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Characterisation of novel alleles and duplication events in the STR DYS19, DYS439, DYS389II and DYS385 loci

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Introduction

The short tandem repeat (STR) loci DYS19, DYS439, DYS389II, DYS393 and DYS385 are commonly used in human parentage testing and evolutionary studies as well as identification (1,2,3,4). The TAGA-repeat locus DYS19 was the first Y-chromosome STR marker to be discovered and amplified with the polymerase chain reaction (PCR) (5,6), while the GATA-repeat locus DYS439 was observed several years later (7). The DYS19 locus is included in the "minimal haplotype", which has been used to generate almost 42,000 profiles in the Y-STR Haplotype Reference Database across almost 360 European, U.S. and Asian populations (www.yhrd.org) (8). DYS19, DYS439, DYS389II and DYS385 loci are included in several commercially available Y-STR multiplex kits (9,10), and presently allelic ladders from PowerPlex® Y System and AmpF*l*STR® Yfiler® PCR Amplification Kit contain 10 alleles ranging from 10 to 19 repeats for the DYS19 locus, 8 alleles ranging from 8 to 15 for the DYS439 locus, 11 alleles ranging from 24 to 34 for the DYS389II locus, 9 alleles ranging from 8 to 16 for the DYS393 locus, and 19 alleles ranging from 7 to 25 for the DYS385 locus.

A review of almost 200 publications containing Y-STR haplotype population information by Butler *et al.* (11) revealed the presence of duplications and triplications at specific loci normally thought of as "single copy". In the present study we characterized additional duplication events at DYS19 and DYS439 as well as a triplication at DYS385. Moreover we sequenced off-ladder PCR products previously unreported at two STRs loci: one of 223bp at locus DYS19 and the other of 193bp at locus DYS439.

Materials and Methods

Buccal cells were collected by oral brushes (Sterile Omni Swab, or Sterile Foam Tipped Swabs, Whatman International Ltd, Maidstone, UK) from 110 unrelated healthy individuals randomly selected from 10 different administrative regions in Central-South Italy: Campania, Apulia, Sicily, Calabria, Lithium, Sardinia, Abruzzi, Basilicata, Marche and Molise. DNA from these samples was extracted using the DNA IQ™ System (Promega Corporation, Madison, WI, USA) according to manufacturer's instructions. The total amount of human genomic DNA extracted was quantitated using the Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Foster City, CA, USA), which employs a TaqMan® MGB Probe-based technology (Applied Biosystems), on an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems). Simultaneous amplifications of Y-STR loci were performed using the PowerPlex® Y System (Promega Corporation) and AmpF*l*STR® Yfiler® PCR Amplification Kit (Applied Biosystems) according to the manufacturer's recommendations.

Separation and detection of amplified products were conducted with the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems), a 16-capillary array system, following the manufacturer's protocol. Data collection was performed with data collection software version 2.0 (Applied Biosystems), and samples were analyzed using the GeneMapper® *ID* software version 3.2 (Applied Biosystems), following the quality control initiatives of the GEDNAP (German DNA Profiling) group (www.gednap.org) (12,13). Alleles were

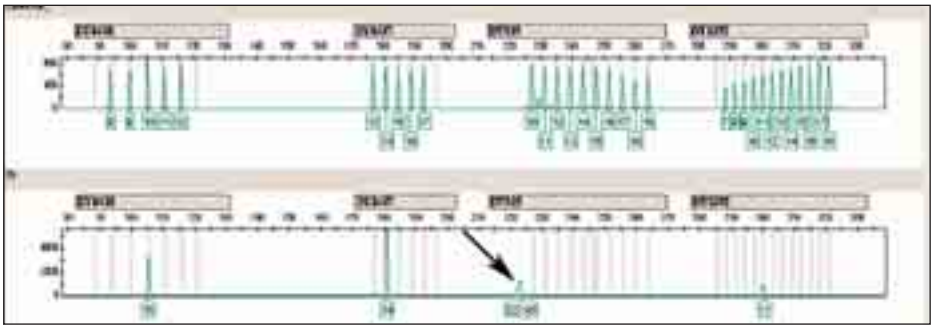

Figure 1: Allele 9 at locus DYS910 (PowerPlex® Y System)

What's inside

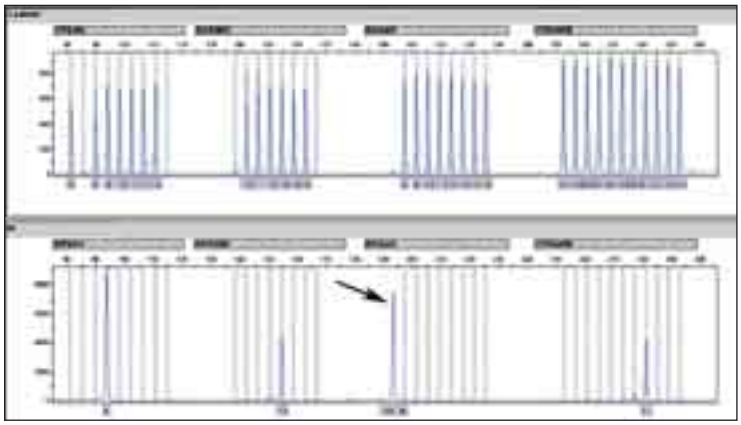

Figure 2: Allele 7 at locus DYS439 (PowerPlex® Y System)

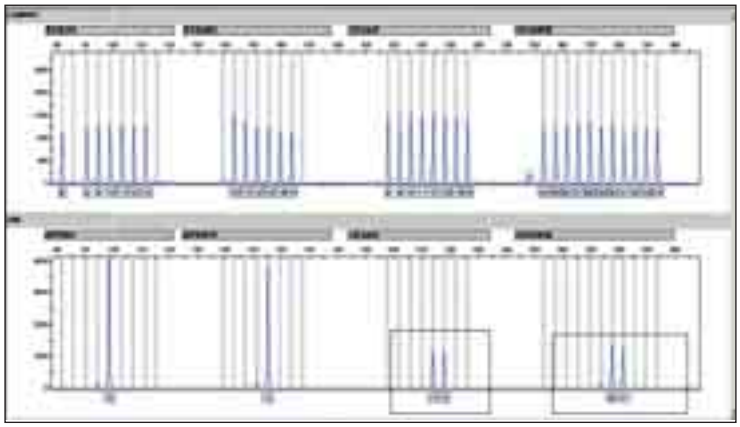

Figure 3: Duplication of alleles at DYS439 and DYS389 II (PowerPlex® Y System)

identified using the PowerPlex® Y allelic ladder and AmpF*l*STR® Yfiler® allelic ladder.

After observation of the novel alleles, we analysed the amplified STR products from these individuals on an ethidium bromide-stained 2% low-melting-point agarose gel for 90 minutes at 80V in 1X TBE Buffer (90mM Tris-borate (pH 8.3), 2mM EDTA). PCR products were visualized with UV light and excised with a razor blade. DNA was extracted using the QIAquick® Gel Extraction Kit (QIAGEN, Hilden, Germany). After gel purification of the bands corresponding to the presumptive novel alleles, the DNA was sequenced using the BigDye® Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems) and the following primers pair: 5´- CTACTGAGTTTCTGTTATAGTGTTTT-3´ and 5´-GTCAATCTCTGCACCTGGAAA-3´ for DYS19 and 5´-TCCTGAATGGTACTTCCTAGGTTT-3´, and 5´-GCCTGGCTTGGAATTCTTTT-3´ for DYS439 (7). Sequencing reaction were carried out in a GeneAmp® PCR System 9600 (Applied Biosystems) thermal cycler according to manufacturer's instructions. Sequencing products were purified by Centri-Sep™ Spin Columns (Applied Biosystems) and separated on an ABI PRISM® 3100-*Avant* Genetic Analyzer (Applied Biosystems), a 4-capillary array system. Data collection was performed with data collection software version 2.0, and sequence data were analyzed by DNA sequencing analysis software version 5.1 (Applied Biosystems) according to manufacturer's recommendations.

To confirm sequence data, samples were replicated and sent to the DNA Human Identity Project Team at the National Institute of Standards and Technology (NIST).

Results and Discussion

Mutations and new alleles were identified with the PowerPlex® Y System and confirmed with the AmpF*l*STR® Yfiler® PCR Amplification Kit. During a study of Y-STR loci frequencies in the central and southern administrative regions of Italy (14), we identified two previously unreported alleles with sizes outside the known allele ranges: allele 9 at locus DYS19 (Figure 1) and allele 7 at locus DYS439 (Figure 2). Sequence analysis revealed a repeat structure of $[TAGA]_3$ tagg$[TAGA]_6$ for the novel DYS19 allele and $[GATA]_7$ for the novel DYS439 allele. Both the DYS19 and the DYS439 nomenclature are based on recommendations set forth by the DNA Commission of the International Society of Forensic Genetics (ISFG) (15).

In the same set of Italian samples, one individual exhibited duplication of alleles at both DYS439 and DYS389II (Figure 3), which are commonly involved in male infertility caused by AZFa region deletions (16).

Another individual was found to possess a triplication at the DYS385 locus using both the PowerPlex® Y System (Figure 4) and AmpF*l*STR® Yfiler® PCR Amplification Kit (data not shown). Off-ladder alleles were verified by sequencing, and duplications were confirmed at NIST. The variant alleles and duplication events observed in this study are summarized in Table 1.

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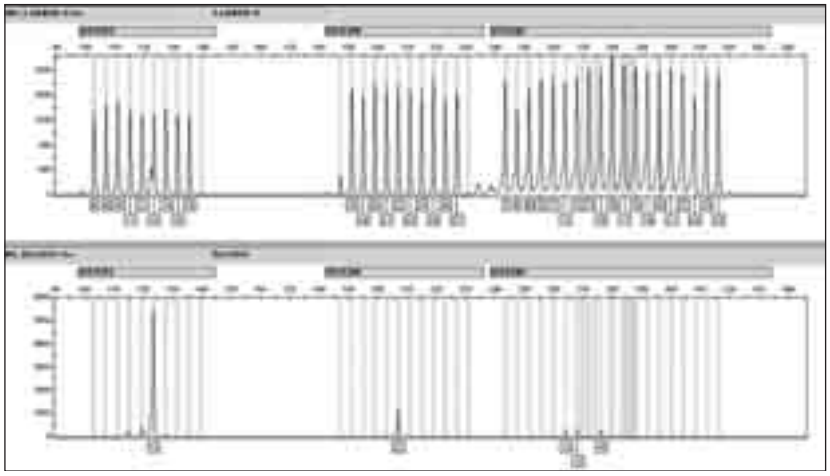

Figure 4: Triplication at the DYS385 locus (PowerPlex® Y System)

Sample ID	DYS391	DYS389I	DYS439	DYS389 II	DYS438	DYS437	DYS19	DYS 392	DYS393	DYS390	DYS385	Italian administrative region
G40	10	13	12,13	30,31	11	14	14	11	13	25	18,19	Sicily
G70	10	13	12	30	10	14	9	11	13,14	24	14,18	Campania
G81	9	13	7	31	10	14	15	11	13	24	17,18	Sicily
G89	10	13	12	30	10	14	9	11	13	24	14,17	Apulia
G93	10	14	12	31	9	15	13	13	13	22	12,13,15	Apulia

Table 1: Observed variant alleles and duplication events.

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27. Spurenworkshop in Frankfurt, Germany

The 27. Spurenworkshop der Deutschen Gesellschaft für Rechtsmedizin was held on February 23–24 2007 in Frankfurt, Germany. More than 300 attendees gathered to discuss the latest developments in the field.

Promega organized a user seminar and presented updates on various technologies.

At the Spurenworkshop, Carsten Hohoff presented the results of the GEDNAP proficiency tests 32 and 33, in which 186 labs from 36 countries participated. In addition, Dr. Lutz Roewer gave an interesting talk about a tricky crime case, which could only be resolved by analysing 21 Y-STR loci. Peter Schneider presented guidelines on how to interpret DNA mixtures. Walther Parson gave an introduction on how to use EMPOP – the forensic mtDNA database, and Burkhard Rolf presented an interesting case in which he could prove that an alleged son of tsar Nicolas II was not related to the Romanov family.

Next year´s meeting will take place mid February in Salzburg, Austria.

Matthias Lindner – new member of the GI Euro team

Matthias is the newest member of the European Genetic Identity team. He joined our team in March 2007 and will be based in Paris, France. Before joining the team Matthias received his Diploma in Biology from the University of Heidelberg, Germany, where he also did his Ph.D. thesis at the German Cancer Research Center. Matthias had been working at the German branch as a Technical Services Scientist since the fall of 2003. In addition to providing technical service for the complete Promega product portfolio, he became more involved in the support of Genetic Identity products, especially the different PowerPlex® Systems. In his new role at Promega, Matthias will be involved in customer training and technical support for Genetic Identity products and applications. He will also develop educational talks and represent Promega at meetings.

In his spare time Matthias enjoys parks and gardens, good food and wine, opera and graphical art. Please join us in welcoming him to the GI team, and feel free to contact him at: matthias.lindner@promega.com

Upcoming Meetings of Interest

ISFG
Copenhagen, Denmark,
August 22–24, 2007
www.isfg2007.org

Fifth ISABS Conference in Forensic Genetics and Molecular Anthropology
Split, Croatia,
September 3–7, 2007
www.isabs.hr

18th International Symposium on Human Identification
Hollywood, California, USA,
October 1–4, 2007
www.promega.com/geneticsymp18/

TECHNICAL CORNER

Removal and fixation of the security anchors for the Maxwell® 16 Instrument

The magnetic rod assembly, plunger bar and platform are anchored in place during shipment to prevent movement of and damage to these parts.

The following exerpt from the instrument manual will help you to remove and set the security anchors of the Maxwell® 16. **For a complete and detailed description please read the Maxwell® 16 Instrument Operating Manual TM274.**

Unpacking instructions

Note: DO NOT plug in or turn on the instrument before removing the shipping anchors. After removing the packaging material in front of the platform, please locate the top magnetic rod assembly shipping anchors. There are a total of 4 anchors: A–D (Figure 1). Once you have loosened anchor B, slide it up to the top of the linear slide to be even with anchors A and D. Retighten anchor B (Figure 2).

Slowly and gently raise the magnetic rod assembly to better visualize shipping anchor C (Figure 3). Once you have loosened anchor C, slide it up to the top of the linear slide to be even with anchors A, B and D. Retighten the anchor C.


Figure 1


Figure 2


Figure 3


Figure 4

Repacking instructions

Slowly extend the platform out of the instrument by hand. Place both pieces of the platform foam packaging on top of the platform (Figure 4).

Carefully push the platform by hand back into the instrument, and gently lower by hand the plunger bar and the magnetic assembly rod.


Figure 5

Loosen anchor C (Figure 5), slide it down the linear slide and tighten it firmly against the top of the plunger bar (Figure 3).


Figure 6

By hand, gently lower the magnetic rod assembly down as far as it will go. The bottom of the magnetic rods should be pressed into the foam packaging material that is sitting on the top of the platform (Figure 6).


Figure 7

Loosen anchor B (Figure 7), slide it down the linear slide and tighten it firmly against the top of the plunger bar (see Figure 8). Finally, replace the foam packaging material in front of the platform. Verify that the magnetic rod assembly, plunger bar, and platform are securely anchored by trying to move these pieces by hand.


Figure 8

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