

1 **Quality standards in Biobanking: authentication by genetic**
2 **profiling of blood spots from donor's original sample**

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21

22 **Abstract**

23 The field of Biobanking requires extensive work in order to maintain traceability of
24 samples. However, sometimes the necessity to authenticate a sample may arise. To
25 address these circumstances, we herein present a method for authenticating derivatives
26 by using a blood spot from each donor, attached to a sample authentication form, by
27 means of genetic profiling. Blood spots are collected at the time a blood sample is
28 donated at a health centre and before processing the blood sample at the biobank. To
29 test the validity of our approach over time, we analyzed 26 blood spots stored at room
30 temperature in our facilities for more than 15 years. DNA was successfully extracted
31 from the three storage materials tested in this study and 15 STR markers plus
32 amelogenin were subsequently analyzed. The storage of a small blood spot attached to a
33 sample authentication form proved to be efficient for genetic profiling and, therefore,
34 may constitute a long-lasting (at least 15 years), cost-effective and effortless approach
35 for genetic authentication of samples in biobanks.

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37 **Keywords:** Biobanking; authentication; traceability; STR; microsatellite; genetic profile

38

39 **Introduction**

40 Traceability of samples constitutes one of the cornerstones in the emerging field of
41 Biobanking, but there are divergent viewpoints within the scientific community
42 regarding the way to implement it efficiently. The development of automatic and
43 robotic procedures has partially helped to overcome this difficulty. Yet, even with the
44 assistance of new high-tech procedures, a biobank may on occasion need to authenticate

45 the origin of a particular sample. Whenever a doubt related to traceability arises in a
46 biobank it is usually very difficult to check whether a sample does belong to a specific
47 donor. In such a case, the biobank will not be able to certify that samples are correctly
48 supplied to other biobanks or research groups. It is therefore crucial for biobanking
49 activities to implement a consistent system to guarantee authentication of biological
50 samples.

51 Genetic identification techniques used in the field of Forensic Genetics might be
52 successfully applied in sample authentication since they provide very high power of
53 discrimination (in the order of 1 in several thousand million). Currently, the most
54 common method utilized for human genetic identification is based on microsatellite or
55 short tandem repeat (STR) loci.¹⁻³ By using less than 5 ng of DNA, up to fifteen
56 autosomal STR loci, plus the amelogenin gender-determining marker, can be
57 simultaneously amplified by means of some commercial multiplex PCR reaction kits
58 with fluorescently labeled PCR primers,⁴ including the 13 STR loci of the CODIS
59 system⁵ which is used worldwide. The main drawback to this genetic profiling system
60 arises when it is necessary to process a large amount of samples as this is an expensive,
61 labour-intensive technique.

62 With the aim of avoiding a genetic profile analysis of every donor when the samples
63 enter a biobank, the DNA Bank of the University of the Basque Country (UPV/EHU)
64 has devised a cost-effective and reliable method for genetic authentication of biological
65 samples through the collection of blood spots from the original donor. These blood
66 spots are available for further analyses if authentication is required. This procedure is

67 applicable to any derivative such as cell collections, biological fluids or DNA obtained
68 from blood samples in biobanks.

69

70 **Materials and Methods**

71 The procedure presented herein is based on the preservation of small blood spots in 10
72 mm discs of storage material. A first blood spot collected for authentication purposes is
73 attached to a sample authentication form by health staff at the time the blood sample is
74 donated and the informed consent is signed (Figure S1). Once the blood sample and all
75 associated information have been received at the biobank, an aliquot is collected from
76 the blood sample to create a second blood spot which is also attached to the sample
77 authentication form to be used by the biobank as an internal control. In order to ensure
78 the privacy of donors, each sample authentication form is linked to information
79 associated with the corresponding donor and every derivative of the blood sample by
80 means of barcode labels generated by a Laboratory Information Management System
81 (LIMS). Blood spots, which are suitably protected from any manipulation and/or
82 contamination (Figure 1), can be stored at room temperatures for long periods of time.

83 The validation of our approach was performed by analyzing 26 blood spots of more
84 than 15 years old stored at room temperature, all of them belonging to 12 people
85 (named S01 to S12; see table 1) who worked in our laboratory and who voluntarily
86 agreed to participate in the study. Genetic profiles of each participant used as reference
87 in the present study were obtained from DNA stored at -20°C. Six out of the 26 blood
88 samples were preserved on absorbent paper, eight in a piece of lint and 12 stored on
89 non-colored cotton fabric. The blood spots collected at the beginning of 1994 were at

90 that time attached to the informed consent of each donor using surgical tape, as
91 described by Rodríguez-Alarcón et al. 1996.⁶ Informed consents were stored at room
92 temperature in a security cabinet, protected from sunlight and at variable moisture
93 levels, according to the season.

94 Blood spot samples were retrieved for DNA extraction in autumn 2008. We used a 3.0
95 mm Whatman Harris punch to obtain a portion of the storage material. This portion was
96 subsequently cut into small pieces using sterile scissors. Samples were incubated
97 overnight at 56°C in 500 µl of lysis solution (NaCl 0.2M, EDTA 10mM, Tris 10mM,
98 SDS 2%, DTT 40mM and 0.8 mg/ml proteinase K as final concentration). DNA
99 extraction was performed using the standard phenol-chloroform procedure.⁷ Firstly, 200
100 µl of phenol/chloroform-isoamyl alcohol in a proportion of 25:24:1 were added to each
101 tube. After centrifugation and recovery of the aqueous phase, two additional washes
102 with chloroform-isoamyl alcohol were carried out to further purify the samples. Once
103 the aqueous phase was recovered, we added NaAc 2M in a proportion of 1/10 to the
104 volume of aqueous phase, 1 µl of glycogen (10mg/ml) and two volumes of cold
105 absolute ethanol (-20°C). To facilitate DNA precipitation, sample tubes containing the
106 precipitation mix were stored at -20°C for two hours. Subsequent centrifugation at
107 14,000 g was carried out to obtain the DNA pellet; this pellet is frequently not visible
108 when extracting DNA from old blood spots. After a final wash with EtOH 70%,
109 samples were introduced into a centrifugal evaporator (DNA mini; Heto Holten,
110 Allerød, Denmark) to get the pellet dried. Finally, samples were resuspended in 20 µl of
111 sterile water.

112 The Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Carlsbad, CA) was used to
113 quantify the extracted DNA. We added 1 µl of each sample to 199 µl of the
114 quantification mix (1/400 PicoGreen and 1/20 Buffer TE 20x) and DNA concentration
115 was obtained in a VersaFluor fluorometer (BioRad, Richmond, CA). Genetic profiles of
116 microsatellite loci for each sample (including reference samples) were obtained by
117 means of PCR amplification in a GeneAmp 9700 Gold (Applied Biosystems, Foster
118 City, CA) using two commercial kits: AmpFISTR® Identifiler™ PCR Amplification
119 Kit and AmpFISTR® SGM Plus™ PCR Amplification Kit (Applied Biosystems). Only
120 2 ng of DNA were used for each amplification reaction. Fragment analysis of PCR
121 products was carried out by capillary electrophoresis using an ABI3130 Genetic
122 Analyzer (Applied Biosystems). Finally, GeneMapper® Software v4.0 (Applied
123 Biosystems) was utilized for allele designation.

124 We conducted a one-way analysis of variance (ANOVA) followed by a Tukey-Kramer
125 multiple-comparison post-hoc test to determine whether mean DNA concentrations
126 obtained for the three different storage materials showed significant differences.

127

128 **Results**

129 The blood spots stored in the three different support media (absorbent paper, lint or non-
130 colored cotton fabric) did not show notable degradation signs, irrespective of the
131 material used. Likewise, oscillations of moisture levels over years did not seem to affect
132 blood spot conservation, since no fungal growth was observed in any of the samples.

133 DNA extraction from blood spots was successful in the three types of storage material
134 tested in our study. Nevertheless, DNA extraction from lint proved to be somewhat
135 complicated since it was rather difficult to manipulate this kind of material, particularly
136 when we attempted to cut it into smaller pieces to facilitate the extraction procedure.
137 Subsequent DNA quantification with PicoGreen confirmed DNA isolation: all the
138 samples rendered concentrations that varied between 1.5 and 28 ng/ μ l of double strand
139 DNA. Mean DNA concentrations (\pm standard error) obtained for lint, absorbent paper
140 and cotton fabric were 7.3 ± 1.6 , 14.8 ± 3.1 and 13.2 ± 1.8 ng/ μ l, respectively.
141 Significant differences between the DNA concentrations obtained in each material were
142 revealed by the ANOVA results ($F = 4.36$; $df = 2, 25$; $p < 0.05$). The Tukey test showed
143 no differences between the DNA concentrations of cotton fabric and absorbent paper,
144 which in turn differed from the concentration obtained in lint ($p < 0.05$).

145 In obtaining the STR profiles, 2 ng of DNA per sample and commercial kit were
146 employed. Table 1 shows the genetic profile obtained for each sample with both
147 commercial kits and the comparison with the corresponding reference profile. Complete
148 and reliable genetic profiles were obtained for each one of the 26 blood spots included
149 in the analysis. Allele genotyping profiles obtained for each sample with the two
150 different commercial kits utilized were coincident for all common STR markers, as
151 shown in Figure S2 for sample S01. This double-checking system constitutes a
152 noteworthy validation step of the method proposed herein. In this sense, no allelic drop-
153 out due to DNA degradation was detected in the 15 year old samples examined, and
154 even fragments of the STR markers with the largest sizes were correctly amplified.
155 Further evidence of the reliability and validity of our method was the fact that the

156 genetic profiles obtained for each sample exactly matched with the genetic profile of the
157 corresponding individual participating in this study.

158

159 **Discussion**

160 Authentication procedures are fundamental to biobanking services, because they must
161 ensure that a specific sample or set of samples come from a specific donor before
162 supplying them to the scientific community. Therefore, biobanks are required for
163 preventing and identifying errors, such as sample misplacement. In fact, an emphasis on
164 high standards in traceability is recommended by many European initiatives for the
165 international networking of Biobanking.⁸⁻¹⁰ Genetic profiling seems to be an efficient
166 approach for sample authentication. Interestingly, there are very few studies focusing on
167 authentication procedures in biobanks by means of genetic profiling, most of them
168 centred on cell line authentication.¹¹⁻¹⁴ Indeed, obtaining the genetic profile for the
169 whole collection of a biobank might be extremely expensive and laborious. To
170 overcome this limitation, we have devised a system based on the conservation of blood
171 spots that allows a biobank to resort to using the genetic profile of the donor only when
172 strictly necessary. The collection of the first blood spot at the time a blood sample is
173 donated at a health centre is crucial in order to carry out our method of authentication.
174 Thus, this blood spot would allow authentication of any derivative to the original donor,
175 whereas the second blood spot would be used to trace sample processing at the biobank
176 as a kind of internal control.

177 There are various commercial systems for fluid storage at room temperature, such as
178 FTA® cards (Whatman®) or IsoCode DNA matrix (Schleicher & Schuell BioScience,

179 Inc.). Yet, biobanks often have to operate within a limited budget in a relatively
180 expensive field and, therefore, implementing low-cost systems may be a valid
181 alternative. In this sense, all the storage materials tested in this study proved to be
182 effective for the conservation of blood spots at room temperature for long periods of
183 time. However, taking into account the DNA concentration obtained for each substrate,
184 non-colored cotton fabric and absorbent paper seem to be the most effective for long-
185 term conservation. It is likely that cotton strands in lint form an excessively open net in
186 which the DNA is more accessible by any degrading agent. It might be advisable to
187 analyze a larger sample set in order to replicate these results. However, even though the
188 DNA Bank of the UPV/EHU accumulates a very large number of samples, only the
189 blood spots from the 12 individuals included in this study were stored exactly in the
190 conditions presented herein and for a period of more than 15 years.

191 The method of genetic profiling presented herein involves the use of two commercial
192 kits. Two main reasons led us to select these kits: 1) the SGM Plus™ Kit shares its 10
193 STR markers with 10 out of 15 microsatellite markers of the Identifiler™ PCR
194 Amplification Kit. Therefore, the genetic profile of each sample is partially double
195 checked, thereby avoiding a potential error in the designation of alleles caused by allelic
196 drop-out due to DNA degradation and, at the same time, improving the quality and
197 reliability of the results; and 2) the use of standardized and validated commercial kits
198 for STR genotyping provides the possibility of managing comparable data¹⁵ between
199 biobanks and conducting inter-laboratory quality controls. In addition, the genetic
200 profiling step would not routinely be carried out, so that the cost increase is not
201 expected to be high.

202 In cases where misplacement is detected, our approach would allow the biobank to
203 confirm a putative traceability gap and, once confirmed, to undertake appropriate
204 corrective actions. It would be advisable to analyze all the derivatives related to the
205 affected sample (plasma, DNA, cell lines, etc.). Thus, the confirmation of a mismatch
206 would involve, at the very least, discarding the “orphan” derivatives from the biobank
207 collection and, therefore, stopping the supply of incorrectly stored derivatives.

208 Concerning the legal and ethical aspects involving sample genotyping for authentication
209 purposes, each biobank must follow the governing laws in the corresponding country.¹⁶
210 The blood preservation method presented herein complies both with Spanish biomedical
211 research law 14/2007¹⁷ on the traceability of data and biological samples and Organic
212 Law 15/1999¹⁸ on personal data protection. According to these laws, all the information
213 related to each sample should be kept at the biobank as classified information. In
214 compliance with these laws, we have developed a sample authentication form
215 specifically designed to store blood spots for long periods of time. This method makes it
216 possible to maintain the anonymity of the donor and, at the same time, make use of a
217 blood spot from the original sample for authentication purposes. To further comply with
218 ethical recommendations about information to donors, notification about the collection
219 and storage of blood spots for sample authentication should be included as part of the
220 informed consent.

221 Further to the above, genetic data generated during the authentication process should be
222 stored at biobanks, bearing in mind that they take responsibility for all data resulting
223 from the collection, storage, processing and shipment of each sample. However, in
224 compliance with the governing laws in many countries such as Spain, upon a donor’s
225 request, a biobank is obliged to destroy the sample and all its derivatives. Our approach

226 conforms to legal requirements as barcode labels generated during sample processing
227 allow us to identify and discard all components of any sample, including any
228 authentication forms.

229 To summarize, the results of the present study obtained from samples which are years
230 old indicate that the storage of small spots of dried blood preserved at room temperature
231 in 10 mm discs of non-colored cotton fabric or absorbent paper, attached to a sample
232 authentication form and conveniently protected from mishandling and/or contamination,
233 could be a long-lasting (at least 15 years), cost-effective and effortless approach for
234 subsequent genetic authentication of biobank samples. In addition, any analysis of
235 genetic profiles through use of commercial, standardized kits for STR typing provides
236 for the quality requirements of a biobank which is an issue of vital importance in the up-
237 and-coming field of biobank networking.

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245 Supplementary information is available at the European Journal of Human Genetics

246 website (<http://www.nature.com/ejhg>).

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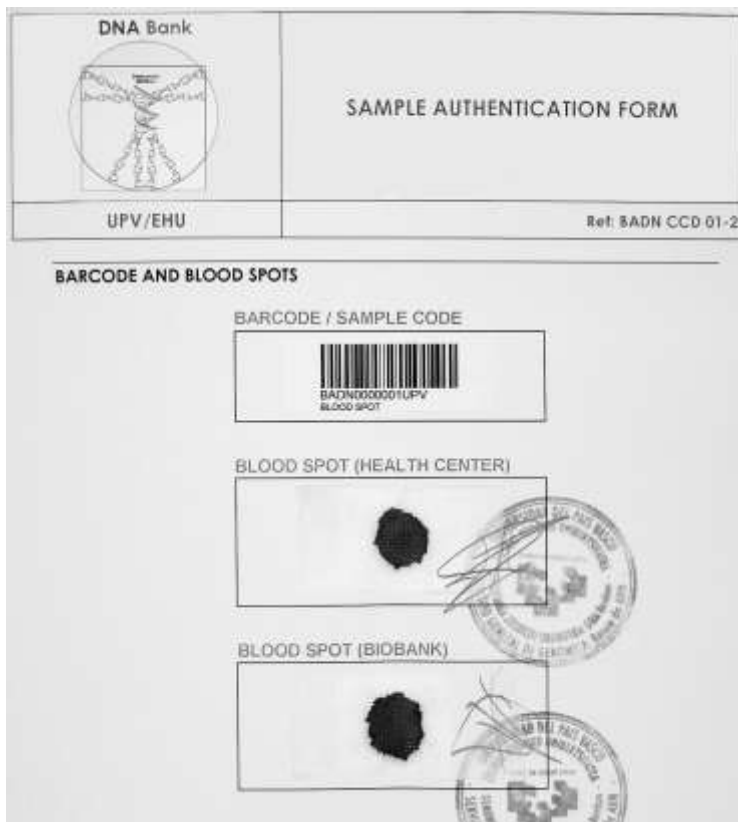
297 **Titles and legends to figures**

298 **Figure 1.** Blood spots attached to a sample authentication form covered with surgical
299 tape. Blood spot collection at the time the sample is donated allows for genetic
300 authentication of samples from the original donor. This collection is certified by the
301 signatures of health staff. The signature of a member of the biobank staff validates the
302 collection of the second blood spot when the sample enters the biobank; this blood spot
303 would be used to trace sample processing in the laboratory. A rubber stamp allows
304 further detection of any uncontrolled manipulation of blood spots.

305

306 **File format:** TIFF image (supplied as separate electronic file)

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308

1 **Tables**

2 **Table 1.** Genetic profiles obtained for all the samples analyzed in this study by means of the
3 AmpFISTR® Identifiler™ and SGM™ PCR Amplification kits.

4

5 (Table supplied as separate electronic file: Table1)

6

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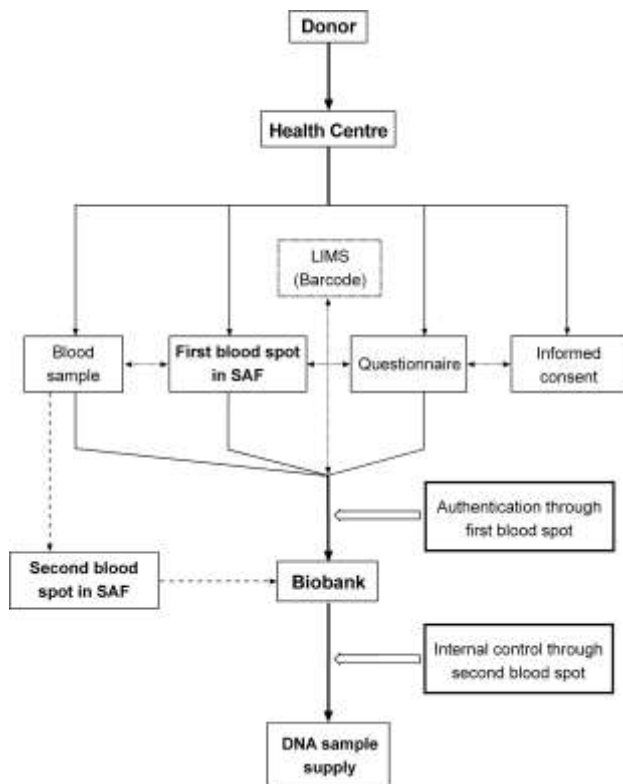
1 **Supplemental Figure**

2 **Figure S1.** Sample processing workflow. Once a donor signs the informed consent at
3 the health centre, associated information as well as the blood sample can be collected by
4 health centre staff. At that time a first blood spot is directly added to the corresponding
5 gap in the sample authentication form (SAF). All these elements make up the sample
6 and are linked through barcode labels. On entering the biobank, an aliquot is collected
7 from the blood sample to attach a second blood spot to the SAF. The first bloodspot is
8 used for sample authentication since it is collected directly from the original donor,
9 whereas the second blood spot allows internal control within the confines of the
10 biobank.

11

12 **File format:** TIFF image (supplied as separate electronic file)

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2 **Figure S2.** Electropherograms show the matching between genetic profiles obtained
3 with AmpFISTR® Identifiler™ (A) and SGM Plus™ (B) PCR Amplification Kits
4 (Applied Biosystems) in one of the samples analyzed in this study (S01).

5

6 **File format:** TIFF image (supplied as separate electronic file)

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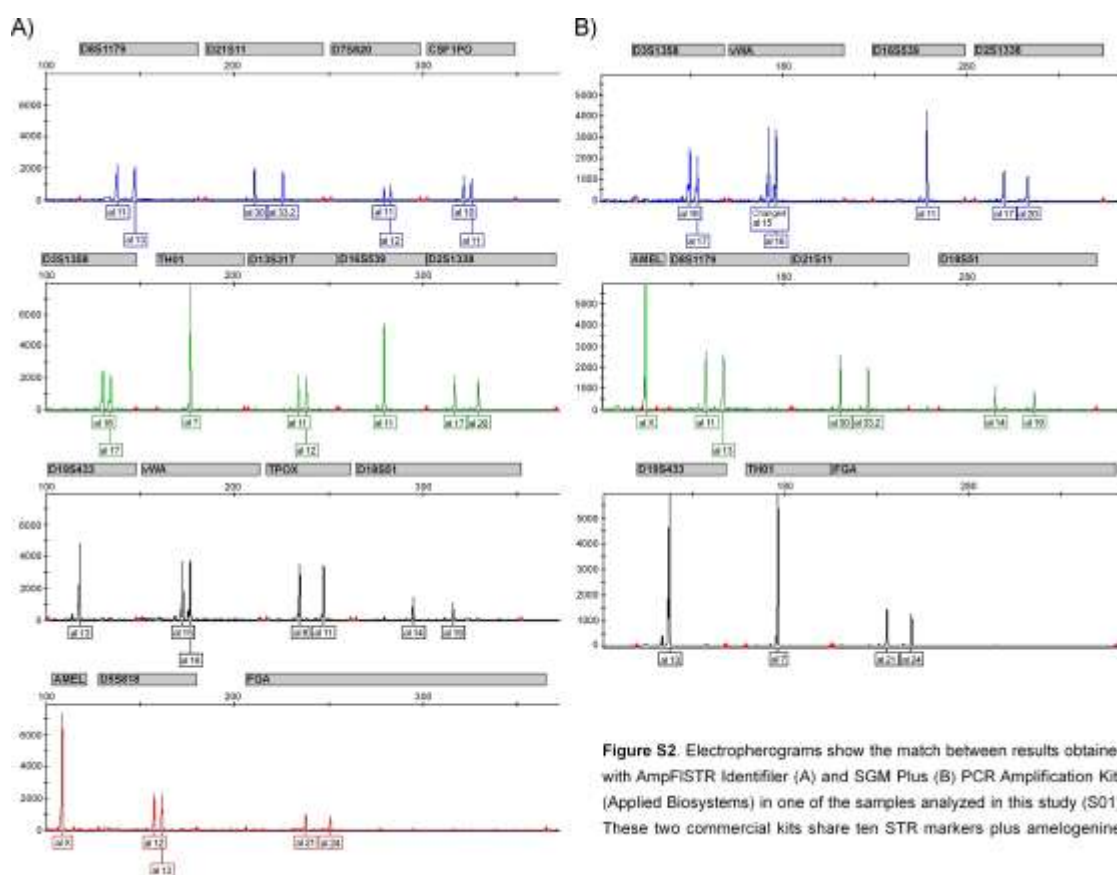
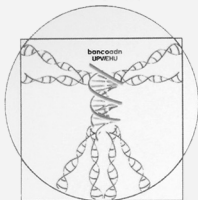


Figure S2. Electropherograms show the match between results obtained with AmpFISTR Identifiler (A) and SGM Plus (B) PCR Amplification Kits (Applied Biosystems) in one of the samples analyzed in this study (S01). These two commercial kits share ten STR markers plus amelogenine.

8

DNA Bank



SAMPLE AUTHENTICATION FORM

UPV/EHU

Ref: BADN CCD 01-2

BARCODE AND BLOOD SPOTS

BARCODE / SAMPLE CODE



BLOOD SPOT (HEALTH CENTER)



BLOOD SPOT (BIOBANK)

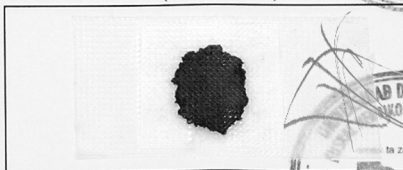


Table 1. Genetic profiles obtained for all the samples analyzed in this study by means of the AmpFISTR® Identifiler™ and SGM™ kits¹.

Sample	Material ²	Alleles observed in each locus															
		D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA	AMEL
S01	A, B, C, D	11 13	30 33.2	11 12	10 11	16 17	7 7	11 12	11 11	17 20	13 13	15 16	8 11	14 19	12 13	21 24	X X
S02	A, B, C, D	13 13	30 30	8 8	12 13	14 18	7 9.3	8 13	12 13	19 23	13 15.2	14 16	8 8	12 14	11 12	21 25	X X
S03	A, B, C, D	11 13	31 32.2	11 13	10 11	16 17	6 9.3	8 11	9 9	17 19	16 16.2	15 18	9 11	14 16	11 12	20 23	X X
S04	A, B, C, D	13 13	30 30	8 11	11 12	14 18	9.3 9.3	9 13	11 12	19 23	13 13	14 16	8 8	11 13	11 12	23 25	X X
S05	A, B, C, D	12 14	30 33.2	11 12	10 11	17 18	6 9	13 14	10 12	17 24	14 15	15 19	8 11	10 19	12 13	22 23	X X
S06	A, B, C, D	12 16	29 32.2	9 11	11 11	15 16	8 9	10 12	11 12	16 20	13 13	17 19	8 12	13 14	11 11	24 25	X X
S07	A, B, C	12 15	28 30	10 11	10 12	15 17	6 9.3	11 11	11 11	17 19	15.2 16	15 18	8 8	14 16	11 11	19 26	X X
S08	A, B, C	10 10	29 30	8 10	10 12	16 16	7 9	11 13	11 11	18 24	13 14	15 18	8 11	13 13	11 14	20 22	X X
S09	A, B	14 15	27 31	9 12	12 14	15 18	9.3 10	12 13	11 13	17 25	14 14	15 17	8 11	12 15	10 12	23 24	X X
S10	A, B	12 15	30 32.2	11 12	12 12	16 18	8 9.3	10 11	13 13	17 19	14 15	17 18	11 11	12 15	11 13	22 26	X Y
S11	A, B	12 14	30 31.2	8 11	10 11	15 15	7 9.3	8 11	11 13	20 24	13 15	16 17	11 12	15 15	9 12	19 24	X X
S12	A, B	13 14	30 30	9 10	11 11	16 18	6 8	8 11	11 12	17 23	13 15	16 17	8 8	13 15	11 13	18 23	X Y

¹Microsatellite loci highlighted in bold are those amplified within the AmpFISTR® SGM Plus™ kit and also analyzed by means of the AmpFISTR Identifiler™ kit. ²Material used for preservation of each sample, A: DNA stored at -20°C; B: non-colored cotton fabric; C: lint; D: absorbent paper.