

**QUALITY ASSURANCE AUDIT
FOR FORENSIC DNA LABORATORIES**

**CONDUCTED IN ACCORDANCE WITH THE QUALITY
ASSURANCE STANDARDS FOR FORENSIC
DNA TESTING LABORATORIES**



**AN AUDIT OF THE NORTH CAROLINA STATE BUREAU
OF INVESTIGATION CRIME LABORATORY
MOLECULAR GENETICS SECTION**

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All answers of No and N/A (not applicable) require further explanation. If the explanation will not fit in the space under the question, then attach additional sheets as required and indicate under the question that the explanation is on another sheet of paper.

PREVIOUS AUDIT

Examine the previous audit

Does the laboratory conduct annual audits in accordance with the DNA Advisory Board outlined standards and state/local standards? Yes
 No

Standard 15.1

Did the previous audit address all areas of the Quality Assurance Audit Checklist and answer all questions? Yes
 No
 N/A

Does the laboratory retain all documentation pertaining to audits in accordance with relevant legal, agency, and state requirements? Yes
 No

Standard 15.1.2

Does a second agency participate in the annual audit at least once every two years? Yes
 No

Are all of the standards which the previous audit found to be out of compliance now in compliance? If not why? Yes
 No
 N/A

Have any other concerns of the last audit been addressed? Yes
 No
 N/A

QUALITY ASSURANCE PROGRAM

Does the laboratory have a well established and maintained quality system that is appropriate to the testing activities and is documented? Yes
 No

Standard 3.1



Does the quality manual address the following (**Standard 3.1.1**):

Mark Y or N

Goals and objectives	<u> Y </u>
Organization and management	<u> Y </u>
Personnel qualifications and training	<u> Y </u>
Facilities	<u> Y </u>
Evidence control	<u> Y </u>
Validation	<u> Y </u>
Analytical procedures	<u> Y </u>
Calibration and maintenance	<u> Y </u>
Proficiency testing	<u> Y </u>
Corrective action	<u> Y </u>
Reports	<u> Y </u>
Review	<u> Y </u>
Safety	<u> Y </u>
Audits	<u> Y </u>

Is there a Quality Assurance Officer or other designated individual(s) who monitors compliance with the quality assurance program? [X] Yes
[] No

Does the Quality Assurance Officer or other designated individual(s) have clearly defined and documented responsibilities with adequate authority to administer the Quality Assurance program? [X] Yes
[] No

ORGANIZATION AND MANAGEMENT

Does the laboratory have a managerial staff with the authority and resources needed to discharge their duties and meet the requirements of the DNA Advisory Board? **Standard 4.1.a** [X] Yes
[] No



Does the laboratory have a technical manager who has overall responsibility for the technical operations? **Standard 4.1.b** Yes
 No

Does the laboratory have a CODIS manager or custodian who is accountable for CODIS operations? Yes
 No

Does the laboratory specify and document the responsibility, authority, and interrelation of all personnel who manage, perform or verify work affecting the validity of the DNA analysis? **Standard 4.1.c** Yes
 No

PERSONNEL

Does the laboratory have a written job description for personnel to include responsibilities, duties, and skills? (See interview worksheets). **Standard 5.1.1** Yes
 No

Does the laboratory have a documented training program for qualifying all technical personnel? **Standard 5.1.2** Yes
 No

Does the laboratory have a documented program to ensure technical qualifications are maintained through continuing education? Yes
 No
Standard 5.1.3

Does the laboratory maintain records on the relevant qualifications, training, skills and experience of the technical personnel? **Standard 5.1.4** Yes
 No



-
- Does the laboratory have a technical manager or leader who meets degree, experience, and duty requirements specified in **Standard 5.2**? [X] Yes
[] No
[] N/A
- Does the laboratory's CODIS Manager or custodian meet the degree, education, and duty requirements specified in **Standard 5.3** of the DNA Database Standards? [X] Yes
[] No
- Do all examiner/analysts meet the education and experience requirements of **Standards 5.3.1 and 5.3.2**; and have they successfully completed a qualifying test before beginning independent casework as required by **Standard 5.3.3**? [X] Yes
[] No
- Do all technicians have on the job training specific to the job function as required in **Standard 5.4.1** and have they successfully completed a qualifying test before participating in forensic DNA typing responsibilities as required in **Standard 5.4.2**? [] Yes
[] No
[X] N/A
- Do laboratory support personnel have the training, education, and experience commensurate with their responsibilities as outlined in their job description and required by **Standard 5.5.1**? [] Yes
[] No
[X] N/A
- FACILITIES**
- Is the laboratory designed to provide adequate security and minimize contamination? **Standard 6.1** [X] Yes
[] No
- Is access to the laboratory controlled and limited? **Standard 6.1.1** [X] Yes
[] No
- Prior to PCR amplification, are evidence examinations, DNA extractions and PCR setup conducted at separate times or in separate spaces? **Standard 6.1.2** [X] Yes
[] No
- Is amplified DNA product generated, processed and maintained in a room(s) separate from the evidence examination, DNA extractions and PCR setup areas? **Standard 6.1.3** [X] Yes
[] No



Does the laboratory follow written procedures for monitoring, cleaning and decontaminating facilities and equipment? **Standard 6.1.4** Yes
 No

Note: Standard 6.1.4 of the DNA Database Standards states that:

A robotic work station may be used to carry out DNA extraction and amplification in a single room, provided it can be demonstrated that contamination is minimized and equivalent to that performed manually in separate rooms.

EVIDENCE CONTROL

Does the laboratory have and follow a documented evidence control system to ensure the integrity of physical evidence? **Standard 7.1** Yes
 No

Is each evidence sample labeled with a unique identifier in accordance with established agency policy? **Standard 7.1.1** Yes
 No

Does the laboratory maintain a chain of custody for all evidence? **Standard 7.1.2** Yes
 No

Does the laboratory follow documented procedures that minimize loss, contamination, and/or deleterious change of evidence? **Standard 7.1.3** Yes
 No

Does the laboratory have secure areas for evidence storage? **Standard 7.1.4** Yes
 No

Does the laboratory retain or return a portion of the evidence sample or extract where possible? **Standard 7.2** Yes
 No

Does the laboratory have a procedure requiring that evidence samples/extract(s) be stored in a manner that minimizes degradation? **Standard 7.2.1** Yes
 No

Does the laboratory have and follow a documented sample inventory control system as defined in **Standard 7.1 of the DNA Database Standards?** Yes
 No



VALIDATION- applies to methods or procedures validated since the last audit.

Does the laboratory use validated methods and procedures for forensic casework analyses? **Standard 8.1** Yes
 No

Have developmental validation studies been conducted and appropriately documented? **Standard 8.1.1** Yes
 No
 N/A

Developmental Validation for Novel Forensic DNA Methodologies

Have novel forensic DNA methodologies undergone developmental validation to ensure the accuracy, precision and reproducibility of the procedure? **Standard 8.1.2** Yes
 No
 N/A

POWERPLEX 1.1

Is there documentation and is it available which defines and characterizes the locus? **Standard 8.1.2.1** Yes
 No
 N/A

Have species specificity, sensitivity, stability and mixture studies been conducted? **Standard 8.1.2.2** Yes
 No
 N/A

Does the laboratory have access to a population data base which is documented and available for use in population statistics? **Standard 8.1.2.3** Yes
 No
 N/A

Does the data base information include allele and genotype distributions for the locus or loci obtained from relevant populations. **Standard 8.1.2.3.1** Yes
 No
 N/A



Where appropriate, has the database been tested for independence expectations? **Standard 8.1.2.3.1** Yes
 No
 N/A

Internal Validation for Existing DNA Methodologies

Has the DNA laboratory completed documented internal validation studies? **Standard 8.1.3** Yes
 No
 N/A

Has the procedure been tested using known and non-probative evidence samples? **Standard 8.1.3.1** Yes
 No
 N/A

Has the reproducibility and precision of the procedure been monitored and documented using human DNA control(s)? **Standard 8.1.3.1** Yes
 No
 N/A

Based on empirical data, have match criteria been established and documented? **Standard 8.1.3.2** Yes
 No
 N/A

Before the introduction of the procedure into casework, has the analyst or examination team successfully completed a qualifying test? **Standard 8.1.3.3** Yes
 No
 N/A

Have material modifications to analytical procedures been documented and subject to validation testing? **Standard 8.1.3.4** Yes
 No
 N/A



Audit of the SBI Molecular Genetics Section

If methods are not specified, does the laboratory, wherever possible, select methods that have been published by reputable technical organizations or in relevant scientific texts or journals, or which have been appropriately evaluated for a specific or unique application? **Standard 8.1.4**

[X] Yes
[] No
[] N/A

Have the unspecified methods or significant deviations from protocol been completely documented?

[X] Yes
[] No
[] N/A

ANALYTICAL PROCEDURES

Does the laboratory have and follow written analytical procedures approved by laboratory management/technical manager? **Standard 9.1**

[X] Yes
[] No

Does the laboratory have a standard operating protocol for each analytical technique used? **Standard 9.1.1**

[X] Yes
[] No

Do the analytical procedures describe reagents, sample preparation, extraction, equipment, and controls which are standard for DNA analysis and data interpretation? **Standard 9.1.2**

[X] Yes
[] No

Does the laboratory have a procedure for the differential extraction of stains which contain semen? **Standard 9.1.3**

[X] Yes
[] No

Does the laboratory use reagents that are suitable for the methods employed? **Standard 9.2**

[X] Yes
[] No

Does the laboratory have written procedures for documenting commercial supplies and for the formulation of reagents? **Standard 9.2.1**

[X] Yes
[] No

Are reagents labeled with the identity of the reagent, the date of preparation or expiration, and the identity of the individual preparing the reagent? **Standard 9.2.2**

[X] Yes
[] No



Are critical reagents* identified and evaluated prior to being placed into service? **Standard 9.2.3**

Yes
 No

*Critical reagents included but are not limited to:

- (a) Commercial kits for performing genetic typing
- (b) Human DNA controls
- (c) Primer sets
- (d) Thermostable DNA polymerase
- (e) Restriction enzyme
- (f) Agarose
- (g) Membranes
- (h) Ladders
- (i) Probes

Does the laboratory have and follow a procedure for evaluating the quantity of human DNA in the sample where possible? **Standard 9.3**

Yes
 No
 N/A

For RFLP casework samples, the presence of high molecular weight DNA should be determined. **Standard 9.3.1**

NA

Does the laboratory monitor the analytical procedures using appropriate controls and standards? **Standard 9.4**

Yes
 No



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Does the laboratory use the following controls for RFLP casework analysis? Yes
Standard 9.4.1 (Put a checkmark by each control used. Answer yes if all controls are checked.) No
 N/A

Quantitation standards which estimate the amount of DNA recovered by extraction **9.4.1.1** _____

K562 as a human DNA control **9.4.1.2** _____

Molecular weight size markers to bracket known and evidence samples **9.4.1.2** _____

Procedure to monitor the completeness of restriction enzyme digestion **9.4.1.3** _____

Does the laboratory use the following controls for PCR casework analysis? Yes
Standard 9.4.2 (Put a checkmark by each control used. Answer yes if all controls are checked.) No
 N/A

Quantitation standards which estimate the amount of human nuclear DNA recovered by extraction **9.4.2.1** ___Y___

Positive amplification controls **9.4.2.2** ___Y___

Negative amplification controls **9.4.2.2** ___Y___

Reagent blanks **9.4.2.3** ___Y___

Allelic ladders and/or internal size markers for variable number tandem repeat sequence PCR based systems **9.4.2.4** ___Y___

Does the laboratory check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST standard reference material or standard traceable to a NIST standard? **Standard 9.5** Yes
 No
 N/A

Are there written general guidelines for the interpretation of data? **Standard 9.6** Yes
 No



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Does the laboratory verify that all control results are within established tolerance ranges? **Standard 9.6.1** Yes
 No

Where appropriate, visual matches shall be supported by a numerical match criterion. **Standard 9.6.2** Yes
 No

Has the 1996 National Research Council report and/or a court directed method been used for the statistical interpretation of a DNA profile for a given population and/or hypothesis or relatedness and are these calculations derived from an established population data base appropriate for the calculation? **Standard 9.6.3** Yes
 No
 N/A

EQUIPMENT CALIBRATION AND MAINTENANCE

Does the laboratory use equipment which is suitable for the methods employed? **Standard 10.1** Yes
 No

Does the laboratory have a documented program for calibration of equipment and instruments? **Standard 10.2** Yes
 No

Has the laboratory identified critical equipment to be calibrated? **DNA Database Standard 10.2.** Yes
 No

Where available and appropriate, are standards traceable to national or international standards used in the calibration of equipment? **Standard 10.2.1** Yes
 No

Where traceability to national standard of measurement is not applicable, does the laboratory provide satisfactory evidence of correlation of results? **Standard 10.2.1.1** Yes
 No

For each instrument requiring calibration, has the frequency of calibration been documented and has such documentation been retained in accordance with applicable Federal or state law? **Standard 10.2.2** Yes
 No

Does the laboratory have a documented program to ensure that instruments and equipment are properly maintained? **Standard 10.3** Yes
 No



Have new instruments and equipment, or instruments and equipment that have undergone repair or maintenance, been calibrated before being used in casework analysis? **Standard 10.3.1** Yes No

Have written records or logs been maintained for maintenance service performed on instrument and equipment and has such documentation been retained in accordance with applicable Federal or state law? **Standard 10.3.2** Yes No

REPORTS

Does the laboratory have and follow written procedures for taking and maintaining case notes to support the conclusions drawn in laboratory reports? **Standard 11.1** Yes No

Does the laboratory maintain in a case record, all documentation generated by examiners related to case analyses? **Standard 11.1.1** Yes No



Audit of the SBI Molecular Genetics Section

Are there written guidelines requiring that the following items be included in reports issued by the laboratory and does the laboratory follow these guidelines? **Standard 11.1.2** (Put a checkmark by each item found in each report and found in written guidelines. Answer yes if all items are checked.) (Also see the interview worksheets for report review).

- | | |
|--|-------------------|
| (a) Case identifier | [X] Yes
[] No |
| (b) Description of evidence examined | __Y__ |
| (c) A description of methodology | __Y__ |
| (d) Locus | __Y__ |
| (e) Results and/or conclusions | __Y__ |
| (f) An interpretative statement (either quantitative or qualitative) | __Y__ |
| (g) Date issued | __Y__ |
| (h) Disposition of evidence | __Y__ |
| (i) A signature and title or equivalent identification of the person(s) accepting responsibility of the content of the report. | __Y__ |

Does the laboratory have written procedures for the release of case report information? **Standard 11.1.3** [X] Yes
[] No

Does the laboratory have an follow written procedures for generating and maintaining documentation for database samples? [X] Yes
[] No
DNA Database Standard 11.1

Does the laboratory have written procedures for the release of database sample information? **DNA Database Standard 11.1.1** [X] Yes
[] No

REVIEW

Does the laboratory conduct administrative and technical reviews of all case files and reports to ensure conclusions and supporting data are reasonable and within the constraints of scientific knowledge? **Standard 12.1** [X] Yes
[] No



Audit of the SBI Molecular Genetics Section

Does the laboratory have a mechanism in place to address unresolved discrepant conclusions between analysts and reviewers? **Standard 12.1.1**

Yes
 No
 N/A

Does the laboratory have and follow a program that documents the annual monitoring of the testimony of each examiner? (See Interview Worksheet) **Standard 12.2**

Yes
 No
 N/A

PROFICIENCY TESTING

Do examiners and other personnel designated by the technical manager/leader who are actively engaged in DNA analysis undergo open external proficiency tests at regular intervals not to exceed 180 days? (See Interview Worksheet) **Standard 13.1**

Yes
 No

Does the laboratory maintain the following records for proficiency tests and is such documentation retained in accordance with applicable Federal or state law? **Standard 13.1.1** (Put a checkmark by each item maintained by the laboratory. Answer yes if all items are checked.)

Yes
 No

- | | |
|---|-------|
| (a) The test set identifier | __Y__ |
| (b) Identity of the examiner | __Y__ |
| (c) Date of analysis and completion | __Y__ |
| (d) Copies of all data and notes supporting the conclusions | __Y__ |
| (e) The proficiency test results | __Y__ |
| (f) Any discrepancies noted | __Y__ |
| (g) Corrective action taken | __Y__ |



Does the laboratory evaluate proficiency tests based upon the following criteria? **Standard 13.1.2** (Put a checkmark by each criteria if used by the laboratory for the evaluation of proficiency tests. Answer yes if all items are checked.)

[X] Yes

[] No

[] N/A

(a) All reported inclusions are correct or incorrect. Y

(b) All reported exclusions are correct or incorrect. Y

(c) All reported genotypes and/or phenotypes are correct or incorrect according to consensus genotypes/phenotypes within established empirically determined ranges. Y

(d) All results reported as inconclusive or uninterpretable are consistent with written laboratory guidelines. The basis for inconclusive interpretations in proficiency tests must be documented. Y

(e) All discrepancies/errors and subsequent corrective actions must be documented. Y

(f) All final reports are graded as satisfactory or unsatisfactory. A satisfactory grade is attained when there are no analytical errors for the DNA profile typing data. Administrative errors shall be documented and corrective actions taken to minimize the error in the future. Y

(g) All proficiency test participants shall be informed of the final test results.

CORRECTIVE ACTION

Does the laboratory have and follow a procedure for taking corrective action whenever proficiency testing discrepancies are detected? **Standard 14.1**

[X] Yes

[] No

Does the laboratory have and follow a procedure for taking corrective action whenever casework errors are detected? **Standard 14.1**

[X] Yes

[] No



Does the laboratory maintain documentation for corrective actions and is such documentation retained in accordance with applicable Federal or state law? **Standard 14.1.1** [X] Yes
[] No
[] N/A

SAFETY

Does the laboratory have and follow a documented environmental health and safety program? **Standard 16.1** [X] Yes
[] No

SUBCONTRACTORS OF ANALYTICAL TESTING FOR WHICH VALIDATED PROCEDURES EXIST

Does the laboratory require certification of compliance with these standards when a subcontractor performs forensic DNA analyses for the laboratory? **Standard 17.1** [X] Yes
[] No
[] N/A

Did the laboratory establish and use appropriate review procedures to verify the integrity of the data received from the subcontractor? **Standard 17.1.1** [X] Yes
[] No
[] N/A
This may include random re-analysis of samples, visual inspection and evaluation of results/data, inclusion of QC samples, on-site visits.

LABORATORY INSPECTION - THE FOLLOWING MATERIAL IS NOT PART OF THE QUALITY ASSURANCE STANDARDS FOR DNA FORENSIC TESTING LABORATORY - BUT IS TO BE USED TO MONITOR COMPLIANCE AND UNDERSTANDING OF THESE STANDARDS

Do the personnel assigned to the DNA analysis laboratory have adequate space to accomplish their assigned tasks? [X] Yes
[] No

Is there sufficient space available for the use and operation of required instrumentation? [X] Yes
[] No



Are the manufacturer's operation manuals readily available to the laboratory personnel? Yes
 No

Is there a secure area for the overnight and/or long term storage of evidence? Yes
 No

Are those reagents currently in use properly labeled? Yes
 No

Is the laboratory designed to provide adequate security and minimize contamination? Yes
 No

Is access to the laboratory controlled and limited? Yes
 No

Are samples believed to contain low DNA levels eg. telogen hairs, old bones, etc) extracted separately in time and/or space from other samples believed to contain high DNA levels? Yes
 No

DNA DATABASE UNIT - THE FOLLOWING MATERIAL IS NOT PART OF THE QUALITY ASSURANCE STANDARDS FOR DNA FORENSIC TESTING LABORATORY - BUT IS TO BE USED TO MONITOR COMPLIANCE AND UNDERSTANDING OF THESE STANDARDS

Does the DNA Database Unit have an operating policy and procedures manual? Yes
 No



Audit of the SBI Molecular Genetics Section

Does this manual cover areas such as lines of authority, sample handling, information access and matching procedures unique to the Database Unit? Yes
 No

Are special provisions of this manual being followed? Yes
 No

Personnel Interviews - THE FOLLOWING MATERIAL IS NOT PART OF THE QUALITY ASSURANCE STANDARDS FOR DNA FORENSIC TESTING LABORATORY - BUT IS TO BE USED TO MONITOR COMPLIANCE AND UNDERSTANDING OF THESE STANDARDS

Are the personnel of the Section aware of the goals, objectives and policies of the Section's Quality Assurance Program? Yes
 No

Is the quality assurance program being followed? Yes
 No

Does the laboratory management provide the staff with an opportunity for continuing education and training? Yes
 No

Is there adequate supervision? YES
 No

Is there adequate technical leadership? Yes
 No

Do approved technical procedures reflect current practices? Yes
 No

Audit of the SBI Molecular Genetics Section

Are copies of the current procedures readily available to personnel performing the procedures? Yes
 No

Is evidence collected, received, handled, sampled and stored so as to preserve the identity, integrity, condition and security of the item(s)? Yes
 No

Does the laboratory policy for "chain of custody" and disposition of evidence reflect current practices? Yes
 No

Are all agent/examiners and DNA Database Analysts aware of the written policies concerning the handling of a discrepancy in a proficiency test? Yes
 No

Is the Safety Manual available to all personnel? Yes
 No

Do all employees have access to the Material Safety Data Sheets? Yes
 No

Are established safety procedures as outlined in the safety manuals being followed? Yes
 No



RECOMMENDATIONS, DEFICIENCIES, SIGNIFICANT FINDINGS.

Timetable for correction

NON-COMPLIANCE ISSUES:

None detected

COMMENTS & RECOMMENDATIONS:

Previous Audit

The schedule of audits exceeds the required standards (DAB 15.2) of once every two years by an external agency.

Quality Assurance Program

CORRECTIVE ACTIONS (DAB 3.1.1)

The DNA QA manual contains comments which give the QA officer the authority to reject chemicals, etc. However, the corrective actions concerning casework, personnel retraining etc. are located in the Crime Laboratory Procedures Manual (CLP). The DNA QA manual should cross reference this additional information.

Organization and Management

Management has been able to successfully obtain grant monies to ensure that adequate monies are available for supplies and equipment to validate new technologies.

Personnel (DAB 5.1.1, 5.1.4, & 5.1.5)

Job descriptions and the QA manual (2.2.2.2) should be revised to include the newly required course work or training in statistics and/or population genetics (DAB 5.3.1).

Continuing Education (DAB 5.1.3)

Potential budget restraints may prevent personnel from attending external training



seminars. In order to comply with the requirement for continuing education and training, it is suggested that those personnel who attend external meetings be required to present information to the Section upon their return to work. An outline of the presentation and attendance can be maintained as documentation of an in-house training session. Another possible way to comply, would be to conduct Journal Article Review sessions.

Validation (PowerPlex 1.1)

It is suggested that notations be made to outline any deviations from the published protocols of Hitachi and Promega, as well as validation work done on these modifications. (DAB 8.1.3.4)

It may be beneficial to have summary sheet(s) on the validations studies for defense discovery motions. Sometimes this will be sufficient and prevent extensive copying and/or visits to your laboratory.

Analytical Procedures (DAB 9.1.2)

The description and formulation of reagents is not found in the analytical procedures but is located in the DNA QA manual. Reference to this location should be made in the Technical Manual.

It would be clearer to add a disclaimer to the Technical Manual that STR Call is not required but is optional for casework.

Remove RFLP sections during the next revision.

A comment should be added to the QA manual (Section 7 pg5) about the required yearly NIST SRM (DAB 9.5). This information is currently located in ADM 98-31)

Release of Casework Information (DAB 11.1.3)

For easier retrieval, a reference to this information in the CLP manual could be made in the DNA QA manual.

Equipment, Calibration & Maintenance (DAB 10.2,10.2.2)

The DNA QA manual does contain documented programs of calibration and their frequency for certain equipment, but not all. The thermometers had a form present but no required time frame. There are not scheduled time references for calibration or checks of other equipment such as centrifuges, hot shakers, autoclaves, etc.

The DNA QA manual should be modified to allow for an increase of tolerance ranges for temperatures of refrigerators and freezers. Currently, several refig./freezers charts show temperatures out of the acceptable +/- 2 degrees.

The thermometers have a tolerance range of +/- 1 degree and calibration documents recorded that #802774 was 2 degrees off and was left in service (though labeled as such).

This is a validation of your procedures which state that it should have been removed.

Database Standard 1.1

This requires written procedures for generating and maintaining documentation for database samples.



This is not as clearly defined as casework, in regards as to which forms, etc. will be found in each gel folder. Also the Laboratory may wish to generate a review check-off list for database samples, especially when sub-contractors are used to perform the analyses.

Review

Although there is clearly an understood policy regards to handling discrepancies between analysts and reviewers, it is not spelled out within the QA Manual. (DAB 12.1.1)

Casework Review

The QA manual states that one should visually assess stutter. How is this performed and agreed upon without a mathematical reference? For consistency within the Laboratory perhaps guidelines should be developed to handle situations of stutter in regards to carry-over in mixed stains.

Perhaps the Laboratory may want to conduct a mini-stutter study using the raw data in the population databases, to demonstrate that your stutter values are in accordance with West Palm Beach. This would just require some number crunching.

Bands occurring in the $n + 4$ locations should also be addressed within the guidelines.

The latest version of the guidelines handles partials profiles nicely by listing the number of bands present and those missing. This better clarifies your results versus just stating that the partial profiles are consistent with a donor.

Personnel Interviews

Information obtained from the personnel interviews demonstrated that the Section had a great sense of team spirit, that they were dedicated to quality while keeping casework turn around times to a minimum, that they felt they were provided a opportunity for training, that supervisor and technical leadership was above adequate, and that they really enjoyed their work.

A general trend in the conversations was that sufficient equipment existed but not enough staff. Other topics on a "wish list" would include:

1. More space for the database operations, possibly even their own lab
2. A position dedicated solely to CODIS operations.
3. Moving non-subject cases to a higher priority perhaps even before the validation of mtDNA analysis. And/or hiring staff dedicated to working non-subject cases.
4. A position whose top priority was handling QA/QC matters, or hiring technicians to handle these duties.
5. Cross-training opportunities between Serology and DNA Units.
6. Some sort of paging system, so that the staff can be easily contacted and located.
7. Additional chemical fume hood for phenol extractions.

Concerns were expressed that the new computer system used for reports was taking longer in the area of review. However, it is new and adjustments are still being worked out.



RESPONSE OF THE DNA UNIT SUPERVISOR

The comments for improving the Quality Assurance Manual found under the topic headings:

Quality Assurance Program
Personnel
Release of Casework Information
Database Standard 1.1
Review
Equipment, Calibration, & Maintenance
Casework Review

are all excellent ones and have been incorporated into the Quality Assurance Manual (see attachments).

The comments for improving the Technical Procedures Manual found under the heading Analytical Procedures, will have also be incorporated in the Technical Procedure Manual (see attachments).

The comments under the heading Validation(PowerPlex 1.1) are somewhat confusing. We already have summary sheets on each set of experiments conducted, however this material is not released for defense review under our statutes. No deviations from protocol established within the unit occurred unless they were part of the actual validation. Any deviations from the validated protocol we use done after the validation experiments have been validated using side-by-side experiments.

The comments under the heading Continuing Education and Personnel Interviews are concurred with and have already been considered.

99memo/auditresponse.wpd



2. Qualification and Training of Personnel

All persons involved in the actual recovery, evaluation, analysis, and interpretation of body fluid identification and DNA evidence shall have a background appropriate to their duties.

All examiners will go through a training process which uses a three-prong test for competence:

- a. Knowledge of the scientific literature and procedures with reference to body fluid identification procedures and/or DNA typing. This will be evaluated by grades received in courses taken or by written tests given as part of an in-house training program.
- b. Skills and mechanical abilities to perform the test which can be evaluated by the observation of qualified personnel and by determining if the proper test results are obtained.
- c. The ability to correctly interpret the test results being of paramount importance. All examiners will undergo proficiency testing periodically to test this skill.

2.1 Job Description

A current copy of all job descriptions within the Molecular Genetics Section will be maintained by the Section SAC in each employee's personnel file.

2.2 Education, Training, and Qualifications of Personnel

2.2.1 Requirements for Individuals Performing Body Fluid Identification Analysis



2.2.1.1 All analysts in the SBI Molecular Genetics Section must possess a minimum of a baccalaureate degree in a biological science (biology, zoology, medical technology, microbiology, biochemistry, etc.).

2.2.1.2 Completion of the Body Fluid Identification Training Program

2.2.1.3 Completion of a series of competency test samples

2.2.2 Requirements for Individuals Performing Forensic DNA Analysis

All analysts will meet the following requirements prior to performing DNA analysis on casework. These requirements will also apply to the Section SAC.

2.2.2.1. A Bachelor's Degree in a biological science and undergraduate and/or graduate level course work in biochemistry, genetics and molecular biology, course work and/or training in statistics and population genetics as it applies to forensic DNA analysis, and 6 months of forensic DNA experience.

2.2.2.2 Molecular biology courses at NC State University (or comparable courses at or another university) to include:

- a. Genetics 501 -Biochemical Genetics - 1 semester hour (Note - Genetics 501 and 502 have been replaced by Genetics 513)**
- b. Genetics 502 - Microbial Genetics - 1 semester hour**
- c. Genetics 560 - Biochemical and Microbial Genetics 3 semester hours credit - Genetics/Biochemistry 561 is a similar course taken by many Section members in the past to meet this requirement. However, this course is no longer taught.**



- 2.2.2.3. Completion of the DNA Training Program
 - 2.2.2.4. Completion of a series of competency test samples
 - 2.2.2.5. Prior to analysis of casework samples, the analyst must have completed the appropriate training programs and competency tests.
- 2.2.3 Requirements for Individuals Performing DNA Database Testing
- 2.2.3.1. A Bachelor's Degree in a biological science
 - 2.2.3.2. Completion of the DNA Database Training Program
 - 2.2.3.3. Completion of a series of competency test samples
 - 2.2.3.4. Database analysts are encouraged to meet the same course requirements set for forensic DNA analysts.
- 2.2.4 Requirements for DNA Technical Manager/Leader (Special Agent In Charge)
- The DNA Technical Manager/Leader will meet the following Quality Assurance Standards For Forensic DNA Testing Laboratories
- 2.2.4.1. A Master's Degree in a biological science or forensic-science related area.
 - 2.2.4.2. A minimum of 12 semester hours in biochemistry, genetics, molecular biology, and statistics or population genetics.



2.2.4.3 A minimum of 3 years of forensic DNA experience.

2.3 Continuing Education

Section analysts must stay abreast of developments within the field by reading current scientific literature and by attendance at seminars, college courses, or professional meetings. Management must provide analysts with an opportunity to comply with the above requirements as resources permit.

2.4 Training Records

Documentation of all training will be maintained in the Section office. A training log is maintained in each employee's personnel file by the SAC.

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Documentation

3.1 Current Procedures Manual

A copy of the current appropriate analytical testing procedure(s) used in the DNA typing of biological material and/or the identification of body fluids will be maintained by each Section analyst.

3.2 Operational Guidelines

3.2.1 Receipt, Identification, Storage and Handling of Evidence Submitted to the Crime Lab

Bureau guidelines for the receipt, identification, storage, and handling of evidence submitted to the Crime Lab are documented in the SBI Crime Lab Procedures Manual. Case samples will be handled and stored under adequate conditions to protect the evidence.

3.2.2 Guidelines Establishing Proper Specimen Criteria

A copy of the SBI case acceptance policy for the DNA typing of biological materials can be found in Appendix A immediately following this section of the Quality Assurance Manual. Internal guidelines for case submissions to the DNA unit are also found in Appendix A.

3.2.3 Storage of Evidence, Destruction and Disposition of Evidence

All evidence submitted for testing will be stored under the appropriate conditions to minimize degradation of the sample.



The nylon membranes generated during the RFLP analysis procedures and slot blots, and any left over extracted DNA from the case samples will be maintained within the Section for a period of at least five calendar years.

Upon completion of DNA analysis, any evidence stains not consumed in analysis will be cut in half; half will be returned with the submission containers to the submitting officer along with a copy of the final report. The other half of the sample and any extracted DNA will be stored frozen for a period of at least five years. After five years, the stored material may be destroyed.

Amplified DNA from STR typing of case samples will be stored frozen for one month, and then discarded.

Any test results and all notes and documentation will be saved in the appropriate file as dictated by the laboratory and Bureau policy.

3.2.4 Guidelines For The Proper Recording Of All Analytical Data From Casework

The following information will be recorded in the permanent file of every case submitted for analysis. The pre-printed forms used can be found in Appendix B immediately following this section.

1. A SBI Physical Evidence Examination Request Form (SBI-5).
2. A sample description, including packaging information.
3. Notes to document all tests performed on each item and those test results.
4. A laboratory case notes cover sheet.
5. A laboratory results computer disposition form.
6. Any documentation or notes relevant to testing procedures.
7. Technical review checklist.
8. Evidence retention log.
9. The final lab report.



In the case of RFLP DNA analysis the following additional information will appear in the permanent case file. Copies of these documents can also be found in Appendix B.

1. A procedure data sheet to document the flow of testing procedures.
2. A sample description form.
3. Results of the estimation of the quality and quantity of the DNA recovered from the yield gel.
4. Results of the restriction digestion of samples which includes the sample protocol and photographs of the results.
5. Analytical gel data.
6. Autoradiography or lumigraph results and the hard copy printout from the video imaging printer, the computer printout of the molecular weights of the bands observed, the population frequency data calculations, percent difference calculations, MJB-K562 quality control sheet, technical review form, computer disposition form, and a copy of the population databases used.

In the case of STR DNA analysis, the following additional documents will appear in the permanent case file. Copies of these documents can be found in Appendix B.

1. A sample description, including packaging information and type of DNA extraction performed.
2. A lumigraph and quantitation sheet.
3. An amplification worksheet.
4. An electrophoresis worksheet and computer generated scans.
5. PopStats sheets reflecting population frequencies and a copy of the databases used (if applicable).



All test results generated within the Section, will be technically reviewed by a qualified analyst and undergo an administrative review by the Section SAC, or his designee.

The following documents will appear in the permanent files of the DNA Database Unit for STR analysis:

1. An extraction worksheet which will provide documentation of the sample bar code number, sex and race. Comments concerning sample quality may also be included.
2. An amplification worksheet.
3. An electrophoresis worksheet and computer generated gel scans.
4. A call sheet generated by the appropriate analysis software or generated manually.
5. A second read sheet.

3.2.5 Data Handling, Storage, and Retrieval

All case files will be maintained by the laboratory Clerical Services Supervisor, the Records Section, or State Archives. If duplicate copies are needed for court, defense experts, etc., they will be made from the originals as needed.

All original autoradiographs, lumigraphs, and optical disks with gel scans and associated workbooks will be stored in a locker in the Section Evidence Vault.

Copies of all quality control and calibration sheets will be maintained by the quality control officers or in the Molecular Genetics Section files.

Validation studies, population studies, and research project results will be maintained in the Molecular Genetics Section.



3.3 Material Safety Data Sheets

Material safety data sheets will be maintained on all chemicals and reagents used in the Molecular Genetics Lab. The Section Safety Officer will file these sheets in a designated place accessible to all personnel.

3.4 Schedules and Procedures for Maintenance, Inspections, Testing and Calibration of Pertinent Equipment

An inventory will be kept of all equipment used in the Section as described in Section 4.1.2 of this document. Anytime a piece of equipment requires service or maintenance, this will be documented.

Calibration instructions and logs will be kept on pertinent equipment items as described in Section 4.1.6 of this document.

3.5 Historical File of All Past Analytical Testing Methods, Procedures and Guidelines

A file will be maintained in the Section office containing all out-dated analytical testing methods, procedures, and operating guidelines. Items in this file will be maintained for five years, or until their usefulness ceases to exist.

3.6 Batch or Lot Number of Materials Considered Critical to DNA Typing

Batch or lot numbers of materials considered critical to the identification or DNA typing method will be maintained by the QC Officer(s). This material will be maintained in a file in the Section office for a period of 5 years.



3.7 Records of Methods Validation

All records of in-house methods validation testing will be maintained in the Molecular Genetics Section.

3.8 Personnel Records

The Section SAC maintains a personnel file on each analyst which is subdivided into the following categories:

1. Personnel history, assignments, promotions, etc.
2. Commendations
3. Complaints and disciplinary action
4. Training
5. Evaluations
6. Equipment issued
7. Job descriptions

The Section SAC also maintains a separate file of proficiency test results from each trainee, and proficiency test results from trained analysts.

3.9 Quality Assurance and Audit Reports

Copies of audit reports will be maintained in section files. Those reports generated by the SBI Inspection Program will be stored according to Bureau procedure. Results from the external audit program, described in Section 10 of this manual, will be maintained by the Section SAC.



3.10 Safety Manuals

Copies of the Safety Manual described in Section 11 of this document will be distributed to every employee of the Molecular Genetics Section.

3.11 Licenses and Certificates

Copies of all licenses and certificates awarded to the Molecular Genetics Section will be maintained in the Section's files.

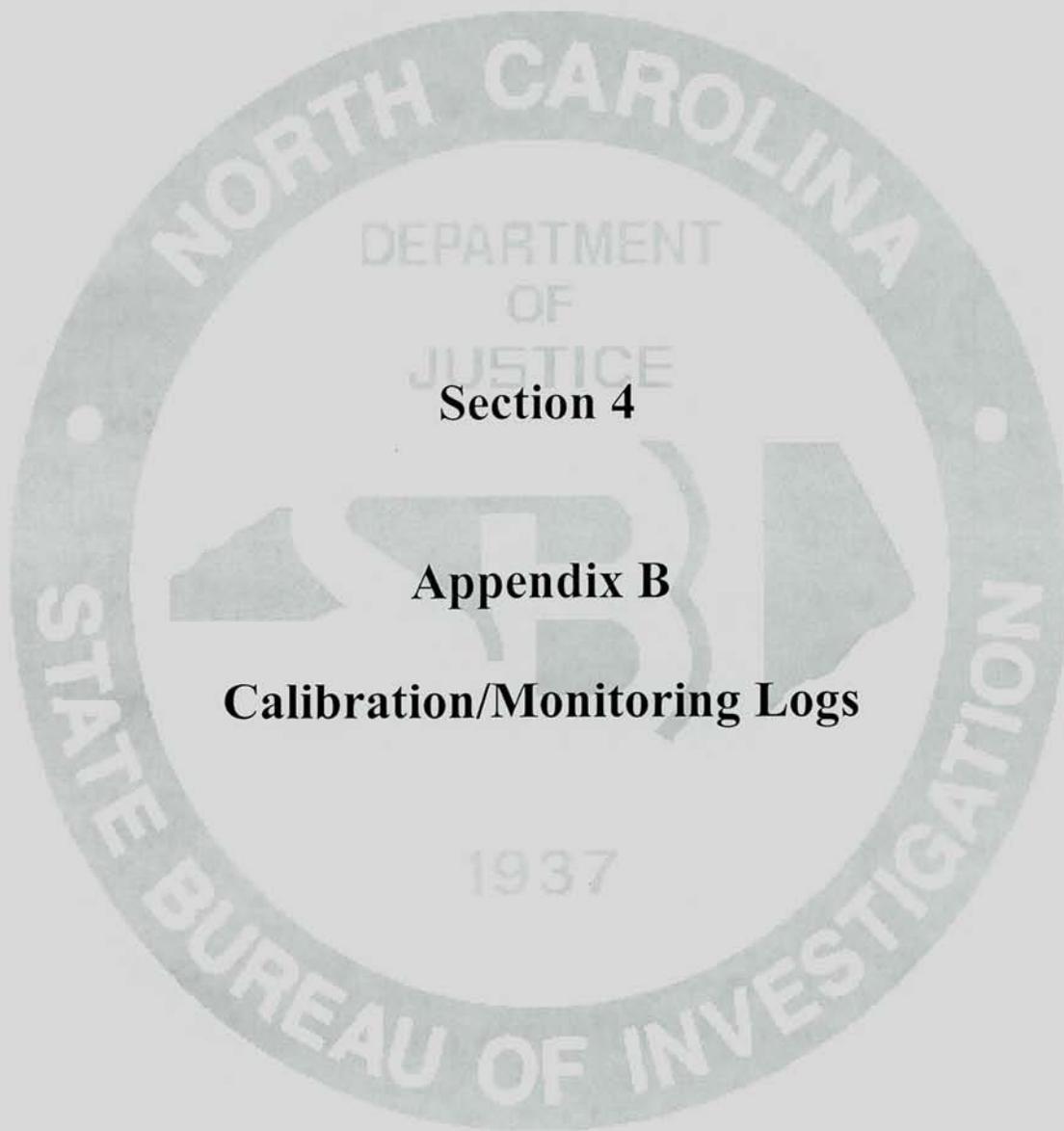
3.12 Population Frequency Data

Data used to create population databases will be maintained in the Molecular Genetics Section files along with the original work sheets and autoradiographs or computer generated scans.

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Section 4

Appendix B

Calibration/Monitoring Logs

Temperature Quality Control Record
Notify the SAC if the temperature is more than
+/- 5 degrees off the set temperature.

Location:

Equipment:

Number:

MOLECULAR GENETICS
SECTION

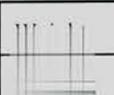


199	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept	Oct.	Nov.	Dec
1												
2												
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Balance Calibration Log

Balance: _____ Number: _____ Location: _____ Year: _____

Month: January Analyst: _____

	1 mg	5 mg	10mg	25mg	50mg	100mg	500mg	1g	5g	10g	25g	50g	100g	150g	200g

Month: February Analyst: _____

Weight	1 mg	5 mg	10mg	25mg	50mg	100mg	500mg	1g	5g	10g	25g	50g	100g	150g	200g
Reading															

Month: March Analyst: _____

Weight	1 mg	5 mg	10mg	25mg	50mg	100mg	500mg	1g	5g	10g	25g	50g	100g	150g	200g
Reading															

Month: April Analyst: _____

Weight	1 mg	5 mg	10mg	25mg	50mg	100mg	500mg	1g	5g	10g	25g	50g	100g	150g	200g
Reading															

Month: May Analyst: _____

Weight	1 mg	5 mg	10mg	25mg	50mg	100mg	500mg	1g	5g	10g	25g	50g	100g	150g	200g
Reading															

Month: June Analyst: _____

	1 mg	5 mg	10mg	25mg	50mg	100mg	500mg	1g	5g	10g	25g	50g	100g	150g	200g

Month: July Analyst: _____

Weight	1 mg	5 mg	10mg	25mg	50mg	100mg	500mg	1g	5g	10g	25g	50g	100g	150g	200g
Reading															

Month: August Analyst: _____

Weight	1 mg	5 mg	10mg	25mg	50mg	100mg	500mg	1g	5g	10g	25g	50g	100g	150g	200g
Reading															

Month: September Analyst: _____

Weight	1 mg	5 mg	10mg	25mg	50mg	100mg	500mg	1g	5g	10g	25g	50g	100g	150g	200g
Reading															

Month: October Analyst: _____

Weight	1 mg	5 mg	10mg	25mg	50mg	100mg	500mg	1g	5g	10g	25g	50g	100g	150g	200g
Reading															

Month: November Analyst: _____

Weight	1 mg	5 mg	10mg	25mg	50mg	100mg	500mg	1g	5g	10g	25g	50g	100g	150g	200g

Month: December Analyst: _____

Weight	1 mg	5 mg	10mg	25mg	50mg	100mg	500mg	1g	5g	10g	25g	50g	100g	150g	200g
Reading															

Temperature Monitoring

The working temperatures of all incubators, water baths, refrigerators, freezers, and heat blocks will be checked either before each use or daily, depending on the application, with a lab or integral digital thermometer. The temperature will be recorded on the Temperature Quality Control Record, and these forms will be maintained by the QC Officer in the Unit.

Lab and integral thermometers will be calibrated yearly at the working temperature against a National Institute of Standards and Technology certified thermometer.

Example Given:

For calibrating a thermometer used in a 56°C water bath:

1. Place both the certified and lab thermometer in the water bath
2. Adjust the temperature of the water bath until the certified thermometer reads 56°C
3. Document the temperature of the lab thermometer
4. If the lab thermometer has a significant difference in temperature with the certified thermometer, the lab thermometer will be discarded

One can also calibrate a thermometer by immersing it in boiling water (100°C) and ice water (0°C). Discard any thermometers that can not be calibrated.

Temperature Verification for the Perkin Elmer Thermocyclers

Use the manufacturer's instructions for each of the different Perkin Elmer thermocyclers in the laboratory.



7. Internal Quality Control and Standards

7.1 Body Fluid Identification

7.1.1 Stain Handling

Stain selection, handling, and/or extraction will be performed as detailed in the appropriate technical procedures.

7.1.2 Known Standards for Tests

1. Preliminary: Phenolphthalein and luminol solutions will be tested prior to use against known bloodstains.
2. Takayama reagent will be made fresh from stock solutions and will be tested against known blood stains.
3. Precipitin antisera will be tested for specificity when received by the Quality Control Officer. For each sample extract tested against known species antiserum, a control will be run against the host serum.
4. Semen identification tests, i.e. acid phosphatase spot plate tests, will be tested simultaneously with a known semen and a blank reagent control.
5. Amylase tests (i.e. Phadebas) will be simultaneously tested against known saliva and a blank reagent control.

7.2 RFLP Controls and Standards

The following controls and standards will be run at each step of the RFLP analysis in accordance with the RFLP Procedure.

7.2.1 Use of Human DNA Controls (RFLP Testing)

For each case, a known human bloodstain from a laboratory donor that has been well characterized will be run at the same time and in concordance with the known samples in that case.



The DNA Database Unit uses a blood sample which has already been typed, but its identity has been encoded. The sample is treated as an internal, open proficiency test.

The searching capabilities of the CODIS system is used to identify the sample. In addition, a known human cell line control K562 will be utilized starting at the yield gel and it will be run along with the samples. Operational monitoring of these controls will verify the procedural method in use.

7.2.2 Procedure for Estimating DNA Recovery

The following methods will be employed to determine the quantity and/or quality of the DNA recovered from specimens.

7.2.2.1 Yield Gels

A visual marker will be placed on each yield gel run to determine if the DNA extracted from the case samples is high molecular weight or degraded DNA, and to monitor the electrophoresis run conditions.

7.2.2.1.2 Yield Calibration Set

An appropriate yield calibration set consisting of DNA of known concentration will be run on each yield gel so that an estimate can be made as to the quantity and quality of DNA present in the case samples.

7.2.3 Test Gels

Digestion of extracted DNA by the restriction enzyme will be demonstrated using a Test Gel which includes the following:



7.2.3.1 Visual Marker

A visual marker will be placed on each test gel run to monitor run conditions.

7.2.3.2 Digestion Control

A pre-digested control from a known human cell line will be run on each test gel to demonstrate the proper level of digested DNA by the restriction enzyme.

7.2.4 Analytical Gel

An analytical gel used to separate the restriction fragment must include the following controls:

7.2.4.1 Molecular Weight Sizing Marker

An appropriate number of the proper molecular weight sizing markers will be run on each analytical gel, so that a determination can be made as to the size or molecular weight of the case sample DNA fragments. Case samples must be bracketed (as defined by the sample lanes) by molecular weight size markers.

7.2.4.2 Visual Marker

A visual marker will be run on each analytical gel. The visual marker monitors the migration of the DNA through the analytical gel and establishes the end point of electrophoresis.



7.2.5 Southern Blot/Hybridization

The performance of these two stages of the RFLP analysis is monitored by the transfer and hybridization of the cell line and size marker controls.

7.2.6 Autoradiography/Chemiluminescence

Exposure intensity will be monitored by the use of multiple films or by successive exposures in order to obtain films of the proper intensity for image analysis.

7.2.7 Image and Data Processing

The functioning of image and data processing is monitored by the human DNA control allelic values.

7.3 STR Controls

The following controls will be run at each step of the STR analysis in accordance with the STR Procedures Manual.

7.3.1 Use of Human DNA Controls

For each case, a known human cell line control K562 will be amplified and run along with the samples. A MJB sample will be extracted and also run with the case samples. Operational monitoring of these controls will verify the procedural method in use.

7.3.2 Extraction Controls

For each set of extractions, a reagent blank will be prepared. This blank will consist of the reagents used in the extraction process and be treated as another sample.



7.3.3 Procedure for Estimating DNA Recovery

An appropriate yield calibration set consisting of DNA of known concentration will be run on each membrane so that an estimate can be made as to the quantity of DNA present in the case samples.

7.3.4 Lumigraphs

Exposure intensity will be monitored by the use of multiple films or by successive exposures, if necessary, in order to obtain films of the proper intensity so that quantitation can occur.

7.3.5 Amplification Control

A reagent blank consisting of sterile water and the amplification reagents will be run at the time of amplification. This amplified sample will be run on the analytical gel as a negative amplification control.

7.3.6 Analytical gel

The analytical acrylamide gel will contain the following controls and markers:

1. K562 and MJB Positive Controls - the DNA Database Unit will utilize only K562
2. Negative Amplification Control
3. Allelic Ladder for fragment allele designation (run with maximum of 2 sample lanes between ladders)
4. Reagent blanks for extraction chemicals



5. Visual marker to allow for the determination of the end of electrophoresis

7.3.7 Image and Data Processing

The functioning of image and data processing is monitored by the human DNA control allelic values.

7.4 NIST Standard Reference Material (SRM)

The DNA Unit will test the current analytical procedure in use against the appropriate NIST SRM kit (or standard(s) traceable to the NIST SRM) on an annual basis. When substantial changes or new procedures are validated, they will be checked against the appropriate NIST SRM as well.

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8. Data Analysis and Reporting

8.1 Independent Analysis of Data

All data, test results, and reports will undergo a technical review by a second qualified analyst. The analyst conducting the technical review will sign the appropriate review sheet. Both analysts must agree on the interpretation of the data to be reported. An administrative review of the case file will be completed by the Section SAC or his designee.

If an analyst and technical reviewer are unable to resolve a technical issue on which they disagree, then the administrative reviewer or the Special Agent In Charge will arbitrate the issue. Issues on how to report complicated mixtures which may fall outside section interpretation/reporting guidelines are often resolved at DNA Unit meetings where input from all analysts can be received. Such periodic meetings provide the basis for modifications and or changes in interpretation/reporting guidelines.

8.2 Tolerance Limits for Matches (RFLP Analysis)

For proper data interpretation, the measured sizes of the restriction fragment bands of the human DNA controls must fall within established tolerance limits.

The unknown or questioned samples will first be compared to the known samples visually for match/non-match status. All visual observations must be confirmed by measurement of the fragment sizes on the imaging system and the sizes of the questioned and known samples must fall within established tolerance limits before a match is determined. These tolerance limits are specified in Appendix A immediately following this section which also describes how results are assessed in forensic casework. The human DNA control as well as DNA controls from blood samples of SBI personnel will be monitored



on each case against established tolerance ranges (see Appendix A).

8.3 Interpretational Guidelines for Matches (STR Analysis)

For proper data interpretation, the allelic values of the human DNA controls must agree with documented allelic values.

The unknown or questioned samples will first be compared to the known samples visually for match/non-match status. All visual observations must be confirmed by a second reader for match status.

These guidelines are specified in Appendix B immediately following this section which also describes how results are assessed in forensic casework. The human DNA control will be monitored on each case against established guidelines (see Appendix B).

8.4 Frequency Determination

Frequency determinations will be made using established databases that are compatible with the DNA procedure in use. A scientifically valid and accepted method for calculating these frequency determinations has been developed by the FBI and will be used in this laboratory. Details of how frequencies are determined are found in Appendixes A and B immediately following this section.

8.5 Report Writing and Review

8.5.1 Report Writing

Lab reports will be issued on all cases and will be prepared in accordance with existing Bureau policy. In addition to the findings and conclusions of the analyst, the DNA locus



(defined by the Nomenclature Committee of the International GeneWorkshop), as identified by a particular probe or primer, as well as the restriction enzyme used to digest the DNA (if applicable), will be included.

8.5.2 Review of Reports

All lab reports will be reviewed by appropriately designated personnel as described in Section 8.1.

8.6 Release of Case Information

Analysts will follow the procedures delineated in the Crime Laboratory Procedures Manual (Section 16) for release of case information.

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STR Interpretation Guidelines

Introduction:

The interpretation of results in casework is necessarily a matter of professional judgement 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 some 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 needs to be re-run. 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. Substrate controls that are submitted to the DNA Unit *may be* run, should the case analyst, the technical reviewer, or the administrative reviewer feel that running them would be helpful in resolving any issues raised by the data.
- 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 consistent results for each sample. Failure to obtain identical 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. However, it is not permissible to "lighten" gel scans to remove bands from the scan.

2 Allele Identification

- 2.1 Analysts will report and record STR DNA types as phenotypes not genotypes.



2.2 Artifacts

2.2.1 From prior experience examining STR scans, it is known that a weak band(s) periodically appears at 4 bp 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 will vary for different loci. The table below lists calculated stutter percent information for PowerPlex 1.1 loci (data provided by the West Palm Beach County Laboratory):

Locus	% Stutter	Locus	% Stutter
CSF1PO	11	D16S539	12
TPOX	7	D7S820	10
THO1	3	D13S17	9
VWA	14	D5S818	11

Analysts should visually evaluate the potential stutter bands seen in light of the data presented above.

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 always has the option of running a 1-D Gel Analysis and Subsequently 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 is only valuable in determining if a possible weak band **IS NOT** stutter, but is not definitive in determining if a weak band **IS** stutter.

Analysts will document on allele call sheets or gel scans which bands they declare stutter or potential stutter bands **BEFORE** comparisons are made to known standards.

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 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. 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.

- 2.3.1.1 Variant alleles that vary by less than the consensus repeat unit will be designated as an integer of that variation (for example THO 1 9.3 allele), as per CODIS recommendations.



2.3.2 When STR scans contain off-ladder bands outside 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 situations 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.

2.3.2.1 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

Whether or not there is a match between patterns produced by the DNA samples extracted from two or more sources is primarily a qualitative judgement 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. A match evaluation applies to the entire pattern taken as a whole.



3.1 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.

3.2 Multiple Contributors

3.2.1 Major/minor contributors - 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:

3.2.1.1 Where two contributors are possible (e.g. victim and suspect), where all bands present in the questioned sample can be accounted for by the standards, there is a clearly predominant profile, and there is band sharing between the two contributors, the laboratory report will state:

The DNA profile obtained from (Item) is indicative of originating from more than one donor and is CONSISTENT WITH A MIXTURE. The predominant profile MATCHED the DNA profile obtained from (Item). The weaker profile is CONSISTENT WITH that of (Item).

3.2.1.2 Where two contributors are possible (e.g. victim and suspect), all bands present in the questioned sample can be accounted for by the standards, and there is no band sharing between the two contributors, the laboratory report will state:



The DNA profile obtained from (Item) is indicative of originating from more than one donor and 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 sample where a match statement is made.

- 3.2.2 There is no major/minor contributor - In the situation where there are no major/minor contributors, and all bands can be accounted for by the standards, then the laboratory report will state:

The DNA profile obtained from (Item) is indicative of originating from more than one donor and is **CONSISTENT WITH A MIXTURE** of the victim and suspect's DNA profiles (Items and , respectively).

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.

- 3.2.3 Partial profiles - In the situation where all the bands present in a mixture obtained from a questioned sample can be accounted for by 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 will be given:



The DNA profile obtained from (Item) is indicative of originating from more than one donor and is **CONSISTENT WITH A MIXTURE**. A partial DNA profile consistent with _____ was obtained where ____ (# of bands) from the total profile of ____ bands were detected, and ____ (# of bands) bands were missing.

- 3.2.4 **Known contributor** - In the case where one of the contributors is known (like the rape victim from a vaginal swab) the genetic profile of the unknown is readily inferred. This can be accomplished by subtracting the known donor's profile from the mixed profile.

4 Possible Conclusions

In a match evaluation, three conclusions are generally possible: 1) the patterns match and there is an inclusion (the patterns are consistent with having come from the same individual), 2) the patterns do not match and there is an exclusion (they are not consistent with having come from the same individual), or 3) the results are inconclusive, give no banding pattern, or are un-interpretable.

Matches and non-matches are determined by careful, objective qualitative and quantitative evaluation of the entire banding pattern produced by the various loci tested.

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 conclusions more typically occur on individual computer scans and may result from, but are not limited to, the following causes:

- a. insufficient amounts of DNA for that locus in one or more of the samples tested;
- b. degradation of one or more of the bands in any sample tested;
- c. preferential amplification due to great differences in amounts of DNA present in a sample from multiple donors,



d. inhibition.

It should be indicated, however, that it is completely acceptable scientifically 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 on only loci which yield conclusive results. An exclusion will be determined if only one locus probed produces exclusionary results.

The following statements are used in laboratory reports:

The DNA banding pattern obtained from (Item) MATCHED the DNA profile obtained from (Item) and DID NOT MATCH the DNA profile obtained from (Item). This match was made for HUMTHO1, HUMTPOX, HUMCSF1PO, HUMVWA, D16S539, D7S820, D13S317, D5S818, and Amelogenin.

The DNA banding pattern obtained from (Item) DID NOT MATCH the DNA profile obtained from the victim (Item) or the suspect (Item). This profile was searched against the casework/convicted offender indexes of the N.C.S.B.I. DNA State Database and no seven locus match was observed.

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 match before further testing will be performed.

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. This data has been entered in 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 The following statement will be used in laboratory reports to report population frequency information:

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 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 always had the legal authority to provide such testimony.

Other analysts in Latent Evidence, Questioned Documents, and Firearms have been offering this type of testimony for years. Now that the main issues dealing with the population genetics of DNA have been laid to rest with the National Research Council's 1996 publication entitled "The Evaluation of Forensic DNA Evidence" and a firm legal foundation for DNA testing is now in place, not only in this state and nation, but world-wide, the time to move forward has arrived.

Analysts are to advise the District Attorney that they will provide opinion testimony as to identity in advance of their testimony, and provide the District Attorney with 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 billion - new estimate just received from the US Census)

Issue Date: December 8, 1999
Prepared By : DNA Unit
Approved By : Mark Nelson
Originating Unit: DNA Unit

Supercedes: April 17, 1998
Date: November 30, 1999
Date: November 30, 1999



9. Proficiency Testing

9.1 Open Proficiency Testing

Each Section analyst conducting DNA testing will complete an open proficiency test every 180 days. The 180 day rule means that an analyst will receive a proficiency test within 180 days of completing the prior test, and that two tests will be completed each year. Each Section analyst conducting only Body Fluid Identification testing will be tested at least once a year with an open proficiency test.

9.2 Blind Proficiency Testing

Blind proficiency test specimens may be submitted to the Section and will be prepared in such a way as to appear to be routine case specimens. These specimens may be prepared internally and/or may be part of an external proficiency testing program.

9.3 Proficiency Test Files

The Section SAC will maintain all proficiency test records, any deficiencies noted, and corrective action taken. When deficiencies are noted, the file will identify the likely cause of the deficiency.

9.4 Proficiency Testing Standards

The Molecular Genetics Section will meet or exceed the proficiency testing requirements specified by ASCLD/LAB and the Quality Assurance Standards For Forensic DNA Testing Laboratories issued by the FBI Director.



9.5 Approved Vendors

Open proficiency tests will be purchased from an ASCLD/LAB approved vendor who prepares proficiency tests in a manner approved by ASCLD/LAB and which meet the Quality Assurance Standards For Forensic DNA Testing Laboratories issued by the FBI Director.

9.6 Corrective Action

Any time there is a discrepancy on a proficiency test, this section will follow the procedures outlined in the Crime Laboratory Procedures Manual (Section 20).

Any time questions arise concerning discrepancies or the efficacy of a technical procedure used in casework analysis, the Special Agent In Charge will immediately investigate the issue.

If the concern is with the efficacy of a technical procedure, this procedure will be suspended from use in case work until the problem has been resolved, and the procedure can be shown to work as expected.

Any time questions arise from case work of analyst competency or performance, the Special Agent In Charge will immediately investigate the issue.

In this instance, the analyst may be suspended from case work analysis until the problem has been successfully resolved. The guidelines offered in the Crime Laboratory Procedures Manual dealing with discrepancies on proficiency tests will be used as a model for corrective action in case work analysis as well.

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Quality Assurance Manual

Section 9

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If the problem is administrative or clerical in nature, it will no require any more than an investigation of the reason for the error, and instructions to the analyst of how to avoid this problem in the future. The guidelines offered in the Crime Laboratory Procedures Manual dealing with discrepancies on proficiency tests will be used as a model for corrective action in case work analysis as well.

Documentation of all corrective actions taken, whether on proficiency tests, case work, or of technical procedures, will be maintained by the Special Agent In Charge.

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Prepared By : DNA Unit

Approved By : Mark Nelson

Originating Unit: Molecular Genetics Section

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Date: November 30, 1999

Date: November 30, 1999

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Information on the formulation, storage conditions, and expiration date for laboratory prepared buffers and solutions can be found in Section 4, Appendix C of the Molecular Genetics Section Quality Assurance Manual.

manuals\procedures\bufferprep.wpd

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STaR CALL

1. Turn on computer by holding down far right button with arrow pointing left on it.
2. Go up to top left corner of screen, click on the "apple".
3. Scroll down to Microsoft Excel alias or use the Microsoft Excel icon on the desktop.
4. New screen appears with a menu bar across the top and a "codisdbf.xls" dialog box at bottom right. (DO NOT CLOSE OUT of this dialog box. It must be opened for STaR CALL to operate. It is the lookup table containing known bp sizes of the standards).
5. Click on StaR Call, then Import STR/RFLP.
6. Chose file to open (xx.dat). Click on open.
7. Click on:
 - a. STR – to calculate bp and genotype
 - b. STR with 1OD values -- to calculate bp, genotype, and % stutter.
8. Go up to STaR CALL (top menu bar).
 - a. Hold down mouse button and go down to "Evaluate STR Data".
 - b. Release mouse button
9. New dialog box opens "STaR CALL. Evaluate STR data".
 - a. Check to ensure your allelic ladders are correct
 - b. Click OK



10. **“STaR CALL - Select Lookup Table” dialog box opens for you to select look up table.**
 - a. **Click on correct table, i.e., .1CH (Channel 1)**
 1. **Choose Powerplex CTTV**
 2. **If samples contain Amelogenin - choose Powerplex + Am**
 - b. **Click OK**
11. **STaR CALL will now perform Genotype Analysis.**
12. **Once analysis is complete, computer asks “Review Lookup Table”. Click NO.**
13. **Go up to FILE**
 - a. **SAVE AS**
 - b. **Name file (eg. DB99-1.1ch.ss or R1999000100.1ch.ss)**
 - c. **Click SAVE**
14. **Go to FILE -- PRINT - OPTIONAL - (It is preferable to print a Landscape version of the data - see below).**
15. **You can have STaR Call reduce your data onto 1 sheet by:**
 - a. **Clicking on STaR CALL**
 - b. **Scrolling down to “View STR (landscape)”**
 - c. **Release mouse button**
 - d. **Go up to FILE**



1. SAVE AS
 2. Name workbook (eg. DB99-1.1ch.wkbk or R1999000100.1ch.wkbk)
 3. Click SAVE
- e. Go to FILE -- PRINT
- f. Close window (click on small box in upper left hand corner of table)
- g. Computer may ask "Save changes in microsoft excel format?" -- Click NO
16. Repeat for Channel 2 data (.2CH.DAT)
17. Once finished go to FILE -- Scroll down and click on QUIT.
18. See merge StaR Call procedure.

**MICROSOFT EXCEL/ STAR CALL PROGRAMS ARE NOW CLOSED.
TO SHUT OFF COMPUTER : GO TO SPECIAL (TOP MENU BAR). SCROLL DOWN
AND CLICK ON SHUT DOWN.**

NOTE: Analysts are not required to make allele calls from StaR Call for case work analysis, but may manually read the gel scans if they prefer.

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Date: November 30, 1999

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COLONEL DAVID B. MITCHELL
SUPERINTENDENT

October 21, 1999

Mark S. Nelson, Special Agent in Charge
Molecular Genetics Section
NC State Bureau of Investigation
3320 Garner Road
P.O. Box 29500
Raleigh, NC 27626-0500

Dear Agent Nelson:

On October 13-15, 1999, I conducted an audit of the Molecular Genetics Section of your Laboratory. The DNA Advisory Board Standards Audit Checklist was used as a guide for the audit. The results of the audit showed that the Molecular Genetics Section is in full compliance with the DNA Advisory Board Standards and to the credit of your laboratory personnel, this service is of the highest quality and is to be commended.

The personnel interviews were very informative and reinforced my opinion that you have a staff very dedicated to quality and who support the Section's goals and objectives. Please convey my gratitude to all members of the Section for their cooperation, assistance and professionalism.

As you know a completed checklist was left at your laboratory. Several recommendations were offered as a mechanism to enhance your existing high quality DNA operations. As always when visiting other laboratories I found many good ideas which I will share with my staff to enhance our operations.

The new facility is very impressive, and your operations are well run with good leadership and expertise. Again, the laboratory is to be commended for its high quality DNA work product.

Sincerely,

Teresa M. Long
Forensic Chemist Manager/Biology Section
Maryland State Police Crime Laboratory

"Maryland's Finest"