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Reduction of stutter ratios in short tandem repeat loci typing of low copy number DNA samples

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ABSTRACT

Increased height of stutter peaks is a phenomenon with low copy number (LCN) short tandem repeat (STR) typing that can impact interpretation. An alternative strategy of lowering the annealing/extension temperature (LT) at 56 °C was designed to attempt to decrease the heights of stutter peaks. STR typing results were generated in terms of stutter ratios using LT-PCR conditions and compared with data obtained using standard (STD) PCR conditions. DNA samples ranging from 100 to 25 pg were amplified using reagents contained in the AmpFℓSTR® Identifiler® PCR Amplification or AmpFℓSTR® Identifiler® Plus PCR Amplification kits with 32 or 34 PCR cycles. Stutter ratios decreased by an average of 14.7%, 14.9% and 18.1% at 100, 50 and 25 pg of template DNA under LT conditions compared with those of STD conditions in the Identifiler® Kit amplified samples. The LT conditions also decreased average stutter ratios by 13.3% compared with those of STD conditions in the Identifiler® Plus Kit amplified samples. The overall PCR efficiency obtained with STD and LT conditions with the two STR kits was comparable in terms of the number of detected alleles, peak heights and peak height ratios. These results support the hypothesis that a lower temperature annealing/extension step reduces the likelihood of slippage during PCR by enhancing the stability of the DNA polymerase/template DNA complex or the stability of the generated duplex than the conditions of the standard extension step. This stability in turn would result in lower stutter ratios.

1. Introduction

Typing of short tandem repeat (STR) loci is the primary technique for human identification because of its high discrimination power and ability to analyze challenged samples. Forensic biological evidence often consists of mixtures which at times are particularly difficult to deconvolve into individual STR profiles due to additive effects, allele masking, and allele drop out. Allele drop out is encountered routinely when small amounts of DNA from any of the contributors are amplified during PCR. In some situations, methods of increasing PCR product yield are attempted to obtain more STR allele data. An increase of the number of PCR cycles is one of various approaches to increase the sensitivity of detection [1,2]. However, stochastic problems, e.g., heterozygous peak height imbalance, can occur. In addition, increased height of stutter peaks can be a problem impacting interpretation, thus complicating determination of true allelic peaks in low copy number (LCN) STR typing.

The stutter phenomenon is caused by slippage that can occur when the DNA polymerase pauses during primer extension [3,4]. Stutter peaks are mostly one repeat unit shorter in length than true allelic peaks, although stutter can occur at lower levels in other positions as well. Most stutter ratios (stutter:true allele height proportions) are below 15% in commercially available STR kits (e.g., AmpFℓSTR® SGM Plus®, Identifiler® and Identifiler® Plus PCR Amplification kits, and PowerPlex® 16 System) under standard PCR conditions using approximately 1 ng of template DNA and 28 PCR cycles [5–8]. Generally, true alleles and stutter peaks are distinguished by approximately <15% stutter ratio values of calling stutter peaks (but the assessment of a peak as stutter can be more difficult when there are alleles from a minor contributor(s) at or below the peak heights of a stutter threshold).

For LCN typing, however, stutter peaks with increased height can be observed as the number of PCR cycles increases. Particularly for mixed LCN DNA samples, higher stutter peaks can complicate interpretation and possibly lead to incorrect interpretation of STR results. Higher stutter peaks may be designated as true alleles and types could be called as heterozygous genotypes. Petricevic et al. [9] observed a larger stutter ratio range in LCN typing with increased PCR cycles, with a maximum of 57% for a stutter ratio.

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They reported that to capture 99% of potential stutter, a stutter interpretation value of 25% should be set for their LCN typing methodology. However, the higher stutter threshold value could result in misassignment of a true allele as stutter; true alleles at heterozygous genotypes are often observed at <25% of peak height ratios in LCN typing. Thus, higher stutter peaks present a challenge for LCN typing.

Stutter ratios tend to increase as the number of uninterrupted core repeat units of an allele increases [10], and repeat units with a high A–T content exhibit increased stutter ratios [4,11]. Higher stutter ratios were shown at the FGA (19.94%) and D18S51 (17.96%) loci with the Identifiler® Plus Kit under standard PCR conditions [12], which is indicative that stutter ratios may increase at these loci as the number of PCR cycles increases in LCN typing. In addition, alleles with lower peak heights had proportionally higher stutters [9]. According to these studies, various factors can affect the yield of stutter peaks.

Decreasing heights of stutter peaks could improve the interpretation of both single source and mixture profiles in LCN typing. Walsh et al. [10] reported that polymerases with high processivity of 50–60 bases may reduce stutter ratios in comparison with a polymerase with low processivity of 5–10 bases. Viguera et al. [13] demonstrated that polymerases with high strand-displacement activity may reduce stutter formation by experiments using a primer extension assay on a single strand DNA template. Most current commercial STR kits already use the AmpliTaq Gold polymerase with 50–60 bases of processivity, and most polymerases with high strand-displacement activity, e.g., phi29 DNA polymerase, are not compatible with the thermal cycling conditions of PCR due to being thermolabile.

We hypothesized that a lower annealing/extension temperature (LT) could reduce yield of stutters using commercial kit reagents. In this study, STR typing results are described in terms of stutter ratios using LT-PCR conditions in comparison with those obtained using standard (STD) PCR conditions (both increased to 32 cycles).

2. Materials and methods

2.1. Sample preparation

Thirteen DNA samples were used for this study. All samples were collected with informed consent and were anonymized to ensure the privacy of the contributing subjects in accordance with University of North Texas Health Science Center IRB. The DNA quantity of the samples was estimated using the Quantifiler® Human DNA Quantification Kit (Life Technologies, Foster City, CA) on an ABI Prism® 7500 Sequence Detection System (Life Technologies) following the manufacturer's protocols [14]. The quantified DNA samples were diluted serially in Tris EDTA buffer (10 mM Tris, 0.1 mM EDTA (TE-4), pH 8.0).

2.2. STR amplification and typing

For STR amplification, 100 pg, 50 pg and 25 pg of genomic DNA were used for each of the 13 samples. PCRs were prepared using the AmpFℓSTR® Identifiler® PCR Amplification Kit (Life Technologies) following the manufacturer's protocols [6]. STD-PCR conditions (except for number of cycles) for the Identifiler® Kit were 11 min at 95 °C for initial incubation, 32 cycles of 1 min at 94 °C for denaturation, 1 min at 59 °C for annealing and 1 min at 72 °C for extension, and 60 min at 60 °C for final extension. LT-PCR conditions were 11 min at 95 °C for initial incubation, 32 cycles of 20 s at 94 °C for denaturation and 3 min at 56 °C for annealing/extension, and 60 min at 56 °C for final extension. For the tests, 13 samples were amplified twice at all DNA quantity levels. Thermal cycling was performed using the GeneAmp® PCR system 9700 (Life Technologies). Typing of PCR products was carried out on a 3500xl Genetic Analyzer (Life Technologies), and the data were analyzed using Genemapper® ID-X (Life Technologies).

Additional tests were performed to determine whether the LT-PCR conditions can reduce stutter ratios using another STR typing kit. For the tests, 13 samples of approximately 25 pg of template DNA were amplified twice using the Identifiler® Plus Kit (Life Technologies). STD-PCR conditions for the Identifiler® Plus Kit were 11 min at 95 °C for initial incubation, 34 cycles of 20 s at 94 °C for denaturation and 3 min at 59 °C for annealing/extension, and 10 min at 60 °C for final extension. LT-PCR conditions were 11 min at 95 °C for initial incubation, 34 cycles of 20 s at 94 °C for denaturation and 3 min at 56 °C for annealing/extension, and 10 min at 56 °C for final extension.

2.3. Data interpretation

The data from the STR results were analyzed with minimum allele peak thresholds of 100 relative fluorescence units (RFU). Stutter peak (−4 bp) height ratios were calculated by dividing peak height of stutters by that of parent alleles. The stutters of ≥25 RFU were used for calculation of stutter ratio. The average peak heights were calculated by dividing sum of heights of the homozygous and heterozygous peaks by the number of detected alleles. The peak height ratios at heterozygote loci were calculated by dividing heights of lower peaks by heights of higher peaks. The homozygous peak was counted as one allele (since the same samples were used in both conditions). At heterozygous genotypes with extreme peak height imbalance (e.g., <15% by dividing one allele by the other allele), only the allele showing higher peak height was called and compared. An unpaired two tail *t*-test was used to determine whether stutter ratios were different between STD and LT conditions at a significance level of 0.05.

3. Results

3.1. Stutter ratios

Three quantities of DNA, 100 pg, 50 pg, and 25 pg, were analyzed under STD and LT conditions with LCN typing conditions of 32 cycles during PCR. The average stutter ratios per locus with the Identifiler® Kit were presented in Fig. 1a–c. For 100 pg of template DNA (Fig. 1a), the trend was that average stutter across the samples was reduced under the LT conditions (5.7 ± 3.1% (standard deviation: SD)) compared with STD conditions (6.6 ± 4.0% (SD)). Of the 15 STR loci, 13 loci showed lower average stutter ratios with LT conditions than with STD conditions. The loci CSF1PO and D13S317 showed higher average stutter ratios with LT conditions. Each locus generally showed similarly high and low stutter ratios across the different PCR conditions. Average stutter ratios decreased by 28.2% at FGA, 5.8% at D19S433, 19.1% at D18S51 and 16.2% at D2S1338 with LT conditions for the loci with on average higher stutter ratios (i.e., >8.0% chosen for demonstration purposes only) under STD conditions. Maximum reduction percentages of average stutter ratios were 28.2% at FGA, 27.6% at TPOX, 22.0% at D7S820 and 19.1% at D18S51 under LT conditions. With the *t*-test comparing the totality of the stutter data for the two PCR conditions, the average stutter ratio of LT conditions was significantly lower than that of STD conditions (p-value = 1.15×10^{-5}).

For 50 pg of template DNA (Fig. 1b), average stutter ratios were 6.2 ± 4.4% (SD) and 7.2 ± 4.4% (SD) with the LT and STD conditions, respectively. Thirteen loci showed lower average stutter ratios under the LT conditions compared with that of the STD conditions. The loci D2S1338 and TPOX showed higher average stutter ratios with the LT conditions. Average stutter ratios decreased by 4.5% at D19S433,

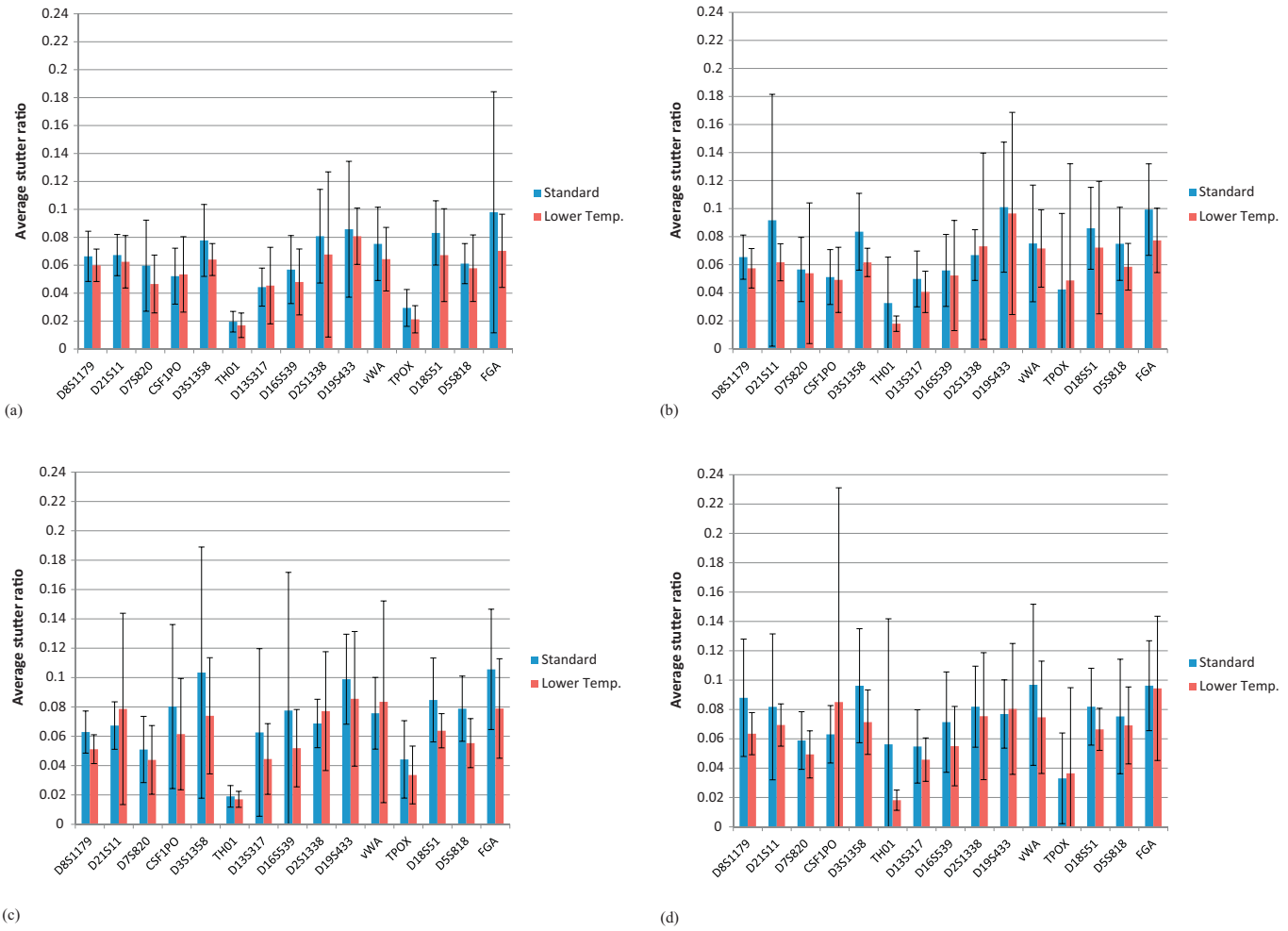

Fig. 1. Average stutter ratios in (a) Identifiler® Kit reactions with 100 pg of template DNA and 32 PCR cycles, (b) Identifiler® Kit reactions with 50 pg of template DNA and 32 PCR cycles, (c) Identifiler® Kit reactions with 25 pg of template DNA and 32 PCR cycles and (d) Identifiler® Plus Kit reactions with 25 pg of template DNA and 34 PCR cycles. Bar stands for standard deviation.

22.1% at FGA, 32.7% at D21S11, 16.0% at D18S51 and 26.2% at D3S1358 under the LT conditions for the loci with on average higher stutter ratios (i.e., >8.0%) under STD conditions. Maximum reduction of average stutter ratios was 45.0% at TH01, 32.7% at D21S11 and 26.2% at D3S1358 loci with LT conditions. Based on the *t*-test, the average stutter ratio of LT conditions was significantly lower than that of STD conditions (p-value = 3.64×10^{-4}).

For 25 pg of template DNA (Fig. 1c), average stutter ratios were $6.3 \pm 4.2\%$ (SD) and $7.7 \pm 5.0\%$ (SD) with LT and STD conditions, respectively. Twelve loci displayed lower average stutter ratios under the LT conditions compared with the STD conditions. The loci D21S11, D2S1338 and vWA showed higher average stutter ratios with the LT conditions. Average stutter ratios decreased by 25.3% at FGA, 28.5% at D3S1358, 13.5% at D19S433, 24.7% at D18S51 and 23.4% at CSF1PO with LT conditions for the loci with on average higher stutter ratios (i.e., >8.0%) under STD conditions. Maximum reduction of average stutter ratios was 33.1% at D16S539, 29.8% at D5S818, 28.9% at D13S317 and 28.5% at D3S1358 loci under LT conditions. Based on the *t*-test, the average stutter ratio of LT conditions was significantly lower than that of STD condition (p-value = 2.05×10^{-4}). These overall results support that the LT-PCR conditions can reduce stutter ratios in LCN typing.

The LT conditions also were tested using the Identifiler® Plus Kit to determine whether stutter ratios can be reduced with a LCN typing approach. With the Identifiler® Plus Kit and 34 PCR cycles, average stutter ratios were lower under LT conditions than under STD conditions. The average stutter ratios were $6.5 \pm 5.1\%$ (SD) and $7.5 \pm 4.2\%$ (SD) with LT and STD conditions, respectively (Fig. 1d). Based on a *t*-test, the average stutter ratio of LT conditions was significantly lower than that of STD conditions (p-value = 0.011). Of 15 STR loci, 12 loci showed average lower stutter ratios with LT conditions than with STD conditions (Fig. 2d). The loci CSF1PO, D19S433 and TPOX showed higher average stutter ratios under LT conditions. Seven loci displayed average stutter ratios > 8.0% (i.e., vWA, FGA, D3S1358, D8S1179, D2S1338, D18S51 and D21S11) in the STD conditions but only 3 loci (i.e., FGA, CSF1PO and D19S433) had high ratios (>8.0%) with LT conditions. There were 13 and 9 stutter peaks exceeding a 15% threshold with STD and LT conditions, respectively (data not shown).

3.2. Higher stutter ratios

Stutter peaks exceeding a 15% threshold were examined (Table 1). There were 11 (stutter ratio ranges: 15.6–63.8%) and 8 (15.1–42.8%) stutter peaks with 100 pg of template DNA under the STD and LT conditions, respectively exceeding the threshold. At 50 pg of template DNA, 14 (15.3–56.8%) and 8 (16.1–44.9%) stutters above the threshold were observed with the STD and LT conditions, respectively. At 25 pg of template DNA, 12 (15.3–51.4%) and 9 (15.1–39.3%) increased stutters observed with the STD and LT conditions, respectively. These results showed that the number of stutters with higher proportion was fewer with the LT

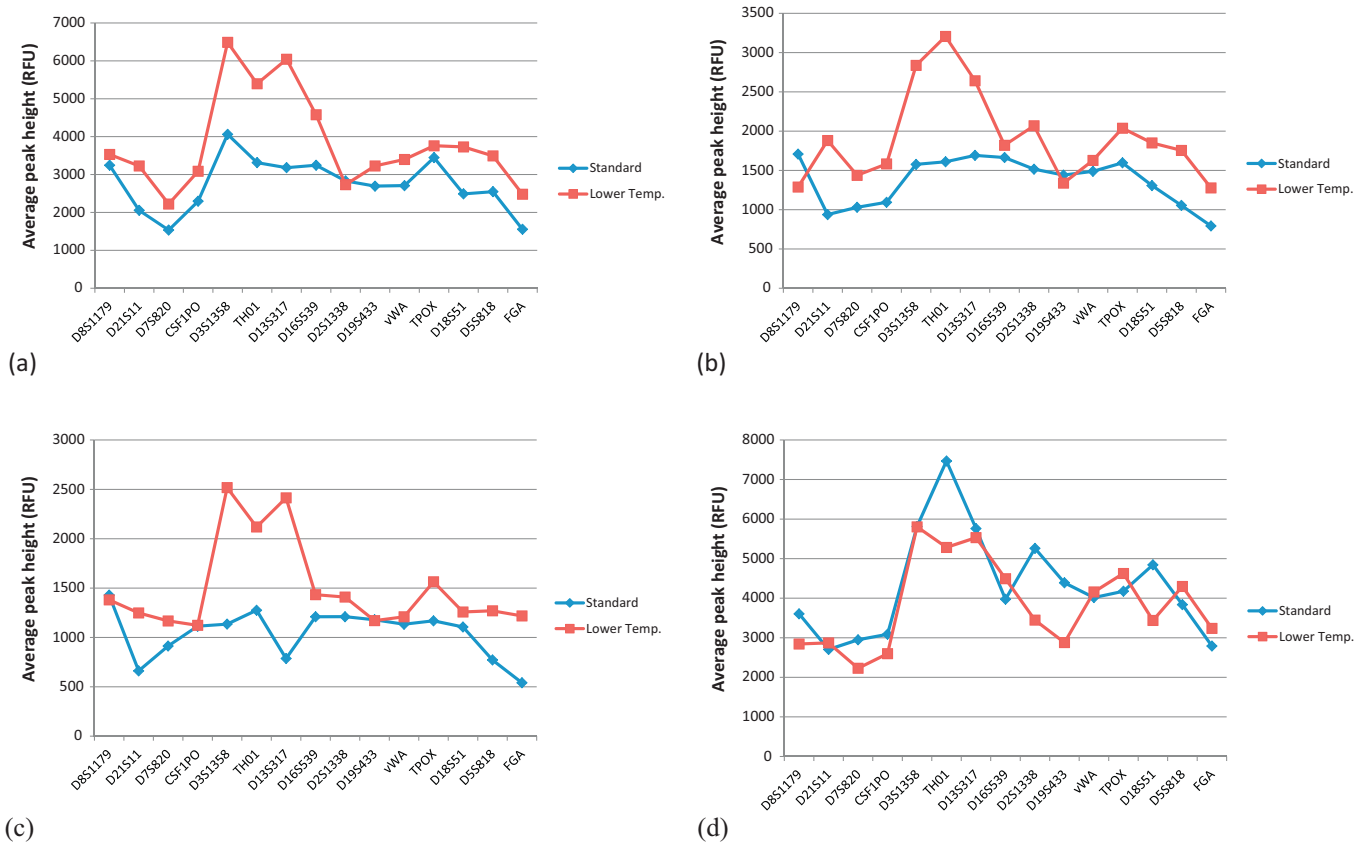

Fig. 2. Average peak height in (a) Identifiler® Kit reactions with 100 pg of template DNA and 32 PCR cycles, (b) Identifiler® Kit reactions with 50 pg of template DNA and 32 PCR cycles, (c) Identifiler® Kit reactions with 25 pg of template DNA and 32 PCR cycles and (d) Identifiler® Plus Kit reactions with 25 pg of template DNA and 34 PCR cycles.

conditions than in the STD conditions at all three quantities of DNA.

For STD conditions, high stutter peaks were observed at least once in 12 loci, except for the D8S1179, D7S820 and TH01 loci, at every DNA concentration level, and frequently observed at the D19S433 (8 occurrences) and FGA (8 occurrences) loci. For LT conditions, high stutter peaks were observed at least once in 13 loci, except for the D8S1179 and TH01 loci, and more frequently observed at the D16S539 (4 occurrences) and D2S1338 (4 occurrences) loci.

The possibility was examined if the occurrence of higher stutter peaks may be related to average stutter ratios of loci. At all DNA concentration levels and both PCR conditions, the D8S1179 locus showed higher average stutter ratios than the TPOX locus. However, the D8S1179 locus did not show high stutter peaks whereas the TPOX locus displayed high stutter peaks (31.1% in STD condition and 43.0% in LT conditions). These results suggested that occurrence of high stutter peaks may not be related to average stutter ratios, but may occur due to stochastic effects.

Table 1
Higher stutter peak of >15% of stutter ratios.

	The number of alleles with higher stutter ratios					
Marker	100 pg DNA		50 pg DNA		25 pg DNA	
	STD	LT	STD	LT	STD	LT
D8S1179						
D21S11		1 (15.4%)	2 (21.0, 56.8%)			1 (38.2%)
D7S820				1 (25.6%)		
CSF1PO					1 (26.9%)	1 (18.4%)
D3S1358	1 (18.4%)		1 (15.3%)		2 (17.5, 47.4%)	1 (21.8%)
TH01						
D13S317		1 (16.8%)			1 (25.8%)	
D16S539	1 (15.8%)	1 (15.2%)		2 (18.0%, 21.1%)	1 (51.4%)	1 (15.4%)
D2S1338	3 (15.6, 18.3, 18.7%)	2 (15.1, 42.8%)		1 (43.8%)		1 (20.9%)
D19S433	1 (35.3%)		5 (15.6, 16.7, 17.1, 17.2, 29.5%)	2 (16.1%, 44.9%)	2 (15.3, 19.0%)	1 (29.0%)
vWA	1 (17.1%)		1 (28.1%)			1 (39.3%)
TPOX			1 (31.1%)	1 (43.0%)		
D18S51	1 (15.7%)	2 (15.7, 22.5%)	2 (15.5, 16.8%)	1 (31.7%)	1 (16.4%)	
D5S818		1 (16.1%)	1 (15.6%)			
FGA	3 (15.6, 17.4, 63.8%)		1 (23.9%)		4 (15.8, 16.0, 19.3, 20.3%)	2 (15.1, 18.7%)

3.3. PCR efficiency under two different PCR conditions

STR typing results with the different PCR conditions using the Identifiler® Kit were analyzed. At 100–25 pg of template DNA, the rates of detected alleles were similar between STD and LT conditions. The percentage of detected alleles was 94.6% and 94.9% at 100 pg of template DNA, 76.6% and 76.1% at 50 pg of template DNA, and 51.6% and 53.4% at 25 pg of template DNA with STD and LT conditions, respectively.

The average peak heights were higher under LT conditions than under STD conditions at all DNA quantity levels with the Identifiler® Kit (Fig. 2a–c). When using LT conditions, the average peak heights increased on average 39.3%, 39.7% and 44.0% at 100 pg, 50 pg and 25 pg of template DNA, respectively. The average peak heights were higher with LT conditions than STD conditions at 14 loci except for the D2S1338 locus at 100 pg of template DNA, at 13 loci except for the D8S1179 and D19S433 loci at 50 pg of template DNA, and at 13 loci except for the D8S1179 and D19S433 loci at 25 pg of template DNA. The average peak height ratios at heterozygous genotypes were 58.0% and 57.3% at 100 pg of template DNA, 61.3% and 57.7% at 50 pg of template DNA, and 63.5% and 61.1% at 25 pg of template DNA under STD and LT conditions, respectively.

Efficiency of STR typing with the different PCR conditions using the Identifiler® Plus Kit also was analyzed. The percentage of detected alleles was higher with LT conditions (46.5%) than with STD conditions (42.1%). The average peak height ratios were 62.5% with STD conditions and 64.8% with LT conditions. In contrast with results obtained with the Identifiler® Kit, the average peak height was 12.0% higher with STD conditions than with LT conditions.

In totality, LT conditions were more effective in reducing stutter ratios than those of STD conditions with both kits and increased PCR cycles. There were no noticeable differences in STR typing efficiency in terms of detected alleles, and average peak height ratios and only slight differences in average peak heights. These results indicated that a lower temperature annealing/extension step may reduce stutter ratios under increased sensitivity of detection typing conditions.

4. Discussion

The use of a lower extension temperature was hypothesized to reduce stutter as it would create a lower stringency environment driving the two DNA strands to be physically closer which in turn would reduce slippage. A lower extension temperature has been shown to be useful for amplification of A/T-rich regions of DNA [15]. Hite et al. [16] reported that a low extension temperature (i.e., 37 °C) used with thermolabile polymerases can decrease the likelihood of dissociation of the 3′ end of the nascent strand, thereby reducing the occurrence of slipped-strand mispairings. Fazekas et al. [17] used decreasing extension temperatures to reduce DNA melting and increase binding affinity of Taq DNA polymerase to mitigate the generation of slipped-strand products.

Given the above studies a lower temperature annealing/extension step may reduce the likelihood of dissociation of polymerase on template DNA during PCR by enhancing the stability of the DNA polymerase/template DNA complex than the standard extension step. This stability in turn would result in lower stutter ratios. For LT conditions, a two-step PCR protocol that combines annealing and extension steps was used under the assumption that the DNA polymerase may be more stable under the condition of no temperature change between annealing and extension steps than under the condition of changing temperatures for the extension step of DNA polymerase. Initially, in our study the annealing/extension temperature that would be effective and not create additional artifacts was determined to be 56 °C. The total annealing and extension time was increased with LT conditions (i.e., 3 min) than with STD conditions (i.e., 2 min) in the Identifiler® Kit test comparisons. While the data appear to support that LT conditions reduce stutter yield, the amplification conditions with Identifiler® Kit study were slightly different; thus one also must entertain the possibility that the LT and annealing and extension time together contributed to the reduced stutter. However, for the Identifiler® Plus Kit data the only difference between the comparisons was the annealing/extension temperature and stutter was reduced under LT conditions. Therefore, the data support that LT conditions were the primary factor in the observed stutter reduction.

The experimental results supported that a lower temperature annealing/extension step can be effective as LT conditions decreased average stutter ratios at all three quantities of template DNA with both kits. The overall PCR efficiency obtained with STD and LT conditions with different two STR kits was comparable in terms of the number of detected alleles, peak heights and peak height ratios. Thus, the altered conditions did not compromise the efficacy of STR typing. The reduced annealing temperature used in the study herein was not predicted to affect reliability of STR typing as variation of PCR thermal conditions is performed under most validation studies often with no deleterious effects observed.

Average stutter ratios increased as the amounts of template DNA decreased from 100 pg to 25 pg for both PCR conditions (i.e., 6.6%, 7.2% and 7.7% in the STD conditions, and 5.7%, 6.2% and 6.3% in the LT conditions, respectively). However, the average stutter ratio with LT conditions at 25 pg of template DNA still was lower than STD conditions at 100 pg of template DNA. These comparison results also supported that LT conditions can generate lower stutter ratios than STD conditions with LCN typing.

Stutters are generated by slippage during primer extension and are a consequence of the presence of repeated sequences [3,4]. Viguera E et al. [13] reported that a polymerase with high strand displacement activity, such as phi 29 polymerase, can proceed through a hairpin and not pause or contribute to slippage. Although phi 29 polymerase may be effective in this aspect, the application under the thermal conditions of PCR is not practical [17]; the polymerase is active at 20–37 °C. Bst DNA Polymerase has strand displacing activity; but it also cannot be applied to the thermal conditions of PCR [18]. The Deep Vent$_R$™ DNA polymerases (both wild and exo-types, New England Biolabs) have relatively high stand displacement activity. They are compatible with the thermal conditions of PCR. In preliminary tests, these polymerases were tested with the Identifiler® Kit reagents to reduce stutter ratios. However, the Deep Vent$_R$ DNA polymerases were not compatible with the Identifiler® Kit reagent formulation, showing substantial allele drop-out (data not shown). In addition, a thermostable DNA polymerase with high processivity has been predicted to reduce stutter in the amplification of repeat sequences [10]. For example, amplification using the Stoffel fragment of Taq polymerase (a processivity of ~5–10 bases) increased stutter ratios over that seen with AmpliTaq DNA polymerase (the processivity of Taq is 50–60 bases) since the lower processivity would allow more opportunity for increased breathing of the DNA strands during PCR. To test whether polymerases with higher processivity could reduce stutter ratios, a polymerase with very high processivity, i.e., Kod Xtreme™ Hot Start Polymerase (Novagen; processivity of 120 bases) was preliminarily evaluated. However, the Kod Xtreme™ Hot Start Polymerase did not decrease stutter ratios, showing a mean stutter proportion of 6.5% higher than stutter generated using AmpliTaq Gold® DNA Polymerase (Life Technologies; processivity of 50–60 bases) (data not shown). Our results were similar with results by Fazekas et al. [17] who reported that quality results from Kapa HiFi (Kapa Biosystems) with increased processivity of the fusion-based enzymes showed no consistent

improvement over AmpliTaq Gold polymerase for samples with mononucleotide repeats greater than 12 bp. These results are consistent with previous work that failed to observe a link between processivity and frameshift error [16]. These preliminary results of alternate thermal stable polymerases and higher processivity polymerases may not indicate the performance of the polymerases as the buffers and cycling conditions were not optimized for the enzymes. More work is warranted regarding more processive polymerases.

5. Conclusion

With LCN typing, increased stutter peaks can complicate true allele designation and possibly lead to incorrect interpretation of STR results. Therefore, if possible, methods should be sought that can reduce stutter ratios. Overall, a lower temperature annealing/extension step was shown to reduce stutter ratios. The approach does provide another direction to reduce stutter as opposed to most previous studies that have focused on high processivity and displacement polymerases. However, the method has some limitation because two-to-three loci showed higher stutter ratios with LT conditions than those with the STD conditions. Further study on optimization of LT conditions with STR buffer formulations and other polymerases may provide better performance for reducing stutter ratios.

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References

[1] B. Budowle, A.J. Eisenberg, A. van Daal, Validity of low copy number typing and applications to forensic science, Croat. Med. J. 50 (2009) 207–217.
[2] P. Gill, Application of low copy number DNA profiling, Croat. Med. J. 42 (2001) 229–232.
[3] G. Levinson, G.A. Gutman, Slipped-strand mispairing: a major mechanism for DNA sequence evolution, Mol. Biol. Evol. 4 (1987) 203–221.
[4] C. Schlötterer, D. Tautz, Slippage synthesis of simple sequence DNA, Nucleic Acids Res. 20 (1992) 211–215.
[5] Applied Biosystems, AmpFlSTR® SGM Plus® PCR Amplification Kit User's Guide, Applied Biosystems, Foster City, CA, 2012.
[6] Applied Biosystems, AmpFlSTR® Identifiler® PCR Amplification Kit User's Guide, Applied Biosystems, Foster City, CA, 2012.
[7] Applied Biosystems, AmpFlSTR® Identifiler® Plus PCR Amplification Kit User's Guide, Applied Biosystems, Foster City, CA, 2012.
[8] B.E. Krenke, A. Tereba, S.J. Anderson, E. Buel, S. Culhane, C.J. Finis, C.S. Tomsey, J.M. Zachetti, A. Masibay, D.R. Rabbach, E.A. Amiott, C.J. Sprecher, Validation of a 16-locus fluorescent multiplex system, J. Forensic Sci. 47 (2002) 773–785.
[9] S. Petricevic, J. Whitaker, J. Buckleton, S. Vintiner, J. Patel, P. Simon, H. Ferraby, W. Hermiz, A. Russell, Validation and development of interpretation guidelines for low copy number (LCN) DNA profiling in New Zealand using the AmpFlSTR SGM Plus multiplex, Forensic Sci. Int. Genet. 4 (2010) 305–310.
[10] P.S. Walsh, N.J. Fildes, R. Reynolds, Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA, Nucleic Acids Res. 24 (1996) 2807–2812.
[11] C. Brookes, J.A. Bright, S. Harbison, J. Buckleton, Characterising stutter in forensic STR multiplexes, Forensic Sci. Int. Genet. 6 (2012) 58–63.
[12] D.Y. Wang, C.W. Chang, R.E. Lagacé, L.M. Calandro, L.K. Hennessy, Developmental validation of the AmpFℓSTR® Identifiler® Plus PCR Amplification Kit: an established multiplex assay with improved performance, J. Forensic Sci. 57 (2012) 453–465.
[13] E. Viguera, D. Canceill, S.D. Ehrlich, In vitro replication slippage by DNA polymerases from thermophilic organisms, J. Mol. Biol. 312 (2001) 323–333.
[14] Applied Biosystems, Quantifiler® Kit User's Manual, Applied Biosystems, Foster City, CA, 2012.
[15] X.Z. Su, Y. Wu, C.D. Sifri, T.E. Wellems, Reduced extension temperatures required for PCR amplification of extremely A + T-rich DNA, Nucleic Acids Res. 24 (1996) 1574–1575.
[16] J.M. Hite, K.A. Eckert, K.C. Cheng, Factors affecting fidelity of DNA synthesis during PCR amplification of d(C-A)n-d(G-T)n microsatellite repeats, Nucleic Acids Res. 24 (1996) 2429–2434.
[17] A. Fazekas, R. Steeves, S. Newmaster, Improving sequencing quality from PCR products containing long mononucleotide repeats, Biotechniques 48 (2010) 277–285.
[18] Bst DNA Polymerase, Large Fragment, (Internet), 2013 (cited: 2013, June 28), available from: https://www.neb.com/products/m0275-bst-dna-polymerase-large-fragment.