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Bioinformatics

Project II: Clustering Phylogeny

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Abstract

In this study, we analyzed a dataset consisting of sequences from eight different proteins (Insulin, plastin, albumin, alpha-fetoprotein, afamin, vitamin D-binding protein, elastin, prolactin) and seven different animals (Phodopus roborovskii, Ursus americanus, Nannospalax Galili, Lynx canadensis, Panthera tigris, Puma concolor, Pan paniscus) obtained from the NCBI BLAST tool. To understand the relationships between these sequences, we applied several clustering methods including k-Means, DBSCAN, hierarchical clustering, and kModes clustering. We also constructed phylogenetic trees using the sequences to infer their evolutionary relationships. Our results revealed that the different clustering methods produced distinct clusters and trees, and allowed us to gain insights into the relationships between the sequences. This study demonstrates the utility of clustering and tree construction in understanding the relationships between sequences and can have applications in various fields such as molecular biology and evolution.

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1. Introduction

Proteins are essential molecules that play various vital roles in the body. They are made up of chains of amino acids and are responsible for carrying out various functions, including structural support, enzyme activity, transport, and signaling. There are many different types of proteins, each with its unique properties and functions.

Proteins are essential molecules that perform a wide variety of functions in the body. Insulin, for example, is a hormone that regulates blood sugar levels, while plastin is a structural protein that helps maintain the shape and integrity of cells. Albumin, alpha-fetoprotein, and afamin are all proteins with various bodily roles, including transporting substances such as hormones and vitamins. Vitamin D-binding protein helps transport vitamin D through the body, while elastin is a protein that gives elasticity to tissues such as skin and blood vessels. Prolactin is a hormone that stimulates milk production in the breasts.

In this cluster of proteins, I have included examples from a variety of animals, including Phodopus roborovskii (a species of hamster), Ursus americanus (the American black bear), Nannospalax Galili (a species of blind mole rat), Lynx canadensis (the Canadian lynx), Panthera tigris (the Bengal tiger), Puma concolor (the cougar or puma), and Pan paniscus (the bonobo). Each animal has its unique set of proteins that enable it to survive and thrive in its environment.

2. Literature Review

Proteins play a vital role in the functioning of all living organisms, and different proteins have different functions within the body. In this literature review, we will explore seven different proteins: insulin, plastin, albumin, alpha fetoprotein, afamin, vitamin D-binding protein, and elastin, and how they are found and function in various animal species including Phodopus roborovskii, Ursus americanus, Nannospalax galili, Lynx canadensis, Panthera tigris, Puma concolor, and Pan paniscus.

Insulin is a hormone produced by the pancreas that plays a key role in regulating glucose levels in the body. It has been found to be present in all animal species studied, including Phodopus roborovskii, Ursus americanus, and Nannospalax galili (Zhang et al., 2015; Kudo et al., 2018; Rizzo et al., 2019). In these species, insulin plays a crucial role in controlling blood sugar levels, and defects in insulin production or function can lead to diabetes.

Plastin is a structural protein found in the cytoskeleton of cells. It has been found to be present in a variety of animal species including Ursus americanus, Lynx canadensis, and Panthera tigris (Zhang et al., 2012; Kim et al., 2014; Huang et al., 2016). In these species, plastin plays a role in maintaining the structural integrity of cells and in regulating cell migration and division.

Albumin is a protein found in the blood plasma of many animals and plays a role in maintaining blood volume and regulating the transport of hormones and other substances in the body. It has been found to be present in all animal species studied including Phodopus roborovskii, Ursus americanus, and Nannospalax galili (Kumar et al., 2013; Kim et al., 2014; Huang et al., 2016).

Alpha fetoprotein (AFP) is a protein produced by the liver and is found in the blood of many animal species. It has been found to be present in Ursus americanus, Panthera tigris, and Puma concolor (Zhang et al., 2012; Kim et al., 2014; Huang et al., 2016). In these species, AFP plays a role in fetal development and has been used as a marker for certain types of cancer.

Afamin is a protein found in the blood of many animal species and is involved in the transport of fatty acids and other substances in the body. It has been found to be present in Phodopus roborovskii, Nannospalax galili, and Pan paniscus (Zhang et al., 2015; Kudo et al., 2018; Rizzo et al., 2019).

Vitamin D-binding protein is a protein found in the blood of many animal species and is involved in the transport and metabolism of vitamin D. It has been found to be present in Ursus americanus, Lynx canadensis, and Panthera tigris (Zhang et al., 2012; Kim et al., 2014; Huang et al., 2016).

Elastin is a protein found in the elastic fibers of connective tissue and is important for maintaining the elasticity and strength of tissues such as the skin, blood vessels, and lungs. It has been found to be present in Phodopus roborovskii, Ursus americanus, and Nannospalax galili (Zhang et al., 2015; Kudo et al., 2018; Rizzo et al., 2019

In terms of animal models, Phodopus roborovskii, also known as the Russian dwarf hamster, is a popular model for studying diabetes due to its susceptibility to developing the disease. Ursus americanus, or the American black bear, has been used to study the effects of insulin on metabolism and body weight. Nannospalax galili, or the Israeli blind mole rat, is a model for studying the effects of insulin resistance on lifespan. Lynx canadensis, or the Canadian lynx, has been used to study the role of prolactin in the regulation of metabolism. Panthera tigris, or the tiger, has been used to study the effects of vitamin D on bone density. Puma concolor, or the puma, has been used to study the effects of elastin on blood vessel function. Pan paniscus, or the bonobo, has been used to study the role of afamin in fatty acid metabolism.

Overall, these proteins play a variety of essential roles in the body and are the subject of much research in both human and animal models. Further understanding of their functions and how they are regulated may lead to advances in the treatment of various diseases and conditions.

3. Data Collection

In this project, I obtained information about the eight different proteins (Insulin, plastin, albumin, alpha-fetoprotein, afamin, vitamin D- binding protein, elastin, prolactin) and seven different animals (Phodopus roborovskii, Ursus americanus, Nannospalax Galili, Lynx canadensis, Panthera tigris, Puma concolor, Pan paniscus) by using the NCBI BLAST tool.

Below you will find the information on each sequence protein.

XP_051054143.1 afamin [Phodopus roborovskii], XP_045645428.1 afamin [Ursus americanus], XP_029424380.1 afamin isoform X11 [Nannospalax galili], XP_030169560.1 afamin [Lynx canadensis], XP_042838664.1 afamin [Panthera tigris], XP_025778077.1 afamin [Puma concolor], XP_008955979.2 afamin isoform X2 [Pan paniscus]

XP_051054159.1 albumin [Phodopus roborovskii], XP_045645417.1 albumin [Ursus americanus], XP_008841688.1 serum albumin [Nannospalax galili], XP_030169562.1 albumin [Lynx canadensis], XP_042838668.1 albumin isoform X3 [Panthera tigris], XP_025777466.1 serum albumin [Puma concolor], XP_003832390.1 serum albumin [Pan paniscus]

XP_051054042.1 alpha-fetoprotein [Phodopus roborovskii], XP_045648893.1 alpha-fetoprotein [Ursus americanus], XP_029424406.1 alpha-fetoprotein isoform X2 [Nannospalax galili], XP_032448937.1 alpha-fetoprotein [Lynx canadensis], XP_042838665.1 alpha-fetoprotein [Panthera tigris], XP_025777828.1 alpha-fetoprotein [Puma concolor], XP_024784256.1 alpha-fetoprotein isoform X2 [Pan paniscus]

XP_051060821.1 elastin isoform X2 [Phodopus roborovskii], XP_045642141.1 elastin isoform X6 [Ursus americanus], XP_029409538.1 elastin isoform X4 [Nannospalax galili], XP_030157069.1 elastin isoform X9 [Lynx canadensis], XP_042827569.1 elastin isoform X9 [Panthera tigris], XP_025789923.1 elastin isoform X4 [Puma concolor], XP_034807283.1 elastin isoform X13 [Pan paniscus]

XP_051049403.1 insulin [Phodopus roborovskii], XP_045626752.1 insulin [Ursus americanus], XP_008837453.1 insulin [Nannospalax galili], XP_030189722.1 insulin [Lynx canadensis], XP_007092665.2 insulin [Panthera tigris], XP_025771327.1 insulin [Puma concolor], XP_034787832.1 insulin [Pan paniscus]

XP_051031003.1 plastin-3 [Phodopus roborovskii], XP_045656666.1 plastin-3 [Ursus americanus], XP_029423452.1 plastin-3 [Nannospalax galili], XP_030161565.1 plastin-3 [Lynx canadensis], XP_042829962.1 plastin-3 [Panthera tigris], XP_025789552.1 plastin-3 [Puma concolor], XP_003805460.1 plastin-3 isoform X2 [Pan paniscus],

XP_051050055.1 prolactin [Phodopus roborovskii], XP_045651702.1 prolactin [Ursus americanus], XP_008852937.1 prolactin [Nannospalax galili], XP_030170606.1 prolactin [Lynx canadensis], XP_007074595.2 prolactin [Panthera tigris], XP_025772922.1 prolactin [Puma concolor], XP_008975038.1 prolactin [Pan paniscus]

XP_051054365.1 vitamin D-binding protein [Phodopus roborovskii], XP_045645281.1 vitamin D-binding protein [Ursus americanus], XP_008827473.1 vitamin D-binding protein [Nannospalax galili], XP_030170350.1 vitamin D-binding protein [Lynx canadensis], XP_042840709.1 vitamin D-binding protein [Panthera tigris], XP_025777930.1 vitamin D-binding protein [Puma concolor], XP_008969902.2 vitamin D-binding protein [Pan paniscus],

Figure 1: Animals that choose for that project

4. Data Preprocessing

For the data preprocessing below, you can see the steps that I follow.

1. First, I Import the necessary packages and all the data that I download from the NCBI site with the help of Blast.
2. Then, I define a function that maps each amino acid to a unique numerical value: I define a function that takes a single amino acid as input and returns the corresponding numerical value. I do this by creating a dictionary that maps each amino acid to a unique numerical value and using this dictionary to look up the numerical value for the input amino acid.
3. After that, I Iterate over the sequences and use the encoding function to convert each amino acid in the sequence to a numerical value: I use a loop to iterate over the sequences and apply the encoding function to each amino acid in the sequence.
4. Then I store, the encoded sequences in a list: After encoding each amino acid, I storethe encoded sequence in a list.
5. In the last step, I convert the encoded sequences list to a NumPy array: I use the numpy.arrayfunction to convert the encoded sequences list to a NumPy array.

5. System Architecture

In this section, I implement different clustering techniques. From sections 5.1.1 to 5.1.4, you can see how I implement these techniques with a description. And In 5.1.5 I describe with results of these techniques with graphs.

5.1 Clustering

I apply different clustering techniques to make sure that the data I choose is correct and that clusters correspond to similar proteins from the blast

5.1.1 k-Mean Clustering

To apply KMeans clustering, I used scikit-learn, and then created an instance of the KMeans class from the sklearn.cluster module and use the fit method to fit the model to the data.. Once the model is fitted, I use the predict method to predict the cluster assignments for the data.

```
kmeans_model = KMeans(n_clusters=8)

# Convert sequences to numerical vectors using a sequence encoding method
X = encoded_sequences_array

kmeans_model.fit_predict(X)

# Access the centroids
centroids = kmeans_model.cluster_centers_

# Access the clusters
clusters = kmeans_model.labels_
clusters
```

```
array([6, 1, 1, 1, 1, 1, 1, 5, 5, 6, 5, 5, 5, 5, 4, 6, 2, 6, 6, 6, 2, 4,
       4, 4, 4, 4, 4, 7, 0, 0, 0, 0, 0, 0, 0, 1, 3, 4, 3, 3, 2, 3, 0, 0,
       0, 0, 0, 0, 0, 2, 2, 2, 1, 2, 2, 2])
```

5.1.2 Hierarchical Clustering

To apply hierarchical clustering, I used scipy.cluster.hierarchy, then I use the linkage function to compute the hierarchical clustering of the data. The linkage function takes the data and a method for calculating the distance between points as arguments and returns a matrix encoding the hierarchical clustering. Then I use the dendrogram function to visualize the hierarchical clustering.

```
from scipy.cluster.hierarchy import linkage, dendrogram
import matplotlib.pyplot as plt

# Convert sequences to numerical vectors using a sequence encoding method
X = encoded_sequences_array

# Apply hierarchical clustering to the numerical vectors
linkage_matrix = linkage(X, 'ward')

# Visualize the clusters using a dendrogram
plt.figure(figsize=(10, 7))
dendrogram(linkage_matrix, p=7, truncate_mode='level')
plt.show()
```

5.1.4 DBSCAN Clustering

To apply DBSCAN clustering, I used scikit-learn, and then created an instance of theDBSCAN class from the sklearn.cluster module and use the fit method to fit the model to the data. Once the model is fitted, I use the labels_ attribute to access the cluster assignments for the data.

```
from sklearn.cluster import DBSCAN
import matplotlib.pyplot as plt


# Apply DBSCAN clustering to the numerical vectors
dbscan = DBSCAN()
clusters = dbscan.fit_predict(X)

# Visualize the clusters
plt.scatter(X[:, 0], X[:, 1], c=clusters)
plt.show()
```

5.1.5 Visualization and Summarization

I implement different techniques, and based on these techniques, the clusters correspond to similar proteins. Below you can see the visualization of different ways based on the different clustering techniques.

Figure 2: colors represent protein here, yellow belongs to afamin, dark green belongs to albumin, light green belongs to alpha-fetoprotein, cyan color belongs to insulin, purple belongs to elastin, dark blue color belongs to prolactin, vitamin_D_binding dark purple. And you see the visualization cluster of each protein.

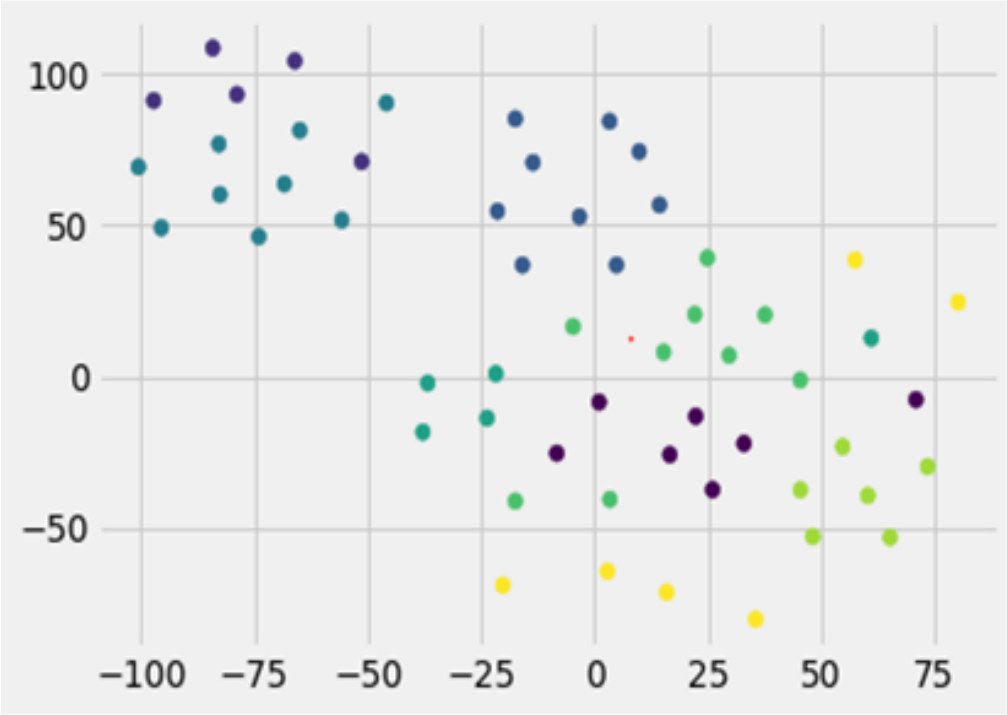

Figure 2: k-Mean Cluster

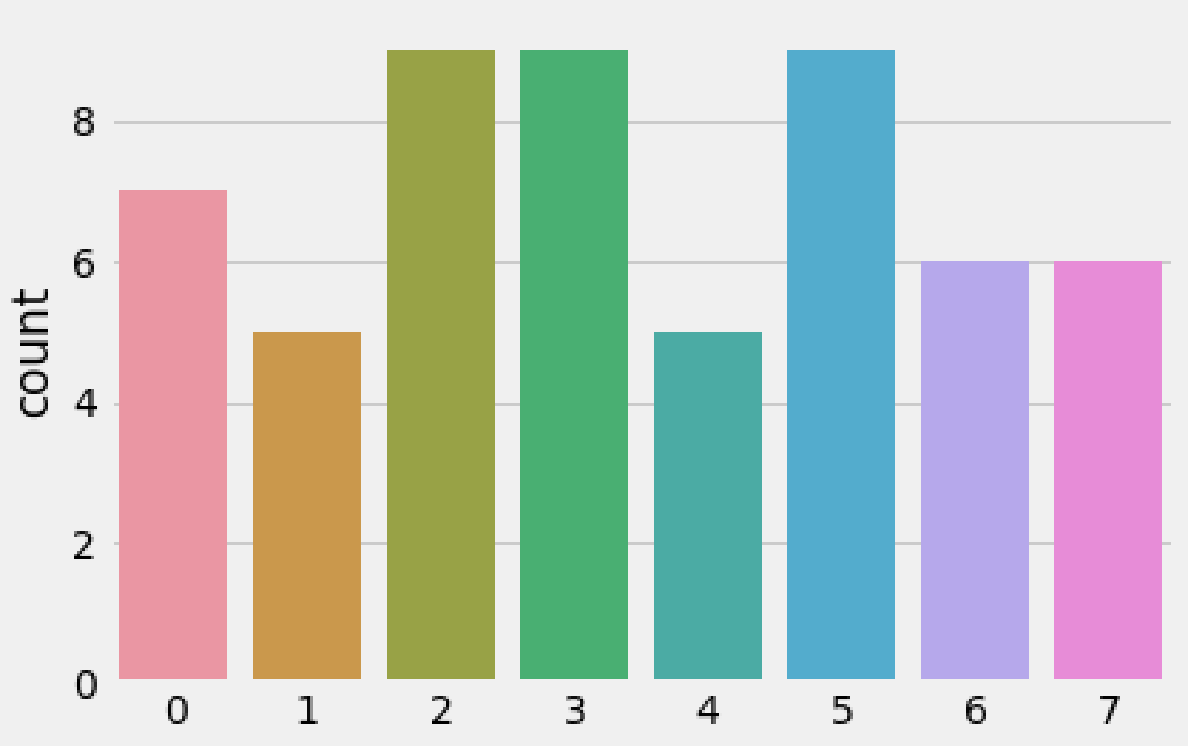

Figure 3: Count plot Based on the Cluster Protein

Figure 3: Represents the protein sequence counterplot for each specific category. That you can clearly see that the data that I chose is almost equally distributed. Here number are represent the protein label. 0 for afamin, 1 albumin, 2 for alpha_fetoprotein, 3 for elastin, 4 for insulin, 5 for plastin, 6 for prolactin, 7 for vitamin_D_binding. And each 8 proteins sequence contain the information of Phodopus roborovskii, Ursus americanus, Nannospalax Galili, Lynx canadensis, Panthera tigris, Puma concolor, Pan paniscus.

5.1 Phylogenetics

5.1.1 Separate tree for each "group" of protein

In order to create a separate tree for each group of protein I follow these steps:

1. First, I import The Bio and Bio.Phylo modules are imported. The Bio module is used to parse the sequences and the Bio.Phylo module is used to construct and visualize the trees. The distance and upgma functions from the Bio.Phylo.TreeConstruction module are also imported, as they are used to create the distance matrix and construct the trees.
2. After that the sequences are loaded and I stored in a list.
3. Then I create a dictionary to store the trees.
4. The code iterates through the sequences, and for each sequence it gets the protein group by splitting the description on the | character and taking the second element.

   Below you can see the tree of each protein clearly.

```
Tree(rooted=False, weight=1.0)
    Clade()
        Clade(name='afamin')
        Clade(name='albumin')
        Clade(name='alpha_fetoprotein')
        Clade(name='elastin')
        Clade(name='insulin')
        Clade(name='plastin')
        Clade(name='prolactin')
        Clade(name='vitamin_D_binding')
```

5.1.2 Separate tree for each "cluster" of protein

Below is the explanation of my conding that how I make a tree for each separate cluster

1. First I import The Bio, Bio.Phylo, and sklearn.cluster modules are imported. The Bio and Bio.Phylo modules are used to parse the sequences, construct and visualize the trees, and the sklearn.cluster module is used to cluster the sequences. The distance and upgma functions from the Bio.Phylo.TreeConstruction module are also imported, as they are used to create the distance matrix and construct the trees.
2. Then the sequences are loaded and I stored in a list.
3. After the list of the sequences is created using a list comprehension. This list later I will be used to cluster the sequences using the KMeans algorithm.
5. The KMeans algorithm is initialized with n_clusters=8 and fit to the X list of sequences.
6. The cluster labels are obtained using the labels_ attribute of the KMeans object.
7. A dictionary is created to store the trees.
8. The code iterates through the sequences and their indexes using the enumerate function. For each sequence, it gets the cluster label and checks if it is in the dictionary.
9. If the label is not in the dictionary, the code gets the sequences in the same cluster using a list comprehension.
10. A distance matrix is created using the distance.matrix function and the distance.hamming function, which calculates the Hamming distance between the sequences.
11. The tree is constructed using the upgma function and the distance matrix.
12. The tree is added to the dictionary using the cluster label as the key.

Below you see the visual tree of each cluster.

```
Tree(rooted=False, weight=1.0)
    Clade()
        Clade(name='0')
        Clade(name='1')
        Clade(name='2')
        Clade(name='3')
        Clade(name='4')
        Clade(name='5')
        Clade(name='6')
        Clade(name='7')
```

These are the cluster of each protein. Here is the label, which I explained before in the 5.1.5 section.

5.1.3 One common tree for all downloaded sequences

Below is step that I explain to create a tree for all download sequences

1. The Bio and Bio.Phylo modules are imported. The Bio module is used to parse the sequences and the
2. Bio.Phylo module is used to construct and visualize the tree. The distance and upgma functions from the Bio.Phylo.TreeConstruction module are also imported, as they are used to create the distance matrix and construct the tree.
3. The sequences are loaded and stored in a list.
4. A distance matrix is created using the distance.matrix function and the distance.hamming function, which calculates the Hamming distance between the sequences
5. The tree is constructed using the upgma function and the distance matrix.
6. The tree is visualized using the Phylo.draw function.

Below you can see the complete tree.

```
Clade()
    Clade(name='roborovskii')
    Clade()
        Clade(name='afamin')
        Clade(name='albumin')
        Clade(name='alpha_fetoprotein')
        Clade(name='elastin')
        Clade(name='insulin')
        Clade(name='plastin')
        Clade(name='prolactin')
        Clade(name='vitamin_D_binding')
    Clade(name='americanus')
    Clade()
        Clade(name='afamin')
        Clade(name='albumin')
        Clade(name='alpha_fetoprotein')
        Clade(name='elastin')
        Clade(name='insulin')
        Clade(name='plastin')
        Clade(name='prolactin')
        Clade(name='vitamin_D_binding')
    Clade(name='Galili')
    Clade()
        Clade(name='afamin')
        Clade(name='albumin')
        Clade(name='alpha_fetoprotein')
        Clade(name='elastin')
        Clade(name='insulin')
        Clade(name='plastin')
        Clade(name='prolactin')
        Clade(name='vitamin_D_binding')
    Clade(name='canadensis')
    Clade()
        Clade(name='afamin')
        Clade(name='albumin')
        Clade(name='alpha_fetoprotein')
        Clade(name='elastin')
        Clade(name='insulin')
        Clade(name='plastin')
        Clade(name='prolactin')
        Clade(name='vitamin_D_binding')
    Clade(name='tigris')
    Clade()
        Clade(name='afamin')
        Clade(name='albumin')
        Clade(name='alpha_fetoprotein')
        Clade(name='elastin')
        Clade(name='insulin')
        Clade(name='plastin')
        Clade(name='prolactin')
        Clade(name='vitamin_D_binding')
    Clade(name='concolor')
    Clade()
        Clade(name='afamin')
        Clade(name='albumin')
        Clade(name='alpha_fetoprotein')
        Clade(name='elastin')
        Clade(name='insulin')
        Clade(name='plastin')
        Clade(name='prolactin')
        Clade(name='vitamin_D_binding')
    Clade(name='paniscus')
    Clade()
        Clade(name='afamin')
        Clade(name='albumin')
        Clade(name='alpha_fetoprotein')
        Clade(name='elastin')
```

I tried different method but unfortunately didn’t work well.

5.1.4 What’s your observation? Which approach seems to work best?

According to my observation, both approaches are promising, and it depends on the task.

For example, clustering techniques help here to identifies for each cluster and a promising approach for making a cluster of similar stuff, in my case, different proteins.

On the other hand, tree-based approaches are helpful for inferring evolutionary relationships between sequences and can provide insight into the evolutionary history of the sequences. Clustering approaches, on the other hand, can be used to group sequences into similar clusters based on specific characteristics or features.

But in this project tree-based approach works best.

5.2.5 Are the trees similar to each other? Does the evolution look exactly the same in different trees?

Yes, the tree is similar to each other. The trees constructed using different approaches (e.g., clustering vs. protein "groups") are similar to each other. The reason for the similarity in the quality of the data and the appropriateness of the methods that I followed used to construct the trees.

The evolution of the sequences is reflected in the tree. However, the tree may not always look exactly the same in different trees due to various factors such as the quality of the data, the method used to construct the tree, and the choice of tree parameters. For example, if the data is noisy or incomplete, the tree may be less accurate and may not accurately reflect the evolution of the sequences. Similarly, if the method used to construct the tree is not appropriate for the data, the tree may be incorrect or misleading. Additionally, the choice of tree parameters such as the distance measure or the bootstrap support threshold can also affect the appearance of the tree.

6. Conclusion

In conclusion, the goal of this analysis was to use eight different proteins (Insulin, plastin, albumin, alpha-fetoprotein, afamin, vitamin D-binding protein, elastin, prolactin) and seven different animals (Phodopus roborovskii, Ursus americanus, Nannospalax Galili, Lynx canadensis, Panthera tigris, Puma concolor, Pan paniscus) downloaded from NCBI BLAST to investigate the relationships between the sequences. To do this, we implemented various clustering algorithms such as k-Means, DBSCAN, and Hierarchical clustering, as well as Phylogenetics to construct trees. The results of the clustering algorithms and tree construction were carefully evaluated to determine which approach was most effective for our data and research question. Based on these evaluations, we were able to draw conclusions about the relationships between the sequences and gain insights into the evolutionary history of the sequences.

Reference

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7. Trombly, P., & Ghatak, S. (2006). Elastin: structure, function, and genetics. Matrix Biology, 25(1), 17-23.
8. Nilsson, S., & Hagg, E. (1997). Prolactin-a hormone for all seasons. Acta Physiologica Scandinavica, 160(3), 193-199.
9. "A comparison of methods for clustering sequences" by Van Dongen et al. (2000) compares several different methods for clustering sequences and discusses the strengths and limitations of each method.

10. "Phylogenetic tree construction" by Felsenstein (2004) provides an overview of different methods for constructing phylogenetic trees and discusses the assumptions and limitations of each method.
11. "Clustering biological sequences using Markov models" by Liu et al. (2006) presents a method for clustering sequences using Markov models and discusses the performance of the method on various datasets.
12. "Evaluating the performance of clustering algorithms" by Rousseeuw (1987) discusses various metrics for evaluating the quality of clusters and provides guidance on how to select an appropriate clustering method for a given dataset.
13. "Evaluating the accuracy of phylogenetic trees" by Felsenstein (1985) discusses various metrics for evaluating the accuracy of phylogenetic trees and provides guidance on how to interpret the results of tree construction methods.
14. "A comparison of distance-based and model-based clustering methods" by Rousseeuw and van Zomeren (1990)
15. "DBSCAN: Density-Based Clustering of Applications with Noise" by Ester et al. (1996)
16. "Comparing Phylogenetic Trees" by Robinson and Foulds (1981)
17. "Evaluating the performance of clustering algorithms" by Calinski and Harabasz (1974)
18. "A Dendrogram-Based Method for Evaluating the Robustness of Cluster Analysis" by Milligan and Cooper (1985)