WIPO National Workshop on Search and Examination of Inventions related to Genetic Resources Manila, Philippines, May 20 to 24, 2019

# Hands-on Exercises <STN>

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### Examples are from "Sequence Motif Searches in CAS REGISTRY<sup>SM</sup>" (https://www.stninternational.org/uploads/tx\_ptgsarelatedfiles/20130730 \_\_\_\_\_\_\_Sequence\_\_Motif\_\_Searches\_\_in\_\_REGISTRY.pdf)



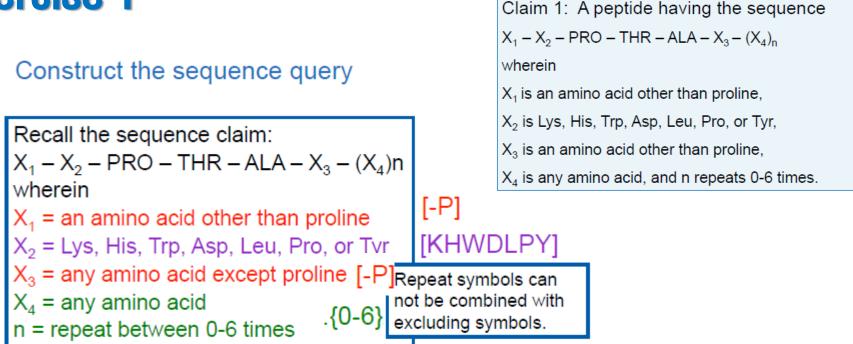
Claim 1: A peptide having the sequence  $X_1 - X_2 - PRO - THR - ALA - X_3 - (X_4)_n$ wherein  $X_1$  is an amino acid other than proline,  $X_2$  is Lys, His, Trp, Asp, Leu, Pro, or Tyr,  $X_3$  is an amino acid other than proline,  $X_4$  is any amino acid, and n repeats 0-6 times.



Select the sequence symbols to use in the query

- Use single letter codes to represent the residues
- Use square brackets to represent possible residues at a specific location
- Use square brackets and minus sign to exclude residues
- Use a period (.) to represent any amino acid
- Use curly braces { } to represent repeats





### Query

### [-P][KHWDLPY]PTA[-P].{0-6}/SQSP

Select the sequence symbols to use in the query

- Use single letter codes to represent the residues
- Use square brackets to represent possible residues at a specific location
- Use square brackets and minus sign to exclude residues
- Use a period (.) to represent any amino acid
- Use curly braces { } to represent repeats





### Query

### [-P][KHWDLPY]PTA[-P].{0-6}/SQSP

To search for	Use this field code	Retrieves	Example
Exact Sequence	/SQEP	Exact match; same length	=> S FCFWKTCT/SQEP
Subsequence	/SQSP	Sequences in which the query sequence may or may not be embedded	=> S LAGLL/SQSP
Exact Family	/SQEFP	Functionally similar amino acids; same length	=> S YGGFL/SQEFP
Subsequence Family	/SQSFP	Functionally similar amino acids; may or may not be embedded	=> S ATCXAWV/SQSFP
Sequence Length	/SQL	Sequences of a certain length	=> S SQL<=10
Annotation	/NTE	Sequences with the search term in the NTE field	=> S MULTICHAIN/NTE



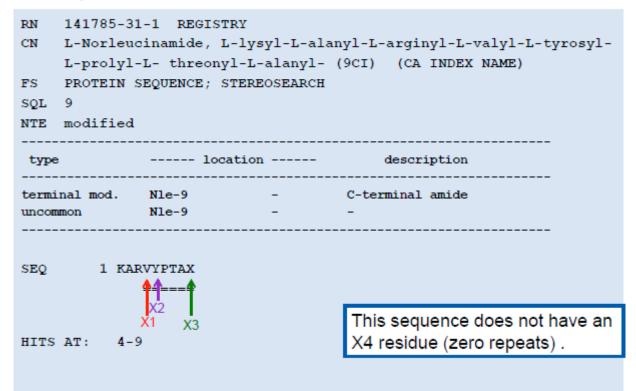
### Exercise 1 Query [-P][KHWDLPY]PTA[-P].{0-6}/SQSP

		To search	Use this for field code	e Retrieves
Run SQSP sequence query in REGIS	STRY	Exact Sequ	ence /SQEP	Exact match; same length
=> FILE REGISTRY				
<pre>=&gt; S [-P][KHWDLPY]PTA[-P].{0-6}/SQSP L1 101893 [-P][KHWDLPY]PTA[-P].{0-6}/SQSP</pre>	Gaps, wild brackets a for a SQE			
=> S L1 AND 6-12/SQL L2 156 L1 AND 6-12/SQL	Refine the with seque (SQL).	e answers ence length		
=> D L2 SQIDE Type D SQID identification the hit seque	information			
		Some display	formats	
		Format 0	Content	
		SQD SQD3 SQIDE SUDE HIT A	10 most recent CA re Sequence data, one-I Sequence data, three	etter codes e-letter codes MF, SR, LC, DT.CA, RL, REF it terms



### Query [-P][KHWDLPY]PTA[-P].{0-6}/SQSP

### Example answer





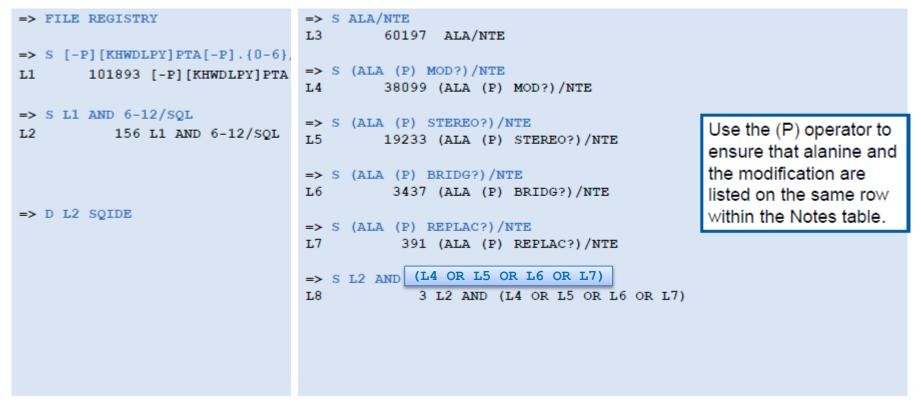
**Exercise 1** 

Dependent Claim 2 requires a modification on the alanine residue in the sequence of Claim 1:

 $X_1 - X_2 - PRO - THR - ALA - X_3 - (X_4)_n$ 



### Run SQSP sequence que Modifications are searched in the NTE field



To search for	Use this field code	Retrieves	Example
Annotation	/NTE	Sequences with the search term in the NTE field	=> S MULTICHAIN/NTE



### $X_1 - X_2 - PRO - THR - ALA - X_3 - (X_4)_n$

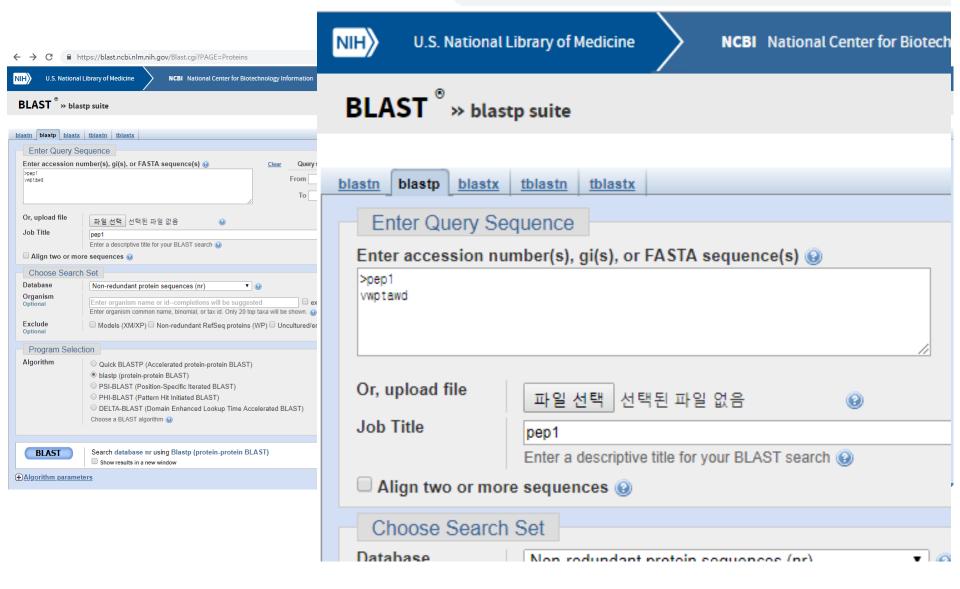
### Information is listed in the NTE table

=> D	SQIDE	Specific modifications can be searched in REGISTRY. See Quick Reference Guide.
RN	365254-57-5 REGIS	
CN	Glycine, L-alanyl-	L-cysteinyl-L-valyl-L-tryptophyl-L-prolyl-L-
	threonyl-3-cyclohe	xyl-D <mark>-alanyl-L-tryptophyl-L-asparaginyl-</mark>
	L-cysteinyl- (9CI)	(CA INDEX NAME)
OTHE	R NAMES:	
CN	42: PN: W00172771 1	FIGURE: 8-2 claimed protein
FS	PROTEIN SEQUENCE; S	STEREOSEARCH
SQL	11	
NTE	modified (modificat	tions unspecified)
type	· loc	cation description
modif	ication Ala-7	- cyclohexyl <chx></chx>
		Alanine is modified by the addition of cyclohexyl.

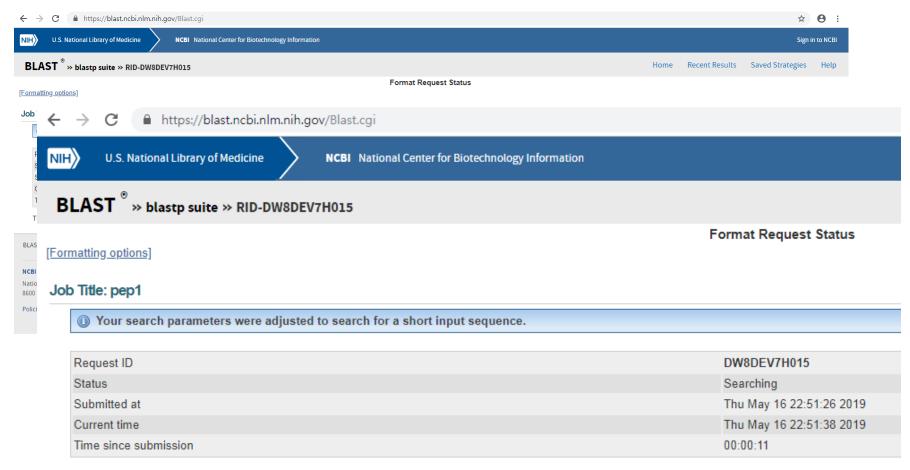




https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins







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☆ \varTheta ← → C 🔒 https://blast.ncbi.nlm.nih.gov/Blast.cgi NIH U.S. National Library of Medicine NCBI National Center for Biotechnology Information Sign in to NCBI BLAST " » blastp suite » RID-DW8G465C015 Home Recent Results Saved Strategies Help **BLAST Results** () Your search parameters were adjusted to search for a short input sequence. Edit and Resubmit Save Search Strategies Formatting options Download NEW Click here to see the new BLAST results page You How to read this page Blast report description Job title: pep1 RID <u>DW8G465C015</u> (Expires on 05-18 10:52 am) Query ID |cl|Query\_122009 Database Name nr Description pep1 Description All non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF excluding environmental samples from WGS projects Molecule type amino acid Program BLASTP 2.9.0+ ▶ Citation Query Length 7 Other reports: > Search Summary [Taxonomy reports] [Distance tree of results] [Multiple alignment] [MSA viewer] Graphic Summary Show Conserved Domains No putative conserved domains have been detected Distribution of the top 101 Blast Hits on 100 subject sequences 😣 Mouse over to see the title, click to show alignments Color key for alignment scores <40 40-50 50-80 80-200 >=200 Questions/comments



← → C 🌐 https://blast.ncbi.nlm.nih.gov/Blast.cgi

#### Descriptions

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download <u>GenPept</u> Graphics Distance tree of results Multiple alignment		
Description	Max Total Query E F Score Score Cover value Id	er. Accession
DUF4982 domain-containing protein [Solitalea canadensis]	29.5 29.5 100% 22 100	.00% <u>WP 0146799</u>
hypothetical protein DMF86_22110 (Acidobacteria bacterium)	29.5 29.5 100% 22 100	.00% <u>PYR72903.1</u>
hypothetical protein DMF85_06420 [Acidobacteria bacterium]	29.5 29.5 100% 22 100	.00% <u>PYR59962.1</u>
Biboflavin transporter (Maliponia aquimaris)	29.5 29.5 100% 22 100	.00% <u>SMX43657.1</u>
DMT family transporter (Maliponia aquimaris)	29.5 29.5 100% 22 100	.00% <u>WP 09402179</u>
9 olycoside hydrolase family 2 protein (Pedobacter sp. PACM 27299)	27.4 27.4 100% 124 85	71% <u>WP 0824615</u>
9/vcoside hydrolase [Pedobacter sp. PACM 27299]	27.4 27.4 100% 124 85	71% <u>ALL08785.1</u>
DUF4982 domain-containing protein [Solitalea longa]	27.4 27.4 100% 124 85	71% <u>WP 1037883</u>
DUF4982 domain-containing protein (Pedobacter antarcticus)	27.4 27.4 100% 124 85	71% <u>WP 0745878</u>
DUF4982 domain-containing protein [Pedobacter antarcticus]	27.4 27.4 100% 124 85	71% <u>WP 0374379</u>
DUF4982 domain-containing protein (Pedobacter antarcticus)	27.4 27.4 100% 124 85	71% <u>WP 0887759</u>
bela-galactosidase [Pedobacter antarcticus]	27.4 27.4 100% 124 85	71% <u>SDL76401.1</u>
ERG4/ERG24 ergosterol biosynthesis protein [Hesseltinella vesiculosa]	27.4 45.8 100% 125 85	71% <u>ORX49430.1</u>
MULTISPECIES: YnfA family, protein [Variovorax]	27.4 27.4 100% 131 85	71% <u>WP 1259506</u>
DNA internalization-related competence protein ComEC/Rec2 [Robbsia andropogonis]	26.9 26.9 100% 175 85	71% <u>WP 0249023</u>
DNA internalization-related competence protein ComEC/Rec2 [Robbsia andropogonis]	26.9 26.9 100% 175 85	71% <u>WP 0527383</u>
DUF4982 domain-containing protein [Mucilaginibacter sp. HYN0043]	26.9 26.9 100% 176 85	71% <u>WP 1194110</u>
gly <u>coside hydrolase family 2 protein [Mucilaginibacter oryzae]</u>	26.9 26.9 100% 176 85	71% <u>WP 1096092</u>
giv <u>coside hydrolase family 2 protein [Cytophagales bacterium B6]</u>	26.9 26.9 100% 176 85	71% <u>WP 0312677</u>
DUF4982 domain-containing protein (Mucilaginibacter sp. RS1)	26.9 26.9 100% 176 85	71% <u>WP 1149402</u>
DUF4982 domain-containing protein [Mucilaginibacter kameinonensis]	26.9 26.9 100% 176 85	71% <u>WP 1125745</u>
DUF4982 domain-containing protein [Mucilaginibacter kamelnonensis]	26.9 26.9 100% 176 85	71% <u>WP 1218090</u>
alpha-galactosidase [Amnibacterium kyongojense]	26.9 26.9 100% 176 85	71% WP 1337659
) hypothetical protein AURDEDRAFT 166132 [Auricularia subglabra TFB-10046 SS5]	26.9 26.9 100% 178 85	71% <u>EJD44945.1</u>
hypothetical protein CANARDRAFT_23100 [[Candida] arabinofermentans NRRL YB-2248]	26.9 26.9 100% 178 85	71% <u>ODV85572.1</u>
DMT family transporter [Synechococcus sp. CB0101]	26.9 26.9 100% 178 85	71% <u>WP 0103079</u>
DMT family transporter [Tropicibacter sp. LMIT003]	26.9 26.9 100% 178 85	71% <u>WP 1216316</u>
RDD family protein [Actinomycetospora chiangmaiensis]	26.9 26.9 100% <b>1</b> 79 85	71% <u>WP 0183335</u>
hypothetical protein B9255 022877 [Caenorhabditis nigoni]	26.9 26.9 100% <b>1</b> 87 85	71% PIC16193.1
hypothetical protein (Phycisphaeraceae bacterium)	26.9 26.9 100% 188 85	Questions



☆ \varTheta :

#### Alignments

#### Bownload ~ GenPept Graphics

DUF4982 domain-containing protein [Solitalea canadensis] sequence ID: <u>WP\_014679911.1</u> Length: 807 Number of Matches: 1 <u>> See 1 more title(s)</u>

### Range 1: 520 to 526 GenPegt Graphics ▼ Next Match ▲ Previous Match Score Expect Identities Positives Gaps 29.5 bits(62) 22 7/7(100%) 7/7(100%) 0/7(0%)

Query 1 VWPTAWD 7 Sbjct 520 VWPTAWD 526

#### Bownload v GenPept Graphics

hypothetical protein DMF86\_22110 [Acidobacteria bacterium] Sequence ID: <u>PYR72903.1</u> Length: 629 Number of Matches: 1

Score		Expect	Identities	Positives	Gaps
29.5	bits(62)	22	7/7(100%)	7/7(100%)	0/7(0%)
Query	1 VWPTAWD	7			
Sbjct	1 VWPTAWD VWPTAWD 565 VWPTAWD	571			

#### Bownload - GenPept Graphics

hypothetical protein DMF85\_06420 [Acidobacteria bacterium] Sequence ID: <u>PYR59962.1</u> Length: 317 Number of Matches: 1

Range	1:2	53 to 259	Ger	Pept	Graphics	
Score				Exp	ect	Identities
29.5	bits(	62)		22		7/7(100%)
Query	1	VWPTAWD	7			

Sbjct 253 VWPTAWD 259

#### Bownload - GenPept Graphics

Riboflavin transporter [Maliponia aquimaris] Sequence ID: <u>SMX43657.1</u> Length: 299 Number of Matches: 1

Range	1:	202	to	208	<u>GenPept</u>	Graphics
-------	----	-----	----	-----	----------------	----------

Score 29.5 bits(6	52)	Expect 22	Identities 7/7(100%)	Positives 7/7(100%)	Gaps 0/7(0%)	
Query 1	VWPTAWD	7				

Positives

7/7(100%)

🔻 Next Match 🔺 Previous Match

Vext Match 🔺 Previous Match

Gaps

0/7(0%)

Shict 202 VWPTAWD 208

#### Bownload v GenPept Graphics

DMT family transporter Malipania accumarial

#### ∃<u>Alignments</u>

#### Bownload v GenPept Graphics

DUF4982 domain-containing protein [Solitalea canadensis] Sequence ID: <u>WP\_014679911.1</u> Length: 807 Number of Matches: 1 <u>See 1 more title(s)</u>

Range	1: 52	20 to 526	GenPept Graph	nics	<b>V</b>	Next Match 🔺 Previou	s Match
Score 29.5		52)	Expect 22	Identities 7/7(100%)	Positives 7/7(100%)	Gaps 0/7(0%)	_
Query	1	VWPTAWD VWPTAWD	7				
Sbjct	520	ÝŴPŤÁŴĎ	526				

#### Bownload v GenPept Graphics

hypothetical protein DMF86\_22110 [Acidobacteria bacterium] Sequence ID: <u>PYR72903.1</u> Length: 629 Number of Matches: 1

Range 1: 565 to 571 GenPept Graphics Vext Match 🔺 Previou								
Score 29.5		52)	Expect 22	Identities 7/7(100%)	Positives 7/7(100%)	Gaps 0/7(0%)		
Query	1	VWPTAWD VWPTAWD	7					
Sbict	565	VWPTAWD	571					

#### Bownload v GenPept Graphics

hypothetical protein DMF85\_06420 [Acidobacteria bacterium] Sequence ID: <u>PYR59962.1</u> Length: 317 Number of Matches: 1

Score			Expect	Expect Identities Posi		Gaps
29.5 b	oits(6	2)	22	7/7(100%)	7/7(100%)	0/7(0%)
Query	1	VWPTAWD	7			
Sbict	253	VWPTAWD VWPTAWD	259			

Bownload v GenPept Graphics

Range 1: 202 to 208 GenPept Graphics

Riboflavin transporter [Maliponia aquimaris] Sequence ID: <u>SMX43657.1</u> Length: 299 Number of Matches: 1

🔻 Next Match 🔺 Previous Match

Score 29.5 bits(62)			Expect 22	Identities 7/7(100%)	Positives 7/7(100%)	Gaps 0/7(0%)
Query	1	VWPTAWD VWPTAWD	7			
Sbjct	202	ŸŴ₽ŦŔŴĎ	208			

Download v GenPept Graphics

DMT family transporter Malipopia aquimarial



Claim 1. Peptides or proteins having the following sequence:

### $X_1 ITIVFX_2 V$

wherein  $X_1$  is an uncommon amino acid, and  $X_2$  is 2-aminobutanoic acid.



#### Common amino acids

1-Letter Code	3-Letter Code	Name		
A	Ala	Alanine		
В	Asx	Aspartic acid or Asparagine		
С	Cys	Cysteine		
D	Asp	Aspartic acid	Uncomm	non amino acids
E	Glu	Glutamic acid		
F	Phe	Phenylalanine	3-Letter	
G	Gly	Glycine	Code	Name
Н	His	Histidine		
I	lle	Isoleucine	Aaa	$\alpha$ -amino acid
J	Xle	Isoleucine or Leucine	Aad	2-aminoadipic acid
K	Lys	Lysine	100	(2-aminohexanedioic acid)
L	Leu	Leucine	Aan	$\alpha$ -asparagine
M	Met	Methionine	Abu	2-aminobutanoic acid
N	Asn	Asparagine		
0	Pyl	Pyrrolysine	Aca	2-aminocapric acid
P	Pro	Proline		(2-aminodecanoic acid)
Q	Gln	Glutamine	Agn	α-glutamine
R	Arg	Arginine	Aib	α-aminoisobutyric acid
S T	Ser	Serine		(a-methylalanine)
Т	Thr	Threonine	Apm	2-aminopimelic acid
U	Scy	Selenocysteine		(2-aminoheptanedioic acid)
V	Val	Valine		
₩	Т-р			
Х	Xxx	Uncommon or Unspecified		
Y	Tyr	Tyrosine		
Z	Glx	Glutamic acid or Glutamine		

Note: The codes B, J, and Z may be used only in subsequence searches (/SQSP and /SQSFP).



Claim 1. Peptides or proteins having the following sequence:

 $X_1ITIVFX_2V$ 

wherein  $X_1$  is an uncommon amino acid, and  $X_2$  is 2-aminobutanoic acid.

Single letter codes and three letter codes can be combined in a query

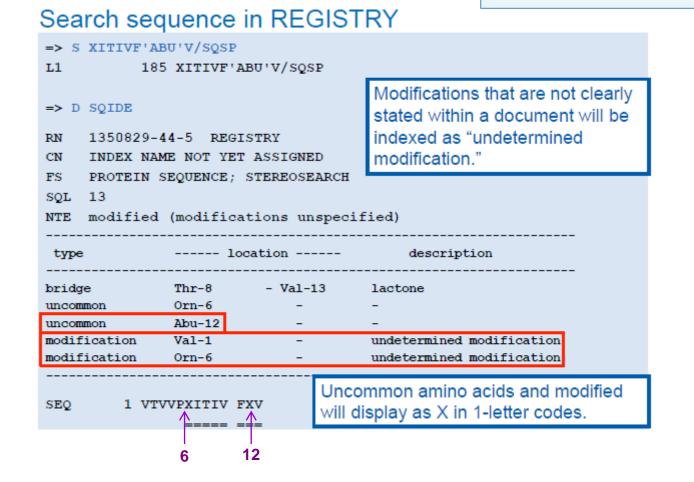
- Use the code X to search uncommon amino acids in REGISTRY
- Place single quotation marks around the threeletter code
  - Search 2-aminobutanoic acid as 'ABU'
- Claimed sequence: X<sub>1</sub>ITIVFX<sub>2</sub>V
- Query sequence: XITIVF'ABU'V/SQSP



Claim 1. Peptides or proteins having the following sequence:

### $X_1 ITIVFX_2 V$

wherein  $X_1$  is an uncommon amino acid, and  $X_2$  is 2-aminobutanoic acid.





Claim 1. A composition comprising a peptide having the following sequence

 $X_1MYX_2KT$ 

wherein X<sub>1</sub> is D-phenylalanine, and

X<sub>2</sub> is D-tryptophan.

Query FMYWKT/SQSP



### Query FMYWKT/SQSP

### D-amino acids are included in the Notes table

=> FILE REGISTRY								
=> S FMYWKT/SQSP	Search in the NTE field with the							
L1 5 FMYWKT/SQSP	(P) operator.							
=> S L1 AND ((PHE (P) D) AND (TRP (P) D))/NTE								
L2 1 L1 AND ((PHE (P) D) AND (TRP (P) D))/NTE								
=> D NTE SEQ								
L2 ANSWER 1 OF 1 REGISTRY								
NTE modified (modifications unspec	ified)							
type location description								
type location	description							
type location bridge Phe-1 - Lys-9								
bridge Phe-1 - Lys-9	covalent bridge							
bridge Phe-1 - Lys-9 stereo Phe-1 -	covalent bridge D							



Claim 1. A peptide comprising a carboxy terminal sequence

# KTDSX<sub>1</sub>VCDS

wherein X<sub>1</sub> is a gap of zero to ten residues, or functional equivalents thereof.

### Query KTDS.<mark>{0-10</mark>}VCDS^/SQ<mark>SF</mark>P

Possible family substitutions for KTDS_VCDS:							
Τ	D	S	V	С	D	S	
Α	В	А	Ι	С	В	Α	
G	Е	G	М		Е	G	
Ρ	Ν	Ρ	L		Ν	Р	
S	Q	Т			Q	Т	
	T A	T D A B	<b>T D S</b> A B A	T         D         S         V           A         B         A         I	T         D         S         V         C           A         B         A         I         C	T         D         S         V         C         D           A         B         A         I         C         B	

Subsequence Family /SQSFP

Functionally similar amino acids; may or may not be embedded



# Query KTDS.{0-10}VCDS^/SQSFP

Possible family substitutions for KTDS_VCDS:							
K	Τ	D	S	V	С	D	S
H	Α	В	Α	Ι	С	В	Α
R	G	Е	G	М		E	G
	Ρ	Ν	Ρ	L		Ν	Р
	S	Q	Т			Q	Т

Claim 1. A peptide comprising a carboxy terminal sequence

### KTDSX<sub>1</sub>VCDS

wherein  $X_1$  is a gap of zero to ten residues, or functional equivalents thereof.

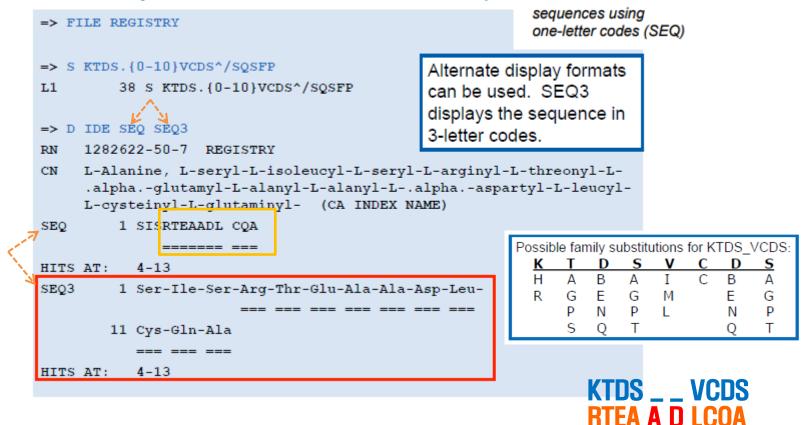
Use this symbol	То	Example			
۸	Require the base occur at	=> S ^GGAAGGG/SQSN			
	the beginning or the end of the sequence	=> S CCTC^/SQSN			

Property	Functionally Similar Amino Acids			
Neutral-Weakly Hydrophobic	Ala,Gly,Pro,Ser,Thr (A, G, P, S, T)			
Hydrophilic-Acid Amine	Asn,Asp,Gln,Glu (N, D, Q, E)			
Hydrophilic-Basic	Arg,His,Lys (R, H, K)			
Hydrophobic	lle,Met,Leu,Val (I, M, L, V)			
Hydrophobic-Aromatic	Phe,Trp,Tyr (F, W, Y)			
Cross-linking	Cys (C)			



# Query KTDS.{0-10}VCDS^/SQSFP

### Use family searches to find functional equivalents





Thank You !

