



Short communication

Flanking region variation of ForenSeq™ DNA Signature Prep Kit STR and SNP loci in Yavapai Native Americans



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ABSTRACT

Massively parallel sequencing (MPS) offers advantages over current capillary electrophoresis-based analysis of short tandem repeat (STR) loci for human identification testing. In particular STR repeat motif sequence information can be obtained, thereby increasing the discrimination power of some loci. While sequence variation within the repeat region is observed relatively frequently in some of the commonly used STRs, there is an additional degree of variation found in the flanking regions adjacent to the repeat motif. Repeat motif and flanking region sequence variation have been described for major population groups, however, not for more isolated populations. Flanking region sequence variation in STR and single nucleotide polymorphism (SNP) loci in the Yavapai population was analyzed using the ForenSeq™ DNA Signature Prep Kit and STRait Razor v2s. Seven and 14 autosomal STRs and identity-informative single nucleotide polymorphisms (iiSNPs), respectively, had some degree of flanking region variation. Three and four of these identity-informative loci, respectively, showed $\geq 5\%$ increase in expected heterozygosity. The combined length- and sequence-based random match probabilities (RMPs) for 27 autosomal STRs were 6.11×10^{-26} and 2.79×10^{-29} , respectively. When combined with 94 iiSNPs (a subset of which became microhaplotypes) the combined RMP was 5.49×10^{-63} . Analysis of length-based and sequence-based autosomal STRs in STRUCTURE indicated that the Yavapai are most similar to the Hispanic population. While producing minimal increase in X- and Y-STR discrimination potential, access to flanking region data enabled identification of one novel X-STR and three Y-STR alleles relative to previous reports. Five ancestry-informative SNPs (aiSNPs) and two phenotype-informative SNPs (piSNPs) exhibited notable flanking region variation.

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1. Introduction

Forensic DNA typing currently utilizes length-based separation of polymerase chain reaction (PCR)-amplified short tandem repeats (STRs) for routine casework. Massively parallel sequencing (MPS) elucidates an additional level of STR motif variation at the sequence level, increasing diversity [1–3]. Sequence-based STR information may add value in kinship analyses and complex

mixture de-convolution efforts. While some commonly used STR loci lack STR-motif sequence variation, they may harbor variation in the flanking regions of the amplicon [3,4]. The same concept for single nucleotide polymorphisms (SNPs) also may apply [5,6]. That is, the current SNPs in MPS kits may indeed be microhaplotypes and their amplicons may contain additional information. While Novroski, et al. [3] described STR flanking region variation in major populations, such variation in Native Americans has yet to be described. Potential genetic variation in flanking regions adjacent to 59 STRs and 172 SNPs in the ForenSeq™ DNA Signature Prep Kit's primer panel was investigated in the Yavapai Native American population. The results show a moderate degree of STR and SNP flanking region genetic variation in the Yavapai population.

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2. Material and methods

MPS data for 27 autosomal, 7 X-chromosomal, and 25 Y-chromosomal STRs, Amelogenin, and 94 identity-informative SNPs (iiSNPs), 56 ancestry-informative SNPs (aiSNPs), and 22 phenotype-informative SNPs (piSNPs) were generated from DNA samples from 62 Yavapai Native Americans [7–9]. The samples and typing methods were described previously by Wendt, et al. [10]. Fastq files were used as standard input for STRait Razor v2s [3,11] to identify flanking region sequence variation.

5X depth of coverage (DoC) and 0.20 allele coverage ratio (ACR) thresholds were applied to the data. In-house Excel-based workbooks and Genetic Data Analysis (GDA) [12] were used to compile length-based and sequence-based allele frequencies, calculate observed and expected heterozygosities (H_o and H_e , respectively), perform tests for departures from Hardy-Weinberg Equilibrium (HWE) and detection of pairwise linkage disequilibrium (LD), compare previously published observed allele frequencies to the Yavapai data using the Chi-Squared Goodness of Fit Test, and generate random match probabilities (RMPs). STRUCTURE v2.3.4 (July 2012) [13–15] was used to assess the genetic structure of the Yavapai relative to the major populations described by Novroski, et al. [3] using length-based and sequence-based autosomal STRs.

A length-based STR allele is defined as only the capillary electrophoresis (CE)-based allele call and a sequence-based STR allele as the repeat motif plus flanking region information, presented herein as full string sequence and condensed nomenclature consistent with the recommendations of Parson et al., 2016 [16]. SNPs do not have a length-based allele call. Here we use the term “SNP” to describe the target SNP marker only or the MPS-based marker with invariable flanking sequences and “microhaplotype” to describe an entire string sequence that includes the target SNP plus adjacent flanking region sequence variation. All SNP and microhaplotype string sequences also are paired with a condensed nomenclature consistent with recommendations by Parson et al. [16].

3. Results and discussion

The total number of loci analyzed herein includes 27 autosomal, 7 X-chromosomal, and 25 Y-chromosomal STRs, Amelogenin, and 94 iiSNPs, 56 aiSNPs, and 15 piSNPs (or piSNP-containing microhaplotypes) [3,11].

3.1. Sequencing performance

Analysis of MiSeq FGx Forensic Genomics System sequence data (with the ForenSeq™ DNA Signature Prep Kit) using STRait Razor v2s and the previously reported ForenSeq™ Universal Analysis Software (UAS) [6] produced slightly different DoCs due to the length differences of the analyzed string sequences. The average DoC and ACR using STRait Razor v2s were $1,600X \pm 1730$ and 0.782 ± 0.147 , respectively, for 59 STRs and Amelogenin (data not shown), and $1,310X \pm 911$ and 0.830 ± 0.132 , respectively, for 165 target SNPs and SNP microhaplotypes (data not shown).

3.2. Flanking region variation

A full discussion of length-based and repeat motif variation for ForenSeq™ DNA Signature Prep Kit STR loci in the Yavapai population can be found in Wendt, et al. [6]. It should be noted that the string sequences analyzed by STRait Razor v2s are operationally defined, based on primer placement, to maximize data recovery from the ForenSeq™ DNA Signature Prep Kit amplicon for each marker [11]. As primer sequences of commercially available MPS library preparation kits are refined or modified, the

genomic coordinates of each amplicon may change, influencing the flanking region variation that can be observed for each target marker.

3.2.1. STRs

Relative to major population groups, few loci in the Yavapai population had flanking region sequence variation. Seven autosomal STRs (D13S317, D16S539, D18S51, D20S482, D2S441, D5S818, and D7S820) exhibited flanking region variation (Tables 1 and 2 and Supplemental Table 1). HWE and pairwise LD p -values for autosomal STRs and all other marker types, where appropriate, are listed in Supplemental Tables 2 and 3. Under the assumption of independence, the combined length-based and sequence-based RMPs for six autosomal STRs with flanking region variation were 1.80×10^{-5} and 7.80×10^{-8} , respectively.

Founder effects and population isolation of Native American populations suggest that these groups may exhibit lower genetic diversity than major population groups. Relative to Novroski, et al. [3], the Yavapai exhibited more loci with repeat region variation only and flanking region variation only than major United States populations but substantially less loci with variation in both regions (Fig. 1). Assuming the populations in Novroski, et al. [3] represent $K=4$ (AFA, CHI, CAU, and HIS), STRUCTURE analysis of length-based and sequence-based autosomal STRs indicate that the Yavapai are most similar to the HIS population (data not shown).

Little flanking region variation was observed in the seven ForenSeq™ DNA Signature Prep Kit X-STR loci (Tables 1 and 2 and Supplemental Table 4). The allele, DXS10074 [CE 15]-GRCh38-ChrX:67757300-67757464 (AAGA)12 AAGG (AAGA)2 67757338-A, was observed once at the DXS10074 locus in the Yavapai. After Bonferroni correction ($p < 0.00238$), two significant pairwise LDs containing DXS10074 (DXS10074/DXS10135 and DXS10074/DXS7132) were observed ($p=0.0015$ and 0.0021 , respectively). While not observed with length-based X-STR alleles [10], significant LD between DXS10074/DXS7132 is not surprising as they are part of a linkage block [18,19].

Minimal variation was observed in the Y-STR flanking regions. Two Y-STRs (DYS389I and DYS389II) exhibited flanking region sequence variation (Tables 1 and 2 and Supplemental Table 5).

One X- and three Y-STR alleles with flanking region variation in the Yavapai population were not reported by Novroski, et al. [3] (Table 2). These novel observations may be the result of sampling error within the populations studied to date; however, given their relatively high frequencies, these alleles may be common variants within Yavapai (or other Native Americans). Future studies of larger Native American cohorts would better characterize the distribution of these alleles within and between Native American groups.

3.2.2. SNPs

To our knowledge, string sequences for target SNPs and microhaplotypes captured by the ForenSeq™ DNA Signature Prep Kit have not been published previously (Supplemental Table 6). Twenty two target iiSNPs had flanking region variation adjacent to the target SNP (Table 1). Eight (8/22) provided no increase in the number of alleles at their respective loci. For example, an iiSNP microhaplotype contains the target SNP rs1015250 (G/C) and a flanking SNP rs6475200 (A/G) (Supplemental Table 6). In this population, the target SNP G allele is always observed with the flanking SNP A allele and the target SNP C allele is always observed with the flanking SNP G allele. Four iiSNP microhaplotypes, containing the target SNPs: rs10776839, rs1109037, rs2830795, and rs876724, substantially increased in diversity compared to the target iiSNPs alone. These markers exhibited an average H_o increase of 0.318 ± 0.190 , with a range of 0.0645–0.500. Assuming

Table 1
Average allele or haplotype frequencies for autosomal, X-chromosomal, and Y-chromosomal short tandem repeat (STR) and identity-informative (iiSNP), ancestry-informative (aiSNP), and phenotype-informative (piSNP) single nucleotide polymorphism (SNP) loci with flanking region sequence variation and their resulting impact on observed (H_o) and expected heterozygosities (H_e), single-locus random match probabilities (RMPs), and haplotype diversity relative to typing length-based STRs or target SNPs only. A comparison of sequence based allele frequencies from Novroski, et al. [3] was performed; p -values for significantly different allele frequencies are <0.05 .

Marker Type	Number of Loci with Flanking Region Variation	Average Frequency	Average H_o Increase	Average H_e Increase	Average Single Locus RMP Decrease	Haplotype Frequency Decrease	Haplotype Diversity Increase	Comparison to Novroski, et al. [3] populations data	Additional Comments
Autosomal STR	7	0.0704 ± 0.0967	0.0553 ± 0.0500	0.0516 ± 0.0483	0.0400 ± 0.0409	–	–	<ul style="list-style-type: none"> • 26 significantly differed from all four populations • D12S391 differed from AFA, CHI, and CAU, but not HIS 	• Greatest RMP decrease for D2S441 (0.13)
X-STR	1	0.0139	0	0.03	–	–	–	<ul style="list-style-type: none"> • DXS10103, DXS10135, DXS7423, HPRTB differed from all four populations • DXS7132 similar to all four populations • DXS10074 similar to HIS • DXS8378 similar to CHI and HIS 	• One novel allele observed (Table 2)
Y-STR	2	0.136 ± 0.0273	–	–	–	0	0	<ul style="list-style-type: none"> • DYF387S1, DYS19, DYS385ab, DYS437, DYS438, DYS481, DYS570, and DYS635 differed from AFA, CHI, CAU, and HIS • DYS389II, DYS505, DYS533, and DYS549 differed from three populations • DYS391, DYS392, DYS448, DYS576, DYS643, and Y-GATA-H4 differed from two populations • DYS389I, DYS390, DYS460, and DYS522 differed from one population • DYS439 was similar to all four 	• Three novel alleles observed at DYS389I and DYS389II in the same three individuals (Table 2)
iiSNP	22	0.121 ± 0.150*	0.109 ± 0.166*	0.0865 ± 0.123*	0.0900 ± 0.115*	–	–	–	• 14/22 provided increased heterozygosity and allele spread
aiSNP	6	0.191 ± 0.215*	0.116 ± 0.144*	0.130 ± 0.171*	–	–	–	–	• 5/6 provided increased heterozygosity and allele spread, two are previously reported [17,18]
piSNP	2	0.0524 ± 0.0627	0.161	0.0895 ± 0.122	–	–	–	–	–

* Reported values are based on the indicated subset of loci showing increase in allele spread.

independence, the combined 14-locus RMPs for iiSNPs and their corresponding iiSNP microhaplotypes were 1.06×10^{-5} and 1.67×10^{-7} , respectively. The flanking region variation of 14 iiSNP

microhaplotypes supports that a relatively small panel of microhaplotypes might be as informative as some low performing STR markers. Many iiSNPs lacked flanking region variation in the

Target Allele	Count	Frequency	String Sequence	Microhaplotype Nomenclature	Count	Frequency
C	94	0.758	AGTACATTTTTGTCAACACTCTGTAACCTGCCTCAGATATTCAAATTTAGTAGATGTAGATA AGTACATTTTTGTCAACACTCTGTAACCTGCCTCAGATATTCAAATTTAGTAGATGTAGACA	rs876724 [CE C]-GRCh38-chr2:114970-115036 rs876724-C; rs300773-T rs876724 [CE C]-GRCh38-chr2:114970-115036 rs876724-C	39	0.314516
Target SNP H ₀ 0.370			Microhaplotype H ₀ 0.651			
Target SNP H _e 0.258			Microhaplotype H _e 0.758			
Target SNP HWE p-value: 0.0263			Microhaplotype HWE p-value: < 0.000532			
rs907100 (n=124)						
Target Allele	Count	Frequency	String Sequence	Microhaplotype Nomenclature	Count	Frequency
C	40	0.323	TGATGCCCTGGCATCAAGAAGGCTCAACTGGCTCTTTCTGTGTTTCCAAGGCTGGAAG TGATGCCCTGGCATCAAGAAGGCTCAACTGGCTCTTTCTGTGTTTCCAAGGCTGGAAG	rs907100 [CE C]-GRCh38-chr2:238654924-238654989 rs907100-C rs907100 [CE C]-GRCh38-chr2:238654924-238654989 rs907100-C; rs11689319-A	5	0.0403
G	84	0.677	TGATGCCCTGGCATGAAGAAGGCTCAACTGGCTCTTTCTGTGTTTCCAAGGCTGGAAG	rs907100 [CE G]-GRCh38-chr2:238654924-238654989 rs907100-G	84	0.677
Target SNP H ₀ 0.441			Microhaplotype H ₀ 0.464			
Target SNP H _e 0.484			Microhaplotype H _e 0.516			
Target SNP HWE p-value: 0.561			Microhaplotype HWE p-value: 0.589			
rs987640 (n=124)						
Target Allele	Count	Frequency	String Sequence	Microhaplotype Nomenclature	Count	Frequency
A	51	0.411	ACAGGTACATTCACCTAACAGGCTCTTTCCACCATGTGAGAAATACAAAAAAGACTTAATACAGACGATGG ACGGTACATTCACCTAACAGGCTCTTTCCACCATGTGAGAAATACAAAAAAGACTTAATACAGACGATGG	rs987640 [CE A]-GRCh38-chr22:33163486-33163560 rs987640-A rs987640 [CE A]-GRCh38-chr22:33163486-33163560 rs1793354-C; rs987640-A	50	0.403
T	73	0.589	ACAGGTACATTCACCTAACAGGCTCTTTCCACCATGTGAGAAATACAAAAAAGACTTAATACAGACGATGG	rs987640 [CE T]-GRCh38-chr22:33163486-33163560 rs987640-T	73	0.589
Target SNP H ₀ 0.488			Microhaplotype H ₀ 0.495			
Target SNP H _e 0.468			Microhaplotype H _e 0.484			
Target SNP HWE p-value: 0.800			Microhaplotype HWE p-value: 0.660			
rs9905977 (n=124)						
Target Allele	Count	Frequency	String Sequence	Microhaplotype Nomenclature	Count	Frequency
A	48	0.387	TGGTGTCCAGGAGGGCTGGGTGACTGTGGTCAAGATTCTTGTCTTTCCCTGCTCCCTCCCTGGCTGTGACGCTTTG TCCTCAGGCTTGGGCTCGTGGCC	rs9905977 [CE A]-GRCh38-chr17:3016058-3016176 rs9905977-A	48	0.387
G	76	0.613	TGGTGTCCAGGAGGGCTGGGTGACTGTGGTCAAGATTCTTGTCTTTCCCTGCTCCCTCCCTGGCTGTGACGCTTTG TCCTCAGGCTTGGGCTCGTGGCC	rs9905977 [CE G]-GRCh38-chr17:3016058-3016176 rs9905977-G; rs28582109-A rs9905977 [CE G]-GRCh38-chr17:3016058-3016176 rs9905977-G	3	0.0242
Target SNP H ₀ 0.478			Microhaplotype H ₀ 0.526			
Target SNP H _e 0.516			Microhaplotype H _e 0.565			
Target SNP HWE p-value: 0.593			Microhaplotype HWE p-value: 0.561			
rs993934 (n=124)						
Target Allele	Count	Frequency	String Sequence	Microhaplotype Nomenclature	Count	Frequency
A	85	0.685	TTTGTCTTGAAGGCAATAGAGCAAGTATTGTGATAACAGCTCCAGAGTATTATTAGCTTAGTTCATAA TTTGTCTTGAAGGCAATAGAGCAAGTATTGTGATAACAGCTCCAGAGTATTATTAGCTTAGTTCATAA	rs993934 [CE A]-GRCh38-chr2:123351571-123351640 rs993934-A rs993934 [CE A]-GRCh38-chr2:123351571-123351640 rs200354-G; rs993934-A	83	0.669
G	39	0.315	TTTGTCTTGAAGGCAATAGAGCAAGTATTGTGATAACAGCTCCAGAGTATTATTAGCTTAGTTCATAA	rs993934 [CE G]-GRCh38-chr2:123351571-123351640 rs993934-G	2	0.0161
Target SNP H ₀ 0.435			Microhaplotype H ₀ 0.456			
Target SNP H _e 0.565			Microhaplotype H _e 0.597			
Target SNP HWE p-value: 0.0184			Microhaplotype HWE p-value: 0.0250			
rs200354 (n=124)						
Target Allele	Count	Frequency	String Sequence	Microhaplotype Nomenclature	Count	Frequency
G	12	0.0968	CAAGCTGCTTGGAACTGGGCTGCCCCATGCACCATGGCCATTGGAAGTGGGTAGTGAAGAGGCTGCCCTGTCCATTGTAGAAATGTTTAA CAGCATCTCTGGAGACTGGCTGCCCCATGCACCATGGCCATTGGAAGTGGGTAGTGAAGAGGCTGCCCTGTCCATTGTAGAAATGTTTAA	rs200354 [CE G]-GRCh38-chr14:98908931-98909052 rs200354-G	12	0.0968
T	112	0.903	CAAGCTGCTTGGAACTGGGCTGCCCCATGCACCATGGCCATTGGAAGTGGGTAGTGAAGAGGCTGCCCTGTCCATTGTAGAAATGTTTAA CAGCATCTCTGGAGACTGGCTGCCCCATGCACCATGGCCATTGGAAGTGGGTAGTGAAGAGGCTGCCCTGTCCATTGTAGAAATGTTTAA	rs200354 [CE T]-GRCh38-chr14:98908931-98909052 rs200354-T; rs200353-G rs200354 [CE T]-GRCh38-chr14:98908931-98909052 rs200354-T	72	0.581
					23	0.185

Yavapai population, however, access to these sequence data may reveal additional variation on a case-by-case basis or in other population groups, defining additional microhaplotypes.

Six aiSNPs had sequence variation adjacent to the target locus with an average frequency of 0.191 ± 0.215 (Table 1 and Supplemental Table 5). Two of the aiSNP microhaplotypes (rs1079597-rs1079598 or “mh11KK-090”; rs870347-870348 or “mh11KK-062” or “PAPD7”) have been described previously [17,18]. One aiSNP microhaplotype (rs1079597-rs1079598; mh11KK-090) provided no increase in the number of alleles at the target rs1079597 locus. The average H₀ and H_e were 0.319 ± 0.237 and 0.343 ± 0.209 for 5/6 aiSNPs, respectively, and 0.435 ± 0.215 and 0.431 ± 0.217 for 5/6 aiSNP microhaplotypes, respectively. Prior to Bonferroni correction, no aiSNP microhaplotypes significantly deviated from HWE expectations. Before and after Bonferroni correction (p < 3.25 × 10⁻⁵), 381 and 22 aiSNP pairwise LDs were observed, respectively. No significant Bonferroni corrected LDs were observed between aiSNPs or aiSNP microhaplotypes on the same chromosome.

A minor increase in variation was observed for two piSNPs when considering flanking region sequences (Table 1 and Supplemental Table 6). Two piSNP microhaplotypes were defined as: mh16-MCR1B and mh16-MCR1C [5]. The average piSNP microhaplotype H₀ and H_e were 0.198 ± 0.213 and 0.190 ± 0.196, respectively. After Bonferroni correction (p < 0.000549), one (rs1805009/mh-16-MCR1B) significant pairwise LD was observed. It is not surprising to observe significant LD between rs1805009 and mh16-MCR1B due to their close physical proximity (~325 basepairs) within the MCR1 gene.

3.3. Bioinformatic concordance

Consistent with the ForenSeq™ UAS and STRbase [10,20], Wendt, et al. [10] did not include the DYS612 [CCT]_a[CTT]_b motif in length-based alleles. Based on Parson, et al. [16] recommendations and Novroski, et al. [3] population data, this region is now included for the length-based alleles at the DYS612 locus.

Compared to frequency data previously published by Wendt, et al. [10], the piSNP N29insA is discordant. The locus was reported as having no observed heterozygosity and yet the allele frequencies are 0.516 and 0.484 for the null and insertion alleles, respectively. By using STRait Razor v2s, a bioinformatic error was discovered. Manual confirmation of the locus in the ForenSeq™ UAS indicated that the error occurred when the “ForenSeqRunStatistics” XML files were offline. Thus, the values reported herein are a discordant due to an operation issue independent of analyses performed with the UAS. The N29insA frequencies reported here (1.00 null and 0.00 insertion) are correct.

4. Conclusion

Twenty-one human-identity markers have been identified in this study of the Yavapai population which contain some degree of flanking region variation, with a wide range of relative frequencies. A small portion of target autosomal STRs and iSNPs exhibited flanking region variation at relatively high frequencies. The human identification marker set captured by the ForenSeq™ DNA Signature Prep Kit produced combined RMPs of 7.66 × 10⁻⁵⁸ and

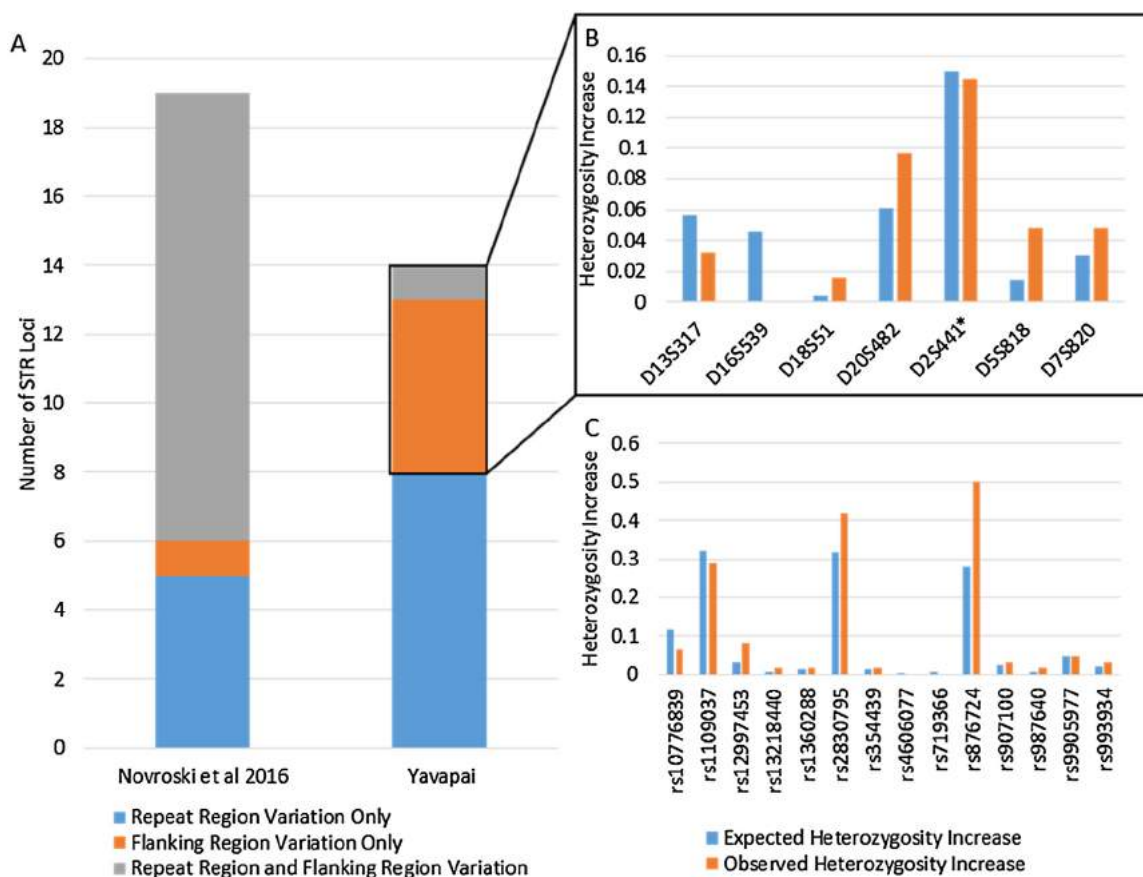


Fig. 1. Sequence variation of autosomal identity-informative markers. (A) Number of short tandem repeat (STR) loci in the Yavapai population and the Novroski, et al. [3] study exhibiting sequence variation; (B) Observed and expected heterozygosity increase of autosomal STR loci with flanking region variation; an asterisk indicates the locus with flanking region and repeat region variation; (C) Observed and expected heterozygosity increase of identity-informative single nucleotide polymorphism containing microhaplotypes.

5.49×10^{-63} , respectively, for a panel of 121 human identification markers (94 iiSNPs or MPS-based iiSNPs/iiSNP microhaplotypes plus 27 length-based or sequence-based autosomal STRs). With such low MPS-based RMPs, additional variation may not seem necessary, however, these results highlight that increased information can be obtained from the full amplicon.

Conflict of interest

The authors report no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fsigen.2017.02.014>.

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