Department of Forensic Science

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FORENSIC BIOLOGY SECTION
PROCEDURES MANUAL

FAMILIAL DNA TESTING
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INTRODUCTION

The concept of familial searching is based upon the genetic principle that close biological relatives, especially first degree relatives, will share recognizable similarities in their DNA profiles. When no direct match is obtained from searching an eligible forensic crime scene profile against a DNA database of offender and arrestee samples, a familial search may provide alternative investigative leads. This alternative approach searches the database for possible relatives of the source of the DNA profile. A likelihood ratio (LR) value is produced which is indicative of the strength of potential parent-offspring (PI) or full sibling (Sib) biological relationships. A ranking of likelihood ratios, based upon either sibling or parent-offspring relationships is used, comparing the probability of the forensic profile and each database profile. Familial search result candidates, by definition, are not direct matches but may provide an investigative lead to law enforcement in cases where other leads have been exhausted. A probative evidentiary DNA profile from a case may be suitable for a familial search if it meets certain criteria, such as being a single-source profile with results at all 13 CODIS core STR loci.

The search conducted by DFS on an evidence profile uses familial search software that generates a list of candidates, ranked from largest to smallest, according to sibling and parent-offspring LRs. The candidate list of parent-offspring relationships is evaluated separately from the candidate list of full siblings. After evaluation of the candidates, which involves applying a threshold based upon modified estimated kinship ratio (mEKR) values for each of the three major population groups, Caucasian, Black and Hispanic, selected male candidates will be further analyzed using Y-chromosome STR profiling\(^1,2\). The Y-chromosome contains Y-STRs that are inherited as a block of loci, called a haplotype\(^3\). While a Y-STR haplotype can include an individual when consistent with an evidentiary profile, it also includes his entire patrilineage of male descendents; however, an exclusion is definitive\(^4\). Y-STR profiles of the selected candidates will be evaluated against the Y-STR profile from the evidence to determine whether further action is warranted.

Familial search results will be reported to the submitting law-enforcement agency as an investigative lead, in a format similar to a DNA Data Bank hit report.

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A familial search cannot be performed on all evidence profiles from every forensic case where no suspect or database match occurs. The allele composition at each locus and the number of loci that generate results in a given DNA profile are important factors in determining whether the profile is suitable for a familial search. The purpose of a familial search is to generate a limited number of potential candidates with high and well correlated LR values, such that an evaluation and determination of likely relative candidates vs. unlikely relative candidates can be accomplished.

1.1 A DNA profile with results at a smaller number of STR loci (less than the 13 CODIS core loci) may not be suitable for a familial search.

1.2 A mixture profile with multiple donors where the major and minor donors cannot be distinguished is not suitable for a familial search.

1.3 A DNA profile with partial results at multiple STR loci is not suitable for a familial search.

1.4 Typically one profile per case will be searched, unless it is clear that multiple perpetrators were involved and an additional qualifying profile is present.

1.5 Y-chromosome STR (Y-STR) analysis results on the forensic evidence sample being searched must be available prior to conducting a familial search, such that potential candidate lists can be properly evaluated.

1.6 Only evidence samples with full single source (or clear single source major profile) Y-STR results, or suitable partial Y-STR results sufficient to evaluate and distinguish potential candidates, will be searched.

1.7 No potential candidate information will be released or reported until Y-STR evaluation of potential candidates is conducted.
2 CONDUCTING A FAMILIAL SEARCH USING THE FAMILIAL SEARCHING SOFTWARE

2.1 The familial search is conducted using custom-designed software. Each evidence profile will be searched against the current set of Virginia DNA Data Bank profiles (consisting of Offender and Arrestee DNA profiles). Refer to the Appendix for software operating instructions to execute a familial search.

2.2 Prior to the search, the previous set of databank profiles shall be removed from the familial search software. Then the current set of databank profiles will be exported from CODIS and imported into the familial search software, thereby making certain that each search encompasses the entirety of the databank profiles.

2.2.1 Any necessary QC synthetic profiles for the quality assurance check will be included in the import.

2.3 The evidence profile to be searched will be exported from CODIS and imported into the familial search software.

2.4 The familial search software is set for the following initial threshold LR cutoff values: 100 for Parent-offspring, 33 for Sibling and 50,000 for Half sibling. This means that a candidate profile will not be displayed if all of the calculated LR values for each population group (Caucasian, Black and Hispanic) for that candidate profile (in relation to the evidence profile for the particular relationship) fall below the respective cutoff values.

2.5 When the familial search is executed, the software produces one unsorted candidate list (in an Excel® file format) that contains all candidates that have LR values which pass the initial threshold LR cutoff value for at least one of the relationship categories of Parent-offspring, Sibling or Half sibling in at least one of the population groups.

2.6 After the familial search of the DNA profile from a forensic case has been performed using the familial searching software, the resulting candidate list Excel® file shall be saved without alteration to an appropriate location. That evidence profile and associated result must then be removed from the familial search software before the evidence profile from the next case is imported and searched.

DFS does not utilize half sibling relationships for candidate selection in familial searches, although the software is designed with this capability. Setting the half sibling initial threshold LR cutoff value to 50,000 effectively removes half sibling relationships as a criterion for candidate selection.
3 Evaluation Of Search Candidate Lists

3.1 The resulting unsorted candidate list from the familial search of an evidence DNA profile will be sorted and evaluated twice, using Excel® workbook macros. The first sorting and evaluation will be based on the parent-offspring (PI) LR result from each of the 3 populations (Caucasian, Black and Hispanic). A separate sorting and evaluation will then be made based on the sibling (Sib) LR result from the 3 populations. The Excel® macros apply a second set of thresholds, based upon modified estimated kinship ratio (mEKR) values specific for each population group, that create ranked-order lists of candidates with LR values above the mEKR-based thresholds. The mEKR formula is listed below:

3.1.1 mEKR = LR (autosomal STRs) x LR (Y-haplotype)/database size (N).

3.1.2 The mEKR must be above 1.0 in one of the population groups and above 0.1 in the two other population groups. For an mEKR value to be greater than 1.0 in a given population group, the PI or Sib LR must be ≥315 for the Caucasian population, 360 for the Black population and 595 for the Hispanic population (assuming a database size of 330,000 and Y-haplotype estimates of 1 in 1065 for Caucasians, 1 in 936 for African Americans and 1 in 561 for Hispanics).

3.2 The outcome of the sorting by PI LR values from the 3 populations (Caucasian, Black and Hispanic) will result in a PI LR ranking list for each population.

3.3 The top 15 candidates from each of the three population lists with the highest PI LR values will be selected for additional testing by Y-STR profiling.

3.4 The outcome of the sorting by Sib LR values from the 3 populations (Caucasian, Black, Hispanic) will result in a Sib LR ranking list for each population.

3.5 The top 50 candidates from each of the 3 population lists with the highest Sib LR values will be selected for additional testing by Y-STR profiling.

3.6 Y-STR analysis will be conducted on the selected candidates in accordance with the DFS Forensic Biology Section Procedures Manual pertaining to Y-STR AmpFℓSTR™ Yfiler™ PCR Analysis.

3.7 Candidates not eliminated by the Y-STR analysis will be considered for reporting to the investigative agency.

3.8 If all candidates are eliminated the case may be subjected to additional familial searches at an interval of approximately every 12 months.

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4 REPORTING A FAMILIAL SEARCH RESULT

4.1 A familial search will typically follow and supplement one or more prior Certificates of Analysis reporting the autosomal STR and/or Y-chromosome STR DNA profile results. The METHODS sections addressing those analyses need not be restated when referencing prior reports.

The Certificate of Analysis addressing a familial search will contain a description of the evidence from which the forensic profile being searched was developed, the polymorphic loci utilized in the search and subsequent comparisons, a description of the search methodology used, the results of the familial search, and follow up explanatory information, requests, and recommendations as appropriate. If a potential relative is reported as an investigative lead, the individual’s identifying information will be included in the report.

4.2 The following exemplars display wording that can be used:

Methods and Results

Description of the evidence:

A DNA profile was previously developed from EVIDENCE (Item #) at the ____ loci (PowerPlex 16), and addressed in the Certificate of Analysis dated _______.

OR

… in the Certificates of Analysis dated _____ and _____. (separate autosomal STR reports)

Methods described within the text:

As requested by your agency, the Virginia Department of Forensic Science has conducted a Familial Search of the Virginia DNA Data Bank for potential first-degree relatives (parent/offspring/full sibling) of the source of this DNA profile using STR-based kinship analysis and Y-STR comparisons.

Results:

Negative Search result:

This search did not identify any such potential relatives in the Virginia DNA Data Bank.

This result does not preclude the possibility that such a biological relative may exist in the Virginia DNA Data Bank; however, identification of that relative through the existing methodology was not feasible. Alternatively, the result may indicate that no such relatives exist in the Virginia DNA Data Bank.

A repeated familial search of the same evidence profile may be conducted, upon written request from your agency, no sooner than 12 months from the date of this Certificate of Analysis.

Positive Search result:

This search identified the following possible relative in the Virginia DNA Data Bank:

Name:

SSN:

DOC #:

DCN #:

SID #:
4 Reporting a Familial Search Result

Date of Birth:
Race:
Gender:

This information is provided only as an investigative lead, to which the following information must be understood and applied in the process of further investigation:

- The named individual is not the source of the forensic unknown DNA profile.
- The DNA results provide an indirect association, based on potential genetic relationships, rather than a direct match between the source of the forensic unknown profile and the named individual.
- This search result only indicates the likelihood of a biological relationship; it does not confirm that the named individual is biologically related to the source of the forensic unknown profile.
- While the DNA results are best explained by a (parent-child or full sibling relationship) other familial relationships could also explain these results.
- Other patrilineal relatives of the named individual, and/or of the source of the forensic unknown profile, are expected to have the same Y-STR haplotype.

Request for Known Samples:

Please contact the Laboratory if there are any questions regarding the familial search, or subsequent submission of known samples.

Termination During Search Process:

The Familial Search of the Virginia DNA Data Bank regarding EVIDENCE (Item #) was discontinued at the request of CHIEF OF LEA/COMMONWEALTH’S ATTORNEY on DATE.
5 FAMILIAL SEARCH DOCUMENTATION AND QUALITY ASSURANCE

5.1 Documentation

5.1.1 The candidate lists for parent-offspring and full sibling that meet the mEKR-based threshold (presented in ‘Evaluation of Search Candidate Lists’) will be placed in the case file.

5.1.2 Lineage marker comparison of the candidate results against the evidence profile will be placed in the case file.

5.1.3 The familial search result (in Excel® format) and the sorted candidate lists will be retained electronically.

5.2 Quality Assurance

5.2.1 Each time a familial search session is performed, a QC search will be conducted using a synthetic profile. This profile will be created to resemble a parent or child of the forensic unknown being searched. The expected synthetic QC candidate(s) must appear in the sorted candidate list, indicating that the software is functioning properly. Synthetic QC candidates will not be counted in the selection of top ranked candidates from the sorted candidate lists.

5.2.2 The results of the QC search will be documented and the documentation paperwork included in the case file.

5.2.3 The mEKR thresholds for the 3 population groups will be recalculated approximately every 6 months to monitor any significant deviation from the mEKR guideline (stated in 3.1.2) and updated in the Excel® workbook macros as necessary. The mEKR used in each case will be recorded in the case file.
APPENDIX: SOFTWARE OPERATING INSTRUCTIONS

The familial search software has two screens for general operation.

1. The Main Menu screen (Figure 1) allows the user to import the databank profiles and the forensic profile to be searched and also to execute the search.

![Figure 1. The Main Menu screen](image)

2. Using the Clean Database button, the first step prior to conducting any searches, is to clean out the previous search results.

3. A new screen, the Clean Up Database screen (Figure 2), will open. This screen allows the selective removal of some or all of the imported DNA profiles, as indicated by the different options available.

![Figure 2. The Clean Up Database screen](image)
4. The **Delete Results** box needs to be checked and this function **ALWAYS RUN** prior to a new familial search. This will remove the results table within the software which contains the previous results.

5. The **Load Offenders** button imports the Databank profiles in either a CODIS CMF32 or Interpol format into the software. This is the database that the evidence profile will be searched against.

6. The **Load Forensic** button imports a forensic profile in either CODIS CMF32 or Interpol format into the software. This is the forensic evidence profile that will be searched.

7. The **Select XML Format** option is to indicate whether the export file from CODIS being imported is in the CMF32 or Interpol format.

8. The **Perform Compare** button executes the familial search.

9. The software indicates when the search is completed, and queries the user regarding where to save the Excel® file results.