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Searching for first-degree familial relationships in California's offender DNA database: Validation of a likelihood ratio-based approach

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ABSTRACT

A validation study was performed to measure the effectiveness of using a likelihood ratio-based approach to search for possible first-degree familial relationships (full-sibling and parent–child) by comparing an evidence autosomal short tandem repeat (STR) profile to California's ~1,000,000-profile State DNA Index System (SDIS) database. Test searches used autosomal STR and Y-STR profiles generated for 100 artificial test families. When the test sample and the first-degree relative in the database were characterized at the 15 Identifiler® (Applied Biosystems®, Foster City, CA) STR loci, the search procedure included 96% of the fathers and 72% of the full-siblings. When the relative profile was limited to the 13 Combined DNA Index System (CODIS) core loci, the search procedure included 93% of the fathers and 61% of the full-siblings. These results, combined with those of functional tests using three real families, support the effectiveness of this tool. Based upon these results, the validated approach was implemented as a key, pragmatic and demonstrably practical component of the California Department of Justice's Familial Search Program. An investigative lead created through this process recently led to an arrest in the Los Angeles Grim Sleeper serial murders.

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1. Introduction

The use of autosomal short tandem repeat (STR) DNA genotyping information to aid in identifying familial relationships is widely accepted in forensic science and is commonly applied to civil and criminal paternity cases, to missing persons cases, and in mass disaster and mass burial situations [1]. There has been interest in applying similar familial searching methods in a systematic way to forensic cases for which an evidence-associated STR profile has been generated but where no matching profile has been found in a relevant database of offender DNA profiles, e.g., in the CODIS (Combined DNA Index System) database [2]. For such circumstances, the use of familial searching techniques could provide investigative leads to potential relatives of the evidence source who may be in the database.

Several recent studies have demonstrated the effectiveness of STR-based likelihood ratio (LR) calculations to search for potential familial relationships in a DNA database [2–5]. Those studies used either large simulated databases or real databases that were orders of magnitude smaller than the California convicted-offender SDIS (State DNA Index System) database. We report here results from a study performed to validate an STR-based familial searching procedure for the California SDIS database, the largest SDIS database in the United States and comprised of ~1,000,000 STR profiles at the time of the study.

Bieber et al. recommended the broad application of familial-searching techniques for all cases in which a direct hit to the database did not occur [2]. This approach is being pursued by the Colorado Bureau of Investigations with some success [6]. In contrast, the California Department of Justice designed a Familial Search Program that would be used only upon the request of law enforcement agencies investigating major violent crimes where there is a serious risk to public safety, and where all other investigative leads have been exhausted. Our goal was to ensure that we were employing an effective investigative tool, given current and readily available technology, as part of a pragmatic program that would strike a balance between privacy concerns and the need to provide information that may solve a violent crime or series of crimes [7]. In addition to the successful detection of authentic relationships, and in recognition of privacy concerns, the program had the important goal of avoiding the further investigation of individuals identified because of coincidental associations.

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2. Material studied, methods, techniques

2.1. The 100 test families

A set of 100 unique, artificial families was created using profiles from a publicly available population database [8,9] of genuine Identifiler® and Yfiler® (Applied Biosystems®, Foster City, CA) profiles. Pairs of profiles were randomly assigned to represent the parents, and artificial matings were performed using Mendelian principles to create two male offspring. No mutations were simulated. For each family, the autosomal STR profile of one offspring was selected as the "test" or "evidence" profile to be searched against the convicted offender SDIS profiles. The STR profiles for the father and the remaining offspring served as positive controls for the "authentic" parent–child and full-sibling relationships, respectively. Although the STR profiles for the father and brother positive-control samples were not actually placed into the SDIS, comparisons to the test sample were performed as if they were in the database (i.e., using the same LR formulae, *vide infra*).

2.2. STR profile comparisons—likelihood ratio calculations

LRs for the autosomal STR test-to-offender comparison were calculated for three racial/ethnic population groups (African American, Caucasian, SW Hispanic) using standard formulae for parent–child and full-sibling duos [10,11] and FBI databases [12,13]. Calculations were limited to first-degree relationships in recognition of the low expectation that more distant relatives would yield LRs sufficient to differentiate them from such a large pool of unrelated individuals [14]. The profiles of our authentic relatives were tested separately as 13-locus profiles (the CODIS core loci required for inclusion in the national database: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, vWA, TPOX, D18S51, D5S818, and FGA) and as 15-locus profiles (the Identifiler® kit loci consisting of the 13 CODIS core loci plus D2S1338 and D19S433) in recognition of the locus-count bimodality in our offender database. Consistent with the approach used in other studies [2,4], no θ-corrections for population substructure were included in the LR calculations.

In a departure from those studies, no attempts were made to correct for the possibility of meiotic mutations or deletions. While our approach to mutations has the intention of simplifying the calculation and avoiding false associations, it does, however, mean that a true parent–child relationship could be excluded. In practice, even when the only relative in the database is a father or son of the perpetrator, only a small proportion of searches should be negatively impacted by this decision. Average meiotic mutation rates for autosomal STR loci [15] are approximately 10^{-3} when testing paternity trios, a comparison that commonly allows for the determination of one specific obligate paternal allele. In parent–child familial searching comparisons, essentially paternity duos, either allele of the evidence profile could be considered obligate. This leaves the possibility that a shared allele identical-by-state between the father and son might mask a mutation in a shared allele identical-by-descent. Similarly, the mutated allele might now be identical-by-state to the maternal allele of the son. Both scenarios suggest that our rate of detecting mutations when performing familial searching should be lower than predicted by paternity trio mutation rates.

Rather than ignore the statistical strength of the Y-STR testing, a Y-STR LR was calculated based upon the known haplotype of the evidence assuming *a priori* that a match to the offender exists. The Y-STR LR is the inverse of the test sample's overall (i.e., "All" population groups combined) Y-haplotype frequency expressed as the upper 95% confidence interval [16] in the US Y-haplotype database [17]. This is combined with the autosomal LR [18,19].

Consistent with the recommendations of Bieber et al. [2], as well as the practices of agencies performing mass disaster identifications [20–22], the overall LR value was adjusted for the size of the database (N). In this case, "$1/N$" is analogous to the approximate prior odds of a database sample being the true relative under the assumption that a relative exists in the database, making the final value proportional to the approximate posterior odds of a Bayesian analysis. It should be noted that, in our approach, familial searches use only California's database of *convicted* offenders. Arrestees collected pursuant to Proposition 69 [23] are not included in a familial search and are not included in N.

$$\text{The final calculation}: \text{"Odds"} = \text{Autosomal STR LR} \times \text{Y} - \text{STR LR} \times 1/N$$

To measure the effect of including the Y-STR LR in the calculation, a comparison was made to "Odds" based solely upon the autosomal STR LR and the database size.

2.3. Autosomal STR allele frequencies for a structured database

For reasons unrelated to this study, the racial/ethnic structure of our database was not assessed. To address this unknown racial/ethnic structure, the "odds" were based upon the minimum LR calculated for the three population groups (African American, Caucasian, SW Hispanic). This approach presupposes that an individual's alleles tend to be more common in their own group than in other groups, and is consistent with other work showing that the use of the cognate ethnic allele frequencies will, on average, give minimum calculated LR values [24]. To measure the effectiveness of using the minimum LR in the calculation, a comparison was made to "Odds" based upon each of the three population groups.

2.4. Statistical thresholds

Statistical thresholds were established to be in line with those recommended by the SWGDAM Ad Hoc Committee on Partial Matches for the investigation of CODIS partial matches [18]. For an offender to be investigated further as a possible familial lead, "odds" for either the parent–child relationship or the full-sibling relationship must be greater than or equal to 1 for at least one population *and* no less than 0.1 for the remaining two populations. Treating 1 and 0.1 as approximate posterior odds, these thresholds mean that at least one population group had attained a posterior probability of relatedness greater than or equal to 50%, and neither of the other two had posterior probabilities lower than 9.1%. The magnitude of these thresholds is appropriate given the intended purpose is to develop a lead for further investigation, not to directly identify an individual.

We acknowledge that including the *same* Y-STR LR and the *same* N for each calculation in a search simply re-scales the LR values. However, combining all of the statistical information in advance allowed us to eliminate associations that would not reach our thresholds even if the test and database samples were to share the same Y-STR profile. While not modeled in this study, in practice the initial Y-STR LR would be recalculated for any offender-to-evidence comparison later observed to have very similar but still discordant Y-STR profiles (e.g., a one-locus discordance within a Yfiler® profile that may be due to a mutation). In such cases, the pair's revised "odds" would be evaluated in relation to the SWGDAM thresholds.

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2.5. Ranking studies

While California's SDIS offender samples are typed for the 13 CODIS core loci required for entry into the national database, current practice is to also type the two additional loci included in the Identifiler® kit. At the time of this study, 15-locus profiles comprised approximately 71% of the samples in the full SDIS test set, while profiles with only 13 loci comprised approximately 29% of the samples and represented the offenders collected during the initial 20 years of California's database. A small fraction (~2%) of the samples in each of these two sets were "missing" one locus (e.g., 14 loci instead of 15 loci), but, for the sake of simplicity, this fraction of samples was kept within the larger set (i.e., 14 loci within the 15-locus set, 12 loci within the 13-locus set).

For each of the 100 test families, the test sample's 15-locus profile was used to calculate full-sibling and parent–child "odds" for each of the *N* database profiles and the authentic father and full-sibling profiles. The number of loci used for each "odds" calculation was also tracked. The "odds" for the database comparisons were listed in descending order. The rankings of the authentic test family "odds" amongst the pool of offenders' "odds" became the primary metric for further evaluation.

As shown in Fig. 1, the ranking studies were performed by organizing the database comparisons into a one-list, two-list, or four-list format. For the one-list format, the database and relatives' "odds" were ranked in descending order based on the larger value: the full-sibling "odds" or the parent–child "odds". For the two-list format, the comparisons were separated into two lists of descending order, one for full-sibling "odds" and one for parent–child "odds". For the four-list format, the comparisons were separated into lists of descending order based on the familial relationship (full-sibling vs. parent–child) and the number of loci used for the "odds" calculation (15 loci or 13 loci). By successively focusing the list down into smaller, more homogenous groups, our intent was to examine whether this could bring more authentic relatives into the top ranks, thereby increasing their detection. For ranking purposes, duplicated database samples were removed. Female offenders were left in place, because the original version of our familial searching software did not record an offender's profile at the gender-informative locus amelogenin.

We also explored an alternate means of compensating for the bimodality in the number of loci compared (15 loci vs. 13 loci). From the test profiles, we estimated the expected D2S1338 and D19S433 LRs for untested parents and full-siblings:

- For each test sample, 500 full-sibling and 500 parent hypothetical 2-locus profiles were created in the manner described by Curran and Buckleton [4]. For each hypothetical profile, alleles not identical by descent with the test sample were selected in proportion with the allele frequencies of one of the three population groups (African American, Caucasian, SW Hispanic) to which the relative would be randomly assigned.
- Regardless of the population group assigned, LRs were then calculated for each test-relative comparison using all three population groups.
- The expected two-locus LR was the geometric mean for each set of 500 LRs.

For each of the 100 test samples, the expected 2-locus LRs (six, representing the two relationships and three population groups) were then multiplied by the respective 13-locus LRs of the authentic father, full-sibling, and that subset (29%) of the offenders not profiled at those loci. Ranking was performed using the one-list format described above.

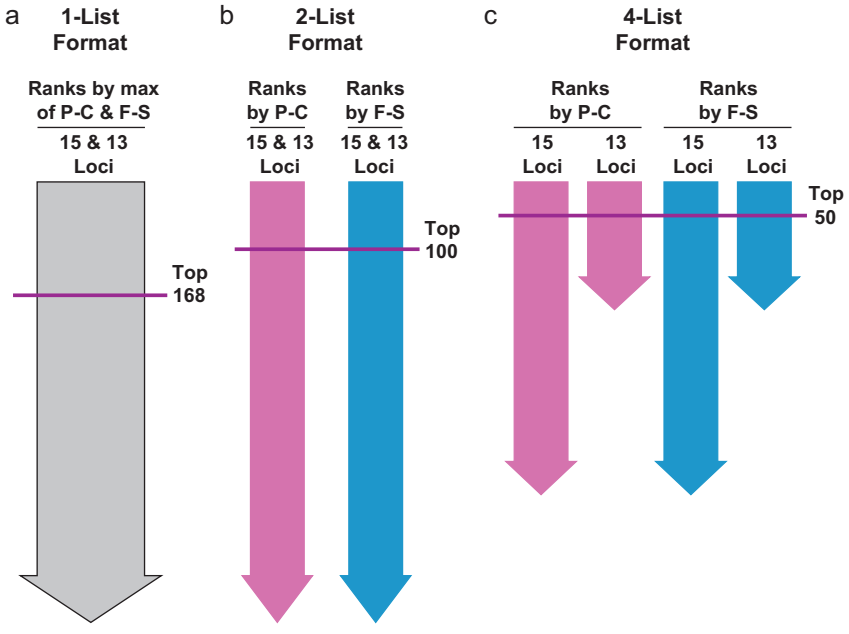

Fig. 1. Three formats for ranking comparisons resulting from a search of an evidence sample with a 15-locus profile against a database of offenders, ~71% of whom have 15-locus profiles, with the remaining ~29% having 13-locus profiles. The arrow lengths are proportional to the percentage of offenders (100%, 71%, or 29%) represented by a specific "odds" calculation. (a) The one-list format ranks order the offenders by the "odds" calculated using the higher of the two LRs, parent–child (P–C) or full-siblings (F-S). Offenders in each list are ranked together in descending order, regardless of the number of loci in their profiles. The first 168 offenders in the list (horizontal bar; corresponding to two 96-well plates) are then sampled for Y-STR testing. (b) The two-list format ranks the offenders two ways: by the "odds" calculated using parent–child LRs; and by the "odds" calculated using full-siblings LRs. All offenders are ranked together in descending order, regardless of the number of loci in their profiles. The first 100 offenders in each list (horizontal bar) are then sampled for Y-STR testing. Editing the sample sets for offenders appearing on both lists generally reduces them to fewer than 168 test samples. (c) The four-list format ranks the offenders four ways: offenders with 15-locus profiles, ranked in descending order by "odds" calculated using parent–child LRs; offenders with 13-locus profiles, ranked in descending order by "odds" calculated using parent–child LRs; offenders with 15-locus profiles, ranked in descending order by "odds" calculated using full-siblings LRs; and offenders with 13-locus profiles, ranked in descending order by "odds" calculated using full-siblings LRs. The first 50 offenders in each list (horizontal bar) are then sampled for Y-STR testing. Editing the sample sets for offenders appearing on multiple lists generally reduces them to fewer than 168 test samples.

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2.6. Ranking results from three real family searches

For initial implementation of the California DOJ familial-searching procedure, the four-list process ranking was chosen. A functional test of the procedure was performed using real Identifiler® and Yfiler® profiles from three male laboratory staff members ("A", "B", and "C") and their first-order relatives. The staff member's autosomal 15-locus profile acted as an evidence test sample and was used to calculate full-sibling and parent–child "odds" for each of the *N* database profiles and his authentic relatives' profiles. Despite our prior knowledge of a female relative's gender, the Y-STR LR calculated from the staff member's Yfiler® profile was included in the "odds" to more accurately replicate the search process. The rankings of the authentic test family members' "odds" amongst the pool of offenders were determined, and Y-STR profiling was performed on the top 50 ranking offenders within each list.

2.7. Software

All comparisons, calculations, and rankings were performed using a combination of in-house Visual Basic® and Excel® (Microsoft®, Redmond, Washington) programs.

2.8. DNA extraction and STR typing

DNA was extracted from buccal collectors or bloodstains using either an automated NaOH-lysis procedure, validated for the California DOJ CAL-DNA data bank program, or a manual "organic" extraction method, validated for the California DOJ casework program. Autosomal and Y-STRs were amplified using the Applied Biosystems® Identifiler® and/or Yfiler® systems according to lab-validated protocols. STRs were resolved and detected on 3130XL Genetic Analyzers (Applied Biosystems®, Foster City, CA) and typed with GeneMapper® ID (Applied Biosystems®, Foster City, CA).

As a result of initial studies, and in consideration of capacity limitations, our laboratory's Familial Search Program has committed to performing Y-STR analysis on a maximum of 168 samples, the number of offender samples that would be analyzed on two 96-well plates (84 offender samples and 12 controls per plate). This practical limitation serves as a reference point for the observations and decisions described below.

Table 1
Likelihood ratios for the authentic relatives and unrelated offenders. All values represent the minimum of the LRs calculated for three population groups (African American, Caucasian, SW Hispanic).

Profile type	Calculation	Authentic relatives		Unrelated offenders
		Low LR	High LR	High LR
15-Locus	Parent–child	3.4×10^{2}	1.7×10^{9}	2.8×10^{7}
	Full-sibling	7.0×10^{-1}	3.7×10^{8}	3.5×10^{6}
13-Locus	Parent–child	5.3×10^{1}	9.7×10^{7}	5.8×10^{5}
	Full-sibling	7.3×10^{-1}	3.7×10^{7}	6.6×10^{5}

3. Results

3.1. Likelihood ratios and odds calculations

Using the minimum LR across the three population groups, the autosomal LRs of authentic relatives varied from a low of 0.7 to a high of 1.7 billion. The highest corresponding LRs for unrelated offender samples fell within this range and exceeded 10^5. (See Table 1 for a summary of the LRs.) Setting the prior odds to 0.5/0.5, as sometimes applied to civil paternity work, rather than 1/*N*, the posterior probabilities of relatedness for the set of unrelated offenders ranged above 99.999%. Such LRs and posterior probabilities are well above thresholds commonly employed by American Association of Blood Banks relationship testing laboratories [15]. While the Y-STR LRs increased the overall LR by a factor of 280–1407, including in the calculation the reciprocal of the database size led to an overall "odds" approximately three orders of magnitude lower than the autosomal LR, and six orders lower when the Y-STR LRs were not included. Overall, the resultant "odds" incorporating the database size appear sufficient to convey the value of the comparison as an investigative lead while tempering the magnitude of results obtainable when making a large number of comparisons to unrelated individuals.

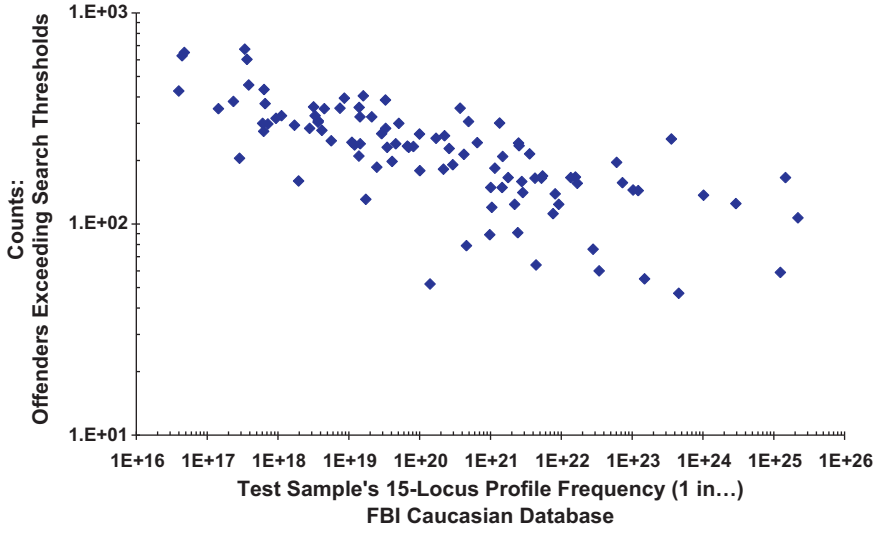

Fig. 2. The number of offenders remaining after the application of statistical thresholds to the calculated "odds". Counts plotted against the rarity of the test sample's 15-locus STR profile.

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3.2. Output filtering based on thresholds

For the 100 test searches, applying our statistical thresholds reduced the ~1,000,000-sample database to lists of 87–1115 candidates, with an average count of 419 and a median count of 405. In other words, on average, the thresholds excluded ~99.96% of the unrelated offender samples. The counts of non-relatives surpassing the thresholds are presented graphically in Fig. 2. In this figure, the number of threshold counts is plotted against the rarity of the 15-locus test profile, which was calculated as the inverse of the random match probability for each test profile using the allele frequencies for the FBI Caucasian population tables (chosen for purposes of comparison, rather than an indication of the population makeup) [12,13]. Not surprisingly, there was a general trend that the more common the autosomal profile of the test sample, the greater were the number of threshold counts due to adventitious allele sharing.

The same thresholds were applied to comparisons of the 100 artificial families' 15-locus test profiles to the 15-locus and 13-locus profiles of their authentic father and full-sibling. When the authentic relative had a 15-locus profile, 100% of father–son relationships and 86% of the full-sibling relationships were included. When the authentic relative's profile was reduced to 13 loci, 96% of the father–son relationships were still included, but the fraction of included authentic full-sibling relationships dropped to 69%. However, the excluded samples represented those relationships that were least strongly linked and were unlikely to stand out against the test comparisons to the large set of unrelated database profiles.

Removing the statistical power of the Y-STR LR from the "odds" calculation had the expected benefit of reducing the number of unrelated offenders who would be subjected to Y-STR testing. A Y-STR LR typically increased the magnitude of the odds by approximately 10^3, and without that increase the vast majority of "odds" for unrelated offenders failed to surpass the SWGDAM statistical thresholds. For the 100 test searches, 51% returned 0 offenders with "odds" exceeding the SWGDAM thresholds, 27% returned 1, 17% returned 2, 4% returned 3, and 1% returned 4, averaging less than 1 per search. However, the percentage of authentic relatives included during a search was also reduced with this format (see Table 2). Comparing the results with or without the Y-STR LR, when the authentic relative had a 15-locus profile, father–son inclusions were reduced by a factor of 2.6 (100/38%) and full-sibling inclusions were reduced by a factor of 3.1 (86/28%). When the authentic relative's profile was reduced to 13 loci, father–son inclusions were reduced by a factor of 6.9 (96/14%) and full-sibling inclusions were reduced by a factor of 4.6 (69/15%). If these results are predictive, searching a large database and applying the SWGDAM thresholds to "odds" that do not include the Y-STR LR would reduce the detection of a true relative in the database to less than half the time. However, fewer unrelated offenders would require additional testing.

Table 2
Percentage of relatives from the 100 artificial families included after applying the SWGDAM Ad Hoc Committee thresholds. All "odds" incorporated the approximate prior odds "1/*N*" and the autosomal STR LR. Results are reported for "odds" calculated with or without the use of the Y-STR LR.

Relative's profile type	Relationship	Percentage of relatives included by SWGDAM odds thresholds	
		Including Y LR (%)	Excluding Y LR (%)
15-Locus	Parent–child	100	38
	Full-sibling	86	28
13-Locus	Parent–child	96	14
	Full-sibling	69	15

3.3. Ranking results from the 100 test family searches

Using only comparisons that exceeded our thresholds, the authentic relatives were ranked against the database of offenders. Fig. 3a (15-locus profiles) and 3b (13-locus profiles) graph the cumulative percentages of authentic relatives included at a given rank (e.g., within the first 50 ranks) using the four-list, two-list, and one-list formats. While fewer than 50% of the authentic relatives achieved the top ranking regardless of list format, the cumulative percentages included rose rapidly when looking deeper into the lists.

The vertical bars on these graphs represent the end-point ranks that would be sampled for Y-STR testing from each list: the top 168 ranks for a one-list format; the top 100 ranks for a two-list format; and the top 50 ranks for a four-list format. It was determined that sampling to these levels yielded, on average, ~168 unique offender samples, the number of offenders that would be analyzed for Y-STRs on two 96-well plates. The difference between 168 and the four-list and two-list maxima of 200 is explained in part by the same offender appearing within the top ranks for both the parent–child and full-sibling lists. Additionally, our exclusion of all parent–child profile comparisons requiring a meiotic mutation led to fewer than the maximum number of candidate offenders in some of the parent–child lists.

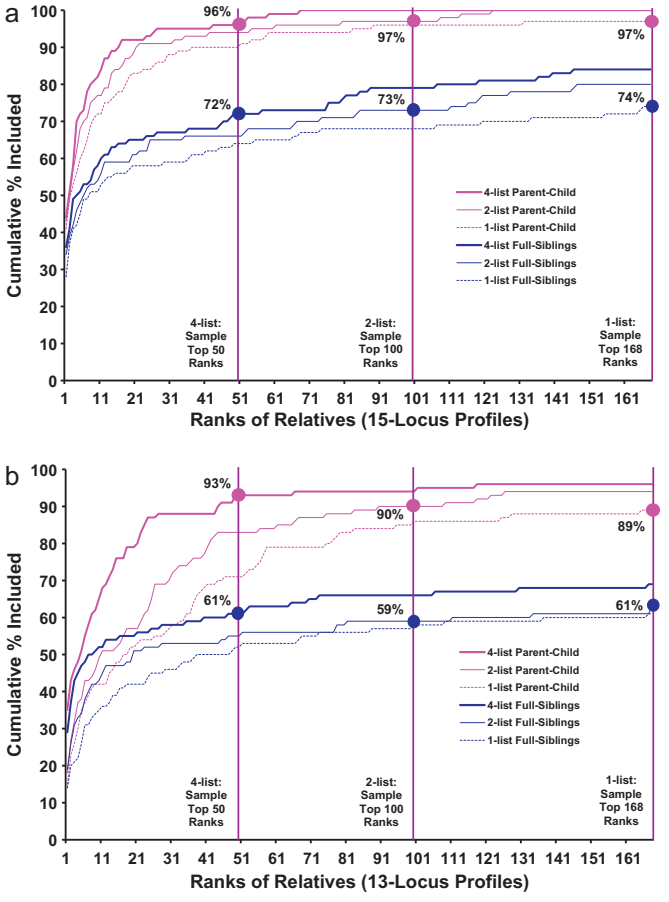

Fig. 3. Comparing the cumulative inclusion percentages of three formats when ranking the true relatives against the database searches. The vertical bar represents the end-point ranks (see Fig. 1) that would be sampled for Y-STR testing from each list: the top 168 ranks for a one-list format; the top 100 ranks for a two-list format; and the top 50 ranks for a four-list format. (a) Relatives with 15-locus profiles. (b) Relatives with 13-locus profiles.

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At their respective rankings for two 96-well plates, the three ranking list formats included 89–93% (13-locus) to 96–97% (15-locus) of the authentic fathers and 59–61% (13-locus) to 72–74% (15-locus) of the authentic full-siblings. The higher inclusion percentages for authentic relatives when using 15-locus profiles vs. 13-locus profiles were expected since D2S1338 and D19S433 are highly polymorphic loci. The number of authentic relatives ranked first increased as the data was partitioned into separate ranking lists, and this coincided with the initial slopes becoming generally steeper, meaning that more relatives were included at an earlier ranking. However, none of the three formats was consistently better than any other at including authentic relatives within our two-plate testing limit. In the context of our Y-STR testing approach, the overall differences between the ranking list formats appear negligible.

As seen in Fig. 4a and b, the use of the LR minimum across the three racial databases generally included more relatives at an earlier ranking than when the "odds" were ranked by a particular ethnicity. Using the four-list format, the difference eventually became negligible.

Enhancing a relative's 13-locus profiles with expected LRs for D2S1338 and D19S433 led to minimum LRs that were overall similar to those for the true 15-locus comparisons (Fig. 5a). While this held true for both relationships, there was less correlation seen in full-sibling comparisons ($R^2 = 0.77$) than in parent–child comparisons ($R^2 = 0.94$). The LR similarities between relatives with true 15-locus profiles and enhanced 13-locus profiles were also seen in the cumulative ranking results evaluated in the one-list format (Fig. 5b). The enhanced 13-locus profiles had higher cumulative ranks, but the overall differences were once again largely negligible in the context of our Y-STR testing approach. When the plots in Fig. 5b are compared to the one-list results described in Fig. 3, where no D2S1338 and D19S433 expected LRs were used, it was seen that inclusions of "enhanced" 13-locus relatives improved but coincided with decreased inclusions of relatives with full 15-locus profiles. This was the unavoidable consequence of also increasing the LRs of unrelated offenders with 13-locus profiles.

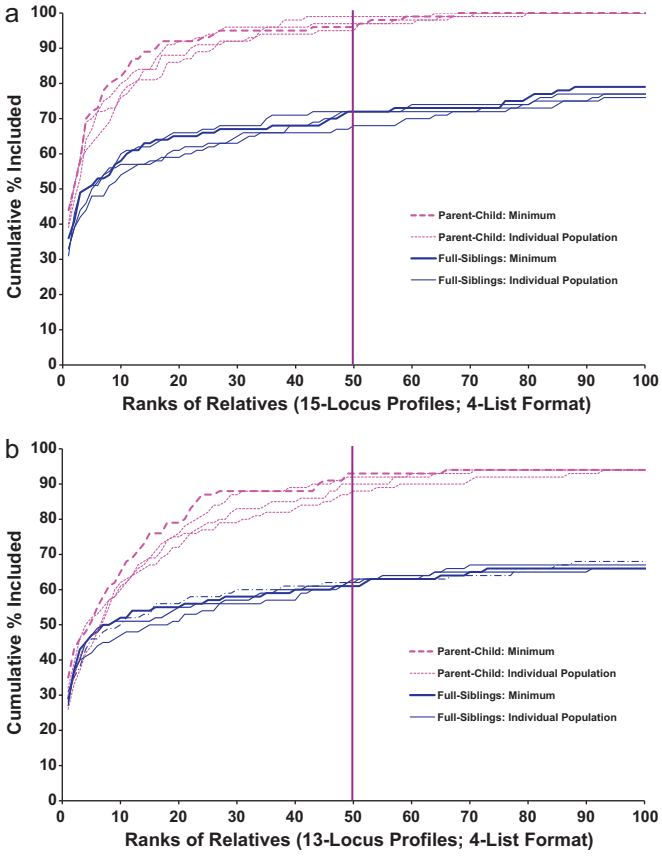

Fig. 4. Comparing the cumulative inclusion percentages when ranking the true relatives against the database searches using "odds" calculated with population-specific LRs (African American, Caucasian, or SW Hispanic) or population-neutral LRs (the minimum value calculated across the three populations). Rankings were performed using the four-list format. The vertical bar represents the end-point top 50 ranks (see Fig. 1) that would be sampled for Y-STR testing from each list. (a) Relatives with 15-locus profiles. (b) Relatives with 13-locus profiles.

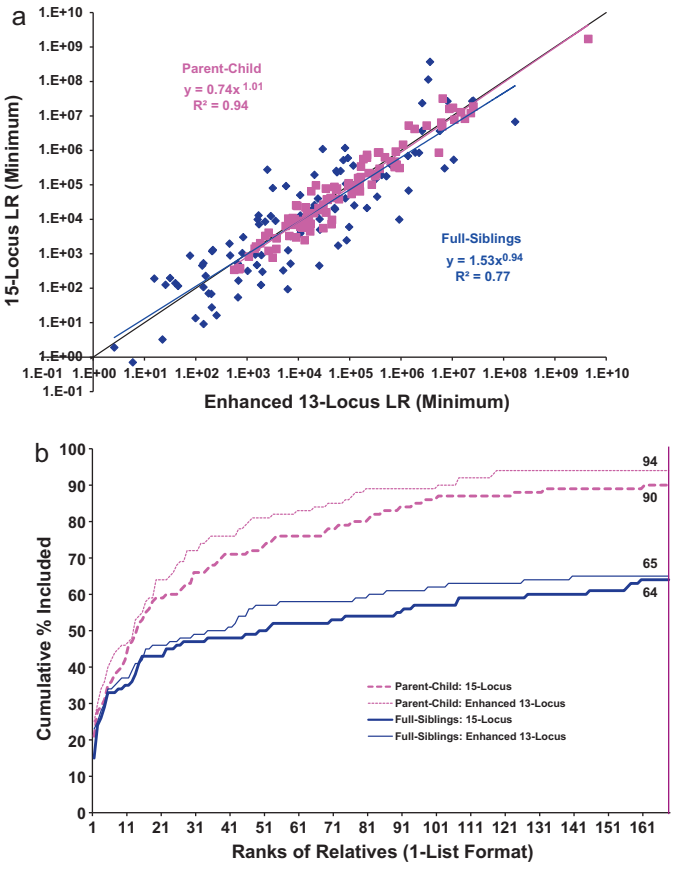

Fig. 5. Comparison of the 15-locus LRs and cumulative inclusion percentages when the "odds" of relative and offender profiles with 13-locus profiles are/are not enhanced with expected LRs for D2S1338 and D19S433 prior to ranking using the one-list format. (a) Minimum LRs across three populations for authentic parent–child and full-sibling pairs: 15-locus vs. enhanced 13-locus profiles. (b) Ranking of all comparisons: 15-locus and enhanced 13-locus profiles.

3.4. Ranking results from three real family searches

Table 3 summarizes the four-list ranking results for the authentic relatives. All of the family members met the laboratory's statistical thresholds, for both 15-locus and 13-locus profiles, with the exception of one female sibling. Also, with that same exception, all family members were ranked within the top 50 when using their 15-locus profile and relationship-appropriate calculation (parent–child or full-siblings). All but two family members were ranked within the top 50 with their relationship-appropriate calculation and the 13-locus profile. No false Y-STR exclusions occurred due to meiotic Y-STR mutations, and no adventitious matches were observed to the Y-STR profiles of unrelated offenders within the top 50 ranks for each search. It is also worth noting that although family member A-1 (sample A's real mother) would be

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Table 3
Ranking results for the authentic relatives (parents, full-siblings, and children treated as "offender" samples) of three staff members when the staff profiles (A–C treated as "evidence" samples) were searched against the California SDIS offender database profiles. Ranks are based upon the four-list format.

Staff member ("Evidence")	Relative of staff member ("Offender")	Rankings using the relatives' 15-locus profiles		Rankings using the relatives' 13-locus profiles	
		Parent–child ranking list	Full-siblings ranking list	Parent–child ranking list	Full-siblings ranking list
A	Mother	1	3	1	4
	Father	2	40	5	29
	Brother	n/a[a]	3	n/a	58
	Sister	n/a	<Thr.[b]	n/a	<Thr.
	Son	1	3	4	40
B	Father	2	3	2	3
	Brother 1	n/a	43	n/a	14
	Brother 2	1	1	1	1
	Son	5	14	5	26
C	Son	15	88	57	131

[a] The "n/a" designation indicates that the "odds" were null for the parent–child comparison, due to the lack of any shared alleles at one or more autosomal STR loci.
[b] The "<Thr." designation indicates that the "odds" did not meet the minimum thresholds for inclusion as a possible relative.

excluded from Y-STR testing based on gender, A-1's ranking and very high "odds" would likely prompt further investigation as a possible female relative of the test sample.

4. Discussion

Previous studies [4,5] demonstrated that an LR-based approach was superior to allele-sharing approaches. Differences between the various LR-based formats we examined were less dramatic. When the cumulative inclusion rates were examined for the rankings that would equate to two 96-well plates of samples for Y-STR testing, the various approaches (minimum LR vs. separate population LRs; one list vs. two lists vs. four lists) led to very similar results in our study of 100 artificial families. For our purposes, our original process utilizing the four-list format ranked by the minimum "odds" appears to be as effective as these other LR formats. Any preference between the formats based upon perceived organizational difficulties, such as ranking the "odds" or combining four or two lists into one set of samples for Y-STR testing, can be neutralized by the use of an Excel® spreadsheet to sort the search results. Moving forward, further comparisons will be made by conducting actual familial searches using both the four-list format and the conceptually simpler one-list format. On the contrary, the approach of using the D2S1338 and D19S433 "expected LR" to enhance the "odds" for 13-locus profiles will not be pursued due to the potential, albeit modest, to negatively impact the ranks of relatives with 15-locus profiles. We know of no other effective approaches to balancing profiles with different locus counts. This issue of multi-modality in locus count may be exacerbated in the future should more loci be added to the profiles of convicted offenders.

The choice of Y-STR haplotyping as a secondary evaluation of highly ranked offenders satisfied multiple desirable criteria: a lineage-specific marker is beneficial when the goal is to discover first-order relatives; the Yfiler® kit is highly discriminating; and Y-STR typing procedures were validated in our laboratory and were largely compatible with the high-throughput protocols already used for autosomal STR typing in the California DOJ Data Bank program. While our search approach focuses on first-order relatives, Y-STR typing would also detect paternal half-siblings that ranked well. Other methods, e.g., autosomal SNP-typing systems, extended STR-typing systems, and mitochondrial haplotyping systems, have also been suggested for this purpose [2,25,26]. Future considerations include incorporating one or more of these approaches, especially as a means of evaluating highly ranked female offenders.

We have developed a procedure that uses STR-based LR calculations, analytical thresholds, and subsequent Y-STR analyses to perform familial searches on an ~1 million profile offender database, attempting to identify potential first-degree relatives to a test/evidence sample while severely limiting the possibility of generating a false lead. Due to practical limitations, we accepted as given that our system would not identify every relative in the database. In our examination of 100 artificially created families, the simple application of our analytical thresholds removed approximately 99.96% of unrelated offenders from further consideration. By limiting to 168 the number of ranked offenders that will be Y-STR typed, the expectation is that a random match between one of the remaining unrelated offenders and the test sample would be an unlikely occurrence. At the same time, our calculation thresholds, our limits on the number of offenders selected for Y-STR testing, and our decision to not allow for meiotic mutations will also lead to true relatives in the database being excluded or undetected. Despite this, our experimental detection of 89–97% of the authentic fathers and 59–74% of the authentic full-siblings, combined with the functional tests of laboratory members and their families, provides confidence that our process will more likely than not detect a true relative should the paternally related full-sibling, parent, or child be present in the database. Our results are consistent with those reported in other studies [2–5].

While additional unrelated offenders would have been excluded by filtering the candidate comparisons based upon an "odds" calculation omitting the potential statistical power of the Y-STR LRs, this approach was less successful at including authentic relatives (less than 40% included). Overall, this option might provide a preferred ratio of "viable leads per sample tested with Y-STRs" for a laboratory choosing to perform mass-screening familial searches on all evidence profiles that do not directly match to an offender during a typical database search. Our approach to calculating "odds" and Y-STR testing appears better suited to our interest in developing viable leads for a very limited set of searches.

As the SDIS database continues to grow in size through the addition of new 15-locus profiles, the need for familial searching will be reduced due to increases in direct matches. Where direct matches do not occur, larger offender database sizes can negatively affect a familial search: smaller "1/*N*" values mean that fewer comparisons to relatives will exceed the laboratory's thresholds; and the ranks of relatives will be depressed in a predictable manner [4] due to an increase in the number of unrelated database samples adventitiously similar to the evidence profile. Decreases in the number of relatives surpassing our thresholds will be somewhat offset by increases in the Y-STR LR as the Y haplotype database size

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increases. Using our four-list format, the relative rankings of 13-locus profiles will be unaffected by increased database size, since all new offenders are entered with 15-locus profiles. The negative effect on the rankings of true relatives with 15-locus profiles will be at least partly offset by the greater likelihood that a true relative is actually included in the database.

To date, 10 familial searches, representing eight separate cases (two cases were searched twice), have been conducted by the California Department of Justice using the validated four-list format. In seven of those cases, no candidate offenders were identified as possible relatives; all of the candidates were excluded through Y-STR screening. The eighth, a Los Angeles serial murder/sexual assault case dubbed the "Grim Sleeper" [27,28], was one of the two subjected to familial searches twice, once in 2008 and again in 2010. No candidate offenders with matching Y-STR profiles were identified in the results of the first search. The second search yielded a Y-STR match to a new highly ranked offender, who entered the California SDIS in 2009. Having passed this first hurdle (i.e., a highly ranked offender having a Y-STR profile that matches the evidence), and following a review by the Familial Search Committee, representatives of the California DOJ Bureau of Investigation and Intelligence conducted a review of available records that could support or dispel the hypothesis that the database offender is related to the perpetrator. In accordance with written DOJ policies, the Familial Search Committee undertook a final review of the available information. By unanimous Committee vote, the convicted offender's name was released to the Los Angeles Police Department. This information led to the analysis of DNA recovered from a piece of pizza and other materials discarded by the offender's father, Lonnie David Franklin [29]. This highlights the final safeguard in the familial searching process: once a suspect is identified, a comparison must still be made between his reference DNA profile and that of the original crime scene evidence. Given the profile of the tested convicted offender, the conditional probability that this offender's untested father's profile would randomly match the perpetrator's profile was equivalent to the numerator of a parent–child autosomal likelihood ratio calculation [10]. This calculation is analogous to equation 4.8 of the National Research Council report [30]. As such, there was an exceedingly low expectation of observing a *random* match across the 15 autosomal loci. After an intensive investigation and the determination that the profile of Lonnie Franklin was identical to the Grim Sleeper evidence profile, Lonnie Franklin was arrested. At the time of submission of this manuscript, he was awaiting trial on 10 counts of murder.

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