

# Bioinformatics

An analysis of alignment-free  
methods using image textures  
from DNA sequences

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# Introduction

## DNA sequence

```
>J01859.1 Escherichia coli 16S ribosomal RNA, complete sequence
AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGT
AACAGGAAGAAGCTTGCTCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAACTGCCTGATG
GAGGGGGATAACTACTGGAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAGAGGGGGACCTTCG
GGCCTCTTGCCATCGGATGTGCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACG
ATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACAGGACACGGTCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTT
CGGGTTGTAAAGTACTTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCG
CAGAAGAAGCACC GGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAAT
TACTGGGCGTAAAGCGCACGCAGGCGGTTTGTAAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAAC
TGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGAAATGCGT
AGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCG
TGGGGAGCAAACAGGATTAGTACCTTGGTAGTCCACGCCGTAACGATGTGCACTTGGAGGTTGTGCC
TTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGAAGGTTAAAACT
CAAATGAATTGACGGGGGCCCCGACAAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCT
TACCTGGTCTTGACATCCACGGAAAGTTTTCAGAGATGAGAATGTGCCCTTCGGGAACCGTGAGACAGGTGC
TGCATGGCTGTCGTGAGTCTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCTTATCCT
TTGTTGCCAGCGGTCCGGCCGGGAACCTCAAAGGAGACTGCCAGTGATAAAGTGGAGGAAGGTGGGGATGA
CGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGA
CCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGATTGGAGTCTGCAACTCGACTCCATG
AAGTCGGAATCGTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCG
CCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGGTTACCACTT
TGTGATTTCATGACTGGGGTGAAGTCGTAACAAGGTAACCGTAGGGGAACCTGCGGTTGGATCACCTCCTT
```

Figure: 16S ribosomal DNA of *Escherichia coli* with FASTA Format.

# Introduction

## DNA sequence

The human genome is made of ~**3.2 billions bp** of DNA.  
~6.4 billions of nucleotides [1].

The HIV-1 genome is made of ~**20k bp** of DNA.  
Meanwhile, the COVID-19 is made of ~**32k bp** [2].

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# Problem

## Phylogenetics steps

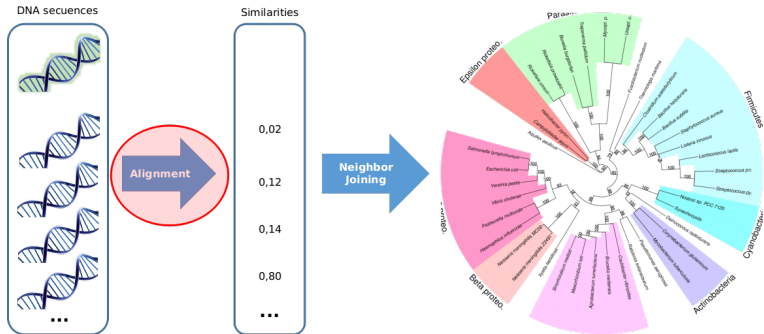


Figure: Steps to visualize phylogenetic trees.

# Problem

## Alignment-based methods

- The most used **alignment-based** method are BLAST and CLUSTALW.



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# Objective

Compare **alignment-free** algorithms based on texture descriptors, against CLUSTALW.

- First-Order Statistics (**FOS**) [3].
- Gray Level Co-occurrence Matrix (**GLCM**) [4].
- Multi-resolution Local Binary Patterns (**MLBP**) [5].

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# FOS

## Mapping function

Each pair of bases have a value from 0 to 15.

$$\alpha = \left\{ \begin{array}{l} AA, AG, AC, AT, GA, GG, GC, GT, \\ CG, CC, CT, CA, TA, TG, TC, TT \end{array} \right\} \quad (1)$$

# FOS

## Image from DNA

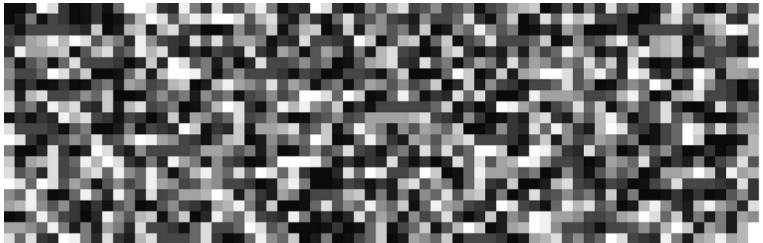


Figure: Textures converted from the DNA sequences of *Bacillus maritimus* 16S ribosomal DNA.



# FOS

## Histogram

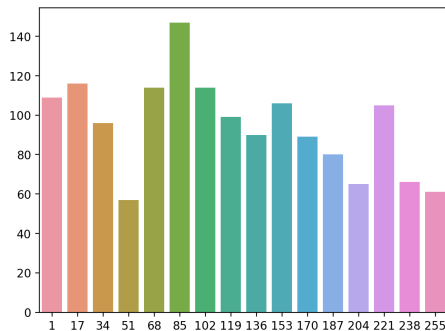


Figure: Histogram of *Bacillus maritimus* 16S ribosomal DNA.

# FOS

## Features

From the histogram, the following features are compute:

- **Skewness** =  $\sigma^{-3} \sum_{i=0}^{G-1} (i - \mu)^3 p(i)$
- **Kurtosis** =  $\sigma^{-4} \sum_{i=0}^{G-1} (i - \mu)^4 p(i) - 3$
- **Energy** =  $\sum_{i=0}^{G-1} p(i)^2$
- **Entropy** =  $-\sum_{i=0}^{G-1} p(i) \lg(p(i))$

Where:

- $p(i) = h(i)/NM$
- $h(i) = \text{histogram}$
- $N$  and  $M$  are image's width and height.
- $\mu = \sum_{i=0}^{G-1} ip(i)$

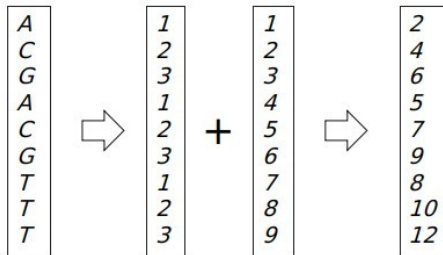
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# GLCM

## Mapping function

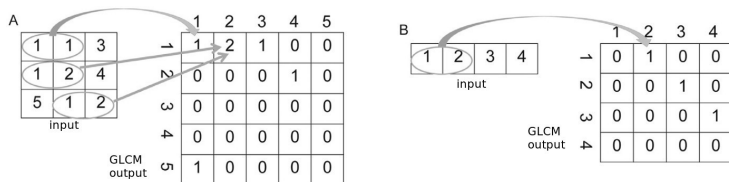
Each base in sequence  $S = \{A, C, G, T\}$  is mapped to the numbers  $S' = \{1, 2, 3, 4\}$ . Then we added to each value the base position.



Then, compute gray-level co-occurrence matrix.

# GLCM

## Mapping function



**Figure:** Examples of GLCM algorithm. Left: GLCM computed from a 2D matrix with intensities from 1 to 5. Right: GLCM computed from a 1D vector with intensities from 1 to 4.

# GLCM

## Features

From the histogram, the following features are compute:

- **Entropy**  $= - \sum_{i=1}^L \sum_{j=1}^L p(i, j) \ln(p(i, j))$
- **Contrast**  $= \sum_{i=1}^L \sum_{j=1}^L (i - j)^2 p(i, j)$
- **Energy**  $= \sum_{i=1}^L \sum_{j=1}^L p(i, j)^2$
- **Correlation**  $= \sum_{i=1}^L \sum_{j=1}^L \frac{(i - \mu_i)(j - \mu_j)p(i, j)}{\sigma_i \sigma_j}$
- **Homogeneity**  $= \sum_{i=1}^L \sum_{j=1}^L \frac{p(i, j)}{1 + |i - j|}$

where,  $p(i, j)$  is the GLCM matrix and  $L$  is the maximun intensity value.

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# MLBP

## Mapping function

Table: Numeric representation for each base used by Kouchaki et al. [5].

Base	Integer	EIIP	Atomic	Real
A	2	0.1260	70	-1.5
T	-2	0.1335	78	1.5
C	-1	0.1340	58	-0.5
G	2	0.0806	66	0.5



# MLBP

## LBP example

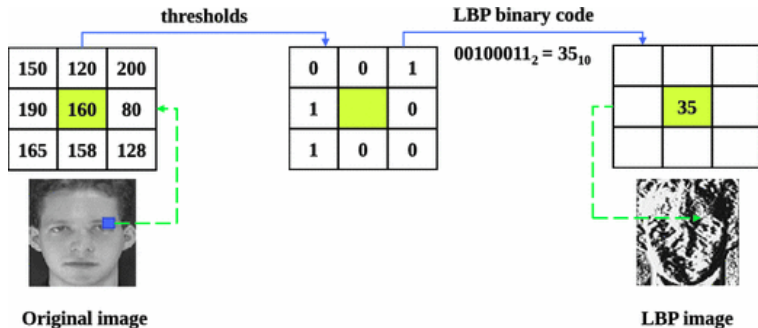


Figure: Example of Local Binary Pattern algorithm.

# MLBP

## Features

$$LBP(x(t)) = \sum_{i=0}^{p/2-1} (Sign(x(t+i-p/2) - x(t))2^i + Sign(x(t+i+1) - x(t))2^{i+p/2}), \quad (2)$$

where  $p$  is the number of neighbouring points and  $Sign$  is:

$$Sign(x) = \begin{cases} 0, & x < 0 \\ 1, & x \geq 0 \end{cases} \quad (3)$$

$$h_k = \sum_{p/2 \leq i \leq N-p/2} \delta(LBP_p(x(i), k)), \quad (4)$$

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# Datasets

Table: 16S ribosomal DNA of 13 bacteria.

Species	Accession Code	Length (bp)
Bacillus maritimus	KP317497	1515
Bacillus wakoensis	NR_040849	1524
Bacillus australimaris	NR_148787	1513
Bacillus xiamenensis	NR_148244	1513
Escherichia coli	J01859	1541
Streptococcus himalayensis	NR_156072	1509
Streptococcus halotolerans	NR_152063	1520
Streptococcus tangierensis	NR_134818	1520
Streptococcus cameli	NR_134817	1518
Thermus amyloliquefaciens	NR_136784	1514
Thermus tengchongensis	NR_132306	1523
Thermus thermophilus	NR_037066	1515
Thermus filiformis	NR_117152	1514

# Datasets

Table: NADH dehydrogenase subunit 4 genes of 12 species genome information from NCBI.

Species	Accession Code	Length (bp)
Macaca fascicularis	M22653	896
Macaca fuscata	M22651	896
Macaca mulatta	M22650	896
Macaca sylvanus	M22654	896
Saimiri sciureus	M22655	893
Chimpanzee	V00672	896
Lemur catta	M22657	895
Gorilla	V00658	896
Hylobates	V00659	896
Sumatran Orangutan	V00675	895
Tarsius syrichta	M22656	895
Human	L00016	896

# Datasets

**Table:** The mitochondrial genome detailed information of 18 eutherian mammals from NCBI database.

Species	Accession Code	Length (bp)
Human	V00662	16569
Pygmy chimpanzee	D38116	16563
Common chimpanzee	D38113	16554
Gorilla	D38114	16364
Orangutan	D38115	16389
Gibbon	X99256	16472
Baboon	Y18001	16521
Horse	X79547	16660
White rhinoceros	Y07726	16832
Harbor seal	X63726	16826
Gray seal	X72004	16797
Cat	U20753	17009
Fin whale	X61145	16397

...

# Mapping functions

Table: Numeric representation for each base.

Base	MAP0	MAP1	MAP2	MAP3	MAP4	MAP5
A	FOS	GLCM	2	0.1260	70	-1.5
T	FOS	GLCM	-2	0.1335	78	1.5
C	FOS	GLCM	-1	0.1340	58	-0.5
G	FOS	GLCM	2	0.0806	66	0.5

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# Results

## Comparison in 16S ribosomal DNA dataset

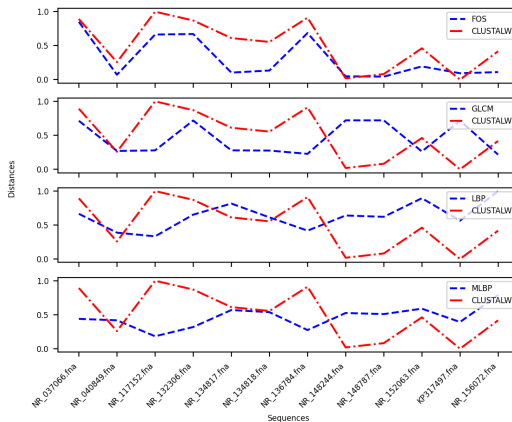


Figure: Euclidean distance of *Escherichia coli* against the rest sequences in 16S ribosomal DNA dataset. We used MEGA. FOS, GLCM, LBP and MLBP.

# Results

## Comparison in NADH dataset

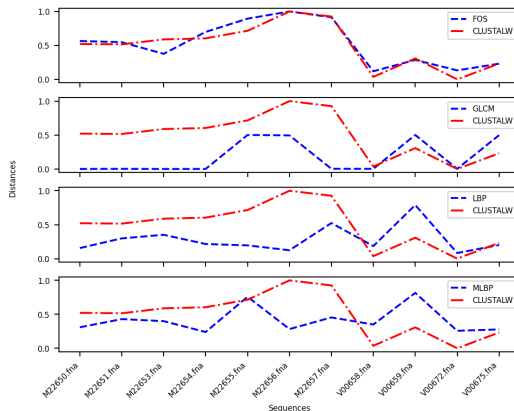
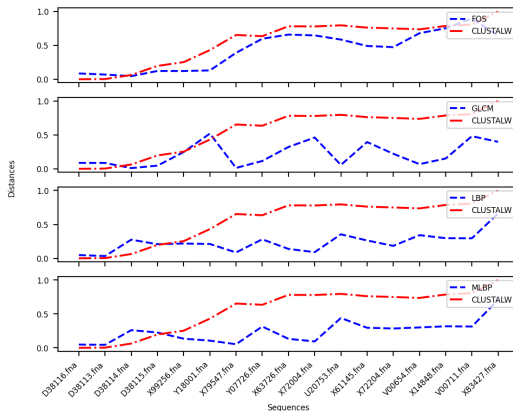


Figure: Euclidean distance of Human against the rest sequences in NADH dehydrogenase protein dataset. We used MEGA. FOS, GLCM, LBP and MLBP.

# Results

## Comparison in the mitochondrial dataset



**Figure:** Euclidean distance of Human against the rest sequences in the mitochondrial genome dataset. We used MEGA. FOS, GLCM, LBP and MLBP.

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# Results

## Comparison of the six mapping functions using FOS algorithm

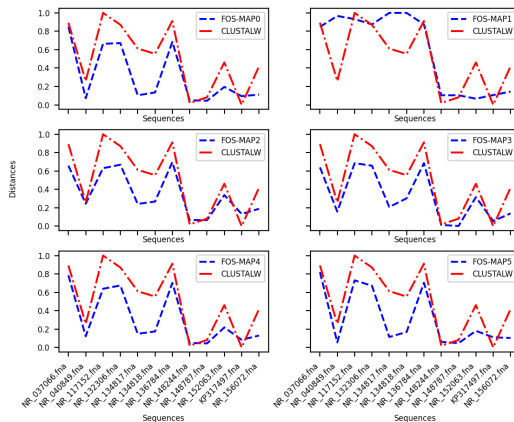


Figure: Comparison of the 6 mapping functions using FOS algorithm over the 16S ribosomal DNA dataset.

# Results

## Comparison of MAP1 mapping function

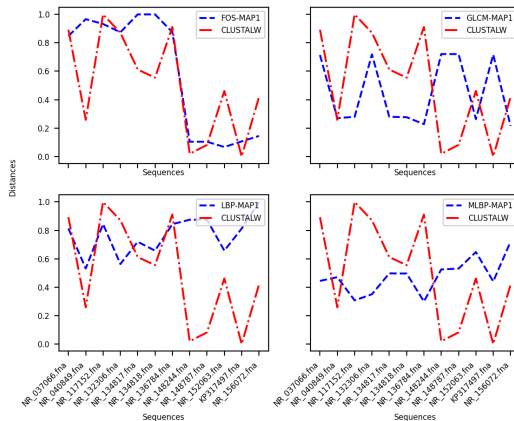


Figure: Comparison of MAP1 mapping function over the 16S ribosomal DNA dataset.

# Results

## Comparison of the six mapping functions using GLCM algorithm

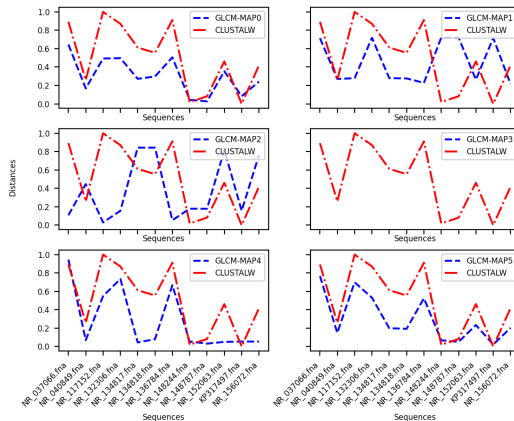
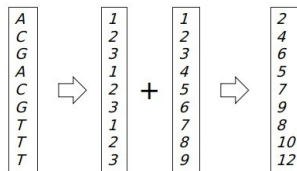


Figure: Comparison of the 6 mapping functions using GLCM algorithm over the 16S ribosomal DNA dataset.

# Results

## Discussion: Problem with MAP1 function

MAP1 function, proposed by Chen at el. [4].



The resultant vector have disperse values and it depends strongly from the sequence's length



# Results

## Comparison of the six mapping functions using LBP algorithm

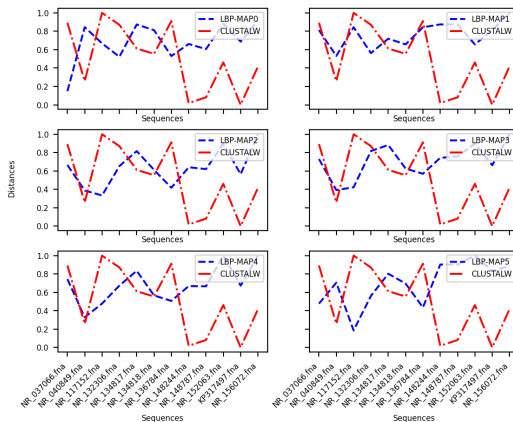


Figure: Comparison of the 6 mapping functions using the LBP algorithm over the 16S ribosomal DNA dataset.

# Results

## Comparison of the six mapping functions using MLBP algorithm

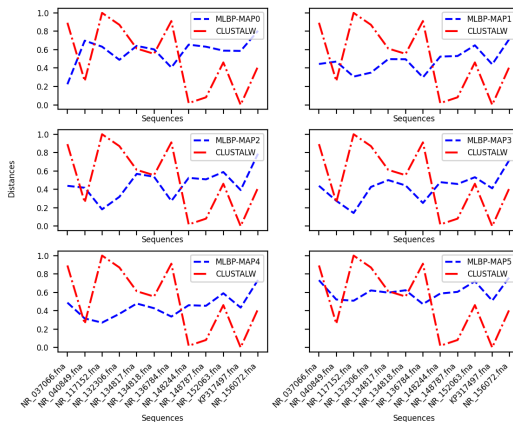


Figure: Comparison of the 6 mapping functions using the MLBP algorithm over the 16S ribosomal DNA dataset.

# Results

Square error of mapping functions and algorithms over the 16S ribosomal DNA dataset

Table: Square error of all mapping functions and the four algorithms over the 16S ribosomal DNA dataset.

Mapping function	FOS	GLCM	LBP	MLBP
MAP0	0.07093	0.07214	0.2498	0.1997
MAP1	0.09229	0.22709	0.2187	0.1805
MAP2	<b>0.04875</b>	0.27343	0.1977	0.1965
MAP3	0.05038		0.2106	0.1848
MAP4	0.06267	0.09814	0.1997	0.1630
MAP5	0.06592	0.06572	0.3395	0.1369

# Results

Square error of mapping functions and algorithms over the NADH dataset

Table: Square error of all mapping functions and the four algorithms over the NADH dataset.

Mapping function	FOS	GLCM	LBP	MLBP
MAP0	<b>0.0103</b>	0.0345	0.0406	0.0711
MAP1	0.1795	0.2279	0.2029	0.0895
MAP2	0.0126	0.0307	0.1682	0.1258
MAP3	0.1642		0.1310	0.1022
MAP4	0.0297	0.0784	0.1410	0.0630
MAP5	0.0865	0.0345	0.0792	0.0452

# Results

Square error of mapping functions and algorithms over the mitochondrial genome dataset

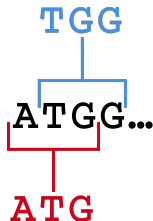
Table: Square error of all mapping functions and the four algorithms over the mitochondrial genome dataset.

Mapping function	FOS	GLCM	LBP	MLBP
MAP0	<b>0.0329</b>	0.0569	0.1693	0.1254
MAP1	0.2439	0.1951	0.1294	0.1465
MAP2	0.0417	0.1567	0.1746	0.1654
MAP3	0.1811		0.0768	0.1094
MAP4	0.0570	0.0731	0.1765	0.1724
MAP5	0.1255	0.0851	0.1622	0.1575

# Results

Discussion: Best mapping function

MAP0 function and histogram proposed by Deliba et al. [3] is very similar to k-mer frequencies. [6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]



ATTTA 762	ATTTG 434	AGCCCA 50	AGCCCG 3
ATTTC 427	ATTTT 830	AGCCCC 34	AGCCCT 36
ACGGGA 40	ACGGGG 17	ATAAAA 312	ATAAAG 418
ACGGGC 7	ACGGGT 25	ATAAAC 382	ATAAAT 441

LBP and MLBP reflects the correlation among pixels within a local area, but the main information in DNA sequences is not related to correlations of neighbor bases.

# Conclusions

- We compared FOS, GLCM, LBP, and MLBP with six mapping functions. We also, compare the phylogenetic trees with Robinson Fould algorithm and Phylo.io.

# Conclusions

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- FOS got the best results. Moreover, MAP1 was the worst mapping function and MAP0 was the best because of its similarity to k-mer method.






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- We compared FOS, GLCM, LBP, and MLBP with six mapping functions. We also, compare the phylogenetic trees with Robinson Fould algorithm and Phylo.io.
- FOS got the best results. Moreover, MAP1 was the worst mapping function and MAP0 was the best because of its similarity to k-mer method.
- LBP and MLBP are not suitable for sequence similarity because they consider the correlation between neighbors.




# Conclusions

- We compared FOS, GLCM, LBP, and MLBP with six mapping functions. We also, compare the phylogenetic trees with Robinson Fould algorithm and Phylo.io.
- FOS got the best results. Moreover, MAP1 was the worst mapping function and MAP0 was the best because of its similarity to k-mer method.
- LBP and MLBP are not suitable for sequence similarity because they consider the correlation between neighbors.
- Furthermore, FOS's tree is the most similar to MEGA's tree for the NADH dehydrogenase and the mitochondrial genomes datasets.




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# Questions?

