SoftGenetics Application Note November, 2006

GeneMarker® Software for Trisomy Analysis

Introduction

Full trisomy of an individual occurs due to non-disjunction during meiosis I or meiosis II of gametogenesis resulting in 24 vice 23 chromosomes in a reproductive cell (sperm or egg) (**1**). Thus, after fertilization, the resulting fetus has 47 chromosomes versus the typical 46. The most common forms of trisomy involve trisomy of chromosome 21 which results in Down Syndrome and trisomy of chromosome 18 which results in Edwards Syndrome. In rare cases, a fetus with trisomy of chromosome 13 can survive. Trisomy 13 is called Patau Syndrome. In all cases there are severe congenital abnormalities and mental retardation associated with the trisomy condition. Often, life expectancy is shortened in trisomy individuals.

Several prenatal tests can be performed on an expectant mother to detect aneuploidy in the fetus. Cells from the fetus' amniotic fluid are collected and analyzed by one of several techniques which include Fluorescence In Situ Hybridization (FISH) karyotyping (**2**), Quantitative Polymerase Chain Reaction (PCR) of Short Tandem Repeats (**3**), Quantitative Fluorescence PCR (QF-PCR) (**4**), Quantitative Real-time PCR (RT-PCR) dosage analysis (**5**), Quantitative Mass Spectrometry of Single Nucleotide Polymorphisms (**6**), and Comparative Genomic Hybridization (CGH) (**7**). GeneMarker is a software tool used in clinical diagnostics and research laboratories across the world to analyze DNA fragments. GeneMarker's new Trisomy Detection function aids clinicians and researchers in analyzing PCR products for aneuploidy cells.

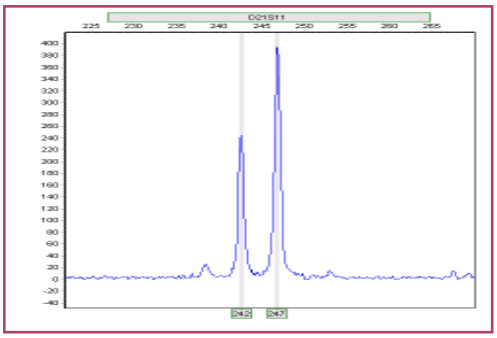
GeneMarker is a robust software for analyzing DNA fragment labeled with MegaBACE™ dyes (Amersham), Big Dye® (Applied Biosystems Inc.) or Beckman dyes from a variety of instrument platforms: ABI Prism DNA Analyzers , Amersham instruments or Beckman instruments. GeneMarker is compatible with files from 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 Li-Cor files.

Procedure

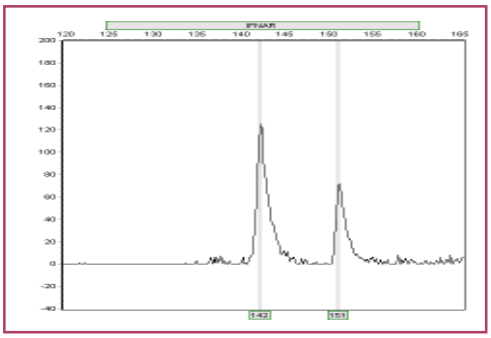
1. Open GeneMarker and upload raw data files
2. Select a Panel, Size Standard and Fragment (Animal) Analysis
3. Use the default settings for Fragment (Animal) Analysis
4. In the *Applications* menu, select *Trisomy Analysis*
5. The *Trisomy Analysis Settings* box appears
6. Adjust settings and click **OK**
7. The *Trisomy Analysis* window appears
8. Click **Print** icon in main toolbar
9. The *Print Preview* window appears with the Trisomy Analysis report
10. Click the **Print** icon to send the report to the printer

Results

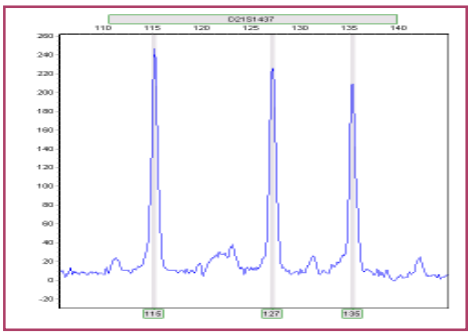
GeneMarker has been designed to accurately detect trisomies using short tandem repeat markers derived from PCR DNA fragments. Trisomy individuals will either show three fragments of equal intensity or two fragments at a 2:1 or 1:2 ratio.



Ratio 1:2



Ratio 2:1



Ratio 1:1:1

The 2:1 Ratio Trisomy

When DNA fragments are run through a PCR reaction, the smaller fragments are preferentially amplified. Electrophoresis injection also holds a bias toward smaller fragments. Subsequently, the smaller fragments' peak intensity in an electropherogram will be higher than the larger fragments in the sample. This is called preferential amplification and it is important in trisomy detection; especially, allele ratios that are 2:1. The 2:1 allele ratio occurs when the individual has 2 alleles in the first position and 1 allele in the second position in a marker. On an electropherogram, the second peak is less intense than the first peak. So the question arises; is this 2:1 imbalance real or is it due to preferential amplification of the first allele?

The GeneMarker Trisomy tool offers two answers to the 2:1 trisomy detection question.
First, in the Ratio Plot in the bottom left corner of the analysis window, the peak intensity ratio of all markers are plotted. A linear regression line is run through the center of the data points and is used to correct for intensity drop due to fragment size increase. The Ratio Plot can be viewed as a linear regression plot or corrected for slope. This method of data correction aids in the detection of 2:1 ratio trisomies.

The second aid in trisomy determination is the trisomy score. First a t-value is determined and defined as the difference between the sample and the expected value divided by the standard deviation. There are two possible t-values for every marker, one is the t-value for heterozygote and the second is for a trisomy. T-Score is the ratio of the heterozygote t-value divided by the trisomy t-value. Therefore, as the T-Score increases, the confidence of the trisomy call also increases. A T-Score greater than 5.0 is a confident trisomy call. A T-Score less than 0.3 indicates a confident heterozygous call.

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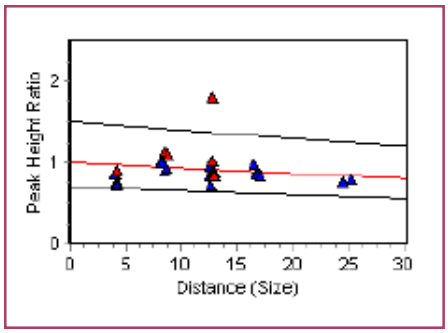
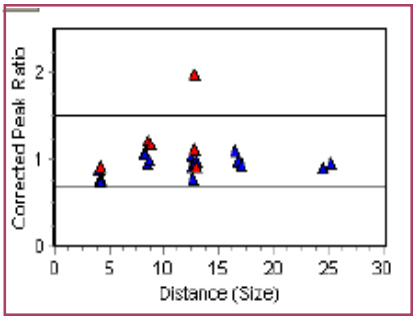
Raw Data Analysis





Corrected Data Analysis

Discussion

GeneMarker Trisomy Analysis function allows users to quickly and accurately detect three types of trisomy in an individual: 1:2, 2:1, and 1:1:1. When problems are encountered during analysis, the source could be one of three things, problems with PCR, capillary electrophoresis effects, and individual variation. Examples of problems with PCR include stutter, incomplete adenylation, and allelic drop-out. Examples of capillary electrophoresis effects include dye blobs, saturation, spikes, and bubbles. GeneMarker automatically corrects for many common PCR and CE problems - instrument spike, color pull-up, peak pull-up, noisy data, saturated peaks and stutter peaks - saving significant analysis time and cost.

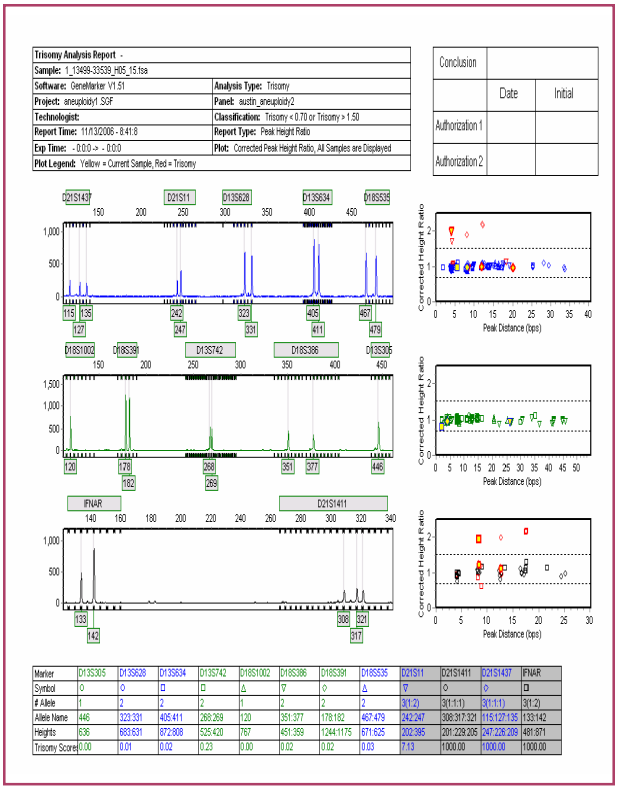
More challenging for the researcher to determine trisomy are aberrations caused by variation within an individual's own cells. Examples of individual variation include mixed samples, mosaicism, and triallelic homozygotes.

MCC

Pregnant women over the age of 35 are screened for trisomy caused syndromes in the fetus because the risk of these syndromes increases as the mother ages. During the procedure whereby cells are collected for analysis, maternal cells can be mixed in with fetal cells. This phenomenon is called Maternal Cell Contamination (MCC). MCC can be recognized when extra alleles appear and by examining inconclusive dosage ratios. When there are three alleles at a locus, the intensity of all three should be essentially equal. An imbalance is said to exist when the intensity ratio between the highest and lowest peak is greater than 60:40. The GeneMarker Trisomy tool identifies loci that contain imbalanced peaks. The user can define, within the settings parameters, the imbalance ratio that is significant for their data.

Mosaicism

Trisomy may not necessarily be present in all cells in an individual. It may be detected in just a specific tissue or within different cells in a tissue. When the presence of chromosomal abnormalities occurs differentially within an individual, it is called chromosomal mosaicism. In general, as we would expect, individuals who are mosaic for a chromosome change tend to have a less severe form of the syndrome present than full trisomy individuals. Critical examples of mosaicism are found in leukemia cases, specifically; chronic lymphocytic leukemia (CLL) which is a trisomy of chromosome 12 and acute myeloid leukemia (AML) prognosis which is a trisomy of chromosome 8. Comparison of normal versus trisomy cells from a single individual is possible with the GeneMarker Trisomy function.



GeneMarker Trisomy Patient Report:
User-definable header with sample traces and report table displayed. Ratio plot represents all samples for comparison.

Triallelic Homozygote

It is possible that an individual with three chromosomes could potentially have the same allele on all three chromosomes. In this instance, the electropherogram trace for this allele would theoretically depict a peak three times the height of a peak with just one allele. Since there is only one allele present in the marker and no other allele for intensity comparison, the analyst must use their own knowledge and experience to determine if the individual is a triallelic homozygote.

Acknowledgment

We would like to thank Austin Diamond at Newcastle University upon Tyne, UK for his collaboration on the development of GeneMarker's Trisomy Detection feature.

References

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