

## FOR THE RECORD

John M. Butler,<sup>1</sup> Ph.D. and Richard Schoske,<sup>1,2</sup> Ph.D.

# U.S. Population Data for the Multi-Copy Y-STR Locus DYS464\*

**POPULATIONS:** U.S. Caucasian, African American, and Hispanic

**KEYWORDS:** forensic science, DNA profiling, short tandem repeats, DNA typing, STR, Y-chromosome, Y-STR, DYS464, multi-copy markers, population genetics

Anonymous liquid blood samples with self-identified ethnicities were purchased from Interstate Blood Bank (Memphis, TN) and Millennium Biotech, Inc. (Ft. Lauderdale, FL). These samples were extracted, quantified, and genotyped with the Identifiler kit as previously described (1) in order to demonstrate that the samples were all from unique individuals. In addition, a set of extracted DNA samples from 20 African American and 20 U.S. Caucasian males were kindly provided by Carll Ladd of the Connecticut Forensic Laboratory (Meriden, CT). John Hartmann of the Orange County Sheriff-Coroner Department (Santa Ana, CA) supplied extracted DNA for 20 Hispanic males. In this study, a total of 679 male U.S. population samples were examined, which included 262 Caucasians, 265 African American, and 152 Hispanic.

The multi-copy tetranucleotide Y-chromosome short tandem repeat (Y-STR) marker DYS464, which was first described by Redd et al. (2), was amplified with the following PCR primers: VIC-CTTTGGGCTATGCCTCAGTTT and GCCATAC-CTGGGTAACAGAGAGAC as part of a multiplex Y-STR assay that has been previously described (3). This single set of primers target (at least) four separate regions of the Y-chromosome (4) and can generate up to four distinct peaks in the size range of 242–286

bp that possess an allele range of 9–20 CCTT repeats (5). Allele calls for the multi-copy DYS464 can be made based solely on the peaks that are present (conservative approach) or a combination of alleles and peak height ratios (expanded typing method) (5). Previous reporting of DYS464 data (2,3,6) has typically involved the conservative approach. In fact, the conservative DYS464 haplotype designations on 647 of these samples were previously reported by Schoske et al. (3).

The VIC<sup>®</sup>-labeled PCR primer was obtained from Applied Biosystems (Foster City, CA) and the unlabeled primer from Qiagen Operon (Alameda, CA). PCR amplification was carried out in 20 µL volumes on a GeneAmp<sup>®</sup> 9700 (Applied Biosystems) using approximately 1 ng of DNA and conditions previously described (3). Amplification products were diluted 1:15 in Hi-Di<sup>™</sup> formamide and GS500-LIZ internal size standard (Applied Biosystems) and analyzed on the 16-capillary ABI Prism<sup>®</sup> 3100 Genetic Analyzer without prior denaturation of samples. POP<sup>™</sup>-6 (Applied Biosystems) rather than POP<sup>™</sup>-4 was utilized for higher resolution separations on a 36 cm array. Samples were injected electrokinetically for 10 s at 3 kV and separated at 15 kV for approximately 45 min. Allele calls were made in Genotyper<sup>®</sup> 3.7 using an in-house macro based on fixed bin allele sizes that were calibrated through DNA sequence analysis of individual alleles (3). Visual examination of peak heights was used to determine the expanded types.

Table 1 includes a summary of the haplotypes observed based on peak height information with the DYS464 locus. Across the 679 samples examined, 179 different expanded types were observed of which 92 occurred only once. If the information in Table 1 is collapsed to only include the peaks observed (i.e., no consideration of the relative peak heights), then 113 conservative types are seen. In 13 individuals, there appears to be more than 4 copies of DYS464 (e.g., 5, 6, or 7 copies) present based on the allele peak heights. In addition, there are 10 individuals that appear based on peak heights to have a deletion of one of the copies (i.e. possess only 3 copies). This phenomenon of Y-chromosomal duplication and deletion has been seen with other Y-STR markers used in human identity testing (7,8). In addition, four microvariant alleles were observed: 13.1, 14.3, 15.1, and 15.3.

<sup>1</sup> Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD 20899-8311.

<sup>2</sup> Current address: Armed Forces Institute of Pathology, Department of Microbiology, Washington, DC 20306.

\* Contribution of the U.S. National Institute of Standards and Technology. Not subject to copyright. Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose. Points of view in this document are those of the authors and do not necessarily represent the official position or policies of the U.S. Department of Justice or the U.S. Department of Defense. Sources of Support: The National Institute of Justice funded this work in part through interagency agreement 1999-IJ-R-094 with the NIST Office of Law Enforcement Standards.

† This work was part of a poster presentation at the International society of Forensic Genetics meeting in September 2003, Archacon, France.



the U.S. Air Force, Air Force Institute of Technology. The technical assistance and suggestions of Peter Vallone, David Duewer, Margaret Kline, and Jan Redman from our group at NIST and Alan Redd from the University of Arizona are gratefully acknowledged.

## References

1. Butler JM, Schoske R, Vallone PM, Redman JW, Kline MC. Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J Forensic Sci* 2003;48(4):908–11. [\[PubMed\]](#)
2. Redd AJ, Agellon AB, Kearney VA, Karafet T, de Knijff P, Park H, Butler JM, Hammer MF. [Forensic value of fourteen novel STRs on the human Y chromosome.](#) *Forensic Sci Int* 2002;130:97–111. [\[PubMed\]](#)
3. Schoske R, Vallone PM, Kline MC, Redman JW, Butler JM. [High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays.](#) *Forensic Sci Int* 2004;139:107–21. [\[PubMed\]](#)
4. Butler JM. Recent developments in Y-short tandem repeat and Y-single nucleotide polymorphism analysis. *Forensic Sci Rev* 2003;15:91–111.
5. Butler JM, Schoske R. Forensic value of the multi-copy Y-STR marker DYS464. *Progress in forensic genetics 10*, Elsevier Science: Amsterdam, The Netherlands, International Congress Series 2004;1261:278–80.
6. Berger B, Niederstatter H, Brandstatter A, Parson W. [Molecular characterization and Austrian Caucasian population data of the multi-copy Y-chromosomal STR DYS464.](#) *Forensic Sci Int* 2003;137:221–30. [\[PubMed\]](#)
7. Butler JM, Decker AE, Kline MC, Vallone PM. Chromosomal duplications along the Y-chromosome and their potential impact on Y-STR interpretation. *J Forensic Sci* 2005;50(4):1–7.
8. Butler JM, Schoske R. [Duplication of DYS19 flanking regions in other parts of the Y chromosome.](#) *Int J Legal Med* 2004;118:178–83. [\[PubMed\]](#)

Additional information and reprint requests:  
 John M. Butler, Ph.D.  
 Research Chemist  
 National Institute of Standards and Technology  
 100 Bureau Dr., Mail Stop 8311  
 Gaithersburg, MD 20899-8311  
 E-mail: john.butler@nist.gov