

## ABSTRACT

Our laboratory is engaged in high-throughput gene mapping of complex traits. Due to confounding issues such as heterogeneity, phenocopies and reduced penetrance, large numbers of samples are required to obtain sufficient power to detect linkage. Processing these numbers of samples is time consuming and error prone. Integration of the Tecan Genesis 200 robotic workstation has automated our laboratory procedures. A custom software application, developed by The Technology Integration Group, was integrated with the protocols to easily overcome alterations in run parameters used to instruct the instrument. Protocols automated include DNA dilution and gridding, Polymerase chain reaction (PCR) setup and pooling of PCR products. DNA protocols with an EXCEL interface dilute DNA at various concentrations to a uniform dilution in a Beckman 96 deep-well plate. PCR reactions are prepared in a 96-or 384-well format. PCR products are pooled into a single plate for subsequent gel loading where the PCR products for one individual are run in a single gel lane. The quality of data generated by automating these procedures has been shown to be reproducible and reliable.

## SOFTWARE



Figure 1a. AGS Main Protocol Screen

The Automated Genotyping System (AGS) software is a Window NT based application that controls a Tecan Genesis 200 to automate the liquid handling for the protocols shown in Figure 1a.

A graphical user interface containing a Genesis worktable image is provided for entering protocol parameters. These parameters are entered by the user or in some protocols, are provided through a tab-delimited ASCII file. Once the parameters are entered, the worktable image is displayed for deck

layout verification, ensuring correct placement of carriers, racks, trays, plates and tubes before proceeding with protocol execution.

The AGS protocols utilize the TECAN Genesis Service and Setup software (version 3.11) and the TECAN Genesis Toolbox Dynamic Link Libraries (DLLs, version 3.11).

The TECAN Setup and Service software is used to “teach” carrier and rack positions located on the worktable and for implementing machine specific XYZ parameters. The TECAN Toolbox DLLs provide an extensive set of both high and low level functions to control the Genesis instrument. Liquid handling parameters, such as aspirate and dispense speeds, are specified in an INI file for each protocol. These parameters are read by the AGS software and are sent to the Genesis when performing liquid handling operations.

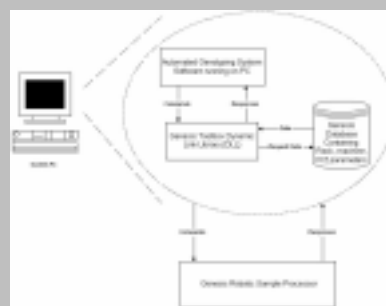


Figure 1b. Software interface between AGS software and Tecan Genesis software

## RESULTS

Figure 5. Gel image illustrates the use of multicolor fluorescent labeling of microsatellite markers and detection with an ABI 377 DNA Sequencer. For each individual the PCR products within a panel are pooled in amounts proportional to their fluorescent dye signal intensity. An internal size standard displayed in red is included in every lane. Gel image represents a panel of 15 markers (4B, 5G, 6Y) used to amplify 48 DNA samples.



Figure 5

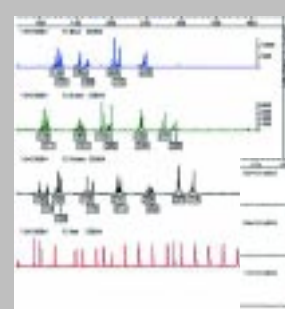


Figure 6a

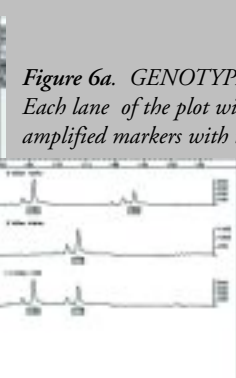


Figure 6b

Figure 6a. GENOTYPER software labels the alleles for each locus. Each lane of the plot window represents the alleles for those amplified markers with the same fluorescent dye label. The last lane of the plot window illustrates the internal lane size standard.

Figure 6b. Nuclear family illustrates the pattern of inheritance of the alleles for a marker. Output shown represents the successful automation of the genotyping process using the Tecan Genesis 200 robotic workstation and the AGS software.

# AUTOMATED GENOTYPING USING A TECAN GENESIS ROBOTIC WORKSTATION

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## MATERIALS AND METHODS

Once the biological samples have been collected and the DNA has been extracted, the process of genotyping complex traits begins with the gridding of DNA samples in a manner suitable for PCR. For each individual the amplified samples are pooled according to panels and run on ABI 377 DNA Sequencers. Gel fragments are analyzed for size. Genotypes are then reviewed in GENOTYPER prior to inheritance checking and subsequent statistical analyses.

### DNA DILUTION AND GRIDDING

The purpose of Protocol F (Figure 2) is to dilute DNA samples from tubes to a uniform concentration (10 ng/ul) in a 96-deep well tray (Beckman, Catalogue # 267007). Six 16-position strip racks are placed on the left of the work surface to hold the DNA tubes (Sarstedt, No. 72.694.006). The DNA samples are placed in the racks in the order that each DNA sample is to be transferred. To the right of the strip racks there are 4 positions available for 96-deep well trays making it possible to create as many as 4 daughter trays of DNA. An adapter plate was designed for each 96-deep well tray to allow for liquid level detection. Due to the variability of the DNA concentration between samples, the AGS software accesses a tab-delimited ASCII file which contains the following information for each DNA sample: family individual number, DNA concentration, amount of DNA for 750ul at 10 ng/ul, the amount of water to add.



Figure 2. Protocol F for making DNA grids from sample tubes.

Alternatively, Protocol L was designed to remove a subset of samples from as many as 14 existing 96-deep well trays and place them into an empty deep well tray. From the user

interface the number of DNA source plates is specified along with the associated data file for each DNA source plate. The family individual numbers are entered for those samples to be transferred. The “Calculate” button determines the total number of DNA samples to transfer.

### PCR CONFIGURATION AND PROTOCOLS

Two reservoirs are positioned to the left of the Genesis work surface. One reservoir is filled with bleach (10%) for washing the tips and the other with deionized water, if needed. Tips are washed using a protocol (18 X 150 ul in 10% bleach followed by a 100 ml flush of deionized water using the fast wash pump at the cleaning station) which was found to be sufficient for eliminating contamination that occurs when using the fixed washable tips. For the PCR Protocols and more specifically Protocol A, (Figure 3), two



Figure 3. Protocol A for PCR setup

Once the parameters are specified within the protocol, the appropriate volume of primer (5 pmol/ul of each F and R) is added to its respective tube containing the PCR reaction mixture. These tubes are then mixed (16 X 200 ul). The reaction mixtures are dispensed into the appropriate PCR plate. The DNA is then transferred to the PCR plates for amplification. DNA is amplified with the cycling conditions found at the following URL: [www.pebio.com/ab/apply/dr/lmsv2/](http://www.pebio.com/ab/apply/dr/lmsv2/). The custom software interface developed by The Technology Integration Group, LLC ([www.ttig.com](http://www.ttig.com)) was combined with the protocols to easily overcome alterations in run parameters used to instruct the instrument. Included are the parameters listed below:

- Number of strip racks
- Number of primer tubes per strip rack
- Volume of primer(s) to add to the PCR reaction mixture(s)
- Volume of AmpliTaq to add to the PCR reaction mixture(s)
- Volume of final PCR reaction mixture to add to wells in PCR plate
- Number of DNA samples to amplify
- Volume of DNA to use in the PCR reactions
- Volume of water to add to the wells in the PCR plate if diluting DNA

### Additional protocols for PCR in the AGS software include:

- Combining grids (Protocol C): DNA samples from up to four 96-deep well trays are combined into a single PCR plate for amplification. The number of DNA samples and the position of the first sample in each tray are specified.
- Duplicating grids (Protocol D): A group of DNA samples from a single 96-deep well tray are added multiple times to the PCR plate for amplification. The number of DNA samples in the group determine the number of times the DNA samples can be dispensed into a single PCR plate. Additionally, the user specifies which primers are used to amplify each group.
- 384-well (Protocols H, J and K): PCR reactions are similarly set up in a 384-well format (384-well plates, PE-Biosystems, Part # 4305505). An adapter plate was designed for the 384-well plate to allow for liquid level detection.

## CONCLUSION

- The Tecan Genesis 200 robotic workstation has enabled our laboratory to automate the genotyping process including DNA dilution and gridding, PCR setup and the pooling of PCR products.
- The AGS software, developed by the Technology Integration Group and integrated with genotyping protocols, allowed our laboratory to easily overcome alterations in run parameters used to instruct the Genesis instrument.
- Protocols established by the laboratory successfully eliminate contamination that can occur between DNA samples when using the fixed washable tips on the Genesis workstation.

18-position strip racks are positioned to the right of the reservoirs to hold individual primer tubes (Sarstedt 1.5 ml screw cap micro tube, No. 72.692) and tubes of AmpliTaq Polymerase. Nine 3-position carriers are placed to the right of the strip racks for placing the following items on the workstation: (1) Pre-made PCR reaction mixture ([www.pebio.com/ab/apply/dr/lmsv2/](http://www.pebio.com/ab/apply/dr/lmsv2/)) is placed in a rack of tubes (Costar cluster tubes, 1.2 ml, Catalogue #4410) (2) DNA samples in a 96-deep well tray (3) PCR plates (96-well plates, PE-Biosystems, Part # N801-0560).

### POOLING OF PCR PRODUCTS

Because our primers are labeled with one of three fluorescent dyes, we pool the PCR products as a panel depending on the dye colors and allele size ranges. Protocol E (Figure 4) was designed to function with two options: (1) the user interface where the parameters are entered by hand or (2) the protocol accesses an EXCEL spreadsheet for the parameter values. An empty plate is placed on the Genesis workstation in the first rack position. The PCR products will be dispensed into this plate. Next, the plates containing the PCR products for any given panel are placed on the work surface in the appropriate order. Regardless of the option chosen, the appropriate volume of PCR product is aspirated from the respective plate and

dispensed into the plate containing the pooled products. If necessary, a final volume of water is added. Finally, the samples are mixed.

Similar to Protocol E, the pooling of PCR products was adapted to the 384-well format (Protocol I). However, the volume of product taken from each 384-well PCR plate is combined into a 96-well plate.



Figure 4. Protocol E for PCR Plate Pooling