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I-DNASE21 system: Development and SWGDAM validation of a new STR 21-plex reaction

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ABSTRACT

I-DNASE21 is a new STR multiplex system that amplifies 21 STR autosomal loci, plus the amelogenin locus in one reaction. This system has been designed to analyze all the STR loci included in the Combined DNA Index System (CODIS), Interpol Standard Set of Loci (ISSL), Extended European Standard Set (ESS-Extended), UK National Criminal Intelligence DNA Database (NDNAD) and German Core loci (GCL). This manuscript presents the validation of the I-DNASE21 system according to the revised guidelines issued by the Scientific Working Group on DNA Analysis Methods (SWGDAM). The results of this validation, added to the extremely high discriminatory power showed, suggest that I-DNASE21 could be a potentially helpful tool for identification and kinship determination even in complex paternity cases.

1. Introduction

Since its first application in a forensic case in 1991 [1], the technology for the analysis of microsatellite markers or Short Tandem Repeats (STRs), has undergone a rapid development establishing itself as the reference technique used for human identification and to determine biological kinship [2–7]. In this sense, a wide range of PCR STR multiplex commercial kits for the amplification of several loci STR in a single reaction have been developed [8–17]. As a result, actually, millions of genetic profiles are obtained each year for different purposes [7].

With the objective of storing and applying all the information contained in these genetic profiles and simultaneously improving the international collaboration on criminalistics, national DNA databases were generated [18–21]. Nowadays, the Combined Index system (CODIS), Interpol Standard Set of Loci (ISSL), Extended European Standard Set (ESS-Extended), UK National Criminal Intelligence DNA Database (NDNAD) and German Core loci (GCL) are the principal DNA national databases. Though all of them share 7 STR core loci, they differ on some specific STR loci and the total number of loci to include [22].

Making an effort to realize the requirements of different sample analyses and with the objective of harmonizing the STR markers used in Forensic Science, international working groups such as EDNAP (European DNA Profiling Group) and ENFSI (European Network of Forensic Science Institute) proposed a serial of recommendations to follow in the design of new STR multiplex reactions. Consequently, several new STR multiplex kits that include more mini-STR loci and the new recommended European Standard Set loci (D1S1656, D2S441, D10S1248, D12S391 and D22S1045) have been validated and commercialized [23–26]. These new reactions have been designed to amplify a wide range of

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STRs in one multiplex reaction, including the 5 new recommended markers, most of them in mini-STR format, but not one of these reactions includes all the STR loci included in the most relevant national DNA databases. This deficiency could be problematic when genetic profiles are obtained from these reactions and must then be compared with the stored information in a specific database. The absence of data for some STRs could result in an insufficient probability of identity limiting the success of the comparison [27,28]. Recently, the life sciences company Applied Biosystems® by Life Technologies™ has developed a new STR multiplex based in a new 6-dye technology called GlobalFiler™ STR Kit designed specifically to resolve this problem. This system will be commercialized soon; even so the requirements of acquiring new equipment for fragment analysis able to detect this new 6-dye technology make difficult that all the laboratories that realize human profiling would be able to adopt this system [29].

In this context, we have designed a new STR multiplex system named I-DNASE21. This system is able to amplify 21 loci STRs (CSF1PO, D5S818, D7S820, D21S11, D2S441, D1S1656, TH01, D16S539, D3S1358, D18S51, D2S1338, TPOX, vWA, D8S1179, D19S433, D12S391, SE33, D13S317, FGA, D22S1045 and D10S1248) plus amelogenin in a unique reaction including all the STRs contained in the above mentioned main international databases (CODIS, ISSL, ECL, NDNAD and GCL) and also including the 5 new recommended loci that constitute the Extended European Standard Set (Extended-ESS) of markers.

In this manuscript, we present the validation of the I-DNASE21 system according to the revised guidelines issued by the Scientific Working Group on DNA Analysis Methods (SWGDAM) [30] that consist on primer set optimization tests, concordance studies with other commercial STR multiplex systems and reproducibility, sensitivity tests, stability studies, database-type sample studies, species specificity tests, determination of stutter percentage, heterozygous peak height ratio (PHR), mixture studies and population studies.

I-DNASE21 has been designed to obtain genetic profiles that can be compared with all the main international DNA databases. Due to the high number of STRs analyzed with this system, I-DNASE21 also shows an extremely high power of discrimination. This, along with the obtained results, demonstrates this system to be an effective, sensitive and robust tool to obtain genetic profiles, as well as, a widely practical solution to forensic science.

2. Materials and methods

2.1. Human DNA control samples

Control DNA 9947A from AmpFLSTR® Yfiler™ PCR Amplification Kit (Applied Biosystems, Foster City, CA), control DNA 007 from AmpFLSTR® NGM™ PCR Amplification Kit (Applied Biosystems, Foster City, CA) and controls DNA K562 and 2800 M from Promega (Promega® Corporation, USA) were used to set up the PCR amplification conditions and run the appropriates sensitivity tests.

All the DNA controls were quantified by Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Foster City, CA) in an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA), according to the manufacturer's recommendations.

2.2. Population samples

Populations samples for the validation of I-DNASE21 were obtained from 895 healthy individuals including, 308 European Caucasoids (Basque Country, Spain) 241 of them from peripheral blood and 67 from buccal swabs, 159 Hispanics and 124 African-Americans from Colombia, all of them from peripheral blood; 160 African-Americans from Haiti and 144 Asians from Thailand from buccal swabs.

The DNA from the Caucasoid individuals was isolated by the salting out extraction method [31].

The samples of Hispanic and Afro-American origin from Colombia were extracted by membrane-based purification with Qiamp DNA Micro Kit (Qiagen, Valencia, CA).

The DNA from Haiti and Thailand was obtained using the Gentra Buccal Cell Kit (Puragene, Gentra Systems, Inc., MN, USA).

Ratios of salt and protein versus DNA extracted were evaluated by spectrophotometry by means of a Scientific NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE) and subsequently DNA extracts were quantified with PicoGreen® (Invitrogen, Carlsbad, CA).

2.3. Primer set optimization

Primer design strategy of the previously validated mini and midi-STR multiplex I-DNA1 [32], which included 14 STR loci plus amelogenin, with amplification products no longer than 300 bp, was used to add the 7 new STRs loci with the aim of not significantly increase the final size of the reaction. In this sense, primers pairs from I-DNA1 were used for the amplification of the STRs CSF1PO, D5S818, D7S820, D21S11, TH01, D3S1358, D18S51, TPOX, vWA, D13S317, FGA and the sex marker Amelogenin. Modifications on the sequence of the reverse primers for D8S1179, D16S539 and D19S433 were made to improve their efficiency with this new combination.

Previous to the design of the new primers, the regions flanking the STRs were studied as described in Odriozola et al. [32]. New primer pairs for the STRs D2S1338, D2S441, D1S1656, D12S391, D22S1045, D10S1248 and SE33 were designed by using the Perlprimer program (http://perlprimer.sourceforge.net/). In this sense and in order to allow the subsequently reaction multiplex, the new pairs of primers were synthesized with melting temperatures as close as possible to those of I-DNA1 primers [32].

A large range of the alleles contained on the STRBase website [33] were taken into account when designing the primers for the new STR loci. The allele range and PCR fragment size range for each STR locus is detailed in Table 1.

Table 1
Allele ranges, PCR fragment sizes and fluorescent dye of the STRs loci included in the design of I-DNASE21.

Locus STR	Allele range	PCR fragment (bp)	Fluorescent Dye
CSF1PO	6–15	75–111	6-FAM™
D5S818	7–17	127–167	
D7S820	6–15	183–219	
D21S11	24–38	240–296	
D2S441	9–16	305–333	
D1S1656	9–21	341–389	
TH01	5–14	57–93	VIC™
D16S539	5–16	105–149	
D3S1358	9–20	161–205	
D18S51	7–27	213–293	
D2S1338	15–28	313–365	
TPOX	6–16	61–101	NED™
vWA	11–24	121–173	
D8S1179	8–20	181–229	
D19S433	5.2–18.2	245–297	
D12S391	14–27	305–357	
SE33	4.2–39.2	372–512	
D13S317	5–17	72–120	PET®
Amelogenin	X,Y	121–127	
FGA	16–51.2	151–293	
D22S1045	8–19	294–327	
D10S1248	8–18	339–379	