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A Modified Median String Algorithm for Gene Regulatory Motif Classification

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Abstract: Consensus string is a significant feature of a deoxyribonucleic acid (DNA) sequence. The median string is one of the most popular exact algorithms to find DNA consensus. A DNA sequence is represented using the alphabet Σ= {*a*, *c*, *g*, *t*}. The algorithm generates a set of all the 4^l possible motifs or *l-mers* from the alphabet to search a motif of length *l*. Out of all possible *l-mers*, it finds the consensus. This algorithm guarantees to return the consensus but this is NP-complete and runtime increases with the increase in *l-mer* size. Using transitional probability from the Markov chain, the proposed algorithm symmetrically generates four subsets of *l-mers*. Each of the subsets contains a few *l-mers* starting with a particular letter. We used these reduced sets of *l-mers* instead of using 4^l *l-mers*. The experimental result shows that the proposed algorithm produces a much lower number of *l-mers* and takes less time to execute. In the case of *l-mer* of length 7, the proposed system is 48 times faster than the median string algorithm. For *l-mer* of size 7, the proposed algorithm produces only 2.5% *l-mer* in comparison with the median string algorithm. While compared with the recently proposed voting algorithm, our proposed algorithm is found to be 4.4 times faster for a longer *l-mer* size like 9.

Keywords: DNA consensus; markov chain; median string algorithm; pattern recognition

1. Introduction

Within the gene, there are short DNA sequences that control the organic phenomenon. These short DNA sequences are known as gene regulatory binding motifs. It is very difficult to identify those short motifs. Those are solely 6–15 nucleotide pairs long. Regulatory motifs don't have any specific point to begin and stop. These motifs can start from and stop anywhere within the sequence. A restrictive motif is indistinguishable from an equivalent random sequence. It is a specific pattern that happens more often than expected beneath the background sequence. The location of such a pattern may differ from one sample to another. In addition, the frequency of a particular pattern may differ from one sequence to another. Discovery of such a regulatory motif from a DNA sequence therefore involves finding such a continuation of the pattern [1]; this can be illustrated in Figure 1.

DNA sequences are composed of the nitrogen bases adenine, cytosine, thiamin, and guanine. These four bases are represented by four symbols *a*, *c*, *g*, and *t*. Finding a specific motif from a DNA sequence incurs a search for a short pattern over the alphabet Σ = {*a*, *c*, *g*, *t*}. The motif search process emphasizes two points: (a) a way to figure out the sequence motif by the application of a proper model; (b) an efficient way to formulate an algorithm for motif finding. The foremost unremarkably used models for motif representation are position weight matrices (PWM) [2] and consensus sequences [3]. The position weight metrics system generally takes a shorter time to report. These metrics use statistical methods but there is no guarantee about finding a global optimum [4–6]. The exact algorithms, which use consensus sequences to represent motifs, are sure to report all (*l*, *d*) motifs by traversing the

full search area. Most of the exact algorithms are driven by patterns. In the case of pattern-driven algorithms, the search space is $O(|\Sigma|^l)$. It rises dramatically with the increment of $|\Sigma|$. As a consequence, most of the existing exact algorithms have been designed only for looking for motifs in deoxyribonucleic acid sequences wherever $|\Sigma|=4$, and that they cannot search low-conserved motifs within a reasonable time within the data sets over larger alphabets, like the protein data sets wherever $|\Sigma| = 20$.

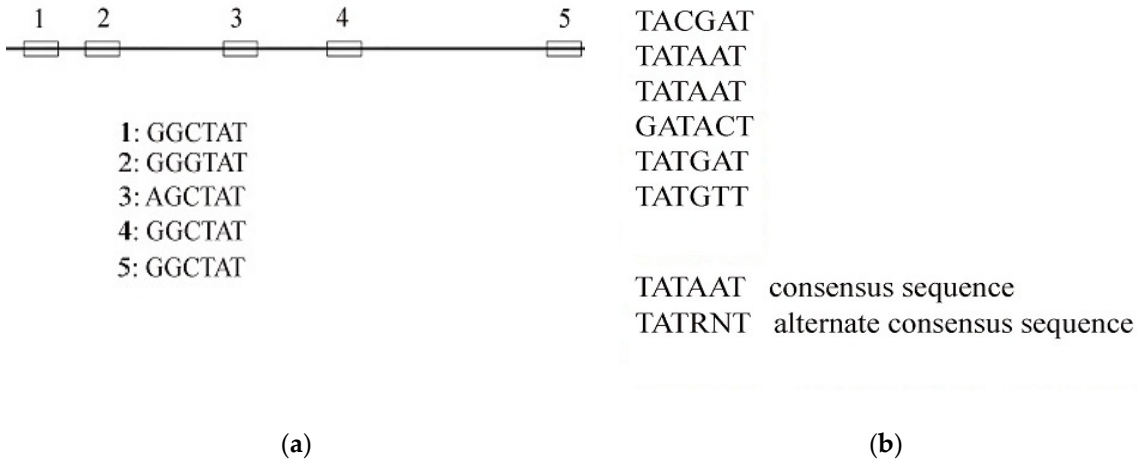


Figure 1. Illustration of consensus string: (**a**) Aligning the DNA sequences; (**b**) The most common and most frequent sequence is the consensus string.

To increase the capability of the exact algorithms for larger alphabets, Ref. [7] proposed the idea of the motif stem within the field of motif search. The consensus is a representative string of a given set S of strings. This is sometimes called the closest string or center string. In the case of multiple string comparison, the consensus is one of the major issues. It has been explored elaborately [8–15] to address several questions arising from computational biology. Though finding the consensus sequence is an NP-complete problem, researchers even have developed fixed-parameter algorithms [10,16–18], approximation algorithms [13,17,19–23] and algorithms for a short range of strings [10,24,25]. All the fixed-parameter algorithm shows how to solve an NP-complete problem in linear time in the case of consensus search. Given k sequences of equal length and a positive integer d, find a "closest string" s such that none of the given sequences has a hamming distance longer than d from s. The closest string is one of the prime obstacles in consensus analysis. Solving the closest string is an NP-complete problem. The authors of [10] and [16] proposed an algorithm to solve this problem in polynomial time. In addition, the authors of [17] proposed an exact algorithm to solve the closest string problem with time complexity $O(n|\Sigma| O(d))$, where Σ is the alphabet. The 1-mismatch problem is the determination of all the maximal sections from an alignment where there exists a "center" string. The inside of the section alignment rows are within Hamming distance 1 from the center. Solving the center string is also NP-complete. The authors of [18] introduced a polynomial-time algorithm to solve the center string problem. In [13], an application of a genetic algorithm to solve the closest string problem was proposed. A polynomial-time approximation algorithm was proposed in [19]. An approximation algorithm for solving center string problem was introduced in [20]. An approximation scheme for the hamming clustering problem was proposed in [21]. A comprehensive study of all recent research in the field of motif search is summarized in [25]. Some new algorithms have been proposed. Among them, oligonucleotide analysis [26], SLI-REST [7], MCES [27], Pruner [28] and Voting [29] algorithms are the most recent.

Among all the algorithms, the median string algorithm provides the highest level of guarantee that it must find the consensus. That is because the median string is a brute force algorithm. The only thing it lacks is that it takes a long time to execute. The median string algorithm searches all the $|\Sigma|^l$ *l-mers*. As the *l-mer* size grows, the execution time grows exponentially. As the size of the alphabet and *l-mer* size both grow, the execution time makes the median sting an inferior algorithm. In this paper,

we used a Markov chain to reduce the search space. As a result, the execution time reduces dramatically. We combined the statistical method to reduce the search space of an exact algorithm. We got the same output within a shorter period. Experiments indicate our proposed algorithm executes faster than the median string algorithm. Furthermore, the paper includes mathematical proof about why this method will produce the same output as that of the brute force algorithm. Finally, we compared the execution time of our proposed algorithm with the execution time of the voting algorithm for motif search. We found our proposed algorithm is faster than the voting algorithm for longer *l-mer* size.

2. Background Information

2.1. Median String Algorithm for Consensus Sequence

The input parameters of the median string algorithm are a set of DNA sequences, number of sequences, number of nucleotides in each sequence, and the length of the motif to be searched. A set of t number of DNA sequences of length n and the length of the motif to be searched l are included. The algorithm produces all possible motifs of length l. Since DNA sequences are composed of four types of nucleotides, the number of all possible *l-mers* is 4^l. It then takes one *l-mer*, compares the nucleotides of the *l-mer* with the l nucleotide starting from the first nucleotide of the first sequence, and calculates the distance. After that, it shifts one nucleotide right, places the *l-mer*, and calculates the distance. The process continues up to the end of the sequence. The minimum distance is the score for the *l-mer* with the sequence. In this way, scores for the *l-mer* with all the sequences are calculated. The sum of scores is the score for the *l-mer* with the set of DNA sequences. The scores for all the *l-mers* are calculated using the same process. The *l-mer* with the lowest score is the consensus string. The algorithm is listed below:

Algorithm 1: Median String Search

```
{
inputs: DNA,t,n,l
output: bestFit_Motif
procedure:
MedianStringSearch (DNA, t, n, l)
        bestFit_Motif ← AAA … A
        bestFit_Score ← ∞
        for each l-mer s from AAA … A to TTT … T
            if TotalFit_Score(s, DNA) < bestFit_Score
          bestFit_Score ← TotalFit_Score(s, DNA)
          bestFit_Motif ← s
         return bestFit_Motif
}
```

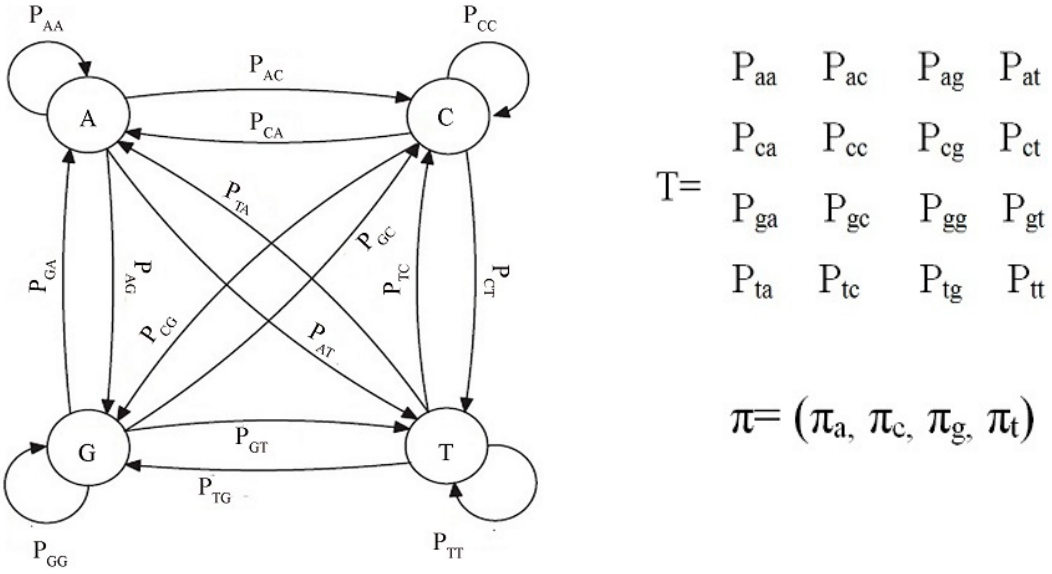
The median string algorithm examines all the 4^l combinations of *l-mers*. Consequently, the number of candidate *l-mers*, as well as execution time, grows exponentially with the rise of l; however, a significant amount of the generated 4^l *l-mers*, will not be the consensus. Even some of them can be absent in a particular sequence. Those motifs were generated as we were interested in generating all possible combinations. For those motifs too, the median string still performs the calculation and contributes to the time complexity. In this paper, we introduced the Markov chain to exclude infrequent candidate motifs. The exclusion of those unnecessary candidate motifs leads to a smaller search space and reduces the time complexity of the median string algorithm.

2.2. Markov Chain

In the case of a series of chance experiments, all the outcomes of the previous experiment could influence the result of the next experiment. The Markov chain can answer questions regarding the

probability of these types of chance experiments. Let S be a set of states where S = {s_1, s_s, ... , s_r}. The process can start from any one of the states and can proceed successively from one position to another. Markov defined these moves as steps. If the current position of the chain is in position s_i and it proceeds to position s_j in the next move, then the probability of this move is expressed by P_{ij}. P_{ij} is called transition probability. The chain can reside in the same position as probability $P_{ii.}$ If we plot the probabilities for all possible moves in a matrix, that matrix is called a transition matrix.

In bioinformatics, DNA sequences can be modeled using Markov chains. A DNA sequence is a series of four-letter alphabet $\sum = \{a, c, g, t\}$. In the case of DNA sequences, a Markov chain is defined by $\sum$ and a probability for transition $P_{ij.}$ The transition probabilities are stored in a transition matrix. A sequence defined by the trajectory of the process through the state space. The scenario is depicted in Figure 2. The diagram exhibits that the sequence can start from any nucleotide and the probability of the next nucleotide is dependent on the current nucleotide. If we start from the state *a*, the next nucleotide can be any one of *a*, *c*, *g*, *t*, and the probability is given by P_{aa}, P_{ac}, P_{ag}, and P_{at}. Starting from another nucleotide will incur another set of transition probabilities. If we start from state *g*, then the probabilities would be P_{ga}, P_{gc}, $P_{gg,}$ and P_{gt}. DNA sequences are defined by Markov Chain in this manner. All the transition probabilities are listed in the transition matrix T and the start state probabilities are given by a vector $\Pi = (\Pi_a, \Pi_c, \Pi_{g,}\Pi_t)$.



(**a**) (**b**)

Figure 2. Markov chain and transition probability: (**a**) Markov chain; (**b**) Transition probability matrix along with start probability vector.

The mathematical definition of entries in the transition matrix is as follows:

$$P_{xy} = p(s_{i+1} = y | s_i = x) \quad (1)$$

This means that the probability of moving from state x to state y is equal to the conditional probability of having state y given that the previous state was x. The probability of the entire sequence can be expressed as a joint probability:

$$P(s) = P(s_1, s_2, \ldots s_n) \quad (2)$$

Simplification of calculation of this probability can be made by factorizing it:

$$P(s) = P(s_n|s_{n-1})P(s_{n-1}|s_{n-2}) \ldots\ldots..P(s_2|s_1)\Pi(s_1) \quad (3)$$

Or

$$P(s) = \Pi(s_1)\prod_{i=2}^{n} P(s_i|s_{i-1}) = \Pi(s_1)\prod_{i=2}^{n} P_{s_{i-1}s_i} \quad (4)$$

where $\Pi(s_1 = x)$ means the probability of having x in location s_1. As a result, we can conclude that the probability of a symbol occurring is dependent only on the previous symbol to simplify the calculation of the joint probability of the entire sequence.

3. Proposed Markov Chain Based Median String Algorithm

Let us consider a database with 10 sample DNA sequences $(S1, S2, \ldots, S10)$. Each sample is composed of 80 nucleotides as in Table 1. Samples are different from each other. The problem is to find whether there is any common motif that exists among the sequences.

Table 1. Sample DNA sequence database.

SID	Sample
S_1	tagtggtcttttgagtgtagatctggagggaaagtatttccaccagttcggggtcacccagcagggcagggtgacttaat
S_2	cgcgactcggcgctcacagttatcgcacgtttagaccaaaacggagttggatccgaaactggagtttaatcggagtcctt
S_3	gttacttgtgagcctggttagacccgaaatataattgttggctgcatagcggagctgacatacgagtaggggaaatgcgt
S_4	aacatcaggctttgattaaacaatttaagcacgtaaatccgaattgacctggtgacaatacggaacatgccggctccggg
S_5	accaccggataggctggttattaggtccaaaaggtagtatcgtaataatggctcagccatgtcaatgtgcggcattccac
S_6	tagattcgaatcgatcgtgtttctccctctggtggttaacgaggggtccgaccttgctcgcatgtgccgaacttgtaccc
S_7	gaaatggttcggtgcgatatcaggccgttctcttaacttggcggtgcagatccgaacgtctctggaggggtcgtgcgcta
S_8	atgtatactagacattctaacgctcgcttattggcggagaccatttgctccactacaagaggctactggtgtgatccgta
S_9	ttcttacacccttctttagatccaaacctgttggcgccatcttcttttcgagtccttgtacctccatttgctctggtgac
S_{10}	ctacctatgtaaaacaacatctactaacgtagtccggtctttcctggtctgccctaacctacaggtcgatccgaaattcg

From this database, we will extract the consensus string using the proposed system. A schematic layout of the proposed system is given in Figure 3.

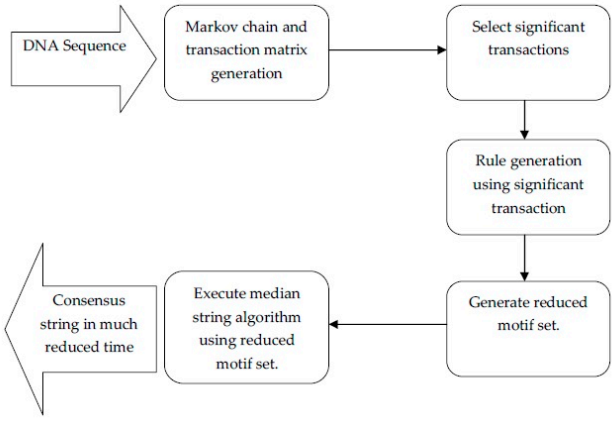


Figure 3. Schematic layout of the proposed system.

3.1. Markov Chain Generation

In the database, there are 10 sequences. Each sequence contains 80 nucleotides. There are $4^2 = 16$ possible two-nucleotide sequences i.e., *aa*,*ac*,*ag*,*at*,*ca*,*cc*,*cg*,*ct*,*ga*,*gc*,*gg*,*gt*,*ta*,*tc*,*tg*, and *tt*. We first count the frequencies of the occurrence of two-nucleotide sequences in the database. From the counts of the two nucleotide sequences, we constructed a Markov chain, which is depicted in Figure 4. According to the

following Markov chain, the frequency of *aa* is 49, *cc* is 46, etc., in the database. Now, we have the counts of 16 possible two nucleotide sequences or di-grams in the database.

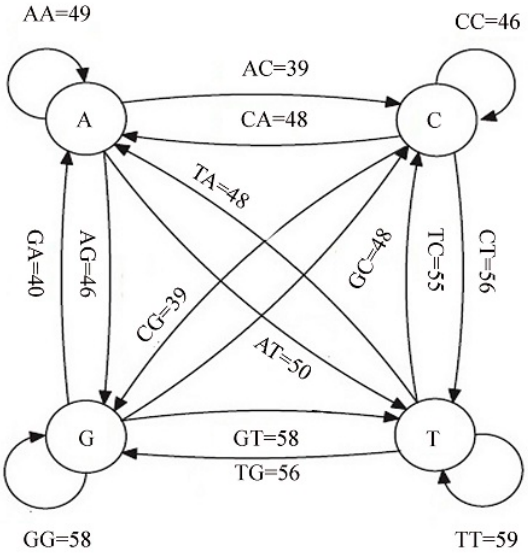


Figure 4. Markov chain generated using data in Table 1.

3.2. Transaction Matrix Creation

We now construct a transition matrix from the Markov chain as in Figure 5. Rows in the matrix indicate the first nucleotide and columns in the matrix indicate the second nucleotide of the two-nucleotide sequences. The first row is for sequences starting with *a*. The first element in this row indicates the counts of *aa* in the database. The second element indicates the counts of *ac*, etc. The next row is for sequences starting with the nucleotide *c*, the third row is for sequences starting with the nucleotide *g*, etc. The transition matrix contains counts for all the 16 two nucleotide sequences in the database.

Transition=

	a	c	g	t
a	49	39	46	50
c	48	46	39	56
g	40	48	58	53
t	48	55	56	59

Figure 5. Transaction matrix using Markov chain.

This matrix helps to infer about the next nucleotide given the staring nucleotide. For example, if we start from nucleotide a, then we can infer P(t|a) > P(a|a) > P(g|a) > P(c|a).

3.3. Rule Generation

In this stage, we tried to find the most frequent transitions as well as the most probable transitions. For this purpose, we sum up all the counts in the transaction matrix. The sum of all the elements of the transaction matrix is 790. To cover 50% of elements, we set the threshold as 790/2 = 395. We start with the highest value in the matrix. The highest value is 59, which represents P(t|t). This element value is

less than the threshold value. We select this element and set the threshold as 395 − 59 = 336. The next highest value in the matrix is 58, which represents P(g|g). This value is less than the threshold value. We select this element and set the threshold as 336 − 58 = 278. The process continues until the element value is greater than the threshold value. This process produces the seven most frequent transitions representing the following probabilities P(t|t),P(g|g),P(c|t),P(t|g),P(t|c),P(g|t),P(a|t). At the end of the process, we will have a matrix with some encircled values as in Figure 6.

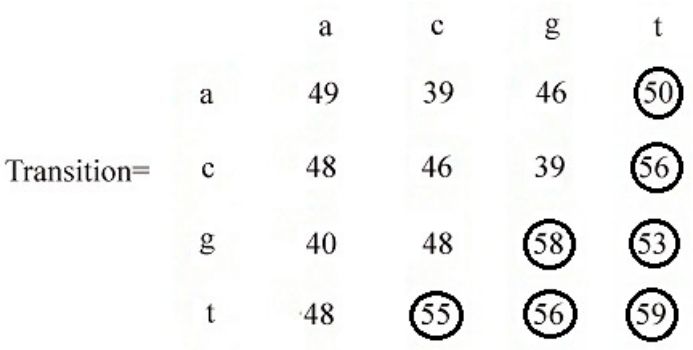


Figure 6. Identification of significant elements in the transition matrix.

The encircled elements are significant; that is, two-nucleotide sequences (*tt*,*ct*,*gg*,*gt*,*tc*,*tg*,*at*) are significant. If we randomly pick any two-nucleotide sequence from the database, there is a more than 50% probability that the sequence will be in the set of (*tt*, *ct*, *gg*, *gt*, *tc*, *tg*, *at*). We now generate rules from this significant combination as in Table 2.

Table 2. Rule generation.

First (l_1)	Second (l_2)	Count	Rule
t	t	59	t→t
g	g	58	g→g
t	g	56	t→g
c	t	56	c→t
t	c	55	t→c
g	t	53	g→t
a	t	50	a→t

3.4. Reduced l-mer Set Generation

According to the rules, we will now generate *l-mers.* For example, let us suppose $l = 4$, then we will generate 4-mers according to these rules. 4-mers can start with any nucleotide at the beginning. If it starts from *a*, we will have a tree generating all the possible 4-mers starting with *a* that follow the rules in Table 2, as in Figure 7. According to rules recorded in Table 2, the next nucleotide after *a* will be *t;* i.e., a two-nucleotide sequence starting with *a* is *at.* After *at*, the next nucleotide can be *t* or *c* or *g*, according to the rules. As a result, we will have 3 three-nucleotide sequences (*att*, *atc*, *atg*), which start with nucleotide *a* and follow the rules. After *atc*, the next nucleotide can be *t* or *c* or *g*, which produces the 4-mers *attt*, *attc*, and *attg.* Symmetrically, from *atc* we have the 4-mer *atct* and from *atg* we have the 4-mers *atgg* and *atgt.* 4-mers also can start with the other three nucleotides *c*,*g*, and *t.* Symmetrically, we will have three other trees for 4-mers starting with *c*,*g*, *and t.* From these trees, we will have all the 4-mers that follow the rules. These are the motifs that we will use instead of all possible 4^l numbers of motifs.

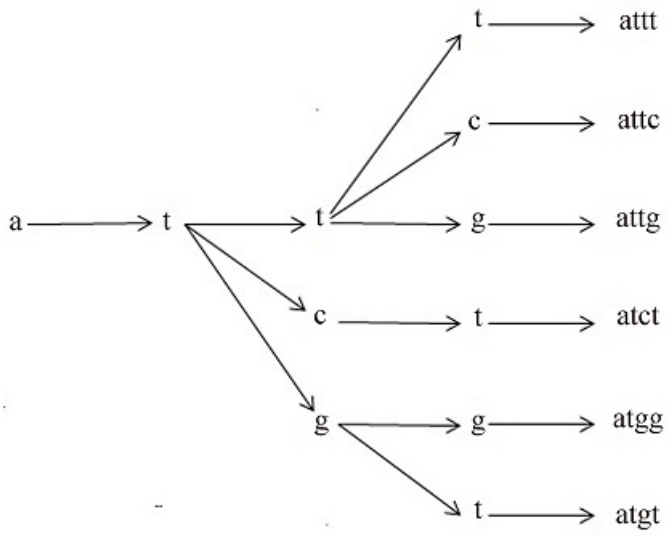


Figure 7. Motif generation tree.

3.5. Proposed Algorithm

Algorithm 2: Markow chain based median string algorithm(DNA,l)

```
{ input:DNA,l
Output: consensus sequence

Modified_Median_String (DNA,l)
{
bestFit_Motif ←AAA . . . A
bestFit_Score ←∞
reduced_motif_set = Reduced_Motif_Set_Generator(DNA,l)
for each l-mer in reduced_motif_set
            if TotalFit_Score(s, DNA) < bestFit_Score
      bestFit_Score←TotalFit_Score(s, DNA)
      bestFit_Motif ← s
return bestFit_Motif
}
Transaction_Matrix_Generator(DNA)
{input:DNA
output:Transition_Matrix
ngram_dict←new dictionary()
i←1
for each sequence in DNA
     for each character in sequence up to sequence length-1
          if character not in ngram_dict.keys()
               ngram_dict[character]←Null
          NextCharacter←Sequence[i + 1]
          ngram_dict[character].append(NextCharacter)
          ←i + 1
TM←empty matrix
      for each key in ngram_dict
     TM ← Counter(ngram_dict(key))
Return TM
}
```

```
Rule_Generator(Transition_Matrix)
{input:Transition_Matrix
output:rule_list
total= ΣTransition_matrix_ij
s ← 0
rule_list ← empty list()
    for each element in Transition_Matrix in descending order
        s ← s+ Transition_matrix element
        if s < total/2
            append a rule in rule_list
        else
            break
    return rule_list
}
Reduced_Motif_Set_Generator(DNA, l)
{input:DNA, length of l-mer
output:Reduced_Motif_Set
TM ← Transition_Matrix_Generator(DNA)
rules ← Rule_Generator(TM)
S1,tmp,tmp2 ← empty list
For each character in {'a', 'c', 'g', 't'}
    if rules.key ==character
        S1 ← character + rules.value
    tmp ← S1
for i = 1 to length of l-mer-2
    for element in tmp
        if rules.key ==last character of element
            tmp2 ← element + rules.value
    tmp ← tmp2
    tmp2 ← null
return tmp
}
```

4. Result and Discussion

In this section, we have analyzed the performance of the proposed method and compared it with state-of-the-art methods in terms of Jupyter Notebook as a Python programming language. We developed the existing method using Algorithm 1. and the proposed method using Algorithm 2. We compared the performance of the proposed system with that of the median string algorithm and voting algorithm. The system configuration is

- Processor: Intel Core i5 CPU
- Clock rate:2.6 GHz
- HardDisk:1000GB
- RAM:4GB

4.1. Comparison with Median String Algorithm

In comparison with the median string algorithm, the proposed algorithm expedites the whole process easier and faster. In this illustration, we have analyzed the number of motifs generated by the Markov chain-based median string algorithm and the median string algorithm for different *l-mer* sizes. A comparison between the proposed algorithm and median string algorithm in terms of the number of generated motifs is listed in Table 3.

Table 3. Comparative interpretation between the proposed algorithm with the median string algorithm in terms of motif generation.

l-mer Size	Proposed Method	Median String	Ratio of Number of Produced Motifs
2	7	16	0.4375
3	17	64	0.2656
4	37	256	0.1445
5	84	1024	0.0820
6	188	4096	0.0458
7	427	16,784	0.0254

The table reports the results of both of the techniques adopted in this paper. In the case of *l-mer* size 2, the proposed method forms 7 motifs whereas the existing algorithm produces 16 motifs. In this case, the number of motifs generated by the proposed algorithm is 43.75% of that of the existing algorithm. The last row depicts that the introduced algorithm produces 427 and the existing algorithm returns 16,784 motifs of length 7. In this case, the proposed algorithm yields only 2.5% motifs while contrasted with that of the existing algorithm. Hence, our proposed algorithm originates fewer motifs in relation to the existing algorithm, which is depicted in Figure 8a. As a result, we claim that our proposed algorithm can reduce the search space significantly.

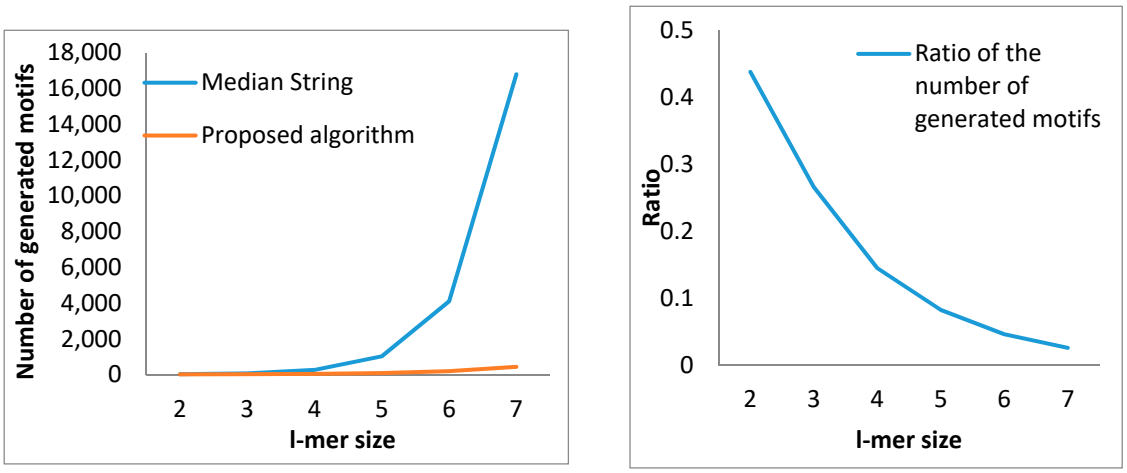


Figure 8. Comparison in terms of motif generation: (**a**) Number of motifs generated by both algorithms; (**b**) The ratio of the number of generated motifs between the proposed algorithm and median string algorithms.

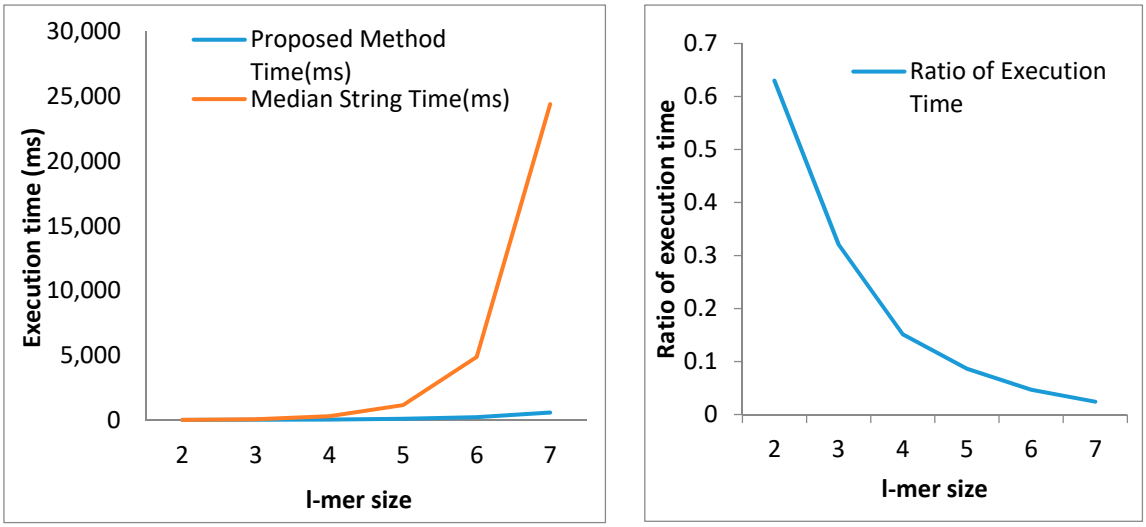
Furthermore, with the increase in *l-mer* size, the proposed algorithm appears to be superior to the median string algorithm in terms of the number of motifs produced. The ratio of the number of produced motifs between the proposed and the existing algorithm for various *l-mer* lengths is recorded in the rightmost column of Table 3. In the case of the *l-mer* of length 2, the ratio is 0.4375 and for *l-mer* of length 7, the ratio is 0.0254. This indicates, with the increase in *l-mer* size, that the ratio is gradually decreasing, which is depicted in Figure 8b. This finding means that the proposed algorithm produces fewer motifs than the existing algorithm. This lower number dramatically reduces with the increase in *l-mer* size (See Appendix A).

We have investigated every level of time consumed by both methods. The introduced approach necessitates a huge time for DNA consensus. The time consumed by these two approaches betokens the robustness of our proposed algorithm. The execution time of both algorithms for different *l-mer* size is listed in Table 4.

Table 4. Comparative interpretation between the proposed algorithm and median string algorithm in terms of execution time.

l-mer Size	Proposed Method Time(ms)	Median String Time(ms)	Ratio of Execution Time
2	10.20	16.10	0.6300
3	21.36	66.6	0.3207
4	44.84	296	0.1514
5	100.18	1160	0.0863
6	228.16	4880	0.0467
7	587.44	24,400	0.0240

Every row of the table demonstrates that the proposed algorithm spent less time than the existing orientation. In connection with the *l-mer* of length 2, the difference in execution time is (16.10 − 10.20) ms = 5.90 ms. It implies that for *l-mer* size 2, the execution time of the proposed method is only 63% of that of the existing algorithm. The time differences have been increased as the *l-mer length* increased. From the last tuple, we notice that for *l-mer* of length 7, the proposed algorithm consumed 587.44 ms, whereas the existing algorithm spent 24,400 ms. In this case, the proposed algorithm's execution time is only 2.4% of the execution time of the median string algorithm. Now, we can claim our proposed algorithm to be faster than the median string algorithm, which is depicted in Figure 9a.



(**a**) (**b**)

Figure 9. Illustration in terms of execution time (**a**) Comparative illustration of execution time between the proposed algorithm and median string algorithm; (**b**) The ratio of execution time between the proposed algorithm and median string algorithm.

With the increase in the length of *l-mer*, the introduced algorithm appears to be superior to the existing algorithm. The ratios of execution time between the proposed and the existing algorithm for *l-mer* of different lengths are listed in the rightmost column of Table 4. In the case of the *l-mer* of length 2, the ratio is 0.63 and for *l-mer* of length 7, the ratio is 0.024. The ratio is gradually decreasing with the increase in *l-mer* size, which is depicted in Figure 9b. This finding means that the proposed algorithm is faster than the median string algorithm. Besides that, it also proves that the fastness of the proposed algorithm rises dramatically with the increase in *l-mer* size.

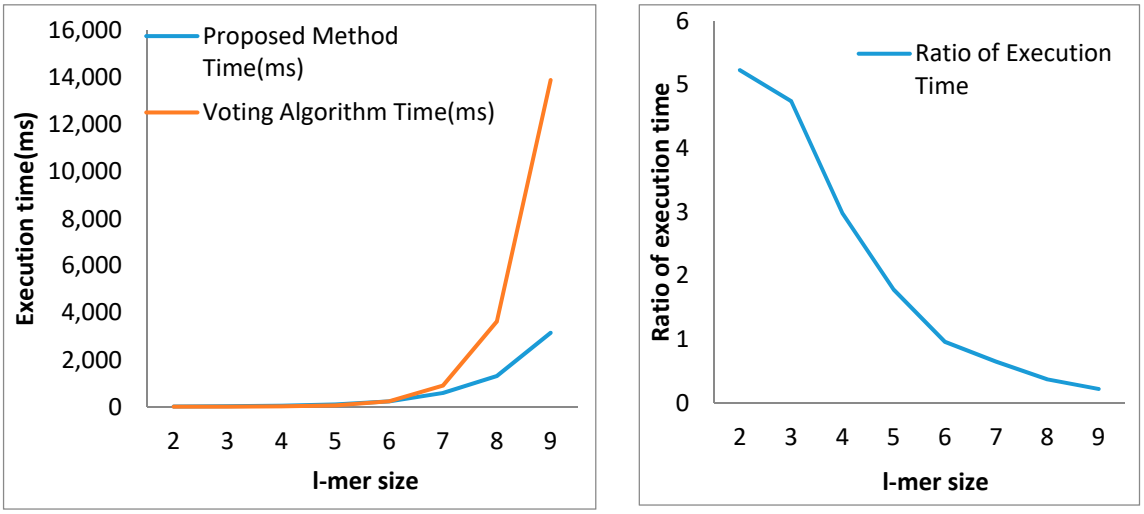
4.2. Comparison with the Voting Algorithm

Finally, we compared the execution time of our proposed algorithm with the execution time of the voting algorithm for *l-mer* of different lengths. The execution times for both algorithms, and their ratios, are given in Table 5.

Table 5. Comparative illustration between the proposed algorithm and voting algorithm in terms of execution time.

l-mer Size	Proposed Method Time (ms)	Voting Algorithm Time (ms)	Ratio of Execution Time
2	10.20	1.95	5.23
3	21.36	4.50	4.74
4	44.84	15.00	2.98
5	100.18	56.00	1.78
6	228.16	236.00	0.96
7	587.44	903.00	0.65
8	1310.00	3630.00	0.37
9	3150.00	13,900.00	0.22

From the table above, we can see for *l-mer* size 2, the execution time of our proposed algorithm is 5.23 times that of the voting algorithm. Though the ratio of execution time is gradually decreasing, it is still above 1 for *l-mer* size 5. This means that, up to *l-mer* size 5, the proposed algorithm remains slower than the voting algorithm;however, from *l-mer* size 6 upward, the execution time ratio becomes less than 1. From this finding, we can claim that our proposed algorithm is faster than the voting algorithm for longer *l-mer* sizes, which is depicted in Figure 10a.



(**a**) (**b**)

Figure 10. Illustration in terms of execution time (**a**) Comparative illustration of execution time between the proposed algorithm and voting algorithm; (**b**) The ratio of execution time between the proposed algorithm and voting algorithm.

Figure 10b depicts the scenario well. The execution time ratio starts at 5.23 for *l-mer* size 2. For *l-mer* size 6, it decreases to less than 1, actually to 0.96. From this level, our proposed algorithm becomes faster. Decreasing gradually, the execution time ratio becomes 0.22 for *l-mer* size 9. From this finding, we can claim that the fastness of the proposed algorithm rises with the increase in *l-mer* size.

5. Conclusions

The median string is an enumeration-based algorithm. The algorithm exhaustively searches the entire search space to find the consensus sequence. As a result, it has become an exponential-time algorithm. The number of candidate *l-mers* increases with the rise of the *l-mer* size. As a result, the execution time also increases with *l-mer* size. As a result, the existing algorithm takes a long time to detect longer l and is inefficient for longer sequences. The only advantage is that it can guarantee that it must find the consensus. Our proposed Markov chain-based median string algorithm produces a subset of the 4^l number of *l-mers*, and does the same operation as the existing algorithm with a reduced number of *l-mers*. Since the search has been reduced, this algorithm takes less time to execute. We compared the proposed algorithm with the recently developed voting algorithm. This comparison illustrates that the proposed phenomena can eradicate the inefficiency of the median string algorithm in the case of longer *l-mer*. The proposed algorithm can guarantee that it must find the consensus; however, it does not use an exhaustive search approach rather a stochastic approach. The execution time of the proposed algorithm does not grow exponentially with the rise of *l-mer* length. In the field of gene regulatory motif search, position weight metric-based algorithms take a shorter time to execute but can not guarantee that they can certainly find consensus. Enumeration-based algorithms can guarantee this, but take exponential time. The proposed algorithm combined both of these approaches and eradicated both method's disadvantages. In this research, we calculated all the samples as a whole. In the future, however, we have the plan to execute an experiment by taking each sample's Markov chain and transition matrix individually, then superimpose the transition matrices one after another. From that superimposed matrix some rules could be generated.

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Appendix A Mathematical Proof That the System Will Work

From the transition matrix in the Figure 8, we have seven encircled elements. There are sixteen elements for sixteen possible transitions. The first element of the first row means there are 49 transitions from nucleotide *a* to *a*, the second element of the first row indicates that there is 39 transitions from nucleotide *a* to *c*. We have seven encircled transition frequencies. These are the top frequencies of transition. Based on these top frequencies, we can generate a probability matrix as follows. Using the encircled frequencies, we generated some transition rules. Using those rules, we generated some motifs. Let us consider our *l-mer* size as 5. Then, our system generates only 84 motifs of length 5. Let us put these motifs in set X = {set of motifs generated by the proposed system}. On the other hand, the median string algorithm generates all the $4^5 = 1024$ motifs of length 5. Let us put these motifs in a set Y = {motifs generated by median string}. Now x is a subset of y. Basically,

$$Y = X \cup W \cup Z \tag{A1}$$

where,

X = {set of motifs generated by the proposed system},
W = {set of motifs generated by the rules based on frequencies which are not encircled},
Z = {set of motifs generated by the rules based on frequencies both encircled and not encircled}.

We can calculate the probability of a particular motif of length 5 using the theory of conditional probability

$$P(X|C_i) = \prod x_k \Big| c_i = P(x_1|c_i) \times P(x_2|c_i) \ldots\ldots\ldots \times P(x_k|c_i) \tag{A2}$$

For *l-mer* of length 5 we can write,

$$P(A \cap B \cap C \cap D \cap E) = P(A) \times P(B\big|A) \times P(C\big|AB) \times P(D\big|ACB) \times P(E\big|ABCD) \tag{A3}$$

Since this equation is a multiplication, and the frequencies that are not encircled are smaller than those that are encircled, the conditional probability of occurrence of any motif from the set $\boldsymbol{W}$ must be less than the probability of occurrence of the motif with the highest conditional probability from the set $\boldsymbol{X}$. In addition, the conditional probability of occurrences of any motif from the set $\boldsymbol{Z}$ will be smaller than the probability of occurrences of the motif with highest conditional probability from the set $\boldsymbol{X}$. The motif with the highest conditional probability will be the consensus. Since the motifs in set $\boldsymbol{X}$ are generated using the most frequent di-grams, the motif with the highest conditional probability will always be found in set $\boldsymbol{X}$.

In our test data set, we found ***ctggt*** as the consensus for *l-mer* size 5 using both methods. Now, according to our claim, ***ctggt*** should have the highest conditional probability. Using Equation (2), we found the conditional probability of ***ctggt*** as follows:

$$P(ctggt) = P(c) \times P(t\big|c) \times P(g\big|ct) \times P(g\big|ctg) \times P(t\big|ctgg) = \ 0.0000112061$$

This is the highest conditional probability, among all the 1024 motifs generated by the median string algorithm. The proposed system generates only 84 motifs including the consensus motif *ctggt.*

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