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Pattern discovery for microsatellite genome analysis

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ABSTRACT

Microsatellite loci comprise an important part of eukaryotic genomes. Their applications in biology as genetic markers are related to numerous fields ranging from paternity analyses to construction of genetic maps and linkage to human disease. Existing software solutions which offer pattern discovery algorithms for the correct identification and downstream analysis of microsatellites are scarce and are proving to be inefficient to analyze large, exponentially increasing, sequenced genomes. Moreover, such analyses can be very difficult for bioinformatically inexperienced biologists. In this paper we present Microsatellite Genome Analysis (MiGA) software for the detection of all microsatellite loci in genomic data through a user friendly interface. The algorithm searches exhaustively and rapidly for most microsatellites. Contrary to other applications, MiGA takes into consideration the following three most important aspects: the efficiency of the algorithm, the usability of the software and the plethora of offered summary statistics. All of the above, help biologists to obtain basic quantitative and qualitative information regarding the presence of microsatellites in genomic data as well as downstream processes, such as selection of specific microsatellite loci for primer design and comparative genome analysis.

1. Introduction

Microsatellites (or simple sequence repeats SSRs) constitute one of the most important classes of genetic markers, widely applied in an array of research areas, such as studies of genetic variation and structure, or construction of genetic maps [1]. Some of the most well known applications in which microsatellites play a key role are paternity testing [2,3], the confirmation of family pedigrees [4] and forensic investigations [5]. The ubiquity of microsatellites within genomes has played an important role in many genetic mapping projects such as in human [6], mouse [7], dog [8], trout [9] and other species.

Recently, the advent of next generation sequencing platforms has produced a wealth of genomic data, permitting a more in depth analysis of microsatellite genome abundance and distribution across different organisms. In this paper we propose a new algorithm for the detection of microsatellite loci in genomic data. The algorithm searches exhaustively for mono-, di-, tri-, tetra-, penta- and hexa-nucleotide microsatellites and for perfect, imperfect, perfect compound and imperfect compound microsatellites simultaneously in one execution. The algorithm has been implemented and is offered in the user friendly application Microsatellite Genome Analysis (MiGA). The MiGA application, the user manual and example datasets are available on http://mlkd.csd.auth.gr/bio/miga/index.html.

The paper is organized as follows. Section 2 provides some necessary background knowledge. Section 3 is dedicated to the detailed description of our approach. This includes the description of the algorithm, the data repository, the front end and the results. Section 4 presents related work and the existing tools. Section 5 presents results of a task oriented user evaluation. In Section 6, as an example, we present a full microsatellite analysis of the genome of *Danio rerio*, (an important vertebrate model organism). The paper is concluded in Section 7.

2. Background knowledge

2.1. Repeated sequences

The entire genome of an organism contains non-coding regions as well as coding regions which are translated into proteins. Big parts of non-coding DNA are organized in repeated sequences. These sequences appear in various sizes and in multiple copies in the genome and it was initially believed that they had no particular role in biological processes. Today, it is accepted that they play a significant role in the structure, the function and the evolution of the genomes and can interact with gene regulatory mechanisms [11,12,13].

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2.1.1. *Simple sequence repeats-SSRs*

Simple Sequence Repeats (SSRs) or *short tandem repeats* (STRs) constitute a very common type of repeated sequences (oligomers) in eukaryotic genomes, e.g. $(A)_n$, $(ACAG)_n$ or $(GGC)_n$. The subscript *n* denotes the number of the repetitions of the sequence in brackets. This repeated sequence is called *motif* or *pattern*. The size of the motif is 1–6 nucleotides long [14]. The number of the repetitions within microsatellite sequences can vary a lot, whereas their length usually varies from 10 to 300 nucleotides [14,15].

2.1.2. *The four types of SSRs*

There are four types of microsatellites. The first type is *perfect SSRs* constituted by only one repeat motif e.g. ATGCATGCATGCATGC or $(ATGC)_4$. The only parameter concerning perfect SSRs is its minimum length. The second type of SSR is *imperfect SSRs* which are the same as perfect, but include some mismatch in the repeat sequence, e.g. $(ATGC)_3G(ATGC)_5$. The third type of SSRs is *perfect compound SSRs*. These are SSRs which constitute of two or more different perfect SSRs e.g. $(ACCG)_4(GGGC)_3$. The last category of SSRs is called *imperfect compound SSRs* and are constituted from two or more imperfect SSRs.

3. The MiGA application

3.1. *Pattern discovery algorithm for SSR extraction*

MiGA′s algorithm uses an exhaustive search in order to identify microsatellites in genomes. The algorithm searches for SSRs in a character string (sequence). It finds the first pattern (1–6 characters) that is repeated and continues by calling itself, with the remaining substring as input, to identify all the patterns and the number of their repeats. This was necessary in order to achieve better execution time. This is essential for complete genome analysis since complete genomes are big datasets and therefore their analysis with desktop computers is prohibitive.

MiGA′s algorithm is modular and is divided in two main parts the *Repeated Pattern Discovery* and the *SSR Assembly*.

3.1.1. *Repeated pattern discovery*

The first part of the algorithm is executed only once during the initial loading of a FASTA file, (the most popular file format in the field of bioinformatics), or during the initial loading of a full genome from the Ensembl database. Its purpose, in general, is to extract all perfect repeats (one to six nucleotides long) from the genomic data. Therefore, the algorithm scans the sequence six times (i.e. each time for a different motif length).

In general, the algorithm places the Beginning Search Position (BSP) at the beginning of the sequence. The algorithm considers as the first candidate motif the *N* long string of nucleotides, which can be found *N* places after the BSP, BSP included. For instance, at the first scan, *N* is equal to one and the candidate pattern is the first nucleotide of the input sequence i.e the BSP. Considering an input sequence such as: *AGGGCT*…, the BSP is nucleotide A, and the candidate mono-nucleotide motif is “A”. In case of scanning for tri-nucleotide long motifs, the candidate motif would be “AGG”.

After the identification of the candidate motif, the algorithm checks whether the *N*-next nucleotides are the same as the nucleotides of the candidate motif, or simply put, if the candidate pattern is repeated. If yes, then the algorithm proceeds, until failure of the comparison and the repeated sequence is subsequently stored in a list along with its start and finish position. The algorithm will then move the BSP at the first position after the ending of the repeated sequence.

Consider for example an input sequence to be the following: *AAAGTTCT*…*CTC*. In the first scan of the sequence, for mononucleotide SSRs, the BSP is at the first nucleotide and the candidate pattern is “A”. The algorithm will check and find this pattern two additional times. After this, the comparison fails, the algorithm saves the pattern “A” in a list, along with the starting and ending position and moves the BSP to nucleotide G, right after the repeated sequence “AAA”. The candidate sequence is now “G”. In case of tri-nucleotide long motifs, the first candidate motif would be AAA. This motif is clearly not repeated, so the BSP would move on to the second A and the pattern “AAG” would become the candidate motif.

Completion of the perfect repeated sequence search is followed by a number of modifications and filtering of results. The most important, is that, to avoid redundancy, the algorithm assigns a repeated sequence as a sequence with the minimum length of motif with the most repetitions. For instance, for a repeated sequence AAAAAAAAAAAA, the algorithm has initially stored this as repetition of motif “A” twelve times, repetition of motif “AA” six times, etc. After filtering, the algorithm stores this only as a twelve times repeated motif “A”.

3.1.2. *SSR assembly*

The second part of the algorithm, depends heavily on the first and is executed only according to a specific search requested from the user. Unlike the first part, it is executed every time there is a new search request, with different parameters. There are four main functions for this part, related to the four microsatellite types (perfect, imperfect, perfect compound and imperfect compound SSRs).

The simplest function is the one concerning perfect SSRs. In order to find the perfect SSRs, the algorithm simply subtracts the end position of a putative SSR from its start position (as available from the first part of the algorithm). The size of a perfect SSR should be greater than the minimum length value, supplied by the user as a parameter. For instance, if the minimum SSR length was set to 16, then the sequence $(ATGC)_3$ would not be considered as a microsatellite. A second function produces sequences assigned to imperfect SSRs. After finding all perfect SSRs the algorithm checks if the distance between consecutive perfect SSRs is even or shorter than the maximum allowed mismatch that has been provided by the user. Our definition of an imperfect microsatellite does not take into consideration the nature of the mutations of the possible mismatch and how they resemble or not the correlated repeat motif. To be assigned as an imperfect SSR, the algorithm also checks if the two patterns of the perfect SSRs belong to the same repeating cycle. Consider the following imperfect SSR: “ATGATG**C**TGATGA”. The algorithm accepts this SSR as an imperfect one, because the pattern TGA and the pattern ATG belong to the same repeating cycle (i.e. patterns ATG, GAT and TGA are equivalent).

The third function assembles perfect compound SSRs. It operates in the same way as with imperfect SSR search, with only small changes to the conditions of acceptance. Firstly, two consecutive SSR sequences should not be equivalent, unlike imperfect SSRs which should belong to the same repeating cycle. Secondly, these two consecutive perfect SSRs should have an (intergenic) distance even or shorter than the one provided by the user and lastly, the length of every individual repeated pattern should be even or greater than the minimum length provided by the user.

The last function, concerning imperfect compound microsatellites operates in the same way as the third function with one difference. At least two of the consecutive repeated sequences must belong to the same repeating cycle, though another repeated consecutive sequence should exist with a different motifs.

3.1.3. *General comment for the algorithm*

We would like to mention the importance of the algorithm′s modular nature. In general, when performing an analysis of a dataset, biologists tend to analyze the same datasets many times with different parameters. Having realized this, we constructed the algorithm in this modular way in order to avoid the repetition of pattern discovery (Section 3.1.1) which is the most time consuming. When there is a new request to analyze the same data with different parameters, only the second part (Section 3.1.2) will be executed.

3.2. *Data repository*

Taking into consideration the size of the data and the information that can be downloaded and analyzed when using the Ensembl database through the program, it became clear that a local *Data Repository* (DR) should be used in order to store all Ensembl originated data. This data can be used in downstream (as well as future) analyses of MiGA. The DR consists of two parts, a database and a collection of files. The first part called LoBiD (LOcal BIology Database), is designed and organized in the same way as commercial databases (Fig. 1). LoBiD consists of five tables which refer to the organism, slices (which are fragments of the DNA sequence), genes, transcripts and exons. The table organism contains the scientific name of the organism, the id of the organism in the Ensembl database, the information whether this organism has an organized karyotype in the Ensembl database or not and the names of the chromosomes in case of karyotype existence. Lastly, this table contains the information about the last update of the particular organism in LoBiD. The remaining four tables contain information about the DNA sequences of this organism.

The second part of the DR consists of files which contain the sequences and the results from the performed analysis. The use of files for storing the DNA sequences was essential, for the efficient and rapid process of the data. The two parts of the database are closely related. In other words, the files contain the raw data of the genome and the database contains all the information about the structure of the genome (i.e. location of genes, names of chromosomes). The existence of the data repository also allows the user to restore the search history and retrieve previous projects and consequently, to perform analysis on previously seen data in a very fast way. The term history has a twofold meaning. Firstly, the application stores the Repeated Pattern Algorithm analysis (for each FASTA file or genome) in binary files in the DR. Therefore, in future SSR discovery analyses, this part of the algorithm does not run again, saving time. Secondly, the application stores the specific analysis parameters, in order to avoid re-execution of the algorithm in case the requested analysis is done on the same data with identical parameters.

3.3. *Functionality overview and front–end description*

3.3.1. *Source of data*

MiGA gives the user the opportunity to choose the source of the data that will be analyzed. MiGA's first window has two tabs which correspond to the two different possible ways for providing data to the application. The first way is to search for microsatellites within user provided data in FASTA format. One single file can contain multiple sequences. Users should specify a name for the project. MiGA will store all results in a folder with the project's name in the data repository. In this step, MiGA also allows the user to retrieve all previous projects in a dropdown menu, in case the user would like to analyze previous data.

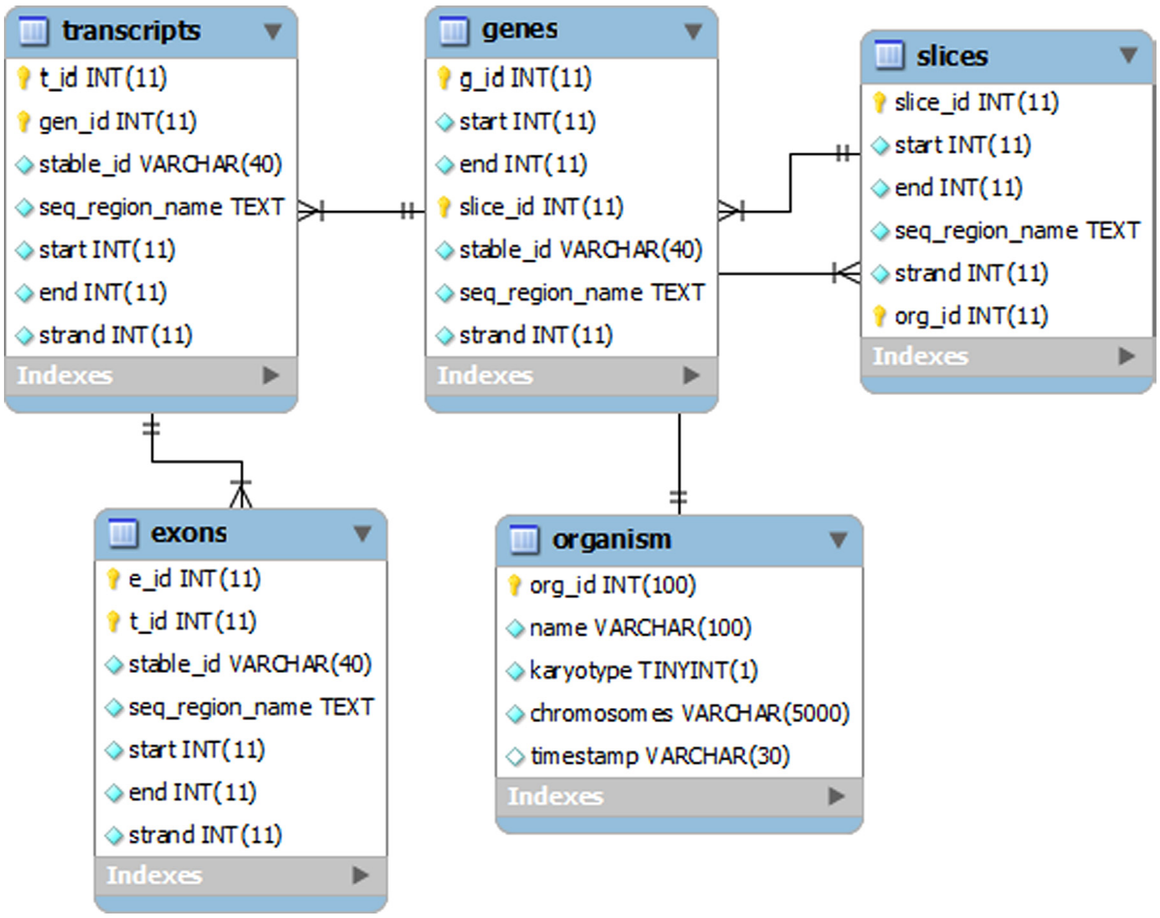
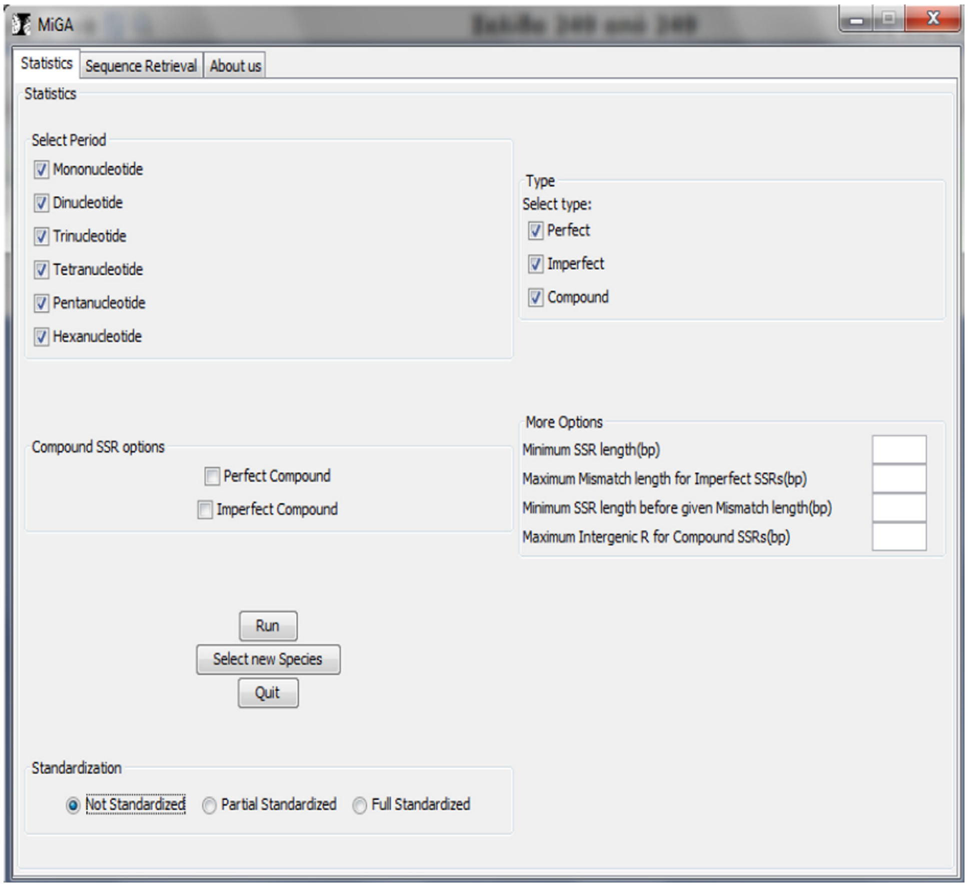


Fig. 1. Schema of the LoBiD Database.

The second option is the analysis of complete genomes. The complete genome analysis is made through the Ensembl database [10], the most comprehensive database for fully annotated eukaryotic genomes. The user can choose from available Ensembl organisms and then MiGA downloads the selected genomes in the DR.



Statistics

Select Period
[✓ Mononucleotide]
[✓ Dinucleotide]
[✓ Trinucleotide]
[✓ Tetranucleotide]
[✓ Pentanucleotide]
[✓ Hexanucleotide]

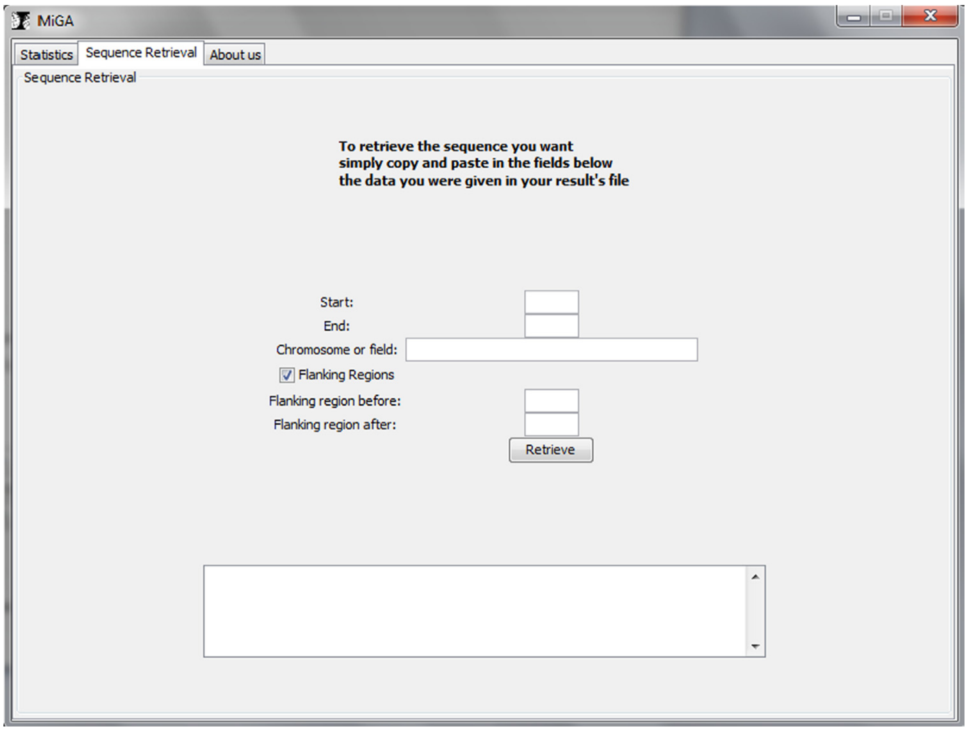
Type
Select type:
[✓ Perfect]
[✓ Imperfect]
[✓ Compound]

Compound SSR options
Perfect Compound
Imperfect Compound

More Options
Minimum SSR length(bp)
Maximum Mismatch length for Imperfect SSRs(bp)
Minimum SSR length before given Mismatch length(bp)
Maximum Intergenic R for Compound SSRs(bp)

Standardization
[✓ Not Standardized] Partial Standardized Full Standardized

Fig. 2. "Search parameters for SSR discovery" tab.



Sequence Retrieval

To retrieve the sequence you want simply copy and paste in the fields below the data you were given in your result's file

Start:
End:
Chromosome or field:
[✓ Flanking Regions]
Flanking region before:
Flanking region after:

Fig. 3. "Sequence Retrieval along with Flanking Regions" tab.

3.3.2. *Parameters for SSR discovery*

MiGA allows the user to search for mono-, di-, tri-, tetra-, penta- and hexa-nucleotide SSRs and for perfect, imperfect, perfect compound and imperfect compound SSRs simultaneously in one execution (Fig. 2). The user should specify the appropriate parameters for each type of SSR. In case of perfect SSRs the parameter is only the minimum SSR length, in base pairs. In case of imperfect SSRs the user should provide (i) the maximum length of the gap/mismatch and (ii) the minimum length of each SSR. For compound SSRs the parameters are (i) the minimum length of the SSRs and (ii) the maximum inter-repeat region (i.e. the region between the two perfect SSRs) that will be allowed.

3.3.3. *Sequence retrieval and flanking regions*

One important aspect when analyzing microsatellite sequences, is to clarify the composition of the flanking regions of each microsatellite locus. The retrieval of those regions is important for primer design for downstream analyses of microsatellite loci, or for comparisons of different loci in different species. There is therefore a tab for sequence retrieval (Fig. 3).

In order to retrieve one SSR sequence, the user has to copy the path, which is the subdirectory from the root folder to the file that contains the SSR, the start and the end of the SSR as given in the results file. The program identifies the SSR and the slice from which it is retrieved in the local database (LoBiD) and provides the user interface with the sequence as it is. In the same way, the program provides the user the additional opportunity of flanking regions retrieval for each SSR. The user should specify the length of the upstream and downstream flanking region.

3.4. *Results and statistics*

At the end of the data analysis, the application produces results and summary qualitative and quantitative statistics, saved in text files. Those files are stored in a local folder with a folder for every different projects in the data repository.

3.4.1. *Results*

Each result file starts with a header which contains the project's name and all the search parameters used. Then, follows information about SSRs found during the analysis. This analytical information includes the motif of the SSR found, the number of repeats for each SSR, the start and end position in the sequence and lastly the path from where the user can retrieve the SSR sequence and its flanking regions.

3.4.2. *Summary statistics*

The application produces a text file, called motif Statistics, which contains analytical information for each exact motif found in the sequences, based on the Repeated Pattern Discovery algorithm (Section 3.1.1). This information includes how many times the motif is found in the genome (count), the total number of the repeats of the motif (repeats), the count of the nucleotides in base pairs (bp) which is the product of the length of the motif multiplied with the repeats, the average microsatellite length (Avg_Length), the standard deviation of the average microsatellite length (SD_Length), the maximum length (Max_Length) and the average motif repeat (Avg_Repeats). Moreover, the application provides the percentage of each nucleotide in a microsatellite motif.

The user can specify whether the program produces standardized, partially standardized or non standardized results. The standardization, partial or not, is a process in which similar, microsatellite motifs are grouped together. More precisely, partial standardization is considered as equivalent motifs that belong to the same repeating cycle. Full standardization takes into account the reverse complements of microsatellite motifs as well, based on the notion that sequencing machines produce DNA sequences of both strands and therefore of unknown direction. The matrices for the standardization process are the same with the Sciroko tool [16].

MIGA also produces a summary table for each SSR type (i.e. perfect, imperfect, perfect compound, and imperfect compound). The statistics offered by the application are the following: The count of each type of SSR, the total size of each type of SSR, the percentage of each nucleotide (A, G, T, C) found in SSRs, the relative frequency of each type of SSR (i.e. count of SSRs/total count of SSRs), abundance (i.e. total size of each type of SSR/total genome length) and relative abundance (i.e. total size of each type of SSR/total length of SSR found in the genome). The text file that contains the statistics is also generated in html format for more usability.

4. Related work

Microsatellites present wide applications in the field of biology. Thus their analysis is a research area with many branches, especially now that genome information is increasingly available. For instance, LobSTR [17] and RepeatSeq [18] are such new applications that aim to infer diploid genotypes in microsatellite loci after full genome sequencing of individuals based on pre-existing reference genomes. Another new branch in microsatellite analysis is to trace microsatellite loci within assembled or non-assembled genomes of any kind of species (non-model included). MiGA belongs to this category.

Several tools already exist in this category based on different approaches and methodologies [19]. Some of the most important tools are TROLL [21], STAR [22], MISA [23], SSRFinder [24], SSRIT [25], TRF [20], Sciroko [16], QDD [26], Sputnik, Modified Sputnik I [27] and Modified Sputnik II [28]. Some tools use a dictionary approach which means that the motifs have to be defined in the program, while others use heuristic approaches. The first algorithms developed to address the problem of finding SSRs in genomes used exhaustive search. In the past, these algorithms were condemned to be applied only in small sequences due to lack of processing power of computers. Nowadays, that this problem has been solved, many new tools [29,30] prefer this methodology in order to be more precise than the heuristic ones. Regardless of the methodology, the purpose of these tools has to be the identification of repeats in a DNA sequence, without a priori knowledge of the repeat unit's composition [20]. Moreover, there should be no limitations in the motif, motif length, and the number of copies that can be detected [19].

In many publications of previously developed programs such as TROLL, STAR and others, comparisons were made using as main criteria only the number of hits and the overall execution time, simplistically concluding that the less execution time and the more hits, the better the tool. In 2009 Jentzsch published her Ph.D. dissertation [19] providing a detailed presentation and comparison of the most important existing tools. One of the key conclusions was that the input parameters should be fine-tuned individually for each program, so that searches can become equivalent; i.e. there is no meaning in comparing different tools, if these tools are not looking for the same thing. This process requires deep knowledge of the various existing programs. An additional main characteristic that Jentzsch considers in her study was the capability of the program to analyze large sequences in the order of millions of nucleotides.

Although, as just stated, a thorough comparison with an existing tool is a difficult process, in this section we are going to provide a qualitative comparison between MiGA and TRF [20] which is the most widely used tool in microsatellite analysis. The first and main difference is that TRF is a heuristic approach which performs approximate search in sequences. On the contrary, MiGA performs an exhaustive search which means that it will discover every microsatellite in a given sequence with respect to the aforementioned definitions. Another difference is that MiGA offers the possibility to run a complete search of perfect, imperfect and compound perfect together or distinctively. TRF does not offer this option. For instance, due to the probabilistic nature of the algorithm TRF cannot search exclusively for perfect repeats. Another difference is that TRF produces some redundant hits. MiGA filters them and presents only SSRs with the minimum motif, as explained earlier. Moreover, MiGA also offers motif standardization, summary statistics, connection to online database and history options, all utilities that TRF does not offer.

Overall, available tools lack some or all of the following characteristics: (i) a graphic user-friendly interface (ii) summary SSR statistics, (iii) connection to an online database, (iv) use of a local database to store and retrieve results and data, (v) full genome SSR search efficiently and fast, and (vi) complete search in one execution. MiGA offers all these characteristics in a very easy way to accomplish.

5. User evaluation

Biologists could be a very diverse group concerning being conversant with computers. A bioinformatics application can be judged inside the scientific community, in terms of success, based on the simplicity and the ease of accomplishing tasks. It is common sense that one of the most important aspects in software design is the usability. Usability can be measured in various ways, but one of the most reliable is through a task oriented evaluation, which is performed by ordinary users who had no involvement in the process of designing and developing the application. Inside knowledge of the whole project, makes designers and developers unsuitable for identification of possible flaws in the design and more generally, for performing an unbiased evaluation of a software′s usability. For all the reasons presented above, we conducted a task oriented user evaluation. The purpose of this evaluation was to identify possible flaws and drawbacks in the design and to measure the application′s usability.

5.1. Evaluation description

Our user evaluation involved 12 users, mostly postgraduate students from the Biology Department of the Aristotle University of Thessaloniki, as well as research staff from our lab (9 biologists and 3 computer scientists). It is important to mention that no user was familiar with the application. The evaluation was designed in a way that simulates a complete microsatellite analysis by a biologist. It consisted of four tasks which were strongly dependent as consecutive parts of the analysis. At the first task users were asked to start the application, load the data for analysis and give a specific project name. The second task was the analysis of the data with some specific parameters. The third task consisted of replying to four questions, based on the results of the analysis. At the last task users were asked to perform the whole analysis from the beginning. The objective of the first three tasks was to evaluate the capability of completing a microsatellite analysis with ease. The last task evaluated the ability of the user to make use of the previous projects without running again the whole process. For each one of the tasks, except the third one, we measured the time needed for the completion. For the third task we measured only the accuracy of the answers. In order to compare MiGA with an existing tool, users were asked to perform the first two tasks with Sciroko, a successful tool in bioinformatics community and one of the few that provide a friendly user interface. The time needed for completion of analysis was again measured.

After evaluation, users were asked to fill a questionnaire. The first part of it, consisted of three questions, comparing the two applications. The second part evaluated some features that MiGA offers. The scoring scale was from 1 which corresponds to useless, to 5 which corresponds to very useful. In order to come to more robust conclusions users were asked to self evaluate themselves with respect to computer familiarity and bioinformatics software.

5.2. Evaluation analysis and conclusions

The scale for the self-evaluation of the users was between 1 and 5. The average score for the computer familiarity was 4.08 and the average score for bioinformatics software familiarity was 2.58. That means that most of them use computers regularly but they are not too familiar with bioinformatics software.

In order to compare MiGA′s and Sciroko′s user interface, a repeated measures T-test was conducted to compare the time needed for the completion of the first two tasks in both applications. *T*-test assumes that the data follow normal distribution. In order to test the normality of the data, we conducted a Shapiro–Wilk test which is appropriate for small sample sizes (<50 samples). The significance values for the Shapiro–Wilk test are 0.378 and 0.639 for MiGA and Sciroko respectively, proving that data follow normal distribution. Results of the repeated measures *T*-test show that there was a significant difference in the scores for MiGA ($M=97.33$, $SD=29.199$) and Sciroko ($M=201.58$, $SD=48.801$) conditions; $t(11)=-7.225$, $p=0.000$. Although these results are based on a limited sample of twelve individuals, they suggest that MiGA does have a more friendly and intuitive user interface.

This result was also confirmed by the questionnaire. All users responded that they found MiGA to be more user friendly. Admittedly the users were familiar with the developing team so the questionnaire comparison might be biased.

Lastly, the analysis of the questionnaire concerning the features offered by MiGA showed that the following were the most important for the users: project history, analysis of whole genomes from Ensembl, the short execution time and the html presentation of the results which offers better visualization of the results.

6. Results of full genome analysis of *Danio rerio*

Zebrafish (*D. rerio*) is a tropical freshwater fish and a model organism for the science of biology [31,33,34]. Its sequencing project started in 2001 by the Sanger Institute and from then on several assemblies have been released. Its full genome was published in 2013 [32]. The assembly of Zebrafish′s genome that has been analyzed was 1.357.051.643 base pairs (bp). The parameters used for the microsatellite analyses, for each type were: (i) perfect SSRs: Minimum SSR length (bp)=12, (ii) imperfect SSRs: Maximum mismatch length for Imperfect SSRs (bp)=4 and Minimum SSR length before given mismatch length (bp)=8, (iii) compound SSRs: Minimum SSR length (bp)=12, Maximum inter-repeat region for Compound SSRs (bp)=12. Additionally motif-statistics were given on a fully standardized search.

The analysis was performed in a typical workstation. RAM: 4GB DDR II. CPU: Intel Core 2 Duo CPU E8500 @ 3.16 GHz 3.17 GHz. OS: 64-bit Operating System Windows 7. The time for finding all SSRs in each category is: 5 min for perfect, 286 min for imperfect and 365 min for compound perfect and imperfect SSRs. Given the fact that this is an exhaustive search, the running time needed is considered logical and certainly non-prohibitive. Memory usage did not exceed 474MB RAM. This is attainable because MiGA does not load the full genome to the RAM memory. The whole genome is processed in slices which are fragments of DNA 25000 nucleotides long. This is why the whole analysis can be performed with a single click (run), and the user does not have to load multiple separate FASTA files or to supervise the whole process.

MiGA application gives four main tables as output which contain basic and analytical information regarding perfect, imperfect, compound microsatellites (Tables 1–3) as well as the nature of the motifs found in these microsatellites (not shown), based on which the most common motifs present in the genome data can be deduced (Table 4). The above information can be used for the subsequent description of the structure of the repeated sequences within genomes [35,36], the comparison among different species [37] and the deduction of microsatellite regions which have been conserved through evolution highlighting possible functional importance [38].

Table 1
Characteristics of Perfect Microsatellites in *D. rerio* genome.

Motif	Count	bp	A%	T%	C%	G%	Relative freq.	Abundance	Relative abundance	Mean length
Mono	211,325	3,337,144	47.65	47.574	2.351	2.425	0.085	0.002	0.067	15.8
Di	481,259	16,698,668	38.156	38.168	11.854	11.822	0.193	0.012	0.335	34.7
Tri	259,406	4,792,107	44.673	44.599	5.342	5.386	0.104	0.004	0.096	18.5
Tetra	55,7039	12,016,836	36.853	36.598	13.054	13.495	0.223	0.009	0.241	21.6
Penta	101,372	2,324,855	43.954	43.557	6.238	6.251	0.041	0.002	0.047	22.9
Hexa	882,942	10,705,368	37.122	36.958	12.964	12.955	0.354	0.008	0.215	12.1
TOTAL	2,493,343	49,874,978	39.152	39.029	10.858	10.962	1	0.037	1	20.0

Table 2
Characteristics of Imperfect Microsatellites in *D. rerio* genome.

Motif	Count	bp	A%	T%	C%	G%	Relative frequency	Abundance	Relative abundance	Mean length
Mono	25,860	568,195	45.942	46.288	3.802	3.968	0.087	0	0.050	22.0
Di	112,669	5,817,335	35.822	35.519	14.449	14.209	0.38	0.004	0.512	51.6
Tri	31,983	1,125,528	46.595	46.491	3.371	3.543	0.108	0.001	0.099	35.2
Tetra	106,968	3,207,631	37.576	36.988	12.176	13.261	0.360	0.002	0.282	30.0
Penta	17,419	592,689	44.811	42.465	6.237	6.488	0.059	0	0.052	34.0
Hexa	1834	49,738	39.209	38.787	10.871	11.132	0.006	0	0.004	27.1
TOTAL	296,733	11,361,116	38.374	37.936	11.733	11.956	1	0.008	1	38.3

Table 3
Characteristics of Compound Microsatellites in *D.rerio* genome.

Type	Count	bp	A%	T%	C%	G%	Relative frequency	Abundance	Relative abundance
Compound perfect	125,797	6,988,308	21.217	20.446	5.675	5.642	0.72	0.005	0.53
Compound imperfect	48,827	6,201,944	17.836	17.548	5.758	5.876	0.28	0.005	0.47
TOTAL	174,624	13,190,252	39.054	37.994	11.433	11.519	1	0.01	1

Table 4
Characteristics of the most common motifs of *D. rerio*'s microsatellites.

Motif	Count	Repeats	bp	Avg_Length	SD_Length	Max_Length	Avg_Repeats
AC	252,968	3,406,857	6,813,714	26.935	21.388	414	13.468
A	201,302	3,177,761	3,177,761	15.786	4.734	488	15.786
AT	180,310	4,402,167	8,804,334	48.829	53.687	928	24.414
AAT	179,137	1,179,720	3,539,160	19.757	11.834	138	6.586
AAAT	164,225	648,037	2,592,148	15.784	9.377	364	3.946
AAAAAT	95,087	192,754	1,156,524	12.163	1.048	66	2.027
AATG	91,116	380,169	1,520,676	16.689	9.869	496	4.172
ATCC	58,307	345,113	1,380,452	23.676	20.389	472	5.919
AG	47,065	533,921	1,067,842	22.689	16.444	246	11.344
AAATAT	45,050	90,725	544,350	12.083	1.000	102	2.014
AAAAAC	42,033	84,523	507,138	12.065	0.706	66	2.011
AGAT	41,675	647,713	2,590,852	62.168	46.907	988	15.542
AAAC	36,880	140,400	561,600	15.228	8.290	124	3.807
ACAG	30,324	190,379	761,516	25.113	21.769	244	6.278
AAAAAG	29,726	59,795	358,770	12.069	0.681	36	2.012
AAAAT	24,570	93,894	469,470	19.107	12.502	155	3.821
AAC	24,297	126,881	380,643	15.666	7.358	144	5.222
AAAATT	23,774	47,822	286,932	12.069	0.812	72	2.012
AAACAC	19,897	40,236	241,416	12.133	1.185	42	2.022
AAAG	19,793	121,139	484,556	24.481	23.681	296	6.120
OTHER SSRs	885,801		12,635,052				

7. Summary and future work

Many programs exist, nowadays, that can detect microsatellites in the genome and some of them are quite successful. However, their drawbacks are numerous. MiGA manages to solve all these problems and moreover it provides functions that have never been offered before. In the future, we plan to further expand the application with new functions such as remote access to the database, in order to make best use of available computer lab resources. MiGA has some features i.e. data repository and modular algorithm that make its expansion possible to more specific directions. We plan to make use of ample features present in genomic databases such as Ensembl, for in-depth comparisons of microsatellites present in specific genes and functional elements. The future goal is to build a robust application, to be used for comparative genomic analyses based on microsatellite information.

Conflict of interest statement

None declared.

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