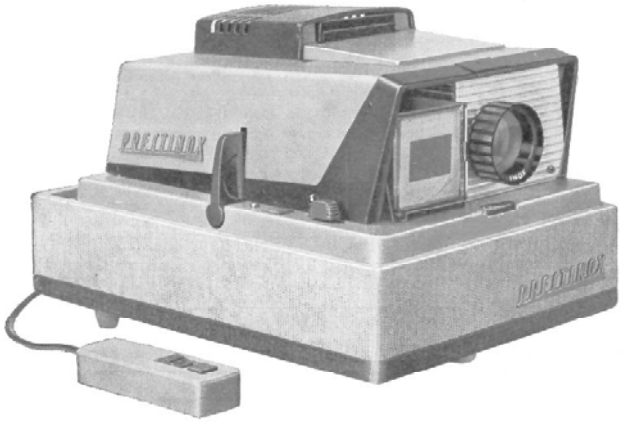
- An article called, "What is Bioinformatics?" is available from the Science Creative Quarterly.

http://www.scq.ubc.ca/what-is-bioinformatics/

DNA Sequencing

Generating Data & Emerging Technologies

Technology



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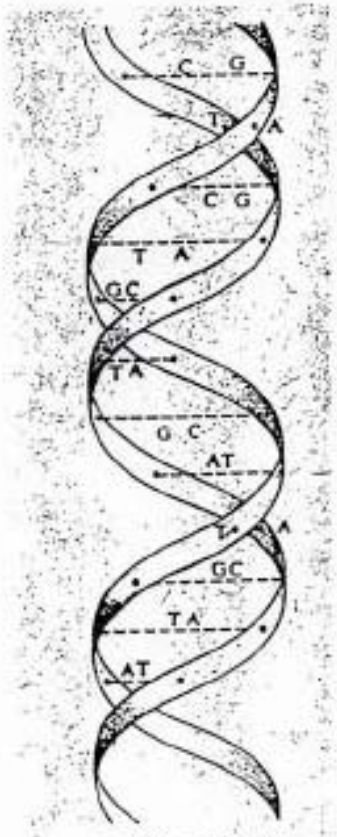
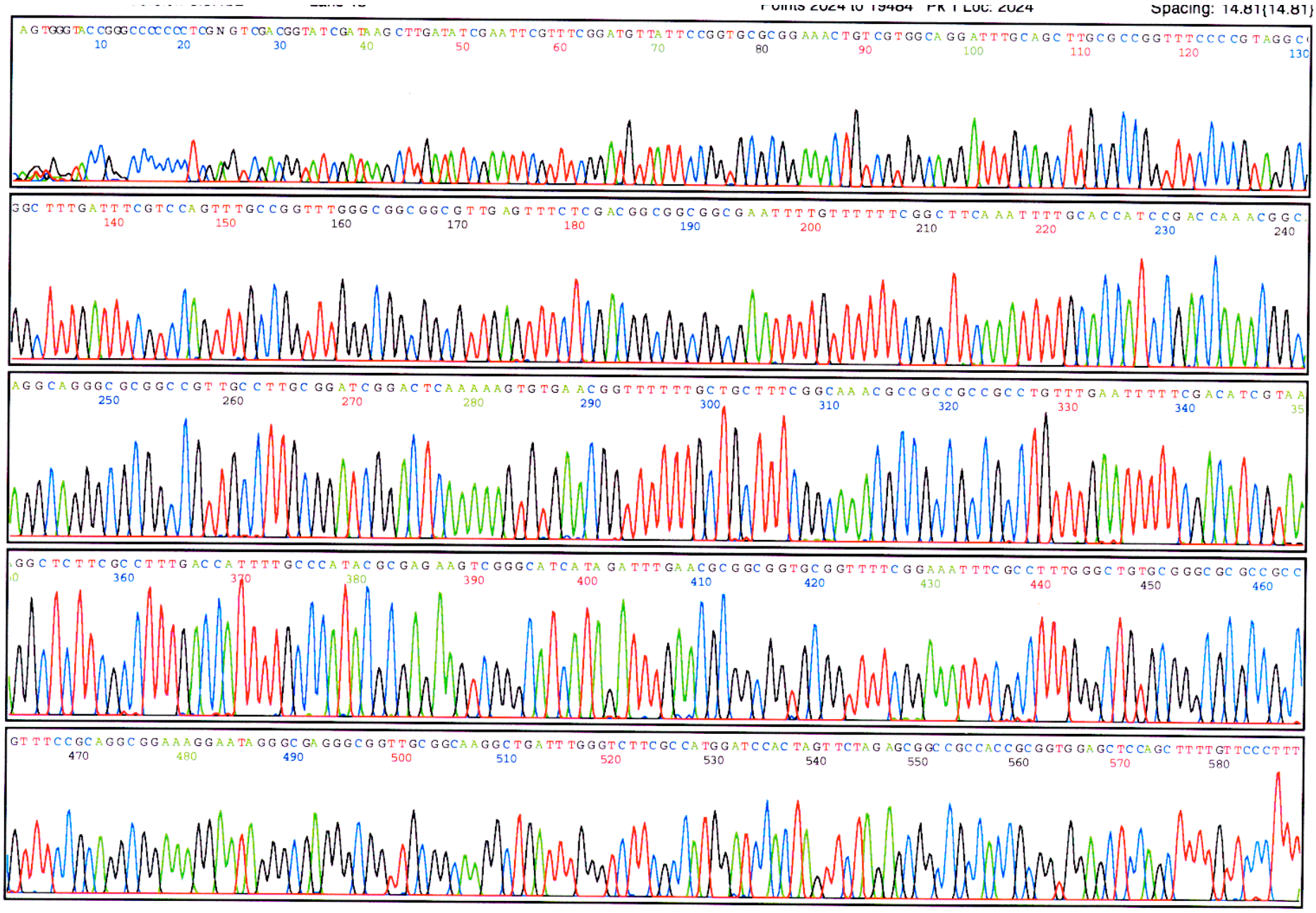


a DNA molecule

= SNOT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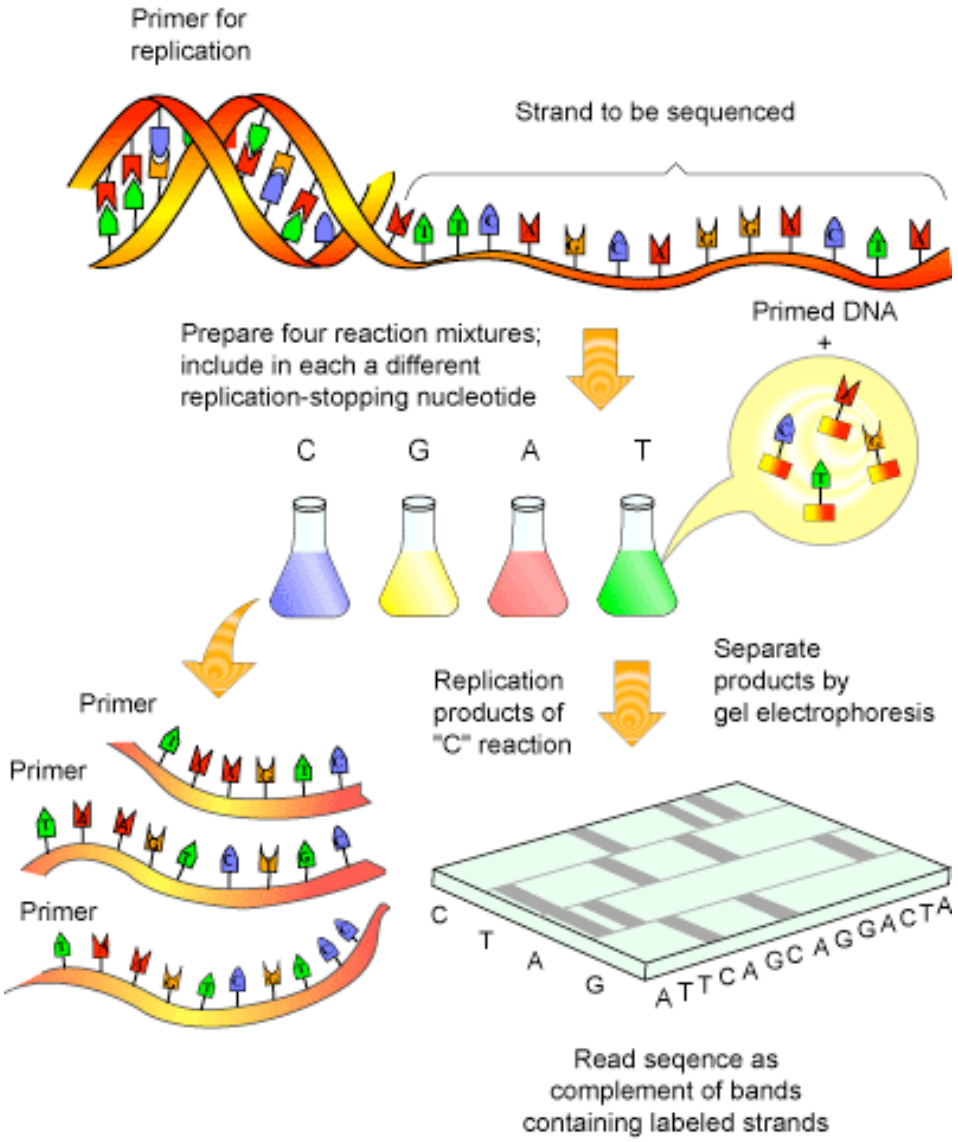


Figure 1. *The Sanger sequencing reaction. Single stranded DNA is amplified in the presence of fluorescently labelled ddNTPs that serve to terminate the reaction and label all the fragments of DNA produced. The fragments of DNA are then separated via polyacrylamide gel electrophoresis and the sequence read using a laser beam and computer.*

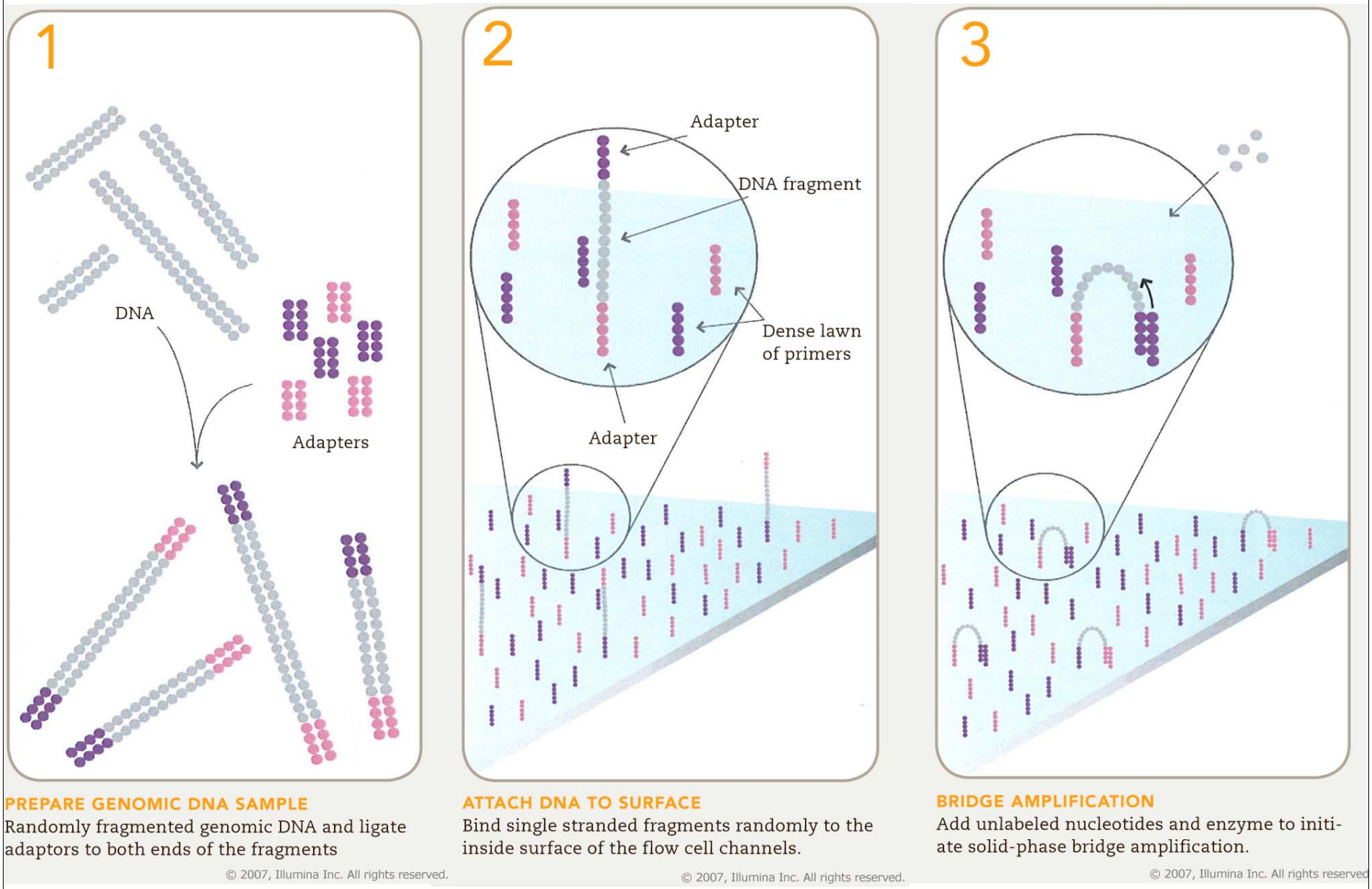
source: http://www.scq.ubc.ca/genome-projects-uncovering-the-blueprints-of-biology/

Every few years, a new technology comes along that dramatically changes how fundamental questions in biology are addressed. The impact of the technology is not always appreciated at first ...

- Stanley Fields

Solexa Technology

- DNA sequencing by synthesis
- approach built around very large number of short sequence reads
- key points:
  - ✓ solid phase amplification = no cloning necessary
  - ✓ reversible chemistry
  - ✓ data generated by imaging
  - ✓ read lengths 30-50 bp
  - ✓ < 1% cost, ultra high-throughput

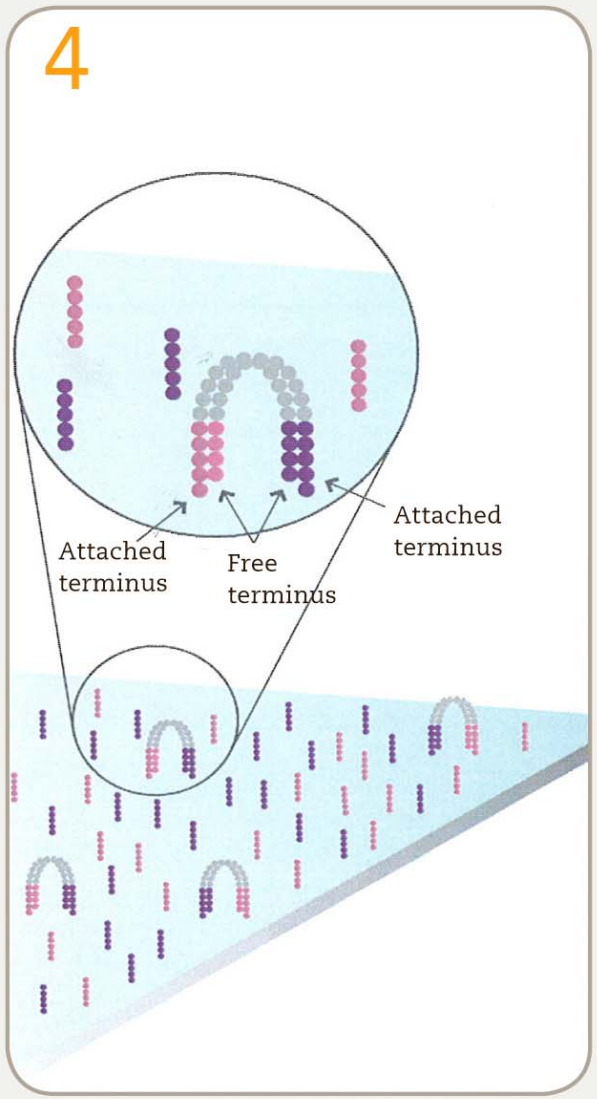


PREPARE GENOMIC DNA SAMPLE
Randomly fragmented genomic DNA and ligate adaptors to both ends of the fragments

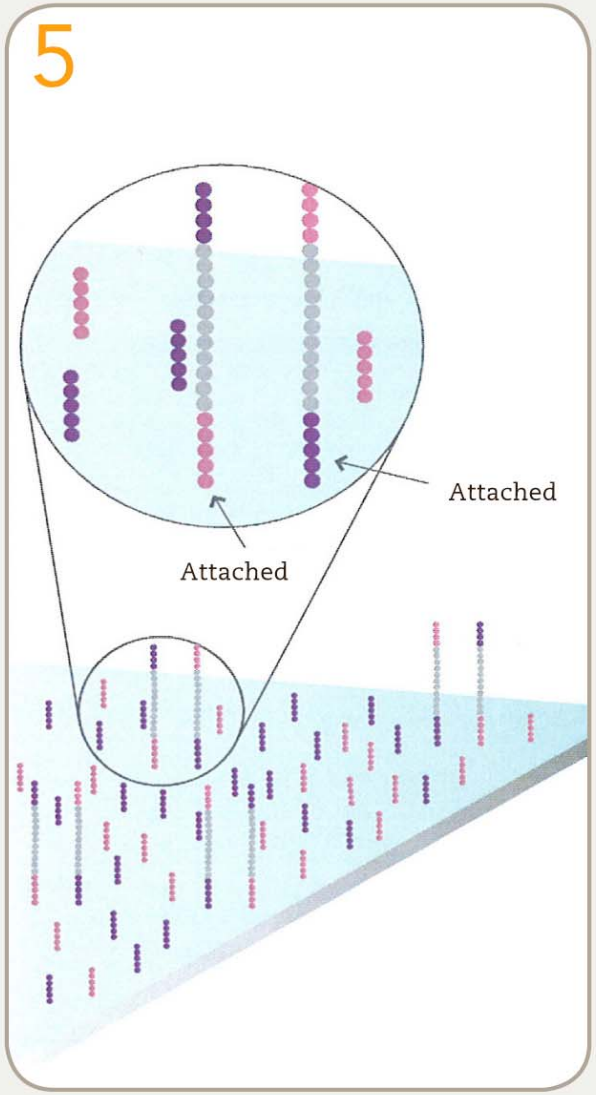
ATTACH DNA TO SURFACE
Bind single stranded fragments randomly to the inside surface of the flow cell channels.

BRIDGE AMPLIFICATION
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

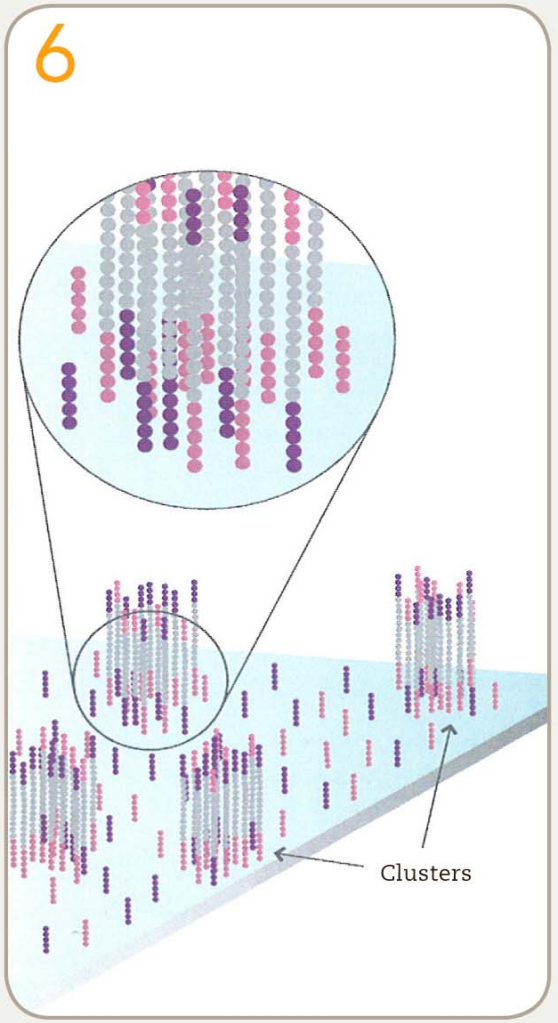
source: http://www.illumina.com/



FRAGMENTS BECOME DOUBLE STRANDED



DENATURE THE DOUBLE STRANDED MOLECULES

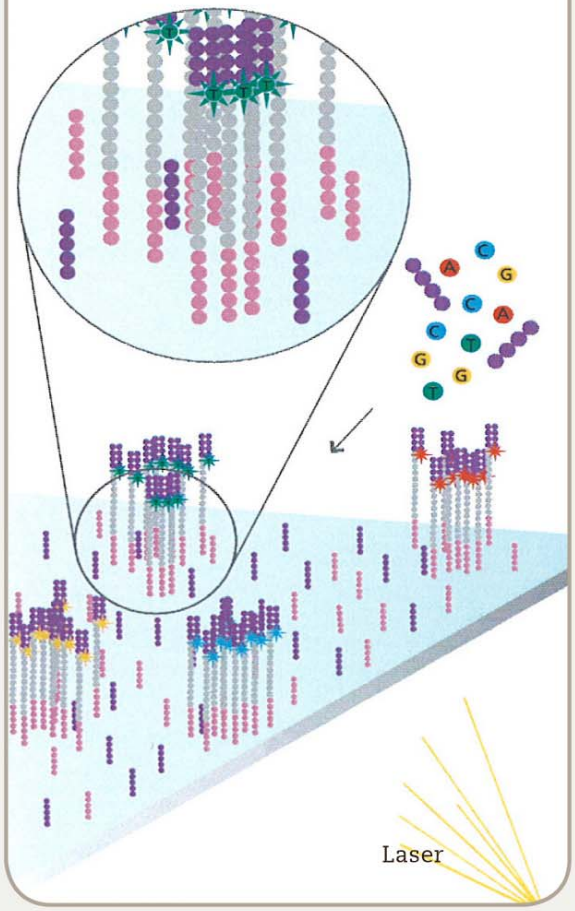


COMPLETION OF AMPLIFICATION

On completion, several million dense clusters of double stranded DNA are generated in each channel of the flow cell.

source: http://www.illumina.com/

7



FIRST CHEMISTRY CYCLE: DETERMINE FIRST BASE

To initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.

8

After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.

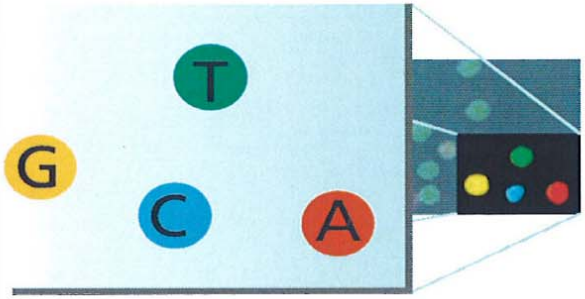
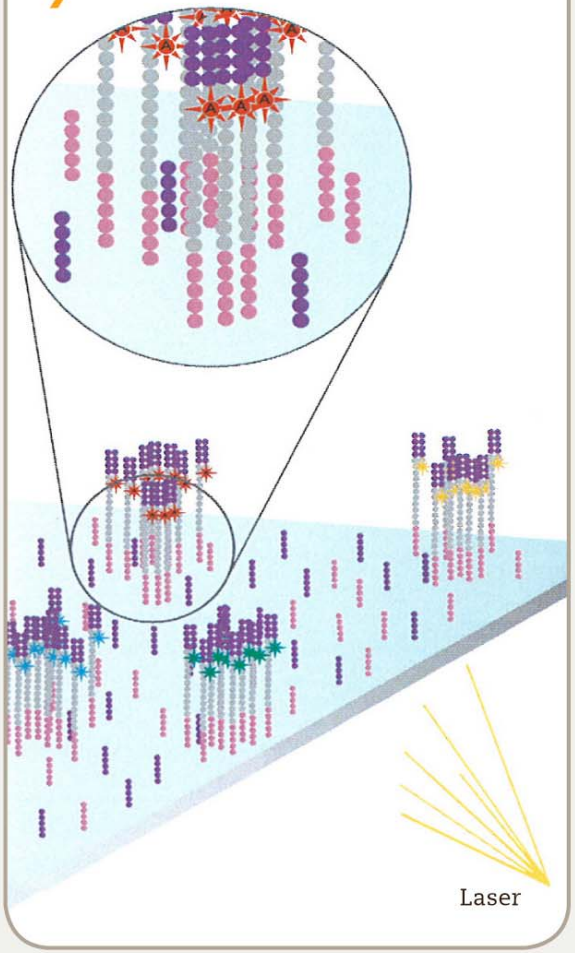


IMAGE OF FIRST CHEMISTRY CYCLE

9



SECOND CHEMISTRY CYCLE: DETERMINE SECOND BASE

To initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.

source: http://www.illumina.com/

10

After laser excitation, collect the image data as before. Record the identity of the second base for each cluster.

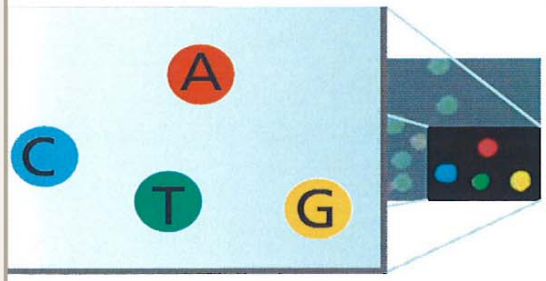
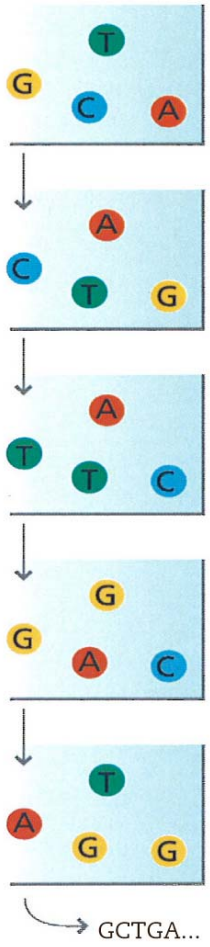


IMAGE OF SECOND CHEMISTRY CYCLE IS CAPTURED BY THE INSTRUMENT.

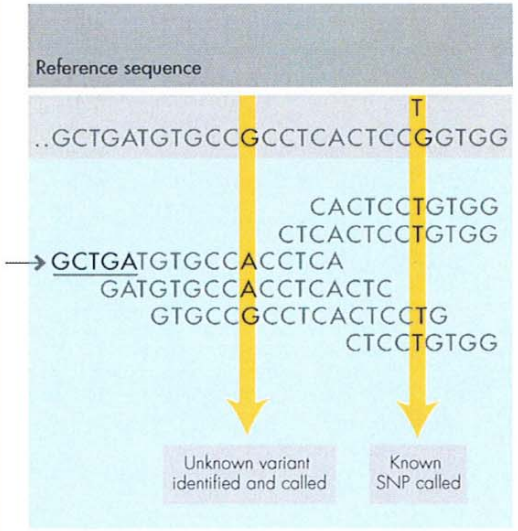
11

Repeat cycles of sequencing to determine the sequence of bases in a given fragment a single base at a time.



SEQUENCE READS OVER MULTIPLE CHEMISTRY CYCLES

12

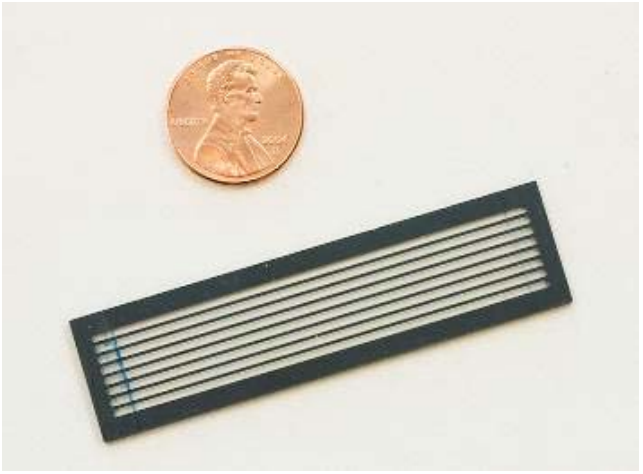


ALIGN THE NEW DATA TO A REFERENCE AND IDENTIFY SEQUENCE DIFFERENCES.

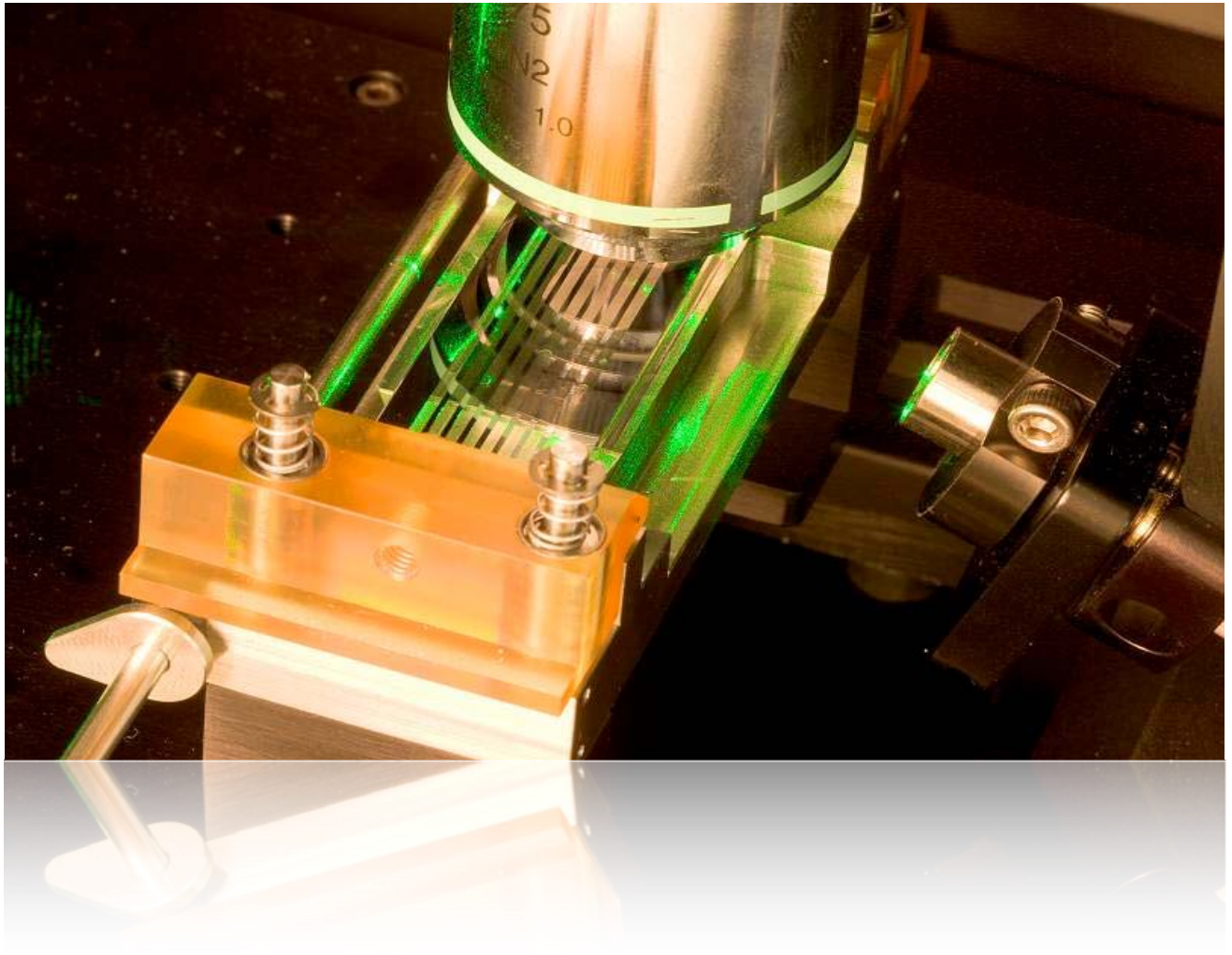
source: http://www.illumina.com/

Illumina/Solexa instrument

- Laser-based TIRF* Optics
- 4-colour Detection
- CCD camera
- 8-channel flow cell
- 1 Gb / run at launch

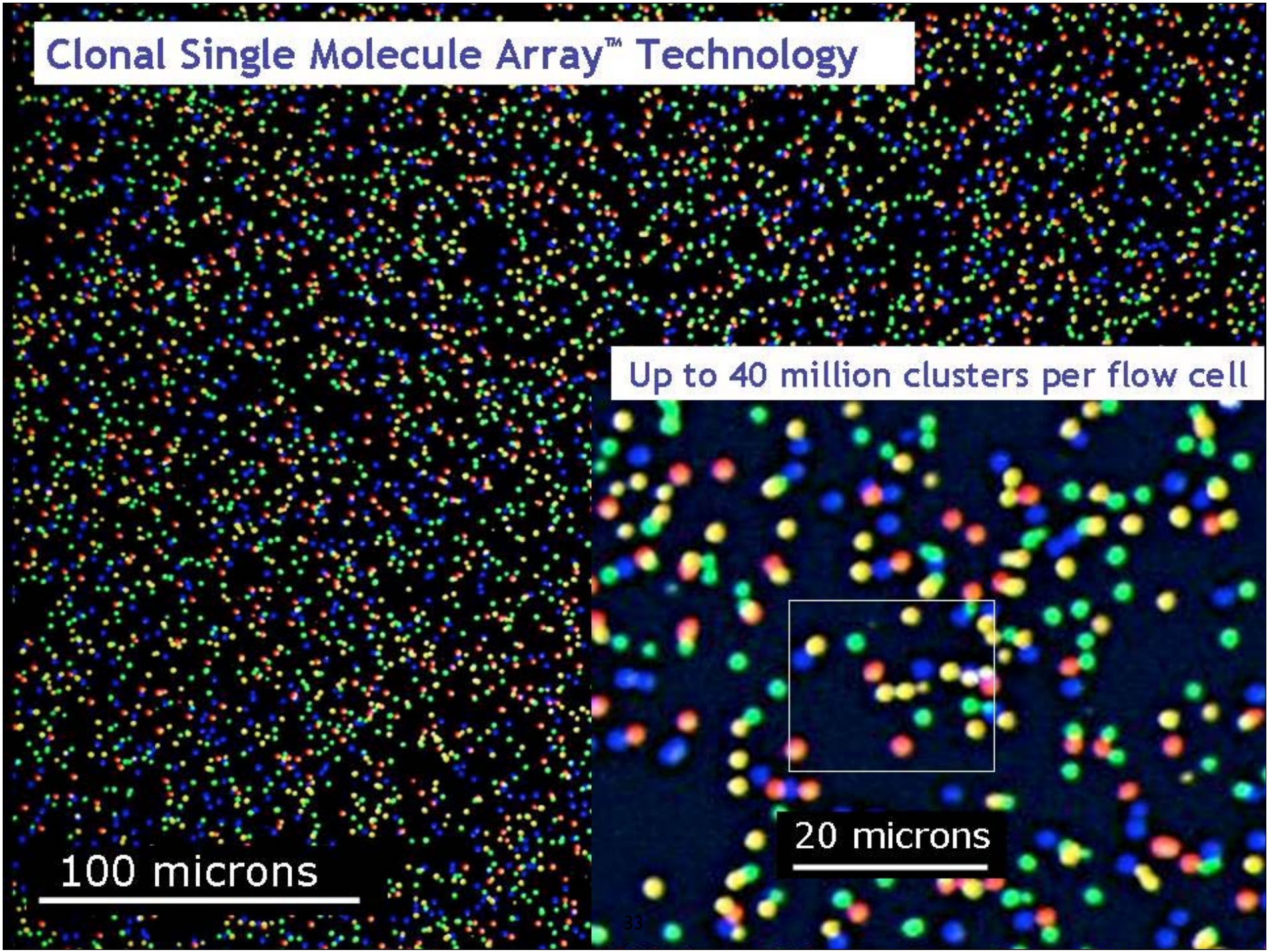


source: Dr. Steven Jones, GSC

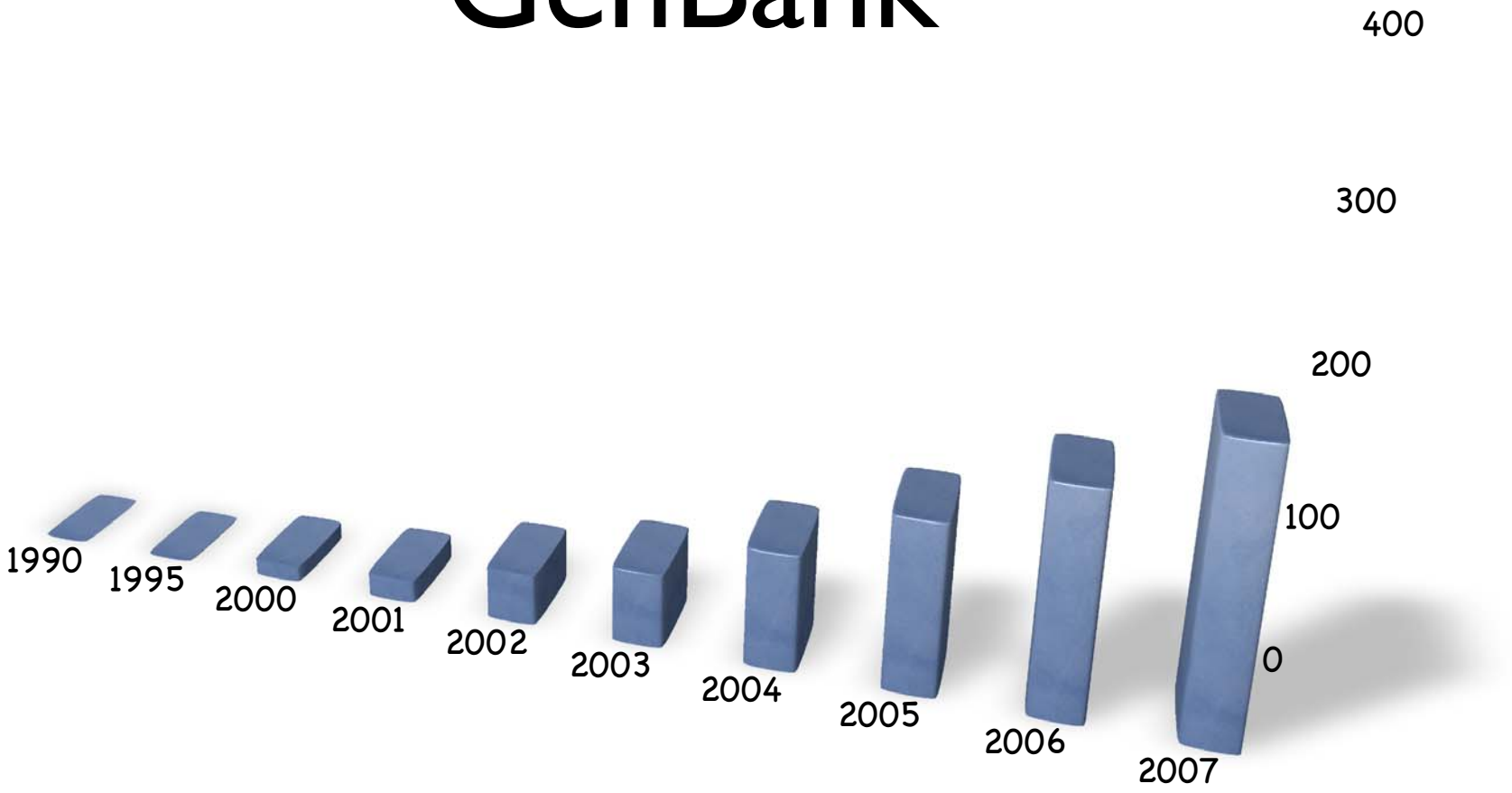


Data acquisition

source: Dr. Steven Jones, GSC


Clonal Single Molecule Array™ Technology
Up to 40 million clusters per flow cell
100 microns
20 microns

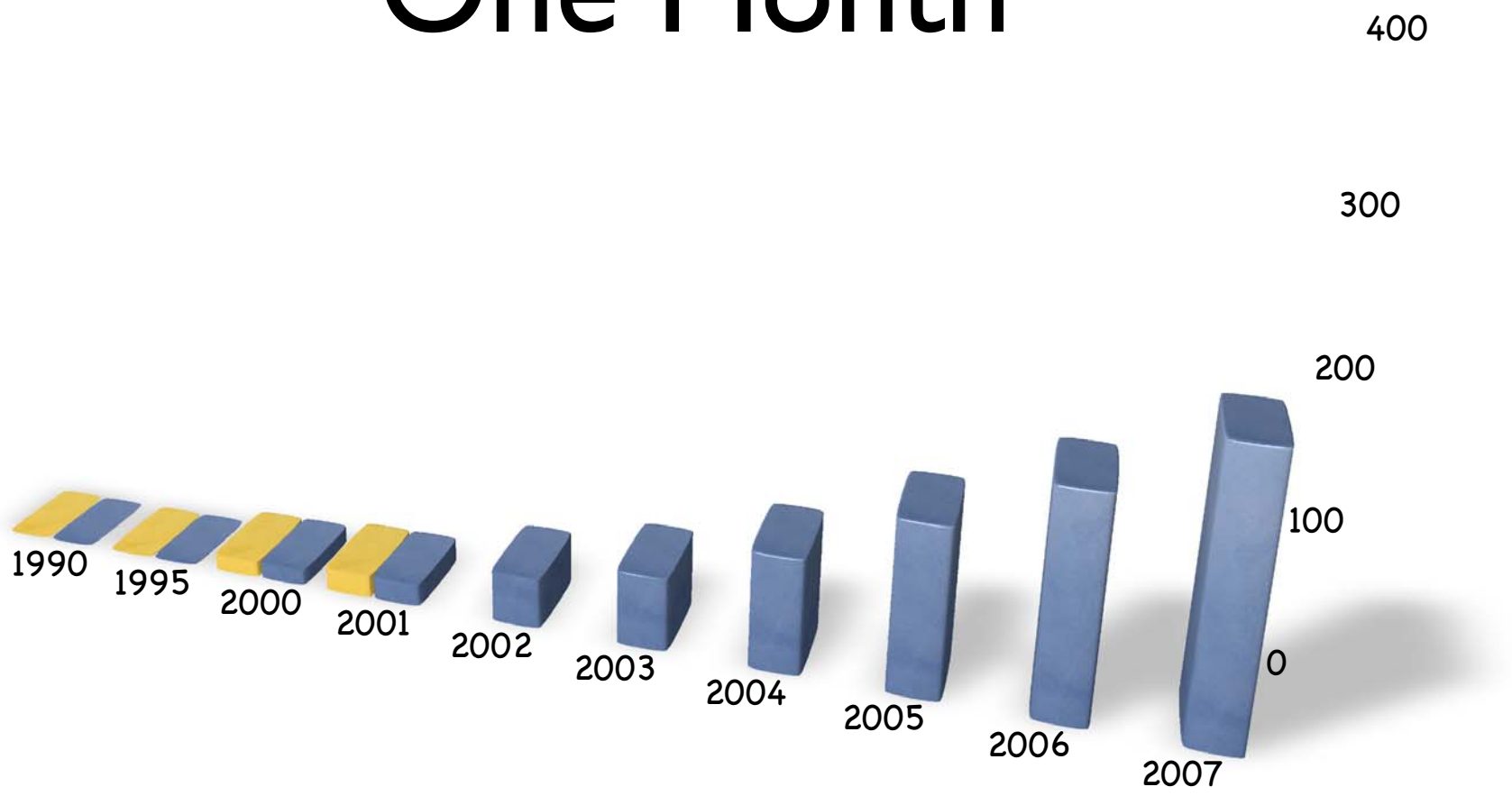
GenBank


400
300
200
100
0
1990
1995
2000
2001
2002
2003
2004
2005
2006
2007

Two Days

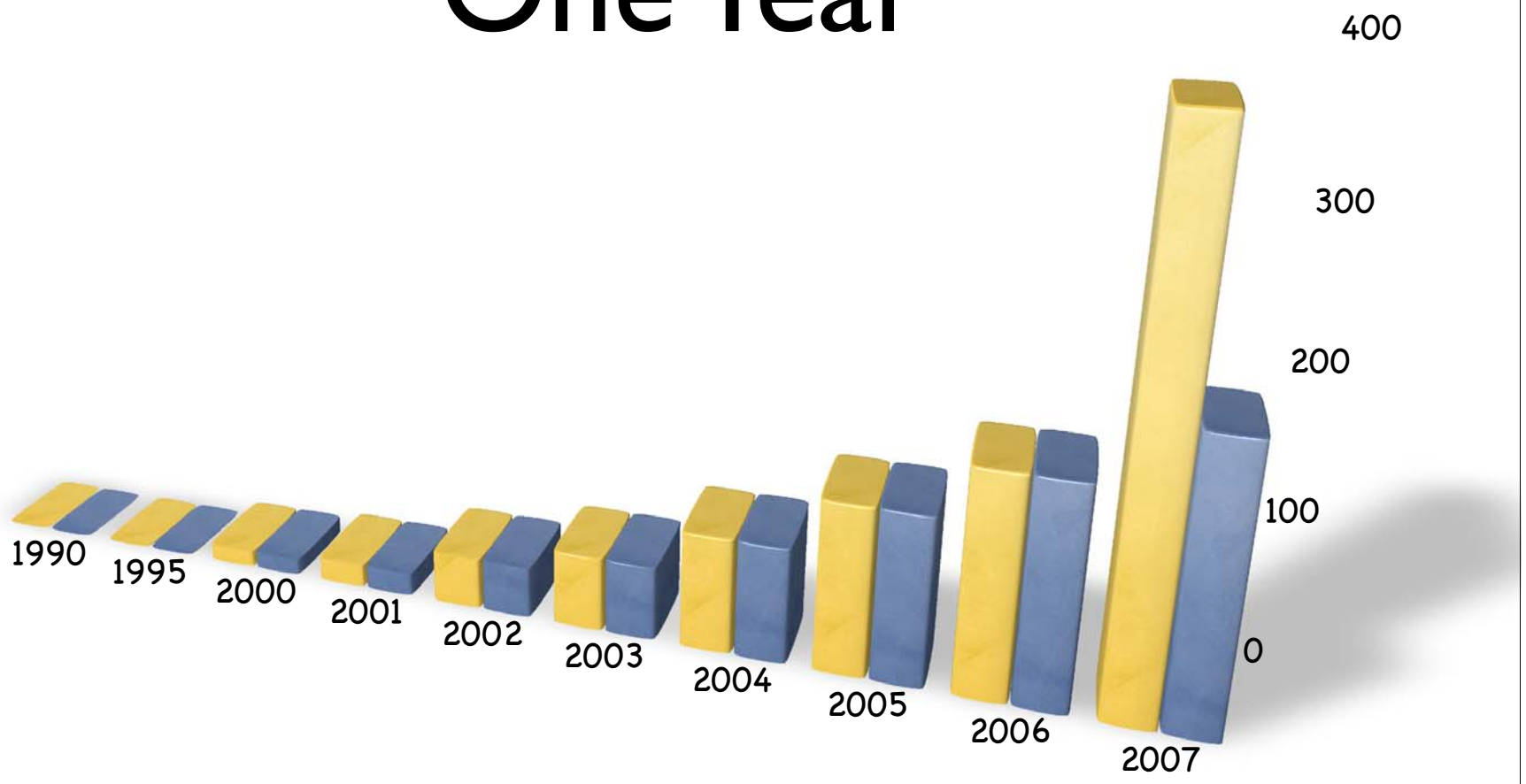
One machine, running for two days, can generate ~1 Gb data.

One Month

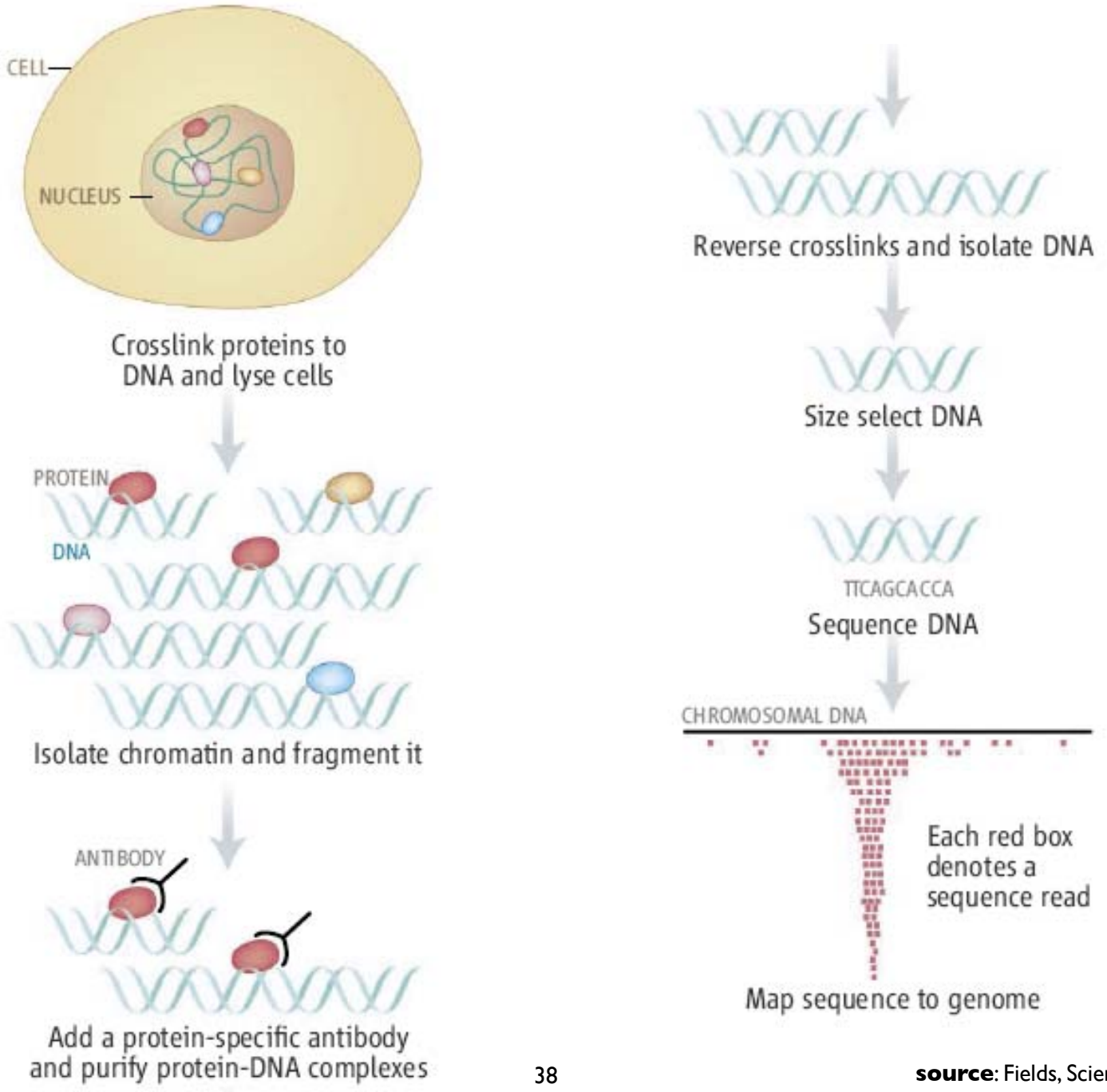


One machine, running for one month, could generate ~15 Gb data.

One Year



Two machines, running for one year, could generate ~365 Gb data.



source: Fields, Science 2007, p1441

Frontiers in Bioinformatics

- ultra high-throughput sequencing
  - DNA binding site identification
  - genome re-sequencing; SNPs, expression
    - ✓ low cost
    - ✓ whole genome
    - ✓ any genome

Credits & References

- Technology Spotlight on DNA Sequencing with Solexa Technology:

http://www.illumina.com/downloads/SS_DNAsequencing.pdf

- Dr. Steven Jones, GSC

several slides/images used with permission

- Stanley Fields, "Site-Seeing by Sequencing", Science, 8 June 2007

Sequence Databases

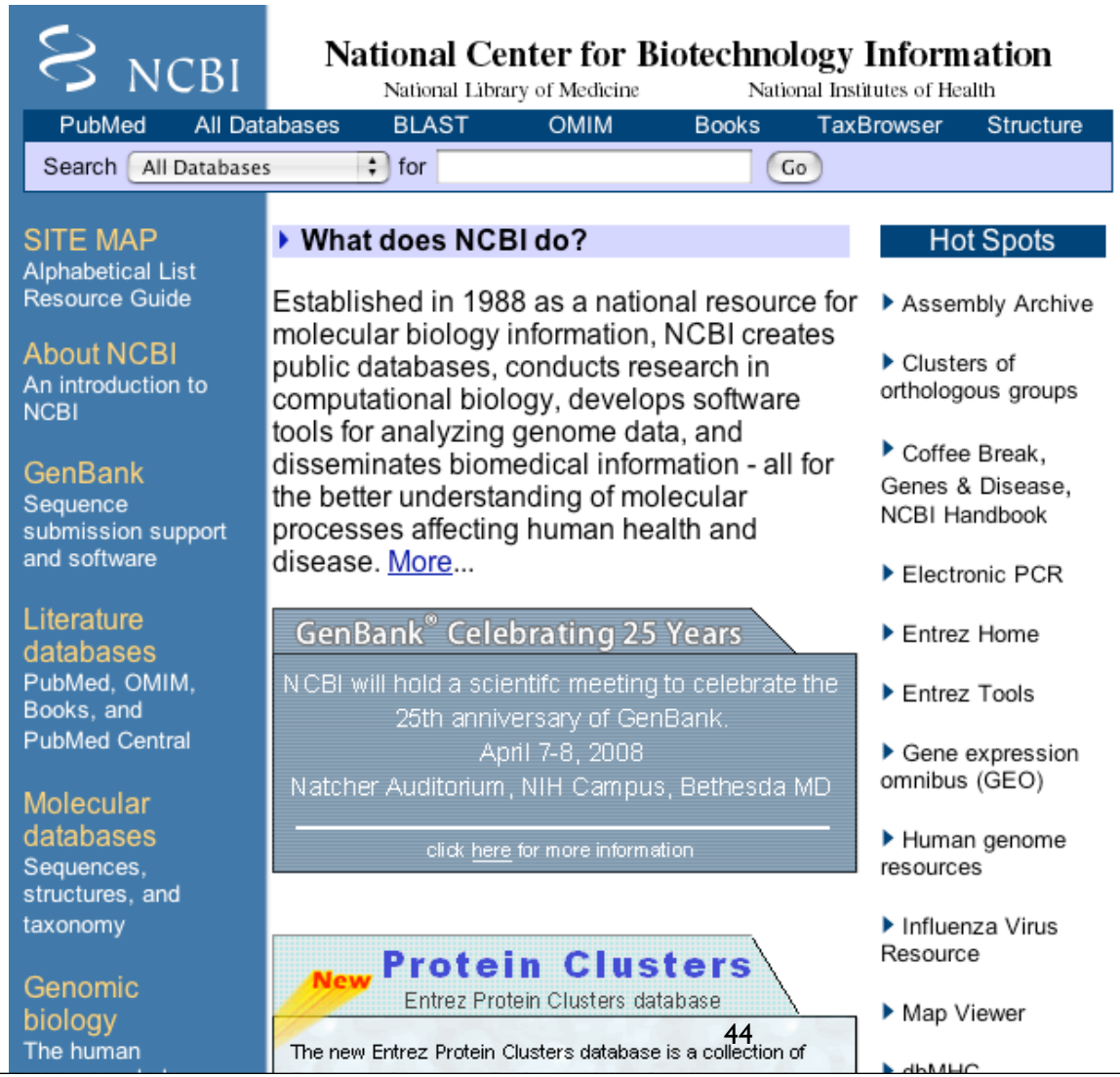
Public Resources at the NCBI

The National Center for Biotechnology Information

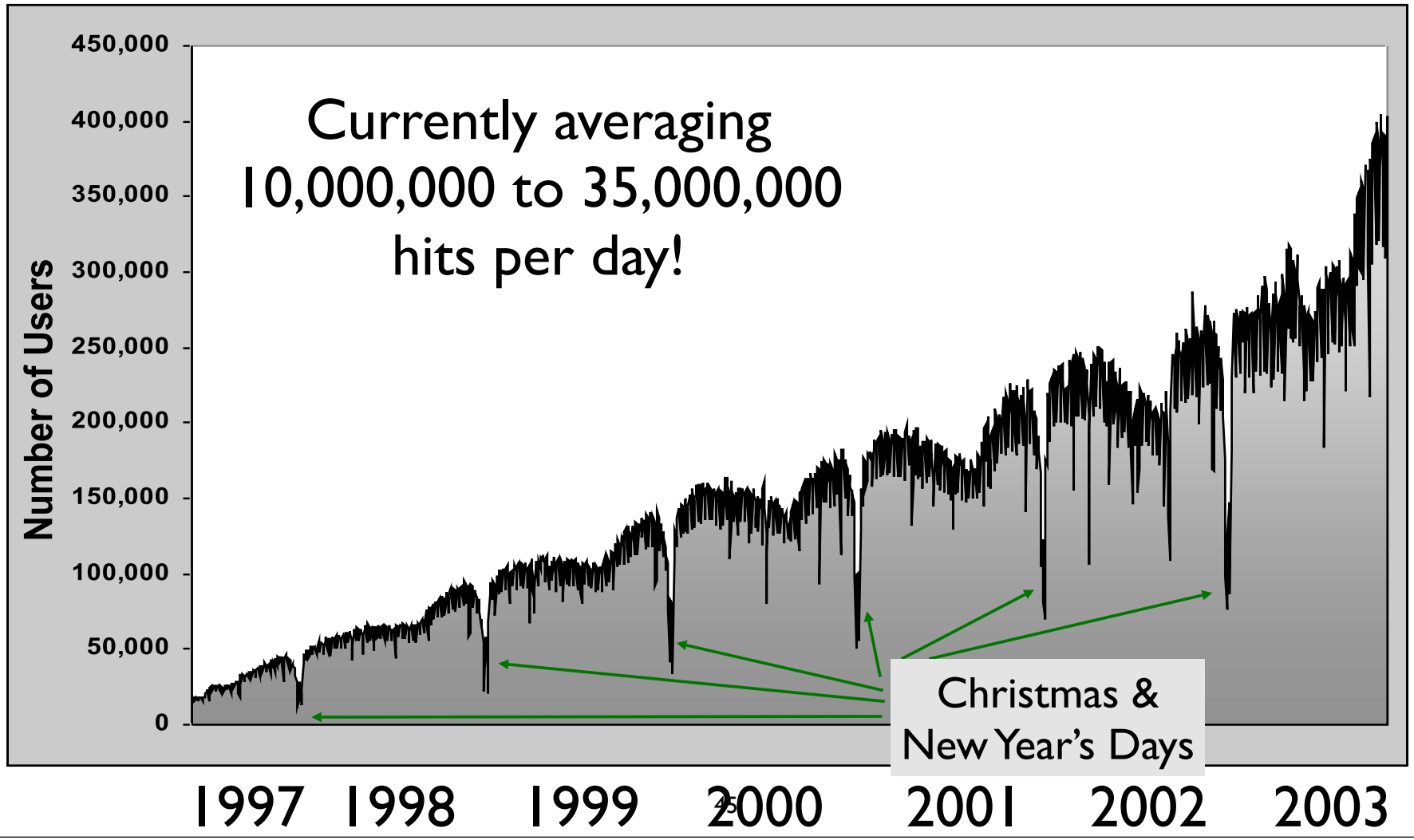
NCBI

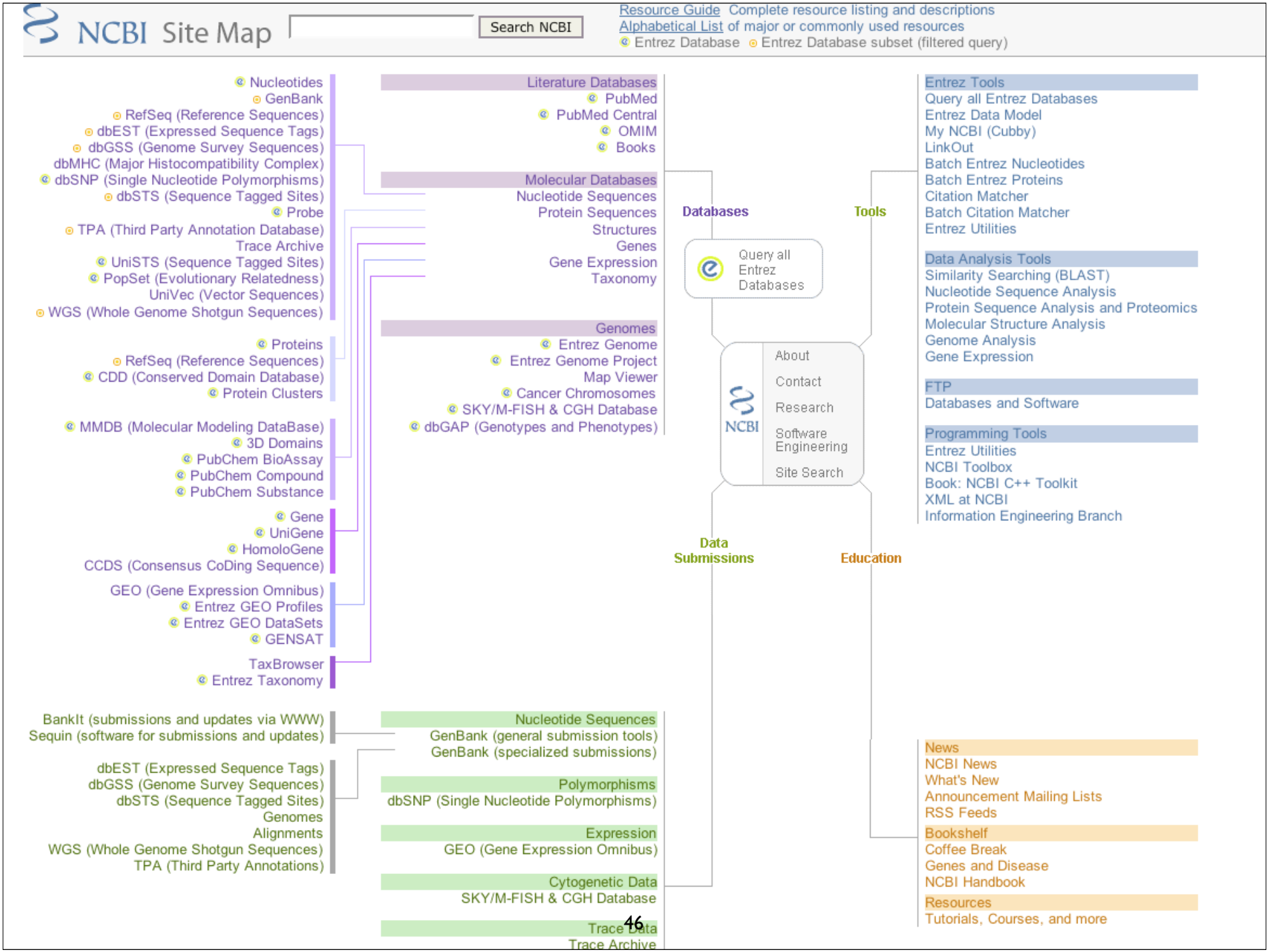
- **Created in 1988 as a part of the National Library of Medicine at NIH**
- Establish public databases
- Research in computational biology
- Develop software tools for sequence analysis
- Disseminate biomedical information

www.ncbi.nlm.nih.gov

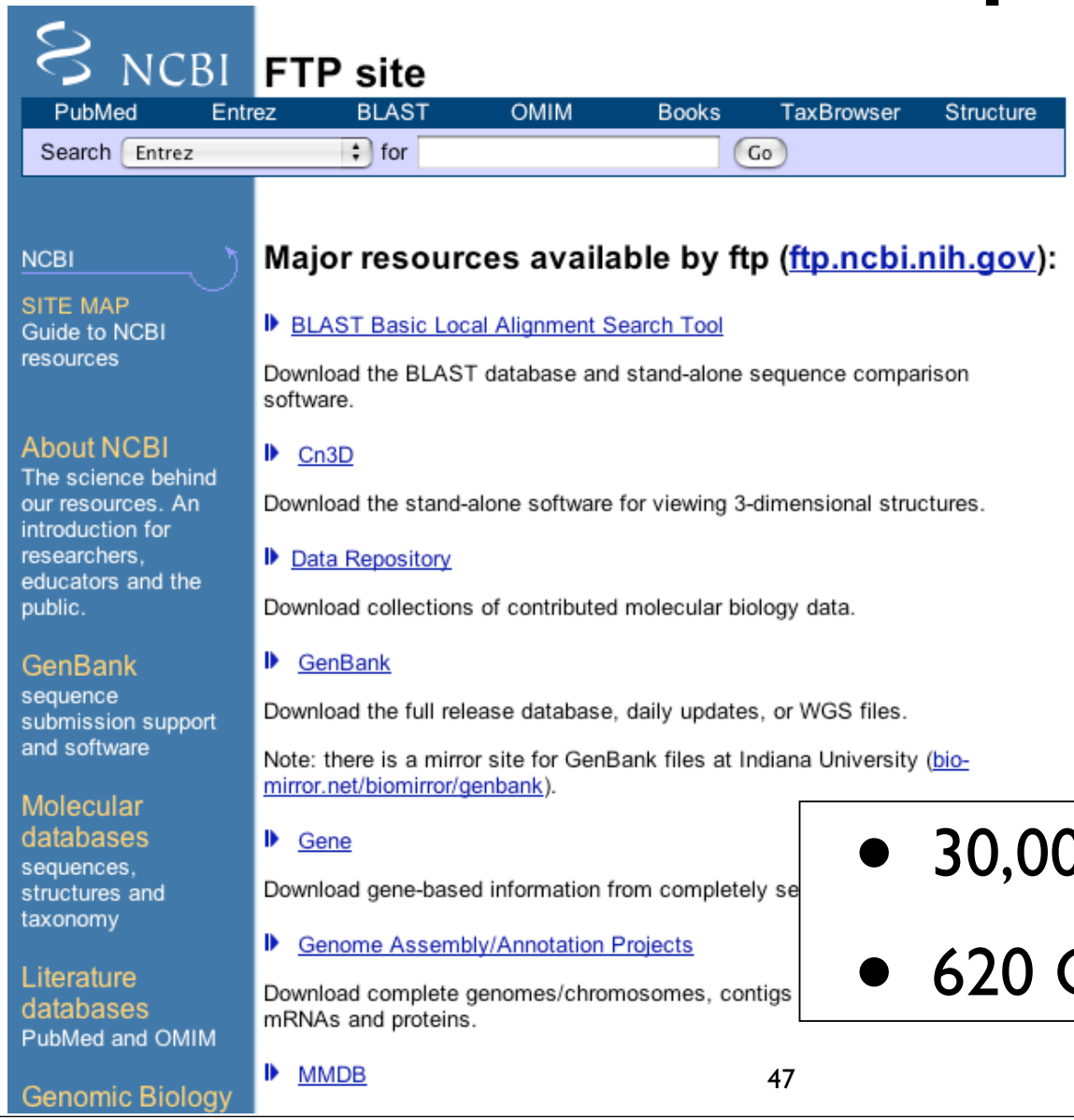


Number of Users and Hits Per Day




NCBI Site Map
Search NCBI
Resource Guide Complete resource listing and descriptions
Alphabetical List of major or commonly used resources
Entrez Database
Entrez Database subset (filtered query)
Nucleotides
GenBank
RefSeq (Reference Sequences)
dbEST (Expressed Sequence Tags)
dbGSS (Genome Survey Sequences)
dbMHC (Major Histocompatibility Complex)
dbSNP (Single Nucleotide Polymorphisms)
dbSTS (Sequence Tagged Sites)
Probe
TPA (Third Party Annotation Database)
Trace Archive
UniSTS (Sequence Tagged Sites)
PopSet (Evolutionary Relatedness)
UniVec (Vector Sequences)
WGS (Whole Genome Shotgun Sequences)
Proteins
RefSeq (Reference Sequences)
CDD (Conserved Domain Database)
Protein Clusters
MMDB (Molecular Modeling DataBase)
3D Domains
PubChem BioAssay
PubChem Compound
PubChem Substance
Gene
UniGene
HomoloGene
CCDS (Consensus CoDing Sequence)
GEO (Gene Expression Omnibus)
Entrez GEO Profiles
Entrez GEO DataSets
GENSAT
TaxBrowser
Entrez Taxonomy
Literature Databases
PubMed
PubMed Central
OMIM
Books
Molecular Databases
Nucleotide Sequences
Protein Sequences
Structures
Genes
Gene Expression
Taxonomy
Genomes
Entrez Genome
Entrez Genome Project
Map Viewer
Cancer Chromosomes
SKY/M-FISH & CGH Database
dbGAP (Genotypes and Phenotypes)
Databases
Query all Entrez Databases
Tools
Entrez Tools
Query all Entrez Databases
Entrez Data Model
My NCBI (Cubby)
LinkOut
Batch Entrez Nucleotides
Batch Entrez Proteins
Citation Matcher
Batch Citation Matcher
Entrez Utilities
Data Analysis Tools
Similarity Searching (BLAST)
Nucleotide Sequence Analysis
Protein Sequence Analysis and Proteomics
Molecular Structure Analysis
Genome Analysis
Gene Expression
FTP
Databases and Software
Programming Tools
Entrez Utilities
NCBI Toolbox
Book: NCBI C++ Toolkit
XML at NCBI
Information Engineering Branch
NCBI
About
Contact
Research
Software Engineering
Site Search
Data Submissions
BankIt (submissions and updates via WWW)
Sequin (software for submissions and updates)
dbEST (Expressed Sequence Tags)
dbGSS (Genome Survey Sequences)
dbSTS (Sequence Tagged Sites)
Genomes
Alignments
WGS (Whole Genome Shotgun Sequences)
TPA (Third Party Annotations)
Nucleotide Sequences
GenBank (general submission tools)
GenBank (specialized submissions)
Polymorphisms
dbSNP (Single Nucleotide Polymorphisms)
Expression
GEO (Gene Expression Omnibus)
Cytogenetic Data
SKY/M-FISH & CGH Database
Trace Data
Trace Archive
Education
News
NCBI News
What's New
Announcement Mailing Lists
RSS Feeds
Bookshelf
Coffee Break
Genes and Disease
NCBI Handbook
Resources
Tutorials, Courses, and more

The NCBI ftp site



- 30,000 files per day
- 620 Gigabytes per day

NCBI Databases & Services

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

Types of Databases

Primary Databases

- ✓ Original submissions by experimentalists
- ✓ Content controlled by the submitter
- ✓ Examples: GenBank, SNP, GEO

Derivative Databases

- ✓ Built from primary data
- ✓ Content controlled by third party (NCBI)
- ✓ Examples: Refseq, TPA, RefSNP, UniGene, NCBI Protein, Structure, Conserved Domain

What is GenBank?
NCBI's Primary Sequence Database

- Nucleotide only sequence database
- Archival in nature
- Historical
- Reflective of submitter point of view (subjective)
- Redundant

GenBank Data

✓ Direct submissions (traditional records)

✓ Batch submissions (EST, GSS, STS)

✓ ftp accounts (genome data)

DDBJ

EMBL

CIB

getentry

SRS

EBI

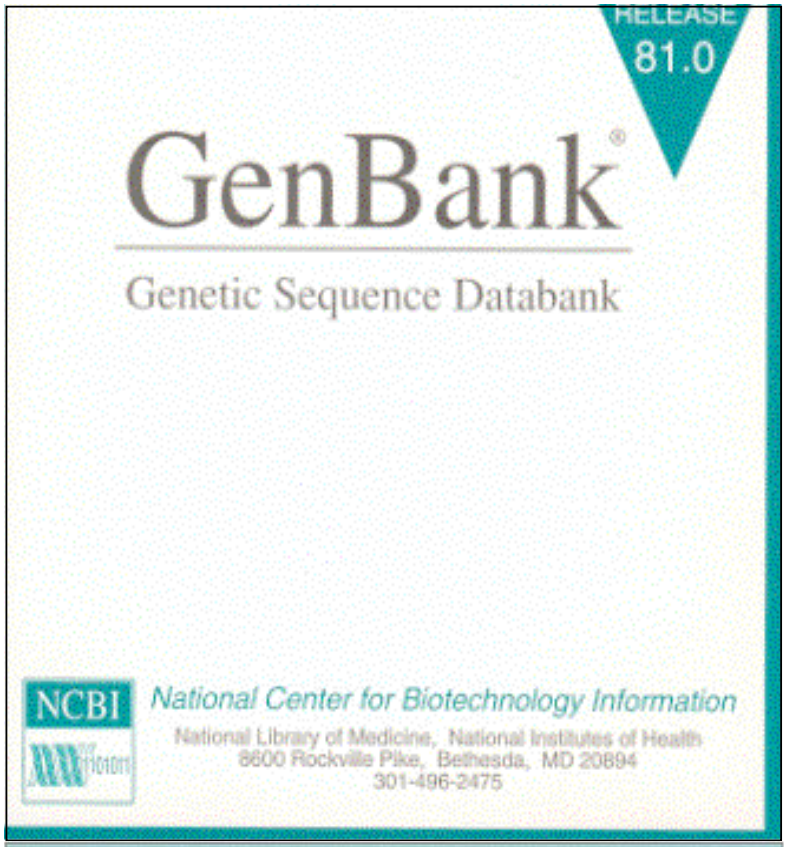
International Sequence Database Collaboration

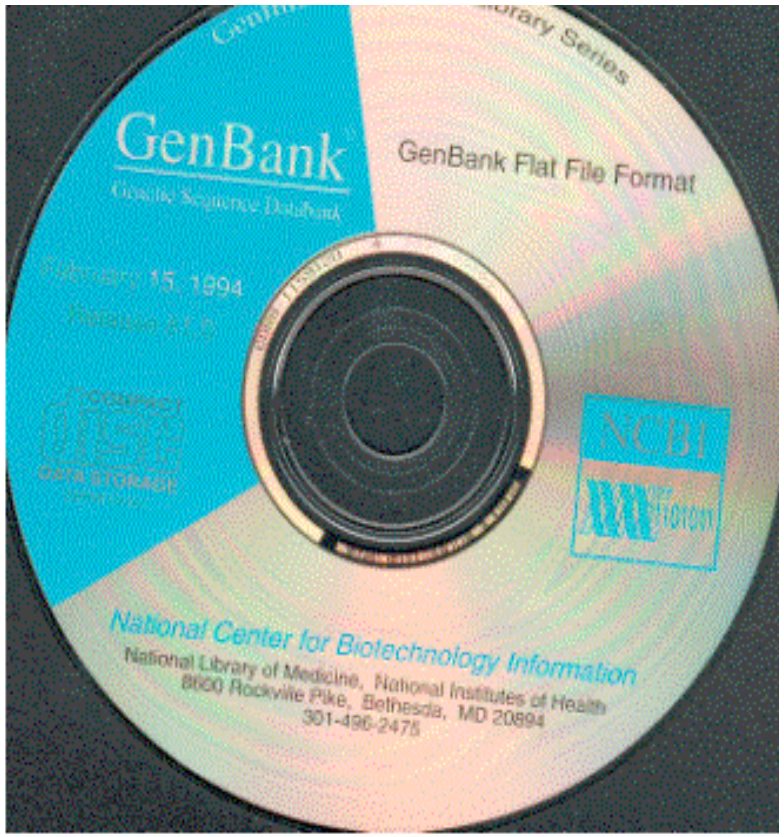
- submit anywhere
- daily updates

GenBank

Entrez

NCBI





GenBank: NCBI's Primary Sequence Database

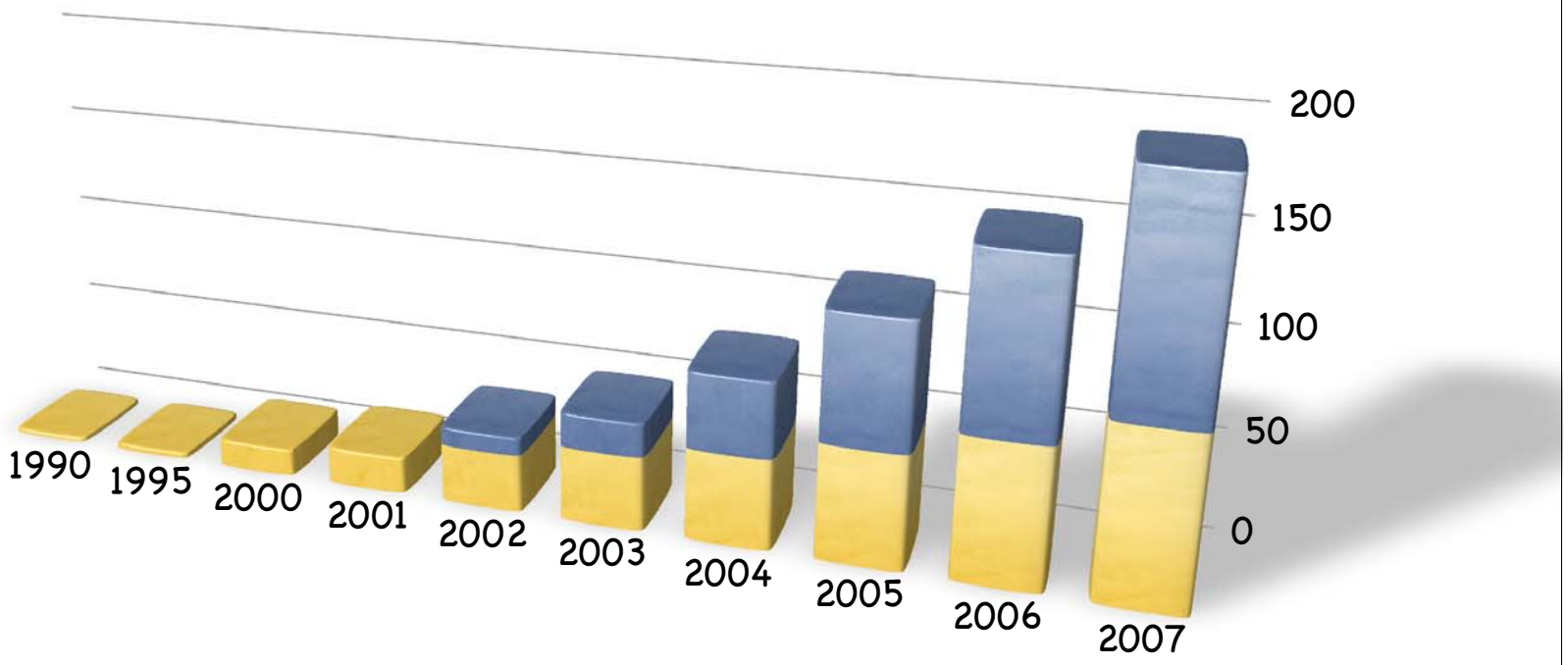
ftp://ftp.ncbi.nih.gov/genbank/

Release 161	August 2007
101,530,711	Records
181,489,883,388*	Total Bases

*includes WGS

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

Growth of GenBank



Current Release 163

Doubling time 12-14 months

Organization of GenBank

Records are divided into 18 Divisions.

12 Traditional

6 Bulk

- Traditional Divisions:

Direct Submissions (Sequin and BankIt)

Accurate

Well characterized

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

Organization of GenBank

Records are divided into 18 Divisions.

12 Traditional

6 Bulk

- BULK Divisions:

  Batch Submission (Email and FTP)

  Inaccurate

  Poorly characterized

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

Entrez query: gbdiv_xxx[Properties]

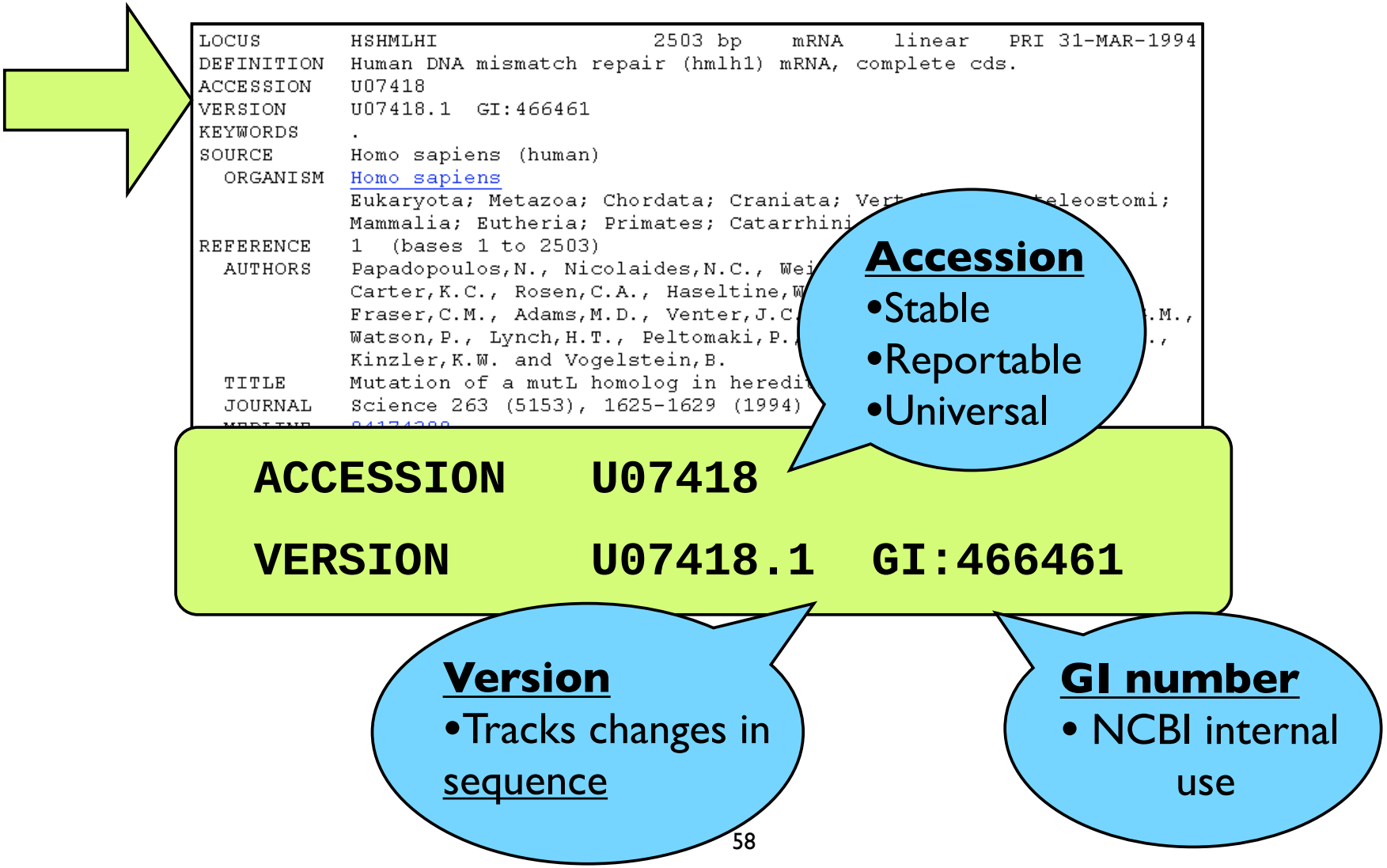
Header

Feature Table

Sequence

A Traditional GenBank Record

Traditional GenBank Record



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well annotated

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      241 ggatcaggaa agaagatctg gatattgtat gtgaaaggtt cactactagt aaactgcagt
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     1921 ctttggaaat tgatgaggaa gggaacctga ttggattacc ccttctgatt gacaactatg
     1981 tgcccccttt ggagggactg cctatcttca ttcttcgact agccactgag gtgaattggg
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     2101 ggaagcagta catatctgag gagtcgaccc tctcaggcca gcagagtgaa gtgcctggct
     2161 ccattccaaa ctcctggaag tggactgtgg aacacattgt ctataaagcc ttgcgctcac
     2221 acattctgcc tcctaaacat ttcacagaag atggaaatat cctgcagctt gctaacctgc
     2281 ctgatctata caaagtcttt gagaggtgtt aaatatggtt atttatgcac tgtgggatgt
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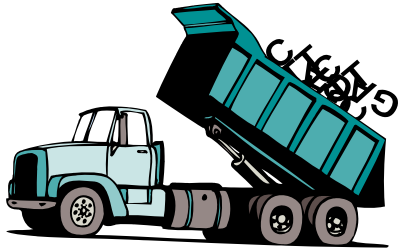
     2461 tacacagtgg attgattata aataaataga tgtgtcttaa cat
//
```

the sequence is the data

Primary vs. Derivative Databases



TATAGCCG

Sequencing Centers

ACGTGC

GenBank

Algorithms

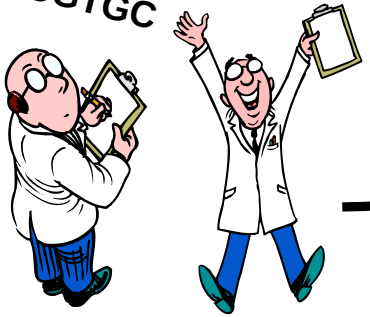
UniGene

Updated continually by NCBI

RefSeq: Annotation Pipeline

RefSeq: Entrez Gene and Genomes Pipelines

ACGTGC



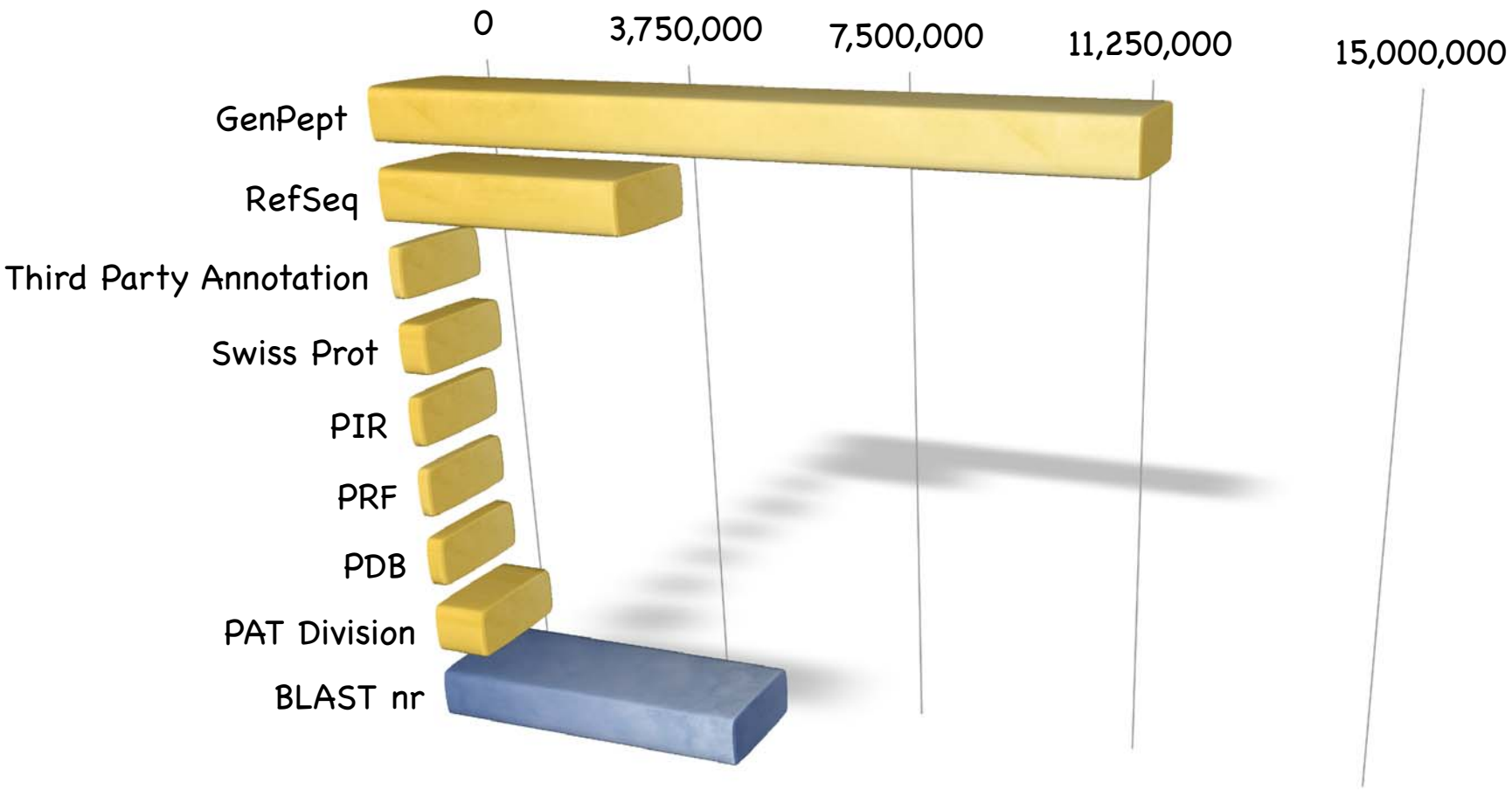
Labs

Curators

TATAGCCG
AGCTCCGATA
CCGATGACAA

Derivative Databases

Entrez Protein



GenPept

- GenBank CDS translations

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                     Number P14161) and S  ococcus pneumoniae (Swiss-Prot
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RefSeq

- The goal is to provide a comprehensive, standard dataset that represents sequence information for a species.
  - transcript, protein, assembled genomic (contigs), and chromosome records
  - known and predicted
  - reviewed
  - human, mouse, rat, fruit fly, zebrafish, arabidopsis, microbial genome (proteins), organelles, and more

RefSeq Accession Numbers

- mRNAs and Proteins

NM_123456	Curated mRNA
NP_123456	Curated Protein
NR_123456	Curated nc RNA
XM_123456	Predicted mRNA
XP_123456	Predicted Protein
XR_123456	Predicted nc RNA

- Genomic Records

NG_123456 Reference Genomic Sequence

- Chromosome

NC_123455 Microbial replicons, organelle, genomes, human chromosomes

- Assemblies

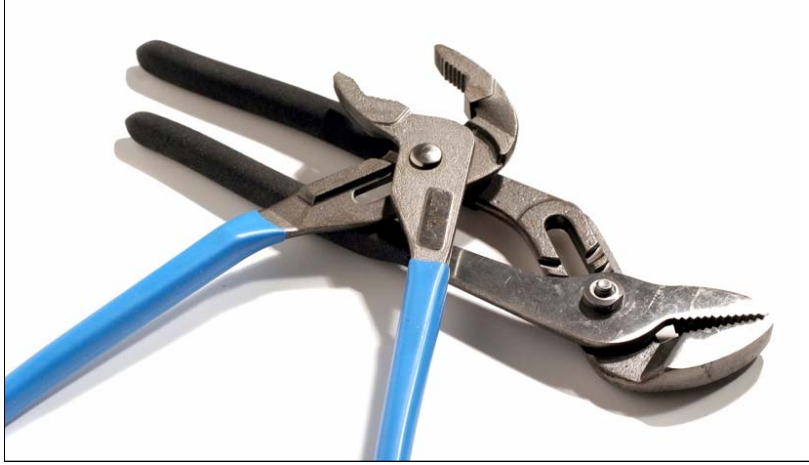
NT_123456	Contig
NW_123456	WGS Supercontig

Other NCBI Databases

Structure:	imported structures (PDB)	Cn3D viewer, NCBI curation
CDD:	conserved domain database	Protein families (COGs and KOGs); Single domains (PFAM, SMART, CD)
dbSNP:	nucleotide polymorphism	
Gene:	gene records	Unifies LocusLink and Microbial Genomes
HomoloGene:	homologs	neighboring function for Gene

Sequence Databases

GUIDED TOUR: Retrieving Data



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

⟷ WWW

⟷ Entrez

⟷ BLAST

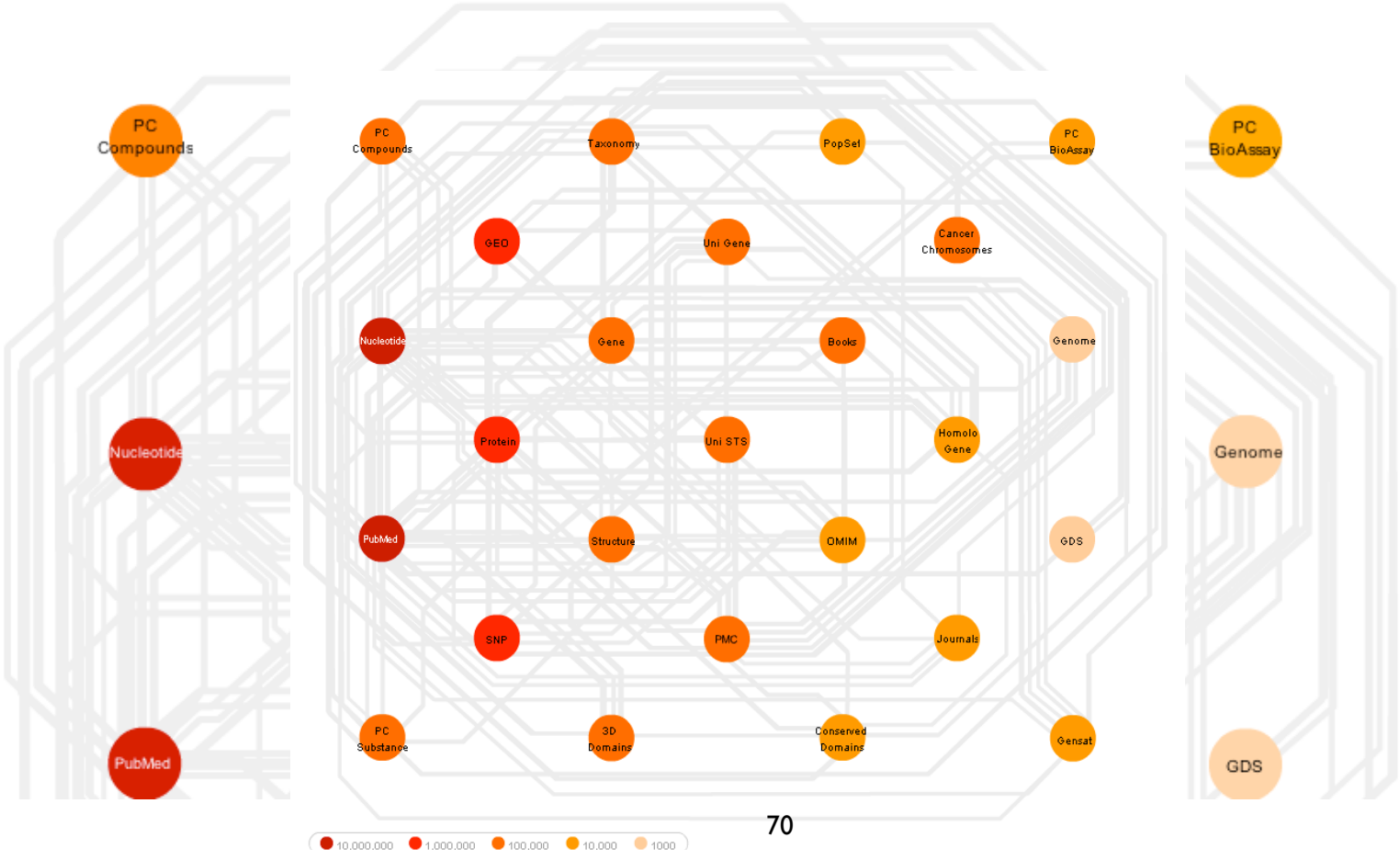
http://www.ncbi.nih.gov/Database/datamodel

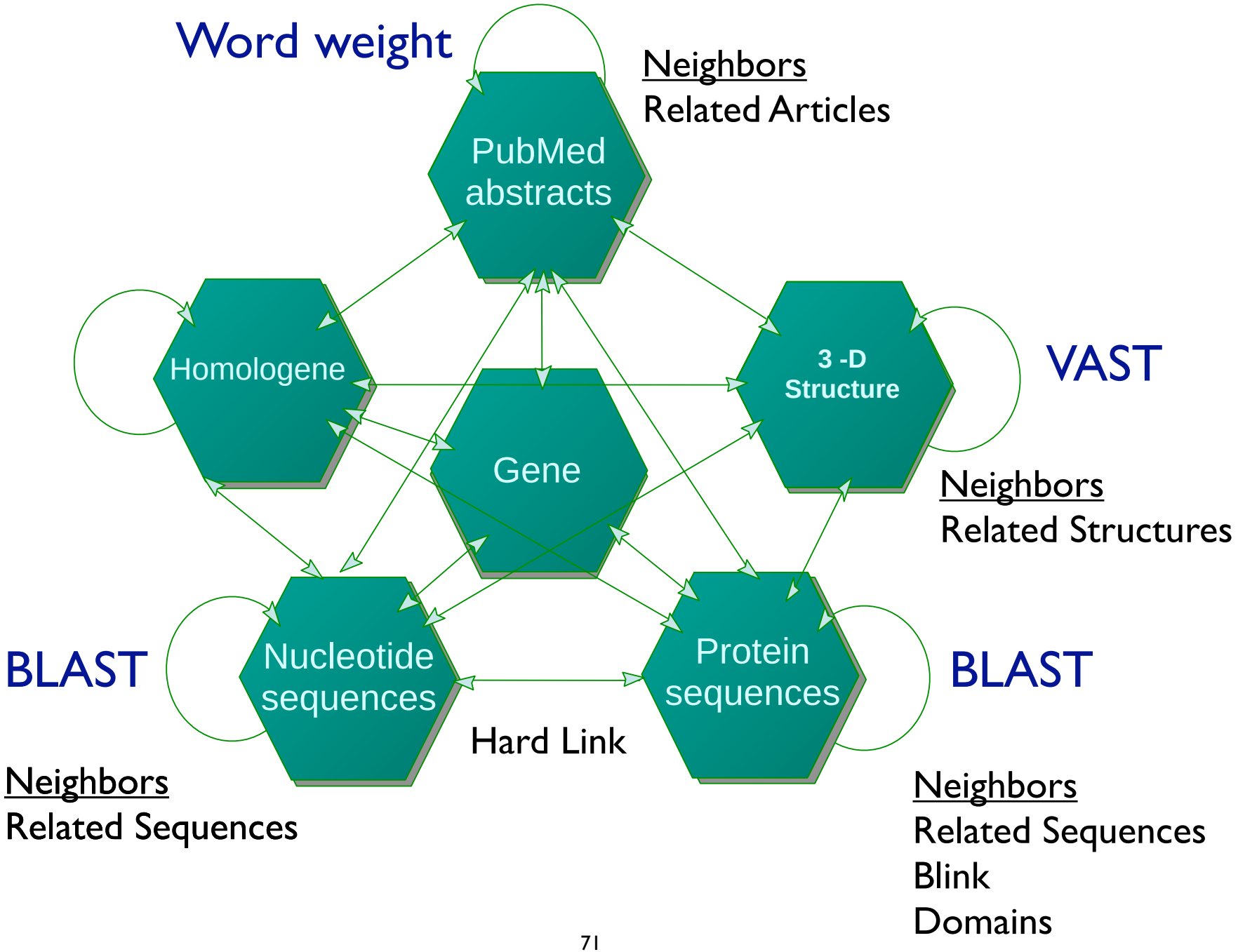
NCBI Model of Entrez Databases

Search Entrez for Go

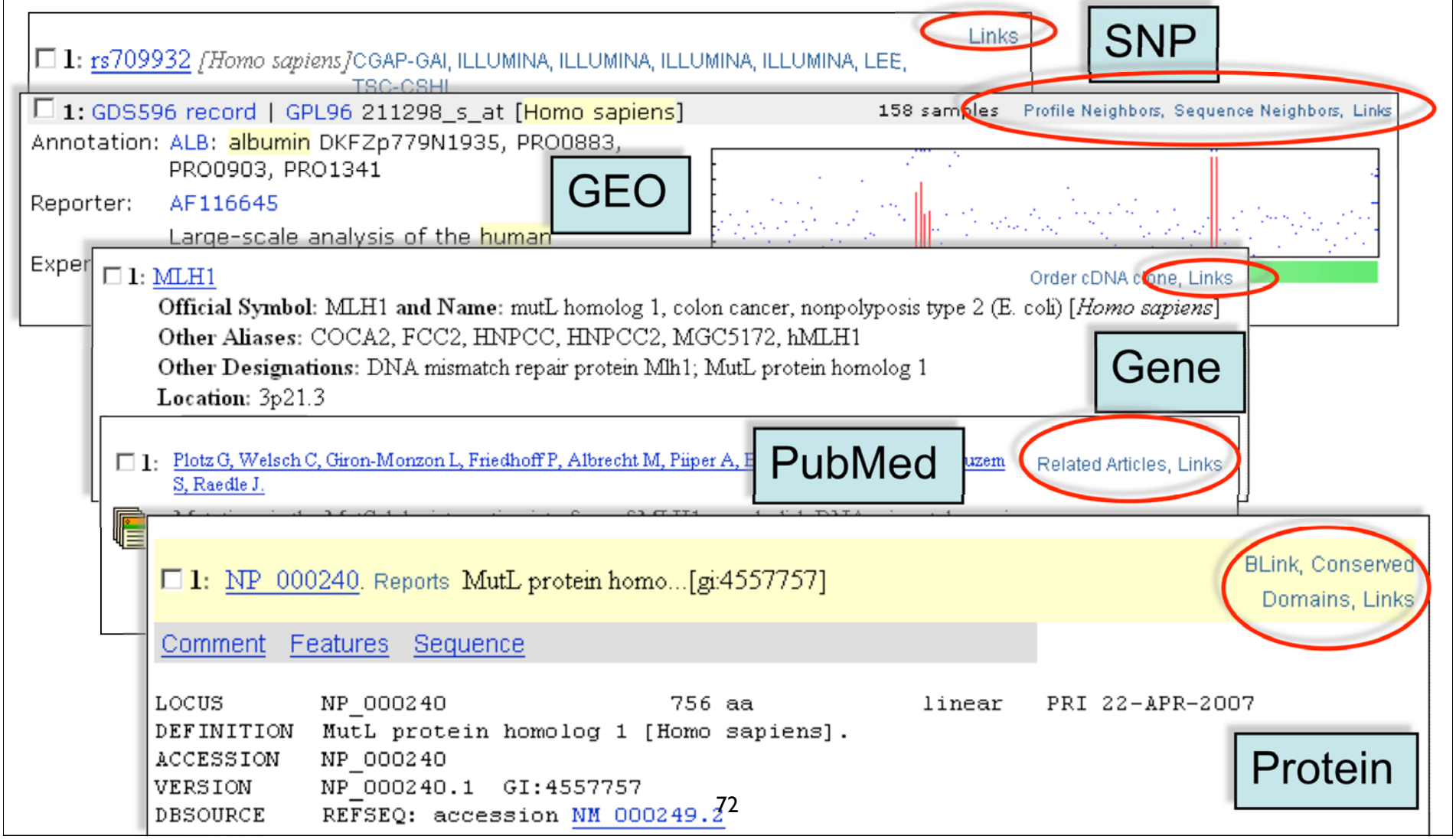
The diagram shows the Entrez databases and the connections between them. Each database is represented by a colored circle, where the color indicates the approximate number of records in the database. Mouse over a circle to see which databases are linked to the one selected, and how many links exist between those databases.

This diagram requires Flash for viewing.

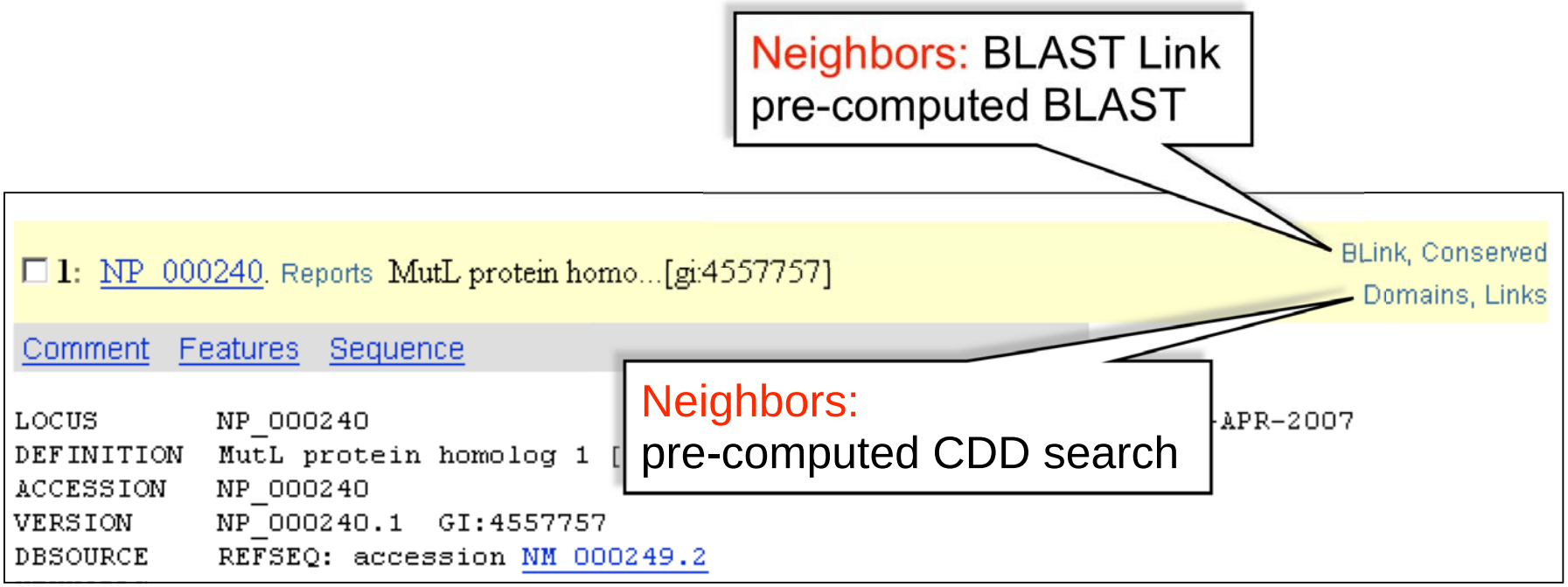



Word weight
Neighbors
Related Articles
PubMed abstracts
Homologene
Gene
3 -D Structure
VAST
Neighbors
Related Structures
BLAST
Nucleotide sequences
Protein sequences
BLAST
Hard Link
Neighbors
Related Sequences
Neighbors
Related Sequences
Blink
Domains

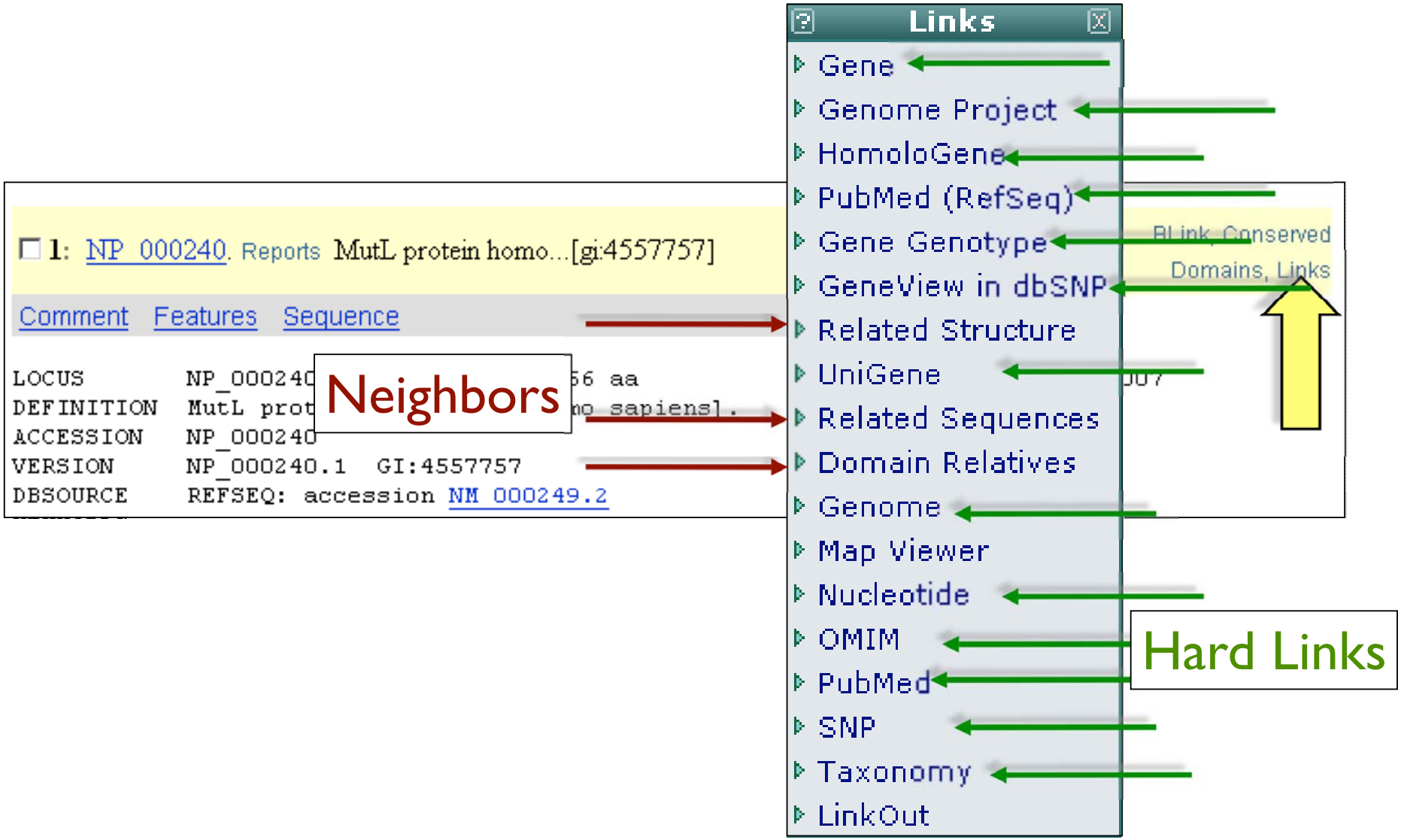
Neighbors in Entrez



Blink & Domains



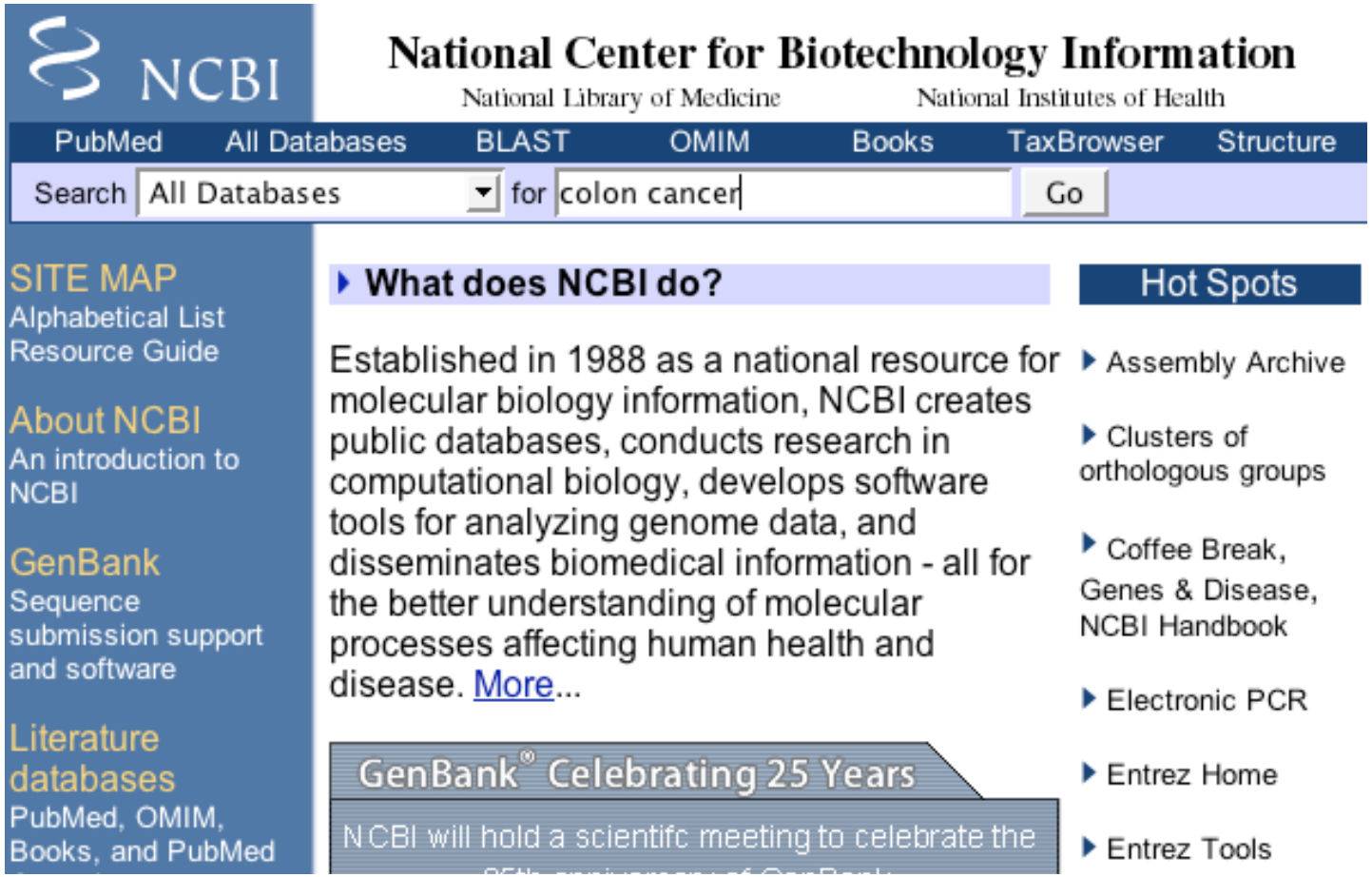
Links


Links
Gene
Genome Project
HomoloGene
PubMed (RefSeq)
Gene Genotype
GeneView in dbSNP
Related Structure
UniGene
Related Sequences
Domain Relatives
Genome
Map Viewer
Nucleotide
OMIM
PubMed
SNP
Taxonomy
LinkOut
Neighbors
Hard Links
1: NP_000240. Reports MutL protein homo...[gi:4557757]
Comment Features Sequence
LOCUS NP_000240
DEFINITION MutL prot
ACCESSION NP_000240
VERSION NP_000240.1 GI:4557757
DBSOURCE REFSEQ: accession NM_000249.2
BLink, Conserved Domains, Links

Database searching with Entrez

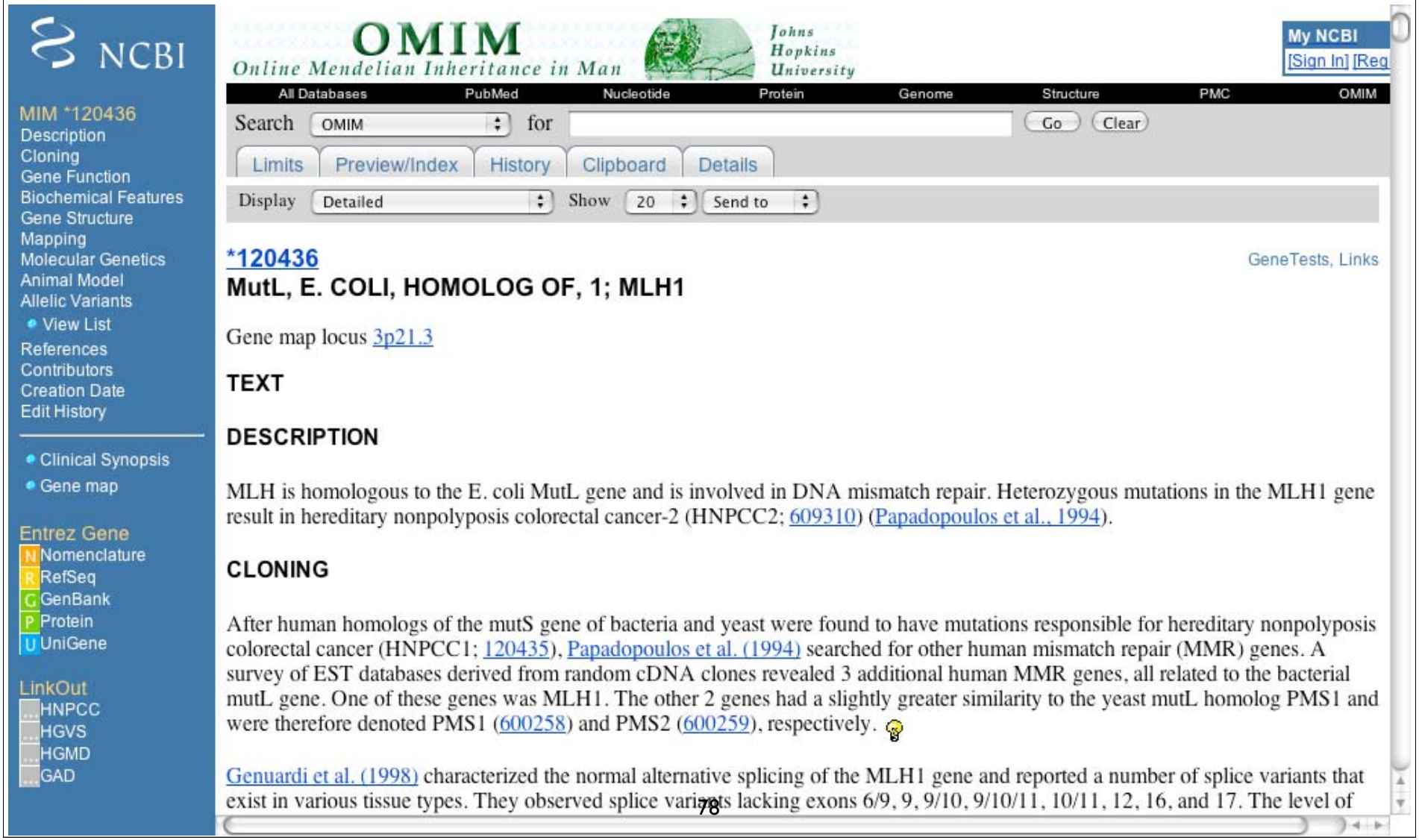
- **Scenario:** Let's find out more about the MutL gene
- Using limits and field restriction to find human MutL homolog
- Linking and neighboring with MutL

Start with a search for "colon cancer"

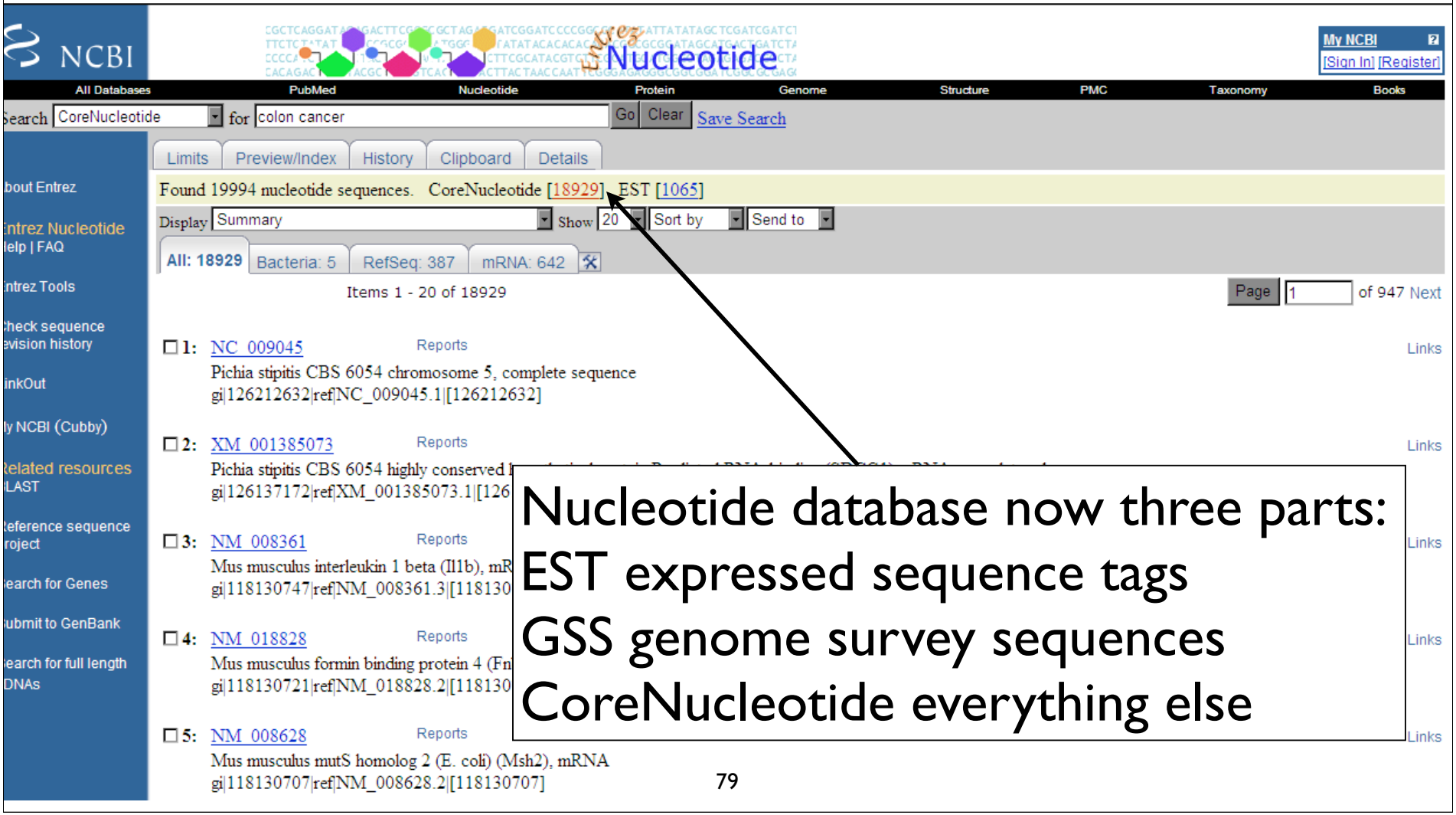



NCBI
Entrez, The Life Sciences Search Engine
HOME
SEARCH
SITE MAP
PubMed
All Databases
Human Genome
GenBank
Map Viewer
BLAST
Search across databases
colon cancer
GO
Clear
Help
- Result counts displayed in gray indicate one or more terms not found
58219 PubMed: biomedical literature citations and abstracts
7197 PubMed Central: free, full text journal articles
7 Site Search: NCBI web and FTP sites
894 Books: online books
374 OMIM: online Mendelian Inheritance in Man
none OMIA: online Mendelian Inheritance in Animals
19529 CoreNucleotide: Core subset of nucleotide sequence records
1156 EST: Expressed Sequence Tag records
none GSS: Genome Survey Sequence records
940 Protein: sequence database
6 Genome: whole genome sequences
2 Structure: three-dimensional macromolecular structures
none Taxonomy: organisms in GenBank
none SNP: single nucleotide polymorphism
493 Gene: gene-centered information
20 HomoloGene: eukaryotic homology groups
2 dbGaP: genotype and phenotype
160 UniGene: gene-oriented clusters of transcript sequences
6 CDD: conserved protein domain database
19 3D Domains: domains from Entrez Structure
34 UniSTS: markers and mapping data
2 PopSet: population study data sets
109008 GEO Profiles: expression and molecular abundance profiles
83 GEO DataSets: experimental sets of GEO data
123 Cancer Chromosomes: cytogenetic databases
4 PubChem BioAssay: bioactivity screens

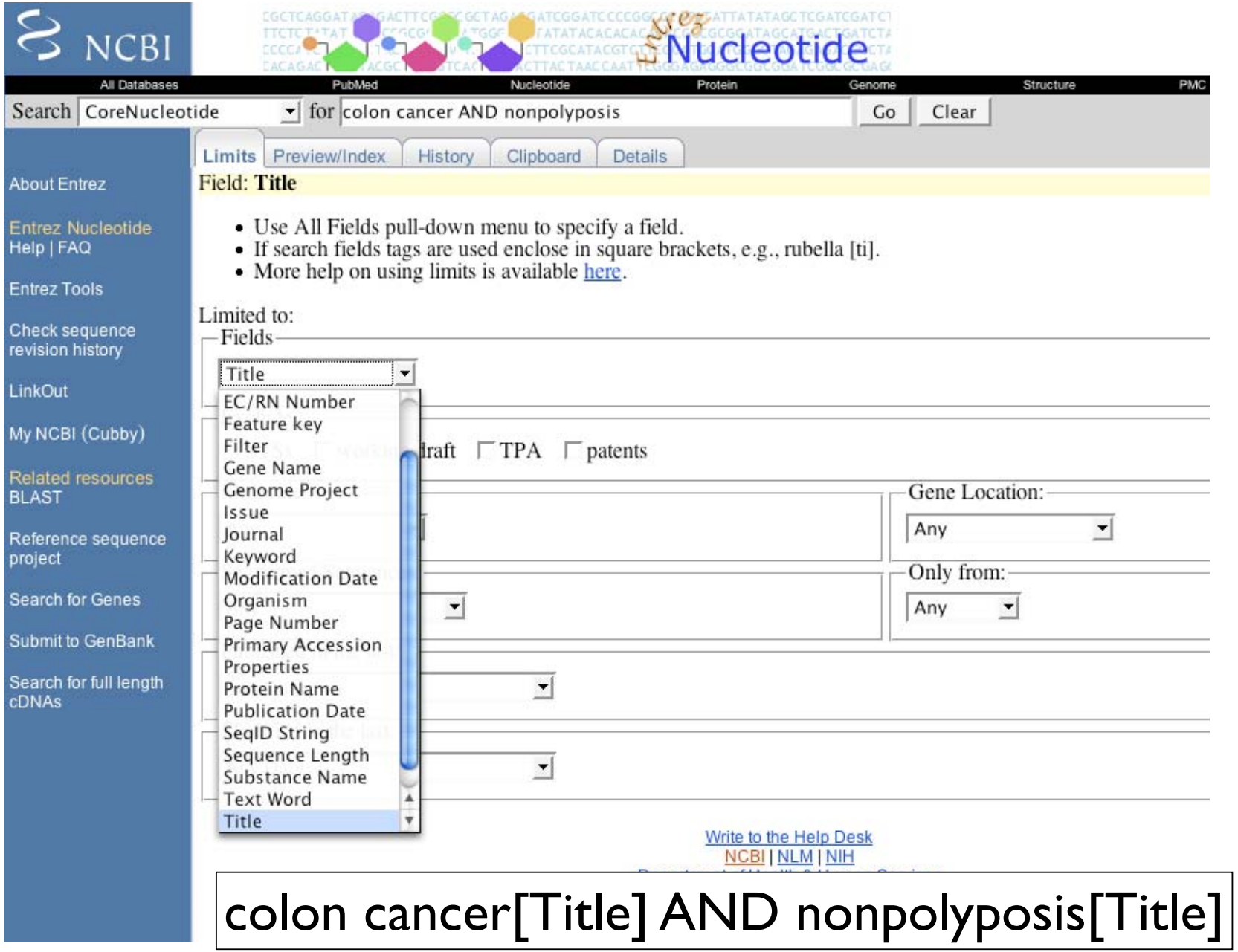
Human Disease Genes



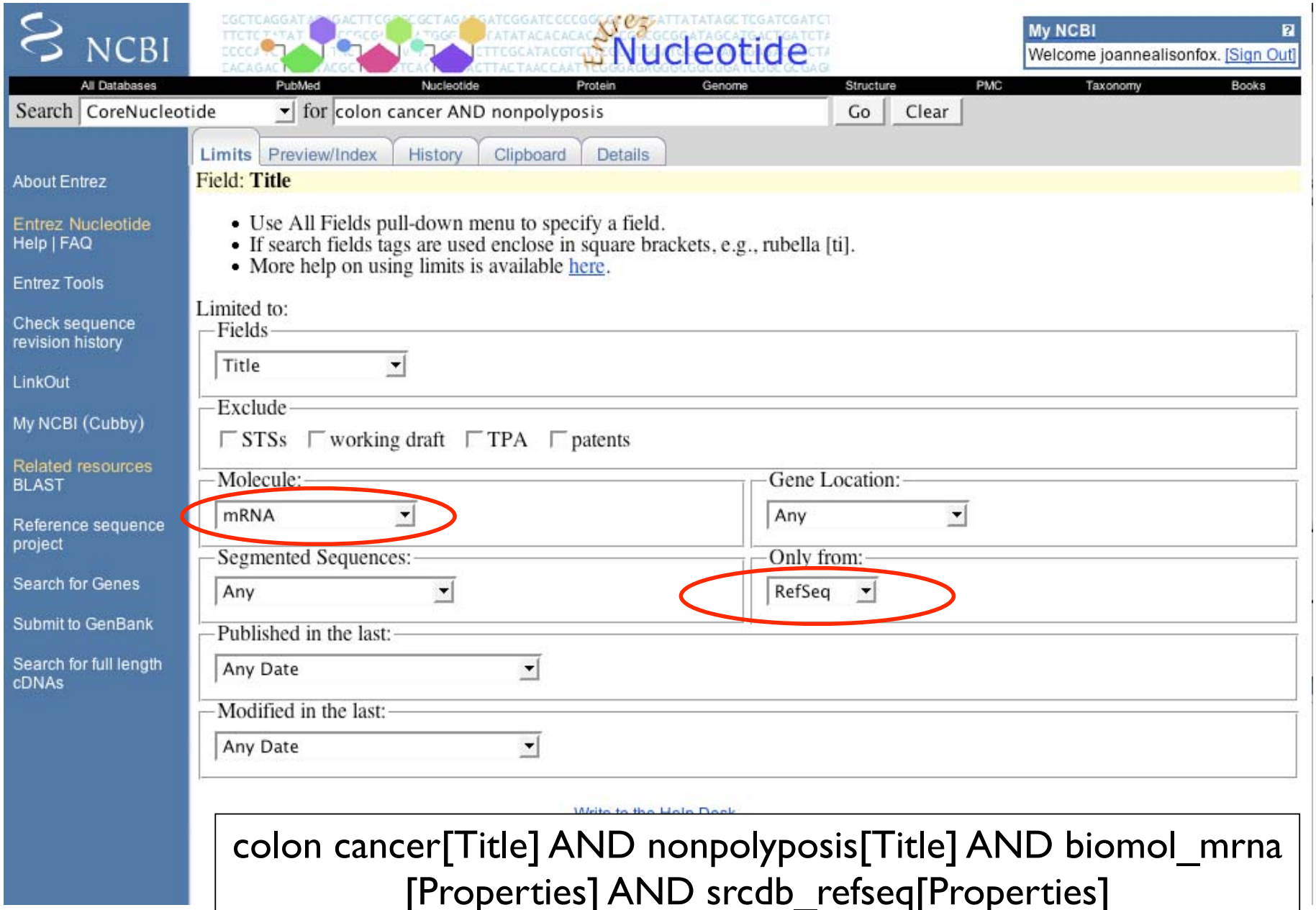
Search CoreNucleotide



Advanced Search Options



colon cancer[Title] AND nonpolyposis[Title]



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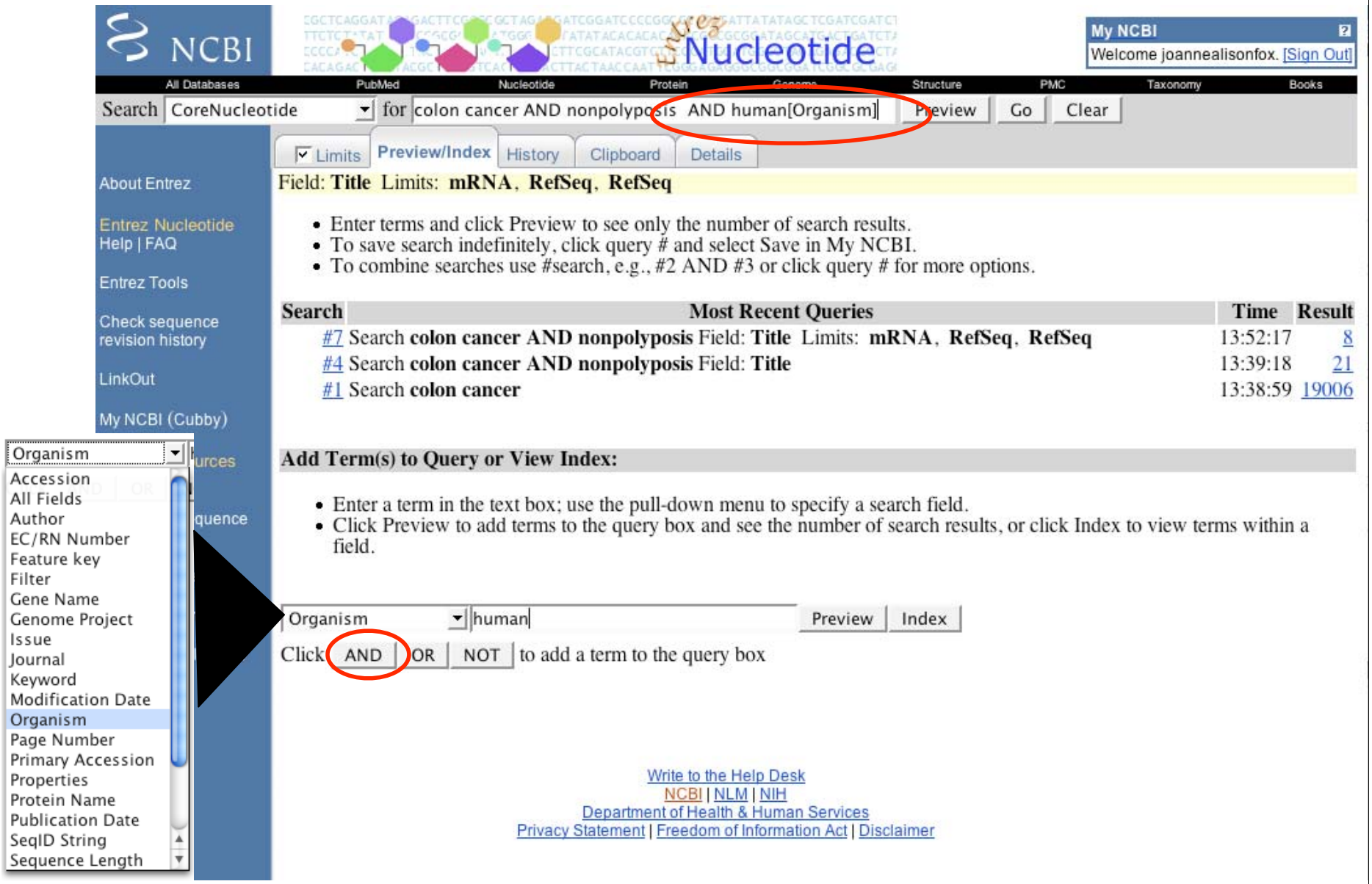
Advanced Search Options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Entrez Nucleotide
My NCBI
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All Databases PubMed Nucleotide Protein Genome Structure PMC Taxonomy Books
Search CoreNucleotide for colon cancer AND nonpolyposis AND human[Organism] Preview Go Clear
Limits Preview/Index History Clipboard Details
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Entrez Nucleotide Help | FAQ
Entrez Tools
Check sequence revision history
LinkOut
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Field: Title Limits: mRNA, RefSeq, RefSeq
Enter terms and click Preview to see only the number of search results.
To save search indefinitely, click query # and select Save in My NCBI.
To combine searches use #search, e.g., #2 AND #3 or click query # for more options.
Search Most Recent Queries Time Result
#7 Search colon cancer AND nonpolyposis Field: Title Limits: mRNA, RefSeq, RefSeq 13:52:17 8
#4 Search colon cancer AND nonpolyposis Field: Title 13:39:18 21
#1 Search colon cancer 13:38:59 19006
Add Term(s) to Query or View Index:
Enter a term in the text box; use the pull-down menu to specify a search field.
Click Preview to add terms to the query box and see the number of search results, or click Index to view terms within a field.
Organism
Accession
All Fields
Author
EC/RN Number
Feature key
Filter
Gene Name
Genome Project
Issue
Journal
Keyword
Modification Date
Organism
Page Number
Primary Accession
Properties
Protein Name
Publication Date
SeqID String
Sequence Length
Organism human Preview Index
Click AND OR NOT to add a term to the query box
Write to the Help Desk
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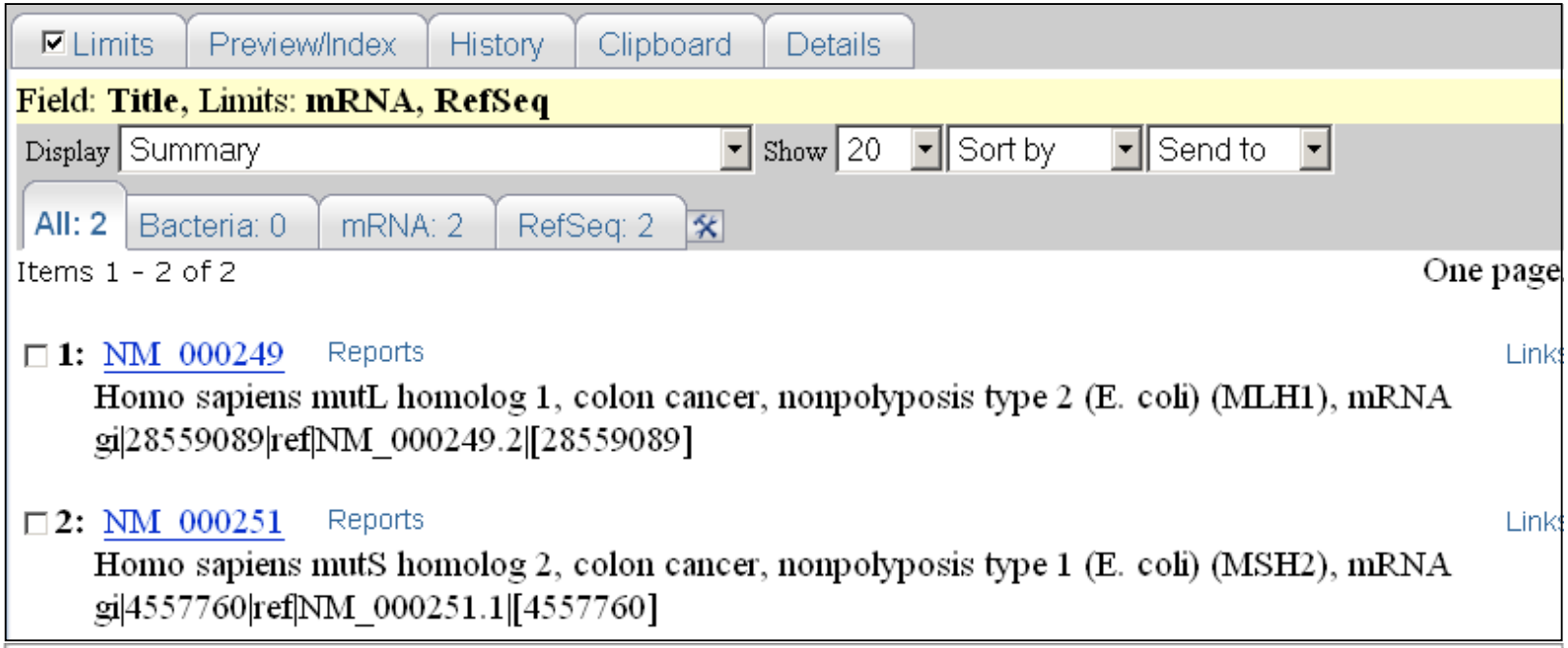
Refining your Search



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

Useful Field Restrictions

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

  `glyceraldehyde 3 phosphate dehydrogenase[Title]`

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

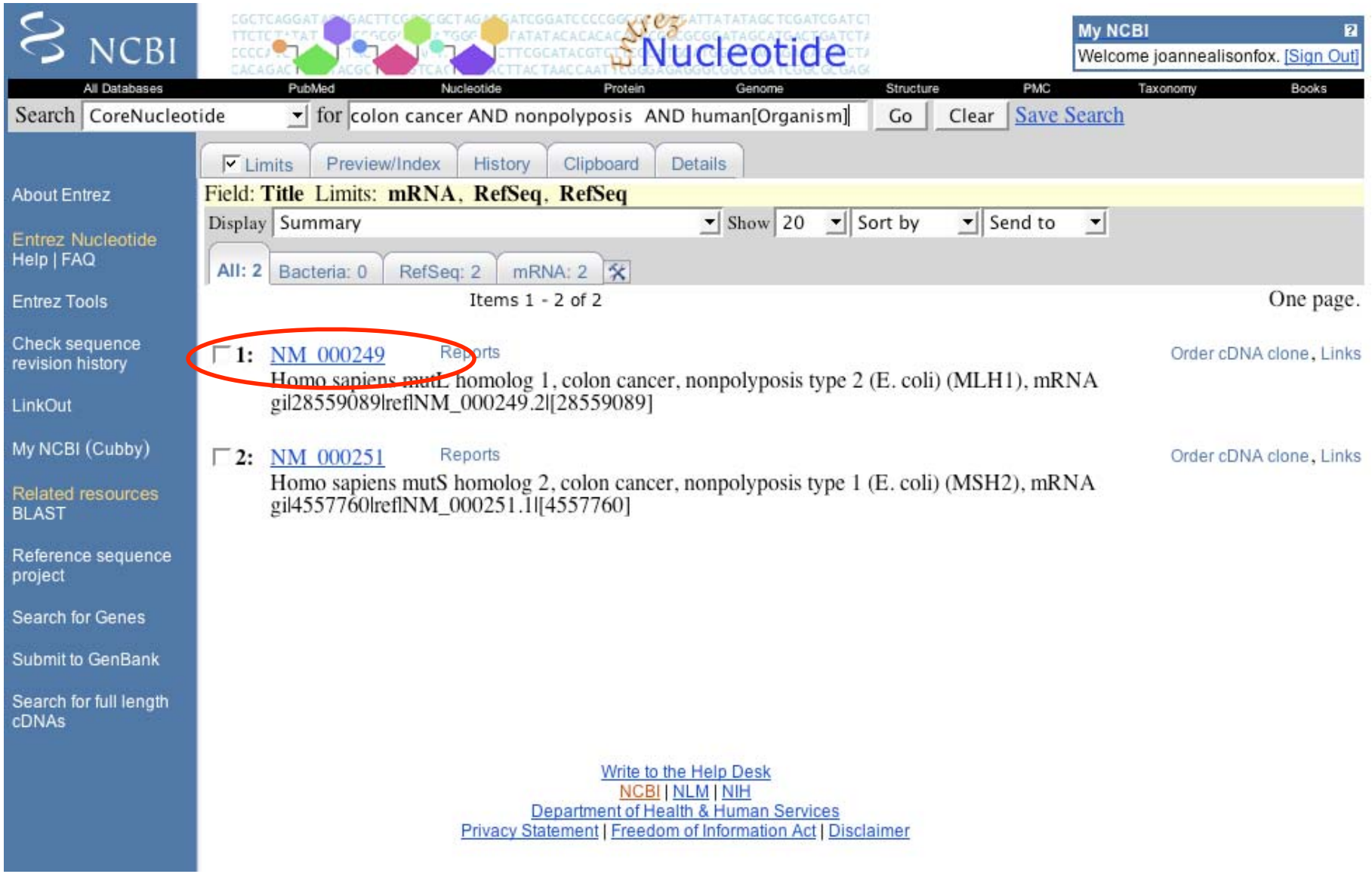
  `mouse[organism]; green plants[organism]; Streptomyces coelicolor [organism]`

- **[Properties]:** molecule type, location, database source

  `biomol_mrna[properties]; biomol_genomic[properties]; gene_in_mitochondrion[properties]; srcdb_pdb[properties]`

- **[Filter]:** subsets of data, Entrez links

  `all[filter]; nucleotide mapview[filter]; nucleotide_omim[filter]`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gi|28559089|ref|NM_000249.2|[28559089]
Order cDNA clone, Links
2: NM_000251 Reports
Homo sapiens mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli) (MSH2), mRNA
gi|4557760|ref|NM_000251.1|[4557760]
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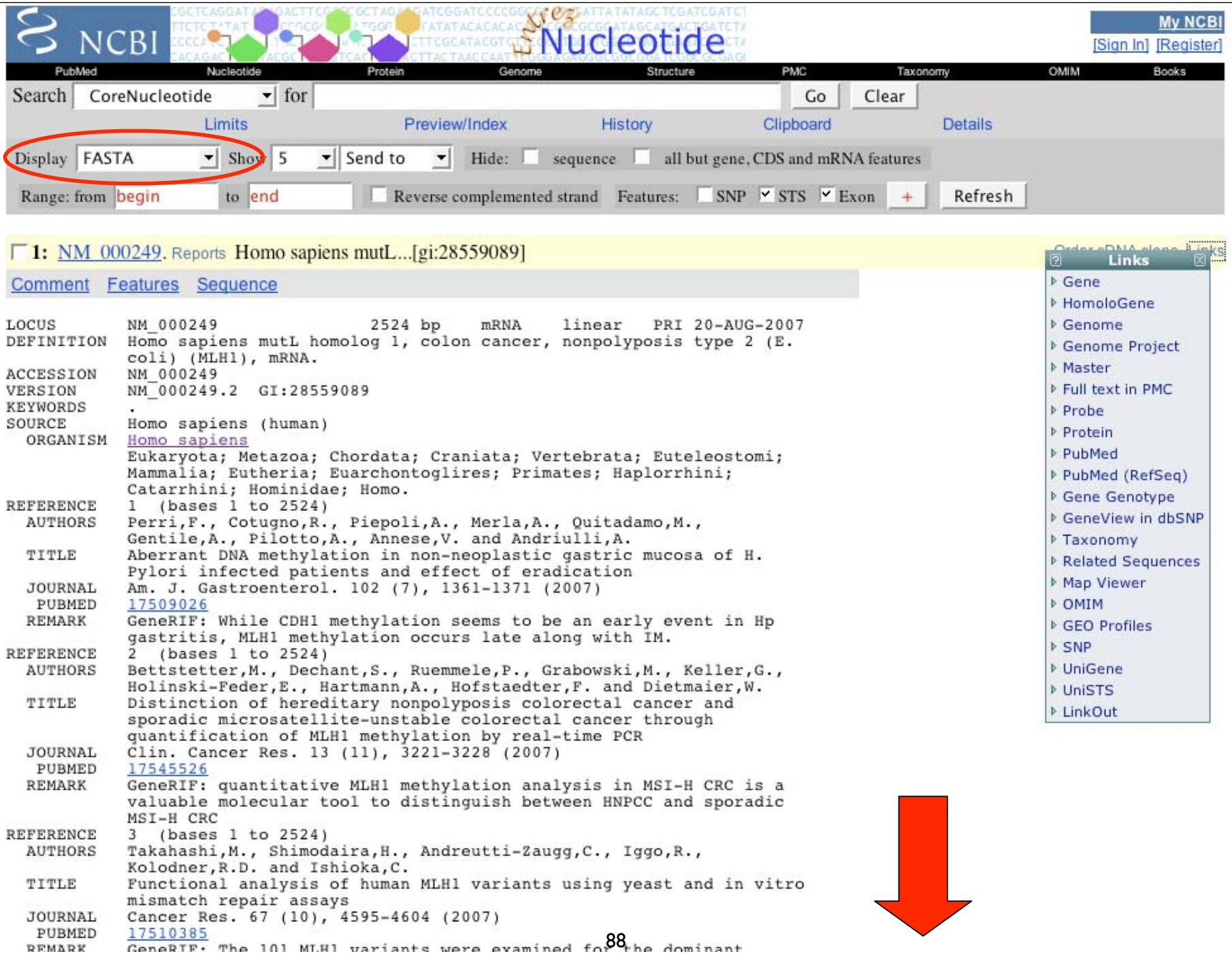
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Display FASTA Show 5 Send to Hide: sequence all but gene, CDS and mRNA features
Range: from begin to end Reverse complemented strand Features: SNP STS Exon + Refresh
1: NM_000249. Reports Homo sapiens mutL...[gi:28559089]
Comment Features Sequence
LOCUS NM_000249 2524 bp mRNA linear PRI 20-AUG-2007
DEFINITION Homo sapiens mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli) (MLH1), mRNA.
ACCESSION NM_000249
VERSION NM_000249.2 GI:28559089
KEYWORDS .
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 2524)
AUTHORS Perri,F., Cotugno,R., Piepoli,A., Merla,A., Quitadamo,M., Gentile,A., Pilotto,A., Annese,V. and Andriulli,A.
TITLE Aberrant DNA methylation in non-neoplastic gastric mucosa of H. Pylori infected patients and effect of eradication
JOURNAL Am. J. Gastroenterol. 102 (7), 1361-1371 (2007)
PUBMED 17509026
REMARK GeneRIF: While CDH1 methylation seems to be an early event in Hp gastritis, MLH1 methylation occurs late along with IM.
REFERENCE 2 (bases 1 to 2524)
AUTHORS Bettstetter,M., Dechant,S., Ruemmele,P., Grabowski,M., Keller,G., Holinski-Feder,E., Hartmann,A., Hofstaedter,F. and Dietmaier,W.
TITLE Distinction of hereditary nonpolyposis colorectal cancer and sporadic microsatellite-unstable colorectal cancer through quantification of MLH1 methylation by real-time PCR
JOURNAL Clin. Cancer Res. 13 (11), 3221-3228 (2007)
PUBMED 17545526
REMARK GeneRIF: quantitative MLH1 methylation analysis in MSI-H CRC is a valuable molecular tool to distinguish between HNPCC and sporadic MSI-H CRC
REFERENCE 3 (bases 1 to 2524)
AUTHORS Takahashi,M., Shimodaira,H., Andreutti-Zaugg,C., Iggo,R., Kolodner,R.D. and Ishioka,C.
TITLE Functional analysis of human MLH1 variants using yeast and in vitro mismatch repair assays
JOURNAL Cancer Res. 67 (10), 4595-4604 (2007)
PUBMED 17510385
REMARK GeneRIF: The 101 MLH1 variants were examined for the dominant
Links
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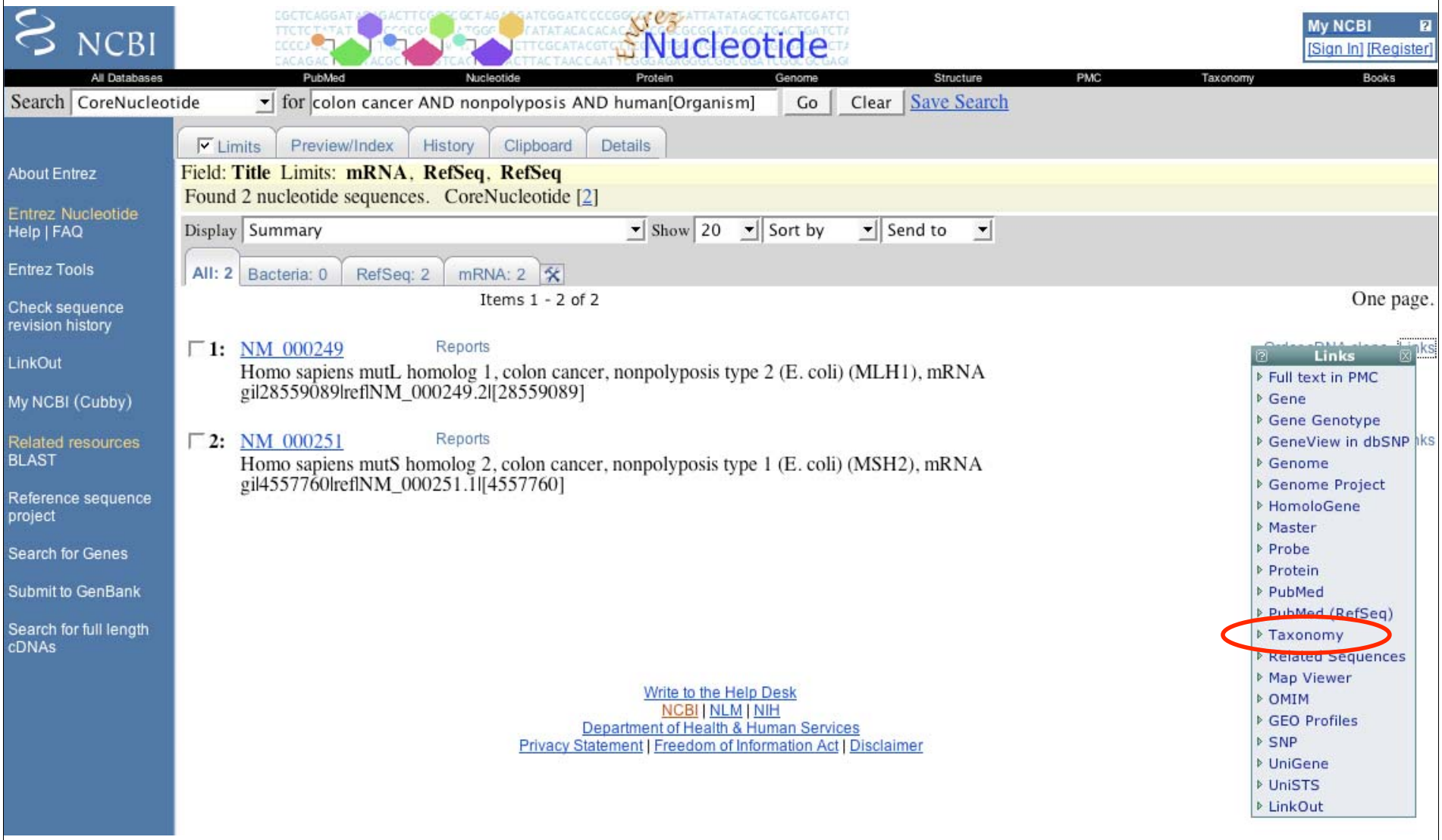
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gi|28559089|ref|NM_000249.2|[28559089]
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Homo sapiens mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli) (MSH2), mRNA
gi|4557760|ref|NM_000251.1|[4557760]
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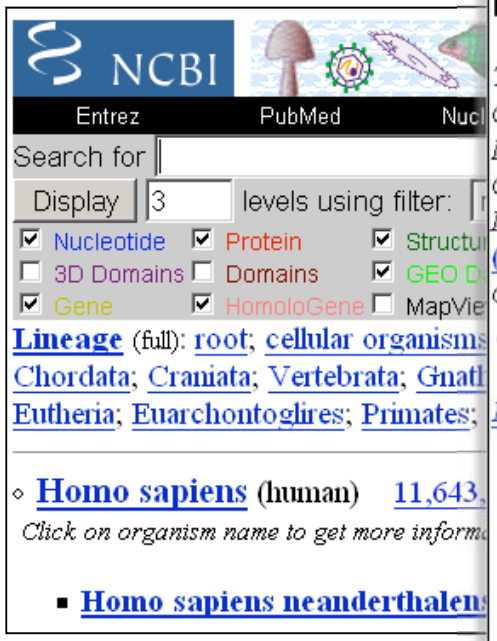
Taxonomy



All molecular databases

Homo sapiens

Taxonomy ID: 9606
Genbank common name: **human**
Rank: species
Genetic code: Translation table 1 (Standard)
Mitochondrial genetic code: Translation table 2 (Vertebrate Mitochondrial)
Other names:
common name: **man**

Lineage (full)
cellular organisms; Eukaryota; Fungi/Metazoa group; Metazoa; Eumetazoa; Bilateria; Coelomata; Deuterostomia; Chordata; Craniata; Vertebrata; Gnathostomata; Teleostomi; Euteleostomi; Sarcopterygii; Tetrapoda; Amniota; Mammalia; Theria; Eutheria; Euarchontoglires; Primates; Haplorrhini; Simiiformes; Catarrhini; Hominoidea; Hominidae; Homo/Pan/Gorilla group; Homo

Entrez records

Database name	Subtree links	Direct links
Nucleotide	11,643,469	11,642,134
Protein	392,990	392,989
Structure	9,472	9,472
Genome Sequences	51	51
Genome Projects	1	1
Popset	20,878	20,878
SNP	11,870,024	11,870,024
3D Domains	35,848	35,848
Domains	19	19
GEO Datasets	3,525	3,525
GEO Expressions	10,649,715	10,649,715
UniGene	124,179	124,179
UniSTS	322,789	322,789
PubMed Central	3,586	3,586
Gene	38,624	38,624
HomoloGene	20,167	20,167
Taxonomy	2	1

Genome Information

See the NCBI Genome homepage
Go to NCBI genomic BLAST page for Homo sapiens

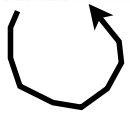
Genome view: 24 chromosomes

Names	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y

Goal: Find MLH1 homologs

- Tip: Use Entrez Gene as your hub to connect to everything else!

Protein



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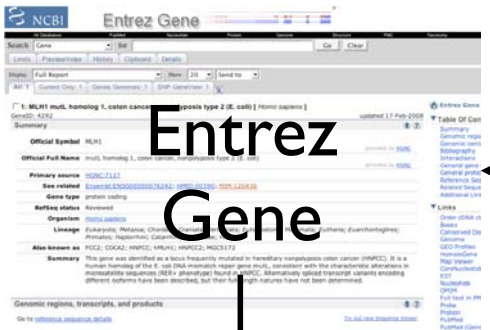
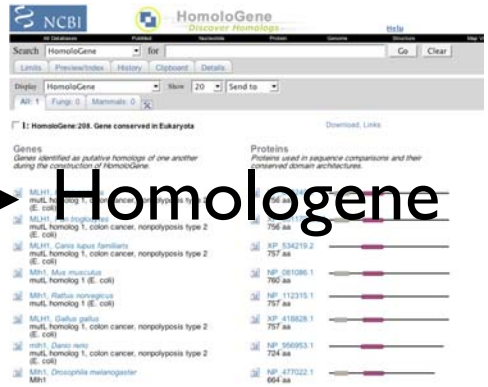


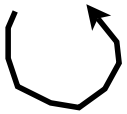
Entrez Gene

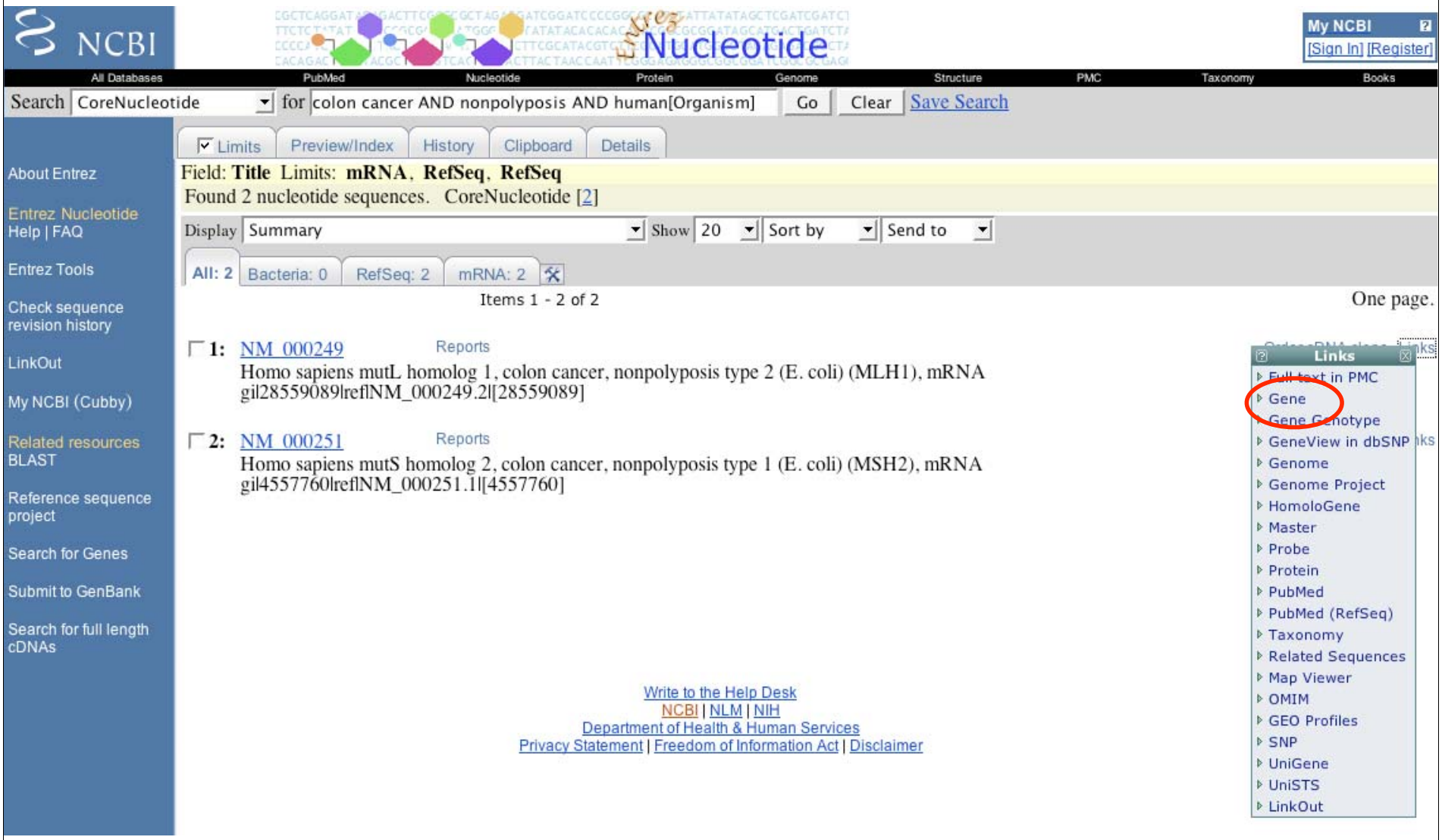
Other Entrez Databases



Homologene

Gene neighbors

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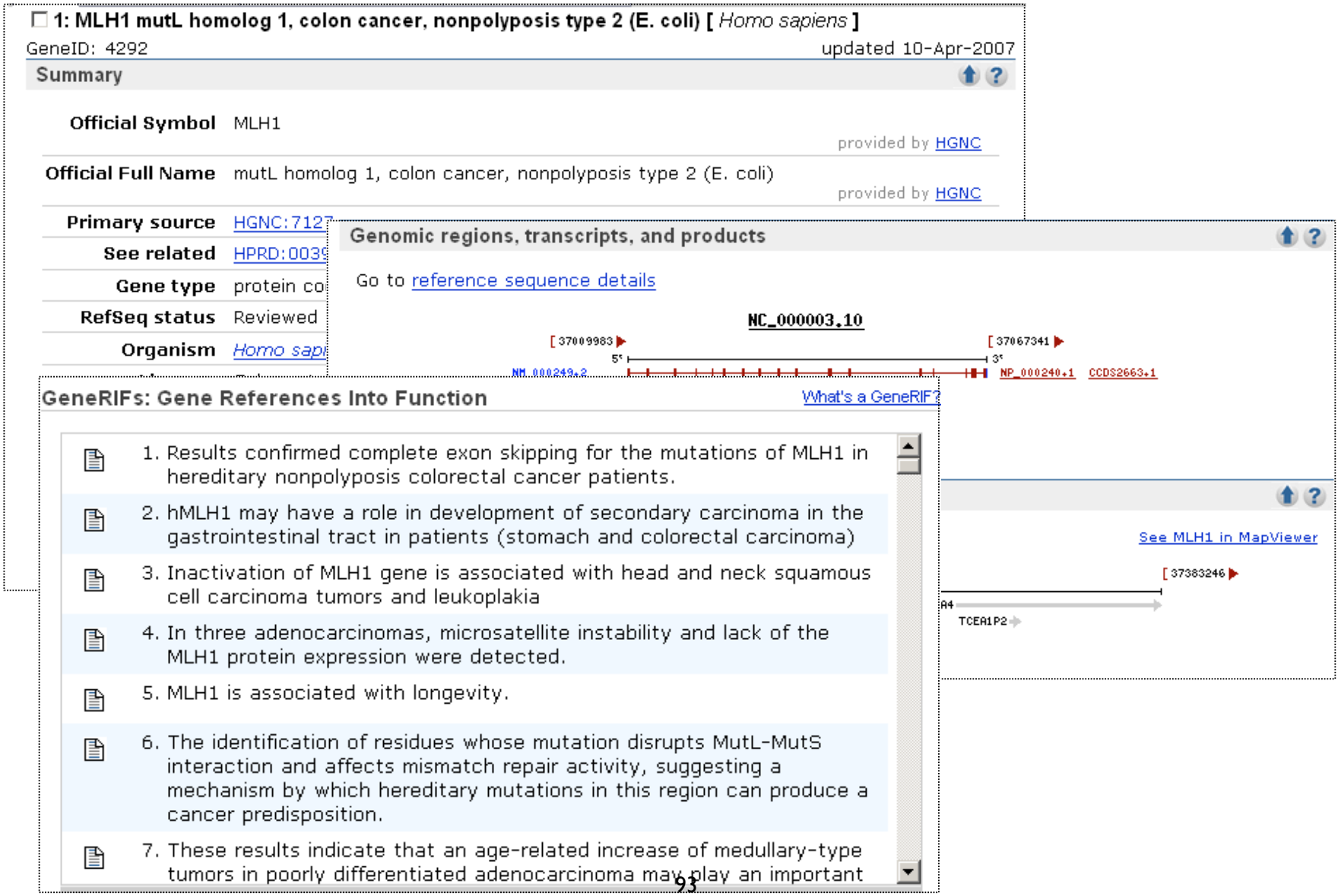
MLH1 Gene Record



Interactions + GO

Interactions

Description .....

Product	Interactant	Other Gene	Complex	Source	P
E2F1 interacts with the MLH1 promoter.					
NC_000003.9	NP_005216.1	E2F1		BIND	
E2F4 interacts with the MLH1 promoter region.					
NC_000003.9	NP_001941.2	E2F4		BIND	
NP_000240.1	NP_000048.1	BLM		HPRD	
MLH1 interacts with BLM.					
NP_000240.1	NP_000048.1	BLM		BIND	
NP_000240.1	NP_009225.1	BRCA1		HPRD	
The exonuclease HEX1 interacts with the mismatch repair protein hMLH1.					
NP_000240.1	NP_003677.3	EXO1		BIND	
The exonuclease hEXO1b interacts with the mismatch repair protein hMLH1.					
NP_000240.1	NP_006018.3	EXO1		BIND	
NP_000240.1	NP_569082.1	EXO1		HPRD	
NP_000240.1	NP_003916.1	MBD4		HPRD	
MLH1 and interacts with MED1.					
NP_000240.1	NP_003916.1	MBD4		BIND	
NP_000240.1	BAA92353.1	MLH3		HPRD	

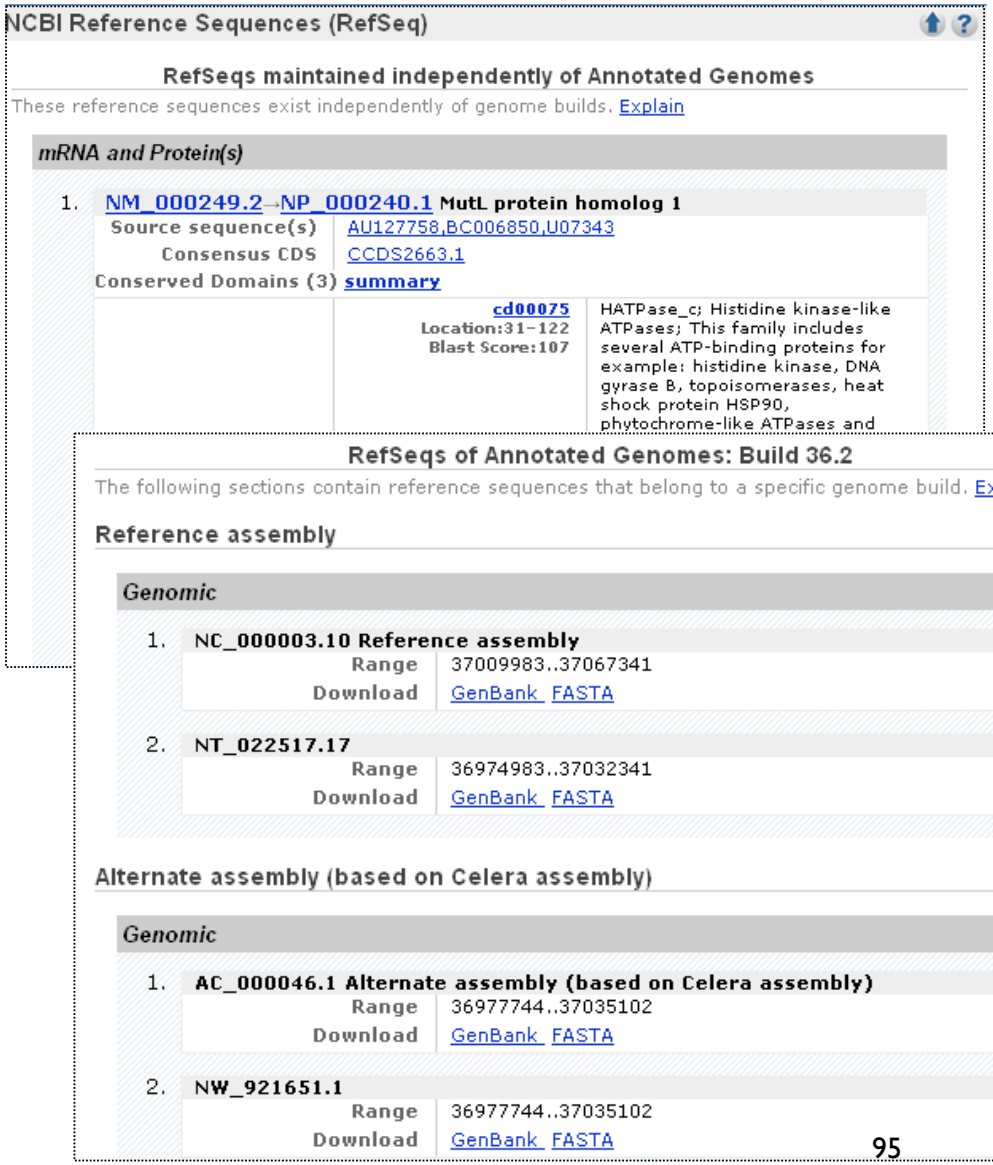
GeneOntology Provided by GOA

Function	Evidence	
ATP binding	IEA	
contributes_to MutSalpha complex binding	IDA	Pubmed
guanine/thymine mispair binding	IMP	Pubmed
guanine/thymine mispair binding	IEA	
mismatched DNA binding	IEA	
protein binding	IPI	Pubmed
contributes_to single-stranded DNA binding	IDA	Pubmed

Process	Evidence	
DNA damage response, signal transduction resulting in induction of apoptosis	IEA	
cell cycle	IEA	
male meiosis chromosome segregation	IEA	
meiotic recombination	IEA	
mismatch repair	IEA	
mismatch repair	TAS	Pubmed
negative regulation of mitotic recombination	IEA	
negative regulation of progression through cell cycle	IEA	

Component	Evidence	
MutLalpha complex	IEA	
condensed chromosome	IEA	
nucleus	IC	Pubmed
nucleus	IEA	
synaptonemal complex	IEA	

Sequences



NCBI Reference Sequences (RefSeq)

RefSeqs maintained independently of Annotated Genomes

These reference sequences exist independently of genome builds. Explain

mRNA and Protein(s)

1. NM_000249.2→NP_000240.1 MutL protein homolog 1

Source sequence(s) AU127758,BC006850,U07343

Consensus CDS CCDS2663.1

Conserved Domains (3) summary

cd00075 Location:31–122 Blast Score:107 — HATPase_c; Histidine kinase-like ATPases; This family includes several ATP-binding proteins for example: histidine kinase, DNA gyrase B, topoisomerases, heat shock protein HSP90, phytochrome-like ATPases and

RefSeqs of Annotated Genomes: Build 36.2

The following sections contain reference sequences that belong to a specific genome build. Explain

Reference assembly

Genomic

1. NC_000003.10 Reference assembly
   - Range 37009983..37067341
   - Download GenBank FASTA
2. NT_022517.17
   - Range 36974983..37032341
   - Download GenBank FASTA

Alternate assembly (based on Celera assembly)

Genomic

1. AC_000046.1 Alternate assembly (based on Celera assembly)
   - Range 36977744..37035102
   - Download GenBank FASTA
2. NW_921651.1
   - Range 36977744..37035102
   - Download GenBank FASTA

Related Sequences

Nucleotide		Protein
Genomic	AC006583.31 (69181..100370, complement)	None
Genomic	AC011816.17 (143145..169313)	None
Genomic	AY217549.1	AAO22994.1
Genomic	AY344475.1	AAQ23474.1
Genomic	AY706914.1	AAU21566.1
Genomic	CH471055.1	EAW64483.1
		EAW64484.1
		EAW64485.1
Genomic	U17839.1	AAA85687.1
Genomic	U17840.1	AAA85687.1
Genomic	U17841.1	AAA85687.1
Genomic	U17842.1	AAA85687.1
Genomic	U17843.1	AAA85687.1
Genomic	U17844.1	AAA85687.1
Genomic	U17845.1	AAA85687.1
Genomic	U17846.1	AAA85687.1
Genomic	U17847.1	AAA85687.1
Genomic	U17848.1	AAA85687.1
Genomic	U17849.1	AAA85687.1
Genomic	U17850.1	AAA85687.1
Genomic	U17851.1	AAA85687.1
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Genomic	U17853.1	AAA85687.1
Genomic	U17854.1	AAA85687.1
Genomic	U17855.1	AAA85687.1
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Genomic	U17857.1	AAA85687.1
Genomic	U40978.1	AAA82079.1
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mRNA	AY517558.1	AAT44531.1
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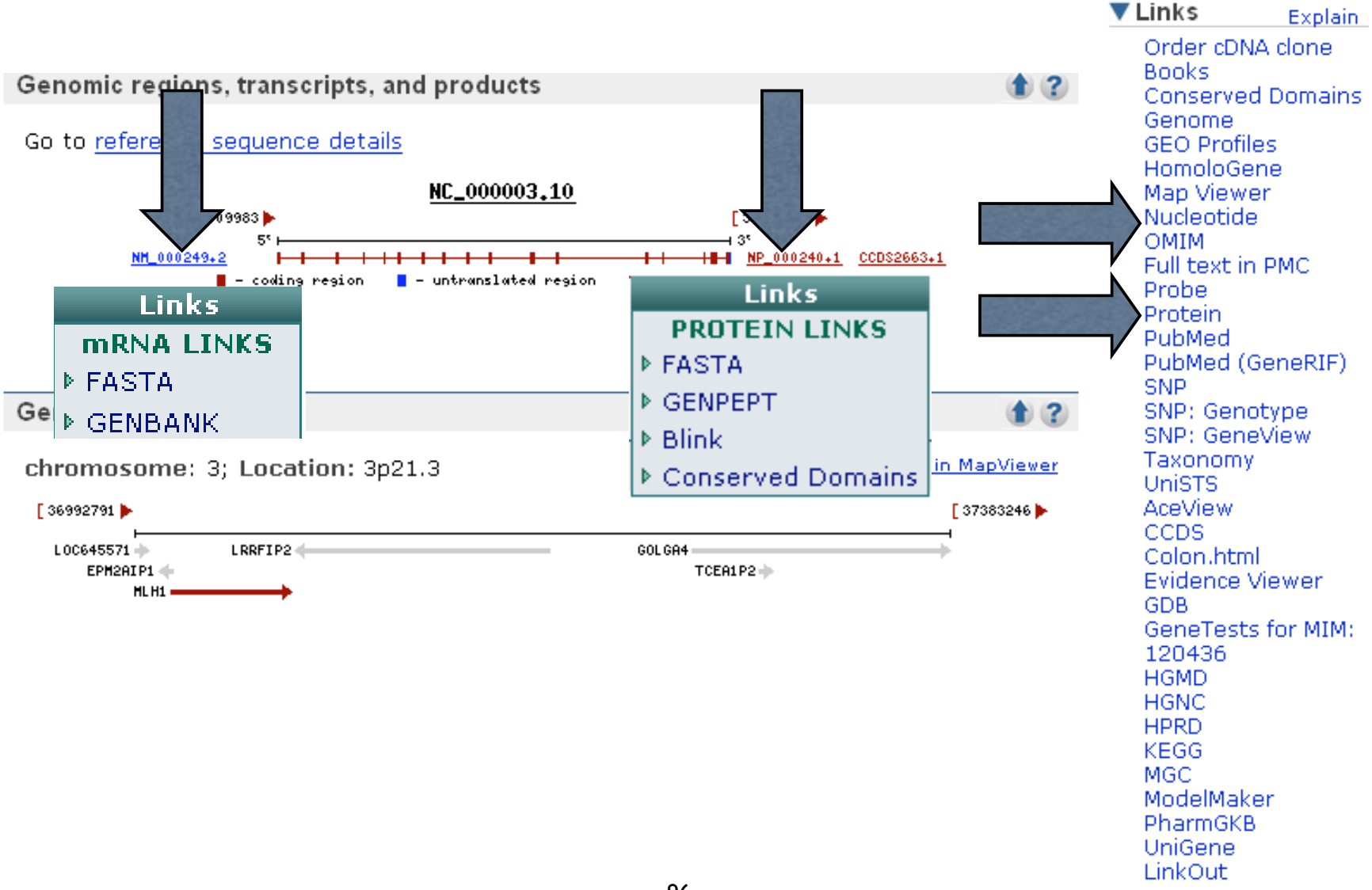
mRNA	BX648844.1	None
mRNA	CR609870.1	None
mRNA	CR617505.1	None
mRNA	DQ648888.1	ABG49483.1
mRNA	DQ648889.1	ABG49484.1
mRNA	DQ648890.1	ABG49485.1
mRNA	DQ648891.1	ABG49486.1
mRNA	DQ648892.1	ABG49487.1
mRNA	DQ648893.1	ABG49488.1
mRNA	S77856.1	AAB34135.1
mRNA	U07343.1	AAC50285.1
mRNA	U07418.1	AAA17374.1

MLH1: Sequence Links

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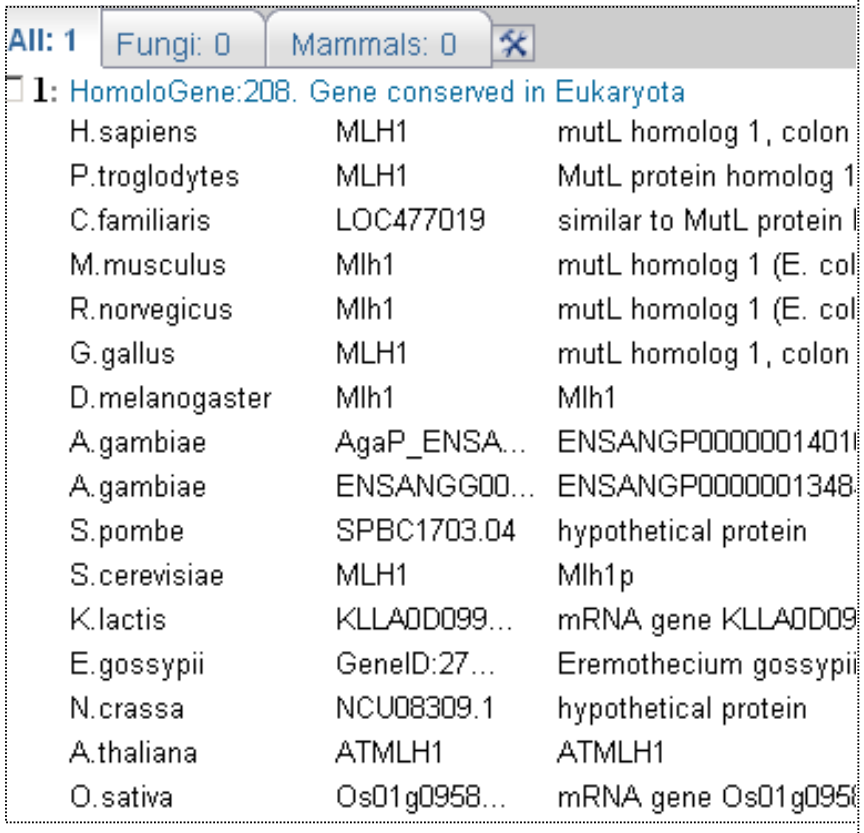
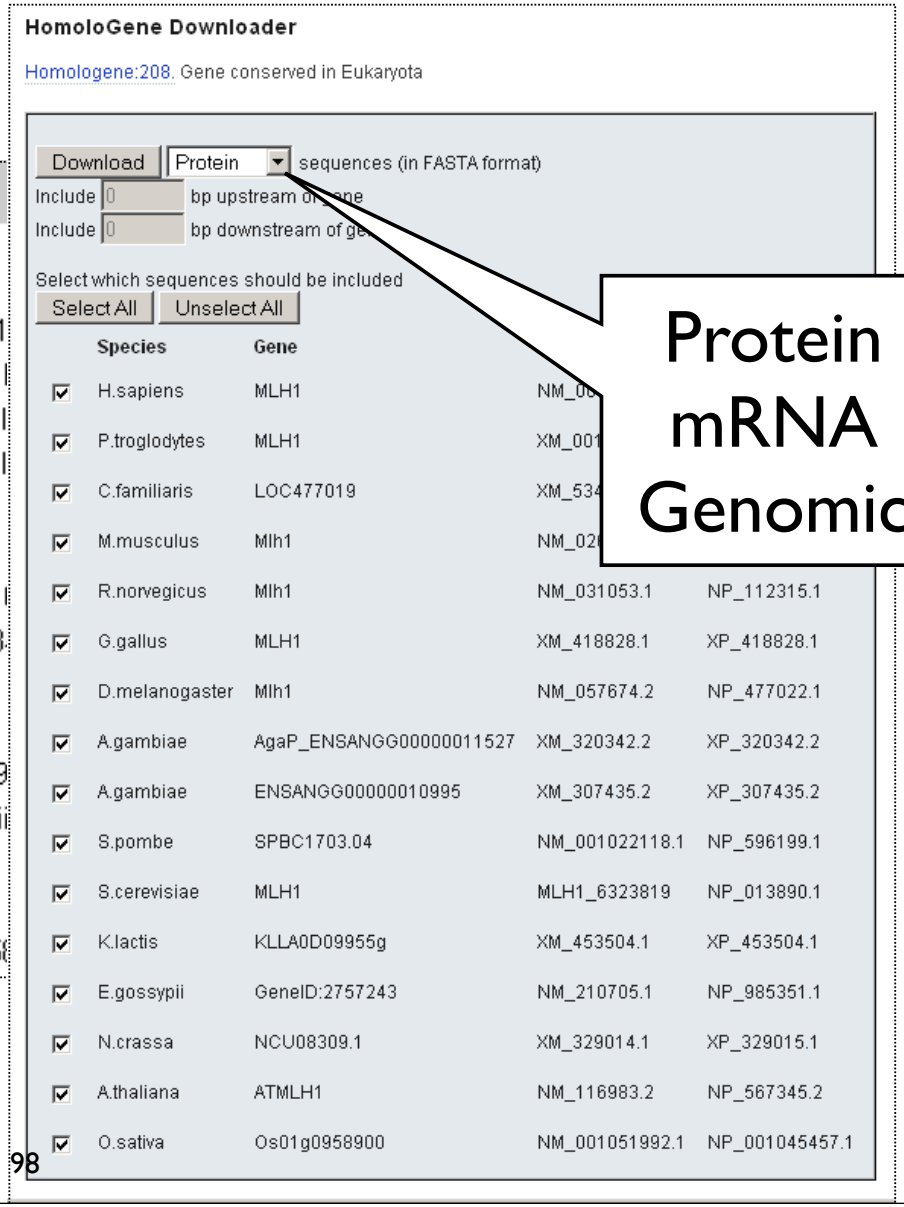
All: 1 Current Only: 1 Genes Genomes: 1 SNP GeneView: 1
1: MLH1 mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli) [Homo sapiens]
GeneID: 4292 updated 16-Sep-2007
Summary
Official Symbol MLH1 provided by HGNC
Official Full Name mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli) provided by HGNC
Primary source HGNC:7127
See related Ensembl:ENSG00000076242; HPRD:00390; MIM:120436
Gene type protein coding
RefSeq status Reviewed
Organism Homo sapiens
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as FCC2; COCA2; HNPCC; hMLH1; HNPCC2; MGC5172
Summary This gene was identified as a locus frequently mutated in hereditary nonpolyposis colon cancer (HNPCC). It is a human homolog of the E. coli DNA mismatch repair gene mutL, consistent with the characteristic alterations in microsatellite sequences (RER+ phenotype) found in HNPCC. Alternatively spliced transcript variants encoding different isoforms have been described, but their full-length natures have not been determined.
Genomic regions, transcripts, and products
Go to reference sequence details
NC_000003.10
Entrez Gene Home
Table Of Contents
Summary
Genomic regions, transcripts...
Genomic context
Bibliography
Interactions
General gene information
General protein information
Reference Sequences
Related Sequences
Additional Links
Links Explain
Order cDNA clone
Books
Conserved Domains
Genome
GEO Profiles
HomoloGene
Map Viewer
CoreNucleotide
EST
Nucleotide
OMIM
Full text in PMC
Probe
Protein
PubMed
PubMed (GeneRIF)
SNP
SNP: Genotype

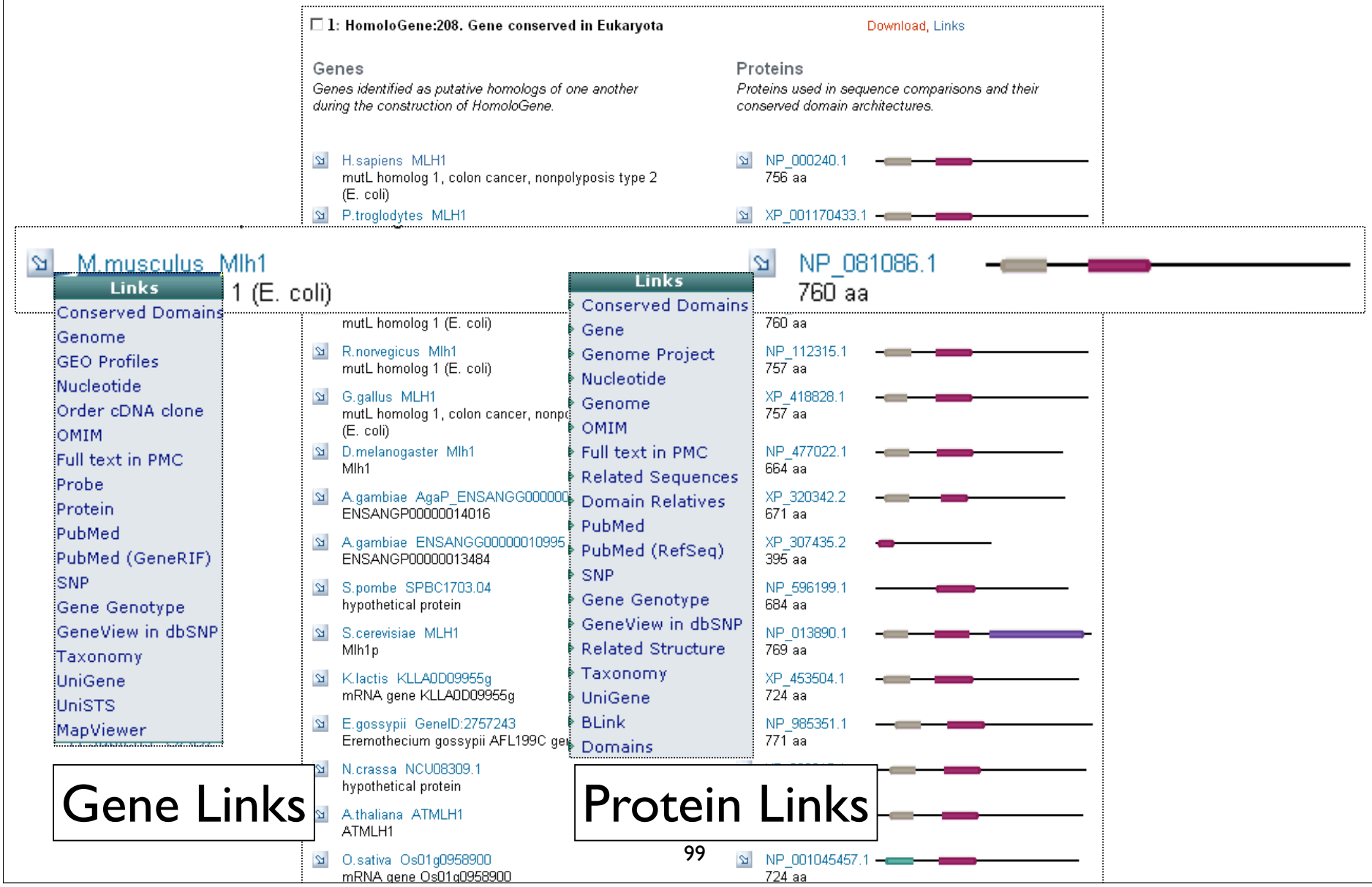
Finding Homologs:



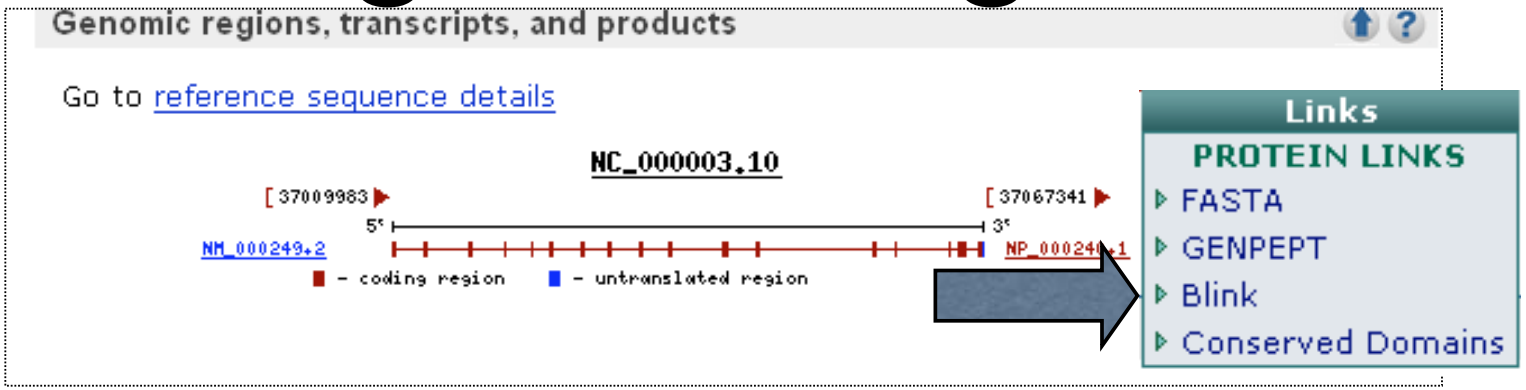


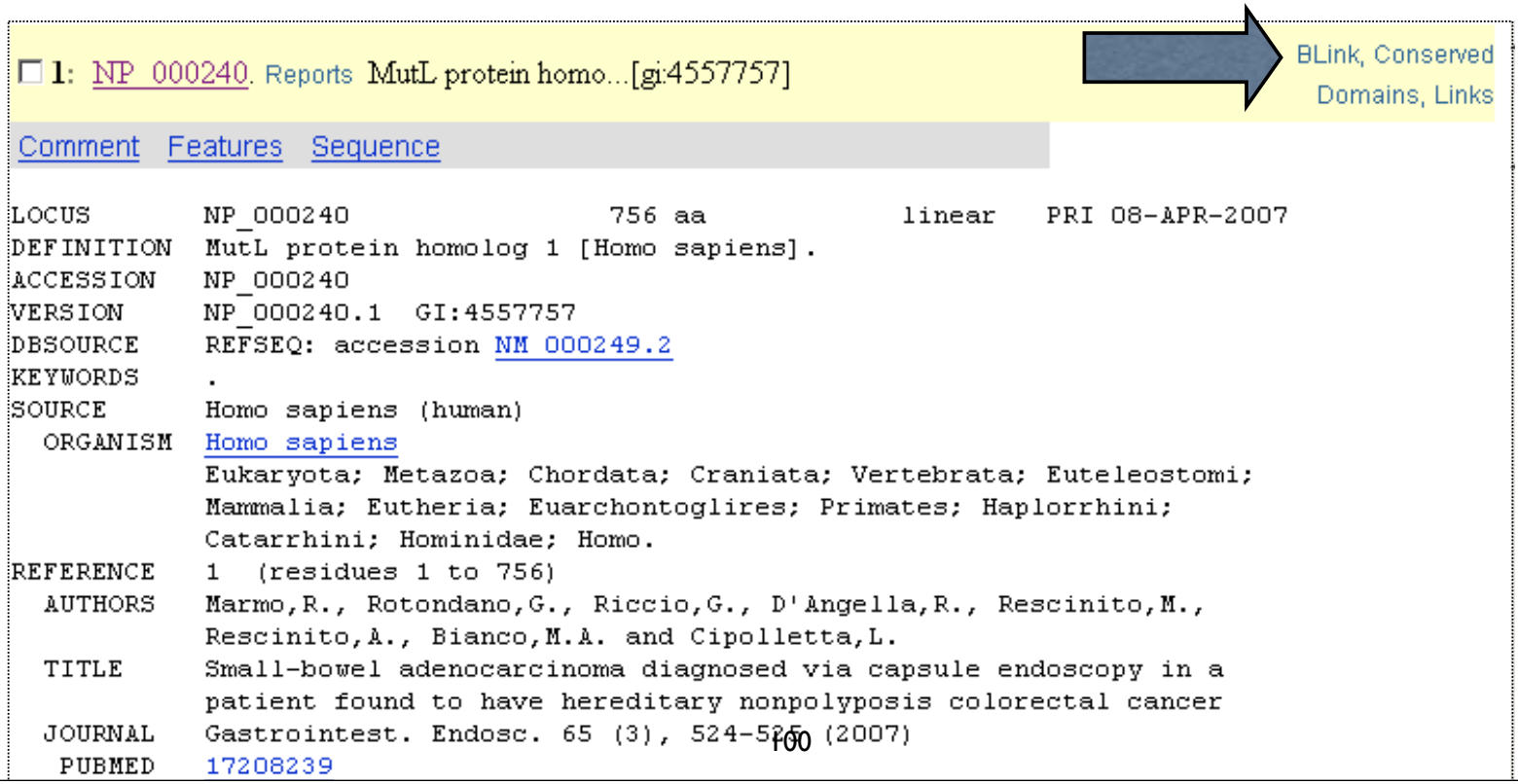
Protein
mRNA
Genomic

HomoloGene Cluster



Finding Homologs 2: BLink





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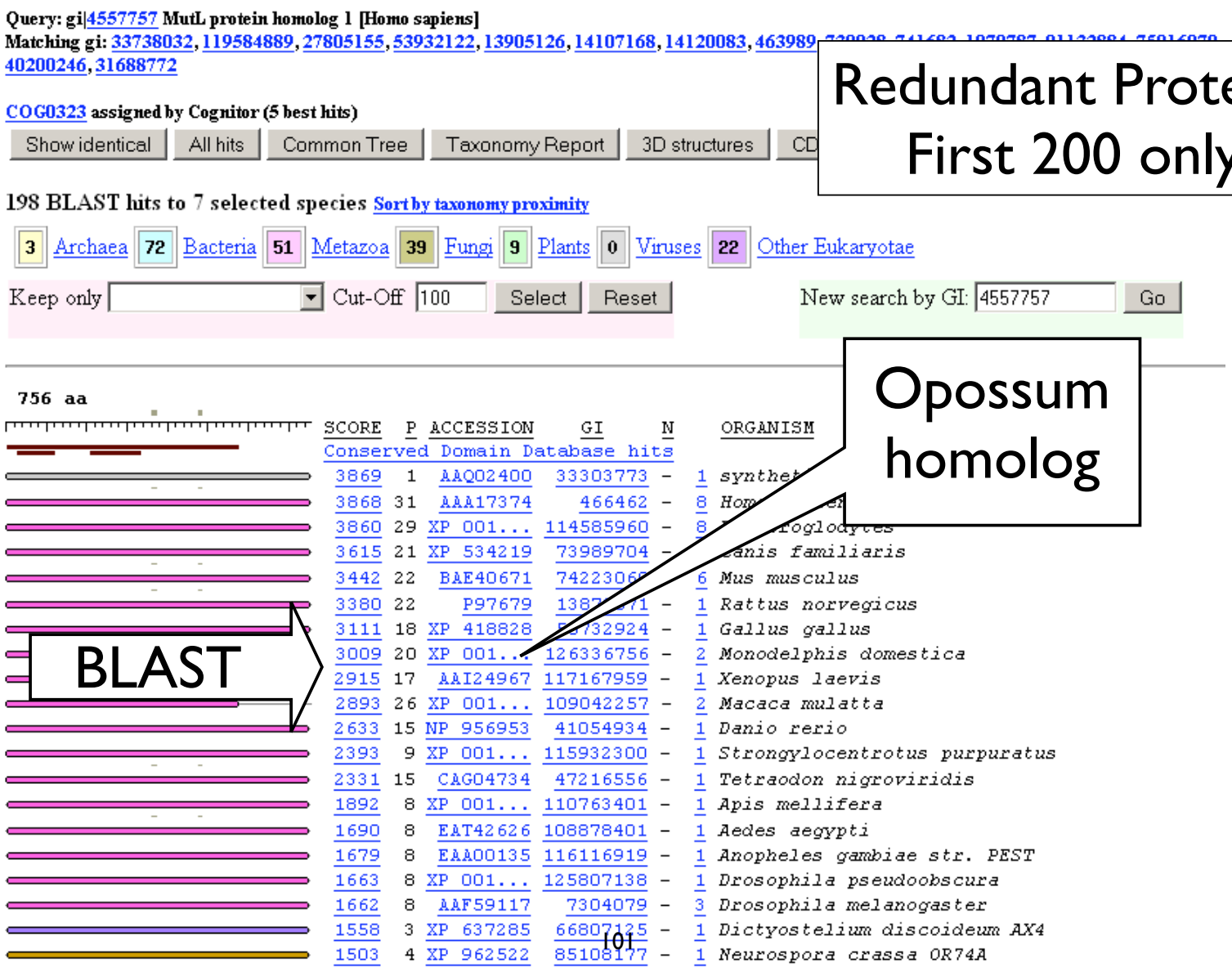
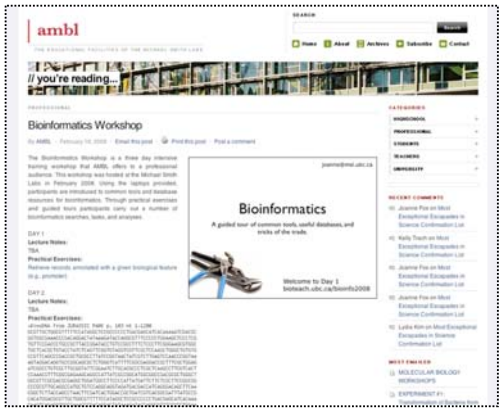


Sequence Databases

PRACTICAL EXERCISES: Navigating Links, Retrieving Data with Entrez, and Searching PubMed

I am studying the regulaton of cancer genes and would like to retrieve all human sequence records associated with cancer that contain a promoter region.

navigate to:
bioteach.ubc.ca/bioinfo2008



Let's compare our results

Follow link to practical exercise page at the NCBI where you'll find step-by-step instructions

Use the preview tab and feature keys

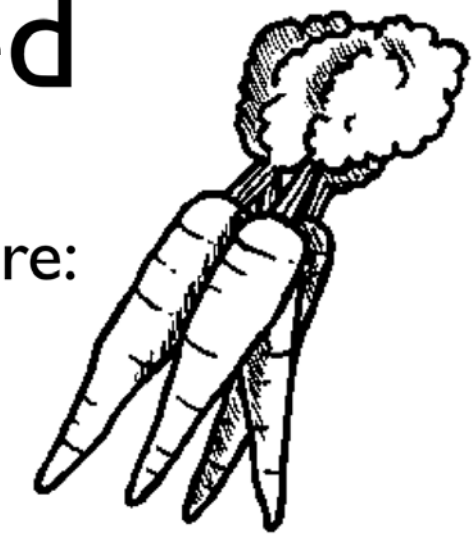
Strategy #1: search nt

Strategy #2: search entrez gene

Check your History

Search	Most Recent Queries	Result
#5	Search #3 NOT #1 (unique hits from Approach B: Entrez Gene to CoreNucleotide)	286
#4	Search #1 NOT #3 (unique hits from Approach A: straight to Entrez CoreNucleotide search)	183
#3	Search #2 AND promoter[Feature key] (limit Approach B search to records with promoter annotated)	329
#2	CoreNucleotide Links for Gene (Search human [Organism] AND cancer[Text Word] AND gene_nucleotide[Filter]) (Approach B: Entrez gene follow link to CoreNucleotide)	53844
#1	Search human[Organism] AND cancer[Text Word] AND promoter[Feature key] (Approach A: Entrez CoreNucleotide search)	226

Searching PubMed



- How many papers in PubMed are there:
  - about cancer?
  - about carrots?
- Using Entrez PubMed, can you see if there is any scientific links between carrots and cancer?
- How many papers are there about "carrots AND cancer"?
- What is the active chemical substance in carrots that may play a role in cancers?

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On call. My 77-year-old father is healthy, but his older brother has just been diagnosed with prostate cancer. Dad says he read that carrot juice will prevent prostate cancer, and he's now drinking it every day. Is he just kidding himself?
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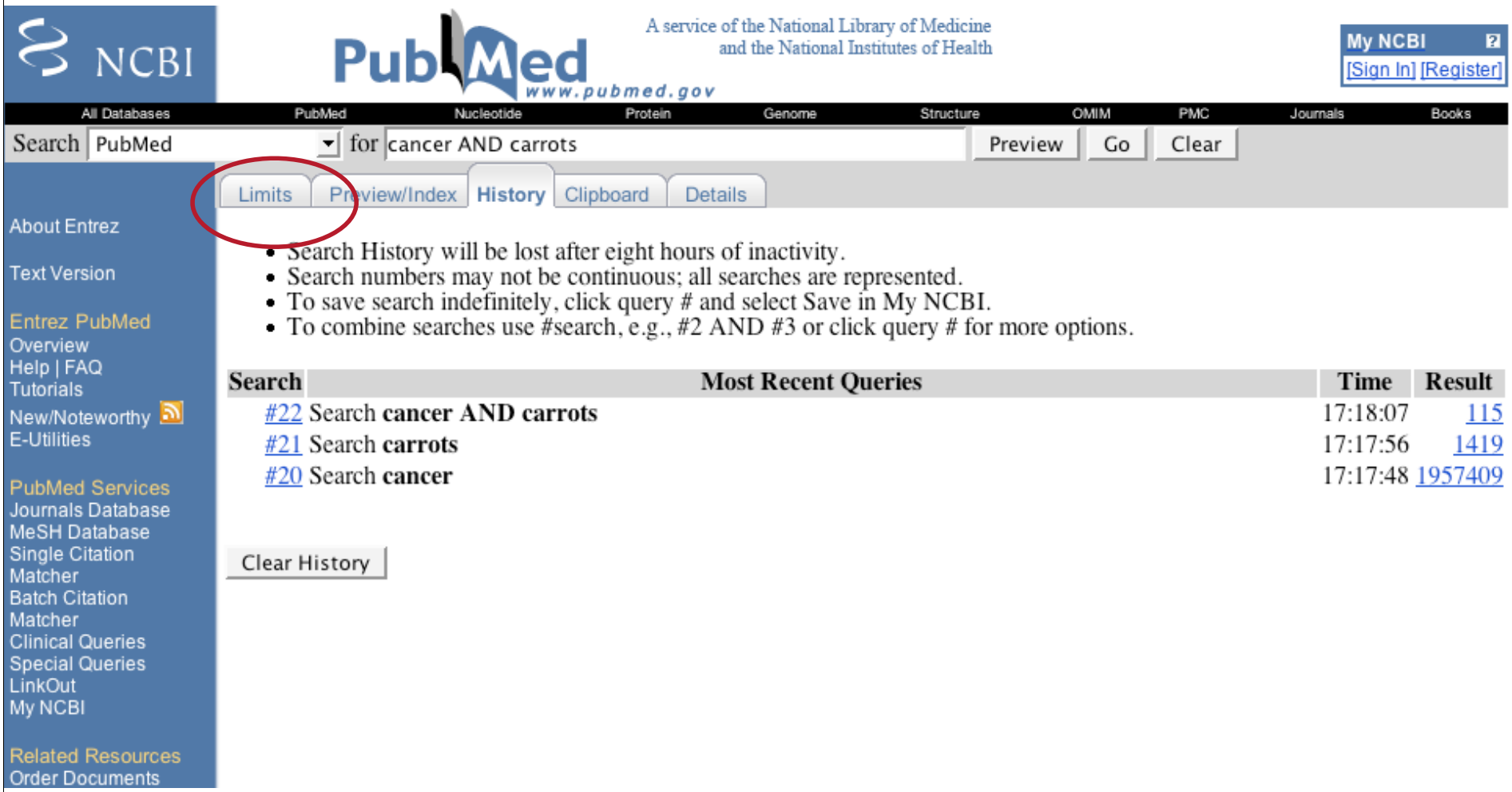
PMID: 16775868 [PubMed - indexed for MEDLINE]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#21 Search carrots 17:17:56 1419
#20 Search cancer 17:17:48 1957409
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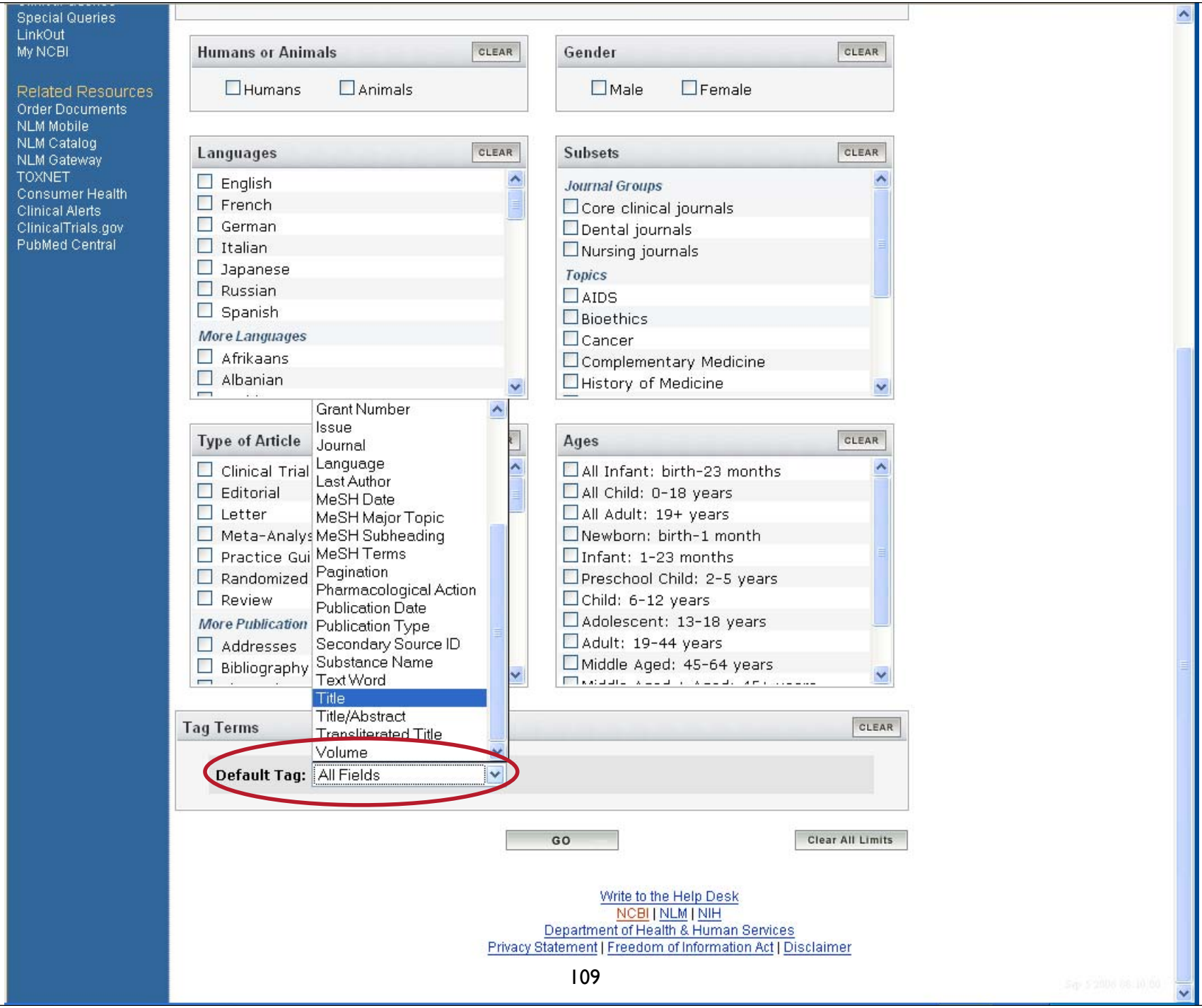
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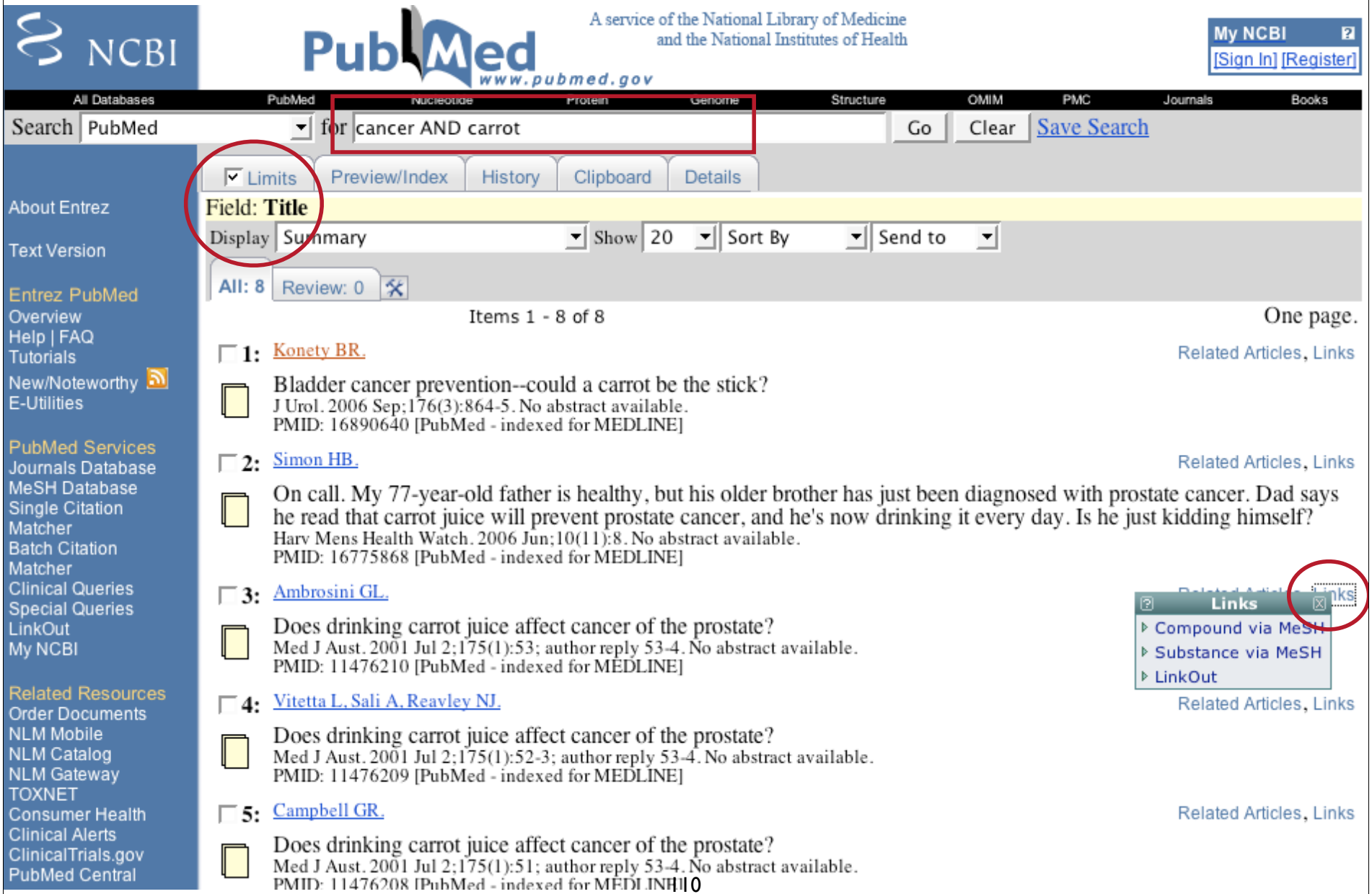
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J Urol. 2006 Sep;176(3):864-5. No abstract available.
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Harv Mens Health Watch. 2006 Jun;10(11):8. No abstract available.
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1: Longnecker MP, Newcomb PA, Mittendorf R, Greenberg ER, Willett WC.
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Intake of carrots, spinach, and supplements containing vitamin A in relation to risk of breast cancer.
Cancer Epidemiol Biomarkers Prev. 1997 Nov;6(11):887-92.
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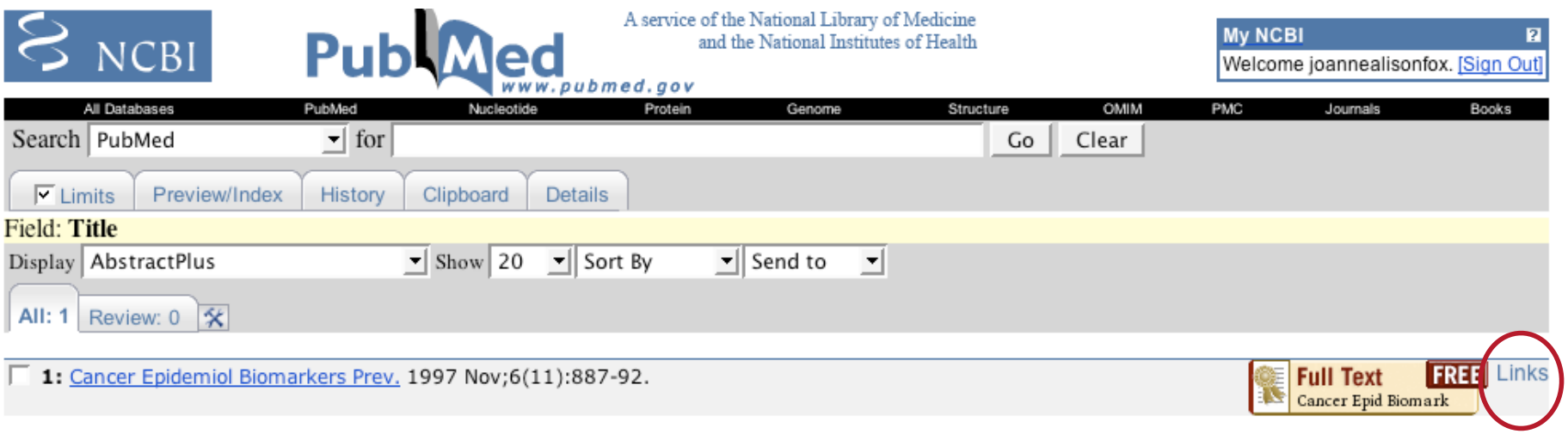
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Longnecker MP, Newcomb PA, Mittendorf R, Greenberg ER, Willett WC.
Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA.
Intake of fruits, vegetables, vitamin A, and related compounds are associated with a decreased risk of breast cancer in some studies, but additional data are needed. To estimate intake of beta-carotene and vitamin A, the authors included nine questions on food and supplement use in a population-based case-control study of breast cancer risk conducted in Maine, Massachusetts, New Hampshire, and Wisconsin in 1988-1991. Multivariate-adjusted models were fit to data for 3543 cases and 9406 controls. Eating carrots or spinach more than twice weekly, compared with no intake, was associated with an odds ratio of 0.56 (95% confidence interval 0.34-0.91). Estimated intake of preformed vitamin A from all evaluated foods and supplements showed no trend or monotonic decrease in risk across categories of intake. These data do not allow us to distinguish among several potential explanations for the protective association observed between intake of carrots and spinach and risk of breast cancer. The findings are, however, consistent with a diet rich in these foods having a modest protective effect.
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Dietary carotenoids and vitamins A, C, and E and risk of breast cancer. [J Natl Cancer Inst. 1999]
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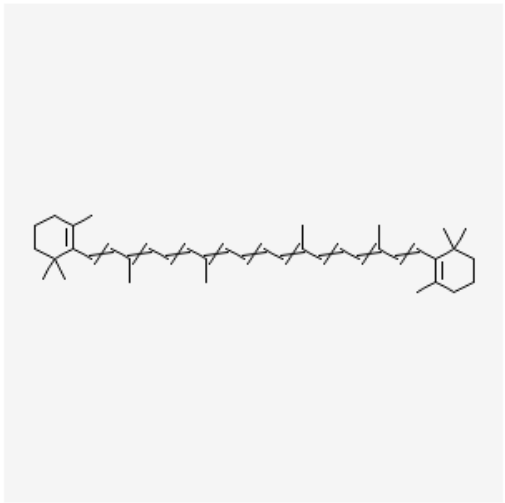

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Medical Subject Annotations: (Total:1)

beta Carotene

A carotenoid that is a precursor of VITAMIN A. It is administered to reduce the severity of photosensitivity reactions in patients with erythropoietic protoporphyria (PORPHYRIA, ERYTHROPOIETIC). (From Reynolds JEF(Ed): Martindale: The Extra Pharmacopoeia (electronic version). Micromedex, Inc, Engewood, CO, 1995.)

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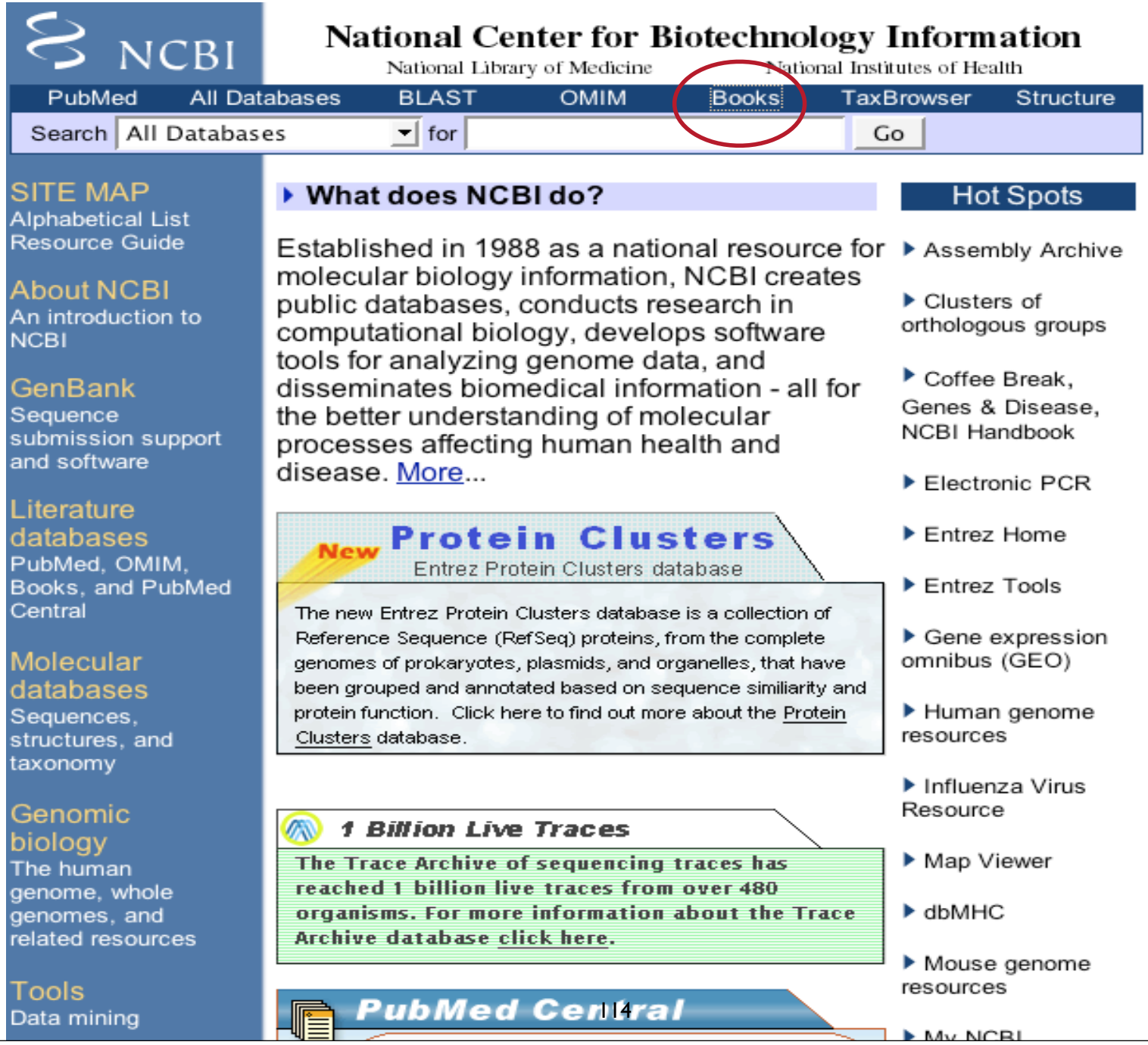
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Kufe, Donald W.; Pollock, Raphael E.; Weichselbaum, Ralph R.; Bast, Robert C., Jr.; Gansler, Ted S.; Holland, James F.; Frei III, Emil, editors.
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Dean T. Jamison, Joel G. Breman, Anthony R. Measham, George Alleyne, Mariam Claeson, David B. Evans, Prabhat Jha, Anne Mills, Philip Musgrove, editors
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2 items in **Alternative Medicine and Rehabilitation: A Guide for Practitioners**
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Holzheimer, Rene G.; Mannick, John A., editors.
Munich: Zuckschwerdt Publishers; c2001.

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Factor, Stewart A.; Weiner, William J.
New York: Demos Medical Publishing, Inc.; c2002

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Current Research

Specific Foods, Nutrients, and Dietary Components Frequently Associated with Cancer Prevention

Many people at risk of cancer focus their attention upon specific foods or nutrients in part because of the extensive marketing of products and publicity generated by the popular press. This tendency is facilitated by the news media when science reporters publicize results of single studies or preliminary findings, often confusing readers with contradictory and conflicting results. The following section briefly summarizes data regarding selected food components or nutrients and may assist the medical practitioner in responding to specific inquires from individuals.
TOP

Vitamins

Vitamin A

Vitamin A is essential for the normal growth and development of epithelial tissues. Vitamin A deficiency is common in many parts of the developing world, but is extremely rare in Americans. Vitamin A is provided in the diet as retinol and its esters, primarily from milk and organ meats, and as β-carotene and a few other provitamin A carotenoids in yellow and leafy green vegetables. Interest in vitamin A and related compounds in the etiology, prevention, and treatment of cancer is rapidly expanding. A protective effect of consuming foods rich in vitamin A has been hypothesized for several types of cancer[1,2,11,16,18,188]; at this time, however, there is no clear evidence that vitamin A supplementation will decrease the risk of cancer in populations or individuals consuming a healthy diet. Although many studies in laboratory models indicate that vitamin A deficiency increases the susceptibility of
risk will be observed
A excess has not
s pharmacologic
s an important area of
s is lacking, the
a large body of

β Carotene

Foods rich in β-carotene, such as many fruits and vegetables, are associated with a lower risk of cancer. However, recent intervention trials with β-carotene clearly question the validity of that hypothesis that the benefits of a plant-based diet can be produced through β-carotene supplements. Two large intervention studies of β-carotene demonstrated a higher risk of lung cancer in smokers.[124,203] Although β-carotene is a potential antioxidant and source of vitamin A, supplements should be discouraged for cancer prevention and dietary sources should be encouraged.
TOP

Very few studies have investigated the role of vitamin E in human cancer [illegible] but several studies suggest a

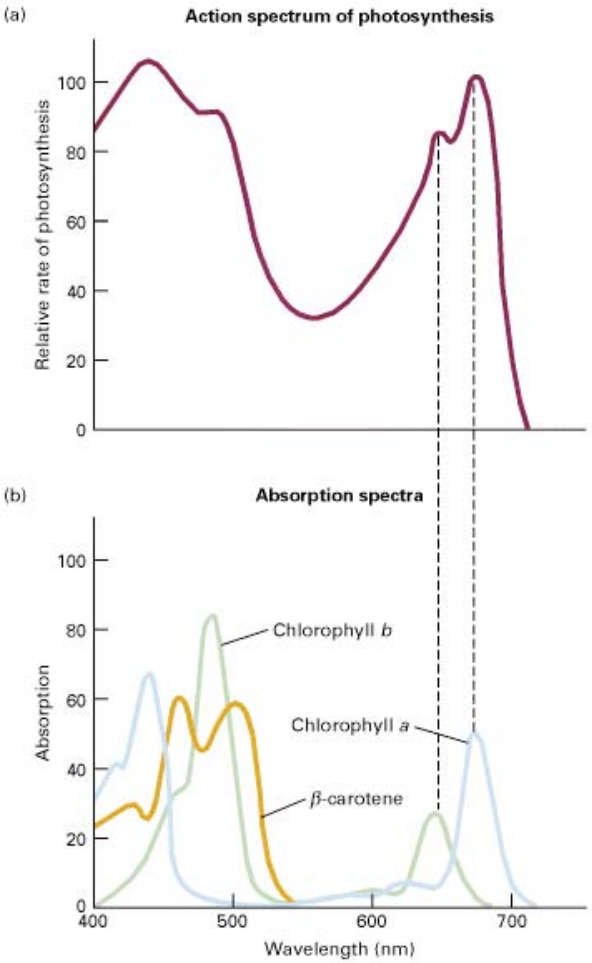

Figure 16-37. Photosynthesis at different wavelengths. (a) The action spectrum of photosynthesis in plants; that is, the ability of light of different wavelengths to support photosynthesis. (b) The absorption spectra for three photosynthetic pigments: chlorophyll *a*, chlorophyll *b*, and β-carotene. Each spectrum shows how well light of different wavelengths is absorbed by one of the pigments. A comparison of the action spectrum with the individual absorption spectra suggests that photosynthesis at 680 nm is primarily due to light absorbed in the

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About this book

16. Cellular Energetics: Glycolysis, Aerobic Oxidation, and Photosynthesis

16.1. Oxidation of Glucose and Fatty Acids to CO_2

16.2. Electron Transport and Oxidative Phosphorylation

16.3. Photosynthetic Stages and Light-Absorbing Pigments

16.4. Molecular Analysis of Photosystems

16.5. CO_2 Metabolism during Photosynthesis

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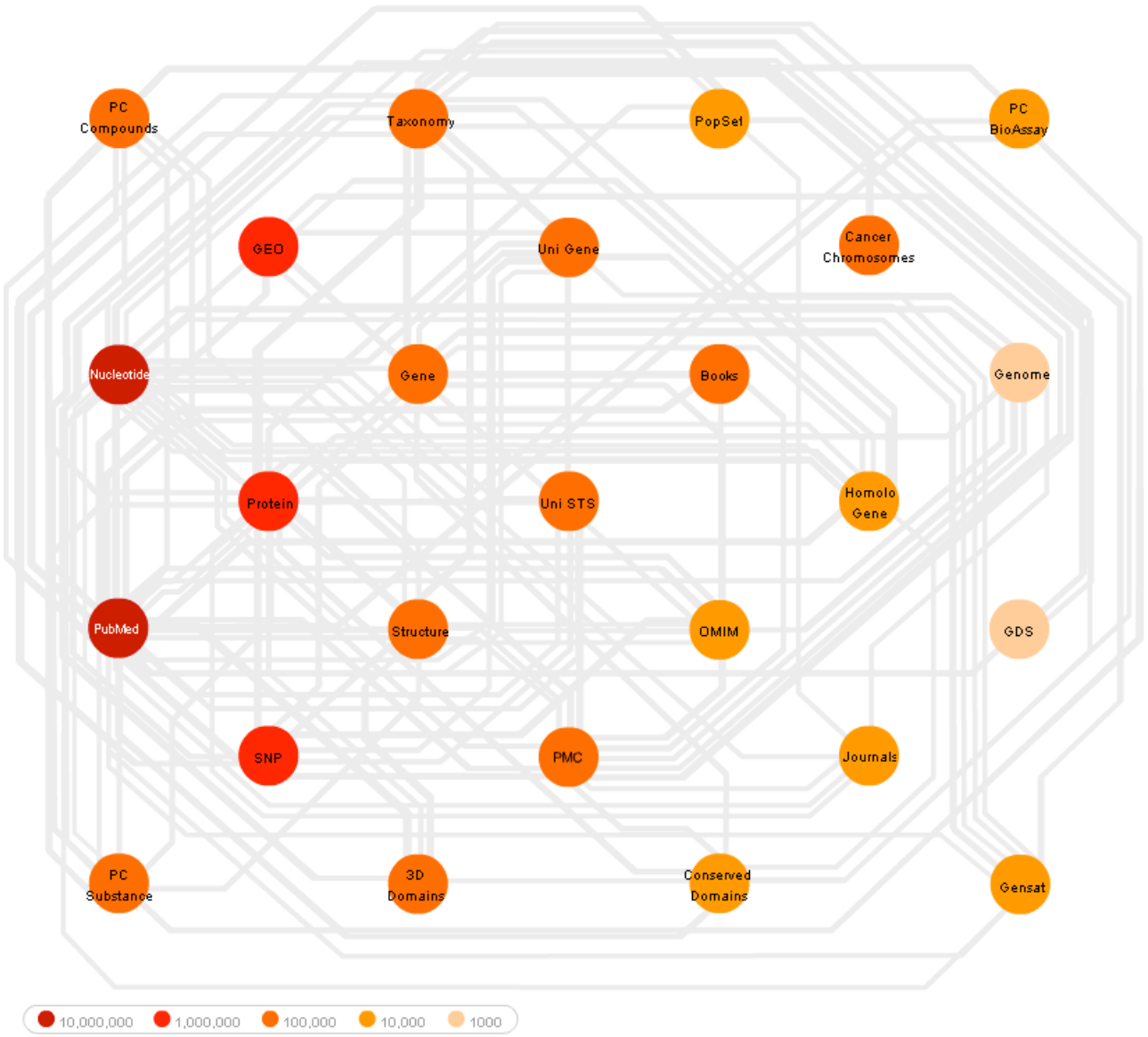
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  http://www.nlm.nih.gov/bsd/pubmed_tutorial/m1001.html

Credits

- Materials for this presentation have been adapted from the following sources:

  NCBI HelpDesk - Field Guide Course Materials

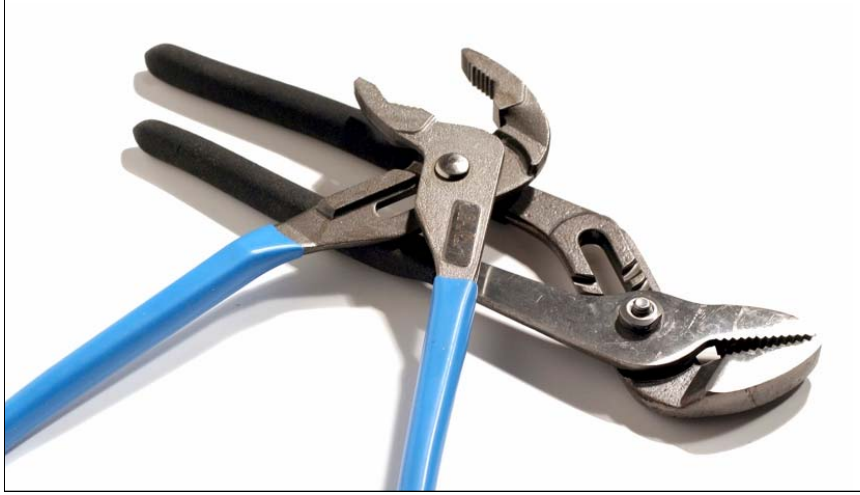
  Bioinformatics: A practical guide to the analysis of genes and proteins

- Questions? Please contact:

Dr. Joanne Fox
Michael Smith Laboratories
joanne@msl.ubc.ca

Let's start at 9:30am

BLAST background, guided tour & practical exercises



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