CG920 Genomics

Lesson 1

Introduction into Bioinformatics

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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

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



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Course Syllabus

□ Chapter 01

Introduction into Bioinformatics

□ Chapter 02

Identification of Genes

□ Chapter 03

Reverse Genetics Approaches

□ Chapter 04

Forward Genetics Approaches



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Course Syllabus

□ Chapter 05

Functional Genomics Approaches

□ Chapter 06

Protein-Protein Interactions And Their Analysis

□ Chapter 07

Current Methods of DNA Sequencing

□ Chapter 08

Structural Genomics



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Course Syllabus

□ Chapter 09

Localization of Genes and Gene Products in The Cell

□ Chapter 10

Genomics and Systems Biology

Chapter 11

Practical Aspects Of Functional Genomics

□ Chapter 12

- Tools Of Systems Biology
- Model Organisms, PCR And PCR Primer Design



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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



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Outline

- Syllabus of thecourse
- Definition of Genomics





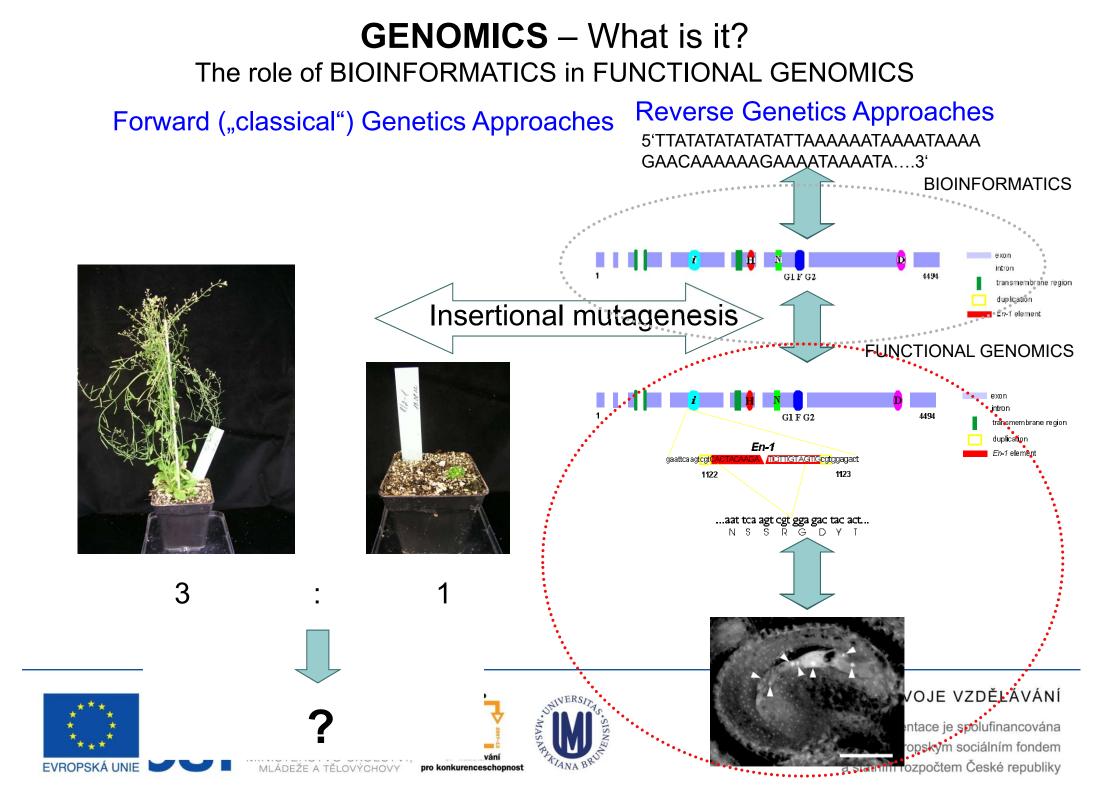
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GENOMICS – What is it?

- 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



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Outline

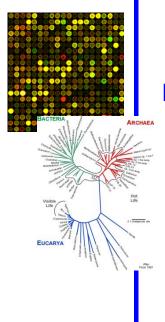
- Syllabus of this course
- Definition of genomics
- Role of BIOINFORMATICS in FUNCTIONAL GENOMICS

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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.





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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



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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



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Spectre of on-line Resources

Vienna Biocenter Austria http://www.at.embnet.org/ REN Belgium http://www.be.embnet.org/ BioBase http://biobase.dk/ Denmark CSC Finland http://www.fi.embnet.org/ http://www.infobiogen.fr/ INFOBIOGEN France GENIUSnet Germany http://genome.dkfz-heidelberg.de/biounit/ IMBB Greece http://www.imbb.forth.gr/ HEN Hungary http://www.hu.embnet.org/

INCBI	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	Sweden	http://www.embnet.se/
SIB	Switzerland	http://www.ch.embnet.org/
SEQNET	UK	http://www.seqnet.dl.ac.uk/

EMBnet Specialist Nodes

EMBnet National Nodes

MIPS	Germany	http://www.mips.biochem.mpg.de/
ICGEB	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

NIH

IBBM	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 Provide	75	
NCBI	USA	http://www.ncbi.nlm.nih.gov/
NLM	USA	http://www.nlm.nih.gov/

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Spectre of on-line Resources

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DNA & RNA genes, genomes & variation	Gene expression RNA, protein & metabolite expression	Proteins sequences, families & motifs	Bioinformatics train	ning
Structures Molecular & cellular structures	Systems reactions, interactions & pathways	Chemical biology chemogenomics & metabolomics		
Contologies taxonomies & controlled vocabularies	Literature Scientific publications & patents	Cross-domain tools & resources	Guide to resources	3
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Spectre of on-line Resources

All Database All D	atabases Search		
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Resources	and health by providing access to biomedical and genomic information.	Bookshelf	
nemicals & Bioassays	About the NCBI Mission Organization Research RSS Feeds	PubMed Central	
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DNA & RNA		BLAST	
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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,
 - http://www.ncbi.nih.gov/Genbank/GenbankSearch.html
 - DDBJ,

http://www.ddbj.nig.ac.jp

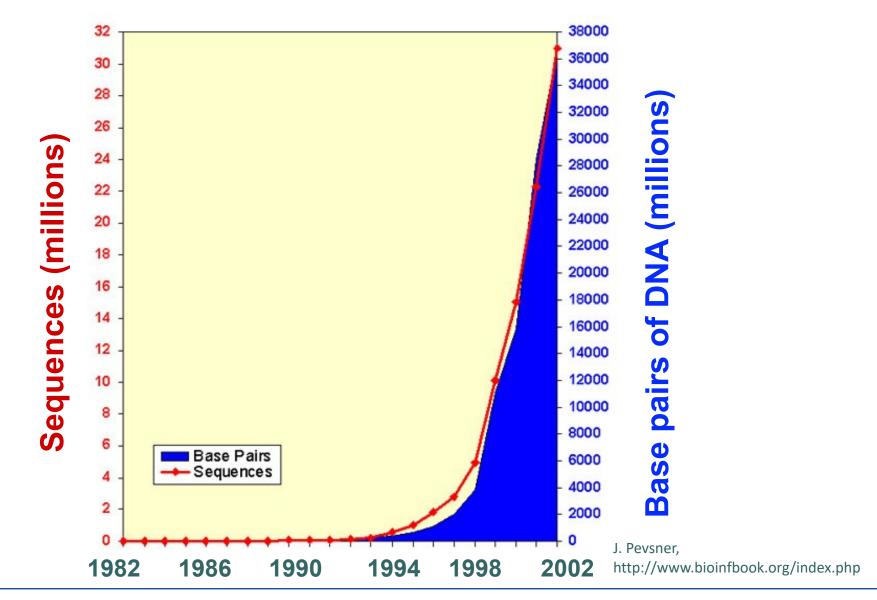
- Daily mutual exchange and backup of data
- Works with large amount of data (capacity and software requirements)
- September 2003 27,2 x 10⁶ entries (approx. 33 x 10⁹ bp)
- August 2005 100 x 10⁹ bp from 165.000 organisms





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Growth of GenBank

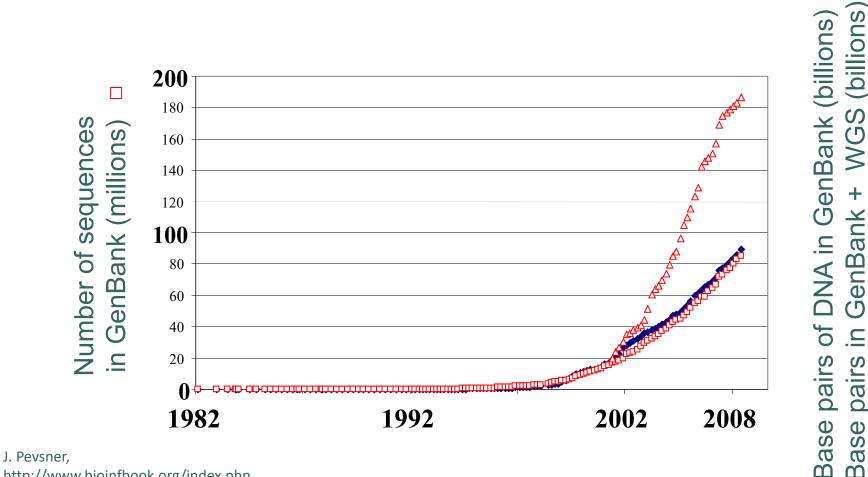


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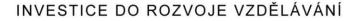
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Growth of GenBank + Whole Genome Shotgun (1982-November 2008): we reached 0.2 terabases

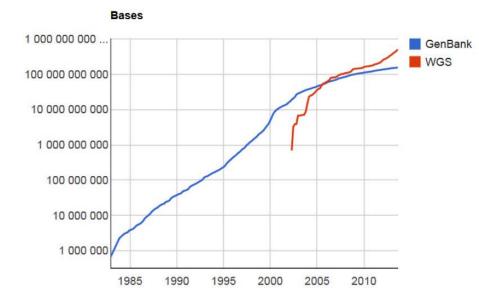


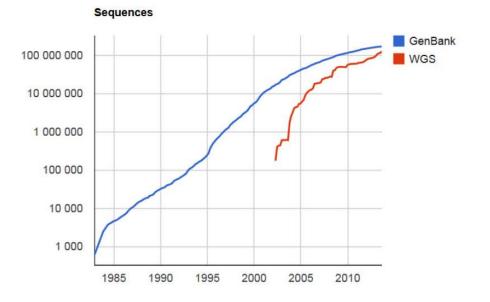
http://www.bioinfbook.org/index.php





Growth of GenBank Feb 15 2013







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WGS

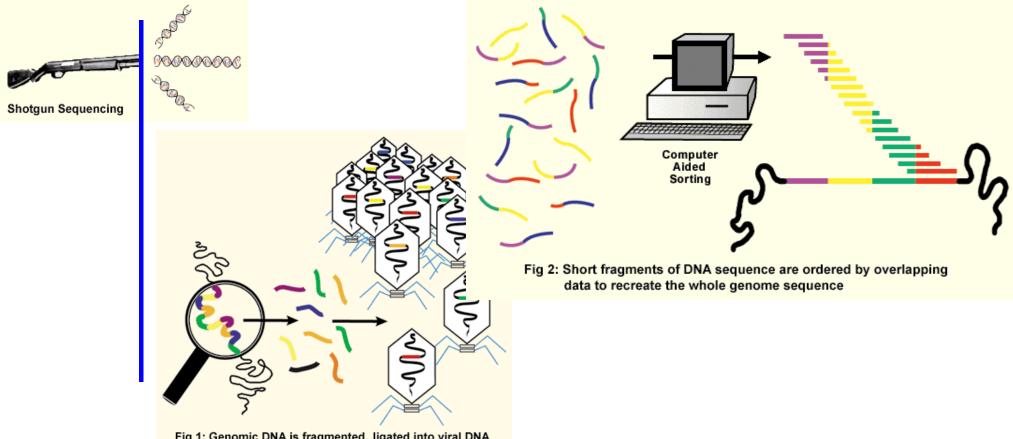


Fig 1: Genomic DNA is fragmented, ligated into viral DNA and packaged into viral particles to create a library

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



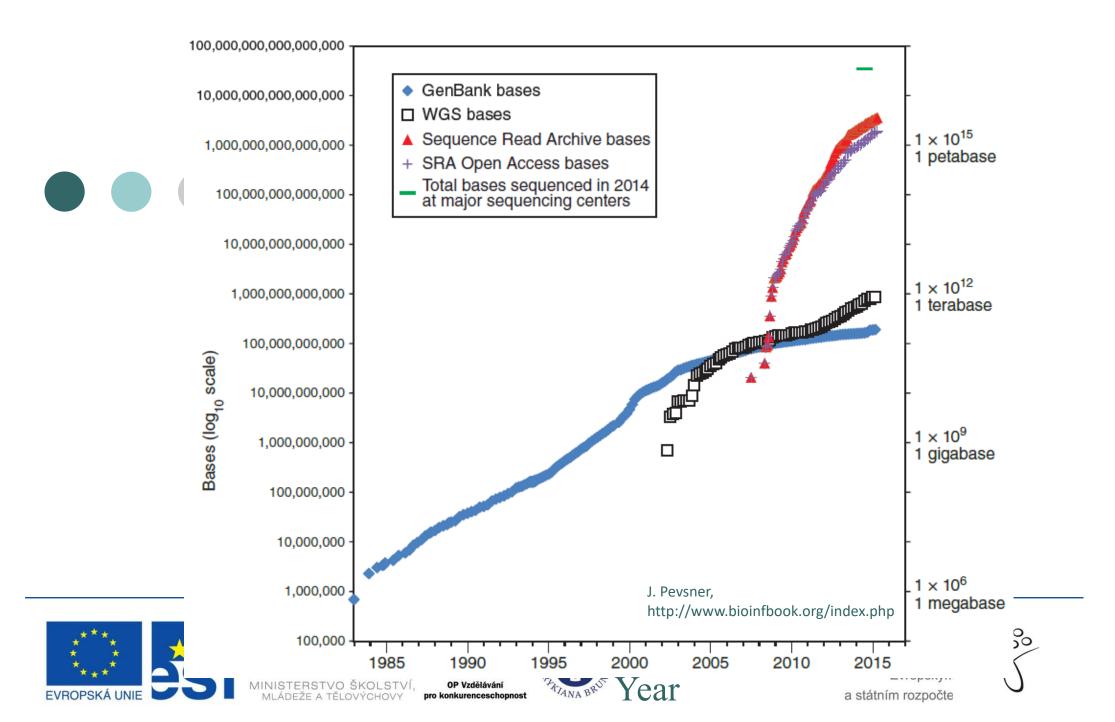




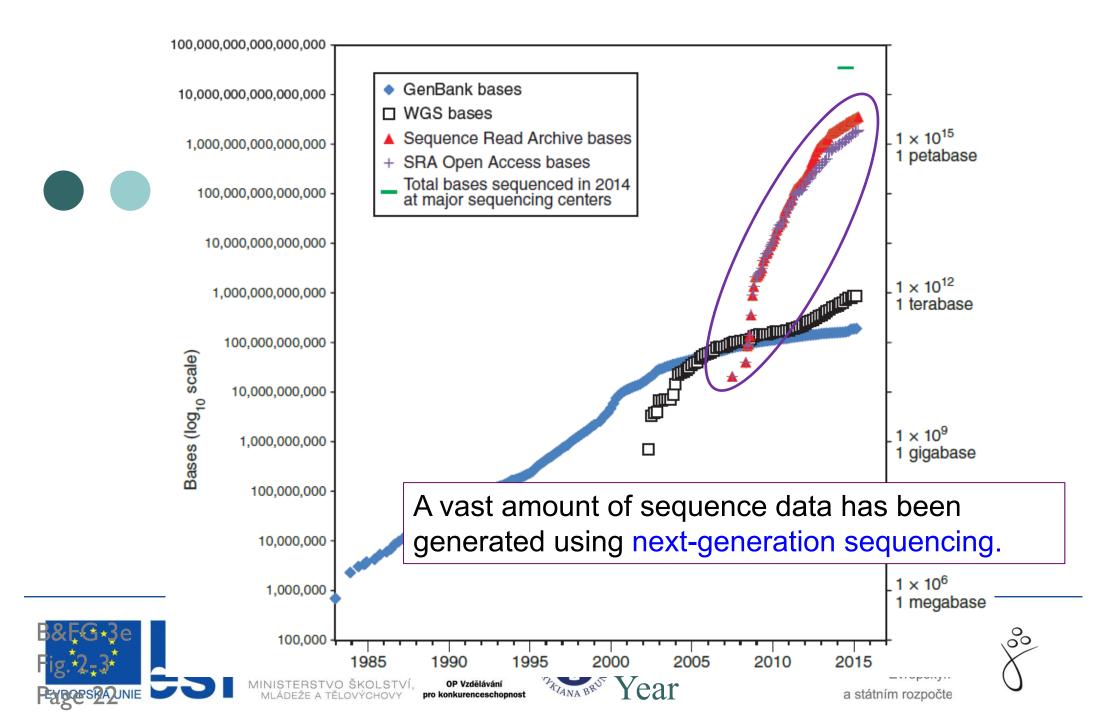


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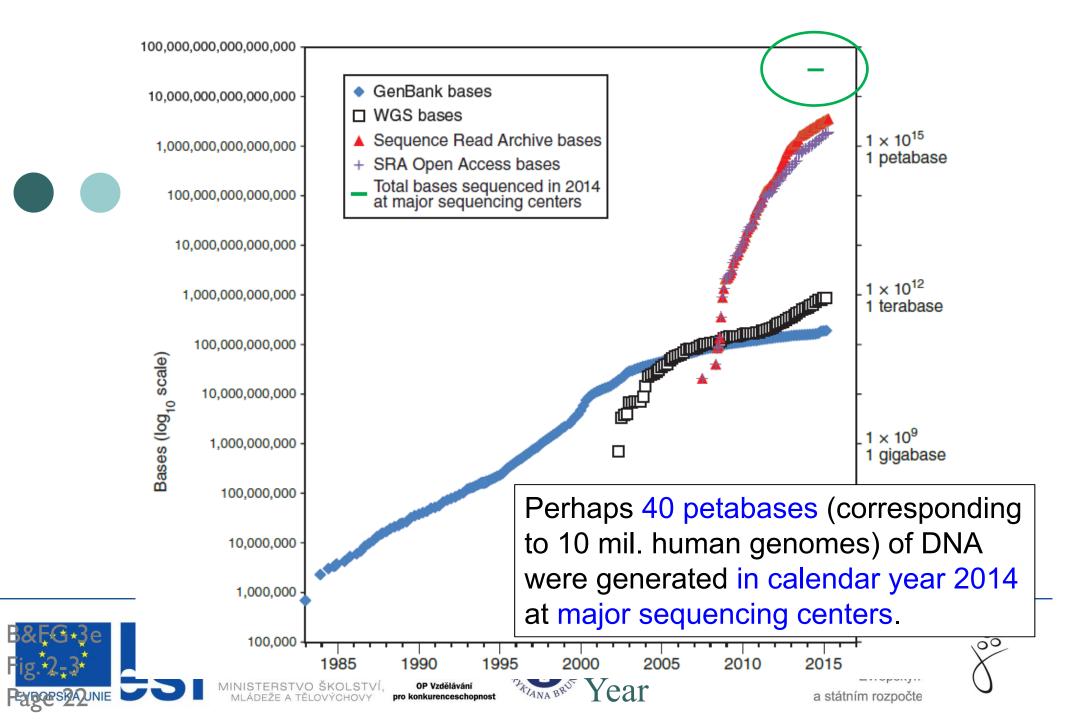
Growth of DNA Sequence in Repositories



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, http://pir.georgetown.edu/
 - □ MIPS, http://www.mips.biochem.mpg.de
 - SWISS-PROT, http://www.expasy.org/sprot/



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- 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)



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

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Biotechnology Information	Search	
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	Genome
Genetics & Medicine	Downloads: 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
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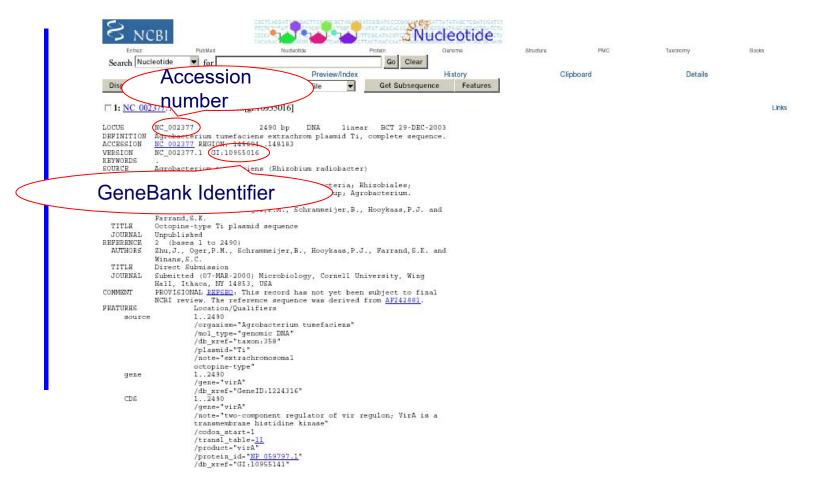
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/translation="MNGRYSPTRQDFKTGAKPWSILALIVAAMIFAFMAVASWQDNAT TQAILSQLRSINADSASLQRDVLRAHTGTVANYRPIISRLGALRKNLEDLKQLFRQSH IVEBENÄAQLLRQLEVSLNSADAAVAAFGAQNVRLQDSLASFTRALSSLPGKASTDQT LEKPTELASMMLQFLRQPSPAISFEISLELERLQKQRGLDEAPVRILAREGPIILSLL POVKDLVNMIQTSDTAEIAEMLQRECLEVYSLKNVEERSARIFLGSASVGLCLYIITL VYRLRKKTDWLARRLDYEELIKEIGVCFEGEAATTSSAQAALRIIQRFFDADTCALAL VDHDRRWAVETFGAKHPKPVWDDSVLREIVSRTKADERATVFRIISSKKIVHLPLEIP GLEILLAHKSTDKLIAVCSLGYQSYRPRPCQGEIQLLELATACLCHYIDVRRKQTECD VLARRLEHAORLEAVGTLAGGIAHEFNNILGEILGHAELAONSVSRTSVTRRYIDYII SSGDRAMLIIDQILTLSRKQERMIKPFSVSELVTEIAPLLRMALPPNIELSFRFDQMQ SVIEGSPLELQQVLINICKNASQAMTANGQIDIIISQAFLPVKKILAHGVMPPGDYVL LSISDNGGGIPEAVLPHIFEPFFTTRARNGGTGLGLASVHGHISAFAGYIDVSSTVGH GTRFDIYLPPSSKEPVNPDSFFGRNKAPRGNGEIVALVEPDDLLREAYEDKIAALGYE PVGFRTFNEIRDWISKGNEADLVMVDQASLPEDQSPNSVDLVLKTASIIIGGNDLKMT LSREDVTRDLYLPKPISSRTMAHAILTKIKT"

ORIGIN

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OP Vzdělávání

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

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







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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:

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

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



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RefSeq

ww.ncbi.nlm. nih.gov /gene/1137489			☆ ♥ C 🛃 = Google	۶
two-component VirA-like sens	sor kinase			See more
NCBI Reference Sequences (R	lefSeq)	×	2	
Genome Annotation				
The following sections contain re	eference sequences that b	elong to a specific genome build. <u>Explain</u>		
Reference assembly				
Genomic				
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Range	180831183332			
Download	GenBank, FASTA, Sequen	ce Viewer (Graphics)		
mRNA and Protein(s)				
		Agrobacterium tumefaciens str. C58]		
UniProtKB/Swiss-Prot Conserved Domains (3) <u>s</u>				
	Location:580 - 694	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		
	Location:466 - 530	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		







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

Accession AC 123456 AP 123456 NC 123456 NG 123456 NM 123456 NM 123456789 NP 123456 NP 123456789 NR 123456 NT 123456 NW 123456 NZ ABCD12345678 Genomic XM 123456 XP 123456 XR 123456 YP 123456 ZP 12345678

Molecule Genomic Protein Genomic Genomic mRNA mRNA Protein Protein RNA Genomic Genomic mRNA Protein RNA Protein Protein

Method Mixed Mixed Mixed Mixed Mixed Mixed Mixed Curation Mixed **Automated** Automated Automated Automated Automated **Automated** Auto. & Curated Automated

Note

Alternate complete genomic Protein products; alternate Complete genomic molecules Incomplete genomic regions Transcript products; mRNA Transcript products: 9-digit Protein products; Protein products: 9-digit Non-coding transcripts Genomic assemblies Genomic assemblies Whole genome shotgun data Transcript products Protein products Transcript products Protein products Protein products J. Pevsner,

http://www.bioinfbook.org/index.php





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Primary Databases

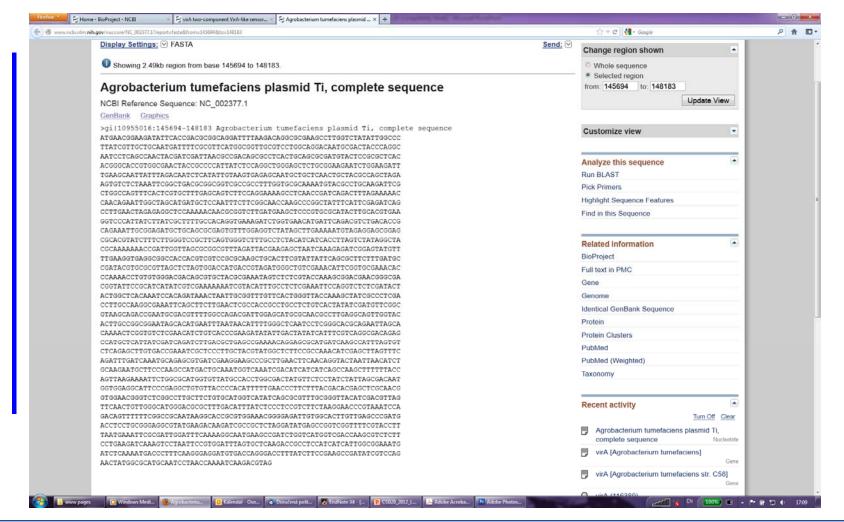
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Genes	Image: NP_059797.1 NP_059797.1: two-component VirA-like sensor kinase total range: NC_002377.1 (145,694148,183) total length: 2,490 strand: plus protein product length: 829 Links & Tools GenBank View: NC 002377.1 (145,694148,183), NP 0597 FASTA View: NC 002377.1 (145,694148,183), NP 059797 BLAST Genomic: NC 002377.1 (145,694148,183) Graphical View: NP 059797.1 BLAST Protein: NP 059797.1 BLINK Results: NP 059797.1	1,400	1,500	1,800	2 K
Bibliography					





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Primary Databases









INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

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

ExPASy Home page	Site Map	Search ExPASy	Contact us	Swiss-Prot	PROSITE	Proteomics tools
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- Databases of functional or structural motifs, acquired by primary data (sequences) comparison
- o PROSITE, http://www.expasy.org/prosite/

>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	-	71B	SpR
726	-	72B	SpK
747	-	749	TeK
794	-	796	SsR
854	-	856	SeK
864	-	866	StR
		B70	
921	-	923	SpK
		959	
		962	
974	-	976	TsK
		999	
1002 .	- 1	1004	TgK
1018 -	- 1	1020	SgK
1031 -	- 1	1033	TqR
1119 -	- 1	1121	SkR



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

>PDOC50109 PS50109 HIS KIN Histidine kinase domain [profile]

402 - 671 NASHDIRGALAGMKGLIDICRDGVKPGSDVDTTLNQVNVCAKDLVALLNSVLDMSKIEGG KMQLVBEDPNLSKLLEDVIDFYHPVAMKKGVDVVLDPHDgavfKFSNVRGDSGRLKQIIN NLVSNAVKFTVD--GHIAVRAWAQrpgsnssvvlasypkgvakfvksmfcknkeesstye teisseirinnanTMEFVFEVDDCKSIFMEMKKSVFRNVQVRELAQGHQGTGLGLGIVQ SLVRLMGGEIRITDKAMGekGTCPQFNVLLTT

>PDOC50110 PS50110 RESPONSE_REGULATORY Response regulatory domain [profile].

987 - 1085 RVLVVDDNFISRKVATGKLKKNGVSeVEQCDSGKKALRLVTEGLtqreeqgsvdklpFDY IFMDCQMFENDGVKATREIRkvekSYGVRTFIIAVSGHD

Graphical summary of hits (java applet)



98 hits with 12 PROSITE entries

ExPASy Home page	Site Map	Search ExPASy	Contact us	Swiss-Prot	PROSITE	Proteomics tools





INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

- 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 SWBS-PROT/TEMBL 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 mutual context provided by motif neighbours. <u>References</u>

New:

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 database code
 By text
 By sequence
 By title
 By number of motifs
 By query language

PRINTS search:

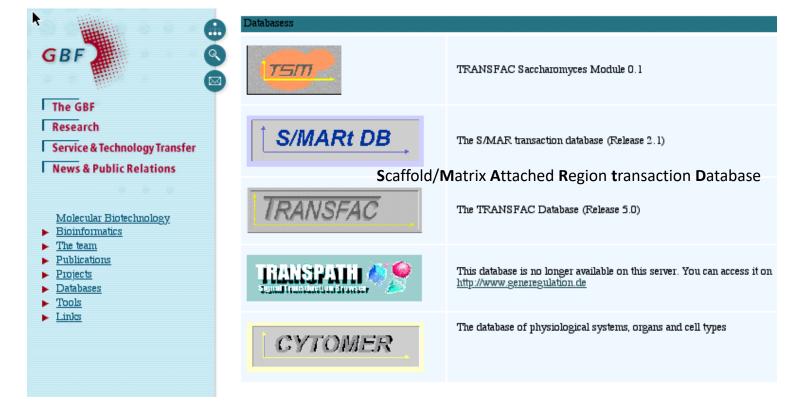
Search PRINTS with NEW <u>FingerPRINTScan</u>
 FPScan
 GRAPHScan
 <u>GRAPHScan</u>
 <u>MUIScan</u>
 <u>MUIScan</u>
 FingerPRINTScan binaries and source are available: <u>contact scordis@bioinf.man.ac.uk</u>





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o TRANSFAC http://www.gene-regulation.com/











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Structural Databases

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











INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Structural Databases

o PDB http://www.rcsb.org/pdb/

Structure Explorer - 1P5Y



Structure Explorer - 1P5Y

View Structure

 Title
 The Structures Of Host Range Controlling Regions Of The Capsids Of Canine and Feline Parvoviruses and Mutants

 Classification
 Virus/Viral Protein

 Compound
 Mol_1d: 1; Molecule: Coat Protein Vp2; Chain: A; Fragment: Sequence Database Residues 190-737; Engineered: Yes; Mutation: Yes

 Exp. Method
 X-ray Diffraction

Summary Information

View Structure

B

Download/Display File

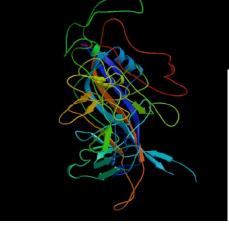
Structural Neighbors

Geometry

Other Sources

Sequence Details

Explore SearchFields



http://www.rcsb.org/pdb/cgi/explore.cgi?job=graphics;pdb1d=1P5Y;page=;pid=173561064349344&bio=1&opt=show&size=500 12/29/2003





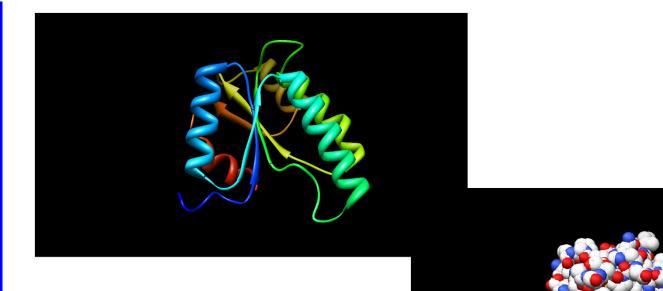




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Structural Databases

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



Pekárová et al., Plant Journal (2011)









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Outline

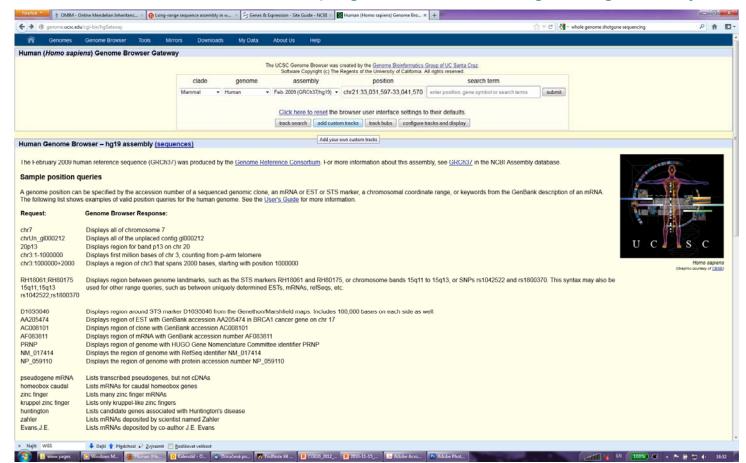
- Syllabus Of The Course
- Definition Of Genomics
- Role Of Bioinformatics In Functional Genomics
- Databases
 - Spectre of "on-line" Resources
 - PRIMARY, SECONDARY And STRUCURAL Databases
 - GENOME Resources





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

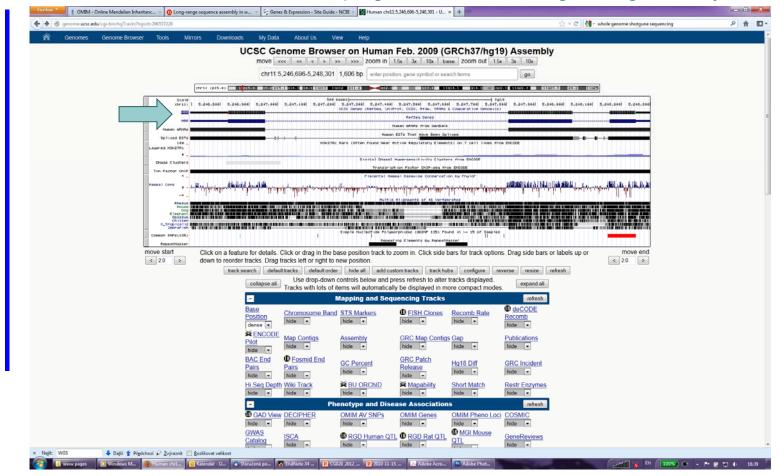






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Human Genome Browser <u>http://genome.ucsc.edu/cgi-bin/hgGateway</u>









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

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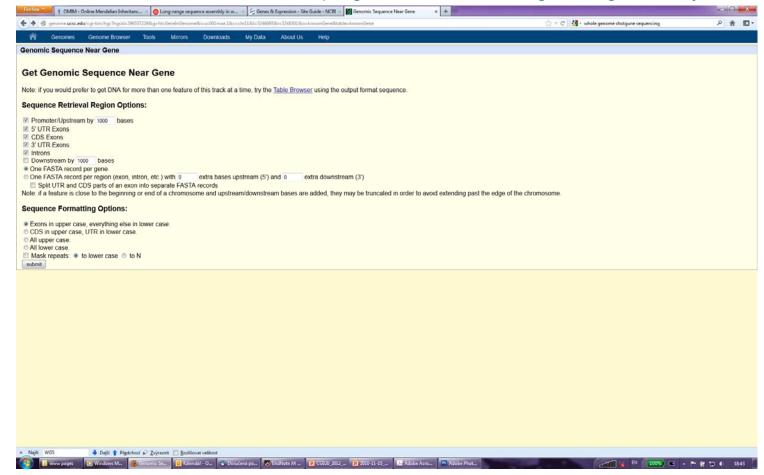






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Human Genome Browser <u>http://genome.ucsc.edu/cgi-bin/hgGateway</u>











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

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









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

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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.

The NEW arabidopsis.org

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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Breaking News

Data Updates Suspended

[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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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
 - Homology Searching



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□ Global versus Local alignment

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

SLAVYTYIEFVRANAPATNIKSECVRAAPIQNYRRVEHVRATAKSETQRYMVIE

Cvrčková, Úvod do praktické bioinformatiky

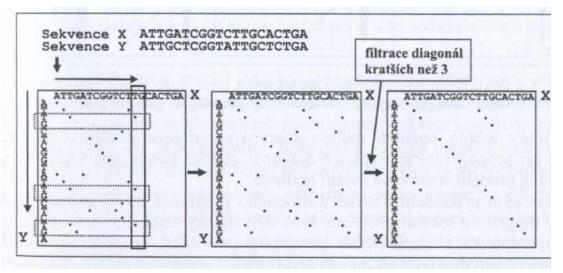
- Global Alignment: only for sequences, which are similar and of a similar length (BUT can insert spaces into one or both sequences)
- Global Alignment is used mainly in case of multiple alignment (CLUSTALW, further in the presentation)
- Local Alignment provides identification and comparison even in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





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□ Choosing the right type of alignment using dotplot



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- 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

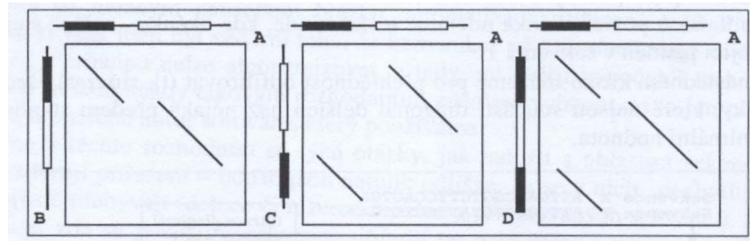






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Examples of sequence alignment using dotplot



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- 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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o BLAST http://ncbi.nlm.nih.gov/BLAST/

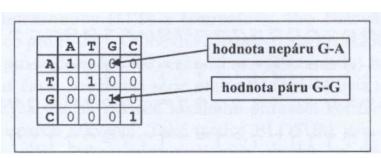
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Choose database 🔟	



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

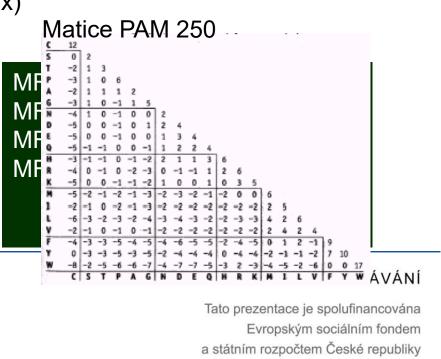
Basic Local Alignment Search Tool

- 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

	E = expectancy $E = expectancy$ $ength = 1793$ $Value$	actin, beta (ACTB), mRNA	œ
	10 bits (560), Expect = 0.0 = 965/1100 (87%) lus / Plus		dsS=1213 E=0.0
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Query: 336 Sbjct: 281	cccattgagcacggtatcgtgaccaactgggacgatat 		
	number with the same	vides the ^{lm} number of exp or higher similarity whe andomly assembled seque	searching in the

the results shows fraction of identical and in case of proteins also

similar sequence positions and/or inserted spaces





OP Vzdělávání pro konkurenceschopnost



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Primary Databases

145,400 145,600 145,800 146 K 146,200 146,400 146,600 146,8	00 147 K	147,200	147,400	147,600
Image: NP_059797.1 NP_059797.1 NP_059797.1 NP_059797.1 total range: NC_002377.1 (145,694148,183) total length: 2,490 strand: plus protein product length: 829 Links & Tools GenBank View: NC_002377.1 (145,694148,183), NP_059 BLAST Genomic: NC_002377.1 (145,694148,183), NP_059 BLAST Genomic: NP_059797.1 BLAST Protein: NP_059797.1 BLAST Protein: NP_059797.1 BLINK Results: NP_059797.1		1,600	1,800	2 K





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Basic Local Alignment Search Tool

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Concerved Domain Database hits	- - 4166 AAK90927 833 two component sensor kinase [Agrobacterium tumefaciens str. C58] 4166 P18540 833 RecName: Full=Wide host range virA protein; Short=WHR virA 4166 AAA79282 833 virA [Plasmid pTiC58]	t range virA protein; Short=WHR virA Ti-SAKURA_p142 [Agrobacterium tumefaciens]
	4166 P18540 833 RecName: Full=Wide host range virA protein; Short=WHR virA 4166 AAA79282 833 virA [Plasmid pTiC58]	t range virA protein; Short=WHR virA Ti-SAKURA_p142 [Agrobacterium tumefaciens]
<u>4166</u> <u>AAK90927</u> 833 two component sensor kinase [Agrobacterium tumefaciens str. C58]	4166 AAA79282 833 virA [Plasmid pTiC58]	TI-SAKURA_p142 [Agrobacterium tumefaciens]
4166 P18540 833 RecName: Full=Wide host range virA protein; Short=WHR virA		
4166 AAA79282 833 virA [Plasmid pTiC58]	<u>4159 NP_053380</u> 833 hypothetical protein pTi-SAKURA_p142 [Agrobacterium tumefaciens]	
		m tumefaciens]
4159 BAA87765 833 tiorf140 [Agrobacterium tumefaciens]	4159 BAA87765 833 tiorf140 [Agrobacterium tumefaciens]	
4153 AAA91590 833 virA [Plasmid Ti]	4153 AAA91590 833 virA [Plasmid Ti]	
<u> 4153 gi[737127</u> 833 virA protein		
4153 CAA34777 833 91.3 kDa protein [Agrobacterium tumefaciens]		
3800 CAA35780 829 virA [Agrobacterium rhizogenes]	3800 CB325700 820 wind [Acceleration whiteseene]	
	<u>5600</u> CAA55780 829 VIR [Agrobacterium rhizogenes]	
<u></u> <u>3718</u> <u>gi 227240</u> 869 virA gene	<u> 3718 gi/227240</u> 869 virA [Agrobacterium rhizogenes]	





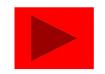




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Specialized Versions

- Currently there exists a lot of specialized versions of BLAST
 - Searching according to source (organism) of sequences, e.g. known genomes of microorganisms
 - 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.





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Specialized Versions

- Currently there exist a lot of specialized versions of BLAST
 - **PSI-BLAST** (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.









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Specialized Versions

- 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



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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].





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 - GENOME Resources
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 - Homologies Searching
 - Searching Of Sequence Motifs, Open Reading Frames, Restriction Sites...



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biology WorkBench toggte between menus and buttons WE Moved! http://workbench.sdsc.edu/
Version 3.2
Session Tools Protein Tools Nucleic Tools Alignment Tools Structure Tools (Alpha)
beta-glucosidase
GBPLN:804655 Hordeum vulgare L. beta-glucosidase (BGQ60) gene, complete cds.
GBPLN:170248 Nicotiana tabacum glucan beta-1,3-glucosidase gene, complete cds.
Select All Deselect All Ndjinn BATCH Add Edit Delete Copy View Download ViewRecords BL2SEQ BL2SEQX BLASTN BLASTX TBLASTX FASTA FASTX FASTY SSEARCH CLUSTALW
CLUSTALWPROF ALIGN LALIGN LFASTA PATTERNMATCHDB PATTERNMATCH TACG PRIMER3
NASTATS BESTSCOR PFSCAN PRIMERCHECK PRIMERTM SIXFRAME REVCOMP RANDSEQ
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Format Fasta Case Upper Change Format Download/view all sequences in text format INEXT1 BOTTOMI Nicotiana tabacum glucan beta-1,3-glucosidase gene, complete cds. GBPLN:170248, 4699 bp > 170248 GAGCTCCCTTGGGGGGGCAAGGGCAAAACTTTTTTGCTAAATGGAAAAATATTATCCAAGTS: GTTACTCAATTTGAATTAACAAAGGGGCAAAATTTTGCCCATTATCCTTTTGGTX ATAAAATATCCCATCCGAAATTCCAAATGGTCCATTATCGGCAAGTAGCTTTCTTT	
INEXT] [BOTTOM] Nicotiana tabacum glucan beta-1,3-glucosidase gene, complete cds. GBPLN:170248, 4699 bp > 170248 GAGCTCCCCTTGGGGGGGCAAGGGCAAAACTTTTTGCTAAATGGAAAAATATTATACCAAGTG? GTTACTCAATTTGAATTAACAAAGGGGCAAAACTTTTTGCTAAATGGAAAAATATTATACCAAGTG? GTTACTCCAATTGGAATTAACAAAGGGGCAAAATTGGCAATTTGCCCTTATATCTTTGGTY ATAAAATATCCCATCCGAAATTCCAAATGGTCCATTATCGGCAAGTAGCTTTCTTT	
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$\label{eq:alpha} ATAAAATATCCCATCCGAAATTCCAAATGGTCCATTATCGGCAAGTAGCTTTCTTT$	
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$ \begin{array}{l} {\tt GTAGAGCCGCCAGTAAAATAAGACCGATCAAATAAAAGCCGCCATTAAAATAATGAATTTTTTGATTGGCACCAGTAAGTGCCAAAACTCTTCCAATACTTTGCTGCAACTTGGGGCTGCTAGGTTCCAGGTTGCAACTTGGGGATATTAAGAAATTTCCGCCTAATTTACATCACCGATACTAAAATTTTAAGAAATTTCCGCCTCGCATGCAT$	
GATTGGCACGTAAGTGCCAAAACTCTTCCAATACTTTGCTGCAACTTGGGGCTGCTAGGTTCCAGGTTAGGATATTGGGATATTTCTAAGTTTAACTCTCCAATATTAAGAAATTTACATCTCAACTAATATTAAGAAATTTCCGGGTCGCTTTGGCACGGTTACTGATTCACTAAGGGGTCGTTTGGTACAAGAAATAATAATAATAATAATTTCGGGGGGGG	
CAGATATGGGATATTICTAAGTTTATCTCCCTAATTTACATCTCAACTAATATTAAGAAATTTACGATATGGGATATTICTAAGGATTTATCTCCTCTAAAGAAGACAATGAATCCGGTTACTGATTCATTGGCCCTCTGCATGCCATATTCACTAAGGGGTCGTTTGGTACAAGAAATAATAATAATAATAATTTCGGGGGAGAATGGAATGCAATTTCAGAAAAATTTTACACACTCACATGTAGAAGGTGGAATGGAATAGCTAATTCCAGATAGCAACTCACATTTTACCACATGTAGAAGGTGGAATGGAATAGCTAATCCACATGAAAATTTTACCACATGTAGAAGGTGGAATGGAATAGCTAATCCACATGAGAAAATTTTACCACATGTTCATAAATCAACTTCATGAGAAAATTTTAGATATGGAATGGAATAAAAATTTAGATATGGAATAGGAATAGAATTTAGATATGGAAAAAA	
CAGCAAATCATAAAATTTTCCTCTAAAGAAGACAATGAATCCGGTTACTGATTCATTGGCCTTCTGCATGCCATATTCACTAAGGGGTCGTTTGGTACAAGAAATAATAATAATAATAATTTCGGGGGGGG	
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$\label{eq:targatical} TAAAAATCAACTATCACATGTAGAAGGTGGAATGGAATAGCTAATCCCATAGCCACTCACATTTATTT$	ATAGAATIT
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CGAGACACTCTTATGGAAGGCGTTGTCTTCAAAGTAGATTCTCATTCAT	
GAGAACTTTCAAATCTCTCAACTACTTTTTAAGGGAATTCAAAATACGACCAATATTTATT	









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Regex pattern:

ctt.{1,32}ctt

0 sequences were searched

1 match was found

Matches are indicated in blue

>170248

GAGCTCC **SCAAGGGCAAAACTTTTTGCTAAATGGAAAAATATTATACCAAGTGTTTGTAAT** GTTACTCAATTTGAATTAACAAAGGGGCAAATTTGACTATTTTGCCCCTTATATCTTTTGGTCACAAAAAC ATAAAATATCCCATCCGAAATTCCAAATGGTCCATTATCGGCAAGTAG TAATTATAGTTAGT GACAAAACACTATCAAGATATCATTATTATAATAATAACT GTAGAGCCGCCAGTAAAATAAGACCGATCAAATAAAAGCCGCCATTAAAATAATGAATTTTAGGACTCTC GATTGGCACGTAAGTGCCAAAACTCTTCCAATACTTTGCTGCAACTTGGGGCTGCTAGGTTCTGAGCTT CAGATATGGGATATTTCTAAGTTTATCTCCTAATTTACATCTCAACTAATATTAAGAAATTAAAC CAGCAAATCATAAAATTTTCCTCTAAAGAAGACAATGAATCCGGTTACTGATTCATTGGCCTTT TCTGCATGCCATATTCACTAAGGGGTCGTTTGGTACAAGAAATAATAATAATAATTTCGGGATAGAATTT GAGATTGCATTTATCTTGTGTGTTTAATTATAAGTATTAGCTAATTTCAGAATAAATTTTACACTAA TAAAATCAACTATCACATGTAGAAGGTGGAATGGAATAGCTAATCCCATAGCCACTCACATAGAATATCC TTATTTATCTCACTATTTTACCAAATGATCGGTTAGTCTTCATGAGAATCCAGTATCCTCAATAAATGC GTAAGAAGTTAGAAAATTTTCATTAAATCAATTCATATAATTTAAAAATATTAGATATGGAGCACTTAAG TGACATC CTATIGTATACTCAAATGAAAGTIIT. GAGAACT TTTAAGGGAATTCAAAATACGACCAATATTTATTA TATAGTTAAATGATATGAATTITATTITAAATTIGAATIGAAAATATTAAATTACTTGATTTAATATA ACAATAGATATCGCTAAGTATTTACCACAAACATGGAGATACTACAGAAGATTTTATTATTTGTAACGAT

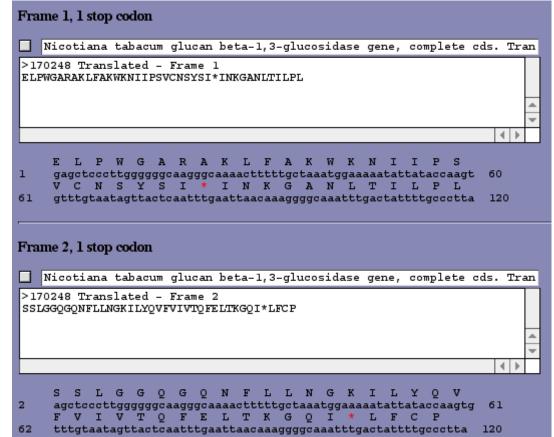








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= Linear Map of Sequence:	
StyI BsaJI CviJI AluI SacI EcoICRI Bsp1286I BsiHKAI BanII BslI SspI	
1 gagetececttgggggggggggggggggaaaatgggtaaaatggtttagagggaaaaatggtttaggttea 1 E L P W G A A L F A W N I I P S S C G Q Q N F L N G I I P S 2 S S L G Q Q N F L N G K I I P S 2 S S L G Q Q N F L N G K I I P S S S G Q Q V S S S G Q Q V S S S G Q Q Q N F L N G K Z X X X X C X Z X X X X X X X X X X X	60
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61 gtttgtaatagttactcaatttgaattaacaaaggggcaaatttgactattttgccctta caaacattatcaatgagttaaacttaattgtttccccgtttaaactgataaaacgggaat 1 V C N S Y S I * I N K G A N L T I L P L 2 F V I V T Q F E L T K G Q I * L F C P * 3 L * * L L N L N * Q R G K F D Y F A L R 4 N T I T V * N S N V F P C I Q S N Q G * 5 T Q L L * E I Q I L L P A F K V I K G K 6 H K Y Y N S L K F * C L P L N S * K A R	120

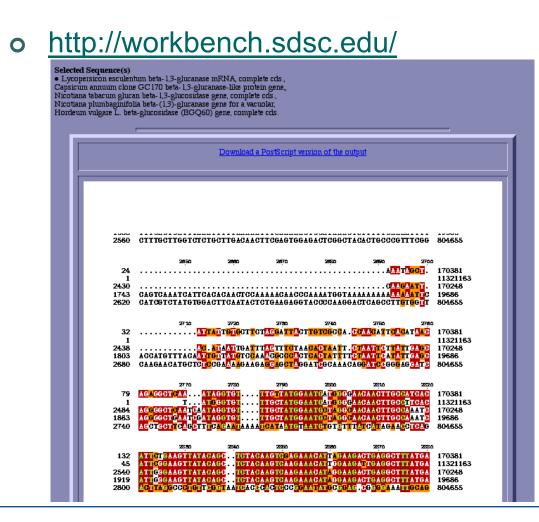








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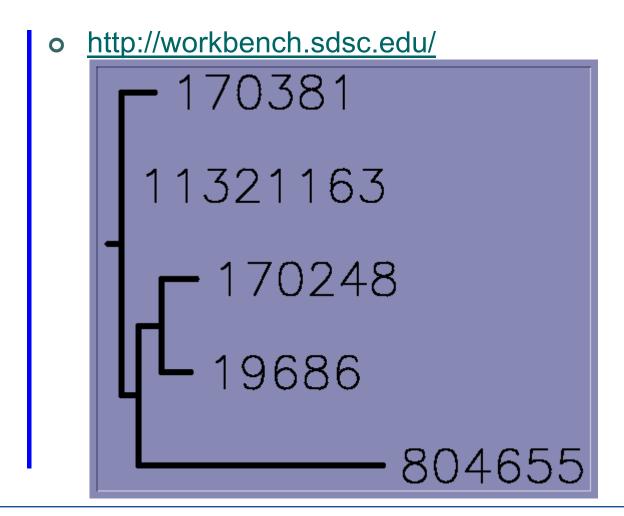


OP Vzdělávání





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• VPCR <u>http://grup.cribi.unipd.it/cgi-bin/mateo/vpcr2.cgi</u>

SEARCH		<u>ABOUT</u>	DOWNLOAD	<u>LINKS</u>
searches Ù NOTE: Abiliti couple thousa: overrepresented	he specified database fo predicts ar ies of VPCR 2.0 are still li nd matches per primer. For l sequences cause problems	r matches to the primers. If uplification. Calculated PCI mited by BLAST espabilities an example, using primers shorter as well. We are now busy solvin have for VPCR 2.0 etc. Curren	sequences (<u>IUB codes</u> allowed for matches are found within 10000 ba R products are displayed within a m d settings, as well as inability of our our or roughly equal to our 11-base word size ag most of these problems, please, he pati thy, this address is for testing VPCR 2.0, <u>0 Homepage</u> .	asës, a PCR simulation model inute. ent software to deal with more than misses most matches. Primers with ent. If you have a minute, please, k
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	Primer 1			
	Primer 2			
	Primer 3			
	Primer 4			
	Primer 5			
	Primer 6			
	Primer 7			
	Primer 8			
	Annealing ter	aperature		
	Do PCR!			12 I





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VPCR <u>http://grup.cribi.unipd.it/cgi-bin/mateo/vpcr2.cgi</u>

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600 619 NC_003076.1 2254473 2253854	
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Read 2 primers, 18 matches and 1 amplicons. TEMP=50oC	
0:1e-06 619 NC_003076.1 2254473 2253854 1 2	



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 - Homologies Searching
 - Searching Of Sequence Motifs, Open Reading Frames, Restriction Sites...
 - Other On-line Genome Tools



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Other On-Line Genome Resources

EVROPSKÁ

TIGR (The Institute for Genomic Research, <u>http://www.tigr.org/software/</u>)

Recently part of the J. Craig Venter Institute

JCVI: Home	× 📮 Diploid Human Genome Browser H 🗵	😔 PHACTR4 phosphatase and actin reg × 🕂		÷
♦ ♦ 8 http://ww	wv.ncbi.nlm. nih.gov/ sites/entrez?Db=gene&Cmd=ShowDetailView8	TermToSearch=65979	C 🚼 - institute for genomic research	۹
	🕑 How To 🖂		My h	NCBI Sign In 📥
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Gene	Limits Advanced		Search	Help =
	Linits Advanced			
Display Settings: 🖂 Ful	I Report	Send to	-	
PHACTR4 phose	hatase and actin regulator 4 [Homo sapiens]		Table of contents	
Gene ID: 65979, update			Summary Genomic context	
2 2			Genomic regions, transcripts, and pro	ducts
🔺 Summary		a de la companya de la	Bibliography	
Official Symbol	PHACTR4 provided by HGNC		Interactions	
	phosphatase and actin regulator 4 provided by HGNC		General gene info	
Primary source			General protein info	
	RP11-442N24A.1		Reference sequences	
	Ensembl:ENSG00000204138; HPRD:07816; MIM:608726		Related sequences	
RefSeq status	protein coding REVIEWED		Additional links	
	Homo sapiens			
		; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini;	Links	
	Hominidae; Homo FLJ13171; MGC20618; MGC34186; DKFZp686L07205; RP11-44	20124 . 6.4	Order cDNA clone	
		lator (PHACTR) family. Other PHACTR family members have been shown to	BioAssay, by Gene target	
,		is gene in the mouse has been shown to interact with actin and PP1. Multiple	BioProjects	
	transcript variants encoding different isoforms have been found fo	r this gene. [provided by RefSeq, Jul 2008]	CCDS	
••••••••••••••••••••••••••••••••••••••			Conserved Domains	
Genomic contes	xt	1	dbVar	
Location : 1p35.3			EST	
Sequence : Chromos	ome: 1; NC_000001.10 (2869609328826881)	See PHACTR4 in MapV	Full text in PMC	
			Genome	
	Chromosome 1 - NG	_UUUUU1.1U [28865708 b	GEO Profiles	
	SESN2 MED18 -> PHACTR4		HomoloGene	
		SNORA73A SNORA73B	Map Viewer	
		RNU105A	Nucleotide	
		RCC1	OMIM	
			Probe	
 Genomic region 	is, transcripts, and products		Protein PubChem Compound	JE VZDI
		Go to reference sequence d		
_			PubMed	
Genomic Sequence N	C_000001 chromosome 1 reference GRCh37.p5 Primary Asse	mbly 🝷	PubMed (GeneRIF)	e je spolu
		Go to nucleotide Graphics FASTA Gen		is je spora

ním fondem bčtem Ceské republiky

Other On-Line Genome Resources

Online Mendelian Inheritance in Man (OMIM)







🕹 Dašší 👚 Předchozí 🖉 Zvýraznik 📃 Bozlišovat veli

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Discussion







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