CG020 Genomika

Lesson 1

Introduction into Bioinformatics

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Functional Genomics and Proteomics of Plants,

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MUNI SCI

Outline

- Syllabus Of The Course
- Definition Of Genomics
- Role Of Bioinformatics In Functional Genomics
- Databases
 - Spectre Of "On-line" Resources
 - PRIMARY, SECONDARY and STRUCURAL Databases
 - GENOME Resources
- Analytical Tools
 - Homologies Searching
 - Searching Of Sequence Motifs, Open Reading Frames, Restriction Sites...
 - Other On-line Genome Tools



Course Syllabus

□ Lesson 01

Introduction into Bioinformatics

Lesson 02

Identification of Genes

□ Lesson 03

Reverse Genetics Approaches

□ Lesson 04

Forward Genetics Approaches



Course Syllabus

□ Lesson 05

RNA Interference and Genome Editing

Lesson 06

Gene Expression and Chemical Genetics

Lesson 07

Protein-Protein Interactions And Their Analysis

Lesson 08

Recent Approaches in DNA Sequencing



Course Syllabus

□ Lesson 09

Structure of Genomes

Lesson 10

Genome evolution

□ Lesson 11

Genomics and Systems Biology

□ Lesson 12

- Practical Aspects Of Functional Genomics
- Model Organisms,
- PCR



Literature

- Literature resources for Chapter 01:
 - Bioinformatics and Functional Genomics, 3rd Edition, Jonathan Pevsner, Wiley-Blackwell, 2015 <u>http://www.bioinfbook.org/php/?q=book3</u>
 - Úvod do praktické bioinformatiky, Fatima Cvrčková, 2006, Academia, Praha
 - Plant Functional Genomics, ed. Erich Grotewold, 2003, Humana Press, Totowa, New Jersey



Outline

- Syllabus of thecourse
- Definition of Genomics

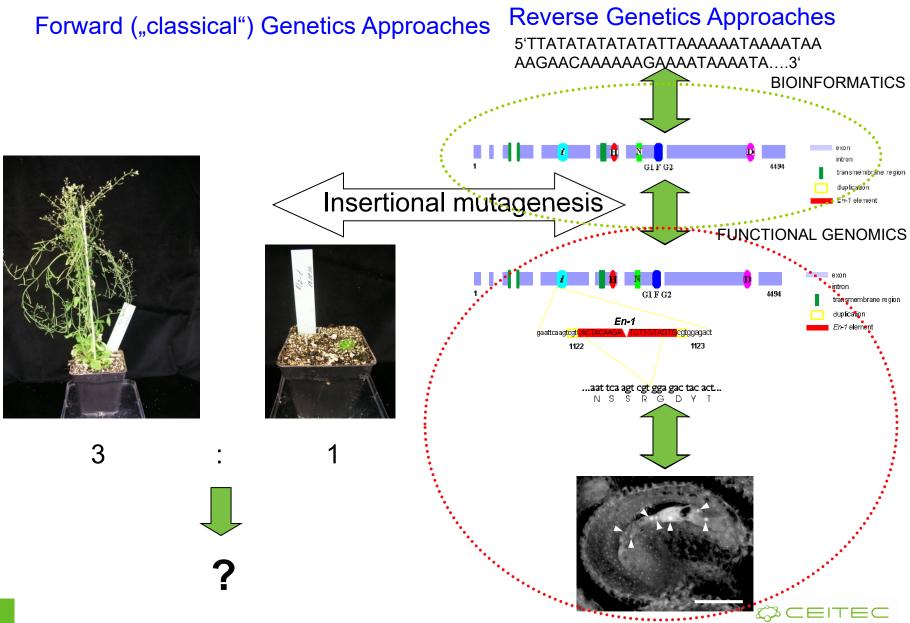


GENOMICS – What is it?

- Sensu lato (in the broad sense) it is interested in STRUCTURE and FUNCTION of genomes
 - Necessary prerequisite: knowledge of the genome (sequence) work with databases
- Sensu stricto (in the narrow sense) it is interested in FUNCTION of INDIVIDUAL GENES – FUNCTIONAL GENOMICS
 - It uses mainly the reverse genetics approaches



GENOMICS – What is it? The role of BIOINFORMATICS in FUNCTIONAL GENOMICS



Outline

- Syllabus of this course
- Definition of genomics
- Role of BIOINFORMATICS in FUNCTIONAL GENOMICS



Bioinformatics

 Definiction of Bioinformatics (according to NIH Biomedical Information Science and Technology Initiative Consortium)

Research, development, or application of computational tools and approaches for expanding the use of biological, medical, behavioral or health data, including those to acquire, store, organize, archive, analyze, or visualize such data.



What is **bioinformatics**?

- Interface between the biology and computers
- Analysis of proteins, genes and genomes using computer algorithms and databases
- Genomics is the analysis of genomes.

The tools of bioinformatics are used to make sense of the billions of base pairs of DNA that are sequenced by genomics projects.

> J. Pevsner, http://www.bioinfbook.org/index.php



Bioinformatics

Bioinformatics in functional genomics

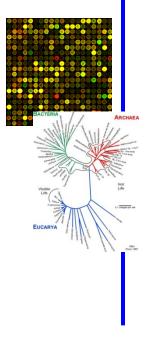
Processing and analysis of sequencing data

- Identification of reference sequences
- Identification of genes
- Identification of homologues, orthologues and paralogues
- Correlative analysis of genomes and phenotypes (incl. human)

Processing and analysis of transcriptional data

- Transcriptional profiling using DNA chips or next-gen sequencing
- Evaluation of experimental data and prediction of new regulations in systems biology approaches
 - Mathematical modelling of gene regulatory networks





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- Databases
 - Spectre of "on-line" resources



Spectre of On Line Resources

EMBnet National Nodes Vienna Biocenter Austria http://www.at.embnet.org/ REN Belgium http://www.be.embnet.org/ BioBase Denmark http://biobase.dk/ CSC Finland http://www.fi.embnet.org/ **INFOBIOGEN** http://www.infobiogen.fr/ France GENTLISnet Germany http://genome.dkfz-heidelberg.de/biounit/ IMBB http://www.imbb.forth.gr/ Greece HEN http://www.hu.embnet.org/ Hungary INCRE Ireland http://acer.gen.tcd.ie/ INN Israel http://dapsas.weizmann.ac.il/bcd/inn.html IEN-ADR Italy http://bio-www.ba.cnr.it:8000/BioWWW/Bio-WWW.htm CADS/CAMM Netherlands http://www.caos.kun.nl/ Bio Norway http://www.no.embnet.org/ IBB Poland http://www.ibb.waw.pl/ IGC Portugal http://www.igc.gulbenkian.pt/ GeneBee Russia http://www.genebee.msu.su/ CNB-CSIC Spain http://www.es.embnet.org/ BMC http://www.embnet.se/ Sweden STR Switzerland http://www.ch.embnet.org/ SEQNET UK http://www.seqnet.dLac.uk/ EMBnet Specialist Nodes http://www.mips.biochem.mpg.de/ MIPS Germany TCGEB Italy http://www.icgeb.trieste.it/ Pharmacia Upjohn Sweden http://www.pnu.com/ F.Hoffmann-La Roche Switzerland http://www.roche.com/ EBI UK http://www.ebi.ac.uk/ HGMP-RC UK http://www.hgmp.mrc.ac.uk/ Sanger UK http://www.sanger.ac.uk/ UMBER UK http://www.bioinf.man.ac.uk/dbbrowser EMBnet Associate Nodes TRRM Argentina http://sol.biol.unlp.edu.ar/embnet ANGES Australia http://www.angis.su.oz.au/ CBI China http://www.cbi.pku.edu.cn/ CIGB Cuba http://bio.cigb.edu.cu/ CDFD India http://salarjung.embnet.org.in/ SANBI South Africa http://www.sanbi.ac.za USA Information Providers NCBI USA http://www.ncbi.nlm.nih.gov/ NLM USA http://www.nlm.nih.gov/

http://www.nih.gov/

NIH

USA



Spectre of On Line Resources

EBI <u>http://www.ebi.ac.uk/services</u>

www.ebiac.uk/services			- d ≤ G -	haplotype	۶ 🖬 -
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DNA & RNA genes, genomes & variation	Gene expression RNA, protein & metabolite expression	Proteins sequences, families & motifs	Bioinformatics train	ing	
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Spectre of On Line Resources

Now easier to use an

format and features c BLAST services. inclu

1.000

□ NCBI <u>http://www.ncbi.nlm.nih.gov/</u>

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National Center for Biotechnology Information	Search	1
NCBI Home	Welcome to NCBI	Popular Resour
Resource List (A-Z)	Search Welcome to NCBI The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information. About the NCBI Mission Organization Research RSS Feeds Description Organization Research RSS Feeds Outloads: Get NCBI Mission Organization Research RSS Feeds Outloads: Get NCBI oftware Downloads: Get NCBI data or software How-To's: Learn how to accomplish specific tasks at NCBI Submissions: Submit data to GenBank or other NCBI databases NCBI YouTube channel Learn how to get the most out of NCBI tools and databases with video tutorials on the NCBI YouTube Channel.	PubMed
All Resources	and health by providing access to biomedical and genomic information.	Bookshelf
Chemicals & Bioassays	About the NCBI Mission Organization Research RSS Feeds	PubMed Central
Data & Software		PubMed Health
DNA & RNA		BLAST
Domains & Structures	Get Started	Nucleotide
Genes & Expression	Get Started B • Tools: Analyze data using NCBI software G • Downloads: Get NCBI data or software G • How-To's: Learn how to accomplish specific tasks at NCBI S • Submissions: Submit data to GenBank or other NCBI databases G	Genome
Genetics & Medicine		SNP
Genomes & Maps		Gene
Homology	and health by providing access to biomedical and genomic informatio About the NCBI Mission Organization Research RSS Feeds Get Started • Tools: Analyze data using NCBI software • Downloads: Get NCBI data or software How-To's: Learn how to accomplish specific tasks at NCBI • Submissions: Submit data to GenBank or other NCBI databases NCBI YouTube channel Learn how to get the most out of NCBI	
Literature		PubChem
Proteins		
Sequence Analysis	NCBI YouTube channel	NCBI Announce
Taxonomy	Learn now to get the most out of NCBI	New version of Gen
Training & Tutorials	on the NCRI YouTube Channel	available
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Taxonomy

PopSet

UniGene

Cancer Chromosomes

HomoloGene

Journals

Genome

GDS



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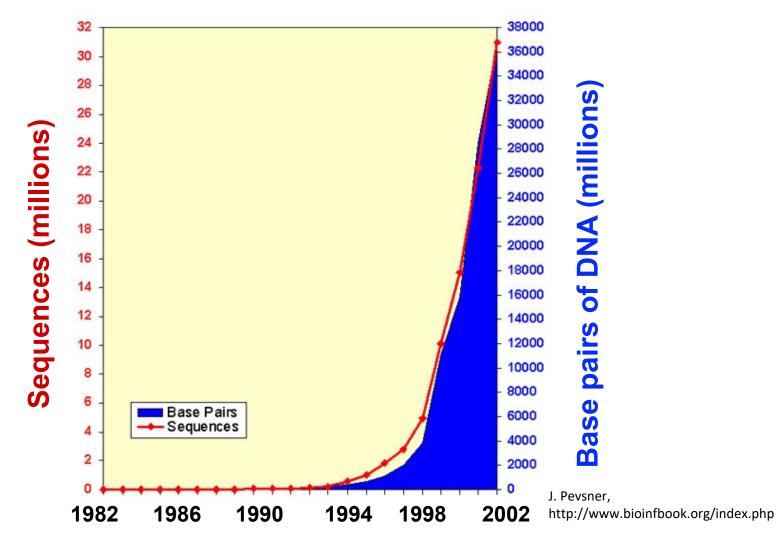


- Include primary datasets <u>DNA</u> and <u>Protein</u> sequences
 - Sequences in databases of "The Big Three":
 EMBL
 - http://www.ebi.ac.uk/embl/
 - GenBank
 - □ <u>http://www.ncbi.nih.gov/Genbank/GenbankSearch.html</u>

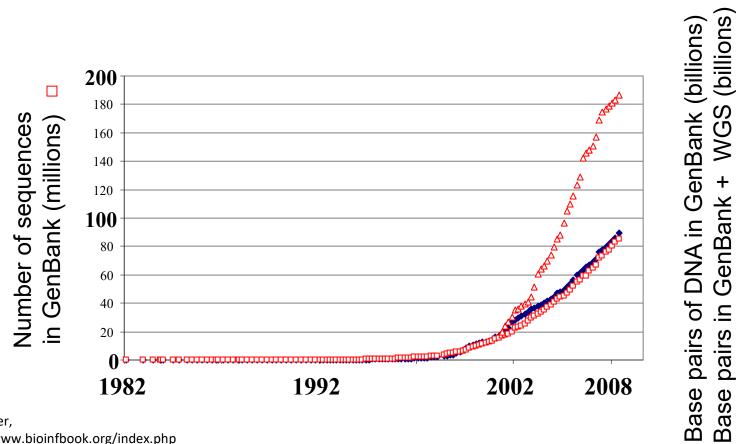
- http://www.ddbj.nig.ac.jp
- Daily mutual exchange and backup of data
- Works with large amount of data (capacity and software requirements)
- September 2003 27,2 x 10⁶ entries (approx. 33 x 10⁹ bp)
- August 2005 100 x 10⁹ bp from 165.000 organisms



Growth of GenBank



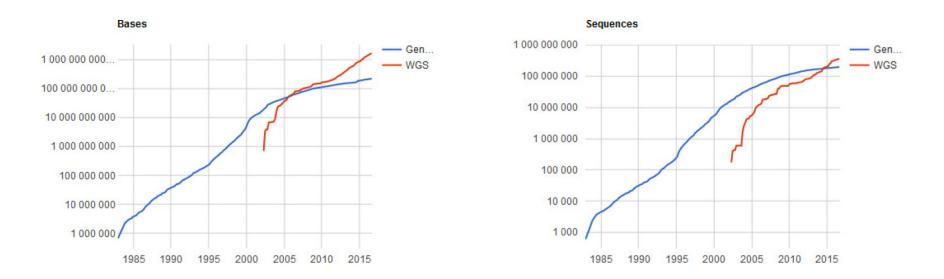
Growth of GenBank + Whole Genome Shotgun (1982-November 2008): we reached 0.2 terabases



J. Pevsner, http://www.bioinfbook.org/index.php

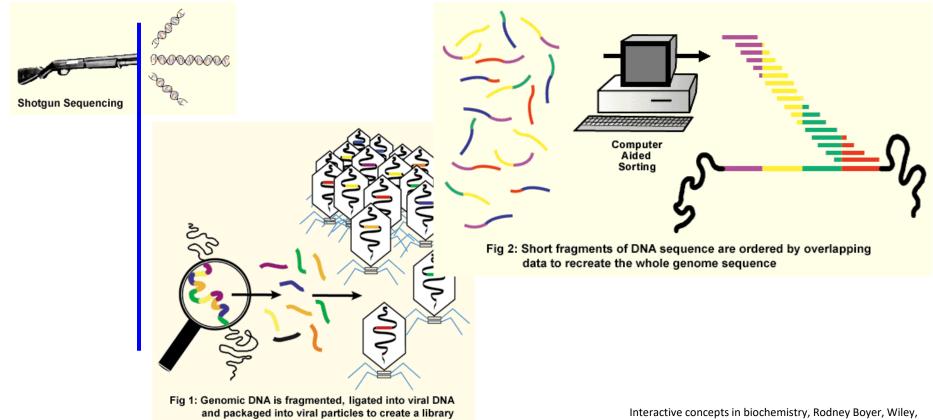


Growth of GenBank Aug 2016



- Dec **1982** 680 338 bp, 606 sequences
- Apr **2002** 19 x 10⁹ bp, 17 x 10⁶ sequences + WGS 692 x 10⁶ bp, 172 768 sequences
- Aug 2016 218 x 10⁹ bp, 196 x 10⁶ sequences + WGS 1,6 10¹² bp, 360 x 10⁶ sequences

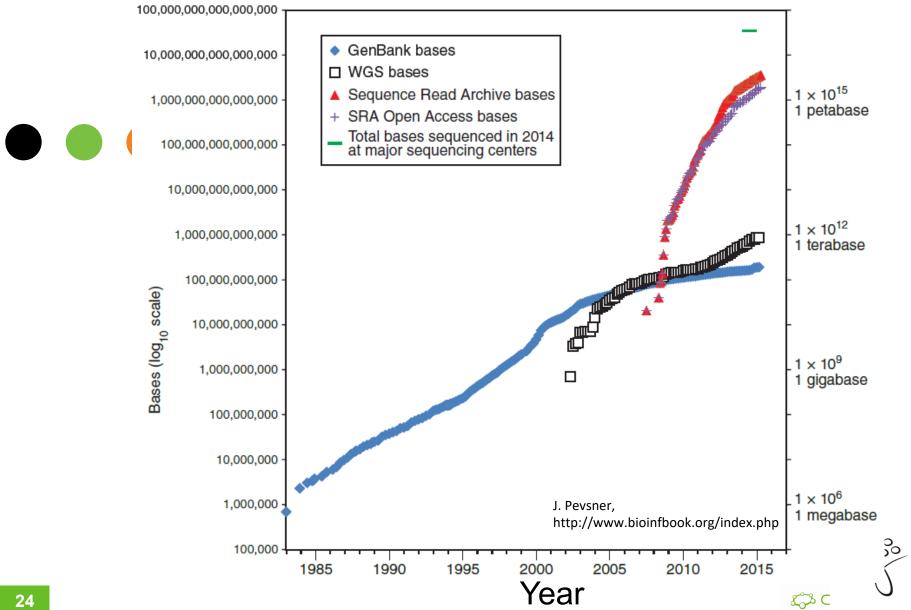
WGS



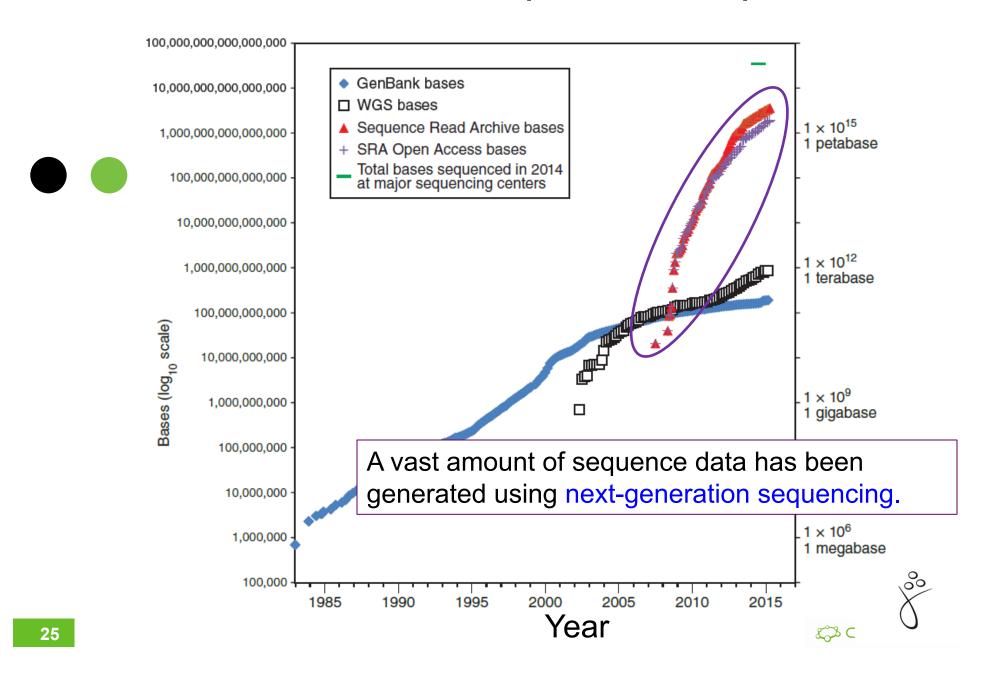
Interactive concepts in biochemistry, Rodney Boyer, Wiley, 2002, http://www.wiley.com//college/boyer/0470003790/



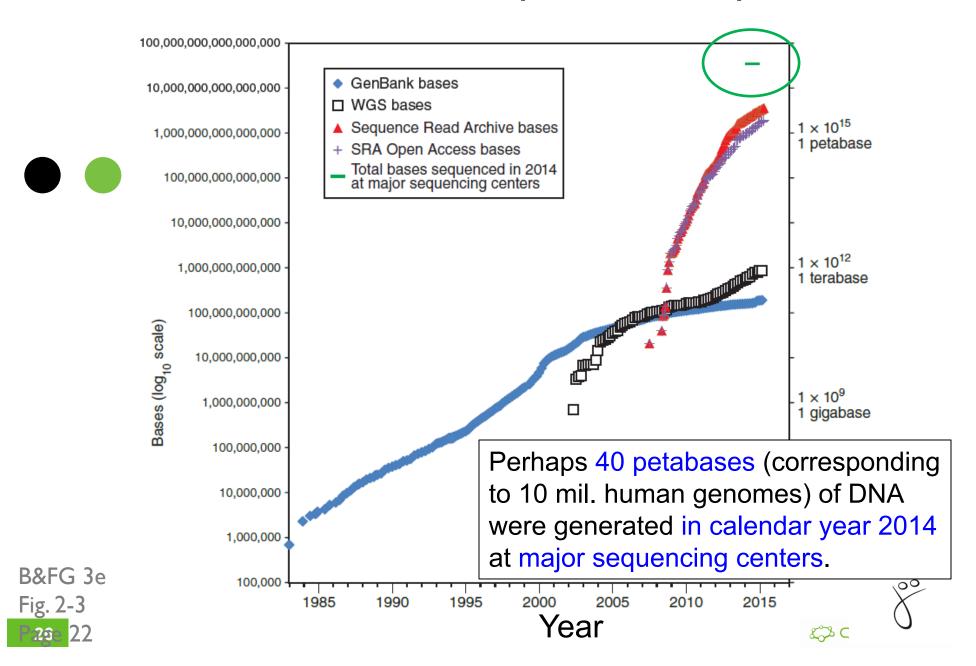
Growth of DNA Sequence in Repositories



Growth of DNA Sequence in Repositories



Growth of DNA Sequence in Repositories



- They include sets of primary data <u>DNA</u> and <u>Protein</u> sequences
 - Protein sequences:
 - PIR, <u>http://pir.georgetown.edu/</u>
 - □ MIPS, <u>http://www.mips.biochem.mpg.de</u>
 - SWISS-PROT, http://www.expasy.org/sprot/



- Types of sequences in primary databases
 - Standard nucleotide sequences acquired by high quality sequencing
 - ESTs (Expressed Sequence Tags)
 - HGTS (High Throughput Genome Sequencing)
 Results of sequencing projects without annotation
 - □ **Reference Sequences** of annotated genomes
 - **TPAs (Third Party Annotation)**
 - sequences annotated by third party (by someone else, not the orginal authors)



GenBank (NCBI) http://www.ncbi.nlm.nih.gov/

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NCBI Home	Welcome to NCBI	Popular Resour
Resource List (A-Z)	The National Center for Biotechnology Information advances science	PubMed
All Resources	and health by providing access to biomedical and genomic information.	Bookshelf
Chemicals & Bioassays	About the NCBI Mission Organization Research RSS Feeds	PubMed Central
Data & Software		PubMed Health
DNA & RNA		BLAST
Domains & Structures	Get Started	Nucleotide
Genes & Expression	Tools: Analyze data using NCBI software Downloads: Get NCBI data or software	Genome
Genetics & Medicine	<u>Downloads</u> : Get NCBI data or software How-To's: Learn how to accomplish specific tasks at NCBI	SNP
Genomes & Maps	Submissions: Submit data to GenBank or other NCBI	Gene
Homology	databases	Protein
Literature		PubChem
Proteins		
Sequence Analysis	NCBI YouTube channel	NCBI Announce
Taxonomy	Learn how to get the most out of NCBI	New version of Gen
Training & Tutorials	tools and databases with video tutorials on the NCBI YouTube Channel.	available
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Gene description two-component VirA-like sensor kinase			Reference sequences	
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What is an **Accession Number**?

An accession number is label that used to identify a sequence. It is a string of letters and/or numbers that corresponds to a molecular sequence.

Examples (all for retinol-binding protein, RBP4):

X02775	GenBank genomic DNA sequence	D 1 1 4
NT_030059	Genomic contig	DNA
Rs7079946	dbSNP (single nucleotide polymorphism)	

N91759.1An expressed sequence tag (1 of 170)RNANM_006744RefSeq DNA sequence (from a transcript)

NP_007635	RefSeq protein	_
AAC02945	GenBank protein	Protein
Q28369	SwissProt protein	
1KT7	Protein Data Bank structure record	L Devener

J. Pevsner, http://www.bioinfbook.org/index.php



NCBI's important RefSeq project: best representative sequences

RefSeq (accessible via the main page of NCBI) provides an expertly curated accession number that corresponds to the most stable, agreed-upon "reference" version of a sequence.

RefSeq identifiers include the following formats:

Complete genome Complete chromosome Genomic contig mRNA (DNA format) Protein

NC_###### NC_###### NT_###### NM_####### e.g. NM_006744 NP_####### e.g. NP_006735

> J. Pevsner, http://www.bioinfbook.org/index.php



RefSeq

two-	-component VirA-like sense	or kinase		ਨੂੰ ⊽ C Soogle	See more.
NCBI R	Reference Sequences (Re	efSeq)	۲	?	
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The foll	lowing sections contain ref	ference sequences that be	elong to a specific genome build. <u>Explain</u>		
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	UniProtKB/Swiss-Prot		.		
	Conserved Domains (3) <u>su</u>				
		<u>cd00075</u> Location:580 – 694 Blast Score: 202	HATPase_c; Histidine kinase-like ATPases; This family includes several ATP-binding proteins for example: histidine kinase, DNA gyrase B, topoisomerases, heat shock protein HSP90, phytochrome-like ATPases and DNA mismatch repair proteins		
		<u>cd00082</u> Location:466 – 530 Blast Score: 144	HisKA; Histidine Kinase A (dimerization/phosphoacceptor) domain; Histidine Kinase A dimers are formed through parallel association of 2 domains creating 4-helix bundles; usually these domains contain a conserved His residue and are activated via		
		PRK13837 Location:14 – 833 Blast Score: 2944	PRK13837; two-component VirA-like sensor kinase; Provisional		
Related	d Sequences		8		



NCBI's RefSeq project: many accession number formats for genomic, mRNA, protein sequences

<u>Accession</u>	<u>Molecule</u>	Method		<u>Note</u>	
AC_123456		Genomic	Mixed	Alternate c	omplete genomic
AP_123456		Protein Mixed		Protein products; alter	nate
NC_123456		Genomic	Mixed	Complete g	genomic molecules
NG_123456		Genomic	Mixed	Incomplete	genomic regions
NM_123456		mRNA Mixed		Transcript products; m	NA
NM_1234567	789	mRNA Mixed		Transcript products; 9-	-digit
NP_123456		Protein Mixed		Protein products;	
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Primary Databases

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CCTTGAACTAGAGAGGCTCCAAAAACAACGCGGTCTTGATGAAGCTCCCGTGCGCATACTTGCACGTGAA GGTCCCATTATCTTATC	Find in this Sequence
CAGAAATTGCGGAGATGCTGCAGCGCGAGTGTTTGGAGGTCTATAGCTTGAAAAATGTAGAGGAGCGGAG	
GCACGTATCTTTCTTGGGTCCGCTTCAGTGGGTCTTTGCCTCTACATCATCACCTTAGTCTATAGGCTA	
CGCAAAAAAACCGATTGGTTAGGCGGCGCGTTTAGATTACGAAGAGCTAATCAAAGAGATCGGAGTATGTT	Related information
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CGATACGTGCGCGTTAGCTCTAGTGGACCATGACCGTAGATGGGCTGTCGAAACATTCGGTGCGAAACAC	Full text in PMC
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CGGTATTCCGCATCATATCGTCGAAAAAAATCGTACATTTGCCTCTCGAAATTCCAGGTCTCTCGATACT	Gene
actggctcacaaatccacagataaactaattgcggtttgttcactgggttaccaaagctatcgccctcga	Genome
CCTTGCCAAGGCGAAATTCAGCTTCTTGAACTCGCCACCGCCTGCCT	Identical GenBank Sequence
GTAAGCAGACCGAATGCGACGTTTTGGCCAGACGATTGGAGCATGCGCAACGCCTTGAGGCAGTTGGTAC	Protein
ACTTGCCGGCGGAATAGCACATGAATTTAATAACATTTTGGGCTCAATCCTCGGGCACGCAGAATTAGCA	
CARAACTCGGTGTCTCGAACATCTGTCACCCGAAGATATATTGACTATATCATTTCGTCAGGCGACAGAG CCATGCTCATTATCGATCAGATCTTGACGCTGAGCCGAAAACAGGAGCGCCATGATCAAGCCATTTAGTGT	Protein Clusters
CTCACAGCTTGTGACCGAAATCGTCCTTGCTACGTATGGCTCTTCCGCCAAACATCGACGTTAGTTTC	PubMed
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GCAAGAATGCTTCCCAAGCCATGACTGCAAATGGTCAAATCGACATCATCATCAGCCAAGCTTTTTTACC	
AGTTAAGAAAATTCTGGCGCATGGTGTTATGCCACCTGGCGACTATGTTCTCCTATCTAT	Taxonomy
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ARCIAIGUCGUAIGUAAICUAAAAATUAAGAUGTAG	
	virA [Agrobacterium tumefaciens str. C58] Gene



- Databases of functional or structural motifs, acquired by primary data (sequences) comparison
- PROSITE, <u>http://www.expasy.org/prosite/</u>

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his program allows to scan a protein sequence (either from <u>Swise-Pret or TrEMBL</u> , or provided by the user) for the occurrence of patterns and profiles stored in the <u>PROSITE</u> database, or to arch protein databases with a user-entered pattern [<u>Reference</u> / <u>Download ps.scan</u> , the standalone version]. The program <u>PRATT</u> can be used to generate your own patterns. You may either: • enter a PROSITE accession number or pattern to search the Swiss-Prof/TrEMBL and/or PDB databases with a pattern, OR • enter a sequence or a Swiss-Prof/TEMBL accession number to scan the sequence with all patterns, profiles and rules in PROSITE, OR • fill in both fields to find all occurrences of a pattern or profile in a sequence.									
Scan a protein for PROSITE matches		Search Swiss-I	Prot with a PROSITE	entry					
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start the scan reset	mate randomize d	h mode greedy, overlaps, no ttabases no	includes (for p	satterns, see <u>help</u>) see <u>help</u>)					



- Databases of functional or structural motifs, acquired by primary data (sequences) comparison
- PROSITE, <u>http://www.expasy.org/prosite/</u>

>PDOC00003 PS00003 SULFATION Tyrosine sulfation site [rule] [Warning: rule with a high probability of occurrence].

571 - 585 nkeesstYeteisns

>PDOC00004 PS00004 CAMP_PHOSPHO_SITE cAMP- and cGMP-dependent protein kinase phosphorylation site [pattern] [Warning: pattern with a high probability of occurrence].

744 - 747 RRvT 814 - 817 KRrS

>PDOC00005 PS00005 PKC_PHOSPHO_SITE Protein kinase C phosphorylation site [pattern] [Warning: pattern with a high probability of occurrence].

148 - 150 SsR 164 - 166 TgR 171 - 173 StK 219 - 221 SkK 369 - 371 TrR 460 - 462 SgK 513 - 515 SgR 585 - 587 SiR 602 - 604 TgK 652 - 654 TdK 716 - 718 SpR 726 - 728 SpK 747 - 749 TeK 794 - 796 SsR 854 - 856 ScK 864 - 866 StR 868 - 870 SeR 921 - 923 SpK - 923 SpK - 959 SvR 957 960 - 962 TgR 974 - 976 TsK 997 - 999 SrK 1002 - 1004 TgK 1018 - 1020 SgK 1031 - 1033 TgR 1119 - 1121 SkR



- Databases of functional or structural motifs, acquired by primary data (sequences) comparison
- PROSITE, <u>http://www.expasy.org/prosite/</u>

>PDOC50109 PS50109 HIS_KIN Histidine kinase domain [profile].

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98 hits with 12 PROSITE entries

La ExPASy Home page Site Map Search ExPASy Contact us Swiss-Prot PROSITE Proteomics tools.							
	A ExPASy Home page	Site Man	Search ExPASy	Contact us	Swiss-Prot	PROSITE	Proteomics tools



- Databases of **functional** or **structural** *motifs*, acquired by primary data (sequences) comparison
- PRINTS, <u>http://www.bioinf.man.ac.uk/dbbrowser/PRINTS/</u>



PRINTS is a compendium of protein fingerprints. A fingerprint is a group of conserved motifs used to characterise a protein family; its diagnostic power is refined by iterative scanning of a SWISS-PROTIFICABL composite. Usually the motifs do not overlap, but are separated along a sequence, though they may be contiguous in 3D-space. Fingerprints can encode protein folds and functionalities more flexibly and powerfully than can single motifs, full diagnostic potency deriving from the matual context provided by motif neighbours. <u>References</u>

New:	
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SPRINT - Search PRINTS-S (relational PRINTS)
 prePRINTS - Search PRINTS' automatic supplement
 InterPro - Search the integrated InterPro family database

Direct PRINTS access:

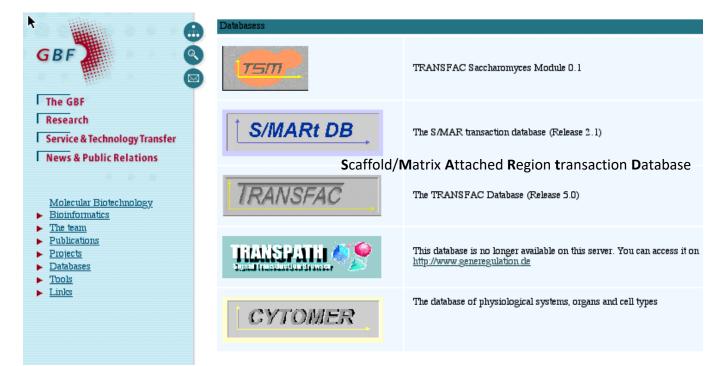
By accession number
 By PRINTS code
 By database code
 By text
 By sequence
 By number of motifs
 By author
 By query language

PRINTS search:





TRANSFAC <u>http://www.gene-regulation.com/</u>





Structural Databases

PDB <u>http://www.rcsb.org/pdb/</u>

structure data.

DEPOSIT data DOWNLOAD files browse LINKS BETA TEST new features BETA mmCIF files

Current Holdings

19623 Structures Last Update: 30-Dec-2002 PDB Statistics



Molecule of the Month: Cytochrome c

The Protein Data Bank (PDB) is operated by Rutgers, The State University of New Jersey; the San Diego Supercomputer Center at the University of California, San Diego; and the National Institute of Standards and Technology -- three members of the <u>Research Collaboratory</u> for <u>Structural Bioinformatics (RCSB)</u>. The PDB is supported by funds from the <u>National Science Foundation</u>, the <u>Department of Energy</u>, and two units of the National Institutes of Health: the



Welcome to the PDB, the single worldwide repository for the processing and distribution of 3-D biological macromolecular

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Structural Databases

• PDB <u>http://www.rcsb.org/pdb/</u>

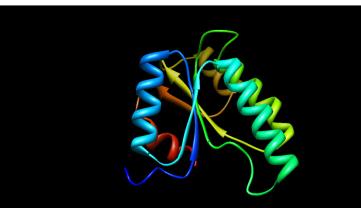
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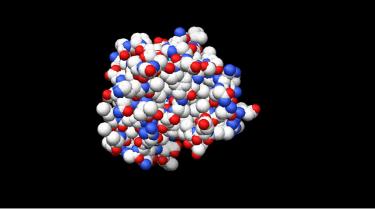
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Structural Databases

• PDB <u>http://www.rcsb.org/pdb/</u>





Pekárová et al., Plant Journal (2011)

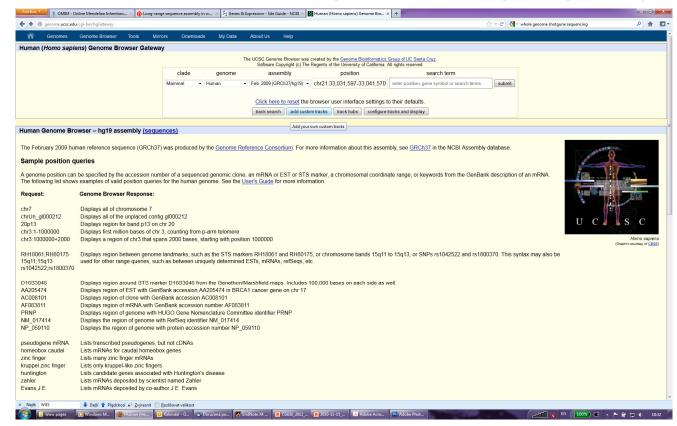


Outline

- Syllabus Of The Course
- Definition Of Genomics
- Role Of Bioinformatics In Functional Genomics
- Databases
 - Spectre of "on-line" Resources
 - PRIMARY, SECONDARY And STRUCURAL Databases
 - GENOME Resources

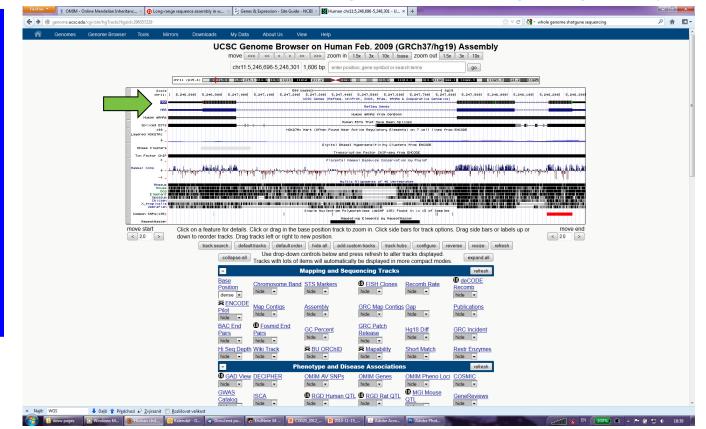


Human Genome Browser <u>http://genome.ucsc.edu/cgi-bin/hgGateway</u>





□ Human Genome Browser http://genome.ucsc.edu/cgi-bin/hgGateway



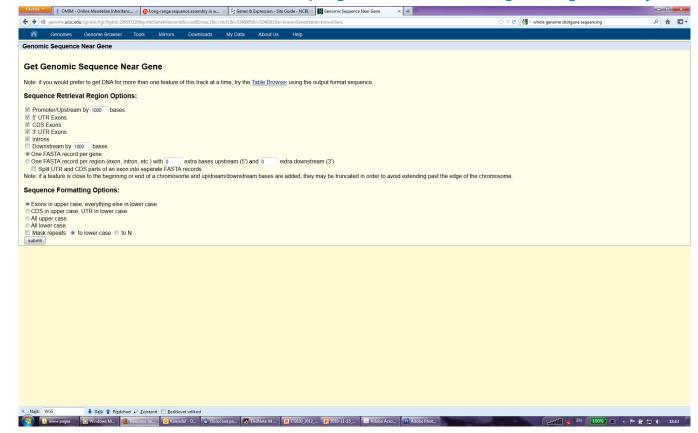


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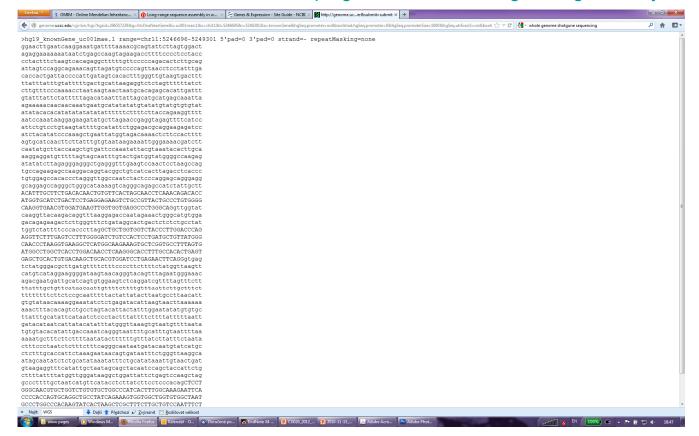


□ Human Genome Browser http://genome.ucsc.edu/cgi-bin/hgGateway



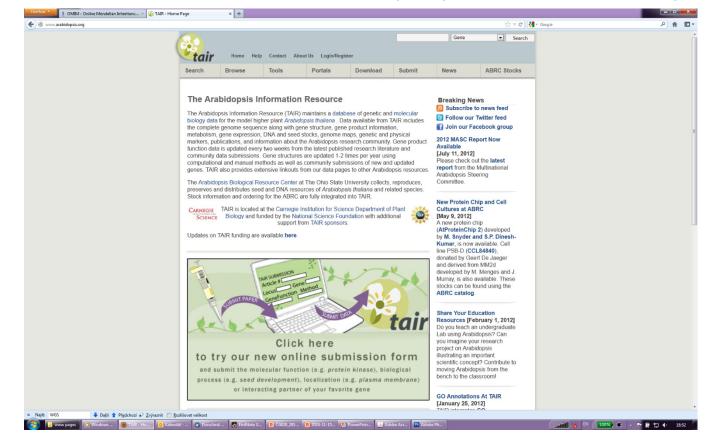


□ Human Genome Browser http://genome.ucsc.edu/cgi-bin/hgGateway





The Arabidopsis Information Resource (TAIR) <u>http://www.arabidopsis.org</u>





TAIR, The Arabidopsis Information Resource, <u>http://www.arabidopsis.org</u>

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The Arabidopsis Information Resource

The Arabidopsis Information Resource (TAIR) maintains a database of genetic and molecular biology data for the model higher plant *Arabidopsis thaliana*. Data available from TAIR includes the complete genome sequence along with gene structure, gene product information, metabolism, gene expression, DNA and seed stocks, genome maps, genetic and physical markers, publications, and information about the Arabidopsis research community. Gene product function data is updated every two weeks from the latest published research literature and community data submissions. Gene structures are updated 1-2 times per year using computational and manual methods as well as community submissions of new and updated genes. TAIR also provides extensive linkouts from our data pages to other Arabidopsis resources.

The Arabidopsis Biological Resource Center at The Ohio State University collects, reproduces, preserves and distributes seed and DNA resources of *Arabidopsis thaliana* and related species. Stock information and ordering for the ABRC are fully integrated into TAIR.

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We've added new dropdown headers and left navigation bars and reorganized our web pages to make it easier to locate information and resources in TAIR. Please contact us if you experience any problems with our new site.

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Data Updates Suspended

Breaking News

[October 19, 2006] Some TAIR data updates, including loading of new ABRC stocks, will be suspended from Oct 20-Nov 17 while we move our servers.

New Phenotype Search

Option [October 15, 2006] Search for genes, germplasms, and polymorphisms using associated phenotype, and see improved phenotype data display in results and detail pages.

ASPB Presentations

[August 15, 2006] Following heavy demand, the TAIR workshop presentations given at the ASPB meeting in Boston have been made available from the TAIR website for download.



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 - GENOME Resources
- Analytical Tools
 - Homology Searching



□ **Global** versus **Local** alignment

Globální přiřazení SLAV-----APATNIK-----PIQNYR-I----AKSETQRYMVIE SLAVYTYIEFVRANAPATNIKSECVRAAPIQNYRRVEHVRATAKSETQRYMVIE

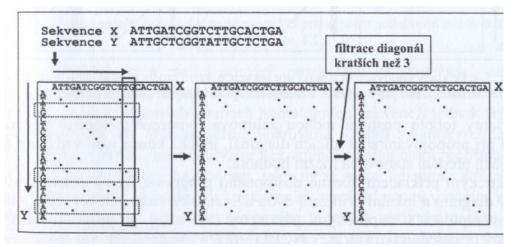
Lokální přiřazení SLAVYTYIEFVRANAPATNIKSECVRAAPIQNYRRVEHVRATAKSETQRYMVIE ------NAPATNIKSECVRA-PIQNYRRVEHVRA------

Cvrčková, Úvod do praktické bioinformatiky

- Global Alignment: only for sequences, which are similar and of a similar length (BUT can insert spaces into one or both sequences)
- Global Alignment is used mainly in case of multiple alignment (CLUSTALW, further in the presentation)
- Local Alignment provides identification and comparison even in case of alignment of regions of sequences with high similarity, e.g. even in case of change of order of protein domains during evolution



□ Choosing the right type of alignment using dotplot

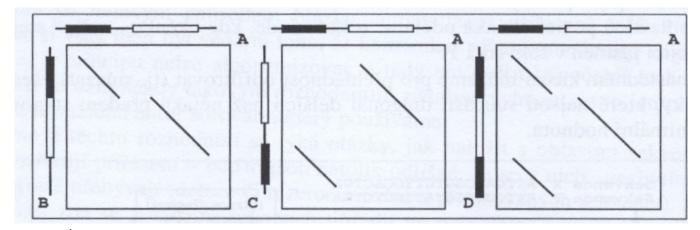


Cvrčková, Úvod do praktické bioinformatiky

- Plotting the sequences against each other (x and y axis)
- Identification of identity in "dot" of specific size (e.g. 2 bp)
- Filtering the diagonals of lengths lower than a treshold



□ Examples of sequence alignment using dotplot



Cvrčková, Úvod do praktické bioinformatiky

- Global Alignment: possible only for sequences A and B
- The rest of the sequences underwent change of order of protein domains and therefore it is neccessary to do a local alignment
- Dotplot can be obtained using BLAST2 (see further in the presentation)



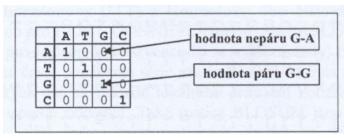
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	<u>Choose database</u>	m		\$					
	Now:	BLAST!	or (Reset quer	y Reset all					

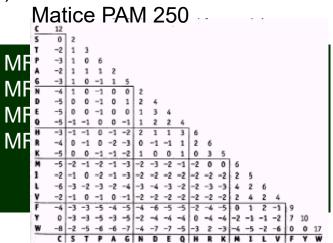


Basic Local Alignment Search Tool

- Word size: 10-11 bp or 2-3 aa
 - Primary similarities (seed matches)
 - Expanding the homology regions to the left and to the right
- Scoring the homology with matrices PAM (Point Accepted Mutation) or BLOSUM (BLOcks Substitution Matrix)
- Showing the results



Cvrčková, Úvod do praktické bioinformatiky





Basic Local Alignment Search Tool

□ > <u>gi 50160</u> L	E= expectancy value actin, beta (ACTB), mRNA	<u>ce</u>
	10 bits (560), Expect = 0.0 = 965/1100 (87%) lus / Plus	dsS=1213 E=0.0
Query: 156 Sbjct: 101	gtcgacaacggetetggeatgtgeaaggeeggatttgeeggagaegatgeteeeegegee 215 	250 1500
Query: 216 Sbjct: 161	gtetteecategattgtgggaegteecegteaceagggtgtgatggteggeatgggeeag 275 	
Query: 276 Sbjct: 221	aaggactegtaegtgggtgatgaggegeagageaagegtggtateeteaceetgaagtae 335 	
Query: 336 Sbjct: 281	cccattgagcacggtatcgtgaccaactgggacgatatggagaagatctggcaccacacc 395 	
	 "expectancy value" provides the^b number of exp number with the same or higher similarity whe database concisiting of randomly assembled sequer 	searching in the

 the results shows fraction of identical and in case of proteins also similar sequence positions and/or inserted spaces



Primary Databases

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Basic Local Alignment Search Tool

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•	4166 AAA79282	833 virA [Plasmid pTiC5	8]	
• <u> </u>	<u>4159</u> <u>NP_053380</u>	833 hypothetical protei	n pTi-SAKURA_p142 [Agrobacterium tumefaciens]	
·	4159 BAA87765	833 tiorf140 [Agrobacte	rium tumefaciens]	
·	4153 AAA91590	833 virA [Plasmid Ti]		
	<u>4153</u> gi 737127	833 virA protein		
•	4153 CAA34777	833 91.3 kDa protein [A	grobacterium tumefaciens]	
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Specialized Versions

- Currently there exists a lot of specialized versions of <u>BLAST</u>
 - Searching according to source (organism) of sequences, e.g. known genomes of <u>microorganisms</u>
 - BLASTP
 - Given the protein query, it returns the most similar protein sequences from the protein database.
 - BLASTN
 - Given the DNA query, it returns the most similar DNA sequences from the DNA database.
 - Other variants, e.g. MEGABLAST, for identification of identical or very similar sequences (searches long similar regions of nucleotide sequences)

BLASTX

• Compares the all possible six-frame translation products of a nucleotide query sequence (both strands) against a protein sequence database.



Specialized Versions

Currently there exists a lot of specialized versions of BLAST

TBLASTN

Compares a protein query against the all six reading frames of a nucleotide sequence database.

TBLASTX

• Translates the query nucleotide sequence in all six possible frames and compares it against the six-frame translations of a nucleotide sequence database.



Specialized Versions

Currently there exist a lot of specialized versions of BLAST

- <u>PSI-BLAST</u> (Position-Specific Iterated Blast)
 - First step: standard BLAST, during which PSI-BLAST identifies a list of similar sequences with E value better than minimal value (standard = 0,005)
 - For every alignment, PSI-BLAST creates so-called PSSM (Position Specific Substitution Matrix)
 - PSSM takes into account relative frequency of specific aminoacid residue in a specific position within sequences identified as similar in first step, which can mean functional conservation.



Specialized Versions

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Currently there exists a lot of specialized versions of BLAST

- PHI-BLAST (Pattern-Hit Initiated BLAST)
 - For identification of specific sequence, e.g. motif (pattern) in sequence of similar protein sequences
 - Sequence of motif must be inserted using special syntax:
 - [LVIMF] means either Leu, Val, Ile, Met or Phe
 - - is spacer (means nothing)
 - x(5) means 5 positions in which any residue is allowed
 - x(3, 5) means 3 to 5 positions where any residue is allowed



Specialized Versions

Example of search by PHI-BLAST

>gi|4758958|ref|NP_004148.1| Human cAMP-dependent protein kinase MSHIQIPPGLTELLQGYTVEVLRQQPPDLVEFAVEYFTRLREARAPASVLPAATPRQSLGHPPPEPGPDR VADAKGDSESEEDEDLEVPVPSRFNRRVSVCAETYNPDEEEEDTDPRVIHPKTDEQRCRLQEACKDILLF KNLDQEQLSQVLDAMFERIVKADEHVIDQGDDGDNFYVIERGTYDILVTKDNQTRSVGQYDNRGS<mark>FGELA LMYNTPRAATIVA</mark>TSEGSLWGLDRVTFRRIIVKNNAKKRKMFESFIESVPLLKSLEVSERMKIVDVIGEK IYKDGERIITQGEKADSFYIIESGEVSILIRSRTKSNKDGGNQEVEIARCHKGQYFGELALVTNKPRAAS AYAVGDVKCLVMDVQAFERLLGPCMDIMKRNISHYEEQLVKMFGSSVDLGNLGQ

[LIVMF]-G-E-x-[GAS]-[LIVM]-x(5,11)-R-[STAQ]-A-x-[LIVMA]-x-[STACV].



Outline

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- Databases
 - Spectre Of "On-line" Resources
 - PRIMARY, SECONDARY And STRUCURAL Databases
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- Analytical Tools
 - Homologies Searching
 - Searching Of Sequence Motifs, Open Reading Frames, Restriction Sites...



https://blog.addgene.org/free-online-molecular-biology-tools

Early Career Researcher Toolbox: Free Online Molecular Biology Tools

By Beth Kenkel



Beth Kenkel September 12, 2023

Share this article

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Primer design. Plasmid mapping. DNA sequence analysis. We all have our favorite tools for tackling these particular tasks, but they tend to be scattered about the internet. To help you keep your virtual molecular biology toolbox organized, today's post features a list of free online molecular biology tools all in one place.

Plasmid mapping

These tools are for viewing, editing or making plasmid maps, but can also analyze and annotate any DNA sequence.

- SnapGene Viewer: The free SnapGene Viewer is great for looking at plasmid maps and viewing sequencing traces, while the paid version provides more tools for plasmid mapping and design (Figure 1).
- Benchling: While you might think of Benchling as an electronic lab notebook, it also has a suite of molecular biology tools and can make plasmid maps. Free for academic users.
- Serial Cloner: Free desktop-based software for plasmid design and mapping.
- ApE (A plasmid Editor): A free, donation-based plasmid analysis tool including editing, annotating, creating maps, and more. This tool is maintained by M. Wayne Davis from the University of Utah.





https://www.snapgene.com/snapgene-viewer/download





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 - Other On-line Genome Tools

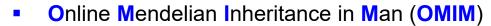


Other On-Line Genome Resources

- TIGR (The Institute for Genomic Research, <u>http://www.tigr.org/software/</u>)
 - Recently part of the J. Craig Venter Institute

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Other On-Line Genome Resources



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Discussion

