INNULs: A Novel Design Amplification Strategy for Retrotransposable Elements for Studying Population Variation

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Key Words

INDELs • INNULs • SNPs • *Alu* • Long interspersed nuclear elements • Genotyping

Abstract

Objectives: Retrotransposable elements (REs), consisting of long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs), are a group of markers that can be useful for human identity testing. Until now, however, due to the inherent size difference (up to 6 kb in some instances) associated with insertion and null alleles (or INNULs), the use of REs for facilitated population studies has not been sought or practical. The size of the insertion elements (from a few hundred to several thousand bp) has proven to limit their utility as a marker because of the inefficient amplicon yield with PCR. A novel primer design now facilitates INNUL marker testing. A preliminary panel of single-locus markers was developed to evaluate the potential of typing these insertion elements. Nine INNULs (5 *Alu* and 4 LINEs) were typed in three major North American populations and analyzed for population genetic features. In addition, the variation of each marker among the sample populations provides insight of its potential use as individual identification or ancestral marker. **Methods:** INNUL markers were

developed into fluorescently labeled single-loci PCR. Nine markers were developed with amplicons that were less than 180 bp in length, and, depending on the locus amplicons of the INNULs, alleles varied in size from 50 to 1 bp. This allele size is noteworthy because the insertion alleles of the 9 loci range in size from 297 to 6,195 bp. The allele distribution of the INNULs was assessed and analyzed in three major North American populations. **Results:** Upon observation of the distribution of the alleles in three major North American populations, the markers generally met Hardy-Weinberg expectations, and there was little evidence of detectable levels of linkage disequilibrium. Due to varying distributions of the alleles in the major population groups tested, some of the markers might be better suited for use as an individual identification marker, while others are better suited for bio-ancestral studies. **Conclusions:** Using the primer design strategy described in our work, SINEs and (for the first time, to our knowledge) LINEs can be utilized as markers for studying population genetic variation that is more amenable to the limitations of the PCR technique. This study lays the foundation for future work of developing a multiplex panel of INNUL markers that can be used as a single-tube assay for human identity testing utilizing small amplicons (<180 bp), which could be useful for ancient or degraded forensic DNA samples.

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Introduction

Short tandem repeat (STR) loci are the primary genetic markers used in human identity testing. These markers are highly polymorphic and afford a high degree of sensitivity of detection such that relatively low quantities (from 1 ng to 250 pg) of template DNA can be analyzed [1–6]. Retrotransposable elements (REs) consisting of long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs) are another group of markers that could be useful for population genetic and evolutionary studies, as well as human identity testing [7–13]. Ustyugova et al. [14] demonstrated that REs could be used for cell line identification. Novick et al. [15] and Mamedov et al. [16] recently described a set of *Alus* (a type of SINE) for paternity testing. Both of these studies intimated that the systems could be applied to forensic analyses. REs have low mutation rates, which make them appealing for population studies and missing persons kinship analyses compared with the less stable STRs. Although REs make up over 40% of the human genome [17] and present myriad possible targets for population studies and potentially human identity testing, these insertion elements have received limited attention for use in human identity testing. We have termed the *Alus* and LINEs as INNULs instead of INDELs as the two allelic states are known to be an insertion and null. Indeed, some INDELs are similar in this regard but may be more difficult to describe in this manner. Thus, INNUL better describes the already well-established allelic states of these markers.

Obtaining a comparable yield of products for both alleles of a heterozygote during PCR typically requires similar amplicon sizes. The difference in size of a null allele and an insertion element generally is quite large from the perspective of PCR efficiency. The preferential amplification of the smaller allele often makes PCR typing of such markers problematic. In addition, some materials such as forensic samples often are compromised in quality and quantity. Degraded samples may contain fragments of DNA that are less than the length of the insertion element [18–21].

REs can range in size from hundreds of (SINEs) to several thousand (LINEs) base pairs (bp) in length [7–13]. Previous attempts to use *Alu* sequences for identity testing capitalized on the size difference between insertion and null alleles by amplifying the entire region with the same forward and reverse primers [15]. The insertion allele would be 200–400 bp larger than the null allele and could be detected electrophoretically based on size differ-

ences. While useful for paternity testing and some population studies where DNA quality is not compromised, the large size difference between amplicons of the null and insertion alleles does impact amplification efficiency during the PCR technique, which is a limitation for the design of efficient genetic typing assays (i.e., multiplexes) and for forensic sample analyses. Thus, the use of REs has not been embraced for routine application diagnostics [22].

A novel primer design described herein and referred to subsequently as Mini-Primer strategy reduces the overall amplicon size as well as the difference in amplicon sizes between the two allelic states of INNULs. Amplification of the two alleles occurs through a common unlabeled forward primer and two fluorescently labeled reverse primers. The labeled reverse primer for the null allele overlaps the insertion site of the RE, and the labeled reverse primer for the insertion allele has an overlap region with the junction and the RE itself, or just inside the RE (fig. 1). With this design, the resulting INNUL allelic amplicons can be designed to differ by as little as 1 bp, and because each allelic-state reverse primer is labeled with a different fluor, the amplicons can even be the same size. Additionally, the amplicon size can be reduced substantially, to a size much smaller than currently used STR markers, such that substantially degraded samples can be typed. With this design, a more simplified and automated technology can be applied for LINE and SINE typing.

Selection criteria for INNUL markers depend on the application. Markers that are highly polymorphic in all major populations (i.e., approaching 50% heterozygosity) are desirable for human identity testing [4, 6, 23–25], while those demonstrating high coefficients of inbreeding (e.g., SNPs in which the different allelic states approach fixation in different populations) can be used for bio-ancestral analyses [26]. To demonstrate the potential of the newly designed primer sets for population studies and potentially for human identity testing, an initial set of INNUL markers based on *Alus* and LINEs was chosen. The *Alu*-based INNUL markers were selected based on molecular characteristics and extant population data [27–29]. There were no available population data on the LINE INNUL markers, so only molecular characteristics were used as selection criteria for this study. The markers' amplicons range in size between approximately 50 and 179 bp, and individual INNULs differ in amplicon size between 1 and 54 bp. Population studies were performed to assess diversity within and among major population groups to determine the applicability of the selected INNULs for either identity testing or bio-ancestral analyses.

Fig. 1. Amplification strategy for INNUL typing. **a** A common forward primer (FC) is used for both insertion and null alleles. A fluorescently labeled 'null-specific' reverse primer (RN) straddles the insertion site of the RE and anneals in the absence of the RE. **b** In instances where the RE is present, the annealing site of the reverse primer is disrupted, and the 'insertion-specific' reverse primer (RI) anneals at the site that overlaps with the insertion site and the adjacent portion of the RE.

Materials and Methods

Marker Selection

Markers were selected from dbRIP, existing literature, and through BLAST sequence analysis [7–16, 27, 30]. After the initial selection, the potential loci were assessed for their suitability for the primer design [22].

Primer Design and Preparation

Primers were designed using Primer3 (input version 0.4.0. <http://frodo.wi.mit.edu/primer3/>). A set of three primers was designed for each marker: one forward primer and two reverse primers, one for the insertion and one for the null allele. All designed primers have T_m values in the range of 58–61°C. The program Reverse Complement from the Harvard Medical Technology Group and Lipper Center for Computational Genomics (arep.med.harvard.edu/) was used. Subsequently, the primers were screened against the GenBank non-redundant database to determine if they were unique DNA sequences.

The fluorescently labeled and unlabeled oligonucleotide primers were synthesized either by Bio-Synthesis, Inc. (Dallas, Tex., USA) or by Integrated DNA Technologies, Inc. (Coralville, Iowa, USA). All lyophilized primers were dissolved in 10 mM TE (Tris and EDTA) buffer (pH 8.0) to a 100 μ M stock concentration (10 $\times$). The stock primers were stored at 4°C until used. Following reconstitution, each primer was diluted using TE buffer to a final concentration of 10 μ M (1 $\times$). Each primer mix consisted of three primers: one unlabeled forward primer and two labeled reverse primers, with twice the amount of the forward primer than the reverse primers (1.0, 0.5, and 0.5 μ l, respectively). All labeled primers were stored in opaque polypropylene tubes to avoid quenching of the fluorescent tags.

Data Analysis for ABI 310

After amplification, samples were prepared by combining 20 μ l of Hi-Di™ formamide, 0.25 μ l of ROX 350 size standard, and 1 μ l of DNA product per reaction. Samples were incubated at 95°C in a water bath for 3 min. Separation and detection of STR amplification products were performed on an ABI Prism® 310 Genetic Analyzer (Life Technologies) using the following parameters for the GS STR POP4 (1 ml) F module: injection at 15 kV for

5 s, separation at 60°C, run time of 24 min. Data were analyzed using the GeneMapper ID-X Software version 3.2 (Life Technologies).

Population Samples

Buccal samples from 279 African-Americans, Caucasians, and southwestern Hispanics were either collected from unrelated individuals residing in Texas or were kindly provided by Genetic Testing Laboratories (Las Cruces, N. Mex., USA). The samples were anonymized and collected in accordance with methods approved by the Institutional Review Board of the University of North Texas Health Science Center in Fort Worth, Tex., USA. Population affinity was determined by self-declaration.

DNA Extraction

DNA was isolated from buccal swabs using either the AutoMate Express® (Life Technologies) or the DNA Investigator® Kit (Qiagen, Hilden, Germany) according to the manufacturers' recommendations. The quantity of DNA was determined by qPCR using the Quantifiler® Quantification Kit and 7500 Real-Time PCR® System (Life Technologies). Samples were then normalized to 250 pg/ μ l and stored at either –20°C or –40°C until amplification.

PCR and Electrophoretic Conditions

For PCR, each locus was amplified as a singleplex assay. A reaction was composed of 12.5 μ l of a 2 $\times$ Amplitaq Gold PCR Master Mix® (Life Technologies): 2.0 μ l of the primer mix (for specifications of primers, see table 1) and 500 pg of template DNA were brought to a final volume of 25 μ l. The resulting reaction mixture was amplified using a Geneamp 9700 PCR System® (Life Technologies) thermocycler. The amplification protocol was an initial step of 94°C for 10 min followed by 32 cycles of 94°C for 1.5 min, 60°C for 1 min, and 72°C for 3 min followed by an extension step of 72°C for 10 min. An additional extension step at 60°C for 60 min was added to promote terminal adenylation.

For capillary electrophoresis, 1 μ l of the amplified product was added to 8.5 μ l of formamide and 0.5 μ l of GS ROX 500 ILS® (Life Technologies), mixed, and snap cooled. Capillary electrophoresis was performed on either a 3500xl or 3130xl Genetic Analyzer® (Life Technologies) capillary electrophoresis instrument.

Table 1. List of INNUL loci, descriptive details of the loci, amplicons, and primer information¹⁻³

Marker name	RE type	Location ¹	dbRIP ID ² (if available)	ALFRED	Insertion size, bp	Insertion amplicon size, bp	Null amplicon size, bp	Primer and sequence	Starting concentration, μ M	Final concentration, μ M
TARBPIR	<i>Alu</i>	Chr 1: 28,104,939– 28,105,312	NA	No	311	50	57	Forward primer: AAGGAGGCAAAAGGAAGAATACA Reverse primer (insertion): CGGCCCATTCATCAGTTTAA Reverse primer (null): CGATGATCCAGTCATTCATCA	10	0.4
Ya5NBC51	<i>Alu</i>	Chr 3: 191,773,344– 191,773,631	1000205	Yes	297	120	119	Forward primer: CGCCATCTCTTCTTCTCTTCA Reverse primer (insertion): ACAGGCGTGAGAAATGCTTTG Reverse primer (null): TGTCAGGGTTAAATGCTTTGT	10	0.4
LC3-2601	LINE	Chr 3: 26,414,512– 26,420,540	2000179	No	6,042	125	179	Forward primer: TTGGCCATAGAAAAACCAGTC Reverse primer (insertion): ACAACCGGAGGAGCCAAAGAT Reverse Primer (null): CCAAGACCCCATTCCTGATTC	10	0.4
CH4-12-7012	LINE	Chr 4: 20,769,969– 20,775,752	2000161	No	6,195	121	149	Forward primer: GGAAAGGTACAAGATGTAATGAGGA Reverse primer (insertion): GAGGAAAAATGGCCAAAGACAA Reverse primer (null): GCCACACCTTGATCTTGA	10	0.4
CH6-28-9163	LINE	Chr 6: 19,873,106– 19,879,163	2000387	No	6,058	113	112	Forward primer: GAGGCTGTGGTGAGGATAA Reverse primer (insertion): GCCATCTTGGCTCCAGTTAG Reverse primer (null): ACATGCCACCATACCCAGTT	10	0.4
Yb7AD155	<i>Alu</i>	Chr 10: 10,493,725– 10,493,824	NA	No	323	99	100	Forward primer: GTACACATTAAAGCACATGGAAGTCA Reverse primer (insertion): GCCCGGCCGTTCTTTTTC Reverse primer (null): GCATGAAATGTTCTTTTTCATCTC	10	0.4
Ya5AC2265	<i>Alu</i>	Chr 13: 102,807,866– 102,808,174	1000063	No	319	100	107	Forward primer: AGAAGAGTGAATGCACATTTATGA Reverse primer (insertion): TAAGAAACTGGCGCGGC Reverse primer (null): TAAGAAACTGAATTCATGACTCC	10	0.4
CH14-50-6236	LINE	Chr 14: 82,705,608– 82,706,236	2000255	No	647	126	176	Forward primer: GCTGGGTAAATGCAAAAGGAA Reverse primer (insertion): TCTGGTATGTGGTGTCCAAAG Reverse primer (null): GACCACACCAAGGCTCTTC	10	0.4
Yb8NBC106	<i>Alu</i>	Chr 21: 40,508,751– 40,509,060	1000917	No	325	122	127	Forward primer: CATCAAACTCCAGAGTTCCTAAG Reverse primer (insertion): GGATTACAGGCGTGAGGATT Reverse primer (null): TGGATTGATGAGGACTCAGG	10	0.4

¹ Benson et al. [28]. ² Wang et al. [27]. ³ Cheung et al. [29].

Samples were injected for 24 s and subjected to electrophoresis at 15 kV in POP4™ (Life Technologies). Electrophoretic data were analyzed using GeneMapper IDX® software (Life Technologies).

Population and Statistical Analyses

Population and statistical analyses, including F_{ST} , were performed with either GDA software [31], Arlequin 3.11 [32], or in-house developed software. Departures from Hardy-Weinberg equilibrium (HWE) and linkage equilibrium were tested using Fisher's exact test. Bonferroni's correction for multiple comparisons was performed according to Weir and Cockerham [33].

Results and Discussion

Marker Selection and PCR Optimization

Initial efforts towards marker selection focused on the set of forensic candidate markers described by Mamedov et al. [16]. Using these markers as a benchmark and the previously described Mini-Primer strategy, an attempt was made to reduce the amplicon size of a subset of markers reported by Mamedov et al. [16]. Primers for 5 markers were designed such that all amplicons were less than 120 bp in size for both the insertion and null alleles (online suppl. table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000343050). Gel electrophoresis was used to visualize the products of the reactions (online suppl. fig. 1). Examples with both insertion and null alleles that differ in size by 7 bp are shown. This result supported the potential of the Mini-Primer strategy. It should be noted that, in this study, each allele was labeled with a different fluor. A long-term goal (beyond the proof-of-concept study herein) would be to label alleles of a locus with the same fluor to facilitate multiplexing and allele balance. However, at this early stage, it was desirable to have an indicator that could designate each allele to evaluate if estimated sizes were indeed those that were observed, and when alleles were similar in size, they could be discerned properly.

Following this initial success, both *Alus* and *LINEs* were chosen from the literature [9, 10, 14–16, 27, 30]. Through trial and error, a set of candidate markers was selected to demonstrate the feasibility of the Mini-Primer approach for typing INNULs. These loci are described in table 1.

The selected markers are 5 *Alus* and 4 *LINEs* with amplicons that are between 50 and 179 bp in length. Figure 2a, b shows example electropherograms of the size range of alleles for two loci (LC3-2601 and CH6-28-9163, respectively) which range from approximately 50 to 1 bp for the candidate set. An electropherogram for a heterozygous individual at the locus with the largest amplicon

is shown in figure 2a (179 bp) and for a heterozygous individual at the locus with the smallest amplicon (TARBP1R; 50 bp) in figure 2c. The insertions and null amplicons were in the position and relative size that were predicted. Thus, it is feasible to generate amplified products of the allelic states of *Alus* and *LINEs* that are more suited for the limitations of the PCR technique. When the size is similar for amplified products of allelic states, assays tend to be more robust and demonstrate less preferential amplification of the smaller-sized allele.

Population Studies

Three North American sample populations (African-American, $n = 93$; Caucasian, $n = 93$; southwestern Hispanic, $n = 93$) were typed for the 9 INNUL loci. The frequencies of the null allele per locus, observed heterozygosity, random match probability, and power of discrimination are listed in table 2. To the best of our knowledge, these are the first population data reported on *LINEs*, and, with the exception of the TARBP1R and Ya5NBC51 loci, these are the first population data generated for the *Alu* markers reported herein. Heterozygosity for the markers ranged from 0.02 to 0.48 in southwestern Hispanics, from 0.03 to 0.59 for Caucasians, and from 0.31 to 0.50 in African-Americans. There was only one departure from HWE detected (at locus CH14-50-6236 in the African-American population). This observed departure was no more than would be expected by chance. When the critical value was adjusted by the Bonferroni correction [33], the departure was no longer significant (table 2).

Departures from linkage equilibrium [i.e., linkage disequilibrium (LD) between pairs of loci] were tested for each of the three populations (online suppl. table 2). Four pairs of loci in the African-American population and one pair in the Caucasian population demonstrated significant LD (at $p < 0.05$ as determined by Fisher's exact test). All of the loci pairs in the African-American population involved locus CH14-50-6236, which was the only locus to demonstrate a detectable departure from HWE (at the 0.05 level). These apparent LDs thus could be associated with the deviation from HWE as described by Falush et al. [34] and Chakraborty [35] or due to substructure in the African-American population. Regardless, when the critical value was adjusted for multiple comparisons (via the Bonferroni correction), none of the loci pairs displayed significant LD.

Wright's F_{ST} was estimated to assess the population substructure [33]. For the three populations, the combined F_{ST} was 0.122 (table 2). Three loci (CH14-50-6236,

Table 2. Population-specific INNUL allele frequencies, other population parameters, and F_{ST} values for alleles across three North American populations (n = 93 each)

Marker	Frequency of null	H_o	HWE (p value) ^{1, 2}	RMP	PD	F_{ST} ³
<i>African-Americans</i>						
LC3-2601	0.5227	0.4545	0.3884	0.3564	0.6436	0.0560
Yac52265	0.7198	0.3407	0.2006	0.4300	0.5700	0.0715
CH14-50-6236	0.4881	0.3571	0.0094*	0.3345	0.6655	0.1766
CH4-12-7012	0.1978	0.3077	0.7372	0.5170	0.4830	0.4831
Ya5NBC51	0.4205	0.4318	0.2722	0.3605	0.6395	0.0680
Yb7AD155	0.5872	0.5000	0.8219	0.3902	0.6098	-0.0030
CH6-28-9163	0.7582	0.3956	0.5825	0.4725	0.5275	0.2553
Yb8NBC106	0.4494	0.4944	1.0000	0.3774	0.6226	0.0368
TARBP1R	0.6833	0.3889	0.3441	0.4052	0.5948	0.0144
Overall				0.0003	0.9997	0.1224
<i>Caucasians</i>						
LC3-2601	0.0163	0.0326	1.0000	0.9369	0.0631	0.0560
Yac52265	0.2473	0.4066	0.5600	0.4691	0.5309	0.0715
CH14-50-6236	0.7258	0.3978	1.0000	0.4416	0.5584	0.1766
CH4-12-7012	0.0215	0.0430	1.0000	0.9177	0.0823	0.4831
Ya5NBC51	0.4674	0.4130	0.0978	0.3450	0.6550	0.0680
Yb7AD155	0.5440	0.5385	0.5338	0.4003	0.5997	-0.0030
CH6-28-9163	0.4674	0.5217	0.8413	0.3887	0.6113	0.2553
Yb8NBC106	0.5000	0.5914	0.1013	0.4332	0.5668	0.0368
TARBP1R	0.4355	0.5269	0.5375	0.3978	0.6022	0.0144
Overall				0.0016	0.9984	0.1224
<i>Southwestern Hispanics</i>						
LC3-2601	0.0110	0.0220	1.0000	0.9570	0.0430	0.0560
Yac52265	0.2778	0.4222	0.7934	0.4440	0.5560	0.0715
CH14-50-6236	0.6611	0.3889	0.2406	0.3899	0.6101	0.1766
CH4-12-7012	0.1222	0.2222	1.0000	0.6373	0.3627	0.4831
Ya5NBC51	0.4167	0.4556	0.5259	0.3696	0.6304	0.0680
Yb7AD155	0.7333	0.4444	0.2922	0.4607	0.5393	-0.0030
CH6-28-9163	0.3278	0.4778	0.4966	0.4240	0.5760	0.2553
Yb8NBC106	0.5889	0.4667	0.8272	0.3758	0.6242	0.0368
TARBP1R	0.6484	0.4835	0.6513	0.4112	0.5888	0.0144
Overall				0.0012	0.0012	0.1224

H_o = Observed heterozygosity; RMP = random match probability; PD = power of discrimination.

¹ α -level of 0.05 is adjusted from 0.05 to 0.005556 when corrected for multiple tests (Bonferroni's correction) [33]. ² Calculated using GDA software [31]. ³ F_{ST} calculated according to Weir and Cockerham [33].

* p value for HWE below the corrected critical value.

Fig. 2. Examples of the range of the amplicon size between markers and alleles. **a** Null and insertion alleles of the marker LC3-2601 that have the largest difference in amplicon size. The difference between the null allele (larger amplicon) and the insertion allele (smaller amplicon) is 54 bp. **b** The amplicon size difference of the alleles of marker CH6-28-9163 is 1 bp. **c** The overall amplicon size of both alleles for the marker shown (TARBP1R) is less than 60 bp (50 and 57 bp for the insertion and null alleles, respectively), which highlights its potential use with degraded samples.

CH4-12-7012, and CH6-28-9163) contributed more than the other loci to the overall F_{ST} . Markers that displayed lower F_{ST} and higher heterozygosities in all three populations would tend to be more useful as individual identification markers among the three populations tested. These loci were Yac52265, Ya5NBC51, Yb7AD155, Yb8NBC106, and TARBP1R. The F_{ST} values for these 5 loci were approximately 0.07 or less. Markers with allele frequencies that differ substantially in one or more of the populations tend to be more useful for bio-ancestral studies. For example, the locus LC3-2601, an L1 LINE, has an allele distribution approaching 50% in the African-American sample population but is almost monomorphic in the Hispanic and Caucasian sample populations. More than 98% of the alleles at this locus were the insertion state in Caucasians and southwestern Hispanics. Panels designed specifically around markers with similar characteristics could be very helpful in determining the ancestral lineage (or admixture) of an individual.

Similar classes of markers are the biallelic insertions and deletions (INDELs). They are appealing in that they have similar properties as INNULs, and there have been some studies describing multiplexes that could be suited for human identity testing [25, 36–42]. Indeed, there is one commercially available kit, the Investigator DIPplex Kit (Qiagen), which enables multiplex typing of 30 INDELs [25, 36, 42]. Another promising INDEL multiplex (with 38 markers) was described by Pereira and colleagues [36, 41]. These INDEL multiplexes show great promise and particularly so because the alleles at a locus can be contained within short amplicons and are different but close in size. Thus, INDELs are appealing as they can be separated by capillary electrophoresis. Both of these multiplexes are informative but display relatively high F_{ST} values and thus are not the best suited for identity testing on a global scale. The selection process to develop INDEL multiplexes with a relatively low average F_{ST} value will require screening a large set of potential markers [see Kidd and colleagues 43, 44]. While there is a wealth of INDELs that can add to or improve upon the potential multiplexes, it may require searching for additional markers beyond known INDELs. Until now, INNULs were not amenable to PCR typing in a potentially robust manner. Because of the size difference of the null and insertion alleles, differential amplification plagued the development of assays with INNULs. This study demonstrates, for the first time, that such loci can be typed in a similar fashion to that of INDELs.

We did not develop a multiplex assay in this study. It did not make sense to devote resources to develop a mul-

tiplex assay when some INNULs will be better suited for identity testing and others will be better suited for bio-ancestral studies. Indeed, 3 of the INNULs were not applicable to a low F_{ST} multiplex. Before making a multiplex that could be used for human identification, it would be desirable to seek INNULs that apply well to human identity testing on a more global basis, that is, demonstrate high discrimination power and low inter-population diversity (e.g., low F_{ST}). The knowledge on INNUL variation in several populations is still nascent. Fortunately, the primer design described herein will facilitate screening sample populations.

Conclusions

This study demonstrates that, by utilizing the Mini-Primer strategy, INNUL markers, i.e., SINEs and LINEs, can be typed in a facile manner regardless of the size of the insertion element. The size of the amplicons for INNULs and the difference between allelic states can be reduced substantially such that these markers have utility for analyzing high- and low-quality human DNA samples in a standard PCR. Depending on the markers selected and the distribution of the alleles in global populations, INNULs can be selected for human identity testing or for bio-ancestral studies. Future work will entail selecting additional markers, developing multiplex assays, and evaluating the system(s) for forensic applications.

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