ADAPTABLE

Tutorial

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ADAPTABLE TUTORIAL – BROWSING INFORMATION ON PEPTIDE SEQUENCES

How to easily retrieve combined information from external databases and from the ADAPTABLE families database

Suppose you want to retrieve information on a Temporin peptide, whose sequence is FLPIVAKLLSGLL. Go to the "BROWSE AMPs & FAMILIES" page and start to type the name (or part of the sequence) in the dedicated box. While typing, all available entries containing the sequence will be displayed.

Screenshot 1.



Just click on the desired peptide (the fifth in *screenshot 1*) and click "Submit". You will see a table as shown in *screenshot 2*.

In the first lines of the table, we learn that your peptide is able to generate a family similar to it (family 2044) but is also a member of other families of the built-in ADAPTABLE families database (f653, f7918, f1651...) that can be also accessed clicking the provided links.

In the field "External database ID" you can see that it has entries in the DBASSP and APD databases (just click on the links to view the related pages of each database). The complete set of information is gathered in the remaining part of the table. We can see if the family generated by our peptide (family 5092) can provide more information related with properties that are still not known for our peptide.

Screenshot 2

AMP properties

Property	Value
Best representing member (father) of the following ADAPTABLE family:	f2044
Member of the following ADAPTABLE families (all_families DB):	I1 I2 I3 I4 I7 I8 I9 110 I11 I15 I16 I17 I8 I19 I26 I27 I28 I29 I30 I33 I34 I35 I36 I39 I40 I49 I54 I59 I75 I79 I80 I87 I88 I95 I99 I100 I102 I110 I112 I117 I124 I12 I3 I49 I159 I160 I178 I181 I185 I186 I186 I192 I203 I205 I250 I289 I329 I342 I369 I372 I379 I397 I399 I469 I496 I565 I569 I573 I612 I634 I653 I671 I672 I676 I678 I691 I717 I749 I758 I793 I805 I811 I882 I1145 I1186 I1651 I1706 I1919 I1954 I1962 I1999 I2028 I2044 [2047 I2132 I2166 I2197 I2293 I2322 I2340 I2370 I2377 I2433 I2446 I2487 I2489 I2508 I2522 I2534 I2550 I2598 I2609 I2614 I2634 I2639 I2650 I2678 I2694 I2728 I2730 I2739 I2749 I2768 I2621 I2865 I2877 I2905 I2925 I2938 I2951 I2953 I2954 I2964 I2977 I3998 I2609 I2614 I2634 I2639 I2650 I2678 I2694 I2728 I2730 I2739 I2749 I2768 I2621 I2865 I2877 I2905 I2925 I2938 I2951 I2953 I2954 I2964 I2977 I3998 I360 I3368 I386 I3450 I3451 I3452 I3475 I3482 I3474 I3125 I3149 I3170 I3711 I3172 I3188 I3242 I3243 I3249 I3250 I3275 I3280 I3290 I3330 I3358 I3360 I3368 I3386 I3450 I3451 I3452 I3475 I3482 I3547 I3579 I3581 I3613 I3615 I3619 I3668 I3668 I3701 I3718 I3758 I3769 I3775 I3801 I3810 I3833 I880 I3881 I3917 I3918 I3969 I4014 I4015 I4041 I4058 I4060 I4078 I4089 I4092 I4134 I4165 I4166 I4181 I4188 I4184 I4221 I4234 I4321 I433 I433 I3473 I4425 I4426 I4433 I1491 I494 I4352 I4525 I4553 I4591 I4601 I4607 I4633 I4034 I4658 I4672 I4743 I4762 I4810 I4489 I4699 I4910 I4951 I5052 I5065 I5098 I5199 I5322 I5330 I5341 I5581 I5582 I5648 I5732 I5734 I5883 I5880 I5910 I5927 I6121 I6318 I6378 I6390 I6757 I6796 I6908 I7076 I7145 I7918 I14649
External database ID	DBAASP10906 APD_AP02977
Sequence	FLPIVAKLLSGLL
Name	Temporin-PE
Source	Pelophylax esculentus; skin secretions, Pelophylax kl. esculentus, Europe
Stereo	L
C Terminus	amidation
PTM	amidation
Target	Lipid Bilayer
Antimicrobial	Yes
Antibacterial	Yes
Antigram Positive	Yes
Antigram Negative	Yes
Antifungal	Yes
Anticancer	Yes
PMID	29191658
Taxonomy	animalia; eumetazoa; edible frog, amphibians, animals; Leu-rich; ; UCLL1c
Targeted Organisms	Candida albicans NCPF 1467 (MIC=4 µM); Staphylococcus aureus NCTC 10788 (MIC=2 µM); Escherichia coli NCTC 10418 (MIC=16 µM); Staphylococcus aureus NCTC 12493 (MIC=4 µM); Enterococcus faaecalis NCTC 12697 (MIC=8 µM); Pseudomonas aeruginosa ATCC 27853 (MIC=128 µM); Human squamous lung carcinoma NCI-H157 (IC50=34.56 µM); Human glioblastoma U251-MG (IC50=25.13 µM); Human prostate adenocarcinoma PC-3 (IC50=38.56 µM); Human breast adenocarcinoma MDA-MB-4355 (IC50=33.23 µM); Staphylococcus aureus (MIC=2 µM); MRSA; E. faaecalis; Candida albicans (MIC=4 µM)
Ribosomal	Yes
Experimental	Yes
Antibiofilm	Yes, against: Candida albicans NCPF; Staphylococcus aureus NCTC; Pseudomonas aeruginosa ATCC

ADAPTABLE- Tutorial

Clicking on f2044 (first line of the table), or typing the sequence of the peptide used as reference in "BROWSE AMPs & FAMILIES" section ("Family overview" subsection), we are redirected to a page (see *screenshot 3*) describing the characteristics of the family constituted by similar peptides (peptides that can be considered as generated by our sequence, with few amino acid modifications).

The page also shows the list of members of the families (*screenshot 4*) with links to the entries of external databases. From the table in *screenshot 3* we discover that antibacterial data are available for over 78% of the members of the family and that indeed our peptide might display antimicrobial activity. We also learn that most of the similar peptides have a wide range of activities including antifungal but they also can be toxic and hemolytic. The button "Show frequency of aminoacids" allows you to review the members of the family highlighting the frequencies of their aminoacids to make motifs more easily viewable.

In conclusion, the ADAPTABLE family database can suggest properties that have never been tested for some specific entries.

Screenshot3.

Family 2044 properties

Family of peptides similar to FLPIVAKLLSGLL

Members of the family

Property		Value (% of members that	could have it depending	on available data per memb	per)	
Activity:						
antimicrobial: (da	ata available for the 100 % of members)	antimicrobial (100.0-100.0	%)			
antibacterial: (da	ta available for the 77.91 % of members)	antibacterial (77.9-100.0 %)			
antigram pos: (d	ata available for the E7.5 % of members)	antigram pos (57 5-100 0	, 4)			
ancigrani_pos. (d		ancigram_pos (57.5-100.0	•)			
antigram_neg: (d	ata available for the 74.58 % of members)	antigram_neg (74.6-100.0	%)			
biofilm: (data ava	ilable for the 55.41 % of members)	staphylococcus (54.2-97.7 ' (21.2-38.3 %); aeruginosa (12.8 %); bm11 (6.7-12.0 % %); nctc (4.2- 7.5 %); enter %)	%); biofilm (55.4-100.0 % 20.8-37.6 %); klebsiella (; cgmcc (4.2- 7.5 %); cow obacter (4.2- 7.5 %); cloa); aureus (53.8-97.0 %); atcc 12.1-21.8 %); pneumoniae (* van (5.4- 9.8 %); 08040724 (acae (4.2- 7.5 %); atcc2592 ((27.9-50.4 %); candida (37.5-67.7 %); 11.7-21.1 %); bacillus (9.6-17.3 %); me (4.6-8.3 %); atcc2002 (4.6-8.3 %); en (4.2-7.5 %); streptococcus (2.9-5.3 %	albicans (37.5-67.7%); pseudomonas egaterium (9.6-17.3%); epidermidis (7.1- terococcus (4.6-8.3%); faecium (4.6-8.3 6); pyogenes (2.9-5.3%); a170 (2.9-5.3
antifungal: (data	available for the 43.75 % of members)	antifungal (43.8-100.0 %)				
antiyeast: (data a	wailable for the 12.08 % of members)	antiyeast (12.1-100.0 %)				
antiviral: (data av	vailable for the 18.33 % of members)	antiviral (18.3-100.0 %)				
antiprotozoal: (d	ata available for the 2.08 % of members)	antiprotozoal (2.1-100.0 %)			
antiparasitic: (da)	ta available for the 2.08 % of members)	antiparasitic (2.1-100.0.%)	,			
anticancer: (data	available for the 5.83 % of members)	anticancer (5.8-100.0 %)				
anticancer_activi members)	ty (μM): (data available for the 0.83 % of	0.00_µm (0.4-50.0 %); 64.3 %)	7_μm (0.4-50.0 %); 2.48 __	_μm (0.4-50.0 %); 2.15_μm ((0.4-50.0 %); 2.20_μm (0.4-50.0 %); 1.	.88_μm (0.4-50.0 %); 1.86_μm (0.4-50.0
anticancer_activi members)	ty_test: (data available for the 0.83 % of	ic50 (0.8-100.0 %); mhc (0	4-50.0 %)			
- cancer_cell_line	: (data available for the 0.83 % of members)	u-937 (0.4-50.0 %); u-943 (0.4-50.0 %); a-549 (0.4-5	50.0 %); 293 (0.4-50.0 %); he	ep3b (0.4-50.0 %); mcf-7 (0.4-50.0 %)	
- cancer_tissue: (data available for the 0.83 % of members)	blood (0.4-50.0 %); lung (0	0.4-50.0 %); renal (0.4-50	.0 %); liver (0.4-50.0 %); bre	east (0.4-50.0 %)	
- cancer_type: (da	ata available for the 0.83 % of members)	ic50 (0.8-100.0 %); lympho 50.0 %); 15251 (0.4-50.0 %	ma (0.4-50.0 %); renal ((); 20231 (0.4-50.0 %); liv	0.4-50.0 %); mhc (0.4-50.0 % er (0.4-50.0 %); 88058 (0.4	6); 000357628 (0.4-50.0 %); 3731 (0.4 -50.0 %); breast (0.4-50.0 %); 86909 (4-50.0 %); lung (0.4-50.0 %); 48573 (0.4- 0.4-50.0 %)
Toxicity:						
toxic: (data availa	ble for the 21.25 % of members)	toxic (21.2-100.0 %)				
cytotoxic: (data a	vailable for the 7.5 % of members)	cytotoxic (7.5-100.0 %)				
hemolytic: (data	available for the 15.83 % of members)	hemolytic (15.8-100.0 %)				
hemolytic activit	v (uM): (data available for the 15 % of	40 µm (5.8-38.9 %): 1.25 µ	ım (5.8-38.9 %): 300 um	(1.7-11.1 %): 5 µm (1.2- 8.	.3 %): 120 µm (0.8- 5.6 %): 0.14 µm (0.4- 2.8 %): 30 µm (0.8- 5.6 %): 75 µm (
members)	Family of peptides similar to FLPIVAKLLSC • Members of the family	0.4- 2.8 %); 95 µm (0.4- 2.8 iLL	ι %); 210 μm (0.4- 2.8 %); 8 μm (0.4- 2.8 %); 14 μm	(0.4-2.8%); 225 µm (0.4-2.8%); 50	µm (0.4-2.8%); 0.05 µm (0.4-2.8%);
	Property	Value (% of members that could ha	Members			
	Activity:		Family 2044.			
	antimicrobial: (data available for the 100 % of members)	antimicrobial (100.0-100.0 %)	Family 2044 has 240 eleme	ents. The best alignment is fou	und with: FLPIVAKLLSGLL	
	antibacterial: (data available for the 77.91 % of members)	antibacterial (77.9-100.0 %)	Colouring by frequency	49.10 5 . 29.10 5 .		
	antigram_pos: (data available for the 57.5 % of members)	antigram_pos (57.5-100.0 %)	1	EI BTUAVI I COLL	father	
	antigram_neg: (data available for the 55.41 % of members) biofilm: (data available for the 55.41 % of members)	antigram_neg (74.6-100.0 %) staphylococcus (54.2-97.7 %); biofil (21.2-38.3 %); aeruginosa (20.8-37.4 12.8 %); bm11 (6.7-12.0 %); cgmcc %); nctc (4.2-7.5 %); enterobacter	2 3 4 5 6	FLPTVAKLLSGLLGRKKRRQRRR FLPTVGKLLSGLL FLPTTAKVLSGLL FLPTTAKLLGGLL FLPTTAKLLSGLL	; Similarity to father=100.0% ; Similarity to father=93.2% ; Similarity to father=91.8% ; Similarity to father=91.8% ; Similarity to father=90.4%	
	antifungal: (data available for the 43.75 % of members)	antifungal (43.8-100.0 %)	8	FLPILGKLLSGLL	; Similarity to father=87.7% ; Similarity to father=87.7%	
	antiyeast: (data available for the 12.08 % of members)	antiyeast (12.1-100.0 %)	10	FLPIVGRLISGLL	; Similarity to father=86.3%	
	antiviral: (data available for the 18.33 % of members)	antiviral (18.3-100.0 %)	12	FLPIVINLLSGLL	; Similarity to father=86.3%	
	antiprotozoal: (data available for the 2.08 % of members)	antiprotozoal (2.1-100.0%)	13	FLPIIGKLLSGIL	; Similarity to father=86.3% ; Similarity to father=86.3%	
	antiparasitic: (data available for the 2.08 % of members)	antiparasitic (2.1-100.0 %)	15 16	FLPTIGULISGIL	; Similarity to father=84.9% ; Similarity to father=84.9% ; Similarity to father=84.9%	
	anticancer: (data available for the 5.83 % of members)	anticancer (5.8-100.0 %)	18	FLPMLAKLLSGFL	; Similarity to father=84.9% ; Similarity to father=83.6%	
	anticancer_activity (pm), (baca available for the 0.83 % of anticancer_activity_test: (data available for the 0.83 % of	ic50 (0.8-100.0 %); mhc (0.4-50.0 %	20. 21. 22.		; Similarity to father=83.6% ; Similarity to father=83.6% ; Similarity to father=82.2%	
	members)		23 24	FFPIVGKLLSGLF-	; Similarity to Tather=82.2% ; Similarity to father=82.2%	
	- cancer_cell_line: (data available for the 0.83 % of members)	u-937 (0.4-50.0 %); u-943 (0.4-50.0	25 26	FLPIVGKLLSGLSGLS	; Similarity to father=82.2% ; Similarity to father=82.2%	
	- cancer_type: (data available for the 0.83 % of members)	ic50 (0.8-100.0 %); lymphoma (0.4-		Close		
	Toxicity	50.0 %); 15251 (0.4-50.0 %); 20231				

Screenshot 4.

Members of family 2044

Properties of the family

Num	Sequence (Click each for more information)	D
1	FLPIVAKLLSGLL	DBAASP10906 APD_AP02977
2	FLPIVAKLLSGLLGRKKRRQRRR	DBAASP10908
3	FLPIVGKLLSGLL	satpdb21344 satpdb21479 hemo1130 DRAMP01342 DRAMP01830 CAMPSQ23 CAMPSQ444 CAMPSQ998 CAMPSQ4071 DBAASP1127 APD_AP00112 ANTISTAPHYStaph_P_13 DADP_2659 DADP_P82848 LAMPL01A000025 ADAM_1230 YADAMP317 uniprotP82848
4	FLPIIAKVLSGLL	satpdb15096 satpdb16614 DRAMP01810 CAMPSQ505 DBAASP3535 APD_AP00866 DADP_P84116 LAMPL01A000541 ADAM_1204 YADAMP307 uniprotP84116
5	FLPIIAKLLGGLL	satpdb27817 DRAMP03682 CAMPSQ9 DBAASP1565 InverPep577 LAMPL01A000011 ADAM_1203 YADAMP306 uniprotP0C1M1
6	FLPIIGKLLSGLL	satpdb22159 satpdb28122 hemo1131 DRAMP01816 CAMPSQ999 CAMPSQ1057 DBAASP1129 DBAASP5609 APD_AP00595 APD_AP02368 InverPep510 InverPep581 ANTISTAPHYStaphP_14 <u>DADP_2660 LAMPL01A003167 ADAM_1210 YADAMP308</u>
7	FLPILGKLLSGLL	satpdb16682 satpdb28993 DRAMP01724 DBAASP6006 LAMPL10D5MTH50 ADAM_1221 YADAMP313
8	FLPIVGKLLSGLF	satpdb11723 satpdb24155 DRAMP01727 CAMPSQ2413 DBAASP1883 APD_AP02056 ADAM_1229 YADAMP316
9	FFPIVGKLLSGLL	satpdb16610 satpdb23945 DRAMP01736 CAMPSQ3637 CAMPSQ4081 DBAASP1864 APD_AP01936 ANTISTAPHYStaphP_303 LAMPL02A001936 ADAM_0968 YADAMP282
10	FLPIVGRLISGLL	satpdb10939 satpdb13725 hemo2483 DRAMP01749 CAMPSQ2921 DBAASP5287 APD_AP00864 DADP_2703 LAMPL02A000864 ADAM_1235 YADAMP318
11	FLPIVTNLLSGLLGK	DRAMP01776
12	FLPIVTNLLSGLL	satpdb13503 satpdb22050 CAMPSQ1004 APD_AP00899 DADP_2690 LAMPL01A003172 ADAM_1238 YADAMP319
13	FLPFVGKLLSGLL	satpdb11702 satpdb17794 DBAASP8038 APD_AP02467 ADAM_1178
14	FLPIIGKLLSGIL	satpdb29086 LAMPL10Q2PGA80 ADAM_1209
15	FLPIIGQLLSGLL	satpdb10701 satpdb21465 hemo1618 DRAMP01760 CAMPSQ2922 DBAASP4222 APD_AP00865 LAMPL02A000865 ADAM_1211 YADAMP309
16	FLPMLAKLLSGFLGK	DRAMP01166 CAMPSQ1319 LAMPL01A002468
17	FLPIVGKLLSGLTGLL	CAMPSQ4078
18	FLPMLAKLLSGFL	satpdb16624 ADAM_1304 Show frequency of aminoacids

ADAPTABLE TUTORIAL - CASE EXAMPLE n.1

Designing new peptides active towards a specific organism and highlighting motifs

The case of Shigella

Suppose you want to design a new antimicrobial peptide active against *Shigella* sp. You want to be inspired by the most active entries in the database, so you look for peptides which are active at concentrations lower than 1 μ M. Go to the "FAMILY GENERATOR" section on the left bar. For the Calculation label, you can choose a name such as "peptides_against_shigella". As you do not want to restrict the generation of families only taking into account "Aurein" peptides (provided as an example), remove all from "Peptide name" field. Go to "Target Organism" and write the name of the organism (or part of it) and the maximum μ M activity value just like in *screenshot 1.1.* You will also need to choose the username ("user" in the example) to be able to access your experiments:

Screenshot 1.1. Family generator

Note: To know more about e	ach field simply hover your mouse over it. in so	me fields the following options are available: y="yes; n="no"; or i=ignored.
Calculation label (mandatory)	peptides_against_shigella	Sample
Username (mandatory)	user	Email notification (mandatory) example@example.org
Append User peptides	1-letter code aminoacid sequence	20 🗠 Create the family of a specific peptide
stional selection criteria (a	run with unspecified criteria might require lo	ong calculation times):
ptide name ^{(empty} if indiferent)	e.g. Aurein	2
quence pattern	e.g. KKV or positional like GL-DIVKKVVGA-GSL	2
rget Organism	shigella	Activity (µM) 1 Activity test i
dvanced (+) Expand Me	le	
Align method: O Simple	ODSSP OSubstitution matrix Blosum45	Minimum % of similarity 40 Sthreshold percentage to group families 75
∽ Simplify aminoacids: 🔘	y 💿 n 🛛 Experimentally validated: 💿 i	y On Include only peptides with data about i

You might want to see a detailed analysis so you can also set the options "Generate additional graphical analysis" and "Run SeqLogo" at the end of the page to yes ("y").

Click on "Submit" at the end of the page. After the calculation is finished, a page summarizing the properties of each generated family will be shown (always accessible from "FAMILY ANALYZER" section). You can also go to the "DOWNLOAD RESULTS" section on the left bar to download all the generated files. In both case you will be asked to type your username:

Screenshot 1.2.

ADAPTABLE

Results

Download your calculation. Please note that experiments will be removed after 6 months.

Note: Here you can find more information on how to extract generated files, in case they don't unpack automatically.

Name Last modified Size Description

peptides_against_shigella/ 2018-11-14 14:49

Open the directory of your calculation "peptides_against_shigella". Here is what you see:

Screenshot 1.3.

				ADAPTABLE	
Results					
ownload your	calculation. Pleas	e note that experim	ents w	ill be removed after 6 months.	
ote: <u>Here</u> you car	n find more informati	ion on how to extract gei	nerated	files, in case they don't unpack automatically.	
	<u>Name</u>	Last modified	<u>Size</u>	Description	
Families/		2018-11-14 14:49	-		
Logos/		2018-11-14 14:49	-		
Graphics/		2018-11-14 14:49	-		
peptides_a	g <u>ainst_shigella-full.t</u>	ar.xz 2018-11-14 14:49	5.4M C	ompressed package to download all the generated output	

You will see a compressed package to download all the generated files, additionally, in the subfolder "Families" you will find the full information for each family (as a whole html file showing different properties and statistics), in the subfolder "Logos" you will find the logos generated by Weblogo and in "Graphics" you will see the generated additional graphical analysis files.

We will go to "FAMILY ANALYZER" section, that includes tools that will allow to parse the generated information in a more detailed way. It will ask for the username and, after that, the following section will be shown to allow choosing the desired user experiment:

Screenshot 1.4.



After going into our experiment, we can see a page that will provide us with some information (Screenshot 1.5).

The run has generated a total of 8 families with more than one member (you can see the rest clicking on "Expand Me to show families with 1 member". Regarding the global properties of the families, you can review a summary and the full html output for each.

It is also shown that this run has generated Logos and Graphical plots that can be accessed directly from here too.

Finally, it's shown that 2 of the 21 generated families are similar among them for the case the user wants to take this into account when reviewing the features of each family.

Going into the "Detailed analysis of family members" we will be able to select the families to analyze for getting more detailed information.

Screenshot 1.5.



Based on sequence alignment, ADAPTABLE has classified all peptides of its database in groups of sequence-related peptides (families), each represented by a single sequence chosen among the members of the family (called the father). The father has the highest resemblance to all other peptides. In this terminology, each family is composed by a father and multiple sons (the other members of the family). This classification is purely based on sequence; it is therefore independent on the activity or other properties.

We will choose to review the members of family 1 and get them shown according to the "Aminoacid frequencies", "Polarity" and type of residue.

Among the 7 members, the first sequence is the one which has been used as reference to build the family, and is therefore called the "father".

Judging by the frequency of occurrence, it seems that some amino acids (in red) are highly repeated in the same position and therefore potentially important for the antimicrobial mechanism of action. The statistical analysis performed at the end of the calculation will help us to better understand this information (see below).

Coloring the sequence alignment by residue type or polarity can sometime reveal other features (as the presence of amphipathic helix).

Screenshot 1.6.

↑	
Family 1:	
Family I has 7 elements. The best alignment is found with: takakakayoudoodakakaka	
Colouring by frequency	
100 % ; 99-80 % ; 79-50 % ; 49-30 % ; 29-10 % ;	
1 EVENUEVESEVENUEVE father	
2. CVKVRVKVGSGVKVRVKVC ; Similarity to father=96.4%	
3. CVKVQVKVGSGVKVQVKVC ; Similarity to father=92.9%	
4. CVKVSVKVGSGVKVSVKVC ; Similarity to father=89.3%	
5. EAKAKAKAGSEAKAKAKAC ; Similarity to father=64.3%	
0. GENERAL STATE STATE STATES IN THE STATES OF A STATE	
Family 1:	
Colouring by polarity	
Non polar ; Essentially non polar ; Polar ; Charged ; Cysteine.	
/ $/ / / / / / / / / / / / / / / / / /$	
1. CVKVKVVKVGSGVKVKVKVC ; father	
CVKVRVKVGSGVKVRVKVC ; Similarity to father=96.4%	
3. CVKVQVKVGS6VKVQVKVC; Similarity to father=92.9%	
4. CVXVSVVVGSCVVVSVVCC; Similarity to Tather=89.3%	
6. CHKEKKEGSGEKEKKEC : Similarity to father=64.3%	
7. CWKWKWKWGSGWKWKWKWC ; Similarity to father=42.9%	
Family 1:	
Colouring by residue type	
Hydrophobic, Aromatic, Fotar, Fositive, Regative, Gytthe, Frotine, Cysteine.	
1. CVKVKVKVGSGVKVKVKVC ; father	
CVKVRVKVGSGVKVRVKVC ; Similarity to father=96.4%	
3. CVKVQVKVGSGVKVQVKVC; Similarity to father=92.9%	
4. CVNVSVNVSSVNVC ; SIMILAFILY LO TALBERES9.3%	
6. CFKFKFKFGSGFKFKFKFC; Similarity to father=64.3%	
7. CWKWKWKWGSGWKWKWKWC ; Similarity to father=42.9%	
	Sequences

In this case, the interpretation is not straightforward. Polar and nonpolar residues do not seem to alternate with the frequency of a helix (3.5 residues per turn). The secondary structure prediction, along with the DSSP structure ("Structure" option) confirms that a helical structure, commonly found in antimicrobial peptides, is not expected in this case:

Screenshot 1.7.

Family 1: Secondary structure prediction Alpha-helix ; Beta-strand; Turn ;
1; father
2; Similarity to father=96.4%
3; Similarity to father=92.9%
4; Similarity to father=89.3%
5. (Interaction); Similarity to father=64.3%
6; Similarity to father=64.3%
7; Similarity to father=42.9%
DSSP structure Alpha-helix ; Alpha-310 ; Alpha-pi ; Beta-bridge; Beta-strand; Turn ; Bend
1; father
2
3
4; Similarity to father=89.3%
5>····>·····; Similarity to father=64.3%
6
7; Similarity to father=42.9%

We can also see a summary of all the information available for the family clicking on "Available data per sequence":

Screenshot 1.8.

Family	1:																																																								
(1 in	red	, fo	or u	ser	sel	ecte	d pr	oper	rtie	s (I	bool	ean	or	not), (orar	ige	for	uns	ele	cted	i bo	ole	an p	prop	ert	ies	, (s	sub-	μM) ·	- (µM	1) - (sub-	- (Mm	(mM)) - (v	ery	wea	k) 1	or	act:	ivit	y va	lue	s, g		n fo	r ur	isel	ect	d no	on-b	oole	an t	info	rmat	ion)
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												t	t n	n				t	t	i	r i	i n					с	е	t	i			- i	t			- r	u	У	u	p i	ı p	а	0		1.1	n .		ι	i	_	×	ι.,			t	
							N C					i :	i t	t	а			i	i	p	y 1	s	a				а	r.	i .	а			с	i	R		u	n	р	g	e	ιl	n	ι					-	v	v	P	ŧ	v	s	i	÷
											s	m I	b i	. i	n	а	а	р	P	ι	p e	e e	n	а	с		n	-	v	n	•	: h	-	v	B	c	m	0	е	_ 1	n (0	t	i			s .		0	i	i ı	r e	£	i	0	v	÷
		s					t t				У	i	a g	g	t	n	n	r	а	a	a i	L c	t	n	е		с	а	i	g)	/ e	а	i	C (e	-	m	r.	d	e i	r 0	i	f		1	¢ .	t	r.	t	r i	i r	t	r	ι,	a i	
		е					e e	•			n	c	c r	ŗ	i	t	t	0	r	S	n s	s t	i	t	ι		е	с	t	i	1	t m	с	t	_ 1	l h	I S	0	t	e	t,	d	0	е			r .	а	g	У	a t	i c	. b	u	u (c t	
		q	s	F		s	r r		с	t	t	r (t a	a	f	i	i	t	а	m	0 1	ı i	c	i	ι	t	r.	t	У	0	•	0	t	У	s 1	ι٥	e	d	е	ι	r I	<u> </u>	x	r.			a	x	а	_	ια	o m	/ i	s	b 1	t y	
		u	0	а		t	mm	1	У	а	h	0	еп	m	u	У	۷	0	s	0	s r	n c	а	t	-	i	-	i	_ `	g 1	t t	t ι	i	-	• _	_ r	n	u	n	i	a	b b	i	а			c .	0	n	v	_ \$	s e	0	-	i :	i _	
		e r	n u	m	g	e	i i		с	r	e	b	r _	-	n	e	i	z	i	d	0 8	a i	n	u	ι	s	t	v	t	e	0 0	э у	v	t	u e	c m	I S	ι	s	v	t i	n r	d	t	D	1	t P	n	i	i	t d	o n	f f	n	1	v t	
		n a	n r	i	е	r	n n	P	ι	g	t	i :	i p	n	g	а	r	0	t	i	m r	1 d	c	m	i	s	У	i	e	n)	к)	(t	i	е	r e	e o	• i	а	i	e	i :	La	а	i	S	p i	и М	0	s	r	en	n t	: i	а	i :	i e	
	I	c n	n c	ι	n	е	u u	т	i	е	i	a	a o	e	а	s	а	а	i	a	i i	i a	e	0	n	u	р	t	s	i 1	i i	l i	t	s	c 1	l n	i n	n	v	r i	n I	ı i	n	v	S	d	r I	m	m	а	s a	a a	ιι,	m	t	t s	1
	D	e e	e e	У	е	0	s s	M	с	t	с	ι	ls	g	ι	t	ι	ι	с	ι	c a	1	r	r	е	е	е	У	t	c (c (c c	У	t	e 1	l e	g	t	е	У	g (j n	t	е	P	b (e D	У	s	ι	t 1	ίl	m	е	y 3	y t	
	1	2 3	3 4	5	6	7	8 9	10	11	12	13 1	4 1	5 16	17	18	19	20	21	22 2	3 2	4 25	5 26	27	28	29	30	31	32 3	33 3	4 35	5 36	5 37	38	39 4	0 43	1 42	43	44	45 4	6 4	7 4	3 49	50	51	52 5	3 5	4 55	56	57	58 !	9 66	9 61	62	63 (64 6	5 66	
1.				0	Θ				1		1	1	1 6	1	0	0	θ	Θ	Θ	0	0 (0	0	0	0	0	θ	θ	0	0 1	1 6	9 1	1		1 (9 0	0	Θ	Θ	0	0 (0	0	Θ		1 (9 1	0	1	0	0 0	9 1		0	S I	1 1	
2.				0	Θ				1		1	1	1 6	1	0	θ	θ	θ	θ	0	0 0	0	θ	0	θ	0	θ	θ	0	0	1 6	9 1	1		1 (9 0	0	θ	Θ	0	0 (0	Θ	θ		1 (9 1	Θ	1	0	0 0	9 1		0	S	1 1	
з.				0	Θ				1		1	1	1 6	1	0	0	θ	Θ	θ	0	0 0	9 0	Θ	0	θ	0	θ	θ	Θ	0 1	1 6	9 1	- 1		1 (9 0	0	Θ	Θ	0	0 (9 0	Θ	Θ		1 (9 1	Θ	1	θ	0 0	9 1		0	S	1 1	
4.				Θ	Θ				1		1	1	1 6	1	θ	0	θ	θ	θ	0	0 0	9 0	θ	Θ	θ	0	θ	θ	0	0 1	1 6) 1	1		1 (9 0	0	θ	Θ	0	0 (0	θ	θ		1 (9 1	Θ	1	θ	0 6	9 1		0	S	1 1	
5.				θ	Θ				1		1	1	1 6	1	θ	θ	θ	θ	θ	0	0 0	0	θ	Θ	θ	θ	θ	θ	θ	0 1	1 6) 1	1		1 (9 0	0	θ	Θ	θ	0 (0	θ	θ		1 (9 1	Θ	1	θ	0 6	9 1		0	S	1 1	
6.				Θ	Θ				1		1	1	1 6	1	0	0	θ	θ	θ	0	0 0	0	θ	0	θ	0	θ	θ	0	0 1	1 6) 1	1		1 (9 0	0	θ	Θ	0	0 (0	0	θ		1 (9 1	θ	1	0	0 0	9 1		0	S	1 1	
7.				θ	Θ				1		1	1 :	1 6	1	θ	θ	θ	θ	θ	0	0 0	0	θ	0	θ	θ	θ	θ	θ	0 1	1 6	9 1	1		1 (9 0	0	θ	Θ	0	0 (0	θ	θ		1 (9 1	Θ	1	θ	0 6	9 1		0	S	1 1	

In this table, each line corresponds to one member of the family and each column corresponds to a property ("1" indicates presence of the property).

But a more readable output showing several statistics and tables can be obtained in the generated HTMLs with the full output as accessed in "Global properties" section:

Screenshot 1.8.

Full output: <u>f1 f2 f3 f4 f5 f6 f7 f8</u>

C Logos were generated for this experiment, click here to review them

In this case, we will click into f1 output and we will see the whole output divided in multiple sections that can be navigated using its left bar to jump from one section to other.

Screenshot 1.9.



Family members

Family members coloured by frequency:

Legend: 100 % 99-80 % 79-50 % 49-30 % 29-10 %

1.	CV <mark>K</mark> VK	<mark>KVGSGV</mark> K	VKV <mark>K</mark> VC	Father
2.	C V <mark>K</mark> V R	<mark>/kv</mark> gsgvk	vrv <mark>kv</mark> c	96.4% similarity to father
з.	c <mark>vkv</mark> q	<mark>/kv</mark> gsgvk	vqv <mark>kv</mark> c	92.9% similarity to father
4.	cv <mark>k</mark> vs	<mark>/kv</mark> gsgvk	vsv <mark>kvc</mark>	89.3% similarity to father
5.	CA <mark>K</mark> AK	A <mark>KA</mark> GSGAK	A <mark>K</mark> A <mark>K</mark> AC	64.3% similarity to father
6.	C F <mark>K</mark> F K	F <mark>K F G S G</mark> F <mark>K</mark>	F K F <mark>K</mark> F <mark>C</mark>	64.3% similarity to father
7.	cw <mark>k</mark> wk	N <mark>KWGSG</mark> WK	w <mark>ĸw</mark> ĸwc	42.9% similarity to father

Family members coloured by residue type:

Legend: Hydrophobic Aromatic Polar Positive Negative Glycine Proline Cysteine

```
1. CVKVKVKVGSGVKVKVKVCFather2. CVKVRVKVGSGVKVRVKVC96.4% similarity to father3. CVKVQVKVGSGVKVQVKVC92.9% similarity to father4. CVKVSVKVGSGVKVSVKVC89.3% similarity to father5. CAKAKAKAGSGAKAKAKAC64.3% similarity to father6. CFKFKFKFGSGFKFKFKFC64.3% similarity to father7. CWKWKWKWGSGWKWKWKWC42.9% similarity to father
```

Family members coloured by polarity:

Legend: Non polar Essentially non polar Polar Charged Cysteine

1. CVKVKVKVGSGVKVKVKVC	Father
2. CVKVRVKVGSGVKVRVKVC	96.4% similarity to father
3. CVKVQVKVGSGVKVQVKVC	92.9% similarity to father
4. CVKVSVKVGSGVKVSVKVC	89.3% similarity to father
5. CAKAKAKAGSGAKAKAKAC	64.3% similarity to father
6. CFKFKFKFGSGFKFKFKFC	64.3% similarity to father
7. CWKWKWKWGSGWKWKWKWC	42.9% similarity to father

Secondary structure prediction for family members:

Legend:



Secondary structure from DSSP for family members:

Legend:



A table is also plotted here showing a summary of all the relevant properties:

Screenshot 1.10.

Detailed properties



We immediately see that these peptides are mostly cyclic, synthetic, actives against gram-negative organisms. We also see that this family tends to be hemolytic and toxic (column 38). Therefore, chemical modifications should be performed to reduce their toxicity.

After that table, some more details are listed:

Screenshot 1.11.



An additional graphical statistical analysis on the amino acid composition and location helps clarifying the properties of this family. The analysis can be found in the subdirectory Graphics in the "DOWNLOAD RESULTS" section on the left bar or simply through the link in "Global properties" section of "Family Analyzer".

Screenshot 1.12.



We find 6 different types of graphs. In graph 1, we can see that the members of family 1 contain on average 5 lysine residues (K) and 4.5 valines (V) but also 2 cysteines (C) per peptide. Hydrophobic and positively charged amino acids dominate the sequence and amino acid side chains tend to be of reduced size.

Screenshot 1.13.



In graph 2, a 3D scheme (its 2D version is in graph 3) shows that the position of valines (green) and of lysines (cyan) along the sequence does not seem to matter. On the contrary, Cysteine (magenta) residues tend to be at the termini. This might suggest the presence of a head to tail disulphide bond (in the "data table" previously examined the peptides are actually reported to be cyclic).



To confirm this hypothesis, we can check the probability to find a cysteine in last position when a Cysteine is present in the first position. This information can be retrieved from graph5 of family 1:





In this graph, different amino acid types have different color code.

When a Cysteine is present in position 1 the probability to find another Cysteine (blue line) 18 amino acids (aa) apart, is 100%. This might sound as trivial information in the present case (these are synthetic peptides) but it might be very useful for peptides whose structure have not been studied yet.

Graph 4 analyzes motifs (groups of amino acids in well-defined relative positions within the sequence), an important information often shedding light on the mechanism of action. Motifs are found by calculating the position-independent probability to find a residue type at a certain distance from a reference residue type (e.g. if you have an Alanine in a peptide of the family, you can calculate which is the probability to find each of the 20 amino acid types n positions apart).

In our case, when C is present, graph 4 states that we have a 100 % probability to find K (green) 2, 6, 12 and 16 positions apart, G (cyan) 8, 10 position apart, S 9 position apart and C 18 positions apart. This means that C-K---K-GSG-K---K-C is a common motif for family 1.





Among this family of peptides highly active against *Shigella* sp., we have highlighted the "father", which is the peptide which better represents the sequences of all other peptides of the family. Can we design a new sequence better resuming the properties of family 1, including the father? Creating a peptide by concatenating the most frequent residue per position is a tempting but inefficient way to solve this problem. The resulting peptide would be a chimera where the synergic action of specific amino acid types in optimal relative positions would be likely lost. However, starting from the most abundant amino acid in each position, we can fill all other positions with the most frequent amino acid found in related peptides. As shown by the logo image, the most abundant amino acid in position 8 is Valine (you can find the logo image in the logos directory of "DOWNLOAD RESULTS" section). This feature of ADAPTABLE is powered by WebLogo/SeqLogo software; http://weblogo.threeplusone.com/ , Department of Plant and Microbial Biology, University of California, Berkeley):





Analyzing all peptides with Valine in position 8 (see graph 6), we can find that the most frequent amino acid in position 1 is C, in position 2 is V and so on. As shown in the figure, we can do the same starting from position 7 or 9 or 10.



The optimal peptide can be found searching for "Best representative peptide".

Screenshot 1.19.

You can also specify more parameters simply clicking on the box to expand it and show them.

Screenshot 1.20.

Specific parameters: (¹⁾ Collapse Me Dispectific parameters: (¹⁾

For example, selecting "Aminoacid frequencies", "Best representing peptide" and "PMID" (the reference to the publication on the selected peptides) you can visualize all this information in one page:

Screenshot 1.21.

Family 1: Family 1 has 7 elements. The best alignment is found with: CVKVKVKVGSGVKVKVKVC	
Calouring by frequency 100 * ; 99-50 * ; 79-50 * ; 49-30 * ; 29-10 * ;	
 CVRVKVRVGESGVRVKVRVE ; father CVRVKVRVGESGVRVKVRVE ; Similarity to father=96.4% CVRVKVRSGESGVRVKVRVE ; Similarity to father=99.3% CVRVSVRVGESGVRVVFVFVF Similarity to father=64.3% CVRVSVRVESGESGRVFVFVF Similarity to father=64.3% CVRVSVRVGESGRVFVFVF Similarity to father=42.9% 	
<pre>Family 1: Best representing sequence is sequence: CVKVKVKVGGGVKVKVKVC (>satpdb28089;hemo2373;DBAASP347;)</pre>	
Secondary structure prediction Alpha-helix ; Beta-strand; Turn ;	
Helix HUMBHRH HUMBHRHH Strand BBBBBBBB - BBBBBBBBB Turn Consensus	
DSSP prediction Alpha-bellx ; Alpha-ji ; Beta-bridge; Beta-strand; Turn ; Bend ;	
<pre>Family 1: PMID: (data available for the 100 % of members) summary: 15328096 (100.0-100.0 %);</pre>	
15328096 (100.0-100.0 %); 1. 15328096;; 3. 15328096;; 4. 15328096;; 5. 15328096;; 6. 15328096;; 7. 15328096;;	
—	Sequences

Using the PMID 15328096 we can check the publication related to the family of peptides and have a confirmation of what we have "discovered". You can also visit the page for getting the details of each family. The template peptide for this family of synthesized peptides, which was not present in the database, coincides with our optimal sequence. The family do has a disulphide bridge connecting the N and C terminus. No helical structure is present.



This image was taken from Frecer, V., Ho, B. & Ding, J. L. De novo design of potent antimicrobial peptides. Antimicrob. Agents Chemother. 48, 3349–3357 (2004).

ADAPTABLE TUTORIAL - CASE EXAMPLE n.2

Predicting the antimicrobial or anticancer activity of a generic peptide sequence

The case of random new peptides

Suppose to have rationally designed an antimicrobial or anticancer peptide targeting microbial or cancerous cell membranes. Your sequence is entirely new and you want to predict its activity based on experimental data on similar peptides. Just for the sake of demonstrating the potentiality of ADAPTABLE in testing new sequences, we will use a completely random sequence: LLKKPPRWQTRGHKWCQRTP.

In order to predict the activity we will tell ADAPTABLE to "Create the family of a specific peptide" using this one as reference. We will give the name "test LLKKPPRWQTRGHKWCQRTP" to calculation.

Screenshot 2.1.

Family gener	ator	
In this page you can cre	ate a family of antimicrobial peptides fo	eaturing user-selected properties.
Please take a look at the <u>F</u>	requently Asked Questions (FAQ) and the tu	itorial.
Note: To know more about e	each field simply hover your mouse over it. in so	me fields the following options are available: y="yes; n="no"; or i=ignored.
Calculation label (mandatory)		
Lisername (mandatory)		Samples
	1-letter code aminoarid sequence	Create the family of a specific pentide If KKPDDW(OTDCHKWCODTD
T Append user peptides	Pretter tode annihold sequence	
Optional selection criteria (a	run with unspecified criteria might require l	long calculation times):
Peptide name ^(empty if indiferent)	e.g. Aurein	a
Sequence pattern	e.g. KKV or positional like GL-DIVKKVVGA-GSI	
Target Organism	e.g. baumannii	Activity (µM) 1 Activity test i
Advanced (+) Expand Me		
Peptide properties (+) Expand N	Ие	
Ξ Align method: ○ Simple	Blosum4	5 Minimum % of similarity 51 SThreshold percentage to group families 75
≁ Simplify aminoacids: ○	y On A Experimentally validated: Oi	○ y ○ n Include only peptides with data about: i
🔀 Run <u>SeqLogo</u> : 🔾 y 💽	n ڬ Generate additional graphical analysis	κ ⊖y ⊚n
Results will be viewable in th	he <u>DOWNLOAD RESULTS</u> section. You can exi	tract meaningful information from the experiment using <u>FAMILY ANALYZER</u> tool too.
Submit Reset		

This tool allows to compare a test sequence (in our case LLKKPPRWQTRGHKWCQRTP), with the full database. In our case, running the prediction did not produce any interesting result. Despite the fact that our sequence contains many amino acids frequently found in antimicrobial peptides (K, A, V, R), the sequence did not resemble any of the peptides of our database. This, of course, does not mean that our random peptide does not exhibit antimicrobial or anticancer properties. However, if active, we can estimate that its mechanism of action is not common in nature.

In order to get more insight in the possible activity of our test peptide, the calculation can be repeated "in simplified space" (setting to "simple" the align method), where residue types are compared by their properties. In this way, hydrophobic residues (A, V, I, L, M) are represented by A,

negative residues (D, E) by D, positive residues (K, R) by K, aromatic residues (W, Y, H, F) by F, polar residues (S, T, N, Q) by S, modified amino acids by (M). Gly, Pro, Cys are treated individually.

Screenshot 2.2.					
Family gener	ator				
In this page you can cre	ate a family of antimicrobial peptides fea	turing user-selected properti	ies.		
Please take a look at the <u>F</u> i	requently Asked Questions (FAQ) and the tuto	rial.			
Note: To know more about e	each field simply hover your mouse over it. in som	e fields the following options are a	vailable: y="yes; n="	"no"; or i=ignored.	
Calculation label (mandatory)	test_LLKKPPRWQTRGHKWCQRTP_				Samples
Lusername (mandatory)	user	Email notification (mandatory)	example@exampl	le.org	
+ Append User peptides	1-letter code aminoacid sequence	≁ 🖉 👬 Create the family of	a specific peptide	LLKKPPRWQTRGHKWCQRTP	
Peptide name(empty if indiferent) Sequence pattern Target Organism CAdvanced (+) Expand Me Peptide properties (+) Expand N	e.g. Aurein e.g. KKV or positional like GL-DIVKKVVGA-GSL e.g. baumannii	λ Activity (μΜ) 1 Activity	y test i v		
Ξ Align method: O Simple	ODSSP OSubstitution matrix Blosum45	▼ Minimum % of similari	ty 51 🕏	Threshold percentage to group families 75	
≁ Simplify aminoacids: ○) y 💿 n 🛛 🗕 Experimentally validated: 🕥 i 📿) y 🔵 n Include only peptides wil	th data about: i	•	
【 Run <u>SeqLogo</u> : ○y On	n 📕 Generate additional graphical analysis:	⊖y © n			
Results will be viewable in th	he <u>DOWNLOAD RESULTS</u> section. You can extra	act meaningful information from	the experiment us	sing <u>FAMILY ANALYZER</u> tool too.	
Submit Reset					

After the calculation, a page summarizing the features of the generated family will be shown. We can analyze the results more deeply by going to the "FAMILY ANALYZER" page and typing our user we can choose our run test_LLKKPPRWQTRGHKWCQRTP_:

Screenshot 2.3.

Family analyzer
Your run with calculation label test_LLKKPPRWQTRGHKWCQRTP_generated a family similar to the peptide LLKKPPRWQTRGHKWCQRTP:
Note: You can download this compressed package 🚯 with all the results.
<u>Global properties:</u>
You can read a <u>summary</u> for the generated family of peptides similar to the one provided by you, or you can read the <u>full output</u> for the experiment.
Your peptide is similar to some fathers of the ADAPTABLE built-in database all_families. Therefore its properties and activities might be similar. You can use this tool to extract relevant information on the similar families. Simply set the calculation label field to all_families and choose the family number (e.g. if your peptide is similar to f14 of all_families, choose 14 to display the properties of family 14).
Families of <i>all_families</i> similar to your peptide and corresponding father (in order of similarity) f16034:DYDWSLRGPPKCATYGQKCRTWSPPNCCWNLRCKAFRCRPR
Detailed analysis of family members: select the families to analyze (a range is noted with numbers separated by hyphens and a list is specified by separated by commas): Calculation label: test_LLKKPPRWQTF List of families to analyze: 1
Aminoacid frequencies Dainity Structure Residue type Best representative peptide Available data per sequence Available data per sequence (as text)
_ Specific parameters: (+) Expand Me
Submit Reset

Our test peptide was able to generate a family of 3 similar peptides (the family can be visualized by clicking on the "Submit" button in the lower part of the page with the option "Aminoacid frequencies" checked). Remember that when running the experiment you can tune the minimum similarity to get peptides included in the family, and change the alignment matrix and method:

Screenshot 2.4.



In the family, the first sequence is our test peptide, followed by similar database sequences with decreasing similarity. We can now click on the "Up arrow" button in the upper part and re-run the analyzing tool to retrieve other information.

In the *Screenshot 2.3*, ADAPTABLE also reports that the test peptide is similar to the father of the family 16064 from "all_families" experiment (a built-in experiment that aims to group all the peptides into families with similar sequence). Therefore, it would be interesting to see its properties. In order to do so, we can use "all_families" as calculation label and we can type "16034" in the "List of families to analyze" field, as in *Screenshot 2.5*.

Screenshot 2.5. Family 16034: Family 16034 has 2 elements. The best alignment is found with: DYDWSLRGPPKCATYGQKCRTWSPPNCCWNLRCKAFRCRPR Colouring by frequency 100 % ; 99-80 % ; 79-50 % ; 49-30 % ; 29-10 % ; 1. DYDWSLRGPPKCATYGQKCRTWSPPNCCWNLRCKAFRCRPR ; father 2. DYDWSLRGPPKCATYGQKCRTWSPRNCCWNLRCKAFRCRPR ; Similarity to father=96.6%

Selecting "Available data per sequence" and clicking on "Submit", we can see that the similar family 16034 is composed of members with antibacterial activity.

To conclude, out test peptide might have antibacterial activity although its sequence is not strikingly similar to any entry of the database.

Let's now try with a different peptide: GFGMAAKLAKK.

In this case, going to the "FAMILY ANALYZER" section and looking for "Aminoacid frequencies" information, we observe something more interesting:

Scre	ens	hot	2.8.

^	r
Family 1:	
Family 1 has 39 elements. The best alignment is found with: GFGMAAKLAKK	
Colouring by frequency	
100 % ; 99-80 % ; 79-50 % ; 49-30 % ; 29-10 % ;	
1. UPUMBAKLAKA 2. GENAVUI VEVU	
3. GENALKLIKK	
4. GEGMALKLXKKVL	
5. GFGMALKLYKKVL	
6. GFGMAÿKLLKKVL	
7. GFGMALKLKKVL	
8. GFGMALKLLKKVL	
9. SFGMALKLIKKVI; Similarity to father=81.0%	
10. GFGMALKLKWVL	
1. GEGRALKYLKKUL ; Similarity to father=/3.0%	
12. grond LLENGL	
14 GGMLKI KVI	
15. GFGMALKLLKKU	
16. GEGMALKLKKVL	
17. GFGMALkLKKVL	
18. GFGMALRLLRRVL	
19. <mark>GFGmALKLKKVL</mark>	
20. A <mark>FGMALKLLKKVL</mark>	
21. <mark>GFkMALKLKKVL</mark>	
22. GEGKALKLLKKVL; Similarity to father=69.8%	
23. aFGMALKLIKKVL	
24. GTGMALKLKKVL	
25. URANIELINVI	
20. LOURALLANCE , Similarity to father=5.1%	
28 FALALKIAKL	
29. GFGMALKXKKUL	
30. aFkMALKLKKVL	
31GMASKLAKVLPHVVKLIK ; Similarity to father=58.7%	
32 <mark>FKLAFKLAKK</mark> AFL	
33. <mark>GFGMALoLL</mark> OO <mark>VL</mark>	
34. GEGVLAKVAAHVVPAIAEHF ; Similarity to father=57.1%	
35. GFKMALKLKKVL ; Similarity to father=57.1%	
30. aproperturber (Similarity to Tather=5).0%	
37. UTUTILTR_LARVANLUSNVAUNUE; SIMILAILUY U TATHET=34.0%	
39 FLARALIKK LIKK LIKK LIKK LIKK LIKK LIKK L : Similarity to father=52.4%	
	Sequences

Our peptide (peptide 1) is quite similar to other peptides in the databases. Some of them (like peptide number 5 or 13) have sequences with non-standard aminoacids. This aminoacids are handled in the same way as regular aminoacids and represented by different characters to recognize them. To try to handle them more easily, we provide a button when necessarily next to the fields/sections that would need it (Screenshot 2.9). The button will open a section (Screenshot 2.10) allowing you to search for characters or aminoacids (pressing Ctrl+F), and to paste those characters in relevant fields when needed (simply clicking on them will paste the characters to the field next to the button used to open the character picker).

Screenshot 2.9.



Screenshot 2.10.

Submit Reset												
	Special ch	aracters for modified aminoa	acids									
	You can click on desired aminoad	You can click on the symbol to select it and use Ctrl+F to search your desired aminoacid										
Family 1: Family 1 has 39 elements. The best alignment is found with: GFGMAAKLAKK	Parent aminoacid	Modified Aminoacid	Symbol									
Colouring by frequency 100 % ; 99-80 % ; 79-50 % ; 49-30 % ; 29-10 % ;	A,ALA	Alanine,_D-	а									
1. GFGMAAKLAKK : father	A,ALA	3-Chloro-D-alanine_Hydrochloride	ъ									
2. GFGMAXKLLKKVL; Similarity to father=82.5%		heta-Cyclohexyl-alapine										
 GFGMALKL\KKVL	7,757	beta-cyclonexyr-atanine	¥									
4. GFGMALKLXKKVL; Similarity to father=82.5%	A,ALA	3-(2-Thienyl)-L-alanine	ю									
5. GFGMALKLYKKVL												
 OF DEPARTMENT	A,ALA	(2S)-2-Amino-3-(ruran-2-yi)propanoic_acid	DI									
8. GFGMALKLLKKVL	A,ALA	3-(3-Pyridyl)alanine	ъ									
9. GFGMALKLIKKVI	A,ALA	3-(1-Benzothiophen-3-Yl)-L-Alanine	щ									
11. GFGMALKXLKKVL												
12. <mark>GFGM</mark> a <mark>LKLLKKVL</mark>	A,ALA	beta-Alanine	×									
13. GEGMALKYLKKVL	A,ALA	2-Naphthylalanine	Φ									
14. OF OPPALLTRY U												
16. GFORMALKLKKVL	A,ALA	2-Amino-3-(1-	F									
17. GFGMALkLLKKVL; Similarity to father=73.0%		haphthyl)propionic_acid_hydrochloride										
18. GFGMALRLLRRVL	A.ALA	(2R)-2-Amino-3-naphthalen-1-	й									
29. AFGMELLENVL		ylpropanoic_acid										
21. GFKMALKLLKKVL	A,ALA	3-(2-Naphthyl)-D-alanine	ö									
22. GFGKALKLKKWL												
23. aFGMALKLERVL	A,ALA	N-Methylalanine	¥									
25. GFKMALKLLKKVL	A,ALA	2-Amino-3-naphthalen-1-ylpropanoic_acid	1									
26. LFGMALKLKKVL		- (
27 FALALKLAKKAL	A,ALA	3-(2-Thienyl)alanine	Pts									
28 <mark>FALALKLAKK</mark> L	A,ALA	4-Methyl-D-leucine	8									
30. a <mark>F</mark> KMALKLKKVL	CCYS	Selenocysteine	r									
31GMASKLAKVLPHVVKLIK ; Similarity to father=58.7%	cjero	Setenocysteme										
32FKLAFKLAKKAFL	C,CYS	S-Methylthiocysteine	д									
34. GF6VLAKVAAHVVPATAEHF; Similarity to father=57.1%	CCYS	Cysteine D-	c									
35. <mark>GfkMALKLLKKVL</mark> ; Similarity to father=57.1%		-,,										
36. a <mark>HKMALKLLKKVL</mark>	C,CYS	Homocysteine	ц									
37. GEGMLFKFLAKKVAKKLVSHVAQKQLE ; Similarity to father=54.0%	CCVS	Ethyl-cysteine	0									
38. SUSALLANARAGUGAVLAVLIIGL; SIMILATILY to Tather=52.4%	C,CTS	Enverysteine	*									
Jos Pregramm eronicamentoric , Jamitority to rather-J2.78		n										

To find more information about the properties of the similar peptides we have several options: rely on FAMILY ANALYZER utilities to review each field we are interested in with full detail, click on "Available data per sequence" to show a table showing a summary or rely on the more readable table provided into the Full output html. We will opt for this last option and, to open it, we simply need to scroll up to "Global properties" section and click on "full output" link:

Screenshot 2.11.

Global properties:

You can read a summary for the generated family of peptides similar to the one provided by you, or you can read the full output for the experiment.

Then, we click into "Property details" and we will see the table.



We discover that the peptides similar to our test sequence are antifungal and anticancer. In the list of similar peptides, the order follows the degree of similarity. This means that the most similar molecule is peptide 2, which is also antimicrobial. Given the sequence similarity we can predict that our peptide is likely to be antifungal and anticancer, possibly also antimicrobial then.

Looking into "Specific parameters" section, we can find more detailed information on a possible "cell_line", including "tissue" and "cancer_type".

Screenshot 2.13.

```
Family 1:
cell_line: (data available for the 33.3333 % of members)
      hela (33.3-100.0 %); ccrf (23.1-69.2 %); ccm (23.1-69.2 %); mcf (10.3-30.8 %); 480 (10.3-30.8 %); bmkc (10.3-30.8 %); 1299 (10.3-30.8 %);
      hela (33.3-100.0%); ccrf-cem (23.1-69.2%); mcf-7 (10.3-30.8%); sw-480 (10.3-30.8%); bmkc (10.3-30.8%); h-1299 (10.3-30.8%); pc-3 (10.3-30.8%);
   2. HeLa:SW:CCRF-CEM::

 HeLa;SW;CCRF-CEM;;

  10
  11. HeLa;SW;CCRF-CEM;;
  12
  13.
14.
  15.
  16
  17.
  18. HeLa;SW;CCRF-CEM;;
  19.
  20. HeLa:SW:CCRF-CEM::
  21
  22.
  23.
  24.
  25. HeLa;SW;CCRF-CEM;;
  26. HeLa;SW;CCRF-CEM;;
27. MCF-7;SW-480;BMKC;H-1299;PC-3;HeLa;;
  28. MCF-7;SW-480;BMKC;H-1299;PC-3;HeLa;;
29. HeLa;SW;CCRF-CEM;;
  30.
  31.
32. MCF-7;SW-480;BMKC;H-1299;PC-3;HeLa;;
  33. HeLa:SW:CCRF-CEM::
  34.
  35.
  36
  37.
  38
  39. MCF-7;SW-480;BMKC;H-1299;PC-3;HeLa;;
Family 1:
 tissue: (data available for the 33.3333 % of members)
      cervix (33.3-100.0 %); colon (33.3-100.0 %); blood (23.1-69.2 %); breast (10.3-30.8 %); skin (10.3-30.8 %); lung (10.3-30.8 %); prostate (10.3-30.8 %);
                                          3-100 0 %), blood (23 1-69 2 %), breast (10 3-30 8 %), skin (10 3-30 8
```

Following the list, we can predict the activity with decreasing accuracy. For example, our compound has relatively good chances to act on HeLa, SW and CCRF-CEM cell lines for Cervix, Colon and Lung cancers (properties of entry 2).

The output (section "Source") also indicates that our compound can be considered part of the family of Macropins peptides:

Screenshot 2.11.

ADAPTABLE TUTORIAL - CASE EXAMPLE n.3

Discovering new activities of pre-existing sequences not tested experimentally

The case of Acinetobacter baumannii

Despite the huge amount of experimental data on antimicrobial peptides, databases contain only a limited number of active peptides against some specific organisms. For example, when searching for peptides active against *A. baumannii*, if we look into peptides with an activity of not more than 0.01μ M to kill the target organisms we obtain only 2 families formed by only 1 aminoacid each.

Screenshot 3.1.

Family gener	ator		
In this page you can crea	ate a family of antimicrobial peptides fe	aturing user-selected propert	ies.
Please take a look at the <u>Fr</u>	equently Asked Questions (FAQ) and the tu	torial.	
Note: To know more about e	ach field simply hover your mouse over it. in so	me fields the following options are a	available: y="yes; n="no"; or i=ignored.
Calculation label (mandatory)	peptides_against_baumannii		Samples
Username (mandatory)	user	Email notification (mandatory)	example@example.org
+ Append User peptides	1-letter code aminoacid sequence	≁ 🗷 🚠 Create the family of	f a specific peptide 1-letter code aminoacid sequence
Optional selection criteria (a Deptide name ^{(empty} if indiferent) Sequence pattern Target Organism Advanced (+) Expand Me Peptide properties (+) Expand M	run with unspecified criteria might require la e.g. Aurein c e.g. KKV or positional like GL-DIVKKVVGA-GSL baumanni	ong calculation times): Activity (µM)	ty test i T
≘ Align method: ○ Simple	ODSSP OSubstitution matrix Blosum4	5 • Minimum % of similar	ity 51 SThreshold percentage to group families 75
≁ Simplify aminoacids: ○	y 💿 n 🛛 Experimentally validated: 🕥 i	⊖ y ⊖ n Include only peptides wi	ith data about.
I Run <u>SeqLogo</u>: ○ y o r	n 陆 Generate additional graphical analysis	:)y O n	
Results will be viewable in th	ne <u>DOWNLOAD RESULTS</u> section. You can ext	ract meaningful information from	n the experiment using <u>FAMILY ANALYZER</u> tool too.
Submit Reset			

A. baumannii has been declared among the most dangerous pathogen in terms of antibiotic resistance by the World Health Organization (WHO). It is therefore very important to find new active compounds which can be used as drugs. To find new peptides that could be similar to this two ADAPTABLE can help in this task. For example, focalizing peptide on 1 (GWLKKIGKKIERVGQHTRDATIQTIGVAQQAANVAATLK), we can use the "Create the family of a specific peptide" feature (see details in the previous example) to find interesting peptides. It allows to compare a given sequence (in our case GWLKKIGKKIERVGQHTRDATIQTIGVAQQAANVAATLK), with our full AMPs database. When running the calculation we obtain 31 potentially active peptides which were never tested with A. Baumannii as can be seen in the summary page that is offered shown after the experiment run. That page summarizes the properties (screenshot 3.2) and lists the members (screenshot 3.3) of family peptides similar the of to GWLKKIGKKIERVGQHTRDATIQTIGVAQQAANVAATLK.

Screenshot 3.2.

Properties of the family of peptides similar to GWLKKIGKKIERVGQHTRDATIQTIGVAQQAANVAATLK

You can visit 'DOWNLOAD RESULTS' section to download all the data.

• Members of the family

Property	Value (% of members that could have it depending on available data per member)								
Activity:	· · · · · · · · · · · · · · · · · · ·								
antimicrobial: (data available for the 96 77 % of members)	antimicrobial (96.8-100.0.%)								
antihactorial: (data available for the 97.09 % of members)	antimicrobid (500-1000 k)								
antibacteriat. (Jata available for the 07.05 % of members)									
antigram_pos: (data available for the 83.87 % of members)									
antigram_neg: (data available for the 83.87 % of members)	antigram_neg (83.9-100.0 %)								
biofilm: (data available for the \$1.61 % of members)	biofilm (51.6-100.9%); staphylococcus (38.7-75.0%); micrococcus (35.5-68.8%); luteus (35.5-68.8%); aureus (35.5-68.8%); pseudomonas (29.0-56.2%); candida (25.8-50.0%); aeruginosa (25.8-50.0%); mycobacterium (25.8-50.0%); smegmatis (25.8-50.0%); albicans (25.8-50.0%); enterobacter (16.1-31.2%); dsm (19.4-37.5%); 511 (19.4-37.5%); 987 (19.4-37.5%); cloacae (16.1-31.2%); bacillus (12.9-25.0%); megtarium (12.9-25.0%); klebsiella (6.5-12.5%); obt); dsm (19.4-37.5%); 511 (19.4-37.5%); 987 (19.4-37.5%); cloacae (16.1-31.2%); bacillus (12.9-25.0%); megmatiae (6.5-12.5%); klebsiella (6.5-12.5%); bp11 (6.5-1								
antifungal: (data available for the 35.48 % of members)	antifungal (35.5-100.0 %)								
antiviral: (data available for the 9.67 % of members)	antiviral (9.7-100.0 %)								
anticancer: (data available for the 3.22 % of members)	anticancer (3.2-100.0 %)								
- cancer_type: (data available for the 3.22 % of members)	leukemia (3.2-100.0 %)								
Toxicity:									
toxic: (data available for the 3.22 % of members)	toxic (3.2-100.0 %)								
cytotoxic: (data available for the 3.22 % of members)	cytotoxic (3.2-100.0 %)								
hemolytic: (data available for the 3.22 % of members)	hemolytic (3.2-100.0 %)								
Source:									
name: (data available for the 100 % of members)	cecropin (71.0-71.0%); sarcotoxin (22.6-22.6%); insects (41.9-41.9%); animals (41.9-41.9%); precursor (29.0-29.0%); lser (19.4-19.4%); cec (9.7-9.7%); altname (9.7-9.7%); full (9.7-9.7%); drosophila (9.7-9.7%); a1a2 (6.5-6.5%); drovi (9.7-9.7%); mdc (6.5-6.5%); cect (6.5-6.5%); lucilin (6.5-6.5%); flags (6.5-6.5%); cec3 (6.5-6.5%); ceca1 (6.5-6.5%); drose (6.5-6.5%); cec2 (6.5-6.5%); gi23259 (6.5-6.5%); sarpe (6.5-6.5%); drosi (6.5-6.5%); cecb (6.5- 6.5%)								
source: (data available for the 96.77 % of members)	fly (77.4-80.0%); drosophila (35.5-36.7%); fruit (38.7-40.0%); lucilia (29.0-30.0%); sericata (22.6-23.3%); peregrina (16.1-16.7%); sarcophaga (16.1-16.7%); flesh (12.9-13.3%); melanogaster (19.4-20.0%); ceratitis (6.5-6.7%); capitata (6.5-6.7%); musca (12.9-13.3%); domestica (12.9-13.3%); blow (19.4-20.0%); morsitans (6.5-6.7%); sechellia (12.9-13.3%); virilis (9.7-10.0%); simulans (9.7-10.0%); tsetse (6.5-6.7%); glossina (6.5-6.7%); medfly (6.5-6.7%); mediterranean (6.5-6.7%); boettcherisca (9.7-10.0%); mauritiana (9.7-10.0%); mojavensis (6.5-6.7%); tik (6.5-6.7%); flies (6.5-6.7%)								
taxonomy: (data available for the 96.77 % of members)	animalia (93.5-96.7%); insects (87.1-90.0%); arthropoda (71.0-73.3%); arthropods (54.8-56.7%); invertebrates (54.8-56.7%); animals (54.8-56.7%); insecta (38.7-40.0%); eumetazoa (41.9-43.3%); diptera (35.5-36.7%); sarcophagi (12.9-13.3%); drosophila (12.9-13.3%); ceropins (19.4-20.0%); sarcophagidae (12.9-13.3%); ceratitis (6.5-6.7%); peregrina (12.9-13.3%); eukaryota (9.7-10.0%); metazoa (9.7-10.0%); cerdysozoa (9.7-10.0%); hexapoda (9.7-10.0%); pterygota (9.7-10.0%); neoptera (9.7-10.0%); holometabola (9.7-10.0%); bachycera (9.7-10.0%); muscomorpha (9.7-10.0%); oestroidea (9.7-10.0%); drosophilidae (9.7-10.0%); melanogaster (9.7-10.0%); cercopin (6.5-6.7%); tephritidae (6.5-6.7%); capitata (6.5-6.7%); boattcharicea (6.5-6.7%);								
Family: (data available for the 54.83 % of members)	cecropin (54.8-100.0 %) Show frequency of aminoacids								

Screenshot 3.5.

Members of the family of peptides similar to GWLKKIGKKIERVGQHTRDATIQTIGVAQQAANVAATLK

Properties of the family

Num	Sequence (Click each for more information)	D
1	GWLKKIGKKIERVGQHTRDATIQTIGVAQQAANVAATLK-	My Peptide
2	GWLKKIGKKIERVGQHTRDATIQTIGVAQQAANVAATLK-	satpdb16356 satpdb18295 canPPD4420 DRAMP03077 CAMPSQ1244 DBAASP11810 APD_AP00135 InverPep97 LAMPL01A002386 ADAM_2948 YADAMP2322
3	GSPEFGWLKKIGKKIERVGQHTRDATIQTIGVAQQAANVAATLKG	satpdb21418 CAMPSQ625 InverPep618 LAMPL01A000665 ADAM_6656
4	GWLKKIGKKIERVGQHTRDATIQTIGVAQQAANVAATLKG	satpdb27249 DBAASP6281 APD_AP02498 InverPep534 LAMPL07APD0040 ADAM_2949
5	GWLKKLGKKIERVGQHTRDATIQTIGVAQQAVNVAATLKG	DBAASP11570 APD_AP03009
6	GWLKKFGKKIERVGQHTRDATIQAIGVAQQAANVAATLKG	satpdb17922 DBAASP8177 APD_AP02503 InverPep539
7	GWLKKIGKKIERVGQHTRDATIQVIGVAQQAANVAATAR-	satpdb21316 DRAMP03102 CAMPSQ126 APD_AP00231 InverPep280 LAMPL01A000138 ADAM_2950 YADAMP2323
8	GWLKKIGKKIERVGQHTRDATIQTIAVAQQAANVAATAR-	satpdb18232 DRAMP03076 CAMPSQ513 APD_AP00132 InverPep96 LAMPL01A000550 ADAM_2947 YADAMP2321
9	GWLKKIGKKIERVGQHTRDATIQVLGVAQQAANVAATARG	satpdb13980 DBAASP8174 APD_AP02500 InverPep536
10	GWLKKIGKKIERVGQHTRDATIQGLGVAQQAANVAATAR-	DRAMP03079 CAMPSQ1265 CAMPSQ2136 LAMPL01A002409
11	GWLKKIGKKIERVGQHTRDASIQAIGIAQQAANVAATARG	satpdb20523 DBAASP8175 APD_AP02501 InverPep537
12	GWLKKIGKKIERVGQHTRDATIQGLGIAQQAANVAATAR-	satpdb10156 satpdb18038 DRAMP03089 CAMPSQ125 CAMPSQ1269 DBAASP1855 APD_AP00230 InverPep279 LAMPL01A000137 ADAM_2945 YADAMP2320
13	GWLRKIGKKIERVGQHTRDATIQVLGIAQQAANVAATAR-	satpdb20196 DRAMP03101 CAMPSQ127 APD_AP00232 InverPep281 LAMPL01A000139 ADAM_2956 YADAMP2326 uniprotP08377
14	GWLKKIGKKIERVGQHTRDATIQGLGIAQQAANVAATARG	satpdb21100 CAMPSQ886 DBAASP5153 InverPep124 LAMPL01A002923 ADAM_2946 YADAMP2367
15	GWLKKIGKKIERIGQHTRDATIQGVGIAQQAANVAATAR-	InverPep121 LAMPL01A002414
16	GWLKKIGKKIERIGQHTRDATIQGLGIAQQAANVAATAR-	DRAMP03114 CAMPSQ1271 InverPep126 LAMPL01A002415
17	GWIRDFGKRIERVGQHTRDATIQTIAVAQQAANVAATLKG	satpdb12541 DRAMP03103 CAMPSQ128 APD_AP00233 InverPep282 LAMPL01A000140 ADAM_2932 YADAMP2365 uniprotP18312
18	-QSEAGWLKKIGKKIERVGQHTRDATIQGLGVAQQAPNVAATAR-	DRAMP03117 CAMPSQ1268 LAMPL01A002412 Show frequency of aminoacids

A quick look to the data table ("Available data per sequence") reveals that some of the new potentially active peptides are not hemolytic. In the table, each line corresponds to one peptide and each column corresponds to a property (1 indicates presence of the property). A "1" in column 37 indicates that the peptide has been tested experimentally for its hemolytic activity, while column 38 expresses whether the peptide is hemolytic (value 1) or not (value 0). In the former case, the intensity of the blue color indicates the extent of the hemolytic activity ((sub- μ M)-(μ M)-(sub-mM)-(mM)-(very weak)).

This way we can select peptides with activities we want or activities we want to skip (like toxic or hemolytic activities).

Screenshot 3.7.

Family	1:																																																					
(1 in	red	, f	or u	ser	-sel	ecte	d p	rope	rti	es (boo	lear	n or	r no	t),	ога	ange	fo	r un	isel	ecte	d b	oole	an p	prope	erti	les,	(su	ıb-μl	M) - (I	.(Mu	(sub	-mM)	- (mM) - (v	ery	weal	() f	or a	ctiv	ity 🛛	/alue	es, 🤇		fo	r un	sele	cted	non	bool	.ean	info	rmati	ion)
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		e					÷ -				v	4	а		a -	÷ ,		1	à	а	à	÷ .		n	•		e (a i			v	e a	4	c .	•		m	- 1			•	f f				+	n 1	t r	4	r .		1	a i	
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		e					e	8			n	c	c	r	г :	1 1	τ.	0		S	n	S	- 1	τ.	L.		e	cτ	1		τ	m c	τ	-	L n	5	0	τı	ε τ	-	a) e				а	g	y a	D	1 1	o u	u	ς τ	
		q	S	F		s	r –	r	c	t	t	r	t	а	a	fi	L i	t	а	m	0	h :	L C	1	ι	t	r i	t y	0		0	o t	У	s	ι ο	е	d	e '	l r	h	_ 1	c r				х	a	_ l	0	m :	s	b '	t y	
		u	0	а		t	m i	n	У	а	h	0	е	m	m i	u y	/ V	0	s	0	s	m e	a	t		i		i	g	t	t	ιi		0	. n	n i	u	n :	i a	0	b :	L a				0	n	v	s	e o		i :	i	
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