International Congress Series 1239 (2003) 609–611

Evaluation of Powerplex™ 16 for typing of degraded DNA samples

B. Glock [a,*], R.B.K. Reisacher [a], S.O. Rennhofer [a], D. Tröscher [a], E.M. Dauber [b], W.R. Mayr [a,b]

[a] *Laboratory for Molecular Biology, Blood Donation Center of the Austrian Red Cross for Vienna, Lower Austria and Burgenland, Nordportalstrasse 248, A-1020 Vienna, Austria*
[b] *Division of Blood Group Serology, University of Vienna, Währinger Gürtel 18-20/4I, A-1090 Vienna, Austria*

Abstract

The performance of the new STR typing kit Powerplex™ 16 was investigated on degraded DNA samples. After optimization of the reaction conditions, comparative typings on six archived whole blood samples and the corresponding plasma retention samples as well as on whole blood DNA, which had been stored at 4 °C and room temperature for 20 days, were performed with Powerplex™ 16 and AmpFlSTR® SGM Plus™. Concerning the loci common in both kits, no differences of performance could be calculated. As expected, allele dropout of loci was observed with increasing fragment length, which unfortunately included some of the most informative marker systems. Inclusion of further loci, which show amplification products smaller than 250 bp, into STR multiplex kits or combination of two different kits might help to increase the information obtainable on degraded DNA samples.

Keywords: Powerplex™ 16; AmpFlSTR® SGM Plus™; Degraded DNA

1. Introduction

The STR multiplex kit Powerplex™ 16 was used on recent whole blood samples as well as on cell-free samples (plasma, fresh frozen plasma = FFP) to optimize the reaction conditions. Subsequently, the performance of the Powerplex™ 16 kit on degraded DNA samples was investigated. Results of archived samples (whole blood and their correspond-

* Corresponding author. Tel.: +43-1-7280199-601; fax: +43-1-7280199-609.
E-mail address: glock@redcross.or.at (B. Glock).

0531-5131/03
PII: S0531-5131(02)00296-0

ing plasma samples stored below −20 °C) were generated taking into account the optimized Powerplex™ 16 conditions and our previously modified AmpFlSTR® SGM Plus™ protocol and compared.

2. Materials and methods

2.1. Samples

Recent EDTA anti-coagulated whole blood, FFP and EDTA plasma were used for the optimization of the Powerplex™ 16 protocol.

Six pairs of archived whole blood samples and the corresponding plasma samples, which were assumed to be degraded after storage at temperatures below −20 °C for 1–3 years, were chosen for the evaluation of Powerplex™ 16 kit.

2.2. DNA extraction

DNA extraction was carried out on 10 μl whole blood or 200 μl plasma using Qiagen DNA Blood Mini Kit® employing a modified protocol: 5 μl Carrier DNA (0.33 μg/μl) was added at the lysis step. Elution at room temperature was performed in buffer AE pre-warmed to 70 °C (200 μl for whole blood and 50 μl for the other samples). Besides immediate STR typing, aliquots of extracted DNA from each whole blood sample were stored at 4 °C and at room temperature for a further 20 days.

2.3. Amplification and detection

Amplification and detection conditions were optimized based on the manufacturer's instructions [1,2].

3. Results and discussion

A modified protocol for the reliable amplification and detection of recent and archived whole blood and cell-free samples using the Powerplex™ 16 kit could be established (two additional cycles in PCR and amplification of whole blood in a final volume of 12.5 μl). According to the validation protocol [3] for Powerplex™ 16, a sizing precision study was carried out.

Ten-fold injections each of an amplified recent whole blood sample and the allelic ladders showed standard deviations of size for the different alleles between 0.00 and 0.07 bp and 0.00 and 0.10 bp for the whole blood samples and the allelic ladders, respectively.

Six whole blood and six corresponding plasma samples as well as aliquots of the six whole blood DNA extracts, which had been stored at 4 °C or room temperature for 20 days, respectively, were subsequently investigated. Eight full profiles could be obtained with Powerplex™ 16, all from whole blood samples, whereas using AmpFlSTR® SGM Plus™ kit, six full profiles could be obtained from whole blood samples. Both kits showed

Table 1
Valid results of degraded DNA samples at the common loci of Powerplex™ 16 and AmpFlSTR® SGM Plus™

	Powerplex 16 (%)	SGM Plus (%)
D3S1358	21/24 (88)	20/24 (83)
vWA	21/24 (88)	24/24 (100)
D16S539	21/24 (88)	23/24 (96)
D8S1179	20/24 (83)	24/24 (100)
D21S11	20/24 (83)	20/24 (83)
D18S51	15/24 (63)	7/24 (29)
TH01	23/24 (96)	20/24 (83)
FGA	14/24 (58)	11/24 (46)
Amelogenin	19/24 (79)	23/24 (96)
All loci	174/216 (81)	172/216 (80)

drop out of single alleles or loci increasing with fragment length. This included the two highly discriminating pentanucleotide loci Penta E and D of the Powerplex™ 16 kit, whose fragment lengths are above 370 bp. Only 50% of the samples (whole blood extracts) showed reliable results at these two loci. No differences could be seen in the performance of the loci occurring in both the Powerplex™ 16 and the AmpFlSTR® SGM Plus™ kit (Table 1). Storage of the degraded whole blood extracts at room temperature revealed slightly better results with both kits than storage at 4 °C. The loss of information compared to the results of the unstored extracts was 38% at 4 °C and 18% at room temperature for Powerplex™ 16 and 23% and 7% for AmpFlSTR® SGM Plus™ kit, respectively.

In summary, the recently launched Powerplex™ 16 kit performed equally well compared to the established AmpFlSTR® SGM Plus™ kit in the typing of degraded DNA samples although slight differences at particular loci could be observed. Accordingly, inclusion of further loci, which show amplification products smaller than 250 bp, into STR multiplex kits or combination of two different kits, might help to increase the information obtained from degraded DNA samples.

References

[1] Gene Print® Powerplex™ 16 System. Technical Manual, Promega.
[2] AmpFlSTR® SGM Plus™ PCR Amplification Kit. User's Manual, Applied Biosystems.
[3] Validation of STR Systems. Reference Manual, Promega.