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UniQ-Typer ™ Y-10 genotyping in South African populations: novel alleles, sequence variation and allelic ladder updates

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A B S T R A C T

DNA profiling the male specific region on the Y-chromosome is fundamental to forensic practise. It is recognised as a powerful tool for sexual assault investigations for resolving the male DNA components, for familial search and as a tool to aid biogeographic inferences. In this study the novel Y-chromosome Short Tandem Repeat (Y-STR) multiplex called the UniQ-Typer ™ Y-10 was utilised for population wide genotyping in South Africa. In this study we provide a significant updated to the genotyping allelic ladder for the panel and report novel variation which gave strong signals to potentially infer biogeographic ancestry.

1. Introduction

In forensic casework, Y-STRs are essential for differentiating between unrelated males and resolving the male component of admixed biological evidence. Its value is also recognized in investigations when a suspect is unknown, whereby information may be obtained regarding the paternal lineage or geographical origin of the DNA evidence [1]. The Y-STR panel of the UniQ-Typer™ Y-10 kit namely (DYS710, DYS518, DYS385ab, DYS644, DYS612, DYS626, DYS504, DYS481, DYS447 and DYS449) was previously shown to improve the discrimination amongst individuals of native populations groups in South Africa (SA) in comparison to the core Y-STRs of commercial kits [2–3].

In this study, population wide genotyping for this panel presented a series of novel alleles, microvariants, duplications and triplications. Genotyping herein for the first time some of the rarely studied population in South Africa we identify a total of 12 novel alleles, 13 duplication events and a triplication which provided a significant update to the UniQ-Typer™ Y-10 ladder. We further report on a series of unique population specific variants which have the potential to assists the prediction of groups and/or geographical origin.

2. Methods

2.1. Sample collection

The DNA samples utilized for this study were obtained by informed consent and ethical approval form the University of the Western Cape (10/3/39) and (15/4/97). A total of 2201 unrelated male DNA samples were collected across nine South African provinces as in [4–6]. A total of fifteen ethnic groups were represented as follows: Afrikaner (161); English (111); Indian (104); Coloured (500); Griequa (68); Nama (47); Pedi (198); Venda (122); Southern Sotho (70); Twana (99); Tsonga (118); Swazi (104); Ndebele (16); Zulu (180) and Xhosa (303).

2.2. PCR amplification

DNA samples were amplified using 10.5 µl UniQ-Typer™ master mix and 2.5 µl of the primer mix (10x) in a 25 µl PCR reaction volume. Thermal cycling conditions included an initial denaturation of 98 °C for 30 s, 30 cycles of denaturation at 98 °C for 5 s, 65 °C annealing/extension for 50 s and a final extension at 72 °C for 2 min.

2.3. Capillary electrophoresis

Amplified loci repeat lengths were analysed with capillary electrophoresis (CE) on the Applied Biosystems (ABI) 3500 Genetic Analyser with GeneMapper® data analysis software ID X. v1.4. Spectral calibration was achieved using an in house developed matrix standard [7].

2.4. Allele cloning

Individual Y-STR alleles were amplified with previously published primer sequences [8] and cloned into linearized pMiniT 2.0 using the NEB cloning kit (NEB #E1202). Plasmids were transformed into NEB 10-beta Competent *E. coli* (NEB #C3019). Colony PCR was conducted using the OneTaq 2x Master Mix using 0,3 µM cloning analysis primer and plasmid DNA extracted using the Zymo Research plasmid miniprep kit (#D4054). Correct allele size was confirmed by PCR amplification

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Table 1
UniQ-Typer allelic ladder composition and updates. Alleles in bold font represent updates provided by this study and previously reported for the panel by [4]. Duplications and triplication events observed are underlined in bold font.

Locus	Allelic Ladder composition (in-house)	Size range (bp)	Allele range
DYS710	**26**, 27, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 36.2, 37, 37.2, 38, 38.2, 39, 39.2, 40, 40.2, 41, 41.2, 42, 42.2, 43, 43.2, 44 **26-33.2, 30-30.2, 30.2-31.2, 30.2-32, 30.2-33, 30.2-34, 31-33, 32.2-33.2, 33-37**	159-224	26-44
DYS518	33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, **41-42**	253-301	28-52.2
DYS385	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, **13.2-16, 10-11-13**	242-295	6-28
DYS644	**10**, 12, 13, 14, 15, 16, 17, 18, 19, **19.4**, 20.4, 21.4, **22.3**, 22.4, **23,3** 23.4, **24.3**, 24.4, **25.3**, 25.4, 26.4, **27.4**, **24.4-25.4**	305-398	10-27.4
DYS612	24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, **31-33**	152-194	24-43
DYS626	23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36	231-276	22-52
DYS504	**9**, 11, 12, 13, 14, 15, 16, 17, 18, 19, **20**	294-338	9-20
DYS481	19, 20, 21, 22, 23, 24, **24.2**, 25, 26, 28, 29, **29.2**, 30, 31, 32, 25-26	108-148	16-34
DYS447	17,19, 20, 21, 22, 22.4, 23, 23.4, 24, **24.4**, 25, 26, 27, 28, 29,30	178-243	15-36
DYS449	24, 25, 26, 27, 29, 31, 33, 34, 35, 36, 24, 25, 28, 30, 32, 33, 34, 35, 36,37, 38	270-328	22-42

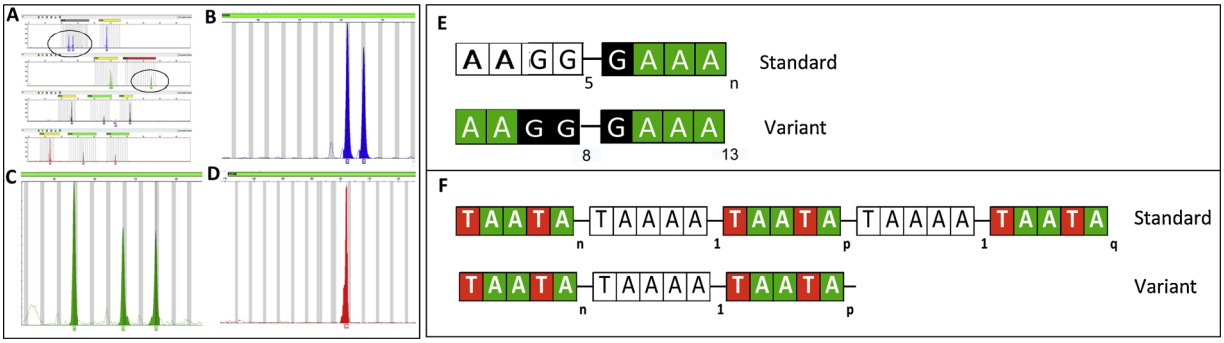

Fig. 1. Novel allele and sequence variation identified in male populations in South Africa.
A) Off-ladder (OL) microvariants observed at locus DYS644 (greed circled) presenting a 1bp deletion within its STR motif. These variants were associated with rare duplication events at locus DYS710 (blue circled). All variants were identified in the Bantu groups Venda and Pedi, in the Khoe group Nama and the admixed Coloured populations from the NE and N South Africa. B) DYS518 duplication (41-42) in Venda, C) DYS385ab triplication (10-11-13) in Pedi, and D) DYS447 microvariant allele (24.4) in Nama. To our knowledge these have not been reported on YHRD, STRbase or in other published population datasets. Atypical sequence structures were identified at E) locus DYS385 for allele 21 presenting 8 tandem [AAGG] repeats in the invariant 5' flanking region. Similar observation previously made by [9–11], suggests that the updated DYS385 structure may be represented as $[AAGG]_{5\text{-}9}[GAAA]_n$. F) Truncated sequence structures (variants) were encountered at locus DYS447 for alleles 17, 19 and 21 which was also previously observed in Korean populations for allele 19 [12]. For (E–F) the clourless boxes [AAGG] and [TAAAA] represents the respective invariant region.

using labelled primers for CE genotyping and unlabeled primers for Sanger sequencing.

2.5. Sanger Sequencing

Cycle sequencing reactions were prepared using the Big Dye Terminator v. 3.1 kit (Thermo Fisher) following the manufacturer recommended conditions. Sequences were aligned to GenBank references AC007972.4, AC010972.3, AC022486.4, AC006462.3, AC006383.2, AC007320.3, AC010972.3, AC016991.5, NT_011875.11 and AC051663.9. All sequenced alleles were submitted previously to GenBank with accession numbers MK005372 - MK005525 [4].

3. Results and discussion

In Table 1, the novel allele 26 at DYS710 was identified in Coloured (admixed) and Nama populations at frequencies of 0,0040 and 0,0213 respectively. The series of novel alleles identified at DYS644 namely (10, 19.4, 22.3, 23.3, 24.3, 25.3 and 27.4) were all identified in the Bantu and Colored (admixed) population groups (see [6] for detailed allele frequencies). The rare allele 20 at DYS504 was observed only in the admixed Griqua and Colored populations with frequencies of 0,0020 and 0,0147 respectively. Rare alleles that were observed only in non-African populations in SA (i.e. European, Asian and Afrikaner) were identified at DYS710 (alleles 41 and 41.2), for DYS518 (alleles 30-33) and at locus DYS626 (alleles 24 and 36). Novel deletion variants at DYS644 [4] were also observed in specific populations and found to have unique association with DYS710 duplications as shown below in Fig. 1.

4. Conclusion

We previously studied a highly informative set of 10 Y-STR markers in order to maximizes male discrimination in South African populations. Implementation of a highly informative male specific Y-STR panel may be crucial for improving conviction rates, exonerating innocent men accused of rape, and alleviating the backlog of sexual assault casework. In this study we describe a series of rare alleles, novel variants and atypical sequences which may potentially aid male identification, exclusion and biogeographic prediction for when a suspect is unknown. These variants were used to update the UniQ-Typer™ allelic ladder.

Declaration of Competing Interest

We declare no conflict of interest

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