

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

Kinship and Parentage Analysis		
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Kinship and Parentage Analysis

1 Kinship and Paternity Analysis

- 1.1 Kinship Analysis tests alternate or competing hypotheses of kinship. In the forensic context, it is useful for determining possible familial relationships. This analysis can be used to assist with: the identification of unknown bodies/human remains, the identification of a donor of a biological stain when the donor/body is missing or unavailable, or the identification of the biological father or mother of products of conception/babies, which result from a sexual assault or are abandoned.
- 1.2 Kinship analysis and statistical calculations are performed to evaluate the strength of proposed familial relationships between individuals. Two individuals are said to be related if they have allele(s) that are identical by descent (IBD); IBD probabilities are utilized by KInCALc, a freely available Microsoft Excel spreadsheet tool with user instructions in the spreadsheet developed by the California Department of Justice.

2 Paternity

- 2.1 The most common kinship calculations performed by the laboratory are paternity, maternity and parentage testing. In cases involving disputed paternity, there may be a requirement to provide a paternity calculation where the genotypes of the mother, child, and alleged father are known. In cases involving disputed identity or identification of human remains, there may be a requirement to provide a parentage calculation where the genotypes of the mother, father, and alleged child are known. The basis of parentage testing is that in the absence of mutation, a child receives one IBD allele from each biological parent at every locus tested. In both applications, the data is initially assessed for this inheritance pattern.
- 2.2 Determining the obligate paternal alleles and comparison
 - 2.2.1 The data from the mother and child should be examined to determine the alleles contributed by the true father. For all loci, the mother and child should share one or both alleles (in the absence of mutation).
 - 2.2.2 In the case where the mother and child share one allele, then the allele found in the child, but not the mother is from the true father. This is referred to as the non-maternal or obligate paternal allele.
 - 2.2.3 In the case where the mother and child are homozygous, this is also the allele received from the biological father.
 - 2.2.4 In the case where both mother and child are heterozygous and share both alleles, then it cannot be determined which allele was received from the biological father. Both alleles are considered obligate paternal alleles.

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- 2.3 The DNA profile of the alleged father should be visually examined to see if he carries the obligate paternal alleles at each location.
- 2.3.1 Due to the possibility of mutation at STR loci between generations, two or more loci of the alleged father must not contain the obligate paternal allele before a conclusion of excluded can be determined.
- 2.3.2 If an exclusion is made by visually examining the profiles, no statistical calculation is required.
- 2.3.3 If an exclusion cannot be declared, then a likelihood ratio must be calculated to assess the strength of the match.
- 2.3.3.1 If only a single location of the alleged father does not contain the obligate paternal allele, this is not considered an exclusion. A second likelihood ratio can be calculated allowing for mutation at the single locus of discordance. Refer to Section 4.
- 2.4 Paternity Index
- 2.4.1 **H1: Prob (Evidence (Child) | Mother, Alleged father)** - This is the probability of obtaining the evidence (i.e. profile of the child), if the mother and the alleged father were the biological parents of the child.
- 2.4.2 **H2: Prob (Evidence (Child) | Mother, Random man)** – This is the probability of obtaining the evidence (profile of the child), if the mother and a random member of the population was the biological father (and not the alleged father).
- 2.4.3 **LR** - is the likelihood ratio of the two probabilities and is also called the paternity index (PI). The PI is calculated for each locus and then multiplied together to determine the combined paternity index (CPI).
- 2.4.3.1 The CPI calculations for the four races (NIST African American, NIST Caucasian, NIST Hispanic, and NIST Asian) are performed using KInCALc. The most conservative LR will be used to make the conclusions.
- 2.5 Probability of paternity (only for criminal paternity/maternity cases)
- 2.5.1 The probability of paternity will be manually calculated from the CPI. This calculation is not performed by KInCALc. A prior probability of paternity of 0.5 (neutral assumption) is assumed for the Bayesian part of the calculation. The probability of paternity is the probability that the alleged father is the biological father of the child given the DNA evidence.
- 2.5.2 Probability of paternity will only be calculated when the CPI is 2000 or greater.

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- 2.5.3 To calculate the probability of paternity from the Combined Paternity Index (CPI), using the neutral assumption, the following formula is used:

$$\text{Probability of paternity} = 1 / (1 + (1/\text{CPI}))$$

3 Kinship

- 3.1 Familial relationships other than parent/child (e.g., full siblings, uncle/aunt/grandparent, first cousin, etc.) are evaluated using KInCALc. Kinship comparison does not require designation of the obligate alleles as not all first and second-degree relatives share an allele(s) at every locus. The genotypes for the tested individuals are entered into the worksheet. Likelihood ratios of relatedness hypotheses are used to determine a kinship index (KI).

3.1.1 Kinship Index

- 3.1.1.1 **H1: Prob (tested profiles|UHR is related to the relative(s) in the pedigree)** - This is the probability of obtaining the profiles generated, if the unidentified decedent is related to the family member(s) in the pedigree as stated.
- 3.1.1.2 **H2: Prob (tested profiles | UHR is unrelated to relative(s) in the pedigree)** – This is the probability of obtaining the profiles generated, if the unidentified decedent is unrelated to the family member(s) in the pedigree as stated.
- 3.1.1.3 **LR** - is the likelihood ratio of the two probabilities and is also called the kinship index (KI). The KI is calculated for each locus, and then multiplied together to determine the combined kinship index (CKI).
- 3.1.1.4 The KPI calculations for the four races (NIST African American, NIST Caucasian, NIST Hispanic, and NIST Asian) are performed using KInCALc. The most conservative LR will be used to make the conclusions.

4 Mutations

- 4.1 There are three mutation approaches:

Approach 0: mutations are not allowed

Approach 1: considers mutations possible at every locus

Approach 2: only applicable to parentage PIs and uses μ/PE

μ = gender specific mutation rate; $\text{PE} = h^2 (1-2hH^2)$ where H is the frequency of homozygosity and h is the frequency of heterozygosity

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- 4.2 Mutation approach 0 will initially be used for all calculations. If a non-zero LR is returned, it will be reported.
- 4.2.1 If an LR of zero occurs for a parentage calculation, the reason for the LR of zero should be explored and documented. (This step may be omitted if the mutation between parent-child is documented by the analyst **prior** to the kinship calculation.) If the interpretation of the data supports relatedness, then usually the LR of zero will be due to discordance at one locus. Highlight the locus where there is discordance, update the mutation approach to 2, and re-run to get an updated PI.
- 4.2.2 If an LR of zero occurs for any other familial relationship comparison, update the mutation approach to 1 and re-run the KI calculation.

5 Silent Alleles

- 5.1 Silent alleles refer to when a primer binding site mutation results in a null allele or false homozygosity. The default silent allele approach within the worksheet is zero, where they are not considered.

6 Custom Pedigrees

- 6.1 Pedigrees that will not fit into the structure of the Pedigree worksheet may be entered under Custom Pedigree. “Manual alternate pedigree?” defaults to “No” and is selected when only one individual is being evaluated (i.e., numerator and denominator are the same except for one person), the individual is considered related in H1 and unrelated in H2, and there are reference samples available for this individual. “Manual alternate pedigree?” can be set to “Yes” if the above does not apply and an alternate pedigree is customized. Custom pedigrees are appropriate for incest cases.
- 6.2 Draw the pedigree(s) first. TEST is always category 17. Follow the instructions on the Custom Pedigree worksheet for adding individuals with references available (1-10) and those with references not available (21-33). When the pedigree is set up, click the button Insert Pedigree(s) Into KI Worksheet and follow the steps below. The drawn pedigree should be included in the case file for technical review.

7 Paternity/Kinship Calculations Using KInCALc 5.0.12

- 7.1 Open KInCALc in MS Excel with macros enabled.
- 7.2 Go to the Kit Conversion worksheet and manually enter the item number and alleles for the first DNA profile. The loci are in the selected kit order.
- 7.2.1 For homozygote genotypes, enter the allele twice. Only enter a single allele if drop-out is possible.

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- 7.2.2 Alleles outside the allelic ladder can be entered as <# or >#. Do not enter OL or OB.
- 7.3 After the profile is entered, click the Transfer to Profiles button.
- 7.4 Repeat step 7.2 for additional DNA profiles needed for the calculation. Note: the order of samples on the Profiles worksheet does not matter.
- 7.5 Go to the Pedigree worksheet and define the pedigree.
- 7.5.1 Click the button “1st: Establish Relationship Categories”. Click on the required cells for people with reference DNA profiles. The macro identifies untested relatives. The person of interest or unknown “Test” is the green cell with an X and does not need to be selected.
- 7.5.2 Enter the biological genders (M/F) based upon the pedigree. The cells will turn green. Note: mutation rates are affected by the gender information so entering this correctly is important.
- 7.5.3 The text at the top of the worksheet will state “Pedigree complete. Ready to insert to KI worksheet.” Click the button “2nd: Insert Pedigree into KI Worksheet”.
- 7.6 On the KI worksheet, use the drop-down menus to select item numbers to associate DNA profiles with the pedigree.
- 7.7 Enter the case number, analyst initials, and date the calculation is performed.
- 7.8 Other settings should be as follows:
- 7.8.1 Select Databases: African American, Caucasian and Hispanic NIST databases (or Asian – this KI will need be calculated separately)
- 7.8.2 General allele probability setting: $x/2N$ (Frequency as observed in the database)
- 7.8.3 Minimum allele probability options: $5/2N$ (ala NRCII: Allele counts < 5 increased to 5)
- 7.8.4 Multiplex for report: Fusion
- 7.8.5 Theta: 0 for parentage (criminal paternity and UHR/MP comparisons), 0.01 for all other calculations
- 7.8.6 Silent alleles: 0 (Default, silent alleles (nulls) not considered unless entered as such in the profile)
- 7.8.7 Mutation: 0 (Default, mutations not allowed)
- 7.8.7.1 If a KI of zero is returned, a mutation has occurred. Generate the KI Report for the original KI, reset to approach 2 for parentage/simple trio and to approach 1 for all other pedigrees. Re-run KI.

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- 7.9 Linkage between vWA and D12S391 may impact the calculation and one of the following approaches shall be followed.
- 7.9.1 Linkage can be ignored for parentage calculations and all loci should be used for the KInCALc. This does not apply to incestuous paternity, which will require the next option.
- 7.9.2 For any other complex or custom pedigrees account for linkage by dropping either vWA or D12S391 KI from the CKI calculation.
- 7.9.2.1 Calculate the KI with all loci, generate the KI report report, and evaluate the locus KIs at vWA and D12S391.
- 7.9.2.2 On the KI worksheet, change the [Omit?] selection to [Yes] for each database for the locus with the higher KI.
- 7.10 Click the KI button to calculate the kinship indices. The worksheet will give the KIs per locus and overall. If a KI of zero is obtained, review the results.
- 7.11 Go to the Report tab. For each KI calculated, pages 1 and 2 must be added to the case file.

8 Conclusions

- 8.1 Parentage (Criminal paternity and Missing Persons/UHR comparisons): The Forensic Biology case report should include all four races used for the calculations, the CPI for each, and the probability of paternity and the assumed prior probability, where applicable. The lowest CPI between the four races will be used to determine the conclusion. The three possible final conclusions are exclusion, inconclusive, or inclusion, based on the resulting CPI.
- 8.1.1 Exclusion: 2 or more loci where the obligate paternal allele is not present in the alleged parent or if the CPI is < 0.1 .
- 8.1.2 Inconclusive - occurs when the combined paternity index is between 0.1 and 10.
- 8.1.3 Inclusion:
- 8.1.3.1 When the CPI is between 11 and 99, the H1 should not be rejected, and should be considered a weak inclusion.
- 8.1.3.2 When the CPI is between 100 and 1999, the H1 is supported by the data.
- 8.1.3.3 When the CPI is 2000 or greater, H1 should be accepted (probability of paternity $> 99.9\%$, 50% prior probability for criminal parentage, paternity practically proven)
- 8.2 Other familial relationships kinship analysis - For sibling(s) and other relative(s) (non-parent) comparisons: The Forensic Biology case report should include the combined kinship index for all

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four races used for the calculations. The lowest CKI between the four races will be used to determine the conclusion reported. The three possible final conclusions are exclusion, inconclusive, or cannot be excluded, based on the resulting CKI.

- 8.2.1 Support for unrelated: CKI is < 1
- 8.2.2 Inconclusive: When the CKI is 1.
- 8.2.3 Cannot be excluded: When the lowest CKI is >1 .
- 8.2.4 When the KI falls between 0.01 and 99, this shows limited support for the respective hypothesis. Therefore, it is recommended to test additional relatives and/or technologies.