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Quality control of PCR primers used in multiplex STR amplification reactions

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Abstract

Reliable amplification of short tandem repeat (STR) DNA markers with the polymerase chain reaction (PCR) is dependent on high quality PCR primers. The particular primer combinations and concentrations are especially important with multiplex amplification reactions where multiple STR loci are simultaneously copied. Commercially available kits are now widely used for STR amplification and subsequent DNA typing. We present here the use of high performance liquid chromatography (HPLC) and time-of-flight mass spectrometry (TOF-MS) methods for characterization of commercially available STR kits.

Keywords: Short tandem repeat DNA typing; Oligonucleotide quality control; HPLC; Mass spectrometry; Multiplex PCR amplification

1. Introduction

Short tandem repeat (STR) markers have become widespread in their use by the forensic DNA typing community due to their ability to provide a high degree of discrimination between individuals in a relatively short period of time and in spite of highly degraded sample material [1–6]. The amplification of multiple STR markers in the same polymerase chain reaction (PCR) tube is made possible by using a combination of oligonucleotide primers that hybridize to multiple regions of genomic DNA [2,3]. Multiplex STR analysis has dramatically improved the power of discrimination with the ability to analyze multiple regions of DNA simultaneously. From a single amplification with less than a nanogram of DNA template, it is possible to obtain random match probabilities of >1 in 100 billion [4–6].

Commercially available kits permit laboratories without extensive molecular biology capabilities to perform DNA typing. Kits are now available that allow a user to simply add a DNA sample in a specified quantity range to an aliquot of premixed PCR reagents [5,6]. Optimized PCR thermal cycling programs are available for these kits to produce well-balanced quantities of alleles from each locus that is amplified.

Commercially available STR multiplex sets vary based on which STR loci are included, the fluorescent dye combinations, the DNA strand that is labeled, allelic ladders present in kits, and most importantly, the primer sequences utilized for PCR amplification (see Table 1). While these STR kits provide a solution to obtain DNA typing results at most of the same DNA markers, the amplicon (PCR product) sizes differ because unique primer sequences are used in the kits.

Good quality control of the PCR primers included in STR multiplex kits will help maintain consistent and reliable results over time as these kits are used to amplify DNA samples from convicted offenders and crime scene samples. While both high performance liquid chromatography (HPLC) [7] and time-of-flight mass spectrometry (TOF-MS) [8] have been previously used for direct detection and accurate genotyping of STR products, this paper focuses on the use of these techniques for measurement of the PCR primers used in multiplex STR amplification reactions. We demonstrate here the ability to separate the various primers

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Table 1
STR loci and dye labels included in commercially available STR multiplex PCR amplification kits[a]

STR markers	Kit A (6 + amel)	Kit B (9 + amel)	Kit C (9 + amel)	Kit D (10 + amel)	Kit E (8 + amel)	Kit F (nine loci)
# Primers expected	14	20	20	22	16 or 18 (w/amel)	18
CSF1PO	JOE	JOE			TMR	
FGA		FAM	FAM	NED		TMR
TH01	JOE	JOE		NED	TMR	Fluorescein
TPOX	JOE	JOE			TMR	TMR
VWA		FAM	FAM	FAM	TMR	TMR
D3S1358	FAM	FAM	FAM	FAM		Fluorescein
D5S818		NED	NED		Fluorescein	
D7S820	NED	NED	NED		Fluorescein	
D8S1179			JOE	JOE		TMR
D13S317		NED	NED		Fluorescein	
D16S539	FAM			FAM	Fluorescein	
D18S51			JOE	JOE		Fluorescein
D21S11			JOE	JOE		Fluorescein
Amelogenin	JOE	JOE	JOE	JOE	TMR	
D2S1338				FAM		
D19S433				NED		
Penta E						Fluorescein

[a] The number of expected primers in each kit ranges from 14 with kit A to 22 with kit D; FAM: 5-carboxy fluorescein (blue); JOE: 6-carboxy-2′,7′-dimethoxy-4′,5′-dichlorofluorescein (green); NED: PE Applied Biosystems proprietary dye (yellow); TMR: tetramethyl rhodamine (red).

in the multiplex kits for the purposes of providing an independent quality control check on each primer.

2. Materials and methods[1]

2.1. Oligonucleotides and STR kit materials

The AmpFlSTR® kits Profiler™, Profiler Plus™, COfiler™, and SGM Plus™ were purchased from Applied Biosystems (Foster City, CA) and the GenePrint® STR kits PowerPlex™ 1.1 and PowerPlex™ 2.1 were obtained from Promega Corporation (Madison, WI). The STR loci present in each kit are listed in Table 1. Individual primer sets for D16S539, D13S317, D7S820, D5S818, CSF1PO, TPOX, TH01, and VWA were also purchased from Promega.

2.2. HPLC separation

The HPLC used for separation and collection of the primers was a Transgenomic Wave® DNA Fragment Analysis System (San Jose, CA, USA) with a DNASep® column (Transgenomic, Inc.) that has a stationary phase consisting of 2 μm nonporous alkylated poly(styrene-divinylbenzene) particles. UV detection was performed at 260 nm. A fluorescence detector was used to identify the dye-labeled primers [9]. The HPLC separations were run at 70°C with a mobile phase consisting of 0.1 M triethylammonium acetate (TEAA; solvent A) and 0.1 M TEAA-25% acetonitrile (solvent B). The separation conditions were a flow rate of 0.9 ml/min and a gradient of 18–50% solvent B over 40 min. Fractions were collected manually and evaporated to dryness in a Savant SPD111V Speed Vac® (Holbrook, NY, USA). Each fraction was reconstituted in 20 μl of deionized water for further characterization by MS.

2.3. Mass spectrometry

DNA samples were spotted on a smooth stainless steel plate using 1 μl sample and 1 μl matrix material. The matrix consisted of a saturated solution of 3-hydroxypicolinic acid (Aldrich Chemical, Milwaukee, WI) in a solvent of 25% acetonitrile and 25 mM ammonium acetate (Sigma Chemical, St. Louis, MO). Mass spectra were collected on a PerSeptive Biosystems Voyager DE time-of-flight mass spectrometer (Framingham, MA) equipped with a pulsed nitrogen laser (337 nm). An acceleration voltage of 25 kV was used with a delayed extraction of 550 ns. The extraction grid voltage was set to 23.75 kV (95%) and the guide wire voltage was 25 V (0.1%). All spectra were taken in positive ion mode with between 100 and 256 laser shots collected on

[1] Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

each sample for signal averaging purposes. Laser power was adjusted with each sample to obtain an optimal sensitivity and resolution. The instrument default calibration was used to mathematically convert spectral data channels from time to mass values. The peak masses described throughout this paper are for the singly charged ions and are in Daltons (Da), where 1 Da is equivalent to 1 g/mol.

2.4. Data analysis

Data points from HPLC chromatograms and mass spectra were plotted in SigmaPlot version 6.0 (SPSS Inc., Chicago, IL). HPLC chromatogram baselines were fitted utilizing a loess algorithm (a locally weighted regression smoothing algorithm that performed a full least-squares fit for each data point) and then subtracted from the raw data. Mass spectra were smoothed and enhanced by the application of matrix convolution filters [10] (ProteoMetrics freeware edition, NY, New York). Both HPLC and MS spectra were normalized to the largest peak in the spectrum as a reference, resulting in normalized intensities between 0 and 100%.

3. Results and discussion

3.1. Impact of oligonucleotide synthesis failure products

Phosphoramidite chemical synthesis of oligonucleotides involves coupling each nucleotide in a specified order. However, failures sometimes occur during this primer synthesis. Since primers are synthesized from the 3′ to 5′ direction with phosphoramidite chemistry, coupling failures at the end of the synthesis cycle result in primer failure products that are shorter at the 5′-end of the primer. These failure products are sometimes referred to as $(n-1)$, $(n-2)$, etc. A high resolution length-based separation with a number of different analytical techniques can provide a direct characterization of the purity of the synthesized oligonucleotide [11–16].

After the primers are chemically synthesized, they are combined to form multiplex amplification sets. For PCR amplification followed by fluorescence detection, each STR marker requires two primers, one that is labeled with a fluorescent dye and one that is not. Different fluorescent dyes are used in order to increase the level of multiplexing possible with multi-color fluorescence detection [2,5,6] (Table 1). Because the two primers define the end of the PCR product, oligonucleotide synthesis failures can impact the length of the subsequent amplicon. In the case of STR amplification, where length measurements are important, an impure primer could potentially impact the overall length of the STR amplicon and affect accurate comparison to allelic ladders or other size standards.

During interlaboratory tests conducted by the European DNA Profiling Group (EDNAP), one laboratory returned larger than expected measurement error at the STR locus D11S554 [17]. Subsequently, it was found that an incorrectly synthesized PCR primer was the source of the result; an extra base had been erroneously attached to the 5′-end of the primer [17]. A quality control check using the methods described here would have been useful to quickly resolve this issue.

3.2. HPLC separation of kit A–D primer sets

The HPLC separation results for four different multiplex STR amplification kits are shown in Fig. 1. The combination of reverse-phase and ion-exchange properties in the HPLC column aid the separation characteristics. Unlabeled primers elute from the column first. The fluorescent dye label attached to one primer in each locus-specific pair was observed to impact the oligonucleotide retention on the HPLC column. This can best be seen in the kit B primer set separation (Fig. 1B). The nine peaks in the time range of 9–15 min are unlabeled oligonucleotides. Peaks 10–17, which pass the detector between 19 and 26 min, are either JOE or 5-FAM dye labeled. Finally, peaks 18–20 are NED-labeled. Thus, the properties of the NED dye are such that oligonucleotides labeled with this dye are retained longer on the HPLC column than 5-FAM and JOE labeled primers. To further confirm which peaks were labeled with fluorescent dyes, a single wavelength fluorescent detector was used in conjunction with the HPLC separation.

With two primers for each STR marker, the seven loci amplified by the kit A kit are expected to have 14 primers. As can be seen in Fig. 1A, 14 peaks were resolved by HPLC. Each HPLC fraction was collected and analyzed using MS. All 14 kit A fractions appear to be unique primers since their masses differ from one another (Table 2).

The peaks for the HPLC chromatograms shown in Fig. 1 are further described in Tables 2–5 in terms of their observed retention time, relative peak heights, measured mass, and peak identity. Peaks were associated with particular primer sequences and STR markers using an integrated approach with HPLC, CE, MS, and informatics that will be described in a future communication (Vallone et al., in preparation). The HPLC separation conditions described here were unable to separate all possible primers in the larger multiplex sets. In fact, peak 8 in both (Fig. 1C) and (Fig. 1D) contain two unresolved primers, which is why its peak height is larger than the other nearby, unlabeled oligonucleotides.

Peaks 16 and 17 (Fig. 1B), peaks 15 and 16 (Fig. 1C), and peaks 18 and 19 (Fig. 1D) are both VWA primers and may be degenerate primers as mentioned by Walsh [18] to correct for the non-amplification of a VWA allele (i.e. null allele) [19]. These primers are both 30 bases in length and possess masses of approximately 9870 Da, contain a peak that migrates between the FAM/JOE labeled and the NED labeled primers. Peak 17 (Fig. 1C) and peak 20 (Fig. 1D) both possess a mass of 9820 Da and do not appear to be associated with any particular STR locus that is amplified in these multiplex kits. This peak has been labeled an ‘‘unknown peak’’ in Tables 4 and 5.

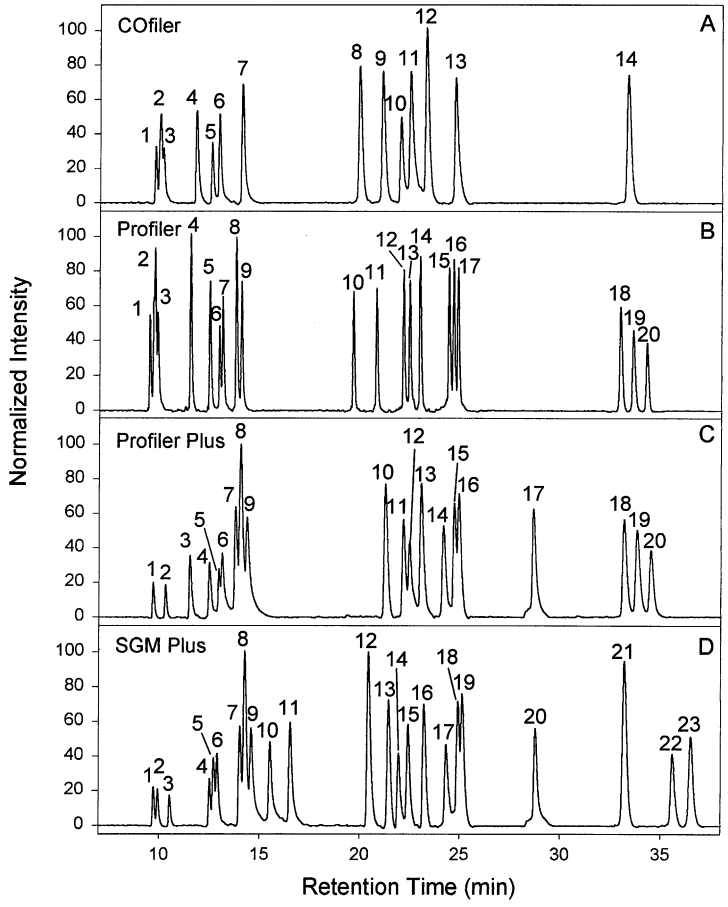

Fig. 1. HPLC chromatograms of 4 STR kit multiplex primer sets: (A) kit A; (B) kit B; (C) kit C and (D) kit D. Peaks are numbered based on their relative retention times, which are listed in Tables 2–5 for each of the primer sets.

Table 2
Summary of peak information from these four HPLC separation[a]

HPLC fraction	Retention time (min)	Peak height (arbitrary units)	Mass (Da)	MS peak label	Peak ID
1	9.7	32.1	5384	A	CSF1PO-R
2	10.0	51.1	5590	B	TH01-F
3	10.1	31.0	5823	C	D3S1358-F
4	11.8	50.5	6513	E	D7S820-R
5	12.6	33.1	6489	D	D16S539-R
6	12.9	49.2	6489	D	TPOX-R
7	14.1	66.1	7429	K	AMEL-R
8	19.9	77.2	6513	E	CSF1PO-F (JOE)
9	21.1	73.9	6830	J	TPOX-F (JOE)
10	22.0	48.0	6655	G	D16S539-F (FAM)
11	22.5	73.6	6715	H	D3S1358-R (FAM)
12	23.3	100.0	8152	L	AMEL-F (JOE)
13	24.7	70.7	6751	I	TH01-R (JOE)
14	33.3	72.6	6612	F	D7S820-F (NED)

[a] HPLC fractions correspond to the peaks shown in Fig. 2A. A total of 14 primers are expected since 7 DNA markers are amplified in this multiplex. The MS peak labels refer to the peaks shown in Fig. 3.

Table 3
Summary of peak information from kit B HPLC separation[a]

HPLC fraction	Retention time (min)	Peak height (arbitrary units)	Mass (Da)	Peak ID
1	9.5	53.9	5377	CSF1PO-R
2	9.7	92.3	5584	TH01-F
3	9.9	55.0	5817	D3S1358-F
4	11.5	100.0	6506	D7S820-R
5	12.5	70.4	6494	TPOX-R, VWA-F
6	12.9	46.7	6009	D13S317-R
7	13.1	63.3	6374	D5S818-R
8	13.8	96.2	7421	AMEL-R
9	14.1	71.4	7123	FGA-R
10	19.6	65.2	6506	CSF1PO-F (JOE)
11	20.8	68.6	6823	TPOX-F (JOE)
12	22.2	78.0	6745	D3S1358-R (FAM)
13	22.4	73.3	6939	FGA-F (FAM)
14	23.0	87.8	8142	AMEL-F (JOE)
15	24.4	79.6	6745	TH01-R (JOE)
16	24.6	83.7	9865	VWA-R1 (FAM)
17	24.9	80.1	9857	VWA-R2 (FAM)
18	33.0	58.4	6608	D7S820-F (NED)
19	33.6	45.6	6708	D13S317-F (NED)
20	34.3	38.2	6848	D5S818-F (NED)

[a] HPLC fractions correspond to the peaks shown in Fig. 2B. A total of 20 primers are expected since 10 DNA markers are amplified in this multiplex.

The primer sets in kits A through D contain a well-balanced mix of primers that appear consistent between multiplex sets (Fig. 1). The primer sequences are kept consistent between kits A, B, C and D that contain the same STR loci [5]. In our studies, the primer sequences and primer concentration ratios appear to remain consistent between these four kits. For example, the same pattern exists for peaks 7, 8, and 9 in kit C (Fig. 1C) and kit D (Fig. 1D).

Table 4
Summary of peak information from kit C HPLC separation[a]

HPLC fraction	Retention time (min)	Peak height (arbitrary units)	Mass (Da)	Peak ID
1	9.7	19.5	5824	D3S1358-F
2	10.3	18.1	5455	D18S51-F
3	11.5	34.7	6513	D7S820-R
4	12.5	31.0	6486	VWA-F
5	13.0	28.2	6012	D13S317-R
6	13.1	36.9	6374	D5S818-R
7	13.8	63.9	7415	AMEL-R
8	14.0	100.0	7042, 7132	D8S1179, FGA-R
9	14.4	57.4	6721	D21S11-F
10	21.3	76.4	7490	D21S11-R (JOE)
11	22.2	55.5	6714, 6714	D8S1179 (JOE), D3S1358-R (FAM)
12	22.5	43.4	6943	FGA-F (FAM)
13	23.1	76.9	8157	AMEL-F (JOE)
14	24.2	52.4	6720	D18S51-R (JOE)
15	24.7	65.9	9882	VWA-R1 (FAM)
16	25.0	70.8	9892	VWA-R2 (FAM)
17	28.7	62.0	9817	Unknown peak
18	33.2	56.5	6600	D7S820-F (NED)
19	33.8	50.1	6694	D13S317-F (NED)
20	34.5	38.3	6851	D5S818-F (NED)

[a] HPLC fractions correspond to the peaks shown in Fig. 2C. A total of 20 primers are expected since 10 DNA markers are amplified in this multiplex.

Table 5
Summary of peak information from kit D HPLC separation[a]

HPLC fraction	Retention time (min)	Peak height (arbitrary units)	Mass (Da)	Peak ID
1	9.7	22.0	5587	TH01-F
2	9.9	19.8	5821	D3S1358-F
3	10.5	16.6	5455	D18S51-F
4	12.5	26.6	6495	VWA-F1
5	12.7	38.4	6490	VWA-F2
6	12.9	40.8	6489	D16S539-R
7	14.0	57.2	7429	AMEL-R
8	14.3	100.0	7042, 7130	D8S1179, FGA-R
9	14.6	55.5	6721	D21S11-F
10	15.5	47.4	7259	D2S1338-R
11	16.6	58.4	7557	D19S433-R
12	20.4	98.3	7074	D2S1338-F (FAM)
13	21.5	71.5	7493	D21S11-R (JOE)
14	22.0	41.2	6652	D16S539-F (FAM)
15	22.4	57.6	6714, 6714	D8S1179 (JOE), D3S1358-R (FAM)
16	23.2	69.3	8155	AMEL-F (JOE)
17	24.3	45.9	6710	D18S51-R (JOE)
18	24.9	71.1	9875	VWA-R1 (FAM)
19	25.1	75.1	9865	VWA-R2 (FAM)
20	28.8	55.4	9819	Unknown peak
21	33.2	94.0	7209	D19S433-F (NED)
22	35.6	40.9	7114	FGA-F (NED)
23	36.5	51.2	6799	TH01-R (NED)

[a] HPLC fractions correspond to the peaks shown in Fig. 2D. A total of 22 primers are expected since 11 DNA markers are amplified in this multiplex.

Likewise for peaks 1, 2, and 3 from kit A (Fig. 1A) and kit B (Fig. 1B) as well as peaks 18, 19, and 20 from Profiler™ (Fig. 1B) and kit C (Fig. 1C) a similar balance and peak pattern are seen. The synthesized primers have been purified and are present in amounts of approximately 10–15 pmol each.

3.3. HPLC separation of kit E and kit F primer sets

HPLC separations of kit E and kit F are shown in Fig. 2. As with the primers included in kits A, B, C and D primer mixes, dye-labeled primers are retained longer on the ion-paired reverse phase separation column. However, there is a much higher degree of peak height variability in the primers detected within the kit E and kit F (Fig. 2) compared to kits A–D (Fig. 1). A closer analysis revealed that the peaks with a higher signal in the chromatogram are for the STR loci that are fluorescein labeled (see Table 1).

The dramatic primer concentration imbalance is due in part to the fact that kits E and F are optimized for use with the Hitachi FMBIO® instrument platform. The FMBIO® uses an excitation laser wavelength of 532 nm to excite fluorescent dyes. Because this laser wavelength is not well optimized for fluorescein, which is best excited near its absorption maximum of 490 nm, the FMBIO® instrument requires a larger amount of fluorescein-labeled PCR products for effective detection [20]. Thus, the fluorescein-labeled primers are in higher concentration in both kit E and kit F in order to generate larger amounts of fluorescein-labeled amplicons compared to TMR-labeled PCR products. While an imbalance of primer amounts is necessary due to detection sensitivity issues, extreme primer imbalances may make quantitative analysis of lower concentration components more difficult.

With the HPLC separation technique used here, we observed multiple peaks for each fluorescein dye-labeled peak used in the kits E and F. To illustrate this point, the HPLC chromatogram for D13S317 monoplex primer set is superimposed (Fig. 2B) on the kit E chromatogram (Fig. 2A). The peaks at 17 and 20 min in Fig. 2B, which correspond to peaks 12 and 15 in the complete kit E primer mixture (Fig. 2A), are both D13S317-R fluorescein-labeled primers.

Similar masses were observed for each pair of fluorescein-labeled primers leading us to the conclusion that likely two different isomers of the fluorescein dye are being detected. Alternatively, the second peak in each pair of fluorescein-labeled primers may result from a synthesis byproduct as was recently described by Wu et al. [21]. Regardless of the exact nature of the dye-oligonucleotide coupling, a single oligonucleotide labeled with two different species of the fluorescein dye generates two peaks that are separated by more than 3 min in an HPLC chromatogram

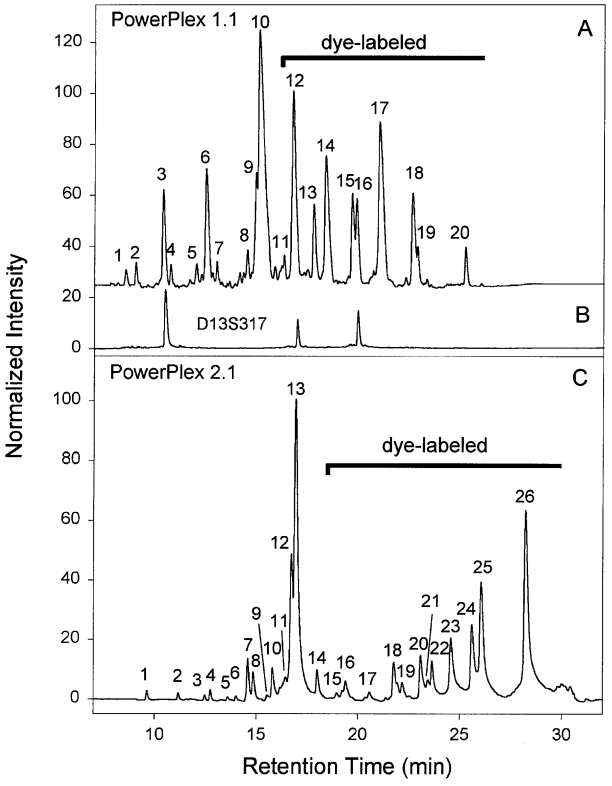

Fig. 2. HPLC chromatograms of 2 STR kit multiplex primer sets and a singleplex primer set: (A) kit E; (B) D13S317 and (C) kit F. Multiple peaks are seen for each of the dye-labeled oligonucleotides (see text for details).

(Fig. 2B). However, while the presence of multiple peaks in the HPLC separation step complicates interpretation of the number of actual primer sequences present in a multiplex STR kit, the impure dye does not appear to impact slab gel separation methods used to detect the PCR products.

Based on mass analysis of collected HPLC fractions for the smaller peaks in the kit E, some of these peaks were determined to be $n-1$ products of other primers or even primers that are missing their dye label. For example, peak 4 in Fig. 2A corresponds to the $n-1$ product of the unlabeled TPOX primer because it possesses a mass of 7167 Da while peak 7 in Fig. 2A is the full length unlabeled TPOX primer with a mass of 7403 Da. In addition, peak 2 in Fig. 2A appears to be the D13S317-R primer but without its dye attached (6150 Da). The full length D13S317-R primer, when it is fully dye labeled, has a mass of approximately 6690 Da.

3.4. Mass spectral information

Multiplex STR primer sets can be subjected to a rapid quality control check by direct analysis with TOF-MS. The presence or absence of a peak at a particular mass can then be used to verify that a particular primer is present in the mix. Fig. 3 is the mass spectrum of the complete kit A primer set produced by spotting 1 μl of sample directly from the primer mix with 1 μl of matrix solution. No other sample preparation is performed in order to obtain these results.

Although 14 primers are expected for the kit A primer set (Table 1), only 12 peaks are seen in Fig. 3 because peaks D and E contain two primers each. The primers within these peaks have similar DNA sequence compositions and thus masses that are not resolvable. For example, peak "D" in Fig. 3 has a mass of 6489 Da and corresponds to the TPOX and D16S539 unlabeled primers.

The signal intensities for the primer peaks in a mass spectrum are not indicative of their concentrations. Primers with a lower mass are selectively ionized more efficiently than those primers with a higher mass. Note that peak "A" is higher than peak "L". Although peak "L" exhibits the lowest sensitivity in the mass spectrum, it is the highest in the HPLC chromatogram (peak 12 in Fig. 1A; Table 2).

For efficient characterization of unknown PCR primer combinations, it is advantageous to use both HPLC and mass spectrometric results. As seen in Fig. 4, the peak positions from HPLC and MS are orthogonal. Thus, if one technique fails to resolve two primers because they have similar sequence compositions or chemical behavior, they may be well separated by the other method. For example, peak "E" in the kit A mass spectrum (Fig. 3) is separated into two

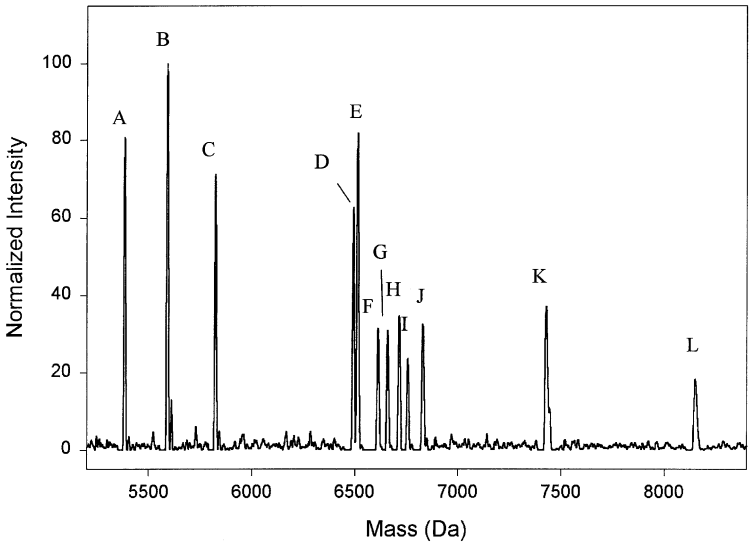

Fig. 3. Mass spectrum of kit A primers. The labeled peak masses are listed in Table 2.

independent primer components (peaks 4 and 8, Fig. 1A) by HPLC.

In general, smaller primers are retained less on the HPLC column demonstrating that this separation technique is size-based in nature (see Fig. 4). The dye-label on the primer though does have a significant impact on the overall separation order. Peak "F" from the kit A mass spectrum (Fig. 3) corresponds to peak 14 in the HPLC chromatogram (Fig. 1A). Likewise, the exchange of dye labels on a primer can dramatically shift its retention time. For example, the FGA-F primer elutes at 22.4 min in the kit B when it is labeled with 5-FAM but is shifted to an elution time of 35.6 min when a NED dye label is placed on the same primer in the kit D.

4. Implications and conclusions

Obtaining consistent multiplex STR amplification results over time is especially important as DNA typing information is being placed into DNA databases around the world [22]. Convicted offender samples may be processed months or even years prior to casework samples that result in a database hit being made. Thus, to obtain consistent multiplex PCR amplification results over time, it is important to have consistent commercially available STR kits with high quality primers and a proper balance between those primers.

The forensic DNA typing community has become increasingly dependent on commercial kits. These commercially available kits quickly dictate which STRs will be used

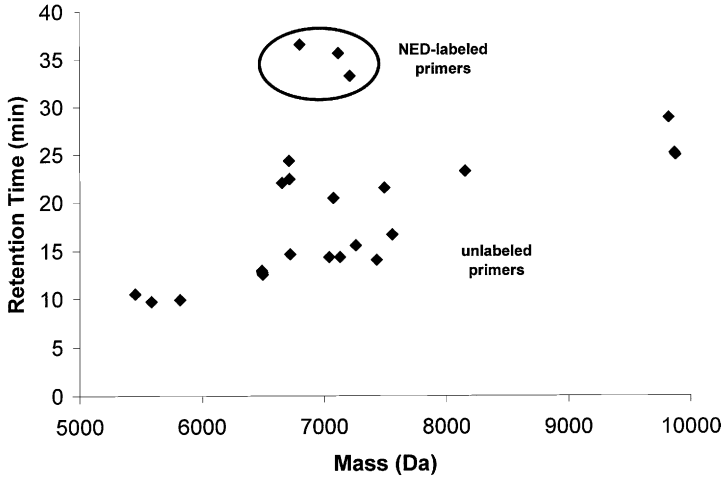

Fig. 4. Comparison of HPLC retention times and primer masses for the kit D multiplex primer set (see Table 5).

by the vast majority of forensic laboratories due to their ease of use [23]. The kits have been extensively tested by their manufacturers and optimized for multiplex amplification reactions. However, the primer sequences present in each multiplex STR kit have not been publicly disclosed. According to the manufacturers, the unique blend of primers present in each kit will not be changed without notifying the users.

The ability to monitor these kits over time may be important to insure that primers are not altered in a way that could impact multiplex PCR amplification performance (e.g. produce null alleles). We propose that a plot of primer masses versus HPLC retention time can serve as a ''fingerprint'' for lot-to-lot variability. Fig. 4 displays this fingerprint for kit D primer sequences and their attached dye labels. Such plots provide a practical alternative to reporting the actual primer sequences. Even a single base modification in a primer will result in a different mass or possibly an altered HPLC retention time compared to a previous primer for a STR marker. In any case, this analytical characterization of the primer sets within commercially available STR kits can serve as a baseline for monitoring future lots of these kits.

In this work, we have demonstrated that MS and HPLC are effective means for measuring the primer sets used by forensic DNA laboratories and monitoring the reagents provided by commercial manufacturers. We have also developed methods for determining individual primer identities using an integrated approach with HPLC, CE, MS, and informatics that will be described in a future communication. The use of both analytical techniques to characterize the primers present in multiplex STR kits can help insure that kits remain consistent and reliable over time.

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