Developmental validation of InnoTyper® 21, a nuclear DNA typing system based on retrotransposable element polymorphisms for degraded forensic samples

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Introduction

The InnoTyper® 21 kit, developed for use in human identification, is a small amplicon DNA typing system for challenging forensic samples that is compatible with currently used PCR/CE instrument platforms. It contains 20 bi-allelic Alu[1] retrotransposon markers and Amelogenin for gender identification. This is the first human DNA typing system that has been developed utilizing di-morphic Alu-insertion polymorphisms[2]. Due to the insertion and null/no-insertion allelic states, the markers have been termed INNUL[3]. INNUL markers are suitable for human identification because they are stable inheritance dependent polymorphisms that are not deleted, and the likelihood of parallel independent insertions is essentially zero. A novel "mini-primer" design strategy (Fig. 1) allows the size differences between insertion and no-insertion alleles to be less than 10 bp, despite the fact that Alu elements are ~300 bp long; also, all 21 markers are between 60 bp to 124 bp, which provides utility for typing degraded DNA samples.

Figure 1. Primer design strategy to type insert and native (or null) alleles with similar but not exact amplicon size differences between the two allelic states. The strategy employs a common forward primer with fluorescent label at the 5′ end and two specific reverse primers to amplify either insertion allele or null allele or both in the case of a heterozygote individual.

The kit is a 5-dye system with 20 Alu markers and Amelogenin labeled in FAM, JOE, TAMRA, and ROX, with TGI-Orange used for the size standard (Fig. 2). The sizes of the resultant alleles range between 63 and 123 bp, making their small size ideal for degraded DNA typing. Comparison with other currently available systems reveals the InnoTyper kit contains a much smaller overall size range (Fig. 3).

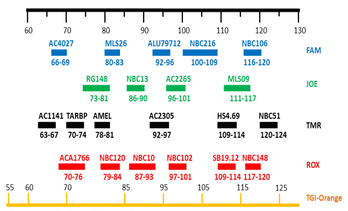


Figure 2. InnoTyper 21 markers and dye configuration.

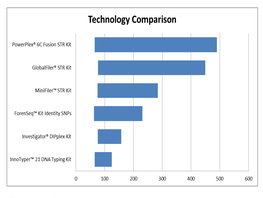


Figure 3. Base pair size comparison with other human identification kits.

Results

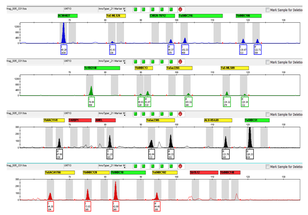
Sensitivity Study

Table 1. Effects of varying input DNA on profile recovery, peak height, and peak height ratio.

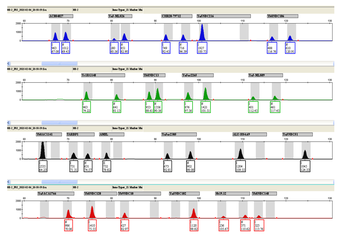
DNA Input (pg)	Mean Profile Recovery (%)		Mean Peak Height (RFU)		Mean Peak Height Ratio
	NIST A	1212	NIST A	1212	
400	100.0 ± 0.0	100.0 ± 0.0	1381 ± 95	1112 ± 216	0.84 ± 0.11
200	100.0 ± 0.0	100.0 ± 0.0	738 ± 40	601 ± 47	0.77 ± 0.14
100	100.0 ± 0.0	100.0 ± 0.0	381 ± 34	301 ± 59	0.66 ± 0.16
50	95.2 ± 2.3	93.7 ± 1.4	183 ± 20	155 ± 13	0.67 ± 0.19
25	79.4 ± 9.9	71.4 ± 10.4	107 ± 12	106 ± 4	0.68 ± 0.20
12.5	40.5 ± 10.4	48.4 ± 9.9	81 ± 4	85 ± 1	0.62 ± 0.11
6.25	11.9 ± 4.1	20.6 ± 5.5	63 ± 11	66 ± 8	N/A

InnoTyper® 21 is highly sensitive and can provide an almost complete 21 marker profile from as low as 50 pg of total DNA

Non Probative Samples Study



50-80Year Old Human Remains
17-locus profile with an average RFU of 285, and a statistical profile frequency of 1 in 14 billion Caucasians and 1 in 254 million African American. (The sample previously produced no result with STR testing and inconclusive result with mtDNA testing.)



2 cm Rootless Hair Shaft
Total DNA = 67 pg
100% Allele Recovery
Degradation Index = 5

Population Database Study

Table 2. InnoTyper® 21 allele frequencies, heterozygosity, random match probability and probability of exclusion for three major population groups. (Additional populations not shown.)

	African American (N=207)					Southwest Hispanic (N=40)					Caucasian (N=301)				
Allele	Freq [1]	H_o	HWE (p-value)[1,2]	RMP	PE	Freq [1]	H_o	HWE (p-value)[1,2]	RMP	PE	Freq [1]	H_o	HWE (p-value)[1,2]	RMP	PE
AC4027	0.53865	0.53623	0.26906	0.37650	0.18675	0.65000	0.35000	0.16031	0.40050	0.17574	0.42857	0.49834	0.81469	0.38030	0.18492
NBC216	0.59662	0.50725	0.17844	0.38490	0.18275	0.53750	0.42500	1.00000	0.37640	0.18679	0.71090	0.35216	0.20531	0.44540	0.15800
MLS26	0.14976	0.23188	0.86844	0.58790	0.11114	0.51250	0.52500	0.56656	0.37520	0.18742	0.38718	0.48837	0.56000	0.40160	0.17528
ALU79712	0.30918	0.43478	0.47125	0.41940	0.16797	0.50000	0.45000	0.36031	0.37500	0.18750	0.48007	0.48173	0.06813	0.37540	0.18730
NBC106	0.57488	0.43478	0.11906	0.38080	0.18466	0.37500	0.65000	0.02531	0.39210	0.17944	0.44020	0.52159	0.34375	0.37870	0.18570
RG148	0.53623	0.50242	1.00000	0.37630	0.18684	0.36250	0.47500	1.00000	0.39610	0.17769	0.30233	0.43854	0.57563	0.42320	0.16644
NBC13	0.21981	0.13333	0.67656	0.49050	0.14208	0.36250	0.47500	1.00000	0.39610	0.17769	0.35714	0.47508	0.59531	0.39790	0.17688
AC2265	0.39130	0.46377	0.77188	0.38770	0.18145	0.76250	0.27500	0.17750	0.47240	0.14830	0.73754	0.35880	0.22750	0.45050	0.15610
ML509	0.23671	0.36715	1.00000	0.47120	0.14803	0.38750	0.47500	1.00000	0.38860	0.18101	0.40532	0.47176	0.72469	0.38440	0.18294
AC1141	0.22947	0.34300	0.68969	0.48030	0.14556	0.71250	0.37500	0.68813	0.43240	0.16288	0.60797	0.52492	0.09125	0.38750	0.18153
TARBP	0.28502	0.37681	0.30751	0.43490	0.16225	0.36250	0.42500	0.73250	0.39610	0.17769	0.37475	0.50498	0.62719	0.38080	0.18467
AC2305	0.30676	0.42995	1.00000	0.42070	0.16744	0.66250	0.37500	0.32063	0.40560	0.17360	0.57807	0.47176	0.56688	0.38130	0.18442
HS4.69	0.31884	0.41546	0.52938	0.41430	0.17001	0.20000	0.30000	0.64031	0.51360	0.13440	0.38704	0.43522	0.14438	0.38870	0.18096
NBC51	0.59420	0.45411	0.38625	0.38480	0.18298	0.53750	0.52500	0.52438	0.37640	0.18679	0.52638	0.48837	0.73500	0.37570	0.18715
NBC102	0.39614	0.45411	0.73250	0.38650	0.18199	0.58750	0.42500	1.00000	0.38300	0.18361	0.40698	0.50166	0.62781	0.38410	0.18310
NBC120	0.59662	0.46860	0.76969	0.38490	0.18275	0.53750	0.37500	0.12281	0.37640	0.18679	0.41030	0.44186	0.15844	0.38340	0.18341
NBC10	0.65942	0.44928	1.00000	0.40430	0.17415	0.48750	0.52500	1.00000	0.37520	0.18742	0.43189	0.51163	0.48511	0.37980	0.18516
ACA1766	0.72222	0.39130	0.47844	0.43900	0.16038	0.80000	0.35000	0.51344	0.51360	0.13440	0.62791	0.45183	0.55438	0.39300	0.17905
SB19.12	0.39614	0.48309	1.00000	0.38650	0.18199	0.17500	0.30000	1.00000	0.54760	0.12353	0.30399	0.40199	0.42969	0.42230	0.16681
NBC148	0.54348	0.51691	0.57969	0.37690	0.18655	0.91250	0.12500	0.25438	0.71890	0.07347	0.87542	0.21595	0.79813	0.63510	0.09717
Overall			**2.47 E-08**	**0.9757**				**3.52 E-08**	**0.9740**				**1.37 E-08**	**0.9784**	

Freq [1]= Frequency of Insertion Allele; Ho= Observed Heterozygosity; RMP= Random Match Probability; PE= Probability of Exclusion
1. α-level of 0.05 is adjusted from 0.05 to 0.0025 when corrected for multiple tests (Bonferroni's correction)
2. Calculated using GDA software[4].

Degradation Study: Effects of Fragmentation Level

Table 3. Effects of degradation on profile recoveries and peak heights of InnoTyper® 21, MiniFiler®, and Identifiler® Plus. 200 pg of degraded DNA samples were amplified in triplicate (based on the "short" quantitation value from InnoQuant®)

Degradation Index (DI)	Mean Profile Recovery (%)			Degradation Index (DI)	Mean Peak Height (RFU)		
	InnoTyper 21	MiniFiler	Identifiler Plus		InnoTyper 21	MiniFiler	Identifiler Plus
0.89	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	0.89	596 ± 20	963 ± 91	530 ± 17
2.16	100.0 ± 0.0	100.0 ± 0.0	64.6 ± 4.8	2.16	346 ± 78	397 ± 58	185 ± 6
2.48	100.0 ± 0.0	100.0 ± 0.0	62.5 ± 0.0	2.48	360 ± 46	336 ± 62	181 ± 19
13.14	99.2 ± 1.4	83.1 ± 5.9	39.6 ± 1.8	13.14	312 ± 34	236 ± 18	137 ± 48
15.16	99.2 ± 1.4	87.0 ± 6.4	33.3 ± 3.6	15.16	335 ± 53	249 ± 18	122 ± 21
62.42	96.8 ± 1.4	72.2 ± 0.0	13.5 ± 1.8	62.42	298 ± 16	218 ± 16	111 ± 14
66.16	98.4 ± 2.7	75.9 ± 3.2	16.7 ± 3.6	66.16	269 ± 33	234 ± 34	102 ± 15
75.16	96.0 ± 3.6	70.4 ± 3.2	14.6 ± 4.8	75.16	317 ± 27	222 ± 13	120 ± 24
241.69	81 ± 7.2	63.0 ± 3.2	4.2 ± 3.6	241.69	249 ± 5	148 ± 11	80 ± 75

Table 4. Heat map of InnoTyper® 21 and Identifiler® Plus degradation study results. Alleles are listed from the smallest to the largest base pair size. The table indicates the number of replicates (out of three replicate amplifications of each sample) exhibiting allele dropout for a particular allele. Green cells indicate no dropout was observed.

InnoTyper® 21

Marker	Allele	Allele Size (bp)	Degradation Index 0.89	2.16	2.48	13.14	15.16	62.42	66.16	75.16	241.69
AC1141	1	**63.25**									
AC1141	N	**66.11**									
AC4027	1	**66.18**									
AC4027	N	**68.57**									
ACA1766	1	**69.32**									
TARBP	1	**70.24**									
RG148	1	**73.38**									
ACA1766	N	**75.11**									
AMEL	X	**77.95**									
NBC120	1	**78.98**									
MLS26	1	**79.92**									
RG148	N	**80.64**									
MLS26	N	**82.38**									
NBC13	1	**85.45**									
NBC13	N	**89.48**									
NBC10	N	**92.87**									
ALU79712	N	**95.48**									
AC2265	1	**96.46**							1		
NBC102	N	**96.86**									
AC2305	N	**97.03**									
NBC216	1	**99.81**									
AC2265	N	**100.56**									1
SB19.12	1	**108.33**									2
HS4.69	1	**108.83**									1
SB19.12	N	**113.25**									2
HS4.69	N	**113.67**									1
NBC106	1	**115.91**						1			2
ML509	N	**116.73**									1
NBC148	1	**117.23**							1		3
NBC51	N	**119.46**									2
NBC106	N	**119.95**				3	1	1	1		3
NBC148	N	**119.95**		1	1				2	1	

Identifiler® Plus

Marker	Allele	Allele Size (bp)	Degradation Index 0.89	2.16	2.48	13.14	15.16	62.42	66.16	75.16	241.69
AMEL	X	**105.67**									1
D19S433	13	**116.79**						1	3	3	3
D3S1358	14	**118.87**						1			3
D3S1358	15	**122.84**						1	2	1	3
D19S433	15	**124.89**					1	3	2	3	3
D8S1179	10	**130.22**					3	1	1	1	3
D8S1179	14	**147.45**			2	1	2	2	2	3	3
D5S818	11	**150.68**				1	3	2	3	3	3
vWA	16	**173.99**			1	1	3	3	3	3	3
vWA	18	**182.07**			2	3	3	3	3	3	3
TH01	9.3	**185.5**				1	3	3	3	3	3
D21S11	28	**199.76**			1	3	3	3	3	3	3
D21S11	32.2	**217.71**		2	2	3	3	3	3	3	3
D13S317	11	**227.94**				3	3	3	3	3	3
FGA	21	**229.72**		3	2	3	3	3	3	3	3
TPOX	11	**241.51**				2	3	3	3	3	3
FGA	24	**241.85**		3	2	3	3	3	3	3	3
D7S820	9	**266.64**		3	3	3	3	3	3	3	3
D7S820	10	**270.67**		3	3	3	3	3	3	3	3
D16S539	11	**275.78**		1	2	3	3	3	3	3	3
D18S51	12	**281.7**		3	3	3	3	3	3	3	3
D16S539	13	**283.83**		1	1	3	3	3	3	3	3
D2S1338	17	**314.23**		3	3	3	3	3	3	3	3
D18S51	20	**314.59**		3	3	3	3	3	3	3	3
D2S1338	19	**322.37**		3	2	3	3	3	3	3	3
CSF1PO	11	**324.11**		3	3	3	3	3	3	3	3
CSF1PO	12	**328.15**		3	3	3	3	3	3	3	3

InnoTyper® 21 was able to recover over 95% of the profile up to a DI of 75.16, which was ~ 1.4 and ~ 6.6 times higher than what could be achieved with MiniFiler® and Identifiler® Plus, respectively.

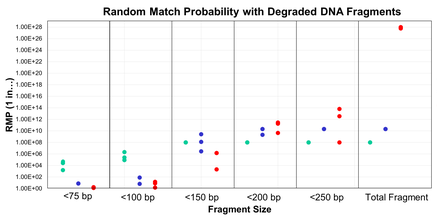


Figure 4. Fragment size versus Random Match Probability for three commercial kits (InnoTyper® 21, GlobalFiler®, and MiniFiler®). A single sample was degraded using a Covaris S2 Sonicator into 75, 100, 150, 200, and 250 bp fragments. Three replicates were run for each commercial kit.[5] (Data courtesy of the NIST Applied Genetics Group.)

Samples degraded to fragments <150 bp in size: InnoTyper® 21 yields the highest RMP

Degradation Study: Effects of Input DNA Amount

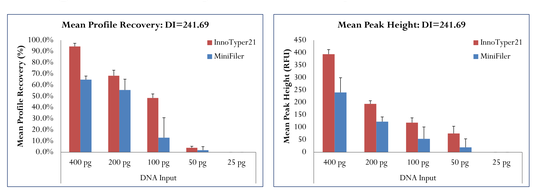


Figure 5. Effects of degradation and template concentrations on profile recoveries and peak heights of InnoTyper® 21 and MiniFiler®.

Materials and Methods

- qPCR quantitation: The InnoQuant® kit was used for all DNA quantitations. The kit contains 2 autosomal targets: a "short" Alu based target of 80 bp in size, and a "long" target from a separate retrotransposon of 207 bp in size. Real-time PCR reactions were processed on the AB 7500 Real-Time PCR System using Agilent Technologies Brilliant Multiplex QPCR Master Mix as follows: 10 min at 95°C; and 32 cycles of: 15 sec at 95°C, and 2 min at 61°C. Degradation Index (DI) was determined by the ratio of the short quantity value and the long quantity value (DI = [short]/[long]).
- InnoTyper® 21 amplification: Primers were designed for 20 Alu markers and AMEL in a multiplex assay. PCR was performed on an ABI 9700 as follows: 15 min at 95°C; 31 or 32 cycles of: 30 sec at 95°C, 30 sec at 58°C and 1 min at 72°C; one cycle of 60°C for 1 hour.
- Capillary electrophoresis: AB 3130 Genetic Analyzer was utilized with POP4. Data analysis was performed using GeneMapper® and/or GeneMapper® ID-X.

Other Results

- Accuracy, Precision, and Reproducibility: The deviations of the sample allele sizes from the allelic ladder sizes were all less than ±0.3 bp. The genotypes of the DNA samples amplified in triplicate produced the same profiles.
- Species Specificity: There were no peaks produced with DNA from non-primate species. Chimpanzee and to a lesser extent orangutan and green monkey yielded partial profiles.
- Inhibition Study: A full profile was obtained in the presence of hematin ≤ 45 μM, melanin up to 10 ng/μL (the highest amount tested), or humic acid ≤ 20 ng/μL with 400 pg of DNA input. A partial profile was observed with hematin up to 60 μM or humic acid up to 30 ng/μL.

Concluding Summary

The InnoTyper® 21 kit is a useful and robust complement to conventional STR kits and is an appropriate alternative when attempting to profile challenging single source samples, such as degraded human remains, hair shafts with no root, paraffin embedded tissues and other sample types where STR testing has failed to generate a useful profile.

Additionally, the InnoTyper® 21 kit is more discriminating than mtDNA sequencing, which until now was one of the few options for characterizing extremely degraded single source samples. Thus, a forensic analyst has alternatives in deciding which typing system to use, especially when confronted with a compromised DNA sample that yields sufficient DNA only for a single analysis.

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