

Automation of the Analysis Procedure of HLA PCR SSP ¹Classification

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The Challenge: To build a program easy to use that eliminates the human error and facilitates the analysis procedure of HLA PCR SSP *Classification.

The Solution: A LabVIEW program running under MS Windows that facilitates the HLA PCR SSP classification by means of many aids not available to the lab technicians when they perform this classification without using this program.

Abstract

The program described here is able to automate the analysis procedure of HLA PCR SSP classification in order to find the alleles of each individual.

Some of the functions that are performed by the instrument are:

- Analyze the image and determine its scale amplification.
 - locate the 5 rows of 24 positions where the genetic material and the reagents are deposited in the gel.
 - Locate the positions of the bands corresponding to amplifications of genetic material.
 - Determine if the intensity of each band is weak or strong.
 - Determine for some bands if its travel was long or short.
 - Search the tables that assigns a band or a combination of them to one group of alleles and display it in the front panel.
 - Display the amplified bands that are not selecting alleles classified by groups.
 - Display the additional bands necessary to complete the groups of the amplified bands that are not selecting alleles.
 - Display all the amplified bands (selected bands) classified by groups.
- and finally print a report.

Introduction

The biologic identity of a particular individual is a consequence of the specific set of genes the individual carries. However, to make a distinction among the members of the specie it is necessary to rely on the presence of genes variable enough to be certain that its possession identifies to every individual in the population. So far, the most variable (polymorphic) genetic system known in humans is the Human Leukocyte Antigen (HLA) system.

Given its power of biologic individual discrimination, HLA gene combinations have been extensively used in forensic and criminanalistic sciences, paternity identification studies, and in selection of donors for organ transplantation.

The most extended method to study the particular HLA combination a given individual carries is the molecular typing. This method is based on a powerful DNA amplification technique known as the Polymerase Chain Reaction (PCR). By this technique, a sequence specific amplification (up to a million fold) of HLA genes is obtained from minute amounts of DNA. The resulting DNA molecules are then resolved in a gel by electrophoresis, photographed and then analyzed by an experimented individual. This procedure of HLA identification relies mostly on the experience of the personnel performing it. Therefore, the automation of the analysis procedure will improve the objectivity, reliability and speed of this process.

¹ HLA - Human Leukocyte Antigen
PCR - Polymerase Chain Reaction.
SSP - Single Stranded Polymorphism.

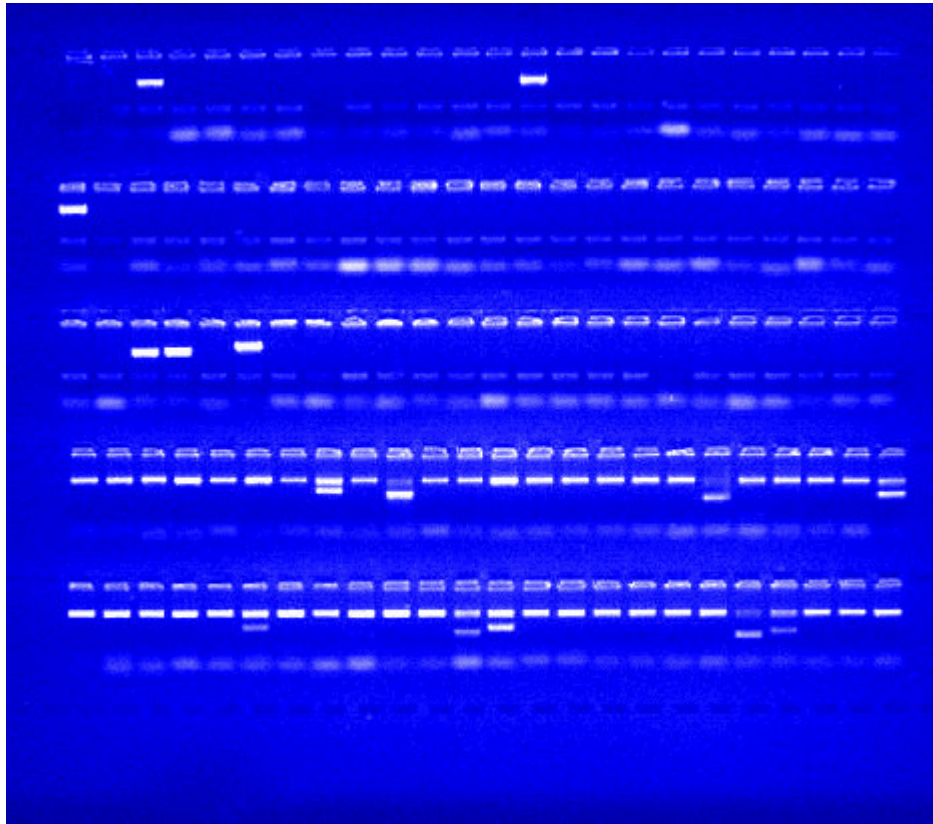


Figure 1. Gel Image

Program Description

The program begins with a front panel where the user enters the personal data of the individual, then selects the photographic image of the electrophoresic analysis. Figure 1

Once the image of the plate is in the front panel the user must mark with the mouse 7 points in the plate that are used by the program to locate the 5 rows of 24 positions where the reagents were deposited. Figure 2.

Then the program locate the amplified bands, calculates the corresponding alleles and display the alleles organized by groups (A1*, B1*, DRB1*, DQB1* y DQA1*) and coded by colors. The program also calculates and displays the amplified bands (selected bands) that are not included in the selected alleles and the groups of bands that contain the not included ones (See figure 2).

After the program calculates and signals the amplified bands by means of a yellow rectangle around them, the user check the bands and proceeds to mark with the mouse the ones that were missed by the program and unmark the ones that the program mistakenly mark.

Each time the user adds or deletes a band, the program updates all the indicators affected by this action.

Finally, the user can print a report. (see figure 3).

Advantages

The main advantage of this program is that it eliminates many errors such as:

- error in counting the position of the bands (for the B1* group, the technician has to count up to 48 positions).
- Error in mapping the alleles based on the amplified bands.

in this procedure is easy to err due to the fact that the tables for the mapping are very complex .

Additional advantages of the program are:

- Saves Time: A well trained technician takes 5 to 10 minutes to make a classification without the aid of this program and about 2 minutes using this program. For a beginner, the times are 30 and 3 minutes respectively.

The speed of the analysis is of great importance because some times the analysis is performed during a surgical operation.

- Less qualified personnel: With this program it is not necessary to have a well trained technician to do this job.

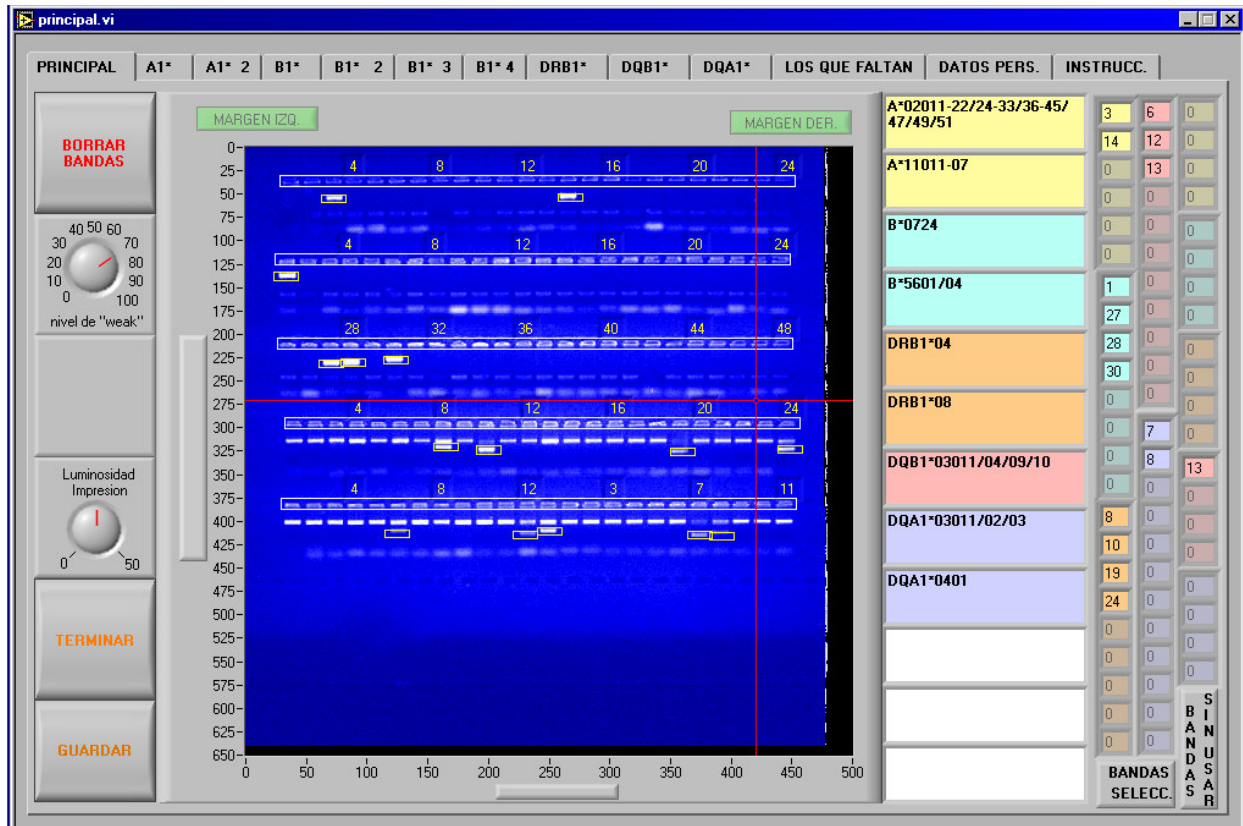


Figure 2. Main User Interface

Characteristics of the software

- Programming language: National Instruments LabVIEW 6.02.
- Operating system: Windows.
- Number of subroutines: 32 created by the author and 22 taken from LabVIEW.
- Number of hours to develop: 500.
- Front panels: 14.
- Indicators: 124.
- Controls: 21.

Concluding Remarks

This program is a valuable tool for all the labs dedicated to this HLA classification procedure because it will improve the objectivity, reliability and speed of this process.

Additional Comments

- The program is available in English and Spanish
- When the reagents are changed ,there is one table in the software that must be updated also.

Translation of terms in figure 2

PRINCIPAL = MAIN

LOS QUE FALTAN = THE MISSED ONES

DATOS PERS. = PERSONAL DATA

INSTRUCC. = INSTRUCTIONS

BORRAR BANDAS = ERASE BANDS

NIVEL DE WEAK = WEAK LEVEL

LUMINOSIDAD IMPRESIÓN = PRINTING LUMINOSITY

TERMINAR = END

GUARDAR = FILE

BANDAS SELECC. = SELECTED BANDS

BANDAS SIN USAR = UNUSED BANDS