

FACULTY OF ENGINEERING AND TECHNOLOGY

Department of Biotechnology

Protein identification and characterization is one of the

important parts of <u>recombinant protein expression</u>.



AACompldent

- 1. Constellation 0: ALL amino acids: Ala, Ile, Pro, Val, Arg, Leu, Ser, Thr, Gly, Met, His, Phe, Tyr, Lys, Asp, Asn, Gln, Glu, Cys and Trp.
- Constellation 1: Ala, Ile, Pro, Val, Arg, Leu, Ser, Asx, Thr, Glx, Gly, Met, His, Phe and Tyr. (Asp+Asn=Asx; Gln+Glu=Glx; Lys, Cys and Trp are not considered).
- Constellation 2: Ala, Ile, Pro, Val, Arg, Leu, Ser, Asx, Lys, Thr, Glx, Gly, Met, His, Phe and Tyr. (Asp+Asn=Asx; Gln+Glu=Glx; Cys and Trp are not considered).
- Constellation 3: Ala, Ile, Pro, Val, Arg, Leu, Ser, Asx, Lys, Thr, Glx, Gly, Met, His and Phe. (Asp+Asn=Asx; Gln+Glu=Glx; Tyr, Cys and Trp are not considered).
- Constellation 4: Ala, Ile, Pro, Val, Arg, Leu, Ser, Asx, Lys, Thr, Glx, Met, His, Phe and Tyr. (Asp+Asn=Asx; Gln+Glu=Glx; Gly, Cys and Trp are not considered).
- Constellation 5: Ala, Ile, Pro, Val, Arg, Leu, Ser, Asx, Lys, Thr, Glx, Gly, Met, His, Phe, Tyr and Cys. (Asp+Asn=Asx; Gln+Glu=Glx; Trp is not considered).

Tagldent

Name of your query (optional):				
pl range: min	max			
Mw (in Daltons, <i>not</i> kD):				
Mw range (in percent): 20 %				

Check for protein sequences
with cysteines in reduced form (-SH)
with cysteines oxidized (-S-S-)

Organism rame or classification or NCBI TaxID (e.g. homo sapiens, eukaryota, 9606):

Keyword from KW lines (for search in Swiss-Prot only):

Tagging

With this option, the first 40 amino acics (or the last 40 aa or the matching internal subsequence) of each protein are printed in the result, in addition to the protein name. You may optionally also erter a tag which will be matched with the sequences. If the tag is found in the displayed sequence, it will be shown in lower case, and the numerical position of the tag in the protein will be given. % can be used to mean 'any amino acid'.
Tag (up to 6 amino acids):

Search for all possible tag permutations.

Display only the sequences matching the tag.

Include scan of your tag against all fragments in UniProtKB matching your organism criteria (and KW terms for Swiss-Prot only). Note: pl and Mw are not computed for fragments.

Display the predicted
N-terminal sequence or the O C-terminal sequence or O the (internal) area of the protein that matches your tag.

Send the result by e-mail

With this opti	on, you will receive the result by e-mail.
Your e-mail:	

O UniProtKE/Swiss-Prot and UniProtKB/TrEMBL

○ UniProtKE/TrEMBL

PROPSEARCH

To find the putative protein family if querying a new sequence has failed using alignment methods

By neglecting the order of amino acid residues in a sequence, it uses the amino acid composition

144 properties like mol.wt, hydrophobicity, average charge etc., are weighted individually and are used as query vector

PROPSEARCH-DISTANCE

TABLE 1: RELIABILITY OF FAMILY IDENTIFICATION AS FUNCTION OF PROPSEARCH-DISTANCE

DIST		RELIABILITY [percent]
between	and	
0.0	1.3	99.9
1.3	7.5	99.6
7.5	8.7	94
8.7	10.0	87
10.0	11.2	80
11.2	12.5	68
12.5	13.7	53
13.7	14.9	41
14.9	16.2	36
16.2	17.5	32
17.5	18.7	25
18.7	19.9	19



PepSea

Tool for identification by peptide mapping or peptide Sequencing

Protein mass range [kDa]: 0 < Mr < 300				
Cleavage agent: Trypsin				
Cysteine is: Carbamidomethyl-Cys 💌				
Oxidized Methionine				
Peptide mass (neutral): 982.6 Monoisotopic mass 💙				
Mass accuracy: 2 Da 🕶				
Peptide sequence tag: (502.2)ptt(813.4) [Syntax: (start mass)XYZ(end mass)]				
Match regions: 1 and 2 and 3 V				
Pattern Match: Search by: Y-type sequence ions 💌				
Only for Edman type searches: N-terminal sequence extension 💌				
Allowed number of errors: 0 💌	On each result page show this number of matches:			
Nominal mass:				
□ 1 = L	Do not search, only compute probability of false-positive match			
	Start PepSea			
Cleavage specificities:	Reset form			
✓ N-terminal specificity (needs to be off for Match Regions = "2 and 3")				
C-terminal specificity (needs to be off for Match Regions = "1 and 2")				

Peptide mass fingerprinting tools

PepMAPPER: Takes peptide mass as the input

Mascot: Can take the following as input,

- 1) Peptide mass fingerprint
- 2) Sequence query
- 3) MS/MS ion search

PepMAPPER

PepMAPPER (1)						
Organism: yeast 🗸	Enzyme Trypsin 🗸					
peptide parameters						
Peptide Masses(m/z)	\mathbf{M} asses are \odot average \bigcirc monoisotopic					
	Charge 💿 Fixed state 🛛 1+ 💌					
	⊙Unknown 1+ ⊻to 2+ ⊻					
~	Missed Cleavages 0 💌 (max possible)					
Error +/- 50 ppm 💙	N-terminal amino acid 🗛 💙					
fixed peptide modifications	protein parameters					
Acetylation (N-term,K) Biotinylated (N-term,K)	Mass Range 0.0 _ 999999.9					
Carbamidomethyl (C) Carbomyl (N-term)	Isolectric point Any 🕶 tol +/- 1 💌					
Report top 10 💌 matches	Search Reset					
[mapper1][mapper2][mapper3][mapper4][mapper5][linkspage]][Help Pages]						



MASCOT

MASCOT Peptide Mass Fingerprint

Your name			Email	
Search title				
Database	MSDB 💌			
Taxonomy	All entries			×
Enzyme	Trypsin 💌		Allow up to	1 💌 missed cleavages
Fixed modifications	Acetyl (K) Acetyl (N-term) Acetyl (Protein N-term) Amidated (C-term) Amidated (Protein C-term)	<	Variable modifications	Acetyl (K) Acetyl (N-term) Acetyl (Protein N-term) Amidated (C-term) Amidated (Protein C-term)
Protein mass	kDa		Peptide tol. ±	1.2 Da 💌
Mass values	⊚мн+ Ом _⊭ Ом-н-		Monoisotopic	⊙ Average ○
Data file		Browse		
Query NB Contents of this field are ignored if a data file is specified.				
Decoy			Report top	AUTO 💌 hits
	Start Search			Reset Form

Transmembrane helices prediction

TMAP TMHMM TMPRED TopPred2 PHDhtm



DAS









MCQs

- 1. A
- 2. A
- 3. A
- 4. A
- 5. A
- 6. A
- 7. A
- 8. A
- 9. A
- 10. A

