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Research paper

The first GHEP-ISFG collaborative exercise on forensic applications of massively parallel sequencing

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ABSTRACT

One of the main goals of the Spanish and Portuguese-Speaking Working Group of the International Society for Forensic Genetics (GHEP-ISFG) is to promote and contribute to the development and dissemination of scientific knowledge in the field of forensic genetics. The GHEP-ISFG supports several Working Commissions which develop different scientific activities. One of them, the Working Commission on "Massively Parallel Sequencing (MPS): Forensic Applications", organized its first collaborative exercise on forensic applications of MPS technology in 2019. The aim of this exercise was to assess the concordance between the MPS results and those obtained with conventional technologies (capillary electrophoresis and Sanger sequencing), as well as to compare the results obtained within the different MPS platforms and/or the different kits/panels and analysis software packages (commercial and open-access) available on the market. The seven participating laboratories analyzed some samples of the annual GHEP-ISFG proficiency test (EIADN No. 27 (2019)), using Ion Torrent™ or MiSeq FGx® platforms. Six of them sent autosomal STR sequence data, five laboratories performed MPS analysis of individual identification SNPs, four laboratories reported MPS data of Y-chromosomal STRs, and X-chromosomal STRs, three laboratories performed MPS analysis of ancestry informative SNPs and phenotype informative SNPs, two labs performed MPS analysis of the mitochondrial DNA control region, and only one lab produced MPS data of lineage informative SNPs. Autosomal STR sequencing results were highly concordant to the consensus obtained by capillary electrophoresis in the EIADN No. 27 (2019) exercise. Furthermore, in general, a high level of concordance was observed between the results of the participating laboratories, regardless of the platform used. The main discordances were due to errors during the analysis process or from sequence data obtained with low depth of coverage. In this paper we highlight some issues that still arise, such as standardization of the nomenclature for STRs analyzed by sequencing with MPS, the universal uptake of a nomenclature framework by the analysis software, and well established validation and accreditation of the new MPS platforms for use in routine forensic case-work.

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1. Introduction

DNA sequencing, notably the technique described by Sanger et al. in 1977 [1], has undergone a constant evolution. The introduction of fluorochrome-labeled ddNTPs detected with the capillary electrophoresis (CE) platforms [2] has increased sensitivity and performance, while simultaneously decreased costs to a level where complete genome sequencing has been possible since 2001 [3,4]. Currently, massively parallel sequencing (MPS) techniques use different optical systems and chemistries which are different to the Sanger method, to provide DNA sequencing data with a capacity, speed and reliability not seen before [5–7], and at a relatively reduced cost [8]. However, these advances still require extensive validation and evaluation, as well as the implementation of bioinformatic support, before they can be applied with full reliability in the forensic field [9].

For some years, the number of laboratories in the field of forensic genetics that are investigating and beginning to implement MPS technologies has increased considerably [10], both for the analysis of established markers of mitochondrial DNA (mtDNA) and Short Tandem Repeats (STRs) and other less widely used markers, including Single Nucleotide Polymorphisms (SNPs) and insertion-deletion markers (InDels) to establish identity, infer ancestry, estimate phenotype and/or body fluid identification [11–13]. Some of the advantages of MPS technology include the ability to simultaneously analyse hundreds or thousands of different DNA markers in a single workflow, and to detect variations at the sequence level [11,13].

Within this scientific framework, the need to contribute to the development and standardization of MPS technologies is highlighted by scientific societies, with the International Society for Forensic Genetics (ISFG) endorsing relevant initiatives [14–16]. As one of the ISFG working groups, the Spanish and Portuguese Speaking Working Group (GHEP, "Grupo de Habla Española y Portuguesa") has developed a range of scientific activities through different working commissions dedicated to specific issues (https://ghep-isfg.org/en/working-commissions). During the 2016 annual assembly of the GHEP-ISFG, members agreed to create a commission to oversee the forensic applications of MPS, with the following aims: (1) to conduct a survey on the implementation of MPS technology in the GHEP-ISFG laboratories and its future perspectives; (2) to organize a collaborative exercise; and (3) to promote population and validation data generation, and consequent collaboration with international forensic databases (STRidER [17], EMPOP [18] and YHRD [19]).

The second initiative of this GHEP-ISFG Working Commission was achieved in 2019, and its main objectives were to give laboratories the opportunity to compare their chosen MPS workflows, as well as to check the concordance of MPS results for DNA markers: autosomal STRs (auSTRs), Y-chromosomal STRs (Y-STRs), X-chromosomal STRs (X-STRs), mitochondrial DNA (mtDNA) Control Region, individual identification SNPs (iiSNPs), lineage informative SNPs (liSNPs), ancestry informative SNPs (aiSNPs) and phenotype informative SNPs (piSNPs), and assess their performance against CE analysis. Moreover, the exercise included an instructional aspect, exploring the limiting factors in the handling of MPS sequence data that could affect the final result (sequence threshold values employed, minimum depth of coverage used, bioinformatic tools applied, etc.). A total of seven GHEP-ISFG laboratories from three different countries were involved in this exercise: five from Spain, one from Colombia and one from Brazil. Most laboratories belong to public institutions (three Justice Administration Labs, one Health and University Lab and two Police Labs), and only one was from a private company. This paper outlines the results and conclusions generated from this first collaborative exercise.

2. Materials and methods

2.1. Samples

Five samples from the GHEP-ISFG program of proficiency testing basic level (EIADN No. 27 (2019)) [20,21] were analyzed in this exercise, including three reference samples, M1 (100 μL blood from a male on an *Ahlstrom-Munksjö GenCollect* card), M2 (100 μL blood from a female on a Blood Stain Storage System (BSSS), *Whatman*® card) and M3 (120 μL saliva from a male on a cotton swab), and two forensic samples, M4 (50 μL of a 1:2 (v/v) mixture of blood from a female and semen (1/7 dilution) from the same donor as M3 on a piece of towel and M5 (telogenic hair shaft from a female donor).

2.2. MPS workflows for library and template preparation, and data analysis

Participating laboratories used different MPS platforms and analysis strategies. This section details the analytical characteristics of each participating laboratory based on data they provided.

2.2.1. Platforms and panels

Four laboratories used the Verogen MiSeq FGx® Forensic Genomics System (*Verogen Inc., San Diego, CA, USA*). The other three laboratories each used a different Ion Torrent™ system (*Thermo Fisher Scientific Inc., Waltham, MA, USA*, herein TFS): one Ion PGM™ System; one Ion S5™ system; and one Ion S5™ XL System. All three laboratories used an Ion Chef™ Instrument to automatically perform certain library preparation and chip loading processes.

The kits and/or marker panels used by each participant are outlined in Table 1. Supplementary Table S1 compiles all markers analyzed in the exercise and Supplementary Table S2 collects the general data on the sequencing run information: chip/flow cell used, total expected and obtained reads by chip/flow cell, total number of samples run by chip/flow cell and the reads per sample in each laboratory. Amongst laboratories using the Verogen platform, two used Primer Mix A of the ForenSeq™ DNA Signature Prep Kit [22] that includes 27 autosomal STRs, 24 Y-STRs, 7 X-STRs, the Amelogenin sex marker, and 94 iiSNPs [23,24]). The other two included Primer Mix B that adds 56 aiSNPs (55 aiSNPs from K. Kidd's lab [25] with the addition of rs1919550) and 22 piSNPs (two aiSNPs are also used for phenotype prediction) [26].

Two laboratories with Ion Torrent™ platforms, used the Precision ID GlobalFiler™ NGS STR Panel v2 [27] comprising 20 CODIS autosomal STRs, 9 novel autosomal Mini-STRs, 2 penta-nucleotide repeat STR markers not in CODIS (Penta D and Penta E), 1 Y-STR (DYS391), and three sex markers (Amelogenin, SRY, rs2032678). Two laboratories used the Precision ID mtDNA Control Region Panel [28,29]. One laboratory used the Precision ID Identity Panel [29] comprising 90 autosomal SNPs [23,24] and 34 upper Y-clade SNPs [30], the Precision ID Ancestry Panel [29] that includes 165 SNPs (55 SNPs from K. Kidd's lab [25] and 123 SNPs from M. Seldin's lab [31], sharing 13 SNPs between them), and the AmpliSeq™ DNA Phenotyping Panel [32] of 23 SNPs and one Indel [26].

2.2.2. MPS workflow

Although the analytical phases carried out in each of the Verogen and Ion Torrent™ platforms have specific steps [13], they can all be summarized as: (1) library preparation; (2) library quantification and/or normalization; (3) template preparation; and (4) sequencing. The following sections specify how each of the participating laboratories applied these processes (Supplementary Fig. S1.1).

(1) Library Preparation. Two of three Ion Torrent™ laboratories used the Ion Chef™ for library preparation. The other laboratories performed manual library preparation.

(2) Libraries Quantification and/or Normalization. All three Ion Torrent™ laboratories quantified their library preparations using the Ion Library TaqMan™ Quantitation Kit [33] in the AB7500 Real-Time PCR system. Verogen laboratories performed a normalization using either bead saturation (three of four), as Verogen recommends [22]; and in one of four, using the Illumina KAPA Library Quantification Kits [34] in the LightCycler® System (*F. Hoffmann-La Roche Ltd., Basel, Switzerland*).

(3) Template preparation. All three Ion Torrent™ laboratories used the Ion Chef™ for template preparation. The Verogen laboratories carried out this process manually.

(4) Sequencing. As described above, four laboratories used the MiSeq FGx® Forensic Genomics System and one laboratory each used the Ion PGM™; Ion S5™; and Ion S5™ XL sequencing system.

2.2.3. Data analysis tools

Reference genome: Regarding nuclear markers, three laboratories used the GRCh38 reference genome build suggested by the ISFG [14] (two Verogen and one Ion Torrent™). Four laboratories used the GRCh37 reference genome build. All laboratories were required to use the revised Cambridge Reference Sequence (rCRS) to characterise mtDNA sequences [35].

The analysis of MPS sequence data can be divided into five phases [9, 13]: (1) raw data reading and/or interpretation; (2) determination of the bases and quality (base calling); (3) sequence alignment to a reference genome; (4) allele/variant calling; and (5) final analysis and reporting.

Laboratories using the Verogen platform performed all the above phases with ForenSeq™ Universal Analysis Software (UAS) [36]. These laboratories used different version 1 releases of the UAS and they did not report having used any other analysis software.

Laboratories using Ion Torrent™ systems performed the first two phases using Torrent Suite™ software (TSS) [37]. Two laboratories performed the sequence alignment and the allele/variant calling by applying specific plugins with TSS: the HID Genotyper Plugin and/or the Torrent Variant Caller. One laboratory also used Converge™ Software v2.1 [38], which analyses results from STRs, SNPs and mtDNA simultaneously. Another laboratory, after TSS analysis applied the open access software STRait Razor v3.0 [39] for STR sequence analysis. Additionally, the Ion Torrent™ laboratories applied IGV [40], toaSTR [41]) and GeneMarker HTS [42].

2.3. Concordance with capillary electrophoresis (CE)

Concordance of genotypes and sequence data for auSTRs, Y-STRs, X-STRs and mtDNA (comprising HV1 nucleotide positions -nps-16,024–16,365; HV2 nps 73–340; and HV3 nps 438–574) was tested using the assigned values established using CE analysis from the EIADN No. 27 (2019) Exercise (GHEP-ISFG proficiency test). For those markers not considered in that exercise, data were only compared between laboratories.

2.4. Depth of coverage (DoC) assessment

Considering the differences between participating laboratories (Supplementary Table S2), in order to make a comparison of the depth of coverage (DoC) which would be as realistic as possible, it was necessary to take into account the output of the chips/flow cell, the number of samples run in the same chip/flow cell, and the number of markers analyzed for each sample in the same run. To take account of these three variables, a correction factor was generated that allowed DoC values to be normalized among the participating laboratories (Supplementary Table S2). This correction factor was constructed as an inverse logarithmic ratio of the number of expected reads per marker and relativized to 1. Those "expected reads per marker" would depend on the following parameters: the total number of reads per chip/flow cell, the number of samples run on this chip/flow cell, and the total number of markers analyzed on this chip/flow cell. Thus, the value 1 of this correction factor would correspond to that laboratory that had the least number of "expected reads per marker", and the lowest value of this correction factor would correspond to that laboratory that had the highest number of "expected reads per marker". To normalize the DoC between laboratories, sequence coverage data for the markers of each laboratory were multiplied by the correction factor calculated individually for each laboratory. The laboratories that analyzed the mtDNA control region were considered independently, since this region does not have a certain number of markers, but rather different overlapping fragments of the

Table 1
Panels and kits used for each of the markers analyzed to perform the GHEP-MPS01 exercise. Light blue shading indicates laboratories using the Ion Torrent™ system (Thermo Fisher Scientific, TFS), and light green shading indicates those using the Verogen system. Markers: autosomal STRs (auSTRs), Y-chromosomal STRs (Y-STRs), X-chromosomal STRs (X-STRs), mitochondrial DNA (mtDNA) Control Region, individual identification SNPs (iiSNPs), lineage informative SNPs (liSNPs), ancestry informative SNPs (aiSNPs) and phenotype informative SNPs (piSNPs).

Lab No.	Platforms	auSTRs	Y-STRs	X-STRs	mtDNA	iiSNPs	aiSNPs	liSNPs	piSNPs
5	Ion S5™ System / Ion Chef™ **(TFS)**	Precision ID GlobalFiler NGS STR Panel v2							
7	Ion S5™ XL System / Ion Chef™ **(TFS)**	Precision ID GlobalFiler NGS STR Panel v2			Precision ID mtDNA Control Region Panel				
11	Ion PGM™ System / Ion Chef™ **(TFS)**				Precision ID mtDNA Control Region Panel	Precision ID Identity Panel	Precision ID Ancestry Panel	Precision ID Identity Panel	Ion Ampliseq DNA Phenotyping Panel
24	MiSeq FGx® **(Verogen)**	ForenSeq DNA Signature Prep Kit Mix B	ForenSeq DNA Signature Prep Kit Mix B	ForenSeq DNA Signature Prep Kit Mix B		ForenSeq DNA Signature Prep Kit Mix B	ForenSeq DNA Signature Prep Kit Mix B		ForenSeq DNA Signature Prep Kit Mix B
33	MiSeq FGx® **(Verogen)**	ForenSeq DNA Signature Prep Kit Mix B	ForenSeq DNA Signature Prep Kit Mix B	ForenSeq DNA Signature Prep Kit Mix B		ForenSeq DNA Signature Prep Kit Mix B	ForenSeq DNA Signature Prep Kit Mix B		ForenSeq DNA Signature Prep Kit Mix B
179	MiSeq FGx® **(Verogen)**	ForenSeq DNA Signature Prep Kit MixA	ForenSeq DNA Signature Prep Kit MixA	ForenSeq DNA Signature Prep Kit MixA		ForenSeq DNA Signature Prep Kit MixA			
265	MiSeq FGx® **(Verogen)**	ForenSeq DNA Signature Prep Kit MixA	ForenSeq DNA Signature Prep Kit MixA	ForenSeq DNA Signature Prep Kit MixA		ForenSeq DNA Signature Prep Kit MixA			

region are analyzed, and both laboratories used the same panel. All DoC figures and data analyses were generated using Microsoft Excel.

3. Results and discussion

3.1. Short tandem repeats

3.1.1. Autosomal STRs

In total, six participating laboratories reported autosomal STR results for samples M1-M4, two using the TFS Precision ID GlobalFiler™ NGS STR Panel v2 [27] (herein GF-NGS) and all four Verogen laboratories using the ForenSeq™ DNA Signature Prep Kit [22] (herein FS) (Table 1). Supplementary Table S3 compiles the autosomal STRs genotypes from each participant.

Concordance with CE data was tested in 23 autosomal STR markers (CSF1PO, D1S1656, D2S1338, D2S441, D3S1358, D5S818, D6S1043, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, FGA, Penta D, Penta E, TH01, TPOX and vWA) plus amelogenin. In the reference samples from single sources (M1, M2, and M3), results were found to be highly concordant (Supplementary Table S3) (98.5 %; 806/818 concordant alleles). A single laboratory (Lab033) presented a locus drop-out at STR D22S1045, which cannot be explained by low coverage and may be due to loss during transcription (clerical error).

It is worth highlighting STR D6S474, which was analyzed by the two laboratories using the GF-NGS panel with concordant results (e.g., reference sample M1, genotype 1414). Nevertheless, discordances were detected against the CE data (e.g., M1, genotype 13) (Supplementary Fig. S2.1), which were reported by 10 laboratories participating in the EIADN No. 27 (2019) using the Qiagen Investigator HDplex kit (*Hilden, Germany*). The manual alignment was performed with the updated Forensic STR Sequence Structure Guide v5 [15] and the sequences obtained with the GF-NGS panel were reviewed with those collected in the *STRSeq catalog* [43] (Supplementary Fig. S2.2), confirming that the allele calling obtained by the GF-NGS panel was correct.

It is interesting to note that there is an isometric heterozygous allele 14 in reference sample M1, because one allele 14 sequence has a SNP in the flanking region (Supplementary Fig. S2.1). This sequence variant was only detected by the laboratories using the GF-NGS panel. The detection of such sequence variants will be of growing interest as certain STRs benefit from the increased genetic diversity detected by sequencing [9,11,13–16,44].

There were 11 STRs without previous consensus CE data: seven (D1S1677, D2S1776, D3S4529, D5S2800, D6S474, D12ATA63, D14S1434) are exclusive to GF-NGS; three (D9S1122, D17S1301, D20S482) are exclusive to FS; and one STR (D4S2408) is common to both panels. In reference samples, concordant results were found between laboratories (Supplementary Table S3), except one. D3S4529 was only typed by two laboratories using the GF-NGS panel, and there were discordances between them. One of the laboratories, used the reverse sequence to define the repeat region variation. One laboratory used STRait Razor [39], which defines the repeat motif as [GATA] (e.g., M1, genotype 1215); while the other laboratory used Converge v2.1 software [38], which defines the repeat motif as [AGAT], giving a 'frame-shift' effect which adds an extra repeat by counting the repeat motifs from a position an extra three nucleotides 5′ upstream (e.g., M1, genotype 13, 16, Supplementary Fig. S3.1). Analysis with STRait Razor is consistent with the repeat motif described in the updated Forensic STR Sequence Structure Guide v5 [15]. While the allele calling using Converge is consistent with alleles collected in the *STRSeq catalog* [43] (BioProject: PRJNA396109) (Supplementary Fig. S3.2). The result from the Converge software is the one that better conforms to the recommendations from the DNA Commission of the ISFG [45], since the motif [AGAT] includes the first 5′-nucleotide defining the repeat motif and corresponds to the original nomenclature described for this marker [46]. Both the Forensic STR Sequence Structure Guide and STRait Razor have adjusted the annotation of D3S4529 to an [AGAT] repeat unit since this exercise was completed (upcoming v6 of the Forensic STR Sequence Structure Guide).

In the case of the mixed-source sample M4, a higher number of discordant results were observed (Supplementary Table S3) (94.2 %; 373/396 concordant alleles). A portion of the discrepancies were due to the reporting of some sequences in stutter positions as real alleles by one laboratory (Lab005), probably due to the lack of well-defined analytical thresholds in the case of mixed profiles. Another laboratory (Lab033) did not report the mixed profile, but the profile of one of the components that matched the sample M3 profile. In this case, the laboratory performed differential lysis reporting the results of the male fraction.

3.1.2. Y-Chromosomal STRs (Y-STRs)

In total, four participating laboratories reported Y-STR results from the Supplementary FS kit (two using Primer Mix A, two using Primer Mix B) (Table 1). Concordance with CE was tested for the following 21 loci (EIADN No. 27 Exercise): DYF387S1, DYS19, DYS385, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS437, DYS438, DYS439 (GATA A4), DYS448, DYS460 (GATA A7.1), DYS481, DYS533, DYS549, DYS570, DYS576, DYS635 (GATA C4), DYS643 and GATA H4. Note that the FS kit genotypes three additional loci (DYS505, DYS522 and DYS612) not analyzed by CE.

Supplementary Table S4 shows the Y-STR typing results in male reference samples M1 and M3, together with the forensic sample M4. The results for sample M1 were completely concordant between the participating laboratories. As expected, the Y-haplotype of the male M3 was detected in sample M4 (DNA mixture from a female and M3 donor). In terms of CE data, concordance was very high (98.2 %; 271/276 concordant alleles). In the case of M3, two different laboratories (Lab033 and Lab179) reported a single drop-out in two different Y-STRs (DYS392 and DYS448, respectively). In the case of M4, two laboratories (Lab179 and Lab265) reported some sequences in stutter positions as real alleles in two different Y-STRs (DYF387S1 and DYS385, respectively). In addition, it is noteworthy that Lab179 reported the same drop-out in DYS448 for samples M3 and M4. The results for the markers analyzed only with MPS were fully concordant.

3.1.3. X-Chromosomal STRs (X-STRs)

The participating laboratories reporting Y-STRs also analyzed X-STRs (using the same Primer Mix A and B; see Table 1). All seven X-STRs included in the FS kit were also analyzed with CE technology: DXS10074, DXS10103, DXS10135, DXS7132, DXS7423, DXS8378 and HPRTB.

X-STR typing results from laboratories analyzing these markers are shown in Supplementary Table S5. Regarding reference samples, near full concordance was observed (99.0 %; 100/101 concordant alleles), except for a minor error in sample M1 from one laboratory (Lab265), reporting sequences in a stutter position as an allele in DXS10135. In the mixed sample M4, one laboratory (Lab033) reported the male component, while two laboratories (Lab179 and Lab265) reported sequences in a stutter position as an allele in DXS7132.

3.2. Mitochondrial DNA control region analysis

The mitochondrial DNA control region (mtDNA-CR) was sequenced by only two laboratories, using the TFS Ion Torrent™ MPS platforms; one using the Ion PGM™ System (Lab011); and another the Ion S5™ XL System (Lab007). Both laboratories used the Precision ID mtDNA Control Region Panel [28,29] (Table 1).

In Supplementary Table S6, the mtDNA-CR haplotypes obtained by both laboratories are shown. A high concordance was observed between methods as insertions after np 309 or 16,193 are not assessed in the reference CE analysis. In the case of sample M1, complete concordance was observed with the consensus haplotypes of the EIADN No. 27 (2019).

In the case of sample M2, one laboratory (Lab007) reported a deletion (29.06 % of the sequences) at np 438 within a polymeric tract of cytosines (C-stretch). When the bam.bai files of both laboratories were analyzed with IGV software [40] (Supplementary Fig. S4.1), it was observed that laboratory Lab007 also had other deleted nucleotides at nps 459 and 498. However, these other deleted positions were not reported by the Converge software as they took place in the sequences of one of the strands and/or did not reach the threshold established by the software. All these deletions, associated with a poly-C homopolymeric sequence segment, appear more or less frequently in the other samples analyzed in this exercise (Supplementary Fig. S4.2). Various studies [47–50] have indicated that these deletion errors in the Ion Torrent™ system occur mostly at the end of homopolymeric tracts, more frequently in those with the highest number of repeated nucleotides and in poly-C tracts [49]. In all cases, none of the sites where a false deletion was detected represented 100 % of the sequence reads [49].

In the case of sample M3, one laboratory (Lab007) reported a deletion at np 309 with a frequency of 34.1 %. The other laboratory also detected this deletion, but with a frequency of 14.3 %. Therefore, the latter laboratory did not report it because of the threshold of 20 % applied to sequence data to define heteroplasmy.

In the case of sample M5 (hair from a female), one (Lab007) also reported the insertion 16193.1C in HV1 at 27.4 % of the sequences (Supplementary Table S6). The interpretation of the sequence patterns between nps 16,183 and 16,193 is usually complex [51,52], since substitutions at A16183C and T16189C generate a homopolymeric tract of 11 Cs, which can generate length heteroplasmy [49]. Analysis of the bam.bai files with IGV (Supplementary Fig. S5.1) indicated an additional C insertion in some sequences at np 16,183 (i.e., 16193.1C, in forensic nomenclature [53]), as well as the A > C transition (Supplementary Fig. S5.2). However, in some of the sequences in which an additional C is not inserted, an A is deleted at np 16,180 (a16183, in forensic nomenclature [53]), at a similar percentage to the C insertion. Therefore, the detection of both the a16183 deletion and the 16193.1C insertion with frequencies around 25 % by Lab007 could be explained by alignment errors. However, it is not possible to rule out real endogenous sequence differences in the sample, since they were different hair shafts from the same individual. In any case, variation at these positions has little value for forensic analysis [53] (despite some contradictory opinions [51]) and they are usually excluded from the mtDNA tree and not considered for phylogenetic reconstruction [54].

3.3. Single nucleotide polymorphisms (SNPs)

3.3.1. Individual identification SNPs (iiSNPs)

In total, 5 participating laboratories reported iiSNP results. One laboratory used the TFS Precision ID Identity Panel [29] (herein Identity Panel) that includes 90 autosomal SNPs [23,24] and 34 upper Y-clade SNPs [30] (results described in the next section); and four laboratories used the FS kit that includes 94 iiSNPs [23,24] (Table 1).

Among the kits of both MPS suppliers, there are 83 iiSNPs in common: 47 from the SNP*for*ID Consortium [23] and 37 from K. Kidd's panel [24], with a single SNP rs2046361 common to both panels. The FS kit also has 11 unique SNPs (rs1294331, rs2399332, rs279844, rs13182883, rs1336071, rs4606077, rs763869, rs2107612, rs2920816, rs8037429, rs8078417), and TFS Identity Panel has seven unique SNPs (rs7520386, rs4847034, rs1872575, rs7704770, rs4288409, rs2016276, rs2292972).

Supplementary Table S7 shows the iiSNP typing results from each participating laboratory. It should be noted that 21 of 83 SNPs common to both kits are typed on different strands (highlighted in yellow in Supplementary Table S7). Sample M5 was not analysed since it was a hair shaft. In general, good concordance was observed, although there were some errors in the single-source samples (M1, M2 and M3) (98.9 %; 1378/1393 concordant loci), reported by laboratories using the FS kit, comprising: genotyping errors, clerical errors and no results. Five genotyping errors were reported by Lab024 (rs1493232 in sample M1; rs1736442 in M1 and M2; and rs1031825 in M3) and Lab179 (rs1294331 in M3). Five clerical errors were reported by Lab179 (rs10773760 and rs576261 in M1; rs2076848 and rs1493232 in M2; and rs1979255 in M3), since, in their original records, one of the alleles of the heterozygous SNPs gives zero reads. At five SNPs, no results were reported by Lab033 (rs1355366, rs2920816 and rs1736442 in M3) and Lab179 (rs1294331 in M1 and M2). For mixed sample M4, Lab011 using the TFS kit did not report SNP results, and Lab033 only gave results for the male component of the mixture (M3), following differential lysis. Only in those SNPs that were heterozygous in M3 and homozygous in M4, could genotyping error be confirmed in rs7041158 and rs1736442 (Lab024 and Lab265); and rs1493232 and rs1031825 (Lab265) due to the lack of the second allele. All mismatches detected were due to the low DoC levels reported by laboratories using the FS kit.

In most cases, several SNPs from the FS kit gave poor sequence data, and thus typing errors: rs1031825, rs1294331, rs1355366, rs1357617, rs1493232, rs1736442, rs2342747, rs2920816, rs321198, rs338882, rs7041158 and rs719366. Low sequence coverage levels were already reported for nine of the above 12 SNPs (rs1031825, rs1294331, rs1357617, rs1736442, rs2342747, rs2920816, rs338882, rs7041158 and rs719366) by Sharma et al. [55]. Hussing et al. [56] previously detected allele dropout in eight SNPs (rs2920816, rs1493232, rs1031825, rs1294331, rs7041158, rs1736442, rs1454361 and rs338882). In this exercise, two additional SNPs (rs1355366 and rs321198) were detected with low sequence coverage levels that were not previously reported. Therefore, these SNPs can be considered unreliable for routine forensic use, and results from the exercise are in agreement with two independent evaluations of forensic identification SNPs typed by MPS [55,56].

3.3.2. Lineage informative SNPs (liSNPs)

In the case of liSNPs, only Lab011 reported results for the 34 upper Y-clade SNPs [30] included in the TFS Identity Panel [29] (Table 1). Supplementary Table S8 outlines the liSNPs typing results obtained for male samples M1 and M3. There are no consensus genotypes as no other laboratory reported this form of SNP typing. Lab011 assigned M1 to haplogroup J and M3 to haplogroup I, using the HID Genotyper Plugin [37] (Supplementary Figs. S6.1 and S7.1). Haplogroup J is widely distributed in southern Europe and central Mediterranean [57], while haplogroup I, close to J, includes about a quarter of all northwest European men [58]. Predictions of Y-chromosome haplogroups from Y-STR data can be compared (e.g., http://www.hprg.com/hapest5/?hapest5, [59,60]), taking into account their limitations [61]. From the Y-STR consensus results for M1 and M3 provided by the organizers of the EIADN No. 27 (2019), predictions are concordant with those obtained using Y-SNPs (Supplementary Figs. S6.2 and S7.2).

3.3.3. Ancestry informative SNPs (aiSNPs)

Three participating laboratories reported aiSNP results (Table 1). One laboratory used the TFS Precision ID Ancestry Panel [29] (herein Ancestry Panel) comprising 165 SNPs (55 K. Kidd's lab [25] and 123 M. Seldin's lab SNPs [31], with 13 in common); and two used the 56 aiSNPs in Primer mix B of the FS kit [22] (which completely overlap with the 55 SNPs from K. Kidd's lab [25] in the Ancestry panel, except rs1919550).

Supplementary Table S9 outlines the aiSNPs typing results from participating laboratories analyzing M1, M2 and M3 samples. In 18 of the 55 shared SNPs the Verogen UAS software reports the reverse strand genotypes (highlighted in yellow in Supplementary Table S9). All results obtained for these 55 shared SNPs were concordant between MPS platforms (100 %; 495/495 concordant loci). For sample M4, Lab011 did not give results, Lab033 gave the same results as for M3, and Lab024 typed this sample as a mixture.

Details of the biogeographical ancestry estimation analyses made by participating laboratories are summarized in Table 2. Samples M2 and M3 were predicted to be European by all laboratories. However,

Table 2
Biogeographical ancestry estimation based on the SNP profiles in Supplementary Table S9. The TFS columns describe results from Lab011, and the Verogen columns those from Lab024 and Lab033. In the Percentage column, those estimates that are >51 % are highlighted (in bold).

Platform	TFS					Verogen	
Sample	**Biogeographical ancestry estimation**	**Percentage(>51 %)**	**Confidence**	**Population**	**Likelihood**	**Biogeographical ancestry estimation**	**Distance to Nearest Centroid**
M1	European	**80**	High	Greeks	3.66E-42	*Admixed American*	*7.8*
	Southwest Asia	15		Finns	1.33E-42		
	Africa	5		Hungarians	6.34E-43		
M2	European	**100**	High	Irish	2.87E-38	European	3.28
				Europeans-HapMap	2.61E-38		
				European Americans	2.89E-39		
M3	European	**95**	High	Irish	4.73E-43	European	3.22
	Southwest Asia	5		Europeans-HapMap	1.58E-44		
				European Americans	3.63E-45		

differences arose for sample M1. The TFS ancestry analysis plugin, which compares the aiSNP profile to data from 65 reference populations, reported: 80 % European, 15 % Southwest Asian and 5% African components of ancestry (Supplementary Fig. S8.1). The TFS ancestry plugin distinguishes seven population groups: Americans, East Asians, Oceanians, Africans, Europeans, South Asians and Southwest Asians. From these 65 populations, M1 was closest to Greeks, Finns and Hungarians, with likelihood ratios of 3.66E-42, 1.33E-42 and 6.34E-43, respectively. The Verogen UAS software [36] classifies the sample M1 as "Admixed American" (Table 2) from the position of the aiSNP profile on a 2D principal component analysis (PCA) plot, which has four reference population groups co-analyzed: European, East Asian, Admixed American and African. The unknown aiSNP profile is compared to the centroids established for the reference clusters on the PCA and the distance between the unknown position and the closest centroid provides an idea of possible admixture (since co-ancestry deflects the unknown profile's position away from the middle of the reference clusters and their centroids). Intermediate points corresponding to 25, 50 and 75 percent admixture between each reference centroid are also provided (Supplementary Fig. S8.2) [36]. The UAS software uses 14 populations from 1000 Genomes SNP data, with four admixed American populations grouped together as "Admixed Americans", comprising: Mexican residents in Los Angeles (USA); Puerto Ricans; Colombians from Medellín and Peruvians from Lima (https://www.internationalgenome.org/data-portal/population). The ancestral composition of the "Admixed American" group consequently has a high European component [62–64]. Despite the initial classification, the M1 sample was also close to the "European" group centroid in the PCA plot.

To address these limitations there are other open-access online tools that allow ancestry to be estimated from SNP data. Snipper (http://mathgene.usc.es/snipper/) [65] is an online suite of tools that allows ancestry estimation through likelihood ratio comparisons and PCA analysis, for both 165 aiSNPs in the Ancestry Panel and 55 aiSNPs in the FS kit. This software has the advantage that it is open and allows the user to upload population data from any other study that may be of interest for analysis of unknown samples. In the case of M1, Snipper software classified the sample as European, in addition to providing a statistical assessment based on likelihood ratios (Supplementary Figs. S8.3 and S8.4). Another alternative is FROG-kb (http://frog.med.yale.edu/FrogKB/) [66,67] from K. Kidd's lab, which can analyse the TFS Ancestry Panel SNPs (comparing 96 populations), or the 55 aiSNPs of the FS kit (comparing 161 populations). Sample M1 was classified as European (Supplementary Fig. S8.5): specifically, with the 165 Ancestry Panel aiSNPs it has the closest relationship with the Basque population [68], and with 55 FS aiSNPs it resembles Finnish and Basque populations. Lastly, the recently developed GenoGeographer open software (http://apps.math.aau.dk/aims/) [69,70] makes likelihood ratio calculations and calculates a z-score to assess the probability that the relevant ancestry population is represented in the reference populations used. GenoGeographer has data for 164 of the 165 Ancestry Panel aiSNPs (rs10954737 missing). Sample M1 was classified as European (Supplementary Fig. S8.6), whether considering the 164 aiSNPs of the TFS Ancestry panel or using the 55 aiSNP subset of the FS kit, with the closest proximity to the Iberian population. When more aiSNPs are considered, the approximation error is reduced (see the error bar plots in Supplementary Fig. S8.6).

3.3.4. *Phenotype informative SNPs (piSNPs)*

Three participating laboratories reported piSNP results. One laboratory used the TFS AmpliSeq™ DNA Phenotyping Panel [32] (herein the Phenotyping Panel) and two laboratories used the FS kit [22] with Primer Mix B (Table 1). Both TFS and FS panels genotype the same 23 piSNPs forming the HIrisPlex system [26], for predicting eye colour and hair colour. Hair shade prediction is also possible using HIrisPlex SNPs but the Verogen UAS software does not make this prediction [36].

Supplementary Table S10 outlines the piSNP typing results from each participating laboratory for M1, M2 and M3. One of the 23 SNPs, rs1800407, is reported from the reverse strand by Verogen (highlighted in yellow in the Supplementary Table S10). Results were concordant for M1 and M2 on both platforms, but in sample M3, a single discordancy was found in rs12896399 (an eye colour predictor) (98.6 %; 213/216 concordant loci). Laboratory Lab011, using the TFS Phenotyping Panel, typed this SNP as G, T (4947 G sequences and 5141 T), while both FS laboratories typed this SNP as T, T (Lab024, 13 sequences of G and 177 of T; Lab033, 107 sequences of T only). Considering the low sequence coverage from Verogen MPS analysis, a typing error cannot be excluded. For sample M4, Lab011 did not report results, Lab033 reported the same results as for M3, and the Lab024 typed this sample as a mixture.

Regarding the phenotypic predictions, it should be noted that the TFS software does not predict phenotypic characteristics but provides SNP genotypes with the Variant Caller plugin [37]. Predictions can be made by uploading the SNP data to the Erasmus HIrisPlex webtool (https://hirisplex.erasmusmc.nl/) [26] for eye and hair colour predictions, or the FROG-kb webtool (http://frog.med.yale.edu/FrogKB/FrogServlet) [66] for eye colour. The Verogen UAS software [36] does make predictions, omitting the option to predict hair shade provided by the Erasmus HIrisPlex webtool (eye colour = blue, brown and intermediate; hair colour = blond, black, brown and red; and hair shade = light and dark). Table 3 outlines the phenotype predictions of the samples analyzed in this exercise. Identical results were obtained for samples M1 and M2: brown eye colour and dark brown hair colour. Slightly different blue eye colour predictive likelihoods were obtained for M3, due to the genotype difference in rs12896399. According to the coordinator of the EIADN No. 27 (personal communication), the M3 donor has hazel or

Table 3
Phenotypic predictions based on the SNP profiles in Supplementary Table S10. The SNP data obtained from each laboratory were uploaded in the "HIrisPlex-S Eye, Hair and Skin Colour DNA Phenotyping Webtool" (https://hirisplex.erasmusmc.nl/) to obtain each prediction. In the case of Verogen labs, this prediction agrees with that made by the UAS software.

	Sample M1			**Sample M2**			**Sample M3**		
Lab	**11**	**24**	**33**	**11**	**24**	**33**	**11**	**24**	**33**
Platform	TFS	Verogen	Verogen	TFS	Verogen	Verogen	TFS	Verogen	Verogen
Blue Eye	0.000			0.002			**0.784**	**0.89**	**0.89**
Intermediate Eye	0.013	0.01		0.017	0.02		0.088	0.05	
Brown Eye	**0.986**	**0.99**	**0.99**	**0.982**	**0.98**	**0.98**	0.128	0.06	
Blond Hair	0.182	0.18		0.145	0.22		0.345	0.35	
Brown Hair	**0.622**	**0.59**	**0.59**	**0.626**	**0.54**	**0.54**	**0.543**	**0.55**	**0.55**
Red Hair	0.022	0.03		0.001	0.00		0.003	0.00	
Black Hair	0.174	0.20		0.228	0.23		0.110	0.09	
Light Hair	0.389			0.403			**0.659**		
Dark Hair	**0.611**			**0.597**			0.341		

amber eyes, i.e. light brown. It is noted that there is an association of the rs12896399-G allele with brown eye colour and the T allele with blue eye colour [71,72]. However, it is not possible to officially confirm the eye colour of the M3 or other sample donors, as they are completely anonymous.

3.4. Depth of coverage (DoC)

It is a complex task to achieve equitable depth of coverage (DoC) assessments when comparing different MPS platforms. We made an approximation by analyzing markers as groups and taking into account as much as possible, the limitations of the type of chemistry for the preparation of libraries and templates, the loading levels of the chips/flow cells of each of the participating laboratories and the number of markers analyzed in any one multiplexed library preparation per panel. To normalize the DoC data from each participating laboratory, a correction factor was calculated and applied individually for each of them (Supplementary Table S2).

Regarding auSTRs assessment within each MPS technique, it is worth mentioning that the average DoC obtained with the TFS GFS-NGS panel was higher than the one obtained with the FS kit (Fig. 1); up to 2.3 times higher with normalized values. Not including the sex markers (Amelogenin, DYS391, SRY and rs2032678), the GF-NGS panel gave average coverage which ranged from 21,125 (TH01) to 799 (Penta D) sequences. While the equivalent range for the FS kit was an average coverage of 8944 (TH01) to 765 (D1S1656) sequences.

Regardless of differences in the chemistry between each platform, it is important to note that the number of markers in each kit differs markedly. The TFS GF-NGS panel, analyzes a total of 35 markers, comprising autosomal STRs plus sex markers. This represents much less markers than those analyzed by the FS kit, regardless of whether Primer Mix A or B is used. In fact, when DoC levels are compared among laboratories using different FS Primer Mix configurations, differences are observed (Fig. 2), with DNA primer Mix A producing twice as much DoC on average (1.9 times higher with normalized values). For primer Mix A (autosomal STRs, Y-STRs, X-STRs and iiSNPs), average coverage ranges from 10,735 (TH01) to 915 (D1S1656) sequences. While Primer Mix B (which includes the above markers and adds aiSNPs and piSNPs) gives average coverage from 4403 (D20S482) to 354 (D1S1656) sequences.

The DoC levels of Y-STRs reflected the observations seen in autosomal STRs. On average, the DoC was slightly higher for Primer Mix A than for Primer Mix B (Fig. 2; 1.3 times higher and 1.2 times higher with normalized values). Primer Mix A gave a DoC range of 10,874 (DYS392) to 290 (DYS389II) sequences, while Primer Mix B, gave a DoC range of 5483 (DYS438) to 195 (DYS522) sequences. The DoC levels of X-STRs again reflect the reduced multiplexing scale when using FS Primer Mix A (Fig. 2), with almost twice the average DoC levels of Primer Mix B (1.8 times higher and 1.7 times higher with normalized values). Primer Mix A gave average coverage ranging from 6817 (DXS7132) to 152 (DXS10103) sequences; and Primer Mix B gave 4192 to 49 sequences for the same STRs, respectively. Fig. 2 gives an overview of the average DoC values with normalized values for all STRs analyzed with the FS kit. In general, regardless of the mix used, greater coverage is observed for X-STRs, followed by autosomal STR markers, with Y-STRs giving the lowest coverage.

Regarding the DoC of mtDNA-CR obtained for each sample, higher average coverage levels were observed by Lab007 using the Ion S5™ XL System (Supplementary Fig. S9.1), which were on average seven times higher than the DoC of Lab011 using the Ion PGM™ System (7.2 times higher with normalized values).

The DoC levels obtained for the iiSNPs in both panels indicate that the TFS Identity panel generates more than five times higher average

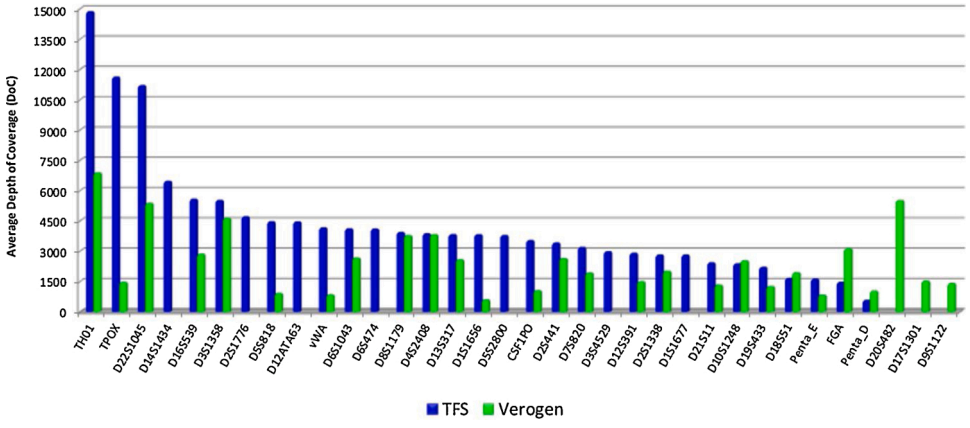

Fig. 1. Average depth of coverage (DoC) obtained for each of the autosomal STRs markers (averaged across participating laboratory data). Dark bars denote TFS GF-NGS panel sequence coverage, and light bars coverage from the FS kit. The average DoC has been normalized based on a correction factor (Supplementary Table S2).

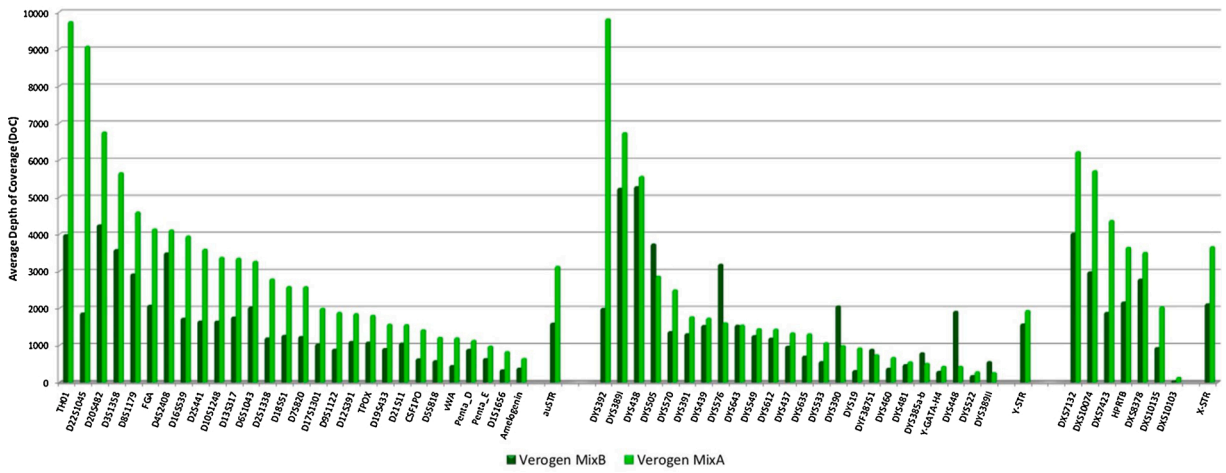

Fig. 2. Average depth of coverage (DoC) obtained per STR marker when typed with FS kit Primer Mix A (light bars), compared with DoC levels when using primer Mix B (dark bars). The average DoC has been normalized based on a correction factor (Supplementary Table S2).

coverage than the FS kit (Supplementary Fig. S9.2; 5.4 times higher and 4.5 times higher with normalized values). The TFS Identity panel gave average coverage ranging from 5978 (rs1058083) to 1909 (rs876724) sequences. While the FS kit gave an average coverage ranging from 4321 (rs1109037 and rs1109037) to 57 (rs1736442) sequences. In addition, there are significant imbalances among different iiSNPs in the FS kit, in some cases reaching ratios greater than 1:200 (Supplementary Table S11). Once again, this may be due to the low overall sequence coverage of the FS kit which may lead to some allelic drop-out [55,56]. The DoC levels for Y-SNPs (only included in the TFS Identity panel) indicated a high average coverage (1823 reads) without significant sequence imbalance between loci, i.e. marker ratios did not exceed 1:3 (Supplementary Fig. S9.3).

The TFS Ancestry Panel DoC levels were on average more than 15 times higher than those from the FS kit (Supplementary Fig. S9.4) (15.4 times higher and 13.1 times higher with normalized values). With the Ancestry Panel, average coverage ranged from 3617 (rs459920) to 1038 (rs13400937) sequences, compared with an average coverage ranging from 1429 (rs1876482) to 102 (rs310644) sequences for the FS kit. Significant imbalances were observed among different aiSNPs in the FS kit, which were lower than the iiSNPs in this kit, but reaching ratios greater than 1:57 (Supplementary Table S11).

The DoC levels obtained for each of the samples, again indicated higher average coverage from the Phenotyping Panel dedicated to these SNPs only, compared to those of the FS kit, where they are part of the enlarged Primer Mix B marker set. On average, TFS gave almost 21 times higher DoC than FS (Supplementary Fig. S9.5) (20.6 times higher and 14.4 times higher with normalized values). The TFS Phenotyping Panel had average coverage ranging from 15,464 (rs28777) to 5068 (rs16891982) sequences. The FS kit had average coverage ranging from 1023 (rs201326893) to 133 (rs12203592) sequences. There was also significant imbalance among the piSNPs in the FS kit, reaching marker ratios greater than 1:33 (Supplementary Table S11), which could result in some allelic drop-out. The imbalances observed could explain the different rs12896399 genotypes recorded amongst laboratories for sample M3.

4. Conclusions

The results presented from the first GHEP collaborative exercise on forensic applications of MPS (GHEP-MPS01) were instigated to evaluate the consistency and robustness of MPS technology for the analysis of forensic DNA markers, which included autosomal STRs, Y-STRs, X-STRs, mtDNA control region, iiSNPs, liSNPs, aiSNPs and piSNPs.

Although only seven laboratories participated in this first set of tests, the results obtained allow interesting conclusions to be drawn regarding the purposes initially sought from the exercise. With respect to STRs, good agreement was found among MPS results using different MPS platforms, as well as by comparing MPS length-based genotypes to previous consensus CE data from single source samples. Only one discrepancy was observed at D3S4529 between MPS length-based results due to a nomenclature issue (different repeat motifs being considered). In D6S474, MPS analysis detects an error in the number of allelic repetitions assigned when using the allelic ladder of the CE-based Investigator HDplex kit. Concordant results were obtained between the two laboratories that analyzed the mtDNA control region, with slight discrepancies in the sequencing and description of C-homopolymeric regions. In general, concordant SNP-MPS results among the different laboratories were observed, with certain genotype discordancies, mainly due to low sequence coverage reported for some loci.

The coverage issue in forensic MPS analysis is of primary importance and should be carefully assessed by forensic laboratories when establishing their sequence analysis workflows. In TFS platforms, the sequence coverage of each marker depends on the efficiency of amplification and the number of wells on the chip used, with the chip efficiency being dictated by the capacity to capture Ionospheres containing the monoclonally amplified target [73]. In contrast, coverage levels using the Verogen system depend on the efficiency of amplification, purification and normalization of the library, and the flow cell used [74]. In addition, the higher the number of markers in the kit used, the greater the impact on the final coverage obtained for each marker. Therefore, a modular system of small-scale multiplexes for a particular forensic test will be more efficient than an all-in-one marker set. Furthermore, it gives greater flexibility to the laboratory, in order to combine only those markers of interest for any case being analyzed. In any case, it is always important to follow the manufacturers' recommendations regarding the suggested optimum number of samples that can be loaded into the chip/flow cell, as well as the internal validation data performed in each laboratory based on their initial validation experiments (modifying or establishing the analysis thresholds for different chip/flow cell loading levels), in order to arrive at a DoC appropriate for the number of analyzed markers and which will avoid genotyping errors due to low coverage.

Although MPS platforms are already widely implemented in many forensic genetics laboratories, there are still some weaknesses that should be considered in this type of DNA analysis. On one hand, there is progress towards standardization of MPS-based STR nomenclature, which is in progress led by the work of the STRAND ISFG Working Group [16]. The uniform availability and application of analysis software can help the harmonization of sequence-based allele nomenclature,

highlighted by the discrepancy that still exists between MPS and CE nomenclature schemes in D6S474. The analysis software associated with each of the two main MPS systems allows easy use and interpretation [9,13,14], with the existence of open-access bioinformatic tools [9,13,26,39–41,65–67,69,70] that can be used in parallel. Forensic laboratories, like those who took part in this first GHEP MPS exercise, are in collaboration with the MPS suppliers developing forensic kits, in order to optimize the analysis workflows and fully record sequence coverage problems that may lead to erroneous typing and consequent misinterpretation of data in critical samples [14]. To finish on a fundamental point: it is crucial that laboratories continue to validate and accredit new MPS techniques in accordance with ISO/IEC 17025:2017, for use in routine forensic casework [13,47,56,68,73–82].

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fsigen.2020.102391.

References

[1] F. Sanger, S. Nicklen, A.R. Coulson, DNA sequencing with chain-terminating inhibitors, Proc. Natl. Acad. Sci. U.S.A. 74 (12) (1977) 5463–5467.
[2] K.R. Mitchelson, The use of capillary electrophoresis for DNA polymorphism analysis, Mol. Biotechnol. 24 (2003) 41–68.
[3] E.S. Lander, L.M. Linton, et al., International human genome sequencing consortium, Initial sequencing and analysis of the human genome, Nature 409 (6822) (2001) 860–921.
[4] J.C. Venter, M.D. Adams, et al., The sequence of the human genome, Science 291 (5507) (2001) 1304–1351.
[5] E.R. Mardis, The impact of next-generation sequencing technology on genetics, Trends Genet. 24 (3) (2008) 133–141.
[6] M.L. Metzker, Sequencing technologies - the next generation, Nat. Rev. Genet. 11 (1) (2010) 31–46.
[7] J.M. Heather, B. Chain, The sequence of sequencers: the history of sequencing DNA, Genomics. 107 (1) (2016) 1–8.
[8] National Human Genome, The Cost of Sequencing a Human Genome, National Institutes of Health (NIH), 2019 (accessed 2020.05.06), https://www.genome.gov/sequencingcosts/.
[9] A. Alonso, P.A. Barrio, P. Müller, S. Köcher, B. Berger, P. Martin, M. Bodner, S. Willuweit, W. Parson, L. Roewer, B. Budowle, Current state-of-art of STR sequencing in forensic genetics, Electrophoresis. 39 (21) (2018) 2655–2668.
[10] A. Alonso, P. Müller, L. Roewer, S. Willuweit, B. Budowle, W. Parson, European survey on forensic applications of massively parallel sequencing, Forensic Sci, Int. Genet. 29 (2017) e23–e25.
[11] B. Budowle, J.D. Churchill, J.L. King, The next State-of-the-art forensic genetics technology: massively parallel sequencing, in: A. Amorim, B. Budowle (Eds.), Handbook of Forensic Genetics. Biodiversity and Heredity in Civil and Criminal Investigations, World Scientific Publishing Europe Ltd., London, 2017, pp. 249–292.
[12] A. Freire-Aradas, V. Lareu, A. Carracedo, Marcadores fenotípicos de interés forense, in: M. Crespillo, P.A. Barrio (Eds.), Genética Forense. Del laboratorio a los Tribunales, Ediciones Díaz de Santos, Madrid, 2019, pp. 269–290.
[13] P.A. Barrio, P. Martín, A. Alonso, Nuevas tecnologías de secuenciación masiva en paralelo (MPS) de uso en genética forense, in: M. Crespillo, P.A. Barrio (Eds.), Genética Forense. Del laboratorio a los Tribunales, Ediciones Díaz de Santos, Madrid, 2019, pp. 319–350.
[14] W. Parson, D. Ballard, B. Budowle, J.M. Butler, K.B. Gettings, P. Gill, L. Gusmao, D. R. Hares, J.A. Irwin, J.L. King, P. Knijff, N. Morling, M. Prinz, P.M. Schneider, C. V. Neste, S. Willuweit, C. Phillips, Massively parallel sequencing of forensic STRs: considerations of the DNA commission of the International Society for Forensic Genetics (ISFG) on minimal nomenclature requirements, Forensic Sci. Int. Genet. 22 (2016) 54–63.
[15] C. Phillips, K.B. Gettings, J.L. King, D. Ballard, M. Bodner, L. Borsuk, W. Parson, The devil's in the detail": release of an expanded, enhanced and dynamically revised forensic STR Sequence Guide, Forensic Sci. Int. Genet. 34 (2018) 162–169.
[16] K.B. Gettings, D. Ballard, M. Bodner, L.A. Borsuk, J.L. King, W. Parson, C. Phillips, Report from the STRAND Working Group on the 2019 STR sequence nomenclature meeting, Forensic Sci. Int. Genet. 43 (2019), 102165.
[17] M. Bodner, I. Bastisch, J.M. Butler, R. Fimmers, P. Gill, L. Gusmao, N. Morling, C. Phillips, M. Prinz, P.M. Schneider, W. Parson, Recommendations of the DNA Commission of the International Society for Forensic Genetics (ISFG) on quality control of autosomal short tandem repeat allele frequency databasing (STRidER), Forensic Sci. Int. Genet. 24 (2016) 97–102. URL, https://strider.online.
[18] W. Parson, A. Dür, EMPOP–a forensic mtDNA database, Forensic Sci. Int. Genet. 1 (2) (2007) 88–92.
[19] S. Willuweit, L. Roewer, International forensic Y chromosome user group, Y chromosome haplotype reference database (YHRD): update, Forensic Sci. Int. Genet. 1 (2) (2007) 83–87.
[20] Proficiency Testing, https://ghep-isfg.org/en/proficiency/, (accessed 2020.05.06).
[21] K. Fernández, J. Gómez, J. García-Hirschfeld, E. Cubillo, C. Sánchez de la Torre, G. Vallejo, Accreditation of the GHEP-ISFG proficiency test: one step forward to assure and improve quality, Forensic Sci. Int. Genet. Supp. Ser. 5 (2015) e515–e517.
[22] ForenSeq™ DNA Signature Prep, Reference Guide. https://verogen.com/wp-content/uploads/2018/08/ForenSeq-DNA-Prep-Guide-VD2018005-A.pdf, June 2018 (accessed 2020.05.06).
[23] C. Phillips, R. Fang, D. Ballard, M. Fondevila, C. Harrison, F. Hyland, E. Musgrave-Brown, C. Proff, E. Ramos-Luis, B. Sobrino, A. Carracedo, F.R. Furtado, D. Syndercombe Court, P.M. Schneider, SNP*for*ID Consortium, Evaluation of the Genplex SNP typing system and a 49plex forensic marker panel, Forensic Sci. Int. Genet. 1 (2) (2007) 180–185.
[24] A.J. Pakstis, W.C. Speed, R. Fang, F.C. Hyland, M.R. Furtado, J.R. Kidd, K.K. Kidd, SNPs for a universal individual identification panel, Hum. Genet. 127 (3) (2010) 315–324.
[25] K.K. Kidd, W.C. Speed, A.J. Pakstis, M.R. Furtado, R. Fang, A. Madbouly, M. Maiers, M. Middha, F.R. Friedlaender, J.R. Kidd, Progress toward an efficient panel of SNPs for ancestry inference, Forensic Sci. Int. Genet. 10 (2014) 23–32.
[26] S. Walsh, L. Chaitanya, L. Clarisse, L. Wirken, J. Draus-Barini, L. Kovatsi, H. Maeda, T. Ishikawa, T. Sijen, P. de Knijff, W. Branicki, F. Liu, M. Kayser, Developmental validation of the HIrisPlex system: DNA-based eye and hair colour prediction for forensic and anthropological usage, Forensic Sci. Int. Genet. 9 (2014) 150–161. URL, https://hirisplex.erasmusmc.nl/.
[27] Precision ID GlobalFiler™ NGS STR Panel v2 with the HID Ion S5™/HID Ion GeneStudio™ S5 System, Application Guide, 2018, 15 November (accessed 2020.05.06), https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0016129_PrecisionIDSTRIonS5_UG.pdf.
[28] Precision ID mtDNA Panels with the HID Ion S5™/HID Ion GeneStudio™ S5 System, Application Guide, 2019, 19 January(accessed 2020.05.06), https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0017770_PrecisionID_mtDNA_Panels_S5_UG.pdf.
[29] Precision ID Panels with the Ion PGM™ System, Application Guide, 2017, 19 July (accessed 2020.05.06), https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0015830_PrecisionID_Panels_IonPGM_UG.pdf.
[30] T.M. Karafet, F.L. Mendez, M.B. Meilerman, P.A. Underhill, S.L. Zegura, M. F. Hammer, New binary polymorphisms reshape and increase resolution of the human Y chromosomal haplogroup tree, Genome Res. 18 (5) (2008) 830–838.
[31] R. Kosoy, R. Nassir, C. Tian, P.A. White, L.M. Butler, G. Silva, R. Kittles, M. E. Alarcon-Riquelme, P.K. Gregersen, J.W. Belmont, F.M. de La Vega, M.F. Seldin, Ancestry informative marker sets for determining continental origin and admixture proportions in common populations in America, Hum. Mutat. 30 (1) (2009) 69–78.
[32] Ion AmpliSeq Community Panels, Ion Ampliseq™ DNA Phenotyping Panel, 2015. March (accessed 2020.05.06), https://www.ampliseq.com/protected/tmpl/view.action?tmplDesignId=87948055#/?listAction=tmplCoverageSummaryList&tmplDesignId=87948055&wrapperId=ajaxTableWrapper.
[33] Ion Library TaqMan® Quantitation Kit, User Guide, 2017, 30 November (accessed 2020.05.06), https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0015802_IonLibrary_Taqman_Quantitation_Kit_UG.pdf.
[34] KAPA Library Quantification Kit for Illumina® Platforms. http://technical-support.roche.com/_layouts/net.pid/Download.aspx?documentID=ff4b26b8-e4d8-e811-df87-00215a9b3428&fileName=00000000000012000019010 6_000_05_005_Native&extension=pdf&mimeType=application%2fpdf&inline=False, 2017 (accessed 2020.05.06).
[35] R.M. Andrews, I. Kubacka, P.F. Chinnery, R.N. Lightowlers, D.M. Turnbull, N. Howell, Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA, Nat. Genet. 23 (2) (1999) 147.
[36] ForenSeq™ Universal Analysis Software Guide, Verogen, 2018. June (accessed 2020.05.06), https://verogen.com/wp-content/uploads/2018/08/ForenSeq-Univ-Analysis-SW-Guide-VD2018007-A.pdf.
[37] Torrent Suite™ Software 5.10, User Guide. https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0017598_TorrentSuiteSoftware_5_10_UG.pdf, 5 June 2018 (accessed 2020.05.06).
[38] Converge™ Software v2.1https://assets.thermofisher.com/TFS-Assets/LSG/Reference-Materials/read-me-converge-v2-1-release-notes.pdf, October 2028 (accessed 2020.05.06).

[39] A.E. Woerner, J.L. King, B. Budowle, Fast STR allele identification with STRait Razor 3.0, Forensic Sci. Int. Genet. 30 (2017) 18–23.

[40] H. Thorvaldsdóttir, J.T. Robinson, J.P. Mesirov, Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration, Brief Bioinform. 14 (2) (2013) 178–192.

[41] S. Ganschow, J. Silvery, J. Kalinowski, C. Tiemann, toaSTR: A web application for forensic STR genotyping by massively parallel sequencing, Forensic Sci. Int. Genet. 37 (2018) 21–28.

[42] GeneMarker® H.T.S., User Manual, Softgenetics. https://softgenetics.com/PDF/GeneMarkerHTS_UsersManual.pdf, 2017 (accessed 2020.05.06).

[43] K.B. Gettings, L.A. Borsuk, D. Ballard, M. Bodner, B. Budowle, L. Devesse, J. King, W. Parson, C. Phillips, P.M. Vallone, STRSeq: a catalog of sequence diversity at human identification Short Tandem Repeat loci, Forensic Sci. Int. Genet. 31 (2017) 111–117. URL, https://www.ncbi.nlm.nih.gov/bioproject/380127.

[44] P.A. Barrio, P. Martín, A. Alonso, P. Müller, M. Bodner, B. Berger, W. Parson, B. Budowle, DNASEQEX Consortium, Massively parallel sequence data of 31 autosomal STR loci from 496 Spanish individuals revealed concordance with CE-STR technology and enhanced discrimination power, Forensic Sci. Int. Genet. 42 (2019) 49–55.

[45] P.J. Lincoln, DNA recommendations – further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems, Forensic Sci. Int. 87 (3) (1997) 181–184.

[46] C.R. Hill, M.C. Kline, M.D. Coble, J.M. Butler, Characterization of 26 miniSTR loci for improved analysis of degraded DNA samples, J. Forensic Sci. 53 (1) (2008) 73–80.

[47] W. Parson, C. Strobl, G. Huber, B. Zimmermann, S.M. Gomes, L. Souto, L. Fendt, R. Delport, R. Langit, S. Wootton, R. Lagacé, J. Irwin, Evaluation of next generation mtGenome sequencing using the Ion Torrent Personal Genome Machine (PGM), Forensic Sci. Int. Genet. 7 (5) (2013) 543–549.

[48] L.M. Bragg, G. Stone, M.K. Butler, P. Hugenholtz, G.W. Tyson, Shining a light on dark sequencing: characterising errors in Ion Torrent PGM data, PLoS Comput. Biol. 9 (4) (2013), e1003031.

[49] S.B. Seo, X. Zeng, J.L. King, B.L. Larue, M. Assidi, M.H. Al-Qahtani, A. Sajantila, B. Budowle, Underlying data for sequencing the mitochondrial genome with the massively parallel sequencing platform ion torrent™ PGM™, BMC Genomics 16 (Suppl 1) (2015) S4.

[50] L. Song, W. Huang, J. Kang, Y. Huang, H. Ren, K. Ding, Comparison of error correction algorithms for Ion Torrent PGM data: application to hepatitis B virus, Sci. Rep. 7 (1) (2017) 8106.

[51] L. Forster, P. Forster, S.M. Gurney, M. Spencer, C. Huang, A. Röhl, B. Brinkmann, Evaluating length heteroplasmy in the human mitochondrial DNA control region, Int. J. Legal Med. 124 (2) (2010) 133–142.

[52] S. Desmyter, S. Dognaux, F. Noel, L. Prieto, Base specific variation rates at mtDNA positions 16093 and 16183 in human hairs, Forensic Sci. Int. Genet. 43 (2019), 102142.

[53] W. Parson, L. Gusmão, D.R. Hares, J.A. Irwin, W.R. Mayr, N. Morling, E. Pokorak, M. Prinz, A. Salas, P.M. Schneider, T.J. Parsons, DNA Commission of the International Society for Forensic Genetics. DNA Commission of the International Society for Forensic Genetics: revised and extended guidelines for mitochondrial DNA typing, Forensic Sci. Int. Genet. 13 (2014) 134–142.

[54] M. van Oven, M. Kayser, Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation, Hum. Mutat. 30 (2) (2009) E386–94.

[55] V. Sharma, H.Y. Chow, D. Siegel, E. Wurmbach, Qualitative and quantitative assessment of Illumina's forensic STR and SNP kits on MiSeq FGx™, PLoS One 12 (11) (2017), e0187932.

[56] C. Hussing, C. Huber, R. Bytyci, H.S. Mogensen, N. Morling, C. Børsting, Sequencing of 231 forensic genetic markers using the MiSeq FGx™ forensic genomics system - an evaluation of the assay and software, Forensic Sci. Res. 3 (2) (2018) 111–123.

[57] F. Di Giacomo, F. Luca, L.O. Popa, N. Akar, N. Anagnou, J. Banyko, R. Brdicka, G. Barbujani, F. Papola, G. Ciavarella, F. Cucci, L. Di Stasi, L. Gavrila, M. G. Kerimova, D. Kovatchev, A.I. Kozlov, A. Loutradis, V. Mandarino, C. Mammi', E. N. Michalodimitrakis, G. Paoli, K.I. Pappa, G. Pedicini, L. Terrenato, S. Tofanelli, P. Malaspina, A. Novelletto, Y chromosomal haplogroup J as a signature of the post-neolithic colonization of Europe, Hum. Genet. 115 (5) (2004) 357–371.

[58] V. Battaglia, S. Fornarino, N. Al-Zahery, A. Olivieri, M. Pala, N.M. Myres, R.J. King, S. Rootsi, D. Marjanovic, D. Primorac, R. Hadziselimovic, S. Vidovic, K. Drobnic, N. Durmishi, A. Torroni, A.S. Santachiara-Benerecetti, P.A. Underhill, O. Semino, Y-chromosomal evidence of the cultural diffusion of agriculture in Southeast Europe, Eur. J. Hum. Genet. 17 (6) (2009) 820–830.

[59] T.W. Athey, Haplogroup prediction from Y-STR values using an allele-frequency approach, J. Genet. Geneal. 1 (2005) 1–7. URL, http://www.hprg.com/hapest5/?hapest5.

[60] T.W. Athey, Haplogroup prediction from Y-STR values using a bayesian-allele-Frequency approach, J. Genet. Geneal. 2 (2006) 34–39.

[61] M. Muzzio, V. Ramallo, J.M. Motti, M.R. Santos, J.S. López Camelo, G. Bailliet, Software for Y-haplogroup predictions: a word of caution, Int. J. Legal Med. 125 (1) (2011) 143–147.

[62] S. Gravel, F. Zakharia, A. Moreno-Estrada, J.K. Byrnes, M. Muzzio, J.L. Rodriguez-Flores, E.E. Kenny, C.R. Gignoux, B.K. Maples, W. Guiblet, J. Dutil, M. Via, K. Sandoval, G. Bedoya, 1000 Genomes Project, T.K. Oleksyk, A. Ruiz-Linares, E. G. Burchard, J.C. Martinez-Cruzado, C.D. Bustamante, Reconstructing Native American migrations from whole-genome and whole-exome data, PLoS Genet. 9 (12) (2013), e1004023.

[63] J.R. Homburger, A. Moreno-Estrada, C.R. Gignoux, D. Nelson, E. Sanchez, P. Ortiz-Tello, B.A. Pons-Estel, E. Acevedo-Vasquez, P. Miranda, C.D. Langefeld, S. Gravel, M.E. Alarcón-Riquelme, C.D. Bustamante, Genomic insights into the ancestry and demographic history of South America, PLoS Genet. 11 (12) (2015), e1005602.

[64] A. Auton, L.D. Brooks, R.M. Durbin, et al., 1000 Genomes Project Consortium, A global reference for human genetic variation, Nature 526 (7571) (2015) 68–74. URL, https://www.internationalgenome.org/data-portal/population.

[65] C. Phillips, A. Salas, J.J. Sánchez, M. Fondevila, A. Gómez-Tato, J. Alvarez-Dios, M. Calaza, M.C. de Cal, D. Ballard, M.V. Lareu, A. Carracedo, SNP*for*ID Consortium, inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs, Forensic Sci. Int. Genet. 1 (3-4) (2007) 273–280. URL, http://mathgene.usc.es/snipper/.

[66] H. Rajeevan, U. Soundararajan, A.J. Pakstis, K.K. Kidd, Introducing the forensic Research/Reference on genetics knowledge base, FROG-kb, Investig. Genet. 3 (1) (2012) 18. URL, http://frog.med.yale.edu/FrogKB/.

[67] H. Rajeevan, U. Soundararajan, A.J. Pakstis, K.K. Kidd, FrogAncestryCalc: A standalone batch likelihood computation tool for ancestry inference panels catalogued in FROG-kb, Forensic Sci. Int. Genet. 46 (2020), 102237.

[68] O. García, J.A. Ajuriagerra, A. Alday, S. Alonso, J.A. Pérez, A. Soto, I. Uriarte, I. Yurrebaso, Frequencies of the Precision ID Ancestry Panel Markers in Basques Using the Ion Torrent PGM™ Platform, Forensic Sci. Int. Genet. 31 (2017) e1–e4.

[69] T. Tvedebrink, P.S. Eriksen, H.S. Mogensen, N. Morling, GenoGeographer – a tool for genogeographic inference, Forensic Sci. Int. Genet. Supp. Ser. 6 (2017) e463–e465.

[70] H.S. Mogensen, T. Tvedebrink, C. Børsting, V. Pereira, N. Morling, Ancestry prediction efficiency of the software GenoGeographer using a Z-Score method and the ancestry informative markers in the precision ID ancestry panel, Forensic Sci. Int. Genet. 44 (2020), 102154. URL, http://apps.math.aau.dk/aims/.

[71] J.D. Andersen, P. Johansen, S. Harder, S.R. Christoffersen, M.C. Delgado, S. T. Henriksen, M.M. Nielsen, E. Sørensen, H. Ullum, T. Hansen, A.L. Dahl, R. R. Paulsen, C. Børsting, N. Morling, Genetic analyses of the human eye colours using a novel objective method for eye colour classification, Forensic Sci. Int. Genet. 7 (5) (2013) 508–515.

[72] V. Kastelic, E. Pośpiech, J. Draus-Barini, W. Branicki, K. Drobnič, Prediction of eye color in the Slovenian population using the IrisPlex SNPs, Croat. Med. J. 54 (4) (2013) 381–386.

[73] M. Eduardoff, C. Santos, M. de la Puente, T.E. Gross, M. Fondevila, C. Strobl, B. Sobrino, D. Ballard, P.M. Schneider, A. Carracedo, M.V. Lareu, W. Parson, C. Phillips, Inter-laboratory evaluation of SNP-based forensic identification by massively parallel sequencing using the Ion PGM™, Forensic Sci. Int. Genet. 17 (2015) 110–121.

[74] J.D. Churchill, S.E. Schmedes, J.L. King, B. Budowle, Evaluation of the illumina® Beta version ForenSeq™ DNA signature prep kit for use in genetic profiling, Forensic Sci. Int. Genet. 20 (2016) 20–29.

[75] O. García, A. Soto, I. Yurrebaso, Allele frequencies and other forensic parameters of the HID-Ion AmpliSeq™ Identity Panel markers in Basques using the Ion Torrent PGM™ platform, Forensic Sci. Int. Genet. 28 (2017) e8–e10.

[76] F. Casals, R. Anglada, N. Bonet, R. Rasal, K.J. van der Gaag, J. Hoogenboom, N. Solé-Morata, D. Comas, F. Calafell, Length and repeat-sequence variation in 58 STRs and 94 SNPs in two Spanish populations, Forensic Sci. Int. Genet. 30 (2017) 66–70.

[77] S. Köcher, P. Müller, B. Berger, M. Bodner, W. Parson, L. Roewer, S. Willuweit, DNASeqEx Consortium, Inter-laboratory validation study of the ForenSeq™ DNA Signature Prep Kit, Forensic Sci. Int. Genet. 36 (2018) 77–85.

[78] P. Müller, A. Alonso, P.A. Barrio, B. Berger, M. Bodner, P. Martin, W. Parson, DNASEQEX Consortium. Systematic evaluation of the early access applied biosystems precision ID Globalfiler mixture ID and Globalfiler NGS STR panels for the ion S5 system, Forensic Sci. Int. Genet. 36 (2018) 95–103.

[79] M.D. Brandhagen, R.S. Just, J.A. Irwin, Validation of NGS for mitochondrial DNA casework at the FBI laboratory, forensic sci, Int. Genet. 44 (2020), 102151.

[80] Scientific Working Group on DNA Analysis Methods, Addendum to "SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories" to Address Next Generation Sequencing. https://1ecb9588-ea6f-4feb-971a-73265dbf079c.filesusr.com/ugd/4344b0_91f2b89538844575a9f51867def7be85.pdf, Approved 04/23/2019 (accessed 2020.05.06).

[81] SWGDAM, Mitochondrial DNA Analysis Interpretation Guidelines, https://1ecb9588-ea6f-4feb-971a-73265dbf079c.filesusr.com/ugd/4344b0_f61de6abf3b94c52b28139bff600ae98.pdf, Approved 04/23/2019 (accessed 2020.05.06).

[82] CNUFADN, Informe y recomendaciones de la CTP sobre las nuevas tecnologas de anlisis gentico y nuevos marcadores de ADN de origen biogeogrfico y de rasgos fenotpicos externos, 2019. November (accessed 2020.05.06), https://www.mjusticia.gob.es/cs/Satellite/Portal/1292429696767?blobheader=application%2Fpdf&blobheadername1=Content-Disposition&blobheadername2=Grupo&blobheadervalue1=attachment%3B+filename%3DInforme_y_recomendaciones_de_la_CTP_sobre_las_nuevas_tecnologias_de_analisis_genetico_y_nuevos_marc.PDF&blobheadervalue2=INTCF.