

FOR THE RECORD

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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[®]-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 μ L volumes on a GeneAmp[®] 9700 (Applied Biosystems) using approximately 1 ng of DNA and conditions previously described (3). Amplification products were diluted 1:15 in Hi-Di[™] formamide and GS500-LIZ internal size standard (Applied Biosystems) and analyzed on the 16-capillary ABI Prism[®] 3100 Genetic Analyzer without prior denaturation of samples. POP[™]-6 (Applied Biosystems) rather than POP[™]-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[®] 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.

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

TABLE 1—Frequency of 179 different expanded *DYS464* types in 679 male samples from 3 different U.S. populations: African Americans (AA), Caucasians (C), and Hispanics (H).

Expanded Type	#AA	#C	#H	Freq	Expanded Type	#AA	#C	#H	Freq	Expanded Type	#AA	#C	#H	Freq
15,15,17,17	11	38	23	0.106	16,16,16,17	3			0.004	13,13,15,16			1	0.001
15,15,16,17	9	25	17	0.075	11,11,14,15	1		1	0.003	13,13,15,16,16,16,18	1			0.001
13,16,16,17	28		1	0.043	11,13,14,15		1	1	0.003	13,13,15,16,16,17	1			0.001
13,16,16,18	21	1	3	0.037	11,14,15,15		2		0.003	13,13,15,16,17,17	1			0.001
12,14,15,16	3	15	3	0.031	12,12,14,15		1	1	0.003	13,13,16,17	1			0.001
14,15,17,17	4	12	1	0.025	12,13,14,15	1			0.003	13,14,14,14		1		0.001
15,15,16,16	7	4	6	0.025	12,13,14,16		1	1	0.003	13,14,14,15		1		0.001
15,15,17,18	5	8	4	0.025	12,14,14,14		1	1	0.003	13,14,15,15		1		0.001
15,16,17,17	6	8	2	0.024	12,14,14,17		2		0.003	13,14,15,15,16	1			0.001
15,16,16,17	5	7	3	0.022	12,14,16,18	2			0.003	13,14,15,16	1			0.001
13,16,17,17	9	2	1	0.018	12,16,16,16	2			0.003	13,14,15,17	1			0.001
13,15,16,16	9		2	0.016	12,16,16,19			2	0.003	13,14,15,18			1	0.001
15,16,17,18	1	7	2	0.015	13,13,13,14		1	1	0.003	13,14,16,16	1			0.001
13,15,16,18	9			0.013	13,13,13,15	1		1	0.003	13,14,3,15,17			1	0.001
13,16,17,18	8			0.012	13,13,14,14		1	1	0.003	13,14,3,16,17	1			0.001
14,15,16,17	1	4	3	0.012	13,13,14,17	1		1	0.003	13,15,15,15			1	0.001
14,15,17,18	2	4	2	0.012	13,13,16,16		1	1	0.003	13,15,15.1,15.1		1		0.001
15,15,15,17	3	3	2	0.012	13,13,16,16,18,19	1		1	0.003	13,15,17,17	1			0.001
13,15,16,17	4	2	1	0.010	13,13.1,15,16,16	1		1	0.003	13,15,18,18	1			0.001
14,16,16,17	2	1	4	0.010	13,14,16,17	1		1	0.003	13,16,16,20	1			0.001
12,14,15,15	1	5		0.009	13,15,15,19		2		0.003	13,16,17,19	1			0.001
12,15,15,16	1	5		0.009	13,15,16,-	2			0.003	13,17,17,20			1	0.001
12,15,16,17	4	2		0.009	13,17,18,18	2			0.003	14,14,14,17			1	0.001
12,15,16,18	6			0.009	15,15,17,-		1	1	0.003	14,14,14,19			1	0.001
13,16,16,16	5		1	0.009	15,15,18,19	1		1	0.003	14,14,15,18	1			0.001
14,14,15,15		4	2	0.009	15,17,17,17			2	0.003	14,14,16,18	1			0.001
15,15,16,18	2	2	2	0.009	17,17,17,17		2		0.003	14,14,16,20	1			0.001
13,16,16,19	4		1	0.007	11,11,12,14,15,15			1	0.001	14,14,3,15,17		1		0.001
14,14,14,15	1	3	1	0.007	11,11,14,14			1	0.001	14,14,3,16,18	1			0.001
14,14,16,17	2		3	0.007	11,11,14,16	1			0.001	14,15,15,18			1	0.001
14,15,16,18	3		2	0.007	11,11,15,16			1	0.001	14,15,16,-			1	0.001
14,16,17,18	4	1		0.007	11,11,16,16	1			0.001	14,15,18,-			1	0.001
15,15,15,16	2	2	1	0.007	11,13,15,15		1		0.001	14,15,18,18		1		0.001
15,16,16,16	3	2		0.007	11,13,15,16			1	0.001	14,15,3,16,16		1		0.001
12,13,13,14		4		0.006	11,13,15,1,16			1	0.001	14,16,16,16,17,17			1	0.001
12,15,15,15	2	2		0.006	11,13,16,16			1	0.001	14,16,16,18	1			0.001
12,15,16,16		4		0.006	11,14,14,14			1	0.001	14,16,17,-	1			0.001
13,15,15,17		3	1	0.006	11,15,15,18		1		0.001	14,17,17,17			1	0.001
13,15,15,18	2	1	1	0.006	11,15,16,17	1			0.001	14,17,18,19	1			0.001
14,16,17,17		2	2	0.006	11,16,16,18	1			0.001	14,3,15,16,17	1			0.001
15,15,18,18	1	1	2	0.006	11,16,18,18	1			0.001	15,-16,16		1		0.001
15,16,16,18		4		0.006	12,12,12,16			1	0.001	15,-,17,17		1		0.001
16,16,17,17	1	2	1	0.006	12,12,13,14			1	0.001	15,15,15,15		1		0.001
11,14,14,15		3		0.004	12,12,14,3,14,3			1	0.001	15,15,15,3,16		1		0.001
11,14,14,16		3		0.004	12,12,15,15,15,16		1		0.001	15,15,16,19		1		0.001
12,13,14,14	1	1	1	0.004	12,12,15,16			1	0.001	15,15,16,20		1		0.001
12,14,14,15	1	2		0.004	12,13,13,13,14			1	0.001	15,15,17,19		1		0.001
12,14,14,16	1	1	1	0.004	12,13,15,16			1	0.001	15,16,16,20		1		0.001
12,16,16,17	3			0.004	12,13,15,17	1			0.001	15,16,17,17,17			1	0.001
13,15,17,18	3			0.004	12,13,15,1,16		1		0.001	15,16,17,19		1		0.001
13,16,18,18	3			0.004	12,14,15,17	1			0.001	15,16,18,18		1		0.001
14,14,14,14		3		0.004	12,14,16,16		1		0.001	15,16,18,20		1		0.001
14,14,15,16		2	1	0.004	12,14,17,17			1	0.001	15,17,18,19		1		0.001
14,14,15,17		2	1	0.004	12,15,15,17			1	0.001	16,16,16,16		1		0.001
14,14,16,16			3	0.004	12,15,15,18	1			0.001	16,16,16,18		1		0.001
14,14,17,17		2	1	0.004	12,15,17,17	1			0.001	16,16,16,19	1			0.001
14,15,15,16	2	1		0.004	12,15,17,18	1			0.001	16,17,17,17	1			0.001
14,15,15,17	2		1	0.004	12,15,17,19	1			0.001	17,17,18,18	1			0.001
14,15,16,16	1	1	1	0.004	13,13,14,-	1			0.001	9,15,16,17			1	0.001
15,17,17,18	2	1		0.004	13,13,14,18			1	0.001	Total	265	262	152	1.00

While the multi-copy nature of *DYS464* may make it difficult to use reliably in forensic casework due to the challenge of deconvoluting a male-male mixture or trying to interpret a degraded DNA profile, *DYS464* is a valuable, highly polymorphic marker that could be useful in paternity testing and genetic genealogy applications. By way of comparison, the next most polymorphic Y-STR marker in our data set is *DYS385*, which has 56 different types in the 679 samples examined.

The complete dataset with *DYS464* and other human identity testing markers on these U.S. samples is available at <http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm>.

Acknowledgments

Much of this work was performed while R.S. was a graduate student at NIST and the American University through funding from

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. [\[PubMed\]](#)

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