National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2395

Human Y-Chromosome DNA Profiling Standard

(In Cooperation with the National Institute of Justice – U.S. Department of Justice)

This Standard Reference Material is intended primarily for use in the standardization of forensic and paternity quality assurance procedures for Polymerase Chain Reaction (PCR)-based genetic testing and for instructional law enforcement or non-clinical research purposes that involve the human Y-chromosome. This SRM can also be used for quality assurance when assigning values to in-house control materials. It is not intended for any human or animal clinical diagnostic use. Additional information on each Y-chromosome marker can be found at a NIST-sponsored database on the Internet: http://www.cstl.nist.gov/biotech/strbase.

This SRM is composed of well-characterized human genomic deoxyribonucleic acid (DNA) in liquid form. A unit of the SRM is composed of 6 frozen components packaged in one box. There are five male samples and one female sample in this SRM. See the section in this certificate entitled *Description of Components* for a complete listing of the components.

Certified and Informational Values: The SRM is certified for genetic loci on the human Y-chromosome [1-12]. Genetic types for loci certified through DNA sequencing, interlaboratory testing, and typing methodologies can be found in Tables 1 through 7. The Tables are organized as follows: Tables 1 and 2 list the genetic types for 22 different Y-chromosome short tandem repeat (STR) markers that have been certified through DNA sequencing of the various alleles; Table 3 describes informational values for 5 additional Y-STR loci (9 allele calls) that have been typed but not yet sequenced; Table 4 contains a summary of the sequence information for the 22 sequenced Y-STR loci; Table 5 summarizes the information gathered at each Y-STR marker for which a value has been assigned; Table 6 describes the different Y-STR assays used at NIST for confirming the allele calls; Table 7 lists the genetic types for 42 different Y-chromosome single nucleotide polymorphisms (Y-SNPs) determined by allele-specific hybridization.

Expiration of Certification: The certification of this SRM is valid until **31 December 2008**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is invalid if the SRM is contaminated or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of certification, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

Storage: Store frozen at a temperature of -20 ºC. **DO NOT** use a self-defrosting freezer because periodic cycling of temperatures may cause shortened shelf life of this SRM.

The overall direction and coordination of the technical activities leading to certification were under the chairmanship of Margaret C. Kline and John M. Butler of the NIST Biotechnology Division.

Analytical determination and technical measurements leading to the certification of this SRM were performed by J.M. Butler, R. Schoske, P.M. Vallone, M.C. Kline, and J.W. Redman of the NIST Biotechnology Division.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by C.S. Davis of the NIST Measurement Services Division.

Vincent L. Vilker, Chief
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Certificate Issue Date: 06 June 2003

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NOTICE AND WARNINGS TO USER

SRM 2395 IS A HUMAN SOURCE MATERIAL. SINCE THERE IS NO CONSENSUS ON THE INFECTIOUS STATUS OF EXTRACTED DNA, HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE.

INSTRUCTION FOR USE

Sample aliquots for analysis should be withdrawn immediately after opening the vials and should be processed without delay for the certified values to be applicable.

Source of Material:[1] Genomic DNA components were extracted from whole blood obtained from Millennium Biotech, Inc., Ft. Lauderdale, FL.

Interlaboratory Analysis: The STR values for this SRM represent the pooled results from analyses performed at NIST, ReliaGene Technologies, Inc. (New Orleans, LA), OligoTrail LLC (Evanston, IL), and the Forensic Laboratory for DNA Research at Leiden University Medical Center (Leiden, The Netherlands).

Description of Components: Six components are included in each unit; all components must be stored at -20 ºC. Components A through F each contain 50 µL of genomic DNA at a concentration of approximately 2 ng/µL.

A	Male Genomic DNA 1
B	Male Genomic DNA 2
C	Male Genomic DNA 3
D	Male Genomic DNA 4
E	Male Genomic DNA 5
F	Female Genomic DNA (to serve as a negative control for Y-chromosome specific assays)

NOTE: DNA concentrations given are nominal values and are not intended for use as concentration standards.

Typing results are shown in Tables 1 through 3. Further characterization of materials through DNA sequencing is summarized in Table 4. Table 5 summarizes the information gathered at each Y-STR marker for which a value has been assigned. Table 6 describes the different Y-STR assays used at NIST for confirming the allele calls. Table 7 lists the genetic types for 42 different Y-chromosome single nucleotide polymorphisms (Y-SNPs) determined by allele-specific hybridization. Figure 1 illustrates a polymorphism within the middle of the DYS390 repeat region for Component C that will not be resolved from a regular 21 repeat allele using a size based STR typing method. Figure 2 displays results from the quadruplicated Y-STR DYS464.

Commercial kits used to obtain typing results used at NIST (see Table 6):

Y-PLEX™ 6 (ReliaGene): DYS19, DYS389II, DYS390, DYS391, DYS393, DYS385 a/b

Y-PLEX™ 5 (ReliaGene): DYS389I/II, DYS392, DYS438, DYS439

PowerPlex® Y prototype (Promega): DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439

Signet™ Y-SNP Identification System (Marligen Biosciences, Ijamsville, MD): 42 Y-SNPs listed in Table 7

Certified and informational values at additional Y-STR and Y-SNP loci will be added in future versions of this Certificate of Analysis.

[1]Certain commercial equipment, instrumentation, or materials are identified in this certificate to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 1. Certified Values for the European Minimal Haplotype and U.S. Core Y-STR Loci

NOTE: These values have all been confirmed through DNA sequencing (see Table 4).

Component ID	Name	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS385 a/b	DYS438	DYS439
A	Male 1	14	13	29	25	11	13	13	12-15	12	12
B	Male 2	14	13	28^	23	11	11	12	14-17	9	12
C	Male 3	16	14	32	21*	12	11	13	17-20	11	11
D	Male 4	15	12	28^	22	10	11	14	14-15	11	11
E	Male 5	17	14	31	24	10	12	14	13-15	10	11
F	Female	--	--	--	--	--	--	--	--	--	--

^ Components B and D both contain allele 28 for DYS389II yet they differ internally. B has 5-10-3-10 and D has 5-11-3-9 (TCTG-TCTA-TCTG-TCTA) repeats (see Table 4).

* Component C has a variant allele at the DYS390 locus. Size-based analytical systems should type this sample as a 21 repeat allele although sequence information reveals a T-to-A polymorphism internal to the repeat (see Figure 1).

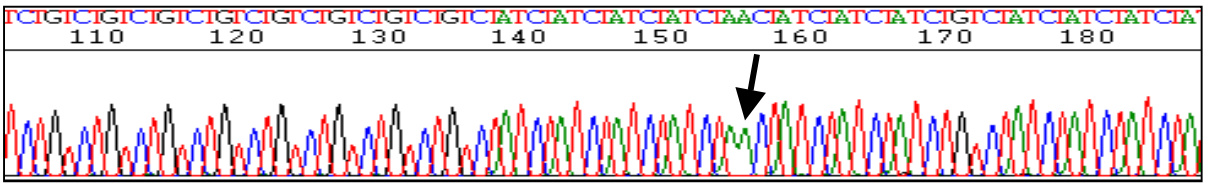

Figure 1. Sequence of DYS390 repeat region for component C.

The arrow indicates a novel variant nucleotide (T-to-A polymorphism) in the center of the regular repeat. Table 4 compares the repeat descriptions for each component at all sequenced Y-STR loci.

Table 2. Certified Values for Other Commonly Used Y-STR Markers

NOTE: These values have all been confirmed through DNA sequencing (see Table 4).

Component ID	Name	DYS388	DYS426	DYS435	DYS436	DYS437	DYS447	DYS448	DYS460	DYS461	DYS462	H4
A	Male 1	12	12	12	12	15	24	19*	11	12	11	12#
B	Male 2	15	11	11	12	14	25^	21*	10	13	11	12#
C	Male 3	12	11	11	12	14	25^	21*	9	13	12	12#
D	Male 4	12	11	11	12	16	23	21*	11	11	13	12#
E	Male 5	13	11	11	12	14	26	20*	11	12	12	11#
F	Female	--	--	--	--	--	--	--	--	--	--	--

^ Components B and C for DYS447 are listed as 25 repeats. However, B contains 9-1-8-1-6 while C is 7-1-8-1-8 in terms of [TAATA]-[TAAAA]-[TAATA]-[TAAAA]-[TAATA] repeats (see Table 4).

* Three different nomenclatures have been published for DYS448 [9]. The one listed here follows Redd *et al* [5].

\# The H4 nomenclature follows Butler *et al.* [4].

Table 3. Informational Values for Additional Y-STR Loci

NOTE: This information has not been fully confirmed through direct DNA sequencing of the SRM components. DYS464 is quadruplicated on the Y-chromosome and can produce up to 4 separate peaks (see Figure 2). Both expanded and conservative methods for calling DYS464 alleles are listed (see [5,10]).

Component ID	Name	DYS450	DYS456	DYS458	DYS464 a/b/c/d (expanded)	DYS464 a/b/c/d (conservative)	YCAII a/b
A	Male 1	10	15	16	14-15-15-17	14-15-17	19-23
B	Male 2	10	15	15	12-13-13-17	12-13-17	19-22
C	Male 3	8	15	17	13-16-16-18	13-16-18	19-19
D	Male 4	9	15	16	13-13-14-14	13-14	20-20
E	Male 5	10	15	16	11-14-14-15	11-14-15	19-21
F	Female	--	--	--	--		--

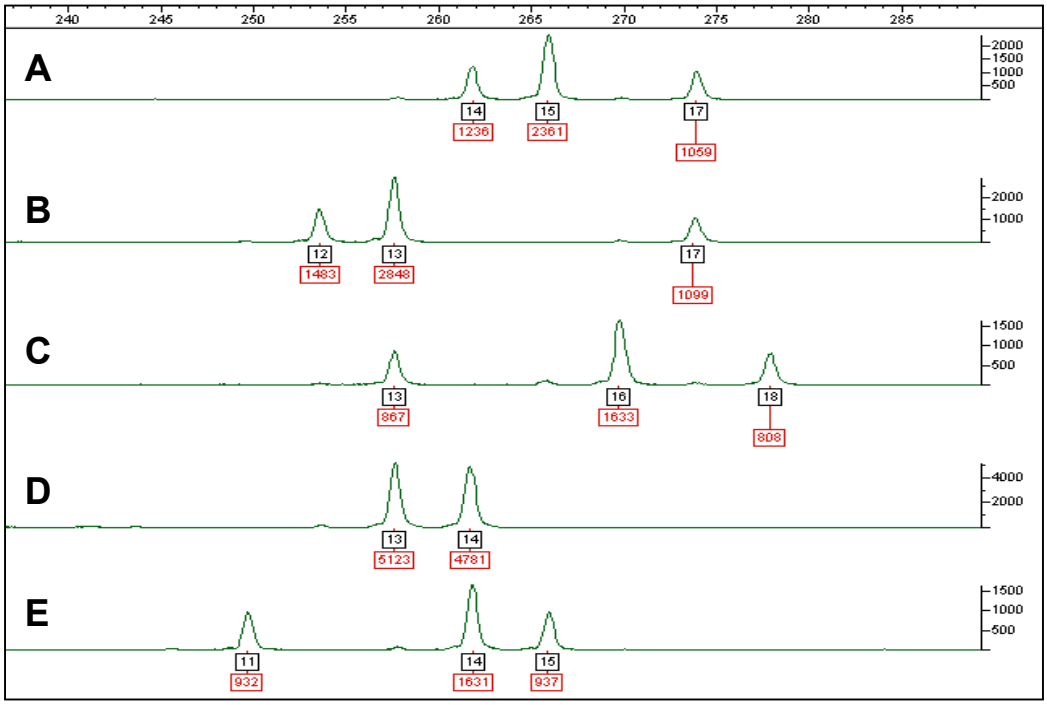

Figure 2. Allele calls (top peak label) and peak heights (bottom label) for the multi-copy Y-STR DYS464, which is quadruplicated on the Y-chromosome.

Table 4. Sequence Data Obtained With Big Dye v. 3 Sequencing Kits

NOTE: All sequencing results were generated at NIST with the exception of DYS462 from Leiden University. Peter de Knijff's group at Leiden also confirmed the NIST sequencing results at DYS19, DYS388, DYS389 I/II, DYS390, DYS391, DYS392, DYS393, DYS460, and DYS461.

DYS19	A	14	$[TAGA]_3$ tagg$[TAGA]_{11}$
	B	14	$[TAGA]_3$ tagg$[TAGA]_{11}$
	C	16	$[TAGA]_3$ tagg$[TAGA]_{13}$
	D	15	$[TAGA]_3$ tagg$[TAGA]_{12}$
	E	17	$[TAGA]_3$ tagg$[TAGA]_{14}$

DYS438	A	12	$[TTTTC]_{12}$
	B	9	$[TTTTC]_9$
	C	11	$[TTTTC]_{11}$
	D	11	$[TTTTC]_{11}$
	E	10	$[TTTTC]_{10}$

DYS389I	A	13	$[TCTG]_3$ $[TCTA]_{10}$
	B	13	$[TCTG]_3$ $[TCTA]_{10}$
	C	14	$[TCTG]_3$ $[TCTA]_{11}$
	D	12	$[TCTG]_3$ $[TCTA]_9$
	E	14	$[TCTG]_3$ $[TCTA]_{11}$

DYS388	A	12	$[ATT]_{12}$
	B	15	$[ATT]_{15}$
	C	12	$[ATT]_{12}$
	D	12	$[ATT]_{12}$
	E	13	$[ATT]_{13}$

DYS439	A	12	$[GATA]_{12}$
	B	12	$[GATA]_{12}$
	C	11	$[GATA]_{11}$
	D	11	$[GATA]_{11}$
	E	11	$[GATA]_{11}$

DYS389II	A	29	$[TCTG]_5$ $[TCTA]_{11}$ … $[TCTG]_3$ $[TCTA]_{10}$
	B	28	$[TCTG]_5$ **$[TCTA]_{10}$** … $[TCTG]_3$ **$[TCTA]_{10}$**
	C	32	$[TCTG]_6$ $[TCTA]_{12}$ … $[TCTG]_3$ $[TCTA]_{11}$
	D	28	$[TCTG]_5$ **$[TCTA]_{11}$** … $[TCTG]_3$ **$[TCTA]_9$**
	E	31	$[TCTG]_5$ $[TCTA]_{12}$ … $[TCTG]_3$ $[TCTA]_{11}$

DYS391	A	11	$[TCTA]_{11}$
	B	11	$[TCTA]_{11}$
	C	12	$[TCTA]_{12}$
	D	10	$[TCTA]_{10}$
	E	10	$[TCTA]_{10}$

DYS460	A	11	$[ATAG]_{11}$
	B	10	$[ATAG]_{10}$
	C	9	$[ATAG]_9$
	D	11	$[ATAG]_{11}$
	E	11	$[ATAG]_{11}$

DYS390	A	25	$[TCTG]_8$ $[TCTA]_{12}$ $[TCTG]_1$ $[TCTA]_4$
	B	23	$[TCTG]_8$ $[TCTA]_{10}$ $[TCTG]_1$ $[TCTA]_4$
	C	21	$[TCTG]_8$ $[TCTA]_5$ **A**CTA $[TCTA]_2$ $[TCTG]_1$ $[TCTA]_4$
	D	22	$[TCTG]_8$ $[TCTA]_9$ $[TCTG]_1$ $[TCTA]_4$
	E	24	$[TCTG]_8$ $[TCTA]_{11}$ $[TCTG]_1$ $[TCTA]_4$

DYS392	A	13	$[TAT]_{13}$
	B	11	$[TAT]_{11}$
	C	11	$[TAT]_{11}$
	D	11	$[TAT]_{11}$
	E	12	$[TAT]_{12}$

DYS461	A	12	$[TAGA]_{11}$CAGA
	B	13	$[TAGA]_{12}$CAGA
	C	13	$[TAGA]_{12}$CAGA
	D	11	$[TAGA]_{10}$CAGA
	E	12	$[TAGA]_{11}$CAGA

DYS447	A	24	$[TAATA]_6$ [TAAAA] $[TAATA]_{10}$ [TAAAA] $[TAATA]_6$
	B	25	**$[TAATA]_9$** [TAAAA] **$[TAATA]_8$** [TAAAA] **$[TAATA]_6$**
	C	25	**$[TAATA]_7$** [TAAAA] **$[TAATA]_8$** [TAAAA] **$[TAATA]_8$**
	D	23	$[TAATA]_6$ [TAAAA] $[TAATA]_9$ [TAAAA] $[TAATA]_6$
	E	26	$[TAATA]_7$ [TAAAA] $[TAATA]_{11}$ [TAAAA] $[TAATA]_6$

DYS393	A	13	$[AGAT]_{13}$
	B	12	$[AGAT]_{12}$
	C	13	$[AGAT]_{13}$
	D	14	$[AGAT]_{14}$
	E	14	$[AGAT]_{14}$

DYS462	A	11	$[TATG]_{11}$
	B	11	$[TATG]_{11}$
	C	12	$[TATG]_{12}$
	D	13	$[TATG]_{13}$
	E	12	$[TATG]_{12}$

DYS448	A	19	$[AGAGAT]_{11}$ N_{42} $[AGAGAT]_8$
	B	21	$[AGAGAT]_{13}$ N_{42} $[AGAGAT]_8$
	C	21	$[AGAGAT]_{13}$ N_{42} $[AGAGAT]_8$
	D	21	$[AGAGAT]_{12}$ N_{42} $[AGAGAT]_9$
	E	20	$[AGAGAT]_{12}$ N_{42} $[AGAGAT]_8$

DYS426	A	12	$[GTT]_{12}$
	B	11	$[GTT]_{11}$
	C	11	$[GTT]_{11}$
	D	11	$[GTT]_{11}$
	E	11	$[GTT]_{11}$

DYS385a	A	12	$[GAAA]_{12}$
	B	14	$[GAAA]_{14}$
	C	17	$[GAAA]_{17}$
	D	14	$[GAAA]_{14}$
	E	13	$[GAAA]_{13}$

DYS437	A	15	$[TCTA]_9$ $[TCTG]_2$ $[TCTA]_4$
	B	14	$[TCTA]_8$ $[TCTG]_2$ $[TCTA]_4$
	C	14	$[TCTA]_8$ $[TCTG]_2$ $[TCTA]_4$
	D	16	$[TCTA]_{10}$ $[TCTG]_2$ $[TCTA]_4$
	E	14	$[TCTA]_8$ $[TCTG]_2$ $[TCTA]_4$

DYS435	A	12	$[TGGA]_{12}$
	B	11	$[TGGA]_{11}$
	C	11	$[TGGA]_{11}$
	D	11	$[TGGA]_{11}$
	E	11	$[TGGA]_{11}$

DYS385b	A	15	$[GAAA]_{15}$
	B	17	$[GAAA]_{17}$
	C	20	$[GAAA]_{20}$
	D	15	$[GAAA]_{15}$
	E	15	$[GAAA]_{15}$

DYS436	A	12	$[GTT]_{12}$
	B	12	$[GTT]_{12}$
	C	12	$[GTT]_{12}$
	D	12	$[GTT]_{12}$
	E	12	$[GTT]_{12}$

GATA-H4	A	12	$[TAGA]_{12}$
	B	12	$[TAGA]_{12}$
	C	12	$[TAGA]_{12}$
	D	12	$[TAGA]_{12}$
	E	11	$[TAGA]_{11}$

Table 5. Results for Various Y-STR Markers From NIST and Interlaboratory Studies
Obtained on SRM 2395 Components A Through E

NOTE: The number of overlapping results obtained by sequencing or typing has been tabulated illustrating that some loci have been evaluated to a greater extent than others. The NIST typing column contains a summary of the number of different results obtained with various assays or commercial kits at NIST (see Table 6).

Marker	Number Overlapping Results	NIST Sequencing	NIST Typing	ReliaGene Typing	OligoTrail Typing	Leiden Sequencing
DYS19	9	X	X (5)	X	X	X
DYS385 a/b	8	X	X (5)	X	X	
DYS389 I	8	X	X (4)	X	X	X
DYS389 II	9	X	X (5)	X	X	X
DYS390	8	X	X (4)	X	X	X
DYS391	9	X	X (5)	X	X	X
DYS392	9	X	X (5)	X	X	X
DYS393	8	X	X (4)	X	X	X
DYS438	6	X	X (4)	X		
DYS439	6	X	X (4)	X		
DYS437	4	X	X (3)			
YCAII a/b	3		X (2)		X	
DYS388	5	X	X (2)		X	X
DYS426	2	X	X (1)			
DYS435	2	X	X (1)			
DYS436	2	X	X (1)			
DYS447	3	X	X (2)			
DYS448	3	X	X (2)			
DYS450	1		X (1)			
DYS456	1		X (1)			
DYS458	1		X (1)			
DYS460 (A7.1)	4	X	X (2)			X
DYS461 (A7.2)	2	X				X
DYS462	1					X
DYS464 a/b/c/d	1		X (1)			
Y-GATA-H4	3	X	X (2)			

Table 6. Comparison of Y-STR Markers Present in Commercial Kits and Multiplex Assays Typed at NIST

NOTE: The dye label for each marker is indicated.

Marker	**Y-PLEX 6 kit** [7]	**Y-PLEX 5 kit**	**PowerPlex Y kit** (prototype)	**OligoTrail Y kit** (prototype)	**NIST 20plex** [4]	**NIST 11plex** [9,10]	**NIST 10plex** [6]
DYS19	6FAM		JOE	ROX	NED		6FAM
DYS385 a/b	TAMRA		TMR	ROX	VIC	VIC	
DYS389 I DYS389 II	6FAM (389II only)	6FAM	FL	6FAM 6FAM	6FAM		
DYS390	TAMRA		TMR	JOE	VIC		
DYS391	TAMRA		FL	TAMRA	6FAM		TET
DYS392		TAMRA	JOE	TAMRA	NED		HEX
DYS393	6FAM		TMR	JOE	VIC		
DYS438		TAMRA	JOE		6FAM		HEX
DYS439		HEX	FL		6FAM		6FAM
DYS437			JOE		6FAM		HEX
YCAII a/b				ROX	VIC		
DYS388				JOE	NED		
DYS426					VIC		
DYS435							6FAM
DYS436							6FAM
DYS447					PET	6FAM	
DYS448					PET	6FAM	
DYS450						NED	
DYS456						NED	
DYS458						NED	
DYS460 (A7.1)					NED		TET
DYS464 a/b/c/d						VIC	
Y-GATA-H4					NED		TET

Table 7. Information Values for 42 Y-SNP Loci

NOTE: SRM components were typed through allele-specific hybridization using the Signet™ Y-SNP Identification System (Marligen Biosciences, Ijamsville, MD) [11]. Derived alleles are shaded that help designate Y-Chromosome Consortium (YCC) haplogroups [12].

	SRM 2395 Components					
Y-SNP Marker	A	B	C	D	E	F
Amelogenin	X,Y	X,Y	X,Y	X,Y	X,Y	X,X
DYS391-C/G	C	C	G	C	C	--
M119-A/C	A	A	A	A	A	--
M11-A/G	A	A	A	A	A	--
M124-C/T	C	C	C	C	C	--
M130-C/T	C	C	C	C	C	--
M146-A/C	A	A	A	A	A	--
M150-C/T	C	C	C	C	C	--
M153-T/A	T	T	T	T	T	--
M157-A/C	A	A	A	A	A	--
M168-C/T	T	T	T	T	T	--
M170-A/C	A	A	A	A	C	--
M172-T/G	T	G	T	T	T	--
M174-T/C	T	T	T	T	T	--
M175- +/-	+	+	+	+	+	--
M18- -/+	-	-	-	-	-	--
M182-C/T	C	C	C	C	C	--
M201-G/T	G	G	G	T	G	--
M207-A/G	G	A	A	A	A	--
M2-A/G	A	A	G	A	A	--
M31-G/C	G	G	G	G	G	--
M32-T/C	T	T	T	T	T	--
M33-A/C	A	A	A	A	A	--
M35-G/C	G	G	G	G	G	--
M37-C/T	C	C	C	C	C	--
M3-C/T	C	C	C	C	C	--
M42-A/T	T	T	T	T	T	--
M45-G/A	A	G	G	G	G	--
M52-A/C	A	A	A	A	A	--
M5-C/T	C	C	C	C	C	--
M60- -/+	-	-	-	-	-	--
M75-G/A	G	G	G	G	G	--
M87-T/C	T	T	T	T	T	--
M89-C/T	T	T	C	T	T	--
M94-C/A	A	A	A	A	A	--
M95-C/T	C	C	C	C	C	--
P25-C/A	A	C	C	C	C	--
P3-C/T	C	C	C	C	C	--
P4-G/A	G	G	G	G	G	--
SRY10831-A/G	G	G	G	G	G	--
SRY465-C/T	C	C	C	C	C	--
SRY9138-C/T	C	C	C	C	C	--
Tat-T/C	T	T	T	T	T	--
YCC Haplogroup	R1b	J2	E3a	G	I	

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