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STRide probes: Single-labeled short tandem repeat identification probes

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ABSTRACT

The demand for forensic DNA profiling at the crime scene or at police stations is increasing. DNA profiling is currently performed in specialized laboratories by PCR amplification of Short Tandem Repeats (STR) followed by amplicon sizing using capillary electrophoresis. The need for bulky equipment to identify alleles after PCR presents a challenge for shifting to a decentralized workflow. We devised a novel hybridization-based STR-genotyping method, using Short Tandem Repeat Identification (STRide) probes, which could help tackle this issue. STRide probes are fluorescently labeled oligonucleotides that rely on the quenching properties of guanine on fluorescein derivatives. Mismatches between STRide probes and amplicons can be detected by melting curve analysis after asymmetric PCR. The functionality of the STRide probes was demonstrated by analyzing synthetic DNA samples for the D16S539 locus. Next, STRide probes were developed for five different CODIS core loci (D16S539, TH01, TPOX, FGA, and D7S820). These probes were validated by analyzing 13 human DNA samples. Successful genotyping was obtained using inputs as low as 31 pg of DNA, demonstrating high sensitivity. The STRide probes are ideally suited to be implemented in a microarray and present an important step towards a portable device for fast on-site forensic DNA fingerprinting.

1. Introduction

Short Tandem Repeat (STR) sequences or microsatellites are highly abundant in human DNA, covering up to 3% of the total genome (Lander et al., 2001). Their important role is exemplified by specific STR loci known to be associated to pathologies, e.g. Huntington's disease (Zoghbi and Orr, 2000). Over the past decades, STRs have been widely used to distinguish individuals based on DNA samples. STR profiling has become the gold standard in forensic DNA research and has proven to be an indispensable piece of evidence in many judicial investigations (Butler, 2005). To obtain a forensic DNA profile, the repeat number of about 20 highly variable STR loci is determined. The repeat number of these STR loci varies between individuals within a population and leads to STR profiles with a strong discriminatory capacity for identification which is recognized worldwide.

Routine forensic STR analysis for human identification is currently almost exclusively performed by polymerase chain reaction (PCR) amplification, followed by amplicon sizing through capillary electrophoresis (CE) (Butler, 2012). These techniques require a specialized laboratory with highly trained staff. Forensic crime scene investigation and disaster victim identification would benefit immensely from quick and portable DNA profiling tools (Bruijns et al., 2016). Assays based on the hybridization of an oligonucleotide probe to the investigated sample are an attractive alternative, as they are simple, relatively cheap, and are ideally suited for implementation in a portable device. Hybridization-based assays, mostly relying on fluorescently labeled probes, are widely established for Single Nucleotide Polymorphism (SNP) genotyping. However, designing hybridization-based assays for STR analysis has proved extremely challenging due to the length of the loci and the similarity between alleles, both caused by the highly repetitive nature of the loci. Hybridization-based STR sizing assays have been described in literature, but have not been adopted by the forensic research community. The best-known example are the HyBeacon™ probes, which were implemented in LGC's ParaDNA device (Blackman et al., 2015; Gale et al., 2008). Suffering from severe probe design restrictions, this technology can only be used for a screening assay targeting five STR loci. Other technologies, e.g. the microarrays described by Kemp et al. (2005) and Herrmann et al. (2010) rely on the introduction of an extra enzymatic step, complicating the proposed workflow and hampering the implementation in a portable device. Halpern and

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Ballantyne (2011) developed an STR melt curve genotyping assay based on fluorescence resonance energy transfer between an intercalating dye and a fluorescent labeled probe, resulting in melting temperature-based, binary match/mismatch results. As the shift in melting temperature caused by a mismatch decreases for longer STR-probes, this genotyping method is challenging for loci characterized by high repeat numbers. There is a clear need for a highly informative, less complicated STR-genotyping assay.

Here, we describe STRide probes (Short Tandem Repeat identification probes), a new hybridization-based STR-genotyping assay, using single-labeled oligonucleotide probes. The goal of this study is to present a detailed report of the working mechanisms of the STRide probes by performing genotyping experiments of synthetically manufactured samples for the D16S539 locus. Moreover, we showcase the utility of STRide probes by genotyping commercially available control DNA and buccal swabs using probes developed for five different CODIS core loci (D16S539, TH01, TPOX, FGA, and D7S820).

2. Material and methods

2.1. Samples

Three commercially available reference samples (9947a, 9948 (OriGene, Rockville, MD, USA), and 2800M (Promega, Madison, WI, USA)), as well as 10 buccal swabs (samples 1–10) and three blood samples (samples 11–13) retrieved from healthy volunteers were used to obtain the results presented in this paper. Ethical approval was obtained from the ethical review board of Ghent University Hospital, all volunteers signed the informed consent. The buccal swabs were immersed in 200 μL of nuclease-free water and vortexed briefly. After an incubation step of 15′, the swab was discarded and the extract, containing about 0.1 ng DNA per μL, was used for downstream analysis without further purification. Blood samples were obtained by a finger puncture using a 21G Minicollect® Lancelino safety lancet with a penetration depth of 2.4 mm (Greiner Bio-One, Kremsmünster, Austria) and collected in a K3E K3EDTA Minicollect® collection tube (Greiner Bio-One, Kremsmünster, Austria). DNA extraction of the blood samples was performed using the DNeasy® Blood and Tissue kit according to the manufacturer's instructions. Besides the reference samples and the buccal swabs, synthetically manufactured samples were analyzed for the D16S539 locus. These oligonucleotides were ordered from Integrated DNA Technologies (Newark, NJ, USA) and comprised all frequently occurring repeat numbers for this locus.

2.2. CE genotyping

Ten buccal swabs were STR-genotyped using CE as a reference. The AmpFlSTR® Identifiler® Plus PCR amplification kit (ThermoFisher Scientific, Waltham, MA, USA) was used according to the manufacturer's protocol. For each buccal sample, 10 μL of the sample was used as input for amplification, whereas for the blood samples, 1 ng of extracted DNA was used. Thermal cycling was performed in a SimpliAmp Thermal Cycler (ThermoFisher Scientific, Waltham, MA, USA) and consisted of an initial denaturation step at 95 °C for 11 min, followed by 28 cycles of 94 °C for 20 s and 59 °C for 3 min. A final elongation step of 60 °C for 10 min was performed. After amplification, CE was performed using the ABI3130xl Genetic Analyzer (ThermoFisher Scientific, Waltham, MA, USA). Analysis of the electropherograms was realized by the GeneMapper ID-x 1.2 software (ThermoFisher Scientific, Waltham, MA, USA). The true genotypes for all samples, as determined using CE, can be found in Supplementary Table S1.

2.3. Asymmetric PCR amplification

Since the presented STR-genotyping method relies on hybridization, an excess of the amplicon strand that is complementary to the probe is required. Presence of the other amplicon strand competitively inhibits the hybridization of sample and probe. The most straightforward method to obtain the desired amplicon strand in excess is asymmetric PCR, where both primers are added at different concentrations. All samples were amplified by singleplex asymmetric PCR in a volume of 50 μL containing $MgCl_2$ at a concentration of 0.5 mM, dNTPs (ThermoFisher Scientific, Waltham, MA, USA) at 200 μM each, 1X Qiagen PCR buffer and 1.3 U HotStarTaq enzyme (Qiagen, Helden, Germany), and a set of primers (IDT, Newark, NJ, USA) per STR as listed in Supplementary Table S2. For the reference samples and the blood samples, 1 ng of input DNA was used, whereas for the buccal swabs 30 μL of sample was added. After an initial denaturation step of 15 min at 95 °C, 60 amplification cycles were performed consisting of denaturation for 60 s at 95 °C, primer annealing for 60 s at 59 °C, and elongation for 80 s at 72 °C.

2.4. STRide probes melting curve analysis

All PCR products were purified after amplification using the DNA Clean & Concentrator-5 (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. The amplicons were eluted in a volume of 45 μL nuclease-free water, to which 5 μL of 10X Qiagen PCR buffer was added after elution. Aliquots of 8.5 μL were dispensed in a qPCR-plate. To each well, 1.5 μL of a specific STRide probe (Table 1) was added, resulting in a final STRide probe concentration of 0.15 μM. Probes were acquired from Integrated DNA Technologies (Newark, NJ, USA) and from Biolegio (Nijmegen, The Netherlands). 10 μL of mineral oil was added to each well to prevent evaporation. Melting curve analysis was performed using a LightCycler 480 (Roche, Basel, Switzerland). After an initial denaturation step of 5 min at 95 °C, the samples were cooled down to 40 °C at a ramp rate of 0.11 °C/s. Following a 10 min hybridization step at 40 °C, melting was performed by heating the sample to 95 °C at a ramp rate of 0.04 °C/s. Fluorescence intensity was continuously measured during melting, resulting in 5 acquisitions per second. Melting curves were constructed by plotting the first derivative of the fluorescence intensity versus temperature. To assess the signal obtained for hybridization of a certain STRide probe with a certain allele, without interference of PCR artefacts, synthetically manufactured oligonucleotides complementary to the STRide probes for the D16S539 locus were used for melting curve analysis. A volume of 10 μL, containing 0.15 μM STRide probes, 1.5 μM synthetic complements (IDT), and 1X Qiagen PCR buffer was subjected to melting curve analysis as previously described.

3. Results and discussion

3.1. STRide probe assay design

To develop the STRide probes, we exploited the intrinsic quenching

Table 1
STRide probe sequences (*For locus FGA, n = 11 corresponds to allele 19, etc.).

Locus	Sequence (5' – 3')	Range of alleles
D16S539	5′GTTTTGTCTTTCAATGA(TATC)$_n$CAC-FAM	n = 9–13
TH01	5′FAM-CCT(CATT)$_n$CACCATGGAGTCTGTGTTCCCTGTG	n = 6–10
TH01 9.3	5′FAM-CCT(CATT)$_3$CAT (CATT)$_6$CACCATGGAGTCTGTGTTCCCTGTG	N/A
TPOX	5′FAM-CCAAA(CATT)$_n$CAGTGAGGGTTCCCTAAGTGC	n = 8–12
FGA	5′TAGTTTCTTTCTTTCTTTTTTCT(CTTT)$_n$CTCCTTC-FAM	n = 11–18*
D7S820	5′FAM-CTAAC (GATA)$_n$GACAGATTGATAGTTTTTTTTTATCTCACTAAATA	n = 8–12

properties of deoxyguanosine nucleotides on fluorescein derivatives, e.g. 6-carboxyfluorescein (FAM). The quenching effect of nucleobases on fluorophores have been described extensively. Seidel et al. (1996) investigated the underlying mechanism of this quenching effect, and found that photo-induced electron transfer plays an important role. Deoxyguanosine nucleotides show the highest quenching efficiency on FAM-fluorophores, which can be attributed to the excellent electron-donor properties of guanine (Mao et al., 2018). Moreover, the context sequence of the guanine residue influences the quenching efficiency (Crockett and Wittwer, 2001). A STRide probe was designed as illustrated in Fig. 1, consisting of an anchor region, a repeat region, and a sensor region. The anchor region is complementary to the region flanking the STR region and should ensure that hybridization occurs in such a way that the first repeat of the probes anneals to the first repeat of the DNA fragments in the sample. The repeat region is complementary to the STR region. For a certain STR locus, a set of probes is designed with repeat numbers matching all commonly occurring STR alleles in the population. The sensor region is complementary to the region flanking the STR, opposite to the anchor region. The sensor region is substantially shorter than the anchor region and has a terminal cytosine residue with a fluorophore attached to it. A detailed list with applied design rules and the corresponding rationale can be found in Supplementary Table S3.

Upon hybridization with a sample containing the exact same repeat number, implying 100% complementarity, a stable homo-duplex is formed and the fluorophore is quenched by one or more guanine residues in the DNA strand of the sample (Fig. 1, A). We hypothesized that if the probe and the sample DNA strand have a different STR repeat number (we refer to this as a mismatch throughout this article), either a less stable hetero-duplex containing a bulge loop (Fig. 1, B), or a hetero-duplex with dangling ends (Fig. 1, C) will be formed. In practice, a combination of both bulge loop and dangling end conformations will occur when there is a mismatch between sample DNA strand and probe. We reasoned that the total fluorescence quenching will be less pronounced in case of a mismatch. We aimed to exploit melting curve analysis for STR genotyping using STRide probes. Melting of a mismatched duplex should result in lower and broader melting peaks, occurring at a lower temperature. The bigger the difference in repeat number between probe and sample, the lower the obtained signal should be. Unlike other hybridization-based STR-genotyping methods, analysis will not solely be performed based on the observed melting temperature. We reasoned that, owing to the strategic positioning of the fluorophore, the shape and the interrelation of the melting peaks will provide valuable information that can aid in unambiguous data interpretation. The developed STRide probes are less complex than other STR-genotyping probes, e.g. HyBeacon™ probes (Gale et al., 2008). Moreover, the severe design restrictions from which the HyBeacon™ probes suffer, do not apply for STRide probes.

3.2. Analysis of synthetic DNA samples for the D16S539 locus

To demonstrate functionality of the STRide probes, an initial characterization experiment was performed for a single locus and under highly controlled conditions, i.e. using commercially acquired, synthetic DNA fragments. Fig. 2 shows the melting curves of all probes for the D16S539 locus after hybridization with different synthetic DNA complements. All possible combinations of probes and complements were analyzed. To facilitate comparison between and within experiments, the melting peaks are normalized with respect to the highest melting peak. For all alleles, a similar melting profile is obtained. The peak corresponding to the matching probe is substantially higher than all other melting peaks, and occurs at a higher temperature. As expected, melting of hetero-duplexes characterized by a single-repeat mismatch results in the highest melting peaks among the hetero-duplexes. Importantly, the shape and interrelation of all melting peaks is very similar across experiments, resulting in characteristic, reproducible melting profiles. Together, these results provide proof of concept that the STRide probes can be used for STR-genotyping by melting curve analysis.

3.3. D16S539, TPOX and D7S820 genotyping of reference DNA samples and buccal swabs after STR PCR

In forensic practice, STR profiling is routinely performed after PCR amplification. Artefacts arising during STR PCR, such as stutter products, present an additional challenge compared to pure, synthetic DNA fragments (Butler, 2005). To test the robustness of the STRide probes, three commonly used, commercially available reference samples (9947a, 9948 and 2800M), 10 buccal swabs, and three blood samples were genotyped after STR amplification by asymmetric PCR. In addition

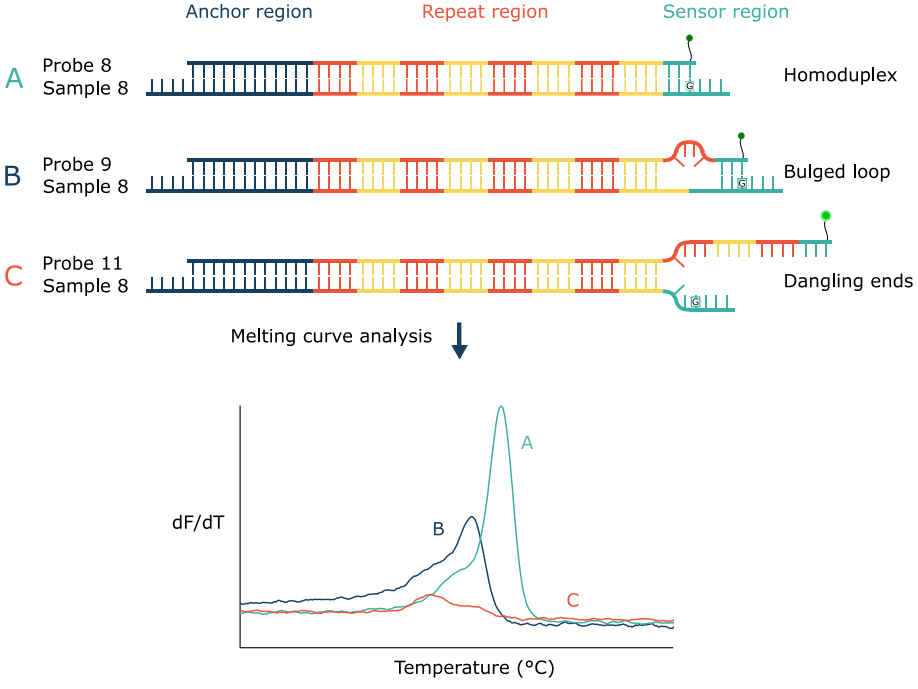

Fig. 1. STRide probe design. The STRide probes consist of an anchor region (blue), a repeat region (red - yellow) and a sensor region (green) terminally labeled with a FAM-fluorophore. Hybridization with the asymmetrically PCR-amplified sample results in three possible duplex conformations. Conformation A occurs upon hybridization with a fully complementary sample, whereas conformations B and C both occur in case of a mismatch. 'A' shows a stable homo-duplex, resulting in quenching of the fluorophore. Melting curve analysis results in high, sharp melting peaks at a high temperature. 'B' shows a hetero-duplex characterized by a bulged loop resulting in quenching of the fluorophore. Melting curve analysis results in lower, broader melting peaks at a lower temperature. 'C' shows a hetero-duplex characterized by dangling ends. No quenching of the fluorophore will occur, and no melting peaks will result from melting curve analysis.

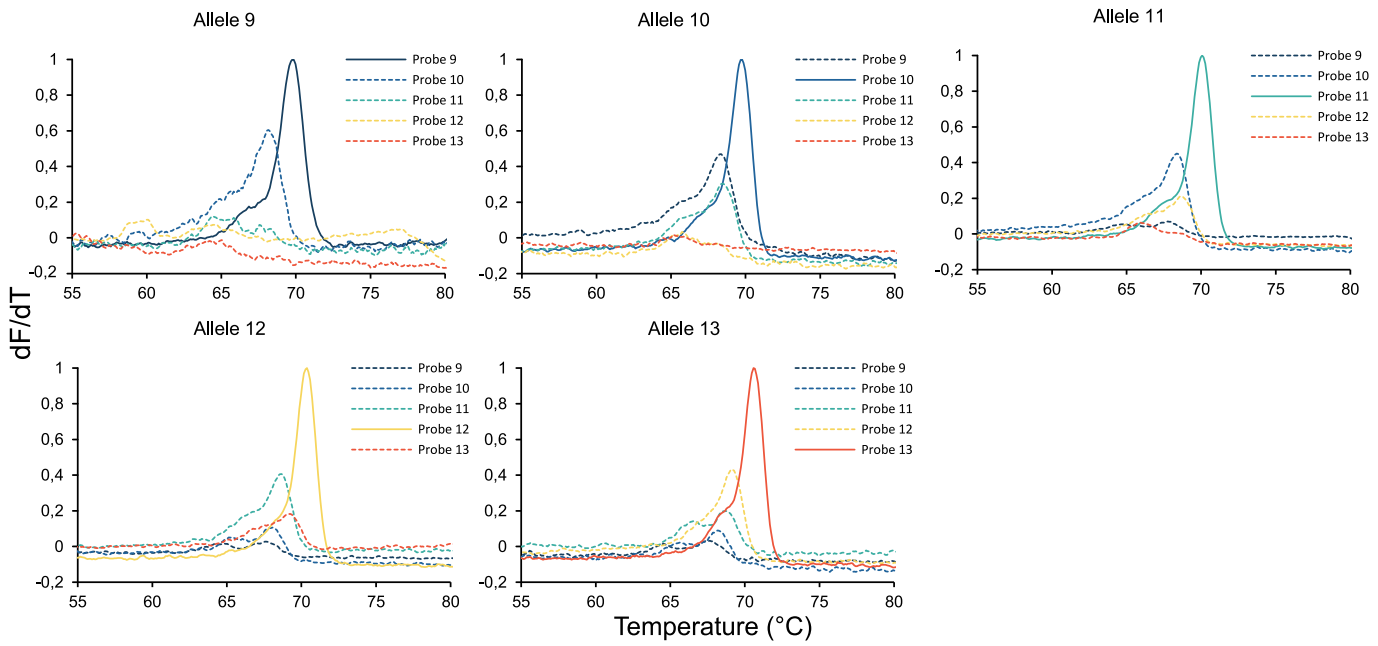

Fig. 2. Locus D16S539 melting profiles. Locus D16S539 melting profiles obtained for five different synthetic DNA samples (alleles 9–13), as indicated above each graph. Melting curves of matching probes are represented as full lines, mismatch probes are represented as dotted lines.

to D16S539, STRide probes were designed for the CODIS core loci TPOX and D7S820. Fig. 3 shows the D16S539 melting profiles for all three reference control DNA samples, Supplementary Fig. S1 shows the D16S539 melting profiles for samples 1–13. The melting peaks shown in these figures are not normalized. For all samples, all probes corresponding to the true allele(s) of the sample result in the highest melting peak(s) and occur at a higher temperature when compared to the mismatch probes. For heterozygous samples, a pronounced melting peak can be observed for both matching probes. As expected, these peaks are about half the height of the matching peak observed in a homozygous sample. Although some allelic imbalance might occur in heterozygous samples, the smallest peak of both true alleles was not lower than 50% of the highest peak in the analyzed samples. Both melting peaks do not necessarily occur at the same temperature, as the probes differ in length.

In some instances, data interpretation is less straightforward. First, SNPs in the sample might influence the melting temperature. The matching peaks for reference sample 2800M (9 and 13) occur in the reversed order as would be expected, which might be explained by the presence of a SNP in the flanking region of allele 13, positioned across the 5′ end of the STRide probe. The presence of this SNP was revealed by in-house sequencing of sample 2800M using the ForenSeq DNA Signature Prep Kit (Verogen Inc., San Diego, CA, USA), according to the manufacturer's instructions. SNPs in the sensor region are more cumbersome, as these will lower the hybridization efficiency of the sensor, and thus lower the peak intensity. Therefore, the sensor region should be designed as short as possible. Second, for some genotypes, the non-specific melting peak might be rather high. For buccal swab N° 3 (alleles 11 and 13), the melting peak of probe 12 is relatively high, as this probe only differs one repeat from both true alleles. This peak occurs at a lower temperature than both matching peaks, indicating the absence of allele 12. For such samples, where peak intensity does not unambiguously reveal the true genotype, other peak parameters aid in data interpretation, being the peak shape and the melting temperature. Third, PCR artefacts can be present in the sample. A minor peak at a higher temperature in the melting curves of probe 10 reveals the presence of stutter artefacts in reference sample 9948. Although these factors might complicate data interpretation, manual data interpretation for single-contributor reference samples remains feasible. Automated interpretation of melting curves, e.g. through machine learning, has already been performed for other applications (Athamanolap et al., 2014), and will be the subject of future research.

The melting profiles for the TPOX and D7S820 loci are also shown in Fig. 3 for the reference samples, and in Supplementary Figs. S2 and S3 for samples 1–13. As opposed to the D16S539 locus, barely any signal can be observed for the mismatch probes in these loci. This discrepancy between loci can be explained by multiple factors influencing the extent of destabilization that a mismatch causes. Examples are differences in length of the sensor region, and differences in GC content of both the repeat unit and the sensor region. Besides that, the number of guanines present in the sample close to the hybridization position of the fluorophore impacts the efficiency of quenching. Notably, the complement of the sensor region of the D7S820 probe contains only one guanine, whereas for all other loci at least two guanine residues are present. Nevertheless, the quenching effect of the single guanine is still sufficient to enable correct genotyping. However, a more extreme allelic imbalance is observed for this locus, e.g. reference sample 2800M. Taken together, these results strongly suggest that STRide probes are sufficiently robust to enable correct STR allele calling even in the presence of PCR artefacts that often arise when amplifying STRs.

3.4. Genotyping of an STR locus characterized by a partial repeat

A subset of STR loci are characterized by imperfect repeats (Butler, 2005). Single-nucleotide deletions in a single repeat of an allele present a particularly challenging problem to CE-based genotyping, since correct allele calling then requires amplicon sizing with very high (≤1 bp) resolution. To evaluate the STRide probes' ability to cope with partial repeats, we designed probes for locus TH01. Fig. 4 shows the melting profiles for locus TH01 for the three reference samples, Supplementary Fig. S4 displays the obtained melting profiles for samples 1–13. A commonly occurring allele of locus TH01 within the population is allele 9.3. This allele consists of 10 repeats, of which the 4th repeat is characterized by a one-nucleotide deletion. Melting profiles of samples containing allele 9.3, e.g. all three reference samples, show characteristic melting peaks with high intensity and high melting temperatures corresponding to the true alleles. As expected, a relatively high peak is also observed for probe 10, albeit at a lower temperature. These results suggest that STRide probes allow STR profiling also in the event of

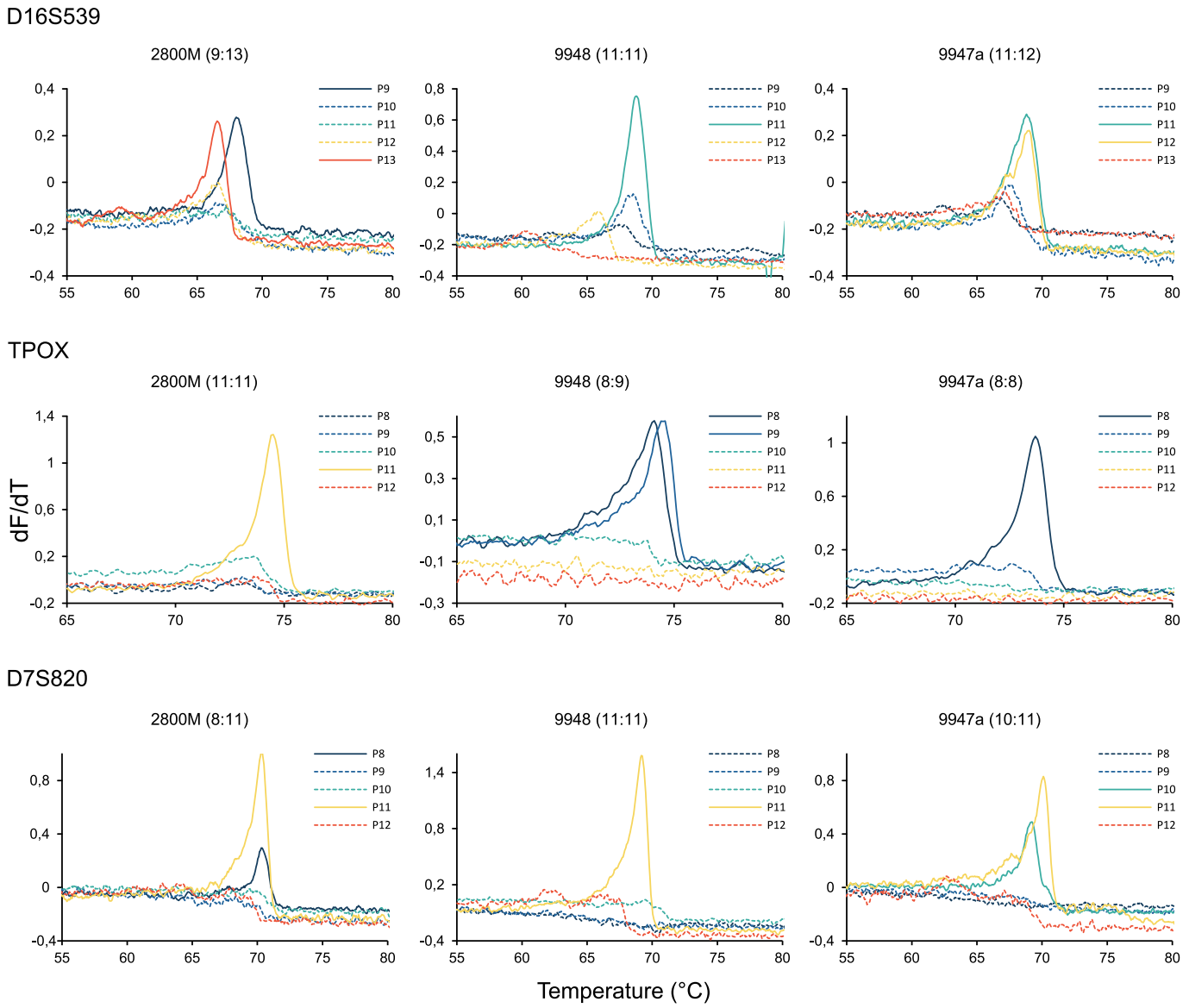

Fig. 3. Locus D16S539, TPOX, and D7S820 melting profiles. Locus D16S539, TPOX, and D7S820 melting profiles obtained for three reference DNA samples 2800M, 9948 and 9947a. Sample names are indicated above each graph, with the true genotype between parentheses. Melting curves of matching probes are represented as full lines, those of mismatch probes are represented as dotted lines.

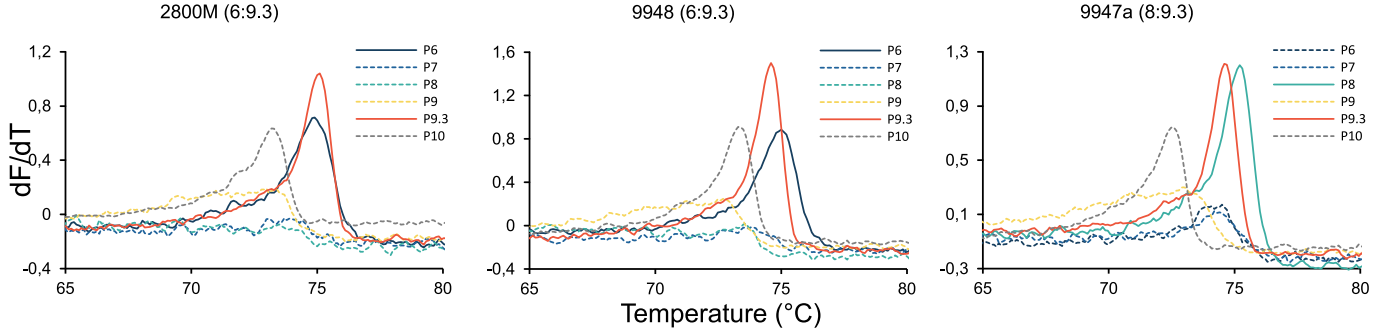

Fig. 4. Locus TH01 melting profiles. Locus TH01 melting profiles obtained for all three reference DNA samples 2800M, 9948 and 9947a. Sample names are indicated above each graph, with the true genotype between parentheses. Melting curves of matching probes are represented as full lines, those of mismatch probes are represented as dotted lines.

partial repeats.

3.5. Genotyping of the highly complex FGA locus

STR loci vary substantially in complexity, both in repeat number and in similarity between repeat region sequence and flanking sequence (Gettings et al., 2015). In this respect, the loci analyzed above (D16S539, TPOX, D7S820 and TH01), while highly relevant for human identification purposes, are of relatively low complexity. In contrast, the FGA locus is one of the most complex STR loci of the CODIS core loci. This locus is not only characterized by a high repeat number, necessitating the use of long probes, but also by a high similarity between the repeat region sequence and the flanking sequence directly next to the repeat region. Consequently, we selected the FGA locus to assess the ability of STRide probes to perform STR profiling of complex loci. STRide probes were designed and evaluated using reference and buccal samples as input for PCR amplification. Although both the high repeat number and the sequence similarity could potentially hamper proper hybridization, successful genotyping could be obtained as shown in Fig. 5 for the reference samples, and Supplementary Fig. S5 for samples 1–13. These results suggest that STRide probes can be used for STR profiling of highly complex genetic loci.

3.6. Sensitivity, specificity, and repeatability assessment

To assess the sensitivity of the developed assay, a dilution series of reference DNA 9947a was genotyped for the TH01 locus. The input for asymmetric PCR ranged from 500 pg to 31 pg DNA. The obtained melting profiles are shown in Supplementary Fig. S6. Successful genotyping was obtained using an input of down to 31 pg of DNA for asymmetric PCR, which is comparable with the sensitivity of commercially available STR kits for CE analysis. These results therefore suggest a high sensitivity for the STRide probes.

To gain insight into the repeatability of the STRide probe assay, sample 13 was analyzed in triplicate, on three different days, using two different LightCycler 480 devices. The obtained results, shown in Supplementary Fig. S7, show reproducible melting profiles. The minor variation in the data results from differences in asymmetric PCR efficiency, and variations in the pipetting steps, which might lead to slightly differing salt concentrations.

The STRide probes should only interact with the amplicons obtained for the corresponding specific locus. Possible interaction with other amplicons present in the sample should not result in any signal upon melting curve analysis. To assess probe specificity, all STRide probes were separately added to mixtures of PCR product obtained from sample 13. These PCR mixtures contain the amplicons from all loci discussed in this manuscript, except the locus corresponding to the investigated STRide probe. The obtained melting curves, shown in Supplementary Fig. S8, show that no non-specific signal is obtained for any of the STRide probes, except a minor peak for the TH01 locus at a low temperature, which does not affect genotyping. This demonstrates a high specificity of the developed assay.

3.7. Comparison to state-of-the-art and future perspectives

Although this research shows that STRide probes are a promising STR profiling method for implementation in a portable device, genotyping all commonly typed STR loci promises to be challenging. Some forensic STR loci, e.g. SE33, are characterized by very high repeat numbers. Longer probes will inevitably result in a smaller destabilizing effect of a mismatch, thereby hampering correct genotyping. Design adaptations, e.g. the introduction of modified nucleotides, might overcome this issue. Moreover, repeat sequence heterogeneity complicates hybridization-based genotyping. For short STR loci, a SNP in the repeat region can potentially provide an extra source of information using STRide probes, as the destabilizing effect of a single-nucleotide mismatch might be observed. For longer loci, this effect may be less pronounced and could hinder straightforward data interpretation. Incorporating universal nucleotides in the STRide probe at a polymorphous position can potentially remediate this issue. Although some challenges remain to be tackled, it seems feasible that STRide probes allow accurate genotyping of most of the original 13 CODIS core-loci, providing prospects for the development of a panel with sufficient discriminatory value, while still being compatible with the currently used databases.

There is a clear need for portable, fast, and particularly flexible solutions to be added to the toolbox of forensic DNA genotyping labs. These could be used at the crime scene to provide fast answers to the investigational team. Moreover, fast identification of a detained suspect could be realized at the police station. Currently, when law enforcement services need fast and reliable answers, urgent samples are prioritized in the forensic laboratory. This disturbs the usual workflow and batching procedure, as CE analysis is most profitable when a 96-wellplate is entirely utilized. STRide probes were designed to be implemented in a portable device as a microarray, which is a surface covered with more than 100 spots, each containing immobilized STRide probes corresponding to a specific STR allele. The development of a chip capable of amplifying and genotyping a sample is the subject of ongoing research. After on-chip amplification, the amplicons will flow towards the microarray cavity, where controlled hybridization and melting curve analysis will be performed. Such a cheap, disposable chip capable of accurately genotyping most of the commonly used forensic STR loci, would rapidly offer sufficient information for an important subset of forensic samples, and thus be a valuable technology complementary to the existing next-generation sequencing and CE technologies.

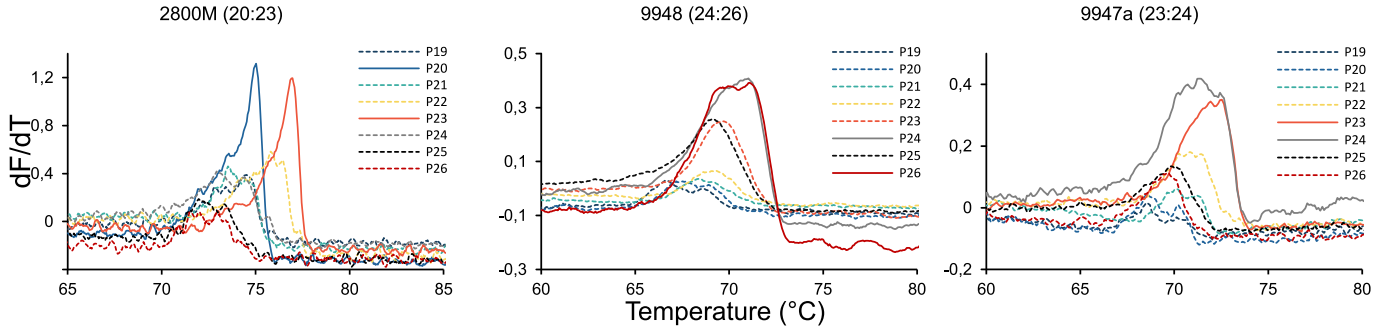

Fig. 5. Locus FGA melting profiles. Locus FGA melting profiles obtained for all three reference DNA samples 2800M, 9948 and 9947a. Sample names are indicated above each graph, with the true genotype between parentheses. Melting curves of matching probes are represented as full lines, those of mismatch probes are represented as dotted lines.

4. Conclusion

A novel hybridization-based STR-genotyping method was developed and validated for five different STR loci. STRide probes, labeled with a single fluorescein derivative, rely on the quenching properties of guanine on these fluorophores. After incubation of these probes with asymmetrically amplified samples, melting curve analysis yields reproducible melting profiles, providing information on the alleles present in the sample. Successful genotyping was obtained for all investigated samples. Moreover, low-input samples could still reliably be genotyped. The TH01 9.3 allele could be distinguished from allele 10, although both alleles only differ one nucleotide in length. STRide probes are ideally suited to be implemented in a portable device, such as a microarray. This is an important step towards fast, portable forensic DNA genotyping.

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CRediT authorship contribution statement

Olivier Tytgat: Writing – original draft. **Maarten Fauvart:** Writing – review & editing, Supervision. **Tim Stakenborg:** Writing – review & editing, Supervision. **Dieter Deforce:** Conceptualization, Writing – review & editing, Supervision. **Filip Van Nieuwerburgh:** Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: O.T., D.D., and F.V.N. are inventors on a pending patent application disclosing the STR-probes described in this publication (PCT/EP2020/076410).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2021.113135.

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