



GelClust: A software tool for gel electrophoresis images analysis and dendrogram generation

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ABSTRACT

This paper presents GelClust, a new software that is designed for processing gel electrophoresis images and generating the corresponding phylogenetic trees. Unlike the most of commercial and non-commercial related softwares, we found that GelClust is very user-friendly and guides the user from image toward dendrogram through seven simple steps. Furthermore, the software, which is implemented in C# programming language under Windows operating system, is more accurate than similar software regarding image processing and is the only software able to detect and correct gel 'smile' effects completely automatically. These claims are supported with experiments.

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1. Introduction

Gaining comprehensive knowledge about constituting elements of individual's genomic DNA is an important and significant goal in molecular biology and related areas. Regarding its aims, a researcher might require having complete or partial knowledge about the DNA under investigation. Complete insight is achievable using expensive and time consuming sequencing techniques such as shotgun sequencing. But, in many cases, like cloning of large plant DNA [1], constructing physiological maps of chromosome [2], identifying restriction fragment length polymorphisms (RFLPs), determining the number and size of chromosomes, and molecular typing [3,4], this is not what the researcher needs. So, there are cheaper methods used for gathering partial knowledge.

Much of the rapid progress that is being made in molecular biology today depends upon the ability to separate, size, and visualize DNA molecules. The most common technique for this purpose is standard agarose gel electrophoresis [5]. Gel electrophoresis was for the first time conducted in 1930s utilizing sucrose gel. Then, starch and acrylamide gels were used respectively in 1955 and 1959. They provided more accurate separation and more control on sized of holes. Then, during the late 1970s, two-dimensional electrophoresis (1975) and agarose gels emerged. This type of gel is the most popular one nowadays and usually is used in Pulse Field Gel Electrophoresis (PFGE), a method introduced in 1983 that is able to separate large DNA molecules.

Gel electrophoresis has different applications in forensics, molecular biology, genetic, microbiology and biochemistry. But, as addressed before, one of the most important

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applications of this method is molecular typing [6,7]. Molecular typing is exploited in epidemiology, finding the food source of pollution, determining the plant pathogenic species penetrated in environment, and specifying a genotype combined with a particular bacterium [8]. Moreover, this method gives us more insight into epidemiological principles and evolution and penetration of many bacterial diseases.

The goal of gel electrophoresis in molecular epidemiology is to investigate the DNA molecules extracted from samples more accurately and to compare them with each other or with a standard sample (i.e., marker). This is accomplished by comparing the size/charge of segments of extracted DNA molecules through three steps of cutting the DNA molecules with restriction enzymes, running the cut molecules of samples in separate columns using electrical current, and comparing the segment maps created for each sample in the spongy tissue of gel with each other.

Amongst three steps of DNA restriction, running segments on gel (agarose or acrylamide), and analyzing the generated map, two first steps are completed inside laboratory. The third step used to be done by human eye, however the process was very time-consuming and error prone. Accordingly, after the demand for gel electrophoresis increased, information technology was exploited to hasten the processing, analysis, and clustering of samples based on the gel image.

2. Related works

A few tools and software has been developed for gel electrophoresis image analysis, but unfortunately, most of them are commercial or banned in Iran. Moreover, some of these software do not fulfill all requirements of users. For example, QIAxcel System [4,9], a product of QIAGEN company, is able to quantize the images but cannot cluster the samples. Another example is Biometra's product called BioDocAnalyze [10]. This software is not easy to work with when processing low quality images. This also applies to Gel Doc EZ System from Bio-Rad Laboratories [11].

In addition to mentioned software, there are other tools with clustering ability. For instance, ClusterVis from SequentiX company [12] is able to draw the phylogenetic tree of samples based on the information provided by GelQuest (another product of SequentiX). Although it is easy to work with GelQuest, but most of the tasks should be carried out manually and this decreases the agility. Moreover, GelScan, a part of

DIAS-II from Serva Electrophoresis [13], Phoretix 1D from Biostep [14], and Quantity One from Bio-Rad [15] are three commercial software sharing all the capabilities of the above mentioned tools (manually or automatic) and also able to cluster samples physiologically. While these tools fulfill most of the requirements of users, however their main problem is their high price, besides the sophisticated usage, which needs the users to be exercised enough [16]. Furthermore, Rementeria et al. [17] and Cardinali et al. [18] has discussed the drawbacks of some commercial packages and discrepancies between them in detecting bands and determining the number of genotypes based on gel images.

With respect to above, some researchers have endeavored to develop their own non-commercial and simple tools for this aim. One example is PyElph [19] which is an open-source software providing all of the abilities of commercial software. This user-friendly software is mainly designed for educational uses and is not so accurate in detecting columns and bands. Other software is GelAnalyzer [20], a java based but not open-source product. Some features of the addressed products are summarized in Table 1.

Having said all these, a need to accurate user-friendly software that decreases the need to interference of user in the analysis process is obvious. In the next sections the proposed software, GelClust, is introduced and its general and specific capabilities are discussed. It is supposed that this software will address some of the issues of the existing tools mentioned above.

3. Methodology

GelClust processes the input image in 5 steps and clusters the samples in 2 steps. The software includes a control window containing a menu bar, user directions, and 'next' and 'previous' buttons. Furthermore, there is an operation window that allows the user to view and edit the results. The below steps are followed during processing the image:

- (1) Loading image and selecting region of interest: software accepts most of the image formats as input. The image file should be imported using the 'File' menu in the menu bar. The user can select a region of interest on which the processing will be conducted in the next steps.
- (2) Automatic or semi-automatic detection of columns (Fig. 1): in this step, columns are automatically detected given two

Table 1 – Some of features of existing gel electrophoresis image processing software.

Software name	Commercial	Output		
		Band correction	Smile effect correction	Dendrogram
QIAxcel System	Yes	Yes (Auto & Manual)	N/A	No
BioDocAnalyze	Yes	Yes (Auto & Manual)	Yes (Manual)	No
Gel Doc EZ System	Yes	Yes (Auto & Manual)	Yes (Manual)	No
Gelquest & ClusterVis	Yes	Yes (Manual)	Yes (Manual)	Yes
GelScan	Yes	Yes (Auto & Manual)	N/A	Yes
Phoretix 1D	Yes	Yes (Auto & Manual)	Yes (Manual)	Yes
Quantity One	Yes	Yes (Auto & Manual)	Yes (Manual)	Yes
GelAnalyzer	No	Yes (Auto & Manual)	Yes (Manual)	No
PyElph	No	Yes (Auto & Manual)	Yes (Manual)	Yes

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