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Testing the behavior of GlobalFiler® PCR amplification kit with degraded and/or inhibited biological samples

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ABSTRACT

The achievement of complete genetic profiles of degraded and/or inhibited biological samples is a challenging task for forensic scientists.

Our aim was to find out the behavior of GlobalFiler® PCR amplification kit in the presence of difficult samples and its ability to substitute or to be complemented by AmpFℓSTR® MiniFiler™ kit.

Degraded bloodstains were extracted by three methods, quantified and amplified with AmpFℓSTR® IdentiFiler™, AmpFℓSTR® MiniFiler™ and/or GlobalFiler® PCR.

In 6 of the 14 analyzed samples, GlobalFiler® kit enabled a complete genetic profile even when the sample had a DNA concentration as low as 0.0049 ng/ml, while in some of these samples Minifiler™ provided a partial profile or didn't amplify any of the markers in study.

1. Introduction

Genetic identification of human body fluids is one of the great advances of modern genetics. This may help identify individuals who left biological material at a crime scene. One of the main challenges of forensic genetics is to be able to recover DNA from degraded samples. UV light, heat, microbial degradation and the presence of inhibitors, are the major factors that interfere with the integrity of DNA which difficult the genetic identification of the sample.

For a successful DNA typing it is important to have sufficient amount of DNA template as well as the absence of PCR inhibitors in the sample.

Our aim was to find out the behavior of GlobalFiler® PCR amplification kit [1] in the presence of difficult samples and its ability to substitute or to be complemented by AmpFℓSTR® MiniFiler™ kit [2].

2. Materials and methods

Blood was collected from three non-related male donors and bloodstains with 30 ml and 50 mm^2 of area were made in denim (g), cotton (a) and lycra [1], previously washed and decontaminated for 20 min with UV light. The bloodstains were dried during 3 days at room temperature before being placed in a fresh water well, inside of a house, buried in sandy, marshy and clay soils and on the soil surface in a forest, during summer and winter seasons and for a maximum of 2 years. Three stains from each donor were collected after 1, 3, 7, 15, 30, 90 days, 6, 12 and/or 24 months, depending on their degradation rate. Graphical registers of temperature and rainfall were obtained from the closest meteorological station.

Bloodstains of each individual in all type of fabrics were used as positive controls. DNA extraction was performed using Chelex 100, QIAamp® DNA Investigator (Qiagen) and DNA IQ™ System (Promega). DNA was quantified with Quantifiler™ Human DNA Quantification kit (Applied Biosystems—AB) (adding 0.5 ul of Bovine Serum Albumin [0.3%]), according to manufacturer's instructions using an ABI Prism®7000 and/or Quantifiler™ Trio DNA Quantification kit (AB), using an Applied Biosystems®7500. Two of these samples were amplified with IdentiFiler (AB), and all the samples were amplified with MiniFiler (AB) and GlobalFiler® PCR Amplification kits (AB) and analyzed with ABI Prism®3130 and/or ABI Prism®3500 Analyzers (AB).

3. Results

For this study 12 samples with quantity of DNA below 0.1 ng/μl and 2 samples (977, 1033) with a partial Identifiler profile were

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Table 1
Quantification results of the selected samples and the profiles obtained with MiniFiler and GlobalFiler PCR Amplification kits. N, O and P are the initials from the donors, l, a and g are the fabric initials (in Portuguese), ↑ means extensively degraded and n.a. means non applicable. [1]Sample 533 was only quantified with Quantifiler Human because the volume of the DNA extraction ended.

Sample	Type of sample	Extraction	ng/μl	IPC	Degradation rate	Minifiler	GlobalFiler®
116	Ol 3 days sandy soil summer	DNAIQ	0.0049	27.29	0.86	Partial 7/9	Complete
129	Na 3 days sandy soil summer	Chelex	0.0248	27.70	2.63	Complete	Partial 23/24
201	Na 7 days sandy soil summer	Chelex	0.0350	27.59	2.54	Complete	Complete
525	Na 15 days sandy soil summer	Chelex	0.0172	27.19	14.68	Complete	Partial 15/24
645	Pa 30 days sandy soil summer	Chelex	0.0290	27.62	↑	No profile	Partial 4/24
977	Pg 3 days sandy soil winter	Chelex	0.1118	27.07	4.09	Complete	Complete
982	Oa 3 days sandy soil winter	Chelex	0.0405	27.23	7.41	Complete	Partial 13/24
1033	Pa 3 days sandy soil winter	QIAamp	0.2355	26.57	2.36	Complete	Complete
1359	Ol 30 days sandy soil winter	Chelex	0.0013	27.26	13.69	Partial 5/9	No profile
360	Oa 7 days clay soil summer	DNAIQ	0.0130	27.49	1.10	Partial 4/9	Complete
533[1]	Nl 7 days clay soil summer	QIAamp	0.0312[1]	28.72[1]	–	Partial 3/9	Complete
1058	Pg 7 days clay soil winter	Chelex	0.0047	27.41	4.15	Complete	Partial 14/24
1439	Og washed with bleach	Chelex	0.0088	26.61	1.50	Complete	Partial 18/24
699	Pa 30 days forest surface summer	Chelex	0.0343	27.38	11.85	Complete	Partial 18/24

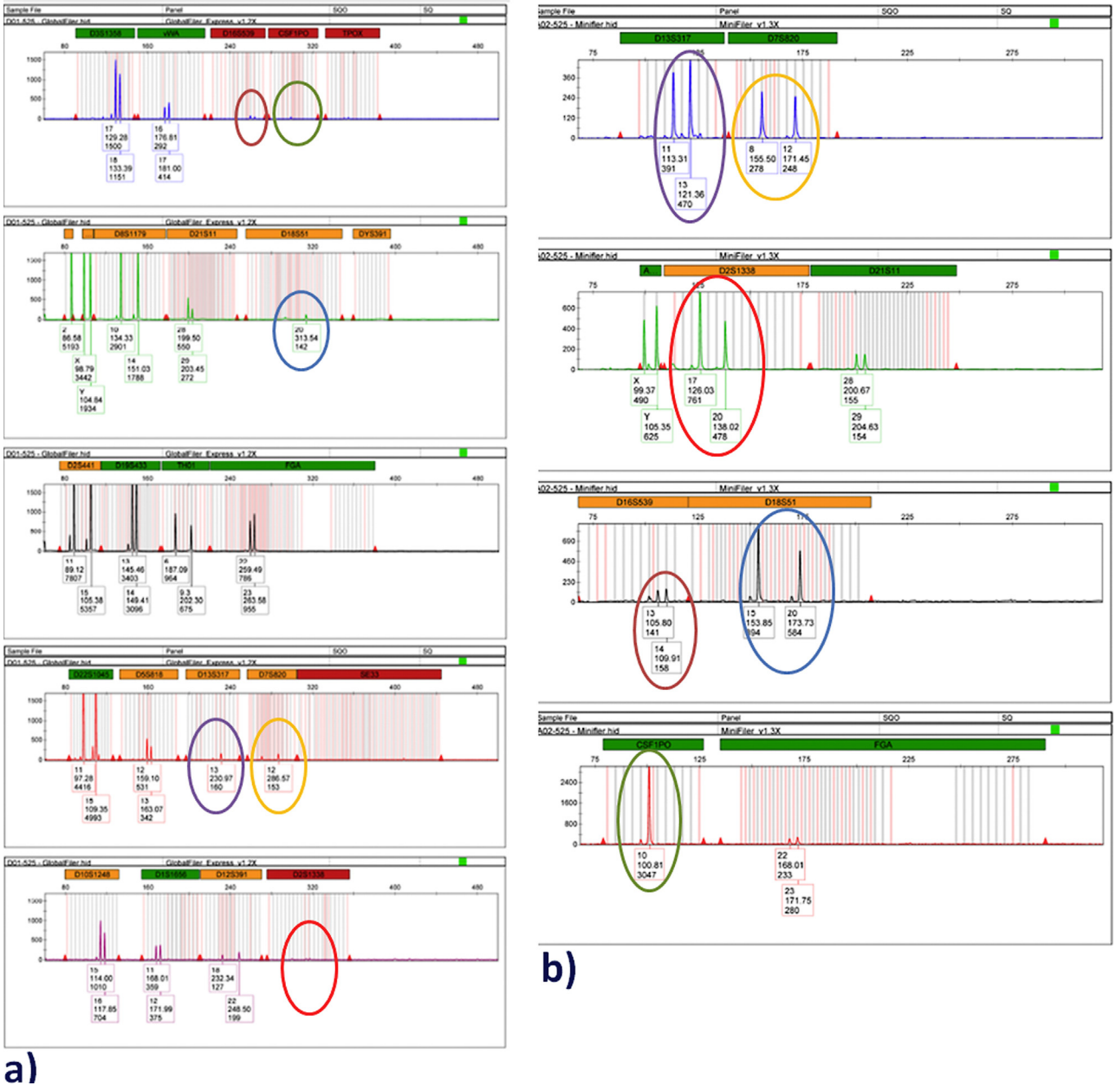

Fig. 1. Genetic profiles of sample 525 obtained with Globalfiler (a) and Minifiler (b).

selected. In Table 1 are gathered the quantification results with Quantifiler Trio and their obtained profiles. In Fig. 1 are presented the genetic profiles obtained for sample 525, with Globalfiler and Minifiler, as an example.

4. Discussion and Conclusion

In 6 of the 14 analyzed samples GlobalFiler kit enabled a complete genetic profile even DNA concentration was as low as 0.0049 ng/μl. In some of these 14 samples, Minifiler provided a partial profile or did not amplify the markers in study (Table 1).

Samples 525 (Fig. 1), 982, 1058, 1439 and 699 were amplified with GlobalFiler provided a partial profile. These samples were also amplified with Minifiler which added more genetic information to the one achieved with GlobalFiler. The larger markers in GlobalFiler, which failed to amplify in these samples, are smaller in size in the Minifiler kit. For this reason, Minifiler enabled some of them to be amplified and be complete, thus complementing the obtained profile with Globalfiler. All these samples have a degradation rate from 1.5–14.68 and a DNA concentration between 0.0047–0.0405 ng/μl.

The sample 1359 when amplified with Minifiler kit enabled only the amplification of 4 markers plus amelogenin while GlobalFiler kit did not enable the amplification of any of the markers in study. This sample is extensively degraded and with a very low DNA concentration which may help to explain the obtained results.

The amplification of sample 645 with GlobalFiler resulted in a partial profile while Minifiler kit was not able to amplify any of the STR markers. This sample besides being degraded may have inhibitors that may have been overcame with Globalfiler.

Samples 977 and 1033 besides having enough DNA concentration to be amplified with Identifiler the obtained profiles were partial. When these samples were amplified with Minifiler or GlobalFiler a complete profile was achieved probably because these kits were able to overcome the presence of inhibitors and degradation.

Although sample 129 resulted in a complete profile with Minifiler and a partial profile with GlobalFiler, Minifiler does not add more information to the profile obtained with Globalfiler.

Samples 116, 360 and 533 were all extracted with extraction kits, have low DNA concentration, probably few inhibitors and no degradation. When these samples were amplified with Minifiler they all resulted in a partial profile but when amplified with GlobalFiler a complete profile was achieved. These results may be due to Globalfiler allowing the amplification with a maximum DNA volume of 15 μl while Minifiler allows only 10 μl, which may have been critical in the amplification of these samples.

In conclusion, Globalfiler can be combined with Minifiler kit when incomplete genetic profiles are achieved in highly degraded and/or inhibited samples.

Role of funding

None.

Conflict of interest

None.

Acknowledgements

None.

References

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[2] LIFE TECHNOLOGIES, GlobalFiler® PCR Amplification Kit User's Guide, 2012.