Implementation of a 21-locus Panel for Human Relationship Testing

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ABSTRACT : In the United States, most relationship testing laboratories utilize markers standardized for forensic use due to the commercial availability of large multiplexes. These systems generally include 13 core CODIS STR loci (D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, CSF1PO, D16S539, D7S820, D13S317 and D5S818) along with the gender determination locus, Amelogenin. In order to increase the power of relationship testing, we evaluated seven STR loci: F13A01, FESFPS, F13B, LPL, Penta C, Penta D and Penta E. Amplification was performed with two fluorescently-labeled multiplexes configured with two overlapping STR loci. The overlapping loci provide a powerful quality control check of the testing process when used in conjunction with two independent DNA extractions. Population studies were performed to further document allele frequencies and to determine the recombination rates for the linked loci Penta E and FESFPS. The recombination rate between Penta E and FESFPS was characterized with >900 cases containing a mother, multiple children, and an alleged father. Penta E-FES haplotype frequency tables have been developed for Caucasians, African Americans, and Hispanics. Results of this 21-locus panel show a significant increase in the typical paternity index over the 13 core STR loci. Utilization of these 21 markers represents a significant increase in genotype information over the 13 core loci.

METHODOLOGY : Buccal swabs were routinely collected as a test sample. DNA was isolated using a proprietary automated magnetic bead extraction. The DNA was quantified with PicoGreen®. Amplification setup was done with automated liquid handlers and consisted of Promega Custom master mixes, water and genomic DNA in 384-well PCR plates. Samples were amplified in Applied Biosystems 9700 thermal cyclers using Promega's PowerPlex® protocol. Cycles were optimized to 10/20. Following amplification, PCR product was mixed with formamide and ILS600 size standard, heat denatured and chilled in ABI 9700 thermal cyclers. Electrophoresis was carried out on Applied Biosystems 3100 genetic analyzers using a 1kV-10sec injection time. Results were analyzed with GeneMapper ID software v3.2.1. Combined Paternity Indices (CPI) values were calculated using proprietary software and LabCorp's in-house allele frequencies. Cases with complete profiles for every individual in all tested loci were used in the analysis. For cases containing mutations, a mutation paternity index was calculated and incorporated into the combined paternity index. The recombination frequency between linked loci FESFPS and Penta E was calculated based upon analysis of informative non-exclusionary cases involving families having multiple children. The percent recombination was determined to be 16% from these data. Haplotype frequencies were developed for African Americans, Caucasians and Hispanics. In calculating the combined CPI, FESFPS and Penta E were treated as linked loci and the developed haplotype frequencies were used (Maha et al, in preparation).

INTRODUCTION : Laboratories today face requests for complex relationship analyses. These more complex relationships include various family studies arising from immigration and probate clients, and increasing pressure to accept cases with only one parent, typically the alleged father and no mother. Testing without the mother is generally not recommended because of the significant loss of power, as also shown in this study. For those cases where the mother is truly not available additional testing may routinely be needed. To handle the increasing complex cases in an efficient manner, LabCorp sought to increase the power of its routine testing panel. LabCorp worked with Promega to develop a powerful custom panel of 20 autosomal loci and Amelogenin, for a 21 panel tests. Also, as a quality control measure LabCorp recognized the importance of using independent extractions to test overlapping loci. LabCorp began this quality control test with the start of its multiplex PCR testing in the early 1990s. As part of this new project LabCorp wished to keep this important quality step. The end product of this project was the use of two multiplexes; one multiplex is the PowerPlex® 16 HS System and the other is a custom hot start multiplex consisting of F13A01, F13B, FESFPS, LPL, Penta C, Penta D, and Penta E. The overlapping loci between PowerPlex® 16 HS and the custom kit are Penta D and Penta E. The use of the apparently linked loci FESFPS and Penta E did require the investigation of the recombination rate and the development of haplotype tables for major racial groups. For racial or ethnic groups without haplotype tables, the use of the higher likelihood ratio of either Penta E or FESFPS can be substituted as a conservative estimate of the paternity index. This is conservative in that the highest individual paternity index is closest to the lowest paternity index obtainable with haplotype tables.

DISCUSSION : Using Promega's 21-locus test battery greatly increased values for the combined paternity index (CPI) and average probability of exclusion (POE), when compared with use of the 13 autosomal CODIS loci. These CPI and POE values are presented in Tables 1and 2, respectively, and subsequently illustrated in various comparisons Figures presented on this poster.

Full trio CPI values for the 20 autosomal loci ranged from a low of 736:1 to a high of 31,479,973,976,492,298,240 to 1. The low CPIs had loci with results consistent with a mutation, with a resulting low CPI. In the actual case work, additional loci were tested to further evaluate the relationship and to possibly improve the CPI or exclude. The high CPI of 31 quintillion is not the norm, although several cases had CPIs in the quintillions. CPIs in the trillions and quadrillions were also seen. Such high numbers have a dramatic impact on the mean CPI value for the 20-locus battery, which, for full trio cases was 1,075,721,614,718,770 to 1 (one quadrillion). Because of this, the median value of 4,468,226,169 to 1 (four billion) becomes a more reasonable assessment of the typical combined paternity index of the 20 autosomal loci; likewise for mother not tested (MNT) cases the median CPI is 3,179,115 to 1. These compare with the CODIS loci median CPI of 1,667,879 to 1 for full trio cases and 14,448 to 1 for MNT cases. See attached figures.

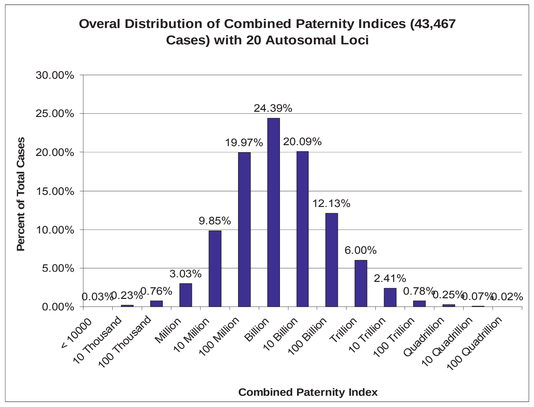
The analysis of average probability of exclusion (POE) values in Table 2 provides insight into the greatly increased discriminating power of the 20 autosomal loci in comparison to the 13 autosomal CODIS loci, Identifiler® and PowerPlex® 16 HS. The POE value of this new test battery was greatest for African Americans and was at least 34-fold more discriminating than the next most powerful system, PowerPlex® 16 HS.

Similarly, the number of exclusions seen increased with the additional loci. A summary of the number of inconsistencies seen for the two test batteries and different case types is found in Table 3. Two cases were seen with 1 inconsistency each and one case with 2 inconsistencies. Additional testing resolved one of these cases as an exclusion and the second case was issued as inconclusive because of the potential involvement of a related man. The third case had two inconsistencies after additional testing, however a brother of the man was submitted who after the same testing had no inconsistencies thus making the interpretation of non-paternity for the man with two inconsistencies reasonable.

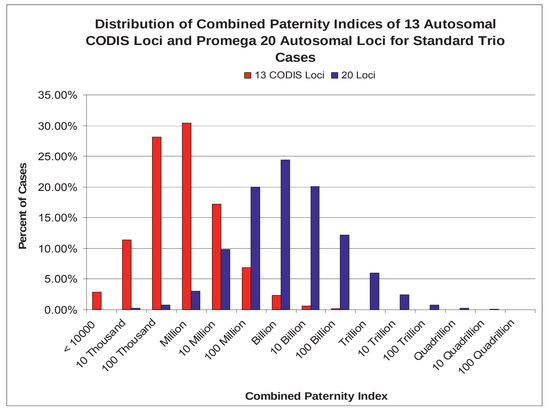
In examining the figures in this presentation the difference between cases with and without the mother are striking. This provides empirical evidence that testing without the mother greatly reduces the power of the test. Based on this evidence, testing without the mother should be reserved for those situations where the mother is truly absent.

In conclusion the 21-locus test panel developed by LabCorp and Promega clearly provides a superior choice for identity testing compared to the CODIS loci alone. Only rarely is the initial testing insufficient to resolve the case, and such cases are largely limited to those in which a possible mutation is present and thus investigation of the possible involvement of a male relative of the tested man must be examined.

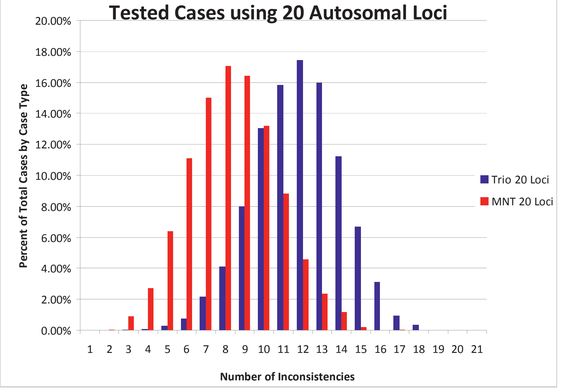
Overal Distribution of Combined Paternity Indices (43,467 Cases) with 20 Autosomal Loci



Distribution of Combined Paternity Indices of 13 Autosomal CODIS Loci and Promega 20 Autosomal Loci for Standard Trio Cases



Number of Inconsistencies with Paternity Seen with Standard Trio Cases and Mother Not Tested Cases using 20 Autosomal Loci



Number of Inconsistencies with Paternity Seen with the 13 Autosomal CODIS Loci versus 20 Autosomal Loci for Standard Trio Cases

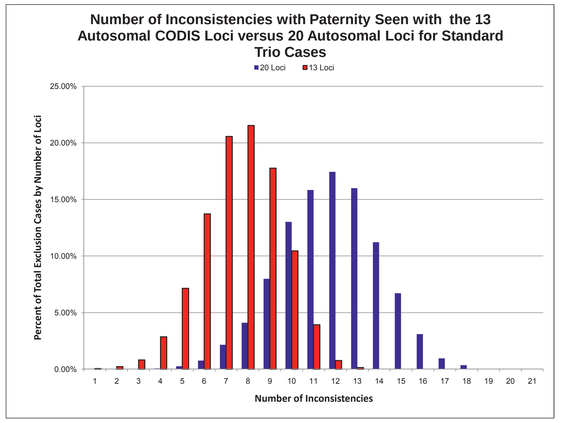


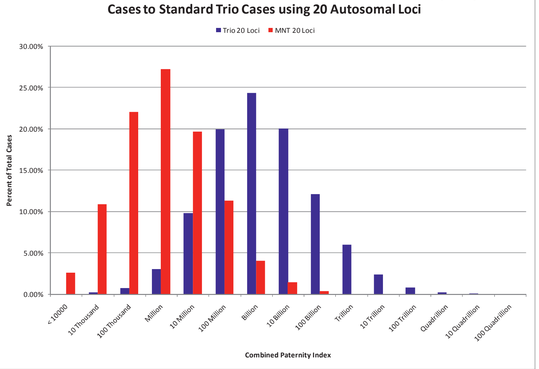
Table 1. Summary of the CPIs from the Testing of Various Numbers of Loci and Types of Cases

	Standard Trio Case (M,C,F)		Mother Not Tested	
	20 Autosomal Loci	13 CODIS Loci	20 Autosomal Loci	13 CODIS Loci
MEAN	1,075,721,614,718,770	2,927,003,917	14,975,249,510	70,752,201
MEDIAN	4,468,226,169	1,677,879	3,179,115	14,448
RANGE	736 to 31,479,973,976,492,298,240	0.111379 to 27,260,717,453,530	0.956189 to 33,770,438,746,313	0.165603 to 392,986,674,084

Table 2. The Average Cumulative Probability of Exclusion (%) and Cumulative Random Man Not Excluded (RMNE) for Various Races and Test Batteries (calculated from LabCorp frequency data)

	AFRICAN AMERICAN		CAUCASIAN		HISPANIC	
LOCI	Cumulative POE%	Cumulative RMNE	Cumulative POE%	Cumulative RMNE	Cumulative POE%	Cumulative RMNE
CODIS 13	99.999550994	0.000004490	99.999398884	0.000006011	99.999304979	0.000006950
20 Autosomal Loci	99.999999385	0.000000006	99.999996749	0.000000033	99.999997684	0.000000023
Identifiler	99.999971415	0.000000286	99.999937189	0.000000628	99.999942046	0.000000580
PowerPlex 16	99.999978464	0.000000215	99.999954852	0.000000451	99.999961314	0.000000387

Comparison of Combined Paternity Indices of Mother Not Tested (MNT) Cases to Standard Trio Cases using 20 Autosomal Loci



Distribution of Combined Paternity Indices of 13 Autosomal CODIS Loci and 20 Autosomal Loci for Mother Not Tested Cases

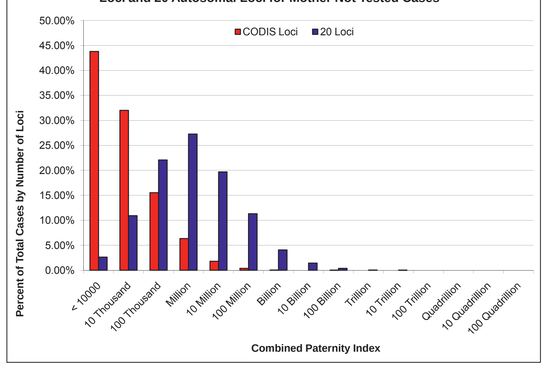


Table 3. Summary of the Number of Inconsistencies from the Testing of Various Numbers of Loci and Types of Cases

	Standard Trio Case (M,C,F)		Mother Not Tested	
	20 Autosomal Loci	13 CODIS Loci	20 Autosomal Loci	13 CODIS Loci
MEAN	11	8	8	5
MEDIAN	12	8	8	5
RANGE	1 to 19	1 to 13	2 to 17	1 to 11

Number of Inconsistencies with Paternity Seen with the 13 Autosomal CODIS Loci versus 20 Autosomal Loci for Mother Not Tested Cases