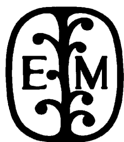

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DNA STR typing for forensic use. Two methods and two instruments in comparison: IR-based sequencer and UV-based sequencer

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Abstract

STR systems and the amelogenin locus system have become the markers of choice for the identification of forensic stains. Nowadays, several commercial kits, which enable the detection of the PCR products by fluorescent chromophores in combination with an UV-based optical system, are available. The use of IR-based automated sequencers in the forensic genetics laboratories, however, has become less frequent after some interesting initial applications [Forensic Sci. Int. 85 (1997) 225., BioTechniques 23 (1997) 942.]. We recently introduced our protocol to analyse the whole core of Combined DNA Index Systems' (CODIS) systems of the FBI, with an infrared automatic DNA sequencer (LI-COR 4200) [Electrophoresis 21 (2000) 3564.]. Preliminary results showed the possibility to investigate degraded forensic materials, like bloodstains, semen stain and saliva. In the present study, we compare the results obtained from particularly degraded samples, using two different methods and two different instruments, based on UV and IR technology.
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Keywords: STR; IR-sequencer; UV-sequencer; Infrared

1. Introduction

We recently reported the use of an IR automated fluorescence monolaser sequencer (LICOR-4200) [1,2] for the analysis of 13 autosomal STR systems (TPOX, D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, TH01, vWA, D13S317, D16S359, D18S51,

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D21S11) and the X–Y homologous genes amelogenin system [3]. These systems are particularly important, because they represent the core of the Combined DNA Index System (CODIS) used by the FBI [4].

The protocol we prepared is based on four new independent multiplex PCR reactions and on the direct labelling of the forward primer of every primer pair, with a new molecule (IRDye™ 800). We standardised two tetraplex systems (MU1: AME, vWA, FGA, D16S359 and MU2: D3S1358, TPOX, TH01, CSF1PO) and two triplex systems (MU3: D8S1179, D21S11, D18S51 and MU4: D7S820, D5S818, D13S317). Some modifications were applied more recently, to improve the efficiency and sensitivity of the method.

We used this protocol to solve paternity testing and to collect data in a Tuscany population of 188 unrelated individuals [5]. Moreover, the four multiplexes were used to analyse forensic samples (blood, saliva, semen and bone) for a collaborative Italian project of Ge.F.I. Since in criminal identifications, it was necessary to exchange data between different laboratories that use the same STR systems, but different technologies, we compare our protocol with a more widespread UV-apparatus with commercially available kits. A total of 8 undegraded and 26 particularly degraded DNA samples previously extracted and typed with UV-technology, were tested with the IR-based protocol and the results were compared.

2. Materials and methods

2.1. DNA extraction

All forensic samples from various sources were extracted using the QIAamp tissue kit (Quiagen, Hilden, Germany).

2.2. Method A: UV-typing

Aliquots of 5 μl of unquantified extracts was analysed with the AmpF*l*STR® SGM Plus™ PCR Amplification kit and ABI Prism® 310 Genetic Analyser (Applied Biosystems), according to the manufacture's recommendation.

2.3. Method B: IR-typing

The same samples were typed with the described protocol [3]. Some modifications were introduced:

the samples were diluted 1:10 and 1 μl was used for PCR;
34 cycles of PCR were realised, with a final extension of 1 h;
in multiplex 2 (MU2), the amplification of TH01 was performed with primers suggested by Edwards et al. [6].
we used a different strategy, to amplify the four loci of the multiplex 2 (MU2). Just a small amount of F-labelled primer was added to a mix containing F- and R-unlabeled primers. The primer's concentration was the following: 0.14 μM for D3S1358/F and R

Table 1
Comparison between results obtained with two methods

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
D5S818	*	*	A B	B	*	–	–	*	–	*	*	*	*	*
D7S820	*	*	A B	–	*	–	B	*	–	*	*	*	*	*
D13S317	*	*	A	B	*	–	–	*	–	*	*	*	*	*
D8S1179	*	*	A	–	*	–	B	*	–	*	*	*	*	*
D21S11	*	*	A B	–	*	–	–	*	–	*	*	*	*	*
D18S51	*	*	A	B	*	–	–	*	–	*	*	*	*	*
D3S1358	*	*	A B	–	*	A	B	*	–	*	*	*	*	*
TH01	*	*	A	–	*	–	B	*	–	*	*	*	*	*
TPOX	*	*	A B	–	*	–	–	*	–	*	*	*	*	*
CSF1PO	*	*	A	–	*	–	–	*	–	*	*	*	*	*
VWA	*	*	A B	B	*	A	B	*	–	*	*	*	*	*
FGA	*	*	A B	–	*	–	B	*	–	*	*	*	*	*
D16S359	*	*	A	–	*	–	–	*	–	*	*	*	*	*
AME	*	*	A B	B	*	A	B	*	–	*	*	*	*	*

	15	16	17	18	19	20	21	22	23	24	25	26	27	28
D5S818	*	A B	*	B	–	B	*	–	–	–	–	*	–	*
D7S820	*	A B	*	B	–	B	*	–	–	–	–	*	–	*
D13S317	*	A B	*	B	–	B	*	–	–	–	–	*	–	*
D8S1179	*	A B	*	B	–	B	*	–	–	–	–	*	–	*
D21S11	*	–	*	B	–	–	*	–	–	–	–	*	–	*
D18S51	*	B	*	B	–	B	*	–	–	–	–	*	–	*
D3S1358	*	A B	*	B	–	B	*	–	–	B	–	*		*
TH01	*	A B	*	B	–	B	*	–	–	–	–	*	–	*
TPOX	*	A B	*	B	–	–	*	–	–	B	–	*	–	*
CSF1PO	*	A B	*	–	–	B	*	–	–	B	–	*	–	*
VWA	*	A B	*	B	–	B	*	–	–	–	–	*	–	*
FGA	*	B	*	B	–	B	*	–	–	–	–	*	–	*
D16S359	*	–	*	B	–	B	*	–	–	–	–	*	–	*
AME	*	A B	*	B	–	B	*	–	–	–	–	*	–	*

"–" = No result, "A" = only results with method A, "B" = only results with method B, " * " = results with both methods.

and 0.01 μM for D3S1358/F-IRDye™ 800, 0.2 μM for TH01/F and R and 0.02 μM for TH01/F-IRDye™ 800, 0.2 μM for TPOX/F and R and 0.02 μM for TPOX/F-IRDye™ 800, 0.4 μM for CSF1PO/F and R and 0.08 μM for CSF1PO/F-IRDye™ 800.

The exact characterisation of the alleles was performed by using ladders from collaborative projects which had been constructed in our laboratory, with specific sequenced alleles and by using positive cell line DNA K562 (Promega, USA).

3. Results

The comparison between the two different methods and instruments showed that the results are very consistent. Most samples gave either complete profiles for both methods, or no results at all (Table 1).

The 8 undegraded samples and 14 degraded samples were typed with success and with identical results from both laboratories, 6 DNA samples were completely negative. For the remaining eight samples, both laboratories obtained only incomplete profiles. The loci, which were successfully typed, varied. However, most problems arose with the largest loci. For method A: D18S51, FGA and D16S359; for method B: D16S359, D18S51 and D13S317. Moreover, one sample was completely typed with method A, but only partially with method B. Vice versa, two samples were almost completely typed by the laboratory that uses method B, while the other laboratory which uses method A produced no results. These results are probably not significant and may be caused by casual pipetting, dilution, etc.

Our data confirm the possibility to use the four multiplexes PCR, in combination with an IR-based automated sequencer use, to analyse the whole set of CODIS loci for forensic purposes. This means that the same instrument can be used both for medical genetics applications, and for forensic genetic purposes.

The comparison with the widespread UV-based DNA technology confirms that both methods are comparably efficient in typing forensic stains. The consistent results suggest that it is possible to exchange data between laboratories that use the same core of markers, but different technologies.

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