Effective Encoding for DNA Sequence Visualization Based on Nucleotide’s Ring Structure

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Abstract: Effective representation of DNA sequences is one of the important tasks in the study of genome sequences. In this paper, we propose a graphical representation of DNA sequences based on nucleotide ring structure. In the proposed representation, we convert DNA sequences into 16 dinucleotides on the surface of the hexagon so that it can preserve nucleotide’s chemical property and positional information. Our approach can provide capability of efficient similarity comparison between DNA sequences and also high comparison accuracy. Furthermore, our approach satisfies uniqueness and no degeneracy of DNA sequences. In the experimental study, we use phylogeny analysis for evolutionary relationship among different species. Extensive performance study shows that the proposed method can give better performance than existing methods in comparison with the degree of similarity.

Keywords: β-globin gene, DNA curve, hexagon, ring structure

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Introduction
The rapid growth of biological sequences, such as of DNA, RNA, and protein, has demanded effective analysis methods for large biological sequences. Additionally, the analysis results are very helpful to biological researchers for predicting genes’ structure and function, as well as similarity comparison between genes and different species.

For biological sequence analysis, two approaches have been mainly used: (i) sequence alignment method and (ii) non-sequence alignment method. The first approach obtains a degree of similarity between DNA sequences by comparing alignment scores of two sequences. This approach suffers from expensive computational cost as the length of sequences grows exponentially. The second approach analyzes DNA sequences by establishing a statistical model or a graphical representation model, or some machine learning model of DNA sequences. Recently, this approach is popularly studied due to the fact that it can give better accuracy and low computational overhead.

In the case of non-sequence alignment method, effective DNA sequence representation or feature selection from DNA sequences is essential for DNA sequence analysis, in areas such as gene prediction, similarity comparison between genes of different species, and finding gene structure and function. For this purpose, several graphical representations have been proposed according to chemical structures of 4 nucleotides, reflecting their distribution with different chemical structure and allowing numerical characterization. As for feature selection, several machine learning techniques are effectively applied such as principal component analysis (PCA), neural network, and several classification models.

In this paper, we propose a graphical representation of DNA sequences based on nucleotide ring structure. In the proposed representation, we convert DNA sequences into 16 dinucleotides on the surface of the hexagon so that the nucleotide’s chemical property and positional information is preserved. Our approach satisfies uniqueness and no degeneracy of DNA sequence is observed. It can also provide capability of efficient similarity comparison between DNA sequences in addition to high comparison accuracy. Extensive performance study shows that the proposed method can give better performance than existing methods in comparison with the degree of similarity.

Related Works
Graphical technique of DNA sequences was first initiated by Hamori and Ruskin. Afterwards, many advances in 2D, 3D, 4D, and 6D representations of DNA sequences were developed. In this type of graphical presentation, nucleotides, dinucleotides, or tri-nucleotides are given a Cartesian coordinate in 2D through to 6D. Then DNA sequences are mapped into a set of Cartesian points and are plotted.

Additionally, there is some research which compares DNA sequences based on several mathematical invariants. For example, Wu et al proposed 10 correlation factors: 4 mononucleotide and 6 dinucleotide factors. Qi et al proposed a graph theory based representation for DNA sequences recently. Word-based measure is one of the most widely used alignment-free approaches for sequence comparison where each sequence is mapped into an n-dimensional vector according to its k-word frequencies/probabilities. Randić et al have considered kinds of condensed matrices. Genomic rules are proposed by Castro-Chavez to compare biological sequences and to find compatible genomes. The classic circular genetic code is used to present the practical aspect of the code rules of variation.

Castro-Chavez proposed natural patterns of symmetry and periodicity for tetrahedral representation of the genetic code. The method is applied to defragged I Ching genetic code and compared to Nirenberg’s 16 × 4 codon table. Those two properties (ie, symmetry and periodicity) act as the harmony between the chosen geometry and the biological reality. Graphical representation of DNA sequences based on mono, di, trinucleotides, etc. need to consider this harmony. Otherwise, it would merely be an instance of displaying the nucleotides (eg, mononucleotide, dinucleotide, codon) which have little biological sense.

When representing DNA sequences graphically, it must be ensured that there is no loss of information due to overlapping, loop, etc. and that the conversion from DNA sequence to graph and graph to DNA sequence should be one to one. However, some representations do not meet these criteria. Therefore, a graphical
representation containing uniqueness and no degeneracy is another contribution in the era of DNA sequence visualization.

On the other hand, methods which are based on non-graphical representation must also ensure that no information is lost. If not, the result would be compatible but not precise with other methods that have no loss in conversion. In this paper, we converted DNA sequences into DNA curves without any loss of information and degeneracy.

**DNA Sequence Visualization**

**by Hexagonal structure**

Chemical structure and classification of DNA bases

As stated previously, DNA sequences are the strings of four bases, that is, A, T, C and G. The core of these bases is heterocyclic organic compound, which forms ring in their chemical structure. Of them purines (A and G) have two rings while pyrimidines (C and T) have one. The chemical ring structures of those four bases are depicted in Figure 1.

The element of these cycles are carbon and nitrogen, hydrogen and oxygen. In Figure 1, sky balls are carbon and blue balls are nitrogen. The hexagonal cycle has nitrogen in positions 1 and 3, and carbon in positions 2, 4, 5, and 6. Other than carbon and nitrogen, the bases have oxygen and hydrogen bonded with carbon and nitrogen in different number. Hence, the bases differ in molecular weight. The molecular weight of A, T, C and G are 135.13, 112.1, 111.1 and 151.13 respectively. Their ascending order in terms of molecular weight is C → T → A → G.

The bases also differ by heterogenic cycle, functionality, and their bonding with hydrogen. A and C fall into the amino category while G and T are in the keto group, based on their functionality. A and T are bonded by three hydrogen bonds, and hence are in strong-H group while G and C are in weak-H group as they are bonded by only two hydrogen bonds.

**Proposed DNA encoding**

The proposed encoding of dinucleotides for DNA sequence visualization is solely based on ring structure of DNA bases and their molecular weight. The bases are paired to make dinucleotides in such a way that their ascending/descending order in terms of molecular weight remains intact. The dinucleotides are placed on the 6 end of the heterocyclic hexagon as well as at the midpoint of each arm of the hexagon. The six dinucleotides which are placed on the 6 ends of the hexagon are in ascending order. The midpoint dinucleotides are positioned by descending order of molecular weight.

We place six ordered dinucleotides on opposite ends of the heterogenic cycle. The opposite ends are 1–4, 2–5, and 3–6. Any class (purine, pyrimidine; amino, keto; strong-H, weak-H) can be positioned at either end of the hexagon. Therefore, there are six possible combinations, as shown in Figure 2. The names of these combinations are Cycle 1, Cycle 2, Cycle 3, Cycle 4, Cycle 5, and Cycle 6 respectively.

In Cycle 1, purines and pyrimidines are positioned at ends 1 and 4 of the hexagon respectively. A and G, the purines, form two dinucleotides: AG and GA. We keep AG on end 1 as it retains the sequence C → T → A → G. For the same reason, CT are placed on end 4, and CA (amino) and TG (keto) are placed on the 2 and 5 ends, respectively, and CG (strong-H) and TA (weak-H) are positioned on the 3 and 6 ends of the hexagon, respectively.

Conversely, midpoint of 2–3, 3–4 and 4–5 arms are determined by the following rule: take the uncommon nucleotides and form a dinucleotide with them such that descending order (G → A → T → C) of molecular weight prevails. As for example, the midpoint of 2–3 arms is GA because the commonality between CA and CG is C. So, G and A are uncommon. This rule is
Fig 3. Cartesian coordinates of 16 dinucleotide in a hexagon.

| Points | Dinucleotide | Cycle 1 | Cycle 2 |
|--------|--------------|---------|---------|
| P₀     | AT           | x=0.5   | x=1     |
| P₁     | TA           | y=1.25  | y=0     |
| P₂     | AC           | z=1     | z=1     |
| P₃     | CG           | -0.5    | 1.5     |
| P₄     | GA           | 2.25    | 1.5     |
| P₅     | AT           | -1.0    | 3.5     |
| P₆     | TG           | 3.75    | 3.5     |
| P₇     | GC           | 0       | 0       |
| P₈     | CA           | 1.0     | 1.5     |
| P₉     | AG           | 2.5     | 2.5     |
| P₁₀    | AG           | 4.0     | 3.5     |

Different for the midpoint of 5–6, 6–1 and 1–2 arms: take the common nucleotide as well as the other which is not available on both ends. For example, the midpoint of 5–6 arms is TC because T is common between TA and TG, while C is neither in TA nor in TG. We follow these simple rules to position the 12 dinucleotides on the hexagon (six dinucleotide on six ends + six dinucleotide on midpoint of each arm of the hexagon). Based on the above discussions, Cycle 1 is drawn in the 2D Cartesian space, shown in Figure 3.

From Figure 3, we can derive the set of position coordinates of 16 dinucleotide: (0, 1.5) → AG, (0.5, 1.25) → AT, (1, 1) → CA, (1, 0) → GA, (1, −1) → CG, (0.5, −1.25) → GT, (0, −1.5) → CT, (−0.5, −1.25) → GC, (−1, 1) → TG, (−1, 0) → TC, (−1, 1) → TA, (−0.5, 1.25) → AC, (0, 1) → AA, (−0.5, 0) → CC, (−1, 0) → GG, (0.5, 0) → TT.

Let $S = \{s₁, s₂, ..., s_N\}$ be a DNA sequence where $s_i \in \sum = \{A, T, C, G\}$ and $i = 1, 2, 3, ..., N$. S is mapped into a series of points $P_1, P_2, ..., P_{N−1}$. We introduce a map function $\phi$ such that $S$ can be formulated as $S = \phi(s_i, s_{i+1})\phi(s_{i+1}, s_{i+2}) ... \phi(s_{N−1}, s_N)$ where,

$$P_i = \phi(s_i, s_{i+1}, i)$$

$$= \phi(x_{s_i, s_{i+1}}, y_{s_i, s_{i+1}}, i), \ i = 1, 2, 3, ..., N-1$$

$x_{s_i, s_{i+1}}$, $y_{s_i, s_{i+1}}$, and $i$ represent the x-coordinate, y-coordinate, and z-coordinate respectively. Thus, we connect the N−1 points from the first one and derive a 3D curve.

To locate the local and global features of the 3D curve as well as to visualize the 3D representation of this curve, we take another numerical representation. Let $X_i = \sum k \ x_k$, $Y_i = \sum k \ y_k$, we derive another mapping function for cumulative feature of the 3D curve such that

$$\lambda(s_i, s_{i+1}) = (X_i, Y_i, i), \ \text{where} \ i = 1, 2, 3, ..., N-1.$$

Connecting N−1 points from the first one, we get the proposed novel 3D zigzag curve.
Example of the proposed method
The following example is used with the arbitrary DNA sequence ATACGATGCAG. The length of the string is 11, hence there are 10 dinucleotide. The 3D coordinate for all cycles of the sequence is shown in Table 1.

The proposed model is useful to show the hidden properties of long DNA sequences which are not seen from the sequence. The pictorial presentation of the proposed method proves that it is very useful to understand the evolutionary similarity/dissimilarity of different species. Figure 5 shows the 3D zigzag curve based on Cycle 1 of first exon of β-globin for 11 different species. The graphical representation clearly shows that:

i. DNA curves of human, gorilla, chimpanzee and lemur are closely similar;
ii. Mouse and rat have also same DNA curves, so as rabbit’s DNA curve;
iii. Goat and bovine are similar; and that
iv. Gallus and opossum seem to be outliers.

Experimental Analysis
Performance metric, dataset and experimental environment
To evaluate the performance, we illustrate the use of the proposed method with an examination of similarities/dissimilarities among the β-globin gene of 11 different species, listed in Table 2, which were also previously studied. The table shows the different important characteristics of the dataset. First, we show the overall performance of the proposed method. To do this, two features are extracted from the DNA curves: (i) geometric center and (ii) mathematical descriptor. Each DNA sequence is finally represented by their mathematical descriptors. These descriptors form six dimensional feature vectors. After that, the Euclidian distance is calculated among feature vectors of the DNA sequences. Secondly, we draw the phylogenetic tree from similarity/dissimilarity matrix using UPGMA method in PHYLIP package. Finally, we compare the proposed method with the already mentioned research works to show its superiority to others.
Figure 4. The graphical representation of the proposed model for the example sequence ATACGATGCAg.

Figure 5. DNA curves of 11 different species.
Our programs were written in Python 2.7, and run with the Windows XP operating system on a Pentium dual-core 2.13 GHz CPU with 2 GB main memory. We used BioPython 1.60 for sequence parsing and also ACD/ChemSketch for drawing the ring structure of nucleotides.

### Numerical analysis of the proposed method

As stated earlier, features from DNA curves are extracted two ways. Firstly, the geometric centers of the curves are calculated using the following equations. Table 3 shows the geometric center of 11 DNA curves.

\[
\begin{align*}
x' &= \frac{1}{N} \sum_{i=1}^{N} x_i, \\
y' &= \frac{1}{N} \sum_{i=1}^{N} y_i, \\
z' &= \frac{1}{N} \sum_{i=1}^{N} z_i
\end{align*}
\]

The significance of the geometric center is that it shows the average value of x, y and z coordinate. Normally, if the geometric centers are plotted, then similar species fall into same cluster. So, the geometric center is an important feature of the DNA curves for the analysis of evolutionary relationship among different species.

To make our system unbiased, we take all the possible rotations (such as Cycle 1, Cycle 2, …, Cycle 6) of hexagonal ring structure and extract the geometric center for each combination.

Secondly, mathematical descriptors are obtained from the first feature, the geometric center, using the following equation. Table 4 shows the mathematical descriptor of the 11 curves.

\[
\rho = \sqrt{u_x^2 + u_y^2 + u_z^2}
\]

We use the Euclidian distance for similarity measurement. Let two different species be i and j. The mathematical descriptor of i are \(p_{1i}, p_{2i}, p_{3i}, p_{4i}, p_{5i}\) and \(p_{6i}\). The same descriptors for species j are \(p_{1j}, p_{2j}, p_{3j}, p_{4j}, p_{5j}\) and \(p_{6j}\). The Euclidian distance of i and j are then calculated using the following equation:

\[
D_{ij} = \sqrt{\left(\frac{p_{1i}}{N+1} - \frac{p_{1j}}{N+1}\right)^2 + \left(\frac{p_{2i}}{N+1} - \frac{p_{2j}}{N+1}\right)^2 + \left(\frac{p_{3i}}{N+1} - \frac{p_{3j}}{N+1}\right)^2 + \left(\frac{p_{4i}}{N+1} - \frac{p_{4j}}{N+1}\right)^2 + \left(\frac{p_{5i}}{N+1} - \frac{p_{5j}}{N+1}\right)^2 + \left(\frac{p_{6i}}{N+1} - \frac{p_{6j}}{N+1}\right)^2}
\]

The similarity/dissimilarity matrix found from the above Euclidian distance metric is shown in Table 5.

Some observations are vividly depicted from Table 5 which are also consistent with the graphical representation portrayed in Section 3.4. They are as follows:

i. The smallest entry is 0.0002 for the pair (human, gorilla), showing that human and gorilla are almost same in terms of evolutionary characteristics. The same is applied for the pair (human, chimpanzee) = 0.0020. Therefore, human, chimpanzee and gorilla are similar species;
Table 3. Geometrical center of 11 different species.

| Species | Cycle 1 | Cycle 2 | Cycle 3 |
|---------|---------|---------|---------|
|         | $u_x$   | $u_y$   | $u_z$   | $u_x$   | $u_y$   | $u_z$   | $u_x$   | $u_y$   | $u_z$   |
| Human   | -4.489  | -15.0275| 46      | -9.81   | -3.75   | 46      | -6.74752| 5.947802| 46      |
| Chimpanzee | -4.7981 | -17.5024| 52.5    | -10.88  | -4.89   | 52.5    | -7.59135| 5.947115| 52.5    |
| Gorilla | -4.5163 | -15.2255| 46.5    | -9.9    | -3.85   | 46.5    | -6.82065| 5.942935| 46.5    |
| Lemur   | -2.3132 | -12.6181| 46      | -6.71   | -2.23   | 46      | -3.65385| 6.035714| 46      |
| Rat     | -6.7802 | -11.8489| 46      | -9.78   | 1.53    | 46      | -3.74176| 10.74725| 46      |
| Mouse   | -5.6882 | -18.2285| 47      | -13     | -1.65   | 47      | -7.8172  | 13.25    | 47      |
| Rabbit  | -2.3736 | -13.6401| 46      | -7.31   | -4.88   | 46      | -5.9011  | 2.239011 | 46      |
| Goat    | -2.7824 | -16.1882| 43      | -9.106  | -4.98   | 43      | -6.68235| 5.679412| 43      |
| Bovine  | -1.7824 | -14.6706| 43      | -7.747  | -4.76   | 43      | -6.06471| 4.447059| 43      |
| Opossum | -1.8571 | -3.8159  | 46       | -1.92   | 0.266   | 46      | 0.489011 | 2.379121 | 46      |
| Gallus  | -2.4615 | -11.5604| 46      | -5.9    | -5.24   | 46      | -4.34615| 0.898352| 46      |

Table 4. Mathematical descriptor of 11 different species.

| Species | Cycle 1 | Cycle 2 | Cycle 3 | Cycle 4 | Cycle 5 | Cycle 6 |
|---------|---------|---------|---------|---------|---------|---------|
|         | $u_x$   | $u_y$   | $u_z$   | $u_x$   | $u_y$   | $u_z$   | $u_x$   | $u_y$   | $u_z$   |
| Human   | 48.60017| 47.18367| 46.87112| 46.98303| 46.55629| 47.55673|
| Chimpanzee | 55.54823| 53.83806| 53.37834| 53.51123| 53.13654| 54.30821|
| Gorilla | 49.13718| 47.69782| 47.37182| 47.48703| 47.06175| 48.07599|
| Lemur   | 47.75529| 46.54027| 46.53795| 46.88248| 47.56467| 47.33033|
| Rat     | 47.98299| 47.05305| 47.38675| 47.01907| 46.94411| 48.01026|
| Mouse   | 50.73099| 48.79265| 49.45373| 49.95977| 48.54146| 49.74948|
| Rabbit  | 48.03838| 46.83215| 46.43098| 46.66777| 46.33272| 46.93223|
| Goat    | 46.03042| 44.23482| 43.88519| 44.35377| 43.81217| 44.79453|
| Bovine  | 45.46871| 43.95081| 43.65269| 44.18473| 43.65795| 44.31434|
| Opossum | 46.19535| 46.04082| 46.06408| 46.03293| 46.24424| 46.36385|
| Gallus  | 47.49423| 46.67191| 46.21359| 46.12239| 46.27557| 46.96276|

Table 5. Euclidian distance among 11 different species.

| Species | Chimpanzee | Gorilla | Lemur | Rat | Mouse | Rabbit | Goat | Bovine | Opossum | Gallus |
|---------|------------|---------|-------|-----|-------|--------|------|--------|---------|--------|
| Human   | 0.0020     | 0.0002  | 0.0124| 0.0109| 0.0336| 0.0116 | 0.0236| 0.0139 | 0.0343  | 0.0189 |
| Chimpanzee | 0.0018 | 0.0138  | 0.0115| 0.0330| 0.0113| 0.0226 | 0.0137| 0.0358 | 0.0201  |        |
| Gorilla | 0.0126     | 0.0109  | 0.0336| 0.0118| 0.0235| 0.0139 | 0.0344| 0.0190  |        |        |
| Lemur   | 0.0134     | 0.0420  | 0.0080| 0.0280| 0.0156| 0.0236 | 0.0114| 0.0051  | 0.0220  |        |
| Rat     | 0.0155     | 0.0174  | 0.0233| 0.0318| 0.0342| 0.0218| 0.0156| 0.0139 | 0.0494  | 0.0368 |
| Mouse   | 0.0443     | 0.0280  | 0.0330| 0.0644| 0.0516| 0.0368 | 0.0088| 0.0037  | 0.0245  | 0.00169|
| Rabbit  | 0.0311     | 0.0186  | 0.0238| 0.0311| 0.0037| 0.0245| 0.00169|
| Goat    | 0.0131     | 0.0494  | 0.0368| 0.0037| 0.0245|
| Bovine  | 0.0370     | 0.0245|
| Opossum | 0.0169     |        |

Figure 6. Phylogenic analysis of 11 different species.

Figure 7. The degree of similarity/dissimilarity of the other 10 species with human.
We see that there is an overall agreement between numerical and phylogenetic analysis. To see it visually, we denote the degree of similarity of the pair (human, chimpanzee, gorilla), (mouse, rat), and (goat, bovine). Conversely, gallus is the outlier and opossum is the remote mammalian species than others.

### Phylogenetic analysis

A phylogeny tree was drawn from the above similarity matrix using the UPGMA method of PHYLIP software package to see the relationship among different species. The tree is shown in Figure 6.

The tree also shows the similarity among (human, chimpanzee, gorilla), (mouse, rat), and (goat, bovine). Conversely, gallus is the outlier and opossum is the remote mammalian species than others.

### Comparison with other methods

We see that there is an overall agreement between numerical and phylogenetic analysis. To see it visually, we denote the degree of similarity of the pair (human, gorilla) as 1 in Table 5, and the results of the examination of the degree of similarity/dissimilarity between human and other several species under the Euclidian measurement are shown in Figure 7. To draw the other curves, we used Table 3 of Qi’s work, Table 7 of Jafarzadeh’s work, Table 2 of Huang, Table 3 of Qi’s work, and Table 4 of Liao’s work. These tables provide the best similarity/dissimilarity value of the research works.

Several reference papers work on same data-set. Of them, the research papers of Qi and Huang were based on dinucleotides, while Jafarzadeh used trinucleotides, and Qi and Liao used single nucleotides. Those works do not reflect the degree of similarity/dissimilarity among different species as accurately as it should be. For example, the difference in degree of similarity/dissimilarity among (rat, opossum), (mouse, opossum), and (goat, opossum) are almost the same in the above listed papers. This, however, is not true in nature as the opossum is the most remote mammal species. Therefore it can be concluded that intra-mammalian degree of similarity/dissimilarity is not properly reflected by the above methods. The proposed method, on the other hand, shows this natural consistency among (rat, opossum), (mouse, opossum), and (goat, opossum) clearly. As the opossum shows the highest peak, this demonstrates that it is not an outlier of the dataset but actually very different from other mammalian species.

The only non-mammalian species, gallus, is not truly represented by the above methods as the difference between peak value of gallus and opossum is not significant. From Table 5, we see that the opossum has variation in similarity score from other species. This is reflected in Figure 7. Gallus has the positive difference of distance in terms of degree of similarity/dissimilarity from all species analyzed, except goat and opossum. For those two species, it maintains negative differences. Therefore, from this analysis it could be concluded that
goat and opossum are either mammals or non-mammals, however it is known that the goat is a mammal. It therefore must be concluded that the opossum is also a mammal, but the most remote species from the remaining mammals. As a result, gallus is the only species that is neither mammalian nor shows score value like opossum. Hence, gallus falls into a single group within the species analyzed: non-mammalian.

Conclusion
A graphical method based on dinucleotides and their positional information is proposed in this research work. Graphical as well as numeric analyses of the model show that the proposed novel method is compatible with the natural consistency in terms of evolutionary relationship of 11 different species. In this paper, DNA sequences are transformed into 3D DNA curves, and features from those curves are then extracted. DNA curves are represented by their feature vector. Subsequently, Euclidian distance is applied to those feature vectors to deduce the evolutionary relationship among 11 different species. Tri-nucleotide based DNA sequence analysis using the proposed method would be one recommended future work.

Author Contributions
Conceived and designed the experiment: AGB, BSJ. Analyzed the data: AGB, MRR, BSJ. Wrote the first draft of the manuscript: AGB, BSJ. Contributed to the writing of manuscript: AGB, MRR, ATI, HJC. Agree with manuscript results and conclusions: AGB, BSJ, HJC. Jointly developed the structures and argument for the paper: AGB, BSJ, HJC. Made critical revisions and approved final revision: AGB, BSJ, MRR, ATI. All authors reviewed and approved of the final manuscript.

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Competing Interests
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