

Forensic Parentage Manual

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### **Section 1 Scope of Work**

The ASCDL performs parentage and relationship testing in criminal cases of the following types:

- a. Forward one-parent paternity - where one biological parent is known and the other (often the father) is in question. Reference samples are analyzed for all three individuals in this scenario.
- b. Forward zero-parent paternity – where one biological parent is in question and the other is not available. Reference samples are collected from the two available individuals in this scenario.
- c. Reverse parentage – where a reference from an unidentified person or remains is compared against a pair of alleged biological parents. Reference samples for all three individuals are analyzed in this scenario.

Cases which fall outside of this scope, particularly those involving close biological relatives (incest), may have laboratory analysis performed at the ASCDL. Cases resulting in exclusions can be resolved; but relevant statistical analyses for inclusions cannot be performed in-house at this time.

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## **Section 2 Parentage Testing Terminology**

For purposes of convenience, many of these definitions are phrased in terms of an alleged father, since that is the most common scenario encountered in forensic paternity work.

**Likelihood Ratio:** the ratio of two probabilities of the same event under different hypotheses. For example, in the case of one-parent paternity testing, the likelihood ratio compares the support of the genetic evidence for the hypothesis that the alleged man is the true biological father, against the support of the biological evidence that a random and unrelated man is the true biological father.

**Paternity Index (PI):** A likelihood ratio at a single genetic locus that compares the probability of the observed genotypes (DNA profiles) if the tested man is the true biological father, to the probability of the observed genotypes (DNA profiles) if a random untested man is the true biological father.

**Combined Paternity Index (CPI):** Because the genetic information at each of the loci is inherited independently, paternity indexes can be multiplied together to get a combined paternity index. The CPI is a measure of the strength of the genetic information from several loci. It indicates whether the hypothesis that the tested man is the father, or the hypothesis that a random untested man is the father, is more supported by the genetic evidence.

**Combined Paternity Index Ranges:** In theory, for a CPI less than one, the genetic evidence is more consistent with non-paternity than paternity. In theory, for a CPI greater than one, the genetic evidence supports the hypothesis that the tested man is the father: "It is XXX times more likely to see the genetic results if the tested man was the true biological father than if an untested random man was the father."

**Probability of Paternity:** A calculation based on both the paternity index and the prior odds. This probability should, in theory, include all evidence in the case, including both the non-genetic information and the genetic information from the DNA paternity test; as such, it is a measure of the weight of all the evidence. In practice, it is common to assume a prior probability of 0.5, or 1:1, when calculating and reporting probability of paternity.

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**Mutation rate:** the rate at which a genetic marker mutates or changes over time. It is the number of mutations per hundreds of generations expressed as a decimal value or a percentage. It indicates how often, on average, one expects a random man in the population, unrelated to the child whose parentage is in question, to appear as if he is the biological father.

**Mean power of exclusion:** the average probability that a random person would have a pattern of genetic information inconsistent with paternity at a particular locus. It indicates how often, on average, one expects a random person in the population, who is unrelated to the child whose parentage is in question, to be correctly excluded as a biological parent.

**One-parent Paternity:** A scenario that includes biological reference samples from a child, one known parent and one alleged parent.

**Zero-parent Paternity:** A scenario that includes biological reference samples from a child and one alleged parent (with no reference from a known biological parent).

**Exclusion:** The obligate parental alleles in the child do not match the alleles in the alleged father in at least four loci. The alleged father is excluded from being the biological father of the child being tested.

**Inclusion (Cannot Exclude):** The obligate parental alleles in the child match the alleles in the alleged father at all (or all but one) of the loci. The alleged father cannot be excluded from (or is consistent with being) the biological father of the child being tested.

**Reverse parentage:** A scenario in which a missing person's reference sample is compared to samples from a pair of alleged biological parents.

**Parentage:** Refers to either paternity or maternity; paternity and parentage are often used interchangeably in genetic testing terminology.

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### **Section 3 Data Interpretation**

On occasion, reference samples may be of poor quality or degraded by environmental conditions. For such samples, loci not deemed complete (due to the possibility of partial or complete allelic drop-out) will not be considered exclusionary solely on the basis of incomplete information. All complete loci will be considered in assessing consistency between reference samples.

- Four or more loci with non-consistent genetic markers – the alleged parent(s) is/are excluded. It is not necessary to calculate CPI or probability of paternity in such cases.
- Two or three loci with non-consistent genetic markers – these results are inconclusive. The CPI and Probability of Exclusion will be calculated and reported, but further testing with additional markers will be recommended to the submitting agency.
- One locus with an inconsistent genetic marker within one repeat unit of the obligate allele – given the possibility of a genetic mutation, this will not be deemed an exclusion. In such a case, the alleged parent(s) cannot be excluded, and CPI and Probability of Exclusion will be calculated and reported.
- All loci have consistent genetic markers – the alleged parent cannot be excluded, and the CPI and Probability of Exclusion will be calculated and reported.

### **Section 4 Significance Estimation**

As with ASCDL casework, calculations will be performed and reported for the following populations: Caucasians, African-Americans, Athabaskans, Inupiat, and Yupiks. Calculations are performed using the U. S. Department of Justice, FBI Popstats Standalone program, version 7.0. The resultant values may be truncated for reporting, but should never be rounded up.

Some assumptions underlying the statistical calculations include:

- Hardy-Weinberg equilibrium
- In a false trio - the biological father is from the same population
- In a false trio – the biological father is unrelated to the mother and child
- In a false trio – the biological father is the same genotype as the tested father
- PI for a mutation is not allele dependent

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For one-parent forward calculations, 0.5 is used for the prior probability.

For all calculations at a locus with possible mutation, Paternity Index = mutation rate for that locus / mean power of exclusion for that locus. Calculations will use mutation rates by locus as reported by NIST, and mean powers of exclusion from the published articles for the respective databases (references listed at the end of this section).

Paternity Index (PI) is calculated for each locus, according to the formulas listed in the tables below. In those tables, capital letters refer to the allele(s) present in each individual tested, and the small letters refer to the frequency of the allele(s).

After PI is calculated (as below) for each locus, the Combined Paternity Index (CPI) is calculated by multiplying together all the individual PIs.

Assuming a prior probability of 0.5, probability of paternity (expressed as a percentage) is calculated as  $(CPI/(CPI+1)) \times 100$ . Probability of paternity is reported to three decimal places (XX.xxx %). These values will be truncated, not rounded, for reporting purposes.

Forward one-parent testing:

Known Parent	Child	Alleged Parent	Paternity Index
BD	AB	AC	$1/2a$
BC	AB	AC	$1/2a$
BC	AB	AB	$1/2a$
BC	AB	A	$1/a$
B	AB	AC	$1/2a$
B	AB	AB	$1/2a$
B	AB	A	$1/a$
AB	AB	AC	$1/[2(a+b)]$
AB	AB	AB	$1/(a+b)$
AB	AB	A	$1/(a+b)$
AB	A	AC	$1/2a$
AB	A	AB	$1/2a$
AB	A	A	$1/a$
A	A	AC	$1/2a$
A	A	A	$1/a$

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Forward zero-parent testing:

Child	Alleged Parent	Paternity Index
AB	AC	$1/4a$
AB	AB	$(a+b)/4ab$
AB	A	$1/2a$
A	AC	$1/2a$
A	A	$1/a$

Reverse parentage testing:

Alleged Parent - 1	Child	Alleged Parent - 2	Paternity Index
BD	AB	AC	$1/8ab$
BC	AB	AC	$1/8ab$
BC	AB	AB	$1/8ab$
BC	AB	A	$1/4ab$
B	AB	AC	$1/4ab$
B	AB	AB	$1/4ab$
B	AB	A	$1/2ab$
AB	AB	AC	$1/8ab$
AB	AB	AB	$1/4ab$
AB	AB	A	$1/4ab$
AB	A	AC	$1/4a^2$
AB	A	AB	$1/4a^2$
AB	A	A	$1/2a^2$
A	A	AC	$1/2a^2$
A	A	A	$1/a^2$

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### **Section 5 Using Standalone Popstats 7.0 for Parentage Statistics**

[NOTE: On occasion, especially when entering mutation rates or toggling between databases, the screen may not automatically refresh to show updated changes. If you do a Print Preview, you can confirm that changes have been applied.]

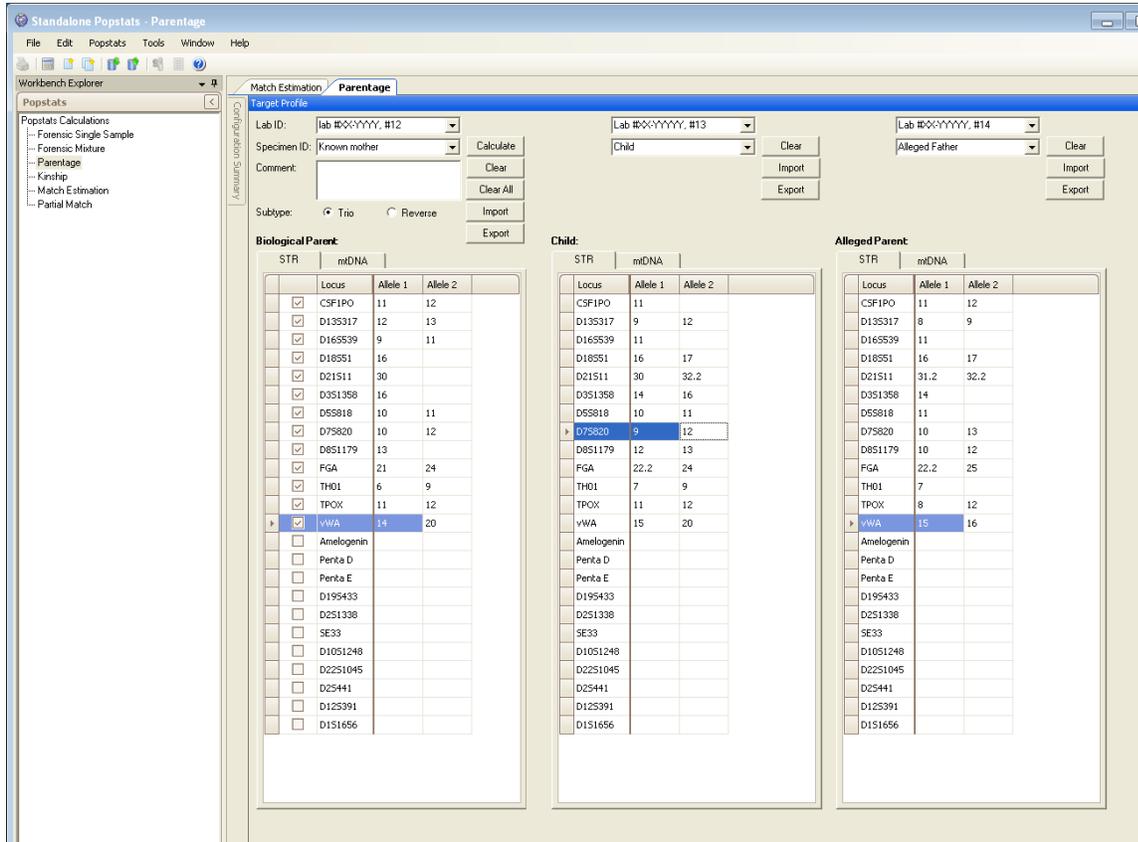
#### **A. One-parent forward paternity (trio):**

1. Open Popstats and choose Parentage from the menu on the left side of the screen.
2. Choose the Parentage tab at the top of the screen.
3. Use the Lab ID field to enter the lab number at least, item number space permitting. If the item numbers do not fit in the typed field they may be hand-written on the printout.
4. Under Subtype, select the Trio button (see following image)
5. Enter STR information from all complete loci (If a locus has partial information, it will not be used for statistics – this includes not only the reference with the partial information, but the same locus in the other references as well).

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6. When the correct information has been entered, select the Calculate button (upper left).
7. If any loci have mutations (mismatched), you will be prompted to enter the mutation rate and the mean power of exclusion (see following image).

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Mutation Rate and Mean Power of Exclusion

Locus: D7S820

Enter Mutation Rate and Mean Power of Exclusion

NOTE: A mutation rate of 0.123% should be entered as 0.00123

Group Name	Mutation Rate	Mean Power of Exclusion
Ath	0.001	0.58
Inu	0.001	0.548
Yup	0.001	0.533

OK Cancel

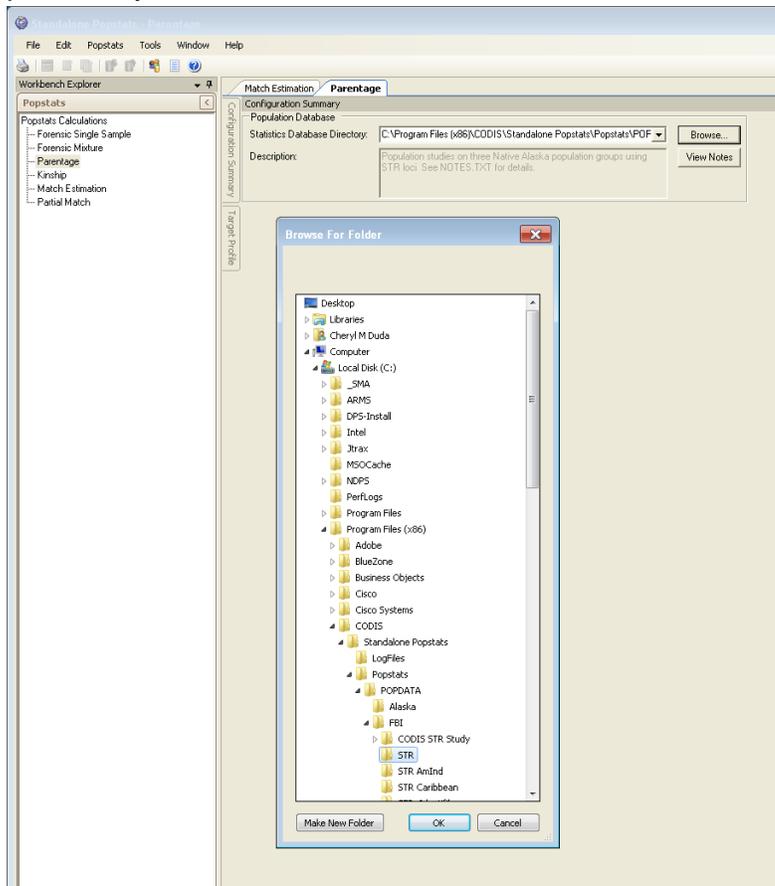
8. Enter the locus-specific mutation rate from the NIST website. A printout of the relevant page is included in this manual.
9. Enter the locus-specific mean probability of exclusion (PE) from the published database references listed at the end of this section of the manual. Please note that these values are NOT the default values included in the Popstats software.
10. Once these values are entered, click OK.
11. Printing: Under the File tab, select Print, then select Parentage Trio Calculations, then select Print.
12. Print out statistic reports for the Caucasian and African-American databases (from the FBI - STR database file) and the Athabaskan, Inupiat, and Yupik databases (from the Alaska database file). In order to toggle between databases, click on the Configuration Summary tab on the left side. Choose Browse to switch between the Alaska and FBI databases (see following image). Once the new database has been selected, click the Target Profile tab to return to the data entry screen, where you will again choose Calculate.

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13. As before, if necessary, you will be prompted to enter mutation rate and mean power of exclusion. When finished, choose OK. Print report as described previously.



B. Reverse parentage (trio):

1. Open Popstats and choose Parentage from the menu on the left side of the screen.
2. Choose the Parentage tab at the top of the screen.
3. Use the Lab ID field to enter the lab number at least, item number space permitting. If the item numbers do not fit in the typed field they may be handwritten on the printout.
4. Under Subtype, select the Reverse button (see following image)

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5. Repeat steps 5-12 from Section A.
6. On reverse parentage trios, Popstats does not allow for the use of a locus with a mutation. In cases with a mutation, omit the locus with the mutation from the Popstats calculation. On the Popstats printout, manually calculate the PI for the locus with the mutation. Use this manual calculation to adjust the CPI and Probability of Paternity calculations as well. Show work clearly.

C. Zero-parent forward:

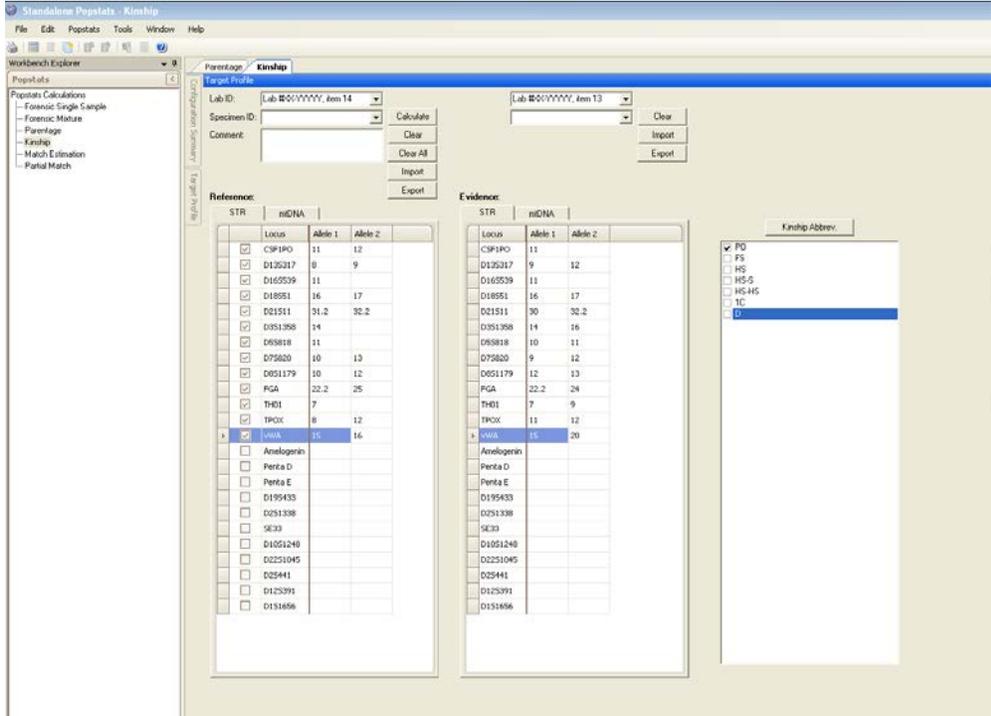
1. Open Popstats and choose Kinship from the menu on the left side of the screen.
2. Choose the Kinship tab at the top of the screen.
3. Use the Lab ID field to enter the lab number (at least, item number space permitting). If the item numbers do not fit in the typed field they may be handwritten on the printout.
4. On the right side of the screen, make sure that only the PO (Parent-Offspring) box is checked (see following image).

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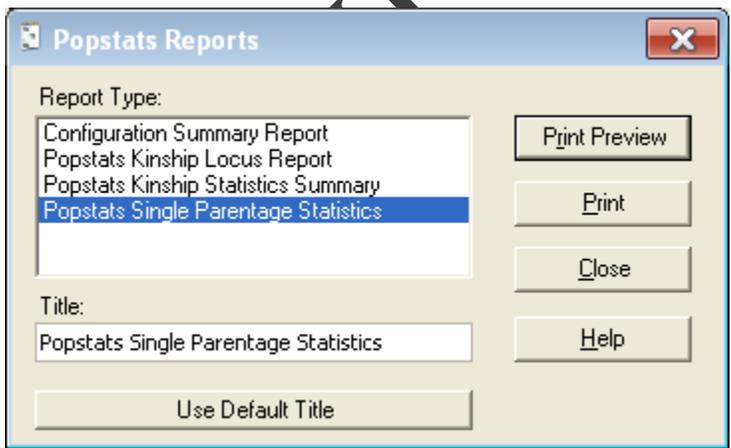
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5. Repeat steps 5-10 from Section A.
6. Printing: Under the File tab, select Print, then select Popstats Single Parentage Statistics, then select Print (see following image).



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7. Print out statistic reports for the Caucasian and African-American databases (from the FBI – STR database file) and the Athabaskan, Inupiat, and Yupik databases (from the Alaska database file). Directions and image for toggling between databases are listed in Section A step 12.

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**Section 6 Sample Report Language**

**Sample report language for one parent forward paternity**

**Inclusion:**

Based on the DNA profiles obtained from the samples listed above (XX core loci), ALLEGED FATHER cannot be excluded as the possible biological father of CHILD'S NAME, assuming MOTHER'S NAME is the biological mother of CHILD'S NAME.

[If applicable: A single genetic inconsistency is observed for the obligate allele at locus\_\_\_\_. This is indicative of a mutation or recombination event in which the allele inherited from the alleged father by the child has been altered.]

<u>Population</u>	<u>Combined Paternity Index</u>	<u>Probability of Paternity</u>
Caucasian		
African-American		
Athabaskan		
Inupiat		
Yupik		

Combined paternity index indicates how many times more likely the observed genetic evidence is if the alleged father is the true biological father of the tested child rather than an unrelated individual from each of the above populations.

Probability of paternity assumes a prior probability of 0.5 and is compared with an untested, unrelated individual from each of the above populations.

**Exclusion:**

Based on the DNA profiles obtained for the samples listed above (XX core loci), ALLEGED FATHER is excluded as a possible biological father of CHILD'S NAME, assuming MOTHER'S NAME is the biological mother of CHILD'S NAME.

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**Inconclusive:**

Based on the DNA profiles obtained for the samples listed above (XX core loci), no conclusions are reported as to whether ALLEGED FATHER could be a possible biological father of CHILD'S NAME, assuming MOTHER'S NAME is the biological mother of CHILD'S NAME. It is recommended that further testing be performed to gain additional information from more genetic loci.

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**Sample report language for zero parent forward parentage**

**Inclusion:**

Based on the DNA profiles obtained for the samples listed above (XX core loci), ALLEGED FATHER cannot be excluded as the possible biological father of CHILD'S NAME.

[If applicable: A single genetic inconsistency is observed for the obligate allele at locus\_\_\_\_. This is indicative of a mutation or recombination event in which the allele inherited from the alleged father by the child has been altered.]

<u>Population</u>	<u>Combined Parentage Index</u>	<u>Probability of Exclusion</u>
Caucasian		
African-American		
Athabaskan		
Inupiat		
Yupik		

Combined parentage index indicates how many times more likely the observed genetic evidence is if FATHER'S NAME is the true biological father of CHILD'S NAME rather than an unrelated individual from each of the above populations.

**Exclusion:**

Based on the DNA profiles obtained for the samples listed above (XX core loci), ALLEGED FATHER is excluded as a possible biological father of CHILD'S NAME.

**Inconclusive:**

Based on the DNA profiles obtained for the samples listed above (XX core loci), no conclusions are reported as to whether ALLEGED FATHER could be a possible biological father of CHILD'S NAME. It is recommended that further testing be performed to gain additional information from more genetic loci.

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**Sample report language for reverse parentage**

**Inclusion:**

Based on the DNA profiles obtained for the samples listed above (XX core loci), ALLEGED CHILD cannot be excluded as the possible biological child of FATHER and MOTHER.

[If applicable: A single genetic inconsistency is observed for the obligate allele at locus \_\_\_\_\_. This is indicative of a mutation or recombination event in which the allele inherited from the parent by the alleged child has been altered.]

<u>Population</u>	<u>Combined Parentage Index</u>	<u>Probability of Exclusion</u>
Caucasian		
African-American		
Athabaskan		
Inupiat		
Yupik		

Combined parentage index indicates how many times more likely the observed genetic evidence is if ALLEGED CHILD is the true biological child of FATHER and MOTHER rather than unrelated individuals from each of the above populations.

**Exclusion:**

Based on the DNA profiles obtained for the samples listed above (XX core loci), ALLEGED CHILD is excluded as a possible biological child of FATHER and MOTHER.

**Inconclusive:**

Based on the DNA profiles obtained for the samples listed above (XX core loci), no conclusions are reported as to whether ALLEGED CHILD could be a possible biological child of FATHER and MOTHER. It is recommended that further testing be performed to gain additional information from more genetic loci.

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## Section 7 References

AABB relationship testing annual reports.

[http://www.aabb.org/Content/Accreditation/Parentage\\_Testing\\_Accreditation\\_Program/Relationship\\_Testing\\_Annual\\_Reports](http://www.aabb.org/Content/Accreditation/Parentage_Testing_Accreditation_Program/Relationship_Testing_Annual_Reports)

Gjertson, D.W. et al. (2007) ISFG: Recommendations on biostatistics in paternity testing. *Forensic Science International Genetics*, 1, 223-231.

Morling, N. et al. (2002) Paternity testing commission of the international society of forensic genetics: Recommendations on genetic investigations in paternity cases. *Forensic Science International*, 129, 148-157.

Thomson, J.A. , et al. (1999) Validation of short tandem repeat analysis for the investigation of cases of disputed paternity. *Forensic Science International*, 100, 1-16.

Jacewicz, R. et al. (2004) Non-exclusion paternity case with a triple genetic incompatibility. *International Congress Series* 1261 511-513.

Mean Power of Exclusion by locus:

Budowle, B. et al. Population data on the thirteen CODIS Core Short Tandem Repeat Loci in African Americans, U.S.Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. (1999) *Journal of Forensic Science*, 44 (6): 1277-1286.

Budowle, B. et al. Population studies on three Native Alaska population groups using STR loci. (2002) *Forensic Science International* 129 51-57.

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**Appendix A: NIST Mutation Rates at STR Loci**

Apparent Mutations Observed at STR Loci in the Course of Paternity Testing*					
STR System	Maternal Meioses (%)	Paternal Meioses (%)	Number from either	Total Number of Mutations	Mutation Rate
<a href="#">CSF1PO</a>	95/304,307 (0.03)	982/643,118 (0.15)	410	1,487/947,425	<b>0.16%</b>
<a href="#">FGA</a>	205/408,230 (0.05)	2,210/692,776 (0.32)	710	3,125/1,101,006	<b>0.28%</b>
<a href="#">TH01</a>	31/327,172 (0.009)	41/452,382 (0.009)	28	100/779,554	<b>0.01%</b>
<a href="#">TPOX</a>	18/400,061 (0.004)	54/457,420 (0.012)	28	100/857,481	<b>0.01%</b>
<a href="#">VWA</a>	184/564,398 (0.03)	1,482/873,547 (0.17)	814	2,480/1,437,945	<b>0.17%</b>
<a href="#">D3S1358</a>	60/405,452 (0.015)	713/558,836 (0.13)	379	1,152/964,288	<b>0.12%</b>
<a href="#">D5S818</a>	111/451,736 (0.025)	763/655,603 (0.12)	385	1,259/1,107,339	<b>0.11%</b>
<a href="#">D7S820</a>	59/440,562 (0.013)	745/644,743 (0.12)	285	1,089/1,085,305	<b>0.10%</b>
<a href="#">D8S1179</a>	96/409,869 (0.02)	779/489,968 (0.16)	364	1,239/899,837	<b>0.14%</b>
<a href="#">D13S317</a>	192/482,136 (0.04)	881/621,146 (0.14)	485	1,558/1,103,282	<b>0.14%</b>
<a href="#">D16S539</a>	129/467,774 (0.03)	540/494,465 (0.11)	372	1,041/962,239	<b>0.11%</b>
<a href="#">D18S51</a>	186/296,244 (0.06)	1,094/494,098 (0.22)	466	1,746/790,342	<b>0.22%</b>
<a href="#">D21S11</a>	464/435,388 (0.11)	772/526,708 (0.15)	580	1,816/962,096	<b>0.19%</b>
<a href="#">Penta D</a>	12/18,701 (0.06)	21/22,501 (0.09)	24	57/41,202	<b>0.14%</b>
<a href="#">Penta E</a>	29/44,311 (0.065)	75/55,719 (0.135)	59	163/100,030	<b>0.16%</b>
<a href="#">D2S1338</a>	15/72,830 (0.021)	157/152,310 (0.10)	90	262/225,140	<b>0.12%</b>
<a href="#">D19S433</a>	38/70,001 (0.05)	78/103,489 (0.075)	71	187/173,490	<b>0.11%</b>
<a href="#">SE33 (ACTBP2)</a>	0/330 (<0.30)	330/51,610 (0.64)	None reported	330/51,940	<b>0.64%</b>

\*Data used with permission from [American Association of Blood Banks \(AABB\) 2003 Annual Report](#).

Includes compilation of multiple years. Information also available on population and allele-specific mutation rates (see <http://www.aabb.org/sa/facilities/Pages/relationshipreports.aspx>). A total of 44 different paternity testing laboratories provided this STR mutation data.

Data from the NIST webpage <http://www.cstl.nist.gov/div831/strbase/mutation.htm>  
(current as of 1/9/14)

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**Appendix B: Revision History**

FPM 2014 R1 Page	FPM 2014 R0 Page	Location	Revision made
6	6	Section 4	<b>Revised</b> the sentence beginning "For all calculations, 0.5 is used...." to "For one-parent forward calculations, 0.5 is used...."
17	17	Section 6	<b>Replaced</b> "paternity" with "parentage" in the title for the zero parent forward sample report.
17	17	Section 6	<b>Replaced</b> "paternity" with "parentage" in the phrase "combined parentage index" in the zero parent forward sample report (2 instances).
17	17	Section 6	<b>Replaced</b> "Probability of Paternity" with "Probability of Exclusion" in the table of the zero parent forward sample report.
17	17	Section 6	<b>Removed</b> the paragraph beginning with "Probability of paternity assumes...." from the zero parent forward sample report.

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