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Allele Frequency of 15 Short Tandem Repeats (Strs) in a Buganda Population (Central Uganda): Forensic Utility and Parentage Testing

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Abstract

The allele frequency of 15 Short tandem repeat (STRs) loci of the Buganda population-Central Uganda was determined in 221 unrelated individuals from the randomly selected 10 districts of the Buganda region. The DNA samples were extracted following Chelex extraction protocol, amplified using the AmpFlSTR® Identifiler Plus™ PCR amplification kit, and separated using capillary electrophoresis on an ABI 3130xl Genetic Analyzer. All loci were in Hardy-Weinberg equilibrium expectations and the Buganda population is heterozygous. When combined, combined power of exclusion (CPE), combined match probability (CMP) and combined power of discrimination (CPD) were 0.99981, 1 in 2.471×10^{17} and 0.999999998 respectively an indication that the loci are highly informative, polymorphic and discriminative useful in paternity and forensic testing in the Buganda population.

Keywords: Short tandem repeats; Allele frequency; Buganda population

Introduction

Autosomal STR markers were first described as effective tools for human identity testing in the early 1990s [1] and have become the common currency of data exchange for human identity testing both in forensic casework and paternity testing [2]. Population databases allow for estimations of how rare or common a DNA profile may be in a particular population [3]. One population for which Short Tandem Repeats (STR) allele frequencies are not available is the Baganda tribe of Uganda. The aim of this work was to generate a Buganda population based 15 STR allele frequency data. We genotyped by multiplex PCR system using AmpF/STR® Identifiler™ Plus PCR Amplification Kit that co-amplifies the 13 Combined DNA Index System (CODIS) STR loci plus additional two tetrameric markers; D2S1338 and D19S433, as well as the amelogenin locus for gender identification. In this study we present the allele frequencies and statistical data of forensic importance in the Buganda population.

Materials and Methods

Ethical clearance was sought from the School of Biomedical Sciences Institutional Review Committee (IRC) College of Health Sciences of Makerere University, (Research file reference: SBS105) and Uganda National Council of Science and Technology (Research file reference: HS 1422). Blood samples were obtained from 221unrelated individuals in the 10 randomly selected districts of the Buganda-Central Uganda who had previously consented. DNA was extracted following Chelex Extraction protocol [4] and amplified using the AmpFlSTR® Identifiler Plus™ PCR amplification kit following the manufacturer's user manual. Separation of the amplicons was done by capillary electrophoresis on an ABI 3130xl Genetic Analyzer using HiDi Formamide to fragment the bases and Gene Scan 500 Liz to size the fragmented bases following Applied Biosystems Instruction Manual (2006): Starting Electrophoresis. The resulting STRs were genotyped using the GeneMapper® ID software V3.2. Allele frequency determination, Exact test probability for Hardy-Weinberg equilibrium (p-value), observed heterozygosity (Ho), expected heterozygosity (He) and inbreeding coefficient expressed as Wright's fixation index (F_{is}) were computed using GenePop v 4.1.3 software. Forensic and paternity efficiency parameters were computed using Promega Powerstat v12 excel spreed sheet.

Results and Discussion

The allele frequency of the 15 STRs generated of the Buganda population (Table 1) was below 50% at all loci as shown by the predominant alleles at each loci reflecting the usefulness and validity of these loci in calculating paternity indices and discriminating individuals [5]. All samples were successfully amplified and genotyped as shown by electropherogram images of the positive control; a quality control measure in genotyping process and selected samples of the Buganda population at the most heterozygous, polymorphic, and discriminating loci namely D21S11, D2S1338, D19S433 and FGA loci (Figure 1). All loci were in Hardy-Weinberg equilibrium (p-values >0.05) with the observed heterozygosity very close to the expected heterozygosity. High levels of observed heterozygosity ranging from 69.23% (D7S820) to 87.78% (D21S11) is an indication that the Buganda population has a high level of genetic variation and this could be successfully utilized in discriminating between individuals [6]. Both the heterozygosity and Fis values indicate that Buganda population is a heterozygous population with limited genetic inbreeding if any [7] and this is attributed to cultural practice of intermarriages among different clans and prohibition of marriages within the same clan.

All loci were highly polymorphic with polymorphic information content (PIC) values ranging from 0.67 at D13S317 to 0.89 at D2S1338 all above 0.5 indicating good informativeness of all the tested STR markers and useful for identification purposes [6]. The power of discrimination (PD) across all tested loci was above 80% (from 0.875 at D13S317 to 0.978 at D2S1338) an indication that all loci are highly discriminating, that is, an innocent person will be excluded as the donor of an evidence unknown sample [6]. The most informative markers were

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Allele	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
6	_	_	_	0.002	_	0.17	_	_	_	_	_	0.081	_	_	_
7	_	_	0.011	0.045	_	0.396	_	_	_	_	_	0.011	_	_	_
8	0.002	_	0.195	0.027	_	0.222	0.02	0.045	_	_	_	0.292	_	0.059	_
9	_	_	0.102	0.138	_	0.161	0.016	0.224	_	0.009	_	0.271	_	0.014	_
9.3	_	_	_	_	_	0.038	_	_	_	_	_	_	_	_	_
10	0.007	_	0.423	0.283	_	0.009	0.032	0.124	_	0.014	_	0.077	_	0.066	_
11	0.048	_	0.172	0.179	_	0.002	0.348	0.303	_	0.088	0.007	0.231	0.002	0.219	_
11.2	_	_	_	_	_	_	_	_	_	0.007	_	_	_	_	_
12	0.138	_	0.086	0.251	0.002	0.002	0.351	0.197	_	0.113	0.002	0.034	0.02	0.342	_
12.2	_	_	_	_	_	_	_	_	_	0.018	_	_	_	_	_
13	0.138	_	0.011	0.068	0.002	_	0.186	0.095	0.002	0.226	0.025	0.002	0.061	0.271	_
13.2	_	_	_	_	_	_	_	_	_	0.086	_	_	0.007	_	_
14	0.33	_	_	0.007	0.095	_	0.045	0.011	_	0.238	0.1	_	0.057	0.025	_
14.2	_	_	_	_	_	_	_	_	_	0.038	_	_	0.007	_	_
15	0.256	_	_	_	0.324	_	0.002	_	_	0.068	0.215	_	0.17	0.002	_
15.2	_	_	_	_	_	_	_	_	_	0.077	_	_	0.007	_	_
16	0.066	_	_	_	0.328	_	_	_	0.07	0.007	0.242	_	0.21	_	_
16.2	_	_	_	_	_	_	_	_	_	0.009	_	_	0.007	_	_
17	0.014	_	_	_	0.204	_	_	_	0.127	_	0.186	_	0.176	0.002	0.002
18	0.002	_	_	_	0.045	_	_	_	0.095	_	0.143	_	0.136	_	0.005
18.2	_	_	_	_	_	_	_	_	_	_	_	_	0.002	_	_
19	_	_	_	_	_	_	_	_	0.145	_	0.061	_	0.068	_	0.043
13.2	_	_	_	_	_	_	_	_	_	0.086	_	_	0.007	_	_
14	0.33	_	_	0.007	0.095	_	0.045	0.011	_	0.238	0.1	_	0.057	0.025	_
14.2	_	_	_	_	_	_	_	_	_	0.038	_	_	0.007	_	_
15	0.256	_	_	_	0.324	_	0.002	_	_	0.068	0.215	_	0.17	0.002	_
15.2	_	_	_	_	_	_	_	_	_	0.077	_	_	0.007	_	_
16	0.066	_	_	_	0.328	_	_	_	0.07	0.007	0.242	_	0.21	_	_
16.2	_	_	_	_	_	_	_	_	_	0.009	_	_	0.007	_	_
17	0.014	_	_	_	0.204	_	_	_	0.127	_	0.186	_	0.176	0.002	0.002
18	0.002	_	_	_	0.045	_	_	_	0.095	_	0.143	_	0.136	_	0.005
18.2	_	_	_	_	_	_	_	_	_	_	_	_	0.002	_	_
19	_	_	_	_	_	_	_	_	0.145	_	0.061	_	0.068	_	0.043
19.2	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0.002
20	_	_	_	_	_	_	_	_	0.063	_	0.02	_	0.041	_	0.036
21	_	_	_	_	_	_	_	_	0.133	0.002	_	_	0.02	_	0.077
22	_	_	_	_	_	_	_	_	0.113	_	_	_	0.005	_	0.231
23	_	_	_	_	_	_	_	_	0.095	_	_	_	0.002	_	0.176
24	_	_	_	_	_	_	_	_	0.072	_	_	_	_	_	0.156
24.2	_	0.002	_	_	_	_	_	_	_	_	_	_	_	_	_
25	_	_	_	_	_	_	_	_	0.043	_	_	_	_	_	0.102
26	_	_	_	_	_	_	_	_	0.025	_	_	_	_	_	0.057
27	_	0.05	_	_	_	_	_	_	0.016	_	_	_	_	_	0.052
28	_	0.242	_	_	_	_	_	_	_	_	_	_	_	_	0.02
29	_	0.226	_	_	_	_	_	_	_	_	_	_	0.002	_	0.018
29.2	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0.002
30	_	0.174	_	_	_	_	_	_	_	_	_	_	_	_	0.005
30.2	_	0.007	_	_	_	_	_	_	_	_	_	_	_	_	0.007
31	_	0.093	_	_	_	_	_	_	_	_	_	_	_	_	_
31.2	_	0.034	_	_	_	_	_	_	_	_	_	_	_	_	0.007
32	_	0.016	_	_	_	_	_	_	_	_	_	_	_	_	_
32.2	_	0.061	_	_	_	_	_	_	_	_	_	_	_	_	0.002
33	_	0.005	_	_	_	_	_	_	_	_	_	_	_	_	_
33.2	_	0.027	_	_	_	_	_	_	_	_	_	_	_	_	_
34	_	0.016	_	_	_	_	_	_	_	_	_	_	_	_	_
34.2	_	0.002	_	_	_	_	_	_	_	_	_	_	_	_	_
35	_	0.029	_	_	_	_	_	_	_	_	_	_	_	_	_
36	_	0.011	_	_	_	_	_	_	_	_	_	_	_	_	_
37	_	0.005	_	_	_	_	_	_	_	_	_	_	_	_	_
H (exp)	0.782	0.843	0.737	0.800	0.737	0.800	0.719	0.794	0.899	0.854	0.828	0.776	0.865	0.755	0.867
H (obs)	0.719	0.878	0.692	0.819	0.747	0.783	0.729	0.760	0.864	0.824	0.805	0.742	0.842	0.697	0.864
Fis	0.041	-0.023	0.083	-0.005	-0.013	-0.036	-0.025	0.020	0.031	0.012	0.028	0.049	0.013	0.044	0.002
P	0.093	0.128	0.183	0.850	0.250	0.543	0.691	0.210	0.351	0.379	0.325	0.066	0.545	0.720	0.790
MP	0.079	0.050	0.105	0.075	0.120	0.114	0.125	0.074	0.022	0.040	0.054	0.091	0.035	0.095	0.033
PD	0.921	0.95	0.895	0.925	0.88	0.886	0.875	0.926	0.978	0.96	0.946	0.909	0.965	0.905	0.967
PIC	0.75	0.82	0.7	0.77	0.69	0.7	0.67	0.76	0.89	0.84	0.8	0.74	0.85	0.71	0.85
PE	0.459	0.75	0.416	0.635	0.504	0.568	0.474	0.527	0.723	0.643	0.609	0.496	0.678	0.423	0.723
TPI	1.78	4.09	1.63	2.76	1.97	2.3	1.84	2.08	3.68	2.83	2.57	1.94	3.16	1.65	3.68

H (exp): Expected heterozygosity
H (obs): Observed heterozygosity
Fis: Wright's fixation index
P: Exact test probability for Hardy-Weinberg equilibrium
MP: Matching Probability
DP: Power of Discrimination
PIC: Polymorphic Information Content
PE: Power of Exclusion
TPI: Typical Paternity Index

Table 1: Allele frequency and Forensic statistical parameters of 15 short tandem repeats loci (STRs) in a Buganda Population-Central Uganda (n =221; n; number of individuals sampled).

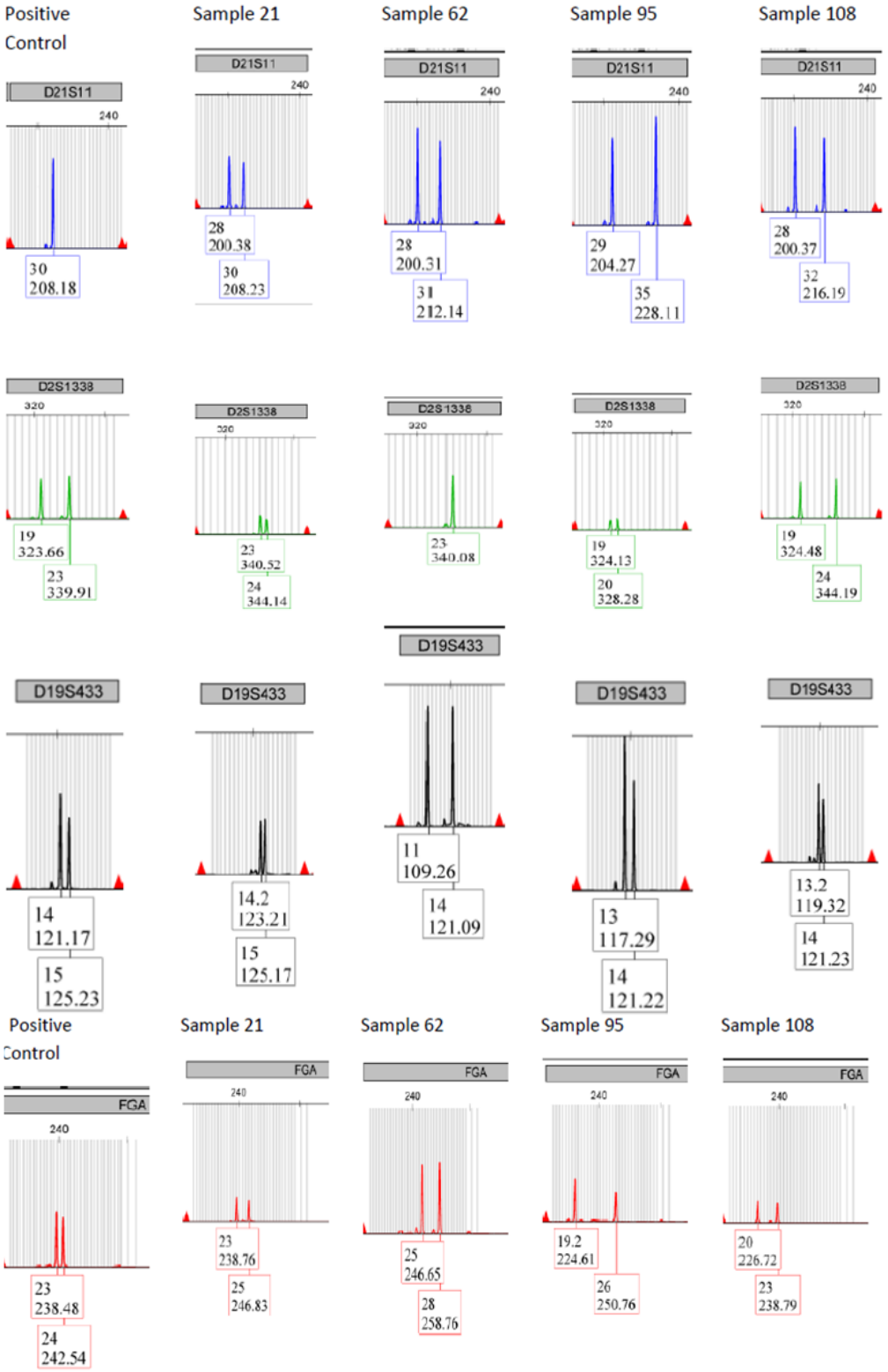

Figure 1: Electropherograms for the positive control and sample 21, sample 62, sample 95 and sample 108 of the Buganda population showing allele call and DNA size in base pairs (bp) at D21S11, D2S1338, D19S433 and FGA loci. The positive control; a quality control measure in genotyping process illustrates that amplification and genotyping were successful. These loci were highly heterozygous and polymorphic with high discriminating (PD) values and power of exclusion (PE) values. Microvariant alleles were observed at some loci in the Buganda population as shown at D19S433 of sample 21 and FGA of sample 95. The DNA molecules are separated according to their respective sizes. So the smallest fragments are chronologically detected first and if two (or more) fragments have the same size, they are distinguished by the fluorescence color. The use of colors (blue, green, yellow and red) with different diffusivity included in the PCR multiplex, allow distinguishing the overlapping fragments (same size).

the most discriminating ones in the Buganda population as it was in the population of Island of Cres, Croatia [7]. The calculated combined match probability (CMP) of 1 in 2.471×10^{17} and the combined power of discrimination (CPD) which was greater than 0.999999998 in the Buganda population will have an important role in the analysis of mixed DNA profiles such as in sexual assault cases as well as matching a control sample to the unknown sample [6].

The typical paternity index values at all loci were greater than 1, indicative of relatedness [8,9]. High paternity exclusion (PE) values ranging from 0.416 (D7S820) to 0.750 (D21S11) were observed for the Buganda population which showed that the loci could powerfully exclude a falsely accused individual as a biological father to a child and the higher the PE value, the more non-fathers are excluded [9]. Combined, the 15 loci yielded a combined typical paternity index (CTPI) of 565,852.41 and a combined power of paternity exclusion (CPE) of 0.99981 (greater than 99%) indicating a high power of exclusion [6].

Conclusion

The Buganda population is genetically diverse, all loci were very informative, highly polymorphic with high power of discrimination and exclusion. When combined, these loci are powerful genetic markers for forensic identification and paternity testing in the Buganda population.

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