Examination of 20 Retrotransposable Polymorphic Insertion/Null (INNUL) markers for their utility in kinship testing using a commercial software program (LSAM)

AnniLauri Villeme*, BS[1,3] Gretchen Bartizal, MS[2,3] Carolyn R. Steffen, MS[3] Michael D. Coble, PhD[3]

[1] The George Washington University, Department of Forensic Sciences, 2100 Foxhall Road, NW, Washington D.C. 20052 (avilleme@gwmail.gwu.edu)

[2] King's College London, Life Sciences & Medicine, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH

[3] National Institute of Standards and Technology, Applied Genetics Group, 100 Bureau Dr., Gaithersburg, MD 20899-8314

Poster B142 – AAFS Annual Meeting
Las Vegas, NV, February 26, 2016

Forensic DNA testing has proven to be a powerful tool for criminal investigations and for identifying human remains via kinship analysis for cases involving mass disasters or missing persons. Short Tandem Repeat (STR) markers most often offer the highest degree of discrimination and speed of analysis by using PCR and capillary electrophoresis to generate DNA profiles. DNA testing for human remains identification are often challenging due to the presence of degraded DNA or inhibitors that affect the PCR reaction. To overcome some of these limitations, miniSTRs have been engineered to decrease the size of PCR amplicons to improve recovery of DNA fragments in the high molecular weight range of standard STR kits [1]. Additional marker systems such as SNPs, Insertion/Deletion markers (InDels), and mitochondrial DNA (mtDNA) have been successfully investigated or used for highly degraded samples.

Retrotransposable Elements (RE) are polymorphisms found in the human genome as RNA elements that have been reverse transcribed into specific loci as cDNA by use of retroposition [2]. RE include the ALU elements short interspersed nuclear elements (SINEs) and long interspersed nuclear elements (LINEs) [3]. These elements can be in two allelic states; either they are present in an individual's DNA as an insertion or absent as a null (INNULs). One advantage for targeting these markers is the ability to create small amplicon sizes for each marker (about 60-125bp in size) [4]. A commercially available kit (InnoTyper 21) containing 20 INNUL markers plus the sex determining marker, Amelogenin, is available (InnoGenomics, New Orleans, LA) and used for this study [4].

We first characterized the allele frequencies and population genetic parameters of the markers in a set of over 600 population samples [5,6]. To demonstrate the utility of the InnoTyper 21 multiplex kit in degraded samples, one sample was degraded into multiple, fixed fragment lengths and then analyzed for their statistical information content using the Random Match Probability for the InnoTyper 21 kit and two commercially available STR kits. Three kinship scenarios, (Two Father/Mother/Child trios and Grandmother/Uncle/Grandchild trio) were genotyped for testing where the father sample (scenarios 1 and 2) and grandchild sample (scenario 3) were artificially degraded using sonication to simulate degraded DNA. Samples were typed with the INNUL markers and a commercially available STR multiplex for determining the Random Match Probability and Kinship Index.

After considering the utility of the INNUL markers in degraded samples and for paternity and kinship analysis, a software package called LSAM - LISA (Laboratory Information System Applications) Statistical Analysis Module from Future Technologies Inc. (Fairfax, VA) was used for direct comparison statistics and to conduct pedigree statistics for the INNULs [7]. The program offered the ability to construct pedigree scenarios for kinship analysis. The results were compared from the software statistics to those determined by hand calculation. Overall, the INNUL markers were able to provide additional genetic information for samples that were highly degraded. The LSAM software program provided strong support for the concordance to produce accurate calculations for kinship analysis compared to hand calculation.

References

1 Coble, M., Butler, J. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. *Journal of Forensic Sciences, 20* (1), 43-53.
2 Cordaux, R., Srikanta, D., Lee, J., Stoneking, M., Batzer, M. (2007). In search of polymorphic Alu insertions with restricted geographic distributions. *Genomics, 90*, 154-158.
3 LaRue, B., Sinha, S., Montgomery, A., Thompson, R., Klaskala, L., Ge, J., King, J., Turnbough, M., Budowle, B. (2012). INNULs: A Novel Design Amplification Strategy for Retrotransposable Elements for Studying Population Variation. *Human Heredity, 74*, 27-35.
4 (2015). InnoTyper™ 21. InnoGenomics Technologies, LLC. Retrieved from http://innogenomics.com/products/innotyper-21/. Accessed July 16, 2015.
5 Bartizal, G. (2015). Population genetics and performance of Retrotransposable Insertion Polymorphism (RIP) markers for forensic testing. Unpublished manuscript.
6 Butler, J., Schoske, R., Vallone, P., Redman, J., Kline, M. (2003). Allele Frequencies for 15 Autosomal STR Loci on U.S. Caucasian, African American, and Hispanic Populations. *Journal of Forensic Science, 48*, (4), 908-911.
7 Future Technologies, Inc. LSAM | Lisa Statistical Analysis Module User Guide
8 Butler, J.M., Hill, C.R., Coble, M.D. (2012) Variability of new STR loci and kits in U.S. population groups. *Profiles in DNA*. Available at http://www.promega.com/resources/articles/profiles-in-dna/2012/variability-of-new-str-loci-and-kits-in-us-population-groups/

Use of NIST data for typing 660 U.S. Population Samples for INNUL Markers

Samples from 660 U.S. individuals were obtained from the Interstate Blood Bank, Inc. (Memphis, TN) and Millennium Biotech, Inc. (Ft. Lauderdale, FL) [5]. Samples were previously extracted and quantified at NIST. These samples were typed using the InnoTyper 21 kit. A DNA concentration of 0.5ng/µl was used in the ABI 9700 Thermocycler following kit protocol. Fragments were separated on the ABI 3130XL Genetic Analyzer, POP-4, 36 cm array, with a standard injection of 13s at 1.2kv. Analysis of the data was completed using GeneMapper ID-X v1.3. Statistical calculations were performed using PowerStats v12 (Promega), to calculate allele frequencies, power of discrimination (PD), polymorphism information content (PIC), typical paternity index (TPI), power of exclusion (PE), and match probability (MP). The Genetic Data Analysis software program, v1.0 (d16c), was used to calculate observed heterozygosity, expected heterozygosity, and the Hardy-Weinberg equilibrium exact test (HWE) [5].

A novel primer design was created to target the insertion/null alleles. There are two different reverse primers that will attach to the target (RN or RI) depending on if the insertion is present or not

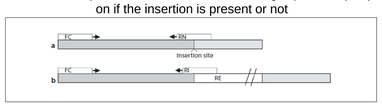


Figure 1: Mini-Primer design, LaRue et. al. (2015) and S. Karger AG, Basel

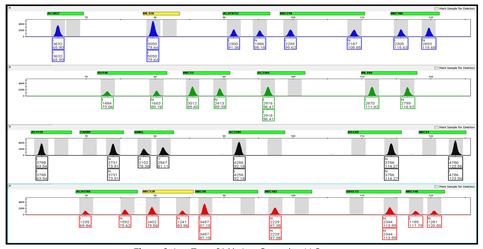


Figure 2: InnoTyper 21 kit, InnoGenomics, LLC

Chromosomal location of the InnoTyper 21 kit

Marker	Chromosome	Band
AC1141	3	3q11.2
AC2265	13	13q33.1
AC2305	13	13q13.3
AC4027	7	7q21.11
ACA1766	8	8q12.1
ALU79712	20	20p12.2
AMEL	X, Y	Xp22.1-22.3 Yp11.2
HS4.69	5	5q34
MLS09	1	1q25.3
MLS26	3	3p22.1
NBC10	4	4q31.21
NBC102	17	17q23.3
NBC106	21	21q22.2
NBC120	22	22q11.21
NBC13	16	16p12.1
NBC148	14	14q31.1
NBC216	7	7p14.1
NBC51	3	3q28
RG148	2	2q23.3
SB19.12	19	19q13.43
TARBP	1	1q42.2

Table1: INNUL chromosome location chart, InnoGenomics Technologies, LLC

Allele frequency tables for 660 U.S. NIST population samples found in the three most common U.S. population groups (African American, Caucasian, Hispanic).

U.S. Caucasian Population

Allele	AC4027	MLS26	ALU79712	NBC216	NBC106	RG148	NBC13	AC2265	MLS09	AC1141	TARBP	AC2305	HS4.69	NBC51	ACA1766	NBC120	NBC10	NBC102	SB19.12	NBC148
I	0.416	0.344	0.485	0.751	0.435	0.324	0.345	0.743	0.377	0.613	0.582	0.504	0.46	0.567	0.63	0.42	0.471	0.446	0.301	0.898
N	0.584	0.656	0.515	0.249	0.565	0.676	0.655	0.257	0.623	0.387	0.418	0.496	0.54	0.433	0.37	0.58	0.529	0.554	0.699	0.102

U.S. African American Population

Allele	AC4027	MLS26	ALU79712	NBC216	NBC106	RG148	NBC13	AC2265	MLS09	AC1141	TARBP	AC2305	HS4.69	NBC51	ACA1766	NBC120	NBC10	NBC102	SB19.12	NBC148
I	0.531	0.182	0.295	0.593	0.566	0.527	0.241	0.373	0.259	0.23	0.29	0.324	0.295	0.542	0.695	0.591	0.678	0.369	0.448	0.515
N	0.469	0.818	0.705	0.407	0.434	0.473	0.759	0.627	0.741	0.77	0.71	0.676	0.705	0.458	0.305	0.409	0.322	0.631	0.552	0.485

U.S. Hispanic Population

Allele	AC4027	MLS26	ALU79712	NBC216	NBC106	RG148	NBC13	AC2265	MLS09	AC1141	TARBP	AC2305	HS4.69	NBC51	ACA1766	NBC120	NBC10	NBC102	SB19.12	NBC148
I	0.521	0.351	0.382	0.514	0.396	0.371	0.396	0.679	0.368	0.682	0.414	0.482	0.339	0.564	0.679	0.536	0.489	0.521	0.275	0.818
N	0.479	0.649	0.618	0.486	0.6	0.629	0.604	0.321	0.632	0.318	0.586	0.518	0.661	0.436	0.321	0.464	0.511	0.479	0.725	0.182
OL					0.004															

I: Insertion; N: no insertion; OL: Off-Ladder

Table 2: INNUL allele frequencies, Bartizal (2015) of 259 African Americans, 261 Caucasians, and 140 Hispanics

Degraded DNA Analysis (single source)

- A single sample was degraded using a Covaris S2 Sonicator into 75, 100, 150, 200, and 250 bp fragments.
- Degraded samples were diluted to the suggested input quantity according to manufacturer's guidelines (InnoTyper 21 kit input value of 0.4ng/µl, GlobalFiler at 1ng/µl, and MiniFiler at 0.5ng/µl).
- Samples were amplified in triplicate with the Applied Biosystems 9700 Thermocycler and fragments were separated on the Applied Biosystems 3500XL Genetic Analyzer
- Results were analyzed on the GeneMapper ID-X software system
- RMP Statistics were calculated for all kits using the LSAM software. Profiles showing a marker with a single allele used the statistical formula "2p".

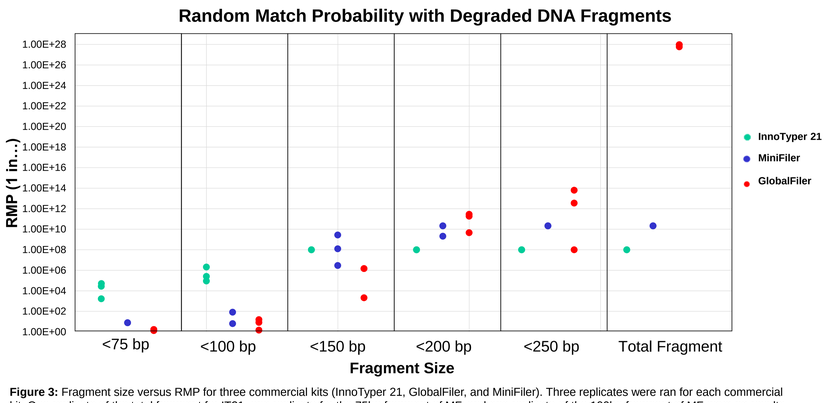


Figure 3: Fragment size versus RMP for three commercial kits (InnoTyper 21, GlobalFiler, and MiniFiler). Three replicates were ran for each commercial kit. One replicate of the total fragment for IT21, one replicate for the 75bp fragment of MF, and one replicate of the 100bp fragment of MF gave no results.

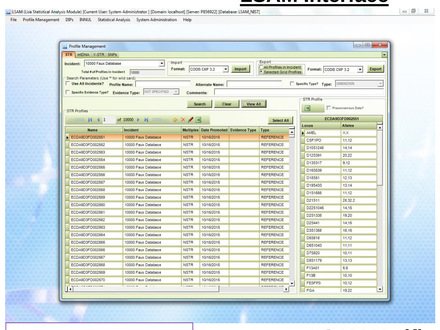
Disclaimer

Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments, or equipment identified are necessarily the best available for the purpose. The authors would like to thank Sudhir Sinha, Gina Pineda, Hiromi Brown, and Jonathan Tabak of InnoGenomics for technical advice and logistics. We also acknowledge the NIST Applied Genetics Group; Kevin Kiesler, Erica Romsos, Katherine Gettings, Margaret Kline, and Peter Vallone for guidance and providing essential data for the research project, as well as providing the initial extraction and quantitation of the NIST U.S. population samples.

LSAM Software system

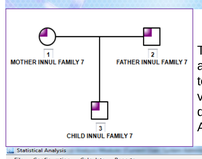
LSAM is the **LISA (Laboratory Information System Applications) Statistical Analysis Module**. It is a highly sophisticated software program that has been designed by Future Technologies Inc. (Fairfax, VA), to be able to store one million profiles of data for statistical and comparison purposes [7]. The software is being used by the Armed Forces DNA Identification Laboratory (AFDIL) for storage of data and analysis in missing persons cases. NIST is using the software program to store all of their published data covering a wide range of genetic marker systems for quick access and statistical calculations. The software program works in a MS Windows based system that was written in C#/.NET and can provide support for autosomal STR, Y-STR, mtDNA, INNUL, InDel, and SNP markers [7]. Capabilities of LSAM include managing profiles, upkeep of population groups, allele frequency studies, lists of multiplex definitions, core statistical calculations, calculations for kinship analysis investigations, direct match comparisons, and much more. Core statistics such as Random Match Probability (RMP), Combined Probability of Exclusion (CPE), and Likelihood Ratios (LR) can be calculated as well as mitochondrial direct match searching against the reference FBI database and Y-STR analysis from LSAM's internal default population database from UNT. Additional mitochondrial and Y-STR databases can be added to the program. LSAM also offers the ability to construct pedigree scenarios for kinship analysis and can incorporate different marker systems into the analysis.

LSAM Interface

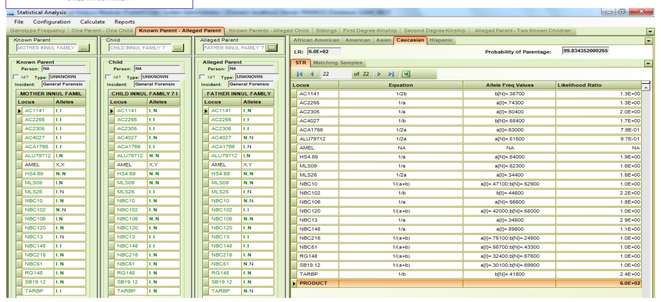


The LSAM interface shows tabs for File, Profile management, DIPs (InDels), INNUL, Statistical Analysis, System Administration, and Help. These tabs can be explored to execute the functionalities that LSAM maintains. Profile Management houses the list of all of the profiles that have been imported into LSAM. Statistical calculations for INNULs can be made under the INNUL tab under Core Stats. Access to the analytical configuration and marker or multiplex information can be found in the statistical analysis tab. The analytical configuration utilized in this study used the Recommendation 4.1 of NRCII for a theta correction of 0.01.

LSAM Verification



The LSAM Family Verification samples were formulated manually to represent a typical mother/father/child family trio. In LSAM, each separate profile was run to determine the genotype frequency and the results of the statistics were verified by hand calculations in Microsoft Excel. Following the genotype determination, the constructed family samples were run as Known Parent-Alleged Parent in LSAM.



EXCEL

	Genotype		Genotype		Genotype		Caucasian			African American			Hispanic		
Locus	Mother		Father		Child		Equation	Allele Freq	LR	Allele Freq		LR	Allele Freq		LR
AC4027	I	I	N	N	I	N	1/Pr(N)	0.416	0.584	1.712	0.531	0.469	2.132	0.521	0.479	2.088
MLS26	I	N	I	N	I	I	0.5/Pr(I)	0.344	0.344	1.453	0.182	0.182	2.747	0.351	0.351	1.425
ALU79712	I	N	I	N	N	N	0.5/Pr(N)	0.515	0.515	0.971	0.705	0.705	0.709	0.618	0.618	0.809
NBC216	I	N	I	N	I	N	1/Pr(N)+Pr(I)	0.751	0.249	1.000	0.593	0.407	1.000	0.514	0.486	1.000
NBC106	I	N	N	N	N	N	1/Pr(N)	0.565	0.565	1.770	0.434	0.434	2.304	0.6	0.6	1.667
RG148	I	N	I	N	I	N	1/Pr(N)+Pr(I)	0.324	0.676	1.000	0.527	0.473	1.000	0.371	0.629	1.000
NBC13	I	N	I	I	I	I	1/Pr(I)	0.345	0.345	2.899	0.241	0.241	4.149	0.396	0.396	2.525
AC2265	I	I	I	I	I	I	1/Pr(I)	0.743	0.743	1.346	0.373	0.373	2.681	0.679	0.679	1.473
MLS09	I	N	N	N	N	N	1/Pr(N)	0.623	0.623	1.605	0.741	0.741	1.350	0.632	0.632	1.582
AC1141	I	I	I	N	I	N	0.5/Pr(N)	0.613	0.387	1.292	0.23	0.77	0.649	0.682	0.318	1.572
TARBP	I	I	N	N	I	N	1/Pr(N)	0.582	0.418	2.392	0.29	0.71	1.408	0.414	0.586	1.706
AMEL	X	X	X	Y	X	Y		NA			NA			NA		
AC2305	I	I	I	I	I	I	1/Pr(I)	0.504	0.504	1.984	0.324	0.324	3.086	0.482	0.482	2.075
HS4.69	N	N	N	N	N	N	1/Pr(N)	0.54	0.54	1.852	0.705	0.705	1.418	0.661	0.661	1.513
NBC51	I	N	N	N	I	N	1/Pr(N)+Pr(I)	0.567	0.433	1.000	0.542	0.458	1.000	0.564	0.436	1.000
ACA1766	I	N	I	N	I	I	0.5/Pr(I)	0.63	0.63	0.794	0.695	0.695	0.719	0.679	0.679	0.736
NBC120	I	N	I	N	I	N	1/Pr(N)+Pr(I)	0.42	0.58	1.000	0.591	0.409	1.000	0.536	0.464	1.000
NBC10	I	N	N	N	I	N	1/Pr(N)+Pr(I)	0.471	0.529	1.000	0.678	0.322	1.000	0.489	0.511	1.000
NBC102	N	N	I	I	I	N	1/Pr(I)	0.446	0.554	2.242	0.369	0.631	2.710	0.521	0.479	1.919
SB19.12	I	N	I	N	I	N	1/Pr(N)+Pr(I)	0.301	0.699	1.000	0.448	0.552	1.000	0.275	0.725	1.000
NBC148	I	I	I	I	I	I	1/Pr(I)	0.898	0.898	1.114	0.515	0.515	1.942	0.818	0.818	1.222
Product										**6.0E+2**			**2.2E+3**			**3.4E+2**
Probability of Paternity										99.834			99.954			99.710

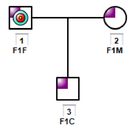
LSAM

	Genotype		Genotype		Genotype		Caucasian			African American			Hispanic		
Locus	Mother		Father		Child		Equation	Allele Freq	LR	Allele Freq		LR	Allele Freq		LR
AC1141	I	I	N	N	I	N	1/2b	b[N]=.38700	1.3E+00	b[N]=.77000		6.5E-01	b[N]=.31800		1.6E+00
AC2265	I	N	I	N	I	I	1/a	a[I]=.74300	1.3E+00	a[I]=.37300		2.7E+00	a[I]=.67900		1.5E+00
AC2305	I	N	I	N	N	N	1/a	a[I]=.50400	2.0E+00	a[I]=.32400		3.1E+00	a[I]=.48200		2.1E+00
AC4027	I	N	I	N	I	N	1/b	b[N]=.58400	1.7E+00	b[N]=.46900		2.1E+00	b[N]=.47900		2.1E+00
ACA1766	I	N	N	N	N	N	1/2a	a[I]=.63000	7.9E-01	a[I]=.69500		7.2E-01	a[I]=.67900		7.4E-01
ALU79712	I	N	I	N	I	N	1/2a	a[N]=.51500	9.7E-01	a[N]=.70500		7.1E-01	a[N]=.61800		8.1E-01
AMEL	I	N	I	I	I	I	NA	NA	NA	NA		NA	NA		NA
HS4.69	I	I	I	I	I	I	1/a	a[N]=.54000	1.9E+00	a[N]=.70500		1.4E+00	a[N]=.66100		1.5E+00
MLS09	I	N	N	N	N	N	1/a	a[N]=.62300	1.6E+00	a[N]=.74100		1.3E+00	a[N]=.63200		1.6E+00
MLS26	I	I	I	I	I	I	1/2a	a[I]=.34400	1.5E+00	a[I]=.18200		2.7E+00	a[I]=.35100		1.4E+00
NBC10	I	N	N	I	I	N	1/(a+b)	a[I]=.47100;b[N]=.52900	1.0E+00	a[I]=.67800;b[N]=.32200		1.0E+00	a[I]=.48900;b[N]=.51100		1.0E+00
NBC102	X	X	X	Y	X	Y	1/b	b[I]=.44600	2.2E+00	b[I]=.36900		2.7E+00	b[I]=.52100		1.9E+00
NBC106	I	I	I	I	I	I	1/a	a[N]=.56500	1.8E+00	a[N]=.43400		2.3E+00	a[N]=.60000		1.7E+00
NBC120	N	N	N	N	N	N	1/(a+b)	a[I]=.42000;b[N]=.58000	1.0E+00	a[I]=.59100;b[N]=.40900		1.0E+00	a[I]=.53600;b[N]=.46400		1.0E+00
NBC13	I	N	N	N	I	N	1/a	a[I]=.34500	2.9E+00	a[I]=.24100		4.1E+00	a[I]=.39600		2.5E+00
NBC148	I	I	I	N	I	I	1/a	a[I]=.89800	1.1E+00	a[I]=.51500		1.9E+00	a[I]=.81800		1.2E+00
NBC216	I	N	I	N	I	N	1/(a+b)	a[I]=.75100;b[N]=.24900	1.0E+00	a[I]=.59300;b[N]=.40700		1.0E+00	a[I]=.51400;b[N]=.48600		1.0E+00
NBC51	I	N	N	N	I	N	1/(a+b)	a[I]=.56700;b[N]=.43300	1.0E+00	a[I]=.54200;b[N]=.45800		1.0E+00	a[I]=.56400;b[N]=.43600		1.0E+00
RG148	N	N	I	I	I	N	1/(a+b)	a[I]=.32400;b[N]=.67600	1.0E+00	a[I]=.52700;b[N]=.47300		1.0E+00	a[I]=.37100;b[N]=.62900		1.0E+00
SB19.12	I	N	I	N	I	N	1/(a+b)	a[I]=.30100;b[N]=.69900	1.0E+00	a[I]=.44800;b[N]=.55200		1.0E+00	a[I]=.27500;b[N]=.72500		1.0E+00
TARBP	I	I	I	I	I	I	1/b	b[N]=.41800	2.4E+00	b[N]=.71000		1.4E+00	b[N]=.58600		1.7E+00
PRODUCT									**6.0E+02**			**2.2E+03**			**3.4E+02**
Probability of Paternity									99.834%			99.954%			99.710%

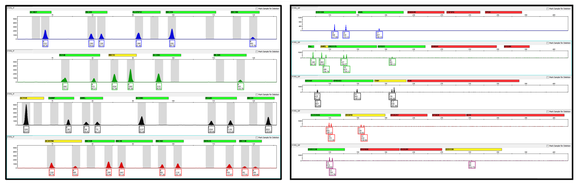
Degraded DNA Analysis – Family Samples (Scenario 1, 2, and 3)

- Eight samples (F1M, F1F, F1C, F2M, F2F, F2C/F3C, F3U, F3G) were diluted to 0.4ng/µl, amplified with InnoTyper 21 in the Applied Biosystems 9700 Thermocycler, and separated in the Applied Biosystems 3500XL Genetic Analyzer
- Results were analyzed on the GeneMapper ID-X software system
- Statistics were calculated in the LSAM software
- Samples: F1F, F2F and F3C were then degraded with the Covaris Sonicator, amplified with InnoTyper 21 and GlobalFiler, and separated.
- Statistics were calculated in the LSAM software to illustrate the effect degradation has the INNUL markers in kinship analysis.

Scenario 1

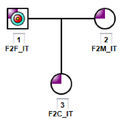


Scenario 1		
System	Total Fragment LR	Degraded Fragment LR
GlobalFiler	2.1E+14	430
InnoTyper 21	120	120

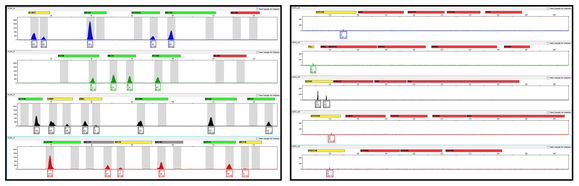


Scenario 1 consists of a Mother/Father/Child Trio. The father sample was degraded using sonication to assess the benefits of INNULs on degraded kinship statistics compared to the standard autosomal STR statistics. The results illustrate that the total fragment INNUL markers provide a combined kinship index (LR) of 120 for the Caucasian population and autosomal STR markers provide a combined kinship index of 2.1E+14. The degraded INNUL markers provide a combined kinship index of 120 and the autosomal STR markers provide a combined kinship index of 430.

Scenario 2

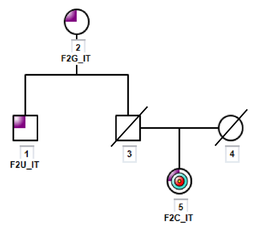


Scenario 2		
System	Total Fragment LR	Degraded Fragment LR
GlobalFiler	8.6E+16	26
InnoTyper 21	12000	3200

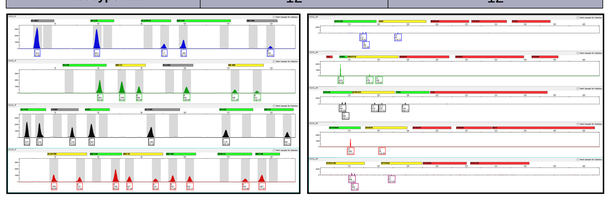


Scenario 2 consists of a Mother/Father/Child Trio. The father sample was degraded using sonication to assess the benefits of INNULs on degraded kinship statistics compared to the standard autosomal STR statistics. The results illustrate that the total fragment INNUL markers provide a combined kinship index of 12000 for the Caucasian population and autosomal STR markers provide a combined kinship index of 8.6E+16. The degraded INNUL markers provide a combined kinship index of 3200 and the autosomal STR markers provide a combined kinship index of 26.

Scenario 3



Scenario 3		
System	Total Fragment LR	Degraded Fragment LR
GlobalFiler	25000	81
InnoTyper 21	12	12



Scenario 3 consists of a situation where the Grandmother, Uncle, and Grandchild are present. In this scenario, the grandchild is the person of interest. The grandchild's sample was degraded using sonication. The results illustrate that the total fragment INNUL markers provide a combined kinship index of 12 for the Caucasian population and autosomal STR markers provide a combined kinship index of 25000. The degraded INNUL markers provide a combined kinship index of 12 and the autosomal STR markers provide a combined kinship index of 81.

Summary

The analysis of the degraded sample in Figure 3 illustrates the random match probability (RMP) of the Caucasian population of the InnoTyper 21, MiniFiler and GlobalFiler kit. InnoTyper 21 proved to be the most discriminative in the samples <75, <100, <150 base pairs with the exception of one replicate of the MiniFiler in the <150 base pair sample. The maximum RMP value that was calculated for the InnoTyper 21 kit was 9.7E+07. This was reached for all samples greater than 150bp. The MiniFiler kit reached a maximum RMP of 2.1E+10 in samples greater than 200bp. The GlobalFiler kit reached a maximum RMP value of 9.4E+27 for only the total fragment sample. The miniSTRs in the MiniFiler kit were useful for providing information in samples degraded at 150bp or greater. With samples degraded to less than 150 bp, the InnoTyper 21 kit produced higher discrimination values than the autosomal STR kits tested.

The analysis of the family samples illustrates the LR for a degraded trio scenario and a degraded complicated kinship scenario. With conventional parent-child trios observed in scenarios 1 and 2, autosomal STRs provided increased LR values compared to the InnoTyper 21 kit. If, the same scenarios are subjected to degradation expected in missing persons cases where the remains have been challenged by microorganisms or the elements, the smaller target amplicons in the InnoTyper 21 kit could provide more information compared to the STR kit. On average, the GlobalFiler kit loses around 13-19 loci. The additional information provided by the InnoTyper 21 kit may be helpful in missing persons, degraded, or complicated DNA scenarios. Research is underway to assess the amount of genetic association between the INNUL markers and forensic STR markers.

LSAM was able to provide accurate and reliable statistics for STR and INNUL calculations in a simple, clear, and consistent format. In the manual calculations, LSAM was able to produce values concordant to those produced by hand. Therefore the system can calculate individual profiles and kinship analysis reliably and efficiently. LSAM has been useful to support and organize all profiles entered into the system accurately.