A Y-chromosome SNP multiplex for haplogroup assignment of West Eurasian men from Tyrol (Austria)

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Introduction Mountain-valleys preset migration routes and define highly structured areas of human settlement. This is likely to result in the formation of complex genetic patterns, reflecting the influence of the landscape's topology. Y-chromosomal data from studies with high geographic resolution are still in demand. In order to gain a better understanding of the genetic landscape of the male population living in Tyrol, we developed a minisequencing assay for the simultaneous analysis of 19 phylogenetically informative single nucleotide polymorphisms (SNPs) on the human Y-chromosome.

Materials and methods A PCR multiplex for the co-amplification of 19 Y-chromosomal SNP markers was developed. The 10 µl amplification reactions comprised 1x KOD buffer (Novagen, Gibbstown, NJ), 2.5 µg non-acetylated BSA, 5 % (w/v) trehalose (both Sigma-Aldrich, St. Louis, MO), 1 mM $MgSO_4$, 200 µM of each dNTP, 0.2 units KOD hot start DNA polymerase (Novagen), and 2 µl gDNA extract. Information regarding PCR primers is given in Fig. 1. 19-plex PCR was carried out in conventional thermal cyclers using an initial heat soak at 94 °C for 2 min, followed by 35 cycles of 95 °C for 15 s, 58 °C for 1 min, and 72 °C for 1 min. The final extension step at 72 °C was extended to 10 min. Assay optimization was aided by the analysis of the PCR products with ion-pair reversed phase high-performance liquid chromatography electrospray ionization mass spectrometry. The 10 µl minisequencing reactions contained 2.5 µl 5x sequencing buffer, 2.5 µl SNaPshot multiplex kit (both Applied Biosystems, AB, Foster City, CA), 400 µM spermidine, 200 µM spermine (both Sigma-Aldrich), 5 % (w/v) trehalose, 15 nM each SBE primer (Table 1), 12 mM $(NH_4)_2SO_4$, and 1 µl of the enzymatically treated (ExoSAP-IT, USB, Cleveland, OH) and 5x diluted PCR-multiplex products. Thermal cycling comprised 30 cycles of 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 30 s. The minisequencing products were enzymatically treated with shrimp alkaline phosphatase (USB), denatured in formamide containing the internal size standard GS120 LIZ (AB), and subjected to laser induced fluorescence capillary electrophoretic separation for allele calling and haplotyping.

Results The 19-plex Y-SNP assay was successfully applied to the Y-chromosomal haplogroup assignment of DNA samples derived from blood donated by 3,401 volunteers born in Tyrol (Austria, Fig. 2). Figure 3 depicts a representative minisequencing electropherogram obtained for a hg J individual. 17 out of the theoretically 22 distinguishable Y-chromosomal haplogroup and subhaplogroup affiliations were found to be present in this large population sample (Figs.4, 5).

Fig. 1 amplicons, PCR primers, and SNP sites

M9 (C>G, hg K-R): 142 bp, F[3]/R[2] primer: 150 nM each
AGGACCCTGAAATACAGAACTGcaaagaaacggcctaagatggttgaatScttctttattttttctttaatttagacatgttcaaacgttcaatgtcttacatacttagttatgtaagtaAGGTAGCGCTTACTTCATTATGCA

M17 (4G>3G, hg R1a1) M18 (A>3AAA) M19 (T>A): 173 bp, F[3]/R[3] primer: 250 nM each
gCCTGGTCATAACACTGGAAATCagattctgtctactcaccagagtttgtggttgctggttgttaScSgggttttttttaagtgaatttttgggggtttgttaagtggccaaactatttttgtgaagactgttgtaTcgtgggtttcagatgtcTCTACATCAGTTTGTGGTCAGCTc

M45 (A>G, hg P): 139 bp, F[3]/R[3] primer: 400 nM each
GAGAGAGGATATCAAAAATTGGCAGTgaaaaattatagataRgcaaaaagctccttctgaggtccaggccaggagatagtaggatttaagaaacaaacaaacaaaaacaacccacaaatGACCTTTGGTGCCACTGTCAc

M78 (C>T, hg E1b1b1a): 156 bp, F[2]/R[4] primer: 400 nM each
gcgTCGACATGAACACAAATTGATACACTTaacaaagatacttctttcYgcccttccaaatatttcaaaataagctggtcatagtacttgcttttcataaaaagatggtaagcttccaatatttagatttAAGGAAAGGTGAAGGAACACTATcgc

M89 (C>T, hg F-R): 165 bp, F/R primer: 300 nM each
AGCTTCCTGGATTCAGCTCTCTTCCTaaggttatgtacaaaaatctYatctctcactttgcctgagttgcagctaccttgctggtgatcctggacccaaagtgtgccagcctctcctgatactctgtgtgtacctgagcagctattcTGCCAAGACTTCACACA

M96 (G>C, hg E): 145 bp, F[3]/R[3] primer: 250 nM each
gTGCCCTCTCACAGAGCACTTtaaagtgagctgtgatgtgtaacttggaaaacaggtctctcataataSgataaaacactcaggtataatattaaaaacctatggcaaaatatatggtccttttaCAAAGCAACAAAGTGGGTGGC

M170 (A>C, hg I): 120 bp, F[3]/R[3] primer: 500 nM each
gCAGCTCTTATTAAGTTATGTTTTCATATTCTGTGcattatacaaatttactatttttatttacttaaaaatcattgttcMctttttttcagtgtggGTTGTGTCTCACTGTAAAATGAGGAC

M173 = P241 (A>C, hg R1): 83 bp, F[3]/R[3] primer: 300 nM each
gTTTTCTTACAATTCAAGGGCATTTAGaacMctttgtcatctgttaatattcagaaaTGATAAGCCAGTGTTTTGTTTTCAGc

M201 (G>T, hg G): 93 bp, F[3]/R[3] primer: 400 nM each
TCAGATCTAATAATCCAGTATCAACTGAGgKctttcgtaataggtacttagtgttggatgaagctgataggaTGCTGGATATGGGATTTGAAC

M223 (C>T, hg I2b): 158 bp, F[2]/R primer: 300 nM each
gCAGCAAGAGTAAGCAAGAGGCACTgagccgctggagtctgcacattgataaatttacttacagtYgtaaataaattgcatcatcttcagctagtaacacagagtctaatttttatagcggcatacttgcctcCACGACTTTCCTAGACACCAGAAAc

Fig. 2 sampling area

Fig. 3 hg J electropherogram

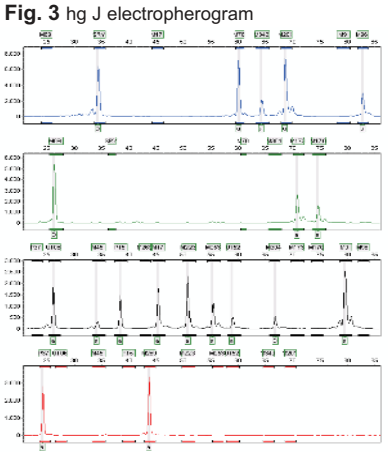


Fig. 1 continued

M253 (C>T, hg I1): 226 bp, F/R primer: 400 nM each
GCTGATCTGTTTCTTTTTGGTGTTactagacaaagctagattttaaaagatgaattaagatgctcagctaactagtcctgtttatagtattgttgatagatagcaagttgaYttctccaggttcttcattgaatgagtccttgtttactatgatgcttgctacatacagttgctacatactactatgtatgagtagttttcTGGTCATAAACTGCATAGAGTTGGAc

M269 (T>C, hg R1b1c): 169 bp, F/R primer: 300 nM each
gCATGCCTAGCCTCATTCCTCtaaaatataatttaaagtggattctgttacatggtatcacaatagaagggaatgatcagggtttggttaatYctggtaaattgaaaacaatttttttttttatcatatgtgcctcagaaggcacacaaAAGAAGTATAGTGGCCGGGC

M304 (A>C, hg J): 112 bp, F[3]/R[3] primer: 300 nM each
gCAAAGAAAAGCAGGAGAGTTTGTAAcaaacagtatgtgggatttttttagatgtgttcaatttgaaagtaacttgtgaMacaaCTGGTGATATTTTGGTATAAGACGTTTc

M343 (C>A, hg R1b): 210 bp, F/R primer: 200 nM each
CTGATTCGCACAAGGCTCAGggtattggtttgaccaggcatgtctttcacgtagccccgagagaaaactggccaccctagccttttaaatatgcaaatgcagagtgccctcgtgttccaMacacctggagatatgtgggggtggctatgctgccaggcacgtgttggggaaagagcaagaggacaaaggtgGGAATCGCCATGTTGAGTGA

P15 (C>T, hg G2a): Y: this study, y: Karafet *et al.* (2008), 203 bp, F[6]/R primer: 200 nM each
CAGAGAGTTTTCTAACAGGGCGtaacaaatagggttaaattatgacttacaggcacactcaacagacagtgaagatgacagtaaagattatcatttcctcacatgaatagagccaatgcttgaggttctgaatcttaYgyctgaaggcagatgaaagttgcaaaagtgatttcccatatttagATAAAGTCCACAAGTGGGCTGG

P37_chrY (T>C, hg D2, hg I2a): 128 bp, F/R primer: 500 nM each
gCATAGTGATAGGGTGGGGATTGGTTcaYagtgtaaaccaaaaataaattTAaaggccccccacaaccatctgaatggattcccgccctttgccagggcaccctaaaACTTAACCTGAGAGTCTGGCTCA

SRY10831 (A>G, hg BR, hg R1a): 152 bp, F[3]/R[3] primer: 400 nM each
gTCATCCAGTCCTTAGCAACCATTAatctggttttagtctctattcatttgccttttcctggatatttcatatacatgggatcattcagtatctggcctcttgtatctgacttttttcacacagtRtaaCATTTTCAAGGTTCACCTATGTGGc

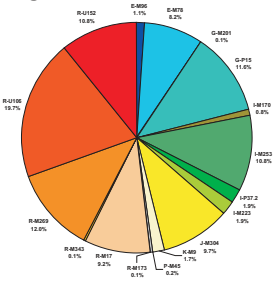
U106 = M405(C>T, hg R1b1b2g): 190 bp, F/R primer: 150 nM each
GACATGCTCTGGTGCATAGGGATTcctgaatagcaaatcccaaagctccaYgggttcaattgcttcttctctgaataacctgccttccccgaccaaacacacaggaagacacatacagctgctaggttttatttccctccaggtgtcatttcaggtccatgacatctcctcTAGGTAGCTTGCATCTGCCA

U152 (G>A, hg R1b1b2h): 164 bp, F/R primer: 200 nM each
GCCTCTTTTTGGCTTCCATAtgatttttaaaatatgttttccaattatctgaagaatgtcaatggtagtttaatgggagtagcattgaatctatacattacttttgagaagtatggRtattttcacaatactcatttttctttatcCTCAAGCGTGGAATGTTTCTC

Table 1 minisequencing primers

Primer	sequence	length
P37sbe_F	CTTAGGGTGGGATTGGTTCA	20 nt
M89sbe_R	TCAACTCAGGCAAAGTGAGAGAT	23 nt
U106sbe_F	(GACT)$_5$GCAAATCCCAAAGCTCCA	26 nt
M45sbe_R [1]	(GACT)$_7$TCTCAGAAGGAGCTTTTTGC	32 nt
SRY1532sbe_F	(GACT)$_7$TCTTGTATCTGACTTTTTCACACAGT	34 nt
P15sbe_F	ACT(GACT)$_5$GACATGCTTGAGGTTCTGAATCTTA	36 nt
M269sbe_F [2]	ACT(GACT)$_5$GAGGAATGATCAGGGTTTGGTTAAT	40 nt
M17sbe_R [1]	(GACT)$_9$GACCAAAATTCACTTAAAAAAACCC	45 nt
M223sbe_F	(GACT)$_6$GACTGCACATTGATAAATTTACTTACAGT	49 nt
M253sbe_F	(GACT)$_7$GTATTGTTGATAGATAGCAAGTTGA	53 nt
U152sbe_F	(GACT)$_6$GACTCTATACATTACTTTGAGAAGTATGG	57 nt
M78sbe_R [3]	(GACT)$_8$GTTTTGAAATATTTGGAAGGGC	58 nt
M343sbe_R	(GACT)$_{11}$TCCACATATCTCCAGGTGT	63 nt
M304sbe_F [2]	(GACT)$_{12}$TGTTCAATTTGAAAGTAACTTGTGA	65 nt
M201sbe_F [4]	(GACT)$_{13}$GATCTAATAATCCAGTATCAACTGAGG	67 nt
M173sbe_F	(GACT)$_{13}$GACTCTTACAATTCAAGGGCATTTAGAAC	69 nt
M170sbe_F [3]	(GACT)$_{13}$CTATTTTATTTACTTAAAAATCATTGTTC	73 nt
M9sbe_F [5]	(GACT)$_{14}$GACGGCCTAAGATGGTTGAAT	77 nt
M96sbe_F	(GACT)$_{17}$CTTGGAAAACAGGTCTCTCATAATA	81 nt

Fig. 5 population study results



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[2] Onofri *et al.*, Forensic Sci Int (2006) 157:23-25
[3] Brión *et al.*, Electrophoresis (2005) 26:4411-4420
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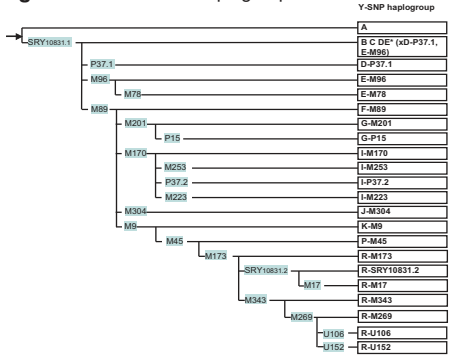
Fig. 4 resolved Y-SNP haplogroups



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