Genes, Genomes and Genomics ©2012 Global Science Books

Khirshyat 1.0: a Simple Micro-Program for Some Molecular Biology Protocols

Muhammad Youssef

Department of Genetics, Faculty of Agriculture, Assiut University, Assiut, postal code 71526, Egypt

Corresponding author: * **mkhirshy@aun.edu.eg, mkhirshy@yahoo.com**

ABSTRACT

Khirshyat 1.0 is a simple, dynamic and informative micro-program designed mainly to provide scientists, researchers and students in research systems and universities in developing countries, a useful tool on some molecular biology protocols. The program includes the most commonly used molecular markers (AFLP, SRAP and RAPD), PCR optimization, oligonucleotide properties, calculations of concentration and quantity using the molecular weight of most commonly used chemicals, plant DNA extraction, DNA concentration and purity, primer preparation, polyacrylamide gel electrophoresis and silver nitrate staining protocols. It accelerates the process of preparing stocks, buffers, solutions as well as provides important tips and general information with simple illustrations about the most common protocols. The program and documentation are freely available online.

Keywords: program, molecular markers, DNA, electrophoresis
Abbreviations: **AFLP**, amplified fragment length polymorphism; **PCR**, polymerase chain reaction; **RAPD**, randomly amplified polymorphic DNA; **SRAP**, sequence related amplified polymorphism

INTRODUCTION

Computational molecular biology consists of the development and use of mathematical and computer science techniques to help solve problems in molecular biology (Setubal and Meidanis 1997). Bioinformatics represents a new field at the interface of the twentieth-century revolutions in molecular biology and computers (Pevsner 2009). Thus, the use of computational methods to mine the biological information in many fields became a fundamental need. Subsequently, several programs for molecular biology, i.e. computer algorithms to analyze proteins, genes, as well as international databases already exist, which facilitate the understanding and ease studying of molecular biology. On the other hand, many protocols for different molecular boilogy techniques are available including, e.g. genomics (Raply 2000; Pereira 2010), proteomics (Walker 2005), electrophoresis (Sheehan and Tyther 2009) and molecular markers (Weising *et al.* 2005). However, there is a lack in basic protocols software, which could help researchers and provide an efficient tool for utilizing the molecular biology protocols. Such simple programs could reduce the time necessitate for hand calculations, and make the research work manageable and effortless. Therefore, the program described here was developed principally as a tool to assist researchers working in molecular biology.

Khirshyat was developed by the author since 2010 and tested by several users to check its simplicity and error-free calculations. The program includes some important protocols for molecular biology with some modifications, which could be used with different plant species. All calculations and technical features included in the program have been checked to avoid the common programming errors. Since the program released online, it seems that increasingly being used by a growing number of students and researchers who utilize some of its features. An overview of the program, its importance and some of its features are described here.

OVERVIEW OF KHIRSHYAT

Program design, download and setup

It is not too easy to find program for a particular task, especially nowadays, scientists are using a computer to help them in their scientific research. If a researcher wants specific program but he cannot find what he wants, subsequently, he has to design and write them using a programming language. Several encoding languages are available, varying in their easiness and utilization. Microsoft® Visual Basic 6, as a one of these languages, was used in designing Khirshyat 1.0. Due to its simplicity and easiness to learn and allow advanced programmers to create powerful Windows applications. On the other hand, a photo editing software tool is required to produce interactive and smart interfaces. Adobe® Photoshop® CS 8 was used for this task in producing backgrounds and graphic designs which provides well designed visual interface making the program enjoyable. Khirshyat runs under Windows® operating system, stands on itself and does not need any supporting programs.

The program can be easily downloaded from the Assiut University web-site or by direct request from the author. It is easy to install this micro-program as any other software by clicking on the "Set up" icon and following the coming menu instructions. The program includes a simple manual and "Read me" file for installation help.

Khirshyat submenus

Khirshyat comes with 25 submenus, in which the user can find some of the most common protocols used in molecular biology. Molecular markers (MM) (i.e. amplified fragment length polymorphism (AFLP, Vos *et al.* 1995), sequence related amplified polymorphism (SRAP, Li and Quiros 2001) and randomly amplified polymorphic DNA (RAPD, Williams 1991)), DNA extraction from plant tissue modified from Dellaporta *et al.* (1983), polyacrylamide gel electrophoresis, and silver nitrate staining (Bassam *et al.* 1991)

Received: 29 April, 2010. Accepted: 1 March, 2011.

Research Note

with some modifications protocols are provided. Moreover, the program gives important tips and general information, which help the user understanding different molecular biology techniques.

Calculations for PCR preparations

Khirshyat offers a dynamic PCR-MIX preparation tool for AFLP, SRAP and RAPD molecular markers according to their protocols, in which the user can modify the stock concentration of the PCR components (*i.e.* dNTPs, $MgCl_2$, Primers,… etc.) to a desired concentration. It enables the user to change the total volume and calculate the PCR-components volumes for any number of samples. Moreover, in the case of trying different primers or different DNA samples, these components can be avoided in calculations to be added separately. Users are able to save or print the thermocycler's program for these molecular markers, which includes its different steps, temperatures and number of cycles. In addition, the program includes a "PCR-optimization" submenu (**Fig. 1**) which can serve for any PCR-based molecular marker, the user needs just to fill stock and final concentrations according to any protocol, then the program gives all calculations in a proper volumes.

Oligonucleotide properties

The program, in addition, provides ' Oligonucleotide' submenu (**Fig. 2**), in which the basic properties of any DNA sequence can be calculated by pasting or entering the sequence followed by clicking 'Calculate'. The program will use the currently entered sequence to calculate the length, molecular weight, %C+G content and the melting temperature (Tm) for the inserted sequence. The calculation of melting temperature for any primer sequence will give a good starting point for determining appropriate annealing temperatures for PCR, RT-PCR, hybridization and primer extension procedures. The equations used to calculate the melting temperature depend on the sequence's length, according to Marmur and Doty (1962) and Wallace *et al.* (1979). Any number of oligonucleotide can be analyzed, by clicking 'Next' all properties of the first sequence will be stored, and another sequence can be entered etc., all entered sequences along with their properties can be saved and/or printed.

Chemicals' concentration and quantity

"Molarity" submenu facilitates users to calculate quantity of chemical (e.g. mg) using its concentration (e.g. mM) and molecular weight in a certain volume, or to calculate concentration using quantity and molecular weight, as well. A list of most common chemicals used in molecular biology and tissue culture is included. In addition, the user can write the molecular weight directly if the chemical is not provided in the list. **Fig. 3** showed the interface of molarity submenu.

DNA Extraction, concentration and purity

A submenu of DNA extraction stocks (i.e. the main stocks, extraction buffer and TE buffer) preparation is included, in which the user can easily get amounts and volumes of different buffers in a simple way. Program provides some important tips, which should be considered to avoid doing mistakes during the preparation of different solutions. It includes calculations of DNA concentration and purity using the spectrophotometer data (**Fig. 4**). DNA samples should be read, at least, at two wavelengths, 260_{nm} and 280_{nm} to get information about its concentration and purity. However, it is recommended to read, additionally, at 230, 270 and 320_{nm} (Stuling and Amberger 1994). The program provides three options to feed the data of spectrophotometer, one, two, or three readings for each sample, and the average of the replicates will be directly calculated. It gives a brief report for sample analysis (i.e. purity: either sample is free from phenols, protein, RNA, or organic compounds as well as its concentration). A sample sheet is provided, in which the results and reports can be stored, to enable the user calculating desired concentrations of DNA samples for further work.

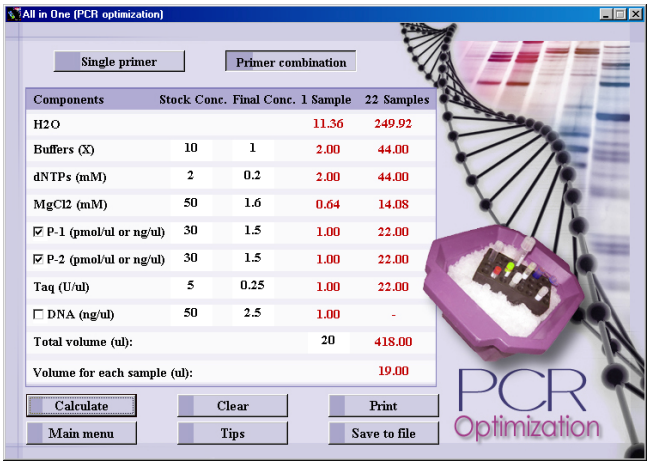

Fig. 1 PCR optimization submenu. Serves for any PCR-based molecular marker technique.

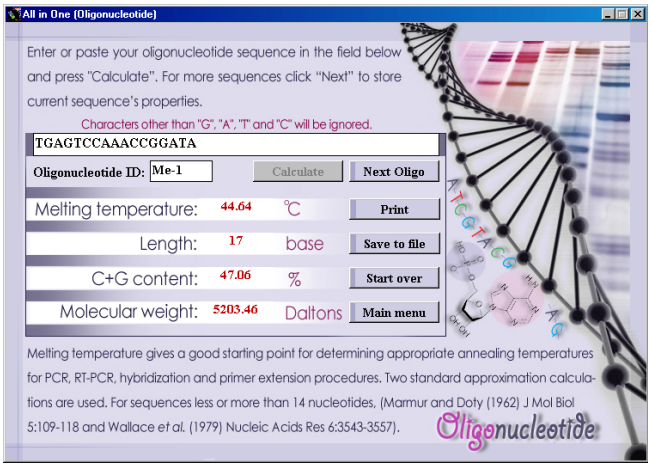

Fig. 2 Oligonucleotide submenu. Helps to get information of any oligonucleotide.

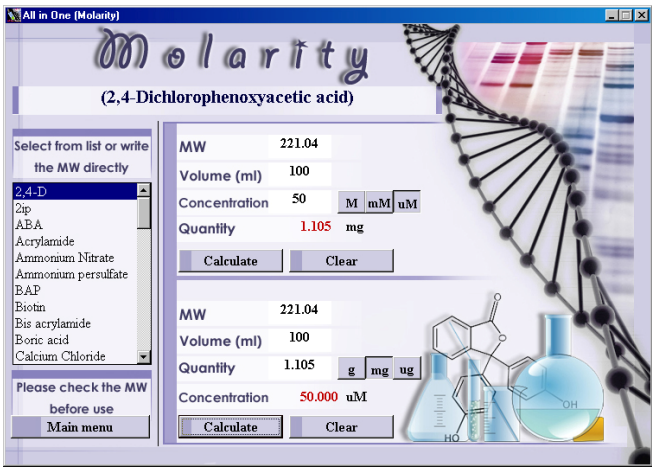

Fig. 3 Molarity submenu. For calculations of chemicals' concentration and quantity.

Primer preparation

Program offers "Primer preparation" submenu (**Fig. 5**), which enables the user to calculate primer stock dilutions and required concentrations in proper volumes using the primer's micrograms or nanomols information given on

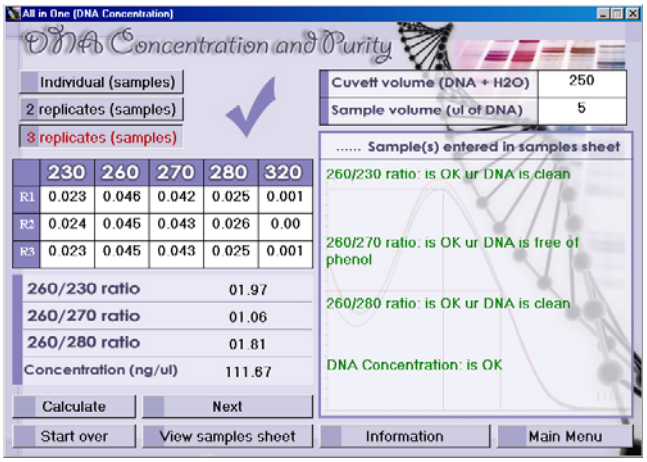

Fig. 4 DNA concentration submenu. Helps to calculate DNA concentration and purity.

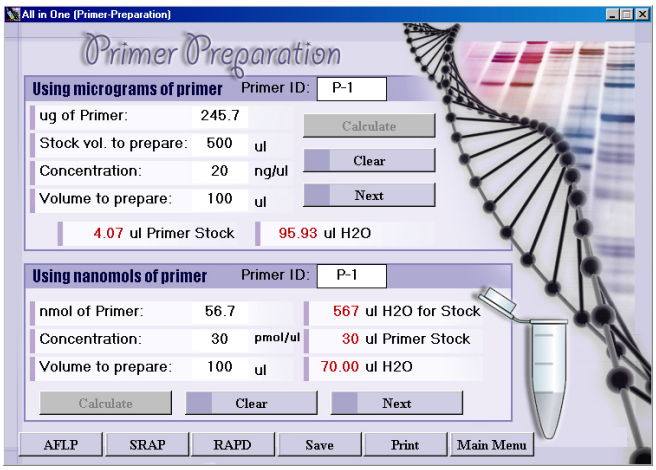

Fig. 5 Primer preparation submenu. Serves for primer preparation using its micrograms or nanomols.

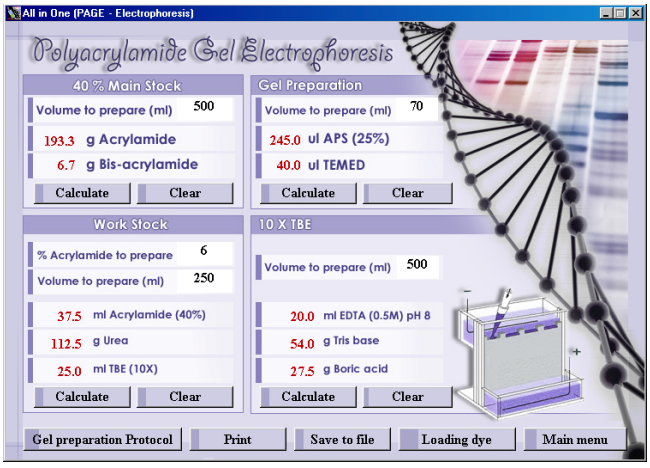

Fig. 6 Electrophoresis submenu. For stocks, solutions preparation and polyacrylamide gel electrophoresis protocol.

primer's vial. The user can make calculations of any number of primers, by clicking 'Next' all information will be stored and another primer can be entered, etc. then, all stored information can be saved or printed.

Polyacrylamide gel electrophoresis

"Electrophoresis" submenu helps the user to prepare the polyacrylamide main stock, the work stock and TBE buffer, used in polyacrylamide gel electrophoresis (**Fig. 6**). The user can determine the required concentration and a volume of acrylamide stock. Depending on gel size, user can enter the volume that he wishes to prepare, and the program will directly calculate the volume and amounts of other components. Moreover, the program provides submenu in which the user easily figure out how to calculate and prepare the loading dyes (following, CIMMYT 2005), with some useful tips.

Time manager

Khirshyat comes with an alarmed countdown timer, which can be work as a stopwatch, in which the user can select the sound file to alert him and a determined number of minutes. This timer can help managing the time during doing experiments.

PROSPECTIVE FOR FUTURE ENHANCEMENT

In near future, the program might be developed to be a comprehensive one by including more protocols and recent ones, e.g. RNA, proteins, isozymes and other recent molecular markers; and a new main menu for tissue culture protocols, including; preparation of the most common used media with different protocols (e.g. micropropagation, somatic embryogenesis, protoplast fusion, cell suspension, etc.) for important plant species. As well as, more useful illustrations and animated tutorial help might be included.

CONCLUSION

Khirshyat micro-program, described here, could be a helpful tool, offers a range of protocols that believed to be welcomed by researchers. Users, especially those who have found the program is useful, are encouraged and welcomed to send me either their comments or suggestions, which will contribute to improve the upcoming versions of this one. Finally, as a personal opinion, the idea of this micro-program can be applied in large-scale protocols covering different scientific research branches.

ACKNOWLEDGEMENTS

The author thanks the kind collaboration and constrictive ideas to Dr. Ahmed Mansour in enhancing this manuscript. Modifications of protocols used in Khirshyat 1.0 micro-program, were made and tested at Dra. Rosa M Escobedo-GM laboratory, Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán. Mérida, Yucatán, México.

REFERENCES

Assiut University web-site: http://www.aun.edu.eg/faculty_agriculture/arabic/genetics/egenactiv.htm

Bassam BJ, Caetano-Anollés G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry* **196**, 80-83

CIMMYT (2005) *Laboratory Protocols* (3rd Edn), CIMMYT Applied Molecular Genetics Laboratory, Mexico, Distrito Federal, 81 pp

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: Version II. *Plant Molecular Biology Reports* **1**, 19-21

Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in *Brassica*. *Theoretical Applied Genetics* **103**, 455-461

Marmur J, Doty P (1962) Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology* **5**, 109-118

Pereira A (2010) *Plant Reverse Genetics: Methods and Protocols*, Humana Press, New Jersey, 282 pp

Pevsner J (2009) *Bioinformatics and Functional Genomics* (2nd Edn), John Wiley and Sons, Inc., Canada, 951 pp

Raply R (2000) *The Nucleic Acid Protocols Hand Book*, Humana Press, New Jersey, 1050 pp

Setubal J, Meidanis J (1997) *Introduction to Computational Molecular Biology*, PWS Publishing Company, Boston, 269 pp

Sheehan D, Tyther R (2009) *Two-Dimensional Electrophoresis Protocols*, Springer Protocols (Vol 519), Humana Press, New Jersey, 545 pp

Stulnig TM, Amberger A (1994) Exposing contaminating phenol in nucleic

acid preparations. *BioTechniques* **16**, 403-404

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research* **23**, 4407-4414

Walker JM (2005) *Proteomics Protocols Hand Book*, Humana Press, New Jersey, 988 pp

Wallace RB, Shaffer J, Murphy RF, Bonner J, Hirose T, Itakura K (1979) Hybridization of synthetic oligodeoxyribonucleotides to phi chi 174 DNA: The effect of single base pair mismatch. *Nucleic Acids Research* **6**, 3543-3557

Weising K, Nybom H, Wolff K, Kahl G (2005) *DNA Fingerprinting in Plants: Principles, Methods and Applications* (2nd Edn), CRC Press, New York, 444 pp

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary Primers are useful as genetic markers. *Nucleic Acids Research* **18**, 6531-6535