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SHORT COMMUNICATION

First experiences using the new Powerplex® ESX17 and ESI17 kits in casework analysis and allele frequencies for two different regions in Germany

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Abstract DNA databases are the most efficient tools in criminal investigations with unknown perpetrators. Due to a significant number of random matches in cross-border DNA profile exchanges, the European Network of Forensic Science Institutes (ENFSI) proposed the addition of further short tandem repeats (STRs) to European DNA databases. Therefore, the new Powerplex® ESX17 and Powerplex® ESI17 kits from Promega comprised the 11 established DNA database STRs and additionally the well-known loci D1S1656 and D12S391, as well as D2S441, D10S1248, and D22S1045. The latter three STRs are thereby established as so-called mini-STRs to fulfill the increasing requirements regarding sensitivity and reproducibility for analysis of minute amounts of DNA. Here, we provide allele frequencies for the five additional STRs from two populations from Germany. A test regarding suitability and robustness of the new kits for routine trace analysis showed that it is more likely to obtain a meaningful profile using Powerplex® ESX17 and Powerplex® ESI17 kits compared to the Powerplex® ES kit. However, for both new kits the range of template DNA amount is rather small, e.g., slightly more DNA than recommended resulted in DNA profiles which could not be reliably evaluated due to allelic drop-in or imbalances and overshoots. In our opinion, the new kits are very promising new tools in forensic trace analysis even though handling and evaluation should yet be carried out with great caution.

Keywords STR · Multiplex PCR · Population data · Germany

Introduction

Short tandem repeat (STR) analysis by commercially available multiplex amplification kits allows genotyping of polymorphic and individual loci relevant to the convicted offender databases of many countries. The first of the national DNA databases was formed in the UK in 1995 and expanded rapidly. By the end of 1999, the database had entries of over 700,000 profiles, achieving around 700 matches each week [1]. By 1998, Austria, Germany, and The Netherlands had successfully introduced their own databases [2].

Today, most countries have introduced legislation for using DNA profiling in criminal investigations and for storing DNA profiles in their national databases. The European Standard Set of STRs (ESS) comprising TH01, HumVWA, FGA, D21S11, D3S1358, D8S1179, and D18S51 was selected by the European Network of Forensic Science Institutes (ENFSI) and forms the core of European database loci. However, depending on (additional) STR loci used for the various databases different kits were developed and employed, e.g., Powerplex® ES (Promega) or Mentype® (Biotype) with the eight German database loci (ESS

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loci plus SE33). Other countries demand additional or different loci, e. g., the 13 CODIS marker in the US including the ESS loci, and D13S317l, D7S820, D16S539, TPOX, CSF1PO, and D5S818.

Due to a significant number of random matches in cross-border DNA profile exchanges, the ENFSI recently proposed the inclusion of further STRs [3]. Other countries also work on the investigation of other STRs [4].

The new Powerplex® ESX17 and ESI17 kits fulfill the ENFSI requirements and provide the additional loci D1S1656 and D12S391, as well as D2S441, D10S1248, and D22S1045, selected by ENFSI because of their shorter PCR products. The employment of STRs with shorter PCR product is necessary due to an increasing number of low template DNA samples. Mini-STRs have been shown to improve the detection threshold and thus increase the number of samples which could be successfully investigated [5–8].

In the past, forensic laboratories used to contribute to the databases with DNA profiles established with a number of common STR loci. With the introduction of the new STR markers, population data for the use of the new STRs in forensic case analysis are missing or rather rare.

We applied the newly developed Promega kits to 639 unrelated individuals from two different German populations (Schleswig-Holstein [SH; northern part of Germany] and Nordrhein-Westfalen [NRW; western part of Germany]) and present the corresponding population data. In addition, the kits were checked for their suitability in forensic case work analysis regarding detection threshold, robustness, and reproducibility by investigating real case work samples.

Material and methods

Population and DNA samples

Buccal swabs or blood samples were collected from 177 men and 162 women born and living in Nordrhein-Westfalen (NRW; western part of Germany) and from 148 men and 152 women born and living in Schleswig-Holstein (SH; northern part of Germany). Samples were obtained and analyzed after advice 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. All samples derived from individuals involved in prior paternity investigations in the two institutes and were thus already genetically investigated using the well-established PCR multiplex-kits Powerplex® 16 kit (Promega) and/or AmpF*l*STR Identifiler® kit (Applied Biosystems).

In addition, 30 randomly chosen swabs from a variety of surfaces (including bottles, screw drivers, steering wheels, tool handles) were investigated which had already been analyzed with the Powerplex® S5 kit (Promega) and the Powerplex® ES kit (Promega) and the results were compared [8].

Different concentrations (range 0.01 to 1.0 ng) from the commercially available cell lines 9947 and 9948 were used for dilution experiments and determination of the detection threshold.

DNA extraction

DNA was extracted from buccal swabs using the innuPrep DNA Mini Kit (Analytik Jena, Jena, Germany) rendering an elution volume of 250 µl DNA-containing solution and from blood using the Qiagen Blood Mini Kit (Qiagen, Hilden, Germany) rendering an elution volume of 50 µl DNA-containing solution. DNA was not routinely quantified prior to PCR.

DNA quantification

In case of problems regarding STR typing, DNA content of the respective samples was measured in a real-time PCR using the Quantifiler® Human DNA quantification kit (Applied Biosystems) according to the manufacturer's instructions.

DNA amplification and fragment analysis

The amplification protocols for the Powerplex® ESX17 and Powerplex® ESI17 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 has been used to save money for this study and the following routine investigations in our laboratories. To be sure that this reduction does not hamper STR amplification and evaluation, the reduced reaction mix was thoroughly and independently tested in both laboratories according to the established quality management systems (using cell line DNAs, blood samples, buccal swabs, and swabs from various traces). There were no evident differences between 12.5 and 25 µl reactions and no relation between the 12.5-µl reaction and hampered STR detection was visible. In each amplification, a positive control (100 pg 9947A) and a negative control (sterile water) were analyzed. Amplification products (0.5 µl in 12 µl sterile water or formamide with 0.3 µl ILS500 standard) were separated and detected on an ABI Prism 310 or on an ABI Prism 3130 Genetic 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 relative fluorescence units (RFUs) and lower or equal to 3,000 RFUs. Each sample was analyzed with the Powerplex® ESX17 kit. Twenty-five percent of samples were additionally investigated with the Powerplex® ESI17 kit.

Control for reproducibility and reliability of the data

The following precautions were applied to ensure reliability of the new data:

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 [9].

All buccal swabs or blood samples analyzed in this study have been investigated previously in prior paternity cases using the Powerplex® 16 and the AmpF*l*STR Identifiler Kit. Since these kits contain multiple loci that are also included in the Powerplex® ESX17 and Powerplex® ESI17, a reliable comparison of STR data was possible. Results from the new STRs were only included in the present study, when the comparable STR data were conclusive and clearly showed the same profile in all kits. Otherwise, analysis was repeated using more or less DNA as template.

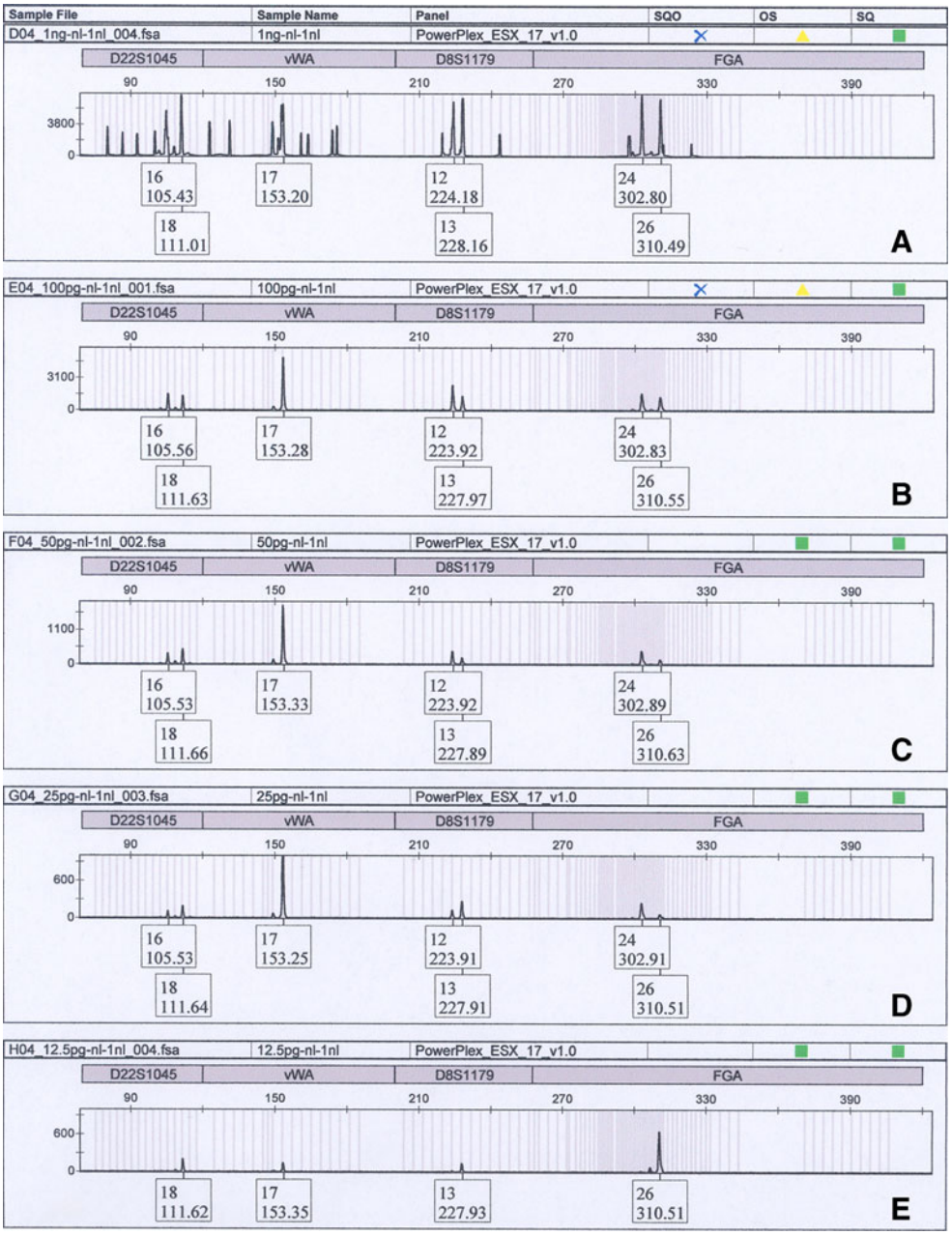

Fig. 1 Detection threshold and robustness of the Powerplex® ESI17 kit to varying DNA concentrations. Exemplary results of employment of different DNA amounts to the multiplex PCR. Electropherogram after capillary electrophoresis on an ABI3130 Genetic Analyzer. DNA from the commercially available cell line 9948 was diluted, and different amounts were subjected to the described multiplex PCR. Named by the Gene Mapper IDv 3.2 are the authentic alleles from the cell line. Panel **a** 1 ng DNA as template, **b** 100 pg, **c** 50 pg, **d** 25 pg, **e** 12.5 pg

Statistical analysis

PowerStats v1.2 was used to evaluate the forensic statistic parameters [10]. Exact test of population differentiation, population pairwise genetic distances F_{ST}, Hardy-Weinberg equilibrium, and pairwise exact test of linkage disequilibrium were all performed using the Arlequin ver. 3.0 software [11].

Results and discussion

Amplification efficiency and reproducibility using the new multiplex kits

Using the described experimental design, both kits showed a detection threshold down to 50 pg. Using less DNA, allelic drop-out or drop-in occurred especially involving the loci D16S539, D18S51, FGA, D12S391, and SE33. Increasing the DNA concentration also led to difficulties in allelic evaluation (Fig. 1).

Real-time PCR and dilution experiments showed that DNA amounts between 0.05 and 0.3 ng usually led to reproducible allelic patterns without difficulties in interpretation. Using more than 0.3 ng DNA resulted in overshoots making especially trace analysis with possible DNA mixtures very difficult to evaluate (Fig. 2).

However, in our laboratories, for example, we did not quantify every DNA sample prior to STR typing, but use the same dilution of every sample for PCR. In routine analysis this procedure usually works quite well using other multiplex PCR kits such as the Powerplex® 16HS kit or the AmpF*l*STR Identifiler® kit. Regarding the new kits, investigation of DNA from blood shows in our experiences that 1 µl of a dilution of 1:350 (equivalent to 0.1 to 2 ng template DNA) gives best results with a failure rate of about 5% due to too high DNA concentrations. An amount of 0.5-µl DNA extracted from buccal swabs was analyzed (dilution of 1:75, equivalent to 0.01 to 1 ng template DNA). Here, we also observed a failure rate of about 5% due to too high or too low DNA content. For these samples the analysis was repeated with less or more DNA. Therefore, the application of the new kits in routine casework with many samples showed that both kits produce reliable results only in a tight range of DNA concentration, in contrast to other multiplex PCR kits

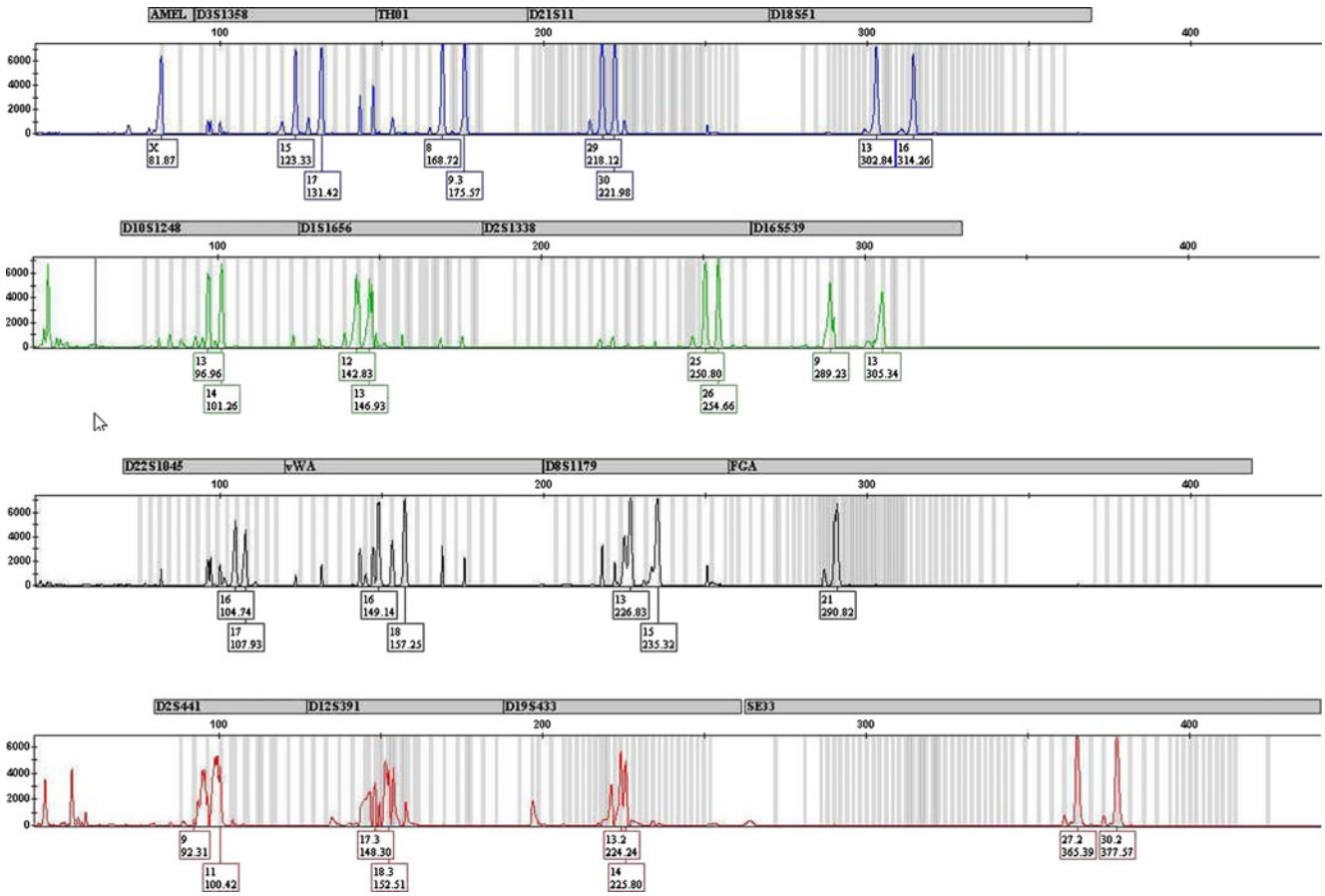

Fig. 2 DNA profile of a DNA extracted from a buccal swap. Exemplary result for a DNA overload. Electropherogram after capillary electrophoresis on an ABI310 Genetic Analyzer

which produce reliable results in a much broader range of DNA concentrations.

Consequently, we recommend a routine DNA quantification at least for trace samples for the use of the new Powerplex® ESX17 and Powerplex® ESI17 kits.

Casework analysis

The analysis of 30 swabs from casework samples showed results similar to those obtained by amplification with the Powerplex® S5 kit [8]. Since the new kits provide additional gene loci it is more likely that in casework samples a meaningful profile could be obtained using the Powerplex® ESX17 and Powerplex® ESI17 kits compared to the Powerplex® ES kit. However, regarding the described overshoot effect the kits should only be applied without DNA quantification in samples that are known to contain only minute amounts of DNA (e. g., swabs from objects, single hairs), albeit with the risk of frequently observed allelic drop-outs. In trace samples with suspected high DNA content, DNA quantification is recommendable. For routine paternity testing, working with a dilution adapted to laboratory settings and thresholds should be sufficient.

Population statistics

Genotyping of both populations from Germany resulted in the allele frequencies and forensic efficiency parameters (Table 1 for the five STRs D1S1656, D2S441, D10S1248, D12S319, and D22S1045, and Tables S1 and S2 for the remaining 11 STRs from the Powerplex® ESX17 and Powerplex® ESI17 kits). D22S1045 is the least informative STR in the two German populations investigated, whereas D1S1656 is the most informative STR of the five "new" STRs in both populations. Alleles were determined in

Table 1 Allele frequencies for the five STRs D1S1656, D2S441, D10S1248, D12S391, and D22S1045 in NRW (n=339) and SH (n=300)

D1S1656			D2S441			D10S1248			D12S391			D22S1045		
Allele	Freq NRW	Freq SH	Allele	Freq NRW	Freq SH	Allele	Freq NRW	Freq SH	Allele	Freq NRW	Freq SH	Allele	Freq NRW	Freq SH
8	0.001	0	9	0.004	0.003	8	0.001	0.002	14	0.001	0.002	10	0.003	0
10	0.001	0.002	10	0.202	0.173	10	0.018	0	15	0.043	0.035	11	0.152	0.127
11	0.083	0.092	11	0.286	0.355	11	0.035	0.003	16	0.032	0.03	12	0.013	0.015
12	0.115	0.11	11.3	0.059	0.057	12	0.035	0.037	17	0.091	0.117	13	0.007	0
13	0.072	0.052	12	0.04	0.027	13	0.257	0.292	17.1	0.003	0.015	14	0.056	0.04
13.3	0.003	0	12.3	0.004	0.002	14	0.324	0.3	17.3	0.015	0.022	15	0.358	0.357
14	0.069	0.092	13	0.038	0.03	15	0.192	0.208	18	0.181	0.17	16	0.333	0.365
14.3	0.009	0.002	13.3	0.004	0	16	0.111	0.12	18.1	0.012	0.023	17	0.065	0.092
15	0.114	0.13	14	0.319	0.307	17	0.025	0.03	18.3	0.022	0.01	18	0.009	0.005
15.3	0.072	0.073	15	0.037	0.04	18	0.001	0.008	19	0.093	0.102	19	0.003	0
16	0.131	0.133	16	0.006	0.007				19.1	0.006	0.01			
16.3	0.053	0.052							19.3	0.013	0.007			
17	0.055	0.068							20	0.128	0.107			
17.1	0	0.003							20.1	0.003	0.007			
17.3	0.133	0.118							20.3	0.012	0.007			
18	0.015	0.018							21	0.117	0.125			
18.3	0.062	0.042							22	0.108	0.103			
19	0.003	0							23	0.052	0.062			
19.3	0.009	0.013							24	0.044	0.038			
									25	0.019	0.007			
									26	0.004	0.003			
PIC	0.90	0.90		0.73	0.7		0.74	0.73		0.89	0.89		0.69	0.67
PD	0.981	0.98		0.914	0.892		0.909	0.903		0.978	0.978		0.882	0.873
PE	0.789	0.802		0.459	0.444		0.626	0.510		0.741	0.735		0.409	0.444
HET_{obs}	0.897	0.903		0.72	0.71		0.814	0.75		0.873	0.87		0.687	0.71

Freq frequency, *PIC* polymorphism information content, *PD* power of discrimination, *PE* power of exclusion, HET_{obs} observed heterozygosity

accordance to the STRbase (http://www.cstl.nist.gov/div831/strbase/). Consequently, the allele description differs in comparison to those originally published by Coble and Butler as follows: in D10S1248 minus one repeat and in D22S1045 plus three repeats [12, 13].

Combined power of discrimination is greater than 0.999999999 for both populations (as expected) and combined power of exclusion is 0.999999936 and 0.999999927 for NRW and SH, respectively. 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 two German populations and compared to other populations [12, 14–28] as far as possible (see Tables S3–S6 for results and references). 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 [14–16]. Therefore we combined .0 alleles and .1 alleles in each population for comparison purposes. No significant differences for the five STRs were found between the two German populations investigated in this study. Deviations in D1S1656 were detected especially to southern European populations and the African populations. Moreover, there are few significant differences for D2S441, D10S1248, and D22S1045 between the populations of this study and the population from Spain [25] or the US Caucasian population [12]. In comparison to Afro–American, US Hispanics, South American, and Asian populations all three mini-STRs displayed deviations.

Conclusion

In summary, our results show that the new Powerplex® ESI17 and ESX17 kits are suitable for STR typing in paternity testing as well as in trace analysis. The inclusion of five more STR loci will certainly improve genetic analysis and cross-border comparisons in Europe. However, in our opinion the robustness of the multiplex PCRs could still be improved since allelic drop-in and drop-out occur in samples with too high or too low DNA content, making DNA quantification prior to genetic analysis—at least in trace work samples with expected high DNA content—recommendable. Furthermore, since the frequencies of the five additional loci have not yet been thoroughly studied, statistical calculations in casework have to be interpreted with caution at this point.

References

1. Martin PD, Schmitter H, Schneider PM (2001) A brief history of the formation of DNA databases in forensic sciences within Europe. Forensic Sci Int 119:225–331
2. Schneider PM (1998) DNA databases for offender identification in Europe—the need for technical, legal and political harmonization, in: proceedings of the 2nd European symposium on human identification, promega corporation. Madison, WI, USA, pp 40–44
3. Gill P, Fereday L, Morling N, Schneider PM (2006) New multiplexes for Europe -amendments and clarification of strategic development. Forensic Sci Int 163:155–157
4. Lu D-J, Liuz Q-L, Zhao H (2010) Genetic data of nine non-CODIS STRs in Chinese Han population from Guangdong Province Southern China. Int J Leg Med. doi:10.1007/s00414-009-0388-x
5. Wiegand P, Klein R, Braunschweiger G, Hohoff C, Brinkmann B (2006) Short amplicon STR multiplex for stain typing. Int J Leg Med 120:160–164
6. Grubwieser P, Mühlmann R, Berger B, Niederstätter H, Pavlic M, Parson W (2006) A new "miniSTR-multiplex" displaying reduced amplicaon lengths for the analysis of degraded DNA. Int J Leg Med 120:115–120
7. von Wurmb-Schwark N, Preusse-Prange A, Heinrich A, Simeoni E, Bosch T, Schwark T (2009) A new multiplex-PCR comprising autosomal and y-specific STRs and mitochondrial DNA to analyze highly degraded material. Forensic Sci Int Genet 3:96–103
8. Poetsch M, Kamphausen T, Bajanowski T, Schwark T, von Wurmb-Schwark N (2010) Powerplex ES versus powerplex S5—casework testing of the new screening kit. Forensic Sci Int Genet. doi:10.1016/j.fsigen.2009.11.002
9. 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
10. Tereba A (1999) Tools for analysis of population statistics, profiles in DNA 3. Promega Corporation Available at: http://www.promega.com/geneticidtools/powerstats/.
11. Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinformatis Online 1:47–50
12. Coble MD, Butler JM (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. J Forensic Sci 50:43–53
13. Butler JM, Coble MD (2007) Authors' response. J Forensic Sci 52:494
14. Schröer K-P, Schmitt C, Staak M (2000) Analysis of the co-amplified STR loci D1S1656, D12S391 and D18S51: population data and validation study for a highly discriminating triplex-PCR. Forensic Sci Int 113:17–20
15. Betz T, Immel U-D, Kleiber M, Klintschar M (2007) "Paterniplex", a highly discriminative decaplex STR multiplex tailored for investigating special problems in paternity testing. Electrophoresis 28:3868–3874
16. 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
17. Wiegand P, Lareu MV, Schürenkamp KM, Brinkmann B (1999) D18S535, D1S1656 and D10S2325: three efficient short tandem repeats for forensic genetics. Int J Leg Med 112:360–363
18. Grubwieser P, Zimmermann B, Niederstätter H, Pavlic M, Steinlechner M, Parson W (2007) Evaluation of an extended set of 15 candidate STR loci for paternity and kinship analysis in an Austrian population sample. Int J Leg Med 121:85–89

19. Nievas P, Prades A, Martinez-Jarreta B (Eds.) (1999) STR loci D1S1656, D18S535 Y D12S391. Nuevos datos geneticos acerca de su distribucion en la poblacion Espanola y rendimiento medico-forense, Vol. IV, Jornadas de Genetica Forense, La Gomera, Spain
20. Lareu MV, Barral S, Salas A, Pestoni C, Carracedo A (1998) Sequence variation of a hypervariable short tandem repeat at the D1S1656 locus. Int J Leg Med 111:244–247
21. Gamero JJ, Romero JL, Gonzalez JL, Arufe MI, Cuesta MI, Corte-Real F, Carvalho M, Anjos MJ, Vieira DN, Vide MC (2000) A study on ten short tandem repeat systems: African immigrant and Spanish population data. Forensic Sci Int 110:167–177
22. Anjos MJ, Andrade L, Carvalho M, Souto L, Corte-Real F, Vieira DN, Vide MC (eds) (1998) Districuicao das frequencies alelicas dos loci D1S1656 e D19S253 numa populacao de Portugal, Vol III. Jornadas de Genetica Forense, Bilbao, Spain
23. Schmid D, Anslinger K, Rolf B (2005) Allele frequencies of the ACTBP2 (=SE33), D18S51, D8S1132, D12S391, D2S1360, D3S1744, D5S2500, D7S1517, D10S2325 and D21S2055 loci in a German population sample. Forensic Sci Int 151:303–305
24. Becker D, Bender K, Edelmann J, Götz F, Henke L, Hering S, Hohoff C, Hoppe K, Klintschar M, Muche M, Rolf B, Szibor R, Weirich V, Jung M, Brabetz W (2007) New alleles and mutational events at 14 STR loci from different German populations. Forensic Sci Int Genet 1:232–237
25. Martin P, Garcia O, Albarran C, Garcia P, Yurrebaso I, Alonso A (2007) Allele frequencies of six miniSTR loci (D10S1248, D14S1434, D22S1045, D4S2364, D2S441 and D1S1677) in a Spanish population. Forensic Sci Int 169:252–254
26. Asamura H, Uchida R, Takayanagi K, Ota M, Fukushima H (2006) Allele frequencies of the six miniSTR loci in a population from Japan. Int J Leg Med 120:182–184
27. 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
28. Vullo C, Borosky A, Romanini C, Catelli L, Yamamoto T (2010) Frequency data for 12 mini STR loci in Argentina. Forensic Sci Int Genet 4:e79–e81