

# Microsatellite Analysis with Linked Pedigree Tool

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## Introduction

Microsatellites or variable tandem repeats (VTRs) are successful markers used for various types of genotyping. Microsatellites are short stretches of repeated DNA found in most genomes that are highly polymorphic in humans and most other species. This variability has made microsatellites a popular genetic marker for genotyping applications such as medical genetics, forensics, genetic mapping, and human and plant population studies.

Microsatellites were originally employed for genetic mapping but now are widely used in oncology research. The polymorphic nature of microsatellites make them useful in linkage studies which attempt to locate genes responsible for various genetic disorders.

Microsatellites also have some advantages, which make them popular markers for research. They are found in large numbers and are relatively evenly spaced throughout the genome. Due to their small size of 2-6 base pairs, microsatellites can be analyzed using polymerase chain reaction (PCR) and accurately sized using electrophoresis systems.

GeneMarker is a unique genotyping tool as it is compatible with files from all major capillary and slab gel electrophoresis systems including ABI files (\*.FSA, \*.AB1, \*.ABI), SCF files, MegaBace files (\*.RSD, \*.ESD), SpectruMedix files (\*.SMD, \*.SMR), Beckman files, and Licor files. GeneMarker is a replacement for such software packages as SAGA from LI-COR, TrueAllele from Cybergenetics, GeneMapper, Genotyper, and GeneScan from Applied Biosystems. GeneMarker can perform fragment analysis on four or five color data sets from any slab gel or capillary electrophoresis system. Additionally, this software automatically corrects for most instrument and chemistry errors, saving significant analysis time and cost.

## Procedure

### Automated Correction

GeneMarker decreases analysis set-up time through automated correction of common genotyping problems including saturated peaks, noisy data, wavelength bleeding, instrument spikes and stutter peaks. GeneMarker's automated Run Wizard is designed to make analysis quick, easy, and accurate. The Data Analysis window features include:

1. **Saturation Correction:** Analysis of saturated data points by creating a synthetic peak based upon the peak shape before and after saturation.
2. **Baseline Subtraction:** The software removes the baseline so that the Y axis is above the noise level.
3. **Pull-up Correction:** This function removes peaks caused by wavelength bleeding.
4. **Spike Correction:** The software automatically removes peaks from voltage spikes caused by micro- air bubbles or debris in the laser path.
5. **Stutter Peak Correction:** The software automatically filters for stutter peaks caused by PCR slippage.

### Analysis Parameters

GeneMarker has flexible settings to allow user manipulation. In order to produce the most accurate results, with low levels of false positives and negatives, the following settings are recommended for fragment analysis.

1. **Analysis Type:** Fragment
2. **Peak Detection Threshold:** Intensity > 100; Percentage > 5 %Max; Local Region > 25 % Local Max.
3. **Stutter Peak Filter[%]:** Left: 90 Right: 30.
4. **Allele Evaluation: Score:** Reject < 1 Check 10 < Pass

### Pedigree

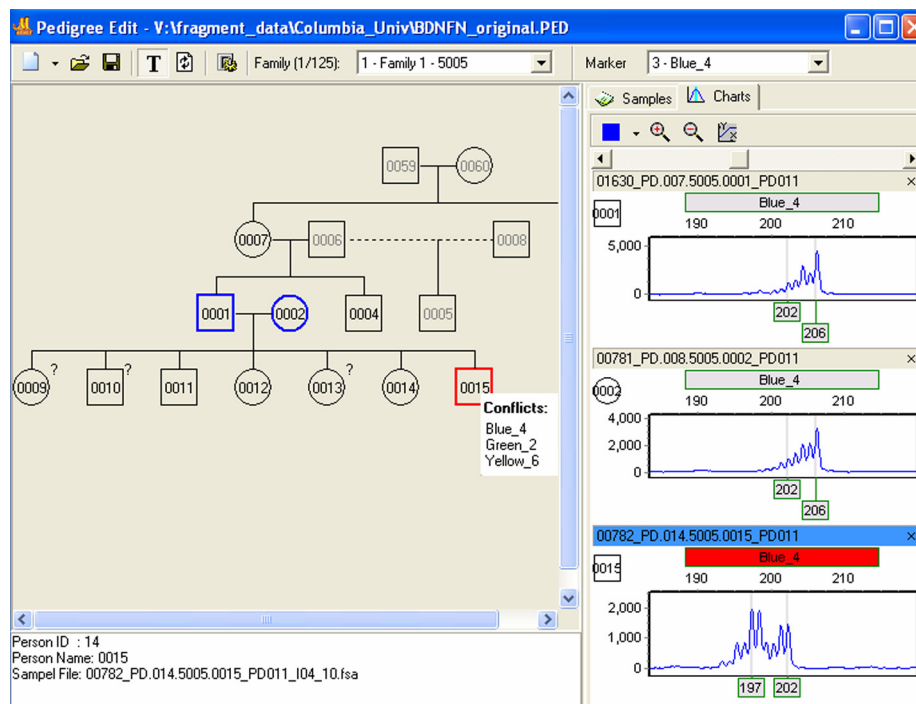
The user can either open an existing pedigree file of type ped or pre or create a new pedigree file in GeneMarker's pedigree tool. The pedigree chart is designed to aid identification of inheritance patterns and abnormalities. All individuals in the pedigree with sample files are directly linked to the corresponding electropherograms with a mouse click, and individuals with illogical or abnormal allele calls are highlighted in red. The link between the pedigree and the electropherograms displaying allele calls for each marker make analysis quick and efficient.

**Existing Pedigree File:** After running sample files in GeneMarker with a size standard and panel, the user must link the sample files to the pedigree file. GeneMarker has incorporated a tool for this function named "Pedigree Filename Match" in the Tools menu. It is necessary to enter the correct character numbers designating family and individual identifiers. In the example, 01\_PD\_5005\_0001\_A01\_07.fsa, the family identifier 5005 would be from 7 to 10 and the individual identifier 0001 from 12 to 15. After linking the files, open the pedigree file in Applications: Family Tree. Load the original pedigree file in the top panel and the linked .SMP file in the bottom panel.

**New Pedigree File:** After running samples in GeneMarker go to Applications: Family Tree and click the New Pedigree File icon located in the toolbar. The user may then create a new multigenerational family. After adding the first individual, it is then possible to add mates, siblings, and children by right clicking on the individual node.

## Results

In the example shown below for Family 5005, there is an inheritance conflict for individual 0015 in marker Blue 3. Allele 197 is called in individual 0015 but not in either parent (0001, 0002). Based upon the results, there are also problems in markers Green2, 3 and Yellow 5 for this individual as indicated in red.



**Figure 1.** Pedigree chart showing inheritance conflicts in Family 5005.

## Discussion

The reasoning behind these conflicts shown in the results may be due to instrument or paternity identification errors. The pedigree chart makes analysis quick by identifying problematic marker regions in red. The user may add, delete or change alleles in the electropherogram by right clicking at the allele location.

The integration of the pedigree tool into genotyping software is very beneficial to track the inheritance of alleles over generations, especially in known genetic disease regions. The identification, presence and/or absence, of alleles is a crucial initial step in the study of disease inheritance patterns within families. GeneMarker's pedigree is a useful tool for the identification and genetic tracking of alleles spanning human chromosomes.

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