STR Interpretation Guidelines

1 DNA Quantitation Interpretation Guidelines

1.1_ Quantiblot

1.1.1 Background:

1.1.1.1 DNA quantitation results are interpreted by comparing the signal intensity for the DNA test sample to the signal intensity obtained for the DNA Standards. The signal intensity for a sample reflects the total amount of DNA spotted on the membrane: 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 ng. The concentration of the Standards are 2, 1, 0.5, 0.25, 0.125, 0.0625, and 0.031 ng/µl respectively.

1.1.1.2 The DNA Quantitation is an estimation of the DNA concentration of the DNA in the Test Samples. There are factors that may affect the ability of a specific sample to quantitate and it is possible for a sample to show no DNA or low levels of DNA on the Quanti-Blot but actually contain enough DNA for PCR amplification. Since this is an estimation of the DNA concentration, all efforts will be made to utilize a specific Quanti-blot so that samples will not be wasted on re-testing.

1.1.2 DNA Calibrators:

1.1.2.1 The DNA Calibrators are used to provide DNA of known concentration to verify that the DNA Standards were correctly diluted and are providing correct results.

1.1.2.2 “DNA Calibrator 1" (stock concentration of 0.7 ng/µl or 3.5 ng/5 µl). The signal for this calibrator should have a signal strength between the 5 and 2.5 ng (1 and 0.5 ng/µl) DNA Standards.

1.1.2.3 "DNA Calibrator 2" (stock concentration of 0.1 ng/µl or 0.5 ng/5 µl). The signal for this calibrator should have a signal strength between the 0.625 and 0.3125 ng (0.25 and 0.1.25 ng/µl) DNA Standards.

1.1.2.4 If the DNA Calibrators do not quantitate correctly with respect to the DNA Standards but at least three (2.0, 1.0, and 0.5 ng/µl) of the DNA Standards can be observed, then the test does not have to be repeated. To save limited DNA test samples for PCR analysis, the quantitation of the DNA test samples may be estimated by comparing the DNA Standards to the DNA Calibrators and determining how far the DNA Standards deviate from the DNA Calibrators and then estimate the concentration of the DNA test sample.

1.1.3 DNA Standards

To save DNA test samples for PCR analysis, the Quantitation does not have to be repeated if the 2.0, 1.0, and 0.5 ng/µl DNA Standards are observed. If the 2.0, 1.0, and 0.5 ng/µl DNA Standards are not observed, increased exposure times for the film should be attempted. If this does not cause the 2.0, 1.0, and 0.5 ng/µl DNA Standards to be observed, then the Quantitation must be repeated.

1.1.4 Estimation of DNA Test Samples:

1.1.4.1 The quantity of DNA test sample spotted on the membrane will be estimated by directly comparing it's signal strength to the intensity of the DNA Standards. The concentration of the DNA Standard that is closest to the intensity of a DNA test sample will be used for calculating the volume needed for PCR analysis.

1.1.4.2 Since it is possible for a DNA test sample to show no DNA or very low levels of DNA on the Quanti-Blot, but actually contain enough DNA for PCR amplification, if a DNA test sample does not show any DNA, then that

sample will still be amplified.

1.2 ABI QUANTIFILER HUMAN KIT

1.2.1 Background:

1.2.1.1 The Quantifiler Human Kit uses a real-time PCR reaction with fluorescent dye chemistry to estimate the quantity of amplifiable DNA in each test sample.

1.2.1.2 DNA quantitation results are interpreted by checking the analysis settings of the ABI 7000 machine, the slope of the standard curve of the DNA standards, and the R^2 value of the DNA standards.

1.2.2 Analysis settings:

1.2.2.1 The correct analysis setting for the ABI 7000 ensure proper analysis and should be set as follows:

Detector: All
Threshold: 0.2
Baseline start (cycle): 6
Baseline end (cycle): 15

1.2.3 Slope:

1.2.3.1 This value indicates the efficiency of the PCR reaction of the quantification assay. A slope value of -3.3 indicates 100% amplification efficiency. The range of values that is acceptable to deem the assay results as valid is -2.9 to -3.3, with the average slope value being -3.1.

1.2.4 R^2 value:

1.2.4.1 This value measures the closeness of fit between the standard curve line and the Ct values of the DNA standard used in the quantification assay. A value of 1.00 indicates a perfect fit between the standard curve line and the DNA standard data points. In order

for the results from a given assay to be valid, the R2 value must be greater than or equal to 0.98

1.2.5 Internal Positive Control:

1.2.5.1 Each reaction in the Human Quantifiler Kit contains an Internal Positive Control (IPC) to help determine whether a sample is a true negative or whether there is inhibition occurring in the PCR reaction. The normal Ct values for the IPC should range from 20 to 30.

1.2.6 Interpreting IPC amplification results:

Quantifiler Human (FAM Dye)	IPC (VIC Dye)	Interpretation
No amplification	Amplification	True negative
No amplification	No amplification	Invalid result
Amplification (low Ct and high delta Rn)	No amplification	Disregard IPC result
Amplification (high Ct and low delta Rn)	No amplification	Partial PCR inhibition

1.2.7 No Template Control (NTC):

1.2.7.1 For each set of samples that an analyst analyzes, they should run a negative control to show that there was no contamination during the setup of the assay. If there is amplification in the negative control, then the results from the assay cannot be used and the assay must be repeated.

2 PCR STR Interpretation

2.1 Introduction:

The interpretation of results in casework is necessarily a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-set rule. It is important that this laboratory develop and adhere to criteria for the interpretation of analytical results.

These criteria are based on our validation studies, literature, and over 10 years of forensic DNA casework experience by this laboratory.

2.2 Preliminary Evaluation of Data

2.2.1 **For Capillary Systems:** It is acceptable to load more than one ladder or 9947A per run. In this case where two ladders or two positive amplification controls are loaded on the same run, it is acceptable for the analyst to choose the best sample to add to the project and not analyze the other one.

2.2.2 **Positive Amplification Control:** Examine the bands/peaks of the positive amplification control. In order to be further assessed, the control must have bands/peaks that are in the proper location relative to the allelic markers. If these characteristic bands/peaks are not in their correct position or are not present (too weak to interpret), that particular locus must be considered inconclusive and must be successfully re-run or re-amplified and analyzed in order to use that locus in the conclusions.

2.2.3 **Negative Controls:**

2.2.3.1 Visually inspect the amplification negative control lane and the reagent control (or Blank) lanes. If any bands/peaks are detected in these lanes, then contamination *may* have occurred and the samples *may* not be interpreted.

2.2.3.2 For Gels: the negative controls should be re-run to check for sample bleed-over being the cause of the bands in the negative control.

2.2.3.3 For Capillary Systems: the negative control(s) may be re-injected if peaks are observed. The sharp spikes that constitute “electrical spikes” and/or dye blobs that are observed in negative samples shall not cause those samples to be reinjected. Those spikes shall be documented in the notes.

2.2.3.4 If bands/peaks are still seen in the negative control lane after the re-run, then all samples MUST be re-amplified.

2.2.4 **Positive Extraction Control:** The known bloodstain from MJB is used primarily as an extraction control and the scans can be interpreted if it fails to amplify at all or any loci.

2.2.5 **Allelic Ladders:** Inspect the bands/peaks in the lanes containing the allelic ladders. The bands/peaks must be of sufficient intensity/peak height to be useful as allelic markers. If regions of the ladder lanes are not present or are too intense, the specimen bands/peaks may not be able to be interpreted in these regions. For Capillary Systems: Check the size standards to ensure that each one is present and is called correctly.

2.2.6 **Samples**

2.2.6.1 Visually inspect the lanes containing the known and questioned samples to assess the quality of the bands/peaks. Gel Scan Analysis: Gel scans are first visualized on the FMBIO analysis software using default settings. The scan may be lightened or darkened as appropriate to bring up the best images possible, and multiple gel scans can be utilized in interpreting the banding patterns obtained. Be aware that band irregularities may signal potential mobility shifts.

2.2.6.2 For Capillary Systems: The set Peak/Height cut off in the GeneMapper™ ID software shall be set at 100 rfu. However, an analyst may use discretion in calling peaks down to 75 rfu or even lower for exclusions. In the case where alleles below 100 rfu are considered for analysis, the noise (background) must be considered. A general rule is that the Peak/Height to Noise (background) ratio should be 3:1. In other words, the Peak/Height should be 3 times greater than the background for a peak to be called.

2.2.6.3 If the questioned sample(s) contain more than two bands/peaks at the same locus, then the results *may* indicate a mixture.

2.2.6.4 Failure of all loci to amplify for a multiplex STR system will not preclude analysts from reporting those that do, even if only one locus amplifies. However, analysts may run the sample a second time to see if more information can be derived from the sample.

2.2.6.4.1 For Gels: the sample(s) may be re-run and a large of the sample can be loaded if a sufficient amount of the sample exists allowing this second attempt in trying to visualize the missing loci.

2.2.6.4.2 For Capillary: the samples(s) may be re-analyzed by the reinjecting for a longer time specified in the DNA Technical Procedures.

2.2.6.5 Analysts using multiple multiplex systems (PowerPlex 1.1 and 2.1) will ensure that the shared loci give concordant results for each sample. Failure to obtain concordant (one system may be more sensitive and a faint mixture may be detected, which is acceptable) results for the shared loci will require that the samples be re-tested for both multiplex systems, starting first with the amplification step and working backwards thru the procedure.

2.3 Allele Identification

2.3.1 Analysts will report and record STR DNA types as phenotypes, not genotypes. Database analysts may record STR DNA types from convicted offenders as either phenotypes or genotypes since CODIS will convert all results to genotype.

2.4 Artifacts

2.4.1 **Stutter**

2.4.1.1 From prior experience examining STR gel scans, it is

known that a weak band(s) periodically appears at 4 base pair below an actual band. It is suspected that this band/peak is the result of stuttering, which is an inherent artifact of PCR amplification. Therefore, the STR scan should not be considered to be inconclusive if weak stutter bands/peaks are present.

2.4.1.2 Stutter bands/peaks are not problematic in samples considered to be from a single source. They can however, prove problematic in samples which appear to be mixtures.

2.4.1.2.1 .For Gels: the analyst has the option of running a 1-D Gel Analysis and StaR Call to get a numerical and relative relationships of band intensities. If a band in a stutter position is greater than the OD cutoff limit, then one would have some confidence in calling it a true allele. However, if a band in the "stutter" position is less than the OD cutoff limit, one can not assume that the band is stutter, because it could be from a weaker profile or the result of differential amplification of alleles within a locus. Therefore, STaR Call may be valuable in determining if a possible weak band IS NOT stutter, but is not definitive in determining if a weak band IS stutter.

2.4.1.2.2 For Capillary Systems: The GeneMapper™ ID software contains designated cutoff for peaks in Stutter positions. These cutoff values shall be used for designating stutter.

2.4.2 Analysts should also keep in mind that an artifact band may appear in the n + 4 position. When an n+4 band is suspected, this should be documented on the allele call sheets or on the gel scan itself.

2.4.3 Variant Alleles/Off-Ladder Alleles

2.4.3.1 **For Gels:** when STR profiles contain variant bands within the region covered by the allelic ladder for any given locus, STaR Call software will be utilized to "size" the allele from the allelic ladders (unless the variant is the TH01 9.3 allele). The analyst *may* also elect to re-run the sample with a molecular weight sizing ladder to determine if the size of the band is consistent with a repeat unit of a possible allele at that locus.

2.4.3.2 **For Capillary Electrophoresis:** The GeneMapper™ID determines the base pair sizes. The analyst may add the correct allele call by typing in the correct allele designation based on the base pair size.

2.4.3.3 Variant alleles that vary by less than the consensus repeat unit will be designated as an integer of that variation (for example TH01 9.3 allele), as per CODIS recommendations.

2.4.3.4 When STR scans contain off-ladder bands/peaks outside the region covered by the allelic ladder for any given locus, the analyst must assign the off-ladder band to the correct locus, if possible. The band/peak in this situation lies between two loci, so the analyst should first determine if the patterns of the two loci are heterozygous or homozygous. If the pattern is heterozygous at one locus and homozygous at the other, then the off-ladder allele is assigned to the homozygous locus (making it heterozygous). If both loci show homozygous patterns, then the only way to determine the correct locus that the off-ladder variant belongs to is to re-test the sample with monoplex primers for the loci involved; or via another typing system (such as the three overlapping loci between PowerPlex 1.1 and 2.1.) **If this is not feasible, then the allele may be called inconclusive.**

2.4.3.5 If an allele falls above the largest value or below the smallest value of the allelic ladder for a locus, the allele will be designated as either greater than (>) or less than (<) their respective allelic ladder, as per CODIS recommendations. The sample may also be re-run using primers for that locus alone to see if the band is truly an allele from that locus.

2.5 Interpretation of Results

2.5.1 Whether or not there is a match between patterns produced by the DNA samples extracted from two or more sources is primarily a qualitative judgment based on careful review by a knowledgeable investigator, utilizing all information pertinent to the tests undertaken. Two or more patterns are considered to match visually if their patterns are qualitatively similar taking into consideration the circumstances of collection and preparation of samples and knowledge of the properties and limitations of the specific techniques used. Matches and non-matches are determined by careful, objective qualitative and quantitative evaluation of the entire banding pattern produced by the various loci tested.

2.5.2 Major/Minor Profiles

2.5.2.1 **For Gels:** In cases where there is clearly a major and minor contributor (dark bands or indicating a major or predominant profile and lighter bands indicating a minor contributor), a predominant DNA profile may be attributed as coming from a known donor in the case of a Match. Minor profiles shall be designated on the Call Sheets by placing parenthesis around the minor allele(s). For example, if the profile at vWA that is a “15,16,17" and has a minor band at “16", the call sheet should look similar to the following: “15 (16) 17".

2.5.2.2 **For Capillary Electrophoresis:** In cases where there is clearly a major and minor contributor (higher peaks indicating a major or predominant profile and lower

peaks indicating a minor contributor), a predominant DNA profile may be attributed as coming from a known donor in the case of a Match. However, since heterozygotes have been shown to have up to a 50% peak/height imbalance, the analyst must use care in designating minor and major alleles based on peak height and take into account any "additive effect" that may occur with shared alleles. Minor profiles shall be designated on the Spread Sheets by placing parenthesis around the minor allele(s). For example, if the profile at vWA that is a "15,16,17" and has a minor band at "16", the call sheet should look similar to the following: "15 (16) 17".

2.5.3 Inconclusive results for an entire case are usually the result of an insufficient quantity of DNA or complete degradation of DNA present in a sample. Inconclusive results may result from, but are not limited to, the following causes:

2.5.3.1 Insufficient amounts of DNA for that locus in one or more of the samples tested.

2.5.3.2 Degradation of one or more of the bands/peaks in any sample tested.

2.5.3.3 Preferential amplification due to great differences in amounts of DNA present in a sample from multiple donors.

2.5.3.4 Inhibition

2.5.4 It is scientifically acceptable for a match or non-match to be determined for a case when one or more of the loci yield inconclusive results. A match will be based only on loci which yield conclusive results. An exclusion will be determined if only one locus probed produces exclusionary results.

2.6 Conclusions

2.6.1 Single Contributor

A sample may be considered to consist of a single contributor based on the expected number of alleles at each locus. All loci are to be evaluated in making this decision.

2.6.2 Match: If the DNA profile from a known sample matches the DNA profile from a single contributor, then the results will be interpreted as follows:

> *"The DNA profile obtained from (Item) **MATCHED** the DNA profile obtained from the blood stain of the , (Item) and **DID NOT MATCH** the DNA profile obtained from the blood stain of the , (Item). The match for Item was made for CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, D5S818, Penta E, D18S51, D21S11, D3S1358, FGA, D8S1179, and Amelogenin* (for PowerPlex 1.1 and 2.1) *or CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX, vWA, and Amelogenin* (for Identifiler)*."*
>
> If only PowerPlex 1.1 was run on (a) certain sample(s) or some loci in the system involved in the match of a second item were inconclusive, then the following statement may be included:
>
> *"The DNA profile obtained from (Item) **ALSO MATCHED** the DNA profile obtained from the blood stain of the , (Item) and **DID NOT MATCH** the DNA profile obtained from the blood stain of the , (Item). The match for Item __ was made for CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, and D5S818. No population frequency data were generated for these Items."*

2.6.3 Non-Match: If the DNA profile from a known sample does not match the DNA profile from a single contributor, then the results will be interpreted as follows:

> "The DNA profile obtained from (Item) **DID NOT MATCH** the DNA profile obtained from the bloodstain of the victim, (Item) or the DNA profile obtained from the bloodstain of the

suspect, (Item).This profile was queried against the casework/convicted offender indexes of the NCSBI State Database and no high stringency match was observed. This profile has been added to a target batch file and will be routinely queried against the NCSBI State Database in search of a match to another forensic case or a convicted offender."

NOTE: Analysts will run all CODIS searches at moderate stringency. Since one would expect one, two, or three locus matches to be somewhat common, this laboratory has set the operating rule that there must be a seven locus high stringency match before further testing will be performed.

2.6.4 Multiple Contributors (Mixtures)

If a DNA profile is observed to have more than two bands/peaks at more than one loci, then there is a high possibility that there is a mixture of two or more individual's DNA profiles (if a three banded pattern is observed at one locus, then there may not be mixture; the individual contributor may have three bands/peaks at that locus). Great care and consideration should be given when interpreting mixtures.

Below is a list of statements that can be used when reporting the results of a completed DNA analysis. This is a general list and may not include all possible scenarios. The statements should be used if the scenario fits. However, other statements may have to be used to fit scenarios not included below. Any additional statement should be consistent with the DNA results and conservative in nature.

2.6.5 Mixtures with Predominant profile - A sample may be considered to consist of a mixture of major and minor contributors if there is a distinct contrast in the banding patterns observed between alleles. All loci must be evaluated in making this determination. If all the bands/peaks present in the mixture are accounted for by the known standards, then the results will be interpreted as follows:

2.6.5.1 Where two contributors are possible (e.g. victim and suspect), all bands/peaks present in the questioned

sample can be accounted for by the standards, a clearly predominant profile is observed, and there is band sharing between the two contributors, the laboratory report may state:

> *"The DNA profile obtained from (Item) is **CONSISTENT WITH A MIXTURE**. The predominant profile **MATCHED** the DNA profile obtained from (Item).*

If a known standard cannot be eliminated as a contributor to the mixture, the laboratory report may additionally state:

> *"Person's name, (Item #) cannot be eliminated as a donor to the mixture."*

If a known standard can be eliminated as a contributor to the mixture, the laboratory report may additionally state:

> *"Person's name, (Item #) was not detected in the mixture."*

2.6.5.2 Where two contributors are possible (e.g. victim and suspect), all bands/peaks present in the questioned sample can be accounted for by the standards, there is a clear predominant profile and there is no band sharing between the two contributors, the laboratory report may state:

> *"The DNA profile obtained from (Item) is **CONSISTENT WITH A MIXTURE**. The predominant profile **MATCHED** the DNA profile obtained from (Item) and the weaker profile **MATCHED** the DNA profile obtained from (Item)."*

In the above situations, it is permissible to calculate population frequency data on any predominant profile where a match statement is made.

2.6.6 Mixture with no major/minor contributor -

2.6.6.1 In the situation where there is a mixture with no major/minor contributors, all bands/peaks can be accounted for by the standards, and one of the contributors is known (i.e. the rape victim from a vaginal swab) the genetic profile of the unknown is readily inferred and can be subtracted from the mixed profile. In this case, the laboratory report may state:

> *"The DNA profile obtained from (Item) is **CONSISTENT WITH A MIXTURE** of the victim and suspect's DNA profiles (Items and , respectively)."*

2.6.6.2 In the situation where there is a mixture with no major/minor contributors, all bands/peaks can be accounted for by the standards, but the sample is not identified as being intimate with one or more contributors (i.e. vaginal swabs, clothing from an individual), then the laboratory report may state:

> *"The DNA profile obtained from (Item) is **CONSISTENT WITH A MIXTURE**. Person's name, (Item #) and Person's name, (Item #) cannot be eliminated as (a) donor(s) to the mixture."*
>
> If a known standard can be eliminated as a contributor to the mixture, the laboratory report may additionally state:
>
> *"Person's name, (Item #) was not detected in the mixture."*

2.6.6.3 In the situation where there are no major/minor contributors, and all bands/peaks can not be accounted for by the standards, then the laboratory report will state:

*"The DNA profile from (Item) is **CONSISTENT WITH** a mixture from multiple contributors and additional bands/peaks were present which can not be accounted for by the standards submitted."*

2.6.7 Partial profiles - In the situation where all the bands/peaks present in a mixture obtained from a questioned sample can be accounted for by one or more of the standards, *but only a partial profile is obtained from one or more individuals (i.e. some of the alleles present in a known standard do not appear in the mixture obtained from the questioned sample),* then the following interpretation(s) may be given:

*"The DNA profile obtained from (Item) is **CONSISTENT WITH A MIXTURE**."*

AND EITHER:

*"The predominant profile **MATCHED** the DNA profile obtained from (Item)"*

OR:

"The (victim and/or suspect), (Item #) was not detected in the mixture."

If a person can be eliminated as a donor to the mixture, the following may be added:

"Name(s), (Item #) was/were not detected in the mixture."

2.6.8 Inconclusive

2.6.8.1 In the case where no profile is obtained or only weak amplification is observed and the analyst does not have enough data to exclude an individual as a possible donor, then the report may state either:

*"No DNA profile was obtained from _____, (Item #) and therefore, **NO CONCLUSION** can be rendered as to the donor of the "Body Fluid*

type" that was observed on the _____(Item #).

OR

"No conclusive DNA profile was obtained from _____, (Item #) and therefore, ***NO CONCLUSION*** *can be rendered as to the donor of the "Body Fluid type" that was* observed on the _____ (Item #)."

2.6.8.2 If no Body Fluid was identified (i.e. looking for epithelial cells on a baseball cap to identify the wearer) and no DNA profile is obtained or only weak amplification is observed and the analyst does not have enough data to exclude an individual as a possible donor, then the report may state either:

No DNA profile was obtained from_____ (Item #).

OR

No conclusive DNA profile was obtained from _____ (Item #).

2.6.9 In the event that a profile is obtained that is consistent with a mixture of the victim and a foreign profile and the victim profile is not the predominant profile, the alleles that are solely from the victim should not be entered into CODIS. Alleles that are shared are to be entered into CODIS and alleles that are solely from the foreign profile should be entered into CODIS as obligate alleles. This will reported as:

The DNA profile obtained from (item) is ***CONSISTENT WITH A MIXTURE*** *of the victim and one unknown contributor. This mixture, with an emphasis on alleles that did not originate from the victim, was queried against the casework/convicted offender indexes of*

the NCSBI State Database and no high stringency match was observed. This mixture profile has been added to a target batch file and will be routinely queried against the NCSBI State Database in search of a match to another forensic case or a convicted offender.

3 Statistical Interpretation

3.1 This laboratory will utilize the North Carolina Databases which have been reviewed by Dr. Bruce Weir. Databases for the North Carolina Caucasian, Black, and Lumbee Indian populations were generated by this laboratory and the North Carolina Hispanic databases were developed by the Charlotte/Mecklenburg Police Department Crime Laboratory. These data have been entered into PopStats. Analysts will use the PopStats program provided with the CODIS software to calculate population frequency information.

3.2 The formulas used in the calculation of the frequency of a DNA profile will be in accordance with those published in the NRC II guidelines and in PopStats.

3.2.1 Heterozygote frequencies - 2pq

3.2.2 Homozygote frequencies - $p^2 + p(1-p)\,\theta$, where $\theta = 0.01$

3.2.3 Multilocus frequencies - the product rule will be used

3.2.4 Minimum allele frequency - 5/2N -

The minimum allele frequency will be used for any allele which is seen less than 5 times in the population frequency database (to include variant and off-ladder alleles).

3.2.5 Mixtures: For DNA mixtures that do not contain a predominant profile or where individuals cannot be excluded, Likelihood Ratios should be utilized for calculating population frequencies. Popstats will allow for the calculation of Likelihood Ratio Data for both single DNA profiles and those involved in mixtures.

3.2.5.1 At a specific locus, if the detected profile consists of overlapping patterns that are consistent with a mixture of the victim and the suspect, the entire DNA profile is considered to be a mixture and the "Likelihood Ratio" frequency (L) is determined by the equation [1]

$$LR = \frac{Pr(Evidence / H_p)}{Pr(Evidence / H_d)}$$

L compares two hypotheses or scenarios - informally they are:

1) What is the probability of the DNA evidence if the prosecution proposition is true?, and

2) What is the probability of the DNA evidence if the defense proposition is true? LR provides a measure of how many times more characteristic of (1) the genetic evidence, than of (2).

Where: H_p the first explanation (the alleles of the mixed DNA profile attributed to unknown contributors under explanation H_p), where P is the number of unknown contributors under explanation H_p.

H_d is the second explanation (the alleles of the mixed DNA profile attributed to unknown contributors under explanation H_d), where D is the number of unknown contributors under explanation H_d.

$Pr(Evidence/H_p)$ is the probability of the DNA profile (Evidence) using explanation H_p

$Pr(Evidence/H_d)$ is the probability of the DNA profile (Evidence) using explanation H_d

NOTE: Because the likelihood ratio calculation

takes into account all possible contributors involved in the mixture and therefore provides a conservative probability, θ is not used in the calculation.

3.2.6 Examples of Mixtures:

3.2.6.1 When the victim and suspect share the same two alleles (i.e., 12,13), if the victim's contribution to the mixture is the minor component throughout the mixture profile and the type that is shared by the victim and suspect is consistent with the major contributor (based upon the intensities of the alleles in relationship to the rest of the DNA profile) the alleles will be considered to have originated from the foreign contributor and may be used in the calculation.

Example: Victim - 12,13
Suspect - 12,13
Evidence - 12,13 [both alleles will be considered as unknowns]

3.2.6.2 If four alleles are observed in a mixture, the alleles that are foreign to the victim will be used in the calculation.

Example: Victim - 5,8
Suspect - 7,9
Evidence - 5,8,(7),(9) [the 7 and 9 alleles should be considered as unknowns]

Note: Alleles in parentheses ( ) are lesser in intensity than the other alleles.

3.2.6.3 If difficulty is encountered in interpreting the results at any locus this locus will not be used in the calculation. However, the results at that locus may be used in the overall decision of an inclusion or an exclusion.

Example: Victim - 10,11

Suspect - 6,8
Evidence - 10,11, (6) [this locus will not be used in the overall calculation of the likelihood ratio frequency]

3.3 The following statements will be used in laboratory reports to report population frequency information:

3.3.1 Match Statement

CONCLUSIONS (Calculation of Likelihood Data):

"This laboratory maintains databases for the N. C. Caucasian, Black, Lumbee Indian, and Hispanic populations, and has access to other population frequency databases which can be used as appropriate.

The DNA profile from (Item) is approximately:

_____ *times more likely to be observed if it came from* _____ *than if it came from another unrelated individual in the N. C. Caucasian population,*

_____ *times more likely to be observed if it came from* _____ *than if it came from another unrelated individual in the N. C. Black population,*

_____ *times more likely to be observed if it came from* _____ *than if it came from another unrelated individual in the N.C. Lumbee Indian population,*

_____ *times more likely to be observed if it came from* _____ *than if it came from another unrelated individual in the N. C. Hispanic population.*"

NOTE : Analysts will report numbers in laboratory reports in excess of a trillion only in terms of trillions (i.e. 1.92 trillion).

3.3.2 Mixture Statement

3.3.2.1 Likelihood ratios will be used to calculate the match probability for the foreign DNA profile when a mixture of DNA profiles from two or more individuals is observed and the entire foreign DNA profile cannot be determined by subtracting the contribution of the known donor from the mixture profile. The likelihood ratio will be reported using wording similar to:

The DNA profile that was obtained from the _____ (Item #) at the genetic loci _____ is approximately:

times more likely if it originated from the SUSPECT and VICTIM than if it originated from the VICTIM and a unknown individual in the Caucasian population.

times more likely if it originated from the SUSPECT and VICTIM than if it originated from the VICTIM and a unknown individual in the Black population.

times more likely if it originated from the SUSPECT and VICTIM than if it originated from the VICTIM and a unknown individual in the Lumbee Indian population.

times more likely if it originated from the SUSPECT and VICTIM than if it originated from the VICTIM and a unknown individual in the Hispanic population.

3.3.2.2 If a statistical calculation is not conducted at a particular locus or loci, this will be indicated in the report using the following

No statistical calculations were conducted for the genetic loci _____.

3.4 Identity Statement

Analysts in the Forensic Biology Section provide opinion testimony as to the uniqueness of a DNA profile in cases which meet the criteria established below. As expert witnesses, these analysts have the legal authority to provide such testimony.

Analysts are to advise the prosecutor in advance of their testimony that they will provide opinion testimony as to identity , and provide the prosecutor with some form of the following question to ask:

"Special Agent _____, based on your professional knowledge, careful reading of the pertinent scientific literature, and years of experience with forensic DNA testing - have you developed an opinion, satisfactory to yourself as to whether or not the stain on State's Exhibit # ____ (description of the State's Exhibit item) could have originated from (the defendant or victim) _____?"

Analysts are to use great care with the wording of their opinion on the uniqueness of the DNA profile and are to paraphrase the following statement as close as possible (to fit the case scenario):

"It is my opinion that it is scientifically unreasonable to expect that the DNA profile derived from the (semen, blood, saliva) stain detected on State's Exhibit # _____ (description of the State's Exhibit item) could

have originated from anyone other than

, unless this individual has an identical sibling. ”

The Forensic Biology Section will not use a specific probability calculation to determine uniqueness, nor will this agency make statements of uniqueness in the laboratory report. Rather, the criteria used will be when population frequency calculations for all population groups exceed the current estimated population of the world (*6.5 billion*).

[1]Weir, BS, Triggs CM, Starling L. Stonewell LI, Walsh KAJ, Buckleton J. Interpreting DNA mixtures. J Forensic Sci 1997:42(2)213-222.

4 Return of unworked evidence

4.1 In the event that analysis of evidence is not needed due to adjudication, the information should be documented on a Stop Analysis Form. The notes will consist of this form and the coversheet. The Administrative and Technical review will be performed by the same reviewer. With regards to the report, the results area will state: The reason being sent back, from what source and the date the information was received.

Revision History		
Effective Date	Revision Number	Reason
NA	00	Original Document
June 21, 2001	01	Procedure Update
November, 2002	02	Procedure Update
June 13, 2003	03	Addition of Mixture Statistics Calculation
November 17, 2003	04	1) Addition of DNA Quantitation Interpretation Guidelines 2) Update to Reporting Guidelines (Section 2.5)
May 21, 2004	05	1) Addition of DNA Interpretation Guidelines for ABI Capillary System.
December 23, 2004	06	Addition of ABI 7000 Interpretation Guidelines

APPROVAL SIGNATURES	Date
Author/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	