UNIVERSITY OF SAO PAULO
SCHOOL OF ARTS, SCIENCES AND HUMANITIES

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Discovery of new epitopes of Trypanosoma cruzi in its interaction with humans.

Sao Paulo
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Translated from Portuguese version

Completion of course work presented to the Undergraduate Course in Biotechnology at the School of Arts, Sciences and Humanities of the University of São Paulo, to obtain the title of Bachelor of Biotechnology.

Advisor: Prof. doctor Joao Carlos Setubal

Co-advisor: Prof. doctor Luciano Antonio Digiampietri

Sao Paulo

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I dedicate this work to all the professors I had, who, without a doubt, made an infinitely greater contribution to this result than myself.

THANKS

First of all, I would like to express my sincere gratitude to my advisor, Prof. doctor João Carlos Setubal, as well as my co-advisor, Prof. doctor Luciano Antonio Digiampietri, and especially my doctoral student supervisor Gianluca Machado Major da Silva for his patience and support given to me throughout the project, without whom I would not have been able to complete this project.

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SUMMARY

Vibanco de Oliveira Neves; Stéffani. Discovery of new epitopes of Trypanosoma cruzi in its interaction with humans. Completion work of the bachelor's degree in biotechnology - School of Arts, Sciences and Humanity, University of São Paulo, São Paulo, 2022.

Large-scale mapping of antigens and epitopes is of fundamental importance for the development of various immunotherapies, but it becomes a major challenge, especially for eukaryotic pathogens, due to their large genomes. In this work, a process flow was developed, from genomic phages, to show that unbiased libraries of the eukaryotic parasite Trypanosoma cruzi allow the identification of antigens by serum samples from patients with Chagas disease. A comprehensive library of Chagas disease antibody response was constructed and validated, with the aim of showing how epitopes of linear and putative conformation (containing many repeating elements), allow the parasite to avoid an accumulation of neutralizing antibodies directed against domains of proteins that mediate the pathogenesis of the infection. Thus, this process flow is a reproducible and effective tool for the identification of epitopes and antigens, not only for Chagas disease, but perhaps also for emerging/reemerging pathogens globally.

Keywords: Antigens. Bioinformatics. Chagas Disease. Epitope. Phage. Immunotherapy.

ABSTRACT

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Large-scale mapping of antigens and epitopes is of fundamental importance for the development of several immunotherapies, but it becomes a great challenge, especially for eukaryotic pathogens, due to their large genomes. In this work, a process flow was developed, starting from genomic phages, to show that unbiased libraries of the eukaryotic parasite Trypanosoma cruzi allow the identification of antigens by serum samples from patients with Chagas disease. A comprehensive library of the Chagas disease antibody response was constructed and validated, with the aim of showing how epitopes of linear and putative conformation (containing many repeated elements) allow the parasite to avoid an accumulation of neutralizing antibodies directed against protein domains. that mediate the pathogeny of the infection.

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SUMMARY

1. INTRODUCTION

1.1. CHAGAS DISEASE

Chagas disease, also known as American trypanosomiasis, is a multisystem disorder that can affect the cardiovascular, digestive, and central nervous systems.[1] Chagas disease is caused by Trypanosoma cruzi, a hemoflagellate parasite that is transmitted by several species of insects hematophagous reduvids (kissing bugs) mainly in endemic areas.[2] The disease was described for the first time by Carlos Chagas in 1909, however Charles Darwin described his encounter with the vector and his own symptoms compatible with the disease, indicating that infection by T. cruzi happened before Dr. Chagas to describe.[3] The World Health Organization (WHO) considers Chagas disease one of the twenty neglected tropical diseases[4], and it is estimated that 6 to 7 million people are infected with T. cruzi worldwide, the vast majority in Latin America.[5]

Chagas disease can be considered a reemerging infection, as areas where there was no locally acquired infection are reporting autochthonous cases.[6]In the United States of America, when testing for Chagas disease in blood donors began in 2008, seropositivity for T. cruzi was 1 in 6,500 donors, with 36% of them having clinical evidence of Chagas cardiomyopathy. In at least 5 of these cases the infection is shown to have occurred as indigenous transmission in Texas.[7] Chagas disease is an important public health problem, affecting multiple systems, including the central nervous system (CNS), the digestive system, the immune system and, mainly, the heart. In Latin America, it is among the most frequent causes of heart failure (HF), and is supposedly responsible for up to 41% of cases in endemic areas.[8]

Parasite stages in mammalian hosts include bloodstream trypomastigotes and intracellular replicative, flagellaless, amastigotes, while vector stages include replicative epimastigotes and infectious metacyclic trypomastigotes. Infections in mammals occur when the parasite is in the trypomastigote stage. Trypomastigotes infect a variety of cells and convert into a replicative amastigote that multiplies in the host cell cytoplasm. The parasitized cells eventually rupture and release trypomastigotes that circulate and can infect other host cells.[9]

T. cruzi is transmitted in endemic areas by several species of three genera of triatomine blood-sucking insects, also known as kissing bugs (Triatoma, Panstrongylus, Rhodnius).[10]The three genera are widely distributed in Latin America, from Mexico to Argentina and Chile, and inhabit both forests and drier areas.[11] However, other infection mechanisms that are important, especially in non-endemic areas, include blood transfusion, organ transplantation, oral ingestion, laboratory accidents, mother-to-child vertical, or shared intravenous needles. Sexual transmission has been reported by in vivo mouse experiments, but no reports in humans are currently available.[12]

1.2.GENETIC DIVERSITY

T. cruzi is a heterogeneous species with seven strains, or discrete typing units (DTU), called TcI, TcII, TcIII, TcIV, TcV, TcVI and Tcbat.[13]This genetic diversity has been related to distribution, pathogenesis, clinical features and response to therapy. The parasites in each DTU are genetically similar and have similar characteristics, including the pathology they cause, biochemistry and immunogenicity, and resistance to treatment.[14]

TcI has a wide distribution, from the southern United States to northern Argentina and Chile, and this DTU is most frequently sampled in sylvatic cycles, but is also frequent in domestic cycles and is the dominant DTU responsible for disease transmission. Chagas disease in endemic countries located north of the Amazon basin. As for TcII, V and VI are more likely to be associated with domestic cycles and patients with chronic Chagas disease in Southern Cone countries and Bolivia. TcIII and IV are mainly sampled in the rainforest. And finally, Tcbat previously identified in bats, was recently found in humans. It's fineIt is known that several DTUs can coexist in the same vector and in a single host.[15]

The genetic variety presented by this parasite is also evident when analyzing the families of multigenes that it possesses. This occurs because the genome of T. cruzi has many repeated sequences, indicating that many of these genes are in linkage disequilibrium and that clonal reproduction of this population occurs. Furthermore, the presence of multigene families is related to its ability to invade cells and present tropism for different tissues, causing different types of heart diseases and mega syndromes associated with Chagas disease. Thus, this genetic variety is associated with the infectivity of T. cruzi, as many of these families code for genes present on the surface of the protozoan, such as trans-sialidases and mucins.

1.3. AUTOIMMUNITY IN CHAGAS DISEASE

T. cruzi has different escape strategies that allow it to evade the host's immune system, allowing its persistence and the establishment of chronic infection that leads to the development of chronic chagasic cardiomyopathy (CCC). The potent immune stimuli generated by the persistence of T. cruzi can result in tissue damage and an inflammatory response. In addition, molecular mimicry between parasite molecules and host proteins can result in cross-reaction with self molecules and, consequently, autoimmune features, including autoantibodies and self-reactive cells. Although controversial, there is evidence that demonstrates a role for autoimmunity in the clinical progression of CCC. Nonetheless,[16]

There are two mechanisms that try to explain autoimmunity in Chagas: one with the activation of B and T lymphocytes in an antigen-independent manner, and the other with molecular mimicry. The first mechanism, together with the presence of parasite antigens, can trigger major tissue damage, surpassing the self-tolerance threshold and inducing the production of autoantibodies. Mimicry, in turn, is due to the similarity between T. cruzi and human antigens, and thus cross-reaction of antibodies occurs.[16]

1.4. PHAGE DISPLAY

The phage display technique was initially developed and used to map antibody epitopes[17], identify antigens involved in diseases such as cancer[18], illnesses[19], and parasitic infections[20], including Chagas disease.[21]Phage Display involves inserting a DNA fragment into a genetically modified bacteriophage, which expresses a peptide on its viral capsid, so that the corresponding peptide (or antibody) encoded by the exogenous DNA fragment is displayed on the surface of the bacteriophage. If the peptide is a fragment of an antigen recognized by a given antibody, the bacteriophage particle can be captured by exposing the protein that interacts with the target ligand.[22]. In this affinity selection process called biopanning, the library is then presented with target molecules, usually immobilized on solid supports. Weak interactions between phage expressing the protein and the target are disrupted by successive washes, while phages containing molecules with high

target affinity are recovered by elution.[23]And thus the antigen is isolated from the pool of phage particles.[24]

1.5. IEDB (IMMUNE EPITOPE DATABASE)

The Immune Epitope Database (IEDB) is a free service with the aim of assisting immunological research. In it, it is possible to find results of more than 1.6 million experiments of adaptive immune response to epitopes, gathered mainly in the literature.[25]These data come from 19,500 publications, including all available literature from the inception of PubMed to the present. Searches are performed on PubMed every two weeks allowing for an update with new content.[25]

The IEDB has a great relevance for this project, it is from it that it becomes possible to validate that our data are about possible epitopes, since it is very likely that we will find epitopes that are already known. In addition, it is possible to have a better understanding of the characteristics of an epitope through a vast database.

1.6. CONTEXT OF THIS WORK

This course completion work is part of a collaborative project between the advisor Professor João Carlos Setubal and Professor Ricardo Giordano. This project, led by Prof. Ricardo, aims to identify T. cruzi epitopes using phage display. In this project, 8 datasets have been analyzed so far (Table 1).

A preliminary analysis of these data sets was published in the article "A refined genome phage display methodology delineates the human antibody response in patients with Chagas disease"[24]by Teixeira et al.,

This course completion work aims to refine the methodology used in the article by Teixeira et al., for a specific data set, the CCC_mild A and B set. This work is also associated with the doctoral work of student Gianluca Machado da Silva, guided by profs. Setubal and Giordano. Gianluca was a co-supervisor of this TCC work.

2. OBJECTIVES

2.1. MAIN GOAL

Generation of a new list of potential epitopes in the T. cruzi-human interaction, based on the analysis of a data set obtained by the Phage Display technique.

2.2. SPECIFIC OBJECTIVES

2.2.1. Familiarization with the following topics:

- Chagas disease and Trypanosoma cruzi;
- Phage display technique;
- Epitope concept;
- setulab computational environment;
- Sequence alignment;
- Sequence clustering;

●BLAST (Basic Local Alignment Search Tool);

●IEDB[25].

2.2.2. For the phage display CCC_mild A and B datasets: ●Extract inserts from reads;

●Comparing DNA sequences to each other for counting of frequency

●Remove inserts that do not map to the T. cruzi genome;

●Determination of the ORFs (Open Read Frame) of each insert;

●Comparison of ORFs with each other to remove duplications;

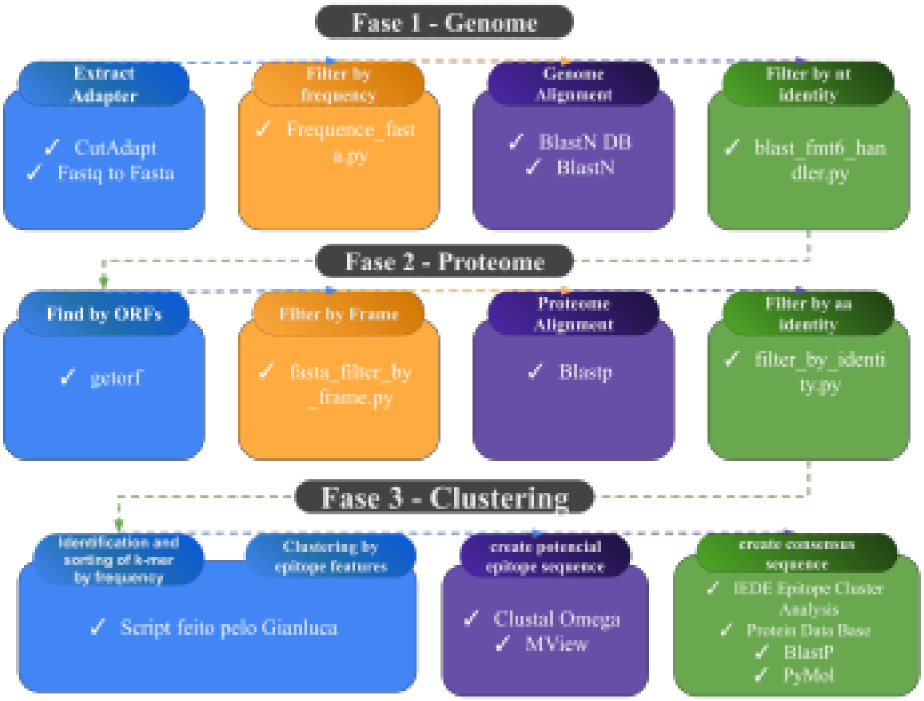
●Comparison of the resulting ORFs with T. cruzi proteome;

●Comparison of the resulting ORFs with T. cruzi epitopes in IEDB.

3. METHODOLOGY

The study was divided into 5 stages: Review and Understanding of the literature (3.1); Genome Treatment (3.2); Proteome Treatment(3.3); Validation (3.4), Clustering (3.5) and Sequence Consensus and Visualization in the protein (3.6). Among these steps, 3.2, 3.3, 3.5 and 3.6 form a pipeline for the identification of epitopes from antigen sequences, as shown in figure 1.

Figure 1: Flow diagram of pipeline steps for epitope identification from antigen sequences obtained using the Phage Display technique.



Source: Stéffani Vibanco de Oliveira Neves (2022).

3.1. LITERATURE REVIEW AND UNDERSTANDING

In order to obtain a better understanding of the processes and themes that were used, a survey of the scientific literature was carried out.

The understanding was fundamentally based on the article “A refined genome phage display methodology delineates the human antibody response in patients with Chagas disease” by Teixeira et al.[24], and also in the article “Protocol for design, construction, and selection of genome phage (gPhage) display libraries.” by Rodriguez Carnero et al..[26]

We decided to approach the issue from a set of data from a Phage Display library (Table 1) from the reference article.[24]With the intention of obtaining a greater genetic variety, the author used, as a source for this library, serum samples from patients contaminated by Chagas disease with different levels of symptoms, and who fit the following requirements.[24]

The. Patients with at least two positive results for the presence of anti-T. cruzi.

B. All candidate patients underwent electrocardiography (ECG) and echocardiography (ECHO) and those with abnormal ECG were classified as having mild cardiomyopathy when the left ventricular ejection fraction (LVEF) was greater than 40% (LVEF > 40%) , or severe cardiomyopathy, when the left ventricular ejection fraction was less than or equal to 40% (LVEF ≤ 40%).

ç. Patients without electrocardiographic changes were considered asymptomatic.

d. Serum samples were pooled into groups of 10 donors to form two independent sets (biological duplicates) for each disease condition:

i. control (2 x 10 donors)

ii. asymptomatic (2 x 10 donors)

iii. mild cardiomyopathy (2 x 10 donors)

iv. severe cardiomyopathy (2 x 10 donors).

Table 1: Available read sets. For each set (line) there are two lots (A and B)

control	control patients
asympto	patients who test positive for Chagas disease but have no symptoms
CCC_mild	patients who test positive for Chagas disease with mild symptoms
CCC_severe	patients who test positive for Chagas disease with severe symptoms

Source: , 2022[24]Stéffani Vibanco de Oliveira Neves

In this project, the set CCC_mild_A (also called K) and B (also called set O) was used.

3.2. DATA PROCESSING

3.2.1. EXTRACTING INSERTS FROM READS

From the download of the data set obtained by sequencing, it was necessary to remove the adapter sequences, these adapters contain the indices (short sequence of bases that identify each sample) and are at the beginning and end of the sequence. It was identified that for this sequencing, the sequence of adapters to be removed is from upstream “ATGACCATGGCAGTAC” and downstream “GTACCCGGTGCGCCGG” and for the removal, the command line tool Cutadapt was used.[27]

In addition, the command line converter from fastq to fasta file was used. In this way, we obtain data containing only the initial sequence of interest containing the possible T. cruzi antigens.

3.2.2. COMPARISON OF DNA SEQUENCES WITH EACH OTHER FOR FREQUENCY COUNTING

The sequences that are part of the set have great diversity. To ensure greater reliability, it was defined that they need to have a minimum frequency of two appearances, this information was observed in the article by Teixeira et al., which cites the reference by Dias-Neto et al, 2009.[28]For this filter, the script in Python was used[29]which checked the appearance of repeated sequences and created a new file containing only sequences with a minimum frequency of two.

3.2.3. REMOVAL OF INSERTS THAT DO NOT MAP IN THE T. CRUZI GENOME

In order to identify the sequences that represent the T. cruzi genome, an alignment of the possible antigen sequences with the T. cruzi sequences (CL Brener, Sylvio X10, DM28c, and Marinkellei strains downloaded from the NCBI[30]). For this, the blastN command line application was used.[31], with the parameters: number of alignments (num_alignments) in ten and maximum number of HSPs (High-Scoring Segment Pairs) in 1.

After the alignment, a Python script was used[32]to verify the percentage of identity of each sequence with the T. cruzi sequences and discard those that obtained a value lower than 90%. Ideally, we would use a value of 100% identity, but due to the variations of T. cruzi strains in the samples, and the one used for alignment, there was a greater tolerance for its identity.

3.3. PROTEOME TREATMENT

3.3.1. DETERMINATION OF ORFS OF EACH INSERT

The sequences that have been obtained so far consist of nucleotides, so for the construction of a proteome it was necessary to use a command line program called EMBOSS GetOrf[33]~~, with it, it was possible to obtain sequences of open reading frames (ORFs).~~

3.3.2. REMOVAL OF INSERTS THAT STARTED WITH A FRAME DIFFERENT FROM TWO

The inserts coming from the DNA phage assume that the correct frame for amino acid translation is frame two positive (+2). Therefore, in this step, it was necessary to discard the open reading frames (ORF) that did not start in frame +2, for which a script in Python was used[34]which filtered the sequences of ORFs that were in frame +2, creating a file with only the desired ones.

3.3.3. REMOVAL OF INSERTS THAT DO NOT MAP IN THE T. CRUZI PROTEOME

In order to identify the sequences that represent the T. cruzi proteome, an alignment of the possible antigen sequences with the T. cruzi sequences (strains CL Brener, Sylvio X10, DM28c, and Marinkellei downloaded from the NCBI[30]~~). For this, the blastP command-line application was used.~~[31]~~, with the parameters: number of alignments (num_alignments) in ten and maximum number of alignments (max_hsps) in 1, the size of matching sequences (word_size) of value 6, number of openings (gapopen) in 13 and the number of alignments that would be expected (evalue) at 100.~~

After the alignment, a Python script was used[35]to verify the percentage of identity of each sequence with the T. cruzi sequences and discard those that obtained a value lower than 70%. This value is due to the 90% filter made in step 3.2.3, since it was used for the nucleotide identity filter, there is a need for greater tolerance when dealing with peptides. This statement is justified by the occurrence of gene families, which may have similar proteins encoded by different genes. It is known that in the T. cruzi genome there are gene families, and therefore, using this tolerance, it was possible to map translated peptides in the proteome taking this phenomenon into account.

3.4. VALIDATION

With the intention of validating that the sequences that passed through the pipeline contain Trypanossoma cruzi epitopes, at this stage of the pipeline all epitope sequences that are present in the database of the IEDB website were downloaded.[reference], with the following parameters: Linear Epitope, Trypanossoma cruzi Organism, Human Host, Chagas disease and other parameters with standard values already inserted by the platform.

After unloading, a Blast database was created with these sequences, and thus the IEDB epitope sequences were aligned with the possible antigen sequences from previous flows, using the BlastP command-line application.[31]

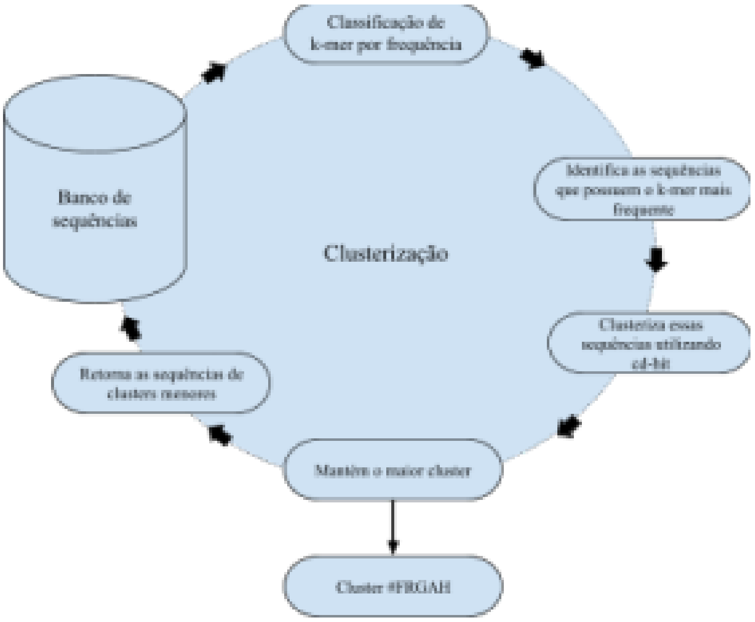
3.5. Clustering

In order to obtain an optimized analysis, it was decided to group the sequences resulting from step "3.3 treatment of the proteome" into clusters. For this, a script in Python was structured[36]which, from the sequence bank, detects subsequences (k-mer) of size eight of each sequence, and orders these k-mers, in descending order, according to their frequency of appearance, from the highest to the lowest. So he uses the cd-hit app[37]~~, which aims to cluster protein sequences with at least 80% identity , using the sequences that have the appearance of this most frequent k-mer.~~

From this, we obtain different clusters arising from the sequences used. The cluster that obtained a greater number of grouped sequences will be considered for final analysis, while the sequences that are part of clusters with a smaller number will return to the sequence bank to be reconsidered by another k-mer, following their frequency order, asfigure 2.

As a result of this script, folders were created for each k-mer sequence, in which there are clusters created by cd-hit, including the cluster with the highest number of sequences. In addition, a log file was created that brings information about the occurrences of the script, a file containing information about the largest clusters of each k-mer.

Figure 2: Clustering process from Python script


Classificação de k-mer por frequência
Identifica as sequências que possuem o k-mer mais frequente
Clusteriza essas sequências utilizando cd-hit
Mantém o maior cluster
Cluster #FRGAH
Retorna as sequências de clusters menores
Banco de sequências
Clusterização

Source: Stéffani Vibanco de Oliveira Neves (2022).

3.6. POSSIBLE EPITOPE SEQUENCE, CONSENSUS SEQUENCE AND VISUALIZATION IN PROTEIN

From the document that contains information about the largest clusters of each k-mer, a cluster was selected in which its possible epitope was already cataloged in the IEDB database.

So, in order to obtain a sequence that possibly represents an epitope of this cluster, the program Clustal Omega was used[38]containing the complete sequences of it. The result was visualized by the other application, Mview[38]. So, in order to verify if this possible epitope is already cataloged in the IEDB, we looked for this sequence in the result of the BlastP with the IEDB (done in step “3.4 - Validation”).

On the other hand, to obtain a consensus sequence of this cluster, the Epitope Cluster Analysis web program was used with the default parameters.[39]~~, containing the fasta with complete sequences of it. The result was the consensus sequence of sequences.~~

Upon obtaining the 100% consensus sequence of this cluster, the BlastP tool was used[31]of the NCBI, using several protein databases, to identify

which protein of the Trypanosoma cruzi organism could correspond to this19 consensus sequence. Then, the protein that contained the greatest correspondence with the possible epitope was selected.

From this, this protein was found in UniProt[40], a protein database that contains, in addition to proteins with validated structures, predictions of non-validated ones. So, we use the PyMol Desktop application[41]for the visualization of this protein, and therefore, the "protein residue sequence selection" function with a single letter code was used to select the part of the protein in which there was an alignment with the consensus sequence, this part was colored for better visualization.

4. RESULTS and DISCUSSION

4.1. SKILLS WITH PROGRAMMING LANGUAGES AND HIGH PERFORMANCE COMPUTING SYSTEMS

Scripts were made in Python that use the argparse modules[42]~~, which make it possible to create user interfaces from the command line. In addition, modules from the biopython library were used.~~[43]~~, which is a tool library for biological data, pandas library modules~~[44]which is used for data manipulation and analysis. The scripts are stored on github by doctoral student Gianluca Major and the author herself, and are being produced throughout doctoral student Gianluca Major's project.

Also, the Google Drive drawing tool was used.[45]to make infographics that facilitated the understanding of the process flows in this project.

4.2. GENOME TREATMENT

Sequences obtained from the library came from patients who tested positive for Chagas disease with mild symptoms. The CCC_mild_A sequence group has 868 thousand sequences, while the CCC_mild_B group has 651 thousand sequences. As the sequences walk through the proposed pipeline, there is a change in the number of sequences in each group, due to filters and alignments that provide sequences that are increasingly close to the epitopes of the Trypanossoma cruzi organism, these changes can be seen in thetable 2.

Initially, the adapters of each sequence were removed, so it was possible to obtain only the nucleotides of interest that represent the possible20 antigens, therefore, sequences that are unique were eliminated, that is, they presented only one recurrence in the entire bank of sequences, and thus errors in the phage display process were reduced, increasing the confidence of the data. At this stage, the number of sequences in group A and B were, respectively, 48435 and 21154 sequences.

Then, after alignment between the sequences of groups A and B, with the sequences of Trypanossoma cruzi already known, provided by the National Center for

Biotechnology Information (NCBI) and filtering by 90% identity, both groups A and group were obtained. B sequences (Table 2) that are more likely to belong to the organism of interest.

4.3. PROTEOME TREATMENT

The sequences, until then of nucleotides, were transformed into Open Reading Frames (ORF), obtaining sequences between the start and stop codons, as the number of sequences in group A and B were, respectively, 414251 and 175888, astable 2. After that, there was a filter in which ORFs that did not start at frame +2 were discarded, which resulted in a large decrease in the number of sequences in groups A and B, respectively. 35231 and 16873.

So, after the alignment between the proteomes of groups A and B, with Trypanosoma cruzi proteomes already known from the NCBI, and a filtering by 70% identity, we obtained 13569 sequences in group A, and 12162 sequences in group B.

Table 2: Sequences of possible Trypanosoma cruzi antigens according to their passage through the pipeline.

Phases	ccc_mild_a (K)	ccc_mild_n (O)
Original Sequences	868305	651503
Deletion of Unique Strings	48435	21154
Sequences with 90% Identity after BlastN	48118	21138
Orfs that start with frame 2	35231	16873
Sequences with 70% Identity after BlastP	13569	13569

Source: Stéffani Vibanco de Oliveira Neves, 2022.

4.4. VALIDATION

The sequence database of groups A and B so far has sequences with possible Trypanosoma cruzi antigens, so an alignment was made with the epitope sequences that are present in the IEDB. As shown in Table 3, we can see that of the sequences in group A that passed through the proposed pipeline, 14.68% have at least one epitope already known by the IEDB, while in group B, there are 77.35%. By excluding repeated epitopes we obtain the amount of unique IEDB epitopes that were found in the sequences of this project. (Table 3)

Table 3: Sequences of possible Trypanosoma cruzi antigens according to their passage through the pipeline.

Description	Total of antigens	Alignment with at least one IEDB epitope with 100% identity	Number of unique epitopes in the IEDB
CCC_mild_a	13569	1993	376
CCC_mild_b	12162	9408	354

Source: Stéffani Vibanco de Oliveira Neves, 2022.

4.5. Clustering

When we passed this project's sequence database through the clustering script explained in Section 3.5, we obtained 637 clusters for group A, and 66 for group B, each with its representative sequence. With this result, we can observe that in group B, there is a greater similarity between the sequences it contains than in group A. As the number of IEDB epitopes that were identified for both groups is similar, after all they are the same biological samples , this result suggests that in group A there are many sequences that are not antigens. Indeed, in the article by Teixeira et al., it is mentioned that this divergence may be due to differences in the phage “input” on the phage display, which was lower for the selection of group B. However, 70% of all epitopes of group B were contained in A,

4.6. POSSIBLE Epitopes

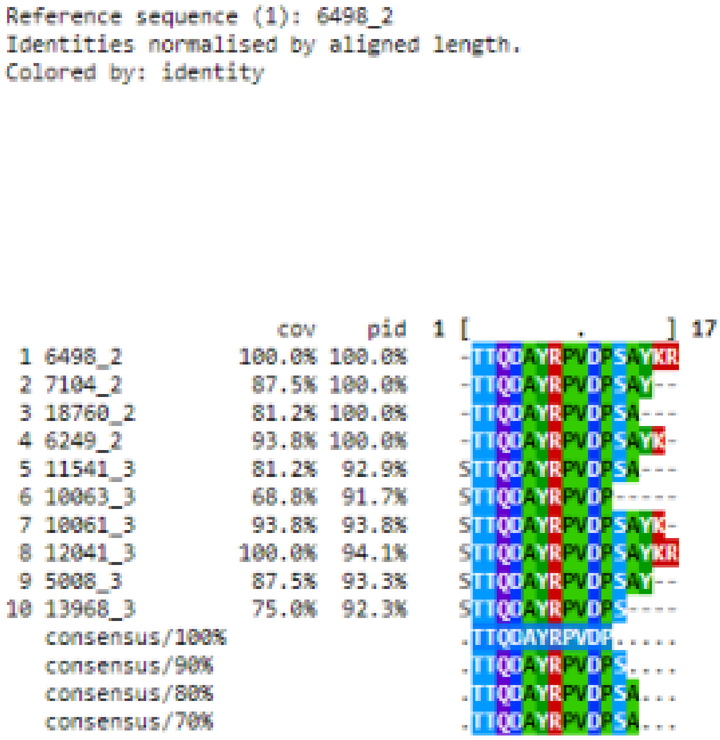
At the end of clustering, we obtained a list of possible epitopes, both from group A and group B. This list is found respectively in appendices A and B of the work.

In these appendices there is a table with several data that are relevant for the selection of clusters and possible epitopes. In the first column of the table found in the appendices, is the K-mer value, which has a chosen size of 8 amino acids that has been listed according to its frequency, the next column is that frequency, then the next column represents the unique sequences that have the k-mer, after that the posterior column represents the amount of sequences that are present in the largest cluster of that k-mer group. The consecutive column has 4 values, represented by the number of sequences with the lowest frequency, the highest frequency, the average size and the standard deviation. In addition, the following columns contain the identification number of the largest Cluster of this k-mer group,

4.7. CONSENSUS SEQUENCE AND VISUALIZATION IN PROTEIN

When obtaining the largest clusters of each k-mer group, a cluster whose possible epitope was already cataloged in the IEDB database was chosen. The selected cluster was the one containing the k-mer “TTQDAYRP”.

In the TTQDAYRP cluster, there are ten sequences that contain your k-mer and ten that have been grouped into a main cluster, that is, all initial sequences. Then, when using Clustal Omega and obtaining a visualization from Mview, we obtained that the closest sequence of an epitope of this cluster is “TTQDAYRPVDP”, asfigure 3.



```
Reference sequence (1): 6498_2
Identities normalised by aligned length.
Colored by: identity



                          cov     pid   1 [        .       ] 17
 1 6498_2            100.0% 100.0%     -TTQDAYRPVDPSAYKR
 2 7104_2             87.5% 100.0%     -TTQDAYRPVDPSAY--
 3 18760_2            81.2% 100.0%     -TTQDAYRPVDPSA---
 4 6249_2             93.8% 100.0%     -TTQDAYRPVDPSAYK-
 5 11541_3            81.2%  92.9%     STTQDAYRPVDPSA---
 6 10063_3            68.8%  91.7%     STTQDAYRPVDP-----
 7 10061_3            93.8%  93.8%     STTQDAYRPVDPSAYK-
 8 12041_3           100.0%  94.1%     STTQDAYRPVDPSAYKR
 9 5008_3             87.5%  93.3%     STTQDAYRPVDPSAY--
10 13968_3            75.0%  92.3%     STTQDAYRPVDPS----
   consensus/100%                      .TTQDAYRPVDP.....
   consensus/90%                       .TTQDAYRPVDPS....
   consensus/80%                       .TTQDAYRPVDPSA...
   consensus/70%                       .TTQDAYRPVDPSA...
```

Figure 3: Result of Multiple Alignment using Clustal Omegaand Mview.

Source: Mview, 2022.

From the comparison made of the closest sequence of an epitope, with the BlastP result of the IEDB epitopes (seen in step 3.4 of Validation), it was possible to identify that this cluster represents an epitope that is already in the IEDB sequence database “TQDAYRPVDPSAYKR” and identification number“397929”, and therefore is valid.

From another perspective, the consensus sequence was found using the Epitope Cluster Analysis web tool.[39]The sequence is configured by “STTQDAYRPVDPSAYKR” as can be seen in thetable 4.

Table 4: Result of Clustering Epitopes of Cluster “TTQDAYRP”

Sequences	Peptide Number	Alignment	Position	Description	Peptide
1.1	**Consensus**	**STTQDAYRPVDPSAYKR**	-	-	-
1.1	1	STTQDAYRPVDPSAYKR	1	12041_3 [2 - 52] | freq=3	STTQDAYRPVDP SA YKR
1.1	two	STTQDAYRPVDPSAYK-	1	10061_3 [2 - 49] | freq=3	STTQDAYRPVDP SA YK
1.1	3	STTQDAYRPVDPSAY--	1	5008_3 [2 - 46] | freq=7	STTQDAYRPVDP SA Y
1.1	4	STTQDAYRPVDPSA---	1	11541_3 [2 - 43] | freq=3	STQDAYRPVDPSA
1.1 1.1 1.1	5 6 7	STTQDAYRPVDPS-- -- STTQDAYRPVDP--- -- -TTQDAYRPVDPSAY KR	1 1 two	13968_3 [2 - 40] | freq=2 10063_3 [2 - 37] | freq=3 6498_2 [2 - 49] | freq=5	STTQDAYRPVDPS STTQDAYRPVDP TTQDAYRPVDPS AY KR

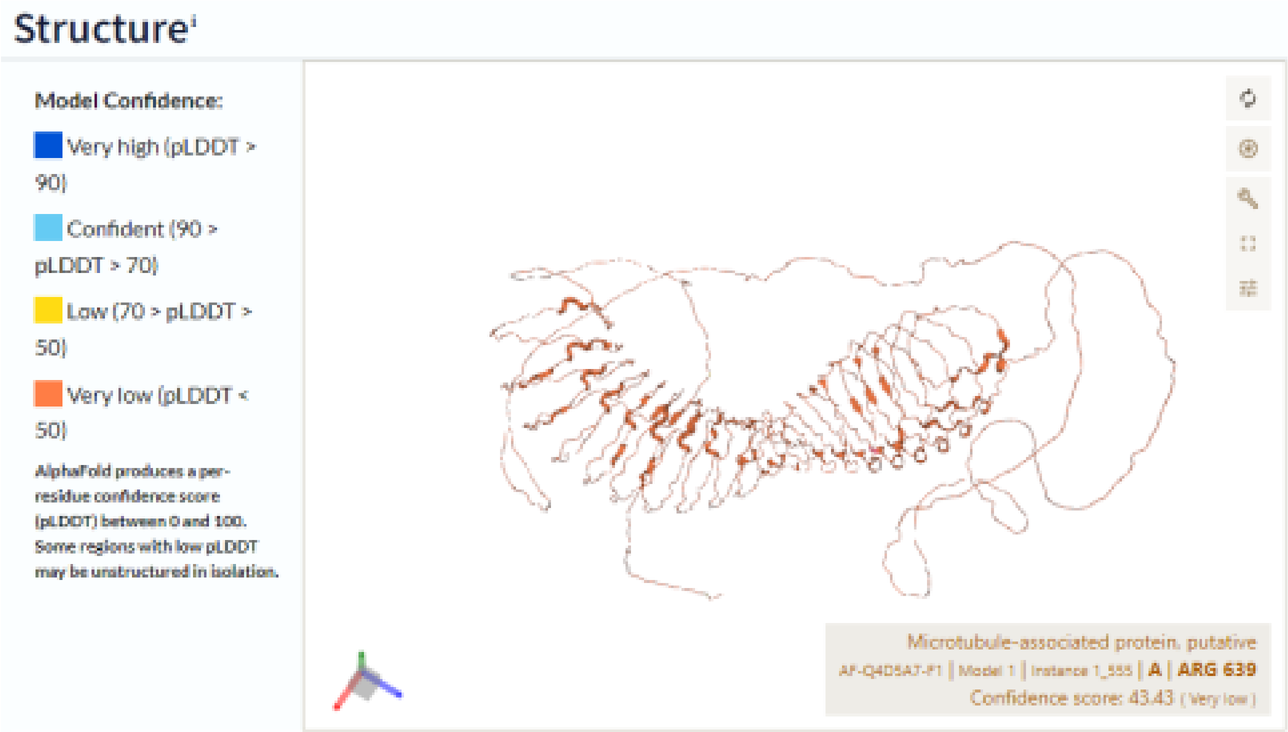
1.1	8	-TTQDAYRPVDPSAYK-	two	6249_2 [2 - 46] | freq=5	TTQDAYRPVDPSAY K
1.1	9	-TTQDAYRPVDPSAY--	two	7104_2 [2 - 43] | freq=5	TTQDAYRPVDPSAY
1.1	10	-TTQDAYRPVDPSA---	two	18760_2 [2 - 40] | freq=2	TTQDAYRPVDPSA

Source: Epitope Clustering, 2022.

From the consensus sequence, the BlastP tool was used[31]from the NCBI, to obtain the proteins that most closely align with this sequence. There were 45 proteins that obtained 100% identity with the sequence, however, from the data obtained from the corresponding epitope found in the IEDB, the protein that best represents this possible epitope was the 6th in the BlastP list, called “microtubule-associated protein” code XP_809567.1, from the strain of T. cruzi CL Brener.

Then the protein was found in the UniProt database, where it was possible observe a structural prediction of the molecule, as it is possible to observe in theFigure 4.

Figure 4: Prediction of the Structure of the Protein “Microtubule Associated Protein”



Structure

Model Confidence:

🟦 Very high (pLDDT > 90)

🟦 Confident (90 > pLDDT > 70)

🟨 Low (70 > pLDDT > 50)

🟧 Very low (pLDDT < 50)

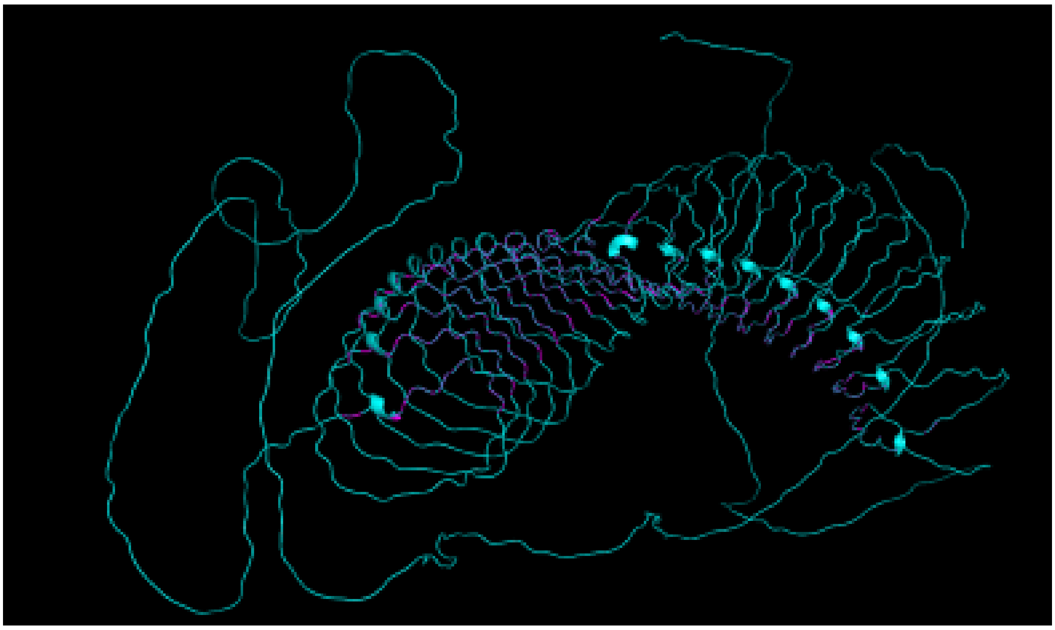
AlphaFold produces a per-residue confidence score (pLDDT) between 0 and 100. Some regions with low pLDDT may be unstructured in isolation.

Microtubule-associated protein, putative
AF-Q4D5A7-F1 | Model 1 | Instance 1_555 | A | ARG 639
Confidence score: 43.43 (Very low)

Source: UniProt 2022 the unreliable in yellow and the very unreliable in orange.

Then, the protein was opened in the PyMol viewer, and the representative part of the “STTQDAYRPVDPSAYKR” consensus sequence that aligns in the protein was colored, as seen in thefigure 5.

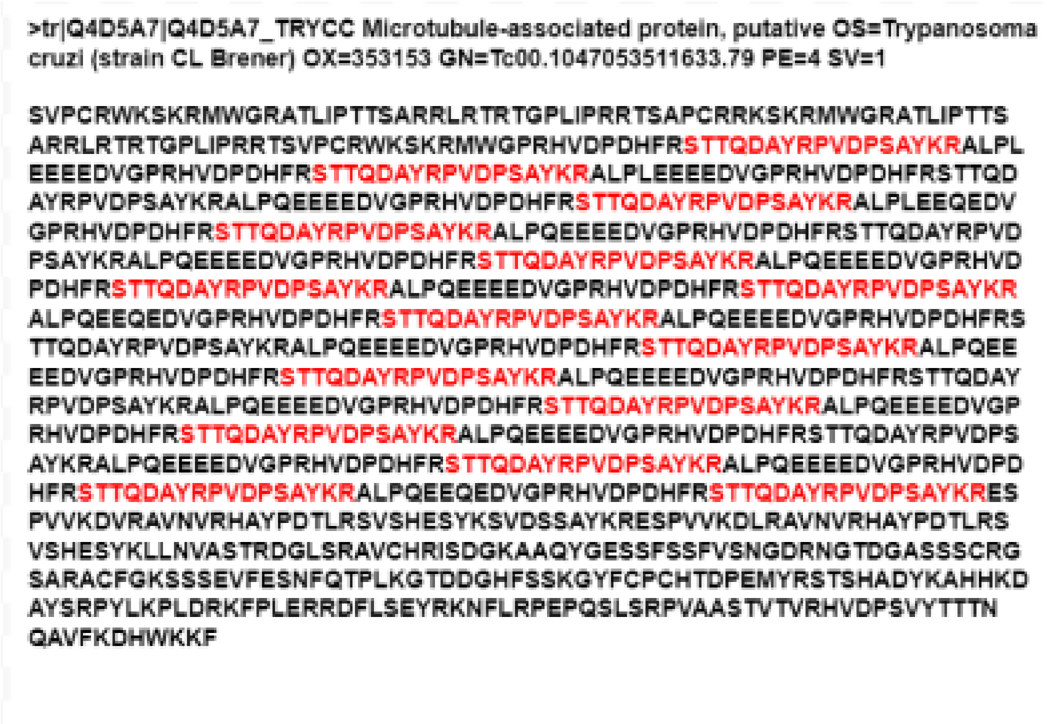
Figure 5: Consensus sequence represented in the molecular structure of its corresponding protein.



Source: Stéffani Vibanco de Oliveira Neves via PyMol, 2022 . Caption: The protein is represented by the cyan color, while the consensus sequence is represented by the magenta color.

Therefore, it is also possible to identify the consensus sequence contained in the complete protein sequence, asFigure 6.

Figure 6 : Complete protein sequence highlighting the location of the consensus sequence



```
>tr|Q4D5A7|Q4D5A7_TRYCC Microtubule-associated protein, putative OS=Trypanosoma
cruzi (strain CL Brener) OX=353153 GN=Tc00.1047053511633.79 PE=4 SV=1

SVPCRWKSKRMWGRATLIPTTSARRLRTRTGPLIPRRTSAPCRRKSKRMWGRATLIPTTS
ARRLRTRTGPLIPRRTSVPCRWKSKRMWGPRHVDPDHFRSTTQDAYRPVDPSAYKRALPL
EEEEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKRALPLEEEEDVGPRHVDPDHFRSTTQD
AYRPVDPSAYKRALPQEEEEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKRALPLEEQEDV
GPRHVDPDHFRSTTQDAYRPVDPSAYKRALPQEEEEDVGPRHVDPDHFRSTTQDAYRPVD
PSAYKRALPQEEEEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKRALPQEEEEDVGPRHVD
PDHFRSTTQDAYRPVDPSAYKRALPQEEEEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKR
ALPQEEQEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKRALPQEEEEDVGPRHVDPDHFRS
TTQDAYRPVDPSAYKRALPQEEEEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKRALPQEE
EEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKRALPQEEEEDVGPRHVDPDHFRSTTQDAY
RPVDPSAYKRALPQEEEEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKRALPQEEEEDVGP
RHVDPDHFRSTTQDAYRPVDPSAYKRALPQEEEEDVGPRHVDPDHFRSTTQDAYRPVDPS
AYKRALPQEEEEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKRALPQEEEEDVGPRHVDPD
HFRSTTQDAYRPVDPSAYKRALPQEEQEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKRES
PVVKDVRAVNVRHAYPDTLRSVSHESYKSVDSSAYKRESPVVKDLRAVNVRHAYPDTLRS
VSHESYKLLNVASTRDGLSRAVCHRISDGKAAQYGESSFSSFVSNGDRNGTDGASSSCRG
SARACFGKSSSEVFESNFQTPLKGTDDGHFSSKGYFCPCHTDPEMYRSTSHADYKAHHKD
AYSRPYLKPLDRKFPLERRDFLSEYRKNFLRPEPQSLSRPVAASTVTVRHVDPSVYTTTN
QAVFKDHWKKF
```

Source: Stéffani Vibanco de Oliveira Neves, 2022. Reference: UniProt

5. CONCLUSION

In summary, a bioinformatics pipeline was used in the samples, obtained from the Phage Display in the article by Teixeira et al, in which there was an initial treatment of the genome, from conversions, frequency filter, nucleotide alignment and identity filter; a treatment of the proteome, from obtaining ORFs, frame filter, protein alignment and identity filter; And finally, a clustering and data analysis, from obtaining k-mers, then clustering and identification of potential epitope sequences and a consensus sequence.

Finally, it is possible to observe that bioinformatics is an invaluable tool for the production of new technologies aimed at human health. We also conclude that the flow traversed in this project has great potential for a possible identification of epitopes.

As for the list of epitopes generated by the pipeline, we can see that there is a large amount of sequences that were not found in the IEDB, showing a possibility of identifying new epitopes.

With regard to the cluster chosen for further analysis, it was possible to notice that despite the epitope being available in the database, there are few tests on its biological and structural function. This pipeline has the ability to illuminate the possible epitopes, calling the attention of the scientific community and instigating in-depth, individual research and possible applications in the health area of each epitope.

6. LIMITATIONS OF THIS WORK

As with any new methodology, complete epitope validation should ideally be confirmed experimentally in an independent patient validation cohort.

Within this framework, the provisional antigens presented (Appendix AandB) should only be considered as candidates until they are unequivocally proven experimentally.

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```
K-mer Frequency Unique Sequences Sequences in TheLlargest Cluster Low Frequency | Bigger Frequency | Medium Size | Standard Deviation Cluster ID Representative Sequence ID Sequence QAAAGDKP 2013 600 582 14|81|51.0|15.76 0 44523_8
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EAAKAVETEKQRAAEAMKVAEAEKQRAAEAMKVAEAEKQKAAEAAKAVETEKQRAAEATKVAEAEKQRAAEAMKVAEAEKQRAAEATKVAEAEKQKA
MRRGQQAQ 945 945 228 21|107|62.0|19.86 2 5433_3 ARESADNMRRGQQAQPHSPHTTRRHPRNEGTQRQQPPTMQEEKTSTTISPSSFPQPQGNGKTPRPPHNAHGASEPSSIHHAAQKFPVIPPSMGHGAQLSAVTRGAPH PQHRPPPT 747 747 745
25|100|71|16.12 0 41354_5 AASTRRPGSPSNKTPPRSPPSTRIRSAQAPQWSPTRSQPHAQTPRACTAGSRCQQTPPQSRRWPQHRPPPTRTAPAPSALTARPSTALRATTAPCRQQTT RGQQAQPH 741 741 174 18|110|57.0|17.7 1 45241_4
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TIQRLVRAMEGATDMPVACTPRVTEGLRLVDGRFSTKMPEERCTPGVFETTGLRLIDDVGSDAVLQW RAQELARE 572 486 339 53|103|76|9.31 1 48266_7
NARAQELAREKKLADRAFLDQKPEGVPLRELPLDDDSDFVAMEQERRQLLEKDPRRNAREIAALEESMNARAQELAREKKLADRAFLDQKPEGVPLRELPLDD
RGQQAQPH 567 567 126 22|104|55.0|20.89 1 45610_3 RERDGVQRIQRERADNMRRGQQAQPHSSHTTRRHPRNEGTQRQQPPTMQEEKASTTISPSSSLQPQGNGQTPRPPQQPHAPANPHPSMTRQQNLLPSRPQWDTE HSSHTTRR 487 487 130
21|99|79.0|17.63 2 17653_5 RSGVAGVSQLISDTEGKEWNERKRDGAQRIQRESADNMRRGQQAQKHSSHTTRRHPREEGTQRQQPPTMQEEKTSTTISPSSFPQPPHNAHGASEPSSI RETEHSGY 448 448 237 12|46|14|2.51 0 31889_3
TGGRETEHSGYSERERRQHEERAAGPATQSTHHETASTRGGHTTPA AKAAAAPA 441 84 83 18|80| 51|15.0 0 9036_3 AAPAKAATAPAKAATAPAKAAAAPAKAAAAPAKAAAAPAKAAAAPAKAAAAPAKAAAAPAKAAAAPAKAAAAPAKAAAAP RGQQAQPH 407
407 91 42|101|80|12.37 1 33559_5 RSGVAGVSQLISDTEGKEWNERERDGAQRIQRESADKMRRGQQAQPHSPHTTRRHPRNEGTQRQQPPTMQEDNTSTTISPSSFPRPPHNAHGAGKPSSIHH
PAAKPAAK 367 198 193 12|70|26|9.33 0 20739_3 GSMPAKSANKPASKPAAKPAAKPAAKAPAPKAEKKGAAKAPAPKAAAPAPKAAAAAPKPAVRDAKQRSDA PAAGGFGS 355 152 138 17|82|52.0|14.66 0 28449_3
ATTTSAPAAGGFGSATTTSAPAAGGFGSAAHTSTPAVGGFGSATTTSAPAAGGFGSATTTSAPAVGGFGSAAHTSTPAAGGG HSSHTTRR 354 354 103 44|89|63|8.68 8 41790_3
RSGVAGVSQLISDTEGKEWNRRERNRAQRIQRERVDNTKRGQQAQPHSSHTTRRHPPPPSPPAHFHNPREMAESPAATTQHTRRKRTLS QPHSSHTT 276 276 74 19|116| 103.5|26.36 1 34204_6
RSGVAGVSQLISDTEGKEWNERERDGAQRIQRERAENMRRRQQAQPHSSHTTRRQPPPSPPAHSRSPGKWPKPRGHHTTHTEQAPTTPATRASEPSSIHDAATKFTAIPPSMGHGA RGQQAQPH 241 241 61 34|99|43|18.56 1 2349_4
VSQKEKEWNRRERDGVQRIQRERADNMRRGQQAQPHSPHTTRGHPRNEGTQRQQPPTMQEEKTSTTISPSSSLQPQGNGQNTAATIPATRASEPSSIND GNAGGLAP 212 212 212 27|75|51.0|5.91 0 44846_5
PQTVPQPAPETKAPPQSPCCDKRAGNAGGLAPHFRFGRPDRKKDTAEETRTGRPRAVCPKHRHGIGESVTLINGC RETEHSGY 211 211 211 15|27|23|1.18 0 34666_1 SVGQKKKNGTGGRETEHSGYSERAQTK EQERRQLL 191 186 146 28|105|59.0|14.62 0
45628_9 QATVNAMPAKKTEESYPYIEANPEGIHKQHLELNKDSKFLALEQERRQLLEKDPRRNAREIAALEESMNARAQELAREKKLADRAFLDQKPEGVPLRELPLDDDS VDPDHFRS 191 162 157 21|82|43|12.49 0 26659_6
PDHFRSTTQDAYRPVDPSAYKRALPQEEEEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKRALPQEEQEDVGPRHVDPDHFRS HSSHTTRR 184 184 92 22|98|63.0|15.79 1 20148_5
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RLAEELEQKAAENEKLADELEQKAAENERLADELEQKAAENEKLADELEQKAAENERLAEELEQKAAENE ANGIRPQP 149 149 60 37|87|65.5|14.44 4 43425_3
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QATVNAMPAKKTEESYPYIEANPEGIHKQHLELNKDSKFLALEQERRQLLEKDPRRNAREIAALEESMNARAQELAREKKLADRAFLDQKPEGVPLRELPLDDDS VDPDHFRS 191 162 157 21|82|43|12.49 0 26659_6
PDHFRSTTQDAYRPVDPSAYKRALPQEEEEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKRALPQEEQEDVGPRHVDPDHFRS HSSHTTRR 184 184 92 22|98|63.0|15.79 1 20148_5
RNRAQRIQRERVDNTKRGQQAQPHSSHTTRRHPPPPSHPAHFHNPREVAKTPRPPHNTHGASEPSVSNDAATKFTLPSSPHWNTEPNPTQPQEKRRGL LEQKAAEN 170 49 49 36|70|55|6.69 0 43642_2
RLAEELEQKAAENEKLADELEQKAAENERLADELEQKAAENEKLADELEQKAAENERLAEELEQKAAENE ANGIRPQP 149 149 60 37|87|65.5|14.44 4 43425_3
RHGPQPGRQLHTSPQTHTQPAVTHQGRRGRENHRHRLQFNPMANGIRPQPRSPHTKHSKQSPKEPHAEAIPHPSTTWRQNFQPFHPK62 0 45628_9
QATVNAMPAKKTEESYPYIEANPEGIHKQHLELNKDSKFLALEQERRQLLEKDPRRNAREIAALEESMNARAQELAREKKLADRAFLDQKPEGVPLRELPLDDDS VDPDHFRS 191 162 157 21|82|43|12.49 0 26659_6
PDHFRSTTQDAYRPVDPSAYKRALPQEEEEDVGPRHVDPDHFRSTTQDAYRPVDPSAYKRALPQEEQEDVGPRHVDPDHFRS HSSHTTRR 184 184 92 22|98|63.0|15.79 1 20148_5
RNRAQRIQRERVDNTKRGQQAQPHSSHTTRRHPPPPSHPAHFHNPREVAKTPRPPHNTHGASEPSVSNDAATKFTLPSSPHWNTEPNPTQPQEKRRGL LEQKAAEN 170 49 49 36|70|55|6.69 0 43642_2
RLAEELEQKAAENEKLADELEQKAAENERLADELEQKAAENEKLADELEQKAAENERLAEELEQKAAENE ANGIRPQP 149 149 60 37|87|65.5|14.44 4 43425_3
RHGPQPGRQLHTSPQTHTQPAVTHQGRRGRENHRHRLQFNPMANGIRPQPRSPHTKHSKQSPKEPHAEAIPHPSTTWRQNFQPFHPK79 1 20148_5
RNRAQRIQRERVDNTKRGQQAQPHSSHTTRRHPPPPSHPAHFHNPREVAKTPRPPHNTHGASEPSVSNDAATKFTLPSSPHWNTEPNPTQPQEKRRGL LEQKAAEN 170 49 49 36|70|55|6.69 0 43642_2
RLAEELEQKAAENEKLADELEQKAAENERLADELEQKAAENEKLADELEQKAAENERLAEELEQKAAENE ANGIRPQP 149 149 60 37|87|65.5|14.44 4 43425_3
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4 47|65|56.0|6.71 5 11612_3 HNKRTHCQQSTTVDKDKKRHTATASSSIPRTTGYGHSPAVNTQRTPSKQSPKEPHAEAIPHPSMT PPPSPPAH 26 26 3 72|94|76|9.57 0 9491_3
DTAREREQTTRGEGSRPSHTVHTPRNGIHERKEHNASSHPPCRRRRHPPPSPPAHSHSQREMAENRGHHTTHTERAPTTPVTRASEPSSIHHAA RGQQAQPH 26 26 4 41|91|68.0|23.57 0 3868_4
EEKECNKRERDGAQRIQRERADKTRRGQQAQPHSLHTTRRHPRNEGTQRQQPPTMQKEQTSTTISQLIPQPTGTGHSPRSPHNAHGASEPS TPTPIEPH 25 25 3 18|52|22|15.17 1 43964_3
TQSNLIYTSQHHSPSNITQSNINTVTINHNLCYITPTPIEPHLPENSANSTK THRHRLQF 25 25 4 54|86|74.5|13.01 1 42220_5 KRHPHSKHTTPVVTHQQGEEVTHRHRLQFSPTDNGIRPQPRSQHTTQTEQAISQRATRRSDSSSIRDEAPHSMRSHGAHHFLNRTH
HRLQFNPT 25 25 2 72|73|72.5|0.5 3 4982_3 TQQTHTPPAATRHGRGEETQRHHRLQFNPTANGIRPQPRSQHTAHTEQAITQRATHAEAIPHPSTTQRPTPSG THTHSPAA 25 25 7 28|74|47|14.7 0 33225_5
DLHSEGHGEQQGRREVRQTHTEGTPHEERVAARHAPYAAHHAQHTLHSTAAGTHTHSPAATPTHTWKREKQTGA VQQARRTG 25 25 19 12|58|16|12.61 0 42724_1 LRVSASPRRKENKTRGKEMCAPPHRPSSHAHTSRLPQRAVRRGTSSCVQQARRTGPSS
TTTTTTTT 25 12 3 41|54|49|5.35 1 27931_3 TTKPPTTTTTTTTKPPTTTTTTTTTAPEAPSITTTETPNTTTTRAPSSIRRIDG PKSAEPKS 24 4 4 48|77|61.5|11.9 0 47942_5
PFTEKKPAKASTATSPSVEHVTTPVATEPKSAEPKSAEPKSAVPKSAEPKSAEPKSAEPKSAEPKSAEPKSAEPKSA FHPSTPLP 24 24 17 27|73|42|12.14 0 10269_5
EASATRDVANDGVSTLPHQTDILMAGDVSSSAARRSSAAHRPPHGNAARPRFHPSTPLPSRTSPLDARLPMEE ERREARER 24 14 14 35|47|42.0|3.36 0 40736_4 REERRGVEERREARERARHEAKERREARERARLQQKLELTVTTTIKD GGRKCPTL 24 24 24
21|81|51.5|15.94 0 36331_5 KQRKKGIAESNSLPDVRRCEGGRKCPTLRPHTPHAFARPHSPSRSSSGSSSLPYAEPASTFAPPSPASRAVPSFPEEVKLP WRWAWHSH 24 24 13 17|46|29|9.16 0 33917_4
SQWRWAWHSHGGAVGAPCSRTATPVGEAAEAHRPRSNATCDRQKHR SHTTRRHP 24 24 4 52|83|70.5|11.51 1 25333_2 ADNMRRGQQAQKHSSHTTRRHPRKEGTQRQQPPCRRRRHPPPSPPAHSRSPREMAKTPRPPHNTHGASKPSSIHHAAQKFPAI
QQITGTHV 23 23 23 31|63|40|7.81 0 42302_4 SYVRPWPSHAEHSTTGAADRHFPAHRHHKHAQQITGTHVRTPNKGGQRQRQTEQRTRTKPTTS YYTNPNRT 23 23 2 36|61|48.5|12.5 1 47345_4
RLKVYHSIHHYIQPYQNHNHKLISYTILYYTNPNRTPPPRDNPHFQPNNVRGRCMNFPPQI HRLQFNPT 23 23 2 30|59|44.5|14.5 4 2506_3 VTHQGRRGRENHRHRLQFNPTANGIQPQPRSQHTKHSKQSPKEPHAEAIPHSTWR
TPTPIEPH 22 223 25|48|31|9.74 1 19664_3 KSINKQNHTKDFITLHPPTTAYITPTPIEPHLPVKSANFQKIMYGGDA SPPESFTA 22 22 15 22|22|22|0.0 1 14447_3 FLFTALRRIDASPPESFTAAPR LTVRHGAH 22 225 24|61|43|13.020 43962_4
TKETDTNSRRRALLLSSPADWRPTLTVRHGAHHPLPSECTQQAGAQSPSCHRKGKNKQSSS HESFLSLE 22 22 15 42|102|85|16.48 0 21607_7
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LDRAIAAAPDAEDGRRLDLRRREALREASKSRWHTLRSELEGTDGNRQHVVRPCLSP NPRQQAHT 22 22 6 30|44|35.0|5.25 6 36711_3 PGMHRTPHTTHDTHSNPRQQAHTHTHSPAATPTHRWRWEKQTGA
QALGRHSH 21 21 17 26|41|32|3.79 1 33861_3 VSTTLPPRHAQGRSQALGRHSHGGRADGYRQQGPLSTVTDA VYEKPHPQ 21 21 21 40|99|65|12.120 19240_7
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YNTKINKYHKPTVYYTNPNRTPPPRKIHGFGRNNVRGRCMNFRPQI THRHRLQF 21 213 25|72|66| 20.892 24074_4 TPSHSAHTAKRHPHSKHTTSVVTHQQGEVTHRHRLQFSPTDNGIRPQPRSQHTTHTEQSPKEPHAEAIPHPS SKQSPKEP 21 214
45|62|54.0|7.795 44733_3 IASSSIPRTTGYGHSPAANTKHSKQSPKEPLAEAIPHPSMTRRQNFQPFQPKWNLMPDSKRS PPPSPPAH 21 213 52|77|63|10.23 1 21141_4
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HEERAAATHAPYAAHHAQHTLQSTAAGTHTHSPAATPTRRWNGEKQTGAAHKAPPHAHTAR PTASTHRE 20 20 10 33|48|39.5|4.32 23265_4 TRTQRMHPQRLPTASTHREVRALEWEGRSRQRTQKEEKHAEPHPSTNS HRLQFNPT 20 20 2 50|57|53.5|3.5 5
47004_3 QQLWRTACVNCETAPTQQTHTPPAATHNGQEATHRHRLQFNPTANGIRPQPAAGTQR AVHHHRPS 20 20 11 27|70|40|11.620 39947_4 HLAPRHAQHSALQRNHARTRVKRNVATEHSRVQRDTATDKLQHSAVHHHRPSTSHHRHEGNAGHNQHGPH
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17 17 17 26|38|26|2.820 10549_3 AEAMKRFATQKEKSEKFIQENLDRQDEAWRRIQELERV LTVRHGAH 17 175 26|44|29|6.692 2951_4 HSRSWQWRPTLTVRHGAHHPLPSERTHQGAQSPSHHRNGETQTI RHPRATRW 17 17 9 25|71|40|12.18 0 27447_4
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17 12 20|39|32.5|5.572 44900_4 SLGEEEVPLTGEAPLGLVHFCFGACGEDAGQKTKVTVKN QPHSSHTT 17 17 4 20|88|43.5|29.69 1 9826_4
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46|80|62|9.96 0 43818_3 RSQSSLERNNTPAQAPRRTQQEFRSGSQGVRRGASHEQRGPQSSTATPSLPGRREVAQGLPHAGFRGQLTRPKNPYAAIA HRHRLQFN 15 15 3 58|85|81|11.9 0 2292_4
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27|44|35.0|5.450 40702_3 TRTRPHHTPVKTFGPSHAKDWSGRTAGKTTPAAMSSPHTPTHRW HLPHHHPF 15 15 10 37|59|45.5|6.930 41381_4 TLLTQSQDPTVPAEAAAAAAPSRRRKQHHHLPHHHPFLVPSLHSQQKSSVPLAHVGYWR THSNPRQQ 15 155
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QTHHHFNQLSIKYHNNNLYYTTKLNHNDINYNNHIHHPYPKMLYYTNPNRTPPPRKNPKIPEIMYG HRQRRPSW 14 146 21|68|39.5|17.530 17755_3 SYSSFGASSSSSAAAVTALHHAPAAITLTEREECVGVCRQHSDRHRQRRPSWHRHPFSTERQPTSLVT
TTPGDSDG 14 14 14 21|25|25.0|1.030 23583_3 AATEKRQNVNNYTTPGDSDGSTAVS TQRQQPPT 14 143 37|70|57|13.574 34008_3 HSPHTTRRHPRNEGTQRQQPPTMQEEKASTTISPSSSLQPQGNGQTPRPPQQPHAPANPRPSITRQQNSL RPASAPCG
14 14 9 35|52|49|6.08 1 10880_5 PSPSSPSCSPVQRTRPASAPCGRRRPQSVTSAHPSRGAAVSATPASSRPHRH THRHRLQF 14 142 30|54|42.0|12.03 20835_3 STQQAHTPPAATHRGQGQEATHRHRLQFNPTANGIRPQPRCQQTQRTPSEQSPK52 1084_3
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30|54|42.0|12.03 20835_3 STQQAHTPPAATHRGQGQEATHRHRLQFNPTANGIRPQPRCQQTQRTPSEQSPK52 1084_3 IQRRGWQLDRGRYHPSPPPPPPPPTTTTTTTTHSHD YYTNPNRT 15 15 1 66|66|66|0.00 37653_6
QTHHHFNQLSIKYHNNNLYYTTKLNHNDINYNNHIHHPYPKMLYYTNPNRTPPPRKNPKIPEIMYG HRQRRPSW 14 146 21|68|39.5|17.530 17755_3 SYSSFGASSSSSAAAVTALHHAPAAITLTEREECVGVCRQHSDRHRQRRPSWHRHPFSTERQPTSLVT
TTPGDSDG 14 14 14 21|25|25.0|1.030 23583_3 AATEKRQNVNNYTTPGDSDGSTAVS TQRQQPPT 14 143 37|70|57| 13.574 34008_3 HSPHTTRRHPRNEGTQRQQPPTMQEEKASTTISPSSSLQPQGNGQTPRPPQQPHAPANPRPSITRQQNSL RPASAPCG
14 14 9 35|52|49|6.08 1 10880_5 PSPSSPSCSPVQRTRPASAPCGRRRPQSVTSAHPSRGAAVSATPASSRPHRH THRHRLQF 14 142 30|54|42.0|12.03 20835_3 STQQAHTPPAATHRGQGQEATHRHRLQFNPTANGIRPQPRCQQTQRTPSEQSPK530
17755_3 SYSSFGASSSSSAAAVTALHHAPAAITLTEREECVGVCRQHSDRHRQRRPSWHRHPFSTERQPTSLVT TTPGDSDG 14 14 14 21|25|25.0|1.030 23583_3 AATEKRQNVNNYTTPGDSDGSTAVS TQRQQPPT 14 143 37|70|57|13.574 34008_3
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30|54|42.0 |12.03 20835_3 STQQAHTPPAATHRGQGQEATHRHRLQFNPTANGIRPQPRCQQTQRTPSEQSPK530 17755_3 SYSSFGASSSSSAAAVTALHHAPAAITLTEREECVGVCRQHSDRHRQRRPSWHRHPFSTERQPTSLVT TTPGDSDG 14 14 14
21|25|25.0|1.030 23583_3 AATEKRQNVNNYTTPGDSDGSTAVS TQRQQPPT 14 143 37|70|57|13.574 34008_3 HSPHTTRRHPRNEGTQRQQPPTMQEEKASTTISPSSSLQPQGNGQTPRPPQQPHAPANPRPSITRQQNSL RPASAPCG 14 14 9 35|52|49|6.08 1
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1 58|58|58|0.00 25282_2 HHTISYCRHLAINTPQQQLNKHLSILTRNHTLYYTNPNRTPPPRKFHKSGKIMYGGDA TRAPSRLR 14 145 26|39|34|4.17 1 20766_3 TSSATTTTTTTKAPITTTTEAPTTTTTTTRAPSRLREID PTQRSHTR 13 132 47|74|60.5|13.50
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13|49|28.0|11.780 38438_4 TSTGTTTAYKSPNNSPGLPAAVVTHTDEQQQHKSARNNTAPHRHHRSHH
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MKVAEAEKQRAAEATKVAEAEKQRAAEATKVAEAEKQRAAEATKVAEAEKRKAAEAAKAMESQKQRFLERFAVLEEEKK LRSQGATF888 33|59|39.5|7.730 28960_5 KTVEVLRSQGATFGPVKAERKGKDAAAPARTEKKPKAAAAAADGAEEEDEAPREKKKPN
MIRENKKK 8 8 6 44| 63|54.5|5.74 1 37355_5 HVVDDALALLYPSVSLQAAETASFSGRREHIEHMIRENKKKKGEKGHETDGKKEETLRQLRED RQLHSIAR885 44|58|52|5.490 26639_3
PHSPPQPGSVARRQLHSIARPTRISEGYLCPPGRRSSTAAEDRCVPRTHTIRTPHTSP HTHTHTHT 8 7 2 39|40|39.5|0.5 1 26802_3 AGMRGTFEQPHRVAAQREHTHTHTHTRKRETEPERGPQEH TVRRSVQK888 48|70|62.0|7.680 16948_6
RPGTHSPTHTGNHTTSNDSSRTVRRSVQKDTARTHEKTAPSPPDRKHSPRDEGSGVEKAGEKKRNNKSHP
SGVRQRGC883 37|43|37|2.83 1 21592_3 PVAECWHAQPAHTSSAHSGVRQRGCRKQQRGTHPREAAEAAVD THTHRERE882 51|53|52.0|1.00 38920_4 VHSTTKSNKKTKQKAEAWDYQRTHTLIAPVKQNTHTHRERERGRPLNAIKTKQ TQQTHTPP882
65|78|71.5|6.50 26010_5 RSSTTHTEETNDRRGKHRQRPHPDRQLHTSPHTHKHTSSRCRQSPGERICSSTQQTHTPPAVTHNARGEATHSHHRLQ SSGERENP885 30|53|47|9.270 12928_6
STNADASRRFSSGERENPPKRRGGGGGCCSEEGFKGSTVRERCLQRSRCTWDS PHTLDKRT886 28|57|47.0|9.910 22102_4 LATVRTPVTPPSPHTLDKRTWAYRPWHAQTHRNKWEENSVQQRPHADTQNHTRHNSG GGAVGVRC885 31|38|33|2.42 1
5341_3 ERRCAVRDGRWSVARCGCGGERRGRTGGAVGVRCGGAE
QQQQQQQQ 8 2 1 61|61|61|0.0 0 41045_4 NIKQQQQQQQQQQPLQQKKKEKDYAAASVSPKGSGGAINTHTHTHTQSTRTHTHKKRERER TTTTTTTT84 1 38|38|38|0.00 7600_3 TTTTTTTTTKAPTTTTTTTTEAPTTTTTHAPSSIRKID HTPIHGSR882
21|40|30.5|9.5 1 15880_3 DTHRGHTTRGEGSSQGCTVRRTPRTTHTPIHGSRHTHTQP
DRVHTARS 8 8 8 29|78|43.5|18.85 0 15747_5 TAMGELTDDSPPPRQITSSQQRTPCEGCQWRSASPDRVHTARSSPMKRTPRGESPARPAWNQNRPTPRVKPTVSAPRT LYYTNPNR88 1 35|35|35|0.00 43633_3
PFHSHSLYYTNPNRTPTSRDNPQFWQNNVRVRCMN PNRTPPPR88 1 34|34|34|0.00 4199_3 HKHAIHNKPKHNHYSLLYYTNPNRTPPPRDNPQN WTRKDSKT 8 8 8 51|103|74.5|15.51 0 44656_8
LELLSGDMQHEAWEKTFSRHPVIHSIAQDMWTRKDSKTEEEVVTIERREREEDEKRMRRMSSSDWRAKFRDRERNPTAEEDEERPIYVIKPETFALLRLQPDV TRSGGSCR887 14|33|18|6.310 6269_1 RRRKPTACSEPLWSCHLRSTRSGGSCRCRRYHA
GPRGGGAE 8 8 8 28|35|30.0|2.28 0 12972_4 GRERRWGPRGGGAETPVRGLGHRLGSNGRRDAAEI
VQSHGQRD 8 8 3 56|59|59|1.41 1 44277_5 TVSQQNAGRRRQPRCHANNSVREPSQRCQSAGERIRPSTQQPHTPPPPPVQSHGQRDAA SMGRRSCD888 25|37|28.0|4.30 14252_5 WMRASSPADVLSMGRRSCDRPVFRPLSQSAPCRRQKE RVTVRCGP 8 8
8 16|26|19.0|2.93 0 20166_3 PPHTRRVTVRCGPPSRADERAEGSSN
KLRLQLRE885 55|70|63|5.9 1 21101_7 AALGEASGKLEAEELQRQLDALRRQKDKLRLQLREARRGEEKLDILRRHNEDLQSRLNDARRGQEKLDAV GGRGGDRG 8 3 3 32|55|38|9.74 0 18702_4
TKIQRTEQAGFRGGNSGGFRGGRGGDRGGFRGGRGGDRGGFHGGRGGDRGGFRGG LWATLPGD888 13|30|19.5|5.320 27923_4 LWATLPGDGGVTKGGIILSGHTDVVPVDGQ SPPSAGRQ776 35|69|53.5|10.95 1 28321_2
SARAHSSKRETTDQQIRRERQRIGVHSLLSPSPPSAGRQRHLLAPPHTKPAAGRDHAKNPQKHRKQTVL GKLRWRFQ 7 7 7 26|35|32|3.36 0 12648_3 VVFCPGTDGKLRWRFQGEKDWRKCARRPEEAPEVN PTQRSHTR772 30|50|40.0|10.03 14578_1
KTRSHHTTHTEQANPHPSITRHKISCHPAINGTRRPTQRSHTRCTTPAGK TRQPVTPH772 30|42|36.0|6.02 28862_4 NLKYTQKIKSIRDICYRGSNSVLGGRRCSTRQPVTPHSAANP YITPTPIE77 1 60|60|60|0.00 38933_4
NYNKFNSKSFLTNSLHNTQPFSKSNSTYKIHRIHFNLTHANPYITPTPIEPHLRVTTPKF RGCFVPQS 7 7 3 36|41|38 |2.05 2 27662_3 LAPKDSRGCFVPQSQWRWAWHSHSSVVGAPCSRTATPVGKT AEGTHQQH 7 7 1 68|68|68|0.0 0 15069_5
RKHPSCPLSSCRTPPRIPSAAERAARDQTLNRTAITSASNIGRHHTNTAPEHAEGTHQQHTASHHHRH RAAHQGSG777 35|56|46|6.170 44782_3 AEVQQTVVRAAHQGSGQNAARDEKKRQQRQQRQQQKQQQSDEMQSGTCDATALDTI ARRGHTPA 7 7 3
40|46|42|2.49 1 18322_4 TTARRGHTPAAHGQSPSSPPLPSLACSLQHKPTPRQRSENKERNTT ARHAPYAA 7 7 2 38|45|41.5|3.5 2 6913_3 PHEERAVARHAPYAAHHGSRHTHTQPCRNTNTQMEEGEANRSGTQ CSRVSRPP773 37|41|40|1.73 10916_4
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TKMPEERSTPGVFETTGLRLIDGVGSDAV PPPPPPTT774 34|36|34.5|0.83 1 25116_3 IQRRGWQLDRGRYHPSPSPPPPPPPTTTTTTTHSHD TAGWRRQG773 43|45|44|0.820 45876_4 ITSFAASGQRGKMERYAENAMPLHNTAGWRRQGSERFYPTLRRGQ
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24|36|30.0|6.05 8136_4 EGERRSAADTARERADKMRRGQQAQPHSSHTTRLGM RHPRNEGT77 1 90|90|90|0.00 19168_3
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MAGGVPSSATRRGSAAHRRPPDNVGRPHFHPSTPLPSRTSPLDARLPMEEDRSFC PSSCVQQA773 21|38|22|7.79 1 28484_1 LSAIKNKTRGKEMCAPPHRPSSRPSSCVQQARRTGPSS RNTGEKGE 7 7 6 28|41|34.0|4.02 1 1677_4
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GKKVRSSTTHTEETNDRREKHRHGPRPGRQLHTLKHTHSQQSPTRDEKEER RRWERRPD776 18|46|34.0|9.670 10334_4 SGWWEPDAERHSRRWERRPDAADGGAAAAVPLGKRPAARHAPELRA LPVPSTGE777 50|80|74|10.180 20957_5
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30708_2 HMRRGQQPGMHRTPHTTHNTHSNPRQHTHTQPCRNTNNTHGRGRSKQERHT QRAKDGQR 7 7 2 56|64|60.0|4.0 2 30035_3 RRTNPGTPIVAEGLTCGFCDSSTVSPTSASRAGHHQKYQTYQRAKDGQRTSASSNDSRSPSAIP PFPPSSSG776
30|35|31.5|1.57 1 31214_4 WGLVGRSPFPPSSSGKAREGSRRIPTADARWLKEA AGHNQHGP776 22|43|34.5|6.80 44773_3 ATDKLQHSAVHHHRPSTSHHGHEGNAGHNQHGPHETQARQHQH CWVRQPPQ777 26 |46|34|6.160 19539_3
QTQASSRKCWSPCCMRDSDRRCWVRQPPQTVATPRSDVTAQHSLAA PNRTPPPR77 1 31|31|31|0.00 5181_3 TFNMFNHKYSNHKPPSLYYTNPNRTPPPRDN

APHRHHRS773 15|30|17|6.652 19695_1 THTDKQQQQHKGAGNNMAPHRHHRSHRHVS WRTARWWS 7 7 7 20|29|27|3.68 0 20834_3 ISFDVAWRTARWWSSHGSQWDTRPAQKSS NNHARWQH 7 7 3 24|38|28|5.89 1 28266_3
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59|64|61.5|2.5 0 4237_4 SPRARGFGPPHNKRTHRQQSPTNEREREETHRHHRLQFSPTANGTRPQPRSQQTQRILSETSGS PAANTQRT772 55|59|57.0|2.0 1 14178_3 RGFGPPHNKRTHRQQPPAMEEEKRPNATIASSSIPRPTRHGHSPAANTQRTPSKLSPKE
LGSRGHQA772 31|55 |43.0|12.0 1 23528_4 RAQTQSQQYRSTADPSERERGAAMPQGITGLGSRGHQAALHGPPAAWRKHRGGHD VAPPSPHT773 34|59|55|10.96 1 31850_3
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YITPTPIE66 1 42|42|42|0.00 15927_3 MPHIIYLSNYHHKFKTNYNESIYITPTPIEPHLPENPAVSLK TLKKGGGG663 43|107|90|27.070 37195_3
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GTHRHKPP 6 6 2 33|94 |63.5|30.5 0 5044_5 AGRDHAKNPQKHRKQTVLQRRSCDSNAKSRSAPTQQPPTHTKRGKGGGGKPAPKINRNGMKRKQCTLHYTSIKCHRSSKHPPPQTGTHRHKPPN RGGRGFGD622 50|56|53.0|3.00 6845_3
VENREENGYNGFGNRGGRGFGDRGGRGFGDRGGHGFGDRGGRGFGDRGGRGFGDRG KASSWMHS663 31|35|33|1.630 25340_1 DRWQKKASSWMHSGPSFRSDCWQTHASSRTCWSPC KECVPGVV663 25|34|29|3.682 4022_3
LHYSGHKGNETLSPDPHRRRMPWTAAKECVPGVV EARRHDGS666 15|19|15.0|1.890 29859_1 WGLREARRHDGSRVSPSGE

EKKLSLGE665 36|88|72|18.620 45297_5 EKMFQIMKPVTIQTLWKEKRAQAEEDDAATGKGAAEEEPQKSDRAKITKRDVTVPEKKLSLGEKLLLKAQERKKRERQERDGATNEEG WANDDRHC 6 6 6 25|72|45.0|15.85 0 19430_3
SHRRSQPRSLAASGMSPSRDGHKDVASNKTFRGTHWANDDRHCGMCGPSAAHLKSHFHASSSSSSGINTPVH RDGAQRIQ663 36|58|40|9.570 46128_3 SQLIRGTEKKEYNKRERDGAQRIQRESRQHGERAAGPAAHFTHHETAPTQGRHTTPAA
KWQHPSSR664 47|57|50.5|3.770 13489_4 SAQTNNTSTQGKSKRRDTAATTAHNHKWQHPSSRSPHTMRQLESVRLQDSHTALQTR LRRGQRSD664 30|43|34.5 |5.320 15763_4 ENAMPLHNTAGWRRQGSERFCPTLRRGQRSDDKACSLGTPNVV
HRLQFNPT 6 6 1 43|43|43|0.0 0 24387_3 NKRTHRQQSSANARGEATHRHRLQFNPTDSGIQPQPAANTQRT LTVRHGAH662 36|42|39.0|3.00 43888_3 TSRLTVRHGAHHPPNSKCNQQVAAQSPSCHCNGEQKQSSSRT RHPRNEGT 6 6 1 83|83|83|0.0 0
9415_4 RRGQQAQPHSSHTTKRHPRNEGTQRQQPPTMQEEKTSTTISPSSSLQPQGNGRNPRPPQNAHRACEPSSIHHAVKTSCHPAIN AGWRERSG663 24|64|43|16 .340 18729_4
GCAIAGWRERSGGKCVSAAVKGRAGCLAVEGISRPGQHRSCRAPSLSLTNPNPNPNPNPNPNPN HCRTGDRS662 27|54|40.5|13.5 1 46007_4 PTPKMSAHLHKREGTKRKGDMIPPSRRGSTLSSTHRHCRTGDRSHIAESAPQCA ERAQRDAG664
39|50|48.0|4.320 23321_5 CGGERRFRDVGGVLRWRDLRGGLRGGRGVDVGERAQRDAGELPVCGRSEP HHLPQLIP 6 6 2 46|52|49.0|3.0 2 36130_2 THHETASTQRRHTTPAATHHAGGQHIHHHLPQLIPAATTQRTRSRQTLIHPS IPAAPGKW662
21|31|26.0|5.03 17951_3 IPAAPGKWQKHRGHHNAHGASEPSSMTRHQN SATPASSR666 30 |68|55.0|13.520 23168_3 HSRTSASAPPSPTSPSCSPGQPMRPASAPCGSRHNQSVPSTHPSRGAATSATPASSRHHRHPAAPFHQ QAYGATRP666
49|80|63.5|12.150 5377_6 EDVLFYSVTTYQAYGATRPLESLRKRGYTEEEELVLGPIKRHARDARRQEQGEGGEEVEEEEEREESADALGGQLFIGDK SSTGRHAP665 30|69|45|12.850 45612_3
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48|49|49.0|0.37 0 20955_4 GHVLESNRHVSRTERNERLRGTKGRERDGEATRNPFPFPSLSPPHPHPP PQPHPPHM666 54|80|61.5|10.230 2501_5
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ERLRNVPG 6 4 3 42|47|47|2.36 0 46231_3 NEHGERLRNVPGERLRSVPGERLRNEHGKRPRNEHGERLRNVPGERL DRYTTHSH 6 6 5 21|36|33|5.27 0 26773_3 TPRQRRVEGKKEKHDRYTTHSHRTKTNGSSPPVATH HTHTHTHT65 1 47|47|47|0.00
16448_2 TTQFTHTHTHTHTHTHRLFTHQRRKPKKSPHAPPCLPKKPQKGKGEGDQ EKQTGAAH662 28|31|29.5|1.5 1 21008_3 PIHGSRHTHTHSPAATPTHRWKREKQTGAAH ELVAPAED632 45|52|48.5|3.50 45289_2
VKELVAPAENVKELVAPAEDVQELVAPAEDVQELVAPAEVVQELVAPAENVK SGDPLINR666 39|56|39.0|6.210 22042_5 DRLLGIHIRSGDPLINRAIGLHYRGGWKPWYNREDPPNSAISSTADEPGQEVGAAY YRRLSCHR666 30|41|33.5|3.530 12432_4
GGKEYRRLSCHRGRPVGAVFTAQKMPRCETEEAVGGFAESV CRGRSQCS 6 6 3 46|59|57|5.72 0 24713_5 SINSFREQPCPHPSVVCRGRSQCSPQRGRKPPGKSCSVDASETVLLHTLSPRHPQNYWK LQRRNHHK664 23|39|29.0|5.740 30330_3
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23|35|29.0|6.0 1 37810_2 QACTVRRTPRTTHTPIHGSRHTHTALPQHQHTDGR HTHSEARR662 37|46|41.5|4 .52 16345_3 HTHSEARRTPSAPLRVHSSGRPVTLAPSQWGNTNDRRRGPSMPLAE PNRTPPPR66 1 28|28|28|0.00 6875_3
NYKLHTYHLYYTNPNRTPPPRKSHGFGQ QQQHKSAR662 26|33|29.5|3.53 48033_3 RYMSSAPSRRHTDEQQQHKSARTNTAPHRHHRS SSHPPCRR662 52|61|56.5|4.50 2133_3
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6421_2 GYDGTDRSLPRMLLPRTDSHDPDTNDADPRSTSSAPSVSTPALSFTTTADALASTPLPNTNPCAAYTWLPDTRSTAPPDATTRALAYNAYSPPNTAASSATPPPTSS THTQRERE662 26|27|26.5|0.54 47049_2 SWRGSKHEHPPAHTHTQRERERERIQKQ
AGAVSGVI666 36|55|47.5|7 .070 1333_4 EYGMDEELGQAGAVSGVISSDHTRRSSLSRWRSNEGPERKMPEESQLQKAGAASG GTRHRRWA664 40|63|55.0|8.790 31578_3
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6 3 2 33|48|40.5|7.5 0 26549_6 STPVDSSAHGTPSTPVDSSAHSTPSTPADSSANGTVLILPDGAALSTF THTHRERE66 1 48|48|48|0.00 16423_5 SIQSKSINKQNHTKDFITLHPPTTPRSSPSLLHRHTHTHRERERERIPL APHHSTQR 6 6 5 36|61|47|8.01 0
25179_2 TGRRVHRQHQLQFRTKFHDHKKHHNPQHRPTPLAPHHSTQRAHTSSTRPGTIIATTPVTCV00 16423_5 SIQSKSINKQNHTKDFITLHPPTTPRSSPSLLHRHTHTHRERERERIPL APHHSTQR 6 6 5 36|61|47|8.01 0 25179_2
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SLHPPTQK664 34|48|42.5|5.580 4331_4 PHSLRHLHSLHPPTQKGGAMGKRSPPHSACIKEYAAATPYTSHCNKEP DSHRTGTH 6 6 6 27|51|39.0|9.09 0 15028_4 THGGNRDVASAGHKLNAKGVPHASIQWRRDSHRTGTHAAASPSQHKTRRPN RTPVAPPS663
47|70|56|9.460 36937_3 PLATVRTPVAPPSPHKHAKVGGEFAAATATRRNPNHTHRNSGASRQSPTQQNNLPGSWPDREIIYGNQTS AAGPAAQF662 32|61|46.5|14.54 18406_4
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RGDERCGR 6 6 4 16|39|19.0|9.42 0 8955_3 MESNRGDERCGRHTHRGHTTRGENSSQACTVRRTPRTTH KAVKETQA664 46|87|78.5|15.880 4085_6
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22626_5 PPLEELRAANGAPIEDGFDAYDRREDDRAARRERVRVVRGELNHPRGKPRQNTILKLDDSDEEK

K-mer Frequency Unique Sequences Sequences in TheLlargest Cluster Low Frequency | Bigger Frequency | Medium Size | Standard Deviation Cluster ID Representative Sequence ID Sequence PAAGGFGS 9979 5103 4924 12|86|42.0|13.92 0 2443_3
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EAQHEATSSPQIQHSPAQTSESESGPVISKQSSSDVIEPFTSAGVGMAEDDSPQNGNTDDQAPQGTSTDVLESVHDEPSN PFGQAAAG 6 4 4 19|33|22.5|5.54 0 15447_5 PPPFGQAAAGEKPPFGQAAAGDKPPPFGQAAAG
THLPPQWW 6 6 6 27|66|35.0|13.28 0 1515_3 RKKKKADRTSAVCTAALTRPLPSCLVAAANLPSTAPPRRTHLPPQWWRPGTAGTPTQDRSCWTPPR YKRALPQE 6 6 3 30|39|34|3.68 0 20273_3 YKRALPQEEEEDVGRATLIPTTSARRLRTRTGPLIPRRT
GGSCRCRR 6 6 6 14|21|18.0|2.67 0 19665_1 WSCHLRSTRRGGSCRCRRCHA VDPDHFRS 6 6 3 32|35| 33|1.25 0 12326_4 ALGQLYEEERERGRSRDVGPRHVDPDHFRSTTQDAY HSSHTTRR 5 5 2 55|67|61.0|6.0 2 1653_4
VDNTKRGQQAQQHSSHTTRRHPPPPSHPAHFHNPREVAKTPRPPHNTHGASEPSVSNGAATKFTLPS
Fgsattts 5 4 3 13 | 44 | 16 | 13.96 0 18860_1 htstpavgfggsatststPavgglalprtlllprtl Regpaqer 5 5 14 | 15 | 0.4 0 12635_1 AREGPAQEREGDRCA SAYKRALP 5 5 3 30 | 34 | 30 | 1.89 1 8456_4
VDPPPSDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD.
YITPTPIE 5 5 3 27|29|28|0.82 1 18591_2 HAYITPTPIEPHLPVTTPNFIEIMYGGDA AEDNRLDI 5 5 5 38|41|38|1.17 0 9638_4 NELAEDNRLDILPGGSPNSLREKTRWNVNTELHPADRAEIG ALAPPSLG 5 5 5 57|73|69|5.73 0 19848_4
ALAPPSLGARWLAALTRHSAARSTKPAVCIIVRKAERIMFVKATTPAPASSTAAMQKYWTWQTPRGGCTAAIR RRQGVAVC 4 4 2 16|66| 41.0|25.0 0 15493_2
RRQGVAVCGAAGVCQPRPQNTTRGKEMCAPPHRPSSHAHTSRLPQRAVRRGPSSCVQQARRTGPSS
MRRGQQAQ 4 4 3 80|88|88|3.77 0 6038_4 VRRKGKEWNERKRDGAQRIQRESADNMRRGQQAQPHSPHTTRRHPRNEGTQRQQPPTMQEEKTSTTISPSSFPRPPHNAHGAGGPSSI PPCRRRRH 4 4 1 96|96|96|0.0 0 9668_1
SRTVHTPRDGIHARKAHNASSHPPCRRRRHPPPSPPAHSRSPREMAKPRGHHTTHTEQANPHPSITRHKITCHPAINGTRSPTQRSHTRCNTPAGK ANGIRPQP 4 4 2 53|78|65.5|12.5 1 5484_3
THTEETNDRREKHRHGPQPGRQLHTSPQTHTQPAVTHQGRRGRENHRHRLQFNPMANGIRPQPRSPHTKHSKQSPKEP GSAAHTST 4 4 3 16|27| 21|4.5 1 17075_4 GSAAHTSTPAAGGGNLGNSASAATSGT YGPLRPTG 4 4 4 48|63|48.0|6.5 0 1322_5
SGSVTSTEPTDGPMEPDYGPLRPTGMWNVEEVVDVKNSTVDFRRIDDVESEVIEALSQPDDAV PHRHHRSH 4 4 4 13|15|13.0|0.87 0 18522_1 NSTAPHRHHRSHHRP
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