

VI. Cvičení sekvence II

RNDr. Karel Berka, Ph.D.

Univerzita Palackého v Olomouci

Sekvence – cvičení 2

- Sekvenátor zpracující lidskou genetickou informaci vám vyplivl sekvenci:
TTCTCAGCCCTCAAGCCCTCCAAGTTG... (viz mail)

Vaším úkolem bude zjistit

- 1) jde o DNA, nebo RNA
- 2) obsahuje tato sekvence nějaké geny?
- 3) pakliže ano, zjistěte, jaký protein dotyčný gen kóduje.
- 4) porovnejte sekvenci proteinu pomocí multiple alignmentu s podobnými proteiny z jiných organismů.
- 5) sestavte fylogenetický strom dotyčného proteinu a zjistěte, který organismus má dotyčný gen nejbližší člověku.
- 6) najděte struktury v pdb databazi, které jsou nalezenému proteinu nejbližší.

P.S.: Webové služby, které se dají pro řešení úkolu například použít:

- Genemark,
- BLAST,
- CLUSTALW

1) DNA

- **TTCTCAGCCCTCAAGCCCTCCAAGTTG...**

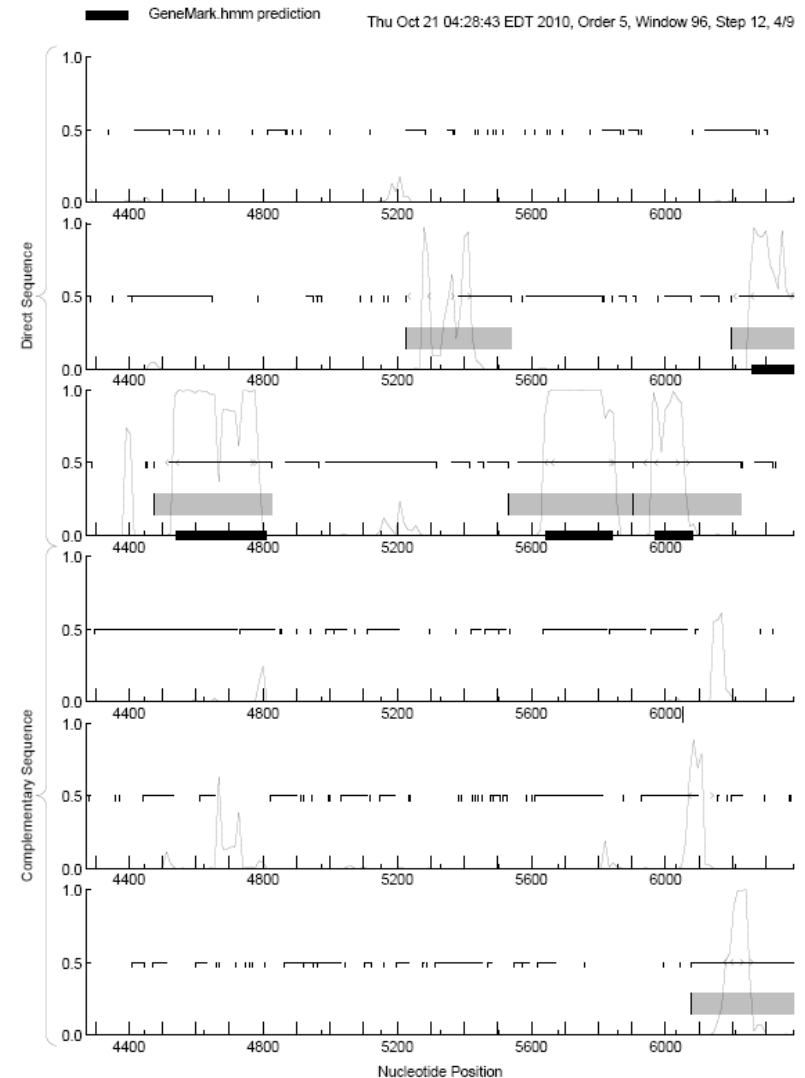
2) hledání genů

GeneMark

<http://exon.biology.gatech.edu/eukhmm.cgi>

- použít eukaryotický a nejlépe lidský model
- získáte proteinovou sekvenci

```
>Thu Oct 21 04:28:43 EDT 2010_1|GeneMark.hmm|gene 1|887_aa  
GLTNQRAQDVLARDGPNALTPPPTPEWVKFCRQLFGGFSILLWIGAILCFLAYGIQAAME  
DEPSNDNLYLGVVLAAVVIVTGCFSYYQEAKSSKIMDSFKNMVPQQALVIREGEKMQINAE  
EVVVGDLEVEVKGDRVPADLR I ISSHGCKVDNSSLTGESEPEQTRSPFTHENPLETRNICF  
FSTNCVEGTARGIV IATGDR TVMGR IATLASGLEVGRTP IAMEIEHFIQLITGVAVFLGVS  
FFVLSLILGYSWLEAVIFLIGIIVANVPEGLLATVTVCLTLTAKRMARKNCLVKNLEAVET  
LGSTSTICSDKTGTLTQNRMTVAHMWFDNQIHEADTTEDQSGATFDKRSPWTALSR IAGL  
CNRVAVFKAGQENISVSKRD TAGDASESALLKCIELSCGSVRKMRDRNPKVAEIPFNSTNKY  
QLSIHEREDSPQSHVLMKGAPERILDRCSTILVQKEIPLDKEMQDAFQONAYMELGGLGE  
RVLGFCQLNLPSPGKFPGRGKFDTDELNFPTEKLCFVGLMSMIDPPRAAVPDAVGKCRSAGI  
KVMVTGDHPITAKAIKGVGII ISEGNETVEDIAARLNIPMSQVNPREAKACVVHGSDLKD  
MTSEQLDEILKNHTEIVFARTSPQQKLIIVEGCQRQGAIVAVTGDGVNDSPALKKADIGIA  
MGISGSDVSKQAADMILLDDNFASIVTGVVEGR LIFDNLKKS IAYTLT SNIPEITPFLLF I  
IANIPLPLGTVTILCIDLGTDMVPAISLAYEAAESDIMKRQPRNSQTDKLVNERLISMAYG  
QIGMIQALGGFFTYFVILAENGF LPSRLLGIRLDWDDRTMNDLEDSYGQEWTYEQRKVVEF  
TCHTAFFASIVVVQWADLIICKTRRNSVVFQQGM
```



3) identifikace proteinu

- BLASTp (protein-protein) <http://blast.ncbi.nlm.nih.gov/>
- záleží na databázi – zkuste použít
 - nonredundant database, nebo
 - reference sequences, nebo pdb
- Jde o Na⁺/K⁺ ATPasu – alpha 2 podjednotku
- Přes Gene se jde dostat na vlastnosti a funkce proteinu

Descriptions

Legend for links to other resources: [U](#) UniGene [G](#) GEO [G](#) Gene [S](#) Structure [M](#) Map Viewer [X](#) PubChem BioAssay

NEW - alignment score below the threshold on the previous iteration
● - alignment was checked on the previous iteration

Run PSI-Blast iteration 2 with max [500]

Sequences producing significant alignments with E-value BETTER than threshold

Accession	Description	Max score	Total score	Query coverage	E value	Links
NEW CAI15272.1	ATPase, Na ⁺ /K ⁺ transporting, alpha 2 (+) polypeptide [Homo sapiens]	1832	1832	100%	0.0	G
NEW NP_000693.1	sodium/potassium-transporting ATPase subunit alpha-2 proprotein [Homo sapiens] >sp P50991	1829	1829	100%	0.0	U G G
NEW BAG52393.1	unnamed protein product [Homo sapiens]	1829	1829	100%	0.0	G
NEW BAA34498.2	KIAA0778 protein [Homo sapiens]	1827	1827	100%	0.0	G
NEW XP_001115318.2	PREDICTED: sodium/potassium-transporting ATPase subunit alpha-2 isoform 1 [Macaca mulatta]	1825	1825	100%	0.0	U G
NEW DAG26951.1	unnamed protein product [Homo sapiens]	1825	1825	100%	0.0	G
NEW XP_545753.2	PREDICTED: similar to Sodium/potassium-transporting ATPase alpha-2 chain precursor (Sodium	1825	1825	100%	0.0	U G
NEW NP_001125304.1	sodium/potassium-transporting ATPase subunit alpha-2 precursor [Pongo abelii] >sp Q5RCD8.1	1820	1820	100%	0.0	G
NEW NP_001165012.1	Na,K-ATPase alpha 2 subunit [Sus scrofa] >gb AD819854.1 Na ⁺ /K ⁺ transporting alpha 2 polyp	1817	1817	100%	0.0	U G
NEW BAC98022.1	mKIAA0778 protein [Mus musculus] >gb EDL39027.1 mCG142115, isoform CRA_b [Mus muscu	1815	1815	100%	0.0	U G
NEW NP_001074993.1	sodium/potassium-transporting ATPase subunit alpha-2 precursor [Bos taurus] >sp A2VDL6.1	1815	1815	100%	0.0	U G
NEW NP_036637.1	sodium/potassium-transporting ATPase subunit alpha-2 precursor [Rattus norvegicus] >ref NP_	1814	1814	100%	0.0	U G
NEW AAH41774.1	Atp1a2 protein [Mus musculus]	1811	1811	99%	0.0	G
NEW XP_001379430.1	PREDICTED: similar to Na,K-ATPase subunit alpha 2 [Monodelphis domestica]	1808	1808	100%	0.0	U G
NEW XP_002929963.1	PREDICTED: LOW QUALITY PROTEIN: sodium/potassium-transporting ATPase subunit alpha-2-li	1806	1806	100%	0.0	G
NEW EFB23738.1	hypothetical protein PANDA_020288 [Ailuropoda melanoleuca]	1801	1801	100%	0.0	G
NEW XP_002715313.1	PREDICTED: ATPase, Na ⁺ /K ⁺ transporting, alpha 2 (+) polypeptide [Oryctolagus cuniculus]	1796	1796	100%	0.0	U G
NEW XP_001915271.1	PREDICTED: similar to KIAA0778 protein [Equus caballus]	1796	1796	100%	0.0	U G
NEW XP_860689.1	PREDICTED: similar to Sodium/potassium-transporting ATPase alpha-2 chain precursor (Sodium	1786	1786	100%	0.0	U G
NEW XP_513921.2	PREDICTED: Na ⁺ /K ⁺ -ATPase alpha 2 subunit proprotein [Pan troglodytes]	1766	1766	100%	0.0	U G
NEW EAW57730.1	ATPase, Na ⁺ /K ⁺ transporting, alpha 2 (+) polypeptide, isoform CRA_b [Homo sapiens]	1762	1762	100%	0.0	G

Entrez gene

Entrez Gene
Genes and mapped phenotypes

Search: Gene

[Limits](#) [Advanced search](#) [Help](#)

[Display Settings](#) Full Report

[Send to](#)

ATP1A2 ATPase, Na+/K+ transporting, alpha 2 polypeptide [*Homo sapiens*]

Gene ID: 477, updated on 29-Sep-2010

Summary

Official Symbol ATP1A2 provided by [HGNC](#)

Official Full Name ATPase, Na+/K+ transporting, alpha 2 polypeptide provided by [HGNC](#)

Primary source [HGNC:800](#)

Locus tag RP11-536C5.4

See related [Ensembl:ENSG0000018625](#), [HPRD:01666](#), [MIM:182340](#)

Gene type protein coding

RefSeq status REVIEWED

Organism [Homo sapiens](#)

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorhini; Catarrhini; Hominidae; Homo

Also known as FHM2; MHP2; MGC58864; ATP1A2

Summary The protein encoded by this gene belongs to the family of P-type cation transport ATPases, and to the subfamily of Na+/K+ -ATPases. Na+/K+ -ATPase is an integral membrane protein responsible for establishing and maintaining the electrochemical gradients of Na and K ions across the plasma membrane. These gradients are essential for osmoregulation, for sodium-coupled transport of a variety of organic and inorganic molecules, and for electrical excitability of nerve and muscle. This enzyme is composed of two subunits, a large catalytic subunit (alpha) and a smaller glycoprotein subunit (beta). The catalytic subunit of Na+/K+ -ATPase is encoded by multiple genes. This gene encodes an alpha 2 subunit. Mutations in this gene result in familial basilar or hemiplegic migraines, and in a rare syndrome known as alternating hemiplegia of childhood. [provided by RefSeq]

Genomic regions, transcripts, and products

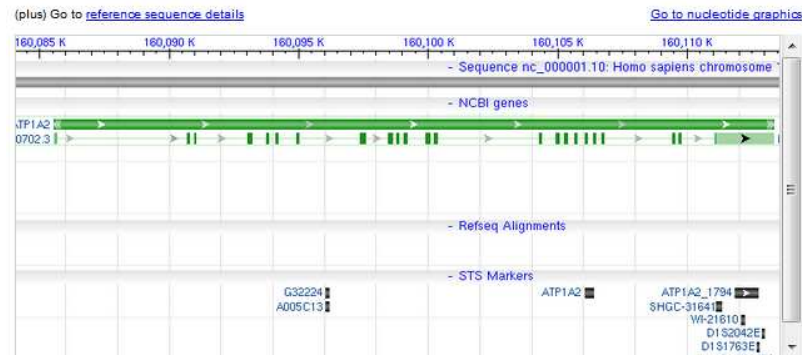


Table of contents

- Summary
- Genomic regions, transcripts, and products
- Genomic context
- Bibliography
- Phenotypes
- Interactions
- General gene info
- General protein info
- Reference sequences
- Related sequences
- Additional links

- Links**
- Order cDNA clone
 - BioAssay, by Gene target
 - BioSystems
 - Books
 - CCDS
 - Conserved Domains
 - EST
 - Full text in PMC
 - GEO Profiles
 - Genome
 - HomoloGene
 - Map Viewer
 - Nucleotide
 - OMIM
 - Peptidome
 - Probe
 - Protein
 - PubChem Compound
 - PubChem Substance
 - PubMed
 - PubMed (GeneRIF)
 - PubMed (OMIM)
 - RefSeq Proteins
 - RefSeq RNAs
 - RefSeqGene
 - SNP
 - SNP: GeneView

Gene Ontology Provided by [GUA](#)

Function	Evidence Code	Pubs
ATP binding	IEA	
ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism	IEA	
cation-transporting ATPase activity	IEA	
hydrolase activity	IEA	
hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances	IEA	
metal ion binding	IEA	
monovalent inorganic cation transmembrane transporter activity	IEA	
nucleotide binding	IEA	
protein binding	IEA	
sodium:potassium-exchanging ATPase activity	IMP	PubMed

Items 1 - 10 Page 1 of 1 Go To Page

Process	Evidence Code	Pubs
ATP biosynthetic process	IEA	
ATP hydrolysis coupled proton transport	IEA	
adult locomotory behavior	IEA	
locomotion	IEA	
negative regulation of heart contraction	IEA	
negative regulation of striated muscle contraction	IEA	
neurotransmitter uptake	IEA	
potassium ion transport	NAS	PubMed
reduction of cytosolic calcium ion concentration	IEA	
regulation of blood pressure	IEA	
regulation of respiratory gaseous exchange by neurological system process	IEA	
regulation of smooth muscle contraction	IEA	
regulation of striated muscle contraction	NAS	PubMed
regulation of the force of heart contraction	IEA	
regulation of vasoconstriction	IEA	
sodium ion transport	NAS	PubMed
visual learning	IEA	

Items 1 - 17 Page 1 of 1 Go To Page

Component	Evidence Code	Pubs
caveola	IEA	
cytoplasm	IDA	PubMed
dendritic spine	IEA	
endosome	IEA	
integral to membrane	IEA	
membrane fraction	IEA	

Position specific iterative BLAST (PSI-BLAST)

- 1) BLAST
- 2) multiple alignment výsledku – nalezení konzervovaných míst
- 3) Tvorba position specific scoring matrix, PSSM (konzervované místa – vyšší skóre)
- 4) Přidání PSSM skóre do výpočtu 2. BLASTu

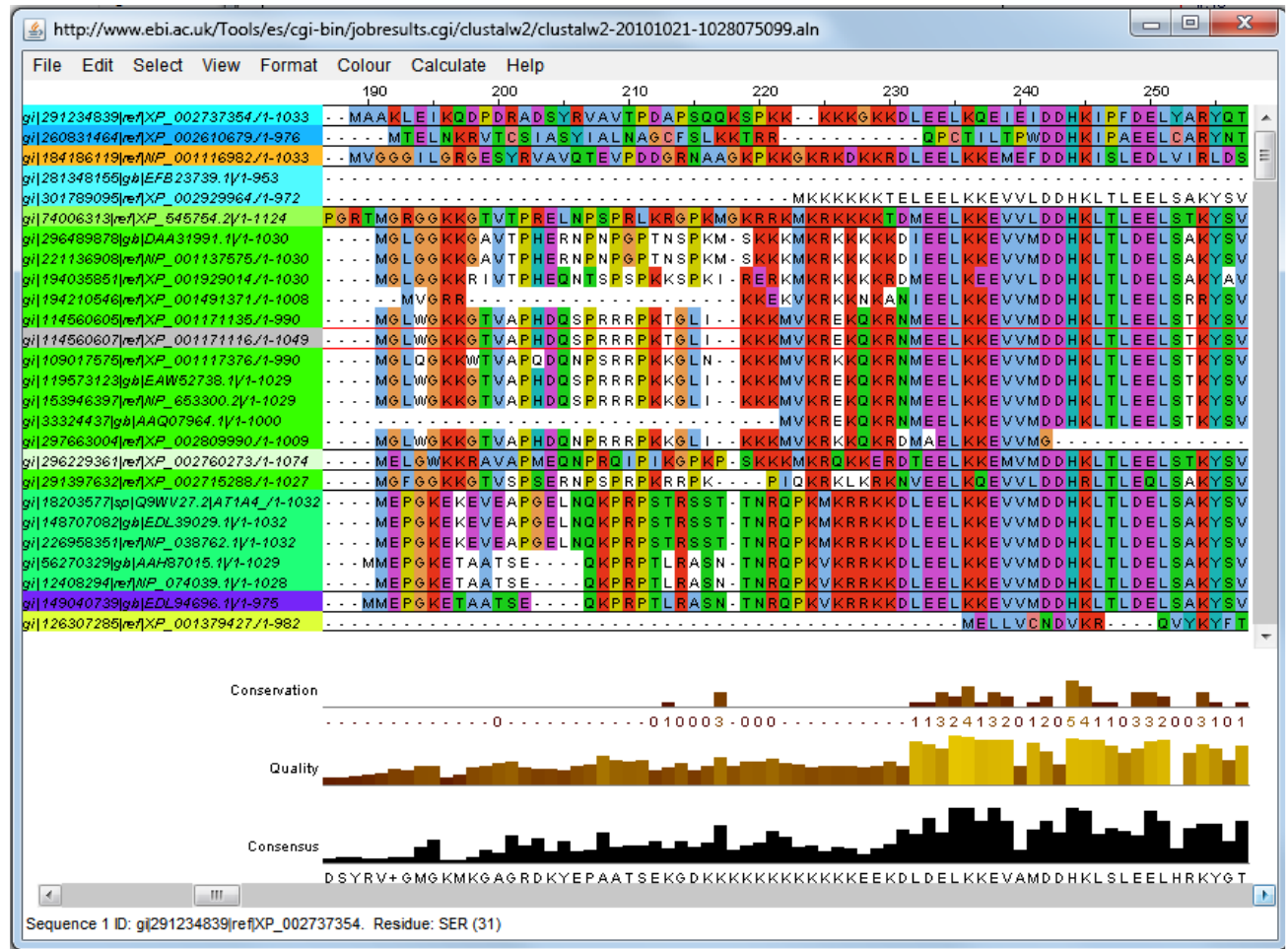
+ zvýšená citlivost

Pattern Hit Initiated BLAST (PHI-BLAST)

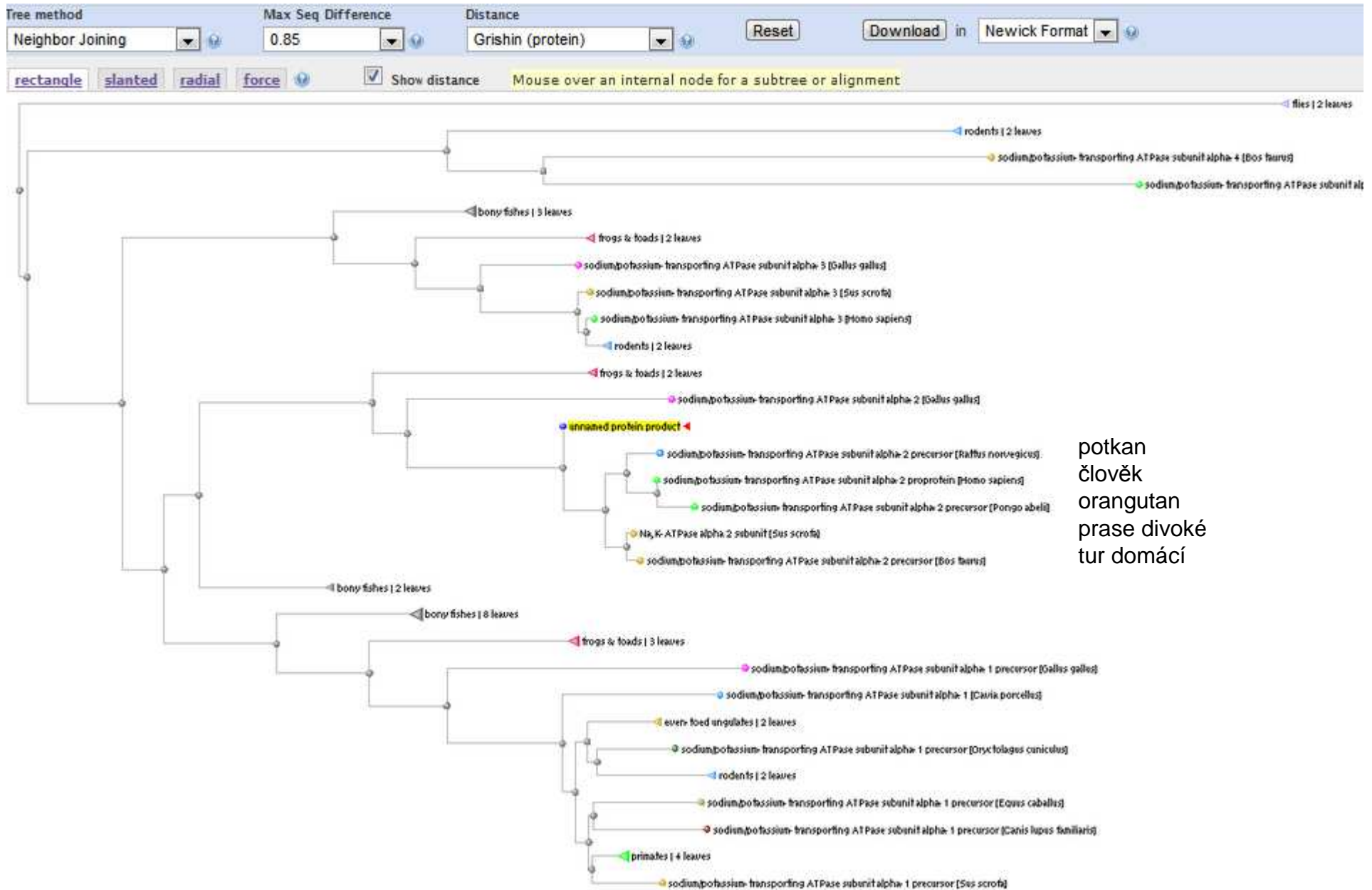
- hledání motivů

4) porovnání sekvencí

- Přímo z BLASTu - COBALT,
- ev. ClustalW (prohlížeč Jalview)



5) fylogenetický strom



6) struktury

- BLASTp proti pdb databázi

lcl|65300unnamed protein product

[3B8E_A](#) Chain A, Crystal Structure Of The Sodium-Potassium Pump

[2ZXE_A](#) Chain A, Crystal Structure Of The Sodium - Potassium Pump In The E2.2k+.Pi State

[1KJU_A](#) Chain A, Ca²⁺-Atpase In The E2 State >pdb|1IWO|A Chain A, Crystal Structure Of The Sr Ca²⁺-Atpase In The Absence Of Ca²⁺

[3BA6_A](#) Chain A, Structure Of The Ca²⁺e₁p Phosphoenzyme Intermediate Of The Serca Ca²⁺-Atpase_

[1MHS_A](#) Chain A, Model Of Neurospora Crassa Proton Atpase

[3B8C_A](#) Chain A, Crystal Structure Of A Plasma Membrane Proton Pump

[1Q3I_A](#) Chain A, Crystal Structure Of Na,K-Atpase N-Domain

[1MO7_A](#) Chain A, Atpase >pdb|1MO8|A Chain A, Atpase