STR Interpretation Guidelines

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.

1 Preliminary Evaluation of Data

1.1 Examine the bands of the K562 control. In order to be further assessed, the control must have bands that visually appear to be in the proper location relative to the allelic markers. If these characteristic bands are not in their correct position or are not visible (too weak to interpret), that particular locus must be considered inconclusive and must be successfully re-amplified and analyzed in order to use that locus in the conclusions. 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.

1.2 Visually inspect the bands in the lanes containing the allelic ladders. The bands must be of sufficient intensity to be useful as allelic markers. If regions of the ladder lanes are not visible or are over-exposed, the specimen bands may not be able to be interpreted in these regions.

1.3 Visually inspect the lanes containing the known and questioned samples to assess the quality of the bands. Be aware that band irregularities may signal potential mobility shifts. If the questioned sample(s) contain more than two bands at the same locus, then the results *may* indicate a mixture.

1.4 Visually inspect the amplification negative control lane and the reagent control (or Blank) lanes. If any bands are detected in these lanes, then contamination *may* have occurred and the samples *may* not be interpreted. The negative controls should be re-run to check for sample bleed-over being the cause of the bands in the negative control. If bands are still seen in the negative control lane after the re-

run, then all samples MUST be re-amplified.

1.5   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, if a sufficient amount of sample exists allowing this second attempt.

1.6   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.

1.7   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.

2   Allele Identification

2.1   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.

2.2   Artifacts

2.2.1   From prior experience examining STR 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 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 are present.

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

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.

2.2.2 When assessing scans with multiple colors, analysts should be cognizant of the possibility of bleed-through of color from one channel to the next and take appropriate steps to identify and eliminate (if possible) bleed thru from the scans.

2.3 Variant Alleles / Off-Ladder Alleles

2.3.1 When STR scans contain variant bands <u>within</u> 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.

2.3.2 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.

2.3.3 When STR scans contain off-ladder bands <u>outside</u> the region covered by the allelic ladder for any given locus, the analyst must assign the off-ladder band to the correct locus. The band in this situation lies between two loci, so the analyst should first determine if the banding patterns in the two loci are heterozygous or homozygous. If the banding 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.

2.3.4 If an allele falls above the largest value or 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.

3   Interpretation of Results

3.1   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.

3.2   In cases where there is clearly a major and minor contributor (dark bands 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.

3.3   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:

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

3.3.2   Degradation of one or more of the bands in any sample tested.

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

3.3.4   Inhibition

3.4   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.

4   Conclusions

4.1   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.

4.1.1   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."*

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 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."*

4.1.2   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.

4.2   Multiple Contributors (Mixtures)

If a DNA profile is observed to have more than two bands 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 is pattern is observed at
one locus, then there may not be mixture; the individual contributor may
have a three bands 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.

4.2.1 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 present in the mixture are accounted for by the known standards, then the results will be interpreted as follows:

4.2.1.1 Where two contributors are possible (e.g. victim and suspect), all bands 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."*

4.2.1.2   Where two contributors are possible (e.g. victim and suspect), all bands 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.

4.2.2   Mixture with no major/minor contributor -

4.2.2.1   In the situation where there is a mixture with no major/minor contributors, all bands 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)."*

4.2.2.2   In the situation where there is a mixture with no major/minor contributors, all bands 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."*

4.2.2.3 In the situation where there are no major/minor contributors, and
all bands 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 were present which can not be*
> *accounted for by the standards submitted."*

4.2.3   Partial profiles - In the situation where all the bands 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 (ie. 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."

4.2.4 Inconclusive

4.2.4.1 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 #)."*

4.2.4.2 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 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 #).*

5   Statistical Interpretation

   5.1   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.

   5.2   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.

       5.2.1   Heterozygote frequencies - 2pq

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

       5.2.3   Multilocus frequencies - the product rule will be used

       5.2.4   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).

       5.2.5   <u>Mixtures</u>: 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.

5.2.5.1 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) is 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, is not used in the calculation.

5.2.6 Examples of Mixtures:

5.2.6.1 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]

5.2.6.2   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.

5.2.6.3   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]

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

5.3.1 <u>Match Statement</u>

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 (eg. 1,920 trillion).

5.3.2 Mixture Statement

5.3.2.1 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:**

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.

5.3.2.2   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 _____.

5.4   Identity Statement

Analysts in the Molecular Genetics 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 (including a close relative) other than _____, unless this individual has an identical sibling."

The Molecular Genetics 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.

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