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Validation and practical experiences with the multiplex kits *gen*RES® MPX-2 (SERAC) and *GenePrint*® Powerplex™ 16 (promega)

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Abstract

Validation studies were carried out using the commercially available multiplex systems *GenePrint*® PowerPlex™ 16 and the *gen*RES® MPX-2. In a first approach, sensitivity and mixture studies were investigated with quantified human DNA. The minimum amount of template DNA that gave a complete DNA pattern ranged between 200 and 500 pg for both multiplex systems. The mixture ratios that were detectable and could be clearly assigned were determined to be 1:5/1:10. In order to get more experience, three pieces of fresh muscle tissue and a 6-month-old microbloodstain on a pacemaker were investigated. The preliminary results of the tissue study revealed that the MPX-2 amplification pattern is more homogeneous than PowerPlex16 and that PowerPlex16 is less robust towards template DNA variation compared to MPX-2 resulting in the increased occurrence of imbalanced peak heights and locus drop-out. As shown in the case of the 6-month-old microbloodstain, the age of the stain material seems to be more crucial for PowerPlex16 than for MPX-2 amplification.

Keywords: *gen*RES® MPX-2; *GenePrint*® PowerPlex™ 16; Validation studies; Forensic material

1. Introduction

In the past, a number of different multiplex systems have been validated and established for forensic identification purposes and are now commercially available. The aim of the establishment was to reduce time, cost and material, and to maximize the forensic power of discrimination. At the beginning of the multiplex era, the systems consisted of no more than three short tandem repeat (STR) systems [1,2]. Meanwhile, the number of systems that can be amplified in a single reaction has increased up to 15 STR systems [3–5]. Before

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application of multiplex systems in routine casework, extensive validation studies have to be performed, e.g. sensitivity and mixtures studies and experimental stain casework, in order to get information about reproducibility, robustness and specificity. Validation studies were carried out with the commercially available multiplex kits *gen*RES® MPX-2 and *GenePrint*® PowerPlex™ 16. The *gen*RES® MPX-2 kit contains eight STRs (TH01, VWA, FGA, ACTBP2, D21S11, D3S1358, D8S1179, D18S51) which are components of the German DNA database and the sex-specific Amelogenin. The *GenePrint*® PowerPlex™ 16 kit co-amplifies 15 STRs+Amelogenin including the 13 CODIS tetranucleotide STR loci and 2 pentanucleotide STR loci. The aim of the study was to perform sensitivity and mixture studies with defined amounts of template DNA and to validate the multiplex systems for forensic casework.

2. Material and methods

2.1. Material

The sensitivity and mixture studies were carried out with quantified human DNA. The case work studies included blood and saliva samples, microbloodstains and tissues samples.

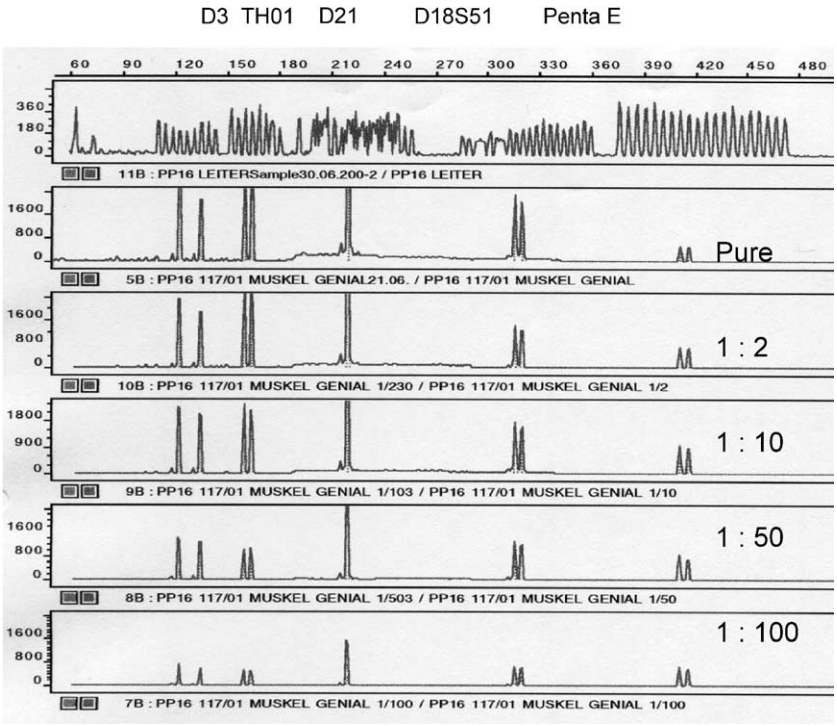

Fig. 1. PowerPlex16: Electropherogram of the results of the dilution series.

2.2. DNA extraction

DNA was extracted using Chelex® 100 for blood and saliva samples or the All-tissue DNA-Kit (GEN-IAL, Troisdorf, Germany) for tissue samples.

2.3. Sensitivity and mixture studies

Sensitivity studies were carried out with different amounts of quantified human DNA ranging from 5 to 0.05 ng. The mixture ratios were 1:1, 1:2, 1:5, 1:10, 1:20, 1:50 and 1:100 with the constant component being 5 ng.

2.4. PCR amplification

All investigations were performed according to the manufacturer's protocol.

2.5. Electrophoresis

The samples were analysed on an ABI Prism 310 Genetic Analyzer using a 50-μm capillary, the polymer POP6 and the module GS STR POP6 (Inj.-T: 30 s, Inj.-V: 2 kV, Run-V: 15 kV) with filter set A. DNA typing and allele assignment were carried out using the GeneScan 2.1 software.

3. Results

3.1. Sensitivity and mixture studies

Sensitivity studies revealed that for both the MPX-2 and the PowerPlex16 kit (Fig. 1), all alleles could be clearly detected and assigned to the allelic ladder at levels between 500 and 200 pg template DNA (Table 1). The phenomenon of allelic drop-out/preferential amplification was observed applying DNA levels below 200 pg. As expected, the sensitivity of the multiplex systems is lower than that of the singleplex amplification. In

Table 1
Results of the sensitivity study for the *gen*RES® MPX-2 kit

ng/system	AM	VWA	SE33	TH01	D21	D8	D3	FGA	D18
5	++	+	+	+	+	+	+	+	+
2	++	+	+	+	+	+	+	+	+
1	++	+	+	+	+	+	+	+	+
0.5	++	+	+	+	+	+	+	+(LB)	+
0.2	++	+	+	(+)PA	+	+	+	+(LB)	+
0.1	++	(+)	(+)PA	(+)	(+)PA	(+)	(+)	(+)LB	(+)PA
0.05	++	(+)	(+)PA	((+))AD	(+)	(+)AD	(+)AD	(+)LB	((+))

(·) Peak height below 200; ((·)) peak height below 100; PA, preferential amplification; AD, allelic drop-out; LB, ladder bands.

studies with different mixing ratios and the constant component being 5 ng, the detection limit of the minor component was heterogeneous ranging from 1:2 (Penta E, PowerPlex16), 1:5 (ACTBP2, MPX-2) and 1:20 (VWA, FGA, MPX-2). In general, reproducible results were achieved with mixing ratios ranging between 1:5 and 1:10.

4. Conclusions

Both multiplex systems showed similar results concerning sensitivity and mixture ratios. As expected, the multiplex systems are less sensitive (200–500 pg) than loci amplified in a singleplex reaction. In general, blood and saliva samples could be typed without problems. Insufficient amplification of ACTBP2 (MPX-2) and of the loci with longer fragment lengths (PowerPlex16) was partly observed. Preliminary results of our investigations on forensic material showed that the MPX-2 amplification pattern is more homogeneous than that of PowerPlex16 and that PowerPlex16 is less robust towards template DNA variation compared to MPX-2 resulting in the increased occurrence of imbalanced peak heights and locus drop-out. As shown in the case of the 6-month-old microbloodstain, the age of the stain material seems to be more crucial for PowerPlex16 than for MPX-2 amplification. Further investigations have to be performed in order to get more experience with these complex multiplex systems.

References

[1] C.P. Kimpton, P. Gill, A. Walton, A. Urquhart, E.S. Millican, M. Adams, Automated DNA profiling employing multiplex amplification of short tandem repeat loci, PCR Methods Appl. 3 (1993) 13–22.
[2] J.M. Wallin, M.R. Buoncristiani, K.D. Lazaruk, N. Fildes, C.L. Holt, P.S. Walsh, TWGDAM validation of the AmpFlSTR blue amplification kit for forensic casework analysis, J. Forensic Sci. 43 (4) (1998) 854–870.
[3] N.J. Oldroyd, A.J. Urquhart, C.P. Kimpton, E.S. Millican, S.K. Watson, T. Downes, P.D. Gill, A highly discriminating octoplex short tandem repeat polymerase chain reaction system suitable for human individual identification, Electrophoresis 16 (1995) 334–337.
[4] E.A. Cotton, R.F. Allsop, J.L. Guest, R.R.E. Frazier, P. Koumi, I.P. Callow, A. Seager, R.L. Sparkes, Validation of the AMPFlSTR SGM Plus system for use in forensic casework, Forensic Sci. Int. 112 (2000) 151–161.
[5] C.J. Finis, Megaplex STR analysis from a single amplification: validation of the PowerPlex® 16 system, Profiles DNA 4 (2) (2001) 3–6.