Automatic Phase Determination of Disease Haplotype from Family Genotyping Data with GeneMarker® 1.90

January 2010

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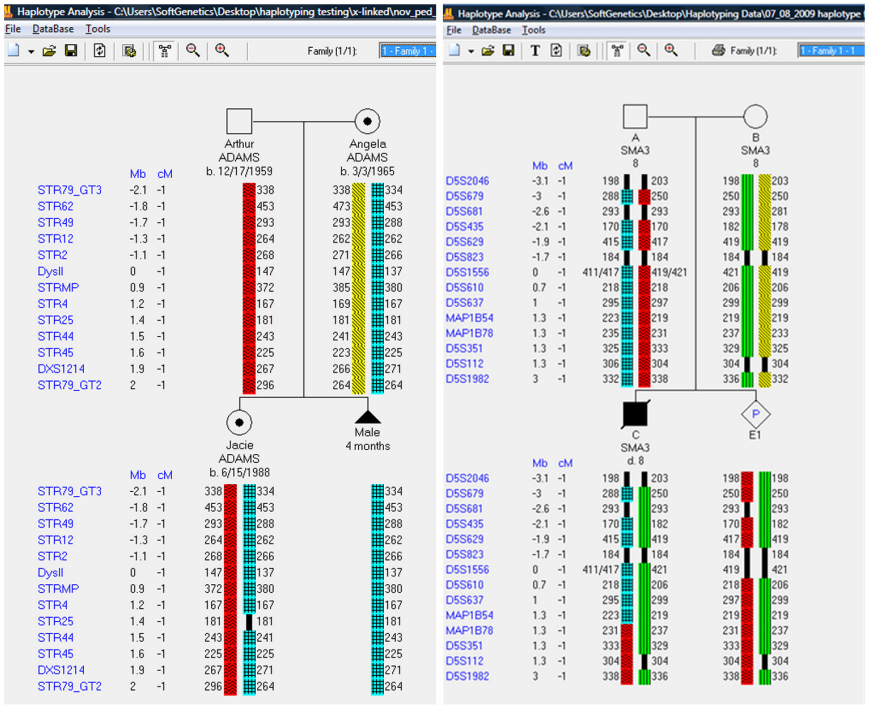
Introduction

Familial DNA fragment data is used for haplotype analysis in areas such as genetic disorder research and preimplantation studies. Commercially available kits with markers for both autosomal and X-linked traits are available for diseases such as cystic fibrosis (CF) and Duchenne muscular dystrophy (DMD). Traditionally, researchers determine the genotype of each family member. This may require using allele calls from two or more kits to obtain the complete allele call information for each person. Researches then draw a pedigree diagram and assign the phase of the alleles, where possible, based on the familial data of parents and child(ren). This manual process is time consuming and subject to error due to data transfer from genotyping software to pedigree analysis software.

In GeneMarker, the Haplotype Analysis application is directly linked to the allele call (genotyping) functionality of the software, eliminating errors. In cases where multiple kits are required to obtain complete genetic profiles, the program automatically combines the results of each person from each kit. GeneMarker Haplotype Analysis contains a 'Family Group Tool' to quickly draw family groups. The software uses the allele calls of children and parents to assign a first-order-approximation phase of the alleles, deducing the haploytpe of the children from the familial data. Whenever the alleles are informative for phase assignment a pattern/color bar is assigned to indicate most probable phase.

Results (figure 1) X-Linked and Autosomal Linkage Examples

X-linked Example:
Personal information
Phase assignment
Carrier status
Standard symbols for male, female, Miscarriage (SAB)



Autosomal linkage Example:
Phase assignment
Cross-over in child 1
Standard symbols for male, female, deceased, pregnancy

Features of GeneMarker's Haplotype Analysis Application

1. Follows Bennett et al. nomenclature for pedigrees
2. X-linked and autosomal pedigree formats based on parent/child(ren) allele calls
3. Edit family and individual information
4. Displays markers, allele calls, personal information in pedigree
5. Control ordering of markers in pedigree by customizing panels
6. Automatically makes first order phase assignment
7. Edit capability for reassigning phase or edit crossover
8. Save pedigree and re-open for adding data and editing
9. Allele conflicts flagged; detects potential uniparental disomy

Procedure

1. Open Data to import data files (.fsa, .rsd, .esd, .scf)
2. Tools →Panel Editor to input Mb and cM. (physical and genetic distances)
3. Run Wizard - set analysis parameters - Run
4. Linked navigation between electropherogram and allele report to verify allele calls
5. Save Allele Report as .txt file
6. Repeat steps 1-5 if a second kit or panel is required for a complete genetic profile of the individuals
7. Applications drop down menu, select Haplotype Analysis
8. Tools or parameter icon to select Haplotype Analysis Settings 
9. Select File to draw a new pedigree or import a previously saved pedigree

Discussion

GeneMarker provides accurate, biologist friendly genotyping with a linked haplotype analysis application. Automating the data transfer from the genotyping analysis to the haplotyping application eliminates the need to use two separate programs. The phase assignment greatly improves efficiency by automating the repetitive steps traditionally manual steps.

The program combines allele calls from multiple kits for each person (figure 2) and automatically makes first order phase assignment based on parent and child(ren) allele calls (figure 1). Edits to individual's information (figure 3), phase assignment, and cross-over (figure 4) are made using straight forward, icon driven selections with the computer's mouse.

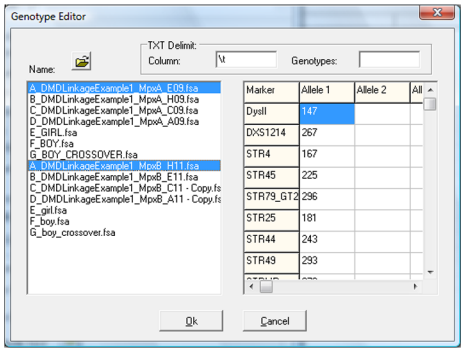


Figure 2. Genotype editor is used to combine allele reports from multiple analyses for each individual; providing a complete genetic profile.

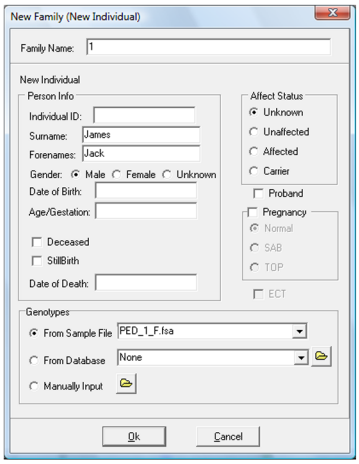


Figure 3. Provides easy entry and edits to information for each individual.

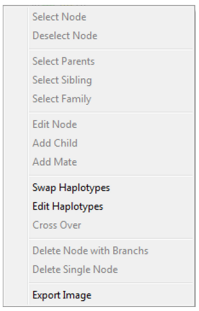


Figure 4. Right click on the pedigree node or haplobar activates a pop-up menu of edit options

GeneMarker has been designed to provide genetic researchers with a biologist friendly genotyping tool; with unique pattern recognition and sizing technology providing >99% accuracy, easy linked navigation, user management control and tracking, exportable LIMS reports, bulk printing capabilities, instrument compatibility with ABI®, MegaBACE™ and Beckman Coulter™. Unique post-genotyping applications in GeneMarker of interest to clinical researchers include Haplotype determination, Trisomy or Aneuploidy analysis, Microsatellite Instability (MSI), Loss of Heterozygosity (LOH) and MLPA® (Multiplex Ligation-dependent Probe Amplification), MS-MLPA.

Acknowledgements

We would like to thank Dr. Pamela Renwick and Dr. Michael Yao, Centre for Preimplantation Genetic Diagnosis, Guys' Hospital, London for their collaboration in development of this application.

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