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SHORT COMMUNICATION

The new Powerplex® ESX17 and ESI17 kits in paternity and maternity analyses involving people from Africa—including allele frequencies for three African populations

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Abstract Paternity and maternity investigations in immigration procedures are frequently done in Germany. Since mostly only one parent and one or more children are investigated, the occurrence of possible mutational events has to be interpreted with great care and the analysis of as many STRs as possible is recommended. The new Powerplex® ESX17 and Powerplex® ESI17 kits from Promega comprising both eleven established STRs and additionally the loci D1S1656, D2S441, D10S1248, D12S391, and D22S1045 (in different order) are potential tools in such paternity or maternity analyses, but only few allele frequency data for the five new loci exist. Here, we provide allele frequencies for the five additional STRs from three different populations from Africa. In addition, we present two maternity cases and one paternity case in which a clear inclusion or exclusion of the alleged parent could only be achieved by the additional application of the new Powerplex® ESX17 kit.

Keywords STR · Multiplex PCR · Population data · Ghana · Madagascar · Morocco

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Introduction

Nowadays, immigration procedures involving family reunions frequently require paternity or maternity investigations with STR analysis being the method of choice. In most of these cases, only the alleged father and the child are included in the investigation, since the maternity of the alleged mother is usually not questioned. Moreover, the mother is frequently absent or not investigated for financial reasons. This may lead to difficult and possibly wrong conclusions, especially if brothers or other relatives of the alleged father may be considered as the true father [1–3] or mutational events occur, even when twelve STRs—as required by the German guidelines—are investigated. Numerous STRs are available these days, but allele frequencies for some of these STRs—especially for newly introduced markers—have only rarely been evaluated in African populations yet.

To enable the use of five only recently available STRs in immigration procedures we applied the newly developed Powerplex® ESX17 and ESI17 kits from Promega to 457

unrelated individuals from three different African populations (Ghana (Gh), Madagascar (Ma), and Morocco (Mo)) and present the corresponding population data. The different groups have been chosen based on their different population history. Despite a colonization by Great Britain the genetic impact of Europeans is rather small in Ghana [4], whereas the influence of France as a colonial power and Spanish and Portuguese people in terms of trade relations is much greater in Morocco. Madagascar has been populated by people from East Africa and Middle East as well as South and South-eastern Asia in 350BC [5, 6] with only little influence from Europe even after its detection by a Portuguese sailor in 1,500.

In addition, we present three maternity and paternity analyses involving people from Africa which could be elucidated by the additional use of the Powerplex® ESX17 kit.

Material and methods

Population and DNA samples

Buccal swabs or blood samples were collected from 100 men and 101 women (randomly selected from a group of 5,100 apparently unrelated adults; 20–48 years old) of the Ashanti population from Ghana, from 53 men and 33 women from Morocco (predominately born and living in Marracesh; 20–52 years old) and from 110 men and 60 women from Madagascar (18–72 years old). Samples were obtained and analyzed after advice of the Committee on Human Research Publication and Ethics (School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana) and of the Medical Ethics Committees of the University of Duisburg-Essen and University Hospital of Schleswig-Holstein in accordance with the declaration of Helsinki. The anonymity of the individuals investigated was preserved corresponding to the rules of data protection of the Human Medical Faculties Essen and Kiel.

Case reports

Case 1: A 15-year-old girl originally coming from Sierra Leone was found without any personal documents at the station in Herne and brought to a children hospital. She claimed that a 30-year-old woman from Sierra Leone, living in Duisburg with four other children was her mother. This woman supported her story, but the immigration department did not believe it. The father of the girl was apparently missing.

Case 2: A 31-year-old man from Cameroon with a German passport applied for the immigration of two children, whom he claimed as his offspring: a 15-year-old girl and a 12-year-old boy. The immigration department requested a paternity analysis, and samples from both children were taken in Cameroon and sent to Germany for genetic analysis. According to the alleged father the mother of the children was dead.

Case 3: A 42-year-old woman from Nigeria with a residence permit for Germany presented three children (a 1-year-old boy, a 6-year-old boy, and a 7-year-old girl) as her offspring at the immigration department and requested residence permit for them. A maternity analysis was performed involving the alleged mother and the three children but without the potential father, who was not available for investigation.

DNA extraction

DNA was extracted from buccal swabs using the innuPrep DNA Mini Kit (Analytik Jena, Jena, Germany) and from blood using the Qiagen Blood Mini Kit (Qiagen, Hilden, Germany).

DNA amplification and fragment analysis

The new Powerplex® ESX17 and Powerplex® ESI17 kits comprise both eleven established STRs and additionally the loci D1S1656, D2S441, D10S1248, D12S391, and D22S1045 (in a different order, with different primers and with different fluorescent labels). The amplification protocols for both kits followed the manufacturer's instructions with a reduced PCR volume of 12.5 µl in the GeneAmp® PCR system 9700 (Applied Biosystems). The employment of this non-standard reaction was done to safe money for this study and the following routine investigations and has been thoroughly and independently tested according to the existing quality managements [7]. In each amplification a positive control (100 pg 9947A) and a negative PCR control (sterile water) were analyzed. Amplification products (0.5 in 12 µl sterile water or formamide with 0.3 µl ILS500 standard) were separated and detected on an ABIPrism310 Analyzer (Applied Biosystems) in comparison to the allelic ladders which are components of both kits. Electrophoresis results were analyzed using the GeneMapper® ID Software v3.2 with the bin set provided by the manufacturer (www.promega.com). Allele peaks were interpreted when greater than or equal to 50 RFUs and lower or equal to 3,000 RFUs. Each sample was analyzed with the Powerplex® ESX17 kit, and 25% of the samples were additionally investigated using the Powerplex® ESI17 kit. All positive controls had to show the expected full STR profile with allelic peaks between 50 RFUs and 3,000

RFUs and without allelic drop out or drop in [8]. All other data were dismissed and analysis was repeated.

Statistical analysis

PowerStats v1.2 was used to evaluate the forensic statistic parameters [9]. Exact test of population differentiation, population pair wise genetic distances F_{ST}, Hardy–Weinberg equilibrium and pairwise exact test of linkage disequilibrium were all performed using the Arlequin ver. 3.0 software [10].

Results and discussion

Reliability of data

All analyzed samples have been investigated using the well-established PCR multiplex-kits Powerplex® 16 kit (Promega) and/or AmpF*l*STR Identifiler® kit (Applied Biosystems) prior to this study. No differences have been found in comparison between the data from the prior analysis and the results from this study. Moreover, no differences could be detected between Powerplex® ESX17 and Powerplex® ESI17 results.

Population statistics

Genotyping of all three populations from Africa resulted in the allele frequencies and forensic efficiency parameters given in Table 1 for the five STRs D1S1656, D2S441, D10S1248, D12S319, and D22S1045, and Electronic supplementary Tables S1, S2, and S3 for the remaining eleven STRs from the Powerplex® ESX17 and Powerplex® ESI17 kits. D22S1045 is the least informative STR in the populations from Madagascar and Morocco, D2S441 in the population from Ghana, whereas D1S1656 is the most informative STR of the five "new" STRs in all populations. Since alleles were determined in accordance to the STRbase (http://www.cstl.nist.gov/div831/strbase/) the allele description differs in comparison to those originally published by Coble & Butler as follows: in D10S1248 minus one repeat and in D22S1045 plus three repeats [11, 12].

No significant deviations from Hardy–Weinberg-expectations were found. Linkage disequilibrium between the STRs on the same chromosome (D2S1338 and D2S441, as well as VWA and D12S391) could not be detected.

Genetic distances were estimated for the five STRs D1S1656, D2S441, D10S1248, D12S319, and D22S1045 for the three African populations and compared to other populations as far as possible (see Electronic supplementary Tables S4–S8 for results and references). We choose populations from Africa (including Afro-Americans), Europe (especially southern Europe), and Asia for comparison due to immigration history of Ghana, Madagascar and Morocco. The allele frequencies for D10S1248 and D22S1045 described by Coble et al. were converted as described above. Regarding D12S391, some authors do not mention x.1 alleles [13]. Therefore, we combined .0 alleles and .1 alleles in each population for comparison purposes. Significant differences were detected in D1S1656 for almost all inter population comparisons with the exception of the comparison between Ghana and an Afro-American population [14] and the comparison between Morocco and a North-African population [15]. A similar picture was found for D2S441. Regarding D10S1248, no deviations were found between Ghana and the Afro-American population [14] between Madagascar and two Asian populations [14, 16]. In addition, the population from Morocco showed a rather great similarity to the other African, Afro-American and even some Asian populations in D10S1248 and D12S319. Significant differences regarding D22S1045 could be detected for all inter population comparisons with the exception of the comparison between Madagascar and China [16].

Case reports

Case 1

A routine investigation using the Powerplex® PP16HS kit (Promega) of the alleged mother and the child revealed three potential exclusions in D7S820 (one repeat difference), D16S539 (one repeat difference), and Penta E (two repeats difference). Three exclusions are the minimal requirements in Germany for the exclusion of paternity or maternity [17]. However, a biological father–child relationship with three mutations has already been reported [18] and in our labs three exclusions are in general not sufficient for the exclusion of the alleged parent from parenthood. An analysis with the AmpF*l*STR Identifiler® kit confirmed the differences in D7S820 and D16S539 and showed no other potential exclusions in the additional loci D19S433 and D2S1338. A verification of the Penta E exclusion was not possible since this locus is not included in the AmpF*l*STR Identifiler® kit. The additional analysis of SE33 and eleven X-STRs (method as described before [19]) did not reveal any more exclusions. The employment of the Powerplex® ESX17 kit confirmed all prior comparable results and revealed two further exclusions in D1S1656 and D10S1248 (one repeat difference each). Therefore, we could successfully exclude the alleged mother from maternity presenting all together five excluding loci after investigation of 23 autosomal markers. Nevertheless, it could be assumed that the woman and the child were closely related.

Table 1 Allele frequencies for the five STRs D1S1656, D2S441, D10S1248, D12S391, and D22S1045 in Ghana (n=201), Madagascar (n=170), and Morocco (n=86)

D1S1656				D2S441				D10S1248				D12S391				D22S1045			
Allele	Freq Gh	Freq Ma	Freq Mo	Allele	Freq Gh	Freq Ma	Freq Mo	Allele	Freq Gh	Freq Ma	Freq Mo	Allele	Freq Gh	Freq Ma	Freq Mo	Allele	Freq Gh	Freq Ma	Freq Mo
10	0.0124	0.0029	0.0116	8	0.0025	0	0	9	0.0149	0.0059	0	13	0.0025	0	0	9	0.002		
11	0.0496	0.1059	0.0349	9	0.05	0	0	10	0.0249	0.0029	0	14	0.01	0.0029	0	10	0.052	0.015	0.006
12	0.0572	0.0265	0.0872	10	0.0597	0.1029	0.0988	11	0.0622	0.0529	0.0116	15	0.0896	0.0706	0.0174	11	0.092	0.209	0.052
13	0.1144	0.1	0.1221	11	0.3706	0.2912	0.2616	12	0.1418	0.0588	0.0872	16	0.0697	0.05	0.0233	12	0.075	0.018	0.017
13.3	0.0025	0.0029	0	11.3	0.0348	0.0971	0.1105	13	0.2711	0.3235	0.2674	17	0.1542	0.1765	0.1802	13	0.005	0.003	0
14	0.2537	0.2088	0.093	12	0.1641	0.2647	0.0349	14	0.2388	0.2441	0.3023	17.3	0.0025	0	0.0058	14	0.197	0.026	0.081
14.3	0.0149	0.0206	0	13	0.0746	0.0441	0.0349	15	0.1493	0.1912	0.2093	18	0.2388	0.1853	0.2093	15	0.241	0.306	0.349
15	0.1468	0.1824	0.1743	13.3	0.0124	0.0029	0.0058	16	0.0796	0.1	0.0988	18.1	0.0025	0	0	16	0.112	0.159	0.314
15.3	0.0274	0.0206	0.0174	14	0.2338	0.1794	0.3547	17	0.0174	0.0176	0.0233	18.3	0.0149	0.0029	0.0233	17	0.206	0.253	0.169
16	0.1045	0.2118	0.1977	14.3	0	0.0029	0	18	0	0.0029	0	19	0.1766	0.15	0.1686	18	0.01	0.012	0.006
16.3	0.102	0.0676	0.0581	15	0.0348	0.0147	0.0988					19.3	0.0124	0	0.0116	19	0.007	0	0.006
17	0.0199	0.0294	0.0233	16	0.0075	0	0					20	0.1244	0.1118	0.1221				
17.3	0.0448	0.0118	0.1105									21	0.0274	0.1118	0.0523				
18	0.005	0	0.0058									22	0.0249	0.0853	0.064				
18.3	0.0398	0.0088	0.0523									23	0.0075	0.0471	0.0523				
19.3	0.005	0	0.0116									24	0.0323	0	0.0349				
												25	0.01	0.0029	0.0291				
												26	0	0	0.0058				
												27	0	0.0029	0				
PIC	0.86	0.83	0.87		0.74	0.76	0.74		0.79	0.75	0.74		0.84	0.86	0.85		0.81	0.74	0.7
PD	0.966	0.967	0.962		0.917	0.922	0.917		0.935	0.913	0.907		0.96	0.963	0.96		0.945	0.911	0.892
PE	0.756	0.599	0.739		0.438	0.588	0.5		0.573	0.621	0.603		0.706	0.844	0.739		0.648	0.485	0.443
HET_{obs}	0.881	0.8	0.872		0.706	0.794	0.744		0.786	0.812	0.802		0.856	0.924	0.872		0.826	0.735	0.709

Freq frequency, *Gh* Ghana, *Ma* Madagascar, *Mo* Morocco, *PIC* polymorphism information content, *PD* power of discrimination, *PE* power of exclusion, HET_{obs} observed heterozygosity

Case 2

The routine investigation using the Powerplex® PP16HS kit and the AmpF*l*STR Identifiler® kit of the alleged father and both children resulted in nine exclusions between potential father and son, as well as a potential exclusion in Penta D (two repeats difference) between the alleged father and daughter. An analysis of eleven X-chromosomal STRs revealed one further potential exclusion in DXS7424 (one repeat difference) between alleged father and daughter (method as described before [19]). The use of the Powerplex® ESX17 kit demonstrated two additional potential exclusions in D1S1656 and D10S1248 (two repeats difference each). Therefore, the alleged father had to be definitely excluded from paternity for both children.

Case 3

The routine investigation using the Powerplex® PP16HS kit of the alleged mother and all three children revealed 15 inclusions for the girl, six exclusions for the older boy, and one potential exclusion in D18S51 (one repeat difference) for the younger boy. An additional analysis using the AmpF*l*STR Identifiler® kit and the Powerplex® ES kit resulted in a potential exclusion in SE33 (one repeat difference) for the girl, two additional exclusions for the older boy and one additional potential exclusion in D2S1338 (two repeats difference) for the younger boy No exclusions could be found for any of the children in eleven X-chromosomal STRs. The application of the Powerplex® ESX17 kit displayed no further exclusions for the girl, two additional exclusion for the older boy and three further exclusions in D1S1656 (two repeats difference), D12S391 (two repeats difference) and D22S1045 (one repeat difference) for the younger boy. In summary, we got one exclusion for the girl, ten exclusions for the older, and five for the younger boy. Consequently, the alleged mother could be excluded from maternity for the two boys, but not for the girl. Here, the one exclusion was handled as a mutational event. The maternity probability (including only the STRs from the AmpF*l*STR Identifiler® kit) was calculated as 99.988% (maternity index $8{,}6418 \times 10^3$, allele frequencies as published by [20]). Regarding the boys, it could be assumed that a sister or half-sister of the alleged mother is the true mother due to the X-chromosomal results.

Conclusion

In summary, our results show that the new Powerplex® ESX17 kit (or the Powerplex® ESI17 kit) is a powerful tool for STR typing in paternity testing in African populations. Our three case reports underline the demand for an extended STR typing program in difficult investigations as it has been proposed by others [18, 21]. The great number of significant differences even between the African populations demonstrates the need for further population studies of other African populations for the five additional STRs.

References

1. von Wurmb-Schwark N, Mályusz V, Simeoni E, Lignitz E, Poetsch M (2006) Possible pitfalls in paternity analysis with related putative fathers—a simulated deficiency investigation in 27 families. Forensic Sci Int 159:92–97
2. Poetsch M, Lüdcke C, Repenning A, Fischer L, Mályusz V, Simeoni E, Lignitz E, von Wurmb-Schwark N (2006) The problem of single parent/child paternity analysis—practical results involving 336 children and 348 unrelated men. Forensic Sci Int 159:98–103
3. Karlsson AO, Holmlund G, Egeland T, Mostad P (2007) DNA-testing for immigration cases: the risk of erroneous conclusions. Forensic Sci Int 172:144–149
4. Davidson B (1978) A history of West Africa 1000–1800. Longman Group UK Ltd., Essex, UK
5. Burney DA, Burney LP, Godfrey LR, Jungers WL, Goodman SM, Wright HT, Jull AJ (2004) A chronology for late prehistoric Madagascar. J Hum Evol 47:25–63
6. Dewar RE, Wright HT (1993) The culture history of Madagascar. J World Prehistory 7:417–466
7. Poetsch M, Bayer K, Ergin Z, Milbrath M, Schwark T, von Wurmb-Schwark N (2010) First experiences using the new Powerplex® ESX17 and ESI17 kits in casework analysis and allele frequencies from two different regions in Germany. Int J Legal Med (in press) PMID: 20567841
8. Szibor R, Edelmann J, Hering S et al (2003) Cell line DNA typing in forensic genetics: the necessity of reliable standards. Forensic Sci Int 138:37–43
9. Tereba A (1999) Tools for analysis of population statistics, Profiles in DNA 3. Promega Corporation. Available at: http://www.promega.com/geneticidtools/powerstats/
10. Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform (Online) 1:47–50
11. Coble MD, Butler JM (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. J Forensic Sci 50:43–53
12. Butler JM, Coble MD (2007) Authors' response. J Forensic Sci 52:494
13. Farfan M-J, Sanz P, Lareu M-V, Carracedo A (1999) Population data on the D1S1656 and D12S391 STR loci in Andalusia (South Spain) and the Maghreb (North Africa). Forensic Sci Int 104:33–36
14. Hill CR, Duewer DL, Kline MC, Sprecher CJ, McLaren RS, Rabbach DR, Krenke BE, Ensenberger MG, Fulmer PM, Storts DR, Butler JM (2010) Concordance and population studies along with stutter and peak height ratio analysis for the PowerPlex® ESX 17 and ESI 17 systems. Forensic Sci Int Genet. doi:10.1016/j.fsigen.2010.03.014
15. Phillips C, Fernandez-Formoso, Garcia-Magarinos M, Porras L, Tvedebrink T, Amigo J, Fondevila M, Gomez-Tato A, Alvarez-Dios J, Freire-Aradas A, Gomez-Carballa A, Mosquera-Miguel A, Carracedo A, Lareu MV (2010) Analysis of global variability in 15 established and 5 new

European standard set (ESS) STRs using the CEPH human genome diversity panel. Forensic Sci Int Genet. doi:10.1016/j.fsigen.2010.02.003
16. Yong RYY, Gan LSH, Coble MD, Yap EPH (2007) Allele frequencies of six miniSTR loci of three ethnic populations in Singapore. Forensic Sci Int 166:240–243
17. Richtlinien für die Erstattung von Abstammungsgutachten der Bundesärztekammer (Deutsches Ärzteblatt, 2002, Heft 10, Seite A665)
18. Mansuet-Lupo A, Henke J, Henke L, Blank C, Ernsting A, Kozlowski P, Rouger P, Van Huffel V (2009) A paternity case with three genetic incompatibilities between father and child due to maternal uniparental disomy 21 and a mutation at the Y chromosome. Forensic Sci Int Genet 3:141–143
19. Poetsch M, El-Mostaqim D, Tschentscher F, Browne ENL, Timmann C, Horstmann RD, von Wurmb-Schwark N (2009) Allele frequencies of 11 X chromosomal loci in a population sample from Ghana. Int J Legal Med 123:81–83
20. Hohoff C, Schürenkamp M, Brinkmann B (2009) Meiosis study in a population from Nigeria: allele frequencies and mutation rates of 16 STR loci. Int J Legal Med 123:259–261
21. Henke L, Henke J (2005) Which short tandem repeat polymorphisms are required for identification? Lessons from complicated kinship cases. Croat Med J 46:593–597