

Aneuploidy Analysis with GeneMarker®

Teresa Snyder-Leiby, David Hulce, Haiguo He, Wan Ning, Rensheng Qi, Jonathan CS Liu

Introduction

Aneuploidy occurs from non-disjunction during meiosis I or meiosis II during gametogenesis and results in an abnormal number of chromosomes in the gametes. Monosomy occurs when one gamete is missing one of the chromosomes; with trisomy, one of the two gametes joined in fertilization contains an extra copy of one of the chromosomes. The most common forms of autosomal trisomy are 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, Patau syndrome, can survive. Autosomal trisomy is frequently associated with severe congenital abnormalities, mental retardation and shortened life expectancy. Aneuploidy of sex chromosomes can also occur: The presence of extra X chromosome(s) causes Klinefelter syndrome in males and Triple X syndrome in females; while monosomy X (45, X) gives rise to females with Turner syndrome.¹ Several prenatal tests can be performed to detect aneuploidy in the fetus. Fetal cells from the amniotic fluid or chorionic villi are collected and analyzed by one of several techniques, many of which involve DNA fragment analysis: Quantitative Polymerase Chain Reaction (PCR) of Short Tandem Repeats (STR)², Quantitative Fluorescence PCR (QF-PCR)³, Quantitative Real-time PCR (RT-PCR) dosage analysis⁴.

GeneMarker is a software tool used in clinical diagnostics and research laboratories across the world to analyze DNA fragments. GeneMarker's Trisomy Detection function aids clinicians and researchers in analyzing QF-PCR products to detect aneuploidy. The software features new reporting options that follow best practice guidelines⁵ including: use of peak height or peak area ratios, and identification of peak ratios that are consistent with designated triallelic (trisomy) or intermediate (inconclusive) ranges. GeneMarker facilitates accurate, rapid analysis of data generated by major QF-PCR kits such as: Aneufast™, Devyser™ and Elucigene™ and is compatible with genetic analyzers: ABI (Life Technologies), MegaBACE® (GE Healthcare), and CEQ (Beckman-Coulter).

Procedure

1. Import data files (.fsa, .rsd, .esd, .scf)
2. Select the Run icon to launch the Run Wizard and review allele calls (figure 1)
3. Select Applications → Trisomy Analysis
4. Analysis Settings → Opens Trisomy Analysis Screen (figure 2)
5. Preview Options → Preview Patient Report (figure 3)
6. Print Preview or Print Patient Report Directly

Results

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. (Figures 2 and 3) Patient report format is flexible and the header box is populated automatically from parameter settings and user management information.

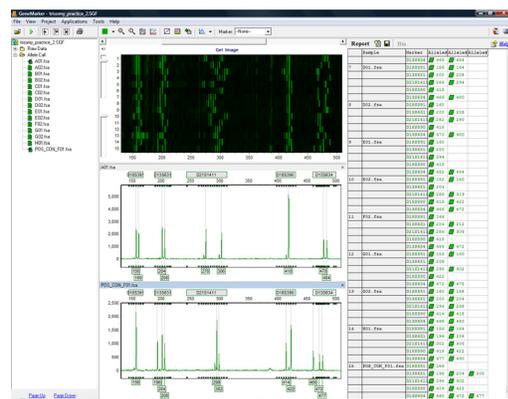


Figure 1: The main analysis screen displays sized data and allele calls. User friendly linked navigation between allele report, electropherogram and synthetic gel image aids in data review.

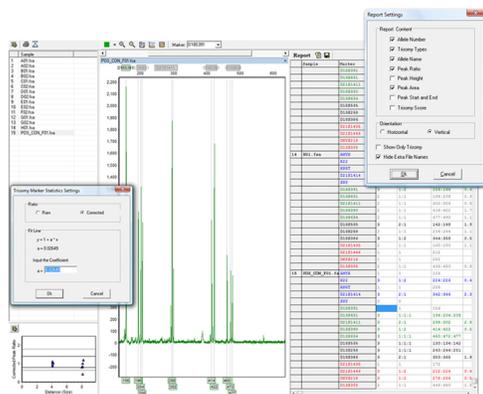


Figure 2: The Trisomy Analysis Screen allele report has linked navigation between the electropherogram and allele report. Dialog boxes allow quick customization of Report Settings and Marker Statistic Settings.

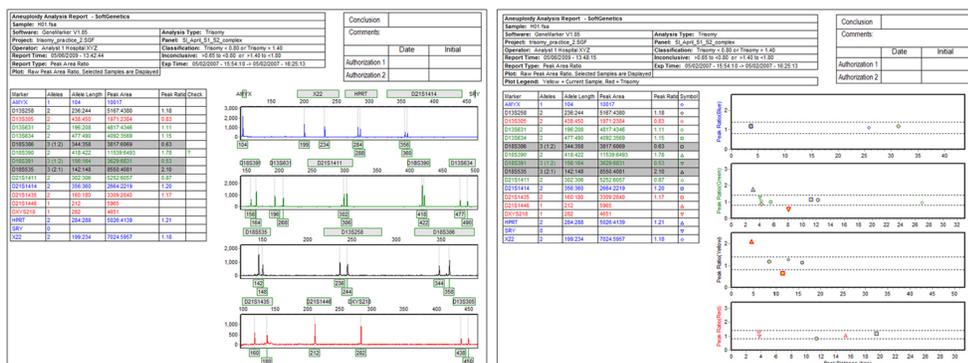


Figure 3: Both pages of the patient report contain a Header with report identification and parameters and a Conclusion/Authorization box. Highlighted files have peak ratios consistent with trisomy. Files marked with a ? have peak ratios consistent with the inconclusive range specified in the parameter settings. The patient report format has an option to display Ratio plots (for the sample or the population) on the second page.

Discussion

Differentiating between preferential amplification and 1:2 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?

To resolve the question of preferential amplification, GeneMarker Trisomy Analysis uses linear regression data correction to aid in detection of 2:1 trisomy. The user has the flexibility to select this option if deemed necessary for the analysis. 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.

Compatibility

GeneMarker is robust software to analyze DNA fragment data - labeled with MegaBACE™ dyes (Amersham), Big Dye® (Applied Biosystems Inc.) or Beckman dyes - from a variety of platforms: ABI DNA Analyzer or Genetic Analyzer, Amersham instruments or Beckman instruments. GeneMarker is compatible with files from all major capillary and slab gel electrophoresis systems including ABI files (*.FSA), *.SCF files, MegaBACE® files (*.RSD), Beckman files (*.ESD) and Li-Cor files. GeneMarker is compatible with major trisomy and aneuploidy kits, including: Aneufast™ QF-PCR, Devyser™ QF-PCR and Eulicigene™ QST*R.

The flexible reporting format allows laboratories to choose among a variety of analysis types and parameters; highlighting samples with peak ratios consistent with trisomy ranges and flag peak ratios that are within the inconclusive ratio range, user management to secure user access and report analyst name/affiliation, choice of peak height or peak area ratios, linear correction of data, display of ratio plots for population or individual sample and a Conclusion/Authorization table.

Acknowledgments

We would like to thank Austin Diamond at Newcastle University upon Tyne, UK for his collaboration; Vincenzo Cirigliano, Genomed Ltd, Kent, UK; Ulf Klangby, Devyser AB, Stockholm, Sweden; and Mike Webb, GenProbe Inc, Abingdon, UK for suggestions and helpful discussion during development of this application.

References

1. Weaver BA, Silk AD, Cleveland DW. 2006. Cell biology: nondisjunction, aneuploidy and tetraploidy. *Nature*. 442(7104) (E9-10).
2. Mansfield ES. 1993. Diagnosis of Down syndrome and other aneuploidies using quantitative polymerase chain reaction and small tandem repeat polymorphisms. *Human Molecular Genetics*. 2(43-50).
3. Ochshorn Y, Bar-Shira A, Jonish A, Yaron Y. 2006. Rapid prenatal diagnosis of aneuploidy for chromosomes 21, 18, 13, and X by quantitative fluorescence polymerase chain reaction. *Fetal Diagnosis and Therapy*. 21(326-331).
4. Tsujie T, Takemura M, Kimura T, Shimoya K, Tsutsui T, Ogita K, Ozaki M, Murata Y. 2006. Rapid detection of trisomy 21 by gene dosage analysis using quantitative real-time polymerase chain reaction. *The Journal of Obstetrics and Gynaecology Research*. 32(368-372).
5. Hamilton, S, and K. Mann. 2007. QF-PCR for the diagnosis of aneuploidy Best Practice Guidelines v2.01. Association for Clinical Cytogenetics.

Trademarks are property of their respective owners.