Applicability of Human-specific STR systems, GlobalFiler™ PCR Amplification Kit, Investigator 24plex QS Kit, and PowerPlex® Fusion 6C in Chimpanzee (Pan troglodytes)

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Research note

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Abstract

Objectives

The human identification systems based on STRs are widely used in human population genetics and forensic analysis. This study aimed to validate the cross-reactivity of three widely known Human-specific STR identification systems i.e. GlobalFiler™ PCR Amplification Kit, Investigator 24plex QS Kit, and PowerPlex® Fusion 6C in Chimpanzee.

Result

The present study revealed the successful amplification of 18 loci using GlobalFiler™ PCR Amplification Kit, 18 loci using Investigator 24plex QS Kit, and 20 loci using PowerPlex® Fusion 6C system. The marker Amelogenin (AMEL) showed differential allele size between male and female revealing the gender identity of Chimpanzees and thus validates their application concerning forensic examination, population estimation, and genetic analysis.

Introduction

Human DNA profiling is a well-known method of identification in the field of forensic science. Earlier, the forensic DNA analysis was used to perform in high profile cases or particularly in cases where the other identification methods such as fingerprinting and anthropometric features failed to produce the identity due to the unavailability of required evidence. With technological inventions and scientific developments, DNA profiling became less needed and rather regularised in general practice. In the light of this practice, several profiling kits based on Short Tandem Repeats (STRs) markers, starting from four (Quadruplex) to 27 (PowerPlex Fusion 6C) markers and more were developed [1]. While developing these marker kits, developmental validations were carried out on several aspects including species specificity [2]. Validation of species specificity is a critical parameter as the forensic casework samples may encounter non-human DNA in an actual or fabricated crime scene and therefore cross-reactivity of markers are critical to assess [3]. The most common non-human DNA which is used for the assessment of cross specificity belongs to Chimpanzees, Orangutans, Bonobos, Gorillas, Macaque, Cat, Horse, Dog, Cow, Sheep, Goat and Pigs [3]. Among all these animals, non-human primates DNA may amplify within the marker range of humans [3]. To assess the extent of amplification of Human STR markers in non-human primates, few earlier studies i.e. Thakur et al. [4] and Singh et al. [5] performed cross-species validation of several commercial forensic STR kits in Chimpanzees. These studies also assessed the power of individual identification of forensically important markers in the case of Chimpanzees. This provides the scope of future application of these marker kits in case of challenging samples and individual identification. Therefore it is essential to validate the cross-reactivity of all the marker sets which are commercially utilized in forensic casework.

In this study, we performed the cross-species assessment of three commercially and widely used Human identification STR marker kits i.e. GlobalFiler™ PCR Amplification Kit, Investigator 24plex QS Kit, and

Methods

Sample collection and DNA extraction

Two male (Chhotu and Mastan) and one female (Buri) Chimpanzee hair samples were received from the Alipore Zoological Garden, Kolkata. These Chimpanzees were juvenile and were confiscated by the officials of the Wildlife Crime Control Bureau (Eastern Unit). Genomic DNA extraction was carried out from the hair follicles using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Germany), following the manufacturer's protocol.

PCR amplification and Genotyping

Three most widely used 6 dye multiplex Human STR systems i.e. GlobalFiler™ PCR Amplification Kit, Investigator 24plex QS Kit, and PowerPlex® Fusion 6C System were used for the genotyping of the samples. The GlobalFiler™ PCR Amplification Kit includes 21 autosomal STR markers i.e. D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338 with Amelogenin (AMEL) as a gender determining marker, DYS391 as Y STR marker and one Y-indel polymorphic marker. The Investigator 24plex QS Kit includes 22 CODIS recommended STR markers TH01, D3S1358, vWA, D21S11, TPOX, DYS391, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, D7S820 including two quality control sensors QS1 and QS2 and a gender determination marker Amelogenin. The PowerPlex® Fusion 6C System following the recommendations of both CODIS and ESS consists of 18 expanded CODIS core loci i.e. CSF1PO, FGA, TH01, vWA, D1S1656, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11 and five highly discriminative loci i.e. Penta D, Penta E, D22S1045, TPOX, and SE33. It also includes two gender determining markers that are Amelogenin and DYS391 and two rapidly mutating Y-STR markers i.e. DYS570 and DYS576. For each marker system, separate PCR reactions were carried out in a total volume of 25 µl with both positive and negative controls on the GeneAmp PCR system 9700 thermocycler (Applied Biosystems, USA) following the manufacturer's protocols. Fragment analyses of the amplified products were carried on ABI 3130 Genetic analyzer (Applied Biosystems, USA).

Data analysis

Genotype calling was performed using GeneMapper ID v.3.2 and the scoring and rearrangement of allelic data were performed in Microsoft Excel. Genetic diversity indices such as Observed heterozygosity (H_o), Expected heterozygosity (H_e), Unbiased heterozygosity (uH_e), Observed number of alleles (N_a), Expected number of alleles (N_e), and Fixation Index (F) were obtained using GENEALLEX v.6.5 [6]. Since the individuals were confiscated animals, therefore genealogical analysis to establish the extent of the

relationship was critical. Hence, the likelihood of relatedness between the individuals was estimated using ML-Relate [7].

Results

The gender determination marker AMEL successfully assigned the gender of known chimpanzees in all the three PCR identification kits with an allele size of 98 in female and 98 and 104 in male (GlobalFiler), 77 in female and 77 and 80 in male (Investigator 24plex), 80 in female and 80 and 86 in male (PowerPlex® Fusion 6C). Out of the total markers tested with all the three kits, three markers i.e. D5S818, SE33, and D12S391 of GlobalFiler, three markers i.e. D12S391, FGA, and D7S820 including QS1 and QS2 of Investigator 24plex, five markers i.e. Penta E, D5S818, SE33, DYS576 and DYS570 of PowerPlex® Fusion 6C failed to amplify in all the individuals while the rest of the markers were observed polymorphic. The genetic diversity analysis based on autosomal STRs revealed a total of 55 alleles ranging from 6 (D1S1656) to 1 (D21S11, TH01, D7S820) with an average of 3.06 ± 0.36 alleles per locus using GlobalFiler, 67 alleles ranging from 6 (D1S1656, D2S441) to 1 (SE33 and D5S818) with an average of 3.72 ± 0.36 alleles per locus using Investigator 24plex, while the PowerPlex® Fusion 6C system revealed a total of 69 alleles ranging from 5 (D1S1656, D2S441, D13S317, D2S1338, D19S433, D22S1045) to 1 (D12S391) with an average of 3.45 ± 0.29 alleles per locus (Table 1–3). The mean H_o i.e. 0.46 ± 0.08 was observed lower than the mean H_e of 0.51 ± 0.07 with an average uH_e of 0.62 ± 0.08 in the GlobalFiler (Table 1). The other two kits i.e. Investigator 24plex and PowerPlex® Fusion 6C system showed higher H_o i.e. 0.63 ± 0.08 and 0.68 ± 0.07 with respect to H_e i.e. 0.61 ± 0.06 and 0.58 ± 0.05 (Table 1&2). Except in the case of Globalfiler amplification kit with a fixation index (F) of 0.06 ± 0.11 , the other two kits revealed negative value of fixation index i.e. -0.05 ± 0.10 (Investigator 24plex) and -0.19 ± 0.08 (PowerPlex® Fusion 6C) (Table 1–3). Further, the genealogical relationship analysis based on 13 loci (D3S1358, vWA, CSF1PO, D16S539, TPOX, D2S441, D8S1179, TH01, FGA, D13S317, D10S1248, D1S1656, D2S1338) in Globalfiler, 16 loci in both Investigator 24plex (TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, D16S539, CSF1PO, D13S317) and PowerPlex® Fusion 6C ((D1S1656, D2S441, D13S317, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D7S820, TPOX, D8S1179, D19S433, D22S1045, FGA) revealed that the examined individuals were genetically unrelated with Delta Ln(L) of 9999 (Table S1).

Table 1
Genetic diversity indices of Chimpanzees with GlobalFiler™ PCR Amplification Kit

Locus	N	Na	Ne	Ho	He	uHe	F
D3S1358	3	4.000	3.600	0.667	0.722	0.867	0.077
vWA	3	3.000	3.000	0.000	0.667	0.800	1.000
D16S539	3	4.000	3.600	0.333	0.722	0.867	0.538
CSF1PO	3	2.000	1.800	0.667	0.444	0.533	-0.500
TPOX	3	2.000	1.385	0.333	0.278	0.333	-0.200
D8S1179	3	5.000	4.500	0.667	0.778	0.933	0.143
D21S11	1	1.000	1.000	0.000	0.000	0.000	#N/A
D18S51	2	2.000	1.600	0.500	0.375	0.500	-0.333
D2S441	3	5.000	4.500	0.667	0.778	0.933	0.143
D19S433	2	2.000	2.000	0.000	0.500	0.667	1.000
TH01	3	1.000	1.000	0.000	0.000	0.000	#N/A
FGA	3	4.000	3.000	0.667	0.667	0.800	0.000
D22S1045	2	2.000	1.600	0.500	0.375	0.500	-0.333
D13S317	3	3.000	2.571	1.000	0.611	0.733	-0.636
D7S820	2	1.000	1.000	0.000	0.000	0.000	#N/A
D10S1248	3	4.000	3.600	0.667	0.722	0.867	0.077
D1S1656	3	6.000	6.000	1.000	0.833	1.000	-0.200
D2S1338	3	4.000	3.600	0.667	0.722	0.867	0.077
Mean (± SE)	2.67 ± 0.14	3.06 ± 0.36	2.74 ± 0.34	0.46 ± 0.08	0.51 ± 0.07	0.62 ± 0.08	0.06 ± 0.11
Na: Observed number of alleles, Ne: effective number of alleles, Ho: observed heterozygosity, He: expected heterozygosity, UHe: unbiased expected heterozygosity, F: fixation index							

Table 2
Genetic diversity indices of Chimpanzees with Investigator 24plex QS Kit

Locus	N	Na	Ne	Ho	He	uHe	F
TH01	3	2.000	1.385	0.333	0.278	0.333	-0.200
D3S1358	3	3.000	2.571	1.000	0.611	0.733	-0.636
vWA	3	3.000	3.000	0.000	0.667	0.800	1.000
D21S11	3	5.000	4.500	0.667	0.778	0.933	0.143
TPOX	3	3.000	2.571	1.000	0.611	0.733	-0.636
D1S1656	3	6.000	6.000	1.000	0.833	1.000	-0.200
SE33	1	1.000	1.000	0.000	0.000	0.000	#N/A
D10S1248	3	5.000	4.500	0.667	0.778	0.933	0.143
D22S1045	3	4.000	3.600	0.667	0.722	0.867	0.077
D19S433	3	5.000	4.500	0.667	0.778	0.933	0.143
D8S1179	3	4.000	3.600	0.667	0.722	0.867	0.077
D2S1338	3	5.000	4.500	1.000	0.778	0.933	-0.286
D2S441	3	6.000	6.000	1.000	0.833	1.000	-0.200
D18S51	3	4.000	3.600	0.333	0.722	0.867	0.538
D16S539	3	4.000	3.600	0.667	0.722	0.867	0.077
CSF1PO	3	2.000	1.800	0.667	0.444	0.533	-0.500
D13S317	3	4.000	3.600	1.000	0.722	0.867	-0.385
D5S818	2	1.000	1.000	0.000	0.000	0.000	#N/A
Mean ($\pm$ SE)	2.83 $\pm$ 0.12	3.72 $\pm$ 0.36	3.41 $\pm$ 0.35	0.63 $\pm$ 0.08	0.61 $\pm$ 0.06	0.73 $\pm$ 0.07	-0.05 $\pm$ 0.10
Na: Observed number of alleles, Ne: effective number of alleles, Ho: observed heterozygosity, He: expected heterozygosity, UHe: unbiased expected heterozygosity, F: fixation index							

Table 3
Genetic diversity indices of Chimpanzees with PowerPlex® Fusion 6C System

Locus	N	Na	Ne	Ho	He	uHe	F
D3S1358	2	3.000	2.667	1.000	0.625	0.833	-0.600
D1S1656	3	5.000	4.500	0.667	0.778	0.933	0.143
D2S441	3	5.000	4.500	0.667	0.778	0.933	0.143
D10S1248	2	3.000	2.667	1.000	0.625	0.833	-0.600
D13S317	3	5.000	4.500	1.000	0.778	0.933	-0.286
D16S539	3	4.000	3.600	0.667	0.722	0.867	0.077
D18S51	3	2.000	1.800	0.667	0.444	0.533	-0.500
D2S1338	3	5.000	4.500	1.000	0.778	0.933	-0.286
CSF1PO	3	2.000	1.800	0.667	0.444	0.533	-0.500
Penta D	3	2.000	1.385	0.333	0.278	0.333	-0.200
TH01	3	3.000	2.000	0.333	0.500	0.600	0.333
vWA	3	4.000	3.000	0.667	0.667	0.800	0.000
D21S11	1	2.000	2.000	1.000	0.500	1.000	-1.000
D7S820	3	4.000	3.000	0.667	0.667	0.800	0.000
TPOX	3	2.000	1.385	0.333	0.278	0.333	-0.200
D8S1179	3	3.000	2.000	0.333	0.500	0.600	0.333
D12S391	1	1.000	1.000	0.000	0.000	0.000	#N/A
D19S433	3	5.000	4.500	0.667	0.778	0.933	0.143
D22S1045	3	5.000	4.500	1.000	0.778	0.933	-0.286
FGA	3	4.000	3.600	1.000	0.722	0.867	-0.385
Mean ($\pm$ SE)	2.70 $\pm$ 0.15	3.49 $\pm$ 0.29	2.95 $\pm$ 0.28	0.68 $\pm$ 0.07	0.58 $\pm$ 0.05	0.76 $\pm$ 0.06	-0.19 $\pm$ 0.08
Na: Observed number of alleles, Ne: effective number of alleles, Ho: observed heterozygosity, He: expected heterozygosity, UHe: unbiased expected heterozygosity, F: fixation index							

Discussion

This study attempted to cross-validate the amplification efficiency and gender determination power of human-specific STRs, included in the most commonly used human STR identification system i.e.

GlobalFiler™ PCR Amplification Kit, Investigator 24plex QS Kit, and PowerPlex® Fusion 6C System. The marker AMEL successfully identified the gender of three known chimpanzees. The genetic diversity analysis and genealogical relationship analysis revealed higher heterozygosity with several alleles and Delta Ln(L) with a value of 9999 indicating that the examined chimpanzees were genetically unrelated.

The three human STR identification systems can be used for the population assessment and genetic identification in non-human primate-like chimpanzees and also can be tested for their applicability on other non-human primates like Orangutans, Bonobos, Gorillas, Macaque and Langur.

Limitations

The study validates the applicability of human-specific STR identification systems in Chimpanzees; however, small sample size was the limitation of this study. Thus we propose further cross-validation on other non-human primates with a large sample size.

Abbreviations

Not applicable

Declarations

Ethics approval and consent to participate:

The study was conducted in compliance with the ethical standards. All the procedures prior to their implementation were approved by the Ethical Committee of State Forensic Science Laboratory, Directorate of Forensic Services, Shimla, Himachal Pradesh with the approval of Zoological Garden, Alipore Kolkata vide letter no 1076-ZGA/17-18 dated 14/03/2018. The written consent for forensic investigation and analysis was provided by Zoological Garden, Alipore Kolkata.

Consent to publish:

Not applicable.

Availability of data and materials:

All the relevant data is provided as the supplementary file. The raw datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests

Funding

Authors' contributions

MT and VS conceived the idea and designed the experiments. **AS, MT** and **VS** performed all the wet lab experiments. **AS, MT, LKS** analyzed data and wrote the manuscript. **VS, KC, DP, AS (Arun Sharma)** contributed in providing materials/analysis tools. All the authors participated in the discussion and provided inputs to improve the content of the manuscript.

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