

PCA deciphers genome

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Abstract

In this paper, we give a tutorial for undergraduate students studying statistical methods and/or bioinformatics. The students learn how data visualization can help in genomic sequences analysis. Students start with a fragment of genetic text of a bacterial genome and analyze its structure. By means of principal component analysis they “discover” that the information in genome is encoded by non-overlapping triplets. Next, they learn to find gene positions. This exercise on principal component analysis and K-Means clustering gives a possibility for active study of the basic bioinformatics notions. In Appendix the program listings for MatLab are published.

Keywords: Principal component analysis; Data visualization; Bioinformatics; Cryptography; Clustering

1 Introduction

When it is claimed in newspapers that a new genome is deciphered, it usually means only that the sequence of the genome has been read, so one can write down a long sequence using four genetic letters: A, C, G and T. The first step in real deciphering is always identification of positions of elementary messages in this text or *detecting genes* in the biological language. Only after one can try to understand what these messages mean for a living cell.

Bioinformatics and genomic sequence analysis in particular is one of the hottest topic in modern science. One can not overestimate usefulness of statistical techniques in these fields. In particular, a successful approach to gene positions identification is statistical analysis of a genetic text composition.

In this exercise we use Matlab environment to convert genetic text into a table of short word frequencies and to visualize this table using principal component analysis (PCA). Students find by themselves that the sequence of letters is not random and that the information in the text is encoded by non-overlapping triplets. Using the simplest K-Means clustering method from Matlab Statistical toolbox, it is possible to detect position of genes in genome and even predict their direction.

2 Required materials

To follow this exercise, it is needed to prepare a genomic sequence. For this activity we propose a fragment of genomic sequence of *Caulobacter Crescentus* bacteria. Other sequences can be downloaded from the Genbank FTP-site [1]. Our procedures work with the files in the *Fasta* format (the corresponding files have *.fa* extension) and limited in practice with 400-500kb length fragments.

Five simple functions are used:

| | |
|---------------------|---|
| <i>LoadFreq.m</i> | loads Fasta-file into Matlab string |
| <i>CalcFreq.m</i> | converts a text into numerical table of short word frequencies |
| <i>PCAFreq.m</i> | visualizes a numerical table using principal component analysis |
| <i>ClustFreq.m</i> | is used to perform clustering with K-Means algorithm |
| <i>GenBrowser.m</i> | is used to visualize the results of clustering onto the text |

All sequence files and the m-files should be placed into the current Matlab working folder.

The sequence of Matlab commands for this exercise is the following:

```
str = LoadSeq('ccrescentus.fa');
xx1 = CalcFreq(str,1,300);
xx2 = CalcFreq(str,2,300);
xx3 = CalcFreq(str,3,300);
xx4 = CalcFreq(str,4,300);
PCAFreq(xx1);
PCAFreq(xx2);
PCAFreq(xx3);
PCAFreq(xx4);
fragn = ClustFreq(xx3,7);
GenBrowser(str,300,fragn,13000);
```

All the required materials can be downloaded from [2].

3 Genomic sequence

3.1 Background

The information that is needed for a living cell functioning is encoded in a long molecule of DNA with only four letters A, C, G and T. The diversity of living organisms and their complex properties is hidden in their genomic sequences. One of the most exciting problem in modern science is to understand organization of living matter by reading genomic sequences.

One distinctive message in a genomic sequence is a piece of text, called *gene*. Genes can be oriented in the sequence in the forward and backward directions (see Fig. 1). The simplified picture with continuous genes is close to reality for bacteria. In highest organisms (humans, for example), the notion of gene is more complex.

It was one of many great discoveries of the 20th century that biological information is encoded in genes by means of triplets of letters, called *codons* in the biological literature. In the famous paper of Crick et al. [3] this fact was proven with use of genetic experiments on bacteria mutants. In this exercise we will prove it by using the genetic sequence only.

In nature there is a special mechanism designed to read genes. It is evident that since the information is encoded by non-overlapping triplets, it is very important for this mechanism to start reading a gene without a shift, from the first letter of the first codon to the last one, otherwise the information decoded will be completely corrupted.

One can find an easy introduction to the modern molecular biology in [4].

3.2 Sequences for the analysis

The activity starts with a fragment of genomic sequence of *Caulobacter Crescentus* bacterium. One can read a short biological description of this bacterium at [5]. The sequence is given as a long text file (300kb), the students are proposed to look inside the file and ensure that there is a text there in which only four letters *a*, *c*, *g* and *t* are used without spaces. It is noticed that although the text seems to be random, it is rather well organized, though without any intention to be read and understood by human mind. Nevertheless, statistical methods help to understand the text organization.

The sequence can be loaded in the Matlab environment by *LoadSeq* function:

```
str = LoadSeq('ccrescentus.fa');
```

4 Converting text to a numerical table

A *word* is any continuous piece of text containing several subsequent letters. Since we do not have spaces in the text, the separation into words is not unique.

The method we utilize is as follows. We clip the whole text into fragments of 300 letters length and calculate frequencies of short words (of length 1-4) inside every

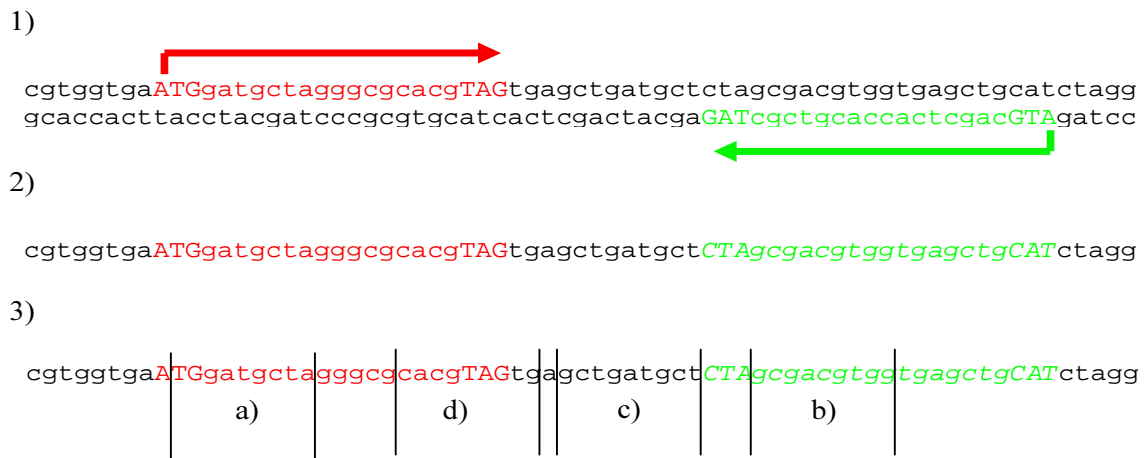


Figure 1: 1) DNA can be represented as two complementary text strings. "Complementary" here means that for every "a", "t", "g" and "c" in one string one has "t", "a", "c" and "g" in the other respectively. Elementary messages or genes can be located in both strings, but in the lower one they are read from right to the left. Genes usually start with "atg" and end with "tag" or "taa" or "tga" words. 2) In databases one obtains only the upper "forward" string. Genes from the lower "backward" string can be reflected on it, but should be read in the opposite direction and changing the letters, accordingly to the complementary rules. 3) If we take a randomly chosen fragment, it can be of one of three types: a) entirely in a gene from the forward string; b) entirely in a gene from the backward string; c) entirely out of genes; d) partially in a gene and partially out.

fragment. This will give us description of the text in the form of a numerical table. There will be four such tables, for every short word length from 1 to 4.

Since we have only four letters, then there are 4 possible words of length 1 (singlets), $16 = 4^2$ possible words of length 2 (duplets), $64 = 4^3$ possible words of length 3 (triplets) and $256 = 4^4$ possible words of length 4 (quadruplets). Then the first table contains 4 columns (frequency of every singlet) and the number of rows equals to the number of fragments. The second table has 16 columns and the same number of rows and so on.

To calculate the tables, students use *CalcFreq.m* function. The first input argument for the function *CalcFreq* is the string containing the text, the second input argument is the length of the words to be counted, the third argument is the fragment length. The output argument is the resulting table of frequencies. Students use the following set of commands to generate tables corresponding to four different word lengths:

```
xx1 = CalcFreq(str,1,300);  
xx2 = CalcFreq(str,2,300);  
xx3 = CalcFreq(str,3,300);  
xx4 = CalcFreq(str,4,300);
```

5 Data visualization

5.1 Visualization

PCAFreq.m function has the only input argument: it is the table obtained at the previous step. It produces a PCA plot for this table. For introduction to Principal Component Analysis, see [6].

By typing this set of commands, students produce four plots for the word lengths from 1 to 4 (see Fig. 2).

```
PCAFreq(xx1);  
PCAFreq(xx2);  
PCAFreq(xx3);  
PCAFreq(xx4);
```

5.2 Understanding plots

The main message in these four pictures is that the genomic text contains information which is encoded by non-overlapping *triplets*, because the plot corresponding to the triplets is evidently highly structured as opposite to the pictures of singlets, duplets and quadruplets. The triplet picture evidently contains 7 clusters.

It is important to demonstrate to students how this 7-cluster structure can be naturally explained from Fig. 1.

We know that the text contains genes and that the information is encoded by non-overlapping subsequent triplets (codons) but we know neither where the genes start and end nor what is the frequency distribution codons are used.

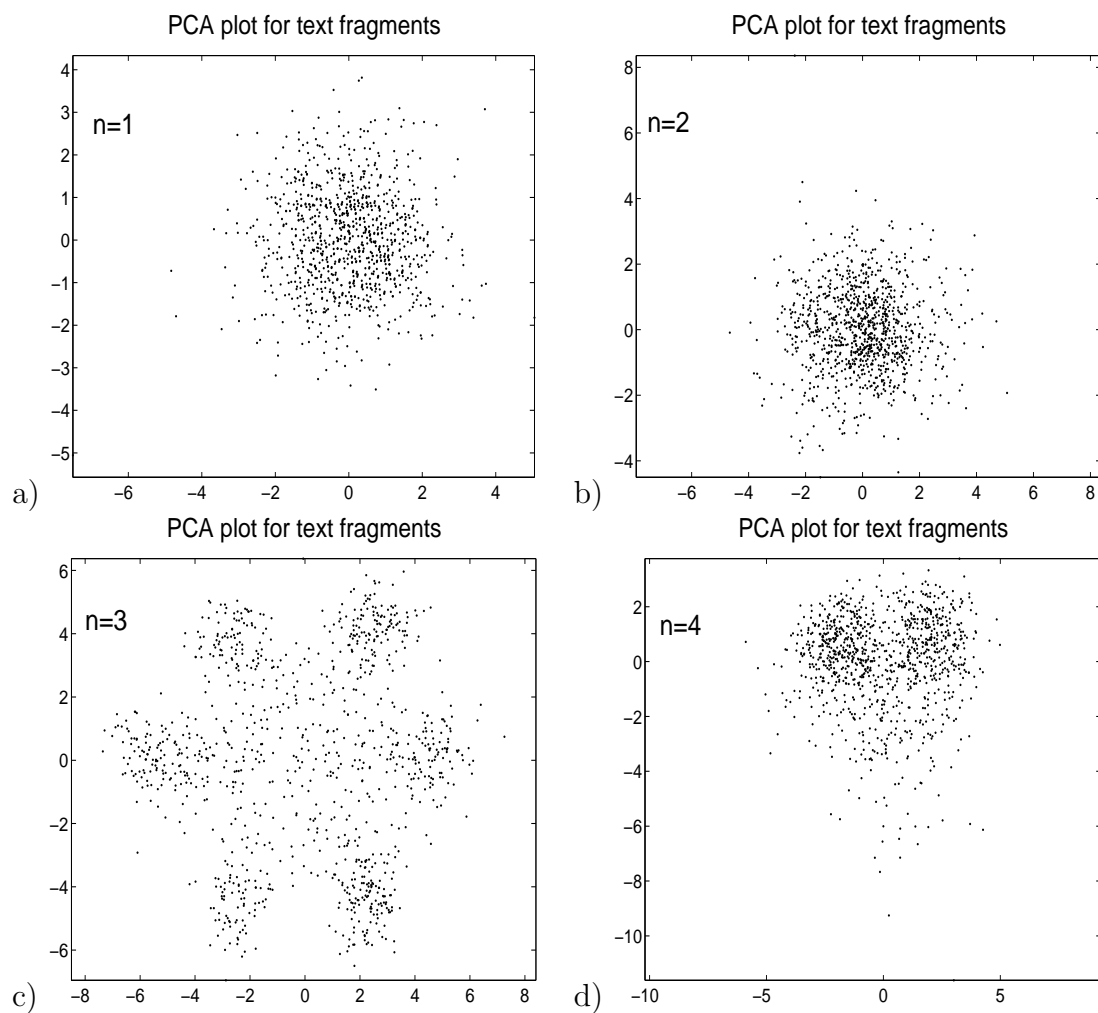


Figure 2: PCA plots of word frequencies of different length. On fig.c) one can see the most structured distribution. The structure is interpreted as existence of non-overlapping triplet code.

Let us blindly cut the text into fragments. Any fragment can contain: a) piece of gene in forward direction; b) piece of gene in backward direction; c) no genes (non-coding part); d) mix of coding and non-coding.

Consider the a) case. The fragment can overlap with a gene in three possible ways, with three possible shifts ($\text{mod}3$) of the first letter of the fragment with respect to gene beginning: if we enumerate the letters in the gene from the first one, 1-2-3-4-..., then the first letter of the fragment can be in the sequence 1-4-7-10-... ($=1(\text{mod}3)$) (a “correct” shift), the sequence 2-5-8-11-... ($=2(\text{mod}3)$), or 3-6-9-12-... ($=0(\text{mod}3)$). If we start to read the information one triplet by another starting from the first letter of the fragment then we can read the gene correctly only if the fragment overlaps with it with a correct shift (see Fig. 1). In general, if the start of the fragment is not chosen deliberately then we can read the gene in three possible ways. Thus, case a) generates three possible frequency distributions, “shifted” one with respect to another.

The b) case is quite analogous and gives also three possible triplet distributions. They are not quite independent from the ones obtained at the step a) for the following reason. The difference is the triplets are read “from the end to the beginning” which produces a kind of mirror reflection of the triplet distributions from the case a).

The c) case will produce only one distribution which will be symmetrical with respect to the “shifts” (or rotations) in the first two cases, and there is a hypothesis that this is a result of genomic sequence evolution. Let us explain it.

Vitality of a bacterium depends on correct functioning of all biological mechanisms. These mechanisms are encoded in genes, and if something wrong happens with gene sequences (for example there is an error when DNA is duplicated), then the organism risks to become non-vital. Nothing is perfect in our world and the errors happen all the time, and in DNA duplication process as well. These errors are called *mutations*.

The most dangerous mutations are those which change the reading frame, i.e. letter deletions or insertions. If such a mutation happens in the middle of a gene sequence, the rest of the gene becomes corrupted: the reading mechanism (which reads the triplets one by one and does not know about the mutation) will read it with a shift. Because of this the organisms with such mutations often die without leaving their off-springs. In opposite, if such a mutation happens in the non-coding part, where there is no genes, this does not lead to a serious problem, and the organism leaves off-springs. Thus such mutations are constantly accumulated in the non-coding part and mixes three shifted triplet distribution in one. The d) case also produces mix of triplet distributions.

As a result, we have three distributions for the case a), three for the case b) and one, symmetrical for the “non-coding” fragments (case c)). Because of natural statistical deviations and other reasons we have 7 clusters of points in the multidimensional space of triplet frequencies.

For more illustrative material see [7, 8, 11, 14].

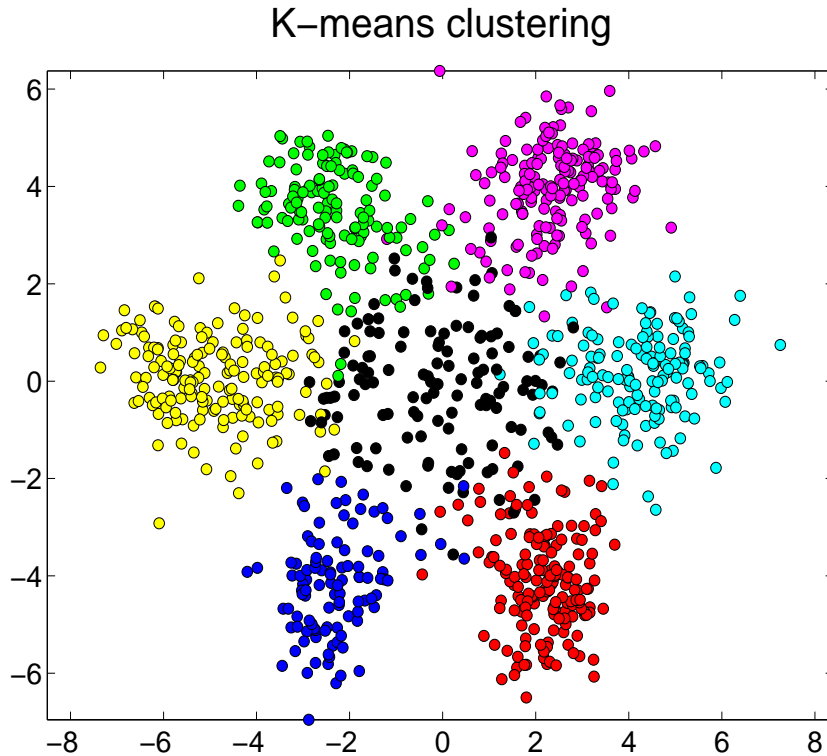


Figure 3: K-Means clustering of triplet frequencies.

6 Clustering and visualizing results

The next step is clustering fragments into 7-clusters. The students are explained that this is a way to classify fragments of text by similarity in their triplet distributions. This is an unsupervised classification (the cluster centers are not known in advance).

Clusterization is performed by K-Means algorithm from Matlab Statistical toolbox and implemented in the *ClustFreq.m* file. The first argument is the frequency table name, the second is the number of clusters proposed. Since we visually identified 7 clusters, in this activity we put 7 as the value of the second argument:

```
fragn = ClustFreq(xx3,7);
```

The function puts different colors onto the cluster points. The cluster which is the closest to the center of the picture is automatically colored in black (see Fig. 3).

After clusterization, every fragment of the text is assigned a cluster label (a color). The *GenBrowser* function put this color back to the text and visualize it. The input arguments of this function are a string with genetic text, the fragment size, a vector with cluster labels and a position in the genetic text to read from (we use 13000 value which can be changed for any):

```
GenBrowser(str,300,fragn,13000);
```


This function implements a simple *genome browser* with information about gene positions.

The students are explained that clusterization of fragments corresponds to segmentation of the whole sequence into homogeneous parts. The homogeneity is understood as similarity of the short word frequency distributions for the fragments of the same cluster (class). For example, for the following text

```
aaaaaaaaatataaaaaattttatttttttttttttggggggggggaagagggggccccccgcctcccccc
```

it is evident that the frequency dictionary of length 1 for a fragment size around 5-10 will be able to separate the text into four homogeneous parts.

7 Task list and further information

Interested students can continue this activity. They can modify the Matlab functions in order to solve the following problems (the first three of them are rather difficult and require programming):

Determine a cluster corresponding to the correct shift. As it was explained in the “Understanding plots” section, some fragments overlap with genes in such a way that the information can be read with correct shift, others contain the “shifted” information. The problem is to detect the cluster corresponding to the correct shift. To give a hint, we can say that the correct triplet distribution, most probably, will contain the lowest frequency of the stop codons TAA, TAG and TGA (see [15, 16]).

Measure information content for every phase. The information of a triplet distribution with respect to a letter distribution is $I = \sum_{ijk} f_{ijk} \ln \frac{f_{ijk}}{p_i p_j p_k}$, where p_i is a frequency of letter i (a genetic text is characterized by four such frequencies), and f_{ijk} is the frequency of triplet ijk . Each cluster is an ensemble of fragments. Each fragment F can be divided on triplets starting from the first letter. We can calculate the information value of this triplet distribution $I(F)$ for each fragment F . Is the information of fragments in the cluster with correct shift significantly different from the entropy of fragments in other clusters? Is the *mean* information value in the cluster with correct shift significantly different from the *mean* information value of fragments in other clusters? Could you verify the hypothesis that in the cluster with correct shift the mean information value is higher than in other clusters?

Increase resolution of determining gene positions. In this exercise we use the same set of fragments to calculate the cluster centers and to annotate the genome. As a result the gene positions are determined with precision equal to the fragment size. In fact, one can calculate the cluster centers with non-overlapping fragments and then scan the genome once again with a sliding window (this will produce a set of overlapping fragments), calculate triplet frequencies for every window and determine the corresponding cluster in the 64-dimensional space. The position in the text is assigned a color corresponding to the content of the fragment centered in this position. For reference, see how it is done in [9, 10, 11, 12].

```

acgatgccgttgcggatgtcgccatcgggaaaaactcgatgccgttgcgggacgttgcgggcccagcgtcttcagctgcaggcgcgcttctcgtcgg
ccacggcgtcgaagccaggcctggccctcggctgggatgttgggtcgccaccggccaggggtgcggtcaggccggcgcaccttgcggccggccgcgcg
aaggcctgcgaagcctgtggcgtggteacctcatggatgaggtgcaggtcgatatagaggatcgcttcgcccctgcttcgctgacgacgtggcgctcc
cagatcttgcgtaaaagggtttgcggacatggcggggatataggggcggatcgtgcgcaaaaatccagtggcgccccaggctcgcgacaatttcttgcg
ccctgtggccttcagctctccgaacagccccgaacgacatagggcgcccatgttgcggcgccctcgcatcgttttcagccttcgggggcccgatgg
gctccgcccgaagagggccgtccactagaaagaagacggtctggaagcccagcgcacaagcgcgggggtggcatcacgttctcacgaaacgctggttgc
tcaatctatgtgtggttgcacccaagcttgaccgtacggcgcgggcaagggcgagggctgttttgacgttgagtggttgcagcgtcgtgaaacgctcct
cgcccttcgacaagctcagggtgagcagactaatggccacggttcgcacgaaatcctcatcctgagcttgcctcccgaggaccgacgcaaggtcggcccg
aggataaaactccggacgaggatctccatccaggctagcgcacaacagccagcgcgaagtgcgcaaaagaaaaaggccccgggtgtctccacggggcctcgt
tcatttcgaaggtcgggaagcttaggcttcggctcgaagccgaagccttcgggcccgcgcgcgctcggcgatacgggcccgaacttacccgcagcgtcgcgc
aggtagtagcagcttggcgcgacggacgacgcgcgacgcttgacttcgatgcttgcgatgctcggcgacagcagcgggaaacagacgctccacgccttcgc
cgaaagaaacttcacggaacctgaagctctcgtgcacgcctcgcggcgcgggcgatgcagacgcttcataggcctgaacgcgctcgcgctcgccttc
cttgatcttcacggttgacgcgcaggggtgcgcccggacggaagtccgggatcgcgcgggcccagcagacgggccgatctcttctgctcgagctgagcg
atgatgttgatcgccatgatcatgtctccttgggcttaccgggttcgggtgcgcttctgcctgttgattggcgagggtgggctccacagagatccgggcg
ccgctcgcgggtcgtttcttcacgcatacgttgcgcatctggctgatctctcttgatgcgcccagcagcagcaccctcggggatgtcgagctcttcgaa
gtccgcggtctcgtgtactgcggatgctcgaggagaccgtcctcgaagcttcttcacgcgtgctctcgatatcccccagaacccccggggcaagtgcga
cgacgcctcgatcagcaaccatcgcgcgcgcttcgccacggcgagtcacggcgtctccgaccgagacctcttcgaaacctcgggctcgagcaccgcgctg
gtccacccccctcgaagcggccgcacagcaccacgatcggggcgcccttggaaccactccctcacgcgcgctgggtcaggggctgcctccggcgctcatg
tcaaaaagcggccgcccacgcgcttcgaggctgtccagcgcgagggcgatcacgtccgctttgagtacggctcctgcgcccaccccgagggtgtcgt
cgaggaagccgcgcttatccttgaaaaagcgcgaatgtccagtgcttccaaacgcacaggtcctcctcctccaggcggtcccgatcatcgagacgcc
gagcgggcccgggaagcctcggggaacatggteaggacgggtggcgggtgaacggcctggttagcggctggaagcctccagagtgogaagcctgc
gcccgcgggggttcateaaagcgcatttccagltgltccacgtcatcggcgaaagtcggcggaacaaactgaggtccacttcgtcccacgcccgaaga
gcgcttctcctcagcgccttagcagggcgctcgtgcttctgcgccgcgctcagagcgtgacgaccggggcgatcgcgatccagttcgcgctgttggc
cctgcgcgctgtcgatcctctctgttggctctctggatgtgggcccctcagcctgcagcggccagatgcaggacgcgctcgatcggcgacccctgatg

```

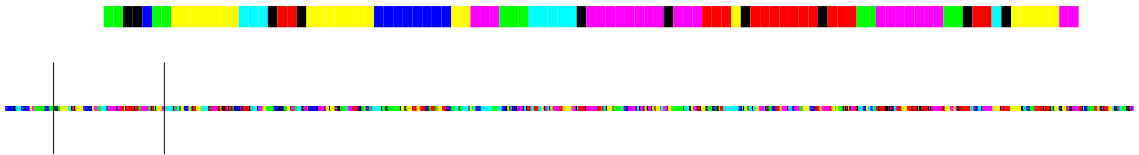


Figure 4: Visualizing results onto the text. There are three scale in the browser. The bottom color code represents the genetic text as whole. The second line shows colors of 100 fragments starting from a position in the text specified as an argument of *GenBrowser.m* function. The letter color code shows 2400 symbols starting from the same position. The black color corresponds to the fragments with non-coding information (the central cluster), other colors correspond to locations of coding information in different directions and with different shifts with respect to randomly chosen division of the text into fragments.

Precise start and end positions of genes. The biological mechanism for genes reading (the polymerase molecule) identifies the beginning and the end of a gene using special signals, specialized codons. Thus, almost all genes start with “ATG” start codon and end with “TAG”, “TAA”, “TGA” stop codons. Try to use this information to precise the beginning and end of every gene.

Play with natural texts. The *CalcFreq* function is not designed specifically for the four-letters alphabet text, it can work with any text. For a natural text, it is also possible to construct local frequency dictionaries and segment it into “homogeneous” (in the meaning of short word frequencies) parts. But be careful with longer frequency dictionaries, for an English text one has $(28 + 1)^2 = 841$ possible duplets!

Students can also look at visualizations of 143 bacterial genomic sequences at [13]. All possible types of the 7-cluster structure have been described in [14]. Non-linear principal manifolds were utilized for visualization of the 7-cluster structure in [16].

8 Conclusion

In this exercise on applying PCA to the analysis of a genomic sequence the students learn basic bioinformatics notions and how to apply PCA for visualization of local frequency dictionaries in genetic text.

9 Appendix. Program listings

```
function str=LoadSeq(fafile)
fid = fopen(fafile); i=1; str = '';
disp('Reading fasta-file...');
while 1
    if round(i/200)==i/200
        disp(strcat(int2str(i),' lines'));
    end
    tline = fgetl(fid);
    if ischar(tline), break, end;
    if(size(tline) =0)
        if(strcmp(tline(1),'>')==0)
            str = strcat(str,tline);
        end;
    end;
    i=i+1;
end
nn = size(str); n = nn(2);
disp(strcat('Length of the string:
',int2str(n)));
```

```

function xx=CalcFreq(str,len,wid)
disp('Cutting in fragments...');
i=1; k=1;nn = size(str);
while i+wid<nn(2)
    if round(k/200)==k/200
        disp(strcat(int2str(k),' fragments'));
    end
    frag = str(i:i+wid-1); vf(k) =
calcf(frag,len);
    i = i+wid; k=k+1;
end
disp('Merging into table...');
names = java.util.Vector; n = 0;
for i=1:size(vf)
    if size(vf(i)) =0
        keys = vf(i).keys;
        while keys.hasMoreElements
            key = keys.nextElement;
            if names.indexOf(key)==-1
                names.add(key);
            end
        end, n=n+1; end, end
xx = zeros(n,size(names));
for i=1:size(vf)
    if size(vf(i)) =0
        if round(i/200)==i/200
            disp(strcat(int2str(i),' points'));
        end
        for j=1:size(names)
            xx(i,j) = getwf(names.elementAt(j-1),vf(i));
        end, end, end
end, end, end

```

```

function vf=calcf(str,num)
vf = java.util.Hashtable; i = 1; nn =
size(str);
while i+num<nn(2)
wrd = str(i:i+num-1); i = i+num;
addwf(wrd,vf,1);
end

function addwf(word,hash,fr)
wf = hash.get(word);
if size(wf)==0 hash.put(word,fr); else
hash.put(word,fr+wf); end

function fr=getwf(word,hash)
wf = hash.get(word);
if size(wf)==0 r=0; else fr=wf; end

function PCAFreq(xx)
% standard normalization
nn = size(xx); n = nn(1) mn = mean(xx);
mas = xx - repmat(mn,n,1); stdr = std(mas);
mas =
mas./repmat(stdr,n,1);
% creating PCA plot
[pc,dat] = princomp(mas);
plot(dat(:,1),dat(:,2),'k.');
```

hold on;

```

set(gca,'FontSize',16);
axis equal; title('PCA plot for text
fragments','FontSize',22);
set(gcf,'Position',[232 256 461 422]);
hold off;
```

```

function fragn = ClustFreq(xx,k)
% centralization and normalization
nn = size(xx); n = nn(1)
mn = mean(xx);
mas = xx - repmat(mn,n,1);
stdr = std(mas);
mas = mas./repmat(stdr,n,1);
% calculating principal components
[pc,dat] = princomp(mas);
% k-means clustering
[fragn,C] = kmeans(mas,k);
% projecting cluster centers into the PCA
basis
XTP = C; temp = size(XTP); nums = temp(1);
X1c = XTP-repmat(mn,nums,1);
X1r = X1c./repmat(stdr,nums,1);
X1P = pc'*X1r'; X1P = X1P';
% marking the central cluster black
cnames = ['k','r','g','b','m','c','y'];
for i=1:k no(i) = norm(X1P(i,1:3)); end
[m,mi] = min(no);
for i=1:size(fragn)
    if fragn(i)==mi fragn(i)=1;
    elseif fragn(i)==1 fragn(i)=mi; end
end
% plotting the result using PCA
for i=1:n
    plot(dat(i,1),dat(i,2),'ko',
        'MarkerEdgeColor',[0 0
0], 'MarkerFaceColor',cnames(fragn(i)));
    hold on;
end
set(gca,'FontSize',16); axis equal;
title('K-means clustering','FontSize',22);
set(gcf,'Position',[232 256 461 422]);

```

```

function GenBrowser(str,wid,fragn,startp)
% we will show 100 fragments in the detailed
view
endp = startp+wid*100; nn = size(fragn); n =
nn(1);
xr1 = startp/(n*wid); xr2 = endp/(n*wid);
cnames = ['k','r','g','b','m','c','y'];
subplot('Position',[0 0 1 0.1]);
for i=1:size(fragn)
    plot(i/n,0, strcat(cnames(fragn(i)),'s'),'MarkerSize',2);
hold on;
end
plot([xr1 xr1],[-1 1],'k'); hold on;
plot([xr2 xr2],[-1 1],'k'); axis off;
subplot('Position',[0 0.1 1 0.1]);
for i=floor(startp/wid)+1:floor(endp/wid)+1
    plot([(i-0.5)*wid (i+0.5)*wid],[0 0],
        strcat(cnames(fragn(i)),'-'),'LineWidth',5);
    hold on;
end
axis off;
subplot('Position',[0 0.25 0.98 0.75]);
xlim([0,1]); ylim([0,1]); twid = 100; nlin =
24; k=startp;
for j=1:nlin
for i=1:twid
    col = cnames(fragn(floor(k/wid)+1));
    h=text(i/twid,1-j/nlin,str(k),'FontSize',8,'FontName','FixedWidth');
    set(h,'Color',col);
    k=k+1;
end end
axis off;
set(gcf,'Position',[64 356 879 195]);

```

References

- [1] Genbank FTP-site: <ftp://ftp.ncbi.nih.gov/genbank/genomes>
- [2] An http-folder with all materials required for the tutorial: <http://www.ihes.fr/~zinovyev/pcadg> .
- [3] Crick F.H.C., Barnett L., Brenner S., Watts-Tobin R.J. General nature of the genetic code for proteins. *Nature*, December 30, 1961. p.1227-1232.
- [4] Clark D., Russel, L. *Molecular Biology Made Simple and Fun*. Cache River Press, 2000.

- [5] *Caulobacter crescentus* short introduction at <http://caulo.stanford.edu/caulo/> .
- [6] Jackson, J. A User's Guide to Principal Components (Wiley Series in Probability and Statistics). Wiley-Interscience, 2003.
- [7] Zinovyev A. Hierarchical Cluster Structures and Symmetries in Genomic Sequences. Colloquium talk at the Centre for Mathematical Modelling, University of Leicester. December, 2004. PowerPoint presentation at <http://www.ihes.fr/~zinovyev/presentations/7clusters.ppt>.
- [8] Zinovyev, A., Visualizing the spatial structure of triplet distributions in genetic texts. 2003. IHES Preprint, M/02/28. Online: <http://www.ihes.fr/PREPRINTS/M02/Resu/resu-M02-28.html> .
- [9] Gorban, A.N., Zinovyev, A.Yu., Popova, T.G. Statistical approaches to the automated gene identification without teacher, Institut des Hautes Etudes Scientifiques. - IHES Preprint, France. 2001. - M/01/34. Available at <http://www.ihes.fr> web-site. (See also e-print: <http://arxiv.org/abs/physics/0108016>)
- [10] Zinovyev, A.Yu., Gorban, A.N., Popova, T.G., Self-Organizing Approach for Automated Gene Identification. *Open Systems and Information Dynamics* **10**(4) (2003), 321–333.
- [11] Gorban, A., Zinovyev, A., Popova, T., Seven clusters in genomic triplet distributions, *In Silico Biology*. **3** (2003), 0039. (e-print: <http://arxiv.org/abs/cond-mat/0305681> and <http://cogprints.ecs.soton.ac.uk/archive/00003077/>)
- [12] Ou, H.Y., Guo, F.B., Zhang, C.T., Analysis of nucleotide distribution in the genome of *Streptomyces coelicolor* A3(2) using the Z curve method, *FEBS Lett.* Apr 10 **540** (1-3) (2003), 188–194.
- [13] Cluster structures in genomic word frequency distributions. Web-site with supplementary materials. <http://www.ihes.fr/~zinovyev/7clusters/index.htm>
- [14] Gorban, A.N., Zinovyev, A.Yu., Popova, T.G., Four basic symmetry types in the universal 7-cluster structure of 143 complete bacterial genomic sequences. *In Silico Biology* **5** (2005) 0025. On-line: <http://www.bioinfo.de/isb/2005/05/0025/>
- [15] Staden, R., McLachlan, A. D. Codon preference and its use in identifying protein coding regions in long DNA sequences. 1982. *Nucleic Acids Res* **10**(1): 141–56.
- [16] Gorban, A.N., Zinovyev, A.Y., Wunsch, D.C., Application of The Method of Elastic Maps In Analysis of Genetic Texts, In *Proceedings of International Joint Conference on Neural Networks (IJCNN)*, 2003, Portland, Oregon, July 20-24.