Introduction to Bio-Informatics

Assignment 1: Sequence Statistics and Gene Finding

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Like humans, Neanderthals had not only nuclear DNA, but also mitochondrial DNA in all their cells. Like humans, the mitochondria of neanderthals contained 13 coding genes and 24 non-coding genes. The neanderthal mitochondrial genome has accession number 'NC_011137'.

Neanderthal mitochondrial genome:

```
data = getgenbank('NC_011137', 'SequenceOnly', true);
data
```

```
data = 
'GATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCTCTCCATGCATTTGGTATTTTCGTCTGGGGGGTGTGCACGCGATAGCATTGCGAGACGCTGGAGCC
```

1. How does the number of genes stated above compare with the number of ORFs you find?

ORFs:

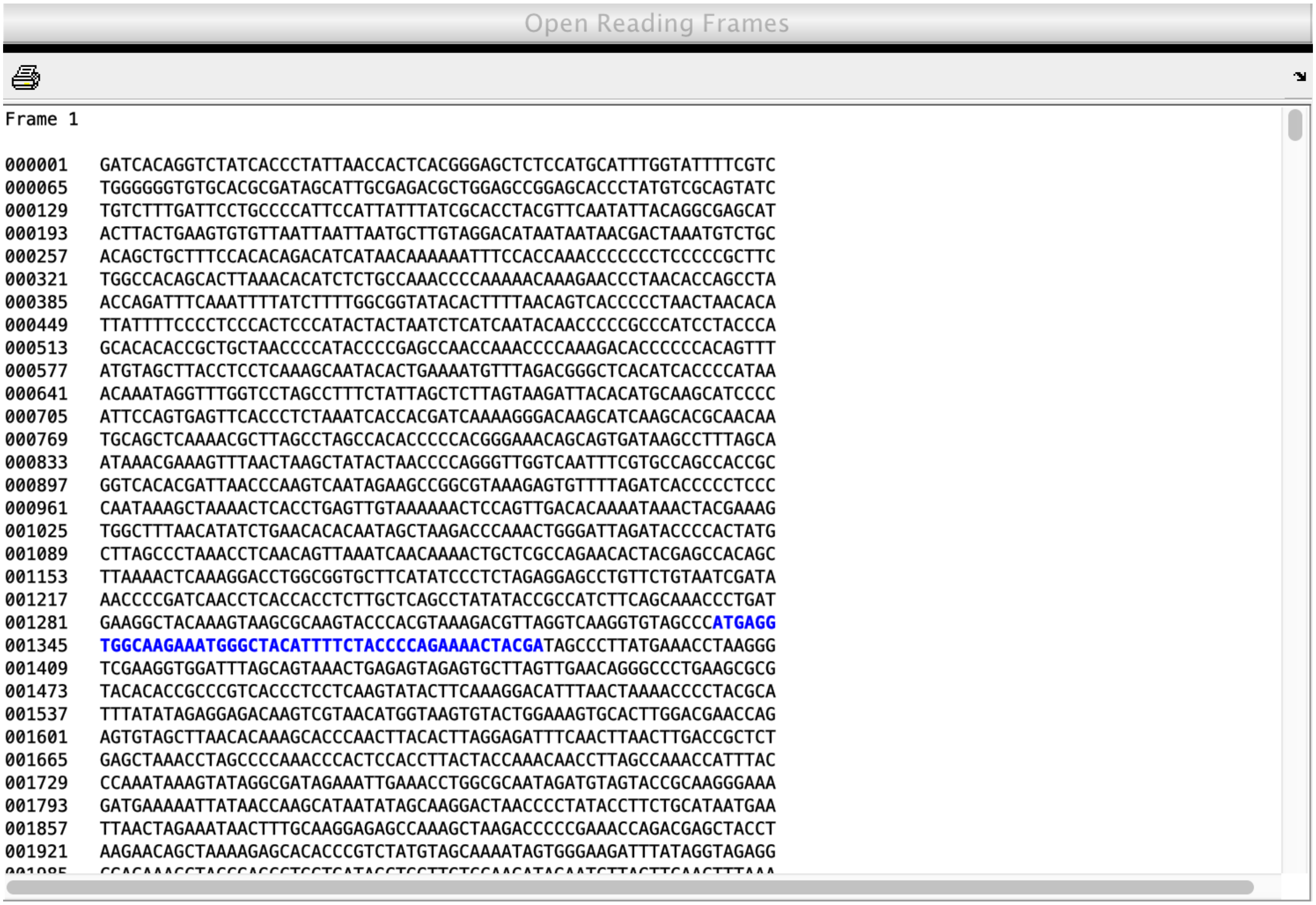
The ORFs of the mitochondria of neanderthals are retrieved throughout function **seqshoworfs**.

It is possible to divide the ORFs in two categories:

1. ORFs that contain real genes
2. ORFs that occur by chance

The second kind of ORFs can be discarded since they are part of the "junk" of the DNA. In order to classify the ORFs, a minum length *k* is calculated. Every ORF of length greater or equal to *k* contains real genes while the remaining candidate genes belong to the second category of ORFs thus, they can be rejected.

```
% Retrive ORFs from the data
structure = seqshoworfs(data);
```



```
Frame 1

000001   GATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCTCTCCATGCATTTGGTATTTTCGTC
000065   TGGGGGGTGTGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTCGCAGTATC
000129   TGTCTTTGATTCCTGCCCCATTCCATTATTTATCGCACCTACGTTCAATATTACAGGCGAGCAT
000193   ACTTACTGAAGTGTGTTAATTAATTAATGCTTGTAGGACATAATAATAACGACTAAATGTCTGC
000257   ACAGCTGCTTTCCACACAGACATCATAACAAAAAATTTCCACCAAACCCCCCCTCCCCCGCTTC
000321   TGGCCACAGCACTTAAACACATCTCTGCCAAACCCCAAAAACAAAGAACCCTAACACCAGCCTA
000385   ACCAGATTTCAAATTTTATCTTTTGGCGGTATACACTTTTAACAGTCACCCCCTAACTAACACA
000449   TTATTTTCCCCTCCCACTCCCATACTACTAATCTCATCAATACAACCCCCGCCCATCCTACCCA
000513   GCACACACCGCTGCTAACCCCATACCCCGAGCCAACCAAACCCCAAAGACACCCCCCACAGTTT
000577   ATGTAGCTTACCTCCTCAAAGCAATACACTGAAAATGTTTAGACGGGCTCACATCACCCCATAA
000641   ACAAATAGGTTTGGTCCTAGCCTTTCTATTAGCTCTTAGTAAGATTACACATGCAAGCATCCCC
000705   ATTCCAGTGAGTTCACCCTCTAAATCACCACGATCAAAAGGGACAAGCATCAAGCACGCAACAA
000769   TGCAGCTCAAAACGCTTAGCCTAGCCACACCCCCACGGGAAACAGCAGTGATAAGCCTTTAGCA
000833   ATAAACGAAAGTTTAACTAAGCTATACTAACCCCAGGGTTGGTCAATTTCGTGCCAGCCACCGC
000897   GGTCACACGATTAACCCAAGTCAATAGAAGCCGGCGTAAAGAGTGTTTTAGATCACCCCCTCCC
000961   CAATAAAGCTAAAACTCACCTGAGTTGTAAAAAACTCCAGTTGACACAAAATAAACTACGAAAG
001025   TGGCTTTAACATATCTGAACACACAATAGCTAAGACCCAAACTGGGATTAGATACCCCACTATG
001089   CTTAGCCCTAAACCTCAACAGTTAAATCAACAAAACTGCTCGCCAGAACACTACGAGCCACAGC
001153   TTAAAACTCAAAGGACCTGGCGGTGCTTCATATCCCTCTAGAGGAGCCTGTTCTGTAATCGATA
001217   AACCCCGATCAACCTCACCACCTCTTGCTCAGCCTATATACCGCCATCTTCAGCAAACCCTGAT
001281   GAAGGCTACAAAGTAAGCGCAAGTACCCACGTAAAGACGTTAGGTCAAGGTGTAGCCCATGAGG
001345   TGGCAAGAAATGGGCTACATTTTCTACCCCAGAAAACTACGATAGCCCTTATGAAACCTAAGGG
001409   TCGAAGGTGGATTTAGCAGTAAACTGAGAGTAGAGTGCTTAGTTGAACAGGGCCCTGAAGCGCG
001473   TACACACCGCCCGTCACCCTCCTCAAGTATACTTCAAAGGACATTTAACTAAAACCCCTACGCA
001537   TTTATATAGAGGAGACAAGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAACCAG
001601   AGTGTAGCTTAACACAAAGCACCCAACTTACACTTAGGAGATTTCAACTTAACTTGACCGCTCT
001665   GAGCTAAACCTAGCCCCAAACCCACTCCACCTTACTACCAAACAACCTTAGCCAAACCATTTAC
001729   CCAAATAAAGTATAGGCGATAGAAATTGAAACCTGGCGCAATAGATGTAGTACCGCAAGGGAAA
001793   GATGAAAAATTATAACCAAGCATAATATAGCAAGGACTAACCCCTATACCTTCTGCATAATGAA
001857   TTAACTAGAAATAACTTTGCAAGGAGAGCCAAAGCTAAGACCCCCGAAACCAGACGAGCTACCT
001921   AAGAACAGCTAAAAGAGCACACCCGTCTATGTAGCAAAATAGTGGGAAGATTTATAGGTAGAGG
001985   CCACAAACCTACCCACCCTCCTCATACCTCCTTCTCCAACATACAATCTTACTTCAACTTTAAA
```

```
structure
```

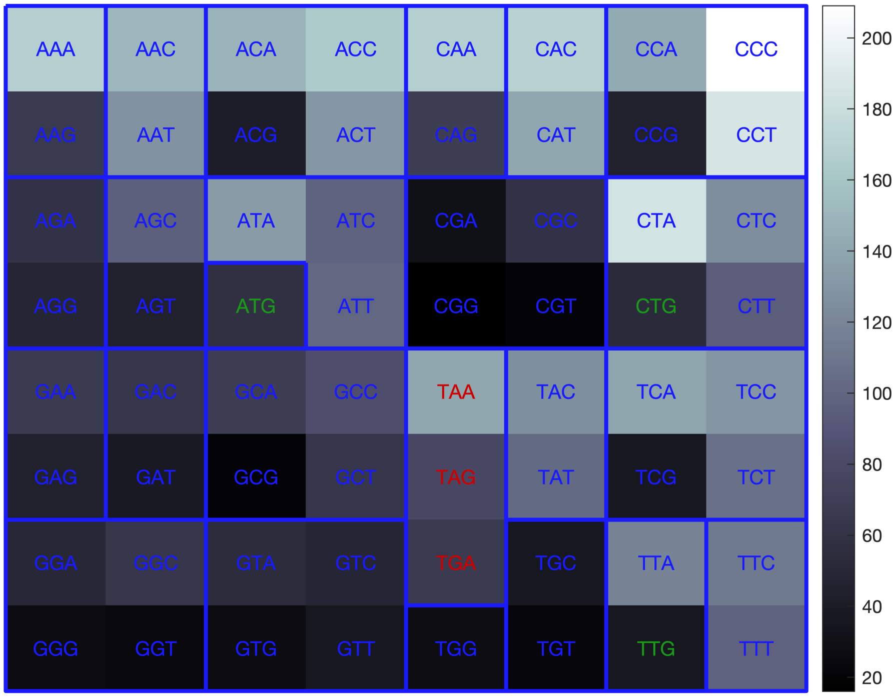
structure = 1×3 struct

Fields	Start	Stop
1	1×26 double	1×26 double
2	1×23 double	1×23 double
3	1×29 double	1×28 double

Codons:

In the first place, the probability of non-stop codons is calculated. TAA, TAG and TGA are stop codons in the DNA. Moreover, using the **codoncount** function, it is possible to observe that the codons are not uniformly distributed since some codons appear more frequently than others.

```
% Plot distribution of Codons
figure;
[codons, codonArray] = codoncount(data, 'figure', true);
```



Genetic Code: Standard

```
codons
```

```
codons = struct with fields:
    AAA: 168
    AAC: 151
    AAG: 66
    AAT: 128
    ACA: 149
    ACC: 164
    ACG: 42
    ACT: 131
    AGA: 61
    AGC: 96
    AGG: 49
    AGT: 45
    ATA: 135
    ATC: 98
    ATG: 58
    ATT: 100
    CAA: 168
    CAC: 170
    CAG: 69
    CAT: 141
    CCA: 142
    CCC: 209
    CCG: 45
    CCT: 187
    CGA: 31
    CGC: 61
    CGG: 16
    CGT: 20
    CTA: 184
    CTC: 125
```

```
CTG: 53
CTT: 95
GAA: 67
GAC: 63
GAG: 45
GAT: 38
GCA: 69
GCC: 84
GCG: 20
GCT: 63
GGA: 50
GGC: 64
GGG: 27
GGT: 25
GTA: 55
GTC: 49
GTG: 27
GTT: 37
TAA: 141
TAC: 126
TAG: 79
TAT: 102
TCA: 139
TCC: 130
TCG: 37
TCT: 107
TGA: 66
TGC: 37
TGG: 28
TGT: 23
TTA: 119
TTC: 113
TTG: 36
TTT: 98
```

Given that, the probability of non-stop codons is calculated as follows:

probability_stop = P(TAA) + P(TAG) + P(TGA)

probability_nonStop = 1 - probability_stop

```
% Calculate total amount of codons
codonVector = reshape(codonArray, [1, 64]);
total_codons = 0;
for i = 1:length(codonVector)
    total_codons = total_codons + codonVector(i);
end
total_codons
```

```
total_codons = 5521
```

```
% Probability of stop codons
probability_stop = (codons.("TAA") + codons.("TAG") + codons.("TGA"))/total_codons
```

```
probability_stop = 0.0518
```

```
% Probability of non-stop codons
probability_nonStop = 1 - probability_stop
```

```
probability_nonStop = 0.9482
```

Moreover, knowing the following inequality:

$$(\text{probability\_nonStop})^{k} < p$$

it possible to retrive the value of *k* for different probabilities *p.* Where *p* is the probability that a real gene is longer that would be expected by chance.

```
% Extract the value of k

% Calculate k with p = 90%
p1 = 0.90;
k1 = log((1 - p1))/log(probability_nonStop)
```

```
k1 = 43.2881
```

```
% Calculate k with p = 95.5%
p2 = 0.955;
k2 = log((1 - p2))/log(probability_nonStop)
```

```
k2 = 58.2998
```

Finally, the length of the ORFs is calculated and compared to the values of *k*. From this comparison we obtain the following information:

- 21 ORFs contain real genes with probability 90%
- 13 ORFs contain real genes with probability 95.5%

From these results, we can conclude that different probabilities lead to different accuracies and numbers of ORFs with real genes. Moreover, with *p* equal to 95.5% the count is equal to the exact number of coding genes of the mitochondria of neanderthals: 13.

```
% Count ORF with length less than k
count1 = 0;
count2 = 0;

for i = 1:3
    start_ORF = structure(i).Start;
    end_ORF = structure(i).Stop;

    for j = 1:length(end_ORF)
        length_ORF = ((end_ORF(j) - start_ORF(j))/3);

        % k with p = 90% is checked
        if length_ORF > k1
            count1 = count1 + 1;

            % k with p = 95.5% is checked
            if length_ORF > k2
                count2 = count2 + 1;
            end
        end
    end
end
```

```
count1
```

```
count1 = 21
```

```
count2
```

```
count2 = 13
```

2. Using what you have learnt above explore the neanderthal mitochondrial genome and find at least one *interesting* feature.

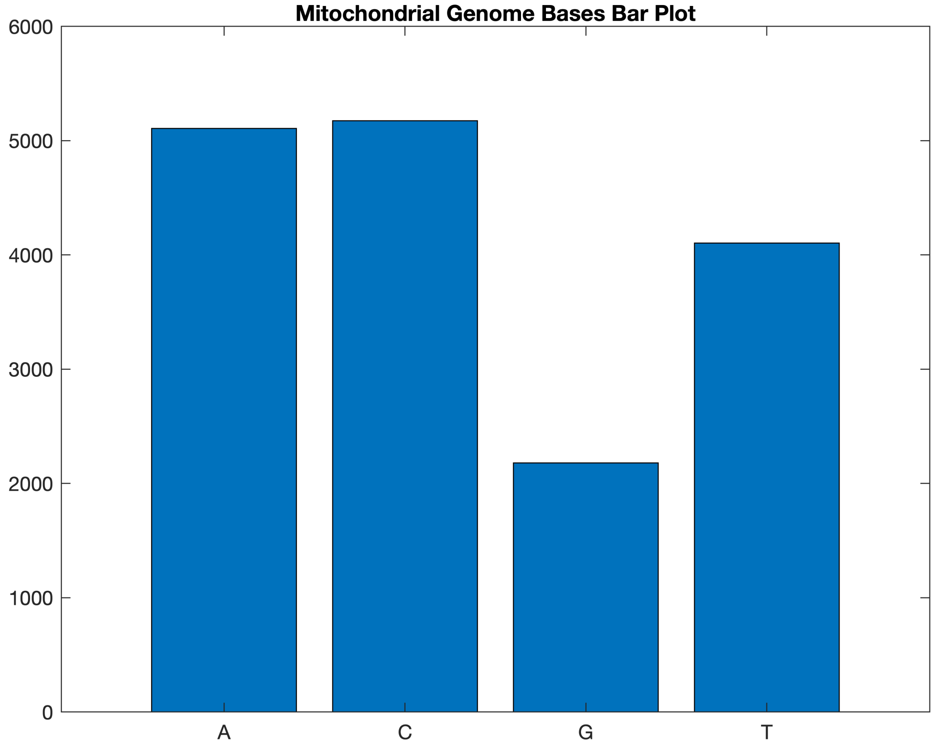
While Codons and ORFs are analysed in the previous section, there exists other features such as Bases, Density and Dimers to be examined in the neanderthal mitochondrial genome.

Bases:

```
% Retrieve bases and possible ambigous elements of the data
bases = basecount(data, 'Ambiguous', 'individual')
```

```
bases = struct with fields:
    A: 5107
    C: 5174
    G: 2180
    T: 4104
    R: 0
    Y: 0
    K: 0
    M: 0
    S: 0
    W: 0
    B: 0
    D: 0
    H: 0
    V: 0
    N: 0
```

```
% Plot of the bases
figure;
X = categorical({'A','C','G','T'});
X = reordercats(X, {'A','C','G','T'});
bar(X, [bases.A, bases.C, bases.G, bases.T]);
title('Mitochondrial Genome Bases Bar Plot');
```

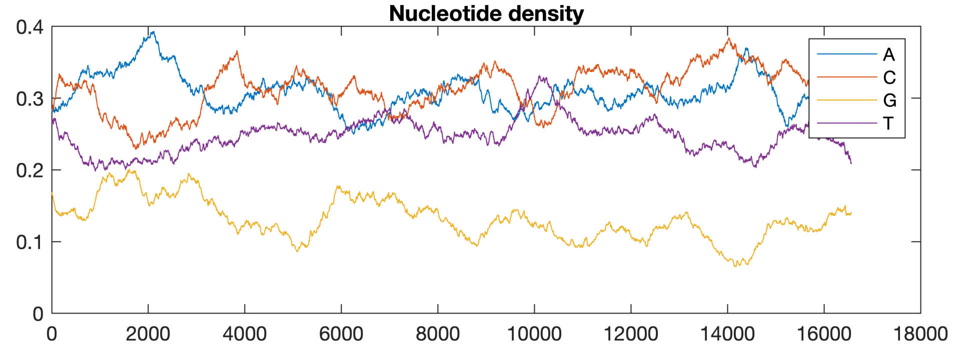


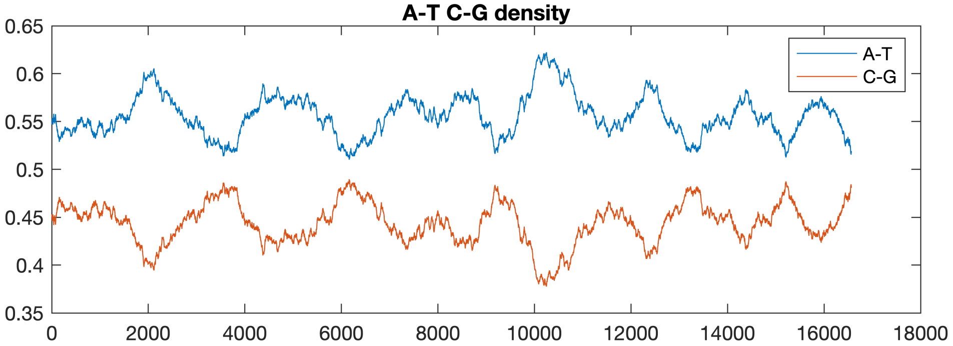
By analysing this plot, it is possible to conclude the following:

- A (Adenine): 5107, C (Cytosine): 5174, G (Guanine): 2180, T (Thymine): 4104
- There is a significant lack of Guanine
- No ambiguous bases are present in the genome

Density:

```
% Retrieve and analyse the density of the bases
figure;
density = ntdensity(data);
```

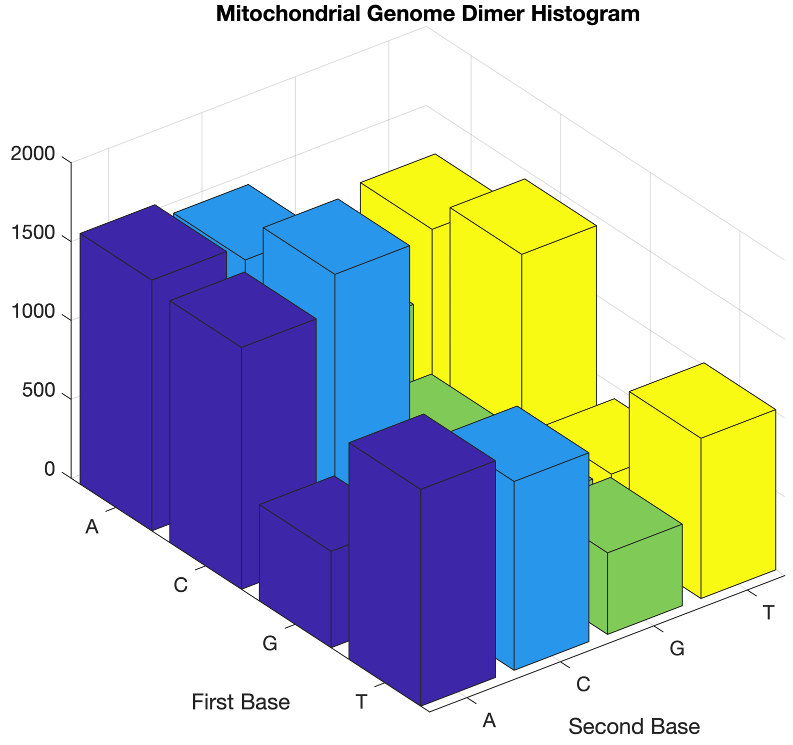




Next, the frequency of the bases is analysed. In these Density plots, there are no particular observations to be made other than the clear lack of Guanine, as also stated in the previous point.

Dimers:

```
% Retrieve and analyse the Dimers
figure;
[dimers, percent] = dimercount(data, 'chart', 'bar');
title('Mitochondrial Genome Dimer Histogram');
```



```
dimers
```

```
dimers = struct with fields:
    AA: 1589
    AC: 1490
    AG: 799
    AT: 1229
    CA: 1533
    CC: 1769
    CG: 431
    CT: 1441
    GA: 611
    GC: 718
    GG: 432
    GT: 418
    TA: 1374
    TC: 1197
    TG: 517
    TT: 1016
```

Finally, the dimers of the genome are studied. From this analysis, it is again clear the lack of Guanine. Moreover, the frequency of the dimers is computed:

```
% Display dimers frequency
figure;
dimers_frequency = array2table(percent);
dimers_frequency.Properties.RowNames = {'A','C','G','T'};
dimers_frequency.Properties.VariableNames = {'A','C','G','T'}
```

```
dimers_frequency = 4×4 table
```

	A	C	G	T
1 A	0.0959	0.0900	0.0482	0.0742
2 C	0.0926	0.1068	0.0260	0.0870
3 G	0.0369	0.0433	0.0261	0.0252
4 T	0.0830	0.0723	0.0312	0.0613

```
% Sum and display frequency of each base
figure;
frequency_sum = sum(percent);
dimers_frequencySum = array2table(frequency_sum);
dimers_frequencySum.Properties.VariableNames = {'A','C','G','T'};
dimers_frequencySum.Properties.RowNames = {'Total'}
```

```
dimers_frequencySum = 1×4 table
```

	A	C	G	T
1 Total	0.3083	0.3124	0.1316	0.2478

From this table we can conclude that Cytosine has the highest frequency and it is the most frequent first base of the dimers.