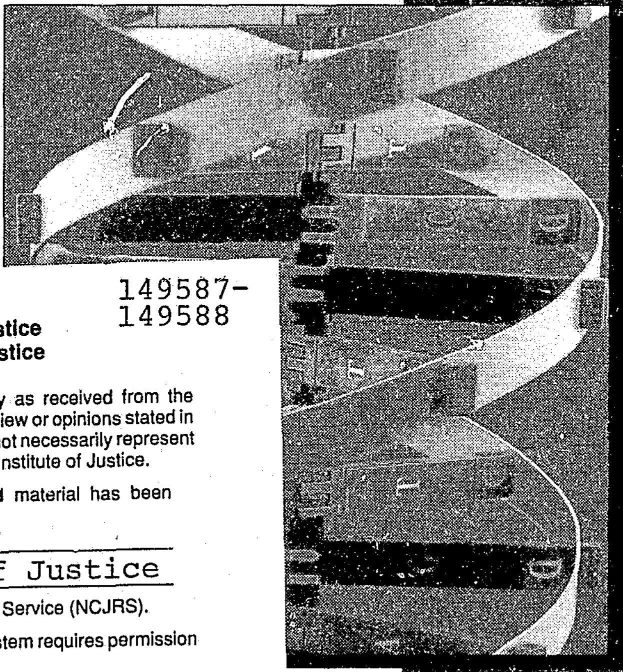
U.S. Department of Justice
Federal Bureau of Investigation

CRIME LABORATORY DIGEST

APRIL 1994

Volume 21, Number 2



The TWGDAM Consensus Approach for Applying the "Ceiling Principle" to Derive Conservative Estimates of DNA Profile Frequencies

Guidelines for DNA Proficiency Test Manufacturing and Reporting

CRIME LABORATORY DIGEST

APRIL 1994

Volume 21, Number 2

ARTICLES

The TWGDAM Consensus Approach for Applying the "Ceiling Principle" to Derive Conservative Estimates of DNA Profile Frequencies 21

149587

Guidelines for DNA Proficiency Test Manufacturing and Reporting 27

149588

DEPARTMENTS

Message from the Assistant Director 18
Editor's Note 33
Book Review 33
Employment Opportunities 34
Meeting Announcements Inside Back Cover

SPECIAL FEATURES

Book Ordering Information 33
Subscriber Information 36

The *Crime Laboratory Digest* (ISSN 0743-1872) is a forensic science journal published by the FBI Laboratory Division in cooperation with the American Society of Crime Laboratory Directors (ASCLD). It is intended to serve as a means of communication between crime laboratories, permitting information of interest and value to be disseminated among crime laboratory scientists.

Postmaster: Send address changes to Editor, *Crime Laboratory Digest*, FSRTC, FBI Academy, Quantico, VA 22135

149587

The TWGDAM Consensus Approach for Applying the "Ceiling Principle" to Derive Conservative Estimates of DNA Profile Frequencies

An alternative method, termed the "ceiling principle," has been devised by the National Research Council (NRC) of the National Academy of Sciences (NRC 1992) for calculating DNA profile frequency estimates. This method provides an even more conservative estimate of the likelihood of occurrence of a DNA profile than the fixed bin or floating bin methods currently employed by the forensic community. The need for the ceiling principle approach was based on a "for the sake of discussion" (NRC Report 1992, p. 80) premise that population substructure might affect the ability to obtain valid estimates of the likelihood of occurrence of a DNA profile when using general population databases.

The "interim" ceiling principle method for deriving very conservative DNA profile frequency estimates is the current approach described in the NRC Report (1992). In the interim ceiling principle, the 95% upper confidence limits of allele frequencies in at least three major US population groups are tabulated. In other countries, the three databases could differ from that required in the United States. The ceiling frequency for each band in a DNA profile is defined as the maximum of the 95% upper confidence limits of the fixed or floating bin frequencies in each general database, or a minimum of 0.100. The frequency of a single locus and/or multiple locus profile is obtained by the product of these interim ceiling frequencies. At such time when data from 15 to 20 "genetically homogeneous" population subgroups become available, the minimum frequency will become 0.050.

The Technical Working Group on DNA Analysis Methods (TWGDAM) cannot recommend the application of the ceiling principle. The basis for the need for a ceiling principle approach is flawed (Budowle and Monson 1993; Budowle *et al.* 1994a, 1994b; Devlin and Risch 1992b; Devlin *et al.* 1993; Latter 1980; Mitton 1978, 1977; Morton 1992; Morton *et al.* 1993; Nei and Roychoudhury 1982; Smouse *et al.* 1982; Weir 1993, 1992b). The need for the ceiling principle is based upon the faulty premise that there is more genetic variation among subgroups within a major population group than between major population groups (Lander 1991; Lewontin and Hartl 1991; NRC Report 1992); the extant data demonstrate the opposite and that the application of the ceiling principle is unnecessary. The current methods employed by forensic scientists have been demonstrated to be robust scientifically (Budowle *et al.* 1994a, 1994b; Chakraborty 1991; Chakraborty and Jin 1992; Chakraborty and Kidd 1991; Chakraborty *et al.* 1993a; Chakraborty *et al.* 1992a, 1992b; Devlin and Risch 1992a, 1992b; Devlin *et al.* 1992; Evett and Gill 1991; Risch and Devlin 1992; Weir 1992a).

While the ceiling principle approach has been criticized severely (Budowle and Monson 1993, 1992; Chakraborty *et al.* 1992b; Devlin *et al.* 1994, 1993; Morton 1992; Weir 1993), it is generally accepted that the interim ceiling principle approach for estimating DNA profile frequency estimates produces conservative results (Budowle and Monson 1993, 1992; Hartl and Lewontin 1993; Krane *et al.* 1992; Lander 1993; NRC Report 1992). This is supported by the observation that ceiling principle estimates of DNA profile frequencies generally are more common estimates than those derived by the already conservative fixed bin approach (Budowle and Monson 1993; Budowle *et al.* 1991; Chakraborty *et al.* 1993b; Devlin *et al.* 1992; Monson and Budowle 1993). For those few situations where the ceiling principle estimate is less common than a fixed bin estimate, the differences in the frequencies are so small as to have no consequence on the inference of the rarity of the DNA profile (Budowle and Monson 1993).

However, for those courts that still prefer DNA profile frequency estimates to be derived using the very conservative interim ceiling principle approach, TWGDAM has developed a consensus approach for the ceiling principle. The attempt is to interpret the intent of the NRC Report (1992) for the use of the interim ceiling principle and to eliminate confusion that some courts may encounter when considering alternate interpretations of the ceiling principle. There are two basic approaches to establishing ceiling principle frequencies. These are based on using either fixed or floating bins to assign allele frequencies. Since both the fixed bin and floating bin ceiling principle approaches, described in the following sections, yield similar DNA profile frequency estimates, either can be employed. It is unnecessary to provide estimates for both approaches. The choice is up to the laboratory.

Fixed Bin Ceiling Approach

1. Fixed bin frequencies will be generated for the appropriate data set as described by Budowle *et al.* (1991). The rebinned format will be used. The data sets to be used are at least three of the four major population groups. These are Caucasians, African Americans, Hispanics, and Orientals (this requirement applies to US laboratories).
2. Global tests for equilibrium will be performed on each population data set (Chakraborty *et al.* 1993a; Devlin and Risch 1992a; Weir 1992a). If all the loci meet Hardy-Weinberg and pair-wise linkage equilibrium expectations (*i.e.*, two-locus independence tests), proceed with calculating the ceiling principle estimate (step 5).
3. If a locus is not in equilibrium, based on a global test, in one of the data sets, a local test (Weir 1992a) for equilibrium will be performed only on the alleles in the particular population sample (this step is required only if the criteria for step 2 are not met). If the loci meet Hardy-

Weinberg and linkage equilibrium expectations at the local test level, proceed with calculating the ceiling principle estimate (step 5).

4. When the criterion in step 3 is not met, the counting method will be used for that locus. The observed number of genotypes with the particular combination of alleles will be used. For the situation where there are no observed genotypes, the 95% upper confidence limit on no observations for those databases with no observed genotypes will be employed. For a database of size n, the formula (Weir 1992a) for calculating the 95% upper confidence limit for no observed genotypes is the following: $1-0.05^{1/n}$. The highest genotype frequency or a minimum of 0.02 (which is the minimum interim ceiling single locus genotype frequency) will be used.

4a. The ceiling principle approach described by the NRC Report (1992) did not describe how to proceed when the alleles in one population are in disequilibrium based on a local test, yet the second and third populations meet equilibrium expectations, and the alleles used in the ceiling principle estimate derive from the second and third populations. It is obvious that there are no tests for determining independence across populations, and using the allele frequencies with the product rule should present no problem. But to avoid confusion, it is recommended to use the genotype counting method for the two alleles in all databases and to use the most conservative estimate or a minimum frequency of 0.02.

4b. The same approach as in 4a would apply when two loci are found to be in disequilibrium by the local test. The counting method will be used for the two loci. The observed number of genotypes with the particular combination of alleles at the two loci will be used to determine a genotype frequency. For the situation where there are no observed genotypes, the 95% upper confidence limit on no observations for each of the databases will be estimated. The highest frequency or a minimum of 0.0004 (which is the minimum interim ceiling two-locus genotype frequency) will be used.

5. The putative bands for estimating a DNA profile frequency generally derive from the evidentiary sample. It also is acceptable to use either the band(s) from the evidentiary sample or the known sample, as long as the more common bin frequency is used. The appropriate bin frequency for the putative band(s) will be determined by establishing a measurement error window (*e.g.*, for the FBI that would be ±2.5%). When the measurement error correction window spans a fixed bin boundary, the larger bin frequency will be used (Chakraborty *et al.* 1993b; Monson and Budowle 1993).

6. The 95% upper confidence limit will be calculated for each of the fixed bin frequencies in each population data set. The 95% upper confidence limit will be derived using the following formula, where p is the bin frequency and n is the number of alleles in the data set: $p+1.645\sqrt{p(1-p)n}$.

7. The largest 95% upper confidence limit value across the data sets at each bin or a minimum of 0.100 will be selected for a ceiling allele frequency estimate.

8. The product rule will be applied. The single-locus estimates for a two-band pattern will be derived using $2p_1p_2$, where p_1 and p_2 are the respective allele frequencies; for single band patterns, 2p will be used. The multi-locus DNA profile frequency estimate will be calculated as the product of the individual locus frequencies.

It should be noted that the fixed bin ceiling approach recommended by TWGDAM differs from the NRC Report (1992) recommendations in two aspects. First, when estimating an allele frequency for a target profile, the TWGDAM fixed bin ceiling approach selects the bin with the higher frequency when the measurement error window spans a bin boundary, instead of summing adjacent bins. Chakraborty *et al.* (1993b) and Monson and Budowle (1993) have demonstrated that selecting the higher frequency of the adjacent bins is sufficiently conservative, and there is no demonstrated need for summing the bin frequencies. Moreover, the floating bin approach, described in the following section, yields very similar ceiling principle estimates to the fixed bin ceiling approach. Second, no interim ceiling frequency estimates will be determined using a Native American database, because each database represents a subgroup and not a major race or population category. The NRC Report (1992) did not describe a method for generating "*general* Native American" databases when applying the interim ceiling principle.

Floating Bin Ceiling Approach

The floating bin ceiling approach recommended by TWGDAM essentially is carried out in the same manner as the fixed bin ceiling approach. There are only two points to consider for floating bins:

1. The size of the floating window should be twice the laboratory's quantitative match criterion. For example, for a match criterion of ±2.5%, the floating window for ceiling principle allele frequencies will be ±5.0%, which is a total width of 10%.
2. The tests for independence can not be done globally. They must be done locally for each case.

It is the opinion of TWGDAM members that previous interpretations for applying the ceiling principle (such as, for example, using ±2.5% floating window instead of a ±5.0% floating window for establishing ceiling principle allele frequencies) also yield conservative DNA profile frequencies. The attempt here is to provide a consensus approach, which does not suggest that previous interpretations of the ceiling principle yielded nonconservative estimates.

References

Budowle, B., Giusti, A. M., Waye, J. S., Baechtel, F. S., Fourney, R. M., Adams, D. E., Presley, L. A., Deadman, H. A., and Monson, K. L. Fixed-bin analysis for statistical evaluation of continuous distributions of allelic data from VNTR loci, for use in forensic comparisons, *American Journal of Human Genetics* (1991) 48:841-855.

Budowle, B. and Monson, K. L. Perspectives on the fixed bin method and the floor approach/ceiling principle. In: *Proceedings from the Third International Symposium on Human Identification 1992*. Promega Corporation, Madison, WI, 1992, pp. 391-406.

Budowle, B. and Monson, K. L. A perspective on the polemic on DNA statistical inferences in forensics. In: *Proceedings from the Fourth International Symposium on Human Identification 1993*. Promega Corporation, Madison, WI, in press.

Budowle, B., Monson, K. L., Giusti, A. M., and Brown, B. L. The assessment of frequency estimates of Hae III-generated VNTR profiles in various reference databases, *Journal of Forensic Sciences* (1994a) (in press).

Budowle, B., Monson, K. L., Giusti, A. M., and Brown, B. L. Evaluation of Hinf I-generated VNTR profile frequencies determined using various ethnic databases, *Journal of Forensic Sciences* (1994b) (submitted).

Chakraborty, R. Statistical interpretation of DNA typing data, *American Journal of Human Genetics* (1991) 49:895-897, 899-903.

Chakraborty, R. and Kidd, K. K. The utility of DNA typing in forensic work, *Science* (1991) 254:1735-1739.

Chakraborty, R. and Jin, L. Heterozygote deficiency, population substructure and their implications in DNA fingerprinting, *Human Genetics* (1992) 88:267-272.

Chakraborty, R., de Andrade, M., Daiger, S. P., and Budowle, B. Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications, *Annals of Human Genetics* (1992a) 56:45-57.

Chakraborty, R., Srinivasan, M. R., Jin, L., and de Andrade, M. Effects of population subdivision and allele frequency differences on interpretation of DNA typing for human identification. In: *Proceedings from the Third International Symposium on Human Identification 1992*. Promega Corporation, Madison, WI, 1992b, pp. 205-222.

Chakraborty, R., Srinivasan, M. R., and Daiger, S. P. Evaluation of standard error and confidence interval of estimated multilocus genotype probabilities and their implications in DNA forensics, *American Journal of Human Genetics* (1993a) 52:60-70.

Chakraborty, R., Jin, L., Zhong, Y., Srinivasan, M. R., and Budowle, B. On allele frequency computation from DNA typing data, *International Journal of Legal Medicine* (1993b) 106:103-106.

Devlin, B. and Risch, N. A note on Hardy-Weinberg equilibrium of VNTR data by using the Federal Bureau of Investigation's fixed-bin method, *American Journal of Human Genetics* (1992a) 51:549-553.

Devlin, B. and Risch, N. Ethnic differentiation at VNTR loci, with special reference to forensic applications, *American Journal of Human Genetics* (1992b) 51:534-548.

Devlin, B., Risch, N., and Roeder, K. Forensic inference from DNA fingerprints, *Journal of the American Statistical Association* (1992) 87:337-350.

Devlin, B., Risch, N., and Roeder, K. Statistical comments on the NRC's report on DNA typing, *Journal of Forensic Sciences* (1994) (in press).

Devlin, B., Risch, N., and Roeder, K. Statistical evaluation of DNA fingerprinting: A critique of the NRC's report, *Science* (1993) 259:748-749, 837.

Evett, I. W. and Gill, P. A discussion of the robustness of methods for assessing the evidential value of DNA single locus profiles in crime investigations, *Electrophoresis* (1991) 12:226-230.

Hartl, D. L. and Lewontin, R. C. Response to Devlin *et al.*, *Science* (1993) 260:473-474.

Krane, D. E., Allen, R. W., Sawyer, S. A., Petrov, D. A., and Hartl, D. L. Genetic differences at four DNA typing loci in Finnish, Italian, and mixed Caucasian populations, *Proceedings of the National Academy of Sciences of the United States of America* (1992) 89:10583-10587.

Lander, E. S. Invited editorial: Research on DNA catching up with courtroom application, *American Journal of Human Genetics* (1991) 48:819-823.

Lander, E. S. DNA fingerprinting: The NRC report, *Science* (1993) 260:1221.

Latter, B. H. D. Genetic differences within and between populations of the major human subgroups, *American Naturalist* (1980) 116:220-237.

Lewontin, R. C. and Hartl, D. L. Population genetics in forensic DNA typing, *Science* (1991) 254:1745-1750.

Mitton, J. B. Genetic differentiation of races of man as judged by single-locus and multilocus analyses, *American Naturalist* (1977) 111:203-212.

Mitton, J. B. Measurement of differentiation: Reply to Lewontin, Powell, and Taylor, *American Naturalist* (1978) 11:1142-1144.

Monson, K. L. and Budowle, B. A comparison of the fixed bin method with the floating bin and direct count methods: Effect of VNTR profile frequency estimation and reference population, *Journal of Forensic Sciences* (1993) 38:1037-1050.

Morton, N. E. Genetic structure of forensic populations, *Proceedings of the National Academy of Sciences of the United States of America* (1992) 89:2556-2560.

Morton, N. E., Collins, A., and Balazs, I. Bioassay of kinship for hypervariable loci in Blacks and Caucasians, *Proceedings of the National Academy of Sciences of the United States of America* (1993) 90:1892-1896.

National Research Council. DNA typing: Statistical basis for interpretation. In: *DNA Technology in Forensic Science.* National Academy Press, Washington, DC, 1992, pp. 74-96.

Nei, M. and Roychoudhury, A. K. Genetic relationship and evolution of human races, *Evolutionary Biology* (1982) 14:1-59.

Risch, N. J. and Devlin, B. On the probability of matching DNA fingerprints, *Science* (1992) 255:717-720.

Smouse, P. E., Spielman, R. S., and Park, M. H. Multiple-locus allocation of individuals to groups as a function of the genetic variation within and differences among human populations, *American Naturalist* (1982) 119:445-463.

Weir, B. S. Independence of VNTR alleles defined as fixed bins, *Genetics* (1992a) 130:873-887.

Weir, B. S. Population genetics in the forensic DNA debate, *Proceedings of the National Academy of Sciences of the United States of America* (1992b) 89:11654-11659.

Weir, B. S. Forensic population genetics and the National Research Council (NRC), *American Journal of Human Genetics* (1993) 52:437-440.

Prepared by the following TWGDAM members:

Jeffrey D. Ban
Virginia Division of Forensic Sciences
Richmond, VA

Charles E. Barna
Michigan State Police
East Lansing, MI

Elizabeth Benzinger
Illinois State Police
Springfield, IL

John H. Bowen
Royal Canadian Mounted Police
Ottawa, Ontario, Canada

Bruce Budowle
FBI Laboratory
Quantico, VA

Eric Buel
Vermont Department of Public Safety
Waterbury, VT

Lucy A. Davis
Kentucky State Police
Frankfort, KY

George Duncan
Broward County Sheriff's Department
Fort Lauderdale, FL

Arthur J. Eisenberg
University of North Texas
Fort Worth, TX

Pamela Fish
Chicago Police Department
Chicago, IL

Ronald M. Fourney
Royal Canadian Mounted Police
Ottawa, Ontario, Canada

Thomas L. Grant
Missouri State Highway Patrol
Jefferson City, MO

John M. Hartmann
Orange County Sheriff-Coroner's Department
Santa Ana, CA

Berch Henry
Washoe County Sheriff's Office
Reno, NV

George L. Herrin
Georgia Bureau of Investigation
Decatur, GA

Lori M. Johnson
South Carolina Law Enforcement Division
Columbia, SC

Roger Kahn
Metro-Dade Police Department
Miami, FL

James J. Kearney
FBI Laboratory
Washington, DC

Kenneth C. Konzak
California Department of Justice
Berkeley, CA

Terry L. Laber
Minnesota Bureau of Criminal Apprehension
Saint Paul, MN

Teresa M. Long
Maryland State Police
Pikesville, MD

Donald C. MacLaren
Washington State Patrol
Seattle, WA

Susan D. Narveson
Arizona Department of Public Safety
Phoenix, AZ

Mark S. Nelson
North Carolina State Bureau of Investigation
Raleigh, NC

Pamela J. Newall
Centre of Forensic Sciences
Toronto, Ontario, Canada

Elaine Pagliaro
Connecticut State Police
Meriden, CT

James M. Pollock
Florida Department of Law Enforcement
Jacksonville, FL

TWGDAM members (continued):

Lawrence A. Presley
FBI Laboratory
Washington, DC

Georgia Sue Rogers
Alabama Department of Forensic Sciences
Birmingham, AL

Clement G. Smetana
US Army Criminal Identification Laboratory
Forest Park, GA

Mohammad A. Tahir
Indianapolis-Marion County Forensic
Services Agency
Indianapolis, IN

Christine S. Tomsey
Pennsylvania State Police
Greensburg, PA

Cecilia von Beroldingen
Oregon State Police
Portland, OR