LOSCHMIDT LABORATORIES

PROTEIN ENGINEERING

2. *IN SILICO* IDENTIFICATION OF PROTEINS

Loschmidt Laboratories
Department of Experimental Biology
Masaryk University, Brno

Outline

- Why to search for new proteins?
- How to acquire new proteins?
  - traditional approach
  - metagenomic approach
  - bioinformatic approach
- Bioinformatic approach
  - Where to find target sequences?
  - How to find target sequences?
  - How to recognize interesting sequences?
- What to keep in mind?

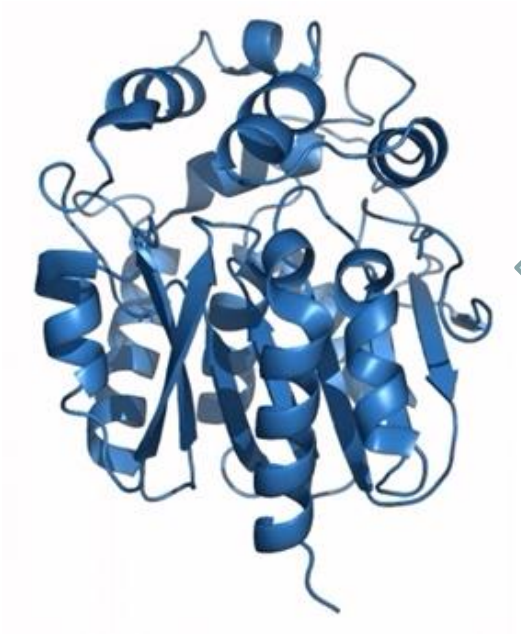
Why to search for new proteins?

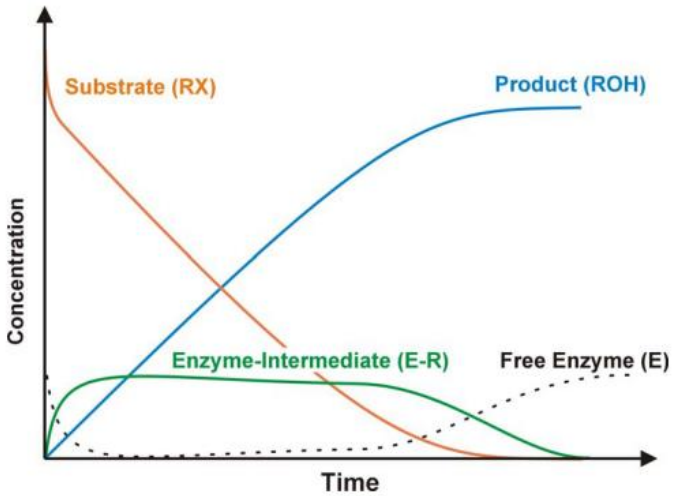
Why to search for new proteins?

- plenty of reasons

Why to search for new proteins?

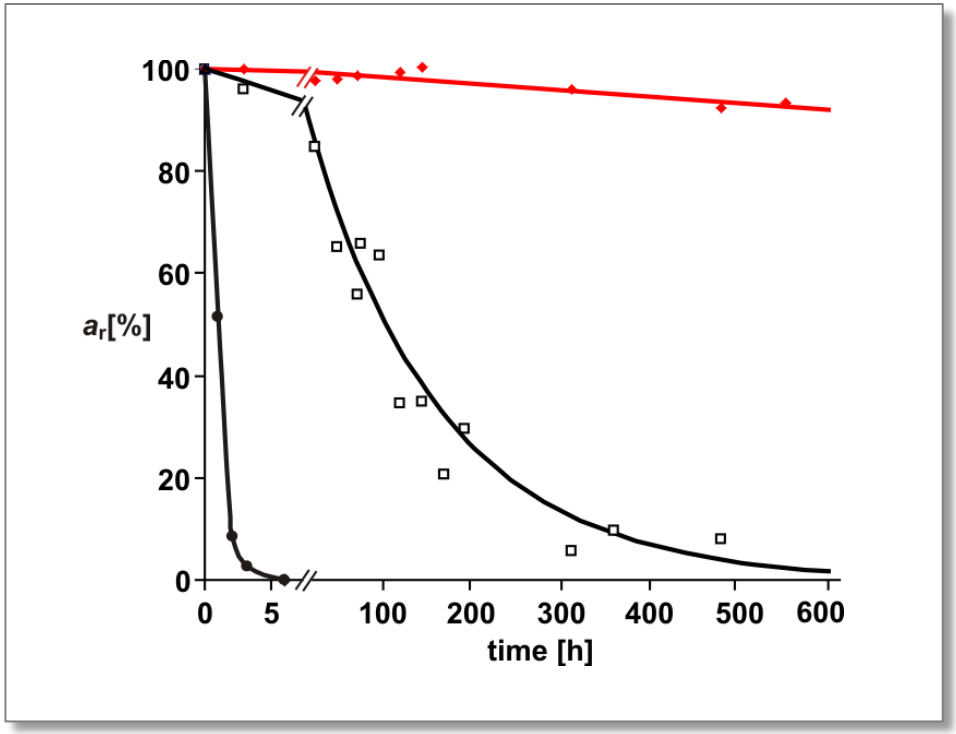
- better understanding of structure-function relationships
  - required for rational design





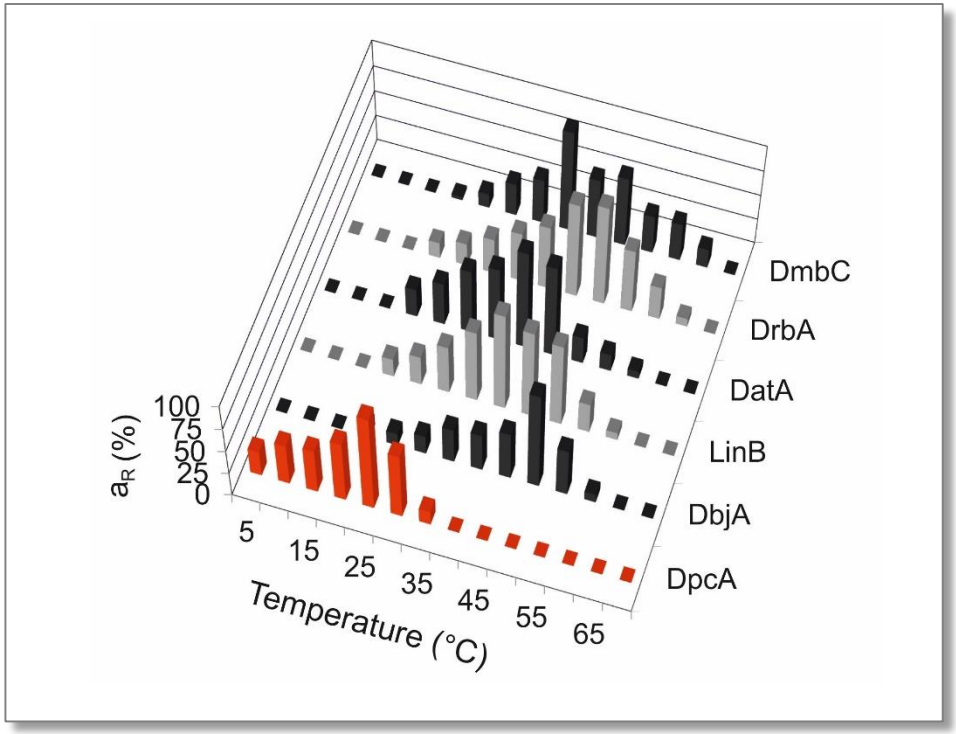
Why to search for new proteins?

- better understanding of structure-function relationships
- novel properties
  - stability



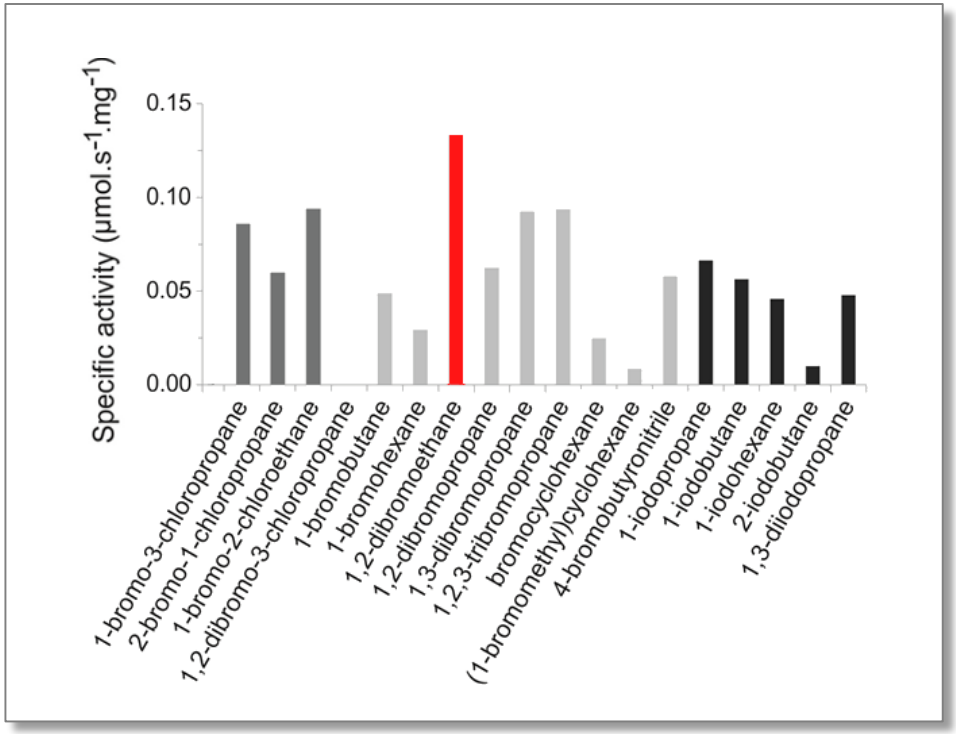
Why to search for new proteins?

- better understanding of structure-function relationships
- novel properties
  - stability
  - temperature profile



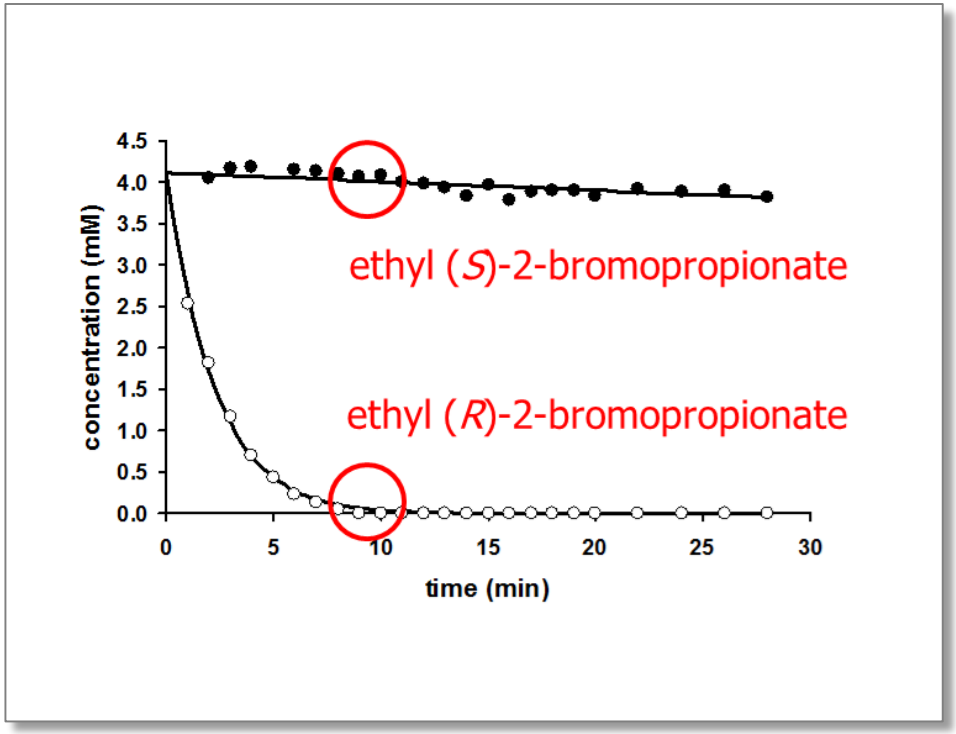
Why to search for new proteins?

- better understanding of structure-function relationships
- novel properties
  - stability
  - temperature profile
  - activity
  - specificity



Why to search for new proteins?

- better understanding of structure-function relationships
- novel properties
  - stability
  - temperature profile
  - activity
  - specificity
  - enantioselectivity



Why to search for new proteins?

- better understanding of structure-function relationships
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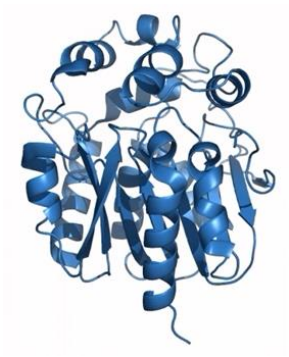
  - …

Why to search for new proteins?

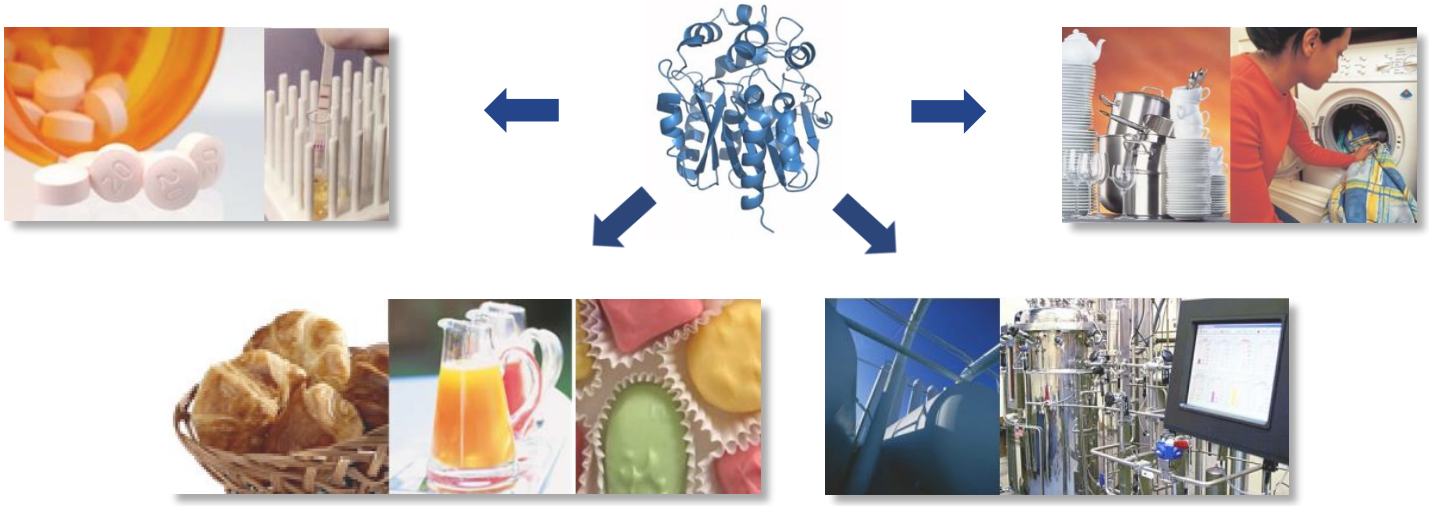
- better understanding of structure-function relationships
- novel properties
- better starting points for protein engineering



Why to search for new proteins?

- better understanding of structure-function relationships
- novel properties
- better starting points for protein engineering

→ proteins with desired properties → practical applications



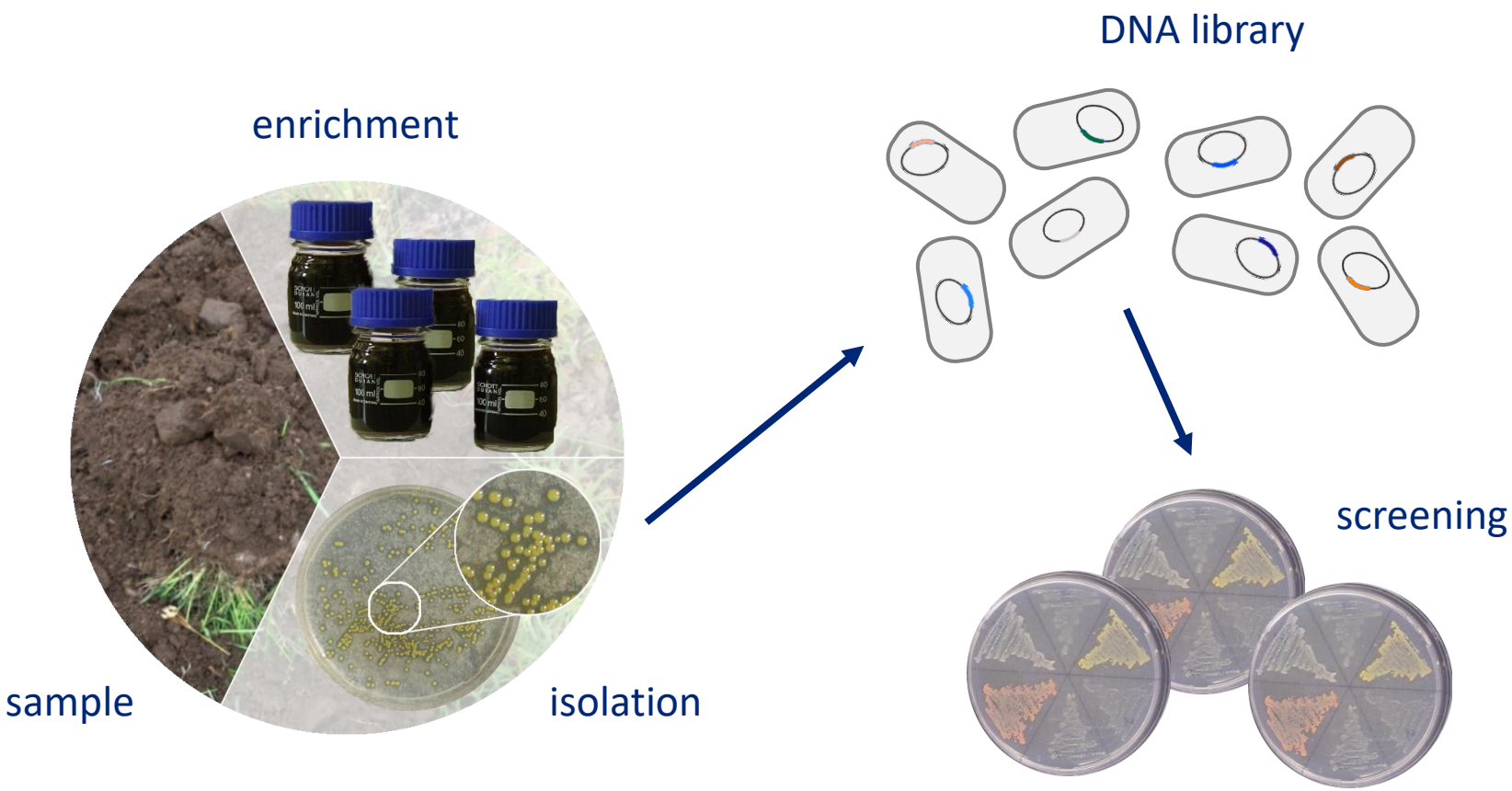
How to acquire new proteins?

How to acquire new proteins?

- traditional approach
- metagenomic approach
- bioinformatic approach

How to acquire new proteins?

- traditional approach

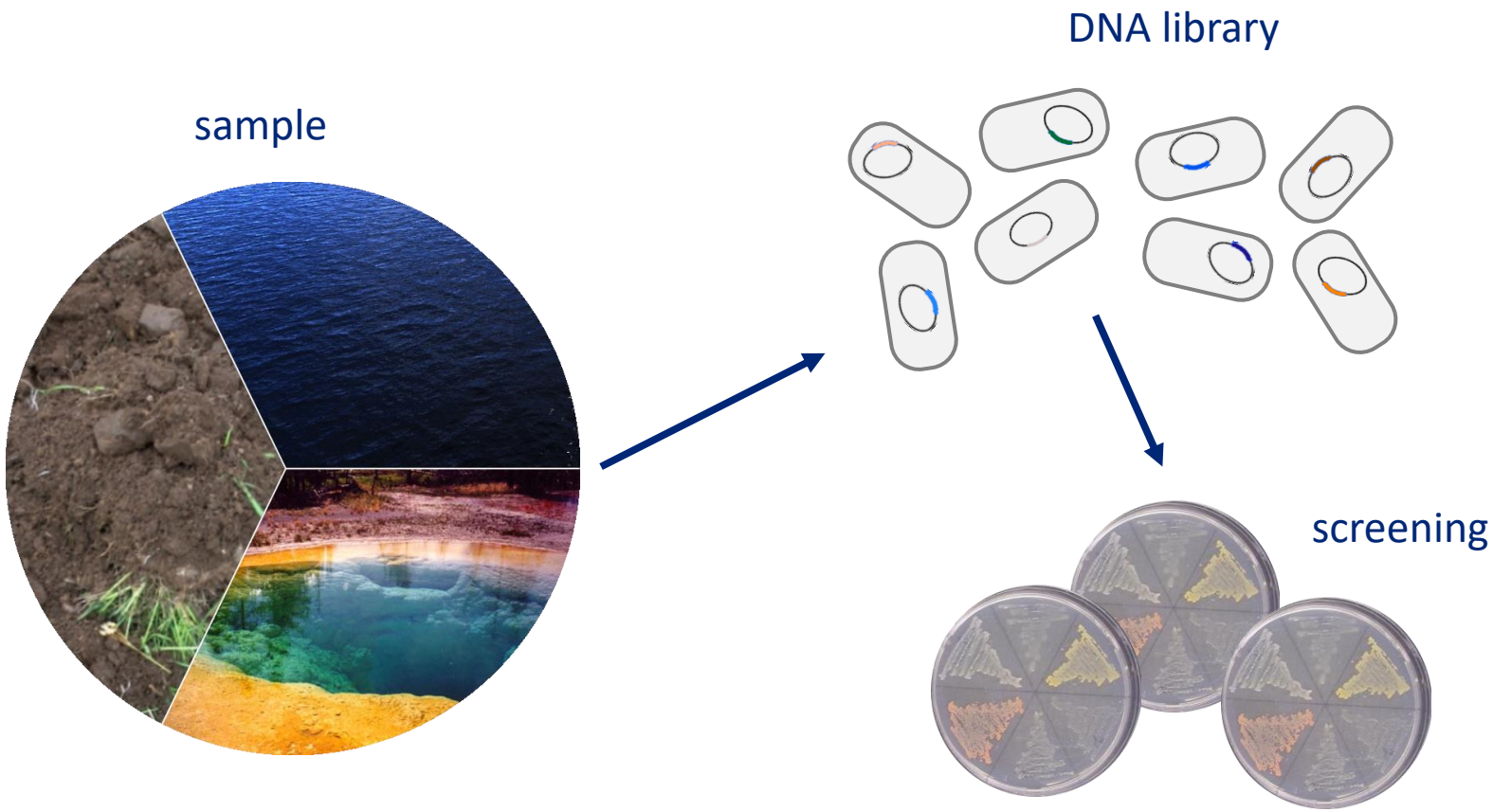


How to acquire new proteins?

- traditional approach
  - microorganisms possessing target activity are enriched from the environment and isolated in pure culture
  - proteins or corresponding genes are recovered from organisms by protein purification, DNA library screening, PCR with specific primers,...
  - ☹ majority of microorganisms (> 99 %) cannot be cultivated using standard techniques → a large fraction of the microbial diversity in an environment is lost

How to acquire new proteins?

- metagenomic approach



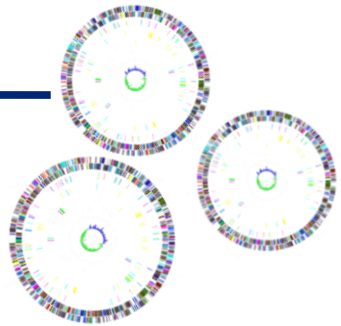
How to acquire new proteins?

- metagenomic approach
  - isolation and cloning of DNA extracted directly from environmental sample (without culturing the present organisms)
  - genes recovered by DNA library screening or PCR with specific primers,...
  - ☺ enables to explore biodiversity of uncultured microorganisms

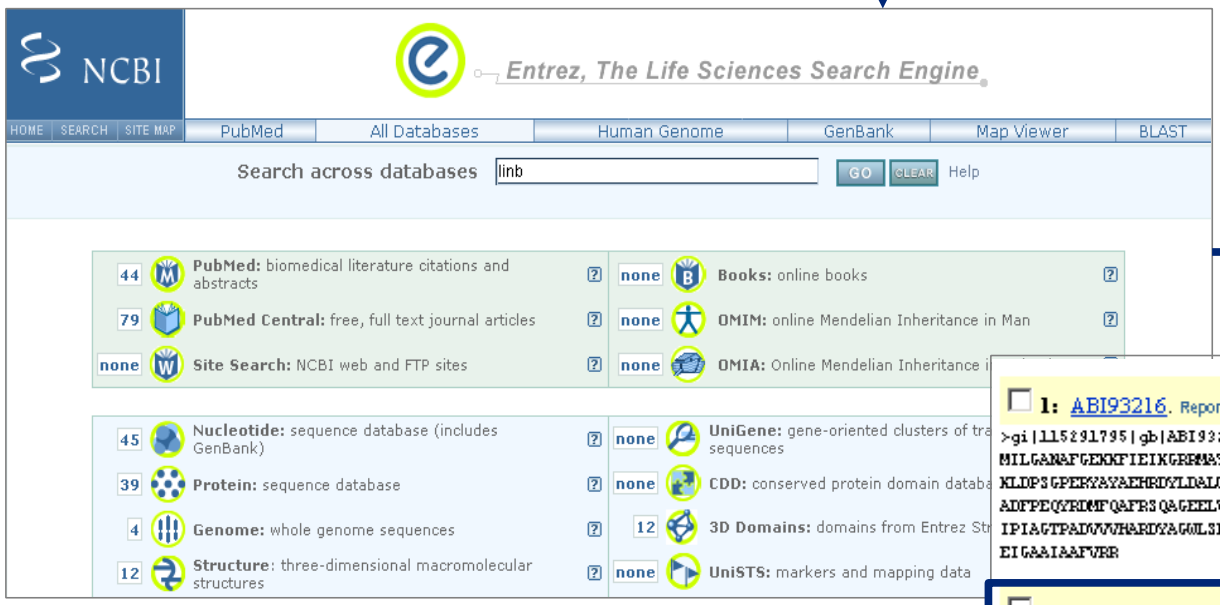
How to acquire new proteins?

- bioinformatic approach

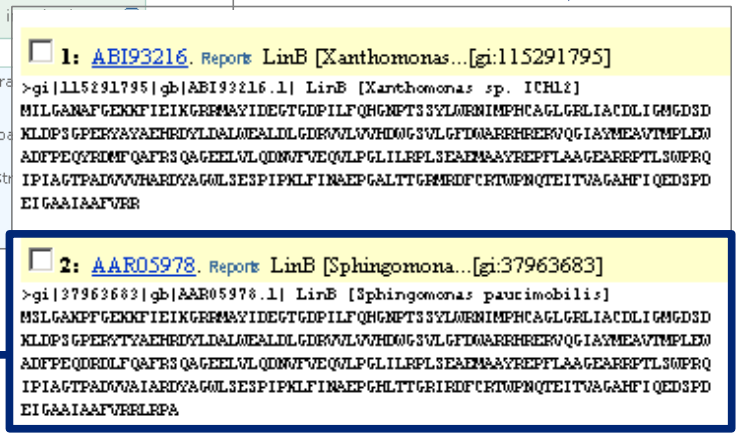
(meta)genomic sequencing projects



sequence database



in silico "screening"



gene synthesis, DNA request

How to acquire new proteins?

- bioinformatic approach
  - sequence data from genomic and metagenomic sequencing projects are stored in sequence databases
  - *in silico* searching of sequence databases

    → ☺ fast and cheap way to identify novel proteins

    → ☹ one cannot find what is not in the database (but there is a lot of data - more than one usually needs ☺)
  - genes are recovered by gene synthesis or obtained from sequencing consortia upon request

Where to find target sequences?

Where to find target sequences?

- databases of nucleotide sequences
- databases of protein sequences

Databases of nucleotide sequences

- GenBank
  - http://www.ncbi.nlm.nih.gov/genbank/
  - provided by NCBI (National Center for Biotechnology Information)

- EMBL-BANK
  - http://www.ebi.ac.uk/embl/
  - provided by EBI (European Bioinformatics Institute)

- DDBJ
  - http://www.ddbj.nig.ac.jp/
  - provided by National Institute of Genetics from Japan

Databases of nucleotide sequences

- GenBank, EMBL-Bank, DDBJ
  - annotated collections of all publically available nucleotide sequences
  - freely available to wide community
  - contain data obtained from genomic centers or research institutions
  - everyday synchronization of new or updated data
  - ☺ contain about 250,000,000 sequences
  - ☹ mostly automatic annotations – lower quality, errors

Databases of protein sequences

- UniProtKB
  - http://www.uniprot.org/
  - provided by EBI, Swiss Institute of Bioinformatics and Protein Information Resource

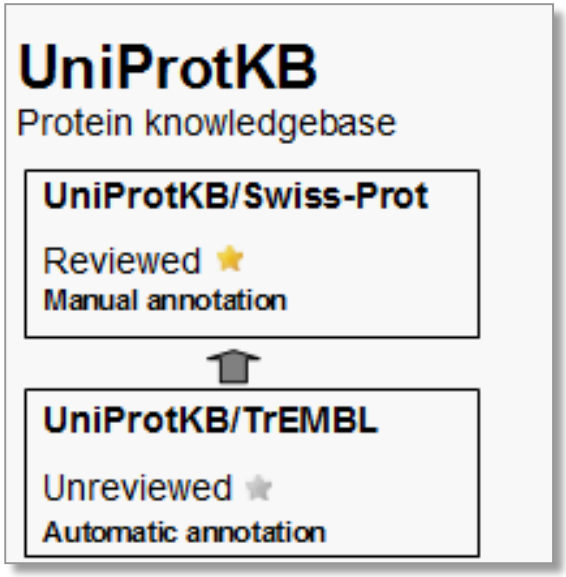
- nr Protein database
  - http://www.ncbi.nlm.nih.gov/protein/
  - provided by NCBI

Databases of protein sequences

- UniProtKB, nr Protein database
  - annotated collections of publically available protein sequences
  - freely available to wide community
  - contain data obtained by conceptual translation of coding sequences from EMBL-Bank/GenBank/DDBJ or provided by research institutions
  - ☺ contain more than 100,000,000 sequences
  - ☹ mostly automatic annotations – lower quality, errors

Databases of protein sequences

- UniProtKB
  - rich annotations (e.g., information about function of protein and individual amino acids, experimental data, biological ontologies, classifications, ...)
  - clear indication of annotation quality (manual vs. automatic)



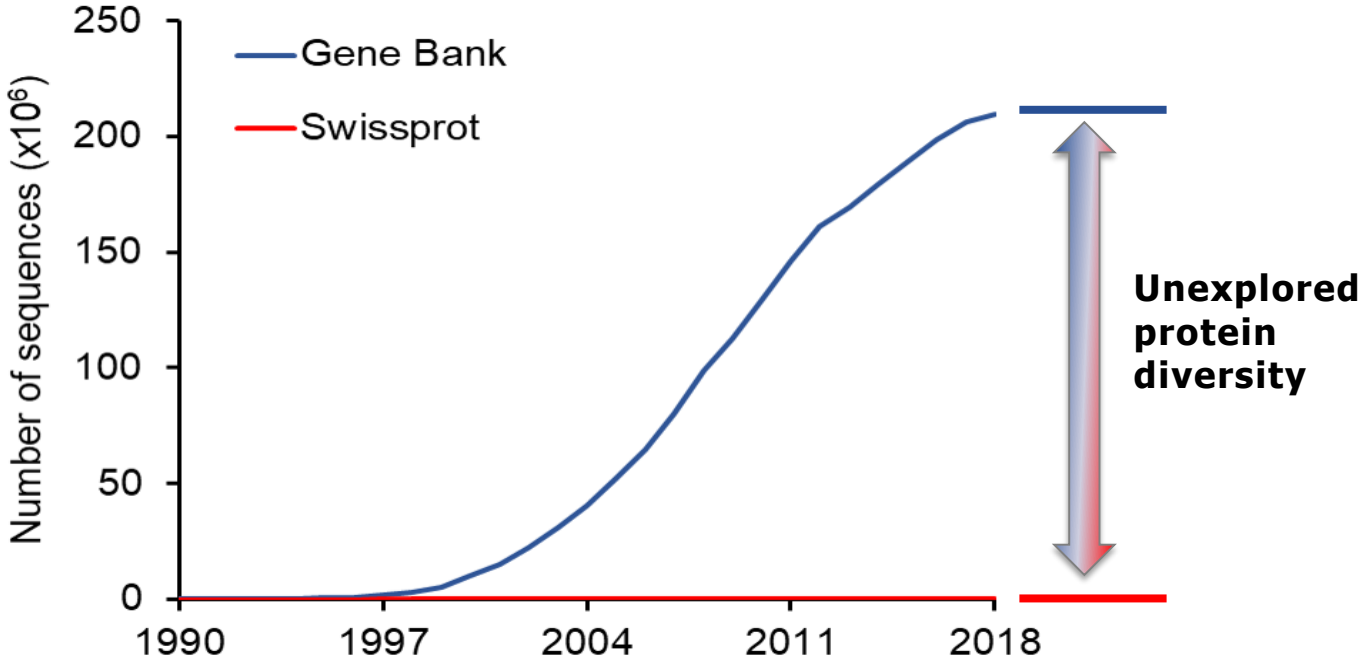
Databases of protein sequences

- UniProtKB/Swiss-Prot
  - high quality annotations, i.e., manually annotated entries or expert-reviewed automatic annotations
  - ☺ source of reliable information
  - ☹ contains “only” ~ 560,000 sequences

- UniProtKB/TrEMBL
  - ☹ automatic annotations – lower quality, errors
  - ☺ contains ~ 180,000,000 sequences

Unexplored protein diversity

- Number of sequences
- Number of characterized proteins



Pitfalls of sequence databases

- large number of errors ☹
  - errors in sequences (wrong base, frameshift errors)
  - wrong positions of genes
  - exon-intron boundary errors
  - errors and inaccuracies in annotations
  - ...

How to find target sequences?

How to find target sequences?

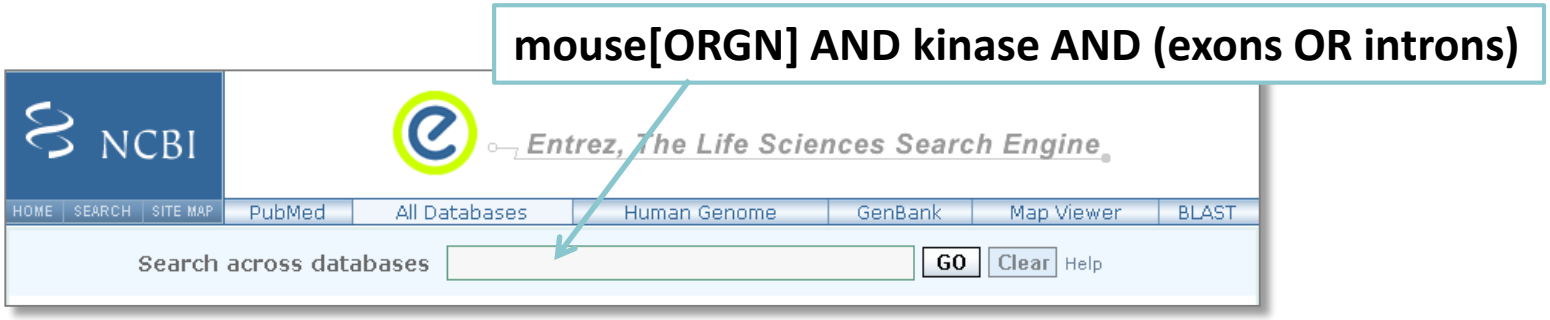
- text-based searches
- sequence-based searches

Text-based searches

- database retrieval systems
  - enable quick and easy search of many databases at the same time
  - specification of queries using logical operators (AND, OR, NOT,...)
  - Entrez (NCBI), SRS (EBI)
- ☹ results dependent on sequence annotations
  - erroneous, inaccurate or too general annotations
  - synonyms
  - misspellings
  - ...

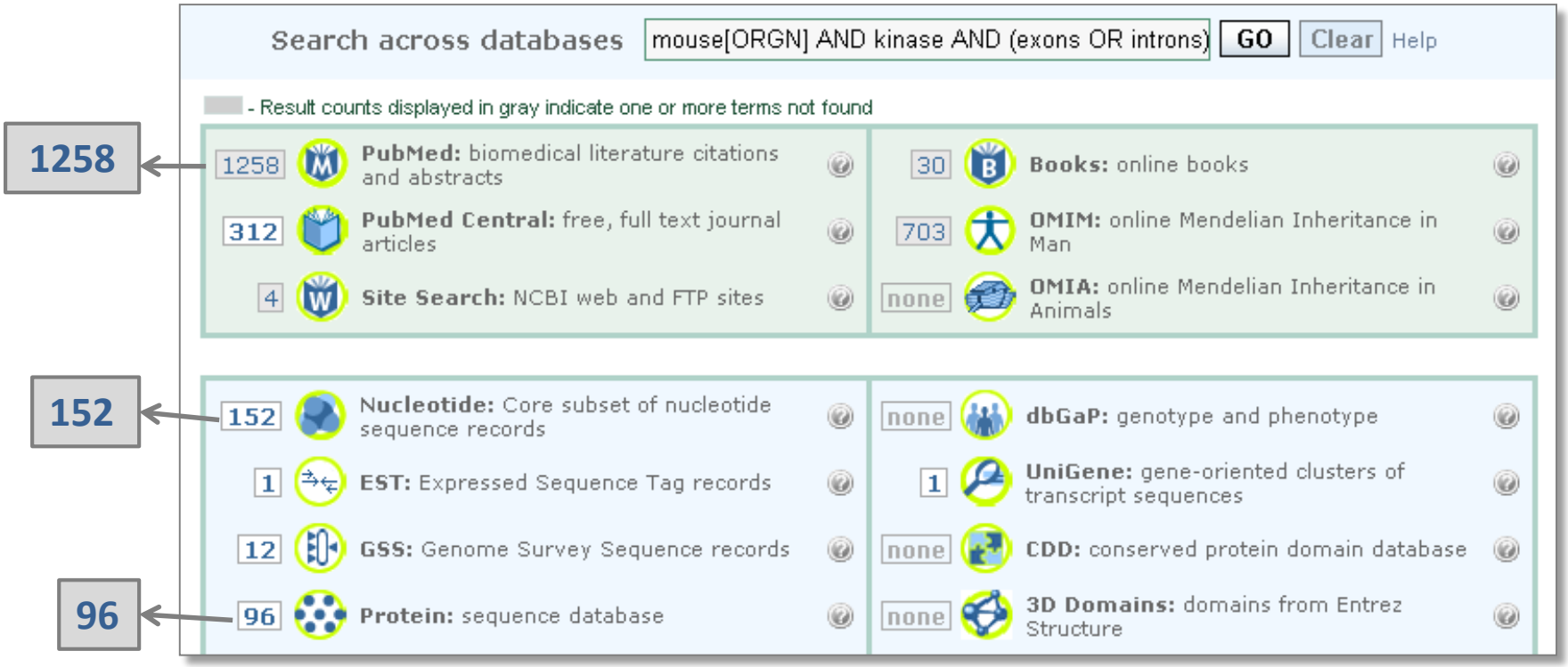
Text-based searches

- database retrieval systems



Text-based searches

- database retrieval systems



Sequence-based searches

- searches based on sequence similarity
  - ☺ results not influenced by sequence annotations
- rely on assumption that proteins with the same function have similar sequence
  - ☹ not always true – close homologs vs. distant homologs vs. analogs

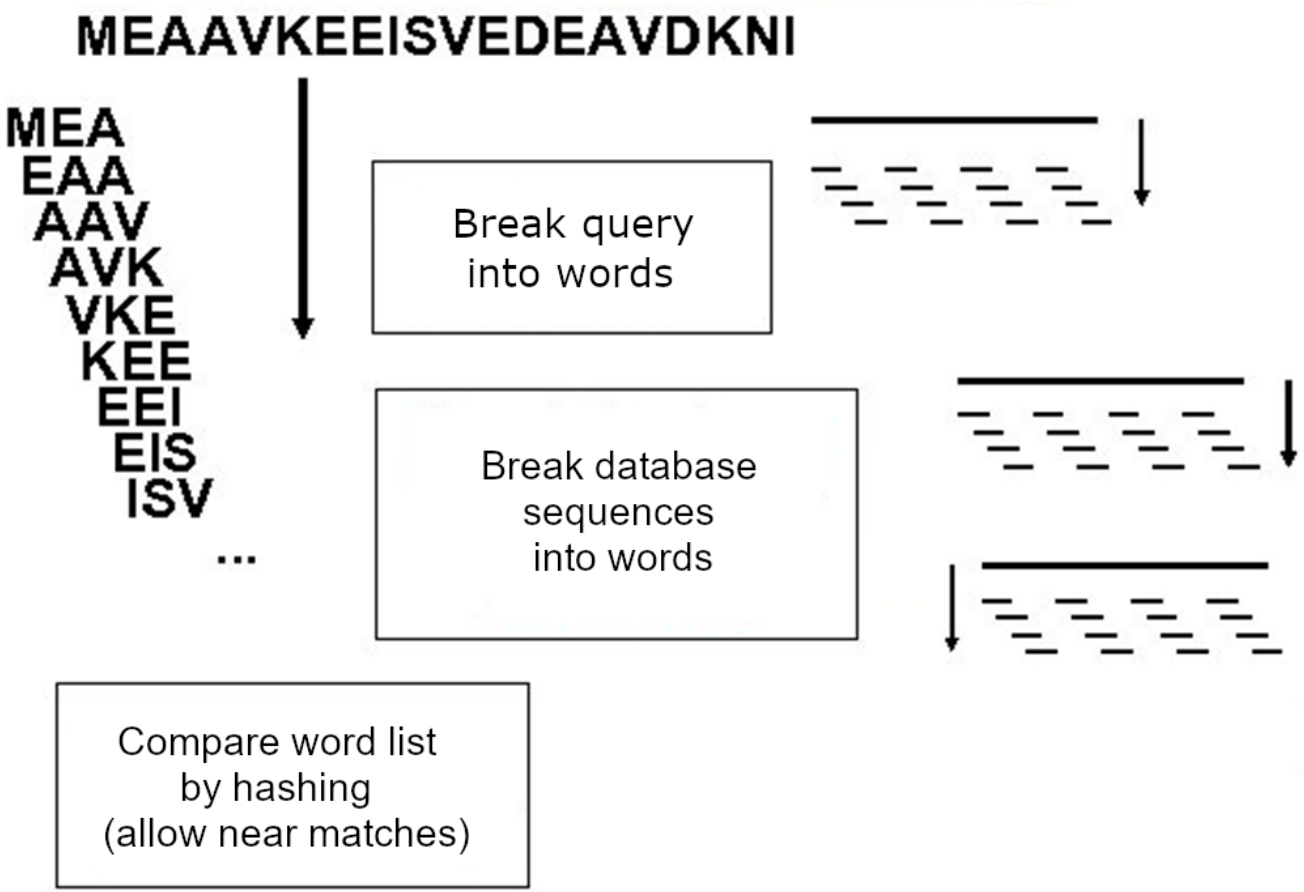
```
1  LSPAEIAAYEAPFPTPDYKAGARAFPALVPTSP
2  LTDAEAAAYGAPFPDQRYKAGVRRFPELVPVSP
3  MSPDECAAYNAPFPDKGHRAALRAFPLMVPESE
4  LSDAERSAYDAPFPDESYKEGARIFPALVPITP
5  VPAGVRAGYDAPFPDKTYQAGARAFPRLVPTSP
6  LSTDVLNAYDAPFPTEAHKAGVRQFPLLVPATT
7  VPAGVRAGYDAPFPDKTYQAGARAFPRLVPTSP
```

Sequence-based searches

- BLAST
  - based on local pairwise alignment
- PSI-BLAST
  - “iterative BLAST” making use of multiple sequence alignment
  - very sensitive search strategy to detect weak but biologically significant similarities between sequences
- ...

BLAST

- Basic Local Alignment Search Tool



BLAST

Basic **L**ocal **A**lignment **S**earch **T**ool

Query sequence: R P P Q G L F

Database sequence: D P **P E G** V V

Exact match is scanned.

Score: -2 **7 7 2 6 1** -1

HSP

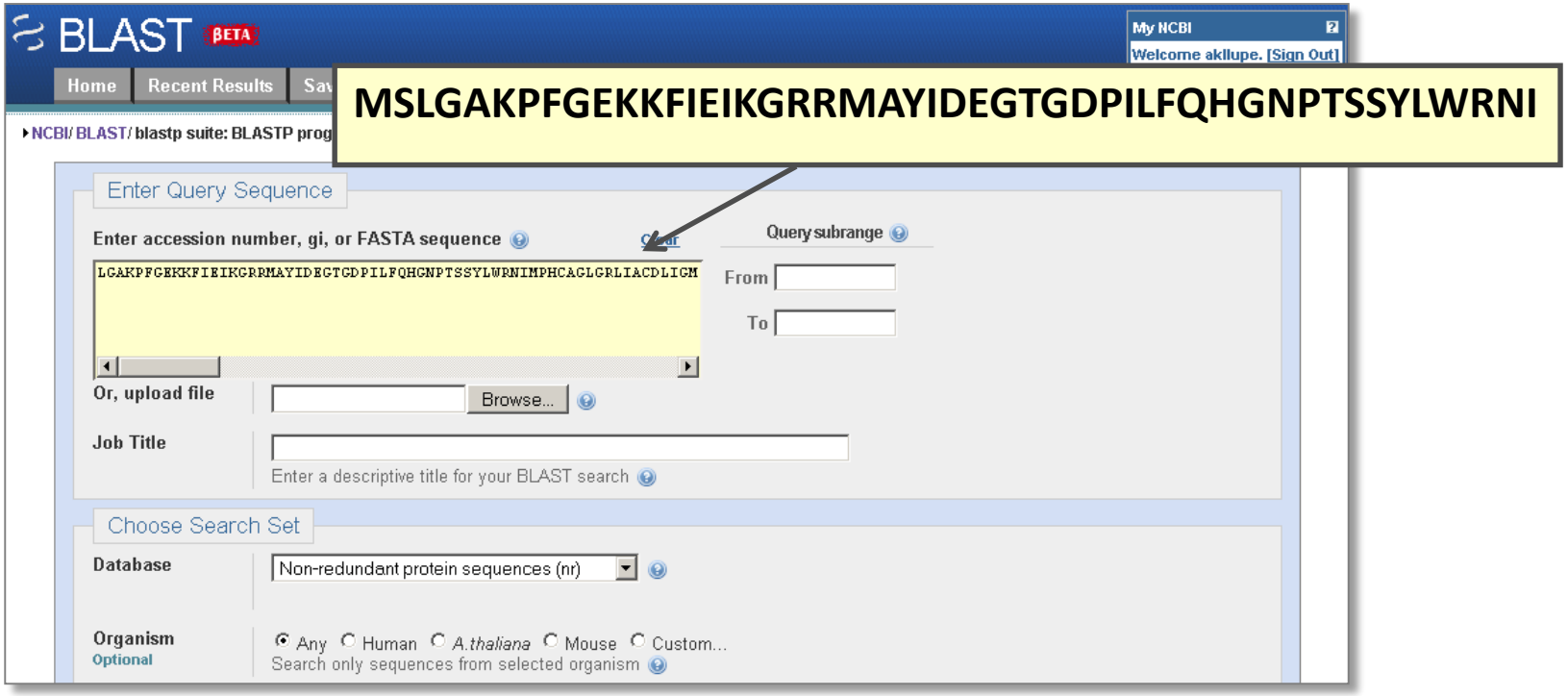
Optimal accumulated score = 7+7+2+6+1 = 23

BLOSUM scoring matrix

	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Ala	4																			
Arg	−1	5																		
Asn	−2	0	6																	
Asp	−2	−2	1	6																
Cys	0	−3	−3	−3	9															
Gln	−1	1	0	0	−3	5														
Glu	−1	0	0	2	−4	2	5													
Gly	0	−2	0	−1	−3	−2	−2	6												
His	−2	0	1	−1	−3	0	0	−2	8											
Ile	−1	−3	−3	−3	−1	−3	−3	−4	−3	4										
Leu	−1	−2	−3	−4	−1	−2	−3	−4	−3	2	4									
Lys	−1	2	0	−1	−3	1	1	−2	−1	−3	−2	5								
Met	−1	−1	−2	−3	−1	0	−2	−3	−2	1	2	−1	5							
Phe	−2	−3	−3	−3	−2	−3	−3	−3	−1	0	0	−3	0	6						
Pro	−1	−2	−2	−1	−3	−1	−1	−2	−2	−3	−3	−1	−2	−4	7					
Ser	1	−1	1	0	−1	0	0	0	−1	−2	−2	0	−1	−2	−1	4				
Thr	0	−1	0	−1	−1	−1	−1	−2	−2	−1	−1	−1	−1	−2	−1	1	5			
Trp	−3	−3	−4	−4	−2	−2	−3	−2	−2	−3	−2	−3	−1	1	−4	−3	−2	11		
Tyr	−2	−2	−2	−3	−2	−1	−2	−3	2	−1	−1	−2	−1	3	−3	−2	−2	2	7	
Val	0	−3	−3	−3	−1	−2	−2	−3	−3	3	1	−2	1	−1	−2	−2	0	−3	−1	4

Sequence-based searches

- PSI-BLAST input



Sequence-based searches

- PSI-BLAST results

Score

E-value

Sequences producing significant alignments Download Manage Columns Show 100

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignmen

Description	Max Score	Total Score	Query Cover	**E value**	Per. Ident	Accession
achaete-scute homolog 2 [Homo sapiens]	373	373	100%	2e-130	100.00%	NP_005161.1
achaete-scute homolog 2 [Pongo abelii]	368	368	100%	3e-128	98.96%	XP_002821424.1
achaete-scute homolog 2 [Nomascus leucogenys]	361	361	100%	2e-125	97.41%	XP_003282133.1
achaete-scute homolog 2 [Macaca nemestrina]	356	356	100%	1e-123	96.37%	XP_011719606.1
achaete-scute homolog 2 [Piliocolobus tephrosceles]	356	356	100%	1e-123	96.37%	XP_023039276.1
achaete-scute homolog 2 [Papio anubis]	297	297	100%	3e-100	95.85%	XP_003909431.1
PREDICTED: achaete-scute homolog 2 [Chlorocebus sabaeus]	297	297	100%	3e-100	95.34%	XP_008003331.1
PREDICTED: achaete-scute homolog 2 [Rhinopithecus bieti]	294	294	100%	3e-99	95.34%	XP_017741776.1
PREDICTED: achaete-scute homolog 2 [Cebus capucinus imitator]	271	271	92%	4e-90	96.07%	XP_017363199.1
PREDICTED: achaete-scute homolog 2 [Callithrix jacchus]	269	269	100%	3e-89	94.82%	XP_009006952.1
achaete-scute homolog 2 [Sus scrofa]	265	265	100%	1e-87	84.97%	NP_001116463.1
PREDICTED: achaete-scute homolog 2 [Capra hircus]	261	261	92%	5e-86	85.39%	XP_017899088.1

hits

Sequence-based searches

- PSI-BLAST results

Sequences producing significant alignments Download Manage Columns Show 100

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignmen

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Sequence-based searches

- PSI-BLAST results

alignment

```
>gb|AAT70109.1|  CurN [Lyngbya majuscula]
Length=341

 Score =  303 bits (777),  Expect = 8e-81, Method: Composition-based stats.
 Identities = 148/297 (49%), Positives = 188/297 (63%), Gaps = 8/297 (2%)

Query  2    SEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYLWRNIIPHV-APSHR  60
             I + FPF    VEV G  + YVD G   G PVLFLHGNPTSSYLWRNIIP+V A  +R
Sbjct  41   LPISSEFPFAKRTVEVEGATIAYVDEG--SGQPVLFLHGNPTSSYLWRNIIPYVVAAGYR  98

Query  61   CIAPDLIGMGKSDKPDLDYFFDDHVRYLDAFIEALGLEEVVLVIHDWGSALGFHWAKRNP  120
             +APDLIGMG S KPD++Y   DHV Y+D FI+ALGL+++VLVIHDWGS +G   A+ NP
Sbjct  99   AVAPDLIGMGDSAKPDIEYRLQDHVAYMDGFIDALGLDDMVLVIHDWGSVIGMRHARLNP  158

Query  121  ERVKGIACMEFIRPI----PTWDEWPEFARETFQAFRTADVGRELIIDQNAFIEGVLPK-  175
            +RV  +A ME + P     P+++         F+  RTADVG ++++D N F+E +LP+
Sbjct  159  DRVAAVAFMEALVPPALPMPSYEAMGPQLGPLFRDLRTADVGEKMVLDGNFFVETILPEM  218

Query  176  CVVRPLTEVEMDHYREPFLKPVDREPLWRFPNEIPIAGEPANIVALVEAYMNWLHQSPVP  235
             VVR L+E EM  YR PF     R P  ++P E+PI GEPA   A V     WL  SP+P
Sbjct  219  GVVRSLSEAEMAAYRAPFPTRQSRLPTLQWPREVPIGGEPAFAEAEVLKNGEWLMASPIP  278

Query  236  KLLFWGTPGVLIPPAEAARLAESLPNCKTVDIGPGLHYLQEDNPDLIGSEIARWLPG  292
            KLLF   PG L P      L+E++PN +   +G G H+LQED+P LIG  IA WL
Sbjct  279  KLLFHAEPGALAPKPVVDYLSENVPNLEVRFVGAGTHFLQEDHPHLIGQGIADWLRR  335
```

Sequence-based searches

- BLAST Score
  - normalized raw score
  - raw score = sum of substitution scores and gap penalties
  - higher is better, but does not adequately represent significance of alignment
- BLAST *E*-value
  - equal to the number of BLAST alignments with a given Score that are expected to be seen simply by a chance
  - indicator of alignment significance
  - results associated with the lowest *E*-values are the best
  - hits with an *E*-value score > 0.01 belong to the "grey zone" – do not trust them

Sequence-based searches

- BLAST alignment
  - identity and similarity level between query and aligned sequence
  - alignment length and coverage of query sequence - the alignment is local, therefore one should always check that the alignment covers a significant portion of the query sequence (e.g., the alignment may involve only few amino acids from the query sequence → not significant hit)

Optimal search strategy

- text-based search
  - good for finding evolutionary “unrelated” proteins with some specific function
  - a large number of false negatives (missed proteins with target function) and false positives (identified proteins with different function) results due to erroneous or inaccurate annotations

Optimal search strategy

- text-based search
- sequence-based search
  - good for finding members of a protein family (i.e., group of evolutionary related proteins sharing some specific function) → not suitable for finding "unrelated" proteins
  - potential false positive results (i.e., proteins belonging to other evolutionary related families)
  - searches using protein sequence queries are generally more sensitive than using nucleotide sequence queries (20 different amino acids vs. 4 different nucleotides)

Optimal search strategy

- text-based search
- sequence-based search
- combination of text-based and sequence-based approaches
  1. text-based search
  2. subdivision of identified sequences into evolutionary related groups
  3. selection of few representatives for each group
  4. sequence-based searches using each representative as a query

  - potential false positive results – should be filtered

How to recognize interesting sequences?

How to recognize interesting sequences?

- sequence clustering
- sequence comparison
- information about host organisms
- automated *in silico* enzyme identification

Sequence clustering

- clustering based on pairwise sequence similarities
  - can be used for a fast and rough classification of sequences in large datasets (thousands of sequences)

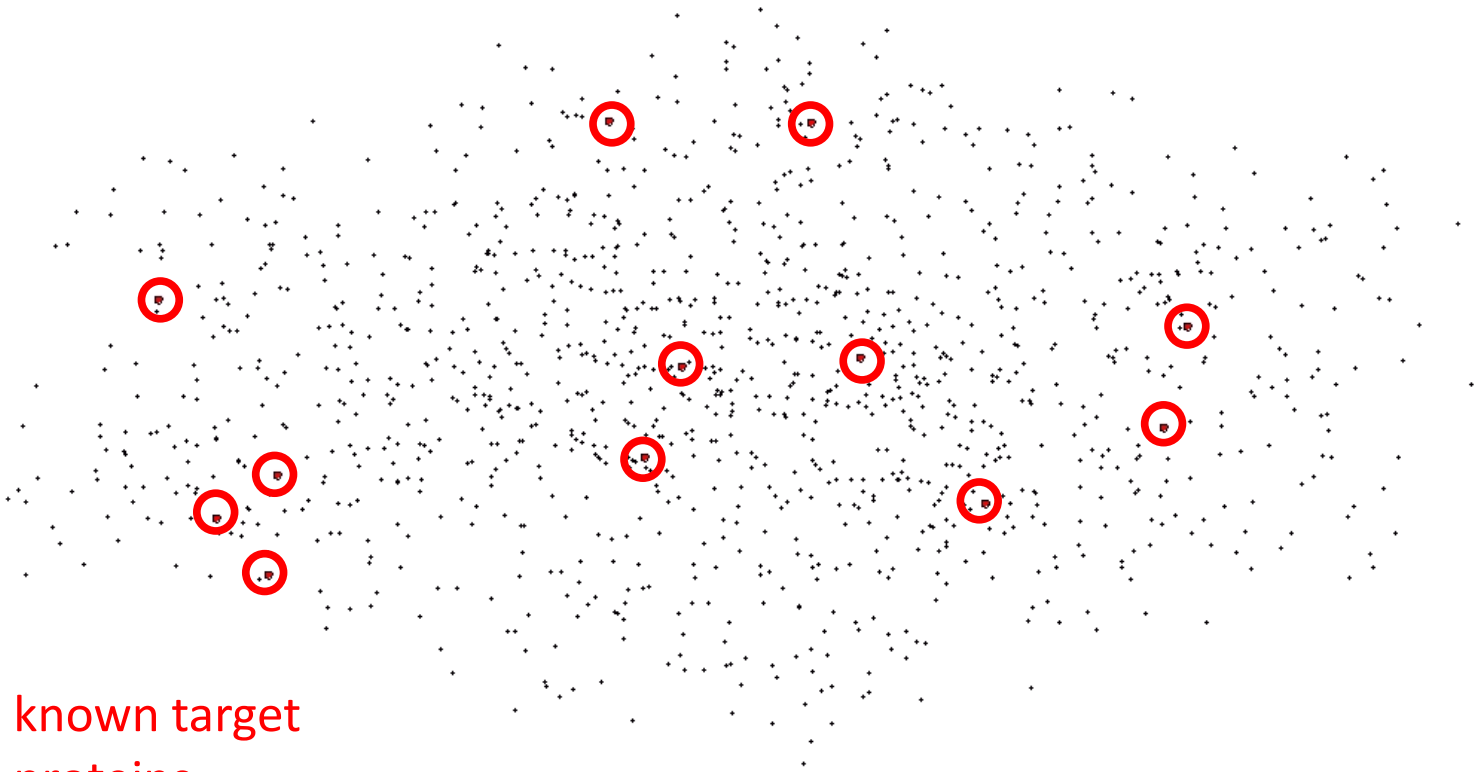
    → effective way to filter results of database searches

    → identification of members of individual protein families

  - CLANS - visualization of pairwise sequence similarities in three-dimensional space → overview of sequence space

Sequence clustering

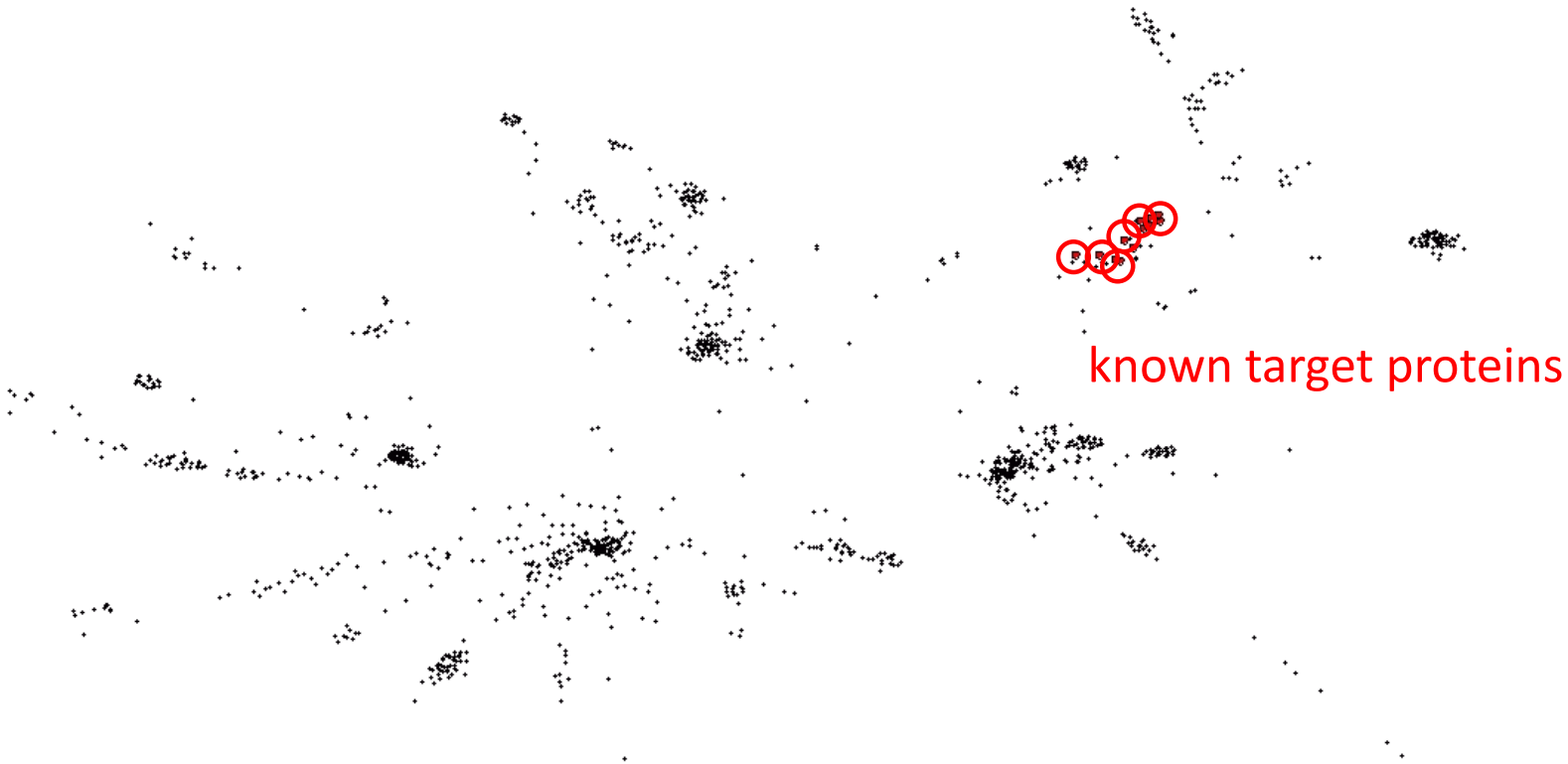
- clustering based on pairwise sequence similarities



known target proteins

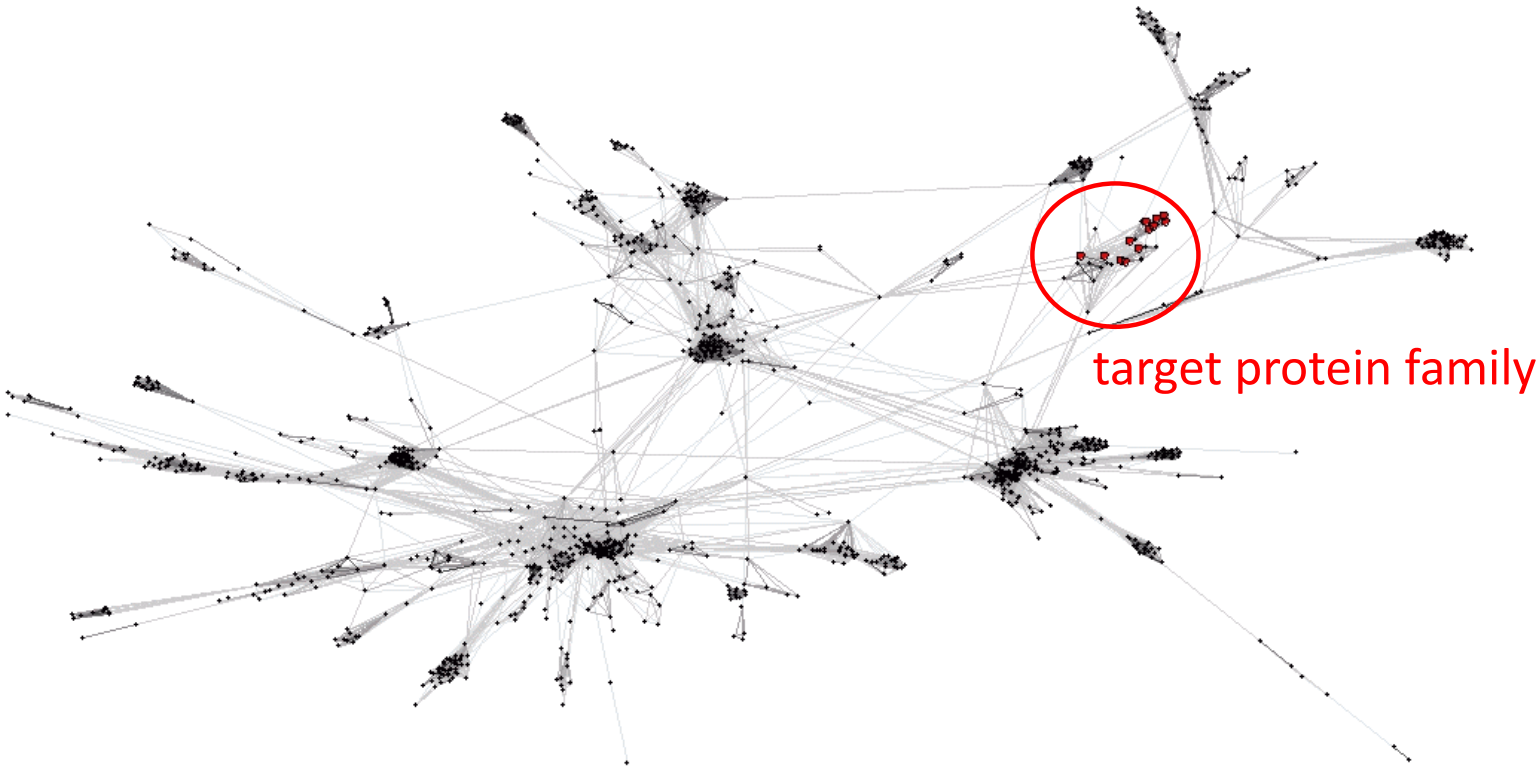
Sequence clustering

- clustering based on pairwise sequence similarities



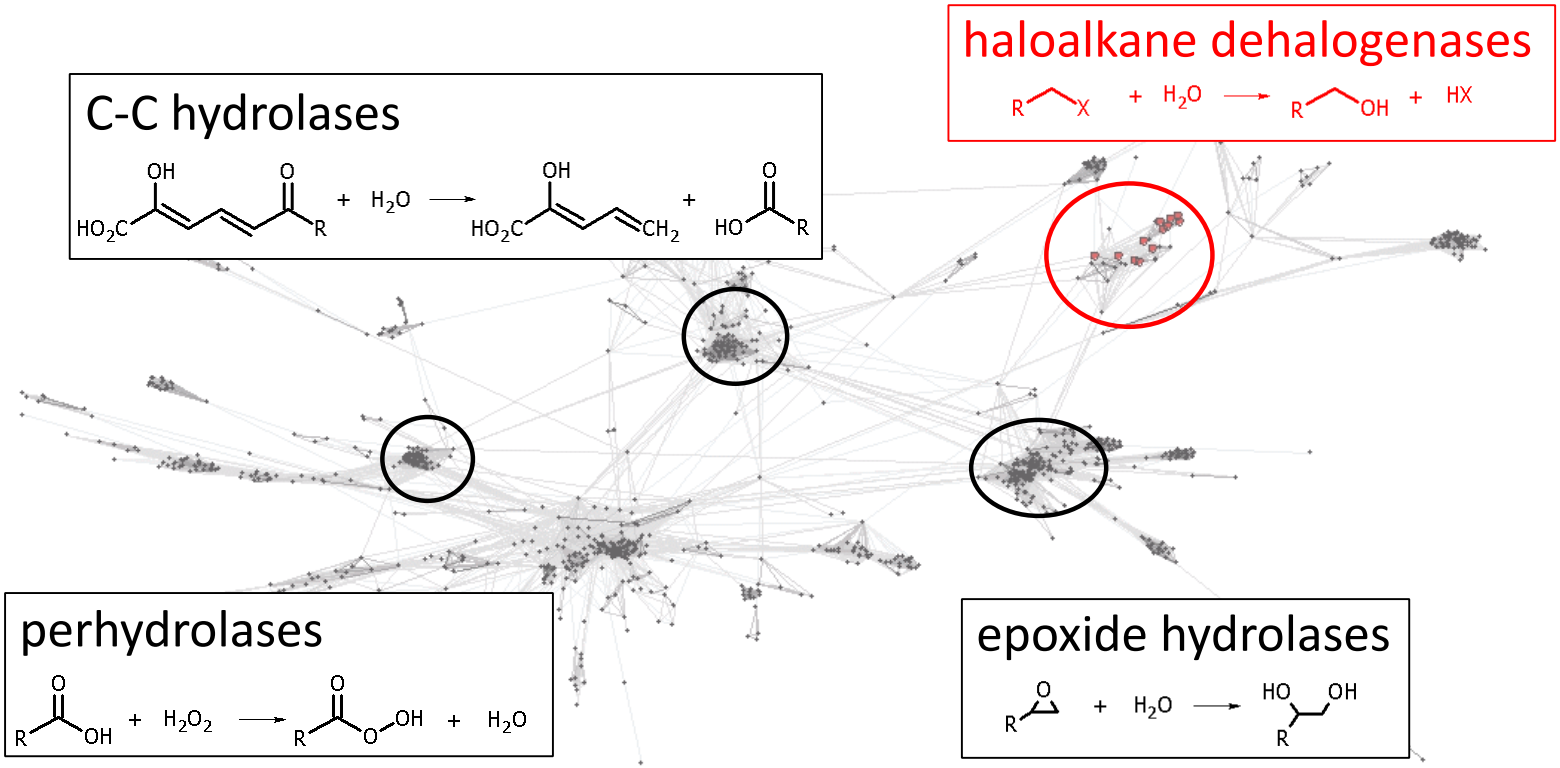
Sequence clustering

- clustering based on pairwise sequence similarities



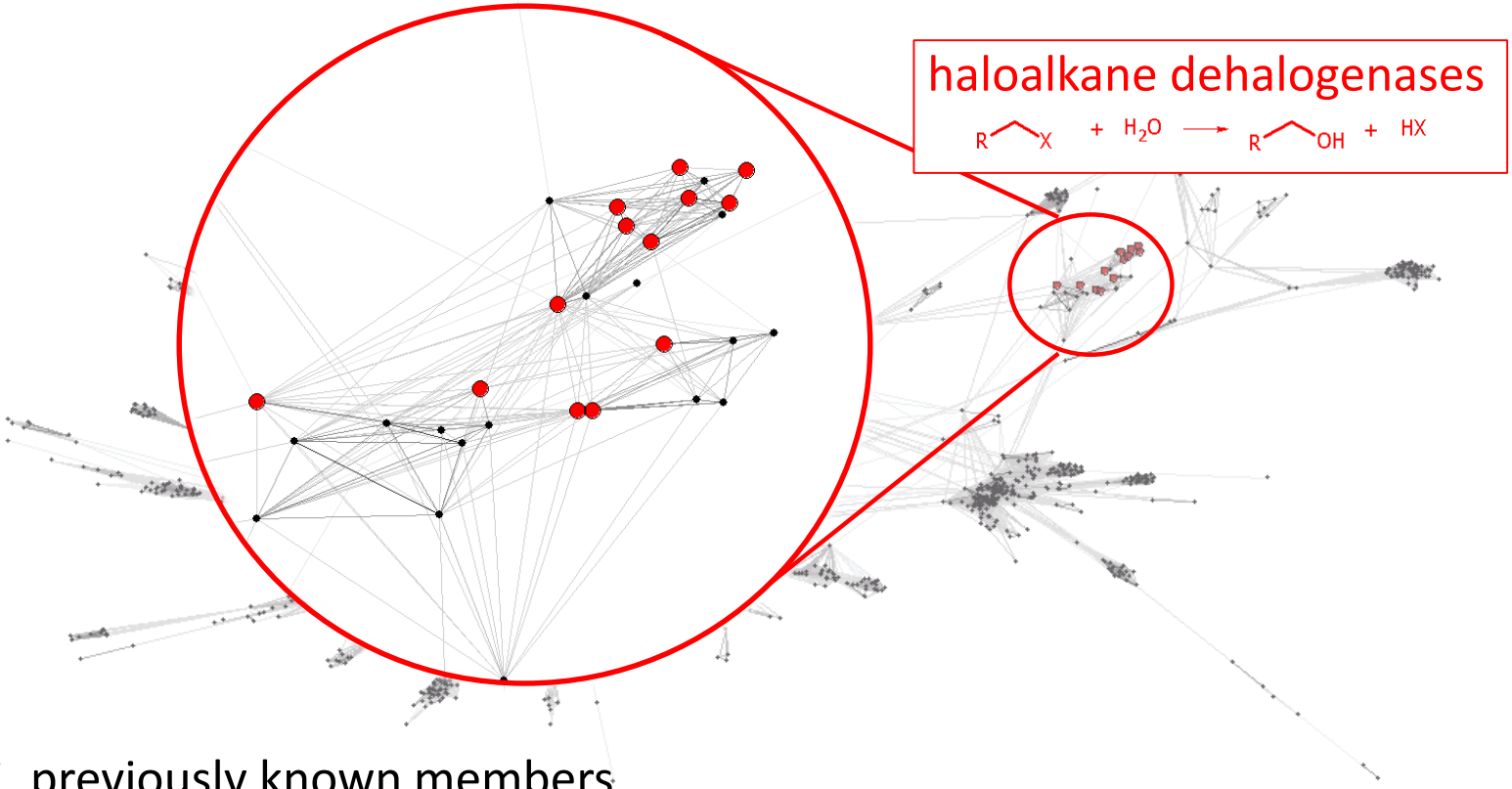
Sequence clustering

- clustering based on pairwise sequence similarities



Sequence clustering

- clustering based on pairwise sequence similarities



- previously known members
- new family members

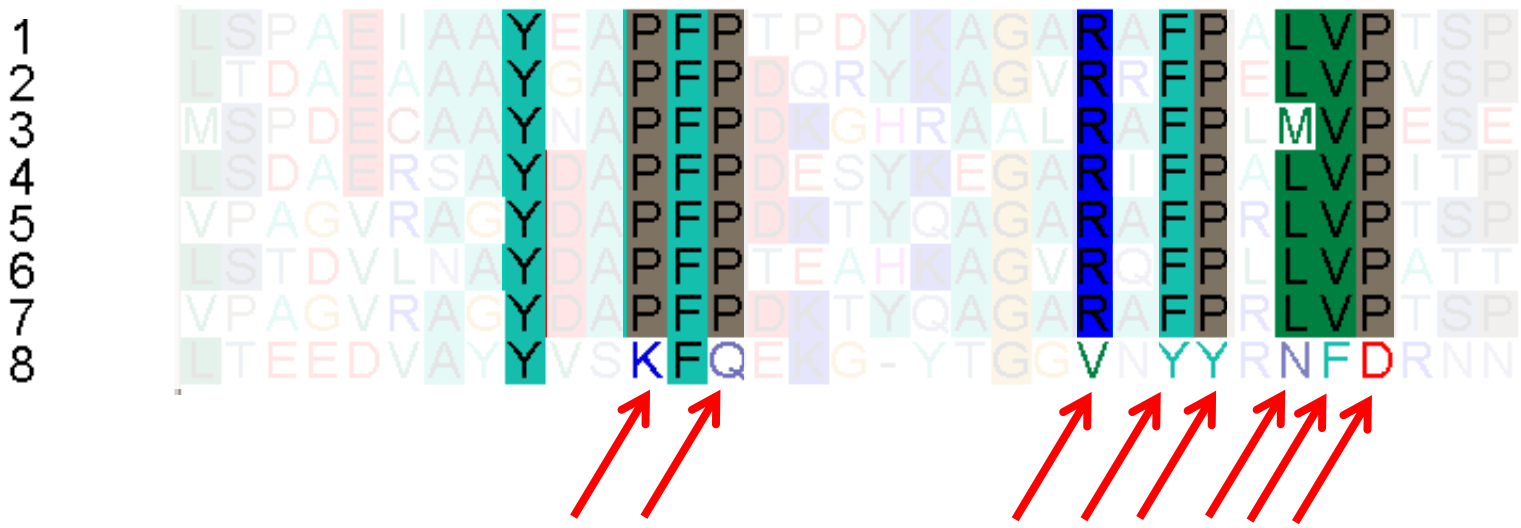
Sequence comparison

- multiple sequence alignment
  - analysis of conserved residues within protein family → identification of protein family members

```
1  LSPAEIAAYEAPFPTPDYKAGARAFPALVPTSP
2  LTDAEAAAYGAPFPDQRYKAGVRRFPELVPVSP
3  MSPDECAAYNAPFPDKGHRAALRAFPLMVPESE
4  LSDAERSAYDAPFPDESYKEGARIFPALVPITP
5  VPAGVRAGYDAPFPDKTYQAGARAFPRLVPTSP
6  LSTDVLNAYDAPFPTEAHKAGVRQFPLLVPATT
7  VPAGVRAGYDAPFPDKTYQAGARAFPRLVPTSP
8  LTEEDVAYYVSKFQEKG-YTGGVNYYRNFDRNN
```

Sequence comparison

- multiple sequence alignment
  - analysis of conserved residues within protein family → identification of protein family members

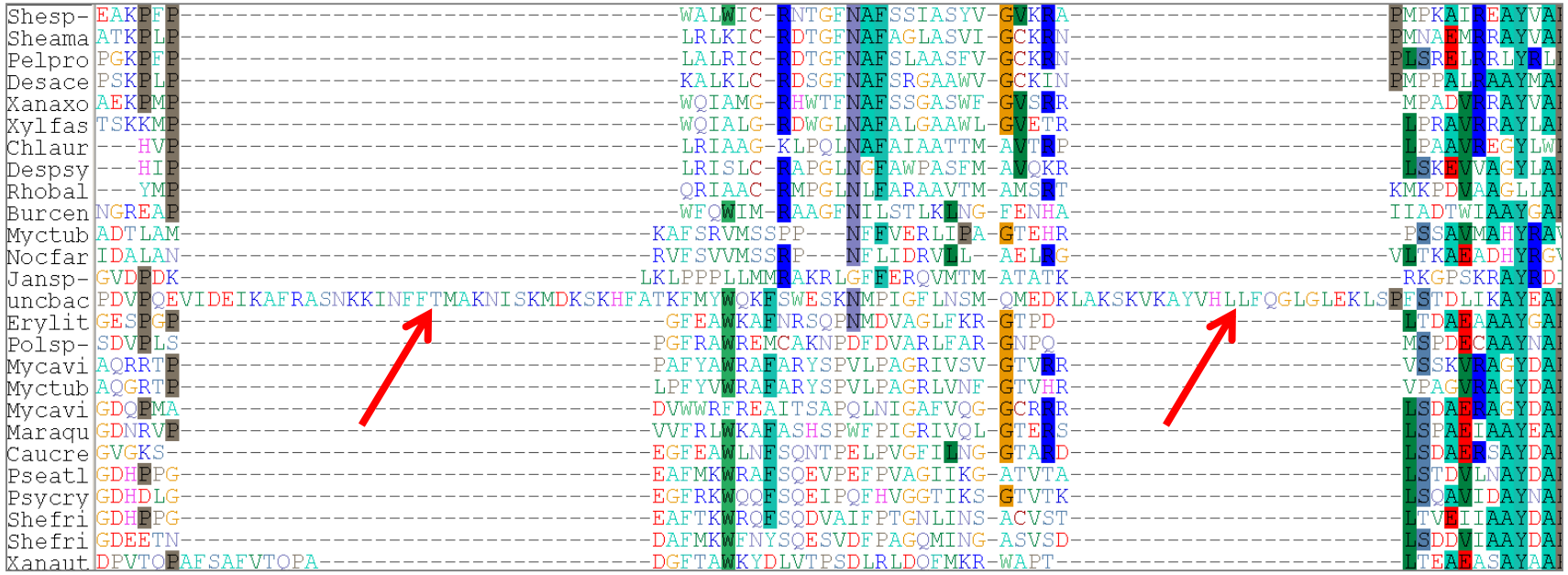


Sequence comparison

- multiple sequence alignment
  - analysis of conserved residues within protein family → identification of protein family members

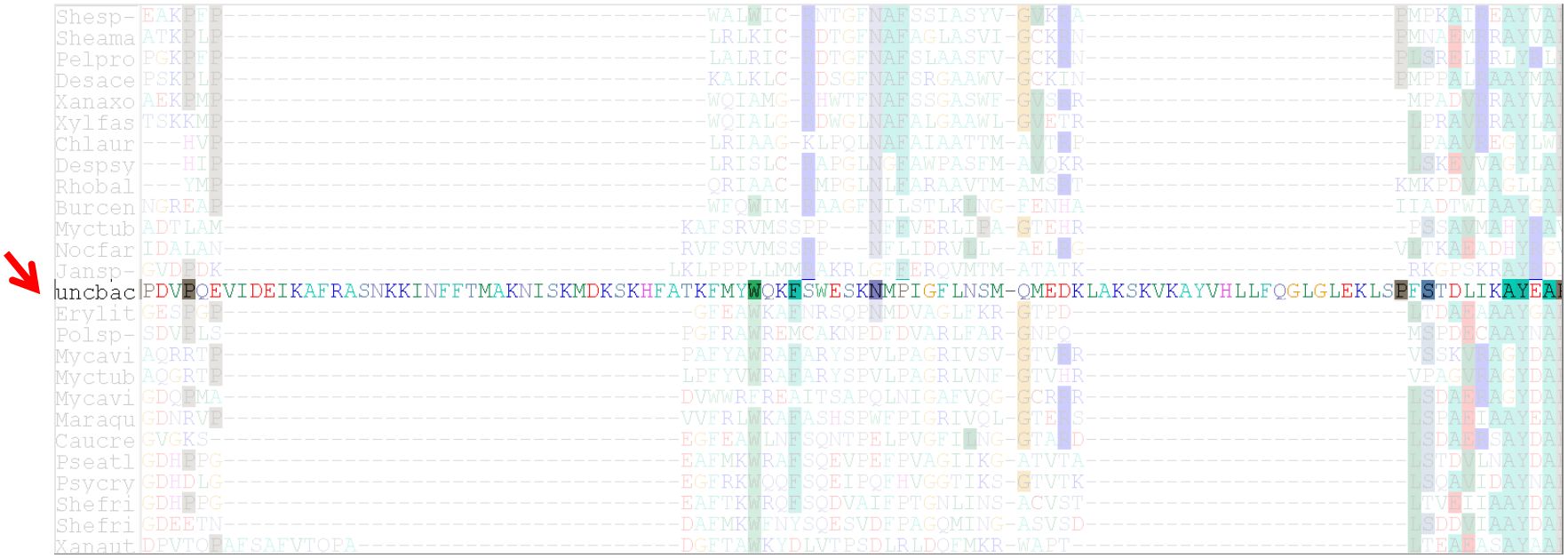
Sequence comparison

- multiple sequence alignment
  - identification of sequences with unique features → proteins with potentially novel characteristics



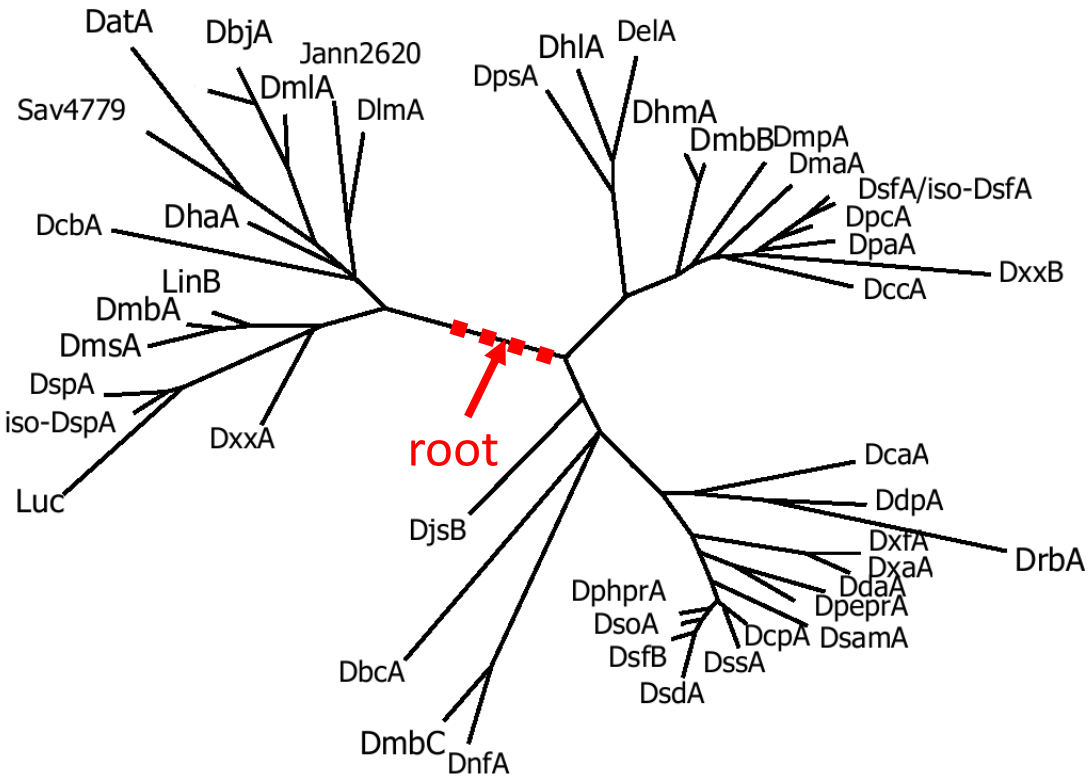
Sequence comparison

- multiple sequence alignment
  - identification of sequences with unique features → proteins with potentially novel characteristics



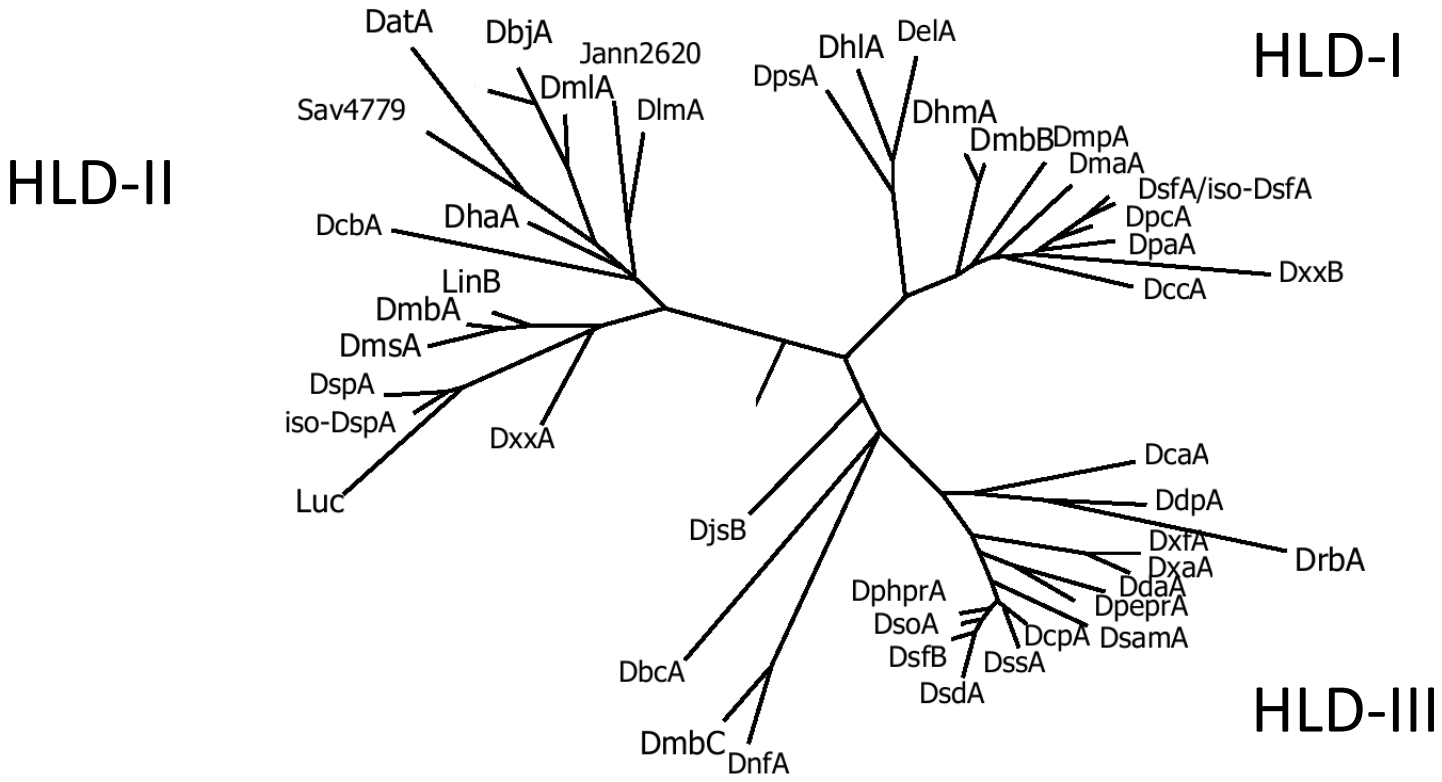
Sequence comparison

- phylogenetics
  - establishment of evolutionary relationships among sequences



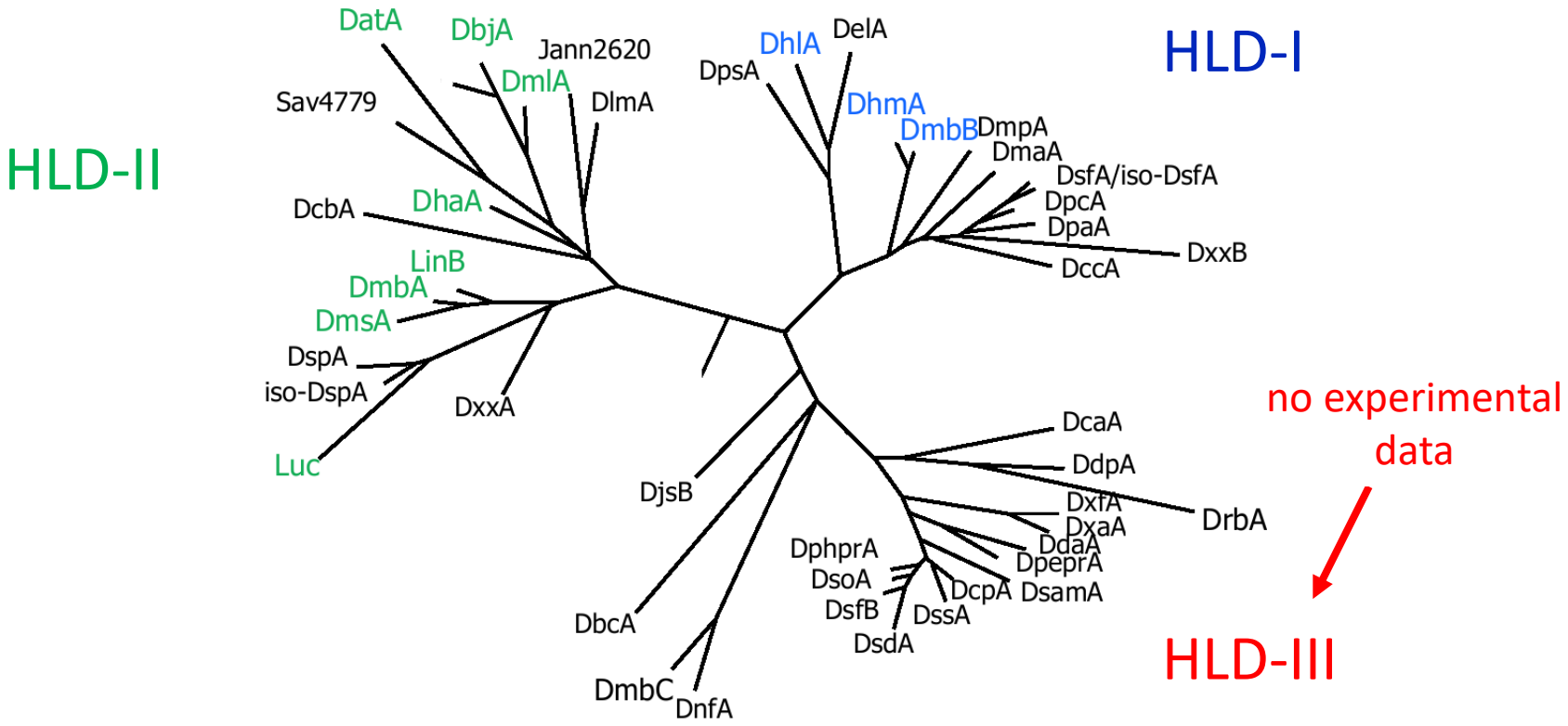
Sequence comparison

- phylogenetics
  - classification of sequences



Sequence comparison

- phylogenetics
  - information about experimental data→ selection of novel proteins



Information about host organisms

- extremophiles - microorganisms living in extreme conditions
  - geochemical extremes (pH, salinity)
  - physical extremes (temperature, pressure)
- proteins from extremophiles
  - often adapted to extreme conditions → unique characteristics, useful for practical applications

Information about host organisms

- Genomes OnLine Database (GOLD)
  - http://www.genomesonline.org/
  - list of complete (>6,000), ongoing (> 27,000) and targeted genome (>1,000) projects
  - information about individual projects and source organisms
- Entrez Genome
  - http://www.ncbi.nlm.nih.gov/sites/genome
  - provided by NCBI
  - data from more than 20,000 finished or ongoing genome projects (includes almost 10,000 organisms)
  - information about genome, source organism, genes, encoded proteins, graphical representations, ...

Information about host organisms

- GOLD

Metagenomes

Classification

- Studies: **370**
- Samples: **2642**

Isolate Genomes

Complete Projects: **4169**

Incomplete Projects: **17714**

Targeted Projects: **1500**

Organism Metadata

MIGS 22	OXYGEN REQUIREMENT	Aerobe
MIGS 37.1	CELL SHAPE	Rod-shaped
MIGS 37.2	MOTILITY	Nonmotile
MIGS 37.3	SPORULATION	
MIGS 37.4	PRESSURE	
MIGS 37.12	TEMPERATURE RANGE	Psychrophile
	SALINITY	Halotolerant
	PH	
MIGS 37.5	CELL DIAMETER	
MIGS 37.6	CELL LENGTH	
MIGS 37.7	COLOR	
MIGS 37.8	GRAM STAINING	
MIGS 15	BIOTIC REALTIONSHIPS	Free living

Information about host organisms

- Entrez Genome

Psychrobacter cryohalolentis

Psychrotolerant organism

Lineage: Bacteria[4049]; Proteobacteria[1682]; Gammaproteobacteria[750]; Pseudomonadales[122]; Moraxellaceae[51]; Psychrobacter[10]; Psychrobacter cryohalolentis[1]

Psychrobacter. These bacteria are commonly isolated from low temperature environments, *Psychrobacter* spp. are cold-adapted organisms that are often isolated from extreme environments such as permafrost or the Antarctic ice.***Psychrobacter cryohalolentis***. *Psychrobacter cryohalolentis*, formerly *Psychrobacter cryopegella* More...

Representative

Community selected, Calculated : Psychrobacter cryohalolentis K5

***Psychrobacter cryohalolentis* K5**. This organism was isolated from saline liquid (12-14%) found 11-24 m below the surface within a forty thousand-year-old Siberian permafrost at the Kolyma-Indigirka lowland in Siberia. This strain will provide insight into growth at extremely low temperatures.

Human Pathogen: no

Type	Name	RefSeq	INSDC	Size (Mb)	GC%	Protein	rRNA	tRNA	Other RNA	Gene	Pseudogene
Chr	-	NC_007969.1	CP000323.1	3.06	42.3	2,467	12	48	6	2,537	4
Plsm	1	NC_007968.1	CP000324.1	0.041221	38.3	44	-	-	-	44	-

Biological Properties

- Morphology
  - Shape : Bacilli
  - Motility : No
- Environment
  - Salinity : ModerateHalophilic
  - TemperatureRange : Psychrophilic
  - Habitat : Multiple

biological properties

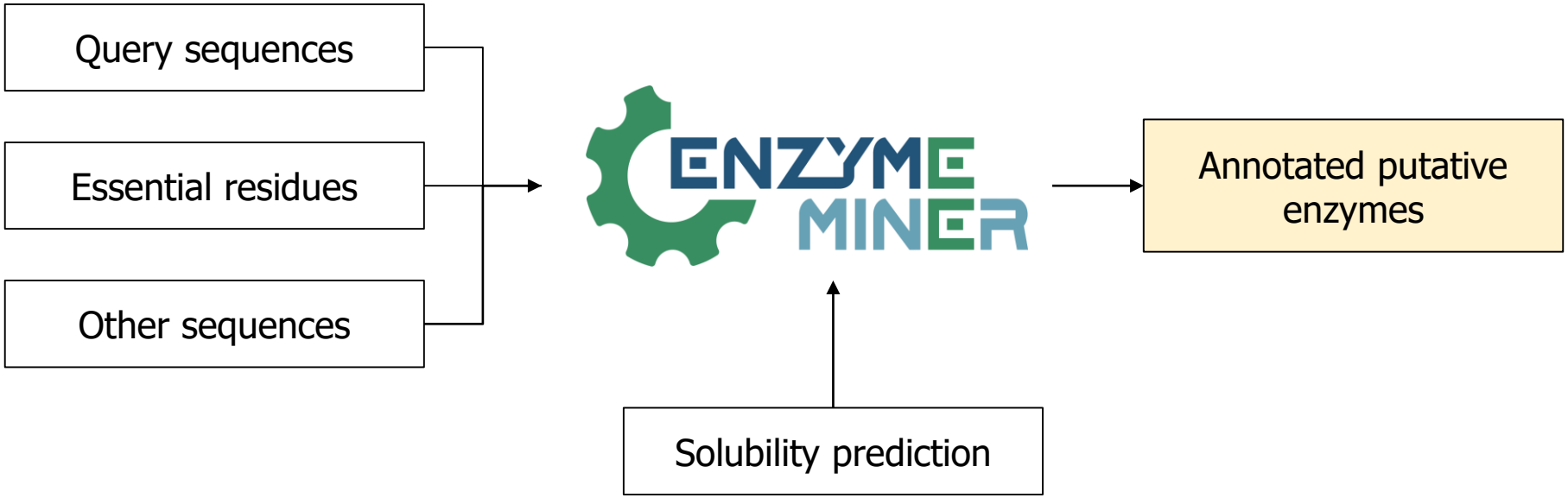
Genome Sequencing Projects

Chromosomes [1] Scaffolds or contigs [0] SRA or Traces [0] No data [0]

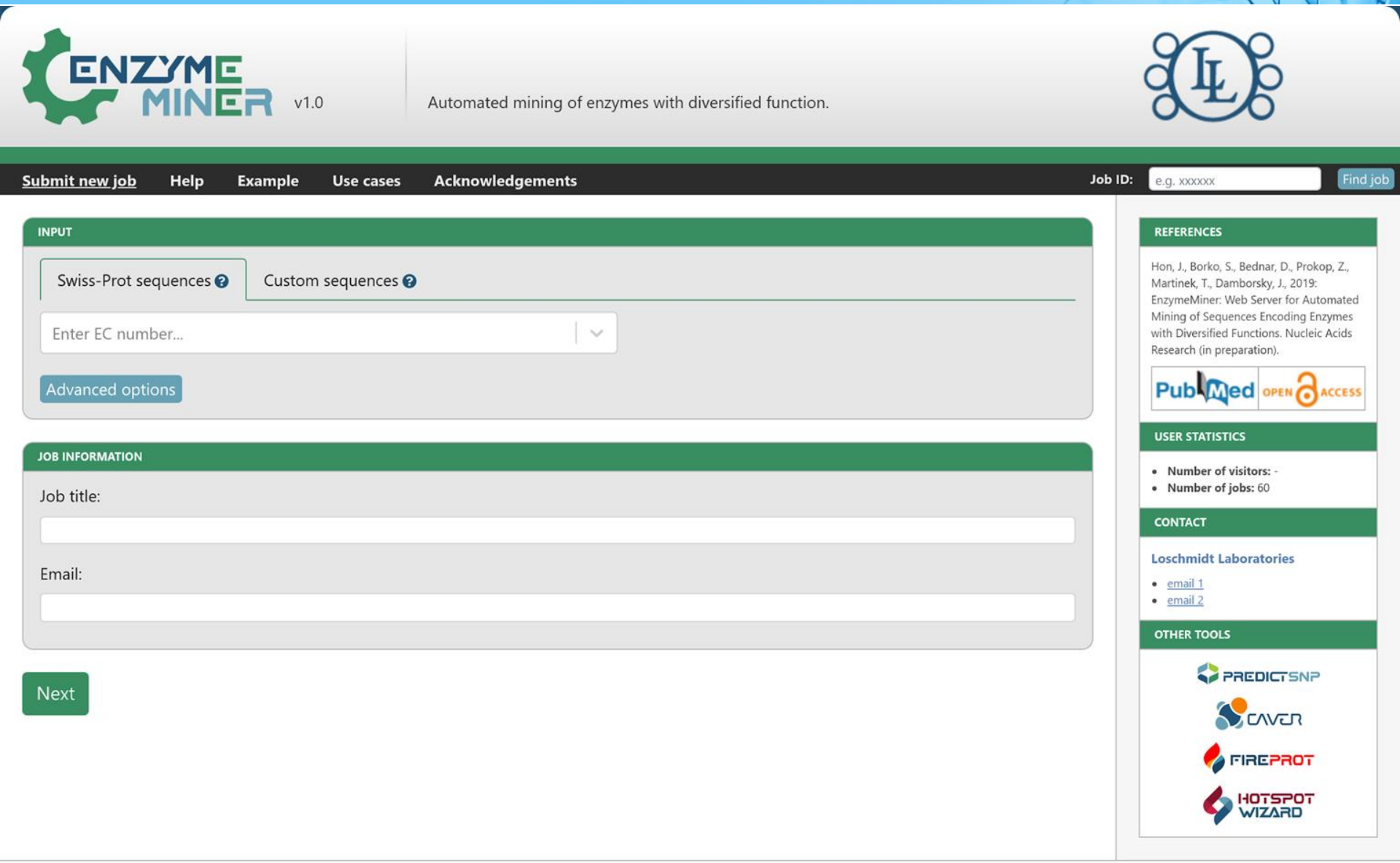
Organism	BioProject	Assembly	Status	Chrs	Plasmids	Size (Mb)	GC%	Gene	Protein
Psychrobacter cryohalolentis K5	PRJNA58373, PRJNA13920	ASM1390v1	●	1	1	3.1	42.2	2,581	2,511

Automated *in silico* enzyme identification

- Enzyme Miner
  - https://loschmidt.chemi.muni.cz/enzymeminer/



Automated *in silico* enzyme identification



INPUT

Swiss-Prot sequences | Custom sequences

Load saved input

Query sequences:

```
>DrbA
MSCRLSSNRRGSSKLAAMTNLASDLFPHPSSELSIDGHTLRYIDTAASSDIPSSAVGSSD
GEPTFLCVHGNPTWSFYYRRIIERYGKQQRVIAVDHIGCGRSDKPSEDEFPYTMAAHRDN
LIRLVDELDLKNVILIAHDWGGAIGLSAMHARRDRLAGIGLLNTAAFPPPYMPQRIAACR
MPVLGTPAVRGLNLFARAAVTMAMSRTKMKPDVAAGLLAPYDNWKNRVAIDRFVRDIPLN
```

Load from file:

Vybrat soubor Soubor nevybrán

Other known sequences:

```
>DmbC
MSIDFTPDPQLYPFESRWFDSSRGRIHYVDEGTGPPILLCHGNPTWSFLYRDIIVALRDR
FRCVAPDYLGFGLSERPSGFGYQIDEHARVIGEFVDHLGLDRYLSMGQDWGGPISMAVAV
ERADRVRGVVLGNTWFWPADTLAMKAFSRVMSSPPVQYAILRRNFFVERLIPAGTEHRPS
SAVMAHYRAVQPNAAARRGVAFMPKQILAARPLLARLAREVPATLGTKPTLLTWGMKDVA
```

Load from file:

Vybrat soubor Soubor nevybrán

Essential residue templates:

Add protein (row) Add residue (column)

Accession	nucleophile	acid1	acid2	base	halide1	halide2	halide3
	D	D, E	D, E	H	H, N, Q, W, Y	H, N, Q, W, Y	H, N, Q, W, Y
DrbA	139	Enter position	272	300	71	140	Enter position
DmbB	123	Enter position	250	279	Enter position	124	164
DmbC	109	Enter position	238	267	43	110	Enter position

JOB OUTPUT INFORMATION

ID: example

Title: Example

Time: 18 12 2019 14:04

Status: Done

Download input file | Re-run job

DOWNLOAD RESULTS

Result table (xlsx)

Result table (tsv)

Raw results

TARGET SELECTION TABLE

Select all | Deselect all | Undo | Redo

Solubility threshold: 0.00

Primary domains:

PF00561 (Abhydrolase_1) ×

Selected | Full Dataset | Extra domain | Known Organism | Temperature | Salinity | Biotic Relationship | Disease | Transmembrane | With Structure

Accession	Annotation	Closest query	Identity closest query	Kingdom	Solubility	Sequence length	Domain
2PSD_A	Chain A, Crystal Stru...	D4Z2G1	41.5	E	0.9735	318	Abh
2PSF_A	Chain A, Crystal Stru...	D4Z2G1	41.5	E	0.9615	310	Abh
2PSJ_A	Chain A, Crystal Stru...	D4Z2G1	41.5	E	0.9614	319	Abh
2PSH_A	Chain A, Crystal Stru...	D4Z2G1	41.2	E	0.9586	319	Abh
WP_071575177.1	haloalkane dehalog...	D4Z2G1	70.8	B	0.9399	270	Abh
3SK0_A	Chain A, structure o...	D4Z2G1	46.2	B	0.9393	311	Abh
4BRZ_A	Chain A, Haloalkane...	D4Z2G1	61.7		0.9357	290	Abh

What to keep in mind?

What to keep in mind?

- sequence databases
  - **nucleotide**: GenBank, EMBL-BANK, DDBJ; **protein**: UniProtKB, nr Protein database
  - **errors** in sequences and annotations
- database searches
  - **text-based:** results influenced by sequence annotations
  - **sequence-based:** identification of family members - BLAST, PSI-BLAST - *E*-value
  - **combination** of both approaches: optimal strategy
  - false positive results: sequences should be filtered
- selection of proteins for experimental characterization
  - **clustering:** classification and filtering of hits from database searches - CLANS
  - **sequence comparison:** classification and identification of unique sequences
  - sequences from **extremophiles:** potentially adapted to extreme conditions
  - **Enzyme Miner:** automated identification of interesting catalysts

What to keep in mind?

- *in silico* identification and analysis of sequences - fast and cheap way to identify new proteins

References

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- Claverie, J-M. and Notredame, C. (2006). **Bioinformatics For Dummies** (2nd ed.). Wiley Publishing, Hoboken, p. 436.
- Steele, H.L. *et al.* (2009). Advances in Recovery of Novel Biocatalysts from Metagenomes. *Journal of Molecular Microbiology and Biotechnoly* **16**: 25–37.
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- Van den Burg, B. (2003). Extremophiles as a source for novel enzymes. *Current Opinion in Microbiology* **6**: 213-218.

LOSCHMIDT LABORATORIES

PROTEIN ENGINEERING

3. PREPARATION OF RECOMBINANT PROTEINS, PROTEIN EXPRESSION AND PURIFICATION

Loschmidt Laboratories
Department of Experimental Biology
Masaryk University, Brno