

Uniparental disomy (UPD) analysis of chromosome 15

Applied Biosystems® 3500xL Genetic Analyzer

Introduction

Researchers at the Laboratory of Medical Genetics in the Department of Biopathology and Imaging Diagnostics, Rome, Italy (led by Professor G. Novelli and supervised by Dr. Emiliano Giardina) are focused on three main areas: molecular diagnostics, research, and forensic analysis. The diagnostics unit includes prenatal and postnatal genetic analysis, as well as genetic screening of frequent mutations in healthy individuals and pharmacogenomic profiling. The main focus of the research unit is the genetic dissection of multifactorial diseases such as psoriasis, age-related macular degeneration (AMD), and atopic eczema. The forensic analysis unit performs paternity tests, DNA profiling, and genetic tests for forensic purposes. This article describes research performed by Dr. Giardina and team using a short tandem repeat PCR (STR-PCR) assay for uniparental disomy (UPD) on the Applied Biosystems® 3500xL Genetic Analyzer with gDNA samples isolated from peripheral blood.



Pictured from left to right: Dr. Stefania Zampatti, Dr. Ilenia Pietrangeli, Dr. Tiziana Lepre, Dr. Raffaella Cascella, Stefano Persico, Dr. Emiliano Giardina (supervisor), Michele Ragazzo, Dr. Laura Stocchi, Dr. Fabiana Taglia, Dr. Cristina Peconi, and Ilaria Parisi.

In human cells, autosomal chromosome pairs are usually inherited from both parents—one chromosome from the mother, and one from the father. In contrast to normal biparental inheritance, UPD occurs when an individual receives two copies of a chromosome, or part of a chromosome, from one parent, and none from the other parent. One of the problems associated with UPD is aberrant gene expression caused by genomic imprinting from the parent's chromosomes. In a deviation from classical Mendelian

genetics, there are some imprinted genes that are expressed from only one parental allele, either maternal or paternal, depending on the gene.

One method used to determine the presence or absence of UPD for the maternal or paternal chromosome is analysis of the genomic DNA (gDNA) from the proband (the first affected family member who seeks medical

attention for a genetic disorder) and from both parents by multiplexed PCR analysis of microsatellites or short tandem repeats (STRs) with fluorescently labeled primers. Laboratories that routinely perform these types of analyses demand high-throughput, efficient, and highly automated solutions. Here we demonstrate the accuracy, ease of use, and throughput capabilities of the Applied Biosystems® 3500xL Genetic Analyzer and Applied Biosystems® GeneMapper® Software (version 4.1) for the analysis of chromosome 15 in UPD research.

The STR-PCR assay for UPD of chromosome 15

To evaluate an STR-PCR assay for UPD research on the Applied Biosystems® 3500 Series Genetic Analyzers, gDNA samples isolated from peripheral blood were assessed by amplifying microsatellite markers on chromosome 15. Microsatellites are polymorphic DNA loci that contain a repeat sequence of 2–7 nucleotides. The ability to choose from such a large selection of highly informative markers has made microsatellite analysis a widely accepted tool for linkage mapping studies, association studies, and the identification of organisms. One of the most common problems encountered when using microsatellite analysis is poor or nonspecific amplification. Microsatellite analysis projects can necessitate the interrogation of hundreds of loci per sample before a robust and informative locus is validated for routine analysis. Managing large numbers of reactions requires efficient primer design and robust universal reaction conditions, which minimize the need for costly reruns caused by failed PCR reactions.

Two multiplex assays containing 4 microsatellite markers were developed by Dr. Emiliano Giardina at the University of Rome “Tor Vergata” in Italy [1]. The first assay contains

markers D15S1007 (NED™), D15S205 (FAM™), D15S1019 (NED™), and D15S130 (FAM™), and the second assay contains markers D15S988 (FAM™), D15S979 (FAM™), D15S128 (NED™), and D15S131 (VIC®). Both assays were employed for these experiments.

STR markers on chromosome 15 were selected based on the expected heterozygosity from the literature and through typing 100 unrelated healthy individuals [1]. To confirm the presence of UPD, at least two fully informative markers showing the absence of transmission of maternal or paternal chromosome need to be observed. A marker is defined as informative when it unambiguously discriminates maternal from paternal chromosomes.

Validation of the STR-PCR assay for UPD on the Applied Biosystems® 3500xL Genetic Analyzer

Following the completion of PCR using the True Allele PCR Premix, aliquots of the resulting dye-labeled amplicons were combined with the GeneScan™ 600 LIZ® Size Standard v2.0. Samples were electrophoresed on the 3500xL Genetic Analyzer using a 50 cm capillary array and Applied Biosystems® 3500 POP-7™ Polymer. The instrument protocol used was the FragmentAnalysis50_POP7 run module with the new peak height normalization feature enabled.

Samples from 17 nuclear families consisting of father, mother, and child were tested for presence or absence of UPD in the child using both multiplex assay mixes. Cases included 16 normal samples and 1 UPD15-positive sample.

Shown in Figures 1 and 2 are electropherograms indicating maternal UPD in a sample as confirmed by 4 different loci for multiplex assay 1, and 3 different loci for multiplex assay 2.

Figure 2 also illustrates uninformative locus D15S988 for multiplex assay 2. A sample demonstrating normal biparental inheritance is shown in Figure 3.

Reverse primer chemistry results in consistent allele calls

One artifact of PCR amplification is the “plus A” peak, which results from nontemplated A nucleotide additions. Plus A artifacts increase the complexity of the peak pattern, making it more difficult to recognize true allele peaks. Reaction conditions can greatly impact these locus-dependent artifacts. Plus A artifacts occur when the polymerase copying a DNA strand adds an additional base (plus A) at the end of the sequence. The percentage of plus A added (0–100%) depends on the last 7 bases of the PCR product. To analyze the result, the plus A peak must be higher than the allele peak. Ambiguity in allele calling can result when the allele and allele plus A peaks are of near-equal height (Figure 4), which occurs for approximately 5–10% of markers.

The patented reverse-primer tailing chemistry of the Custom Tailed Primer Pair improves allele-calling efficiency by eliminating the problems associated with nontemplated nucleotide addition. Primer tailing works by controlling the sequence context at the point where the polymerase binds to the end of double-stranded DNA and adds the nontemplated nucleotide. The tailed reverse primer contains a sequence of 7 bases that generates close to 100% of plus A.

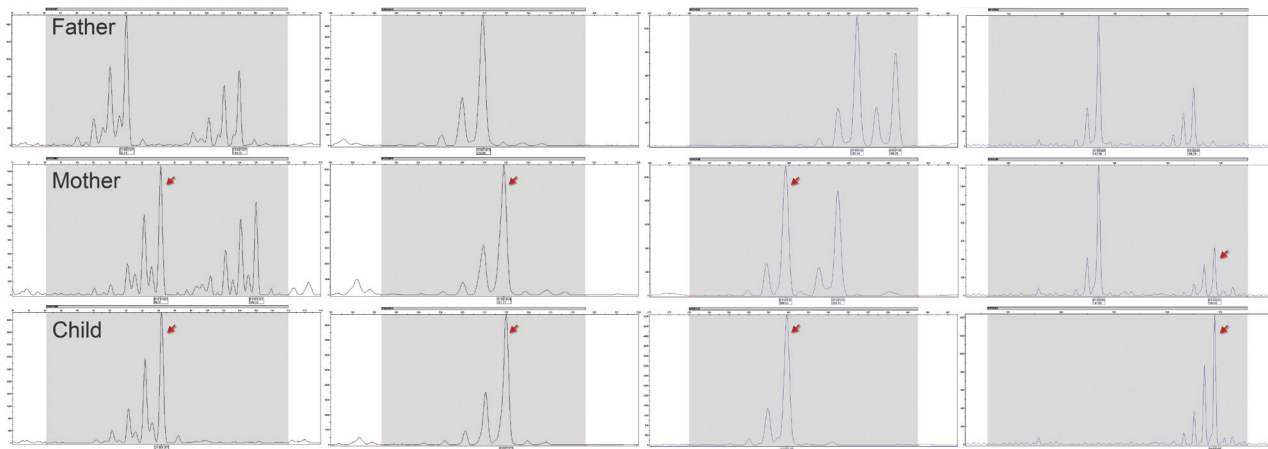


Figure 1. Electropherograms illustrating a positive maternal UPD15 sample using multiplex assay 1. Left to right, markers D15S1007 (NED®), D15S205 (FAM®), D15S1019 (NED®), and D15S130 (FAM®). Note that for each marker the child inherited one allele from the mother (red arrows), but no alleles from the father.

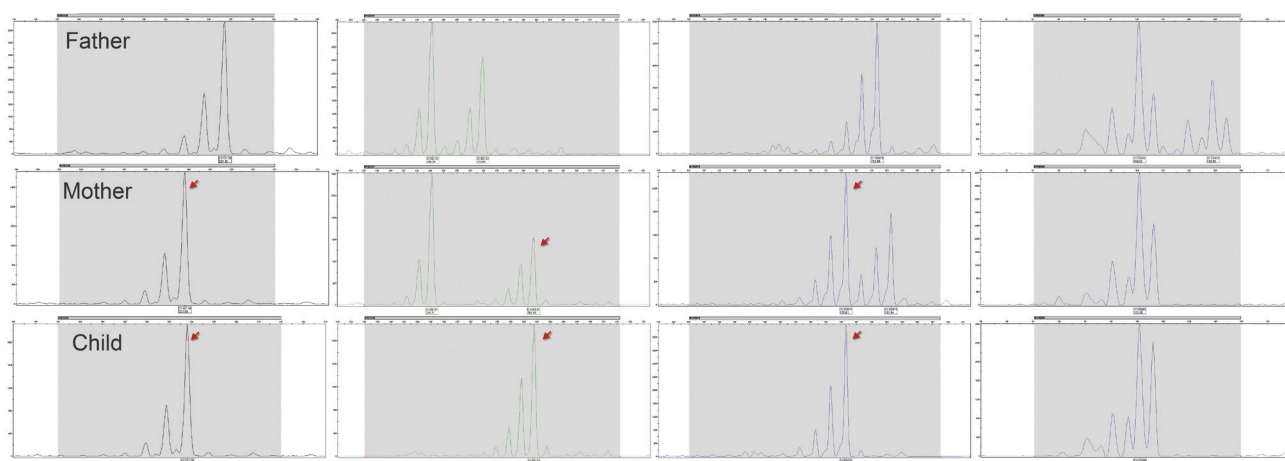


Figure 2. Electropherograms illustrating a positive maternal UPD15 sample using multiplex assay 2. Left to right, markers D15S128 (NED®), D15S131 (VIC™), D15S979 (FAM®), and D15S988 (FAM®). Note that for markers D15S128, D15S131, and D15S979 the child inherited one allele from the mother (red arrow), but no alleles from the father. Marker D15S988 is uninformative because both paternal and maternal inheritance of the allele is possible.

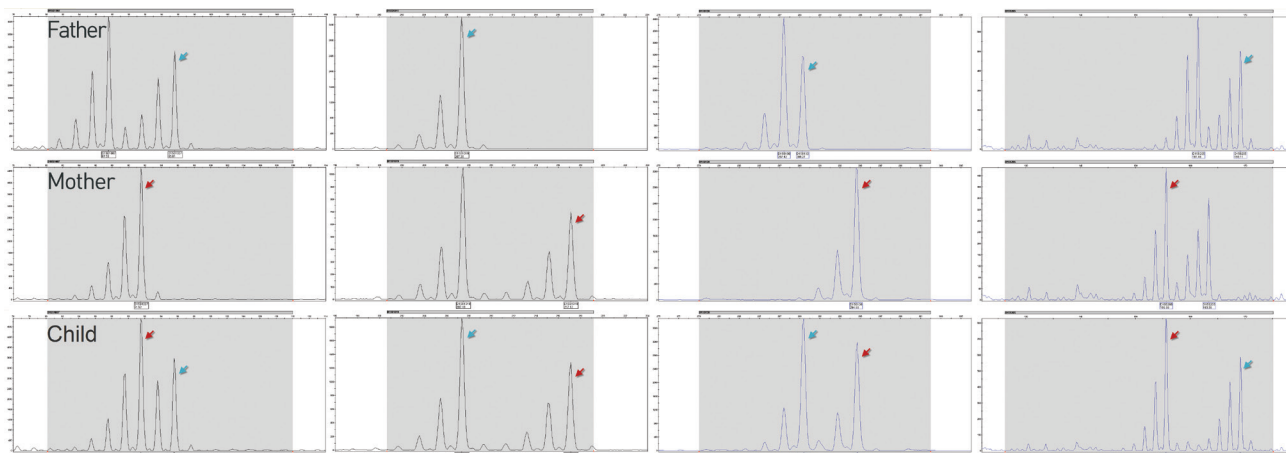


Figure 3. Electropherograms illustrating normal biparental inheritance for a sample using multiplex assay 1. Left to right, markers D15S1007 (NED®), D15S205 (FAM®), D15S1019 (NED®), and D15S130 (FAM®). Note that for each marker the child inherited one allele from the mother (red arrows), and a second allele from the father (blue arrows).

Applied Biosystems® GeneMapper® Software filters out stutter artifacts

A second well-characterized artifact of PCR amplification of di-, tri-, or tetranucleotide microsatellite loci is stutter, which is observed as multiple peaks preceding the true allele peak by 1–4 repeats (Figure 4). Stutter peaks are generated during the PCR reaction when the polymerase, while copying the DNA and the di-, tri-, or tetranucleotide repeat, shifts and loses 1, 2, or 3 repeats. The number of peaks and their intensities are proportional to the length and number of repeats in the PCR product. In the case of a dinucleotide repeat, the stutter peaks generated will have 2, 4, and 6 bases less than the allele peak. For a trinucleotide repeat, the stutter peaks will have 3 and 6 bases less than the allele peak.

Applied Biosystems® GeneMapper® Software filters out stutter peaks automatically, resulting in accurate identification of true alleles. Additionally, GeneMapper® Software uses genotyping quality (GQ) scores to flag lower-quality sample files for manual review, and produces tables that can be sorted in a format that is easy to interpret. This software can be configured to provide reports and calculations, offering user-customized tools for reporting the results from multiplexed STR-PCR assays for UPD.

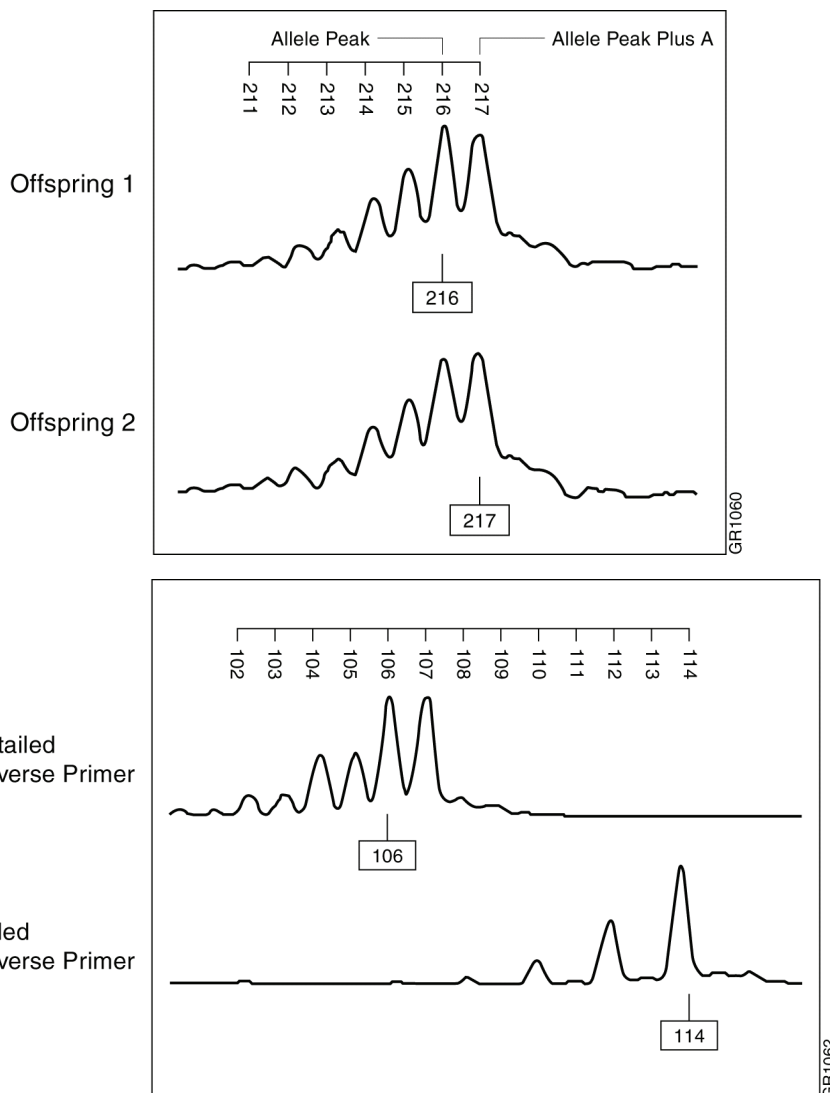


Figure 4. Reverse-primer tailing chemistry improves allele calling. (A) Illustration of two individuals with the same genotype analyzed for the same dinucleotide repeat marker using untailing primer. Di-, tri-, and tetranucleotide repeats tend to generate complex patterns due to the combination of stutter and the plus A artifact. In this example, the stutter peaks are 214 and 212, and the plus A artifacts are peaks 217, 215, and 213. Peak 216 is the correct allele peak for both samples. GeneMapper® software might not correctly call the alleles even though the pattern is visible for both offspring because the allele peak (216) is the highest peak for offspring 1, whereas the allele plus A peak (217) is the highest peak for offspring 2. Data of this type require manual editing to avoid missed or incorrect allele calls when reverse-primer tailing chemistry is not employed. (B) In this example, the 106 peak is the allele peak in the untailing product. The 114 peak is the allele peak plus A in the tailed product, which is 8 bases longer because it includes the 7-base tail and the additional A. Since GeneMapper® Software filters out stutter peaks automatically, elimination of the plus A problem results in easily called alleles.

Conclusion

The advanced capabilities of the Applied Biosystems® 3500 Series Genetic Analyzers, including new thermal control systems, enhanced optical detection, and new consumables designs, provide an easy-to-use platform for the detection and analysis of multiplexed STR-PCR assays for UPD research. Particularly important for UPD analysis are the benefits incorporated in the Custom Tailed Primer Pair, such as high spectral resolution and reverse-primer chemistry (tailing), which

result in consistent allele calls and streamlined data analysis. Using the Custom Tailed Primer Pair addresses genotyping variables and, with the elimination of the plus A problem, results in accurate genotyping. In addition, the advanced algorithms in GeneMapper® Software recognize and filter amplification chemistry artifacts, including plus A and stutter peaks, by differentiating among microsatellite repeats of varying lengths. Use of this software allows researchers to review large amounts of microsatellite data accurately and rapidly.

Acknowledgments

This collaboration was led by Rosella Petraroli, Falcon Team, Europe.

3500 Series Genetic Analyzers

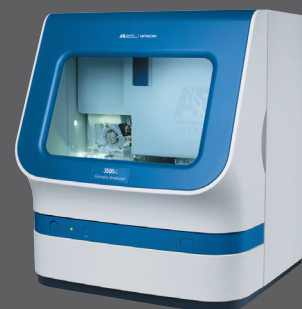
3500 and 3500xL Systems are designed to deliver consistently reliable results, critical for demanding fragment analysis applications, and offer:

- Optimized instrument run modules and implementation of the GeneScan™ 600 LIZ® Size Standard v2.0 for improved signal normalization, especially when comparing capillary-to-capillary, injection-to-injection, or instrument-to-instrument within an association of investigators who run samples on multiple identical instruments
- New thermal sub-system design for improved temperature stability
- 3500 Data Collection Software with an intuitive workflow that performs size calls and applies quality control flags to alert the user to off-scale data, broad peaks, and interfering artifacts or “pull-up”

The 3500 Series Genetic Analyzers are available in two throughput options: the 8-capillary 3500 system, and the 24-capillary 3500xL system. Sample analysis is fully

automated from the moment each 96- or 384-well plate is placed on the instrument and the run is initiated. Easy-to-use wizards for instrument operation and maintenance help ensure predictable, hassle-free performance. And, with recent improvements in the instrument run module, the majority of applications can be analyzed on a single configuration of POP-7™ Polymer with a 50 cm capillary array.

The 3500 Series Systems feature simplified and easy-to-install consumables. The Anode and Cathode Buffer Containers are supplied as ready-to-use 1X Genetic Analysis Buffer formulations. The Automated Polymer Delivery System has been improved with the introduction of polymer pouches, available in 96- and 384-sample sizes, which significantly reduce setup time. Radio frequency identification (RFID) tagging of buffers, polymer, and arrays enables automated electronic tracking of lot number, usage, and expiration date information during analysis.



Also, by employing a single-excitation line solid-state laser, the 3500 Series System has a more compact overall footprint than previous genetic analyzers and operates using a standard power supply. The smaller footprint and standard power supply mean that 3500 and 3500xL Genetic Analyzers fit in more places and don't require ducting for heat removal.

Ordering information

Product	P/N
Reagents and primers	
Custom Tailed Primer Pair: 10,000 picomoles each	4304981
Custom Tailed Primer Pair: 80,000 picomoles each	4304982
Custom Tailed Primer Pair: 300,000 picomoles each	403061
True Allele® PCR Premix	4408399
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Software	
GeneMapper® Software v4.1	4366925

References

1. Giardina E [2008] A multiplex molecular assay for the detection of uniparental disomy for human chromosome 15. *Electrophoresis* 29:4775–4779.

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